

Mechanism of Skeletal Muscle Contraction

The sliding filament theory: This is the accepted theory for the basic mechanism of muscle contraction. Two groups in England, A.F. Huxley and Niedergerke (1954), and H.E. Huxley and Hanson (1954), are the founders of the theory. Their classical experiments are shown in Figures ME1 and ME 2.

Fig. ME1 shows a living frog muscle fiber under the interference microscope. A bands appear dark, I bands are light. The length of the sarcomeres, A and I bands were measured on densitometer tracings. The fiber was stimulated electrically and it was allowed to shorten. On the left side for each frame two numbers are given. The upper numbers indicate the sarcomere length, which in successive times during contraction decreased from 3.10 to 2.93 to 2.70 to 2.37 micron. The lower numbers indicate A band length, 1.43, 1.45, 1.50, and 1.48 micron, that means, it was unchanged during contraction.

Fig. ME2 shows the pictures of glycerol extracted psoas fibrils under a phase contrast microscope. A bands are dark, I bands are light; in the middle of I bands the Z lines are seen. Four sarcomeres of one fibril are pictured during ATP induced contraction. A bands did not change in length, but the I bands shortened, and, in the last frame the I bands disappeared.

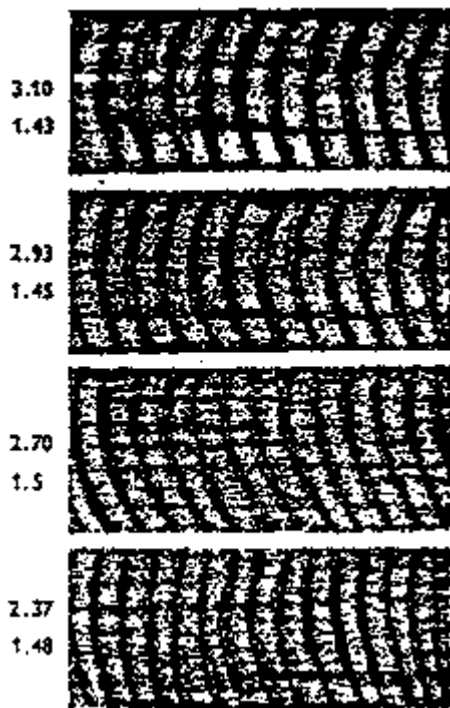


Fig. ME1. Changes in sarcomere length and in I band length during contraction of an electrically stimulated frog fiber (From A.F. Huxley and Niedergerke, 1954).

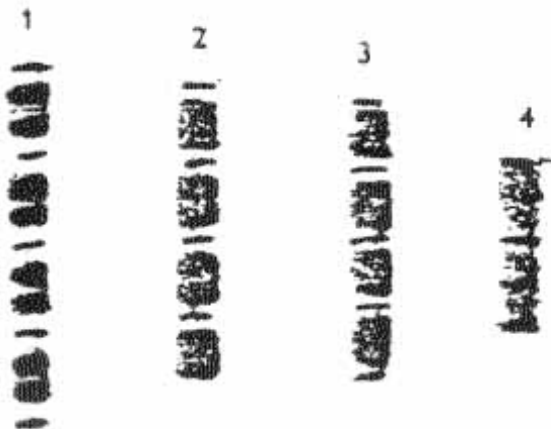


Fig. ME2. Changes in sarcomere length and in I band length during contraction of a glycerol-extracted fibril contracted by ATP (From H.E. Huxley and Hanson, 1954).

Further experimentation revealed that during contraction the length of the actin containing thin filaments and the length of the myosin containing thick filaments remained constant. Thus, during contraction the length of the sarcomere and I band decrease, the overlap between thick and thin filaments increases, the length of the thick and thin filaments remains unchanged. Consequently, the filaments must slide past each other.

