Sarcomeric Myosin Isoforms: Fine Tuning of a Molecular Motor

Carlo Reggiani, Roberto Bottinelli, and Ger J. M. Stienen

Sarcomeric or striated muscle myosins are the molecular motors whose fine tuning is best known. Sarcomeric myosin isoforms convert chemical into mechanical energy at very different rates without losing efficiency. Availability of the amino acid sequences offers for the first time the chance to understand the molecular basis of the versatility of these molecular motors.

Myosin is the best-known molecular motor. Molecular motors are proteins that are able to convert chemical energy into mechanical energy (chemomechanical transduction) through a structural change (working stroke). The working stroke, amplified by the ordered organization of an ensemble of motors, can give rise to microscopically, or even macroscopically, visible movements of cells or parts of cells.

At present, three molecular motors are known: myosin, kinesin, and dynein. The molecular motor that satisfies the energy need for muscular contraction is myosin, or more precisely, class II myosin, which is one of the 15 distinct classes of myosins presently identified. After many years as the only known molecular motor, class II myosin still represents the motor whose fine tuning is best understood. In multicellular organisms, class II myosins are present not only in muscle cells but also in platelets, epithelial gastroenteric cells, and neurons. In unicellular organisms, such as the amoeba and dictyostelium, they play a central role in cytokinesis.

Class II myosins are hexamers composed of two myosin heavy chains (MHC), two essential myosin light chains (MLC), and two regulatory MLC (see Fig. 1). Each MHC (molecular mass 220 kDa) is formed by a globular head and a long tail. The globular head (~900 amino acids) contains the catalytic site for ATP hydrolysis and the surface for actin binding. Its detailed structure has been recently resolved (6) and is shown in Fig. 1A. The head domain can be divided into three parts by papain digestion: 25-kDa, 50-kDa, and 20-kDa domains. Flexible loops (loop 1 and loop 2) sensitive to enzymatic digestion connect the three domains. The 20-kDa domain is largely formed by a 8.5-nm-long α-helix that protrudes from the head, forming a shaft around which one essential and one regulatory MLC are wrapped. This shaft continues, after a sharp bend, with a very long α-helical rod (150 nm long, ~1000 amino acids), indicated as the myosin tail. Pairs of MHC dimerize by forming coiled structures with their tails, which are the backbone of the thick filament.

Thick filaments of sarcomeric or striated muscles are ~1.6 μm long and are composed of two symmetrical portions, each containing ~300 myosin molecules. Force and/or shortening are generated by cyclic interactions of the myosin heads protruding from the thick filament with the adjacent actin thin filaments. Energy is provided by ATP hydrolysis. ATP binds to the catalytic site on the myosin head and is quickly hydrolyzed. After ATP hydrolysis, myosin attaches to the actin filament, undergoes the structural changes (working stroke), thus producing an elementary displacement \( D \) of the thin filament, and then releases the hydrolysis products (ADP and inorganic phosphate). Finally, myosin detaches from the actin filament. The repeated working strokes associated with cyclic attachment and detachment of myosin to actin drive the axial sliding of the filaments in the direction of shortening. The speed of shortening can be calculated as \( V = D_{\text{on}}/t_{\text{on}} \), where \( t_{\text{on}} \) is the time spent attached to actin. The ratio \( t_{\text{on}}/t_{\text{total}} \), where \( t_{\text{total}} \) is the total duration of the ATP hydrolysis cycle, is called the duty ratio and corresponds to the fraction of myosin heads attached at each instant to the thin filament.

Myosin isoforms

The range of rates at which sarcomeric myosins are able to convert chemical energy into work is surprisingly large when various animal species and, in each species, different types of muscles are compared. This is related to the functions that are accomplished by striated muscles, from maintaining posture to moving the body in space, from chewing food to pumping blood. The requirements of these functions in terms of mechanical power are very different within each animal species and vary further from species to species.

