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Tale of the Good and the Bad Cdk5: Remodeling of the Actin Cytoskeleton in the Brain

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Abstract

Cdk5 kinase, a cyclin-dependent kinase family member, is a key regulator of cytoskeletal remodeling in the brain. Cdk5 is essential for brain development during embryogenesis. After birth, it is essential for numerous neuronal processes such as learning and memory formation, drug addiction, pain signaling, and long-term behavior changes, all of which rely on rapid alterations in the cytoskeleton. Cdk5 activity is deregulated in various brain disorders including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and ischemic stroke, resulting in profound remodeling of the neuronal cytoskeleton, loss of synapses, and ultimately neurodegeneration. This review focuses on the "good and bad" Cdk5 in the brain and its pleiotropic contribution in regulating neuronal actin cytoskeletal remodeling. A vast majority of physiological and pathological Cdk5 substrates are associated with the actin cytoskeleton. Thus, our special emphasis is on the numerous Cdk5 substrates identified in the past two decades such as ephexin1, p27, Mst3, CaMKv, kalirin-7, RasGRF2, Pak1, WAVE1, neurabin-1, TrkB, 5-HT6R, talin, drebrin, synapsin I, synapsin III, CRMP1, GKAP, SPAR, PSD-95, and LRRK2. These substrates have unraveled the molecular mechanisms by which Cdk5 plays divergent roles in regulating neuronal actin cytoskeletal dynamics both in healthy and diseased states.

Keywords

Cdk5; p25; p35; β-Amyloid; Neuronal cytoskeleton; Actin; Microtubules; Remodeling; Cdk; Cyclins; Neurodegeneration; Alzheimer's disease; Parkinson's disease; Dendritic spines; Neurotransmitters; Synaptic plasticity; Learning and memory; Neuronal migration; Axonal growth; Neurotransmitters; Synaptogenesis; Drug addiction; Ephexin1; p25; Mst3; CaMKv; Kalirin-7; RasGRF2; Pak1; WAVE1; Neurabin-1; TrkB; 5-HT6R; Talin; Drebrin; Synapsin I; Synapsin III; CRMP1; GKAP; SPAR; PSD-95; LRRK2

Introduction

Cyclin-dependent kinases (Cdks) are proline-directed serine/threonine kinases that are essential for cell cycle progression. Currently, more than 20 Cdk members are known [1]. Cdks bind specific protein partners, "cyclins," for activation, which also determine their

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substrate specificity. Cdks are additionally regulated via phosphorylation by CAK, Myt1, and Wee1 kinases. Myt1 and Wee1 phosphorylate Cdks at conserved T14 and Y15 residues (human Cdk1 numbering) within the active site, respectively, which interferes with proper ATP binding, resulting in their inhibition. Conversely, CAK phosphorylates Cdks at T161 (human Cdk1 numbering) in the T-loop, which stabilizes Cdk-cyclin complexes and improves substrate binding, resulting in their full activation. Cdk-cyclin complexes also interact with a number of Cdk inhibitor proteins (CKIs), such as p21Cip1, p27Kip1, p57Kip2, and Ink4 family members, which impede their kinase activities under non-optimal conditions during the cell cycle [2].

Cyclin-dependent kinase-5 (Cdk5) shares high homology with other family members; however, it differs from them by adopting unique activation strategies and distinctive cellular functions [3–5]. Cdk5 is not activated by classical cyclins such as cyclin A, cyclin D, and cyclin E but binds to its own specific protein partners, p35, p39, and cyclin I [6–8]. Cyclin I has not been shown to activate any other Cdks, suggesting it might be a unique Cdk5 activator.

Likewise, Cdk5 interacts with its own set of negative regulators and is not inhibited by p27Kip1 (aka p27), p21Cip1, or p57Kip2, which inhibit other family members. Instead, Cdk5 activity is inhibited by binding GSTP1, cyclin D1, and cyclin E [9–11]. Furthermore, Cdk5 is not phosphorylated at T14, and phosphorylation at Y15 by EphA4, c-Abl or Fyn has been shown to cause Cdk5 activation, instead of inhibition observed for other family members [12–14]. Importantly, one study reported that phosphorylation at Y15 has no impact on Cdk5 activity [15].

Cdk5 also has distinct functions in a variety of neuronal and non-neuronal tissues. The Cdk5 activators, p35 and p39, are highly expressed in the central nervous system, particularly in postmitotic neurons. As a result, Cdk5 plays a vital role in neuronal signaling, where other Cdks are minimally active or expressed. During embryogenesis, Cdk5 plays a central role in brain development by regulating neuronal migration, neurite outgrowth, axon guidance, and synapse formation [16]. After birth, Cdk5 activity is crucial for higher cognitive functions such as learning and memory formation, synaptic plasticity and homeostasis, drug addiction, and long-term behavioral changes, which rely on fast neuronal cytoskeletal adaptations [17–20].

Cdk5 activity is tightly regulated temporally and spatially in cells under physiological conditions. Both inactivation and hyperactivation of Cdk5 are neurotoxic, which give rise to various neurodevelopmental and neurological disorders. For example, reduction or loss of Cdk5 activity is associated with epilepsy, attention-deficit/hyperactivity disorder (ADHD), mental retardation, and schizophrenia [21–24]. In contrast, hyperactivation of Cdk5 occurs in many neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), ischemic stroke, and amyotrophic lateral sclerosis (ALS) and is highly destructive [25–27].

Multiple neurotoxic signals including β-amyloid (Aβ), excitotoxicity, ischemia, and oxidative stress increase intracellular calcium levels in neurons, causing the activation of

calpain, which cleaves p35 into p25 and p10 [28]. p25 has a much longer half-life compared to p35 and lacks the membrane anchoring signal. This results in the constitutive activation and mislocalization of the Cdk5-p25 complex, which results in the phosphorylation of a variety of non-physiological targets, culminating in neuronal death [29–35].

This review highlights the functions of the "good and bad Cdk5 in the brain" with specific emphasis on its direct targets and the molecular mechanisms by which it regulates neuronal actin cytoskeleton in the brain.

Cdk5 and Neuronal Cytoskeleton

The neuronal cytoskeletal network is made of actin filaments, microtubules, and neurofilaments. These cytoskeletal polymers differ in diameter, subcellular localization, mechanical stiffness, polarity, assembly dynamics, and the molecular motors they associate with, which results in their unique architecture and functions in neurons [36].

Actin filaments form sheet-like structures and are highly enriched in the leading edge of dendrites and axons including the lamellipodia and filopodia (Fig. 1a). Microtubules (MTs) form a stiff track-like structure in the axon and transport materials between the cell body and axon terminals at the synapse. Neurofilaments form a structural matrix in the axon, which nestles MTs and resists mechanical stresses [36]. Active Cdk5 is highly enriched in the neuronal cytoskeletal fractions and is a major orchestrator of actin, microtubule, and neurofilament dynamics [5]. This review focuses on the molecular mechanisms by which Cdk5 regulates the pleiotropic neuronal processes that involves reorganization of the actin cytoskeleton. We will also briefly discuss the consequences of Cdk5 deregulation in eliciting actin cytoskeletal abnormalities in various neurological disorders.

