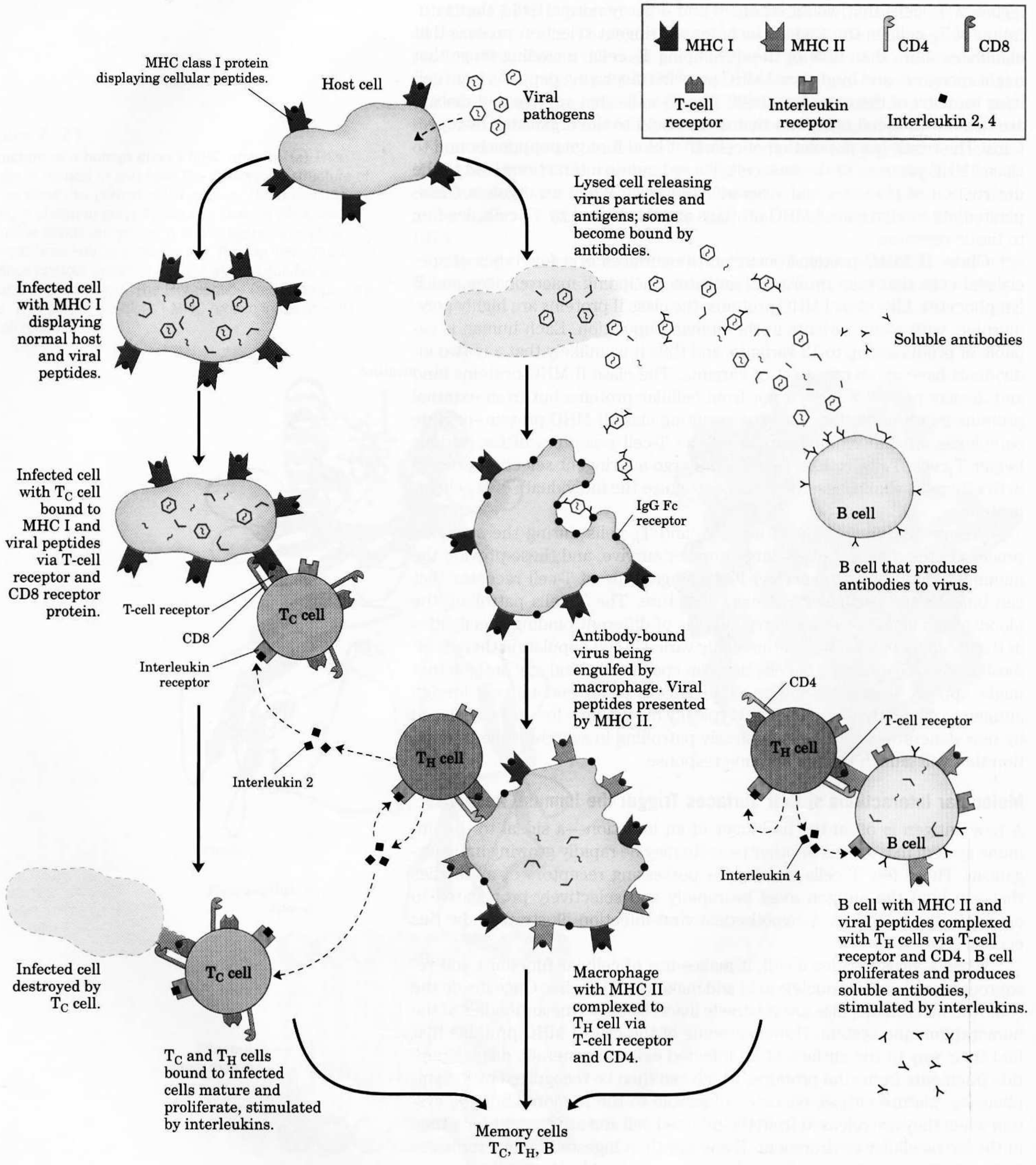

figure 7–22

Overview of the immune response to a viral infection. The individual steps are described in the text.

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The class I MHC protein–peptide complexes on infected cells are recognized as foreign and bound by those T_C cells with T-cell receptors having the appropriate binding specificity. The T-cell receptors respond only to peptide antigens that are complexed to class I MHC proteins. The T_C cells have an additional receptor, **CD8,** also called a coreceptor, that enhances the binding interactions of T-cell receptors and MHC proteins (Fig. 7–22, middle left). The T_C cells live up to the name killer T cells by destroying the virally infected cell to which they are complexed through their T-cell receptors. Cell death is brought about by a number of mechanisms, not all well understood. One mechanism involves the release of a protein called **perforin,** which binds to and aggregates in the plasma membrane of the target cell, forming molecular pores that destroy the capacity of that cell to regulate its interior environment. T_C cells also induce a process called **programmed cell death,** or **apoptosis** (most commonly pronounced app′-a-toe′-sis), in which the cells complexed to T_C cells undergo metabolic changes that rapidly lead to the demise of the cell.

T_C cells with the proper specificity must proliferate selectively if large numbers of virus-infected cells are to be destroyed. To this end, T_C cells complexed to an infected cell generate cell-surface receptors for signaling proteins called **interleukins.** Interleukins, secreted by a variety of cells, stimulate the proliferation of only those T and B cells bearing the required interleukin receptors. Because T and B cells produce interleukin receptors only when they are complexed with an antigen, the only immune system cells that proliferate are those few that can respond to the antigen. The process of producing a population of cells by stimulated reproduction of a particular ancestor cell is called **clonal selection.**

The peptides complexed to class II MHC proteins and displayed on the surface of macrophages and B lymphocytes are similarly bound by the appropriate T-cell receptors of T_H cells. The T_H cells also have a coreceptor, called **CD4,** that enhances the binding interactions of the T-cell receptors. This overall binding interaction, in concert with secondary molecular signals that are currently being identified, activates the T_H cells. A subpopulation of activated T_H cells secrete a small signal protein called interleukin-2 (IL-2; M_r 15,000), which stimulates proliferation of nearby T_C cells and T_H cells having the appropriate interleukin receptors. This greatly increases the number of available immune system cells capable of recognizing and responding to the antigen. Another subpopulation of activated T_H cells complexed to macrophages or B lymphocytes secrete interleukin-4 (IL-4; M_r 20,000), which stimulates the proliferation of B cells that recognize the antigen (Fig. 7–22, bottom right). Proliferation of the responding B, T_C, and T_H cells continues as long as the appropriate antigen is present.

The proliferating B cells promote the destruction of any extracellular viruses or bacterial cells. They first secrete large amounts of soluble antibody that binds to the antigen. This bound antibody recruits a cellular system of about 20 proteins collectively called **complement** because they complement and enhance the action of the antibodies. The complement proteins disrupt the coats of many viruses or, in bacterial infections, produce holes in the cell walls of bacteria, causing them to swell and burst by osmotic shock.

Unlike T cells, B cells do not undergo selection in the thymus to eliminate those producing antibodies that recognize host (self) proteins. However, B cells do not contribute significantly to an immune response unless they are stimulated to proliferate by T_H cells. The T_H cells *do* undergo selection in the thymus, leaving no T_H cells capable of stimulating B cells that produce antibodies potentially dangerous to the host.

The T_H cells themselves participate only indirectly in the destruction of infected cells and pathogens, but their role is critical to the entire immune

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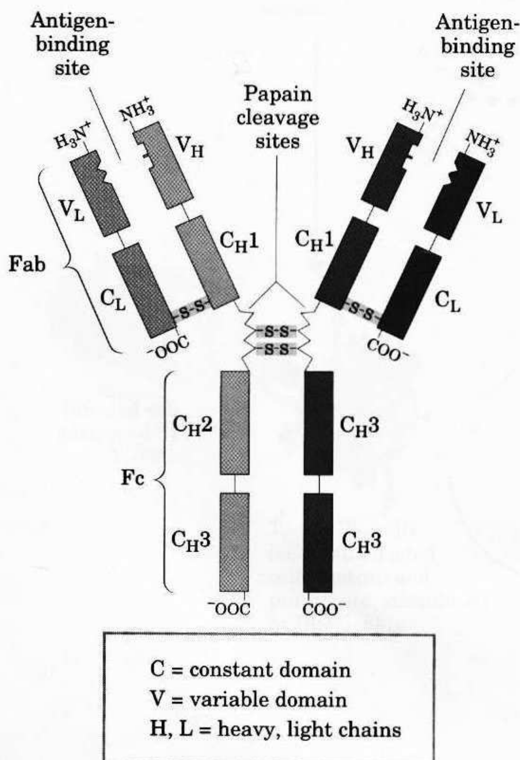
response. This is dramatically illustrated by the epidemic produced by HIV (human immunodeficiency virus), the virus that causes AIDS (acquired immune deficiency syndrome). The primary targets of HIV infection are T_H cells. Elimination of these cells progressively incapacitates the entire immune system.

