Striated Muscle—Contractile and Control Mechanisms

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A new era in muscle research had its beginning in the early and mid-1950s. The new structural and biochemical techniques that became available to muscle-cell biologists were combined with clever physiological experiments to provide a rigorous and fresh new approach, the hallmark of which has been the understanding of close relationships between function and underlying structure. As a result, striated muscle has become more than a black box containing springs and viscocities and delimited by a membrane with unusual electrical properties. Within a muscle fiber, specific patches of membrane have specific functions in the control of muscle activity, enzymes are suitably located for their functional contribution, and the contractile material is disposed in a highly organized array of interacting filaments and bridges. Springs and viscocities have been moved to the description of muscle contractility at the molecular level. Beyond these successes, what has been learned from muscle is applied to many other biological phenomena, e.g., excitation-secretion coupling in secretory cells and motility in many kinds of nonmuscle cells.

In this chapter, we attempt to review that progress. Of necessity, we have limited our coverage to certain topics and our references to a few key papers and reviews. In general, we have attempted to summarize and to simplify, and in doing so we may be guilty of obscuring controversies and omitting details. The following three sections follow the historical development of modern research on muscle. In the second part of the paper, current knowledge on selected topics is considered. Personal biases, as well as the purpose of this volume, encourage us to emphasize structural findings in this review.

Background

Some of the structural information underlying modern theories of muscle function was available by the early part of the century. However, the light microscope was being used at the limits of its resolution, and relatively crude fixation techniques were known to produce potentially severe artifacts. As a result, there seemed to be little hope of discovering which of several views of muscle structure was correct. Considerable imagination came into play, and resulting theories were often contradictory (cf. 1 and 2). Perhaps because of this, in the words of A. F. Huxley, “microscopy really went out of fashion from 1900 until after World War II.”

From the vantage point of today’s knowledge, we can select some authors whose views we now recognize to be remarkably correct. We will discuss here some that are relevant to the rest of this chapter.

Filaments: Cross-striations of myofibrils have been known since Bowman (1840). Constancy of A-band length with shortening of the sarcomere was demonstrated subsequently (3). Birefringence of the A band had been noted and correctly interpreted as caused by the presence of longitudinally oriented protein rodlets. High-angle X-ray diffraction failed to produce evidence for changes in helical structure of muscle proteins during rigor, as expected from several contemporary theories of contraction, in which the emphasis had been on shortening of individual filaments rather than on interaction between filaments. It seemed clear to some workers, notably to H. E. Huxley, that the interesting things were happening at a level larger than the protein intramolecular structure (1 nm or less). Early, low-angle X-ray diffraction detected the regular arrangement of filaments and striking differences in equatorial reflections of active and rigor muscles, at the beginning of the modern era (4).

Proteins and Contraction: The two major contractile proteins, actin and myosin, had been separated from each other. Their association in the absence of adenosine triphosphate (ATP), to form “myosin B” or actomyosin, and their dissociation followed by superprecipitation in the presence of magnesium and ATP, had been demonstrated (5). Myosin was known to be an ATPase. A fortuitous biochemical observation later became an important technique in the study of the myosin molecule. Myosin incubated in trypsin shows a dramatic fall in viscosity and forms fragments, among which are a light fragment (light meromyosin [LMM]) and a heavy fragment (heavy meromyosin [HMM]) (6). HMM shows ATPase and actin-binding activity. LMM shows neither of these activities, and it is insoluble at normal ionic strength.

Initial evidence for a role of calcium in the activation of the contractile material was given (7).

Fenn and Marsh (8) described the relationship between two important mechanical parameters: load applied to the muscle and the velocity of shortening. Maximum velocity of shortening is a useful parameter for classification of fibers.
membranes: Reticular networks were a favorite subject of 19th century light microscopists, and often were rejected as artifacts (1). Some, however, were real. The black reaction of Golgi provided an Italian biologist of the turn of the century with “images of noteworthy subtilety and elegance” (11). Veratti’s description of a fine reticular network in muscle cells, later to be identified with the network of transverse tubules, suffered almost complete oblivion until it was rediscovered and republished in a special issue of The Journal of Biophysical and Biochemical Cytology in 1961 (11).

The other internal membrane system in muscle fibers is the sarcoplasmic reticulum (SR). Virtually unknown in the classical literature, it has been described in detail for a variety of species and fiber types during the period we will review. Marsh (reviewed in reference 12) discovered that the supernate of muscle homogenate, later recognized to contain the microsomal fraction, induces relaxation of a suspension of myofibrils. This effect of the “Marsh” factor was to occupy numerous investigators and to result in the definition of the role of the sarcoplasmic reticulum in the relaxation of muscle.

In The Beginning

SLIDING-FILAMENT MODEL OF CONTRACTION: In 1954 A. F. Huxley and Niedergerke (13), and H. E. Huxley and Hanson (14) simultaneously and independently proposed a theory of contraction that has become known as the sliding-filament model. It proposes that changes in the length of the sarcomere are caused by longitudinal sliding of two sets of filaments relative to each other, without changes in the length of the filaments themselves (Fig. 1).

COMPOSITION OF THE SARCOMERE: A rigorous comparison of the protein mass contained within the A band (measured by interference microscopy) with the amount of myosin extracted from the fibers, showed that the major component of the A band is myosin (15). The second set of filaments, which remained after extraction of myosin, was shown to contain actin.

Fibrils contain a large concentration of a few proteins for which good purification techniques were soon developed. Thus, muscle fibers were among the first cells to which the technique of immunocytochemistry was successfully applied. By this method, the location of actin and myosin in thin and thick filaments, respectively, was directly confirmed (16; see also review in reference 17).

The immediate source of energy for contraction was proven to be ATP in the careful biochemical study by Davies in 1964 (18). Thus splitting of ATP by myosin was confirmed to be the primary event in the interaction of actin and myosin.

FILAMENTS: In 1957, H. E. Huxley (19) published a complete description of the double hexagonal array of filaments forming the fibrils. The major structural features of the filaments and their disposition were beautifully illustrated (Figs. 2 and 3). Thick, myosin-containing filaments occupy the length of the A band, in the center of the sarcomere. Thin, actin-containing filaments attach at the Z line and interdigitate with the thick filaments at the borders of the A band. Both thick and thin filaments are well aligned and have a uniform length. The central region of the A band, which contains only thick filaments, is the H zone, observed earlier by light microscopy. The filaments form a highly ordered, double-hexagonal array (Fig. 3). Thus the stage is set for an interaction of a myosin filament with six adjacent thin filaments, and of each thin filament with three thick filaments (1:2 ratio). Bridges project out of the thick filament shaft all the way to its tapered ends, and seemingly attach to the thin filament. The central region of the thick filament has a bridge-free region (about 1,200 Å wide).

A MECHANICAL MODEL OF BRIDGE ACTION: A. F. Huxley (20) published a theoretical treatment of the sliding-filament model in which myosin cross-bridges, capable of moving around an equilibrium site, attach and detach from appropriate sites on the actin filaments according to a simple kinetic scheme. Cross-bridge action is a repetitive cycle of attachment, relative motion of the two filaments, and detachment. This early model could account quite well for such mechanical parameters as the force-velocity relationship and also for the relationship between load and energy utilization.

MENBARNE STRUCTURE: Cell biologists were attracted...
by the elegant disposition of membranes in the form of a sarcoplasmic reticulum (the SR) with a repeating pattern relative to that of the sarcomere (21). Fittingly, the first full description of the SR was part of a series describing the structure and disposition of a cytoplasmic vesicular system, the endoplasmic reticulum, in a variety of cells. Porter and Palade (22) described the SR as an intracellular membrane system, separating its internal compartment from the rest of the sarcoplasm. The SR network is continuous transversely, but is longitudinally segmented at periodic intervals. Different muscle fibers contain a somewhat different expression of the SR, but with comparable structural characteristics. The most remarkable structural feature of the SR is the triad, formed by the apposition of two identical-looking sacs of the SR and an intervening intermediate element. In most muscles, triads are precisely located relative to the bands of the sarcomere (Fig. 4).

