The 55th Annual Meeting and Symposium of the Society of General Physiologists

Molecular Motors

(Organized by H. Lee Sweeney, Erika L.F. Holzbaur, and E. Michael Ostap)

Muscle contraction and cell motility arise from the conversion of chemical energy, in the form of ATP hydrolysis, into mechanical work. The conceptual scheme for this chemomechanical energy conversion is the Lymn-Taylor four-state scheme (Fig. 1), which for the last 30 years, or so, has formed the conceptual underpinnings for current work in muscle contraction (and, with some generalization, all motor proteins).

It generally is believed that Fig. 1 correctly describes the overall features of muscle contraction (and molecular motors in general); but it has proven difficult to visualize the actual movement of the myosin cross-bridge, which still has to be inferred. Nevertheless, the molecular/structural basis for chemomechanical energy conversion is emerging thanks to advances in structural biology and mechanical force measurements—together with detailed kinetic analyses and studies on the regulation of motor assembly.

The recent advances in elucidating the structure, assembly, and function of molecular motors were the focus of the 55th Annual Meeting of the Society of General Physiologists, which took place in Woods Hole, MA, September 5–8, 2001. H. Lee Sweeney, Erika L.F. Holzbaur, and E. Michael Ostap from the University of Pennsylvania organized the symposium on Molecular Motors, which highlighted how progress in structure determination and force measurements are revolutionizing the field. With more than 140 participants, and 80 abstracts covering a broad range of topics, the meeting was lively and the major issues were put in perspective for all.

As a backdrop for the meeting, there are a total of 40 known or predicted human myosin heavy chain genes (J.S. Berg, B.C. Powell, and R.E. Cherry. 2001. *Mol. Cell. Biol.* 12:780). About one third of these are conventional (Class II myosins), similar to skeletal muscle myosin, the rest are unconventional (nonmuscle) myosins, such as the monomeric Class I myosins and a wide variety of other types. Similarly, there are >40 genes in the kinesin and >10 in the dynein superfamilies, respectively (information provided in presentations by N. Hirokawa and T. Hays). These superfamilies are distantly related, meaning that general principles learned in one system are likely to apply to the other systems as well. The multitude of genes, and even larger diversity of proteins (not to mention the further diversity arising from the number of light chains and other subunits), serves to illustrate the multitude of functions that depend on molecular motors. It also emphasizes the challenges involved in deducing mechanism and function. How is chemomechanical transduction achieved? What distinguishes motors that move forward (that move toward the plus end of actin filaments or microtubules) from those that move backward? How do motors recognize their cargo? How is motor function regulated?

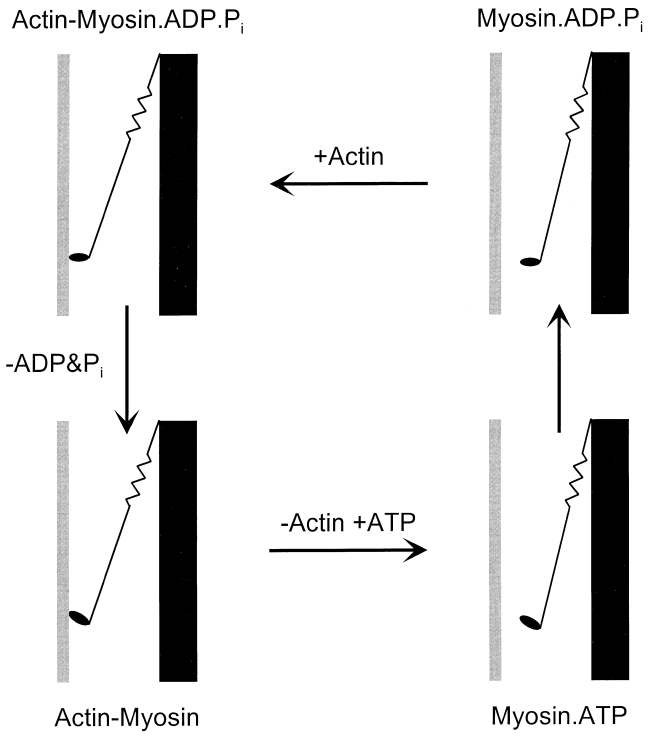

Figure 1. The Lymn-Taylor cycle (*Biochemistry.* 1971. 10:4617). In the absence of ADP or ATP (bottom left), the myosin head binds strongly to the actin filament (gray). ATP binding to myosin causes a rapid dissociation of the actin–myosin complex (bottom right). The subsequent hydrolysis of ATP leads to a stable myosin.ADP.P_i complex (top right) in which the head has "swung" back (ready to recombine with actin [top left]). The mechanical movement of the myosin filament relative to the actin filament occurs as ADP and P_i dissociate from the actin.myosin.ADP.P_i complex (top left to bottom left). Modified after M.A. Geeves and K.C. Holmes (1999. *Annu. Rev. Biochem.* 68:687).

