Chapter 2
Myofibers

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Abstract Muscle tissue is a highly specialized type of tissue, made up of cells that have as their fundamental properties excitability and contractility. The cellular elements that make up this type of tissue are called muscle fibers, or myofibers, because of the elongated shape they have. Contractility is due to the presence of myofibrils in the muscle fiber cytoplasm, as large cellular assemblies. Also, myofibers are responsible for the force that the muscle generates which represents a countless aspect of human life. Movements due to muscles are based on the ability of muscle fibers to use the chemical energy procured in metabolic processes, to shorten and then to return to the original dimensions. We describe in detail the levels of organization for the myofiber, and we correlate the structural aspects with the functional ones, beginning with neuromuscular transmission down to the biochemical reactions achieved in the sarcoplasmic reticulum by the release of Ca^{2+} and the cycling of crossbridges. Furthermore, we are reviewing the types of muscle contractions and the fiber-type classification.

Keywords Skeletal muscle · Myofiber · Myofibril · Sarcomere · Slow-contracting muscle fiber · Fast-contracting muscle fiber

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2.1 General Description of Skeletal Muscle Structure

Movement is one essential characteristic of living creatures, its forms becoming varied and highly complex in the humans for which it is specific. Due to active movements, humans gain greater independence toward changes in their environment. Motor actions, results of contractions and relaxations of the muscles, represent the expression of the volitional aspect of the act of communication, while mimic muscles, voice, and writing express aspects of the human personality. In this sense, the nervous and muscular systems form a functional unit.

In the human body, the skeletal muscles represent about 40% of the total weight, being the most abundant tissue. Skeletal muscles are specially designed to perform contractions based on their characteristic properties such strength, flexibility, and plasticity [1]. They allow various actions to be taken from writing to weight lifting or jumping. Muscle contraction is involved in a series of important physiological processes such as breathing or heat generation, in maintaining normal body temperature. Human skeletal muscles are made up of muscle fibers (myofibers) and other different types of cells (adipocytes, fibroblasts, satellite cells, smooth and endothelial cells which are part from the vessel walls, neurons, and Schwann nerve cells) [2]. The main source of energy that provides ATP for contraction is glycogen. After contraction, there are three major systems for the replenishment of ATP: the phosphagen system (ATP–creatine phosphate system), the glycolytic system, and the mitochondrial oxidative phosphorylation system [3].

2.1.1 Embryology and Postnatal Development of the Myofibers

Skeletal muscles are derived from the paraxial mesoderm, along the embryonic development being divided into somites [4]. Each group is divided into three divisions: sclerotome (vertebrates), dermatome (which forms the skin), and myotome (which forms muscles) [5]. During development, myoblasts (muscle progenitor cells) that originated from mesenchymal stem cells may remain in somites to compose muscles of the spine; otherwise they participate in the formation of other muscles [6]. In the development of striated muscle fibers of the postnatal period, the satellite cells are also involved, and they are also responsible for the regeneration of the muscles in the adult [7, 8]. Skeletal muscle fibers develop through the fusion of myogenic progenitors (myoblasts) forming muscles in a process known as myogenesis [9]. Myogenesis is regulated by a series of transcription factors, including Pax 3, Pax 7, and Gli, and four myogenic regulatory factors: MyoD, Myf-5, myogenin, and MRF-4 [10, 11].

2.1.2 Organizational Hierarchy of Skeletal Muscle

Skeletal muscles are hierarchically comprised of muscle fascicles and muscle fibers, which are made of myofibrils (arranged in parallel), are further divided into myofilaments and sarcomeres (arranged in series), and are ultimately broken down into structural proteins. In skeletal muscles, there is a close relationship between the muscle fibers and the connective tissue responsible for providing the nourishment of the muscle and the transmission of the force. Thus, each striated muscle is surrounded on the outside by a fibrous structure called fascia (dense lamellar connective tissue), which is anchored by epimysium (dense semi-coordinated connective tissue) [12]. The epimysium, consisting of collagen, reticular, and elastic fibers, provides the shape of the muscle and contains blood vessels and nerves. From the epimysium start connective septa – perimysium – which delimits and wraps muscle bundles. The internal perimysium envelops the primary muscles, and the external perimysium covers the secondary and tertiary muscle bundles [13]. Several muscle fibers form a primary fascicle, some primary fascicles form a secondary fascicle, and some secondary fascicles form a tertiary fascicle. In the connective tissue of perimysium, there are vessels, nerves, and proprioceptors (neuromuscular spindles, Vater-Pacini corpuscles, Ruffini corpuscles). Each muscle fiber is wrapped in endomysium, composed mainly of reticulin fibers (type III collagen) and rare type I collagen fibers. Endomysium contains numerous blood capillaries and nerve fibers, but there are no lymph capillaries (Fig. 2.1). All these connective structures represent 10–15% of the volume of the muscle and form a sort of "skeleton" of the muscle that modulates and controls its activity [14]. The number of fibers ranges from several hundred in small muscles to >1 million in large muscles. Muscle fibers are innervated by somatic efferent (motor) neurons which participate in the formation of a motor unit consisting of axonal terminals and skeletal muscle fibers that it innervates [15]. Each muscle is formed by tens or hundreds of motor units, each with own specificity that allows the same muscle from the same species and in different species to be used for various tasks [16]. These vary from continuous low-intensity activities, like posture keeping in humans and supporting their body weight, to performing movements in a large variety of situation (e.g., locomotion) that involve repeated submaximal contractions and fast and strong maximal contractions (jumping, kicking) [16]. To deal with these divergent activities, muscle cells have been provided with large differences in their contractile properties and metabolic profile, the nerve activity being a major determinant of the fiber-type profile [16].

2.1.3 Skeletal Muscle Cells: General Characteristics and Morphological Aspects

The skeletal muscle fiber is a cylindrical cell, with a length that can range from 2–3 cm up to 50 cm (with an average of 10 cm in men) and a thickness between 10 and 100 μm. From the ultrastructural point of view, skeletal striated muscle fibers

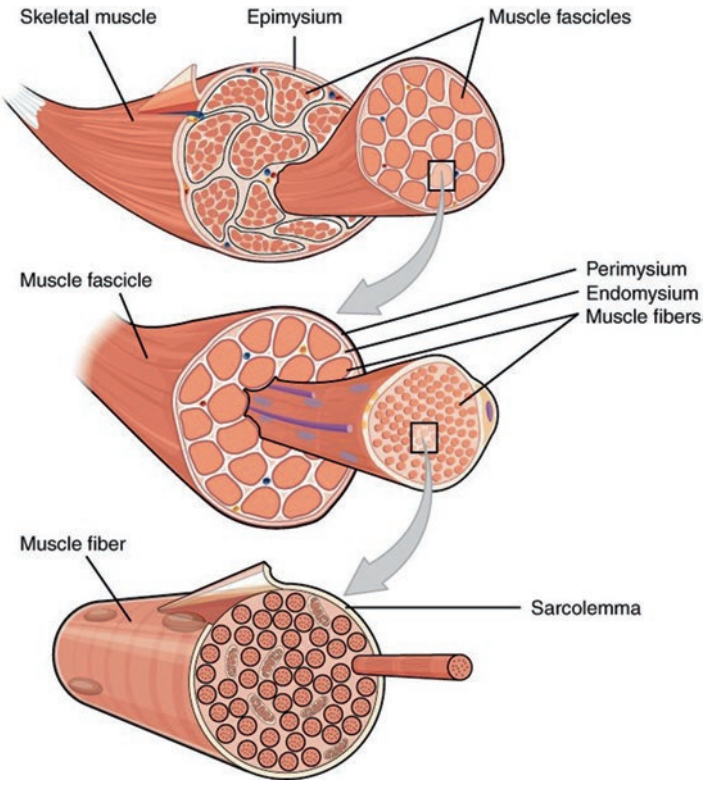

Fig. 2.1 The three connective tissue layers of a skeletal muscle. The muscle is surrounded by a connective tissue sheath called epimysium. Bundles of muscle fibers, called fascicles, are covered by the perimysium. Each skeletal muscle fiber is covered by the endomysium. (Image credit: download for free at http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@8.119)

describe all three classical components of a cell: membrane (sarcolemma), cytoplasm (sarcoplasm), and numerous peripheral nuclei. The myofiber contains up to 100–200 nuclei representing the largest cell in the body. Each myofiber contains long, thin, cylindrical rods, called myofibrils, usually 1–2 μm in diameter, which run parallel to the long axis of the muscle fiber occupying most of the intracellular space [17]. As a consequence, cell organelles, like mitochondria and nuclei, are pushed to the periphery of the sarcoplasm. Myofibrils are about 2500 per fiber, and each one contains approximately 8000 repetitive units called sarcomeres (2.7 μm in length for the human muscle), which are joined end to end [18]. Each sarcomere is delineated between two Z lines and is made up of myofilaments comprised of thick and thin filaments (Fig. 2.2), the thick one consisting in myosin and the thin composed of actin, troponin, and tropomyosin [19]. In fact, sarcomere periodicity is responsible for the distinctive banding pattern of striated muscle, which can be observed in light and electron microscopy. Myofibrils are specific contractile

