

# Elucidation of the *Drosophila* Myosin Filament Binding Protein Flightin: An Electron Microscopic Approach

Lori Nyland\*, Jim Vigoreaux\*, and Teresa Ruiz\*\*

\* Dept. of Biology, University of Vermont, Burlington, VT 05405

\*\* Dept. of Molecular Physiology and Biophysics, University of Vermont, Burlington, VT 05405

Muscle contraction is a cellular phenomenon that involves the coordinated movement of the molecular assemblies of myosin thick filaments and actin thin filaments sliding past each other within sarcomeres. Even though myosin is the most prevalent protein in muscle, little is known about the proteins coupled to myosin thick filaments. Within *Drosophila* flight muscle it has been shown that the myosin thick filament coupled protein, flightin, is necessary for maintaining the structural integrity of the filaments and also for regulating their length (1). It has also been shown that flightin, a 20kD protein, binds to the rod portion of myosin (2). However, the structure and distribution of flightin along the myosin thick filament has yet to be definitively determined. A high resolution three-dimensional reconstruction would allow us to elucidate the relationship between flightin and the myosin thick filament and possibly provide a better understanding of the connection other myosin thick filament coupled proteins have with the myosin thick filament.

In this study, we have acquired single axis tomography tilt series of negatively stained *Drosophila* indirect flight muscle myosin thick filaments in order to obtain a reference volume. Alignment is facilitated by the presence of ordered myosin heads. We used both blebbistatin and alkaline phosphatase to order myosin heads along the filaments, which has been shown effective for vertebrate and invertebrate myosin thick filaments (3, 4) and is the first time it has been demonstrated in myosin thick filaments from *Drosophila* indirect flight muscle. The tomographic reconstruction will serve as a reliable reference for the alignment of 2D images of myosin thick filaments possessing ordered heads. These images will permit us to obtain a 3D reconstruction of the filament with higher resolution. Furthermore, we anticipate being able to interpret unique features of the myosin thick filaments from *Drosophila* as we proceed.

## References:

1. Contompasis et al. (2010), J. Mol. Biol. 395:340-348.
2. Ayer et al. (2003), Cell Biochem. Biophys. 38:41-54.
3. Zhogbi et al. (2008), PNAS 105:2386-2390.
4. Alamo et al. (2008), J. Mol. Biol. 384:780-797.



Figure 1: Tomographic slices from *Drosophila* flight muscle myosin thick filaments. Scale bars = 25 nm. (A) Effect of no treatment with blebbistatin and alkaline phosphatase. Note the disordered myosin heads projecting away from the filament. (B) Effect of treatment with blebbistatin and alkaline phosphatase. Note the ordered heads lying along the filament.