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A tale of the good and bad: remodeling of the neuronal microtubules in the brain by Cdk5

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Abstract

Cdk5, a cyclin dependent kinase family member, is a global orchestrator of neuronal cytoskeletal dynamics. During embryogenesis, Cdk5 is indispensable for brain development. In adults, it is essential for numerous neuronal processes, including higher cognitive functions such as learning and memory formation, drug addiction, pain signaling and long term behavior changes through long term potentiation and long term depression, all of which rely on rapid alterations in the cytoskeleton. Cdk5 activity becomes deregulated in various brain disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease, attention-deficit hyperactivity disorder, epilepsy, schizophrenia and ischemic stroke; these all result in profound remodeling of the neuronal cytoskeleton. This Commentary specifically focuses on the pleiotropic contribution of Cdk5 in regulating neuronal microtubule remodeling. Because the vast majority of the physiological substrates of Cdk5 are associated with the neuronal cytoskeleton, our emphasis is on the Cdk5 substrates, such as CRMP2, stathmin, drebrin, dixa1, axin, GKAP, kinesin-5, tau and PSD-95, that have allowed to unravel the molecular mechanisms through which Cdk5 exerts its divergent roles in regulating neuronal microtubule dynamics, both in healthy and disease states.

Keywords

Cdk5; p25; p35; microtubules; cytoskeleton; neurodegeneration; Alzheimer disease

Introduction

Cyclin dependent kinases (Cdks) are proline-directed serine/threonine kinases that play critical roles in cell cycle progression. Cdks bind to cyclins, their specific protein partners, for activation. Their activity can be further regulated through phosphorylation by the kinases Cdk-activating kinase-1 (Cak1), membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase isoform 1 (Myt1) and Wee1. Phosphorylation of Cdks by Cak1 at T161 (Cdk1 numbering) results in activation, whereas phosphorylation by Myt1 and Wee1 at T14

and Y15 respectively inhibit their activity. The activity of Cdk is also inhibited by binding of the cell cycle proteins Cyclin-Dependent Kinase Inhibitor 1 (CDKN1A) and Cyclin-Dependent Kinase Inhibitor 1B (CDKN1B) (henceforth referred to as p21Cip1 and p27Kip1, respectively).

Cdk family members—The Cdk family member Cdk5 (Gene ID: 1020, mapped at chromosome 7q36) shares a high homology with other Cdks; however, it is distinguished from those by possessing unique activation pathways and distinct cellular functions [1, 2]. Unlike other Cdks, Cdk5 does not participate in cell cycle regulation in proliferating cells, although it can aberrantly activate different components of the cell cycle in post-mitotic neurons, resulting in death under pathological conditions [3]. Furthermore, Cdk5 is not activated by the canonical cyclins (cyclin A, cyclin D and cyclin E), but binds to its own specific partners, CDK5R1 and CDK5R2 (henceforth referred to as p35 and p39, respectively) [4, 5]. Expression of p35 is nearly ubiquitous, whereas p39 is exclusively expressed in the central nervous system (CNS). Cdk5 is also activated by cyclin I (CCNI) in post mitotic cells (e.g. neurons and podocytes) [6]. Cyclin I does not activate any other Cdks, suggesting it might be a Cdk5-specific activator. Thus, Cdk5 has vital role in CNS where it is the only Cdk expressed.

Cdk5 Regulators—Similar to its unique activation partners, Cdk5 also possesses its own set of negative regulators and is not inhibited by p21Cip1 and p27Kip1, which inhibit other family members. Instead, glutathione S-transferase P (GSTP1), cyclin D1 (CCND1) and cyclin E (CCNE) inhibit Cdk5 activity [7–9]. Likewise, phosphorylation of Cdk5 at Y15 by the kinases Abl, Ephrin receptor A (Eph A) or Fyn does not inhibit its activity as has been observed for other Cdks. Instead, phosphorylation of Cdk5 at Y15 has been shown to either increase or has no effect on its activity [10–12].

Role of Cdk5 in neuronal development and learning and memory formation—Cdk5 also differs from other family members in that it has distinct functions in a variety of neuronal and non-neuronal tissues. Although most of the Cdk/cyclin complexes shuttle between the cytoplasm and the nucleus, the majority of active Cdk5-p35 complexes reside in close proximity to the membrane and cytoskeletal elements. Although Cdk5 is a cytosolic kinase, both p35 and p39 have membrane-targeting motifs and this alters the subcellular localization of active Cdk5 complex [13]. The vast majority of the physiological Cdk5 substrates are associated with the neuronal cytoskeleton. This, in turn, allows Cdk5 to play a pivotal role in neuronal development by regulating neuronal migration, neurite outgrowth, axon guidance, and synapse formation [14]. Associated processes during development, such as the establishment of polarity, migration, neurite outgrowth, and synaptogenesis all depend on cytoskeletal dynamics. In adults, higher cognitive functions such as learning and memory formation also rely on rapid cytoskeletal alterations, making Cdk5 activity crucial for neurotransmission, synaptic plasticity and homeostasis, drug addiction and long-term behavioral changes [15–19].

Role of Cdk5 in neurodevelopmental and neurodegenerative diseases—In several brain disorders, such as mental retardation, attention-deficit/hyperactivity disorder

(ADHD) [20], epilepsy [21] and schizophrenia [22], Cdk5 activity is significantly reduced and is believed to play a causal role in their development. By contrast, Cdk5 is also hyperactivated in many neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and ischemic stroke, and it is destructive and neurotoxic ([23–26]. A variety of neurotoxic insults such as exposure to β -amyloid ($A\beta$), excitotoxicity, ischemia and oxidative stress disrupt the intracellular calcium homeostasis in neurons, thereby leading to the activation of calpain, which cleaves p35 into p25 and p10. Cdk5-p25 complex exhibits higher kinase activity in vitro than Cdk5-p35 complex. Furthermore, p25 has a six-fold longer half-life compared to p35 and lacks the membrane anchoring signal, which results in its constitutive activation and, most importantly, mislocalization of the Cdk5-p25 complex to the cytoplasm and the nucleus. There, Cdk5-p25 is able to access and phosphorylate a variety of atypical targets, such as tau, GM130, peroxiredoxins, lamin A and lamin B, triggering a cascade of neurotoxic pathways that culminate in neuronal death [27–31]. Interestingly, Maccioni et al revealed that Cdk5 can also be deregulated via increased stability of Cdk5-p35 complex when exposed to fibrillary β -amyloid toxicity [32].

