Review

Neuronal Ca^{2+}/Calmodulin-Dependent Protein Kinase II—Discovery, Progress in a Quarter of a Century, and Perspective: Implication for Learning and Memory

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Much has been learned about the activity-dependent synaptic modifications that are thought to underlie memory storage, but the mechanism by which these modifications are stored remains unclear. A good candidate for the storage mechanism is Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II). CaM kinase II is one of the most prominent protein kinases, present in essentially every tissue but most concentrated in brain. Although it has been about a quarter of a century since the finding, CaM kinase II has been of the major interest in the region of brain science. It plays a multifunctional role in many intracellular events, and the expression of the enzyme is carefully regulated in brain regions and during brain development. Neuronal CaM kinase II regulates important neuronal functions, including neurotransmitter synthesis, neurotransmitter release, modulation of ion channel activity, cellular transport, cell morphology and neurite extension, synaptic plasticity, learning and memory, and gene expression. Studies concerning this kinase have provided insight into the molecular basis of nerve functions, especially learning and memory, and indicate one direction for studies in the field of neuroscience. This review presents the molecular structure, properties and functions of CaM kinase II, as a major component of neurons, based mainly developed on findings made in our laboratory.

Key words Ca^{2+}/calmodulin-dependent protein kinase II; memory; protein phosphorylation; Ca^{2+} signaling; plasticity; postsynaptic density

1. INTRODUCTION

In the central nervous system (CNS), the synapse is a specialized junctional complex by which axons and dendrites emerging from different neurons intercommunicate. Changes in the efficiency of synaptic transmission are important to a number of aspects of neural function. Synaptic transmission occurs through neurotransmitters. Synaptic plasticity is defined as an activity-dependent change in synaptic transmission. Transient modifications of synapses have been associated with short-term memory and more lasting changes have been associated with long-term memory in the mature neuron.

Neurotransmitters and other signaling molecules bind to cell surface receptors, and regulate cellular functions. Signal transduction mechanisms at the plasma membrane lead to the generation of intracellular signals and second messengers. These molecules interact with specific target molecules to initiate a cascade of biochemical events and lead to a change in cellular function. Calcium ion (Ca^{2+}) is an universal second messenger in eukaryotic cells. Ca^{2+} plays an essential role in the basic operation of neurons through synaptic communication. Cells typically maintain an intracellular Ca^{2+} level of 10^{-7} M, which is 10^4 times lower the level outside the cells. The intracellular Ca^{2+} level rapidly increases up to 10^{-4} M derived from extracellular and intracellular sources in response to extra cellular stimuli. The predominant intracellular receptor of Ca^{2+} is calmodulin, a EF-hand family Ca^{2+}-binding protein. Calmodulin is a highly conserved Ca^{2+} sensor that is ubiquitously expressed in mammalian cells, and its major targets are protein kinases.

Protein phosphorylation is a major regulatory mechanism of signal transduction. A large number of extraceluular signalings have been shown to regulate protein phosphorylation in the nervous system. Neuronal CaM kinase II became known as a memory molecule with the report that transgenic mice lacking the α isoform of this kinase are defective in long-term potentiation (LTP, a potential cellular mechanisms of learning and memory) and spatial learning,[1] and interest has been directed foward the molecular mechanism of learning and memory through the action of this kinase. There are many interesting features of this kinase. This review will focus on various aspects of neuronal CaM kinase II. Figure 1 shows a schematic representation of the activation of CaM kinase II and of the regulation of neuronal functions. Several other reviews on CaM kinase II provide additional perspectives for the interested reader.[2—8]

2. DISCOVERY OF CaM KINASE II

2.1. Calmodulin-Dependent Protein Phosphorylation in the Brain When cAMP-dependent protein phosphorylation was demonstrated in the regulation of tyrosine hydroxylase, a rate limiting enzyme of catecholamine,[9,10] protein phosphorylation was noticed as the important regulatory mechanism in the nervous system. Ca^{2+}-dependent phosphorylation of particulate proteins in the rat brain and other tissues was found to be stimulated by a calcium-dependent regulator, later named calmodulin.[11,12] These reports did not pay attention to the kinase itself.

Independently, role of cAMP and Ca^{2+} in the regulation of catecholamine and serotonin biosynthesis was investigated through activation of their rate limiting enzymes, tyrosine hydroxylase and tryptophan hydroxylase, respectively (Table 1). cAMP activates tyrosine hydorxylase, which is simultaneously phosphorylated by cAMP dependent protein kinase (PKA), in the adrenal medulla.[13] Meanwhile, Ca^{2+} activates both tyrosine and tryptophan hydroxylases in the brainstem. Ca^{2+}-dependent activation also requires ATP and calmod-

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ulin, suggesting that a Ca^{2+}-dependent protein kinase is involved in the activation of these hydroxylases.[14—16] Furthermore, protein phosphorylation activity, especially Ca^{2+}-dependent activity, is much stronger in the brain than in other tissues of the rat.[14] Most of the Ca^{2+}-dependent endogenous phosphorylation of rat brain cytosol proteins require calmodulin.[17] These observations reinforce the idea that there is a new Ca^{2+}-calmodulin-dependent protein kinase in the brain which has a broad substrate specificity and phosphorylates many proteins. At that time, only two kinases, myosin light chain kinase and phosphorylase kinase, were known as calmodulin-dependent protein kinases. These two kinases are specific to myosin light chain and phosphorylase b as a substrate, respectively.

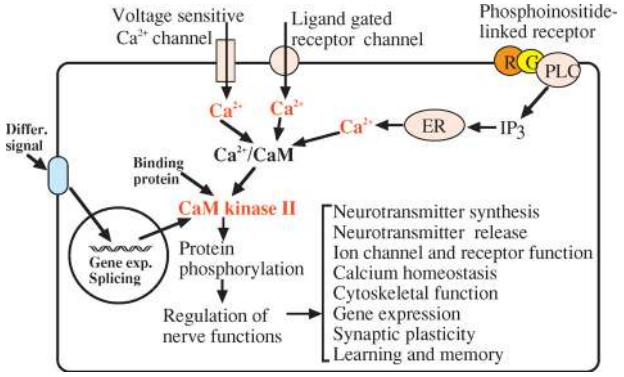

Fig. 1. Schematic Representation of Activation and Role of CaM Kinase II in Neuronal Cells

Intracellular Ca^{2+} is increased by extracellular stimuli, binds to calmodulin, and activates CaM kinase II. CaM kinase II phosphorylates various kinds of proteins and regulates physiological processes. CaM kinase II protein is induced by the stimuli of differentiation.

Table 1. Effect of cAMP and Ca^{2+} on the Activation of Tyrosine Hydroxylase and Tryptophan Hydroxylase in the Presence of ATP

Tissue	Enzyme	cAMP[a)]	$Ca^{2+a)}$
Brainstem	Tyrosine hyrdoxylase	Yes	Yes
	Tryptophan hydroxylase	No	Yes
Adrenal medulla	Tyrosine hydroxylase	Yes	No

a) Yes, activation of enzyme; no, no activation of enzyme.

2.2. Identification of CaM Kinase II CaM kinase II is the first demonstrated in 1980 by gel filtration of calmodulin-dependent protein kinases of rat brain monitoring the activation of tryptophan hydroxylase and the phosphorylation of endogenous proteins. It was the second Ca^{2+}/CaM-dependent protein kinase peak eluted from a sizing column used to fractionate the calmodulin-dependent protein kinases from rat brain cytosol (Fig. 2, left panel).[18] The basic properties of this enzyme are a high concentration in the brain, wide substrate specificity, and size of about 500 kDa. On the other hand, the same kinase phosphorylates the 2nd site of protein I, later named synapsin,[19] but the substrate of this kinase was reported to be restricted to synapsin at that time.

2.3. Purification and Cloning of CaM Kinase II CaM kinase II was purified from rat forebrain using ammonium sulfate fractionation, chromatography on Sepharose CL-4B, calmodulin-Sepharose 4B and phosphocellulose, and its molecular and catalytic properties have been investigated extensively.[20] The kinase absolutely requires calmodulin and Ca^{2+} for its activity. Its molecular mass and sedimentation coefficient are 540 kDa and 16.5 S, respectively. CaM kinase II is composed of two distinct but closely related protein subunits of 50 kDa (α) and 60 kDa (β). It forms large oligomeric assemblies which are composed of either single or multiple iso-

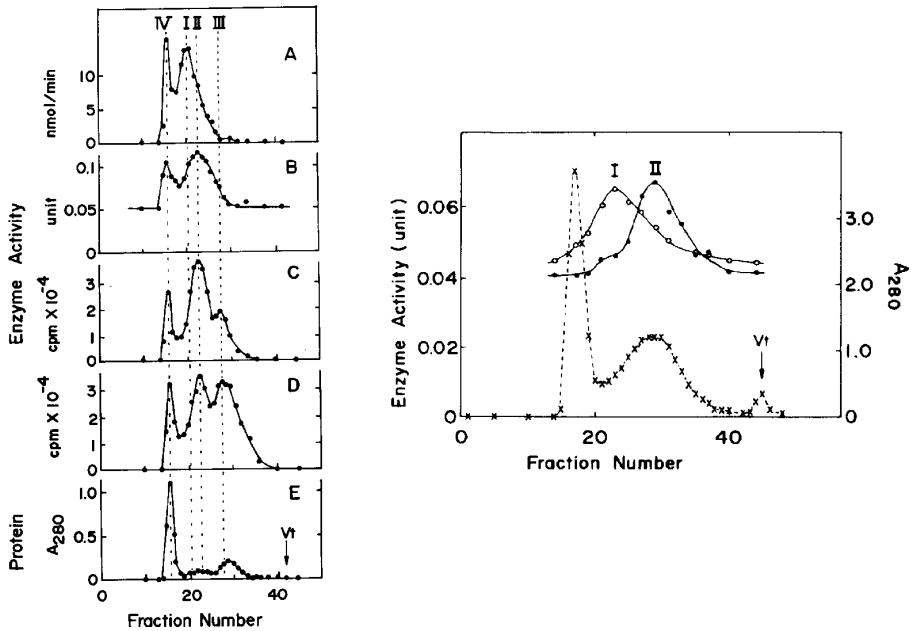

Fig. 2. Left. Identification of CaM Kinase II by Gel Filtration on Sepharose CL-6B Column[18]

Calmodulin binding proteins from rat forebrain are fractionated by gel filtration, and protein kinase activity is assayed using various proteins as substrates. (A) Phosphorylase kinase activity; (B) CaM kinase II activity; (C) casein kinase activity; (D) myosin light chain kinase activity; (E) protein; vt, column volume.

