Cell. Mol. Life Sci. (2013) 70:4039–4046
DOI 10.1007/s00018-013-1319-z

Cellular and Molecular Life Sciences

REVIEW

Going vertical: functional role and working principles of the protein Inscuteable in asymmetric cell divisions

Simone Culurgioni · Marina Mapelli

Received: 24 December 2012 / Revised: 27 February 2013 / Accepted: 4 March 2013 / Published online: 21 March 2013
© Springer Basel 2013

Abstract Coordinating mitotic spindle dynamics with cortical polarity is essential for stem cell asymmetric divisions. Over the years, the protein Inscuteable (Insc) has emerged as a key element determining the spindle orientation in asymmetric mitoses. Its overexpression increases differentiative divisions in systems as diverse as mouse keratinocytes and radial glial cells. To date, the molecular explanation to account for this phenotype envisioned Insc as an adaptor molecule bridging between the polarity proteins Par3:Par6:aPKC and the spindle pulling machines assembled on NuMA:LGN:Gαi. However, recent biochemical and structural data revealed that Insc and NuMA are competitive interactors of LGN, challenging the simplistic idea of a single apical macromolecular complex, and demanding a revision of the actual working principles of Insc.

Keywords Inscuteable · Asymmetric cell division · Spindle orientation · Cellular polarity · NuMA · LGN

Introduction

During development and in adulthood of multicellular organisms, asymmetric cell divisions (ACDs) balance proliferation with differentiation. At any given embryonic stage, stem cells undergo either symmetric divisions to increase the stem cell number or ACDs to generate one daughter endowed with self-renewing capabilities identical to the mother and one committed to differentiation. The combination of intrinsic and extrinsic factors dictating the symmetric versus asymmetric outcome of stem cell divisions is a fascinating and intense object of current research. Evidence gathered during the past years converge on the notion that the molecular machinery executing ACDs is conserved between vertebrate and invertebrate stem cells. In particular, coupling of mitotic spindle orientation to cortical polarity cues has emerged as a hallmark of all ACDs, and in most of the studied systems this process impinges on a conserved player named Inscuteable (Insc). In several stem cell types, ablation of Insc increases symmetric divisions, while its exogenous over-expression drives apico–basal asymmetric divisions. Despite its fundamental role, the molecular mechanism accounting for Insc functions in mitosis remains poorly understood. In this review, we summarize what is known about the biological relevance of Insc for stem cell biology, and about the mechanisms underlying Insc function, with particular emphasis on recent data describing how Insc interacts with the mitotic spindle orientation machinery.

S. Culurgioni · M. Mapelli (✉)
Department of Experimental Oncology, European Institute of Oncology, Via Adamello 16, 20139 Milan, Italy
e-mail: marina.mapelli@ieo.eu

Discovery and characterization of Inscuteable in *Drosophila* melanogaster

The first report on Insc dates back to 1996, when Kraut and Campos-Ortega identified it as a novel neural precursor gene in fruitflies. Insc expression was observed in embryos at sites of morphological changes or cell movements such as neuroblasts, midgut cells, tracheal branches, and pupal wing epithelia [1]. Based on its localization at the plasma membrane, Insc was initially classified as a protein involved in cytoskeleton–membrane association. Homozygous null mutations of the Insc locus are lethal in flies, while heterozygous fly mutants present defects in the organization of neuroblasts, sense organs and muscle fibers, indicating a key role of Insc for correct morphogenesis. Remarkably,

more than a decade later, this is most of what we know about the biological function of Insc in multicellular organisms.

The greatest insight into the molecular mechanism of Insc activity came from studies in neuroblasts [2], the stem cells of the *Drosophila* central nervous system. Neuroblasts always divide asymmetrically with the spindle axis perpendicular to the plane of the neuroepithelium from which they have delaminated (Fig. 1a). The cortical polarization of mitotic neuroblasts is driven by contacts that they retain to the neuroepithelium. Here, an apical domain is assembled by the polarity proteins Par3(Bazooka in flies)/Par6/aPKC. Such apically localized Par complex orchestrates the basal confinement of so-called 'fate determinants' such as Numb, Prospero, and Brat [3] to establish apico–basal polarity. Alignment of the mitotic spindle with the polarity axis ensures unequal segregation of Par proteins and fate determinants upon citokynesis, and this is exactly the process that requires Insc activity. In neuroblasts, Insc is recruited apically by direct interaction with Bazooka [4, 5], and contributes to the basal localization of Numb and Prospero [1]. The molecular connection between the Bazooka:Insc complex and the spindle tethering machinery is the switch-like protein Pins. Pins colocalizes with Insc in mitotic neuroblasts, and is required for all mitotic functions of Insc [6, 7]. Intriguingly, the mitotic apical localization of Pins and Insc is mutually dependent, and requires Bazooka. The molecular reasons for this behavior are not yet clear. Insc mutant neuroblasts misorient the spindle relative to the neuroepithelium [2]. However, they are still able to organize polarized cortical domains cell-autonomously, and to coordinate the spindle in order to divide asymmetrically, a phenomenon known as *telophase rescue* [8]. While Insc localization depends on actin function [2], telophase rescue has been reported to impinge on microtubule dynamics and on the mitotic kinesin Khc-73 (GAKIN in vertebrates) [8]. Whether a mechanism analogous to the telophase rescue exists in vertebrates is unknown.

