Neuronal Migration: An Overview of Modes, Molecular Mechanisms and Model Systems

Introductory article

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Jaroslav Icha, *Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*

Caren Norden, *Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*

A century ago, Santiago Ramón y Cajal had already postulated that neurons born in specific proliferative zones have to migrate to ensure correct neocortical layering. Today we know that neuronal migration is a common phenomenon in many brain regions and that neurons are often born micrometres to centimetres away from the place they fulfil their function. Mutations that lead to defects in neuronal migration can have devastating consequences like lissencephaly, autism and schizophrenia in humans. With these conditions in mind, neuronal migration has been an area of intense research for more than 50 years. Additionally, neuronal migration phenomena are outstanding models to understand basic themes in cell biology.

The rodent neocortex has been a very popular system to study different modes of neuronal migration. More recently, additional model tissues and organisms have been examined, which help to further expand our knowledge.

The authors provide an overview on different migratory modes and then focus on the cell biology that enables migration in different contexts. They further discuss some experimental challenges that need to be overcome in order to understand neuronal migration in even more depth.

Introduction

The ways neurons reach their final position within the neuronal network can differ depending on neuronal cell type, its location within the CNS and developmental stage of the organism. Two basic types of migration exist: (a) radial and (b) tangential migration. Their definition is based mainly on the orientation of neuronal movements. Radially migrating neurons are born by asymmetric division of radial glia progenitors and move from the apical (ventricular) to the basal (pial) side of the developing cortex (i.e. perpendicular to the ventricular zone (VZ)). Tangentially migrating neurons move perpendicular to the apicobasal axis of the tissue (i.e. parallel to the VZ) (**Figure 1a**) (Marin *et al*., 2010). Most neurons use more than one migratory mode before reaching their final location. **See also**: Lissencephaly, Genetics of; Ramón y Cajal, Santiago

Another feature that differs between the two migratory modes is their speed. On average, tangential migration is faster (approximately $50\,\mu m\,h^{-1}$) than radial migration (approximately $10\,\mu m\,h^{-1}$) (Kriegstein and Noctor, 2004). Movement of neurons in general is rather slow compared with cells that are primarily adapted for migration, for example, neutrophils, which migrate at approximately $600\,\mu m\,h^{-1}$. The reason is that neuronal migration is not optimised for speed but rather for fidelity. It allows input from multiple cues and can be corrected on the way (Rørth, 2011).

Neuronal migration has been intensely studied in the rodent cerebral cortex. Generally, migration of cortical projection neurons can be subdivided into three stages (Kriegstein and Noctor, 2004): (1) multipolar/free migration, (2) glial-guided migration and (3) terminal translocation (**Figure 1b**). These modes will be discussed in detail in the following sections. The authors then concentrate on how forces for different modes of migration are generated. Here the authors focus on vertebrate models, the rodents, mouse and rat, as well as the zebrafish. Finally, they discuss the different experimental setups that are used and are being developed to meet future challenges in the neuronal migration field. One trend they emphasise is to conduct experiments in the true *in vivo* situation, that is, observing neuronal migration in the intact developing embryo. **See also**: Mouse as a Model for Human Diseases; Zebrafish Embryo as a Developmental System

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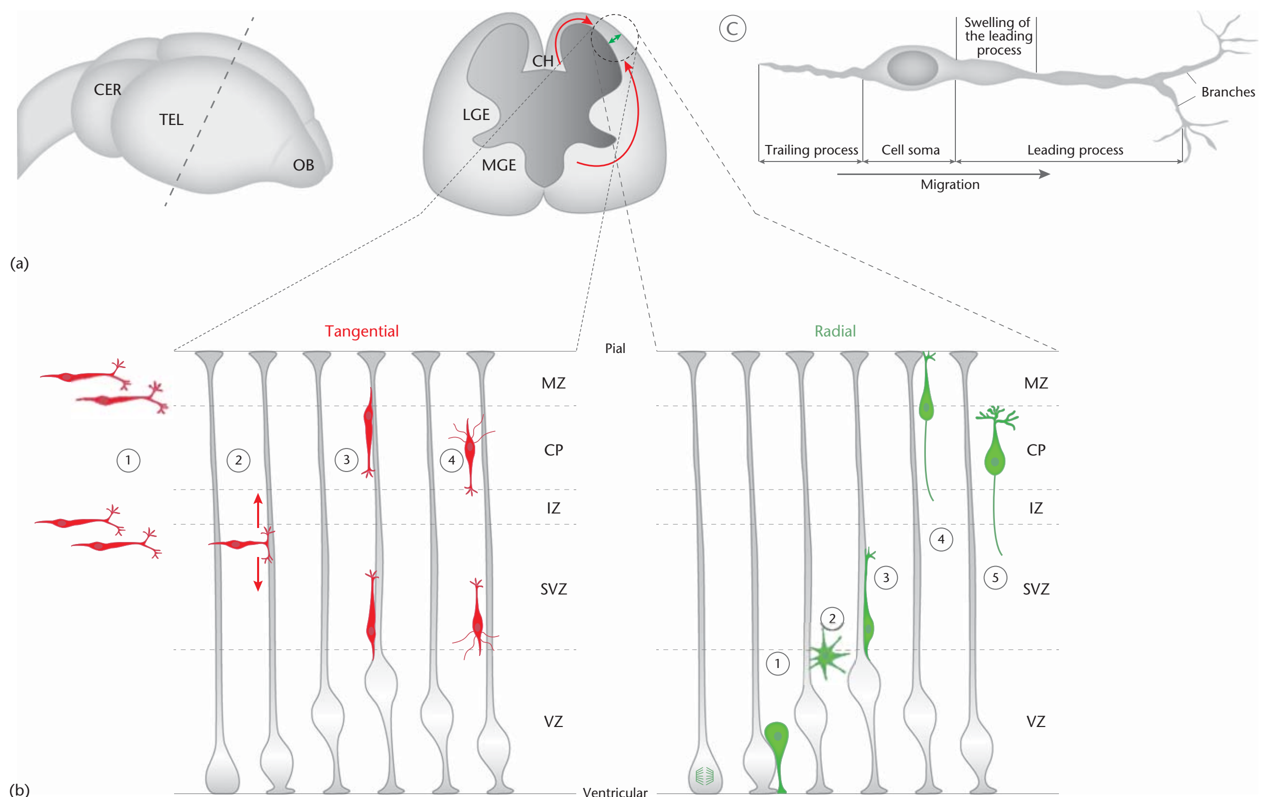

