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FIBER TYPES IN MAMMALIAN SKELETAL MUSCLES

Stefano Schiaffino and Carlo Reggiani

Venetian Institute of Molecular Medicine, Department of Biomedical Sciences, University of Padova, Consiglio Nazionale delle Ricerche Institute of Neurosciences, and Department of Human Anatomy and Physiology, University of Padova, Padova, Italy

Schiaffino S, Reggiani C. Fiber Types In Mammalian Skeletal Muscles. *Physiol Rev* 91: 1447–1531, 2011; doi:10.1152/physrev.00031.2010.—Mammalian skeletal muscle comprises different fiber types, whose identity is first established during embryonic development by intrinsic myogenic control mechanisms and is later modulated by neural and hormonal factors. The relative proportion of the different fiber types varies strikingly between species, and in humans shows significant variability between individuals. Myosin heavy chain isoforms, whose complete inventory and expression pattern are now available, provide a useful marker for fiber types, both for the four major forms present in trunk and limb muscles and the minor forms present in head and neck muscles. However, muscle fiber diversity involves all functional muscle cell compartments, including membrane excitation, excitation-contraction coupling, contractile machinery, cytoskeleton scaffold, and energy supply systems. Variations within each compartment are limited by the need of matching fiber type properties between different compartments. Nerve activity is a major control mechanism of the fiber type profile, and multiple signaling pathways are implicated in activity-dependent changes of muscle fibers. The characterization of these pathways is raising increasing interest in clinical medicine, given the potentially beneficial effects of muscle fiber type switching in the prevention and treatment of metabolic diseases.

I. INTRODUCTION

Mammalian skeletal muscles are heterogeneous in nature. The functional unit of the motor system, the motor unit, is composed of a motor neuron and a bunch of muscle fibers with similar, if not identical, structural and functional properties. To form a muscle, many (from tens to hundreds) motor units are assembled together, and each brings its own specific and distinct contribution. The selective recruitment of motor units enables a muscle to respond in the best manner to the functional demands. The heterogeneity of the muscle fibers is the base of the flexibility which allows the same muscle to be used for various tasks from continuous low-intensity activity (e.g., posture), to repeated submaximal contractions (for example, locomotion), and to fast and strong maximal contractions (jumping, kicking). In addition, the structural and functional properties of the fibers, which are generally referred to as fiber phenotype, can change in response to hormonal and neural influences, nerve-activity being a major determinant of the fiber type profile. This property is defined muscle plasticity or malleability. The molecular mechanisms of the heterogeneity and plasticity of muscle fibers have been the object of intensive investigation, and several signaling pathways have been identified that appear to mediate the emergence of specific muscle phenotypes. The field of study is no longer confined to basic science, but its applications are spreading from sport sciences to clinical medicine and are likely to expand in the near future. Actually, muscle activity has a beneficial effect in the prevention of many chronic diseases, and a better understanding of the mechanism of activity-dependent muscle changes may help to identify potential therapeutic targets.

This review focuses on mammalian muscle fiber heterogeneity, with the goal to relate the new mechanistic studies on signaling pathways to an integrative description of the function and structure of different types of skeletal muscle fibers. We first review the different aspects of muscle fiber specialization from excitability to metabolism, from contraction mechanism to calcium kinetics aiming to an integrated view. We then consider the variations in fiber type profile in relation to species, gender, and individual polymorphism; the process of fiber diversification during development; and the fiber type remodeling in adult skeletal mus-

cle. Finally, we consider selected signaling pathways, leading to muscle fiber specialization and the underlying adaptive changes in muscle plasticity. Many other reviews in the last few years have covered various aspects of this subject, and the readers are referred also to them (42, 59, 489, 605, 704, 715, 755).

II. FIBER TYPE HETEROGENEITY IN MAMMALIAN SKELETAL MUSCLE

A. Four Major Fiber Types With Distinct Myosin Composition

Scientists have been aware that skeletal muscles can be distinguished on the basis of their color as red or white and their contractile properties as fast and slow since the first half of the 19th century. However, it is during the last 40 years that the notion of muscle fiber type diversity has rapidly progressed leading to the identification of four major fiber types in adult mammalian skeletal muscles. Until around 1968–1970, the common view of diversity in mammalian skeletal muscles was based on the old classification of fast-twitch muscles, characterized by glycolytic metabolism and specialized for phasic activity, generally identified with white muscles, and slow-twitch muscles, rich in myoglobin and oxidative enzymes and specialized for more continuous activity, also called red muscles (562). The mechanical and the biochemical approach found their meeting point in the relation between the actin-activated ATPase activity of myosin and the speed of muscle shortening which holds through the whole range of variations of different muscles and different species (47).

A more complex scheme emerged at the end of the 1960s, mainly as the result of four independent lines of evidence: *1*) correlated histochemical and physiological studies of individual motor units, *2*) electron microscopy of fast and slow skeletal muscles, *3*) novel procedures for myosin ATPase histochemistry, and *4*) biochemical studies on oxidative and glycolytic enzymes in different muscles. Edstrom and Kugelberg (200) stimulated single motor axons in rat fast tibialis anterior (TA) muscle to characterize single motor units with respect to twitch properties, then induced glycogen depletion in the corresponding muscle fibers to identify these fibers in cryosections by periodic acid-Schiff (PAS) staining and applied enzyme histochemistry for succinate dehydrogenase (SDH) activity, reflecting oxidative metabolism, in serial sections. Using this approach, they were able to show that *1*) motor units are homogeneous in fiber type composition, based on SDH staining; *2*) units composed of small SDH-positive fibers or large SDH-negative fibers are both fast-twitch units; but *3*) they differ with respect to resistance to fatigue. Units composed of SDH-negative fibers undergo rapid decline in tension following repetitive stimulation, whereas units composed of SDH-positive fibers show no force drop for several minutes. A spectrum of units with intermediate SDH staining and resistance to fatigue was detected in TA muscle. A similar spectrum of fibers with variable SDH and myoglobin staining was seen in the rat extensor digitorum longus (EDL), a muscle known to contain essentially only fast-twitch motor units (157), and by electron microscopy these fibers were found have a variable mitochondrial content, with mitochondrial volume ranging from $<5\%$ to $>25\%$ of total fiber volume (710). Both small mitochondria-rich and large mitochondria-poor muscle fibers in rat EDL muscle showed a richly developed sarcoplasmic reticulum (SR), in contrast to the poorly developed SR seen in the slow-twitch soleus muscle fibers (710). On the other hand, sarcomeres in mitochondria-poor muscle fibers of fast muscle have a thin Z line, whereas both mitochondria-rich fibers in fast muscle and slow soleus muscle fibers have a thick Z line. It was thus proposed that muscle fiber structure is the expression of two distinct physiological parameters: speed of contraction, correlated with SR development, and resistance to fatigue, correlated with mitochondrial content and thickness of the Z line (710). Another line of evidence pointing to the heterogeneity of fast muscle fibers was the development of enzyme histochemical procedures for myosin ATPase that led to the identification of two fiber populations, called type 2A and 2B, which are abundant in fast-twitch muscles and distinct from the type 1 fibers predominant in slow-twitch muscles (105, 305). Physiological-histochemical correlations using both myosin ATPase and SDH staining in cat muscle confirmed the existence of two types of fast-twitch motor units, fast fatigable (FF) units composed of 2B fibers staining weakly for SDH and fatigue-resistant (FR) units composed of 2A fibers staining strongly for SDH, in addition to the slow-twitch motor units (S) (117). It should be stressed that the resistance to fatigue of FR motor unit is lower compared with that of S motor units. A further contribution to the characterization of these three major fiber populations was the recognition, based on biochemical studies on muscles composed predominantly of one or another fiber type, that both 2A and 2B fibers have high levels of glycolytic enzymes, in spite of the different oxidative enzyme complement; this led to the classification of slow oxidative (type 1), fast-twitch oxidative glycolytic (2A), and fast-twitch glycolytic fibers (2B) (599). More precise analyses of metabolic properties were subsequently made possible by the introduction of the microdissection of single fibers (209, 493, 754) that showed wide variation in enzyme activities within the different fiber populations.

A further step in the characterization of the major fiber types present in mammalian skeletal muscle took place around 1988–1994 with the discovery of a third fast fiber type with myosin heavy chain (MyHC) composition different from 2A and 2B fibers. Monoclonal antibodies against MyHCs led to the identification of fibers called type 2X (706, 709, 714), and independent studies using an im-

proved electrophoretic procedure led to the identification of a distinct type 2D MyHC band (46, 789). The identity of 2X and 2D MyHC was demonstrated by Western blotting (455); however, it was not established whether this MyHC is a different isoform or results from posttranslational modification of other MyHCs until definitive evidence for the presence of a distinct MyHC-2X transcript in type 2X fibers was obtained (184). Motor units composed of type 2X fibers have twitch properties (contraction and half-relaxation time) similar to those of 2A and 2B units, and their resistance to fatigue is intermediate between that of 2A and 2B units (463). In rat skeletal muscle, type 2X fibers have moderate to strong SDH staining (463, 709), and their maximal velocity of shortening is intermediate between that of 2A and 2B fibers (89, 94). Immunohistochemical and in situ hybridization analyses of muscle sections (184, 278) and biochemical and physiological studies of single fibers (89, 603) confirmed the existence of a spectrum of fiber types with pure or hybrid MyHC composition, according to the scheme: 1 ↔ 1/2A ↔ 2A ↔ 2A/2X ↔ 2X ↔ 2X/2B ↔ 2B. However, this pattern of MyHC gene expression is not obligatory; for example, fibers coexpressing type 1 and 2X but not 2A MyHCs have been detected both in normal muscles (121) and in slow muscles stimulated with a fast impulse pattern (706). A fiber type profile similar to that present in rat skeletal muscle has been observed in different mammalian species including mouse, rat, rabbit, and guinea pig (278); however, in human muscles, MyHC-2B is not detectable, although the corresponding *MYH4* gene is present in the genome, and fibers typed as 2B based on ATPase staining are in fact 2X fibers based on MyHC composition (744). It is worth noting that, in contrast to rat and mouse 2X fibers, human 2X fibers have the lowest level of SDH activity compared with all other fiber types. Therefore, it is not justified to extrapolate results from transgenic mice to human muscles assuming that each fiber type has similar properties in different species (see, for example, Ref. 32).

The four major fiber types discussed above are variously distributed in body muscles of mammals, including limb, trunk, and head muscles. The relative proportion of any fiber type may vary according to species (see sect. IV*A*) and anatomical site. For example, the diaphragm, a continuously active respiratory muscle, is a fast muscle in rat and mouse but a slow muscle in large mammals, such as the cow. In the rat, the diaphragm consists predominantly of type 2X fibers and lacks type 2B fibers that are abundant in leg muscles (709). In leg muscles, the most studied muscles of the body, slow type 1 fibers are more abundant in the posterior compartment, where the typical slow soleus muscle is also located, in relation with the greater postural role of posterior muscles. Finally, in many species, type 2 fibers are more numerous in forelimbs than in hindlimbs and, accordingly, in humans upper limb muscles are faster than lower limb muscles (320, 531).

The central role of myosin as molecular motor in muscle cell physiology and the existence of several MyHC isoforms differentially distributed in various fibers makes MyHC the best available marker for fiber typing **(FIG. 1)**. Specific MyHC isoforms are also present in developing muscle, thus providing a useful marker for regenerating fibers, and in specific anatomical districts, such as head muscles, where unique fiber types have been identified, as discussed below. Clearly, a large number of proteins are differentially expressed in the various muscle fiber types, and it has been proposed that the simple classification based on myosin composition and level of oxidative enzymes is only convenient for communication until more complete profiles of gene expression at the transcript and protein level will become available (see Ref. 755). However, microarray analyses have not much contributed so far to help in better delineating fiber types in skeletal muscle. One possible reason is that transcriptomic comparison has been carried out on RNA prepared from whole muscles. In any muscle, different fiber types coexist and, besides muscle fibres, other cell types, as fibroblasts in the connective layers (perimysium and endomysium), endothelial and smooth muscle cells in the vessel walls, Schwann cells around the axons, and blood cells are present (126, 877). Microarray analysis of single muscle fibers is required and is presently possible with microgenomics approach. A first step in this direction is the recently published analysis of transcriptome of single type 1 and type 2B fibers isolated from murine muscles (144).

B. Minor Fiber Types With Restricted Distribution in Skeletal Muscles

The model based on four major fiber types (slow and three fast fiber types) has been until now the main reference to study fiber heterogeneity and plasticity in mammalian muscles. Such a model is representative of limb and trunk muscles, which have generally been considered the paradigm for studies of muscle fiber diversity. There are, however, several other muscle groups with functional specializations and fiber types distinct from those seen in limb muscles. In particular, atypical muscle fibers are present in head and neck muscles, including the extraocular muscles (EOMs), jaw muscles, middle ear muscles, and laryngeal muscles, as well as in the muscle spindles.

1. Head and neck muscles

Specific embryological origin (see sect. V) and highly specialized functions cooperate in determining the structural and functional specificity of many head and neck muscles.

EOMs, placed around the eye ball, are responsible for stabilization of the eye and for several distinct voluntary and reflex movements: very fast saccadic movements, slow pursuit and vergence movements, vestibulo-ocular reflex and opto-kinetic reflex, and accomodation. There are six EOMs

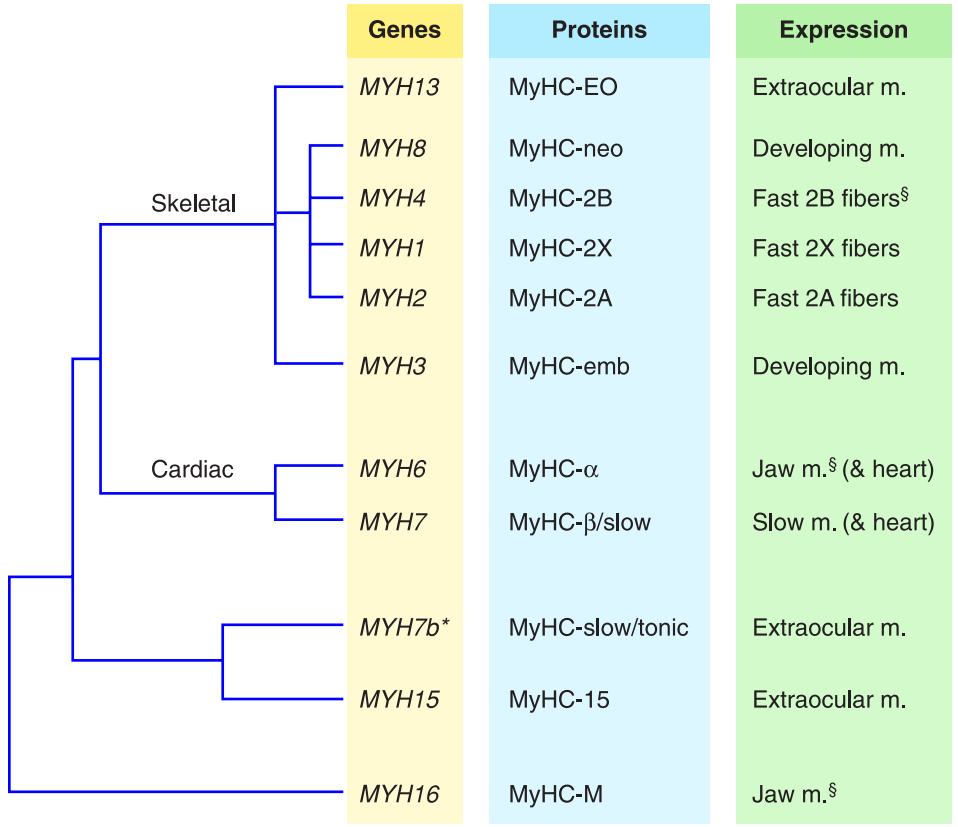


FIGURE 1. The complete panel of sarcomeric *MYH* genes in mammals with the corresponding protein products and their expression pattern. The evolutionary relationship between *MYH* genes is indicated in the phylogenetic tree on the left. Spacing and length of the branches do not reflect actual scale in this simplified scheme. Only extrafusal muscle fibers are considered for the expression pattern. §Expression only in some mammalian species. **MYH7b*, also referred to as *MYH14*, is expressed in both slow muscles and heart at the transcript level, but only in extraocular muscles at the protein level (661). [Scheme modified from Rossi et al. (661).]

in mammals: rectus superior, rectus lateralis, rectus inferior, rectus medialis, superior oblique or trochlearis, and inferior oblique. In EOMs, the fibers present in the peripheral, orbital layer are thinner than fibers in the internal, global layer and differ in many structural and functional properties. The contractile properties of the extraocular muscles differ significantly from typical skeletal muscles. Their specific tension is lower than that developed by limb muscles (503), although some contrasting results have been reported (239). At variance with limb muscles, a very high speed of contraction (159, 616) coexists with a relatively high resistance to fatigue (240) correlated with high SDH activity. Adult EOMs express most known striated muscle myosin isoforms, including MyHC-slow tonic, coded by the *MYH7b* (*MYH14*) gene (661), MyHC-EO coded by *MYH13* and the developmental MyHC-emb and -neo isoforms. In addition, a novel MyHC isoform, coded by the *MYH15* gene, has been recently shown to be expressed selectively in the orbital layer of EOMs (661). Many EOM fibers synthesize multiple MyHC isoforms and localize them differentially along the length of the fiber (843, 864). Moreover, in both the global and orbital layers of the EOMs, multiple innervated fibers are found, a condition totally unusual in trunk and limb adult mammalian skeletal muscles (332), where multiple innervation is restricted to intrafusal fibers. Combined acetylcholinesterase and antimyosin staining showed that these fibers express the MyHC-slow tonic isoform (85, 86). Expression profile studies based on microarrays have provided a detailed characterization of the specific gene expression pattern in EOMs compared with limb skeletal muscles and cardiac muscle in the rat (405) and mouse (617). EOMs, like cardiac muscle, do not seem to follow the skeletal muscle pattern of dependence upon glycogen as a metabolic fuel and express nonskeletal muscle isoforms of several glycolytic enzymes, which may contribute to the relative sparing of these muscles in the metabolic myopathies (617). The expression of nearly all skeletal muscle myosin isoforms is confirmed by microarray studies which in addition point to the expression of specific cardiac isoforms as cardiac α-actin and cardiac troponin T. As far as excitation-contraction coupling, microarrays show a surprising low expression of the γ subunit of dihydropyridine receptor (DHPR), which is essential for the channel inactivation properties, and of mitsugumin 29 (Mg29), but a high expression of both isoforms of calsequestrin and the presence of both SERCA1 and SERCA2.

The low levels of adult myomesin and CK-M transcripts in EOMs have found support in immunoblotting and electron microscopy, which has shown a postnatal disappearance of the M-band in the fibers of the orbital layer (618, 865).

Laryngeal muscles comprise in mammals five distinct muscles: *1*) thyroarytenoid, *2*) lateral cricoarytenoid, *3*) interarytenoid, *4*) posterior cricoarytenoid, and *5*) cricothyroid. Their origin can be traced to fourth and sixth branchial arches. Laryngeal muscles have three types of functions: airway protection, respiration, and phonation. Taking into account their main functions, the laryngeal muscles can be divided into *1*) adductors, comprising thyroarytenoid, lateral cricoarytenoid, and interarytenoid, which close the glottis; *2*) abductor, comprising the posterior cricoarytenoid, which opens the glottis; and *3*) vocal fold tensor, the cricothyroid. Thyroarytenoid, and in particular its medial division, generally indicated as vocalis, plays a role in modulating the tension of the vocal fold. Each muscle has a specific fiber type composition, and further differences have been described among species (see Ref. 344 for a comprehensive review). Fast fibers are predominant over slow fibers, which are virtually absent in many laryngeal muscles of small mammals as rats (883). In all animal species analyzed (rat, rabbit, dog, human), the proportion of slow fibers is greater in posterior cricoarytenoid than in cricothyroid, whereas it is very low in thyroarytenoid (344). Different types of fast fibers can be identified based on the expression of various MyHC isoforms: 2A, 2X, and 2B are expressed not only in the rat (883) and rabbit (103) but also in the dog (70, 801). In addition, the expression of MyHC-EO and of developmental isoforms has been documented in the rabbit (103) and rat (650). The existence of fibers expressing a specific laryngeal myosin isoform has been reported in a number of studies, but its identification is still controversial (176, 344, 883). There are also contrasting evidence in favor of the expression of the slow tonic isoform, although this does not bear a direct relation with the presence of multiple innervation (344, 748). Actually, many fibers of thyroarytenoid and lateral cricoarytenoid show several neuromuscular junctions spread along their length, but each fiber seems to be in contact with only one motor neuron (481). From the metabolic point of view, all laryngeal fibers, regardless of the type of myosin they express, are very resistant to fatigue, and this is in agreement with the high SDH activity (344, 527).

Middle ear muscles consist of two small muscles: the stapedius, which pulls the head of the stapes backward, and the tensor tympani, which draws the tympanic membrane medialward. Their embryological origin can be traced in the first branchial arch for the tensor tympani and in the second arch for the stapedius. Their fibers are very thin and often lacking M-band (217). Whereas stapedius has a quite constant fiber type composition among species, with predominance of fast 2A and 2X fibers (178, 387, 823), tensor tympani is very variable in its fiber type composition. Slow tonic fibers and MyHC-slow tonic have been detected in tensor tympani of several carnivore species together with fiber-expressing masticatory myosin (217, 526, 666), whereas slow and 2A fibers often coexpressing neonatal myosin have been found in large mammals such as cow and pig (702). Fast fibers, mainly 2A and 2X and in some cases expressing also neonatal myosin, are predominant in the rat (387). There is still controversy as to the presence of multiply innervated fibers (217, 526).

Jaw muscles or masticatory muscles are devoted to the movement of the jaw mainly in relation to food assumption and mastication. Most jaw muscles share a common embryological origin in the first branchial arch: masseter, temporalis, pterygoideus medialis and lateralis, tensor veli palatini, tensor tympani, anterior digastricus, and mylohyoideus. The structural and functional features of the muscle fibers composing masticatory muscles are very different in relation to life-style, diet, and eating habits, which are much more variable among species compared with locomotory demands. As proposed by Hoh (345), masticatory muscles of mammals can be classified into two groups. The first group includes those species where the masticatory muscles share the same fiber types present also in trunk and limb muscles. Ruminants and most rodents belong to this first group. The jaw closers of the rat are composed of the four fiber types found in limb muscles (160, 726), with predominance of fast 2A and 2X fibers, while the jaw-closer muscles of sheep and cattle are homogeneously slow (394, 610). It is quite obvious to find a relation between the type of slow and continuous mastication and rumination of the cattle and the predominance of slow and fatigue-resistant fibers or a relation between the gnawing of the rodents and the need of fast and powerful fibers. The second group includes species where additional and, in some case, unique fiber types are present in the masticatory muscles. In this group there are kangaroos whose jaw closers are homogeneously composed of fibers containing α-cardiac myosin (346), rabbits whose jaw closers contain α-cardiac fibers in addition to slow and 2A fibers (99, 206), humans whose jaw closers comprise fibers coexpressing α-cardiac and neonatal MyHCs in addition to slow, 2A, and 2X fibers (432, 721). Carnivores, primates (except humans), some chiroptera, and some marsupials are characterized in their masticatory muscles by the presence of fibers that express a jaw-specific MyHC, referred to as MyHC-M or masticatory isoform (666), which is coded by a distinct *MYH16* gene (631). The presence of this myosin, often incorrectly referred as "superfast," is likely important to give to the fibers a contractile behavior characterized by high force development and moderately fast speed shortening, ideal for reaching high power level during contraction (799). The presence in fibers expressing *MYH16* of a well-developed SR is in full agreement with the fast kinetics of the contractile cycle (666, 785). The absence of *MYH16* expression in human masti-

catory muscles deserves a comment. As in all primates, the gene coding for *MYH16* is present in human genome but is not expressed due to frameshift mutation (760). The typical fiber type pattern of human jaw closer muscles is a combination of abundant and thick slow fibers with less abundant and thinner fast fibers, expressing MyHC-2A, -2X, -neo, and α-cardiac (721). In those species that have specialized fiber types in masticatory muscles, MyHC isoforms are not the only distinctive molecular sign of specialization. Other myofibrillar proteins such as myosin light chain 2 (630), tropomyosin (393, 666) and myosin-binding protein C (MyBP-C) (393, 882, 891) are present in those fibers with isoforms specific for masticatory muscles.

2. Muscle spindles

Muscle spindles are proprioceptive sensory organs present in skeletal muscles that act as stretch-sensitive mechanoreceptors able to monitor changes in muscle length: the proprioceptive signals generated in the spindles are transmitted by afferent neurons to the spinal cord, where they elicit appropriate contractile responses, including stretch reflexes mediated by direct monosynaptic connections between sensory neurons and α-motor neurons. The muscle spindles consist of a few short and thin muscle fibers, called intrafusal fibers, innervated by sensory neurons (group-Ia afferent neurons) in the central or equatorial region and by γ-motor neurons in the polar regions (see Ref. 57). The spindles are wrapped by a capsule derived from terminal Schwann cells of sensory axons. The intrafusal fibers have been traditionally classified as nuclear-bag and nuclear-chain fibers on the basis of the distribution of the myonuclei in the equatorial region, and bag fibers were further subdivided into nuclear-bag$_1$ and nuclear-bag$_2$ fibers by myosin ATPase histochemistry (579) and physiological properties (96, 246). Bag fibers are slow fibers with some unique properties not shared by extrafusal slow fibers, in particular they react with a sustained contracture rather than a twitch to succinylcholine (746) and give only nonpropagated potentials in response to γ-axon stimulation (461): in this respect, bag fibers are similar to the slow-tonic fibers of amphibians and birds and to the multiply innervated slow-tonic fibers present in EOMs of mammals. This analogy was confirmed by the finding that bag fibers are reactive with polyclonal antibodies to avian slow myosin, which stain slow-tonic fibers of amphibians and multiply innervated slow-tonic fibers in mammalian EOMs but not slow-twitch mammalian muscle fibers (85, 86). This immunoreactivity is shared by some monoclonal anti-chicken myosin antibodies, the most specific being S46 (748), and has been widely used as a specific intrafusal fiber marker in studies of muscle spindle development (see sect. V). Definitive evidence for the existence of slow-tonic MyHC was provided by the demonstration that a mammalian gene (called *MYH7b* or *MYH14*), homologous to avian slow myosin 2, is expressed at the protein level in bag fibers of muscle spindles and multiply innervated fibers of EOMs (661). Nuclear-bag fibers also express MyHC-β/slow and MyHC-α (445, 593), whereas nuclear-chain fibers express embryonic MyHC (665) in addition to a limited amount of fast MyHC-2A and -2B (445). The complexity of the MyHC composition of intrafusal fibers is further increased by the existence of variations in MyHC distribution along the length of both bag and chain fibers, possibly in relation to a local influence of sensory innervation in the equatorial region and motor innervation in the polar region (see Refs. 592, 843). The discovery of a novel MyHC-15 isoform, coded by the *MYH15* gene, has recently enriched the collection of MyHCs present in intrafusal fibers, and also provided a further demonstration of the uneven myosin distribution in these fibers. In mammalian skeletal muscle, the *MYH15* gene, homologous to the gene coding for avian ventricular MyHC, is expressed exclusively in the extracapsular region of bag muscle fibers, as well as in the orbital layer of extraocular muscles (661).

C. Functional Significance of Diversity Between Fiber Types

The ability of skeletal muscles to generate force and movement is exploited by the nervous system for a variety of motor tasks. For the sake of simplicity, motor tasks can be reduced to three main types: *1*) postural joint stabilization, *2*) long-lasting and repetitive activities like respiration or locomotion or chewing, and *3*) fast and generally powerful actions such as jumping, kicking, or biting. Muscle fibers suited to each different task have been developed by evolution leading to the present heterogeneity that can be recognized in all vertebrates from fish to human beings. The connection of the various fiber types with motor neurons with specific activity (discharge pattern or firing pattern) is not only instrumental to the correct use of the functional properties of each fiber, but also a major factor in fiber type remodeling in adult muscles (see sect. VI).

In mammals, the neuromuscular system is functionally organized into discrete units, the motor units, each consisting of a motor neuron and all the muscle fibers that it exclusively innervates. Motor neurons display striking differences in the firing patterns. Two classes of motor neurons, "tonic" and "phasic," have been first described 50 years ago by Granit et al. (280), and a continuous spectrum of motor neurons with distinct subgroups, the key variable being cell size, has been later described by Henneman et al. (329). Continuous electromyography (EMG) recording of single motor units based on fine electrodes chronically implanted in muscles has made possible the identification of three distinct firing patterns in the rat (330) **(FIG. 2)**. A first pattern is typical of motor units comprising slow and fatigue-resistant fibers. It is characterized by high amount of impulse activity (300,000–500,000 over 24 h), with long-lasting trains (300–500 s) and relatively low frequency of firing (~20 Hz). In contrast, motor units comprising fast fibers show higher frequency of firing and can be divided in

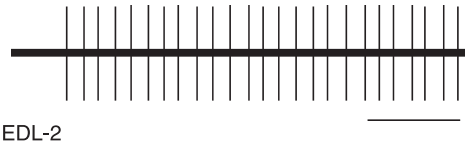
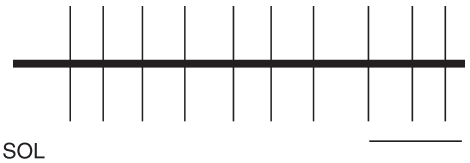
Firing patterns	Median firing frequency (Hz)	Longest train duration (s)	Impulses per 24 hrs	Time on per 24 hrs
	69-91	0.8-3.9	2600-11200	0.5-3 min
	48-83	59-141	89500-243100	23-72 min
	18-21	290-548	309500-495800	5.3-8.4 hrs

FIGURE 2. Firing patterns of three classes of motor units identified in rat fast extensor digitorum longus (EDL) and slow soleus (SOL) muscles by continuous recording in vivo. EDL-1 and EDL-2 presumably correspond to units composed of 2B and 2A muscle fibers, respectively. [Data from Henning and Lomo (330). Scheme modified from Lomo (490).]

two distinct subgroups: one characterized by very modest amount of activity per day (3,000–10,000 impulses), high discharge frequency (70–90 Hz), and short duration of the trains (<3 s), presumably corresponding to 2B fibers. The other subgroup is not very different in average discharge rate (50–80 Hz), but has much greater activity per day (90,000–250,000 impulses) and relatively long train duration (60–140 s) and presumably corresponds to 2A and 2X fibers.

The three subgroups of motor units characterized for their discharge pattern correspond quite well to the three groups of motor units identified on the basis of fatigue resistance, time parameters of the twitch and histochemical properties in rat muscles (447) and later in cat muscles (116, 117). Thus, at an integrative level, there is a perfect matching between the nervous side (discharge pattern) and the muscle side (contraction time parameters and fatigue resistance) of the motor units. It will be the aim of the following section (sect. III) to discuss the available information and the present understanding of how muscle fibers achieve a degree of specialized molecular structure and physiological parameters to perfectly match the demands of the motor neurons. In conclusion, the heterogeneity of skeletal muscle fibers reflects primarily an adaptation to the different patterns of activity, which can be quantified in terms of impulse rate and total number of impulses per day. Different activity patterns dictate not only the specializations in membrane properties, calcium shuttling mechanisms, and contractile machinery, but also in the structure of the cytoskeleton, possibly in relation to the variable load imposed to the muscles, and in energy metabolism, to match energy consumption with energy supply.

Fiber type diversification may also reflect an adaptation to whole body metabolism. Two aspects of this relation can be considered. First, skeletal muscle is the main protein reservoir in the body, and amino acid release from muscle during prolonged starvation is essential to maintain plasma glucose concentration via gluconeogenesis in the liver and for new protein synthesis. During fasting or exposure to glucocorticoids, as well in other conditions of muscle wasting, such as cancer cachexia or sepsis, type 2 glycolytic (2B) muscle fibers show greater atrophy than the type 1 oxidative fibers (479, 529). The greater sensitivity of the type 2B fibers may be due to their lower content of PGC1α, as suggested by the finding that PGC1α overexpression partly prevents muscle atrophy by reducing the FoxO-dependent upregulation of ubiquitin ligases atrogin-1 and MuRF1 (692). A second aspect of the relation between muscle fiber types and whole body metabolism concerns plasma glucose disposal. Skeletal muscle is the major sink for plasma glucose after a meal, a function mediated by the insulin-dependent translocation of the glucose transporter GLUT4 to the sarcolemma. Hyperglycemia occurs when glucose uptake by muscle fibers is defective, such as seen in insulin resistance and type 2 diabetes. As discussed in section III*D*, slow-oxidative muscle fibers are more active than fast-glycolytic fiber in removing glucose from blood, and it has been suggested that an altered fiber type distribution with prevalence of fast-glycolytic fibers, often associated to obesity and due to genetic factors and/or lack of physical activ-

ity, may contribute to insulin resistance and type 2 diabetes (492, 590).

III. MUSCLE FIBER DIVERSITY IN SUBCELLULAR FUNCTIONAL COMPARTMENTS

Most studies on muscle fiber heterogeneity have focused on two main areas of diversity: contractile response and metabolism. However, the diversity between muscle fibers is not restricted to myofibrillar proteins (myosin isoforms, in the first place) and metabolic enzymes (predominance of glycolytic or mitochondrial activities), but extends to any subcellular system, including transmembrane ionic fluxes and intracellular calcium signalling. The existence of wide variability among muscle fibers in all these domains may suggest that muscles consist of a continuous spectrum of fibers rather than distinct fiber types. However, when specific parameters are measured and even more clearly when two or more parameters are considered together, clusters of values will often appear rather than a continuous distribution, a strong indication that fiber types do exist **(FIG. 3)**. This is especially obvious when type 1/slow and type 2/fast fibers are compared, whereas a more continuous distribution of values is often observed with the different subsets of type 2 fibers. Preferential combinations of specific molecular and functional properties presumably reflect the need to obtain consistent values of specific functions, for example, matching energy production with energy consumption, calcium release with calcium uptake. Fiber types can, therefore, be viewed as the result of preferential combinations of gene expression profiles that must be compatible with the constraints imposed by electrical and mechanical influences, namely, membrane excitability must be matched to

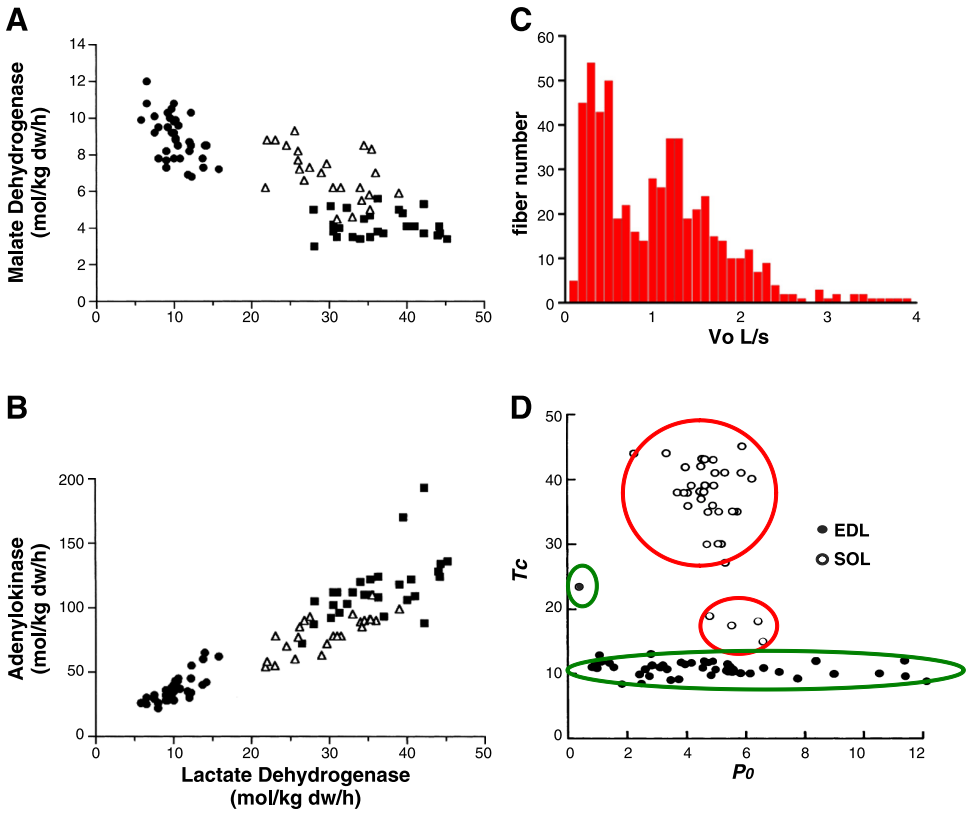


FIGURE 3. Clustering of functional parameters in skeletal muscle fibers. *A* and *B*: clustering of enzymatic activities determined in single human muscle fibers. In each individual fiber, two activities were determined, and each fiber was classified on the basis of myofibrillar ATPase reaction as type 1/slow (circles), fast 2A (triangles), and fast 2X (squares). Note the inverse relation between the activities of malate dehydrogenase and lactate dehydrogenase and the direct relation between adenylokinase and lactate dehydrogenase. *C*: distribution of unloaded shortening velocities (V_o) determined at 12°C in a population of 587 single fibers from vastus lateralis muscles of 25 healthy subjects. Note the bimodal distribution with the peak on the left corresponding to type 1/slow fibers and the second peak to type 2 fast fibers. (Courtesy of R. Bottinelli.) *D*: clustering of motor unit distribution with respect to twitch contraction time (T_c, in ms) and maximum isometric tetanic tension (P_o, in g) in 36 motor units from rat soleus and 47 motor units from rat EDL. Soleus consists of a majority of slow-twitch units and a minor group of relatively faster units, corresponding to type 2A muscle fibers. EDL consists essentially of units with similar fast-twitch properties but variable force, corresponding to the variable size of type 2 fibers, plus a single slower unit, corresponding to the rare type 1 fibers present in this muscle. [Modified from Close (157), with permission from Wiley-Blackwell.]

motor neuron firing and contractile activity to force transmission to tendons. On the other hand, gene expression profiles must also guarantee consistency between functions in different cellular compartments. Fiber type heterogeneity will be hereafter discussed in relation to four major functional compartments according to the schema shown in **FIGURE 4,** which follows the sequence of events leading from neural stimulation to force and movement generation.

A. Generation and Spreading of Action Potential

1. Transmission of nerve impulse to muscle fibers

The neuromuscular junction (NMJ) represents the communicative link between motoneurons and muscle fibers. Synaptic transmission at the NMJ is highly efficient and reliable because *1*) the amount of transmitter released per nerve impulse is greater than that required to trigger an action potential, and *2*) the number of receptors activated by released acetylcholine (ACh) is in excess of that required to reach threshold. This allows a margin of safety to guarantee neuromuscular transmission both during fast, high-frequency bursts and during prolonged, low-frequency activation of muscles. A parameter indicated as "safety factor" can be calculated as the ratio of the estimated amplitude of the endplate potential to the depolarization required to reach threshold for an action potential or as the ratio of ACh receptors activated by released ACh to those required to reach threshold (see Ref. 873). To ensure efficient and reliable transmission in front of highly different discharge patterns, NMJs of fast and slow fibers have developed specialized structural and functional adaptations.

Measurements of the synaptic area reveal significant differences among muscle fibers, with NMJ area varying in proportion to fiber size. Thus, in the rat, synaptic area is greater in soleus slow fibers than in EDL fast fibers (839, 874), but the ratio between NMJ area and fiber size is very similar in both muscles. The size of the vesicular pool is accordingly larger in soleus than in EDL. Combined electrophysiological recordings and determination of the loss of the fluorescent membrane dye FM1–43 loaded into synaptic vesicles yield values of 178,000 vesicles for the EDL fibers and 252,000 vesicles for the soleus fibers (73). In rat diaphragm, slow fibers are innervated by the smallest axons, whereas type 2A, 2X, and 2B fibers receive progressively larger axons (622). Absolute areas of nerve terminals and endplates progressively increase from type 1, 2A, 2X, to 2B fibers; however, when normalized for fiber diameter, the areas of nerve terminals are largest in type 1 fibers, with no difference among type 2 fibers (622).

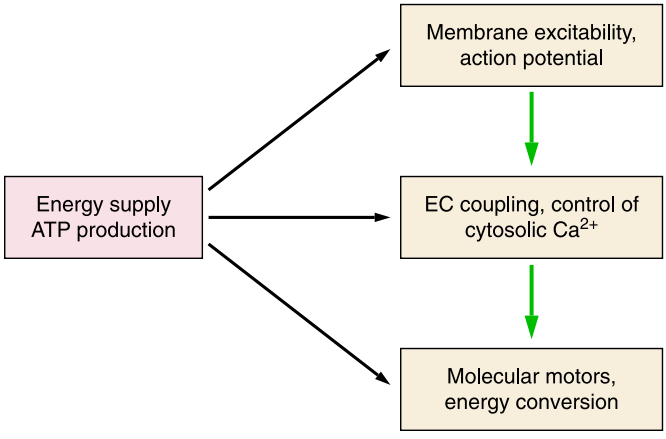


FIGURE 4. Muscle contractile function requires the coordinated activity of four major cellular functional compartments (membrane excitation, excitation-contraction coupling, contraction, and energy supply) whose molecular composition and functional properties diverge among muscle fiber types (see sect. III).

