Neural Progenitor Nuclei IN Motion

Elena Taverna[1] and Wieland B. Huttner[1,*]
[1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
*Correspondence: huttner@mpi-cbg.de

Interkinetic nuclear migration (INM), the movement of neuroepithelial and radial glial cell nuclei along the apical-basal axis in concert with the cell cycle, underlies the pseudostratification of the ventricular zone (VZ). Recent studies provide insight into the molecular mechanisms of INM and its effects on neural progenitor cell fate determination. Moreover, INM not only has a key role in increasing the VZ progenitor pool, but also may have set the stage for the evolution of subventricular zone progenitors implicated in cortical expansion.

The primary stem cells of the vertebrate CNS, the neuroepithelial cells, exhibit two remarkable features that, intriguingly, are interrelated—*pseudostratification* and *interkinetic nuclear migration* (INM). Pseudostratification refers to the fact that although all neuroepithelial cells extend from the lumenal (apical) surface of the neuroepithelium to the basal lamina throughout their cell cycle, their nuclei are found at various positions along this apical-basal axis resulting in a multilayer appearance (Baye and Link, 2008; Miyata, 2008; Sauer, 1935) (Figure 1A). INM refers to the fact that mitosis of neuroepithelial cells occurs at (or very close to) the apical surface of the neuroepithelium, whereas S phase usually takes place at a more basal location, with apical-to-basal nuclear migration in G1 and basal-to-apical nuclear migration in G2 (Baye and Link, 2008; Miyata, 2008; Sauer, 1935) (Figure 1A). Hence, INM is responsible for the pseudostratified appearance of the neuroepithelium. INM was first proposed in 1935 by Sauer, who deduced its existence from a histological analysis of the pseudostratified neuroepithelium (Sauer, 1935). INM can be regarded as a highly specialized form of the evolutionary conserved process of nuclear migration and positioning (Morris et al., 1998; Reinsch and Gönczy, 1998).

The existence of INM has raised several questions. First, what are the links between INM and cell polarity? Second, what are the links between INM and cell cycle progression? Third, what is the molecular machinery underlying INM? Fourth, what is the role of INM for neural stem and progenitor proliferation versus differentiation? Fifth, how does INM impact on cellular architecture? Sixth, what is the relationship of INM to the nuclear movements in the neural progenitors generated from neuroepithelial cells? And seventh, what is the role of INM in cortex evolution? Here, we will discuss recent insight into these issues.

INM and Cell Polarity

Neuroepithelial cells, like other epithelial cells, exhibit apical-basal polarity, with their apical plasma membrane lining the lumen of the neural tube and their basal plasma membrane contacting the basal lamina (Götz and Huttner, 2005; Huttner and Brand, 1997) (Figure 1A). Neuroepithelial cells are highly elongated and have a bipolar morphology with a "pearl-on-a-string" shape. In line with the pseudostratified appearance of the neuroepithelium, the part of the cell harboring the nucleus at any given time is by far the thickest, with much thinner apical and basal processes extending to the neural tube lumen and the basal lamina, respectively (Götz and Huttner, 2005; Miyata, 2008). The ratio of apical-basal length to the average thickness of these processes is extreme and often exceeds a value of 100 to 1. This, together with the occurrence of junctional complexes at the apical-most end of the lateral plasma membrane (Farkas and Huttner, 2008; Marthiens and ffrench-Constant, 2009) and the relative scarcity of apical microvilli (Marzesco et al., 2005), implies that the apical plasma membrane and the basal plasma membrane proper (that is, the membrane contacting the basal lamina) each constitute only a minor proportion of the neuroepithelial cell surface (Farkas and Huttner, 2008; Kosodo et al., 2004). These geometrical aspects are significant when considering the molecular machinery underlying INM, the role of INM for neural stem and progenitor cell fate, and the impact of INM on cellular architecture, as will be discussed below.

As pointed out by Sauer in his classical study, "*the mitoses are confined to the region of the lumen not because only nuclei of that region divide, but because a nucleus that is about to divide moves to the region of the lumen to do so*" (Sauer, 1935). But why does the nucleus of neuroepithelial cells migrate toward the apical cell surface for mitosis? A major reason lies in the fact that the apical plasma membrane localizes, throughout interphase, an organelle with a key role for setting up the mitotic spindle—the centrosomes (Chenn et al., 1998; Farkas and Huttner, 2008; Tamai et al., 2007). This is so because in neuroepithelial cells, like in other vertebrate epithelial cells, the primary cilium (1) protrudes from the apical plasma membrane, (2) persists through the cell cycle until the onset of mitosis, and (3) provides, via its basal body and associated second centriole, the centrosomes for the future mitotic spindle poles (Dubreuil et al., 2007; Santos and Reiter, 2008) (Figure 1A). For the ease of presentation, from now on, when referring to "apical centrosomes," we include the ciliary basal body.