Not enough was known at the time to allow precise speculation on the function of the SR. However, two important points were made. First, the SR membrane allows its content to have a composition different from the cytoplasm that bathes the fibrils. Second, because of its continuity, the SR could be involved in the transmission of impulses within the muscle fiber. A first suggestion that this role might be played by a component of the triad came from the elegant serial-sectioning study of mouse skeletal muscle by Andersson-Cedergren (23). She established that the central elements of the triads form networks in a plane transverse to the fiber-long axis. These networks are called the transverse tubular system (transverse or T tubules being the individual segments of the network), and they are not part of the SR. Andersson-Cedergren recognized the T tubules as the obvious candidates for carrying excitation to the fiber's interior, and calculated that they should be able to conduct the impulse to the center of the fiber fast enough to account for known excitation-contraction (e-c) coupling delays. Further, she suggested that, as a result of the stimulus propagated along the T tubules, an activator substance is liberated from the adjacent SR and diffuses to the fibrils. A set of questions for further research was thus proposed.

**E-C COUPLING:** Much of the work done since 1955 on muscle membrane systems has been an effort to solve the problem of e-c coupling proposed by Hill (24). The entire cross section of the fiber is activated during a single twitch. The delay between depolarization of the surface membrane of a muscle fiber and its peak twitch tension is very short (a few to a few hundred milliseconds). Calculations showed that an activator diffusing from the surface membrane to the fiber's interior would take longer than the time available to reach a significant concentration at the center of the fiber. Thus, simple diffusion cannot account for the rapid activation of the fiber's entire cross section.

A. F. Huxley and co-workers set themselves the task of defining the link between excitation of the surface and contraction, in relation to the underlying structure (25). This work is a triumph of the concept of structure-function correlation. In these experiments (Fig. 5), a small patch of surface membrane was depolarized by an external electrode closely applied to the fiber surface. The tip of the pipette and the fiber's

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**FIGURE 2** Disposition of thin and thick filaments is demonstrated in this longitudinal section of a single sarcomere from a rabbit muscle, fixed in rigor. Bridges, joining thick (myosin) to thin (actin) filaments are visible. The center of the thick filament is bridge-free. Images like these were of great importance in confirming the sliding filament model. Reprinted from reference 19.

**FIGURE 3** Cross-section through several myofibrils, at the level of the A band, illustrating the relative disposition of thin and thick filaments in the sarcomere. At right, thin filaments occupy a trigonal position in the hexagonal lattice of thick filaments. At left, in the H zone, only thick filaments are present. Cross-links join thick filaments to each other at the M line (top, left), located in the center of the sarcomere. X 33,000.
Figure 4 Longitudinal section of a fast-acting muscle from a fish. Numerous elements of the sarcoplasmic reticulum occupy the spaces between the fibrils. Triads (triple arrows) are located at regular intervals. Small arrows point to feet, joining SR to T tubules. The central element of the triad belongs to the transverse tubular (T) system. The precise disposition of membranes relative to the cross-striation suggested their role in the control of fiber activity to Porter and Palade (22). X 71,000.

Figure 5 Local stimulation experiments in frog twitch fibers. The pipette is applied closely to the surface of the fiber (before, top and bottom) and current passed to it (during, top and bottom). When the pipette is opposite the A band, nothing happens (top). When it is centered over the I bands, a local, transversely spreading contraction of the two adjacent half-sarcomeres results from the depolarization (below, right). Inward spread of contraction coincides with the location of the transverse tubules. Reprinted from reference 26.

Striations were visualized by interference or polarization light optics. In frog-twitch fibers, when the depolarized patch was at the level of the A band, no contraction was ever seen. On the other hand, when depolarization was at the level of the Z line, a small localized shortening of the two adjacent half-I bands was sometimes seen. Larger pulses produced a contraction that traveled further into the fiber in a transverse direction, i.e., in the plane of the Z lines. Muscle fibers from lizards and crabs produced a local, transversely spreading contraction when stimulated at the level of the A-I junction, and not at the Z line. Simultaneously with these experiments, electron microscopy showed that triads and T tubules are located at different levels of the sarcomere in different fibers: some fibers, as in the frog, have triads at the Z line (Fig. 6); some, as in the lizard, have triads at the A-I junction (Fig. 4), correlating with the location of sensitive spots. Crabs have dyads—structures that are similar to triads—near the A-I junction (see Fig. 21), again where sensitive spots are found (27). These results clearly suggest that some component of the triad and dyad must be involved in the inward spread of excitation.

Using single fibers, Hodgkin and Horowicz (28) established that, above a threshold value, a steep relationship exists between membrane potential and tension, and thus, presumably, between membrane potential and the release of an activator substance to the fibrils. These and the local stimulation experiments of A. F. Huxley and co-workers established the value of single fibers for observation of contraction by light-optical methods or measurement of mechanical performance not af-
Triumphant Confirmations

The next stage in the development of muscle research saw a number of significant contributions in morphology, biochemistry, and physiology. These provided confirmatory evidence for the theories of muscle contraction and its control, described in the previous section.

Filaments: Native thin filaments and filaments formed by polymerization of actin in vitro were examined by Hanson and Lowy (29) (Fig. 7). The two types of filaments are apparently identical (but see section on Regulatory Proteins). Individual actin monomers are visible as small, approximately spherical structures, forming long chains with a subunit repeat of 5.5 nm. Two chains form a helical structure. The helical repeat varies somewhat with preparative procedures, showing that the two chains can unwind. In most preparations, the repeat is 36–37 nm (the helix is not integral).

Individual myosin molecules are easily damaged by negative staining, but they can be examined by shadow-casting techniques (31). They are very elongated and are made of two parts: a rod and a head region, with a total length of 150 nm (156 nm in the best recent estimate). The myosin head is globular and is approximately 5 x 10 nm (Fig. 8).

The best understanding of the structure of the thick filament and its relationship to the role of myosin in contraction came from examination of tryptic fragments (HMM and LMM) and of natural and reconstituted filaments and aggregates (31). HMM, which maintains the actin-binding and ATPase activity of the entire molecule, consists of the head and a short portion of the tail. LMM forms the rest of the tail and retains the self-aggregating characteristics of the entire molecule, forming paracrystals at low ionic strength. The length of LMM is estimated to be 96 nm.

The first model of a complete myosin filament was constructed bearing in mind LMM’s self-assembly properties and the appearance of isolated myosin molecules (Fig. 9) (31). It was built by assuming that myosin molecules assemble in a tail-to-tail configuration in the center of the filament and then add on in a tail-to-head configuration in either side. The model fully accounts for structural and functional properties of the filament. (a) The central bare region, which is visible in thin sections of intact sarcomeres, does not bear bridges. (b) The two side regions bear bridges, and these are composed of the head portions of the myosin molecules that interact with actin. (c) More importantly, the model has a polarity that reverses in the center, because the myosin molecules in the halves of the filament point in two different directions. This satisfies a requirement of the sliding filament model: forces pulling the thin filaments of the two halves of a sarcomere toward the center must be in opposite directions on either side of the H zone.

Antibody-staining techniques add details to the basic model of the thick filament. For example, variations in the availability to staining of LMM sites with overlap of the filaments are interpreted as caused by changes in interfilament spacings at different sarcomere lengths. These produce a loosening of the LMM rod packing, as the bridges reach further from the thick filament shaft (33).

Sliding-Filament Model: The sliding-filament model requires the thin filaments to have a polarity that reverses at the Z line. The existence of this polarity was demonstrated by allowing actin and myosin to interact, in the absence of ATP, to form the so-called “decorated” thin filaments (31). When thin filaments are exposed to either intact

Figure 6. Longitudinal section at the periphery of a frog twitch fiber. Extracellular spaces and the transverse tubules are filled by an electron-dense tracer. By this means, continuity of extracellular spaces and lumen of T tubules is demonstrated. Opening of a T tubule (arrow), within the I-band region, coincides with the location of sensitive spots in local stimulation experiments. × 220,000.
myosin molecules or HMM, the entire actin filament is covered by myosin, forming an arrowhead configuration. When an entire I segment, consisting of a Z line and the two sets of thin filaments attached to it on either side, is decorated, the arrowhead tips point away from the Z line on both sides, thus revealing an inherent polarity in the thin filament array.

Since its first application in fibroblasts by Ishikawa, Bischoff, and Holtzer in 1968, the “decoration” technique has become widely used in all types of cells (34), and it is now one of the standard tools for identifying actin-containing filaments in nonmuscle cells.

The expectations of the sliding-filament model also were confirmed by physiological and X-ray diffraction experiments in living muscle fibers.

