Published in final edited form as:

Trends Neurosci. 2009 November ; 32(11): 575–582. doi:10.1016/j.tins.2009.07.002.

Making a Neuron: Cdk5 in Embryonic and Adult Neurogenesis

Sebastian Jessberger¹, Fred H. Gage², Amelia J. Eisch³, and Diane C. Lagace⁴

¹Institute of Cell Biology, Department of Biology, ETH Zurich, Schafmattstrasse 18, 8093 Zurich, Switzerland ²Laboratory of Genetics, The Salk Institute for Biological Studies, 10010 N Torrey Pines Rd, Lab Genetics, La Jolla, CA 92037, USA ³Department of Psychiatry, University of Texas Southwestern Medical Center, NC 6.506E, 5323 Harry Hines Blvd, Dallas, TX 75390, USA ⁴Department of Cellular and Molecular Medicine and Neuroscience Program, 451 Smyth Road, Ottawa, ONT, Canada

Abstract

Cyclin-dependent kinase 5 (Cdk5) has been previously implicated in the migration, maturation, and survival of neurons born during embryonic development. New evidence suggests that Cdk5 has comparable – but also distinct – functions in adult neurogenesis. Here we summarize the accumulating research on the role of Cdk5 in the regulation of cell cycle, migration, survival, maturation, and neuronal integration. We specifically highlight the many similarities, and few tantalizing differences, in the roles of Cdk5 in the embryonic and adult brain. We discuss the signaling pathways that may contribute to Cdk5's action in regulating embryonic and adult neurogenesis, stressing future research directions that will help clarify the mechanisms underlying lifelong neurogenesis in the mammalian brain.

Introduction

The discovery that neurons are born throughout life in restricted brain areas of mammals, including humans, adds to the challenge of understanding adult brain function and raises questions about how embryonic and adult neurogenesis compare ¹. In both the embryo and adult brain, neurogenesis refers to the complex process of the division of neural stem cells (NSCs) and progenitor cells into daughter cells that migrate and give rise to new neurons. During embryonic development, cells are generated in massive waves by neuroepithelial and radial glial NSCs ^{2, 3}. Subsequently, newborn cells migrate through “developing” tissue in an orchestrated fashion to reach their final destination in the brain. In the adult brain, neurogenesis is a more rare event and initiates in either the subventricular zone (SVZ) of the lateral ventricle or the subgranular zone (SGZ) of the adult hippocampal dentate gyrus ^{1, 4}.

There are fundamental differences between neurogenesis in the embryonic brain and in the adult brain. In the embryo, the “niche”, or cellular environment, of NSCs (and their immediate progeny) is remarkably specialized to support proliferation, differentiation and eventually migration. In striking contrast, adult hippocampal SGZ neurogenesis, our focus for the context

© 2009 Elsevier Ltd. All rights reserved.

Correspondence should be addressed to: S.J. (jessberger@cell.biol.ethz.ch), F.H.G. (gage@salk.edu), A.J.E. (amelia.eisch@utsouthwestern.edu), or D.C.L. (dlagace@uottawa.ca).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

of adult neurogenesis in this review, takes place in an environment that not only has to support neurogenesis but also is required for the proper functioning of a fully mature network of preexisting neurons¹. In addition, adult hippocampal NSCs and their progeny at various stages of maturation co-exist in close proximity within the dentate gyrus, in contrast to the embryonic brain, where relatively clear borders define distinct germinal zones⁵. Thus, while some mechanisms involved in NSC proliferation, differentiation, migration, and neuronal maturation are shared between the embryonic and adult brain, the differences in the structures in which embryonic and adult neurogenesis take place encourage careful consideration of factors that might also play distinct regulatory mechanisms in embryonic versus adult neurogenesis.

Here we review evidence that the cyclin-dependent kinase Cdk5 plays key roles in both embryonic and adult neurogenesis (Figure 1A). As a unique member of the cyclin-dependent kinase family due to its reliance on non-cyclin cofactors p35 or p39, Cdk5 was first identified to be critical for correct migration during embryonic cortical development⁶. Moreover, a number of recent studies have further supported similar but also distinct roles for Cdk5 in adult neurogenesis^{7, 8}. We highlight these findings and review the potential mechanisms for Cdk5's action in the processes of embryonic and adult neurogenesis, as well as the technical challenges associated with selective manipulation of gene activity in newborn cells of the adult brain.

Role for Cdk5 in Cell Cycle Regulation

Cdk5 was initially characterized to have no role in the cell cycle since, unlike traditional members of the cyclin family, ectopic expression of Cdk5 does not promote cell cycle progression in yeast or in mammalian cells⁹. This finding was in agreement with the observation that Cdk5 expression and activity occur almost exclusively in postmitotic neurons in the embryo¹⁰ and in postmitotic neurons in the neurogenic regions of the adult brain⁸. Thus, although Cdk5 can regulate several cell cycle proteins^{11–13} – most notably, phosphorylating retinoblastoma (Rb) protein, a critical step in cell cycle exit^{13, 14} – the lack of Cdk5 activity in dividing cells in the CNS suggests that it does not have a classical role in cell cycle regulation, which is obviously a critical step in embryonic but also adult neurogenesis.

