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David Aitchison Smith
Department of Physiology, Anatomy and Microbiology
La Trobe University
Melbourne, VIC, Australia

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For Nigel, Pamela, Samuel and Audrey

Preface

Perhaps no field of scientific endeavour has been quite like muscle contractility for bringing together people with different backgrounds and expertise and for establishing the bona fides of biophysics as a coherent synthesis of biology and physics. The somewhat mysterious mechanism of muscle action has attracted physicists for over 50 years, and many theories of muscle action have been proposed, only to fall by the wayside as they were refuted by new experimental data. Many monographs and textbooks on muscle have been published; why should yet another one see the light of day? Most monographs concentrate on experimental studies, which range widely from muscle physiology to its biochemistry and the atomic structure of the proteins. There are fewer books on the theory of muscle action, which to some extent has lagged behind the experimental advances. Why this is so forms part of the narrative of this book. It appears that a unified theory of muscle contraction at any level of activation is finally within our grasp, despite the difficulties of marrying the biochemical actin-myosin cycle to mechanical events at the molecular level. The theory is necessarily mathematical, and mathematical developments are presented in enough detail that they can be verified without resorting to the literature.

What distinguishes a theory from a model? In the past, many models of muscle dynamics have been invented for particular purposes, primarily to interpret new experimental data and to show that the interpretation is consistent with other aspects of muscle behaviour. As the quantity and scope of new data grew, so did the variety of models constructed to accommodate them, with the result that no unified model was apparent. Any model which can accommodate the whole range of mechanochemical observations might deserve to be known as a theory, but this is not a sufficient criterion. The study of muscle contraction has suffered from a surfeit of "data-driven models whose structure and parameters lack physical meaning", to quote O'Shaughnessy and Pollard (2016). This book is an account of the search for a unified theory which embodies the true molecular mechanism of muscle action, tested against the wealth of available data.

The presentation is suitable for biophysicists and mathematicians with a basic knowledge of graduate-level mathematics and chemical physics. No prior knowledge of muscle structure or function is assumed, but the selection of topics is tightly focussed on molecular mechanisms of contractility. This focus covers a fraction of the phenomena of interest to muscle researchers, ranging from the mechanism of excitation-contraction coupling and the regeneration of ATP by mitochondria to the growth of muscle cells, the phylogeny of muscles in mammals, vertebrates and invertebrates, cardiac muscle and the law of the heart, muscular dystrophy and cardiac myopathies, muscle fatigue and ageing, steroids, sports medicine and the treatment of injuries, and more.

Melbourne, VIC, Australia David Aitchison Smith
June 2018

Acknowledgements

I am grateful to my colleagues and many people who encouraged my involvement in muscle research for the last 30 years. My interest started with the realization that mechanisms of cell motility were poorly understood and might be tested by appropriate modelling. This soon turned into a gut feeling that force generation by myosin S1 in muscle could be the driver of motile slime filaments and amoeboid cells. This insight turned out to be largely incorrect; we now know that the movements of many motile cells are driven by actin polymerization. However, I was by then committed to exploring theories of muscle action. I am very grateful to Prof. Mike Geeves for allowing me to do this during a 6 month sabbatical at the University of Bristol: my collaboration with Mike on different aspects of muscle contraction has proved very fruitful.

I am also much indebted to Prof. Bob Simmons of King's College London for inviting me to join his laboratory with John Sleep and Walter Steffen at King's College in 1995. At that time, the group was building an optical trap system for detecting the working stroke of a single myosin molecule, and my contribution was the development of software for automatic detection of stroke events in the presence of Brownian noise. Success arrived just in time for the new millennium, and results obtained with this highly position-stabilized system showed that the tethered myosin was binding to target zones separated by 36 nm, the periodicity of the actin filament. However, optical-trap systems developed by Justin Molloy at York, David Warshaw at Vermont and Amit Mehta at Stanford all gave the same result; the working stroke of myosin S1 from a fast muscle was 5–6 nm, whereas atomic structures indicated that the length of the lever arm was 10 nm. The reason for this discrepancy is described in Chapter 6.

My other areas of activity while at King's College (1995–2002) were thin-filament regulation and the development of the chain model for linked tropomyosins, in collaboration with Mike Geeves and coworkers, and the perennial problem of finding a structurally-based working model of muscle contraction at the level of the half-sarcomere. All three projects were supported by project grants from the Wellcome Trust. The third project continued in Australia, courtesy of an NIH-supported

collaboration with Dr. Srba Mijailovich which led, inter alia, to two papers in 2008 entitled “Towards a Unified Theory of Muscle Contraction”. The title proved prophetic; in the words of A. V. Hill, “the lifetime of theories in the area of muscle contraction is generally rather short”. A major change to the 2008 model was the belated recognition that the structuralists were correct in insisting that the myosin working stroke is triggered by the release of phosphate from its active site. Doubtless, more changes will be incorporated as muscle modelling is further refined.

More recently, my collaboration with Prof. George Stephenson at La Trobe University, Melbourne, led to papers on the mechanism of spontaneous oscillatory contractions at low calcium and the stability of the filament lattice in striated muscle. I am grateful to Mike Geeves and John Sleep for discussions and advice over many years and for helpful discussions with Kevin Burton, Gerald Elliott, Gerald Offer, David Morgan, K. W. Ranatunga and many others at Biophysical Society meetings and elsewhere. I also wish to thank Mike Geeves, John Sleep and Gerald Offer for their comments on the manuscript.

The mysterious aspect of muscular contractility is why it has taken so long to build a working model from our current understanding of the mechanism, even with the exquisitely detailed information provided by atomic structures of the proteins. The molecular mechanism (or mechanisms?) of contractility is still a subject of abiding interest, particularly now that 35 different kinds of myosin motor have been identified. Most of them have roles to play in non-muscle cells.

Contents

Chapter 1
Introduction

There are more things in heaven and earth, Horatio, than are dreamt of in your philosophy.
William Shakespeare, Hamlet, Act I, scene 5.