The secret of the extraordinary ability of the myosin motor to comply with very different functional requirements resides in the existence of multiple isoforms of both MHC and MLC. Isoforms are proteins very similar to each other and able to replace each...
other. Slight diversity in their amino acid sequences gives rise to different structural and functional properties. In the genome of each species, a number of genes code for MHC and MLC isoforms; further variations are produced during mRNA maturation by alternative splicing (for review, see Ref. 8).

Information about the sarcomeric muscle myosin isoforms and their relationship with contractile muscle performance are available for several species, from invertebrates (Drosophila) to fishes (carp, cod), amphibians (toad, frog), birds (chicken), and several mammals (mouse, rabbit, rat, human, horse). However, the

FIGURE 1. Structure of myosin molecule and location of clusters of molecular diversity. A: tertiary structure of S1 myosin fragment with a schematic reference to complete molecule and to filament lattice. Functionally relevant regions are indicated. Essential and regulatory myosin light chains (MLC) are blue. Myosin heavy chain (MHC) is drawn with a smooth spectrum of colors from blue (NH₂ terminus and the 25-kDa domain) to green and yellow (50-kDa domain) and red (20-kDa domain—including the shaft—and COOH terminus). Figure was prepared using Rasmol and coordinates of myosin S1 from Ref. 6. B: schematic representation of primary structure of complete MHC molecule, with indication of functionally relevant sequences and, below, localizations of regions where diversity among isoforms tends to cluster (black bands) in paired comparison between MHCα and MHCβ/slow, from Ref. 15.
largest body of information presently available on myosin isoforms and their properties related to chemomechanical transduction concerns sarcomeric myosins of mammals and particularly of the rat, rabbit, and human. In these species, the sequences of large portions and, in some cases, of whole MHC and MLC genes have been provided by clone isolation and sequencing (for review, see Ref. 15), whereas detailed functional characterization of biochemical, energetic, and mechanical parameters have been obtained from studies on single-fiber preparations and on purified myosin preparations (for review, see Ref. 8).

In the above-mentioned mammals, at least eight distinct isoforms of class II MHC encoded by eight separate genes are expressed in sarcomeric or striated muscle cells. They combine with several MLC isoforms to generate myosin molecule isoforms (isomysins) (8). The eight known sarcomeric MHCs are as follows: MHC1 or MHCβ/slow, expressed in ventricular myocardium and in slow skeletal muscle fibers; MHCα, expressed in atrial myocardium and occasionally in skeletal muscle fibers; MHC2A, MHC2X, and MHC2B, which are the three fast isoforms expressed in fast skeletal muscle fibers; MHCexoc, a fast isoform expressed only in extraocular muscles; and MHCemb and MHCneo, which are two isoforms expressed during embryonic and perinatal development. As a general rule, in fast muscle fibers a fast MHC isoform associates with a fast regulatory isoform (MLC2f) and with one of the two fast essential MLC isoforms (MLC1f or MLC3f). Interestingly, these two isoforms originate from the same gene through a process of alternative splicing. In slow fibers, the slow MHCβ binds a slow regulatory isoform (MLC2s) and a slow essential MLC, either MLC1sa or MLC1sb. In atrial myocardium, a specific atrial isoform of regulatory MLC (MLC2a) and a developmental form of essential MLC (MLC1a-emb) are associated with MHCα. Finally, in ventricular myocardium, MHCβ or MHCα is expressed together with the slow isoforms MLC2s and MLC1sb.

