© 2014. Published by The Company of Biologists Ltd | Journal of Cell Science (2014) 127, 2391–2400 doi:10.1242/jcs.147553

COMMENTARY

Cdk5 activity in the brain – multiple paths of regulation

Kavita Shah[1,*] and Debomoy K. Lahiri[2]

ABSTRACT

Cyclin dependent kinase-5 (Cdk5), a family member of the cyclin-dependent kinases, plays a pivotal role in the central nervous system. During embryogenesis, Cdk5 is indispensable for brain development and, in the adult brain, it is essential for numerous neuronal processes, including higher cognitive functions such as learning and memory formation. However, Cdk5 activity becomes deregulated in several neurological disorders, such as Alzheimer's disease, Parkinson's disease and Huntington's disease, which leads to neurotoxicity. Therefore, precise control over Cdk5 activity is essential for its physiological functions. This Commentary covers the various mechanisms of Cdk5 regulation, including several recently identified protein activators and inhibitors of Cdk5 that control its activity in normal and diseased brains. We also discuss the autoregulatory activity of Cdk5 and its regulation at the transcriptional, post-transcriptional and post-translational levels. We finally highlight physiological and pathological roles of Cdk5 in the brain. Specific modulation of these protein regulators is expected to provide alternative strategies for the development of effective therapeutic interventions that are triggered by deregulation of Cdk5.

KEY WORDS: Cdk5, p25, p35, β-Amyloid, Excitotoxicity, Cdk, Cyclins, Neurodegeneration, Alzheimer's disease, Synaptic plasticity, Learning and memory, Pain signalling, Neuronal migration, Axonal growth, Synaptogenesis

Introduction

Cyclin-dependent kinases (Cdks) are Ser/Thr protein kinases that associate with specific cyclin subunits in order to be activated. This family currently includes 11 classic Cdks (Cdk1 – Cdk11) and two new family members (Cdk12, Cdk13) (Malumbres et al., 2009). All Cdks phosphorylate Ser-Pro or Thr-Pro sites, with a preference for the basic residues Lys and Arg at proximal upstream and downstream positions. Cdks are highly expressed in proliferating cells and play indispensable roles in specific phases of cell cycle.

The first human Cdk was cloned in 1987 by using functional complementation in yeast, and was termed cell division cycle 2 (Cdc2; now officially known as Cdk1) because of its high homology with fission yeast kinase Cdc2 (Lee and Nurse, 1987). The second human homolog of the Cdk family to be cloned from a human cDNA library was kinase Cdk2 (Elledge and Spottswood, 1991; Ninomiya-Tsuji et al., 1991; Tsai et al., 1991). The discovery of human Cdk1 and Cdk2 paved the way for the identification of Cdk5 (Gene ID: 1020); and in 1992, five independent laboratories reported Cdk5 as a new member of the Cdk family, although each study gave it a different name, i.e. tau kinase II (Ishiguro, et al., 1992), neuronal Cdc2 like kinase (nclk) (Hellmich et al., 1992), brain proline-directed kinase (Lew et al., 1992), PSSALRE (Meyerson et al., 1992) and Cdk5 (Xiong et al., 1992).

Cdk5, although highly homologous to its relatives, is a unique Cdk family member (Dhavan and Tsai, 2001). Cdk5 is ubiquitously expressed, but its functions are vital in post-mitotic neurons, where other Cdks are not expressed or active. In contrast to other Cdks, Cdk5 does not participate in cell cycle progression in proliferating cells, but can aberrantly activate various components of cell cycle when it is deregulated in post-mitotic neurons, leading to cell death (Chang et al., 2012). For its activation Cdk5 also requires its specific binding partners CDK5R1 and CDK5R2 (hereafter referred to as p35 and p39, respectively) (Tsai et al., 1994; Tang et al., 1995). Furthermore, unlike other family members, Cdk5 is not regulated by the cyclin-dependent kinase inhibitors CDKN1A and CDKN1B (hereafter referred to as p21Cip1 and p27Kip1, respectively) that, thus, have crucial roles in the regulation of Cdks.

Accumulating evidence has revealed a plethora of Cdk5 functions in neuronal and non-neuronal cells. During embryogenesis, Cdk5 is indispensable for normal brain development (Ohshima et al., 1996), whereas – in adult brains – it regulates neuronal survival, synaptic plasticity, learning and memory formation, pain signaling, drug addiction and long-term behavioral changes (Bibb et al., 2001; Fischer et al., 2002; Pareek et al., 2006). Cdk5 is known to be deregulated in several neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD), which causes neurotoxicity. Consequently, although the deregulation of other Cdks commonly occurs in many types of cancer, Cdk5 is emerging as a potential clinical target to treat multiple neurodegenerative diseases (Cheung and Ip, 2012). Thus, Cdk5 is unique among the Cdks in that it employs its own activation strategies, and has widely diverse functions in neuronal and non-neuronal tissues with different clinical implications (Hisanaga and Endo, 2010). In this Commentary, we discuss the mechanisms through which Cdk5 is regulated by binding to its protein partners in normal and diseased brains. In addition, we describe the mechanisms by which Cdk5 and p35 are regulated at transcriptional, post-transcriptional and post-translational levels. Finally, we briefly highlight the various physiological and pathological functions of Cdk5 in the brain that might be exploited in therapeutic interventions.

Regulation of Cdk5 activity by protein partners

As noted above, Cdk5 is activated by binding it specific activators p35 and p39 (and their cleaved products, p25 and p29, respectively) (Fig. 1). Recent findings have further identified cyclin I (CCNI) as an activator of Cdk5 (Brinkkoetter

[1]Department of Chemistry, 560 Oval Drive, West Lafayette, IN 47907, USA. [2]Laboratory of Molecular Neurogenetics, Departments of Psychiatry and of Medical & Molecular Genetics, Indiana University School of Medicine, Institute of Psychiatric Research, Neuroscience Research Building, 320 W. 15th St., Indianapolis, IN 46202, USA.

*Author for correspondence (shah23@purdue.edu)

et al., 2009) (Fig. 1). Cyclin I is not known to activate other Cdk family members, suggesting it might be a Cdk5-specific activator. In contrast, Cdk5 is inhibited by cyclin D1 (CCND1) (Modi et al., 2012), cyclin E (CCNE) and glutathione S-transferase P (GSTP1) (Sun et al., 2011) (Fig. 1).

p35

p35 was the first Cdk5 activator to be identified (Fig. 1). It forms a tertiary structure that is similar to the cyclin-box fold domain that is required for Cdk activation, although the primary sequence of p35 is distinct from that of cyclins (Tang et al., 1997). In fact, the Cdk5–p35 complex has been shown to adopt a ternary structure that is similar to the complex between Cdk2 and cyclin A (Cdk2–cyclin-A) (Tarricone et al., 2001).

p35 is a membrane-anchored protein that comprises two parts, an N-terminal region encompassing the p10 component and a C-terminal region containing p25. The binding of p25 to Cdk5 leads to its activation even in the absence of p10. Notably, p10 contains the myristoylated region that is important for the membrane targeting of p35. p10 also contains a signal for p35 degradation through the ubiquitin-proteosome pathway. Consequently, p35 has only a short half-life ($t_{1/2}$=20–30 minutes) and is rapidly degraded by the proteosome.

Active Cdk5 also autoinhibits its activity by directly phosphorylating p35. Rapid degradation of p35 is observed in neurons that express Cdk5, which can be inhibited by expression of a dominant-negative or kinase-dead Cdk5 mutant, or by treatment with the Cdk5 inhibitor Roscovitine (Wei et al., 2005). Cdk5 primarily phosphorylates p35 at amino acid residues Ser8 and Thr138 (hereafter S8 and T138, respectively), two phosphorylation events that are differentially regulated and have different consequences (Kamei et al., 2007). The phosphorylation level of S8 remains constant during development, whereas T138 phosphorylation is highest in fetal brains and undetectable in adult brains (Fig. 2). Because both sites are phosphorylated by Cdk5, the differential phosphorylation levels are believed to be due to increased dephosphorylation of T138 in adult brain by protein phosphatase 1 (PP1) or protein phosphatase 2A (PP2A) (Kamei et al., 2007). However, further studies are needed to confirm this possible mechanism.

Cdk5-mediated phosphorylation of p35 at S8 is required to retain p35 in the cytoplasm (Asada et al., 2012). Consequently, a phosphomimetic p35 S8E mutant shows cytoplasmic distribution, whereas a phosphoresistant p35 S8A mutant accumulates in the nucleus (Asada et al., 2012) (Fig. 2).

Importantly, phosphorylation of p35 at T138 by Cdk5 has three functions. First, it prevents the intramolecular cleavage of p35 to p10 and p25 by calpain, thereby preventing the formation of p25 during brain development. As p25 formation is highly neurotoxic (as discussed below), fetal brains might employ T138 phosphorylation to protect against neurotoxicity when Cdk5 activity is high (Fig. 2). Second, phosphorylation of p35 at T138 inhibits its microtubule (MT) polymerization activity (He et al., 2008). A large amount of p35 in the cell binds MTs through its N-terminal domain and promotes MT bundling (He et al., 2008) (Fig. 2). Binding of p35 to MTs removes it from Cdk5 and renders the kinase inactive. Cdk5-mediated phosphorylation of p35 at T138 inhibits its binding to MTs, thereby preventing MT bundling and neurite outgrowth (Fig. 2). Third, it increases proteosomal degradation of p35, thereby tightly regulating its levels. This, in turn, allows Cdk5 to autoregulate its own activity. Collectively, these findings demonstrate that Cdk5 regulates the stability and subcellular localization of p35 through direct phosphorylation, which in turn allows active Cdk5 to spatiotemporally regulate its own activity in the cells.

p25 and p10

p25, the truncated form of p35, is also a neuron-specific activator of Cdk5 (Lew et al., 1994; Fig. 1). p25 is generated from p35 under a variety of neurotoxic conditions, such as exposure to amyloid β peptide (Aβ), excitotoxicity, ischemia and oxidative stress (Patrick et al., 1999; Sahlgren et al., 2006). In this context, it is important to mention that AD is characterized by accumulation of Aβ peptide as neuritic plaques and of hyperphosphorylated tau as neurofibrillary tangles (NFT), as well as by oxidative stress, all of which are targeted for therapeutic interventions in AD (Lahiri et al., 2003; Lahiri, 2011; Lahiri et al., 2014; Perez et al., 2014; Rosenmann, 2013). Notably, these neurotoxic insults disrupt the intracellular Ca^{2+} homeostasis in neurons, thereby promoting activation of calpain, which in turn cleaves p35 into p25 and p10 (Lee et al., 2000). In agreement with this finding, calpains – particularly μ- and m-calpain – are abnormally activated in AD (Saito et al., 1993). Similarly, calpastatin, an endogenous inhibitor of calpains, is substantially decreased in AD (Nixon, 2003), which could lead to