Actin Regulates Cdk5 Activity

Interestingly, while Cdk5 controls actin dynamics by phosphorylating many actin-associated proteins, actin too regulates Cdk5 activity by directly associating with it. Actin consists of monomeric globular (G) actin and polymeric filamentous (F) actin. The Cdk5 activators, p35 and p39, bind to F-actin, which recruits Cdk5 to the perinuclear region and the peripheral lamellipodia, respectively [37]. Furthermore, p35 binding to F-actin promotes the formation of actin bundles, which stabilizes F-actin against dilution-induced depolymerization [38]. However, binding of F-actin to p35 or p39 does not alter the activity of Cdk5-p35 or Cdk5-p39 complexes. This is in sharp contrast with G-actin, which binds to Cdk5 directly and inhibits its activity, despite the fact that it does not perturb the formation of Cdk5-p35 or Cdk5-p25 complexes [39]. Although the functional consequences of G-actin-mediated inhibition of Cdk5 activity remains unclear in vivo, F-actin polymerization by p35 with a concomitant decrease in the G-actin pool appears to be an alternate mechanism to regulate Cdk5 activity spatially and temporally in neurons.

Cdk5 and Remodeling of the Actin Cytoskeleton

The actin cytoskeleton regulates numerous processes including cell division, protein trafficking, cell migration, and cell morphogenesis. In developing neurons, the actin

cytoskeleton is crucial for neurite formation and branching, as well as for synaptogenesis. G-actin is distributed uniformly throughout neurites, whereas F-actin is highly enriched in axonal and dendritic growth cones of developing neurons. In growth cones, filopodia undergoing dynamic retraction and extension predominantly contain bundled F-actin fibers, while lamellipodia contain a cross-linked actin meshwork [40] (Fig. 1a, b).

In mature neurons, F-actin is highly abundant at both the presynaptic and the postsynaptic terminals (Fig. 1c). F-actin serves as a major scaffold for the organization of the presynaptic components and is essential for the formation and function of the presynaptic active zone and synaptic vesicle pools. Actin remodeling at the presynaptic terminal is crucial for vesicle mobilization, trafficking, and neurotransmitter release [41]. In the postsynaptic terminal, actin is highly expressed in dendritic spines, which are major sites of information handling and storage in the brain [42]. Dendritic spines are highly dynamic membranous protrusions on postsynaptic dendrites, which have constricted necks with either mushroom-shaped big heads or thin smaller heads (Fig. 1c, d). Dendritic spines are believed to originate from highly motile dendritic filopodia during early postnatal life, which upon appropriate synaptic contact with the axon can morphologically and functionally transform into more stable mushroom spines [43]. In the young brain, dendritic filopodia and spines are very dynamic, causing either synapse formation or elimination. During adulthood, the extent of spine dynamics is reduced and they acquire a more stable structure; still, physiological and pathological conditions can spontaneously induce rapid changes in spine size and spine numbers [44]. Postsynaptic density (PSD) is a thickening at the head of dendritic spines which clusters hundreds of proteins including a variety of signaling proteins, receptors (such as NMDA, AMPA, and metabotropic glutamate receptors), adhesion molecules, scaffolding protein PSD-95, and ion channels [45, 46] (Fig. 1d). The PSD, thus, plays a pivotal role in the formation and maintenance of dendritic spines.

F-actin filaments are concentrated in the head, neck, and periphery regions of dendritic spines (Fig. 1d). Structural changes in dendritic spines rely profoundly on actin remodeling, which in turn form the cellular basis for learning and memory formation. Particularly, actin polymerization is associated with spine enlargement/formation during long-term potentiation (LTP) and actin depolymerization leads to spine shrinkage during long-term depression (LTD), making dendritic spines central hubs for information processing in the brain [40]. Actin remodeling also regulates PSD, trafficking of synaptic cargos, protein synthesis, and diffusion of receptors within the plasma membrane [46]. As a result, the process of actin polymerization is critical for synaptogenesis, synaptic plasticity, neuronal motility, and navigation of growth cones.

Cdk5 along with its activators, p35 and p39, is highly localized on actin filaments in the growth cone of developing neurites [47, 48]. Cdk5 is also highly concentrated in mature synapses, particularly in dendritic spines [49]. Cdk5 controls several aspects of actin dynamics by directly phosphorylating several actin-associated proteins and by regulating Rho family GTPases.

Cdk5 Controls Both Upstream Regulators and Downstream Effectors of Rho GTPases: Positive and Negative Regulation

Rho family GTPases are key regulators of actin dynamics [50]. GTPases are molecular switches, which are active in the GTP-bound form and are inactive when bound to GDP. Their activities are controlled by guanine nucleotide exchange factor (GEF) and GTPase-activating protein (GAP), which function to activate or inactivate them, respectively. In humans, 20 Rho GTPases have been discovered so far, of which RhoA, Rac, and Cdc42 remain the best explored [51]. Not surprisingly, Cdk5 exerts its major influence on actin dynamics by regulating all three major Rho GTPases— RhoA, Rac, and Cdc42. Cdk5 phosphorylates numerous upstream regulators and downstream effectors of Rho GTPases, which translates into a multifaceted role for Cdk5 in synaptic plasticity, long-term behavior changes, drug addiction, and learning and memory formation.

Importantly, RhoA, Rac, and Cdc42 elicit opposite effects on axonal growth. Active Rac1 and Cdc42 trigger polymerization at the leading edge, coordinating the formation of lamellipodia, peripheral actin microspikes, filopodia, and dendritic spines [52]. By contrast, active RhoA causes retraction of the leading edge, leading to growth cone collapse, axonal growth inhibition, and loss of dendritic spines [53, 54]. Cdk5 is known to both activate and inhibit Rac and RhoA signaling, but it is only known to activate Cdc42 signaling.

Cdk5 and RhoA

Depending upon the cellular context, Cdk5 can either activate or inhibit RhoA signaling by phosphorylating different up-stream regulators. Thus, it is capable of promoting either growth cone collapse or axonal growth and migration (Table 1).

Cdk5 Activates RhoA via Ephexin1 and CaMKv

Cdk5 facilitates RhoA-mediated growth cone collapse in hippocampal neurons in response to ephrin-A1 [12]. Ephrin-A1 activates the EphA4 receptor, which increases Cdk5 activity by Tyr15 phosphorylation. Active Cdk5 phosphorylates ephexin1, a Rho GEF, which activates RhoA, leading to dendritic spine retraction and growth cone collapse during axon guidance ([12], Fig. 2, Table 1).

In dendritic spines, Cdk5 activates RhoA via an alternate pathway which involves a pseudokinase of the CaMK family named calmodulin kinase-like vesicle-associated (CaMKv) [55]. CaMKv mRNA localizes at dendrites, and its synthesis is induced by neuronal activity and sensory experience. CaMKv is essential for activity-dependent dendritic spine maintenance. Accordingly, CaMKv knockdown in mouse hippocampal CA1 pyramidal neurons deteriorates synaptic plasticity in vivo, resulting in enhanced locomotor activity and impaired spatial memory. In response to AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor activation and Ca^{2+} influx, CaMKv inhibits RhoA by binding its exchange factor—Lfc, resulting in increased spine density. Ephrin-A1-activated Cdk5 phosphorylates CaMKv on Thr345 and inhibits its binding to Lfc. Lfc is then free to stimulate RhoA, resulting in dendritic spine loss ([55], Fig. 2, Table 1).