Unfortunately, complete sequences are available only for few sarcomeric MHC isoforms. For many other MHC isoforms only portions of the sequences are known. On the basis of available data, the degree of homology between corresponding MHC isoforms in different species (orthologous MHC isoforms) is between 90 and 95%; in other words, only 95–190 out of 1900 amino acids are different. If only nonconservative changes, i.e., replacements of one amino acid with another one belonging to a different group, are considered, the homology arrives at 98–99%; only 20–30 amino acids are different. The degree of homology between different MHC isoforms in one species (paralogous MHC isoforms) is lower; the percentage of nonidentical amino acids can reach 20% (15). The percentages of nonidentical amino acids are not substantially different in the tail and in the head domain. Most of the nonidentical amino acids are clustered in few regions (15): the two flexible loops, the MLC binding regions, the S2 subfragment, the hinge region, and the tail (Fig. 1). Some other regions, like the ATP-binding pocket or the actin-binding surface, are virtually identical in all isoforms. Particularly interesting are the two flexible loops connecting the 25-kDa and 50-kDa domains and the 50-kDa and 20-kDa domains, respectively. The lack of conservation in the two loops might simply reflect lack of functional constraints. It is, however, also possible that these two loops are capable of functional modulation (see below). Loop 1 is located near the entrance of the ATP-binding site, and loop 2 is located on the actin-binding surface (see Fig. 1A).

The expression of MHC and MLC genes during development and adult life is controlled by several factors, including motoneuron discharge patterns, loading conditions, and hormones. Although coexpression of two or more MHC genes is possible and even frequent during development or changes in activity patterns, it is a general rule that each cell expresses only one MHC gene associated with two MLC genes (8). This creates a heterogeneous population of muscle fibers, each containing a specific combination of MHC isoforms and MLC isoforms. These conditions offer us the chance to study the chemomechanical transduction features of various isoforms by simply analyzing the functional behavior of individual muscle fibers.

**Single-fiber studies**

In the last decade, several studies have analyzed the correlations between myosin isoform composition and functional properties of mammalian skeletal muscle fibers (for review, see Ref. 8). The novelty of these studies has been represented by the determination of the mechanical and energetic parameters related to chemomechanical energy transduction and of the protein isoform composition in single muscle fibers. This has been made possible by a more powerful resolution of electrophoretical and immunological identification of protein isoforms and by the technical improvement of functional characterization of demembranated fibers, i.e., fibers in which sarcolemma and other membranes are solubilized or removed and only the myofibrils with the contractile proteins are functionally intact.

In muscle fibers, the conversion of chemical into mechanical energy is defined by 1) the amount of chemical energy released (G), which is obtained by multiplying the number of ATP moles consumed by the energy released from each mole (~50 kJ/mol, under the conditions present in muscle cells) and 2) the work generated as measured from the product of force and movement. Only part of the chemical energy can be actually converted into work (W); another part is converted into heat (H) and a third part is absorbed by the reaction itself. The efficiency of the chemomechanical transduction is defined as thermodynamic efficiency = W/G or as mechanical efficiency = W/(W+H). Instead of work and the amount of energy released, their respective time derivatives are more often used because they are direct expressions of the instantaneous properties of the motor function: power (W'), rate of energy released (E'), estimated from the rate of ATP hydrolysis multiplied by the ATP energy equivalent, and the sum of W' and the rate of heat production (H').

The power output (W') of a muscle shortening against a load equals the product of force developed and velocity of shortening.
Examples of force-velocity curves of two fiber populations, one expressing the slow MHCβ and the other expressing the fast MHC2B, are shown in Fig. 2A. Maximum shortening velocity (V_0), i.e., the shortening velocity at zero load, is about three times higher in the fast than in the slow fibers. Power-velocity curves are shown in Fig. 2B. The peak power, W'_{max}, is reached at a velocity (V_\text{opt}) approximately corresponding to 1/3 of V_0. Figure 2C shows the rates of energy release as a function of shortening velocity. During shortening against a load, ATP consumption increases in direct relation to velocity of shortening. This increase is closely proportional to the rate of ATP consumption during isometric contraction (Fig. 2C), being therefore much higher in muscle fibers that contain fast MHC than in muscle fibers that contain slow MHC isoforms (7). However, because fast fibers also develop much higher power than slow fibers (see Fig. 2B), thermodynamic efficiency is only slightly greater for slow than for fast fibers (see Fig. 2D). The optimum efficiency is reached at approximately the same speed of shortening as power (V_\text{opt}). V_\text{opt} varies widely among muscle fibers and depends on MHC isoforms. Recruitment of muscle fibers containing distinct MHC isoforms enables muscles to optimize power production and efficiency when movements are performed at different speeds. In other words, muscles in vivo optimize their power and efficiency by shortening at V_\text{opt}. Thus V_\text{opt} is possibly one of the most physiologically relevant contractile parameters of muscle fibers.