Electron microscopy studies show that postsynaptic folding does not change in relation to fiber type (874) or is greater in the NMJs of fast than slow fibers (573, 581). A greater fold density, which implies an increased area for Na channel accumulation, is consistent with the higher density of postsynaptic voltage-gated sodium channels reported at NMJs in fast fibers (541, 673) (see also section III*A*2D). Moreover, in rat diaphragm (519), interposition of mitochondria or myonuclei between motor endplate and myofibrils can be frequently observed in type 1 and 2A fibers, while NMJs of type 2X and 2B fibers are larger and show more direct apposition of motor endplates and myofibrils.

At the onset of stimulation trains, transmitter release is greater in fast fibers than in slow fibers (645), and the amplitude of the endplate potential is thus accordingly greater (874). During repetitive activation, NMJ of both fast and slow fibers exhibit a significant synaptic depression, which is much greater in fast EDL **(FIG. 5)**. The amplitude of spontaneous postsynaptic potential (miniature endplate potentials, mepps) remains constant, showing that the depression reflects a reduction in the number of synaptic vesicles released per stimulus and not in the amount of neurotransmitter in each vesicle (645). The synaptic depression is responsible for a significant difference between fiber types since the safety factor for neuromuscular transmission (see above) at the NMJ of slow fibers of rat soleus is low but remains stable with repetitive stimulation, whereas in fast EDL fibers is high at the beginning of the stimulation but sharply decreases with repeated stimulation (266).

A further interesting diversity is related to the enzyme acetylcholinesterase (AChE), which removes the neurotransmitter from the NMJ. In the rat, AChE activity in fast muscles is several times higher than in the slow soleus muscle (741). The content of AChE in slow fibers is about one-fourth of that in fast fibers and changes in relation to

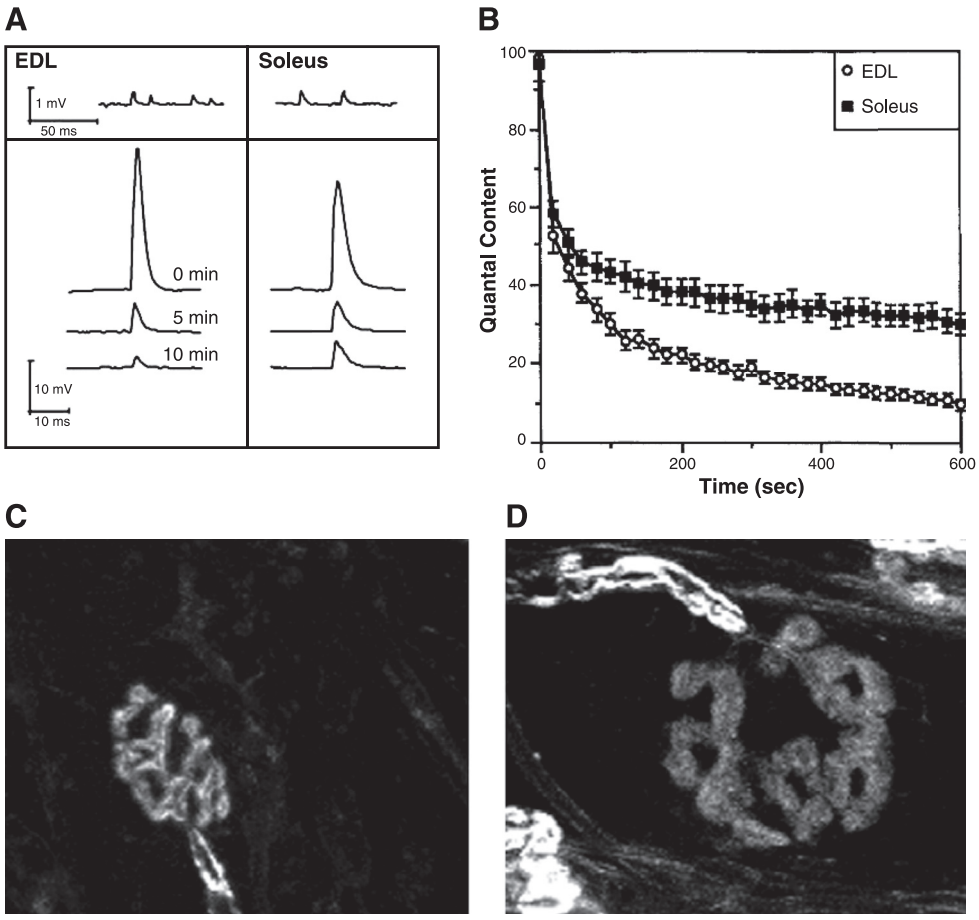


FIGURE 5. Differences in the neuromuscular junctions between fast and slow muscle fibers. The amplitude of the potential evoked by nerve stimulation is greater in fast EDL fibers than in slow soleus fibers and decreases during repeated stimulation (*A*), but the quantal content of the release is better maintained in slow than in fast fibers (*B*). [From Reid et al. (645).] The neuromuscular junction size is greater in fast (*D*) than in slow (*C*) rat diaphragm fibers. [From Mantilla et al. (519), with permission from Elsevier.]

electrical activity and load (623). It has been hypothesized that specific neural impulse patterns in fast or slow muscles are responsible for different AChE activities. AChE is coded by a single gene from which two splicing variants, H and T, are generated. Oligomerization of the T variant gives origin to symmetric forms (G1, G2, G4), while association with the collagen subunit ColQ gives origin to asymmetric forms (A4, A8, A12), (see Ref. 471). Globular G1 and G4 forms of AChE are present in fast fibers, and their expression is reduced by low-frequency electrical stimulation (741). In the synaptic clefts of NMJs of slow fibers, the smaller A8 and A4 forms are predominant (95). It has been proposed that adult fast fibers constitutively express a basic profile of AChE molecular forms of the type displayed by slow fibers, and varying levels of G4 are added in proportion to the amount of phasic activity performed by the muscles (95). Extrajunctional expression of AChE occurs in slow but not in fast muscle fibers in relation to the difference in the extrajunctional expression of ColQ between fiber types (169).

Taken together, the pre- and postsynaptic specializations are likely aimed to achieve a greater safety of transmission in NMJ of fast motor units and to resist synaptic depression during prolonged repetitive stimulation in NMJ of slow motor units. In fast fibers, a higher quantal content of each evoked ACh release from the nerve terminal, a greater density of ACh receptors, a greater density of voltage-dependent sodium channels in the postsynaptic folds and in the surrounding region contribute to raise the safety factor. In slow fibers, the lower synaptic depression helps to continue to respond to motor neurons during sustained repetitive discharge.

In contrast to most muscle fibers, which have a single NMJ with *en plaque* nerve ending, a number of fibers in EOMs have multiple NMJs along their length with *en grappe* endings. These fibers display slow-tonic contractile properties and contain a unique slow-tonic MyHC (see sects. II*B* and III*C1*). The NMJs of singly innervated fibers express only the adult epsilon subunit, not the fetal gamma subunit of the ACh receptor (AChR), whereas the NMJs of the multi-

ply innervated fibers expressed only the gamma subunit, and not the epsilon subunit (228).

2. Ionic channels and membrane excitability

A) INTRODUCTION. The sarcolemma is the barrier that limits muscle fiber intracellular environment. Sarcolemma permeability to ions and various compounds plays an essential role in the origin of electrical potential and in the metabolic activity related to energy production. In addition, sarcolemma is connected on one side to intracellular cytoskeleton and on the other side to extracellular matrix and represents the way to transmit tension from one fiber to adjacent fibers (547, 767). While this latter aspect of the sarcolemma will be considered below in the section devoted to cytoskeleton (see sect. III*C2*) and the transport of metabolically relevant compounds will be discussed in section III*D*, the present section will deal with permeability to ions which is the basis for muscle fiber excitability and is essential to the cytosol composition homeostasis. As discussed in the following paragraphs, the permeability of the sarcolemma of fast and slow fibers must match at least three distinct requirements related with the firing properties of the motor neurons:

1) Ionic permeability should be such to electrically stabilize the membrane at rest and, at the same time, to allow the membrane electrical activity (action potentials) to follow closely the discharge pattern of the motor neuron.

2) Water permeability should guarantee the constant osmolarity in front of large increases in concentration of some molecules as phosphate, creatine, and lactate during prolonged contractile activity.

3) Substrate permeability should be adequate to allow entry of energy-rich compounds, glucose in the first place, and release of by-products, lactate in the first place, although the role played by lactate may be very different in specific fiber types as described below (see sect. III*D*).

Comparative studies of slow and fast muscle fibers consistently reveal a significant diversity in their electrophysiological properties. The resting membrane potential is more negative in fast than slow fibers in rat (842) and human muscles (670, 673), with potential ranges between −80 and −85 mV in slow fibers and between −90 and −95 mV in fast fibers. The membrane capacitance per unit area is similar in fast and slow fibers: for example, values of 3.82 versus 3.76 μF/cm^2 have been calculated in slow and fast human muscle fibers, respectively (673). In contrast, the membrane resistance per unit area is lower in fast than slow fibers (for example, 358 Ω/cm^2 in fast rat fibers versus 588 Ω/cm^2 in slow fibers), and the difference has been attributed to a greater expression of chloride channels in fast muscle fibers (see below).

B) CHLORIDE CHANNELS. The conductance of muscle fiber membrane at rest is dependent for 70% on chloride ions and only for 30% on potassium ions (102). Cl conductance plays an important role in determining resting potential and stabilizing the membrane, thus regulating excitability. At least two isoforms of chloride channels are expressed in muscles: the isoform ClC1, which is muscle specific, and the ubiquitous isoform ClC2 (626). Mutations in the gene encoding ClC1 lead to myotonia, i.e., muscle hyperexcitability, in humans (426), mice (296), and other animals (65). Studies carried out in rat and mouse muscles show that chloride conductance is higher in fast compared with slow muscle, and this is due to the higher level of expression of ClC1 in fast fibers, but also to the greater inactivation of ClC1 mediated by protein kinase C (PKC) phosphorylation in slow fibers (109, 612). Several conditions that induce a fiber type transition are associated with the expected changes in ClC1 expression (611) and with changes in PKC-mediated inactivation (612).

C) POTASSIUM CHANNELS. In skeletal muscle fibers K outward currents are relatively small (17), and this implies that *1*) K current contributes to resting potential less than Cl current (102), *2*) K current contributes to repolarization less than Na current inactivation, and *3*) the local increase of extracellular K following repetitive stimulation is likely limited. Several types of K channels have been described in mammalian muscle fibers: inward-rectifying, voltage-gated, Ca-activated, and ATP-sensitive K channels. The ATP-sensitive K channels or K-ATP, i.e., the channels opening in response to a reduction of ATP/ADP ratio, probably represent the most abundant type of K channels active in the skeletal muscle fibers (7). The density of K-ATP current is higher in fast-twitch muscles compared with the slow-twitch muscles. The K-ATP channels are hetero-octamers comprising two subunits: the pore-forming subunit, either Kir6.1 or Kir6.2, which belongs to the inward rectifier K channel family, and the regulatory subunit sulfonylurea receptor SUR (SUR1 or SUR2), which belongs to the ATP-binding cassette protein superfamily. The density of K-ATP currents in skeletal muscle fibers might be correlated with the level of Kir6.2 expression, which is higher in flexor digitorum brevis (FDB) and TA muscles than in EDL and soleus (810). SUR2A is the most abundant subunit expressed in all muscles, whereas the SUR2B subunit is expressed at lower levels. A significant expression of SUR1 has also been reported in fast-twitch muscles (810). The physiological role of K-ATP may be related (647) to *1*) a protective action based on sensing ATP levels during fatigue and tetanus as reduced intracellular ATP levels lead to an K current and membrane hyperpolarization, *2*) a regulation of the extracellular K concentration which has an influence on membrane resting potential and excitability as well as on local vasodilation, and *3*) modulation of glucose uptake (542).

Two functionally different Ca-activated K channels (BKCa) have been found in skeletal muscle. The BK of fast muscles, as FDB, is sensitive to calcium and to acetazolamide, whereas the BK of slow muscles, as soleus, is less sensitive to calcium and resistant to acetazolamide (809). During slow-to-fast phenotype transition, the BK channels of soleus acquire properties similar to those of FDB. Finally, potassium delayed outward current differs between fast and slow fibers of the rat: two components (one slow and one fast) are detectable in slow fibers, whereas only the fast component is present in fast fibers (195).

D) SODIUM CHANNELS. Excitability of the membrane is determined by the number of Na channels and by the fraction of them that is not inactivated. Two isoforms of the pore-forming α subunit of the sodium voltage-gated channels, Nav 1.4 and Nav 1.5, also indicated as SKMI and SKMII, are expressed in skeletal muscle (136) in association to the ubiquitous β-1 subunit and exhibit different electrophysiological properties (890). Expression of Nav 1.5 is restricted to immature and denervated muscles (389), while Nav 1.4 is expressed in adult muscles both in slow and fast fibers. Na channel density is two to three times higher in fast than in slow fibers in humans (673) as in other mammals (125, 185). This is consistently supported by evidence obtained in electrophysiological studies as well as in saxitonin binding experiments (317). No difference is detectable in unitary conductance and open probability of single channels from fast and slow fibers (185).

Na current density is 3–10 times higher at the border of the endplate than away from it (62, 672), and this uneven distribution is instrumental to increase efficiency of the neuromuscular transmission. Very high concentrations of channels are present in the membrane in the depths of the folds under the NMJ (125, 223), with the likely goal to amplify the effect of the released transmitter or to reduce the threshold for nerve-evoked action potential (743). The gradient in Na current moving away from the endplate is much lower in slow fibers (673).

Recent work has shown that, in rat muscles, maximal Na current ($I_{Na,max}$) and maximal Na conductance ($g_{Na,max}$) increase from slow to fast fiber types, following the scheme $1 < 2A < 2X < 2B$, and that the curve of voltage dependence of activation is shifted toward positive potentials from type 1 to 2A, 2X, and finally 2B fibers (641) **(FIG. 6)**. Inactivation voltage and time dependence also vary in relation to fiber type. Importantly, inactivation, i.e., the transient decrease of Na conductance which follows a change in membrane potential, has two components: slow inactivation has a time constant in the order of 20–30 s (736), whereas fast inactivation is much faster (time constant in the order of 0.1–1 ms) and occurs at less negative potentials. Comparing fast and slow fibers, fast inactivation acts at potentials less negative in slow ($V_{h1/2} > -65$ mV) than in

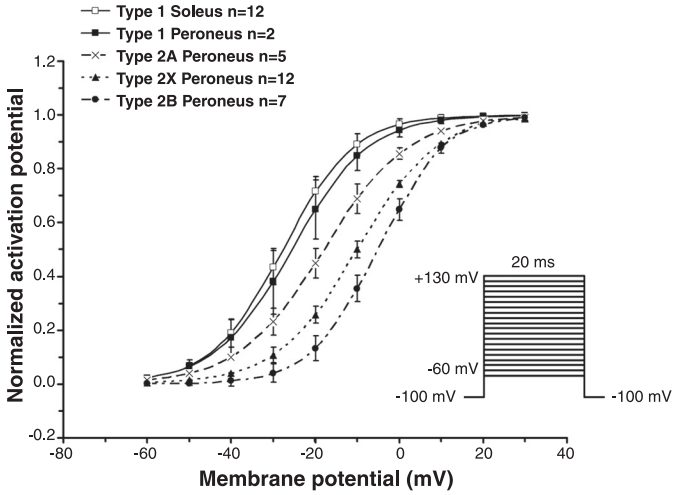


FIGURE 6. Activation curves of voltage-gated sodium channels, in single fibers from fast peroneus longus and slow soleus rat muscles, identified according to their myosin heavy chain isoform content. Curves were obtained by fitting the data with the Boltzmann equation. [Modified from Rannou et al. (641), with permission from John Wiley and Sons.]

fast fibers ($V_{h1/2} < -65$ mV), and the same is true for slow inactivation ($V_{s1/2} \approx -70$ mV in slow and ≈ -95 mV in fast fibers) (185, 671, 673). The molecular basis of such kinetic diversity among fiber types is still uncertain since, as stated above, only one isoform of the pore forming α subunit and one isoform of the auxiliary β subunit are expressed in both slow and fast adult skeletal muscle fibers. The relation between changes in kinetic properties of Nav 1.4 and Nav 1.5 and their interaction with calmodulin (890) points to a possible activity-dependent regulation mechanism.

In conclusion, the greater density of Na channels allows fast fibers to respond following the high discharge rate of the motor neurons and thus generate strong but short-lasting contractile responses. Such fast responses are not required for slow-twitch fibers. Actually, the greater influx of sodium in fast fibers might contribute to their fatiguability as sodium influx can exceed the capacity of the Na^+-K^+-ATPase to extrude it from the cytoplasm and prevent alterations of the intracellular milieau. The diversity in slow inactivation might have an important effect on resistance to fatigue (670). Actually, in fast fibers, the more negative value of $V_{s1/2}$ can lead to loose excitability in the presence of the modest depolarization which could occur during repetitive stimulations. This is avoided in slow fibers as their $V_{s1/2}$ is further from the resting potential. Thus slow inactivation, possibly together with other mechanisms that reduce membrane excitability, may protect fast fibers from prolonged activations at high frequency. The relative resistance of slow fibers to slow inactivation of Na channels combined with the relatively good ability of slow fibers to remove potassium from the extracellular space (for example, greater capillary-to-fiber ratio) may enable slow fibers to be tonically active at low frequency (10–20 Hz) for long periods (670).

E) CALCIUM CHANNELS. L-type voltage-gated calcium channels (also called DHPR or L-type calcium channels, LTCC) are composed of different pore-forming α1 subunit isoforms named Cav1.1 (α1S), Cav1.2 (α1C), Cav1.3 (α1D), and Cav1.4 (α1F) associated with auxiliary subunits, α2, β, γ, δ. The muscle specific isoform of the pore-forming subunit α 1s or Cav1.1 plays the role of voltage sensor and triggers the opening of the ryanodine receptor (RyR) channels in the terminal cisternae of the SR (783), whereas the isoforms α1C and α1D are widely present in cardiomyocytes where they contribute to E-C coupling. In skeletal muscle fibers, the contractile response does not disappear even when extracellular calcium is removed with EGTA (34). Accordingly, the inward calcium current through the DHPR has been calculated to contribute for <5% to the intracellular calcium transient, on the basis of experiments showing that removal of extracellular calcium (191) or addition of calcium channel blockers (191, 274) do not significantly decrease the contractile response in skeletal muscle cells. There is, however, evidence that in soleus and diaphragm the cardiac isoform of DHPR with the subunit Cav1.2 is present at mRNA and protein level, while it is virtually absent in fast muscles (238, 595). This observation points to a fiber-type-dependent diversity in the contribution given by calcium influx to the calcium transient. It has been shown that calcium removal from the bathing medium causes a decrease in force output in a large fraction of fibers from FDB from aged mice, but not from young mice (591). This suggests that calcium influx becomes relevant to the contractile response during aging. Importantly, the density of DHPR per volume unit is three- to fivefold greater in fast-twitch than in slow-twitch fibers and, in accordance, charge movement is much greater in fast than in slow fibers, as will be discussed in more detail below in relation to the role in E-C coupling.

At the end of fetal life, two types of calcium currents can be recorded in myofibers: L-type, high-voltage-activated (HVA) calcium currents and T-type, low-voltage-activated (LVA) calcium currents (63, 730, 769). In mouse skeletal muscles, T-type calcium current shows a peak (3 pA/pF) at the age of 16 days pc, and there is evidence that the α1H subunit generates functional T-type calcium channels in developing skeletal muscle fibers and that T-type channels are involved in the early stages of muscle differentiation (71, 75).

More recently, the issue of a calcium entry pathway activated by repetitive or long-lasting depolarization has been reinvestigated (147), and it has been shown that skeletal muscle DHPR provides under specific conditions a mechanism for calcium permeation, indicated as ECCE (excitation-coupled calcium entry). The precise molecular identification is still controversial (see Ref. 186), and no data are available to support fiber-type-dependent variations of ECCE.

In skeletal muscle fibers, as in most cells, depletion of calcium from intracellular calcium stores causes calcium entry across the plasma membrane, a process generally termed capacitative calcium entry or SOCE (store-operated calcium entry) (586, 628). The presence of SOCE in skeletal muscle fibers has been demonstrated (449, 818). Recent data identify the molecular basis of SOCE in the interaction between the endoplasmic/sarcoplasmic reticulum calcium sensor protein STIM1 and the transmembrane protein Orai (see Ref. 186). No evidence of differences in SOCE among fiber types is presently available.

In addition to voltage-gated channels and store-operated channels, calcium enters muscle fibers via stretch-activated channels (SAC) (323), stretch-inactivated channels (SIC), and leak channels (349). Calcium entry at rest through the sarcolemma is greater in fibers from the slow soleus muscle compared with the fast EDL (229). The difference has been attributed to a greater expression or activity of calcium-permeable SAC and contributes to determine higher resting cytosolic calcium concentration in slow than in fast fibers (see sect. III*B1*). The slow-to-fast transition induced by hindlimb suspension is accompanied by a reduction of calcium permeability and by a decrease of resting cytosolic calcium (229).

F) SODIUM-POTASSIUM PUMP. The Na-K pump is a tetramer, composed by two α subunits and two β subunits. In rat and mouse, the β1 subunit is more abundant in slow and fast oxidative fibers and the β2 subunit in fast fibers (363, 515a). The β2 subunit is not detected in human skeletal muscle (364). The Na-K pump controls changes of intracellular sodium and potassium concentration during repetitive electrical activity. Actually, any action potential is associated with Na influx and K efflux. The measurements of the density of the Na-K pump based on oubain binding indicates that, at least in rat muscles, fast fibers contain ~20% more pumps than slow fibers (214). This would compensate the Na influx during action potentials, which is expected to be greater in fast than in slow fibers in relation to the greater density of Na channels (see above). The muscle electrical activity per se causes an activation of the Na-K pump, and the increase of intracellular Na concentration which may accompany electrical muscle activity further enhances pump-mediated transport rates. The response of Na-K pump to electrical activity and to increase of intracellular Na is three times greater in slow fibers. Thus, during repetitive activity, Na-K pump is more active in slow than in fast fibers (214).

G) CALCIUM PUMP. Plasma membrane calcium/calmodulin-dependent ATPases (PMCAs) are present in all cells, including muscle fibers. PMCA isoforms 1, 4, and 3 are expressed in skeletal muscles of the rat (289), and PMCA isoforms 1 and 4 have been demonstrated also in human skeletal muscles (759). The plasma membrane calcium pumps contrib-

ute to the export of calcium with high affinity and low capacity and are regulated by calmodulin- and protein kinase A (PKA)-dependent phosphorylation (768). No indications of a differential distribution or role of PMCAs in slow and fast fibers are available.

H) SODIUM/CALCIUM EXCHANGER (NCX) AND SODIUM/HYDROGEN EXCHANGER (NHE). Three mammalian isoforms of the NCX protein, products of three different genes, have been cloned and appear to have very similar properties: NCX1, the predominant isoform in cardiac muscle, is also expressed in skeletal muscles, where it coexists with the other two isoforms NCX2 and NCX3. NCX is mainly localized at the t tubule and contributes to calcium extrusion after contraction (151, 679). Actually, due to its lower affinity (K_m ~3 μM) and high capacity, NCX cannot have a significant impact on resting cytosolic calcium concentration (~50 nM), but can contribute to export calcium during caffeine or tert-nutyl-hydroquinone (TBQ)-induced contracture (43). The genetic ablation of NCX3 has been shown to cause fiber necrosis and functional impairment in murine skeletal muscles (749). There are indications that its role may be more important in slow-twitch and -tonic fibers than in fast fibers (77). In agreement with this view, contraction can be induced by external sodium removal without depolarizing the surface membrane in slow fibers but not in fast fiber (475). NHE is present in skeletal muscles with the ubiquitous isoform NHE1. There is evidence that, in rat muscles, expression of NHE1 is higher in fast than in slow fibers and can be modified during fiber type transitions (385).

I) CONCLUSION. To establish a full consistency between motor neuron and muscle fibers, a first essential condition is that muscle fiber electrophysiological properties are compatible with the discharge pattern of the motor neuron. For example, in rodent fast motor unit, the discharge rate can reach peaks above 100 s^{-1}, and this implies repolarization and refractory period must be complete within 10 ms. This is not a requirement in a slow motor unit where such high discharge rates are never reached. In a slow motor unit, however, fibers need to be able to compensate the effects of long-lasting stimulations such as accumulation of ions which might alter resting membrane potential and lead to ion channel inactivation. Compared with slow fibers, the sarcolemma of fast fibers shows higher ionic conductance at rest (Cl and K, also in relation to a more negative membrane potential) and during activity (Na). Also Ca channels (DHPR) are more abundant in fast fibers, and this is related to a more efficient coupling between membrane depolarization and calcium release (see next section). Ca entry, however, is greater in slow compared with fast fibers both at rest and during depolarization. Among the active ion transsarcolemmal transporter, Na/K pump is more active in fast than slow fibers, but such ratio can be inverted during prolonged activity due to the more effective activation of the pump in slow fibers. Among the sarcolemma exchangers, available evidence indicates that NCX is more important in slow fibers, while NHE is more abundant in fast fibers, possibly in relation to proton generation during contractile activity.

B. Excitation-Contraction Coupling: Ca^{2+} Shuttling Between Sarcoplasmic Reticulum and Myofibrils

1. Introduction

Calcium represents a powerful intracellular messenger in skeletal muscle fibers, being able not only to trigger contractions via binding to troponin, but also to activate protein phosphorylation or dephosphorylation via binding to calmoldulin and to activate proteolysis via calcium-dependent proteases. On this ground, it is easy to understand that the concentration of intracellular cytosolic free calcium concentration needs to be controlled precisely. This task is accomplished by the SR through calcium release and uptake systems with important contributions of cytoplasmic calcium buffers, mitochondria and sarcolemma. Skeletal muscle fibers show a pronounced specialization in the control of intracellular calcium, as different types of fibers are characterized by distinct levels of resting calcium concentration and specific kinetics of the calcium transients that accompany action potentials.

Importantly, calcium transient kinetics have a direct impact on the dynamic properties of the muscle fibers. The faster time course of calcium transients contributes, together with the faster cross-bridge kinetics, to determine the shorter time to peak and time to half relaxation of the isometric twitch of fast fibers (see Ref. 156 for a classical review). While tetanic tension sees its major determinant in the cross-bridge density and in the fraction of attached force-generating cross-bridges, the amplitude of the twitch is determined by the amplitude of the calcium transient and by the calcium sensitivity of the myofibrils. Thus the greater values of the twitch-to-tetanus ratio in fast compared with slow fibers likely find their explanation in the greater size of the calcium transient in fast fibers. The calcium sensitivity of the myofibrils also plays an important role in determining force development in a twitch: for example, the changes of twitch amplitude with repetitive stimulation (posttetanic potentiation, see below) are attributed to changes in myofibrillar calcium sensitivity, caused by myosin phosphorylation. The contribution of various processes in controlling resting calcium concentration and amplitude and kinetics of calcium transients will be discussed here below.

2. Resting calcium and calcium transients

The cytosolic free calcium concentration at rest is higher in slow than fast muscle fibers (132, 133, 229, 230, 250). In

rodent muscles, values close to 50–60 nM have been reported in slow fibers versus 30 nM in fast fibers. The influence of resting calcium concentration on the determination and maintenance of fiber phenotype has been first hypothesized by Sreter (757), and experiments on rabbit myotube cultures (443) have confirmed the hypothesis showing that the increase of resting calcium concentration induced by a calcium ionophore is followed by a fast-to-slow transformation (see sect. VII).

The cytosolic calcium transient following a single action potential has different features in slow and fast skeletal muscle fibers (61, 133, 212, 241), with further diversity existing between different types of fast fibers (124) **(FIG. 7)**. In spite of the discrepancy of about one order of magnitude between the calculated peak amplitudes of the cytosolic calcium transient likely attributable to the calcium indicator used (61), there is substantial agreement that calcium transients reach a higher peak in fast than in slow fibers: for example, 19 versus 9 μM in rat fibers analyzed with furaptra (61) or 2.4 versus 1.3 μM in mouse fibers with fura 2 (486). The rate of decline of the calcium transient is approximately two times faster in fast than in slow fibers, with rate constants ranging from 63 versus 24 s^{-1} in rat fibers loaded with aequorin (212) to 40 versus 23 s^{-1} in rat fibers loaded with fura (133), 92 versus 45 s^{-1} in mouse fibers loaded with fura 2 (486) and 213 versus 106 s^{-1} in mouse fibers loaded with furaptra (61) **(FIG. 7, *A* AND *B*)**. The kinetics of the calcium transients, determined using mag-fluo 4-AM as calcium indicator, have been studied in murine single muscle fibers classified as slow or fast (2A, 2X, or 2B) with myosin isoform electrophoresis (124). The results have shown that the kinetic parameters of the calcium transients form a continuum where two main clusters of values can be detected, corresponding to slow and fast 2A fibers (decay rate 9–12 s^{-1}) and to fast 2X and 2B (decay rate of 40–50 s^{-1}), respectively **(FIG. 7*C*)**.

3. SR volume and surface area

Given the central role of the SR in E-C coupling, it is not surprising that the relative extension of the SR is a major determinant of the contraction and relaxation kinetics by controlling both calcium release, via the SR terminal cister-

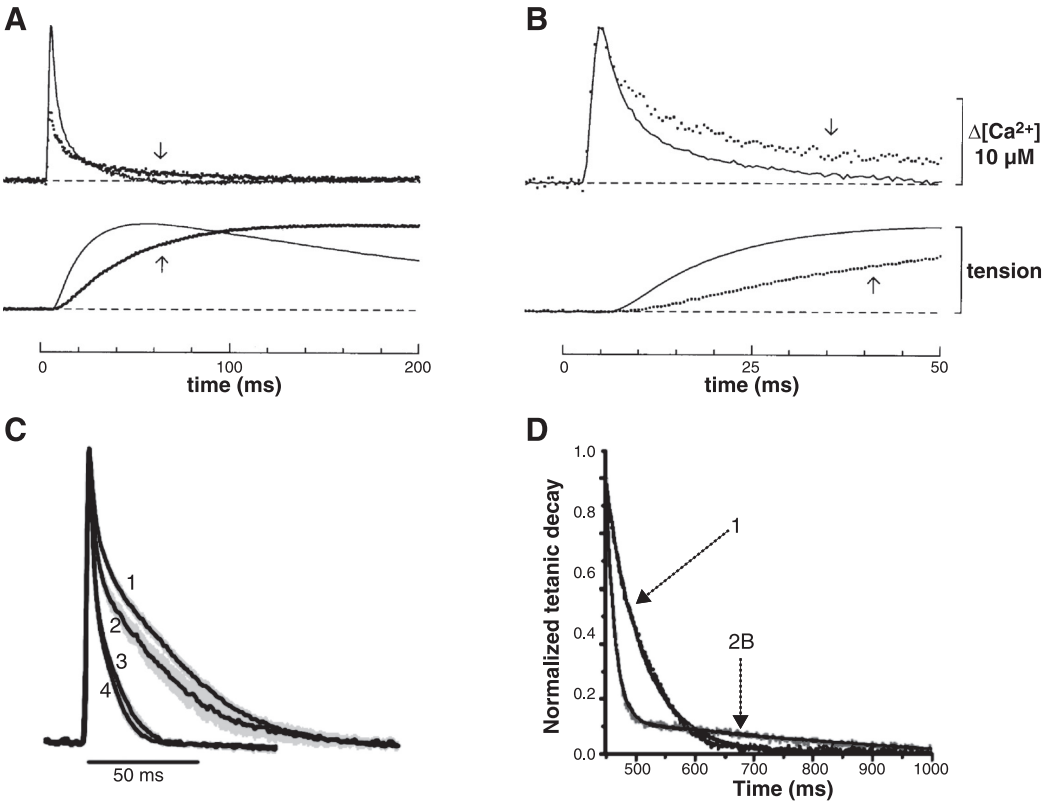


FIGURE 7. Diversity in cytosolic Ca^{2+} transients in fast and slow muscle fibers. *A*: Ca^{2+} transients (*top panel*) and tension development (*bottom panel*) in rat soleus (arrows) and EDL fibers. Note the slower kinetics of the slow fibers. *B*: same as *A*, with amplified time scale. [From Baylor and Hollingworth (61), with permission from John Wiley and Sons.] *C*: Ca^{2+} transients in single murine muscle fibers identified according to their myosin heavy chain composition (1, slow; 2, 2A; 3, 2X; 4, 2B fibers). Note the similarity between type 1 and 2A fibers, and between 2X and 2B fibers. [From Calderon et al. (124), with permission from John Wiley and Sons.] *D*: decay phase of Ca^{2+} transients at the end of tetanic stimulation in murine type 1 and 2B fibers. Note the typical tail in type 2B fibers attributed to the presence of parvalbumin. [Modified from Calderon et al. (124), with permission from John Wiley and Sons.]

nae, and calcium uptake, via the SR longitudinal tubules. The greater development of the SR in fast compared with slow muscle fibers was first detected by electron microscopy in rat (711) and mouse (496) muscles, and it was noted that the SR is richly developed in both mitochondria-poor (now identified as type 2B) and mitochondria-rich (now identified as type 2A and 2X) fast muscle fibers (711). The differences were confirmed by morphometric analyses in different mammalian species (202, 496). An inverse relation between fraction volume of terminal cisternae and time to peak tension has been obtained in a comparative analysis of rat motor units (448); thus the shorter time to peak typical of fast fibers is associated with a larger size of SR terminal cisternae.

4. Calcium release from SR

The calcium release channel/RyR represents the most effective mechanism to raise cytosolic calcium concentration in a very short time, by virtue of its direct connection to the voltage sensor DHPR in the specialized triad architecture. The direct coupling between DHPR and RyR is a guarantee that action potential and t-tubule depolarization are immediately followed by a large and fast release of calcium from the SR, leading to a calcium transient sufficient to trigger the contractile response of the myofibrils. Although most of the differences in the kinetics of the time course of the calcium transients evoked by action potential are likely dependent on the mechanisms of cytosolic calcium buffering and removal (see below), a diversity in calcium release between slow and fast fibers also finds supportive evidence. For example, Ca release from SR in slow fibers is less inhibited by cytosolic Mg (761), and this might contribute to a lower sensitivity to fatigue as an increase of intracellular Mg concentrations occur during advanced stages of fatigue. The response to caffeine, which offers a convenient way to bypass voltage-controlled calcium release and activate directly calcium release from RyR, is greater in slow than in fast muscles (369). The greater responsiveness to caffeine of slow compared with fast muscle fibers has been confirmed on glycerol-treated single fibers (229, 689). The measurement of cytosolic free calcium concentration with the photoprotein aequorin shows that the first detectable increases in intracellular calcium concentration occur in the presence of 0.2 mM caffeine in soleus and 1 mM in EDL (241). In fast fibers, but not in slow fibers, the threshold for caffeine response becomes lower when SR is maximally loaded with calcium and the ratio between the thresholds at high and low SR calcium content has been used as a tool to classify single muscle fibers with respect to SR characteristics (88). The determination of the threshold for caffeine contracture in vitro is an accepted test of malignant hyperthermia (MH) diagnosis in human muscles (205): in slow fibers the threshold is 5.9 $\pm$ 1.8 mM, whereas in fast fibers it is 10.4 $\pm$ 2.6 mM (4) and is considerably decreased (below to 2 mM) in muscles from MH subjects. Finally, neonatal muscles are more responsive to caffeine than adult muscles (72).

The reasons for the diversity in calcium release properties are not completely clear. The SR calcium release channel is present in mammalian skeletal muscle fibers with distinct isoforms coded by two genes: RyR1 is the dominant one, whereas expression of RyR3, after a wide distribution during the postnatal period, is restricted to a few muscles, and the cardiac isoform RyR2 is never expressed (72, 752). In adult rodents (mouse and rat) and rabbits, RyR3 is expressed in a fraction of fibers in the diaphragm and in cephalic muscles, mainly expressing slow myosin (166, 222, 663). The expression of RyR3 in slow fibers of diaphragm and head muscles, but not in slow fibers of limb and trunk muscles, differs from the expression of other SR proteins (for example, calsequestrin or SERCA) that is more strictly fiber-type dependent, irrespective of the muscle considered. This suggests that several control mechanisms, developmental, muscle-specific, and fiber type-specific, operate independently in regulating RyR3 expression. Thus the difference in calcium release between fiber types cannot be attributed to differential expression of RyR1 and RyR3, although this may contribute in some cases. In particular, the presence of RyR3 is relevant for determining the features of the calcium release during postnatal development (72). In developing muscle fibers, which lack a well-developed t-tubule system (496, 711), the expression of RyR3 channels may help to accelerate the spread of calcium release from the periphery to the center of myotubes or myofibers following plasma membrane depolarization. A relation between RyR3 expression and resting cytosolic calcium levels has been demonstrated in 1B5 myotubes in culture and in murine fibers during early postnatal development (596). Since the expression of specific RyR isoforms does not seem to be directly relevant, the fiber type-related diversity in calcium release might be determined by other factors, such as *1*) posttranslational modifications, as RyR is target for phosphorylation by PKA (668) and is affected by oxidative state (370); *2*) interactions with other proteins including DHPR, calmodulin, FKBP-12, and calsequestrin; and *3*) amount of calcium available in the terminal cisternae, as SR is maximally loaded in slow but not in fast fibers (see below). The DHPR, i.e., the L-type voltage-gated calcium channel which acts as a voltage sensor during E-C coupling, has been considered in the previous section (see sect. III*A*) and might contribute to the slow versus fast diversity.

It is generally accepted that the amount of calcium released by a single action potential is greater in fast than in slow fibers: for example, 350 μmol/l fiber volume in murine fast fibers versus 120 μmol in slow fibers (61). Such quantitative difference can be related to the abundance of the RyR channels, which is more than twice in fast than in slow fibers (227) and the different proportion of RyR channels coupled with DHPR channels. Actually, in connection with the lower density of DHPR in slow compared with fast fibers, the ratio DHPR to RyR is lower in slow than in fast fibers

(457, 521). This implies that the proportion of RyR directly controlled by DHPR through the charge movement is lower in slow than in fast fibers (61, 183). Thus calcium-induced calcium release might play a greater role in slow than in fast fibers, and E-C coupling in slow fibers might therefore be reminiscent of that typical of cardiac muscle. An indirect support to this view is given by the presence of cardiac type L calcium channels in soleus and diaphragm but not in EDL (595). A decrease of the ratio between DHPR and RyR has been observed during aging (648), and this would lead to fewer RyRs available for calcium release and a greater percentage of uncoupled RyRs, resulting in a reduction in voltage-activated calcium release from the SR.

Interestingly, despite the large diversity in the amount of calcium released by an action potential, the rate of decline of the amount released in repeated action potentials is similar in fast and slow fibers, thus suggesting that the inactivation kinetics of the RyR channels is not different (61). For example, the fifth depolarization in a high-frequency train of action potentials will release only the amount of calcium corresponding to one-fifth of the calcium released by the first action potential in a slow fiber and to one-sixth in a fast fiber (61).

RyR channel activity is also influenced by intraluminal proteins. Calsequestrin (CASQ) is the most important calcium binding protein inside SR and is mainly located in the terminal cysternae in close proximity to RyR. In skeletal muscles, calsequestrin exists in two isoforms, CASQ1 and CASQ2. Both isoforms can be found in slow fibers, whereas only CASQ1 is expressed in fast fibers (177, 680). Calsequestrin content is greater in fast than in slow fibers (466) and a quantitative analysis on single murine fibers (554) points to a concentration of 36 μM in fast fibers (only CASQ1) and 11 μM in slow fibers (CASQ1 and CASQ2). CASQ plays two important roles: *1*) calcium buffer since, due to the large number of acidic residues, each CASQ1 molecule binds up 80 calcium ions and each CSAQ2 up to 60 calcium ions, and *2*) modulator of calcium release due to its interaction with RyR (64).

Together with junctin and triadin, CASQ and RyR form a quaternary complex whereby CASQ helps to accumulate a high amount of calcium near the luminal side of the RyR channels through a protein bridge formed by junctin and triadin (751). Calmodulin and FKBP-12 bind directly to RyR and contribute to modulate its calcium channel activity (36, 784). Other proteins are involved in the architecture of junctional complexes between surface membrane/t-tubule system and terminal cisternae of the SR, namely, Mg29 and junctophilin 2. These latter proteins play not only a structural role but are also relevant for calcium control, as suggested by the changes in resistance to fatigue detectable in muscles of mice carrying null mutations (339). No evidence for fiber type-specific isoforms or for differential contribution of these proteins to the E-C coupling and calcium release in slow and fast fibers is presently available.

5. Cytosolic calcium buffers

The faster decline of the calcium transient in fast compared with slow fibers is determined by the activity of *1*) calcium removal mechanisms (present in SR membranes, mitochondria, and sarcolemma) and *2*) binding to cytoplasmic buffers. The major cytosolic calcium buffers in muscle are parvalbumin, C-troponin, and calmodulin and, as discussed here below, they provide a more effective buffering action in fast than in slow fibers.

Parvalbumin is a cytosolic calcium buffer that is expressed in fast fibers at concentrations of ~1 mM, whereas it is virtually absent in slow fibers (126, 137, 301). Immunocytochemical analyses showed that parvalbumin is specifically expressed in type 2B and 2X fibers, not in type 2A and slow type 1 fibers; interestingly, its content correlates with the type of myosin but not with SDH levels (243). The important role of parvalbumin in regulating the speed of relaxation is supported by the finding that muscles of parvalbumin-null mice show a marked prolongation of the calcium transient (720). Taking into account the fibre-type specific distribution, parvalbumin will contribute to quickly lower cytosolic calcium concentration after the increase caused by a release from SR only in fast muscle fibers. Parvalbumin-bound calcium will be returned to the SR slowly after the end of the mechanical response, giving origin to the tail of the calcium transient (420). Actually, such tail is detectable only in fast muscle fibers **(FIG. 7*D*)** (124).