To understand the molecular basis for motor func-

tion, one needs both structural information and force measurements. The need for both types of information was a key theme—evident even in the Symposium's organization—as there were two Keynote speakers, who summarized the advances in each area. K.C. Holmes (Max-Planck-Institute for Medical Research, Heidelberg, Germany) started out with a structuralist's view of muscle contraction, which summarized an amazing amount of work from many different laboratories. Atomic resolution structures show that myosin can exist in at least two forms, similar to what has been observed for G-proteins and a number of other ATPases: (1) a CLOSED structure in which the nucleotide-binding site is partly occluded; and (2) an OPEN structure in which the nucleotide-binding site is more accessible. Based on these structures, myosin can be described (Fig. 2) as being composed of a (catalytic) motor domain that through a short converter domain is connected to the light chain–binding lever domain, which, in turn, is connected to the coiled-coil dimerization domain.

Taking the known CLOSED and OPEN atomic resolution myosin structures as the starting point, one can model these structures unto lower resolution structures (obtained by cryoelectron microscopy and image reconstruction) of myosin-decorated actin filaments; and, thus, obtain molecular models for what might transpire as the motor moves. This approach has dangers since actin binding has large effects on myosin, but it is capable of producing a remarkably detailed, if complex, picture of muscle contraction—and it suggests how the molecular motion underlying muscle contraction can occur by a "swing" of the myosin lever arm rather than by the motor domain (Fig. 3).

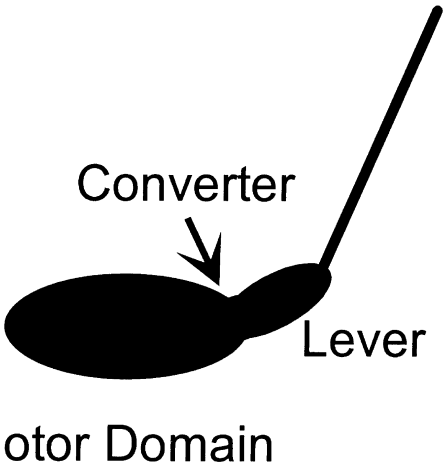

FIGURE 2. Domain organization of a muscle myosin. The motor domain is composed of an actin attachment component and a catalytic component. The motor domain is connected to the light chain binding lever domain by a short converter domain. The lever domain finally is connected to the coiled-coil dimerization domain (thin straight line).

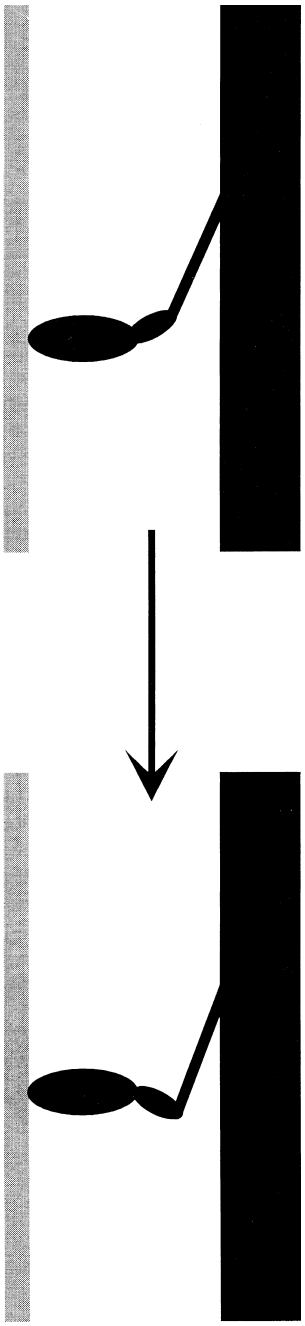

FIGURE 3. Swinging lever arm model of muscle contraction. The myosin motor domain remains almost invariant in relation to its attachment to the actin filament (gray). The motion (and force generation) arises from a 60° rotation of the lever domain. Modified after M.A. Geeves and K.C. Holmes (1999. *Annu. Rev. Biochem.* 68:687).

