Review

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Mechanisms controlling arrangements and movements of nuclei in pseudostratified epithelia

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During development, cells undergo complex rearrangements that contribute to the final tissue architecture. A characteristic arrangement found in rapidly expanding, highly proliferative tissues is pseudostratified epithelium, which features notably elongated cells with varied nuclear positions along the cell axis. Although anomalies in its structure are implicated in diseases like microcephaly, how pseudostratification is formed and maintained remains elusive. In this review, we focus on a typical feature of pseudostratified epithelia called interkinetic nuclear migration (INM), which describes dynamic movements of nuclei within the elongated cell bodies. We provide an overview of cytoskeletal components underlying INM in different systems, discuss current understanding of its kinetics and timing, and evaluate how conflicting results could be explained through developmental and evolutionary considerations.

Pseudostratification and INM: an overview

Epithelia are ancient tissue types that function as protective and selective barriers between the external world and the body interior, as well as between distinct body compartments. Subcategories of epithelia are defined by cell morphology (Figure 1) as squamous, cuboidal, or columnar and also by number of cell layers as simple, stratified, or pseudostratified. Pseudostratified epithelia comprise a single layer of elongated cells; however, their nuclei are arranged in a manner that gives a stratified (multilayered) appearance (Figure 1c). This distinct epithelial arrangement is widely conserved and has been identified in various tissues with high proliferative turnover. Examples of pseudostratified epithelia in invertebrate tissues are the *Drosophila* wing disc and *Nematostella* ectoderm (see Glossary) and vertebrate tissues include nose, trachea, larynx, lung-buds, liver-buds, male reproductive, urinary, and digestive systems, and the developing central nervous system [1–3]. Interestingly, pseudostratification has also been described in case studies of certain cancers [4], underscoring the notion that epithelial pseudostratification is a hallmark of highly proliferative tissues. Abnormal pseudostratification has been implicated in brain defects such as microcephaly [5–7], suggesting that properly

Glossary

Actin: a globular protein featuring an ATPase activity (G-actin) that assembles into microfilaments (F-actin). Hydrolysis of ATP into ADP reduces its binding affinity, leading to depolymerization. Actin filaments are polarized, with a more dynamic actin plus, or barbed, end that depolymerizes and an ATP-bound minus, or pointed, end. Actin polymerization provides forces for cell motility, and contractility of actin networks has been implicated in cell division and cell shape changes. For example, actomyosin contracts as a ring-like structure during cytokinesis, whereas contractions along the whole cortex play a role in different morphological processes (for more details, see [34]).

Apical/basal in comparison to ventricular/pial surface: the apical surface is defined as the side of an epithelium that faces the lumen, whereas the basal surface faces the basal lamina. In pseudostratified epithelia, thin elongated apical and basal processes maintain attachment to these surfaces. Hallmarks of the apical surface are the centrosome, adherens and tight junctions, and the apical polarity complex. Characteristic hallmarks of the basal surface are less pronounced, but the proteins lethal giant larvae, Scribble, and Discs-Large, whose exact functions are not fully understood, localize specifically to this side. In the neuroepithelium of the rodent neocortex, the apical surface of the cells is often referred to as the ventricular surface because it lines the ventricle (a brain cavity), whereas the basal surface is called the pial surface because it is attached to the pial basal lamina.

Centrosome: centrosomes comprise a pair of centrioles linked by the pericentriolar matrix (PCM). Centrioles comprise cylindrical arrays of triplet microtubules organized with ninefold radial symmetry; the PCM comprises a meshwork of various proteins interlaced in a well-organized pattern. The centrosomes are the major microtubule-organizing center (MTOC) in most cells and play a role in cell division, cell-cycle progression, polarity, and ciliogenesis (for more details, see [45,46]).

***Drosophila* imaginal disc:** imaginal discs are larval tissues forming pockets of undifferentiated cells that give rise to the respective adult structures, such as antenna, eyes, wings, and legs. Imaginal discs comprise pseudostratified and opposing squamous cell layers.

Dynein/Dynactin complex: cytoplasmic Dynein is a microtubule motor protein with an ATPase activity that is required to transport molecules towards the (–) end of microtubules. Dynactin forms a large complex that functions as a Dynein activator. Together, Dynein/Dynactin play critical roles in vesicular transport and cell division.

Kinesins and Kif1A: Kinesins are a 'super-family' of proteins that function as microtubule motors. There are 14 classes of Kinesins identified so far that can act as monomers or dimers and can transport cargo towards the (–) end and/or (+) end. Kif1A is a (+) end-directed member of the Kinesin 3 family that is highly expressed in brain and to a less extent in other tissues like spinal cord, digestive system, and liver. Kif1A is found as a monomer in solution, but can also appear as a dimer, which is highly processive.

Lis1: an atypical microtubule-binding protein. It regulates Dynein motor function and mutations in this gene are implicated in lissencephaly, an inherited genetic disease that leads to a 'smooth brain' lacking the typical invagination of a gyrencephalic brain.

Microtubules: comprise α- and β-tubulin, which polymerize into protofilaments. Dimers of 13 linear protofilaments are assembled around a hollow core in a parallel head-to-tail fashion to form microtubules. Microtubules are cable-like polarized structures with a dynamic, 'growing' (+) end and a more static (–) end. The minus end is often, but not always, attached to the MTOC. Organelle and vesicle transport occurs along microtubules with the help of motor proteins. Microtubules play a crucial role in cell division and are important structural components for cell morphology.

***Nematostella* ectoderm:** the ectoderm of *Nematostella* comprises a pseudostratified epithelium from which the first neural structures arise during gastrulation. These ectodermal cells exhibit INM [9,47].

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Neuroepithelia cells: neuroepithelia are widespread in brain development, appearing in retina, hindbrain, cortex, and other brain structures. These cells are purely proliferative (self-renewing, generating more progenitors) early in development and enter neurogenesis later in development. Neuroepithelia comprise a single layer of cells that appear stratified. They are highly polarized along their apical–basal axis and include typical apical and basal plasma membrane domains.

Non-muscle Myosin II: this Myosin II is the major contractile Myosin found in non-muscle tissue. It plays a crucial role in contracting the cytokinetic furrow during cell division and maintaining tension or contraction within the cell cortex. Myosin II acts as a dimer in which both head regions bind to opposing actin filaments and thereby crosslink them. It is activated by phosphorylation of its light and heavy chains, which then pull actin filaments toward each other.

