X-Ray and Cryo-EM Structures Reveal Mutual Conformational Changes of KIF5C and GTP Microtubules upon Binding

The molecular motor KIF5C is an ATP-driven intracellular transporter, moving preferentially along GTP microtubules in axons. However, the initial state of KIF5C stepping on GTP microtubules has yet to be solved and how KIF5C distinguishes microtubules is poorly understood at the atomic level. We herein present the crystal structure of nucleotide-free KIF5C, and the cryo-electron microscopic structure of nucleotide-free KIF5C with GTP microtubules. Structural and functional analyses collectively reveal that the charged hydrophobic residues in KIF5C loop11 induce conformational changes and enable higher specificity to GTP microtubules than to GDP microtubules, explaining the preferential binding of KIF5C to GTP microtubules and the mechanism of directional transport.

Kinesin superfamily proteins (KIFs) are molecular motors that are responsible for transporting various molecular cargoes along microtubule (MT) tracks from one region of the cell to each of the destinations [1]. Our previous study revealed that GTP-MTs are enriched in the axon compared with dendrites [2]. The motor domain of the conventional type of KIF, KIF5C, selectively moves towards the axon and plays significant roles in axonal transport [3, 4]. KIFs convert chemical energy of ATP into mechanical stepping towards the MT plus-end. In dimeric KIF5C, two motor domains are linked to each other by a neck-linker, and alternatively attach to and detach from MTs through the cycle of ATP hydrolysis [5-10]. Detachment occurs in collaboration with the hydrolysis of ATP, and attachment occurs by the release of ADP, i.e. in the nucleotide-free state. To elucidate the molecular mechanisms of processive stepping of KIF5C on MT, it is necessary to clarify the KIF5C structure in the nucleotide-free state. We therefore set out to solve the crystal structure of KIF5C without any bound nucleotide.

We crystallized the KIF5C motor domain (mouse KIF5C residues 1–334 with a 7-His-tag in C-terminal) and determined the structure at the resolution of 2.9 Å (**Fig. 1A**) [11]. After trials of several additives such as EDTA or apyrase, we finally found that the C-terminal peptide of KIF5C accelerates the release of ADP from the nucleotide-binding pocket. In comparison with the previously solved structures, the switch II and neck-linker of the nucleotide-free KIF5C adopts a conformation that is similar to that of KIF5 in the ATP state (ATP-like conformation), whereas the switch I conformation adopts an ADP-like conformation (**Fig. 1B**) and is slightly different from the ATP-like conformation (**Fig. 1C**) [5, 12]. In the ADP-AlF$_4$ bound state (red model), switch I completely closes the nucleotide-binding pocket with the formation of Mg-stabilizer between Arg191 in switch I and Asp232 in switch II, which retains the nucleotide in the pocket. In the nucleotide-free state (gray model), however, switch I helix α3 rotates 12 degrees so that the C-terminal region of α3 and the following loop L9 move away from the nucleotide-binding pocket (arrows). The rotation of α3 and L9 breaks the Mg-stabilizer, thus Mg-ADP is destabilized, and the pocket is opened to facilitate the exchange of ADP with Mg-ATP [13].

In silico docking of this crystal structure into the cryo-EM structure of KIF5C complexed with MT solved by the real space helical reconstruction method [14], at 8.1 Å resolution, revealed the mutual conformational changes of KIF5C and MT (**Fig. 2A**). Nucleotide-free KIF5C complexed with MT polymerized with GTP non-hydrolyzable analogue GMPCPP (GMPCPP-MT) acquires a new conformation where switch II helix α4 exists distal from the nucleotide-binding pocket and switch I, coupled

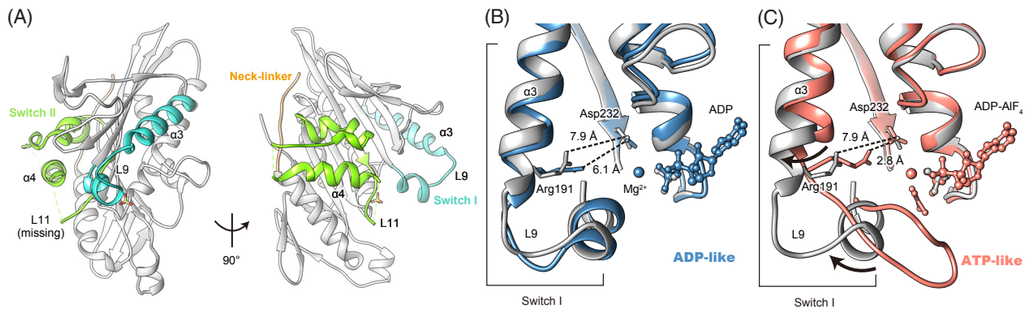

Figure 1: (A) Overall crystal structure of nucleotide-free KIF5C. **(B, C)** Close-up view of nucleotide-binding pocket of nucleotide-free KIF5C (gray), in comparison with ADP-like structure (blue; PDB ID: 1BG2) **(B)** and ATP-like structure (red; PDB ID: 4HNA) **(C)**.