1.1 Historical Perspectives

Muscular contraction is a unique method of movement and locomotion common to all higher life forms, from primitive fishes to invertebrates, mammals and mankind. Although the Greek philosophers speculated at length about the nature of matter and living things, the first systematic attempt to understand muscle action seems to be due to the Roman physician Galen, circa 300 A.D. Galen systematically dissected all kinds of muscle, and understood that muscles exert a tensile force: his treatise "De Motu Musculorum" (Goss 1968) includes a discussion of muscle tone and its relation to blood supply. In Renaissance times, Leonardo da Vinci catalogued human anatomy and attempted to push the boundaries of the human musculoskeletal system by constructing a winged flying machine (the "ornithopter"). By this time it was known that muscles were composed of fibres. In 1664, William Croone published a short treatise "On the Reason of the Movement of Muscles" (Maquet et al. 2000) which attempted a mechanical explanation of contraction. Croone thought that because the volume of an intact muscle stays constant as it is activated, the axial contraction could be explained in terms of inflatable bladders. In 1682, Leeuwenhoek wrote that the fleshy fibres were composed of globules; this seems to be the first observation of the transverse striations of vertebrate muscle.

In 1791, Galvani made a frog's legs twitch by passing a current generated by an electric battery (the newly-discovered Voltaic pile), thus discovering what he thought was "animal electricity." Volta disagreed, thinking correctly that the electricity was generated by his pile. In 1902, Veratti observed in the optical microscope that muscles contained a delicate network of longitudinal and transverse filaments, the latter being the T-tubule system (Martonosi 2000), and ideas of muscle action formed around the hypothesis that muscles contract because the filaments shrink.

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In the first half of the twentieth century, biochemists began to explore the chemical events accompanying contraction. Exhaustive stimulation of a muscle caused an increase in the free concentration of phosphate ions (PO_4) and lactic acid, leading to the nostrum that muscle fatigue was caused by an excess of lactic acid. The muscle proteins myosin and actin which form the filaments were isolated. The co-enzyme ATP (adenosine triphosphate) was identified, and contraction was shown to involve the hydrolysis of ATP in which the terminal phosphate group is split off by an OH^- ion. The Lohmann reaction, by which ATP is regenerated by phosphocreatine, was discovered and shown to occur in vivo. The discovery of 'superprecipitation' by Szent-Gyorgi in 1943 gave a strong clue to the action of these ingredients: when solutions of myosin and actin were mixed together, an insoluble actomyosin precipitate was formed, and the addition of ATP caused the precipitate to dissolve, indicating that ATP breaks myosin-actin bonds. The monograph of Dorothy Needham (1971) gives a full account of the early history of muscle structure, function and chemistry.

In 1935, a model for shortening muscle was proposed by A.V. Hill, in which a contractile element with viscoelastic properties was coupled to a series elastic component (SEC); although empirical in nature, this model had the virtue of being quantitative and therefore testable. In fact, the contractile element (myosin) works as a spring whose resting length is reset by a configurational change. It was natural to investigate the SEC by subjecting a muscle to a sudden length change and observing the subsequent behaviour of the tension. Much later on, the same length-step protocol was used to devastating effect in unmasking the mechanism of contraction, but Hill's length steps were not as fast as the mechanism under investigation and his results were consequently misleading (Hill 1953). The hypothetical series elastic component was subsequently abandoned, and it is ironic that it resurfaced when the distributed elastic compliance of muscle filaments was observed with X-ray diffraction in 1994. Nevertheless, A.V. Hill's lasting contribution to muscle science is his equation for the steady-state relation between tension and shortening velocity, viewed as a statement of energy conservation and supported by a combination of mechanical and heat measurements. Hill's equation is described in the next chapter.

1.1.1 The Sliding Filament Model

The modern approach to contractility started in 1953 when the filament structure of vertebrate muscle was observed in the optical microscope, showing two arrays of interdigitating filaments which slide into each other rather than shrink (Fig. 1.1). This discovery was made independently by Huxley and Niedegerke (1954) and Huxley and Hanson (1954), and the sliding-filament model survives to this day as the template on which all models of muscle contraction in striated muscle must build. Major experimental advances since then include the following:

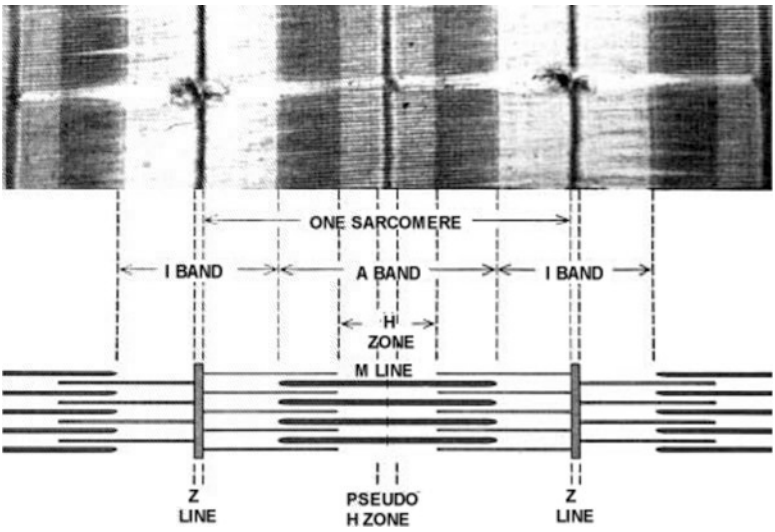

Fig. 1.1 The longitudinal structure of vertebrate muscle, showing the striations which define sarcomeres and interdigitating filaments in each sarcomere (Huxley 2004). With permission of John Wiley and Sons

1. X-ray determinations of muscle ultrastructure by Hugh Huxley, Gerald Elliott and John Squire; the lattice structure of striated muscle, the periodicities of myosin dimers on thick filaments and actin monomers on the thin filament, which is a double helix. Huxley's contribution is described in an obituary (Hitchcock-DeGregori and Irving 2014). See also Elliott et al. (1965), Squire (1981).
2. The tension-length curve of striated muscle and its interpretation: myosin heads are independent force generators (Gordon et al. 1966)
3. The biochemistry of the interactions between actin, myosin and ATP (Lymn and Taylor 1971), following the isolation of myosin and actin from thick and thin filaments.
4. Mechanical evidence that myosin-S1 on actin generates force and movement by making a working stroke (Huxley and Simmons 1971).
5. The atomic structures of actin (Kabsch et al. 1990) and myosin (Rayment et al. 1993), followed by confirmation of the myosin working stroke from atomic structures of myosin-S1 (M), M.ATP, and M.ADP with various phosphate analogues.
6. The mechanism of thin-filament regulation by calcium ions, starting from X-ray studies leading to the steric blocking model (Haselgrove and Huxley 1972), and culminating in cryo-EM determinations of the locations of troponin, tropomyosin on filamentary actin under different conditions (Vibert et al. 1997). The atomic structure of troponin-C (Herzberg and James 1988; Sundaralingam et al. 1985) with and without calcium, and of the core of the troponin complex TnT-TnC-TnI (Takeda et al. 2003).

