Review

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Cdk5: A New Player at Synapses

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Cdk5 · p35 · Synapse · NMJ · Cyclin

Abstract
Cdk5 is a member of the cyclin-dependent kinase family. Unlike other conventional Cdks that are major regulators of eukaryotic cell cycle progression, Cdk5 displays diverse functions in neuronal as well as non-neuronal tissues. In particular, accumulating evidence points to the roles of this kinase in CNS development and other cellular processes. In this article, we summarize the functional roles of Cdk5 pertaining to the formation and functions of synapse, a specialized structure for the fundamental functions of neurons.

Introduction

Cyclin-dependent kinase 5 (Cdk5) is a unique member of the Cdk family, playing key roles in a number of cellular processes rather than the control of cell cycle progression. Since the discovery of Cdk5 in the early 1990s, an explosion of studies has unraveled many remarkable properties of this kinase. Whereas the activity of other members in the Cdk family depends on the association with another family of regulators, the cyclins, Cdk5 interacts with and is activated by its non-cyclin activators, p35 or p39 [1–3]. Although Cdk5 is ubiquitously expressed in most tissues, its kinase activity is largely restricted in the nervous system, owing to the specific localization of its activators [4]. The best-characterized function of Cdk5 is to regulate neuronal migration, mainly based on the studies of null mice of Cdk5 and its activators [5–7]. Moreover, several lines of evidence also point to the roles of Cdk5 in neurite outgrowth and cytoskeleton dynamics [8–10]. Intriguingly, deregulated Cdk5 activity is also linked to neurodegenerative diseases such as Alzheimer's disease [11, 12] and amyotrophic lateral sclerosis [13].

Cdk5 Is Not a Typical Cyclin-Dependent Kinase

Cdk5, also known as neuronal Cdc2-like kinase (NCLK), was initially identified by biochemical purification from bovine brain [14]. Although Cdk5 shows a high sequence homology to Cdc2 and Cdk2 (~60% identity in the mammalian system), there are striking differences between Cdk5 and other Cdks. Cdks are so named because their activity depends on association with a family of regulatory factors, the cyclins [15]. Surprisingly, although Cdk5 can associate with cyclin D1 and cyclin E, these Cdk5/cyclin complexes cannot phosphorylate histone H1 [16, 17]. The activity of Cdk5 depends on association with non-cyclin activators, p35 or p39, indicating

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that Cdk5 is not cyclin-dependent. Notably, Cdk5 activity is in large part restricted to neurons because of the spatial expression of its activators. p35, also known as neuronal Cdk5 activator, was originally identified as Cdk5 binding partner in brain extract by two different groups [1, 2]. Another identified Cdk5 activator, p39, also known as neuronal Cdk5 activator isoform, shares 57% amino acid identity with p35 [3]. Interestingly, although p35 and p39 show little sequence similarity to cyclins, it has been reported that p35 may adopt a cyclin-like structure for Cdk5 activation [18, 19]. Whereas Cdk5 is present in both cytoplasm and membrane, p35 and p39 are enriched in plasma membrane [20–22], owing to an N-terminal myristoylation signal motif [10]. Recent studies also reveal the expression of Cdk5 and p35 in the nucleus [23, 24].

The difference between Cdk5 and other Cdks is also exhibited in the regulation of its activity by phosphorylation and association with inhibitors. Whereas the conventional Cdks require Thr160 phosphorylation in their T-loop by Cdk-activated kinase (CAK) for maximal activation [25], this site is dispensable for Cdk5 full activation, though Cdk5 contains the equivalent Ser159 residue [26, 27]. On the contrary, the structural analysis of p25/Cdk5 predicts that upon phosphorylation on Ser159, the activity of Cdk5 would probably be inhibited [28]. Furthermore, dual-specificity kinases Wee1 and Myt1 phosphorylate Thr14 and Tyr15 of conventional Cdks and subsequently inhibit their activities [25]. However, although Thr14 and Tyr15 are conserved in Cdk5, Wee1 cannot phosphorylate Cdk5 in vitro [26]. Bovine thymus contains an inhibitory kinase that inactivates Cdk5 activity in vitro, but its identity still remains a mystery [29]. Unexpectedly, a novel adaptor protein, Cables, links Cdk5 to the non-receptor tyrosine kinase, c-Abl, which phosphorylates Tyr15 of Cdk5 and subsequently enhances its activity [30]. In addition, while Cdk-inhibitory subunits (CKIs) such as p21 and p27 are efficient inhibitors for other Cdks, they cannot inhibit Cdk5 effectively, suggesting that Cdk5 has unique mechanisms for the regulation of its activity [31].