With the onset of neurogenesis, neuroepithelial cells transform into radial glial cells, which are highly related to neuroepithelial cells and like the latter exhibit apical-basal polarity but are even more elongated (Götz and Huttner, 2005; Kriegstein and Götz, 2003) (Figure 1B). This elongation pertains primarily to the basal process of radial glial cells, which maintains its contact with the basal lamina and hence grows in length concomitant
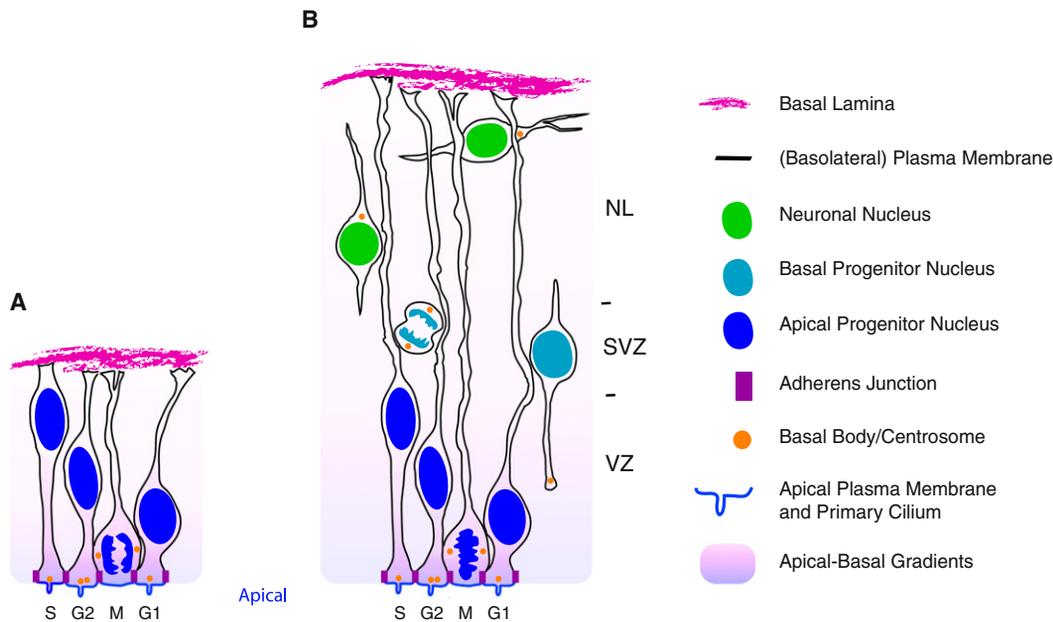
Figure 1. INM of Neural Progenitors

INM and apical-basal polarity of neuroepithelial (A) and radial glial (B) cells, collectively referred to as apical progenitors (APs). APs exhibit apical-basal polarity, with (i) the apical plasma membrane lining the lumen of the neural tube, (ii) the primary cilium protruding from the apical plasma membrane, (iii) interphase centrosomes (including the basal body) located at the apical plasma membrane, (iv) adherens junctions at the apical-most end of the basolateral plasma membrane, and (v) the basal plasma membrane contacting the basal lamina. AP nuclei occupy different positions along the apical-basal axis depending on the phase of the cell cycle. AP mitosis (M) occurs at the apical surface, whereas S phase takes place at a more basal location, with apical-to-basal nuclear migration in G1 and basal-to-apical nuclear migration in G2. In neuroepithelial cells (A), INM may extend over their entire apical-basal axis. By contrast, in radial glial cells (B), INM is confined to the portion of the cell residing in the ventricular zone (VZ) and, when present, the subventricular zone (SVZ) constituted by basal progenitors (BPs), and does not extend into the neuronal layers (NL). Besides underlying pseudostratification and reserving the limited apical space for AP mitoses, an emerging function of INM is to influence AP fate by controlling the exposure of AP nuclei to different, proliferative versus neurogenic, signals localized along the apical-basal axis.

with the formation of the neuronal layers and the resulting thickening of the neural tube wall. The transformation from neuroepithelial to radial glial cells, however, is associated with a major change with regard to INM. Whereas in neuroepithelial cells INM may extend over their entire apical-basal axis (Figure 1A), INM in radial glial cells does not extend into the portion of the cell that traverses the neuronal layers, but is confined to the portion of the cell residing in the ventricular zone (VZ) and, when present, the subventricular zone (SVZ) (Götz and Huttner, 2005) (Figure 1B). Possible reasons for this restriction are discussed below.

As in neuroepithelial cells, the primary cilium of radial glial cells, in line with their epithelial nature, is located at the apical plasma membrane (Dubreuil et al., 2007), and hence, of relevance for INM, these cells also undergo mitosis at their apical surface (Schenk et al., 2009) (Figure 1B). It is for this reason that neuroepithelial and radial glial cells are collectively referred to as apical progenitors (APs). The nuclear movements in the recently characterized outer SVZ (OSVZ) progenitors, which can be regarded as delaminated radial glial cells (Fietz et al., 2010; Hansen et al., 2010), will be addressed below.

