Muscle Contraction

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Whilst muscular contraction as a general phenomenon is familiar through everyday experience, relatively few people are aware that it depends on complex interactions between highly ordered polymers of very large molecules (proteins) organized into regular arrays of filaments which slide past each other. It may also come as a surprise to many that the detailed molecular changes that occur during contraction have still not been fully elucidated, although a great deal is now known about many aspects of the process.

All muscles are believed to contract by the same basic mechanism, which may also be involved in movement, shape change and internal transport in non-muscle cells. The muscles which have been studied most extensively are the voluntary, skeletal muscles of vertebrates. These can generate forces up to 3 kg/cm^2 of their cross-section, can shorten by up to a third of their length or more at velocities equivalent to several times their own length per second, and can be switched fully on or fully off in a small fraction of a second, or even within a few milliseconds in some cases. Frogs have been a favourite source of preparations for physiologists, whilst rabbits and chickens have provided most of the material for biochemists.

Studies by X-ray diffraction and electron-microscopy have shown that in these muscles the two principal protein molecules involved in contraction, actin and myosin, are ordered into separate but overlapping arrays of filaments of fixed length (Fig. 1). The myosin filaments form a hexagonal array about 400 Å apart, and where overlap occurs, the actin filaments are located symmetrically between each set of three neighbouring myosin filaments. The space between the filaments is occupied by a dilute solution of salts, metabolites and some soluble proteins. Filaments in each array are held in register by cross-connecting structures at their mid-points, which form lateral bands known as Z-lines (for the actin filaments) and M-lines (for the myosin filaments). The myosin filaments are about 1.5 μm long and have a diameter of 100-150 Å, while the actin filaments are somewhat less than 100 Å in diameter and extend about 1 μm on either side of the Z-lines. Thus although the filaments themselves are too thin to be resolved in the light microscope, the arrays that they form are readily visible, and the pattern resulting from their partial overlap gives the muscle its characteristic striated appearance which also shows up particularly well in electron-micrographs (Fig. 2).

Changes in the striation pattern during contraction, and the behaviour of the filaments themselves have shown that active shortening of the muscle is brought about by a process in which the actin and myosin filaments slide past each other at essentially constant length. The sliding force is believed to be generated by the action of so-called "crossbridges", which project out sideways from the backbone of the myosin filaments and make repetitive attachments to the actin filaments. The crossbridges represent the enzymatically active part of the myosin molecules, *i.e.* the part that catalyses the chemical reaction (hydrolysis of adenosine triphosphate-(ATP)) which provides the energy for contraction. Another part of each myosin molecule is built into the backbone of the thick filaments, holding the inner ends of the crossbridges in place. Each myosin molecule has a molecular weight of about 500000, and the crossbridges occur in groups of three at intervals of about 143 Å along the thick filaments.

A plausible model for the contractile process supposes that upon activation of the muscle (when the arrival of nerve impulses leads to the rapid release of calcium ions throughout the fibres) a crossbridge attaches to actin, undergoes some structural rearrangement so that it pulls the actin filament along in the appropriate direction for a distance of the order of 40-120 Å, and then releases again, returning to its original configuration so that it is ready to begin another cycle. Similar cycles of action are taking place asynchronously at all the other crossbridges within the region of overlap of the actin and myosin filaments and their combined action results in the steady force and shortening generated by the muscle as a whole. When activation ceases, the crossbridges all detach from actin and the muscle relaxes; it can be stretched out to its original length by a very small force.

This general model accounts very well for virtually all the characteristic properties of muscle and also seems to be perfectly feasible in terms of the properties of the constituent protein molecules. The wide range of characteristic shortening velocities of different types of muscle can be correlated with different cycling rates of the enzymatic cycle, and the fact that most muscles develop very similar maximum tensions (at zero shortening velocity) follows from the fact that sarcomere structures are virtually identical so that similar numbers of crossbridges will contribute their tensions in parallel. (The maximum force generated by each crossbridge will depend on the energy derived from splitting one molecule of ATP and the length of the force-generating stroke; it is not likely to be a sharp function of the maximum possible enzymatic cycling rate in that species of muscle.)

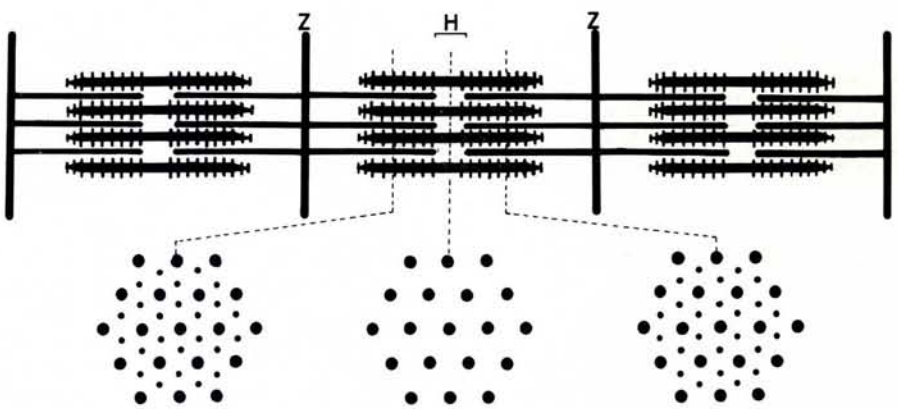

Fig. 1 — Diagrammatic representation of the structure of a myofibril in striated muscle, showing overlapping arrays of actin- and myosin- containing filaments. For convenience of representation, the structure is presented with considerable longitudinal foreshortening: with the filament diameters and side-spacings as shown, the filament lengths should be about five times longer than in the diagram.

