46 Muscle Contraction: Molecular and Cellular Physiology

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46.1 Introduction

Skeletal muscles are by far the largest organ of the human body, accounting for up to 40%–50% of the body weight. In the past much of our knowledge on muscle contraction and relaxation was obtained from experiments with isolated frog muscle or single muscle fibers stimulated with electric pulses. If a vertically suspended muscle is fixed at the upper end and the lower end is loaded with a weight, the muscle fibers become stretched; however, if they are stimulated with a single electric pulse, the muscle fibers respond with an isotonic twitch, thereby lifting the load and preserving mechanical work. If, on the other hand, both ends of the muscle are firmly held, the stimulated muscle fibers cannot shorten, but instead generate force in an isometric contraction. The duration of the twitch may vary, depending on whether fast- or slow-twitch muscle fibers have been used for the experiment. If the stimulation is repeated at short intervals of, say, 50 ms, twitches superimpose and even fuse to a smooth tetanus, developing a force of up to 20–30 N per cm\(^2\) fiber cross-section. As the muscle contracts it produces heat, which arises from the chemical reactions that take place, in particular the splitting of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and phosphate (Pi). The hydrolyzed ATP is almost immediately regenerated from phosphocreatine, which phosphorylates ADP as it breaks down to form creatine. Thus, the net concentration of ATP in muscle cells remains practically constant (about 3–5 mM), even in fatigued muscle. At the onset of contraction the rate of phosphocreatine breakdown and ATP usage increases tremendously [16], perhaps by a factor of 100 or more, which means that the ATPase activity of the contractile proteins becomes enormously activated. Activation occurs very rapidly. Thus the interval between the first stimulus and the beginning of isometric tension rise, i.e., the latent period (electromechanical latency), lasts only about 10–15 ms in a frog sartorius muscle at 0°C, but may of course be much shorter at higher temperatures. According to Hill [36], an “active state” (see Chap. 45) is then set up very rapidly, even before muscle force fully develops.

Energy and Signal Transduction. The chain of events that occurs in the latent period and that ultimately leads to the activation of the contractile proteins has been referred to as excitation–contraction coupling (EC coupling). These signal transduction processes as well as contraction itself are now gradually being understood on the subcellular and molecular level and have been reviewed recently (see, for example [6,11,71,83,86,97]). It is clear that the primary event is the release of Ca\(^{2+}\) ions from cellular stores to activate the molecular motors, the “swinging” crossbridges between actin and myosin filaments that make the filaments slide. Both the performance of the contractile machinery and the events associated with EC coupling are quicker in fast than in slow muscle, but in the latter they are more economical in the sense that they use less energy for contraction (Sect. 46.3.4). Thus, we use these muscles for holding a load or simply for standing upright, whereas fast muscles are used to power the movements of our limbs.

Some muscles of the body are extremely powerful; the muscles of a racing horse, for example, may generate up to 15 HP (horsepower; 1 HP: 746 W) by hydrolyzing ATP, the immediate source of energy. Thus, muscles are essentially machines that transform the chemical energy released by the hydrolysis of ATP into mechanical energy. They are, as it were, highly efficient chemomechanical energy transducers, transforming as much as 40%–50% of the available chemical energy into mechanical work and the remainder into heat (see Chap. 45).
46.2 Molecular and Structural Basis of Contraction

The molecular motors of muscle have been identified as the “heads” of myosin molecules, which hydrolize ATP and interact with another protein, actin, thereby causing the sliding of actin filaments past myosin filaments during muscle shortening (see Chap. 45).

46.2.1 Contractile Proteins

Myosin and actin interact in vitro and combine to form actomyosin. Ever since the discovery that gel-like protein threads made from actomyosin contract when ATP is added, actin and myosin have been referred to as contractile proteins. Therefore, in order to understand contraction on a molecular level, we must begin with a description of the basic properties of these proteins.

Actin. Extraction of acetone-dried muscle powders by water yields actin in its monomeric form, known as globular actin or G actin. It has a molecular mass of 42 kDa and readily polymerizes to form a fibrous structure known as fibrillar actin or F actin when salt is added. The atomic structure of G actin has been determined [59]. The molecule (see Fig. 46.1A) consists of a single polypeptide chain of 375 amino acid residues that are organized into two domains, the small one containing subdomains 1 and 2 and the large one containing subdomains 3 and 4. The N-terminal and C-terminal end of the chain are located near to each other in subdomain 1, and the center of the molecule contains ATP or ADP as well as a divalent cation (Ca\(^{2+}\) or Mg\(^{2+}\)). ATP is reversibly bound in G actin, which upon polymerization becomes hydrolyzed to ADP and trapped. Because of the asymmetry of each of the actin monomers, F actin shows polarity. In nonmuscle cells, an F actin filament may grow at the pointed end (plus end) by incorporating an actin monomer, but at the same time it may lose a monomer at the minus end by depolymerizing, giving rise to the phenomenon of “treadmilling.” The filament structure is more stable in skeletal muscle. It consists of a double-stranded helix, each turn of the helix containing 13 monomers. The large actin domains lie on the inside of the helix, which constitutes the backbone of the thin filaments [39]. In addition, the filaments also contain two other proteins, troponin and tropomyosin (Fig. 46.2C).

![Fig. 46.1A,B. Thin filament proteins. A The atomic structure of actin (from [39]) showing a large and small domain, separated by a central cleft containing bound ATP and Ca\(^{2+}\)). B The structure of troponin C (ribbon band representation) elucidated by X-ray crystallography (from [35]). Ca\(^{2+}\)-binding sites I and II are regulatory; III and IV are higher-affinity sites; A–H are helical regions](image-url)
Myosin. This protein is the major constituent of thick filaments (which also contain C protein; see Table 46.1). Myosin has a molecular mass of approximately 520 kDa. It is a hexameric molecule consisting of two heavy chains and four light chains. The two myosin heavy chains form a coiled rod-like alpha-helical structure and bear two globular heads at their ends (Fig. 46.2A). In muscle fibers, most of the long alpha-helical structure is woven into the thick filament, from which the two globular heads project as crossbridges. These heads are pear-shaped, about 16 nm in length and 6 nm wide, and may be cleaved from the rest of the protein by proteolytic digestion (with papain). The proteolytic fragment obtained is known as subfragment 1 (S1) of myosin. Other proteolytic fragments of myosin are subfragment 2 (S2), light meromyosin (LMM), and heavy meromyosin (HMM), a larger fragment containing both S1 and S2 (Fig. 46.2A). LMM subfragments of myosin readily interact with each other. It is this structure, therefore, which causes the self-assembly of myosin molecules in the form of bipolar myosin filaments [47] and anchors the myosin molecule to the shaft of the filament (Fig. 46.2B).

The myosin light chains are peptide chains that are noncovalently attached to the myosin heads. One type of light chain is called the “essential” or “alkali” light chain, as it can be readily removed under alkaline conditions. The other type of light chain may be removed by the SH group reagent dithionitrobenzene (DTNB) and is therefore called DTNB light chain. This light chain may be phosphorylated by myosin light chain kinase (see below). While slow muscle contains only one type of essential light chain (M, 27000), fast-twitch muscle has two isoforms, LC1 (M, 21000) and LC2 (M, 16000), but only one DTNB light chain, LC (M, 20000). As a single myosin head contains only one DTNB light chain and one alkali light chain (either LC1 or LC2), the double-headed myosin molecule may exist in three isoforms, namely as a heterodimer containing LC1 and LC2, or as one of two homodimers, containing either LC1 or LC2.

The fact that myosin is involved in contraction became apparent very early when it was found to hydrolyze ATP [7] and when it was shown that ATPase activity was increased by actin. When myosin is digested into fragments, all the ATPase activity and actin-binding activity may be restored in the S1 fraction.

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**Table 46.1. Myofibrillar proteins**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular mass (Da)</th>
<th>Content (wt %)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin</td>
<td>520000</td>
<td>43</td>
<td>A band (thick filament)</td>
</tr>
<tr>
<td>Actin</td>
<td>42000</td>
<td>22</td>
<td>I band (thin filament)</td>
</tr>
<tr>
<td>Tropomyosin</td>
<td>2 x 33000</td>
<td>5</td>
<td>Thin filament</td>
</tr>
<tr>
<td>Troponin C</td>
<td>18000</td>
<td>1</td>
<td>Thin filament</td>
</tr>
<tr>
<td>Troponin I</td>
<td>21000</td>
<td>1</td>
<td>Thin filament</td>
</tr>
<tr>
<td>Troponin T</td>
<td>31000</td>
<td>2</td>
<td>Thin filament</td>
</tr>
<tr>
<td>Myomesin</td>
<td>185000</td>
<td>&lt;1</td>
<td>M line</td>
</tr>
<tr>
<td>M protein</td>
<td>165000</td>
<td>2</td>
<td>M line</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>42000</td>
<td>&lt;1</td>
<td>M line</td>
</tr>
<tr>
<td>C protein</td>
<td>135000</td>
<td>2</td>
<td>A band (thick filament)</td>
</tr>
<tr>
<td>α-Actinin</td>
<td>2 x 95000</td>
<td>2</td>
<td>Z line</td>
</tr>
<tr>
<td>β-Actinin</td>
<td>37000</td>
<td>1</td>
<td>Z line</td>
</tr>
<tr>
<td>Titin</td>
<td>2500000</td>
<td>10</td>
<td>A and I bands</td>
</tr>
<tr>
<td>(connectin)</td>
<td></td>
<td></td>
<td>I band</td>
</tr>
<tr>
<td>Nebulin</td>
<td>800000</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Based on Bagshaw [6]
Many enzyme kinetic studies have been done using $S_i$ rather than myosin, because the latter is less soluble. $S_i$ has been crystallized, and its three-dimensional atomic structure (shown in Fig. 46.9) has been completely solved by using X-ray diffraction techniques [82]. It is now clear that the pear-shaped myosin head consists of three domains, an N-terminal 25-kDa domain and a 50-kDa domain, which together form the thick part of the head, and an elongated 20-kDa domain (10 nm long), which joins the 50-kDa domain of the myosin head to the myosin tail. The actin-binding domain is in the 50-kDa “front region” of the head. ATP binds to the 25-kDa domain, whereas the light chains are attached to the 20-kDa domain. The role of these various parts of the myosin head region in energy transduction will be described in more detail below (Sect. 46.2.4).

