Neuronal Motility and Structure: Cdk5 Pathways

B A Samuels and L-H Tsai, Massachusetts Institute of Technology, Cambridge, MA, USA

Introduction to Cdk5

Cdk5 and the Cyclin-Dependent Kinase Family

Cdk5 is an atypical cyclin-dependent kinase Cyclin-dependent kinase 5 (Cdk5) was first isolated based on its homology to other Cdks, specifically human cell division control kinase 2 (Cdc2/Cdk1). The typical role of a Cdk is to phosphorylate substrates that drive progression through the cell cycle. Cdc2, for example, is the catalytic subunit of the maturation promoting factor (MPF) that is necessary for the G2/M phase transition. Cdk5, however, is an atypical Cdk. There is no strong evidence that Cdk5 is functionally involved in promoting the cell cycle. In fact, the principal associated kinase activity of Cdk5 is detected in the central nervous system, where the protein is enriched in postmitotic neurons. Although this signifies a functional distinction from other Cdks, Cdk5 does share a few very basic characteristics with members of the Cdk family that are important for understanding its various roles.

p35 and p39 are essential activators of Cdk5 Monomeric forms of Cdks do not display any kinase activity; instead, association with an activator is required. Most Cdks associate with and are activated by cyclins, a family of unstable proteins whose expression level during the cell cycle is phase dependent. For example, MPF is achieved through the association of Cdc2 with cyclin B, a protein whose expression rises through the cell cycle until M phase, when it is abruptly degraded. Such regulation of activator expression and turnover is a major mechanism underlying the regulation of Cdk activity. Cdk5 activity is achieved through association with one of two activators, p35 or p39. Although p35 and p39 are very similar to each other, they have little sequence similarity to cyclins. However, the tertiary structure of a portion of p35 bears some resemblance in that it adopts a cyclin-like fold. Another similarity to cyclins is that p35 is very unstable. Ubiquitination of p35 is readily detectable, and the protein is rapidly targeted for degradation by the proteasome, retaining only a 20–30 min half-life in primary cultured cortical neurons.

Posttranslational modifications of Cdk5 In addition to the turnover rate of the activators, Cdk5 activity, like that of other Cdks, can be regulated by posttranslational modifications such as phosphorylation. Phosphorylation of Cdk5 on tyrosine 15 enhances Cdk5 activity. Cdk5 associates with the c-Abelson tyrosine kinase (c-Abl) through a linker protein termed Cables, and this association stimulates phosphorylation of Cdk5 on Y15 in a c-Abl-dependent manner. Cdk5 may also be phosphorylated on serine 159 by casein kinase I. Structural studies suggest that this phosphorylation event would inhibit Cdk5 activity by preventing association with its activators.

Consensus site for phosphorylation by Cdk5 The only other similarity to the Cdk family is that Cdk5 shares the consensus phosphorylation site on its substrates. Cyclin-dependent kinases are proline-directed serine/threonine kinases, phosphorylating substrates at the minimal consensus motif S/TP. Cdc2, Cdk2, and Cdk5 also prefer a basic residue in the +3 position, making the consensus sequence for phosphorylation (S/T)PX(K/H/R).

Cdk5 and Its Activators

Cdk5 activity is defined by the distribution of p35 and p39 Whereas Cdk5 protein is enriched in postmitotic neurons, its expression is widespread in nonneuronal tissues. Importantly, Cdk5 activity is primarily restricted to the nervous system because p35 and p39 are detected at high levels exclusively in neurons. The intracellular distribution of the activators is likely also important for regulating Cdk5 activity and restricting the kinase to its physiological substrates. In this regard, p35 contains an N-terminal myristoylation site that helps target kinase activity to membrane fractions. Cdk5 and its activators are also distributed to detergent-insoluble cytoskeleton fractions and both pre- and postsynaptic compartments. The spatial distribution pattern of Cdk5 activity defines its chief physiological roles, which seem to be regulating cytoskeletal elements during developmental neuronal migration and synaptic function in the mature nervous system. A diverse group of identified Cdk5 substrates have corroborated these functions and also have implicated altered regulation of the kinase in the pathology of neurodegeneration.

Cdk5 Regulates Neuronal Migration

Mouse Models of Cdk5 Deficiency

Cerebral cortex The best-understood role of Cdk5 is in the regulation of the cytoarchitecture of the central nervous system. The characteristic phenotype of mice deficient in Cdk5 activity is an inverted laminar organization of pyramidal neurons in the cerebral cortex. Mice deficient in Cdk5 and double knockouts of p35 and p39 display indistinguishable phenotypes, suggesting that p35 and p39 are the only activators of Cdk5 during development. p35 knockout mice display a similar but less severe phenotype and survive into adulthood.

