Thick Filaments Consist of Myosin

The major protein components of striated muscle are listed in Table 34-4. Vertebrate thick filaments are composed almost entirely of a single type of protein, myosin, which occurs in virtually every vertebrate cell. Myosin molecules consist of six highly conserved polypeptide chains: two 220-kD heavy chains and two pairs of different light chains, the so called essential and regulatory light chains (ELC and RLC), that vary in size between 15 and 22 kD depending on their source. Myosin is an unusual protein in that it has both fibrous and globular properties (Fig. 34-50). The N-terminal half of its heavy chain folds into an elongated globular head, around $55 \times 200$ Å, whereas its C-terminal half forms a long fibrous $\alpha$-helical tail. Two of these $\alpha$-helical tails associate to form a left-handed parallel coiled coil.

![Diagram of muscle structure](image1)

**FIGURE 34-48.** An electron micrograph of parts of three myofibrils in longitudinal section. The myofibrils are separated by horizontal gaps. A myofibril's major features, as indicated in the accompanying interpretive drawings, are the light I band, which contains only hexagonally arranged thin filaments; the A band, whose dark H zone contains only hexagonally packed thick filaments, and whose even darker outer segments contain overlapping thick and thin filaments; the Z disk, to which the thin filaments are anchored; and the M disk, which arises from a bulge at the center of each thick filament. The myofibril's functional unit, the sarcomere, is the region between two successive Z disks. [Courtesy of Hugh Huxley, Brandeis University.]

**TABLE 34-4. PROTEINS OF STRIATED MUSCLE**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Mass (kD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin</td>
<td>540</td>
</tr>
<tr>
<td>Heavy chain</td>
<td>230</td>
</tr>
<tr>
<td>Essential light chain (ELC)</td>
<td>$\approx 20$</td>
</tr>
<tr>
<td>Regulatory light chain (RLC)</td>
<td>$\approx 20$</td>
</tr>
<tr>
<td>G-Actin</td>
<td>42</td>
</tr>
<tr>
<td>Tropomyosin</td>
<td>33</td>
</tr>
<tr>
<td>Troponin</td>
<td>72</td>
</tr>
<tr>
<td>TnC</td>
<td>18</td>
</tr>
<tr>
<td>TnI</td>
<td>23</td>
</tr>
<tr>
<td>TnT</td>
<td>31</td>
</tr>
<tr>
<td>$\alpha$-Actinin</td>
<td>200</td>
</tr>
<tr>
<td>Desmin</td>
<td>50</td>
</tr>
<tr>
<td>Vimentin</td>
<td>52</td>
</tr>
<tr>
<td>Titin</td>
<td>$\approx 3600$</td>
</tr>
<tr>
<td>Nebulin</td>
<td>$\approx 800$</td>
</tr>
<tr>
<td>Dystrophlin</td>
<td>427</td>
</tr>
<tr>
<td>C-Protein</td>
<td>150</td>
</tr>
<tr>
<td>M-Protein</td>
<td>100</td>
</tr>
</tbody>
</table>

![Deep-etched, freeze-fractured myofibril](image2)

**FIGURE 34-49.** Electron micrograph of deep-etched, freeze-fractured myofibril showing its alternating thick and thin filaments. The knobs (cross-bridges) projecting from the thick filaments are helically arrayed. [Courtesy of John Heuser, Washington University School of Medicine.]
coil, yielding an ~1600-Å-long rodlike segment with two globular heads. The amino acid sequence of myosin's α-helical tail is characteristic of coiled coils: It has a seven-residue pseudo-repeat, a-b-c-d-e-f-g, with nonpolar residues concentrated at positions a and d. Thus, much like in the coiled coils of the fibrous protein keratin (Section 7-2A) and leucine zippers (Section 33-3B), the myosin helix has a hydrophobic strip along one side that promotes its lengthwise association with another such helix. One of each type of light chain is associated with each of the heavy chain dimer's globular heads.

Myosin only exists as single molecules at low ionic strengths. However, under physiological conditions, these proteins form aggregates that resemble thick filaments. Natural thick filaments consist of several hundred myosin molecules with their rodlike tails packed end-to-end in a regular staggered array (Fig. 34-51). The thick filament is therefore a bipolar entity in which the globular myosin heads project from either end, leaving a bare central region. It is these myosin heads that form the cross-bridges that interact with the thin filaments in intact myofibrils.

In addition to its structural function, the myosin heavy chain is an ATPase. It hydrolyzes ATP to ADP and P_i in a reaction that powers muscle contraction. Muscle is therefore a device for transducing the chemical free energy of ATP hydrolysis to mechanical energy. The myosin light chains, through their level of phosphorylation, are thought to modulate the ATPase activity of their associated heavy chains.

FIGURE 34-50. The myosin molecule. (a) Electron micrograph showing that the myosin molecule is a fibrous entity with two globular heads. [Courtesy of Henry Slayter, Harvard Medical School.] (b) Its rod-shaped tail is formed by the two extended α-helices, one from each of its two identical heavy chains, that wrap around each other to form a parallel coiled coil. One of each type of myosin light chain, an essential light chain (ELC) and a regulatory light chain (RLC), is associated with each of myosin's identical globular heads.

FIGURE 34-51. The thick filament of striated muscle. (a) An electron micrograph showing the myosin heads projecting from the thick filament's outer segments and its bare central zone. [From Trinick, J. and Elliott, A., J. Mol. Biol. 131, 135 (1977).] (b) A thick filament typically contains several hundred myosin molecules organized in a repeating staggered array such that the myosin molecules are oriented with their globular heads pointing away from the filament's center.
FIGURE 34-52. The enzymatic cleavage pattern of myosin.

Myosin

Light meromyosin (LMM)

Heavy meromyosin (HMM)

trypsin

papain

S2

S1

S1

FIGURE 34-53. The X-ray structure of chicken muscle myosin subfragment-1 (S1). (a) A ribbon diagram in which the heavy chain's 25-, 50-, and 20-kD segments are green, red, and blue, respectively, and its essential and regulatory light chains, RLC and ELC, are purple and yellow. Residue numbers are indicated at various positions, with 2000 and 3000 being added to those of the RLC and ELC to distinguish them from the heavy chain. A sulfate ion, shown in space-filling form (red), is bound near the confluence of the three heavy chain fragments, where it is thought to occupy the binding site of ATP's β-phosphate group (sulfate is a competitive inhibitor of myosin's ATPase function). An RLC-bound Ca²⁺ ion (lower left) is represented by a gray ball. (b) A space-filling representation of S1, colored and oriented similarly to that in Part a. Note the prominent vertical cleft that divides the 50-kD fragment.

