Indian Institute of Science
Centre for BioSystems Science and Engineering

BSSE Colloquium

27 October 2020, 04:00 PM, Virtual

Single molecule imaging of cytoplasmic dynein in vivo reveals the mechanism of cargo capture and motor activation

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ABSTRACT:

In the crowded confines of the eukaryotic cell, where large cargo (> 100 nm) are diffusionally constrained, intracellular transport by motor proteins plays a crucial role in the exchange of material between various compartments of the cell and maintaining cellular homeostasis. Aberrant intracellular transport has been implicated in several disease states, including neurodegenerative disorders like Alzheimer's and Parkinson's disease. Cytoplasmic dynein and the kinesin family of motor proteins drive cargo movement on microtubules. Eukaryotes contain ~ 40 different types of kinesins with most carrying specific cargo towards the plus ends of the microtubules. While there are minus end directed kinesin motors (like kinesin-14 involved in mitosis), in many cell types cytoplasmic dynein is the only minus end directed motor protein and thus understanding how a single motor can transport various kinds of cargo remains an interesting question. Recent studies have revealed that dynein is inactive by default and is activated upon the formation of a tripartite complex containing dynein, the dynein regulator dynactin, and the cargo (via a cargo adaptor such as BicD2). However, how processive complexes come together in the complex in vivo milieu is unknown. In this talk, I will present my research over the past five years to understand how single molecules of dynein are activated to transport intracellular cargo.

We first improved HILO microscopy to visualize fluorescently-labeled single dynein molecules in HeLa cells and observed that only ~30 % of the dyneins binding to the microtubule moved in a processive fashion

towards the minus ends. Moreover, the dynein molecules detached from the microtubule after a residence time of ~ 0.7s. We hypothesized that the processive dynein molecules represented dyneins that were activated upon binding to cargo-dynactin complexes that were anchored to microtubules and that the cargo-dynactin-dynein complex moves in short bursts. To test this hypothesis, we first focused on understanding how dynein binds to dynactin in cellulo. A prevalent idea is that the +TIP protein EB1 drives the formation of dynactin clusters at the microtubule plus ends which then recruit dynein onto them. These clusters of dynein-dynactin at the cell periphery have been proposed to bind incoming cargo, thereby achieving motor activation and cargo transport. We reinvestigated the localization of the p150 subunit of dynactin in live cells using confocal microscopy and observed that unlike dynein, dynactin was persistently bound to microtubules. Further, we employed high-resolution microscopy and image analysis to unveil that a majority of dynactin is bound along the microtubule lattice and that plus end clustering of dynein is unlikely. Together, this indicated that stochastic binding of dynein along the microtubule length enables its interaction with dynactin and cargo to form the tripartite complex and thereby effect motor activation. Accordingly, when we depleted the p150 sub-unit of dynactin using RNA interference, we observed that the proportion of minus-end directed runs reduced to ~ 19% with a concomitant increase in the that of plus-end directed runs to ~ 30%.

Next, we utilized a three-pronged approach to probe the interaction of dynein with the last component of the tripartite complex – the cargo. (i) We used high resolution confocal microscopy to verify that endosomal cargo were closely associated with the microtubules. (ii) We visualized the movement of endocytosed dextran and observed that ~ 80 % of the dextran vesicles moved less than 1 μm within a 10 s interval. Moreover, the dextran vesicles underwent short runs with a run time of ~ 0.3 s, similar to that of single molecules of dynein. (iii) Finally, we employed high speed dual-channel imaging to visualize dynein and endocytosed cargo simultaneously. We observed that binding of a single dynein molecule to a stationary cargo on the microtubule was sufficient to bring about a short minus-end directed run. Moreover, we estimated ~3-4 dynein molecules on the dextran vesicles inside the cell. This represents the first visualization of motor-cargo interactions and activation of dynein at the single molecule level inside mammalian cells.

Taken together, we propose that dynein activation and cargo transport is achieved in living cells when single molecules of dynein stochastically bind to cargo-dynactin complexes that are anchored to microtubules.