# The Role of the MAGUK Protein CASK in Neural Development and Synaptic Function

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**Abstract:** CASK, which belongs to the family of membrane-associated guanylate kinase (MAGUK) proteins, is recognized as a multidomain scaffolding protein highly expressed in the mammalian nervous system. MAGUK proteins generally target to neuronal synapses and regulate trafficking, targeting, and signaling of ion channels. However, CASK is a unique MAGUK protein in several respects. It not only plays a role in synaptic protein targeting but also contributes to neural development and regulation of gene expression. Several CASK-interacting proteins have been identified from yeast two-hybrid screening and biochemical isolation. These proteins, whose interactions with CASK are reviewed here, include the Parkinson's disease molecule parkin, the adhesion molecule neurexin, syndecans, calcium channel proteins, the cytoplasmic adaptor protein Mint1, Veli/mLIN-7/MALS, SAP97, caskin and CIP98, transcription factor Tbr-1, and nucleosome assembly protein CINAP. More important, CASK may form different complexes with different binding partners and perform different functions. Among these interactions, CASK, Tbr-1, and CINAP can form a transcriptional complex regulating gene expression. Reelin and NMDAR subunit 2b (NR2b) genes have been identified as Tbr-1 target genes. Reelin is critical for neural development. NR2b is an important subunit of NMDAR, which plays important roles in neural function and neurological diseases. Regulation of reelin and NR2b expression suggests the potential roles of the Tbr-1-CASK-CINAP complex in neural activity, development, and disease. The functions of these CASK protein complexes are also discussed in detail in this review.

**Keywords:** Synapse, postsynaptic density, neural development, transcription, gene expression, cerebral cortex, hippocampus.

## 1. INTRODUCTION

MAGUK proteins play important roles in targeting, anchoring, and signaling of ion channels at synapses and in regulation of neural activity. The most well-studied MAGUK proteins belong to the PSD-95 subfamily, and detailed information about this subfamily can be found in previous reviews [1, 2]. The current review focuses on CASK, which belongs to the p55 subfamily of MAGUK proteins. CASK was originally identified by yeast two-hybrid screening of a rat brain library using the C-terminal region of neurexin as bait [3]. Unlike PSD-95, a neuronal-specific MAGUK protein, CASK is expressed in but not restricted to neurons, though the expression levels of CASK are 3-5-fold higher in brain than in other organs [3, 4]. In addition to playing an important role in the nervous system, CASK has also been suggested as having a role in establishing epithelial cell polarity in mammals [reviewed by 5]. In *C. elegans*, the CASK homolog LIN-2 is also critical for vulval induction from epithelial cells [6]. These studies imply the importance of CASK in epithelial cells.

Although CASK stands for calcium/calmodulin-dependent serine protein kinase and it contains a CaMK-like domain, it does not possess calcium/calmodulin-dependent kinase activity; the important residues at the active site of the CaMK-like domain are mutated [4]. Like other MAGUK proteins, CASK functions as a multidomain scaffolding protein. However, unlike members of the PSD-95 subfamily, CASK not only contributes to ion channel trafficking and transmembrane protein anchoring at synapses but also enters the nuclei of neurons and regulates gene expression. In addition, targeted mutation of PSD-95 or PSD-93 does not affect mouse development; both knockout mice are healthy [7, 8]. In contrast, CASK is an essential gene. Both insertional mutation in the CASK gene [9] and targeted knockout of the CASK gene [10] result in lethality in mice within 1-2 days after birth. Although the reason for lethality is not clear, cleft palate occurs in both types of mutant mice. These findings suggest that CASK is important in development.

Because CASK acts as a multidomain adaptor protein, to elucidate the biological significance of CASK, I first review the protein domain structure of CASK, summarize the interacting proteins of CASK, and extend our understanding about CASK function from its protein-protein interaction in this review. Although CASK is widely distributed in different tissues, I then focus on the function of CASK in the nervous system because the bulk of information regarding CASK comes from studies of the nervous system.

## 2. CASK AND OTHER MAGUKS

### 2.1. Domain Structures of the MAGUK Protein Family

MAGUK proteins consist of several modular protein-protein interacting domains (Fig. **1**). The PDZ, Src homolog 3 (SH3), and guanylate kinase (GK) domains are the characteristic protein domains shared in all MAGUK proteins. Based on sequence similarity and organization of the protein domain, the proteins are classified into the PSD-95 and p55 subfamilies. Some MAGUK proteins, such as MAGI-1 [11] and CARMA1 [12], possess special domain organization or unique protein domains and therefore cannot

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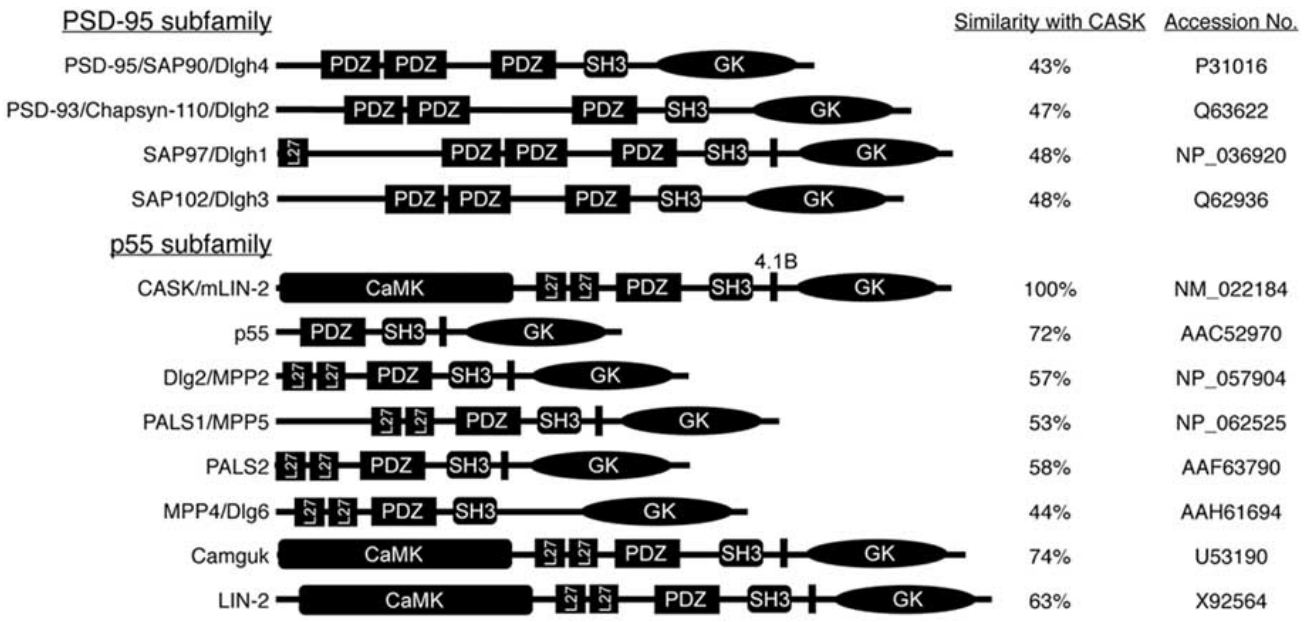


**Fig. (1).** Schematic domain organization of MAGUK proteins.

The members of the PSD-95 and p55 subfamilies are shown. The amino acid sequence similarities of the other MAGUK proteins with that of CASK are listed, as indicated. For p55 and the members of PSD-95 subfamily, the similarities are compared from the third or the single PDZ domain to the C-terminal end. For camguk and LIN-2, full-length proteins are compared. For the remaining members of the p55 subfamily, the sequences are compared from the L27 domain to the C-terminal end. The GenBank Accession numbers for sequence information are listed.

be classified into these two subfamilies. In the PSD-95 subfamily, three PDZ domains are clustered at the N-terminal half followed by the SH3 and guanylate kinase domains (Fig. **1**). In addition to the PDZ, SH3, and GK domains, the members of the p55 subfamily contain one or two L27 domain preceding the single PDZ domain (Fig. **1**). CASK is a special member of the p55 subfamily because an additional CaMK-like domain is present at its N-terminal region (Fig. **1**). CASK is an evolutionarily conserved gene. Rat CASK protein shares 99% amino acid sequence similarity with mouse, 98% with human, 97% with *Xenopus*, and 94% with zebrafish. Even the *Drosophila* and *C. elegans* CASK homologs, respectively camguk and LIN-2, share 74% and 63% similarity with the rat CASK protein, respectively. The features of each domain of the CASK protein are described below.

### 2.2. The CASK CaMK-Like Domain

The CaMK-like domain of the CASK protein shares ~66% similarity with CaMKII in the amino acid sequence. Because the important residues at the active site are mutated, the CaMK-like domain of CASK is not expected to have kinase activity [4]. Instead, the CASK CaMK-like domain functions as a protein-protein interacting domain, which binds to several cellular proteins, such as Mint1/X11 [13, 14], Caskin 1 [15], CIP98 [16], and Carom [17]. The function of the interaction between CASK and CIP98 or Carom is unclear. The biological significance of the interaction between Mint1 and CASK will be further illustrated in the following section. Caskin 1 is also expected to act as an adaptor protein because it contains six N-terminal ankyrin repeats and a single SH3 domain [15]. Interestingly, Caskin 1 competes with Mint1 to interact with the CaMK domain of CASK [15], suggesting that CASK forms two distinct protein complexes with Mint1 and Caskin 1. Thus far, it is unclear whether or not Carom or CIP98 also competes with Mint1 to bind CASK.

Although CASK does not possess kinase activity, *Drosophila* camguk complexes to CaMKII in the presence of calcium/calmodulin [18]. This finding suggests the possibility that the active postsynaptic pool of CaMKII can be controlled locally to differentiate active and inactive synapses *via* interaction with camguk. In mammals, it is not clear if CASK also binds to CaMKII and localizes the activity of CaMKII. In addition to interacting with CaMKII, Drosophila camguk has been shown to play a critical role in regulation of neurotransmitter vesicle release [19] and modulation of surface expression and phosphorylation of potassium channel [20]. In mammal, CASK is also implied in regulation of neurotransmission (see details in Section 4).

