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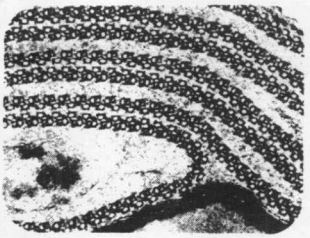

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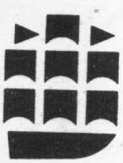

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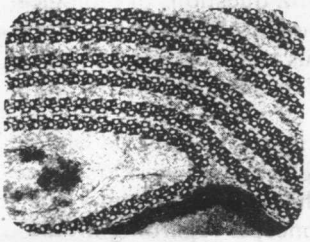


Preface

Since the introduction of the earliest microscopes, biologists have been fascinated by the movement of cells. The frenetic swimming motions of spermatozoa, the streaming of plant cell protoplasm and the peripatetic gliding movements of amoebae were all extensively documented long before the turn of the century. Despite the limitations imposed by the resolution of their instruments, many of the observations have proved extremely accurate and moreover were interpreted with considerable insight, so much so, that they have often provided the basis for subsequent studies.

With the advent of the electron microscope a new dimension to the study of cell motility was introduced. Now it became possible, for the first time, to look inside the cell and to identify structures associated with cell movements. Initially progress was steady rather than spectacular; many problems relating to fixation and specimen preservation had to be overcome. However, a picture gradually emerged in which two classes of minute fibrous structures, referred to as *microtubules* and *microfilaments*, were almost invariably found in the region of the cell in which movement occurred. Concurrently with this increase of information regarding cell ultrastructure, a rapid improvement in the technology of cell fractionation and purification laid cell motility open to the biochemists, and our understanding of its molecular basis rapidly advanced.

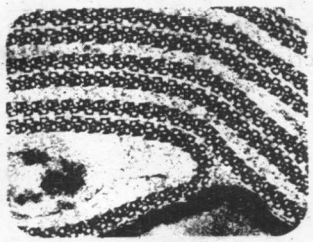
It is now clear that there are many more compelling reasons to study cell movement than the natural beauty and fascination which attracted the first microscopists. Such problems as sperm function and fertility, the development and function of the nervous system and the invasive

nature of cancer cells are three of the central questions of biological and medical sciences which fall within its compass. For this reason cell motility has in recent years seen an enormous accumulation of interest, to the extent that it is without doubt one of the most productive areas of modern cell biology.

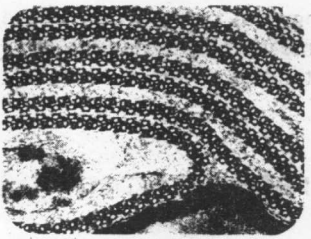
Our intention in producing this text is to provide students with an introduction to many of the problems which concern workers in the area of cell motility, to describe some of the systems currently being investigated, and to discuss the various concepts regarding motile mechanisms which have so far emerged.

We are indebted to all the authors who have generously supplied illustrations which are so essential for a text of this nature.

H. Stebbings and J. S. Hyams
Exeter, Devon. 1978



Contents



Chapter 1

Structure, biochemistry and function of striated muscle

At first glance it may seem incongruous to begin a volume devoted to the movement of non-muscle cells in its many guises with a consideration of the mechanism of muscle contraction. Virtually all the examples of cell motility which we shall consider in this book, however, share one common feature, namely they require the conversion of chemical energy, synthesised by metabolic activity and stored primarily in the form of adenosine triphosphate (ATP), into mechanical work. In striated muscle this transduction of chemical energy into force production has evolved to a staggering efficiency. The flight muscles of insects, for example, are capable of producing a power output comparable to that of certain internal combustion engines and can exert a tension equivalent to several kilograms for each square centimetre of their cross-sectional area. Increasingly, however, the organisation and function of striated muscle have come to be regarded as highly specialised manifestations of a property which is fundamental to many other less specialised cells. Hence, although the force generation required to propel an amoeba on its gliding meanderings across its substrate, or to constrict an animal cell at cytokinesis, is infinitesimal when compared to the violent contraction of muscle fibres, the proteins involved and the mechanisms by which they interact may be analogous. The arguments for and against this view are considered in some detail in subsequent chapters. What is without doubt is that our current understanding of cell motility in its many and diverse forms has been profoundly influenced and perhaps even biased by the classical and elegant studies of muscle contraction.