Once antigen is depleted, activated immune cells generally die in a matter of days by programmed cell death. However, a few of the stimulated B and T cells mature into **memory cells.** These are long-lived cells that do not participate directly in the primary immune response when the antigen is first encountered. Instead they become permanent residents of the blood, ready to respond to a reappearance of the same antigen. Memory cells, when subsequently challenged by the antigen, can mount a secondary immune response that is generally much more rapid and vigorous than the primary response because of prior clonal expansion. By this mechanism, vertebrates once exposed to a virus or other pathogen can respond quickly to the pathogen when exposed again. This is the basis of the long-term immunity conferred by vaccines and the natural immunity to repeated infections by the same strain of a virus.

Antibodies Have Two Identical Antigen-Binding Sites

Immunoglobulin G (IgG) is the major class of antibody molecule and one of the most abundant proteins in the blood serum. IgG has four polypeptide chains: two large ones, called heavy chains, and two light chains, linked by noncovalent and disulfide bonds into a complex of M_r 150,000. The heavy chains of an IgG molecule interact at one end, then branch to interact separately with the light chains, forming a Y-shaped molecule (Fig. 7–23). At the "hinges" separating the base of an IgG molecule from its branches, the immunoglobulin can be cleaved with proteases. Cleavage with the protease papain liberates the basal fragment, called **Fc** because it usually *c*rystallizes readily, and the two branches, which are called **Fab,** the *a*ntigen-*b*inding fragments. Each branch has a single antigen-binding site.



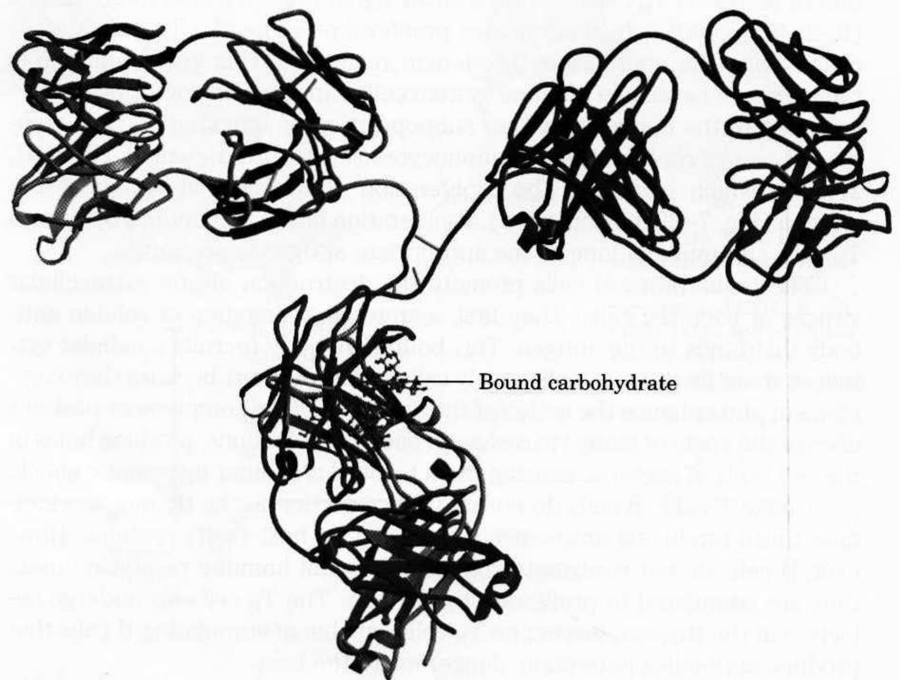


figure 7–23
The structure of immunoglobulin G. (a) Pairs of heavy and light chains combine to form a Y-shaped molecule. Two antigen-binding sites are formed by the combination of variable domains from one light (V_L) and one heavy (V_H) chain. Cleavage with papain separates the Fab and Fc portions of the protein in the hinge region. The Fc portion of the molecule also contains bound carbohydrate. **(b)** A ribbon model of the first complete IgG molecule to be crystallized and structurally analyzed. Although the molecule contains two identical heavy chains (two shades of blue) and two identical light chains (two shades of red), it crystallized in the asymmetric conformation shown. Conformational flexibility may be important to the function of immunoglobulins.

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The fundamental structure of immunoglobulins was first established by Gerald Edelman and Rodney Porter. Each chain is made up of identifiable domains; some are constant in sequence and structure from one IgG to the next, others are variable. The constant domains have a characteristic structure known as the **immunoglobulin fold,** a well-conserved structural motif in the all-β class. There are three of these constant domains in each heavy chain and one in each light chain. The heavy and light chains also have one variable domain each, in which most of the variability in amino acid residue sequence is found. The variable domains associate to create the antigen-binding site (Fig. 7–24).

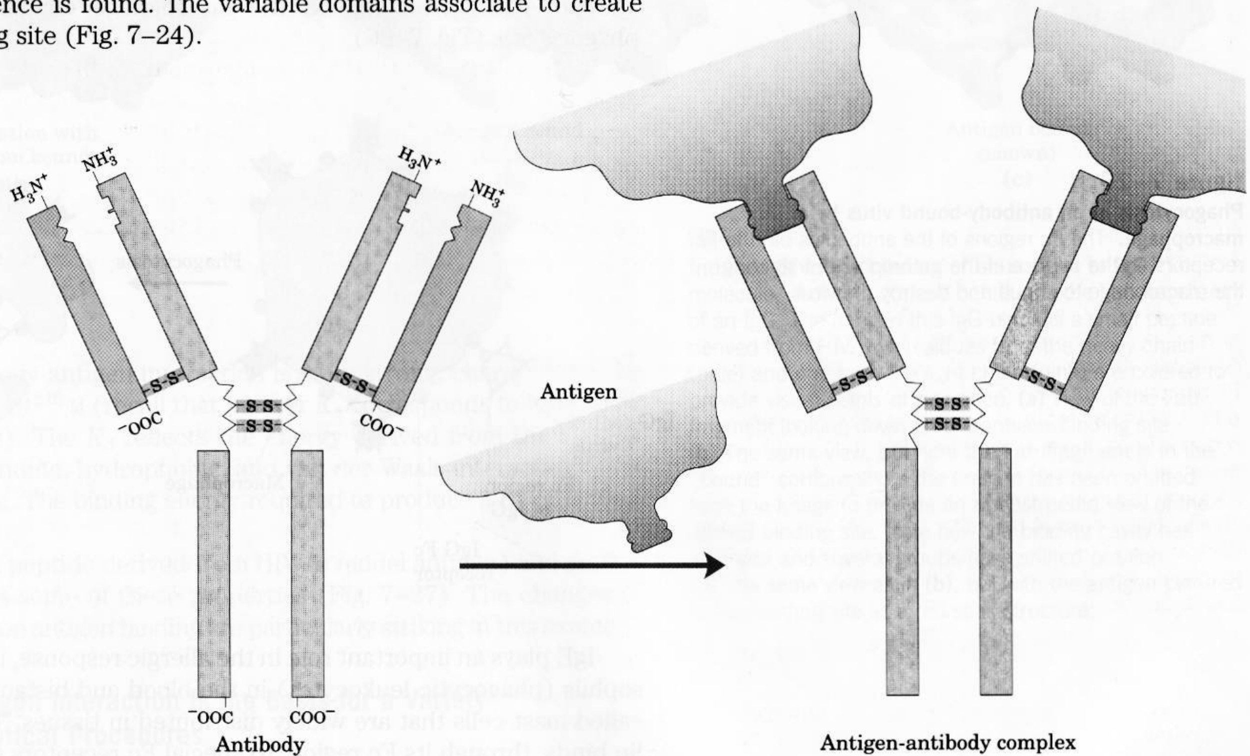

figure 7–24
Binding of IgG to an antigen. To generate an optimal fit for the antigen, the binding sites of IgG often undergo slight conformational changes. Such induced fit is common to many protein-ligand interactions.

