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Neuronal Migration

Introductory

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Cell migration is a major event in neural development and its abnormalities cause human diseases. Cell–cell interactions are important for neuronal migration. Secreted proteins can guide the direction of neuronal migration through intracellular signal transduction pathways that regulate cytoskeletal organization.

Introduction

Nerve cells, or neurons, are distributed in distinct layers in the brain. The formation of neuronal cell layers, or lamination, is important for the proper wiring of the neuronal circuitry, and is thus crucial for information processing in the nervous system. In early embryos, neurons originate from a single layer in the cerebral cortex where they proliferate and are then positioned into distinct layers. The exquisitely precise positioning of the neurons in the cortex and elsewhere requires active migration of neurons from their origin.

Observations made more than a century ago by classical embryologists led to the suggestion of neuronal migration in the developing central nervous system (CNS). The question of whether cells truly migrate or whether cells formed earlier are simply displaced by later-born cells was clarified by observations of changes in cell position in a direction opposite to that expected from cell displacement through histological examinations in the spinal cord of chick embryos, and more convincingly through autoradiographic tracing in the cerebrum of rodent embryos. Electron microscopic examination and reconstruction uncovered strong evidence for the migration of

neuronal cell bodies. Together, these results demonstrated that there exists active cell migration rather than simple physical displacement during the development of the CNS.

In vitro cultures of neurons and glia isolated from live brain tissues (referred to as primary cultures) allowed the direct observation of neuronal migration. A considerable amount of work using histology, autoradiography, retroviral tracing, dye labelling and modern imaging techniques has established that the majority of neurons migrate throughout the developing CNS.

Radial Migration Versus Tangential Migration

Two modes of neuronal migration have been documented during CNS development. Radial migration is often thought to be the 'classical' form of neuronal migration. This type of migration is best exemplified during the development of the mammalian neocortex, when neuronal precursors from the ventricular zone migrate radially along radial glial fibres towards the surface of the brain to form the cortical plate, eventually resulting in a mature cortex (Figure 1). Different cell types travel different distances from their origin, resulting in the formation of cortical layers termed lamina; the formation of the lamina is called lamination. Perhaps all precursors for projection neurons in the neocortex migrate radially. Cells from the external granular layer (EGL) of the cerebellum and the ventricular zone of the spinal cord also undergo radial migration.

In the cerebral cortex, radial migration can be further divided into locomotion and somal translocation. These are distinguished by the dynamic properties and morphological features of migrating cells. During translocation, the entire cell body moves: a cell with a leading process (a thin and distinct protrusion at the cell front) terminating at the pial surface advances its soma toward the pial surface, the leading process becomes thicker and progressively shorter while its tip remains attached to the pial surface. Cells that undergo locomotion have short, unbranched, pial-directed but unattached leading processes. Growth cone-like structures, which are dynamic structures usually found at the tip of an axon, are frequently associated with the leading process. Cells undergoing locomotion resemble features of glia-guided migrating neurons and many of them undergo rapid translocation during the final stages of migration.

The second mode of neuronal migration is tangential migration. The existence of tangential migration was suggested from observations using Golgi-staining, [^{3}H]thymidine labelling and electron microscopy of brain preparations. This mode of migration is characterized by the movement of neurons parallel to the brain surface (Figure 1). The presence of tangentially oriented cells in the developing cortex in brain sections, retroviral lineage tracing and slice culture further demonstrated the significance of tangential migration. In the mammalian telencephalon, cells that follow the tangential migratory pathways usually give rise to interneurons and oligodendrocytes. Most of the neuronal precursors develop into γ-aminobutyric acid (GABA)ergic neurons, while some become dopaminergic or cholinergic neurons.

The major sources of neurons that undergo tangential migration are the ventral telencephalon and the upper rhombic lip in the developing cerebellum. The ventral telencephalon contains three proliferating zones, named the ganglionic eminence. Neuronal precursor cells from the ventricular zone of the lateral ganglionic eminence (LGE) in the embryo and the subventricular zone of the neonatal LGE migrate to the olfactory bulb; cells from the medial ganglionic eminence (MGE) migrate to the neocortex; and cells from the caudal ganglionic eminence may migrate to the posterior dorsal telencephalon. Unlike radial migration, tangential migration is not dependent on glial fibres. In the adult rostral migratory stream (RMS), astrocytes, a population of 'star-shaped' glia, have been implicated in the formation of tubular structures through which chains of neurons migrate, hence termed chain migration. *In vitro*, neuronal precursor cells from the anterior subventricular zone (SVZa) can migrate individually in a three-dimensional matrix containing collagen (referred to as collagen matrix) without relying on other cells.