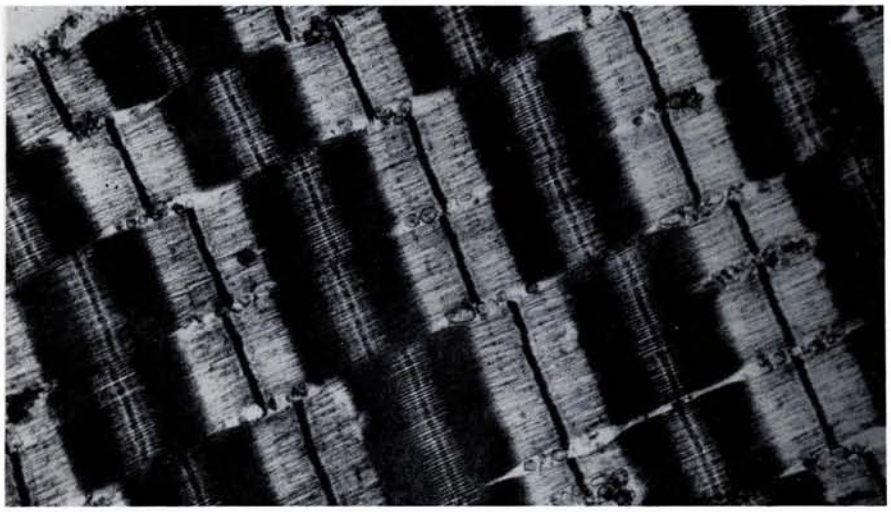

Fig. 2 — Electron micrographs of a longitudinal section of vertebrate striated muscle (from rabbit). Within the repeating pattern of sarcomeres, one can see the denser A-bands formed from the in-register arrays of myosin filaments, partially overlapped by the arrays of actin filaments whose ends are attached to the narrow dense structures (Z-lines). Between the Z-lines and the ends of the A-bands the actin filaments form the lighter bands of the sarcomere (see Fig. 1).

The central problem in understanding the contraction mechanism has been, for some years now, to establish whether in fact the crossbridges do undergo the kind of cyclical behaviour which has been postulated, and to elucidate the details of the structural and biochemical changes taking place within them if indeed this general type of model is correct. Unless such direct experimental evidence is available, it always remains possible in principle for a different mechanism to exist.

Structural evidence about crossbridge behaviour during contraction has been obtained from low angle X-ray diffraction diagrams from muscle (Fig. 3). These arise from the very regular arrangement of the actin and myosin filaments in a hexagonal lattice in the overlap region and from the regular arrangement of the protein molecules within their respective filaments (Fig. 4). The patterns are relatively weak when obtained using normal laboratory X-ray tubes, but the recent development of facilities where the intense synchrotron radiation from electron storage rings can be used as an X-ray source has increased the available flux by a factor of 1000 or more and has made it possible, using electronic X-ray detectors, to carry out time resolved X-ray diffraction measurements during different phases of muscle activity.

These show substantial changes in the equatorial pattern, taking place slightly ahead of tension development and reversing during relaxation, which could be accounted for if the crossbridges hinged outwards from the myosin filament backbone and became closely applied to the actin filaments when the muscle was in the active state. Changes in other parts of the X-ray pattern, and the behaviour of the reflections when rapid mechanical transients (e.g. small sudden length changes) are applied to a contracting muscle, support the general idea of a repetitive attachment-detachment crossbridge cycle taking place in a contracting muscle, with a crossbridge remaining attached to a particular site on an actin filament as the filament slides along past the myosin filament for about 100 Å. The crossbridge then rapidly detaches and reattaches again, further out along the actin filament.

However, what the X-ray results have not been able to do so far is to provide a definite model for the structural behaviour of the actin-crossbridge complex which enables the myosin head to remain attached to the actin over the 100 Å sliding distance, and which allows an active force to be developed over some part at least of the range of attachment. Moreover, no other experimental technique has so far provided the necessary information. This is in considerable part because of the technical difficulty of extracting information about dynamic behaviour involving complicated submicroscopic structural changes, some of which may be very rapid (i.e. taking place in $< 10^{-3}$ s). Although we understand many basic phenomena, the actual physical details of the various mechanisms involved are in most cases quite unknown, and this ignorance is a particularly serious drawback when the essence of the process is a structural change rather than a particular combination of chemical events. Whereas there is a good deal of evidence for flexibility and relative movement in several parts of the molecules involved in energy transduction in muscle, we have no clear picture at present of how the ensemble of molecules and their interactions will be affected by Brownian motion and by the electrical charges that they carry, and how these effects will overlay the production of directed force and movement. We are dealing with physical and chemical phenomena at levels of size and of energies at which the basic laws of classical physics will in general apply but where the actual behaviour of such systems is not known properly either experimentally or theoretically.