ATP hydrolysis occurs in the CLOSED structure, and the OPEN→CLOSED transition acts as a switch to trigger a 60° rotation of the converter domain between the motor (or catalytic) domain and the myosin lever (Fig. 3), such that a fairly modest 5–6-Å movement of the switch is converted into a 110-Å movement at the distal end of the myosin lever arm. The distance traversed by the lever do-

main is in good agreement with what has been inferred by single-molecule mechanical measurements.

Myosin is a product-inhibited ATPase, meaning that the catalytic rate (and the rate of cross-bridge turnover) is limited by the rate of ADP and P_i dissociation, a point that was pursued in several talks. A. Houdusse (CNRS Institute Curie, Paris, France) summarized structure–function studies on *Dictyostelium* myosin, which described a possible path for P_i dissociation. The proposed path was validated by examining the structural features and kinetic properties of the wild-type and selected mutant myosins. M.A. Geeves (University of Kent, Kent, UK) described experiments that elucidated the importance of ADP dissociation. Not only is the cross-bridge kinetics dependent on the rate of ADP release, but also the rate of ADP release is stress-dependent, as would be expected for a motor. Moreover, the two myosin heads differ in their ADP affinity differs by a factor of 20 for binding of the first ($K_d \approx 1$ μM) and the second ($K_d \approx 20$ μM) ADP molecule. This result provides further support for a reciprocal coupling between myosin ATPase activity and mechanical activity. Altogether, one needs to consider explicitly the chemomechanical coupling when developing models for molecular motors.

This point was pursued further by J. Howard (Max-Planck-Institute for Molecular Cell Biology and Genetics, Dresden, Germany), who summarized an extensive analysis of kinesin using a microtubule gliding assay in which the kinesin–microtubule interactions are examined by following the movement of microtubules over a surface that is sparsely covered with kinesin molecules. At sufficiently low surface densities of kinesin, the microtubules interact with only a single kinesin molecule, and the gliding assay provides direct information about the kinesin motor. A surprising feature of these measurements is that the velocity with which the microtubules move along the surface is independent of the microtubule length. This means that the velocity is not limited by viscous drag, but rather by the kinetics of the kinesin–microtubule cross-bridge turnover. It also means that the force generated by a single kinesin cross-bridge must be much larger than the hydrodynamics drag. The effect of force on the individual steps in the cross-bridge turnover cycle can be visualized in a conventional reaction diagram (Fig. 4), which illustrates how an applied force ($= -dG/dx$, where G and x denote the free energy and distance, respectively).

Conceptually, there is no difference between how a mechanical force alters the rate of motion and how an applied electric field alters the rate of ion channel gating, but the mechanical experiments are technically more demanding. Nevertheless, it is possible to deduce a complete kinetic model and estimate all the parameters in the model, which leads to the conclusion that each "elementary" transport event (a complete cycle) is

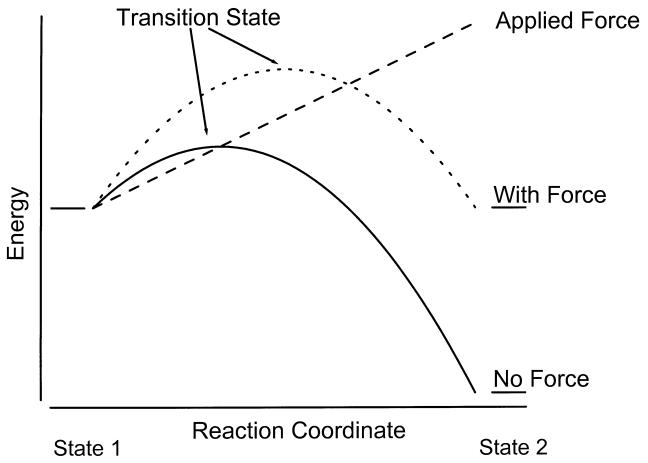

FIGURE 4. Reaction diagram for chemomechanical conversion. The diagram depicts a motor that can exist in two states (State 1 and State 2). In the absence of an applied force, the conversion of State 1 to State 2 is favored. The rate of the conversion occurs as a diffusive passage across an energy barrier separating the two states: first into the Transition State corresponding to the barrier peak; and then into the final passage. This passage across the energy barrier corresponds to the physical movement in the system, the movement of the motor protein. When a mechanical force is applied to the system, work will be done when protein moves and the energy profile will be the sum of the profile with no applied force and the energy profile due just to the movement of the protein. (For simplicity, the figure is drawn assuming a linear mapping of the reaction coordinate unto the mechanical coordinate.) As a result, the free energy difference between States 1 and 2 will vary as a function of the applied force, as will the position of the Transition State relative to States 1 and 2 and the energy difference between the Transition State and States 1 and 2, which in turn will alter the rate of the State 1→State 2 conversion.