Length-tension relationship: The physiological interpretation of the sliding filament theory was tested by measuring the tension of a single muscle fiber at different sarcomere length (Gordon et al., 1966). Figure ME3 illustrates the experiment. Maximum tension was obtained at rest length, between 2.0-2.25 micron, when all crossbridges were in the overlap region between thick and thin filaments. When the muscle fiber was stretched so that the sarcomere length increased from 2.25 to 3.675 micron and consequently the number of crossbridges in the overlap region decreased from maximum to zero; the tension fell from 100% to 0.

The crossbridges are uniformly distributed along the thick filaments with the exception of a short bare zone in the middle. The crossbridges seem to be identical and are the site of the interaction between thick and thin filaments. The tension is the algebraic sum of the tension produced at each individual site. At or above rest length the tension is directly proportional to the number of crossbridges in the overlap region between thick and thin filaments.

Below rest length, when the thin filaments meet in the center of the A band or they start to interact with the oppositely directed crossbridge sites past the bare zone (in the middle of the sarcomere), tension drops off.

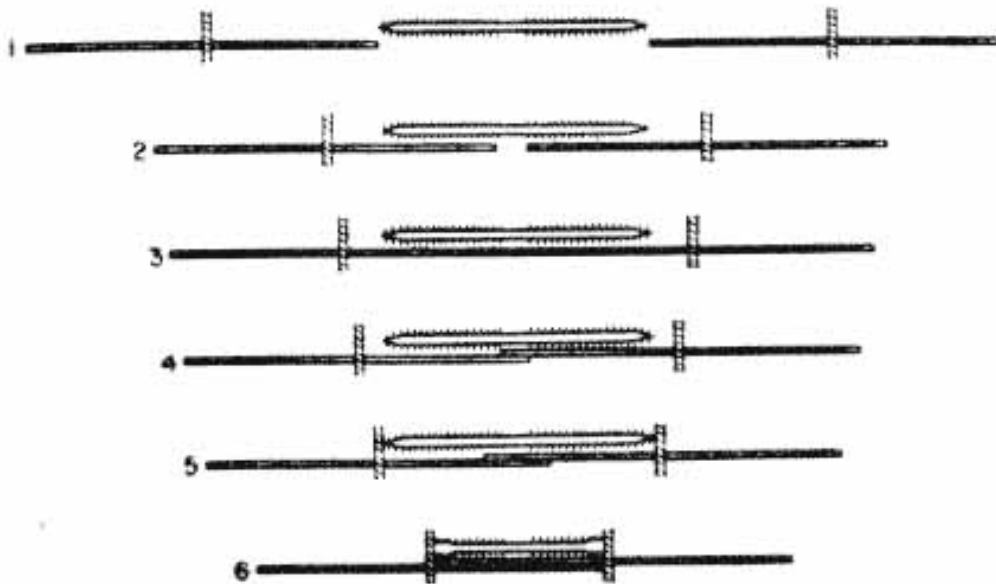
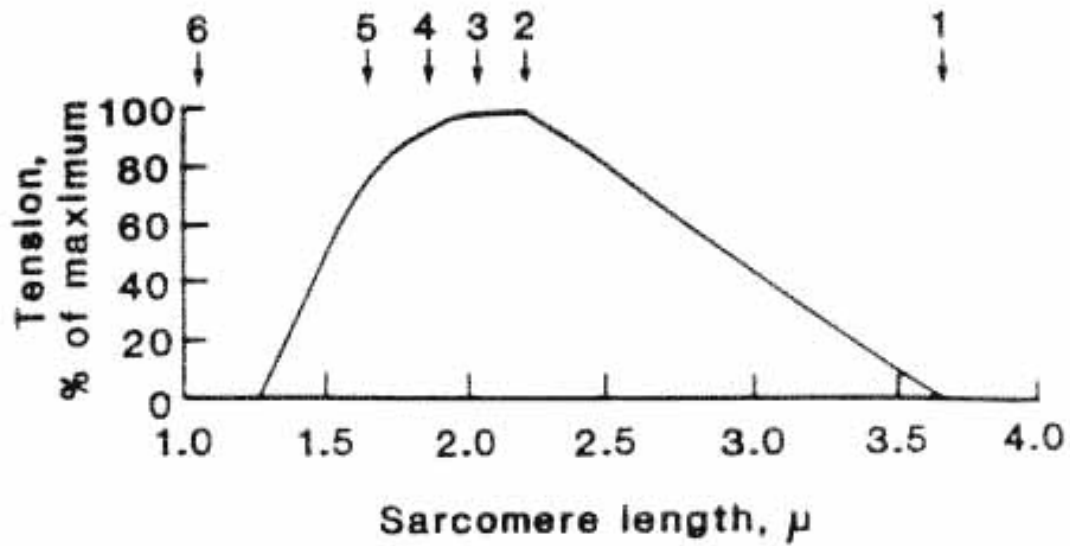