Right. Resolution of Activating Activity of Tryptophan Hydroxykase into Two Components on Sepharose CL-6B[32)]

○, each fraction is assayed in the presence of fraction II; ●, each fraction is assayed in the presence of fraction I; ×, A280. Fraction I, CaM kinase II; Fraction II, activator protein; vt, column volume.

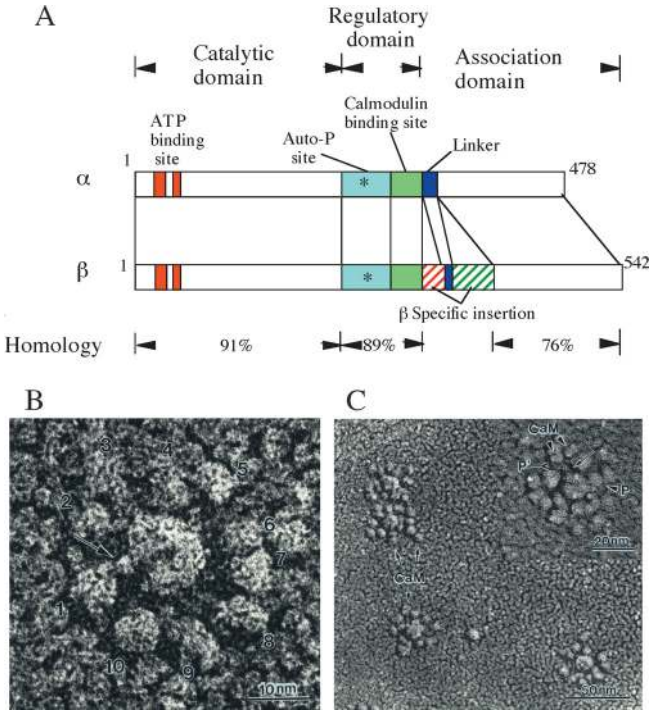

Fig. 3. Structure of CaM Kinase II

(A) Domain structure of α and β CaM kinase II. CaM kinase II is composed of three distinct functional domains, catalytic, regulatory, and association domains. Two isoforms are highly conserved. (B) A hig magnification electron micrograph of α CaM kinase II having 10 peripheral particles.[54] An arrow indicates a thin projection linking peripheral and central particles. (C) Binding of α CaM kinase II with calmodulin.[54] Calmodulin molecules (CaM) associated with the peripheral particles (P) from the outside. (Inset) Two molecules of calmodulin are observed covering a peripheral particle (P′).

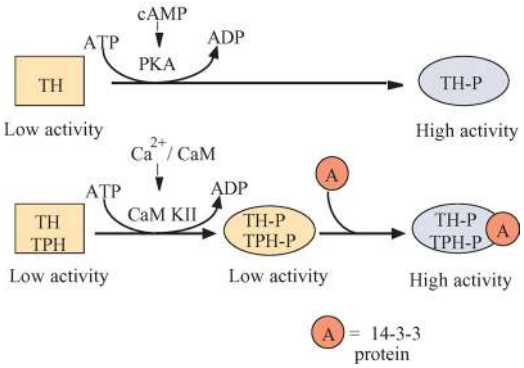

Fig. 4. Two Mechanisms of Activation of the Enzyme through Phosphorylation

Upper, one-step mechanism. Tyrosine hydoxylase (TH) is phosphorylated and simultaneously activated by PKA. Lower, two-step mechanism. First, TH and tryptophan hydroxylase (TPH) are phosphorylated by CaM kinase II, and second, phosphorylated TH and TPH are activated by interaction with the activator protein. TH-P and TPH-P, phosphorylated tyrosine hydrozylase and tryptophan hydroxylase, respectively. A, activator/14-3-3 protein.

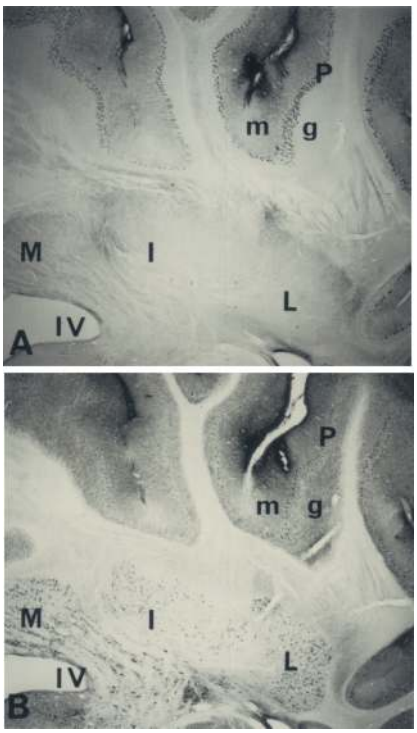

Fig. 5. Differential Localization of α and β Isoforms in Rat Cerebellum[61]

(A) α Immunoreactivity is present in the molecular layer (m) and Purkinje cell layers (p), and fibers between the medial (M) and interposed (I) cerebellar nuclei and beneath the medial cerebellar nucleus. Note the absence of immunoreactivity in the granular cell layer (g). (B) β Immunoreactivity is present in the molecular, Purkinje cell and granule cell layers and all cerebellar nuclei. IV, 4th ventricle; L, lateral cerebellar nucleus.

forms. The same kinase was purified independently using synapsin I or microtubule proteins as a substrate by two laboratories in 1983,[21,22] but was not noticed as the same enzyme as CaM kinase II, because the substrate specificity and molecular size were not well characterized in these reports. Later, synapsin I kinase 2 was shown to be the same enzyme as CaM kinase II, through comparison with a reference of glycogen synthase kinase[23,24] Therefore, the kinase has the same number, CaM kinase II. Several groups have purified the kinase from various tissues and species.[6]

2.4. Cloning of CaM Kinase II cDNA cDNA of the α and β subunits was cloned from rat brain and sequencing revealed that the subunits are the products of two highly homologous transcription units and contain catalytic, regulatory, and association domains (Fig. 3A).[25,26] Two other subunits, γ and δ, were cloned from rat brain.[27,28] These cDNA clones were obtained from 4 distinct gene products, thus, CaM kinase II has 4 distinct isoforms, α, β, γ, and δ. Each isoform has several alternatively spliced forms.[6] CaM kinase II comprises a family of isoforms derived four closely related genes. The corresponding kinase isoforms from mammalian species are highly conserved. The coding region of rat α mRNA is 93% identical to the human mRNA sequence. At the protein level, the human and rat α isoforms are identical in amino acid composition. On the other hand, *Drosophila* CaM kinase II has only one gene and some alternatively spliced forms.[29,30] The amino-terminal half, including the catalytic domain and calmodulin binding sites of *Drosophila* CaM kinase II, is 85% identical to that of the rat kinase polypeptides, and the carboxyl-terminal association domain is 65—70% identical. *Caenorhabditis elegans* CaM kinase II (unc-43) also has one gene with several splice variants.[31]

3. DISCOVERY OF A NEW ACTIVATOR PROTEIN AND TWO STEP MECHANISM FOR ENZYME REGULATION

The function of CaM kinase II was first demonstrated in the regulation of monoamine biosynthesis in the brain. Rat

brain tryptophan hydroxylase is activated by incubation with ATP, Mg^{2+}, calmodulin and Ca^{2+}.[32] The activating activity resolves into two distinct peaks upon gel filtration; one, CaM kinase II, and the other, a heat-labile activator protein. CaM kianse II phosphorylates tyrosine hydroxylase and tryptophan hydroxylase, the rate limiting enzymes of the biosynthesis of monoamines, but these enzymes are not activated by phosphorylation alone. The activation requires another protein, an activator protein (Fig. 2, right panel). Thus, the activation occurs in two steps, and a new mechanism of enzyme regulation is proposed; first, phosphorylation of tyrosine hydroxylase and tryptophan hydroxylase by CaM kinase II, and second, activation of the phosphorylated enzymes by the activator protein (Fig. 4).[32,33] A similar two-step mechanism was proposed much later and it is now well known that Src homology domain 2 (SH2) proteins bind to phospho-tyrosine in the protein, previously phosphorylated by tysoine kinase in response to extra-cellular stimuli.[34,35] Currently, these mechanism are recognized as important to cellular signal transduction.

The new activator protein is purified to homogeneity from rat brain using calmodulin-Sepharose, Sephadex G-150, and phenyl-Sepharose column chromatography.[32] It is composed of two subunits and has a molecular mass of 70 kDa and sedimentation coefficient of 4.3 S. The activator protein is present in large amounts in all tissues including the brain. Therefore, it is suggested that the activator protein has many functions other than the regulation of monoamine biosynthesis.[32] A schematic representation of the activation of tyrosine hydroxylase and tryptophan hydroxylase is shown in Fig. 4.

About 6 years after its discovery, we demonstrated that the activator protein is the same protein as 14-3-3.[36] 14-3-3 protein is a brain protein which had been numbered based on gel electrophoresis. Thus, the purification, characterization, and functional mechanisms of 14-3-3 protein are first demonstrated as the activator protein for tyrosine hydroxylase and tryptophan hydroxylase. Currently, 14-3-3 protein is well known to be a regulator of the signal transduction/phosphorylation mechanism.[37]

4. CHARACTERISTICS OF CaM KINASE II

4.1. Enzymatic Properties CaM kinase II has 4 isoforms (α, β, γ and δ), with α and β the two major isoforms expressed almost exclucively in the nervous system. The molecular properties of α and β CaM kinase II are listed in Table 2. Kinetic properties were obtained from the enzyme expressed by cloned cDNA in Chinese hamster ovary cells.[38] These isoforms show similar catalytic properties except that the affinity for calmodulin of α CaM kinase II is lower than that of the β isoform. CaM kinase II has a broad substrate specificity and phosphorylates a variety of proteins in the nervous system as well as in various tissues described below. The major consensus phosphorylation site for CaM kinase II is RXXS/T.[39] CaM kinase II also phosphorylates Ser or Thr of the sequence S/TXD, which is a completely different sequence from the major consensus site.[40] Therefore, CaM kinase II has extremely broad substrate specificity, phosphorylates a broad range of brain proteins, and plays many important roles in the functions of the brain.[6,7]

4.2. Autophosphorylation Autophosphorylation provides critical regulation of CaM kinase II. The interest in CaM kinase II has been fueled by its fascinating regulatory properties, which are based on autophosphorylation.