In many vertebrates, IgG is only one of five classes of immunoglobulins. Each class has a characteristic type of heavy chain, denoted α, δ, ϵ, γ, and μ for IgA, IgD, IgE, IgG, and IgM, respectively. Two types of light chain, κ and λ, occur in all classes of immunoglobulins. The overall structures of **IgD** and **IgE** are similar to that of IgG. **IgM** occurs in either a monomeric, membrane-bound form or a secreted form that is a cross-linked pentamer of this basic structure (Fig. 7–25). **IgA,** found principally in secretions such as saliva, tears, and milk, can be a monomer, dimer, or trimer. IgM is the first antibody to be made by B lymphocytes and is the major antibody in the early stages of a primary immune response. Some B cells soon begin to produce IgD (with the same antigen-binding site as the IgM produced by the same cell), but the unique function of IgD is less clear.

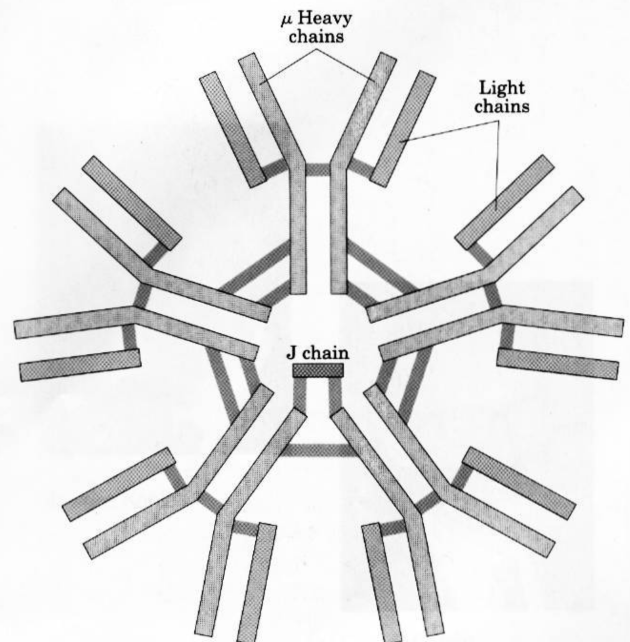

figure 7–25
IgM pentamer of immunoglobulin units. The pentamer is cross-linked with disulfide bonds. The J chain is a polypeptide of M_r 20,000 found in both IgA and IgM.

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The IgG described above is the major antibody in secondary immune responses, which are initiated by memory B cells. As part of the organism's ongoing immunity to antigens already encountered and dealt with, IgG is the most abundant immunoglobulin in the blood. When IgG binds to an invading bacterium or virus, it not only activates the complement system, but also activates certain leukocytes such as macrophages to engulf and destroy the invader. Yet another class of receptors on the cell surface of macrophages recognizes and binds the Fc region of IgG. When these Fc receptors bind an antibody-pathogen complex, the macrophage engulfs the complex by phagocytosis (Fig. 7–26).

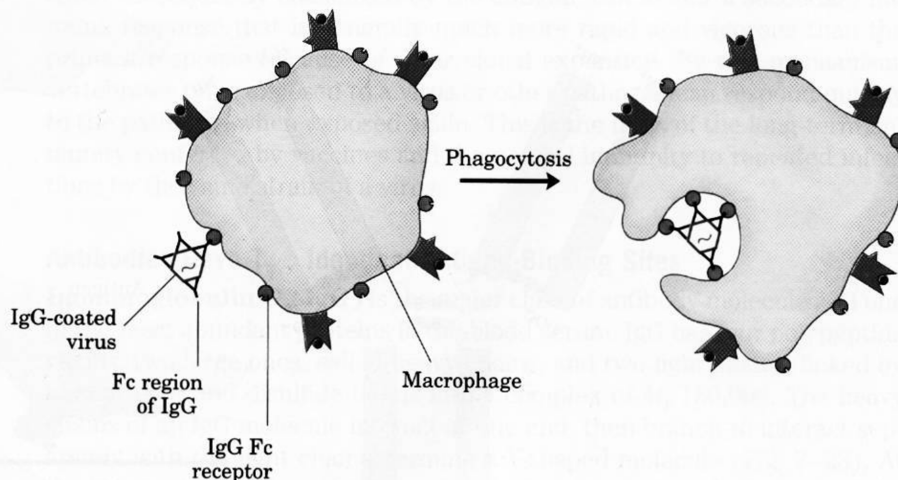

figure 7–26
Phagocytosis of an antibody-bound virus by a macrophage. The Fc regions of the antibodies bind to Fc receptors on the surface of the macrophage, triggering the macrophage to engulf and destroy the virus.

IgE plays an important role in the allergic response, interacting with basophils (phagocytic leukocytes) in the blood and histamine-secreting cells called mast cells that are widely distributed in tissues. This immunoglobulin binds, through its Fc region, to special Fc receptors on the basophils or mast cells. In this form, IgE serves as a kind of receptor for antigen. If antigen is bound, the cells are induced to secrete histamine and other biologically active amines that cause dilation and increased permeability of blood vessels. These effects on the blood vessels are thought to facilitate the movement of immune system cells and proteins to sites of inflammation. They also produce the symptoms normally associated with allergies. Pollen or other allergens are recognized as foreign, triggering an immune response normally reserved for pathogens.

Antibodies Bind Tightly and Specifically to Antigen

The binding specificity of an antibody is determined by the amino acid residues in the variable domains of its heavy and light chains. Many residues in these domains are variable, but not equally so. Some, particularly those lining the antigen-binding site, are hypervariable—especially likely to differ. Specificity is conferred by chemical complementarity between the antigen and its specific binding site, in terms of shape and the location of charged, nonpolar, and hydrogen-bonding groups. For example, a binding site with a negatively charged group may bind an antigen with a positive charge in the complementary position. In many instances, complementarity is achieved interactively as the structures of antigen and binding site are influenced by each other during the approach of the ligand. Conformational changes in the antibody and/or the antigen then occur that allow the complementary groups to interact fully. This is an example of induced fit (Fig. 7–27).

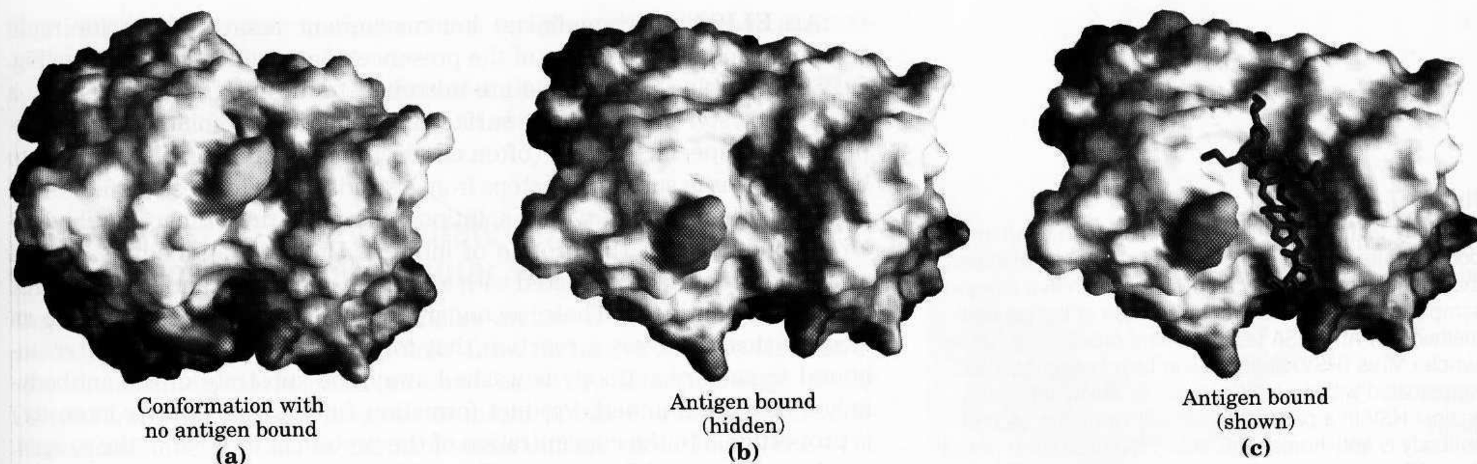

figure 7–27
Induced fit in the binding of an antigen to IgG. The molecule, shown in surface contour, is the Fab fragment of an IgG. The antigen this IgG binds is a small peptide derived from HIV. Two residues from the heavy chain (blue) and one from the light chain (pink) are colored to provide visual points of reference. **(a)** View of the Fab fragment looking down on the antigen-binding site. **(b)** The same view, but here the Fab fragment is in the "bound" conformation; the antigen has been omitted from the image to provide an unobstructed view of the altered binding site. Note how the binding cavity has enlarged and several groups have shifted position. **(c)** The same view as in **(b),** but with the antigen pictured in the binding site as a red stick structure.