Whereas other Cdks are the major regulators in the control of cell cycle progression, there is no evidence that Cdk5 participates in the regulation of cell cycle, which represents another significant difference between Cdk5 and other Cdks. In contrast, Cdk5 is involved in many other cellular processes, particularly in the nervous system. During the last decade, the knowledge of Cdk5 has been accumulated dramatically, partially as a result of the identification and characterization of its substrates. To date, more than 20 substrates for Cdk5 have been identified [32]. Interestingly, most of the proteins are not substrates for other Cdks, indicating that Cdk5 has distinct characteristics from other Cdks.

Well-Established Role of Cdk5 in Neuronal Migration

The best-characterized functional role of Cdk5 is to regulate cytoarchitecture in the central nervous system (CNS), which is revealed from the studies of Cdk5 knockout mice. In the adult mouse brain, six layers reside in the outer surface of the brain, cerebral cortex, which is established by neuronal migration in an inside-out fashion during development: the neurons in the outer layer come from the later born cells and migrate through previously formed layers to reach their final destination. Whereas Cdk5 knockout mice show perinatal lethality [5], the anatomical study reveals the disruption of neuronal layering in many brain regions, including cerebral cortex, hippocampus, cerebellum and olfactory bulb, pointing to a role of Cdk5 in neuronal migration [5, 33, 34]. p35 null mice display a similar inverted layering of cortical neurons. However, they exhibit mild disruptions in the hippocampus and cerebellum, possibly due to the compensation of p39 [6, 35]. Unlike Cdk5 knockout mice, p35 null mice are viable and fertile. While p39-deficient mice display normal phenotype, p35/p39 double-mutant mice exhibit indistinguishable characters as Cdk5 null mice, suggesting that p35 and p39 are necessary and sufficient for Cdk5 activation [7]. All the evidence above indicates that Cdk5 is important for neuronal positioning.

While Cdk5 displays critical functions in neuronal migration, various studies also unravel the significant roles of Cdk5 in axon guidance, cytoskeleton dynamics, membrane transport and neurodegenerative diseases [32, 36]. In addition, recent studies also provide evidence that Cdk5 activity can regulate the synaptic functions in the CNS as well as the neuromuscular junction.

Central Synapse and Neuromuscular Junction

Synapse is a specialized structure where neurotransmission occurs either between neurons or between neuron and effector cell. The neurotransmitter release represents one of the pivotal events in synaptic transmission. At the synapse, neurotransmitters are stored in the synaptic vesicles at the presynaptic sites. When depolarization signals arrive at the presynaptic terminals, neurotransmitters are

released to the synaptic cleft, bound to the receptors present on the postsynaptic membrane, and trigger the opening of ion channels, which results in either depolarization or hyperpolarization of the postsynaptic cells [37]. During the formation of synapses, the pre- and postsynaptic cells undergo complex modifications in order to mediate rapid and accurate neurotransmission. The presynaptic differentiation is characterized by accumulations of synaptic vesicles at the presynaptic terminals as well as the vesicle recycling through endocytosis [38]. On the other hand, the postsynaptic differentiation is characterized by the clustering of neurotransmitter receptors and postsynaptic proteins, e.g. postsynaptic density protein 95 (PSD-95), in the postsynaptic sites. Neurons in the CNS communicate at the central synapse, whose formation and maintenance are largely unknown, owing to its inaccessibility and complexity. Most of the knowledge about synapse formation is derived from the study of one specialized synapse, the neuromuscular junction (NMJ), where the motor neuron communicates with the muscle fiber [39]. The NMJ is an intricate structure composed of the presynaptic motor nerve terminal ensheathed by the Schwann cell, the postsynaptic muscle fiber, and the synaptic cleft, which is occupied by the basal lamina. The presynaptic specialization in the NMJ involves the accumulation of synaptic vesicles containing neurotransmitters, such as acetylcholine (ACh), at the active zones of nerve terminals, while the postsynaptic specialization of the muscle fibers is well characterized by the clustering of a number of postsynaptic proteins, including acetylcholine receptor (AChR), and the clustering of nuclei at the subsynaptic region (the subsynaptic nuclei) that contributes to selective gene transcription [40]. Two major nerve-derived molecules, agrin and neuregulin (NRG), have been identified to induce the postsynaptic specialization via different molecular mechanisms. Agrin mediates the clustering of pre-existing AChRs and other postsynaptic proteins on the muscle fibers by activation of the receptor tyrosine kinase, MuSK [41–44]. On the other hand, NRG increases the local transcription of AChR subunits in the subsynaptic nuclei of the muscle fibers through stimulation of the receptor tyrosine kinase, ErbB [45–48].

Accumulating evidence indicates that Cdk5 is a new player at synaptic sites, both the central synapse and the NMJ [49]. Cdk5 and p35 are expressed at the growth cones of the neurons [9, 50]. Moreover, Cdk5 is localized in both pre- and postsynaptic terminals of the neurons, revealed by immunogold labeling [51]. Subcellular fractionation experiments show that Cdk5 and its activators are present in the synaptosomes [20]. Cdk5 is involved in neurosecretion at the presynaptic terminal [32]. Furthermore, Cdk5 modulates dopamine signaling and affects NMDA receptor-mediated induction of long-term potentiation (LTP) in the postsynaptic sites of the central synapses [51]. In addition, Cdk5 activity is potentially involved in the NMJ formation and maintenance [22].