Table 2. Comparison of Two Major Brain Isoforms α and β CaM Kinase II

Properties	α Isoform	β Isoform	Ref.
Molecular size	500 kDa	500 kDa	20
Subunit size	54 kDa	60 kDa	20
Composition of subunit	α 10	β 8	54
Distribution	FB> BS>Ce	Ce>FB=BS	79
Development	Immature≪mature	Immature≪mature	79
Subcellular distribution	Cytosol, PSD	Cytoskeleton	71
Catalytic activity[a]			
K_m for MAP2	0.74 μM	0.81 μM	38
K_m for ATP	14 μM	13 μM	38
K_a for CaM	111 nM	21 nM	38

a) Assayed for MAP2 as substrate. Abbreviations: FB, forebrain; BS, brainstem; Ce, cerebellum.

4.2.1. Activation Autophosphorylation results in the formation of Ca^{2+}-independent enzymes.[41—43] Initial autophosphorylation is completely dependent on the presence of Ca^{2+} and calmodulin. The autoinhibitory domain of the kinase is disrupted by the binding of calmodulin at its C-terminal end, which leads to de-inhibition of the kinase. The autoinhibitory domain can be further disrupted by phosphorylation of a key threonine residue common to all isoforms (Thr286 of the α isoform).[44—46] This phosphorylation converts the kinase to a Ca^{2+}-independent enzyme. Autophosphorylation is a reaction between two proximate subunits on the same holoenzyme[47,48] Autophosphorylation increases the affinity of the kinase for calmodulin several hundred fold by reducing the dissociation rate.[49] The Ca^{2+}-independent activity of the enzyme prolonging the Ca^{2+} action transiently increased in response to nerve stimuli, involved in LTP, a basic process of learning and memory.[3]

4.2.2. Inactivation Autophosphorylation also results in inactivation of the enzyme.[50,51] Autophosphorylation of Thr305 and Thr306 on α and β CaM kinase II, respectively, occurs after phosphorylation at Thr286 (α) or Thr287 (β) in a Ca^{2+}-independent manner.[52] This is responsible for the loss of ability of CaM kinase II to bind Ca^{2+}/calmodulin, resulting in a reduction in the kinase activity. The physiological significance of the inactivation by autophosphorylation is not well understood. Recently, phosphorylation of Thr305 of CaM kinase II is demonstrated to be increased in Angelman mental retardation syndrome,[53] as described below (Section 11.2). This phosphorylation results in the inactivation of the kinase, and in the reduction of PSD CaM kinase II. Therefore, misregulation of CaM kinase II function may cause the neurological symptoms in Angelman's syndrome.

5. MOLECULAR CONFORMATION

The molecular conformation of CaM kinase II was studied by electron microscopy using a quick-freezing technique (Fig. 3B).[54] The molecule is composed of two kinds of particles, with one large central particle and smaller peripheral particles, and has a shape resembling that of a flower with 8 or 10 petals. Each peripheral particle binds to the central particle through a thin linker structure. The 8- and 10-petal molecules are octamers and decamers of CaM kinase II subunits,

respectively, each assembled with the association domains of subunits gathered in the center, and the catalytic and regulatory domains in the peripheral particles. Each peripheral particle binds with calmodulin (Fig. 3C). In forebrain enzyme, the majority of molecules possess 10 petals, which consists of the α subunit alone. The majority of cerebellum enzyme has 8 petals, consisting of the β subunit alone. These observations indicate an elegant, polarized organization for the holoenzyme.

The three-dimensional structure of α CaM kinase II was calculated using three-dimensional electron microscopy.[55] The structure consist of 12 subunits that are organized in two stacked hexameric rings with 622 symmetry. The core of α CaM kinase II is gear shaped, consisting of six slanted flanges, with six foot-like processes attached by narrow appendages to both ends of the flanges. In the model, the catalytic domain is contained within the peripheral foot as described previously.[54] The crystal structure of the association domain of CaM kinae II was reported.[56] The association domain forms a hub-like assembly, composed of two rings of seven protomers each, which are stacked head to head and held together by extensive interfaces. However, the crystal structure of the holoenzyme has not been elucidated.

6. DISTRIBUTION IN THE BRAIN

6.1. Immunocytochemistry Immunoreactivity to CaM kinase II is present in neurons throughout the brain. CaM kinase II composes up to 1% of total protein in the forebrain and 2% of that in the hippocampus.[57] The ratio of α to β isoforms is about 3 : 1 and 1 : 4 in the adult forebrain and cerebellum, respectively.[58,59] The distribution of α and β isoforms in the forebrain was investigated using monoclonal antibodies specific to each isoform.[60] Immunoreactivity to α and β was detected in most neurons, including the hippocampus, but not in glial cells. Light and electron microscopic analyses showed a difference in the regional and subcellular distribution of each isoform in the forebrain. α Immunoreactivity was more homogeneously distributed through the cellular layers (layers II to IV) than β immunoreactivity. Neurons in the globules pallidus were immunostained by the anti-β antibody more than the anti-α antibody. Marked differences between α and β immunoreactivity were not observed in the hippocampal formation.

A different distribution of α and β CaM kinase II is observed in the cerebellum (Fig. 5).[61] The α isoform is present selectively in Purukinje cells; perikarya, dendrites with their spines, axons and their terminal in the cerebellar cortex, cerebellar nuclei and lateral vestibular nuclei. A majority of the β isoform distributes in granular cells and neurons in the cerebellar nuclei. The β isoform is present in Purukinje cells at low levels. Regional differences between the immunohistochemical distribution of CaM kinase II α and β isoforms were also shown in the brainstem and retina of rats.[60,62] The retinal enzyme is composed of the α isoform alone.[63] The distribution of α CaM kinase II immunoreactivity corresponded with that of choline acetyl transferase (ChAT), suggesting that the kinase participates in the regulation of the cholinergic system, especially the 'light OFF' system in the retina.[64] Immunoreactivity for the β isoform is also detected in the retina.[64] A different distribution of α and β isoforms is also examined in spinal cord of rat and monkey.[65] Both dorsal and ventral corticospinal tract fibers are strongly immunoreactive for the α-antibody. In these regions, very weak immunoreactivity is observed for the β-antibody. β-Immunoreactivity is distributed in the neuropil of the gray matter.

6.2. Subcellular Distribution The subcellular localization and compartmentalization of specific proteins generally play a significant role in the functioning of signal transduction. CaM kinase II is present in very high concentrations in both soluble and particulate fractions from the brain.[21,23] In brain tissue, α CaM kinase II is found in the cytosolic fraction and postsynaptic density (PSD). α CaM kinase II is one of the major proteins in the PSD[66—68] and the significance of its distribution in the PSD is discussed below (Section 9.5). Early subcellular fractionation identified a significant cytoskeletal localization of CaM kinase II.[69] In the hippocampus, β CaM kinase II is associated with actin filaments.[70]

The subcellular distribution of α and β isoforms was investigated using cells overexpressing CaM kinase II. The distribution of the α and β isoforms differs.[71] α CaM kinase II is mainly distributed in the cytosolic fraction, whereas the β isoform is obtained in the particulate fraction on subcellular fractionation.[71] Deletion analysis revealed that the second part of the β-specific insertion and oligomeric form are important to the particulate distribution of β CaM kinase II.[72]

6.3. Dendritic Distribution of mRNA mRNA of α CaM kinase II is distributed in dendrites of the cerebral cortex.[73] The 3′-noncoding region of α CaM kinase II mRNA is important to the dendritic distribution of the kinase.[74] mRNAs of some neuronal proteins, including microtubule associated protein 2 (MAP2), calmodulin, and activity-regulated cytoskeleton-associated protein (Arc), are also found in dendrites.[75] The translation of dendritic mRNAs may be regulated by signaling events at synapses, and local protein production during long-term synaptic plasticity has focused attention on the mechanism involved.[75—77]

7. DEVELOPMENT

The expression level of α and β proteins of CaM kinase II depends on the stage of development. There are two developmentally regulated isoforms of the kinase in the rat forebrain with $\alpha:\beta$ ratios for 10-d and adult enzymes of 1 : 1 and 2.3 : 1, respectively.[58,59,78] Developmental changes in CaM kinase II have been immunochemically examined in the forebrain, cerebellum and brainstem of rats.[79] The concentration of α and β proteins varies markedly in brain regions with age in the postnatal period. In early postnatal brain, the concentration of these proteins is low, and increases 20—60 fold between day 10 and 30 dependent on the region. These results indicate that the kinase is specifically induced in the most active period of synaptic network formation. Similar results have been reported.[80,81]

Developmental change is also examined using cultured neuroblastoma cell lines. CaM kinase II is induced by neural differentication of P19 embryonic carcinoma cells and neuroblastoma cells.[82,83] The δ isoform of the kinase is the major isoform in these cells and the splicing pattern of this isoform changes during differentiation. Cell type-distinctive

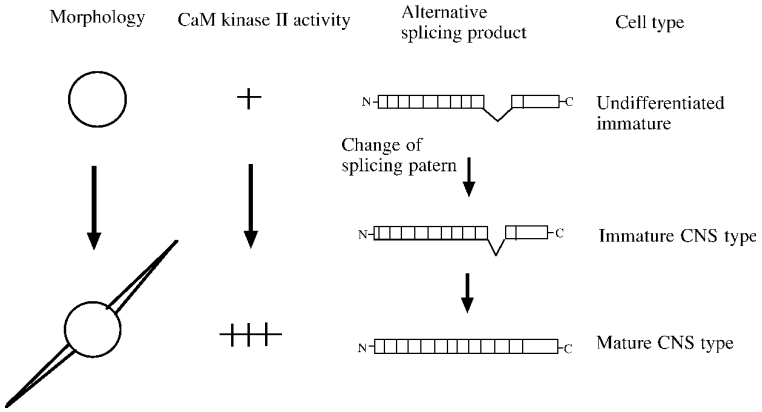

Fig. 6. Schematic Representation of Phenotypic Changes during Differentiation

Changes in cell morphology, CaM kinase II activity, splicing pattern, and neuronal cell types are shown. Undifferentiated cells are round and have low CaM kinase II activity and the smallest splice variant. During differentiation, cells form processes, and have increased CaM kinase II activity and large splice variants. + to +++, increased CaM kinase II activity. The longest splice variant is the major splice variant in mature brain.

change in the splicing pattern is shown in cultured neuronal cell as well as forebrain and cerebellum of rats. Neuronal cells may be characterized into three groups by alternative splicing: cells of a mature central nervous system, cells of an immature central nervous system and differentiated neuroblastoma, and cells of an undifferentiated neuroblastoma (Fig. 6).