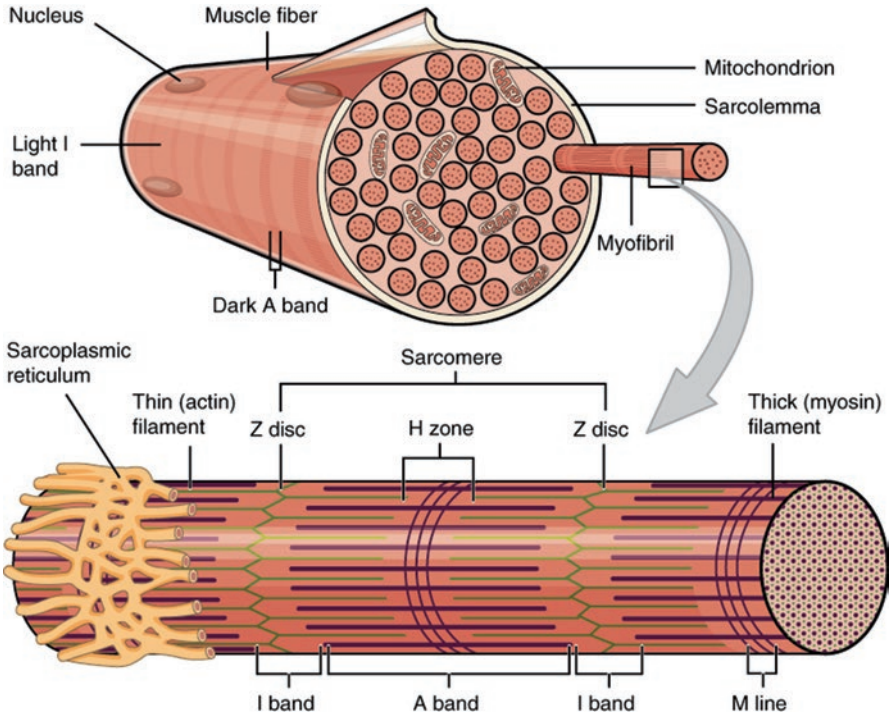

Fig. 2.2 Muscle fiber. A skeletal muscle fiber is surrounded by a plasma membrane called the sarcolemma, which contains sarcoplasm, the cytoplasm of muscle cells. A muscle fiber is composed of many myofibrils, which give the cell its striated appearance. Each myofibril is a succession of sarcomeres. Each sarcomere is delineated between two Z lines. (Image credit: download for free at http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@8.119)

organelles, arranged parallel to each other and to the longitudinal axis of the muscle fiber. They can take up between 80 and 86% of the cell volume. Myofibrils are composed of thin and thick myofilaments, parallel to each other. Myofilaments are accompanied by regulatory proteins (tropomyosin and troponin) and stabilizing proteins [17].

In a longitudinal section, skeletal muscle fibers appear as parallel, organized, multinucleated structures (plasmodial aspect), with hundreds of fallen, pliable nuclei distributed across the length of the fiber and placed subsarcolemmally. Sometimes the round-oval nuclei of the satellite cells can be seen outside the myofiber [20]. Sarcoplasm is almost entirely occupied by striated myofibrils. These are parallel to the long axis of the skeletal muscle fiber and placed so that all the clear and dark disks overlap perfectly, giving the fiber the striated appearance (Fig. 2.3a). These transverse strains are less obvious in the usual staining techniques but readily detectable with Heidenhain's hematoxylin. By this method, it is possible to emphasize, especially in the immersion objective, the alternation of clear I band bisected

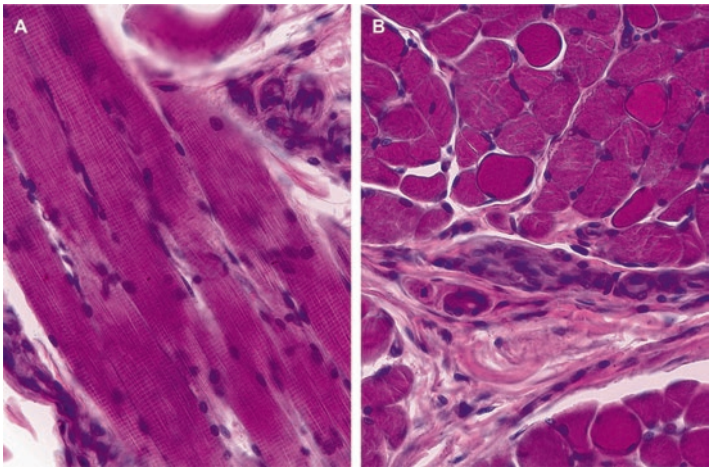

Fig. 2.3 Light microscope slide of skeletal muscle stained by H&E. (**A**) Longitudinal section depicting the A bands which are stained dark and the I bands which are lighter forming the so-called striations. (**B**) A cross section of skeletal muscle – one cannot see the striations, but in the bundles of circles that contain mosaic-like figure formed by a group of myofibrils separated by a clear interstitial substance called "Cohnheim fields," you can identify the peripherally located nuclei (dense purple spots around the large pink fibers). Courtesy of Dr. Adrian Dumitru

by Z line (for Zwischen-Scheibe meaning interim disk) and dark A band containing the clear H band (for HelleScheibe), halved by M line (for mittel – middle). The myofibrils are grouped in bundles called Leydig colonnettes (Koelliker) separated from each other by acidophilic sarcoplasm [21].

In the cross section, the muscle fibers have a polygonal contour (due to tight wrapping of the cells) or round-oval, with 1–3 nuclei surprised in the section field, and there is a punctual aspect given by the organized myofibrils in the Cohnheim areas or fields (clusters of points delimited by clear spaces) in the cytoplasm (Fig. 2.3b). The cross-sectional area of an individual muscle fiber ranges from approximately 2000 to 7500 μm^2.

As observed in the transmission electron microscope, sarcolemma has the classical structure of a plasmalemma and is surrounded by a glycoprotein/glycosaminoglycan layer similar to a basal lamina of epithelia. Reticular fibers are also present in its structure, mingled with those from the endomysium. At each end of the muscle fiber, this surface layer is lost between the tendinous fibers with which it merges. Satellite cells are located between the basal lamina of the muscle fiber and sarcolemma, closely intimate with the muscle fiber whose sarcoplasm is deformed to the inside by the satellite cells, the outer surface of the fiber being not deformed [22, 23].

Sarcolemma has inward extensions (invaginations) into the sarcoplasm and forms the T (transverse) tubule system – T system:

– It builds a very branched network filled with extracellular fluid that prolongs the extracellular space in the depth of the cell up to the vicinity of the contractile structures; this system together with a pair of terminal cisterns of the sarcoplasmic reticulum forms triads [24]; T tubules penetrate to all levels of the muscle fiber.
– It is perpendicular to the plane of the membrane at the junction where the A and I bands of the myofibrils overlap and where a mesh surrounding each myofibril is formed. In this way, ions and signal molecules can reach up to the contractile structures [25].
– Sarcolemma of the T tubules is intertwined with a large number of L-type calcium channels, designed to propagate the potential of action initiated at the neuromuscular junction within the muscle fiber.

Sarcolemma itself contains the integral proteins and ion pumps (ATPase, adenylate cyclase, 5′-nucleotidase) to control plasma ATP concentration. Also, at the level of the sarcolemma are described the costameres – structural-functional components. Costameres are subsarcolemmal assemblies of proteins aligned across the circumference of the skeletal fiber at the Z lines and have the role of physically coupling the force generated by sarcomeres with sarcolemma, tethering the sarcomere to the cell membrane [26–28]. The DAG (dystrophin-associated glycoprotein) complex contains various integral and peripheral proteins, such as dystroglycan and sarcoglycan, which are thought to be responsible for the connection between the internal cytoskeletal system of myofibers (actin) and the structural proteins within the extracellular matrix (such as collagen and laminin) [29]. Through this complex, sarcolemma ensures the binding of the sarcomere to the extracellular connective tissue. If the complex comes to be associated with desmin, the respective regions turn out to be involved in signaling. Proteins associated with dystrophin-glycoprotein complex might be dysfunctional, leading to myopathies, which manifest by progressive muscle damage and impairments in regeneration [29]. Caveolae are sarcolemmal invaginations existing in the regions of the membrane microdomains rich in caveolin-3 and organized into multilobed structures which provide a large reservoir of surface-connected membrane underlying the sarcolemma. Besides acting as cellular devices involved in the concentration and functional regulation of various signal molecules [30], caveolae can protect the muscle sarcolemma against damage in response to excessive membrane activity [31].