Despite the adverse effects when it is misregulated Cdk5 is a vital component of ongoing healthy neuronal function. Here, we highlight the functions of Cdk5 when it is “good” and when it is “bad” in neuronal signaling, with a specific emphasis on its direct targets and the molecular mechanisms by which it regulates MT cytoskeleton in the brain.

Similar to in any eukaryotic cell, the neuronal cytoskeletal network includes actin filaments and tubulin microtubules (MTs) but also contain neurofilaments (NFs), a class of intermediate filaments that are unique to neuronal cells. Actin, tubulin and neurofilaments differ physiochemically in protein constituents, subcellular localization, diameter, mechanical stiffness, polarity, assembly dynamics and the kind of molecular motors they associate with, which translate to their unique architecture and functions in the neurons [33]. Actin filaments form sheet-like structures and are highly enriched at the leading edge of dendrites and axons, including the lamellipodia and filopodia (Fig. 1). MTs are dynamic tubulin polymers, which switch stochastically between shrinking and growing phases. MTs form track-like structures in the axon and transport materials from the cell body to the axon terminals at the synapse [34] (Fig. 1). Neurofilaments form a structural matrix in the axon that nestles MTs and resists mechanical stresses [35]. This review focuses on Cdk5-mediated regulation of microtubules and we will also briefly discuss how Cdk5 deregulation causes cytoskeletal abnormalities in various neurological disorders.

Role of Cdk5 in MT dynamics

Microtubules consist of 10–15 (usually 13) protofilaments of α/β -tubulin heterodimers that associate laterally to form a polarized hollow tube approximately 24 nm in diameter. The “plus” end of MTs favor polymerization and their “minus” end disassembly, resulting in MTs usually growing from their plus ends. Their polarity enables MTs to enter various subcellular locations and form productive interactions with various signaling molecules. MTs can rapidly switch from growth mode to shrinkage and vice versa. Thus, MTs are constantly assembling and disassembling, which allows them to play a major role in

intracellular transport, organelle distribution, mitosis, and growth and maintenance of axons and dendrites (Fig. 1) [34].

MTs are present in both axons and dendrites, where they however differ in two major aspects. First, axonal MTs have a unidirectional arrangement, with their plus ends always facing the growth cone, thereby facilitating directional axonal growth, whereas mammalian dendritic MTs have a mixed orientation (Fig. 1). Second, despite a variety of microtubule-associated proteins (MAPs) present in both axons and dendrites, dendritic MTs are mostly associated with MAP2 and axonal MTs with tau. MAPs not only regulate MT dynamics, but also aid in MT-mediated long-range transport of cellular cargos. Cdk5 has been shown to be particularly enriched in MT fractions and employs pleiotropic mechanisms to promote axonal generation and axonal transport [36, 37].

Cdk5 directly influences MT dynamics by regulating the acetylation levels of α -tubulin, the monomer of MTs. Mechanistically, α -tubulins are acetylated and deacetylated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively [38]. Acetylation of α -tubulin promotes MT stability and favors axonal growth, whereas deacetylation blocks growth cone dynamics. Cdk5 phosphorylates Sirtuin-2 (SIRT2), an HDAC, inhibiting its activity (Fig. 2) (Table 1). This event increases the pool of acetylated α -tubulin in neurons, leading to axonal growth and maintenance [39].

Cdk5-mediated regulation of MT dynamics through MAPs

The dynamic polymerization and depolymerization of MTs is crucial for their biological functions as it allows them to rapidly reorganize and probe the surrounding space in response to guidance cues. Cdk5 has been shown to affect the association between MTs and MAPs by phosphorylating a number of MAPs, including collapsing response mediator protein 2 (CRMP2), axin, stathmin, doublecortin, TPPP, tau and MAP1B (Fig. 2 and Table 1) as discussed below.

Cdk5 and CRMP2—CRMP2 is highly enriched in dendrites and axons and mediates signals from several environmental cues, such as ephrins, semaphorins and neurotrophins. It facilitates MT assembly, cell migration [40], axonal growth and guidance [41], dendritic spine development [42] and synaptic plasticity [43].

Depending on the cellular context, Cdk5 can either activate or inhibit CRMP2, and so promote either axonal elongation or growth cone collapse, respectively (Fig. 2, Table 1). For instance, in dorsal root ganglion neurons, Cdk5 promotes Semaphorin 3A (Sema3A)-induced growth cone collapse through inhibiting CRMP2 and alpha2-chimaerin [44], a GTPase activating protein (GAP) for Rac1. Active alpha2-chimaerin acts as an adaptor protein and recruits CRMP2 through interactions with its SH2 domain and Cdk5-p35 through its GAP domain, which in turn allows Cdk5 to phosphorylate CRMP-2 at serine 522. CRMP2 phosphorylation at S522 primes it for Glycogen synthase kinase- β (GSK3 β) kinase-mediated phosphorylation at T514 and S518, resulting in inactivation and triggering growth cone collapse (Fig. 2). Importantly, Cdk5-mediated CRMP2 phosphorylation at S522 is essential for proper organization of the dendritic field and the precise bifurcation of apical dendrites of CA1 pyramidal neurons *in vivo* [42, 45].

In a reverse mechanism, in response to neurotrophins, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT3), Cdk5 promotes axonal growth by inhibiting the inactivation of CRMP2 through directly phosphorylating Axin at T485 [46] (Fig. 2). Axin is a scaffolding protein; upon phosphorylation, it binds to and inhibits the activity of GSK3 β , thereby increasing the pool of non-phosphorylated active CRMP2 in the growth cone and promoting axonal growth. Cdk5 also indirectly inhibits GSK3 β activity via inactivating protein phosphatase 1 (PP1) [47]. PP1 activates GSK3 β by dephosphorylating it at S9. Thus, Cdk5-mediated inhibition of GSK3 β activity promotes axonal growth. GSK3 β functions as a negative regulator of axon formation [48]. Intriguingly, a recent study showed that p25 (cleaved product of p35) preferentially binds GSK3 β compared to Cdk5, which not only activates GSK3 β , but also alters its substrate specificity [49]. By contrast, GSK3 β does not bind p35. These findings suggest that p25 upregulation may in part promote neurotoxicity via elevated GSK3 β activity.