Troponin C (TnC), which triggers contraction on binding calcium ions, is present in the myofibrils at the concentration of ~60 μM with two different isoforms; as reported below (see sect. III*B8*), TnC-fast expressed in fast fibers has four calcium-binding sites (two high affinity and two low affinity), whereas TnC-cardiac-slow expressed in slow fibers lacks one of the low-affinity sites. Thus the contribution of TnC to cytoplasmic buffer capacity for calcium is greater in fast compared with slow fibers (240 versus 180 μM).

Calmodulin is more abundant in fast than slow fibers, although the difference (about twofold) is less than that reported for parvalbumin (126). Calmodulin contributes, upon calcium binding to the regulation of contractile function via myosin light chain (MLC) kinase. This pathway is typical of fast fibers because of the higher expression of MLC kinase in fast fibers (97). Myosin phosphorylation via MLC kinase increases force development at submaximal calcium concentration, which corresponds to a shift of the force-pCa curve towards lower calcium concentrations (see sect. III*B8*). Taking into account that fast fibers are more prone to fatigue than slow fibers, the force enhancement produced by myosin phosphorylation after repeated stimulation

might represent a useful mechanism to counteract the quick reduction in force caused by fatigue. Calmodulin also contributes to the activation of calcium-dependent signaling pathways involved in muscle gene regulation (see sect. VII).

6. Calcium uptake by SR

Longitudinal tubules, which contain the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps, form the compartment of the SR specialized in taking up calcium from the cytosol (322). Calcium uptake by the SR has been analyzed using membrane fractions enriched in SR vesicles obtained by differential centrifugation of muscle homogenates. Calcium uptake is faster in fast than in slow fibers (522, 688, 756), and this is determined partly by the greater SR volume or SR surface area and partly by the presence of specific transport proteins.

The active calcium transport mechanism into SR is based on SERCA pumps, which exist in distinct isoforms coded by three different genes, each of them giving origin to variants by alternative splicing (see Ref. 597). The SERCA1 gene is exclusively expressed in fast skeletal muscle fibers with two developmentally regulated variants generated by alternative splicing: an adult isoform or SERCA1a and a neonatal isoform or SERCA1b (514). The SERCA2 gene is expressed in slow skeletal muscle, cardiac muscle, smooth muscle, and nonmuscle tissues. Among the splicing variants, the isoform SERCA2a is specific for muscle cells, while SERCA2b is a "housekeeping" isoform, ubiquitously expressed in all cells (507). When studied in muscle fibers (512), SERCA isoforms show a pronounced diversity in calcium leakage and ADP sensitivity, which may impair the refilling of the SR and become relevant during muscle fatigue when ADP concentration rises. Slow fibers show a smaller ADP-induced reduction in SR calcium-pump rate and a lower increase in SR calcium-leak rate, thus contributing to the greater resistance to low-frequency fatigue typical of slow fibers (511) (see below). However, when expressed in COS cells, SERCA1a and SERCA2a show identical kinetics and transport rate (508); thus the diversity in uptake rate between slow and fast fibers might arise from other factors: *1*) the density of the pumps, *2*) the presence of regulatory subunits, or *3*) the free calcium gradient.

1) The density of the pump is much greater (5- to 7-fold) in fast than in slow fibers (rabbit, Ref. 467; human, Ref. 213; rat, Ref. 881). Among fast fibers, the density is higher in 2B than in 2A fibers both in rat and rabbit muscles (440). This is likely due to the fact that SR is more developed in fast than in slow fibers in terms of fractional volume and surface area (see above), but may also reflect a greater concentration of the pump per unit area of the SR (190).

2) Calcium uptake in SR is affected by extraluminal regulatory proteins bound to the pumps and by intraluminal proteins. In large mammals, but not in rodents, SERCA2a activity is modulated by the phosphorylation state of the regulatory subunit phospholamban (409, 465, 778, 821). Phospholamban likely plays an inhibitory role in slow skeletal muscle fibers as it does in cardiac muscle. The regulatory role of sarcolipin, which is also able to reduce calcium pump activity and is associated with SERCA1 in fast fibers of many species including rodents, is less known (821).

3) An essential contribution to calcium storage in SR is given by intraluminal calcium-binding proteins, which allow calcium to be stored keeping the free calcium concentration low. As mentioned above, CASQ is the most important calcium binding protein inside SR. Due to the larger SR volume (202) and the greater abundance of CASQ (466, 554), the SR of fast fibers is only filled with calcium for 35% of its capacity at resting concentrations of cytosolic free calcium: thus any increase of cytosolic free calcium will be followed by a fast and effective resequestration into the SR with little increase of intrareticular free calcium concentration (128, 242). In contrast, in slow fibers, the SR is completely saturated with calcium at resting concentrations of cytosolic free calcium (242); thus during the reuptake phase of a cytosolic calcium transient the saturation of SR will slow down the removal of calcium from cytosol by back-inhibition of the Ca^{2+} pump (367).

7. Contribution of sarcolemma and mitochondria to calcium homeostasis

Trans-sarcolemmal influx and efflux of calcium occur in adult mammalian skeletal muscle fibers, although to a very limited extent. As discussed in section III*A2*, in skeletal muscle fibers, the inward calcium current through the DHPR is unlikely to contribute more than 5% to the intracellular calcium transient, with a possible greater contribution in slow compared with fast muscles and in aged compared with young muscles. In addition to voltage-gated channels, calcium might enter muscle cells via SOCE (449), ECCE (147), SAC (323), SIC (772), and leak channels (349). Little is known about possible fiber type-related diversity, with the exception of some data suggesting a possible greater activity of SAC in slow compared with fast fibers (229). Among active calcium extrusion mechanisms, adult muscle fibers also express plasma membrane calcium pumps or PMCA and NCX, whose functional relevance has been discussed in section III*A2*.

In addition to their important role as energy suppliers, mitochondria may play an important role in contributing to modulate the cytosolic free calcium. In skeletal muscle fibers, mitochondria are either clustered beneath the sarcolemma (subsarcolemmal mitochondria) or embedded among the myofibrils (intermyofibrillar mitochondria) (575). The latter are mainly located in transversal rows in close proximity to the calcium release units, at the I-A band edge, with an additional location in longitudinal rows in oxidative (slow or fast 2A) fibers. The main mechanism of

calcium entry into mitochondria is the mitochondrial calcium uniporter (MCU), a calcium channel (185a), whereas NCX takes care of extrusion. In spite of the low affinity of the uniporter (1–10 μM), due to specific localization of mitochondria close to the SR calcium release channels and the formation of microdomains with high calcium concentration (654), mitochondria can take up calcium once released from RyR (226, 662). The use of specific probes for mitochondrial calcium (667) has unambiguously demonstrated that, in mammalian skeletal muscles, mitochondria take up calcium during contraction and rapidly release it during relaxation. The mitochondrial calcium increase is delayed by a few milliseconds compared with the cytosolic calcium increase and occurs both during single twitches and tetanic contractions. Calcium entry is followed by activation of three dehydrogenases in the mitochondrial matrix, and this results in increases in mitochondrial NADH/NAD ratios, ATP levels, and increased substrate uptake by mitochondria (265, 700). Calcium is, thus, an important signal for mitochondrial function, but at the same time mitochondria may contribute to cellular calcium homeostasis either playing the role of calcium buffer or regulating calcium release via control of reactive oxygen species (ROS) production. Actually, a retrograde signal from the mitochondria to the adjacent SR calcium release units is believed to be relevant to the suppression of the local calcium release events, termed "sparks," in adult mammalian skeletal muscle (370; for a review, see Ref. 187). Although calcium uptake by mitochondria has been demonstrated both in slow soleus and in fast EDL fibers of the mouse (108), there is some evidence of a diversity between slow and fast fibers in the role played by mitochondria in calcium homeostasis. Pharmacological inhibition of mitochondrial calcium uptake has shown that the active role of mitochondria in promoting relaxation is negligible in fast fibers, but significant in mitochondria-rich slow fibers (268). A study on parvalbumin knockout mice (145) has reported that in fast fibers the lack of parvalbumin induces mitochondrial biogenesis leading to a twofold increase in mitochondrial volume. Accordingly, knockout of the major SR calcium binding protein CASQ leads to an increase in mitochondrial volume (584). These findings are suggestive of a role of mitochondria as a compensatory calcium sink. Proteomic analysis provides evidence that the mitochondrial protein complement differs between slow and fast fibers, suggesting that mitochondria can specialize not only for energy metabolism but also calcium regulation (632).

8. Response of myofibrils to calcium

A) MECHANISM OF MYOFIBRILLAR ACTIVATION. The reversible binding of calcium ions to TnC represents not only one calcium buffering mechanism, but also the way through which the increase of cytosolic calcium (calcium transients) triggers the contraction. Actually TnC, together with troponin T (TnT) and troponin I (TnI) and tropomyosin (TM), forms a regulatory unit that controls seven actin molecules along the thin filament. In the absence of calcium, TnI and TM keep low the availability of actin binding site for myosin (blocked state) with the exception of a few sites where weak electrostatic interactions are allowed. The availability of the actin molecule to form strong binding cross-bridges with myosin is increased upon binding of TnC to calcium. Two key steps are involved as calcium binding to TnC results in *1*) an increased affinity of TnC for TnI which releases its inhibitory control on actin and *2*) a partial release of the binding of TnT on TM to allow a TM rotational movement on the actin filament surface by ~10°. In such conditions, actin molecules become partially available to the formation of strong hydrophobic myosin binding (closed state), and such binding drives a further movement (~25°) of tropomyosin on the filament surface which makes all binding sites on actin open to myosin (open state) (535). Whereas the regulatory mechanism itself has been the subject of a specific review (276), the focus of the present review is restricted on the structural and functional diversity among muscle fiber types. Such diversity can arise from the transcriptional regulation of the six molecular players (three Tn subunits, tropomyosin, actin and myosin heavy chain) and their possible posttranslational modifications (e.g., phosphorylation). The structural and functional diversity has an impact on the amount of force developed at submaximal activations and on the rate of tension development during contraction and decline during relaxation.

B) ISOFORMS OF TROPOMYOSIN AND TROPONIN SUBUNITS. All components of the regulatory complex exist in distinct isoforms expressed in skeletal muscle fibers (for a review, see Ref. 713). Briefly, TnC is coded by two genes, TnC-cardiac/slow expressed in slow fibers and TnC-fast expressed in fast fibers. The main difference between the two isoforms is the number of the low-affinity calcium binding sites that are relevant for regulation: whereas TnC-fast has two high-affinity and two low-affinity calcium binding sites, TnC-cardiac/slow has only one low-affinity site in addition to two high-affinity sites. TnI also exists in two isoforms, TnI-slow and TnI-fast, expressed, respectively, in slow and fast fibers.

For both TnC and TnI there is a very tight coexpression rule with MyHC isoforms and thus with fiber type. For example, in a sample of 390 fibers from rat muscles studied individually, slow isoforms of TnC and TnI were associated without exception with slow MyHC, whereas all fast MyHC isoforms were coexpressed with fast isoforms of the two troponin subunits (107). A similar tight coupling has been demonstrated in human muscle fibers (687). The association rule is, however, less binding during fiber type transitions and muscle regeneration (208).

TnT and tropomyosin exhibit much more complex combinations of isoforms. Two genes expressed in skeletal muscles code for TnT subunit, and each gene gives origin to a

number of isoforms via alternative splicing. The TnT-fast gene can potentially produce up to 64 variants due to the alternative splicing of five exons at the 5' end and one exon at the 3′ end. However, only a limited number of splicing variants actually occur in mammalian muscles: for example six in fast rabbit muscles (EDL) and four to five in rat muscles (104, 127). These isoforms are generally identified with numbers increasing with decreasing molecular weight: TnT1f, TnT2f, TnT3f, TnT4f, etc. Preferential associations between TnT1f and MyHC-2X, TnT3f and MyHC-2A, and TnT4f and MyHC-2B have been reported in rat single fibers (127, 252). The presence of four major variants of TnT fast has been demonstrated also in human limb skeletal muscles (26, 678). The TnT-slow gene can also produce some variants by alternative splicing. In rat, rabbit, and human muscles, two isoforms (TnT1s and TnT2s) have been identified in slow fibers (249, 691, 716). Three slow splicing variants (sTnT1, sTnT2, and sTnT3) were described in mouse muscles, and a new variant, with a molecular weight slightly higher than that of sTnT3, was found in rat muscles (414). Other splicing variants are detectable in developing skeletal muscles when also cardiac TnT isoform is expressed (681). Finally, further heterogeneity of TnT isoforms is created by phosphorylation (326).

Tropomyosin is a rod-shaped coiled-coil dimer that forms a head-to-tail polymer along the length of an actin filament. The dimers can be either hetero- or homodimers. Three genes coding for tropomyosins are expressed in skeletal muscles: *TPM1* (also called α) which codes TM-α-fast, *TPM2* (or β) which codes for TM-β, and *TPM3* (or γ) which codes for TM-α-slow (see Refs. 303, 304). Coordinated expression of TM isoforms with MyHC isoforms has been reported in the rat (179), where TM-α-slow is expressed in slow fibers, TM-α-fast in fast 2B fibers, whereas all three isoforms seem to be present in intermediate fast fibers (2A and 2X). In human muscles, slow fibers express TM-α-slow and TM-β, whereas fast fibers generally express TM-α-fast along with TM-β (93). Recently, the association between MyHC slow and TM-α-slow has been confirmed also in bovine muscles (572). Tropomyosin is also the target of posttranslation modification, such as phosphorylation, which can further increase heterogeneity.

C) TENSION-PCA CURVE. The most popular and simple way to express the response of the myofibrillar apparatus to variations of calcium concentration is the relation between tension developed and free calcium concentration (in logarithmic form pCa) indicated as tension-pCa (T-pCa) relationship **(FIG. 8*A*)**. The T-pCa experimental data are interpolated with the Hill equation: $P/P_o = 1/[1 + 10^{n(pK - pCa)}]$, where P/P_o is the normalized tension, n is the Hill coefficient, and K is the apparent dissociation constant ($pK = -\log K = pCa_{50}$, where pCa_{50} is pCa necessary to develop 50% of the P_o). The value of pCa_{50} or pK is an index of myofibrillar affinity for calcium, whereas n is an index of the steepness of the relationship and therefore of the level of cooperativity in the myofibrillar activation. The sensitivity of myofibrillar apparatus to calcium is generally studied in skinned fibers by measuring the isometric force developed at steady state in activating solutions of different calcium concentrations (188). However, force-pCa curves were also obtained in intact muscle fibers (12).

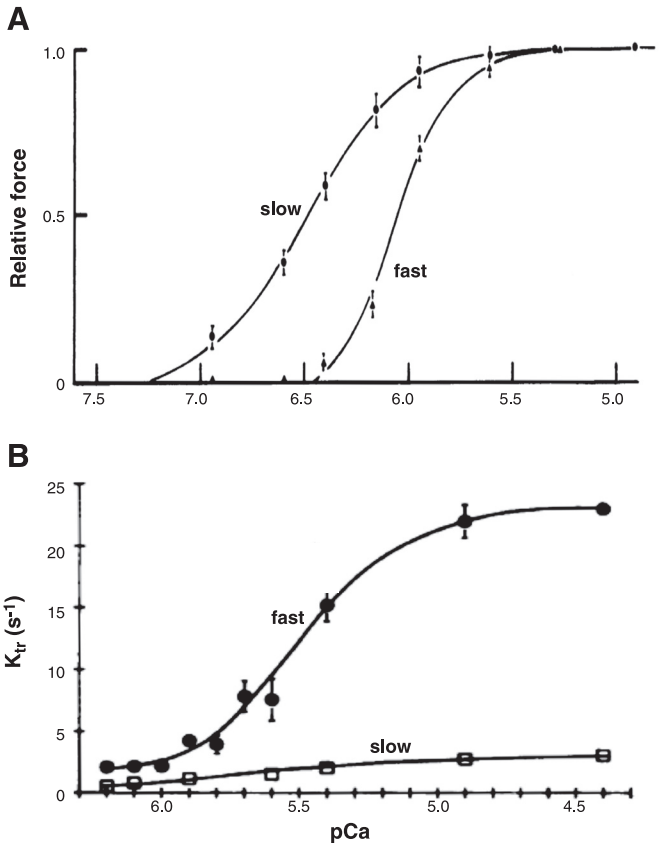


FIGURE 8. Modulation of active tension and rate of tension development by free calcium concentration (expressed as pCa = −log [Ca^{2+}]). *A*: relative steady-state force-pCa relations of rat skinned fibers at 22°C. A fast fiber from rat EDL and a slow fiber from rat soleus are shown. Note that slow fibers have a lower threshold and lower slope compared with fast fibers. [Modified from Stephenson and Williams (762), with permission from Wiley-Blackwell.] *B*: variations of the rate constant of tension redevelopment (K_{tr}) with pCa in rabbit fast and slow skinned fibers at 15°C. Values are means ± SE, with 7–20 observations per point. Note that at each pCa, the value for fast fibers is significantly greater than the value for slow fibers and that the calcium regulation of K_{tr} occurs in a range (pCa 6.5–4.5) higher than the range of force regulation (pCa 7.5–5). [Modified from Metzger and Moss (539), with permission from The American Association for the Advancement of Science.]

The response of myofibrils to activator calcium shows clear variations among different types of mammalian skeletal muscle fibers. Results obtained in many species, including rabbit (404), rat (762), guinea pig (779), and humans (219, 669), consistently indicate that the threshold for activation is lower in slow than in fast muscle fibers, whereas the Hill coefficient n is higher in fast than in slow fibers **(FIG. 8*A*)**.

Some disagreement exists about p*K* values. At room temperature, rat type 1 fibers have higher p*K* (i.e., higher calcium sensitivity) than 2X fibers, with 2A fibers being intermediate (179, 264, 762). Measurements in human muscle fibers are not completely consistent with this view as some studies reported no significant differences in p*K* at 22°C (219, 504), whereas others reported higher calcium sensitivity for type 1 than for type 2A human fibers at 15 and 22°C (669, 863). Finally, Bottinelli et al. (93) reported higher calcium sensitivity for type 2A and 2A-2X than for type 1 human fibers at 12°C. Such discrepancies might be accounted for, at least in part, by the experimental conditions, such as temperature and sarcomere length. The response of myofibrils to calcium is particularly sensitive to temperature (270, 762), and this can contribute to determine the variation with temperature of the amplitude of the twitch compared with tetanus (twitch-to-tetanus ratio) (638, 640).

The diversity in p*K* between slow and fast fibers becomes especially evident if strontium is employed to activate myofibrils instead of calcium: when activated with strontium, slow fibers are >10-fold (more than 1 unit of p*K*) responsive than fast fibers (188) (219). Such diversity is based on the distinct affinity of slow and fast TnC isoform for strontium and has become a popular method for fiber type classification in full agreement with MyHC isoform identification, given the tight coupling between TnC isoforms and MyHC isoforms (87).

If the myofibrillar function is measured in terms of ATP hydrolysis rate, a sigmoid curve similar to T-pCa curve can be obtained by plotting ATPase activity versus pCa. Not only steady-state myofibrillar activity is controlled by calcium, as depicted by T-pCa or ATPase-pCa curves, but also some kinetic parameters show a clear dependence on free calcium. In particular, the rate of tension redevelopment (often expressed in terms of k_{TR}) after a quick shortening-relengthening maneuver or Brenner's maneuver (100) is strictly dependent on calcium, whereas maximum shortening velocity (V_o) is relatively independent of calcium (see Ref. 276). The k_{TR}-pCa relationship is shifted to the right of the relative tension-pCa relationship, indicating that the concentration of Ca^{2+} necessary for half-maximal activation of k_{TR} is markedly greater than that for steady-state isometric tension (100). Thus the relation between k_{TR} and P_o is nonlinear and exhibits an exponential shape. In this regard, slow and fast fibers differ not only in the absolute magnitude of k_{TR} but also in the shape of the k_{TR}-force relationship, as in slow fibers the increase in k_{TR} with increasing force is much less pronounced **(FIG. 8*B*)** (539).

D) MOLECULAR DETERMINANTS OF CALCIUM SENSITIVITY AND COOPERATIVE ACTIVATION. Calcium sensitivity, as measured by the parameter p*K* or pCa_{50}, of the force-pCa curve can be affected by a number of factors, including temperature, pH, sarcomere length, ionic strength, interfilament spacing, concentrations of P_i, MgATP, MgADP, and posttranslational modification of myofibrillar protein (e.g., phosphorylation of the myosin regulatory light chains). With regard to interfiber diversity, the major role in controlling calcium sensitivity is played by the presence of specific isoforms of regulatory proteins tropomyosin and troponin. However, other proteins may be involved as well. Since binding of myosin S1 fragment to regulated actin facilitates further binding and stabilizes the thin filament in a state of higher calcium sensitivity (290), one might expect that MyHC isoforms that have distinct kinetics may contribute to generate heterogeneity in calcium sensitivity. However, a recent paper (718) has provided evidence that this is not the case at least in vitro. Regulatory myosin light chain (MyLC) phosphorylation is known to increase calcium sensitivity, i.e., p*K* values (see Ref. 774). Since MyLC phosphorylation is typical of fast fibers where expression of MLCK is higher (97), this mechanism may contribute to increase the responsiveness of fast fibers to submaximal calcium (see sect. III *C1*F). After repetitive stimulation, twitch peak tension is increased in fast muscles, a phenomenon named "posttetanic potentiation" (40), which finds explanation in MyLC phosphorylation and increased calcium sensitivity of the myofibrils. Posttetanic potentiation does not occur in slow muscles, which even show a "posttetanic depression" (113). The association between fast fiber phenotype, higher phosphorylation of MyLC2, and posttetanic potentiation may be of physiological relevance, as it represents a mechanism to counteract the loss of force due to fatigue during prolonged repetitive stimulation. Among thick filament proteins, a possible role for myosin binding protein C (MyBP-C) in determining calcium sensitivity is suggested by the observation that extraction of MyBP-C decreases force at submaximal calcium concentrations (341).

The dissection of the specific contribution of the isoforms of each troponin subunit and of tropomyosin is very difficult. However, comparison between fibers with different isoform composition, studies of naturally occurring or induced mutations, and experiments based on exchanging various components of the regulatory system have given some clue.

Unambiguous evidence demonstrates that TnC isoforms alone are the determinant of the different responsiveness to strontium of slow and fast fibers (571). Less clear is the role played by TnC alone in determining the myofibrillar responsiveness to calcium, whereas the replacement of the whole fast troponin complex with the slow troponin complex is definitely sufficient to lower the threshold and increase the sensitivity to calcium (614).

Cooperativity in myofibrillar activation is the determinant of the slope of force-pCa curve. In the early model of Hill et al. (335), cooperativity was related to activity within the functional unit of seven actin-TM-Tn. Nearest neighbor

interactions between the units based on TM-TM overlap causes transmission of conformational changes along the filament. A subsequent model has identified the cooperative unit in the number of actin subunits trapped in the open state by one myosin head bound (263). Tropomyosin isoforms might play a role in determining the size of the cooperative unit on the basis of their actin affinity; the higher the affinity, the smaller the number of myosin heads required for full activation (798). It is still to be clarified whether tropomyosin isoforms give distinct contribution to the cooperative activation.

9. Conclusions

The traditional classification of fast-twitch and slow-twitch muscles is based on simple physiological parameters, such as time to peak tension and half-relaxation time, and reflects mainly basic differences in the E-C coupling process between fast-twitch and slow-twitch muscle fibers. Compared with slow-twitch, fast-twitch fibers are characterized by the following: *1*) lower cytosolic free calcium concentration as a result of more powerful cytosolic buffering (parvalbumin, troponin C) and reduced calcium entry from extracellular space; *2*) ability to release and take up larger amount of calcium in shorter time, thanks to a more developed SR and a greater abundance of calcium release channels (RyRs), calcium pumps (SERCA), and intraluminal buffers (calsequestrin), which allow to accumulate a greater amount of calcium in the SR; and *3*) greater cooperativity in response to the increase of cytosolic calcium concentration, due to the presence of specific troponin and tropomyosin isoforms, leading to a steeper tension-pCa curve.

Taken together, such features of the fast fiber phenotype allow the generation fast and large calcium transients but would not be sufficient to meet the need of a strong and fast contractile performance if the contractile machinery was unable to respond to calcium transients with fast and powerful molecular motors. As described in the next section, fast fibers have indeed a contractile machinery endowed with high shortening velocity and power output matching the fast and large calcium transients. Interestingly, the twitch parameters (time to peak tension and half-relaxation time) show a close correlation with the reciprocal of shortening velocity: the faster the shortening velocity, the shorter the time parameters (158, 643).

C. Muscle Contraction: Myofibrillar Motors and Cytoskeletal Scaffolds

The contractile machinery of skeletal muscles is organized in highly ordered supramolecular structures, the sarcomeres, which are associated in series, to form the myofibrils, and in parallel, thus generating the striated pattern of muscle fibers. Each sarcomere consists of overlapping arrays of thick or myosin filaments and thin or actin filaments, which represent the contractile machinery proper, and of a cytoskeletal scaffold. The sarcomeric cytoskeleton is composed of transversal structures, the Z disks and M bands, and longitudinal filaments composed of the giant proteins titin and nebulin, running in parallel with thin and thick filaments. Contractile and cytoskeletal components are connected by multiple links, for example, titin and myosin interact both directly and via the myosin binding protein C, and nebulin filaments are closely associated with actin filaments. The sarcomere also provides the scaffold for the association of other proteins involved either in energy metabolism, e.g., creatine kinase associated to the M band, or signaling, e.g., calsarcin/FATZ involved in calcineurin signaling.

The basic structure of the sarcomere is very similar in different types of striated muscles, including cardiac muscle, except for minor variations detectable by electron microscopy, as Z disk thickness, M band ultrastructure, and actin filament length (see below). However, many contractile and cytoskeletal proteins exist as multiple isoforms **(FIG. 9)**, which are responsible for the wide variations in both active and passive mechanical properties of the myofibrils in different types of muscle fibers. The contractile function of the sarcomere is mediated by the interaction of actin and myosin filaments, which is controlled by calcium through the regulatory protein complex associated to the thin filaments, namely, the troponin-tropomyosin complex. In contrast to the wealth of molecular variants of regulatory proteins (see sect. III*B8*) and of motor proteins (myosin, see below), the thin filament basic components are very similar in all muscle fibers. Actin is the main structural component of the thin filaments which form the trail along which myosin motors work. Two isoforms of actin can be expressed in mammalian skeletal muscle fibers: α-skeletal and α-cardiac isoforms, which differ by only four amino acids and are encoded by two different genes. While in rat and mice skeletal muscles only α-skeletal actin is expressed, the two isoforms coexist in human muscles (302). There are, however, no indications of a functional impact of such heterogeneity.

1. Myosin isoforms and contractile properties

A) INTRODUCTION. Although all structural and functional features of the muscle fibers are shaped to contribute to their specialized performance, the major determinants of the ability to correctly respond to the demands of the nervous system are the molecular motors, i.e., the proteins able to convert chemical energy into work. The molecular motors that power skeletal muscle contraction belong to class II myosins, which also include the motors of cardiac and smooth muscle contraction, and some nonmuscle myosins (see a complete list in Ref. 69). Myosin molecules expressed in cardiac and skeletal muscles, i.e., in striated muscles, are called sarcomeric myosins as they are part of the highly ordered supramolecular structure indicated as sarcomere. As all class II myosins, sarcomeric myosins are hexamers

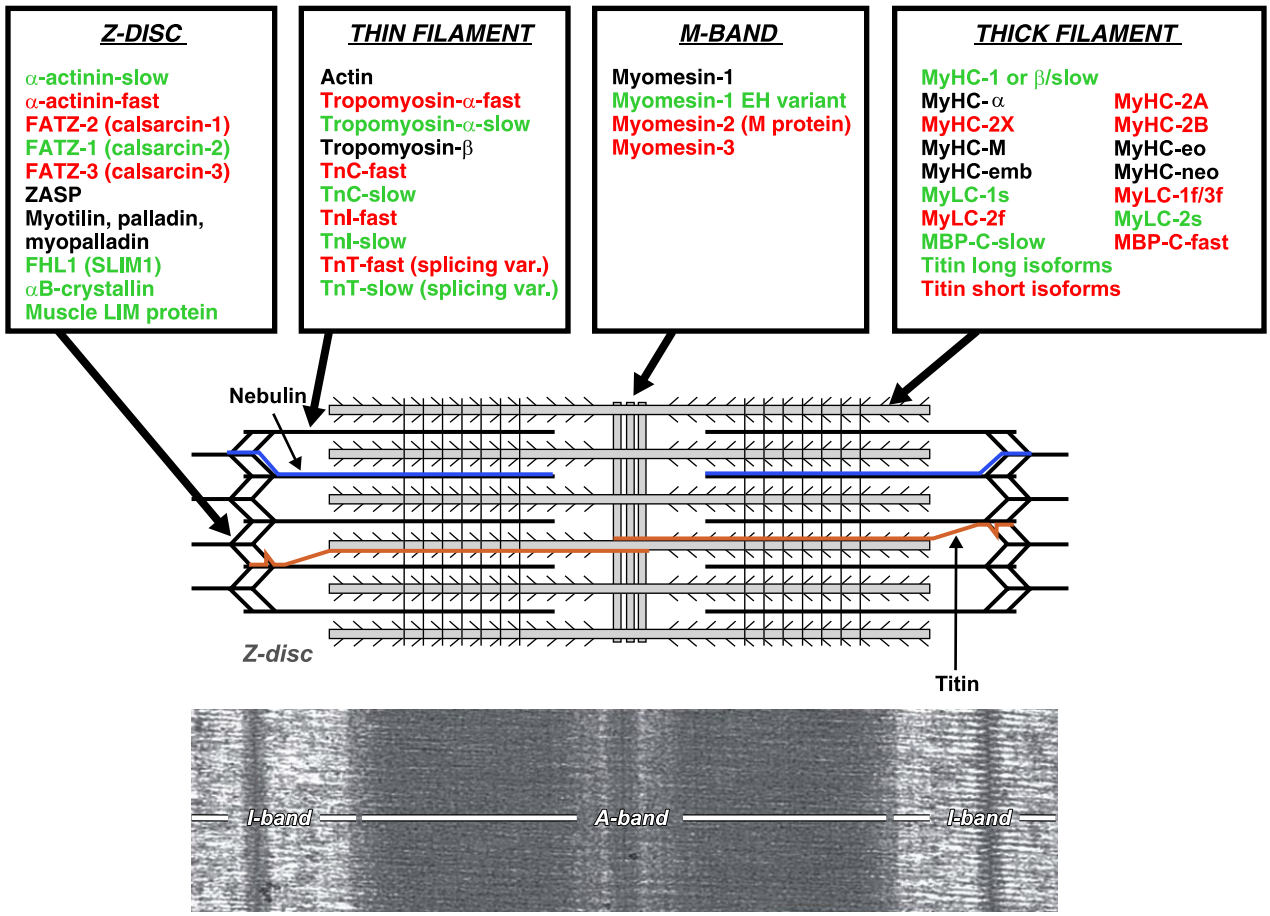


FIGURE 9. Schematic diagram showing the main components of the striated muscle sarcomere (*top panel*) and electron micrograph showing the appearance of the sarcomere in longitudinal section (*bottom panel*). The molecular components that are known to exist as multiple isoforms with differential distribution in the slow (green) and fast (red) fibers are indicated. [Modified from Luther (499).]

consisting of two MyHC and two pairs of MyLC, called essential or alkali MyLC and regulatory or phosphorylatable MyLC, respectively.

B) MYOSIN ISOFORMS. Sarcomeric myosins are shaped as elongated molecules, characterized by two globular domains called "heads" and a long filamentous portion called "tail." Each globular head is formed by the NH_2-terminal domain of the MyHC comprised of ~840 amino acids with high homology to the motor domains of all other members of the myosin superfamily. Both the ATP- and actin-binding sites are located in the myosin head, which therefore assumes the function of motor domain, able to hydrolyze ATP and generate force and displacement. At the COOH terminus, the head ends with a long α-helix, the so-called "neck," which contains two IQ domains that bind the two MyLCs (one essential and one regulatory for each head). Further distally, the MyHC molecule stretches to an α-helical domain of ~1,100 amino acids. Such long tail domain contains heptad repeat sequences that form an α-helical coiled coil that allows heavy chain dimerization. Through a process of self-assembly, the myosin tails create the "thick" or myosin filament decorated with hundreds of myosin heads responsible for force generation and movement at the supramolecular level. The tail represents a unique portion of the sarcomeric myosin molecule with three important tasks: *1*) dimerization of the MyHC, *2*) thick-filament assembly, and *3*) MyBP aggregation.

Both MyHCs and MyLCs comprise multiple isoforms with distinct fiber type specific distribution. Indeed, MyHC isoforms provide the basis of the more common classification of fiber types. Mammalian genomes contain 11 sarcomeric *MYH* genes, highly conserved in vertebrate evolution, each coding for a different MyHC isoform (69) (see **FIG. 1**). Most MyHC genes are associated in two clusters, one containing the isoforms expressed in cardiac muscle, MyHC-α and MyHC-β/slow (or MyHC-1, coded by *MYH6* and *MYH7*), and another containing the three isoforms expressed in adult skeletal muscle fibers (MyHC-2A, MyHC-2X, and MyHC-2B, coded by *MYH2*, *MYH1*, and *MYH4*), two developmental isoforms expressed in embryonic and neonatal muscles (MyHC-emb and MyHC-neo, coded by *MYH3* and *MYH8*), and one isoform expressed exclusively in extraocular muscles (MyHC-EO, gene *MYH13*). Three additional MyHC isoforms (MyHC-slow tonic, MyHC-15 and

MyHC-M, coded by *MYH7b*, *MYH15,* and *MYH16,* respectively) are expressed exclusively in some head and neck muscles (see sect. II*B*). Species variations in the expression of MyHC are detailed in section V. Intrafusal muscle fibers retain also in adult muscles the ability to express developmental isoforms together with adult isoforms (see sect. II*B5*).

Both light myosin subunits, i.e., the essential MyLC also called MyLC1 and the regulatory or phosphorylatable MyLC or MyLC2, also exist in several isoforms. The issue has been described in detail in several reviews (74, 713, 795) and can be summarized as follows. At least four isoforms of MyLC2, each coded by a distinct gene, are expressed in skeletal muscle fibers: MyLC2s (gene *MYL2*) expressed in slow fibers and in ventricular myocardium, MyLC2f (gene *MYLPF*) expressed in fast fibers, and MyLC2m (gene *MYL5*) expressed in masticatory muscles in association with MyHC-M, and MyLC2a (gene *MYL7*) specific to atrial cardiomyocytes. Essential or alkali MyLC also exists in at least four different isoforms: MyLC1sa (gene *MYL6B*) present in slow fibers and nonmuscle cells, MyLC1sb/sv (gene *MYL3*) expressed in slow fibers and ventricular cardiomyocytes, MyLC1f and MyLC3f which are splicing variants of the *MYL1* gene expressed in fast skeletal muscle fibers and MyLC1a/emb (gene *MYL4*) expressed in developing skeletal muscles and in atrial myocardium. Rules of coexpression dictate which MyLC isoform combines with a given MyHC isoform, giving origin to a large number of myosin isoforms.

C) MYOSIN BINDING PROTEINS. The structure of thick filament is created by the close and extremely regular association of myosin molecules with the cytoskeletal proteins titin and myomesin or M protein (see below) and with the MyBP-C. MyBP-C or C-protein binds myosin at regular intervals along the thick filament with binding sites for the myosin rod and for titin at the COOH terminus (247, 577) and a second binding site for myosin subfragment 2 (S2) at the NH_2 terminus (298). The interactions with myosin and titin allow MyBP-C to contribute to the stabilization and maintenance of the sarcomeric A-band and possibly to modulation of contractile response. Three isoforms of MyBP-C, namely, MyBP-C-fast, MyBP-C-slow, and MyBP-C-cardiac, are encoded by separate genes (851). While the expression of the cardiac isofom is restricted to cardiomyocytes, MyBP-C-fast is present in fast fibers, either alone or in association with the slow isoform that is specifically expressed in slow fibers (259). The rules of coexpression between MyHC isoforms and MyBP-C isoforms are less clear in masticatory and extraocular muscles (418, 891). Recently, four distinct splicing variants of MyBP-slow have been identified (for a review, see Ref. 2). MyBP-C slow variant-1 is expressed in all skeletal muscles both during development and at maturity and is localized at the periphery of the M band where it can interact with obscurin (429). Slow variant-2 is preferentially expressed in fast muscles, whereas slow variant-3 is specific to slow muscles and slow variant-4 is widely but nonubiquitously expressed.

D) CONTRACTION MECHANISM. The basic mechanism which leads, through cyclical interactions between myosin heads and actin filaments to force or displacement generation, accompanied by ATP splitting, is essentially similar in all sarcomeric myosin isoforms (for recent reviews, see Refs. 262, 276, 894). Upon binding of ATP, myosin can dissociate from actin breaking the acto-myosin "rigor complex," and this dissociation is quickly followed by ATP hydrolysis to P_i and ADP. Hydrolysis is accompanied by a conformational change (reverse stroke) and then followed by myosin binding to actin and subsequent release of P_i. Release of P_i is followed by a conformational change in the converter (inside the myosin head) amplified by the lever arm and transmitted to the actin filament as force or displacement (power stroke). The subsequent release of ADP leads to the formation of a new acto-myosin rigor complex, which will be in turn dissociated by ATP binding, thus starting a new cycle. The kinetic parameters of the above cycle, and possibly also the size of the power stroke, i.e., the unitary force or displacement step (130), differ among myosin isoforms, and this represents a major determinant of the diversity in contractile properties of muscle fibers (see below). For example, the rate of the ATP hydrolysis step can vary sixfold between 22 s^{-1} in slow soleus myosin and 131 s^{-1} in fast 2X psoas myosin (368). Under conditions of low or zero load, the rate of ADP release is the rate-limiting step of the cycle and thus a major determinant of the speed of filament sliding (735, 854) (see **FIG. 10*D***). The kinetics of ADP release, however, reveal another important difference among isoforms. The sensitivity of the detachment kinetics to the applied load or strain leads to classify myosin isoforms in two main types, called strain sensors and fast movers, respectively, based on the comparison between their ADP affinity and thermodynamic coupling between ADP and actin affinity (569). Fast sarcomeric myosin isoforms are the typical fast movers, while slow sarcomeric myosin isoforms can be considered close to the group of strain sensors. The notion of strain sensor was first introduced in studies on smooth muscle (class II) or brush-border (class I) myosins, which differ from fast sarcomeric myosin in that they show an ADP-induced tail swing detectable by electron microscopy (862), a double step in the laser trap (824), a small free energy change associated with ADP release from actomyosin, and a weak coupling between the affinity of myosin for ADP and actin (K_{AD}/K_D) (see Ref. 569 for a review). While in all myosin isoforms the large free energy drop associated with P_i release drives the power stroke of the cross-bridge to generate force and displacement, in strain sensor myosins a further swing of the converter and neck of the cross-bridge (in the same direction as the power stroke) is required before the nucleotide pocket opens to allow ADP release. If load is applied on the cross-bridge, ADP released is post-

poned and the attached state is prolonged, thus generating force without further ATP consumption (569). There is evidence, however, that a double step in the power stroke is detectable in all sarcomeric myosins (130), and the thermodynamic coupling in slow sarcomeric myosin isoforms is not far from that of smooth muscle myosin (81).

E) REGULATION OF CONTRACTILE PROPERTIES BY SELECTIVE MYOSIN ISOFORM EXPRESSION. The expression of a given specific myosin isoform is the major determinant of the contractile performance and of ATP consumption of a muscle fiber. In addition, contractile performance is modulated by post-translational modification of myosin: phosphorylation (774) and glycosylation (410, 635). Finally, calcium-based regulation plays a switch on-switch off role on the contraction mechanism by controlling the availability of the actin binding sites on the thin filament (see sect. III*B8*).

I) *Contractile performance (power, force, and velocity).* The mechanical performance of an activated muscle fiber can be best characterized in terms of power, i.e., mechanical energy generated or released per unit of time. Mechanical power is directly related to chemical energy released by ATP hydrolysis, with the ratio between the two (i.e., power/chemical energy release rate) being the thermodynamic efficiency. Mammalian skeletal muscle fibers show a large variability in the mechanical parameters that define their contractile performance. Comparing adult muscle fibers that express either slow myosin or one of the fast isoforms (2A, 2X, 2B), peak power values increase orderly from slow fibers to fast 2A, fast 2X, and fast 2B fibers which exhibit the highest values, in all mammalian species and all muscles examined up to now (91, 94, 269). The range is up to 10-fold when fibers are studied in vitro at low temperature, as can be seen for human fibers in **(FIG. 10*A*)**. The mechanical power generated by a muscle fiber is determined by the maximum velocity of unloaded shortening (indicated as V_{max} when extrapolated from the force velocity curve and V_o when determined with slack test protocol), the isometric force (P_o, the maximum isometric force per unit cross-sectional area, or specific tension), and the curvature of the relationship, given by the dimensionless parameter a/P_o (or b/V_{max}). The less the curvature, the greater the force sustained at a particular velocity and, therefore, the greater is the power generated.

