Cortical development: **Cdk5 gets into sticky situations**

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Cyclin-dependent kinase 5 (Cdk5) is much more than its name implies; it plays a role in neuronal migration, neurite outgrowth and degeneration. Recent evidence suggests that Cdk5 regulates neuronal adhesion and cytoskeletal dynamics.

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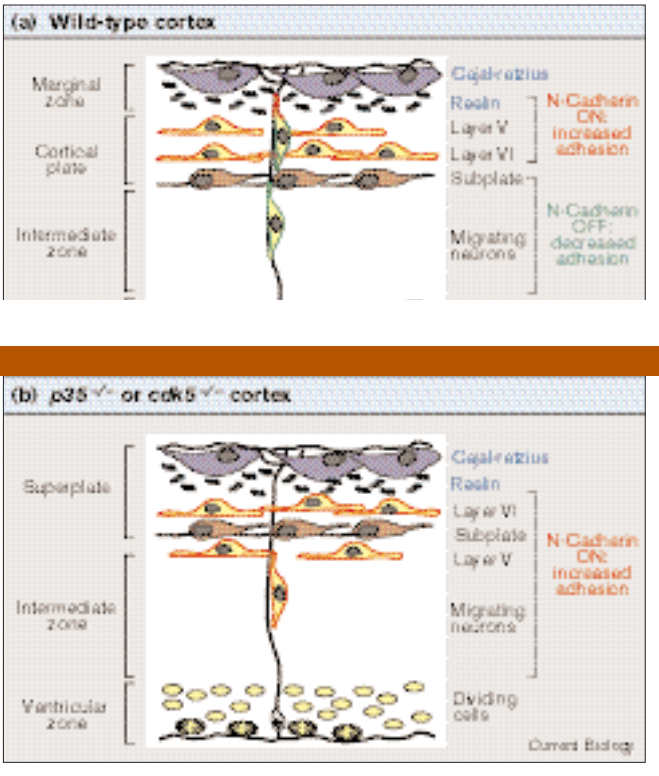
Cyclin-dependent kinase 5 (Cdk5) was identified independently by two groups based on its biochemical and sequence similarities to $p34^{cdc2}$ protein kinase, a well-known regulator of mitosis [1,2]. Unlike $p34^{cdc2}$, however, Cdk5 kinase activity is not detected in dividing cells. In fact, the catalytically active form of Cdk5 is present only in differentiated neurons of the brain, where it associates with a neuron-specific 35 kDa regulatory subunit, p35. Cdk5 expression gradually increases during neurogenesis until it reaches maximum levels in the adult brain [3]. The p35/Cdk5 kinase plays a variety of roles in the developing

a critical role in the regulation of N-cadherin-mediated cell adhesion during cortical development.

Insights into the function of Cdk5 and p35 came from studies on mice with targeted disruption of the gene encoding either Cdk5 or p35 [5,6]. The two mutants were found to exhibit similar abnormalities in the laminar structure of the cerebral cortex. But the *cdk5*$^{-/-}$ mice had additional defects in other brain structures and died shortly after birth. A small fraction of *p35*$^{-/-}$ mice died after spontaneous seizures, and greater than 50% died after seizure-inducing treatments that are not fatal in normal mice. The differences between the phenotypes of *cdk5*$^{-/-}$ and *p35*$^{-/-}$ mice may be due to the presence of other Cdk5 regulatory subunits in the brain. Thus far, several other Cdk5 regulators have been identified, including p39, Munc18 (p67) and a truncated form of p35, p25 [7–9]. At least in the forebrain, both p35 and Cdk5 play an essential role in the formation of the cortical layers during development.