In the following sections, we make no attempt to provide a

comprehensive review of muscle physiology but rather to introduce concepts and principles which, as will become increasingly apparent in later chapters, are vital to a clear understanding of the motility of non-muscle cells.

1.1 Structure of vertebrate striated muscle

Vertebrate striated muscle has been much more extensively studied than other muscle types, largely because the regularity of its structure makes it particularly suitable for ultrastructural investigation, but also, since it comprises the large lifting and running muscles of vertebrates, it is available in large quantities for biochemical studies. Striated skeletal muscle consists of three distinct components:

1. Multinucleate cylindrical myofibres which are the contractile machinery of the muscle. (These range from 10–100 μm in diameter and extend up to several centimetres in length.)
2. Mitochondria which produce ATP, the fuel for contraction.
3. The sarcotubular system which is a system of membrane-bound channels thought to coordinate the contraction of the fibres.

See Fig. 1.1.

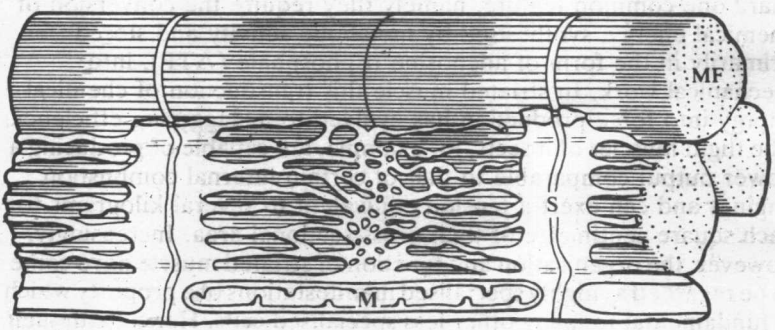


Fig. 1.1 Diagram illustrating the essential elements of striated skeletal muscle. The diagram shows the arrangement of the banded myofibrils (MF), mitochondria (M) and sarcotubular system (S).

Myofibres in turn are composed of a system of elongated elements 1–2 μm in diameter and called *myofibrils*. These are the basic contractile units of the muscle fibre and the term 'striated' derives from the in register periodic repeats which they exhibit along their length. Examination of striated muscle by light microscopy reveals that each myofibril is subdivided into repeating units known as *sarcomeres*,

which are separated by regularly spaced dense Z lines (from German *zwichen* = between). The myofibrils also show a regular pattern of banding which is seen particularly clearly using polarising microscopy. At either end of a sarcomere is a less dense region forming the I band (as it appears isotropic) and between these is the A band (anisotropic) which has greater density except for a central H zone (from German *hell* = clear).

Electron microscopy has shown that the basis of the banding lies in a highly ordered arrangement of myofilaments within each sarcomere (Fig. 1.2). The filaments are of two kinds – thick (10–12 nm in