V_{max} and V_o are strictly determined by the type of myosin that is present in the fiber and increase in the order slow $<$ 2A $<$ 2X $<$ 2B (91, 94, 646, 775), as shown in **FIGURE 10*B*** for murine and human muscle fibers. The parallel determination of V_o and V_f (filament sliding velocity in in vitro motility assay, IVMA), gives support to the view that myosin isoforms alone determine the speed at which actin filaments are translocated by myosin motors. As shown in **FIGURE 10*C***, there is a close proportion between V_f determined on purified myosin isoforms in IVMA and V_o in muscle fibers containing the same myosin isoforms (594). In terms of acto-myosin kinetics $V_o = d/t_{on}$, where d is the displacement produced during a single interaction and t_{on} is the duration of the interaction, or the time spent in attached state. At the low temperature at which muscle fibers are generally studied in vitro (12–20°C), the detachment rate that is the reciprocal of t_{on} is determined by the rate of cross-bridge dissociation by ATP in fast fibers, while the ADP release rate k_{AD} limits the velocity of slow fibers (570). Extrapolation to 37°C based on temperature sensitivity of each specific kinetic parameter suggests that at physiological temperature the rate of ADP dissociation may limit V_o for both slow and fast fibers (570) **(FIG. 10*D*)**. To summarize, the expression of a given myosin determines the speed of filament sliding by dictating the rate of cross-bridge detachment (or the duration of the cross-bridge life, which is the reciprocal) through the control of ADP release rate. Although among the subunits of myosin, MyHC isoforms are the main determinants of maximum shortening velocity, there are indications that MyLC isoforms play a modulatory role. The alkali or essential MyLC1s and MyLC1f possess a long NH_2-terminal extension, able to reach and bind thin filaments, whereas MyLC3 lacks such an extension (for a review, see Refs. 796, 797). In fast fibers that express either MyLC1f or MyLC3, maximum shortening velocity increases in proportion to the amount of MyLC3f expressed in the fiber (90). This effect can be explained by a "braking action" played by the long NH_2-terminal extension of MyLC1f interacting with actin filaments.

The diversity in curvature of the force velocity relation first observed in intact muscles (111, 639) has been confirmed in single muscle fibers. The curvature is less pronounced in fast than in slow fibers (94, 269, 325). In contrast, isometric tension is greater in fast than in slow fibers (484, 594, 800). The two factors together contribute to enhance the diversity in peak power between slow and fast fibers. Isometric tension reaches very high values in fibers of jaw closer muscles expressing MyHC-M, and this feature allows those fibers to develop high power, although their shortening velocity is not very fast (799). Two factors contribute to determine the higher tension development in fibers expressing fast MyHC isoforms compared with those expressing slow isoforms, namely, a larger fraction of strongly attached force-generating myosin heads and a higher force per head (484).

When distinct fiber types are compared, two parameters that deserve attention are the values of shortening velocity and force at which peak power is reached: such values are often indicated as optimal velocity and optimal force. Optimal velocity (V_{opt}) represents a virtually constant fraction of V_o in fast fibers, ~20%. Thus V_{opt} is higher in fast 2B than in fast 2X or 2A fibers. Due to their greater curvature, in slow fibers V_{opt} is ~15% V_o, and this implies a further difference with fast fibers (91, 269). Evi-

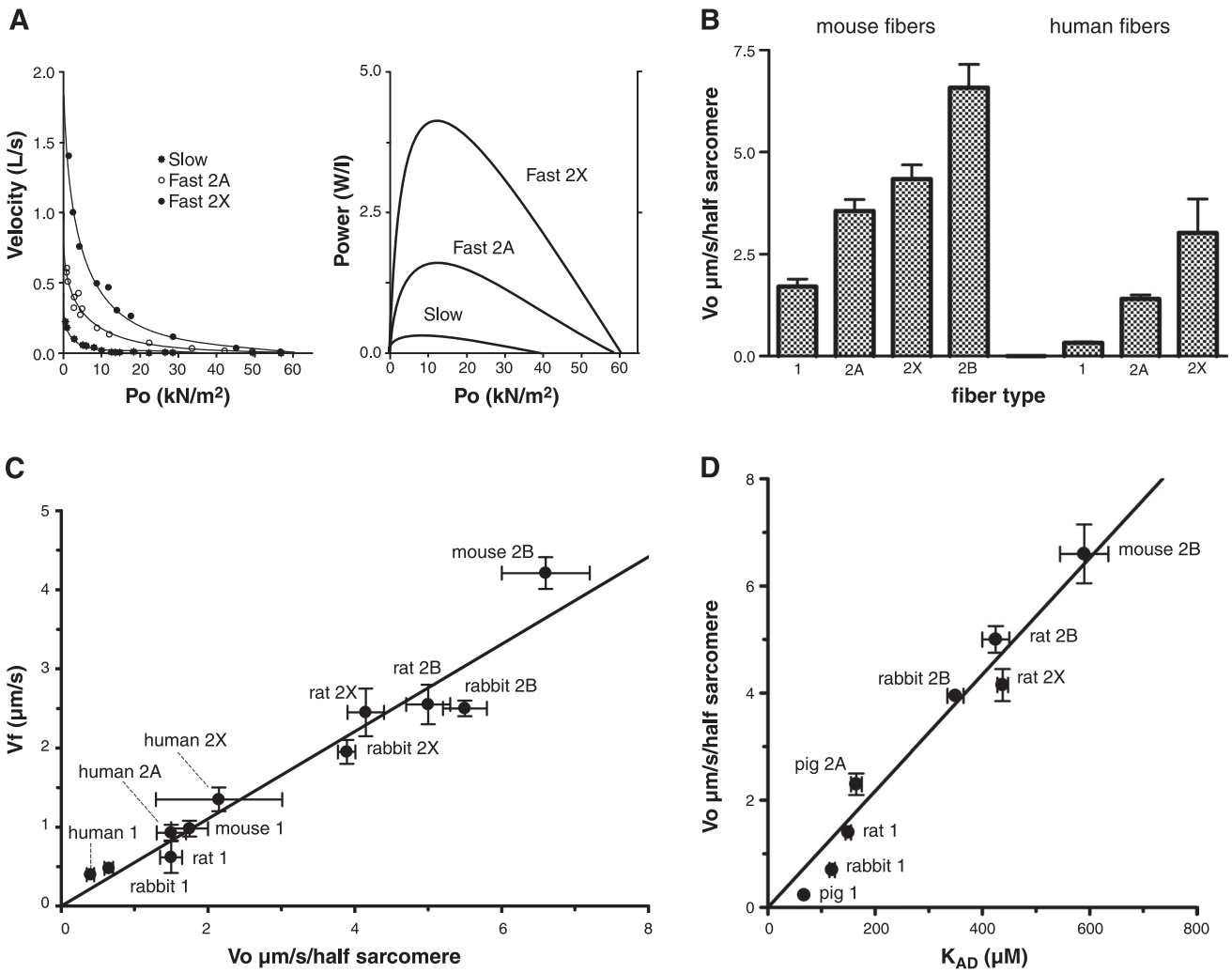


FIGURE 10. Contraction kinetics of slow and fast fibers. *A*: representative force-velocity (*left panel*) and force-power (*right panel*) curves of three single human muscle fibers (slow, fast 2A, and fast 2X) permeabilized and maximally activated at 12°C. *B*: comparison of unloaded shortening velocity values (V_o) of mouse and human muscle fibers classified according to myosin heavy chain composition. Each column represents the average of V_o values obtained with the slack test protocol on 6–25 single fibers, permeabilized and maximally activated at 12°C. *C*: correlation between sliding filament velocity of purified myosins (V_f) determined by in vitro motility assay at 25°C and unloaded shortening velocity of corresponding isolated single fibers (V_o) permeabilized and maximally activated at 12°C. Fibers were obtained from four different species. [Modified from Pellegrino et al. (594).] *D*: correlation between unloaded shortening velocity of isolated single muscle fibers (V_o) and ADP affinity (K_{AD}) of corresponding myosins from different muscles and different species. [Modified from Nyitrai et al. (570), with permission from Elsevier.]

dence from in vivo studies and theoretical analysis show that V_{opt} is the shortening velocity preferentially adopted during movement (502, 657). Thus fibers are recruited not only in view of the need to develop force but also in relation to the speed at which a movement must be performed, and recruitment of fibers is modulated to reach the required power output.

Diversity among fiber types is present also when they resist to elongation applied during contraction, i.e., in the negative region of the force-velocity relation. Force enhancement during lengthening of an active muscle is a condition that normally occurs during locomotion in vivo, and is attributed to recruitment of myosin heads that attach to and detach quickly from actin in cycles that do not require hydrolysis of ATP. The extent of recruitment is larger in slow fibers than in fast fibers, and this implies that, for each given lengthening velocity, fast and slow fibers develop similar steady force and power output (484).

A further parameter relevant in isometric condition is the rate at which tension can be developed once maximal and steady activation is achieved. Such rate is often expressed as the rate constant (k_{TR}) of tension development (101) and is calculated during a maneuver of fast shortening and fast reelongation (Brenner's maneuver). The value of k_{TR} is related with the rate of cross-bridge formation and varies in relation to myosin isoform composition (539), being greater in fast than in slow fibers.

An interesting approach to analyze cross-bridge kinetics in isometric conditions is based on the determination of the rate constants of a tension transient triggered by a small stretch during an isometric contraction and often indicated as stretch activation (251). The diversity among skeletal muscle fibers has been thoroughly investigated with such an approach, and the results show a strong correlation between MyHC isoform composition and kinetics of stretch activation, with rate constants decreasing in the order: MyHC-2B > MyHC-2X > MyHC-2A > MyHC-1 (252).

Sinusoidal oscillation analysis provides an effective approach to characterize all elementary steps of the cross-bridge cycle including the force-generation step. Such analysis has been successfully applied to study single muscle fibers from rabbit muscles expressing slow or fast myosin isoforms (253, 846). The rate constants of virtually all elementary steps are greater in fast than in slow fibers, the largest difference being in the force-generating step. The affinity constants for ATP and ADP decrease progressively from slow to fast 2A, 2X, and 2B fibers.

Rapid temperature jumps (T-jumps with a rise time of 0.2 ms) represent a powerful perturbation technique to analyze the temperature-sensitive steps in the cross-bridge cycle. The net rise in tension after a T-jump is biexponential characterized by two time constants (τ_2 and τ_3) that have lower values in fast than in slow fibers. In both fibers, τ_2 corresponding to endothermic force generation becomes faster with an increase in temperature, whereas τ_3 is temperature sensitive in slow fibers but not in fast fibers (637).

II) Myosin ATPase activity. Muscle contraction requires ATP, and myosin is a major utilizer of ATP in skeletal muscle fibers. During an isometric twitch, the energy consumed by myosin is ~60–70% of the total consumption (55) and during steady maximal isometric contraction can be above 75% (777). Besides ATP hydrolysis catalyzed by myosin, ATP is used in muscle fibers for maintaining transmembrane gradients, for energy cost of activation, i.e., calcium release and uptake from SR, and for energy required for metabolic reactions.

Measurements of steady-state ATPase activity in permeabilized fibers during isometric contraction in vitro have shown that ATP hydrolysis rate increases from fibers expressing slow myosin to fibers expressing fast 2A, fast 2X, and reaches its highest value in fast 2B fibers (92, 313, 325, 764). Although a difference is present in the amount of developed tension (see above), the tension cost, i.e., the amount of ATP consumed per unit time and per unit tension, is also lower in slow than in fast myosin (92, 764), thus making slow fibers an energy-saving motor suitable for maintaining tension in isometric conditions as in postural activity. This important feature can be related to the specific kinetics of slow myosin as strain sensor (see above): if load is applied and movement is not allowed, the cross-bridge life is prolonged and tension is developed with reduced ATP consumption (81, 569).

During shortening ATP hydrolysis rate increases in all fiber types, and this represents the so-called Fenn effect (for a review, see Ref. 745). The ATP hydrolysis rate increases both in slow and fast fibers in direct relation to the speed of shortening. The rate of ATP hydrolysis during active shortening is proportional to power output, which is different depending on fiber type. The proportion between mechanical power and ATP hydrolysis rate implies that efficiency of the chemomechanical transduction is virtually identical in slow and fast fibers (325, 642). The issue is, however, controversial as there is evidence that efficiency during shortening is higher in slow than in fast mouse muscle fibers (51, 53).

The presence of slow myosin gives a substantial advantage to slow fibers in isometric conditions as, in such conditions, tension cost is much lower than in any type of fast fibers (see, e.g., Ref. 92). This is particularly relevant when fibers are engaged in postural duties. When external work is performed, the advantage is reduced or even disappears depending on whether efficiency is higher in slow fibers (53), or equal (325).

f) MYOSIN PHOSPHORYLATION AND GLYCOSYLATION. Phosphorylation is the best-known posttranslational modification of the myosin molecule. The site of phosphorylation is represented by the regulatory or phosphorylatable MyLC (also called MyLC2) and is catalyzed by a calmodulin-dependent kinase (myosin light-chain kinase or MLCK) that is activated by the increase in cytosolic calcium (83). Thus a repetitive or tetanic stimulation causes a transient increase of phosphorylation of regulatory MyLC. Phosphorylation is then removed by protein phosphatase 1 (PP1) associated with MYPT2, a targeting subunit specific to skeletal muscle MyLC (549). Myosin phosphorylation enhances force development at submaximal calcium concentration (776), i.e., causes a shift of the T-pCa curve (598) (see section III*B8*) towards lower calcium concentrations and, through this mechanism, offers a plausible explanation for the phenomenon of the posttetanic potentiation of the twitch (355). Phosphorylation level is higher in fast than in slow muscles (548); decreases in fast muscles with chronic low-frequency stimulation, which induces a fast-to-slow transformation; and increases in slow muscles with hindlimb unloading, which causes a slow-to-fast transformation (97, 98). The difference among fiber types is attributable to a transcriptional regulation of MLCK, more abundant in fast than in slow fibers (97). Myosin heavy chains are also the target of nonenzymatic glycosylation, which has been demonstrated to be restricted to slow fibers of aged rats, and thus to MyHC-1 isoform. Myosin glycosylation is able to modify significantly contractile parameters, in particular maximum shortening velocity and speed of actin filament transloca-

tion in in vitro motility assay (635). Also MyHC-M, expressed in jaw muscles, is a target of glycosylation, but no functional implications are known (410).

G) CONCLUSION. Some mechanical parameters of fast and slow muscle fibers, such as maximum shortening velocity, reflect directly the activity of the molecular motors, i.e., myosin isoforms, which power muscle contraction. The properties of the various myosin isoforms are closely matched to the calcium kinetics and calcium sensitivity of the myofibrils so that other muscle mechanical parameters (time to peak tension, time to half relaxation) are in fact a combined and coordinated result of E-C coupling mechanisms and molecular motor activity. On the other hand, the mechanical performance has a counterpart in energy metabolism, as tension development and power generation have a cost in terms of ATP consumption. Slow fibers contract more slowly and generate less mechanical power but also spend less ATP, particularly in relation to tension development. Fast fibers can produce higher mechanical power and contract more quickly, but have a higher ATP expenditure. The two aspects, mechanical and energetic, together make slow fibers more suitable for low-intensity and long-lasting activity, whereas fast fibers are best for short and strong contractile performance. In both cases, a full consistency is required between the properties of the molecular motors and the metabolic processes aimed to ATP regeneration.

2. Cytoskeleton

A) INTRODUCTION. The cytoskeleton is a cellular scaffolding responsible of determining the shape and size (length) of muscle fibers and transmitting the force generated by the actin-myosin interaction to the extracellular fibrous skeleton. Myofibrils themselves can be considered as a specialized component of the cytoskeleton. Myofibrils run along the fiber major axis from one end to the other, ending with a specialized connection with the sarcolemma called myotendinous junction (MTJ), through which tension generated by the contraction is transferred to the tendons. The lateral or transversal transmission of force is not less important, and lateral connections keep in register fibers even in the presence of different contractile activity (547). Lateral connections are based on protein complexes called costameres, located over the Z lines of the underlying myofibrils and thus equally spaced along the sarcolemma. Costameres form a linkage between the extramyofibrillar cytoskeletal proteins, including actin, desmin, and spectrin, which in turn are connected to sarcomeric structures and the interstitial connective tissue which at the surface of the fiber is specialized as basal lamina or basement membrane. Basal lamina is rich in laminins, proteoglycans, and collagen IV and is anchored to the interstitial extracellular matrix or ECM through a network rich in collagen VI. Importantly, several signaling proteins are embedded into the cytoskeleton, which acquires in this way a role in several signaling pathways.

We will briefly consider here the sarcomeric and extrasarcomeric cytoskeleton, including the membrane cytoskeleton, in the perspective of skeletal muscle fiber diversity.

B) Z-DISC. The Z-disc appears as a dense line that sets the sarcomere boundaries in striated muscles. The first function of the Z-disc is to provide a mechanical link transmitting force longitudinally between adjacent sarcomeres and transversally, through the extramyofibrillar cytoskeleton to adjacent myofibrils and to costameres. A second, and likely not less important, role of Z-discs is to play as a force sensor linking tension along the myofibrils with signaling mechanisms (629). The width of the Z-disc depends on fiber type, being narrow in fast fibers (~30–50 nm) and wide in slow fibers and cardiac myocytes (~100 nm) **(FIG. 11)** (202). The molecular architecture of the Z-disc is very complex. The basic structure is represented by a lattice or network of α-actinin filaments which form links (Z links) between thin filaments, whose barbed ends are covered by cap protein, and bind the two giant proteins titin and nebulin **(FIG. 9)**. In addition, the actinin filament network hosts a very large number of other proteins that are generally classified into three families: myotilin, FATZ, and enigma (for a review, see Refs. 499, 836).

α-Actinin is a rod-shaped protein, 35 nm long, which forms antiparallel homodimers and exists in two isoforms coded by distinct genes (66): α-actinin 2 coded by *ACTN2* expressed in all muscle fibers and α-actinin 3 coded by *ACTN3* with expression restricted to a subset of fast fibers. Human ACTN3 gene has recently received attention because of a frequent polymorphism: the variant R577X has a premature stop codon that causes deficiency of α-actinin 3 in XX homozygotes (568). There is evidence of a positive association between the presence of the R allele and the capacity to perform high-power muscle contractions, while the X allele might predispose for better endurance exercise performance (509). In human skeletal muscle, α-actinin 3 is more abundant in type 2X than in type 2A fibers, and a positive association between the ACTN3 RR genotype and the proportion of fast, glycolytic fibers (2X) has been demonstrated (831).

In transversal sections, the lattice formed by the Z links (α-actinin filaments) may occur in two distinc appearances, a "basket weave" and "small-square" form. It has been proposed that the transition between the small-square form and the basket weave form is related to the transition between rest and contraction (271). In longitudinal sections, the Z-disc width is correlated with the number of layers of Z-links (α-actinin) connecting the anti-parallel actin filament ends that overlap in the Z-disc. The number of layers is different in relation to fiber type, giving an explanation of

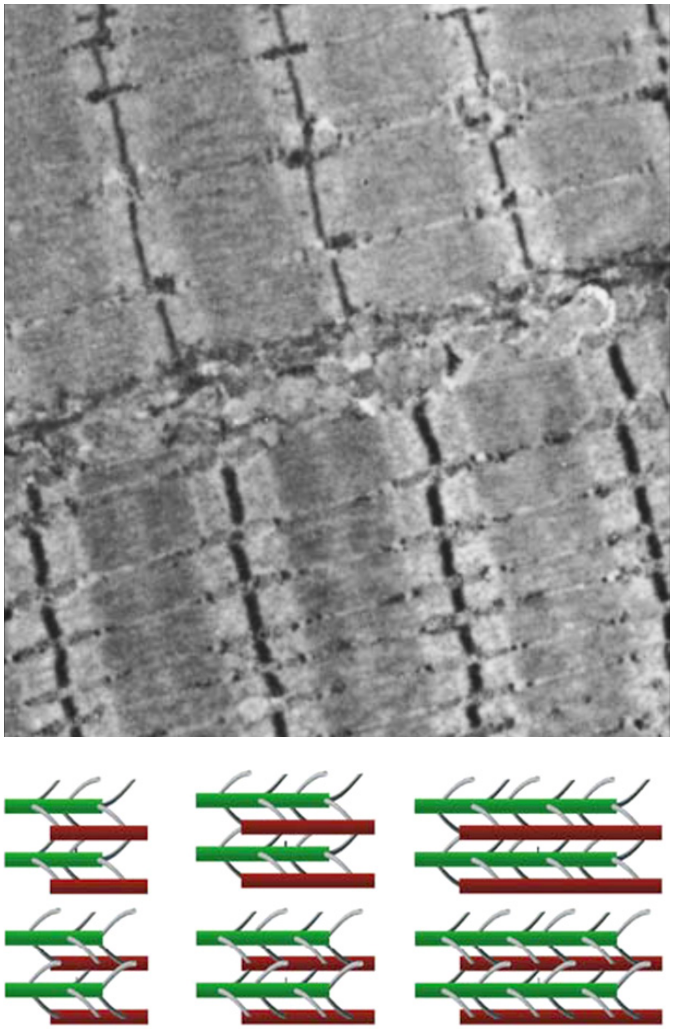


FIGURE 11. Z-disc thickness varies among different fiber types. *Top panel*: electron micrograph of a longitudinal section of rat EDL, a fast muscle: note the difference in Z-disc appearance between the mitochondria-rich fiber (*bottom*, probably a type 2A fiber) with thick Z-discs and the mitochondria-poor fiber (*top*, probably a type 2B fiber) with thin Z-discs. [Modified from Schiaffino et al. (710).] *Bottom panel*: 3-dimensional models of Z-disc structures postulating the existence of different layers of α-actinin (gray) cross-linking oppositely oriented actin filaments (red and green). Up to 6 layers of α-actinin were identified in bovine slow skeletal muscles with thick Z-bands. [Modified from Luther (499).]

the difference in Z-band thickness **(FIG. 11)**. The number of layers is two, three, and four in various vertebrate fast muscles and up to six in mammalian slow muscle fibers (see Refs. 499, 501).

Anchored to the network of proteins formed by actinin, actin and the giant proteins, titin and nebulin, a high number of other proteins are harbored in the Z-discs, some of which exist as distinct isoforms. This is the case of FATZ (filamin-, actinin-, and telethonin-binding protein of the Z-disc), also called calsarcin or myozenin, which includes three isoforms expressed exclusively in striated muscle. Calsarcin-1 (FATZ 2) and calsarcin-3 (FATZ 3) are expressed predominantly in fast fibers, while calsarcin-2 (FATZ 1) is expressed in slow fibers (233, 234). FATZ/calsarcin/myozenin have different binding partners, including not only myotilin, filamin, telethonin, α-actinin, and ZASP but also calcineurin. Importantly, calsarcin binding to calcineurin plays a negative regulatory action as double knockout of calsarcin 1 and 2 causes an increased activity of calcineurin signaling and a consequent shift from fast to slow fiber (231, 232) (see sect. VII*B*).

The enigma family of proteins is characterized by a PDZ domain at the NH_2 terminus and a variable number of LIM domains at the COOH terminus. Cypher/ZASP (Z-band alternatively spliced PDZ-containing protein)/oracle is the best known enigma family member (216, 899). Six isoforms of ZASP, generated by alternative splicing, have been described in the mouse, with one being specific to cardiac muscle and the other ones expressed in skeletal muscle (see Ref. 896). Cypher/ZASP binds to α-actinin via its PDZ domain and may be involved in signaling as it binds protein kinase C via its LIM domain. The myotilin family includes myotilin, palladin, and myopalladin. These proteins have immunoglobulin domains and bind α-actinin, filamin, and FATZ. Myotilin is linked to signaling networks by binding to the ubiquitin ligases Murf-1 and Murf-2 and indirectly via FATZ (calsarcin/myozenin) to calcineurin. Other proteins present in the Z discs exhibit fiber type-dependent variations in the level of expression. For example, four-and-a-half LIM domain 1 (FHL1 or SLIM1), α_B-crystallin and cysteine and glycine-rich protein 3 (CSRP3 or muscle LIM protein, MLP), are expressed at higher levels in slow than in fast fibers (126, 877).

C) M-BAND. The M-band is the cytoskeletal structure that provides a link between myosin tails and titin filaments in the middle of the sarcomere. Two possible mechanical functions have been proposed: *1*) transversal stabilization of the A band, by contrasting the expansion of the thick filament lattice during contraction, and *2*) longitudinal stabilization of the two hemisarcomeres, by compensating possible force unbalance of the opposing thick filaments in cooperation with titin. The fact that A band is strained by tension unbalance between the two hemisarcomeres suggests a potential role as mechanical sensor of tension generated during contraction. In this regard, M-band could be in a different condition compared with Z-disc, which is equally strained by active force generated during contraction and passive force applied to resting muscle fiber. Finally, the presence of creatine kinase gives to M band a role in metabolic supply to muscle contraction.

The molecular composition of the M-band varies considerably in relation to fiber type. The most important constituents of M-bands include myomesin 1, myomesin 2 or M protein, myomesin 3, and creatine kinase (CK-MM) (*5*). In addition, alternative splicing of the myomesin 1 gives origin to an 100-amino-acid longer splice variant known as EH-

myomesin, the sum of EH and non-EH variants of myomesin 1 being approximately constant. While myomesin 1 is ubiquitously expressed in all striated muscles, the longer EH variant of myomesin is typically present in slow fibers where longer variants of titin are also expressed (see below), thus suggesting the organization of a more compliant myofibrillar cytoskeleton in that particular fiber type. Myomesin 2 or M protein is associated with the fastest 2B fibers, and myomesin 3 is associated with fast intermediate 2A and 2X fibers (717). Extraocular muscles show a diversity between orbital and global layer, as the latter layer is characterized by expression of myomesin 2 and more ordered filament lattice, whereas the former layer is characterized by lack of detectable M band, poor lattice filament order, and expression of myomesin 1, particularly in its EH variant (865).

Electron microscopy shows that in longitudinal sections of a myofibril, the M-band consists of three to five parallel electron-dense lines in the central zone of the A-band. They are designated M6', M4', M1, M4, and M6, with M1 being in the center of the sarcomere. Diversity among fiber type can be detected at the ultrastructural level, as fast fibers have a three-line pattern (M4′-M1-M4′ with M6 and M6′ lines missing), slow fibers have a four-line pattern (M6′-M4′–M4-M6 with M1 line missing), and fast intermediate fibers exhibit a five-line pattern (740). In addition, some types of striated muscle, such as the embryonic heart or extraocular muscle, do not display any electron-dense M-band (27). According to an accepted ultrastructural model (500), the M-band is composed by a web of elastic M filaments oriented longitudinally and transversally between thick filaments with a triangular symmetry. The discovery of the ability of the major protein constituents of the M band, the myomesins, to bind titin and form antiparallel dimers has provided the molecular basis to such ultrastructural model (458). According to such model, myomesin dimers are the molecular components of both the longitudinal M filaments together with titin NH_2 terminus and the transversal M filaments binding to myosin tails.

D) GIANT PROTEINS. Titin (3.2–3.8 MDa), nebulin (0.6–0.9 MDa), and obscurin (~0.65–0.75 MDa) are three large size proteins that contribute to the myofibrillar cytoskeleton. Each of them is coded by a single gene and is expressed in several variants with a fiber type specific and muscle specific distribution.

Titin (848), first described as connectin (524), is the largest protein (molecular mass >3 MDa) expressed in skeletal muscle fibers and exists in different isoforms generated by alternative splicing from a single titin gene (453). Titin molecules extend from the Z-disc, where the NH_2-terminal region interacts with actinin and telethonin, to the M-band, where the COOH-terminal region binds to the M filaments and contains a specific domain endowed with kinase activity. Titin isoforms differ in molecular mass and length and contribute to the resting tension. Whereas the titin segment in the A band is embedded in the thick filaments and is stiff, the segment present in the I band behaves as a molecular spring, with several variants of different size and elastic properties (see Refs. 281, 429). In skeletal muscles, so-called "N2A-titin" contains two segments that vary in length in the different isoforms: a tandem Ig region, proximal to the Z-disk, and the PEVK domain (Fig. 12*A*). As a general rule, longer titin isoforms are expressed in muscles rich in slow fibers or where slow myosin is abundantly expressed (621). Titin is a major determinant of passive tension as discussed below, and the expression of longer titin variants implies a lower level of passive tension or a higher muscle fiber extensibility (see below).

Obscurin (890a) is concentrated at the periphery of M-bands and by binding a small ankyrin 1 variant, an integral component of the SR membrane, may mediate interactions between the sarcoplasmic reticulum and myofibrils (38, 430). Several transcript variants exist for this gene. Comparison among rabbit muscles (621) shows that the obscurin variant size is positively correlated with titin variant size and with the abundance of slow myosin.

Nebulin runs along the length of the thin filaments (452). Its COOH-terminal region is an integral part of the Z-disc structure, while the NH_2-terminal region projects into the I-band and forms a template for thin filament. Most of the nebulin sequence consists of repeated copies of 35-residue modules which are in turn grouped into super-repeats of seven-modules. Each nebulin super-repeat corresponds to the 38.5-nm repeat of actin filament, each comprising seven actin monomers, one tropomyosin and one troponin complex (608). This structure supports the idea that nebulin filaments are the template for thin filament organization. Actually, skeletal muscles of mice lacking nebulin show shorter thin filaments and reduced active force production (871). It is, however, clear that nebulin-independent mechanisms also contribute to regulate thin filament length. Tropomodulin (Tmod), the pointed end capping protein, is considered responsible for thin filament length control. Mice lacking Tmod do not form striated myofibrils and die before birth (237). As discussed in a recent review (485), nebulin could act as a stabilizer of a long segment of the thin filament, while the nebulin-independent mechanism would regulate pointed-end dynamics, and the specified filament lengths would be sensitive to Tmod capping activity. There are differences in nebulin size among animal species (442) and splicing variants derived from a single nebulin gene in each species. Larger variants are expressed in muscles rich in slow fibers (621), in accordance with the expression of larger titin variants. The diversity in nebulin and titin size has an impact on the active and passive tension length relations as discussed below.

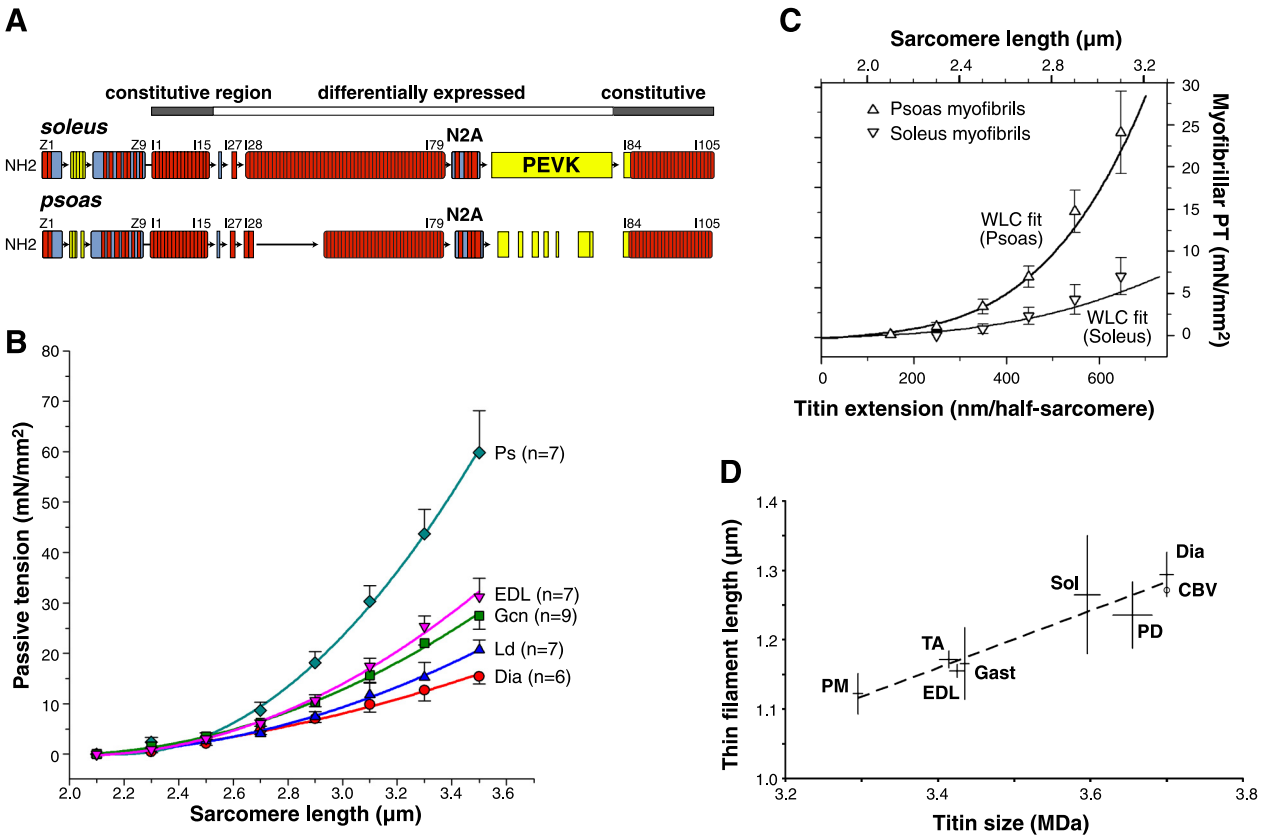


FIGURE 12. Diversity of sarcomeric cytoskeleton and resting mechanical properties in different rabbit muscle types. *A*: schematic representation of the I-band domain of titin in different isoforms. The fast psoas fibers have fewer Ig repeats (orange bands) and shorter PEVK elastic domain (yellow band) compared with slow soleus. [Modified from Granzier and Labeit (282), with permission from John Wiley and Sons.] *B*: passive tension curves of single fibers from various rabbit muscles. Note that fast psoas (Ps) fibers exhibit the highest passive tension. [Modified from Prado et al. (621). Copyright 2005 Rockefeller University Press.] *C*: passive tension of myofibril from fast psoas and slow rabbit soleus is determined by titin elastic properties. Symbols represent experimental data obtained on myofibrils; curves represent the prediction based on WLC (worm-like chain) model of titin. [Modified from Prado et al. (621). Copyright 2005 Rockefeller University Press.] *D*: thin filament length measured as the distance between Z-disc and tropomodulin is linearly related to the size of titin in different muscle types. [Modified from Castillo et al. (135), with permission from Elsevier.]

e) EXTRAMYOFIBRILLAR CYTOSKELETON. Desmin, γ-actin, and filamin C are the basic components of the extramyofibrillar cytoskeleton that connects neighboring myofibrils, nuclei, mitochondria, and sarcolemma. Desmin is the classical type III intermediate filament (IF) protein of striated and smooth muscle cells. Organization of the IF system depends on the proper interaction of desmin with its binding partners, plectin and αB-crystallin. Plectin plays an essential role in linking IF filaments with their targets, with specialized isoforms for linking nucleus (plectin 1), costameres (plectin 1f), Z bands (plectin 1b), and mitochondria (plectin 1d) (428). Plectin role cannot be replaced by other proteins connected to IF such as synemin and syncoilin. The importance of the mitochondria-cytoskeleton docking makes slow oxidative fibers more sensitive to the lack of plectin than fast glycolytic fibers (428). Accordingly, plectin is more abundantly expressed in mitochondria-rich slow oxidative type I, compared with glycolytic type II muscle fibers (293). γ-Actin filaments are present both around the myofibrils at the Z-disc level and in subsarcolemmal location likely contributing to link myofibrillar networks to the membrane systems. γ-Actin filaments bind nonmuscle tropomyosin isoform coded by the γ-*TM* gene, which localizes to the sarcolemma and to a region adjacent to the Z-line in skeletal muscle fibers (see Ref. 399). Filamins are cytoskeletal proteins that link actin filaments into either parallel bundles or three-dimensional meshwork. Filamin C (formerly also called filamin 2, γ-filamin, or ABP-L) is the muscle-specific filamin isoform. It is mainly localized at the periphery of Z-disks and interacts with myotilin and FATZ/calsarcin/myozenin, while at the sarcolemma it interacts with γ- and δ-sarcoglycan in costameres and with Xin in myotendinous junctions (814, 815)

f) COSTAMERES. Costameres are subsarcolemmal protein assemblies, in register with the Z-disc of peripheral myofibrils, which form a link between sarcomeres and sarcolemma in striated muscle cells (585). Subsarcolemmal cytoskeleton also contains many proteins in register with the M line and longitudinal strands that connect components lo-

cated at the M-band with costameres. The canonical focal adhesion protein vinculin is an essential component of the costameres. Two major transsarcolemmal protein complexes are involved in costameres: integrins and the dystrophin-glycoprotein complex (DGC). Integrins bind to specific ligands in the extracellular matrix and recruit cytoskeletal and cytoplasmic proteins with the final results to stabilize the cell and provide a communication between the outside and inside of the cell. Integrins are heterodimers formed by α and β subunits. Among the many isoforms of the α and β subunits, $\alpha7\beta1$ is the predominant integrin in adult skeletal muscle. The $\alpha7$ subunit binds to laminin in the basal lamina surrounding muscle fibers, whereas the $\beta1$ subunit participates in linking with actin several subsarcolemmal proteins, including α-actinin, talin, vinculin, paxillin, and tensin (see Refs. 80, 530). The DGC links the cytoskeleton of muscle fibers to their extracellular matrix. Dystrophin, sarcoglycans, dystrobrevins, syntrophins, and sarcospan gather around the transsarcolemmal components, α- and β-dystroglycans. While dystroglycans bind to laminin in the basal membrane, dystrophin forms a mechanically strong physical linkage with the γ-actin filaments of the extramyofibrillar cytoskeleton. Additional components in the DGC in skeletal muscle include α- and β-dystrobrevin, the syntrophins, neuronal nitric oxide synthase (nNOS), and caveolin 3 (see Ref. 460). Little is known about heterogeneity of costameric proteins and their supramolecular aggregations in relation to fiber type. However, focal adhesion kinase (FAK), which is associated with costameres, shows more obvious sarcolemmal immunoreactivity in the slow type 1 and fast type 2A fibers in rat soleus muscle compared with EDL fast type 2B fibers (224). Cross-reinnervation experiments confirm a direct relation between FAK abundance and fiber contractile activity.

G) MYOTENDINOUS JUNCTION. The myotendinous junction (MTJ) is a specialized structure responsible for the transmission of force from muscle contractile and cytoskeletal proteins to the structural proteins of the tendon (417, 794). Force transmission is based on chains of proteins including vinculin, talin, integrin, fibronectin, and collagen. The $\alpha7\beta1$ integrin is enriched at the MTJ (45). A nebulin-like protein, able to bind actin filament, indicated as N-RAP, has been localized at the MTJ. Based on its structural organization, actin binding properties, and tissue localization, N-RAP appears to be part of a complex of proteins that anchor the actin filaments of the terminal sarcomeres to the membrane and transmit tension from the myofibrils to the extracellular matrix (497). An additional contribution is likely given by proteins involved in cell adhesion. Myonexin and dystrophin, muscle-specific proteins found at MTJs, may also be involved in MTJ force transmission. MTJ membrane shows typical folding, which may play a mechanically important role by reducing membrane stress. Disuse atrophy is associated with decreased MTJ membrane folding and, therefore, an increase in MTJ stress during subsequent reloading. This decrease in membrane folding may be the basis of increased tears in atrophied muscle. No information on diversity of MTJ between slow and fast fibers is presently available. It is, however, known that the collagen content in tendon and muscle ECM is greater in soleus than in tibialis anterior, likely in relation to their different activity (433), and that the increase in response to acute exercise is more pronounced in red than in white muscles (828).

H) FUNCTIONAL IMPLICATIONS OF CYTOSKELETON DIVERSITY. Four specific issues related with the structure and function of the cytoskeleton are worth to be considered in the perspective of fiber type diversity.

I) Slack length and passive/resting tension. From slack length, i.e., the sarcomere length at which muscle fibers settle when left without any strain, and corresponding to sarcomere length between 1.9 and 2.4 μm, resting fibers can be elongated virtually without any resistance in a relaxed muscle fiber by ~0.5 μm/sarcomere. Above that elongation, fibers resist to stretch and generate tension, called resting or passive tension. Resting tension in whole muscles depends partly on the properties of the fibers and partly on the extracellular matrix properties (endomysium and perimysium). Although the level of resting tension observed in mammalian muscle fibers is not strictly a function of fiber type, available data indicate that resting tension is higher in fast than in slow fibers (352, 558, 621) **(FIG. 12, *B* AND *C*)**. Intracellular cytoskeleton is the main determinant of resting tension with minor contribution of the sarcolemma and basal lamina (285, 621). Among cytoskeleton component, titin plays a major role as indicated by the reduction in resting tension upon removal of titin by tripsinization and by the similarity between the stress strain properties of single titin molecule and those of muscle fibers where titin is expressed (402, 621). As discussed above, titin exists in several isoforms with longer isoforms expressed in slow fibers or in proportion with the expression of slow myosin isoforms (621). Longer isoforms of the "N2A-titin" are characterized by greater size of the two variable segments, the tandem Ig region and the PEVK domain, and the expression of longer and more extensible titin isoforms gives a molecular explanation for the lower resting tension in slow fibers.

The contributions of intermediate filaments to muscle passive tension have been analyzed in experiments based on sequential extraction of various cytoskeleton proteins in psoas fiber segments (847) and cardiac myocytes (284). The data show that, in skeletal muscle fibers, the IF network contributes to passive tension only at extreme sarcomere lengths (~5 μm), while in cardiomyocytes it contributes also in the physiological sarcomere length range. Interestingly, fast muscles express lower levels of desmin than slow muscles (149). The results obtained in desmin-deficient

mice, however, are difficult to interpret as variable resting tension changes have been reported (25, 727).