Other brain regions In addition to the inverted neocortex, mice lacking Cdk5 activity display widespread cytoarchitectural abnormalities in many brain regions. The pyramidal neurons of the cornus ammonus in the hippocampus form only diffuse laminae with cell-free rifts between clusters of cells. Furthermore, the dentate gyrus of the hippocampus is imperceptible in Cdk5-deficient mice. The cerebellum of Cdk5-deficient mice is also strikingly abnormal because it completely lacks foliation (the several folds or gyri normally seen on the cerebellar surface), is much smaller in size, and does not have a Purkinje cell layer. There are also major disruptions in the olfactory bulb, thalamus, and brain stem. Furthermore, motor neurons exhibit ballooned perikarya suggestive of chromatolysis, where the cell body swells, Nissl bodies disperse, and the nucleus becomes displaced peripherally in the cell. Chromatolysis, also known as the 'axonal reaction,' is commonly seen upon damage or lesion to an axon.

Brief Introduction to Neocortical Development

Different neurons in the neocortex come from distinct places Pyramidal neurons and interneurons are the principal types of neurons comprising the neocortex, but these different classes of neurons take distinct paths to their final destination. The pyramidal neurons are generated in the dorsal ventricular zone and migrate in a radial manner toward the pial surface before reaching their final cortical position. Interneurons, on the other hand, are produced in the ventral telencephalon and must migrate dorsally by taking a tangential route. Consequently, the distance traversed by the tangentially migrating interneurons is much longer than that of the radially migrating pyramidal neurons (Figure 1).

Formation of cortical laminae Classic studies of corticogenesis have shown that the earliest born cortical neurons align above the ventricular zone to form the preplate, which signifies the first distinct event in the histogenesis of the neocortex. The next group of neurons born in the ventricular zone migrates radially to split the preplate into the superficial marginal zone and the deeper subplate, forming the cortical plate in between. The assembly of the cortical plate proceeds in an 'inside-out' manner, in which each cohort of neurons migrates past its cortical plate predecessors to form a more superficial layer (closer to the eventual pial surface). Ultimately, the six cortical laminae can

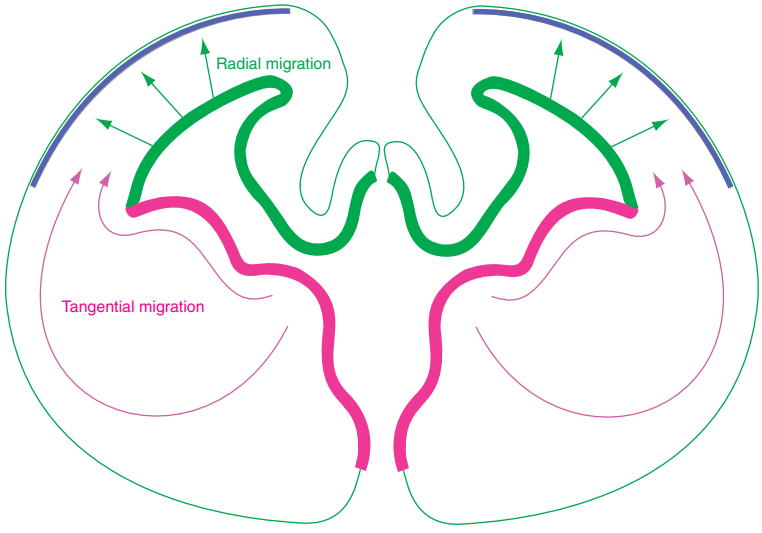

Figure 1 Two distinct routes of neuronal migration. Pyramidal neurons are produced along the ventricular surface in the dorsal telencephalon (heavy green band) and migrate in a radial direction (green arrow) to the outermost layer of the developing cortex (heavy blue band). Interneurons are produced in the ventral telencephalon (light red band) and migrate along a much longer route in a tangential manner (red arrow).

be distinguished by the earliest born neurons residing in the deepest layers of the neocortex and the latest born neurons forming the most superficial layer (Figure 2).

Cdk5 Activity Is Essential for Proper Neocortical Lamination

Formation of cortical laminae in p35 knockout mice
Neuronal birth dating and histological experiments demonstrated that in p35-deficient mice early born neurons are capable of splitting the preplate. However, unlike normal inside-out cortical plate assembly, each subsequent cohort of neurons in p35-deficient mice is unable to migrate past its predecessors. Therefore, without proper Cdk5 activity, corticogenesis proceeds in an erroneous 'outside-in' manner, resulting in an inverted laminar structure.

Reelin Signaling Also Regulates Neuronal Migration

The phenotype of the mutant mice has cemented the Cdk5 pathway as a major regulator of neuronal migration during development of the central nervous system. However, to fully grasp the importance of Cdk5 in brain development, it is necessary to also consider another classical neuronal migration pathway that involves Reelin signaling. Upon gross inspection, there are many similarities in the abnormal neuronal cytoarchitecture between Cdk5 and Reelin mutant mice.