However, accumulating evidence indicates that Cdk5 activity in postmitotic neurons is involved in suppressing cell cycle reentry. As reviewed by Herrup and Yang¹⁵, postmitotic neurons appear capable of reentry into the cell cycle, especially under conditions of stress or models of neurodegeneration. It was initially thought that Cdk5 might be involved in inhibiting cell cycle reentry, since mitotic cells were found abnormally in the cortical plate and intermediate zone in Cdk5-deficient embryos¹⁶. The abnormal presence of mitotic cells concurred with enhanced cell death with no significant increase in cell number, supporting that reentry into the cell cycle resulted in cell cycle-related neuronal death¹⁷.

Mechanistically, it appears that Cdk5 activity and/or Cdk5 subcellular localization are important for preventing re-activation of the cell cycle in postmitotic neurons (Figure 1B). In wild-type embryonic cells, blocking Cdk5 activity via roscovitine treatment did not prevent reentry¹⁶, whereas the subcellular distribution of Cdk5 from the nucleus to the cytoplasm could prevent reentry¹⁷. In contrast, roscovitine (which is a pharmacological inhibitor of Cdk5) treatment prevented postmitotic cell cycle reentry in other neuronal models, such as cultured cortical neurons treated with either amyloid-beta or prion peptides, or primary cerebellar granule neurons treated with DNA-damaging agents^{18, 19}. In the latter case, Cdk5 directly phosphorylates and activates the phosphatidylinositol-3-kinase-like kinase ATM (ataxia-telangiectasia mutated) preferentially in the nucleus. Notably, among ATM's functions, its activation is necessary to initiate DNA damage-induced neuronal cell cycle reentry and subsequent neuronal death¹⁸. These findings raise the possibility that targeting Cdk5 could provide a new pharmacological treatment for blocking ectopic cell cycle reentry in

neurodegenerative disease. It remains to be determined how these findings will apply to postmitotic neurons in the adult brain *in vivo* under naïve conditions. Recently, conditional mouse model systems for the targeted removal of Cdk5 from the adult brain have been created, 7, 8, 20–22 and ectopic cycling postmitotic cells have not been reported therein. However, future examination of cell cycle reentry in naïve and pathological conditions is warranted.

Cdk5 and Neuronal Migration

Upon cell cycle exit, newborn cells have to find their appropriate position through neuronal migration. During embryonic development, the mammalian neocortex is structured in an “inside out” layering, with the earliest-born pyramidal cells in the deepest layers and later-born neurons migrating past older layers to generate superficial neuronal layers. Migration of pyramidal cells toward the pial surface is thought to occur along a radial glial scaffold²³. Cdk5 mutant mice show severe defects in the lamination of a number of cortical structures, including the neocortex, hippocampus and cerebellum. As newborn cells appear to get “stuck” and are unable to migrate through earlier-generated neurons, these data suggest a critical role for Cdk5 in neuronal migration during embryonic cortical development^{24–27}. In contrast to the neocortex, the excitatory granule cells of the hippocampal dentate gyrus become layered in an “outside in” mode; the oldest neurons form the outermost layers in the vicinity of the molecular layer, and granule cells that are born later remain in the inner parts of the granule cell layer (GCL)²⁸. NSCs reside in the SGZ, and their progeny – immature newborn neurons – migrate only short distances into the GCL, where they become stably integrated following an activity-dependent selection process^{29, 30}. Similar to neocortical development, a glial scaffold might also underlie migration of new neurons born in the adult dentate gyrus³¹. Adult-born neurons deficient in Cdk5 activity due to cell-selective overexpression of a well-characterized dominant-negative mutant (DNCdk5) fail to move toward the GCL, suggesting that Cdk5 is required for proper migration during not only embryonic but also adult neurogenesis⁷. Interestingly, Cdk5 appears to also be critically involved in the migration of neuroblasts from the SVZ to the olfactory bulb during early postnatal development^{21, 32}.

How might Cdk5 regulate neuronal migration in the embryonic and adult brain? One possibility is that Cdk5 affects microtubule dynamics by phosphorylating a number of proteins that are important for the nucleokinesis underlying certain forms of neuronal migration, such as Nudel1 (Ndel1), focal adhesion kinase (FAK), the serine/threonine kinase Pak1, p27/Kip1, and the microtubule-associated protein doublecortin (DCX)^{33–38}. In fact, recent evidence suggests that Ndel1 is important for neuronal migration (and maturation) in adult hippocampal neurogenesis³⁹. Based on the DCX expression pattern in newborn granule cells⁴⁰, it seems plausible that Cdk5-dependent phosphorylation of DCX might also be critical for neuronal migration in the adult dentate gyrus (Figure 1B).

Clearly, there is much that is unknown about neuronal migration in the adult hippocampus. Future studies will have to address the exact relationship of immature neurons with a potential glial scaffold, the mode of neuronal migration (using longitudinal observation of newborn cells with time lapse imaging), and the molecular pathways in which Cdk5 is involved. Nevertheless, genetic deletion during embryogenesis and retrovirus-based inhibition of Cdk5 activity in the adult hippocampus suggest similar roles for Cdk5 in neuronal migration in both the embryonic and adult brain.