Effect of Cdk5 on other MAPs—Cdk5-mediated phosphorylation of MAPs often affects their affinity of MT binding (Table 1). For instance, in cultured cerebellar macro neurons, Cdk5-mediated phosphorylation of MAP1B enhances its MT-binding affinity and so promotes axonal elongation [36]. In contrast, Cdk5-mediated phosphorylation of the MAP doublecortin at S297 reduces its affinity towards MTs *in vitro*, thereby reducing its effect on MT polymerization and neuronal migration (Fig. 2) [50] (Table 1). Accordingly, mutations in the doublecortin gene in humans result in similar cortical lamination defects in the developing brain as those seen in Cdk5-null mice. These mice exhibit severe abnormalities in lamination in the hippocampus, cerebral cortex and cerebellum.

Likewise, Cdk5 phosphorylates tubulin polymerization-promoting protein (TPPP) at multiple sites *in vitro* and *in vivo*, which results in the loss of its MT-assembling activity [51] (Fig. 2). TPPP and tubulin are enriched in aggresomes and Lewy body in brain tissues from patients with synucleinopathies [52], suggesting that Cdk5-mediated phosphorylation of TPPP may prevent the formation of these inclusions in pathological states.

Of particular interest in neurodegenerative pathology, Cdk5-mediated phosphorylation of tau reduces its ability to bind to and stabilize MTs during axonal growth. This has important implications, as during brain development, tau is phosphorylated by the Cdk5-p39 complex at Ser-202 and Thr-205, but not by Cdk5-p35 [53]. Because p35 and p39 display differential expression patterns in the developing brain, Cdk5-mediated phosphorylation of tau promotes neuritic growth in a region-specific and developmentally regulated manner in developing axons. Consequently, at the period of brain development, phosphorylated tau is only present abundantly during strong neuritic outgrowth, whereas it diminishes to undetectable levels during neurite stabilization and synaptogenesis [54]. In the developing brain, the expression level of p39 is elevated in embryonic spinal cord, hind brain and postnatal cerebral cortex, whereas p35 is highly abundant in cerebral cortex.

In a different mechanism, Cdk5 phosphorylates the MAP stathmin at Ser38, which results in axonal growth (Fig. 2 and Table 1) [55]. Stathmin sequesters free tubulin, thereby inhibiting MT growth. Cdk5-mediated phosphorylation of stathmin releases bound tubulin, thereby facilitating axonal growth and branching. A recent study demonstrated that stathmin-

dependent changes in MT stability are crucial for synaptic function and memory formation [56]. Learning induces stathmin phosphorylation, which in turn regulates MT dynamics and long term memory formation.

p35 can also act as a MAP; it binds to MTs through its N-terminus and promotes MT assembly and bundling of MT filaments [57]. Interestingly, the MT-binding and polymerizing activities of p35 are regulated both in a Cdk5-dependent and -independent manner. In the mechanism that is independent of Cdk5, p35, through its N-terminal domain, binds preferentially to calmodulin (CaM)-binding protein in the presence of Ca^{2+} , and not to MTs, thereby inhibiting MT assembly. In the other mechanism, Cdk5 directly phosphorylates p35 at T138 and so inhibits its microtubule polymerization activity (Fig. 2) [58]. As T138 phosphorylation is highest in fetal brain and undetectable in adults, this suggests that Cdk5-mediated phosphorylation of p35 in developing brains may be required for the highly dynamic reorganization of the MT network by promoting both the rapid association and dissociation of MAPs, thereby allowing for a high plasticity during brain development.

Role of Cdk5 in neuronal migration

Although Cdk5 is known to have more than two dozen substrates that are associated with neuronal migration, we focus here on those substrates that promote migration predominantly through modulating MT organization.

The first step in neuronal migration is the extension of the neurite, which is followed by movement of the soma and the nucleus (nucleokinesis) into the leading process with simultaneous retraction of the trailing process. Cdk5 plays a key role in nucleokinesis by phosphorylating focal adhesion kinase (FAK) at S732 *in vitro* and in the developing brain, which results in its binding to specific MTs that originate from MT-organizing centers (MTOCs). Expression of phospho-resistant S732A FAK results in disorganization of MTs between the nucleus and the centrosome causing migration defects. This phenotype suggests that phosphorylation of FAK by Cdk5 at least in part promotes the proper organization of MTs that link the nucleus and the centrosome, thereby facilitating nucleokinesis and thus neuronal migration [59] (Fig. 3).

Another important substrate of Cdk5 is disrupted in schizophrenia 1 (DISC1), whose gene mutations are strong risk factors for several psychiatric disorders, such as autism, schizophrenia, depression and bipolar disorder. In neurons, DISC1 is involved in multiple processes, including MT-mediated transport and regulation of dendritic spines [60]. During embryonic neocortical development, DISC1 regulates several steps of neurogenesis, including cell proliferation, cell migration, axon and dendrite formation. In developing cerebral cortex, Cdk5 phosphorylates DISC1 at Ser713, which increases its affinity towards two centrosomal proteins, Bardet-Biedl syndrome (BBS)1 and BBS4, resulting in increased neuronal migration, while concurrently decreasing neuronal proliferation (Fig. 3) [61]. Thus, Cdk5-mediated phosphorylation of DISC1 acts as a developmentally regulated switch that, through increasing neuronal migration, attenuates the proliferation of progenitor cells and allow them to move towards their correct locations in the developing cerebral cortex. Cdk5

also indirectly regulates DISC1-mediated neuronal migration through phosphorylating DIX domain containing-1 protein (Dixdc1) at Ser250, which facilitates the association of nude nuclear distribution E homolog (Ndel1) to DISC1-Dixdc1 complex resulting in increased neuronal migration via modulating the actin and MT cytoskeleton (Fig. 3). Ndel1 is necessary for normal migration of neocortical projection neurons, and its ablation significantly diminishes this developmental process [62]. In the absence of Dixdc1 phosphorylation by Cdk5, Ndel1 does not bind to DISC1-Dixdc1 complex resulting in cell proliferation. Thus, the authors proposed that Cdk5-mediated phosphorylation of Dixdc1 is an alternate mechanism, which switches the involvement of DISC1 from progenitor cell proliferation to neuronal migration [63].