**Actin Activation of Myosin ATPase.** The interaction of actin and myosin, a fundamental event in muscle contraction, may be studied by investigating the activation of the myosin ATPase by actin. Under the conditions existing in muscle (low ionic strength, magnesium and ATP equimolar), the ATPase activity of myosin has an extremely low value, which may account for the low rate of energy expenditure in resting muscle. In contracting muscle the ATP splitting may be 100 times higher. A similarly high rate of ATP splitting can be achieved with myosin in vitro when the myosin molecules are activated by F actin [19].

ATP hydrolysis involves several steps [6,101]:

- Binding of ATP to myosin to form the enzyme-substrate complex
- Hydrolysis of ATP into ADP and Pi
- Formation of the enzyme–product complex
- Dissociation of this complex.

It is now clear that the low activity of myosin is not due to the slowness of the hydrolytic step. Thus, when ATP is suddenly mixed with $S_i$, the enzyme–substrate complex is rapidly formed and hydrolyzed to form the enzyme–product complex (MA-DP-Pi), which, however, decomposes extremely slowly, at a rate of less than 0.1 per s. Unless the products are released, a new molecule of ATP cannot be bound. Hence, the rate of continued hydrolysis is extremely low. Actin activates the ATPase because it accelerates the product release step, thereby enhancing the rate of ATP splitting (Fig. 46.3A).

After the release of the product, a new molecule of ATP is bound and hydrolyzed. The bound ATP also dissociates actin and myosin (Fig. 46.3B). Thus, ATP has two forms of action:

- It serves as a substrate and energy source
- It breaks the links between actin and myosin, thereby dissociating the actomyosin complex into actin and myosin (as shown in Fig. 46.3B).

Both reactions, the dissociation of actomyosin by ATP and the hydrolysis of ATP followed by actin-accelerated decomposition of the enzyme–product complex, occur in sequence, as depicted in Fig. 46.3C. The scheme of events is more complex, however, as ATP may also be hydrolyzed by actomyosin prior to or even without dissociation (nondissociating pathway) and also because of the complex nature of product release. Thus, phosphate may be released before ADP, a reaction which is associated in vitro

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**Fig. 46.3A-C.** Mechanism of adenosine triphosphate (ATP) hydrolysis (see [6]). ADP, adenosine diphosphate. A ATP hydrolysis by myosin ($M$; pathway I) and actomyosin (AM; pathway II). B Dissociation of actomyosin by ATP. C Interactions between myosin intermediates ($M$–ADP-P; $M$-ADP-P; $M$–ADP-P) and actin during ATP hydrolysis (schemes A and B combined). Note that AM–ATP may dissociate [3] or may be hydrolysed to AM–ADP–P [7] (nondissociating pathway).
with a large drop in free energy and which is therefore fairly irreversible. In intact muscle fibers, by contrast, the situation may be different, because the drop in chemical free energy associated with Pi release is compensated by a gain in mechanical energy that, under isometric conditions, is stored in the crossbridge (see Sect. 46.2.4).

**Regulatory Proteins: Troponin and Tropomyosin.** Troponin and tropomyosin are proteins of the thin filament that regulate actin–myosin interaction. The high level of actin-activated and Mg\(^{2+}\)-dependent ATPase activity of myosin can be inhibited in vitro by troponin, provided that the Ca\(^{2+}\) concentration is below about 10^{-7}M. The low Ca\(^{2+}\) concentration is increased, the divalent cation is bound to troponin, thereby derepressing its inhibitory action. This effect is greatly amplified by tropomyosin. Thus, troponin apparently has three functions [17]:

- Ca\(^{2+}\) binding
- An inhibitory action on the actin-activated myosin ATPase
- Cooperation with tropomyosin

These functions can be located in different subunits of troponin, designated troponin C, troponin I, and troponin T (TnC, TnI, and TnT). The 18-kDa protein troponin C, which has a dumbbell appearance (Fig. 46.1B) [35], consists of a single peptide chain of 159 amino acid residues containing four Ca\(^{2+}\)-binding sites, two of which bind with a very high affinity, and two regulatory sites. Troponin I (21 kDa, consisting of 179 residues) interacts with the N-terminal sequence of actin, thereby interfering with actin activation. This inhibitory interaction is released when it interacts with troponin C in the presence of Ca\(^{2+}\) rather than with actin. Troponin T (31 kDa) is an elongated molecule of 259 amino acid residues that binds to troponin C and I, but also at its N-terminal end to tropomyosin. The latter is a rod-like, 41-nm-long molecule that contains two identical helical peptide chains of 33 kDa oriented in parallel and organized into 14 negatively charged repeats that interact with actin. Within the thin filament, each tropomyosin molecule spans seven actin monomers while running along the grooves of the thin filaments (Fig. 46.2C). The role of these regulatory proteins in the regulation of muscle contraction will be described in more detail below (Sect. 46.3.3).

### 46.2.2 Structure of Myofibrils and Myofilaments

The myofibrils are the contractile organelles in the muscle fibers. They are surrounded by curtains of sarcoplasmic reticulum and are filled with contractile proteins. Myosin and actin are the major components of the myofibrils, making up 45% and 22%, respectively, but they also contain regulatory proteins and cytoskeletal proteins (listed in Table 46.1). Myofibrils are 1–2 μm in width and cross-striated, as they are divided into innumerable sarcomeres that are connected in series.

**Sarcomeres.** These elementary contractile units have a banded structure (Fig. 46.4) and are separated from each other by Z disks, to which the actin filaments are attached. The latter extend by about 1 μm towards the middle of the sarcomere, where they overlap with the 1.6-μm-long and 10-nm-thick myosin filaments. These form the dark, anisotropic band of the sarcomere seen in the light microscope, the A band, which is flanked on either side by isotropic light zones, the I bands. The I bands and the region of overlap contain the thin filaments made of actin. The zone of overlap appears to be distinctly darker in the light microscope than the central zone, the H zone, in which there are no actin filaments. Many electronmicrographs of this zone reveal a very narrow, dark band in the middle of the sarcomere, the M line. It contains a network of supporting proteins such as myomesin and M protein (see Table 46.1) that hold the bundle of myosin filaments together.

Sarcomeres of resting muscle are approximately 2 μm in length, but may be extended considerably up to a length of 3.6–4 μm when the muscle is stretched. When this happens, the actin filaments are pulled out from the array of the thick filaments so that the A band width stays constant while the width of the I band and of the H zone increases. The actin–myosin overlap zone decreases [43,53], but the myosin filaments remain centered due to huge, elastic stabilizing molecules known as titin that attach to the "bare zone" of the myosin filament in the middle of the sarcomere and reach all the way along to the Z line. Because of the elasticity of these huge molecules, any attempt to stretch the myofibril is met with resistance, and considerable passive resting tension develops when myofibrils become stretched [104].

**Crossbridges.** Thick and thin filaments are adjacent and may be connected by crossbridges. Cross-sections through the sarcomere reveal a highly ordered filament array [46]. Thus, in the region of overlap each thick filament is surrounded by six actin filaments in a hexagonal lattice,
whereas each actin filament is surrounded by three myosin filaments (Fig. 46.5a). Thick and thin filaments of vertebrate skeletal muscle are hexagonally arranged in two sets of planes, denoted 1.1 and 1.0. When muscle fibers are exposed to parallel beams of X-rays, these two different planes give rise to two diffraction spots on the equator of the X-ray diffraction pattern (Fig. 46.5b). The 1.0 equatorial reflections arise predominantly from the thick myosin filaments, while the intensity of the 1.1 reflections depends on the mass of both thick and thin filaments. Thus, the intensity ratio of 1.1 and 1.0 reflections may be taken as a rough measure of the ratio of the mass of thick and thin filaments. In the absence of ATP, in the so-called rigor state, as well as in contraction, this intensity ratio is much larger than in relaxed resting muscle fibers, indicating that mass must have been shifted from thick filaments towards thin filaments. This has been taken to indicate that, in contraction, the projections of the thick filaments, the crossbridges, swing out towards the thin filaments and attach [50, 52] (see [6] for further discussion). Crossbridges are formed by the 20-nm-long ovate heads of myosin molecules, which, as shown in electronmicrographs (longitudinal sections), attach to thin filaments in a very regular manner in rigor (see Chap. 45): the angle of attachment appears to be approximately 45°, thus forming an arrowhead structure pointing towards the M line in the middle of the sarcomere; in relaxed resting muscle, however, the crossbridge angle appears to be more like 90° [84]. It was this finding that inspired the swinging crossbridge model of muscle contraction (see below).

46.2.3 Sliding Filament Mechanism:
Generation of Motion

In an isotonic contraction, muscles shorten as the result of the shortening of countless sarcomeres in the myofibrils. During this contraction, the thin filaments slide over and past the thick filaments, moving towards the middle of the sarcomere while the filament length remains constant [53], [43]. Thus, during shortening, the A band width stays constant, while both the I band and the H band become narrower. The sliding filament theory also explains the way in which sliding is brought about by the molecular movement of myosin heads. As already mentioned, the myosin molecules are assembled in the thick filaments to give a bipolar filament structure. The actin filaments also exhibit polarity that is reversed at the Z line. During the contraction process, each myosin head or crossbridge links the myosin filament to an adjacent actin filament. According to H.E. Huxley [48], attaching heads tilt or bend to an angle of about 45° to form arrowhead structures (pointing towards the M line) similar to the structure occurring in “rigor.” The tilting of the crossbridges then causes the sliding of actin past myosin filaments.

Swinging Crossbridge Hypothesis. Owing to the bipolarity of the filament, tilting crossbridges pull the actin filaments of both half-sarcomeres in opposite directions and make them slide by about 10 nm towards the M line in each crossbridge stroke [48]. In the course of each stroke, an individual sarcomere, with an approximate length of
be sufficiently slow to rate limit the speed of unloaded muscle shortening [92].