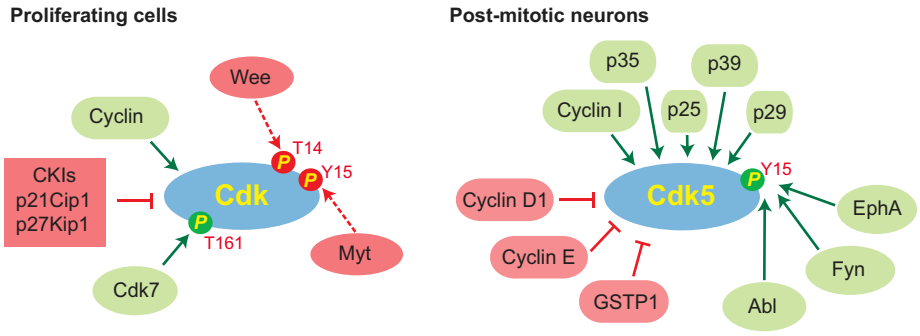

Fig. 1. Regulation of Cdk5 differs from that of other Cdk family members. (Left) Regulators of Cdk family members other than Cdk5. In proliferating cells, these cyclin-dependent kinases are activated by classic cyclins (but not cyclin I) and inhibited by Cdk-dependent inhibitors (CKIs), e.g. p21Cip1 and p27Kip1. Myt1 and Wee1 kinases inhibit Cdks by phosphorylation of amino acid residues T14 and Y15, respectively. (Right) Regulators of Cdk5 (in post-mitotic neurons). Specific binding partners (e.g. p35, p25, p39, p29 or cyclin I) can activate Cdk5, whereas GSTP1, cyclin D1 or cyclin E inhibit it. Cdk5 is also differentially regulated through phosphorylation of Y15, which increases its activity – although not by Myt1 and Wee1, but by Eph, Abl or Fyn. Green arrows indicate positive regulation of Cdk activity, red dashed arrows indicate negative regulation through phosphorylation. Green ovals indicate activating proteins, red ovals or boxes indicate inhibiting proteins.

Journal of Cell Science (2014) 127, 2391–2400 doi:10.1242/jcs.147553

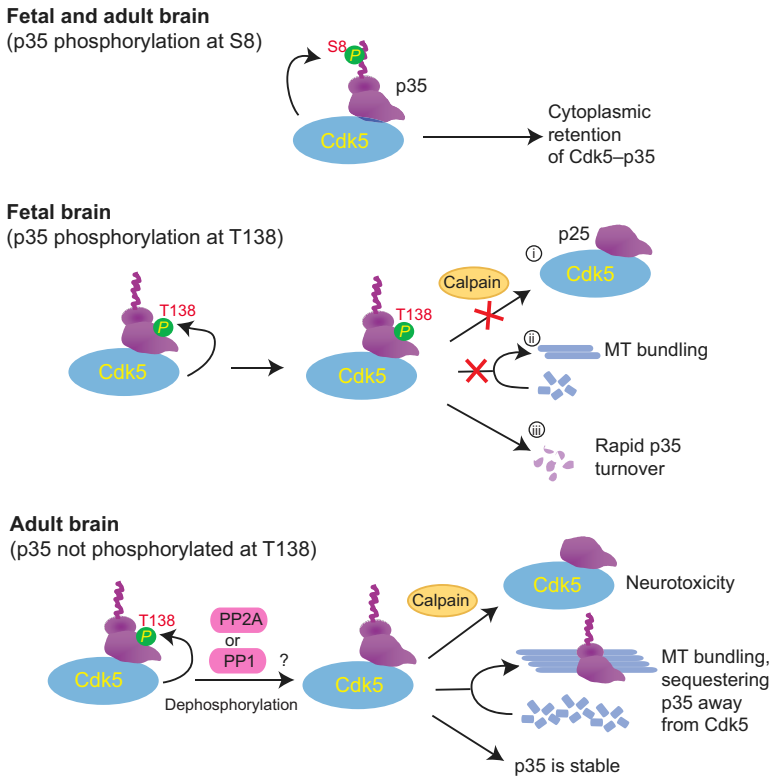

Fig. 2. Regulation of the subcellular localization and stability of p35 in response to Cdk5-mediated phosporylation. (Top) In fetal and adult brains p35 is phosphorylated by Cdk5 at amino acid residue S8, which promotes a cytoplasmic localization of the Cdk5–p35 complex. (Middle) Fetal brain has high levels of p35 phosphorylated at T138 mediated through Cdk5. This has three known consequences: (i) Inhibition of calpain-mediated cleavage of p35 to p25. In fetal brain, neurotoxicity is, thereby, prevented despite a high activity of Cdk5. (ii) Inhibition of the ability of p35 to bind MTs. p35-induced MT bundling and neurite outgrowth in neurons is, thereby, prevented. (iii) The level of degradation of p35 is increased, thereby reducing Cdk5 activity. (Bottom) In adult brain, p35 phosphorylation at T138 is not detected anymore, presumably because this residue becomes dephosphorylated by PP1 or PP2A. p35 that is not phosphorylated at T138 (regardless of S8 phosphorylation) is susceptible to calpain-mediated cleavage, yielding p10 and p25, which then leads to the formation of the neurotoxic complex Cdk5–p25. Furthermore, p35 not phosphorylated at T138 binds to microtubules (MTs), leading to their bundling and to neurite outgrowth. It also sequesters p35 away from Cdk5, thereby reducing its activity. Moreover, p35 not phosphorylated at T138 has a half-life longer than its phosphorylated counterpart at T138.

deregulation of Cdk5 through increased formation of p25 (Sato et al., 2011).

Although a few *in vitro* studies have shown that the complex between Cdk5 and p25 displays a higher activity than that between Cdk5 and p35, other studies have reported comparable activities (Peterson et al., 2010). The relative catalytic activities of the Cdk5–p35 and Cdk5–p25 complexes *in vivo* are not known. Nevertheless, because p25 is resistant to ubiquitin-mediated proteolysis and has a fivefold longer half-life than p35 (Patrick et al., 1999), it substantially extends the activation period of Cdk5, leading to hyperphosphorylation of its normal physiological targets, which is detrimental to the cells. For example, in developing axons, tau is phosphorylated by Cdk5–p39, but not by Cdk5–p35, resulting in neuritic growth in a region-specific manner that is regulated during development (Takahashi et al., 2003). However, under neurotoxic conditions, Cdk5–p25 hyperphosphorylates tau, which aggregates to form neurofibrillary tangles in AD. Likewise, under physiological conditions, Cdk5 phosphorylates neurofilament heavy chain (NF-H) at its tail domain on Lys-Ser-Pro motifs. This promotes the assembly of neurofilaments in the axon, resulting in axonal support and neurite outgrowth while reducing axonal transport (Shea et al., 2004). However, in several neurological disorders, they are hyperphosphorylated and aggregate in cell bodies. Several kinases including deregulated GSK3B, PKA, MAPK, CaMK2 or Cdk5–p25 are known to substantially contribute to aberrant phosphorylation of NFs in diseased neurons (Shea et al., 2004). Thus, owing to the prolonged half-life of p25 compared with p35, the Cdk5–p25 complex hyperphosphorylates its physiological targets leading to neuronal toxicity.

Recent studies have further highlighted that Cdk5–p35 and Cdk5–p25 complexes display both overlapping and distinct subcellular distribution. Whereas the Cdk5–p35 complex mainly localizes to the perinuclear region and at the plasma membrane with less distribution in the nucleus (Fu et al., 2006; Asada et al., 2008), the Cdk5–p25 complex is largely cytosolic and nuclear. Thus, the mislocalization of the Cdk5–p25 complex to the cytoplasm and the nucleus is considered to be another crucial factor that leads to Cdk5-induced neurotoxicity. For example, the mislocalization allows the access of Cdk5–p25 to a variety of new targets, e.g. GM130, peroxiredoxin 1, peroxiredoxin 2, lamin A, lamin B1, Cdc25A, Cdc25B and Cdc25C, which causes neurotoxicity (Chang et al., 2010; Chang et al., 2011; Chang et al., 2012; Sun et al., 2008a; Sun et al., 2008b; Sun et al., 2009). The Cdk5-p25 complex causes aberrant Golgi fragmentation by phosphorylating GM130 and promotes oxidative stress by phosphorylating peroxiredoxins (Sun et al., 2008a; Sun et al., 2008b). Cdk5–p25 triggers dispersion of the nuclear envelop by phosphorylating lamins, and promotes aberrant activation of the cell cycle by phosphorylating Cdc25A, Cdc25B and Cdc25C (Chang et al., 2011; Chang et al., 2012). Thus, the Cdk5–p25 complex is believed to cause neurotoxicity, both through hyperphosphorylation of its physiological targets and phosphorylation of non-physiological targets.

Interestingly, p10, the other cleavage product of p35, was assumed to have no function, as it is rapidly degraded by ubiquitylation. However, a recent study showed that p10 possesses a unique pro-survival domain that is essential for the physiological functions of Cdk5–p35 in neurons. Indeed, loss of the p10 domain results in Cdk5–p25-mediated toxicity and neurodegeneration *in vivo* (Zhang et al., 2012). These findings suggest that p10 imparts a distinct substrate specificity to the Cdk5–p35 complex compared with Cdk5–p25. More interestingly, ectopic expression of p10 alone (without the p25 domain) can protect neurons from Cdk5–p25-induced toxicity; however, p10 does so without any association with the Cdk5–p25

complex. This finding suggests that other cellular factors work with p10 in inhibiting Cdk5–p25-mediated neurotoxicity, but future studies are needed to unravel this mechanism. Notably, the pro-survival function of p10 augurs well for developing interventions against deregulated Cdk5-mediated signaling pathways.

p39 and p29

p39 is an isoform of p35 and another neuron-specific activator of Cdk5 (Fig. 1), but the pattern of p39 expression is distinct from that of p35. Whereas p35 is highly expressed in the cerebral cortex of developing brains, p39 is prominently expressed in embryonic and adult hind brains, and the postnatal cerebral cortex (Zheng et al., 1998; Jeong et al., 2003; Takahashi et al., 2003). Genetic studies have shown that, although functions of p39 largely overlap with those of p35, it also has several unique functions. $Cdk5^{-/-}$ mice display perinatal lethality with extensive defects in several brain regions (Ohshima et al., 1996), whereas p39 deficiency does not result in any apparent abnormalities. However, p35 and p39 double-knockout mice display a phenotype that is identical to that of $Cdk5^{-/-}$ mice (Ko et al., 2001), highlighting p35 and p39 as the main Cdk5 activators in the brain.