In granular neurons, Cdk5 employs another indirect mechanism to regulate Ndel1-mediated neuronal migration by acting through Aurora A kinase. Although the exact mechanism remains to be elucidated, it appears that cyclin-dependent kinase 5 regulatory-associated protein 2 Cdk5RAP2, a centrosomin family protein, mediates the interaction between Aurora A and Cdk5, which is critical for the correct centrosomal targeting of Aurora A. In turn, Aurora A phosphorylates Ndel1 at S251, resulting in MT remodeling and neuronal migration (Fig. 3) [64]. Depletion of Cdk5 reduced the centrosomal targeting of Cdk5RAP2, phosphorylation of Aurora A at T288, and that of Ndel1 at S251, which resulted in impaired neuronal movement. In addition, Cdk5RAP2 may have the role of inhibition of centrosomal Cdk5 during neurogenesis, perhaps taking part in a direct feedback loop with Cdk5 and may participate in regulation of brain size [65].

A recent study further revealed that Cdk5-mediated phosphorylation of receptor tyrosine-protein kinase erbB-4 (ErbB4) at Tyr1056, a docking site for PI3-kinase, is necessary for tangential migration of interneurons both toward and within the cerebral cortex [66] (Table 1). Importantly, phosphorylated ErbB4 reside in close proximity to MTs in adult interneurons, suggesting that Cdk5-directed migration of cortical interneurons likely occurs through MT cytoskeletal remodeling. Taken together it is clear that Cdk5 utilizes several strategies to direct MT dynamics and neuronal migration.

Dynamic MTs meet actin filaments in growth cones via Cdk5

Dendritic spines are highly dynamic membranous protrusions on post-synaptic dendrites. Structural changes in dendritic spines form the basis for learning and memory formation, making them central hubs for the processing and storage of information in the brain [67]. F-actin filaments are highly concentrated in the head, neck and periphery regions of dendritic spines, whereas stable MTs are confined mainly to the axons and dendritic shafts. As a result, until recently, actin polymerization was deemed to be the underlying cause for the formation or enlargement of the spine during long term potentiation (LTP), whereas actin depolymerization has been linked to spine shrinkage observed during long term depression (LTD) [68, 69]. Recent studies, however, have revealed that dynamic MTs can polymerize directly into the dendritic spines, resulting in spine enlargement and increase in post synaptic density protein 95 (PSD-95) levels [70–72]. As PSD-95 upregulation is strongly associated with enhanced synaptic strength, it suggests that MTs invasion directly promotes structural alterations in dendritic spines. More importantly, inhibition of MT dynamics alters spine

morphology through the actin cytoskeleton, suggesting that the MT and actin cytoskeleton in fact act together in dendritic spines to regulate synaptic plasticity [73].

Of particular interest, Cdk5 appears to be responsible for coupling actin filaments to MTs through an F-actin-binding protein, drebrin, in primary cortical neurons [74] (Fig. 3). Drebrin is enriched in growth cones of developing neurons and promotes neuronal migration and neuritogenesis [75]. In mature neurons, it is highly expressed in dendritic spines and is essential for actin remodeling underlying memory [76]. Loss of drebrin in dendritic spines is causal to memory loss in mild cognitive impairment and AD [77]. In growth cones, drebrin was shown to couple dynamic MTs to actin through microtubule-binding +TIP protein EB3 [78]. Worth et al showed that Cdk5-mediated phosphorylation of drebrin at Ser142 is responsible for this event. The authors proposed that drebrin phosphorylation by Cdk5 induces a conformational change that allows it to bind or bundle F actin, which in turn interacts with EB3 located at the +end of a MT invading the filopodium, and thus couple F-actin to MTs [74]. Furthermore, a recent study reported that Cdk5-p35 mediated phosphorylation of drebrin E at S142 and drebrin A at Ser142 and Ser342 promotes radial migration of neurons in embryonic cortex [79]. Drebrin E is embryonic form that is ubiquitously expressed, whereas drebrin A is adult brain-specific isoform. As neuronal migration requires highly coordinated regulation of actin and MT cytoskeleton, Cdk5 may regulate this process through dynamic reorganization of actin and MT network by phosphorylating drebrin (Fig. 3).

Cdk5 and axonal transport: role in brain development

During development, various axonal proteins, organelles and lipids are synthesized in the neuronal cell soma and delivered to growing axon terminals by anterograde axonal transport. Likewise, retrograde axonal transport is essential for transducing extracellular signals and recycling misfolded proteins from the nerve terminals to cell soma, thus avoiding the build-up of toxic aggregates. Axonal transport requires the cytoskeletal MT scaffold to serve as tracks, and the motor proteins kinesin and dynein that exert mechanical force to move cargoes anterogradely and retrogradely, respectively [80].

Cdk5 and Kinesins

Cdk5 orchestrates anterograde and retrograde axonal transport by regulating both kinesin and dynein, respectively. Cdk5 indirectly drives kinesin-induced axonal transport by inhibiting the activity of GSK-3 β through Ser9 phosphorylation (Fig. 4). GSK3 β inhibits kinesin-mediated anterograde vesicle transport by releasing its cargo [81]. Cdk5 also directly phosphorylates the kinesin-3 family member 13B (KIF13B) at Thr506, which allows it to bind to transient receptor potential vanilloid 1 (TRPV1)-containing vesicles and deliver them to the membrane surface (Fig. 4). TRPV1 channels are important at the surface of primary sensory neurons as they regulate heat sensitivity. Accordingly, Cdk5-mediated increase in the surface localization of TRPV1s contributes to heat hyperalgesia [82]. Further, a recent study has revealed that Cdk5 directly phosphorylates kinesin-5 (also known as Eg5) at Thr926, which is essential for its association with MTs (Fig. 4) [83]. Kinesin-5 is a homotetrameric motor protein, which is ~100 times slower than dynein. As a result, it acts as

a molecular ‘brake’ that can effectively halt the movement of MTs by other motors, resulting in reduced neuronal growth [84]. Depletion or inhibition of kinesin-5 in neurons results in rapid but random axonal growth. Thus, the authors suggested that Cdk5 regulates the rate and directionality of neuronal growth and migration by phosphorylating kinesin-5 [83].

