

Doubling Up on Microtubule Stabilizers: Synergistic Functions of Doublecortin-like Kinase and Doublecortin in the Developing Cerebral Cortex

Dynamic regulation of neuronal cytoskeletal machinery in response to extracellular cues enables distinct changes in neuronal development in the cerebral cortex. In this issue of *Neuron*, three related studies on doublecortin-like kinase, a microtubule-associated protein related to doublecortin, by Shu et al., Koizumi et al., and Deuel et al., provide evidence that doublecortin-like kinase is essential for proper neurogenesis, neuronal migration, and axonal wiring.

Construction of functional synaptic circuits in the mammalian cerebral cortex relies on finely synchronized proliferation, migration, and differentiation of neurons during development. Molecular analysis of human cortical developmental disorders indicate that every aspect of neuronal development in cerebral cortex is influenced by dynamic rearrangement of the neural cytoskeleton, whether it is coordination of the spindle alignment during mitosis in the ventricular zone, maintenance of cellular polarity during neuronal migration, dendritic/axonal elaboration once a cell has reached its final destination, or synaptogenesis.

Mutations in the gene doublecortin (*DCX*), a microtubule-associated protein in migrating neurons, leads to X-linked lissencephaly (also called double-cortex or subcortical band heterotopia) in humans. In these patients, neurons that migrated aberrantly are deposited in a broad band in subcortical layers and thus may cause severe mental retardation, seizures, and decreased lifespan in affected individuals (des Portes et al., 1998; Gleeson et al., 1998). *DCX* is critical for the stabilization of the microtubule network (Caspi et al., 2000; Feng and Walsh, 2001; Francis et al., 1999; Gleeson et al., 1999; Tanaka et al., 2004; Taylor et al., 2000).

Unlike affected humans, mice with a targeted deletion in *DCX* have a much less severe phenotype, with a relatively preserved neocortex (Corbo et al., 2002). Surprisingly, RNAi-mediated knockdown of *DCX* in rat resulted in migrational arrest and formation of the characteristic subcortical band of ectopic neurons seen in the humans with double-cortex syndrome (Bai et al., 2003). Neuronal migration was affected in a cell-autonomous as well as a non-cell-autonomous manner by the decrease of *DCX* levels. More importantly, these studies raised the possibility that *DCX* might be functionally compensated for by other related genes during brain development. One such *DCX* ortholog is doublecortin-like kinase (DCLK or *Dcamk1l*). Like *DCX*, the *Dclk* gene also functions as a microtubule-associated protein (MAP), with the N-terminal domain binding to and stimulating microtubule polymerization (Burgess et al., 1999). Unlike *DCX*, the *Dclk* has varying levels of expression throughout embryonic and adult life through regulated expression of multiple splice variants, including a full-length isoform (DCLK), a *DCX*-domain isoform (DCLK *DCX*-like), a kinase

domain only isoform (CPG16), and a CaMK-related peptide (CARP) (Burgess and Reiner, 2002; Silverman et al., 1999; Vreugdenhil et al., 2001). In this issue of *Neuron*, three groups investigated the function of DCLK and provide novel insights into multiple, related functions of this protein in neurogenesis, neuronal migration, and axonal outgrowth in the developing cerebral cortex.

The work of Shu, Tsai, and colleagues (Shu et al., 2006) describes the role of DCLK in mitotic spindle formation and the regulation of cell fate determination in cortical neural progenitors. Using a screen to identify MAPs enriched in developing CNS tissue, they identified doublecortin-like kinase as a MAP enriched in regions of active neurogenesis in cerebellum and cerebral cortex. In neuronal progenitor cells in vitro and in vivo, DCLK is associated with mitotic spindles, polymerize microtubules in a Lis1 and dynein-dependent fashion, and the N-terminus of DCLK plays a role in mitotic arrest at prometaphase. In utero gain-of-function expression of DCLK resulted in a substantial decrease in the number of neural progenitors in the VZ and formation of an ectopic layer of neurons in the IZ, which prematurely expressed *Tbr-1*, a marker of differentiated glutamatergic neurons in layer 5/6. In contrast, in vivo loss of function of DCLK led to cell-cycle exit, premature commitment of neural progenitors to a neuronal fate, and a resultant elevation of post-mitotic neurons within the SVZ/VZ compartment. Alterations in the levels of DCLK appear to result in premature differentiation of SVZ/VZ progenitors into neurons.

