

# An Integrated View of Insect Flight Muscle: Genes, Motor Molecules, and Motion

David W. Maughan and Jim O. Vigoreaux

*Substituting an alanine for serine in the regulatory subunit of the motor protein myosin dramatically alters Drosophila's flight ability. Power output, at all levels of the flight system, is reduced. This example of deciphering a protein's function by producing malfunctions illustrates the broadening use of molecular genetics in integrative biology.*

For decades biologists have sought to understand how the mechanical and metabolic demands of animal flight are met. This has been especially true of insects, whose flight systems face an enormous task of generating and transmitting enough power to overcome gravity, air resistance, and drag. A major challenge has been to identify experimental parameters that allow function to be linked at all levels of structural organization.

## The "power of ten" in structure and function

The fruit fly *Drosophila melanogaster* is a powerful experimental model in which to study integrated function, especially the structural and functional basis of flight. In Fig. 1 we illustrate the different organizational levels of the *Drosophila* flight system, separated by powers of ten. At the top is the whole fly (x1) and its behavior, which, in the case illustrated, is flight toward a strong attractant (x0.1). Continuing clockwise, the fly's thorax houses the flight system (x10), which consists of the wings, wing hinges, thoracic cuticle, and underlying musculature, including two sets of large power muscles oriented more or less perpendicularly to one another (x100). For simplicity, somatic sensory organs and components of the nervous system that initiate activity and enervate the muscles are not included in the illustration. Small control muscles are also not shown. Because the power muscle cells are attached to the cuticle and are only connected indirectly to the wings, they are called "indirect" flight muscles (IFM). The IFM are structurally and function-

ally distinct from the control muscle cells, most of which attach directly to the wing hinge and help modulate flight (3).

To achieve enough power for lift, the fruit fly must beat each wing up to 240 times/s over a span of  $\sim 170^\circ$ . Mechanical power is proportional to the cube of both wing beat amplitude and frequency, both of which are large in this case. The typical power generated, 80 W/kg muscle mass (2), is close to the maximum power available from fast muscle relying largely on aerobic metabolism ( $\sim 100$  W/kg; Ref. 6). The power generated by the whole fly,  $\sim 31$   $\mu$ W, is sufficient to propel the fly upward without difficulty (flight ability index, 5.8 of 6.0). Fast cameras and charge-coupled optical devices are used to measure the wing beat amplitude and frequency, from which mechanical power is calculated.  $\text{CO}_2$  gas analyzers are used to measure energy consumption, from which metabolic power output is calculated. Flight efficiency (ratio of mechanical to metabolic power) is 10%, about the same value as reported for other insects (7).