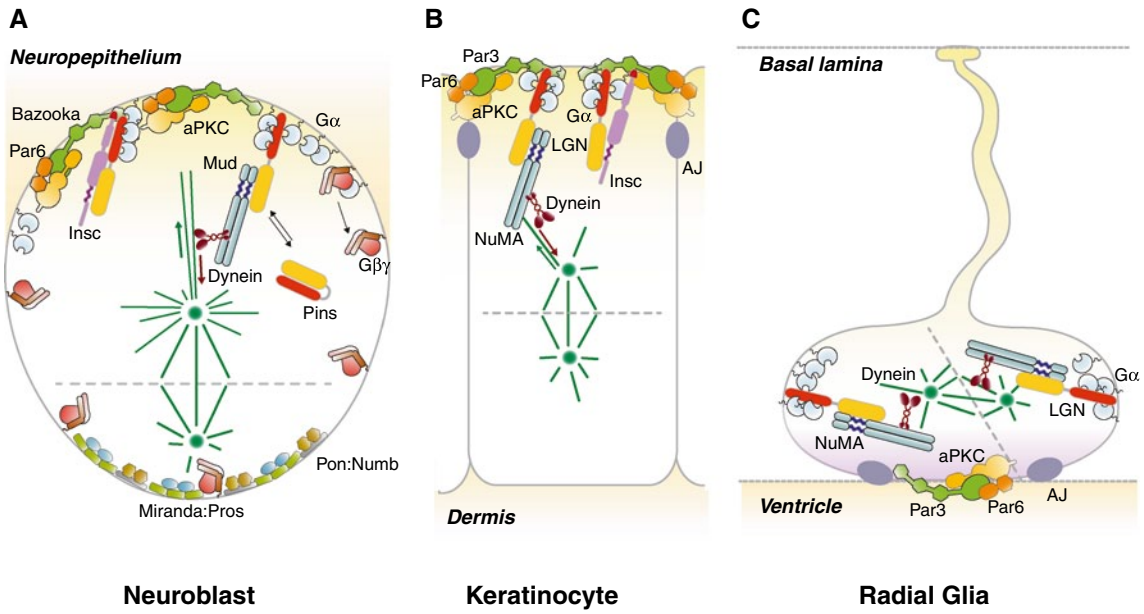

Fig. 1 Schematic description of the organization of cortical polarity and mitotic spindle in asymmetric metaphases. **a** *Drosophila* neuroblasts delaminate from a neuroepithelium to which they remain in contact with a region containing the polarity proteins Bazooka:Par6:aPKC. In mitosis, the switch-like protein Pins undergoes a major conformational rearrangement enabling the binding to Mud and to three $G\alpha i^{GDP}$ subunits dissociated from $G\beta\gamma$ and anchored at the apical membrane via myristoyl groups. The interaction of Mud with the microtubule-motor Dynein is responsible for the membrane-directed pulling forces exerted by cortical Mud:Pins:Gαi complexes on astral microtubules. Mitotic Pins:Gαi also form a complex with Bazooka-bound Insc, whose interplay with the Mud:Pins:Gαi pathway remains unclear to date. At the opposite site of the neuroblast cortex, Miranda and Pon assist the basal localization of the fate determinants Pros and Numb, respectively. **b** In mammals, keratinocytes divide vertically to stratify the skin. Apico–basal polarity is established by localization of Par3:Par6:aPKC above the adheres junctions (AJ) stabilizing the epithelial architecture. Apical Par3 recruits Insc:LGN:Gαi complexes at the apical site, in conjunction with the spindle-tethering devices NuMA:LGN:Gαi, physically linked to Dynein. **c** In the ventricular zone of the developing neocortex, vertebrate radial glial cells organize a tiny apical domain with Par proteins opposite to the basal process protruding to the basal lamina. The force generators NuMA:LGN:Gαi are distributed at the plasma membrane in an equatorial belt that maintains the spindle parallel to the ventricular surface. A diffuse staining for Insc towards the apical side has been reported (*violet shading*). In this configuration, a minor tilt in the spindle axis is sufficient to determine the unequal segregation of Par proteins and of the basal process upon cytokinesis

In interphase, apical Insc also associates with the dsRNA-binding protein Staufen, which in mitosis assists the basal localization of Pros and Pros mRNA [5, 9]. It will be interesting to explore whether the vertebrate homologue of Insc works in the same way to ensure the asymmetric localization of Staufen2 in mouse radial glial cells [10].

The *Drosophila* model system was also used to demonstrate for the first time that ectopic expression of Insc is sufficient to reorient the spindle and to induce orthogonal divisions in epithelial cells that normally divide planarly [2]. Later on, this remarkable function of Insc was found conserved in chick and mouse neural progenitors [11, 12], as well as in mouse skin progenitors [13, 14]. In the *Drosophila* sensory organ precursor lineage, Insc is specifically expressed only in the pIIb cells, where it regulates the distribution of Miranda and Pros [15]. Intriguingly, when ectopic expression of Insc is induced during the first division of PI cells, planar polarity is reversed with anterior relocalization of Bazooka with Pins, and posterior relocalization of Numb [16].