Figure 1 Two modes of neuronal migration. (a) Depiction of a mouse brain at E13.5. The dashed line indicates the plane of coronal section in the middle panel. The brain ventricle is shown in dark grey. The upper red arrow represents tangential migration of Cajal–Retzius cells from the cortical hem. The lower red arrow represents interneuron migration from the medial ganglionic eminence to the cortex. The green arrow represents radial migration of projection neurons from the ventricular to the pial side of the cortex. CER, cerebellum; CH, cortical hem; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; OB, olfactory bulb; TEL, telencephalon. (b) Zoom into dashed circle in (a). Tangential migration of interneurons (red) is shown in four steps: (1) migration towards the cerebral cortex in two streams parallel to the ventricular and pial surfaces, (2) interneurons reaching the cortex associate with radial glia cells (grey), (3) radial movement within the cortex by glial-guided migration and (4) differentiated interneurons at their final position. Radial migration of projection neurons (green) is shown in five steps: (1) the neuron is born at the ventricular surface, (2) multipolar phase of migration, (3) radial movement by glial-guided migration, (4) terminal translocation and (5) differentiated projection neuron at its final position. MZ, marginal zone; CP, cortical plate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone. (c) General anatomy of a radially migrating neuron. The nucleus is depicted in dark grey. The arrow indicates the direction of migration.

Migration Modes

Radial migration

Radial migration of neurons is observed during development of many central nervous system (CNS) regions, like the cerebral and cerebellar cortices or the spinal cord. Radial migration can be subdivided into two distinct types: (1) somal translocation and (2) glial-guided migration (sometimes also referred to as locomotion; Nadarajah *et al.*, 2001).

A characteristic feature of somal translocation is the anchorage of the leading process of migrating neurons to other cells or to extracellular matrix (ECM) components in basal laminae. This anchorage defines the direction of movement and is already established before the onset of migration. Subsequently, the neuron translocates its soma, the nucleus and other organelles, and coordinates its movement with detachment of the trailing process. Somal translocation is, for example, exhibited by early-born neurons in the cortex at developmental stages at which the cortex is still relatively thin spanning only some hundred micrometres. At this developmental stage, neurons cross the whole cortical width by somal translocation. Later in development, when the cortex is several millimetres thick, neurons use glial-guided migration (described below) in combination with somal translocation. Somal translocation further allows the final phase of migration once the leading process gets anchored in the marginal zone (MZ) (**Figure 1b**). This last phase of movement is called terminal translocation (Nadarajah *et al.*, 2001). Other neurons that use somal translocation to reach their final position in the CNS include spinal motor neurons (Bron *et al.*, 2007) and retinal ganglion cells, the most basal neurons in the retina (Randlett *et al.*, 2011).

Glial-guided migration is exhibited, for example, by cortical projection neurons and was discovered in the series of Golgi and electron microscopic studies of rhesus macaque cerebellum and neocortex (Rakic, 1971, 1972). During glial-guided migration, neurons attach to radial

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glia cells, which span the whole width of the cortex. Glial-guided migration starts in the subventricular zone (SVZ), where neurons attach to radial glia cells and use them as 'tracks' from the ventricular to the pial surface to travel towards their appropriate cortical layer. Cells that undergo glial-guided migration feature two processes: a leading process that points to the direction of movement and a trailing process at the other end of the cell (**Figure 1c**). Glial-guided migration is dictated by movement of the biggest organelle, the nucleus, a phenomenon called nucleokinesis. Nucleokinesis is saltatory, that is, forward movement of the nucleus is interrupted by periods of stalling (Nadarajah *et al*., 2001; Solecki *et al*., 2004). Owing to this nuclear stalling, glial-guided migration is on average two times slower than somal translocation (Nadarajah *et al*., 2001). Ultimately, the trailing process of radially migrating cells elongates and transforms into the axon. Distances travelled via glial-guided migration at later stages of cortical development can reach millimetre scales. The molecular mechanisms of glial-guided migration will be described in a later section. **See also**: Glial Cells in Neural Development