Cdk5 and Dynein

Cdk5 also indirectly acts on dynein and promotes its function through phosphorylating Ndel1, a dynein-interacting protein [85]. Cdk5 phosphorylates Ndel1 at S197, T219 and S231, which enables it to bind to 14-3-3e, an adaptor protein, which protects it from dephosphorylation as well as strengthens its interaction with Lis1, another dynein-binding factor (Fig. 4) [86]. Ndel1 and Lis1 are believed to regulate the processivity of dynein complex. Inhibition of Cdk5 has been shown to alter the localization of Ndel1 and its affinity towards the dynein complex, resulting in axonal swellings [87]. More recently, another study found that expression of a dominant-negative form of Cdk5 or a mutant of Ndel1 that cannot be phosphorylated by Cdk5 in adult axons not only completely abrogated retrograde transport, but also severely reduced anterograde flux of acidic organelles [88], suggesting that there is a Cdk5-dependent switch that regulates Lis-Ndel1-Dynein-dependent transport in adult axons. In this model, Lis1 and unphosphorylated Ndel1 interact only weakly and although they bind to dynein inhibit its capacity to move cargo. Phosphorylation of Ndel1 by Cdk5 turns the switch “on”, triggering activation of the Lis1-Ndel1-dynein complex and stimulating the ability of dynein to cargo transport (Fig. 4). Therefore, Cdk5 appears to employ several direct and indirect strategies to recruit different motor proteins and direct MT dynamics, neuronal migration and axonal transport.

Reduced activity and hyperactivity of Cdk5 are each potentially neurotoxic: implications for neurodevelopmental disorders and neurodegenerative diseases

A variety of neurological disorders display profound cytoskeletal abnormalities, including AD, ALS, Huntington’s disease (HD), PD, and Down’s syndrome. In addition, several memory disorders and psychiatric illnesses such as mental retardation and schizophrenia also involve defects in the regulation of the MT cytoskeleton [89, 90]. Although many Cdk5 substrates are known to promote neurotoxicity, below we mainly focus on the brain disorders that involves deregulation of the MT cytoskeleton caused either by reduced activity or hyperactivity of Cdk5 (Fig. 5).

Several neurological disorders originate due to loss or reduction in Cdk5 activity. In patients with NF1 microdeletion syndrome, lack of one copy of *Cdk5R1* (p35), leads to severe mental retardation [91]. Similarly, in schizophrenia patients, p35 expression is reduced in specific brain regions. Importantly, mimicking an analogous reduction in p35 levels in heterozygous mice revealed similar cognitive deficits suggesting that impaired Cdk5 activity plays a causal role in schizophrenia [22]. p35 knockout mice have also been shown to exhibit spontaneous epileptic seizures [21]. Dredrup et al further observed that p35 knock-out mice display behavioral phenotypes reminiscent of ADHD [20]. In addition, as noted before, Cdk5 directs tangential migration of interneurons by activating ErbB4/PI3K

pathway, dysfunction of which is repeated linked to neurodevelopmental disorders such as autism and schizophrenia [64]. The authors indeed show that loss in Cdk5 activity in p35 knockout mice causes permanent reduction in the final number of specific types of interneurons, which in turn may alter neuronal circuit formation, thereby increasing the risk of neurodevelopmental disorders.

In HD, most of the studies also favor a neuroprotective role of Cdk5. Reduced Cdk5 and p35 levels were reported in the brains of HD patients [92], however, one study showed increased p25/p35 levels in HD patients [93]. Future studies are needed to unravel the mechanism of Cdk5 deregulation in this disease. Nevertheless, Cdk5 inhibits aggregation of huntington (htt) by phosphorylating it at Ser434, which impairs its degradation by caspases [92]. Cdk5 also prevents aggregation of mutant htt by disrupting the MT network [94]. Intact MT cytoskeleton is required for aggregation of mutant htt [95]. Further, DNA damage induced Cdk5 activation triggers the phosphorylation of htt at Ser1181 and Ser1201, which protects cultured striatal neurons from mutant htt-induced neurotoxicity [96]. Collectively, these findings underscore an essential role of normal Cdk5 activity for healthy neuronal functions.

By contrast, hyperactivation of Cdk5 is highly detrimental in several neurodegenerative diseases (Fig. 5). Mice that are transgenic for p25 show deregulated Cdk5 activity and display axonal swelling, cytoskeletal disorganization and unusual clustering of lysosomes and mitochondria, all features that are consistent with the loss of a functioning MT network [97]. Disturbances in neuronal cytoskeletal organization observed were similar to those in several neurodegenerative diseases including AD [98]. Deregulation of Cdk5 in AD leads to loss of dendritic spines, with subsequent synaptic dysfunction and memory loss [99]. Similarly, it has been shown that hyperactivation of Cdk5 in the striatum of animal models reduces dendritic spine density and causes impaired motor coordination and decreased locomotor sensitization to cocaine [100].

Role of Cdk5 in neurofibrillary tangles (NFT)

At the molecular level, Cdk5 deregulation not only results in hyperphosphorylation of several of its physiological substrates, but also in the phosphorylation of non-physiological targets that cause neurotoxicity. For example, during development, tau is phosphorylated by Cdk5-p39 but not by Cdk5-p35 in axons, resulting in axonal growth in a region-specific manner [53]. However, upon neurotoxic insults, such as excitotoxicity or β -amyloid stimulation, formation of p25 from p35, hyperactivates Cdk5, which in turn hyperphosphorylates tau, which aggregates to form the neurofibrillary tangles observed in AD. NFTs contribute to axonal degeneration by disrupting mitochondrial transport in AD [101]. Likewise, under physiological conditions, Cdk5 phosphorylates neurofilament heavy chain (NF-H) at KSP motifs in its tail domain, which results in axonal support and neurite outgrowth although it reduces axonal transport ([102]. However, in several neurological diseases, NFs are hyperphosphorylated and aggregate in cell bodies. Many kinases, including deregulated GSK3 β , PKA, MAPK, CaMK2 or Cdk5-p25, are known to substantially contribute to aberrant phosphorylation of NFs in diseased neurons [101]. Similarly, under physiological conditions, Cdk5-mediated direct or indirect regulation of CRMP2 is involved in axonal elongation or growth cone collapse. However, in AD,

hyperphosphorylation of CRMP2 causes its aggregation and so contributes to NFT formation. Therefore, owing to hyperactivation and mislocalization, the Cdk5–p25 complex hyperphosphorylates several of its physiological targets, thereby leading to neuronal toxicity. Interestingly, hyperphosphorylation of CRMP2 in AD patients is observed prior to formation of amyloid plaques and NFTs, suggesting it might be early event in AD pathogenesis [103].

