Potential mechanisms of mutations that affect neuronal migration in man and mouse

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Mutations in the genes that encode filamin-1, Lis1 and doublecortin are responsible for X-linked lissencephaly in man, whereas mutations in the genes that encode Cdk5, its activator p35 and the reelin-signaling pathway disturb migration and architectonic development in mice. To understand the action of genes that control neuronal migration and the phenotype of corresponding defects, it might be as important to consider the positioning of the nucleus as it is to consider the guidance of the leading process.

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Abbreviations

CDK	cyclin-dependent kinase
FLN1	filamin-1
Lis1	lissencephaly 1
PAFAH1β1	platelet-activating factor acetylhydrolase β1
VZ	ventricular zone

Introduction

The long-range migration of neurons in the developing brain is a process that is unusually amenable to genetic analysis, so that the past two years have witnessed a small explosion in our understanding. During development, neurons are generated in specialized proliferative zones along the ventricular cavities, called ventricular zones (VZs). Postmitotic neurons then migrate through the parenchyma over variable distances before they settle and form early architectonic patterns. Although neuronal migration occurs throughout the nervous system, it is best known and studied in the cerebral cortex, the hippocampus, the olfactory bulb, and the cerebellar cortex.

The most familiar type of neuronal migration proceeds radially from the VZs to the pial surface. The leading process — which represents the dendritic tip that will become the dendritic pole — as well as the cell body and the trailing process are all closely apposed to and follow radial neuroepithelial and, later, glial fibers. Thus this migration has been termed giophilic [1]. More recently, the known variety of tangential migration, parallel to the pial surface, has been greatly expanded. Examples are the migration of external cerebellar granule cells [2], or of γ amino butyric acid (GABA)ergic neurons from the ganglionic eminence to the cortex [3–5]. In tangential migrations, the leading process is capped with a growth cone that is similar to axonal growth cones and migration proceeds perpendicularly to the radial fibers, often, though not always, in apposition to other neurons; hence, it is sometimes called neuronophilic. More recently, a third type of neuronal migration, called chain migration, has been introduced to describe migration from the subventricular zones to the olfactory bulb and rostral forebrain [5,6]. Neurons appear to move within a glial tunnel and their leading processes have features of growth cones [7]. How chain migration differs from gliophilic and neuronophilic migration remains to be assessed. This review discusses recent progress in the understanding of genes that are required for neuronal migration, and our attempts to fit these genes into biochemical and cellular pathways.

Events in neuronal migration

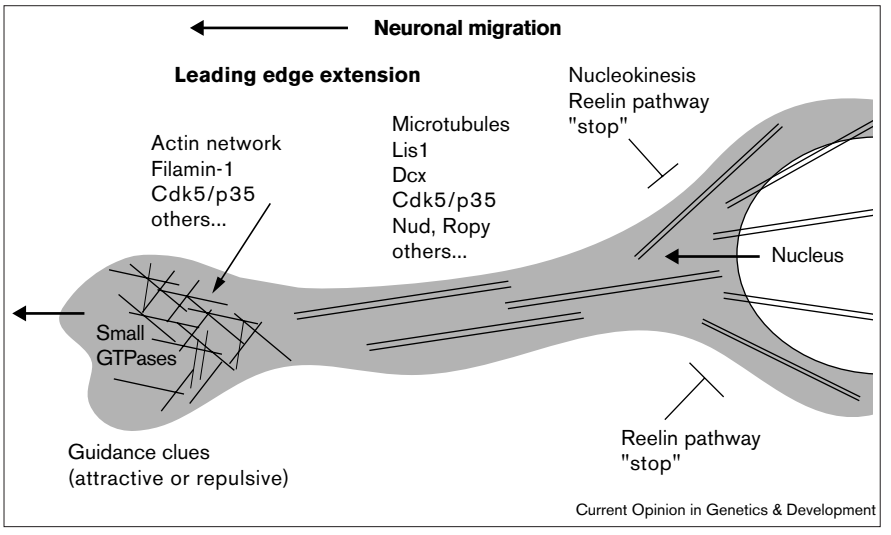
Studies of migrating cells in culture [8], define three main events: first, migration is initiated at the cell's leading edge through formation of protrusions and formation of adhesion plaques; this is controlled by GTPases of the Rho family, such as Cdc42 and Rac, that regulate the polymerization and reorganization of actin microfilaments; second, contractile forces behind the leading edge drive movement of the cell body, particularly displacement of the nucleus (nucleokinesis), there is evidence that microtubules are involved in this process and in regulating the turnover of adhesions; third and finally, adhesions are removed at the cell rear. Although the extension of cellular processes and displacement of the nucleus are not completely independent, genes that disrupt neuronal migration appear to affect these two processes differently.

To consider that neuronal migration is not solely guidance of the leading process but also nucleokinesis is less trivial than it seems, as illustrated with a few examples. The idea that migration to the cortex could be partly explained by perikaryon displacement in a cytoplasmic furrow is far from new and has generated heated debate [9]. Although the concept fell slightly out of fashion, recent observations suggest that it could indeed play a part in early cortical development [10].