Tangential migration

Tangential migration was discovered using lineage studies of rat cerebral cortex neurons. It was observed that cells of a specific clone are not always found in a column within the cortex but can be dispersed throughout the tissue (Price and Thurlow, 1988; Walsh and Cepko, 1988). These findings indicated that some subtypes of neurons move perpendicular to the orientation of radial glia cells. It was later shown that these tangentially migrating cells in the cortex are interneurons originating from the ganglionic eminences in the ventral VZ of the telencephalon (Wichterle *et al*., 2001; **Figure 1a**). Cortical interneurons travel via two streams: through the SVZ and the most superficial layer of the developing cortex, the MZ (**Figure 1b**). As opposed to cells migrating along glia, tangentially migrating cells move independently of underlying cells but often rely on the sensing of molecular gradients. Cells detect these cues using their heavily branched leading process that screens the environment. Selective stabilisation of one of the branches enables cells to move in a defined direction, whereas the other branches are retracted. Then the stabilised process branches again and the cycle repeats (Martini *et al*., 2008; **Figure 2a**). Most tangentially migrating neurons switch to radial migration during the last step of their translocation and thereby reach their final position within the layers of the respective tissue (Marin *et al*., 2010; Faux *et al*., 2012; **Figure 1b**). However, they can also travel along axons of surrounding cells or via brief interactions with radial glia (Faux *et al*., 2012).

Tangential migration is also used by Cajal–Retzius cells that migrate as the first cell type into the cortex and form the preplate that hosts the earliest-born projection neurons. Cajal–Retzius cells in the cortex originate mainly from the cortical hem (Takiguchi-Hayashi, 2004; **Figure 1a**). They then migrate along the cortical surface using factors from the brain meninges as guidance cues and ultimately populate the whole tissue (Borrell and Marín, 2006).

A special type of tangential migration is neuronal chain migration. Here, a pioneer neuron is followed by other neurons that adhere to each other and thereby migrate towards the same direction. A well-studied example of tangential chain migration is the rostral migratory stream. In this case, a chain of GABAergic interneurons migrates from lateral ganglionic eminence to the olfactory bulb. In rodents, this migratory stream persists even in adulthood (Alvarez-Buylla and García-Verdugo, 2002). Neuronal chain migration also occurs in the developing zebrafish nervous system in which facial branchiomotor neurons (Wanner and Prince, 2013) or cerebellar granule neurons translocate by this migratory mode (Rieger *et al*., 2009).

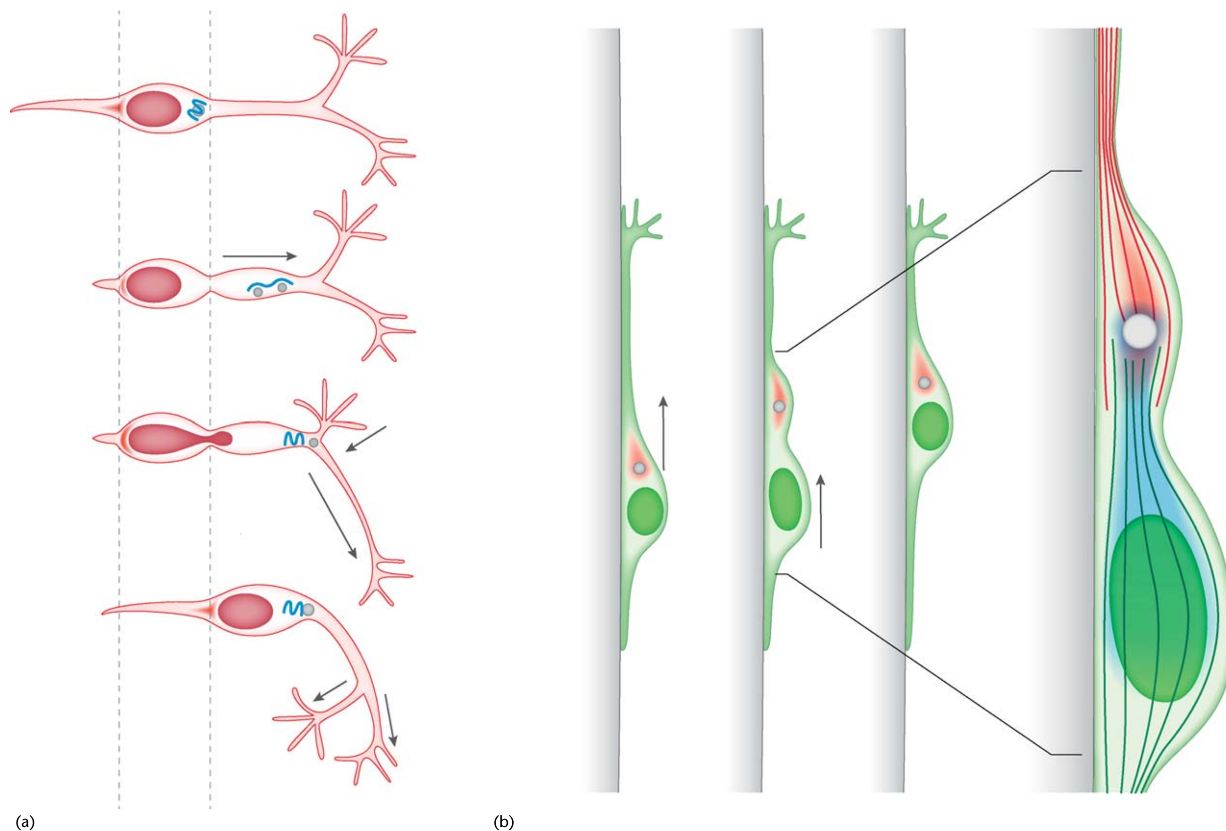
Multipolar migration

Right after cortical neurons are born at the ventricular surface of the developing cortex, they enter a stage of multipolar migration. Neurons migrate to the SVZ independently of radial glia cells (**Figure 1b**). Their speed is much lower than that during the later occurring glial-guided movement. Moreover, migration paths are less oriented. The most likely reason for this is that in the multipolar phase, directionality of neurons is not restricted by any attachment. Therefore, they can move in radial as well as tangential direction (Tabata and Nakajima, 2003), a useful ability if obstacles block their migratory path.