Molecular Mechanisms for Directional Guidance of Neuronal Migration

An important question for a neuron undergoing migration is in which direction should it migrate? In radial migration, neurons that migrate along glial fibres need to follow one of the two directions along the glial fibre. In tangential migration, the question of directional guidance is even more acute.

Guidance cues provide a solution to the problem. They serve as molecular 'road signs' that direct neurons to their destinations. Recent discoveries have provided a basic understanding of the molecular mechanisms for directional guidance of neuronal migration.

During development of the mammalian olfactory bulb, neuronal precursors from the SVZa migrate rostrally along the RMS into the olfactory bulb. Using a collagen matrix co-culture system, Hu and Rutishauser demonstrated that there exists a repulsive activity in the septum for the SVZa cells. The molecular nature of this repulsion is due to a secreted protein, Slit. Slit was first found in *Drosophila* and was shown to be a repellent for axons; it functions through the transmembrane receptor Roundabout (Robo). *Drosophila slit* mutants exhibit defects in the midline, a region that separates the CNS bilaterally into two halves. Projection of a group of axons termed commissural axons was abnormal: instead of crossing the midline once, then projecting longitudinally as in wild-type animals, the commissural axons collapsed at the midline in *slit* mutants. The Robo receptor was discovered in a genetic screen for mutations affecting axon pathfinding in *Drosophila*. *robo* mutants exhibit increased numbers of axons crossing and recrossing the ventral midline – hence the name Roundabout. The receptor encodes a single-pass transmembrane protein with a long cytoplasmic domain that has been shown to be important in mediating repulsion. Co-culture experiments of Slit-expressing cells and explants from different brain regions in collagen matrices showed that Slit can direct axon navigation and neuronal migration; it repels several groups of axons including that from the olfactory bulb and motor neurons, and repels SVZa cells. Among three mammalian Slit genes, two are expressed in the septum. *In vitro* co-culture experiments showed that Slit functions as a chemorepellent: it repels SVZa cells in a concentration gradient-dependent manner.

Experiments on brain slices provided evidence that Slit can regulate migration of SVZa cells in their natural migratory pathway. Functional blocking of Slit using the extracellular part of Robo reduced the repulsive effect of the septum on SVZa cells, indicating that Slit contributes to the endogenous repulsive activity in the septum. Together, these data have established the role of Slit as a chemorepellent molecule that directs tangential SVZa cell migration during development of the olfactory bulb. Data from the authors' laboratory have also shown that Slit can repel LGE cells from the embryonic neocortex in a collagen co-culture system, and that the repelled cells are GABAergic (Figure 2).

The signal transduction pathway mediating neuronal migratory responses to Slit is only beginning to be understood. The intracellular part of Robo interacts with proteins that regulate the Rho family of guanosine triphosphatases (RhoGTPases). The RhoGTPases are well known regulators of a diverse array of cellular activities, particularly regulation of the cytoskeleton including the actin and microtubule networks, and affect cell motility. The best characterized members of the RhoGTPases include Rho, Rac and Cdc42, all of which have been demonstrated to be important regulators of the cytoskeleton. GTPases cycle between an active guanosine triphosphate (GTP)-bound state and an inactive guanosine diphosphate (GDP)-bound state. GTPase-activating proteins (GAPs) catalyse the intrinsic hydrolytic rate of the GTPase, converting the GTP-bound form into the GDP-bound form, thereby inactivating it. srGAPs, a novel family of GAP domain-containing proteins, have been implicated in Slit–Robo signalling. One of these proteins, srGAP1, has been shown to inactivate the RhoGTPase, Cdc42. Extracellular interaction of Slit with Robo increases the intracellular binding of Robo to srGAP1, leading to inactivation of Cdc42 in SVZa neurons. Reversal of Cdc42 inactivation by the introduction of a constitutively active mutant of Cdc42 eliminates the repulsive effect of Slit. A model for the Slit signal transduction pathway has been proposed that starts from extracellular Slit to intracellular cytoskeletal reorganization.

