Myosin isoforms in mammalian skeletal muscle

STEFANO SCHIAFFINO AND CARLO REGGIANI

Department of Biomedical Sciences and Consiglio Nazionale delle Ricerche Unit for Muscle Biology and Physiopathology, University of Padova, 35121 Padua; and Department of Human Physiology, University of Pavia, 27100 Pavia, Italy

Schiavino, Stefano, and Carlo Reggiani. Myosin isoforms in mammalian skeletal muscle. J. Appl. Physiol. 77(2): 493-501, 1994.—Skeletal muscles of different mammalian species contain four major myosin heavy-chain (MHC) isoforms: the “slow” or β-MHC and the three “fast” IIA-, IIX-, and IIb-MHCs; and three major myosin light-chain (MLC) isoforms, the “slow” MLC1s and the two “fast” MLC1f and MLC3f. The differential distribution of the MHCs defines four major fiber types containing a single MHC isoform and a number of intermediate hybrid fiber populations containing both β/slow- and IIA-MHC, IIX- and IIX-MHC, or IIb- and IIb-MHC. The IIA-, IIX-, and IIb-MHCs were first detected in neonatal muscles, and their expression in developing and adult muscle is regulated by neural, hormonal, and mechanical factors. The transcriptional mechanisms responsible for the fiber type-specific regulation of MHC and MLC gene expression are not known and are presently being explored by in vivo transfection experiments. The functional role of MIIC isoforms has been in part clarified by correlated biochemical-physiological studies on single skinned fibers. These studies, in agreement with results from in vitro motility assays, indicate that both MHC and MLC isoforms determine the maximum velocity of shortening of skeletal muscle fibers.

myosin heavy chains; myosin light chains; muscle fiber types; muscle fiber differentiation; velocity of muscle shortening

THE STRUCTURAL and functional diversity of skeletal muscles reflects a variety of myosin isoforms. The myosin molecule is composed of two myosin heavy chains (MHCs), the carboxy-terminal portions of which associate to form an α-helical coiled-coil rod (myosin tail), whereas the amino-terminal portions are separated and form two elongated globular domains (myosin heads). At the head-tail junction each MIIC associates with two myosin light chains (MLCs), one belonging to the alkali or MLC1 family and one to the regulatory or MLC2 family. Both MHCs (mol mass ~220 kDa) and MLCs (mol mass 17–23 kDa) exist in multiple isoforms that are differentially distributed in the various fiber types. Our understanding of the complexity of myosin diversity and of the functional significance of MHC and MLC isoforms has rapidly progressed during the last few years. Major advances in the study of mammalian skeletal muscle myosins have been the recognition of a new “fast-type” MHC isoform that is widely distributed in most skeletal muscles, the characterization of the developmental MHC transitions that lead to the emergence of the adult myosin isoform profile, and the demonstration that both MHCs and MLCs influence the velocity of muscle shortening.

In this brief review we focus on three major aspects: 1) the fiber type distribution of MHC isoforms in mammalian skeletal muscles and their developmental changes, with particular reference to the “fast” or type II MHCs; 2) the myosin gene families and the regulation of their expression; and 3) the functional role of MIIC and MLC variants. Our aim is not to present a comprehensive review of myosin diversity but rather to illustrate recent important advances, discuss open issues, and show the direction of ongoing research.

SARCOMERIC MIIC AND MLC ISOFORMS ARE THE PRODUCT OF MULTIGENE FAMILIES

The catalog of myosin isoforms so far identified is still incomplete: most studies have focused on a few muscles of selected mammalian species, and additional myosin variants are probably present in fibers so far considered to be homogeneous in terms of myosin composition (e.g., the slow fibers; see below). On the basis of available information, it is useful to distinguish between major isoforms, which are widely distributed in most muscles and most animal species, and minor isoforms, which have a restricted distribution in specialized muscles. A list of the major MHC and MLC isoforms identified in rat skeletal muscle is shown in Table 1. Similar forms exist in other...
mammalian species, such as mouse, guinea pig, and rabbit. The structure of each MHC or MLC isotype is generally conserved between species, whereas greater sequence divergence may be found between different isoforms within the same organism.