To explore the function of DCLK in cerebral cortical development, groups led by Gleeson and Walsh generated *Dclk*-deficient mice through targeted deletions of different functional domains of the gene. Due to the alternative splicing of *Dclk*, it is difficult to generate a null that lacks all three isoforms of the gene. The study presented in this issue by Koizumi, Gleeson, and colleagues (Koizumi et al., 2006) describes a mouse model in which targeted deletion of exon 3 of *Dclk* disrupts both the full-length *Dclk* as well as the *Dclk Dcx*-like isoform, leaving the *CPG16* isoform intact. Although loss of *Dclk* results in a viable and fertile strain of mice with no detectable lethality and intact cortical lamination, there was a near-complete ablation of the corpus callosum (CC), with an increased appearance of Probst bundles. Intermediate differences were noted in the heterozygotic littermates, suggesting a gene dosage effect. The corticothalamic fibers, where DCLK is also expressed, appear not to be affected in DCLK mutants, suggesting that DCLK plays a specific role in regulating the extension and decussation of axons that cross midline. The fiber-tract deficiencies in DCLK mutants appear not to be dependent on strain background. To determine whether DCLK functionally compensates for *DCX* during cortical development, these investigators generated DCLK/*DCX* double-null mice. Compound nulls display disrupted cortical lamination and complete loss of corpus callosum, anterior, and hippocampal commissures not seen in single nulls. RNAi-mediated inactivation of DCLK results in cortical migration deficits similar to the one seen with *DCX* inactivation.

In a closely related study, Deuel, Walsh, and colleagues analyzed DCLK function using DCLK mutant mice with targeted deletion of exons 9-11, which results in the elimination of all kinase domain-containing isoforms of DCLK but leaves the CARP isoform intact. These

mice, like those generated by Koizumi et al. (2006), were viable and fertile and had preserved neocortical lamination, with no evidence of subcortical heterotopias. Similar to Koizumi et al.'s observations, mice mutant for both DCX and DCLK displayed profound disorganization in cortical layering and widespread axonal defects in the corpus callosum, anterior commissure, subcortical fiber tracts, and internal capsules. However, in contrast to Koizumi et al.'s results, less severe phenotypes were noticed in the corpus callosum, suggesting potentially divergent functions of DCLK, DCLK-DCX-like, CARP, and CPG16 isoforms in distinct axonal pathways. Interestingly, human neurons deficient in DCX displayed axon-growth defects similar to those seen in DCLK/DCX double-null mice. Disruption of DCLK and DCX function in neurons *in vitro* led to abnormal dendritic development, shorter axons and dendrites, and disrupted axonal transport of synaptic vesicle proteins during axon growth. Together, the studies by Koizumi et al. and Deuel et al. strongly suggest that DCLK and DCX play a synergistic role in neuronal migration and axon growth in embryonic cortex.

The diverse function of DCLK evinced from these three studies highlights the significance of dynamic rearrangement of microtubule cytoskeleton during corticogenesis and raises the intriguing question of how the functions of MAPs, like DCLK and DCX, are orchestrated during distinct stages of neuronal development in cerebral cortex. Clearly, understanding the complimentary as well as unique functions of DCLK and DCX during neurogenesis, neuronal migration, and neuronal differentiation will be critical. The differences in DCLK expression noticed in different domains of the developing cerebral wall in these three studies suggest that DCLK expression may change from a restricted expression within the ventricular zone during early stages of neurogenesis to an increasingly more restricted expression in postmitotic, migrating neurons and postmigratory, differentiating neurons as cerebral cortical development unfolds. It is attractive to hypothesize that this change in DCLK expression pattern during development may serve to coordinate neurogenesis with appropriate placement and connectivity of neurons. Considering the striking differences in phenotypes observed between DCX null mice and RNAi-mediated DCX knockdown, further analysis of neural precursor proliferation in DCLK, DCX mouse mutants described by Koizumi et al. and Deuel et al. will be informative. These mouse models will also be eminently useful in determining whether DCLK functions to regulate the neuronal fate of all dividing ventricular progenitors in a generic manner or whether it differentially regulates the fate of symmetrically and asymmetrically dividing neural precursors.

