Chapter 9
The Spindle Orientation Machinery Beyond Mitosis: When Cell Specialization Demands Polarization

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Abstract Mitosis is a process requiring strict spatial organization of cellular components. In particular, the orientation of the mitotic spindle with respect to the tissue defines the division plane. In turn, the orientation of cell division can regulate tissue morphology or the fate of daughter cells. While we have learned much about the mechanisms of mitotic spindle orientation, recent studies suggest that the proteins implicated can also play important roles in post-mitotic cells. Interestingly, post-mitotic protein function often involves polarizing the cell cytoskeleton during differentiation, mirroring its ability to orient the mitotic spindle during division. This review focuses on alternative functions of the spindle orientation machinery after division, when the cell undergoes a specialization process associated with differentiation or mature function, and discusses diseases associated to those alternative functions.

Keywords Cell polarity • Oriented cell division • Mitotic spindle • Cytoskeleton polarization • Post-mitotic cell morphogenesis • Inscuteable • LGN (leu-gly-asn) / Gpsm2 (G-protein signalling modulator 2) • Guanine nucleotide binding protein (G protein) • Alpha inhibiting

The control of cell proliferation, cell fate and cell organization in a tissue are major biological requirements at every stage of life. In the last 20 years, the regulation of cell division orientation has emerged as a prominent level of control in this context. On the one hand, the plane along which cells divide impacts tissue structure. The positioning of the two daughter cells is largely determined by the cleavage plane during cytokinesis, itself instructed by the orientation of the microtubule-based mitotic spindle. In an epithelium, for example, divisions along the apico-basal axis increase tissue thickness, while orthogonal divisions increase epithelial surface. Failure to properly regulate this process results in altered epithelial morphogenesis [1, 2],

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and was proposed to contribute to diseases such as polycystic kidney disease, microcephaly or lissencephaly [1–3]. On the other hand, the orientation of cell division can directly determine cell fate, at least in invertebrates. Since cellular components are not necessarily evenly distributed throughout the mitotic cell, their inheritance can be variably biased depending on the orientation of the mitotic spindle, influencing daughter cells' behavior. For example, stem cells can self-renew while generating a daughter cell with more restricted fate, and this asymmetric outcome involves asymmetric inheritance of RNA or protein fate determinants, membrane domains or organelles. In many cases, cell fate and tissue architecture are hard to separate however, as when failure to maintain planar division gives rise to delaminated cells that have lost their epithelial characteristics and become mesenchymal-like, which can promote proliferation and possibly tumor development [4, 5]. Although the importance of spindle orientation in strictly driving binary cell fate decision in vertebrates is still debated, it is clear that spindle misorientation can alter the total proliferative potential and cell type composition of a tissue.

The importance of proper control over the division plane has motivated a large-scale effort to identify and functionally characterize the molecular constituents of the machinery that orients the mitotic spindle. This prolific field of research has made tremendous strides in the recent past, and has been extensively reviewed already [1, 2, 6–11]. By contrast, our goal here is to spotlight the emergent, less well-known examples where key proteins regulating spindle orientation were found to take on different roles in post-mitotic cells. Analogous to their role during cell division, they largely appear to influence cytoskeleton polarization, and participate in specialized subcellular processes associated to cell differentiation or mature cell function. While such examples are still relatively scarce, their growing significance is underscored by relevance to disease like hearing loss and drug-seeking behavior.

9.1 The Core Machinery Behind Oriented Divisions

To provide context and draw parallels with their post-mitotic functions discussed further below, we will begin with a brief overview of the central players regulating mitotic spindle orientation. Generally speaking, these proteins become enriched at specific regions of the cell cortex in prometaphase dividing cells, guided by canonical markers of cell polarity. These regulators then locally recruit partner proteins that capture and pull on astral microtubules, the microtubules that emanate from each centrosome but do not participate in chromosome segregation. In essence, proteins of the core machinery are cortical landmarks used as reference to ensure that the mitotic spindle becomes aligned with the polarity of the cell, and that the resulting daughter cells are situated correctly within the tissue. The orientation machinery is strikingly conserved across tissues and organisms, and has been studied in a wide variety of model systems, including the first divisions of the *C. elegans* zygote [12–14], neuroblast lineages in the fly (see below), the murine embryonic epidermis [15–18], and neuroepithelial cells in the vertebrate central nervous system [19–22],

to name only a few. Of note, however, there is much variation in the way the core spindle orientation proteins operate among different systems, a topic outside the scope of this chapter.

The initial discovery and much of the pioneering work addressing mitotic spindle orientation has been done in *C. elegans* and *Drosophila* [23]. In *Drosophila* embryonic and larval neuroblasts, which have become a choice model of self-renewing asymmetric stem cell division, the Par complex localized apically in the neuroectoderm is carried over when the neuroblast delaminates basally [24, 25]. This complex composed of Par-3, Par-6, and the atypical kinase aPKC is known as a master regulator of apico-basal polarity [26]. Par3 recruits the adapter protein Inscuteable (Insc; mInsc in mammals) to the apical cell cortex [27–29], and mInsc in turn binds to the TPR repeats of Partner of Inscuteable (Pins; LGN, mPins or Gpsm2 in mammals) [30–32] (Fig. 9.1a). Pins/LGN is further stabilized at the cortex through interaction of its GoLoco domains with GDP-bound Gα_i (Gα_i-$_{GDP}$) anchored at the membrane via myristoylation [33]. As a result, Insc-Pins/LGN-Gα_i colocalize in a crescent at the cell cortex during prophase and metaphase. This core spindle orientation complex then recruits the large coiled-coil protein Mud (NuMA in vertebrates) [34–38]. The transition is proposed to occur through a switch mechanism whereby Mud/NuMA replaces Insc, as both proteins compete for the TPR motifs in Pins/LGN and cannot bind simultaneously [39–41]. Mud/NuMA provides a link to the astral microtubules since it directly binds the Dynein-Dynactin motor complex [42]. Overall, the spindle becomes anchored to the cell cortex in a polarized manner, and pulling forces align the mitotic spindle to ensure apico-basal divisions where the apical daughter retains neuroblast identity and the basal daughter inherits basally located fate determinants, adopting a more restricted fate.

Biochemically, LGN and other GoLoco-containing proteins act as G protein dissociation inhibitors (GDI), effectively competing with Gβγ and preventing guanine nucleotide exchange by stabilizing G$\alpha_{i\text{-}GDP}$ [43–45]. In principle, this activity is known to uncouple trimeric G proteins from GPCRs at the membrane and reduce signaling, while potentially also prolonging stimulation of Gβγ-dependent effectors. Interestingly however, there is only limited evidence that Gα_i proteins relay GPCR signaling during spindle orientation [46]. While cell-autonomous guanine exchange factors (GEFs) have been implicated [47–51], it is generally accepted that LGN-G$\alpha_{i\text{-}GDP}$ is the active signaling complex acting on the spindle.

9.2 Roles of the Core mInsc-LGN-Gα_i Complex Beyond Spindle Recruitment

A number of studies recently proposed that mInsc-LGN-Gα_i proteins locally regulate cytoskeleton rearrangement in specialized cells, a fundamental role falling in line with their better-known ability to recruit the mitotic spindle during division.

We discuss below interesting novel findings where this protein complex is involved in such diverse post-mitotic processes as neuronal synaptic function,

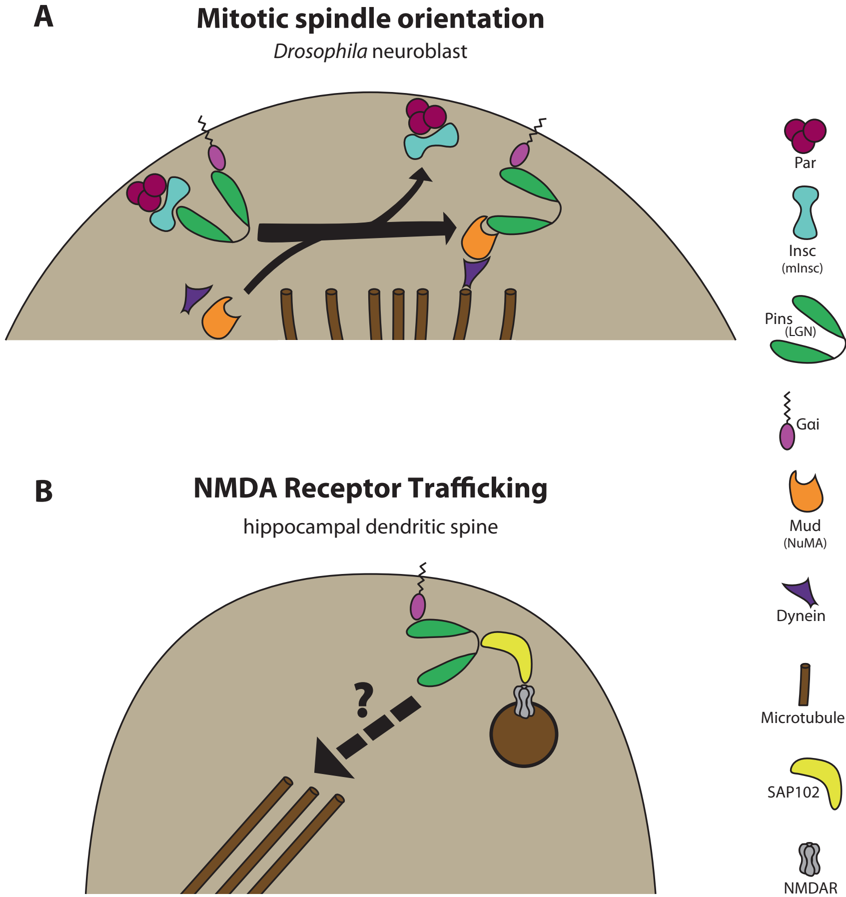

Fig. 9.1 Comparison of LGN's roles in mitotic spindle orientation and NMDA receptor trafficking. (**a**) In the *Drosophila* neuroblast, LGN is recruited to the membrane by the Par complex, $G\alpha_i$, and Insc. NuMA then displaces Insc from LGN, and NuMA's association with dynein recruits astral microtubules to the cortex. (**b**) In hippocampal dendritic spines, SAP102 binds LGN and NMDA receptors. By analogy with (**a**), LGN could provide a link to microtubules in order to help locally deliver NMDAR vesicles to the cell surface. See text for additional details

chemotaxis, and the generation of intrinsic cytoskeleton asymmetry in developing hair cells, a cellular patterning event crucial for sensory perception in the inner ear. Both the mitotic and post-mitotic actions of this complex are schematized in Fig. 9.2.

9.2.1 Modulating Neuronal Function

Components of the spindle orientation machinery have been shown to regulate the function of neuronal synapses. The NMDA receptor (NMDAR) is a glutamate receptor that is critical for proper neural development, learning and memory, affect,

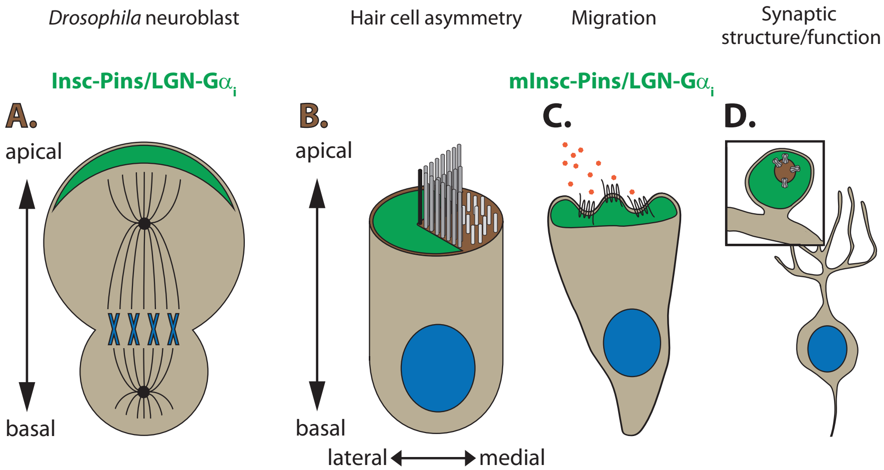

Fig. 9.2 The roles and localization of the Insc-Pins/LGN-Gα_i complex in polarized cell activities in dividing and post-mitotic cells. (**a**) In *Drosophila* neuroblasts, Insc-Pins-Gα_i (*green*) colocalize at the apical cell cortex and help orient the mitotic spindle along the apico-basal axis. (**b**–**d**) Functions of mInsc-LGN-Gα_i in post-mitotic cells. (**b**) mInsc-LGN-Gα_i localize to the "bare zone", a lateral subset of the apical membrane devoid of microvilli in inner ear hair cells. mInsc-LGN-Gα_i were proposed to help define the lateral edge of the stereocilia bundle. Stereocilia and microvilli are depicted in *dark and light grey*, respectively, and the primary cilium, or kinocilium, is shown in *black*. (**c**) mInsc-LGN-Gα_i are found at the leading edge of chemotaxing neutrophils, where they signal downstream of GPCRs to stabilize actin-based pseudopods. (**d**) Within the dendritic spines of neurons, Insc-Pins/LGN-Gα_i interact with NMDA receptors, potentially influencing their delivery to the plasma membrane and influencing synaptic function

and cognition [52]. In experiments designed to elucidate the regulation of glutamate receptor trafficking, LGN was found to bind SAP102 (Dlg3) [53], a member of the MAGUK protein family important for scaffolding proteins at neuronal synapses [54]. Overexpression of LGN in cultured hippocampal neurons leads to changes in both number and morphology of dendritic spines [53]. LGN and SAP102 also bind NMDA receptor subunits and G$\alpha_{i\text{-GDP}}$, forming an NMDAR-SAP102-LGN-Gα_i complex, which was proposed to be important for proper NMDAR trafficking [53]. Similar to its role in recruiting astral microtubules to the cell cortex during mitosis, in this model LGN could regulate receptor trafficking by acting as a bridge between microtubules and receptor-containing vesicles [55] (Fig. 9.1b). Collectively, these results suggest that the LGN-Gα_i complex acts in multiple ways to influence synaptic signaling, as both spine morphology and NMDAR dynamics are mediators of synaptic plasticity [56, 57]. In hippocampal neurons, LGN also modulates current through the G protein-activated inwardly rectifying potassium channel (GIRK) [58]. In this study, the authors suggest that, under basal conditions, LGN enhances GIRK current by binding and stabilizing G$\alpha_{i\text{-GDP}}$, enhancing the activity of G$\beta\gamma$, which then activates GIRK. Following GPCR stimulation, however, LGN actually

reduces GIRK current, likely by uncoupling $G\alpha_i$ from the GPCR [58]. By acting as a GDI, LGN was thus shown to modulate GPCR signaling and regulate neuronal excitability.

Interestingly, the vertebrate Pins homolog and LGN paralog protein AGS3 has been more tightly associated to non-mitotic functions than to spindle orientation [59]. Changes in the expression of *Ags3* could contribute to the alterations in G-protein signaling efficacy caused by chronic cocaine exposure and, intriguingly, *Ags3* antisense nucleotides infused into the prefrontal cortex block the reinstatement of drug-seeking behavior following cocaine withdrawal [60]. Similarly, *Ags3* antisense oligonucleotides administered into the core of the nucleus accumbens prevented reinstatement of heroin-seeking behavior [61]. AGS3 can also increase protein surface expression, exemplified by the Kir2.1 potassium channel [62]. It probably does this by regulating protein transit between the trans-Golgi network and plasma membrane [62]. As Kir2.1 can strongly affect resting membrane potential [63], this finding suggests that AGS3, like LGN, could regulate synaptic plasticity. It remains uncertain whether AGS3 helps to deliver cargoes to the cell membrane by coupling to the cytoskeleton, as suggested above for LGN and the NMDAR.

9.2.2 Regulating Cellular Movement

Interestingly, mInsc can drive polarized responses in post-mitotic cells downstream of G-protein coupled receptor (GPCR) signaling. Neutrophils must chemotax toward the source of chemoattractants in order to help mediate immune responses. This directed motility is achieved by polymerization of filamentous actin at the leading edge of the cell and contraction at the opposite end of the cell mediated by myosin II [64]. Neutrophils express GPCRs that are locally activated by chemoattractants and, via coupling specifically to the Gαi family of heterotrimeric G proteins [65] at the leading edge, activation results in the generation of $G\alpha_{i\text{-}GTP}$ and free Gβγ, which play separate but complementary roles in directed migration. Much work has focused on the role of free Gβγ, which promotes motility via activation of molecules including PI3K [66]. Recently, it has also been suggested that Gβγ-free $G\alpha_{i\text{-}GDP}$ produced by hydrolysis of $G\alpha_{i\text{-}GTP}$ plays an important role in maintaining appropriate directionality during chemotaxis [67]. Strikingly, this pathway uses many of the proteins involved in orienting the mitotic spindle: $G\alpha_{i\text{-}GDP}$ probably generated downstream of GPCR activation by chemoattractants recruits LGN/AGS3, which recruits mInsc and subsequently the Par complex to the leading edge [67] (Fig. 9.2c). Depletion of mInsc affects only directionality during chemotaxis, and not overall motility [67], suggesting that mInsc does not affect Gβγ function. It remains unclear, however, how $G\alpha_{i\text{-}GDP}$-LGN/AGS3-mInsc-Par stabilize the directionality of migrating neutrophils.

LGN can also control changes in cellular shape. Recent work suggests that LGN regulates sprouting angiogenesis, perhaps via destabilization of cell-cell and cell-matrix adhesions downstream of altered microtubule dynamics in endothelial cells [68].

9.2.3 Regulating Hair Cell Morphogenesis in the Inner Ear

We and others have discovered a surprising new role for the mInsc-LGN-Gα_i complex during early hair cell differentiation in the inner ear [69–71]. Here, these proteins are involved in organizing the apical membrane of hair cells, the highly specialized cells ensuring the detection of sounds, acceleration, and gravity.

Hair cells are crowned with a bundle of apical protrusions, termed stereocilia, that respond to mechanical deflection by modulating electric currents in the cell. Stereocilia derive from microvilli that initially cover the apical membrane and, under largely unknown influence, grow in girth and length. The stereocilia bundle is characterized by a strong radial asymmetry along the epithelial plane in each cell. Asymmetry is manifested notably by the V-or arched shape of the bundle, and the staircase-like organization of stereocilia, which align into rows harboring graded heights (Fig. 9.3a). Cytoskeleton polarization is also manifested at the tissue level. In the cochlea, hair cells are organized in four rows (Fig. 9.3b), and all cells adopt a strikingly uniform planar orientation of their bundle. This occurs by the planar cell polarity (PCP) pathway, which is generally responsible for the coordinated orientation of cells along the epithelial plane [72]. Cell-intrinsic and tissue level polarization are essential for sensory function, and notably account for direction-sensitivity to stimuli: hair cells only respond to bundle deflections toward or away from the tallest stereocilia row, while orthogonal deflections have no effect [73].

As in dividing progenitors, mInsc-LGN-Gα_i colocalize as a protein complex in early post-mitotic hair cells [69–71] (Fig. 9.3a–c). This complex is asymmetrically enriched in the plane, forming a lateral crescent at the apical membrane. mInsc-LGN-Gα_i both label and are required to generate a patch of membrane devoid of microvilli, which we termed the "bare zone" [70]. As the hair cell develops, this region expands and closely abuts the lateral edge of the forming bundle, which hosts the tallest stereocilia. Disrupting the protein complex reduces or eliminates the bare zone, leading to severe stereocilia placement defects. It thus appears that mInsc-LGN-Gα_i act by defining an exclusion zone for microvilli as a strategy to define the contour of the forming bundle.

The influence of mInsc-LGN-Gα_i is not limited to regulating the placement of actin-based stereocilia. Early during differentiation, the hair cells' one true cilium, the kinocilium, moves from the cell center to the periphery (Fig. 9.3c). Although the underlying mechanism remains obscure, the eccentric shift is required for bundle morphogenesis [74], and its normal lateral direction depends on tissue-level planar cell polarity (PCP) [75]. Since mInsc-LGN-Gα_i recruit astral microtubules during mitosis, it is tempting to speculate that these proteins could pull on microtubules connected to the basal body nucleating the kinocilium to trigger the shift. Accordingly, one study proposed that the shift depends on Gα_i signaling based on results in organotypic culture [69], although off-center kinocilium shifts were still observed when Gα_i inactivation was achieved in vivo [70]. Later during hair cell differentiation, LGN and Gα_i also play an important role to ensure the precise localization of the kinocilium in the center of the arched stereocilia bundle

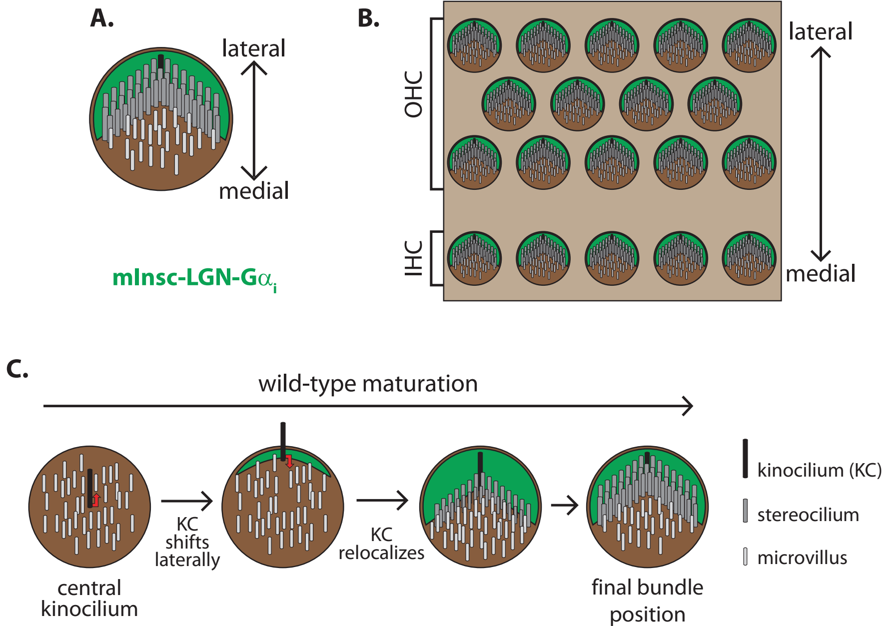

Fig. 9.3 Hair cell organization in the mammalian cochlea. (**a**) At the single cell level, each hair cell is highly asymmetric along the planar axis. mInsc-LGN-Gα_i (*green*) localize to a lateral crescent at the apical surface and mark a region devoid of microvilli (the "bare zone"). The mechanosensitive stereocilia bundle grows in a chevron pattern in the central region of the apical membrane. Stereocilia and microvilli are depicted in *dark* and *light grey*, respectively, and the primary cilium, or kinocilium, is shown in *black*. (**b**) At the tissue level, cochlear hair cells are organized in four rows (OHC: outer hair cells; IHC: inner hair cells). Hair cells are uniformly oriented, with the chevron shape of the bundle, the tallest stereocilia row and the mInsc-LGN- Gα_i crescent facing the lateral edge. (**c**) During early hair cell differentiation, the kinocilium (KC) or primary cilium is first observed at the center of the cell, amid a full covering of microvilli. The kinocilium then shifts laterally as mInsc-LGN-Gα_i become detectable at the lateral edge. mInsc-LGN-Gα_i expand medially, creating the microvilli-free bare zone, and the kinocilium relocalizes more centrally. At the same time, select microvilli grow into stereocilia that become precisely aligned and adopt graded heights to form the mature bundle