Primary cilium: a microtubule-based 'antenna-like' structure that emanates from the apical surface of pseudostratified cells. Primary cilia are responsible for receiving mechanical and chemical signals from surrounding cells. Additional functions and the significance of this structure remain under investigation. Note: *Drosophila* pseudostratified epithelia do not feature primary cilia.

Radial glia cells (RGCs): the remaining apically dividing cells in late neocortex development. They have residual neuroepithelial as well as astroglial properties. RGCs represent more fate-restricted progenitors than neuroepithelial cells [11] and successively replace them. They can directly give rise to neurons; however, most give rise to basal progenitors that then differentiate into neurons after one more round of division.

Ventricular zone: although the neocortical cell length extends to approximately 250 μm, INM occurs only in a restricted zone (the first ~100 μm from the apical/ventricular surface) called the ventricular zone. What restricts nuclear movements to this zone is unknown; however, it is possible that the presence of other cell types in the remainder of the cell length forms a physical barrier that excludes efficient INM.

formed pseudostratified epithelia serve crucial, although not well understood, functions in animal development.

Pseudostratification is marked by the dense packing of elongated cells, resulting in the nuclei assuming varied positions along the length of the cells. This arrangement differs from other simple (single-layered) columnar epithelia, which have rather uniform nuclear positioning within a plane parallel to the apical and basal surfaces (Figure 1a). Thus, the number of cells occupying a given volume of columnar epithelia is limited by the planar circumference of the nuclei. In a pseudostratified epithelium, however, the nuclei fill the entire depth of the tissue by adopting a wide range of positions along the apicobasal axis (Figure 1c). In this way (Figure 1c), pseudostratification allows a greater cell density (number of cells/given width) (Figure 1, red line) by multiple nuclei sharing the same planar circumference. This efficient packing order of pseudostratified epithelia has been suggested to promote rapid tissue expansion [2,8]; however, this idea has not been rigorously tested. Thus, the reverse is also possible: that rapid tissue expansion leads to dense packing of nuclei and cells. In either case, pseudostratified epithelia are often found in areas of substantial cell proliferation, such as intestinal epithelia and neuroepithelia [1,8–10].

Pseudostratification often occurs transiently in the course of development. For example, liver- and lung-buds lose pseudostratification and lengthen into simple columnar structures [3,8]. Similarly, neuroepithelial cells lose pseudostratification as they differentiate into neurons and migrate towards their final location [11,12]. It is not yet known which signals trigger these cells to initially adopt pseudostratified arrangements and how these signals are terminated. Elucidating these processes may hint at the function and dynamics of pseudostratification.

A characteristic phenomenon in all pseudostratified epithelia is INM (also abbreviated as IKNM in the literature). INM was first identified in 1935 through a detailed cytological study of fixed chick and pig neuroepithelia [13], describing the constant movements of the nuclei along the length of the cells during 'interkinetic' (i.e., non-mitotic)

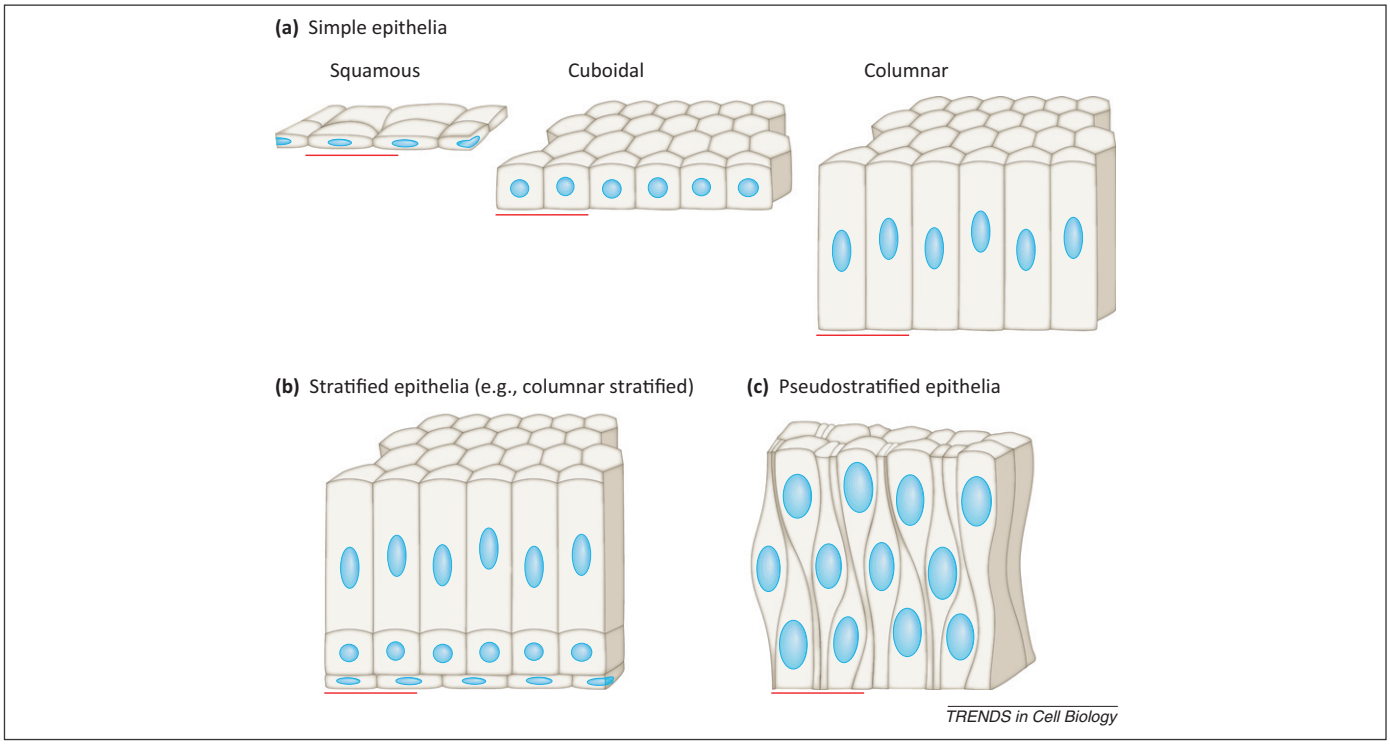

Figure 1. Epithelial categories. Epithelia are categorized by their shape (squamous, cuboidal, columnar) and the number of cell layers. **(a)** Single-layered epithelia are called simple epithelia. **(b)** Multilayered epithelia are known as stratified epithelia. Here, only one type of stratified epithelia is depicted as an example. **(c)** Pseudostratified epithelia are elongated and simple (one cell layer), but the arbitrary position of the nuclei gives them a stratified (multilayered) appearance. This also allows more nuclei to occupy a given width/area/volume, compared with simple columnar epithelia (red line).

