THE ROYAL SOCIETY

Published online 27 December 2004

Introduction

In 1674 van Leeuwenhoek used his simple microscope to discover the myofibrils and cross-striations in muscle fibres. He wrote in a letter (originally in Dutch) to Robert Hooke at The Royal Society:

> I could distinctly see that the fleshy fibres, of which the greater part of a muscle consists, were composed of globules.

He presented a drawing that clearly shows the cross-striations that delineate the 'globules' i.e. sarcomeres. Considering that the sarcomeres are only *ca*. 2.5 μm long, this was an amazing achievement. (Some 50 years later this work was published in translation in the Philosophical Transaction of The Royal Society.)

In a typical 25 cm long human muscle, each muscle fibre may contain 100 000 sarcomeres arranged in series with each other. Based on these observations Croone suggested in 1675–1680, that the sarcomeres ('globules') delineated by cross-striations may serve as units of contraction. This is indeed true. However, the origins and significance of the cross-striations were to remain enigmatic for another 280 years, until the papers by Andrew Huxley and Rolf Niedegerke and by Jean Hanson and Hugh Huxley in *Nature* in 1954 (Hanson & Huxley 1954; Huxley & Niedergerke 1954) showed how the sarcomere was built up and how it worked. A full appreciation of the underlying components of the cross-striations is actually the main topic of the present discussion meeting!

The protein myosin is the main component of muscle. It was discovered in 1864 by Willie Kühne in Leipzig by treating minced muscle with salt. He named it 'myosin'. In 1939 Engelhardt and Ljibimova showed that myosin could hydrolyse adenosine triphosphate (ATP). A little later Albert Szent-Györgyi and Straub showed that Kühne's myosin was really two proteins, actin and myosin. Albert Szent-Györgyi was also able to show that fibres of actin and myosin contract on adding ATP. This discovery was the harbinger of modern muscle research. Muscle is a machine for turning chemical energy into mechanical work at high efficiency and at constant temperature. To date, no nanotechnology comes close to emulating this process. Therefore we can learn much by studying the molecular mechanism of muscle.

Fifty years ago came the sliding-filament hypothesis. This maintained that each sarcomere consists of overlapping sets of filaments, thick and thin. During a contraction the thin filaments move past the thick filaments so that the sarcomere shortens and therefore the muscle shortens. Hugh Huxley and Jean Hanson used phase-contrast light microscopy and electron microscopy to examine changes in the structure of individual rabbit muscle myofibrils at various stages of contraction (a myofibril is a substructure of a muscle fibre: as indicated in van Leeuwehoek's drawing, internally a muscle fibre is composed of many parallel, identical myofibrils; figure 1). They also managed to dissolve out the myosin to show that the thick filaments were made of myosin and the thin filaments of actin. The sarcomere is delineated by the Z-line. The actin filaments bind to the Z-line and reverse polarity on both sides of the Z-line. The myosin molecules are arranged in bipolar thick filaments. Andrew Huxley and Rolf Niedergerke used a specially developed interference light microscope to observe the behaviour of intact frog muscle fibres during contraction. Both teams showed that when muscle contracts, the filaments keep a constant length and therefore during shortening the filaments must slide.

The sliding-filament hypothesis did not find immediate acceptance. The then current view was that myosin was a long negatively-charged polypeptide without much structure that shortened down on the addition of Ca^{2+} ions. The fact that there was practically no evidence to support this model did not detract from its wide acceptance. It was argued that the cross-striations of skeletal muscle could not be of great significance because smooth muscle contracted without having them. Moreover, despite Engelhardt and Ljubimova's paper in *Nature* it was not widely accepted that myosin was an ATPase. Myosin was a structural protein and had no business being an enzyme. This point of view was held by no less than the joint Nobel Prize winners A.V. Hill and Otto Meyerhof (the co-discoverer of ATP). However, H.E. Huxley's electron microscopy removed any lingering doubts that when cross-striated muscle contracts the two sets of interdigitating filaments, made of myosin and actin, slide past each other without either altering its length significantly. Moreover, about the same time soluble fragments of myosin were prepared that contained the ATPase. These parts of the myosin molecule were then shown to contain the myosin crossbridge, an entity that binds and releases the actin filament cyclically while hydrolysing ATP. It also undergoes a conformational change during its combination with actin that 'rows' the actin filament past the myosin filament—the swinging crossbridge. Final direct proof that filaments really do slide was provided more recently by '*in vitro* motility assays', showing for example single fluorescently-labelled actin filaments sliding over a bed of myosin in the presence of ATP (Kron & Spudich 1986). Two recent historical reviews may be consulted for more detail (Cooke 2004; Szent-Gyorgyi 2004).