Cdk5 in Neurotransmitter Release and Endocytosis

One of the most prominent roles of Cdk5 at the presynaptic terminal is the regulation of neurotransmitter release (fig. 1). The presynaptic terminal is characterized by the accumulation of numerous synaptic vesicles, which are filled with neurotransmitters. A number of proteins are involved in the process of transmitter release from the synaptic vesicles, including ATPase, *N*-ethylmaleimide-sensitive fusion protein (NSF), soluble NSF attachment proteins (SNAPs), the SNAP receptors (SNAREs) integral to either synaptic vesicles (v-SNAREs; i.e. vesicle-associated membrane protein (VAMP)) or target membrane (t-SNAREs; i.e. syntaxin and synaptosome-associated protein of 25 kDa (SNAP-25)). For the release of neurotransmitter, v-SNAREs and t-SNAREs are associated to create a 7S core complex that further recruits SNAPs and NSF to form a 20S complex, which is required to bring the donor and target membranes to close proximity and become fusion-competent when NSF hydrolyses ATP [52–56]. The process of membrane fusion is regulated by Munc-18. Munc-18 interacts with one of the t-SNAREs, syntaxin 1A, preventing the interaction of syntaxin 1A with v-SNAREs, which is required for secretory vesicles to achieve competency for membrane fusion at the presynaptic terminals. Interestingly, association of Cdk5/p35 with Munc-18/syntaxin 1A is likely to occur at the presynaptic terminals in the absence of ATP. In the presence of ATP, Cdk5 phosphorylates Thr574 of Munc-18 and subsequently disassembles Munc-18/syntaxin 1A complex, allowing the interaction between dissociated syntaxin 1A and v-SNAREs, which leads to increased neurotransmitter release [57, 58]. This finding indicates that Cdk5 activity can enhance neurotransmission through modulation of Munc-18/syntaxin 1A interaction. On the contrary, Cdk5 also negatively regulates secretory responses through modulation of the interaction of SNARE proteins with the calcium channels. The voltage-dependent calcium channels (VDCCs) are involved in the regulation of neurotransmitter release at the presynaptic terminals, and P/Q-type VDCCs are highly concentrated at central syn-