A typical antibody-antigen interaction is quite strong, characterized by K_d values as low as 10^{-10} M (recall that a lower K_d corresponds to a stronger binding interaction). The K_d reflects the energy derived from the various ionic, hydrogen-bonding, hydrophobic, and van der Waals interactions that stabilize the binding. The binding energy required to produce a K_d of 10^{-10} M is about 65 kJ/mol.

A complex of a peptide derived from HIV (a model antigen) and an Fab molecule illustrates some of these properties (Fig. 7–27). The changes in structure observed on antigen binding are particularly striking in this example.

The Antibody-Antigen Interaction Is the Basis for a Variety of Important Analytical Procedures

The extraordinary binding affinity and specificity of antibodies makes them valuable analytical reagents. Two types of antibody preparations are in use: polyclonal and monoclonal. **Polyclonal antibodies** are those produced by many different B lymphocytes responding to one antigen, such as a protein injected into an animal. Cells in the population of B lymphocytes produce antibodies that bind specific, different epitopes within the antigen. Thus, polyclonal preparations contain a mixture of antibodies that recognize different parts of the protein. **Monoclonal antibodies,** in contrast, are synthesized by a population of identical B cells (a **clone**) grown in cell culture. These antibodies are homogeneous, all recognizing the same epitope. The techniques for producing monoclonal antibodies were developed by Georges Köhler and Cesar Milstein.

Georges Köhler

Cesar Milstein

The specificity of antibodies has practical uses. A selected antibody can be covalently attached to a resin and used in a chromatography column of the type shown in Figure 5–18c. When a mixture of proteins is added to the column, the antibody will specifically bind its target protein and retain it on the column while other proteins are washed through. The target protein can then be eluted from the resin by a salt solution or some other agent. This is a powerful tool for protein purification.

In another versatile analytical technique, an antibody is attached to a radioactive label or some other reagent that makes it easy to detect. When the antibody binds the target protein, the label reveals the presence of the protein in a solution or its location in a gel or even a living cell. Several variations of this procedure are illustrated in Figure 7–28.

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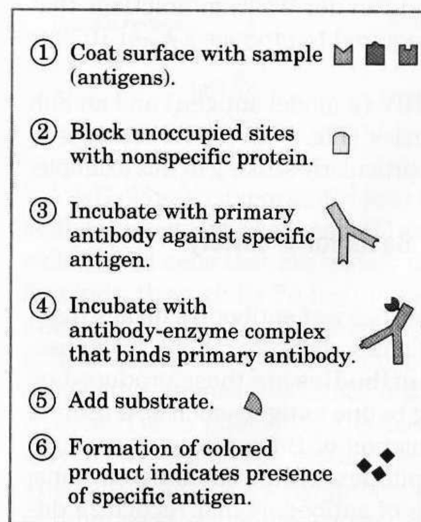
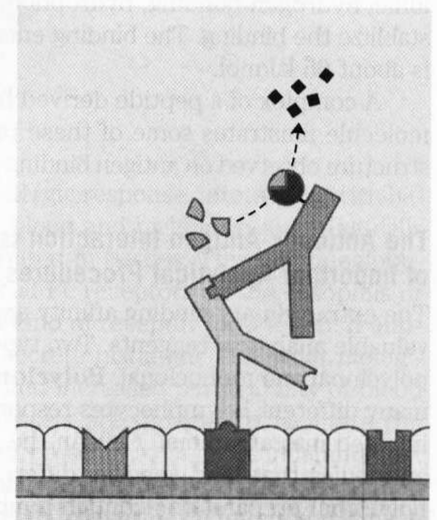
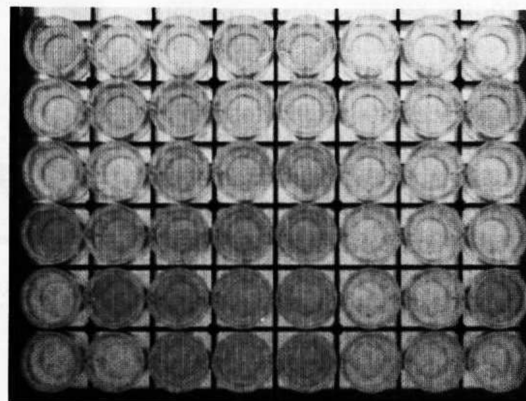
An **ELISA** (enzyme-linked immunosorbent assay) allows for rapid screening and quantification of the presence of an antigen in a sample (Fig. 7–28b). Proteins in a sample are adsorbed to an inert surface, usually a 96-well polystyrene plate. The surface is washed with a solution of an inexpensive nonspecific protein (often casein from nonfat dry milk powder) to block proteins in subsequent steps from also adsorbing to these surfaces. The surface is then treated with a solution containing the primary antibody—an antibody against the protein of interest. Unbound antibody is washed away and the surface is treated with a solution containing antibodies against the primary antibody. These secondary antibodies have been linked to an enzyme that catalyzes a reaction that forms a colored product. After unbound secondary antibody is washed away, the substrate of the antibody-linked enzyme is added. Product formation (monitored as color intensity) is proportional to the concentration of the protein of interest in the sample.

In an **immunoblot assay** (Fig. 7–28c), proteins that have been separated by gel electrophoresis are transferred electrophoretically to a nitrocellulose membrane. The membrane is blocked (as described above for ELISA), then treated successively with primary antibody, secondary antibody linked to enzyme, and substrate. A colored precipitate forms only along the band containing the protein of interest. The immunoblot allows

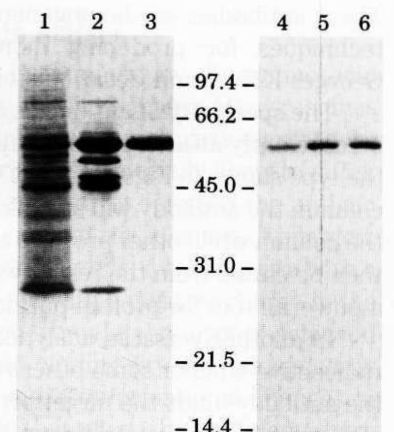




(a)



ELISA assay
(b)



(c)

figure 7–28
Antibody techniques. The specific reaction of an antibody with its antigen is the basis of several techniques that identify and quantify a specific protein in a complex sample. **(a)** A schematic representation of the general method. **(b)** An ELISA testing for the presence of herpes simplex virus (HSV) antibodies in blood samples. Wells were coated with an HSV antigen, to which antibodies against HSV in a patient's blood will bind. The second antibody is anti–human IgG linked to horseradish peroxidase. Blood samples with greater amounts of HSV antibody turn brighter yellow. **(c)** An immunoblot. Lanes 1 to 3 are from an SDS gel; samples from successive stages in the purification of a protein kinase have been separated and stained with Coomassie blue. Lanes 4 to 6 show the same samples, but these were electrophoretically transferred to a nitrocellulose membrane after separation on an SDS gel. The membrane was then "probed" with antibody against the protein kinase. The numbers between the gel and the immunoblot indicate M_r ($\times 10^{-3}$).

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the detection of a minor component in a sample and provides an approximation of its molecular weight.

We will encounter other aspects of antibodies in later chapters. They are extremely important in medicine and can tell us much about the structure of proteins and the action of genes.

Protein Interactions Modulated by Chemical Energy: Actin, Myosin, and Molecular Motors

Organisms move. Cells move. Organelles and macromolecules within cells move. Most of these movements arise from the activity of the fascinating class of protein-based molecular motors. Fueled by chemical energy, usually derived from ATP, large aggregates of motor proteins undergo cyclic conformational changes that accumulate into a unified, directional force—the tiny force that pulls apart chromosomes in a dividing cell, and the immense force that levers a pouncing, quarter-ton jungle cat into the air.