Though it is evident that DCLK and DCX can synergistically act during neuronal migration and differentiation, the nature of this interaction remains unclear. Is DCX a substrate for doublecortin-like kinase and what are the endogenous substrates for DCLK? Are the microtubule binding activities of DCX and DCLK differentially regulated? Do different DCLK isoforms have distinct patterns of developmental coexpression with DCX within neural progenitors and postmitotic cortical neurons? Does DCLK interact differentially with known DCX interactors such as AP-1, neurofascin, Lis1, MARK, cJNK, JIP, neurabin II, and Cdk5? Additionally, the suggestion

that the DCX/DCLK pathway might regulate the vesicle trafficking (Deuel et al., 2006) needed to support membrane expansions during neuronal motility and process growth adds a new twist to DCX/DCLK's function in cerebral cortex. While these three studies provide novel and exciting perspectives on the role of DCLK in cerebral cortex, how doublecortin domain-containing proteins orchestrate their multiple functions during cortical development remains a challenging question.

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Selected Reading

- Bai, J., Ramos, R.L., Ackman, J.B., Thomas, A.M., Lee, R.V., and LoTurco, J.J. (2003). *Nat. Neurosci.* 6, 1277–1283.
- Burgess, H.A., and Reiner, O. (2002). *J. Biol. Chem.* 277, 17696–17705.
- Burgess, H.A., Martinez, S., and Reiner, O. (1999). *J. Neurosci. Res.* 58, 567–575.
- Caspi, M., Atlas, R., Kantor, A., Sapir, T., and Reiner, O. (2000). *Hum. Mol. Genet.* 9, 2205–2213.
- Corbo, J.C., Deuel, T.A., Long, J.M., LaPorte, P., Tsai, E., Wynshaw-Boris, A., and Walsh, C.A. (2002). *J. Neurosci.* 22, 7548–7557.
- des Portes, V., Francis, F., Pinard, J.M., Desguerre, I., Moutard, M.L., Snoeck, I., Meiner, L.C., Capron, F., Cusmai, R., Ricci, S., et al. (1998). *Hum. Mol. Genet.* 7, 1063–1070.
- Deuel, T.A.S., Liu, J.S., Corbo, J.C., Yoo, S.-Y., Rorke-Adams, L.B., and Walsh, C.A. (2006). *Neuron* 49, this issue, 41–53.
- Feng, Y., and Walsh, C.A. (2001). *Nat. Rev. Neurosci.* 2, 408–416.
- Francis, F., Koulakoff, A., Bouchner, D., Chafey, P., Schaar, B., Vinet, M.C., Friocourt, G., McDonnell, S.K., Reiner, O., Kahn, A., et al. (1999). *Neuron* 23, 247–256.
- Gleeson, J.G., Allen, K.M., Fox, J.W., Lamperti, E.D., Berkovic, S., Scheffer, I., Cooper, E.C., Dobyns, W.B., Minnerath, S.R., Ross, M.E., and Walsh, C.A. (1998). *Cell* 92, 63–72.
- Gleeson, J.G., Lin, P.T., Flanagan, L.A., and Walsh, C.A. (1999). *Neuron* 23, 257–271.
- Koizumi, H., Tanaka, T., and Gleeson, J.G. (2006). *Neuron* 49, this issue, 55–66.
- Shu, T., Tseng, H.-C., Sapir, T., Stern, P., Zhou, Y., Sanada, K., Fischer, A., Coquelle, F.M., Reiner, O., and Tasai, L.-H. (2006). *Neuron* 49, this issue, 25–39.
- Silverman, M.A., Benard, O., Jaaro, H., Rattner, A., Citri, Y., and Seger, R. (1999). *J. Biol. Chem.* 274, 2631–2636.
- Tanaka, T., Serneo, F.F., Higgins, C., Gambello, M.J., Wynshaw-Boris, A., and Gleeson, J.G. (2004). *J. Cell Biol.* 165, 709–721.
- Taylor, K.R., Holzer, A.K., Bazan, J.F., Walsh, C.A., and Gleeson, J.G. (2000). *J. Biol. Chem.* 275, 34442–34450.
- Vreugdenhil, E., Engles, B., Middleburg, R., von Koningsbruggen, S., Knol, J., Velduisen, B., and de Kloet, E.R. (2001). *Brain Res. Mol. Brain Res.* 94, 67–74.

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Cannabinoids in Microglia: A New Trick for Immune Surveillance and Neuroprotection

Microglia are the resident immune cells of the brain, and they are under permanent activity to patrol the