One contribution of 14 to a Discussion Meeting Issue 'Myosin, muscle and motility'.

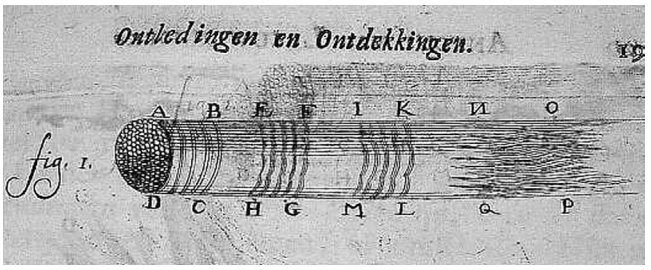

Figure 1. A drawing sent to Robert Hooke by Antonie van Leeuwenhoek in 1682 showing the cross-striations that he was able to see on a muscle fibre from a cow. The distance between the striations is *ca*. 2.5 μm. The striations delineate the sarcomeres characteristic of skeletal muscle. The myofibril substructure of the muscle fibre is also visible.

Myosin is a very large molecule with an α-helical coiled-coil tail and two globular 'heads'. The tails of the molecules pack together to form the thick filaments, while the heads—the crossbridges—stick out from the thick filaments and cyclically interact with the actin filaments, moving them along, by a kind of rowing action. The fuel for this process is provided by the hydrolysis of ATP. Each interaction produces *ca*. 10 nm of sliding movement or, if the muscle is constrained, a few piconewtons of force.

The whole process works in a cycle. The myosin crossbridge initially binds ATP and splits it into ADP (adenosine diphosphate) and phosphate, but the products of the reaction are not released from the crossbridge. However, this reaction primes the crossbridge, which then attaches to a neighbouring actin site. Binding to actin causes release of products. The phosphate is released first from the crossbridge and the crossbridge changes its shape in order to pull on the actin causing the *ca*. 10 nm movement, the 'power stroke' or 'working stroke'. At the end of the power stroke ADP is released, which allows a new ATP molecule to bind to the myosin. This brings about a rapid release of the crossbridge from the actin filament and the cycle starts again. This is the Lymn–Taylor model of the crossbridge cycle (Lymn & Taylor 1971). In the absence of ATP (*rigor mortis*) the crossbridge binds tightly to actin in the post-power-stroke conformation. This state is only transitorily present in the Lymn–Taylor cycle.

Over the past 50 years, the structures of the myosin cross-bridge and actin filaments have been elucidated. It has become clear that actin is the passive partner—it provides binding sites for crossbridges, and in most muscles actin has associated proteins, troponin and tropomyosin, that regulate muscle contraction: the actin filament is 'turned on' when calcium binds to troponin. For studies of the underlying basis of the power stroke the focus is firmly on myosin. A great deal is known about the steps in the biochemical reaction of ATP breakdown by myosin and how these relate to the production of force by the crossbridge. X-ray crystallographic studies showed the crossbridge to have a long α-helical tail that looks like a lever arm. This lever arm binds two 'light chains', low molecular weight proteins that apparently stabilize the α-helix to make it stiff. Moreover, this lever arm was found in two conformations 60° apart that appear to be the two ends of the power stroke. This and many other experiments have led to the consensus that most of the movement occurring during the power stroke arises from a rotation of the lever arm, which, when the crossbridge attaches to actin, lies distal to the actin filament (the swinging-lever-arm hypothesis). In addition, recombinant DNA technology may be used to change the crossbridge structure in defined ways. Most dramatically, using recombinant DNA technology to alter the length of the lever arm produces a sliding velocity proportional to the length of the lever arm.