Isometric length-tension curve. If the force-generating elements are the individual bridges, then it is expected that the force of an isometric contraction would be proportional to the number of bridges capable of interacting with actin and, therefore, to the amount of overlap between thin filaments and the bridge-bearing regions of thick filaments. The isometric length-tension curve produced in 1940 by Ramsey and Street for single, isolated muscle fibers generally agreed with these predictions. One discrepancy in particular remained and was later clarified: A. F. Huxley and Peachey found that a small, residual, isometric tension produced by fibers stretched to the point where no overlap should exist arose from shorter sarcomeres, which still had some overlap near the myotendon junctions (2).

The entire length-tension curve (Fig. 10) was obtained in a series of elegant experiments, in which the effects of the ends of the fibers were minimized by a complex apparatus that allowed only a central portion to be studied (35). The results were sufficiently precise that each segment and inflection point in the curve could be related to some feature of filament position in the sarcomere length (Figs. 10 and 11). The length-tension relationship established in these experiments was in astoundingly good agreement with expectations, and produced a strong argument in favor of the sliding filament model.

X-ray diffraction patterns of living muscle. The portion of the X-ray pattern containing meridional and offmeridional reflections with an axial periodicity of 42.9 nm is dominated by the contribution of the cross-bridges (36). Early X-ray diffraction observations showed that these reflections are not altered during passive stretches. Improvements in X-ray diffraction cameras in the mid-1960s (36) allowed the first recording of diffraction patterns from stimulated muscles. It was found that axial periodicities of actin and myosin were virtually unchanged in isometric and isotonic contractions, i.e., no changes in the length of the filaments accompanies either the production of force or the shortening of the sarcomere (4, 37).

Tubules and E-C Coupling: T tubules are obvious candidates for the intracellular conduction of excitation, as demonstrated by the local activation experiments. However, this role would require the T tubules to have a functional, if not a direct, anatomical connection with the surface membrane.
of the T tubules was demonstrated directly in frog fibers by subsequently form long chains, penetrate into the fiber, and eventually form contacts with the SR (40). These sub- size of ferritin diffuse into the T tubules. The SR is not directly since been used in a variety of muscles (38), but rarely in vertebrates. A new fixative, glutaraldehyde, finally allowed preservation of continuities between the T-tubule membrane and the plasma membrane in a fish muscle (39). In frog muscle, as a result of the tortuosity of the most peripheral segment of the tubules, openings are difficult to see. To date, even though numerous single openings have been seen (Fig. 6), the exact relationship between individual openings and the location of sensitive spots in local stimulation experiments is not clear.

Free exchange between extracellular fluids and the content of the T tubules was demonstrated directly in frog fibers by allowing the T tubules to be infiltrated by a fluorescent dye and by ferritin (40, 41). A number of different tracers have been used in a variety of muscles (see Figs. 6 and 17–21), and the general conclusion is that molecules at least up to the size of ferritin diffuse into the T tubules. The SR is not directly infiltrated, and thus it is an intracellular compartment.

T tubules develop during muscle differentiation from initial small inpocketings of the plasmalemma (caveolae). These subsequently form long chains, penetrate into the fiber, and eventually form contacts with the SR (40).

**Tubules and Electrical Parameters:** Even before the real nature of the T tubules was established, specific models were proposed to explain the results of impedance measurements in muscle. Falk and Fatt (42) formulated the first equivalent circuit model for muscle that took into account the contribution of the transverse tubules. It was shown that a model in which T-tubule resistance and capacitance are in parallel to those of the plasmalemma could roughly account for the low frequency impedance data. The calculated surface area of transverse tubules in frog twitch and slow fibers (43, 44) and in crustacean muscles (27) agrees with the capacitance data, assuming that the specific capacity of the muscle membrane is roughly the same as that of the nerve membrane.

**SR and Relaxation:** The function of SR in relaxation was defined in studies of isolated vesicles and in “skinned” fiber experiments. Once it was known (12) that the supernate of a muscle homogenate is composed of vesicles of SR origin (microsomal fraction, relaxing factor, muscle grana, vesicles), research started in earnest. Hasselbach and Makinose (reviewed in references 45 and 46) defined the properties of the calcium pump: the pump accumulates calcium into the SR against a concentration gradient, using ATP as a source of energy. The ATPase activity of the pump is stimulated by calcium and it requires magnesium. Weber and co-workers (47, 48) demonstrated that the relaxing effect of SR vesicles is uniquely attributable to their ability to reduce calcium concentration to below $10^{-7}$ M. At that calcium concentration fibrils are relaxed. One curious observation, which later acquired significance, was made in these studies: some actomyosin preparations could not be made to relax by lowering the calcium concentration. The explanation for this phenomenon is to be found in the Control Proteins section.

The presence of a calcium sink in the intact fiber was indirectly demonstrated by cleverly designed experiments using skinned fibers (49). More directly, when either skinned fibers or muscles fixed in glutaraldehyde were exposed to oxalate, calcium oxide deposits were located within the SR (40, 50). Oxalate is currently used to locate calcium stores in other cell types.

**Comparative Morphology:** A good correlation has been established between contractile activity of different muscle fibers and their content of SR and T tubules. The astounding variety of functional and morphological adaptations in the muscles of arthropods led G. Hoyle to suggest that these, rather than vertebrate muscles should be the primary target of modern research (51). Particularly beautiful is the disposition of SR and T tubules in fast-acting fibers of arthropods (reviewed in reference 40).

**Myosin**

In the words of Lowey (30), myosin is “an unusual protein; it cannot be classified as either a globular enzyme or a fibrous, structural protein. Rather it combines both classes of molecules in a functional, covalently linked unit.”

A single myosin molecule has two heads connected to a common, double-stranded, α-helical, rodlike tail (Fig. 8). The two heads have equivalent ATPase activity (30).

Papain digestion separates the head regions, called subfragment 1, or S1, from the tail portion of HMM, called S2. S1 maintains the ATPase and actin-binding abilities of the entire molecule, and thus S1 must be located within the bridge, or, better, must form the bulk of the bridge. The tail portion of the HMM is thought to perform the function of allowing the S1 to reach actin filaments by swinging out from the thick filament shaft (52). The trypsin-sensitive region joining HMM to LMM is thought to act as a hinge. The ability of myosin to move out from the shaft of the filament is required by the constant volume behavior of the sarcomeres, which results in a variable separation between the peripheries of actin and myosins at different sarcomere lengths.
Under a variety of denaturing conditions, low molecular-weight subunits (light chains) separate from the myosin molecule. Myosin of "fast" muscle fibers contains three light chains. Two of the chains are related by a single identical thiol sequence and have been called alkali light chains (A1 and A2). Their removal affects myosin's ATPase activity. A third light chain has a different amino acid sequence; it can be removed without affecting ATPase, and has been called the dithionitrobenzene (DTNB) light chain (53). There are two DTNB light chains per mole of myosin and one per mole of S1. The molar ratio of A1 and A2 to myosin is not an integral number, but the ratio of the sum of the two is 2:1. Each S1 subfragment of myosin (i.e., each head) contains one DTNB light chain and either an A1 or an A2. A1 and A2 are the products of two related genes. Both isoenzymes are present in an individual muscle fiber, in its fibrils and filaments, and even, sometimes, within the same myosin molecule (51).

**Myofibrils and Bridges**

The basic model of thick filament structure proposed by H. E. Huxley in 1963 remains unchallenged in its main outline (reviewed in references 17, 33, and 54). Further details of the arrangement of the individual molecules to form the filaments are under close scrutiny. One question concerns the packing of the LMM subunits into the shaft of the thick filament. In one model (17, 33), LMMs are thought to lie parallel to the filament axis and to be staggered in a manner that brings HMM portions of the molecule out at appropriate intervals to form the bridges. The length of LMM (96 nm) in an alternate model, LMMs are thought to be tilted around the long axis of the filament, rather than being parallel to it (54). Ultimately, a detailed model of the thick filaments should provide a useful description of the exact number and disposition of the force-generating portions of the molecule, i.e., the HMM bridges. As a first approximation (6), X-ray diffraction diagrams of frog muscles are interpreted as showing that the arrangement of myosin heads is a helix with a repeat of 43 nm and a translation of 14.3 nm between adjacent levels. Every 14.3 nm, two bridges arise on opposite sides of the filament: successive levels rotate by 60° around the filament axis. The structural regularity extends to a hexagonal superlattice, in which the nearest neighbors are not identical, but the next ones are.