Similar to p35, p39 possesses a N-terminal myristoylation site, although p39 has a longer half-life than p35 (Minegishi et al., 2010). p39 is also cleaved by calpain to yield p10 and p29 under neurotoxic conditions (Patzke and Tsai, 2002). p39 contains a small insertion at the C-terminus (amino acids 329–366 – that is absent in p35) – which binds the intracellular protein muskelin, recruiting it to the cell periphery where it regulates cell adhesion and cytoskeletal organization (Ledee et al., 2005). Muskelin also facilitates transport of the GABA receptor along actin and MT networks through different motor protein complexes (Heisler et al., 2011), suggesting that p39-mediated recruitment of muskelin at the cell periphery is essential for its physiological function.

In vitro, the Cdk5–p39 complex displays a similar enzymatic activity and substrate specificity to that of the Cdk5–p35; however, compared with Cdk5–p35 signaling, little is known about the activity of Cdk5–p39 *in vivo*, presumably because it has been difficult to isolate an active Cdk5–p39 complex. The catalytic activity of Cdk5–p39 is instantly abolished when it is extracted from the cell membrane (Ko et al., 2001). It has also proven challenging to reconstitute Cdk5–p39 *in vitro*, as the detergent that can stabilize Cdk5–p35 destabilizes the Cdk5–p39 complex (Yamada et al., 2007). Similar to p35, Cdk5 also regulates the cytoplasmic retention of p39 by direct phosphorylation of p39 at S8 (Asada et al., 2012). Likewise, Cdk5 also phosphorylates p39 at S173 (equivalent to T138 for p35; Fig. 2); however, whether this phosphorylation regulates p39 stability has not been analyzed. In addition, Cdk5 phosphorylates p39 at T84, a unique site not shared by p35. Compared with p35, p39 has a higher tendency to accumulate in the nucleus, which is inhibited by Cdk5 through phosphorylation of T84 (regardless of whether S8 is phosphorylated or not) (Asada et al., 2012). These results suggest that the subcellular localization of the different Cdk5-activator complexes is determined by the kinase activity of Cdk5.

Cyclin D

Initial studies showed that cyclin D1 and cyclin D3 associate with Cdk5 in human diploid fibroblasts, but have no influence on its activity (Xiong et al., 1992; Bates et al., 1994). However, Guidato et al. reported that cyclin D2 modulates the activity of the Cdk5–p35 complex, although the specific mechanism was not investigated in this study (Guidato et al., 1998). In agreement, a recent study revealed that cyclin D1 modulates Cdk5–p35 activity, possibly by competing with p35 binding to Cdk5 (Modi et al., 2012). Importantly, unlike cyclin D2, cyclin D1 is expressed at high levels in adult mouse brain, particularly in hippocampal pyramidal neurons (De Falco et al., 2004). Cyclin D1 can be further upregulated upon exposure to Aβ (Modi et al., 2012), suggesting that similar to cyclin E, it might be a physiologically or pathologically relevant inhibitor of Cdk5 (Fig. 1). A recent work uncovered a role for cyclin D2 as a cardiogenic coactivator of GATA4 and suggests a paradigm for cell-specific effects of cyclin D proteins (Yamak et al., 2014).

Cyclin E

Cyclin E is normally expressed in proliferating cells, where it binds and activates Cdk2 (Koff et al., 1991). The Cdk2-cyclin E complex is critical for G1/S transition of the cell cycle and DNA synthesis (Tsai et al., 1993). Recent studies have shown that cyclin E is also highly expressed in adult brain where it forms a complex with Cdk5 (Odajima et al., 2011). Interestingly, instead of activating Cdk5, the binding of cyclin E inactivates Cdk5 and sequesters it away from its normal activators p35 and p39 (Odajima et al., 2011). Moreover, cyclin-E-mediated sequestering of Cdk5 promotes dendritic growth, synaptic plasticity and memory formation in transgenic animal models, suggesting that cyclin-E-mediated inhibition of Cdk5 is physiologically relevant (Fig. 1).

Cyclin I

Cyclin I was initially isolated from human forebrain; however, because cyclin I failed to associate with any of the classic Cdks, its function was not investigated at the time (Nakamura et al., 1995). Recently, reduced Cdk5 activity has been observed in cyclin-I-deficient mice (Brinkkoetter et al., 2009). The authors further noticed that the Cdk5–cyclin-I complex predominantly exists in post-mitotic cells (e.g. neurons and podocytes). However, a recent study reported that cyclin I is also involved in cell cycle progression (Nagano et al., 2013).

In post-mitotic cells, the Cdk5–cyclin-I complex induces cell survival through transcription of the anti-apoptotic proteins B-cell lymphoma 2 (Bcl2) and B-cell lymphoma-extra large (Bcl-XL; officially known as BCL2L1) (Brinkkoetter et al., 2009; Brinkkoetter et al., 2010). In contrast, the Cdk5–p35 complex does not induce the transcription of Bcl2 and Bcl-XL, suggesting that cyclin I binding either recruits Cdk5 to different pool of substrates or alters it substrate specificity. Future studies are needed to evaluate this mechanism. Likewise, it will be interesting to see whether an alteration in cyclin I levels contributes to the Cdk5 deregulation observed in neurodegenerative diseases. A potential decrease in cyclin I levels might allow Cdk5 to bind other activators (such as p35 or p25), thereby resulting in a certain phenotype and vice versa.

GSTP1

The aberrant formation of p25 in neurodegenerative neurons is regarded as the primary mechanism that leads to the deregulation of Cdk5 in neurological disorders (Patrick et al., 1999; Tseng et al., 2002; Cruz et al., 2003). However, several research groups have reported unaltered or slightly reduced levels of p25 in AD patients when compared with age-matched unaffected individuals (Taniguchi et al., 2001; Yoo and Lubec, 2001; Tandon et al., 2003), suggesting that other regulatory factors for Cdk5 exist or

that there are alternative molecular mechanisms for the deregulation of Cdk5.

Recently, we have shown that glutathione-S-transferase P (GSTP1) is a regulator of Cdk5 in neuronal cell lines, cancer cell lines and AD brains (Sun et al., 2011; Fig. 1). GSTP1 is a detoxifying enzyme that eliminates free radicals by using the reduced glutathione motif. GSTP1 is widely expressed, with especially high levels in the brain (Berhane et al., 1994). GSTP1 activity and levels are reduced in AD neurons, suggesting that GSTP1 downregulation acts as a trigger that promotes oxidative stress and causes neurodegeneration in AD (Lovell et al., 1998). Our study revealed that GSTP1 inhibits the activity of Cdk5 through two mechanisms. First, it competes with p35 and p25 for Cdk5 binding. Second, GSTP1 scavenges molecules associated with oxidative stress, a potent activator of Cdk5 activity. Most importantly, we observed an inverse correlation between GSTP1 levels and the activity of Cdk5 in various cancers, as well as in neuronal cell lines and AD brains, suggesting that GSTP1 is a common regulator of Cdk5 that inhibits its activity in different tissues (Sun et al., 2011).

Transcriptional regulation

Transcriptional regulation of p35

Transcription of p35 is promoted by several stimuli, including nerve growth factor (NGF) (Harada et al., 2001), brain-derived neurotrophic factor (BDNF) (Lim et al., 2008), retinoic acid (Lee and Kim, 2004), interferon γ (IFNG) (Song et al., 2005), 1,25-dihydroxyvitamin D3 (Chen et al., 2004), tumor necrosis factor alpha (TNFα) (Utreras et al., 2009) and laminin (Paglini et al., 1998). These factors activate extracellular regulated MAP kinases 1 and 2 (MAPK1 and MAPK3, hereafter referred to as Erk1/2) signaling, which upregulate p35 gene expression through the participation of the transcription factor early growth response protein 1 (EGR1) (Fig. 3). Fas engagement also activates Erk1/2 signaling leading to p35 transcription in dorsal root ganglia; however, whether this is due to EGR1 upregulation is not known (Desbarats et al., 2003).

Apart from stimulating the Erk1/2–EGR1 pathway, which increases p35 transcription, TNFα also activates the p38 MAPK (MAPK14), Jun N-terminal kinase (JNK) and NF-κB pathways, all of which inhibit the transcription of p35 (Utreras et al., 2009) (Fig. 3). In contrast, BDNF stimulates the Erk1/2–EGR1 pathway to increase p35 transcription, as well as the phosphoinositide-3 kinase (PI3K) pathway to increase p35 protein levels in striatal neurons (Bogush et al., 2007) (Fig. 3). It is not known whether P13K-mediated increase in p35 levels is at transcriptional or post-translational level (Bogush et al., 2007) (Fig. 3).

The transcription factors Brn-1 and Brn-2 (POU3F3 and POU3F2, respectively) also participate in the gene expression of p35 and p39. Similar to *Cdk5*-knockout mice, Brn-1 and Brn-2 deficient mice exhibit severe cortical lamination defects (McEvilly et al., 2002). These mice lack p35 and p39 mRNAs in migrating neurons of the cortex and hippocampus, thereby strongly indicating that these transcription factors regulate the transcription of p35 and p39 (Fig. 3). In addition, heat shock factor Hsf2 binds to the p35 promoter increasing p35 expression (Chang et al., 2006). Accordingly, the cerebral cortex of $Hsf2^{-/-}$ mice displays a mispositioning of neurons within superficial layers, a phenotype that is consistent with p35 deficiency (Chang et al., 2006).

