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Myosin Isoforms, Muscle Fiber Types, and Transitions

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ABSTRACT Skeletal muscle is an extremely heterogeneous tissue composed of a variety of fast and slow fiber types and subtypes. Moreover, muscle fibers are versatile entities capable of adjusting their phenotypic properties in response to altered functional demands. Major differences between muscle fiber types relate to their myosin complement, i.e., isoforms of myosin light and heavy chains. Myosin heavy chain (MHC) isoforms appear to represent the most appropriate markers for fiber type delineation. On this basis, pure fiber types are characterized by the expression of a single MHC isoform, whereas hybrid fiber type express two or more MHC isoforms. Hybrid fibers bridge the gap between the pure fiber types. The fiber population of skeletal muscles, thus, encompasses a continuum of pure and hybrid fiber types. Under certain conditions, changes can be induced in MHC isoform expression heading in the direction of either fast-to-slow or slow-to-fast. Increased neuromuscular activity, mechanical loading, and hypothyroidism are conditions that induce fast-to-slow transitions, whereas reduced neuromuscular activity, mechanical unloading, and hyperthyroidism cause transitions in the slow-to-fast direction. *Microsc. Res. Tech. 50:500–509, 2000.*

INTRODUCTION

One of the unique features of skeletal muscle is its fiber architecture. The highly organized arrangement of muscle fibers contributes to a variety of functional capabilities. In addition, the existence of numerous types of fibers adds to the heterogeneous nature of skeletal muscle as a tissue. Muscle fiber types can be delineated according to differences in their structural and functional properties. Of the multitude of classification schemes that have appeared in the literature, only a few have turned out to be widely used (Pette and Staron, 1990).

To date, the most informative methods to delineate muscle fiber types are based on specific myosin profiles, especially the myosin heavy chain (MHC) isoform complement. Methods such as myofibrillar adenosine triphosphatase (mATPase) histochemistry, immunohistochemistry using antibodies specific to MHC isoforms, and electrophoretic analysis of MHC isoforms in microdissected single fibers have increased our understanding of the diversity of fiber types and their dynamic nature. Collectively, these various methodological approaches have revealed the existence of muscle fibers that either contain a single MHC isoform ("pure fiber types") or two or more MHC isoforms ("hybrid fiber types").

According to the major MHC isoforms found in adult mammalian skeletal muscles, the following pure fiber types exist: slow type I with MHCIβ, and three fast types, namely type IIA with MHCIIa, type IID with MHCIId (MHCIId and fiber type IID are considered to be equivalent to MHCIIx and fiber type IIX, respectively; Pette and Staron, 1990; Schiaffino and Reggiani, 1994, 1996), and type IIB with MHCIIb. The coexpression of specific pairs of these major MHC isoforms results in the formation of hybrid fibers, which can be subdivided based on the predominant MHC isoform. Accordingly, the following hybrid fiber types can be distinguished: type I/IIA, also termed IC (MHCIβ>MHCIIa); type IIA/I, also termed IIC (MHCIIa>MHCIβ); type IIAD (MHCIIa>MHCIId); type IIDA (MHCIId>MHCIIa); type IIDB (MHCIId >MHCIIb), and type IIBD (MHCIIb>MHCIId).

Fiber type-specific programs of gene expression are not restricted to the MHC isoforms, but exist for many other muscle proteins (Pette and Staron, 1997; Schiaffino and Reggiani, 1996). For example, fiber type-specific isoforms exist for the essential and regulatory myosin light chains (MLC), the three troponin subunits, tropomyosin, α-actinin, and various Ca^{2+}-regulatory proteins (e.g., sarcoplasmic reticulum Ca^{2+}-ATPase, calsequestrin, and the α-subunit of the dihydropyridine receptor) (for reviews see Pette and Staron, 1990, 1997). In addition to these qualitative differences, the expression levels of some proteins vary in a fiber type-specific manner. These quantitative differences may either be graded (e.g., the fast Ca^{2+}-ATPase isoform SERCA1, which is expressed at higher levels in type IIB fibers compared to type IIA fibers), or all or none (e.g., phospholamban in type I but not in type II fibers or parvalbumin in type II but not in type I fibers).