### 2.3. The CASK L27 Domains

The L27 domain was originally identified from LIN-2/CASK and LIN-7/Veli/Mals, which is a novel protein-protein interacting domain with approximately 50-60 amino acid residues [reviewed by 21]. Veli/MALS/LIN-7 is a small polypeptide containing one L27 domain followed by one class I PDZ domain. In CASK/LIN-2, there are two L27 domains localized between the CaMK-like and PDZ domain (Fig. **1**). The first L27 domain, namely the L27A or L27N domain, interacts with the unique L27 domain of SAP97 [22], another MAGUK protein belonging to the PSD-95 subfamily (Fig. **1**). The second L27 domain, namely the L27B or L27C domain, interacts with the N-terminal L27 domain of

Veli/MALS/LIN-7 [13, 14, 21, 23]. Formation of the heteromeric L27 complexes is highly specific. The CASK L27N domain only interacts with SAP97 but not with Veli/Mals/mLIN-7. Conversely, the CASK L27C domain specifically recognizes Veli/Mals/mLIN-7 but not SAP97. High-resolution NMR analysis further demonstrated that L27 domains form a heterotetrameric complex [24, 25]. Each L27 domain contains three α helices. The first two helices of CASK L27A and SAP97 L27 domains are packed together to form a four-helical bundle in the heterodimer. The third helix forms another four-helical bundle that assembles two heterodimers into a tetramer [24]. The CASK L27B and Veli L27 domain form a similar structure [25]. The specificity of L27 complex formation is determined by the third α helix [25].

### 2.4. The CASK PDZ Domain

The PDZ domains, consisting of approximately 90 amino acid residues, are specialized for binding to short peptide motifs at the extreme C-terminal tail of their binding partners [reviewed by 26], although other modes of interaction also occur [reviewed by 27]. PDZ stands for the first three identified PDZ proteins: PSD-95 (postsynaptic density-95), Dlg (Disc large), and ZO-1 (zonula occludens-1). Three types of PDZ domains have been classified based on their preference of binding sequence. The consensus sequence for the recognition of class I PDZ domains is X-S/T-X-V or X-S/T-X-L. X can be any amino acid residue. The PDZ domains of the PSD-95 subfamily all belong to this class. The class II PDZ domains prefer to bind to the consensus sequence X-Φ-X-V/A/I. Φ represents a hydrophobic amino acid, such as Tyr, Phe, Val, or Ile. The PDZ domains of the p55 subfamily belong to class II. The class III PDZ domain interacts with the sequence X-D-X-V. The PDZ domain of nNOS is a class III PDZ protein.

The structure of the third PDZ domain of PSD-95 has been determined using X-ray crystallography approaches [28]. This work revealed that the PDZ domain forms a hydrophobic pocket, which allows the free C-terminal residue to fit in and form a stable interaction. The PDZ ligand engages the PDZ domain *via* antiparallel main chain interactions with a β sheet of the domain. The specific side-chain interactions determine the selective recognition of the C-terminal consensus sequence. The structure of the CASK PDZ domain has also been solved [29]. The result showed that class II PDZ domains differ from class I domains by formation of a second hydrophobic binding pocket, which interacts with the hydrophobic side chain of the -2 residue of the class II PDZ ligands.

For CASK, several cellular proteins have been identified as binding partners *via* the PDZ domain, including neurexin (C-terminus: -E-Y-Y-V [3]), syndecans (C-terminus: -E-F-Y-A [30], parkin (C-terminus: -W-F-D-V [31], and SynCAM (C-terminus: -E-Y-F-I [32]). The biological significance of these interactions will be discussed in Section 4.

### 2.5. The CASK SH3 Domain

The Src homolog 3 (SH3) domain originally identified from Src protein typically interacts with a proline-rich sequence. It has been shown that the CASK SH3 domain interacts with a strong class I SH3 domain-binding consensus R-Q-L-P-Q-T-P-L-T-P-R-P present in the $\alpha_{1B1}$ subunit of the N-type $Ca^{2+}$ channel [33, 34]. The $\alpha_{1B1}$ subunit of the N-type $Ca^{2+}$ channel not only interacts with the CASK SH3 domain *via* its proline-rich motif but also binds to the first PDZ domain of Mint1 through its C-terminal tail of the D-H-W-C sequence [34]. The recombinant CASK and Mint1 colocalize with N-type $Ca^{2+}$ channels at synapses. Moreover, mutation on the CASK or Mint1 binding site impairs the synaptic distribution of the N-type $Ca^{2+}$ channel. These findings support the idea that interactions between the N-type $Ca^{2+}$ channel and CASK/Mint1 are critical for synaptic targeting of the N-type channel (Fig. **2b**) [33].

In addition to interaction with the proline-rich motif, the CASK SH3 domain mediates interaction with the GK domain [35]. This interaction can occur intra- or inter-molecularly. CASK protein can therefore form a homodimer *via* the inter-molecular SH3-GK interaction. Because the CASK SH3 domain also recognizes the GK domain of other MAGUK proteins, such as p55, CASK also forms heterodimers with other MAGUK proteins *via* an inter-molecular SH3-GK interaction [35]. This kind of SH3-GK interaction is not restricted to the p55 subfamily. It also happens in the PSD-95 subfamily, including PSD-95, Chapsyn-110, and SAP97 [36, 37]. Therefore, it has been hypothesized that MAGUK proteins may form their large scaffold complexes *via* their SH3-GK interaction [35].

### 2.6. The Hook (Protein 4.1 Binding) Motif

The protein 4.1 binding motif or so-called "Hook motif," a lysine-rich short stretch, is present in some MAGUK proteins, including CASK, p55, and SAP-97 (Fig. **1**) [4, 38, 39]. In CASK, the protein 4.1 binding motif is in the amino acid sequence K-K-K-K-Q-Y-K-D-K. The *in vitro* pull-down and overlay assay demonstrated that this short sequence interacts with protein 4.1 [4]. The function of protein 4.1 is to interact with actin molecules and promote formation of actin/spectrin microfilaments. A biochemical reconstitution assay has demonstrated that CASK binds a brain-enriched isoform of protein 4.1 and nucleates local assembly of actin/spectrin filaments. This complex also recruits a CASK-binding membrane protein, neurexin [40]. The evidence suggests that CASK acts as an adaptor protein linking transmembrane proteins to cytoskeleton.

### 2.7. The CASK GK Domain

Guanylate kinase catalyzes the reversible phosphoryl transfer from ATP to GMP in the presence of $Mg^{2+}$ [41]. It plays an important role in the synthesis of the nucleotide precursors for nucleic acids. Although the rat CASK GK domain shares 51% similarity with rat guanylate kinase1 in the amino acid sequence, thus far, there is no evidence indicating that the CASK GK domain carries the phosphoryl transfer activity. Instead, like other CASK domains, the GK domain also functions as protein-protein interaction domain. By yeast two-hybrid screening, several GK domain interacting proteins have been identified, including T-box transcription factor Tbr-1 (T-brain-1) [42], nucleosome assembly protein CINAP (CASK Interacting Nucleosome Assembly Protein)[43], and a zinc finger protein, Bcl11A/Evi9/CTIP1 [44]. Interestingly, all the identified CASK GK interacting proteins are nuclear proteins. This

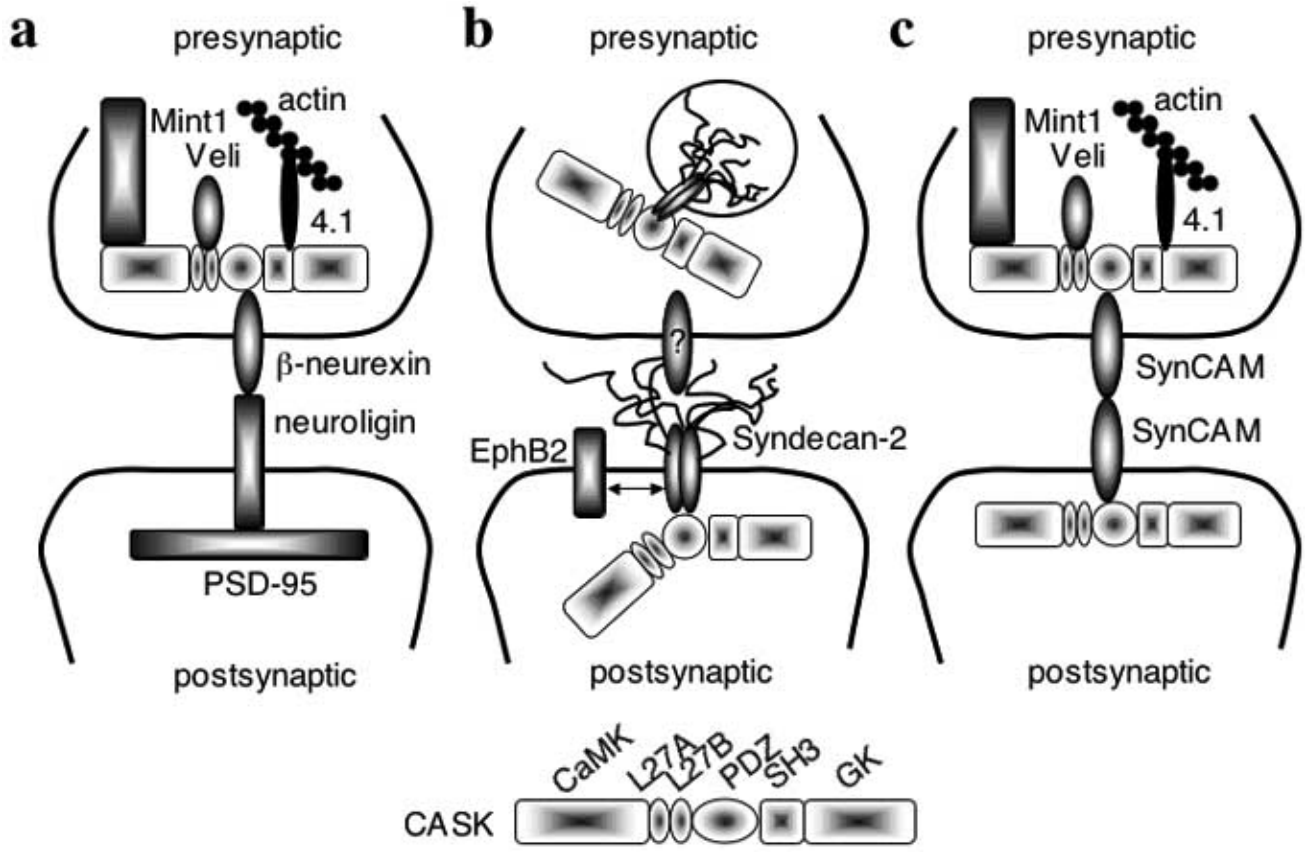


**Fig. (2).** The CASK protein complex in protein trafficking and synaptic targeting.

(**a**) The CASK-Mint1-Veli complex is involved in NMDAR trafficking from soma to synapse. *Via* the interaction between the Veli PDZ domain and the C-terminal tail of NR2b and the binding between the Mint1 PDZ domain and the C-terminal tail of KIF17, the CASK-Mint1-Veli complex links NMDAR to vesicle transport cargo. (**b**) Synaptic targeting of the N-type calcium channel is mediated by both Mint1 and CASK. The N-type calcium channel interacts with both Mint1 and CASK through different motifs. Abolishment of the interactions with CASK and Mint1 inhibits the synaptic distribution of the N-type calcium channel.

property of CASK is very different from that of PSD-95, which interacts with another adaptor protein, GKAP [45], and the signaling molecule SPAR [46]. The interaction between PSD-95 and GKAP further links PSD-95 to another adaptor protein, Shank [47]. The interaction between PSD-95 and SPAR couples PSD-95 to a signaling pathway important for dendritic spine formation [48]. CASK's distinct property of interacting with nuclear proteins suggests a function of CASK in the nucleus. The detail nuclear function of CASK will be discussed in Section 4.