Other cell types that have been shown to undergo multipolar migration are the two types of retinal interneurons – amacrine and horizontal cells (Poché and Reese, 2009). They are multipolar, that is, they do not feature an apical or a basal process during most of their migration but instead have multiple protrusions emanating into different directions. In the case of amacrine cells, it is assumed that they migrate basally towards the amacrine cell layer in a rather directed fashion (Hinds and Hinds, 1978). Horizontal cells migrate in two phases, first they move basally together with amacrine cells, but hours later they change their path and migrate back apically towards the horizontal cell layer where they differentiate (Boije *et al*., 2009). Neither the necessity of initial basal migration nor the triggers needed to invert the direction of their migration are known at this point. **See also**: Visual System Development in Vertebrates

Molecular Mechanisms of Neuronal Migration

Unravelling the molecular cascades responsible for the types of neuronal migration described has been a constant focus in this field. Various cytoskeletal components generate forces that are needed to move the nucleus and other organelles during neuronal migration depending on the organism and cellular context. Here, the authors give a



(a) (b)

Figure 2 Molecular mechanisms of neuronal migration. (a) Cellular morphology of tangentially migrating interneuron and its organelle arrangement. The image shows interneuron migrating from left to right; stages of movement are depicted from top to bottom. First, the centrosome (grey circle) and the Golgi apparatus (blue) move into the swelling of the leading process. During this movement, the centrosome splits and the Golgi apparatus straightens in direction of migration (grey arrow). Subsequently, the nucleus (dark red) travels towards the centrosome/Golgi. In some cases, the nucleus deforms and moves due to forces exerted by actomyosin contractions (light red) at the cell rear (see main text for details). Leading process morphology: one of the interneuron leading processes branches, gets stabilised and determines the direction of migration for the next migratory cycle; other branches are retracted and new ones are created (grey arrows). (b) Cellular morphology of neurons undergoing glial-guided migration. Neuronal migration is depicted from bottom to top. The sequence of events is depicted from left to right. First, the centrosome (grey circle) is pulled into the swelling of the leading process by actomyosin contractions (light red). Then the nucleus (green) translocates towards the centrosome (arrow). The zoomed in view of the migrating neuron on the right shows the detailed arrangement of the microtubule cytoskeleton: Microtubules (lines) form a cage-like structure around the nucleus. The microtubules in the leading process (red lines) are anchored to the centrosome and acetylated and thereby stabilised. The microtubules forming the cage around the nucleus (dark green lines) are not anchored to the centrosome. They are tyrosinated and thereby dynamic (see 'Actin and microtubules'). Dynein (blue) is also localised around the centrosome as well as in the proximal leading process during glial-guided migration.

brief overview of the major molecular mechanisms playing a role in migration of interneurons and cerebellar granule neurons, which are the two most thoroughly investigated models to date (**Figure 2**).

Neuronal migration and the cytoskeleton

Actin and microtubules

One of the first detailed descriptions of neuronal migration on a molecular level was a seminal report in 1995, which studied mouse cerebellar granule neurons cultured on glial cells (Rivas and Hatten, 1995). This work suggested that the highly dynamic leading process hosts the force-generating entities that enable neurons to move along glia cells, whereas the trailing process passively follows. Supporting this idea, both actin and microtubules were reported to be concentrated in the leading process; microtubules formed a dense cage-like structure around the nucleus oriented in the direction of migration, and the highest actin concentration was found in a swelling of the leading process approximately 10 μm in front of the soma (Rivas and Hatten, 1995; **Figure 2b**). Correspondingly, neuronal movements decreased on addition of drugs that interfered with actin or microtubule dynamics (Rivas and Hatten, 1995). **See also**: Actin and Actin Filaments; Tubulin and Microtubules

Later studies extended these findings and demonstrated that migration occurs as a two-stroke movement (Bellion *et al.*, 2005; Solecki *et al.*, 2004): First, the centrosome, the major cellular microtubule-organising centre (MTOC), moves into the swelling of the leading process followed by nucleokinesis in the same direction (**Figure 2b**). Before nucleokinesis commences, centrosome and nucleus can be tens of micrometres apart (Tsai *et al.*, 2007). Once the

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general arrangement of the microtubule cytoskeleton was discovered, microtubule dynamics and turnover rates were explored. In granule cells of mouse cerebellar slices, microtubules that wrap the nucleus are mainly tyrosinated, which implies that they are dynamic (Umeshima *et al.*, 2007). This finding was corroborated by fluorescent recovery after photobleaching (FRAP) experiments on cultured mouse cerebellar granule neurons, which revealed high turnover rates of cage microtubules (Solecki *et al.*, 2004). However, the microtubule bundles that emanate into the leading process contain a significant amount of acetylated tubulin, indicating that these microtubules are stabilised (Umeshima *et al.*, 2007; **Figure 2b**). Specific disruption of dynamic microtubules around the nucleus did not interfere with migration, indicating that the stable acetylated microtubules can be sufficient to drive nucleokinesis (Umeshima *et al.*, 2007). Furthermore, the acetylation of α-tubulin by histone acetyltransferase complex called Elongator is necessary for normal migration and branching of projection neurons in the mouse cerebral cortex. This suggests a special relevance of stable microtubules during migration (Creppe *et al.*, 2009).