The Links between Cell Cycle Progression and INM

Given that in the various phases of the cell cycle, AP nuclei occupy different positions along the apical-basal cell axis, what then are the links between cell cycle progression and INM? Several lines of evidence indicate that INM is not required for cell cycle progression and that cell cycle progression can be uncoupled from INM. First, upon treatment of various neuroepithelial systems, notably the chick neural tube including the retina, with cytochalasin B, which inhibits F-actin polymerization (but also glucose uptake), mitotic figures, rather than being confined to the apical surface, are observed throughout the neuroepithelium (Messier, 1978; Murciano et al., 2002). This has been taken to indicate that cell cycle progression continues, and AP nuclei can progress to mitosis when INM is inhibited (Baye and Link, 2008; Murciano et al., 2002). While this is a reasonable conclusion, it should be pointed out, however, that the data reported would also allow an alternative interpretation. That is, there exists a second class of neural progenitors, called basal progenitors (BPs) (Haubensak et al., 2004) or intermediate progenitors (Noctor et al., 2004), that are known to divide away from the apical surface, i.e., at a basal location (Figure 1B). Even though BPs are typically found in the mammalian telencephalon (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004), it cannot strictly be excluded that the reported cytochalasin B treatment, rather than inhibiting basal-to-apical INM of APs, resulted in the generation of BPs, which lack basal-to-apical INM (Attardo et al., 2008; Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004). Even if such a scenario may seem unlikely, it is interesting to note that the cell fate changes seen upon cytochalasin B treatment
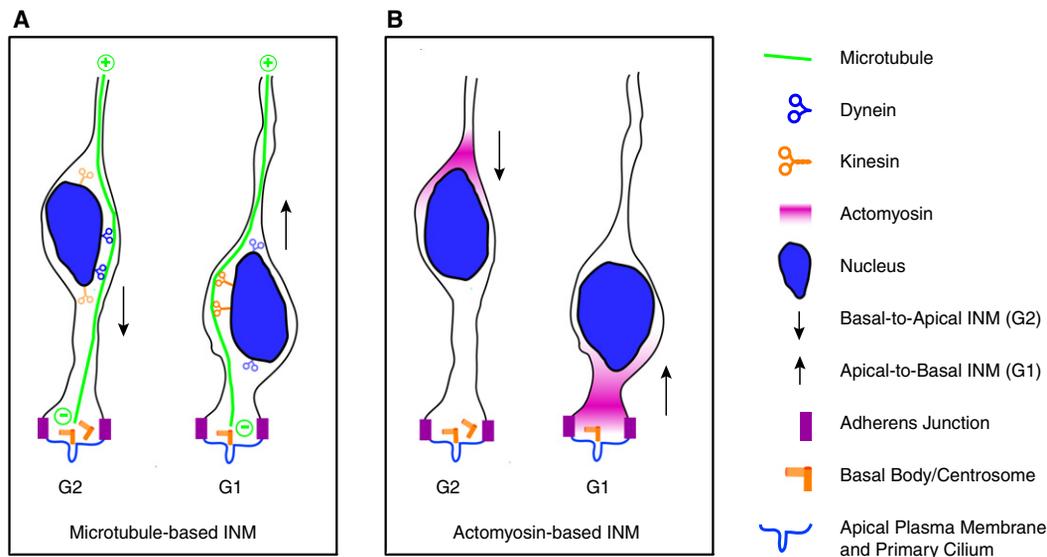
Figure 2. Microtubule-based and Actomyosin-based INM
(A) Ad-centrosomal, microtubule minus end-directed, dynein-based basal-to-apical INM in G2; ab-centrosomal, microtubule plus end-directed, kinesin-based apical-to-basal INM in G1. (B) Basal-to-apical INM in G2, and apical-to-basal INM in G1, both driven by directional actomyosin constriction.

(Murciano et al., 2002) would be consistent with this alternative possibility.

A recent study reports unequivocal evidence that inhibition of INM does not affect cell cycle progression. Specifically, upon interference with INM in the developing mouse cortex using the myosin II inhibitor blebbistatin, no difference in the length of the various cell cycle phases and in progression through mitosis of progenitors in the VZ was observed (Schenk et al., 2009). On a general note, the findings that INM is dispensable for cell cycle progression of APs is, perhaps, not surprising, given the existence of other neural progenitors that progress through the cell cycle without INM.

Notably, the converse does not hold true, that is, INM has been found to depend on cell cycle progression, as shown in several studies of developing mouse cortex. Specifically, pharmacological treatments inducing S phase or G2/M arrest resulted in concomitant inhibition of INM in the basal and apical region of the VZ, respectively (Baye and Link, 2008; Ueno et al., 2006). Moreover, acceleration and retardation of cell cycle progression by overexpression and RNA interference, respectively, of cyclins/cyclin-dependent kinases did not appear to alter the position of S phase and mitosis of APs, which still occurred in the basal region of the VZ and at the ventricular surface, respectively (Lange et al., 2009; Pilaz et al., 2009). This suggests that APs adapted the overall speed of their INM to that of the various cell cycle phases in order to maintain the synchrony of INM with cell cycle progression. Together, these data support the concept that the master regulators of cell cycle progression also control INM. It will be important to dissect the pathway(s) via which this control is achieved.

Molecular Machinery of INM
Over the past years, substantial progress has been made regarding the molecular machinery of INM. Before describing these insights, we would like to mention some general considerations as to the phenotypes one can anticipate upon inhibition of INM.