The expression of many protein substrates has also been examined after phosphorylation of the soluble and particulate fractions from rat brain by CaM kinase II using two dimensional gel electrophoresis. The expression of protein substrates are regulated developmentally, and many protein substrates are increased from neonates to adults according to the increase in the level of CaM kinase II.[84)]

8. GENE STRUCTURE AND GENE EXPRESSION

The gene encoding the α isoform of rat CaM kinase II has been cloned, and its exon-intron organization analyzed.[85)] The coding region of cDNA consists of 18 exons spanning more than 50 kilobase pairs. Each discrete functional unit, such as the ATP-binding site, autophosphorylation site responsible for Ca^{2+}-independent activity, calmoduln binding site, and link structure is encoded by a single exon. The largest and smallest exons consist of 229 and 41 base pairs, respectively. All splice junction sequences flanking the introns conform to the consensus splice junction sequence and the GT-AG splice rule. The transcription initiation site is T at -149 or A at -147 from the first ATG.[86)]

The genomic organization of the β isoform is also demonstrated.[87)] The gene consists of 21 exons spanning more than 80 kbp and the coding sequence is made up of 20 exons. The structure of the exon/intron junction is completely conserved between the α and β genes except for the β-specific insertion and around the stop codon, although the size of introns is very different between the two genes. The transcription initiation site is G at -78 from the first ATG. *Drosophila* CaM kinase II has only one gene and consists of at least 16 exons spaning approximately 20 kbp.[29,30)] Splice sites of these kinase genes are highly conserved, although the size of introns is different (Fig. 7).[30,85,87)] The kinase has some alternatively spliced forms.[6)]

The expression of neuronal CaM kinase II increased most rapidly in postnatal brain during the most active phase of synapse formation.[79—81)] Elucidating its transcription control mechanism is of interest in order to explain these biological features of the CaM kinase II gene. Neuronal cell type-specific promoter activity and silencer elements are found in the 5′-upstream regions of α and β genes, although sequence homology is not found between the two genes.[87—89)] CaM kinase II genes are transcribed from a tissue-specific promoter which is under intense negative control (Fig. 8).

Proteins that interact with the promoter region of α CaM kinase II have been demonstrated in nuclear extract of rat forebrain and cerebellum by electric mobility shift assay.[86)] A protein bound to β CaM kinase II promoter is also demonstrated.[87)] However, properties of these proteins are not characterized yet. Although the transcriptional regulation of many eukaryotic genes has been extensively investigated, neuronal cell type-specific gene expression of CaM kinase II is not known at all. Identification of the promoter and silencer elements and the trans-acting factors that interact with those elements may provide insight into the neuronal cell type- and temporal-specific expression of CaM kinase II genes.

9. ROLE OF CaM KINASE II

Neuronal CaM kinase II regulates important neuronal functions, including neurotransmitter synthesis, neurotransmitter release, modulation of ion channel activity, cellular transport, cell morphology and neurite extension, synaptic plasticity, learning and memory, and gene expression. Some examples are described.

9.1. Substrate of CaM Kinase II CaM kinase II has broad substrate specificity and phosphorylates various kinds of proteins, more than 50, including enzymes, receptor proteins, channel proteins, cytoskeletal proteins, transcription factors, adaptor proteins, and membrane proteins.[4)] This kinase alters the function of these substrate proteins by phosphorylation. Moreover, more than 25 neuronal protein substrates have also been found in the PSD fraction of the rat using proteomic analysis, including receptor proteins, ion channel proteins, scaffold proteins, enzymes and cytoskeletal

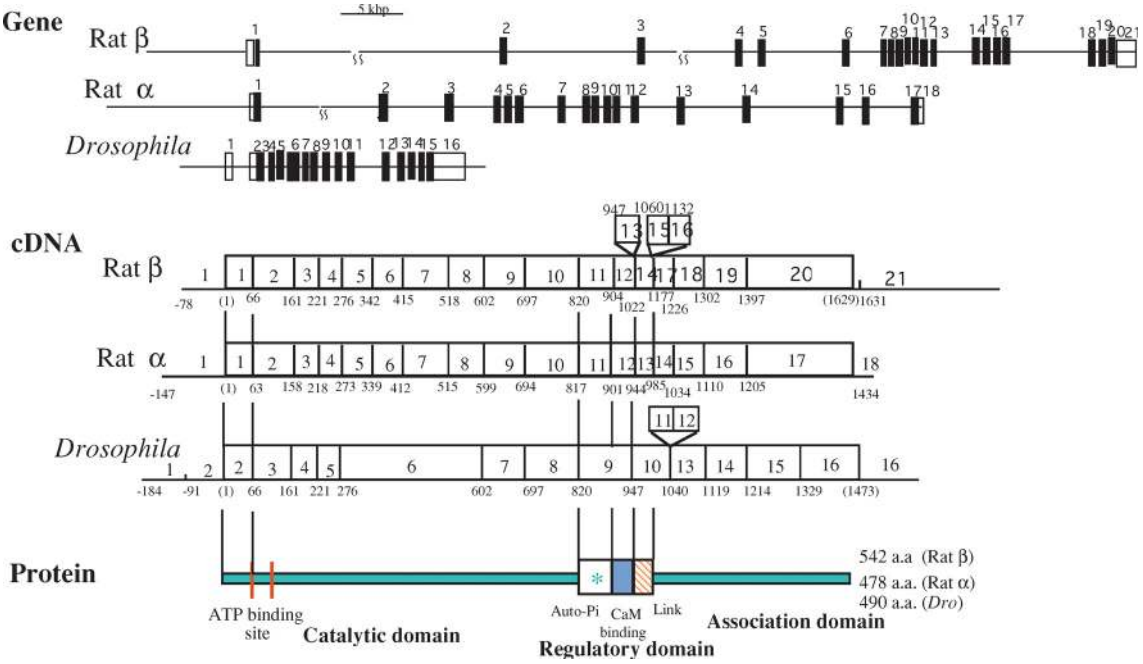

Fig. 7. Organization of Gene, cDNA, and Protein of CaM Kinase II from Rat Brain and *Drosophila*

Top, genes and exons. Exons are indicated by black bars, and their numbers are shown in the middle panel. *Middle*, cDNA. The numbers below the boxes of cDNA show the position of the first nucleotide of each exon, with the first ATG of cDNA assigned as +1. *Bottom*, protein. Three functional domains and functional units are shown. Red bar pair, ATP binding site; ∗, Autophosphorylation site (Auto-Pi site); dotted box, calmodulin-binding site; striped bar, link structure.

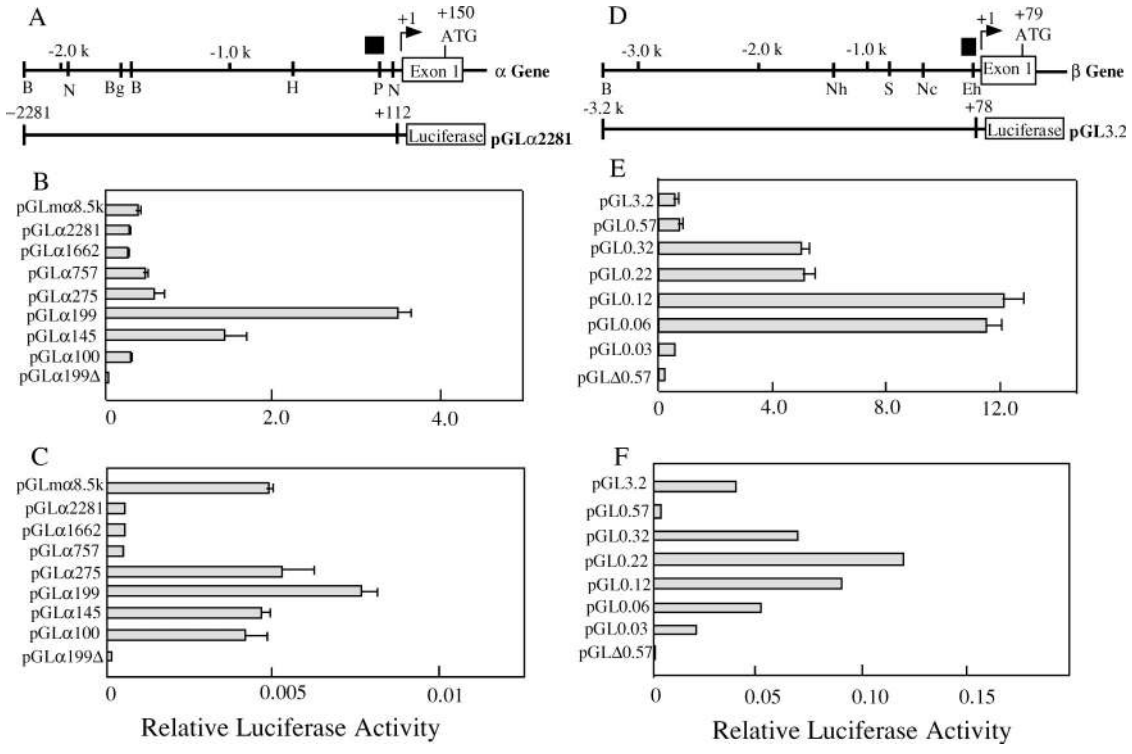

Fig. 8. Promoter-Luciferase Reporter and Deletion Analysis of CaM Kinase II Promoter Activity[89)]

(A) A schematic representation of the 5′ flanking region of the α CaM kinase II gene (upper) and one of the α CaM kinase II promoter-luciferase reporters (lower). The sequence is numbered with respect to the transcription initiation site (indicated as +1) shown by an arrow. The box represents exon 1 and the translation initiation site is shown by ATG and +150. A black box shows the neuronal cell type-specific promoter region. Restriction enzyme sites and their positions are shown; B, BamHI; Bg, BglII; H, HindIII; N, NcoI; P, PstI. (B) and (C) Deletion analysis of α CaM kinase II promoter activity in transiently transfected neuronal and non-neuronal cells. Neuronal cells, NG108-15 (B), non-neuronal cells, BALB/c3T3 (C). (D) A schematic representation of the 5′ flanking region of the β CaM kinase II gene (upper) and one of the β CaM kinase II promoter-luciferase reporters (lower). The sequence is numbered as in (A). A black box shows the neuronal cell type-specific promoter region. Restriction enzyme sites and their positions are shown; B, BamHI; Eh, EheI; Nc, NcoI; Nh, NheI; S, Sac I sites. (E) and (F) Deletion analysis of β CaM kinase II promoter activity in transiently transfected cells. Neuronal cells NG108-15 (E), and non-neuronal cells BALB/c3T3 (F).

proteins.[90] According to the broad substrate specificity, CaM kinase II plays multifunctional roles in living cells, especially in the nervous system.