Quantitative differences can also be used to delineate fiber types, but result in less defined categories compared to qualitative differences. Different metabolic enzyme activity levels, especially those of aerobic-oxidative and anaerobic pathways of energy metabolism, have been used to delineate fast fiber subtypes both histochemically (Peter et al., 1972) and biochem-

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TABLE 1. Myosin heavy chain isoforms identified in adult extrafusal fibers of mammalian skeletal muscles[1]

Designation	Nomenclature	Muscle/fiber location
Fast-twitch	MHCIIb	Fiber types IIB, IIBD, IIAB
Fast-twitch	MHCIId	Fiber types IID, IIBD, IIDA
Fast-twitch	MHCIIa	Fiber types IIA, IIAB, IIDA, IIC, IC
Fast-twitch	MHC_{eom}	Extraocular and laryngeal muscles
Fast-twitch	$MHCII_{m}$	Masticatory muscles
Slow-twitch	MHCIβ	Fiber types I, IC, IIC
Slow-twitch	MHCIα	Extraocular, diaphragm, masseter muscles, fast-to-slow transforming fibers
Slow-twitch	MHCIa	Plantaris, soleus, slow-to-fast transforming fibers
Slow-tonic	$MHCI_{ton}$	Extraocular, laryngeal, and tensor tympani muscles
Embryonic	MHC_{emb}	Extraocular muscles
Neonatal	MHC_{neo}	Extraocular, masseter muscles

[1] Modified from Table 1 in Pette and Staron (1997).

ically (Lowry et al., 1978). However, according to quantitative measurements of metabolic enzyme activities, they are expressed in a continuum and do not appear to be strictly co-ordinated with the myofibrillar protein isoforms, especially MHCs. Indeed, large overlaps in enzyme activity levels can be found between fast fiber subtypes and between fast and slow fibers defined by their MHC isoform profiles (Pette and Staron, 1990). As may be expected, energy supply for the chemomechanical transformation underlying the contractile process varies considerably depending on specific functional requirements, e.g., short-term high-intensity vs. sustained low-intensity. Therefore, adjustments in energy metabolism need not be synchronized in a rigid manner to adjustments in the contractile machinery.

IMPORTANCE OF MYOSIN-BASED FIBER TYPE CLASSIFICATION

Myosin, the most essential part of the contractile machinery in muscle, exists in multiple isoforms which contribute to the functional diversity of muscle fibers. Methods that are able to assess these differences in the myosin complement can yield important information concerning muscle fiber heterogeneity and functional specialization. Major functional differences of myosin isoforms reside in the heavy chain portion of the myosin molecule (Weiss et al., 1999a). A good example is ATPase activity, which is associated with the globular head region of the myosin heavy chain. Single fiber analysis has revealed that mATPase-based fiber types correlate with specific MHC profiles. As such, mATPase histochemistry has proven to be useful for the delineation of muscle fiber types. However, this histochemical method results in a qualitative assessment of the myosin complement and, therefore, the ability to delineate the multitude of potential hybrid fibers is limited. Immunohistochemistry, which is also qualitative, appears to offer an advantage over mATPase histochemistry because this method allows the detection, at least in most cases, of hybrid fibers. However, the most informative method for the assessment of MHC isoform profiles is single fiber electrophoresis. This method yields important quantitative information, especially pertinent when two or more MHC isoforms are expressed in varying ratios, and also allows the detection of "special" MHC isoforms, e.g., MHCIa (Fauteck and Kandarian, 1995; Galler et al., 1997a).

MULTIPLICITY OF MYOSIN ISOFORMS

Myosin is a hexameric protein composed of heavy and light chains. The heavy chains are encoded by a multigene family (Leinwand et al., 1983; Weiss et al., 1999a). To date, a total of 11 MHC isoforms have been identified in adult extrafusal fibers (Table 1).Some of these isoforms appear to be expressed in a muscle-specific manner ($MHCII_{m}$, MHC_{eom}, $MHCI_{ton}$, MHCIα, MHCIa, MHC_{emb}, MHC_{neo}), while others are more widely distributed in a variety of skeletal muscles (MHCIβ, MHCIIa, MHCIId, MHCIIb). In addition, species differences exist in the expression of some of these isoforms. For example, body size appears to correlate with the relative concentrations of MHCIβ, MHCIIa, MHCIId, MHCIIb. Studies on specific skeletal muscles have revealed that as body mass increases, expression levels of the slower isoforms increase at the expense of faster isoforms (Aigner et al., 1993; Hämäläinen and Pette, 1995). Indeed, muscles in human and other large mammals do not appear to express MHCIIb under normal conditions. In this context, fibers previously classified as type IIB in human muscle have more recently been renamed type IID based on their MHC complement that resembles MHCIId of the rat (Ennion et al., 1995; Smerdu et al., 1994). Interestingly, a separate gene encoding for MHCIIb has been identified in the human (Weiss et al., 1999a).