The skeletal muscle fiber contains numerous nuclei (30–40 nuclei/cm long), oval-elongated (8–10 μm) and rich in heterochromatin. The nuclei are disposed in the peripheral sarcoplasm immediately beneath the sarcolemma, with their long axis parallel to the fiber and in alternate positions. Their number is higher at the level of the motor end plates and the myotendinous junctions, where they form agglomerations [12].

Sarcoplasm is a component found among myofibrils and can vary in quantity depending on the type of skeletal fiber in which it is found (red muscles, rich in cytoplasm; white muscles, little sarcoplasm) [32]. It also contains common and specific organelles and various inclusions (glycogen, lipid, pigments).

Common Organelles Mitochondria are located in the sarcoplasm in the vicinity of the nucleus or among the bundles of myofibrils – intermyofibrillar [33]. The number of mitochondria is higher at the Z line and in the I band where they have a long axis parallel to the long axis of the muscle fiber and are very numerous in high-speed skeletal fibers.

Specific Organelles Sarcoplasmic reticulum (SR) can be considered as a muscle-specific organelle, although it is, actually, the smooth endoplasmic reticulum specialized in calcium release/storage [34]. The sarcoplasmic reticulum describes a dilated portion (junctional SR) in contact with the T tubules and a binding portion (free SR). In the SR lumen, calcium is linked to calsequestrin and has a concentration of 10^4–10^5 times higher than cytoplasmic calcium. The action potential of the sarcolemma is led up to the neighborhood of the SR through the T-tubes and determines the release of calcium from SR cisterns through membrane ion channels. The calcium concentration in the sarcoplasm increases from 10^{-7} to 10^{-6} and triggers the contraction. Calcium reuptake is performed by an enzyme, the Ca^{2+} pump, with ATP consumption, against the concentration gradient, the consequence being the decrease of calcium in the sarcoplasm followed by relaxation [35].

Muscle contraction is triggered by electrical activity induced at the level of the transverse tubules and the membrane cell surface. The scientific research is currently focusing on the correlation between two major components, respectively, SR and T tubules. This interaction is mediated by the dihydropyridine receptors (DHPRs) and by ryanodine receptors (RyRs). These channels are implicated in calcium release mechanism. Optimal functioning of the skeletal muscles requires three essential processes, respectively, storage, discharge, and recovery of calcium. In these mechanisms are implicated three classes of SR calcium-regulatory proteins: luminal calcium-binding proteins, SR calcium release channels, and sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps. The first category includes calsequestrin, histidine-rich calcium-binding protein, junctate, and sarcalumenin and is involved in calcium storage, while the second category (type I ryanodine receptor or RyR1 and IP3 receptors) is implicated in calcium release. Calcium recovery is provided by SERCA pumps [36]. Triads are specialized complexes consisting of a centrally located T tubule and flanked by two junctional sarcoplasmic reticulum cisterns [37, 38]. They are located adjacent to the boundary between A and I bands and are designed to ensure a smoothing of muscle fiber contraction.

Myofibrils are the specific contractile organs parallel to each other and the longitudinal axis of the muscle fiber, occupying between 80 and 86% of the cell volume. Myofibrils are composed of thin and thick myofilaments, parallel to each other, and are responsible for the striated nature of the muscle fiber. The skeletal fiber-specific band (cross striations) can be seen in optical microscopy as an alternation between dark A bands (anisotropic under polarized light, dark in phase contrast) and bright I bands (isotropic under polarized light, bright in phase contrast). In the middle of the bright bands, the narrow, dense lines, the Z lines or Z disks, can be seen (Fig. 2.4). The orderly arrangement of myofibrils is conferred by solidarization, by means of

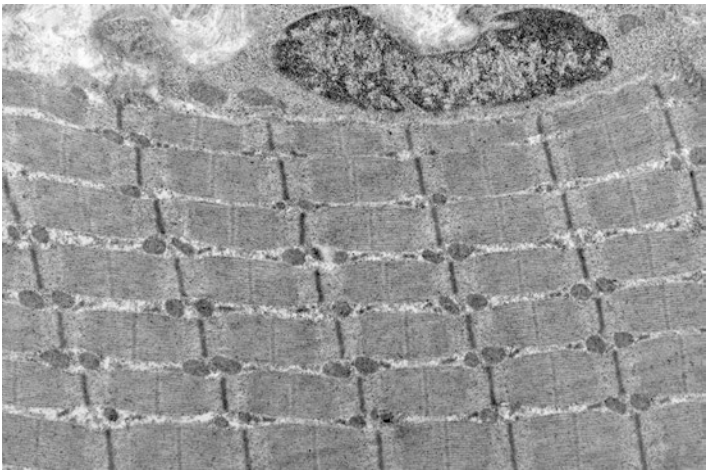

Fig. 2.4 Transmission electron micrograph (TEM) of a longitudinal section through the skeletal muscle. The striations are due to the presence of sarcomeres consisting of the darker bands – A bands (includes a lighter central zone, called the H band) – and the lighter bands, I bands. Each I band is bisected by a dark transverse line called the Z line flanked by mitochondria. Paired mitochondria are on either side of the electron opaque Z line. The Z Line marks the longitudinal extent of a sarcomere unit

intermediate filaments of desmin. The Z disks are solidarized between the adjacent myofibrils via plectin. The segment comprised of two Z-membranes (disks) is a sarcomere (the Krause muscular box) – the morpho-functional unit of the ribbed myofibril. The sarcomere is the functional unit of the myofibril and consists of an A band and two clear halves of I band and has a length of 2–3 μm. In electron microscopy, it is observed that the A band (1.5 μm long) is electron-dense and is crossed through by a clear area – H band (Hensen) through which a fine membrane passes – the M line (Mittel – middle line), hard to observe in optical microscopy. The I band (0.8 μm long) is transparent to the electron beam. The middle of clear bands is crossed by a thin membrane – Z (Stria Amici or Krause's membrane) membrane. Myofilaments include:

– Thick filaments, ~ 1.500 per sarcomere (15 nm in diameter and 1.5 μm long), disposed in the middle of the sarcomere and forming the A band.
– Thin filaments, ~3000/sarcomere (7 nm in diameter and 1.0 μm long), form the I band but also participate in A band formation.

While A band contains thick and thin filaments (a thick filament is surrounded by six thin filaments), I band is formed only from thin myofilaments. The H band is composed only of thick myofilaments solidified at the M band by cytoskeletal filamentous proteins. The Z band consists of actin-like filament anchor proteins: α-actinin, CapZ, and nebulin.

2.1.4 Molecular Organization of Myofilaments in Striated Muscle Fiber

The myofibrils are composed of proteinaceous structures, called myofilaments, which are different in size. Myofilaments are the actual contractile-specific organelles of striated muscles, made of individual filamentous polymers of myosin II (thick filaments) and actin and specifically associated proteins.

Thin Filaments Thin myofilaments contain actin, tropomyosin, troponin, and other associates. The thin filaments are mostly made up of a globular monomeric protein called G-actin (**g**lobular) – about 300 individual molecules. They measure 8 nm in diameter and extend from the Z line for a length of ~ 1.0 μm [19]. The G-actin monomers combine to form a long polymer chain F-actin (filamentous). Each G-actin molecule of the thin filaments has a myosin-binding site, which in resting stage is protected by tropomyosin molecule. Because all the actin monomers are oriented in the same direction, actin filaments have a distinct polarity and their ends (called the plus and minus ends). Two such actin polymers intertwine in a helical fashion to form a thin filament strand. Thin filaments are oriented in opposite directions at each Z line of a sarcomere, which is essential for the production of contractile forces [39]. Tropomodulin is intended to cover the end of the actin by preventing the addition of new actin G monomers. The F-actin filament has a specific polarity with a tropomodulin-coated end that penetrates the thick filaments which is called minus (−) end and a plus (+) end that anchors to the Z membrane by the CapZ protein when the filament reaches the right length. Then, the plus end of each filament is bound to the Z line by α-actinin (bundles thin filaments into parallel arrays and anchors them at the Z line) with nebulin assistance [40]. The minus end extends toward the M line and is protected by tropomodulin, an actin capping protein. Nebulin anchors through the terminal carboxyl-terminus at the Z lines and with the amino-terminal ends at the A band [41]. Nebulin is an inelastic filamentous protein that twists around the actin filament by packing with actin, troponin, and tropomyosin molecules [41]. The nebulin is linked with thin filaments through tropomodulin and Z line proteins, being involved in establishing their length [26].