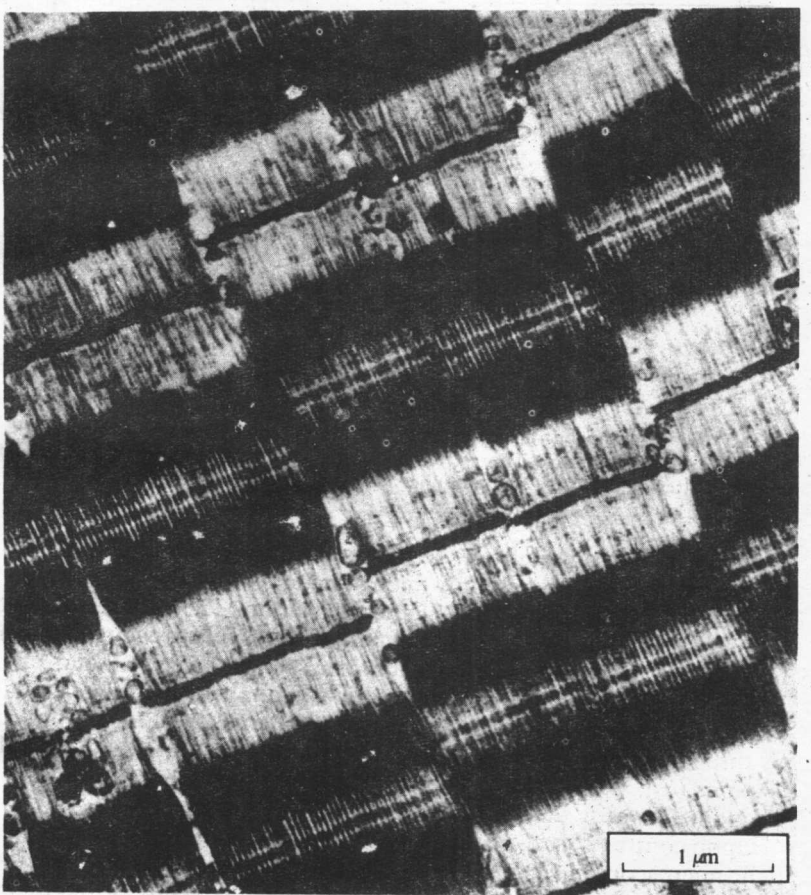


Fig. 1.2 Longitudinal section of a number of myofibrils showing the distinct sarcomeric structure. (Courtesy of Dr H. E. Huxley.)

diameter) and thin (5–8 nm), the banding resulting from the degree of overlap of the in register filaments (Fig. 1.3).

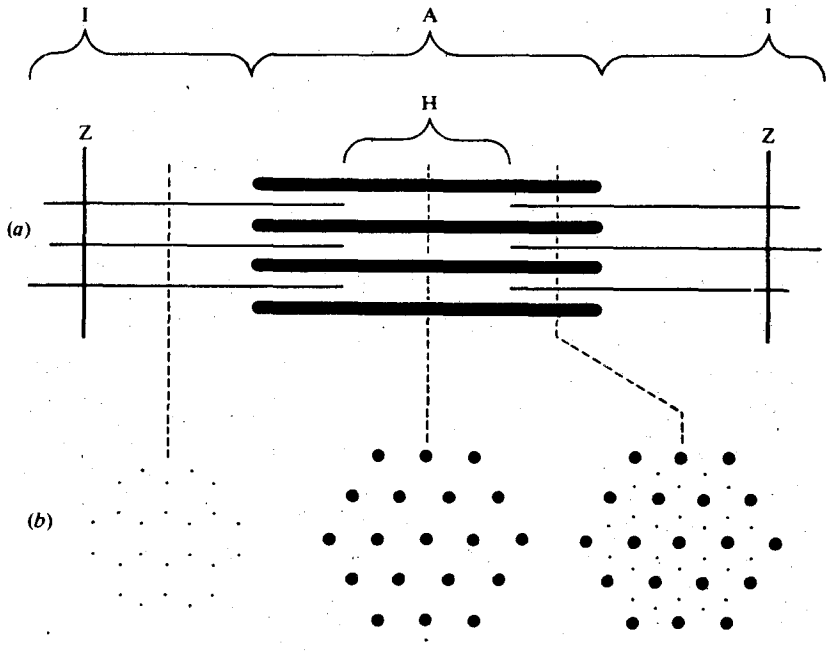


Fig. 1.3 Diagram showing the arrangement of thin and thick filaments within a single sarcomere of striated muscle. The overlapping of filaments gives rise to the characteristic banding pattern and can be seen in either longitudinal (*a*) or transverse (*b*) section.

Hence, as we can see from the figure, the I bands contain only thin filaments, and the H bands only thick filaments, while the A bands are regions where thick and thin filaments overlap. The geometric arrangement of the two types of filament with respect to each other can be discerned most clearly from transverse sections (Figs. 1.3 and 1.4). These show that each thick filament is surrounded by an hexagonal array of thin filaments and that each thin filament is therefore bounded by three thick ones. Furthermore, in both transverse and longitudinal sections (Fig. 1.5), bridges can be seen extending from the thick filaments towards the thin filaments.