phases. These movements always conclude with mitosis at the apical surface, facing the internal cavity of the polarized epithelium. Thus, apically directed INM (henceforth referred to as 'apical INM', as opposed to 'basal INM' in the opposite direction) is thought to be required for cell division. In line with this, arresting apical INM by depleting Dynein or its interactors Lis1 and NudC blocked cell division in rodent neocortex [5,14,15]. However, the significance of the apical division in the development of a pseudostratified tissue is not yet understood and, thus, the function and significance of INM also remains elusive. Nevertheless, the importance of INM in development is underlined by the fact that it appears in all pseudostratified epithelia examined so far, from invertebrates like *Nematostella* and *Drosophila* [9] to vertebrate tissues like the rodent gut [8,16], retina, and neocortex [12,17]. Further, abnormalities in INM have been linked to maintaining progenitors [18,19] and diseases like torsion dystonia [20]. Studies so far have focused on the molecular mechanisms that drive these nuclear movements, particularly the cytoskeletal components and associated motors. A significant amount of evidence demonstrated the microtubule dependency of INM, but recent studies have also added a role for actomyosin, opening new avenues to fully understand INM mechanics. Additionally, new findings regarding the kinetics and the timing of apical or basal INM have suggested specific links between cell-cycle phases and cytoskeletal forces.

In this review, we discuss progress in understanding the characteristics of nuclear movement within pseudostratified epithelia and its relationship with the phases of the cell cycle. Also, we summarize the latest developments in our understanding of cytoskeletal components and how they might generate forces for controlled INM. We further propose that evolutionarily divergent pseudostratified epithelia with distinct cellular dimensions might reflect genuine variations in the mechanisms that regulate INM in these tissues.

Kinetics of INM

The first description of INM kinetics from a 1935 publication by F.C. Sauer stated: '...as the nuclei grow after a division they are migrating, first away from the lumen, and later toward the lumen...They then pass through the mitosis and again recede from the lumen during the next interkinetic stage' [13]. Since then, the timing of INM and its underlying mechanisms have been investigated in various tissues.

Several studies explored the links between cell-cycle stage and nuclear position in fixed rodent neuroepithelia. These neuroepithelia comprise highly elongated (up to 250 μm) radial glia cells that can give rise either to basal progenitors or, less frequently, directly to neurons [11]. INM occurs only within an apical zone of approximately 120 μm in length called the ventricular zone (Figure 2b and Table 1). In these cells, S-phase nuclei locate at predominantly basal positions [21,22] of the ventricular zone, M-phase nuclei at the apical surface, and G1 and G2 nuclei at positions spanning the whole length of the ventricular zone. These observations supported the idea that nuclei undergo apically and basally directed translocation in concert with cell-cycle stage: basal movements during the G1 phase, S phase at the basal end of the epithelium, and apical movements during G2, followed by division at the most apical positions (Box 1 Figure I).

Recent time-lapse analysis supplemented these findings. Nuclei in zebrafish neuroepithelia, comprising cells that are less elongated (about 50 μm) (Table 1) and undergo INM throughout the length of the epithelium, migrate in a rapid apically directed manner only during G2 and thereby reach the apical surface, where they undergo mitosis. Nuclei in the G1 and S phases of the cell cycle exhibited stochastic motion in the apical and basal direction [10,23]. Similarly, time-lapse imaging of microbeads introduced in slice cultures of mouse neocortex (extremely elongated, ventricular zone INM) demonstrated largely stochastic basal movements [24]. From the stochastic nature of basal INM, both studies concluded that the source of nuclear displacement in the basal direction is likely to be due to the passive displacements induced by apically migrating neighbor nuclei. Supporting this idea, blocking apical INM using G2-phase inhibitors consistently showed significant reduction of stochastic nuclear movements [23,24]. This suggests that stochastic movements are dependent on apical G2 movements. However, to determine whether G1 or G2 movements are active or passive, it is necessary to analyze carefully the nuclear movements in real time, in the context of the densely packed tissue. Because the nuclei of pseudostratified epithelia are in close proximity (Figure 1c), the migratory tracks and divisions of each nucleus are likely to influence close and more distant neighboring nuclei and are also affected by tissue growth. This could be measured through 3D imaging of all nuclei undergoing INM within local cell domains. Such information would significantly add to current knowledge, which represents only the randomly selected individual nuclear trajectories, and would likely contribute to new hypotheses about the active or passive nature of INM and the role of nuclear position in tissue growth and differentiation.

Further, the studies outlined above indicated that cell-cycle progression is required for INM. This hypothesis was substantiated by reports (Box 1) [23–25] in both rodent and zebrafish neuroepithelia, which demonstrated that pharmacological inhibition of S- or G2/M-phase progression arrests INM. However, many questions remain in this regard. For example, at what point in the cell cycle is apical movement initiated and what initiates it? Zebrafish neuroepithelial cells that finish S phase still do not initiate migration upon G2/M arrest [23], suggesting that the trigger for apical INM resides in G2 phase. Interestingly, these results were consequences of Cdk1 inhibition [23], raising the possibility that Cdk1 may act as a trigger. Further, we do not understand how the timing mechanism for apical INM is linked to the generation of apically directed forces. To answer these questions, we must first understand the arrangements of cytoskeletal components during INM and the associated motors that provide directionality to the nuclear movements. This has been a prolific area of research, as we discuss in the next section.

