Cdk5 in Neuroskeletal Dynamics

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Key Words

Cdk5 · Tau · MAP2 · Nudel · Amyloid precursor protein · Presenilin-1 · β -Catenin · Pak1 · CAMKII · Cables · Disabled · Amphiphysin 1 · Synapsin 1

Abstract

Signal transduction in a broad sense refers to intracellular, molecular changes triggered by cues outside the cell in order to bring about behavioral responses such as migration, differentiation, secretion, or death. Until recently, the best-studied end targets of signal transduction pathways were transcriptional regulators that contribute to changes in gene expression. However, it is clear that many pathways can act through mechanisms other than, or in addition to, transcription control. The three major cytoskeletal systems, microtubules, intermediate filaments, and filamentous actin, are targets for this kind of regulation. Acute changes in cytoskeletal organization are particularly important during embryonic development, when many cells are motile and undergo dramatic changes in shape. Cytoskeletal dynamics are also important in motile cells in adult systems and, in a more limited way, in cells that carry out rapid, regulated secretion. Even changes in synaptic efficacy may involve structural modifications requiring changes in the neuroskeleton. Cytoskeletal reorganization can itself be viewed as signal transduction, producing changes in

molecular trafficking and interactions. This review considers evidence that cyclin-dependent kinase 5 is a modulator of neuroskeletal dynamics.

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Introduction

The term ‘cytoskeleton’ evokes a rigid structure that maintains the cell’s shape, much like the bones of an animal. It stems from early microscopic studies in which the filaments in fixed samples appeared static. In the last 3 decades, this purely structural view of the cytoskeleton has been displaced by evidence that cytoskeletal systems are highly dynamic and can undergo rapid reorganization globally or locally, depending on the age, the environment, or the function of a particular cell. In neuronal development, for example, establishment of polarity, migration, neurite outgrowth, and synaptogenesis depend on cytoskeletal dynamics. Some of the dynamic processes that have been documented are listed in figure 1. Modifications of these processes probably function in mature systems and may influence synaptic function and neuronal survival. Large numbers of proteins are being identified that have roles in regulating cytoskeletal dynamics during mitosis and migratory behavior [1–3]. Many of these are targeted by cyclin-dependent kinase (Cdk) family members [4], including Cdk5. The Cdk5 activity de-

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Fig. 1. The importance of neuroskeletal dynamics. Various degrees of cytoskeletal reorganization are accomplished through a group of related but distinct mechanisms (top box). The regulation of these events is critical for virtually all aspects of neuronal development and function (bottom box). The molecular machinery regulating neuroskeletal dynamics must be tightly regulated, and CDK5 stands poised to be a part of the orchestration.

Fig. 2. Cdk5 is intimately linked to receptor systems and substrates that can impact neuroskeletal dynamics. In pink are proteins that can act as Cdk5 substrates. In green are proteins that are linked to cdk5 regulation or that may be influenced by Cdk5 activity by virtue of their interaction with Cdk5 substrates. Proteins are placed in categories according to which neuroskeletal network they are most notably associated with, although substantial cross talk occurs across categories.

depends not on cyclins, like the activity of other Cdk family members, but on two largely neuronal proteins, p35 and p39 [5]. Because of this, the Cdk5 activity is most prominent in developing neurons, where it functions in neuronal migration and neuritic growth and synaptogenesis. Some of the known and suspected neuroskeletally related

Cdk5 substrates are collected in figure 2. In adult animals, Cdk5 may modulate synaptic transmission [6]. Although Cdk5 is vital during development, its activity at the wrong place or at the wrong time in mature systems is implicated in neurodegenerative disorders like Alzheimer's disease [7] and amyotrophic lateral sclerosis (ALS) [8].

A number of cell surface receptors involved in adhesion, pathfinding, and neurotransmission have been linked with Cdk5 activity [5]. Some, including integrin receptors, plexins, neurotrophin receptors, and neurotransmitter receptors, regulate the kinase. Others respond to Cdk5 activity. Cell surface receptors often interact directly or indirectly with cytoskeletal elements, and because many Cdk5 substrates are associated with cytoskeletal dynamics, it is worth considering a role for Cdk5 in the communication between receptors and neuroskeletal systems. Among known or suspected Cdk5 substrates are microtubule-associated proteins (MAPs), including tau [9], MAP2 [Tsai, personal commun.], and the dynein-associated protein, Nudel [10]. Cdk5 substrates also include a long list of proteins related to actin dynamics, such as CaMKII [11], the Abl-interacting protein, Cables [12], Pak1 [13], amphiphysin [14], and synapsin 1 [15]. Finally, Cdk5 can use two of three neuronal intermediate filament subunits, NF-H and NF-M, as substrates. It is not too far-fetched, therefore, to speculate that a component of Cdk5's signaling function may be to coordinate motile behaviors through its impact on, or sensitivity to, cytoskeletal dynamics.