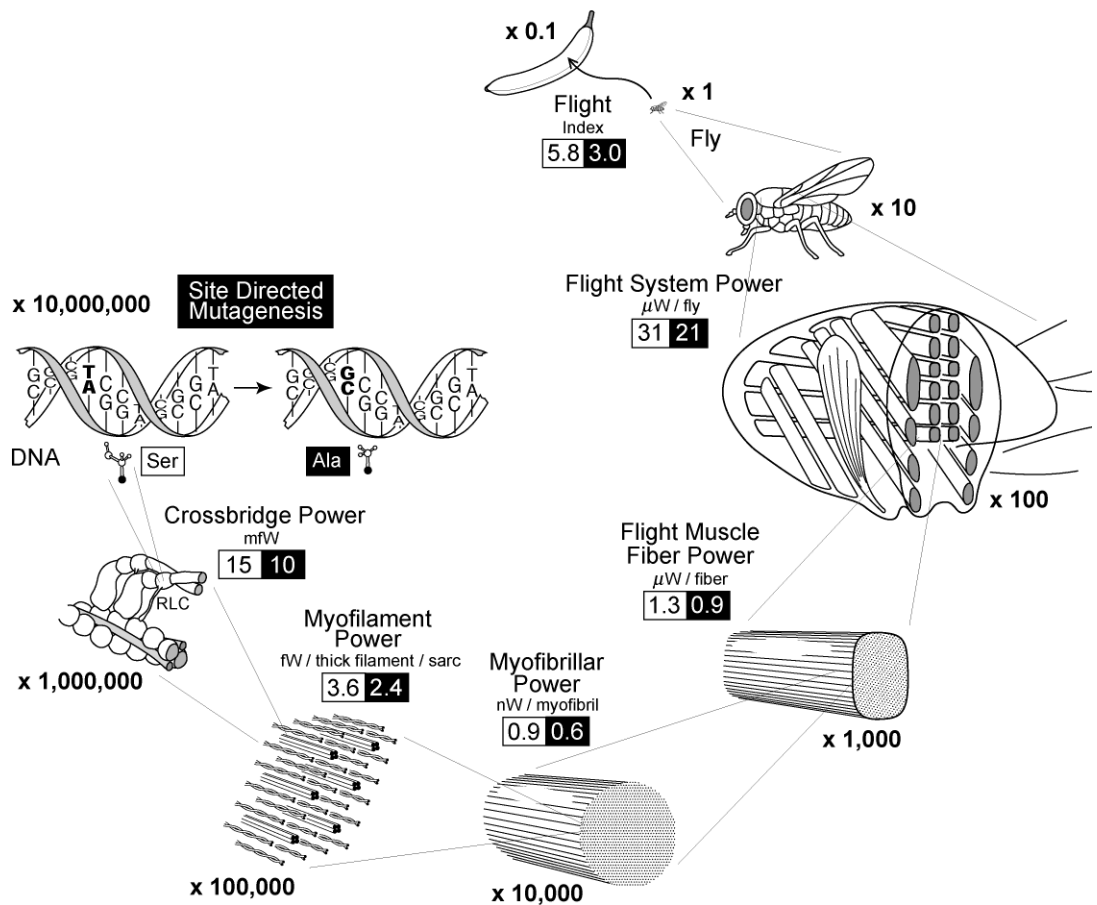
The two sets of IFM, the dorsal ventral muscles (DVM) and the dorsal longitudinal muscles (DLM), are primed with intracellular  $\text{Ca}^{2+}$  released from calcium stores by intermittent nervous stimulation. Just before flight, the fly jumps abruptly and becomes airborne. The jump is caused by the contraction of the tergal depressor of the trochanter (TDT), the triangular muscle underlying the IFM in Fig. 1. The TDT not only sets the fly in motion, but it also lengthens the DLM oriented perpendicular to it (and shortens the parallel DVM). The DLM responds to stretch with a delayed rise in tension (the stretch activation response), causing it to contract. This in turn causes a reciprocal stretch and delayed contraction in the DVM. As the two sets of muscles alternately contract, the thorax oscillates rapidly, caus-

---

*"...the fruit fly must beat each wing up to 240 times/s. ..."*

---

*D. W. Maughan and J. O. Vigoreaux are in the Department of Molecular Physiology & Biophysics and the Department of Biology, respectively, of the University of Vermont, Burlington, VT 05405, USA.*