It is worth pointing out that, while Insc over-expression couples the mitotic spindle axis to apico–basal polarity, it is not sufficient per se to promote fate changes in cells that divide symmetrically, as has been demonstrated in the *Drosophila* optic lobe neuroepithelium [17]. Most likely, this is due to the inability of these cells to localize fate determinants asymmetrically.

mInsc

Studies of vertebrate Insc lagged behind the characterization of the *Drosophila* gene, partly because of poor sequence conservation [18] but also because of a general delay in the development of genetic and imaging tools to study asymmetrically dividing stem cells in vertebrates.

Most of what we know about vertebrate Insc comes from studies in mouse keratinocytes of the developing skin and in neural progenitors, the two stem cell systems where mouse Insc (mInsc hereon) was first discovered [19, 20]. mInsc is endowed with properties similar to that of fly Insc: it is part of a mitotic complex with Par3 and LGN (the vertebrate counterpart of Pins), it localizes in an apical crescent together with Par3 in embryonic skin keratinocytes, and finally it contributes to setting up apico–basal asymmetric divisions in the developing mouse epidermis [14].

The role of mInsc during keratinocytes divisions has been addressed at different embryonic stages by elegant experiments based on inducible over-expression followed by imaging [13]. About 8 h after a short burst of induction at embryonic stage E14.5, 90 % of mInsc-expressing cells undergo vertical asymmetric divisions compared to 70 % of wild-type. These data are consistent with the notion that the presence of mInsc is sufficient to reorient the spindle apico–basally. Surprisingly, after 3 days of the induction burst, the proportion of spindles perpendicular to the epidermis decreases to 53 % and no morphological changes are observed in the epidermal architecture. This suggests that robust feedback mechanisms control the orientation of the division plane through regulation of mInsc. Under these conditions, apical crescents of mInsc and LGN are also visible when keratinocytes divide with a planar spindle. However, these crescents do not include NuMA, the Dynein-associated LGN interactor responsible for the traction forces on astral microtubules needed to orient the spindle [21] (Fig. 1b). No molecular explanation has been proposed to account for these phenotypes, which imply more than the mere existence of an apical multi-subunit complex containing both mInsc and NuMA.

The other system in which mInsc has been characterized extensively is that of radial glial cells, the mouse neural progenitors. At E13, radial glia cells start neurogenic asymmetric divisions to generate either a glial cell and a neuron (direct neurogenesis) or a glial cell and an intermediate basal progenitor that divides a few times to produce 2–4 neurons (indirect neurogenesis). Ablation of mInsc in radial glia cells decreases direct neurogenesis; conversely, its over-expression expands the neuronal pool prematurely [12]. Although these results are consistent with the role reported for mInsc in other cellular systems, they fall short of a molecular description of the spindle dynamics subtending non-planar divisions in radial glia. In fact, immunostaining data showed that, in mitotic chicken neuroepithelial cells, NuMA and LGN localize in an equatorial belt above the spindle poles [22], while mInsc localizes to the apical emisphere of mouse radial glia [12]. A peculiar feature of radial glial cells is that they organize a tiny apical domain, termed apical end-foot, in which Par proteins localize. In mitosis, a minor tilt in the spindle axis is sufficient to determine the asymmetrical segregation of the apical end-foot between daughters [23, 24] (Fig. 1c). In this scenario, it is difficult to envision how mInsc can affect spindle movements. Results of mInsc over-expression experiments performed in the developing chick neocortex mirror the ones obtained in mice [11].

Recently, mInsc has been imaged apically in distal embryonic lung epithelial cells [25] and muscles [26], although in these systems the only information available is that mInsc colocalizes with LGN and NuMA. An overview of the localizations and fate changes observed upon genetic manipulation of Insc/mInsc in various tissues is reported in Table 1.

Structure function analysis of Insc

Considerable effort has been spent to understand which domains of *Drosophila* Insc are responsible for its activities.

