Nucleokinesis illuminated

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Newborn neurons in the cerebellum migrate along radial glial processes through a series of distinct steps. A report in this issue uses live imaging to grant us a close-up view of the cytoskeletal structures and regulating proteins involved in this migration.

Cortical neurons in the brain reach their final position through a highly organized migration in either the radial (from ventricular to pial surface) or tangential (parallel to the ventricular surface) direction[1]. Most radially migrating neurons move along the processes of radial glia cells through a series of sequential steps. They first extend a leading process in the direction of migration, which is followed by 'nucleokinesis', the saltatory forward movement of the nucleus into the leading process. Nucleokinesis critically depends on the microtubule network of the migrating neuron[2]. Microtubule-dependent nuclear positioning also occurs in many other cell types and cellular processes, including developing photoreceptors of the *Drosophila melanogaster* eye[3] and P-cell migration in *Caenorhabditis elegans*[4], and often depends on an interaction of the microtubule organizing center (MTOC) with the nucleus[5]. In neurons, disruption of nuclear translocation leads to defects in neuronal migration[6–8]. There is also strong evidence for an essential interaction between the centrosome (MTOC) and nucleus during nucleokinesis, mediated by a specialized network of perinuclear microtubules. Most studies that have described these microtubules in neurons have used static imaging[6,8,9], so knowledge of perinuclear microtubule dynamics during migration has been limited. In this issue, Hatten and colleagues make a substantial contribution to the understanding of the cell biology of nuclear translocation by developing a system to image the cytoskeleton in migrating neurons[10].

Static images of perinuclear microtubules have revealed a cage- or fork-like structure[8,9]. The differences reported are likely to result from the use of different types of neurons or from the fact that these perinuclear microtubules are delicate and difficult to label. The authors overcome this experimental constraint by infecting cerebellar granule cells migrating in culture with a retroviral construct encoding α-tubulin tagged with the Venus variant of yellow fluorescent protein (YFP)[10]. Their extraordinary series of movies shows that the perinuclear microtubule cage, labeled with the fluorescent Venus, undergoes substantial changes during nucleokinesis. As nucleokinesis begins, the microtubule cage becomes stretched. The cage and nucleus then move forward together, compressing the microtubule network. Finally, as the nucleus comes to a halt, the cage regains a compact form (**Fig. 1**). During the entire movement sequence, the microtubule cage enwraps the nucleus. Stationary cells also have a perinuclear microtubule cage, but it remains mostly stagnant, suggesting that dynamic alterations in the cage structure underlie nuclear translocation.

If the dynamic alterations of this specialized microtubule network are the basis of nuclear translocation, it is easy to imagine that mutations disrupting perinuclear