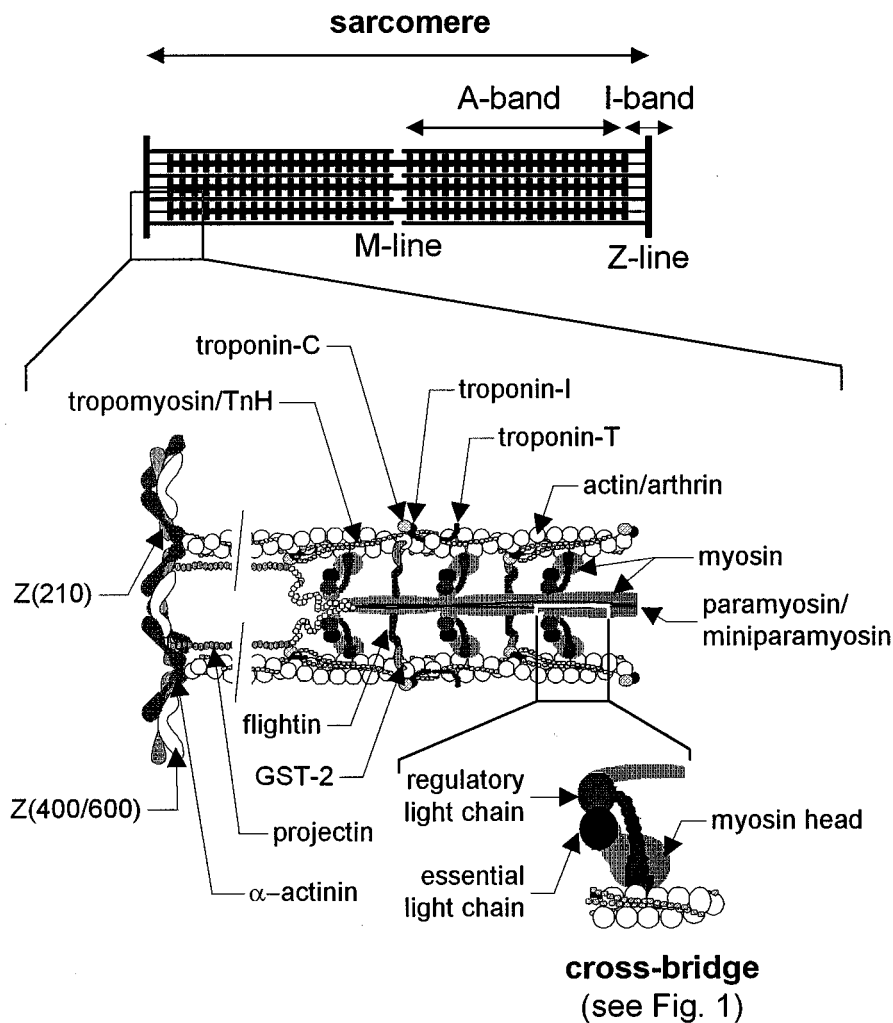


**FIGURE 1.** Flight system of *Drosophila melanogaster*, in powers of ten. At the level of whole fly behavior (x0.1 and x1), wild-type flies have a flight index of 5.8, where 6 denotes straight-up flight in every trial and 0 denotes complete loss of flight ability (2). Resonant flight system (x10) consists of wing, wing hinges, cuticle, and underlying musculature of thorax. Muscles shown (x100) include 12 dorsal longitudinal muscle cells (DLM) and 12 dorsal ventral muscle cells (DVM) that are oriented roughly perpendicularly to the more medially located DLM. Not shown are control muscles involved in steering and 2 minor DVM cells (3). By deforming thorax, DLM undergoing contraction move wings downward while DVM contraction moves wings upward. Tergal depressor of trochanter (triangular muscle between DVM and DLM) kick-starts oscillation, like initial whack of a tuning fork. High oscillatory power output of flight muscle fibers (x1,000) and myofibrils (x10,000) is achieved through a combination of evolutionary modifications of myofilament lattice (x100,000) and contractile proteins (x1,000,000), including motor molecule myosin and its associated regulatory light chain (RLC). Genetically engineered single-amino acid changes in RLC (x10,000,000), combined with *in vivo* expression of altered protein by germline transformation, produces impaired flight via reduction of power output at many levels in flight system. In case illustrated (alanine substitution of a phosphorylatable serine), reduced power output stems from either reduced number of power-producing cross bridges or reduction in power output per cross bridge (2). Nos. in open boxes are indexes of wild-type flies; nos. in filled boxes are indexes of flies in which RLC has been altered by substituting alanine for serine 66 (RLC mutant flies). Flight indexes from Ref. 2. Cross-bridge power is calculated on the basis that all cross bridges contribute equally to power production; however, probably <1/4 do so (15). Assumptions used for power calculations: 1) power output, 80 W/kg for wild-type IFM and 54 W/kg for “indirect” flight muscles (IFM) of RLC mutant flies (2); 2) 0.3 kg IFM/kg fly (2); 3) 1.3 mg/fly (average of 15 female flies); 4) 24 IFM fibers/fly; 5) 1,500 myofibrils in typical IFM [myofibrillar diameter: 1.8  $\mu\text{m}$ ; fiber diameter: 100  $\mu\text{m}$  (9); fraction of fiber cross section containing myofibrils: 0.5]; 6) 900 thick filaments/myofibril (9), 7) average length of myofibril (DLM): 1 mm; 8) average sarcomere length: 3.7  $\mu\text{m}$ ; 9) average thick filament length (minus bare zone): 3.1  $\mu\text{m}$ ; and 10) total no. of cross bridges per nm thick filament: 0.276 (based on 4-start helix with cross bridges on each strand, spaced 14.5 nm apart, that realign axially every 116 nm). Using “skinned” DLM fibers with length perturbations of 0.25% muscle length (10-fold smaller than those estimated *in vivo*), Dickinson et al. (3) observed maximal power outputs of 24 nW/mm<sup>3</sup> for wild-type and 11 nW/mm<sup>3</sup> for RLC mutant flies.