The free energy of ATP hydrolysis under the ionic conditions existing in muscle has been estimated to be about 60 kJ/mol. This energy is, however, released only to a very small degree during the hydrolysis of the so-called energy-rich phosphate bond. Instead, it is transferred to an energy-rich intermediate, the actin–myosin–product state (AMDP). It is the release of inorganic Pi which is associated with the liberation of over 50% of the total free energy. This may be transformed into mechanical energy (work) when the crossbridge assumes an angled attitude, thereby displacing an actin filament. Alternatively, it may be degraded into heat when crossbridges detach without doing work.

### 46.2.4 Generation of Force

In a strictly isometric contraction of sarcomeres, myofilaments need not slide, and no external work is being done by crossbridges when force is produced and maintained; thus all the energy of ATP (60 kJ per mol) is eventually degraded into heat (heat of maintenance, see Chap. 45). Using strong synchrotron radiation, it has become possible to follow structural changes in the crossbridges during contraction, as revealed by changes in the X-ray diffraction pattern [52,54]. During the development of isometric force, the intensity ratio of the 1.1 and 1.0 reflections seen in low-angle X-ray diffraction patterns increases [50,51,62,69], suggesting that crossbridges are transferred to the actin filament. Force is then generated when crossbridges attach to the thin filament, first weakly and then strongly. According to the theory proposed by Huxley and Simmons [44], force generation requires attached crossbridges to tilt to an angle of approximately 45°, thereby stretching an elastic element in the crossbridge [42]. The elastic element may be in the neck of the crossbridge (Fig. 46.7A) or elsewhere. Alternatively, the globular heads may become strained (Fig. 46.7B).

During maintained contraction, crossbridges are in a dynamic state, continuously turning over and cycling between detached or weakly attached states and strongly attached conformations, thereby splitting ATP. The kinetics of these processes are governed by two apparent rate constants, $f_{app}$ and $g_{app}$ (or simply $f$ and $g$), that describe the rate at which crossbridges enter or leave the force-generating state [10,11]. The rate of crossbridge cycling corresponds to the rate of ATP splitting and is proportional to $fg(f + g)$. Force, on the other hand, is dependent on the fraction of cycling crossbridges attached at any one moment, which depends on $f(f + g)$. It also depends on crossbridge force and on the number of crossbridges that actually can attach and cycle and hence on the degree of actin–myosin filament overlap and the actual length of the sarcomere (see Chap. 45).

**Mechanochemical Coupling.** Since cycling crossbridges use ATP, the rate of ATP consumption and heat production increases with force development. The quotient of

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**Fig. 46.6.** Swinging crossbridge model (see [48]) and correlation of chemical and mechanical crossbridge states during the crossbridge cycle. A, actin; M, myosin; T, adenosine triphosphate, ATP; D, adenosine diphosphate, ADP; P, phosphate, Pi.

2.0 μm, will thus shorten by about 1% of its length. However, sarcomeres of lightly loaded muscle may shorten by about 0.4 μm in 0.3 s [58]. To achieve this, crossbridges would have to perform their pulling action not once, but 20 times in this length of time, much like a team of people pulling a long rope hand over hand. Thus, it is clear that extensive shortening can only be produced by the cyclic action of crossbridges. Each crossbridge cycle may involve at least four stages (Fig. 46.6):

- Attachment
- Tilting movement or power stroke
- Detachment
- Recovery movement.

The energy required for cycling is released by the hydrolysis of ATP, which is split by the myosin head. Presumably one ATP molecule is required per crossbridge cycle.

**Adenosine Triphosphate Powers Crossbridge Cycles.** Figure 46.6 shows how the different chemical states of the crossbridge cycle may be coupled to the structural and mechanical states [6]. After each power stroke, one molecule of ATP is bound to the crossbridge, which then becomes dissociated from actin. Without ATP, crossbridges would remain permanently attached in their arrowhead configuration. This is the case in rigor mortis [84]. As soon as ATP is bound, it is hydrolyzed to the products ADP and Pi, resulting in the formation of the myosin–product complex MDP (M, myosin crossbridge; D, ADP; P, Pi). This presumably causes the recovery movement of the detached crossbridge, which then reattaches to actin, first weakly and then strongly. Weakly attached crossbridges are possibly in rapid equilibrium with detached ones. The point of no return in a crossbridge cycle during filament sliding is initiated by the release of inorganic phosphate, which causes strong crossbridge interaction followed by a power stroke of the crossbridge and the release of ADP. Then a new molecule of ATP is bound and, with the detachment of a crossbridge, a new crossbridge cycle begins; this chain of events is repeated again and again. The release of ADP may
ATP consumption rate and tension is therefore fairly constant in a given muscle and is referred to as “tension cost.” Yet it may vary in different kinds of muscle and under different conditions. It is related to the apparent detachment rate [11]. Clearly, if the latter is reduced, the life span of attached crossbridges and the duration of a crossbridge cycle is prolonged. It seems intuitively clear that crossbridges cycling at a lower speed use less ATP and may hold force in a more economical manner (rigor mortis being an extreme example of this). The question arises as to how the different chemical states of ATP hydrolysis are related to the different crossbridge states, and a plausible mechanism is explained in Fig. 46.7A [6,11].

The energy released from ATP hydrolysis is not used for external work, but for doing internal work during force maintenance. According to the Huxley–Simmons model [44], this is because crossbridges attach and then bend in each crossbridge cycle, thereby stretching a passive element of the crossbridge possibly located in the neck region between the myosin head and the myosin rod (Fig. 46.7A).

If, as proposed, this movement is associated with the release of bound Pi [40], a considerable amount of chemical free energy is released. However, the total free energy of the attached crossbridge hardly changes, because chemical energy is transformed into mechanical energy, i.e., the potential energy stored when the passive elastic crossbridge element is strained [11,40]. Assuming that the passive element behaves like a linear elastic spring, force is proportional to the distance (Δx) by which the element is strained, and the mechanical potential energy is proportional to ½Δx² [37]. This is illustrated in Fig. 46.7C, showing the change in free energy of the AMD crossbridge state when the mechanical strain is increased. Thus, under isometric conditions, the total free energy (chemical and mechanical) of the (unstrained) AMD state and of the strained AMD* crossbridge state may be nearly equal, so that the two states are in rapid equilibrium and actually fluctuate [6], thereby trading chemical free energy for mechanical energy and vice versa.

Because of the reversibility of the Pi release step, the accumulation of inorganic Pi such as occurs in muscle fatigue (Sect. 46.3.2) will therefore drive the reaction backward, thereby converting strongly attached force-generating bridges in the AMD* state into weakly attached ones that are in the AMD state. This is why force declines when inorganic Pi accumulates [87,112]. The reversibility of the Pi release reaction has been demonstrated directly by showing that added 32P-labeled inorganic Pi binds to the crossbridge (presumably in the AMD* state) and is incorporated back into ATP, perhaps via the non-dissociating pathway depicted in Fig. 46.3 (ATP-Pi medium exchange) [40,93,105,106,112].

It is clear from the above data that the affinity of crossbridges for ADP must also depend on crossbridge strain. The energy-rich crossbridge state (AMD* state) loses its energy when the actin filament is allowed to slide (see Fig. 46.7A). The attached crossbridges then snap back because the spring tension in their elastic element is discharged so that the free energy of the crossbridge decreases.

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Fig. 46.7A–C. Mechanism of force generation. A Huxley–Simmons model [44], highly simplified. a, Crossbridge attaches; b, crossbridge tilts, thereby stretching a passive elastic element; the actin filament is pulled by the elastic force in the spring; c, as the actin filament moves, the elastic element recoils and muscle shortens. Note that states a (AMDP state) and b (AMD* state) are almost isoenergetic, as explained in Fig. 46.7C. B Alternate model of force generation (see [11,95]). a, Weak crossbridge attachment; b, formation of strongly attached, force-generating crossbridges; c, crossbridges tilt (power stroke) when actin filaments move and muscle shortens. C Basic free energy profile of a crossbridge in two different attached states (AMD and AMD-P, see Fig. 46.7A) (according to Bagshaw [6]; see also [93]). X is the relative position of the thin filament, and Δx the filament displacement caused by fiber extension or shortening while the crossbridge is attached. Note that the total basic free energy of the crossbridge is the sum of the chemical and mechanical potential energy and hence depends on x, when the crossbridge (AMD state) is linked to a passive elastic element (as shown in the inset) stretched by Δx, the increase in mechanical potential energy will be proportional to ½Δx² (e.g., from c to b). Thus, the strained AMD* crossbridge state (b) has nearly the same basic free energy as the unstrained AMD-P state (a), but it loses its stored mechanical potential energy when the strain is released (c). The energy released (from b to c) that may be converted to work is about half of the free energy of adenosine triphosphate (ATP) hydrolysis (ΔG ATP = −60 kJ).
and may be converted into external work. However, if the crossbridge detaches before doing work, the spring recoils and all the energy stored in the crossbridge is dissipated into heat [6].

**Mechanical and Structural Analysis.** As already mentioned, force may be developed by attaching crossbridges that move or bend from an orientation perpendicular to the filament axis to a tilted configuration (45°), thereby stretching a passive elastic element within the crossbridge by about 5–10 nm. This model was originally proposed by Huxley and Simmons [44] on the basis of mechanical experiments and was later supported by structural studies [56]. Muscle fibers contracting isometrically were quickly released by a length step of about 5 nm per half-sarcomere. Tension suddenly fell to almost zero (T₁ force) as the strained elastic element became discharged (Fig. 46.8). Within 2 ms following the quick release (quick phase), force partially recovered (T₂ value), and some tension recovery was even observed when the release was as large as 1% of the fiber length. Since this corresponds to a filament displacement of about 11 nm, this experiment suggested that (synchronized) attached crossbridges that rotate or bend to an angle of 45° during the quick phase may take up the slack caused by the release and that, therefore, the total crossbridge movement must have been of the order of about 11 nm. This movement probably also accounts for quick recovery of force (quick phase) and for the structural changes detected in the X-ray diffraction pattern during the quick phase by Irving et al. [56]. They found a change in the meridional 14.3-nm reflections, suggesting that crossbridges tilt during the force recovery in the quick phase [cf. also 54].