9.2. Neurotransmitter Synthesis and Neurotransmitter Secretion The nervous system makes use of two main classical substances for signaling: small-molecule transmitters and neuroactive peptides. Neurotransmitters are contained in vesicles, which release their contents by an exocytotic mechanism. Catecholamine and serotonine transmitters are synthesized from the essential amino acids tyrosine and tryptophan. The regulation of catecholamines and serotonin synthesis by CaM kinase II is described above (Section 3). Tyrosine hydroxylase and tryptophan hydroxylase are phosphorylated by CaM kinase II. Tyrosine hydroxylase is phosphorylated at Ser-19,[91] and tryptophan hydroxylase, at Ser-58 and Ser-260.[92] Phosphorylated enzymes are activated by activator protein/14-3-3 protein (Fig. 4). Since these monoamines are known to be involved in normal mental function or in some neurological diseases, CaM kinase II may play an important role in mental function.

When an action potential reaches a neuron's terminal, Ca^{2+} enters the presynaptic terminal. The rise in intracellular Ca^{2+} concentration causes the vesicles to fuse with the presynaptic membrane and thereby release their neurotransmitter *via* an exocytitic mechanism. The interaction of α CaM kinase II with syntaxin was demonstrated using isolated proteins from the rat brain.[93] Syntaxin is a key component of the exocitotic molecular machinery. The binding is Ca^{2+} and ATP dependent. A CaM kinase II–syntaxin complex forms in the presynaptic terminal. Microinjection of the CaM kinase II-binding domain peptide of syntaxin specifically resulted in a decrease in the frequency of exocytosis in chromaffin cells and in neuron, causing interference with the endogenous CaM kinase II–syntaxin complex. The Ca^{2+}/ATP-dependent binding of CaM kinase II to syntaxin is an important step in the regulation of exocytosis.

9.3. Regulation of Cytoskeleton Microtubule proteins, including tubulin, high molecular weight microtubule-associated proteins (MAPs) and low molecular weight MAP, tau, are suitable substrates for CaM kinase II. Phosphorylation of MAP2 leads to a reduction in affinity for tubulin, and induces microtubule disassembly.[94] Phosphorylation of MAP2 also inhibits actin polymerization by MAP2, indicating that it regulates microtubule-microfilament interaction.[95] Tau is also phosphoryalted by CaM kinase II and the phosphorylation results in a slight reduction in affinity for tubulin.[96] The microtubule network plays an important role in maintaining cellular morphology, in membrane interaction, in intracellular trafficking, and in establishing neurite outgrowth of differentiating neurons.[97] Thus, CaM kinase II is involved in dynamic processes in the nerve cells.

9.4. Neurite Extension To elucidate the functional role of CaM kinase II in synapse formation of neuronal cells, a cell culture model was developed by overexpressing the kinase in neuroblastoma cells. α and β isoforms of CaM kinase II and its mutant were expressed in Neuro2a (Nb2a) and NG108-15 neuroblastoma cells, and phenotypic effects of overexpression of these kinases were investigated.[98,99] Expression of α and β isoforms of CaM kinase II stimulated neurite outgrowth and growth cone motility in these neuronal cells. The neurite extension was much greater by the action of the β isoform than that of the α isoform (Fig. 9). Although the sequence of the two isoforms is highly conserved, α CaM kianse II is present in the cytosolic fraction, while β CaM kinase II is associated with subcellular structures and is fractionated in the particulate fraction. β CaM kinase II has a β-specific insertion on the C-terminal side of the calmodulin-binding site. Deletion analysis of the β-specific insertion demonstrated that the insertion is involved in the subcellular distribution of the kinase (Urushihara and Yamauchi, 2001). These results indicated that the subcellular distribution of the kinase is important in neurite extention.

The effect of α and β isoforms of CaM kinase II on neurite outgrowth is enhanced by the selective protein kinase C (PKC) inhibitor, H-7.[99] PKC stimulates cell growth and proliferation.[100] Thus, these results suggest that proriferation and differentiation of neuronal cells are regulated by the activity of CaM kinase II and PKC. Figure 9 is a schematic representation of the action of CaM kinase II and PKC.

Autophosphorylation of Thr268 of α CaM kinase II is an essential event for this kinase to exert cellular functions efficiently.[98,101] Stimulation of Nb2a cells overexpressing α CaM kinase II results in an increase in neurite length, phosphorylation of Thr268 and conversion of Ca^{2+}-independent enzyme. The effect of the mutation of an autophosphorylation site (Thr286 to Ala or Asp) was examined. αT286A (Thr286 to Ala) kinase is not converted to a Ca^{2+}-independent form and αT286D (Thr286 to Asp) kinase has Ca^{2+}-independent activity. Cells expressing αT286A kinase do not form neurites and cells expressing αT286D kinase have much longer neurites. These results indicate that the Ca^{2+}-independent activity of the kinase autophosphoryalted at Thr-286 is involved in neurite outgrowth.

9.5. Role of CaM Kinase II in the PSD

9.5.1. Molecular Constituents of PSD Important mechanisms for synaptic regulation, including LTP and long-term depression (LTD), may be based on the PSD (for reviews; refs. 102—107). The PSD is a tiny, amorphous structure located beneath the post-synaptic membrane and is visible under the electron microscope as tight complexes of post-synaptic junctional proteins. It is a disc-shaped subcellular organelle about 50 nm thick and 100—900 nm in diameter apposed to postsynaptic membranes. The most prominent PSDs are Type I PSDs associated with excitatory glutamatergic synapses (Fig. 10A).[107] CaM kinase II constitutes up to 1% of total brain protein and 2% of hippocampal protein, and has been implicated in the modification of nerve functions, including learning and memory[4,5,7] It is one of the major proteins in the PSD of the cerebral cortex and hippocampus (Fig. 10B).[66—68,108] Many attempts have been made to identify and characterize the molecular constituents of the PSD, but, not all constituents are known.[103,107] Recently, molecular constituents were analyzed using an integrated liquid chromatography-based protein identification system, and results provide a catalogue of the major protein sets associated with the PSD (Fig. 10C).[109] PSD contains various proteins involved in signal transduction, including receptors, ion channel proteins, protein kinases and phosphatases, G-protein and related proteins, scaffold proteins, and adaptor proteins. Structural proteins, including membrane proteins involved in cell adhesion and cell–cell-interaction, proteins involved in endocytosis, motor proteins, and

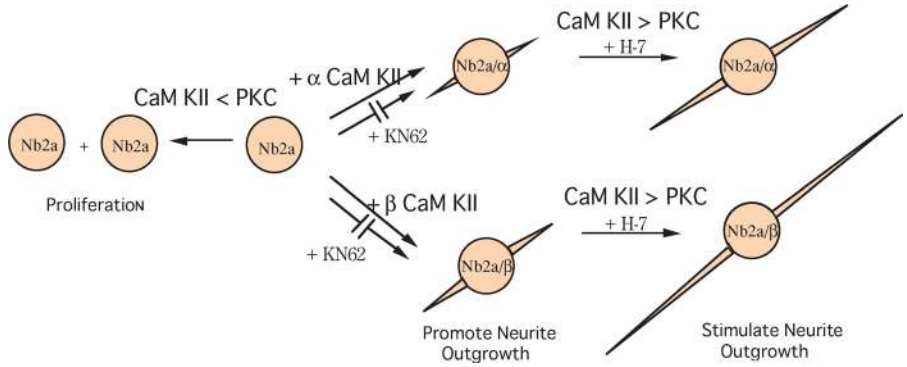

Fig. 9. Schematic Representation of Changes in Cell Morphology of Nb2a Cells

Wild-type Nb2a cells have relatively strong PKC activity than CaM kinase II activity, and proliferate. By the expression of α or β CaM kinase II, Nb2a cells form neurites. Formation of neurites is inhibited by KN62, CaM kinase inhibitor. H-7, selective PKC inhibitor, stimulates neurite extension induced by CaM kinase II. β CaM kinase II has a greater effect on neurite extension than α.

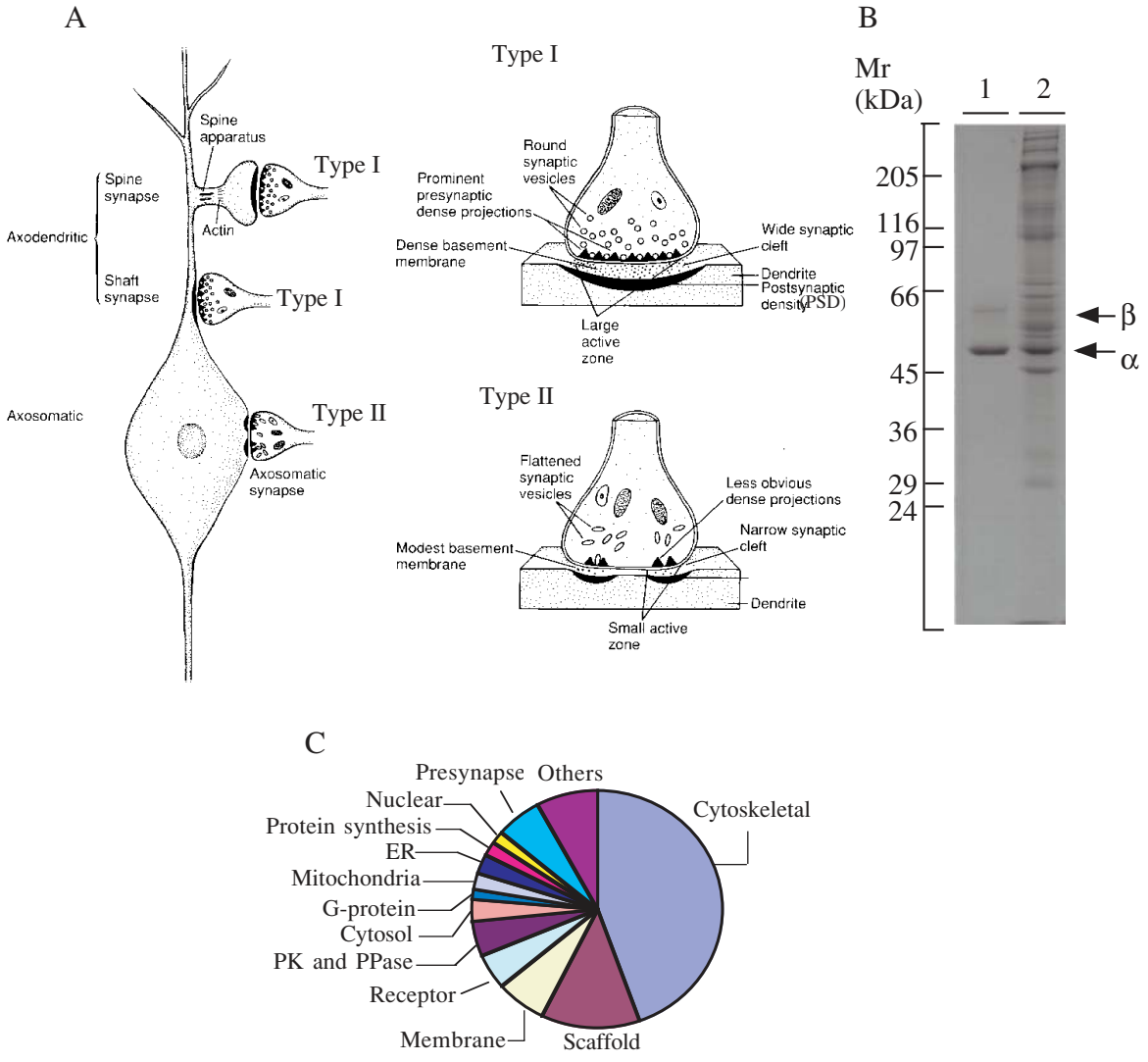

Fig. 10. Characterization of PSD

(A) The two most common types of synapses in the central nervous system. Type I synapses are usually excitatory, as exemplified by glutamatergic synapses. Type II synapses are usually inhibitory, as exemplified by GABAergic synapses.[107] (B) SDS-PAGE of CaM kinase II (lane 1) and PSD proteins (lane 2).[108] Lower and upper arrows indicate α and β isoforms of CaM kinase II, respectively. One of the major PSD proteins is α CaM kinase II. (C) Grouping of PSD proteins based on their contents calculated from the data of Yoshimura *et al.*[109]