The mammalian cortex is assembled through a choreographed series of events that ultimately result in the segregation of neurons with similar properties into six layers (Figure 1a) [10]. In the earliest phase of development, the preplate, composed of Cajal-Retzius and subplate neurons, is formed between the pial surface and the ventricular zone (VZ), where cells are actively dividing. Cells exit the cell cycle in the VZ and migrate radially outward towards the pial surface along glial fibers. The first-born neurons migrate past the subplate, displacing this layer away from the Cajal-Retzius cells in an area known as the marginal zone. Splitting of the preplate requires Reelin, a large extracellular protein secreted by Cajal-Retzius cells. The next wave of post-mitotic neurons migrates along the same fibers, past the subplate and the older neurons in the cortical plate, before inserting beneath the marginal zone.

Figure 1



A model of cortical development. **(a)** In wild-type cortex, post-mitotic neurons (yellow) express p35/Cdk5 kinase and down-regulate N-cadherin mediated adhesion (green), allowing them to migrate past cortical plate cells that exhibit N-cadherin mediated adhesion (red). Reelin (light blue), secreted by the Cajal-Retzius cells (purple), inhibits the p35/cdk5 kinase pathway in migrating cells. Inhibition of p35/cdk5 kinase, in turn, activates N-cadherin mediated adhesion to terminate migration and initiate homotypic adhesion between the new arrivals and neurons resident in the cortical plate. **(b)** In *p35*$^{-/-}$ or *cdk5*$^{-/-}$ cortex, migrating neurons cannot down-regulate N-cadherin, hence they are not able to bypass the cortical plate neurons to take up residence beneath the subplate.

In this manner, the classical inside-out pattern of the neocortex arises in which the sequential generation of layers II–VI occurs beneath the marginal zone (future layer I).

In the cortices of both *p35*$^{-/-}$ and *cdk5*$^{-/-}$ mutant mice, the marginal zone is unaffected and Reelin expression is normal [11,12]. The preplate splits in *p35*$^{-/-}$ and *cdk5*$^{-/-}$ brains, and the first-born population of neurons — the future layer VI — appears to migrate correctly past the subplate cells. But the migration of the later-born neurons is impeded, in the mutants, such that the second wave of neurons fails to migrate past the established layer of cortical neurons and, instead, accumulates below the subplate. On the basis of these observations, it has been proposed that p35 and Cdk5 are required for neurons to bypass one another during corticogenesis, although they are not required for splitting of the preplate.

To form distinct neuronal layers in the developing cortex, one would expect migrating neurons to recognize and preferentially adhere to their cohorts in the respective layers. Indeed, in aggregation assays, early-born cortical neurons selectively associate with one another [13]. A good candidate molecule to mediate this cell–cell adhesion is the Ca^{2+}-dependent neuronal adhesion molecule N-cadherin. N-cadherin is expressed transiently throughout the developing cortical plate, but it persists only in the deepest layers of the postnatal brain [14]. Thus, it is possible that N-cadherin plays a role in maintaining the integrity of the cortical plate at the time newly generated neurons migrate past. This model presents a paradox, however: how can migrating neurons expressing the homophilic protein N-cadherin bypass cortical plate neurons that also express N-cadherin?

The recent work of Kwon *et al.* [4] on p35-interacting proteins has shed some light on this subject. They identified a novel interaction between p35 and the intracellular regulator of N-cadherin, β-catenin. Expression of Cdk5 and p35 in cultured cells was found to decrease the association between β-catenin and N-cadherin, resulting in decreased cell adhesion. Conversely, in neuronal aggregation assays, loss of Cdk5 kinase activity by pharmacological inhibition or ablation of p35 resulted in increased cell adhesion. These results provide important insights into the molecular mechanism by which Cdk5 and p35 regulate neuronal migration in the developing cortex.