Cdk5 Inhibits RhoA Signaling via p27 and Mst3

In a contrasting mechanism, Cdk5 downregulates RhoA signaling via p27 (p27Kip1) stabilization, promoting neuronal migration in the developing cerebral cortex [56] (Fig. 2). As noted before, p27 is an inhibitor of Cdk-cyclin complexes. However, independent of its cell cycle functions, p27 also promotes neuronal migration by inhibiting RhoA [57]. Cdk5-mediated phosphorylation of p27 at Ser10 stabilizes and increases its levels. Consequently, p27 binds RhoA and prevents its activation by GEFs. Inhibition of RhoA prevents ROCK1 and LIM-kinase-1 (LIMK1) activation. Active LIMK1 is known to phosphorylate cofilin at Ser3, which impedes its actin severing activity (Fig. 2). Thus, Cdk5-mediated inhibition of LIMK1 via the p27 pathway activates cofilin, facilitating its actin severing function, resulting in neuronal migration.

In a similar mechanism, Cdk5 promotes neuronal migration in developing neurons by inhibiting RhoA via Mst3 kinase [58]. Cdk5-mediated phosphorylation of Mst3 at Ser79 causes Mst3 to phosphorylate RhoA at Ser26, inhibiting its activity (Table 1, Fig. 2). Modulation of Rho activity promotes radial neuronal migration and final neuronal positioning in the developing mouse neocortex. Thus, Cdk5 phosphorylates several upstream regulators of RhoA to regulate actin dynamics, resulting in either growth cone collapse or neuronal migration.

Cdk5 and Rac

As noted above, Rac1 activation leads to axon outgrowth and extension. Similar to RhoA regulation, Cdk5 can both activate and inhibit Rac signaling by phosphorylating either its upstream regulators (kalirin-7, RasGRF2, and neurabin-1) or its downstream effectors (Pak1 andWAVE1) (Table 1, Fig. 3).

Cdk5 Activates Rac Signaling via Kalirin-7

Kalirin-7 is a brain-specific guanine nucleotide exchange factor (GEF) for Rac, which is highly expressed in the hippocampus and cerebral cortex [59]. Expression of kalirin-7 is barely discernible at birth, but it increases in parallel with increased synaptogenesis postnatally [60]. It localizes at the PSD in dendritic spines and plays a crucial role in regulating dendritic spine morphogenesis, synaptic plasticity, and synaptic pathology [61, 62]. Overexpression of kalirin-7 in cortical neurons promotes the formation of dendritic spines, whereas its knockdown prevents it [59, 60]. Not surprisingly, kalirin-7 knockout mice exhibit a deficiency in long-term potentiation (LTP) and a decreased spontaneous excitatory postsynaptic current (sEPSC) frequency, which correlates with decreased spine length, synapse number, and PSD size in their hippocampal CA1 pyramidal neurons [63, 64].

Cdk5 activates the exchange activity of kalirin-7 by phosphorylation at Thr1590, thereby triggering Rac1 activation and dendritic spine stabilization (Fig. 3, Table 1) [65]. GTP-bound Rac1 is known to promote F-actin polymerization and dendritic spine stabilization through the activation of downstream effector p21-activated kinase-1 (Pak1). Active Pak1 in turn phosphorylates LIMK1, activating it. LIMK1 subsequently phosphorylates cofilin at

Ser3, which inhibits its actin severing ability, causing F-actin polymerization and increased dendritic spine density [66]. The significance of Cdk5-kalirin-7 signaling was recently demonstrated in rats, where this pathway promotes the induction of contextual locomotor conditioning during repeated amphetamine exposure, presumably by stimulating drug-induced neuroadaptations in dendritic spines [67].

Cdk5 Inhibits Rac Signaling via RasGRF2, Pak1, WAVE1, and Neurabin-1

Cdk5 inhibits Rac activation by phosphorylating its exchange factor—Ras guanine nucleotide releasing factor 2 (RasGRF2), at Ser737, which reduces its exchange activity [68] (Table 1, Fig. 3). RasGRF2 is a dual Ras/Rac guanine nucleotide exchange factor that is essential for LTP in situ. Cdk5 also inhibits Rac1 by phosphorylating neurabin-1 at Ser95, an F-actin-binding protein. Cdk5-mediated phosphorylation of neurabin-1 decreases its association with F-actin, leading to the speculation that neurabin-1 may regulate levels of active Rac1 at the F-actin cytoskeleton, enabling controlled neurite outgrowth. Cdk5-mediated regulation of neurabin-1 during developmental processes is critical for establishing correct neuronal morphology and migration [69] (Fig. 3).

In another mechanism, Cdk5 inhibits Rac1 signaling by phosphorylating its downstream effector Pak1 (Table 1). p35 directly binds to GTP-bound active Rac1, recruiting the Cdk5-p35 complex to Rac1 and associated Pak1, which allows Cdk5 to directly phosphorylate Pak1, rendering it inactive [70]. As noted above, active Pak1 phosphorylates and activates LIMK1, which inhibits cofilin [66]. Consequently, Cdk5-mediated Pak1 inactivation has the opposite effect, facilitating cofilin-mediated actin severing, leading to axonal outgrowth and neuronal migration (Fig. 3).

Thus, Cdk5 regulates actin depolymerization and polymerization by activating (via p27, Pak1, and RasGRF2 phosphorylation) and inhibiting cofilin (via phosphorylation of kalirin-7), respectively, thereby maintaining the appropriate balance of Ser3 phosphorylation of cofilin. Regulation of cofilin is a vital control point in the full cycle of actin polymerization and depolymerization, required for proper cortical neuronal migration. Cdk5-mediated inactivation of Pak1 also promotes cell spreading via myosin light-chain kinase (MLCK). Pak1 inhibits MLCK activity via phosphorylation and thus blocks cell spreading [71] (Fig. 3).

Cdk5 inhibits another Rac effector, Wiskott-Aldrich syndrome protein (WASP)-family verprolin homologous protein 1 (WAVE1), by phosphorylating it at Ser310, Ser397, and Ser441. WAVE1 is present in dendrites, dendritic spines, and axonal growth cones and plays a crucial role in dendritic spine formation by promoting Arp2/3 complex-dependent actin polymerization. Cdk5-mediated phosphorylation of WAVE1 inhibits its activity, resulting in decreased spine growth [72].

Importantly, WAVE1 is also essential for activity-dependent mitochondrial trafficking to dendritic spines and filopodia through the actin cytoskeleton [73]. While microtubules are required for long-distance mitochondrial movement, the actin cytoskeleton is important for short-distance mitochondrial trafficking [74]. Under basal conditions, WAVE1 associates with the outer mitochondrial membrane, but it is largely inactive due to Cdk5-mediated

phosphorylation. However, repetitive depolarization of hippocampal neurons reduces p35 levels, which in turn decreases Cdk5 activity. Consequently, the active pool of WAVE1 increases, which promotes mitochondrial movement into dendritic spines and filopodia during spine morphogenesis [73].

Cdk5 and Cdc42

Activation of Cdc42 promotes axonal and dendrite outgrowth and extension. Cdk5 indirectly activates Cdc42 via TrkB (tropomyosin receptor kinase B), leading to actin polymerization and dendrite outgrowth (Fig. 4). BDNF (brain-derived neurotropic factor)-induced TrkB activation facilitates Cdk5 activation via Tyr15 phosphorylation, and Cdk5 in turn phosphorylates TrkB at Ser478 in the juxtamembrane region (Table 1). Subsequently, TrkB activates Cdc42, causing dendrite outgrowth and extension [75].