Transcriptional regulation of *Cdk5*

Expression of *Cdk5* is also upregulated through the Erk1/2 pathway but is mediated by the transcription factors Fos and cAMP-responsive element binding protein (CREB) (Lee and Kim, 2004) (Fig. 4). Accordingly, inhibition of Erk1/2 prevents retinoic-acid-induced expression of *Cdk5*, whereas constitutively active MEK1 induces *Cdk5* expression in neuroblastoma cells (Lee and Kim, 2004). Interestingly, Cdk5 also inhibits the gene expression of p35 though a feedback mechanism that downregulates activation of Erk1. Here, Cdk5 directly phosphorylates MEK1 (MAP2K1) – the

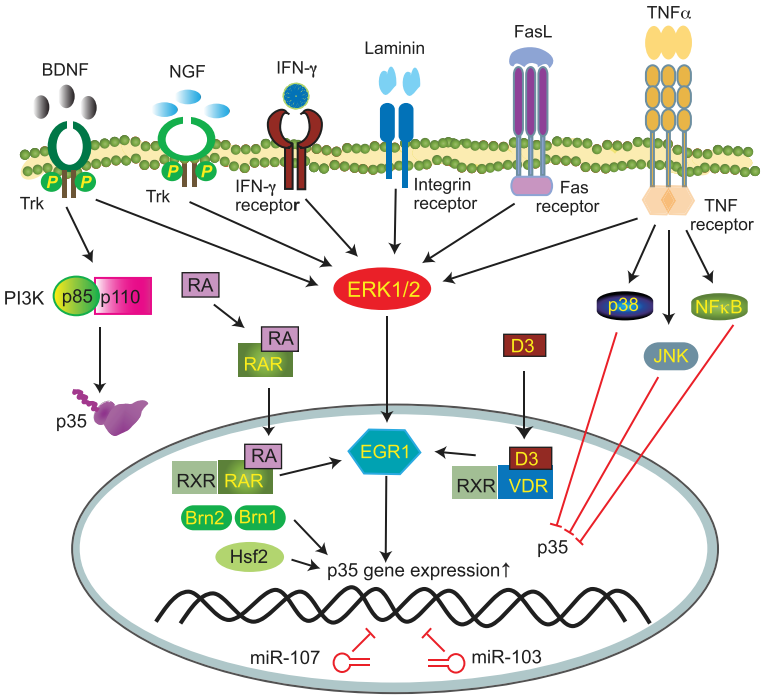

Fig. 3. Transcriptional regulation of p35. Several stimuli, including BDNF, NGF, interferon-γ (IFN-γ), laminin, retinoic acid (RA), 1,25-dihydroxyvitamin D3 (D3) and TNFα, activate Erk1/2, which upregulate the transcription of p35 through the transcription factor EGR1. FasL engagement also increases the transcription of p35 through the Erk1/2 pathway, but it is unknown whether this occurs through upregulation of EGR1. In addition to the Erk1/2 pathway, BDNF also activates the PI3K pathway leading to increased levels of p35 protein, although it is unclear whether this is mediated at mRNA or protein level. TNFα increases transcription of p35 through the Erk1/2 pathway, but inhibits it through the activation of the p38, JNK and NFκB pathways. miR-103 and miR-107 inhibit the expression of p35 post-transcriptionally. RAR, retinoic acid receptor; VDR, vitamin D3 receptor; RXR, retinoid X receptor.

Journal of Cell Science (2014) 127, 2391–2400 doi:10.1242/jcs.147553

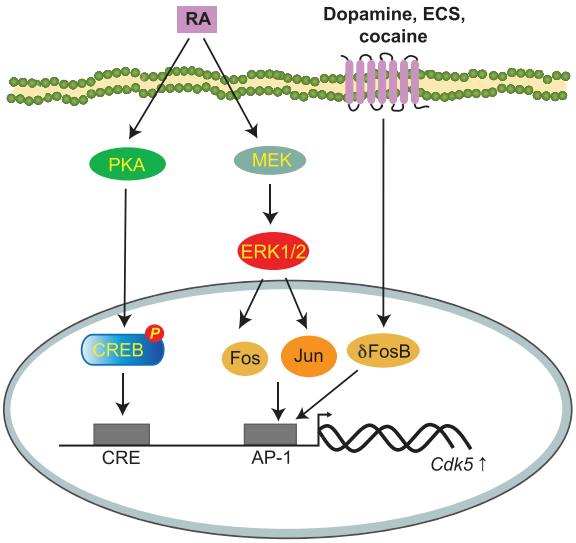

Fig. 4. Transcriptional regulation of Cdk5. During neuronal differentiation, retinoic acid (RA) increases transcription of *Cdk5* through at least two mechanisms: (1) RA-mediated increase of ERK1/2 activity leads to upregulation of the transcriptional factors Fos and Jun; they then bind to the AP-1-binding site within the *Cdk5* promoter, thus inducing transcription of *Cdk5*. (2) Treatment with RA increases PKA activity, resulting in phosphorylation of CREB, which, in turn, results in its binding to the cAMP response element (CRE) at the *Cdk5* promoter inducing *Cdk5* expression. Moreover, Cdk5 expression is also enhanced in response to chronic cocaine use and electroconvulsive seizures (ECS) through the transcription factor δFosB.

upstream activator of Erk1 – and represses its activity, leading to repression of p35 transcription (Sharma et al., 2002).

Transcription of *Cdk5* is also stimulated by δFosB, a *FosB* gene product and a member of the Jun family of transcriptional factors. δFosB-overexpressing transgenic mice have increased levels of Cdk5 mRNA and protein (Chen et al., 2000; Bibb et al., 2001). Dopamine, cocaine and electroconvulsive seizures, all of which increase δFosB levels promote the transcription of *Cdk5* (Fig. 4).

Post-transcriptional regulation of p35 and Cdk5

MicroRNAs (miRNAs) are emerging as an important regulator for various pathways and have been shown to be involved in several CNS diseases, including neurodegenerative disorders (Long and Lahiri, 2012). For example, several miRNA species, such as miR-101, miR-153 and miR-339-5p are dysregulated in AD (Long and Lahiri, 2011a; Long and Lahiri, 2011b; Long et al., 2012; Long et al., 2014). In this context, p35 expression can be post-transcriptionally suppressed by miRNAs 103 and 107 (miR-103 and miR-107, respectively) (Moncini et al., 2011). miR-103 and miR-107 directly bind to 3′UTR of p35 and repress its translation (Fig. 3). Other miRNAs, such as miR-26b, participate in nuclear export and activation of Cdk5 (Absalon et al., 2013). Thus, the regulation of Cdk5 activity by specific miRNAs might be important both under physiological and pathological conditions.

Post-translational regulation of Cdk5

Although binding to a protein partner is obligatory for Cdk5 activation, its activity can be further tweaked by phosphorylation or S-nitrosylation.

Phosphorylation

Cdk5 possesses three conserved phosphorylation sites that are common among the Cdk family: Thr14, Tyr15 and Ser159. But the mode of Cdk5 activation differs from that of its family members. The kinases Wee1 and Myt inhibit the activity of other Cdks by dual phosphorylation at, for example, Thr14 and Tyr15 in the case of Cdk1; however, these kinases are unable to phosphorylate Cdk5 at either position (Fattaey and Booher, 1997) (Fig. 1). Instead, the kinases Abl, ephrin receptor A (EphA) and Fyn phosphorylate Cdk5 at Tyr15, leading to its activation (Fig. 1), thereby promoting neuronal migration, neurite outgrowth and synaptogenesis (Zukerberg et al., 2000; Sasaki et al., 2002; Cheng et al., 2003). Abl-mediated phosphorylation of Tyr15 can also hyperactivate Cdk5, resulting in neurodegeneration (Lin et al., 2007; Cancino et al., 2011).

Another difference between Cdk5 and other Cdks is the effect of Cdk7 on the latter. Cdk7 is known to phosphorylate Cdks in the T loop, e.g. Thr160 in Cdk2 and Thr161 in Cdk1, which strongly activates them (e.g. a 200-fold activation of Cdk2) (Fig. 1). However, Cdk7 is unable to phosphorylate the corresponding Ser159 residue in Cdk5 (Poon et al., 1997). It remains unclear whether Cdk5 is phosphorylated at Ser159 in cells; however, the corresponding phospho-mimetic Cdk5 mutant (S159E) is defective in binding p35, suggesting that Cdk5 phosphorylation at this site inhibits its activity (Tarricone et al., 2001).

S-Nitrosylation

Cdk5 is S-nitrosylated at Cys83 and Cys157 when treated with S-nitrosocysteine or S-nitrosoglutathione *in vitro* (Qu et al., 2011). Interestingly, nitrosylating agents at low concentration (200 µM) augment the activity of Cdk5 (Qu et al., 2011), whereas they inhibit it at high concentration (1 mM) (Zhang et al., 2010). In neurons, Cdk5 is S-nitrosylated by neuronal nitric oxide synthase (NOS1), which in turn transnitrosylates the mitochondrial fission protein Drp1, leading to mitochondrial fission in dendritic spines (Qu et al., 2011). Thus, S-nitrosylated Cdk5 not only has higher kinase activity but also acts as a nascent transnitrosylase. Importantly, S-nitrosylation of Cdk5 is enhanced in the presence of Aβ and N-methyl-D-aspartate (NMDA) (Qu et al., 2011; Qu et al., 2012), suggesting that deregulated Cdk5 also induces neurotoxicity through aberrant S-nitrosylation reactions under pathological conditions.

Cdk5 in the brain

Cdk5 and synaptic plasticity

Notably, transgenic animal models have revealed contrasting roles of Cdk5 in learning and the formation of memory. A positive role of Cdk5 in promoting synaptic plasticity was identified in $p35^{-/-}$ mice, which display depotentiation – the erasure of long-term potentiation (LTP) – and the defective induction of long-term depression (LTD) (Ohshima et al., 2005). Likewise, Cdk5 is transiently upregulated in mice that are exposed to stress and facilitates context-dependent fear conditioning (Fischer et al., 2002). Similarly, Cdk5f/f/T29 mice – in which Cdk5 is selectively ablated in the CA1 pyramidal neurons of the hippocampus – revealed impaired memory function and synaptic plasticity (Guan et al., 2011).

In contrast, Hawasli et al. demonstrated that the initial loss of Cdk5 in Cdk5 conditional knockout mice results in enhanced LTP, NMDA-receptor-mediated synaptic plasticity and improved performance in hippocampal behavioral learning tasks, which highlights a negative role of Cdk5 in learning and memory

formation (Hawasli et al., 2007). Likewise, tau-tubulin kinase-1 (TTBK1)-overexpressing transgenic mice, which display elevated Cdk5 activity, expedite calpain-mediated degradation of the NMDA receptor subtype 2B (NR2B), causing learning deficits (Sato et al., 2008). Taken together, these findings imply that Cdk5 not only promotes LTP and LTD, but also counteracts changes induced by LTP and LTD to maintain neuronal network stability, thereby functioning as a homeostatic regulator of synaptic plasticity.

Cdk5 and pain

Cdk5 facilitates heat hyperalgesia-associated inflammatory pain in the dorsal root ganglion (DRG) of mice and rats (Pareek et al., 2006; Pareek et al., 2007; Yang et al., 2007). Cdk5 and p35 are abundantly expressed in the DRG, trigeminal ganglion and spinal cord. Their expression further increases during peripheral inflammatory response, followed by subsequent cleavage of p35 to p25, leading to hyperactivation of Cdk5 (Pareek et al., 2006). In agreement with this molecular pathway, p35-knockout mice display pain hyposensitivity to the thermal stimuli when compared with wild-type mice, whereas overexpression of p35 has the opposite effect (Pareek et al., 2006). Similarly, Cdk5 conditional knockout mice display palpable hypoalgesia (Pareek et al., 2007).