Myosin II

Similar to microtubule dynamics, actomyosin contractility in the leading process has been linked to nucleokinesis (Solecki *et al.*, 2009). In rat cortical slices, myosin II activity is important for nuclear, but not for centrosomal, movement, indicating that these two processes can be uncoupled (Tsai *et al.*, 2007). Another hypothesis resulting from experiments on cerebellar granule neurons plated on glia cells suggests that the actomyosin cytoskeleton first pulls the centrosome into the leading process, which is followed by microtubule-dependent nucleokinesis (Solecki *et al.*, 2009, 2006). Granule neurons display an actin mesh embedding the centrosome in the leading process (**Figure 2b**), and FRAP measurements showed that the leading process is a place of high actin turnover. **See also**: Myosins

Unlike cerebellar granule cells that exhibit actomyosin enrichment in the leading process, interneurons and explanted cortical cells seeded in 3D matrigels display actomyosin accumulation at the rear of the nucleus prior to nuclear movements (**Figure 2a**; Bellion *et al.*, 2005; Martini and Valdeolmillos, 2010). In cortical interneurons, actomyosin contractions behind the nucleus are thought to depend on calcium transients (Martini and Valdeolmillos, 2010). However, actomyosin-dependent contractility alone is not sufficient for migration and hence movement additionally depends on a functioning microtubule cytoskeleton. Therefore, it has been speculated that the role of myosin II is to align the nucleus on the same axis as the centrosome/MTOC and to retract the trailing process (Bellion *et al.*, 2005; Martini and Valdeolmillos, 2010).

Microtubule-associated motors

The major motor protein complex that transports cargo towards the minus end of microtubules is the dynein/dynactin complex. During glial-guided radial migration, dynein was shown to localise to the swelling of the leading process and to the centrosome in cerebellar granule cells (Solecki *et al.*, 2004) and cortical projection neurons (Tsai *et al.*, 2007; **Figure 2b**). Consequently, downregulation of dynein as well as the dynein activator Lis1 by RNA interference led to stalling of centrosomal as well as nuclear movements. These results imply that dynein/dynactin activity is important for both steps of nucleokinesis, the movement of the centrosome into the cytoplasmic swelling as well as ensuing nucleokinesis (Tsai *et al.*, 2007). A different set of experiments on granule cells in cerebellar slices argues that interference with Lis1 activity blocks nuclear migration without affecting microtubule–centrosome coupling (Umeshima *et al.*, 2007). The reasons for this discrepancy are not clear at this point.

Members of the other major microtubule motor family, kinesins, are also involved in neuronal migration. For example, the plus end-directed motor kinesin-5 that is otherwise mainly known as a regulator of mitotic spindle length was implicated in nucleokinesis of migrating rat cerebellar granule neurons (Falnikar *et al.*, 2011). When kinesin-5 is downregulated, cultured granule neurons move faster but less directed, whereas they move slower when kinesin-5 is overexpressed. This led to the hypothesis that kinesin-5 can act as a 'brake' on microtubule–microtubule interactions and thereby regulate the directionality of migration (Falnikar *et al.*, 2011). A more recent study investigated the role of another kinesin, kinesin-6 (Falnikar *et al.*, 2013), which has so far mainly been studied during cytokinesis. In migrating mouse cortical neurons, kinesin-6 is concentrated in the leading process and its downregulation leads to multipolar instead of bipolar neurons, whose movement is less directed. Additionally, downregulation of kinesin-6 results in a more dispersed localisation of actin into several processes, instead of accumulation in the swelling of the leading process. The fact that interference with MgcRacGAP, a downstream factor of kinesin-6 during cytokinesis, leads to similar phenotypes made the authors speculate that maintenance of bipolar morphology during neuronal migration has some similarities to mechanisms important for cytokinesis (Falnikar *et al.*, 2013). **See also**: Dynein and Kinesin

In summary, both the microtubule and the actin cytoskeleton as well as their associated motor proteins are crucial players of all neuronal migration phenomena studied so far. However, their exact contribution, interplay, dynamics and location of force generation can vary between different types of cells. **See also**: Motor Proteins

The role of the centrosome in neuronal migration

It has been suggested that forward movement of the centrosome into the cytoplasmic swelling of the leading process is crucial for subsequent nucleokinesis (Bellion *et al.*, 2005; Solecki *et al.*, 2006; Tsai *et al.*, 2007; Tsai and Gleeson, 2005). According to these studies, the centrosome pulls microtubules and nucleus in the direction of the

leading process. As a consequence, the distance between centrosome and nucleus increases and decreases during migration (**Figure 2a** and **2b**). However, a recent report shows that in tangentially migrating interneurons in the mouse cortex, the distance between nucleus and centrosome (or Golgi) can remain relatively constant over the course of migration (Yanagida *et al*., 2012).