Table 1 Phenotypic effects of genetic alterations of Insc on ACDs

Cellular system	Genetic alteration	Spindle axis	Localization	Daughter cells fate	Reference
Drosophila neuroblasts	Homozygous null mutation	Misoriented	Numb, Prospero and Pins mislocalized; Baz properly localized apically. Miranda, PON mislocalized at metaphase.	Telophase rescue occurs: cell fate determinants correctly segregated in the two daughter cells.	[2], [4], [6], [42]
				Defects in cell fate specification in cells born from early GMC (in NB1-1, NB4-2, and NB7-1 lineages).	[43]
	InscCEN(252–615) overexpression	Unaltered	InscCEN apical		[28]
	InscCEN(252–578) overexpression	Unaltered	InscCEN apical; Numb and Miranda properly localized.		[27]
Drosophila ectodermal cells	Ectopic expression of Insc	Apico-basally reoriented			[2]
Drosophila epithelial cells	Ectopic expression of Insc	Apico-basally reoriented	Pins relocalization from lateral to apical cortex.		[6]
Drosophila sensory organ precursor cells (pI)	Ectopic expression of Insc		Baz anterior relocalization (from posterior) and Numb posterior relocalization (from anterior)		[16]
Drosophila sensory organ precursor cells (pIIB)	Ablation		Miranda partially mislocalized apically; Prospero segregated in the apical daughter cell.	pIIIb daughter cells not correctly specified.	[15]
Mouse embryonic and adult keratinocytes	K14-driven mInsc-GFP overexpression		LGN, Par3 and mInsc colocalized apically opposite to integrins.		[19]
	Inducible K14-driven mInsc overexpression	Increased vertical orientation		Transient but not long-term increase in ACDs; overall skin architecture unaltered.	[13]
	Lentiviral YFP-mInsc overexpression	Increased vertical orientation	LGN and mInsc apically colocalized.	Increased ACDs; thickening of the spinous layer.	[14]
Mouse neural progenitors	Ablation	Decreased oblique/vertical orientation		Fewer Intermediate Progenitors and Outer Radial Glial cells; decreased thickness of the intermediate zone and the cortical plate; enlarged ventricles.	[12]
	HA-mInsc overexpression	Increased vertical orientation	HA-mInsc localizes apically	Enhanced number of PAX6-positive Radial Glial cells outside the ventricular zone.	[11]
	mInsc-GFP expression	Increased oblique and vertical orientation		Increased number of Intermediate Progenitors and Outer Radial Glial cells; enhanced thickness of the intermediate zone and of the cortical plate.	[12]
Rat embryonic retinal neuroepithelial precursors	Ablation	Decreased vertical orientation		Increased progenitors proliferation and reduced differentiation into photoreceptors; cell fate specification defects.	[20]
Chicken dermomyotome	mInsc overexpression	Increased vertical orientation		Increased ACDs and cell progeny specification defects.	[26]

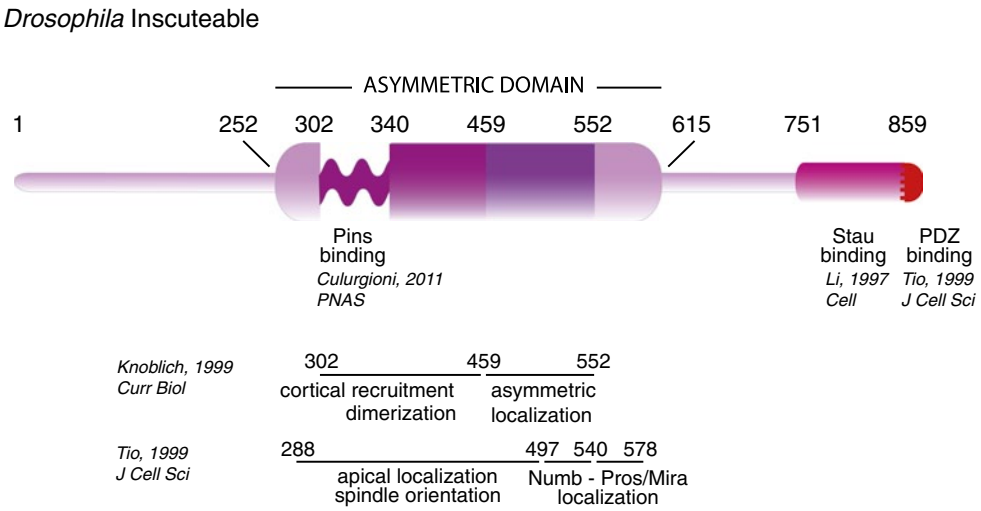

Fig. 2 Domain structure of *Drosophila* Insc. Protein fragments whose function has been established by in vivo analysis or in vitro binding assays are reported in different shades of *purple* accompanied by the corresponding references. The putative *C*-terminal PDZ binding motif is shown in *red*

Structure–function analyses conducted in *Drosophila* neuroblasts and RP2 cells led to the identification of a central portion of Insc encompassing residues 252–615 able to rescue Insc functions in neuroblasts lacking Insc, and ectopically reorienting epithelial cells divisions [27, 28] (Fig. 2). Based on these properties, this portion of Insc was termed *asymmetric domain,* and from here on it will be referred to as Insc^{ASYM}. Insc^{ASYM} is predicted to contain several Ankyrin repeats, which fold as antiparallel-helical motifs and are often found in protein–protein interaction scaffolds. Analyses of the phenotypic behavior of constructs shorter than Insc^{ASYM} were used to map individual Insc functions within this central domain. In particular, it was found that residues 302–459 drive cortical recruitment, while residues 436–552 are essential for the asymmetric distribution of Insc in the apical domain [28]. The full fragment 302–459 is sufficient to sustain spindle orientation, but cannot ensure the correct basal localization of fate determinants, which requires the additional residues 497–540 in the case of Numb, or 540–578 for Miranda and Prospero. An intriguing property of Insc$^{302-459}$ resides in its ability to dimerize with full-length Insc [28], a feature whose biological relevance remains elusive. Interestingly, the portion of Insc harboring the Staufen binding site is not included in Insc^{ASYM} but maps to the last 200 amino acids of the protein [9]. This implies that the mRNA-transport activities of Staufen associated with Insc are not essential for asymmetric neuroblast divisions. Primary sequence analysis of fly Insc also revealed the existence of a proline-rich motif in the *N*-terminal half of the protein, and a hydrophobic carboxy-terminal stretch (ESVF) matching the consensus for binding to PDZ domains. Whether and how these modules are used for binding interactions in living cells is not known. Of note, *C*-terminal tagging of Insc might have hindered the functional role of the evolutionary conserved PDZ-binding consensus motif.