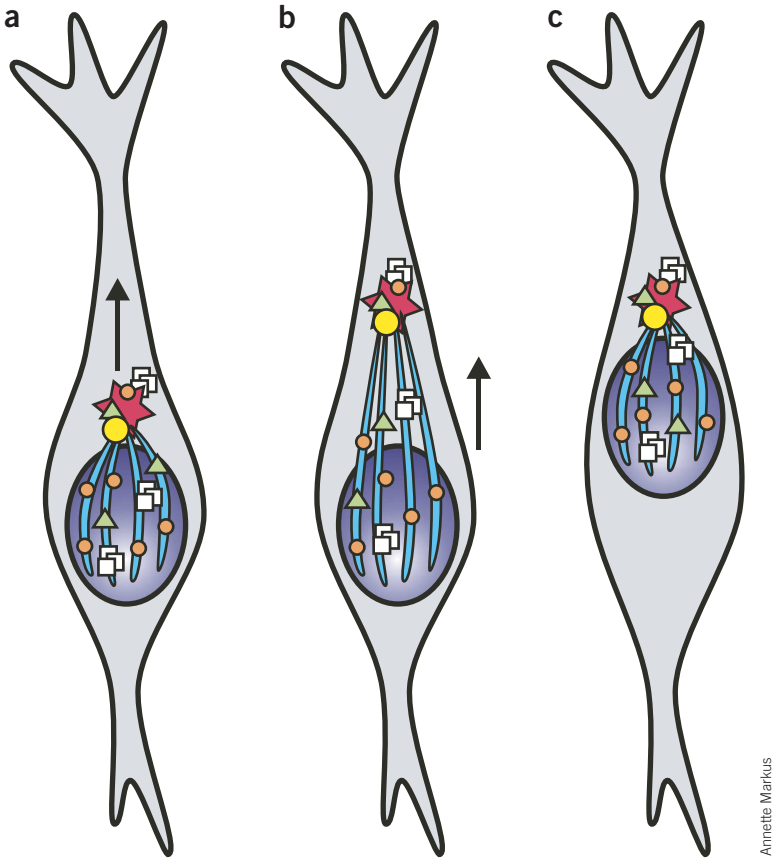

Figure 1 Function of the perinuclear cage in nucleokinesis. (**a**) Nucleokinesis initiates when the centrosome (red) begins movement into the leading process. This step depends on the function of mPar6α (yellow) and possibly Cdk5-phosphorylated FAK (green triangles). (**b**) After the centrosome moves, the structure of the perinuclear microtubule cage changes. A functional dynein-Lis1-Nudel complex (white) maintains tight coupling of the centrosome and nucleus by moving toward the minus end of the microtubules situated at the centrosome. This movement may pull the nucleus toward the centrosome through unknown proteins anchored in the nuclear membrane, resulting in directional nuclear translocation. (**c**) After nuclear movement, the microtubule cage regains its original form. Throughout nucleokinesis, Dcx (orange) regulates microtubule integrity[6].

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microtubules will have drastic consequences during nucleokinesis and neuronal migration. We are only now beginning to understand the components of the perinuclear microtubule network from a molecular standpoint. However, it is known that *Lis1* and *Dcx* (doublecortin), two proteins that when mutated result in lissencephaly, decorate perinuclear microtubules that extend to the centrosome[6,8]. Intriguingly, the homolog of *Lis1* in the fungus *Aspergillus nidulans*, *NudF*, is essential for proper distribution of nuclei. Two Lis1 binding partners, dynein and Nudel, also have homologs that cause abnormal nuclear distribution when mutated in *A. nidulans*[11]. Sure enough, Nudel and dynein are also components of the neuronal perinuclear microtubule network, as is Cdk5-phosphorylated focal adhesion kinase (FAK)[8]. Overexpression of a FAK mutant that cannot be phosphorylated by Cdk5, or depletion of any of the proteins in the dynein-Lis1-Nudel complex, disrupts perinuclear microtubules and results in nuclear translocation and neuronal migration defects[7,8]. Thus, properly organized perinuclear microtubules seem to be important for nuclear translocation. It has also been suggested that the neuronal migration defects seen in type I lissencephaly are really defects in nuclear migration and/or translocation[2,11]. Taking this hypothesis a step further, it is likely that defects in the perinuclear microtubule cage result in failure of nuclear translocation and improper neuronal migration.

To understand exactly what this microtubule network does, it is necessary to consider the other key player in nucleokinesis, the centrosome itself. In many organisms and cell types, nuclear positioning is dependent on the MTOC. Several examples suggest that when the MTOC and the nucleus are experimentally separated, the MTOC will be positioned correctly but the nucleus will not[5]. In their study, Hatten and colleagues identified a previously unrecognized component of the neuronal centrosome, mPar6α, through immunostaining and biochemical fractionation. The mPar6 proteins, which form scaffolding complexes for different forms of protein kinase C, are the mammalian homologs of a protein that is required for the generation of anterior-posterior polarity in *C. elegans* embryos[12]. The mPar6α-PKCζ complex interacts with GSK-3β to promote polarization of the centrosome and to control the direction of cell protrusion in astrocytes[13]. Using a Venus-tagged version of mPar6α, Hatten and colleagues were able to image the centrosome relative to the nucleus in a migrating neuron. As has been shown before, throughout the movement, the centrosome was tightly coupled to the nucleus and remained on the side of the nucleus proximal to the leading process[14]. However, the authors found that the movement of the centrosome preceded that of the nucleus. To confirm this finding, they used another Venus-tagged centrosome protein, p50 dynactin of the dynein complex[15]. Again, the Venus-dynactin–labeled centrosomes initiated movement in the direction of the leading process just before nuclear translocation. Just as nuclear positioning depends on the MTOC in many other cell types and organisms, these data demonstrate that in neurons as well, the centrosome establishes polarity by moving first and leading the nucleus in the direction of migration.