Cdk5 and drug addiction

Cocaine enhances synaptic dopamine levels in the striatum by inhibiting dopamine re-uptake at axon terminals. An acute dose of cocaine increases dopamine levels, which activates PKA. PKA phosphorylates protein phosphatase 1 regulatory subunit 1B (DARPP-32) at residue T34, which potentiates dopamine signaling (Nishi et al., 2000). In contrast, chronic exposure of cocaine upregulates mRNA levels of *Cdk5* and p35 in reward-associated brain regions by persistent upregulation of the transcription factor δFosB (Bibb et al., 2001; Kelz and Nestler, 2000; Fig. 4). Increase in Cdk5 activity and protein levels results in elevated levels of DARPP-32 phosphorylated at T75 and inhibition of PKA activity, resulting in depotentiation of dopamine signaling (Bibb. et al., 2001). Cdk5, thus, elicits an adaptive homeostatic response that irreversibly dedicates the affected neurons to a process of cytoarchitectural changes, leading to addiction.

Cdk5 in neurological diseases

Significantly higher activity of Cdk5 has been observed in brains of AD patients compared with non-demented individuals (Lee et al., 1999). In a mouse model, intracerebroventricular injection of the Aβ(1–40) peptide hyperactivates Cdk5 because of the formation of p25, and causes tau hyperphosphorylation, aberrant cell cycle induction, synaptotoxicity and neuronal loss (Lopes et al., 2010). Transgenic mice that express tetracycline-inducible p25 show substantial brain atrophy, synaptic dysfunction and NFT formation (Cruz et al., 2003). Wen et al. further showed that overexpression of p25 increases Cdk5-mediated transcription of BACE1, leading to aberrant Aβ processing (Wen et al., 2008). Therefore, Cdk5 inhibitors might prevent Aβ and neurofibrillary pathology and synaptic loss in AD patients (Monaco, 2004; Glicksman et al., 2007).

In mouse models of PD, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treatment results in higher levels of the Cdk5–p25 complex in dopaminergic neurons, leading to neuronal death (Smith et al., 2003). MPTP treatment destroys dopaminergic neurons in the substantia nigra of the brain, causing irreversible symptoms of PD. Cdk5 also induces autophagy in the PD model through the phosphorylation of endophilin B1 (Wong et al., 2011). Deregulation of autophagy has been linked to PD pathogenesis, and autophagosome accumulation occurs in the brains of both PD patients and PD mouse models (Anglade et al., 1997; Zhu et al., 2003).

In stroke-affected patients, levels of phosphorylated Cdk5, p35 and p25 are substantially increased in the affected but not in healthy neurons, suggesting a link between Cdk5 and the pathogenesis of ischemia (Mitsios et al., 2007). Cdk5 inhibition confers neuroprotection in *in vivo* models of ischemia and stroke, underscoring its crucial role in disease progression (Menn et al., 2010). In the case of HD, Cdk5 deregulation is linked to striatal neuronal death (Paoletti et al., 2008).

Cdk5 as a drug target

Based on these different roles of Cdk5 in the brain, Cdk5 is considered an attractive drug target in order to treat multiple neurological disorders that involve learning and memory deficits, and defective motor functions. Although there are several known high-potency Cdk5 inhibitors, they concurrently show poor selectivity and specificity because they inhibit other Cdks with equal or higher potency. As a result, despite their potential, Cdk5 inhibitors have – owing to serious side effect – thus far not been successful in clinical trials. Given the pivotal role of Cdk5 in the brain, it would be beneficial to develop Cdk5-specific inhibitors, or engineer inhibitors that selectively abrogate the interaction of Cdk5 with p25 but not with p35. In this respect, the finding regarding the pro-survival function of p10 (the cleaved product of p35) is encouraging and can potentially be used to selectively abrogate Cdk5–p25-mediated neurotoxicity (Zhang et al., 2012). Likewise, Cdk5 inhibitory peptide (CIP), a 125 amino acid fragment of p25 (Zheng et al., 2002), effectively and specifically inhibits the hyperactivation of Cdk5–p25 *in vivo* (Sundaram et al., 2013). In CIP-p25-transgenic mice, expression of CIP rescues the neurodegenerative pathologies caused by deregulation of Cdk5–p25 without compromising the normal neurodevelopment. Such an approach would allow to retain the physiological functions of Cdk5 while putting a brake on its neurotoxic behavior.

Conclusions

Research over the past 25 years has not only uncovered numerous physiological and pathological roles of Cdk5, but also revealed an increasing number of Cdk5 regulators. Whereas earlier studies mainly focused on p35 and p39 as the predominant regulators of Cdk5 in the CNS, emerging studies have identified multiple mechanisms that regulate Cdk5 at transcriptional, post-transcriptional and post-translational levels. Recent studies have also identified several so far unknown protein regulators of Cdk5, including cyclin D1, cyclin E, cyclin I and GSTP1. Whereas cyclin D1, cyclin E and GSTP1 inhibit Cdk5 activity, cyclin I is an activator of Cdk5. A retrospective analysis of these modulators in normal and diseased tissue specimens might uncover alternate modes of Cdk5 deregulation in various neurological disorders. It is also likely that many more of Cdk5 regulators exist, which might affect Cdk5 localization, substrate specificity, activity and levels. Targeting these regulators might provide an alternative strategy for the development of effective therapeutic interventions that arise from deregulation of Cdk5 in conditions such as AD, HD, PD, ischemia and stroke, frontotemporal dementia, amyotrophic lateral sclerosis and Niemann-Pick disease type C.

Competing interests
The authors declare no competing interests.

Funding
We sincerely appreciate the grant support from the National Institute on Aging, National Institutes of Health to K.S. and D.K.L., and the Alzheimer's Association (IIRG) and CTSI- Indiana University to D.K.L. Deposited in PMC for release after 12 months.

References

Absalon, S., Kochanek, D. M., Raghavan, V. and Krichevsky, A. M. (2013). MiR-26b, upregulated in Alzheimer's disease, activates cell cycle entry, tau-phosphorylation, and apoptosis in postmitotic neurons. *J. Neurosci.* **33**, 14645-14659.

Anglade, P., Vyas, S., Javoy-Agid, F., Herrero, M. T., Michel, P. P., Marquez, J., Mouatt-Prigent, A., Ruberg, M., Hirsch, E. C. and Agid, Y. (1997). Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. *Histol. Histopathol.* **12**, 25-31.

Asada, A., Yamamoto, N., Gohda, M., Saito, T., Hayashi, N. and Hisanaga, S. (2008). Myristoylation of p39 and p35 is a determinant of cytoplasmic or nuclear localization of active cyclin-dependent kinase 5 complexes. *J. Neurochem.* **106**, 1325-1336.

Asada, A., Saito, T. and Hisanaga, S. (2012). Phosphorylation of p35 and p39 by Cdk5 determines the subcellular location of the holokinase in a phosphorylation-site-specific manner. *J. Cell Sci.* **125**, 3421-3429.

Bates, S., Bonetta, L., MacAllan, D., Parry, D., Holder, A., Dickson, C. and Peters, G. (1994). CDK6 (PLSTIRE) and CDK4 (PSK-J3) are a distinct subset of the cyclin-dependent kinases that associate with cyclin D1. *Oncogene* **9**, 71-79.

Berhane, K., Widersten, M., Engström, A., Kozarich, J. W. and Mannervik, B. (1994). Detoxication of base propenals and other alpha, beta-unsaturated aldehyde products of radical reactions and lipid peroxidation by human glutathione transferases. *Proc. Natl. Acad. Sci. USA* **91**, 1480-1484.

Bibb, J. A., Chen, J., Taylor, J. R., Svenningsson, P., Nishi, A., Snyder, G. L., Yan, Z., Sagawa, Z. K., Ouimet, C. C., Nairn, A. C. et al. (2001). Effects of chronic exposure to cocaine are regulated by the neuronal protein Cdk5. *Nature* **410**, 376-380.

Bogush, A., Pedrini, S., Pelta-Heller, J., Chan, T., Yang, Q., Mao, Z., Sluzas, E., Gieringer, T. and Ehrlich, M. E. (2007). AKT and CDK5/p35 mediate brain-derived neurotrophic factor induction of DARPP-32 in medium size spiny neurons in vitro. *J. Biol. Chem.* **282**, 7352-7359.

Brinkkoetter, P. T., Olivier, P., Wu, J. S., Henderson, S., Krofft, R. D., Pippin, J. W., Hockenbery, D., Roberts, J. M. and Shankland, S. J. (2009). Cyclin I activates Cdk5 and regulates expression of Bcl-2 and Bcl-XL in postmitotic mouse cells. *J. Clin. Invest.* **119**, 3089-3101.

Brinkkoetter, P. T., Pippin, J. W. and Shankland, S. J. (2010). Cyclin I-Cdk5 governs survival in post-mitotic cells. *Cell Cycle* **9**, 1729-1731.

Cancino, G. I., Perez de Arce, K., Castro, P. U., Toledo, E. M., von Bernhardi, R. and Alvarez, A. R. (2011). c-Abl tyrosine kinase modulates tau pathology and Cdk5 phosphorylation in AD transgenic mice. *Neurobiol. Aging* **32**, 1249-1261.

Chang, Y., Ostling, P., Akerfelt, M., Trouillet, D., Rallu, M., Gitton, Y., El Fatimy, R., Fardeau, V., Le Crom, S., Morange, M. et al. (2006). Role of heat-shock factor 2 in cerebral cortex formation and as a regulator of p35 expression. *Genes Dev.* **20**, 836-847.

Chang, K. H., de Pablo, Y., Lee, H. P., Lee, H. G., Smith, M. A. and Shah, K. (2010). Cdk5 is a major regulator of p38 cascade: relevance to neurotoxicity in Alzheimer's disease. *J. Neurochem.* **113**, 1221-1229.

Chang, K. H., Multani, P. S., Sun, K. H., Vincent, F., de Pablo, Y., Ghosh, S., Gupta, R., Lee, H. P., Lee, H. G., Smith, M. A. et al. (2011). Nuclear envelope dispersion triggered by deregulated Cdk5 precedes neuronal death. *Mol. Biol. Cell* **22**, 1452-1462.

Chang, K. H., Vincent, F. and Shah, K. (2012). Deregulated Cdk5 triggers aberrant activation of cell cycle kinases and phosphatases inducing neuronal death. *J. Cell Sci.* **125**, 5124-5137.