Cdk5 Involvement in Dendrite Extension and Synapse Formation

In both the embryonic and adult brain, newborn neurons start to extend neurites roughly coincidentally with neuronal migration⁴¹. These neurites have to accomplish two tasks: growth/extension and proper pathfinding. There is vast evidence that extracellular cues – including chemoattractants and chemorepellants such as semaphorins, Eph and their ephrin

receptors, reelin, and neurotrophins – are fundamentally important for the extension and pathfinding of dendritic and axonal processes during embryonic development⁴². Intriguingly, Cdk5 plays a key role in these signaling cascades during embryonic development, with Cdk5 deficiency leading to mere growth impairment and/or incorrect patterning of dendritic processes (Figure 1B). For example, BDNF-stimulated dendritic growth of hippocampal neurons *in vitro* requires Cdk5 activity, as Cdk5 directly phosphorylates the cognate BDNF receptor TrkB⁴³. In line with a role for Cdk5 in dendritic development, mice with attenuated Cdk5 activity show impaired dendritic development and altered dendritic pathfinding during embryonic development and in a number of *in vitro* models^{44–46}.

Strikingly, inhibition of Cdk5 using a DNCdk5 or shRNA against the mRNA coding for Cdk5 at least partially mimics this embryonic phenotype also in the adult: newborn dentate gyrus granule cells with reduced Cdk5 activity show reduced growth of dendritic processes⁷. More importantly, Cdk5 deficiency impairs pathfinding of dendrites, leading to ectopically projecting dendrites extending from newborn granule cells. Further supporting a role for Cdk5 in dendritic pathfinding of dentate gyrus granule cells are findings from mice with a null mutation of p35, one of Cdk5's co-factors. P35 null mice, unlike classic knock-outs of Cdk5, survive embryonic development, but their dentate gyrus granule cells extend aberrant dendrites in the absence of p35. Notably, this aberrant pathfinding is present irrespective of the seizure activity that spontaneously occurs in p35 mutant mice^{47, 48}.

Thus, it appears that dendritic growth and pathfinding require Cdk5 activity for proper development in the embryonic and adult brain. In addition to impaired extracellular signaling, it will be interesting to determine the intracellular consequences of attenuated Cdk5 function, especially in the context of adult neurogenesis. Promising candidates that might underlie impaired dendritic maturation with Cdk5 deficiency could be members of the family of small RhoGTPases, such as Rac1, RhoA and cdc42, all of which are key regulators of cytoskeletal dynamics⁴⁹. Cdk5 phosphorylates (and thereby inhibits) a number of guanine-exchange factors (GEFs) that regulate the activity of RhoGTPases^{36, 50–52}.

In addition to the putative mechanisms contributing to Cdk5-induced regulation of dendritic pathfinding, small RhoGTPases could also be critical for the function of Cdk5 in spine formation and synaptogenesis (Figure 1B). First identified to be involved in synapse formation during embryonic development at the neuromuscular junction^{53, 54}, Cdk5 has been shown to regulate the localization and/or activity of a number of pre- and postsynaptic proteins, such as ErbB receptors, ephexin1, PSD-95, Plk2, and CASK^{52, 54–57}. Inhibition of Cdk5 activity during embryonic development or in primary neurons *in vitro* reduces the number of formed spines and synapses, suggesting a role for Cdk5 in synapse formation and/or maintenance.

In contrast to embryonic development, where spines arising from many neurons are formed more or less simultaneously, new neurons that are born in the adult hippocampus have to integrate synaptically in an environment that is already filled with numerous spines and synaptic connections of preexisting neurons⁵⁸. One related hypothesis that has recently gained significant attention is that newborn granule cells compete for presynaptic partners to survive an activity-dependent selection process⁵⁸. Inhibition of Cdk5 activity using the DNCdk5 impairs the formation of mature mushroom spines (and thus potentially synapse formation, as spines are the major sites for synapses formed with dentate granule cells) of newborn cells in the adult hippocampus⁷. The effect of Cdk5 deletion on mature granule cells remains unclear. However, hyperactivation of Cdk5 by ectopic expression of p25 leads to a transient increase in the spine formation of hippocampal neurons, which is subsequently followed by reduced spine numbers. Thus, complementing what was previously known in the embryonic brain, these recent findings confirm a role for Cdk5 in spine formation/maturation also in the adult brain⁵⁹.

Cdk5 in Neuronal Survival and Cell Death

Removal of Cdk5 from the embryonic and adult brain implicated Cdk5 as a critical regulator of neuronal survival. In Cdk5-deficient embryos, swelling of the cell soma and nuclear margination in the brainstem and spinal cord occur²⁷. Recently it has been shown *in vivo* that retinal neurons and developing new neurons of the dentate gyrus require Cdk5 activity for survival^{8, 60}. The pro-survival actions of Cdk5 are mediated by the various substrates that are phosphorylated within pathways crucial for survival or inactivation of apoptosis (Figure 1B). For example, Cdk5 phosphorylates i) neuregulin receptor ErbB2 and activates the neuregulin/phosphatidylinositol 3 kinase (PI3K/Akt) survival pathway⁶¹; ii) c-Jun N-terminal kinase 3 (JNK3) to suppress apoptotic pathways⁶²; and iii) mitogen-activated protein kinase kinase (MEK)1 to prevent apoptosis induced by sustained activation of the mitogen-activated protein kinase (MAPK) family^{63–65}. In addition, the anti-apoptotic protein Bcl-2 (B-cell lymphoma protein 2) has recently been identified as a substrate of Cdk5 that can alter neuronal survival; specifically, Cdk5 phosphorylation of Bcl-2 contributes to cell survival and inhibition of phosphorylation promotes apoptosis⁶⁰.