The importance of this result is emphasized by recent studies that have assayed centrosome-nucleus coupling in relation to neuronal migration[6,7]. Microtubule-dependent nuclear positioning involves a tight association between the centrosome and the nucleus, and disruption of this coupling in neurons results in migration defects. For example, mice with a reduction in Lis1 dosage display a disorganization of neuronal cytoarchitecture throughout the brain, and cultured neurons from *Lis1*$^{+/-}$ mice show an increased separation between the nucleus and centrosome during migration[6]. Likewise, Lis1, dynein or Nudel loss of function results in defects of centrosome-nucleus coupling and neuronal migration[6,7]. Hatten and colleagues show that overexpression of mPar6α results in loss of centrosome and microtubule integrity. Intriguingly, depletion of mPar6α by short hairpin RNA (shRNA) resulted in a motionless centrosome. This suggests that the dosage of mPar6α is crucial for regulating movement of the centrosome, which itself is an initial step in nucleokinesis.

With the demonstration by Hatten and colleagues that centrosome movement precedes that of the nucleus, taken together with previous studies demonstrating the importance of centrosome-nucleus coupling and describing the perinuclear microtubule network, the function of the microtubule cage is becoming clear (**Fig. 1**). First, mPar6α, a protein important for establishing polarity, regulates the movement of the centrosome[10]. When mPar6α is overexpressed, the centrosome disintegrates and the microtubule network cannot be sustained, resulting in uncoupling of the centrosome and the nucleus. Furthermore, when mPar6α is depleted, the centrosome fails to move. Cdk5-phosphorylated FAK also may regulate centrosome movement, as overexpression of the nonphosphorylatable mutant FAK results in a prominent centrosome that is still adjacent to the nucleus, but loss of integrity of the perinuclear microtubules and faulty nuclear translocation[8]. Upon forward movement of the centrosome, the perinuclear microtubule network that extends from the nucleus to the centrosome becomes stretched[10]. The function of the cage at this point is to maintain the tight association between the nucleus and the centrosome, perhaps by triggering a functional dynein-Lis1-Nudel complex to move in a retrograde fashion along the cage toward the centrosome. Depletion of any of these molecules will result in uncoupling of the centrosome and the nucleus, loss of integrity of the microtubule cage and abnormal nuclear translocation[7]. A functional dynein-Lis1-Nudel complex, however, may associate with unknown proteins that pull the nucleus toward the centrosome by anchoring themselves in the nuclear membrane. As the nucleus moves in the direction of migration, the perinuclear microtubule network regains its original shape, and tight centrosome-nucleus coupling is maintained. Throughout this process, doublecortin regulates the integrity of the microtubule cage and centrosome-nucleus coupling[6].

Although several remaining molecular components—and the mechanistic properties that allow for interplay between all of these steps—remain to be worked out, Hatten and colleagues have added some important pieces to the puzzles that still remain in the neuronal migration field. A more advanced framework for understanding nucleokinesis is now in place.

1. Gupta, A. *et al. Nat. Rev. Genet.* **3**, 342–355 (2002).
2. Lambert de Rouvroit, C. & Goffinet, A.M. *Mech. Dev.* **105**, 47–56 (2001).
3. Mosley-Bishop, K.L. *et al. Curr. Biol.* **9**, 1211–1220 (1999).
4. Malone, C.J. *et al. Development* **126**, 3171–3181 (1999).
5. Reinsch, S. & Gonczy, P. *J. Cell. Sci.* **111**, 2283–2295 (1998).
6. Tanaka, T. *et al. J. Cell. Biol.* **165**, 709–721 (2004).
7. Shu, T. *et al. Neuron* **44**, 263–277 (2004)
8. Xie, Z. *et al. Cell* **114**, 469–482 (2003).
9. Rivas, R.J. & Hatten, M.E. *J. Neurosci.* **15**, 981–989 (1995).
10. Solecki, D.J. *et al. Nat. Neurosci.* **7**, 1195–1203 (2004).
11. Morris, N.R. *et al. Trends. Cell. Biol.* **8**, 467–470 (1998).
12. Wodarz, A. *Nat. Cell. Biol* **4**, E39–E44 (2002).
13. Etienne-Manneville, S. *et al. Nature* **421**, 753–756 (2003).
14. Rakic, P. *Experientia* **46**, 882–891 (1990).
15. Echeverri, C.J. *et al. J. Cell. Biol.* **132**, 617–633 (1996).