Cytoskeletal components enabling INM

The microtubule and actomyosin cytoskeletons have been implicated in nuclear positioning and migration in various

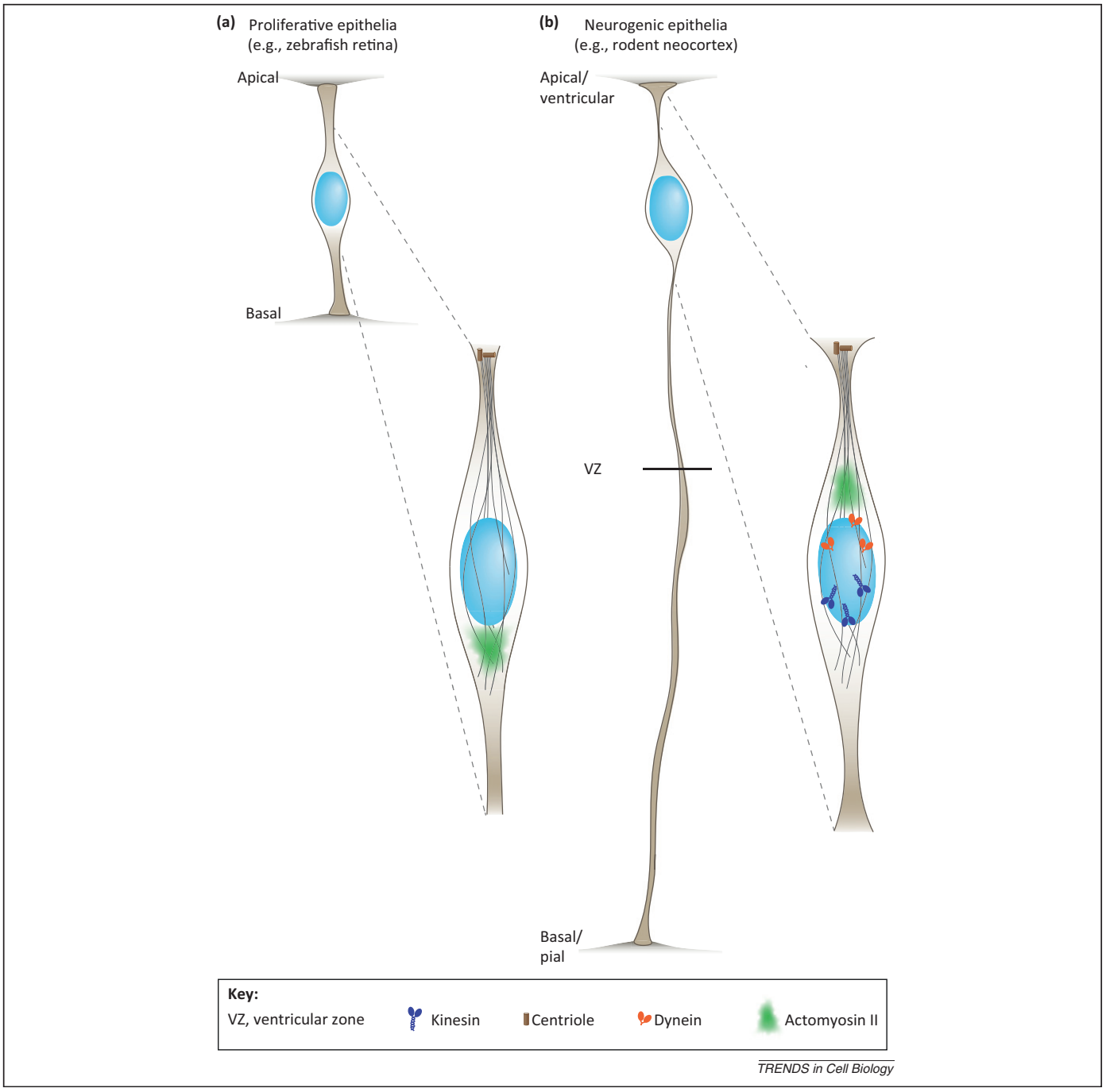

Figure 2. Molecular regulators of interkinetic nuclear migration (INM). **(a)** Representative depiction of pseudostratified epithelia undergoing self-renewing divisions, as observed in zebrafish retinal and hindbrain progenitors, mouse hindbrain progenitors, *Drosophila* wing disc cells and *Nematostella* ectoderm cells. These cells span approximately 50 μm and have thicker processes than those of the very elongated cortical cell depicted in **(b)**. These cells feature a microtubule cage like cells in the neocortex and require basal actomyosin constrictions for INM (green cloud). (b) Representative depiction of pseudostratified epithelia with more restricted potential, as observed in a rodent neocortex apical progenitor. The cell length is approximately 250 μm; the ventricular zone, within which nuclei undergo INM, spans approximately 120 μm. Microtubules emanate from the centrosome in a polarized apicobasal fashion and surround the nucleus. Nuclei migrate apically via Dynein and basally via a Kinesin, Kif1a. It has further been proposed that actomyosin plays a role in the basal movement of nuclei (green cloud).

cell types from yeast to neurons [26–29] and, due to this fact, researchers have intensely investigated whether and to what extent these structures might also act as the subcellular force generators in pseudostratified epithelia INM. Here, we provide a brief summary of these findings organized by tissue type (Figure 2) and refer readers to Box 2 and other reviews for a detailed overview of how researchers interfered with cytoskeletal components to understand their role in INM [1,12].

Observations in rodent neocortex

In the rodent neocortex, the subcellular arrangements of microtubules and how these might lead to nuclear migration have been studied extensively. Microtubules polymerize with apical to basal directionality in this system, with microtubule minus ends tethered to the apically located microtubule organizing center, the centrosome, and growing plus ends moving away from the centrosome towards the basal surface [14]. Interestingly, these microtubules

Table 1. Comparison of INM in various epithelia from different species

	Tissue	Approximate cell length (μm)	Cytoskeleton used in INM	Developmental timing observed	Refs
Pig	Neural tube	50	NA	Proliferative	[13]
Mouse	Neocortex	120[a]	MT, actin	Neurogenic	[19,24]
	Hindbrain	50[a]	NA	Proliferative/neurogenic	[37]
	Retina	70	NA	Neurogenic	[17]
	Gut	NA	NA	Proliferative	[8]
	Hepatoendoderm	NA	NA	Proliferative	[3]
Zebrafish	Hindbrain	50	Actin	Proliferative	[23]
	Retina	50	Actin	Proliferative	[10,17,23]
Fruit fly	Wing discs	50	Actin	Proliferative	[9]
Anemone	Ectoderm	50	Actin, MT	Proliferative	[9,47]

MT, microtubule; NA, not analyzed.