1.1.2 New Experimental Techniques

Prompted by the initial breakthrough in 1953, research on the mechanism of muscle action became highly active, spawning many new experimental techniques and several technological breakthroughs. After building models of muscle action, it is necessary to test them against the outcomes of experiments made with the new techniques. Here is a sample:

(a) The use of fluorescent labelling, and spin labelling with electron spin resonance (Fajer et al. 1998), to probe molecular enviroments and dynamic changes in real time. FRET (fluorescent exchange transfer) has also been widely used to measure distances of up to 10 nm between excimer states on labelled atoms, for example by Suzuki et al. (1998).
(b) The motility assay. Following the visualization by Yanagida of single fluorescent actin filaments, Kron and Spudich (1986) observed their motion over a lawn of molecules of heavymeromyosin fixed to a glass surface.
(c) Single-molecule mechanics, with a single myosin motor interacting with an optically-tethered actin filament (Finer et al. 1994). Subsequent variations include the use of evanescent-wave spectroscopy (Funatsu et al. 1995), the use of quantum dots as markers of local filament movements, and a cantilevered myosin walking on actin filaments (Kitamura et al. 1999).
(d) Ultrastructure in X-ray diffraction; observation of fringe patterns and their interpretation as interference between diffracted beams from the two halves of the same sarcomere (Linari et al. 2000). The authors claimed that these patterns can be used to detect the myosin working stroke.
(e) The atomic structure of chicken-gizzard myosin-S1 (Rayment et al. 1993) was obtained only after a chemical trick (methylation of lysine residues), amid rumours of a shortage of chickens in Wisconsin.

1.1.3 Models of Contractility

Theoretical contributions to the mechanism of contraction in striated muscle started with A.F. Huxley (1957), who proposed a highly simplified mechanochemical model based on sliding filaments and cyclic attachment of myosin-S1 heads to actin. Huxley's model has attracted very wide acceptance from muscle physiologists, probably because of the lack of simple competitors, but the underlying mechanism is hidden. The accepted mechanism for force generation in muscle is related to the atomic structure of myosin-S1: myosin has a lever-arm which swings to make a different angle with the globular part bound to actin, creating a working stroke. The swinging-lever-arm mechanism has been challenged by models which postulate quite different mechanisms for generating force and movement. These are as follows:

1. Iwazumi (1970) proposed a model in which actin filaments are drawn into the lattice of myofilaments by electrostatic attraction. This model does not require that myosin-S1 heads bind to actin.
2. A water-jet model in which water expelled from myosin by hydrolysis of ATP was directed to cause contraction (Oplatka 1997). Apart from the difficulty of generating sufficient axial momentum transfer, this model can be discounted because Lymn and Taylor showed that ATP hydrolysis takes place when myosin is not bound to actin.
3. The impulse model of Elliott and Worthington (2001), in which tension was created when ATP binds to the actomyosin complex. This model can be dismissed for the same reason; ATP rapidly dissociates myosin from actin after force was generated, a statement confirmed by single-myosin optical trap data.
4. Soliton waves have been postulated as a means of contraction (Yomosa 1985). The proposed mechanism posits a coupling between ATP hydrolysis and vibrational excitation in the alpha-helical rod linking myosin heads to their myofilament backbone. It is claimed that this vibration can create a solitary wave which bends the backbone, causing myosin heads to graze the actin filament.
5. Ratchet models (Astumian 1997).

These alternatives are mostly of historical interest (Ingels 1979), but ratchet models are alive and well. They postulate that a motor molecule (such as myosin-S1) sees its complementary filament (actin) as a periodic sequence of asymmetric potential wells. Brownian motion in such a structure is not unidirectional, but if a conformational change in the motor (such as the hydrolysis of ATP) changes the bias of each well, then unidirectional walking motion is possible. In a sense, this mechanism is not just a fantasy created by theoretical physicists; there are many kinds of molecular motor which are processive, meaning that they make large numbers of walking steps on their cofilaments. Examples include kinesin on microtubules and various DNA walkers. That stepping requires a conformational change in the motor or cofilament is well understood, and synthetic walking motors, including light-driven motors (Credi et al. 2014), have been created.

In Chap. 6, ratchet models are discussed in the context of an experimental tour-de-force from the laboratory of Toshio Yanagida, in which a single myosin molecule walks for several steps along a bundle of actin filaments (Kitamura et al. 1999). It now appears that the small number of steps seen in this experiment is a consequence of the geometry of binding sites on the actin double-helix, regardless of whether myosin makes a working stroke on binding to actin.

Finally, there are ‘black-box’ models of contraction, which aim to derive Hill’s equation from a macroscopic model. The model of Baker and Thomas (2000) is of this kind; muscle is treated as a single entity operating near equilibrium and working against a fixed load. The argument is essentially thermodynamic, and does not provide a systematic way of including the deviations required to set up steady-state ATP hydrolysis. However, their novel approach suggests that a macroscopic description in terms of linear irreversible thermodynamics is possible.

Unavoidably, this survey has used various technical terms and concepts which, for the sake of brevity, have not been defined. These deficiencies are remedied as we proceed.

1.2 A Short Guide to Contractile Behaviour

Any theory of muscle contraction is motivated, and tested, by observations of the behaviour of activated muscle. There are striated muscles, which are composed of parallel fibres and show repeated striations in the optical microscope, and smooth muscles, which are spindle-shaped with filaments arranged obliquely. Skeletal muscles are the striated muscles which link the bones of vertebrates through tendons and ligaments, and are under the control of the central nervous system. Cardiac muscle which drives the heart is also striated, but has a different structure and a different tension-length curve.