In Table 1, mechanical and energetic parameters of muscle fibers from three mammalian species are reported. Fibers are identified on the basis of their MHC isoform composition. Data come from previously published work of our group and refer to demembranated fiber segments maximally activated at 12°C. The MLC isoforms that are most frequently associated with the MHC are indicated in some cases. The variations in contractile and biochemical properties among muscle fibers are extremely large, both comparing various isoforms in the same species (paralogous) and corresponding isoforms in different species (orthologous). MHC isoform diversity generates large variations in V_0 (2, 3, 11), W'_{max}, V_\text{opt}, rate of ATP splitting in isometric conditions (A_0) (4, 5, 9), and in the rate of tension rise (not shown). On the contrary, the variability in isometric tension (P_0) is only loosely related to the presence of different MHC isoforms. Because ATP consumption in isometric conditions is substantially higher in muscle fibers containing fast MHC isoforms than in muscle fibers containing the slow MHC (4, 9), the amount of ATP needed to maintain a given amount of tension (tension cost) is much higher in fast than in slow muscle fibers.

MLC isoforms also contribute significantly to modulating motor properties. Fast MHC isoforms are associated with two essential MLC isoforms: MLC1f and MLC3f. In the rat and the rabbit, the relative proportion of these two isoforms appears to significantly affect V_0 through a complex interplay with MHC isoforms: V_0 increases in proportion to the amount of MLC3f present (2, 11). ATPase activity and other parameters are not affected (4). Furthermore, MLCs provide the only myosin-based posttranslational regulatory mechanism identified until now: phosphorylation of regulatory MLC enhances force development of submaximally activated muscle fibers.
Inspection of Table 1 shows that orthologous isoforms in different species exhibit different values of the kinetic parameters. As a general rule, the values of the kinetic parameters are higher in the rat than in the rabbit and in the human. This has offered a new and definitive explanation of a classic observation of A. V. Hill on limb muscle performance of different animal species. To achieve a comparable speed of locomotion, small animals need faster muscles than large animals. This goal can be reached in two alternative ways, either with a high proportion of fast fibers or with a higher speed of shortening of both fast and slow fibers. The precise identification of MHC isoforms has shown that both mechanisms cooperate to create the difference in speed of shortening between muscles of different animal species. For example, mouse muscles are richer in fast MHC2B than corresponding muscles of rat, and these in turn express fast MHC2B more than corresponding muscles of the rabbit. This fast and expensive MHC isoform is virtually not expressed in human muscles (8). Moreover, it is true that orthologous MHC have different functional properties in different species. Each MHC isoform is associated with a higher speed of shortening and power output in small animals than in large animals (Table 1 and Fig. 3A). The same holds true for energetic properties: each MHC isoform hydrolyzes more ATP and consumes more energy for tension development in small than in large animals. This fits well with the high general metabolic activity typical of small mammals and their large body surface-to-volume ratio, which promotes heat loss.