Sarcomeric MHCs and MLCs are the product of three multigene families, each presumably derived from a single ancestor gene. The MHC gene family comprises several genes, each coding a distinct isoform, that are located in two clusters. The β/slow-MHC gene is closely linked to the cardiac α-MHC gene on chromosome 14 in both human and mouse (42, 58, 80). The other skeletal muscle isoforms, including the embryonic and neonatal (or perinatal) isoforms and the adult fast MHCs, are clustered on human chromosome 17 and mouse chromosome 11 (36, 80). At least six MHC genes, some of which are still unidentified, are present in the human chromosome 17 cluster (69, 85). At variance with the MHC genes, the MLC genes are dispersed in different chromosomes. Each sarcomeric MLC is coded by a distinct gene, with the exception of the MLC1f and MLC3f isoforms, which originate from a single gene by alternative promoters and alternative splicing of the first exons (for review see Ref. 5).

The distribution of the various MHC and MLC isoforms is not always strictly confined to specific muscle fibers or specific stages of development. For example, the embryonic and neonatal MHCs persist in adult extraocular muscles (62, 83) and intrafusal nuclear chain fibers (56), as well as in the adult masseter muscle (10, 18), which also contains the embryonic MLC1 (68). Embryonic and neonatal MHCs and embryonic MLC1 are also reexpressed in regenerating (13, 61) and denervated muscle (65). Furthermore, the MLCs typical of fast muscles can be detected in a number of type I (slow) fibers, and, conversely, MLCs typical of slow muscles are found in some type II (fast) fibers. MHC and MLC isoforms can in fact associate in various combinations, generating a large number of myosin molecules (see Ref. 48).

In addition to the major isoforms indicated in Table 1, other MHC and MLC variants have been identified in certain muscles and in certain species. A specific MHC and specific MLC1 and MLC2 isoforms have been detected in the fast-contracting jaw-closing muscles and in

### TABLE 1. Major MHC and MLC isoforms in rat skeletal muscle

<table>
<thead>
<tr>
<th>Isoforms Expressed Mainly in</th>
<th>Developing muscles</th>
<th>Fast muscles (type II fibers)</th>
<th>Slow muscles (type I fibers)</th>
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<tr>
<td>MHC</td>
<td>Emb-MHC</td>
<td>IIα-MHC</td>
<td>β/Slow-MHC</td>
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<td></td>
<td>Neo-MHC</td>
<td>IIβ-MHC</td>
<td>Ix MHC</td>
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<td>MLC1</td>
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<td>(alkali)</td>
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<td>MLC2</td>
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MHC, myosin heavy chain; MLC, myosin light chain; emb, embryonic; neo, neonatal.

the tensor timpani muscle of some carnivores and primates (44, 57). The cardiac α MHC protein and the corresponding mRNA are expressed in the rabbit masseter muscle (see Ref. 17). A unique MHC form, coded by a specific gene, is present in most type II fibers of extraocular muscles (62, 83). A number of type I fibers in extraocular muscles, as well as the bag fibers of muscle spindles, contain an MHC form antigenically different from the β/slow-MHC present in normal slow-twitch fibers and similar to that present in the “slow-tonic” fibers of amphibians and birds (50). However, this slow-tonic MHC has not yet been characterized, and it is not known whether it derives from a distinct gene. Other β/slow-MHC isoforms are probably present in mammalian skeletal muscle. According to a recent report, three antigenically different slow MHC isoforms are sequentially expressed in developing mammalian muscle (28). Two types of MHC transcripts, differing in the 3′ untranslated region, have been recognized by in situ hybridization in two populations of type I fibers present in human skeletal muscle (V. Smerdu, I. Karsch-Mizrachi, M. Campione, L. Leinwand, and S. Schiaffino, unpublished data). A second form of MLC1s, called MLC1sa while the major isoform is also referred to as MLC1sb, was initially identified in rabbit slow skeletal muscles (78). The corresponding gene has recently been identified and found to be expressed in both muscle and nonmuscle tissues (26).