Precise alignment of thick and thin filaments and accessory proteins in the sarcomere produce fine cross periodicities (Fig. 12). At the M line, in the center of the sarcomere, the thick filaments (in vertebrates) are bound to each other by cross-links, organized in three to five lines. The light (L) zone on either side of the M line is caused by the bridge-free regions of the thick filaments. In well-fixed material, the edges of the bridge-free regions, and those of the A bands are very sharp, indicating good alignment of bridges in adjacent filaments and precise uniformity in the length of the thick filaments. The disposition of bridges along the length of the filaments is not uniform: the absence of a single set of bridges near the ends forms a highly visible gap. The A band is crossed by lines with an approximate repeat of 43.0 nm. Eleven of these are most prominent and are probably the result of the contribution of the bridges reinforced by the presence of accessory proteins, the C and H proteins (55). A 40-nm period in the I band is caused by the presence of control proteins in the thin filaments.

Excellent preservation of bridges and beautiful micrographs were obtained from asynchronous flight muscles of an insect. In relaxed muscles, the pattern is dominated by the 14.3-nm repeat of the bridges arising from the thick filaments, and the helical arrangement can be determined quite accurately. The pattern from muscles put into rigor with low concentrations of ATP is dominated by the 2 x 38.5-nm repeat of the actin helix, which restrains the attachment sites of the bridges (56). Computer modeling gives the best fit with the data when the bridges are allowed considerable freedom, i.e., they have the flexibility of rotating around the thick filament by 30° and of moving up to 14.0 nm axially (57). In contracting muscles, the bridges may have a corresponding amount of freedom in the search for an actin attachment site. Such freedom of movement may be required by the known mismatch in the longitudinal periodicities of thick and thin filaments.

**X-ray Diffraction**

Striated muscle is admirably suited for studies by low-angle X-ray diffraction because most of the relevant periods are in the 10-40 nm range. Muscle is unique as a tissue, in that it can be examined usefully by X-ray diffraction in its living state both at rest and during activity.

X-ray diffraction studies have gone through several stages, encouraged by technical innovations and improvements toward
obtaining better results. Initially, the extremely long exposure times required reduced their application to the study of dead specimens. Improved cameras and sources allowed use of living muscle, but with the limitation that only steady-state situations, i.e., muscle in rigor or rest, could be used. Out of these observations came the first description of the hexagonal arrangement of thick filaments and of changes in intensity of equatorial reflections with the state of the muscle, later to be attributed to movement of the bridges relative to the thick filament. The constant volume behavior of sarcomeres with changes in length, i.e., the fact that interfilament distance is inversely proportional to the square root of sarcomere length is an important observation of x-ray diffraction, which must be kept in mind when considering bridge activity (36, 58).

In 1964–65, further improvements allowed the first experiments on contracting muscles. From work of this period came two very important confirmations of the sliding model: (a) the 14.3-nm bridge period does not change during contraction; and (b) arrangement of bridges changes in contracting muscles (4, 37).

Correlation with electron micrographs was used to assign meridional and off-meridional reflections to details of the architecture of thin and thick filaments. Direct evidence for the existence of bridges projecting from the surface of the thick filaments was found in X-ray diffraction of living muscle. Evidence of a high degree of order, rarely preserved when tissue is prepared for electron microscopy, was also demonstrated. Of great interest were comparisons between X-ray diffraction of intact muscle and light optical diffraction of electron micrographs, in the orderly muscles of an insect. These still represent the best evidence for a high order of arrangement of bridges and for changes in their positions at rest and in rigor.

In recent times, further improvements came with the introduction of electronic position-sensitive X-ray detectors in place of the far less sensitive photographic plates. With this technique, exposure times are greatly reduced, and appropriate synchronization allows time resolution of changes in intensity ratio of 1.0 and 1.1 equatorial reflections during the time-course of a contraction. Direct evidence for lateral movement of the bridges, as an event which precedes or coincides with onset of tension is thus obtained. A proportionality exists between the number of bridges that have shifted toward the actin filaments and tension produced (59).

**Myosin, Actomyosin, ATP, and Bridge Cycles**

Research on the biochemistry of the interaction of myosin (as an ATPase), ATP, and actin has reached very sophisticated levels. Interest in this research goes beyond muscle contractility. In most cells there are proteins which have intrinsic ATPase activity and a mechanochemical coupling role, e.g., dynein in cilia and all types of myosin in nonmuscle cells. Current interpretation of their function relies heavily on actomyosin models.

ATP has a dual effect on actomyosin. (a) It reduces affinity between the two molecules, thus allowing dissociation of actin from myosin. This step is considered equivalent to the detachment of bridges from actin and thus to the change between rigor and relaxation (60, 61). The physiological substrate for actomyosin ATPase in MgATP, which is present in the intact muscle filament in the millimolar range. Binding of MgATP to actomyosin and dissociation of actomyosin both are very rapid steps (62). (b) ATP is the substrate for hydrolysis by myosin ATPase. The reaction involves several intermediate steps. The scheme outlined below was initially proposed by Lynn and Taylor (62) and has been confirmed and subsequently refined by the work of Trentham and co-workers (reviewed in references 60 and 63–66). Step 4 is rate-controlling (63):

\[
\begin{align*}
  M + \text{ATP} & \rightleftharpoons M\cdot\text{ATP} \rightleftharpoons M^*\text{ATP} \rightleftharpoons M^*\text{ADP}\cdot\text{P}_i \\
  & \rightleftharpoons M^*\text{ADP}\cdot\text{P}_i \rightleftharpoons M^*\text{ADP} + \text{P}_i \rightleftharpoons M\cdot\text{ADP} \rightleftharpoons M + \text{ADP}.
\end{align*}
\]

Actomyosin ATPase has a much higher steady-state rate of hydrolysis than does ATPase in pure myosin. Hydrolysis of ATP by actomyosin is considered to be a simple extension of the myosin-ATPase cycle, as follows:

\[
\begin{align*}
  \text{AM} + \text{ATP} & \rightleftharpoons \text{AM} \cdot \text{ATP} \rightleftharpoons \text{A} + \text{M}^*\text{ATP} \\
  & \rightleftharpoons \text{A} + \text{M}^*\text{ADP}\cdot\text{P}_i \rightleftharpoons \text{AM} \cdot \text{ADP}\cdot\text{P}_i \rightleftharpoons \text{AM} + \text{ADP} + \text{P}_i.
\end{align*}
\]

The rate-limiting step is bypassed, allowing for a much more rapid turnover. This scheme includes the dissociating effect of ATP on actomyosin, ATP hydrolysis, and the cyclic association and dissociation of the two proteins.

Interest in this research stems from the assumption that the biochemical steps are related to the cycle of activity of the bridges (Fig. 13). Fibrils deprived of ATP are in rigor, i.e., the bridges are all attached and the fibril is inextensible, but it does not produce active tension. After ATP is added, fibrils initially become extensible, i.e., they are in a relaxed state because the bridges are detached. Hydrolysis of ATP follows immediately. The bridges then bind to actin (step 4 in the kinetic scheme above) and release the reaction products and energy (producing either tension or movement). In the presence of ATP, detach-
ment and another cycle quickly follow. In the absence of ATP, the bridges remain in rigor. The position of a bridge in the absence of ATP is thus considered equivalent to the position assumed by the bridge after having released its energy. Under physiological conditions, ATP is present in millimolar concentrations, and thus the bridges are continuously cycling as long as the calcium concentration is sufficient to remove the control proteins’ inhibition of the activity.

Final understanding of mechanochemical transduction by the bridge requires information on the actual shape changes of the myosin molecule, which allow it to produce tension and/or movement. Interestingly, the most detailed theory available to date is derived from studies of the mechanical, rather than the biochemical, performance of the bridges. Precise experiments were designed to measure the transient force response of an isometrically contracting muscle, which is subjected to a very rapid, small change in length. The basic setup is the same as that used for the exact measurement of length-tension relationship (2, 68, 69). The response to a length decrease consists of four phases: (1) an initial, instantaneous tension drop; (2) rapid recovery to almost, but not quite, the initial tension; (3) a slower, plateau, or slight decrease in tension; (4) a slow redevelopment of tension to final values. Phase 1 is consistent with the existence of an undamped-series elastic element within the bridge, possibly in S2. The rapid recovery of phase 2 may be caused by an internal rearrangement in the position of S1 relative to actin. This is possible if the bridge can rock among three different positions (possibly attachment angles) that reflect different states of the myosin molecule. Phases 3 and 4 are explained as results of detachment of some bridges, those that have rocked to their farthest position, and their subsequent reattachment. In an overall scheme, the bridge (HMM) may comprise a hinge region at its junction to LMM (52), which allows it to acquire different positions relative to the thick filament shaft, an elastic segment (S2), and a head (S1), which, in turn, can take different angles relative to S2 and the actin filaments (68).