Shortening velocity and ATP hydrolysis rate appear to vary in proportion, comparing both paralogous MHC isoforms in one species and orthologous MHC isoforms in different species. In his influential study, Barany (1) showed, 30 years ago, that in 16 different muscles of various animal species there was a correlation between shortening velocity and ATPase activity of purified myosin activated by actin or by calcium over a range of variation of 200 times. The recent data obtained in single fibers confirm that a correlation exists between V₀ and ATP utilization rate during isometric contraction (A₀; see Fig. 3B). In the single-fiber studies, both kinetic parameters are measured on the same preparations under identical experimental conditions. Furthermore, molecular characterization allows precise definition of the fiber type, i.e., which MHC isoform is present. The general correlation between A₀ and V₀ is in accordance with Barany’s findings, but some interesting deviations occur. For example, the orthologous βslow or type 1 MHC isoforms in rat, rabbit, and human, respectively, are associated with different speeds of shortening but similar isometric ATPase activity values. Among fast fibers, fibers containing MHC2X and MHC2B of rat and rabbit have similar shortening velocities but differ significantly in ATP-splitting rate. Interestingly, the correlation, which can be seen when groups formed with fibers all containing the same MHC isoforms are compared (Fig. 3B), does not always show up when individual fibers within each group are considered. For example, in a population of rat fibers containing MHC2B, V₀ and A₀ are not correlated. This can be explained by assuming that MLC exerts a powerful influence.
on $V_0$ that is not accompanied by a corresponding effect on $A_0$ (4). Finally, a dissociation between $V_0$ and $A_0$ appears to occur when temperature is changed, because $V_0$ shows a higher thermal sensitivity than $A_0$ (3, 9). It is worth noting that the correlation between $V_0$ and $A_0$ is consistent with the observation that efficiency is virtually constant. Actually, the slope of the correlation between $V_0$ and $A_0$ (or the ratio $V/A_0$) is comparable to efficiency. Efficiency is the ratio between power output and energy released by ATP hydrolysis during loaded shortening. As discussed above, ATPase rate during shortening increases in close proportion to its value in isometric conditions ($A_0$), and $V_0$ is a major determinant of $W_{\text{max}}$.

The picture emerging from the results obtained in skinned fiber studies points to an important conclusion. The diversity between myosin isoforms produces large variations in several kinetic parameters, such as $V_0$, $W_{\text{max}}$, and tension cost, whereas other parameters, such as efficiency, ratio of $V_0$ to $A_0$, and $P_0$ show much smaller variations. This can be explained by assuming that all sarcomeric myosins share a common conserved basic mechanism of chemomechanical transduction on which the mechanisms of modulation of the kinetic properties are superimposed.

**New insights with in vitro motility assay**

Single-fiber studies find their limits in that they cannot unambiguously distinguish whether the diversity of parameters such as $V_0$ or $W_{\text{max}}$ arise from differences in rate constants of the actomyosin interaction cycle or from differences in the elementary mechanical events. For example, $V_0$ is equal to $D_{\text{uni}}t_{\text{on}}$ under conditions of zero load. As stated above, $D_{\text{uni}}$ is the elementary displacement produced by myosin when attached to actin and $t_{\text{on}}$ is the time spent by a myosin in the attached state. Mechanical analysis of single fibers cannot distinguish whether the variations in $V_0$ are caused by variations of $D_{\text{uni}}$ or $t_{\text{on}}$. The in vitro motility assay has recently offered an exciting approach to extend the knowledge of functional differences between myosin isoforms to the molecular level (14).

Purified myosin isoforms translocate actin filaments at speeds proportional to their actin-activated ATPase activity. Mixtures of two myosin isoforms move actin filaments at speeds intermediate between those produced by the faster and the slower myosin alone (see Ref. 14). Analysis of the motion shows that the two myosins in the mixture interact mechanically, and their behavior can be predicted either by simple force-velocity analysis or by the use of a kinetic model of the actomyosin interaction. Not only MHC isoforms but also essential MLC isoforms are able to influence speed of actin translocation. In particular, the speed of actin movement increases in relation to the amount of MLC3f, in agreement with the results obtained in muscle fibers (reviewed in Ref. 14).

