Regulation of Neuronal Migration in the Developing Brain

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Peering down a microscope at a mouse cerebral cortex reveals a fascinating structure. Cells are organized in three easily detectable zones: a dense ventricular zone, a sparse intermediate zone and a cortical plate in which cells are precisely aligned in six layers. This accurate layering and with it the ability of the cortex to form complex tasks are consequences of a complex phenomena known as neuronal migration. The cortex is not the only laminar brain structure that it formed by neurons that migrate away from their place of birth. The cellular architecture of the cerebellum, hippocampus, and colliculi is the product of coordinated neuronal journey and the olfactory bulbs are constantly being replenished by neurons form the rostral migratory stream. However the neurons in the cortex by far make the longest and the most interesting journey. Two types of neuronal migrations occur during embryonic development: radial migration and tangential migration. Radially migrating neurons will be the inhibitory pyramidal neurons and will comprise roughly 75% of the cortical cells population. Radially migrating neurons travel perpendicular to the ventricular surface outwards towards the pial surface. The radially migrating neurons are guided by a scaffold of specialized cells called radial glia. In the cortex, radial glial cells are the source of new neurons and neuronal progenitors, have a cell body in the ventricular zone and a process that reaches the surface of the brain. Post mitotic neurons rap around them and leave them when they reach the correct layer, which they will occupy. The moving cell extends a leading neurite, the centrosome and later the nucleus translocates forward, and a trailing process grows to become a future axon. Cells usually detach from their guiding glia cell during migration for a short period of time during which they move slowly and extend neurites to different directions. This is an exploratory behavior known as multipolar migration.

The tangential migration route supplies the cortex with its excitatory neurons. These are born in the ganglionic eminences, migrate parallel to the brain surface and later intercalate in the forming cortical layers. Unlike radial migration, tangential migration does not rely on radial glia and instead neurons attach to each other and relay on extracellular guidance cues to reach their final destination.

How do cells know when to leave the ventricular zone and commence their journey? What are the forces that allow cells to move and how are they generated on a molecular level? How does a cell acquire its polarity and directionality during the migration? These are all questions that entertain us and many researchers in the field. Our lab was established on the pioneer discovery of the first gene to cause a neuronal migration disease: LIS1 (Reiner et al., 1993). Mutations in LIS1 cause a devastating disease known as lissencephaly (smooth brain). Lissencephaly is a disorder of both tangential migration and radial migration, is characterized by a cortex that has only four layers and a brain that appears smooth with an absence of gyri and sulci and enlarged ventricles (Fig. 1).

Affected individuals suffer from severe mental retardation and usually a short life span. Our lab was the first to point to the importance of the microtubule cytoskeleton during migration. We showed that LIS1 binds and directly affects the dynamic behavior of this important biopolymer (Sapir et al., 1999a; Sapir et al., 1997). These finding were later supported by a plethora of studies the most recent of which is the finding that mutations in the GTPase binding site of α-tubulin cause abnormalities in neuronal migration.

We have created a mouse model for the disease in which the targeted deletion involves the first coding exon, such that a shorter version of the protein was expressed from the mutated allele. The protein ability to form homodimers was compromised and resulted in a delay in cortical neuron migration and an abnormal radial glia scaffold (Cahana et al., 2001; Reiner et al., 2002). Interestingly, a similar mutation exists in a human patient. We have studied what are the biochemical and cellular consequences of lissencephaly causing mutations (Caspi et al., 2003; Reiner and Coquelle, 2005; Sapir et al., 1999b; Sapir et al., 2000). We have extended our studies to a second gene that causes lissencephaly in males or subcortical heterotropia (double cortex),

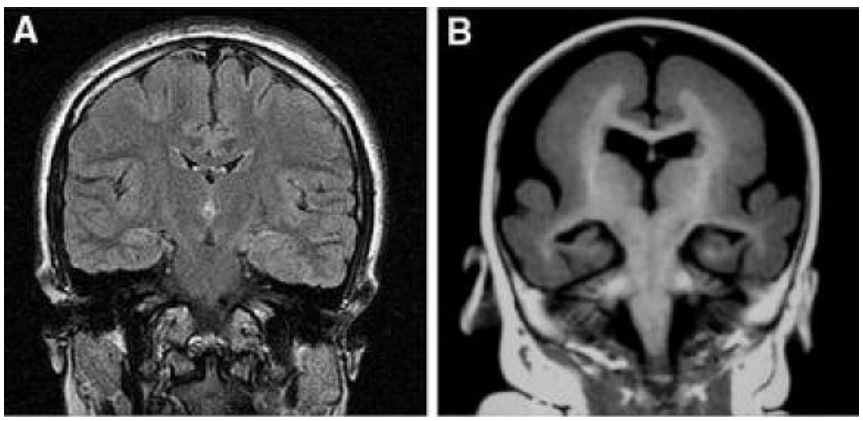

Fig. 1 *MRI of normal and lissencephalic brain. The outcome of neuronal migration defect is a smooth cerebral cortex with large ventricles. Affected individual suffer from severe mental retardation and reduced lifespan (from: Mamm Genome 2007 July; 18(6): 425–430).*