Upon contraction, the organisation of the sarcomere as seen in the light microscope undergoes pronounced changes. While the A band remains constant the I band is reduced proportionately to the degree of shortening. As we shall see in more detail later, these observations

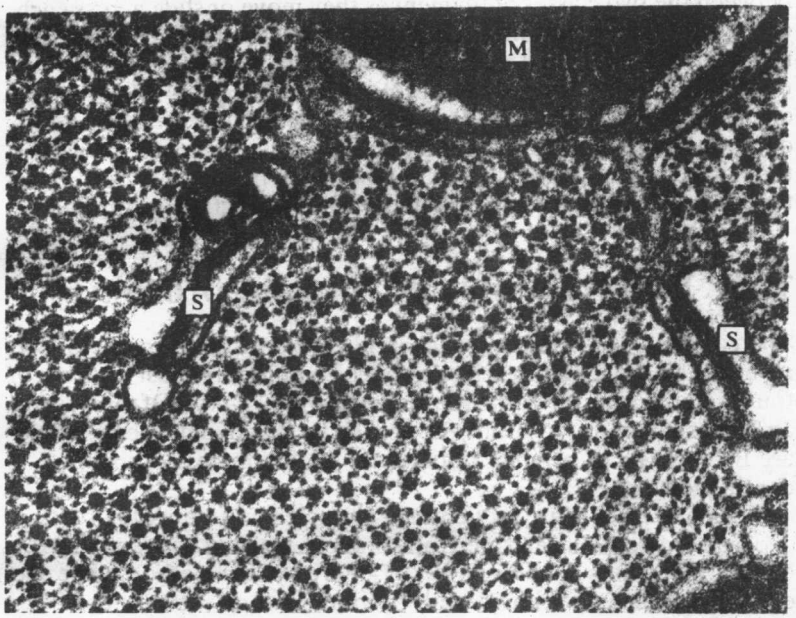


Fig. 1.4 Transverse section of a myofibril showing the hexagonal packing of thin and thick filaments. (M) mitochondrion; (S) sarcotubular system.

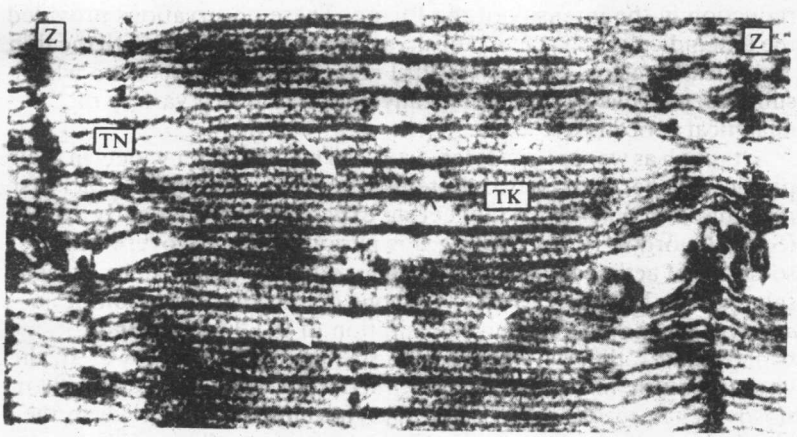


Fig. 1.5 High magnification of a single sarcomere. Bridges can be clearly seen extending between the thick (TK) and the thin (TN) filaments (arrows). (Courtesy of Dr H. E. Huxley.)

have been interpreted as reflecting alterations in the degree of overlap between the two classes of filament as they move or slide across each other while undergoing no change in length themselves. If correct, this mechanism implies that contraction of the muscle fibre can be understood in terms of the molecules which comprise the structural elements of the sarcomere. It is therefore pertinent to ask several questions: what are the thick and thin filaments composed of? what is the nature of the bridges extending between them? how do the two types of filament interact? and how is the interaction controlled?