However, during force development under strictly isometric conditions, alterations of the meridional X-ray diffraction reflections, as observed by Irving et al. [56], could not be seen, suggesting that force generation may be caused by processes occurring prior to the tilting of crossbridges [13]. As changes in the equatorial reflections did occur [13,51], Squire [95] (see also [13,18]) proposed an alternative theory of force generation: crossbridges attach to actin first weakly and then strongly, but in a perpendicular manner; they are then in an internally strained state, i.e., they are transformed into a new elastic body behaving like a strained leaf spring and exerting a force on the actin filament (Fig. 46.7B). The force-generating crossbridges still attached to actin at a 90° angle are in a state of high potential mechanical energy as they are restrained from adopting the preferred 45°-angle attached state. However, after release or under isotonic conditions, when the actin filament is free to move and slide, the attached crossbridge changes shape and displaces the actin filament by some 10 nm, thus leading to the preferred configuration (45°-angle attachment); the crossbridge is then no longer mechanically strained. Thus, the flexed, strained configuration has been transformed into a released configuration, much like a compressed spiral spring that elongates or like a bent leaf spring that extends when released. This configurational change may well correspond to the “shape change” deduced from modeling the myosin head structure [81] (see Sect. 46.2.5). The initial transition from weak binding to strong binding that precedes this shape change however, leads to a distinct change in the X-ray diffraction pattern (in the intensity ratio of the equatorial 1.1 and 1.0 reflections), suggesting that it may be due to a change in the actin–myosin interface rather than to an angle change within the crossbridges [13] like the gross conformational change of S₁ proposed by Rayment et al. [81].

### 46.2.5 The Crossbridge as a Molecular Motor

We have already seen that the crossbridges that generate force and movement consist of myosin heads that hydrolize ATP, thereby generating motion. Much insight into the mechanism of this motor has been gained recently from the detailed knowledge of the atomic structure of actin [59] and of the head region of the myosin molecule [82] shown in Fig. 46.9.

**Structure of Myosin Heads.** The myosin head contains three domains, which are interconnected by flexible links of about five to ten residues that are highly sensitive to proteolytic enzymes. Tryptic digestion produces an N-terminal 25-kDa domain, a 50-kDa domain, and a 20-kDa domain that is C-terminal and that connects to the S₁ fragment of myosin. The myosin head is very elongated because of the nearly 10-nm-long alpha-helical structure of the 20-kDa domain that stretches from the thick front part of the head down to the hinge region connecting the head and the S₁ fragment of myosin. It is stabilized along its entire length by two light chains that are noncovalently bound. This part of the molecule acts as a kind of conformational coupler linking the ATP-hydrolizing motor end of the myosin head with the S₁ region and thus with the myosin filament. It is thus a molecular lever, as it were, amplifying small conformational changes of the motor region. The latter comprises the actin-binding site in the 50-kDa domain, which is separated into an upper and lower part by a gap, the actin cleft that extends from the actin interface to the ATP-binding pocket (the active site cleft) and is formed by the interfaces of all three domains. This
lever, the 10-nm-long 20-kDa domain, thereby performing a power stroke, as shown in Fig. 46.10.
To summarize, it is the closure of the actin cleft during product release that results in the power stroke of the crossbridge that propels the actin filaments by about 6 nm in each crossbridge cycle. Remarkable, it is the small movement in the ATP-hydrolyzing motor region that generates the motion, and this motion is greatly amplified by the elongated head (the 20-kDa region of S,) and then transformed into gross muscular movement by the repetitive cycles of innumerable crossbridges. In some insect muscles the movement may occur at extremely high frequencies and may even be synchronized. In midges, for example, the oscillatory movement of the indirect flight muscle produces a wing beat at a frequency of more than 1000 Hz.

**Actin–Myosin Interface.** A cyclic interaction of crossbridges requires that the affinity of myosin for actin is high during the power stroke (strongly bound state), but weak during the return movement of the crossbridge. Thus the actin–myosin interface is a focus of great interest. The amino acid residues contributing to strong actin–myosin interaction are near residue 333 on actin, which is situated extremely close to residue 407 in a loop region near the actin-binding cleft of the 50-kDa domain. Another region of contact is actin residue 149 and myosin residue 545, which is also in the 50-kDa domain. Thus it is mainly subdomain 1 of actin to which the 50-kDa domain of S, attaches, thereby forming strongly binding crossbridges. However, a flexible loop region containing residues 626–647, which forms the junction between the 50- and 20-kDa domains, may interact weakly with the N terminus of actin (residues 1–4 and 24–25) [81,91]. These weak interactions are mainly electrostatic, due to the positive and negative charges of the myosin- and actin-binding sites. The respective binding sites are possibly the first to become involved when the crossbridges attach to actin loosely and weakly. This early step may be followed by enlargement of the binding interface, thereby forming strong stereospecific bonds and creating a strained, force-generating state when Pi is released. The linker sequence may therefore determine the rate at which crossbridges attach strongly and may influence ATPase activity, as the latter is rate limited by the apparent attachment constant \( f \) [12]. Indeed, if this critical region is replaced by a fragment derived from a slow myosin (having low ATPase activity), the chimeric myosin produced by this genetic engineering has a low ATPase activity. In contrast, a loop region borrowed from a fast myosin (having high ATPase activity) confers high ATPase activity to the chimeric myosin [108]. Interestingly, these manipulations do not affect the power stroke and hence the rate at which sliding actin filaments are pulled. The sliding velocity is decreased, however, in a mutant myosin obtained from cardiac muscles of patients suffering from a hereditary cardiomyopathy. In the mutant myosin, residue 403, which is involved in strong myosin–actin interaction, is altered [15] (see also Chap. 47).

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**Fig. 46.9.** Structure of the myosin head (subfragment S, of myosin, ribbon band representation) solved by X-ray crystallography; 2000 and 3000 have been added to the residue numbers of the regulatory light chains (RLC, light chain-2) and the essential light chain (ELC) to distinguish these from the heavy chain. (From [82])

A Hypothesis About Conformational Changes During the Crossbridge Cycle. Based on the atomic structure of actin and myosin, Rayment et al. [81] proposed a working model of the myosin motor as follows (Fig. 46.10): at the start of a crossbridge cycle, ATP slips into the ATP pocket, with the gamma phosphate group entering first; it becomes hydrolyzed while the actin-binding site remains stationary. This causes an opening of the actin cleft and lowers the affinity of the myosin head to actin so that the crossbridge detaches. The subsequent movement of the 50-kDa domain, however small, might produce a movement of the alpha-helical lever (the 20-kDa domain) by about 0.6 nm. Such a movement has been observed by X-ray scattering experiments on S, during ATP hydrolysis in solution [111]. It is reversed when the products of ATP hydrolysis, ADP and Pi, are released. The actin cleft closes. The movement of the 50-kDa domain is then amplified by the molecular pocket is a V-shaped, 1.3-nm-deep cleft opposite the actin-binding region [82] (see Fig. 46.9).
Fig. 46.10. Hypothetical scheme [82] explaining the possible conformational changes of myosin heads (subfragment S1 of myosin) that occur when crossbridges attach, detach, and reattach during a crossbridge cycle. Note that the power stroke displacing the actin filament towards the M line occurs after phosphate (Pi) release and may possibly be associated with the opening of the adenosine triphosphate (ATP)-binding pocket. This hypothetical conformational change is preceded by the closure of the actin cleft that occurs when Pi is released. ADP, adenosine diphosphate. (From [83])

Removal of the myosin light chains also considerably reduces the sliding velocity [65], perhaps suggesting that light chains might affect actin–myosin interaction (see Sect. 46.3.3). However, it must be remembered that the light chains also stabilize and stiffen the 20-kDa domain that forms the molecular lever connecting the motor domain of S1 with the S2 fragment. Therefore removal may simply result in a collapse of the helical lever structure, thus causing a smaller working stroke and hence a slower sliding velocity, while the ATPase activity is not impaired. A method for determining the molecular movement associated with power stroke and the rate of filament sliding has been made possible by recent advances in the techniques for performing in vitro motility assays.

In Vitro Motility Assays; Single Crossbridge Recording. To demonstrate the movement of myosin molecules or even isolated myosin heads in vitro [97,107,115], these molecules are attached to a solid support, such as the surface of a cover slip, that can be viewed by a microscope. The cover slip is then covered with a layer of buffer solution containing fluorescently labeled actin filaments. Because of the labeling, Brownian random movement can easily be seen under the microscope. As soon as ATP is added, the sliding movement becomes directed and is greatly enhanced, because myosin heads now attach and pull the filaments in a cyclic manner. Remarkably, the velocity at which the filaments slide is quite similar to the sliding velocity of actin past myosin filaments in intact muscle fibers shortening under no load [109]. Actin filaments may also be attached to a force transducer. Thus, when attaching, each myosin molecule pulls, thereby generating force. With only a few molecules attaching, force fluctuates because of the cyclic interaction of the bridges [109] [57]. Finer and colleagues [22] (also see [115]) even succeeded in measuring the force of a single myosin molecule attaching to a single actin filament that was fixed to a force transducer. Under these quasi-isometric conditions, the filament was not able to slide. Instead, the attaching myosin molecules produced brief force transients averaging 3–4 pN, followed by pauses due to the detachment of crossbridges and then by another transient. The frequency of these repetitive force transients corresponds to the turnover of the ATPase. Thus the interval between crossbridge force transients presumably reflects the duration of the crossbridge cycle. These experiments therefore directly demonstrate that crossbridges act in a cyclic manner by attaching, moving, and detaching. The crossbridge movement was measured under lightly loaded isotonic conditions that allowed the myosin molecule to pull the actin filament. This occurred in steps of about 10 nm, which were obviously due to the bending of a crossbridge, as described in the preceding paragraph.