Tropomyosin is a fibrous protein consisting of rods (40 nm each) linked head-tail and is located in the grooves of the double helix of actin F. Tropomyosin has two α-helical polypeptides that bind laterally to seven contiguous actin subunits as well as head to tail to neighboring tropomyosins, forming a continuous strand along the whole thin filament. Troponin is a complex oligomeric protein and has three components: troponin C (Ca^{2+}-binding), troponin I (inhibitory), and troponin T (tropomyosin binding) [42].

In striated muscles, the concentration of Ca^{2+} influences the complex formed from tropomyosin molecules and troponins; thus at low calcium concentration,

muscles do not contract. If the level of Ca^{2+} is higher, muscle contraction is initiated [26, 43].

Thick Filaments These filaments are 12–16 nm in diameter and ~ 1.6 μm long and are packed in a hexagonal array on 40–50 nm centers throughout the A bands [19]. Each thick myofilament contains approximately 250 myosin II molecules arranged antiparallel and associated with myomesin, titin, and protein C. The myosin II class includes various muscle myosins and cytoplasmic myosins that also have two heads and long coiled tails. The assembly of tails into bipolar filaments allows myosin II to pull together oppositely polarized actin filaments during muscle contraction. Myosin II, a 510 kDa, long, rod-shaped, actin-associated motor protein, is an asymmetric dimer composed of two heavy polypeptide chains (222 kDa each) and four light chains (two regulatory chains and two essential chains). Heavy chains form a structure called a tail or stick, twisted in the form of a helix, but it also enters the constitution of a large part of the globular ends. The ends of the myosin molecule contain, besides heavy chains, the associated light chains, one of 20 kDa (LC20) and one of 17 kDa (LC17). LC20 comprises the phosphorylation site by MLCK (myosin light chain kinase).

Myosin molecules in striated muscle aggregate tail to tail to form bipolar thick myosin filaments; the tails overlap so that the globular heads protrude from the thick filament at regular intervals to form transverse bridges. In the middle of the filament, there are not any globular projections.

The regions of the myosin heads contain distinct actin-binding sites, ATP hydrolysis, and association of light chain subunits. By limited proteolysis, myosin can be divided into two functional domains due to the presence of protease-sensitive sites in the hinge region and the head-tail junction. Under the controlled action of trypsin, light meromyosin (LMM) is formed – the region in which myosin molecules interact to form filaments – and heavy meromyosin (HMM) is the transverse bridge (the tail and the two globular ends). HMM can be cleaved under the action of papain in two subfragments: S2 representing the rest of the tail and S1 (representing the two globular ends) containing the ATP and actin-binding sites.

Several accessory proteins stabilize thick filaments. The M line in the center of the sarcomere is a three-dimensional array of protein cross-links that maintains the precise registration of thick filaments. M line proteins include myomesin, M protein, obscurin, and muscle creatine phosphatase. The interaction between the heavy and light chains determines the speed and strength of muscle contraction. The myosin head has two specific binding sites, one for ATP with ATPase activity and one for actin [26].

Myomesin is a protein that solidarizes the filaments at the level of line M. The protein C binds to the myosin in the vicinity of the M line at the end of the thin filament at the intersection of A and I bands.

Titin is a large (2500 kDa) protein, which spans half of the sarcomere, and is responsible for the axial periodicity of myofilaments because it maintains

three-dimensional relationships by keeping the thick and thin filaments in proper alignment. Titin is named after the mythological giants, due to its remarkable size: more than 30,000 amino acids folded into a linear array of 300 immunoglobulins and fibronectin II measuring more than 1.2 μm long. The amino terminus end of the titin molecule completely crosses the Z lines and is anchored to α-actinin. At the Z band, the titin molecules in the adjacent sarcomeres overlap. The carboxy terminus end traverses the entire M line and overlaps the titin molecules in the other half of the sarcomere and binds to the myomesin. At I band, titin interacts with actin molecules and at A band interacts with protein C. If titin molecules are broken experimentally, thick filaments slide out of register toward one Z disk during contraction.

Desmin helps to align the sarcomere laterally by linking each Z disk to its neighbors and to specialized attachment sites on the plasma membrane (intermediate filaments that interconnect adjacent myofibrils).

The interaction of these myofibrillar proteins allows muscles to contract.

2.2 Skeletal Muscle Contraction Mechanism

2.2.1 Neuromuscular Transmission

Skeletal muscle works under voluntary control. Muscles will contract or relax when they receive signal from the nervous system. The control of skeletal muscle fibers is performed by alpha motor neurons located in the anterior horns of the spinal cord and in motor nuclei of the origin of the cranial nerves. A neuron, along with the specific muscle fibers that it innervates, is called a motor unit. The axons of the neurons branch as they are adjoining the muscle, giving rise to terminal branches that end on individual muscle fibers. The neuromuscular junction is the site of the signal exchange where synaptic bulb of an axon and a muscle fiber connect. The axon ending is a typical presynaptic structure which contains numerous mitochondria and synaptic vesicles that contain the neurotransmitter acetylcholine (ACh). The neuron that carries the action potential is known as the presynaptic cell and the cell receiving it (muscle cell) as the postsynaptic cell. The neurotransmitter is released in the synaptic cleft, the space between the axon terminal and the muscle cell (the space contains amorphous basal lamina matrix). Motor end plate is a region of the sarcolemma that participates in the synapse having ACh receptors. The nicotinic ACh receptor in striated muscles is a transmitter-gated Na^+ channel. Binding of ACh opens Na^+ channels, causing an influx of Na^+ into striated muscle cell. These channels are not voltage-gated, and they will open only when the ACh attaches to them. Once open, they will allow the passage of sodium ions into the muscle cell, down their electrochemical gradient.

2.2.2 Excitation-Contraction Coupling (Exposure of Active Sites)

When sarcolemma is depolarized, an action potential (AP) is generated and triggers muscle cell contraction. The AP initiated on the membrane surface spreads radially in all directions, spanning the entire surface and then penetrating deep into the cell via T tubule (invaginations of the sarcolemma). Due to these tubules, the action potential can spread along the muscle cell evenly and quickly [44]. As the AP reaches the membrane of the sarcoplasmic reticulum, it makes it permeable to calcium ions. Once the calcium is inside the cytosol, it can interact to thin filaments to initiate contraction. T tubules show numerous L-type voltage-dependent Ca^{2+} channels. The change in potential difference opens the Ca^{2+}channels and allows the calcium to penetrate into the cell according to the concentration gradient. This type of calcium channels is also called dihydropyridine (DHP)-dependent channels because they can be blocked by dihydropyridine. The amount of Ca^{2+} penetrated through these channels is small and incapable to trigger muscle fiber contraction. However, activation of these dependent Ca^{2+} DHP channels is mandatory in triggering the contraction. Activation of Ca^{2+} L-type-dependent channels (DHP dependent) drives two mechanisms:

- The flow of Ca^{2+} through the channel produces conformational changes in the subunits that compose it. Through the proximity of the T tubule with the sarcoplasmic reticulum within the triad, intimate contact is allowed between the dependent DHP channels and the Ca^{2+} channels of the sarcoplasmic reticulum and the RyRs-dependent channels. Activating dependent Ca^{2+} DHP channels activates RyRs-dependent channels [45].
- The release of Ca^{2+} from the sarcoplasmic reticulum increases the concentration of Ca^{2+} approximately 10^{-7} to 10^{-5} M. The bond between troponin-tropomyosin complex and actin becomes weak. The action potential causes a short-lived conformational change in DHP receptors that is transmitted directly to the associated RyRs Ca^{2+} release channels. Cytoplasmic Ca^{2+} binds to troponin C. Troponin changes position, pulling tropomyosin away from the active sites. This shift increases the probability that myosin-ADP-Pi heads will bind to the thin filament, dissociating their bound Pi and producing force. Ca^{2+} binds to troponin C rapidly (milliseconds) but dissociates slowly (tens of milliseconds) [46].