In this context, it is important to note that neuronal migration and nucleokinesis do not always depend on a leading centrosome. For example, in radially migrating granule cells of mouse cerebellar slices, the centrosome can frequently be overtaken by the nucleus that has no effect on the direction of nuclear movement or neuronal migration (Umeshima *et al*., 2007). Similar findings were made for two different neuronal subtypes in the developing nervous system of the zebrafish (Distel *et al*., 2010; Randlett *et al*., 2011). In neurons derived from the zebrafish rhombic lip, which is an embryonic cerebellar structure, the centrosome is regularly overtaken by the nucleus during cell migration (Distel *et al*., 2010). Although the centrosome moves into the leading process in front of the nucleus after the apical (trailing) process detaches from the apical lamina upon neurogenesis, once nucleokinesis commences, it often stays behind the nucleus. Additionally, the nucleus moves in a saltatory manner, whereas the speed of centrosomal movement stays constant. Together, these findings argue that the movement of these two organelles is uncoupled (Distel *et al*., 2010). Retinal ganglion cells, the most basal neurons in the retina whose axons form the optic nerve, migrate through somal translocation and the centrosome stays in the process apical of the nucleus (which would be the trailing process for other neurons) during the whole course of migration (Randlett *et al*., 2011).

Adhesion and deadhesion of neurons to and from substrates

Many adhesion proteins, such as connexins, *N*-cadherin, integrins, astrotactin, JAM-C or TAG-1, were found to mediate the interaction between neurons and their substrates (Solecki, 2012). Glial-guided migration in particular depends on the attachment and detachment of neurons to and from the underlying radial glia cells. Understanding the interplay and redistribution of adhesion proteins during glial-guided migration has, therefore, garnered much attention in the field. **See also**: Integrin Superfamily

The role of the endocytic machinery

Endocytosis is known to play a role in release of cellular adhesion in many different contexts, including neural development (Wong *et al*., 2012). Controlled endocytosis is also important for glial-guided migration that depends on sequential adhesion and deadhesion of neurons from the glia cells. A study on mouse cortical neurons showed that inhibition of clathrin or dynamin, two upstream components of the endocytic pathway, results in disturbed migration of neurons from cortical explants (Shieh *et al*., 2011). Organotypic slices of mouse cortex further revealed that clathrin-mediated endocytosis components are enriched at the swelling of the leading process at times when nucleokinesis starts (Shieh *et al*., 2011). In this system, blocking endocytic pathways entirely by knockdown of the upstream component dynamin resulted in complete stalling of neuronal migration (Kawauchi *et al*., 2010). When focussing on distinct vesicular trafficking pathways using dominant negative approaches in slice culture, it became clear that interfering with the early endosome component Rab5 as well as the recycling endosome component Rab11 perturbed trafficking of *N*-cadherin and thereby neuronal migration per se. This suggests that endocytosis in general and the Rab5 pathway in particular affect cell adhesion between migrating neurons and underlying glia (**Figure 3**; Kawauchi *et al*., 2010). *N*-cadherin-mediated adhesion is also important for stable attachment of the leading process during somal translocation (Franco and Müller, 2011). Furthermore, *N*-cadherin trafficking in neurons of the cortical intermediate zone seems to play a role once the neuron transforms from multipolar to bipolar state (Jossin and Cooper, 2011). So far, our knowledge on how endocytosis influences neuronal migration is limited to a few factors and cell types. How this machinery influences different modes of neuronal migration and developmental systems is not well explored at this point. **See also**: Clathrin-coated Vesicles and Receptor-mediated Endocytosis

The role of gap junction proteins

Gap junctions represent specialised connections between cells enabling intercellular communication and adhesion. Gap junctions consist of varying connexin proteins, abbreviated Cx. Connexins have been studied in rat neocortical slice cultures, in which Cx26 as well as Cx43 is concentrated at contact points between radial glia and migrating projection neurons (**Figure 3**; Elias *et al*., 2007). Knockdown of these connexins led to multipolar morphology of neurons and severe migration defects. Similar results were obtained in Cx43 knockout mice (Cina *et al*., 2009). The exact mechanisms of how these connexins influence neuronal migration seem to differ for Cx43 and Cx26. Cx43-mediated adhesion is important for stabilisation of leading process branches, whereas Cx26 provides adhesion required for the centrosomal movement (Elias *et al*., 2007). Importantly, neuronal migration could be restored by introducing Cx43, or Cx26, constructs that rescued only adhesion but not pore formation between cells. This supports the notion that connexins are responsible for adhesion between neurons and glia and thereby mediate radial glial-guided migration (Elias *et al*., 2007).