Mouse Models of Reelin Deficiency

Multiple mutant mice deficient in Reelin signaling have been described, including naturally occurring mutants such as *reeler*, *scrambler*, and *yotari*, as well as genetically modified mice such as Dab1, VLDLR, and ApoER2. In *reeler* mice, first described approximately 50 years ago for their ataxic 'reeling' gait, the early born neurons intended to form the cortical plate fail to split the preplate. Subsequent waves of migrating neurons are unable to pass their predecessors and accumulate in gradually deeper positions. Therefore, similar to Cdk5 mutants, *reeler* mice have an inverted laminar organization of the neocortex. The defective migration in these mice is attributable to a deficiency in the protein Reelin, a very large secreted extracellular matrix protein. The other naturally occurring or genetically modified mice with identical phenotypes have defined a pathway in which Reelin binds to VLDLR and ApoER2 receptors on the surface of migrating cells, which triggers a signaling cascade mediated by tyrosine phosphorylation of Disabled-1 (Dab1), the gene that is mutated in *scrambler* and *yotari* mice.

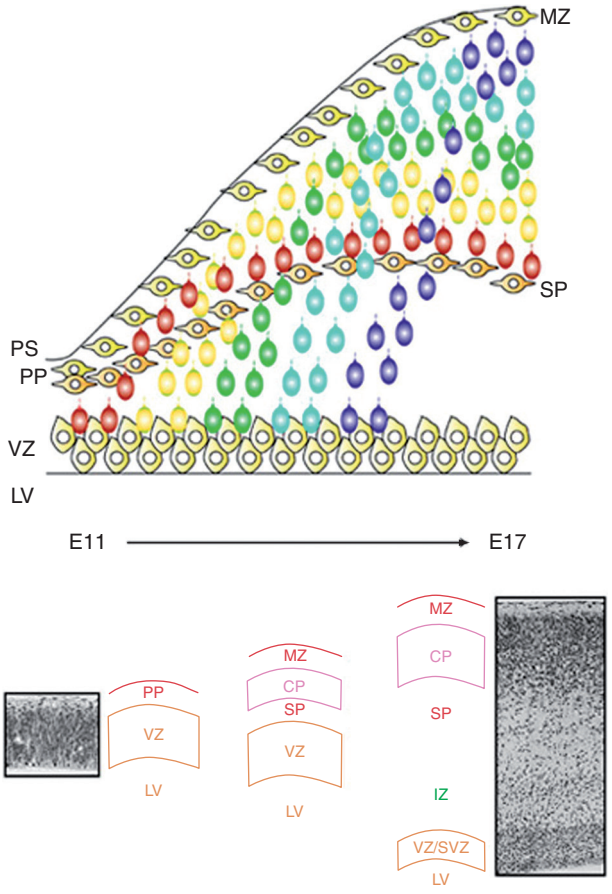

Figure 2 Development of the mouse cerebral cortex via radial migration from E11 to E17. (Top) Corticogenesis proceeds in an 'inside-out' manner. Neuronal progenitors are present in the ventricular zone (VZ) along the surface of the lateral ventricle (LV). The earliest born neurons align above the VZ and form the preplate at the young pial surface (PS). The next wave of neurons (red) to be born splits the preplate into the marginal zone and subplate, forming the cortical plate in between. The following cohort of neurons (yellow) migrates past their cortical plate predecessors (red) to form a more superficial layer. Successive waves of neurons then continue to migrate past their predecessors into six distinct laminae, with the earliest born cortical plate neurons residing in the deepest layer. This is described as inside-out formation of the cerebral cortex. Also labeled are the preplate (PP), the superficial marginal zone (MZ), and the subplate (SP). (Bottom) Distinct cortical zones during development. The VZ exists along the surface of the LV. The first wave of neurons, the PP, aligns above the VZ. The next wave of neurons forms the cortical plate, splitting the PP into the superficial MZ (at the pial surface and composed mostly of Cajal–Retzius cells) and the SP. As the brain matures, neurons must migrate longer distances, including through a cell body-sparse intermediate zone (IZ) composed mostly of an increasing number of projecting axons.

Distinctions between Cdk5 and Reelin Mutant Mice

Formation of the Preplate

The similarities between mice deficient in Reelin and Cdk5 signaling have fueled much research aimed at

determining if there is any potential cross-talk between these two pathways. However, time has demonstrated that it is really the distinctions, not the similarities, between these mutant mice that are more useful in defining the relative importance of the pathways and understanding the developmental neuronal migration program. One major difference is that early born cortical plate neurons in Cdk5-deficient mice migrate fairly normally and split the preplate into the superficial marginal zone and the deeper subplate. However, in Reelin-deficient mice these early born neurons intended to form the cortical plate fail to split the preplate, instead accumulating in a deep position, beneath the preplate.