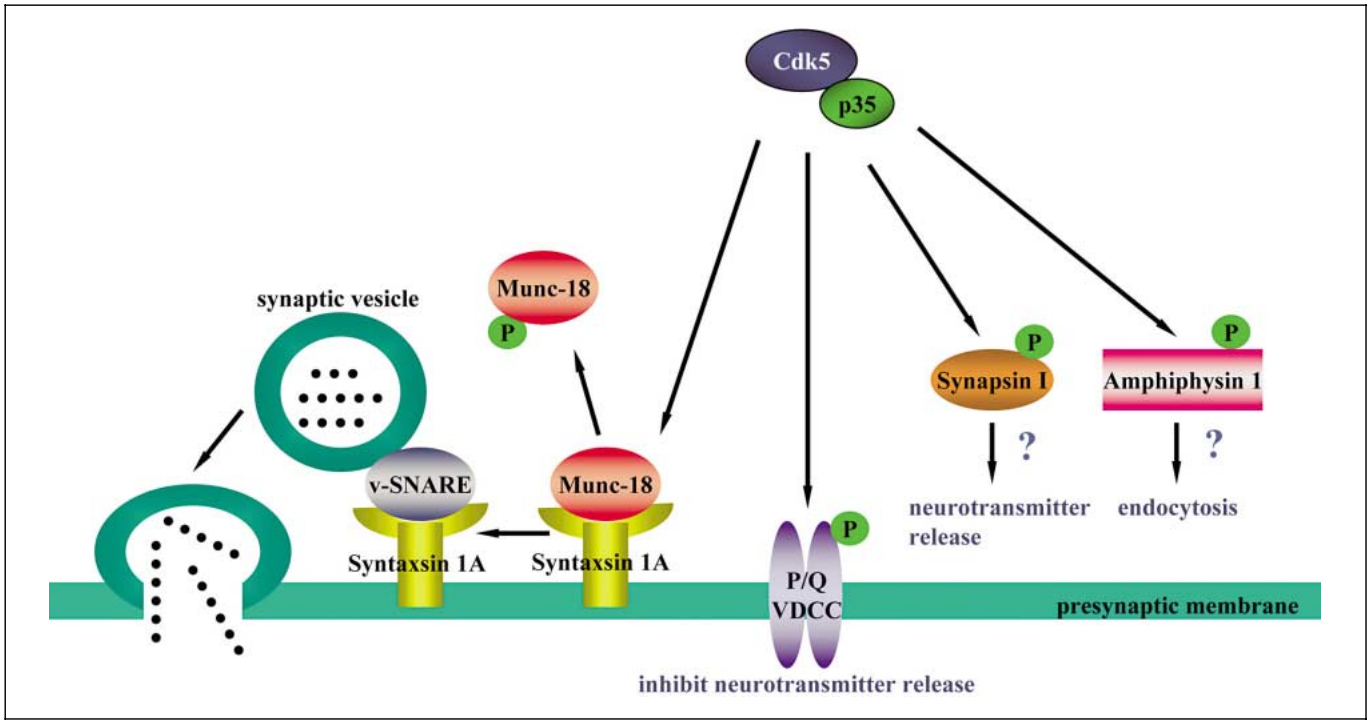

Fig. 1. Cdk5 in neurotransmitter release and endocytosis at the presynaptic terminals. In the absence of Cdk5, Munc-18 binds syntaxin 1A, interfering the interaction between syntaxin 1A and v-SNAREs, which is required for synaptic vesicle to gain competency for membrane fusion. Upon phosphorylation by Cdk5, Munc-18 is dissociated from syntaxin, allowing the syntaxin 1A/v-SNAREs interaction and leading to enhanced neurotransmitter release. In contrast, Cdk5 inhibits neurotransmitter release through phosphorylation of P/Q type voltage-dependent calcium channel (VDCC), resulting in the dissociation of VDCC from SNAREs, which attenuates the efficiency of neurotransmission. Cdk5 also phosphorylates synapsin I and amphiphysin 1, which are potentially involved in neurotransmitter release and endocytosis, respectively, although the physiological significance remains to be determined.

apses [59, 60]. Cdk5 is able to phosphorylate the intracellular loop connecting domains II and III (L_{II-III}) of P/Q-type VDCCs, and the phosphorylation subsequently disrupts the interaction between VDCCs and SNARE proteins, such as SNAP-25 and synaptotagmin, which is required for efficient neurotransmission [59, 61]. Moreover, Cdk5 inhibitor roscovitine enhances neurotransmitter release by increasing the EPSP slope and Ca^{2+} influx of P/Q-type VDCCs, suggesting that Cdk5 also suppresses calcium channel activity [61]. Finally, one of the major phosphoproteins of synaptic vesicles at the nerve terminals, synapsin I, is thought to be involved in the regulation of neurotransmitter release [62]. It has been reported that Cdk5 phosphorylates Ser551 and Ser553 of synapsin I, although the physiological significance of the phosphorylation is still unknown [63]. Taken together, these studies show that Cdk5 can regulate neurotransmitter release both positively and negatively through different molecular mechanisms.

The efficient neurotransmission is regulated by two presynaptic cycles, the neurotransmitter cycle and the synaptic vesicle cycle [64]. The neurotransmitter cycle involves transmitter biosynthesis, storage, reuptake and degradation, while the synaptic vesicle cycle involves targeting to the nerve terminal, docking, fusion, endocytosis and recycling [64]. These two cycles coordinate and form the basis of neurotransmitter release at the presynaptic terminals. During high rates of neurotransmitter release, the nerve terminals maintain a relatively constant surface area by endocytosis. Actually, exocytosis is followed rapidly by endocytosis, which is necessary for recycling synaptic vesicles at the nerve terminals [64]. Clathrin and the clathrin adaptors such as AP180 and AP-2 are the main components in the process of endocytosis. The recycling

of synaptic vesicles at the presynaptic terminal depends on the formation of clathrin coat on nascent endocytotic vesicles. Other accessory proteins are also implicated in the process of synaptic vesicle endocytosis, including dynamin, synaptojanin, endophilin and amphiphysin [65]. Based on a yeast two-hybrid screen, a number of proteins that are involved in the process of endocytosis have been identified to interact with p35 [66, 67, unpubl. observation]. Amphiphysin 1, an abundant phosphoprotein in the nerve terminals of mature neurons, belongs to a protein family conserved from yeast to human, whose members play critical roles in endocytosis possibly by interacting with dynamin, AP2 and clathrin [68–72]. Amphiphysin 1 knockout mice exhibit defects in synaptic vesicle recycling, indicating an important endocytotic function of amphiphysin 1 [73]. Mammalian amphiphysin 1 interacts with p35 and can be phosphorylated by Cdk5 at the sites including Ser272, Ser276 and Ser285 [66, 67]. Interestingly, the yeast homologue of amphiphysin 1 (Rvs167) binds the p35 counterpart Pcl2 in yeast, and is a substrate of Pho85, the Cdk5 homologue, indicating a conserved function of the protein networks from yeast to human [74]. Strikingly, Pcl2, Pho85, and Rvs167 mutations exhibit similar defects in endocytosis and actin function in yeast [74]. Although the physiological function of amphiphysin 1 phosphorylation by Cdk5 remains to be elucidated, the similarity between p35/Cdk5/amphiphysin 1 and Pcl2/Pho85/Rvs167 protein networks suggests a potential role of Cdk5 in endocytosis at synapses (fig. 1).