### 2.8. Alternative Splicing of CASK

The first paper showing the alternative splicing of CASK was contributed by Joanna Wilson's laboratory [9]. They analyzed the phenotype of a transgenic mouse carrying an insertional mutation in the CASK gene. Their study revealed that mutation in the CASK gene, which is X-linked, results in sex-linked cleft palate. When they analyzed the mRNA of murine CASK, they found that the murine CASK gene expresses two splicing forms, mCASK-A and mCASK-B. The short form, mCASK-A, contains only the CaMK, L27, and PDZ domains; the long form, mCASK-B, is virtually identical to rat and human CASK. Another study analyzing the alternative splicing mediated by Nova in the brain also showed that CASK is one of the genes in the brain regulated by alternative splicing [49]. Although the evidence supports the alternative splicing of CASK in the brain, it is not clear whether any specific signal or physiological situation regulates mRNA splicing of CASK in the brain or what the consequence of CASK alternative splicing is. More detailed analyses need to be performed to address this issue.

## 3. REGIONAL AND SUBCELLULAR DISTRIBUTION OF CASK IN BRAIN

The results of biochemical and immunohistochemical studies have shown that CASK proteins are widely distributed in different regions of the rat brain, including the cerebral cortex, hippocampus, thalamus, cerebellum, and brain stem [30]. These findings suggest that CASK may play an essential role in different brain functions. Higher magnification imaging using confocal and electric microscope (EM) was also performed to analyze the subcellular distribution of CASK proteins. In adult rat brain, CASK proteins are mainly somatodendritic. Confocal analysis showed a punctate staining pattern of CASK [30], suggesting a synaptic distribution of CASK proteins.

Immunogold EM further demonstrated that CASK is present at both presynaptic sites and associated with postsynaptic density (PSD) [30]. Biochemical fractionation demonstrated that CASK proteins are enriched at PSD [30]. At pre- and post-synaptic sites, CASK may associate with different molecules (see details in Section 4). Although CASK is enriched at synapses, a significant amount of CASK is also present in the cytoplasm [30], indicating that CASK not only functions as an adaptor protein at the synapse but also plays a role in the cytoplasm of mature neurons.

**Table 1. Summary of CASK Interacting Proteins**

| CASK domain | Interacting protein | Function of the interaction | Reference |
|---|---|---|---|
| CaMK domain | Mint1/X11 | synaptic protein trafficking and targeting | [33, 34, 55] |
| | Caskin1 | competes CASK binding with Mint1 | [15] |
| | CIP98 | unknown | [16] |
| | Carom | unknown | [17] |
| | liprin | synaptic vesicle cycling | [52] |
| L27A domain | SAP97 | links to Inward rectifier potassium channel | [65, 66] |
| | liprin | synaptic vesicle cycling | [52] |
| L27B domain | Veli/mLIN-7/Mals | synaptic protein trafficking and targeting | [55] |
| PDZ domain | Neurexin | synaptic interaction | [3] |
| | syndecan-2 | synaptic interaction and formation | [30, 64] |
| | parkin | unknown | [31] |
| | SynCAM | synaptic interaction | [32] |
| SH3 domain | N-type Ca channel | synaptic targeting | [33, 34] |
| | GK domain of MAGUKs | dimerization | [35] |
| GK domain | Tbr-1 | transcriptional regulation; brain development | [42, 43, 75] |
| | CINAP | transcriptional regulation; synaptic response | [43] |

At the embryonic stage, although the majority (~80%) of CASK proteins are still present in the cytoplasm, a portion (~20%) of CASK is detected in the nuclei of embryonic neurons [42]. This observation suggests a nuclear function of CASK in embryonic neurons. Indeed, nuclear CASK in embryonic neurons interacts with T-box transcription factor Tbr-1 and enhances the transcriptional activity of Tbr-1 [42]. Tbr-1 is an important transcription factor in cerebral cortex development. The interaction with Tbr-1 and enhancement of its transcriptional activity suggest a role for CASK in development during the embryonic period.

At the postnatal stage (before postnatal day 21), a robust CASK immunostaining signal is present in axonal pathways, including the corpus callosum (an axonal pathway connecting the two hemispheres of the cerebral cortex), the fimbria and alveus of the hippocampal formation, the white matter of the cerebellum, and the axon tracts running through the thalamus [50]. At maturation of rat brain (at the end of three weeks after birth), axonal CASK signal is greatly reduced, and CASK expression shifts to a somatodendritic pattern like that of the adult brain [50].

The above studies demonstrate a developmental regulation of the CASK expression pattern in the brain, suggesting versatile roles for CASK at different stages of brain development. In the embryonic period, some CASK proteins enter the nuclei of neurons to regulate gene expression; in the juvenile brain, CASK can distribute into axons and may contribute to axonal outgrowth or pathfinding; in the adult brain, the synaptic distribution of CASK indicates that CASK acts as an adaptor protein and is involved in synaptic organization and synaptic protein targeting. These speculations based on CASK distribution are further confirmed by studies of CASK-interacting proteins, which are described in detail in Section 4.

## 4. THE BIOLOGICAL SIGNIFICANCE OF CASK IN THE BRAIN

### 4.1. Genetic Mouse Models

A mouse mutant line harboring a transgenic encoding the Epstein-Barr virus latent membrane protein-1 in the CASK gene exhibited the phenotype of sex-linked cleft palate [9, 51]. All transgenically positive males died within 24 hours of birth because of the inability to suckle. Both males and females displayed an altered cranial morphology, indicating an essential role of CASK in development. Also, spinal kinks were observed in transgenic females, suggesting a critical neural function of CASK. These phenotypes were not observed in any other transgenic lines expressing the same transgene, supporting the inference that disruption of the CASK gene results in the described phenotype.

Gene targeting approaches have been used to generate CASK traditional knockout mice [10]. As with the transgenic CASK mutant mice, CASK knockout mice die within a few hours of birth and also exhibit the cleft palate phenotype. In addition, neural apoptosis is increased in CASK-deficient mice. A decrease in the frequency of inhibitory mini-events and an increase in the frequency of excitatory spontaneous mini-events were observed in CASK knockout mice, indicating that the balance of excitatory-inhibitory synapse numbers is altered. The findings from these two genetic mouse models support the role of CASK in development and neural activity.

### 4.2. The Protein Complex of Mint1/LIN-10, CASK/LIN-2, and Veli/LIN-7 IN *C. Elegans* and in Mammalian Brain

In *C. elegans*, the CASK homolog LIN-2 was identified from a screening that isolated mutants with a defect in vulval differentiation [6]. From the screening, mutation in another two genes, LIN-7 and LIN-10, was also found to elicit the same phenotype as the lin-2 mutant. Genetic studies indicate that LIN-2, LIN-7, and LIN-10 work together downstream of the LET-23 (EGFR) receptor tyrosine kinase pathway involved in induction of vulval development [6]. After identification of the genes, biochemical analyses revealed that LIN-2, LIN-7, and LIN-10 form a protein complex *via* interactions between LIN-2/LIN-7 and LIN-2/LIN-10 [23]. LIN-7 and LIN-10 are also PDZ-containing adaptor proteins. The C-terminal tail of LET-23 carries a class I PDZ binding site (E-T-C-L), which interacts with the PDZ domain of LIN-7. LIN-7 is a small protein, containing only an L27 domain and a PDZ domain [13, 14, 23]. The L27 domain interacts with the LIN-2 L27B domain. LIN-10 consists of a LIN-2 binding domain at the N-terminus followed by a PTB (phosphotyrosine binding) domain and two PDZ domains [13, 14, 23]. Mutation of LIN-2, LIN-7, and LIN-10 leads to mislocalization of LET-23, which cannot properly receive the LIN-3 (EGF) signal delivered from the anchoring cell. Meanwhile, Mint1 (munc-18 interacting protein 1) and mLIN-7/Veli/Mals (mammalian LIN-7/vertebrate LIN-7) were identified as mammalian homologs of LIN-10 and LIN-7, respectively [13, 14].

The biochemical studies also demonstrated that, similar to *C. elegans* homologs, mammalian CASK, Veli, and Mint1 also form a protein complex in neurons [13, 14]. However, the biological function of the Mint1-CASK-Veli complex may not be identical to that of the LIN-10-LIN-2-LIN-7 complex. First of all, because Mint1 is a neuron-specific gene, the Mint1-CASK-Veli complex only performs its function in neurons, although CASK and Veli may still form a complex and work together in other tissues. Secondary, mammalian EGFR may not be regulated by the CASK-Veli-Mint1 protein complex. In mammals, there are four members of the EGFR tyrosine receptor kinase family, including EGFR (ErbB1), ErbB2, ErbB3, and ErbB4. The C-terminal tails of these members are F-I/S-G-A (EGFR/ErbB1), D-V-P-V (ErbB2 isoform 1), W-L-C-S (ErbB2 isoform 2), A-Q-R-T/I (ErbB3), and N-T-V-V (ErbB4), which are not identical to the C-terminal tail of LET-23. Although ErbB4 carries a class I PDZ binding site, no results from biochemical studies support that there is an interaction between the CASK-Veli-Mint1 complex and ErbB4. Therefore, instead of localizing EGFR members at specific subcellular regions, the CASK-Veli-Mint1 complex may have a different function in mammalian brain. Indeed, this protein complex has been shown to play roles in synaptic organization, synaptic protein targeting, and regulation of neurotransmission. For instance, it has been recently shown that the protein complex containing Veli/MALS, CASK, and liprin-α is suggested to play a role in presynaptic vesicle cycling [52]. In this complex, both the CaMK and the first L27 domain of CASK are required for the direct interaction with the first SAM domain of liprin-α. In *Drosophila*, the mutant lacking of liprin-α exhibits a concomitant decrease in synaptic transmission [53]. Moreover, targeting mutation of all three Veli/MALS genes in mice leads to abnormal presynaptic vesicle cycling and therefore reduces excitatory postsynaptic currents [52]. These studies indicate a role of CASK-Veli-Mint1 complex in neurotransmission.