In striking contrast to Cdk5 activity promoting survival, dysregulation of Cdk5 can induce neuronal death following cellular perturbation⁶⁶. These effects are mainly associated with the calpain-mediated proteolytic cleavage of the Cdk5 coactivators p35 or p39 into p25 and p29, respectively, which are more stable proteins that induce prolonged Cdk5 activity^{66, 67}. Recently, the role of p25/Cdk5 has been linked through various experimental approaches to neurodegenerative diseases, either causing or accelerating the formation of early neurofibrillary tangles that are composed mainly of hyperphosphorylated and aggregated tau^{66–68}. Of additional importance to neurodegenerative disease states, Cdk5 activity has recently been identified to be critical for mitochondrial dysfunction and Golgi fragmentation through its ability to phosphorylate peroxiredoxin 1 and 2 (Prx1, Prx2) and a cis-Golgi matrix protein known as GM130, respectively^{69–71}. Furthermore, a range of neurotoxic insults (including ischemia, oxidative stress, and nerve injury) generate a calpain-dependent increase in p25 activity that subsequently contributes to neuronal death. The cellular mechanisms that underlie Cdk5/p25 involvement in neuronal death in these models are beginning to be understood. For example, Cdk5 phosphorylates NMDA receptors to amplify calcium influx required for induction of cell death following ischemia in hippocampal neurons^{72, 73}, and Cdk5 phosphorylates myocyte enhancer factor (MEF2) to protect primary neurons from neurotoxin-induced apoptosis⁷⁴. p25 can directly interact and inhibit HDAC1 to induce DNA damage, aberrant expression of cell-cycle activity, and double-stranded DNA breaks leading to neurotoxicity⁷⁵. Taken together, these data strongly support the hypothesis that Cdk5 activity is critical for the life/death decision in the embryonic and adult neuron, and they have shed new light on the complex mechanisms involved in Cdk5's role in various forms of pathology.

The Challenge of Selective Targeting of Newborn Cells in the Adult Brain

Recent studies showed that Cdk5 is critically involved in adult neurogenesis, and precise genetic manipulation of the Cdk5 gene during the process is important for elucidating its functions^{7, 8}. As only small fractions of cells within the adult dentate gyrus are “newborn” at a given time, it is technically challenging to analyze the function of a given gene in NSCs and their progeny. At this time, there are basically two different strategies to obtain cell-type specific data in the context of adult neurogenesis (Figure 2). First, retroviral vectors that require breakdown of the nuclear membrane and thus specifically integrate into the DNA of dividing cells have turned out to be a powerful tool to i) visualize newborn cells in the adult dentate gyrus, making them accessible for morphological and electrophysiological analyses; and ii) manipulate gene expression and/or protein activity^{76–78}. Using retroviruses expressing either DNCdk5 or an shRNA targeting Cdk5 mRNA, it was found that Cdk5 is critical for neuronal

migration, dendrite extension, and dendritic pathfinding of newborn granule cells in a cell-autonomous fashion⁷. A second method to delete specific genes in newborn cells of the adult brain is the use of floxed mouse mutants crossed with transgenic mice conditionally expressing Cre recombinase under NSC-specific (or at least enriched) promoters, such as the nestin promoter^{8, 79}. This approach has several advantages compared to virus-based approaches but also has certain limitations. Retroviral vectors stoichiometrically integrate into dividing cells. If the hypothesis is correct that some NSCs are largely quiescent, then this population can be easily missed by injection of retroviruses^{80, 81}, making the analysis of gene function in some early events of adult neurogenesis difficult. Recombination systems using inducible Cre such as nestin-CreERT2 circumvent this problem. In addition, a much larger number of cells gets recombined using, for example, the nestin-CreERT2 system, allowing population analyses of recombined cells in contrast to only single-labeled cells using virus-based approaches. However, this population approach could also be a potential disadvantage. For instance, genetic deletion of Cdk5 using nestin-CreERT2 resulted in dramatic reduction of neuronal survival, which only occurred mildly using viral expression of DNCdk5. There are several possible explanations for this discrepancy. Expression of DNCdk5 or shRNA against Cdk5 might not fully inhibit Cdk5 activity, which remains sufficient to allow the cells to survive, and reveals a function of Cdk5 in neuronal migration/neurite pathfinding in the adult brain. In addition, there seems to be a non-cell autonomous role for Cdk5 in regulating adult neurogenesis, as discussed above⁸. Thus, Cdk5 deletion in the whole population of newborn cells at the time of tamoxifen-induced recombination could lead to non-cell autonomous effects on newborn “neighboring” cells. Therefore, the ideal experimental set-up would be the combination of virus-based approaches (to understand the strictly cell-autonomous function of genes in newborn cells) with inducible Cre-mediated recombination in NSCs and their progeny (to analyze gene function in NSCs themselves and to analyze the effects of gene deletion on a population level). To determine the role of Cdk5 in adult neurogenesis, future studies will have to analyze the effects of Cdk5 deletion in single newborn cells using a Cre-expressing retrovirus to understand if the observed differences are due to levels of knockdown or non-cell autonomous effects of Cdk5 deletion.