2.2.3 The Main Steps Involved in Muscle Contraction

The interaction between myofibrillar proteins myosin (the thick filament) and actin (the thin filament) allows muscles to contract. This fact was demonstrated long before the fine structure of the myofibril became known. In 1954, the mechanism of muscle contraction, based on muscle proteins that slide past each other to generate movement, was suggested by Andrew F. Huxley and is known as the sliding

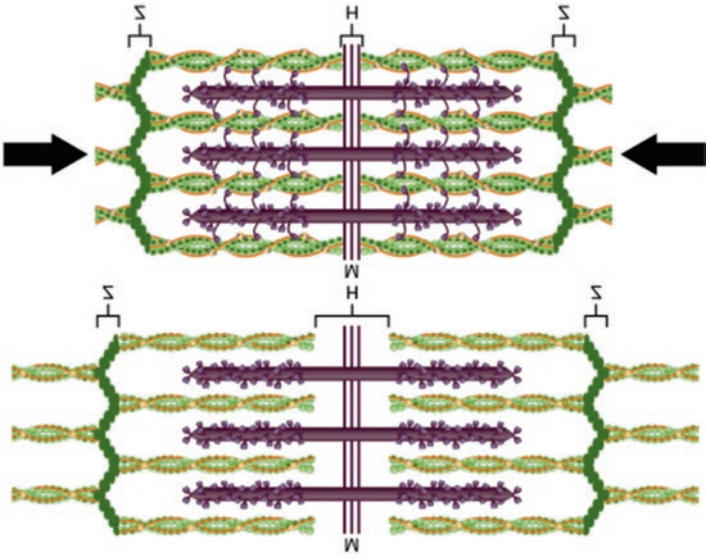

Fig. 2.5 The sliding filament model of muscle contraction. When a sarcomere contracts, the Z lines move closer together, and the I band becomes smaller. The A band stays the same width. At full contraction, the thin and thick filaments overlap completely. (Image credit: download for free at http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@8.119)

filament model of contraction [47–49] (Fig. 2.5). The movement of muscle in mammalian species is directly dependent on the hydrolysis of ATP as its source of energy [1]. The first step is represented by the exposure of actin active sites. In a second step, myosin crossbridges bind to actin active sites. ATP binds to myosin head and induces conformational changes of the actin-binding site. The third step is represented by cycles of the myosin heads. The light chain enzyme of the myosin head allows ATP cleavage in ADP and Pi. As a result of the dissociation of the macroergic bond, part of the energy is released, and the head of myosin bends from an angle of 90 degrees to an angle of 45 degrees with the advancement of the actin filaments by 11 nm [50]. After crossbridge attachment, the energy is released as the myosin head pivots toward the M line. This action is called the power stroke. When adenosine diphosphate (ADP) and Pi are released, both products remain bound to the myosin head. The fourth step consists of the detachment of crossbridges [51]. Another ATP binds to the myosin head, and the link between the actin active site and myosin head is broken. The active site is now exposed and able to interact with another crossbridge. When a muscle is stimulated to contract, the myosin heads start to walk along the actin filaments in repeated cycles of attachment and detachment. During each cycle, a myosin head binds and hydrolyzes one molecule of ATP. Myosin molecule moves the tip of the head along the actin filaments toward the plus end. This movement, repeated with each round of ATP hydrolysis, propels the myosin molecule unidirectionally along the actin filament. In the last step, the reactivation of myosin occurs when myosin heads split ATP and myosin head is in the resting position (Fig. 2.6). The contraction stops by Ca^{2+} returning to the sarcoplasmic

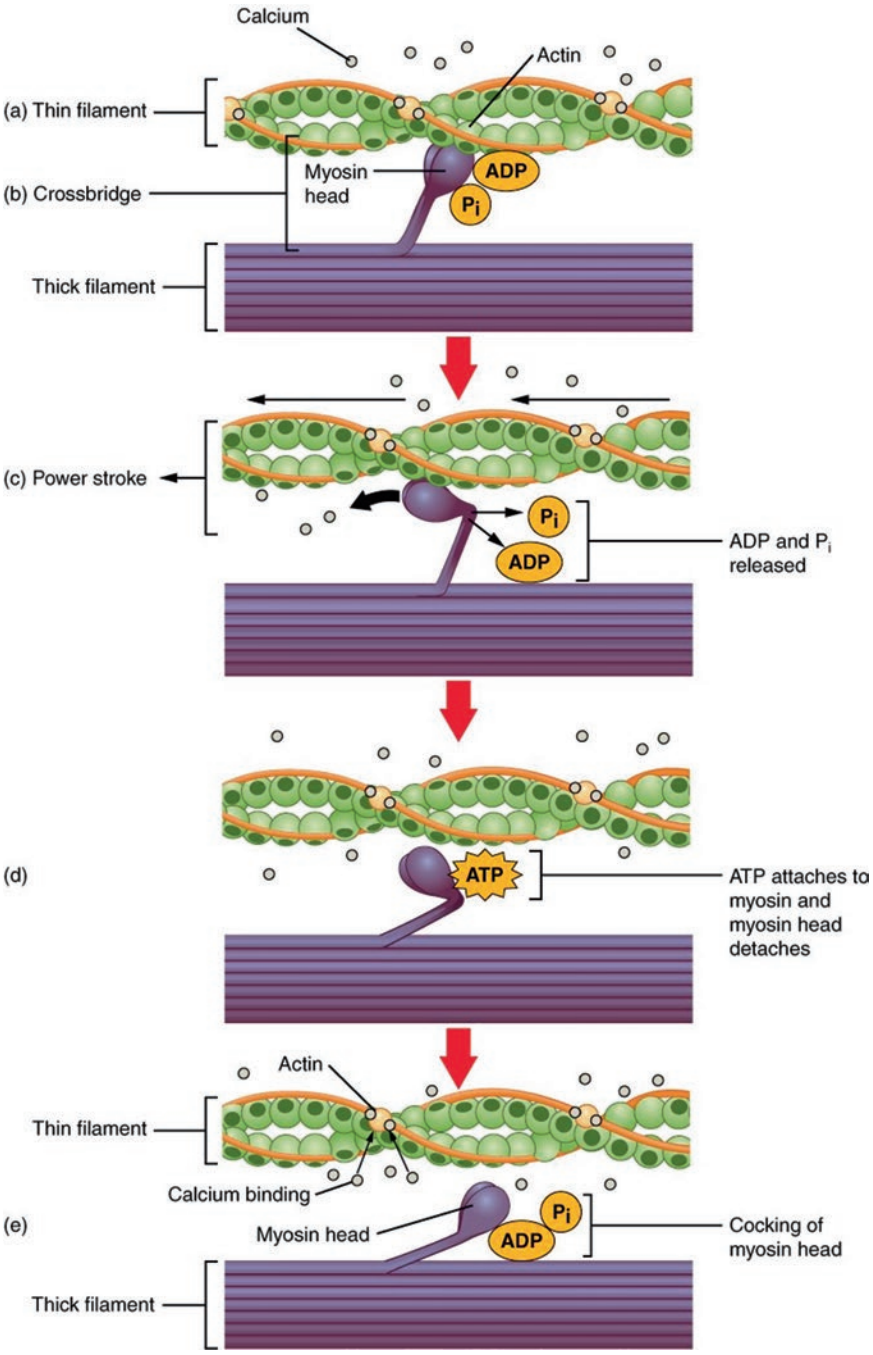

Fig. 2.6 (**a**) The active site on actin is exposed as calcium binds to troponin. (**b**) The myosin head is attracted to actin, and myosin binds actin at its actin-binding site, forming the crossbridge. (**c**) During the power stroke, the phosphate generated in the previous contraction cycle is released. This results in the myosin head pivoting toward the center of the sarcomere, after which the attached ADP and phosphate group are released. (**d**) A new molecule of ATP attaches to the myosin head, causing the crossbridge to detach. (**e**) The myosin head hydrolyzes ATP to ADP and phosphate, which returns the myosin to the cocked position. (Image credit: download for free at http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@8.119)

reticulum via the SERCA pump. The SERCA pump is found in the membrane of the sarcoplasmic reticulum and plays a role in pumping Ca^{2+} against the concentration gradient. Pump activity is controlled by phospholamban, regulated in turn by β-adrenergic receptors. β-Adrenergic stimulation is followed by phosphorylation of phospholamban (activated form) followed by inhibition of Ca^{2+} pumps with increased concentration in the cytoplasm and increased contraction force.

Because all the sarcomeres contract together, the entire muscle shortens at the same rate. When a skeletal muscle fiber contracts the H bands and I bands get smaller, the overlapping zones get larger, the Z lines move closer together, and the width of the A bands remains constant. The contraction ends once the fiber has shortened by 30% (elimination of the I bands) [52, 53].