Muscles vary in their speed of response to stimulation. Striated muscles may be fast or slow, because individual fibres may have different myosin isoforms. Slow-twitch fibres (type-I myosin) maintain tension and and appear red because of high myoglobin content, while fast-twitch fibres are of type-IIA, which appear pink and have an oxidative metabolism, or type-IIB, which appear white, have a glycolytic metabolism and fatigue easily. Slow fibres hold tension, while fast fibres are required for movement and locomotion. The fastest fibres are found in the swimbladder muscle of the toadfish (Rome 2006), the throat muscles of two songbirds (the European starling and the zebra finch), and the wing-beat muscles which propel flying insects such as the gnat. Slow muscles are better at holding structures in place, and there is a trade-off between efficiency and the speed of unloaded contraction. Smooth muscles are there to hold tension, and are present in the walls of blood vessels, where they serve to regulate blood flow. In invertebrates, slow muscles are the norm, but they are not universal. In bivalves such as clams, the adductor muscle is composed of smooth muscle tissue which holds the valves shut (‘catch muscle’) and striated muscle which can open the valves. For more details, see Carlson and Wilkie (1974), Squire (1981), MacIntosh et al. (2006) or Ruegg (2017). In this book, only striated muscles are considered.

How does a fast striated muscle behave when it is stimulated by nerve impulses? This question can be answered in different ways, depending on what is observed and at what scale. One can begin by treating a muscle as a black box supplied with nerves and blood vessels. In the next section, we look at what lies inside the box.

Consider a fast striated muscle innervated by nerve axons. When axons deliver a single action potential (which lasts for a few milliseconds), the muscle is observed to twitch; it shortens momentarily unless it is held in place by an external load which prevents shortening. In that case the tension rises over about 50 ms before decaying back to zero; the time course of muscle tension can be measured with a transducer which senses the small length change induced within. The twitch lasts about 0.2 s.

As this book is primarily concerned with the mechanism of contraction, the way in which an intact muscle responds to nervous stimulation is peripheral to the main inquiry. However, it is worth noting that individual fibres within the muscle are generally not all activated at the same time. The muscle is innervated at multiple points along its length, each of which is under the control of efferent nerves from the central nervous system. The region of fibre that can be activated by just one such innervation defines a motor unit. Efferent nerve axons innervate different parts of the muscle, and more motor units will be recruited as the number of active nerve axons transmitting action potentials, or the frequency of action potentials in each axon, is increased, so the net tension is a smoothly graded function of the overall frequency of nervous stimulation. On top of that, afferent nerve axons sense the level of activation and relay these signals back to the central nervous system, creating a feedback loop for exquisite control of tension and movement (Katz 1966). It is desirable to study muscular contraction in isolation from its nervous inputs and outputs, and this is often done by extracting individual fibers.

If a string of action potentials is delivered with a frequency above 100 Hz, the potential spikes merge to give a tetanus, and the unloaded muscle remains in a state of full activation while shortening at a speed which depends on the type of muscle. For fast muscles, the unloaded shortening speed v_0 is typically about 2 muscle lengths/second; for slow muscles the speed of shortening may be 4–5 times smaller. With a transducer attached, the tension rises to a steady level which is the isometric tension T_0. For undamaged frog sartorius muscle at 3 °C or rabbit psoas muscle at 12–15 °C, isometric tension is typically 2–3 $\times$ 10^5 Pascals (Newtons/m^2). However, the cross-section is not uniform; a muscle is generally thinner at the ends where it is attached to tendons. Measurements were made on intact single fibres which were electrically stimulated, or skinned fibres activated by calcium ions in Ringer's solution.

If a muscle is tetanised and forced to shorten at a fixed velocity v, the tension quickly adjusts to a steady value $T(v) < T_0$ until shortening stops, for structural reasons if no other. Conversely, if the muscle is attached to an external load $F < T_0$, it will shorten at a speed $v(F)$ which should be the solution of the equation $T(v) = F$. However, muscle stiffness, measured by applying a small high-frequency length oscillation during shortening, also falls with shortening velocity but remains finite when the tension has dropped to zero. For frog muscle, this behaviour is illustrated in Fig. 1.2. The rate of ATP consumption increases with the speed of shortening, and can rise to a factor of 3–6 times the isometric rate.

Somewhat opposite behaviour is observed in stretching. When striated muscle is stretched at constant speed, tension rises and the rate of ATP turnover falls to a very low level. A very limited amount of stretch (about 5%) is possible before the filaments move out of overlap, but by then the tension may have reached a steady-state value in excess of T_0. Thus stretching muscle acts a brake. The steady-state tension rises with the speed of stretching and saturates at somewhere between $1.2T_0$ and $2T_0$, depending on conditions (Lombardi and Piazzesi 1992). If the tensile load is increased above this saturation value, the muscle cannot hold and stretches rapidly. Figure 1.3 shows steady-state tensions for shortening and stretching.

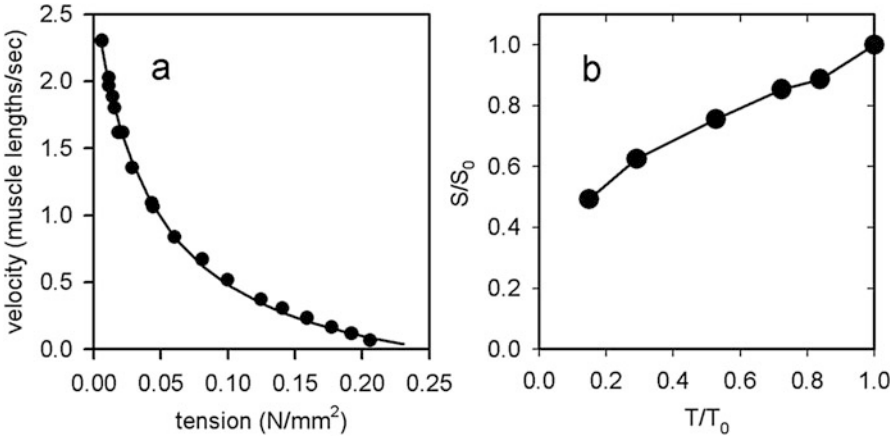

Fig. 1.2 The shortening behaviour of frog muscle at 0–3 °C: (**a**) Shortening velocity as a function of load (Edman 1979). With permission of John Wiley and Sons Inc. and K.A.P. Edman. (**b**) The high-frequency stiffness as a function of load (Piazzesi et al. 2007), normalised to isometric tension T_0 and isometric stiffness S_0. In their experiments, the averaged values were $T_0 = 240$ kPa and $S_0 = 50.3$ kPa/nm. Note that 1 N/mm^2 = 1000 kPa. With permission of Elsevier Press and G. Piazzesi