Fig. ME3. Length-tension relationship of a single frog *semitendinosus* muscle fiber (From Gordon et al., 1966). The numbers 1 through 6 on the length tension curve correspond to the numbers on the schematic diagram of thick and thin filament arrangement. In this way the relationship between thick and thin filaments can be compared to the tension at various sarcomere lengths.

Crossbridge cycle and its relation to actomyosin ATPase: A scheme for the coupling of ATP hydrolysis to the crossbridge cycle is shown in Fig. ME4. The following major steps are involved:

1. ATP dissociates actomyosin into actin and myosin; i.e. the thick filaments will be detached from the thin filaments. ATP binds to the myosin head in the thick filaments.
2. ATP is hydrolyzed by myosin; the products ADP and P_i are bound to myosin. The energy released by the splitting of ATP is stored in the myosin molecule. The myosin.ADP. P_i complex is a high-energy state; this is the predominant state at rest.
3. Upon muscle stimulation, the inhibition of actin-myosin interaction, imposed by the regulatory proteins, is lifted and consequently the myosin with bound ADP and P_i attaches to actin. It is believed that the angle of crossbridge attachment is 90° .
4. The actin-myosin interaction triggers the sequential release of P_i and ADP from the myosin head, resulting in the working stroke. It is thought that the energy stored in the myosin molecule brings about a conformational change in the crossbridge tilting the angle from 90° to 45° . This tilting pulls the actin filament about 10 nm toward the center of the sarcomere, while the energy stored in myosin is utilized.

With a new ATP a new cycle may begin and the cycling may continue until the regulatory mechanism stops the interaction of actin and myosin. As shown in Fig. ME4, ATP is needed for step 1; that is for the detachment of myosin from actin. In case of ATP depletion, the cycle is arrested. When actin and myosin are permanently bound in the absence of ATP, the muscle becomes rigid. This state is called *rigor mortis*.

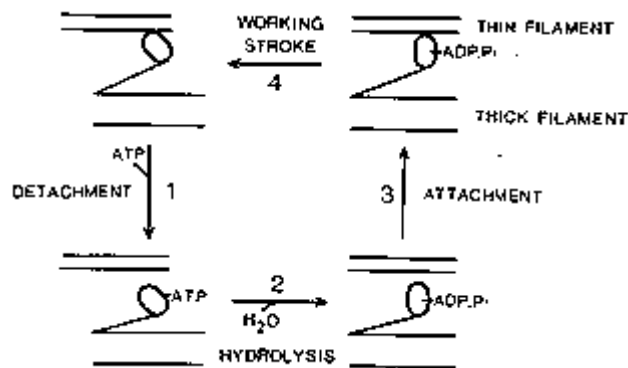


Fig. ME4. Crossbridge cycle and its relation to actomyosin ATPase (Courtesy of Dr. Jack Rall).

In Summary: As discussed in the Regulation of Muscle Contraction chapter, Contraction is initiated by depolarization of the sarcolemma. The action potential spreads inside along the T-tubules. The signal is transmitted from T-tubule to terminal sacs of sarcoplasmic reticulum (SR). Calcium is released from (SR) into sarcoplasm.