Migratory Mode

Further distinctions in Cdk5 and Reelin mice have come from analysis of the behavior of migrating neurons at the cellular level *in vivo*. Neurons that migrate radially to form the neocortex (i.e., the pyramidal neurons born in the dorsal ventricular zone) display one of two characteristic migratory modes.

Somal translocation Early born neurons migrate primarily by extending a leading process that attaches to the nearby pial surface. The cell body of the migrating neuron then moves in a rather continuous manner toward the pial surface, with the leading process shortening progressively as the cell traverses. This migratory mode is called somal translocation, and it is likely used by the early born neurons because they have the shortest distance to migrate and are capable of maintaining their leading process and its attachment to the pial surface throughout their journey. Importantly, somal translocation requires only a single step of continuous nuclear movement and consequent shortening of the leading process.

Locomotion Later born neurons that must traverse longer distances display a distinct and more complex migratory behavior described as locomotion. These neurons extend a leading process into the subventricular zone, which they move into and then pause to become multipolar. Some of these neurons then reverse direction and move back into the ventricular zone before reversing polarity, and all of these neurons ultimately use a pia-directed leading process to migrate along their mother radial glia cell scaffold toward their final cortical position. Neurons that are undergoing locomotion display a short leading process that is not attached to the pial surface and migrate in a saltatory manner characterized by short bursts of movement followed by long stationary phases. In the final stage of migration, when their leading process has reached the pial surface and there is only a short distance to traverse, neurons switch from locomotion to somal translocation. This complex migratory behavior of later born neurons, which consists of multiple switches in polarity and repeated bursts of movement, likely requires much more intricate cytoskeletal rearrangement than that of the early born neurons that move by one step of somal translocation.

Migratory modes in Cdk5 and Reelin mutant mice As might have been predicted, the cellular behavior in mice with decreased Cdk5 activity (p35−/− mice) displays mostly normal somal translocation of early born neurons that first form the cortical plate properly by splitting the preplate. However, when the migratory mode should switch to that involving locomotion, p35−/− neurons fail to remain attached to their mother radial glia guide and start to move by an abnormal branched migration mode in which there is repeated extension of a branched leading process and subsequent zigzag movement of the soma to different branch points. This phenotype is in sharp contrast with mice that have a deficiency in the Reelin signaling pathway (scrambler mice). First, early born scrambler neurons fail to attach the endfeet of their leading process within the preplate. More important, however, scrambler neurons seem unable to dissociate from their mother radial glia at the completion of the migratory journey. This rather major distinction between Cdk5- and Reelin-deficient migrating neurons suggests that the two pathways mediate different cellular functions.

Compound Mutant Mice

Further evidence that Cdk5 and Reelin are operating in parallel pathways to regulate neuronal migration comes from studies of compound mutant mice. Mice that are deficient in ApoER2 or Dab1 and p35 exhibit an exacerbated cytoarchitectural phenotype in the hippocampus and neocortex suggestive of synergistic contributions of each pathway to brain development. Taken together, all of these studies suggest that proper lamination of the neocortex requires distinct functions of Cdk5 and Reelin signaling.

Doublecortin and Dynein Pathways in Neuronal Migration

Doublecortin

In addition to those directly involved in Cdk5 and Reelin signaling, several proteins that are also important for proper corticogenesis have been discovered through analysis of mutant mice and human diseases. Most of these proteins are important for cytoskeletal functions or cell adhesion. Missense mutations in

Doublecortin (DCX) can result in an X-linked form of lissencephaly (smooth brain) in humans. Furthermore, silencing of DCX expression with RNAi in rats, or genetic knockout of DCX and DCLK (a related protein) in mice, results in neuronal migration defects. DCX is expressed only in postmitotic neurons and is a microtubule-associated protein containing two tandemly repeated 90-amino acid domains that are capable of binding purified tubulin. Analysis of disease-causing human mutations showed that several of the missense mutations cluster in the tandem repeats. It is believed that DCX normally functions to stabilize microtubules.

Dynein, Lis1, and Ndel1 (Nudel)

Another pathway involved in neuronal migration involves the function of the minus-end microtubule motor dynein and two dynein interacting proteins, Lis1 and Ndel1 (also referred to as Nudel). In addition to DCX, human lissencephaly-causing mutations have been linked with the LIS1 gene. Furthermore, mouse models of Lis1 deficiency show a dosage-dependent effect on neuronal positioning. Ndel1 was first cloned as a Lis1-binding protein, but it can also associate directly with dynein. Further evidence has suggested that both Lis1 and Ndel1 positively regulate dynein function and maintain microtubule networks essential for migration. RNAi-mediated knockdown in embryonic mouse brain of dynein heavy chain, Lis1, or Ndel1 results in severe neuronal positioning defects.

