

## SANFORD I. BERNSTEIN

### Molecular Genetic Analysis of Muscle Gene and Protein Function During *Drosophila* Development

We use the fruit fly, *Drosophila melanogaster*, to explore gene expression and protein function in muscle cells. Defects in contractile proteins cause human cardiac and skeletal muscle disease. Therefore, it is useful to pursue an understanding of contractile protein function in an organism that is readily manipulated by genetic and transgenic means. Our integrative approach allows us to study muscle from the molecular level (using biochemical and biophysical assays), through the cellular level (using microscopy and fiber mechanical assays) to the level of whole organism muscle function.

The molecular motor of muscle is myosin heavy chain, a protein that interacts with actin to cause muscle contraction. We determined that *Drosophila* possesses a single myosin heavy chain gene, and that multiple forms of the myosin protein are produced by the process of alternative RNA splicing. Specific regions of the myosin RNA are included in specific muscle types to yield myosin protein isoforms. The regions that differ among the isoforms must be critical for the myosin-mediated ultrastructural and physiological differences among muscle types. In collaboration with Dr. Ron Milligan (the Scripps Research Institute), we determined the location of the isoform differences on the three dimensional map of the myosin molecule. Using these data, we developed hypotheses regarding how these differences can affect myosin function.

To experimentally test our hypotheses on myosin isoform function, we developed transgenic strains of *Drosophila* that express the wrong isoforms in particular muscle types. As an initial approach, we showed that the normal myosin gene can rescue muscle defects associated with myosin null mutations. Interestingly, additional copies of the myosin gene result in defective muscles as well, due to an overabundance of myosin-containing thick filaments. We next succeeded in expressing the embryonic form of myosin in adult flight muscle. To our surprise, the adult muscle assembles normally using the embryonic protein, indicating that structural properties of the muscle are not affected by the isoform of myosin present. However, the flight muscles do not function when the embryonic myosin substitutes for the normal form, indicating that myosin isoforms are functionally different.

As a result of our efforts, we created a fly line that expresses a single myosin isoform in all its muscle

types. We have produced additional lines that express other myosin isoforms. Currently, we are isolating single isoforms of the myosin protein from these lines to determine the biochemical and biophysical properties that are encoded by particular variable regions of the protein, e.g. actin binding ability, ATPase kinetics, *in vitro* thin filament motility, *in vitro* force generation. Transient kinetic studies are performed in collaboration with Dr. Michael Geeves (University of Kent at Canterbury) while single myosin molecule assays are performed in collaboration with Dr. Justin Molloy and Dr. John Sparrow (University of York). Muscles with altered myosin isoforms are studied in collaboration with Dr. David Maughan (University of Vermont) to assess how mechanical properties are affected by the alterations. Overall our approach should lead to a complete understanding of the *in vitro* and *in vivo* properties imparted by particular regions of the myosin molecule

We are pursuing similar functional studies on alternative forms of the thick filament protein paramyosin. We discovered that the gene encoding this alpha-helical protein also produces a novel muscle protein, miniparamyosin. By mutational analysis, isoform substitution, biochemical studies and the use of the yeast two-hybrid interaction assay, we intend to define the role of paramyosin and miniparamyosin in determining muscle structure and function.

We are studying elements responsible for tissue-specific transcription of contractile protein genes during muscle development. We attach a putative transcriptional promoter region to a "reporter gene" that is then returned to the *Drosophila* genome. The transgenic lines readily indicate the location of transgene expression upon histochemical treatment. We then produce deletions of the suspected transcriptional elements in the construct and make additional transgenic lines to find which muscle-specific transcriptional elements we have deleted. We are using both genetic and biochemical approaches to discover the trans-acting factors that activate contractile protein genes by binding to the transcriptional elements. We are also interested in the regulation of post-transcriptional contractile protein gene expression, i.e. the elements governing alternative RNA splicing. Using an *in vitro* splicing system, production of transgenic lines containing *in vitro* manipulated genes, as well as *Drosophila* genetics, we are identifying cis-acting signals and trans-acting factors responsible for the tissue-specific regulation of RNA splicing. Our studies on the molecular biology of contractile protein genes yield insight into how gene expression is regulated during organismal development.

## Representative Publications

Wells, L., K.A. Edwards and S.I. Bernstein. Myosin heavy chain isoforms regulate muscle function but not myofibril assembly. *EMBO J.* 15: 4454-4459 (1996).

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Kronert, W.A., A. Acebes, A. Ferrús and S.I. Bernstein. Specific myosin heavy chain mutations suppress troponin I defects in *Drosophila* muscles. *J. Cell Biol.* 144: 989-1000 (1999).

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