[Courtesy of Ivan Rayment and Hazel Holden, University of Wisconsin.]
In 1953, Andrew Szent-Györgi demonstrated that limited trypsin digestion cleaves myosin into two fragments (Fig. 34-52):

1. **Light meromyosin (LMM)**, a 950-Å long α-helical rod that aggregates to form filaments but lacks both ATPase activity and the ability to associate with light chains.

2. **Heavy meromyosin (HMM)**, which has a rodlike tail and two globular heads, does not aggregate but has ATPase activity and binds to light chains.

HMM can be further split by treatment with papain to yield two identical molecules of **subfragment-1 (S1)** and one of the rod-shaped **subfragment-2 (S2)**. The 130-kD S1, which contains myosin’s ATPase activity and its thin filament-binding site, consists of a 95-kD heavy chain fragment and one molecule each of ELC and RLC. S1 is a pear-shaped molecule that electron microscopy studies indicate is 190 Å long and 50 Å wide at its widest point. Further tryptic digestion of S1 yields three fragments: a 25-kD N-terminal segment that binds nucleotide (ATP or ADP), a central 50-kD segment, and a 20-kD C-terminal segment.

**X-Ray Structure of Myosin Subfragment-1**

The X-ray structure of chicken muscle S1, determined by Ivan Raymert and Hazel Holden, reveals it to be nearly 50% α helical (Fig. 34-53). However, the core of the molecule consists of a mostly parallel seven-strand β sheet whose strands are contributed by all three tryptic segments. Perhaps the most conspicuous structural feature of S1 is an 85-Å-long helix that extends from the thick part of the head, the so-called motor domain, to the protein’s C-terminal region at its narrow end. The motor domain contains the binding site for ATP as well as that for the protein actin, the thin filament’s major component (see below).

The ATP-binding site, which is located in a 13-Å-deep V-shaped pocket at the point where the three tryptic segments come together, was identified from its resemblance to such sites in certain other nucleotide-binding proteins and from the positions of amino acids previously known to be at myosin’s ATP-binding site. The tight binding of ATP to myosin (association constant, $K = 3 \times 10^{11} M^{-1}$) suggests that this pocket must close around the ATP. This notion is supported by the observation that the particularly reactive Cys 697 and Cys 707 residues can be cross-linked by bifunctional sulphydryl reagents whose reactive groups are 3 to 14 Å apart only when nucleotide is bound to S1. These residues, which lie near the C-terminal ends of two consecutive and highly conserved α helices, are 18 Å apart in the nucleotide-free X-ray structure of S1. The site that is implicated in binding actin is located on the opposite side of the S1 globule from the nucleotide-binding site and is formed by the 50-kD tryptic fragment. The nucleotide and actin binding sites are connected by a deep cleft that divides the 50-kD fragment into two domains, the so-called actin cleft. The way in which myosin and actin interact is considered below.

The two light chains share both sequence and structural homology with calmodulin (CaM; Section 17-3C). However, RLC contains only one of CaM’s four Ca²⁺-binding motifs and ELC has none. ELC embraces the middle region of myosin’s long helix (Fig. 34-53a) in a manner resembling the way that CaM interacts with the CaM-binding helix of myosin light chain kinase (Fig. 17-17). RLC also clasps the long helix, but near its C-terminus and in a manner different from that of ELC (Fig. 34-53a).

**Thin Filaments Consist of Actin, Tropomyosin, and Troponin**

Actin, a ubiquitous and highly abundant eukaryotic protein, is the major constituent of thin filaments. At low ionic strengths, actin occurs as 375-residue bilobal globular monomers called G-actin (G for globular) that normally bind one molecule of ATP each. Under physiological conditions, however, G-actin polymerizes to form fibers known as F-actin (Fig. 34-54; F for fibrous), a process that hydrolyzes the ATP to ADP, which remains bound to the F-actin monomer unit. F-actin forms the core of the thin filament.

**FIGURE 34-54. F-actin.** (a) An electron micrograph of a thin filament from striated muscle. [Courtesy of Hugh Huxley, Brandeis University.] (b) An actin fiber (red) as visualized through image analysis of cryoelectron micrographs. Note the bilobal appearance of each monomeric (repeating) unit. The tropomyosin binding sites (see text) are blue. The F-actin helix has a maximum diameter of ~100 Å, 2.17 actin monomers per left-handed helical turn (13 subunits in 6 turns), and a rise per turn of ~60 Å. [Alternatively, F-actin may be described as a double (two-start) helix with 13 subunits per right-handed turn of each strand and a pitch of 720 Å.] [Courtesy of Daniel Safer, University of Pennsylvania, and Ronald Milligan, The Scripps Research Institute.]
Each of F-actin’s monomeric units is capable of binding a single myosin S1 head. Electron micrographs of S1-decorated F-actin have the appearance of a series of head-to-tail arrowheads (Fig. 34-55a). F-actin must therefore be a polar entity; that is, all of its monomer units have the same orientation with respect to the fiber axis (Fig. 34-55b). The “arrowheads” in S1-decorated thin filaments that are still attached to their Z disk all point away from the Z disk indicating that the thin filament bundles extending from the two sides of the Z disk have opposite orientations.

Myosin and actin, the major components of muscle, account for 60 to 70% and 20 to 25% of total muscle protein, respectively. Of the remainder, two proteins that are asso-

![Image](image-url)

**FIGURE 34-55.** (a) An electron micrograph of a thin filament decorated with myosin S1 fragments. Note its resemblance to a series of arrowheads all pointing in the same direction along the filament. [Courtesy of Hugh Huxley, Brandeis University.] (b) Image reconstruction of S1-decorated actin filaments at a resolution of ~30 Å. The actin is colored green, the S1 fragments are pink, and the bound troponin (see text) is orange. The helical filament has a pitch of 370 Å. [After a drawing provided by Ronald Milligan, The Scripps Research Institute, and Paula Flicker, University of California at San Francisco.]