Outlook

There are numerous physiological processes in the embryonic and adult brain in which Cdk5 seems to play a critical role, including neuronal migration, neurite growth, neuronal survival, and synaptic plasticity. Cdk5 does not contribute to the normal cell cycle regulation as a typical cyclin in either the embryonic or adult brain⁶; however, Cdk5 has recently been discovered to be crucial to cell cycle suppression in embryonic post-mitotic neurons and neuronal cell lines. This function of Cdk5 may be unique and specific to embryonic neurogenesis, since current *in vivo* Cdk5 adult knockout models have failed to report these effects^{7, 8, 20–22}, raising the need for more investigation into these paradoxical findings. Within both embryonic and adult neurogenesis, many lines of evidence support a critical and similar role for Cdk5 in neuronal migration, neurite outgrowth and neuronal survival^{6, 43, 82}, in contrast to other factors, such as disrupted-in-schizophrenia 1 (DISC1), that have opposite effects on neuronal migration during embryonic and adult neurogenesis^{39, 83}. These similarities and differences highlight the need to continue to dissect the fundamental and distinct regulatory mechanisms in embryonic versus adult neurogenesis, which may be important for many adult-onset neurological diseases.

Mechanistically, studies continue to identify many proteins and signaling pathways with which Cdk5 is linked. As for other key players in cellular signaling, such as MAP kinases, future studies are needed to understand the cellular circumstances under which certain functions of Cdk5 become active and important. In the context of adult neurogenesis, it will be important to identify the targets that lead to reduced survival and impaired migration/dendrite extension.

This identification is complicated by the fact that biochemical analysis of knockout cells *in vivo* is extremely difficult, given the fact that the relative number of newborn cells is only a small fraction of the tissue within the hippocampal dentate gyrus. Therefore, current approaches only try to phenocopy the effects of Cdk5 inhibition, e.g., with the deletion of DCX or other promising target genes. Furthermore, there is a strong need for better culturing methods of adult NSCs. Current approaches using neurosphere or monolayer cultures of NSCs have been extremely useful in identifying signaling pathways regulating adult NSC proliferation or neuronal differentiation⁸⁴, but it is challenging to use these *in vitro* approaches to assess neuronal migration, directed neurite growth, or later steps of neurogenesis such as survival or synapse formation, since these processes are highly dependent on the integrity of the neurogenic niche. Therefore, the causal linkage of Cdk5 with up- and downstream pathways requires advanced *in vitro* models of adult neurogenesis mimicking the niche of the adult brain and cell-specific manipulations of Cdk5 activity within the adult brain.

Acknowledgments

We would like to thank Mary Lynn Gage for editing this manuscript. Our research is supported by the NCCR Neural Plasticity and Repair, Swiss National Science Foundation (3100A0-117744/1), ETH grant (ETH-0108-1), Théodore Ott and Novartis Foundation (to SJ), NIH R21 DA023701 and K02 DA023555 (to AJE), and salary support from the Heart and Stroke Foundation Centre for Stroke Recovery (DCL). The authors declare they have no conflict of interest.

References

1. Zhao C, et al. Mechanisms and functional implications of adult neurogenesis. *Cell* 2008;132:645–660. [PubMed: 18295581]
2. Farkas LM, Huttner WB. The cell biology of neural stem and progenitor cells and its significance for their proliferation versus differentiation during mammalian brain development. *Current opinion in cell biology* 2008;20:707–715. [PubMed: 18930817]
3. Noctor SC, et al. Neural stem and progenitor cells in cortical development. *Novartis Foundation symposium* 2007;288:59–73. [PubMed: 18494252]discussion 73-58, 96-58
4. Alvarez-Buylla A, Lim DA. For the long run: maintaining germinal niches in the adult brain. *Neuron* 2004;41:683–686. [PubMed: 15003168]
5. Kempermann G, et al. Milestones of neuronal development in the adult hippocampus. *Trends in neurosciences* 2004;27:447–452. [PubMed: 15271491]
6. Dhavan R, Tsai LH. A decade of CDK5. *Nat Rev Mol Cell Biol* 2001;2:749–759. [PubMed: 11584302]
7. Jessberger S, et al. Cdk5 Regulates Accurate Maturation of Newborn Granule Cells in the Adult Hippocampus. *PLoS biology* 2008;6:e272. [PubMed: 18998770]
8. Lagace DC, et al. Cdk5 is essential for adult hippocampal neurogenesis. *Proceedings of the National Academy of Sciences of the United States of America* 2008;105:18567–18571. [PubMed: 19017796]
9. Meyerson M, et al. A family of human cdc2-related protein kinases. *The EMBO journal* 1992;11:2909–2917. [PubMed: 1639063]
10. Tsai LH, et al. Activity and expression pattern of cyclin-dependent kinase 5 in the embryonic mouse nervous system. *Development (Cambridge, England)* 1993;119:1029–1040.
11. Alexander K, et al. Cellular senescence requires CDK5 repression of Rac1 activity. *Mol Cell Biol* 2004;24:2808–2819. [PubMed: 15024070]
12. Yang HS, et al. ERM proteins and Cdk5 in cellular senescence. *Cell Cycle* 2003;2:517–520. [PubMed: 14504464]
13. Yang HS, Hinds PW. Phosphorylation of ezrin by cyclin-dependent kinase 5 induces the release of Rho GDP dissociation inhibitor to inhibit Rac1 activity in senescent cells. *Cancer research* 2006;66:2708–2715. [PubMed: 16510591]
14. Hamdane M, et al. p25/Cdk5-mediated retinoblastoma phosphorylation is an early event in neuronal cell death. *J Cell Sci* 2005;118:1291–1298. [PubMed: 15741232]
15. Herrup K, Yang Y. Cell cycle regulation in the postmitotic neuron: oxymoron or new biology? *Nature reviews* 2007;8:368–378.