Crossbridge Kinetics. Single-molecule recording of unitary movement and force is reminiscent of and analogous to single-channel recording of unitary conductance, something which was made possible by the patch-clamp...
the technique of Sakmann and Neher [88]. In much the same way as the kinetics of single-channel opening and closing can now be analyzed in terms of "open probability" and probability of being closed, we may now wish to describe the properties of the force generators in terms of "off probability" and "on probability." The latter is equal to \( f/(f + g) \), where \( f \) and \( g \) are rate constants for crossbridge attachment and detachment, respectively; it is about 0.07 or 0.4 under isotonic or isometric conditions, respectively, and it corresponds to the so-called "duty ratio" of the crossbridge cycle.

If we consider the ATPase activity of \( S_0 \) or heavy meromyosin (HMM) to be about ten molecules of ATP hydrolyzed per molecule of myosin per s, the duration of a crossbridge cycle will be approximately 100 ms; the duration of a movement step of a single crossbridge, as measured by Finer et al. [22], is of the order of 7 ms under unloaded conditions, suggesting that during isotonic shortening the crossbridges pull actively on the actin filament only during about 7% of the cycle time and pause during 93% of the time. The duty ratio then is 0.07 [57,107], but may be up to 0.4 under isometric conditions. It is the fraction of time during which the myosin head is attached and generating force during the crossbridge cycle. This means that cycling crossbridges develop on average only about 40% (1–2 pN) of the peak force developed during the power stroke, as found by Finer et al. [22].

The probability of being in the "on" state, i.e., of being attached, is therefore about 0.4, as it is also related to the fraction of crossbridges that are strongly attached to actin and generating force at any one moment, as mentioned above (Sect. 46.2.4). This fraction and hence also force depend on the rate constants \( f \) and \( g \) (or more correctly \( f_{\text{app}} \) and \( g_{\text{app}} \), and these rate constants may therefore be deduced from motility assays.

In the two-state crossbridge model of A.F. Huxley [41], the constant \( g \) depends on the mechanical strain on the crossbridges. Thus under isometric conditions when the strain is high the constant \( g \) is low, while during unloaded shortening the detachment rate is high, since crossbridges are not strained; they may even be compressed, thus producing negative force. According to the model suggested by Ishijima et al. [57], this kind of strain dependence of crossbridge rate constants would best account for the single-molecule motility behavior of myosin. Indeed, as already mentioned, Finer et al. [22] showed that under isotonic conditions when the actin filaments are pulled under low load, the "on" state is much shorter than under strictly isometric conditions while force is developed; this shortening of the "on" state may be due to an increase in the detachment rate constant that reduces the lifetime of an attached crossbridge. The detachment rate constant \( g \) may actually rate limit the speed of muscle shortening under unloaded conditions.

**Skinned Fiber Studies.** The rate of crossbridge detachment was also determined in muscle fibers under isometric conditions and shown to be dependent on ATP. In order to free muscle cells from ATP, the surface membrane of the fiber had to be removed or made highly permeable by membrane skinning, while the contractile system was left intact. Skinned fibers are prepared by mechanical dissection or by treatment with glycerol and/or detergents. They have proved to be very useful models to study the role of ATP. Thus, it was shown by Weber and Portzehl [113] that such muscle fibers become stiff and rigor-like when depleted of ATP, whereas Reedy et al. [84] showed that crossbridges attach to actin in rigor but cannot detach again unless ATP is also present. Indeed, addition of ATP causes relaxation of muscle fibers that are in a state of rigor contraction, provided that reattachment is inhibited. Relaxation is, however, very slow, since ATP diffusion in the fiber is time-consuming, but this diffusional difficulty can be overcome by letting skinned fibers equilibrate with a photolabile, but biologically inert ATP precursor (caged ATP), which, upon a brief flash of ultraviolet (UV) light, is immediately transformed into ATP. This then produces a sudden relaxation with a rate constant corresponding to \( g_{\text{app}} \), the rate of crossbridge detachment [27,40]. The time course is similar to that of relaxation of intact muscle fibers. ATP binding, not ATP hydrolysis, was required for this relaxation, since actin and myosin dissociated before ATP hydrolysis occurred about 20 ms after the light flash. This relaxation, however, occurred only at very low Ca\(^{2+}\) concentrations (<10\(^{-5}\)M), since under these conditions the attachment of crossbridges was inhibited. The addition of Ca\(^{2+}\), on the other hand, stimulated contraction. If Ca\(^{2+}\) is suddenly released by flash photolysis of "caged" Ca\(^{2+}\), e.g., 1-[2-amino-5-(1-hydroxy-1-[2-nitro-4,5-methylenedioxyphenyl]-methyl)-phenoxy]-2-(2' -amino-5'-methylphenoxy)-ethane-N, N', N'-tetraacetic acid (Nitr 5), the rise in force may be as fast as that in living muscle following stimulation [4,40], depending on the sum of the rate constants \( f_{\text{app}} \) and \( g_{\text{app}} \).

The development of isometric force by skinned or permeabilized fibers suspended in ATP salt solution is Ca\(^{2+}\)-dependent. The relationship between Ca\(^{2+}\) concentration and force is fairly steep (Fig. 46.11). Thus, fibers contract maximally at 10\(^{-5}\)M Ca\(^{2+}\), but relax after the addition of a Ca\(^{2+}\) chelator (ethylene glycol tetra-acetic acid, EGTA) which reduces the free Ca\(^{2+}\) concentrations to a value below 10\(^{-7}\)M [21,29,34]. Because of the slow diffusion of the chelator, relaxation is necessarily slow. To investigate the true kinetics of relaxation, single-skinned fibers that contracted in ATP were therefore preincubated with a "caged" Ca\(^{2+}\) chelator (caged 1,2-bis-(o-aminophenoxy)-ethane-N,N',N'-tetraacetic acid, BAPTA) that is immediately transformed into a chelator when it is illuminated with a flash of UV light. The rate of relaxation observed when Ca\(^{2+}\) was suddenly removed was similar to that characteristic of living muscle fibers during relaxation and comparable to the net crossbridge detachment rate [40]. It may be concluded, therefore, that contracted muscle fibers relax when crossbridges detach, but are prevented from reattachment and force generation by the tropomin–tropomyosin system at low Ca\(^{2+}\) concentrations. These regulatory processes will be described in detail below.
46.3 Regulation of Contraction: Excitation-Contraction Coupling

The muscles of our body may be activated extremely fast. Thus when a muscle is stimulated, it contracts after a latency of only a few milliseconds. During this period the action potential generated by a nerve impulse or by direct electrical stimulation is transmitted into the interior of a muscle fiber along invaginations of the plasma membrane, the T tubules, and may reach intracellular Ca\(^{2+}\) stores, i.e., the sarcoplasmic reticulum [28,45]. The immediate release of Ca\(^{2+}\) from these stores that follows initiates a transient rise in intracellular Ca\(^{2+}\) concentration. This Ca\(^{2+}\) transient is the signal that elicits a twitch. This was demonstrated in experiments using aequorin, which was injected into muscle fibers as a Ca\(^{2+}\) indicator [8]. Aequorin is a bioluminescent protein isolated from certain jellyfish that emits light when it binds to Ca\(^{2+}\) ions. Significantly, the glow of light emitted from aequorin-injected muscle fibers always preceded the twitch contraction and fell off as the Ca\(^{2+}\) concentration fell again just prior to muscle relaxation. The intracellular processes that are initiated by the excitation of the plasma membrane and that lead to the release of the stored Ca\(^{2+}\) and to contraction are referred to as excitation-contraction coupling [86].

The importance of Ca\(^{2+}\) in activating contraction has already been demonstrated by showing that:

- The injection into muscle fibers of Ca\(^{2+}\) in millimolar or even micromolar concentrations elicits contraction of these fibers [33,79].
- Ca\(^{2+}\) activates demembranated (skinned) muscle fibers that are fuelled by ATP [21,34].

In fact the contractile force of these fibers depends on the negative logarithm of the Ca\(^{2+}\) concentration (pCa) in a sigmoidal manner, reaching a half-maximum value between pCa6 and pCa5 (Fig. 46.11). In the following, we shall first address the question of how Ca\(^{2+}\) is stored in relaxed muscle fibers and then how the Ca\(^{2+}\) signal is generated by the release of intracellularly stored Ca\(^{2+}\) and how the Ca\(^{2+}\) activator switches the myofilaments on, thereby causing crossbridges to become active.

46.3.1 Storage of Ca\(^{2+}\) in Relaxed Muscle

In relaxed muscle the free Ca\(^{2+}\) concentration in the myoplasm is about 10\(^{-7}\)M, whereas the total Ca\(^{2+}\) content is more than 1 μmol per g tissue. Thus most of the Ca\(^{2+}\) is either bound to soluble proteins of the cytoplasm such as parvalbumin or it may be stored in a special compartment, the sarcoplasmic reticulum, but partly also in the mitochondria.

T Tubules and Sarcoplasmic Reticulum. As shown in Fig. 46.12, the sarcoplasmic reticulum is an internal membrane system which forms a network of tubules lying longitudinally with respect to the fiber axis between the myofibrils. These tubules, which form the longitudinal system (L system), end in blind sacs filled with Ca\(^{2+}\), the lateral (i.e., terminal) cisternae. These are closely apposed to the membrane of other tubules that are oriented perpendicularly to the longitudinal system and that usually surround the myofibrils at the level of the Z line (amphibian muscle) or in the region of the I band (muscles of higher vertebrates). These transverse tubules, the T tubules (T system), originate from invaginations of the outer cell membrane and, unlike the L system, they actually communicate with the extracellular space [75]. In a structural and functional sense this T tubular system bridges the gap between the plasma membrane and the sarcoplasmic reticulum. Thus the membrane of the T system is primarily involved in transmitting the action potential to the interior of the muscle fiber and in particular to the Ca\(^{2+}\) release site at the lateral cisternae [28,45]. Both the T system and the L system are thus particularly abundant in fast muscles, which need to be activated quickly. The L system may occupy 5%-15% of the fiber volume or even 30% in the most rapid muscles, those of the swim bladder of tad fish, whereas the T system amounts to less than 1%.