### 4.3. NMDAR Trafficking and the Veli-CASK-Mint1 Complex

In the CASK-Mint1-Veli complex, Veli/Mals/mLIN-7 contains one class I PDZ domain, which shares 55% and 63% amino acid sequence similarity with the first and second PDZ domains of PSD-95, respectively. It also shares the same binding specificity. Like the first and second PDZ domains of PSD-95, the PDZ domain of Veli/Mals/mLIN-7 binds the C-terminal tail of the NMDAR subunit 2b (NR2b) [54]. In the CASK-Mint1-Veli complex, Mint1 interacts with KIF17, a dendritic-specific motor protein, *via* its first PDZ domain and the C-terminal tail (G-E-P-L) of KIF17 [55]. Therefore, the CASK-Mint1-Veli complex links NMDAR to KIF17 vesicle transport cargo and mediates NMDAR transport along microtubules [55]. KIF17 is critical for NR2b transportation from soma to synapses because cellular knockdown or functional blockade of KIF17 significantly impairs NR2b expression and its synaptic localization in cultured hippocampal neurons [56]. These studies are the first demonstration that the Mint1-CASK-Veli complex plays a role in protein trafficking in neurons (Fig. **2a**).

### 4.4. Neurexin-Neuroligin in Synaptic Interaction

Mammalian CASK was first identified with a yeast two-hybrid screening using β-neurexin as bait [3]. CASK PDZ domain interacts with the C-terminal tail (E-Y-Y-V) of β-neurexin. β-neurexins are neuronal cell-surface adhesion molecules, which tightly bind to neuroligins, another class of neuronal cell surface receptor at the postsynaptic site [reviewed by 57]. The evidence indicates that CASK or perhaps the CASK-Mint1-Veli complex binds to the C-terminal end of β-neurexin at the presynaptic site [3, 13], and neuroligin interacts with PSD-95 *via* its C-terminal tail and the third PDZ domain of PSD-95 at the postsynaptic site (Fig. **3a**) [58, 59].

More interesting, overexpression of β-neurexin in PC12 cells induced PSD-95 clustering in contacting dendrites of hippocampal neurons. This effect is specific to β-neurexin and was not observed with other synaptic cell adhesion molecules such as N-cadherin or SynCAM [60]. Expression of a dominant-negative neuroligin-1 lacking the C-terminal PSD-95 binding site in cultured neurons markedly reduced the sizes and densities of PSD-95 puncta induced by β-neurexin [60]. Treatment of cultured neurons with a fusion protein containing the ectodomain of β-neurexin reduces the number of both excitatory and inhibitory synapses [61], suggesting a critical role of β-neurexin in synaptic formation. Because the β-neurexin/neuroligin junction has been implicated in synaptic interaction and the CASK-Mint1-Veli complex acts as an adaptor protein complex interacting with β-neurexin, the current model proposes that the CASK-Mint1-Veli complex functions as a nucleation site for the assembly of proteins involved in synaptic junctions and synaptic vesicle exocytosis (Fig. **3a**).

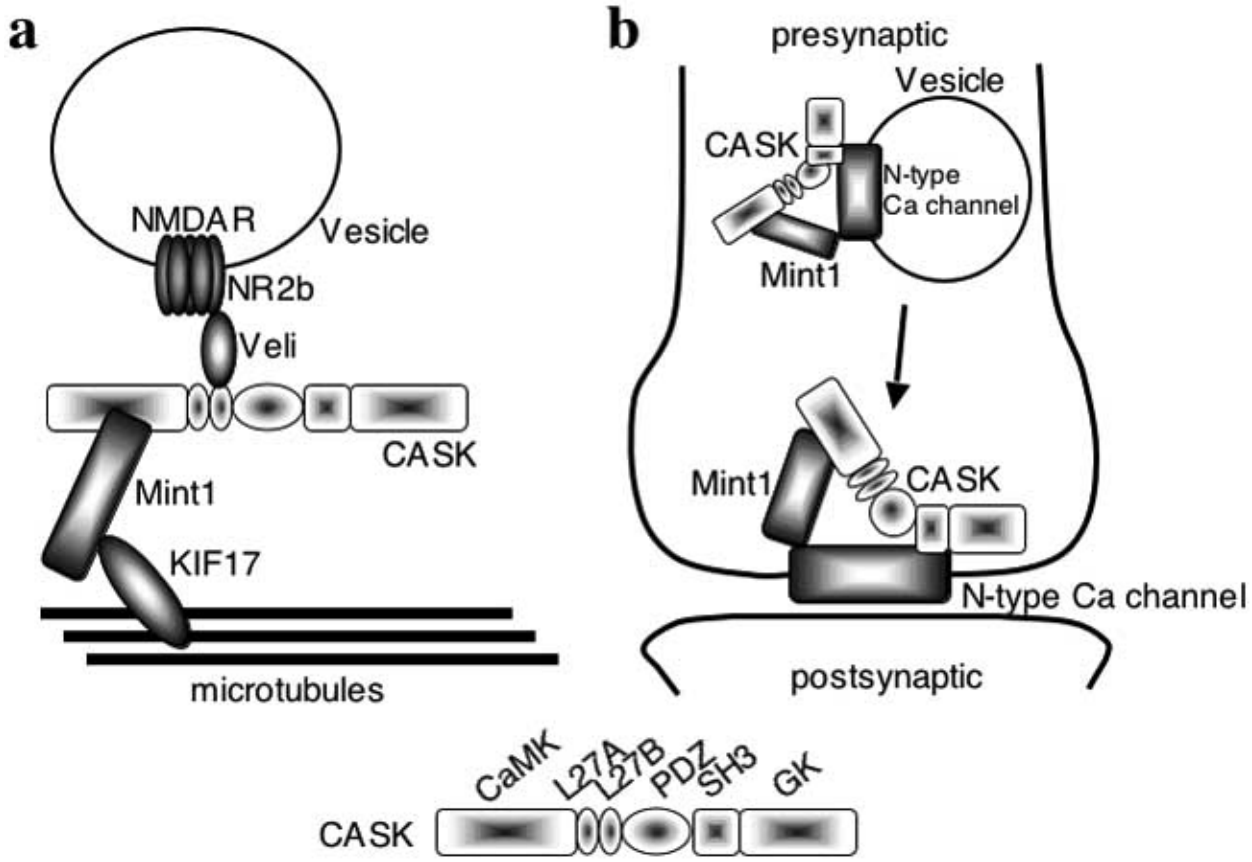


**Fig. (3).** The role of CASK in synaptic interaction and formation.

CASK involvement in synaptic interaction and formation is mediated by interaction with (**a**) neurexin, (**b**) syndecan-2, and (**c**) SynCAM. (**a**) At the presynaptic site, CASK not only recognizes β-neurexin but also forms a complex with Mint1, Veli, and protein 4.1. Protein 4.1 further links the complex to the actin cytoskeleton. The extracellular domain of β-neurexin binds neuroligin located at the postsynaptic site. PSD-95 is the adaptor protein for neuroligin. (**b**) Syndecan-2 is present at both the postsynaptic site and presynaptic vesicle. It is likely that syndecan-2 is also present at the presynaptic plasma membrane. The ligand for syndecan-2 at the postsynaptic site is still unknown. Because syndecans may mediate homophilic interactions, if syndecan-2 also present at the presynaptic plasma membrane, postsynaptic syndecan-2 might interact with presynaptic syndecan-2 and promote synaptic interaction and formation. Both phosphorylation by tyrosine kinase receptor EphB2 and the interaction with CASK are required for the synaptogenesis activity of syndecan-2. (**c**) SynCAM can also form a homophilic interaction between psot- and pre-synaptic sites. CASK binds the C-terminal tail of SynCAM. However, it is unclear if CASK is required for SynCAM activity at both post- and pre-synaptic sites.

### 4.5. Syndecan-2 and CASK in Dendritic Spine Formation

Using yeast two-hybrid screening, syndecan-2 was identified as a CASK PDZ domain interacting protein [4, 30]. Syndecan-2 belongs to the syndecan heparan sulfate proteoglycan (HSPG) family, which is the major transmembrane HSPG. In mammals, there are four members in this family: syndecan-1, syndecan-2, syndecan-3, and syndecan-4. Because these four members all share the identical C-terminal sequence E-F-Y-A, they all interact with CASK [30, 50]. Expression and distribution of syndecans are tightly controlled by several physiological conditions (such as tumorigenesis, angiogenesis, and wound healing), developmental stages, and cell type [reviewed by 62, 63]. In adult rat brain, all four syndecans can be detected by in situ hybridization as having different expression patterns [50]. Syndecan-1 is restricted to the granular cell layer of the cerebellum and is almost undetectable in forebrain. Both syndecan-2 and syndecan-3 are widely expressed in different regions of rat brain, although syndecan-2 exhibits slightly more expression in the striatum, and expression levels of syndecan-3 are slightly increased in thalamus when these two syndecans are compared. Syndecan-4 is mainly restricted to glial cells. Therefore, syndecan-2 and syndecan-3 are the two major neural syndecans in rodent [50].

The results of immunohistochemistry studies further indicate a differential subcellular distribution of syndecan-2 and syndecan-3 in neurons. Syndecan-3 is more highly expressed in developing brain and concentrated in axons [50]. In contrast, syndecan-2 is highly restricted at synapses. Expression levels of syndecan-2 are gradually increased during neural maturation both *in vitro* and *in vivo*, which correlates with synaptogenesis [30, 50, 64]. A shift of CASK distribution from an axonal pattern to somatodendritic localization during brain developmental processes (details in Section **3**) is consistent with the differential expression of syndecan-2 and syndecan-3 in the brain. These findings indicate that CASK interacts with syndecan-3 along axons in

juvenile brain (before postnatal day 21) and binds to syndecan-2 at synapses in adult brain [30, 50].