Clearly, in an intact muscle with hundreds of millions of cross-bridges it is difficult to synchronize their activity for experimental purposes. Therefore, experiments on single myosin molecules can be very revealing. By using 'laser-traps' as micro-forceps and very sensitive force transducers one can indeed measure the tiny amounts of sliding and force produced when this ultimate 'nanomotor' interacts with an actin filament.

There are many different isoforms of myosin. The class containing myosins found in skeletal muscle (cross-striated) is known as myosin II. Heart muscle is also cross-striated and also contains myosin II isoforms. Smooth muscle (found in arteries, bladder, the gut and the uterus) differs microscopically from skeletal muscle in having no cross-striations. The myosin (another myosin II isoform) associates into irregular thick filaments and the actin filaments are attached to dense bodies rather than to regular Z-lines as in cross-striated muscle. The control of contraction is exercised by calcium-dependent phosphorylation of the light chains rather than by binding calcium to troponin on the thin (actin) filament, as is the case in cross-striated muscle. Otherwise the mechanism of the power stroke appears to be very similar.

Outside muscle, most cells in the body display a dozen or more myosin isoforms (often referred to as myosin motors), which are involved in many processes involving transport and motility. Myosins even play a crucial role in hearing. All myosins interact with actin, but usually the myosin is not packed into thick filaments as in cross-striated muscle. However, the core mechanism of all myosin crossbridges does seem to be invariant, so that information from any myosin isoform helps to solve the general problem of how myosin works.

As one might anticipate from its function, the myosin crossbridge has a rich repertoire of conformations and the structures of a number of different conformers have been characterized by X-ray crystallography. However, structural data can only yield the stable end states of a dynamic process. Moreover, there are no X-ray crystallographic data on the actin–myosin complex so that electron microscopy must be used to study the actin–myosin interaction. These data are of limited resolution and can only be interpreted by combining them with high-resolution structural data derived from myosin crossbridges not bound to actin. Furthermore, it is necessary to assign a particular crossbridge structure to a particular biochemical state. Therefore, what actually happens in the power stroke after myosin binds to an actin filament has to be inferred by combining physiological and biochemical measurements with structural information. This was a major theme of the present discussion meeting.

As summarized by Ken Holmes (Holmes *et al.* 2004) the myosin crossbridge has an elongated head, containing a 7-stranded β-sheet and associated α-helices forming a deep cleft at one end of the crossbridge. The cleft separates two

parts of the molecule, which are referred to as the upper 50K, and lower 50K domains, both of which are involved in actin binding. The ATP-binding site, which lies close to the apex of the cleft, consists of a 'P-loop' motif flanked by switch 1 and switch 2 elements, similar to those found in the G-proteins. The switch 2 element connects with the 'relay helix'. The proximal end (as seen from the actin helix) of the lever arm is anchored in the converter domain that is attached to the relay helix.

Most crystal structures of the myosin head fall into two classes depending on whether the relay helix has a kink at its middle point or not. The two states are known as pre-power-stroke state and post-rigor state. The post-rigor state was the first structure of the myosin cross-bridge solved and since in this crystal there was no nucleotide in the active site it was thought to be the true rigor state. As is explained by Lee Sweeney (Sweeney & Houdusse 2004) the recently solved structure of a nucleotide-free myosin V construct appears to be closer to the rigor state (the rigor-like state): the commonly occurring post-rigor state has another role in the crossbridge cycle, namely to be the form of myosin after the rebinding of ATP at the end of the power stroke that rapidly releases from actin. Thus the two structure classes now need to be increased to three. These three frequently occurring conformers of the myosin cross-bridge are presently referred to as the pre-power-stroke, rigor-like, and post-power-stroke conformations (this unified nomenclature arose out of the discussion meeting).

The kink produced in the relay helix leads to a rotation of the distal end of the relay helix that in turn rotates the attached converter domain through *ca*. 60°. The rotation of the converter in turn leads to a 60° rotation of the lever arm. Removing the kink causes the lever arm to rotate back by 60°, which is the elementary structural event in the power stroke. The mechanism by which actin binding brings about the straightening of the kinked relay helix becomes a central issue in understanding muscle contraction.