Thus the study of such a system as muscle, which despite the inherent difficulties I have mentioned does offer many experimental advantages, is particularly fascinating at the present time, and many very interesting techniques are being applied to learn more about it. Some of these involve the measurement of relatively simple parameters such as the tension generated by a muscle, but using preparations in which the fluid medium surrounding the filaments in a normal intact muscle is replaced by one whose composition can be varied. In some recent experiments this has been done using an intense laser flash to release ATP photochemically from an inactive precursor, so that the whole population of crossbridges can be activated almost simultaneously. In other experiments electron paramagnetic re-

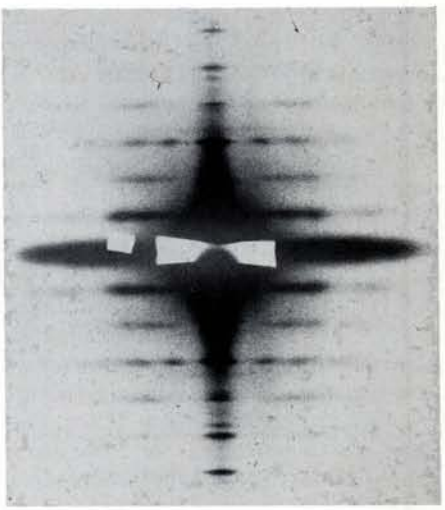

Fig. 3 — Low-angle X-ray diffraction diagram from live frog sartorius muscle, showing pattern of layer-lines corresponding to 429 Å helical repeat of myosin crossbridges on thick filaments. The sampling of the pattern produced by the lateral register of crossbridges in the filament array can be seen, together with prominent 143 Å meridional reflection from the axial repeat of groups of crossbridges.

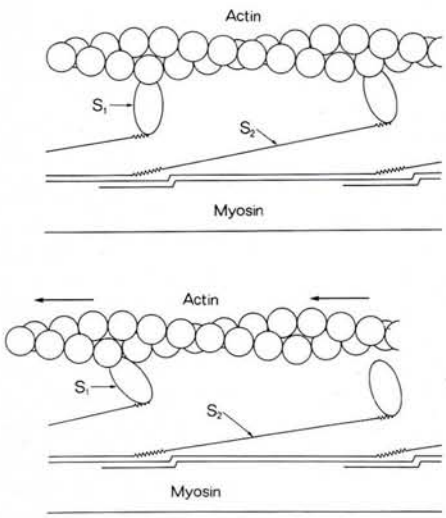

Fig. 4 — Diagrammatic representation of some aspects of filament and crossbridge structure in muscle. The globular heads (S_1) of the myosin molecules are attached to the backbone of the myosin filaments by α-helical rod portions of the molecule (S_2), allowing interaction with actin to take place over a range of sidespacings and of positions on the actin helix. A possible mechanism for contraction involves changes in shape or angle of attachment of the myosin heads, coupled to repetitive cycles of attachment to and detachment from actin, so that progressive relative sliding movement of the actin filaments takes place.

sonance measurements are used to follow the orientation and mobility of spin labels attached to specific sites on the myosin head. Nuclear magnetic resonance experiments have detected particularly mobile regions in some of the muscle proteins and the technique is being used to explore their behaviour during ATP hydrolysis by the actin-myosin complex and to attempt to identify some of the amino-acids involved. Attempts are also being made to freeze contracting muscles within a millisecond or less by slamming them against liquid helium cooled copper blocks, and then examine their crossbridge structure by electron microscopy.

Another recent development is the direct visualization in the light microscope of the movement of tiny plastic beads (as small as 0.5 μm in diameter), to which myosin molecules have been attached, along oriented bundles of actin filaments in the presence of ATP. This preparation is the synthetic analogue of a type of movement used to transport organelles in a number of cells and provides a particularly clear demonstration of sliding movement as the basic interaction between myosin and actin even in the absence of an organized sarcomere structure. The same sliding principle has also been demonstrated in motility phenomena involving another type of cell organelle, the microtubules, which are capable of effecting movement of cell organelles over long distances (*e.g.* down nerve fibres) as well as participating in the relative sliding movement between neighbours which gives rise to ciliary motion.

Thus the study of muscle contraction provides an entry not only to a fascinating new range of physical phenomena involved in the biochemical energy transduction processes, but also helps to advance our understanding of the movements within cells, and between one cell and another, which are equally necessary for life.

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