The biological significance of the interaction between CASK and syndecan-3 along the axon has not been fully explored. It is possible that CASK links syndecan-3 to the actin cytoskeleton *via* protein 4.1 and contributes to axon outgrowth. The role of the interaction between CASK and syndecan-2 at synapses is much clearer (Fig. **3b**). Syndecan-2 is not only present at synapses but also is important for dendritic spinogenesis. Dendritic spine formation typically occurs after 14 DIV (day *in vitro*) in cultured hippocampal neurons. Syndecan-2 is expressed late (after 14 DIV) in cultured hippocampal neurons, which coincides with dendritic spine formation in mature neuronal cultures [64]. Overexpression of syndecan-2 starting at 1 DIV accelerates spine formation in hippocampal neurons to 8 DIV [64]. The C-terminal CASK binding site of syndecan-2 is essential for promoting spinogenesis; the syndecan-2 mutant lacking the last three amino acids loses the ability to promote dendritic spine formation [64]. These data suggest that CASK may be involved in spinogenesis *via* interaction with syndecan-2, although direct evidence supporting this point needs to be obtained by knocking out or knocking down CASK in mice or cultured neurons.

### 4.6. SynCAM and Synaptic Interaction and Function

SynCAM (Synaptic Cell Adhesion Molecule) was originally identified by a database search with the criteria that the protein contains an extracellular immunoglobulin (Ig) domain and an intracellular PDZ binding motif [32]. SynCAM functions as a homophilic cell adhesion molecule through its extracellular Ig domain. *Via* its C-terminal E-F-Y-I motif (a class II PDZ binding site), SynCAM interacts with the CASK PDZ domain [32]. Like CASK, SynCAM is present at both the pre- and post-synaptic sites. Overexpression of full-length SynCAM in hippocampal neurons increases the frequency of spontaneous miniature synaptic currents (minis). Because the mini frequency recorded in a postsynaptic neuron depends primarily on the number of synapses and their release probability, an increase in the mini frequency by overexpression of SynCAM indicates that SynCAM regulates synaptic formation and/or synaptic function. Indeed, co-expression of SynCAM and glutamate receptor GluR2 in HEK239 cells induces co-cultured neurons to form a synapse at the contact sites between HEK239 cells and neurons. This synaptic contact is functional because the presynaptic vesicle can be released upon stimulation [32]. Overexpression of the full-length cytoplasmic region of SynCAM as a dominant-negative mutant reduced synaptic activity in neurons; however, the cytoplasmic mutant construct lacking the CASK binding site did not affect synaptic activity. These findings indicate that the C-terminal CASK PDZ binding site of SynCAM is important for the function of SynCAM in synaptic formation and function. Therefore, the function of the interaction between SynCAM and CASK is similar to that of CASK-syndecan-2 and CASK-neurexin interaction, which is critical for synaptic interaction, formation, and function (Fig. **3c**).

### 4.7. The Interaction Between CASK and Parkin

In addition to the transmembrane proteins syndecan-2, neurexin, and SynCAM, the CASK PDZ domain has been shown to interact with a cytoplasmic protein, parkin [31], which is a RING-type E3 ubiquitin ligase involved in the ubiquitin-dependent proteasomal degradation pathway. Mutations in the parkin gene cause an autosomal recessive juvenile-onset form of Parkinson's disease, which involves the selective degeneration of dopamine neurons in the midbrain. Through the C-terminal W-F-D-V motif, parkin interacts with the CASK PDZ domain [31]. Thus far, the biological significance of the interaction between CASK and parkin is unclear. Because parkin functions as an E3 ligase, its interaction with CASK might regulate CASK protein stability; however, the *in vitro* ubiquitination assay has demonstrated that parkin does not ubiquitinate CASK [31]. Therefore, the interaction between CASK and parkin does not target regulation of CASK protein levels. It is possible that parkin ubiquitinates CASK-interacting proteins *via* interaction with CASK. Alternatively, parkin may target to synapse *via* the interaction with CASK. More experiments need to be performed to elucidate the function of the CASK-parkin interaction.

### 4.8. The Interaction Between CASK and Other MAGUK or PDZ Proteins

CASK can interact with other MAGUK proteins *via* two different mechanisms. One is through the interaction between the L27A domain of CASK and the N-terminal L27 domain of SAP97; the other is *via* the SH3-GK interaction (see Section **2**). Because different MAGUK proteins recognize various binding partners, the interaction with other MAGUK proteins provides a more complex protein-protein interaction network, further enhancing the biological importance of CASK. For instance, proteomic studies have shown that CASK associates with inward rectifier Kir2 potassium channels [65, 66] and serotonin receptor 5-HT2C [67]. In the Kir2 study, CASK, SAP97, Veli, and Mint1 all are present in the pull-down complex using Kir2 fusion proteins. The C-terminal tail of Kir2 contains a class I PDZ domain binding site. Therefore, the interaction between the PDZ domain of SAP97 or Veli and the C-terminal tail of Kir2 is suggested to mediate the interaction between Kir2 and the CASK-SAP97-Mint1-Veli complex [66]. For serotonin receptor 5-HT2C, CASK, Mint1, and Veli are also present in the precipitate using an 5-HT2C fusion protein [67]. The C-terminal I-S-S-V sequence of 5-HT2C binds to the Veli PDZ domain [68]. Because the C-terminal PDZ binding domain of 5-HT2C is essential for receptor phosphorylation and resensitization of 5-HT2C [69], the interaction between 5-HT2C and the Veli-CASK-Mint1 complex may play a critical role in 5-HT2C signaling.

In addition to MAGUK proteins, we recently identified GRIP1, a multiple PDZ protein, as a CASK-interacting protein from a proteomic study [70]. The CASK protein complexes were isolated from rat brain extracts, separated by 2-dimensional electrophoresis, and analyzed by MALDI-TOF mass spectrometry. GRIP1 is one of the CASK-associated proteins isolated from the analysis. Co-immunoprecipitation was further performed to confirm the interaction between CASK and GRIP1 and to identify that the GRIP1 PDZ6 domain is the associating domain for the CASK PDZ domain [70]. Based on X-ray crystallography studies, the PDZ6 domain of GRIP1, also a class II PDZ domain, forms a multimer through a PDZ-PDZ interaction

[71]. Therefore, the GRIP1 PDZ domain may use the same mechanism to interact with the CASK PDZ domain.

Since GRIP1 was originally identified as glutamate receptor interacting protein, which specifically binds to the C-terminal tails of AMPA type glutamate receptor GluR2 and GluR3, the association between CASK and GluR2/3 in rat brain was also examined. Indeed, CASK antibody could also co-precipitate GluR2/3 from rat brain extracts [70]. The results show that this interaction with GRIP1 links CASK to the AMPA receptor. Because the synaptic function of GluR2/3 is tightly regulated by plasma membrane insertion and endocytosis and the CASK protein complex has been implicated in protein trafficking, it will be interesting to examine whether or not the interaction between CASK and GRIP1 is involved in protein trafficking of GluR2/3.

### 4.9. The Nuclear Function of CASK in Neurons

In the embryonic period, confocal analysis indicates that approximately 20% of CASK proteins are present in the nuclei of neurons in the cerebral cortex. Biochemical fractionation also confirms the presence of CASK in the nuclear fraction of cerebral cortex and hippocampus [42]. In the nucleus, CASK interacts with nuclear proteins and regulates gene expression. Yeast two-hybrid screening helped to identify three CASK-interacting proteins using the CASK GK domain as bait. These three proteins are Tbr-1 [42], CINAP [43], and Bcl11A/Evi9/CTIP [44]. The Tbr-1 gene is highly expressed in embryonic brain, and expression gradually decreases during development but is still present in significant amounts in the adult brain [42, 72]. Its expression is restricted to the cerebral cortex, the hippocampus, and the olfactory bulb [72, 73]. Knocking out the Tbr-1 gene in mice results in abnormal lamination of the cerebral cortex and defective axonal projections between the cerebral cortex and the thalamus [74]. The mutant mice die within one to two days after birth.

In rodent cerebral cortex, neurons are divided into 6 layers based on their morphology and function. Axons of each layer of neurons target specific regions of brain or spinal cord. Disruption of the migration of specific neurons to their final destination (namely lamination) interrupts the formation of the normal neural circuit. The phenotype of Tbr-1 knockout mice in lamination indicates an essential role of Tbr-1 in cerebral cortex development. Interaction with CASK enhances the transcriptional activity of Tbr-1 [42]. Thus, CASK may play a role in cerebral cortex development *via* the interaction with Tbr-1 (Fig. **4a**). This idea is consistent with the phenotype of smaller brain observed in CASK insertional mutant mice (described in Section 4.1).

To further elucidate the role of Tbr-1 and CASK in brain development, the downstream target genes of the Tbr-1/CASK complex were identified initially from a computerized search using the Tbr-1 binding sequence from GenBank [75]. More than 60 genes containing a Tbr-1 binding site in the promoter region were identified from the search. Around 20 are potential Tbr-1 target genes because of their expression in brain. Among these genes, reelin and NMDA receptor subunit 2b (NR2b) are particularly interesting. The reelin gene encodes a large extracellular matrix protein, which is important in lamination of cerebral cortex [76-78]. This function is consistent with the lamination phenotype of Tbr-1 knockout mice. NR2b is a subunit of an important ion channel NMDA type glutamate receptor (NMDAR). The unique capabilities of NMDAR to gate $Ca^{2+}$ ions and link to $Ca^{2+}$-dependent intracellular signaling such as LTP have indicated the important roles of NMDAR in synaptic plasticity and learning. In addition, NMDAR also plays important roles in neural development, such as axonal guidance and neural circuit formation [79-81], and pathophysiological conditions, such as schizophrenia, Parkinson's disease, Hungtington's disease, Alzheimer's disease, drug abuse, and excitotoxicity [reviewed by 82]. NMDAR is a heteromeric ion channel composed of two NR1 subunits and two NR2 subunits [83]. There are four NR2 subunits, NR2a, NR2b, NR2c, and NR2d, and their expression is regionally and developmentally regulated [84-87]. NR2c is specifically expressed in cerebellum, and NR2d is highly concentrated in midbrain structures. NR2a and NR2b are two major NR2 subunits in the forebrain: NR2b expression starts in the embryonic period, peaks around postnatal day 20, and gradually decreases; NR2a is first detected near birth and gradually increases toward maturation of brain development. Among these four NR2 subunits, NR2b is particularly interesting because the NMDAR containing the NR2b subunit has a longer duration for allowing more $Ca^{2+}$ influx upon synaptic stimulation. Therefore, regulation of NR2b expression and function is the important issue, although little is currently known about regulation of NR2b gene expression [reviewed by 88]. NR2b regulation by the Tbr-1 and CASK complex is the first suggestion of a sequence-specific transcription factor that regulates NR2b expression [75]. Because NR2b is expressed embryonically and involved in axonal outgrowth and neural map formation, it may mediate the function of Tbr-1 in axonal projections between the cerebral cortex and the thalamus. Although Tbr-1 is important for cerebral cortex development, it may also play a role in adult brain because it still expresses a significant amount in adult brain. Conditional Tbr-1 knockout mice will be helpful in addressing the function of Tbr-1 in adult brain and in investigating whether Tbr-1 also regulates NR2b expression in adults.