Cdk5 Pathways Controlling Neuronal Migration

Since the phenotype of Cdk5-deficient mice is identical to the double knockout of its activators, p35 and p39, it is Cdk5 kinase activity that is essential for neuronal migration. Therefore, elucidation of relevant Cdk5 substrates will provide the underlying molecular mechanisms by which this kinase regulates neuronal migration. Furthermore, since Cdk5-deficient neurons display irregular migratory behavior that likely requires massive cytoskeletal rearrangement, logic dictates that Cdk5 substrates would regulate cytoskeletal functions during migration.

Cdk5 Phosphorylation of Focal Adhesion Kinase

S732-phosphorylated Focal Adhesion Kinase regulates centrosome-associated microtubules The first Cdk5-dependent phosphorylation event demonstrated to be essential for neuronal migration was phosphorylation of focal adhesion kinase (FAK) at serine 732. FAK was identified as a tyrosine kinase that localizes to focal adhesions, which are structures linking the extracellular matrix to the actin cytoskeleton. Interestingly, Cdk5-dependent phosphorylation of FAK is actually more important for microtubule organization during neuronal migration. S732-phosphorylated FAK, which is abolished in Cdk5-deficient mice, is enriched along a centrosome-associated microtubule fork/cage-like structure that abuts the nucleus in wild-type neurons. In Cdk5-deficient neurons, and in neurons overexpressing the S732A nonphosphorylatable FAK mutant, there is no discernible organization to this microtubule fork/cage structure.

The role of the cytoskeleton in migrating neurons A general hypothesis to explain the role of the cytoskeleton during neuronal migration relies on a simplified model based mainly on *in vitro* experiments in which the movement of a neuron consists of a few steps. First, there is extension of a leading process from the cell soma in the direction of migration, an event that primarily relies on the actin cytoskeleton. At this point, the centrosome, which is the major microtubule-organizing center, is located ahead (in the direction of migration) of the cell soma in the leading process. Second, microtubules linking the centrosome and the nucleus, which occupies most of the cell soma, drive nuclear movement into the leading process. This second step is commonly referred to as nucleokinesis. A third step is retraction of the trailing process. Successive cycles of leading process outgrowth and nucleokinesis will result in a migrating neuron. Importantly, this model suggests that different components of the cytoskeleton have distinct cellular functions during migration (Figure 3).

S732-phosphorylated FAK and nucleokinesis It was hypothesized that the microtubule fork/cage structure decorated with Cdk5-phosphorylated FAK, linking the centrosome to the nucleus, and disorganized in the cases of FAK–S732A expression or Cdk5 deficiency, would be essential for proper nucleokinesis. Indeed, live imaging of neurons migrating *in vitro* showed impaired nuclear movement. When the portion of the nucleus distal to the leading process began to translocate in the direction of migration, the proximal pole of the nucleus failed to move in tandem, resulting in a more rounded nuclear morphology. Furthermore, overexpression of FAK–S732A in embryonic brain by *in utero* electroporation resulted in neuronal migration defects with a characteristic rounded nuclear morphology phenotype that was very similar to what was observed in Cdk5-deficient brains (Figure 4). Therefore, Cdk5 phosphorylation of FAK is important for the maintenance of an essential microtubule network that mediates nucleokinesis. Although the discovery

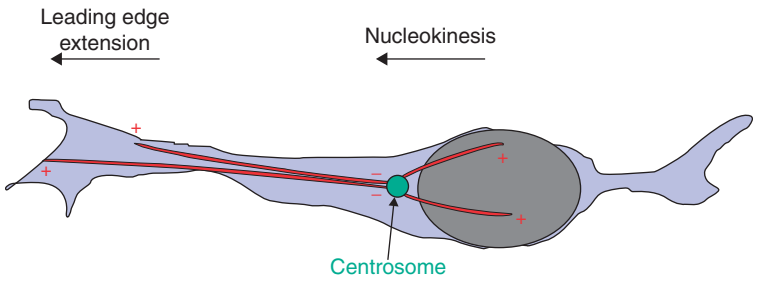

Figure 3 The role of the cytoskeleton in neuronal migration. Neurons first extend a leading process in the direction of migration (leading edge extension), an event primarily dependent on the actin cytoskeleton. Microtubules (red) emanating from the centrosome (green) and abutting the nucleus then drive nucleokinesis, the movement of the nucleus (dark gray) toward and into the leading process.