(Fig. 9.3c). Together, these results suggest that the mInsc-LGN-Gα_i complex is required to spatially coordinate apical membrane domains with both the microtubule- and actin-based cytoskeleton.

As mInsc-LGN-Gα_i work at the single cell level, their activity must somehow become coordinated with the PCP pathway to ensure that all hair cells orient their asymmetric bundle in the same planar direction. Interestingly, inactivating Gα_i signaling results not only in bundle defects in single hair cells, but also in hair cell misorientation [70]. This suggests the intriguing possibility that Gα_i signaling could link cell-intrinsic morphogenesis with PCP signaling initiated at apical junctions by cell–cell interactions.

The emerging molecular function of mInsc-LGN-Gα_i in hair cells is of particular interest since LGN mutations were recently shown to underlie congenital hereditary hearing loss in multiple human families [71, 76–80]. Loss-of-function mutations in *LGN* (*GPSM2*) were originally identified in patients classified as having nonsyndromic hearing loss [79, 80]. Mutations in *LGN* were subsequently identified in patients with Chudley-McCullough syndrome [76–78], a condition first described in 1997 [81] where profound congenital hearing loss coincides with partial agenesis of the corpus callosum, grey matter heterotopia, and often hydrocephaly [82]. Interestingly, the authors then expanded their analyses to the first reported *LGN* pedigrees and identified subclinical brain malformations consistent with Chudley-McCullough syndrome [78]. As mice expressing a truncated LGN protein are profoundly deaf [71], it is now tempting to speculate that hearing loss stems from defective apical cytoskeleton polarization in hair cells during development. In contrast, brain malformation could result from defects in mitotic spindle orientation, as described in the spinal cord and the cortex in model animals [19, 20]. If true, it could seem curious that mutations in a core mitotic spindle protein would have hearing loss as their most severe clinical presentation. However, hair cells are highly specialized, and many proteins that generate or compose their unique stereocilia bundle appear essential for this task in particular, resulting in non-syndromic hearing loss when defective (for review, see [83]). In contrast, given the importance of keeping cell proliferation and tissue architecture in check in all tissues, mitotic spindle orientation must be particularly robust mechanistically.

To date, no association to disease has been made for *mInsc*. Given that there are no clear paralogs of *mInsc*, mutations could be incompatible with life. However, *mInsc* knockout mice are viable and display no gross phenotypes [21, 67, 70], which does not support this idea. Rather, since *mInsc* mutation mildly affects hair cell morphology compared to disruption of *Lgn* or Gα_i [70], mutations may not lead to clinically noticeable phenotypes. It remains possible, however, that more subtle issues exist, such as reduced immune response due to defective neutrophil chemotaxis [67]. Gα_i proteins are involved in a multitude of signaling functions, making any particular connection between mutation and defects in cytoskeleton polarity challenging.

9.3 Further Evidence: Examples of Partner Proteins with Post-Mitotic Functions

9.3.1 Canoe/Afadin

Some proteins with well-established roles in mitotic spindle orientation in *Drosophila* were first studied in a post-mitotic context in vertebrates prior to being implicated in mitosis. For example, the *Drosophila* protein Canoe helps mediate spindle orientation [84] by binding Pins and helping recruit Mud (NuMA homolog),

thus providing a link between Pins and microtubules [85]. A role for Canoe's mammalian homolog, Afadin/AF-6, in orienting the mitotic spindle has only recently been demonstrated. Studies in human cell lines suggest that Afadin is important for recruiting LGN to the cortex and providing a bridge to F-actin [86, 87]. Post-mitotically, Afadin is directly involved in the formation and/or maintenance of cellular junctions, including adherens junctions, tight junctions [88], and neuronal synapses [89]. Afadin is also important in remodeling the architecture of dendritic spines downstream of NMDA receptor activity [90]. Reminiscent of the role of mInsc in neutrophil chemotaxis, Afadin specifically regulates the directionality but not the overall motility of NIH3T3 cells [91]. In addition, Canoe can affect axon pathfinding by regulating Slit/Robo signaling at the *Drosophila* CNS midline [92].

