MAR10-2009-020080

Abstract for an Invited Paper
for the MAR10 Meeting of
the American Physical Society

Molecular approach to intracellular cargo transport

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Landmark discoveries in the study of cytoplasmic motors have been made through advances in single molecule biophysics and detailed mechanistic models exist for kinesin and dynein. However, the function of motors in physiological conditions has not been carefully tested. In cells, more than few dyneins can attach to the same cargo and interact with the opposite polarity motors of kinesin. To study the molecular crosstalk between the motors, we have used intraflagellar transport (IFT) in *Chlamydomonas reinhardtii* as a model system. Ultrahigh spatio-temporal tracking of single cargo movement showed that IFT particles move for long distances unidirectionally with 8 nm increments, agreeing with measured step sizes of kinesin and dynein. To measure how many motors transport each cargo, we have linked large polystyrene beads to internal IFT particles through a transmembrane protein. Force measurements indicated that, on average, 3-4 motors transport cargoes in each direction. The results showed that IFT motors are tightly coordinated and might be involved in recycling each other to the appropriate end of the flagellum.