Regulatory Proteins

Extensively purified preparations of actin and myosin are fully turned on (hydrolyze ATP at a fast rate), as long as MgATP is present in sufficient concentrations. “Natural” actomyosin and intact myofibrils, on the other hand, have an ATPase activity that is regulated by the calcium concentration in the medium. At calcium concentrations below approximately $10^{-7}$, natural actomyosin’s ATPase is inhibited (turned off), and at higher calcium concentrations, it is turned on. The difference is caused by the presence of additional proteins called regulatory proteins. The regulation by “natural tropomyosin” of the interaction of actin and myosin was first described by Ebashi (70). On further analysis, this “natural tropomyosin of Bailey,” was found to have two major components: tropomyosin (TM) and troponin (TN) (71, 72). TM mediates association of TN with actin.

TN is a globular protein with the highest affinity for calcium in the contractile system, and is composed of several fractions, with different properties. In time, three components were recognized (73), and they now have specific roles attached to each. Following Greaser and Gergely’s nomenclature, these are TN-T, TN-I, and TN-C (74). TN-T is responsible for attaching the TN group to TM. TN-I inhibits actomyosin ATPase, in concert with TM and the other TN subunits. TN-C is the calcium-binding subunit (64). Occupation of all Ca-binding sites of TN-C results in a release of TN-I inhibition of the actomyosin ATPase and thus in activation of the contractile material (Fig. 14) (60).

Tropomyosin is a rod-shaped molecule, 40 nm long (75). Three-dimensional reconstruction of the density profile of thin filaments and thin filaments containing tropomyosin (76), indicate that tropomyosin is located in the grooves of the actin helix. A pair of tropomyosin molecules cover the length of seven pairs of actin monomers (Fig. 15). Troponin binds at a specific site along the tropomyosin molecule. In the intact fibril, this produces a “40-nm period,” distinctly different from the period of the actin helix (58, 77). Because there is one mole of troponin for one of tropomyosin, two troponin complexes are attached at 400-Å intervals along the thin filaments, one for each tropomyosin (Fig. 15).

Three lines of experimental evidence have been consolidated into a scheme for the mechanism of interaction between contractile and regulatory proteins. (a) Optical reconstructions of decorated thin filaments indicate that S1 penetrates fairly deeply into the groove of the actin helix (78). Thus, the binding site between actin and myosin is thought to be located within the groove, close to the position occupied by the string of tropomyosin molecules. (b) Intensity changes in x-ray-diffraction reflections attributed to the thin filaments may indicate a movement of the tropomyosin nearer to the center of the groove in conditions where myosin bridges and actin filaments are interacting (79). (c) An apparent cooperative effect between actin monomers is revealed in the presence of “rigor complexes,” i.e., in the presence of S1 bound to actin at low ATP concentrations (80, reviewed in reference 67). A rigor complex is able to override the inhibitory action of Ca-free troponin, possibly by obliging TM to move deeper into the actin groove and to reveal myosin-binding sites on the actin monomers. By this mechanism, the effect of rigor complexes and of Ca binding to troponin are both amplified by tropomyosin, to cover a range of 400 Å, or seven actin monomers.

A second mechanism of regulation exists in some invertebrate muscles: calcium acts directly on the myosin molecule to regulate its rate of ATP splitting (81). Finally, some muscles possess both myosin-based and tropomyosin-troponin-based regulation.

Electrical Parameters

Electrical parameters of muscle have been most extensively investigated for frog-twitch fibers (reviewed in references 50, 82–84).

A variety of equivalent circuits have been studied as representations of the contribution of the surface and transverse tubular membranes to the overall electrical properties of the muscle fiber. Initially, based on AC impedance analysis, the contribution of the T system was thought to be well represented by a single capacitance in series with a resistance (lumped model [42]). Later papers have demonstrated a tendency toward a more realistic representation of the T system as capacitance and resistance spread along the radius of the fiber (distributed model), or as a distributed system with an extra resistance (“access” resistance) in series with the tubules near the surface of the fiber (reviewed in references 82, 84, and 85). In general, there is agreement that the capacities per unit area of transverse tubules and surface membrane are the same and slightly less than 1 μF/cm².

More directly related to muscle function have been attempts to relate electrical potential changes in the T tubular membrane...
Both calcium ions and ATP are needed to turn on the complete contractile system, which is composed by myosin and actin-tropomyosin-troponin filaments. Myosin-ADP complex is indicated by a black dot on the bridge. In the absence of calcium ions, the bridge is either in the relaxed or rigor state in the presence or absence of ATP, respectively. In the presence of calcium, the bridge is in an active state in the presence of ATP (see Fig. 13), and in rigor in its absence. Reprinted from reference 67.

In a native actin filament, tropomyosin molecules occupy both grooves of the actin helix, providing a scaffolding for the periodic attachment of troponin molecules. Reprinted from reference 72.

to the activation of contraction. Adrian et al. (86) determined the decrement in potential along the tubules and, from this, a length and time constant for the network. These experiments were done on fibers in tetrodotoxin-containing solutions, to inhibit action potentials, and, therefore, dealt with a passive T tubule.

Subsequently, Costantin (87) showed that, in the absence of tetrodotoxin, depolarization sometimes resulted in the contraction of centrally located fibrils and not of the peripheral ones. From these experiments and from earlier measurements of the speed of inward spread of excitation by Gonzales-Serratos (reviewed in reference 80), it was concluded that, in frog-twitch fibers, an action potential is the normal mode in which the T system conducts depolarization into the fiber. A series of cleverly designed experiments followed, confirming the existence of an action potential in the transverse tubules of frog-twitch fibers (reviewed in reference 81).

Peachey and Adrian (84) computed action potentials spreading along the fiber surface and into the T network, using a Hodgkin-Huxley scheme for the active changes in membrane conductance. A visible hump at the beginning of the after potential in the computed action potential is a result of activity in the T tubules.

Excitation-Contraction Coupling

The term, as initially defined by Sandow (88, reviewed in references 46, 50, 89, and 90), indicates the steps between excitation of the surface membrane and contraction of the myofibrils. Similar concepts are now extended to coupling, in other cell types, between events at the surface membrane and intracellular events (i.e., excitation-secretion coupling at nerve endings and in secretory cells in general). In muscle, it includes several steps, only one of which still is totally unclear in its mechanism. Successive steps are as follows (see 49).

1. SPREAD OF DEPOLARIZATION ALONG THE SURFACE: In twitch-muscle fibers, a sufficiently large excitatory postsynaptic potential generated at one or two large (en plaque) end plates initiates a self-regenerating action potential. The action potential spreads without decrement along the muscle fiber, essentially as in an axon. The mechanical response to a normal action potential is an all-or-none twitch. Experimentally, the magnitude and time-course of muscle fiber depolarization can be varied, and, under these conditions, a twitch fiber produces a mechanical response of variable magnitude and duration. From this, one concludes that the intact twitch fiber acts in all-or-none fashion because the action potential is all-or-none, but that later events in e-c coupling can be graded.

2. THE ROLE OF T TUBULES: Where present, T tubules are obligatory intermediates between external surface membrane depolarization and the subsequent steps in e-c coupling. This is most directly demonstrated by the local stimulation experiments and by glycerol treatment. The latter is a procedure that either disrupts the T tubules or interrupts their continuity (reviewed in references 40 and 83). In glycerol-treated twitch-muscle fibers, even though excitation of the surface membrane is unaltered, no contraction follows, because spread of depolarization along the T tubule network is impeded.