The mechanical properties of resting intact muscle fibers cannot be described only in terms of resting or passive tension, as the tension response to a ramp elongation consists of three components: a viscous (P1), a viscoelastic (P2), and an elastic (P3) component. The three components can be identified on the basis of their sensitivity to stretch velocity. The viscosity coefficient derived from the first component (P1) and attributed to the interfilamentary viscous resistance to stretch within sarcomeres is about two times larger in slow than in fast fibers. The relaxation time of the viscoelasticity (P2) is also significantly larger in slow compared with fast muscle fibers (557). It has been proposed that viscoelasticity reflects properties of the titin molecules with their variants expressed in slow and fast muscles (556).

II) Active tension-length curve. While the bell-shape of the tension-sarcomere length curve is common to all striated muscles, the sarcomere lengths at which maximum tension is generated varies between species and between muscles. For example, the optimal sarcomere length lies between 2.0 and 2.2 μm in fast frog skeletal muscle fibers (see Ref. 277), but is shifted to higher values (2.3–2.6 μm) in mouse and rat skinned fibers (763) and intact fibers (198). In fish muscles, a remarkable difference between slow and fast muscle fibers has been reported (283). The optimum length corresponds to the sarcomere length where the filament overlap is greatest, i.e., in the range comprised between the sarcomere length equal to the sum of thin filament lengths and the sarcomere length equal to the sum of thin filament lengths plus bare zone. Since the thick filament structure and length is conserved in all vertebrates (582), the length of the thin filament becomes the major determinant. As discussed above, thin filament length is determined by the interplay between the stabilizing role of nebulin and the control of actin polymerization at the pointed end operated by tropomodulin (135). According to this view, the difference in the active tension-length curve between slow and fast fibers in the fish is explained by the different thin filament length (1.24 versus 0.96 μm in fast versus slow fibers, respectively) (283) and the difference between frog and mammalian muscles by the difference in filament length (1 μm in frog and 1.1–1.25 μm in mammalian muscles). Very careful measurements of filament length, defined as the distance from Z-disc to tropomodulin, in different fiber types in the rabbit have shown that a good correlation exists between thin filament length and titin length as determined by molecular weight **(FIG. 12*D*)** (135). This implies that slow fibers, which have longer titin isoforms, also have longer thin filaments, with the consequence that their operational range is shifted towards longer sarcomere length compared with fast fibers.

III) Force deficit after eccentric contractions. While inactive fibers are not damaged by lengthening, provided that it does not exceed thick and thin filament overlap, elongation of a contracting muscle or single muscle fiber induces a damage that can be detected from the loss of isometric force development and from ultrastructural alterations (see Ref. 624). In humans and in other mammals, fast fibers are more susceptible than slow fibers to the damage induced by eccentric contractions. This has been observed in permeabilized rat fibers (515), as well as in human muscles after treadmill walking (383) and in rat (829) and rabbit muscles (480). However, unloading induced by hindlimb suspension in small rodents makes slow fibers more vulnerable than fast fibers upon subsequent reloading (830). It remains to be seen whether this effect is due to alterations in the cytoskeleton. One should also take into account that unloading induces a transformation of slow fibers into fast.

There is no definitive answer to the question why fast fibers are more susceptible to damage due to eccentric contractions. A possible suggestion comes from the ultrastructural observations that fast fibers appear to have narrower Z-discs compared with slow fibers (see above), which reflect the lower amounts of proteins connecting thin filaments of the adjacent sarcomeres, in the first place α-actinin, and also narrower M-bands (see above). Some studies have underlined that desmin plays an important role in stabilizing muscle fibers and intermediate desmin filaments are disrupted by eccentric contractions (50). As mentioned above, the desmin content is approximately double in slow compared with fast fibers (149). Finally, it is also possible that the longer titin isoforms expressed in slow fibers (see above) can better withstand stress during eccentric contraction due to their greater compliance.

IV) Force sensor localization. Although it is accepted that tensions applied and/or developed by skeletal muscle fibers are relevant for regulation of muscle fiber size, the localization of the sensors for mechanical load, i.e., the proteins that are able to convert mechanical forces into biochemical signals (mechanochemical transduction) is still debated. While mechanosensitive ion channels (see sect. III*A2*) might provide one important and relatively well-known mechanism of mechanochemical transduction, there is evidence that some cytoskeleton components play also an important role. Available evidence point to FAK, a kinase associated with the integrin complex, thus to transversal force transmission, as an important player in the mechanochemical transduction (193, 424). Other studies, however, have suggested the localization of the load sensor in the myofibrillar cytoskeleton, Z-discs and M-bands being the most likely candidate, thus in relation to longitudinal force transmission. The strain-dependent activation of the titin COOH-terminal kinase domain (TK) at the sarcomeric M-band (625) and some of the signaling proteins in Z-discs (48, 49) have been proposed as possible sites of the mechano-chemical transduction.

I) CONCLUSION. The cytoskeleton scaffold differs in fast and slow muscle fibers both with respect to the relative amount of some components and their molecular composition. These differences are consistent with the properties of the contractile machinery and are specifically correlated with the mechanical tension generated by contraction. The major differences concern the myofibrillar cytoskeleton: Z-discs, M-bands, and sarcomeric giant proteins (titin and nebulin). Continuously active slow fibers have longer and more extensible titin isoforms, thus can withstand passive elongation with less mechanical resistance (different resting tension-length curve and viscoelasticity). Slow fibers have longer nebulin and actin filaments; thus active tension-length curve and optimal length are shifted towards longer sarcomere length. Finally, slow fibers have thicker Z-discs and M-band, a feature which is probably related to the ability to withstand active force. In this respect, it is important to underline that maximal isometric force is higher in fast than in slow fibers, but active forces are generated for longer time in slow than in fast fibers. In addition, the difference in active force disappears during lengthening, and the highest forces are generated just during eccentric contractions. In such conditions, the ability to withstand tension without damage is greater in slow than in fast fibers.

D. Energy Supply: Glycolytic and Oxidative Metabolism

1. ATP consumption and regeneration

All energy required for cellular functions, including contractile activity, is provided by ATP hydrolysis to ADP and P_i. For this reason, the energy consumed by a muscle fiber can be measured in moles of ATP consumed or in energy units (calories or Joules). It is generally accepted that the hydrolysis of 1 mol ATP releases between 50 and 60 kJ under physiological conditions (see Ref. 10 and for a critical discussion Ref. 745). At rest as well as during contraction, the most relevant parameter for muscle energetics is "power": metabolic power, i.e., moles of ATP/unit time generated or consumed and thermodynamic power, i.e., calories or Joules/unit time. A number of distinct approaches allow the determination of the energy consumption rate in muscle fibers, ranging from experiments on permeabilized fibers (325, 619) to microchemical determination of high-energy phosphate in muscle biopsy samples (170, 292), enthalpy determination from heat and mechanical measurements (51), measurement of oxygen consumption (327), and ^{31}P magnetic resonance spectroscopy in vivo (78, 165). In contrast, the term capacity is generally adopted to indicate the total amount of energy stored in muscle fibers in the form of ATP or released during a given period of activity. It is worth to recall that substrate or ATP concentrations are expressed either in mmol/l fiber volume, i.e., Molar units, or mmol/kg dry wt, the ratio between the two units being ~5.

A unique feature of skeletal muscle fibers is the fact that they can undergo extremely large and sudden changes in energy consumption when switching from rest to contractile activity. Energy expenditure in muscle at rest is low: in humans, values of 0.008 mM/s have been determined with NMR spectroscopy in forearm muscles (78) and between 0.004 and 0.008 mM/s in hand and leg muscles (18). ATP turnover at rest is governed by the plasma membrane Na^+-K^+-ATPase and by protein synthesis, two parameters which are not expected to vary greatly among muscle fibers (656). Energy consumption during contraction has two main components: ATP consumed by myofibrillar ATPase (~70%) and ATP consumed by ionic transport (~30%), calcium in the first place (see Ref. 348 and sect. III*C1*E). The myofibrillar ATP consumption rate in physiological conditions (35°C) in human fibers during maximal isometric contraction has been calculated (684) on the data obtained in single human permeabilized fibers in vitro (764) ending with the following values (in mmol·kg dry $wt^{-1}·s^{-1}$): type 1 fibers, 6.5; type 2A, 17.6; type 2X, 26.6. These values should be increased by 30–40% to account for ionic transport, in the first place calcium uptake by SR (765), leading to values ranging from 1.7 to 4.7 and 7.2 mM/s for human slow, type 2A, and type 2X fibers, respectively. NMR spectroscopy-based measurements of ATP consumption in vivo on human adductor pollicis have yielded values of 4.4 mM/s during maximal isometric contraction and 9.7 mM/s during loaded shortening (811). Calculations based on microchemical analysis on single human muscle fibers have shown that during 32 s of electrically induced intermittent contractions, the rate of ATP consumption is 1.2 and 2.4 mM/s in slow and fast fibers, respectively (292). In murine muscles, total energy turnover has been measured from heat production and mechanical work (i.e., enthalpy output) in vitro at 20°C. In EDL, the transition from rest to full activity (maximal isometric contraction) is accompanied by an increase from 1.5 (54) to 134 $mJ·g^{-1}·s^{-1}$ (51), which corresponds to 0.04–2.8 mM/s of ATP. In mouse soleus, enthalpy output is 2.5 $mJ·g^{-1}·s^{-1}$ at rest (54) and 28 $mJ·g^{-1}·s^{-1}$ in full contraction (51).

To face such huge variations in ATP consumption, fast and efficient mechanisms of ATP resynthesis are needed. Three main mechanisms provide ATP resynthesis in muscle fibers: creatine kinase (CK) activity, glycolysis, and mitochondrial oxidative phosphorylation. Phosphocreatine (PCr) serves as a high-energy phosphate reservoir for the rapid regeneration of ATP by CK, thus avoiding ADP accumulation. The PCr-CK system is localized close to the sites of ATP consumption (myofibrils and SR) and represents a high-power and low-capacity ATP regeneration process. A minor amount of ATP is resynthesized via adenylate-kinase: from two ADP molecules one ATP and one AMP are generated. Glycolysis is responsible for glycogen and glucose metabolism leading to pyruvate or lactate production and represents a second source of ATP with lower power but greater

capacity compared with PCr-CK system. Pyruvate, fatty acids, and ketone bodies provide the supply of acetyl-CoA which is the substrate for the mitochondrial oxidative processes characterized by an even lower power but a very high capacity in ATP regeneration. Obviously, energetic substrates and oxygen must be available in muscle fibers or supplied via microcirculation, and removal of by-products, such as lactate and carbon dioxide, is required to keep all metabolic process steadily working.

Slow and fast muscles differ in the relative role of glycolysis and oxidative phosphorylation. Slow muscles are able to generate all ATP they need by oxidative mitochondrial processes; actually, their ATP consumption during contraction is not that high, and this contributes to their ability to maintain contractile activity for long time without showing fatigue. Fast muscles rely upon glycolytic processes to generate ATP very rapidly, and this sets a limit to the duration of their contractile activity. The diversity in metabolic properties, initially observed among red and white muscles, was extended at fiber level by the development of the histochemistry (574, 580). Based on their biochemical properties, fibers were classified as S (slow), FOG (fast oxidative glycolytic), and FG (fast glycolytic) (58, 599). Combined with the physiological analysis of individual motor units, muscle fiber histochemistry revealed that all fibers within a motor unit share similar oxidative capacity (SDH activity), which is among the major determinants of the resistance to fatigue (116, 117, 447, 564). The subsequent development of microchemical methods at the scale of single fibers allowed the determination of enzyme activities and the quantitative description of the metabolic properties of individual muscle fibers (209, 210, 493, 644). Later, ^{31}P NMR spectroscopy opened a new approach to study muscle metabolism in vivo at rest and during contraction making detectable the diversity related to fiber type composition (587, 819). In the magnetic resonance spectra, the shift of the P_i peak with change in pH gives a direct indications of the activation of three distinct fiber type populations (587, 819).

Available evidence shows that metabolic diversity among muscle fibers involves a number of aspects of muscle fiber structure and function, which will be discussed hereafter as follows: *1*) structural basis of metabolic diversity, *2*) substrate transport through sarcolemma, *3*) high-energy phosphate and ATP regeneration from PCr, *4*) glycolytic versus mitochondrial ATP regeneration, and *5*) overview and conclusion.

2. Structural basis of metabolic diversity of muscle fibers

Muscle fibers adapt their size or thickness, their internal structure (relative abundance of relevant intracellular components such as mitochondria), and their extracellular surroundings (for example, capillary density) to better cope with their metabolic activities.

A) FIBER SIZE. Fiber thickness is in general inversely related to the aerobic oxidative activity or to mitochondrial density. Such inverse relation holds inside each muscles, but the same fiber type with identical metabolic properties may have different size in different muscles. For example, in rat gastrocnemius, there is an inverse relationship between SDH activity (indication of mitochondrial density) and fiber size and a positive correlation between α-glycerophosphate dehydrogenase (GPD, indication of glycolytic activity) and fiber size (653). A similar inverse relation between SDH activity and fiber size has been found in rat diaphragm (734), although all diaphragm fibers are thinner than gastrocnemius fibers. Accordingly, there are striking differences between various muscles; for example, slow muscle fibers in the rat soleus are much bigger than slow fibers present in rat fast muscles. Thus both fiber type and muscle of origin are relevant for fiber size, although in some cases, a consistent difference can be found between oxidative and glycolytic fibers. For example, a recent comparative analysis on seven distinct muscles of the dog has shown that, in spite of intermuscle variations, the oxidative slow and 2A fibers are thinner than the glycolytic 2X fibers (1). In most human muscles, with the exception of the jaw closer muscles (721), type 2 fibers are larger than type 1 fibers (see Ref. 686 for a large compilation of morphometric determinations).

B) CAPILLARY DENSITY AND ARTERIOLE RESPONSIVENESS. The number of capillary vessels surrounding a muscle fiber is higher for slow than for fast fibers. For example, in rat muscles, the capillary to fiber ratio is 2.85 in soleus (slow muscle) and 1.46 in peroneus (fast muscle) (67), and the number of capillary per fiber actually perfused at rest is 0.98 in soleus and 0.66 in fast superficial part of tibialis anterior (182). In human muscles, capillary-to-fiber ratio is 4.92 for slow fibers, 4.52 for fast 2A, and 3.52 for fast 2X fibers (23, 739). In dog muscles, capillary density decreases from 3.2 in slow fibers to 2.2 in fast 2X fibers (1).

There is an active debate as to whether in skeletal muscles all capillaries are perfused at rest or there exists a reserve of unperfused capillaries that are recruited during activity (152). Obviously, there is no doubt that blood flow in muscles increases several times during exercise. In this frame, available evidence is in favor of a diversity in the ability of arterioles to dilate and allow the increase of blood flow during exercise. First-order arterioles in rat soleus (mainly slow) express more endothelial nitric oxide synthase (eNOS) (875) and are more responsive to acetylcholine (875, 887) when compared with their counterparts in gastrocnemius (mainly fast). This suggests that arterioles from muscles composed of slow fibers are more prone to vasodilate compared with arterioles from muscle composed of fast fibers. The diversity in vasodilatory response is likely the basis of the different pattern of changes in local oxygen partial pressure during contractile activity (67). In rat mus-

cles, during repetitive stimulation, local oxygen partial pressure declines from resting values close to 30 mmHg, with a shorter delay and a shorter time constant in fast than in slow muscles, and reaches steady-state levels lower in fast (10 mmHg) than in slow muscles (15–20 mmHg). This implies that oxygen supply is better preserved in slow than in fast muscles in the presence of similar contraction patterns (534).

c) MITOCHONDRIAL VOLUME AND STRUCTURE. Mitochondrial content varies significantly in relation to fiber type. In human fibers, the mitochondrial volume varies from 6% in type 1 fibers to 4.5% in type 2A fibers and 2.3% in type 2X fibers (356). In the rabbit, mitochondrial content is 14.5 ± 1.2 mg/g wet wt in soleus versus 5.3 ± 0.6 mg/g wet wt in gracilis muscle, with a 2.7-fold difference (372). In the rat, the fraction volume occupied by mitochondria ranges from 2.2% in FG fibers of gastrocnemius to 10% in SO fibers of soleus (202) and even more in 2A and 2X fibers (710).

Beside their density, mitochondria of slow and fast fibers also differ in their ultrastructure as slow fiber mitochondria exhibit more densely packed crista (260, 372) and in their enzyme activity. In isolated mitochondrial preparations, citric acid cycle enzyme activities are approximately twice higher and electron transport chain (ETC) exhibits twice the capacity for coenzyme oxidation in mitochondria from slow muscles compared with those from fast muscles of the rabbit (372). Mitochondrial generation of hydrogen peroxide is two- to threefold higher in white fast glycolytic muscles than in slow oxidative soleus of the rat (24). Further diversity, including the high concentration of mitochondrial glycerol-3-phosphate dehydrogenase (GPDH) in glycolytic fibers, will be discussed in section III*D5*.

Two distinct mitochondrial subpopulations exist as a continuous reticulum in slow and fast fibers with distinct subcellular localization, morphology, and biochemical properties (356, 411). Subsarcolemmal mitochondria show a large, lamellar shape, whereas intermyofibrillar mitochondria are smaller and more compact and located between the myofibrils in close proximity to the triads where calcium is released from SR. In rat muscles, subsarcolemmal mitochondria are more abundant in oxidative (slow and 2A) fibers than in fast glycolytic fibers and are more responsive to variation of activity, such as disuse and training (441), and to thyroid hormones (41) than intermyofibrillar mitochondria.

d) GLYCOGEN AND LIPID STORES. Stores of glycogen and lipids to be used as substrates for energy production are present in all muscle fibers. Glycogen content at rest is higher in fast than in slow fibers, with fast fibers having 16% (833) to 31% (292) more glycogen than slow fibers. During exercise, glycogen concentration decreases first in slow and thereafter in fast fibers; this probably reflects the orderly recruitment of motor units (833). Lipid content is greater in slow than in fast fibers: in human muscles, triglyceride concentration is 7 mM in slow and 4.2 mM in fast fibers (273), and lipid droplets represent 0.5% of fiber volume in slow type 1 and <0.1% in fast 2X fibers (356).

3. Trans-sarcolemmal substrate transport

Since intracellular substrate stores can only support the regeneration of a limited amount of ATP, muscle fiber energy metabolism depends strongly on substrate trans-sarcolemmal transport. In this regard, great diversity exists between fast and slow fibers. The available data point to a privileged conditions of slow fibers that are equipped with greater abundance of transport mechanisms for lactate, glucose, and fatty acids (FA). With reference to a widely used model, i.e., the comparison between sarcolemmal vesicles prepared from pooled red and pooled white muscles of the rat, lactate and glucose uptake is about twice as great, and FA transport threefold greater in red compared with white muscles (138, 386, 436). Such preparations of pooled muscles are still heterogeneous in fiber type composition, but pooled red muscles mainly contain slow and fast 2A fibers, whereas pooled white muscles contain mainly fast 2X and 2B fibers. On the whole, the data obtained in vitro suggest that red oxidative muscles receive an overall substrate supply more than twice as great as white glycolytic muscles. The difference is further emphasized by the enhancement of transport due to insulin and to contractile activity.

a) LACTATE TRANSPORT. Both at rest and during exercise, skeletal muscle is a major site of lactate oxidation as well as production. According to an accepted view, fast glycolytic fibers produce and extrude lactate that is taken up and oxidized by slow oxidative fibers (cell-to-cell lactate shuttle hypothesis) (106, 609). Lactate released from fast glycolytic fibers can be either used by slow oxidative fibers within the same muscle in which it was produced or can be transported via blood circulation to other muscles or to the liver where lactate is converted to pyruvate and then into glucose by gluconeogenesis. The intracellular shuttle hypothesis (106), which assumes that lactate is directly translocated from cytosol to mitochondria and there converted into pyruvate and oxidized, is more controversial (682).

Lactate is transported through the sarcolemma by a lactate/H^+ cotransport mechanism, whose capacity is greater in red than in white rat muscles; this difference is associated with a higher maximal transport rate (V_{max}) in red muscles, whereas the K_m is similar in the two muscle types (386). Such diversity may be due to a different abundance of transporters and to the presence of specific isoforms. Indeed, lactate transport is mediated by monocarboxilic acid transporters (MCT) which exist in several distinct isoforms. In human skeletal muscles (220), MCT1 expression is high in slow fibers, lower in fast 2A fibers, and almost absent in fast 2X fibers. In contrast, MCT4 expression is low in type 1

fibers and higher in most fast oxidative and in all fast glycolytic fibers, and MCT2 expression is restricted to a subset of slow fibers, which varies from <5 to 40%, depending on the specific muscle considered. In porcine muscles (723), MCT1 has been found in all muscle fibers in small but variable amounts, whereas MCT2 is more abundant in the glycolytic than in the oxidative muscles and MCT4 is abundant and equally distributed. A study on rat muscle fibers (321) has shown that *1*) MCT1 has both sarcolemmal and intracellular localization in slow and fast oxidative fibers, *2*) MCT4 is highly expressed in the sarcolemma of fast fibers but poorly expressed in slow fibers, and *3*) MCT2 is scarcely present at the sarcolemma of oxidative fibers. While the comparison with the results in human and porcine fibers points to interspecies diversity, the presence of MCT inside muscle fibers and its colocalization with cytochome oxidase staining (321) supports the view that MCT is present on the mitochondrial membrane and is involved in intracellular lactate shuttle.

In terms of muscle metabolism, the differential cellular localization and relative abundance of MCT isoforms contribute to the cell-to-cell lactate shuttle. Lactate formed in fast fibers with high glycolytic activity could be released via MCT4 and transported into oxidative fibers via sarcolemmal MCT1 lactate transporters (609). In the frame of the intracellular lactate shuttle hypothesis, imported lactate could be readily taken up and oxidized by subsarcolemmal mitochondria. However, no definitive evidence about the role of each specific isoform is presently available.

B) GLUCOSE TRANSPORT. Glucose is a major substrate for energy production in skeletal muscles, and muscles are the greatest consumers of glucose in the body. For this reason, skeletal muscles play an active part in regulation of plasma glucose concentration, by taking up glucose under the effect of insulin or contractile activity. The facilitated transport of glucose across the plasma membranes of mammalian skeletal muscle fibers is catalyzed by a family of glucose transporter (GLUT) proteins. In striated muscles, the expression of GLUT isoforms depends on the developmental stage. While the insulin-dependent transporter GLUT4 is continuously expressed from fetal life through mature adult muscle, GLUT1 and GLUT3 are found in fetal muscle but disappear around birth and are reexpressed only in regenerating fibers (256, 257). Recently, expression of GLUT11 has been demonstrated in slow-twitch fibers, where it seems to be associated more with intracellular structures than to sarcolemma (258). This pattern of localization is strikingly different from that of GLUT4, which is associated almost exclusively with the plasma membrane in all fiber types (520). The amount of GLUT4 on the plasma membrane is subjected to a fast regulation based on translocation from intracellular vesicular pools and to a slow regulation based on transcription (see Ref. 357). Insulin and contractile activity play a major regulatory role in promoting GLUT4 translocation to the membrane. Insulin acts via the PI3K-Akt pathway on a GTPase activating protein, TBC1D4/AS160, and Rab proteins, while contraction acts mainly via the CaMKII and AMPK, which also act on TBC1D4/AS160 (357, 808).

In rodents, glucose uptake capacity is greater in slow and fast oxidative muscle fibers than in fast glycolytic muscle fibers (275). Single fiber studies in rabbit showed that GLUT4 expression is higher in slow and fast oxidative fibers (427). A direct relation between insulin-stimulated glucose uptake and proportion of type 1 fibers has been demonstrated in bundles of human fibers in vitro (900), although the difference between type 1 and type 2 fibers is less pronounced in human muscle (181).

C) FATTY ACID TRANSPORT. FA enter muscle fibers by protein-mediated transmembrane transport, which likely coexists with passive diffusional uptake. The membrane-associated proteins involved are plasma membrane fatty acid-binding protein (FABPpm), fatty acid translocase (FAT/CD36), and two isoforms of the fatty acid-transport protein family (FATP1 and FATP6) (431). FAT/CD36 colocalizes with caveolin, thus suggesting that caveolae might be involved in FA uptake (832).

FA transport has been analyzed in rat skeletal muscles comparing a pool of red muscle (red gastrocnemius and red vastus lateralis) and a pool of white muscles (white gastrocnemius and white vastus lateralis) (138). Palmitate transport rate has been found to be two to three times higher in red than in white muscles, and this is accompanied by a greater abundance of FAT/CD36 both at mRNA and protein level. This fiber-type specific distribution is reminiscent of that of GLUT4, and this similarity is further supported by the increase in FA uptake and oxidation in skeletal muscle during exercise (84) and by the demonstration of contractile activity-dependent translocation of FAT/CD36 to the membrane (347). In rat skeletal muscles, uptake of palmitate into sarcolemmal giant vesicles increases 50–75% after contractions and correlates with expression of FAT/CD36. In addition, muscle training induces increased FA uptake and utilization (see Ref. 406). It is important to take into account that membrane transport might not be the rate-limiting step and FA uptake might be regulated by their utilization by the muscle fibers (406). Interestingly, in diabetic rats and in obese and diabetic humans, the rate of FA transport into skeletal muscle is markedly increased (see Ref. 138). Two lipases contribute to make FA available as a substrate: the endothelial lipoprotein lipase (LPL) acting on circulating lipoproteins and the sarcoplasmic hormone-sensitive lipase (HSL) acting on intracellular lipids. Both enzymes show a fiber-type-dependent distribution, being more expressed in slow-oxidative than in fast glycolytic muscles (312, 459).

In human skeletal muscles, FAT/CD36 is most abundantly expressed in capillary endothelium, but is also present in muscle fibers where it colocalizes with caveolin-3, thus confirming that FAT/CD36 is localized in the sarcolemma, or in close vicinity (401). In accordance with the distribution observed in rat muscles (see above), in human muscles FAT/CD36 is more abundant in the sarcolemma of type 1 or slow muscle fibers, and much less abundant in the sarcolemma of type 2 or fast muscle fibers (401).

D) MYOGLOBIN AND OXYGEN TRANSPORT. Large amounts of oxygen are required by mitochondria during sustained contractile activity. Oxygen travels along partial pressure gradients by simple diffusion and by myoglobin facilitated diffusion (for review, see Ref. 297). Myoglobin is an iron- and oxygen-binding protein expressed in muscles with a major role in oxygen storage and in oxygen diffusion. It may also play a role as an effective scavenger of nitric oxide and thus participates in the control of mitochondrial respiration (328). In most animals, including rat, guinea pig, rabbits, and cats, myoglobin levels are high in red muscles where type 1 and 2A fibers are predominant and very low in white muscles where 2X and 2B fibers are predominant. Myoglobin expression decreases in the order type 1 > 2A > 2X and is virtually absent in type 2B fibers (578). In human muscles, however, the content of myoglobin in human muscle does not differ greatly between fiber types, with oxidative fiber containing ~50% more myoglobin than glycolytic fibers (376, 563).

E) AQUAPORIN AND WATER PERMEABILITY. The exchange of ions and metabolic relevant molecules through the sarcolemma is accompanied by exchange of water, and this allows a redistribution of water and ions between intra- and extracellular compartments particularly during repeated contractile activity (435, 813). During contraction, water moves from the plasma into the interstitial and intracellular compartments of muscle fibers. The main driving force for the net flux of water into contracting muscle is the increase in intracellular osmolarity mainly due to P_i accumulation resulting from rapid hydrolysis of ATP and to lactate accumulation resulting from the increased rate of glycolysis. Water permeation across the sarcolemma of skeletal muscle is mediated by aquaporins (AQP). AQP exist in several isoforms, among which AQP1, -3, and -7 are expressed in adult rat skeletal muscles (382, 840, 841) with no difference among fiber types. In contrast, expression of AQP4 is restricted to fast-twitch fibers of mammalian skeletal muscles, and such selective expression might account for the faster osmotic response of rat EDL compared with soleus (236). The ability to change quickly the volume to preserve a constant osmolarity may be important to fast fibers when large amount of lactate and P_i are generated during intense contractile activity.

4. High energy phosphate and ATP regeneration by creatine kinase and adenylate kinase

In human muscles, ATP concentration at rest is very similar in slow and fast fibers, with a range of 23.7–25.5 mmol/kg dry wt (4.7–5.1 mM) (396), or slightly higher in fast than in slow fibers (27 versus 22 mmol/kg dry wt) (695). In other species, such as the rat, differences are more pronounced: 18.3 mmol/kg dry wt in slow versus 30.3 mmol/kg dry wt in fast fibers (336). In the mouse, ATP concentration has been found to be 1.6 mM in slow soleus versus 2.5 mM in fast tibialis anterior (632). Such values are obtained in whole muscle or single fiber homogenates and hence reflect spatially averaged distribution across specific fiber compartments, thus leaving open the possibility of heterogeneous ATP distribution inside each fiber. ATP concentrations are continuously adjusted using the available store of PCr, the conversion (ADP + PCr ↔ ATP + Cr) being catalyzed by CK. PCr provides the fastest and most effective way to regenerate ATP during brief bouts of contractile activity but also plays the role of a shuttle between the site of ATP synthesis at the mitochondria and the sites of ATP utilization, myofibrils and SR in the first place (826). Different isoenzymes catalyze the generation of PCr from ATP at the mitochondrial site (CK-MB and CK-mi) and generation of ATP from PCr at the myofibrillar location (CK-MM) (see Ref. 826).

In human muscles at rest, PCr content is slightly higher in fast than slow fibers, the concentrations ranging from 60–70 mmol/kg dry wt (12–14 mM) to 80–90 mmol/kg dry wt (16–18 mM) in slow and fast fibers, respectively (396, 683, 695). Higher values of PCr concentration are reported in other studies: for example, 26 mM (165). The total activity of CK is equal in fast and slow fibers (29) or slightly higher in fast fibers (375, 888), whereas the activity of CK-MB is higher in slow fibers and approximately proportional to citrate synthase (CS) activity (29, 375, 888). The greater PCr content and the higher activity of CK-MM suggest that ATP regeneration from PCr is likely more effective in fast than in slow fibers. In general terms, a more effective buffering action of the CK system on ATP is typical of fast and glycolytic fibers, whereas slow and oxidative fibers are characterized by a lower buffering action and more efficient transfer of high-energy phosphates between mitochondria and energy utilization sites (826, see also sect. III*D5*).

Among the by-products of ATP hydrolysis, total ADP concentration in muscle fibers at rest is ~2–3 mmol/kg dry wt (i.e., 0.4–0.6 mM), but free ADP concentration represents a small fraction, in the range of 10–20 μM. P_i content is higher in slow than fast fibers, ranging in rodent muscles between 0–2 mM in fast muscles and 3.6–6.7 mM in slow muscles (450). In human muscles at rest, P_i concentration varies between 1.5 and 3 mM, increasing in proportion to the percentage of slow fibers (820). Thus P_i/PCr ratio is definitely higher in slow than in fast muscle fibers, and such

a ratio has been proposed as a criterion to distinguish muscle fiber types and evaluate their proportion in muscles using NMR (450). Concentrations of AMP and IMP (which derives from AMP by deamination) are very low at rest (5–20 μM) (747), but may significantly increase during contraction, as discussed below.

PCr is the main source of ATP supply in mammalian skeletal muscles during the transition from rest to activity. Under such circumstances, PCr is rapidly consumed in the attempt to keep constant ATP and its resynthesis follows later at the end of the contractile activity or when other metabolic pathways are activated. PCr depletion is, however, unevenly distributed among fiber types (68). If we take as an example a strenuous short-duration exercise in humans (<30 s, such as the all-out cycling performed during Wingate test), the fast progressing reduction in mechanical power output can be related to deep depletion of high-energy phosphate in different fiber types (397) **(FIG. 13)**. Actually, in a short time, both ATP and PCr quickly decrease, while ADP concentration raises up to 10 times (683). Fatigue under such conditions, measurable as the decrease in power output, may be explained as the consequence of the metabolic challenge that involves a relatively small population of fast fatigue-sensitive 2X fibers (696). In human skeletal muscles, ATP depletion to 30% of resting values occurs after just 10 s of maximal exercise in 2X fibers and is accompanied by an even greater reduction of PCr content. Since 2X fibers generate high peak power and contract with high shortening speed, their energetic failure will imply a great decrease in muscle power output, especially at fast movement rates (397, 696). The fact that depletion is more pronounced in 2X fibers is likely due to their greater ATP consumption rate (see sect. III*D1*). The view that the initial development of fatigue at the start of a series of contractions is due to a mismatch between the rate of energy supply and the rate of energy use has been confirmed also in vitro in isolated murine soleus and EDL with a completely independent approach based on heat production measurements (52).

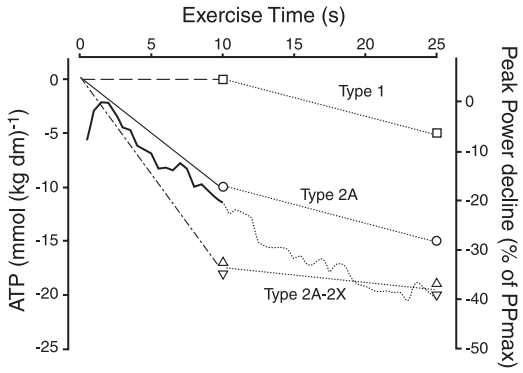


FIGURE 13. ATP concentration declines in different human muscle fibers during during a brief maximal exercise. Mean ATP concentration values are shown at 10 and 25 s for type 1 (squares), 2A (circles), and 2A/2X (triangles) fibers. The continuous noisy line represents the decline of the muscle power in one subject. [Modified from Karatzaferi et al. (397).]

Significant accumulation of by-products occurs during short-duration intense exercise. P_i concentration increases up to eightfold have been observed by NMR (819), whereas free ADP concentration can reach values up to 67 μM in rat gastrocnemius (150) and 200 μM or even 300 μM in rabbit muscles (141). Such increase in ADP concentration plays an important and fiber-type-specific role in activation of mitochondrial respiration (see below). ADP can also support directly ATP regeneration through a reaction catalyzed by adenylate kinase (AK), which converts 2 ADP molecules in 1 ATP and 1 AMP. The AK reaction might contribute to 15% of ATP turnover during short exercise (196). Due to AK activity, during the first phases of an intense contractile activity the concentration of AMP increases, and this is followed by accumulation of IMP (up to 3–10 mmol/kg dry wt), which derives from AMP by deamination (683, 747). The activity of AMP deaminase (AMPD), the enzyme which catalyzes the conversion of AMP to IMP, is higher in fast glycolytic fibers, and this has been explained with expression of fiber specific isoforms (550). The increase of IMP is inversely related to glycogen concentration (683) and might represent a signal to activate glycogenolysis (30, 292). AMP accumulation also plays an important function as it can activate via AMP kinase (AMPK) both GLUT4 and CD36 translocation and fatty acid utilization in mitochondria (see Refs. 866, 869) and control gene expression (see sect. VII*D1*).

5. Glycolytic ATP regeneration

Large diversity exists among muscle fibers in the rate (or power) and in the capacity of ATP generation through glycolytic anaerobic and, respectively, oxidative aerobic metabolism **(FIG. 14)**. In spite of some overlap between the activity spectra of various enzymes of anaerobic and aerobic metabolism in slow and fast fibers, two major fiber groups can be distinguished by some key enzyme activities, determined either with histochemical methods on muscle cryosections or on single fibers with microchemical methods **(FIG. 3, *A* AND *B*)**.

Anaerobic glycolysis is the catabolic pathway from glucose to pyruvate associated with the regeneration of 2–3 mol ATP/mol glucose. Glucose can either derive from transsarcolemmal transport (higher in slow fibers, see sect. III*D3*) or from glycogen stores (greater in fast fibers, see sect. III*D2*). Glycogen breakdown is activated by the increase of AMP and IMP concentrations which follows the start of contractile activity. In mixed human muscles in vivo, the rate of glycogen breakdown is estimated to be 1.9–3.4 mmol·kg dry $wt^{-1}\cdot s^{-1}$ (expressed in glucosyl units) (142, 143) with peaks of 7.5 mmol·kg dry $wt^{-1}\cdot s^{-1}$ after short high-intensity exercise (845). In human muscles, a pronounced difference in glycogenolysis rate has been re-

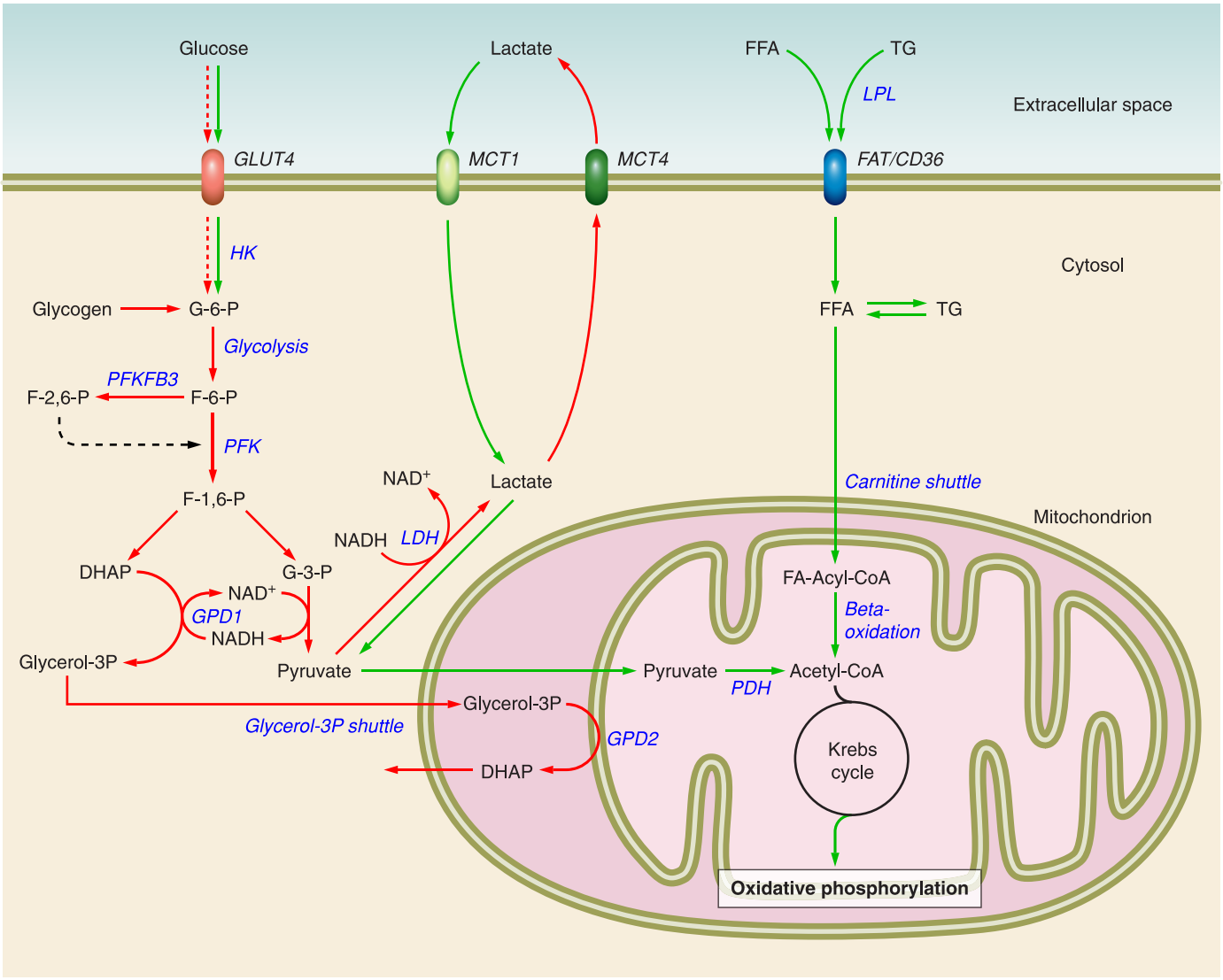


FIGURE 14. Scheme showing some differences in glucose, lactate, and fatty acid metabolism between fast and slow muscle fibers. Pathways prevalent in fast or slow muscle fibers are shown as red or green arrows, respectively. DHAP, dihydroxyacetone phosphate; GLUT4, glucose transporter 4; F-6-P, fructose-6-phosphate; FAT/CD36, fatty acid translocase; FFA, free fatty acids; F-1,6-P, fructose-1,6-bisphosphate; F-2,6-P, fructose-2,6-bisphosphate; G-3-P, glyceraldehyde-3-phosphate; G-6-P, glucose-6-phosphate; GPD1, glycerolphosphate dehydrogenase 1 (cytoplasmic); GPD2, glycerolphosphate dehydrogenase 2 (mitochondrial); HK, hexokinase; LDH, lactate dehydrogenase; MCT1, monocarboxilic acid transporter 1; MCT4, monocarboxilic acid transporter 4; PDH, pyruvate dehydrogenase; PFK, phosphofructokinase; PFKFB3, phosphofructokinase-fructose bisphosphatase 3; TG, triglycerides.

ported between slow and fast fibers during maximal contractions: 0.35 and 0.52 mM/s in type 1 and type 2 fibers, respectively (834), or 0.18 and 3.54 mmol·kg dry wt^{-1}·s^{-1} (corresponding to 0.035 and 0.7 mM/s) in type 1 and type 2 fibers, respectively (292). The higher glycogenolytic activity in fast compared with slow fibers is due partly to the higher phosphorylase content and partly to the more effective stimulation induced by the faster accumulation of AMP and IMP in fast fibers. Actually, with a stronger stimulation obtained with contractions in ischemic conditions, glycogenolysis can increase from 0.18 to 2.05 mmol·kg dry wt^{-1}·s^{-1} in slow fibers but only from 3.54 to 4.32 mmol·kg dry wt^{-1}·s^{-1} in fast fibers (292). Also, epinephrine-stimulated glycogen breakdown in vitro is higher in fast than in slow fibers (377). Thus the pattern of glycogen depletion during muscle contractile activity is highly fiber-type dependent. In physiological conditions of contraction, however, the main determinant of glycogen breakdown is not represented by fiber intrinsic properties, but by motor unit recruitment sequence (see sect. IIC). For this reason, in human muscles, glycogen decreases first in type 1 and type 2A fibers and then in type 2X fibers (272, 833). Glycogen depletion is followed by glycogen resynthesis. The full recovery after a strenuous exercise can require even 24 h, the initial rate (first 3–5 h) being faster in slow fibers. (134, 787).