In the developing brain, neuronal precursors from the MGE undergo tangential migration into the neocortex and form the striatal–neocortical pathway. Both Slit and another guidance cue, Netrin-1, can repel MGE cells. Netrin-1 also plays an important role in cerebellar development. The group of neurons that reside in the outermost layer in the developing cerebellum is termed the external granular layer (EGL). However, this group of cells originates from a region named the rhombic lip. Cerebellar precursors from the rhombic lip migrate tangentially along the surface of the developing cerebellum and form the EGL. When co-cultured with explants derived from embryonic day 12–14 (E12–14) lower rhombic lip (LRL), Netrin-1 attracted these migrating neurons. Interestingly, data from the authors' laboratory have shown that Netrin-1 attracts cells from E15 upper rhombic lip (URL) and repels cells from E16 URL. In postnatal cerebellum, Netrin-1 repels the migrating granule cells from explants of the EGL. The mechanism(s) underlying the spatial and temporal control of cerebellar neurons in response to Netrin-1 remains to be investigated.

In radial migration the molecular mechanisms for mediating cell–cell interaction between neurons and glial cells have been studied. Hatten and colleagues carried out a screen for molecules involved in neuron–glial association and discovered astrotactin, a cell surface glycoprotein present on granule neurons. In microcultures of cerebellar cells, the presence of an antiastrotactin antibody blocked the association between

astroglia and neurons. Interestingly, the level of astrotactin expression was greatly reduced in the mouse mutant *weaver*, which exhibits a failure of glial-guided neuronal migration.

The molecular mechanisms underlying directional guidance of radial migration is not well understood. *In vivo*, glia-dependent migration is directional from the ventricular zone to the cortical plate, whereas *in vitro* dissociated granule cells can migrate in both directions along the same glial fibres. It is likely that there may also be guidance molecules in the ventricular or marginal zones for glia-dependent radial migration in the CNS. A potential molecule for directing radial migration is the Netrin-like molecule. Mice mutants of *UNC-5*, which encodes a receptor for Netrin, exhibit abnormalities in lamination in the rostral cerebellar cortex. The expression of *unc-5* is consistent with a potential role in granule cell migration in the cerebellum. Netrin-1 is unlikely to guide glia-dependent neuronal migration in the cerebellar cortex as no recognizable migration defects in this region has been found in Netrin-1 knockout mice. Other UNC-5 ligands, possibly Netrin-like molecules, may serve as guidance molecules for radial migration in the cerebellum. Future experiments with explants may help to provide direct evidence to establish whether putative Netrin-like molecules or other molecules identified from genetic defects in mice and humans can direct glia-dependent migration in the nervous system.

Genetic Studies of Neuronal Migration in Mice

Studies in mice exhibiting neuronal migratory abnormalities have not only provided information on the *in vivo* function of molecules, but have also served as powerful animal models that allow the uncovering of mechanisms involved in the control of neuronal migration.

The mouse mutant *reeler* has provided a focal point for intensive genetic studies of cortical lamination and neuronal migration over the past 50 years. The complementary deoxyribonucleic acid (cDNA) for *reeler* was cloned in 1995 and it encodes a large secreted protein, Reelin (Reln). Loss of function mutations in the *reeler* gene cause defects in CNS lamination, including a failure of neurons in the cerebral cortex to align in an inside-out fashion. These phenotypes suggest a role for Reelin in the control of cell–cell interactions critical for cell positioning. Recently, an autosomal recessive lissencephalic (smooth brain) disorder exhibiting cerebellar hypoplasia has been shown to be associated with Reelin mutations in humans.

The receptors for Reelin were also identified genetically; mice lacking both of these receptors exhibit defects identical to those in *reeler* mice. Expression of both genes is detected in Reelin target cells, and correlates spatially and temporally with the function of Reelin in directing cell positioning.

Interestingly, the mouse mutant *scrambler* also exhibits a *reeler*-like phenotype with defects in neuronal positioning. Genetic evidence suggests that *scrambler* functions downstream of the Reelin signalling pathway, and the gene mutated in *scrambler* has been mapped to the gene Disabled-1 (Dab1). Disabled-1 encodes a protein that contains domains for protein–protein interaction with nonreceptor tyrosine kinases. Tyrosine phosphorylation (the addition of a phosphate group on to a tyrosine residue, a common posttranslational modification of proteins in the regulation of signal

transduction) of Dab1 is important in Reelin signalling and its function in cell positioning, because substitution of these tyrosine residues with phenylalanine results in defects in neuronal migration that closely resemble those in *reeler* and *dab1* mutants.