[a]Length of the ventricular zone, in which INM occurs.

are stabilized and surround nuclei during INM, which led to the hypothesis that nuclei travel apically and basally within a polarized microtubule 'cage' via microtubule-associated motor proteins [6]. Several studies tested this idea by disrupting centrosome structures, microtubule dynamics, or the function of microtubule-associated motor proteins, using either genetic or pharmacological methods [5–7,14,24,30] (Box 2). Together, these reports illustrated that nuclei are moved apically before mitosis via Dynein and subsequently towards basal locations by the plus end-directed motor Kif1A, in agreement with the apicobasal polarity of the microtubule cage [14] (Figure 2b inset and Box 2 for experimental details). In this context, anchorage of the nuclear envelope to microtubules seems necessary, because interfering with the SUN/KASH complex members Sun1/2 and Syne1/2, which connect the nuclear envelope to the microtubule cytoskeleton, disrupt apical INM [31,32].

Other studies have examined the role of the actomyosin contractile system in neocortical INM, producing somewhat inconsistent results. One study found that using low doses of the specific Myosin II inhibitor Blebbistatin reduced INM only in the basal direction [19]. By contrast, another study showed that neither apical nor basal INM is affected by Blebbistatin or by Myosin II RNAi treatment [14]. The reason for this discrepancy is unclear at this time.

Observations in non-mammalian neuroepithelia

Stable, polarized microtubule cages also exist in the neuroepithelial cells of the zebrafish retina [10], but interfering with these microtubule arrangements through depolymerization of microtubules, centrosome–microtubule uncoupling, or expression of a dominant-negative Dynein/Dynactin construct had little or no effect on apical INM [10]. Another report demonstrated that apical INM is slowed down, although not completely abolished, in a *dynactin-1* (mok) mutant retinal neuroepithelium [18]. Together, these data suggest that the Dynein/Dynactin machinery may not be an exclusive driving force behind apical nuclear movement in G2. However, both studies showed that, with reduced Dynein/Dynactin activity, nuclei migrate to significantly more basal positions compared with the wild type conditions. This raises the possibility that Dynein/Dynactin prevents the nucleus from traveling too far basally in G1 and S phase, potentially by stabilizing nuclear position during interphase. The possible mechanism and significance of this await further investigation.

Two studies on zebrafish neuroepithelia reported that rapid apical INM in G2 seems to depend on the actomyosin cytoskeleton [10,23]. Myosin II inhibition abolished apical nuclear migration and severely reduced the stochastic nuclear movements in G1 and S phases. Interestingly, these stochastic movements, but not the apical INM, reemerged with the expression of constitutively active Myosin Regulatory Light Chain (MRLC) in endogenous Myosin II-inhibited embryos (Myosin inhibitor BDM inhibits the ATPase domain of Myosin, thus constitutively active MRLC is not affected) [10]. This raises the possibility that the apical directionality comes from endogenous regulation of Myosin II activity. However, it is unknown where apically directed cues, if they exist, are derived from. To this end, an initial observation hints at the break of symmetry in Myosin II activity at the onset of rapid apical migration [23]. However, what might account for the actomyosin asymmetry is currently unknown. To address this, one must first understand how Myosin II activity is regulated during INM and whether these regulators localize and function in an asymmetric manner. Furthermore, to understand the role of actomyosin at a single-cell level, it will be essential to use non-drug-related approaches to disrupt actomyosin activity in a temporally and spatially controlled manner; for example, via expressing inducible dominant-negative constructs of proteins that regulate actomyosin contractility.

A recent study in the chick neural tube demonstrated that INM could be achieved in a two-step process. First, INM is initiated through microtubule-dependent apical movements and completed with actomyosin forces that drive cell rounding [33], demonstrating that, in this tissue, apical INM is a joint effort by two cytoskeletal systems.

Observations in non-neural tissues

The role of the microtubule and the actomyosin cytoskeleton in INM was also assessed in the *Drosophila* wing imaginal disc and the larval ectoderm of the sea anemone *Nematostella vectensis* [9]. In these tissues, parallel microtubules span the length of the epithelia. In *Drosophila*, disrupting these microtubule arrangements had little effect on the apical localization of prophase nuclei, suggesting that apical INM is not regulated by microtubules in this

Box 1. Interference with the cell cycle and subsequent effect on INM

In the last decade, whether cell cycle progression is a prerequisite for INM has been a prolific topic of investigation. In mouse telencephalon, two different drugs were used to investigate this: 5-azacytidine, leading to G2/M arrest, and cyclophosphamide, which causes S-phase arrest [25]. The effects of these drugs on cell cycle-dependent nuclear positioning were evaluated by BrdU labeling, which showed that nuclei accumulated at positions normally occupied by cells in G2/M or S phase, respectively. 5-Azacytidine caused nuclear accumulation at the ventricular/apical surface without entering mitosis, whereas cyclophosphamide treatment resulted in nuclear accumulation at the pial/basal surface. This analysis supported the idea that cell-cycle inhibition can induce INM arrest. Similarly, another report on mouse cortical slice culture used hydroxyurea to block nuclei in S phase, which ceased apical INM [24]. In the same tissue, genetically arresting the cell cycle in G1 by overexpressing the cyclin-dependent kinase (Cdk) inhibitor $p18^{Ink4c}$ caused basal accumulation of nuclei [24]. The effects of cell-cycle phase progression on INM kinetics were further confirmed by live-imaging experiments in zebrafish retina and hindbrain [23]. Blocking S-phase progression using hydroxyurea/aphidacolin stalled nuclear movements in both directions, whereas the Cdk1 inhibitor RO-3306 abolished rapid apical migration of nuclei [23].