In another mechanism, Cdk5was shown to activate Cdc42-mediated neurite outgrowth by phosphorylating the 5-hydroxytryptamine-6 receptor (5-HT6R, also known as serotonin 6 receptor) at Ser350 in its C-terminus (Fig. 4, [76]). 5-HT6R is a G protein-coupled receptor (GPCR), which upon agonist binding activates adenylate cyclase, triggering the production of second messenger cyclic AMP (cAMP) and subsequent activation of protein kinase A (PKA). Interestingly, Cdk5 associates constitutively at the C-terminus of 5-HT6R under basal conditions, which is independent of agonist activation. Consequently, treatment with 5-HT6R agonists did not amplify neurite outgrowth; however, exposure to 5-HT6R antagonists abrogated its interaction with Cdk5, inhibiting neurite outgrowth. This finding suggests that antagonist binding induces a conformational change in 5-HT6R that results in Cdk5 dissociation. Importantly, Cdk5-mediated phosphorylation at Ser350 was found to be indispensable for the association of Cdc42 with 5-HT6R and subsequent neurite outgrowth; however, the exact mechanism leading to Cdc42 activation remains unclear. Similarly, phosphorylation of 5-HT6R by Cdk5 was essential for the expression of voltage-gated calcium channels (VGCCs), although whether this expression is downstream of Cdc42 needs to be addressed (Fig. 4). Increased neurite outgrowth and expression of VGCCs are hallmarks of neuronal differentiation (Fig. 4).

Collectively, these findings show that Cdk5 acts as a major orchestrator of actin remodeling by regulating the Rho family of GTPases at multiple levels.

Other Cdk5 Substrates Involved in Actin Dynamics (Talin, Drebrin, CRMP1, Synapsin I and Synapsin III)

Cdk5 also directly phosphorylates a number of actin-binding proteins to regulate neuronal migration (Fig. 5). Cdk5 promotes neuronal migration via phosphorylation of talin, an actin- and β-integrin tail-binding protein (Fig. 5). The talin head domain activates integrin and localizes to focal adhesions and maintains cell edge protrusions. However, it is rapidly degraded by Smurf1, an E3 ligase. Cdk5 directly phosphorylates the talin head at Ser425, inhibiting its interaction with Smurf1, thus stabilizing the talin head domain and promoting overall cell migration [77].

Recent studies showed a pivotal role for Cdk5 in organizing F-actin in dendritic spines by phosphorylating drebrin at Ser142, an F-actin-binding protein. Cdk5-mediated phosphorylation of drebrin connects dynamic MTs to F-actin in growth cone filopodia and arranges F-actin in dendritic spines, promoting neuronal migration [78, 79]. Drebrin is highly expressed in growth cones of developing neurons and in dendritic spines of mature neurons.

Cdk5 also promotes semaphorin 3A-induced growth cone collapse by phosphorylating CRMP1 at T509 and S522, which is a member of the collapsin response mediator protein family (CRMPs). Cdk5-mediated phosphorylation of CRMP1 decreases its affinity towards actin, which is believed to promote growth cone collapse and contributes to regeneration after rat sciatic nerve crush [80].

Recently, Cdk5 was shown to phosphorylate synapsin III at S404, which is an atypical member of the synapsin family of synaptic vesicle-associated proteins [81]. Synapsin III localizes at the synapses in adult brains, similar to its family members— synapsin I and synapsin II. However, it is mainly expressed in the cell body and growth cones of developing neurites [82]. Synapsin III is also expressed very early in the neurodevelopmental process and is downregulated in adults. Synapsin III thus plays a pivotal role in early stages of neurodevelopment including neuronal survival, neuritic outgrowth, and polarization; all of these processes are dependent on phosphorylation by Cdk5 (Fig. 5) [81]. Similarly, another study revealed that semaphorin-3A (Sema3A)-induced activation of Cdk5 causes it to phosphorylate synapsin III, which regulates radial migration and orientation in vivo [83] (Fig. 5). Although a direct role of synapsin III in regulating actin dynamics was not demonstrated in any of these studies, synapsin III is present at the growth cone, where intense actin remodeling occurs during neuritic outgrowth and migration. Furthermore, synapsin III shares a high degree of homology with the actin binding domain of synapsin I [84], suggesting that synapsin III-mediated neuronal morphological maturation occurs via direct regulation of actin cytoskeleton.

Cdk5 at the Presynaptic Terminal

Neurotransmitters are released by Ca^{2+}-triggered exocytosis of synaptic vesicles, followed by clathrin-mediated endocytosis and finally uncoating of clathrin-coated vesicles. The actin cytoskeleton is a key regulator of synaptic vesicles trafficking at presynaptic nerve terminals [41]. Cdk5 regulates the presynaptic vesicle pool by phosphorylating a number of substrates involved in exocytosis, endocytosis, and synaptic vesicle recycling and depending upon the context can either inhibit or promote neurotransmitter release. In mature synapses, synapsin I regulates the trafficking of synaptic vesicles and neurotransmitter release by interacting with actin [85, 86]. Cdk5 directly phosphorylates synapsin I at Ser549 and Ser551, which increases its binding to F-actin, thereby favoring the reclustering of recycled synaptic vesicles and contributing to Cdk5-mediated homeostatic scaling ([87], Fig. 5). Cdk5 also facilitates the release of neurotransmitters by phosphorylating Munc-18 (p67). Cdk5-mediated phosphorylation of Munc-18 diminishes its affinity towards syntaxin 1, which in turn associates with SNAP receptors to form the vesicle SNAP receptors (v-SNARE)/

syntaxin complex, leading to the membrane fusion between secretory vesicles and presynaptic terminals, initiating the release of neurotransmitters [88].

Deregulated Cdk5 and Actin Cytoskeleton Abnormalities

Multiple neurological disorders including AD, ALS, PD, and Down's syndrome exhibit profound cytoskeletal abnormalities. Several memory disorders such as mental retardation also involve defects in regulation of the actin cytoskeleton [89]. A recent study implicated that mutations and polymorphisms in Cdk5 and p35 genes may contribute to the onset of the non-syndromic intellectual disability phenotype [90]. Abnormalities of ion channel trafficking is often linked to epilepsy and autism [91]. Cdk5 contributes to these diseases in several ways including regulation of the neuronal cytoskeleton and the trafficking of neuronal components. Similarly, in animal models, inducible activation of Cdk5 in the striatum reduces dendritic spine density, causing impaired motor coordination and decreased locomotor sensitization to cocaine [92].

Cdk5 and the PSD

As noted before, PSD is under the surface membrane of spine heads and serves as a postsynaptic organizing hub, where it assembles a variety of signaling proteins including receptors, channels, and adhesion molecules [46]. Disassembly of the PSD results in the loss of dendritic spines and subsequent decrease in synapse numbers, which correlates well with the onset of cognitive decline in AD pathogenesis.

Cdk5 phosphorylates many PSD-associated proteins and is a crucial regulator of synaptic functions under physiological conditions. PSD-95 (postsynaptic density-95), the most abundant scaffolding protein in PSDs, is a potent regulator of synaptic strength [93]. Approximately 200–300 PSD-95 molecules are present in a typical PSD, where they link NMDA and AMPA receptors to the cytoskeleton and other signaling molecules [94, 95]. Cdk5 was shown to phosphorylate PSD-95 at three sites, Thr19, Ser25, and Ser35, in PSD fractions, which reduces the ability of PSD-95 to multimerize, resulting in decreased NMDAR clustering (Table 2). This finding suggested that Cdk5 may regulate rapid changes in the density of NMDAR, AMPAR, and ion channels at the synapses under physiological conditions. Upon beta-amyloid stimulation, Cdk5 deregulation reduces PSD-95 levels with concomitant decrease in surface expression of AMPAR glutamate receptor subunit 2 [96] (Table 2).