This review will concentrate on Cdk5 data related to each of the three cytoskeletal networks, considering them separately with the understanding that a large degree of molecular cross talk occurs between the different networks.

The Microtubule Connection

The microtubule network can be stabilized or broken down rapidly (catastrophe). The rate of polymerization of α - and β -tubulin dimers can also be regulated. Microtubule stability is supported by several MAPs [2]. The well-known MAPs, MAP2 and tau, bind to the surface of the microtubule, bridging tubulin subunits, and may neutralize the negative charge on the microtubule surface. Members of the XMAP215/Stu2p/TOG family promote microtubule polymerization at 'plus' ends and regulate the catastrophe frequency [16]. The microtubule-destabilizing factor katanin severs microtubules [17], while depolymerizing kinesins of the KinI family may cause protofilament peeling [18]. Op18/Stathmin may sequester tubulin dimers or promote GTP hydrolysis to destabilize microtubules [19]. Lis1 interacts with the microtubule motor, cytoplasmic dynein, and can reduce microtubule catastrophe frequency [20] and impact microtubule organization [21]. Cytoplasmic dynein also promotes transport of

assembled microtubule segments. This function is particularly prominent in axons [22]. There is evidence that dynein can also directly influence the polymerization dynamics of microtubules. At the vertebrate kinetochore, for example, motors interact directly with microtubule ends and modulate polymerization dynamics to orchestrate chromosome movements during mitosis [23]. The Lis1-associated protein Nudel may impact dynein behavior and microtubule organization [10, 24]. End-binding proteins emphasize and make use of the intrinsic polarity of tubulin polymers [25, 26]. CLIP-170 and EB1 copolymerize with new tubulin subunits by binding to a special configuration of the microtubule plus end. They also seem to serve as links between growing microtubules and kinetochores or focal adhesions centers at the cell cortex through proteins such as APC and β -catenin [27, 28]. Moreover, EB1 interacts with cytoplasmic dynein, and binding of β -catenin to the cytoplasmic dynein/dynactin motor complex at microtubule ends and cortical sites or kinetochores can stabilize microtubules [28]. Many of the factors regulating the microtubule stability are themselves regulated by kinases and phosphatases. New findings point to a molecular interrelationships between different skeletal systems. In this first section, we will concentrate on some of these microtubule-regulatory proteins and their relationship to Cdk5.

Tau

In 1993, Baumann et al. [9] described a link between Cdk5 and microtubules [9]. Cdk5 copurified with known MAPs and the kinase was capable of phosphorylating tau, a fairly well-studied and somewhat infamous MAP. Abnormally hyperphosphorylated forms of tau were known to form paired helical filaments typical of neurofibrillary tangles in the brains of Alzheimer's disease patients [9]. Over the last decade, a strong link has been forged between Cdk5 deregulation and Alzheimer's disease [7]. Neurotoxic induction of calpain leads to the proteolytic cleavage of p35 to p25 [29]. This product maintains its ability to activate Cdk5, but is more stable and less membrane associated [30]. Interestingly, Alzheimer's-like tau phosphorylation is more apparent when Cdk5 is paired with p25. Cotransfection of Cdk5 and p25 resulted not only in a dramatic increase in tau phosphorylation and in the disorganization of microtubules, but was more toxic than the coexpression of Cdk5 and p35 [31]. The brains of mice in which a p25 transgene was expressed showed increased Cdk5 activity, increased immunostaining for hyperphosphorylated tau, axonal swelling, and disorganization of the cytoskeleton [32].

Based on these data, most models predict that Cdk5 phosphorylates tau only under pathological conditions when p25 is generated. However, healthy neurons may have use for tau phosphorylation by Cdk5 in a spatially or temporally limited manner. So what does tau do in healthy neurons? Tau was originally characterized as a MAP that promoted the assembly of 6S tubulin dimers into 36S tubulin rings [33]. Tau was then shown to suppress microtubule catastrophe in vitro [34] and in cultured fibroblasts [35]. Overexpression of tau in CHO cells had dramatic effects on cellular systems that were thought to be dependent on the function of the microtubule-based motor protein kinesin [36]. These effects were related to the finding that tau overexpression affected the frequency of motor attachment and detachment to microtubule tracks and had an impact on vesicle minus to plus end reversal with a net effect of increasing the directional bias towards minus ends [37]. Tau overexpression also inhibited axon transport in neurons [38].

Despite the obvious impact of tau on microtubules and motors in vitro and in cultured cells, the nervous system of tau^{-/-} mice appears to be normal, and axonal elongation is unaffected in cultured neurons [39]. In some small-caliber axons, however, the microtubule stability is decreased, and the microtubule organization is significantly altered. An observed increase in expression of another MAP, MAP1A, in these mice suggested that this protein might compensate for the functions of tau in the unaffected large-caliber axons.