Phosphofructokinase (PFK) is the rate-limiting enzyme of glycolysis. In mammalian skeletal muscles, only one PFK-M muscle specific isoform is expressed, as a homotetramer, and tends to localize at the membrane in proximity of caveolin-3 (753) and in the sarcomere I band (437). PFK enzyme activity is higher in fast-glycolytic than in slow-oxidative fibers (754). PFK activity has often been taken as discriminative marker between different fiber types, but all enzymes of the glycolytic pathway, including LDH (see **FIG. 3**), exhibit higher activity (up to 9–10 times: 0.3 to 3 mol·kg dry $wt^{-1}\cdot h^{-1}$) in fast than in slow fibers (493). The differential expression of glycolytic genes in fast and slow muscles has been confirmed by PCR and microarray studies in both mouse and human (144, 615). Fast fibers achieve a higher glycolytic power, i.e., can synthetize ATP via glycolysis at higher rate than slow fibers, not only because of the higher level of glycolytic enzymes, but also due to the existence of three additional mechanisms essential to guarantee an efficient glycolytic flux **(FIG. 14)**. Lactate dehydrogenase activity and the glycerol-3-phosphate shuttle (see below) allow the rapid oxidation of NADH produced by glycolysis. In addition, fast muscles have a higher level of phosphofructokinase/fructose-bisphophatase 3 (Pfkfb3) (our unpublished observations), an enzyme responsible for the generation of fructose-2,6-bisphosphate, a potent activator of PFK and thus of glycolysis (331).

The glycerol-3-phosphate shuttle reveals an important difference between mitochondria of fast and slow fibers, namely, the differential distribution of mitochondrial glycerol-3-phosphate dehydrogenase (GPDH), which is more abundant in fast muscles and can be considered as a marker of glycolytic metabolism (599). NADH cannot traverse the inner mitochondrial membrane, and shuttles are used to transfer the reducing equivalents to mitochondria. In skeletal muscle, the glycerol-3-phosphate shuttle is much more important than the aspartate-malate shuttle. Dihydroxyacetone phosphate produced by aldolase serves as acceptor of reducing equivalents generated in the cytoplasm in a reaction catalyzed by cytoplasmic GPDH. The resulting glycerol-3-phosphate can cross the outer mitochondrial membrane and is reoxydized to dihydroxyacetone phosphate by mitochondrial GPDH, a flavin adenine dinucleotide-linked dehydrogenase located on the outer surface of the inner mitochondrial membrane, that transfers the electrons to the respiratory chain. The specific activity of mitochondrial GPDH is highly correlated with its cytosolic counterpart and with the other glycolytic enzymes. Histochemical staining for GPDH, presumably reflecting the activity of mitochondrial GPDH, shows a 2B > 2X > 2A > 1 fiber type expression pattern (311).

The end product of the glycolysis is pyruvate, which can be converted to lactate by lactate dehydrogenase (LDH) with reoxidation of NADH. There are two LDH genes (LDH-A and LDH-B) whose products, M (muscle) and H (heart), combine to form five isoenzymes: LDH-1 (H4), LDH-2 (H3M1), LDH-3 (H2M2), LDH-4 (H1M3), and LDH-5 (M4). These isoforms differ in K_m pyruvate and K_m lactate, the prevalence of M isoforms being associated with pyruvate to lactate conversion and the prevalence of the H isoforms with lactate to pyruvate conversion (523). The distribution of LDH isoenzymes varies according to fiber type, with type 1 fibers displaying a predominance of LDH-1, -2, and -3, whereas the majority (80%) of type 2 fibers contain only LDH-5 (468, 600). About 20% of the fast fibers contain, in addition to LDH-5 as the main component, small amounts of LDH-4 and -3, but no clear-cut differences in LDH isoenzyme expression can be found among subtypes of fast fibers (2A and 2X). The distribution of LDH isoforms is thus consistent with the tendency for the conversion of pyruvate to lactate in fast fibers and the conversion of lactate to pyruvate in slow fibers.

Alternatively, pyruvate can enter the citric acid cycle via the pyruvate dehydrogenase (PDH) complex, which in skeletal muscle controls the glucose oxidation pathway by catalyzing the irreversible decarboxylation of pyruvate to acetyl coenzyme A. PDH is negatively regulated by phosphorylation mediated by pyruvate dehydrogenase kinases (PDK1 to -4). Thanks to its inhibitory control on PDH, PDK acts as switch of fuel oxidation from carbohydrate to fat. PDK activity is less active in glycolytic than in oxidative muscles (601). Two isoforms (PDK4 and PDK2) are expressed in mammalian skeletal muscles, and PDK4 expression is regulated in fast fibers in relation to the feeding state, being upregulated by fasting (770). PDH activity is enhanced by a pyruvate dehydrogenase phosphatase (PDP), which is also more abundant and more active in slow oxidative fibers (469). Interestingly, PDK4 expression is coactivated by PGC1-α, which is one of the main positive regulator of genes related to oxidative metabolism (856).

The glycolytic power, i.e., rate of ATP regeneration based on glycolysis has been calculated to be 2.5 mM/s in fast fibers and 1.2 mM/s in slow fibers during short intense contractile activity in human muscles in vivo (292). The difference is the result of the higher activity of the glycolytic enzymes in fast fibers and implies that, in some fibers, the glycolytic power can be greater than the power of the oxidative phosphorylation (see below).

6. Mitochondrial ATP regeneration

ATP resynthesis via mitochondrial oxidative phosphorylation utilizes acetyl-CoA derived either from pyruvate generated by the activity of PDH or from FA via β-oxidation and can provide 18 ATP per acetate with a consumption of oxygen in a P/O ratio of 2.6.

Substrate availability for mitochondria differs among fiber types. Acetyl-CoA is provided by two sources: *1*) from FA via activation to acyl-CoA, transfer into the mitochondria

via carnitine-acyl-transferase and degradation to acetyl-CoA via β-oxidation, and *2*) from pyruvate, derived from glycolysis via PDH. As mentioned above, PDH is regulated by a number of factors, among them the action of the kinase PDK and the phosphatase PDP. The contribution of FA from β-oxidation to the tricarboxylic acid (TCA) cycle is higher in slow than fast fibers. In fact, type 1 fibers have greater availability and higher utilization of free fatty acids than type 2 fibers. This is based on greater abundance of intracellular lipid stores (see sect. III*D2*), more active HSL and more effective trans-sarcolemmal fatty acid transport (see sect. III*D3*). The mitochondrial carnitine shuttle has an obligatory role in β-oxidation by permitting acyl-CoA translocation from the cytosol. Carnitine palmitoyltransferase I (CPT I), which spans the outer mitochondrial membrane, catalyzes the initial step by transferring acyl groups from CoA to carnitine, and carnitine palmitoyltransferase II (CPT II) regenerates acyl-CoA from acyl-carnitine and transfers the fatty acyl groups into the mitochondrial matrix. CPT I activity is regulated by the concentration of malonyl-CoA, and the isoform of the skeletal muscle CPT I (or CPT I-β) expressed in slow fibers is more active likely because it is less sensitive to the physiological inhibition operated by malonyl-CoA (407). Finally, the activity of 3-hydroxy-acyl-CoA dehydrogenase (HAD), a key enzyme of β-oxidation, is higher in slow than in fast fibers.

As mentioned in section III*D2*, mitochondrial volume varies in different fiber types; thus it is not surprising that mitochondrial enzyme activities measured in single fibers, either histochemically or microchemically, are different (337, 493). Such measurements are generally performed under standard, and very often optimal, conditions so that they can very well show the interfiber diversity, but they say little about the actual contribution of this pathway to ATP supply in different fiber types in physiological conditions. Isolated mitochondria also represent a nonphysiological preparation. Attempts to evaluate the activity of the mitochondrial system in physiological conditions, both at rest and during contractions, have followed three alternative approaches: *1*) analyses in saponin-permeabilized fibers; *2*) biopsy studies on muscle before, during, and after contraction with microchemical analysis and correlations with oxygen consumption; and *3*) NMR possibly combined with optical methods to assess oxygen consumption (noninvasive). The paragraphs below discuss the three approaches in relation to fiber metabolic heterogeneity.

1) Slow and fast fibers permeabilized with saponin analyzed at low temperature (22–25°C) show marked diversity in oxygen consumption when maximally stimulated with ADP: for example, 0.048 mM/s in rat soleus versus 0.022 mM/s in rat gastrocnemius (41, 451, 825). Such values are approximately in proportion to mitochondrial content and to CS activity, although the difference is not completely abolished by normalization to CS activity (299, 300). Actually, studies on single fibers have revealed a difference in regulation of mitochondrial activity between slow and fast fibers in that slow fibers display a much higher apparent K_m of oxidative phosphorylation for ADP than fast fibers (114, 300, 451, 825) **(FIG. 15*A*)**. Such different K_m values have been attributed to specific permeability of outer mitochondrial membrane to ADP (685); for example, in rabbit muscles, values range between 212 μM in slow versus 8 μM in 2X fibers, and in rat muscles between 350 μM in soleus and 8–20 μM in fast muscles (451). Studies on human muscle samples with mixed composition indicate values close to 140 μM, which implies that, at rest with ADP concentration of 10–20 μM, the respiratory rate of the mitochondria is <10% of the maximal (844). In addition, mitochondria in slow fibers are more sensitive to creatine concentration than those of fast fibers, which further increases ADP-stimulated respiration. The greater sensitivity of mitochondria of slow fibers is believed to be due to functional coupling between mitochondrial CK, adenine nucleotide translocase (ANT), and porin/VDAC (11): such coupling does not exist

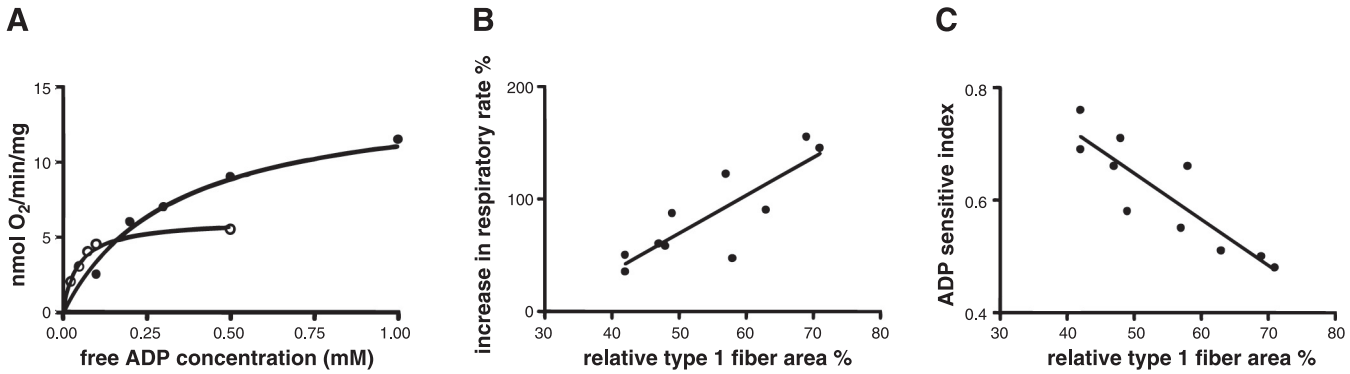


FIGURE 15. Diversity in mitochondria response to variations of ADP and creatine concentration between fast and slow muscle fibers. *A*: dependence of mitochondrial respiration rate on ADP concentration differs between slow rat soleus (black dots) and fast gastrocnemius single fibers (open circles). [Redrawn from Kuznetsov et al. (451).] *B* and *C*: the increase in respiration rate induced by creatine is greater in slow fibers (*B*), whereas the response to increase of ADP concentration is greater in fast fibers (*C*), as indicated by the linear relation between the response and the proportion of slow fibers present in bundles of human skinned fibers. [Redrawn from Tonkonogi et al. (803), with permission from John Wiley and Sons.]

in fast fibers (825). Comparative analysis of human fiber bundles with variable percentage in fast and slow fibers shows that the ADP sensitivity is negatively correlated with type 1 fiber area and oxidative potential, as indicated by CS activity, whereas creatine sensitivity is directly correlated with type 1 fiber area (803, 804) **(FIG. 15, *B* AND *C*)**.

2) The results of the studies in vitro on permeabilized fibers are strengthened by the validation obtained in vivo in different assay conditions, aimed at understanding to which extent is mitochondrial ATP generation activated. Oxygen consumption during contraction increases in both slow and fast muscles: for example, in the rat repetitive contractions (2 min at 1 Hz) in vivo causes an increase from 22 to 141 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ in soleus and from 10 to 72 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ in fast peroneus muscle, values equivalent to 0.016 and 0.007 mM/s of oxygen at rest and 0.104 and 0.050 mM/s of oxygen during activity in slow and fast muscle, respectively (533). Such values are comparable with those observed in human muscles: oxygen consumption increases from 0.006 mM/s at rest to 0.132 mM/s during intense contractions (44), eventually reaching a maximal value of 0.26 mM/s (82). It has been calculated that the oxygen consumption of 0.26 mM/s corresponds to a TCA cycle rate of 0.07 mM/s, which is comparable with the maximal enzyme activity of oxoglutarate dehydrogenase and is well below the maximal activity of other TCA enzyme such as SDH (82). With the assumption of a generation of 18 mol ATP/mol acetate, the yield of TCA cycle, maximally stimulated in physiological conditions, will be 1.37 mM ATP/s, in a mixed human muscle (vastus lateralis). Such rate of ATP generation implies a coupling of ATP molecule synthesized per oxygen atom consumed (P/O coupling) of 2.63. It is possible to hypothesize that such value will result from a higher ATP generation rate in slow fibers and a lower ATP generation rate in fast fibers. It is important to underline that, in slow human fibers, total ATP consumption rate has been calculated at 1.2 (292) or 1.7 (683) mM/s, which could be completely covered by ATP generation in mitochondria. This will never be feasible in fast fibers where ATP consumption rate is much higher (see above).

3) The in vivo approach, based on infrared spectrometry and NMR spectroscopy, has been applied only in resting conditions and confirms the value of oxygen uptake at rest (~0.0015 mM/s) and ATP regeneration (~0.007–0.008 mM/s) (18). Surprisingly, this approach shows that the coupling of oxidation to phosphorylation is higher in fast (P/O 2.7) than in slow fibers (P/O 2.0) (18). The partial uncoupling in slow fibers has been tentatively related to proton leakage or to activation of uncoupling proteins (UCP) and the ANT and is thought to reduce ROS production by reducing the mitochondrial membrane potential (164). Determination, based on NMR, of oxidative phosphorylation during prolonged submaximal contraction of vastus lateralis (electrical stimulation for 2 min at 3 Hz) gives values of 1.1 mM/s (165).

A further difference between slow and fast fibers can be localized in "metabolic inertia," i.e., in the temporal delay in activation of mitochondrial ATP regeneration during a sustained contractile activity. In a classical study, Bangsbo et al. (44) showed that in healthy human skeletal muscles PCr hydrolysis and glycolysis provide 80% of the total ATP generated during the initial 30 s of high-intensity exercise, 45% during the subsequent 60–90 s, and ~30% after 120 s of exercise. The decrease in anaerobic ATP regeneration is covered by a parallel increase in aerobic ATP resynthesis. At least three distinct factors are considered to play a role in determining such delay or "metabolic inertia," and all three differ among fiber types. First, a lag in muscle blood flow may limit oxygen delivery at the onset of exercise, although recent studies (286, 287) have reduced the weight of this factor. As discussed in section III*D*2B, oxygen supply to fast and slow muscle fibers differs in amount and kinetics. Another factor is the activation of mitochondrial respiration by ADP and creatine, which requires a time sufficient for accumulation of these by-products of anaerobic ATP production. Both ADP- and creatine-dependent activation are different in slow and fast fibers as discussed above. Finally, the availability of the acetyl group is limited at the onset of contractile activity (acetyl group deficit) due to a delay in activation of the PDC, which likely represents a rate-limiting step in the rate of rise in oxygen-dependent ATP production (291). Regulation of PDC varies in relation to fiber types, as discussed above.

7. Conclusions

1) The metabolic diversity among muscle fibers shows up as soon as their contractile activity starts. Upon activation, the energy expenditure in muscle fiber increases many times, due to ATP hydrolyzed by both myofibrillar and SR ATPase. Both components vary markedly in relation to fiber type. During high-intensity contractions in human muscles in vivo (35°C), the ATP consumed is 1.5 mM/s in slow fibers and up to 7 mM/s in fast fibers. In murine muscles in vitro (20°C), ATP consumption during isometric contractions is 0.5 mM/s in slow soleus and 2.7 mM/s in EDL. The available stores of ATP and PCr provide energy to support contractile activity for only few seconds, and definitely for a period longer in slow than in fast fibers. Thus, even on a very short time base, although fast fibers can generate more power, they can only contract for a shorter time compared with slow fibers.

2) Immediate ATP regeneration is supported by CK and partly (up to 15%) by AK, which is more active and thus leads to greater AMP accumulation in fast than in slow fibers. AMP is a substrate for AMP deaminase, which is also more abundant in fast fibers where, by generating IMP, causes greater activation of glycogenolysis. On the other

hand, AMP activates AMPK, which is more active in slow fibers and thus causes greater glucose uptake and β-oxidation in slow than in fast fibers.

3) ATP regeneration through the glycolytic pathway is more effective in fast than in slow fibers as the glycolytic activity is about two times higher in fast fibers. In both fiber types this is not sufficient to cope with maximal ATP consumption, thus setting a limit to the use of this source for contractile activity. The pyruvate produced can be either converted to lactate and exported or decarboxylated to acetyl-CoA, the balance between the two alternatives being shifted towards acetyl CoA in slow fibers and towards conversion to lactate in fast fibers. The presence of different LDH isoforms and PDH regulation via kinase (PDK) and phosphatase (PDP) control the switch between the two alternative pathways.

4) ATP regeneration based on TCA cycle is more effective in slow than in fast fibers due to the greater mitochondrial density and greater TCA cycle fuelling based on β-oxidation, which is about three times higher in slow than in fast fibers. Diversity is also present in the regulation of mitochondrial activity between slow and fast fibers, with different effects of the stimulation due to ADP, more effective in fast fibers, and the stimulation due to creatine, more effective in slow fibers. Importantly, once mitochondrial respiration and ATP regeneration are activated, in slow fibers a complete energy balance can be achieved, i.e., consumption is covered by regeneration, also in view of the relatively low ATP consumption. Such condition of balance is never achieved in fast fibers.

5) As discussed in section II, the total activity of muscle fiber, calculated for example on a daily or weekly basis, differs markedly depending on motor unit activity **(FIG. 2)**. In this perspective, the energy need over a long period, for example, 24 h, can be estimated on the basis of the recorded electrical activity, which is 100–200 times greater in rat slow than in fast muscles, and the ATP consumption during contraction, which is four to five times greater in fast muscles than in slow muscles. The calculations point to a daily energy consumption even 40 times greater in slow than in fast fibers. The difference is likely somewhat lower as, due to different firing rate of motor neurons, activation level is likely lower in slow than in fast muscles. A more conservative calculation might lead to the conclusion that the overall daily energy expenditure in a slow oxidative muscle is likely 10–20 times greater than that of a fast glycolytic muscle. This implies another important diversity between slow and fast fibers: slow fibers are able to take up 20 times more substrates (glucose, fatty acids, amino acids, and lactate) than fast fibers. The diversity is relevant for the energy economy of the whole organism, and a different proportion of slow and fast fibers in muscles can be anticipated to be relevant for the many other organs and tissues. The contribution of muscle to global energy is important also when comparing animal species with different muscle fiber composition, such as mouse and human, or individuals with different fiber type distribution.

IV. VARIATIONS IN MUSCLE FIBER TYPES IN RELATION TO SPECIES, GENDER, AND INDIVIDUAL POLYMORPHISM

A. Species Diversity

Specialized fiber types are present in skeletal muscles from all mammalian species, and several general aspects are common. For example, fast and slow fibers, as well as fibers with variable oxidative enzyme content, are easily identified in most species. There are, however, significant variations among species, which emerged during evolution to accommodate specific functional demands. Body size has a major role in determining the functional demands on skeletal muscles. Muscles from small mammals like mice consist predominatly of 2X and 2B fibers with abundant oxidative enzymes, whereas muscles from large mammals like humans have lower levels of oxidative enzymes and are mainly composed of type 1 and 2A fibers **(FIG. 16*A*)**. Body size is relevant for energy metabolism and for locomotion. Energy metabolism for unit body mass is inversely related to body size (Kleiber's law), basal or resting metabolic rate per unit mass (BMR) varying with the three-fourth power of body mass (419). This implies that the metabolic activity of skeletal muscle, which represents the most abundant tissue of the body, is higher in smaller species than in large animals. BMR varies with the three-fourth power of body mass (419), and this implies that metabolic activity is higher in muscle fibers of small than large animals. BMR, however, is determined not only by skeletal muscle metabolism, but also by heart and liver metabolism. On the other hand, maximal metabolic rate per unit mass (MMR), i.e., the metabolic rate during a very high intensity aerobic exercise is virtually determined by muscle fiber metabolism. Also MMR scales with body size with a power below one or, in other words, decreases with body size when normalized to unit of mass (853). This is the result of a higher activity of the mitochondrial processes of substrate oxidation **(FIG. 16*B*)** and can be related to the volume occupied by mitochondria in muscle fibers and to the density of capillary vessels (see Ref. 351 and for a more general discussion Refs. 350, 853). In the common shrew, the smallest mammalian species, whose muscles are composed exclusively of type 2X and 2B fibers (701), mitochondrial volume density can be more than 35% of the fiber volume, as in the diaphragm of *Sorex minutus* (528), compared with values of ~2–5% of the fiber volume in human skeletal muscle fibers (356). Muscle fiber thickness is lower in small compared with large mammals; for example, the average fiber diameter is 18 μm in the shrew diaphragm (only 2X and 2B fibers), whereas it is 44 μm in bovine diaphragm (slow fibers) (261).

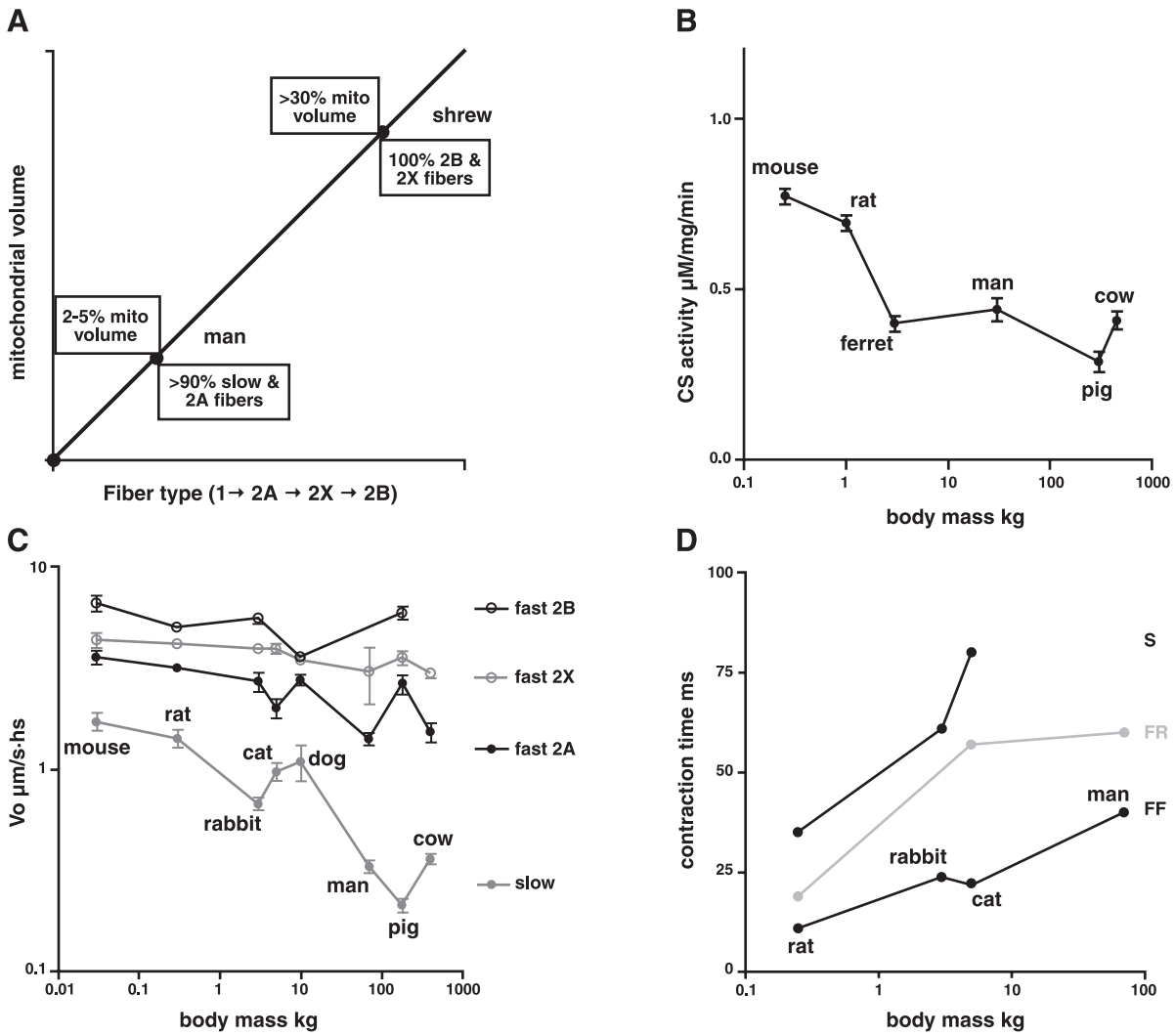


FIGURE 16. Scaling of different muscle metabolic and contractile parameters with body mass in mammals. *A*: increase in body mass (human vs. shrew) correlates with increase in type 1/slow fibers and decrease in mitochondrial volume. *B*: mitochondrial citrate synthase (CS) activity decreases with increasing body mass in different mammalian species. [Redrawn from Hodge et al. (340).] *C*: unloaded shortening velocity (V_o) of single type 1 and 2A fibers decreases in proportion with body mass in different species, whereas 2X and 2B fibers show only minor variations. V_o was determined in single fibers permeabilized and maximally activated at 12° in the same conditions. [Data from Toniolo et al. (801).] D: contraction time increases in proportion with body mass in FF, FR, and S motor units in different species. [Data from Close (157) for rat, Bagust (39) for rabbit, Burke (115) for cat, and Fuglevand et al. (245) for human.]

Although body size among terrestrian mammals can vary over 6 order of magnitude (5–5,000,000 g), the essential conditions for feeding, hunting, and reproduction require that locomotion speed becomes virtually independent of body size, considered either as mass or limb length. As pointed out by A. V. Hill (334), this implies that muscles from small-size animals must be able to shorten faster than those from large-size animals. This requirement is met through two distinct specializations. The first is that the proportion of fast fibers (especially 2X and 2B) in each muscle, and also in the whole body, is higher in small than in big animals. For example, fast fiber proportion in the "slow" soleus muscle is 100% in the shrew, 60% in the mouse, 20% in the rat, and virtually 0 in the rabbit. In addition, fibers expressing MyHC-2B, which are abundant in rat and mouse muscles, are absent in most skeletal muscles of large mammals, including bovine (782), horse (652), goat (33), baboon (495), and human (22, 744), except in specialized muscles, such as bovine eye muscles (510) and dog eye and laryngeal muscles (801). Muscle fibers expressing MyHC-2B transcripts but not protein were detected in human eye and jaw muscles (354). However, the relation between presence of MyHC-2B and body size is not absolute, as MyHC-2B is widely expressed in guinea pig (278), rabbit (838), pig (470), and llama skeletal muscles (288) but not in cat (780) and dog muscles (1, 801). Muscle fibers containing MyHC-2B have likewise been detected in different marsupial species, independently of body size (898).

The second specialization is that the maximum velocity of shortening in each specific fiber type decreases from small to big animals (525, 594, 658, 722, 800). For example, slow fibers from human skeletal muscle are slower than slow fibers from mouse muscle (594). This point is illustrated in **FIGURE 16*C***, where all data are obtained under the very same experimental conditions. Note that the negative slope of the double logarithmic relation is more evident in slow fibers and much less in fast fibers. Importantly, the variations in maximum shortening velocity are the direct effect of variations in functional properties of myosin paralogs, as a similar trend is detectable also when speed of actin of translocation measured by in vitro motility assay is plotted versus body mass (594, 802). For example, rat slow myosin moves actin filaments at higher speed than human slow myosin; however, the corresponding MyHCs are very similar, showing only 14 nonconservative substitutions, most of which are located in domains so far not assigned a functional role (129). Interestingly, in some of these positions the rat MyHC-β/slow has the same residues found in fast skeletal MyHCs. However, the correspondence between maximal velocity of shortening and body mass is not absolute. For example, in the horse, type 1 or 2A fibers are faster than the corresponding human fibers in spite of a fivefold greater body mass in the horse (525).

Not only shortening velocity but also tension development is faster in muscles of small mammals. The time parameters that characterize the kinetics of isometric twitch are much faster in small than in big animals **(FIG. 16*D*)**, and this is true for both fast and slow motor units. As observed several years ago by Close (158) and discussed in section III*B1*, there is a close relation between maximum shortening velocity and contraction time, as a result of the relation between kinetics of cross-bridge formation and kinetics of calcium release and reuptake. The stimulation frequency necessary to obtain fused tetanic contractions is, accordingly, higher in small than in large animals. For example, maximal force is attained at a frequency of ~50 Hz in human muscles in situ (513), while rat fast motor units reach tetanic fusion only above 100 Hz and slow motor units above 70 Hz (613).

The relation between shortening velocity and body size is, however, made more complex by the interaction of body size with at least three other relevant factors: length of limbs (see Ref. 525), type of locomotion (biped versus quadruped, digitigrad versus plantigrad), and lifestyle. The maximal shortening velocity of skeletal muscles is "optimized" so that, to quote the classical A. V. Hill's paper (334), a cheetah should be able to produce enormous power over a very short period while a gazelle should be able to cruise efficiently at high speed but with less capacity to accelerate. Lifestyle also refers to feeding habit so that masticatory muscles of ruminants must be suited to long-lasting low-intensity activity, while jaw closer muscles of the carnivores must develop force and power sufficient to kill the prey and tease apart muscles and bones. Unique features of muscle properties may be related to the specific habitat of certain mammalian species. For example, skeletal muscles of diving marine mammals, such as dolphins and whales, have a much darker color than terrestrial mammals due the very high myoglobin concentration, required to sustain aerobic metabolism during diving hypoxia (see Ref. 391).

The impact of the diversity in lifestyle is obvious in masticatory muscles. The functional demands on these muscles vary greatly with the lifestyle, diet, and eating habits of the animal. While the locomotory demands are satisfied simply by changing the proportions of the four major fiber types in limb muscles and/or by changing the contractile properties of each fiber type, masticatory muscles of different species are extremely divergent in fiber type composition. For example, the jaw-closer masseter muscle is homogeneously composed of fast type 2 fibers in mouse, rat, and guinea pig (705) but contains only slow type I fibers in cattle and sheep. The jaw closers of carnivores and several other orders of mammals, including primates but not humans, are mainly composed by fibers expressing a jaw-specific MyHC coded by *MYH16* (see sect. III*C1*) (666). A still different composition in fiber types is found in rabbits, in which jaw closers comprise muscle fibers containing α-cardiac MyHC in addition to type 1 and 2A fibers (99, 206) and in kangaroos whose jaw closer muscles are homogeneously composed of fibers expressing α-cardiac MyHC (346). Various combinations of MyHCs, including α-cardiac, β/slow, 2A, 2X and developmental MyHCs, are often coexpressed in human jaw closer muscle fibers (758); a distinctive feature of human jaw closer muscles is the small size of type 2 fibers, possibly related to the mutation of *MYH16* gene (760).

B. Sexual Dimorphism and Gender Diversity

Although the fiber type composition of most skeletal muscles is essentially similar in males and females, some muscles display a striking sexual dimorphism in certain species. This is true for jaw muscles, such as temporalis and masseter muscles. In the guinea pig, the temporalis is a "fast-white" muscle in the male but a "fast-red" muscle in the female (307), and both the histochemical and the contractile protein phenotypes can be reversed by castration in the male or by testosterone injection in the female (307, 506). The mouse masseter, in addition to a major MyHC-2X component, contains fibers reactive for MyHC-2B, which are more abundant in the male, and fibers reactive for MyHC-2A, which are more abundant in the female (197); accordingly, the rate of force generation and relaxation is slower in female masseter in parallel with the reduced proportion of type 2B fibers (180). While castration results in an increase in the expression of type 2A fibers in males, ovariectomy has no effect on the fiber type composition in females; thus testosterone appears to play a major role in the maintenance

of MyHC expression in the adult male mouse masseter (197). The rabbit masseter contains type 1 fibers, coexpressing MyHC-β/slow and MyHC-α, and type 2A fibers, the latter being more abundant in the male (206). Sexual dimorphism in fiber type composition is also present in jaw muscles of other species, including rat and macaque. An extreme form of androgen dependency is seen in some perineal muscles, such as the rat levator ani: this muscle undergoes complete involution and disappears after birth in female rats unless the animals are injected with testosterone (306).

In humans, like in most mammals, there is obvious gender diversity in skeletal muscle mass, which is dependent on the increased testosterone levels induced in men after puberty. The fiber type profile in terms of MyHC composition is essentially similar in men and women; however, there is a greater ratio of type 2 to type 1 fiber mass in men, possibly due to greater hypertrophy of type 2 fibers induced by testosterone (see references in Ref. 855). Thus, in men, the levels of MyHC-slow transcripts are lower than in women and the levels of MyHC-2A and -2X transcripts are higher (855). Other sex-related differences in gene expression have been detected by microarray analysis in human skeletal muscle, some of which may contribute to the sex difference in muscle mass (855). Significant sex differences are also seen with respect to some metabolic properties, such as lipid metabolism (406). Intramuscular triacylglycerol content and fatty acid translocase (FAT/CD36) protein levels are higher in women than in men, and women use more lipids during exercise than men. The factors responsible for these gender differences in muscle metabolism are not known.

C. Individual Variability

Significant individual variations can be detected in the fiber type composition of human skeletal muscles. For example, the proportion of type 1 fibers in the human vastus lateralis muscle was found to vary from 15 to 85% in a large cohort of individuals, including both sedentary and physically active individuals of both sexes, 25% of individuals having either less than 35% or more than 65% type I fibers (738). Analyses of muscle biopsies from monozygotic and dizygotic twins indicate that almost 50% of this variance is associated with genetic factors (737). Muscle biopsies of athletes also show marked variations in the fiber type profile, with a tendency for type 2 fiber predominance in sprint athletes and slow type I fiber predominance in endurance athletes (168). It is not known to what extent these variations are produced by training or simply reflect the genetic endownment (21). The molecular basis of genetic variation remains to be established, but the rapid progress in the sequencing of the individual genomes should lead to the identification of single nucleotide polymorphisms (SNPs) or possibly copy-number variants of specific genes implicated in defining the fiber type profile of each person.

An interesting type of polymorphism has been detected in a contractile protein, α-actinin-3, which is normally expressed only in type 2 fibers (567) and is especially abundant in human type 2X (831) and mouse type 2B fibers (540) (see Z-disc proteins in sect. IIIC2). This protein is surprisingly absent in a substantial percentage of the population (~18% of the population in Europe) because of homozygosity for a mutation (R577X) leading to a premature stop codon (568). This mutation does not result in any disease phenotype; in contrast, it may predispose to better endurance exercise performance, as shown by the slightly higher frequency of the X variant in endurance elite athletes and of the R variant in elite sprint athletes, a difference that is statistically significant in females but not in males (889). Another study has confirmed the existence of a significant association between the R577X genotype and strength in women but not in men, suggesting that *ACTN3* is one of many genes contributing to genetic variation in muscle performance and adaptation to exercise (154). The finding that the number of type 2X fibers is greater in the RR than in the XX genotype group has suggested that this mutation may be associated with fiber type distribution (831). How could α-actinin polymorphism affect the muscle fiber type profile? One possibility is that this effect is mediated by calcineurin signaling, which is involved in fiber type specification (see sect. VII). Like other Z-disk components, α-actinins interacts with calsarcins/myozenin/FATZ (see sect. IIIC2), a family of three calcineurin-binding proteins expressed exclusively in striated muscle which negatively modulate calcineurin function (233, 234). Given the role of calcineurin in promoting slow-oxidative phenotype, it is not surprising that the lack of calsarcin-1, expressed in slow fibers, leads to an enhanced calcineurin signaling with consequent increase in slow fibers (231) and that lack of calsarcin-2 expressed predominatly in fast fibers leads to an increase of type 2A fast oxidative fibers and improved fatigue resistance in fast muscles (232).

V. FIBER TYPE DIVERSIFICATION DURING MUSCLE DEVELOPMENT AND REGENERATION

A. Fiber Types in Trunk and Limb Muscle During Embryonic Development

Muscle fiber type diversification is observed since early developmental stages in different vertebrate classes. This initial fiber diversification phase is independent of neural influence and reflects an intrinsic heterogeneity of myoblast lineages. The notion that embryonic skeletal muscles derive from distinct myoblast lineages committed to form different fiber types independently of neural influence was first proposed by Stockdale, based on the finding that two distinct myoblast clones can be isolated from embryonic chick muscle, one expressing both fast and slow isoforms and another one express-

ing only fast myosin isoforms (766). Accordingly, chick muscles with predominant fast or slow myosin composition were detectable in vivo even when neuromuscular transmission was completely blocked by curare since early embryonic stages (171). In chick embryos, nerve-independent diversification of muscle fibers occurs concomitantly with muscle-independent diversification of motor neuron, supporting the hypothesis that different classes of motoneurons innervate selectively muscle fibers of the appropriate type during early neuromuscular development (633).

A precocious diversification of fast and slow myogenic lineages is seen in zebrafish, with a subpopulation of adaxial cells of the paraxial mesoderm becoming committed to the slow lineage under the influence of the transcriptional repressor Prdm1/Blimp1, which is activated in response to Hedgehog signaling: Prdm1 inhibits directly the fast muscle genes while promoting indirectly the slow gene program by inhibiting the transcriptional repressor Sox6 (60, 835).

In mammals, there is no direct evidence for heterogeneity of muscle cell lineages at earliest stages of embryonic myogenesis. In the mouse, before E16 all muscle fibers express both MyHC-emb and MyHC-β/slow (162, 505, 560), and both fast- and slow-type isoforms of many other muscle genes (565). Fiber type diversification is first observed at E16, with some primary generation fibers losing MyHC-slow expression and acquiring MyHC-neo. The two populations of primary generation fibers expressing either MyHC-emb and -neo or MyHC-emb and -β/slow will give rise to fast or slow muscle fibers, respectively (162). In the same time, a new wave of muscle fibers (secondary generation fibers) is formed, which express MyHC-emb and -neo but not MyHC-β/slow, although in slow muscles they also tend to switch to MyHC-β/slow expression. Which signaling pathways control fiber type diversification at these stages? Six factors appear to control the fast gene program, as *Six1-Six4* double knockout mice show specific downregulation of many fast-type genes already in E10.5 somites (565). The transcriptional repressor Sox6 is also involved, as mutant mice lacking a functional *Sox6* gene show upregulation of slow- and downregulation of fast-type genes already at E15.5 (309, 310). The transcription factor Nfix, whose expression is induced by Pax7 in fetal muscle, activates the transcription of fetal specific genes such as muscle CK while repressing embryonic genes such as MyHC-β/slow, thus has been proposed to control the switch from embryonic to fetal myogenesis (538). MyHC-β/slow is upregulated in embryonic muscles of *Nfix* knockout mice, but downregulated in transgenic mice overexpressing Nfix. This effect appears to be mediated by a transcription factor of the NFAT family, NFATc4, whose expression is negatively regulated by Nfix and is known to control MyHC-β/slow expression like other members of this family (123).

Also in mammals, fiber type diversification is independent of neural influence, as shown by experiments using β-bungarotoxin to destroy motor neurons in rat fetuses prior to hindlimb muscle innervation (163). Although muscle growth was dramatically affected by loss of motor neurons, aneural muscles in E20 fetuses contained fibers expressing either MyHC-neo or -β/slow with differential distribution (163). These observations support the view that muscle fiber type identity is established independently of innervation, and some form of matching of the types of motor neurons and muscle fibers might be occurring during early synaptogenesis. This interpretation is consistent with the finding that early motor units are largely homogeneous with respect to fiber type even during the period in which they are polyneuronally innervated (792).