2.2.4 Types of Muscle Contractions

Single direct electrical stimulation of a muscle, or indirect through the motor nerve, with a constant current of a certain intensity and duration, causes a muscular twitch (rapid shortening followed by a return). Twitch is an elemental, biologically active functional manifestation of muscle contractility consisting of its shortening and tension development. Twitches can be experimentally produced by applying an electric current to a motor nerve. Under physiological conditions, there are no twitches. Shiver, contraction of extraocular muscles, and other types of contractions, even if they are short-duration contractions, require a short-term discharge of a large number of nerve impulses [54].

During twitch, a series of steps are described that follow the unique stimulation of the fiber muscle:

- There is a latency phase of approximately 5 ms from the initiation of the process to the beginning of the contraction. This is given by the time required to propagate the action potential and the time required to mobilize Ca^{2+} from the sarcoplasmic reticulum.
- There is a contraction phase of about 15 ms when the increased concentration of Ca^{2+} in the cytosol allows actin-myosin coupling that corresponds to muscle shortening and muscular force generation.
- There is relaxation phase, longer than 25 ms, in which the Ca^{2+} concentration in the cell slowly decreases by pumping it into RS, followed by the decrease of the actin-myosin bridges.

Physiologically, all contractions of the skeletal muscles are done by tetanus contraction. Tetanus contraction is a summary of twitches. Strong, efficient, variable-duration contraction is achieved. The contraction of the heart muscle is a response to a single stimulus, but due to the long duration of the action potential, the cardiac twitch is entirely different from the skeletal muscle. Increasing the frequency of stimulation of the muscle fiber generates a continuous and stronger contraction than the twitch. When the stimulus frequency is low during the contraction period,

incomplete relaxation periods will occur, and muscle tension will be inconsistent. This type of contraction is called incomplete tetanus. If the stimulation frequency does not allow relaxation periods during muscle contraction, a plateau of muscle tension appears, and the contraction is called complete tetanus. The developed force is maximal, superior to both twitch and incomplete tetanus contraction [54].

Muscle fiber generates tension through the action of actin and myosin cross-bridge cycling. While under tension, the muscle may lengthen, shorten, or remain the same. Muscle activity in the body is a combination of the isometric, isotonic, and auxotonic forms of contractions. An isometric contraction occurs when the contracting muscle is fixed to both extremities. Thus, the length of the fibers does not change during contraction, but the increase in muscle tension occurs [55]. The anti-gravity muscles, those which maintain the posture, and the masticatory muscles used in the process of crushing food perform isometric contractions. Isotonic contraction is performed by the muscle that raises a weight. During contraction, its length is reduced, but the tension is remaining unchanged. Isotonic contractions are characteristic of the movement of limbs in the process of walking or lifting of constant weight [56]. There are two types of isotonic muscle contraction: concentric and eccentric muscle contraction. In concentric muscle contraction, muscle fibers shorten as tension in the muscle increases, as when lifting a weight. In eccentric muscle contraction, although the actin and myosin filaments within the muscle fibers contract (to produce the force needed), the fibers themselves also slide alongside each other resulting in the overall lengthening of the muscle [57]. Muscle lengthens as tension in the muscle increases, as when slowly lowering a weight. Auxotonic contraction is an intermediate functional manifestation. During the contraction, the muscle shortens but with the progressive increase of the tension. Auxotonic contractions are combined with the previous ones in the work process when the superior muscular force defeats a growing external force [58].

2.3 Biochemical Diversity of Skeletal Muscle

In the last decade, the biochemical, structural, and functional properties of myofibers were intensively studied, but understanding molecular processes regulating fiber-type diversity is still poorly understood, due to the heterogeneity of cell types present in the skeletal muscle organ [2].

Skeletal muscle is a complex and versatile tissue composed of a variety of functionally diverse myofibers which reach their normal length at puberty (13–15 years). Regarding the mean fiber diameter in normal muscles, there are no significant differences between the three muscle fiber types which are less than 12% [59]. Gender difference shows larger myofibers in men than women for type I and type II. In women, type I fibers are larger than type II, while in men these dimensions are reversed. The muscle mass begins to decrease between 20 and 80 years by reducing the number of myofibers by 30–40% [60].

Skeletal muscle tissue is a very heterogeneous one, composed of a bundle of muscle cells which are implicated in a series of activities appropriate to each animal species. To deal with divergent activities, muscles are composed of muscle cells with large differences in metabolic profile and contractile properties, found under the influence of hormonal and neural systems. Moreover, it seems that nerve activity plays a major role in the determination of the fiber type [16]. Skeletal muscle fibers can be classified based on their color (red, high in myoglobin; white, low myoglobin), on their speed (slow, fast, intermediate), on their fatigability (fatigue resistant and fatigable), or on their myosin isoforms.

At the beginning of the nineteenth century, based on their *speeds of shortening*, muscle fibers were defined as slow or fast [61]. In the mid-twentieth century, by refining certain techniques for *myosin ATPase (mATPase) histochemistry* and electron microscopy and by advanced biochemical studies regarding oxidative and glycolytic enzymes, skeletal muscle cells were characterized in much more details. The combination of histochemical analysis for myofibrillar actomyosin ATPase (myosin ATPase) and for enzymes of energy metabolism gives rise to the fiber nomenclature. Also, the speed of contraction is dependent on how quickly the ATPase of myosin can hydrolyze ATP to produce crossbridge action. Based on these criteria, there are three main types of skeletal muscle fibers (cells): slow oxidative (type I), fast oxidative (type IIa), and fast glycolytic (type IIb) [62]. Fast fibers hydrolyze ATP approximately twice as quickly as slow fibers. The fast-twitch muscle fibers are known as the white muscle, while the slow-twitch muscle fibers are known as red muscle. Based on their fatigability, fast-twitch motor units can be categorized as fast-twitch fatigue resistant (type FR), fast-twitch fatigue intermediate (type FInt), and fast-twitch fatigable (type FF) [63].

Slow-contracting muscle fiber (type I) is characterized by (a) low myosin ATPase activity (compared with type II fibers), (b) high capacity for ATP production via oxidative phosphorylation (aerobic cellular respiration), (c) very dense capillary network, (d) high levels of intracellular myoglobin (predominant color is red), and (e) function for long periods without fatigue.

Fast-contracting muscle fiber (type IIa) is characterized by (a) higher myosin ATPase activity than type I fibers, (b) high capacity for ATP production via oxidative phosphorylation (aerobic cellular respiration), (c) dense capillary network, (d) high levels of intracellular myoglobin (predominant color is red), and (e) being more fatigue resistant than type IIb fibers.

Fast-contracting muscle fiber (type IIb) is characterized by:

(a) Higher myosin ATPase activity than type I fibers.
(b) Lower capacity for ATP production via oxidative phosphorylation than “red” fibers (anaerobic glycolysis); muscle fatigue occurs sooner.
(c) Sparser capillary network.
(d) No intracellular myoglobin (predominant color is white).
(e) These fibers fatigue quickly.

Type IIb fibers can be converted into type IIa fibers by resistance training.

Details about all these fibers can be found in Table 2.1.

Table 2.1 Comparison between the three main types of skeletal muscle fibers

Characteristic	Red/slow (type I) slow-twitch fibers	Red/fast (type IIa) fast oxidative fibers	White/fast (type IIb) fast glycolytic fibers
Color	Red	Red	White
Contraction speed	Slow	Fast	Very fast
Oxidative capacity	High	High	Low
Resistance to fatigue	High	Medium (intermediate)	Low
Diameter (of muscle fiber)	Small	Medium (intermediate)	Large
Capillary density	High	Medium (intermediate)	Low
Mitochondrial density	High	High	Low
Glycogen reserves	Low	Intermediate	High
Myosin ATPase activity	Low	High	High
Main (metabolic) pathway for production of ATP	Aerobic cellular respiration – final stage: oxidative phosphorylation	Both aerobic and anaerobic metabolic pathways	*Only* anaerobic metabolism, esp. anaerobic glycolysis
Anaerobic enzyme content	Low	Medium	*High*
Force production (i.e., force produced by muscle)	Low	Medium-high	Very high
Example of typical use	Repeated low-level contractions, e.g., walking or low-intensity cycling for 30 mins.	Used primarily for movements, such as walking (require more energy than postural control but less energy than sprinting). Activities involving speed, strength, and power	Used to produce rapid, forceful contractions to make quick, powerful movements. Short, fast, bursts of power such as heavy weight training, power lifting, and sprints
Examples of skeletal muscles with this type of fiber	Postural muscles of the neck and spine, leg muscles (type I and type IIa fibers)	Leg muscles (large quantities of both type I and type IIa fibers)	Arm muscles