ing deformations at the wing hinges that make the wings beat at the resonant frequency of the flight system. The estimated power output of a single flight muscle fiber (Fig. 1, x1,000) is ~1.3  $\mu\text{W}$ .

Recent advances in microfabrication and instrumentation have allowed muscle biologists to take their mechanical and biochemical studies to even more basic levels of organization—that is,

to myofibrils and single molecules. In *Drosophila*, a typical IFM fiber consists of ~1,500 myofibrils (Fig. 1, x10,000). The myofibrils are ~1.8 mm in diameter, and they span the thorax (the tendons end within the cuticle). Taking muscle mechanics to the myofibrillar level will allow us to measure performance indexes, like power output, under conditions in which the ionic environment of the



**FIGURE 2.** Structural organization of *Drosophila* flight muscle sarcomere. Basic structure of myofibril is remarkably similar to that of striated muscle from other species (including human hearts). However, multiple (probably redundant) modifications of sarcomere have evolved to fulfill high power demands of flight system. These include (but are not limited to) modifications of conserved sarcomeric proteins (e.g., myosin, actin, tropomyosin, troponin), presence of flight muscle-specific proteins [e.g., flightin, troponin H (TnH)], or presence of proteins with novel functions [e.g., glutathione-S-transferase (GST-2)]. For clarity, titin is not included in illustration.

motor proteins and their attachments to the measuring instruments are rigorously controlled. Although myofibrillar power has not yet been measured, the predicted value is of the order of 0.9 nW. Novel technologies may be required to measure such small values accurately. Promising approaches include nanofabricated transducers and adaptations of atomic force microscopy (AFM). Already, AFM has been used to image *Drosophila* myofibrils in solution and to probe the myofibril's mechanical properties (8).

At the most basic levels of structural organization, the myofibrils are shown to consist of interdigitating thick and thin filaments, i.e., myofilaments (Fig. 1, x100,000). Each myofibril consists of ~1,000 thick filaments and 3,000 thin filaments, organized into structures called sarcomeres (Fig. 2). The thick filament consists primarily of the motor molecule myosin. Myosin is a hexamer whose two globular heads interact with the

thin filament's actin molecules to produce an oscillatory sliding motion between the two sets of filaments. In this way, power developed by the thick-thin filament interaction is transmitted to the wings. For each sarcomere, oscillatory power per thick filament is predicted to be of the order of 3.6 fW (Fig. 1). Although the basic design of the myosin head and actin is conserved across species, other adaptive features have evolved that "fine tune" the contractile proteins' interactions. Figure 2 illustrates the major myofibrillar proteins identified to date in *Drosophila* IFM, some of which are conserved proteins that have unusual extensions (e.g., myosin regulatory light chain and troponin T). Other proteins are unique to the IFM of insects (e.g., flightin). The presence of novel proteins or conserved proteins with unusual extensions or amino acid sequence differences presumably increases the synchrony of the unitary events underlying the response to

---

*"Each myofibril consists of ~1,000 thick filaments and 3,000 thin filaments..."*

---

muscle stretch. These specializations may also help stiffen the myofilament lattice to increase the efficiency of power delivery to the ends of the fiber and to structurally reinforce the sarcomeres to withstand high stress.