Interfering with INM—General Considerations
Bearing in mind that the AP cell cycle progresses upon inhibition of INM (Baye and Link, 2008), one would expect that if the *basal-to-apical INM during G2* is specifically inhibited, mitoses would now occur away from the ventricular surface, in a more basal location within the neuroepithelium/VZ. An important corollary to this prediction is that the centrosomes have to move from the apical surface, where they usually reside, to a more basal location because they are required to set up the mitotic spindle. A second interesting aspect of this scenario is the issue of S phase location of the resulting daughter cells, which might then occur more basally than usual. Conversely, if the *apical-to-basal INM during G1* is specifically inhibited, the prediction is that the location of mitoses at the ventricular surface would not be affected whereas S phase should now occur in a more apical location.

A Special Centrosome-Nucleus Liaison
The spatial relationship between the AP nucleus and centrosomes has a bearing on the molecular machinery of INM. The localization of the AP primary cilium, and hence the centrosomes, at the apical plasma membrane throughout the cell cycle, except for mitosis, implies that the INM during G1 is directed away from the centrosome (ab-centrosomal INM), and that during G2 is directed toward the (by then duplicated) centrosomes (ad-centrosomal INM) (Figures 1 and 2). In other words, there is a lack of physical proximity between nucleus and centrosomes in APs in interphase. This distinguishes these pseudostratified epithelial neural progenitors from their progeny, that is, BPs, OSVZ progenitors and neurons, which also show nucleokinesis but in which the centrosome(s) are located in the perinuclear area (Farkas and Huttner, 2008; Fietz et al., 2010;

Hansen et al., 2010; Higginbotham and Gleeson, 2007; Tsai and Gleeson, 2005) (Figure 1B). Despite the lack of nucleus-centrosome physical proximity in interphase APs, several lines of evidence indicate that the centrosome has a functional role in ad-centrosomal INM. First, the centrosomal proteins Cep120, TACCs, and Hook3 were found to be necessary for basal-to-apical INM of mouse cortical APs (Ge et al., 2010; Xie et al., 2007). Second, in the developing rat cortex lacking the transcription factor Pax6, the centrosomes in interphase APs were not always found in their normal position at the apical plasma membrane but were occasionally observed in a slightly abventricular location, and this was correlated with perturbed basal-to-apical INM (Tamai et al., 2007).

What, then, is the nature of the functional link between the apical centrosomes of APs and their ad-centrosomal INM? Given (1) the role of the centrosome as microtubule organizing center (MTOC), (2) the well-known function of microtubules in nuclear positioning (Reinsch and Gönczy, 1998), and (3) the early observation that intact microtubules are required for INM (Messier, 1978), microtubules and their associated motor proteins have become a major focus of the research aiming to dissect the molecular machinery of INM.

Microtubule-Dependent Basal-to-Apical INM

Indeed, the requirement of the centrosomal proteins Cep120, TACCs, and Hook3 for basal-to-apical INM is thought to reflect their role in organizing the microtubules at the centrosome (Ge et al., 2010; Xie et al., 2007). These data are consistent with the concept that, in basal-to-apical INM, the AP nucleus is moved, as a cargo, along microtubule tracks (Figure 2A), as is known to be the case for other membrane-bound organelles (Tolić-Nørrelykke, 2008). As the orientation of microtubules is with their minus-ends toward the centrosome (Norden et al., 2009; Reinsch and Karsenti, 1994), such nuclear movement would be expected to be mediated by minus-end-directed microtubule-based motor proteins (Baye and Link, 2008) (Figure 2A).

Evidence that this is so has come from studies of the gene *LIS1*, mutations in which cause lissencephaly, a disorder of human brain development characterized by an almost complete absence of *gyri* (Vallee et al., 2001). Specifically, the Lis1 protein interacts with, and regulates, the minus-end-directed motor protein dynein (Figure 2A), in particular with regard to transporting high-load cargo such as a nucleus (McKenney et al., 2010; Tanaka et al., 2004; Wynshaw-Boris and Gambello, 2001). Upon reduction of Lis1 levels in the developing rodent cortex, basal-to-apical INM is inhibited, and mitoses are no longer confined to the apical surface but observed throughout the VZ (Gambello et al., 2003; Tsai et al., 2005).

Further support for dynein motors mediating the basal-to-apical INM has come from studies of the developing fish CNS. In the zebrafish retina, a mutant in dynactin-1, a component of the dynactin complex that mediates the interaction of dynein with its cargo (Schroer, 2004), shows perturbed basal-to-apical INM and, consequently, mitoses throughout the VZ (Del Bene et al., 2008). A similar phenotype has been observed in the neural tube of a medaka laminin γ1 mutant, in which the dynein-dynactin system is thought to be perturbed due to defective signaling involving the focal adhesion kinase (Tsuda et al., 2010).

Together, these studies provide compelling evidence that the microtubule minus-end-directed dynein motor system is involved in basal-to-apical INM. Thus, the available data are consistent with the concept that the AP nucleus is moved by dynein motors along microtubule tracks toward the apical centrosomes (Figure 2A), although it cannot be excluded that the observed effects on INM reflect a more indirect role of the dynein system.