The second essential property of the myosin crossbridge is the strong negative coupling between ATP binding and actin binding. In the absence of ATP the crossbridge binds with high affinity to the actin filament (rigor—strong binding). The binding of ATP to the myosin crossbridge leads to a rapid release from actin via formation of the post-rigor structural state (weak binding). Furthermore, the rebinding of the myosin crossbridge in the pre-power-stroke state, carrying the products of hydrolysis (ADP and phosphate), to actin leads to the release of products.

Anne Houdusse and Lee Sweeney (Sweeney & Houdusse 2004) explained how the study of unconventional myosins was aiding our understanding of the crossbridge cycle since the varying kinetic properties lead to different conformers being the dominant species. They have studied in particular myosin V and myosin VI. Myosin V is an unconventional myosin involved in transporting vesicles along actin cables in the cell. Like myosin II it is a two-headed molecule but has a long lever arm (six IQ motifs) and a binding site for its cargo at the C-terminus. It appears to proceed along the actin filament by a 'hand-over-hand' mechanism so that at any one time at least one head is attached to the actin filament (processive). Myosin V is kinetically tuned to allow movement along actin filaments: nucleotide-free myosin V appears to be constitutively in the strong binding form (which is not the case for myosin II). Moreover, the structure of the apomyosin V crossbridge shows the cleft between the upper and lower 50K domains to be shut. The structural effects of cleft
closure appear to include the opening of switch 1, which opens the nucleotide-binding pocket, and a twist of the central β-sheet, which is associated with a large movement of the P-loop that destroys the nucleotide-binding site.

Incubating actin filaments with isolated crossbridges without ATP produces the rigor complex known as 'decorated actin'. Decorated actin provides a model system for studying the strong interaction between actin and myosin. Ken Holmes described how cryo-energy-filter electron microscopy has recently yielded a 14 Å resolution map of rabbit skeletal actin decorated with chicken skeletal S1 (Holmes *et al.* 2004). These studies showed that the cleft in the actin-binding site is closed on strong binding to actin. Moreover, the myosin V atomic model can be fitted without deformation into the electron microscope 3D reconstruction. Thus myosin V appears to be structurally the strong binding form and may therefore be taken as a model of myosin in the rigor complex (near-rigor). This allows a detailed description of the actin–myosin interface and also suggests a mechanism (by twisting the central β-sheet) whereby actin binding may straighten the relay helix and bring about the power stroke. Although the apomyosin V structure has a straight relay helix and the lever arm is in the end-of-power-stroke configuration, the switch 2 element is 'closed'. In the post-power-stroke state the switch 2 element is 'open', which had been taken to mean that the opening of switch 2 was strongly linked to the execution of the power stroke. The present results indicate that the switch 2 element is closed and remains closed during strong binding to actin. The straightening of the relay helix comes about through the twisting of the β-sheet. If the cleft closure at the actin-binding site causes the twisting of the β-sheet then one has a mechanism for actin binding driving the power stroke.

In the course of the studies that led Hugh Huxley to the sliding-filament model he observed the rich low angle X-ray diffraction pattern arising from the crystalline lattice of thick and thin filaments in muscle. The myosin filaments give rise to a series of meridional reflections in the X-ray diagram of muscle that arise from the regularly repeating crossbridges along the filament. The regular repeat of myosin crossbridges in each half of a bipolar filament produces an X-ray interference effect that manifests itself by splitting each of the strong meridional reflections into two subsidiary peaks. During a contraction these interference peaks move. This allows the axial motions of the cross-bridges to be followed in an intact muscle fibre with a precision of *ca*. 1 Å. Working with isolated frog muscle fibres Malcom Irving and his collaborators (Lombardi *et al.* 2004) used this effect to measure the unitary working stroke of myosin crossbridges *in situ*, as they pull the actin filaments towards the centre of the myosin filament during muscle shortening. At low load (0.25 times the isometric force) the average working stroke was 12 nm, consistent with crystallographic studies. The working stroke was smaller and slower at higher load.