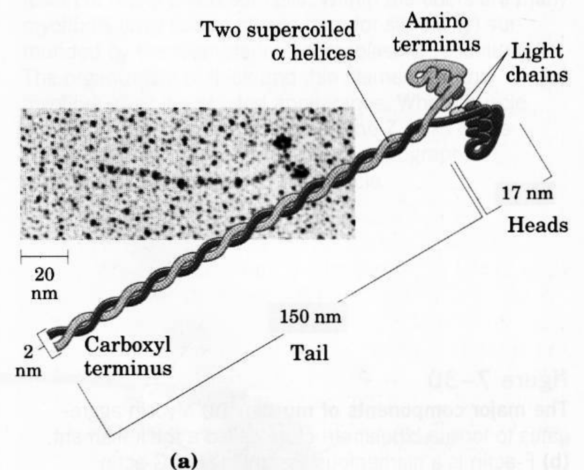
The interactions among motor proteins, as you might predict, feature complementary arrangements of ionic, hydrogen-bonding, hydrophobic, and van der Waals interactions at protein binding sites. In motor proteins, however, these interactions achieve exceptionally high levels of spatial and temporal organization.

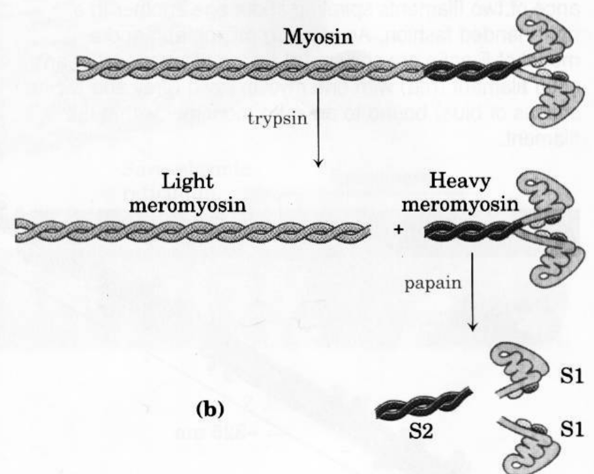
Motor proteins underlie the contraction of muscles, the migration of organelles along microtubules, the rotation of bacterial flagella, and the movement of some proteins along DNA. As we noted in Chapter 2, proteins called kinesins and dyneins move along microtubules in cells, pulling along organelles or reorganizing chromosomes during cell division (see Fig. 2–19). An interaction of dynein with microtubules brings about the motion of eukaryotic flagella and cilia. Flagellar motion in bacteria involves a complex rotational motor at the base of the flagellum (see Fig. 19–32). Helicases, polymerases, and other proteins move along DNA as they carry out their functions in DNA metabolism (Chapter 25). Here, we focus on the well-studied example of the contractile proteins of vertebrate skeletal muscle as a paradigm for how proteins translate chemical energy into motion.

The Major Proteins of Muscle Are Myosin and Actin

The contractile force of muscle is generated by the interaction of two proteins, myosin and actin. These proteins are arranged in filaments that undergo transient interactions and slide past each other to bring about contraction. Together, actin and myosin make up over 80% of the protein mass of muscle.

Myosin (M_r 540,000) has six subunits: two heavy chains (M_r 220,000) and four light chains (M_r 20,000). The heavy chains account for much of the overall structure. At their carboxyl termini, they are arranged as extended α helices, wrapped around each other in a fibrous, left-handed coiled coil similar to that of α-keratin (Fig. 7–29a). At its amino termini, each heavy chain has a large globular domain containing a site where ATP is hydrolyzed. The light chains are associated with the globular domains.





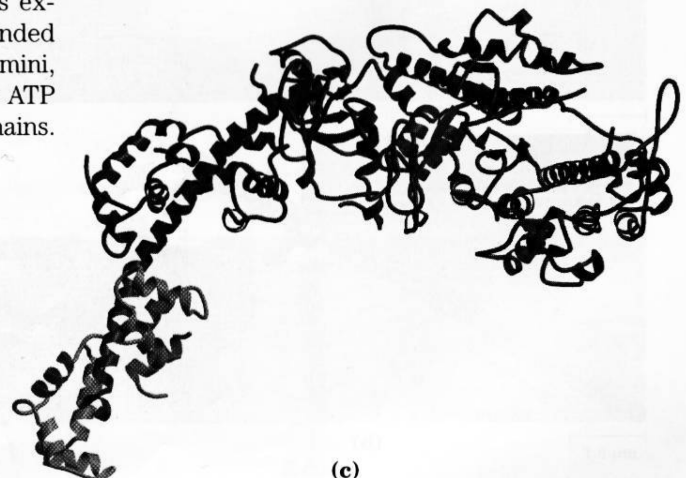
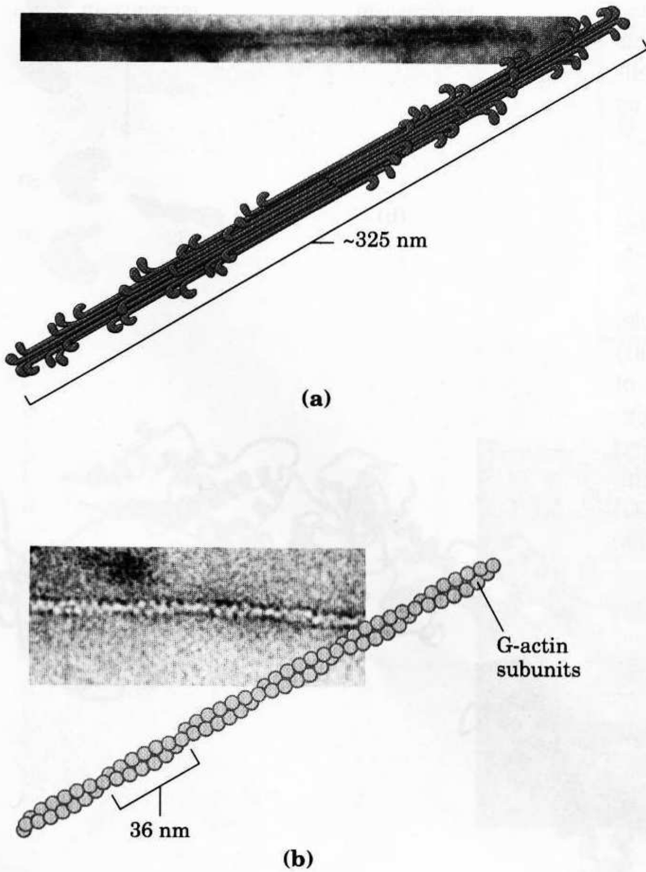


figure 7–29
Myosin. (a) Myosin has two heavy chains (in two shades of pink), the carboxyl termini forming an extended coiled coil (tail) and the amino termini having globular domains (heads). Two light chains (blue) are associated with each myosin head. **(b)** Cleavage with trypsin and papain separates the myosin heads (S1 fragments) from the tails. **(c)** Ribbon representation of the myosin S1 fragment. The heavy chain is in gray, the two light chains in two shades of blue.

When myosin is treated briefly with the protease trypsin, much of the fibrous tail is cleaved off, dividing the protein into components called light and heavy meromyosin (Fig. 7–29b). The globular domain, called myosin subfragment 1, or S1, or simply the myosin head group, is liberated from heavy meromyosin by cleavage with papain. The S1 fragment produced by this procedure is the motor domain that makes muscle contraction possible. S1 fragments can be crystallized, and their structure has been determined. The overall structure of the S1 fragment as determined by Ivan Rayment and Hazel Holden is shown in Figure 7–29c.

In muscle cells, molecules of myosin aggregate to form structures called **thick filaments** (Fig. 7–30a). These rodlike structures serve as the core of the contractile unit. Within a thick filament, several hundred myosin molecules are arranged with their fibrous "tails" associated to form a long bipolar structure. The globular domains project from either end of this structure, in regular stacked arrays.

The second major muscle protein, **actin,** is abundant in almost all eukaryotic cells. In muscle, molecules of monomeric actin, called G-actin (*g*lobular actin; M_r 42,000), associate to form a long polymer called F-actin (*f*ilamentous actin). The **thin filament** (Fig. 7–30b) consists of F-actin, along with the proteins troponin and tropomyosin. The filamentous parts of thin filaments assemble as successive monomeric actin molecules add to one end. On addition, each monomer binds ATP, then hydrolyzes it to ADP, so all actin molecules in the filament are complexed to ADP. However, this ATP hydrolysis by actin functions only in the assembly of the filaments; it does not contribute directly to the energy expended in muscle contraction. Each actin monomer in the thin filament can bind tightly and specifically to one myosin head group (Fig. 7–30c).