If the effect of dynein is direct, the question arises how the dynein system is linked to the nuclear envelope. Intriguingly, integral membrane proteins of the nuclear envelope that exert this role have recently been identified. Specifically, in the developing zebrafish retina, knockdown of the KASH-domain-containing protein Syne2a, which spans the outer nuclear membrane, has been reported to phenocopy the dynactin-1 mutant with regard to INM (Del Bene et al., 2008). Syne2a directly interacts with dynactin and thus anchors the nucleus to the dynein motor system (Zhang et al., 2009). In the developing mouse cortex, Syne2a knockdown results in perturbed basal-to-apical INM and, consequently, mitoses throughout the VZ (Zhang et al., 2009). Moreover, Syne2a also directly interacts with the SUN-domain-containing proteins SUN1 and SUN2, which span the inner nuclear membrane. In the SUN1/2 double knockout, INM is perturbed in essentially the same way as upon Syne2a knockdown (Zhang et al., 2009). Taken together, these data indicate that APs possess a protein-protein interaction network that links the nucleus to microtubules via the dynein motor system (Figure 2A). This network provides a mechanistic explanation of how the force exerted by the microtubule minus-end-directed dynein motors moves the nucleus, as a cargo, along microtubule tracks toward the apical centrosomes.

Actomyosin-Dependent Basal-to-Apical INM

The involvement of the dynein system in basal-to-apical INM does not rule out a role of other classes of motor proteins, notably actomyosin (Figure 2B). Consistent with this possibility, inhibition of the small GTPase Rac1 results in retarded basal-to-apical INM (Minobe et al., 2009). Direct evidence that actomyosin plays a role has recently come from a study on the zebrafish retina, in which the mechanism underlying basal-to-apical INM has been analyzed primarily by tracking neuroepithelial cell nuclei via live imaging, with three principal findings (Norden et al., 2009). First, during most (90%) of the cell cycle, nuclear movement is a largely stochastic process, similar to Brownian motion. Persistent directed movement of the nucleus was observed only immediately before and after mitosis. Second, pharmacological inhibition of myosin II function prevented basal-to-apical INM; the only mitoses observed in this condition were confined to the apical surface. Third, upon interference with dynactin and microtubule function, basal-to-apical INM was largely unaffected, suggesting that in this system microtubule minus-end-directed dynein motors play only a minor role (Norden et al., 2009).

Each of these findings is remarkable on its own and deserves comment. First, the largely stochastic nuclear movement observed in the zebrafish retina is clearly different to the mainly directed nuclear movement reported in previous live imaging studies in other systems, notably the developing rodent cortex (Attardo et al., 2008; Haubensak et al., 2004;

Miyata et al., 2004; Noctor et al., 2004), but also the mouse (Saito et al., 2003) and chick (Pearson et al., 2005) retina and the medaka neural tube (Tsuda et al., 2010). Possible explanations for these divergent observations include the following. One is related to the cytoarchitecture of the different systems used. Specifically, in the zebrafish retina at the developmental stage studied, the apical-to-basal extension of the neuroepithelium corresponded to 3–4 nuclear diameters (Norden et al., 2009), which is substantially less than, for example, in the rodent cortex (Attardo et al., 2008; Haubensak et al., 2004; Miyata, 2008; Miyata et al., 2004; Noctor et al., 2004). It is thus possible that certain parameters of tissue architecture such as cell elongation or volume ratio of nucleus to surrounding cytoplasm determine whether INM is largely stochastic or directed. Another, not mutually exclusive, possible explanation is related to the proportion of the cell cycle taken up by S phase versus G1 and G2 phase. Specifically, the published data do not exclude the possibility that, assuming that stochastic nuclear movements typically occur during S phase whereas directed nuclear movements are characteristic of G1 and G2 phase, S phase takes up a greater proportion of the neuroepithelial cell cycle in the zebrafish retina than in the other systems studied. The occurrence of directed nuclear movement just before and after mitosis (Norden et al., 2009) is consistent with this scenario.

Second, the absence of abventricular mitoses upon inhibition of basal-to-apical INM is puzzling. This is in contradiction not only with previously reported data (Del Bene et al., 2008; Gambello et al., 2003; Tsai et al., 2005), but also with the concept that the cell cycle is thought to progress in the absence of INM (Baye and Link, 2008; Murciano et al., 2002; Schenk et al., 2009), and thus on slowing-down of basal-to-apical INM mitoses should have occurred before nuclei were able to reach the apical surface. Perhaps the class of motor system targeted by the inhibitory manipulation and its relative contribution to basal-to-apical INM make the difference. Specifically, one may speculate that if this INM is largely microtubule motor-based (Del Bene et al., 2008; Gambello et al., 2003; Ge et al., 2010; Tsai et al., 2005; Xie et al., 2007), its inhibition may allow the centrosomes to dissociate from the apical plasma membrane and hence abventricular mitoses to occur, whereas upon inhibition of actomyosin as the main INM machinery in the zebrafish retina (Norden et al., 2009), this is not the case. In addition, the data of the latter study imply that cell cycle progression, and especially the onset of mitosis, adapted to the impaired INM. Perhaps, comparison of the zebrafish retina (Norden et al., 2009) with other systems such as the developing rodent cortex (Schenk et al., 2009) will shed light on how cell cycle progression is, or is not, coupled to INM.

Third, the minor contribution of microtubules and minus-end-directed dynein motors in basal-to-apical INM in the zebrafish retina (Norden et al., 2009) is in contrast to previous data (Del Bene et al., 2008; Gambello et al., 2003; Tsai et al., 2005). This discrepancy may reflect differences in the species studied (fish versus rodents), the tissue analyzed (retina versus cortex), the type of AP present (neuroepithelial versus radial glial cell), or the technology of analysis used (high versus low temporal resolution of time lapse).