### TYPE IIα-, IIβ-, AND IIX-MHCS DEFINE DISTINCT FIBER POPULATIONS IN MAMMALIAN SKELETAL MUSCLE

Three major populations of type II fibers, including a novel type IIX subset distinct from the IIα and IIβ fibers, have been identified in rat skeletal muscle by immunohistochemical staining with different anti-MHC monoclonal antibodies (23, 66, 67). The IIX fibers are not easily distinguished from IIα and IIβ fibers by the usual adenosinetriphosphatase (ATPase) histochemical reactions and in previous studies were generally confused with the IIβ fibers. The IIx fibers are numerous in most leg muscles and are especially abundant in the diaphragm (66), are rich in oxidative enzymes (67), and belong to motor units relatively resistant to fatigue (34). Immunoblotting analyses showed that muscles rich in IIx fibers contain a IIX-MHC isoform distinct from IIα- and IIβ-MHCs (66). Three type II MHC isoforms were independently identified by gel electrophoresis (3). Single-fiber studies showed that the band of intermediate mobility between the IIα- and IIβ-MHCs, which is called IIId-MHC, is distributed in a specific type II fiber population (72). Because IIx-MHC was also separated and identified by immunoblotting as a band of intermediate electrophoretic mobiliy (31), it appears that IIx- and IIId-MHCs correspond to the same isoform (Fig. 1).

Definitive evidence that the novel MHC is a distinct protein and not the product of posttranslational modification of other MHCs was recently obtained with the isolation of cDNAs corresponding to the specific IIX-MHC mRNA (19). Type IIα-, IIβ-, and IIX-MHC cDNAs display different restriction endonuclease maps, thus must derive from different genes, and have unique 3′
FIG. 1. Identification of 4 myosin heavy-chain (MHC) isoforms in rat skeletal muscle by electrophoresis and immunoblotting. MHCs present in diaphragm (DIA) and tibialis anterior (TA) muscles of adult rat were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and either silver stained (A) or transferred to nitrocellulose and stained with monoclonal antibody BF-32, which reacts with \( \beta \)/slow- and Ila-MHC (B). This same nitrocellulose sheet was subsequently incubated with another monoclonal antibody, RT-D9, which reacts with IIX- and IIb-MHC (C). [From La Framboise et al. (31).]

untranslated sequences that can be used to generate specific probes. In situ hybridization studies using these probes show that Ila-, IIb-, and IIX-MHC mRNAs are differentially distributed in the corresponding fiber types identified by staining of serial sections with specific anti-MHC antibodies (Fig. 2). In agreement with the results of immunocytochemical (66) and electrophoretic studies (72), in situ hybridization also shows that, in addition to fibers containing a single type of MHC mRNA, there are fibers coexpressing different MHC genes, i.e., fibers containing both \( \beta \)/slow- and Ila-MHC, Ila- and IIX-MHC, or IIX- and IIb-MHC. These hybrid fibers are very numerous in rat muscles; for example, the extensor digitorum longus muscle contains \( \sim 26\% \) pure IIX fibers, \( 10\% \) Ila/IIX hybrid fibers, and \( 20\% \) IIX/IIb hybrid fibers (19).