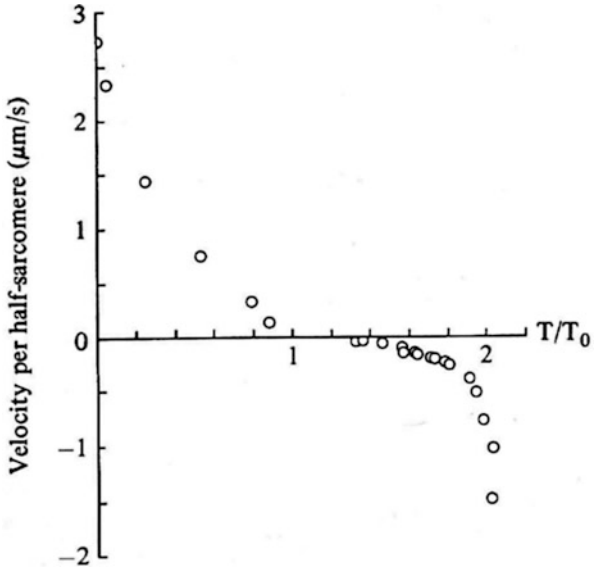

Fig. 1.3 Steady-state tension versus velocity for shortening and lengthening frog muscle on the plateau of the tension-length curve (Lombardi and Piazzesi 1992). Note the apparent change in slope at zero velocity. With permission, Cambridge University Press

This discussion shows that activated muscle is almost never in thermodynamic equilibrium, even when no mechanical work is generated. The maintenance of isometric tension is accompanied by steady heat production, which is a signature of the consumption of chemical energy. In fact the energy comes from the hydrolysis of adenosine triphosphate (ATP), which in turn is generated by glycogen from

carbohydrates. Viscous damping ensures that there is mechanical equilibrium of internal forces, but there is no chemical equilibrium. Even in an isometric tetanus, ATP hydrolysis dissipates energy at a constant rate, staying close to a steady state for a period of about 1 s, when the products of hydrolysis (ADP and inorganic phosphate) start to build up. Many mechanisms, including a build-up of lactic acid, have been proposed to explain muscle fatigue after repeated stimulation, but it now seems that the main culprit is inorganic phosphate rather than the reduction in pH (Westerblad et al. 2002). Equilibrium conditions are approached during rapid stretching, when ATP consumption is minimal, and when the ATP supply is cut off, after which rigor mortis sets in. Some tension remains in rigor, which is best likened to a glassy state in which some non-equilibrium conditions are frozen in.

1.3 The Structure of Skeletal Muscle

Skeletal muscles in vertebrates are a variety of striated muscle under the control of the nervous system. The most important structural feature is the presence of periodically repeating striations, spaced by about 2 μm, which span the cross-section. The region between adjacent striations defines the sarcomere, which can (somewhat simplistically) be considered as a one-dimensional unit cell for contractile behaviour. However, the three-dimensional structure of muscle has several layers of complexity, including the transverse tubules which distribute the action potentials arriving at nerve terminals and convert them into calcium ions, through a mechanism known as excitation-contraction coupling. Calcium ions are responsible for switching on the molecular mechanism of contraction, and the way in which this occurs is the subject of Chap. 7. A top-down description of muscle structure will be familiar to many readers, so the following presentation is intended as an outline of what has been studied in depth.

Skeletal muscle is made up of bundles of cylindrical fibres (Fig. 1.4), enclosed in a sheath (fascia) of connective tissue (collagen). Biologists identify each fibre as a multi-nucleated cell, called a myocyte, and the membranous wall of the cell is called the sarcolemma. The fibres are mainly aligned parallel to each other, although not necessarily along the axis of the muscle. However, spaces must be left for other structures, notably mitochondrial cells, fed by internal blood vessels, which maintain the supply of ATP. An individual fibre may be as small as 0.2 mm in diameter, and can with a little practice be dissected from the muscle. At each striation the transverse-tubule system invaginates the membrane and conducts depolarising axon potentials to terminal cisternae, where they are converted to calcium ions by the ryanodine receptor, which implements excitation-contraction coupling. Calcium ions are then fed to the sarcoplasmic reticulum, a network of longitudinal tubes within each striation (Peachey 1965; Ruegg 2017). The whole structure is designed to convert action potentials efficiently and quickly to calcium ions and distribute them throughout the interior of the fibre (the sarcoplasm), without having to diffuse

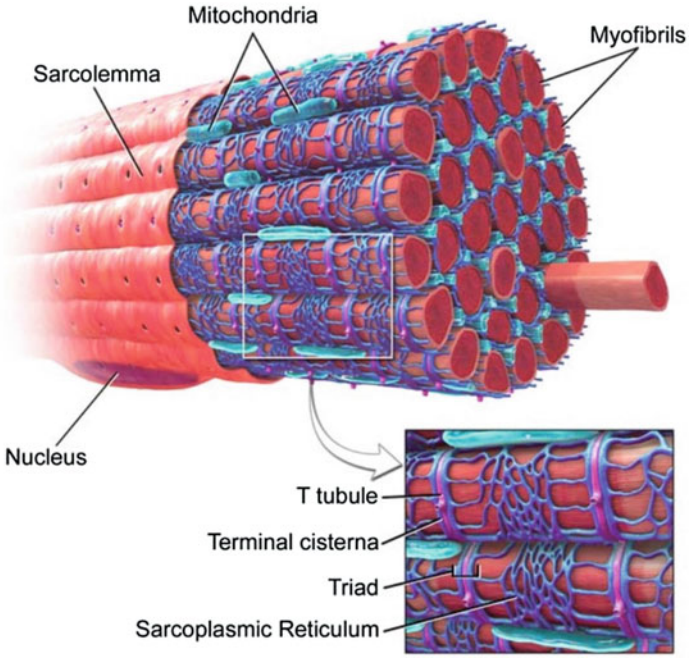

Fig. 1.4 Ultrastructure of a striated muscle fibre (diameter ~ 1 mm) and its sarcolemma membrane, with myofibrils (diameter ~ 1 μm), and the transverse tubule network. (Blausen 0801 Skeletal Muscle.png, from www.teachmeanatomy.info)

over long distances. After stimulation ceases, an ionic pump removes calcium ions to the reticulum so that the muscle can relax.

Quid pro quo, each fibre is composed of parallel bundles of myofibrils. The diameter of a myofibril is typically about 1 μm, and individual myofibrils can be isolated with a mechanical blender. Finally, each myofibril is made up of parallel myofilaments, arranged over the cross-section in a regular two-dimensional lattice. The lattice spacing is typically between 40 and 50 nm.