Chen, J., Zhang, Y., Kelz, M. B., Steffen, C., Ang, E. S., Zeng, L. and Nestler, E. J. (2000). Induction of cyclin-dependent kinase 5 in the hippocampus by chronic electroconvulsive seizures: role of [Delta]FosB. *J. Neurosci.* **20**, 8965-8971.

Chen, F., Wang, Q., Wang, X. and Studzinski, G. P. (2004). Up-regulation of Egr1 by 1,25-dihydroxyvitamin D3 contributes to increased expression of p35 activator of cyclin-dependent kinase 5 and consequent onset of the terminal phase of HL60 cell differentiation. *Cancer Res.* **64**, 5425-5433.

Cheng, Q., Sasaki, Y., Shoji, M., Sugiyama, Y., Tanaka, H., Nakayama, T., Mizuki, N., Nakamura, F., Takei, K. and Goshima, Y. (2003). Cdk5/p35 and Rho-kinase mediate ephrin-A5-induced signaling in retinal ganglion cells. *Mol. Cell. Neurosci.* **24**, 632-645.

Cheung, Z. H. and Ip, N. Y. (2012). Cdk5: a multifaceted kinase in neurodegenerative diseases. *Trends Cell Biol.* **22**, 169-175.

Cruz, J. C., Tseng, H. C., Goldman, J. A., Shih, H. and Tsai, L. H. (2003). Aberrant Cdk5 activation by p25 triggers pathological events leading to neurodegeneration and neurofibrillary tangles. *Neuron* **40**, 471-483.

De Falco, M., Fedele, V., De Luca, L., Penta, R., Cottone, G., Cavallotti, I., Laforgia, V. and De Luca, A. (2004). Evaluation of cyclin D1 expression and its subcellular distribution in mouse tissues. *J. Anat.* **205**, 405-412.

Desbarats, J., Birge, R. B., Mimouni-Rongy, M., Weinstein, D. E., Palerme, J. S. and Newell, M. K. (2003). Fas engagement induces neurite growth through ERK activation and p35 upregulation. *Nat. Cell Biol.* **5**, 118-125.

Dhavan, R. and Tsai, L. H. (2001). A decade of CDK5. *Nat. Rev. Mol. Cell Biol.* **2**, 749-759.

Elledge, S. J. and Spottswood, M. R. (1991). A new human p34 protein kinase, CDK2, identified by complementation of a cdc28 mutation in Saccharomyces cerevisiae, is a homolog of Xenopus Eg1. *EMBO J.* **10**, 2653-2659.

Fattaey, A. and Booher, R. N. (1997). Myt1: a Wee1-type kinase that phosphorylates Cdc2 on residue Thr14. *Prog. Cell Cycle Res.* **3**, 233-240.

Fischer, A., Sananbenesi, F., Schrick, C., Spiess, J. and Radulovic, J. (2002). Cyclin-dependent kinase 5 is required for associative learning. *J. Neurosci.* **22**, 3700-3707.

Fu, X., Choi, Y. K., Qu, D., Yu, Y., Cheung, N. S. and Qi, R. Z. (2006). Identification of nuclear import mechanisms for the neuronal Cdk5 activator. *J. Biol. Chem.* **281**, 39014-39021.

Glicksman, M. A., Cuny, G. D., Liu, M., Dobson, B., Auerbach, K., Stein, R. L. and Kosik, K. S. (2007). New approaches to the discovery of cdk5 inhibitors. *Curr. Alzheimer Res.* **4**, 547-549.

Guan, J. S., Su, S. C., Gao, J., Joseph, N., Xie, Z., Zhou, Y., Durak, O., Zhang, L., Zhu, J. J., Clauser, K. R. et al. (2011). Cdk5 is required for memory function and hippocampal plasticity via the cAMP signaling pathway. *PLoS ONE* **6**, e25735.

Guidato, S., McLoughlin, D. M., Grierson, A. J. and Miller, C. C. (1998). Cyclin D2 interacts with cdk-5 and modulates cellular cdk-5/p35 activity. *J. Neurochem.* **70**, 335-340.

Harada, T., Morooka, T., Ogawa, S. and Nishida, E. (2001). ERK induces p35, a neuron-specific activator of Cdk5, through induction of Egr1. *Nat. Cell Biol.* **3**, 453-459.

Hawasli, A. H., Benavides, D. R., Nguyen, C., Kansy, J. W., Hayashi, K., Chambon, P., Greengard, P., Powell, C. M., Cooper, D. C. and Bibb, J. A. (2007). Cyclin-dependent kinase 5 governs learning and synaptic plasticity via control of NMDAR degradation. *Nat. Neurosci.* **10**, 880-886.

He, L., Hou, Z. and Qi, R. Z. (2008). Calmodulin binding and Cdk5 phosphorylation of p35 regulate its effect on microtubules. *J. Biol. Chem.* **283**, 13252-13260.

Heisler, F. F., Loebrich, S., Pechmann, Y., Maier, N., Zivkovic, A. R., Tokito, M., Hausrat, T. J., Schweizer, M., Bähring, R., Holzbaur, E. L. et al. (2011). Muskelin regulates actin filament- and microtubule-based GABA(A) receptor transport in neurons. *Neuron* **70**, 66-81.

Hellmich, M. R., Pant, H. C., Wada, E. and Battey, J. F. (1992). Neuronal cdc2-like kinase: a cdc2-related protein kinase with predominantly neuronal expression. *Proc. Natl. Acad. Sci. USA* **89**, 10867-10871.

Hisanaga, S. and Endo, R. (2010). Regulation and role of cyclin-dependent kinase activity in neuronal survival and death. *J. Neurochem.* **115**, 1309-1321.

Ishiguro, K., Takamatsu, M., Tomizawa, K., Omori, A., Takahashi, M., Arioka, M., Uchida, T. and Imahori, K. (1992). Tau protein kinase I converts normal tau protein into A68-like component of paired helical filaments. *J. Biol. Chem.* **267**, 10897-10901.

Jeong, Y. G., Rosales, J. L., Marzban, H., Sillitoe, R. V., Park, D. G., Hawkes, R. and Lee, K. Y. (2003). The cyclin-dependent kinase 5 activator, p39, is expressed in stripes in the mouse cerebellum. *Neuroscience* **118**, 323-334.

Kamei, H., Saito, T., Ozawa, M., Fujita, Y., Asada, A., Bibb, J. A., Saido, T. C., Sorimachi, H. and Hisanaga, S. (2007). Suppression of calpain-dependent cleavage of the CDK5 activator p35 to p25 by site-specific phosphorylation. *J. Biol. Chem.* **282**, 1687-1694.

Kelz, M. B. and Nestler, E. J. (2000). deltaFosB: a molecular switch underlying long-term neural plasticity. *Curr. Opin. Neurol.* **13**, 715-720.

Ko, J., Humbert, S., Bronson, R. T., Takahashi, S., Kulkarni, A. B., Li, E. and Tsai, L. H. (2001). p35 and p39 are essential for cyclin-dependent kinase 5 function during neurodevelopment. *J. Neurosci.* **21**, 6758-6771.

Koff, A., Cross, F., Fisher, A., Schumacher, J., Leguellec, K., Philippe, M. and Roberts, J. M. (1991). Human cyclin E, a new cyclin that interacts with two members of the CDC2 gene family. *Cell* **66**, 1217-1228.

Lahiri, D. K. (2011). An integrated approach to genome studies. *Science* **331**, 147.

Lahiri, D. K., Farlow, M. R., Sambamurti, K., Greig, N. H., Giacobini, E. and Schneider, L. S. (2003). A critical analysis of new molecular targets and strategies for drug developments in Alzheimer's disease. *Curr. Drug Targets* **4**, 97-112.

Lahiri, D. K., Maloney, B., Long, J. M. and Greig, N. H. (2014). Lessons from a BACE1 inhibitor trial: Off-site but not off base. *Alzheimers Dement.* [Epub Ahead of Print]

Ledee, D. R., Gao, C. Y., Seth, R., Fariss, R. N., Tripathi, B. K. and Zelenka, P. S. (2005). A specific interaction between muskelin and the cyclin-dependent kinase 5 activator p39 promotes peripheral localization of muskelin. *J. Biol. Chem.* **280**, 21376-21383.

Lee, J. H. and Kim, K. T. (2004). Induction of cyclin-dependent kinase 5 and its activator p35 through the extracellular-signal-regulated kinase and protein kinase A pathways during retinoic-acid mediated neuronal differentiation in human neuroblastoma SK-N-BE(2)C cells. *J. Neurochem.* **91**, 634-647.

Lee, M. G. and Nurse, P. (1987). Complementation used to clone a human homologue of the fission yeast cell cycle control gene cdc2. *Nature* **327**, 31-35.

Lee, K. Y., Clark, A. W., Rosales, J. L., Chapman, K., Fung, T. and Johnston, R. N. (1999). Elevated neuronal Cdc2-like kinase activity in the Alzheimer disease brain. *Neurosci. Res.* **34**, 21-29.

