Structural Analysis of the Mammalian Cell Polarity Protein Complex mInsc and LGN

Interaction between the cell polarity proteins mInsc (mammalian homolog of Inscuteable) and LGN (Leu-Gly-Asn repeat-enriched protein) plays a crucial role in mitotic spindle orientation during asymmetric cell division. We determined the crystal structure of the LGN-binding domain (LBD) of mInsc in complex with the N-terminal tetratricopeptide repeat (TPR) motifs of LGN at 2.6 Å resolution. In the complex, mInsc-LBD adopts an elongated structure that runs antiparallel to LGN along the concave surface of the superhelix formed by the TPR motifs. Structural and biochemical analyses provide new insights into the control of mitotic spindle orientation by the mInsc-containing complex.

In mitotic cells, orientation of the mitotic spindle defines the direction of cell division and the position of two daughter cells. The mitotic spindle orientation is controlled by the evolutionarily conserved protein complex containing LGN (Leu-Gly-Asn repeat-enriched protein), NuMA (nuclear mitotic apparatus protein), and the Gαi subunit of trimeric Gi proteins. In LGN, an intramolecular interaction between its N- and C-termini occurs, bringing this protein into an autoinhibitory conformation. The asymmetric division requires the establishment of apical–basal polarity in epithelial cells; the Par3-containing protein complex is specifically localized to the apical cortex for polarity establishment. The adaptor protein mInsc (mammalian homolog of Inscuteable) simultaneously binds to Par3 and LGN to provide a physical link between the two complexes; the link thereby couples cortical cell polarity and spindle orientation for asymmetric cell division [1]. LGN and its paralog AGS3 consist of an N-terminal domain comprising eight tetratricopeptide repeat (TPR) motifs and a C-terminal region harboring four GPR (G-protein regulatory) motifs that bind to the Gαi subunit of trimeric G proteins (Fig. 1). LGN interacts with multiple partners including mInsc, NuMA, and the C-terminal region of LGN via its TPR motifs. mInsc contains an N-terminal region highly homologous to the asymmetry domain of Pins, a fruit-fly homolog of LGN, which is responsible not only for apical cortical localization and mitotic spindle orientation along the apical–basal axis but also for interacting with Pins. However, the molecular basis for the mInsc-mediated regulation of the Par3-containing cell polarity complex and the mitotic spindle orientation regulating Gαi–LGN–NuMA complex remains elusive.

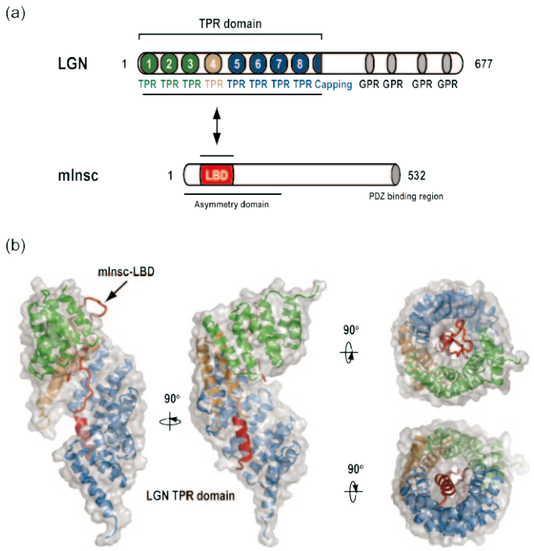

Figure 1
Structure of mInsc and LGN. (a) Domain architecture of LGN and mInsc. Human mInsc of 532 amino acids harbors an N-terminal LBD, which is located in a region corresponding to the asymmetric localization domain of *Drosophila* Insc, and a region for binding to the Par3 PDZ domains. LGN of 677 amino acids harbors an N-terminal domain composed of eight TPR motifs and a C-terminal region of four GPR motifs. (b) Orthogonal views of the overall structure of mInsc-LBD in complex with LGN TPR domain. mInsc-LBD is colored in red. The TPR domain of LGN is shown in a surface representation with the secondary structure indicated in green (TPR1-3), in orange (TPR4), and in blue (TPR5-8 and the capping helix).

