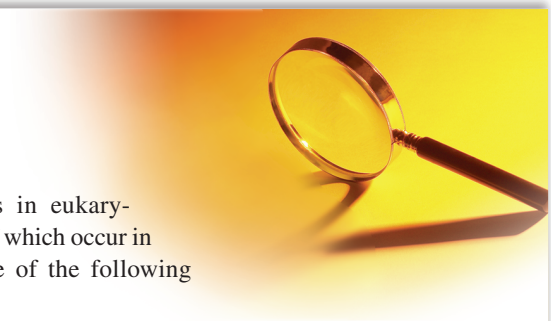
BIOCHEMISTRY IN PERSPECTIVE

Molecular Machines



How do living organisms utilize chemical bond energy to perform the thousands of tasks required to sustain life? Purposeful movement is the hallmark of living organisms. This behavior takes myriad forms that range from the record-setting 110 km/h chasing sprint of the cheetah to more subtle movements such as the migration of white blood cells in the animal body, cytoplasmic streaming in plant cells, intracellular transport of organelles, and the enzyme-catalyzed unwinding of DNA. The multisubunit proteins responsible for these phenomena (e.g., the muscle sarcomere and various other types of cytoskeletal components, and DNA polymerase) function as biological machines. Machines are defined as mechanical devices with moving parts that perform work (the product of force and distance). When machines are used correctly, they permit the accomplishment of tasks that would often be impossible without them. Although biological machines are composed of relatively fragile proteins that cannot withstand the physical conditions associated with human-made machines (e.g., heat and friction), the two types do share important features. In addition to having moving parts, all machines require energy-transducing mechanisms; that is, they convert energy into directed motion.

Despite the wide diversity of motion types in living organisms, in all cases, energy-driven changes in protein conformations result in the accomplishment of work. Protein conformation changes occur when a ligand is bound. When a specific ligand binds to one subunit of a multisubunit protein complex, the change in its conformation will affect the shapes of adjacent subunits. These changes are reversible; that is, ligand dissociation from a protein causes it to revert to its previous conformation. The work performed by complex biological machines requires that the conformational and, therefore, functional changes occur in an orderly and directed manner. In other words, an energy source (usually provided by the hydrolysis of ATP or GTP) drives a sequence of conformational changes of adjacent subunits in one functional direction. For example, the DNA replication machine DNA polymerase III incorporates nucleotides into a new DNA strand at the rate of 9000 nucleotides per minute. The directed functioning of this and other biological machines is possible because nucleotide hydrolysis is irreversible under physiological conditions.

Motor Proteins

Despite their functional diversity, all biological machines possess one or more protein components that bind nucleoside triphosphates (NTP). These subunits, called NTPases, function as mechanical transducers or **motor proteins**. The NTP hydrolysis–driven changes in the conformation of a motor protein trigger ordered conformational changes in adjacent subunits in the molecular machine. NTP-binding proteins perform a wide variety of functions in eukaryotes, most of which occur in one or more of the following categories.

1. **Classical motors**. Classical motor proteins are ATPases that move a load along a protein filament, as shown earlier (Figure 2.4). The best-known examples include the **myosins**, which move along actin filaments, and the kinesins and dyneins, which move vesicles and organelles along microtubules. **Kinesins** walk along the microtubules toward the (+) end, away from the centrosome (the microtubule organizing center). **Dyneins** walk along the microtubules toward the (–) end, toward the centrosome.
2. **Timing devices**. The function of certain NTP-binding proteins is to provide a delay period during a complex process that ensures accuracy. The prokaryotic protein synthesis protein EF-Tu (Biochemistry in Perpective Box EF-TU: A Motor Protein, Ch. 19) is a well-known example. The relatively slow rate of GTP hydrolysis by EF-Tu when it is bound to an aminoacyl-tRNA allows sufficient time for the dissociation of the complex from the ribosome if the tRNA-mRNA base sequence binding is not correct.
3. **Microprocessing switching devices**. A variety of GTP-binding proteins act as on-off molecular switches in signal transduction pathways. Examples include the β-subunits of the trimeric G proteins. Numerous intracellular signal control mechanisms are regulated by G proteins.
4. **Assembly and disassembly factors**. Numerous cellular processes require the rapid and reversible assembly of protein subunits into larger molecular complexes. Among the most dramatic examples of protein subunit polymerization are the assembly of tubulin and actin into microtubules and microfilaments, respectively. The slow hydrolysis of GTP by tubulin and ATP by actin monomers, after the incorporation of these molecules into their respective polymeric filaments, promotes subtle conformational changes that later allow disassembly.