The idea that nuclear position during INM is linked to cell-cycle phase was already noted in Sauer's original publication in 1935 [13]. Since then, researchers have been interested in testing this notion and visualizing the cell-cycle timing of nuclear movements (Figure I). Nuclear position and cell-cycle stage have been correlated by consecutive BrdU/EdU labeling of fixed tissues [19,48]. Recently, it also became possible to assess cell-cycle stages by live imaging. Live cell-cycle markers often include the fluorescent ubiquitination-based cell-cycle indicator (FUCCI) system, which distinguishes G1 nuclei from the rest of the cell cycle [49], or the fluorescently tagged proliferating cell nuclear antigen (PCNA) DNA clamp, which reports cell-cycle phase-specific localization patterns [23].

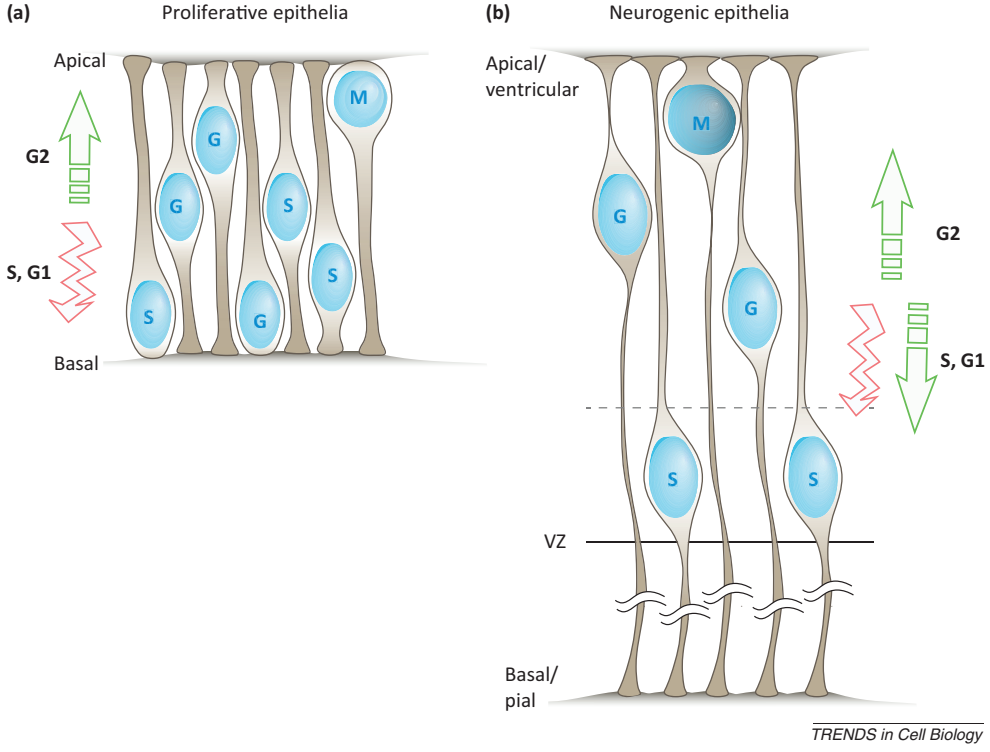

Figure I. Modes of interkinetic nuclear migration (INM) kinetics. Left panel: In shorter pseudostratified epithelia, nuclei of G1, S, and G2 phase are found along the whole length of pseudostratified cells. Directed movement toward the apical surface occurs in G2. G1 and S phase are characterized by stochastic movements. Right panel: In the neocortex, cell-cycle phase correlates with nuclear positioning. Nuclei are found in basal-most positions during S phase, in apical-most positions during M phase, and in middle regions during G1 and G2 phase. Apical INM in these cases is directed, whereas basal INM has been shown to be directed and/or stochastic.

tissue. Of note, microtubule stabilization by Taxol treatment had a stronger effect on nuclear positioning than did destabilization by colchicine (Box 2), arguing that microtubule disassembly might be an important prerequisite for apical nuclear migration. However, disruption of actin dynamics prevented apical mitosis. Further, inhibition of Rho kinase, a Myosin II activator, led to severe disruption of apically directed prophase movements [9], suggesting that Rho kinase is an upstream activator of actomyosin contractility during INM. However, how Rho kinase achieves asymmetric actomyosin activity and how it interacts with cell-cycle regulators is unknown. In *Nematostella*, disrupting either microtubules or actomyosin blocked INM, suggesting that diverse mechanisms may underlie this process in different types of pseudostratified epithelia [9]. We discuss this idea in more detail in the next sections.

Remaining questions about force generation during INM

Although we have made significant strides towards identifying the cytoskeletal elements involved in INM, it is still not well understood how force is transduced from the cytoskeleton to move the bulky nucleus through a viscous cytoplasm in a tightly packed epithelium. For instance, studies of neocortex INM demonstrated that basal-to-apical movement is significantly faster than apical-to-basal movement [6,14,24]. This raises intriguing questions. What accounts for the difference in the rate of nuclear movements? Do nuclei assume apically directed movements with higher

Box 2. Summary of drug and genetic approaches to interfere with different cytoskeletal systems

Since early studies of INM, researchers have aimed to elucidate which cytoskeletal structures are responsible for the nuclear movements and which motor proteins help to transduce the force from cytoskeleton to nuclei (Figure 2). To do this, perturbation of cytoskeletal systems was achieved either by drug treatment or by genetic protein knockdown and mutant approaches. Here we summarize the results of these studies.

Drug approaches

Microtubule destabilization

In an early study of chick neuroepithelium, still images obtained after treatment with dithiodiglycole (inhibits microtubule polymerization)/cold or monoiodoacetamide (prevents microtubule repolymerization)/formamide (disturbs microtubule structure) showed stalled INM [50]. By contrast, a study using live-imaging of the zebrafish retina found that treatment with colcemide does not affect rapid apical INM [10]. Similarly, treating *Drosophila* imaginal discs with another microtubule-destabilizing drug, colchicine, did not affect nuclear positioning, whereas the same drug caused mitotic figures to appear at slightly more basal positions in *Nematostella* ectoderm [9].

Microtubule stabilization

So far, the effect of microtubule stabilization on INM has been studied only in *Drosophila* imaginal discs. Paclitaxel addition caused slightly more basally located mitotic nuclei than colchicine treatment, and mitotic arrest. Thus, it has been speculated that the disassembly of interphase microtubules might be important for apical INM (see main text) [9].

