MYOSIN-VB CONTINUES TO BIND ACTIN DURING MICROTUBULE-BASED TRANSPORT

John A. Mercer and D. William Provance Jr.

McLaughlin Research Institute, Great Falls, MT 59405, USA

Cellular organization involves the intracellular trafficking of components through both actin- and microtubule (MT)-based movement. Sensitized mutant (and wild-type control) myosin-Vb was tagged with eGFP, expressed in HeLa cells and imaged before and after the microinjection of N6-phenylethyl ADP (PE-ADP), which selectively and specifically inhibits the sensitized mutant Myo5b by inducing its tight binding to actin (Provance et al., PNAS 101:1868, 2004). Two prominent patterns of myosin-Vb localization were observed: tubulovesicular structures in the cell periphery and discrete vesicles. The peripheral structures had highly dynamic edges from which vesicles budded. The discrete vesicles exhibited both actin- and microtubule-based movement, categorized on the basis of their speeds, trajectories, and sensitivity to latrunculin A or nocodozole treatment. In cells expressing the sensitized mutant myosin-Vb and injected with PE-ADP, all movement ceased within 2 min. The tubulovesicular peripheral structures became static and spherical. Vesicles also were no longer observed to originate from them and other vesicles froze within the cytoplasm. None of these effects were observed under control conditions consisting of wild-type myosin-Vb with PE-ADP, wild-type myosin-Vb injected with tracer only, and sensitized mutant myosin-Vb with tracer only. We conclude that the cessation of movement under experimental conditions is a direct result of sensitized mutant myosin-Vb binding to actin. Our data strongly support a model in which myosin-Vb cooperates with MT-based motors on peripheral endosomes to create opposing forces necessary for vesicle formation. Furthermore, those vesicles, which continue to be labeled by eGFP-tagged myosin-Vb, are constantly capable of interacting with actin through myosin-Vb activity, even as passengers undergoing MT-based transport. In addition, we plan to present data on the function of myosin-Vb in hippocampal neurons.
jam@mri.montana.edu
http://www.montana.edu/wwwmri/mercer.html