Because the CASK protein itself does not possess any known transcriptional co-activation motif and mainly functions as an adaptor protein to link functional related proteins together, it is very likely that CASK couples Tbr-1 and other transcriptional modulators, thus regulating transcriptional activity of Tbr-1. The second CASK GK interacting protein, CINAP, may play a role. CINAP contains a conserved nucleosome assembly protein (NAP) domain. More than a dozen NAPs have been identified. They act as histone chaperones, which deposit histones on the newly synthesized chromosomal DNA or remove histones from the chromosome during transcription [reviewed by 89, 90].

CINAP is widely distributed in different tissues with a higher expression level in brain. In mature neurons-non-dividing cells-CINAP cannot contribute to chromosomal DNA synthesis, and it may primarily regulate gene expression. *Via* interaction with CASK, CINAP may modulate chromosomal structure flanking the Tbr-1 binding sites and regulate expression of Tbr-1 downstream target genes. Indeed, in the presence of CASK, CINAP co-

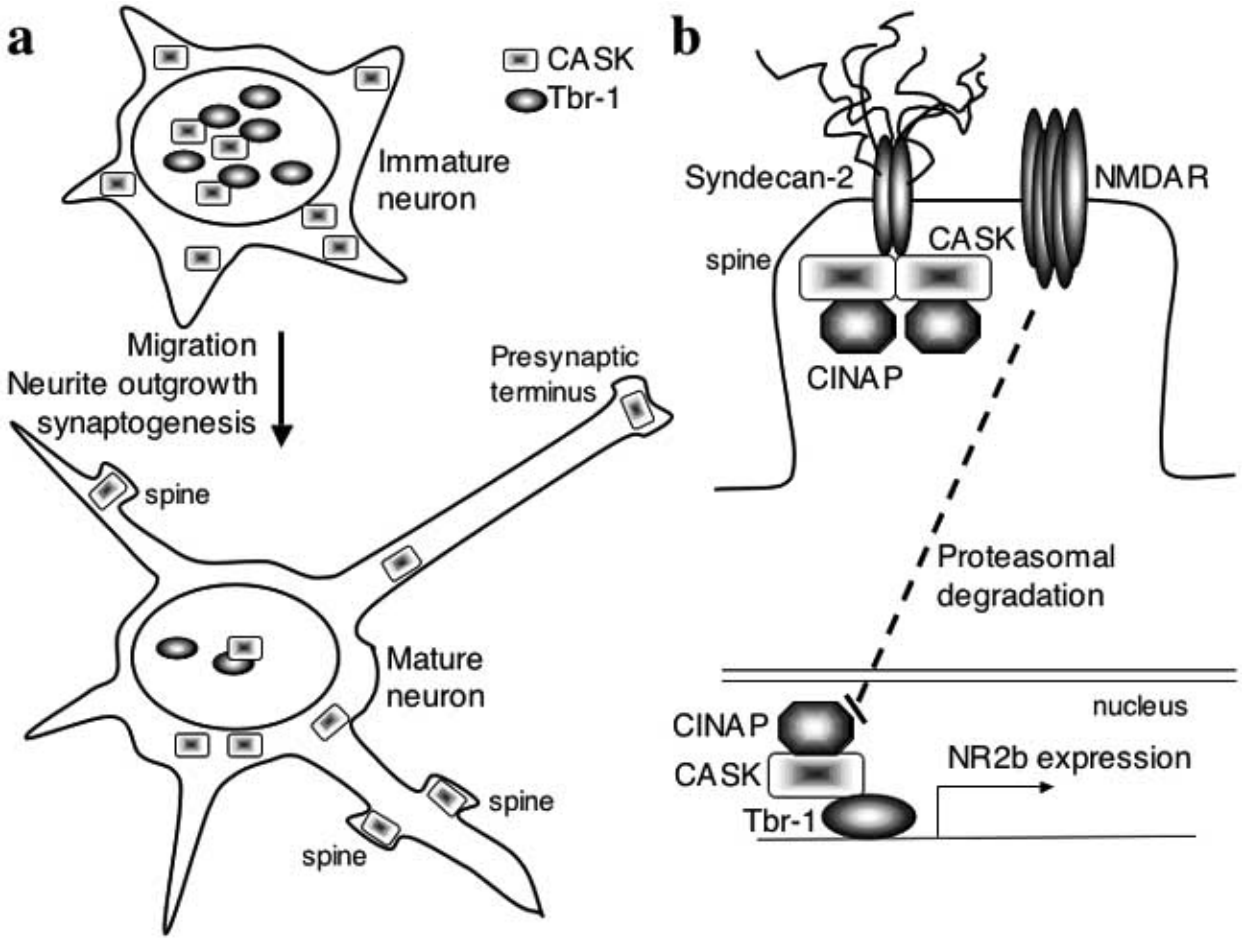


**Fig. (4).** The nuclear function of CASK in the brain.

(**a**) The potential role of the Tbr-1-CASK complex in neural development. In immature neurons, a significant portion of CASK enters the nucleus of neuron, interacts with Tbr-1, and activates expression of genes involved in neural development. Several processes are involved in neural development, including neural migration, neural outgrowth, and synaptogenesis. Based on the phenotype of Tbr-1 knockout mice, the Tbr-1-CASK complex may regulate expression of genes, such as reelin and NR2b, that contribute to migration and axonal outgrowth. In mature neurons, the majority of CASK is present in the cytoplasm, dendrites, dendritic spines, and axonal termini. Expression of genes involved in development controlled by the Tbr-1-CASK complex is therefore reduced. (**b**) The function of the CINAP-CASK-Tbr-1 complex in synaptic response. Through the interaction with CASK, Tbr-1 further forms a complex with CINAP. The CINAP-CASK-Tbr-1 complex regulates NR2b expression. Because CINAP protein levels are controlled by a proteasomal degradation pathway activated by NMDAR, the CINAP-CASK-Tbr-1 complex mediates a negative feedback mechanism by which NMDAR activation downregulates NR2b expression. In addition, a portion of CINAP overlaps with CASK at the synapse. It is unclear if the synaptic CASK-CINAP complex can be translocated into the nucleus upon an unknown signal or whether the synaptic CASK-CINAP complex can perform a function distinct from regulation of gene expression.

immunoprecipitated with Tbr-1 in heterologous cells. The results of immunofluorescence staining indicate that CINAP, CASK, and Tbr-1 colocalize in the nuclei of neurons in layer 6 of the cerebral cortex in postnatal day 1 rat brain (Fig. **4b**) [43]. More important, according to the results of chromatin immunoprecipitation, all Tbr-1, CASK, and CINAP associate with the Tbr-1 binding site of the NR2b promoter, indicating that CASK, CINAP, and Tbr-1 form a complex on the NR2b promoter (Fig. **4b**) [43]. The luciferase reporter assay further supports that CINAP is required for NR2b expression; knocking down of endogenous CINAP reduced the activity of the NR2b promoter in cultured hippocampal neurons [43].

CINAP is an unstable protein. Its protein levels are regulated by a proteasomal degradation pathway downstream of NMDAR [43]. Therefore, the CINAP-CASK-Tbr-1 protein complex provides a negative feedback mechanism with which synaptic NMDAR activation downregulates NR2b expression (Fig. **4b**). This finding helps explain why synaptic activation inhibits NR2b expression and synaptic blockade increases NR2b expression in neurons. This study is also the first to provide an example of a nucleosome assembly protein regulating expression of specific gene *via* a specific protein-protein interaction.

CINAP protein levels are regulated by synaptic activity *via* a proteasomal degradation pathway [43], indicating a role of CINAP in synaptic response. Like CASK, CINAP is also

widely distributed in different regions of rodent brain [91]. We noticed that the immunoreactivity of CINAP in the nuclei of hypothalamus, including the paraventricular nucleus (PVN), arcuate nucleus, suprachiasmatic nucleus, and supraoptic nucleus, is very prominent. The hypothamalus is the center controlling many physiological responses, such as osmoregulation, growth, metabolism, stress response, and circadian rhythm. To further explore the role of CINAP in synaptic responses *in vivo*, systematic saltwater administration was used to modulate neural activity in the PVN [91]. Total immunoreactivity of CINAP in the PVN is around 2-3-fold higher in mice administered saltwater compared with control mice. In addition, more neurons in the PVN containing nuclear CINAP were observed [91]. The results support that both CINAP total protein levels and nuclear translocation are controlled by synaptic activity. It will be intriguing to further explore if CINAP is required for synaptic response, if modulation of CINAP protein levels or nuclear translocation modifies neural activity, and whether the interaction with CASK is involved in the function of CINAP in neural activity.

Because Tbr-1 is restricted in the cerebral cortex, hippocampus, and olfactory bulb and CASK and CINAP are widely distributed in different regions of brain [30, 91], the nuclear function of CASK and CINAP in other regions can not be mediated by Tbr-1. By yeast two-hybrid screening, Bcl11A/Evi9/CTIP was identified as the third nuclear protein interacting with CASK [44]. Like CASK and CINAP, Bcl11A is broadly distributed in different regions of brain [44]. It will be interesting to investigate if Bcl11A works together with CASK and CINAP in the same manner as Tbr-1. Because Bcl11A has been shown to act as a transcriptional repressor *via* the interaction with a sequence-specific transcription factor, COUP-TF [92], it is also possible that Bcl11A represses the activity of CINAP or Tbr-1 through interaction with CASK. More experiments need to be performed to address whether CASK forms different transcriptional regulatory complexes *via* interactions with different nuclear proteins.