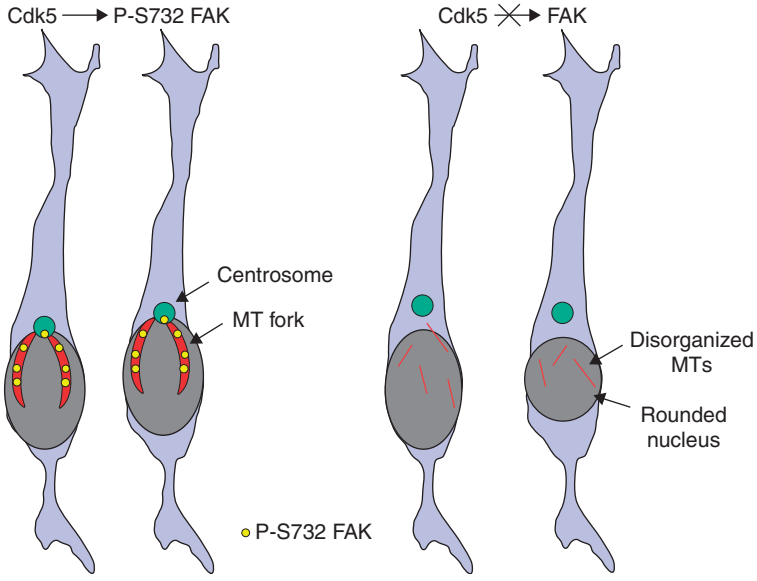

Figure 4 Cdk5 phosphorylation of FAK at S732 regulates nucleokinesis. (Left) Microtubules (red) emanating from the centrosome (green) and abutting the nucleus (dark gray) in a fork-like configuration are decorated with Cdk5-phosphorylated FAK (yellow). Nucleokinesis occurs as the nucleus moves into the leading process (compare nuclear position in the two cells). (Right) In cases of Cdk5 deficiency or expression of a nonphosphorylatable form of FAK, microtubules are severely disorganized. The pole of the nucleus proximal to the leading process fails to move, whereas the distal pole does move. This aberrant coupling of movement results in a more rounded nuclear morphology and a failure of nucleokinesis.

of FAK–S732 phosphorylation represented an important step in understanding the mechanisms that underlie Cdk5-mediated neuronal migration, recent work has suggested that a few other substrates are also important for implementing the complete downstream effects of Cdk5.

Cdk5 Phosphorylation of Doublecortin

An essential neuronal migration protein, DCX, is also a Cdk5 substrate. DCX has been shown to regulate the distance of nuclear-centrosome coupling in neurons, and it is localized to the microtubule fork/cage-like structure linking the centrosome and nucleus. Phosphorylation of DCX at serine 297 by Cdk5 regulates the interaction between DCX and microtubules, and mutation of S297 blocks the effects of DCX in a migration-dependent cellular reaggregation assay in a manner similar to pharmacological inhibition of Cdk5 activity, suggesting a role in nucleokinesis.

Cdk5 Phosphorylation of Ndel1

Cdk5 can also phosphorylate Ndel1, an essential member of the dynein/Lis1/Ndel1 complex. Indications that this complex is involved in nucleokinesis during neuronal migration first came from counterparts in *Aspergillus nidulans*, in which mutations in the orthologs of dynein (NudA, NudC, and NudG), Lis1 (NudF), and Ndel1 (NudE) all result in abnormal nuclear distribution ('Nud'). Furthermore, dynein, Lis1, and Ndel1 all are localized to the microtubule fork/cage that links the centrosome to the nucleus, and RNAi-mediated knockdown of these proteins caused a disruption of microtubule bundles. Live imaging of RNAi transfected cultured neurons demonstrated that

nuclear movement is also disrupted; rather than moving continuously toward the centrosome in the leading process, the movement of the nucleus oscillated back and forth. Cdk5-phosphorylated Ndel1 is capable of interacting with 14–3-3ε, a gene that is deleted along with Lis1 in severe cases of lissencephaly (e.g., Miller–Dieker syndrome). Interestingly, mice deficient in 14–3-3ε display a mislocalization of Ndel1 and Lis1 and a mild cortical distortion. Cdk5-dependent phosphorylation of Ndel1 also facilitates an interaction with the microtubule severing protein katanin/p60, and dominant negative mutants of p60 result in defective migration.

Cdk5 Phosphorylation of p27 (kip1)

Whereas the majority of Cdk5 research aimed at understanding migration has suggested a critical role for the kinase in regulating microtubules, the description of p27 (kip1) as a novel substrate opened up the possibility that Cdk5 can also regulate the actin cytoskeleton in migrating neurons. Cdk5 phosphorylates p27 on serine 10, an event that is important for stabilizing p27 levels. Once stabilized, higher p27 levels result in the activation of cofilin, an actin-severing protein. Although p27-deficient mice do not have any obvious neuronal migration phenotype, RNAi-mediated knockdown of p27 in embryonic brains results in impaired migration of cortical neurons and decreased amounts of F-actin in neuronal processes.