Looking along a single fibre reveals the periodic striations and the structure of each sarcomere (Fig. 1.1). Each striation appears as a dark region, known as the Z-line. Each sarcomere is seen to contain bundles of thick filaments in the central region, called the A-band, and interdigitating thin filaments centred on the Z-lines. Thick filaments do not extend to the Z-line, and the intervening spaces which contain only thin filaments are the I-bands, which appear much lighter in an optical microscope with phase contrast. There is also a small lighter region, the bare zone, in the centre of each sarcomere. Extraction and analysis revealed that the thick filaments are made of myosin and the thin filaments are of actin. In each half of every sarcomere, thin filaments are attached to their Z-line, a polymeric trellis which locates them in the cross-section of each myofibril and defines the lattice structure.

Over the cross-section of the whole fibre, the Z-lines may be staggered, but the Z-line is normally continuous over each myofibril unless the fibre has been damaged.

The pivotal discovery made simultaneously by Hugh Huxley and Andrew Huxley in 1953 was that the thick and thin filaments slide against each other as the length of the muscle, and hence the length of each sarcomere, is changed. As the muscle was shortened, the width of the I-bands in every sarcomere diminished but the width of each central region (the A-band) stayed constant. At a stroke, this 'Eureka moment' put paid to a century of speculation based on the hypothesis of shrinking filaments, not to mention centuries-old notions based on blood flow and the swelling of imaginary bladders. From then on, it looked as if the filaments could be regarded as rigid structures. A new era of muscle research then began in earnest, with X-ray diffraction employed by Hugh Huxley, Gerald Elliott, John Squire and others to establish the structures of the myosin and actin filament lattices. The information required was the crystal structure of the two-dimensional lattices, the lattice spacings, and the axial periodicities and structures of the filaments. The history of this period is well documented by Hitchcock-DeGregori and Irving (2014).

Meanwhile, a decade passed before the connection between sliding filaments and the isometric tension-length curve was made by Gordon et al. (1966). For a single fibre of frog sartorius muscle, the isometric tension stayed constant on a plateau for sarcomere lengths of 2.05–2.25 μm, dropping linearly as length was increased from 2.20 to zero tension at 3.65 μm (Fig. 1.5). Tension also decreased at lengths below 2.05 μm, slowly at first and then more steeply for lengths below 1.65 μm. As the filaments slide against each other, this information enables us to deduce the lengths of the thick and thin filaments (1.60 μm and 2.05 μm) and the width of the bare zone (0.2 μm). Thus maximum tension was achieved when thin filaments completely overlapped the two non-bare sections of each thick filament, and tension on the descending limb was proportional to overlap length despite the concomitant changes in lattice spacing. As is now well-known, sarcomeric tension is created by individual force-generating units (myosin crossbridges) periodically distributed along the non-bare portions of thick filaments, so that tension is proportional to the number of force-generating units in overlap with the thin fiament. Figure 1.5 also shows that the decrease in tension at sarcomere lengths below 2.05 μm is due to actin filaments poking into the other half of the sarcomere.

1.3.1 Muscle Ultrastructure

Looking inside the sarcomeres of striated muscle shows a rich structure associated with myosin and actin filaments. Some key results of two decades of X-ray and EM studies, up to but not including atomic-structure determinations of the 1990's, can be summarized as follows:

1. In many skeletal muscles, the cross-sectional lattice of myosin filaments is hexagonal, having a rhombohedral unit cell with myosin filaments at each vertex

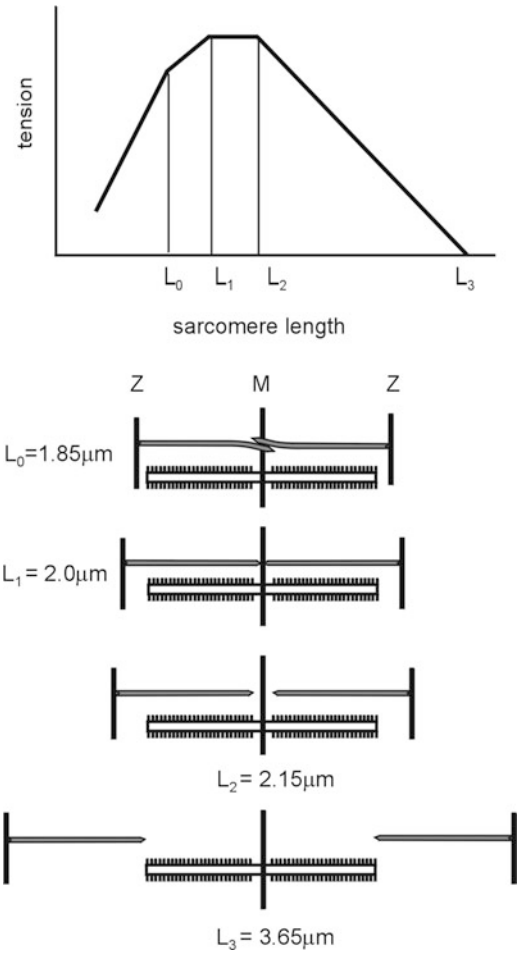

Fig. 1.5 The shape of isometric tension as a function of sarcomere length is explained by interdigitating filaments (Gordon et al. 1966). If myosin and actin filaments in each half-sarcomere have lengths L_M, L_A and the half-width of the bare zone is L_B, then $L_0 = 2(L_A - L_B)$, $L_1 = 2L_A$, $L_2 = 2(L_A + L_B)$ and $L_3 = 2(L_A + L_M)$

(Fig. 1.6). In the A-band where myosin and actin filaments overlap, each actin filament is located in the centroid of its three neighbouring myofilaments, so that there are two actin filaments per unit cell. Equatorial X-ray reflections showed that, in skinned fibres of frog semitendinosus muscle, the myosin-myosin lattice spacing d_M of the relaxed fibre was 48 nm, reducing to 44 nm when activated (Matsubara et al. 1984). However, an intact muscle maintains constant volume when activated because the sarcolemma is relatively impermeable and inelastic.