In the MAGUK family, CASK is not the only member that enters the nuclei of cells. The tight junction-associated MAGUK protein ZO-1 also plays a role in the nucleus. In subconfluent MDCK and LLC-PK1 cells, ZO-1 is present in the nuclei. However, ZO-1 is restricted at the cell junction of the confluent culture [93]. In the nucleus, ZO-1 interacts with the Y-box transcription factor, ZONAB (ZO-1-associated nucleic acid binding protein), and regulates expression of an EGFR member ErbB2 [94]. ZONAB is critical for regulation of MDCK cell proliferation because reduction of ZONAB expression by RNA interference or antisense approaches strongly reduces proliferation of MDCK cells [95]. These studies indicate a role of the ZO-1-ZONAB tight junction complex in regulation of epithelial cell proliferation. The examples of CASK and ZO-1 imply a role for MAGUK proteins in the communication between cell junction and the nucleus.

## 5. CONCLUSIONS AND FUTURE STUDIES

In summary, CASK performs its multiple functions *via* interactions with different binding partners. Three major functions can be inferred for the CASK protein complexes. The first function is synaptic interaction and formation (Fig. **3**). The interactions with neurexin, syndecan-2, and SynCAM imply a role for CASK in synaptic interaction and formation. In this respect, CASK may function at both pre- and post-synaptic sites because syndecan-2 and SynCAM are expressed at both sites. Abolishment of these interactions by removing the CASK PDZ binding site from these CASK binding partners impairs the synaptic formation ability of these molecules. However, the direct evidence that CASK itself is required for synaptic formation is still missing. Dominant-negative mutants or RNA interference will be useful approaches to address the function of CASK in this regard.

Second, CASK is also involved in protein trafficking and synaptic targeting (Fig. **2**). The Mint1-CASK-Veli protein complex is important for transportation of NMDAR from soma to synapses. Also, both Mint1 and CASK control synaptic targeting of the N-type calcium channel. Because CASK interacts with many synaptic proteins, it is possible that CASK is involved in synaptic targeting of other proteins, such as the GRIP1-GluR2/3 complex or serotonin receptor 5-HT2C. Thus far, KIF17 has been identified as a cargo molecule for the Mint1-CASK-Veli complex. It will be interesting to explore whether other cargo molecules are involved in the transportation mediated by the CASK protein complex.

The third function of CASK is to regulate gene expression and neural development (Fig. **4**). Through interaction with Tbr-1 and CINAP, CASK contributes to both neural development and the response from the synapse to the nucleus in neurons. It is still unclear whether CASK can directly transfer from synapses to the nucleus. Because CASK is quite abundant and stable in neurons, it is also possible that synaptic and nuclear CASK proteins are actually two independent pools and that they individually perform different functions in different subcellular compartments. Time-lapse imaging to monitor the movement of CASK in neurons may provide the answer.

CASK is widely distributed in different subcellular regions, interacts with many proteins, and performs multiple functions. It is intriguing to explore whether any signal regulates the subcellular distribution and protein-protein interaction of CASK. Because protein phosphorylation is one of most common posttranslational modifications modulating protein function, we have been interested in identifying which protein kinase phosphorylates CASK and thus regulates CASK function. From our screening, PKA is one of the protein kinases phosphorylating CASK. PKA phosphorylation of CASK proteins enhances the complex formation of Tbr-1, CASK, and CINAP [96]. It will be intriguing to further investigate whether other protein kinases can also phosphorylate CASK and regulate interactions between CASK and other binding partners. Understanding the regulation of CASK protein-protein interactions will help to dissect the detailed functions of CASK.

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## REFERENCES


[1] Kim, E.; Sheng, M. *Nat. Rev. Neurosci.*, **2004**, *5,* 771.
[2] Montgomery, J.M.; Zamorano, P.L.; Garner, C.C. *Cell Mol. Life Sci.*, **2004**, *61,* 911.
[3] Hata, Y.; Butz, S.; Sudhof, T.C. *J. Neurosci.*, **1996**, *16,* 2488.
[4] Cohen, A.R.; Woods, D.F.; Marfatia, S.M.; Walther, Z.; Chishti, A.H.; Anderson, J.M.; Wood, D.F. *J. Cell Biol.*, **1998**, *142,* 129.
[5] Caruana, G. *Int. J. Dev. Biol.*, **2002**, *46,* 511.
[6] Hoskins, R.; Hajnal, A.F.; Harp, S.A.; Kim, S.K. *Development*, **1996**, *122,* 97.
[7] Migaud, M.; Charlesworth, P.; Dempster, M.; Webster, L.C.; Watabe, A.M.; Makhinson, M.; He, Y.; Ramsay, M.F.; Morris, R.G.; Morrison, J.H.; O'Dell, T.J.; Grant, S.G. *Nature*, **1998**, *396,* 433.
[8] McGee, A.W.; Topinka, J.R.; Hashimoto, K.; Petralia, R.S.; Kakizawa, S.; Kauer, F.W.; Aguilera-Moreno, A.; Wenthold, R.J.; Kano, M.; Bredt, D.S. *J. Neurosci.*, **2001**, *21,* 3085.
[9] Laverty, H.G.; Wilson, J.B. *Genomics*, **1998**, *53,* 29.
[10] Atasoy, D.; Schoch, S.; K.A. Nadasy, K.A.; Ho, A.; Liu, X.; Nosyreva, E.D.; Fernandez-Chacon, R.; E.T. Kavalali, E.T.; Sdhof, T.C. *Annual Meeting, Society for Neuroscience, 2005*, **2005 of Conference**, Program No. 964.20. 2005 Abstract Viewer/Itinerary Planner.
[11] Dobrosotskaya, I.; Guy, R.K.; James, G.L. *J. Biol. Chem.*, **1997**, *272,* 31589.
[12] Gaide, O.; Favier, B.; Legler, D.F.; Bonnet, D.; Brissoni, B.; Valitutti, S.; Bron, C.; Tschopp, J.; Thome, M. *Nat. Immunol.*, **2002**, *3,* 836.
[13] Butz, S.; Okamoto, M.; Sudhof, T.C. *Cell*, **1998**, *94,* 773.
[14] Borg, J.P.; Straight, S.W.; Kaech, S.M.; de Taddeo-Borg, M.; Kroon, D.E.; Karnak, D.; Turner, R.S.; Kim, S.K.; Margolis, B. *J. Biol. Chem.*, **1998**, *273,* 31633.
[15] Tabuchi, K.; Biederer, T.; Butz, S.; Sudhof, T.C. *J. Neurosci.*, **2002**, *22,* 4264.
[16] Yap, C.C.; Liang, F.; Yamazaki, Y.; Muto, Y.; Kishida, H.; Hayashida, T.; Hashikawa, T.; Yano, R. *J. Neurochem.*, **2003**, *85,* 123.
[17] Ohno, H.; Hirabayashi, S.; Kansaku, A.; Yao, I.; Tajima, M.; Nishimura, W.; Ohnishi, H.; Mashima, H.; Fujita, T.; Omata, M.; Hata, Y. *Oncogene*, **2003**, *22,* 8422.
[18] Lu, C.S.; Hodge, J.J.; Mehren, J.; Sun, X.X.; Griffith, L.C. *Neuron*, **2003**, *40,* 1185.
[19] Zordan, M.A.; Massironi, M.; Ducato, M.G.; Te Kronnie, G.; Costa, R.; Reggiani, C.; Chagneau, C.; Martin, J.R.; Megighian, A. *J. Neurophysiol.*, **2005**, *94,* 1074.
[20] Marble, D.D.; Hegle, A.P.; Snyder, E.D., 2nd; Dimitratos, S.; Bryant, P.J.; Wilson, G.F. *J. Neurosci.*, **2005**, *25,* 4898.
[21] Doerks, T.; Bork, P.; Kamberov, E.; Makarova, O.; Muecke, S.; Margolis, B. *Trends Biochem. Sci.*, **2000**, *25,* 317.
[22] Lee, S.; Fan, S.; Makarova, O.; Straight, S.; Margolis, B. *Mol. Cell Biol.*, **2002**, *22,* 1778.
[23] Kaech, S.M.; Whitfield, C.W.; Kim, S.K. *Cell*, **1998**, *94,* 761.
[24] Feng, W.; Long, J.F.; Fan, J.S.; Suetake, T.; Zhang, M. *Nat. Struct. Mol. Biol.*, **2004**, *11,* 475.
[25] Feng, W.; Long, J.F.; Zhang, M. *Proc. Natl. Acad. Sci. USA,* **2005**, *102,* 6861.
[26] Sheng, M.; Sala, C. *Annu. Rev. Neurosci.*, **2001**, *24,* 1.
[27] Hung, A.Y.; Sheng, M. *J. Biol. Chem.*, **2002**, *277,* 5699.
[28] Doyle, D.A.; Lee, A.; Lewis, J.; Kim, E.; Sheng, M.; MacKinnon, R. *Cell*, **1996**, *85,* 1067.
[29] Daniels, D.L.; Cohen, A.R.; Anderson, J.M.; Brunger, A.T. *Nat. Struct. Biol.*, **1998**, *5,* 317.
[30] Hsueh, Y.P.; Yang, F.C.; Kharazia, V.; Naisbitt, S.; Cohen, A.R.; Weinberg, R.J.; Sheng, M. *J. Cell Biol.*, **1998**, *142,* 139.
[31] Fallon, L.; Moreau, F.; Croft, B.G.; Labib, N.; Gu, W.J.; Fon, E.A. *J. Biol. Chem.*, **2002**, *277,* 486.
[32] Biederer, T.; Sara, Y.; Mozhayeva, M.; Atasoy, D.; Liu, X.; Kavalali, E.T.; Sudhof, T.C. *Science*, **2002**, *297,* 1525.
[33] Maximov, A.; Bezprozvanny, I. *J. Neurosci.*, **2002**, *22,* 6939.
[34] Maximov, A.; Sudhof, T.C.; Bezprozvanny, I. *J. Biol. Chem.*, **1999**, *274,* 24453.
[35] Nix, S.L.; Chishti, A.H.; Anderson, J.M.; Walther, Z. *J. Biol. Chem.*, **2000**, *275,* 41192.
[36] McGee, A.W.; Bredt, D.S. *J. Biol. Chem.*, **1999**, *274,* 17431.
[37] Shin, H.; Hsueh, Y.P.; Yang, F.C.; Kim, E.; Sheng, M. *J. Neurosci.*, **2000**, *20,* 3580.
[38] Lue, R.A.; Marfatia, S.M.; Branton, D.; Chishti, A.H. *Proc. Natl. Acad. Sci. USA,* **1994**, *91,* 9818.
[39] Marfatia, S.M.; Lue, R.A.; Branton, D.; Chishti, A.H. *J. Biol. Chem.*, **1994**, *269,* 8631.
[40] Biederer, T.; Sudhof, T.C. *J. Biol. Chem.*, **2001**, *276,* 47869.
[41] Agarwal, K.C.; Miech, R.P.; Parks, R.E., Jr. *Methods Enzymol.*, **1978**, *51,* 483.
[42] Hsueh, Y.P.; Wang, T.F.; Yang, F.C.; Sheng, M. *Nature*, **2000**, *404,* 298.
[43] Wang, G.S.; Hong, C.J.; Yen, T.Y.; Huang, H.Y.; Ou, Y.; Huang, T.N.; Jung, W.G.; Kuo, T.Y.; Sheng, M.; Wang, T.F.; Hsueh, Y.P. *Neuron*, **2004**, *42,* 113.
[44] Kuo, T.-Y.; Hsueh, Y.-P. *Annual meeting, Society for Neuroscience, 2005*, **2005 of Conference**, Program No. 727.7. 2005 Abstract Viewer/Itinerary Planner.
[45] Kim, E.; Naisbitt, S.; Hsueh, Y.P.; Rao, A.; Rothschild, A.; Craig, A.M.; Sheng, M. *J. Cell Biol.*, **1997**, *136,* 669.
[46] Pak, D.T.; Yang, S.; Rudolph-Correia, S.; Kim, E.; Sheng, M. *Neuron*, **2001**, *31,* 289.
[47] Naisbitt, S.; Kim, E.; Tu, J.C.; Xiao, B.; Sala, C.; Valtschanoff, J.; Weinberg, R.J.; Worley, P.F.; Sheng, M. *Neuron*, **1999**, *23,* 569.
[48] Pak, D.T.; Sheng, M. *Science*, **2003**, *302,* 1368.
[49] Ule, J.; Ule, A.; Spencer, J.; Williams, A.; Hu, J.S.; Cline, M.; Wang, H.; Clark, T.; Fraser, C.; Ruggiu, M.; Zeeberg, B.R.; Kane, D.; Weinstein, J.N.; Blume, J.; Darnell, R.B. *Nat. Genet.*, **2005**, *37,* 844.
[50] Hsueh, Y.P.; Sheng, M. *J. Neurosci.*, **1999**, *19,* 7415.
[51] Wilson, J.B.; Ferguson, M.W.; Jenkins, N.A.; Lock, L.F.; Copeland, N.G.; Levine, A.J. *Cell Growth Differ.*, **1993**, *4,* 67.
[52] Olsen, O.; Moore, K.A.; Fukata, M.; Kazuta, T.; Trinidad, J.C.; Kauer, F.W.; Streuli, M.; Misawa, H.; Burlingame, A.L.; Nicoll, R.A.; Bredt, D.S. *J. Cell Biol.*, **2005**, *170,* 1127.
[53] Kaufmann, N.; DeProto, J.; Ranjan, R.; Wan, H.; Van Vactor, D. *Neuron*, **2002**, *34,* 27.
[54] Jo, K.; Derin, R.; Li, M.; Bredt, D.S. *J. Neurosci.*, **1999**, *19,* 4189.
[55] Setou, M.; Nakagawa, T.; Seog, D.H.; Hirokawa, N. *Science*, **2000**, *288,* 1796.
[56] Guillaud, L.; Setou, M.; Hirokawa, N. *J. Neurosci.*, **2003**, *23,* 131.
[57] Missler, M.; Fernandez-Chacon, R.; Sudhof, T.C. *J. Neurochem.*, **1998**, *71,* 1339.
[58] Irie, M.; Hata, Y.; Takeuchi, M.; Ichtchenko, K.; Toyoda, A.; Hirao, K.; Takai, Y.; Rosahl, T.W.; Sudhof, T.C. *Science*, **1997**, *277,* 1511.
[59] Song, J.Y.; Ichtchenko, K.; Sudhof, T.C.; Brose, N. *Proc. Natl. Acad. Sci. USA,* **1999**, *96,* 1100.
[60] Nam, C.I.; Chen, L. *Proc. Natl. Acad. Sci. USA,* **2005**, *102,* 6137.
[61] Levinson, J.N.; Chery, N.; Huang, K.; Wong, T.P.; Gerrow, K.; Kang, R.; Prange, O.; Wang, Y.T.; El-Husseini, A. *J. Biol. Chem.*, **2005**, *280,* 17312.
[62] Tkachenko, E.; Rhodes, J.M.; Simons, M. *Circ. Res.*, **2005**, *96,* 488.
[63] Couchman, J.R. *Nat. Rev. Mol. Cell Biol.*, **2003**, *4,* 926.
[64] Ethell, I.M.; Yamaguchi, Y. *J. Cell Biol.*, **1999**, *144,* 575.
[65] Leonoudakis, D.; Conti, L.R.; Anderson, S.; Radeke, C.M.; McGuire, L.M.; Adams, M.E.; Froehner, S.C.; Yates, J.R., 3rd; Vandenberg, C.A. *J. Biol. Chem.*, **2004**, *279,* 22331.
[66] Leonoudakis, D.; Conti, L.R.; Radeke, C.M.; McGuire, L.M.; Vandenberg, C.A. *J. Biol. Chem.*, **2004**, *279,* 19051.
[67] Becamel, C.; Alonso, G.; Galeotti, N.; Demey, E.; Jouin, P.; Ullmer, C.; Dumuis, A.; Bockaert, J.; Marin, P. *EMBO J.*, **2002**, *21,* 2332.
[68] Becamel, C.; Gavarini, S.; Chanrion, B.; Alonso, G.; Galeotti, N.; Dumuis, A.; Bockaert, J.; Marin, P. *J. Biol. Chem.*, **2004**, *279*(19), 20257.
[69] Backstrom, J.R.; Price, R.D.; Reasoner, D.T.; Sanders-Bush, E. *J. Biol. Chem.*, **2000**, *275,* 23620.
[70] Hong, C.-J.; Hsueh, Y.-P. *Annual Meeting, Society for Neuroscience, 2005*, **2005 of Conference**, Program No. 727.6. 2005 Abstract Viewer/Itinerary Planner.
[71] Im, Y.J.; Park, S.H.; Rho, S.H.; Lee, J.H.; Kang, G.B.; Sheng, M.; Kim, E.; Eom, S.H. *J. Biol. Chem.*, **2003**, *278,* 8501.
[72] Bulfone, A.; Smiga, S.M.; Shimamura, K.; Peterson, A.; Puelles, L.; Rubenstein, J.L. *Neuron*, **1995**, *15,* 63.

