Neuronal migration illuminated
A look under the hood of the living neuron

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During vertebrate brain development, migration of neurons from the germinal zones to their final laminar positions is essential to establish functional neural circuits.1-3 Whereas key insights into neuronal migration initially came from landmark studies identifying the genes mutated in human cortical malformations,4 cell biology has recently greatly advanced our understanding of how cytoskeletal proteins and molecular motors drive the morphogenetic cell movements that build the developing brain. This Commentary & View reviews recent studies examining the role of the molecular motors during neuronal migration and critically examines current models of acto-myosin function in the two-step neuronal migration cycle. Given the apparent emerging diversity of neuronal sub-type cytoskeletal organizations, we propose that two approaches must be taken to resolve differences between the current migration models: the mechanisms of radial and tangential migration must be compared, and the loci of tension generation, migration substrates and sites of adhesion dynamics must be precisely examined in an integrated manner.

Basic Mechanisms of Neuronal Migration: A Multistep Process

Since the first time-lapse studies documented cerebellar granule neuron (CGN) migration along Bergmann glial fibers,5 in vitro and ex vivo imaging has revealed many features of migrating neurons that are conserved throughout the nervous system.6-10 Most migrating neurons extend a dynamic leading process from the cell body to sample the surrounding microenvironment while the soma and nucleus remain largely stationary. Leading processes differ in morphology across classes of neurons, but all leading processes require microtubule stability and efficient membrane trafficking for their extension and function. After the leading process commits to a single direction, the cell body and nucleus translocate forward in a two-step process termed nucleokinesis. During the first step, a distal swelling appears in the proximal leading process and the centrosome and Golgi apparatus move toward it.10-13 These two events are thought to be strong predictors of the final step of migration, in which the nucleus and cell body translocate, occasionally leaving behind a trailing process.

Our understanding of the mechanisms of migration has advanced greatly during the past five years, with improved imaging techniques and reagents. Neuronal migration is now known to be a highly orchestrated event involving intricate interplay between microtubules, actin and their associated motor proteins. To better understand how neurons move, it is important to know where cytoskeletal elements are located and how they interact during the migratory cycle, which is perhaps best investigated using drugs that alter the natural state of the cytoskeleton and motor proteins. In cytoskeletal perturbation experiments, 50 μM blebbistatin, which inhibits ATPase activity of the A and B isoforms of non-muscle myosin ii, halted somal translocation without affecting leading process advancement, whereas 100 μM blebbistatin completely arrested

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Abbreviations: CGN, cerebellar granule neuron; SVZa, anterior subventricular zone; FRAP, fluorescence recovery after photobleaching; RMS, rostral migratory stream; EGL, external granule layer; ML, molecular layer; IGL, internal granule layer; MGE, medial ganglionic eminence

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migration of anterior subventricular zone (SVZa) cells. Conversely, depolymerization of microtubules via nocodazole treatment did not affect cell body movement but halted the advance of the leading process. Whereas such studies demonstrate that the acto-myosin and the microtubule cytoskeletal components have different functional requirements during discrete migration stages, the spatio-temporal sites of action of these components remains a topic of much debate.

**Nucleokinesis**

Centrosome movement into the dilated region is commonly used as a predictor of subsequent nuclear movement. In recent slice experiments, dynein was shown to be responsible for centrosome and nuclear movement into the proximal leading process, as knockdown of LIS1 (a dynein regulator) or dynein itself inhibited centrosomal displacement, whereas blebbistatin did not affect centrosome movement.10 However, we suspect that the concentration of blebbistatin in these experiments may have been insufficient to affect centrosome movement; subsequent studies demonstrated that addition of blebbistatin to purified CGNs did in fact arrest centrosomal movement, as did jasplakinolide, an actin-anchoring drug.14

Many previously believed that the nucleus is tethered to the centrosome and that the centrosomes’ movement into the proximal region of the leading process induces forward nuclear movement.15 Umeshima et al. confirmed that the nuclei of CGNs migrating in ex vivo slice preparations are surrounded by a cage of microtubules,13 as had been observed in previous in vitro studies.15 Interestingly, the cage is composed of different populations of microtubules, most of which are tyrosinated and are thought to be dynamically unstable. However, the microtubules around the anterior surface of the nucleus were found to be acetylated, a post-translational modification associated with stability. The acetylated microtubules extended from the nucleus toward the proximal leading process and were not associated with the centrosomes as previously thought. In live imaging experiments ex vivo and in vitro, they observed that the nucleus, powered by cytoplasmic dynein, frequently moved ahead of the centrosome. In fact, the microtubules emanating from the anterior portion of the nucleus tended to bypass the centrosome and extend into the leading process. This population of microtubules was shown to be essential for migration of the nucleus, possibly because they provide a direct bridge between the microtubules surrounding the nucleus and the tension in the leading process provided by acto-myosin.