A current model for Reelin signalling is that, by binding to its receptors, Reelin becomes internalized and in turn activates a tyrosine kinase signalling cascade that includes tyrosine phosphorylation of Dab1. Several possible functions have been proposed for Reelin, among which the stop signal hypothesis is particularly attractive. However, the precise role of Reelin remains controversial.

Studies of the mouse mutant lacking a protein called cyclin-dependent kinase 5 (Cdk5) have revealed a role for this protein in regulating the cytoskeleton in migrating neurons. Cdk5 knockout mice exhibit defects in neuronal migration in the cerebral cortex, hippocampus and cerebellum. Neurons in the Cdk5 mutant seem to have difficulties in migrating over long distances or through previously formed cortical layers. Mice mutants for Cdk5-interacting proteins exhibit similar migration defects to that of Cdk5 mutants, indicating the importance of this signalling pathway in neuronal migration.

Genetic Defects Affecting Neuronal Migration in Humans

Genetic studies in humans have not only led to the identification of molecules involved in neuronal migration, but have also helped to define steps in neuronal migration.

Genetic defects of neuronal migration in humans have been interpreted as distinct events in neuronal migration, including: (1) acquisition of a 'go' signal; (2) regulation of cell–cell and cell–extracellular component adhesion; (3) directional guidance of cell movement; and (4) 'braking' when arriving at the correct position. Most of the molecules identified from genetic studies are intracellular molecules, including cytoskeleton-associated proteins important for the regulation of actin or microtubule organization.

Studies of humans with genetic disorders in neuronal migration allowed the identification of an intracellular protein, Filamin-1 (Fln1). Fln is an actin-associated protein required for cell migration, and has been shown to be crucial for neuronal migration. Mutations in human Fln1 cause periventricular heterotopia, characterized by the formation of islands of ectopic neurons close to the ventricle, as a result of defective radial migration. In individuals with this disorder, neurons in the ventricular zone fail to leave the ventricle, suggesting an important role of filamin in actin-based motility and the initiation of neuronal migration.

Microtubule-associated proteins have also been identified as critical components for neuronal migration. LIS1 and Doublecortin (Dcx) are microtubule-stabilizing proteins whose mutations have been linked to genetic diseases known as lissencephalies. Characteristic abnormalities of a lissencephalic brain include an absence or reduction of the normal cerebral convolutions and disrupted lamination of the cerebral cortex. Microtubules may be important for nuclear migration and establishment of cell polarity in certain cell types. Studies in the fungus *Aspergillus* showed that nudF, an

evolutionarily conserved fungal counterpart (or orthologue) of Lis1, is important for nuclear translocation. The mammalian NudC, whose orthologue in *Aspergillus* is important for nuclear migration and viability, is reported to interact with Lis1 and a microtubule-based motor complex at the leading pole of migrating neurons. The migration defects in *lis1* and *dcx* mutants may result from aberrant microtubule organization and failure of nuclear translocation. Studies of the regulation of microtubule dynamics in migrating neurons and their reorganization in response to extracellular guidance cues may allow better understanding of the mechanisms underlying neuronal migration.

Summary

Despite our knowledge of the importance of neuronal migration, understanding of the mechanisms underlying neuronal migration remains limited. Studies using *in vitro* cell culture, molecular biology, genetics, and imaging techniques have provided insights into neuronal migration during the development of the nervous system, and have unveiled possible molecular mechanisms underlying the sculpting of the brain.

We still know little about what determines the initiation or the speed of neuronal migration. Further investigations are also needed for dissecting intracellular signal transduction mechanisms regulating the migration of the leading process and the cell body of a neuron. It remains elusive to us how cell migration is precisely choreographed by the interplay of multiple factors.