In the Niemann–Pick type C (NPC) mouse model, deregulated Cdk5-p25 hyperphosphorylates several neuronal cytoskeletal proteins, including neurofilaments, tau and MAP2, at 4 weeks of age, the earliest time point studied here, resulting in the formation of axonal spheroids, suggesting a causal role of Cdk5 in NPC pathogenesis. The severity of these cytoskeletal abnormalities rapidly spread to the other parts of the brain resulting in neurodegeneration in NPC [104]. An environmental trigger for late-life Cdk5-mediated tau hyperphosphorylation may be early-life exposure to heavy metals, such as lead (Pb). Developmental (post-natal days 1–20) exposure to Pb in drinking water resulted at 24 months of age in greater levels of Cdk5 and tau protein and mRNA and of tau hyperphosphorylation in mouse brains. These changes did not occur in animals only exposed later in life to Pb (7–9 months age) [105], which is consistent with the previously proposed LEARN model for late-life neuropsychiatric disorders [106].

Conditions caused by axonal transport defects upon Cdk5 deregulation

Failure of axonal transport is one of the crucial triggers in the onset and progression of several neurodegenerative disorders, including injury and motor neuron diseases [107, 108]. Defects in transportation often result in the accumulation of abnormal organelles (such as damaged mitochondria) or protein aggregates that cause swollen axons or spheroids [109]. In AD, hyperphosphorylation of tau and CRMP2 by Cdk5 not only lead to the formation of NFTs, they also significantly impair axonal transport, causing neuronal death [110]. Similarly, deregulation of Cdk5 by ectopic expression of p25 results in increased pausing of mitochondria in neurons [111]. The resulting mitochondrial ‘traffic jam’ causes a drop in ATP levels, resulting in synaptic dysfunction and ultimately neuronal death [112].

A recent study reported that A β inhibits kinesin-5 and so causes a reduced transport of neurotrophin and of neurotransmitter receptors to the cell surface, which results in neuronal death; however, a potential role of Cdk5 was not examined in this work [113]. As Cdk5 is known to phosphorylate kinesin-5 at Thr926, which enhances its MT-binding ability [83], it is likely that Cdk5 hyperactivation in A β -exposed neurons could result in hyperphosphorylation of kinesin-5, thereby completely halting axonal transport. Future studies are needed to unravel the underlying mechanism. In summary, while physiological Cdk5 activity is crucial for promoting axonal transport, deregulated Cdk5 elicits defects in axonal transport through the hyperphosphorylation of several of its substrates that are involved in axonal transport.

Conclusions

Research over the past two decades has uncovered numerous physiological and pathological roles of Cdk5 in various tissue types. Under physiological conditions, Cdk5-p35 acts as a major orchestrator of neuronal cytoskeletal dynamics. As a result, Cdk5 activity is essential

for normal brain development during embryogenesis and, in adults, it is important for all aspects of synaptic signaling as well as for drug addiction, pain signaling and long term behavior changes. At the other end of the spectrum, deregulation of Cdk5 triggers a number of degradative pathways, which result in profound remodeling of neuronal cytoskeleton, loss of synapses and ultimately neurodegeneration. In this context it is worth noting that hyperactivation of Cdk5 not only results in the hyperphosphorylation of many of its physiological targets that are associated with the cytoskeleton, but also in its mislocalization, which allows it to access and phosphorylate multiple non-physiological, cytoplasmic and nuclear targets, thus causing further toxicity.

Consequently, Cdk5 is an attractive drug target for multiple neurological disorders. Although there are many known Cdk5 inhibitors that exhibit high potency, these suffer from poor selectivity and inhibit other Cdks with equal or higher potency. Therefore, despite their potential, Cdk5 inhibitors have thus far not been successful in clinical trials due to serious side effects. Given the pivotal contribution of Cdk5 in the brain, it would be beneficial to develop specific Cdk5 inhibitors or to engineer inhibitors that selectively abrogate the interactions of Cdk5 with p25, but not with p35. Such an approach would allow to target the deleterious effects of Cdk5, while retaining its physiological and beneficial functions in the brain.

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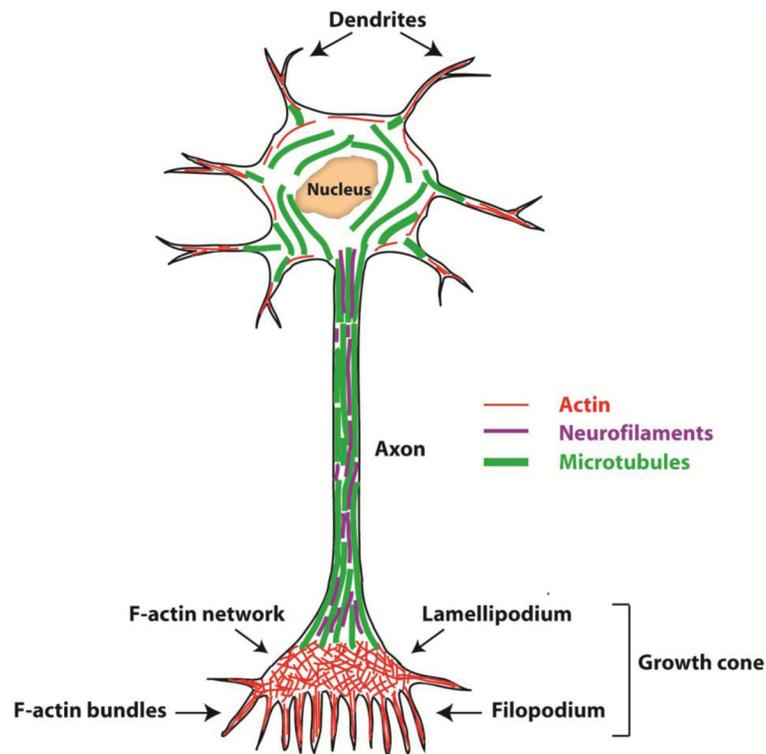


Fig. 1. Components of neuronal cytoskeleton

The neuronal cytoskeleton is composed of actin filaments (red), microtubules (MTs; green) and neurofilaments (NFs; purple). The axonal growth cone is comprised of lamellipodia and filopodia, and is highly enriched in actin filaments. Lamellipodia consist of a dense F-actin network, and filopodia contain bundled F-actin, whereas MTs emanate from axons. Axonal microtubules have an unidirectional arrangement with their plus ends facing the axon tip, thus facilitating directional axonal growth, whereas dendritic microtubules have a mixed orientation. NFs are highly enriched in the axons and maintain axonal integrity, caliber and conduction velocity. MTs are approximately three times thicker in diameter compared to NFs, which are three times thicker than actin.