**FIGURE 34-56.** A model of the striated muscle thin filament based on the 15-Å resolution X-ray structure of tropomyosin and electron micrographic studies of F-actin. Tropomyosin, a coiled coil of two α-helical subunits, wraps in the groove of the F-actin helix (large blue spheres) in a head-to-tail manner such that one tropomyosin molecule contacts seven consecutive blobal actin monomer units (the red and blue regions of tropomyosin identify the seven homologous segments that are presumed to form its actin-binding site). Each tropomyosin molecule binds a single troponin molecule at its head-to-tail joint (left, the small white spheres represent bound Ca\(^{2+}\) ions). The tropomyosin chain winding about the opposite side of the F-actin helix from the tropomyosin shown has been omitted for clarity. [Courtesy of George N. Phillips, Jr., Rice University.]
associated with the thin filaments are particularly prominent (Fig. 34-56):

1. **Tropomyosin**, a homodimer whose two 284-residue \( \alpha \) helical subunits wrap around each other to form a parallel coiled coil (Fig. 34-57). These 400-Å-long rod-shaped molecules are joined head-to-tail to form cables wound in the grooves of the F-actin helix such that each tropomyosin molecule contacts seven consecutive actin monomers in a quasi-equivalent manner.

2. **Tropinin**, which consists of three subunits: TnC, a \( \text{Ca}^{2+} \)-binding protein (Fig. 34-58) that is 70% homologous to CaM; TnI, which binds to actin; and TnT, an elongated molecule, which binds to tropomyosin at its head-to-tail junction.

The tropomyosin–tropinin complex, as we shall see, regulates muscle contraction by controlling the access of the myosin S1 cross-bridges to their actin-binding sites.

**X-Ray Structure of Actin**

The tendency of G-actin to polymerize has thwarted its crystallization in a manner suitable for X-ray crystallographic analysis. Curiously, however, it binds pancreatic deoxyribonuclease I (DNase I) in a 1:1 complex that, together with a \( \text{Ca}^{2+} \) ion and a molecule of either ATP or ADP, form crystals whose X-ray structures were determined by Wolfgang Kabsch and Kenneth Holmes (Fig. 34-59). The actin monomer consists of two domains that, for historical reasons, are referred to as the small and large domains, even though the former is only slightly smaller than the latter. Each domain has extensive secondary structure and is divided into two subdomains. The ATP and ADP bind in a cleft between the two domains, where the \( \text{Ca}^{2+} \) ion is liganded by the protein as well as by the \( \beta \)-phosphate of ADP or the \( \beta \) and \( \gamma \)-phosphates of ATP. The two actin structures are otherwise very similar. Two of the subdomains, one on each domain, contain a five-stranded \( \beta \) sheet consisting of a \( \beta \) hairpin motif followed by a right-handed \( \beta \) hairpin motif (Fig. 17-15), suggesting that these subdomains arose by gene duplication even though their amino acid sequences exhibit no significant similarity.

The atomic model of ADP–actin has been used to generate a model of F-actin that fits the observed X-ray fiber

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**FIGURE 34-57.** The low-resolution X-ray structure of rabbit cardiac muscle tropomyosin. Its two identical 284-residue polypeptides (gold and light blue) form a parallel coiled coil of \( \alpha \) helices. Only the residues at the ends of the chains are not in \( \alpha \) helices. [Based on an X-ray structure by George N. Phillips, Jr. and Carolyn Cohen, Brandeis University.]

**FIGURE 34-58.** The X-ray structure of chicken skeletal muscle TnC. Its two globular domains, which are connected by an \( \alpha \)-turn \( \alpha \) helix, can each bind two \( \text{Ca}^{2+} \) ions. Under the conditions of the crystal structure determination, however, only the \( \text{Ca}^{2+} \)-binding sites of the C-terminal (lower) domain are occupied (silver spheres). These latter sites remain occupied at the lowest physiological \( \text{Ca}^{2+} \) concentrations in muscle so that the regulatory effects exerted by TnC must arise from the binding of \( \text{Ca}^{2+} \) to the N-terminal (upper) domain. Note the resemblance of the TnC structure to that of the homologous \( \text{Ca}^{2+} \)-binding regulatory protein calmodulin (Fig. 17-15). [Based on an X-ray structure by Muttiliya Sundaralingam, University of Wisconsin.]
largest polypeptide known (it was only recently discovered because it does not enter polyacrylamide gels), extends from the thick filament to the Z disk, where it is thought to act as a spring to keep the thick filament centered in the sarcomere. Nebulin, which is also extremely large (~800 kD, ~7000 residues), consists, over at least 80% of its length, of a repeating 35-residue actin-binding motif that is predicted to be α helical (although it lacks the heptad repeat of α helices that form coiled coils). The observations that the

**FIGURE 34-59.** The X-ray structure of rabbit skeletal muscle actin-Ca²⁺-ATP in its complex with bovine pancreatic DNase I. The protein is shown in ribbon form with subdomains 1 and 2, which together form the small domain, purple and light blue, and subdomains 3 and 4, which together form the large domain, orange and yellow. The ATP is shown in stick form (C green, N dark blue, O red, and P gold) and the bound Ca²⁺ ion is represented by a silver sphere. Note the structural similarity between subdomains 1 and 3. [Based on an X-ray structure by Wolfgang Kabsch and Kenneth Holmes, Max-Planck-Institute für medizinische Forschung, Germany.]

Diagram from oriented gels of F-actin (Fig. 34-60). The monomer has a specific orientation with its small domain at a high radius from the F-actin helix axis and with all four subdomains making contacts with neighboring monomers. This model is in excellent agreement with three-dimensional maps of vertebrate muscle thin filaments obtained by cryoelectron microscopy and image reconstruction (Fig. 34-54): The two studies agree in terms of filament polarity, monomer orientation, and the three-dimensional location of the C-terminus of the actin monomer.

**Minor Muscle Proteins Control Myofibril Assembly**

The Z disk, which anchors two sets of oppositely oriented thin filaments (Fig. 34-48), is an amorphous entity that contains several fibrous proteins. For instance, α-actinin, which binds to the ends of F-actin filaments *in vitro*, is localized in the Z disk's interior (Fig. 34-61a). α-Actinin is therefore thought to attach thin filaments to the Z disk. Two other proteins, desmin and vimentin, largely occur at the Z disk periphery (Fig. 34-61b), where they apparently act to keep adjacent myofibrils in lateral register. Titin, whose ~3600-kD molecular mass (~33,000 residues) makes it the

**FIGURE 34-60.** A model of the actin filament based on fitting the known X-ray structure of the actin monomer to the X-ray fiber diffraction pattern of F-actin. Actin monomers are shown in space-filling representation, in alternating blue, red, and white, with each amino acid residue represented by a sphere. The lowest monomer shown is oriented identically to that in Fig. 34-59. The residues which cross-linking studies indicate form the myosin binding site are green. [Courtesy of Wolfgang Kabsch and Kenneth Holmes, Max-Planck-Institute für medizinische Forschung, Germany.]
aggregate length of these putative α helices is approximately that of a thin filament, that nebulin antibodies label the thin filament, and that this labeling pattern is fixed with respect to the Z disk suggest that nebulin winds along the ~1 μm full length of a thin filament, thereby controlling this length. Titin may similarly control the length of thick filaments.