To gain insights into the molecular mechanisms underlying Insc functions in neuroblasts, a search for Insc^{ASYM} interacting partners was conducted. Yeast two-hybrid screens revealed that Insc directly associates with the *N*-terminal tetratrico-peptide repeats (TPRs) of Pins, a result confirmed and refined by in vitro pull-down experiments with Insc$^{288-479}$ and Pins^{TPR} [29]. The prominent role of the Insc:Pins interaction for spindle alignment in neuroblasts was demonstrated by the fact that, upon Pins ablation, the correct apical localization of Insc is lost, the division plane is randomized, and the daughter size asymmetry is compromised [7, 29]. The ability of mouse LGN to rescue Pins-null phenotypes in *Drosophila* neuroblasts hinted for the first time at the conservation of the spindle orientation machinery from invertebrates to mammals [29]. This is even more remarkable considering that no orthologs of fly Insc were known at that time.

The very poor sequence conservation of Insc between flies and vertebrates does not allow mapping on the mouse protein of a functional domain corresponding to the *Drosophila* Insc^{ASYM}. Until recently, the only mInsc mutants characterized were *N*-terminal truncations shown to impair binding to LGN for deletions longer than 29 residues [20].

Major insights into the topology of the Insc:Pins and mInsc:LGN assemblies came from the determination of the crystallographic structures of the *Drosophila,* human and mouse complexes [30–32]. The structures revealed that the LGN-binding site of Insc consists of an evolutionary conserved peptide (Fig. 3). The dimer architecture is peculiar in that the flexible Insc fragment binds with an extended conformation to the inner side of the α-solenoid formed by the *N*-terminal TPR domain of Pins/LGN. The binding interface is modular, mostly contributed by an invariant Triptophan on the initial α-helix of Insc (Trp-313 of fly Insc and Trp-31 of mInsc), and a Glu-X-Glu (EXE) triplet in the middle. The