It is now possible to elucidate mechanical and biochemical events associated with a single cross bridge (Fig. 1,  $\times 1,000,000$ ), the collision complex between myosin and actin. This interaction is coupled to the hydrolysis of MgATP (reviewed in Ref. 5). Recent experiments on isolated muscle cells suggest that rotation of the myosin heads with respect to the filament axis is directly linked to force generation and filament sliding (5). Remarkable advances in optical "tweezers" technology have allowed researchers to measure changes in force and displacement that constitute the unitary "power stroke" (11, 12). Although unitary power has not yet been measured in *Drosophila*, predicted oscillatory power is  $\sim 15 \times 10^{-18}$  W per cross bridge. Thus, to produce the 31  $\mu$ W generated during flight,  $\sim 2$  trillion ( $= 31 \times 10^6$  W/fly,  $15 \times 10^{-18}$  W/cross bridge) double-headed myosin molecules must work together. This work is produced at an astonishing rate, comparable to a reciprocating engine operating at 12,000 rpm (i.e., 200 Hz, the average wing beat frequency).

### The "power of genetics"

At the base of the organizational ladder is the cell's genome, which contains the DNA instructions (Fig. 1,  $\times 10,000,000$ ) encoding the protein parts that constitute the muscle machine. Protein engineering using recombinant DNA technology has become a powerful tool in understanding the relationship between molecular structure and function. In *Drosophila*, as in a handful of other multicellular eukaryotes, this approach has the added advantage that engineered proteins can be expressed in the living animal.

One of the most direct ways to understand the function of a protein is to examine the properties (phenotype) of cells in which expression of the protein has been obliterated. This can be achieved genetically by generating a null mutation (i.e., a mutation that either blocks transcription of the gene or translation of its mRNA). Null mutations can be obtained by random mutagenesis, assuming a specific phenotype can be identified. More recently, targeted mutagenesis systems have been developed in a variety of organisms, including flies, whereby specific genes can be excised or knocked out.

The use of null mutants to study protein function has one potentially severe limitation. Knocking out expression of a protein (or a critical part of the protein) may kill the cells in which the pro-

tein is expressed, affect viability of the organism, or render the organ so dysfunctional that it is nearly impossible to establish the specific effect of the mutation. The latter problem is acute for contractile and other structural proteins where functional units require the interaction of many protein parts and where the absence of any one component may wreak havoc.

The *Drosophila* flight system offers a partial solution to this problem. In many cases, null mutations of the major contractile proteins can be obtained that do not result in the fly's death. This arises from the fact that some protein isoforms are encoded by IFM-specific genes (e.g., actin), whereas others are the product of an IFM-specific alternative splicing pathway (e.g., myosin heavy chain). Because flight is not essential for viability of flies maintained in lab cultures, flies can be mutagenized at random and then selected for flightlessness. In this way, null alleles of contractile protein genes can be selected that affect IFM function but leave essential muscles unscathed.

In *Drosophila*, null mutants have been identified and characterized for many of the IFM contractile proteins (Fig. 2; Ref. 1). Despite the shortcomings mentioned above, these mutants have proven exceedingly useful for two reasons. First, they can be used to dissect the process of myofibrillar assembly. For example, studies of actin nulls and myosin heavy chain nulls have shown that thin filament assembly takes place independently of thick filament assembly (1). Second, null mutants serve as convenient transformation hosts for gene replacement. Even if the endogenous gene is present but nonfunctional, one can introduce into the cell a new version of the gene that is fully expressed.