Ca\(^{2+}\) Transport ATPase. Ca\(^{2+}\) is stored within the longitudinal system mainly in the lateral cisternae (terminal cisternae) [67], where it is bound to the protein calsequestrin and from where it is released during activation. The membranes of the sarcoplasmic reticulum contain Ca\(^{2+}\) pumps that pump Ca\(^{2+}\) into the tubules by an ATP-dependent, active transport mechanism. Even in vitro Ca\(^{2+}\) is pumped back into purified subcellular fractions of sarcoplasmic reticulum vesicles when ATP is present as an energy donor [31,32]. The molecular structure of these pumps is quite similar to the Ca\(^{2+}\) pump in the plasma membrane [66]. The domains involved in ATP binding and splitting seem to be situated in a loop region of the cyto-
Fig. 46.12. Internal membrane system in striated muscle and structures involved in excitation–contraction coupling. TT, transverse tubules invaginating the cell membrane; LT, longitudinal system of the sarcoplasmic reticulum; TC, terminal cisternae (lateral cisternae). Ca\(^{2+}\) released from the terminal cisternae interacts with troponin (Tn) in thin filaments containing actin, tropinin, and tropomyosin (Tm). (From [86] and [29])

plasmic site of the sarcoplasmic reticulum pump, whereas the Ca\(^{2+}\)-binding residues are located in the membrane-spanning channel region. The sarcoplasmic–endoplasmic reticulum ATPase (SERCA) is activated by Ca\(^{2+}\) and, in slow, cardiac, and smooth muscle, inhibited by the protein phospholamban. When it is dephosphorylated, phospholamban interacts with the Ca\(^{2+}\) pump and inhibits its ATPase activity [61]. Phospholamban may be phosphorylated by a cyclic adenosine monophosphate (cAMP)-dependent protein kinase which derepresses Ca\(^{2+}\) transport activity [60].

Structure and Function of Triads. In electron micrographs of fiber cross-sections, a T tubule and the two lateral cisternae flanking it appear as three swellings described as a “triad.” The membranes of the T tubules and of the lateral cisternae are closely apposed. The 15-nm gap between the T-tubular membrane and the junctional membrane of the terminal cisternae is bridged by electron-dense particles called “junctional feet” which form a regular tetragonal lattice of about 800 feet per \(\mu\)m\(^{2}\) junctional membrane in frog muscle. Here the junctional membrane comprises about 27% of the cisternal membrane and amounts to up to 1500 cm\(^{2}\)/g muscle [24]. These feet are particularly important in the communication between the T system and the sarcoplasmic reticulum, and they may in fact represent channels that release the Ca\(^{2+}\) stored in the lateral cisternae (Fig. 46.13). The detailed structure of the feet is quite similar in different muscles. On freeze fracture electron micrographs they appear on the surface of the sarcoplasmic reticulum membrane as square particles consisting of four subunits. Each unit contains pores functioning as Ca\(^{2+}\) release channels extending up to 12 nm from the surface of the sarcoplasmic reticulum membrane into the gap between the T-tubular membrane and the sarcoplasmic reticulum. On the surface of the former and apposing the feet there are groups of four particles called “tetrads” that are located in the T-tubular membrane [9]. These structures also represent a kind of Ca\(^{2+}\) channel, the dehydroprypidine (DHP) receptor, that senses the membrane potential in the T tubules, and in muscle activation they are involved in the coupling of signal transmission between the excitable T-tubular membrane and the Ca\(^{2+}\) release channels of the sarcoplasmic reticulum [85].

46.3.2 Ca\(^{2+}\) Release from the Sarcoplasmic Reticulum

Ca\(^{2+}\) release from the sarcoplasmic reticulum takes place through the calcium release channels in the foot region of the junctional membrane of the lateral cisternae. As these channels are sensitive to micromolar concentrations of the alkaloid ryanodine, which they bind tightly, they are also called ryanodine receptors. The ryanodine-binding property greatly facilitated the isolation of the channel protein [55], which was subsequently cloned and sequenced [99,116]. With a molecular mass of more than 400 kDa, these channels are very large molecules containing more than 5000 amino acid residues. When the purified protein was incorporated into lipid layers, it formed a channel with similar Ca\(^{2+}\) conductance properties as the native channel [94] located in the foot region that couples the terminal sac (lateral cisternae) of the sarcoplasmic reticulum to the T tubules. Both the feet-like structures and the isolated protein have a similar structure, exhibiting fourfold symmetry, which suggests that the feet are identical with the channels [110]. Thus, as mentioned already, ryanodine receptors form square particles, tetrameres, which include...
a central pore with four perpendicular channels leading into the pore. Upon stimulation and depolarization of the T-tubular membrane, \( \text{Ca}^{2+} \) is released at a high rate, i.e., up to 0.2 µmol \( \text{Ca}^{2+} \) released in 1 ml cytoplasm within a few milliseconds [85,71].

**Coupling of T System and Sarcoplasmic Reticulum.** A basic question that arises is how depolarization of the plasma membrane and of the T-tubular membrane opens the \( \text{Ca}^{2+} \) channels of the terminal cisternae of the sarcoplasmic reticulum. In cardiac and smooth muscle, the release channels seem to be opened by second messengers, \( \text{Ca}^{2+} \) and inositol trisphosphate, respectively (see Chaps. 93 and 44). While both second messengers may play a modulatory role in skeletal muscle, they do not seem to be primarily involved in channel opening according to recent research. Rather, T tubules and \( \text{Ca}^{2+} \) release channels may be mechanically coupled, as originally suggested by Schneider and Chandler [89]. These researchers proposed that, following depolarization of the plasma membrane, a charged structure of the T-tubular membrane moved (charge movement), thereby “pulling” and opening the \( \text{Ca}^{2+} \) release channel. This theory has been supported by recent experiments (Fig. 46.14) showing that charge movement and rate of \( \text{Ca}^{2+} \) release depend in the same manner on the membrane potential, suggesting that charge movement and the gating of the \( \text{Ca}^{2+} \) release channel are tightly coupled [72]. The charge movement within the T-tubular membrane is probably associated with the gating of voltage-dependent \( \text{Ca}^{2+} \) channels [20] shown to exist in the T-tubular membrane [3].

T-tubular \( \text{Ca}^{2+} \) channels bind DHP. Hence, they are often referred to as DHP receptors. However, unlike in cardiac muscle, these receptors do not actually function as channels, but merely act as voltage sensors and signal transducers in excitation–contraction coupling. Thus, although the DHP receptor has a structure and pharmacological properties characteristic of a L-type \( \text{Ca}^{2+} \) channel and even \( \text{Ca}^{2+} \) conductance properties, the latter do not seem to be essential for excitation–contraction coupling. Charge movement and contraction were elicited even when no \( \text{Ca}^{2+} \) current was flowing, for instance in the absence of external \( \text{Ca}^{2+} \) or when \( \text{Ca}^{2+} \) influx was blocked by cobalt or cadmium salts. Therefore, in contrast to cardiac muscle (see Chap. 93), \( \text{Ca}^{2+} \) influx into the cell does not seem to be necessary for signal transmission from the T tubules to the \( \text{Ca}^{2+} \) release channels. On the other hand, as shown by studies using dysgenic mice (see Chap. 47), DHP receptors are essential for excitation–contraction coupling [100], but they apparently send their information on the voltage of the T tubules not by opening a pore for the influx of external \( \text{Ca}^{2+} \), but in some other way. These might involve conformational changes of proteins bridging the gap between the T system and the sarcoplasmic reticulum or even a direct mechanical interaction between DHP receptors and \( \text{Ca}^{2+} \) release channels, as originally suggested by Schneider and Chandler and depicted in Fig. 46.13.
**Generation of the Ca²⁺ Signal.** After brief electrical stimulation the Ca²⁺ channel may open only for a few milliseconds [90]. Since, therefore, Ca²⁺ is released in a burst-like manner, the free Ca²⁺ level increases rapidly but transiently as the released Ca²⁺ is quickly bound to troponin and to the Ca²⁺-binding protein parvalbumin or to the Ca²⁺-transporter proteins of the sarcoplasmic reticulum, which pump the Ca²⁺ back into the lateral cisternae. This generates a brief Ca²⁺ transient [8], which can be measured using Ca²⁺ indicators such as aequorin, fura-2, or indo-1 (Fig. 46.15A). While the upstroke of a transient may largely depend on the rate of Ca²⁺ release, the downstroke is mainly, but not exclusively, controlled by the rate at which Ca²⁺ is taken up by the sarcoplasmic reticulum. This rate is itself a function of myoplasmic Ca²⁺ concentration, since saturation of the Ca²⁺ pump proteins depends on the free Ca²⁺ concentration in the range of 0.1–1.0μM. In slow muscle fibers, Ca²⁺ reuptake by the sarcoplasmic reticulum is also slow because of the low activity of the Ca²⁺ ATPase (SERCA). Thus, the Ca²⁺ transient and the twitch contraction are prolonged.

If the electrical stimulation is repeated at a high rate, e.g., 20 per s in the case of frog muscle fibers, the Ca²⁺ transients fuse, indicating that the concentration of intracellular free Ca²⁺ remains high [8]. This is because there is not enough time during the interval between stimuli for the Ca²⁺ pump to take the Ca²⁺ back into the sarcoplasmic reticulum. Under these conditions, therefore, tension is also maintained as twitchs fuse to a tetanus (see Chap. 45). In fact, as each stimulus releases more Ca²⁺, the intracellular Ca²⁺ levels actually rise and may eventually reach a much higher value than in a single twitch (Fig. 46.15B). This may be one of the
reasons why tetanus force of mammalian muscle fibers is larger than twitch force. However, it must also be considered that, during the brief activation of a muscle at the beginning of a single twitch, there may simply not be enough time for all Ca\(^{2+}\)-activated crossbridges to attach in a force-generating manner. However, when the state of elevated Ca\(^{2+}\) concentration lasts longer, as in tetanus, additional crossbridges may have time to attach so that force increases [23]. In frog muscle, the latter mechanism may even predominate, as a single stimulus may cause the release of a supramaximal amount of Ca\(^{2+}\), thereby increasing the free Ca\(^{2+}\) concentration to as much as 10 \(\mu\)M [1] (see Fig. 46.15B).