**Conflicting Acto-Myosin Models: Engine in the Leading Process or Cell Rear?**

While a central role for acto-myosin dynamics in driving cell migrations has been established for nearly every cell type examined,16 there is considerable diversity in cytoskeletal architecture or location of motor components for many models of cell motility. For example, blood cells possess prominent accumulation of acto-myosin at the cell rear7 while epithelial cells or migrating fibroblasts possess significant myosin ii accumulation in the lamellum forward of the nucleus.18

Given the apparent cytoskeletal diversity observed in non-neuronal cells it is no surprise that differences in acto-myosin function have been observed in migrating neurons (Fig. 1). Initial studies suggested that acto-myosin contractility is localized at the rear of migrating neurons.9,10,12,19 This theory, based largely on Medial Ganglionic Eminence (MGE) interneurons migrating in cortical slice preparations or in vitro cultures2 and SVZa precursors migrating in 3D matrigel matrix,9 surmises that myosin ii-based contraction at the rear of migrating neurons functions to squeeze the contents of the neuron forward during somal translocation and nucleokinesis (Fig. 1A).

More recently, our laboratory and the laboratory of Xiaobing Yuan, have shown the leading process of radially migrating CGNs contains a high concentration of F-actin and myosin ii motors, with very little acto-myosin located in the cell body rear or trailing process (Fig. 1B).14,20 Fluorescence recovery after photobleaching (FRAP) analysis also showed the leading process is the region of highest acto-myosin turnover while pulse chase experiments further consolidated these findings, showing that F-actin flows from the cell soma toward the direction of migration.14,20 The co-localization of F-actin and myosin ii strongly suggests that acto-myosin contractility events play an important role in the cytoplasmic dilation and distal leading process of CGNs. Further, we have observed that leading process acto-myosin contractility is required for translocation of the centrosome14 and Golgi (unpublished data) into the leading process during CGN migration. Thus, acto-myosin tension anterior to the cell body may be required for organelle positioning events prior to the final stage of movement and may “prime” the cell for somal/nuclear translocation.

While the observed differences in migration mode between MGE interneurons, SVZa cells and CGNs may represent bona fide cell type-specific differences in acto-myosin organization, we’d like to note there were significant differences in experimental conditions and how acto-myosin organization was assessed. For example, migration substrates significantly differed: MGE migration was assayed in both ex vivo slices or cortical mixed cultures, SVZa migration was assayed in matrigel matrix and CGN migration was assayed in neuron-glial co-cultures that recapitulate radial migration. Moreover, most of the studies demonstrating the localization of myosin ii at the cell rear lack correction for changes in cytoplasmic volume and have not directly probed cytoskeletal dynamics using techniques like FRAP or photoactivation. Thus, it is still unclear if the cell rear is a site for specific acto-myosin accumulation (i.e., specifically concentrated relative to cytoplasmic volume) or if actin undergoes rapid myosin ii-dependent turnover in the cell rear in MGE interneurons or SVZa cells.

Local inactivation of myosin ii motor activity in the leading process and at the cell rear would shed much needed light on this controversy and would be a significant advance compared to the bath application of myosin ii inhibitors used in previous studies.9,12,14 Recently, He and colleagues have re-examined acto-myosin localization in migrating CGNs and performed local inactivation of myosin ii and f-actin dynamics.20 These studies confirmed the high-leading process and low cell rear distribution of myosin ii. Moreover, focal
inactivation of acto-myosin activity in the leading process but not cell rear slowed somal translocation. Similar studies will be needed in MGE interneurons or SVZa neurons to confirm the proposed differences in subcellular acto-myosin function or if leading process acto-myosin function has been underestimated in these cells.

**Toward Resolving Model Differences: Tangential vs. Radial Migration**

Whereas at first it appears difficult to resolve the apparent cytoskeletal diversity of migrating neurons, closer inspection reveals that these neurons differ in their migration pathways and morphology. Acto-myosin organization has been closely examined in two tangentially migrating neuronal types to date: MGE interneurons and SVZa cells\(^9,12,19\) and radially migrating CGNs (by time-lapse imaging).\(^14,20\) SVZa cells migrate as coherent chains of neuroblasts\(^21\) that require laminin recognition.
by β1 integrin receptors to reach the olfactory bulb. While tangentially migrating MGE cells also require an integrin receptor to migrate, these neurons do not use laminin substrates or migrate as chains; rather, they interact with multiple migratory pathways including axons, dendrites and eventually radial glia. In contrast, radially migrating neurons are β1 integrin-independent and use a different set of molecules to adhere to glial fibers as they migrate to a final laminar position: CGNs use astrotactin and cortical pyramidal neurons use connexin.