Further Reading

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Figure 1#Examples of radially and tangentially migrating neurons in the neocortex. Shown in blue are radially migrating cells that move along glial fibres (brown lines in the inset) from the ventricular zone towards the pia. Shown in red are tangentially migrating cells that migrate from the ganglionic eminence to the neocortex. V, ventricle. Adapted from Lumsdem A and Gulisano M (1997) *Science* **278**: 402–403.
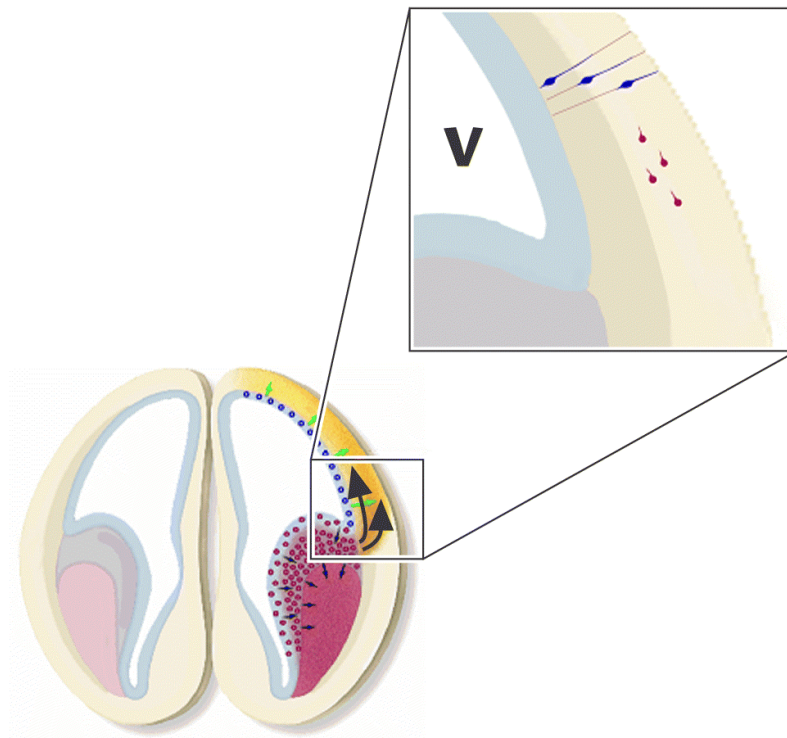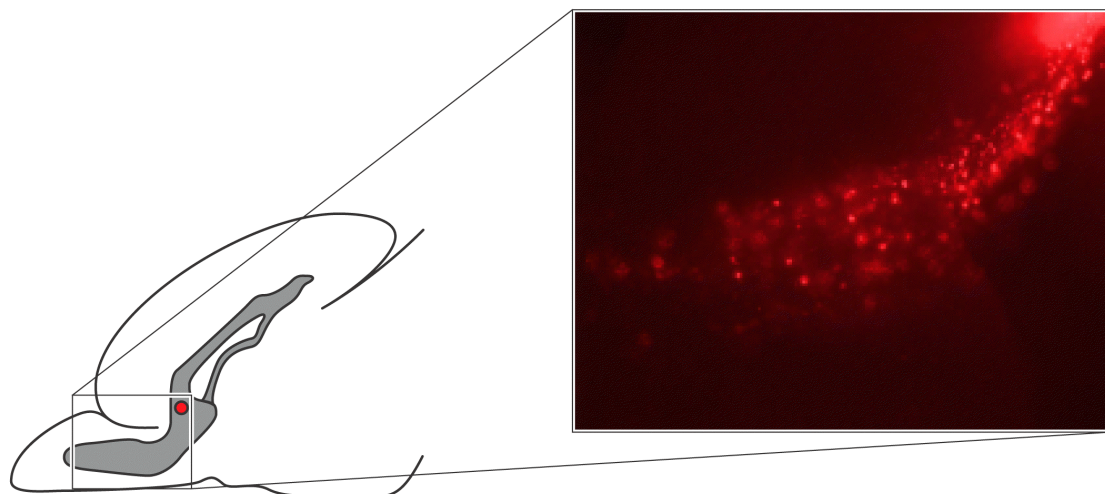

Figure 2#Neuronal migration in the rostral migratory stream (RMS) revealed by labelling with a lipophilic dye (DiI). A piece of DiI crystal (indicated by a red dot) was placed in the SVZa region in a live forebrain slice. After overnight culture, labelled cells migrated tangentially into the olfactory bulb in the RMS as observed under a fluorescent microscope.

Glossary

Actin#An abundant cytoskeletal protein that forms actin filaments by polymerization.

Ganglionic eminence#Thickening of the lateral ventricular wall in the ventral telencephalon; gives rise to the basal ganglia (medial and lateral ganglionic eminences).

Glia#Support cells associated with neurons; do not conduct electrical signals; include astrocytes and oligodendrocytes.

Interneurons#Neurons that branch locally to innervate other neurons.

Microtubule#A major filamentous component of the cytoskeleton composed of tubulin.

Neurons#Cells specialized for the conduction and transmission of electrical signals in the nervous system.

Pial surface#The innermost layer of the external covering of the brain (meninges).

Rhombic lip#A specialized germinative epithelium located along the edge between the neural tube and the roofplate of the fourth ventricle.

Soma#Cell body of a nerve cell.