9.3.2 Myosin VI

Myosin VI may be more accurately categorized as an "effector" rather than a "regulator" of spindle orientation. In *Drosophila* neuroblasts, Myosin VI targets the protein Miranda [93] and cell fate determinants Prospero, Brat, and Numb to the basal portion of the cell [9]. Myosin VI has not been associated to mitotic spindle orientation in vertebrates, but has interesting post-mitotic functions. In spite of being widely expressed in animal tissues [94] and the sole characterized minus end-directed myosin [95], Myosin VI predominantly causes deafness when absent [96], an interesting parallel to the case of LGN described above. Following up on this discovery, human deafness has also been linked to mutations in *MYO6* [97, 98]. In *Myo6* mutant mouse cochlear hair cells, stereocilia fuse together into giant stereocilia [99]. In addition, Myosin VI is also required at the basal end of hair cells to generate the ribbon synapses, a subtype of synapse specialized for fast, sustained, and graded neurotransmitter release, which transmit sound information to ganglion neurons [100]. Furthermore, like LGN and Afadin, Myosin VI is involved in neuronal synaptic function. Myosin VI is enriched at the postsynaptic density, and *Myo6* mutant hippocampal neurons have fewer dendritic spines and synapses and impaired internalization of AMPA receptors [101]. Strikingly, like mInsc and Afadin, Myosin VI was also proposed to regulate the directionality of cell migration without affecting overall motility by regulating transport of epidermal growth factor receptor to the leading edge [102]. Accordingly, Myosin VI is found at the leading edge of growth factor-stimulated fibroblasts [103] and is important for motility of *Drosophila* border cells [104].

9.3.3 Additional Candidates

The recurring patterns of protein function discussed above suggest that future work will uncover more links between the spindle orientation machinery and polarized responses in post-mitotic cells. For instance, the $G\alpha_i$ guanine nucleotide exchange

factor Ric8 not only helps orient the mitotic spindle [47–51, 105], but it is also implicated in *Dictyostelium* chemotaxis by amplifying Gα_i signal initiated downstream of chemoattractant receptor signaling [106]. Additionally, huntingtin appears to regulate protein transport in mitotic and non-mitotic contexts. It mediates cortical localization of dynein-dynactin-LGN-NuMA in dividing cells, thus helping to orient the spindle [107, 108]. Huntingtin also regulates apical localization of Par3-aPKC during mouse mammary epithelial morphogenesis [109] and microtubule-based transport in neurons [110–112].

9.4 Summary

In conclusion, proteins that orient the mitotic spindle are emerging as also playing a variety of essential roles in post-mitotic cells. Examples detailed above represent relatively disparate systems and processes, suggesting they could be the tip of the iceberg. In these alternate contexts, mInsc-LGN-Gα_i and partners appear to use their ability to mark and organize subcellular domains for a wide variety of processes. They generally act by scaffolding partner proteins together and/or by regulating the cytoskeleton. We thus anticipate that several additional processes relying on mInsc-LGN-Gα_i will be uncovered in the future when their role is progressively studied in new post-mitotic contexts. In addition, new or known partners of mInsc-LGN-Gα_i in the spindle orientation machinery will be obvious candidates to pursue in these novel contexts. Finally, the large body of knowledge gathered over the years by studying spindle orientation will be invaluable to accelerate the understanding of normal biological processes and disease mechanisms where spindle proteins play a post-mitotic role.

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