論文の内容の要旨

論文題目 Cryo-Electron Microscopy Study of KIF and Microtubule Complex
（KIF-微小管複合体のクライオ電子顕微鏡構造解析）

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Kinesin superfamily proteins (KIFs) are microtubule (MT)-based molecular motors that play fundamental roles in intracellular transport. Kinesins use energy from ATP to move along the MTs. Structural studies of kinesin motility have suggested that the energy generated by the hydrolysis of ATP is used for the active detachment from the MT track, whereas the energy produced by the binding to the MT, coupled with ADP release, allows the plus-end directed movement. Based on this alternating attachment and detachment of a kinesin catalytic head, the hand-over-hand model has been proposed to explain the processive, cooperative movement of dimeric kinesin. According to this model, the two catalytic heads alternately "step" so that kinesin is always attached to the MT. This means that one head cannot hydrolyze ATP to detach from the MT until the other head has released ADP to attach strongly to the MT. Hence, the mechanical process in which the catalytic head attaches to the MT, coupled with ADP release, defines the coordination between the two heads.

To elucidate the molecular mechanisms of the fundamental processive movement of kinesin on MT, kinesin structures before and after ADP release, i.e., the Mg-ADP state and the nucleotide-free state, are necessary. Cryo-EM structure of nucleotide-free kinesin complexed with taxol-stabilized GDP-MT (GDP-taxol-MT) was reported for some types of kinesins, KIF5 (kinesin-1/conventional kinesin) and Kar3 (kinesin-14). Both forms, however, take very different conformations, especially at the switch II element. The switch II conformation of KIF5 is similar to the crystal structure in the Mg-ADP state (ADP-like conformation), whereas the interface of Kar3 differs from

both the Mg-ATP form (ATP-like conformation) and the ADP-like conformation. The reason for the difference between KIF5 and Kar3 is still debated and the high-resolution structure remains elusive.

Understanding this key mechanical step in KIF5 motility on MT has attracted the attention of cell biologists and neuroscientists. In neurons, KIF5 transports several types of cargo to the axon, not to the dendrites. This polarized transport in neurons is possible because, in axons, GTP-MTs are enriched over GDP-MTs and are preferentially "searched for" as landmarks by KIF5. This high-affinity binding of KIF5 to GTP-MTs guides various cargoes to their correct neuronal destinations. Recently, the structure of GTP-MT was reported and the characteristic feature of KIF5 binding site was suggested to account for the different affinity between KIF5 and GDP-/GTP-MTs. To further understand the molecular mechanism of the fundamental relationship between kinesins and MTs, elucidation of the structures of KIF5 at the beginning of stepping, at the nucleotide-free state and complexed with GTP-MT, is necessary.

Here I describe the successful cryo-EM structure of nucleotide-free KIF5C complexed with GTP-state MT at 8.9 Å resolution. GTP-state MT was stabilized using GTP analog guanylyl 5'-a, b-methylenediphosphonate (GMPCPP). This analog promotes the polymerization of normal microtubules with the same polymerization rate to GTP, while the depolymerization rate is extremely slow, so that GMPCPP-MTs are mimic of GTP-state MTs. In silico docking of crystal structures with the cryo-EM structure revealed the mutual conformational changes of KIF5C and GMPCPP-MT. Nucleotide-free KIF5C complexes with GMPCPP-MT acquires a new conformation that I termed the "rigor conformation", by analogy with myosin. This conformation not only provides an important missing link in the structural analysis of kinesin, but also elucidates the molecular mechanism of the preferential binding to the GTP-MT. Furthermore, this is the first observation of the conformational change in the GMPCPP-MT induced by KIF5C binding. GMPCPP-MT predominantly changes its surface conformation of α- and β-tubulins with strengthening of the longitudinal contacts. These structural and functional analyses provide the molecular mechanism of the preferential binding of KIF5 to GTP-state microtubules.