Deregulated Cdk5 also phosphorylates Guanylate kinase-associated protein (GKAP) at Ser77 and Ser111, a PSD scaffolding protein, triggering its degradation and collapse of the synaptic actin cytoskeleton [97] (Table 2). These findings underscore a key role of hyperactivated Cdk5 in mediating Aβ-induced PSD disassembly and synapse loss via remodeling of the actin cytoskeleton.

Cdk5 also phosphorylates PSD-95-interacting protein Spine Associated RapGAP (SPAR), resulting in its degradation ([98], Table 2). SPAR is a Rap-specific GTPase-activating protein (RapGAP), which organizes the actin cytoskeleton and recruits PSD-95 to F-actin. Not surprisingly, SPAR expression in dendrites results in the enlargement of spine heads

[99]. Cdk5 phosphorylates SPAR at Ser1328, which creates a binding site for Plk2, which further phosphorylates it, resulting in its degradation and subsequent synaptic scaling. Although the clinical significance of this pathway in AD pathogenesis is not completely understood, Aβ-induced Cdk5 deregulation indeed increased the phosphorylation at S1328 in cultured hippocampal neurons, suggesting that Cdk5-mediated degradation of SPAR may be one of the mechanisms that lead to synaptic dysfunction in AD.

Leucine-rich repeat kinase 2 (LRRK2) is a commonly mutated protein in both inherited and sporadic forms of PD [100]. It was recently reported that the mutation R1628P in LRRK2 generates an adjacent phosphorylation site for Cdk5. Phosphorylation of this site (Ser1627) by Cdk5 increases LRRK2 activity ([101], Table 2). Interestingly, LRRK2 R1628P increases the susceptibility of WT, but not $Cdk5^{-/-}$ neurons, to cell death when treated with a bioactive metabolite of the toxin MPTP, underscoring a crucial role of Cdk5 in PD pathogenesis. Furthermore, another study showed that a toxic gain of function mutation in LRRK2 increases the activation of ERM proteins, leading to abnormal accumulation of F-actin in filopodia and subsequent neurite outgrowth defects [102]. ERM proteins link cytoplasmic membrane proteins to the actin cytoskeleton. Although the exact mechanism remains undetermined, it appears that deregulated LRRK2 likely hinders the regeneration of neurites in the PD brain, thereby accelerating neuronal degeneration in PD.

Conclusion

In summary, Cdk5 is a crucial regulator of neuronal actin dynamics both under physiological and pathological conditions. The “good Cdk5” regulates neuronal migration, neurotransmission, synaptogenesis, synaptic plasticity, learning and memory formation, neuronal motility, and navigation of growth cones via remodeling of the actin cytoskeleton. On the other hand, the “bad Cdk5” triggers multiple neurodegenerative pathways including loss of synapses, cognitive deficits, and impaired motor functions in various neurological disorders via deregulation of actin dynamics. As a result, Cdk5 is an important therapeutic target for several neurological diseases. Although recent studies have identified several new regulators of Cdk5, increased p25 level is still considered to be the key mechanism that leads to Cdk5 deregulation in various human diseases. Thus, it would be highly beneficial to identify drugs that selectively kill the “bad Cdk5 in complex with p25” and retain the “good Cdk5 in complex with p35” in the brain for retaining its physiological and beneficial functions in the brain.

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Abbreviations

Aβ	β-Amyloid
CAK	Cdk-activating kinase
CaMKv	Calmodulin kinase-like vesicle-associated

Cdk	Cyclin-dependent kinase
Cdk1	Cyclin-dependent kinase-1
Cdk2	Cyclin-dependent kinase-2
Cdk5	Cyclin-dependent kinase-5
CKI	Cdk inhibitor protein
CRMP1	Collapsin response mediator protein-1
EphA	Ephrin receptor A
GAP	GTPase-activating protein
GEF	Guanine nucleotide exchange factor
GSTP1	Glutathione S-transferase pi 1
5-HT6R	5-Hydroxytryptamine-6 receptor
LIMK	LIM kinase
LTD	Long-term depression
LTP	Long-term potentiation
MLCK	Myosin light-chain kinase
MT	Microtubule
Myt1	Membrane-associated tyrosine- and threoninespecific cdc2-inhibitory kinase isoform 1
NMDA	*N*-Methyl-D-aspartate
NMDAR	*N*-Methyl-D-aspartate receptor
Pak1	p21 activated kinase
p21Cip1	Cyclin-dependent kinase inhibitor 1A
p27Kip1	Cyclin-dependent kinase inhibitor 1B
PD	Parkinson’s disease
TrkB	Tropomyosin receptor kinase B
VGCC	Voltage-gated calcium channels
WAVE1	Wiskott-Aldrich syndrome protein (WASP)-family verprolin homologous protein 1

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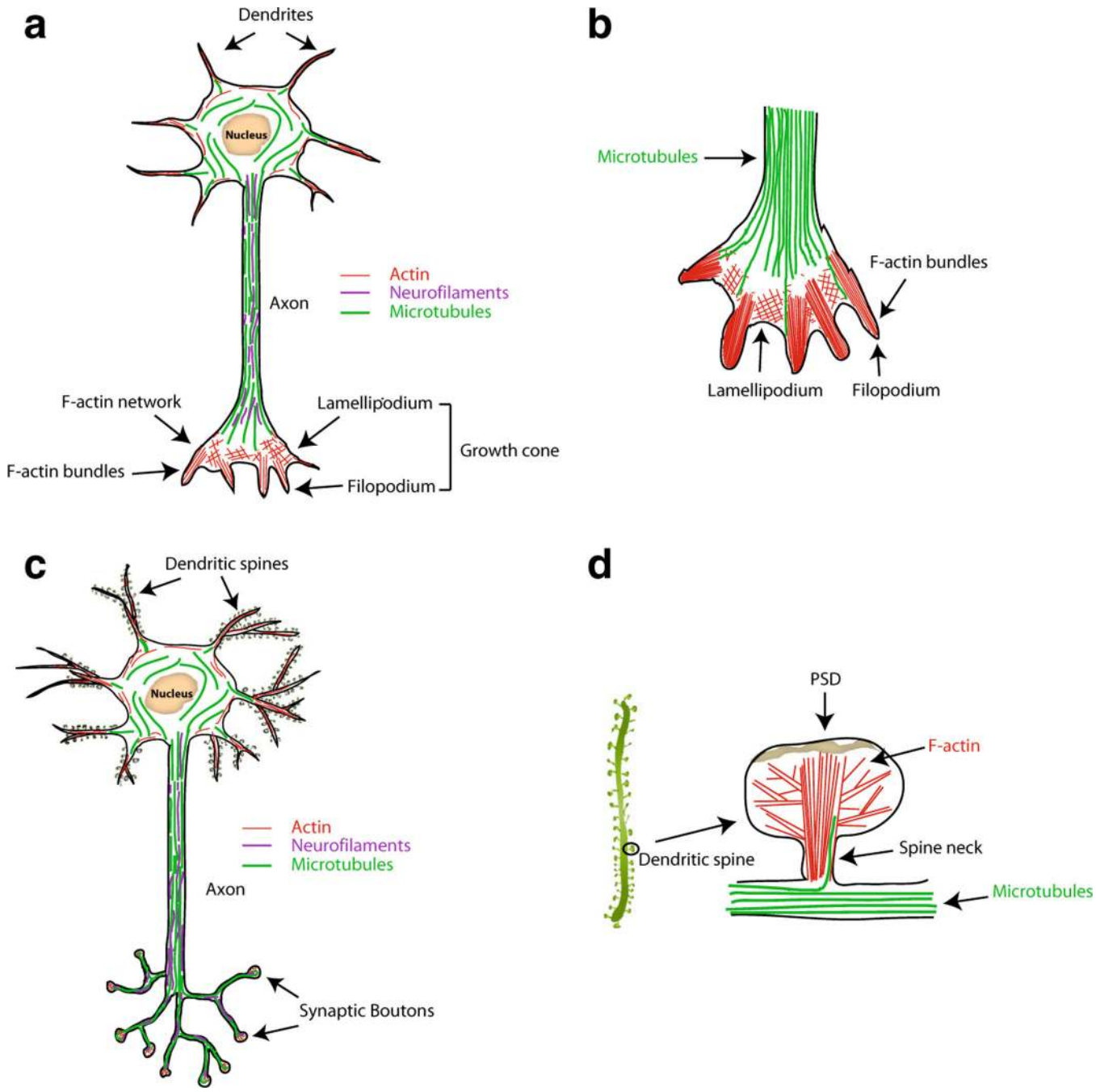