1.2 Biochemistry of muscle proteins

The complex, three-dimensional architecture of the sarcomere revealed by the electron microscope is composed almost entirely of four proteins, *actin*, *myosin*, *tropomyosin* and *troponin*. Of these, actin and myosin account for some 80% of the total and on this basis alone are likely to be of primary importance to the process of muscle contraction.

Actin and myosin are sequentially extracted when muscle fibres are exposed to fairly concentrated salt solutions, usually between 0.3–0.6 M KCl. Myosin is solubilised very rapidly by this method while the extraction of actin is achieved only by more prolonged treatment. The removal of the two proteins is accompanied by characteristic changes in the pattern of striations of the sarcomere seen in the light microscope. Separation of myosin results in the disappearance of the A band while the displacement of actin is contiguous with a marked reduction in the intensity of the I band. These observations provided the first indication that actin and myosin could be equated with the thin and thick filaments of the myofibril respectively, a conclusion which subsequently has been emphatically confirmed by a variety of analytical procedures.

Although as we shall see later, actin and myosin differ widely in their physical properties, the two proteins interact very strongly, a fact first recognised in a series of pioneering experiments in the early 1940s (Szent-Györgyi, 1951). During this period it was discovered that solutions of actin and myosin, mixed together in the presence of high salt, rapidly became extremely viscous due to the formation of an *actomyosin* complex. Upon the addition of ATP (adenosine triphosphate), the fuel by which muscle contraction is powered, the viscosity of the mixture at first declined to its original level but then slowly began to rise again. ATP therefore appeared to reverse the physical association between actin and myosin while itself being consumed by the reaction. The obvious inference of such a finding is that the actomyosin mixture possessed *ATPase activity*, that is it

contained an enzyme capable of cleaving the terminal phosphate group of ATP and hence liberating energy for mechanical work.

With increasing sophistication in the technology of preparing the muscle proteins it was clearly established that this enzymatic activity was an integral property of the myosin molecule. However, the rate at which myosin catalysed the hydrolysis of ATP in the presence of magnesium, the most abundant sarcoplasmic ion, was found to be extremely slow, some hundredfold less than other enzymes of this type and therefore far too inefficient to account for the rapid consumption of ATP during muscle activity. At least a partial resolution of this dilemma was provided by the key discovery that in the presence of actin, myosin ATPase was dramatically stimulated, sufficient to now suggest that the hydrolysis of ATP by myosin, *in association with actin*, is the force-generating reaction of contraction.

Further evidence for this possibility was obtained experimentally by Szent-Györgyi in a very direct way. By extruding actomyosin solutions from a fine capillary into dilute salt solutions he formed slender, filamentous precipitates which he called 'threads'. Incredibly, upon the addition of ATP, these actomyosin threads were seen to contract, or in other words, mixtures of purified actin and myosin were capable of generating movements reminiscent of the muscle fibre from which they were derived.

That the properties of the actomyosin threads corresponded in some meaningful way to the situation in the muscle fibre was clearly suggested by the behaviour of glycerinated myofibrils. Exposure of isolated myofibrils for several days at 0°C to an aqueous 50% glycerol solution resulted in the extraction of the surrounding membrane and all soluble proteins, leaving behind essentially a cytoskeletal framework of actin and myosin molecules. As with the actomyosin threads, these glycerinated 'models' also contracted upon the addition of ATP. Both of these artificial preparations, however, differed from living muscle in one important aspect, namely, that once contracted they were not capable of subsequent relaxation. Both therefore can be regarded as being in *rigor*, a condition associated with muscle in death (hence rigor mortis) where the loss of ATP causes the fibres to become rigid and inextensible.