composed of several smaller steps. The overall step size is invariant, ~80 Å, but, the rate-limiting step (and step length) varies with the ATP concentration and imposed load: at low ATP concentrations, the rate-limiting step is ~28 Å; at high load, the rate-limiting step is ~52 Å.

K. Hirose (National Institute of Advanced Interdisciplinary Research, Tsukuba, Japan) summarized recent progress in elucidating the structural basis for kinesin movement. The approach was similar to that described for the actin-myosin motors, namely to combine atomic resolution structures of the individual molecules with lower resolution information on kinesin-decorated microtubules. Kinesins occur as dimers, but only one of the motor domains (heads) in a dimer appears to be bound to microtubules. Addition of adenine nucleotides has modest effects on the microtubule-attached head, but causes the free head to move in such a manner that kinesins that are highly processive show large changes in the position of the free head, whereas less processive kinesins display little change in the position of the free head. S. Endow (Duke University) continued the discussion of kinesin processivity by comparing the kinesins and the so-called ncd motors (so named

from their initial discovery in nonclaret disjunctional *Drosophila* mutants), which are members of the kinesin superfamily but support retrograde movement—meaning opposite directionality to that of the conventional kinesins. Using chimeric constructs, all kinesin-related motor domains turns out to have an intrinsic positive directionality (moving toward the microtubule plus end, similar to conventional kinesins); directionality is determined by the neck/stalk domain corresponding to the myosin lever domain.

Motor directionality is an issue not only in the kinesins. H.L. Sweeney (University of Pennsylvania) summarized results on myosin VI, which is a processive myosin that also serves as a stereocilia anchor. Myosin differs form other myosins by having a large insert in the converter domain. Myosin VI moves backward, and chimeras between myosin VI and the conventional myosin II show that the converter domain is a necessary, but not sufficient, determinant of directionality. The "missing elements" that determine directionality remain to be fully defined; but myosin VI differs from conventional myosins in other ways: the step length is ~300 Å and myosin VI will not move on a single actin filament. Using a dual-beam laser trap, the step size turns out to be broadly distributed (with forward as well as backward steps), and the step size increases as the load is increased. The results raise the question whether the coiled coil that stabilizes the myosin VI dimer can uncoil? J. Molloy (University of York, York, UK) provided further information about the mechanisms underlying myosin movement through single-molecule studies on the monomeric myosin I. Using careful measurements of position and force generation, the cross-bridge stiffness is increased during the power stroke, which is inferred to result from a combination of a rocking motion of the motor domain on the actin filament and a swing of the lever.

T. Yanagida (Osaka University, Osaka, Japan) presented results that further shifted the focus from the lever domain to the motor domain. The experiments used myosin V, which has an exceptionally long lever domain and a 360-Å step size. When the lever domain is shortened, however, the step size is not altered, and the processive velocity is similar for the full-length and the domain-shortened myosins. These results with myosin V differ from those obtained by others, but on other myosins. What is going on? One possibility is that myosin movement indeed proceeds by a combination of motor domain rocking and lever swinging; but that the relative importance of the these two contributions varies among various myosin forms. In any case, Yanagida notes that the step size that is observed with myosin V corresponds to the helical pitch of the actin filament.

The role of molecular motors in cell function was the focus of the second half of the symposium. E.L.F. Holzbaur (University of Pennsylvania) began by describing the third superfamily of molecular motors, the dyneins, which are important for (retrograde) intracellular vesicle transport, specifically axonal transport, mitotic spindle assembly, and microtubule–kinetochore interactions. Dyneins occur as huge complexes of ~15 proteins, including the cocomplex dynactin that is composed of ~10 proteins. 50% of dynein is cytosolic, which complicates localization studies; but dynein also is localized at adherens junction, where they bind to β-catenin and tether microtubules to the adherens junctions, which could be important for rapid communication between the plasma membrane and the nucleus. R. Vallee (University of Massachusetts Medical School) described the pathophysiology associated with dynein-related gene product, LIS1. Human lissencephaly is a serious developmental disorder where the neuronal distribution in the cortex is grossly disrupted and there is no higher brain function. The disorder results from a haplo-insuffiency at the LIS1 locus. LIS1 binds to dynein and dynactin, and may serve as a "platform" that organizes the dynein complex and enables it to serve as an efficient carrier of a wide variety of cargoes. The complex regulation of the dynein complex and its targeting was further highlighted by K. Vaughan (University of Notre Dame), who showed that the targeting of the complex to the plus end of microtubules is disrupted by phosphorylating the $p150^{Glued}$ component of the dynactin complex, and that this regulation further depends on the state of phosphorylation of the dynein intermediate chain.