Fig. 1.

Actin cytoskeletal network in growth cones and dendritic spines. **a** Components of the neuronal cytoskeleton in a developing neuron. The neuronal cytoskeleton comprises of actin filaments (*red*), microtubules (*green*), and neurofilaments (*purple*). **b** The axonal growth cone is comprised of lamellipodia and filopodia, both of which are highly enriched in actin filaments. Lamellipodia consist of a dense F-actin network, and filopodia contain bundled F-actin, whereas MTs emanate from axons. **c** A mature neuron showing dendritic spines and

synaptic boutons. **d** Dendritic spines are highly dynamic membranous protrusions on postsynaptic dendrites, which have a rich F-actin cytoskeleton

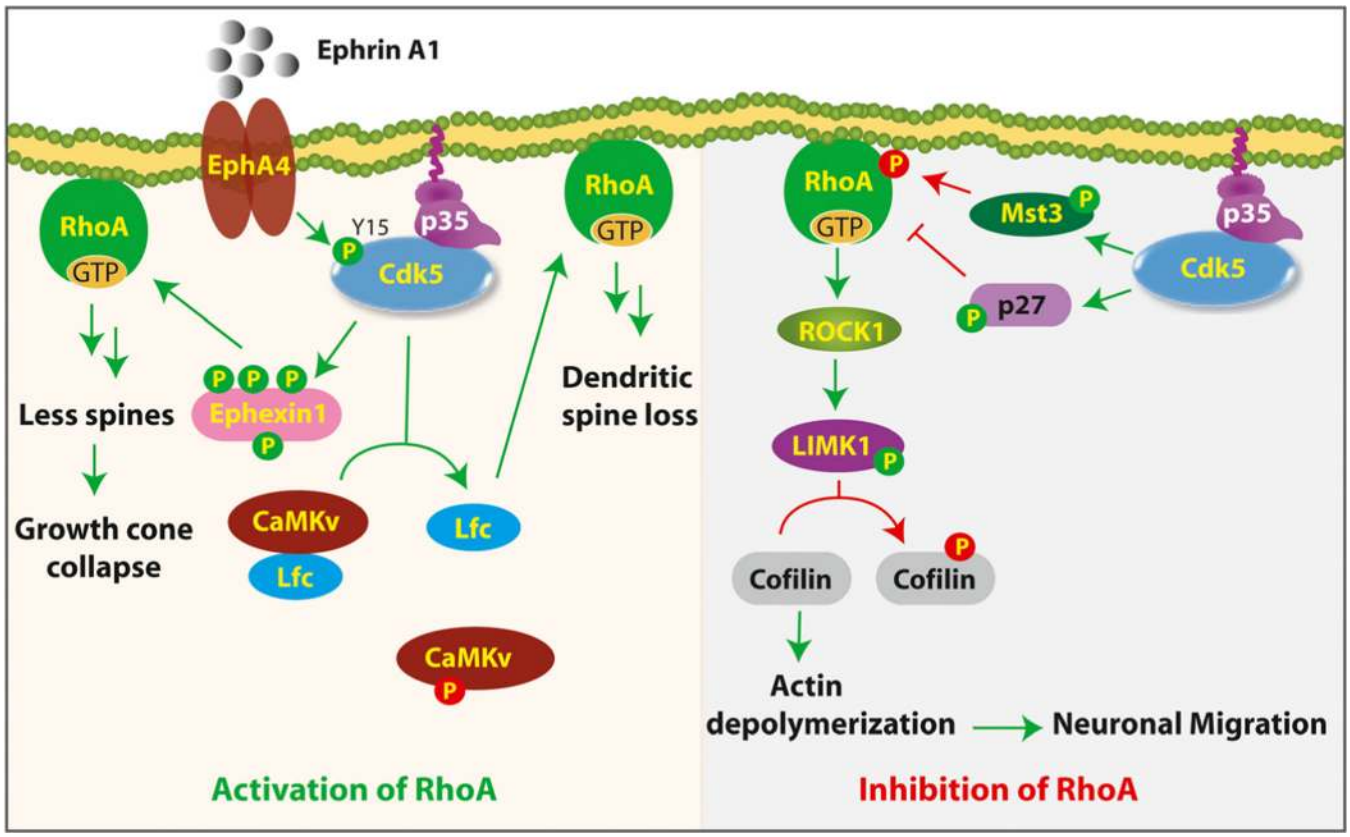

Fig. 2.

Cdk5 regulates actin dynamics by phosphorylating several upstream regulators of RhoA GTPase: Cdk5 can both positively (*left panel*) and negatively (*right panel*) regulate RhoA signaling depending upon the context. *Left panel*: Cdk5 activates RhoA via the phosphorylation of ephexin1, resulting in growth cone collapse. Similarly, Cdk5-mediated phosphorylation of CaMKv dissociates the Lfc-CaMKv complex, releasing Lfc. Lfc is a RhoA GEF, which activates RhoA, resulting in dendritic spine loss. *Right panel*: Cdk5 inhibits RhoA via phosphorylation of p27 and Mst3. Cdk5-mediated phosphorylation of p27 increases its levels, which in turn binds Rho, inhibiting its activity. Similarly, Cdk5 activates Mst3 by phosphorylating Ser79, which inactivates Rho by phosphorylating it at Ser26. Inhibition of RhoA inhibits LIMK1 activation and consequently results in active cofilin, causing actin depolymerization and neuronal migration. *Red* and *green circles* show activating and inactivating phosphorylation, respectively. *Red* and *green arrows* represent activating and inactivating pathways, respectively