A major step toward understanding whether the diversity between myosin isoforms must be attributed to the rate constants or to the elementary mechanical events has been provided by the optical laser traps. With this new technique, the first measurements of elementary mechanical events of single myosin molecules were obtained. Recent comparison (10) of unitary force and displacements between MHCα and MHCβ/slow purified from rat myocardium indicates that the size of the unitary displacement (9–10 nm) as well as of unitary force (~2 pN) is similar for both isoforms. The durations of both force and movement events are about four times longer for MHCβ/slow than for MHCα. Interestingly, for both isoforms the duration of the displacement events is only 60% of that of force events. This suggests that the two isoforms also share a similar strain sensitivity (or load sensitivity) of the kinetic rate constants for attachment to and detachment from actin. Because force developed in elementary actin-myosin interaction is similar, as is the force developed by maximally activated skinned preparations containing either MHCα or MHCβ/slow, no difference in the duty ratio (or in the instantaneous fraction of attached cross bridges) can be expected.
A clear conclusion seems to emerge from the results obtained in experiments on single myosin molecules. In fast and slow MHC isoforms, the size of the elementary mechanical events (both of force and displacement) are similar, and the diversity resides in attachment and/or detachment rate constants. This conclusion is also in agreement with the results obtained by comparing skeletal muscle myosin with smooth muscle myosin (14).

Molecular diversity between myosin isoforms

The molecular basis of the kinetic diversity must be localized in those amino acids that form the structural difference between isoforms. As stated above, a large fraction of the nonidentical amino acids is clustered in few regions. Among them are the two flexible loops of the MHC head. The hypothesis that these two loops represent hypervariable regions that regulate ATPase activity and shortening velocity was introduced a few years ago.

The role of loop 2 in regulating ATPase activity has received support from the experimental work of Uyeda et al. (13). Loop 2 of Dictyostelium myosin II was replaced with loops of other conventional myosins. The actin-activated ATPase activities of the chimeric myosins were found to correspond to those of the donor myosins. The role of loop 1 is still more uncertain; in smooth muscle, an insert of seven amino acids in loop 1 seems sufficient to influence speed of shortening. In a very recent study (12), loop 1 of smooth muscle myosin was replaced with the corresponding sequences of a number of other myosins (including three sarcomeric MHC). The changes of length and charge in loop 1 were found to modify the rates of ADP release, ATPase activity, and actin filament translation in vitro. However, no clear evidence of a direct relation between loop 1 features and kinetic properties of donor myosin were found. This suggests that loop 1 alone cannot determine any of the kinetic properties of the myosin. The diversity between isoforms is not restricted to the two loops but is also present in other parts of the molecule. For example, it is known that clusters of amino acid diversity are also located in the myosin tail (see Fig. 1B). No information, however, is available on their possible functional impact.

Although most of the work aimed at establishing the molecular basis of the functional differences among isoforms is focused on MHCs, the observation that essential MLC isoforms also affect V₀ should not be overlooked. The two essential MLCs only differ in the NH₂-terminal region. Compared with MLC3f, MLC1f has a long NH₂-terminal extension that allows contact with actin. Through this contact with actin, MLC1f might regulate V₀ by strengthening actomyosin interaction. In agreement with this, S1-MLC1f shows a greater affinity for actin than S1-MLC3f. Alternately, it has been suggested that the MLC isoforms that are wrapped around the α-helical shaft extending from the globular portion of S1 might modulate its flexibility (for further discussion, see Ref. 8).

**Conclusion and future perspectives**

Skeletal muscle fibers have their cytosol filled with myosin molecules (almost 50% of the muscle fiber proteins) arranged in a highly ordered way along the thick filaments in the myofibrils. For this reason, they offer a unique opportunity for investigating the operation of molecular motors by studying energetic and mechanical properties of the whole cell.

The study of the relationship between myosin variants and diversity of energetic and mechanical performance has found a convenient model in demembranated segments of skeletal muscle fibers. Limb muscles of rabbit, rat, mouse, and human have been carefully studied. The results have provided a rather complete picture of how mechanical parameters, such as shortening velocity, power, and energy release rate, vary largely in relation to myosin and particularly MHC isoforms. Other parameters, such as efficiency of chemomechanical conversion, remain relatively constant. More recently, the methods of molecular mechanics have been applied to isolated myosin motors. The first results indicate that isoform diversity influences the kinetics but not the amplitude of the elementary force and movement events. In parallel, the understanding of molecular diversity between myosin isoforms is growing. The sequences of MHC and MLC isoforms have become available and have shown that nonidentical amino acids tend to cluster in defined regions of the molecule. These regions are likely endowed with regulatory properties. Further support to the identification of the functionally relevant amino acid sequences has been provided by mutational analysis.