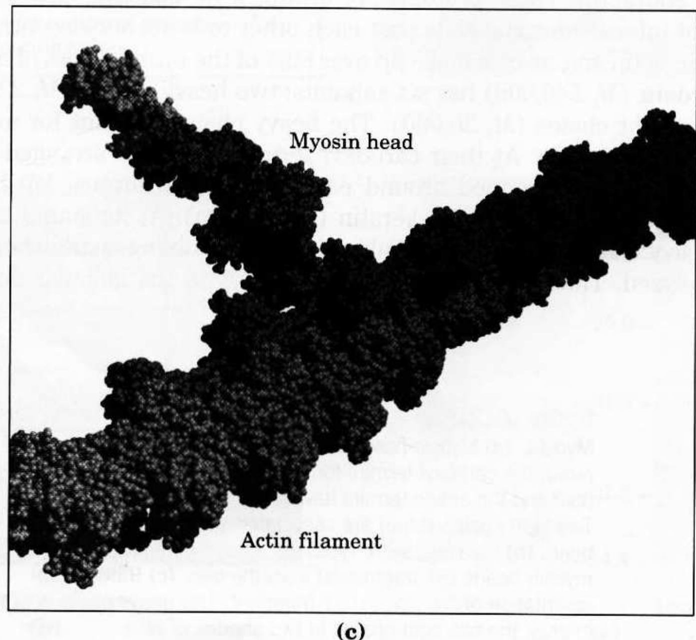


figure 7–30
The major components of muscle. (a) Myosin aggregates to form a bipolar structure called a thick filament. **(b)** F-actin is a filamentous assemblage of G-actin monomers that polymerize two by two, giving the appearance of two filaments spiraling about one another in a right-handed fashion. An electron micrograph and a model of F-actin are shown. **(c)** Space-filling model of an actin filament (red) with one myosin head (gray and two shades of blue) bound to an actin monomer within the filament.

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Additional Proteins Organize the Thin and Thick Filaments into Ordered Structures

Skeletal muscle consists of parallel bundles of **muscle fibers,** each fiber a single, very large, multinucleated cell, 20 to 100 μm in diameter, formed from many cells fused together and often spanning the length of the muscle. Each fiber, in turn, contains about 1,000 **myofibrils,** 2 μm in diameter, each consisting of a vast number of regularly arrayed thick and thin filaments complexed to other proteins (Fig. 7–31). A system of flat membranous vesicles called the **sarcoplasmic reticulum** surrounds each myofibril. Examined under the electron microscope, muscle fibers reveal alternating regions of high and low electron density, called the **A** and **I bands** (Fig. 7–31b,c). The A and I bands arise from the arrangement of

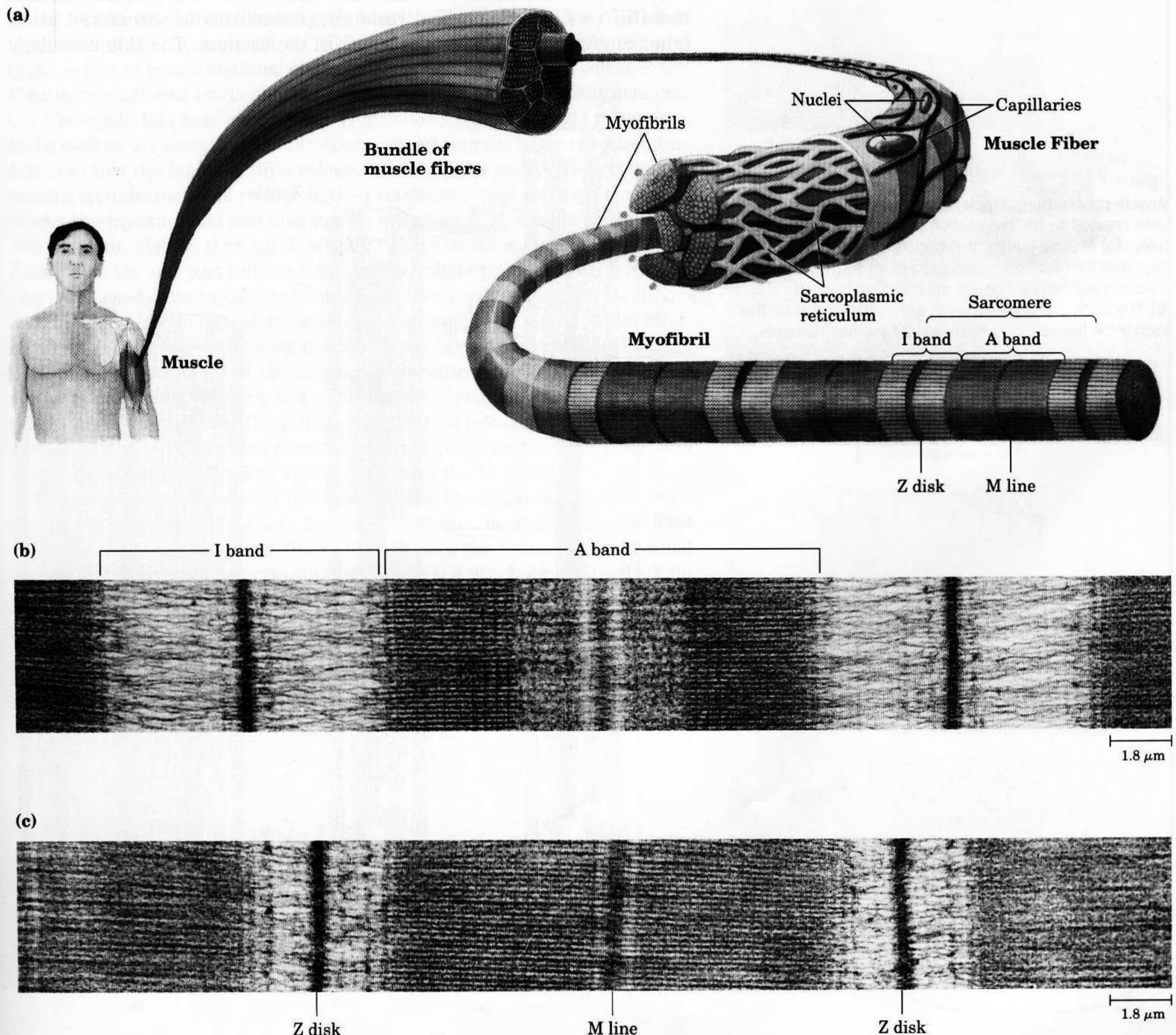

figure 7–31
Structure of skeletal muscle. (a) Muscle fibers consist of single, elongated, multinucleated cells that arise from the fusion of many precursor cells. Within the fibers are many myofibrils (only six are shown here for simplicity) surrounded by the membranous sarcoplasmic reticulum. The organization of thick and thin filaments in the myofibril gives it a striated appearance. When muscle contracts, the I bands narrow and the Z disks come closer together, as seen in electron micrographs of relaxed **(b)** and contracted **(c)** muscle.

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thick and thin filaments, which are aligned and partially overlapping. The I band is the region of the bundle that in cross section would contain only thin filaments. The darker A band stretches the length of the thick filament and includes the region where parallel thick and thin filaments overlap. Bisecting the I band is a thin structure called the **Z disk,** perpendicular to the thin filaments and serving as an anchor to which the thin filaments are attached. The A band too is bisected by a thin line, the **M line** or M disk, a region of high electron density in the middle of the thick filaments. The entire contractile unit, consisting of bundles of thick filaments interleaved at either end with bundles of thin filaments, is called the **sarcomere.** The arrangement of interleaved bundles allows the thick and thin filaments to slide past each other (by a mechanism discussed below), causing a progressive shortening of each sarcomere (Fig. 7–32).