The M disk (Fig. 34-48) arises from the local enlargement of in-register thick filaments. The two proteins that are associated with this structure, C-protein and M-protein, probably participate in thick filament assembly. Invertebrate thick filaments contain a core of paramyosin which, in some muscles, is the dominant component.

Duchenne muscular dystrophy (DMD) and the less severe Becker muscular dystrophy (BMD) are both sex-linked muscle-wasting diseases. In DMD, which has an onset age of 2 to 5 years, muscle degeneration exceeds muscle regeneration causing progressive muscle weakness and ultimately death, usually at around age 20. In BMD, the onset age is 5 to 10 years and there is an overall less progressive course of muscle degeneration and a longer (sometimes normal) lifespan than in individuals with DMD.

The ~2500-kb gene responsible for DMD/BMD, which contains at least 70 introns (the most known in a single gene; Section 33-2F), encodes a 3685-residue protein named dystrophin. However, dystrophin has numerous isoforms that differ at their C-termini through alternative mRNA splicing, as well as at their N-termini through alternative transcriptional initiation (Section 34-3C). Dystrophin appears to be a member of the family of flexible rod-shaped proteins that includes the actin-binding cytoskeletal components spectrin (Section 11-3C) and α-actinin, each of which contains segments homologous to portions of dystrophin. Subcellular fractionation and immuno-fluorescence studies reveal that dystrophin, which has a normal abundance in muscle tissue of 0.002%, is associated with the inner surface of the muscle plasma membrane where it probably functions to anchor specific membrane glycoproteins, much as do spectrin and ankyrin in the erythrocyte (Fig. 11-35d).

The dystrophin gene in most individuals with DMD/BMD contains deletions or, less frequently, duplications of one or more exons. Individuals with DMD usually have no detectable dystrophin in their muscles, whereas those with BMD mostly have dystrophins of altered sizes. Evidently, the dystrophins of individuals with DMD are rapidly degraded, whereas those of individuals with BMD are semia functional.

B. Mechanism of Muscle Contraction

So far we have simply described the components of striated muscle. Now, like good engineers, we must ask how do these components fit together and how do they interact? In other words, how does muscle work?

Thick and Thin Filaments Slide Past Each Other during Muscle Contraction

Physiologists have long known that a contracted muscle is as much as one third shorter than its fully extended length. Electron micrographs have demonstrated that this shortening is a consequence of a decrease in the length of
the sarcomere (Fig. 34-62). Yet, during muscle contraction, the thick and the thin filaments maintain constant lengths as is indicated by the observations that the width of the A band as well as the distance between the Z disk and the edge of the adjacent H zone do not change. Rather, sarcomere contraction is accompanied by equal reductions in the widths of the I band and the H zone. These observations were independently explained by Hugh Huxley and Jean Hanson and by Andrew Huxley and R. Niedergerke who, in 1954, proposed the sliding filament model: The force of muscle contraction is generated by a process in which interdigitated sets of thick and thin filaments slide past each other (Fig. 34-62).

**Actin Stimulates Myosin’s ATPase Activity**

The sliding filament model partially explains the mechanics of muscle contraction but not the origin of the contractile force. Albert Szent-Györgi’s work in the 1940s pointed the way towards the elucidation of the contraction mechanism. The mixing of solutions of actin and myosin to form a complex known as actomyosin is accompanied by a large increase in the solution’s viscosity. This viscosity increase is reversed, however, when ATP is added to the actomyosin solution. Evidently, ATP reduces myosin’s affinity for actin.

Further insight into the role of ATP in muscle contraction was provided by kinetic studies. Isolated myosin’s ATPase function has a turnover number of $\sim$0.05 s$^{-1}$, far less than that in contracting muscle. Paradoxically, however, the presence of actin increases myosin’s ATP hydrolysis rate to the physiologically more realistic turnover number of $\sim$10 s$^{-1}$, a rate enhancement of $\sim$200 (indeed, actin was so named because it activates myosin). This is because isolated myosin rapidly hydrolyzes ATP

$$\text{ATP}^{\text{ads}} + \text{H}_2\text{O} \rightarrow \text{ADP}^{\text{ads}} + \text{HPO}_4^{2-} + \text{H}^+$$

but only slowly releases the products ADP + Pi, as is indicated by the observation that myosin-catalyzed ATP hydrolysis begins with a rapid burst of H$^+$, whereas free ADP and Pi appear much more slowly. Actin enhances myosin’s ATPase activity by binding to the myosin–ADP–Pi complex and stimulating it to sequentially release Pi, followed by ADP. The myosin–ADP–Pi complex cannot be formed by simply mixing myosin, ADP, and Pi, which suggests that this complex is a “high-energy” intermediate in which the free energy of ATP hydrolysis has somehow been conserved.

The foregoing observations led Edwin Taylor to formulate a model for actomyosin-mediated ATP hydrolysis (Fig. 34-63):

1. **Step 1** ATP binding to the myosin component of actomyosin results in the dissociation of actin and myosin.

2. **Step 2** The myosin-bound ATP is rapidly hydrolyzed to form a stable “high-energy” myosin–ADP–Pi complex.

3. **Step 3** Actin binds to the myosin–ADP–Pi complex.

4. **Step 4** In a process accompanied by a conformational relaxation to its resting state, the actin–myosin–ADP–Pi complex sequentially releases Pi, followed by ADP yielding actomyosin that can undergo another round of ATP hydrolysis.

This ATP-driven alternate binding and release of actin by myosin provides, as we shall see, the vectorial force of muscle contraction.

**A Structure-Based Model for the Interaction of Actin and Myosin**

Rayment, Holden, and Ronald Milligan have formulated a model for the so-called rigor complex of the myosin and thin filaments remain constant upon myofibril contraction. The interpenetrating sets of thick and thin filaments must therefore slide past each other, as drawn. [Courtesy of Hugh Huxley, Brandeis University.]
S1 head and F-actin (Fig. 34-64; the rigor complex is that taken up by ATP-deprived muscle, which occurs in rigor mortis, the temporary rigidity of muscles after death). This was done, with an estimated accuracy of 5 to 8 Å, by fitting the X-ray structure of the myosin S1 head (Fig. 34-53) and the X-ray fiber structure of F-actin as derived from the X-ray structure of G-actin (Fig. 34-60) to the electron density map obtained from the electron microscopy–based image of this complex (Fig. 34-55). The bulky motor domain of S1 binds tangentially to the actin filament at a 45° angle to the filament axis. Its extension, the narrow S1 tail,

FIGURE 34-63. The reaction sequence in actomyosin-catalyzed ATP hydrolysis.