Fibers showing an immunoreactivity profile characteristic of type IIX-MHC are numerous in mouse and guinea pig muscles (23), and fibers containing the type IId-MHC

FIG. 2. Distribution of type IIb- (B), IIX- (X), and Ila-MHC (A) mRNAs, determined by in situ hybridization, matches that of corresponding protein isoforms, determined by immunoperoxidase staining. Serial cryosections of rat extensor digitorum longus muscle were incubated with \(^{35}\)S-labeled cRNA probes specific for Ila- (a), IIX- (b), and IIb-MHC mRNA (c); reaction was revealed by autoradiography and visualized by dark field microscopy. Serial cryosections were incubated with monoclonal antibodies reactive with IIX-MHC (d), all MHCs but IIX-MHC (e), and IIb-MHC (f). Bound antibodies were revealed by peroxidase-conjugated secondary antibodies. Hybrid fiber coexpressing IIX- and IIb-MHC mRNA is marked by an open circle.
MYOSIN ISOFORM SWITCHING AND FIBER TYPE DIVERSIFICATION DURING DEVELOPMENT

The development of fast skeletal muscles is accompanied by the progressive substitution of the embryonic and neonatal MHC isoforms with the adult type II MHC isoforms (82). As discussed above, the appearance of the definitive β/slow-MHC is also apparently preceded by two antigenically distinct “slow” isoforms; however, these MHCs have not yet been characterized biochemically (28). A simplified scheme of muscle fiber diversification in rat muscles, based on major and well-documented MHC isoform transitions, is shown in Fig. 3. The first fetal stage is characterized by the differentiation of fibers strongly expressing β/slow-MHC, derived from primary generation fibers, and fibers strongly expressing neonatal MHC, derived from both primary and secondary generation fibers (15, 40, 46). The appearance of the three type II MHC mRNAs, which are first detected a few days after birth in rat hindlimb muscles, defines a second neonatal phase in muscle fiber differentiation (19). Interestingly, the various mRNAs display since the earliest appearance a specific fiber distribution; for example, many fibers of soleus muscle contain IIa- but not IIX- or IIb-MHC transcripts, and many fibers of tibialis anterior contain IIb- but not IIa- or IIX-MHC transcripts, whereas other fibers express IIX-MHC mRNA exclusively. The distribution of the various transcripts prefigures the pattern found in adult muscles. During postnatal development the embryonic and neonatal MHC isoforms are progressively eliminated at various time periods according to muscle type and fiber type (65), and further MHC transitions occur with age in different muscles. For example, a switch from IIa-MHC to β/slow-MHC expression is found in the rat soleus muscle during early postnatal development (11), and a progressive disappearance of β/slow-MHC is seen in mouse fast leg muscles (81). In old rat muscles there is a relative decrease of IIb-MHC and a corresponding increase in IIX-MHC (33, 70). Accordingly, type IIX motor units become the predominant motor unit type in the tibialis anterior muscle of old rats, apparently as a result of an age-related transition from IIb to IIX motor units (32).

Muscle fiber diversification is also accompanied by changes in MLC composition during fetal and postnatal development. MLC1f and MLC1emb are the major transcripts in both developing fast and slow fetal muscles, whereas MLC1s transcripts are first detected at relatively later fetal stages and are exclusively expressed in prospective type I fibers, namely, the same fibers showing greater accumulation of β/slow-MHC (40, 46). Postnatal development is characterized by the disappearance of MLC1emb, which is accompanied by a progressive increase in MLC3f in fast muscles and a switch from the mixed MLC1f/MLC1s profile to the pure MLC1s profile in slow muscles (43). Electrophoretic analyses on single fibers indicate that MLC1f and MLC3f generally coexist within the same type II fibers. In rat skeletal muscles, the relative proportion of the two isoforms is variable but MLC3f is significantly more abundant than MLC1f in IIX fibers, with the lowest concentrations being found in IIX fibers (6, 77).