**Muscle Fatigue.** In muscle fatigue (see Chap. 45), contractile force declines even at high frequencies of stimulation, because less and less Ca\(^{2+}\) is released so that the Ca\(^{2+}\) level declines. The tension fall in fatigue may actually be even greater that expected from the decline in the free Ca\(^{2+}\) level, since the Ca\(^{2+}\) responsiveness of the myofilaments is also decreased. This Ca\(^{2+}\) desensitization may be due to metabolic factors such as a decrease in pH or an increase in the concentration of inorganic Pi, which is known to accumulate in fatigued muscle [2]. During muscle fatigue, the speed of relaxation is also reduced, since the ATP-dependent Ca\(^{2+}\) reuptake into the sarcoplasmic reticulum is inhibited while the Ca\(^{2+}\) transients are prolonged. Interestingly, the prolongation of the relaxation time has the effect of allowing twitches elicited by single stimuli to summate and to reduce the tetanus fusion frequency to a value lower than when the muscle is resting (see Chap. 45).

### 46.3.3 Ca\(^{2+}\) Activation of Actin–Myosin Interaction

It is now well established that Ca\(^{2+}\) release by the sarcoplasmic reticulum is the trigger of contraction. As already mentioned, Ca\(^{2+}\) activates the contractile structure in the narrow concentration range of about 10\(^{-6}\) to 5 x 10\(^{-4}\) M Ca\(^{2+}\). The molecular switches that are involved in this Ca\(^{2+}\) regulation are located in the thin actin filaments. These are troponin, which forms two long strands running along the two grooves along the double-stranded actin filament, as well as troponin, which binds to troponymosin at intervals of about 40 nm [68,78]. Thus, one troponin molecule controls a segment of seven actin monomers (as shown in Fig. 46.2C).

**Role of Thin Filament Proteins.** The transient rise in intracellular Ca\(^{2+}\) from 0.1 to 1 or even 10 \(\mu\)M Ca\(^{2+}\) during muscle activation is sensed by the C subunit of troponin as it binds Ca\(^{2+}\) in this range of concentration. Ca\(^{2+}\) binding to TnC is therefore the first and essential step leading to the activation of the contractile proteins. TnC molecules of fast skeletal muscle fibers have four Ca\(^{2+}\)-binding sites, namely two nonspecific, high-affinity sites and two regulatory sites that bind Ca\(^{2+}\) in a physiological concentration range. These binding sites are formed by helix–loop–helix mo-

tives (called “E-F hand”), with Ca\(^{2+}\) binding coordinated between amino acid residues in the loop regions flanked by the helices [29]. TnC of slow muscle, on the other hand, has only one specific Ca\(^{2+}\) site and two nonspecific ones.

When Ca\(^{2+}\) binding takes place, there is a rearrangement of helical regions of the TnC molecule, thus exposing the central region of the molecule, which then interacts more strongly with TnnI. This interaction actually causes TnnI to move away from its actin-binding site by about 4 nm [25]. In this way the interaction of TnnI with actin is weakened, so that the inhibitory effect of TnnI on actin-activated myosin ATPase is reversed. The conformational changes occurring in TnnI as well as in TnnT may then allow the tropomyosin to move on the actin filament from an inhibitory position at the edge of the filament to an activating position in the center of the groove, as shown by X-ray diffraction studies [78] and by cryoelectron microscopy [73]. Thus, at low Ca\(^{2+}\) concentrations the high affinity of TnnI for actin holds troponin in a position which prevents strong crossbridge binding to actin, while still permitting weak binding. When Ca\(^{2+}\) binding occurs, troponin releases tropomyosin, which moves away from its blocking position along the outer surface of the actin helix, allowing crossbridges to attach in a strongly binding, force-generating state (Fig. 46.16).

![Fig. 46.16. Ca\(^{2+}\) regulation in the thin filament of vertebrate striated muscle, illustrating the changes in the position of troponymosin (TM) in relaxed muscle (top) and contracted muscle (bottom). The diagram shows the cross-section of a thin filament with interacting myosin subfragment 1 (S-1). A, actin. Position as indicated in the reconstruction of Milligan and Flicker [73]. The angular change in the position of troponymosin is calculated from X-ray diffraction changes in the second actin layer. The tropomyosin position in the relaxed state allows weak binding to subfragment 1, but retards stronger attachment unless the TM moves following Ca\(^{2+}\) activation, as indicated in the bottom diagram. (From [29])](image-url)
A positive feedback mechanism is involved in Ca\(^{2+}\) activation that causes an extremely steep dependence of force on Ca\(^{2+}\) concentration. This is because crossbridge attachment is cooperative [5,29,97], and attaching crossbridges also move the tropomyosin away from the blocking position while at the same time increasing—by yet unknown mechanisms (S, TnC feedback)—the affinity of TnC for Ca\(^{2+}\) [30,102]. In summary, the troponin–tropomyosin system constitutes a Ca\(^{2+}\) switch allowing the interaction of two Ca\(^{2+}\) ions with one troponin molecule to regulate actin–myosin interaction in a highly cooperative manner along a 40-nm-long segment of the thin filaments containing several actin monomers.

Activation of Crossbridges. According to the classical “steric blockage hypothesis,” tropomyosin sterically hinders crossbridge attachment in relaxed muscle, but moves out of the way when muscle is activated [49,74]. However, in relaxed muscle most detached crossbridges are already in rapid equilibrium with weakly attached ones. Rather than promoting attachment, Ca\(^{2+}\) occupancy of TnC and the conformational changes in the thin filament that follow promote the transition of weak crossbridge binding states to strongly binding crossbridges. This transition is governed by the apparent rate constant \(f_{app}\) and, according to Brenner [10], it is mainly the increase in this rate constant that is induced by Ca\(^{2+}\) activation and leads to an enhanced force development and contractility in the muscle (Fig. 46.17). This is because under isometric conditions force development is proportional to the quotient \(f_{app}/(f_{app} + g_{app})\), while the detachment rate \(g_{app}\) is not dependent on Ca\(^{2+}\). As the crossbridge step controlled by \(f_{app}\) is associated with Pi release, which rate limits ATPase activity, the rate of ATP splitting by myofibrillar ATPase will also be enhanced by increasing the intracellular free Ca\(^{2+}\).

In conclusion, Ca\(^{2+}\) occupancy of TnC seems to accelerate a kinetic step of the crossbridge cycle that is associated with Pi release rather than “recruiting” more crossbridges. This is consistent with the concept that crossbridges bind weakly to actin but—provided that Ca\(^{2+}\) is present—move into a strong binding position in concert with tropomyosin movement. In relaxed muscle, tropomyosin lies close to the myosin-binding site of actin [81]. According to a modified steric blocking model, therefore, tropomyosin may sterically hinder the transition from a weak to a strong binding state of the myosin head in the absence of Ca\(^{2+}\), but not in its presence, because the azimuthal movement of tropomyosin into the actin groove would then unblock the strong myosin-binding site of actin [29]. Time-resolved X-ray diffraction studies of frog muscles actually indicated that such a tropomyosin movement does occur and that it occurs before crossbridge attachment and tension development [62,63]. In addition, the tropomyosin movement may even allow detached, noncycling crossbridges that are sterically blocked from reacting with actin [70] to enter the crossbridge cycle. If this is so, this conformational change would then increase force by regulating crossbridge attachment (as suggested by the steric blocking model) as well as by controlling the transition of crossbridges from a weakly bound state to a strongly bound conformation [95].

Modulatory Role of Light Chain-2 Phosphorylation. The apparent rate constant \(f\) controlling the formation of the strongly bound crossbridge state may also be increased by phosphorylation of light chain-2 [98] at a given submaximal Ca\(^{2+}\) concentration. This would also increase force development, indicating that Ca\(^{2+}\) responsiveness or Ca\(^{2+}\) sensitivity must have been increased, thus resulting in a leftward shift of the pCa force relation. In other words, a higher force is developed at a given submaximal Ca\(^{2+}\) concentration than in unphosphorylated preparations or, conversely, a lower Ca\(^{2+}\) concentration is required for half-maximal force activation. However, light chain-2 phosphorylation is itself dependent on Ca\(^{2+}\). This is because the phosphorylation is catalyzed by myosin light chain kinase, which is activated by calmodulin, if occupied with Ca\(^{2+}\). It follows that Ca\(^{2+}\) must have a dual effect:

- A fast regulatory action on the thin filament due to Ca\(^{2+}\) occupancy of TnC.
- A slow, indirect and modulatory effect on the thick filament mediated by a Ca\(^{2+}\)-dependent phosphorylation of light chains attached to the myosin heads.

The increase in Ca\(^{2+}\) responsiveness (Ca\(^{2+}\) sensitivity) due to light chain-2 phosphorylation may well be a phenomenon that may account for “post-tetanic potentiation” (see Chap. 45), explaining why a twitch elicited after a prolonged tetanus may be much larger than a twitch elicited prior to tetanic stimulation [76,96]. In addition, Ca\(^{2+}\) binding to the light chains may also play a role in modulating crossbridge attachment [38], as in the case of molluscan muscle [114].

Cessation of Actin–Myosin Interaction: Relaxation. In a tetanus, crossbridges are continuously cycling, detaching,
and reattaching, thereby hydrolyzing ATP as long as the intracellular Ca\textsuperscript{2+} level is kept high. When stimulation stops and intracellular Ca\textsuperscript{2+} concentration falls (as shown in Fig. 46.15B), the cycling crossbridges continue to detach, but fail to reattach firmly because of the inhibitory action of the troponin–tropomyosin system. If the rate of crossbridge detachment exceeds that of crossbridge reattachment, net detachment occurs and muscle relaxes. Muscle force and stiffness then decline with a rate determined mainly by the process of net crossbridge detachment, but also by the rate at which Ca\textsuperscript{2+} dissociates from TnC and is pumped back into the sarcoplasmic reticulum or bound to parvalbumin, the soluble Ca\textsuperscript{2+}-binding protein of the myoplasm [26]. Relaxation is an energy-requiring process, as ATP is used both for the active transport of Ca\textsuperscript{2+} into the sarcoplasmic reticulum as well as for crossbridge detachment caused by the dissociation of actin and myosin. Thus, when the energy supply to the muscle is limited, relaxation may be slowed or it may even fail. After death, at extremely low ATP concentrations, a state of rigor develops (rigor mortis) in which crossbridges become firmly attached (untill autolysis).