One might ask the question whether developing fibers expressing MyHC-emb and/or -neo represent a particular "fiber type" with specific contractile properties or are just transitional structures preceding the emergence of the definitive fiber types. Several lines of evidence indicate that contractility is required in developing muscle both at the single-cell level for muscle cell differentiation and for maturation of NMJs, and at whole body level for embryonic development. Muscle cell contraction is required for correct myofibrillar growth in developing muscle fibers (634) and for the stabilization of synaptic acetylcholine receptor at the NMJ and suppression of ectopic synapse formation (374). On the other hand, embryonic motility has an essential role in the development of the musculoskeletal system. In the chick embryo, curare or botulinum toxin causes muscle atrophy as well as impaired formation of some cartilages and of joint cavities leading to ankylosis of joints (189, 456, 555). Given the crucial role of embryonic muscle contractile function, one may ask whether embryonic and neonatal myosins have functional properties that are appropriate for this role. It has been suggested that load-bearing may be a physiological stimulus for the developmental transitions of muscle protein isoforms, embryonic and neonatal myosins being formed during a prenatal developmental phase when essentially no load is presented to the muscle and adult myosins during the load-bearing postnatal phase (131). Interestingly, developmental MyHC isoforms persist in extraocular muscles, which contract against very low load, at variance with other adult muscles (698, 864). However, there are no functional studies on embryonic and neonatal MyHCs to support this hypothesis.

B. Diversification of Head and Neck Muscle Fibers

Head muscles do not derive from the somites like trunk and limb muscles but from presomitic cranial mesoderm (see Ref. 566). Cranial mesoderm cells populate the branchial arches, also known as pharyngeal arches, the templates of the adult craniofacial structures, where they are closely associated with cranial neural crest cells. The origin of all head muscles has not been completely defined, and various head muscles originate in distinct anlagen: jaw muscles, as well as inner ear and most pharyngeal and laryngeal mus-

cles, derive from the mesoderm of the pharyngeal arches, but extraocular muscles develop from the mesoderm in the periocular region. In contrast, the tongue and other muscles in the neck are derived from myoblasts originating in the most anterior set of somites (occipital somites). The complexity of the mesodermic fields giving origin to head muscles is highlighted by the finding that cranial mesoderm fields overlap with the lateral splanchnic mesoderm, which has cardiogenic potential. In particular, cells expressing Islet1 (Isl1), a marker of the secondary heart field, contribute not only to the anterior pole of the heart, but also to the myogenic mesoderm of the first branchial arch (see Ref. 812).

Head muscles also differ from trunk and limb muscles with respect to the transcription factors involved in myogenesis (see Ref. 110). In the somites, myogenesis is governed by the four members of the basic helix-loop-helix (bHLH) family muscle regulatory factors Myf5, Mrf4, Myod, and myogenin and by the paired-box transcription factors Pax3 and Pax7. Myf5, Mrf4, and Myod are responsible for the commitment of myogenic precursors, as shown by the complete loss of myogenic precursors when all three factors are missing, whereas myogenin is an essential differentiation factor. Pax3 and Pax7 are also essential for somitic myogenesis, as shown by the loss of myogenic precursors in Pax3:Pax7 double-mutant somites. In contrast, Pax3 is not expressed and Pax7 is expressed only at later stages in head muscle precursor cells, which therefore differentiate in the absence of Pax3 and Pax7. Whereas somitic muscles can be formed in the absence of Mrf4 and Myf5, due to the activation of Myod by Pax3, extraocular muscles are entirely dependent on Mrf4 and Myf5. Other genes, including the bicoid-related homeodomain gene *Pitx2* and the Tbox containing gene *Tbx1,* are important in head muscle formation (690). *Pitx2* null embryos lack EOMs (248, 415), and branchial arch myogenesis is impaired in *Tbx1* mutants (380, 403). Two related bHLH proteins, MyoR (Musculin or Msc) and capsulin (Tcf21), are involved in the formation of jaw muscles derived from the first branchial arch: masseter and temporalis muscles are completely absent in *MyoR* and *capsulin* double null mice (494). The different origin of trunk and head muscles is also reflected in the satellite cells associated with these muscles. Whereas satellite cells in trunk and limb muscles can be identified by the expression of Pax3, and later Pax7, cranial satellite cells derive from separate genetic lineages, defined by *MesP1* and, in some muscles like the masseter, by *Isl1* (319). Satellite cells were isolated from jaw or EO muscles and analyzed in culture or after transplantation into limb muscles to determine whether the unique contractile protein isoforms present in these muscles reflects a distinct myoblast lineage. The presence of specific MyHC, MBP-C, and tropomyosin isoforms appears to be due to a cell autonomous property of the jaw-specific myogenic lineage, as satellite cells isolated from cat jaw muscles express in culture these unique myofibrillar proteins (393). However, rat EOM satellite cells were not found to express EOM-specific MyHC-EO and MyHC-15 in culture, and MyHC-EO was not detected when EOM satellite cells were transplanted into limb muscles (690).

C. Differentiation of Intrafusal Fibers in Muscle Spindles

The development of muscle spindles has been analyzed in detail in the rat and mouse: in these species spindles are formed during late fetal and early postnatal development through complex bidirectional interactions between nerves and muscle fibers (see Refs. 146, 454) **(FIG. 17)**. The pioneering studies of Zelena showed that sensory, not motor, innervation is essential for the differentiation of the muscle spindles (892, 893). Accordingly, sensory but not motor denervation in developing muscle prevents the appearance of slow tonic myosin in the intrafusal fibers (444). Sensory neurons are also responsible for blocking the growth of intrafusal fibers: these undergo marked hypertrophy resembling extrafusal fibers after nerve lesion in 4- to 7-day-old rats, which causes disappearance of sensory nerve terminals in most spindles, typical thin intrafusal fibers being only found in the rare spindles with sensory terminals (712). It has been suggested that intrafusal fibers are maintained in an immature state by sensory innervation, both with respect to growth and MyHC complement (843). However, the view that slow-tonic MyHC is a slow-developmental isoform widely expressed in most embryonic muscles (843) has not been confirmed in more recent studies (661); in addition, both α-cardiac MyHC and MyHC15 are not expressed in embryonic muscle. Therefore, intrafusal muscle fibers appear to be specialized fibers that share some features of immature myofibers but have also unique properties not present in developing muscle and presumably related to their specialized function.

The factors implicated in the early contacts between sensory neurons and primary myotubes, which in the mouse occur at E13–14, have not been identified, and it is debated whether muscle fibers destined to become intrafusal are induced by sensory innervation or are already predetermined. One factor produced by sensory nerves, neuregulin 1 (Nrg1), appears to be essential for the induction of muscle spindle (338) through the activation of ErbB2 tyrosine kinase receptors on primary myofibers (28, 477). Among the transcription factors induced in myotubes under the influence of Nrg1 shortly after myotubes are contacted by Ia afferents, the zinc finger transcription factor Egr3 (early growth response gene 3) is especially important because in *Egr3*-deficient mice spindles are formed during embryogenesis but disappear after birth and intrafusal muscle fibers that express slow-tonic MyHC fail to differentiate (806, 807). Thus it appears that Egr3 has an essential role in regulating genes required for the transformation of undifferentiated myotubes into intrafusal fibers. Egr3 is also es-

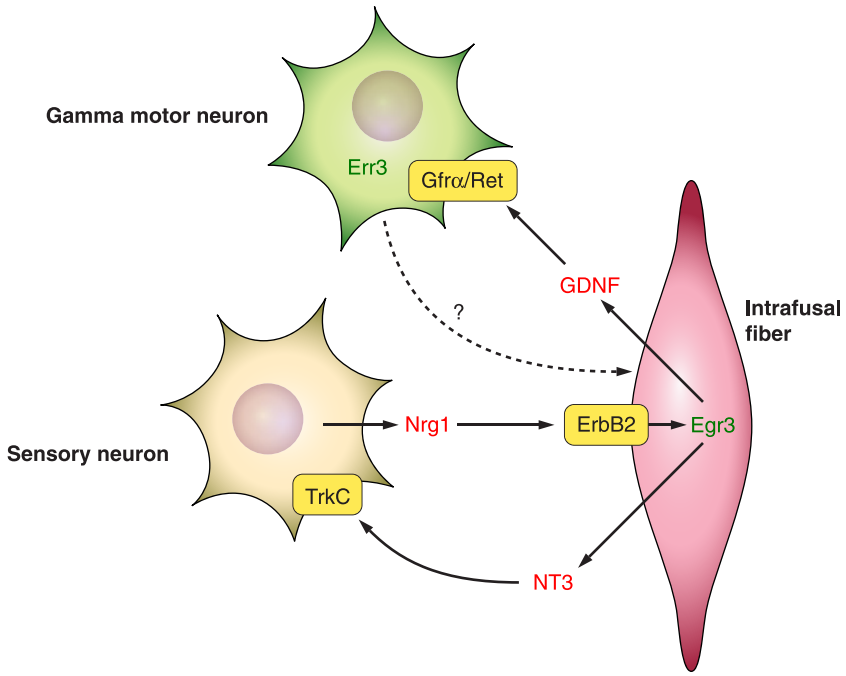


FIGURE 17. Bidirectional interactions between intrafusal muscle fibers and neurons during muscle spindle development. Neuregulin 1 (Nrg1), derived from sensory neurons and required for the differentiation of intrafusal fibers, and the neurotrophins NT3 and GDNF, produced by intrafusal fibers and required for the differentiation and survival of sensory and γ motor neurons, respectively, are indicated in red. The cognate membrane receptors are boxed in yellow. The transcription factors Egr3, present in intrafusal fibers and involved in the production of NT3 and GDNF, and Err3, expressed selectively in γ motor neurons but whose function is not known, are indicated in green.

sential for the upregulation of neurotrophin 3 (NT3) by differentiating precursors of intrafusal fibers: NT3, by interacting with receptor tyrosine kinase TrkC present in sensory neurons, is required for the survival of these neurons during the neonatal and early postnatal stages and consequently for the maintenance of developing spindles (207, 421, 790). NT3- and TrkC-deficient mice show uncoordinated, ataxic movements due to loss of proprioceptive signaling. TrkC- or NT3-deficient mice show a complete absence of muscle spindles in limb muscles but not in head muscles, like the masseter, where other neurotrophins appear to compensate for the loss of NT3 (215). Intrafusal fibers also produce another neurotrophin, glial cell line-derived neurotrophic factor (GDNF), which is essential for the postnatal survival of γ-motor neurons by signaling through a receptor complex, common to most GDNF family ligands (GFLs), comprising GFL-specific co-receptors (GFRα) and the RET tyrosine kinase (279, 731). GDNF, like NT3, is preferentially expressed by intrafusal fibers in E18.5 muscle. GDNF might be involved in the differentiation of γ-motor neurons, which are characterized by high levels of the transcription factor Err3 and low levels of the neuronal DNA binding protein NeuN, whereas α-motor neurons, innervating fast and slow extrafusal fibers, have an opposite distribution of these markers (235). The restriction of Err3 expression to gamma motor neurons occurs over the period when functional sensory-motor circuits are established, suggesting that signals from muscle spindles are required to support the differentiation of $Err3^{on}/NeuN^{off}$ γ-motor neurons.

D. Muscle Fiber Types During Postnatal Development and Aging

In many mammalian species, such as rat and mouse, skeletal muscles are still immature at birth, and important changes in the fiber type profile take place during early stages of postnatal development, as a result of the maturation of the neuromuscular system, in particular the disappearance of the polyneuronal innervation, and under the influence of the thyroid hormone, which increases after birth. Four major changes take place in the fiber type and MyHC isoform composition of rat and mouse muscles during the first weeks after birth. The first is the progressive disappearance of embryonic and neonatal MyHC, which are lost more slowly in type 2A fibers (708). The second is the upregulation and adult fast MyHC genes and the progressive accumulation of MyHC-2A, -2X, and -2B in specific fast fiber subpopulations (184). Still another switch occurs in mouse fast muscles, such as tibialis anterior and plantaris, which at birth contain a small but significant proportion of type 1 fibers that disappear completely or almost completely during postnatal development (6, 858). Finally, another switch takes place in slow rat muscles, such as soleus, with the progressive transformation of type 2A into type 1 fibers, a

process that continues throughout postnatal development with a concomitant transformation of type 2 into type 1 motor unit (446). The progressive transition towards adult fiber type composition is mirrored by the changes in maximum shortening velocity and in time to peak tension. Maximum shortening velocity increases and time to peak tension decreases in rat EDL during the first 3 mo after birth, whereas the opposite changes take place in rat soleus (155).

The postnatal changes in fiber type profile can be explained by the combined action of an intrinsic genetic program and of extrinsic factors, such as hormonal and neural influence, acting on the myonuclei of the myofibers themselves (see below). However, one should also consider the possibility of developmental changes of the satellite cells that are continuously incorporated into the growing myofibers of neonatal muscle. Direct evidence for satellite cell transformation was recently reported with respect to the role of the transcription factor Pax7. The *Pax7* gene was inactivated at various developmental periods using an inducible knockout approach (476). The striking and unexpected finding was that muscle regeneration was severely compromised when *Pax7* was inactivated at P7–11, in agreement with previous studies using a traditional knockout model. However, the survival of satellite cells and the muscle regenerative capacity is maintained when the *Pax7* gene is inactivated in the adult, myogenic precursors becoming independent of *Pax7* during the second to third week after birth. These experiments appear to indicate that the transcriptional program of satellite cells undergoes drastic changes during a critical period of early postnatal development.

The role of neural and hormonal cues, and probably mechanical load, in the postnatal maturation of the fiber type profile has been studied in mouse and rat muscles. Rat slow muscles like soleus display after birth a progressive accumulation of MyHC-slow and concomitant downregulation of embryonic and neonatal MyHCs (119). Rat and mouse hindlimb muscles destined to become fast muscles undergo a switch from embryonic/neonatal to adult fast MyHC expression during the first 2–3 postnatal weeks. The transcripts of MyHC-2B, -2X and -2A are first detected by days 2–5 after birth in rat hindlimb muscles and display from their first appearance a specific spatial pattern of expression corresponding to the distribution of these myosins in adult skeletal muscles: for example, in the tibialis anterior muscle MyHC-2B transcripts and proteins are mostly expressed in superficial areas, whereas MyHC-2A transcripts are present only in deep regions (184). A similar temporal pattern of expression is seen for the corresponding proteins in mouse skeletal muscle (6, 16) and for both transcripts and proteins in rabbit skeletal muscle (537). Interestingly, the spatiotemporal pattern of developmental expression of adult fast MyHCs, as well as the downregulation of the embryonic and neonatal isoforms, is unchanged in mice null for MyHC-2B or MyHC-2X (16), although at later postnatal stages there are compensatory adaptations, with MyHC-2A being upregulated in the MyHC-2X null mice (699). On the other hand, a severe loss of muscle fibers occurs in the MyHC-2B knockout (14).

The developmental MyHCs disappear during the first 2–4 postnatal weeks, first in type 2B and 2X, later on in type 2A muscle fibers (708), with significant variations among different muscles (6, 174). The novel MyHCs coded by *MYH7b* (*MYH14*), corresponding to MyHC-slow tonic, and *MYH15* genes display unique developmental patterns of expression: the MyHC-slow tonic is detected in all rat EO muscle fibers in the fetus but disappears from most fibers, except the slow-tonic fibers, after birth; in contrast, MYH15 is absent in embryonic and fetal muscle and is first detected after birth in the orbital layer of EO muscles (661).

Three major factors are responsible for the perinatal changes in MyHC gene expression and the emergence of the adult fiber type profile. The first is the maturation of NMJs (742) with elimination of polyneuronal innervation, which is lost during the second postnatal week in the mouse, and the formation of the definitive motor units (221, 374). Fast and slow motor neurons probably undergo a parallel maturation process in the same time period, and specific motor neurons firing patterns are established so that the properties of fast and slow motor neurons are closely matched to those of the muscle fibers they innervate. While α-motor neurons can be distinguished from γ-motor neurons by their Err3^{off}/NeuN^{on} profile (see sect. V*C*), no specific marker is available to identify fast and slow α-motor neurons. The lack of specific markers has hindered the study of slow/fast α-motor neuron diversification and the identification of the signals, including those derived from the muscle fibers, and signaling pathways involved in this process (395). The role of innervation in muscle fiber type differentiation is especially evident in the postnatal development of slow muscle fibers: after neonatal denervation, slow myosin progressively declines in the rat soleus and is no longer detectable after 4 wk (254). In contrast, the postnatal upregulation of MyHC-2B takes place also in rat fast muscle denervated at birth (118), and in mouse muscles treated with β-bungarotoxin at fetal stages (857), although the innervation is required for the downregulation of neonatal MyHC (676). A second related factor is the mechanical load imposed on neonatal limb muscles by the transition from the intrauterine environment to the postnatal environment in which weight-bearing activity is imposed especially in postural muscles like the soleus. Indeed, 7-day-old rats exposed for 16 days in a weightlessness spaceflight environment showed markedly reduced expression of MyHC-slow in soleus without significant MyHC changes in fast muscles (3). It remains to be established whether the effects of reduced weight-bearing are due to a reduction of motor neuron activity or reflect also a direct effect of unloading on muscle

fibers. The first alternative would appear more likely, as unloading induced in adult rats by hindlimb suspension causes a drastic decrease in activity detected by quantitative electromyography in both fast and slow muscles (79) and alters the functional properties of hindlimb motor neurons (167). A third major factor involved in postnatal muscle fiber type differentiation is the level of thyroid hormone, which is low until birth, then increases to reach its maximum value at 2–3 wk after birth (254). The thyroid hormone has a role in fast myosin induction: the postnatal transition from neonatal to adult fast MyHCs is delayed by hypothyroidism and accelerated by hyperthyroidism (173, 254). The effect of thyroid hormone on developing muscle seems to be direct and independent of the nerve, as adult MyHC-2B is precociously induced in neonatal muscles by thyroid hormone injection even in the absence of the nerve (675).

The fiber type profile of skeletal muscles undergoes significant changes throughout subsequent stages in postnatal life and aging. For example, most mouse fast muscles contain a significant proportion of slow type 1 fibers during the first postnatal week that are subsequently transformed into type 2 fibers (860) with concomitant disappearance of MyHC-slow (6). On the other hand, the proportion of slow fibers may increase in slow muscles, e.g., the neonatal rat soleus contains only ~50% slow fibers that increase up to 80–90% in the adult, due to a progressive transformation of fast 2A into type 1 fibers (119). The postnatal switch in the fiber type profile must be taken into account for a correct interpretation of fiber type changes in transgenic mice, such as those overexpressing activated calcineurin or PGC-1α (see sect. VII).

Further fiber type transitions occur during aging. Rat skeletal muscles undergo a type 2B to 2X switching in aging fast muscles and a type 2A to type 1 switching in slow soleus (462–464). The histochemical fiber type profile does not appear to change with age in human skeletal muscle; however, the relative proportion of fast MyHCs is reduced in elderly subjects (423, 733) due to greater atrophy of type 2 fibers (478). The greater susceptibility of type 2B fibers (2X fibers in human muscle) to aging may be due to neurogenic changes leading to selective denervation of these fibers, reflected by fiber type-specific endplate fragmentation (773). The fiber type transition during aging is accompanied by a loss of mitochondrial ATP production resulting from reduced abundance of mitochondrial DNA and reduced content of several mitochondrial proteins (732). These changes appear to affect especially type 2 fibers, as noninvasive spectroscopic analyses of human muscles in vivo indicate that mild mitochondrial uncoupling, and thus reduced ROS production, contribute to preserve mitochondrial function in muscles richer in type 1 fibers compared with muscles rich in type 2 fibers (18).

E. Fiber Types in Regenerating Skeletal Muscle

Regenerating muscle provides an interesting model to study developmental changes of the fiber type profile in adult animals. Although it is frequently stated that regeneration "recapitulates" embryonic myogenesis, it should be stressed that the satellite cells responsible for muscle regeneration differ from the muscle precursor cells present at embryonic and fetal stages of development, and differ even from the satellite cells involved in muscle growth during early postnatal development (see below). During regeneration, developmental MyHC isoforms are first expressed (175, 697, 707), then a switch to adult isoforms takes place under the control of nerve activity. In a widely used model, the rat soleus muscle undergoes a rapid regeneration process following injury and in the presence of innervation acquires within a week the typical slow profile, whereas the denervated regenerating muscle activates the default switch to a fastlike gene program (211, 859) **(FIG. 18)**. Functional innervation of regenerating myofibers starts between days 3 and 5 after bupivacaine-induced injury and leads to a rapid downregulation of MyHC-2B and -2X and upregulation of MyHC-slow (378) and MyLC1s (379) transcripts. This rapid switch in MyHC gene expression is induced by the specific impulse pattern delivered by slow motor neurons, since it can be reproduced by low-frequency stimulation of denervated regenerating muscle (388). The regenerating soleus muscle provides an ideal system to dissect the signaling pathways that mediate the effect of specific activity patterns on muscle gene regulation. By transfecting regenerating muscle with constitutively active or dominant interfering mutants of different signal transducers, it has been possible to identify some pathways involved in activity-dependent fiber type specification (see sect. VII). A limited degree of activation of the slow gene program is also observed when regenerating fast muscle is stimulated with the same slow-type impulse pattern (388, 602), suggesting that regenerating muscle has a greater plasticity compared with adult muscle. However, the finding that regenerating fast and slow skeletal muscle show a different MyHC composition when stimulated with the same impulse pattern in the absence of innervation point to intrinsic differences between muscle types, probably reflecting intrinsically different satellite cells (388). The notion of satellite cell heterogeneity is supported by the finding that satellite cells from adult mammalian muscle tend to form fibers with a phenotype reflecting their fiber type of origin (56, 194, 358, 660, 852). However, clonal analysis of satellite cells isolated from adult human skeletal muscle revealed no evidence for the existence of different fast and slow satellite cell lineages (199), possibly reflecting the importance of permissive culture conditions.

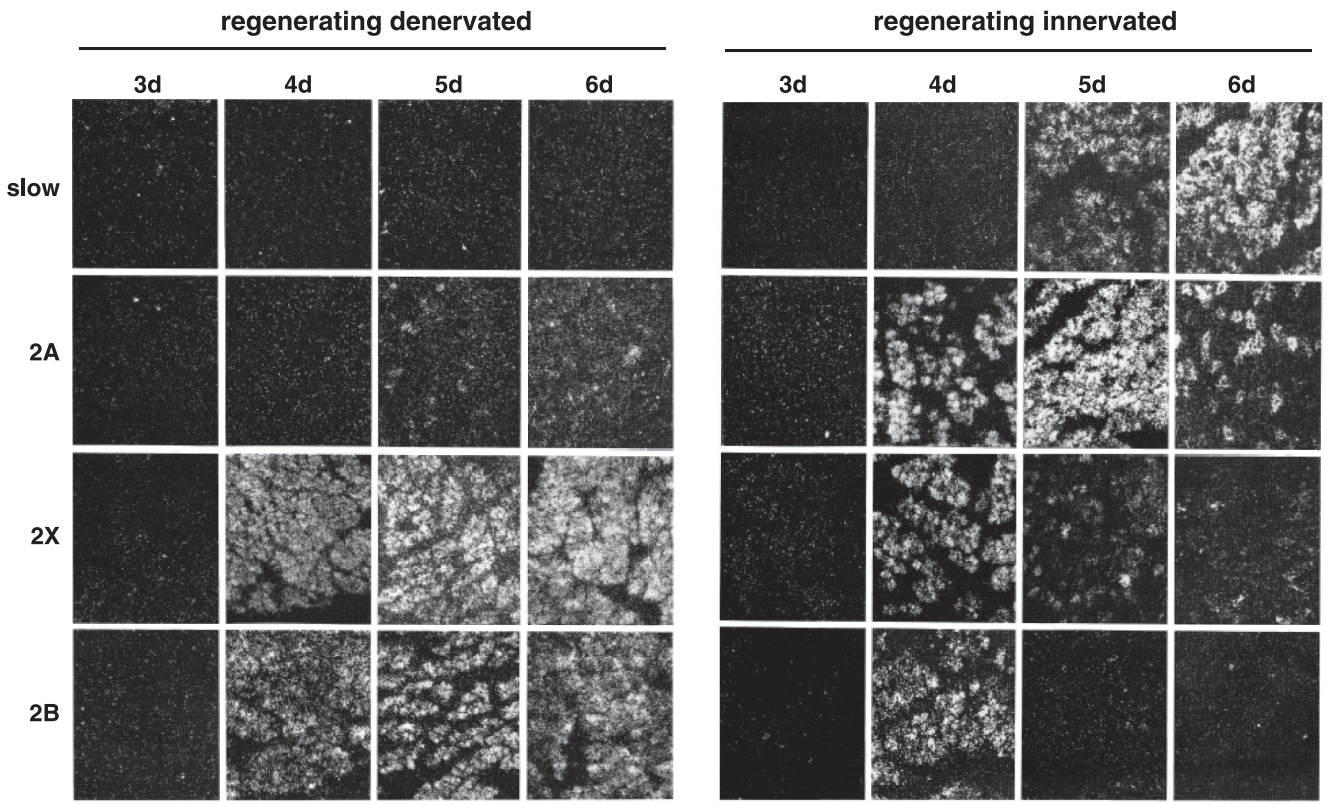


FIGURE 18. Fiber type switching induced by slow nerve activity in regenerating rat soleus muscle. Serial sections of regenerating soleus from day 3 to day 6 after bupivacaine injury were processed for in situ hybridization and autoradiography with ^{35}S-labeled riboprobes specific for the adult fast (2A, 2X, 2B) and slow myosin heavy chain (MyHC) transcripts (positive reaction: white signal against black background). Until day 3, only MyHC-emb and -neo, not detected by the probes used, are present in regenerating muscle. MyHC-2X and -2B transcripts are detected at day 4 after injury in both innervated and denervated regenerating fibers by in situ hybridization. Reinnervation of regenerating muscle starts by day 4, and at day 5, a fast-to-slow switch in MyHC gene expression is induced by slow nerve activity, with downregulation of MyHC-2X and -2B and upregulation of MyHC-slow transcripts. In contrast, the denervated regenerating muscle maintains the default fast-type gene program. [Modified from Jerkovic et al. (378).]

VI. FIBER TYPE REMODELING IN ADULT SKELETAL MUSCLE

Skeletal muscles have a remarkable capacity to undergo adaptive changes in response to use and disuse, including changes in fiber size (e.g., muscle hypertrophy) and fiber type (e.g., fast-to-slow fiber type switch), and correlated changes in muscle force and resistance to fatigue (see Ref. 604). The role of exercise in remodeling fiber type properties is a central issue in general physiology and sport sciences and has emerged as a hot topic in the field of metabolic diseases and diabetes. Several factors, in particular nerve activity patterns, mechanical loading, and circulating factors, including different hormones, are known to affect muscle fiber type composition, but it is outside the scope of this review to cover this complex field. We will hereafter discuss only the general aspects of muscle fiber type plasticity, focusing mainly on the role of nerve activity, and in next section will deal with the signaling pathways involved in fiber type regulation in adult skeletal muscle.

A. Range of Fiber Type Transformations in Adult Muscles

Fiber type switching can be induced in adult skeletal muscle by changes in nerve activity or loading or by hormonal influences. The role of the nerve in affecting the fast/slow properties of skeletal muscles was first demonstrated in cats by Buller et al. (112), who showed that slow muscles become faster when reinnervated by a fast nerve and fast muscles become slower when reinnervated by a slow nerve. The model of cross reinnervation has been successfully applied in other species, such as rat (342), and the factors responsible for the distinct responsiveness of various muscles have been analyzed (111, 791). Subsequent studies based on electrical stimulation of innervated or denervated muscles, using different impulse patterns to reproduce the firing pattern of fast and slow motor neurons, showed that the effect of the nerve is mediated by specific impulse patterns (see Refs. 490, 607). A slow-to-fast switch in the direction 1 → 2A → 2X → 2B can be induced by phasic high-frequency electrical stimulation, resembling the firing

pattern of fast motor neurons, and by muscle unloading caused by hindlimb suspension, as well as by hyperthyroidism. A fast-to-slow switch in the opposite direction 2B → 2X → 2A → 1, can be induced by tonic low-frequency electrical stimulation, resembling the firing pattern of slow motor neurons, and by muscle overloading caused by ablation of synergistic muscles, as well as by hypothyroidism. The actual changes seen in any given muscle under different experimental conditions are dictated by *1*) the fiber type composition of the muscles at the beginning of the experiment and *2*) the existence of intrinsic differences between muscles that limit the range of possible adaptations. This can lead to changes in opposite directions of the same MyHC/fiber type in different muscles. For example, MyHC-2A expression is decreased in rat soleus but increased in fast EDL or TA muscle in response to low-frequency stimulation (35, 788) or hypothyroidism (371). The antithetical regulation of the same MyHC gene in the two muscles can be explained if we consider that the rat soleus is composed essentially of type 1/slow fibers with a minor component of 2A fibers, while EDL contains only 2B > 2X > 2A fibers and only rare type 1 fibers. Therefore the switch in the direction 2B → 2X → 2A → 1 will necessarily cause a decrease in the 2A component in soleus but an increase in EDL, also due to an intrinsic resistance in activation of the slow program in fast muscles. It has been suggested that each muscle has a "adaptive range" of possible transformations in response to different activity patterns (see Ref. 490), e.g., fast muscles have the capacity to adapt mostly in the range 2B ↔ 2X ↔ 2A and slow muscles in the range 1 ↔ 2A ↔ 2X (35) **(FIG. 19)**. However, changes in the thyroid state can expand this range; for example, the combined effect of hyperthyroidism and mechanical unloading can induce significant expression of MyHC-2B in slow muscles (120), while hypothyroidism combined with chronic low-frequency stimulation or overloading can induce significant expression of MyHC-slow in fast muscles (122, 412). Species differences may be important, chronic stimulation in the rabbit being apparently more efficient in inducing slow myosin in fast muscles (606). However, the finding of fiber degeneration and regeneration after low-frequency stimulation of fast rabbit muscles (517) complicates the interpretation of these experiments, suggesting that the fast-to-slow conversion is not exclusively caused by adult fiber transformation, but may also result from the differentiation of newly formed muscle fibers. The time factor is also important in promoting the completion of fiber type transitions by hyperactivity or inactivity. Low-frequency stimulation for 2 mo does not lead to significant expression of MyHC-slow in fast rat muscles (35, 788), but a fast-to-slow switch has been detected after 4 mo (870). A MyHC switch in the direction 2B → 2X → 2A → 1 is observed in different models of reduced muscle activity (780). A complete or almost complete disappearance of type 1 fibers has been demonstrated in human skeletal muscle after long-term (several years) spinal cord injury (295) and in rat fast and slow muscles after long-term (90 days) spinal cord isolation (360, 897).

The notion of adaptive range of fiber type transformations, controlled by neurogenic factors that modulate muscle plasticity and intrinsic myogenic constraints that limit the range of possible adaptations, can be expanded to take into account specialized head and neck muscles. For example, some laryngeal muscles express MyHC-EO, also expressed in EOM and characterized by very fast contractile properties (see sect. II*B1*). Cross-reinnervation experiments between the rat laryngeal muscle thyroarytenoid and the pharyngeal muscle sternohyoid were carried out to determine the relative role of myogenic versus neurogenic control of muscle fiber type specification (649). The thyroarytenoid originates from the sixth branchial arch and is composed of muscle fibers coexpressing MyHC-2B and MyHC-EO (650, 884), while the sternohyoid is derived from somitic meso-

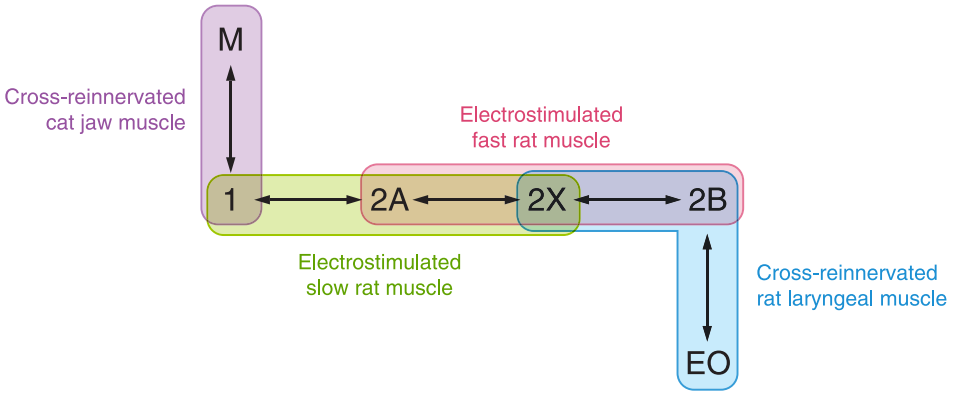


FIGURE 19. Myogenic and neurogenic control of fiber type specification in skeletal muscles. The scheme illustrates the adaptive range of MyHC transformations observed in different muscle systems after experimental interventions aimed at dissecting the relative contribution of neurogenic factors that induce muscle plasticity and intrinsic myogenic constraints that limit the range of possible adaptations. Based on results from Ausoni et al. (35) for electrostimulated fast and slow rat limb muscles, from Rhee and Hoh (649), Rhee et al. (650), and Wu et al. (884) for reinnervated rat laryngeal (thyroarytenoid) muscle, and from Kang and Hoh (392) for reinnervated cat jaw muscle.

derm and consist, like limb muscles, of type 2B, 2X, 2A, and rare type 1 fibers, but does not express MyHC-EO. After cross-innervation with the thyroarytenoid nerve, the sternohyoid showed an increased proportion of 2X fibers but no expression of MyHC-EO. On the other hand, after cross-innervation with the sternohyoid nerve, the thyroarytenoid maintained its fiber type composition with most fibers coexpressing MyHC-2B and -EO, with some MyHC-2X mostly concentrated in the vocalis division of the muscle. Thus it appears that in this case the adaptive range is between MyHC-2X/2B and MyHC-EO **(FIG. 19)**. Interestingly, MyHC-slow and -2A were not induced in this muscle by hypothyroidism (883). Cross-reinnervation combined with muscle transplantation experiments were performed with cat jaw muscles to determine whether the expression of jaw-specific myofibrillar proteins is maintained following transplantation into a limb muscle bed (343, 392). The cat temporalis was induced to regenerate into the bed of the fast EDL muscle, after elimination of EDL. When reinnervated by the fast nerve, the regenerated temporalis was found to express jaw-specific MyHC, tropomyosin, and myosin-binding protein C but not the fast adult MyHC normally present in limb muscles. In contrast, after transplantation into the slow soleus muscle bed and reinnervation by the slow nerve, there was a transient expression of jaw-specific MyHC followed by a progressive transition to the slow-MyHC. Thus it appears that in this case the adaptive range is between the type 1/slow and jaw-specific contractile proteins **(FIG. 19)**.

Muscle plasticity may be restricted not only by intrinsic differences between muscles but also by intrinsic differences among muscle fibers within the same muscle, including fibers with apparently identical phenotype. For example, MyHC-2A is induced after denervation or by unloading in some but not all type 1 soleus muscle fibers. How to explain this differential response? One possibility is that phenotypically similar type I fibers differ in their developmental history, some being derived from primary generation fibers that never express fast-type MyHC while others derive from secondary generation fibers that switch from type 2A to type 1 during postnatal development (see sect. V*D*). Although this issue has not been specifically addressed, it is tempting to speculate that MyHC-2A is only expressed after denervation in secondary generation fibers. If this interpretation is correct, one can envisage that there are intrinsic differences among various fiber types with respect to the activity-dependent type 1 ↔ 2A switch: *1*) in soleus secondary generation fibers, the switch can take place easily, e.g., 2A → 1 switch during postnatal development, 1 → 2A switch after short-term inactivity; 2) in soleus primary generation fibers, the 1 → 2A switch requires longer inactivity periods, such as long-term spinal cord injury in humans or spinal cord isolation in rats, or as a result of more drastic experimental conditions, e.g., switch induced by high-frequency stimulation; and 3) finally, as discussed above, this switch is apparently even more difficult for fast muscles as 2A → 1 switch is rare and seen only after very long periods of low-frequency stimulation especially in combination with hypothyroidism.

Hybrid fibers with mixed MyHC composition are very frequent in muscles undergoing fiber type transformation, also due to the slow protein turnover of myosin. MyHC-slow is still present in most rat soleus fibers 1 mo after inducing hyperthyroidism combined with unloading (120) and even 2 mo after denervation followed by high-frequency electrical stimulation, when most fibers are hybrid fibers containing ~85% MyHC-2X and 15% MyHC-slow (35, 706). In humans, muscle fibers containing type 1 and 2A fibers are increased in endurance-trained individuals, whereas fibers containing type 2A and 2X MyHC are increased in sedentary individuals (422). The much shorter half-life of the MyHC transcripts can lead to a mismatch between a given MyHC protein detected by immunocytochemistry and the corresponding transcript detected by in situ hybridization in the same fiber (19, 20). It is not clear whether a mismatch between MyHC mRNA and protein levels, which has also been detected in denervated muscle (359), may reflect a posttranslational control of MyHC expression. The half-life of MyHCs may also change due to increased protein degradation: the E3 ubiquitin ligase MuRF1 is activated in atrophying muscle and is responsible for MyHC degradation (153, 161, 218); however, there is no evidence for isoform-specific accelerated degradation of MyHC in muscles undergoing fiber type transition.

B. Muscle Plasticity and Motor Neuron Plasticity

As discussed in section V, during postnatal development and regeneration, as originally proposed by Butler-Browne and Whalen (119) and by Esser et al. (211), a default nerve activity-independent pathway of muscle fiber differentiation, which is controlled by thyroid hormone, leads to the activation of a fast gene program. In contrast, the induction of the slow gene program during postnatal development and regeneration is dependent on slow motor neuron activity. Once established, the slow gene program is not easily reversed in adult muscle fibers and tends to persist for a long time even in denervated muscle. Thus a slow-to-fast fiber type switch, which can lead to the disappearance of the slow fibers, can only be induced by long-term complete inactivity, e.g., after spinal cord isolation in mice or spinal cord injury in humans. A fast-to-slow switch, usually incomplete, can be induced by chronic electrical stimulation or overloading induced by ablation of synergistic muscles, especially when combined with hypothyroidism. However, physiological adaptations to increased activity, such as different types of exercise in humans and running or swimming in rats or mice, induce a switch to a more oxidative fast phenotype (2X → 2A in humans, 2B → 2X → 2A in rats

and mice) but no significant switch to the type 1/slow profile. For example, a significant increase in the percentage of fibers expressing MyHC-2A and a concomitant decrease in the percentage of fibers expressing MyHC-2B was observed in mouse fast muscles (gastrocnemius and tibialis anterior) after some weeks of voluntary wheel exercise (13). The switch in the range 2B ↔ 2X ↔ 2A in mouse and rat muscle or in the range 2X ↔ 2A in human muscle likely reflects the total amount of activity, as shown by the finding that electrical stimulation of denervated fast rat muscles with bursts of high-frequency trains every 15 min leads to a high level of MyHC-2B expression, while the same high frequency trains given every 15 s induce high level of MyHC-2A and -2X expression (35).

One might speculate that under physiological conditions muscle plasticity reflects motor neuron plasticity and that there are intrinsic differences in the firing pattern of fast and slow motor neurons that restrict the adaptive range of muscle fiber type transitions. Motor neurons may change the total amount of activity, e.g., fast motor neurons innervating 2B fibers can increased their activity during an endurance training protocol and thus entrain the corresponding muscle fibers into a 2B → 2X → 2A transition. On the other hand, motor neurons probably cannot change their frequency pattern; therefore, fast motor neurons cannot change into slow or slow motor neurons turn fast, although motor neuron firing pattern has not been examined to our knowledge in cross-reinnervation experiments. Slow motor neuron may drastically reduce or stop firing, e.g., after spinal cord injury, thus releasing the default fast pattern in the corresponding muscle fibers. In addition, the finding that regenerating fast and slow muscles respond differently to the same stimulation pattern suggests that there are intrinsic differences between muscle cell precursors that may also restrict muscle plasticity (388).

C. Coordinated Changes in Different Functional Compartments During Fiber Type Switching

The transcription of different fiber type-specific genes must be precisely coordinated in skeletal muscle fibers to maintain muscle function during the transition phase. Available evidence from exercise and electrical stimulation studies indicates that fiber type transformation involves changes in all muscle cell compartments, including components of the excitation-contraction coupling, cell metabolism, and contractile machinery (see Ref. 604). The time course of changes may vary in the different compartments, though, and it was initially suggested that activity-dependent fiber type switching involves sequential changes in metabolic properties preceding changes in contractile components. However, it was subsequently shown that some transcriptional changes in MyHCs may take place very early in response to activity/inactivity: for example, an abrupt MyHC-2B to MyHC-2X transcript switch can be induced by slow-type electrical stimulation in rat fast muscles (413) and MyHC-2X transcripts are rapidly (within 12–24 h) downregulated after exercise in humans (J. L. Andersen, personal communication). On the other hand, a dissociation between metabolic and contractile adaptive response can occur if the intensity of imposed activity is modest: thus moderate endurance training can induce only metabolic changes, such as increase in oxidative enzymes without concomitant changes in MyHC composition. These finding may suggest that metabolic and contractile properties are controlled by distinct signaling pathways (see sect. VII).