Cdk5 in Receptor Signaling

To accomplish the efficient reception of neurotransmitter signals, the postsynaptic terminal is specialized by the clustering of a number of proteins, including specific neurotransmitter receptors, ion channels, signaling molecules, adaptor and scaffold proteins, protein kinases, protein phosphatases, cytoskeletal and adhesion proteins. Different receptors (e.g. glutamate receptors and dopamine receptors) clustered at synapses are responsible for ligand activation and signal transduction, which form the basis of the normal synaptic functions. For example, at glutamatergic synapses in the CNS, glutamate receptors are clustered on the postsynaptic membrane, including *N*-methyl-*D*-aspartate receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), and metabotropic glutamate receptor (mGluR) [75]. Increasing evidence demonstrates that Cdk5 can modulate various receptor signaling at the synapse (fig. 2).

NMDA Receptor Signaling

Glutamate is the major neurotransmitter that mediates most of the excitatory neurotransmission. Glutamate receptors are classified as ionotropic receptors or metabotropic receptors (mGluRs). Ionotropic glutamate receptors are further divided into NMDA receptors, AMPA receptors, kainate receptors and δ receptors. In the CNS, NMDAR is important for learning, memory and CNS development [76, 77]. NMDAR is an NR1/NR2 heteromeric complex composed of the receptor subunit NR1 and the regulatory subunits NR2A–NR2D [78]. The activation of NMDAR correlates with the induction of long-term synaptic plasticity including LTP [79]. Adult NR2A-deficient mice show defective LTP and impaired spatial memory, suggesting a critical role of NR2A in LTP induction [80]. Recent evidence indicates a direct linkage between Cdk5 and NMDAR: Cdk5 associates with NR2A and phosphorylates Ser1232 of NR2A both in vitro and in intact cells, and the phosphorylation is attenuated in Cdk5 null mice. Notably, the Cdk5 inhibitor roscovitine blocks LTP induction as well as NMDA-evoked currents in rat CA1 hippocampal neurons, suggesting an involvement of Cdk5 in the regulation of NMDAR in LTP induction, which might contribute to memory and learning [51].

Interestingly, a recent report also correlates Cdk5 function with NMDAR through identification of two interacting proteins of Cdk5 activator, α-actinin-1 and α-subunit of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII$_\alpha$) [81]. CaMKII$_\alpha$, α-actinin-1 and Cdk5 activator, p35 or p39, are present in a complex and the association of these proteins is stimulated by Ca^{2+}. In addition, the NMDAR antagonist MK801 reduces the association between p35 and CaMKII$_\alpha$ to basal levels, suggesting that the glutamate-mediated increase of the interaction between Cdk5 activator and CaMKII$_\alpha$ is mainly regulated by NMDAR signaling [81]. These observations indicate a possible cross talk between Cdk5 and CaMKII pathways mediated by NMDARs, which might represent a critical mechanism underlying synaptic plasticity, learning and memory.

mGluR Signaling

Another type of glutamate receptors, the mGluR, is a member of the superfamily of seven transmembrane segment G protein-coupled receptors that exert their effects via direct modulation of ion channels or formation of second messengers [82, 83]. (S)-3,5-dihydroxyphenylglycine