If, depending on the system, both microtubule-based motors and actomyosin contribute to basal-to-apical INM, albeit to a differential extent, how may this contribution be integrated? A possible clue has come from studies on the outer nuclear envelope proteins Syne. Syne2 has been reported to interact not only with the dynein system (Del Bene et al., 2008; Starr, 2007; Zhang et al., 2009) as discussed above, but also, via its N-terminal calponin-homology domain, with actin (Starr and Han, 2003). This raises the intriguing possibility that Syne2 may constitute a point of convergence between actomyosin and the dynein system.

Actomyosin-Dependent Apical-to-Basal INM

Apical-to-basal INM has historically received less attention. One reason for this may lie in the fact that, in contrast to basal-to-apical INM which is specific to APs, apical-to-basal nuclear migration does not *only* occur in INM. Rather, apical-to-basal nuclear migration is a feature that APs share with other neural progenitors and with newborn neurons, all of which delaminate from the apical surface (see below) (Figure 1B); this puts an additional challenge to dissecting apical-to-basal nuclear migration specifically in APs. Another reason may be the fact that interference with the microtubule and actomyosin system perturbs progression of APs through mitosis and cytokinesis. This renders the analysis of the cytoskeletal basis of apical-to-basal INM, which in contrast to basal-to-apical INM occurs *after* AP mitosis, more difficult.

Using the specific myosin II inhibitor blebbistatin at a concentration at which progression of APs through cytokinesis was not impaired, evidence has been reported that actomyosin contractility is important for apical-to-basal INM of APs in the developing mouse neocortex (Schenk et al., 2009) (Figure 2B). Interestingly, at the relatively low blebbistatin concentration used, no perturbation of basal-to-apical INM was observed (Schenk et al., 2009). Consistent with the observed concentration of actomyosin at the apical cell cortex and its weakening upon blebbistatin treatment, it was proposed that the actomyosin-based apical-to-basal INM is mechanistically different from the microtubule-based basal-to-apical INM in that the nucleus is not moved as a cargo but, rather, via directional myosin-II-dependent constriction (Schenk et al., 2009) (Figure 2B).

Microtubule-Dependent Apical-to-Basal INM

Two considerations, when taken together, have led to the proposal that plus end-directed microtubule-based motors of the kinesin type may be involved in apical-to-basal INM (Baye and Link, 2008) (Figure 2A). First, the outer nuclear envelope protein Syne2 interacts not only with dynein complexes (Del Bene et al., 2008) and actin (Starr, 2007; Starr and Han, 2003), but also with kinesin complexes (Zhang et al., 2009). Second, the microtubules in APs are oriented parallel to the apical-basal axis, with the plus ends directed away from the centrosomes (Norden et al., 2009), thus providing a possible track to move the nucleus via a plus end-directed motor. Indeed, a recent RNAi screen has led to the identification of a nonconventional kinesin that specifically mediates apical-to-basal INM in rodent cortical APs (J.-W. Tsai and R. Vallee, personal communication) (Figure 2A).

Directionality of INM

Thus, the picture emerges that both microtubule-based motors and actomyosin participate in either direction of INM, although

to a different extent depending on the system. This raises the issue of the control of directionality of INM. In the case of microtubule-based INM, given the apical localization of the centrosomes and the uniform orientation of the microtubules, directionality of INM can be achieved by a cell cycle phase-dependent (G1 versus G2) switch in the plus end- versus minus end-directed motor protein used to move the nucleus (kinesin versus dynein) (Baye and Link, 2008) (Figure 2A). In contrast, in the case of actomyosin constriction, given that the same motor protein is being used for apical-to-basal as well as basal-to-apical INM, directionality can only be achieved if actomyosin constriction is regulated to progress along the apical-basal axis of APs with the proper orientation depending on the phase of the cell cycle (Figure 2B).

Passive Components in INM
The findings that motor protein activities underlie basal-to-apical as well as apical-to-basal INM does not mean that every nuclear movement in INM is necessarily motor protein-mediated (Norden et al., 2009). Rather, it is possible that certain nuclear movements are the result of a passive, nonautonomous cell behavior. Specifically, given the tight packing of nuclei in the neuroepithelium, the active migration of the nucleus of one AP in, say, the basal-to-apical direction may result in the passive displacement of the nucleus of a neighboring AP in the opposite, apical-to-basal, direction, thereby maintaining local nuclear density. It will be important to develop experimental approaches for investigating a possible passive component of INM.

Harmonizing INM
INM simultaneously occurs in a population of cells and thus needs to be harmonized between these cells in space and time in order to maintain the proper tissue architecture. It has been shown that APs are coupled via gap junctions (Bittman et al., 1997; LoTurco and Kriegstein, 1991) and that calcium signals can propagate between coupled cells (Owens and Kriegstein, 1998; Pearson et al., 2004). Indeed, evidence consistent with a role of gap junctions and calcium signaling in the intercellular harmonization of INM has been reported. Specifically, in the developing chick retina (Pearson et al., 2005) and mouse neocortex (Liu et al., 2010), interference with gap junctions and calcium signaling has been found to impair INM. Given the involvement of actomyosin contractility in INM (Norden et al., 2009; Schenk et al., 2009) and the regulation of this contractility by calcium (Marston, 1995), the intriguing possibility arises that gap junction-mediated calcium signals contribute, via their effects on actomyosin, to harmonizing INM within the AP population.