Actin depolymerization

In chick neuroepithelium, the application of cytochalasin D caused INM arrest and induced ectopic mitotic figures to appear at basal positions [22]. Similarly, this drug prevented apical translocation of nuclei in *Nematostella* ectoderm. Another actin-depolymerizing drug, latrunculin A, caused basal accumulation of nuclei in *Drosophila* imaginal discs [9].

Inhibition of Myosin II

Studies in cortical mouse slices showed that Blebbistatin treatment did not affect apical or basal nuclear movement [14], whereas another study reported using a lower dose of the drug in mouse hemisphere rotation culture and finding a specific inhibitory effect on the basal movement of nuclei [19]. In zebrafish retina, both Blebbistatin and BDM severely inhibited apical and basal INM [10].

Inhibition of Rho kinase, an upstream regulator of Myosin II

In *Drosophila* imaginal discs, treatment with a Rho-associated kinase inhibitor, Y-27632, led to basal accumulation of nuclei [9].

Considerations when using drug approaches

The advantage of drug approaches is that they are readily available and their effects can be controlled temporally and, to a varying extent, at different doses. By contrast, inducible knockdown constructs are not as easily accessible and subtle differences in protein function are difficult to tease out in a dose-dependent manner using knockdown techniques. Nonetheless, drug approaches have important limitations to consider. Their effects *in situ* are global and cannot be turned on and off at a single-cell level. Therefore, when interfering with essential components not specific to INM, such as microtubules, actin, and their associated motor proteins, drug experiments have to be very well controlled. In addition, high doses of cytoskeleton-destabilizing drugs can lead to epithelial collapse, in which the apical, basal, or both processes retract. In such cases, ectopic nuclear localization or division is difficult to attribute to aberrant INM, because it might instead be caused by the disturbed morphology of the epithelium. Thus, careful calibration of drug concentration that allows cytoskeletal inhibition without affecting epithelial integrity is necessary. Likewise, epithelial integrity is an essential factor to monitor in genetic studies of INM. In summary, results acquired using drug treatments should further be validated by genetic approaches as outlined below, using either dominant-negative constructs or inducible knockdown techniques.

Genetic approaches

Microtubule-associated proteins

In cortical mouse and rat slices, Lis1 and NudC knockdown treatments abolished apical INM and Dynein RNAi resulted in a dose-dependent reduction of apical INM [5,14,15]. Kif1A RNAi resulted in reduced rates of basal movement and nuclei stayed in close proximity to the apical surface for prolonged periods [14]. In zebrafish retinal neuroepithelium, the mok mutant (a mutation in p150glued/dynactin) showed more pronounced basal translocation of nuclei and a reduction of apical migration [18]. The expression of a dominant-negative form of p150glued/dynactin also led to more pronounced basal translocation of nuclei, but showed no effect on rapid apical migration [10].

Actin-associated proteins

In cortical rat slices, Myosin II RNAi treatment had no effect on apical or basal nuclear migration [14].

speed, more persistent forward movements, or both? Could the difference in the rate of apical versus basal INM represent mechanistic details of how the respective motors function in this system or of the types of forces and resistances on each nucleus in either direction? Similarly, it is unclear how nuclei-moving forces are generated by actomyosin during INM. Myosin II is known to be able to constrict actin networks at the cell cortex underlying the plasma membrane or to provide pinching forces through a contractile ring [34,35]. Thus, it seems possible that nuclei are indirectly moved through spatiotemporally controlled cortical or actomyosin-ring contractions that generate cytoplasmic flow. However, this awaits further investigation.

Carefully monitoring nuclear shape changes during INM may also provide insights into the types of force that move the nuclei. A recent study in *Drosophila* oocytes demonstrated that nuclear shape changes can reflect mechanisms by which nuclei are moved [36]. Interestingly, Sauer had already noted in 1935 that nuclei assume 'pointed droplet' morphology when they migrate toward the apical lumen, instead of the ovoid shape they take in other phases of movement [13]. The pointed end aims towards the apical direction, which might suggest a combination of pulling and pushing forces that act on nuclei; for instance, apical pulling might lead to the pointed appearance, whereas basal pushing might flatten the basal side of the nucleus. Further analysis of nuclear shape changes during INM, accompanied by analysis of the stiffness or compliance of nuclei and the viscosity of the cytoplasm, might add to our understanding of the balance between forces applied to the nuclei.

Finally, there are many pseudostratified epithelia whose INM mechanics have not yet been examined, like the mammalian lung, liver, and gut epithelia, as well as self-renewing rodent hindbrain neuroepithelia. A more comparative understanding of INM over many different tissues and species will expand our understanding of INM as a general phenomenon and may hint at interesting hypotheses about the functional significance of different cytoskeletal mechanisms across diverse organisms and tissues. In this regard, we suggest models that may reconcile existing evidence in the next section.

Hypotheses to reconcile different mechanisms leading to INM in diverse systems

Current evidence indicates that microtubule- versus actomyosin-dependent modes of INM vary depending on model organism or tissue, suggesting that the mechanics of INM are likely to be not identical in all pseudostratified epithelia. Instead, specific tissue architectures and different developmental stages may require divergent mechanisms to generate suitable types of force to move nuclei. For instance, the epithelia that predominantly rely on actomyosin forces have shorter apical-to-basal cell lengths and thicker shapes and belong to more primitive tissues compared with the relatively newly emerged mammalian neocortex, in which microtubules seem to be mainly responsible for nuclear movements (Table 1).

In zebrafish neuroepithelia, *Drosophila* imaginal disc, and *Nematostella* ectoderm, INM depends on actomyosin activity and the nuclear movements span almost the entire length of the cell, typically around 50 μm. These cells also contain significant amounts of cytoplasm within their relatively thick apical and basal processes (Figure 2a). The same seems to be true for cells within the developing mouse neural tube [37]. On the other hand, the neuroepithelial cells of the evolutionarily younger mammalian neocortex assume a particularly elongated shape with extremely thin apical and basal processes that appear almost devoid of cytoplasm (Figure 2b). These cells show INM only along a portion of the cell length within the ventricular zone (Figure 2b), which spans more than 100 μm in length in radial glial cells. Interestingly, the nuclei in these cells require microtubules and their associated motor activity for INM. Therefore, it seems possible that such morphological differences contribute to how cytoskeletal forces can function. One could imagine that, in shorter and wider cells, constrictions by the actomyosin cytoskeleton may be able to efficiently move nuclei apically, whereas in the thin and more elongated cells of the rodent cortex, actomyosin-dependent forces might not be able to support persistent movements over long distances and thus nuclear movement requires microtubules and their associated motor proteins.