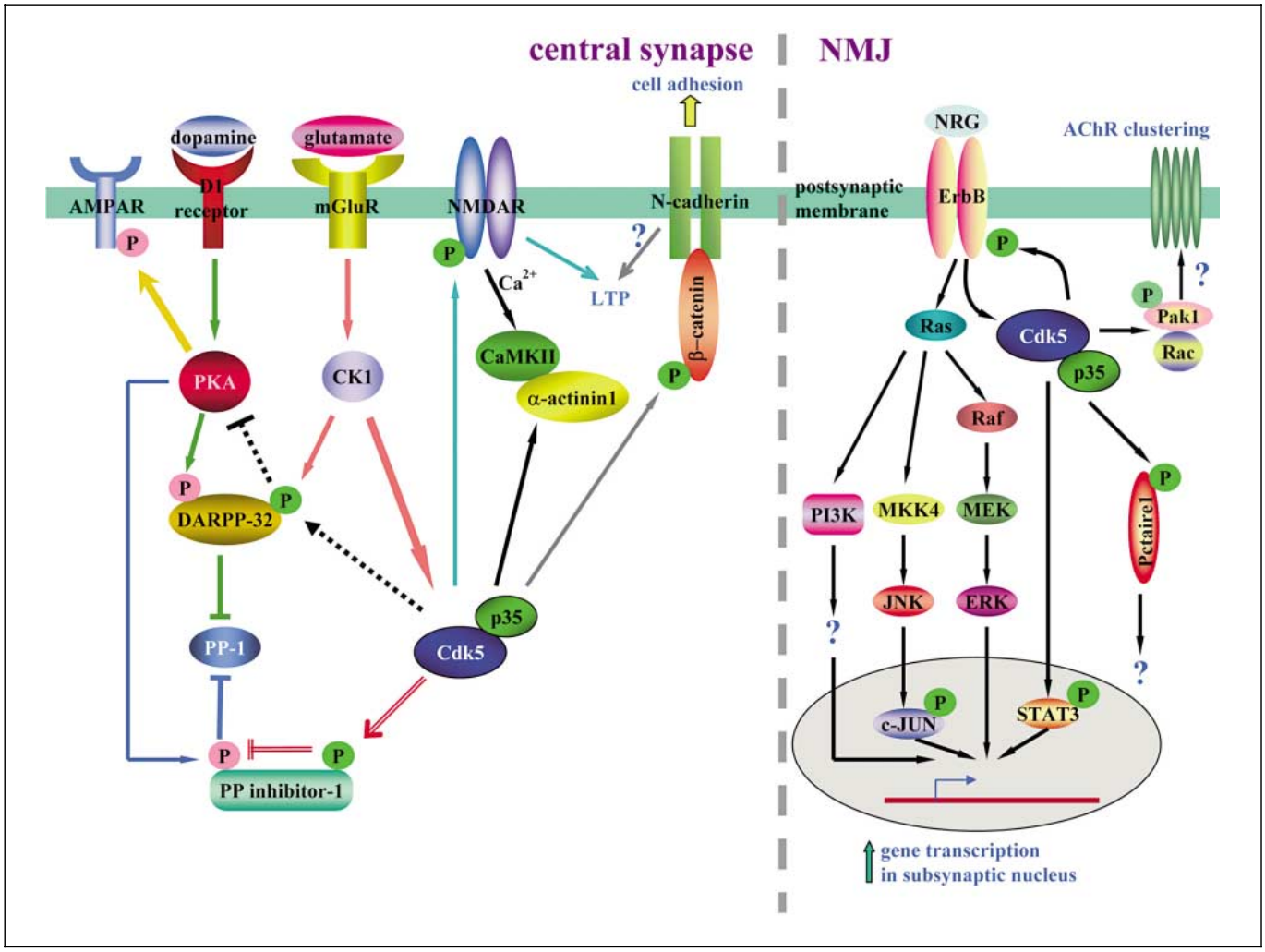

Fig. 2. Involvement of Cdk5 in the signal transduction at synapses. Cdk5 phosphorylates DARPP-32, a modulating protein in the D1 dopamine receptor-PKA pathway. The phosphorylation of DARPP-32 by Cdk5 converts it into an inhibitor of PKA, whereas the phosphorylation of DARPP-32 by PKA renders it an inhibitor of PP-1. Moreover, Cdk5 phosphorylates PP inhibitor-1 and makes it a poor substrate of PKA, which in turn attenuates the inhibitory ability of PP inhibitor-1 to PP-1. Furthermore, activated mGluR induces CK1 and subsequently Cdk5 activities, which contribute to DARPP-32 phosphorylation. In addition, Cdk5 phosphorylates NR2A subunit of NMDA receptor (NMDAR), leading to LTP induction. Conversely, NMDAR-induced Ca^{2+} influx stimulates the association of p35/CaMKII/α-actinin1 complex. Cdk5 also inhibits cell-cell adhesion through phosphorylation of β-catenin, dissociating β-catenin from N-cadherin, an adhesive molecule that is also involved in LTP induction. At the postsynaptic region of the NMJ, NRG-ErbB signaling induces Cdk5 activity, which in turn phosphorylates ErbB receptors, leading to increased transcription of AChR. Cdk5 also induces phosphorylation of STAT3 and gene transcription in subsynaptic nucleus. Furthermore, Cdk5 phosphorylates Pctaire1 and upregulates its activity in muscle, although the physiological significance is not clear. In addition, Cdk5 interacts with Rac and Pak1 and phosphorylates Pak1 in a Rac-dependent manner, raising an intriguing possibility that Cdk5 might regulate AChR clustering at the postsynaptic site of the NMJ.