Table 2.2 Panel of sarcomeric MHC genes with the corresponding protein products and their location

Gene	Proteins	Expression
MYH13	MyHC-EO	Extraocular muscle
MYH8	MyHC-neo	Developing muscle
MYH4	MyHC-2B	Fast 2B fibers
MYH1	MyHC-2X	Fast 2X fibers
MYH2	MyHC-2A	Fast 2A fibers
MYH3	MyHC-emb	Developing muscle
MYH6	MyHC-α	Jaw muscle and heart
MYH7	MyHC-β/slow	Slow muscle and heart
MYH7b	MyHC slow/tonic	Extraocular muscle
MYH15	MyHC-15	Extraocular muscle
MYH16	MyHC-M	Jaw muscle

Another classification system is based on *myosin heavy chain (MHC) isoforms*, and the heterogeneity of myosin isoform expression dates back to 30 years ago [64, 65]. Originally, four major myosin isoforms were identified: MHCI, MHCIIa, MCHIIx, and MHCIIb [66–68]. Recently, myosin ATPase histochemical staining allows the description of some other types, such as Ic, IIc, IIac, and IIab, based on the intensity of staining at different pH levels [69, 70]. Several isoforms of MHC are known to exist in mammalian skeletal muscle including IIm, alpha, neonatal, embryonic, and extraocular. These isoforms can be determined using anti-myosin antibodies or by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [71]. Nowadays, one knows that these MHC isoforms are first established by intrinsic myogenic control mechanisms during embryonic development and are later modulated by neural and hormonal factors [9]. According to a study conducted by Schiaffino, in any muscle, different fiber types coexist. One can observe in Table 2.2 the complete panel of sarcomeric MHC genes with the corresponding protein products proposed by Schiaffino in mammalian species extrafusal muscle fibers [16].

2.4 Conclusion

Skeletal muscle physiology is complex, and there are many functional differences between fiber types starting with neuromuscular transmission, excitation-contraction coupling, and cycling of crossbridges and finishing with ATP consumption. Gene and protein expressions depending on the type of fiber are still at the beginning regarding their importance in several conditions leading to muscle atrophy.

References

1. Roman W, Gomes ER (2017) Nuclear positioning in skeletal muscle. Semin Cell Dev Biol. https://doi.org/10.1016/j.semcdb.2017.11.005
2. Chemello F, Bean C, Cancellara P, Laveder P, Reggiani C, Lanfranchi G (2011) Microgenomic analysis in skeletal muscle: expression signatures of individual fast and slow myofibers. PLoS One 6(2):e16807. https://doi.org/10.1371/journal.pone.0016807
3. Baker JS, McCormick MC, Robergs RA (2010) Interaction among skeletal muscle metabolic energy systems during intense exercise. J Nutr Metab 2010:905612. https://doi.org/10.1155/2010/905612
4. Buckingham M, Bajard L, Chang T, Daubas P, Hadchouel J, Meilhac S, Montarras D, Rocancourt D, Relaix F (2003) The formation of skeletal muscle: from somite to limb. J Anat 202(1):59–68
5. Coalson RE, Tomasek JJ (2012) Musculoskeletal System. In: Embryology (Oklahoma Notes), 2nd edn. Springer, New York
6. Braun T, Bober E, Rudnicki MA, Jaenisch R, Arnold HH (1994) MyoD expression marks the onset of skeletal myogenesis in Myf-5 mutant mice. Development 120(11):3083–3092
7. Yin H, Price F, Rudnicki MA (2013) Satellite cells and the muscle stem cell niche. Physiol Rev 93(1):23–67. https://doi.org/10.1152/physrev.00043.2011
8. Boonen KJ, Post MJ (2008) The muscle stem cell niche: regulation of satellite cells during regeneration. Tissue Eng Part B Rev 14(4):419–431. https://doi.org/10.1089/ten.teb.2008.0045
9. Bentzinger CF, Wang YX, Rudnicki MA (2012) Building muscle: molecular regulation of myogenesis. Cold Spring Harb Perspect Biol 4(2). https://doi.org/10.1101/cshperspect.a008342
10. Francetic T, Li Q (2011) Skeletal myogenesis and Myf5 activation. Transcription 2(3):109–114. https://doi.org/10.4161/trns.2.3.15829
11. Collins CA, Gnocchi VF, White RB, Boldrin L, Perez-Ruiz A, Relaix F, Morgan JE, Zammit PS (2009) Integrated functions of Pax3 and Pax7 in the regulation of proliferation, cell size and myogenic differentiation. PLoS One 4(2):e4475. https://doi.org/10.1371/journal.pone.0004475
12. Krause WJ (2005) Krause's essential human histology for medical students 3rd. Universal Publishers, Boca Raton
13. Gartner LP, Hiatt JL, Strum JM (2011) BRS review series cell biology and histology. Lippincott Williams & Wilkins, Baltimore
14. Korthuis RJ (2011) Skeletal Muscle Circulation. Morgan & Claypool Life Sciences, San Rafael
15. Brooks SV (2003) Current topics for teaching skeletal muscle physiology. Adv Physiol Educ 27(1–4):171–182. https://doi.org/10.1152/advan.00025.2003
16. Schiaffino S, Reggiani C (2011) Fiber types in mammalian skeletal muscles. Physiol Rev 91(4):1447–1531. https://doi.org/10.1152/physrev.00031.2010
17. Boncompagni S (2012) Severe muscle atrophy due to spinal cord injury can be reversed in complete absence of peripheral nerves. Eur J Transl Myol 22(4):161–200
18. Infantolino BW, Ellis MJ, Challis JH (2010) Individual sarcomere lengths in whole muscle fibers and optimal fiber length computation. Anat Rec (Hoboken) 293(11):1913–1919. https://doi.org/10.1002/ar.21239
19. Metzler DE (2003) The chemical reactions of living cells. In: Metzler DE (ed) The chemical reactions of living cells, 2nd edn. Academic Press, San Diego, pp 1088–1128
20. Kadi F, Thornell LE (2000) Concomitant increases in myonuclear and satellite cell content in female trapezius muscle following strength training. Histochem Cell Biol 113(2):99–103
21. Bagshaw CR (1982) Outline studies of biology: muscle contraction. Chapman and Hall, London
22. Morgan JE, Partridge TA (2003) Muscle satellite cells. Int J Biochem Cell Biol 35(8):1151–1156

23. Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, Morgan JE (2005) Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell 122(2):289–301. https://doi.org/10.1016/j.cell.2005.05.010
24. Al-Qusairi L, Laporte J (2011) T-tubule biogenesis and triad formation in skeletal muscle and implication in human diseases. Skelet Muscle 1(1):26. https://doi.org/10.1186/2044-5040-1-26
25. Bennett PM, Maggs AM, Baines AJ, Pinder JC (2006) The transitional junction: a new functional subcellular domain at the intercalated disc. Mol Biol Cell 17(4):2091–2100. https://doi.org/10.1091/mbc.E05-12-1109
26. Henderson CA, Gomez CG, Novak SM, Mi-Mi L, Gregorio CC (2017) Overview of the muscle cytoskeleton. Comp Physiol 7(3):891–944. https://doi.org/10.1002/cphy.c160033
27. Bloch RJ, Capetanaki Y, O'Neill A, Reed P, Williams MW, Resneck WG, Porter NC, Ursitti JA (2002) Costameres: repeating structures at the sarcolemma of skeletal muscle. Clin Orthop Relat Res 403(Suppl):S203–S210
28. O'Neill A, Williams MW, Resneck WG, Milner DJ, Capetanaki Y, Bloch RJ (2002) Sarcolemmal organization in skeletal muscle lacking desmin: evidence for cytokeratins associated with the membrane skeleton at costameres. Mol Biol Cell 13(7):2347–2359. https://doi.org/10.1091/mbc.01-12-0576
29. Gawor M, Proszynski TJ (2018) The molecular cross talk of the dystrophin-glycoprotein complex. Ann N Y Acad Sci 1412(1):62–72. https://doi.org/10.1111/nyas.13500
30. Fridolfsson HN, Roth DM, Insel PA, Patel HH (2014) Regulation of intracellular signaling and function by caveolin. FASEB J 28(9):3823–3831. https://doi.org/10.1096/fj.14-252320
31. Lo HP, Hall TE, Parton RG (2016) Mechanoprotection by skeletal muscle caveolae. BioArchitecture 6(1):22–27. https://doi.org/10.1080/19490992.2015.1131891
32. Flucher BE, Takekura H, Franzini-Armstrong C (1993) Development of the excitation-contraction coupling apparatus in skeletal muscle: association of sarcoplasmic reticulum and transverse tubules with myofibrils. Dev Biol 160(1):135–147. https://doi.org/10.1006/dbio.1993.1292
33. Ferreira R, Vitorino R, Alves RM, Appell HJ, Powers SK, Duarte JA, Amado F (2010) Subsarcolemmal and intermyofibrillar mitochondria proteome differences disclose functional specializations in skeletal muscle. Proteomics 10(17):3142–3154. https://doi.org/10.1002/pmic.201000173
34. Takekura H, Sun X, Franzini-Armstrong C (1994) Development of the excitation-contraction coupling apparatus in skeletal muscle: peripheral and internal calcium release units are formed sequentially. J Muscle Res Cell Motil 15(2):102–118
35. Stokes DL, Wagenknecht T (2000) Calcium transport across the sarcoplasmic reticulum: structure and function of Ca2+-ATPase and the ryanodine receptor. Eur J Biochem 267(17):5274–5279
36. Rossi AE, Dirksen RT (2006) Sarcoplasmic reticulum: the dynamic calcium governor of muscle. Muscle Nerve 33(6):715–731. https://doi.org/10.1002/mus.20512
37. Flucher BE (1992) Structural analysis of muscle development: transverse tubules, sarcoplasmic reticulum, and the triad. Dev Biol 154(2):245–260
38. Franzini-Armstrong C (1972) Studies of the triad. 3. Structure of the junction in fast twitch fibers. Tissue Cell 4(3):469–478
39. Ono S (2010) Dynamic regulation of sarcomeric actin filaments in striated muscle. Cytoskeleton 67(11):677–692. https://doi.org/10.1002/cm.20476
40. Ottenheijm CA, Granzier H (2010) New insights into the structural roles of nebulin in skeletal muscle. J Biomed Biotechnol 2010:968139. https://doi.org/10.1155/2010/968139
41. Labeit S, Ottenheijm CA, Granzier H (2011) Nebulin, a major player in muscle health and disease. FASEB J 25(3):822–829. https://doi.org/10.1096/fj.10-157412
42. Johnston JR, Chase PB, Pinto JR (2018) Troponin through the looking-glass: emerging roles beyond regulation of striated muscle contraction. Oncotarget 9(1):1461–1482. https://doi.org/10.18632/oncotarget.22879

43. Ohtsuki I (2002) Calcium regulation by troponin and its genetic disorder in striated muscle contraction. Nihon yakurigaku zasshi Folia pharmacologica Japonica 120(1):20P–23P
44. Gordon AM, Homsher E, Regnier M (2000) Regulation of contraction in striated muscle. Physiol Rev 80(2):853–924. https://doi.org/10.1152/physrev.2000.80.2.853
45. Ohtsuki I (2005) Molecular basis of calcium regulation of striated muscle contraction. Adv Exp Med Biol 565:223–231.; discussion 397-403. https://doi.org/10.1007/0-387-24990-7_17
46. Chalovich JM (2002) Regulation of striated muscle contraction: a discussion. J Muscle Res Cell Motil 23(4):353–361
47. Huxley HE (1953) Electron microscope studies of the organisation of the filaments in striated muscle. Biochim Biophys Acta 12(3):387–394
48. Huxley H, Hanson J (1954) Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. Nature 173(4412):973–976
49. Huxley AF, Niedergerke R (1954) Structural changes in muscle during contraction; interference microscopy of living muscle fibres. Nature 173(4412):971–973
50. Payne MR, Rudnick SE (1989) Regulation of vertebrate striated muscle contraction. Trends Biochem Sci 14(9):357–360
51. Grigorenko BL, Rogov AV, Topol IA, Burt SK, Martinez HM, Nemukhin AV (2007) Mechanism of the myosin catalyzed hydrolysis of ATP as rationalized by molecular modeling. Proc Natl Acad Sci U S A 104(17):7057–7061. https://doi.org/10.1073/pnas.0701727104
52. Yanagida T, Esaki S, Iwane AH, Inoue Y, Ishijima A, Kitamura K, Tanaka H, Tokunaga M (2000) Single-motor mechanics and models of the myosin motor. Philos Trans R Soc Lond Ser B Biol Sci 355(1396):441–447. https://doi.org/10.1098/rstb.2000.0585
53. Huxley AF (2000) Mechanics and models of the myosin motor. Philos Trans R Soc Lond Ser B Biol Sci 355(1396):433–440. https://doi.org/10.1098/rstb.2000.0584
54. Mann MD (2011) Muscle contraction: twitch and tetanic contractions. In: Mann MD (ed) The nervous system in action. http://michaeldmann.net/mann14.html. Last visited 7 Aug 2018
55. Sejersted OM, Hargens AR, Kardel KR, Blom P, Jensen O, Hermansen L (1984) Intramuscular fluid pressure during isometric contraction of human skeletal muscle. J Appl Physiol Respir Environ Exerc Physiol 56(2):287–295. https://doi.org/10.1152/jappl.1984.56.2.287
56. Lee SC, Becker CN, Binder-Macleod SA (1999) Catchlike-inducing train activation of human muscle during isotonic contractions: burst modulation. J Appl Physiol 87(5):1758–1767. https://doi.org/10.1152/jappl.1999.87.5.1758
57. Sargeant AJ, Dolan P (1987) Human muscle function following prolonged eccentric exercise. Eur J Appl Physiol Occup Physiol 56(6):704–711
58. Burghardt TP, Sun X, Wang Y, Ajtai K (2017) Auxotonic to isometric contraction transitioning in a beating heart causes myosin step-size to down shift. PLoS One 12(4):e0174690. https://doi.org/10.1371/journal.pone.0174690
59. Dumitru D, Amato AA, Zwarts MJ (2002) Electrodiagnostic medicine. Hanley & Belfus, Philadelphia
60. Lexell J (1995) Human aging, muscle mass, and fiber type composition. J Gerontol A Biol Sci Med Sci 50 Spec No:11–16
61. Needham DM (1926) Red and white muscles. Physiol Rev 6:1–27
62. Enad JG, Fournier M, Sieck GC (1989) Oxidative capacity and capillary density of diaphragm motor units. J Appl Physiol 67(2):620–627. https://doi.org/10.1152/jappl.1989.67.2.620
63. Sieck GC, Fournier M, Prakash YS, Blanco CE (1996) Myosin phenotype and SDH enzyme variability among motor unit fibers. J Appl Physiol 80(6):2179–2189. https://doi.org/10.1152/jappl.1996.80.6.2179
64. Zhang M, Gould M (2017) Segmental distribution of myosin heavy chain isoforms within single muscle fibers. Anat Rec (Hoboken) 300(9):1636–1642. https://doi.org/10.1002/ar.23578
65. Quiroz-Rothe E, Rivero JL (2004) Coordinated expression of myosin heavy chains, metabolic enzymes, and morphological features of porcine skeletal muscle fiber types. Microsc Res Tech 65(1–2):43–61. https://doi.org/10.1002/jemt.20090

66. Fitts RH, Widrick JJ (1996) Muscle mechanics: adaptations with exercise-training. Exerc Sport Sci Rev 24:427–473
67. Zhan WZ, Miyata H, Prakash YS, Sieck GC (1997) Metabolic and phenotypic adaptations of diaphragm muscle fibers with inactivation. J Appl Physiol 82(4):1145–1153. https://doi.org/10.1152/jappl.1997.82.4.1145
68. Hansen G, Martinuk KJ, Bell GJ, MacLean IM, Martin TP, Putman CT (2004) Effects of spaceflight on myosin heavy-chain content, fibre morphology and succinate dehydrogenase activity in rat diaphragm. Pflugers Arch: Eur J Physiol 448(2):239–247. https://doi.org/10.1007/s00424-003-1230-9
69. Staron RS (1997) Human skeletal muscle fiber types: delineation, development, and distribution. Can J Appl Physiol = Revue canadienne de physiologie appliquee 22(4):307–327
70. McComas AJ (1996) Skeletal muscle: form and function, 2nd edn. Human Kinetics Publishers, Champaign
71. Pette D, Peuker H, Staron RS (1999) The impact of biochemical methods for single muscle fibre analysis. Acta Physiol Scand 166(4):261–277. https://doi.org/10.1046/j.1365-201x.1999.00568.x