Hugh Huxley (2004) described his measurements of the interference peaks from intact frog muscle. By using an

intact muscle and the most intense available synchrotron X-ray source he was able to observe the interference fringes with millisecond time resolution. The changes in the time-resolved interference fringes provide a new source of structural information about crossbridge movement during mechanical transients and during steady shortening from intact muscle. With some assumptions, many observations can be interpreted very satisfactorily by the tilting-lever-arm model. In isometric contraction the lever arms are in an orientation near the start of the working stroke. Upon rapid release of 10–12 nm, they move to the end of the stroke with a delay of 1–2 ms. This delay must represent additional processes that have to occur during contraction even in tension-generating heads. In muscles shortening at moderate loads the mean position of the heads moves only 2–3 nm closer to the M-line than in the isometric case.

Clive Bagshaw (Zeng *et al.* 2004) spoke about the dynamics of actomyosin interactions in relationship to the crossbridge cycle. Transient kinetic measurements of the actomyosin ATPase provided the basis of the Lymn–Taylor model for the crossbridge cycle, which underpins current models of contraction. Following the determination of the structure of the myosin motor domain, it has been possible to introduce probes at defined sites and resolve the steps in more detail. Probes have been introduced in myosin II motor domain expressed in *Dictyostelium discoideum* (cellular slime mould):

(i) single tryptophan residues at strategic locations throughout the motor domain
(ii) green fluorescent protein fusions at the N and C termini and
(iii) labelled cysteine residues engineered across the actin-binding cleft.

These studies confirm that in solution the tryptophan (W501) in the relay loop senses the switch 2 movement at the active site (open to closed) that is loosely coupled to the hydrolysis reaction. (One should note that the studies on myosin V and the strong binding to actin cited above appear to show that the switch 2 remains closed for the whole of the power stroke. Thus in the actin bound form the W501 fluorescence will respond to the position of the converter domain rather than the opening or closing of switch 2.) Cleft closure and the coupled switch 1 opening appear to be a key step in the actin dissociation and subsequent activation of product release.

There are two translational steps in the actomyosin crossbridge cycle, the working stroke, whereby an attached myosin crossbridge moves relative to the actin filament, and the repriming step, in which the crossbridge returns to its original orientation. John Sleep reported on his investigation of crossbridge movement (using optical tweezers) at the single molecule level resulting from the binding to actin of a variety of myosin intermediate states produced by the binding of ATP analogues (Steffen & Sleep 2004). With the exception of M·ADP·P_i, all states that might be regarded as product-like give a working stroke of zero, from which he concludes that these all bind to actin in a way that cannot go through the power stroke because they are already at the end of the power stroke, i.e. a post-power-stroke state. Apparently for the same reason, ATP-like states, namely M·GTP, M·ITP and M·ATPβS, also give no measurable working stroke. Only the transient state, M·ADP·P_i that binds during the ATPase cycle gives a working stroke, indicating that it alone must bind in a pre-power-stroke state. The observation that states mimicking M·ATP bind in a post-power-stroke conformation implies that the repriming step, a post-to-pre transition, must take place while the myosin is dissociated from actin.

To achieve the observed efficiency of muscle, crossbridges cannot dissociate from actin once the working stroke has started. Present data suggest that the full strength of actin binding, and the corresponding slow rate of actin dissociation, is not achieved until late in the ATPase cycle, which poses problems regarding the ordering of P_i release and the opening of the switch 1 and switch 2 elements.

Justin Molloy and his co-workers (Batters *et al.* 2004) used an optical tweezers-transducer to measure the force and movement produced by a single-headed myosin molecule while it interacts with actin. They found that Myo1c (which is a myosin isoform implicated in hearing and also studied by Gillespie (2004)) produced a power stroke of *ca.* 3.4 nm and that movement was generated in two phases (a similar two phase power stroke has already been demonstrated in another myosin isoform, Myo1b). The overall lifetime of binding events showed a biphasic exponential distribution. This indicates that there are two detachment pathways from the actomyosin bound crossbridge state. There is a fast, ATP-independent, detachment pathway and a much slower, ATP-dependent pathway. They suggest that the fast phase involves attachment and detachment of myosin with no net ATP breakdown whereas the slow population represents normal crossbridge cycling. The fast population would not be expected to contribute to the power stroke and would therefore reduce the average observed step size of Myo1c. However, these crossbridges would produce a viscous drag force that might be physiologically relevant.