Genetic interactions among extant mutants and screens for modifiers (enhancers and suppressors) of extant mutants provide another avenue of investigation in muscle biology. Analysis of heteroallelic mutant combinations can reveal interactions where none were thought to be present. The presence of a recessive phenotype in a double-mutant heterozygous fly when neither single heterozygous fly expresses the phenotype is evidence that the products of the mutant genes interact functionally (4). Although this genetic interaction does not constitute proof of protein-protein interaction, it does provide insight into the molecular requirements of a functional unit. On the other hand, characterization of intergenic, allele-specific modifiers can provide information on direct protein-protein contact sites that will prove valuable in understanding how proteins interact in a functional assembly. Thus the combination of in vitro mutagenesis of cloned genes and trans-

---

*"...targeted mutagenesis systems have been developed in a variety of organisms..."*

---

posable element-mediated germline transformation, now both routine procedures, together with classical genetic techniques to investigate functional units in novel ways, provide a powerful one-two-three punch for the molecular dissection of protein function.

Recent site-directed mutagenesis of regulatory light chain (RLC) in *Drosophila* (2) illustrates the kind of information that can be obtained from an integrated, genetic approach to understanding muscle function (Fig. 1). *Drosophila* RLC is encoded by a single gene that yields a polypeptide of 222 amino acids that is present in all muscles throughout all developmental stages (1). *Drosophila* RLC closely resembles the RLC of vertebrate species, but it also has an unusual 47-amino acid NH<sub>2</sub>-terminal extension that has correlates elsewhere in the animal kingdom. Homologous extensions are also found in certain isoforms of the essential light chain (ELC) in vertebrate cardiac and skeletal muscle (see Ref. 13 for a review of the literature). In *Drosophila* RLC, substituting phosphorylatable serine residue 66 with an alanine (a nonphosphorylatable residue of comparable size) has little or no effect on the fly's viability, IFM development, and myofibrillar structure (2). However, the substitution does result in marked impairment of flight ability. The flight index drops from 5.8 (wild type) to 3.0 (mutant) on a scale of 0–6 (Fig. 1). The ratio of maximum force to body weight also drops, from 1.35 to 1.08. The drop in flight ability and force-to-body weight ratio is strongly associated with marked reductions in the fly's mechanical power output (Fig. 1). The oscillatory power of IFM that have been "stripped" of their membranes and Ca<sup>2+</sup> activated is also diminished (2). The root cause of this loss of power capability throughout the organizational tree is a 50% reduction in amplitude of stretch activation of the IFM (2), the heightened response to muscle stretch referred to earlier. The kinetics of the cross bridges, however, appear to be unaltered (2), which suggests that the altered power is caused by a reduction in the number of power-producing cross bridges (e.g., a reduction from 2 power-producing heads to 1 per myosin). Alternatively, it is plausible that the absence of the phosphorylatable serine alters the regional elasticity of the regulatory domain (10), thereby altering myosin stiffness and power output (Fig. 1). It is interesting to note that the RLC serine substitution in *Drosophila* results in a dynamic response to stretch that more closely resembles the much smaller responses of vertebrate striated muscles lacking the homologous extension. This raises the possibility that the serine mutation influences myosin stiffness and power output by altering RLC's postulated contact with actin (2, 10, 14).

These results indicate a strong link among flight ability, flight system power, flight muscle power, and, not surprisingly, the number of working cross bridges or their power output, all modulated by RLC phosphorylation. The big question in the larger context of fly behavior is, Why is it necessary to modulate cross-bridge power in this way? One possible answer may lie in the behavioral requirements of the fly. In *Drosophila*, wing movement plays a dual role: in flight and courtship. In flight, enough power needs to be developed to lift the fly's body and overcome drag. In courtship, the male needs to develop just enough power to beat its wings so it can attract the female—but not so powerfully as to cause him to fly away. Perhaps RLC serine phosphorylation/dephosphorylation allows full expression of both behaviors by modulating power output.

Having reviewed current technologies and approaches in *Drosophila* muscle biology, what is on the horizon? Mutating specific regions of the molecules will continue to provide information about the respective roles of each molecule. The Holy Grail of muscle biology, however, is to understand how muscle functions as a whole. The quest translates into understanding how different proteins interact and how they functionally integrate into a unit. As discussed here, the genetic approach will continue to prove invaluable, not only in the *Drosophila* model, but in other animal models as well. New technologies or unusual combinations of existing technology will continue to propel research. New advances in obtaining X-ray diffraction patterns of IFM from living flies, for example, may allow us to monitor structural changes of flight muscle proteins during flight, thereby realizing a complete integration of structure and function covering many powers of ten.