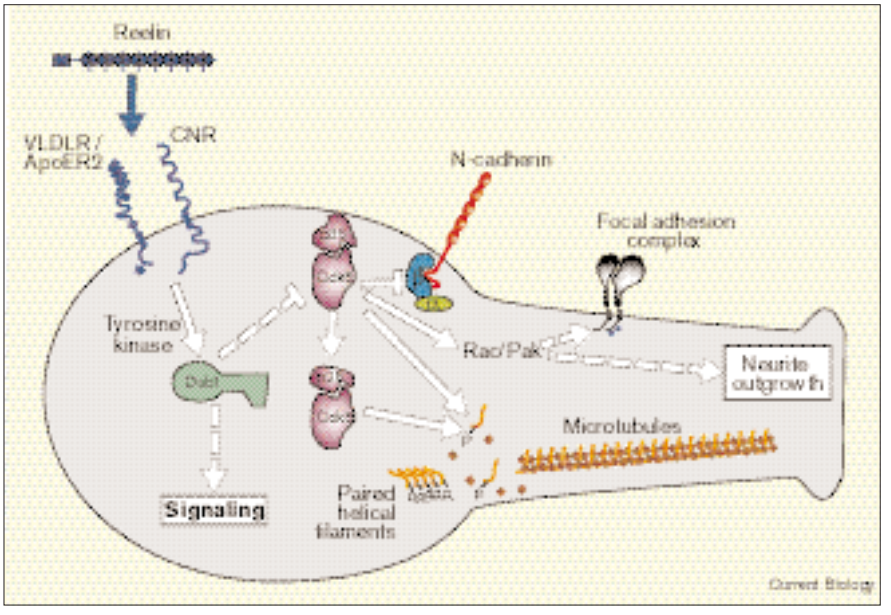
On the basis of these findings [4] a new molecular model can be put forward to describe cortical development (Figure 1a). As neurons exit the cell cycle, they express p35, which activates Cdk5, causing down-regulation of N-cadherin-mediated cell adhesion. This allows the neurons to migrate past their predecessors in the cortical plate, which express large amounts of N-cadherin. In *cdk5*$^{-/-}$ and *p35*$^{-/-}$ brains (Figure 1b), the migrating neurons are unable to down-regulate N-cadherin, so they cannot bypass the earlier-born cortical plate neurons. This may explain why later-born neurons accumulate beneath the subplate in these mutant mice.

One remaining question is how does a migrating neuron that has reached the marginal zone down-regulate the p35/Cdk5 kinase pathway and reactivate N-cadherin mediated adhesion? Reelin has been proposed to function as a stop signal for migrating neurons [15]. It is possible that Reelin acts as a stop cue by inhibiting the p35/Cdk5 kinase pathway. Thus, Reelin may activate N-cadherin-mediated cell adhesion to terminate migration and initiate homotypic adhesion between the new arrivals and neurons resident in the cortical plate. Recent results by Hiesberger *et al.* [16] support this hypothesis. They demonstrated that tau, a substrate of Cdk5, is hyperphosphorylated in *reeler* mutant brains. One explanation for this observation is that Reelin down-regulates p35/Cdk5 kinase activity under normal conditions. Therefore, it is possible that, in *reeler* mice, p35/Cdk5 kinase remains active in migrating neurons, resulting in a failure of post-mitotic neurons to adhere to one another in the presumptive marginal zone. This may in part explain the disruption of cortical layers observed in *reeler* brains.

There is accumulating evidence indicating that p35 and Cdk5 play an important role in the outgrowth and maintenance of neuronal axons. Both p35 and Cdk5 colocalize with actin filaments in growth cones of developing neurons in culture [17,18]. Importantly, expression of p35/Cdk5 kinase in transfected neurons resulted in extension of neurites, whereas expression of a dominant-negative mutant form of Cdk5 abolished neurite outgrowth. More recently, Nikolic *et al.* [19] showed that p35/Cdk5 kinase colocalizes with Rac, a member of the Rho family of GTPases, and a Rac effector, Pak1 kinase. Both Rac and Pak1 are involved in regulation of focal adhesion complexes and the actin cytoskeleton. The association of p35/Cdk5 kinase with Rac and Pak1 results in hyper-phosphorylation and inactivation of Pak1 kinase activity. Pak1 plays a complex role in the regulation of cytoskeletal dynamics. Although Cdk5 inhibits Pak1 kinase activity, kinase-deficient Pak1 can still induce neurite extension in PC12 cells [20]. The relationships among p35/Cdk5 kinase, Rac and Pak1 are intriguing. It would be interesting to determine whether Rac or Pak1 mediates the effect of p35/Cdk5 kinase on neurite outgrowth in neurons.