16. Cicero S, Herrup K. Cyclin-dependent kinase 5 is essential for neuronal cell cycle arrest and differentiation. *J Neurosci* 2005;25:9658–9668. [PubMed: 16237170]
17. Zhang J, et al. Nuclear localization of Cdk5 is a key determinant in the postmitotic state of neurons. *Proceedings of the National Academy of Sciences of the United States of America* 2008;105:8772–8777. [PubMed: 18550843]
18. Tian B, et al. Phosphorylation of ATM by Cdk5 mediates DNA damage signalling and regulates neuronal death. *Nature cell biology* 2009;11:211–218.
19. Lopes JP, et al. Cdk5 acts as a mediator of neuronal cell cycle re-entry triggered by amyloid-beta and prion peptides. *Cell Cycle* 2009;8:97–104. [PubMed: 19158499]
20. Hawasli AH, et al. Cyclin-dependent kinase 5 governs learning and synaptic plasticity via control of NMDAR degradation. *Nat Neurosci* 2007;10:880–886. [PubMed: 17529984]
21. Hirasawa M, et al. Perinatal abrogation of Cdk5 expression in brain results in neuronal migration defects. *Proceedings of the National Academy of Sciences of the United States of America* 2004;101:6249–6254. [PubMed: 15067135]
22. Benavides DR, et al. Cdk5 modulates cocaine reward, motivation, and striatal neuron excitability. *J Neurosci* 2007;27:12967–12976. [PubMed: 18032670]
23. Noctor SC, et al. Neurons derived from radial glial cells establish radial units in neocortex. *Nature* 2001;409:714–720. [PubMed: 11217860]
24. Ko J, et al. p35 and p39 are essential for cyclin-dependent kinase 5 function during neurodevelopment. *J Neurosci* 2001;21:6758–6771. [PubMed: 11517264]
25. Gilmore EC, et al. Cyclin-dependent kinase 5-deficient mice demonstrate novel developmental arrest in cerebral cortex. *J Neurosci* 1998;18:6370–6377. [PubMed: 9698328]
26. Chae T, et al. Mice lacking p35, a neuronal specific activator of Cdk5, display cortical lamination defects, seizures, and adult lethality. *Neuron* 1997;18:29–42. [PubMed: 9010203]
27. Ohshima T, et al. Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. *Proceedings of the National Academy of Sciences of the United States of America* 1996;93:11173–11178. [PubMed: 8855328]
28. Altman J, Bayer SA. Mosaic organization of the hippocampal neuroepithelium and the multiple germinal sources of dentate granule cells. *J. Comp. Neurol* 1990;301:325–342. [PubMed: 2262594]
29. Tashiro A, et al. NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. *Nature* 2006;442:929–933. [PubMed: 16906136]
30. Kempermann G, et al. Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development (Cambridge, England)* 2003;130:391–399.
31. Shapiro LA, et al. GFAP-expressing radial glia-like cell bodies are involved in a one-to-one relationship with doublecortin-immunolabeled newborn neurons in the adult dentate gyrus. *Brain research* 2005;1040:81–91. [PubMed: 15804429]
32. Hirota Y, et al. Cyclin-dependent kinase 5 is required for control of neuroblast migration in the postnatal subventricular zone. *J Neurosci* 2007;27:12829–12838. [PubMed: 18032654]
33. Tanaka T, et al. Cdk5 phosphorylation of doublecortin ser297 regulates its effect on neuronal migration. *Neuron* 2004;41:215–227. [PubMed: 14741103]
34. Schaar BT, et al. Doublecortin microtubule affinity is regulated by a balance of kinase and phosphatase activity at the leading edge of migrating neurons. *Neuron* 2004;41:203–213. [PubMed: 14741102]
35. Kawauchi T, et al. Cdk5 phosphorylates and stabilizes p27kip1 contributing to actin organization and cortical neuronal migration. *Nature cell biology* 2006;8:17–26.
36. Nikolic M, et al. The p35/Cdk5 kinase is a neuron-specific Rac effector that inhibits Pak1 activity. *Nature* 1998;395:194–198. [PubMed: 9744280]
37. Xie Z, et al. Serine 732 phosphorylation of FAK by Cdk5 is important for microtubule organization, nuclear movement, and neuronal migration. *Cell* 2003;114:469–482. [PubMed: 12941275]
38. Niethammer M, et al. NUDEL is a novel Cdk5 substrate that associates with LIS1 and cytoplasmic dynein. *Neuron* 2000;28:697–711. [PubMed: 11163260]
39. Duan X, et al. Disrupted-In-Schizophrenia 1 Regulates Integration of Newly Generated Neurons in the Adult Brain. *Cell* 2007;6:1146–1158. [PubMed: 17825401]