(DHPG), an agonist for group I mGluRs, induces both casein kinase 1 (CK1) and Cdk5 activities in neostriatal neurons [84]. Both CK1 and Cdk5 phosphorylate a signaling molecule in dopamine signaling cascade, dopamine and cyclic AMP-regulated phosphoprotein with molecular mass 32 kDa (DARPP-32) [84, 85]. CK1 phosphorylates Thr137 of DARPP-32, while Cdk5 phosphorylates Thr75 of DARPP-32. Moreover, Cdk5 inhibitor butyrolactone selectively blocks Thr75 phosphorylation of DARPP-32, whereas CK1 inhibitors CK1–7 and IC261

abolish DHPG-evoked Cdk5 activity as well as the phosphorylation of both Thr137 and Thr75 of DARPP-32, suggesting that Cdk5 resides downstream of CK1 in the mGluR-CK1-Cdk5-DARPP-32 cascade [84]. Interestingly, application of DHPG induces Ca^{2+} currents in neostriatal neurons and the enhancement is greatly diminished in the presence of CK1–7 or butyrolactone, as well as in DARPP-32–/– neurons, indicating that mGluR-mediated upregulation of Ca^{2+} currents potentially involves CK1, Cdk5 and DARPP-32 [84].

Dopamine Signaling

DARPP-32, an important signaling molecule identified by Greengard's group, displays critical roles in dopamine signaling pathway, which is implicated in several neurological diseases such as Parkinson's disease and schizophrenia [86]. At dopaminergic synapses, the neurotransmitter dopamine activates D1 dopamine receptor and triggers a signaling cascade involving cyclic AMP-dependent protein kinase (PKA), DARPP-32, and type 1 protein phosphatase (PP-1). The activation of D1 dopamine receptor induces PKA phosphorylation on Thr34 of DARPP-32, which converts DARPP-32 into a PP-1 inhibitor, attenuating dephosphorylation of PKA substrates and increasing the efficacy of dopamine signaling [87, 88]. However, when DARPP-32 is phosphorylated at Thr75 by Cdk5, DARPP-32 is converted into an inhibitor of PKA, and subsequently reduces phosphorylation of downstream PKA substrates, including GluR1 subunit of the AMPA receptor [85]. Therefore, the Thr75 phosphorylation of DARPP-32 by Cdk5 inhibits PKA signaling cascade. Furthermore, PKA and Cdk5 can regulate PKA cascade in antagonistic manners through phosphorylation of protein phosphatase inhibitor-1 (PP inhibitor-1) at different residues. Thr35 phosphorylation of PP inhibitor-1 by PKA makes it a potent inhibitor of PP-1, whereas Ser67 phosphorylation of this inhibitor by Cdk5 renders it a poor substrate for PKA, although the Ser67 phosphorylation itself does not affect the inhibitory ability of PP inhibitor-1 [89]. These observations suggest that Cdk5 and PKA can regulate dopamine signaling with opposing effects through phosphorylation of DARPP-32 and PP inhibitor-1. In addition to the regulation of the signaling molecules, a recent study also demonstrates an involvement of Cdk5 induction in cocaine addiction mediated by the transcription factor, ΔFosB, thereby further supporting a role of Cdk5 in the modulation of dopamine signaling [90]. The findings that Cdk5 is involved in both mGluR and dopamine signalings also raise the possibility of a cross talk between these two pathways.

Cadherin-Mediated Signaling

Cadherins are a family of Ca^{2+}-dependent adhesion molecules that are stabilized at the plasma membrane, including neural- (N-) and epithelial- (E-) cadherins. β-Catenin is a cytoplasmic protein that links cadherins to cytoskeleton [91]. The isolation of β-catenin as a p35-interacting protein provides the first demonstration of a role of Cdk5 in cell-cell adhesion, a possible mechanism leading to neuronal migration. β-Catenin is a substrate of Cdk5 and the phosphorylation by Cdk5 dissociates β-catenin from N-cadherin, leading to attenuated cell-cell adhesion [92, 93]. Strikingly, cadherins have been reported to be critical in the induction, but not the maintenance, of LTP in hippocampal slices, which can be significantly blocked by either antibodies or antagonistic peptides of cadherin [94, 95]. A recent study suggests that Cdk5 may regulate β-catenin/cadherin affinity as well as β-catenin localization at synaptic sites through modulation of Tyr654 phosphorylation on β-catenin, which can be diminished by Cdk5 inhibitor roscovitine, although the detailed mechanism remains to be determined [96]. Therefore, the participation of Cdk5 in cadherin signaling suggests yet another potential mechanism of Cdk5 in LTP induction.

ErbB Signaling at the NMJ

In addition to the diverse functional roles of Cdk5 at the central synapse, a report also indicates remarkable functions of Cdk5 in the formation and maintenance of the NMJ. Recently, Cdk5 has been reported to play an important role at the postsynaptic region of the NMJ, involving in the NRG-ErbB signaling pathway [22]. Cdk5 and p35 are highly expressed in embryonic muscle and localized at the NMJ during early postnatal stages of muscle development. NRG, a factor that induces the synapse-specific transcription of AChR subunits at the postsynaptic sites through the ErbB-Ras-Raf-MEK-ERK, ErbB-Ras-MKK4-JNK or ErbB-PI3K pathway [97–99], stimulates p35 and Cdk5 expression as well as Cdk5 activity in C2C12 myotubes. In addition, Cdk5 phosphorylates ErbB2/3, suggesting a positive feedback mechanism. Moreover, the inhibition of Cdk5 activity by roscovitine, dominant-negative Cdk5 construct, or antisense oligonucleotides diminishes the NRG-evoked ErbB activity and the downstream MEK-ERK phosphorylation, accompanied by decreased transcription of luciferase reporter gene driven by the AChRε promoter. In contrast, overexpression of p35 in both C2C12 cells and tibialis anterior muscle induces AChRε promoter activity without NRG treatment [22]. Taken together, these observations demon-