Dietmar Manstein (2004) described how protein engineering provides an excellent tool to investigate how structural features relate to mechanism. Expression of myosin II from *Dictyostelium discoideum* in plasmids allows great freedom of design for both point mutations and fusion proteins (myosin II in *Dictyostelium* is not involved in a muscle function; it plays an important role in cytokinesis). In addition to studies aimed at dissecting the communication pathways in myosin and other enzymes, it is possible to generate enzymes with increased catalytic efficiency and specifically altered or newly introduced functions. Manstein described a specifically designed construct that rotated the lever arm through 180°. This myosin indeed moves actin in the opposite direction to normal and is apparently a model for myosin VI, a naturally occurring myosin that moves backwards.

Mike Geeves (Nyitrai & Geeves 2004) discussed the strain sensitivity of ADP release in myosin motors. The release of ADP from the actomyosin crossbridge plays a crucial role in the ATP driven crossbridge cycle. In fast-contracting muscle fibres the rate at which ADP is released from the crossbridge correlates with the maximum shortening velocity of the muscle fibre, and in some models the rate of ADP release defines the maximum shortening velocity. Indeed, it has long been thought that the rate of ADP release could be sensitive to the load on the cross-bridge and thereby provide a molecular explanation of the Fenn effect (the fact that total energy production is linked to the amount of work done). However, direct evidence of a strain-sensitive

ADP release mechanism has been hard to come by for fast muscle myosins. The recently published evidence for a strain-sensing mechanism involving ADP release for slower muscle myosins and in particular non-muscle myosins is more compelling and is at the heart of models of myosin V processivity. A strain-sensitive mechanism of ADP release appears to be universal for all myosins, but the basic mechanism has evolved in different ways for different types of myosin. Furthermore, this strain-sensing mechanism provides a way of coordinating the action of multiple myosin motor domains, either in a single myosin molecule or in complex assemblies of myosins.

Yale Goldman (Takagi *et al.* 2004) discussed the dynamics of both conventional and unconventional myosins as measured on single molecules. Using single-molecule fluorescence polarization (to measure the angle change of the lever arm) and nanometre localization (to measure displacement), he and his co-workers recently studied angle changes and dynamics of chicken myosin V molecules while translocating processively along actin. The results provided strong support for a hand-over-hand mechanism of processive motility. With conventional myosin, an isometric force clamp was used to maintain the position of an actin filament constant in response to a myosin interaction. The results suggest that that mechanical work done by actomyosin occurs before phosphate release, that the work can be reversed by an applied load, and that phosphate release is load dependent.

Avril Somlyo (Somlyo *et al.* 2004) summarized the special properties of smooth muscle myosin. An important property of smooth muscles is the ability to maintain force at low levels of ATPase activity and shortening velocity, which reflects the very high affinity of smooth muscle myosin for ADP. Novel ATP/ADP-fluorescent analogues were used to measure directly ADP release and binding kinetics during the crossbridge cycle in phasic and tonic smooth muscles. ADP release was significantly faster in phasic than tonic smooth muscles. Phosphorylation of the regulatory light chains that control smooth muscle activity increased and strain decreased the release rate approximately twofold. One concluded that the strain- and dephosphorylation-dependent high affinity of ADP to attached crossbridges and slow ADP release from smooth muscle myosin prolongs the fraction of the duty cycle occupied by strongly bound actomyosin·ADP state(s) and contributes to the high economy of force production.