40. Couillard-Despres S, et al. Doublecortin expression levels in adult brain reflect neurogenesis. *The European journal of neuroscience* 2005;21:1–14. [PubMed: 15654838]
41. Higginbotham HR, Gleeson JG. The centrosome in neuronal development. *Trends in neurosciences* 2007;30:276–283. [PubMed: 17420058]
42. Barnes AP, et al. New insights into the molecular mechanisms specifying neuronal polarity in vivo. *Current opinion in neurobiology* 2008;18:44–52. [PubMed: 18514505]
43. Cheung ZH, et al. Cdk5 Is Involved in BDNF-Stimulated Dendritic Growth in Hippocampal Neurons. *PLoS biology* 2007;5:e63. [PubMed: 17341134]
44. Cheung ZH, Ip NY. The roles of cyclin-dependent kinase 5 in dendrite and synapse development. *Biotechnology journal* 2007;2:949–957. [PubMed: 17526057]
45. Sasaki Y, et al. Fyn and Cdk5 mediate semaphorin-3A signaling, which is involved in regulation of dendrite orientation in cerebral cortex. *Neuron* 2002;35:907–920. [PubMed: 12372285]
46. Ohshima T, et al. Cdk5 is required for multipolar-to-bipolar transition during radial neuronal migration and proper dendrite development of pyramidal neurons in the cerebral cortex. *Development (Cambridge, England)*. 2007
47. Wenzel HJ, et al. Dentate development in organotypic hippocampal slice cultures from p35 knockout mice. *Developmental neuroscience* 2007;29:99–112. [PubMed: 17148953]
48. Wenzel HJ, et al. Abnormal morphological and functional organization of the hippocampus in a p35 mutant model of cortical dysplasia associated with spontaneous seizures. *J Neurosci* 2001;21:983–998. [PubMed: 11157084]
49. Luo L. Rho GTPases in neuronal morphogenesis. *Nature reviews* 2000;1:173–180.
50. Kim Y, et al. Phosphorylation of WAVE1 regulates actin polymerization and dendritic spine morphology. *Nature* 2006;442:814–817. [PubMed: 16862120]
51. Kesavapany S, et al. p35/cyclin-dependent kinase 5 phosphorylation of ras guanine nucleotide releasing factor 2 (RasGRF2) mediates Rac-dependent Extracellular Signal-regulated kinase 1/2 activity, altering RasGRF2 and microtubule-associated protein 1b distribution in neurons. *J Neurosci* 2004;24:4421–4431. [PubMed: 15128856]
52. Fu WY, et al. Cdk5 regulates EphA4-mediated dendritic spine retraction through an ephexin1-dependent mechanism. *Nat Neurosci* 2007;10:67–76. [PubMed: 17143272]
53. Lin W, et al. Neurotransmitter acetylcholine negatively regulates neuromuscular synapse formation by a Cdk5-dependent mechanism. *Neuron* 2005;46:569–579. [PubMed: 15944126]
54. Fu AK, et al. Cdk5 is involved in neuregulin-induced AChR expression at the neuromuscular junction. *Nat Neurosci* 2001;4:374–381. [PubMed: 11276227]
55. Morabito MA, et al. Cyclin-dependent kinase 5 phosphorylates the N-terminal domain of the postsynaptic density protein PSD-95 in neurons. *J Neurosci* 2004;24:865–876. [PubMed: 14749431]
56. Seeburg DP, et al. Critical role of CDK5 and Polo-like kinase 2 in homeostatic synaptic plasticity during elevated activity. *Neuron* 2008;58:571–583. [PubMed: 18498738]
57. Samuels BA, et al. Cdk5 promotes synaptogenesis by regulating the subcellular distribution of the MAGUK family member CASK. *Neuron* 2007;56:823–837. [PubMed: 18054859]
58. Toni N, et al. Synapse formation on neurons born in the adult hippocampus. *Nat Neurosci* 2007;10:727–734. [PubMed: 17486101]
59. Fischer A, et al. Opposing roles of transient and prolonged expression of p25 in synaptic plasticity and hippocampus-dependent memory. *Neuron* 2005;48:825–838. [PubMed: 16337919]
60. Cheung ZH, et al. Cyclin-dependent kinase 5 supports neuronal survival through phosphorylation of Bcl-2. *J Neurosci* 2008;28:4872–4877. [PubMed: 18463240]
61. Li BS, et al. Cyclin-dependent kinase-5 is involved in neuregulin-dependent activation of phosphatidylinositol 3-kinase and Akt activity mediating neuronal survival. *The Journal of biological chemistry* 2003;278:35702–35709. [PubMed: 12824184]
62. Li BS, et al. Cyclin-dependent kinase 5 prevents neuronal apoptosis by negative regulation of c-Jun N-terminal kinase 3. *The EMBO journal* 2002;21:324–333. [PubMed: 11823425]
63. Zheng YL, et al. Cdk5 Modulation of mitogen-activated protein kinase signaling regulates neuronal survival. *Molecular biology of the cell* 2007;18:404–413. [PubMed: 17108320]