Functional Aspects of INM
Contrary to the frequently held view that the function of INM has been largely enigmatic, we believe that the primary function of INM has been noted by Smart in 1972, that is, to achieve pseudostratification of the VZ and thus to maximize mitoses of APs (Fish et al., 2008; Smart, 1972a, 1972b). In addition, recent studies have revealed a role of INM in cell fate determination.

Maximizing AP Mitoses
As pointed out above, the persistence of the AP primary cilium thoughout interphase necessitates that their mitoses occur apically (Figure 1). By translocating AP nuclei in interphase away from, and back to, the apical surface, INM serves to maximize the number of AP mitoses per available apical space (Fish et al., 2008; Smart, 1972a, 1972b). The resulting pseudostratification allows an increase in the number of APs that can be accommodated per apical surface area. Thus, INM is instrumental for the expansion of the AP pool, that is, of radial units (Rakic, 1988), a process that is at the core of cortical expansion (see below). Consistent with these considerations, interference with INM has been found to reduce the AP pool (Ge et al., 2010; Xie et al., 2007). On a more general note, given (1) that many of the genes affected in primary microcephaly encode centrosomal proteins (Bond et al., 2002; Bond and Woods, 2006) and (2) the pivotal role of centrosomes in INM (Ge et al., 2010; Xie et al., 2007), it will be interesting to explore whether perturbed INM contributes to the microcephaly phenotype (Buchman et al., 2010).

Influencing AP Fate
Given the highly polarized nature of APs, their apical versus basal intracellular microenvironment is likely to be different (Figure 1). A corollary of this is that INM is responsible for the extent to which an AP nucleus becomes exposed to these different microenvironments. Indeed, it has been proposed that INM influences AP fate by determining the time the AP nucleus spends at any given location along the apical-basal axis during the cell cycle (Baye and Link, 2008; Del Bene et al., 2008; Murciano et al., 2002). We will refer to this hypothesis to as the "*nuclear residence hypothesis*."

The nuclear residence hypothesis predicts that a signal influencing AP fate in an INM-dependent manner should be highly polarized along the apical-basal axis (Figure 1). Indeed, in the developing retina of chick and zebrafish, Notch signaling, which is known to prevent progenitor cells from differentiating, predominantly localizes to the apical side of the neuroepithelium (Del Bene et al., 2008; Murciano et al., 2002) and regulates its nuclear targets as the AP nuclei migrate apically (Del Bene et al., 2008). Upon inhibition of basal-to-apical INM by interference with actomyosin (Murciano et al., 2002) or dynein (Del Bene et al., 2008), the retinal progenitors exit the cell cycle prematurely and differentiate into early-born retinal ganglion cells. Moreover, in the developing zebrafish retina, the more basal the AP nucleus moves, the more likely it is to undergo a neurogenic division (Baye and Link, 2007). In the developing mouse cortex, slowing-down of apical-to-basal INM by myosin II inhibition has been found to result in a cell fate change, in this particular case yielding more neurogenic BPs at the expense of proliferative APs (Schenk et al., 2009). In conclusion, one emerging function of INM is to control the exposure of AP nuclei to proliferative versus neurogenic signals along the apical-basal axis, thereby influencing AP fate (Figure 1).

In this context, an important aspect of INM concerns the factors that determine how far basal an AP nucleus migrates. The extent of basally directed nuclear migration can be highly variable in a seemingly homogeneous population of neuroepithelial cells (Baye and Link, 2007, 2008), as is also indicated by the relative broad distribution of APs in S phase that is observed in the non-apical portion of the VZ in certain systems (Baye and Link, 2007; Schenk et al., 2009). Besides concerning the issue of the possible significance of differential S phase location for

AP fate, this broad distribution also raises the question whether there is variability in G1 and G2 length between individual APs with differential S phase location. Moreover, the extent of basally directed nuclear migration is markedly different between neuroepithelial cells (Figure 1A) and radial glial cells (Figure 1B), as INM does not extend into most of the basal process of the latter, as mentioned above. Little is known about the mechanisms underlying these differences, but two principal possibilities can be envisaged. First, determinants for the maximum basal position of the AP nucleus could be cell intrinsic, exerting either a positive role (e.g., the degree of basal extension of the INM-supporting cytoskeletal system) or a negative role (e.g., some kind of physical restriction in the basal process). Second, the determinants could be cell-extrinsic, that is, for example, physical barriers imposed by structural aspects of surrounding cells.