3. CHARGE MOVEMENTS: Schneider and Chandler (91, reviewed in reference 92) have explored the electrical properties of muscle fibers exposed to solutions designed to eliminate all voltage-dependent permeability changes, and depolarized to a
The effect of the transmission would be to open channels that intramembranous charge movement within the T tubule's account most of the data currently available. One (91) assumes potential range as e-c coupling, it is speculated that it is a movement of charge within the membrane's dielectric. This current is most likely to be capacitative, i.e., caused by the inward current above that measured with a similar step potential within the contraction threshold range. Under these conditions, a depolarizing step produces an extra, small outward current above that measured with a similar step performed at membrane potentials near the resting level. The current is most likely to be capacitative, i.e., caused by the movement of charge within the membrane's dielectric. This study is significant for two reasons. (a) It is the first measurement of a nonlinear capacitative (gating) current. (b) Because the measured charge movement occurs in the same membrane potential range as e-c coupling, it is speculated that it is a necessary step in the coupling (91).

4. T TO SR COUPLING: It is calculated that the amount of calcium needed for activation is far more than that entering the fibers during an action potential (50). The SR is an obvious source for this calcium. At triads, dyads, and peripheral couplings, a small gap separates SR and T tubules (or surface membrane). It is generally accepted that transmission from surface membrane or T tubules to SR occurs at those junctions, although the mechanism of this transmission is unknown.

Two current hypotheses of transmission at the triad take into account most of the data currently available. One (91) assumes that intramembranous charge movement within the T tubule's membrane facing the SR is the initiating step, and that its effect is directly transmitted to the SR membrane by links located in the junctional feet joining the apposed membranes. The effect of the transmission would be to open channels through which Ca can exit the SR, following its chemical gradient.

A second hypothesis (93) proposes that a small ionic current flows between the lumina of T tubules and SR during excitation. This is a result of the temporary opening of channels across the T-tubule membrane, which is strongly dependent on voltage. A regenerative change in SR’s permeability to calcium and Ca release follows.

5. MECHANISM OF CA RELEASE: Even though little is directly known about transmission at the triad, a good deal can be deduced by observing the results of the coupling, i.e., the release of calcium. Autoradiographic studies of calcium distribution reveal the presence of two calcium pools in the SR: an uptake and a storage pool. The latter, located in the triad, may coincide with the release sites (94). Direct evidence for calcium release from the SR was obtained following recent refinements in the technique of electron microprobe analysis of frozen sections (95). Interestingly, the measured amount of calcium release exceeds that needed to saturate fully troponin.

Ca-sensitive probes are employed to detect appearance of calcium in the sarcoplasm. Murexide was the first to be used, whereas the bioluminescent protein, aequorin, and others were subsequently introduced (96). By use of calcium indicators, it was determined that a steep relationship exists between calcium transients and the intramembranous charge movements (97).

A second stimulus capable of producing a rapid release of calcium from SR of skinned fibers is a sudden change in ionic composition of the medium (e.g., a change from the impermeant sulfate to the permeant chloride ion), which presumably produces a change in SR membrane potential (reviewed in reference 50). There is evidence that a change in SR membrane potential accompanies (but does not necessarily precede) calcium release. Results were obtained from the study of optical signals. These can be used to measure changes in electrical potential across membranes not accessible to microelectrodes. The technique is currently used in a variety of cell types. Baylor and Oetliker (99) have measured birefringence changes from muscle fibers, and Bezanilla and Horowicz (100) have measured a fluorescence change in fibers treated with Nile Blue A, a penetrating lipid-soluble dye. In both cases, in addition to signals assigned to surface and T-tubule membranes, a large component of probable SR origin was seen.

T Tubules and SR

GENERAL DISPOSITION: Different portions of SR and T tubules have special structural and functional characteristics, the former being detectable either in thin sections or in freeze-fracture replicas. In addition, both systems vary a great deal in size and distribution in different fiber types. Individual variables have been extensively described and reviewed in the past (40, 41, 51, 101–109). In general, a good correlation exists between content of SR and T tubules and the speed of a muscle fiber's activity cycle (Figs. 16 and 17). The relationship is quite complex, because there are several morphological parameters to be considered: amount and distribution of T tubules; number and disposition of dyads; triads, and peripheral junctions; amount of calcium-pumping SR and its content of Ca pump and Ca-binding proteins. Additionally, factors other than the membranes affect the overall speed of a muscle fiber.

High-voltage electron microscopy of thick slices of muscle fibers, in which either SR or T tubules, or both, are selectively "stained" by electron-dense substances has recently contributed the most complete views of the two membrane systems (Fig. 18).

T Tubules: There are two basic components of the T tubules' network: (a) narrow-diameter tubules with a round cross section, which do not participate in junctions with the SR; (b) flat tubules and cisternae, which form junctions with the SR. The difference between the two is most obvious after glutaraldehyde fixation, and it is emphasized by infiltration of the network with electron dense tracers. In vertebrate-twitch fibers (Figs. 18–20), the transverse net-
FIGURE 16  Cross-section through a fast-acting muscle from a fish. Fibrils are small and completely separated by SR and T tubules. Most of the section is at the level of triads, and the rows of junctional feet joining SR to T tubules are visible (arrows). × 18,000.

FIGURE 17  Cross-section of a slow, tonic fiber from the frog. Fibrils are large and often not entirely separated from each other at the A-band level. Triads (arrows) are fairly numerous, but they do not cover as much of the T network as in twitch fibers (see above). They also have a different orientation. × 23,000.
works of tubules that penetrate across the fibers at periodic intervals are composed of alternate sections of flat tubules and round tubules. The fraction of round tubules within the transverse network varies among different fiber types. Longitudinal extensions of the network exist in all types of fibers. Network parameters have been quite precisely worked out in the case of frog twitch fibers.

In slow fibers of vertebrates and in most fibers from invertebrates, the T-tubule networks are less precisely arranged transversely, and in some cases run as frequently in the longitudinal as in the transverse direction. The networks are composed of alternate round tubules and flat, pancakelike cisternae (Fig. 21). The latter forms dyads and triads with the SR.

High-voltage electron microscopy has revealed a new feature of the fibers examined; an area of the fiber was found where the network is not transverse, but in the form of a helicoid, with a pitch equal to the sarcomere length (110). Helicoids also have been seen in this way in rat twitch fibers. At the center of each helicoid is a dislocation in the cross-striation, and in this region longitudinally oriented T tubules are most numerous. Even though the presence of helicoids does not alter the basic concepts of e-c coupling described above, it results in a rather more complicated T network than thus far envisaged.

SR: In twitch fibers from higher vertebrates, the SR is segmented by the penetration of the T-tubule network across the fiber. Generally, the SR is composed of distinct regions (Fig. 4): (a) the lateral sacs of the triad on either side of the T tubules; (b) intermediate cisternae and longitudinal tubules, which join the lateral sacs of the triad to (c) a fenestrated collar adjacent to the middle of the A band. In fibers with two triads per sarcomere, a fenestrated collar is situated opposite the I bands.

In slow and cardiac muscle fibers from vertebrates and in skeletal and cardiac muscle of invertebrates (Fig. 21), the SR is more continuous because junctions with the T tubules are in a longitudinal or oblique, rather than transverse, plane (dyads and triads). The fenestrated portion of the SR is more prominent and the longitudinal elements less so. Flattened regions of the SR participate in the formation of dyads. Other components of the SR form flat cisternae, which form junctions (peripheral couplings) with the surface membrane. Peripheral couplings are likely to be functionally equivalent to dyads and triads.

The amount of SR and T tubules has been estimated for a number of fibers (43, 44, 102). As an example, in a 100-μm diameter frog twitch fiber, the T-network surface area is approximately seven times the external surface area, and the total SR surface area is almost 20 times larger than that of the T tubules.

Composition: Purified microsomal fractions from muscle homogenate contain vesicles of SR origin and a small number of proteins. The most abundant are: a membrane protein, the calcium-activated ATPase, and an internal calcium-binding protein, calsequestrin (reviewed in reference 111). In smaller amounts are acidic proteins with high affinity for calcium (contained within the lumen), two intrinsic proteins of 30,000 and 34,000 mol wt, and a third small (M55) protein.

Vesicles with a dense content are identified as originating from the lateral sacs of the triad, and they contain the calsequestrin (Fig. 22).

The light SR fraction is identified with the longitudinal elements of the SR. It has an extremely simple protein composition. More than 90% of the protein is the calcium pump ATPase. M55 protein is also present. Because of this very pure composition, the light SR fraction is ideal for basic studies on the structure and interactions of functional intrinsic proteins with their surrounding lipids.