Journal of Cell Science

Lee, M. S., Kwon, Y. T., Li, M., Peng, J., Friedlander, R. M. and Tsai, L. H. (2000). Neurotoxicity induces cleavage of p35 to p25 by calpain. *Nature* **405**, 360-364.
Lew, J., Beaudette, K., Litwin, C. M. and Wang, J. H. (1992). Purification and characterization of a novel proline-directed protein kinase from bovine brain. *J. Biol. Chem.* **267**, 13383-13390.
Lew, J., Huang, Q. Q., Qi, Z., Winkfein, R. J., Aebersold, R., Hunt, T. and Wang, J. H. (1994). A brain-specific activator of cyclin-dependent kinase 5. *Nature* **371**, 423-426.
Lim, J. Y., Park, S. I., Oh, J. H., Kim, S. M., Jeong, C. H., Jun, J. A., Lee, K. S., Oh, W., Lee, J. K. and Jeun, S. S. (2008). Brain-derived neurotrophic factor stimulates the neural differentiation of human umbilical cord blood-derived mesenchymal stem cells and survival of differentiated cells through MAPK/ERK and PI3K/Akt-dependent signaling pathways. *J. Neurosci. Res.* **86**, 2168-2178.
Lin, H., Lin, T. Y. and Juang, J. L. (2007). Abl deregulates Cdk5 kinase activity and subcellular localization in Drosophila neurodegeneration. *Cell Death Differ.* **14**, 607-615.
Long, J. M. and Lahiri, D. K. (2011a). MicroRNA-101 downregulates Alzheimer's amyloid-β precursor protein levels in human cell cultures and is differentially expressed. *Biochem. Biophys. Res. Commun.* **404**, 889-895.
Long, J. M. and Lahiri, D. K. (2011b). Current drug targets for modulating Alzheimer's amyloid precursor protein: role of specific micro-RNA species. *Curr. Med. Chem.* **18**, 3314-3321.
Long, J. M. and Lahiri, D. K. (2012). Advances in microRNA experimental approaches to study physiological regulation of gene products implicated in CNS disorders. *Exp. Neurol.* **235**, 402-418.
Long, J. M., Ray, B. and Lahiri, D. K. (2012). MicroRNA-153 physiologically inhibits expression of amyloid-β precursor protein in cultured human fetal brain cells and is dysregulated in a subset of Alzheimer disease patients. *J. Biol. Chem.* **287**, 31298-31310.
Long, J. M., Ray, B. and Lahiri, D. K. (2014). MicroRNA-339-5p downregulates protein expression of the β-site amyloid precursor protein cleaving enzyme 1 (BACE1) in human primary brain cultures and is reduced in brain tissue specimens of Alzheimer's disease subjects. *J. Biol. Chem.* **289**, 5184-5198.
Lopes, J. P., Oliveira, C. R. and Agostinho, P. (2010). Neurodegeneration in an Abeta-induced model of Alzheimer's disease: the role of Cdk5. *Aging Cell* **9**, 64-77.
Lovell, M. A., Xie, C. and Markesbery, W. R. (1998). Decreased glutathione transferase activity in brain and ventricular fluid in Alzheimer's disease. *Neurology* **51**, 1562-1566.
Malumbres, M., Harlow, E., Hunt, T., Hunter, T., Lahti, J. M., Manning, G., Morgan, D. O., Tsai, L. H. and Wolgemuth, D. J. (2009). Cyclin-dependent kinases: a family portrait. *Nat. Cell Biol.* **11**, 1275-1276.
McEvilly, R. J., de Diaz, M. O., Schonemann, M. D., Hooshmand, F. and Rosenfeld, M. G. (2002). Transcriptional regulation of cortical neuron migration by POU domain factors. *Science* **295**, 1528-1532.
Menn, B., Bach, S., Blevins, T. L., Campbell, M., Meijer, L. and Timsit, S. (2010). Delayed treatment with systemic (S)-roscovitine provides neuroprotection and inhibits in vivo CDK5 activity increase in animal stroke models. *PLoS ONE* **5**, e12117.
Meyerson, M., Enders, G. H., Wu, C. L., Su, L. K., Gorka, C., Nelson, C., Harlow, E. and Tsai, L. H. (1992). A family of human cdc2-related protein kinases. *EMBO J.* **11**, 2909-2917.
Minegishi, S., Asada, A., Miyauchi, S., Fuchigami, T., Saito, T. and Hisanaga, S. (2010). Membrane association facilitates degradation and cleavage of the cyclin-dependent kinase 5 activators p35 and p39. *Biochemistry* **49**, 5482-5493.
Mitsios, N., Pennucci, R., Krupinski, J., Sanfeliu, C., Gaffney, J., Kumar, P., Kumar, S., Juan-Babot, O. and Slevin, M. (2007). Expression of cyclin-dependent kinase 5 mRNA and protein in the human brain following acute ischemic stroke. *Brain Pathol.* **17**, 11-23.
Modi, P. K., Komaravelli, N., Singh, N. and Sharma, P. (2012). Interplay between MEK-ERK signaling, cyclin D1, and cyclin-dependent kinase 5 regulates cell cycle reentry and apoptosis of neurons. *Mol. Biol. Cell* **23**, 3722-3730.
Monaco, E. A., III (2004). Recent evidence regarding a role for Cdk5 dysregulation in Alzheimer's disease. *Curr. Alzheimer Res.* **1**, 33-38.
Moncini, S., Salvi, A., Zuccotti, P., Viero, G., Quattrone, A., Barlati, S., De Petro, G., Venturin, M. and Riva, P. (2011). The role of miR-103 and miR-107 in regulation of CDK5R1 expression and in cellular migration. *PLoS ONE* **6**, e20038.
Nagano, T., Hashimoto, T., Nakashima, A., Hisanaga, S., Kikkawa, U. and Kamada, S. (2013). Cyclin I is involved in the regulation of cell cycle progression. *Cell Cycle* **12**, 2617-2624.
Nakamura, T., Sanokawa, R., Sasaki, Y. F., Ayusawa, D., Oishi, M. and Mori, N. (1995). Cyclin I: a new cyclin encoded by a gene isolated from human brain. *Exp. Cell Res.* **221**, 534-542.
Ninomiya-Tsuji, J., Nomoto, S., Yasuda, H., Reed, S. I. and Matsumoto, K. (1991). Cloning of a human cDNA encoding a CDC2-related kinase by complementation of a budding yeast cdc28 mutation. *Proc. Natl. Acad. Sci. USA* **88**, 9006-9010.
Nishi, A., Bibb, J. A., Snyder, G. L., Higashi, H., Nairn, A. C. and Greengard, P. (2000). Amplification of dopaminergic signaling by a positive feedback loop. *Proc. Natl. Acad. Sci. USA* **97**, 12840-12845.
Nixon, R. A. (2003). The calpains in aging and aging-related diseases. *Ageing Res. Rev.* **2**, 407-418.
Odajima, J., Wills, Z. P., Ndassa, Y. M., Terunuma, M., Kretschmannova, K., Deeb, T. Z., Geng, Y., Gawrzak, S., Quadros, I. M., Newman, J. et al. (2011). Cyclin E constrains Cdk5 activity to regulate synaptic plasticity and memory formation. *Dev. Cell* **21**, 655-668.
Ohshima, T., Ward, J. M., Huh, C. G., Longenecker, G., Veeranna, Pant, H. C., Brady, R. O, Martin, L. J. and Kulkarni, A. B. (1996). Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. *Proc. Natl. Acad. Sci. USA* **93**, 11173-11178.
Ohshima, T., Ogura, H., Tomizawa, K., Hayashi, K., Suzuki, H., Saito, T., Kamei, H., Nishi, A., Bibb, J. A.., Hisanaga, S., Matsui, H. and Mikoshiba, K. (2005). Impairment of hippocampal long-term depression and defective spatial learning and memory in p35 mice. *J. Neurochem.* **94**, 917-925.
Paglini, G., Pigino, G., Kunda, P., Morfini, G., Maccioni, R., Quiroga, S., Ferreira, A. and Cáceres, A. (1998). Evidence for the participation of the neuron-specific CDK5 activator P35 during laminin-enhanced axonal growth. *J. Neurosci.* **18**, 9858-9869.
Paoletti, P., Vila, I., Rifé, M., Lizcano, J. M., Alberch, J. and Ginés, S. (2008). Dopaminergic and glutamatergic signaling crosstalk in Huntington's disease neurodegeneration: the role of p25/cyclin-dependent kinase 5. *J. Neurosci.* **28**, 10090-10101.
Pareek, T. K., Keller, J., Kesavapany, S., Pant, H. C., Iadarola, M. J., Brady, R. O. and Kulkarni, A. B. (2006). Cyclin-dependent kinase 5 activity regulates pain signaling. *Proc. Natl. Acad. Sci. USA* **103**, 791-796.
Pareek, T. K., Keller, J., Kesavapany, S., Agarwal, N., Kuner, R., Pant, H. C., Iadarola, M. J., Brady, R. O. and Kulkarni, A. B. (2007). Cyclin-dependent kinase 5 modulates nociceptive signaling through direct phosphorylation of transient receptor potential vanilloid 1. *Proc. Natl. Acad. Sci. USA* **104**, 660-665.
Patrick, G. N., Zukerberg, L., Nikolic, M., de la Monte, S., Dikkes, P. and Tsai, L. H. (1999). Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. *Nature* **402**, 615-622.
Patzke, H. and Tsai, L. H. (2002). Calpain-mediated cleavage of the cyclin-dependent kinase-5 activator p39 to p29. *J. Biol. Chem.* **277**, 8054-8060.
Perez, F. P., Bose, D., Maloney, B., Nho, K., Shah, K. and Lahiri, D. K. (2014). Late-Onset Alzheimer's Disease, Heating up and Foxed by Several Proteins: Pathomolecular Effects of the Aging Process. *J. Alzheimers Dis.* **40**, 1-17.
Peterson, D. W., Ando, D. M., Taketa, D. A., Zhou, H., Dahlquist, F. W. and Lew, J. (2010). No difference in kinetics of tau or histone phosphorylation by CDK5/p25 versus CDK5/p35 in vitro. *Proc. Natl. Acad. Sci. USA* **107**, 2884-2889.
Poon, R. Y., Lew, J. and Hunter, T. (1997). Identification of functional domains in the neuronal Cdk5 activator protein. *J. Biol. Chem.* **272**, 5703-5708.
Qu, J., Nakamura, T., Cao, G., Holland, E. A., McKercher, S. R. and Lipton, S. A. (2011). S-Nitrosylation activates Cdk5 and contributes to synaptic spine loss induced by beta-amyloid peptide. *Proc. Natl. Acad. Sci. USA* **108**, 14330-14335.
Qu, J., Nakamura, T., Holland, E. A., McKercher, S. R. and Lipton, S. A. (2012). S-nitrosylation of Cdk5: potential implications in amyloid-β-related neurotoxicity in Alzheimer disease. *Prion* **6**, 364-370.
Rosenmann, H. (2013). Immunotherapy for targeting tau pathology in Alzheimer's disease and tauopathies. *Curr. Alzheimer Res.* **10**, 217-228.
Sahlgren, C. M., Pallari, H. M., He, T., Chou, Y. H., Goldman, R. D. and Eriksson, J. E. (2006). A nestin scaffold links Cdk5/p35 signaling to oxidant-induced cell death. *EMBO J.* **25**, 4808-4819.
Saito, K., Elce, J. S., Hamos, J. E. and Nixon, R. A. (1993). Widespread activation of calcium-activated neutral proteinase (calpain) in the brain in Alzheimer disease: a potential molecular basis for neuronal degeneration. *Proc. Natl. Acad. Sci. USA* **90**, 2628-2632.
Sasaki, Y., Cheng, C., Uchida, Y., Nakajima, O., Ohshima, T., Yagi, T., Taniguchi, M., Nakayama, T., Kishida, R., Kudo, Y. et al. (2002). Fyn and Cdk5 mediate semaphorin-3A signaling, which is involved in regulation of dendrite orientation in cerebral cortex. *Neuron* **35**, 907-920.
Sato, S., Xu, J., Okuyama, S., Martinez, L. B., Walsh, S. M., Jacobsen, M. T., Swan, R. J., Schlautman, J. D., Ciborowski, P. and Ikezu, T. (2008). Spatial learning impairment, enhanced CDK5/p35 activity, and downregulation of NMDA receptor expression in transgenic mice expressing tau-tubulin kinase 1. *J. Neurosci.* **28**, 14511-14521.
Sato, K., Minegishi, S., Takano, J., Plattner, F., Saito, T., Asada, A., Kawahara, H., Iwata, N., Saido, T. C. and Hisanaga, S. (2011). Calpastatin, an endogenous calpain-inhibitor protein, regulates the cleavage of the Cdk5 activator p35 to p25. *J. Neurochem.* **117**, 504-515.
Sharma, P., Veeranna, Sharma, M., Amin, N. D., Sihag, R. K., Grant, P., Ahn, N., Kulkarni, A. B. and Pant, H. C. (2002). Phosphorylation of MEK1 by cdk5/p35 down-regulates the mitogen-activated protein kinase pathway. *J. Biol. Chem.* **277**, 528-534.
Shea, T. B., Yabe, J. T., Ortiz, D., Pimenta, A., Loomis, P., Goldman, R. D., Amin, N. and Pant, H. C. (2004). Cdk5 regulates axonal transport and phosphorylation of neurofilaments in cultured neurons. *J. Cell Sci.* **117**, 933-941.
Smith, P. D., Crocker, S. J., Jackson-Lewis, V., Jordan-Sciutto, K. L., Hayley, S., Mount, M. P., O'Hare, M. J., Callaghan, S., Slack, R. S., Przedborski, S. et al. (2003). Cyclin-dependent kinase 5 is a mediator of dopaminergic neuron loss in a mouse model of Parkinson's disease. *Proc. Natl. Acad. Sci. USA* **100**, 13650-13655.
Song, J. H., Wang, C. X., Song, D. K., Wang, P., Shuaib, A. and Hao, C. (2005). Interferon gamma induces neurite outgrowth by up-regulation of p35