The road to achieving a full understanding of the structure-function relationships of molecular motors is still long. Important steps forward can be made by determining complete amino acid sequences of many orthologous and paralogous MHC isoforms and by analyzing the correlations with functional properties of muscle fibers and isolated myosin molecules. Actually, for many myosin variants even in the restricted field of the mammalian muscles, little or no functional data are available. No less important, however, is the solution to many still-open issues regarding the basic force generation mechanism, such as the communication between the ATP-binding site and the actin-binding surface, the changes in myosin head configuration during the power stroke, and the coordination between the two myosin heads.

This work was supported by the European Union (Human Capital and Mobility Grant ERBCHRXCT 940606) and by MURST-Italy (Cofin-1998). The authors regret that many important contributions could not be cited due to space restrictions.

**REFERENCES**


First, attempts were made in the late 1960s to develop techniques that would allow the measurement of membrane potential from multiple neighboring cells. Hence, optical recording became possible, providing new insights on cell communication and the trigger for important cellular events. Spatiotemporal heterogeneities of action potentials (APs) now govern propagation, repolarization, and AV node activity more accurately than ever before.

Voltage-sensitive dyes and imaging techniques offer new perspectives on how heterogeneous properties are governed by fiber structure, intercellular coupling, and APs with spatially varying properties. The next step was to stain excitable cells with a dye and record the resulting optical action potentials (APs).

Very poor signal-to-noise ratios (S/N) were encountered when using the light scattering and birefringence of isolated nerve cells and skeletal muscles. This was because the loss of dye over time made it difficult to maintain a signal. Only dye molecules bound to excitable membranes could transduce potential changes to an optical response. The larger the fractional absorption and/or fluorescence of the dye, the greater the voltage "sensitivity" of the dye. These dyes have spectral properties that depend on the local environment and are absorbed in the cytosol, making it possible to record optical APs for 2–4 h.

The first measurements of optical APs from heart muscle in vivo were obtained with merocyanine 540 (M-540) (13). Dyes with larger voltage-dependent responses and less phototoxic damage to the muscle were preferred. However, the difficulties in maintaining optical AP recordings across the heart made the next step impractical.

The loss of dye over time made it difficult to maintain a signal. Only dye molecules bound to excitable membranes could transduce potential changes to an optical response. The larger the fractional absorption and/or fluorescence of the dye, the greater the voltage "sensitivity" of the dye. These dyes have spectral properties that depend on the local environment and are absorbed in the cytosol, making it possible to record optical APs for 2–4 h.

After bathing the muscle with M-540, the stimulus produced a rapid increase in fluorescence followed by a slower response caused by the movement artifact. The first measurements of optical APs from heart muscle in vivo were obtained with merocyanine 540 (M-540) (13). Dyes with larger voltage-dependent responses and less phototoxic damage to the muscle were preferred. However, the difficulties in maintaining optical AP recordings across the heart made the next step impractical.

The next step was to use a dye that could bind tightly to the myocyte membrane and not seem to be internalized in the myosin subfragment-1 (S1). The myosin subfragment-1 (S1) is a molecular motor that is involved in the movement of sarcomeres. It is made up of two myosin heavy chains and two myosin light chains. Myosin isoform composition in skeletal muscle fibres of the rat was studied using myosin light chain and heavy chain variations. Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol. Rev. 76: 371–423, 1996.

The optical trap technique was used to measure the movement of myosin isoforms. Enzymatic activities correlate with chimaeric substitutions at the actin-binding face of myosin. Nature 368: 567–569, 1994.

In vitro motility assays were developed to study the movement of myosin isoforms. This technique provided a clearer picture of myosin molecular motor. News Physiol. Sci. 11: 1–7, 1996.


References: