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Doublecortin and a Tale of Two Serines

Doublecortin (DCX) is a microtubule-associated protein that interacts with and regulates the microtubule cytoskeleton and is required for neuronal migration in the cortex. Two papers in this issue of *Neuron* (Schaar et al. and Tanaka et al.) demonstrate a role for phosphorylation in the regulation of Doublecortin. Together with recent results showing that Doublecortin may play a role regulating the morphology of migrating neurons, these findings provide new insight into the mechanisms governing neuronal migration.

The textbook image of cortical neurons migrating along radial glia with a simple and uniform bipolar morphology is an oversimplification. New neocortical neurons actually undergo a series of complex morphological changes as they migrate. In addition to extending a leading process and translocating along radial glia, recent time-lapse imaging studies indicate that migrating neocortical neurons progress through a series of global changes in cellular morphology and display a remarkable variety of behaviors (Noctor et al., 2004). Such cellular dynamics indicate that migrating neurons in neocortex undergo considerably more cytoskeletal restructuring than previously recognized and suggest that many spatially and temporally regulated protein-protein interactions may be necessary to coordinate the complex cellular program of migration.

Genetic evidence has been extremely useful in identifying key proteins essential to migraton in neocortex, and many of these proteins either directly or indirectly regulate the cytoskeleton (Gupta et al., 2002). Notably, mutations in a microtubule binding protein, doublecortin (DCX), cause X-linked lissencephaly and double cortex syndrome in humans (des Portes et al., 1998b; Gleeson et al., 1998). More recently, RNAi of DCX has been shown to cause migrating neurons to adopt multipolar morphologies not typical for radially migrating neurons (Bai et al., 2003). Two papers in this issue of *Neuron* now suggest specific biochemical mechanisms by which DCX may dynamically regulate cytoskeletal structure (Schaar et al., 2004; Tanaka et al., 2004). These two new studies show that the affinity of DCX for tubulin and its ability to stabilize microtubules is altered by the phosphorylation of two serines by different kinase systems. One of these kinases, Cdk5/p35, has been previously shown to be essential to neuronal migration in neocortex, while others, which phosphorylate a different serine, include kinases previously associated with cellular polarization in *Drosophila* and *C. elegans*. DCX may therefore sit at the center of a general cellular program of morphological change engaged as neurons migrate through developing neocortex.

Migration and DCX

In order to ultimately match biochemical mechanism to neuronal migration, it is necessary to understand, in some detail, the specific cellular phases of migration. Time-lapse imaging studies of neurons in slices of developing neocortex show that neurons can migrate by three modes: somal translocation, cellular locomotion (Nadarajah et al., 2001), and a more recently defined multipolar mode (Tabata and Nakajima, 2003). In a new imaging study, Noctor et al. (2004) show by long-term time-lapse imaging that each migrating neocortical neuron transitions through multiple phases of migration that may constitute all three modes of migration. As sumarized in Figure 1A, this new imaging data indicates that neuroblasts exit from the cell cycle as simple bipolar cells, then become highly multipolar as they move out of the ventricular zone (VZ), and then become bipolar again as they progress through the intermediate zone (IZ) and enter the cortical plate (CP). The new multipolar mode of migration had been previously characterized as having unpredictable behavior likened to the behavior of growth cones at decision points, and cells can migrate in any direction while in this mode (Tabata and Nakajima, 2003). Noctor and colleagues now show that this multipolar mode is typically associated with distinct behaviors that include significant and complex changes in morphology and in migration direction. Most cells in the multipolar phase extend a process toward the VZ surface, reverse direction, and then finally adopt a bipolar morphology as they migrate through the IZ. This new mode indicates that radially migrating neocortical neurons progress through a stage in which they lose and then regain their radially directed migration path.

The anatomical phenotype of double cortex syndrome in humans indicates that DCX is essential for neurons to migrate successfully through the IZ of developing cortex (des Portes et al., 1998a; Gleeson et al., 1998). An open question was which mode(s) of migration is DCX essential for. Genetic deletion of Dcx in mice failed to produce a neocortical phenotype (Corbo et al., 2002), but recent development and application of in utero RNAi approaches in rat have made it possible to directly examine the role of DCX in radial migration and associated cellular morphologies through cortical development. Bai et al. (2003) showed that DCX is in fact required for all radially migrating neocortical neurons to enter the CP from the IZ (Bai et al., 2003). More informatively, cells stalled in the IZ after RNAi treatment attain a multipolar morphology similar to the multipolar morphologies recently described by Noctor et al. (2004) and Tabata and Nakajima (2003). In addition, the only neurons that extended long apical neurites into the cortical plate or adopted a classic bipolar morphology were the few neurons that still expressed DCX protein and presumably escaped the direct effects of RNAi (Bai et al., 2003). Such observations suggest that DCX is necessary for cells to successfully transition from the multipolar phase of migration to the bipolar radially migrating phase.

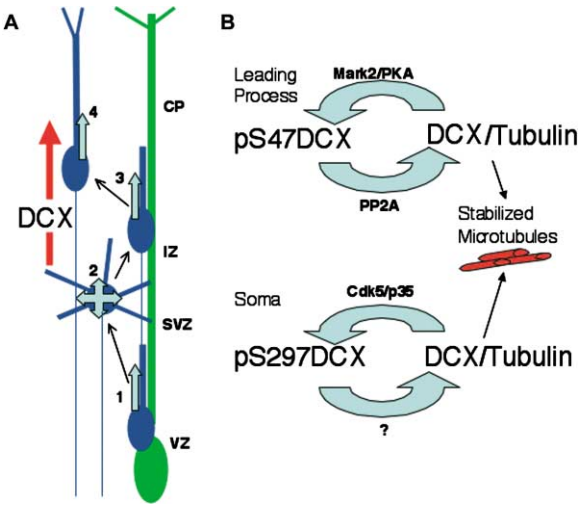

Figure 1. Migration and DCX Regulation

(A) Phases of radial migration in neocortex: (1) bipolar in the ventricular zone (VZ); (2) multipolar in the subventricular zone (SVZ) and intermediate zone (IZ); and (3 and 4) bipolar in the upper IZ and through the cortical plate (CP).

(B) DCX binding to tubulin is regulated by phosphorylation and dephosphorylation of two different serine residues. Phosphorylation of DCX at either serine leads to a lowered affinity for microtubules and decreased microtubule stabilization.

Serine 47 and 297

DCX protein directly binds to and stabilizes microtubules (Francis et al., 1999; Gleeson et al., 1999). If DCX binding to and stabilization of microtubules is involved in the complex transition from a multipolar cell to a bipolar radially migrating neuron, then these functions might be expected to be dynamically regulated and to operate in multiple cellular compartments. Two papers in this issue of *Neuron* show these two new features of DCX function. First, a paper by McConnell and colleagues presents results indicating that a cycle of dephosphorylation and phosphorylation regulates binding and unbinding of DCX to microtubules in growing neurites (Figure 1B). Inhibition of Protein Phosphatase 2A (PP2A) by Okadaic acid causes a rapid dissociation of DCX protein from microtubules at the growing ends of neurites, and similarly, phosphorylation of DCX at serine 47 by either Protein Kinase A (PKA) or MARK kinases reduces the affinity of DCX for microtubules (Figure 1B). In a second paper from the Gleeson lab, a different serine residue, serine 297, was found to be critical to the association of DCX protein with microtubules in the cell soma. As with serine 47, phosphorylation of serine 297 reduces the affinity of DCX for microtubules but it is phosphorylated by a different kinase system, Cdk5/p35 (Figure 1B). In addition, experiments using a series of serine deletion mutations indicate that there are several other serine residues in DCX that may be phosphorylated, raising the possibility that additional kinases may also regulate DCX function. Taken together, these two papers show that the association of DCX with microtubules is dynamically regulated by differential action of phosphatases and kinases within multiple compartments of migrating neurons.

Matching Modes and Mechanism

The stage is now set to match specific migration phases in neocortex with phosphorylation of different residues of DCX. Phosphorylation and dephosphorylation of serine 297 may regulate somal movements, while phosphorylation and dephosphorylation of serine 47 may be more important for directed growth of leading processes. Loss of both of these residues might then be expected to result in multipolar cells that fail to extend a single leading process or to translocate their nuclei along any process. Alternatively, phosphorylation of the two serines may not serve separable functions but rather both be necessary for migrating neurons to establish polarity required for continued radial migration. Answers to these questions will require combining live imaging in slices with cellular genetic modification with RNAi and mutant DCX.

A more general question is the significance of the multipolar phase of neuronal migration. A particularly appealing possibility, analogous to the behavior of growth cones at choice points, is that during the multipolar phase migrating cortical neurons search the local environment for signals that will determine whether new neocortical neurons progress directly radially to the necortex or diverge from the mother radial glia and migrate tangentially. Consistent with this idea, a recent paper has shown that loss of Cdk5 function causes radially migrating neurons to disperse tangentially from the mother radial glia (Gupta et al., 2003). Moreover, as DCX appears to be a target for multiple kinase systems, it appears to be ideally situated to integrate multiple signals from extracellular sources. In this case, the reason that loss of DCX function, either in humans or in RNAi experiments, causes stalled migration in the IZ is because neocortical neurons without functioning DCX are incapable of progressing through an important choice point.