The thin actin filaments are attached at one end to the Z disk in a regular pattern. The assembly includes the minor muscle proteins **α-actinin, desmin,** and **vimentin.** Thin filaments also contain a large protein called **nebulin** (~7,000 amino acid residues), thought to be structured as an α helix long enough to span the length of the filament. The M line similarly

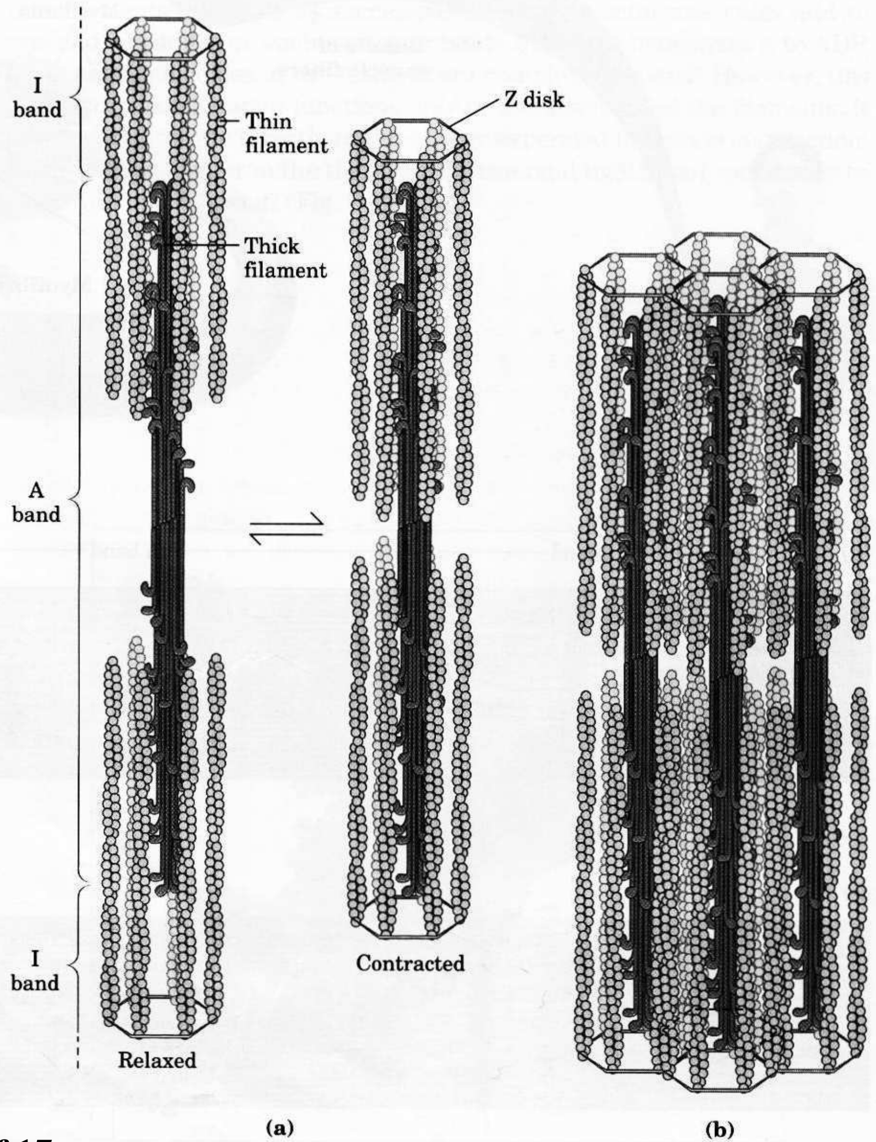

figure 7–32
Muscle contraction. Thick filaments are bipolar structures created by the association of many myosin molecules. **(a)** Muscle contraction occurs by the sliding of the thick and thin filaments past each other so that the Z disks in neighboring I bands approach each other. **(b)** The thick and thin filaments are interleaved such that each thick filament is surrounded by six thin filaments.

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organizes the thick filaments. It contains the proteins **paramyosin, C-protein,** and **M-protein.** Another class of proteins called **titins,** the largest known single polypeptide chains (the titin of human cardiac muscle has 26,926 amino acid residues), link the thick filaments to the Z disk, providing additional organization to the overall structure. Among their structural functions, the proteins nebulin and titin are believed to act as "molecular rulers," regulating the length of the thin and thick filaments, respectively. Titin extends from the Z disk to the M line, regulating the length of the sarcomere itself and preventing overextension of the muscle. The characteristic sarcomere length varies from one muscle tissue to the next in a vertebrate organism, attributed in large part to the expression of different titin variants.

Myosin Thick Filaments Slide along Actin Thin Filaments

The interaction between actin and myosin, like that between all proteins and ligands, involves weak bonds. When ATP is not bound to myosin, a face on the myosin head group binds tightly to actin (Fig. 7–33). When ATP binds to myosin and is hydrolyzed to ADP and phosphate, a coordinated and cyclic series of conformational changes occur in which myosin releases the F-actin subunit and binds another subunit farther along the thin filament.

The cycle has four major steps (Fig. 7–33). ① ATP binds to myosin, and a cleft in the myosin molecule opens, disrupting the actin-myosin interaction so that the bound actin is released. ATP is then hydrolyzed (step ②), causing a conformational change in the protein to a "high-energy" state that moves the myosin head and changes its orientation in relation to the actin thin filament. Myosin then binds weakly to an F-actin subunit closer to the Z disk than the one just released. As the phosphate product of ATP hydrolysis is released from myosin in step ③, another conformational change occurs in which the myosin cleft closes, strengthening the myosin-actin binding. This is followed quickly by the final step, ④, a "power stroke" during which the conformation of the myosin head returns to the original resting state, its orientation relative to the bound actin changing so as to pull the tail of the myosin toward the Z disk. ADP is then released to complete the cycle. Each cycle generates about 3 to 4 pN (piconewtons) of force and moves the thick filament 5 to 10 nm relative to the thin filament.

Because there are many myosin heads in a thick filament, at any given moment some (probably 1% to 3%) are bound to the thin filaments. This prevents the thick filaments from slipping backward when an individual myosin head releases the actin subunit to which it was bound. The thick filament thus actively slides forward past the adjacent thin filaments. This process, coordinated among the many sarcomeres in a muscle fiber, brings about muscle contraction.

The interaction between actin and myosin must be regulated so that contraction occurs only in response to appropriate signals from the nervous

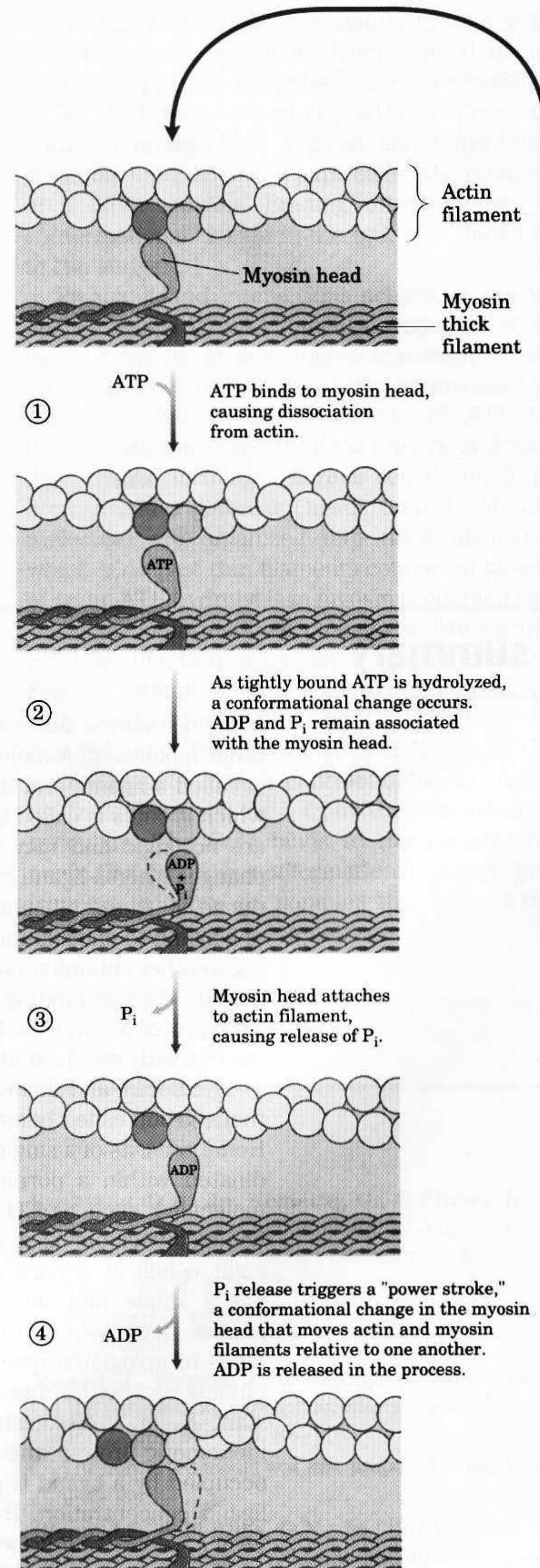

figure 7–33
Molecular mechanism of muscle contraction. Conformational changes in the myosin head that are coupled to stages in the ATP hydrolytic cycle cause myosin to successively dissociate from one actin subunit, then associate with another farther along the actin filament. In this way the myosin heads slide along the thin filaments, drawing the thick filament array into the thin filament array (see Fig. 7–32).