The binding of Ca^{2+} to troponin results in a cooperative conformational change in the troponin-tropomyosin system. The inhibition of actin and myosin interaction is released. Crossbridges of myosin filaments are attached to actin filaments. Tension is exerted, and/or the muscle shortens by the sliding filament mechanism.

Relaxation will occur when Ca^{2+} is pumped back into the (SR). Subsequently, crossbridges are detached from the thin filaments and the troponin-tropomyosin regulated inhibition of actin and myosin interaction is restored. Finally, active tension disappears and the rest length is restored. This completes the contraction-relaxation cycle.

X-ray Diffraction Studies of Muscle and the Crossbridge Cycle

X-ray diffraction is a very powerful technique to study the mechanism of muscle contraction, because it probes the molecular structural changes at the level of intact muscle, while the muscle produces tension or shortens and relaxes (Squire and Knupp, 2005) Basic concepts of muscle x-ray diffraction were discussed in the [Actin-Myosin Interaction](#) chapter.

Electron position storage rings provide x-ray beams which allow the study of transient events of muscle contraction. Thus, it is possible to record information about the structure in the meridional reflections from contracting muscle. Furthermore, with the very high flux of protons it is possible to record such patterns with millisecond time resolution (Huxley, 2004).

Studies of interference effects on the meridional reflections from the myosin filaments have revealed important new details about the configuration of the myosin heads and their changes during rapid mechanical transients (for references see Huxley, 2004). Lombardi et al. (2004) applied length changes of a few nanometers on single muscle fibers which were completed in about 0.1 msec. They could resolve the structural changes associated with the length step from those associated with the about 1000 s^{-1} force recovery step that followed it. This single fiber technique became feasible as a result of further developments of brighter synchrotron x-ray beams and new signal averaging methods. Thus, the changes in the intensity of the myosin M3 reflections in response to rapid length steps could be analyzed.

Linari et al., (2000) established x-ray interferometry as a new tool for studying the motions of myosin heads during muscle contraction. Figure ME5 shows the axial x-ray pattern from resting and active single muscle fibers. At rest [Fig. ME5, Part A and C (black)] the M3 reflection was dominated by a peak at 14.35 nm. During active isometric contraction, the M3 reflection was split into two peaks of roughly equal intensity at 14.46 and 14.67 nm [Fig. ME5 Part C (red)].