VII. SIGNALING PATHWAYS CONTROLLING MUSCLE FIBER TYPE-SPECIFIC GENE PROGRAMS

Before considering the specific pathways involved in fiber type regulation, we need to address two general issues. The first concerns the relative role of a signaling pathway in inducing a phenotype during development and in maintaining or switching this phenotype in the adult. Traditional transgenic or knockout studies may not provide unambiguous conclusions about this problem, as discussed elsewhere (see Ref. 704). Inducible models are required to determine the role of a pathway in the adult, but few studies are available. An alternative method is to use in vivo transfection by electroporation, that allows to explore the effect of gain-of-function or loss-of-function approaches in adult or regenerating muscles. A second general problem concerns the coordinated regulation of fast and slow gene programs, as discussed below.

A. Mechanisms Controlling the Coordinated Regulation of Fast and Slow Gene Programs

Muscle fiber type switching involves the coordinated antithetic regulation of different fast and slow gene programs, for example, the activation of the fast gene program occurs simultaneously with the repression of the slow gene program, and vice versa. A vivid illustration of this regulation is provided by the MyHC transitions induced by innervation in regenerating slow muscles, as described in section V. Following the developmental to adult fast MyHC switch, the default fast program of gene expression persists in the absence of the nerve, but a striking change is rapidly induced by slow nerve activity, with the simultaneous upregulation of MyHC-slow and downregulation of MyHC-2X and -2B **(FIG. 18)**. A similar switch is observed in adult muscles in response to electrical stimulation or thyroid hormone. One can envisage several possible mechanisms to account for the coordinated up- and downregulation of different fast and slow muscle genes, but available evidence supports the following: *1*) transcription factors acting as

both activators and repressors; *2*) bidirectional promoters generating both sense and antisense transcripts; and *3*) miRNAs hosted in MyHC genes **(FIG. 20)**.

The first mechanism involves the role of transcription factors, such as the calcineurin-dependent nuclear factor of activated T-cells (NFAT) transcription factors (see below), that may act as activators or repressors of distinct MyHC genes **(FIG. 20*A*)**. In other cell systems, the transcription factors of the NFAT family are also known to act as both activators and repressors of gene expression (e.g., see Ref. 366). In skeletal muscle, in vivo transfection with a constitutively active mutant of NFATc1 activates MyHC-β/slow but deactivates MyHC-2B promoter activity (532). Accordingly, the NFAT inhibitor VIVIT activates MyHC-2X gene expression in regenerating and adult slow muscle while blocking the induction and maintenance of MyHC-β/slow (532). Studies on fast troponin I gene regulation show that direct binding of NFAT to the fast troponin I intronic regulatory element mediate the activity-dependent transcriptional repression of this element (636).

A second mechanism, operating in *cis*, involves the role of bidirectional promoters located between closely associated MyHC genes **(FIG. 20*B*)**. Most sarcomeric MyHC genes are organized in two clusters, one comprising the MyHC-α and MyHC-β/slow and another the 2A-2X-2B genes, as well as other sarcomeric MyHC genes. Baldwin and colleagues have identified intergenic bidirectional promoters that can regulate the expression of neighboring MyHC genes by generating a sense transcript from the downstream gene and an antisense transcript from the upstream gene. Antisense transcription is common in the regulation of mammalian genes and antisense transcripts frequently link neighboring genes into transcriptional units (324, 398). Expression of an antisense to *Myh7* gene coding for MyHC-β/slow was found to increase in the mouse heart during postnatal development and in response to thyroid hormone, thus matching changes in MyHC-β/slow expression under these conditions (308). However, the mechanism of the postulated inhibitory effect of the antisense RNA on MyHC-β/slow gene transcription is not known. Similar bidirectional promoters were identified in the intergenic region between the

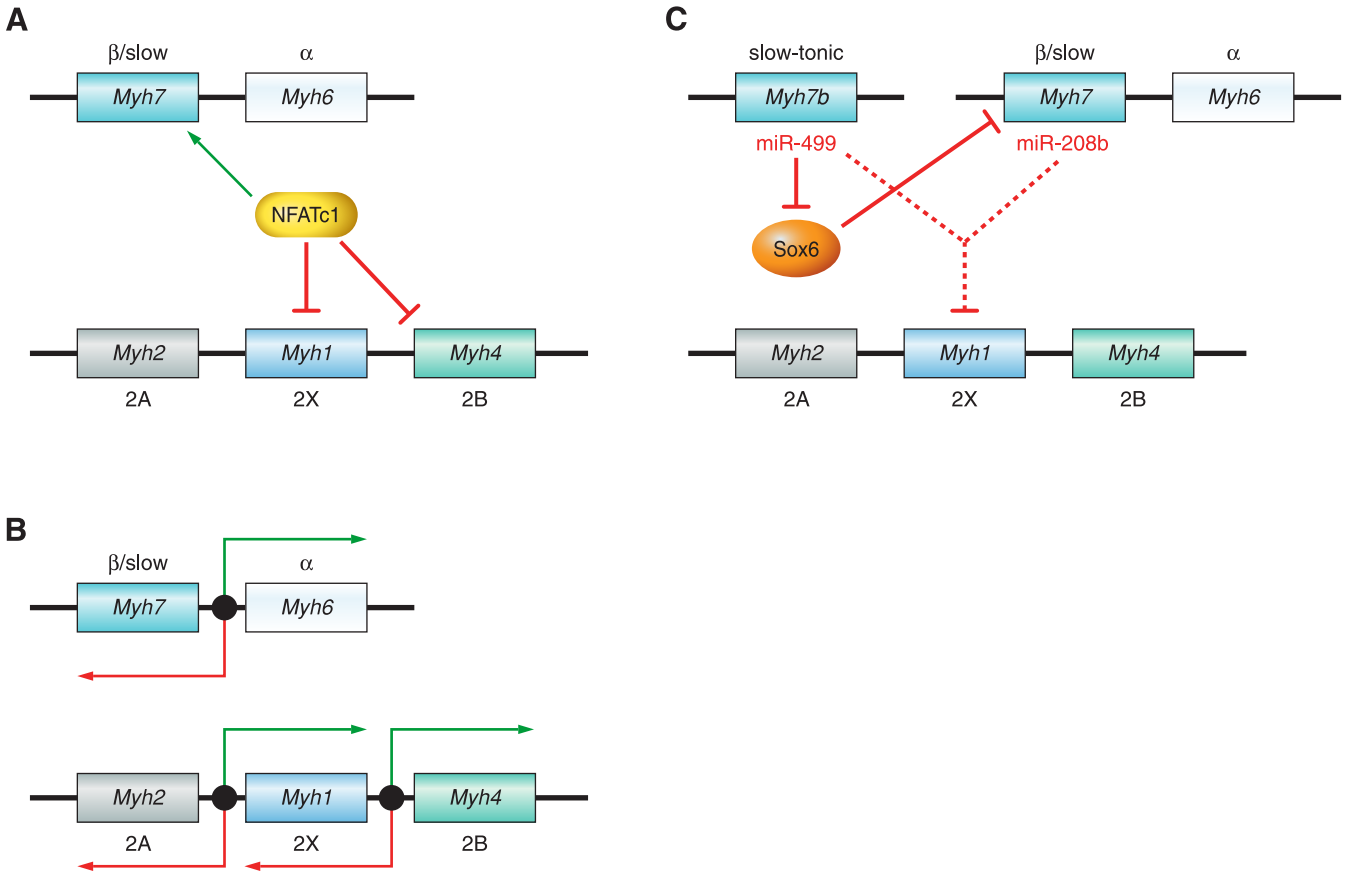


FIGURE 20. Antithetic control of different *Myh* genes during muscle fiber type transitions. Three mechanisms can account for the coordinated regulation of different *Myh* genes during fiber type switching: *A*) transcription factors acting as both activators (green) and repressors (red) of *Myh* genes, such as NFATc1 (see Ref. 532); *B*) bidirectional promoters generating both sense (green) and antisense (red) transcripts of tandem genes (see Refs. 308, 651); *C*) microRNAs hosted in *Myh* genes, like miR-499 embedded in *Myh7b* and mir-208b embedded in *Myh7*, which inhibit transcriptional repressors, like the transcription factor Sox6. Dotted lines indicate that the mechanism of repression of fast *Myh* genes by miR-499 and miR-208b is indirect and as yet undefined (see Refs. 309, 310, 817).

Myh2 and *Myh1* genes coding for MyHC-2A and -2X, respectively (583), and between the *Myh1* and *Myh4* genes coding for MyHC-2X and -2B, respectively (651). Expression of antisense to *Myh1* was downregulated in response to resistance exercise, thus matching the upregulation of MyHC-2X observed in this experimental model, associated with a rapid shift from MyHC-2B to -2X expression. It will be of interest to determine whether the working of the bidirectional promoter in the intergenic region between *Myh2* and *Myh1* genes is coordinated with that located between *Myh1* and *Myh4* genes, so to explain the sequential order of MyHC isoform expression 2A ↔ 2X ↔ 2B. The regulatory elements and transcription factors that control the activity of these bidirectional promoters have not been identified, although a binding site for nuclear factor 1 (NF1) seems important in the MyHC-β/slow antisense promoter regulation (267).

A third mechanism for coordinated regulation of fast and slow gene programs involves some muscle-specific microRNAs (miRNAs) that are located within *MYH* genes and thus coexpressed with these genes (817) **(FIG. 20*C*)**. These include miR-208a and miR-208b, encoded by introns of *Myh6 and Myh7* genes, coding for MyHC-α and -β/slow, respectively, and miR-499, located in an intron of *Myh7b*, also called *Myh14*, and coding for MyHC-slow tonic (816). Targeted deletion experiments have shown that miR-208b and miR-499 play redundant roles in the specification of muscle fiber identity by activating slow and repressing fast myofiber gene programs: deletion of either miR-208b or miR-499 did not alter the fiber type profile, whereas double knockout mice showed a marked slow-to-fast switch in fiber type profile and MyHC expression (817). Conversely, muscle-specific overexpression of miR-499 leads to a complete fast-to-slow switch in the MyHC profile of soleus and a switch from MyHC-2B- to -2X in fast muscles (817). Different targets appear to mediate the effects of these miRNAs, including the transcriptional repressor Sox6, as shown by the finding that *1*) the 3′ UTR of Sox6 mRNA contains four evolutionarily conserved target sites for miR-499, *2*) a reporter construct containing this UTR is repressed by miR-499, and *3*) Sox6 mRNA levels are reduced in skeletal muscles of miR-499 transgenic mice. The role of Sox6 in controlling the slow gene program is supported by the finding that mutant mice that lack a functional Sox6 gene show a marked fast-to-slow switch in myosin gene expression (309, 310, 631a). Sox6 has been shown to repress slow gene expression in zebrafish muscle (835), and overexpression of Sox6 leads to reduced expression of MyHC-β/slow in mouse muscles (817). On the other hand, expression of fast contractile proteins is not activated by Sox6 overexpression, suggesting that other targets of miR-499 or miR-208b are required for activation of the fast gene program. The transcriptional repressors Purβ and Sp3, which were previously shown to repress MyHC-β/slow expression in mouse skeletal muscle (381), are also targets of miR-499 and miR-208b (817). An open issue here is whether this mechanism involving muscle-specific miRNAs and Sox6, which controls fiber type diversification in developing muscle, as revealed by transgenic and knockout experiments, is also involved in the maintenance of fibre type properties in adult skeletal muscle.

B. Calcineurin-NFAT Signaling

Calcineurin is a calcium/calmodulin-regulated protein phosphatase that acts on the transcription factors of the NFAT family inducing their translocation to the nucleus and transcriptional activation. Calcineurin is a heterodimer of catalytic (CnA) and regulatory (CnB) subunits. Both calcineurin subunits and NFATs comprise various isoforms. Skeletal muscles express CnB1, but not CnB2, and both CnAα and CnAβ, but not CnAγ. All the four calcineurin-dependent NFAT isoforms, called NFATc1-c4, are expressed in skeletal muscle. Several studies using gain-of-function and loss-of-function approaches support a major role of the calcineurin-NFAT pathway in activity-dependent fiber type specification in skeletal muscle. Fast muscles from transgenic mice overexpressing activated calcineurin under the control of muscle CK promoter have a slightly increased number of slow fibers (561) and an increased proportion of fibers staining for MyHC-2A with a concomitant decrease of type 2B fibers (139). Electrophoretic analyses showed that these muscles have decreased MyHC-2A and increased MyHC-slow in the soleus, but decreased MyHC-2B and increased MyHC-2X, -2A, and -slow in the fast plantaris muscle (781). As discussed in section VI*A*, the antithetical regulation of the MyHC-2A gene in different muscles can be interpreted in terms of initial fiber type composition and adaptive ranges of MyHC gene expression. The finding of a slight increase in slow fibers in fast muscles of calcineurin transgenic mice is not necessarily the result of a transformation of fast into slow fibers but may simply reflect a block in the postnatal slow-to-fast fiber type switching (see section VI. D). Indeed, in the plantaris muscle from calcineurin transgenic mice the amount of MyHC-β/slow was about 6% compared with about 1% in controls (781), and MyHC- β/slow is known to decrease in the same muscle from about 6% in 7-day-old mouse to ~1% in adult (6).

Overexpression of activated calcineurin in skeletal muscle also shows changes in the metabolic profile of fast muscles with increased expression of myoglobin (561) and of enzymes responsible for mitochondrial oxidative phosphorylation and lipid metabolism (491, 677). The expression of transcription factors PPARα and PPARβδ and of the transcriptional coactivator PGC-1α, which are involved in the regulation of oxidative enzymes (see below), is also increased in calcineurin transgenic mice (491).

The physiological relevance of calcineurin in vivo is demonstrated by several studies using loss-of-function ap-

proaches. A partial slow-to-fast fiber type switching is induced in the adult soleus muscle of rats treated with the calcineurin inhibitor cyclosporin A (CsA) (148). CnAα and CnAβ double null mice show a downregulation of the slow-oxidative gene program in skeletal muscles (589) and mice with muscle-specific deletion of the calcineurin regulatory subunit CnB1 show impaired fast-to-slow fiber type switching following functional overloading of the fast plantaris muscle (588). The upregulation of MyHC-slow induced by slow motor neurons or by low-frequency stimulation in regenerating rat soleus muscle is prevented by the calcineurin inhibitors Cyclosporin A and FK506, and by transfection with the calcineurin inhibitory protein domain from cain/cabin-1 (724). Calcineurin is also involved in the maintenance of the slow muscle gene program because transfection with cain causes a switch from MyHC-slow to MyHC-2X and -2B gene expression in adult rat soleus muscle, as determined by in situ hybridization analysis (724). In addition, the activity of a MyHC-slow promoter-reporter construct is inhibited in vivo by cyclosporin A and FK506 (724).

The role of calcineurin activity in the activity-dependent regulation of the slow gene program is supported by the effect of the calcineurin modulatory proteins RCAN1 (also know as MCIP1, calcipressin, and DSCR1) and calsarcins (also know as FATZ and myozenin). Overexpression of RCAN1 inhibits calcineurin signaling (244, 664) through direct binding of its COOH-terminal domain to the enzyme active site (140). Transgenic mice overexpressing RCAN1 in skeletal muscle since early developmental stages show a normal muscle fiber type profile at birth (576). However, they begin losing slow fibers at postnatal day 7, and by day 14, all type 1 fibers in the soleus muscle have switched to type 2A. This striking result suggests that calcineurin signaling is not required for the initial diversification of fast and slow fibers in the embryo but is necessary for the nerve activity-dependent maintenance of slow fibers in postnatal life. On the other hand, no significant change was detected in the type 2A/2X/2B profile in fast muscles of transgenic mice, nor in the myoglobin, cytochrome *c*, and PGC-1α expression in both fast and slow muscles, suggesting that muscle oxidative capacity is largely independent of calcineurin activity (576). How can one reconcile this finding with the previously reported observations of increased expression of oxidative enzymes induced by activated calcineurin? One possibility is that the basal expression of oxidative enzymes in the various fast and slow fibers is controlled by calcineurin-independent mechanisms and that the effect seen in calcineurin transgenic mice results from a nonphysiological overexpression of activated calcineurin. It would be of interest to know whether the increase in oxidative enzymes induced by exercise or functional overload is blunted in animals overexpressing RCAN1.

Another open issue concerns the role of calcineurin in controlling the phenotype of the fast oxidative type 2A fibers, which is apparently unaffected by RCAN1 overexpression. Studies on cultured myotubes showed that the MyHC-2A promoter is strongly dependent on calcium and calcineurin activity (15). It has been suggested that the type 1 and 2A phenotypes are both dependent on calcineurin but display a differential sensitivity to calcineurin inhibition, MyHC-β/slow expression being especially sensitive to calcineurin inhibition with cyclosporine A or cain (724). Interpretation of RCAN1's role in skeletal muscle is complicated by the finding that double knockout of RCAN1 and -2 display decreased calcineurin activity, suggesting that when expressed at normal levels these proteins act as physiological facilitators of calcineurin in vivo. Loss of RCAN1 and -2 did not affect basal MyHC-β/slow protein expression in skeletal muscle but only exercise-induced expression (694), whereas CnAβ-null and CnB1-null mice each showed dramatic reductions in basal expression (588, 589). The calcineurin-interacting proteins calsarcins colocalize with calcineurin at the Z-disc of the myofibrils, and their expression pattern is fiber type specific, with calsarcin-1 being expressed in slow fibers and calsarcin-2 and -3 in fast fibers. Calsarcin-1-deficient mice show an increase in calcineurin activity and in the number of slow fibers (231), while calsarcin-2-deficient mice show an increase of type 2A fast oxidative fibers and improved fatigue resistance in fast muscles (232).

The transcription factors of the NFAT family act as nerve activity sensors in skeletal muscle and control activity-dependent fiber type specification, as shown by four major lines of evidence: *1*) expression of NFATc1-GFP fusion protein, *2*) NFAT reporters, *3*) constitutively active NFAT mutants, and *4*) NFAT inhibitory peptides. First, an NFATc1-GFP fusion protein expressed in isolated fibers from adult mouse flexor digitorum brevis, a predominantly fast-twitch muscle, showed a cytoplasmic localization in unstimulated fibers but a nuclear localization in fibers stimulated with a low-frequency pattern typical of slow motor units (487). In vivo studies showed that NFATc1-GFP has a predominantly cytoplasmic localization in the fast tibialis anterior muscle but a predominantly nuclear localization in the slow soleus muscle (805). NFATc1 nuclear import is rapidly induced in fast tibialis anterior muscle fibers by low-frequency but not high-frequency electrical stimulation, whereas nuclear export is rapidly induced in slow soleus muscle fibers by inactivity consequent to denervation or anesthesia (805). Glycogen synthase kinase 3β (GSK3β), casein kinase (CK) 1 and 2, and dual-specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) appear to be the major kinases that regulate NFATc1 nuclear export after muscle activity in isolated muscle fibers from adult skeletal muscle (728). Second, NFAT transcriptional activity monitored by different NFAT reporters is higher in slow than in fast muscles (532, 589). Furthermore, NFAT activity is decreased by denervation in slow muscles and is in-

creased by electrostimulation of denervated muscles with a continuous low-frequency impulse pattern, mimicking the firing pattern of slow motor neurons, but not with a phasic high-frequency pattern typical of fast motor neurons (532). Third, constitutively active NFATc1 is able to induce MyHC-slow expression in regenerating denervated soleus muscle and also, though with less efficiency, in regenerating EDL muscle (532). Constitutively active NFATc1 stimulates a MyHC-β/slow promoter and inhibits a MyHC-2B promoter in adult fast muscle fibers. However, MyHC-slow expression is not induced by this mutant.

NFATc2-null and NFATc3-null mice have essentially normal fiber type composition (353, 400); however, this may be due to a redundant role of different NFAT isoforms, as suggested by transfection experiments with the peptide inhibitor VIVIT, which blocks the interaction of all NFATs with calcineurin. VIVIT blocks the expression of MyHC-slow induced by slow motor neuron activity in regenerating rat soleus muscle and causes a rapid downregulation of MyHC-slow and upregulation of MyHC-2X and -2B transcripts in adult rat soleus (532). Selective knockdown of single members of the NFAT family in adult skeletal muscle indicates that NFATs may be also involved in the regulation of fast 2B, 2X, and 2A gene programs (123). Thus MyHC-2B promoter activity is inhibited by NFATc4 RNAi, but not by NFATc1, -c2, or -c4 knockdown, whereas MyHC-2X and -2A promoters are inhibited by NFATc2, -c3, or -c4 RNAi, but not by NFATc1 knockdown. Accordingly, NFATc4 has a constitutively nuclear localization in all fast fiber types both in stimulated and unstimulated muscles, whereas NFATc3 undergoes nuclear translocation in response to both a fast- and slow-type impulse pattern and NFATc1 only in response to a slow pattern.

C. MEF2, HDAC, and CaMK

MEF2 transcription factors are major regulators of muscle differentiation and have been recently involved also in activity-dependent muscle fiber type remodeling. There are four MEF2 genes (MEF2a, -2b, -2c, and -2d), and further variants are generated by alternative splicing (76). The protein levels of MEF2a, MEF2c, and MEF2d are similar in fast and slow mouse muscles (620). A MEF2-dependent reporter, made by three tandem MEF2 binding sites from the desmin gene promoter linked to LacZ, which is not expressed in adult transgenic mice except in 10–15% of soleus muscles, can be activated in both slow and fast muscles by running exercise (880). This effect was blocked by CsA and was interpreted as resulting from a direct interaction of calcineurin with MEF2a leading to dephosphorylation and enhanced transactivator function of MEF2 (880). A crosstalk between the calcineurin-NFAT and MEF2 pathways was suggested by the finding that MEF2 activity was strongly activated by calcineurin (879). A role of MEF2 genes in the regulation of the fiber type profile in vivo is supported by the finding that the proportion of type 1 fibers is decreased in mice with muscle-specific knockout of *Mef2c* or *Mef2d*, but not *Mef2a*, and is increased by overexpression of an activated *Mef2c* (MEF2c-VP16) (620). MEF2c-VP16 also upregulated PGC-1α, but not PGC-1β, in skeletal muscles. In cultured muscle cells, the activity of MEF2 is controlled by class II histone deacetylases (HDACs), HDAC4 and HDAC5, that bind to MEF2 and repress MEF2-dependent genes: MEF2 activity is induced by HDAC kinases, such as calcium- and calmodulin-dependent protein kinase (CaMK), that cause phosphorylation and nuclear export of HDAC (536). In adult muscle, transcript levels of class II HDACs, including HDAC4, HDAC5, and HDAC7, are higher in slow than fast muscles, but protein levels are higher in fast muscles because these proteins are selectively ubiquitinated and degraded by the proteasome in slow fibers (620). Low-frequency electrostimulation of isolated adult fast fibers causes nuclear export of HDAC4-GFP, but not HDAC5-GFP, which is blocked by the CaMK inhibitor KN-62 (488). Mice lacking individual class II HDACs display a normal fiber type profile; however, *Hdac5* and *Hdac9* double-knockout mice showed increased proportion of slow fibers in soleus and increased levels of MyHC-β/slow and MyHC-2A transcripts in both soleus and plantaris (620). On the other hand, the increase in type 1 and 2A fibers induced by 4-wk running in gastrocnemius and plantaris was prevented in transgenic mice bearing an inducible signal-resistant mutant of HDAC5 (620).

A role of CaMK in mediating the effect of activity on muscle fiber type profile was first suggested by the finding that overexpression of a constitutively active form of CaMKIV selectively in skeletal muscle causes mitochondrial biogenesis and upregulation of oxidative enzymes (878). However, CaMKIV is not expressed in skeletal muscle, and CaMKIV null mice have normal fiber type profile and display expected fiber type transitions in response to stimulation (9). On the other hand, CaMKII is expressed in skeletal muscle and is stimulated by muscle activity (659). Electrostimulation of adult isolated fibers with low-frequency 10-Hz trains, which leads to HDAC4 nuclear export and MEF2 reporter activation, also induces autophosphorylation and nuclear translocation of CaMKII that are blocked by KN-62 (488); however, the KN-62 inhibitor is not specific and definitive evidence for a role of CaMKII is lacking.

Another protein kinase involved in HDAC export is protein kinase D1 (PKD1), which is not directly regulated by calcium but can be activated through protein kinase C (PKC) mediated phosphorylation. PKD1 is expressed at higher levels in slow compared with fast muscles, and overexpression of a constitutively active PKD1 mutant leads to markedly reduced muscle size and increased type I and IIA fibers but no change in metabolic enzyme and PGC-1α levels (408).

However, no difference in fiber type composition was observed in muscles with muscle-specific PKD1 knockout (408).

D. AMPK, PPARβ/δ, and PGC-1

AMPK is a heterotrimer composed of a catalytic α subunit and regulatory β and γ subunits and is a major regulator of muscle energy metabolism acting as a fuel and energy status sensor (see Ref. 318). Each AMPK subunit is present in different isoforms, including α1 and α2, β1 and β2, as well as γ1, γ2, and γ3. In rat skeletal muscle, AMPK protein levels are highest in the slow-twitch oxidative soleus, slightly less in the red gastrocnemius, and lowest in the white gastrocnemius, and immunohistochemical analyses indicate that α1 and α2 are especially abundant in type 2A fibers (627). On the other hand, the β2 and γ2 isoforms are most highly expressed in white quadriceps, and γ3 in red quadriceps and soleus (192). AMPK activity is activated in response to exercise and muscle contraction via the increased AMP concentration secondary to ATP consumption in both rodents (365, 822, 867) and humans (872). Two upstream kinases, LKB1 and CaMKK, are involved in the activation of AMPK (318). Mice expressing a dominant negative form of AMPK are intolerant to exercise (551), and the same is true for mice with muscle-specific LKB1 deficiency, which causes lower levels of cytochrome *c* and PGC-1α protein expression in the red region of the quadriceps (793). Conversely, AMPK activation by the AMP mimetic drug AICAR leads to increased levels of mitochondrial oxidative enzymes (868) and enhances running endurance (559). This effect is probably mediated via PGC-1α, as mice expressing a dominant-negative mutant of AMPK in muscle show no change in PGC-1α and mitochondrial content after treatments that lead to AMPK activation and increased PGC-1α and mitochondrial content (901), and the effect of AMPK on mitochondrial gene expression is drastically reduced in mice deficient in PGC-1α (373). AMPK phosphorylate PGC-1α at two specific residues, and these phosphorylations are required for PGC-1α-dependent induction of the PGC-1α promoter (373).

AMPK activation or inhibition can also affect MyHC gene expression and fiber type profile (655). Mice carrying a mutation in the AMPKγ1 subunit leading to activation of AMPK show an increase in MyHC-2A and -2X in the triceps muscle with a corresponding increase in PGC-1α (but not PGC-1β) and CS activity. In contrast, mice expressing a muscle-specific AMPKα2 inactive subunit show decreased PGC-1α and CS activity. However, the response of these mice to exercise training suggests that AMPK is involved in MyHC but not in mitochondrial changes. In fact, the shift from MyHX-2B to -2A and -2X induced by running exercise was reduced in mice expressing a muscle-specific AMPKα2 inactive subunit, but mitochondrial markers, such as citrate synthase and succinate dehydrogenase, increased similarly in wild-type and transgenic mice (655). This conclusion is supported by the finding that exercise-dependent upregulation of oxidative enzymes is unaffected by AMPKα2 knockout, suggesting that AMPKα2 may not be essential for metabolic adaptations of skeletal muscles to exercise training (384).

The peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily that bind DNA as heterodimers with retinoid X receptors (RXRs). The three PPAR isoforms in mammals, α (NR1C1), β/δ (NR1C2), and γ (NR1C3), are activated by lipids and affect lipid metabolism in different tissues. PPARβ/δ, which is severalfold more abundant than either PPARα or PPARγ in skeletal muscle (552), is expressed at higher levels in slow/oxidative compared with fast/glycolytic muscles (850) and is induced by endurance training in mice (498) and by acute exercise in humans (516). PPARβ/δ is activated by polyunsaturated fatty acids, and the rise in serum free fatty acid induced by a high-fat diet has been shown to cause increased PPARβ/δ binding to the PPAR response element of promoters of the carnitine palmitoyltransferase 1 (255). This leads to an increase in PGC-1α protein levels and in mitochondrial proteins (314). PGC-1α physically associates with PPARβ/δ in muscle tissue and can powerfully activate it even in the absence of ligands (849).

Muscle-specific overexpression of wild-type or constitutively active PPARβ/δ leads to a more oxidative fiber type profile with increased mitochondrial DNA, upregulation of some slow contractile protein genes, and increased resistance to fatigue (498, 850). These effects appear to be a direct effect of PPARβ/δ activation, as levels of PGC-1α remain unchanged (850). The physiological relevance of PPARβ/δ is supported by the finding that muscle-specific knockout of PPARβ/δ causes a fiber type switching toward a less oxidative fiber type profile and downregulation of some slow contractile protein genes (719). PGC-1α was also downregulated in muscles of PPARβ/δ null mice. Upregulation of genes associated with an oxidative fiber type profile, such as muscle carnitine palmitoyltransferase 1 and PDK4, was also produced in mouse fast muscles by treatment with the specific PPARβ/δ agonist GW1516 (559). However, mitochondrial DNA content was not changed by this treatment, and running performance was likewise unchanged. On the other hand, GW1516 administration combined with exercise training was found to increase mitochondrial DNA content and resistance to fatigue, as well as many genes involved in fat metabolism, such as the fatty acid transporter CD36, fatty acyl coenzyme A synthase, and LPL (559). Interestingly, a number of genes activated by GW1516 plus exercise but not by either GW1516 or exercise alone are strikingly similar to those upregulated by constitutively active PPARβ/δ even in untrained mice, suggesting their primary dependence on PPARβ/δ. A similar

synergistic effect was observed by combined treatment with GW1516 plus the AMPK activator AICAR.

Peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), which was originally identified as an interactor of PPARγ in brown adipose tissue, is a transcriptional coactivator that does not bind DNA but can potently stimulate transcriptional activity by interacting with transcription factors and basal transcriptional machinery. PGC-1α stimulates mitochondrial biogenesis and oxidative enzymes in different cell types both by inducing the expression of nuclear respiratory factors (NRF)-1 and -2, which control the transcription of many mitochondrial genes, and coactivating their transcriptional activity (see Ref. 703). NRF-1 and -2 are in turn able to stimulate the expression of the mitochondrial transcription factor A (mtTFA), a mitochondrial protein essential for the replication and transcription of mitochondrial DNA.

PGC-1α is expressed at higher levels in slow than fast muscles and is readily induced by exercise in both rodents and humans (37, 674, 786, 886). Exercise can also induce a rapid activation of PGC-1α possibly due to p38 mitogen-activated protein kinase (MAPK)-dependent phopshorylation, that leads to nuclear translocation of PGC-1α and increased transcription of mitochondrial enzymes even prior to an increase in PGC-1α expression level (876). The role of exercise appears to be in part mediated by the sympathetic nervous system and β2 adrenergic receptor stimulation because exercise-induced upregulation of PGC-1α is partially inhibited by β2 adrenergic receptor specific inhibitors (545). Two new splicing products of the PGC-1α gene, PGC-1α-b and PGC-1α-c, different at the NH_2 terminus from the previously described PGC-1α-a transcript, are specifically induced by exercise and by the β2-specific agonist clenbuterol, and the exercise-induced upregulation of PGC-1α-b and PGC-1α-c is partially inhibited by β2 adrenergic receptor specific inhibitors (543).

The transcription of PGC-1α is regulated by calcium-dependent pathways, such as CaMK and calcineurin (316), by energy deprivation via AMPK activity (771, 786, 901) and by p38 MAPK via the transcription factor ATF2 (8). Calcineurin-dependent coactivators of cAMP responsive element binding protein (CREB), called TORCs, are potent activators of PGC-1α (885), and the transcription factor MEF2 was also found to control the activity of the PGC-1α promoter (172) (see below). Posttranscriptional mechanisms may be involved in PGC-1α gene expression, as shown by the finding that activation of PPARδ,β induced by a high-fat diet causes increased levels of PGC-1α protein in the absence of any increase in PGC-1α mRNA (314).

Transgenic mice overexpressing PGC-1α show increased mitochondrial content and increased levels of oxidative enzymes in fast muscle fibers, as well as an increase in the proportion of type 1 and 2A fibers in fast muscles, thus making the muscles more resistant to fatigue (482, 483, 544). However, mitochondrial dysfunction leading to ATP depletion and muscle atrophy has also been reported in these muscles (546). No significant difference in fiber type distribution was observed in the soleus muscle from PGC-1α knockout mice, but the level of transcripts of mitochondrial ATP synthase and cytochrome oxidase subunits was decreased. Fast muscles from knockout mice showed an increased level of slow genes, such as slow troponin I and SERCA2; this paradoxical effect was interpreted as the result of the hyperactivity of these animals secondary to alterations in the central nervous system (31). If this interpretation is correct, this would rule out a significant role of PGC-1α in mediating activity-dependent changes in fiber type profile. Slow muscles in another PGC-1α knockout model, in which mice have reduced locomotor activity, show diminished mitochondrial number and respiratory capacity; however, the fiber type profile was not analyzed (474). A subsequent study showed that the levels of different mitochondrial proteins were reduced in resting muscles of PGC-1α null mice; however, their increase in response to exercise training was unchanged (472). Muscle-specific PGC-1α gene knockout caused reduction in mitochondrial gene expression, partial fiber type shift from type 2A to type 2X and 2B, and reduced exercise tolerance (315).

PGC-1β, a homolog of PGC-1α, also binds NRF-1 and is a coactivator of NRF-1 target genes. PGC-1β is expressed at high levels in skeletal muscle and seems to be especially expressed in type 2X fibers (32). In contrast to PGC-1α, PGC-1β expression is not changed by exercise or denervation (434). Overexpression of PGC-1β in transgenic mice stimulates mitochondrial biogenesis and oxidative enzymes with an increase in type 2X fibers (32). PGC-1β null mice show reduced expression of oxidative phosphorylation genes and mitochondrial content in soleus muscle; however, the fiber type profile in skeletal muscle was not examined (473, 750, 827). It remains to be established whether the lack of major changes in mitochondrial gene expression in either PGC-1α or PGC-1β null mice is due to overlapping function of the two coactivators.

E. Other Pathways and Transcription Factors

Different MAPKs, including the ERK1/2, the p38 MAPK, and the JNK pathway, are activated during exercise and also during contraction of isolated muscle, which points to local cell-autonomous rather than systemic non-cell-autonomous inducing factors (438). A role of ERK1/2 in the regulation of fiber type-specific gene programs was suggested by the observation that a constitutively active Ras mutant, which is known to selectively activate ERK1/2, can induce MyHC-slow expression in regenerating denervated

rat soleus muscle (553). In contrast, a Ras mutant that selectively activates the PI3K-Akt/PKB pathway, affects cell growth but not fiber type specification in the same system (553). When adult muscles are denervated and electrostimulated for 24 h with a low-frequency (20 Hz) impulse pattern that mimics the firing pattern of slow motor neurons, a sixfold increase in ERK activity can be observed with respect to denervated unstimulated muscles (553). In contrast, a fast nerve-like stimulation (150 Hz) did not have any effect. A role of ERK1/2 in promoting the slow gene program is supported by the finding that pharmacological blockade of the ERK pathway in primary muscle cultures decreases MyHC-β/slow and increases MyHC-2X and -2B (333). However, opposite results were reported in another study in C2C12 muscle cells, showing that the activity of a MyHC-β/slow promoter/reporter is increased by an inhibitor of the ERK pathway and decreased by constitutively active ERK, while a MyHC-2B promoter is repressed by ERK inhibitors and activated by constitutively active ERK (729).

The muscle-specific bHLH transcription factors of the MyoD family (MyoD, Myf5, myogenin, and Mrf4) bind a specific element called E-box present in the promoter of muscle genes and play a fundamental role in muscle differentiation during embryonic development (see sect. V). MyoD and myogenin decrease in skeletal muscle after birth and are differentially expressed in different fiber types of adult muscles, with MyoD being prevalent in fast and myogenin in slow muscles (362, 837). This finding has suggested that the two transcription factors may be involved in fiber type regulation; however, it is unlikely that they are part of an activity-dependent signaling pathway. In fact, both MyoD and myogenin are rapidly upregulated by denervation, and this increase is prevented by electrical stimulation (201). In addition, changes in the fast/slow fiber type profile induced by hypothyroidism or low-frequency stimulation do not closely correlate with the relative expression of MyoD and myogenin (439). An E-box within the MyHC-2B gene promoter is bound by MyoD and is required for gene expression in fast muscle: MyoD strongly activates the MyHC-2B promoter in a E-box-dependent manner, whereas myogenin activates this promoter to lesser extent and in a E-box-independent manner (861). Mice lacking functional MyoD show changes in MyHC composition that vary according to the muscle type, e.g., MyHC-2B is decreased in the diaphragm but increased in the masseter, and the upregulation of MyHC-2B in the unloaded soleus is blunted in MyoD-null mice (725). Slow-type electrical stimulation induces phosphorylation of MyoD at T115, and overexpression of MyoD mutated at T115 leads to increased proportion of fast fiber types in muscles receiving normal slow activity (204). Myogenin overexpression induces a shift from glycolytic to oxidative metabolism in muscles of transgenic mice without significant changes in MyHC gene expression (361), and a similar effect was observed after somatic gene transfer (203). Myogenin null mice die when the gene is deleted during embryonic or fetal development, but survive if the gene is deleted at birth or in adult; in this case, skeletal muscles are apparently normal (425). However, surprinsingly, myogenin-deleted mice perform better than control in treadmill running and voluntary wheel running and show an increased proportion of type 1 slow fibers and SDH-positive fibers in the gastrocnemius muscle after long-term exercise (225).

The homeodomain transcription factor Six1 and its transcriptional coactivator, the protein phosphatase Eya1, are enriched in the nuclei of type 2B and 2X muscle fibers, and forced expression of these genes in slow-twitch muscle leads to a fiber type switch from type 1 and 2A to 2X and 2B, and upregulation of genes of the glycolytic metabolic pathway, such as aldolase and enolase (294). It is not known whether the expression of the Six1-Eya1 system is controlled by activity patterns and whether the fiber type profile is altered by knockout or knockdown of these factors. The proposed role for six factors in embryonic muscle has been discussed in section V.

The FoxO transcription factors, in particular FoxO3, control muscle atrophy by activating the ubiquitin-proteasome (693) and the autophagy-lysosome pathways (518, 895). However, there is some evidence that they may also be involved in fiber type regulation. FoxO1 is more abundant in slow muscles and FoxO4 in fast muscles (416). Transgenic mice overexpressing FoxO1 show not only muscle atrophy but also a significant decrease in type I fibers (390). Paradoxically, a similar slow-to-fast fiber type switch is produced by muscle-specific FoxO1 knockout (416). This effect has been interpreted as the result of accelerated differentiation of MyoD-containing myoblasts during embryonic development.

VIII. CONCLUSIONS AND PERSPECTIVES

During the last few years a more complete picture of muscle fiber type diversity has emerged. We can identify four major areas in which research is rapidly progressing, and further advances are predictable in the near future. First, it is now clear that muscle fiber diversity involves not only myosin composition and metabolic profile, the two traditional types of markers used for fiber typing, but all aspects of cell structure and function, from surface membrane properties to calcium homeostasis. Although global gene expression patterns have not yet provided a coherent picture of fiber type profiles, we may predict that single fiber analyses, or analyses of homogeneous fiber populations, will overcome the limitations due to whole muscle studies. Another major progress has been the recognition of the basic diversity between trunk/limb muscles and head/neck muscles based on their different origin, the differential role of various transcriptional regulators during embryonic development, and

the expression of specific markers, such as MyHC isoforms, whose pattern of expression has now been completely defined. Recent experiments of cross-reinnervation and cross-transplantation with head/neck muscles suggest that the notion of distinct adaptive ranges of fiber type transitions, derived from electrical stimulation studies on fast- and slow-twitch limb muscles, can also be applied to head muscles. Thus it appears that specific patterns of nerve activity can modulate the muscle fiber phenotype but only within a predetermined range of possible adaptations dictated by intrinsic myogenic constraints. A third related area of rapid progress is the study of the myogenic populations that participate in the building of skeletal muscles, their intrinsic differences in relation with fiber type, stage of development and aging, and their differentiation potential. In the near future we should expect to have a final answer to long-standing open questions, for example, whether satellite cells associated with fast and slow limb muscle fibers are intrinsically different or not. The explosive growth of stem cell studies, boosted by the expectations of stem cell therapy, should accelerate the progress in this field. Finally, signaling biology should define the pathways that control fiber type diversification during development and those responsible for the maintenance of the fiber phenotypes in the adult. A better knowledge of the mechanisms that control adaptive changes in fiber types will be important to promote the remodeling the muscle phenotype, an objective with potential implications in clinical medicine. Exercise has an obvious beneficial effects on human health; however, the mechanism of this effect is not clear. It is likely that changes induced by exercise in skeletal muscle, in addition to systemic effects, may be relevant, as shown by the finding that some of the effects of muscle activity, for example, increased insulin sensitivity, can be reproduced by activation of specific signaling pathways in skeletal muscles of transgenic mice. These results suggest that promoting muscle fiber type switching may help in preventing some deleterious consequences of the metabolic syndrome and type 2 diabetes in humans, and possibly other chronic diseases linked to inactivity.

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Address for reprint requests and other correspondence: S. Schiaffino, Venetian Institute of Molecular Medicine, Via Orus 2, 35129 Padova, Italy (e-mail: stefano.schiaffino@unipd.it); C. Reggiani, Dept. of Human Anatomy and Physiology, Univ. of Padova, via Marzolo 3, 35131, Padova, Italy (e-mail: carlo.reggiani@unipd.it).

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