REGULATION OF FIBER TYPE-SPECIFIC MYOSIN GENE EXPRESSION

Multiple mechanisms, including myoblast predetermination, neuronal influences, and thyroid hormone, regulate muscle fiber diversification and myosin gene expression during development (for review see Ref. 25). The expression of myosin genes can be further modulated in the adult stages by a variety of factors, such as
innervation (see Ref. 49), thyroid hormone (see Ref. 30), electrical stimulation (see Refs. 2 and 73), and mechanical factors (see Ref. 37). For example, IIx-MHC can be induced in the rat soleus muscle, in which this isoform is normally absent, by either thyroid hormone treatment, electrical stimulation at high frequency, or mechanical unloading after hindlimb suspension (2, 12, 19). The MHC transitions appear to reflect an obligatory pathway of MHC gene expression in the order I \( \rightarrow \) IIa \( \rightarrow \) IIx \( \rightarrow \) IIb (64). Changes in the pattern of activity or in thyroid hormone levels can push the transformation of the fiber phenotype to the right or to the left along this pathway. This interpretation helps to explain the apparently paradoxical finding (30) that the IIa-MHC gene is upregulated by thyroid hormone in the soleus muscle, which contains a majority of type I fibers, but is downregulated in the extensor digitorum longus muscle, which is composed almost exclusively of type II fibers. It remains to be established whether the specific patterns of MHC transitions reflect specific rules in the transcriptional regulation of MHC genes and whether MHC gene regulation is affected by the chromosomal organization of the MHC gene clusters.

The regulatory DNA sequences and the transcription factors responsible for fiber type-specific myosin gene expression are not known. It has been suggested (29, 76) that the transcription factors MyoD and myogenin, which are known to play a role in muscle cell differentiation during development (for review see Ref. 79), may be implicated in differential muscle gene expression. MyoD mRNAs are more abundant in type II muscle fibers, whereas myogenin mRNAs are more abundant in type I fibers, and the transformation of fiber types induced by thyroid hormone or by cross-reinnervation is accompanied by a corresponding alteration in the MyoD and myogenin mRNA levels. However, at present there is no direct evidence for a specific role of MyoD and myogenin in the differential regulation of myosin genes in skeletal muscle fibers.

In vitro transfection assays, which are generally used to study muscle gene regulation, are not appropriate to identify fiber type-specific sequences because cultured muscle cells do not undergo fiber type differentiation. On the other hand, in transgenic animals the pattern of transgene expression may differ from that of the corresponding endogenous gene. For example, a transgene made of regulatory sequences of the MLC1f/3f gene linked to a bacterial reporter gene is expressed in fast but not in slow muscle fibers like the corresponding endogenous gene. However, at variance with the endogenous gene, the level of expression of the transgene varies according to the type II fiber subtype, in the order IIb > IIx > IIa fibers (20). Similar variations in level of expression between type II fiber subtypes have been observed with a quail fast troponin I transgene (27). It has been suggested that the IIb > IIx > IIa expression hierarchy may represent a fundamental pattern, also possibly involved in the regulation of MHC genes, whereas additional, as yet unidentified, elements would be responsible for enhancing the expression in IIx and IIa fibers, leading to the homogeneous expression of the endogenous gene in the three type II fiber populations (27). An alternative interpretation is that the abnormal pattern of expression of these transgenes results from differences in genomic imprinting, possibly due to methylation processes, between transgenes and their endogenous counterparts; genomic imprinting has been implicated in the generation of the aberrant rostrocaudal gradient of MLC1f/3f transgene expression (21). It will be of interest to determine whether similar variations in gene expression are seen using alternative procedures of in vivo transfection, such as intramuscular injection of plasmid DNA into skeletal muscles of adult animals (84). The efficiency of gene transfer is very low in normal rat muscles, but the level of expression of the transgene can be increased by two orders of magnitude by DNA injection into regenerating muscles (75). We are presently using this experimental model to identify the regulatory sequences responsible for the fiber type-specific expression of muscle genes. In a recent study plasmids containing fragments of the 5'-flanking region of the mouse MLC1s gene linked to bacterial reporter genes showed a much higher level of expression in slow than in fast muscles, like the corresponding endogenous gene, and a specific regulatory element has been identified by deletion analysis in the MLC1s gene promoter (74).
FIG. 5. Influence of MHC and MLC isoform composition on myofibrillar ATPase activity was determined in isolated fibers from NADH oxidation enzymatically coupled to ATP regeneration, as described by Potma et al. (51). A: differences in ATP hydrolysis rate during isometric contractions between 4 sets of fibers, each containing single MHC isoform. Differences between IIb and IIx and between IIb and IIA fibers were statistically significant, whereas difference between IIA and IIx fibers was not significant. B: \( V_o \) and ATPase activity plotted vs. relative content of MLC3f in set of 18 fibers containing IIb-MHC. Regression analysis (regression lines are shown) showed that \( V_o \) (solid line) was significantly related to MLC3f content, whereas ATPase (dashed line) was not.