cytoskeletal proteins are also abundant. Similar results were reported somewhat later.[110] It is now known that the PSD contains various signaling proteins and pathways. Many proteins in the PSD are phosphorylated and regulated by various protein kinases, (Fig. 11).[107] The presence and organization of the signaling machinery vary among different synaptic

types. This information is crucial because the complement of signaling complexes at a synapse determines the rules by which it interacts and encodes information.

9.5.2. Translocation of CaM Kinase II to the PSD Enzymatic properties of PSD CaM kinase II are somewhat different in the stability of Ca^{2+}-dependent activity after autophosphorylation.[108] Autophosphorylation-dependent reversible translocation of α CaM kinase II to the PSD was demonstrated using isolated PSD and purified CaM kinase II.[111,112] When CaM kinase II is autophosphorylated in the presence of Ca^{2+} and calmodulin, the kinase associates with PSD to form PSD–CaM kinase II complex. CaM kinase II is recruited to the *N*-methyl-D-aspartate (NMDA) receptor NR2B subunit through cytoplasmic carboxyl-terminal domain of the subunit. After dephosphorylation by the action of protein phosphatase 1 (PP1), CaM kinase II is released from the PSD.[113]

9.5.3. Phosphorylation of PSD Proteins CaM kinase II of PSD–CaM kinase II complex is active and has Ca^{2+}-independent activity. It can phosphorylate many PSD proteins in both the presence and absence of Ca^{2+}. Most substrates have been identified by proteomic analysis.[90,114] These substrates include receptor and ion channel proteins, scaffold and adaptor proteins, motor proteins, cytoskeletal proteins, enzymes, and membrane proteins. Potential substrates are various glutamate receptors, synaptic GTPase activating protein (SynGAP), and PSD-95/Disc-large/ZO-1 (PDZ) proteins including PSD-95 and SAP-97. The level of CaM kinase II in the PSD can affect LTP and hippocampal-dependent learning. Thus, α CaM kinase II is a key player in the regulation of plasticity.

9.5.4. Regulation of Ion Channel Activity PSD contains various types of glutamate receptors, including NMDA receptors, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, and metabotropic glutamate receptors (mGluR). These receptors are a substrate of CaM kinase II.[90] The AMPA receptor subunit GluR1 contains a single phosphorylation site, Ser831, that when phosphorylated by CaM kinase II, enhances channel function.[115] Biochemical studies have shown that receptor phosphorylation occurs during LTP, as its induction increases ^{32}P incorporation into the GluR1 subunit by the CaM kinase II.[116] The phosphorylation of Ser831 alters LTP induction which which would be expected to increase the AMPA channel conductance, and this has been directly observed.[117] The NMDA receptor NR2B subunit is phosphorylated at Ser1303 by CaM kinase II.[118] Glutamate receptors are phosphorylated by various kinds of protein kinases, including PKC, PKA, CaM kinase II and fyn tyrosin kinase, and regulated by phosphorylation.[107]

Members of the *Shaker* Kv channel family are localized to pre- and postsynaptic components, and possible targets for CaM kinase II phosphorylation. Kv1.4, a rapidly inactivating Kv channel, was demonstrated to be phosphorylated and inactivated by CaM kinase II.[119] CaM kinase II phosphorylates an amino-terminal residue Ser123. This phosphate is dephosphorylated by calcineurin (phosphatase 2B). This Ca^{2+}-sensitive phosphorylation/dephosphorylation of Kv1.4 has profound functional consequences for the inactivation.

9.5.5. Potentiation of CaM Kinase II Action by Arc Activity-regulated cytoskeleton-associated protein (Arc) is rapidly induced by LTP, and Arc mRNA is prominent in den-

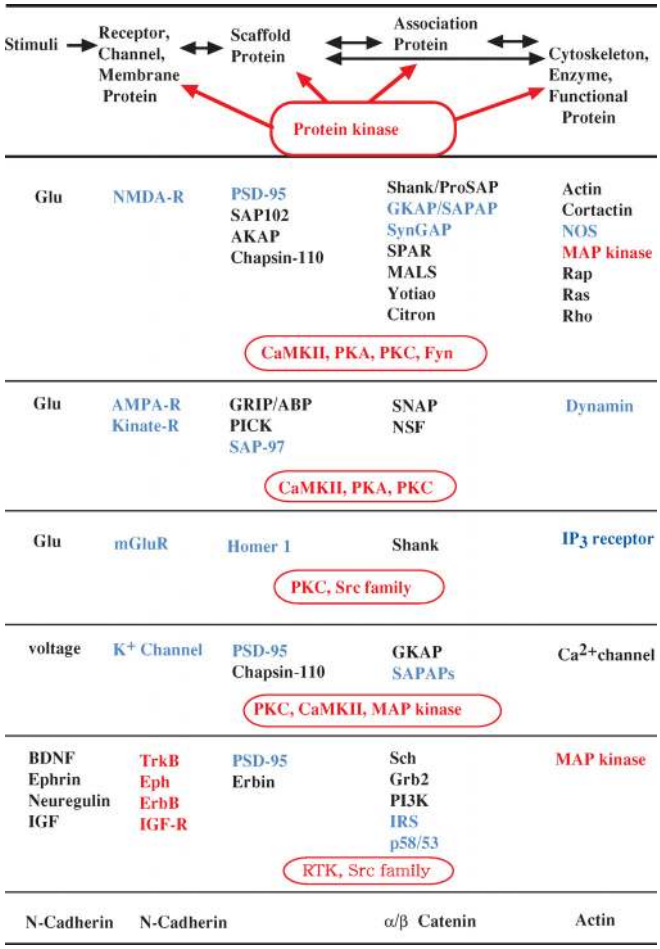

Fig. 11. Signaling and Regulatory Molecules in the PSD

There are various stimuli and signaling systems in the PSD. Signal is transduced by protein–protein interactions, and regulated by phosphorylation–dephosphorylation through the actions of several protein kinases. Red letter, protein kinase; blue letter, substrate of protein kinase.

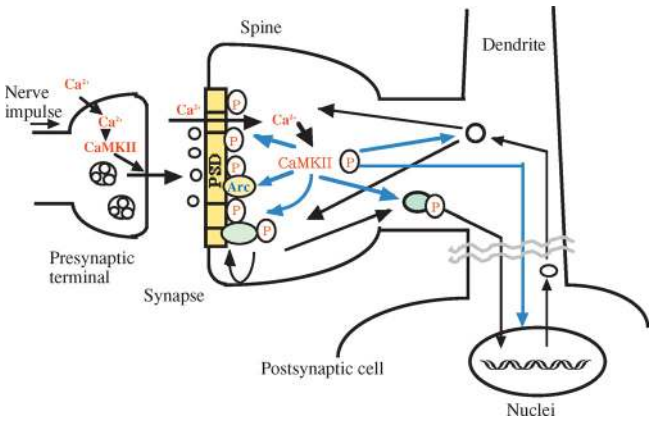

Fig. 12. Schematic Representation of the Regulation of PSD and Post synaptic Signaling by CaM Kinase II

When a nerve impulse reaches nerve terminals and local Ca^{2+} increases, glutamate is released into the synaptic cleft. Glutamate binds to the NMDA receptor, and Ca^{2+} enters postsynaptic cells. Ca^{2+} binds calmodulin and activates CaM kinase II. The activated CaM kinase II is autophosphorylated at Thr286 and translocated to the PSD, and then phosphorylates various PSD proteins. At the same time, the activated CaM kinase II also phosphorylates various proteins in postsynaptic cells. Arc potentiates CaM kinase II function. Phosphorylated proteins change their activity and regulate signaling pathways, and then new synapses are formed, resulting in a change of synaptic activity.

drites.[120] mRNA expression is strongly induced by synaptic activation which evokes LTP.[120,121] The function of Arc is not known, although it may play a role in stabilizing activity-dependent changes in synaptic efficacy. The translation of dendritic mRNAs may be regulated by signaling events at synapses, and local protein production during long-term synaptic plasticity has focused attention on the mechanism involved.[75—77] Arc protein is concentrated in the PSD.[122] Arc and CaM kinase II accumulated after electroconvulsive treatment. The relationship between CaM kinase II and Arc was investigated using neuroblastoma cells as a model system. Neuroblasotma cells expressing both Arc and CaM kinase II had longer neurites than those expressing CaM kinase II alone. Arc itself did not promote neurite outgrowth. Thus, Arc potentiated the action of CaM kinase II for neurite extension, suggesting that Arc and CaM kinase II in the PSD play an important role in activity-induced synaptic modification.[122]

10. MOUSE GENETIC APPROACHES FOR STUDYING LEARNING AND MEMORY

To investigate the physiological role of CaM kinase II *in vivo*, the knock-out of α CaM kinase II provides a new insight in neuroscience.[1] Null mutants are severely impaired in LTP of the hippocampus and in spatial learning in the Morris water maze.