In most neurons (e.g. cortical neurons, Purkinje cells), the nucleus clearly does not move into the elongating axon; however, in some instances (e.g. peripheral sensory neurons) it does migrate for a limited distance. In tangentially migrating cells, nucleokinesis is actually what defines migration itself. Interestingly, in neurons that migrate from the rhombic lip, the nucleus moves into a long tangential process that resembles axons but clearly lacks many axonal biochemical characteristics [11•]. In radially migrating neurons, the leading tip is guided by clues that must be different from those that guide tangential migration, although common mechanisms

Figure 1

Neuronal migration requires the extension of the leading edge (dendritic tip or axonal growth cone) in response to guidance clues, as well as nucleokinesis, and both processes could be independently regulated. Leading edge extension requires integration of extracellular clues by small GTPases, the actin network and possibly microtubules, whereas microtubules are necessary to allow nucleokinesis. Mutations known to affect the microfilament system have been demonstrated in genes encoding filamin-1 and Cdk5/p35. Mutations in Lis1, Dcx, Cdk5/p35, Nud and Ropy genes disturb microtubules and nucleokinesis. A function of the reelin pathway (Reln) could be to stop nucleokinesis.



are used in response to these different cues; the nucleus consistently closely follows the extension of the leading tip.

These examples illustrate that studies of neuronal migration should address two complementary aspects. First, what are the processes that regulate directional growth of the leading tip: growth cone elongation in tangential migration and dendritic tip extension in radial migration. Second, what are the mechanisms that control the engagement and movement of the nucleus in the leading process, and the place where it stops and becomes positioned. We would like to suggest that, in order to explain the action of the genes that control neuronal migration and the phenotype of corresponding defects, the positioning of the nucleus may be as important as the guidance of the leading process (Figure 1).

Potential disorders of actin and leading processes

Human X-linked periventricular heterotopia is a disorder of radial migration that results in the formation of islands of ectopic neurons that stay close to the ventricle [12,13]. It is caused by mutations in the gene encoding filamin-1 (FLN1; also known as FLNA and actin binding protein 280 [ABP-280]), which encodes an actin-cross-linking phosphoprotein that transduces ligand–receptor binding into actin reorganization, and is required for locomotion of many cell types [14], and for filopodia extension. As, in this condition, neurons fail to migrate altogether, FLN1 appears to be necessary for neurons to leave the ventricular zone. The requirement for actin reorganization in filopodia extension is well known and the role of filamins in this process is well documented. At present it seems most likely that filamin acts by regulating actin cross-linking.

Potential disorders of microtubules

Reorganization of the microtubule network of a cell is also crucial for migration and two genetic migratory disorders in humans have been indirectly linked to aspects of microtubule function. In man, heterozygous mutation or deletion of the gene encoding the β subunit of platelet-activating factor acetylhydrolase (PAFAH1β1, also known as LIS1) is associated with type I lissencephaly of the Miller-Dieker type [15]. Mice with one inactive *Lis1* allele display cortical, hippocampal and olfactory bulb disorganization, and mice with further reduction of Lis1 activity display more severe brain disorganization as well as cerebellar defects [16]. The Lis1 protein has a stabilizing action on microtubules and reduces microtubule catastrophe events [17]. Another radial migration disorder causes X-linked double cortex syndrome in carrier females and lissencephaly in affected males, and is due to defective function of a protein dubbed doublecortin or Dcx [18,19]. In humans, the migrational abnormalities caused by to *LIS1* and *DCX* mutations are remarkably similar [20]. Like LIS1, Doublecortin binds to and stabilizes microtubules [21•,22•,23].

Recent work in the filamentous fungus *Aspergillus nidulans* suggests that LIS1 may function in relationship to translocation of the nucleus [24•]. LIS1 is homologous to the NUDF protein, which is required for nuclear translocation in *Aspergillus* [25]. As *Aspergillus* NudF interacts genetically with tubulin, dynein, and other elements of microtubule motors, it provides additional evidence that LIS1 might promote neuronal migration by regulating nucleokinesis via microtubules. Several genes that control the placement of nuclei in cells in *Saccharomyces cerevisiae*, *Aspergilius nidulans* and *Neurospora* crassa also have microtubule-associated functions [24•,26]. Studies in *Drosophila*, *Caenorhabditis elegans* and on the movement of *Xenopus* oocyte pronuclei similarly suggest that nuclear movement is at least partly governed by the microtubule organizing centers [24•,26,27]. These data suggest the possibility that LIS1 and DCX may govern microtuble reorganization required for leading edge

extension and, perhaps even more importantly, for nuclear displacement or nucleokinesis.