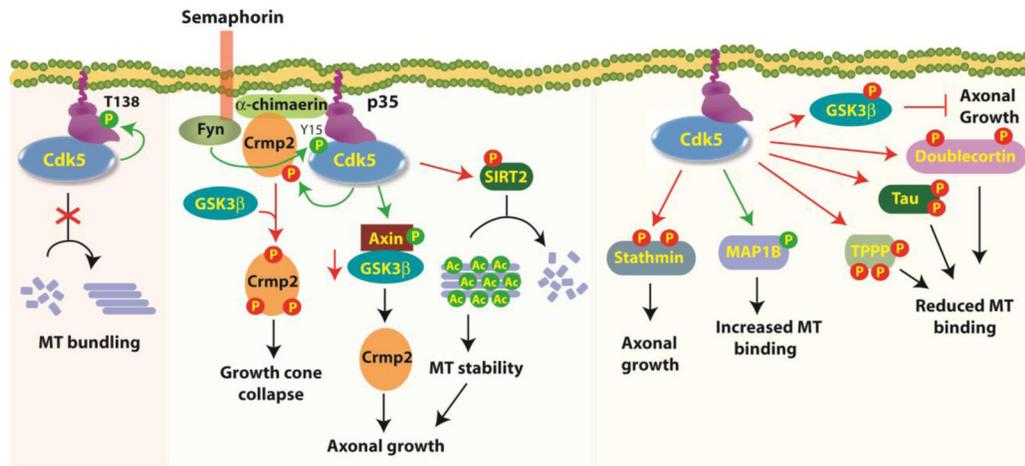


Fig. 2. Cdk5-mediated regulation of MT dynamics and axonal growth

Cdk5 regulates MTs dynamics by phosphorylating several MT-associated proteins (MAPs), such as p35, CRMP2, axin, stathmin, doublecortin, TPPP, tau, and MAP1B. As shown on the left, Cdk5 phosphorylates p35 at T138 in fetal brains, which inhibits its MT polymerization activity and results in the dynamic reorganization of the MT architecture. Furthermore, Cdk5 regulates CRMP2 through several direct and indirect mechanisms (shown in the middle). Direct phosphorylation of CRMP2 by Cdk5 primes it to be phosphorylated by GSK3 β , which inactivates CRMP2, resulting in growth cone collapse. Indirectly, Cdk5 activates CRMP2 by phosphorylating Axin and GSK3 β . Phosphorylated axin binds to GSK3 β , which inhibits its activity. Cdk5 also directly inhibits GSK3 β through phosphorylation, which increases the pool of non-phosphorylated, active CRMP2, thus promoting axonal growth. Cdk5 increases the pool of acetylated α -tubulin by inhibiting SIRT2 resulting in MT stability and axonal growth. Moreover, Cdk5-mediated phosphorylation of stathmin prevents sequestration of free tubulin, facilitating axonal growth (shown on the right). Cdk5-mediated phosphorylation of MAP1B enhances its MT-binding affinity, whereas Cdk5-mediated phosphorylation of doublecortin, tau and TPPP lower their affinity towards MTs. Red and green arrows represent activating and inactivating pathways, respectively.

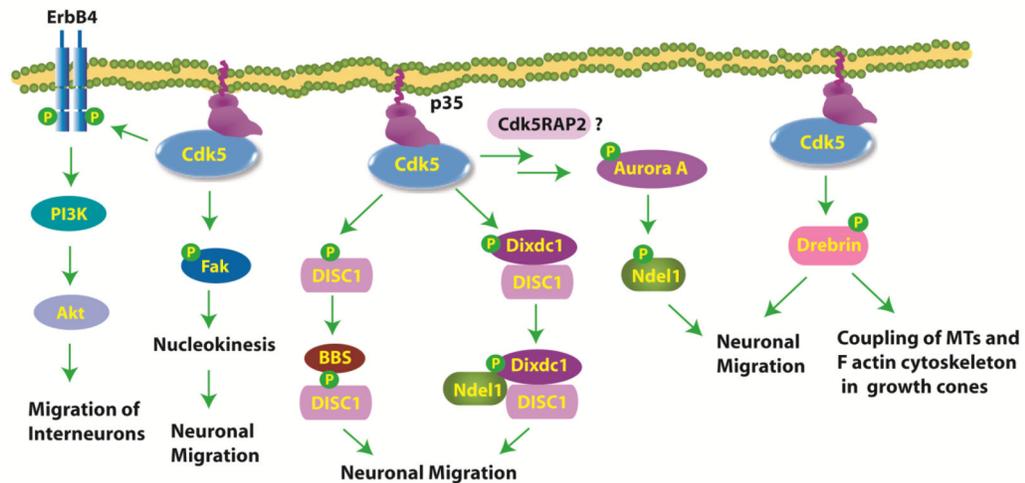


Fig. 3. Role of Cdk5 in neuronal migration

Cdk5-mediated phosphorylation of ErbB4 activates PI3K/Akt pathway leading to tangential migration of interneurons toward and within the cerebral cortex. Cdk5 promotes nucleokinesis by phosphorylating FAK at S732. Phosphorylation of FAK by Cdk5 is believed to promote the proper organization of MTs that link the nucleus and the centrosome, thereby facilitating nucleokinesis and thus neuronal migration. During neocortical development, Cdk5, through DISC1, attenuates the proliferation of progenitor cells, while concurrently directing them towards correct locations by increasing neuronal migration. Cdk5 phosphorylates DISC1 at Ser713, which increases its affinity towards BBS1 and BBS4, resulting in increased neuronal migration. Cdk5 also indirectly regulates DISC1-mediated neuronal migration by phosphorylating Dixdc1 at Ser250, which facilitates the formation of the DISC1-Dixdc1-Ndel1 complex that is essential for neuronal migration as it modulates the actin and MT cytoskeleton. In granular neurons, Cdk5 employs an indirect mechanism to activate Ndel1. Cdk5 activates Aurora A kinase through Cdk5-RAP2, which in turn phosphorylates Ndel1, resulting in MT remodeling and neuronal migration. In addition, Cdk5-mediated phosphorylation of Drebrin at Ser142 enables it to couple dynamic MTs to F-actin through MT-binding +TIP protein EB3 in growth cone resulting in increased neuronal migration.