It is likely that p35/Cdk5 kinase regulates other major cytoskeletal components in neurons such as intermediate filaments and microtubules. Cdk5 phosphorylates neurofilament proteins, the major constituents of intermediate filaments. Phosphorylation of neurofilament proteins directs them to axons, where they affect axonal transport and conduction velocity [21]. In *cdk5*$^{-/-}$ brains, there is a dramatic

Figure 2



Summary of neuronal functions of Cdk5. Cdk5 is recruited to the membrane and activated by the p35 regulatory subunit. The active p35/Cdk5 kinase inhibits N-cadherin-mediated cell adhesion by disrupting the interaction between β-catenin and N-cadherin. It is possible that this effect is inhibited by the Reelin signaling pathway during corticogenesis. Reelin binds to lipoprotein receptors, including the very low density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2), and to a family of cadherin-related neuronal receptors (CNRs) [16,23,24]. Reelin activates a signaling cascade, resulting in the tyrosine phosphorylation of Dab1 in neurons, In mice lacking either Reelin or both VLDRL and ApoER2, tau is hyperphosphorylated, suggesting that the Reelin signaling pathway inhibits p35/Cdk5. The p35/Cdk5 kinase also forms a complex with Rac and Pak1. This association may mediate the effect of p35/Cdk5 on neurite extension. On the other hand p35/Cdk5 kinase may regulate focal adhesion by association with Rac and Pak1. Finally, Cdk5 is one of the major kinases that phosphorylates the microtubule-associated protein tau. Cleavage of p35 into the cytosolic p25 isoform results in the constitutive activation of Cdk5 and aberrant phosphorylation of tau. Hyperphosphorylated tau loses its affinity to bind to microtubules and aggregates into paired helical filaments.

loss of phosphorylated neurofilament proteins in axons [6]. Cdk5 is also one of the two major kinases that have been shown to phosphorylate the microtubule-associated protein tau [22]. Tau stabilizes microtubules and regulates neurite extension and axonal transport. When hyperphosphorylated, tau loses its ability to bind to microtubules and aggregates into paired helical filaments in the somatodendritic region of neurons. Paired helical filaments are components of neurofibrillary tangles associated with Alzheimer's disease and other neurodegenerative disorders.

There is now compelling evidence that Cdk5 plays a role in Alzheimer's disease. Patrick *et al.* [9] recently showed that the truncated form of p35, p25, accumulates in neurofibrillary tangles in brains of Alzheimer's disease patients. Unlike the membrane-bound p35, p25 resides in the cytoplasm. It has a much longer half-life than p35 and it constitutively activates Cdk5. Transfection of p25 and Cdk5 into cultured cells leads to phosphorylation of tau, resulting in decreased association with microtubules. In culture, expression of p25/Cdk5 kinase caused neurons to retract their axons and undergo apoptosis. These results imply that aberrant regulation of Cdk5 may underlie certain aspects of neurodegeneration.

Increasing evidence points to the fact that Cdk5 is a key regulator of neuronal function (Figure 2). It modulates cell adhesion and cytoskeletal dynamics, processes that are essential during development and in the adult nervous system. Not surprisingly, deregulation of Cdk5 has severe consequences for brain development, causing disruption of cell positioning and perinatal lethality. In the adult nervous system, aberrant activation of Cdk5 by p25 is associated with neurodegeneration. Cdk5 family members play critical roles in the control of cell division and they have attracted attention as a target for anti-cancer therapies. Perhaps Cdk5 plays a similarly critical role in postmitotic, developing and adult neurons. It may also attract attention as a therapeutic target for neurodegenerative diseases.

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