CONTRACTILE PROPERTIES OF MHC-BASED FIBER TYPES

Correlations between contractile properties and myosin isoforms were initially demonstrated by the work of Bárány (1967) and by physiological measurements of histochemically defined motor units (Close, 1967). The establishment of single fiber biomechanics has extended these correlations and made it possible to assign specific contractile properties to specific MHC-based fiber types (Lännergren, 1987; Reiser et al., 1985; Sweeney et al., 1986). Skinned muscle fibers, identified by either immunohistochemistry (Bottinelli et al., 1991) or by single fiber electrophoresis (Galler et al., 1994; Larsson and Moss, 1993), have shown a correlation between MHC-based fiber type and maximum unloaded shortening velocity. Velocity is lowest in type I fibers, and although considerable overlap of V_{max} exists within the fast fiber population, the type IIB fibers are the fastest with types IID and IIA displaying

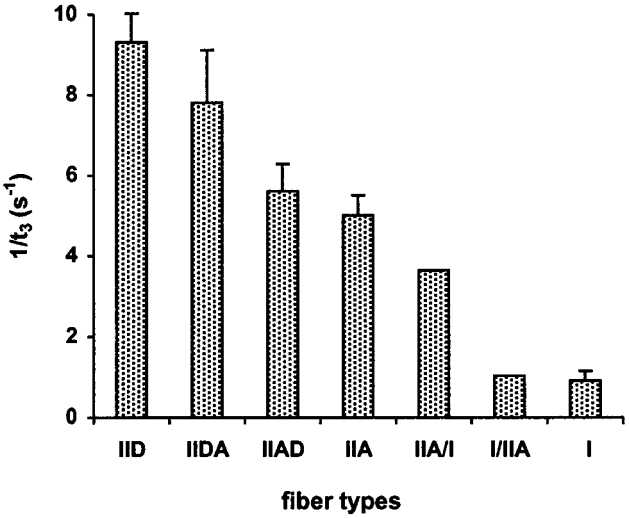

Fig. 1. Comparison of contractile properties of human muscle fiber types as determined by measurements of stretch activation. Single fibers were classified as pure (I, IIA, IID) or hybrid (I/IIA = MHCIβ>MHCIIa, IIA/I = MHCIIa>MHCIβ, IIAD = MHCIIa>MHCIId, IIDA = MHCIId>MHCIIa) according to their electrophoretically determined myosin heavy chain complement. Stretch activation properties are expressed as reciprocal of t_3-values, i.e., the time elapsed between the beginning of the stretch and the peak value of the delayed force increase. (Modified from Hilber et al., 1999.)

V_{max} values similar to each other but lower than type IIB. This overlap in the shortening velocities between the fast fiber populations appears to be due to the existence of hybrid fibers and to various MLC combinations (Bottinelli et al., 1994a; Bottinelli and Reggiani, 1995; Galler et al., 1994; Larsson and Moss, 1993).

One disadvantage of the measurement of V_{max} is the inability to detect the specific contribution of MHC hybrids vs. specific MLC complements to contractile properties. On the other hand, stretch activation measurements on single fibers from rat, rabbit, and human muscles have successfully demonstrated the impact of MHC complement on specific contractile properties. The increase in stretch-induced delayed force appears to be caused by a simultaneous power stroke of stretch-synchronized myosin heads. According to these measurements, a strong correlation exists between the MHC complement of a fiber and its stretch-activation kinetics. Fiber types IIB, IID, and IIA can thus be separated without overlap by differences in their stretch-activation kinetics. In addition, values obtained from hybrid fibers fall between the pure fibers and are spread out according the specific ratios of the coexisting MHC isoforms (Fig. 1). With regard to stretch activation, pure and hybrid fibers form a continuum spanning from one extreme to the other (Galler et al., 1994, 1996, 1997b; Hilber et al., 1999; Hilber and Galler, 1997).

Interestingly, the gradient in contractile velocity of the various fiber types corresponds to a similar gradient in tension cost, i.e., the ratio between ATPase activity and isometric tension. Type IIB fibers display the highest tension cost, types IID and IIA are intermediate, and type I fibers are the lowest (Bottinelli et al., 1994b). Likewise, measurements of the energy potential as reflected by the $[ATP]/[ADP_{free}]$ ratio, in single muscle fibers demonstrate a similar gradient spanning from type IIB to type I (Conjard et al., 1998).