The evidence for a nonpathological role for Cdk5 in tau biology is not extensive. However, tau phosphorylation at known Cdk5 sites is decreased in Cdk5^{-/-} mouse brains. This is not the case in p35^{-/-} mouse brains, suggesting that p39 might play a role [40]. Cdk5 is anchored to microtubules through binding to unphosphorylated tau; phosphorylation of tau results in the dissociation of Cdk5. The phosphorylation of tau by Cdk5/p39 in vitro also decreased tau's affinity for microtubules [40]. Thus, although it has been difficult to pin a physiological function on tau, Cdk5 could conceivably affect normal neuronal cytoskeletal dynamics and motor protein function by regulated posttranslational modification of the protein.

MAP2

Brain MAP2 was one of the first MAPs to be isolated [41–43]. Whereas tau is preferentially localized to axonal compartments, MAP2 tends to be dendritic. Both proteins are thought to confer stability to microtubules in a phosphorylation-sensitive manner. Like tau, MAP2

can be phosphorylated in vitro by a number of protein kinases, including protein kinases A and C (PKA, PKC) [44], calcium calmodulin dependent kinases (CAMKs) [45], MARK kinases [46], Cdc2 [44], MAP/ERK kinases [47–49], and glycogen synthase kinase 3 β [50]. Cdk5 can also phosphorylate MAP2 [Tsai, personal commun.]. In general, MAP2 phosphorylation seems to interfere with microtubule association and to decrease the microtubule stability, but the specific effects of individual kinases in particular cellular contexts are not completely understood.

Postsynaptic MAP2 is a target of signaling pathways activated by glutamate [51]. Glutamatergic regulation of MAP2 phosphorylation by CAMKII and PKC may transduce neural activity into modifications in dendritic structure. Indeed, there is a reduction in microtubule density in dendrites and a reduction of dendritic length in MAP2-deficient mice [52]. Freda Miller and colleagues [49] recently showed that neuronal activity promoted reversible dendrite formation accompanied by an increased association of MAP2 with microtubules and an increased microtubule stability. Inhibition of either CaMKII or the MEK/ERK pathways was required for dendrite formation, while inhibition of both pathways caused dendrites to retract.

Does Cdk5 play a role in these glutaminergic responses? Cdk5 is enriched in postsynaptic membranes. Moreover, calcium increase or activation of glutamate receptors stimulates an interaction between CAMKII α - and Cdk5-regulatory subunits [11]. Inhibition of CAMKII activation diminishes this interaction. Together, these data suggest the intriguing possibility that cross talk between the Cdk5 and CAMKII signal transduction pathways may be a component of the complex molecular/cytoskeletal mechanisms contributing to synaptic plasticity. It remains to be determined whether Cdk5 is involved in MAP2 regulation in dendrites.

Amyloid Precursor Protein (APP)

Another link between Alzheimer's disease and Cdk5 was forged by the demonstration that A β , the proteolytic peptide derived from APP, can induce calpain-dependent cleavage of p35 and stimulate tau hyperphosphorylation and neurotoxicity [7]. Interestingly, blocking microtubule dynamics with taxol inhibits this A β -dependent pathway and stabilizes the normally short-lived p35 regulatory subunit [53]. Taxol is not sufficient to protect primary cortical neurons from constitutive Cdk5 activation following overexpression of p25, however. A nonpathological connection between APP/A β and Cdk5 has not been

described, but there is evidence for APP's involvement in microtubule-based transport. Recent data suggest that it functions as a kinesin-I cargo receptor, mediating the targeting of several synaptic proteins to the nerve terminal [54–57]. Axonal transport of APP is mediated by direct binding to the light-chain subunit of kinesin-I [58]. Moreover kinesin-mediated axonal transport of a membrane compartment containing β -secretase and presenilin-1 (PS1) requires APP [59]. Indeed, profound loss of immunoreactivities for the presynaptic proteins synaptophysin and synapsin 1, as well as the dendritic marker MAP2, was reported in many brain areas of APP $^{-/-}$ mice [60]. APP, PS1, synapsin 1, and MAP2 are all likely substrates for Cdk5. Could there be a role for the kinase in microtubule motor-based trafficking of synaptic proteins?

