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BSSE Annual Work Presentation

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Functional regulation of cytoplasmic dynein in vivo

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ABSTRACT

Cytoplasmic dynein is a motor protein that plays a crucial role in various cellular functions like cell division, centrosome assembly and intracellular transport. Intracellular cargo transport is essential for cell survival and its perturbation has been implicated in neurodegenerative diseases. In many cell types, cytoplasmic dynein is the only minus-end directed motor protein transporting intracellular cargo. Therefore, understanding the function and regulation of dynein is of substantial importance. In vitro experiments on dynein have provided insight into dynein's structure, stepping and force generation on microtubules. Recently, dynein's interaction with its regulator dynactin and cargo adapters, specifically BicaudalD2(BicD2) has been studied at the single molecule level in vitro conditions and it has been observed that the formation of dynein-dynactin-cargo adapter complex is a prerequisite for processive motion. However, given dynein's diverse interactions within the cell, how dynein's function is spatio-temporally regulated in vivo is not understood. In this talk, I will describe the progress we have made towards understanding the same. To visualize dynein's interaction with the microtubules and its subsequent movement at the singlemolecule level we modified Highly Inclined Laminated Optical (HILO) sheet microscopy which allowed us to image a small region of the cell within a single plane and along its height thereby effectively countering photobleaching of the complete cell and allowing long duration imaging (~30s) at high frame rates (~25fps). Our imaging revealed that while a large fraction of the dynein inside a cell is freely diffusing in the cytosol, with binding events to microtubule visible distinctly, only a fraction of dynein that bound from the cytoplasm to the microtubule displayed net processive motion towards the minus end of the microtubules. Interestingly, we observed that in a region which was 1 μm higher than the lowest region of the cell, the fraction of dynein binding events that resulted in net processive motion was also higher. In very thin (~300nm) sections of cells, we observed dynein working in groups to transport cargo indicating the coexistence of different dynein behaviors within the same cells. Our results indicate that in the thinnest regions of the cell where diffusion is sterically hindered, dynein perhaps relies on EB1/Dynactin mediated accumulation at the plus tips of the microtubules to encounter cargo. On the other hand, in thicker regions of the cell where diffusion is not hindered, dynein might interact with cargo by independently binding to the microtubules from the cytoplasm. Our current experiments are focussed towards understanding how multiple modes of dynein-driven transport might arise as a consequence of the distinct differences in dynein behavior within the same cell.