The best-characterized motor protein is myosin. A brief overview of the structure and function of myosin in the molecular events in muscle contraction is provided.

Myosin

The myosins are a family of motor proteins that transduce ATP bond energy into unidirectional movement along actin filaments. Found in all eukaryotic cells, the myosins are involved in a wide variety of cellular movements. The role of myosin in movement

is best understood in skeletal muscle contraction. Skeletal muscle myosin, referred to as myosin II, consists of two heavy chains and two light chains (Figure 5A). The N-terminal globular head domain (Figure 5B) of the heavy chain possesses separate binding sites for ATP and actin. A long α-helix extending from the head domain forms the flexible neck region and the C-terminal tail. The actin-binding cleft and ATP-binding site are on opposite sides of the myosin head domain. These sites are connected by so-called switch helices. As a result, the ATP-hydrolyzing activity of the ATP-binding site is activated when the myosin head binds to actin. In the *swinging neck–lever model* of the actin-myosin crossbridge cycle, ATP-induced changes in myosin conformation cause a leverlike swinging motion in the neck relative to the catalytic domain. The relative motion of thick (myosin filaments) and thin filaments (actin polymers complexed with several other proteins) in the muscle sarcomere (the functional unit of skeletal muscle) is caused by the swinging stroke of the myosin neck domain (Figure 5C).

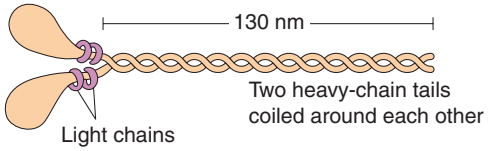


FIGURE 5A

Myosin II Structure

A myosin II molecule is composed of two heavy chains, each of which contains a globular head, a hinge region and a long rodlike tail, as well as four light chains. Two small light chains are wrapped around each myosin head.

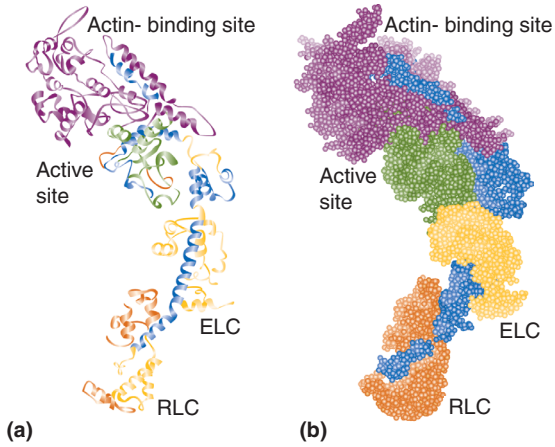


FIGURE 5B

Myosin II Head Domain Structure

(a) Ribbon model and (b) space-filling model: green, heavy-chain residues 4–204; red, heavy-chain residues 216–626; purple, heavy-chain residues 647–843; yellow, essential light chain (ELC); orange, regulatory light chain (RLC). The light chains encircle and stabilize the long α-helix formed by the heavy chain.

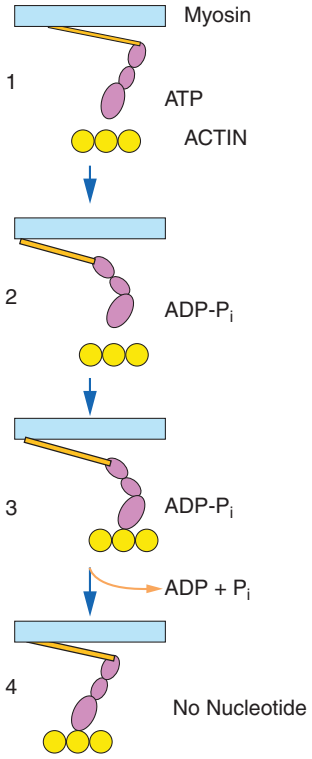


FIGURE 5C

The Actomyosin Cycle in Skeletal Muscle

(Step 1:) The myosin head has bound an ATP within the nucleotide-binding site and has detached from actin. (Step 2:) ATP hydrolysis causes the myosin head to become "cocked." (This is the energy-transducing event.) (Step 3:) Myosin binds weakly to actin. (Step 4:) Release of ADP and P_i causes the myosin head to bind tightly to actin, which is followed by the power stroke. The conformational change in the myosin head (the leverlike swinging motion) causes the myosin filament to move along the actin filament.

SUMMARY: Molecular machine function is made possible by conformational changes triggered by the hydrolysis of nucleotides bound to protein subunits called motor proteins.