The fact that actomyosin-dependent INM is observed in phylogenetically diverse systems, whereas microtubule-based motors appear to be crucial INM force generators in the more evolved mammalian neocortex, suggests that actomyosin-dependent INM may represent an evolutionarily more ancient mode of pseudostratified epithelial nuclear movement (Table 1). In addition to the differences mentioned above, the mammalian neocortical epithelium is also unique in having a strict spatial separation between the space where S-phase nuclei reside, in the basal area of the ventricular zone (Box 1), and where nuclei in all other phases reside, in more apical areas. In other epithelia, like the *Drosophila* imaginal disc [9], zebrafish retina and hindbrain [23], mouse retina [17], and mouse hindbrain [37], S-phase nuclei are observed along the whole length of the apical–basal axis. Interestingly, it has been suggested that, in the zebrafish retina, the basal position of S-phase nuclei correlates with the onset of neurogenesis [17]. Taken together, it seems possible that, during the evolution of the brain, new specialized features, such as further cell elongation, basal localization of S-phase nuclei, and microtubule-dependent movements, arose that affect INM mechanisms and dynamics. To test these ideas, it will be important to analyze INM in other mammalian tissues with less elongated cells and non-neural epithelia that are more ancient (Table 1).

The developmental stage and state of progenitor cell commitment may also be crucial to the type of INM. For example, studies in zebrafish, *Drosophila*, and *Nematostella* mainly concentrated on early progenitor cells engaged in symmetric, self-renewing divisions, whereas studies in rodents have mainly focused on progenitors that are undergoing neurogenic divisions. It will therefore be interesting to test whether progenitors of early cortical neuroepithelium, which are shorter in length and undergoing self-renewing divisions, might depend on actomyosin.

Possible functions and significance of INM

The function and significance of INM and apical division during pseudostratified tissue development remains unclear. Apical mitosis may serve as a means to organize all dividing cells spatially, to ensure orderly and efficient proliferation. For example, mitotic spindles occupy more than twice the space of interphase nuclei [13,23] and may require a designated space to maintain order in growing tissues. Thus, it seems possible that apical INM is a way to utilize this space for expansive cell division. Related to this, it remains unclear whether apical migration functions as a licensing step for cell division; in other words, whether cells must reach the apical surface to enter mitosis. Additionally, spindle orientation and subsequent division-plane orientation have been proposed to play a role in keeping the precise balance between proliferative (maintaining progenitor pool) versus neurogenic (giving rise to cells with restricted/differentiated potential) divisions of neural progenitors. Supporting this, uneven distribution of apical membrane has been shown to promote neurogenesis and increasing apical divisions expand the progenitor pool [30,38,39]. Techniques to induce subapical cell division will enable us to understand how the division plane is affected in this condition and whether this indeed leads to premature reduction of the progenitor pool. Another possibility is that apical nuclear migration is a consequence of the predetermined apical position of the centrosome [10,14,40]. In neuroepithelial cells, the centrosome is thought to localize at the apical surface to support its function as a basal body for the primary cilium during the interphases [41]. Because centrosomal position is set, nuclei may be required to migrate apically before mitosis to access centrosomes that organize spindles during chromosome segregation. Interestingly, pseudostratified epithelia that do not have primary cilia, like the *Drosophila* imaginal disc, still feature apical centrosomes and similar INM kinetics [9], suggesting there may be additional factors that account for the apical localization of the centrosome. To this end, studies have investigated whether detaching centrosomes from the apical surface affects INM. Conditional deletion of Cdc42 in the rodent neocortex results in centrosome mispositioning and an increase in basal mitosis. However, because this condition also causes apical process retraction, it is unclear whether ectopic basal cell division is specifically due to centrosome mispositioning within a polarized cell or a

consequence of apical process retraction [42]. Furthermore, the exact composition of factors that hold centrosomes apically is unknown and we do not understand whether apical centrosomal positioning is required for INM in an intact, polarized pseudostratified epithelia. Identifying anchors that tether the centrosome apically may allow specific mispositioning of centrosomes to address whether apical INM and cell division still occur. It would also be interesting to elucidate whether the centrosome itself is vital to INM. Therefore, testing how ablating or increasing centriole number physically or genetically affects INM would provide an important insight into this problem.

Identifying the regulators of initiation and termination of pseudostratification will reveal the function and significance of this tissue architecture. For example, it is unknown what molecular mechanisms are needed for pseudostratification and subsequent INM, and when these mechanisms are established. Although polarity proteins and Notch signaling have been shown to play a role in the formation of pseudostratified epithelia [18,22,43], the detailed mechanisms remain unclear, and it is unknown how these signals could be related to the asymmetry that provides directionality to an actively migrating nucleus. Likewise, identifying the terminating signal to pseudostratification and analyzing the consequence of maintaining pseudostratification in tissue development will be important aspects to understand the contribution of INM.

Concluding remarks

Many molecular and mechanistic details of INM are still not fully understood, partly due to the fact that many regulators of INM are also required for other processes like cell division or cell-polarity maintenance. However, we hope that this review points out interesting new avenues to obtaining further insights into the mechanisms and function of this fascinating and conserved feature of pseudostratified epithelia. We would like to conclude by pointing out that INM also shares important features with processes that are typical of all cell divisions. For example, cells in culture and in tissue constrict cortical actin to round upon entry into mitosis [44] and INM has been proposed to be a manifestation of cell rounding in the context of polarized epithelial tissues [9,33]. In other words, although pseudostratified epithelial cells maintain contacts with apical and basal surfaces, mechanisms similar to cell rounding may regulate the cortical tension and cell shape changes during INM. Thus, aside from understanding pseudostratified epithelial biology, INM might also provide an excellent platform to understand other important cell biological processes that use similar mechanisms to achieve slightly different outcomes.

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