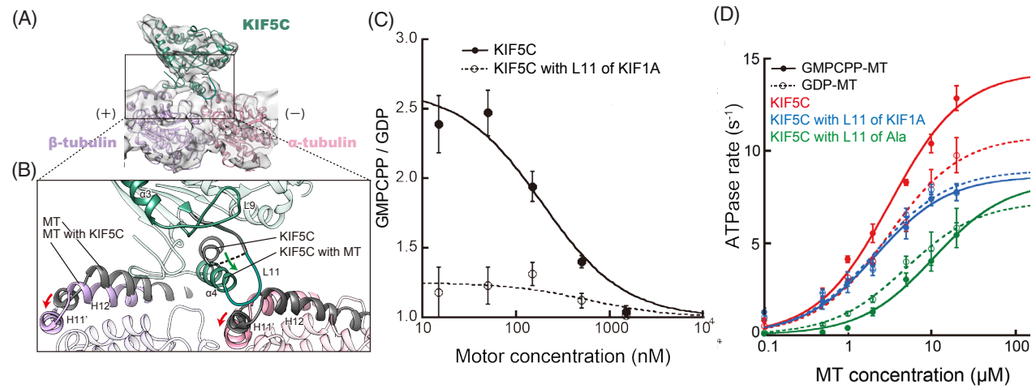

Figure 2: (A) Cryo-EM map of nucleotide-free KIF5C and GMPCPP-MT complex (gray), and the docked models. **(B)** Mutual conformational changes of KIF5C and GMPCPP-MT upon their binding. **(C)** Binding assays of KIF5C to MTs. The affinity of KIF5C for GMPCPP-MT (solid line) was approximately three times higher (Kd = 190 ± 70 nM) than that for GDP-MT (Kd = 500 ± 170 nM). However, mutant KIF5C with L11 of KIF1A (swap mutant) completely abolished the substrate specificity, reflected in the similar dissociation constants for GMPCPP- and GDP-MT (Kd = 590 ± 170 nM vs. Kd = 730 ± 210 nM, respectively) (broken line). These results showed that KIF5C uses L11 to discriminate MT structures. The mean and SEM were measured from 120 MTs at each point. **(D)** ATPase activities of the wild-type KIF5C (red), the L11 swap mutant (blue), and the Ala mutant (a triple alanine mutation introduced into the three charged regions in L11) (green), with GMPCPP-MT (solid lines) and taxol stabilized GDP-MT (broken lines). The swap mutation abolished the difference between GMPCPP-MT and GDP-MT and the Ala mutation lessened the difference, suggesting that at least one of these residues contributes to substrate specificity. Also, the alanine mutations increased the $K_{M, MT}$ for both GMPCPP-MT and GDP-MT, indicating that one, if not all, of these residues is involved in the interface common to GMPCPP-MT and GDP-MT.

with the elongation of L11 (**Fig. 2B**, green arrow). The direct binding of KIF5C L11 pushes the surface domains of α-tubulin and β-tubulin downward (**Fig. 2B**, red arrows), in comparison with the naked GMPCPP-MT structure [15]. These conformational changes affect the interaction of both inter-tubulin-dimer contact and intra-tubulin-dimer contact, stabilizing the longitudinal lattice of GMPCPP-MT. These observations may provide the structural key to solving the molecular mechanism of the cooperative binding of KIFs to MTs [16]. Functional experiments of KIF5C using L11 mutants revealed that the binding between charged hydrophobic residues in L11 and the specific surface structure in the GMPCPP-MT accomplishes a higher specificity than that with GDP-MT (**Figs. 2C** and **2D**), suggesting L11 is essential in preferential transport of KIF5C to axons where GTP-MT is enriched over GDP-MT. To further understand the molecular mechanism and structural background of the fundamental relationship between KIFs and MTs, elucidation of the structures at higher resolution is necessary.

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