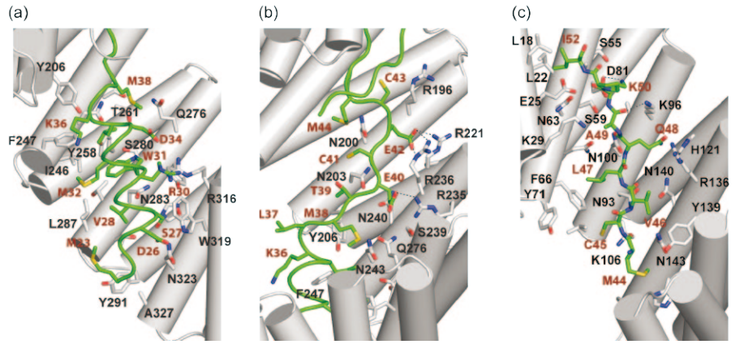

Figure 2
Interactions between mInsc-LBD and LGN-N. Recognition of the α-helix region of mInsc-LBD (a), the extended region of mInsc-LBD (b), and the β-sheet region of mInsc-LBD (c). mInsc-LBD is colored in green and LGN-N represents the cylinder model colored in gray. Residues involved in mInsc-LGN interaction are labeled.

We first mapped the regions required for the interaction between mInsc and LGN using truncated forms of mInsc and LGN. Binding experiments between mInsc and LGN revealed that the LGN-binding domain of mInsc (mInsc-LBD, residues 23-69) is sufficient for interaction with the LGN TPR motifs (LGN-N, residues 13-414). To understand the molecular mechanism underlying the interaction between mIncs-LBD and LGN TPR motifs, we determined the crystal structure of the LGN-N–mInsc-LBD complex at 2.6 Å resolution [2]. In the complex, mInsc-LBD forms an elongated structure containing an α-helix, and an antiparallel β-sheet linked by an extended region. LGN-N contains eight TPR motifs with a C-terminal capping helix (17 anti-parallel α-helices in total), which adopt a solenoid-like superhelix structure (Fig. 1). mInsc-LBD runs antiparallel along the concave surface of the TPR superhelix of LGN and displays an electrostatic surface complementary to the LGN groove formed by the TPR motifs. mInsc-LBD can be roughly divided into three distinct binding regions: an α-helix, an extended region, and an antiparallel β-sheet, from which strands β1 and β2 are connected through a 13-residues loop. All of these three regions have distinctive features in LGN binding (Fig. 2). The large interacting surface area buried at the mInsc-LGN interface is approximately 4,200 Å^2, suggesting a stable interaction between mInsc and LGN. Indeed, mInsc bound to LGN with an estimated K_D value of approximately 2.4 nM. Because residues critical for mInsc–LGN interaction are completely conserved, key features of the mInsc-LBD–LGN-N complex structure appear to be maintained throughout the evolution of animals from insects to mammals.

Structural and biochemical analyses also helped to define residues that are crucial for mInsc-LGN interaction and revealed that the LGN TPR domain directly binds to the AGS3-binding protein Frmpd1 and its relative protein Frmpd4 in a manner similar to, but distinct from that of mInsc, whereas NuMA and the C-terminal region of LGN bind to the LGN TPR domain in a fashion different from that of mInsc and Frmpd proteins. To elucidate the relationship between mInsc and other LGN binding partners, we performed competition assays between mInsc and other partners. Interestingly, mInsc binds to LGN with the highest affinity among the partners investigated and efficiently replaces not only the Frmpd proteins but also NuMA and the C-terminal region of LGN, suggesting the priority of mInsc in binding to LGN. The competition between mInsc and NuMA to LGN may regulate spindle orientation during mitosis.

REFERENCES

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