Nudel

A link between cytoplasmic dynein and tubulin dynamics has been demonstrated in the filamentous fungus *Aspergillus nidulans* [61]. Temperature-sensitive loss-of-function dynein mutants (*NUDA*) in *Aspergillus* showed a lower frequency of microtubule catastrophe, a lower rate of shrinkage during catastrophe, and a lower frequency of rescue. These results indicate that cytoplasmic dynein affects microtubule dynamics in vivo. Loss-of-function mutation in a dynein-interacting protein (*NUDF*) had similar effects. Haploinsufficiency of the gene encoding the human homolog of NUDF (resulting in a 50% reduction in protein expression) causes the majority of the known cases of a rare, but severe, developmental brain abnormality termed classical lissencephaly [61]. In animal models of lissencephaly (Lis1 $^{+/-}$ mice or flies with Lis1 disrupted in neurons), the neuronal defects are multifold, ranging from mitotic abnormalities in precursors to defects in migration and neuritic growth [62, 63]. Purified brain Lis1 has been shown to cosediment with microtubules and to reduce microtubule catastrophe events in vitro [20]. Furthermore, changes in the expression level of Lis1 in cultured nonneuronal cells has a dramatic effect on microtubule organization [21]. Dynein and Lis1 are linked to Cdk5 through a protein called Nudel [10, 24] and probably also through a related protein, mNudE [64]. Nudel can be phosphorylated by Cdk5 in vivo and in vitro. Moreover, phosphospecific antibodies do not recognize Nudel in Cdk5 $^{-/-}$ brain extracts [Tsai, personal commun.]. It is not yet clear if and how Lis1 and Nudel regulate dynein function, but it is not out of the question that they regulate dynein's impact on the microtubule stability. Thus Cdk5 might work through Nudel to impact microtubule dynamics.

Lis1 also seems to be involved in some mitotic roles of dynein [65]. Very recently, phosphorylation of Nudel and mNudE by Cdc2, a mitotic kinase and a close homolog of Cdk5, was implicated in the translocation of dynein and spindle assembly checkpoint proteins away from kinetochores [66]. The kinase may be regulating a checkpoint-inactivating event that allows chromosome segregation to occur only when spindles are properly attached. It would be interesting if there were some kind of a parallel checkpoint system that functions to regulate microtubule dynamics during migration.

PS1 and β -Catenin

PS1 [see 67 for a recent review] is a component of an enzymatic activity, γ -secretase, implicated in proteolysis of APP and generation of a form of A β peptide abundant in senile plaques. PS1 is produced as a holoprotein, then processed to amino- and carboxy-terminal fragments that are incorporated into stable, high molecular mass complexes. Presenilins function both in Notch signaling (acting as a protease) and in regulating β -catenin stability in response to Wnt signaling.

Three fairly recent findings are more to the point of this review. PS1 reportedly is a substrate of Cdk5 [68]. Cdk5/p35 phosphorylates a threonine within the carboxyl-terminal domain of PS1 both in vitro and in vivo and stabilizes the carboxy-terminal fragment. Second, PS1 binds to the microtubule plus-end-capping protein, CLIP-170 [69]. PS1/CLIP170 interactions have a regulatory effect on β -amyloid secretion and uptake, but the mechanism is not clear. The authors suggest a scenario in which PS1 guides microtubule binding to specific membrane subdomains possibly containing amyloidogenic fragments. An interesting twist is that CLIP-170 interacts with the Nudel-binding partner, Lis1, in mitotic cells; Lis1 is apparently responsible for CLIP-170 accumulation at kinetochores [70, 71]. It is not known whether Lis1 and CLIP-170 interact in neurons. PS1 also interacts with β -catenin [72], another Cdk5 substrate [73]. Cdk5 apparently phosphorylates β -catenin to regulate binding of β -catenin to PS1, but also regulates β -catenin's interaction with N-cadherin cell adhesion molecules. The likely influence of Cdk5 is downregulation of N-cadherin at the cell surface [74, 75]. Interestingly, an intracellular pool of N-cadherin is tightly associated with the microtubule network [151]. Moreover, expression of N-cadherins in centrosome-free cytoplasts increased levels of microtubule polymer and also changed the behavior of microtubules from treadmilling to dynamic instability [152]. N-cadherins thus appear to initiate a signaling pathway that alters

microtubule organization by stabilizing microtubule ends. Cdk5 may directly or indirectly impact these interactions through regulating β -catenin's interactions and changing N-cadherin function. Interestingly, Lis1 and β -catenin both interact with cytoplasmic dynein and may serve to stabilize microtubules at cortical sites [28, 76, 77]. A potential role for Cdk5 in a neuronal version of 'microtubule capture' at leading edge or growth cone plasma membranes is intriguing.

Schuman and Murase [75] recently demonstrated that Cdk5 may regulate a β -catenin/N-cadherin interaction in synapses as well. In hippocampal neurons, depolarization caused a decrease in Cdk5 kinase activity and a redistribution of EGFP- β -catenin from dendritic shaft into spines, where cadherins are highly concentrated. Decreasing the Cdk5 kinase activity with the inhibitor roscovitine caused a similar redistribution of EGFP- β -catenin into spines and increased the amount of β -catenin coimmunoprecipitating with cadherin. A point mutation in tyrosine 654 of β -catenin blocked its movement into spines in response to Cdk5 inhibition and prevented it from interacting with cadherin.