Diversity in migration paths or substrates can have important implications for cytoskeletal organization. For example, in recent studies variation in the migratory environment’s geometry (one-, two-, or three-dimensional) dramatically altered the position of actin or myosin II motors in fibroblasts. Fibroblasts normally harbor myosin II at the cell rear, but they localized it forward of the nucleus when they migrate along Bergmann glial fibers. Because parallel fibers are anchored in the soma migrates forward. Remarkably, they must trail an extensive axonal structure from the rear of the cell body as the soma migrates forward. Remarkably, very little is known about how granule neurons elaborate this axonal process as they migrate along Bergmann glial fibers. Because parallel fibers are anchored in the ML, complete de-adhesion of the trailing process, as seen at the rear of migrating fibroblasts or as proposed for tangentially migrating neurons, would likely result in misplacement or loss of contact between the granule neuron soma and parallel fiber axons. In contrast, both MGE interneurons and SVZa neurons possess a less extensive trailing process and do not trail an axon.

Thus, extensive retraction of the trailing process could create the appearance of “squeezing at the rear” of these cells. It will be of interest to examine whether other types of neurons that undergo radial migration and possess an axonal trailing process, like cortical pyramidal neurons, possess an acto-myosin organization like that of CGNs. If tangentially migrating cells generally show prominent “squeezing at the rear” whereas radially migrating neurons do not, the controversy surrounding the two opposing models will be somewhat resolved. In that case, the cerebellar granule neuron would be an invaluable tool to discover the molecular mechanisms controlling the switch in cytoskeletal organization, as these cells undergo two migration phases: tangential migration near the cerebellar surface followed by radial migration away from the EGL after they cross the ML and enter the internal granule layer.

### Adhesion Dynamics and Myosin II Motors

Most neuronal migration studies to date have indirectly examined sites of myosin II function by using movement of the cytoplasmic organelles, nucleus or soma as surrogate indicators. We believe that the site of action of acto-myosin-based contraction can be firmly established only by local determination of myosin II function. Historically, myosin II activity has long been believed to power migration by squeezing cytoplasm toward the front of the cell, shaping the cell rear and breaking adhesions in the trailing portion of migrating cells. Recent advances in general cell motility suggest that myosin II motors not only act in the cell rear, as older studies suggested, but also play a direct role in the leading portion of the cell where myosin II-based contractions generate force of the nucleus is required for efficient adhesion and cell motility.

### Does Myosin II or Dynein Power the First Step?

The largest remaining discrepancy between the “reach and pull” and “squeezing at the rear” models is the identity of the motor(s) that power the first step of the two-stroke movement cycle. Whereas it is well established that cytoplasmic dynein powers nuclear translocation in migrating neurons and regulates the positioning of microtubule arrays, recent studies suggest...
that acto-myoosin also plays an important role in centrosome positioning. Piel et al. have shown that the actin cytoskeleton is needed to attract daughter centrioles to the future site of mid-body abscission during mitosis.38 Rosenblatt et al. have shown that cortical myosin II motors are essential to control mitotic spindle rotation.40 Our studies using blebbistatin and the F-actin stabilizer jasplakinolide strongly suggest that myosin II provides the force needed to move centrosomes and thus coordinate the first step of the migration cycle.14 We therefore propose that myosin II in the leading process plays a significant role in positioning organelles within the actin-rich cytoplasmic dilation before nuclear/somal translocation.

As both dynein and myosin II are required for centrosome positioning events, further studies are required to dissect the functions of these motor components. It is possible that dynein and myosin II cooperate to power organelle positioning events before somal/nuclear translocation. For example, an intact actin cytoskeleton is required for dynein transport of microtubule fragments into axons during slow axonal transport.41 How could acto-myoosin and dynein cooperate during the first step of the migration cycle? Dynein interacts with the plasma membrane of a variety of cell types in an actin-dependent manner via a linkage between dynactin and scaffolding molecules like β-catenin38 and PLAC-24.42 Interestingly, β-catenin and PLAC-24 recruitment to the cell cortex depend on an intact actin cytoskeleton and myosin II motors.42,43 Therefore, interaction of dynein with the actin-rich leading process cortex via molecules like β-catenin or PLAC-24 could link acto-myoosin flow to microtubule movement associated with centrosome positioning. Studies under way in our laboratory are examining whether inhibition of myosin II motor activity or actin cytoskeletal dynamics in live CGNs alters the localization of dynein or dynactin in the leading process or alters their turnover to begin to directly test this hypothesis.

**Concluding Remarks**

Examination of the genes and signaling pathways regulating neuronal migration has led to the discovery of a host of cytoskeletal components required for neuronal migration. The remarkable successes of the past decade have created a new challenge—that of weaving the ever-increasing array of diverse cytoskeletal regulators required for neuronal migration into an unbiased and integrated model of neuronal migration. Meeting this challenge in a manner that allows a better understanding of the pathology of neuronal positioning disorders and perhaps eventually repair defective migration will require careful consideration of the differences in neuronal migration model systems and an integrated approach to the function of the neuronal migration machinery, as opposed to the study of cytoskeletal components in isolation.

**Future Directions**

Whereas cell type-specific variations in migration substrate and morphology may explain some differences in migration mode and cytoskeletal organization, more important factors, such as the true site of action of acto-myoosin-based tension and the functional cooperation of actin- and microtubule-based motors in generating the two-stroke migration cycle, remain to be fully elucidated.

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