Gene targeting is used to create hippocampal area CA3-restricted null mutants of α CaM kinase II. Several lines of transgenic mice have been generated to increase CaM kinase II activity levels, such as transgenic Tg (T286D) mutants that constitutively overexpress mutated α CaM kinase II mimicking the autonomous activityof Thr286 autophosphorylated kinase. Autophosphorylation of α CaM kinase II is essential for the induction and maintenance of LTP.[7] α CaM kinase II mRNA in dendrites and new CaM kinase protein synthesized in dendrites are also required for late-phase LTP.[74] Some mutants were also generated by knock-in technology to investigate the physiological importance of α CaM kinase II autophosphorylation and of local protein synthesis. Various mutants have been generated and mouse genetic approaches have provided us with a wealth of information on the role of α CaM kinase II and cognition (see review; ref. 8).

11. RELATION TO NEURONAL DISEASE

11.1. Alzheimer's Disease Alzheimer's disease, a progressive neurodegerative disorder, is characterized by the formation of neurofibrillary tangles (NFTs) and amyloid plaques.[123,124] The tangles are composed of straight and paired helical filaments (PHFs), with a major component being an aberrantly hyperphosphorylated form of the microtuble-associated protein tau, normally expressed in axons. Abnormal phosphorylation of tau is related to PHF formation in Alzheimer's brain. A number of protein kinases can phosphorylate tau.[125] CaM kinase II phosphorylates tau at the site of Alzheimer's disease brain tau.[96,126] Recently, other sites of tau were demonstrated to be phosphorylated by CaM kinase II.[127] Thus, about one-forth of the phosphorylation sites of Alzheimer's brain tau is phosphorylated by CaM kinase II, suggesting that the kinase is involved in the abnormal phosphorylation of tau in Alzheimer's disease brain. Increased phosphorylation of tau alone does not induce cell death in neuroblastoma cells overexpressing tau. Some additional stimuli may be required to induce the cell death associated with abnormal phosphorylation of tau in Alzheimer's disease.[128]

11.2. Angelman's Mental Retardation Syndrome Angelman's mental retardation syndrome is a disorder of human cognition characterized by severe mental retardation and epilepsy. The *Ube3a* gene is identified as the genetic locus for Angelman's syndrome.[129] The *Ube3a* gene encodes for an E6-AP ubiquitin ligase, an enzyme involved in protein degradation through the ubiquitin-associated proteosome-mediated pathway. The behavioral phenotype of mice with a maternal deficiency for *Ube3a* resembles Angelman's syndrome, manifesting motor dysfunction, inducible seizures, and context-dependent association learning deficit.[53] LTP is also severely impaired in the mice. Angelman's syndrome model animals exhibit a significant increase in phosphorylation at Thr268 and Thr305, with no corresponding change in the levels of total CaM kinase II. Phosphorylation at Thr305 reduces the CaM kinase II activity and its affinity for the PSD. These observations confirm that the phosphorylation at Thr305 is involved in the inhibition of synaptic response. This phosphorylation may also be related to LTD, although the possibility is yet to be examined. Thus, misregulation of α CaM kinase II function may cause the neurological symptoms in Angelman's syndrome. The misregulation of CaM kinase II is probably caused by the decreased protein phosphatase PP1/PP2 activity.[53]

12. PERSPECTIVE

Plasticity in the CNS is clearly associated with short-term and long-term changes in the cellular and subcellular architecture. Our understanding of synaptic plasticity and memory formation has been greatly enhanced by a quarter of a century of discoveries concerning the role and regulation of CaM kinase II in the nervous system. CaM kinase II plays important roles in synaptic plasticity and learning and memory, and is now recognized to be one of the most important memory molecules (Fig. 12). On the other hand, there are many other molecules for which similar evidence has been found. In the protein kinase family alone, mitogen activated protein kinase (MAP kinase), PKA, PKC, cyclin-dependent rpotein kinase 5 (cdk5), and receptor and non-receptor tyrosine kinases have all been shown to be required for the formation of some type of memory. A more germane question is whether there actually are memory molecules at all. Memory is formed and maintained by neural networks through the action of these memory molecules in the brain. Misregulation of these signaling systems and molecules may be related to neuronal disease. Studying the regulation and cross-talk of memory molecules and their signaling systems will be intimately tied to understanding the molecular mechanism of synaptic plasticity and neuronal diseases.

REFERENCES

1) Silva A. J., Paylor R., Wehner J. M., Tonegawa S., *Science*, **257**, 206—211 (1992).
2) Hanson P. I., Schulman H., *Ann. Rev. Biochem*., **61**, 559—601

(1992).
3) Lisman J., *Trends Neurosci.*, **17**, 406—412 (1994).
4) Braun A. P., Schulman H., *Annu. Rev. Physiol.*, **57**, 417—445 (1995).
5) Soderling T. R., *Curr. Opin. Neurobiol.*, **10**, 375—380 (2000).
6) Hudmon A., Schulamn H., *Ann. Rev. Biochem.*, **71**, 437—510 (2002).
7) Lisman J., Schulman H., Cline H., *Nature Rev. Neurosci.*, **3**, 175—190 (2002).
8) Elgersma Y., Sweatt J. D., Giese K. P., *J. Neurosci.*, **24**, 8410—8415 (2004).
9) Harris J. E., Morgenroth V. H. III, Roth R. H., Baldessarini R. J., *Nature* (London), **252**, 156—158 (1974).
10) Morgenroth V. H. III, Hegstrand L. R., Roth R. H., Greengard P., *J. Biol. Chem.*, **250**, 1946—1948 (1975).
11) Schulman H., Greengard., *Nature* (London) **271**, 478—479 (1978).
12) Schulman H., Greengard P., *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 5432—5436 (1978).
13) Yamauchi T., Fujisawa H., *Biochem. Biophys. Res. Commun.*, **82**, 514—517 (l978).
14) Yamauchi T., Fujisawa H., *Arch. Biochem. Biohys.*, **198**, 219—226 (1979).
15) Yamauchi T., Fujisawa H., *Biochem. Biophys. Res. Commun.*, **90**, 28—35 (1979).
16) Yamauchi T., Fujisawa H., *Biochem. Int*., **1**, 98—104 (1980).
17) Yamauchi T., Fujisawa H., *Biochem. Biophys. Res. Commun.*, **90**, 1172—1178 (1979).
18) Yamauchi T., Fujisawa H., *FEBS Lett.*, **116**, 141—144 (1980).
19) Kennedy M. B., Greengard P., *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 1293—1297 (1981).
20) Yamauchi T., Fujisawa H., *Eur. J. Biochem.*, **132**, 15—21 (1983).
21) Bennett M. K., Erondu N. E., Kennedy M. B., *J. Biol. Chem.*, **258**, 12735—12744 (1983).
22) Goldenring J. R., Gonzalez B., McGuire J. S., Jr., DeLorenzo R. J., *J. Biol. Chem.*, **258**, 12632—12640 (1983).
23) McGuinness T. L., Lai Y., Greengard P., Woodgett J. R., Cohen P., *FEBS Lett.*, **163**, 329—334 (1983).
24) Woodgett J. R., Cohen P., Yamauchi T., Fujisawa H., *FEBS Lett.*, **170**, 49—54 (1984).
25) Bennett M. K., Kennedy M. B., *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 1794—1798 (1987).
26) Lin C. R., Kapiloff M. S., Durgerian S., Tatemoto K., Russo A. F., Hanson P., Schulman H., Rosenfeld M. G., *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 5962—5966 (1987).
27) Tobimatsu T., Kameshita I., Fujisawa H., *J. Biol. Chem.*, **263** 16082—16086 (1998).
28) Tobimatsu T., Fujisawa H., *J. Biol. Chem.*, **264** 17907—17912 (1989).
29) Cho K.-O., Wall J. B., Pugh P. C., Ito M., Mueller S. A., Kennedy M. B., *Neuron*, **7**, 439—450 (1991).
30) Ohsako S., Nishida Y., Ryo H., Yamauchi T., *J. Biol. Chem.*, **268**, 2052—2062 (1993).
31) Relner D. J., Newton E. M., Tian H., Thomas J. H., *Nature* (London), **402**, 199—203 (1999).
32) Yamauchi T., Nakata H., Fujisawa H., *J. Biol. Chem.*, **256**, 5404—5409 (1981).
33) Yamauchi T., Fujisawa H., *Biochem. Biophys. Res. Commun.*, **100**, 807—813 (1981).
34) Sadowski I., Stone J. C., Pawson T., *Mol. Cell. Boil.*, **12**, 4396—4408 (1986).
35) Koch C. A., Anderson D., Moran M. F., Ellis C., Pawson T., *Science*, **252**, 668—674 (1991).
36) Ichimura T., Isobe T., Okuyama T., Yamauchi T., Fujisawa H., *FEBS Lett.*, **219**, 79—82 (1987).
37) Berg D., Holzmann C., Riess O., *Nature Rev. Neurosci.*, **4**, 752—762 (2003).
38) Yamauchi T., Ohsako S., Deguchi T., *J. Biol. Chem.*, **264**, 19108—19116 (1989).
39) Kennelly P. J., Krebs E. G., *J. Biol. Chem.*, **266**, 15555—15558 (1991).
40) Ando S., Tokui T., Yamauchi T., Sugiura H., Tanabe K., Inagaki M., *Biochem. Biophys. Res. Commun.*, **175**, 955—962 (1991).
41) Saitoh T., Schwartz J. H., *J. Cell Biol.*, **100**, 835—842 (1985).
42) Lai Y., Nairn A. C., Greengard P., *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 4253—4257 (1986).
43) Miller S. G., Kennedy M. B., *Cell*, **44**, 861—870 (1986).
44) Fong Y.-L., Taylor W. L., Means A. R., Soderling T. R., *J. Biol. Chem.*, **264**, 16759—16763 (1989).
45) Hanson P. I., Kapiloff M. S., Lou L. L., Rosenfeld M. G., Schulman H., *Neuron*, **3,** 59—70 (1989).
46) Ohsako S., Nakazawa H., Sekihara S., Ikai A., Yamauchi T., *J. Biochem.* (Tokyo), **109**, 137—143 (1991).
47) Hanson P. I., Meyer T., Stryer L., Schulman H., *Neuron*, **12**, 943—956 (1994).
48) Mukherji S., Soderling T. R., *J. Biol. Chem.*, **269**, 13744—13747 (1994).
49) Meyer T., Hanson P. I., Stryer L., Schulman H, *Science*, **256**, 1199—1202 (1992).
50) Kuret J., Schulman H., *J. Biol. Chem.*, **260**, 6427—6433 (1985).
51) Yamauchi T., Fujisawa H., *Biochem. Biophys. Res. Commun.*, **129**, 213—219 (1985).
52) Colbran R. J., Soderling T. R., *J. Biol. Chem.*, **265**, 11213—11219 (1990).
53) Weeber E. J., Jiang Y. H., Elgersma Y., Varga A. W., Carraquillo Y., Brown S. E., Christian J. M., Mirnikjoo B., Silva A., Beaudet A. L., Sweatt J. D., *J. Neurosci.*, **23**, 2634—2644 (2003).
54) Kanaseki T., Ikeuchi Y., Sugiura H., Yamauchi T., *J. Cell Boil.*, **115**, 1049—1060 (1991).
55) Kolodziej S. J., Hudmon A., Waxham M. N., Stoops J. K., *J. Biol. Chem.*, **275**, 14354—14359 (2000).
56) Hoelz A., Narin A. C., Kuriyan J., *Mol. Cell*, **11**, 1241—1251 (2003).
57) Erondu N. E., Kennedy M. B., *J. Neurosci.*, **5,** 3270—3277 (1985).
58) McGuinness T. L., Lai Y., Greengard P., *J. Biol. Chem.*, **260**, 1696—1704 (1985).
59) Miller S. G., Kennedy M. B., *J. Biol. Chem.*, **260**, 9039—9046 (1985).
60) Ochiishi T., Terashima T., Yamauchi T., *Brain Res.*, **659**, 179—193 (1994).
61) Ichikawa T., Sekihara S., Ohsako S., Hirata Y., Yamauchi T., *J. Chem. Neuroanat.*, **5**, 383—390 (1992).
62) Ochiishi T., Terashima T., Yamauchi T., *Brain Res.*, **790**, 129—140 (1998).
63) Bronstein J. M., Wasterlain C. G., Farber D. B., *J. Neurochem.*, **50**, 1438—1446 (1988).
64) Ochiishi T., Terashima T., Sugiura H., Yamauchi T., *Brain Res.*, **634**, 257—265 (1994).
65) Terashima T., Ochiishi T., Yamauchi T., *J. Comp. Neurol.*, **340**, 469—479 (1994).
66) Kennedy M. B., Bennett M. K., Erondu N. E., *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 7357—7361 (1983).
67) Goldenring J. R., McGuire J. S., Jr., DeLorenzo R. J., *J. Neurochem.*, **42**, 1077—1084 (1984).
68) Kelly P. T., McGuinness T. L., Greengard P., *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 945—949 (1984).
69) Sahyoun N., LeVine H., Bronson D., Siegel-Greenstein F., Cuatrecasas P., *J. Biol. Chem.*, **260**, 1230—1237 (1985).
70) Shen K., Teruel M. N., Subramanian K., Meyer T., *Neuron*, **21**, 593—606 (1998).
71) Yamauchi T., Sekihara S. Ohsako S., *FEBS Lett.*, **266**, 55—58 (1990).
72) Urushihara M., Yamauchi T., *Eur. J. Biochem.*, **268**, 4802—4808 (2001).
73) Burgin K. E., Waxham M. N., Rickling S., Westgate S. A., Mobley W. C., Kelly P. T., *J. Neurosci.*, **10**, 1788—1798 (1990).
74) Miller S., Yasuda M., Coats J. K., Jones Y., Martone M. E., Mayford M., *Neuron*, **36**, 507—519 (2002).
75) Steward O., Schuman E. M., *Annu. Rev. Neurosci.*, **24**, 299—325 (2001).
76) Steward O., *Neuron*, **18**, 9—12 (1997).
77) Steward O., Worley P. F., *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 7062—7068 (2001).
78) Kelly P. T., Shields S., Conway K., Yip R., Burgin K., *J. Neurochem.* **49**, 1927—1940 (1987).
79) Sugiura H., Yamauchi T., *Brain Res.*, **593**, 97—104 (1992).
80) Rostas J. A. P., Seccombe M., Weinberger R. P., *J. Neurochem.*, **50**, 945—953 (1988).
81) Brock L., Srinivasan M., Shulman H., *J. Neurosci.*, **15**, 6797—6808 (1995).
82) Donai H., Murakami T., Amano T., Sogawa Y., Yamauchi T., *Mol. Brain Res.*, **85**, 189—199 (2000).