**BOTH MHC AND MLC ISOFORMS DETERMINE MUSCLE CONTRACTILE PROPERTIES**

Since the early studies of Close (14) and Barany (4), the maximum velocity of shortening of fast and slow skeletal muscles has been correlated with the presence of myosin isozymes having high and low ATPase activity, respectively. The higher efficiency in chemomechanical conversion of the slow myosin isozymes has been considered responsible for the greater economy in tension maintenance of slow muscles compared with fast muscles (see Ref. 16). Subsequent studies have shown that shortening velocity may also differ between muscles containing different fast-type myosins. For example, the rat extensor digitorum longus muscle, containing predominantly IIb-MHC, has a higher maximum shortening velocity than the soleus muscle chronically stimulated at high frequency, which contains predominantly IIx-MHC (63). However, interpretation of studies at the whole muscle level is complicated by the heterogeneous composition of most skeletal muscles. Single-fiber studies have shown that the presence of slow MHCs and MLCs is correlated with a lower shortening velocity, a lower power output, and a higher curvature of the force-velocity relationship than fast myosin isoforms (9, 24, 54, 71). The relative role of the MHC and MLC isoforms in determining these differences has not been clearly established. MHC isoforms have been considered as major determinants of the different speed of fast and slow muscle fibers (54); however, MLCs may be important as well, as suggested by the finding that human type IIA fibers that contain exclusively MLC2f have a higher shortening velocity than hybrid IIA fibers containing both MLC2f and MLC2s (35).

The functional role of fast-type MHC and MLC isoforms has been investigated in a number of correlated biochemical-physiological studies on mammalian single fibers. Fibers containing IIA-MHC were found to display a lower maximum velocity of shortening than fibers containing IIB-MHC (9, 22, 35, 55, 71). Fibers containing IIx-MHC are more similar to IIA fibers with respect to shortening velocity, whereas they are similar to IIb fibers with respect to power output and curvature of the force-velocity curve (9). On the other hand, a role of the alkali MLCs was suggested by the finding that the maximum velocity of shortening is higher in fibers that contain higher amounts of MLC3f (22, 24, 71). Interpretation of these results is complicated by the preferential association of MLC1f with IIA-MHC and MLC3f with IIb-MHC (6, 41, 60, 77). Therefore it is not clear whether IIb fibers are faster because they contain IIb-MHC or because they contain more MLC3f. The relative role of MHC and MLC isoforms can only be established by comparing the contractile properties of fibers containing the same MHC isoform but differing in MLC3f relative content or in fibers with similar MLC3f content but different MHC isoform composition. This has been done in two recent studies on single human (35) and rat muscle fibers (6). By using rat muscle fibers containing a single type of MHC, and thus avoiding the further complication of the
coexistence of multiple MHCs (7), Bottinelli et al. (6) found that the maximum shortening velocity is closely related to the relative content of MLC3f. This finding is in agreement with previous results in skinned fibers in which MLC1f was partially substituted with MLC3f (45). However, as shown in Fig. 4, the impact of MLC3f is more pronounced in IIb fibers, where a 10% variation in MLC3f content gives a 16% change in maximum shortening velocity, than in IIa and IIX fibers, where the same 10% variation induces a 4 and 8% change, respectively, of the mechanical parameters. Thus it appears that in rat muscle fibers both MHCs and alkali MLCs determine the maximum shortening velocity: at a given value of MLC3f relative content, velocity depends on MHC composition, and vice versa. On the other hand, Larsson and Moss (35) were not able to find any correlation between maximum shortening velocity and MLC3f relative content in human muscle fibers. However, as suggested by the same authors, interpretation of these results is complicated by the frequent coexistence of fast and slow MLC2 isoforms in a large proportion of type II human fibers and the aforementioned possible influence of MLC2 isoforms in determining the maximum shortening velocity.