Although a connection with microtubules in dendritic spines is not likely, Cdk5 is also intimately involved in actin regulation, and β -catenin interacts with actin through α -catenin.

The Actin Connection

Although Cdk5 in brain extracts cosediments with microtubules after a series of polymerization-depolymerization steps, the Cdk5 immunoreactivity coincides best with regions rich in filamentous actin, such as leading edges of growth cones [78]. Moreover, the Cdk5 activity in nonneuronal cells stimulates changes in actin organization. One of Cdk5's activating partners, p39, colocalizes with actin in cultured cells and coimmunoprecipitates with actin in brain lysates [79]. Disruption of the actin cytoskeleton alters the subcellular distribution of p39 [79].

Like microtubules, actin filament formation and stability is regulated by an extensive collection of actin-binding proteins (over 150) and signal transduction pathways [80]. Some actin-binding proteins maintain the population of assembly-ready actin monomers (profilin). Others bind to and block the growing ends of actin filaments (gelsolin). Actin-binding proteins can nucleate actin assembly (gelsolin, Arp2/3, cofilin), sever actin filaments (gelsolin, ADF/cofilin), or cross-link actin filaments (Arp2/3). The

activity of these proteins is regulated by several signaling pathways, most prominently those involving the RHO GTPases. The members of this family of biological switches impact many aspects of actin dynamics and are important in a wide range of cell behaviors [81]. The identification of several Cdk5-interacting proteins/substrates related to actin prompts speculation about a role for Cdk5 activity in regulating actin dynamics or actin-microtubule interactions. We will consider each of the actin-related Cdk5 links in turn to try to shed some light on how these may relate to the function of the kinase.

Pak1

The first link between Cdk5 and actin was the demonstration that the kinase phosphorylates Pak1 in a Rac1-dependent manner and inhibits Pak1 activity [13]. Rac1 is strongly implicated in actin dynamics and neurite growth, and one of its primary effectors is Pak1 [82]. Strangely, few actin-related targets of Pak1 have been identified, although a recent report [83] suggested that Pak1 could phosphorylate actin directly, resulting in the dissolution of stress fibers and redistribution of microfilaments. Pak1 also targets myosin light-chain kinase and decreases its activity which may have an impact on actin dynamics in migrating cells and growth cones [84]. Finally, Pak1 increases the activity of LIM kinase towards the actin-regulatory protein, cofilin [85].

Although Rac signaling is mainly associated with actin, there is growing evidence that Rac1 influences actin in response to microtubule dynamics [86]. For example, the growth of microtubules induced in fibroblasts by removal of the microtubule destabilizer nocodazole activates Rac1 GTPase, leading to the polymerization of actin in lamellipodial protrusions [87]. It was also recently shown that the Pak activity might inhibit a microtubule-destabilizing protein, Op18/Stathmin [88], so Rac/Pak signaling could influence microtubule dynamics as well. Pak1 phosphorylated on T212 (the Cdk5 phosphorylation site) is enriched in axonal growth cones and colocalizes with small peripheral bundles of microtubules [89]. Cortical neurons overexpressing a Pak1 mutated at T212 display a tangled neurite morphology which suggests that the microtubule cytoskeleton is affected. In the developing cortex and in cultured fibroblasts, phosphorylated Pak1 T212 is enriched in microtubule-organizing centers and along parts of mitotic spindles. Indeed, cyclin B1/Cdc2 phosphorylates Pak1 during mitosis. Banjeree et al. [90] showed that, in living cells, a peptide mimicking phosphorylated T212 accumulates at the centrosomes and spindles and causes an increased length of astral microtubules during meta-

phase or following nocodazole washout. Staining of fibroblasts extracted with detergent using anti-Pak1 antibodies revealed that a small subpopulation of this kinase remained tightly bound to the mitotic spindles. Exposure to a Pak1 peptide phosphorylated by Cdk5, but not unphosphorylated peptide, reduced the pool of microtubule-bound Pak1. Significant levels of the phosphorylated peptide were tightly bound to the cytoskeleton, suggesting that it competes with endogenous Pak1 for microtubule association.

Thus, accumulating data suggest that reciprocal Cdk5 and Pak1 activity could be important in regulating actin/microtubules, but the specifics of this cooperation are not yet well understood.

α -Actinin and CaMKII

CaMKII is localized in the postsynaptic density (PSD) and is necessary for LTP induction. The autophosphorylated state of CaMKII binds tightly to NMDA receptors and to α -actinin [91]. α -Actinins are primarily thought of as actin cross-linking proteins that tether cell surface receptors, ion channels, and adhesion complexes to the cortical actin cytoskeleton [92–97]. α -Actinin is present in PSDs and dendritic spines [98]. Disruption of the actin cytoskeleton attenuates NMDA-receptor-mediated transmission [99] and causes the loss of CaMKII at the PSD and α -actinin at spines [100].

Cdk5/p35 and Cdk5/p39 can complex with both CaMKII and α -actinin-1 in a calcium-sensitive manner [11]. Glutamate can strengthen the interaction, to a large extent through NMDA receptors. The association of CaMKII and Cdk5 with α -actinin-1 may serve to anchor both kinases to the subsynaptic cytoskeleton and perhaps modulate their proximity to the NMDA receptor and other substrates at the PSD. Alternatively, neurotransmission may trigger changes in these interactions in order to regulate the underlying actin skeleton. Further studies will be needed to make this distinction. Interestingly, changes in expression or activity of the myosin light-chain kinase, a downstream target of Pak1 kinase, influence NMDA receptor neurotransmission, probably by indirectly regulating actin and myosin [101]. Because the Pak1 activity can be regulated by Cdk5, it could impact this pathway.

Cables

Cables is a substrate of the c-Abl tyrosine kinase that promotes the interaction between c-Abl and Cdk5 [12]. Active c-Abl kinase leads to Cdk5 tyrosine phosphorylation, and this phosphorylation is enhanced by Cables. Phosphorylation of Cdk5 by c-Abl occurs on tyrosine 15

(Y15) which is stimulatory for the p35/Cdk5 kinase activity. Expression of antisense Cables in primary cortical neurons inhibits neurite outgrowth. Furthermore, expression of active Abl resulted in lengthening of neurites.

How does this relate to actin dynamics? Actin organization can impact c-Abl activity [102]. c-Abl tyrosine kinase is inhibited by F-actin, and this inhibition can be relieved through mutation of its F-actin-binding domain. In a reciprocal manner, C-Abl can impact the actin skeleton. c-Abl stimulates the formation of actin microspikes in fibroblasts spreading on fibronectin and F-actin branches in neurites of rat embryonic cortical neurons [103]. These functions of c-Abl are dependent on its kinase activity, but not on its F-actin-binding domain. It is interesting to speculate that these actions of c-Abl might work through its ability to regulate Cdk5.

Disabled 1

Enabled (Ena) and disabled (Dab) are genetic modulators of the phenotype observed in flies null for the Abl tyrosine kinase gene [104]. Cdk5 phosphorylates the murine homolog of the *Drosophila* disabled protein, mDab1, on serine 491 in vitro and in vivo [105]. Mutations in mouse equivalent of disabled have brain abnormalities similar but not identical to Cdk5^{-/-} mice [106, 107]. Although the functional significance of mDab1 phosphorylation by Cdk5 is not clear, there are interesting links between mDab1 and another Cdk5 substrate, APP. The cytosolic domain of APP interacts with mDab1 and another protein, FE56 [108, 109]. Fe65 functions in the regulation of actin cytoskeleton and cell movement [110]. This may be related to the direct interaction of Fe56 with domains with the proline-rich repeats in the mouse homolog of the *Drosophila* enabled protein (Mena) [111]. Mena is a member of a family of actin-binding proteins that promote or inhibit actin-dependent processes by antagonizing various capping proteins at the barbed end of actin filaments [112]. APP and FE65 colocalize with actin and Mena in lamellipodia of migrating MDCK cells and with β 1-integrin in focal complexes [110]. Overexpression of APP accelerates cell migration in an MDCK cell wound-healing assay [110]. Coexpression of APP and FE65 dramatically enhances the effect of APP on cell movement, probably by regulating the amount of APP at the cell surface.

In a related role, Fe65 may serve as a linker between another actin-binding protein, c-Abl, and APP. Fe65 binds to the active form of Abl, and APP is tyrosine phosphorylated on Tyr682 in cells expressing this form of Abl [113]. Active Abl and tyrosine-phosphorylated APP are

present in coimmunoprecipitated complexes. Together, these findings suggest that Abl, Mena, and mDab1 are involved in APP biology. It will be interesting to determine if and how Cdk5 activity impacts these interactions in neurons.

Amphiphysin 1

Amphiphysin 1 is generally associated with clathrin-mediated endocytosis, but recently has been linked with the actin skeleton, albeit somewhat tenuously [114]. Amphiphysin 1 colocalizes with actin and p35 in neuronal growth cones and is phosphorylated by Cdk5/p35 [14]. Amphiphysin 1 oligonucleotides caused growth cone collapse and disruption of the actin cytoskeleton in cultured hippocampal neurons without affecting receptor-mediated endocytosis. This suggests that amphiphysins could have actin-related functions in growth cones independent of clathrin-mediated endocytosis. In support of this, amphiphysins interact with neuronal isoforms of focal adhesion kinase (FAK) [115]. FAK is a nonreceptor tyrosine kinase that has been shown to be a major mediator of integrin signal transduction pathways. FAK and amphiphysins are associated in nerve terminals, where FAK also interacts with components of the actin cytoskeleton such as talin, α -actinin (another Cdk5 substrate), and paxillin and is part of the complex that anchors the F-actin cytoskeleton to the plasma membrane [116]. The tangled web gets more tangled when we consider that both FAK and Cdk5 kinase activities can be stimulated by integrin signaling. Is it possible that both FAK and Cdk5 impact cytoskeletal dynamics through amphiphysin 1 and α -actinin in response to extracellular matrix interactions? Or does Cdk5 function upstream or downstream of FAK?

Synapsin 1

Cdk5 reportedly can also phosphorylate synapsin 1 [15], a member of a family of five neuron-specific phosphoproteins associated with the membranes of synaptic vesicles [117]. Synapsins are implicated in controlling neurotransmitter release by tethering vesicles to actin filaments in a phosphorylation-dependent manner, thus regulating the number of vesicles available for release. In living hippocampal terminals, green fluorescent protein labeled synapsin 1a dissociates from synaptic vesicles and disperses into axons during action potential firing, then reclusters to synapses after the cessation of synaptic activity [118]. Synapsin 1 also participates in the formation and maintenance of synaptic contacts in hippocampal neurons. Retarded outgrowth of predendritic neurites

and axons from cultured hippocampal neurons was reported in Synapsin 1^{-/-} mice [119]. Furthermore, the synapse formation was significantly delayed, and this impairment was found to be caused by postsynaptic changes [120].

Synapsin 1 can bind to actin [121–128], microtubules [129–132], and neurofilaments [133] *in vitro*. When bound either to synaptic vesicles or to pure phospholipid vesicles, synapsin 1 exhibits phosphorylation-dependent actin-nucleating activity. Synaptic vesicles depleted of endogenous synapsin 1 decrease rate and extent of actin polymerization. Synapsin 1 is also reportedly an actin-bundling protein; this activity is also controlled by phosphorylation [124], but it may be unrelated to the Cdk5 activity [15]. Therefore, the consequence of Cdk5 activity towards synapsin 1 is not clear and will require further study. If indeed synapsin 1 is intimately related to all three neuroskeletal elements, then the possibility exists that it functions in some sort of integrative capacity that could be modulated by Cdk5.

The Neurofilament Connection

Intermediate filament (IF) proteins form the largest family of cytoskeletal proteins in mammalian cells [3]. IF family members include nuclear lamins, vimentin, desmin, glial fibrillary acidic protein, and type I and II keratins. The function of these medium-sized polymers has generally been considered to be structural, but evidence that IFs can modulate essential cell processes and signal transduction events has broadened this view [3].

In neurons, the most prominent IFs are composed of three ‘neurofilament’ proteins [for two recent reviews see 134, 135]. These are termed NF-H, NF-M, and NF-L to signify high (H), medium (M), and low (L) molecular weight subunits. Neurofilaments possess side arms composed of the COOH domains of NF-M and NF-H; that of NF-H is heavily phosphorylated in axons. Axonal NF-H becomes highly phosphorylated in maturing systems and is hyperphosphorylated in some neurodegenerative diseases. Neurofilaments have a well-established role in the control of axon caliber. There is also growing evidence based on transgenic mouse studies that neurofilaments can affect the dynamics of microtubules and actin filaments. Neurofilaments contain phosphorylation sites for a large number of protein kinases, including PKA, PKC, ERK, GSK-3 β , and the stress-activated protein kinase gamma [136]. Cdk5 also figures prominently as a neurofilament kinase [135]. Cdk5 target phosphorylation sites,

located in tail regions of NF-M and NF-H, consist of the repeat sequence motif, Lys-Ser-Pro (KSP).

Degenerative disorders of motor neurons include a range of progressive fatal diseases such as ALS. Although the causative genetic alterations are known for some cases, the molecular basis of most ALS cases is unknown. However, ALS is characterized by depositions of neurofilaments in the perikarya and proximal axons, and variant alleles of the neurofilament heavy-subunit gene (NF-H) have been found in some ALS patients [136]. Moreover, transgenic mice that overexpress neurofilament proteins show motor neuron degeneration and defects in the axonal transport of neurofilament and other proteins, including tubulin and actin [137]. Mutations in copper/zinc superoxide dismutase 1 (SOD1) can cause ALS through an unidentified mechanism. Two lines of transgenic mice with SOD1 mutations linked to familial ALS showed a reduced transport of selective cargoes, especially tubulin, that arose months before neurodegeneration. For one mutant, this transport defect represents the earliest detectable abnormality [138].