INM and Cellular Architecture

These considerations lead us to reflect, in general terms, on the impact of INM on the intracellular architecture of APs. EM analyses show that the distance between the outer nuclear envelope and the lateral plasma membrane is very small, often only in the 100 nm range. In other words, there is, relatively speaking, very little cytoplasmic space between the nucleus and the plasma membrane. One may say that in INM, the nucleus moves through the cytoplasm a bit like a teflon pestle does in a Potter-Elvehjem homogenizer (Figure 1). This implies that during INM most, if not all, cytoplasmic organelles will be squeezed passed the nucleus. Although little, if any, experimental data exist regarding this issue, one may predict that INM requires a near-complete rearrangement of cytoplasmic structures. One, though by no means the only, cytoplasmic structure affected by INM is likely to be the endoplasmic reticulum, an endomembrane network typically in continuity with the nuclear envelope. On a general note, dissecting the cell biological basis of INM-associated cytoplasmic rearrangements can be expected to become an emerging topic of research.

Nuclear Migration in Delaminating Neural Progenitors and INM

During CNS development, nuclear migration events take place not only in APs (i.e., INM), but also in other cell types, of which downstream neural progenitors generated from APs are of particular relevance in the present context. Are the latter forms of nuclear migration related to INM, and if so, how? We would like to address this question with regard to two AP-derived neural progenitor types, (1) BPs, which form the SVZ in rodents (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004), and (2) OSVZ progenitors (Smart et al., 2002), which have recently been characterized in human and ferret (Fietz et al., 2010; Hansen et al., 2010). Two principal states with regard to the nucleus-centrosome relationship can be distinguished as these two cell types originate from APs. Initially after their birth, as shown for BPs (Miyata et al., 2004) and as is likely the case also for OSVZ progenitors, these cells may still have apical contact, and their apical-to-basal nuclear migration resembles apical-to-basal INM of APs in that it is ab-centrosomal. Subsequently, after delamination of these cells from the apical adherens junctions and retraction of their apical process (Figure 1B),

the centrosomes are found in the perinuclear area (Farkas and Huttner, 2008; Fietz et al., 2010; Hansen et al., 2010), and their further apical-to-basal nuclear migration is reminiscent of that of migrating newborn neurons (Higginbotham and Gleeson, 2007; Tsai and Gleeson, 2005).

In line with these considerations, in the developing mouse cortex, the apical-to-basal nuclear migration of newborn BPs involves actomyosin contractility (Schenk et al., 2009). AP-derived neural progenitors destined for the SVZ thus seem to exploit the same machinery for this process as APs use for apical-to-basal INM. Basal-to-apical nuclear migration is not observed to any significant extent in rodent BPs (Attardo et al., 2008; Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004) and human OSVZ progenitors (Farkas and Huttner, 2008; Fietz et al., 2010; Hansen et al., 2010), which presumably reflects the retraction of their apical process and the eventually perinuclear location of their centrosomes. This in turn is consistent with the notion that basal-to-apical nuclear migration is specific to APs and that this leg of INM reflects the apical location of their centrosomes (Cappello et al., 2006).

INM and Cortex Evolution

Regarding the expansion of the cerebral cortex during mammalian evolution, INM has been instrumental in at least two ways. One is to reserve the limited apical space for AP mitoses and thereby to allow expansion of the AP pool. An interesting aspect here is the modification that the INM machinery presumably underwent as the degree of pseudostratification of the VZ in the dorsal telencephalon increased during evolution to reach the optimal level with regard to mitotic efficiency of APs (Smart, 1972a, 1972b). Such modification may concern, for example, motor protein systems as such, their regulation, and synergies between distinct systems.

A second crucial aspect concerns the use of a seemingly very similar, if not identical, machinery for apical-to-basal INM of APs and apical-to-basal nuclear translocation of the progenitors destined to the SVZ (Schenk et al., 2009). The actomyosin-based machinery used in both nuclear translocation processes does not require the centrosomes to remain located at the apical surface, in contrast to the microtubule-based machinery of the AP-specific basal-to-apical INM. This may have facilitated the transition from expanding the AP pool to generating BPs and OSVZ progenitors (Fietz et al., 2010; Hansen et al., 2010) that apparently occurred in evolution as the pseudostratification of the VZ had reached the optimal level and any further increase in neural progenitor mitotic efficiency necessitated the formation of a second, non-apical, germinal layer, the SVZ (Smart, 1972a, 1972b). The expansion of the SVZ, and notably of OSVZ progenitors, is thought to be a major underlying cause of cortical expansion (Abdel-Mannan et al., 2008; Fish et al., 2008; Kriegstein et al., 2006). Therefore, it will be exciting to dissect the evolutionary changes in the machineries mediating nuclear translocations in neural progenitors that underlie this expansion.

Conclusions

Consistent with the epithelial nature of APs, notably apical primary cilia and hence apical centrosomes in interphase, these progenitors undergo mitosis apically. By moving interphase

nuclei of APs away from, and back to, the apical surface in concert with the cell cycle, that is, by causing pseudostratification, INM serves to increase the number of AP mitoses and to expand the AP pool. In addition, INM controls the extent to which AP nuclei are exposed to the different, proliferative versus neurogenic, environments along the apical-basal axis of APs, thereby influencing their fate. INM, which is likely to have a major impact on cellular architecture, is brought about by the interplay of two polarized cytoskeletal machineries, (1) the apical centrosome-dependent, microtubule-based minus-end- and plus-end-directed motor systems, and (2) directional actomyosin contractility. The use of the latter also in the nuclear movements of neural progenitors derived from APs and destined for the SVZ is thought to have facilitated cortical expansion during evolution.

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