The same connexins, Cx43 and Cx26, have also been investigated in the context of tangential migration of interneurons (Elias *et al*., 2010). Although they are not required for migration from the ganglionic eminences into the cortex, Cx43 plays a role in the last radial phase of interneuron migration. This last phase of migration is

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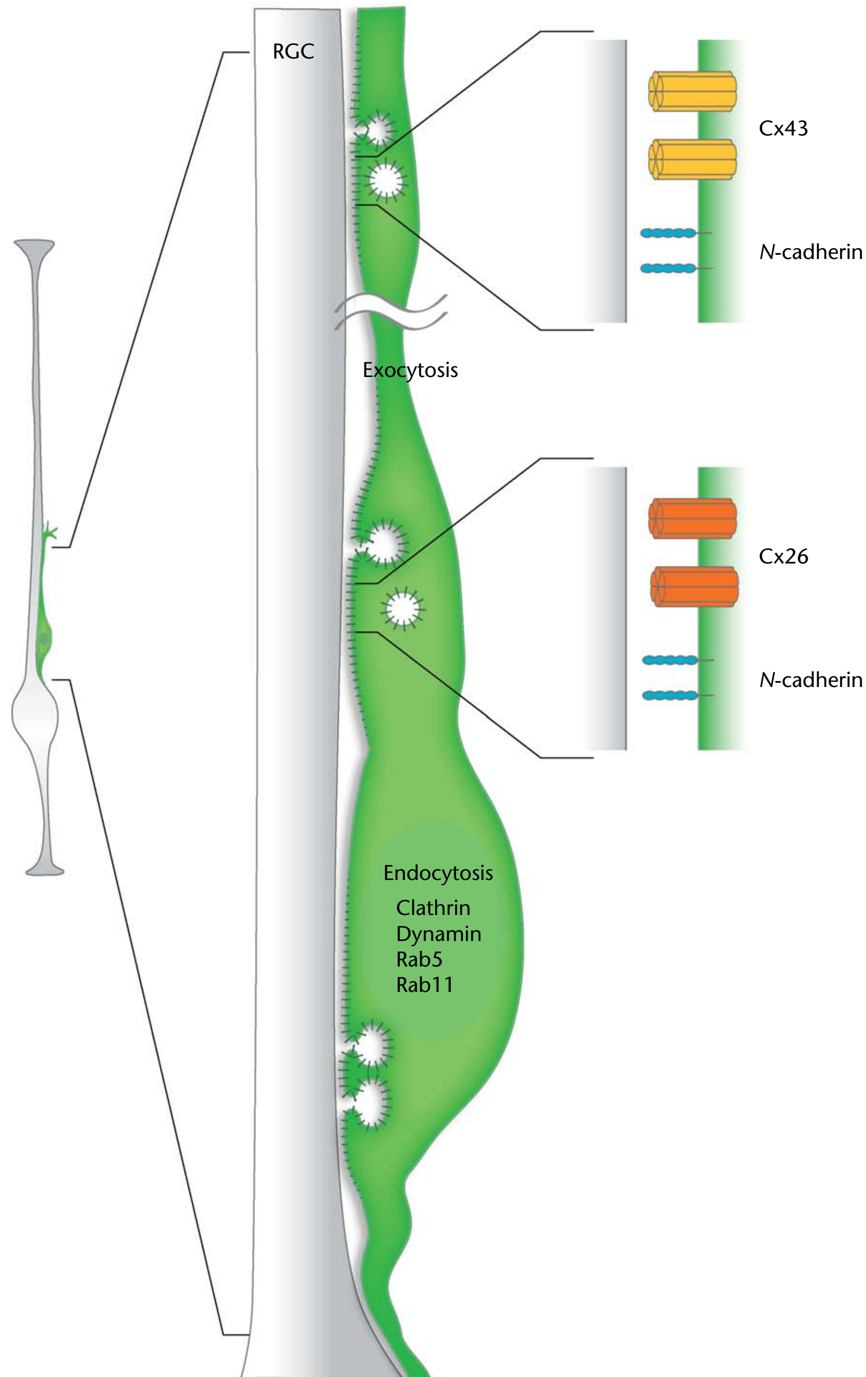

Figure 3 Turnover of adhesion molecules during glial-guided migration. Before nucleokinesis, adhesion between neuronal soma (green) and glial cells (grey) is weakened by endocytosis of connexins and *N*-cadherin (small bars in the invaginating vesicle). Endocytosis depends on clathrin, dynamin and the Rab family proteins Rab5 and Rab11. Later, connexins and *N*-cadherin (bars in vesicles) are exocytosed in the leading process of the neuron. The distribution of adhesion molecules is not uniform. Cx26 (red) is enriched in the swelling of the leading process, whereas Cx43 (yellow) is enriched in the tip of the leading process and its branches. The adhesion molecules in the membrane of RGC were omitted due to the lack of experimental data, but it has been speculated that they form homotypic interaction with adhesion molecules of the neuron. RGC, radial glia cell; Cx26, connexin 26; Cx43, connexin 43.

dependent on glial adhesion (Faux *et al*., 2012), underlining the fact that the connexin adhesive properties are important for directed neuronal migration along glia cells (Elias *et al*., 2010). Similar results were obtained for another protein regulating cell adhesion, the focal adhesion kinase (FAK) (Valiente and Marín, 2010). FAK is important for glial-guided migration but dispensable for somal translocation and tangential migration. On the

molecular level, FAK is required for Cx26 aggregation at the adhesion sites in the swelling of the leading process (Valiente and Marín, 2010). **See also**: Gap Junctions and Connexins: The Molecular Genetics of Deafness

More studies are needed to establish further candidates and molecular pathways that play a role in neuronal glial adhesion. It will additionally be important to understand how those pathways ensure communication between migrating cells and the underlying glia scaffold.