83) Donai H., Nakamura M., Sogawa Y., Wang J. K. T., Urushihara M., Yamauchi T., *Neurosci. Lett.*, **293**, 111—114 (2000).
84) Sugiura H., Yamauchi T., *Brain Res.*, **659**, 42—54 (1994).
85) Nishioka N., Shiojiri M., Kadota S., Morinaga H., Kuwahara J., Arakawa T., Yamamoto S., Yamauchi T., *FEBS Lett.*, **396**, 333—336 (1996).
86) Sunyer N., Sahyoun N., *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 278—282 (1990).
87) Donai H., Morinaga H., Yamauchi T., *Mol. Brain Res.*, **94**, 35—47 (2001).
88) Mima K., Deguchi S., Yamauchi T., *Neurosci. Lett.*, **307**, 117—121 (2001).
89) Mima K., Donai H., Yamauchi T., *Biol. Proced. Online.*, **3**, 79—90 (2002).
90) Yoshimura Y., Shinkawa T., Taoka M., Kobayashi K., Isobe T., Yamauchi T., *Biochem. Biophys. Res. Commun.*, **290**, 948—954 (2002).
91) Itagaki C., Isobe T., Taoka M., Natsume T., Nomura N., Horigome T., Omata S., Ichinose H., Nagatsu T., Greene L. A., Ichimura T., *Biochemistry*, **38**, 15673—15680 (1999).
92) Jiang G. C.-T., Yohrling G. J., Schmitt I. V. J. D., Vrana K. E., *J. Mol. Biol.*, **302**, 1005—1017 (2000).
93) Ohyama A., Hosaka K., Komiya Y., Akagawa K., Yamauchi E., Taniguchi H., Sasagawa N., Kumakura K., Mochida S., Yamauchi T., Igarashi M., *J. Neurosci.*, **22**, 3342—3351 (2002).
94) Yamauchi T., Fujisawa H., *Biochem. Biophys. Res. Commun.*, **110**, 287—291 (1983).
95) Yamauchi T., Fujiswa H., *Biochim. Biophys. Acta*, **968**, 77—85 (1988).
96) Singh T. J., Wang J.-Z., Novak M., Kontzekova E., Grundke-Iqubal I., Iqubal K., *FEBS Lett.*, **387**, 145—148 (1996).
97) Drubin D. G., Nelson W. J., *Cell.*, **84**, 335—344 (1996).
98) Goshima Y., Ohsako S., Yamauchi T., *J. Neurosci.*, **13**, 559—567 (1993).
99) Nomura T., Kumatoriya K., Yoshimura Y., Yamauchi T., *Brain Res.*, **766**, 129—144 (1997).
100) Nishizuka Y., *Science*, **258**, 607—614 (1992).
101) Sogawa Y., Yoshimura Y., Otaka A., Yamauchi T., *Brain Res.*, **881**, 165—175 (2000).
102) Kennedy M. B., *Trends Neurosci.*, **20**, 264—268 (1997).
103) Ziff E. B., *Neuron*, **19**, 1163—1174 (1997).
104) Kennedy M. B., *Science*, **290**, 750—754 (2000).
105) Luscher C., Nicoll R. A., Malenka R. C., Muller D., *Nature Neurosci.*, **3**, 545—550 (2000).
106) Sheng M., *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 7058—7061 (2001).
107) Yamauchi T., *Mass Spectrometry Rev.*, **21**, 266—286 (2002).
108) Ochiishi T., Sugiura H., Yamauchi T., *Brain Res.*, **610**, 97—107 (1993).
109) Yoshimura Y., Yamauchi Y., Shinkawa T., Taoka M., Donai T., Takahashi N., Isobe T., Yamauchi T., *J. Neurochem.*, **88**, 759—768 (2004).
110) Peng J., Kim M. J., Cheng D., Duong D. M., Gygi S. P., Sheng M., *J. Boil. Chem.*, **279**, 21003—21011 (2004).
111) Strack S., Choi S., Lovinger D. M., Colbran R. J., *J. Biol. Chem.*, **272**, 13467—13470 (1997).
112) Yoshimura Y., Yamauchi T., *J. Biol. Chem.*, **272**, 26354—26359 (1997).
113) Yoshimura Y., Sogawa Y., Yamauchi T., *FEBS Lett.*, **446**, 239—242 (1999).
114) Yoshimura Y., Aoi C., Yamauchi T., *Mol. Brain Res.*, **81**, 118—128 (2000).
115) Barria A., Derkach V., Soderling T., *J. Boil. Chem.*, **272**, 32727—32730 (1997).
116) Barria A., Muller D., Derkach V., Griffith L. C., Soderling T. R., *Science*, **276**, 2042—2045 (1997).
117) Benke T. A., Luthi A., Isaac J. T. R., Collingridge G. L., *Nature* (London), **393**, 793—797 (1998).
118) Omkumar R. V., Kiely M. J., Rosenstein A. J., Min K. T., Kennedy M. B., *J. Biol. Chem.*, **271**, 31670—31678 (1996).
119) Roeper J., Lorra C., Pongs O., *J. Neurosci.*, **17**, 3379—3391 (1997).
120) Lyford G. L., Yamagata K., Kaufmann W. E., Barnes C. A., Sanders L. K., Copeland N. G., Gilbert D. J., Jenkins N. A., Lanahan A. A., Worley P. F., *Neuron*, **14**, 433—445 (1995).
121) Steward O., Wallace C. S., Lyford G. L., Worley P. F., *Neuron*, **21**, 741—751 (1998).
122) Donai H., Sugiura H., Ara D., Yoshimura Y., Yamagata K., Yamauchi T., *Neurosci. Res.*, **47**, 399—408 (2003).
123) Spillantini M. G., Goedert M., *Trends Neurosci.*, **21**, 428—433 (1998).
124) Selkoe D. J., *Physiol. Rev.*, **81**, 741—766 (2001).
125) Lovestone S., Reynolds C. H., *Neuroscience*, **78**, 309—324 (1997).
126) Steiner B., Mandelkow E.-M., Biernat J., Gustke N., Meyer H. E., Schmidt B., Mieskes G., Soling H. D., Drechsel D., Kirschner M. W., Goedert M., Mandelkow E., *EMBO J.*, **9**, 3539—3544 (1990).
127) Yoshimura Y., Ichinose T., Yamauchi T., *Neurosci. Lett.*, **353**, 185—188 (2003).
128) Yoshizaki C., Tsukane M. Yamauchi T., *Neurosci. Res.*, **49**, 363—371 (2004).
129) Albrecht U., Sutcliffe J. S., Cattanach B. M., Beechey C. V., Armstrong D., Eichele G., Beaudet A. L., *Nature Genet.*, **17**, 75—78 (1997).