strate a novel role of Cdk5 in the NRG-ErbB signaling at the NMJ. In addition to the modulation of the ErbB receptor, Cdk5 also regulates a downstream signaling molecule in NRG pathway, the transcription factor STAT3 [24]. Interestingly, Cdk5 activity induces phosphorylation of STAT3 and its transcriptional activity in C2C12 myotubes, further supporting a role of Cdk5 in regulating gene expression at the NMJ [24]. Similar regulation on ErbB and STAT3 by Cdk5 is also observed in the CNS [unpubl. observation]. Moreover, NRG and ErbB are also essential for neuronal development in the CNS. Although their precise functions in the adult CNS are unknown, a report demonstrates that ErbB4 associates with PSD-95, and that the expression of PSD-95 can enhance the NRG-mediated activation of ErbB4 and MAP kinase. In addition, NRG suppresses LTP induction in the hippocampal CA1 region without affecting basal synaptic transmission, suggesting that NRG-ErbB signaling in the central synapse may modulate synaptic plasticity [100]. It remains to be determined whether Cdk5 is also involved in the NRG-ErbB signaling at central synapses.

Other Synapse-Related Functions of Cdk5

While the functions of Cdk5 at the NMJ are still to be further explored, a p35-interacting protein in muscle, Pctaire1, has been isolated from a yeast two-hybrid screen (fig. 2). Pctaire1, a Cdk-related kinase, interacts with p35 and can be phosphorylated by Cdk5 on Ser95. Although the significance of this phosphorylation in muscle remains to be determined, it enhances Pctaire1 activity in vitro [101]. Recently, a report indicates that Pctaire1 can regulate neurite outgrowth in Neuro2A cells, suggesting a possible link between Pctaire1 and cytoskeleton [102]. These findings raise a possibility that Cdk5 may regulate the clustering of the postsynaptic proteins via p35-Pctaire1 interaction and Pctaire1 phosphorylation, which might be involved in the regulation of cytoskeleton. Notably, Cdk5/p35 colocalizes and associates with small GTPase Rac and its effector Pak1, the important players in actin polymerization. Furthermore, Cdk5 causes Pak1 hyperphosphorylation in a Rac-dependent manner, which results in decreased Pak1 activity [9]. Because the Rho family of GTPases and Pak kinases are implicated in actin polymerization [103, 104], the modification of Pak1 by Cdk5 is likely to affect the actin network, which might result in the clustering of the postsynaptic proteins. Additionally, Rac has been reported to mediate agrin-induced AChR clustering in muscle [105, 106]. Moreover, Pak1 interacts with Dvl, a MuSK interacting protein, and can be activated by agrin in a Dvl-dependent manner, leading to AChR clustering [107]. Inhibition of Cdk5 regulates the agrin-mediated Pak1 phosphorylation [108]. Taken together, these observations raise a possibility that Cdk5/p35 might play a role in the regulation of AChR clustering in muscle through interaction with Rac and phosphorylation of Pak1 (fig. 2).

Moreover, several lines of evidence also link Cdk5 to synaptic activity. For example, nerve injury induces Cdk5 and p35 expression as well as Cdk5 activity in skeletal muscle, whereas the treatment of sciatic nerve with tetrodotoxin (TTX), a blocker of voltage-gated Na^+ channels, results in increased p35 expression in muscle, indicating that nerve activity regulates Cdk5 activity at the postsynaptic regions [109]. Depolarization experiments in hippocampal neurons also reveal that neural activity inhibits Cdk5 activity, suggesting that Cdk5 activity is highly regulated at synapses [96].

Conclusion

Cdk5 has been identified as a neuronal Cdc2-like kinase a decade ago. Unlike other members of the Cdk family, Cdk5 is neither cyclin-dependent, nor responsible for the control of cell cycle progression. Although the best-known function of Cdk5 is in neuronal migration and the development of the CNS, increasing evidence unravels additional roles of this kinase in neuronal and non-neuronal systems. The involvement of Cdk5 in the central synapse and the NMJ, largely based on the identification of a number of interacting proteins as well as substrates for Cdk5/p35, reveals novel functions of this enzyme, including neurotransmitter release and various receptor signalings, which might contribute to, at least in part, the basic functions of the brain and muscle. Further study of Cdk5 will undoubtedly provide more exciting insights into the mechanisms of these processes.

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