In summary, then, muscle relaxation depends on many processes occurring in sequence: Ca\textsuperscript{2+} sequestration into the sarcoplasmic reticulum, Ca\textsuperscript{2+} dissociation from TnC, movement of tropomyosin into the “off” position on the thin filament [62], and crossbridge detachment.

### 46.3.4 Excitation–Contraction Coupling in Fast- and Slow-Twitch Fibers

Mammalian twitch muscle may respond with brief, powerful efforts or with sustained activity by using fast- and slow-twitch fibers, respectively (see Chaps. 45, 49). These fiber types are quite distinct (Table 46.2).

**Diversity of Fiber Types.** Slow-twitch fibers (type-1 fibers) are red, as they are rich in mitochondria and myoglobin. The fast response is produced by type-2B fibers (fast, white, glycolytic) or type-2A fibers (fast, pink, oxidative). The rate of rise and fall of twitch tension may differ by a factor of 2–4 in fast and slow fibers, suggesting that the rate of Ca\textsuperscript{2+} release and delivery to the myofilaments as well as the rate of Ca\textsuperscript{2+} reuptake into the sarcoplasmic reticulum differs accordingly. Indeed, the Ca\textsuperscript{2+} transients are much slower in slow than fast muscle. This is because the former have a less elaborate T system and sarcoplasmic reticulum, as well as a less active SERCA (see Table 46.3). Thus, in a twitch the Ca\textsuperscript{2+} concentration in the myoplasm is lowered more slowly than in fast muscle, which therefore relaxes at a higher rate. However, rapid Ca\textsuperscript{2+} binding to the protein parvalbumin in the myoplasm and a high rate of crossbridge detachment may also contribute to the more rapid relaxation of fast than slow muscle [26].

Twitch fibers of the glycolytic type (2B fibers) fatigue rapidly, since they depend on anaerobic glycolysis for energy provision and since the rate of ATP consumption by crossbridges and SERCA is quite high. Due to the lower Ca\textsuperscript{2+}-transporter ATPase activity of slow muscle, these fibers use less ATP for Ca\textsuperscript{2+} handling than fast muscle in a given period of time. However, type-1 fibers also use less ATP for crossbridge cycling than type 2B fibers, because they express myosin heavy chains with low ATPase activity that are different from the enzymically highly active heavy chains of fast muscle. These muscles are also rich in mitochondria and therefore have a high capacity to generate ATP in aerobic metabolism. Thus, both type-1 fibers and type-2A fibers are fairly resistant to fatigue.

Among the fastest muscle fibers are those of the larynx muscles, such as the cricoarytenoid muscle. The other extremes are the slow tonic fibers of extraocular muscles, which are innervated at many points (mononeural, multiterminal innervation). Unlike twitch fibers, these tonic fibers do not respond to nervous stimuli with either all or no action potentials, but by a very slow membrane depolarization caused by summating end plate potentials that elicit a slow but graded contraction, a contraction. Force is regulated on the cellular level by the degree of the long-lasting membrane potential change causing a graded increase in the level of intracellular free Ca\textsuperscript{2+}.

**Plasticity of Muscle Fibers.** At birth there is very little difference in the contraction speed of muscle fibers that will

<table>
<thead>
<tr>
<th>Feature</th>
<th>Twitch fibers</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>IIB (FF)</td>
<td>IIA (FR)</td>
<td>I (S)</td>
</tr>
<tr>
<td>Color</td>
<td>White</td>
<td>Red or pink</td>
<td>Red</td>
</tr>
<tr>
<td>Contraction</td>
<td>Fast twitch</td>
<td>Fast twitch</td>
<td>Slow twitch</td>
</tr>
<tr>
<td>Fatigue resistance</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Glycolytic</td>
<td>Glycolytic and oxidative</td>
<td>High</td>
</tr>
<tr>
<td>Lactic dehydrogenase</td>
<td>High</td>
<td>Medium or high</td>
<td>Low</td>
</tr>
<tr>
<td>activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>activity</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FF, fast fatiguing; FR, fast, fatigue resistant; S, slow

*See [86]*
become fast or slow later in life. It is only after these fibers receive their postnatal innervation that fast- and slow-fiber types emerge within the first few weeks of life. The importance of innervation in differentiation into fiber types was demonstrated by the cross-innervation experiments carried out by Buller and Eccles [14], in which the motor nerve fibers innervating a fast- or a slow-twitch muscle were cut and exchanged when reimplanting them into the muscles. After a few weeks, when cross-innervation became established, the originally fast muscles became slow and vice versa. However, a fast muscle may also be transformed into a slow one by chronic stimulation with electrical pulses at a frequency of 10 Hz [64] or following denervation.

When a fast muscle is denervated, the structural features characteristic of fast excitation–contraction coupling change to the slow type concomitantly with the mechanical properties, whereas slow-type myosin isoforms emerge much later [77]. This suggests that it is the characteristics of excitation–contraction coupling rather than the properties of the contractile proteins that determine the time course of an isometric twitch. On the other hand, it is also clear that the characteristics of excitation–contraction coupling and the performance of the contractile machinery must be matched. For instance, a high rate of Ca\(^{2+}\) release and delivery to the myofilaments would be of little use if it were not matched by an equally fast response of the contractile machinery [86]. Thus, slow and fast striated muscle fibers do indeed differ not only in the rate at which Ca\(^{2+}\) is supplied to and removed from the contractile structures, but also with regard to the kinetics of the crossbridge cycle. Not surprisingly, then, the properties of the motor nerves are also closely matched to the types of muscle fibers which they innervate. Thus, the speed of nerve conduction is correlated with contraction speed in different fibers.

### Table 46.3. Comparison of mammalian fast- and slow-twitch fibers in the guinea pig

<table>
<thead>
<tr>
<th>Feature</th>
<th>Fiber type</th>
<th>Slow red (soleus), type I</th>
<th>Fast white (vastus), type IIIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>T system (% fiber volume)</td>
<td></td>
<td>0.14</td>
<td>0.27</td>
</tr>
<tr>
<td>SR total (% volume)</td>
<td></td>
<td>3.15</td>
<td>4.59</td>
</tr>
<tr>
<td>Maximal rate of Ca(^{2+}) uptake (nmol Ca(^{2+}) per mg SR protein per s)</td>
<td></td>
<td>5.8</td>
<td>22.5</td>
</tr>
<tr>
<td>Maximal Ca(^{2+}) uptake (µmol Ca(^{2+}) per mg SR protein)</td>
<td></td>
<td>2.8</td>
<td>5.5</td>
</tr>
<tr>
<td>Half-time of relaxation (t(_{1/2}) ms)</td>
<td></td>
<td>113</td>
<td>21</td>
</tr>
<tr>
<td>Time to peak contraction (ms)</td>
<td></td>
<td>82</td>
<td>22</td>
</tr>
<tr>
<td>Actomyosin ATPase activity (µmol P per min per mg protein)</td>
<td></td>
<td>0.05</td>
<td>0.13</td>
</tr>
</tbody>
</table>

SR, Sarcoplasmic reticulum

*After [86]*

### 46.4 Conclusions and Outlook

The elucidation of the atomic structure of actin and myosin and the recording from a single myosin molecule have been a landmark in the endeavors to understand how the crossbridges, the molecular motors of muscle, transform chemical energy released from ATP hydrolysis into mechanical energy. Another matter of great interest is the problem, presently still unsolved, of how an electric signal, the action potential, is transduced into an intracellular Ca\(^{2+}\) signal that elicits a mechanical response. Indeed, Ca\(^{2+}\) is pivotal in the regulation of muscle contraction, as the force of the contractile system depends on the free Ca\(^{2+}\) concentration surrounding the myofilaments. In intact fibers of skeletal muscles, unlike smooth or cardiac muscle, force does not, however, seem to be graded by a variation of the intracellular Ca\(^{2+}\) concentration since—at least in frog muscle fibers—an action potential usually triggers the release of a supramaximal amount of Ca\(^{2+}\), which saturates the Ca\(^{2+}\)-binding protein troponin C fully. Therefore, and because of the “all or nothing” nature of the twitch, contraction force of the intact muscle in situ seems to be increased by enhancing the frequency of impulses conducted by the innervating nerve fibers as well as by recruiting more and more motor units rather than by increasing the intracellular free Ca\(^{2+}\) concentration in a graded manner, as in the case of tonic fibers and cardiac muscle (see also Chap. 93). A motor unit consists of a motoneuron and the group of muscle fibers it innervates, all of the same type, either fast or slow. In a voluntary contraction of muscle, motor units containing slow-twitch fibers are usually recruited first, while larger motor units consisting of fast, rapidly fatiguing muscle fibers (type 2B) will often be called into action only during maximal efforts when a high power output is required.

As we have seen, fast- and slow-twitch muscles have somewhat different contractile and regulatory proteins, and there is also a complex relationship between innervation and the physical stresses acting on muscle on the one hand and the expression of muscle enzymes and regulatory or contractile proteins on the other. Changing the electrical stimulation pattern, for instance, may transform a fast, rapidly fatiguing skeletal muscle into a slow, fatigue-resistant one that can be made to contract rhythmically for indefinite periods when suitably stimulated by a pacemaker. After surgical reposition, such transformed muscles may then, for instance, be used to assist a failing heart (cardiac assist). Though the contractile and regulatory proteins expressed in fast and slow muscle fibers, respectively, may be quite similar in general, they may differ in specific, functionally important regions. Molecular genetic approaches (such as site-directed mutagenesis) may thus be used to change single-amino acid residues or to cut out and exchange certain parts of the contractile proteins in order to examine the functional consequences [108]. Such studies may eventually lead to a molecular understanding of how muscle adapts to the specific functional demands of the body. As remarked by Prosser [80], “no other tissue may
illustrate as well as muscle the theme of the common mechanisms with variations adapted to specific functions.”

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