Other Substrates of Cdk5 Potentially Important for Neuronal Migration

One intriguing candidate to play a role in neuronal migration is Pak1, a small GTPase effector that regulates both the microtubule and the actin cytoskeleton. Cdk5 can phosphorylate Pak1 at threonine 212, and expression of a nonphosphorylatable form of Pak1 results in severe neuritic patterning defects. Pak1 phosphorylation at T212 by Cdc2 regulates microtubules in dividing cells, so it is possible that the altered patterning in cortical neurons is due to a microtubule defect. Another microtubule-associated protein involved in neuronal migration that can be phosphorylated by Cdk5 is MAP1B. Stimulation of cultured cortical neurons with reelin or netrin increased MAP1B phosphorylation in a GSK3- and Cdk5-dependent manner, but Cdk5 does not appear to phosphorylate MAP1B in nonstimulated brain slices. *In vitro* experiments have also demonstrated that Cdk5 activity can regulate N-cadherin/β-catenin-mediated complexes, indicating a direct role for Cdk5 in cell adhesion. Finally, Cdk5 is capable of phosphorylating Dab1, a critical mediator of Reelin signaling, at serine 491. Interestingly, although this phosphorylation event occurs *in vivo*, it seems to be completely independent of Reelin signaling. It will ultimately be important to determine if phosphorylation of Dab1 impacts on neuronal migration (Figure 5).

Cdk5 and Axon Guidance

Corpus Callosum Defects in p35-Deficient Mice

In addition to neuronal migration, there is strong evidence that Cdk5 plays an important role in guidance for the correct establishment of axon/target connections. The first indication of this role of Cdk5 came from studies of the corpus callosum in p35-deficient mice, in which callosal axons display an abnormal projection pattern. Tracer injection and axonal staining experiments performed in p35−/− mice demonstrated a general failure of projecting cortical axons to assimilate into the body of the corpus callosum, suggesting a defect in fasciculation between axons.

Cdk5 and Semaphorin 3A

Further experiments have placed Cdk5 into a pathway with semaphorins, a well-established, large family of

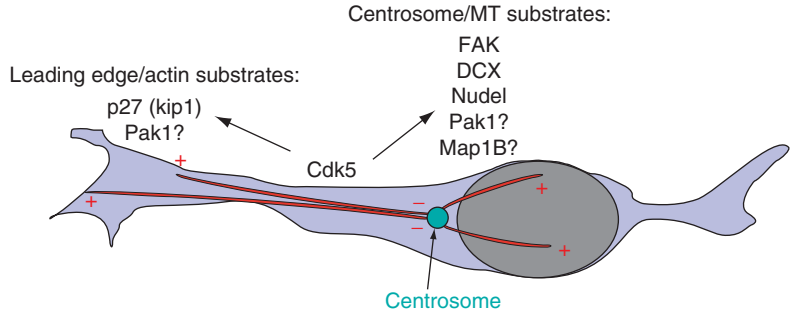

Figure 5 Cdk5 substrates involved in cytoskeletal regulation during neuronal migration. The majority of Cdk5 substrates understood to be involved in migration localize to the centrosome and microtubules, and they are known to regulate microtubule function and nucleokinesis. These include FAK, doublecortin (DCX), and Nudel. MAP1B phosphorylation, if important for migration, likely also regulates microtubule function. The first Cdk5 substrate shown to regulate the actin cytoskeleton during neuronal migration, and likely leading edge extension, was p27 (kip1). Phosphorylation of Pak1 may regulate actin or microtubule function.

secreted and membrane-bound proteins that can function as chemorepulsive/attractive cues. In cell culture, Sema3A normally induces collapse of growth cones through a large signaling pathway that includes functional roles for collapsin response-mediator proteins (CRMP), Rac, and LIM-kinase. Interestingly, dorsal root ganglia growth cones from Cdk5-deficient mice fail to collapse in response to Sema3A. Likewise, a dominant negative Cdk5 mutant attenuates Sema3A-induced collapse. *In vitro* kinase assays demonstrated that Cdk5 is capable of phosphorylating CRMP-2 at serine 522. Importantly, overexpression of a CRMP-2 alanine 522 mutant inhibited Sema3A-induced collapse. Mechanistically, it seems that Cdk5-dependent phosphorylation primes CRMP-2 for phosphorylation by GSK-3β, another event necessary for growth cone collapse. Therefore, the essential role of Cdk5 in Sema3A-induced growth cone collapse is likely mediated in part through phosphorylation of CRMP-2.