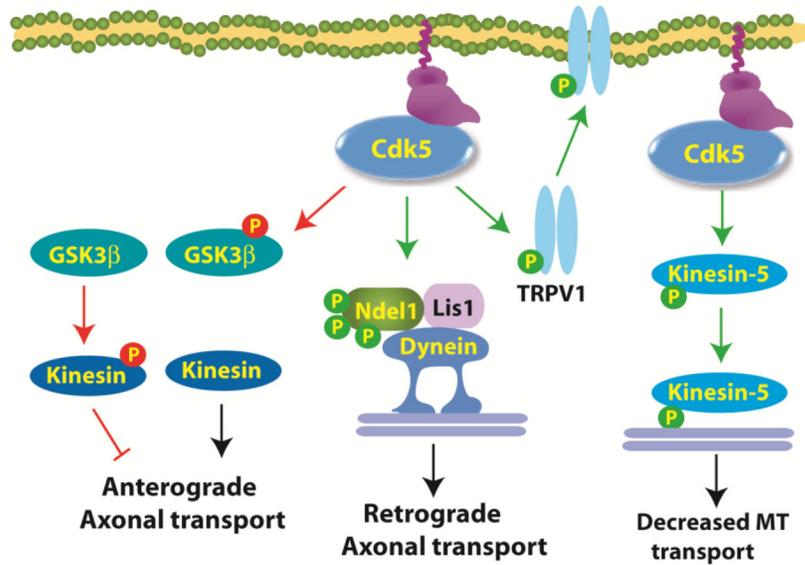


Fig. 4. Cdk5-mediated regulation of axonal transport

Cdk5 orchestrates anterograde and retrograde axonal transport by regulating kinesin and dynein, respectively. Cdk5 drives kinesin-induced axonal transport by inhibiting GSK-3 β activity and facilitates dynein function by phosphorylating Ndel1, thereby strengthening its interaction with Lis1. Ndel1 and Lis1 both bind to dynein and stimulate its cargo transport capacity. Cdk5 also regulates the rate and directionality of neuronal growth and migration by inducing the association of kinesin-5 with MTs through phosphorylation of Thr926.

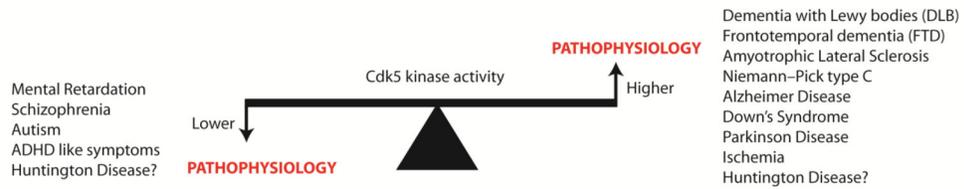


Fig. 5. Reduced activity and hyperactivity of Cdk5 are each potentially neurotoxic
In healthy cells, Cdk5 activity is exquisitely controlled. Increase in Cdk5 activity or loss of Cdk5 activity, both can give rise to neurodevelopmental or neurological disorders.

Table 1
Overview of the Cdk5 substrates that are involved in MT dynamics

First column lists the direct substrates of Cdk5 involved in MT dynamics. Second column lists the consequences of Cdk5-mediated phosphorylation on MT dynamics. Column 3 lists the functional outcomes of Cdk5-mediated phosphorylation of different substrates on MT dynamics.

Direct Substrates of Cdk5 in MT Dynamics		Functional Outcomes	References
Axin	Activates CRMP2	Axonal Elongation	Fang et al., 2011
CRMP2	Inhibits CRMP2	Growth cone collapse	Brown et al., 2004
GSK3B	Activates CRMP2	Axonal Elongation	Jiang et al., 2005
MAP1B	Increases MT affinity	Axonal Elongation	Paglioni et al., 1998
Doublecortin	Reduces MT affinity	Inhibits neuronal migration	Tanaka et al., 2004
TPPP	Loss in MT assembly		Hlavanda et al., 2007
Tau	Reduces MT affinity	Axonal Elongation	Takahashi et al., 2003
Stathmin	Inhibits tubulin sequestration	Axonal Elongation	Hayashi et al., 2006
p35	Inhibits MT polymerization	MT Remodeling	He et al., 2008
SIRT2	Decreased tubulin deacetylation	Axonal growth	North et al., 2003
Dixdc1	DISC1/Dixdc1/Nde1 formation	Increased neuronal migration	Singh et al., 2010
DISC1	Increases affinity for BBS1 & BBS4	Increased neuronal migration	Ishizuka et al., 2011
Fak	Organization of MTs	Nucleokinesis	Xie et al., 2003
Drebrin	Couples dynamic MTs to F-actin	Neuronal migration	Worth et al., 2013
ErbB4	Modulates ErbB4/PI3K signaling	Tangetial migration of interneurons	Raki et al., 2015
GSK3B	Inhibits GSK3B	Kinesin mediated axonal transport	Morfini et al., 2004
Nde1	Facilitates dynein function via Lis1	Dynein mediated retrograde transport	Toyo-oka et al., 2003
Kinesin-5	Triggers association with MT	Decreased transport and neuronal growth	Kahn et al., 2015
KIF13B	Deliver TRPV1 to membrane surface	Heat hyperalgesia	Xing et al., 2012
MAP2	Cytoskeletal abnormalities	Formation of axonal spheroids	Bu et al., 2002