John Kendrick-Jones (Roberts *et al.* 2004) presented the myosin family tree as it is known today (some 18 classes of myosin have been discovered so far). He then went on to discuss myosin VI. Unlike almost all the other myosins so far studied, myosin VI moves towards the minus end of actin filaments. In the cell it has been localized in membrane ruffles at the leading edge, in the Golgi complex and in clathrin-coated pits/vesicles, indicating that it functions in a wide variety of intracellular pathways. Although previous studies indicated that myosin VI might be a dimer, biochemical characterization and electron microscopy reveal that myosin VI as isolated in Cambridge is a monomer. Using an optical tweezers force transducer it was observed that myosin VI is non-processive (it appears to have only one head) and produces a large working stroke of 18 nm. This result is difficult to comprehend within the current models of lever arm movement because myosin IV has a rather short lever arm.

For the last paper in the meeting, as an example of the rich repertoire of myosin functions, Peter Gillespie (2004) described myosin and mechanical transduction by the inner ear. Twenty years ago, the description of hair-cell stereocilia as actin-rich structures led to speculation that myosin molecules were important for mechanical transduction in the inner ear. Howard & Hudspeth (1987) proposed specifically that a myosin I might mediate adaptation of the transduction current. Movement of stereocilia produces strain in so-called tip links, joining adjacent stereocilia at their tips, and in turn this opens strain-sensitive ion channels and leads to transduction. However, sustained deflection of stereocilia results in a decrease in transduction, arising from (as Howard and Hudspeth proposed) myosin molecules linked to each tip link that slide along the sterocilia and lower the strain on the tip link. Gillespie has exploited the voluminous myosin literature to design tests of this hypothesis and to pinpoint the responsible isoform, myosin-1c. The identification of this myosin as the adaptation motor would have been impossible without an understanding of other myosins, particularly muscle myosins. The sliding-filament hypothesis for muscle contraction has thus led, through a circuitous pathway, to a deep understanding of the behaviour of the inner ear.

In the past 50 years we have come a long way towards understanding muscle contraction in purely physicochemical terms. The technology used has often been highly innovative. Indeed, an important side effect of this research has been technological spin-off. In many cases techniques pioneered for muscle and myosin research are now in general use. Important advances in electron microscopy from thin sectioning to three-dimensional image reconstruction and high-resolution cryo-electron microscopy were largely made to understand pressing problems in actin and myosin structure. The use of synchotron radiation as an intense X-ray source was initiated in order to observe the changes in the low-angle X-ray scattering from muscle fibres that report crossbridge movement. Later, this technology made it possible to solve the crystal structure of myosin. Elucidating the biochemical pathway of the actomyosin ATPase, high time-resolution mechanics, *in vitro* motility assays and single molecule studies were all initiated to understand muscle. Myosin research included the discovery of the many classes of non-muscle myosin that play an important role in cell biology. Last, but not least, are the intellectual and technological innovations that have provided a detailed and consistent theory of the actomyosin interaction. We expect that well within the next 50 years a complete understanding of the structural events behind the power stroke will emerge. Improved molecular dynamic approaches will make it possible to simulate the actomyosin interaction from a knowledge of static structures. At the moment muscle provides the prime example of the success of the reductionist approach to biology. The question then arises as to where one should stop. Should we strive for a detailed, quantum-mechanical solution? Perhaps more difficult are the biological question concerning the origins of muscle. An evolutionary understanding of the origin of myosin and of the different myosin isoforms will surely be forthcoming. There remains the conundrum of the coevolution of muscle and the central nervous system. This may prove even more

challenging than the reduction of voluntary movement, considered by the ancients to be the epitome of life, to mere physical chemistry.

K. C. Holmes *November 2004*
Max-Planck-Institute fur Medizinische Forschung, Jahnstrasse 29, Heidelberg D-61920, Germany

REFERENCES

Batters, C., Wallace, M. I., Coluccio, L. M. & Molloy, J. E. 2004 A model of stereocilia adaptation based on single molecule mechanical studies of myosin I. *Phil. Trans. R. Soc. B* **359**, 1895–1905. (doi: 10.1098/rstb.2004.1559)

Cooke, R. 2004 The sliding filament model: 1972–2004. *J. Gen. Physiol.* **123**, 643–656.