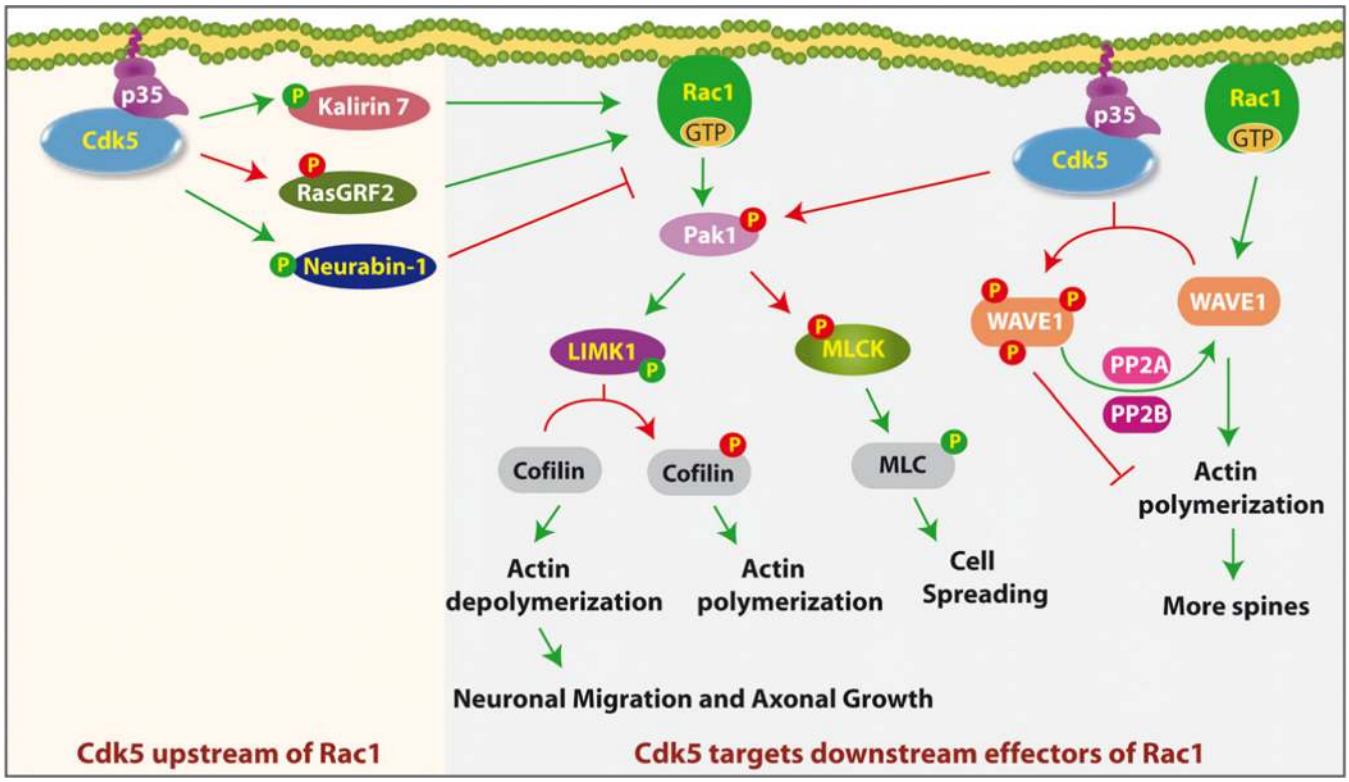

Fig. 3.

Cdk5 regulates actin dynamics by phosphorylating multiple upstream regulators (*left panel*) and downstream targets of Rac GTPase (*right panel*): Similar to RhoA, Cdk5 can either activate or inhibit the Rac1 pathway using alternate substrates, thereby controlling both actin polymerization and depolymerization. Cdk5 activates Rac signaling via its upstream regulator kalirin-7, but inhibits it by phosphorylating other upstream regulators—neurabin-1 and RasGRF2 (*left panel*). Cdk5 also regulates Rac signaling by phosphorylating its downstream effectors— Pak1 and WAVE1. Pak1 activates LIMK1 kinase, which inactivates cofilin, resulting in actin polymerization. Cdk5 reverses this regulation by inactivating Pak1 via phosphorylation, resulting in LIMK1 inhibition and cofilin activation, promoting actin depolymerization. Cdk5 phosphorylates Rac effector WAVE1, which inhibits its activity, resulting in decreased spine density. In contrast, PP2A and PP2B-mediated dephosphorylation of WAVE1 increases its activity, leading to enhanced spine density. *Red* and *green circles* show activating and inactivating phosphorylation, respectively. *Red* and *green arrows* represent activating and inactivating pathways, respectively

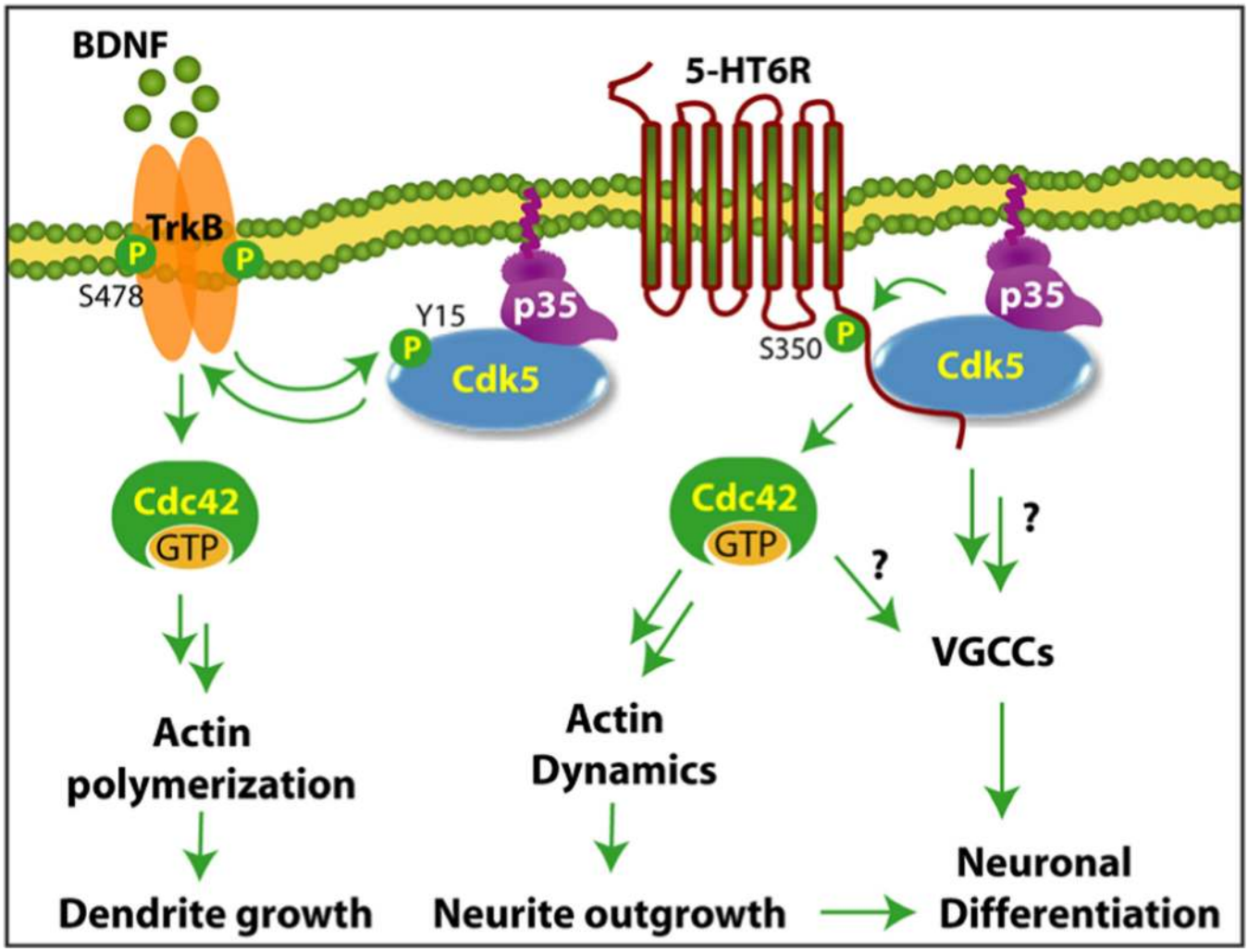

Fig. 4.