*We thank our many teachers, students, and colleagues who have been instrumental in helping us develop the Drosophila muscle model in our laboratories. We also thank present members of our laboratories for their helpful comments.*

*This work was supported by the National Institutes of Health, the Office of Naval Research, and the National Science Foundation.*

## References

1. Bernstein, S. I., P. T. O'Donnell, and R. M. Cripps. Molecular genetic analysis of muscle development, structure, and function in *Drosophila*. *Int. Rev. Cytol.* 143: 63–152, 1993.
2. Dickinson, M. H., C. J. Hyatt, F. Lehmann, J. R. Moore, M. C. Reedy, A. Simcox, R. Tohtong, J. O. Vigoreaux, H. Yamashita, and D. W. Maughan. Phosphorylation-dependent power output of transgenic flies: an integrated study. *Biophys. J.* 73: 3122–3134, 1997.

---

*"... wing movement plays a dual role: in flight and courtship."*

---

3. Dickinson, M. H., and M. S. Tu. The function of *Dipteran* flight muscle. *Comp. Biochem. Physiol.* 116A: 223–238, 1997.
4. Homyk, T., and C. P. Emerson. Functional interactions between unlinked muscle genes within haploinsufficient regions of the *Drosophila* genome. *Genetics* 119: 105–121, 1988.
5. Irving, M., and G. Piazzesi. Motions of myosin heads that drive muscle contraction. *News Physiol. Sci.* 12: 249–254, 1997.
6. Josephson, R. K. Contraction dynamics and power output of skeletal muscle. *Annu. Rev. Physiol.* 55: 527–546, 1993.
7. Lehmann, F.-O., and M. H. Dickinson. The changes in power requirements and muscle efficiency during elevated force production in the fruit fly, *Drosophila melanogaster*. *J. Exp. Biol.* 200: 1133–1143, 1997.
8. Nyland, L., J. Peterson, and D. Maughan. Functional and morphometric study of *Drosophila* indirect flight muscle using atomic force microscopy (Abstract). *Biophys. J.* 72: A102, 1997.
9. Peckham, M., J. E. Molloy, J. C. Sparrow, and D. C. S. White. Physiological properties of the dorsal longitudinal flight muscle and tergal depressor of the trochanter muscle of *Drosophila melanogaster*. *J. Muscle Res. Cell Motil.* 11: 203–215, 1990.
10. Poetter, K., H. Jiang, S. Hassanzadeh, S. R. Master, A. Chang, M. C. Dalakas, I. Rayment, J. R. Sellers, L. Fananapazir, and N. D. Epstein. Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle. *Nature Genet.* 13: 63–69, 1996.
11. Simmons, R. M., J. T. Finer, S. Chu, and J. A. Spudich. Quantitative measurements of force and displacement using an optical trap. *Biophys. J.* 70: 1813–1822, 1996.
12. Swank, D. M., M. L. Bartoo, S. I. Bernstein, J. E. Molloy, and J. C. Sparrow. In vitro measurements of velocity, step size and attached lifetime of *Drosophila* myosins (Abstract). *Biophys. J.* 74: A261, 1998.
13. Sweeney, H. L. Function of the N terminus of the myosin essential light chain of vertebrate striated muscle. *Biophys. J.* 68, Suppl. 4: 112S–119S, 1995.
14. Trayer, I. P., H. R. Trayer, and B. A. Levine. Evidence that the N-terminal region of A1-light chain of myosin interacts directly with the C-terminal region of actin: a proton magnetic resonance study. *Eur. J. Biochem.* 164: 259–266, 1987.
15. Tregear, R. T., R. J. Edwards, T. C. Irving, K. J. V. Poole, M. C. Reedy, H. Schmitz, and E. Towns-Andrews. X-ray diffraction indicates that active cross-bridges bind to actin target zones in insect flight muscle. *Biophys. J.* 74: 1439–1451, 1998.