FIGURE 34-64. A space-filling atomic model of the myosin–actin interaction constructed by fitting the atomic models of myosin S1 (Fig. 34-53) and the actin filament (Fig. 34-60) to the electron microscopy–based image of S1-decorated actin filaments (Fig. 34-55). The myosin S1 is rotated about its long axis relative to the view in Fig. 34-53; its tryptic fragments and light chains are similarly colored to those in that figure. [Courtesy of Ivan Rayment and Hazel Holden, University of Wisconsin.]
which contains the two light chain binding regions, projects
tangentially away from the filament axis at ~90°, an ori-
entation that permits S1 to impose tension on the rodlike
myosin tail that associates with other such tails to form the
thick filament. The myosin head appears to interact with
actin via ion pairing involving several Lys residues on myo-
sin and several Asp and Glu residues on actin. These inter-
actions are bolstered by what appears to be a stereospecific
association between juxtaposed surface-exposed patches of
hydrophobic residues on actin and myosin.

Myosin Heads “Walk” Along Actin Filaments

In order to complete our description of muscle contraction
we must determine how ATP hydrolysis is coupled to the
sliding filament model. If the sliding filament model is
correct then it would be impossible for a myosin cross-
bridge to remain attached to the same point on a thin fila-
ment during muscle contraction. Rather, it must repeatedly
detach and then reattach itself at a new site further along
the thin filament towards the Z disk. This, in turn, suggests
that muscular tension is generated through the interaction of
myosin cross-bodies with thin filaments.

The X-ray structure of myosin S1 (Fig. 34-53) together
with the model of its rigor complex with actin (Fig. 34-64)
suggests how ATP hydrolysis is coupled to myosin’s confor-
mational change. Despite the excellent fit of the X-ray struc-
tures of actin and myosin S1 to the image of their rigor
complex (Fig. 34-55), the resulting atomic model of the
rigor complex (Fig. 34-64) contains a steric clash between
residues at the actin–myosin contact region. However, it
seems quite plausible that, upon ATP binding, this clash is
relieved by the opening of the actin cleft, the cleft that di-
vides the 50-kD segment into two domains (Fig. 34-526).
Rayment, Holden, and Milligan therefore postulated that
the closure of the actin cleft under the impetus of the release
of ADP from the nucleotide binding site is responsible for
the conformational change that produces myosin’s “power
stroke” in muscle’s contractile cycle. This has led to the
following variation of the widely accepted “rowboat”
model for the contractile cycle (Fig. 34-65):

1. ATP binds to the S1 head in a manner that opens up the
actin cleft. This, in turn, causes S1 to release its bound
actin.
2. The active site cleft (distinct from the actin cleft) closes
about the ATP in a manner that catalyzes its hydrolysis.
This process “cocks” the myosin molecule; that is, puts
it into its “high energy” state in which its S1 head is
approximately perpendicular to the thick filament.
3. The S1 head binds weakly to an actin monomer that is
closer to the Z disk than the one to which it had been
bound previously.
4. S1 releases P, which causes its actin cleft to close,
thereby increasing S1’s binding affinity for actin.
5. The resulting transient state is immediately followed by
the power stroke, a conformational shift that sweeps S1’s
C-terminal tail by an estimated ~60 Å towards the Z
disk relative to the motor domain, thus translating the
attached thin filament by this distance towards the M-
disk. It seems likely that the ~85-Å-long helix that con-
nects S1’s motor domain to its C-terminal tail is the
conformational coupler in this energy transduction step.

6. ADP is released, thereby completing the cycle.

The cyclic nature of this process is necessary to prevent this
molecular motor from reversing its power stroke while still
bound to the actin which, if it occurred, would result in no
net movement of the myosin head relative to the actin fila-
ment. The ATP-driven active transport of ions across a
membrane is a similarly cyclic vectorial process (Sections
18-3A and B).

The ~500 S1 heads on every thick filament asynchronously
cycle through this reaction sequence about five times
per second each during a strong muscular contraction. The
S1 heads thereby “walk” or “row” up adjacent thin fila-
ments towards the Z disk with the concomitant contraction
of the muscle.

A remarkably similar model of actomyosin to that in Fig.
34-64 has been derived by fitting the X-ray structures of
myosin S1 and F-actin to the image reconstruction of rabbit
F-actin decorated with Dictyostelium discoideum (slime
mold) myosin S1. The observation that Dictyostelium
myosin binds to rabbit F-actin with the same affinity as
does rabbit muscle myosin indicates a high degree of con-
servation at the interface between these two proteins and
thus supports the forgoing mechanistic model for the con-
tractile cycle.

Myosin Light Chains Function to Increase the Rate of
Muscle Contraction

The function of the myosin light chains in vertebrate
skeletal muscle had, for many years, been enigmatic. Re-
cently, however, a motility assay has demonstrated that the
rate at which myosin heavy chains slide along actin fila-
ments is reduced 10-fold when the light chains are
removed, even though removing the chains does not signifi-
cantly reduce myosin’s ATPase activity. It is therefore
suggested that the 85-Å-long α helix to which both light
chains bind (Fig. 34-53) and which is thought to act as the
leverage arm that amplifies the conformation change in myo-
sin’s motor domain to produce the power stroke, is stabi-
lized by its bound light chains. Indeed, a bare α helix is
rarely observed in proteins in aqueous solution. Thus, in
the absence of light chains, the long α helix is likely to
collapse leading to a smaller power stroke.

C. Control of Muscle Contraction

Striated muscles are, for the most part, under voluntary
control; that is, their contraction is triggered by motor
nerve impulses. How do these nerve impulses trigger mus-
cle contraction? To answer this question, let us begin at
the level of the myofibril and work up.
FIGURE 34-65. The mechanism of force generation in muscle. The myosin head “walks” up the actin thin filament through a cyclic vectorial process that is driven by ATP hydrolysis. Only one of myosin’s two independent S1 heads is shown. The narrow cleft that splits the 50-kD segment of the S1 head into two domains (Fig. 34-53b) is represented by a horizontal gap perpendicular to the thin filament (although in the actual atomic model, Fig. 34-64, this gap is inclined by about 30° to the thin filament, thereby obscuring it in that figure). The actin monomer to which S1 was bound at the beginning of the cycle is more darkly colored for reference. [After Raymont, I., and Holden, M., Curr. Opin. Struct. Biol. 3, 949 (1993).]