The density of filaments in a myofibril follows from the lattice spacing, which supplants older estimates based on muscle mass and molecular weights. For $d_M = 48$ nm, the area of the rhomboid unit cell is $\sqrt{3}d_M^2/2 = 2.0 \times 10^{-15}$ m^2. As there is one myofilament per unit cell, the density of myofilaments is 0.5×10^{15} per m^2. The density of actin filaments is twice that number. In a

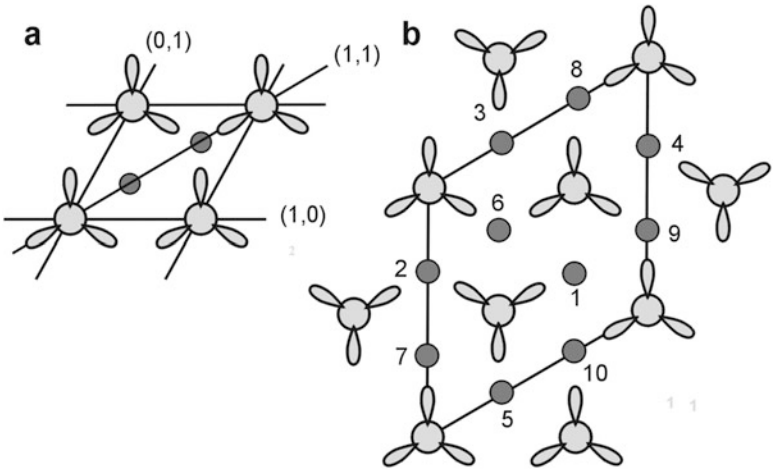

Fig. 1.6 The unit cell of muscle lattices in cross-section, with crowns of three myosin dimers on each myofilament. (**a**) The rhomboidal unit cell of the lattice of bony fish muscle, with one myofilament and two actin filaments per cell. In X-ray diffraction patterns, the (1,0) and (0,1) reflections detect myofilaments while the (1,1) reflection also includes contributions from actins. (**b**) For tetrapods, some myosins in that unit cell are oppositely oriented, giving the supercell proposed by Luther and Squire (1980)

fibre, about 85% of the cross-section is filled with myofibrils, the rest being taken up by mitochondria and other vesicles.

2. The actin filament (F-actin) is a double helix of repeating monomers of G-actin (Fig. 1.7). This double helix has a half-repeat of 36.0 nm, so that each strand has a periodicity of 72 nm. These repeats show up as layer lines on the meridian of X-ray diffraction patterns. There are 13 monomers per 72 nm repeat, so that the monomer spacing is 72/13 = 5.54 nm and the right-handed rotation per monomer is 360/13 = 27.7°. Comparing the two strands, adjacent monomers are spaced by half that distance, so the strands appear staggered by 2.77 nm. The rotation required from one monomer to its neighbour on the other strand is −166.15°, because two such rotations bring us back to 27.7°. Thus the actin double helix is actually left-handed (Fig. 1.7b). After the atomic structure of G-actin was discovered, Holmes et al. (1990) showed how these monomers could bind to generate the structure of F-actin. The outer radius is about 4.5 nm.
3. The thick filament is composed of a backbone woven with myofilaments, which project radially from the axis at intervals of 14.3 nm (Fig. 1.7c, d), giving a prominent layer-line reflection on the meridian in X-ray observations. The projecting part of each myofilament consists of a rod structure, designated S2, which is a double coiled-coil with a length of 60–100 nm. Each terminal of this double helix is flexibly joined to the C-terminal of one molecule of myosin-S1 (Fig. 1.8). Myosin-S1 (or just S1) is a large molecule with a molecular weight of 120 KiloDaltons. It has a globular motor domain of roughly 5 nm in diameter, coupled to a 10 nm-long neck made from a heavy chain of amino-acid residues. It

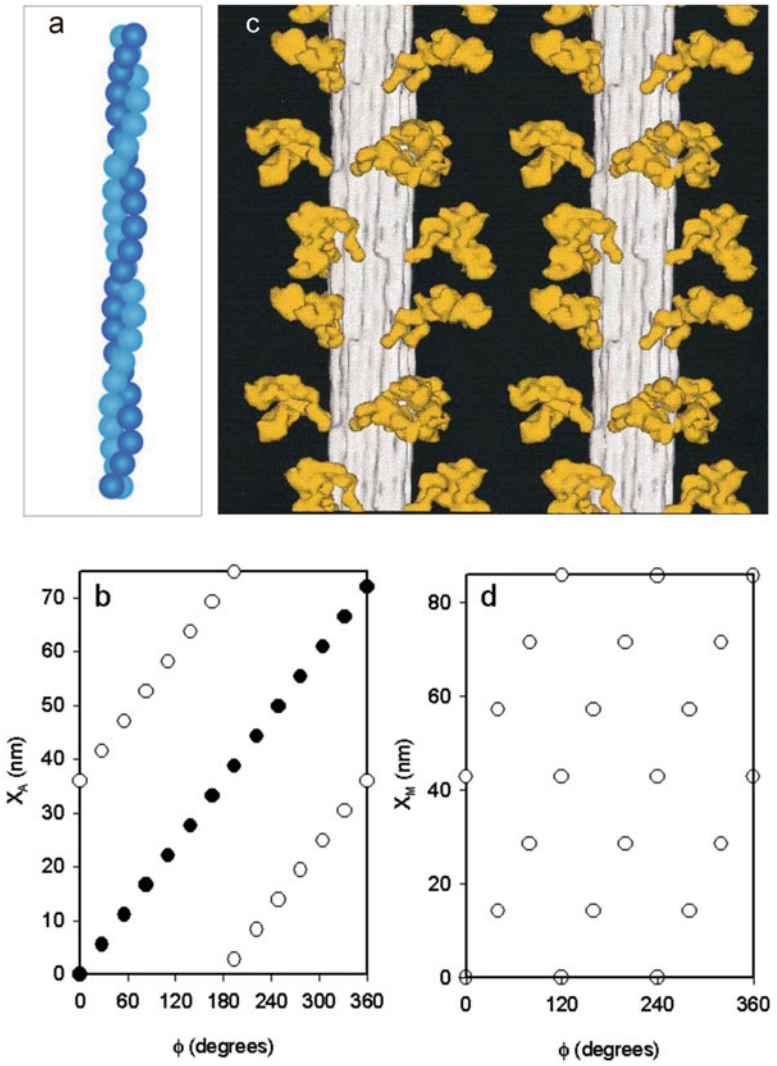

Fig. 1.7 (**a** and **b**) The double-helical structure of the actin filament, with a period of 72 nm and thirteen monomers per period on each strand. (**c**) myosin-S1 heads on the thick filament. With crowns of three dimeric myosins per layer and layers spaced by 14.3 nm (Hudson et al. 1997, with permission of Elsevier Press). (**d**) Dimers on adjacent layers are rotated by 40°, defining three helices with periods of 3 × 42.9 nm

is the distal (C-terminal) end of this neck that is joined to one terminal of S2, while the motor domain is free to bind to F-actin. In this book we refer to myosin-S1 as a head, on the understanding that this includes the motor domain and the neck.

