

Journal Club

CDK5

The “pathfinder” for new born neurons in adult hippocampus?

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Neurogenesis takes place in the mammalian hippocampus throughout the whole life and deficient adult hippocampal neurogenesis has been related to neurological conditions like Alzheimer’s disease (AD), Parkinson’s disease (PD) and epilepsy. The molecular mechanisms by which immature neurons and their extending neurites find their appropriate position and target area remain largely unknown. Recent work by Jessberger et al.¹ examines the role of Cdk5 in normal adult neurogenesis by a retroviral knock-down approach. Cdk5 is shown to be implicated in the migration of newborn neurons into the granule cell layer (GCL), as well as, in correct targeting of dendrites from newborn granule cells (GC) into the molecular layer (ML) of the dentate gyrus (DG). The study also shows that aberrant dendrites still seem to become synaptically integrated into the existing circuitry thereby suggesting a mechanistic dissociation between accurate dendritic targeting and subsequent synapse formation. The finding of Cdk5 guiding this integration of new born neurons at the physiologically appropriate place is an important step towards understanding adult neurogenesis that may help to overcome problems with the restorative use of neural stem cells in present grafting approaches in neurological diseases.

Introduction

In contrast to the leading opinion in brain research for more than a hundred years, it has been well established in the past 20 years that neurogenesis in the mammalian brain continues during adulthood. Neural stem (NSC) and progenitor cells (NPCs) persist in the subventricular zone (SVZ) of the lateral ventricles, as well as in the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus. These are the two sites where adult neurogenesis has been shown to take place and intensely studied in

this context.²⁻⁴ New neurons are added into the dentate circuitry throughout life, with the hippocampal NPCs giving rise to only one neuronal subtype: the highly polarized, excitatory dentate GCs. Glutamatergic neurons receive their main excitatory input onto dendrites extending into the molecular layer (ML) and send out axons towards area CA3 targeting pyramidal cells, inhibitory basket cells, interneurons and excitatory mossy cells. It has been shown that, after undergoing morphological and electrophysiological maturation, newborn GCs are incorporated into the functional network of adult dentate gyrus.³

Structural changes in the hippocampus—through synaptic plasticity and generation of new neurons and their integration into the circuitry—are widely accepted to be the underlying mechanisms of memory and learning.³⁻⁶ Using an elegant new strategy for engineering strains of mutant mice, a recent study by Itaru Imayoshi⁷ and his colleagues now provide strong evidence that continuous generation of new neurons is critical for brain function, and that the newly-generated cells perform two distinct roles. In the olfactory system, the new neurons are critical for tissue maintenance whereas in the hippocampus, new born neurons are important for the formation of associative and spatial memory. Deficit in hippocampal neurogenesis has been associated with several neurological diseases, including AD, major depression^{4,8,9} and epilepsy.^{10,11} However, the regulatory genes required by newborn neurons in adult brain for migration, and pathfinding still remain largely unknown. Jessberger et al.¹ hypothesize that the critical factors for correct integration of newborn neurons in the adult brain might be different from the ones necessary during embryogenesis or early post-natal development. Instead, they argue that previously suggested models of axonal growth which predict a concerted repulsion by chemorepellents expressed at a certain time postnatally, are difficult to apply to the mechanisms of pathfinding and integration of newborn neurons in the mature hippocampus,¹² mainly because of different environmental cues in a mature adult brain as compared to the developing brain. Their findings shed some light on the molecular mechanisms by which new neurons are integrated in the adult brain and have potential implications for improving cell replacement therapies in the treatment of neurological diseases.

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Cdk5—The Black Sheep of the Cyclin-Dependent Kinases' Family...