Ca²⁺ Regulates Muscle Contraction in a Process Mediated by Troponin and Tropomyosin

It has been known since the 1940s that Ca²⁺ is somehow involved in controlling muscle contraction. It was not until the early 1960s, however, that Setsuro Ebashi demonstrated that the effect of Ca²⁺ is mediated by troponin and tropomyosin. He did so by showing that actomyosin extracted directly from muscle, and therefore bound to troponin and tropomyosin, contracts in the presence of ATP only when Ca²⁺ is also present, whereas actomyosin prepared from purified actin and myosin contracts in the presence of ATP regardless of the Ca²⁺ concentration. The ad-
dition of troponin and troponin to the purified actomyosin system restored its sensitivity to Ca$^{2+}$. Indeed, it was through these experiments that troponin was discovered.

The TnC subunit of troponin (Fig. 34-58) is the only Ca$^{2+}$-binding component of the tropomyosin–troponin complex. Tropomyosin, as we saw, binds along the thin filament groove in relaxed muscle (Fig. 34-56), where it apparently blocks the attachment of S1 myosin heads to seven consecutive actin units. X-ray diffraction studies indicate that when the [Ca$^{2+}$] reaches a critical level, an allosteric interaction between Ca$^{2+}$–troponin and troponymosin causes troponin to move ~10 Å deeper into the thin filament groove (Fig. 34-66). This movement, it is thought, uncovers the actin's myosin-binding sites, thereby switching on muscle contraction. This switching mechanism may well be cooperative, in that the binding of a myosin head to an actin subunit might push troponin away from neighboring myosin-binding sites in a conformational change that could also increase the Ca$^{2+}$-binding affinity of its associated TnC subunit.

Nerve Impulses Release Ca$^{2+}$ from the Sarcoplasmic Reticulum

In order to understand how a nerve impulse affects the [Ca$^{2+}$] in a myofibril we must further consider the anatomy of striated muscle fibers. A nerve impulse arriving at a neuromuscular junction is transmitted directly to each sarcomere by a system of transverse or T tubules, nervelike invaginations of the muscle fiber's plasma membrane that surround each myofibril at its Z disk (Fig. 34-67). The nerve impulse transmission is the subject of Section 34-A. All of a muscle's sarcomeres therefore receive the signal to contract within a few milliseconds of each other so that the muscle contracts as a unit. The electrical signal is transferred, in a poorly understood manner, to the sarcoplasmic reticulum (SR), a system of flattened membranous vesicles derived from the endoplasmic reticulum that surround each myofibril rather like a net stocking. The SR membrane, which is normally impermeable to Ca$^{2+}$, contains a transmembrane Ca$^{2+}$-ATPase (Section 18-3B) that pumps Ca$^{2+}$ into the SR so as to maintain the cytosolic [Ca$^{2+}$] of resting muscle below $10^{-3}M$, whereas that in the SR is over $10^{-3}M$. The SR's ability to store Ca$^{2+}$ is enhanced by the presence of a highly acidic (37% Asp + Glu) 55-kD protein named calsequestrin, which has >40 Ca$^{2+}$-binding sites.

The arrival of a nerve impulse renders the SR permeable to Ca$^{2+}$ which, in a few milliseconds, diffuses through specific Ca$^{2+}$ channels into the myofibril so as to raise its internal [Ca$^{2+}$] to ~$10^{-3}M$. This Ca$^{2+}$ concentration is sufficient to trigger the conformational change in troponin–tropomyosin that permits muscle contraction. Once nerve excitation has subsided, the SR membrane again becomes impermeable to Ca$^{2+}$, so Ca$^{2+}$ inside the myofibril is pumped back into the SR. Tropomyosin therefore resumes its resting conformation causing the muscle to relax.

**FIGURE 34-66.** The control of skeletal muscle contraction by troponin and troponyosin. (a) In contracting muscle, here diagrammed in cross-section, the myosin S1 heads freely interact with and thereby “walk” up the F-actin filaments (A). (b) Muscle relaxes when Ca$^{2+}$ dissociates from troponin's TnC subunit thereby allosterically moving the tropomyosin (TM) molecules to positions which sterically block myosin-actin interactions. [After Zot, A.S. and Potter, J.D., Annu. Rev. Biophys. Biochem. 16, 555 (1987).]

**D. Smooth Muscle**

Vertebrates have two major types of muscle besides skeletal muscle: cardiac muscle and smooth muscle. Cardiac muscle, which is responsible for the heart’s pumping action, is striated, indicating the similarity of its organization to that of skeletal muscle. Cardiac and skeletal muscle differ mainly in their metabolism, with cardiac muscle, which must function continuously for a lifetime, being much more dependent on aerobic metabolism than is skeletal muscle. Vertebrate heart muscle contraction is also spontaneously initiated by the heart muscle itself rather than through external nervous stimuli, although the nervous system can influence this contractile response. Smooth muscle, which is responsible for the slow, long-lasting, and involuntary contractions of such tissues as the intestinal walls, uterus, and large blood vessels, has a quite different organization from that of striated muscle. Smooth muscle consists of spindle-shaped, mononucleated cells whose
thick and thin filaments are more or less aligned along the cells' long axes but which do not form myofibrils.

Smooth muscle myosin, a genetically distinct protein, is functionally distinct from striated muscle myosin in several ways:

1. Its maximum ATPase activity is only ~10% of that of striated muscle.
2. It interacts with actin only when one of its light chains is phosphorylated at a specific Ser residue.
3. It forms thick filaments whose cross-bridges lack the regular repeating pattern of striated muscle and are distributed along the thick filament's entire length.

**Smooth Muscle Contraction Is Triggered by Ca^{2+}**

The thin filaments of smooth muscle contain actin and tropomyosin but lack troponin. Smooth muscle contraction is nevertheless triggered by Ca^{2+} because myosin light chain kinase (MLCK), an enzyme that phosphorylates myosin light chains and thereby stimulates smooth muscle to contract, is enzymatically active only when it is associated with Ca^{2+}—calmodulin (Fig. 34-68, bottom; myosin light chain phosphorylation in skeletal muscle appears to modulate the degree of tension produced by contraction). The mechanism whereby Ca^{2+}—CaM activates MLCK is discussed in Section 17-3C.