Neurofilament protein is transported in axons as a polymer [139]. Movements are fast and bidirectional, but interspersed with long pauses. Kinesin and cytoplasmic dynein are involved in transporting neurofilaments in axons. Several recent findings have linked motor neuron disease with microtubule motors. Inhibition of dynein-mediated axonal transport by postnatal transgenic overexpression of a component of the dynactin regulatory complex in mouse motor neurons causes neurodegeneration [140]. Interestingly, a missense mutation in another subunit of dynactin has been identified in a human kindred displaying spinal-bulbar muscular atrophy [141]. Missense point mutations in mice that exclusively perturb neuron-specific functions of cytoplasmic dynein result in progressive motor neuron degeneration and Lewy-like inclusion bodies [142]. Finally, mutations in the anterograde kinesin motor, KIF1B, can lead to Charcot-Marie-Tooth disease, a slowly progressive motor neuropathy [143].

A recent analysis of neurofilament movement in transfected living neurons [144] demonstrated that phosphorylation of NF-H side arms by Cdk5/p35 influenced the transport of neurofilaments through axons. Phosphorylation effectively slowed neurofilament transport due to increased pausing of neurofilaments. The Cdk5/25 activity is associated with the hyperphosphorylation of tau and neurofilament proteins in mice expressing a mutant SOD1. It has been suggested that perikaryal accumulations of neurofilament proteins in motor neurons may

actually lessen the ALS pathogenesis by acting as a phosphorylation sink for this aberrant Cdk5 activity, reducing detrimental hyperphosphorylation of tau and other substrates [145]. However the link between Cdk5 and neurofilament transport suggests that deregulated Cdk5 might be the cause of accumulations of neurofilaments. The verdict, then, is still out on a normal physiological role for Cdk5 in neurofilament dynamics.

Concluding Remarks

The aim of this review is to focus attention on the relationships between Cdk5 and the neuroskeleton. An emerging theme in cytoskeletal dynamics in all cells is the interrelated nature of the three distinct cytoskeletal elements. For example, we generally think of cell motility as membrane protrusion powered by turnover of adhesion complexes that link the actin cytoskeleton to the extracellular matrix [146]. Directional polarity however, is likely attained by microtubules that modulate Rho GTPase activity and actin behavior at adhesion sites. This site-specific targeting by microtubules may bring enzymatic complexes (bound to microtubules or MAPs) in close proximity to adhesion complexes to promote complex disassembly and remodeling of the actin cytoskeleton [86].

A requirement for this type of communication would not be unique to neurons, and if we want to make the argument that Cdk5 serves in an integrative capacity to coordinate neuroskeletal dynamics, we need to explain how these events are coordinated in nonneuronal cells which, for the most part, lack Cdk5 activity. The obvious candidate for a Cdk5 correlate is the 'mitotic' kinase, Cdc2, which shares a large degree of homology and substrate specificity with Cdk5. So, does Cdc2 participate in cytoskeletal dynamics?

There are actually many parallels. For example, the integrin-mediated Cdc2 activity has been shown to be important for the migratory activity in prostate cancer cells [147]. Ectopic expression of cyclin A, an activator of Cdc2, can confer anchorage-independent mitosis, i.e., uncouple adhesion and cytoskeletal dynamics [148]. Cdc2 can actually exert an effect directly on actin dynamics, through a protein called caldesmon, which inhibits binding of Arp2/3 to F-actin, reducing Arp2/3-mediated nucleation [149]. Phosphorylation of caldesmon with Cdc2 inhibits its ability to interact with actin and reverses its inhibitory effect on Arp2/3-induced actin polymerization. It is not known whether a similar role is played by Cdk5 in

neurons. Cdc2 affects MAP2-induced microtubule polymerization in vitro [44] and can phosphorylate Nudel and Pak1 in mitotic cells to regulate dynein and microtubules, respectively [66, 90]. Cdc2 is also an important regulator of the breakdown of the nuclear membrane in prometaphase. It exerts an impact at the level of nuclear IFs called lamins [150].

We are just beginning to unravel the complexities of coordinated cellular responses to extracellular signals. Our continued effort over the next several years is likely to reveal the extent to which these two Cdk family mem-

bers influence cytoskeletal dynamics, and will hopefully shed some light on when and how they do so. Is it a coordinated sweep of enzymatic activity that targets a multitude of substrates in response to specific signals? If so, does it switch rapidly on and off, and do the target substrates fluctuate in response to changing extracellular cues? Alternatively, are cytoskeletal dynamics producing the signals that these kinases are responding to? Answers to these questions will no doubt require the sophisticated proteomic and imaging techniques that are currently being developed and improved at a rapid pace.

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