The importance of kinesin for maintaining flagellar structure was emphasized by W. Marshal (Yale University), who used GFP-labeled tubulin to show how a kinesin II–driven intraflagellar transport (IFT) is necessary for maintaining flagella at full length after their initial assembly. The results indicate that the rate of flagellar microtubule disassembly occurs at a constant rate, and that the rate of reassembly is determined by the rate at which tubulin is delivered to the flagella tip by the IFT system. Considering the large diversity of cargoes that are moved by a given type of molecular motor, how is specificity obtained? One possibility is that cargo-specific adaptor proteins provide the specificity, and L.J. Megeath (Harvard Medical School) showed how a novel kinesin-associated (adaptor) protein Milton was required for axonal transport of mitochondria into the presynaptic nerve terminals in *Drosophila* photoreceptors.

The challenges involved in targeting many different cytoplasmic and membrane constituents to their correct destinations were highlighted by L.S B. Goldstein (University of California at San Diego). Using *Drosophila* genetics, a number of adaptor proteins have been identified, one of them is the amyloid precursor protein (APP) that plays a key role in the pathogenesis of Alz-

heimer's disease. APP interacts with the light chain subunit of kinesin I and is necessary for vesicular axonal transport. APP deletion disrupts the vesicular transport and "clogs up" the cell with organelles. Similarly, overexpression of APP decreases the axonal transport machinery and causes neurodegeneration in a manner reminiscent of Alzheimer's disease. The power of *Drosophila* genetics was demonstrated also by T. Hays (University of Minnesota at Minneapolis), who again emphasized the importance of adaptor proteins for dynein-mediated cargo transport. A point that was reemphasized by N. Hirokawa (University of Tokyo, Tokyo, Japan).

Most myosins are dimers with two motor domains, but a large class of myosins (Class I myosins) occur as monomers. Class I myosins are the classic nonmuscle myosins, and they are present in all cells. E.M. Ostap (University of Pennsylvania) summarized their importance for membrane trafficking. Using GFP-labeled myosin Ib and ratio-imaging methods, the staining is highest in areas with high turnover (and high turnover of the actin cytoskeleton), such as membrane ruffles and endosomal membranes. Myosin Ib does not associate with mature, tropomyosin-containing actin filaments; but as might be expected from the accumulation areas where the actin filament turnover is high, myosin Ib binds to dynamic (new) actin filaments that do not contain tropomyosin. M. Mooseker (Yale University) summarized results on the role of another Class I myosin, brush border myosin I (BBMI), for intestinal brush border structure and function. BBMI forms a link between the microvillar membrane and the underlying actin core in microvillus. BBMI contains multiple calmodulin light chains, and BBMI has been proposed to be important for targeting membrane components to microvilli, and for mechanoregulation of nutrient transport especially the vitamin D–dependent Ca^{2+} uptake. However, when BBMI is knocked out, there is no overt phenotype—at least no phenotype that would be predicted based on the functions that have been proposed for BBMI. The brush border structure is seemingly normal, except for some herniation of the plasma membrane up into the microvillar region—and, surprisingly, reduced levels of other brush border–associated myosins. However, laboratory mice live under conditions that differ substantially from those of free-living mice in the wild. If the BBMI-knockout mice are stressed, by fasting and refeeding or by exposure to sodium dextran sulfate, which produces chemical injury of the intestinal epithelium, the microvilli vesiculate and the brush border structure is effectively denuded. Quite apart from the implications these results have for role of BBMI in microvillar stability, they demonstrate that conclusions about (the lack of) phenotypic changes in transgenic animals often will require studies that monitor how the animals respond to adverse conditions.