Gillespie, P. G. 2004 Myosin I and adaptation of mechanical transduction by the inner ear. *Phil. Trans. R. Soc. B* **359**, 1945–1951. (doi:10.1098/rstb.2004.1564)

Hanson, J. & Huxley, H. E. 1954 Changes in the cross striations of muscle during contraction and stretch and their structural interpretation. *Nature* **173**, 973.

Holmes, K. C., Schröder, R. R., Eschenburg, S., Sweeney, H. L. & Houdusse, A. 2004 The structure of the rigor complex and its implications for the power stroke. *Phil. Trans. R. Soc. B* **359**, 1819–1828. (doi: 10.1098/rstb.2004.1566)

Howard, J. & Hudspeth, A. J. 1987 Mechanical relaxation of the hair bundle mediates adaptation in mechanoelectrical transduction by the bullfrog's saccular hair cell. *Proc. Natl Acad. Sci. USA* **84**, 3064–3068.

Huxley, A. F. & Niedergerke, R. M. 1954 Structural changes in muscle during contraction. Interference microscopy of living muscle fibres. *Nature* **173**, 971–973.

Huxley, H. E. 2004 Recent X-ray diffraction studies of muscle contraction and some of their implications. *Phil. Trans. R. Soc. B* **359**, 1879–1882. (doi: 10.1098/rstb.2004.1556)

Kron, S. J. & Spudich, J. A. 1986 Fluorescent actin filaments move on myosin fixed to a glass surface. *Proc. Natl Acad. Sci. USA* **83**, 6272–6276.

Lombardi, V. (and 10 others) 2004 X-ray diffraction studies of the contractile mechanism in single muscle fibres. *Phil. Trans. R. Soc. B* **359**, 1883–1893. (doi: 10.1098/rstb.2004.1557)

Lymn, R. W. & Taylor, E. W. 1971 Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry* **10**, 4617–4624.

Manstein, D. J. 2004 Molecular engineering of myosin. *Phil. Trans. R. Soc. B* **359**, 1907–1912. (doi: 10.1098/rstb.2004.1565)

Nyitrai, M. & Geeves, M. A. 2004 ADP and strain sensitivity in myosin motors. *Phil. Trans. R. Soc. B* **359**, 1867–1877. (doi: 10.1098/rstb.2004.1560)

Roberts, R., Lister, I., Schmitz, S., Walker, M., Veigel, C., Trinick, J., Buss, B. & Kendrick-Jones, J. 2004 Myosin VI: cellular functions and motor properties. *Phil. Trans. R. Soc. B* **359**, 1931–1944. (doi:10.1098/rstb.2004.1563)

Somlyo, A. V., Khromov, A. S., Webb, M. R., Ferenczi, M. A., Trentham, D. R., He, Z.-E., Sheng, S., Shao, Z & Somlyo, A. P. 2004 Smooth muscle myosin: regulation and properties. *Phil. Trans. R. Soc. B* **359**, 1921–1930. (doi: 10.1098/rstb.2004.1562)

Steffen, W. & Sleep, J. 2004 Using optical tweezers to relate the chemical and mechanical cross-bridge cycles. *Phil. Trans. R. Soc. B* **359**, 1857–1865. (doi: 10.1098/rstb.2004.1558)

Sweeney, H. L. & Houdusse, A. 2004 The motor mechanism of myosin V: insights for muscle contraction. *Phil. Trans. R. Soc. B* **359**, 1829–1842. (doi: 10.1098/rstb.2004.1576)

Szent-Gyorgyi, A. G. 2004 The early history of the biochemistry of muscle contraction. *J. Gen. Physiol.* **123**, 631–641.

Takagi, Y., Shuman, H. & Goldman, Y. E. 2004 Coupling between phosphate release and force generation in muscle actomyosin. *Phil. Trans. R. Soc. B* **359**, 1913–1920. (doi: 10.1098/rstb.2004.1561)

Zeng, W., Conibear, P. B., Dickens, J. L., Cowie, R. A., Wakelin, S., Málnási-Csizmadia, A. & Bagshaw. C. R. 2004 Dynamics of acto-myosin interactions in relation to the crossbridge cycle. *Phil. Trans. R. Soc. B* **359**, 1843–1855. (doi: 10.1098/rstb.2004.1527)