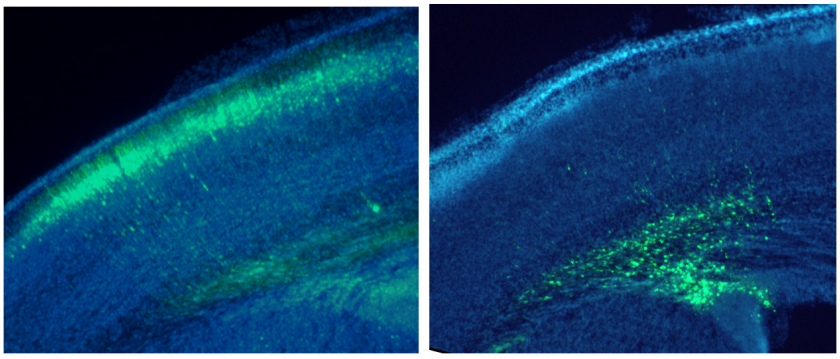

Fig. 2 *Manipulation of the mouse brain allows dissection of signaling pathway leading to neuronal polarity and migration. Introduction of specific shRNA in E14.5 mouse embryos (green cells, right panel) causes cells to stall close to the ventricular zone while control cells (green cells, left panel) make it all the way out to their final destination in the outer layers of the cortical plate.*

DCX. We defined this gene product as a microtubule binding protein with two microtubules binding domain in which many of the disease mutations cluster (Francis et al., 1999; Horesh et al., 1999; Sapir et al., 2000). Furthermore, we uncovered the interaction between DCX and LIS1, which was later also shown by genetic means (Caspi et al., 2000). DCX undergoes multiple posttranslational modifications, which regulate its activity and interaction with other proteins. More specifically DCX undergoes phosphorylation by JNK, interacts with JIP-1/-2 (JNK interacting protein), and with neurabin-2 (Gdalyahu et al., 2004; Reiner et al., 2004). Part of the phospho-DCX sites are dephosphorylated by PP1, and this dephosphorylation is mediated by neurabin-2 (Shmueli et al., 2006).

Interestingly a DCX mouse model failed to mimic the human phenotype and only defects in non-radial migration events were reported. The role of DCX during migration was uncovered only following acute reduction of DCX *in utero* was employed. This method is currently used routinely in our laboratory. Using this technique we were able to demonstrate the involvement of a signaling pathway known to contribute to the establishment of cellular polarity during migration (Fig. 2).

Watching live sections allowed us to monitor the translocation of the centrosome along the leading edge and to identify the substrate that mediates the kinase activity during migration.

Our study includes other members of the DCX family. We have demonstrated some of DCLK1 cellular roles (Burgess et al., 1999; Burgess and Reiner, 2000; Burgess and Reiner, 2001; Burgess and Reiner, 2002; Shu et al., 2006). Using bioinformatics tools we defined new members of the family and showed that they have common as well as differentiating characteristics (Coquelle et al., 2006; Reiner et al., 2006). One particular member of the family has a pivotal role during mitosis and it is now under extensive study (Fig. 3).

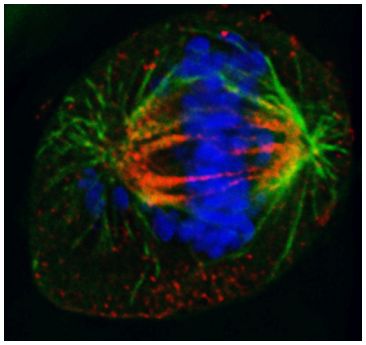

Fig. 3 *Studying the cellular role of proteins from the DCX family. HeLa cells undergoes mitosis DCX containing protein (red) is colocalized with microtubules (green), chromosomes (blue) are aligned before being separated to the forming daughter cells.*

The molecular motor dynein has been shown to be extremely important in generating the forces needed for nuclear translocation during migration. LIS1 has a conserved role in relation to dynein regulation (Morris et al., 1998). Furthermore, we identified and studied the intricate relationships between LIS1, CLIP-170, and dynein (Coquelle et al., 2002). In a recent study we have uncovered a novel role for Ndel1, a LIS1 binding partner, in regulating the motor activity and in keeping the balance between the function of motors that ride the microtubule in opposite direction (Fig. 4). By combining animal models with cellular studies, bioinformatics and cutting edge imaging technologies we are aiming at uncovering more of the molecular mechanism that underlie this fascinating process of corticogenesis.

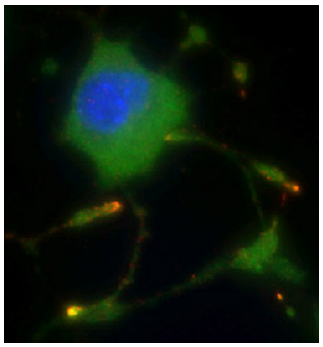

Fig. 4 *Localization of LIS1 (red) to the tip of CAD cells depends on the balanced activity of + and – end directed molecular motors. LIS1 and Ndel1 participate in regulating Dynein, a minus end motor.*

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