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Context Matters

Midbrain dopamine neurons are thought to encode the difference between predicted and actual reward on conditioning tasks. Successful models assumed a simple form of prediction that depended only on currently available information. In this issue of *Neuron*, Nakahara and colleagues record from dopamine neurons in alert monkeys and show that the neurons can encode predictions that are not so restricted, taking into account the context of past trends.

Bad predictions can have bad consequences. Consider the case of Ivan Rodriguez, starting catcher for the World Series champion Florida Marlins. As a free agent, he is looking for a contract worth ~$10 million a year (i.e., 400 NIH modules) for four years. However, no team has signed him yet. The problem is that the reward he predicts for himself is substantially greater than the reward he is likely to get. His asking price might accurately reflect past contracts for players with similar abilities but neglects recent context: baseball's highest salaries peaked a few years ago and since then have gone into a predictable decline. In this issue of *Neuron*, Nakahara and colleagues report that, happily, neurons that predict reward are more accurate than baseball superstars and their agents and take into account factors like predictable trends in the recent past (Nakahara et al., 2004).

Nakahara and colleagues studied neurons in the substantia nigra pars compacta, one of two midbrain regions (the ventral tegmental area is the other) with neurons that release the neurotransmitter dopamine (DA). These regions project diffusely to several targets including the frontal cortex, the striatum, and the nucleus accumbens. Their function is suggested by several well-documented observations: degeneration of DA neurons gives rise to Parkinson's disease; DA neurons are particularly effective targets for electrical self-stimulation; DA receptor blockers can disrupt learning; and addictive drugs like cocaine, amphetamine, and opiates prolong the effects of DA (Wise, 1996, 2002). Thus, midbrain DA neurons appear to play a central role in computations that incorporate reward, goal-directed behavior, and learning.

One critical computation thought to be carried out by DA neurons is to compare predictions of reward with actual rewards received (Fiorillo et al., 2003). This reward prediction error is like a running commentary on how well expectations are being met by reality. As such, DA neural responses range from "pleasantly surprised" (a strong, phasic excitation corresponding to an unexpected reward) to "adequate" (no response, corresponding to a predicted reward) to "disappointed" (a phasic depression corresponding to no reward delivery at a time it was expected). For example, consider a DA neuron in a monkey trained to release a bar when a light is flashed in order to receive a juice reward. Before training, the reward is unexpected whenever it is given, so the DA neuron gives a phasic response upon reward delivery. In contrast, after training, if the light is flashed, the bar is released, and juice is delivered, then DA activity is unchanged because reward and prediction match. If the light is flashed but no juice is delivered, DA activity is depressed.

Computational theories from machine learning have helped guide our understanding of these reward prediction error signals. One algorithm, called temporal difference (TD) learning, has been particularly effective at describing these kinds of signals and placing them in a functional context (Sutton and Barto, 1981). The TD algorithm is a way of learning associations between events (stimuli and rewards) that can occur spread out in time (for an intuitive explanation of TD learning, see Sutton and Barto, 1998). A key feature is that like other algorithms, including a precursor to TD learning called the Rescorla-Wagner rule, the difference between a predicted reward and the actual reward drives learning. Here is the obvious link to DA neurons; this is exactly the signal they are thought to compute (Schultz, 2002).

Nakahara and colleagues sought a better understanding of how the reward prediction error encoded by DA neurons is computed. Most previous studies of DA neurons in primates used tasks in which reward probability depended only on the sensory information in the given trial. In such studies, if a stimulus led to a reward with a certain probability on one trial, then the same stimulus led to that reward with the same probability on another trial (analogously, if a baseball player expects a certain contract one year, then a player with the same ability would expect the same contract any other year). This assumption is consistent with the TD model and makes the computational problem of predicting rewards more tractable, but it is restrictive. Reward probabilities can be varied trial-to-trial (or season-to-season), and taking this information into account can help to make more accurate predictions. Nakahara and colleagues tested whether the prediction error signals represented in DA responses account for this "context"—information from the past—that can be used to improve reward predictions.

They used two behavioral tasks that associated visual stimuli with water rewards. The tasks differed in how the probability of getting a reward on a given trial depended on the recent history of rewards. For the first task, the probability of reward on a given trial was independent of whether the previous trials were rewarded. For the second task, the probability of reward depended critically on the past history, dropping to its lowest point