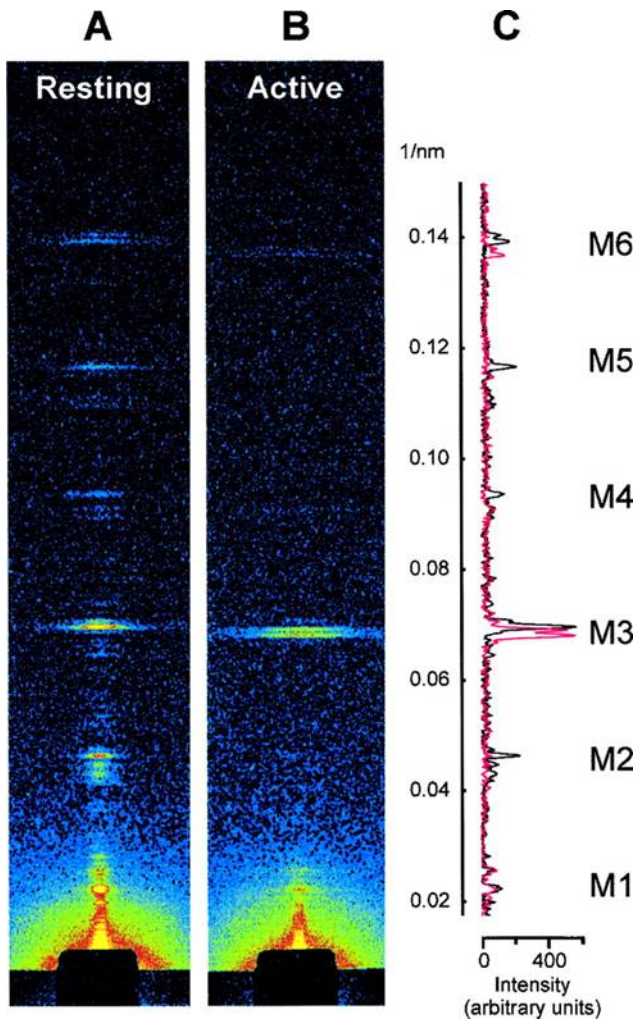


Fig. ME5. Axial x-ray diffraction patterns from a frog single muscle fiber at rest (A) and at the plateau of an isometric tetanus (B), and comparison of respective axial intensity distributions (C; resting, black; active, red). (Reprinted with permission from Linari, M., Piazzesi, G., Dobbie, I., Koubassova, N., Reconditi, M., Narayanan, T., Diat, O., Irving, M., and Lombardi, V. Figure 1 in their paper entitled: Interference fine structure and sarcomere length dependence of the axial x-ray pattern from active single muscle fibers. *Proc. Natl. Acad. Sci. USA*, **97**, 7226-7231, 2000. Copyright 2000, National Academy of Sciences, USA).

The explanation of the fine structure of the M3 reflections was tested by stretching muscle fibers to different sarcomere length before stimulating them (Linari et al., 2000). As the sarcomere length is increased, the actin filaments are pulled out from the myosin filament array, so that the region of overlap between the myosin and actin filaments is shorter, and the myosin heads near the center of the myosin filaments can no longer bind to actin. Thereby, the active force generated by the fiber is reduced, in proportion to the degree of overlap between the filaments. The intensity of the M3 reflection during active contraction also decreased in proportion to filament overlap (Figure ME6), suggesting that the heads that can not bind to actin during active contraction are disordered, and make little contribution to the M3 reflection.

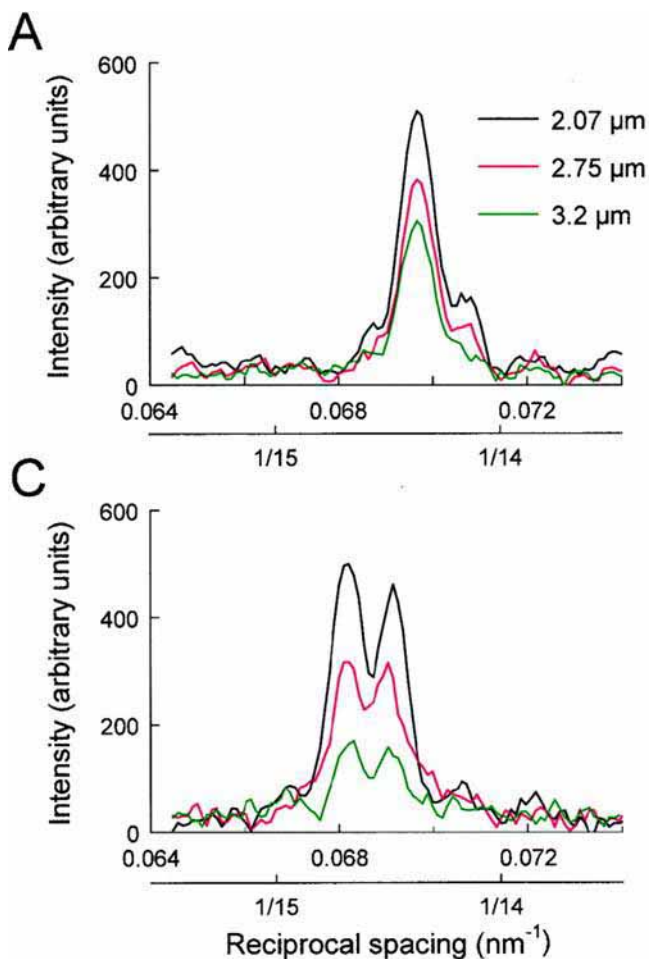


Fig. ME6. Sarcomere length-dependence of the axial intensity distribution of the M3 reflection at rest (A) and at the isometric tetanus plateau (C). The color code for the sarcomere length is indicated in the upper right corner of (A).

