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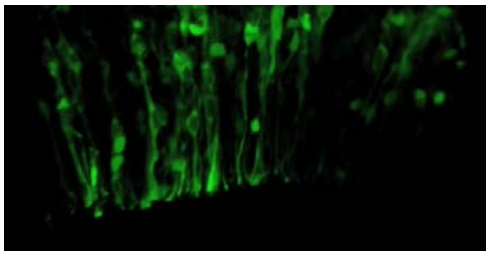
Pushing in, crowding out: Dual directional mechanisms of interkinetic nuclear migration

August 5, 2011 – The surface, or cortex, the mammalian brain is formed from highly organized layers comprising distinct types of neural cells. This cellular diversity, and the underlying order that informs the overall cortical structure, are largely the result of routines that play out during the brains development in embryonic stages. Important among these is a process called asymmetric cell division, in which progenitor cells give rise to daughter cells of different fates. Interestingly, neural progenitors remain on the move during this process, shuttling their nuclei back and forth between the apical and basal sides of the developing cortex in an action known as interkinetic nuclear migration (INM). These movements are timed in sync with the cell cycle, but just how this is accomplished has remained enigmatic.



Neural progenitors undergo dynamic oscillations of nuclei, called interkinetic nuclear migration, and divide facing the ventricular zone (apical side). In this movie, cells with nuclear GFP migrate basally at G1 phase while those in G2 phase (cytoplasmic GFP) migrate apically.

Now, Yoichi Kosodo and others in the Laboratory for Cell Asymmetry (Fumio Matsuzaki, Group Director) have identified a pair of mechanisms that couple INM to the mitotic cycle. In an article published in *The EMBO Journal*, the group shows that cell-autonomous basal-to-apical nuclear migration proceeds under the control of the microtubule-associated protein Tpx2, which drives migration of the nucleus in the opposite direction via a displacement, or "crowding out," effect.

The group began by developing a system to allow them to track nuclear movement during INM in living tissue, enabling them to identify a number of novel features of this behavior and its coordination with various stages of the cell cycle. To test whether interkinetic nuclear migration in fact depends on cell cycle progression, they used an inhibitor to arrest neural progenitors at the G1 phase, and found that this prevented the apical migration of nuclei at the start of INM. Previous studies had raised the possibility that this linkage might rely on the function of Tpx2, a microtubule nucleating protein, the expression of which is regulated by the cell cycle. Monitoring of a fluorescent-tagged version of the protein revealed that it associates with microtubules in the apical processes of neural progenitor cells.

Kosodo next interfered with Tpx2 function using RNAi to examine whether it is involved in apical nuclear migration, and found that its knockdown resulted in a significant decrease in this aspect of INM; specifically, that loss of Tpx2 function caused a slowdown in the speed of nuclear movement toward the apical region. Observations of microtubule distribution in wildtype and Tpx2-inhibited cells indicated that the factor works to localize microtubules in the apical process of neural progenitor cells. Taken together, these results suggest that basal-to-apical nuclear migration during the G2 phase is dependent on the reorganization of microtubules by Tpx2.

What then of nuclear migration in the opposite direction? In G1-arrested neural progenitor cells, the group observed that nuclei accumulated basally, raising the possibility of a separate mechanism behind this. Using a magnetic fluorescent microbead assay, they tracked the

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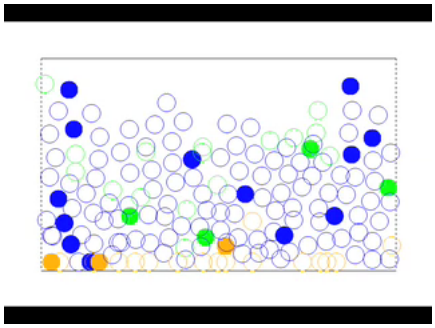
motion of apically-located beads in cultured brain slices, and found that this drew them to the basal side, from which they did not subsequently return to the apical region, even when nuclei continued to migrate in that direction. It appeared that their translocation was therefore non-cell-autonomous, but the mechanism remained in doubt.



A computer modeled simulation faithfully mimics the behavior of apical to basal nuclear migration of neural progenitors. The model assumes that G1 nuclei move such that the local nuclear density is minimized, whereas G1 nuclei apically migrate at a constant speed (1μm/min)

One possibility was that the active migration in the basal-to-apical direction increases nuclear density in the apical region, which might lead to a "crowding out" effect. Kosodo et al. tested this by arresting the cell cycle at S phase by drug treatment and observing the effects on nuclear migration over time. Cell cycle arrest causes a drop in the number of apically migrating nuclei. They found that this consequently reduced the rate at which nuclei moved in the basal direction as well. The same phenomenon was observed in observations of microbeads, suggesting that it was not due to some unknown bioactive property of the mitotic inhibitor, but that basal movements of nuclei or beads are tightly linked with apically directed nuclear movements. To further test their hypothesis that apical-to-basal nuclear migration is the result of displacement, the group, in collaboration with Akatsuki Kimura at National Institute for Genetics, constructed a computational simulation of INM to model conditions that might influence nuclear migration in the apical-to-basal direction, and found that their in silico predictions jibed well with their in vivo observations, strongly suggesting that this aspect of INM is driven by physical displacement of nuclei from the basal region.

"In the developing brain, a huge number of neural progenitors must engage in highly dynamic movements in order to give rise to its myriad neurons," says Matsuzaki. "What we have discovered is that the independent but linked mechanisms that drive movements in opposing directions help to avoid extreme deviations or collisions of progenitors and maintain the structural order of the brain's morphology. Indeed, this may represent a fundamental strategy for maintaining order in dynamic cell populations."

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