[73] Bulfone, A.; Wang, F.; Hevner, R.; Anderson, S.; Cutforth, T.; Chen, S.; Meneses, J.; Pedersen, R.; Axel, R.; Rubenstein, J.L. *Neuron*, **1998**, *21,* 1273.
[74] Hevner, R.F.; Shi, L.; Justice, N.; Hsueh, Y.; Sheng, M.; Smiga, S.; Bulfone, A.; Goffinet, A.M.; Campagnoni, A.T.; Rubenstein, J.L. *Neuron*, **2001**, *29,* 353.
[75] Wang, T.F.; Ding, C.N.; Wang, G.S.; Luo, S.C.; Lin, Y.L.; Ruan, Y.; Hevner, R.; Rubenstein, J.L.; Hsueh, Y.P. *J. Neurochem.*, **2004**, *91,* 1483.
[76] Ogawa, M.; Miyata, T.; Nakajima, K.; Yagyu, K.; Seike, M.; Ikenaka, K.; Yamamoto, H.; Mikoshiba, K. *Neuron*, **1995**, *14,* 899.
[77] Rice, D.S.; Curran, T. *Annu. Rev. Neurosci.*, **2001**, *24,* 1005.
[78] Tissir, F.; Goffinet, A.M. *Nat. Rev. Neurosci.*, **2003**, *4,* 496.
[79] Dickson, K.S.; Kind, P.C. *Curr. Biol.*, **2003**, *13,* R920.
[80] Herkert, M.; Rottger, S.; Becker, C.M. *Eur. J. Neurosci.*, **1998**, *10,* 1553.
[81] Behar, T.N.; Scott, C.A.; Greene, C.L.; Wen, X.; Smith, S.V.; Maric, D.; Liu, Q.Y.; Colton, C.A.; Barker, J.L. *J. Neurosci.*, **1999**, *19,* 4449.
[82] Loftis, J.M.; Janowsky, A. *Pharmacol. Ther.*, **2003**, *97,* 55.
[83] Premkumar, L.S.; Qin, F.; Auerbach, A. *J. Gen. Physiol.*, **1997**, *109,* 181.
[84] Monyer, H.; Burnashev, N.; Laurie, D.J.; Sakmann, B.; Seeburg, P.H. *Neuron*, **1994**, *12,* 529.
[85] Akazawa, C.; Shigemoto, R.; Bessho, Y.; Nakanishi, S.; Mizuno, N. *J. Comp. Neurol.*, **1994**, *347,* 150.
[86] Wenzel, A.; Fritschy, J.M.; Mohler, H.; Benke, D. *J. Neurochem.*, **1997**, *68,* 469.
[87] Portera-Cailliau, C.; Price, D.L.; Martin, L.J. *J. Neurochem.*, **1996**, *66,* 692.
[88] Myers, S.J.; Dingledine, R.; Borges, K. *Annu. Rev. Pharmacol. Toxicol.*, **1999**, *39,* 221.
[89] Adams, C.R.; Kamakaka, R.T. *Curr. Opin. Genet. Dev.*, **1999**, *9,* 185.
[90] Gruss, C.; Sogo, J.M. *Bioessays*, **1992**, *14,* 1.
[91] Lin, C.W.; Huang, T.N.; Wang, G.S.; Kuo, T.Y.; Yen, T.Y.; Hsueh, Y.P. *J. Comp. Neurol.*, **2006**, *494,* 606.
[92] Avram, D.; Fields, A.; Pretty On Top, K.; Nevrivy, D.J.; Ishmael, J.E.; Leid, M. *J. Biol. Chem.*, **2000**, *275,* 10315.
[93] Gottardi, C.J.; Arpin, M.; Fanning, A.S.; Louvard, D. *Proc. Natl. Acad. Sci. USA,* **1996**, *93,* 10779.
[94] Balda, M.S.; Matter, K. *EMBO J.*, **2000**, *19,* 2024.
[95 Balda, M.S.; Garrett, M.D.; Matter, K. *J. Cell Biol.*, **2003**, *160,* 423.
[96] Huang, T.-N.; Chang, H.-P.; Wang, G.-S.; Hsueh, Y.-P. *Annual Meeting, Society for Neuroscience, 2005*, **2005 of Conference**, Program No. 727.8. 2005 Abstract Viewer/Itinerary Planner.

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