The alkali MLC isoform composition does not seem to play a correspondingly important role in the ATP consumption and economy of tension development of isometrically contracting fast fibers. As shown in Fig. 5A, during isometric contraction the ATPase activity is significantly higher in rat type II than in type I fibers and, among type II fibers, in fibers containing IIb-MHC than in fibers containing IIa- or IIx-MHC (8). However, no significant relationship was observed between ATPase activity and the relative proportion of MLC3f (Fig. 5B). This conclusion is in agreement with the results of studies on avian fast myosins: the actin-activated ATPase activity of myosin from chicken pectoralis muscle is only little decreased by removal of alkali MLCs (38), and no significant difference in ATPase activity is found between two myosin fractions containing either MLC1f or MLC3f (47). Lowey et al. (38, 39) have also explored the functional role of MLCs using an in vitro motility assay, i.e., a reconstituted actomyosin system made of fluorescent actin filaments sliding over chicken pectoralis myosin adhered to nitrocellulose-coated coverslips. The velocity of actin filaments, which can be considered an analogue of unloaded velocity of shortening of muscle, is markedly decreased by removal of MLCs (38) and is significantly higher in myosin containing MLC3f than in myosin containing MLC1f (39). Using the same approach it was found that myosins from embryonic and early posthatch chicken pectoralis, which contain similar MLC complement but different MHCs, translocate actin at different velocities, suggesting that the heavy chain is the principal determinant of the lower velocity of embryonic compared with neonatal and of neonatal compared with adult muscle myosin (39).

In conclusion, it appears that both MHC and MLC isoforms are responsible for determining the maximum velocity of shortening, whereas only MHCs determine the ATP consumption and the isometric tension cost. This conclusion is consistent with current molecular models of muscle contraction based on a variety of biochemical and biophysical approaches and confirmed by the recent crystallographic reconstruction of the myosin S1 subfragment (52, 53). The structural model clearly shows that the ATPase site and the actin binding region are located on opposite sides of the distal globular part of the myosin head, which represents the motor domain. This domain undergoes small conformational changes in relation to the hydrolytic cycle; these changes are amplified and transmitted to the thick filament through a 85-A-long α-helix, around which are wrapped the regulatory and alkali MLCs (light-chain binding domain). Within the framework of the new structural information, one can now ask specific questions concerning the properties of the various myosin isoforms. 1) Which sequences of the MHC are critical for the molecular events that determine the speed of movement or the ATPase hydrolysis rate? 2) Is the lower shortening velocity associated with MLC1f due to changes in the physical properties of the 85-A MHC α-helix (possibly caused by the long amino-terminal extension of MLC1f) or to a direct interaction between MLC1f and actin that could place a mechanical load on the cycling cross bridges? Several converging lines of research, including molecular biology studies providing the complete sequence of various MHCs, crystallographic studies, and in vitro motility assays with recombinant normal and mutated MHCs and MLCs, should contribute to answering these and similar questions and to further progress in defining the functional role of the different myosin isoforms present in mammalian skeletal muscle.

Our research was supported by grants from Ministero dell’Università e della Ricerca Scientifica e Tecnologica of Italy (to S. Schiaffino and C. Reggiani), Agenzia Spaziale Italiana (to S. Schiaffino), and Telethon-Italia Grant 343 (to C. Reggiani).

Address for reprint requests: S. Schiaffino, Dept. of Biomedical Sciences, Via Trieste 75, 35121 Padova, Italy.

REFERENCES