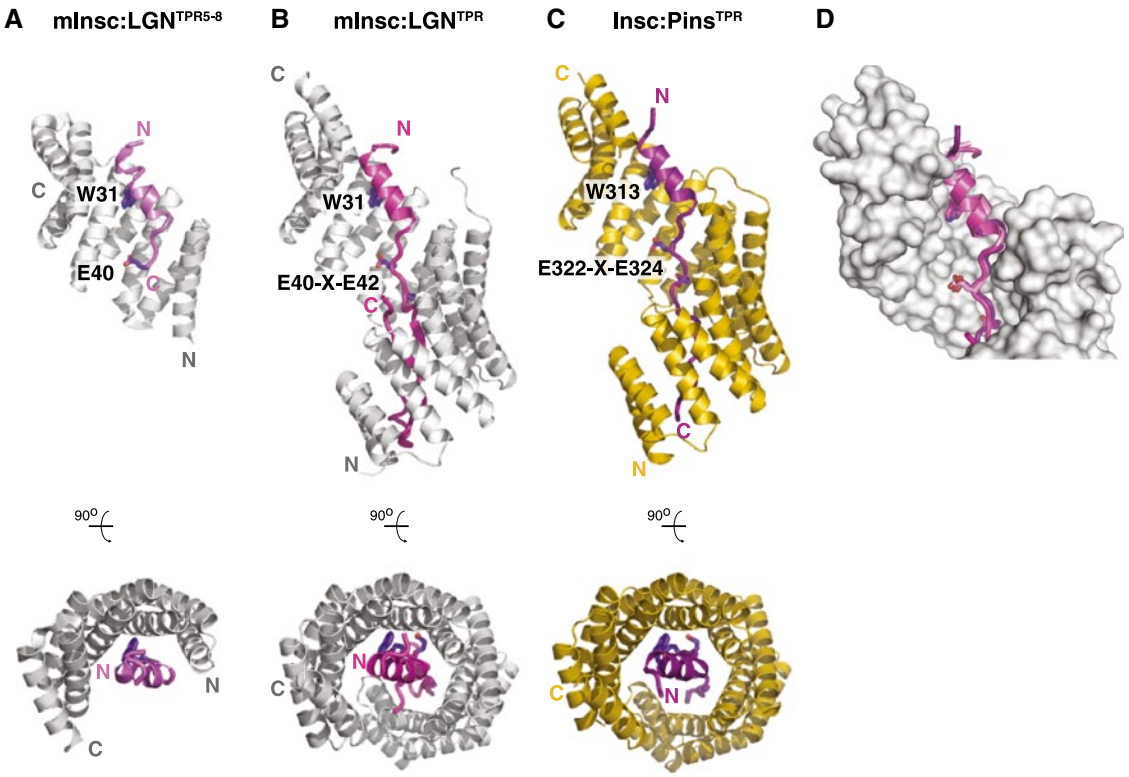

Fig. 3 Three-dimensional architecture of mInsc:LGN and Insc:Pins complexes. **a** Cartoon representation of mouse mInsc:LGN determined using constructs containing only the *N*-terminal α-helix of mInsc and TPR5-8 of LGN, encompassing residues 191–350. The mInsc peptide is colored in *pink* with the Trp-31 and the Glu-40 depicted in *purple balls-and-sticks* (PDB-ID 3RO3). **b** Structure of human mInsc$^{23-69}$ (*magenta*) in complex with LGN$^{13-414}$ (*gray*). The mInsc peptide lines up in the inner groove of the TPR α-solenoid, almost parallel to the superhelical axis. The Trp-31 on the α-helix of mInsc and the central E40-E42 are depicted in *purple balls-and-sticks*. **c** The *Drosophila* Insc:Pins^{TPR} assembly is shown with the same orientation and features of the human counterpart, with the invariant Trp-313, Glu-322 and Glu-324 of Insc in *purple* (PDB-ID 4A1S). In the *bottom row* of **a**–**c**, views of the dimers rotated 90° are reported. **d** Close-up of the structural superposition of the three complexes showing how the topology of the interaction between mInsc/Insc and LGN/Pins is conserved throughout evolution. Superposed Insc peptides are displayed on the surface of the TPR scaffold as in the individual panels

extended dimer interface accounts for a nanomolar affinity between these proteins, as measured in all three species. In fruitflies, the Pins-binding peptide maps at position 302–340 of full-length Insc, within the portion of Insc^{ASYM} required for cortical recruitment in neuroblasts and for dimerization. Biochemical studies also revealed that the association of mInsc to LGN displaces the *C*-terminus of LGN from its own TPR domain, suggesting that mInsc cannot bind to the close form of LGN [30]. The functional implications of this finding for spindle orientation remain to be explored.

Relationship between Insc and NuMA in the spindle orientation pathway

The most striking result emerging from the recent structural and biochemical analyses is that mInsc and the microtubule-associated protein NuMA (named Mud in *Drosophila*) are mutually exclusive interactors of LGN, with mInsc displaying higher affinity. Binding of cortical LGN:Gαi to NuMA controls spindle positioning in systems as diverse as HeLa cells [33], *C. elegans* embryo [21], *Drosophila* neuroblasts [34–36], as well as mouse skin [19] and neural progenitors [22]. Molecularly, LGN-bound NuMA mediates the cortical recruitment of the microtubule motor Dynein, whose minus-end-directed movement establishes directional pulling forces on astral microtubules [21]. To date, it was believed that, in asymmetrically dividing cells, the function of mInsc was to recruit Dynein-associated NuMA:LGN:Gαi complexes at polarity sites in conjunction with Par proteins in order to align the spindle to the polarity axis. This simplistic model rested on the assumption that mInsc and NuMA could simultaneously associate with the same LGN molecule, as suggested by co-localization and co-immunoprecipitation experiments in neuroblasts [6, 36] and skin keratinocytes [19]. However, such a model is not compatible with the competitive binding of mInsc and NuMA to LGN. Structural data have revealed that NuMA and mInsc occupy the same inner groove of the LGN^{TPR} domain, sharing a

common consensus motif (more details on the structural similarities between mInsc:LGN^{TPR} and NuMA:LGN^{TPR} assemblies are discussed in a recent review by Mapelli and Gonzalez [37]).

This evidence highlights the need of a revised molecular model to explain spindle coupling to cortical polarity in vertical asymmetric mitoses. LGN could bind sequentially first to mInsc and then to NuMA [30, 38]. This hypothesis provides a rationale for correct recruitment of force generators at the polarity sites, and is consistent with the higher affinity of LGN to mInsc as compared to that of NuMA. However, it raises the questions of how LGN is transferred form mInsc to NuMA to generate microtubule-pulling forces, and of how NuMA:LGN:Gαi complexes are maintained in a restricted cortical domain near Par proteins. Intriguingly, opposite to what has been observed in fly neuroblasts and skin progenitors, in radial glial cells LGN and Par proteins do not colocalize. Whether Insc is differentially regulated in these diverse cellular settings is unclear and debated.

Conclusions

Insc/mInsc has emerged as a key regulator of spindle alignment in asymmetrically dividing cells. Its ablation reduces spindle tilting along the polarity axis thus decreasing the proportion of ACDs, while its ectopic over-expression has the opposite effect. The molecular mechanism accounting for these phenotypes is still unclear. mInsc directly binds to Par3 and to LGN in competition with NuMA, but how these interactions are orchestrated in space and time to properly position the spindle remains unknown. There is evidence that NuMA and LGN are phosphorylated by mitotic kinases such as Aurora-A [39], Plk1 [33], aPKC [40], and Abl-1 [41]. However, no description of how these post-translational modifications can impact on the binding of LGN to mInsc has been reported. In addition, no post-translational modifications of mInsc have been documented. We cannot exclude that yet-uncharacterized ancillary components might regulate the interactions of mInsc in mitosis. Alternatively, the Insc dimerization reported in flies might serve a similar purpose [28]. Little is known on how mInsc binds to Par3, a critical regulator of the asymmetric localization of Insc at the cortex. In summary, fundamental aspects of mInsc functioning are still unclear and need to be investigated to gain a deeper understanding of how the protein works in living cells. Future studies will benefit from detailed information provided by crystallographic structures and biochemical data, and from the recently acquired possibility of visualizing mInsc and the spindle orientation machinery during ACDs. We are confident that learning about the mechanistics underlying asymmetric divisions will prove fundamental for the understanding of stem cell biology.

Acknowledgments We are grateful to members of the Mapelli group and to Thomas Vaccari for critical reading of the manuscript. Mapelli's laboratory is funded by the Italian Association for Cancer Research (AIRC) and the Italian Ministry of Health; S.C. is a FIRC postdoctoral fellow.

References

1. Kraut R, Campos-Ortega JA (1996) Inscuteable, a neural precursor gene of *Drosophila*, encodes a candidate for a cytoskeleton adaptor protein. Dev Biol 174:65–81
2. Kraut R, Chia W, Jan LY, Jan YN, Knoblich JA (1996) Role of Inscuteable in orienting asymmetric cell divisions in *Drosophila*. Nature 383:50–55
3. Neumuller RA, Knoblich JA (2009) Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer. Genes Dev 23:2675–2699
4. Schober M, Schaefer M, Knoblich JA (1999) Bazooka recruits Inscuteable to orient asymmetric cell divisions in *Drosophila* neuroblasts. Nature 402:548–551
5. Wodarz A, Ramrath A, Kuchinke U, Knust E (1999) Bazooka provides an apical cue for Inscuteable localization in *Drosophila* neuroblasts. Nature 402:544–547
6. Yu F, Morin X, Cai Y, Yang X, Chia W (2000) Analysis of partner of inscuteable, a novel player of *Drosophila* asymmetric divisions, reveals two distinct steps in inscuteable apical localization. Cell 100:399–409
7. Parmentier, M.L., Woods, D., Greig, S., Phan, P.G., Radovic, A., Bryant, P., and O'Kane, C.J. (2000). Rapsynoid/partner of inscuteable controls asymmetric division of larval neuroblasts in *Drosophila*. J Neurosci 20, RC84
8. Siegrist SE, Doe CQ (2005) Microtubule-induced Pins/Galphai cortical polarity in *Drosophila* neuroblasts. Cell 123:1323–1335
9. Li P, Yang X, Wasser M, Cai Y, Chia W (1997) Inscuteable and Staufen mediate asymmetric localization and segregation of prospero RNA during *Drosophila* neuroblast cell divisions. Cell 90:437–447
10. Vessey JP, Amadei G, Burns SE, Kiebler MA, Kaplan DR, Miller FD (2012) An asymmetrically localized staufen2-dependent RNA complex regulates maintenance of Mammalian neural stem cells. Cell Stem Cell 11:517–528
11. Konno D, Shioi G, Shitamukai A, Mori A, Kiyonari H, Miyata T, Matsuzaki F (2008) Neuroepithelial progenitors undergo LGN-dependent planar divisions to maintain self-renewability during mammalian neurogenesis. Nat Cell Biol 10:93–101
12. Postiglione MP, Juschke C, Xie Y, Haas GA, Charalambous C, Knoblich JA (2011) Mouse inscuteable induces apical-basal spindle orientation to facilitate intermediate progenitor generation in the developing neocortex. Neuron 72:269–284
13. Poulson ND, Lechler T (2010) Robust control of mitotic spindle orientation in the developing epidermis. J Cell Biol 191:915–922
14. Williams SE, Beronja S, Pasolli HA, Fuchs E (2011) Asymmetric cell divisions promote Notch-dependent epidermal differentiation. Nature 470:353–358
15. Orgogozo V, Schweisguth F, Bellaiche Y (2001) Lineage, cell polarity and inscuteable function in the peripheral nervous system of the *Drosophila* embryo. Development 128:631–643
16. Bellaiche Y, Radovic A, Woods DF, Hough CD, Parmentier ML, O'Kane CJ, Bryant PJ, Schweisguth F (2001) The Partner of Inscuteable/Discs-large complex is required to establish planar polarity during asymmetric cell division in *Drosophila*. Cell 106:355–366
17. Egger B, Boone JQ, Stevens NR, Brand AH, Doe CQ (2007) Regulation of spindle orientation and neural stem cell fate in the *Drosophila* optic lobe. Neural Develop 2:1