(Reprinted with permission from Linari, M., Piazzesi, G., Dobbie, I., Koubassova, N., Reconditi, M., Narayanan, T., Diat, O., Irving, M., and Lombardi, V. Part A and C of Figure 4 in their paper entitled: Interference fine structure and sarcomere length dependence of the axial x-ray pattern from active single muscle fibers. *Proc. Natl. Acad. Sci. USA*, **97**, 7226-7231, 2000. Copyright 2000, National Academy of Sciences, USA).

The position of the interference fringes on the M3 myosin meridional reflection at 14.56 nm in contracting muscle provides an extremely sensitive measure of the changes in the axial position of the center of scattering mass of the myosin heads attached to actin. It is capable of detecting movements of 1-2 Å. Measurement of the ratio of the intensities of the two major peaks of the M3 reflection, is a convenient way of measuring the relative movement of the sampling fringes and, hence the myosin heads (Huxley, 2004). Many of these observations can be interpreted satisfactorily by the tilting lever arm model of muscle contraction (Huxley, 2004).

Dickinson et al., (2005) conducted beautiful studies on the flight muscles of *Drosophila* actually flying in the synchrotron x-ray beam. They observed major oscillatory changes in the 145 Å and 72 Å meridional reflections and in the 387 and 195 Å actin layer lines. The 145 Å and 72 Å reflections increased in intensity during the upbeat of the wing (muscle stretch) and reduced on the downbeat (muscle shortening). The 195 Å layer line behaved the same way. These observations were interpreted largely in terms of coordinated myosin lever arm movements.

Griffiths et al., (2002) followed changes in myosin head (S1) orientation and force induced by a temperature increase. They used time-resolved x-ray diffraction to measure

M3 meridional intensity during sinusoidal oscillations of force produced by frog skeletal muscle. This technique allows definition of S1 orientation relative to the axis of the muscle fiber. M3 intensity changes were approximately sinusoid at low temperature but became increasingly distorted with increasing temperature. The increased distortion was explained by a shift in the orientation of the tail domain of actin-bound S1 toward the orientation at which M3 intensity is maximal. This is consistent with a tail domain rotation model of force generation in which the tail approaches a more perpendicular projection from the thin filament axis at higher temperatures.

Previous x-ray diffraction studies described the 429 Å axial repeat from the myosin heads and the 360-370 Å repeat from actin. Recent studies revealed the axial reflection from C-protein at 440 Å and from troponin at 385 Å (Squire et al., 2004). Importantly, the later studies described reflections not seen before, indicating unknown components in the A-band.

Yagi (2003) used x-ray diffraction to study early structural changes during contraction of frog skeletal muscle stimulated with a single electrical pulse of 1 ms duration and with a time resolution of the diffraction pattern of 0.53-1.02 ms. Tension began to drop at 6 ms (latency relaxation), reached a minimum at 8 ms, and then twitch tension developed. The intensity of the meridional reflection at 385 Å from troponin on the thin filament began to increase at 4-5 ms and reached a maximum at ~12 ms. The meridional reflections of the myosin 430 nm repeat began to decrease when the tension began to develop. The peak position of the myosin M3 reflection began to shift toward the higher angle at ~5 ms, reached a maximum shift at 10 ms, and then moved toward the lower angle. The intensity of the second actin layer line at 180 Å in the axial direction started to rise at 5 ms, whereas the latency relaxation started at 3.5 ms. The results suggested that the Ca²⁺-induced structural changes in both the thin and thick filaments have already taken place during latency relaxation. .

In summary, the high resolution, time-resolved x-ray diffraction of the 21st century contributed greatly to our understanding of the molecular mechanism of muscle contraction, that is how force and movement are generated. In addition, the technique revealed new structures in the sarcomere, the identification of which will be the subject of a new research.

Suggested readings: Squire and Knupp, (2005); Linari et al, (2000); Lombardi et al., (2004); Huxley, (2004).

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