M.A. Titus (University of Minnesota at Minneapolis) presented results on the nonmuscle myosin VII, which is important for cell–substrate adhesion, such as in phagocytosis and cell migration. Myosin VII knockout animals have defective muscle cell adhesion to the hypodermis; but the phenotype could be largely rescued by overexpressing just the coiled-coil tail. In fact, it remains unclear whether the motor function is primary or whether myosin VII is primarily a structural protein, which organizes plasma membrane receptors in the plasma membrane. T. Hasson (University of California at San Diego) continued describing the functions of a myosin VII (myosin VIIa) and another nonmuscle myosin (myosin VI), which together are important in maintaining normal hearing and control of balance. These myosins are important in membrane turnover events: myosin VIIa is important for phagocytosis and is targeted to adhesion domains; and myosin VI seems to be involved in endocytosis. Myosin VIIa also is important for spermatogenesis, which involved a partial phagocytosis of the germ cell by Sertoli cells. Another unconventional myosin that is involved in hearing is myosin Ic. P. Gillespie (Vollum Institute) presented an ingenious set of experiments that probed the role of myosin Ic in the slow adaptation that occurs in auditory mechanotransduction. This slow adaptation is believed to result from a myosin Ic–dependent resetting of the mechanotransducer. Using a strategy pioneered in studies on protein kinases, myosin Ic was mutated ($Tyr^{61} \rightarrow G$) so as to increase the size of the nucleotide binding site. The mutant myosin had normal ATP hydrolysis rates, but bound ADP analogues with bulky substituents in the adenine ring (e.g., N^6(2-methylbutyl)ADP)—and that did not bind to the wild-type myosin. When the mutant myosin was expressed as a transgene in mice, the protein was expressed in hair cells. When examined under standard whole-cell clamp, the transgenic hair cells exhibited normal electrophysiological properties; however, when N^6(2-methylbutyl)ADP was present in the patch pipette, the normal adaptation process was blocked, as would be expected if the N^6(2-methylbutyl)ADP had bound to the Y61G-myosin Ic, induced rigor and thereby disrupted the normal adaptation process!

A traditional feature of the symposia organized by the Society of General Physiologists is the New Ideas/New Faces sessions, where the speakers are chosen by the organizers based on the free abstracts submitted to the meeting. This is, indeed, where the new ideas are presented. At this meeting, the speakers were supported by a grant from the Keith R. Porter Endowment, which aims to encourage (usually) young investigators to undertake exciting new projects—and to talk about them. In addition to presentations mentioned above (by Gillespie, Marshall, Megeath, and Vaughan), the follow-

ing individuals were Porter Endowment speakers: R. Adelstein (role of nonmuscle myosin II in development); E. de la Cruz (kinetics of myosin V dissociation from actin); J. Kull (atomic resolution structure of a myosin I); B.H. LaMonte (dynamitin and motor neuron disease); G.I. Mashonov (visualizing single myosin I molecules in living cells); S. Rosenfeld (ATP-induced reorientation of the kinesin neck linker); P. Selvyn (the role of actin and the S2 rod in myosin conformation); G. Skiniotis (the "bridge" in dimeric kinesin); A.M. Sokac (role of myosin 1b in meiotic maturation); and Y. Takagi (isometric force production by rabbit skeletal myosin I).

The capstone on the meeting was the final Keynote lecture by S.M. Block (Stanford University) who gave a sweeping overview of the universe of molecular motors, which is even larger than could be covered in this meeting—and includes the processive nucleic acid enzymes, ATP synthases, and bacterial rotary motors. For all of these systems, an important (rate-limiting) step in understanding their function is the development of robust single-molecule assays, including force clamps, which allow for a detailed kinetic analysis of the effect of load on the movement of single motors. This is important because despite the insights that have been obtained from through structural biology, biochemical kinetics, genetic engineering, or single-molecule fluorescence, neither of these methods probe directly the effect of load. Using kinesin as an example, it was shown how single-molecular force measurements (under a wide variety of experimental conditions) allow for the deduction of an underlying kinetic model. The range of topics that was covered was enormous; but the bottom line remains that central elements in the chemomechanical transduction remain unknown, as single-molecule methods do not directly probe the underlying structural changes.

Altogether, the meeting amply demonstrated the methodological and conceptual sophistication underlying current research in molecular motors. It also illustrated the need for more direct probes of the link between structure and load, a point that was made by both Keynote speakers and many of the intervening presentations. The technical challenges to this quest are enormous; but the advances that have occurred over the last decade suggest that the challenges, eventually, will be overcome. The frontier is moving forward at a brisk pace.

Olaf S. Andersen
Editor
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