ECM proteins

Other important factors to ensure attachment and detachment of neurons during migration are the different components of the ECM. Generally, ECM molecules are responsible for structural support of cells in the nervous system and most other tissues within organisms. So far, different ECM components, including laminins, tenascins and proteoglycans, have been shown to be involved in neuronal migration. Another recent review has focused on this topic and the authors refer the reader to this work for a more detailed description (Franco and Müller, 2011).

The most thoroughly investigated ECM component that regulates neuronal migration is reelin (Zhao and Frotscher, 2010). It is a large ECM glycoprotein secreted by Cajal–Retzius cells in the MZ of the developing cortex (Zhao and Frotscher, 2010). The role of reelin in neuronal migration was discovered in a spontaneous mouse mutant called reeler that displayed poor motor coordination due to disorganised cortical layering and reduced cerebellum size (D'Arcangelo *et al*., 1995). Reelin is most important for the late steps of cortical migration. It acts as a stop signal for migrating neurons and influences the tip of their leading process, by which it controls the final positioning of the neuron during terminal translocation (Franco and Müller, 2011). Reelin signalling also leads to detachment of neurons from their radial glia tracks. When reelin is overexpressed, it stimulates glial-independent migration of neurons already at early stages of migration. These neurons thereby prematurely undergo modes of migration more similar to terminal translocation (Kubo *et al*., 2010). It will now be interesting to link the knowledge we acquired on the reelin protein to other components of the ECM and also investigate the role of reelin in different parts of the brain other than the cortex and in model systems other than rodents.

Neuronal Migration *In Vivo*

In the developing nervous system, neurons migrate through complex tissue environments. This means that multiple signals have to be integrated, including the existence of neighbouring cells, ECM components, molecular gradients and tissue stiffness. Although studies on cultured neurons and organotypic tissue slices are indispensible for understanding general mechanisms of neuronal migration, these experimental conditions might not fully resemble the *in vivo* situation. Therefore, observing neuronal movements in the intact embryo is important to understand migration in its full intricacy.

Microscopes suitable for *in vivo* imaging

In vivo imaging of neuronal migration continues to be improved by the use of more advanced microscopes. The most commonly used systems are point scanning and two-photon confocal systems. These allow good signal-to-noise ratios and deep tissue penetration. However, due to the high amount of light that is used during image acquisition, caveats of these methods are phototoxicity and photobleaching. Furthermore, acquisition times are in the range of minutes and thereby rather slow in relation to subcellular events like cytoskeletal dynamics. Spinning-disc confocal microscopes, however, induce less phototoxicity and allow acquisition times in the order of seconds. A different imaging approach that is becoming more and more popular is light sheet microscopy (Huisken, 2004). The advantages of this method are mainly the characteristic massive reduction of phototoxicity and photobleaching and the option to image at very short intervals. Additionally, improved mounting strategies allow more natural development and growth of the animal during the course of an experiment (Kaufmann *et al*., 2012). Owing to these advances, imaging is possible for days or even weeks, which in principle allows following neurons from birth to their final location. **See also**: Confocal Microscopy

Vertebrate model organisms suitable for *in vivo* studies

In vivo experiments are becoming easier to pursue and developing embryos are becoming more accessible due to the outlined advances in microscopy and the growing ease by which neurons and subcellular structures can be labelled with fluorescent proteins. However, different model organisms display different advantages and disadvantages when it comes to studies of neuronal migration in the intact embryo.

The vertebrate that offers the best imaging conditions due to its embryonic transparency is the zebrafish. This is one reason for its increasing popularity for *in vivo* time-lapse imaging of neuronal migration in different parts of the developing brain (e.g. Distel *et al*., 2010; Rieger *et al*., 2009; Stockinger *et al*., 2011; and others). One big advantage of the zebrafish is that its nervous system develops very fast within the first 5 days after fertilisation. However, the disadvantage is its relatively simple brain structure. Zebrafish do not develop a neocortex and therefore only less derived modes of neuronal migration can be explored.

To understand neuronal migration in a mammalian system, the mouse embryo has been shown to be a suitable albeit challenging *in vivo* model (Ang *et al*., 2003; Yanagida *et al*., 2012; Yokota *et al*., 2007). Owing to the *in utero* development of mouse embryos combined with the fact that the majority of neuronal migration occurs before

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birth, imaging in this system has to bypass multiple obstacles. A further challenge is the poor transparency of mouse embryos. However, recent pioneering studies enabled imaging of interneurons migrating close to the surface of the cerebral cortex *in utero*. This setup allowed new observations, including random movements in all directions during final positioning of the interneurons Ang *et al*., 2003; Yokota *et al*., 2007). Even subcellular resolution of interneurons has recently been achieved by the Murakami group in a study that analysed the dynamics of leading process, nucleus and the Golgi apparatus (Yanagida *et al*., 2012).

Concluding Remarks

The authors summarise here their current knowledge on how neurons move to find their final location and ensure correct wiring of complex neuronal networks in the brain. It is clear that there is not one underlying mechanism by which neurons migrate. Rather, cells adapt to their environmental conditions. Some of the differences in migratory modes and underlying machinery might result from varying distance cells have to travel to reach their final location. For example, in the retina, cells move only five to ten times their nuclear lengths, whereas in the rodent or primate cortex this often becomes 100 nuclear lengths (Tsai and Gleeson, 2005). It is, therefore, conceivable that the latter requires stricter coordination of migration. To correlate neuronal migration modes and mechanisms used for nucleokinesis with the distance a neuron has to travel *in vivo* will be an interesting field for future research.

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