Cdk5 may regulate both actin and microtubules

Although cyclin-dependent kinase (Cdk)5 is structurally homologous to other Cdks, it is unique in functioning primarily in postmitotic neurons and may represent a link between actin and microtubules in migrating neurons. In Cdk5$^{-/-}$ mice, there are widespread deficits of migration in several parts of the brain, including the cerebral cortex, hippocampus, cerebellum and brainstem, as well as axonal alterations, particularly in brainstem [28,29]. Cdk5 is activated by a co-factor called p35, and the migration defect in *p35*$^{-/-}$ mice is rather similar to the Cdk5$^{-/-}$ phenotype, yet more subtle [30]. The milder *p35*$^{-/-}$ phenotype probably reflects the presence in brain of other Cdk5 activators, particularly p39, which can compensate partly for lack of p35. Interestingly, in the cerebral cortex, neither Cdk5 or p35 affect the formation of the preplate or of the early cortical plate [28]. Migration is very defective, however, when neurons need to migrate through previously deposited cortical layers or over relatively long distances in brainstem and cerebellum. As a result, the gradient of formation of the cortex is abnormal, and the cerebellum, brainstem and several other structures develop poorly.

Studies of *p35/Cdk5* kinase expression and function suggest prominent roles for these genes in actin regulation. Both proteins concentrate at the leading edges of axonal growth cones and regulate neurite outgrowth in cortical neurons in culture [31]. Cdk5 associates directly with Rac and Pak1 kinase. The active p35/Cdk5 kinase causes Pak1 hyperphosphorylation which results in downregulation of Pak1 kinase activity [31]. The modification of Pak1 may affect the dynamics of the reorganization of the actin cytoskeleton, thus promoting neuronal migration and neurite outgrowth [31]. FLN1 also contains multiple consensus sequences for phosphorylation by Cdk5 [14], although direct phosphorylation of FLN1 by Cdk5 has not yet been demonstrated. The phenotypes of FLN1 and Cdk5/p35 defective brains point to the role of actin microfilaments in promoting radial migration, presumably at the leading edge, in response to unknown guidance clues. In addition to its action on the actin network, however, Cdk5/p35 may also regulate microtubules, as it is a major kinase that phosphorylates tau [32–34].

Antimigratory genes of the Reelin family may inhibit both actin and microtubules

Regarding neuronal migration as leading-edge extension plus displacement of the nucleus may also help explain the action of the Reelin pathway — mutations of the genes encoding this pathway generate the *reeler* phenotype in mice [35]. The *reeler* mice have defective organization of neuronal cell patterns that reflects defects at the very end stages of radial migration. Reelin is a secreted extracellular matrix glycoprotein. In the embryonic brain, it is made by some neurons in the marginal zone of the cortex and hippocampus, by cerebellar granule cells, mitral cells, and diffusely distributed neurons at all levels of the brain, including spinal cord. The neurons that are affected in *reeler* mice are the cortical plate in neocortex and hippocampus, Purkinje cells, inferior olivary cells, cranial nerve nuclei and several others. Interestingly, the affected neurons do not make Reelin, but they express two members of the lipoprotein receptor family, VLDLR (very low density lipoprotein receptor) and ApoER2 (apolipoprotein E receptor type 2, also named LRP8) as well as the intracellular adaptor protein Dab1 (disabled 1) [36••,37]. Remarkably, mutations in the gene encoding Dab1 [37–39], as well as simultaneous mutations in *VLDLR* and *ApoER2* genes [36••], produce a migratory phenotype indistinguishable from the *reeler* phenotype. Recently, direct binding of Reelin to VLDLR and ApoER2 has been shown [40•,41••], and binding of Reelin to these receptors regulates the phosphorylation of Dab1 [41••,42•], suggesting that these proteins form part of a signaling pathway that regulates the end stages of migration [43]. Reelin also binds to the protocadherin protein CNR1, which itself docks to the intracellular tyrosine kinase Fyn, suggesting that CNR1 may be an additional part of a Reelin receptor complex and that Fyn may be implicated in Reelin signaling [44••].

The other members of the Reelin pathway, and thus the putative action of Reelin on target cells, are presently unknown; however, observations on *reeler* mice and studies of Reelin and Dab1 expression in embryonic brains of mammals and other amniotes [45,46•] suggest different, not necessarily exclusive, possibilities. It was proposed long ago that Reelin may trigger homotypic adhesion mechanisms among target neurons — a mechanism that receives some support from the recently described interaction between Reelin and CNR1 [44••]. Another, not exclusive possibility would be that Reelin influences the position of target cells by providing a 'stop signal' [47] —not to the leading edge but to the perikaryon — preventing the nucleus from moving and settling into Reelin-rich zones. This hypothesis predicts an action of the Reelin pathway on the microtubule cytoskeleton that counters that of Doublecortin or Lis1, which promote nucleokinesis, as explained above. Although such a mechanism is compatible with available observations, there is thus far little evidence to support it, apart from the intriguing observation that the microtubule associated protein tau appears to be hyperphosphorylated in mice deficient in the Reelin pathway [41••].

Conclusions

Studies of man and mouse mutations converge with previous work in less complex organisms and point to the importance of the actin and microtubule cytoskeleton in the regulation of neuronal migration. We speculate that the mechanisms involved are best understood by considering that neuronal migration is not solely extension at the leading edge but also displacement of the nucleus, and that both phenomena are controlled individually. Further studies are needed to evaluate that hypothesis.

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