neuron-specific cyclin-dependent kinase 5 activator via activation of ERK1/2 pathway. *J. Biol. Chem.* **280**, 12896-12901.

Sun, K. H., de Pablo, Y., Vincent, F., Johnson, E. O., Chavers, A. K. and Shah, K. (2008a). Novel genetic tools reveal Cdk5's major role in Golgi fragmentation in Alzheimer's disease. *Mol. Biol. Cell* **19**, 3052-3069.

Sun, K. H., de Pablo, Y., Vincent, F. and Shah, K. (2008b). Deregulated Cdk5 promotes oxidative stress and mitochondrial dysfunction. *J. Neurochem.* **107**, 265-278.

Sun, K. H., Lee, H. G., Smith, M. A. and Shah, K. (2009). Direct and indirect roles of cyclin-dependent kinase 5 as an upstream regulator in the c-Jun NH2-terminal kinase cascade: relevance to neurotoxic insults in Alzheimer's Disease. *Mol. Biol. Cell* **20**, 4611-4619.

Sun, K. H., Chang, K. H., Clawson, S., Ghosh, S., Mirzaei, H., Regnier, F. and Shah, K. (2011). Glutathione-S-transferase P1 is a critical regulator of Cdk5 kinase activity. *J. Neurochem.* **118**, 902-914.

Sundaram, J. R., Poore, C. P., Sulaimee, N. H., Pareek, T., Asad, A. B., Rajkumar, R., Cheong, W. F., Wenk, M. R., Dawe, G. S., Chuang, K. H. et al. (2013). Specific inhibition of p25/Cdk5 activity by the Cdk5 inhibitory peptide reduces neurodegeneration in vivo. *J. Neurosci.* **33**, 334-343.

Takahashi, S., Saito, T., Hisanaga, S., Pant, H. C. and Kulkarni, A. B. (2003). Tau phosphorylation by cyclin-dependent kinase 5/p39 during brain development reduces its affinity for microtubules. *J. Biol. Chem.* **278**, 10506-10515.

Tandon, A., Yu, H., Wang, L., Rogaeva, E., Sato, C., Chishti, M. A., Kawarai, T., Hasegawa, H., Chen, F., Davies, P. et al. (2003). Brain levels of CDK5 activator p25 are not increased in Alzheimer's or other neurodegenerative diseases with neurofibrillary tangles. *J. Neurochem.* **86**, 572-581.

Tang, D., Yeung, J., Lee, K. Y., Matsushita, M., Matsui, H., Tomizawa, K., Hatase, O., Wang, J. H. (1995). An isoform of the neuronal cyclin-dependent kinase 5 (Cdk5) activator. *J. Biol. Chem.* **270**, 26897-26903.

Tang, D., Chun, A. C., Zhang, M. and Wang, J. H. (1997). Cyclin-dependent kinase 5 (Cdk5) activation domain of neuronal Cdk5 activator. Evidence of the existence of cyclin fold in neuronal Cdk5a activator. *J. Biol. Chem.* **272**, 12318-12327.

Taniguchi, S., Fujita, Y., Hayashi, S., Kakita, A., Takahashi, H., Murayama, S., Saido, T. C., Hisanaga, S., Iwatsubo, T. and Hasegawa, M. (2001). Calpain-mediated degradation of p35 to p25 in postmortem human and rat brains. *FEBS Lett.* **489**, 46-50.

Tarricone, C., Dhavan, R., Peng, J., Areces, L. B., Tsai, L. H. and Musacchio, A. (2001). Structure and regulation of the CDK5-p25(nck5a) complex. *Mol. Cell* **8**, 657-669.

Tsai, L. H., Harlow, E. and Meyerson, M. (1991). Isolation of the human cdk2 gene that encodes the cyclin A- and adenovirus E1A-associated p33 kinase. *Nature* **353**, 174-177.

Tsai, L. H., Lees, E., Faha, B., Harlow, E. and Riabowol, K. (1993). The cdk2 kinase is required for the G1-to-S transition in mammalian cells. *Oncogene* **8**, 1593-1602.

Tsai, L. H., Delalle, I., Caviness, V. S., Jr, Chae, T. and Harlow, E. (1994). p35 is a neural-specific regulatory subunit of cyclin-dependent kinase 5. *Nature* **371**, 419-423.

Tseng, H. C., Zhou, Y., Shen, Y. and Tsai, L. H. (2002). A survey of Cdk5 activator p35 and p25 levels in Alzheimer's disease brains. *FEBS Lett.* **523**, 58-62.

Utreras, E., Futatsugi, A., Rudrabhatla, P., Keller, J., Iadarola, M. J., Pant, H. C. and Kulkarni, A. B. (2009). Tumor necrosis factor-alpha regulates cyclin-dependent kinase 5 activity during pain signaling through transcriptional activation of p35. *J. Biol. Chem.* **284**, 2275-2284.

Wei, F. Y., Tomizawa, K., Ohshima, T., Asada, A., Saito, T., Nguyen, C., Bibb, J. A., Ishiguro, K., Kulkarni, A. B., Pant, H. C. et al. (2005). Control of cyclin-dependent kinase 5 (Cdk5) activity by glutamatergic regulation of p35 stability. *J. Neurochem.* **93**, 502-512.

Wen, Y., Yu, W. H., Maloney, B., Bailey, J., Ma, J., Marié, I., Maurin, T., Wang, L., Figueroa, H., Herman, M. et al. (2008). Transcriptional regulation of beta-secretase by p25/cdk5 leads to enhanced amyloidogenic processing. *Neuron* **57**, 680-690.

Wong, A. S., Lee, R. H., Cheung, A. Y., Yeung, P. K., Chung, S. K., Cheung, Z. H. and Ip, N. Y. (2011). Cdk5-mediated phosphorylation of endophilin B1 is required for induced autophagy in models of Parkinson's disease. *Nat. Cell Biol.* **13**, 568-579.

Xiong, Y., Zhang, H. and Beach, D. (1992). D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. *Cell* **71**, 505-514.

Yamada, M., Saito, T., Sato, Y., Kawai, Y., Sekigawa, A., Hamazumi, Y., Asada, A., Wada, M., Doi, H. and Hisanaga, S. (2007). Cdk5—p39 is a labile complex with the similar substrate specificity to Cdk5—p35. *J. Neurochem.* **102**, 1477-1487.

Yamak, A., Latinkic, B. V., Dali, R., Temsah, R. and Nemer, M. (2014). Cyclin D2 is a GATA4 cofactor in cardiogenesis. *Proc. Natl. Acad. Sci. USA* **111**, 1415-1420.

Yang, Y. R., He, Y., Zhang, Y., Li, Y., Li, Y., Han, Y., Zhu, H. and Wang, Y. (2007). Activation of cyclin-dependent kinase 5 (Cdk5) in primary sensory and dorsal horn neurons by peripheral inflammation contributes to heat hyperalgesia. *Pain* **127**, 109-120.

Yoo, B. C. and Lubec, G. (2001). p25 protein in neurodegeneration. *Nature* **411**, 763-764, discussion 764-765.

Zhang, P., Yu, P. C., Tsang, A. H., Chen, Y., Fu, A. K., Fu, W. Y., Chung, K. K. and Ip, N. Y. (2010). S-nitrosylation of cyclin-dependent kinase 5 (cdk5) regulates its kinase activity and dendrite growth during neuronal development. *J. Neurosci.* **30**, 14366-14370.

Zhang, L., Liu, W., Szumlinski, K. K. and Lew, J. (2012). p10, the N-terminal domain of p35, protects against CDK5/p25-induced neurotoxicity. *Proc. Natl. Acad. Sci. USA* **109**, 20041-20046.

Zheng, M., Leung, C. L. and Liem, R. K. (1998). Region-specific expression of cyclin-dependent kinase 5 (cdk5) and its activators, p35 and p39, in the developing and adult rat central nervous system. *J. Neurobiol.* **35**, 141-159.

Zheng, Y. L., Li, B. S., Amin, N. D., Albers, W. and Pant, H. C. (2002). A peptide derived from cyclin-dependent kinase activator (p35) specifically inhibits Cdk5 activity and phosphorylation of tau protein in transfected cells. *Eur. J. Biochem.* **269**, 4427-4434.

Zhu, J. H., Guo, F., Shelburne, J., Watkins, S. and Chu, C. T. (2003). Localization of phosphorylated ERK/MAP kinases to mitochondria and autophagosomes in Lewy body diseases. *Brain Pathol.* **13**, 473-481.

Zukerberg, L. R., Patrick, G. N., Nikolic, M., Humbert, S., Wu, C. L., Lanier, L. M., Gertler, F. B., Vidal, M., Van Etten, R. A. and Tsai, L. H. (2000). Cables links Cdk5 and c-Abl and facilitates Cdk5 tyrosine phosphorylation, kinase upregulation, and neurite outgrowth. *Neuron* **26**, 633-646.

Journal of Cell Science