The impact of the MLC complement on specific contractile properties, first illustrated by Greaser et al. (1988), has been firmly established by motility assays of MLC-free and MLC-reconstituted myosin molecules (Lowey et al., 1993). According to these studies, removal of the light chains has little or no effect on the ATPase activity, but the alkali (essential) light chains markedly modulate V_{max}. Moreover, an increased MLC3f to MLC1f ratio enhances V_{max}. The same effects have been shown in studies on single rat muscle fibers defined by their MHC and MLC complements (Bottinelli et al., 1994a). Taken together, the different MHC and MLC combinations form a large number of isomyosins that contribute to the creation of a smooth continuum of functional properties.

FIBER TYPE TRANSITIONS

Muscle fibers are dynamic structures capable of altering their phenotype under various conditions, e.g., increased or decreased neuromuscular activity, mechanical loading or unloading, altered hormonal profiles (especially of the thyroid hormones), and aging. The changes in MHC isoforms tend to follow a general scheme of sequential and reversible transitions from fast-to-slow and slow-to-fast:

$$MHCI\beta \leftrightarrow MHCIIa \leftrightarrow MHCIId \leftrightarrow MHCIIb.$$

These sequential MHC isoform transitions appear to be related to gradual differences in the energy cost of force production (Bottinelli et al., 1994b) and differences in ATP phosphorylation potentials of the various fast and slow fiber types (Conjard et al., 1998).

Neuromuscular Activity

Neuromuscular activity is important for the establishment of specific muscle fiber phenotypes during development (e.g., Pette and Vrbová, 1985; Rubinstein and Kelly, 1978), and for the subsequent maintenance of their phenotypic properties. The impact of neural activity on muscle phenotype has been demonstrated in numerous denervation experiments (e.g., Carraro et al., 1985; d'Albis et al., 1994; Gutmann et al., 1972; Jakubiec-Puka et al., 1990; Schiaffino et al., 1988). In the absence of innervation, slow muscles become faster and fast muscles become slower. This results from decreases in the relative concentrations of MHCI and MHCIIb in combination with concomitant increases in MHCIIa and MHCIId (Huey and Bodine, 1998; Jakubiec-Puka et al., 1999). Similarly, the cross-reinnervation model has highlighted the importance of neural activity by demonstrating the ability to change muscle fiber phenotypes (Buller et al., 1960). Fast muscles turn slow when reinnervated by a slow nerve, and slow muscles turn fast when reinnervated by a fast nerve (reviewed by Pette and Vrbová, 1985). These cross-reinnervation-induced changes primarily relate to specific neural impulse patterns delivered to the muscle.

