Structural Basis of Arf6-MKLP1 Complex Formation on the Flemming Body Responsible for Cytokinesis

Cytokinesis is the process of cell division in which a single cell is divided into two daughter cells. Arf6 is a small GTPase involved in cytokinesis by localizing on the Flemming body (the midbody). In addition, vesicle transport from cytosol to Flemming body is also important for the completion of cytokinesis. MKLP1 (mitotic kinesin-like protein 1) is a family of kinesins involved in vesicle transport during cytokinesis, but it remains unknown how Arf6 and MKLP1 contribute to cytokinesis. Here, we show from a structural point of view how the formation of Arf6-MKLP1 complex is critical for the proper completion of cytokinesis.

Cytokinesis is the final stage of cell division. Before cytokinesis, a contractile ring constricts the plasma membrane in the equatorial region of a dividing cell to form a cleavage furrow, while an overlapping region of antiparallel microtubules from the central spindle gradually forms the Flemming body (the midbody) in the middle of the cleavage furrow. Finally, the cell undergoes abscission on either side of the Flemming body during cytokinesis. The cell shape drastically changes and membrane fission occurs during cytokinesis.

Arf (ADP-ribosylation factor) is a family of small GTPases that regulate membrane traffic by varying their GTP/GDP binding states. Arf6 can be localized to the plasma membrane and the endocytic system. It has been suggested that Arf6 is essential for the final stage of cytokinesis in mammalian cells. During cytokinesis, Arf6 transiently localizes to the Flemming body. Our previous study suggested that Arf6 is recruited to the Flemming body independently of Rab11- and FIP3-containing endosomes [1]. In this study, we determined the crystal structure of a complex between Arf6 and the C-terminal domain of MKLP1, which together with MgcRacGAP/Cyk4 constitutes the central spindlin complex at the Flemming body. Furthermore, our structure-based mutagenesis and siRNA-mediated knockdowns allowed us to critically test models of Arf6 recruitment to the Flemming body. Our findings demonstrate that the formation of Arf6-MKLP1 complex is crucial for the completion of cytokinesis [2].

The 3.0 Å crystal structure of the complex consisting of Arf6 full length (Q67L, GTP-bound form mutant) and the C-terminal domain (residues 690–807) of MKLP1 (cMKLP1) reveals a 2:2 Arf6-MKLP1 heterotetramer complex (Fig. 1). A unique extended β-sheet formed of 22 strands spans the entire Arf6-MKLP1 complex. The structure of Arf6 in the complex is similar to that of monomeric Arf6-GTPγS (PDB: 2J5X). On the other hand, the Dali server search revealed no solved structures similar to the present cMKLP1. Monomeric cMKLP1 is composed of five strands and a long loop including the short sheet region involved in the complex formation. Additionally, we confirmed the formation of this Arf6-MKLP1 complex in solution by a combination of SAXS measurements and cross-linking experiments.

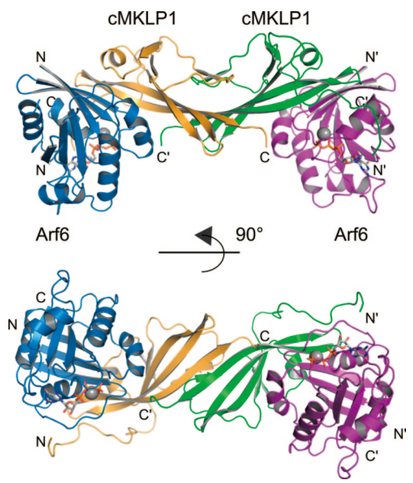

Figure 1
Overall structure of Arf6-MKLP1 complex. Cartoon representations of the Arf6-cMKLP1 complex are shown in two orthogonal views (side and bottom views, for upper and lower panels, respectively). The two Arf6 molecules are colored in skyblue and purple, and the two cMKLP1 molecules are colored in orange and green, respectively.

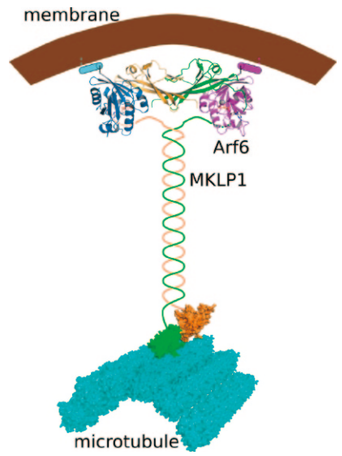

Figure 2
Model for membrane and microtubule interactions of the Arf6-MKLP1 complex. Arf6 molecules are colored in skyblue and purple with schematically represented N-terminal helices, and their myristate extensions are represented as dotted lines. MKLP1 molecules are colored in green and orange, respectively.

We have found that Arf6 is required for the binding of cMKLP1 in a GTP-dependent manner. The interface between Arf6 and cMKLP1 in the complex structure explains the GTP-dependent interaction between Arf6 and cMKLP1. Interestingly, the conformation of Arf6 changes significantly depending on its GTP/GDP-bound state. The conformational change of the switch 1 and switch 2 regions changes with a two-residue register shift in the interswitch region [3]. The β2 strand interacts with the β5 strand of cMKLP1 in the complex structure (GTP-bound form). In the GDP-bound form of Arf6, the switch 1 region is retracted. As a consequence, a new β-strand of Arf6, which is overlapping with the β5 strand of cMKLP1, is formed next to the β2 strand of Arf6 in a GDP-bound state. Taken together, these data strongly suggest that this is the reason why Arf6 can interact with MKLP1 only in its GTP-bound state.

The additional hydrogen bond formed between Tyr77^{Arf6} and Tyr754^{cMKLP1} in a hydrophobic pocket common among most small GTPases was important for the interaction between Arf6 and cMKLP1. Indeed, Arf6 (Q67L/Y77A) mutant abolished the interaction between Arf6 and cMKLP1, as shown by pull-down assay using the GST-tagged cMKLP1. Moreover, our mutant analysis confirmed that the interaction between Tyr77^{Arf6} and Tyr754^{cMKLP1} is critical for the proper completion of cytokinesis. Arf6 (Q67L/Y77A)-cMKLP1 (wild type) or Arf6 (wild type)-cMKLP1 (Y754A) mutant could not be co-localized on the Flemming body. Furthermore, cMKLP1 (Y754A) mutant marginally rescued the multinucleate phenotype, different from MKLP1 (wild type), in HeLa cells with depletion of MKLP1 by siRNA treatment.

We therefore propose that two activated Arf6 molecules bind to each homodimer of MKLP1 to form the presently solved complex structure on the Flemming body for a higher fidelity of completion of cytokinesis (Fig. 2).

REFERENCES

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BEAMLINES

5A, 10C and AR-NW12A

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