64. Sharma P, et al. Phosphorylation of MEK1 by cdk5/p35 down-regulates the mitogen-activated protein kinase pathway. *The Journal of biological chemistry* 2002;277:528–534. [PubMed: 11684694]
65. Harada T, et al. ERK induces p35, a neuron-specific activator of Cdk5, through induction of Egr1. *Nature cell biology* 2001;3:453–459.
66. Cheung ZH, Ip NY. Cdk5: mediator of neuronal death and survival. *Neuroscience letters* 2004;361:47–51. [PubMed: 15135890]
67. Cruz JC, Tsai LH. Cdk5 deregulation in the pathogenesis of Alzheimer's disease. *Trends Mol Med* 2004;10:452–458. [PubMed: 15350898]
68. Dhariwala FA, Rajadhyaksha MS. An unusual member of the Cdk family: Cdk5. *Cellular and molecular neurobiology* 2008;28:351–369. [PubMed: 18183483]
69. Sun KH, et al. Novel genetic tools reveal Cdk5's major role in Golgi fragmentation in Alzheimer's disease. *Molecular biology of the cell* 2008;19:3052–3069. [PubMed: 18480410]
70. Sun KH, et al. Deregulated Cdk5 promotes oxidative stress and mitochondrial dysfunction. *J Neurochem* 2008;107:265–278. [PubMed: 18691386]
71. Qu D, et al. Role of Cdk5-mediated phosphorylation of Prx2 in MPTP toxicity and Parkinson's disease. *Neuron* 2007;55:37–52. [PubMed: 17610816]
72. Wang J, et al. Cdk5 activation induces hippocampal CA1 cell death by directly phosphorylating NMDA receptors. *Nat Neurosci* 2003;6:1039–1047. [PubMed: 14502288]
73. Li BS, et al. Regulation of NMDA receptors by cyclin-dependent kinase-5. *Proceedings of the National Academy of Sciences of the United States of America* 2001;98:12742–12747. [PubMed: 11675505]
74. Gong X, et al. Cdk5-mediated inhibition of the protective effects of transcription factor MEF2 in neurotoxicity-induced apoptosis. *Neuron* 2003;38:33–46. [PubMed: 12691662]
75. Kim D, et al. Deregulation of HDAC1 by p25/Cdk5 in neurotoxicity. *Neuron* 2008;60:803–817. [PubMed: 19081376]
76. van Praag H, et al. Functional neurogenesis in the adult hippocampus. *Nature* 2002;415:1030–1034. [PubMed: 11875571]
77. Zhao C, et al. Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *J Neurosci* 2006;26:3–11. [PubMed: 16399667]
78. Toni N, et al. Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nat Neurosci* 2008;11:901–907. [PubMed: 18622400]
79. Lagace DC, et al. Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. *J Neurosci* 2007;27:12623–12629. [PubMed: 18003841]
80. Doetsch F, et al. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell* 1999;97:703–716. [PubMed: 10380923]
81. Suh HK, et al. In Vivo Fate Analysis Reveals the Multipotent and Self-Renewal Capacities of Sox2 + Neural Stem Cells in the Adult Hippocampus. *Cell Stem Cell* 2007;1:515–528. [PubMed: 18371391]
82. Tanaka T, et al. The doublecortin and doublecortin-like kinase 1 genes cooperate in murine hippocampal development. *Cereb Cortex* 2006;16:i69–i73. [PubMed: 16766710]
83. Chubb JE, et al. The DISC locus in psychiatric illness. *Molecular psychiatry* 2008;13:36–64. [PubMed: 17912248]
84. Lie DC, et al. Wnt signalling regulates adult hippocampal neurogenesis. *Nature* 2005;437:1370–1375. [PubMed: 16251967]

Figure 1. Cellular and molecular functions of Cdk5 in embryonic and adult neurogenesis
(A) Cdk5 was initially identified to be critical for proper neuronal migration and neuronal process extension during embryonic development (left part of the panel). During embryonic cortical development newborn neurons (green) migrate along radial glial fibers (blue) to build the cortex. Cdk5 deficiency impairs radial migration of neurons, leading to severe defects in the layering of the cortex. In addition, Cdk5 deficiency results in abnormal dendritic morphology. Recent studies suggest that Cdk5 also regulates neuronal migration and neuronal process extension in the context of adult neurogenesis (right part of the panel). In addition, Cdk5 is critically involved in spine and synapse formation of newborn hippocampal granule cells of the adult brain and regulates the survival of newborn neurons within the adult dentate

gyrus as Cdk5-knockout cells show high levels of cell death (represented by red cell in the figure). **(B)** This scheme depicts the most prominent signaling pathways in which Cdk5 is involved. Cdk5 regulates neuronal migration (pink), dendritic growth and spine formation (green), survival/apoptosis, and cell cycle (blue). Note that this list of interaction partners and molecular targets of Cdk5 is not complete and is constantly growing.

Figure 2. Strategies to analyze gene function in adult neurogenesis

(A) The effect of gene deletion on certain aspects of adult neurogenesis can be analyzed with the use of conditional mutants crossed with a transgenic line conditionally expressing Cre-recombinase under a “neural stem cell” promoter. (B) Alternatively, the use of retroviral vectors either expressing the gene of interest, a shRNA directed against this gene, or Cre-recombinase (when injected in conditional mouse mutants) is a powerful approach to characterize gene function in adult neurogenic zones. The advantages and potential disadvantages are listed.