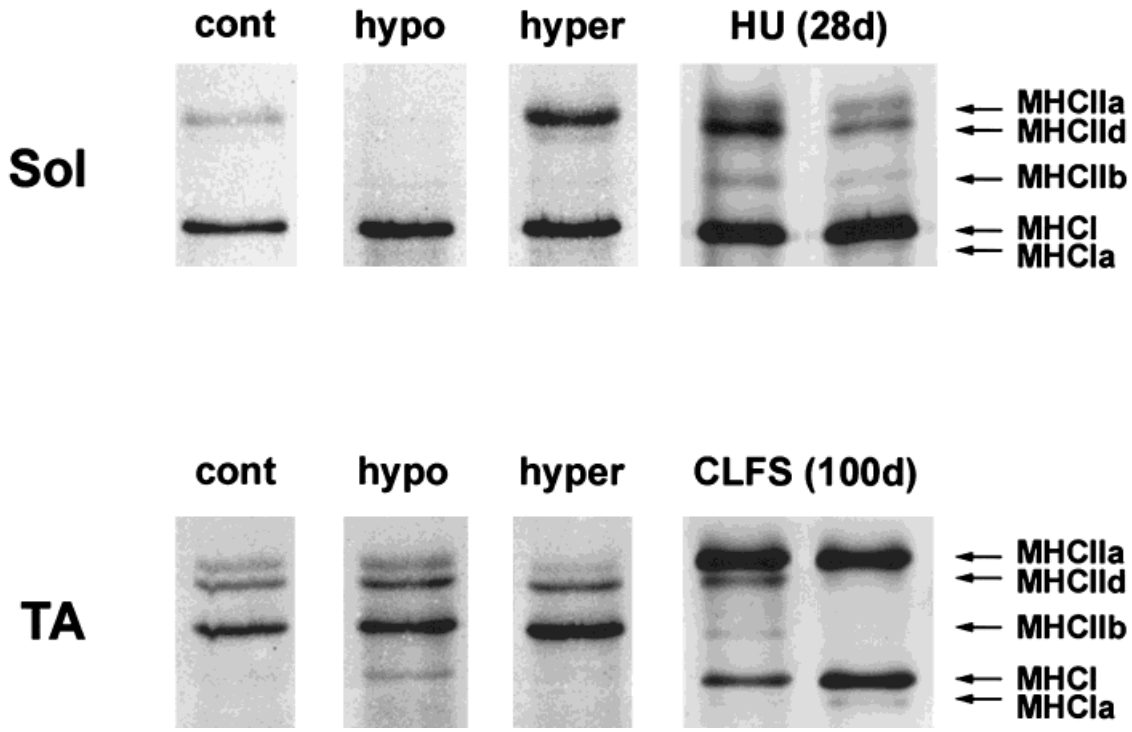

Fig. 2. Electrophoretic separations of myosin heavy chain isoforms from rat soleus (SOL) and tibialis anterior (TA) muscles under various conditions. cont, control; hypo, hypothyroid; hyper, hyperthyroid; HU (28d), unweighting by hindlimb suspension for 28 days; CLFS (100d), chronic low-frequency stimulation for 100 days. Two muscles are shown for both the unweighted and the stimulated animals. Note the variable responses of two animals under the same conditions. Also note the slow-to-fast transitions under conditions of hyperthyroidism and unloading, and the fast-to-slow transitions under conditions of hypothyroidism and CLFS. Data for HU (Stevens et al., 1999a), data for cont, hypo, hyper, and CLFS (Gohlsch et al., unpublished data).

Thus, the application of slow or fast motoneuron-specific impulse patterns by artificially stimulating muscles will also elicit a set of orchestrated changes in muscle phenotype (for reviews, see Pette and Staron, 1997; Pette and Vrbová, 1992, 1999).

The most frequently used model to modify neural activity delivered to a muscle is via electrical stimulation. According to the nature of slow and fast motoneuron-specific firing patterns, two protocols of electrical stimulation have evolved. Chronic low-frequency stimulation (CLFS) mimics the tonic low-frequency impulse pattern normally delivered to a slow-twitch muscle (Salmons and Vrbová, 1969), and phasic**,** high-frequency stimulation mimics the pattern normally delivered to a fast-twitch muscle (Lömo et al., 1974).

In numerous studies performed with CLFS, the stimulation protocol consists of a 10-Hz impulse pattern delivered to specific fast-twitch hindlimb muscles [extensor digitorum longus (EDL), tibialis anterior (TA)] via electrodes implanted lateral to the common peroneal nerve (Salmons and Vrbová, 1969; Simoneau and Pette, 1988). Compared to other models that also increase neuromuscular activity such as exercise, CLFS offers distinct advantages, e.g., (1) a reproducible and standardized regimen of contractile activity, (2) activation of all motor units in a specific target muscle, (3) use of the contralateral muscle as a control, (4) specific dose-response relationships can be established by varying the amount of contractile activity, and (5) assessment of the limits of adaptation to persistently increased contractile activity.

CLFS induces major changes in myosin that encompass a replacement of fast by slow isoforms. Inter- and intraspecies differences exist with regard to the extent of CLFS-induced fast-to-slow transitions. Some species (e.g., rabbit) are able to undergo a greater amount of fast-to-slow transition compared to other species (e.g., rat and mouse). Also, differences can be seen with regard to the extent of fast-to-slow transition between animals of the same species (Fig. 2). In the fast EDL and TA muscles of the rabbit where MHCIId is the predominant fast isoform, the fast-to-slow changes in MHC isoform expression occur in the following order: MHCIId $\rightarrow$ MHCIIa $\rightarrow$ MHCIβ. During the final step from MHCIIa to MHCIβ, there is a transient upregulation of MHCIα (Peuker et al., 1999). In rat EDL and TA muscles where MHCIIb predominates (Fig. 2), the transformation sequence begins with MHCIIb (Jarvis et al., 1996; Jaschinski et al., 1998; Termin et al., 1989; Windisch et al., 1998). To date, the involvement of MHCIα in the final transition from MHCIIa to MHCIβ has not been confirmed in the rat (Putman et al., 1999).