Cdk5 is a highly versatile kinase that requires association with its regulatory partner p35 for activation.¹³ Despite of its classification as a member of the family of cyclin-dependent kinases ("cdk-s") because of its sequence homology of >60% to, for e.g., the mammalian Cdk2, it is neither "dependent on cyclin" for its activation, nor implicated in the cell cycle regulatory functions, as the other members of this family are.¹⁴

As a Prime Suspect for Proper Neurogenesis in the Adult Brain

Instead, Cdk5 plays a role in a variety of neurobiological processes, such as homeostatic synaptic plasticity, neuronal degeneration, dopamine signaling, and learning & memory.^{15,16} Moreover, it has been reported to be crucial for proper embryonic corticogenesis and neuronal migration, and for proper neurite development^{17,18} and synaptogenesis.¹⁴ However, as mentioned earlier, such implication during development does not inevitably allow to deduce a similar role of Cdk5 in neuronal development of the adult brain, as in the adult DG the new born neurons will have to integrate into a fully mature and functional environment of pre-existing neural circuitries. Besides, its indispensable role during early years of life, Cdk5 has also been implicated in normal adult neurophysiology, for its inhibition in hippocampus profoundly impairs associative learning and memory.¹⁹ Studies have also implicated Cdk5 in neurodegenerative diseases, like Alzheimer's disease²⁰ and amyotrophic lateral sclerosis,²¹ Parkinson's disease,²² to name a few.

The authors' interest in *cdk5* was raised when it turned up in a genetic screen of chromosome regions involved in adult neurogenesis. In this study, by analyzing quantitative trait loci (i.e., the quantitative variance of a phenotypic characteristic attributable to the interaction between two or more loci on the genome), the authors reveal critical implication of a region on the chromosome 5 of the mouse genome, containing the *cdk5* gene, in adult neurogenesis.

Incriminating *cdk5* by Retroviral Strategy

Using a retroviral approach, Jessberger et al.¹ injected a retrovirus construct into the hippocampus of adult mice, carrying—along with GFP—either a transgene of *cdk5* to induce *cdk5* overexpression ("CDK5"), or a dominant-negative form of *cdk5* ("DN*cdk5*") to induce a knock-down, or a control virus harboring the GFP gene only (control). GFP fills soma as well as the processes, making even structural analysis possible.³ Because the retroviral vector CAG-GFP is replication incompetent, only the very few dividing cells at the time of surgery were infected and subsequently expressed GFP, making possible to track the fate and localization of new born cells.

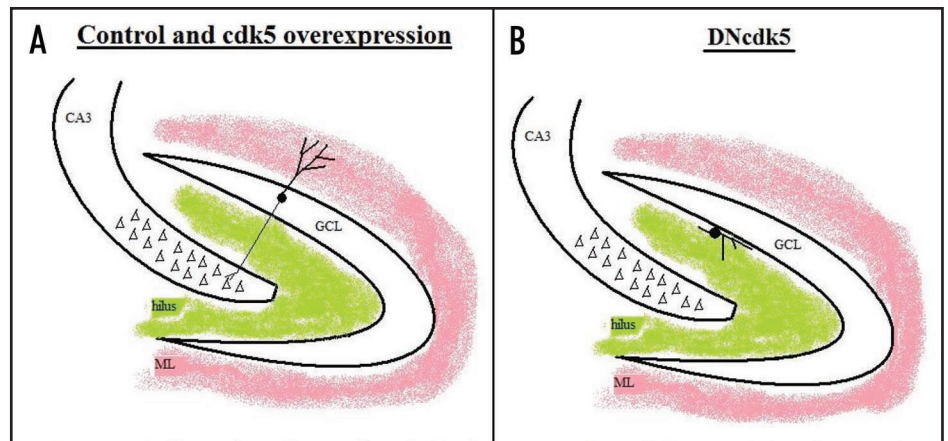


Figure 1. (A) Illustration of a normally migrated neuron in the granular cell layer (GCL) and correctly targeted dendrites into molecular layer (ML) as observed in control or Cdk5-overexpression. (B) Cartoon depicting of a similar neuron from animal lacking Cdk5 due to viral injection (DN*cdk5*): GC progenitor did not properly migrate and exhibited dendrites targeted along GCL or towards hilus due.