Relaxation was shown by Ebashi in the 1960s to depend upon the introduction of yet another factor, extracted from muscle by treatment with dilute salt solutions. This fraction, which had first been isolated twenty years previously and designated 'native tropomyosin', allowed the contracted system to relax but only if the level of calcium in the reaction solution was lowered to below 10^{-6} M. At the same time the presence of native tropomyosin inhibited the syneresis, and hence the ATPase activity, of actomyosin unless the calcium ion concentration was once again raised above 10^{-5} M. In the jargon of the biochemist,

native tropomyosin is therefore said to confer *calcium sensitivity* to the actin–myosin–ATP interaction. Hence contraction proceeds at elevated levels of calcium while relaxation requires the level of calcium ions to be lowered, the transition being under the direct influence of native tropomyosin. Subsequently it has been shown that native tropomyosin is in fact a complex of two proteins, tropomyosin and troponin, and the role of these molecules in the regulation of muscle contraction *in vivo* has been amply confirmed.

1.2.1 Thick filament

The thick filament of the myofibril is composed exclusively of a single protein, myosin, which comprises some 50% of the total structural protein of striated muscle. Myosin is a complicated molecule, sufficiently large to be visualised in the electron microscope where it is seen to consist of a long rod-like tail and a bilobed globular head (Fig. 1.6). The size of the molecule is reflected in its enormous molecular weight of 470,000 daltons. This is composed of two heavy chains each of approximately 200,000 daltons plus four light chains, each around 20,000 daltons, although the precise details may vary among different types of muscle. The heavy chains constitute the helical tail of the molecule together with most of the globular head region while the light chains are confined exclusively to the head.

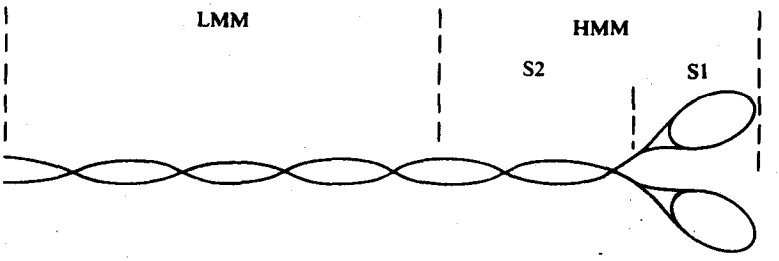


Fig. 1.6 Diagram of a myosin molecule showing the position of enzymatic cleavage into light meromyosin (LMM) and heavy meromyosin (HMM) and the subfragments of HMM, S1 and S2.

Treatment of myosin with the proteolytic enzyme trypsin, digests the molecule into two fragments. *Heavy meromyosin* or HMM consists of the two globular heads plus about a third of the tail portion and contains all the ATPase activity of the protein. *Light meromyosin* or LMM is an α helical, rod-shaped molecule consisting of the rest of the myosin tail. HMM may be further cleaved either by extended trypsin treatment or by another protease, papain, into two smaller fragments designated S1 and S2 (for subfragment 1 and 2; Fig. 1.6). S1 consists of just the head of the myosin molecule while S2 is the small portion of

the tail associated with HMM. Enzymatic activity has been found to be associated only with S1 and this has led to the hypothesis that in the thick filament the myosin molecules are organised in such a way that this portion of the molecule projects towards the thin filament with which it undergoes cycles of attachment and detachment, causing the two filaments to slide over one another (Section 1.3). S2 has different chemical properties from the rest of the myosin tail (LMM) and probably is also important in this cross-bridge reaction, possibly acting as a swivel upon which the S1 component pivots.

The possible involvement of S1 in a reversible interaction with the thin filament has been emphatically and elegantly demonstrated by Huxley who showed that either HMM or S1 would interact with purified actin filaments *in vitro* to form a highly specific and characteristic *arrowhead* complex which is clearly revealed by negative staining (Fig. 1.7) and results from the attachment of the myosin