The transitions in MHC isoforms along the fast-to-slow transformation sequence coincide with pronounced increases in hybrid fibers. As the transformation proceeds, hybrid fibers with faster MHC isoforms

are eventually replaced with slower isoforms (Conjard et al., 1998). Similar to the alterations in MHC isoform expression, the MLC pattern also undergoes fast-to-slow transitions. The light chain transitions, however, do not occur in synchrony with the changes in MHC isoforms. This results in combinations of both fast and slow light chains with fast heavy chains, especially MHCIIa. Thus, the induced fast-to-slow transition creates conditions that greatly increase the number of potential isomyosins (Leeuw and Pette, 1996).

CLSF can also be applied to a slow muscle, but will have no effect (Pette et al., 1975, 1999) unless the muscle is denervated. Under denervation conditions, the slow phenotype can be maintained by CLFS. As such, the tonic low-frequency impulse pattern counteracts the denervation-induced atrophy and slow-to-fast changes in MHC isoform expression (Gorza et al., 1988). Conversely, phasic high-frequency stimulation of denervated rat soleus muscle elicits slow-to-fast transitions in myosins (Gorza et al., 1988; Gundersen et al., 1988), including the induction of MHCIId and even MHCIIb (Ausoni et al., 1990; Hämäläinen and Pette, 1996). Nevertheless, the slow-to-fast transitions induced by phasic high-frequency stimulation of denervated soleus muscle are incomplete. Even after relatively long stimulation periods, a complete transformation of all fibers does not occur and significant amounts of MHCI are still expressed (Ausoni et al., 1990; Gorza et al., 1988; Gundersen et al., 1988; Hämäläinen and Pette, 1996).

Mechanical Loading and Unloading

Similar to CLFS, stretch and mechanical loading cause fast-to-slow transitions. However, unlike CLFS these models do not appear to increase neuromuscular activity (Pattullo et al., 1992). Stretch-overload produced by immobilization of fast muscles in a lengthened position has been shown to cause an increase in the fraction of slow fibers (Pattullo et al., 1992) and fast-to-slow transitions in MHC isoform expression (Goldspink, 1999; Goldspink et al., 1991, 1992; Loughna et al., 1990; Loughna and Morgan, 1999).

The compensatory hypertrophy model has also been used to study the effects of functional overload on skeletal muscle (Goldberg, 1967). Changes elicited by this type of overload are similar to those induced by stretch-overload, but are of greater magnitude. Characteristic changes include pronounced increases in slow fibers (Ianuzzo et al., 1976, 1989; Noble et al., 1983; Roy et al., 1985), as well as significant increases in MHCI at both the protein and mRNA levels (Gregory et al., 1986, 1990; Kandarian et al., 1992; Morgan and Loughna, 1989; Periasamy et al., 1989; Tsika et al., 1987).

The effects of mechanical unloading have been studied using various models including tenotomy, immobilization in a shortened position, hindlimb suspension, and microgravity (for review, see Pette and Staron, 1997). Under these conditions, unloaded slow muscles become faster (Vrbová, 1963). These changes result from decreases in type I fibers (Booth and Kelso, 1973; Caiozzo et al., 1996; Desplanches et al., 1990; Ohira et al., 1992; Riley et al., 1990; Templeton et al., 1988; Thomason and Booth, 1990), which correspond to decreases in the relative concentration of MHCI and increases in MHCIIa and MHCIId (Caiozzo et al., 1994; Campione et al., 1993; Diffee et al., 1993; Oishi, 1993; Oishi et al., 1994; Thomason et al., 1987). More recently, these slow-to-fast transitions have been shown to extend to MHCIIb (Fig. 2) (Fauteck and Kandarian, 1995; Saitoh et al., 1999; Staron et al., 1998; Stevens et al., 1999a; 1999b). Unloading a fast muscle induces less obvious changes that can be characterized as fast-to-faster transitions (Jänkälä et al., 1997). As such, unloaded fast muscles appear to be less affected compared to unloaded slow muscles.

Hormones

Some hormones have a profound influence on the muscle fiber type composition of specific muscles. For example, testosterone has been shown to have a significant effect on the fiber type composition of guinea pig temporalis muscle (Gutmann and Hanzlíková, 1970; Lyons et al., 1986), laryngeal muscle fibers of the frog (Catz et al., 1995), and perhaps rabbit masseter muscle (English et al., 1999). An extreme example of sexual dimorphism is the levator ani muscle of the rat. This muscle disappears during development of the female, but can be maintained by testosterone administration (Hanzlíková et al., 1970; Joubert et al., 1994). Hormonal differences, especially testosterone, may also contribute to the gender differences in specific fiber type sizes that ultimately affect the relative concentrations of MHC isoforms (Staron et al., 2000).