Membrane Architecture: It is postulated that the SR membrane facing directly toward the T tubules (junctional SR, or jSR) must be the sensor that detects the level of T-tubule membrane depolarization (112). The remainder of the SR membrane, facing toward the fibrils and called free SR (fSR), does not participate in the formation of any junction with other membrane systems. In twitch fibers of the frog, there is approximately 10 times more free than junctional SR. By
FIGURES 19 AND 20  Cross-section of rat twitch fibers, stained by the black reaction of Golgi, seen by light and electron microscopy. Both images show the completeness of the T tubule. In Fig. 19, wider and thinner sections of T tubules correspond to junctional and nonjunctional regions of the network, respectively. Fig. 19, x 11,000. Fig. 20, x 340.

FIGURE 21  In this muscle fiber from a crab, both T tubules and SR have been “stained” by the black reaction of Golgi. The T tubules (arrows) are mostly located over the A bands and consist of alternated thin-diameter tubules, and flat, junctional cisternae. The SR forms a longitudinally continuous fenestrated network, with intercalated flat cisternae (white asterisk). SR and T tubule cisternae are closely apposed to form dyads and triads (not seen in this illustration). x 11,000.
contrast, amounts of fSR and jSR are about equal in insect fibrillar flight muscles.

Structural differences between jSR and fSR membranes are visible after freeze fracture. The free SR is a markedly asymmetric membrane (Fig. 23). It contains intramembraneous particles in its cytoplasmic leaflet (P face), and it has a luminal leaflet (E face) which shows barely visible indentations (presumably a negative image of the particles). The particles are aggregates of Ca pump proteins (reviewed in references 111 and 113). On the average, there are three to four ATPase molecules for each intramembraneous particle (see reference 112). The Ca ATPase is located deep within the SR membrane, and it extends into the lumen and into the cytoplasm. The free SR has a remarkably uniform distribution of particles along its entire surface although the specific density of particles per unit area of SR varies in fibers of different types (reviewed in reference 114). This suggests that the calcium-pumping capacity per unit surface area of SR is uniform throughout a muscle fiber.

In proximity to the T tubules, the fSR shows an abrupt transition into jSR.

Two regions of the membrane delimiting the T tubules are defined. One faces toward the SR (jT) and the other away from it (fT). fT membrane has very few particles on its cytoplasmic leaflet and has either none or very few particles (depending on species) on the luminal leaflet. Its structure is quite different from that of jT membrane to be described below.

**STRUCTURE OF TRIADS (AND HOMOLOGOUS ORGANELLES):** At triads and dyads, the membranes of transverse tubules and SR are separated by a narrow (about 10 nm) junctional gap. The surface of the SR is dimpled, and the
dimples are attached to electron-dense "feet," which cross the junctional gap and join SR and T tubule membranes (reviewed in reference 114). The feet have variable appearances within the same section, and this has resulted in a variety of descriptions in the literature. There is, however, general agreement that they are arranged in a tetragonal disposition, forming either two or multiple parallel rows (Fig. 16). In freeze-fracture replicas, specializations are found both in the jSR and jT membranes. However, the structure of these two membranes is somewhat difficult to describe because they usually do not present the striking arrangement of intramembranous particles that are characteristic of membranes forming special junctions between cells (i.e., gap and tight junctions). The T to SR junction is unique in that the two apposed membranes have different structure from each other, thus making the junction asymmetric. So far, descriptions of the junction have provided no obvious clue to its function. There is, however, some evidence that intramembranous components bear some relationship to the junctional "feet" and thus that the feet may have some direct role in the coupling of the two membranes.

Special Types of Fibers

Special biological requirements have resulted in the evolution of types of fibers with distinctive structural and functional characteristics. As examples from two extremes, this section describes the very fast asynchronous, fibrillar flight muscles of insects and the slow fibers of vertebrates.

**Fibrillar Flight Muscles**: Fibrillar flight muscles are found in selected insect orders. They are recognizable as a distinct fiber type on the basis of several unusual functional and morphological details (reviewed in references 41 and 109). The name fibrillar derives from the appearance in cross sections, where relatively large fibrils (1–5 microns diam) are easily seen in the light microscope because they are separated by numerous, large mitochondria. Mitochondria, as in all insect flight muscles, occupy a large percentage of the fiber's volume.

The structure of the sarcomere is highly organized. Alignment of thick filaments is precise, and thin filaments lie rigidly halfway along the line joining two adjacent thick filaments. This disposition results in a 3:1 thin-to-thick filament ratio, different from the 2:1 ratio of vertebrate muscles. Because of this precise arrangement, this muscle has proved to be ideal for the study of bridges.

The most striking morphological feature of the membrane system is the scarcity of selected components of the SR. It consists of little more than the vesicles forming dyads with the T tubules. Thus fSR, the calcium-pumping portion of the SR membrane, is reduced relative to most other types of fibers, whereas jSR is not. T tubules are numerous, and indeed their frequency of motor-nerve impulses and electrical events at the surface membrane do not correspond on a one-to-one basis to the mechanical events (hence the name "asynchronous," by which these muscles are known). Special properties of the fibrils, when activated, are responsible for the oscillation. Briefly, the effect is caused by a delayed change in tension after a step change in length. Necessary small amplitude length changes are imposed on the muscles by the wing and elastic ligaments in the insect's thorax. Nerve impulses simply activate the muscle (presumably, by conventional e-c coupling). A high-frequency oscillatory contraction then follows, which lasts as long as the fibrils are activated. Thus, in this muscle a sufficient sarcoplasmic concentration of calcium must be maintained for a prolonged period of time. Paucity of Ca-pumping fSR is consistent with this requirement. Adequate supplies of T tubules, jSR, and mitochondria are consistent with requirement for a rapid turn-on and for an adequate supply of ATP.

**Slow Fibers**: Function of slow fibers is thought to be the maintenance of a prolonged state of contraction with the least expenditure of chemical energy. In the frog, for example, such a contraction is needed during the mating season, when the male's amplexus may last uninterrupted for many days.

The following is a description of slow fibers in frogs. Others may vary slightly from this prototype (reviewed in references 104, 106, 107, and 116). Slow fibers differ from fast fibers in the following properties. They do not conduct an action potential, and they respond to application of acetylcholine or potassium with a slow prolonged contracture. The latter phenomenon suggests that prolonged depolarization does not result in inactivation of some step in e-c coupling, as in twitch fibers. The response to stimulation through the nerve is also graded and slow.

Two details of structure and function of slow fibers may be directly correlated to the lack of an action potential: (a) multiple innervation, which allows postsynaptic potentials to be produced at frequent intervals along the length of the muscle fiber; (b) large membrane resistance, which makes the fiber a more effective, if slow, cable.

The morphological differences between slow and twitch fibers in the frog are quite prominent. End plates are small and multiple (en grappe). Myofibrils are large and often incompletely separated by a layer of SR. Z lines are wider than in twitch fibers, and with a different structure; M lines are apparently absent. The thin filaments have variable lengths. T tubules, SR, and their junctions are present, but in amounts and disposition different from twitch fibers (see Fig. 17). The T tubules mostly course at the level of the Z line, but not as precisely as in twitch fibers. Longitudinal extensions are more numerous. The SR is less abundant than in twitch fibers, mostly because it forms an incomplete cuff around the fibrils. In addition, it has far fewer intramembranous particles per unit area of membrane. Triads and dyads are irregularly oriented, but they are composed of essentially the same structural elements as triads in twitch fibers. Peripheral junctions between SR and plasmalemma are numerous.

The morphology of the membrane system indicates a basic similarity of function with that of twitch fibers. This conclusion is largely confirmed by the following physiological data. In skinned fibers, the contraction produced by direct application of calcium to the fibrils has a slow time-course, indicating that the slow onset of tension production is mostly caused by slow cycle rather than by a large delay in e-c coupling (reviewed in reference 50). Activation kinetics, as indicated by strength-duration curves that correlate pulse duration with membrane potentials in voltage-clamped fibers, imply a similar mechanism of calcium release in slow and in twitch fibers (reviewed in reference 117). Slow onset of tension and metabolic economy are thus mostly results of the slow rate of bridge cycling.
Types of Twitch Fibers

In this section, we consider the differences within a restricted group of fibers: the twitch-type fibers that compose the voluntary musculature of mammals. In the study of “fiber types,” various techniques are integrated. Initial approaches included histochemistry, electron microscopy, and physiological studies of mechanical properties and how innervating axons affect them. More recently, various combinations of sophisticated physiological studies of individual motor units, quantitative electron microscopy, immunocytochemistry, microbiochemistry, experiments with cross innervation and chronic stimulation in vivo and in vitro, and the study of differentiation during development have brought us to the threshold of a complete understanding of the factors controlling differentiation of fiber types and of the close match between the properties of a motor neuron and the fibers it innervates. Muscle pathology has greatly benefited from these studies, and, in turn, it has contributed clarifying information, in the form of diseases whose expression depends on the type of fiber affected.

