McGill Faculty of Engineering

RESEARCH SEMINAR

Collective dynamics of kinesin and dynein in intracellular transport

Dr. Adam G. Hendricks
Pennsylvania Muscle Institute and Department of Physiology
University of Pennsylvania

Cytoplasmic dynein and members of the kinesin superfamily drive the long-range transport of proteins, mRNA, organelles, and signaling molecules along the microtubule cytoskeleton. Active transport is particularly critical for highly polarized cells such as neurons, which can exceed one meter in length. Accordingly, defects in kinesin or dynein cause neurodegenerative disease in humans and mouse models, and impairment of axonal transport has been identified in models of many neurodegenerative diseases including amyotrophic lateral sclerosis, Alzheimer's, and Huntington's diseases. The single-molecule properties of these motor proteins has been well-characterized using biochemical and biophysical methods. However, in the cell, plus- and minus-end directed motors function in teams and interact on the same cargo to drive bidirectional transport. Motor function is regulated by binding partners and effectors. In addition, the motors must navigate a complex cytoskeletal network, decorated with associated proteins, through the crowded and viscoelastic cytoplasm.

To probe the collective dynamics of an endogenous complement of motors, we examined the forces on bidirectional cargoes in living cells. We developed methods to calibrate optical traps in living cells, taking into account the local viscoelastic environment of each cargo. Using these techniques, we examined forces on latex beads taken up into mammalian macrophages, which are encased in native phagosomes that are transported by kinesin-1, kinesin-2, and dynein motors. Forces exerted by kinesin-1 and kinesin-2 are indicative of 1-3 motors which often detach at forces below the unitary stall force of ~5 pN. In contrast, multiple peaks in the dynein force histogram at 1-2 pN intervals suggest collective transport by many motors (up to 13). During high-force (|F| > 10 pN) events driven by multiple motors, the cargoes often advanced in 8-nm steps, suggesting that multiple kinesin and dynein motors step in a correlated manner at high loads. We compared forces in living cells to those produced by isolated latex bead-containing phagosomes along taxol-stabilized microtubules in vitro. The force histograms display similar peaks, indicating that individual motors produce similar forces in vitro and in living cells. However, forces up to ~ +/-20 pN were observed in living cells compared to maximum forces of ~ +/-12 pN in vitro, suggesting that for the same type of cargo, more motors are engaged in the

cell. The dense and viscoelastic cellular environment may promote motor binding by constraining diffusion, and/or by allowing motors on a single cargo to interact with multiple microtubules.

Future work will focus on how motor dynamics are modulated and regulated by the cellular environment. In particular, I propose developing novel methodologies including: (1) FRET-based sensors to measure the time-varying number of motors on a cargo that are engaged to the microtubule and their mechanochemical state, (2) optical trapping techniques to measure the forces on cargoes in living cells and on extracted cytoskeletal networks, and (3) tracking in living cells with high temporal (<10 μs) and spatial (<1 nm) resolution based on dark field imaging of gold nanospheres. These studies will result in novel insights into the function of motor proteins in intracellular transport.

Thursday, March 28
2 p.m.
MD 267