Of all hormones, thyroid hormones appear to have the greatest effect on muscle fiber phenotypes. In general, hypothyroidism causes fast-to-slow transitions (Fitts et al., 1980; Ianuzzo et al., 1977; Nwoye and Mommaerts, 1981), while hyperthyroidism elicits transitions in the reverse direction (for review, see Pette and Staron, 1997). With regard to myosin expression (Fig. 2), low levels of thyroid hormones cause fast-to-slow shifts in MHC isoform expression: MHCIIb $\rightarrow$ MHCIId $\rightarrow$ MHCIIa $\rightarrow$ MHCIβ, whereas high levels of thyroid hormones cause slow-to-fast shifts in MHC isoform expression: MHCIβ $\rightarrow$ MHCIIa $\rightarrow$ MHCIId $\rightarrow$ MHCIIb (Caiozzo et al., 1992; Canepari et al., 1998; d'Albis and Butler-Browne, 1993; Fitzsimons et al., 1990; Izumo et al., 1986; Larsson et al., 1994; Li et al., 1996). Depending on the initial fiber type composition, each muscle begins the transformation process at a specific starting point along the sequence. In addition to their impact on adult muscle fiber phenotypes, thyroid hormones play an important role during muscle development and maturation. Low thyroid hormone levels inhibit or delay the appearance of the adult fast MHC isoforms, whereas high levels accelerate the transition from the developmental MHC isoforms to the adult fast isoforms (Adams et al., 1999; Butler-Browne et al., 1987; Butler-Browne and Whalen, 1984; d'Albis et al., 1987, 1990; Gambke et al., 1983; Mahdavi et al., 1987; Russell et al., 1988; Sugie and Verity, 1985).

Aging

In addition to muscle atrophy, it has been suggested that aging causes fast-to-slow transitions (for review, see Larsson and Ansved, 1995). Cross-sectional and longitudinal studies on slow and fast rat muscles have

demonstrated age-related changes in fiber type and MHC isoform composition. In fast muscles of the rat, there is an apparent age-dependent decrease in the relative concentration of MHCIIb with a concomitant increase in MHCIId**,** and potentially MHCIIa and MHCI (Danieli-Betto et al., 1995; Larsson et al., 1991; 1993; Škorjanc et al., 1998; Sugiura et al., 1992; Sullivan et al., 1995). In addition, a decrease in the relative concentration of MHCIIa concomitant with an increase in MHCI has been reported in rat soleus muscle (Larsson et al., 1995; Sugiura et al., 1992; Sullivan et al., 1995). This slowing of the aged soleus muscle may, however, also be due to age-related changes in the properties of the slow myosin (Hook et al., 1999).

Because extrinsic and intrinsic factors may play a role in the process of aging, it is difficult to decide whether changes in fiber type composition and MHC isoform profiles represent primary or secondary events. Thus, degenerative processes in the central (selective loss of fast α-motor neurons) and/or peripheral nervous system, as well as inactivity and altered thyroid hormone levels, may all contribute to the observed atrophy and/or loss of fast motor units or fast fibers. For example, increases in fiber type grouping of aged muscle suggest denervation/reinnervation processes (Larsson et al., 1991). Also, there appears to be a reduced number of motor units with increased size in aging muscles (Booth et al., 1994).

SEQUENCE OF MYOSIN ISOFORM TRANSITIONS

Fast-to-slow and slow-to-fast transitions in MHC isoforms appear to follow the orderly sequence MHCIIb ↔ MHCIId ↔ MHCIIa ↔ MHCIβ. This sequence matches the hierarchy of specific ATPase activities and tension costs (Bottinelli et al., 1994b). Thus, adaptive responses in MHC isoform expression in either direction occur in a graded manner. It is tempting to speculate that this orderly sequence in MHC transitions relates to their gene organization. For mouse and human, gene organization of the fast MHC isoforms is in the order of MHCIIa, MHCIId, MHCIIb (Weiss et al., 1999b). Likewise, single fiber studies have shown that the ATP phosphorylation potential, as reflected by the [ATP]/[ADP_{free}] ratio, increases from MHCI to MHCIIb (Conjard et al. 1998). This suggests that sequential activation or inactivation of the MHC genes is somehow related to changes in the ATP phosphorylation potential (Conjard et al., 1998; Green et al., 1992).