Cdk5 Surely Shows the Way

In case of DN*cdk5*, Jessberger et al.¹ found more than 50% of the neurons not extending their dendrites into ML, but along GCL or even towards the inferior hilus (HL), while control or CDK5 never developed a dendrite extending towards HL, but correctly targeted ML (Fig. 1A). Also, interestingly Cdk5 overexpression had no effect on dendritic complexity. By measuring the total dendritic length and number of branching points 4 wk after virus injection, the authors found that Cdk5 inhibition (DN*cdk5*) reduced both the number of dendritic branching points and total dendritic length irrespective of the position of dendrites within the dentate area, whereas retroviral overexpression of *cdk5* had no effect on dendritic architecture. They further show that Cdk5 is not only essential for correct dendritic targeting of new born granule cells, but also for proper migration of the entire cell, as DN*cdk5* newborn granule cells did not migrate into GCL (Fig. 1B), whereas control cells did significantly migrate into GCL showing the potential of becoming integrated into the circuitry at the same level as already existing granule cells. The authors conclude that aberrant dendrites are rather due to a cell-autonomous effect and not due to a modification of the neurogenic niche, which they were not able to exclude in one of their previous studies on epilepsy.¹¹ Additionally, the authors suggest that the improper connections formed by these cells could interfere with information processing in the hippocampus. In one of their previous work,¹¹ they have shown epilepsy to be associated with increased neurogenesis in the hippocampus along with impairment in correct targeting of the dendrites. In this context, a follow up study using behavioral testes to provide definitive evidence that *cdk5* inhibition impairs memory formation would provide a better insight into hippocampal dependent learning and memory functions.

Cdk5 Critical for Spine Maturation

Using fluorescent confocal microscopy and electron microscopy, the authors found that aberrantly targeted dendrites still

showed spines—although significantly decreased in number and maturation, as measured by the number of mushroom spines. Notably, Cdk5 inhibition caused no significant effect on the spine density of the correctly targeted (towards the ML) dendrites extending from the *DNcdk5*-expressing neurons, thereby suggesting that *cdk5* deficiency itself does not reduce the number of spines. However, *cdk5* inhibition impaired the maturation of dendritic spines into mushroom spines in wrongly as well as in correctly targeted dendrites, indicating it to be an important target for spine maturation. Furthermore, Jessberger et al.¹ found that mushroom spines of misguided dendrites, were in close proximity to the pre-synaptic marker synapsin, suggesting the functional integration into the circuitry of those aberrantly targeted dendrites. A question then arises that,¹⁵ whether the ectopic synapses have any functional significance. It would, therefore, be interesting to know if synaptic connections with the wrong targets, shown to persist for one year are actually functional and whether they are similar to dentate glutamatergic synapses in terms of receptor expression, electrical properties etc.

Without any alteration in the fate of newborn neurons, Jessberger et al.¹ have provided strong evidence for the role of Cdk5 in correct integration of the newborn neurons into the neuronal circuitry of DG. For newborn neurons, Cdk5, therefore, is critically involved for correct migration, for appropriate targeting of their dendrites into ML as well as in accurate maturation of the dendritic spines. From correctly and aberrantly targeted dendrites, which both showed functional synaptic integration, the authors concluded distinct regulatory processes for dendritic targeting and synaptic integration, the latter not being dependent on Cdk5 and maybe solely dependent on the apposition of filopodia and synaptic buttons. Furthermore, they are suggesting different potential downstream targets getting phosphorylated by Cdk5, possibly causing the morphological changes in the dendrites, for example, DCX²³ (a microtubule associated protein) or semaphorin-3A²⁴ (known to be necessary for the apical dendrite extension during cortical development).

As the field advances, it is now established that newborn neurons in adult hippocampus are essential for associative and spatial memory,⁷ as well as for normal pattern separation function in the DG of adult mice.²⁵ Present work by Jessberger et al.¹ could have significant consequences for neural tissue transplantation strategies aiming at restorative brain therapy following brain injuries or neurodegenerative diseases. It shows that inactivating a specific gene in adult NSC or NPC in the hippocampus makes emerging neurons form wrong connections. This reflects the need for therapeutic approaches assuring that cells used in regenerative medicine are put in a position to make appropriate rather than promiscuous connections. The clinical benefits of experimental transplants have been inconsistent and largely disappointing to date, with most transplanted neurons unable to integrate into existing brain circuits.

Whatever the precise mechanisms, the discovery of *cdk5*'s role in guiding new neurons to their proper place improves the understanding of adult neurogenesis, boosts its contribution to cognitive function and brightens the future for brain cell therapy.

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