Myosin motors: Live cell imaging and state of the art enzymology

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Myosins are molecular motors that perform mechanical work along actin filaments which is then coupled to diverse cellular processes. Myosins carry the ability to couple ATP hydrolysis to produce mechanical work via the conserved chemomechanical transduction pathway. In this presentation, we focus on the function of a recently discovered human Myo19 to localize mitochondria to filopodia tips under stress conditions. Notably, we observed that Myo19 localization to filopodia tips with the mitochondria displays directional movement on actin filaments, characteristic of active motor transport.

To investigate how Myo19 unique enzymatic adaptation are coupled to its cellular function as an active transporter of mitochondria to filopodia we studied the effect of point mutations in a highly-conserved residue at position Trp^{140} in the motor domain (compared to Val & Glu found in 33/38 human myosin family members). We identified that converting Myo19 unique Trp^{140} to Val or Glu inhibited localization of the mitochondria to the filopodia tips. Our *in vitro* detailed kinetics analysis of WT Myo19-3IQ and a specific mutant, $Myo19^{W140V}$-3IQ, revealed altered steady state parameters with a 5-fold weaker $K_{m,ATPase}$ for the mutant with a bit faster k_{cat}. We then utilized transient kinetics to reveal that $Myo19^{W140V}$ loss of function is due to the mutant's lowered duty ratio by 7-fold compared to WT. This provides evidence that alteration of the mutant duty ratio by point mutation strongly hindered its ability to propel mitochondria motility in cells. The lower duty ratio suggests that actin based mitochondrial motility is carried out as an ensemble of motors rather than single motors.

Finally, by perturbing myosins cellular function with strategic point mutations in the motor domain hindering specific signaling of their chemomechanical transduction pathway, combined with *in vitro* transient kinetics provide very powerful tool to study molecular motors of the cytoskeleton.