The orderly sequence of MHC isoform transitions has been deduced from the time-dependent exchanges of faster with slower MHC isoforms on muscles undergoing fast-to-slow conversion (Jaschinski et al., 1998; Leeuw and Pette, 1993; Staron et al., 1987; Termin et al., 1989). Similarly, single fiber studies on transforming muscles have confirmed time-dependent increases in hybrid fibers, indicating sequential shifts from fast-to-slow (Conjard et al., 1998) and also from slow-to-fast (Stevens et al., 1999b). However, atypical MHC isoform combinations, e.g., MHCI/MHCIIb, MHCI/MHCIIa/MHCIIb, MHCI/MHCIId/MHCIIb, have been reported for the combined conditions of mechanical unloading and hyperthyroidism (Caiozzo et al., 1998). This appears to conflict with the proposed sequential order of MHC transitions. Because mRNA/protein mismatches may result from inadequate resolution of the applied methods, especially at the protein level, this possibility must be excluded (Pette et al., 1999; Peuker and Pette, 1997). One possible explanation for the observed atypical MHC combinations is the possibility of nonuniform isoform expression along the length of transforming fibers (Staron and Pette, 1987). Atypical MHC isoform combinations at the mRNA level, but not at the protein level, have been observed in unloaded rat soleus muscle (Stevens et al., 1999b). In view of these observations, it must be taken into account that MHC isoform expression is not only under transcriptional, but also under translational, and possibly posttranlational control. An uncoupling of these different control levels results in mRNA/protein mismatches (Andersen et al., 1999; Andersen and Schiaffino, 1997; Peuker et al., 1998; Peuker and Pette, 1997; Stevens et al., 1999b) . Interestingly, the frequency of such mismatches appears to be greatly increased under conditions of unweighting where slow-to-fast transitions in MHC expression coincide with pronounced atrophy.

POSSIBLE MECHANISMS INVOLVED IN FIBER TYPE TRANSITIONS

Fiber type transitions not only involve changes in myosin expression, but include alterations in the isoform profiles of a multitude of sarcomeric proteins. Therefore, fiber type transformation represents highly coordinated processes of gene upregulation and downregulation. The exchange of protein isoforms, or changes in specific protein amounts, involve all levels of control, namely transcription, translation, and proteolysis. Altered transcriptional and translational activities are responsible for gene induction and repression, whereas proteolysis plays an important role in the exchange of newly synthesized protein isoforms with their preexisting counterparts. These coordinated processes raise the question as to the existence and nature of superior control elements capable of exerting synergistic and antagonistic effects. In this context, changes in the phosphorylation potential of the muscle fiber may play an important role because such changes may be transmitted to various signal pathways related to the control of gene expression, e.g., AMP-activated protein kinase and/or Ca^{2+}dependent protein kinase cascades. A decrease in the ATP phosphorylation potential impairs the function of the sarcoplasmic reticulum Ca^{2+}-ATPase and, thus, has an immediate impact on Ca^{2+}-sequestration (Läuger, 1991). Indeed, CLFS has been shown to lead to pronounced increases in free Ca^{2+} in fast-twitch fibers of rabbit muscle (Sréter et al., 1987). This has been confirmed by recent studies that have demonstrated twofold increases in free intracellular Ca^{2+} in single fibers from low-frequency stimulated rat muscle (Carroll et al., 1999). This elevation in free Ca^{2+} is of interest with regard to its generally accepted role as a versatile second messenger in the control of gene expression (Hardingham and Bading, 1999). In addition, the calcineurin-dependent pathway has been implicated in the control of gene expression in fast and slow muscle fibers (Chin et al., 1998; Naya et al., 2000). For such a regulatory pathway, changes in the resting Ca^{2+} levels may be an important trigger for

fiber type transitions. In support of this, increasing intracellular Ca^{2+} levels in myotube cultures has been shown to enhance the expression of slow myosin (Kubis et al., 1997).

CONCLUSIONS

The reversible fiber type transitions described in this article clearly demonstrate that gene expression of a fully differentiated postmitotic cell can be altered by neuromuscular activity, mechanical loading and unloading, hormonal factors, and age. A large amount of literature over the past three decades has elucidated the basic phenomena of fiber type transitions. Future research will be needed to elucidate in more detail the molecular elements and mechanisms underlying fiber type determination and transformation. However, it is already clear that the dynamic nature of skeletal muscle fibers is an important evolutionary achievement that can be regarded as a significant contribution to improve survival.

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