Abstract

Molecular motors are essential for multiple cellular functions, such as intracellular cargo transport, cell division, cell motility, cytoskeleton arrangement, etc. There are three classes of molecular motors: kinesin, dynein, and myosin. While kinesin and dynein are microtubule-based motors, myosin is actin-based. All molecular motors drive their movement by conversion of chemical energy into mechanical work. Malfunction of these molecular motors has been implicated in multiple disease conditions, such as neurodegeneration and cancer. Kinesin-3 is one of the largest families, consisting of KIF1, KIF13, KIF14, KIF16B, and KIF28 subfamilies. Kinesin-3 motors are inherently fast, superprocessive, and have increased microtubule landing rate. Kinesin-3 motors are crucial for various cellular processes and possess unique motility properties. Despite having significant physiological and clinical importance, the underlying chemomechanical properties and cellular functions of kinesin-3 motors remain poorly understood. In this dissertation, I aim to investigate molecular mechanisms underlying the unique properties of kinesin-3 motors using multiple *in vitro* and *in vivo* approaches.

Members of kinesin-3 family are inherently fast and superprocessive and exhibit increased microtubule landing rate, compared to other kinesin families. However, the mechanochemical properties of kinesin-3 family motors that enable their superprocessivity and higher microtubule landing remains poorly understood. Full-length and constitutively active kinesin-3 motors were purified from the Sf9-baculovirus expression system. Single-molecule motility analysis using Sf9-purified motors showed fast, superprocessive motility of kinesin-3 motors along the microtubules. Using colorimetric ATPase assays, we demonstrate that constitutively active kinesin-3 motors possess 10-fold higher ATPase activity compared to the well-studied

constitutively active kinesin-1. In contrast, full-length kinesin-3 motors are inactive and exhibit minimal basal activity. Intriguingly, the velocities of kinesin-3 family motors inversely correlate with their microtubule-binding affinity. Furthermore, microtubule gliding assays of constitutively active kinesin-3 motors revealed smooth microtubule sliding and efficient crossing over. Moreover, microtubules propelled by kinesin-3 motors showed enhanced bending of microtubules without apparent change in the gliding velocities. These findings unveil the effect of enzyme catalysis and microtubule affinity on motor processivity. Thus, we propose that a fine balance between ATP hydrolysis rate and nature of motor interaction with the microtubule together can achieve family-specific mechanical outputs.

Membrane tubulation is a hallmark of recycling endosomes (RE), mediated by KIF13A, a kinesin-3 family motor. KIF13 motors are uniquely regulated by the presence of a proline residue located at the interface of neck-coil (NC) and CC1 domains. This proline induces a kink and favors intramolecular interaction between NC and CC1, which retains the motor in a monomeric and inactive state. Interestingly, deletion of proline (ΔP390) releases this restraint and results in a stable straight conformation, allowing motor dimerization and subsequent motility along the microtubule. Although wild type KIF13A is inactive *in vitro*, it plays an essential role in recycling endocytosis. Understanding the regulatory mechanism of KIF13A in RE tubulation and cargo recycling is of fundamental importance but is overlooked. Here, we report a unique mechanism of KIF13A dimerization modulated by Rab22A, a small GTPase, during RE tubulation. Remarkably, Rab22A plays an unusual role by binding to NC-CC1 domains of KIF13A, relieving proline-mediated inhibition, and facilitating motor dimerization. As a result, KIF13A motors produce balanced motility and force against multiple dyneins in a

molecular tug-of-war to regulate RE biogenesis and homeostasis. Together, our findings demonstrate that KIF13A motors are tuned at a single-molecule level to function as weak dimers on the cellular cargo.

Next, we investigated whether KIF13B that belongs to same subfamily as KIF13A plays any role in recycling endocytosis. Interestingly, we report for the first time that KIF13B forms recycling endosome tubules, indicating that two different motors (KIF13A and KIF13B) are involved in the same process. We show that KIF13A is loaded onto recycling endosome before KIF13B and is able to overcome inactivity of KIF13B. In contrast, KIF13B was not able to overcome KIF13A inactivity. Interestingly, KIF13B forms more RE tubules compared to KIF13A. Results show that Rab10 relieves proline-mediated inhibition in KIF13B via binding to its NC-CC1 domains. We also demonstrate the role of CAP-Gly, an unusual domain, in KIF13B regulation. We conclude that although two motors are involved in the same cellular process, they both serve at different time and rate to ensure efficient cargo recycling. Overall these studies provide in-depth understanding of kinesin-3 motors regulation and functioning and pave way for future investigations regarding role of these motors in disease conditions.