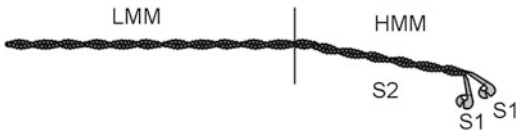

Fig. 1.8 The myosin filament (molecular weight 520,000), and its sections determined by tryptic digestion. The tail is woven into the myofilament backbone. The projecting part is lightmeromyosin (M.W. 160,000), hinge-jointed to heavymeromyosin (M.W. 340,000) which consists of the S2 rod (M.W. 100,000) and two myosin-S1 heads of M.W. 120,000, each joined to one strand of S2. All parts except S1 are α-helical coiled coils

How myosin-S1 interacts with actin to generate force and movement is the central business of theories of muscle contraction; the now-dominant explanation is that the neck acts a lever-arm. However, early studies of this interaction focussed on the radial spacing between F-actin and myosin heads. In relaxed muscle, the heads of each dimer fold up against the thick filament, either as an intimate pair (Hudson et al. 1997) or in opposite directions (Offer et al. 2000). In rigor muscle, all heads are bound to actin (Cooke and Franks 1980) and there is a jumble of orientations as all heads locate actin sites on a double helix, some with favourable axial positions or azimuthal orientations, some not. A myosin head bound to actin is often called a crossbridge. In activated muscle, not all heads are bound, and the (1,0) and (1,1) equatorial X-ray reflections provide a convenient way of monitoring how heads move radially towards actin in the presence of calcium. On going from relaxed to rigor muscle, the intensity of the (1,0) reflection, which is generated by myofilaments only, is unchanged, while that of the (1,1) reflection, which includes myosin and actin filaments, increases (Fig. 1.6a).

4. In the sarcomere, the myosin filament backbone is bipolar, and so are the actin filaments. There is a mirror symmetry between the two halves of every sarcomere, first seen by H.E. Huxley (1963) from electron-microscope (EM) studies of isolated myosin filaments as arrowheads pointing away from the central M-band in each half-sarcomere. Isolated actin filaments decorated with myosin-S1 show the same arrowhead struture (Moore et al. 1970), confirming that F-actin is also a polar filament. Actin filaments have a a barbed end (the ‘plus’ end) and a pointy end (or ‘minus’ end), and uncapped filaments polymerize more rapidly at the +end (Howard 2001). In striated muscle, the +ends are attached to Z-lines so the -ends point towards the M-band. As a contracting muscle shortens, myosin heads detach from actin and rebind to actin sites closer to the Z-line, pulling it towards the centre. Thus myosin-S1 is a +end motor. Here we are talking about muscle myosin, namely myosin-II in the phylogenetic tree. Most, but not all, myosins created by evolution are +end motors.

   This bipolarity enables us to make sense of the tension-length characteristic of isometric muscle at sarcomere lengths below the plateau (Fig. 1.5) When actin filaments are pushed into the ‘wrong’ half of the sarcomere, myosin heads must do an ‘about-turn’, so to speak, to be able to bind to actin filaments of the wrong

polarity for their preferred orientation. Consequently, their affinity for actin is reduced and this is reflected as reduced isometric tension relative to the plateau, where all heads can bind to actin sites of the right polarity. However, these heads retain the ability to hydrolyse ATP as before (Stephenson et al. 1989).

5. The azimuthal orientations of myosin heads on the thick filament has been established by a combination of X-ray diffraction and electron microscopy of thin sections (Squire 1981). A crown of three myosin dimers, attached to three S2 rods, project out from the thick filament every 14.3 nm, generating a prominent layer-line X-ray reflection. The azimuthal orientations of the three dimers on the crown are separated by 120°. Adjacent crowns along the backbone are rotated by 40°, so that the orientations of crowns are repeated with a period of $3 \times 14.3 = 42.9$ nm (Fig. 1.7). In one half-sarcomere, the overlap section of a myofilament of frog sartorius muscle has a length of 700 nm, which means that it holds $3 \times 700/14.3 = 147$ dimers.

   The starting orientations of the dimers on the first crown after the bare zone are not necessarily the same on all myofilaments. This has been studied by Luther and Squire (1980) by looking at myofilament orientations in the bare zone. In muscles of bony fish, sharks, rays and sturgeons, all myofilaments have the same starting orientation, However, in tetrapods (mammals, reptiles, birds and amphibians), the starting orientations of adjacent myofilaments in the lattice may be equal or opposite, according to the following rules: the orientations of three adjacent myofilaments on a triangle cannot be the same, and neither can the orientations of three adjacent colinear myofilaments (Luther 2004). This has the effect of creating a superlattice whose unit cell is three times the area of the rhombohedral cell (Fig. 1.6b).

   Actin filaments also have starting orientations. Considering that the orientations of myosin heads at the pointy end of F-actin varies with sarcomere length, it probably doesn't matter very much what those orientations are. Nevertheless, Hirose and Wakabayashi (1988) have shown that they are the same for all F-actins in the lattice.

6. Myofilaments and F-actins are not the only filaments in the sarcomere. In each half-sarcomere there are extensible filaments that connect the M-band to the Z-line and stop the sarcomeres from disassembling when stretched beyond the point of zero overlap. These are titin filaments; titin is a giant polymeric molecule made of repeating units (either PVEK or fibronectin) connected by elastic links. Their tension-length curve shows hysteresis; under tension these links may unwind and then wind up again, so that a fibril stretched beyond overlap will contract in discrete steps (Miklos et al. 1997; Rief et al. 1997; Tskhovrebova et al. 1997). Titin filaments are responsible for the tension-length characteristic of relaxed muscle, which in skeletal muscle appears as an exponential rise starting near the bottom of the descending limb.

   While titin filaments, and perhaps nebulin filaments also, are responsible for maintaining the integrity of sarcomeres pulled beyond zero filament overlap, the M-band and Z-line structures play a role in stabilising the lattice spacing of relaxed muscle in the zero-overlap region. However, under physiological