The intracellular [Ca^{2+}] varies with the permeability of the smooth muscle cell plasma membrane to Ca^{2+} which, in turn, is under the control of the autonomic (involuntary) nervous system. When the [Ca^{2+}] rises to ~10^{-6}M, smooth muscle contraction is initiated as described. When the [Ca^{2+}] falls to ~10^{-7}M through the action of the plasma membrane's Ca^{2+}-ATPase, the MLCK is deactivated, the myosin light chain is dephosphorylated by myosin light chain phosphatase, and muscle relaxation ensues. Thus, Ca^{2+}, like cAMP, is a second messenger that transmits extracellular signals within the interior of a cell. In the many situations in which Ca^{2+} is a second messenger, calmodulin or a calmodulin-like protein is invariably the intracellular signal receiver.

**Smooth Muscle Activity Is Hormonally Modulated**

Smooth muscles also respond to hormones such as epinephrine (Fig. 34-68, top). The binding of epinephrine to its plasma membrane—bound receptor activates adenylate cyclase. The cytosolic cAMP that is thereby generated binds to and causes the dissociation of the regulatory dimer, R_2, of an inactive protein kinase, R_kC_2, yielding active catalytic
subunits, C, that phosphorylate MLCK. Phosphorylated MLCK binds Ca²⁺-calmodulin only weakly, so the extracellular presence of epinephrine causes smooth muscles to relax. Note the resemblance of this system to that controlling glycolgen metabolism in skeletal muscle (Section 17-3). Asthma, a breathing disorder caused by the inappropriate contraction of bronchial smooth muscle, is often treated by the inhalation of an aerosol containing epinephrine, thereby relaxing the contracted bronchi.

The sequence of events culminating in smooth muscle contraction are inherently much slower than those leading to skeletal muscle contraction. Indeed, the structure and regulatory apparatus of smooth muscle suits it to its function: the maintenance of tension for prolonged periods while consuming ATP at a much lower rate than skeletal muscle performing the same task. The structural and functional resemblance of TnC to CaM therefore suggests that TnC is a CaM variant that has evolved in skeletal muscle to provide a rapid response to the presence of Ca²⁺.

E. Actin and Myosin in Nonmuscle Cells

Although actin and myosin are most prominent in muscle, they also occur in other tissues. In fact, actin is ubiquitous and is usually the most abundant cytoplasmic protein in eukaryotic cells, typically comprising 5 to 10% of their total protein. Myosin, in contrast, is usually present in only about one tenth the quantity of actin. This ratio reflects the fact that actin, in addition to its role in actomyosin-based
contractile systems, participates in several myosin-independent motility systems as well as being a principal cytoskeleton component.

Actin Forms Microfilaments

Actin in muscles is entirely in the form of thin filaments. Nonmuscle actin, however, is about equally partitioned between soluble G-actin and F-actin fibers known as microfilaments. The actin content of microfilaments was established both through the immunofluorescence microscopy of living cells (e.g., Fig. 34-69) and because microfilaments can be decorated with S1 myosin heads to form arrowhead structures that are visually indistinguishable from those formed by muscle thin filaments (Fig. 34-55a). Such decoration is possible because actin is highly conserved throughout the eukaryotic kingdom. For instance, slime mold and rabbit muscle actins differ at only 17 of their 375 residues. Actin in vitro is monomeric at low temperatures, low ionic strengths, and alkaline pH's. Under physiological conditions, G-actin polymerizes in a process that is accelerated by the presence of ATP. In vivo, microfilament assembly and disassembly is also influenced by numerous actin-binding proteins. For example, profilin, a 16-kD protein, binds G-actin in a 1:1 profilactin complex so as to prevent actin polymerization. A dramatic example of the effect of profilin occurs in many invertebrates upon the encounter of sperm and egg. A sea urchin sperm, for example, contains a reservoir of profilactin in its acrosome, a vesicle that lies just beneath the front of the sperm's head. Contact with the egg's jelly coat triggers a reaction that dissociates the profilactin by raising the acrosomal pH. The newly liberated G-actin undergoes "explosive" polymerization so as to erect, in a matter of seconds, a 90-μm long bundle of F-actin filaments, the acrosomal process, that is projected outwards from the sperm head (Fig. 34-70). It is the acrosomal process that penetrates the egg's jelly coat to initiate the fusion of sperm and egg.

There is clear evidence that the assembly and disassembly of actin filaments plays an important role in such cellular motility processes as ameboid locomotion, phagocytosis, cytokinesis (the separation of daughter cells in the last stage of mitosis), and the extension and retraction of various cellular protuberances, such as microvilli (fingerlike projections of cell surfaces) and neuronal axons. This evidence was obtained largely through the use of drugs that interfere with actin aggregation. For example, the fungal alkaloid cytochalasin B

![Cytochalasin B](image)

**FIGURE 34-69.** The microfilaments in a fibroblast resting on the surface of a culture dish as revealed by indirect immunofluorescence microscopy using anti-actin antibody. When the cell begins to move, the filaments disassemble to form a diffuse mesh, thereby suggesting that actin plays a central role in cellular movement. [Courtesy of Elias Lazarides, California Institute of Technology.]
FIGURE 34-70. A series of light micrographs showing the elongation of the acrosomal process in a sea urchin sperm. The photographs were taken at 0.75-s intervals beginning 2 s after the sperm was artificially stimulated to begin the acrosomal reaction. The arc to the right of the sperm head is the sperm tail that has curved around outside the field of the micrograph. The acrosomal process’ final length (bottom frame) is ~70 µm. [From Tilney, L.G. and Inoué, S., J. Cell Biol. 93, 822 (1982).]

(which we have seen inhibits Na⁺-independent glucose transport; Section 18-4A) blocks actin polymerization by specifically binding to the end of a growing F-actin filament (the “barbed” end of S1-decorated filaments) so as to inhibit actin polymerization from that end. In contrast, phalloidin,

\[
\text{Phalloidin}
\]

a bicyclic heptapeptide produced by the poisonous mushroom Amanita phalloides (which also synthesizes the chemically similar eukaryotic RNA polymerase inhibitor α-amanitin; Section 29-2F), blocks microfilament depolymerization by specifically binding to its actin units.

ATP accelerates actin polymerization by activating G-actin to add preferentially to a particular end of a growing actin filament. The ATP is eventually hydrolyzed, but not until after polymerization has occurred. This vectorial process has the consequence that there is a certain “critical” G-actin concentration at which activated monomer units add predominantly (although not exclusively) to their preferred end of an actin filament at the same rate that monomer units dissociate predominantly from the opposite end of the filament. Under these conditions, the actin filament neither grows nor shrinks; rather, it assumes a steady state in which, through this treadmill process, actin monomer units are continually translocated from one end of the actin filament to the other (Fig. 34-71).

Actomyosin Has Contractile Functions in Nonmuscle Cells

Myosin is not so well conserved a protein as is actin. Nevertheless, nonmuscle myosin forms thick filaments that participate in contractile processes with microfilaments. One of the best characterized such processes occurs during cytokinesis (cytoplasmic division) in animal cells and protozoa. In the final stages of mitosis, a cleavage furrow forms around the equator of the dividing cell in the plane perpendicular to the long axis of the mitotic spindle. Immunofluorescence microscopy demonstrates that the cleavage furrow is lined with an actomyosin belt (Fig. 34-72). Cytokinesis is accomplished through the tightening of this so-called contractile ring, which disperses once cleavage has occurred. Blood platelets also contain actomyosin, which, upon blood clot formation (Section 34-1), contracts so as to strengthen the clot. The contraction is initiated by the Ca²⁺-CaM activation of MLCK as occurs in smooth muscle.

F. Ciliary Motion and Vesicle Transport

Eukaryotes have two nearly ubiquitous but unrelated types of motility systems:

1. Microfilament-based systems, such as muscle, which contain actin.
2. Microtubule-based systems, such as cilia (see below), which contain the protein tubulin.
FIGURE 34-71. The "treadmilling" of actin monomer units along an actin filament. Actin monomers continually add to the left end of the filament, with eventual ATP hydrolysis, but dissociate at the same rate from the right end, so the filament maintains a constant length while its component monomer units translocate from left to right.

FIGURE 34-72. An indirect immunofluorescence micrograph, using anti-myosin II antibodies (red), of a dividing Dictyostelium (slime mold) amoeba showing that its contractile ring contains myosin II (a double-headed protein such as muscle myosin). The cell has also been treated with anti-myosin I antibodies (green: myosin I is a single-headed protein that has ~40% sequence identity with myosin II), thereby demonstrating that myosin I is localized at the leading edges of the daughter cell's lamellipodia (sheetlike extensions of the cell surface that participate in cell locomotion). [Courtesy of Edward Korn, National Institutes of Health, and Yoshio Fukui, Northwestern University Medical School.]
Microtubules (Fig. 34-73), as their name implies, are tubular structures, ~300 Å in diameter, which form a class of cytoskeletal components distinct from the ~70 Å in diameter microfilaments and the 100- to 150-Å-diameter intermediate filaments (the cytoskeleton's third major component, which apparently has only a structural role; Section 1-2A). Microtubules comprise the major components of such cellular organelles as the mitotic spindle and cilia, and are thought to form the framework that organizes the cell.

**Microtubules Are Composed of Tubulin**

Microtubules are polymers of tubulin, a dimer of globular α- and β-tubulin subunits (55 kD each). Each of these types of subunits are highly homologous throughout the eukaryotic kingdom and, to a lesser extent, with each other. At low temperatures, and in the presence of Ca²⁺, tubulin assumes a soluble protomeric form (the αβ dimers dissociate only in the presence of denaturing agents). Under physiological conditions, however, tubulin polymerizes to microtubules through a process in which each tubulin molecule binds 2 GTPs and hydrolyzes one of them to GDP + Pₐ, during or shortly after the incorporation of the αβ dimer into a microtubule. Electron microscopy and X-ray studies indicate that microtubules consist of 13 parallel but staggered protofilaments arranged about a hollow core (Fig. 34-74). The protofilaments, which consist of alternating head-to-tail...
linked α- and β-tubulin subunits, all run in the same direction. Consequently, microtubules, like microfilaments, are polar entities: The end that grows most rapidly is called the plus end, whereas the other end is called the minus end. Microtubules are generally oriented in a cell with their minus ends towards a centrosome (the organizing center from which they emanate; Fig. 34-73), and their plus ends towards the cell periphery.

Microtubules undergo continuous assembly and disassembly. Indeed, in a given population of microtubules, some may grow while others simultaneously shrink. This dynamic instability occurs because if the second GTP in a tubulin subunit at the microtubule’s plus end becomes hydrolyzed to GDP before it is “capped” by another tubulin subunit, the resulting GDP–subunit rapidly dissociates from the microtubule. The balance between net microtubule growth or shrinkage in a cell therefore depends on the rate that tubulin hydrolyzes its second bound GTP together with the availability of GTP–tubulin subunits. Even when microtubules maintain a constant length, they are by no means static. They undergo GTP-driven treadmilling in which tubulin subunits add to the plus end at the same rate that they dissociate from the minus end. By regulating the rate of tubulin polymerization, cells presumably vary their shapes and induce the formation and dissolution of such cellular apparatus as the mitotic spindle.

**Antimitotic Drugs Inhibit Microtubule Formation**

Colchicine,

\[ \text{Colchicine} \]

an alkaloid produced by the meadow saffron, inhibits microtubule-dependent cellular processes by inhibiting the polymerization of tubulin protomers. For example, colchicine arrests mitosis in both plant and animal cells at metaphase (when the condensed and replicated chromosomes line up on the cell’s equator; Section 27-1A) by preventing the formation of the mitotic spindle. It also inhibits cell motility.

Colchicine has been used for centuries to treat acute attacks of gout (which result from elevated uric acid levels in body fluids; Section 26-5B). The lysosomes of the white cells that engulf urate microcrystals are ruptured by these needle-shaped crystals, causing cell lysis and triggering the local acute inflammatory reaction responsible for the exquisite pain characteristic of gout attacks. Colchicine, it is thought, slows the

ameboid movements of white cells by inhibiting their microtubule-based systems.

**The vinca alkaloids, vinblastine and vincristine,**

\[ \text{Vinblastine: R} = \text{CH}_3 \]
\[ \text{Vincristine: R} = \text{CHO} \]

products of the Madagascan periwinkle *Vinca rosea*, also inhibit microtubule polymerization by binding to tubulin. These substances are widely used in cancer chemotherapy since blocking mitosis preferentially kills fast-growing cells. Curiously, colchicine is not selectively toxic to cancer cells.

**Cilia and Eukaryotic Flagella Contain Organized Sheaves of Microtubules**

Cilia are the hairlike organelles on the surfaces of many animal and lower plant cells that function to move fluid over the cell’s surface or to “row” single cells through a fluid. In humans, for example, epithelial cells lining the respiratory tract each bear ~200 cilia that beat in synchrony to sweep mucus-entrained foreign particles towards the throat for elimination (Fig. 34-75; individuals with the in-