Cdk5 and GDNF/GFRα1

A second guidance pathway that seems to be associated with Cdk5 activity involves the function of GFRα1, a GPI-anchored receptor for glial cell line-derived neurotrophic factor (GDNF) that can be secreted or exist as a membrane-bound protein. Treatment of the motor cell line MN1 with GDNF in the presence of exogenous GFRα1 induced an increase in Cdk5 activity. Most interestingly, use of the Cdk inhibitor roscovitine or a dominant negative Cdk5 blocked GDNF/GFRα1-stimulated neurite outgrowth and guidance in explanted sympathetic ganglia.

Cdk5 Pathways Control Neuronal Motility and Structure

Taken together, all these findings suggest that Cdk5 is directly upstream of substrates that regulate the cytoskeleton during neuronal migration, and it specifically maintains the integrity of a microtubule network that links the centrosome with the nucleus and is essential for nucleokinesis. It is possible that Cdk5 activity is a signaling mediator during neuronal migration, relaying extracellular cues about the migration status to the cytoskeleton to permit the necessary remodeling. Multiple types of cues may converge on Cdk5 to translate their message into cytoskeletal rearrangement. Furthermore, Cdk5 is emerging as an important regulator of axon guidance, suggesting that the functions of Cdk5 in orchestrating brain development are manifold.

See also: Actin Cytoskeleton in Growth Cones, Nerve Terminals, and Dendritic Spines; Axon Guidance: Building Pathways with Molecular Cues in Vertebrate Sensory Systems; Axon Guidance: Guidance Cues and Guidepost Cells; Axonal and Dendritic Transport by Dyneins and Kinesins in Neurons; Cerebral Cortex; Cytoskeletal Interactions in the Neuron; Neocortex: Origins; Neuronal Motility and Structure: MARK and GSK Pathways.

Further Reading

Dhavan R and Tsai LH (2001) A decade of CDK5. *Nature Reviews Molecular Cell Biology* 2: 749–759.

Feng Y and Walsh CA (2001) Protein–protein interactions, cytoskeletal regulation and neuronal migration. *Nature Reviews Neuroscience* 2: 408–416.

Gupta A, Tsai LH, and Wynshaw-Boris A (2002) Life is a journey: A genetic look at neocortical development. *Nature Reviews Genetics* 3: 342–355.

Kawauchi T, Chihama K, Nabeshima Y, and Hoshino M (2006) Cdk5 phosphorylates and stabilizes p27kip1 contributing to actin organization and cortical neuronal migration. *Nature Cell Biology* 8: 17–26.

Kriegstein AR and Noctor SC (2004) Patterns of neuronal migration in the embryonic cortex. *Trends in Neurosciences* 27: 392–399.

Kruger RP, Aurandt J, and Guan KL (2005) Semaphorins command cells to move. *Nature Reviews Molecular Cell Biology* 6: 789–800.

Lambert de Rouvroit C and Goffinet AM (2001) Neuronal migration. *Mechanisms of Development* 105: 47–56.

Ledda F, Paratcha G, and Ibanez CF (2002) Target-derived GFRalpha1 as an attractive guidance signal for developing sensory and sympathetic axons via activation of Cdk5. *Neuron* 36: 387–401.

Marin O and Rubenstein JL (2003) Cell migration in the forebrain. *Annual Review of Neuroscience* 26: 441–483.

Niethammer M, Smith DS, Ayala R, et al. (2000) NUDEL is a novel Cdk5 substrate that associates with LIS1 and cytoplasmic dynein. *Neuron* 28: 697–711.

Rice DS and Curran T (2001) Role of the reelin signaling pathway in central nervous system development. *Annual Review of Neuroscience* 24: 1005–1039.

Ross ME and Walsh CA (2001) Human brain malformations and their lessons for neuronal migration. *Annual Review of Neuroscience* 24: 1041–1070.

Sasaki Y, Cheng C, Uchida 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.

Tanaka T, Serneo FF, Tseng HC, et al. (2004) Cdk5 phosphorylation of doublecortin Ser297 regulates its effect on neuronal migration. *Neuron* 41: 215–227.

Toyo-oka K, Shionoya A, Gambello MJ, et al. (2003) 14–3-3epsilon is important for neuronal migration by binding to NUDEL: A molecular explanation for Miller–Dieker syndrome. *Nature Genetics* 34: 274–285.

Walsh CA and Goffinet AM (2000) Potential mechanisms of mutations that affect neuronal migration in man and mouse. *Current Opinion in Genetics & Development* 10: 270–274.

Xie Z, Samuels BA, and Tsai LH (2006) Cyclin-dependent kinase 5 permits efficient cytoskeletal remodeling – A hypothesis on neuronal migration. *Cerebral Cortex* 16(supplement 1): i64–i68.

Xie Z, Sanada K, Samuels BA, Shih H, and Tsai LH (2003) Serine 732 phosphorylation of FAK by Cdk5 is important for microtubule organization, nuclear movement, and neuronal migration. *Cell* 114: 469–482.