18. Katoh M (2003) Identification and characterization of human Inscuteable gene in silico. Int J Mol Med 11:111–116
19. Lechler T, Fuchs E (2005) Asymmetric cell divisions promote stratification and differentiation of mammalian skin. Nature 437:275–280
20. Zigman M, Cayouette M, Charalambous C, Schleiffer A, Hoeller O, Dunican D, McCudden CR, Firnberg N, Barres BA, Siderovski DP et al (2005) Mammalian inscuteable regulates spindle orientation and cell fate in the developing retina. Neuron 48:539–545
21. Kotak S, Busso C, Gonczy P (2012) Cortical dynein is critical for proper spindle positioning in human cells. J Cell Biol 199:97–110
22. Peyre E, Jaouen F, Saadaoui M, Haren L, Merdes A, Durbec P, Morin X (2011) A lateral belt of cortical LGN and NuMA guides mitotic spindle movements and planar division in neuroepithelial cells. J Cell Biol 193:141–154
23. Kosodo Y, Roper K, Haubensak W, Marzesco AM, Corbeil D, Huttner WB (2004) Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells. EMBO J 23:2314–2324
24. Marthiens V, ffrench-Constant C (2009) Adherens junction domains are split by asymmetric division of embryonic neural stem cells. EMBO Rep 10:515–520
25. El-Hashash AH, Warburton D (2011) Cell polarity and spindle orientation in the distal epithelium of embryonic lung. Dev Dyn 240:441–445
26. Ben-Yair R, Kahane N, Kalcheim C (2011) LGN-dependent orientation of cell divisions in the dermomyotome controls lineage segregation into muscle and dermis. Development 138:4155–4166
27. Tio M, Zavortink M, Yang X, Chia W (1999) A functional analysis of inscuteable and its roles during *Drosophila* asymmetric cell divisions. J Cell Sci 112(Pt 10):1541–1551
28. Knoblich JA, Jan LY, Jan YN (1999) Deletion analysis of the *Drosophila* Inscuteable protein reveals domains for cortical localization and asymmetric localization. Curr Biol 9:155–158
29. Yu F, Morin X, Kaushik R, Bahri S, Yang X, Chia W (2003) A mouse homologue of *Drosophila* pins can asymmetrically localize and substitute for pins function in *Drosophila* neuroblasts. J Cell Sci 116:887–896
30. Culurgioni S, Alfieri A, Pendolino V, Laddomada F, Mapelli M (2011) Inscuteable and NuMA proteins bind competitively to Leu-Gly-Asn repeat-enriched protein (LGN) during asymmetric cell divisions. Proc Natl Acad Sci USA 108:20998–21003
31. Yuzawa S, Kamakura S, Iwakiri Y, Hayase J, Sumimoto H (2011) Structural basis for interaction between the conserved cell polarity proteins Inscuteable and Leu-Gly-Asn repeat-enriched protein (LGN). Proc Natl Acad Sci USA 108:19210–19215
32. Zhu J, Wen W, Zheng Z, Shang Y, Wei Z, Xiao Z, Pan Z, Du Q, Wang W, Zhang M (2011) LGN/mInsc and LGN/NuMA Complex structures suggest distinct functions in asymmetric cell division for the Par3/mInsc/LGN and Galphai/LGN/NuMA Pathways. Mol Cell 43:418–431
33. Kiyomitsu, T., Cheeseman, I.M. (2011). Chromosome- and spindle-pole-derived signals generate an intrinsic code for spindle position and orientation. Nat Cell Biol 14:311–317
34. Izumi Y, Ohta N, Hisata K, Raabe T, Matsuzaki F (2006) *Drosophila* Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization. Nat Cell Biol 8:586–593
35. Bowman SK, Neumuller RA, Novatchkova M, Du Q, Knoblich JA (2006) The *Drosophila* NuMA Homolog Mud regulates spindle orientation in asymmetric cell division. Dev Cell 10:731–742
36. Siller KH, Cabernard C, Doe CQ (2006) The NuMA-related Mud protein binds Pins and regulates spindle orientation in *Drosophila* neuroblasts. Nat Cell Biol 8:594–600
37. Mapelli M, Gonzalez C (2012) On the inscrutable role of Inscuteable: structural basis and functional implications for the competitive binding of NuMA and Inscuteable to LGN. Open Biol 2:120102
38. Mauser JF, Prehoda KE (2012) Inscuteable regulates the Pins-Mud spindle orientation pathway. PLoS ONE 7:e29611
39. Johnston CA, Hirono K, Prehoda KE, Doe CQ (2009) Identification of an Aurora-A/PinsLINKER/Dlg spindle orientation pathway using induced cell polarity in S2 cells. Cell 138:1150–1163
40. Hao Y, Du Q, Chen X, Zheng Z, Balsbaugh JL, Maitra S, Shabanowitz J, Hunt DF, Macara IG (2010) Par3 controls epithelial spindle orientation by aPKC-mediated phosphorylation of apical Pins. Curr Biol 20:1809–1818
41. Matsumura S, Hamasaki M, Yamamoto T, Ebisuya M, Sato M, Nishida E, Toyoshima F (2011) ABL1 regulates spindle orientation in adherent cells and mammalian skin. Nat Commun 3:626
42. Wang H, Cai Y, Chia W, Yang X (2006) Drosophila homologs of mammalian TNF/TNFR-related molecules regulate segregation of Miranda/Prospero in neuroblasts. EMBO J 25(24):5783–5793
43. Udolph G, Rath P, Tio M, Toh J, Fang W, Pandey R, Technau GM, Chia W (2009) On the roles of Notch, Delta, kuzbanian, and inscuteable during the development of Drosophila embryonic neuroblast lineages. Dev Biol 336:156–168