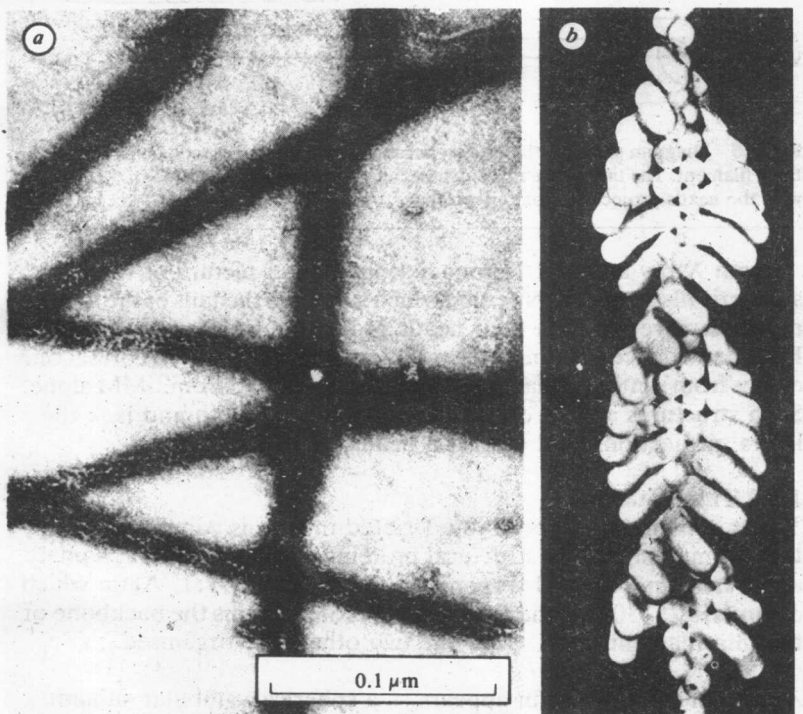


Fig. 1.7 Decoration of actin filaments with HMM. (*a*) negatively stained preparation showing the arrowhead complexes and (*b*) a three-dimensional model showing the attachment of the HMM to the actin helix. ((a) Courtesy of Dr H. E. Huxley; (b) from H. E. Huxley, *Proc. Roy. Inst. Gr. Br.*, **44**, 274 (1970).)

fragment at a periodicity of 36 nm. More importantly, in the presence of ATP, which causes the detachment of cross-bridges during muscular contraction, the binding of the arrowheads was reversed. So specific is the arrowhead decoration of actin filaments by HMM or S1 that this technique has become widely used as a cytological probe for the identification of actin filaments (microfilaments) in a wide variety of non-muscle cells.

Under physiological conditions, myosin molecules will spontaneously assemble into bipolar aggregates resembling the thick filaments of the myofibril (Fig. 1.8). These consist of a bare central shaft, 150 nm long by 2–3 nm in diameter, with projections containing the ATPase activity and actin binding sites at each end.

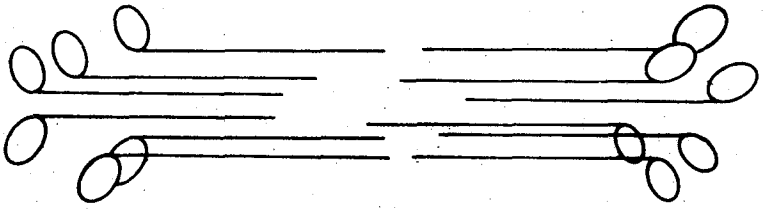


Fig. 1.8 Diagram showing the bipolar arrangement of myosin molecules within the thick filament. The tails of the myosin molecules make up the backbone of the filament with the heads projecting from either end.

From X-ray and electron microscope data a picture of these synthetic filaments has emerged which supposes the tails of the myosin molecules overlap with their heads projecting from opposite ends. Further evidence that this interpretation may in fact be the correct one comes from synthetic filaments similarly assembled from LMM alone. Such structures are, as expected, completely smooth and lack the lateral projections of the myosin heads.

1.2.2 Thin filament

The thin filament of vertebrate striated muscle is composed of the three remaining major structural proteins of the sarcomere, namely, actin, tropomyosin and troponin in the ratio of 7:1:1. Actin which comprises 25–30% of the total muscle protein forms the backbone of the thin filament upon which the two others are organised.

Actin In solution, actin appears as a spherical, globular subunit approximately 5.5 nm in diameter. The protein is composed of a single polypeptide chain of 376 amino acids whose sequence has been completely determined (Elzinga and Collins, 1973) and from which a