Cdk5 activates the Cdc42 pathway via TrkB and 5-HT6 receptor phosphorylation, leading to dendrite growth and neuronal differentiation, respectively. BDNF-induced TrkB activation facilitates Cdk5 activation via Y15 phosphorylation, which in turn phosphorylates TrkB at S478 in the juxtamembrane region. Subsequently, TrkB activates Cdc42, causing dendrite outgrowth and extension. Cdk5 constitutively associates with the C-terminal domain of 5-HT6R and phosphorylates it at S350, leading to the activation of the Cdc42 pathway, promoting neurite outgrowth. This phosphorylation event also increases the expression of VGCCs, although it remains undetermined whether it is a Cdc42-dependent or Cdc42-independent process. Both neurite outgrowth and VGCC upregulation are hallmarks of neuronal differentiation

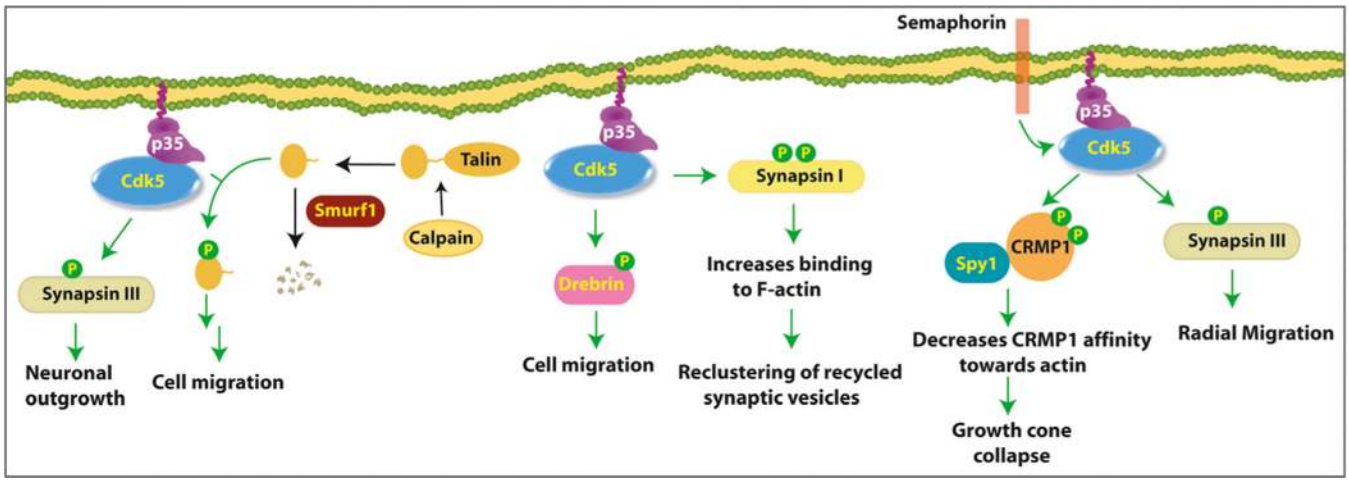

Fig. 5.

Cdk5 regulates actin remodeling by phosphorylating many actin-binding proteins independent of RhoA, Rac, and Cdc42 GTPase signaling, leading to cell migration. Cdk5 promotes cell migration by phosphorylating talin. Smurf1 degrades the head domain of talin, which is inhibited by Cdk5-mediated phosphorylation, resulting in neuronal migration. Similarly, Cdk5-mediated phosphorylation of drebrin increases cell migration by organizing F-actin cytoskeleton. Cdk5 phosphorylates synapsin I at S549 and S551, which increases its affinity towards F-actin, which promotes reclustering of recycled synaptic vesicles, thereby regulating synaptic scaling. Semaphorin-3A (Sema3A)-induced activation of Cdk5 leads to phosphorylation of CRMP1, which decreases its affinity towards actin, resulting in growth cone collapse. Similarly, Sema3A-induced active Cdk5 also phosphorylates synapsin III at S404, which regulates radial migration and orientation in vivo. Synapsin III phosphorylation by Cdk5 also regulates neuronal survival, neuritic outgrowth, and polarization

Table 1

Overview of the "good Cdk5" substrates that are involved in actin dynamics. The first column lists the direct substrates of Cdk5 involved in actin dynamics. The second column lists the phosphorylation sites on each of these substrates. The third column highlights the consequences of Cdk5-mediated phosphorylation on actin dynamics. The fourth column lists the functional outcomes of Cdk5-mediated phosphorylation of different substrates on actin dynamics

Direct substrates of "good Cdk5" in actin dynamics			**Functional outcomes**
Ephexin1 (Rho GEF)	T41, T47, S57, S139	Activates RhoA	Growth cone collapse
CaMKv	T345	Activates RhoA	Decreased dendritic spine density
p27	S10	Inhibits RhoA	Neuronal migration
Mst3	S79	Inhibits RhoA	Radial neuronal migration
Kalirin-7 (Rac GEF)	T1590	Activates Rac	Increased dendritic spine density and drug conditioning
RasGRF2 (Rac GEF)	S737	Inhibits Rac	Accumulation of MAP1B in cell body
Neurabin-1	S95	Inhibits Rac	Neurite outgrowth
Pak1 (Rac effector)	Not known	Inhibits Rac	Axonal outgrowth, cell migration and spreading
WAVE1 (Rac effector)	S310, S397, S441	Inhibits Rac	Decreased spine density
TrkB	S478	Activates Cdc42	Dendrite outgrowth and extension
5-HT6R	S350	Activates Cdc42	Neurite outgrowth and VGCCs expression
Talin	S425	Stabilizes the head domain of talin	Cell migration
Drebrin	S142	Connects dynamicMTs to F-actin	Organizes F-actin in dendritic spines
CRMP1	CRMP1	Decreases its affinity towards actin	Growth cone collapse
Synapsin III	S404	Regulation of actin dynamics?	Neurite outgrowth and axonal elongation
Synapsin I	S549, S551	Increases its binding to F-actin	Reclustering of recycled synaptic vesicles

Table 2

Overview of the “bad Cdk5” substrates that are involved in actin dynamics. The first column lists the direct substrates of Cdk5 involved in actin dynamics. The second column lists the phosphorylation sites on each of these substrates. The third column highlights the consequences of Cdk5-mediated phosphorylation on these substrates. The fourth column lists the functional outcomes of Cdk5-mediated phosphorylation of different substrates

Direct substrates of “bad Cdk5” in actin dynamics		**Functional outcomes**	
PSD-95	T19, S25, S35	PSD-95 degradation	Decreased surface expression of AMPAR subunit 2
GKAP	S77, S111	GKAP degradation	Collapse of the synaptic actin cytoskeleton
SPAR	S1328	SPAR degradation	Synaptic dysfunction?
LRRK2	S1627	Increases LRKK2 activity	Neuronal death