Twitch fibers are all capable of a propagated action potential, to which they respond with an all-or-none twitch. Repetitive stimulation at appropriate frequency produces a tetanus. Innervation is at one or two large (en plaque) end plates. Within this general scheme, there are variations in details, such as the intrinsic speed of shortening, the frequency of stimulation necessary to produce a fused tetanus, and fatigability, i.e., failure of the muscle fiber to produce tension as a result of repetitive stimulation. A fiber possessing a particular set of characters is assigned to a “fiber type.” Such classification is very useful, as long as it is kept in mind that the boundaries between different fiber types are not always clear-cut. Fibers with virtually any possible mixture of characters may exist. In addition, many of the characters do not vary in stepwise fashion, but form a continuum between two extremes.

In the leg muscles of cats, three distinct types of fibers are defined on the basis of interrelated morphological biochemical and functional characters (118): fast fatigable (FF), fast fatigue resistant (FR), and slow fatigue resistant (SR). Fast fatigable fibers have high myofibrillar ATPase, a high content of glycolytic enzymes, and few mitochondria. They produce a fast twitch, have a high fusion frequency, and fatigue rather rapidly. FR fibers have a high, acid-unstable ATPase, a good supply of glycolytic enzymes, and fairly numerous mitochondria. They have a fast twitch and a moderately high fusion frequency, and are innervated by axons of fast conduction velocity. They are relatively resistant to fatigue. SR fibers have low ATPase, a large number of mitochondria, and low glycolytic enzyme activity. Their twitch is definitively slower than that of fibers in other fiber types. They are relatively resistant to fatigue. SR fibers have low ATPase, a large number of mitochondria, and low glycolytic enzyme activity. Their twitch is definitively slower than that of fibers in either fast category. They are relatively resistant to fatigue. SR motor units are used for tonic, postural movements, FR for rapid movements of some duration, and FF are probably used only for very rapid movements, such as those involved in an escape reaction.

It is likely that the above classification can be applied to leg muscles of most mammals, even though details vary with species (reviewed in reference 106, 119, and 120), and further subdivisions may need to be included. When one examines muscles responsible for more delicate and complex movements, classification becomes far more difficult. On the basis of morphology alone, for example, at least five categories of fibers are described in extraocular muscles.

There are three metabolic categories of fibers: predominantly oxidative, oxidative glycolytic, and glycolytic. These correlate very well with the relative content of mitochondria (highest in oxidative fibers, Fig. 24). Content of oxidative and glycolytic enzymes varies in a continuous, not a stepwise fashion, and so does the relative volume of mitochondria (102). Fibers rich in mitochondria contain the oxygen-carrying pigment myoglobin and are associated with a rich network of capillaries. For that reason, the portions of muscles containing them have a deeper color. Fibers rich and poor in mitochondria are often referred to as red and white, respectively (reviewed in reference 121). No direct correlation exists between the pattern of metabolic activity and the speed of contraction. Muscles that are purely fast may contain red as well as white fibers. On the other hand, a correlation exists between mitochondrial content and fatigability: red fibers are more resistant to fatigue than are white fibers. Metabolic properties are, at least partially, under the influence of exercise.

Intrinsic speed of shortening is a property of the myofibrils and is related to the rate of ATP splitting by actomyosin ATPase (122). There are two genetically distinct types of myosin, one contained in fast-twitch fibers and the other in slow-twitch fibers. Fibers of intermediate speed contain a mixture of the two (Figs. 25 and 26). The two myosins differ in the number and type of light chains associated with S1: "slow" myosin has only one type of alkali light chain with a molecular weight different from either of the two alkali light chains of "fast" myosin. The two types of myosin are antigenically distinguishable, and they can be related by immunofluorescence (see Figs. 25 and 26). The study of the distribution of myosins in different fiber types in the adult and developing muscles and in experimentally altered muscles is one of the most active current fields of muscle research. A good collection on papers on this subject has been published recently (123).

A muscle's well-being depends on innervation. A motor neuron and the muscle fibers it innervates comprise a motor unit, which is composed of the same types of fibers. A motor neuron that innervates a slow-twitch motor unit produces a steady, low-frequency discharge (10–20 per second). A fast motor axon fires isolated bursts of action potentials at a higher frequency (30–60 per second). When the motor nerve that serves a muscle predominantly composed of fast twitch fibers is crossed with that of a predominantly slow-twitch fibers, the mechanical properties of the two muscles are, at least in part, reversed, as is the myosin ATPase. If, as is often the case, the muscles used for cross-innervation have fibers with different mitochondrial contents and calcium-pumping activity of SR, then this also is reversed. It is now established that, following cross-innervation, the fibers gradually change their content of myosin (and other proteins) from the fast to the slow type and vice versa. Under the influence of the steady activity of a slow motor neuron, a fast fiber suppresses the genes responsible for the synthesis of fast myosin. Over a period of time, which is presumably dependent on the turnover rate of the myosin molecule, the fiber then gradually acquires a content of slow myosin.

Chronically implanted electrodes, producing direct excitation of the muscle at a steady rate of 10 per second over a period of 20–40 days, induce the change of a fast to a slow muscle as effectively, or more so, than does cross-innervation. Muscle fibers can be caught in the process of transforming, at a stage when they possess both types of myosin simultaneously.

In early stages of differentiation, all muscle fibers synthesize fast myosin. If left alone, or if innervated by a fast axon, they will continue to do so. Appearance of slow myosin in future
FiGURE 24  Cross section of the "red" portion of a rat muscle. The small-diameter muscle fibers, probably of a nonfatigable type, have a large content of mitochondria located between the fibrils and in aggregates at the periphery of the fiber. Numerous capillaries surround each muscle fiber. × 3,200.

FiGURES 25 AND 26  Serial sections of the rat extensor digitorum longus muscle, stained with affinity purified antibodies against rabbit "fast" and "slow" myosin, respectively. Some heterogeneity in staining with antifast is observed, and few fibers are not stained. In Fig. 26, those fibers which fail to stain with antifast do stain intensely with antislow. Additionally, a number of fibers that stain with slightly less than maximum intensity with antifast also stain lightly with antislow. These fibers probably are intermediate in speed of shortening between the other two. Reprinted from Rubinstein, N., and A. M. Kelly. Manuscript submitted for publication.
slow-twitch fibers is detectable at stages when muscle becomes innervated. Thus, either the steady firing of the slow motor neuron, some trophic factor, or a combination of the two, are responsible for inducing the synthesis of slow myosin. This may be at the basis of the higher sensitivity of slow-type fibers to denervation and for the prevention of their atrophy by activity.

Conclusion

This chapter considers only those aspects of the skeletal muscle fiber that distinguish it most from other cells: its elaborate contractile machinery and the accessory structures responsible for the control of activity. Great progress has been made in the understanding of these structures. Most of the steps in excitation-contraction coupling are well identified. Remaining questions on the basic mechanism of contraction are at the molecular level. Factors responsible for the exquisite adaptation of fibers to varying functional demands and for the integration of motor neuronal and muscle-fiber properties are established. We are at the threshold of a complete understanding of what makes us move—and how.

It should not be forgotten that, even though highly specialized, a muscle fiber shares properties with other cells, and that much has been learned in the study of muscle applies to other cell types, as well. The muscle fiber should be thought of as an extreme state of differentiation—a modulation and augmentation of basic cellular structure and functions.

So that the reader should not be misled into thinking we have reviewed all that is known about muscle, we should mention that several broad areas of past and current interest in the study of the cell biology of muscle have not been covered at all: among these are structure and function of smooth and cardiac muscle; muscle metabolism, structure, function, and development of end plates; the integration of nerve and muscle function and their feedback control; response of muscle to training and disease; differentiation and regeneration; and muscle pathology.

ACKNOWLEDGMENTS

Supported in part by the Muscular Dystrophy Association (Henry M. Wattis Neuromuscular Disease Center) and National Institutes of Health (HL-15835, Pennsylvania Muscle Institute). We thank Miss Denah Appelt for help with the illustrations.

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