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system. The regulation is mediated by a complex of two proteins, **tropomyosin** and **troponin.** Tropomyosin binds to the thin filament, blocking the attachment sites for the myosin head groups. Troponin is a Ca^{2+}-binding protein. A nerve impulse causes release of Ca^{2+} from the sarcoplasmic reticulum. The released Ca^{2+} binds to troponin (another protein-ligand interaction) and causes a conformational change in the tropomyosin-troponin complexes, exposing the myosin-binding sites on the thin filaments. Contraction follows.

Working skeletal muscle requires two types of molecular functions that are common in proteins—binding and catalysis. The actin-myosin interaction, a protein-ligand interaction like that of immunoglobulins with antigens, is reversible and leaves the participants unchanged. When ATP binds myosin, however, it is hydrolyzed to ADP and P_i. Myosin is not only an actin-binding protein, it is also an ATPase—an enzyme. The function of enzymes in catalyzing chemical transformations is the topic of the next chapter.

summary

Protein function often entails interactions with other molecules. A molecule bound by a protein is called a ligand, and the site on the protein to which it binds is called the binding site. Proteins are not rigid and may undergo conformational changes when a ligand binds, a process called induced fit. In a multisubunit protein, the binding of a ligand to one subunit may affect ligand binding to other subunits. Ligand binding can be regulated. Oxygen-binding proteins, immune system proteins, and motor proteins are useful models with which to illustrate these principles.

Myoglobin and hemoglobin contain a prosthetic group called heme to which oxygen binds. Heme consists of a single atom of Fe^{2+} iron coordinated within a porphyrin. Some other small molecules, such as CO and NO, can also bind heme. Myoglobin is a single polypeptide with eight α-helical regions connected by bends. It has a single molecule of heme, located in a pocket deep within the polypeptide. Oxygen binds to myoglobin reversibly. Simple reversible binding can be described by an association constant K_a or a dissociation constant K_d. For a monomeric protein, the fraction of binding sites occupied by a ligand is a hyperbolic function of ligand concentration. Because O_2 is a gas, the term P_{50}, which describes the partial pressure of oxygen at which an O_2-binding protein is half saturated with bound oxygen, is used in place of K_d. The entry and exit of O_2 depend upon small molecular motions, called "breathing," of the myoglobin molecule.

Normal adult hemoglobin has four heme-containing subunits, two α and two β. They are similar in structure to each other and to myoglobin. Strong interactions occur between unlike (α and β) subunits. Hemoglobin exists in two interchangeable states, called T and R. The T state is stabilized by several salt bridges and is most stable when oxygen is not bound. Oxygen binding promotes a transition to the R state.

Oxygen binding to hemoglobin is both allosteric and cooperative. Binding of O_2 to one binding site of hemoglobin affects binding of O_2 to other such sites, an example of allosteric binding behavior. Conformational changes between the T and R states, mediated by subunit-subunit interactions, give rise to a form of allostery called cooperative binding. Cooperative binding results in a sigmoid binding curve and can be analyzed by a Hill plot. Two major models have been proposed to explain the cooperative binding of ligands to multisubunit proteins. In the concerted model, all subunits are in the same conformation at any given time, and the entire protein undergoes a reversible transition between two possible conformations. Successive binding of ligand molecules to the low-affinity conformation facilitates transition to the high-affinity conformation. In the sequential model, individual subunits can undergo conformational changes. Binding of a ligand to one subunit alters that subunit's conformation, facilitating similar changes in, and binding of additional ligands to, the other subunits.

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Hemoglobin also binds H^+ and CO_2. In both cases, binding results in the formation of ion pairs that stabilize the T state and O_2 binding is weakened, a phenomenon called the Bohr effect. The binding of H^+ and CO_2 to hemoglobin in the tissues promotes the release of O_2, and the binding of O_2 to hemoglobin in the lungs promotes the release of H^+ and CO_2. Oxygen binding to hemoglobin is also modulated by 2,3-bisphosphoglycerate, which binds to and stabilizes the T state.

Sickle-cell anemia is a genetic disease caused by a single amino acid substitution (Glu to Val) at position 6 in the β chains of hemoglobin. The change produces a hydrophobic patch on the surface of the protein that causes the hemoglobin molecules to aggregate into bundles of fibers. These bundles give the erythrocytes a sickle shape. This homozygous condition results in serious medical complications.

The immune response is mediated by interactions among an array of specialized leukocytes and their associated proteins. T lymphocytes produce T-cell receptors. B lymphocytes produce immunoglobulins. All cells produce MHC proteins, which display host (self) or antigenic (nonself) peptides on the cell surface. Helper T cells induce the proliferation of those B cells and cytotoxic T cells producing immunoglobulins or T-cell receptors that bind to a specific antigen, a process called clonal selection.

Humans have five classes of immunoglobulins, each with different biological functions. The most abundant is IgG, a Y-shaped protein with two heavy and two light chains. The domains near the upper ends of the Y are hypervariable within the broad population of IgGs and form two antigen-binding sites. A given immunoglobulin generally binds to only a part, called the epitope, of a large antigen. Binding often involves a conformational change in the IgG, an induced fit to the antigen.

Protein-ligand interactions achieve a special degree of spatial and temporal organization in motor proteins. Muscle contraction results from choreographed interactions between myosin and actin, coupled to the hydrolysis of ATP by myosin. Myosin consists of two heavy and four light chains, forming a fibrous coiled coil (tail) domain and a globular (head) domain. Myosin molecules are organized into thick filaments, which slide past thin filaments composed largely of actin. ATP hydrolysis in myosin is coupled to a series of conformational changes in the myosin head, leading to dissociation of myosin from one F-actin subunit and its eventual reassociation with another F-actin subunit farther along the thin filament. The myosin thus slides along the actin filaments. Muscle contraction is stimulated by the release of Ca^{2+} from the sarcoplasmic reticulum. The Ca^{2+} binds to the protein troponin, leading to a conformational change in a troponin-tropomyosin complex that triggers the cycle of actin-myosin interactions.

further reading

Oxygen-Binding Proteins

Ackers, G.K. & Hazzard, J.H. (1993) Transduction of binding energy into hemoglobin cooperativity. *Trends Biochem. Sci.* **18,** 385–390.

Changeux, J.-P. (1993) Allosteric proteins: from regulatory enzymes to receptors—personal recollections. *Bioessays* **15,** 625–634.

An interesting perspective from a leader in the field.

Dickerson, R.E. & Geis, I. (1982) *Hemoglobin: Structure, Function, Evolution, and Pathology,* The Benjamin/Cummings Publishing Company, Redwood City, CA.

di Prisco, G., Condò, S.G., Tamburrini, M., & Giardina, B. (1991) Oxygen transport in extreme environments. *Trends Biochem. Sci.* **16,** 471–474.

A revealing comparison of the oxygen-binding properties of hemoglobins from polar species.

Koshland, D.E., Jr., Nemethy, G., & Filmer, D. (1966) Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry* **6,** 365–385.

The paper in which the sequential model is introduced.

Monod, J., Wyman, J., & Changeux, J.-P. (1965) On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* **12,** 88–118.

The concerted model was first proposed in this landmark paper.

Olson, J.S. & Phillips, G.N., Jr. (1996) Kinetic pathways and barriers for ligand binding to myoglobin. *J. Biol. Chem.* **271,** 17,593–17,596.

Perutz, M.F. (1989) Myoglobin and haemoglobin: role of distal residues in reactions with haem ligands. *Trends Biochem. Sci.* **14,** 42–44.

Perutz, M.F., Wilkinson, A.J., Paoli, M., & Dodson, G.G. (1998) The stereochemical mechanism of the cooperative effects in hemoglobin revisited. *Annu. Rev. Biophys. Biomol. Struct.* **27,** 1–34.

Immune System Proteins

Blom, B., Res, P.C., & Spits, H. (1998) T cell precursors in man and mice. *Crit. Rev. Immunol.* **18,** 371–388.

Cohen, I.R. (1988) The self, the world and autoimmunity. *Sci. Am.* **258** (April), 52–60.

Davies, D.R. & Chacko, S. (1993) Antibody structure. *Acc. Chem. Res.* **26,** 421–427.

Davies, D.R., Padlan, E.A., & Sheriff, S. (1990) Antibody-antigen complexes. *Annu. Rev. Biochem.* **59,** 439–473.

Davis, M.M. (1990) T cell receptor gene diversity and selection. *Annu. Rev. Biochem.* **59,** 475–496.

Dutton, R.W., Bradley, L.M., & Swain, S.L. (1998) T cell memory. *Annu. Rev. Immunol.* **16,** 201–223.

Life, Death and the Immune System. (1993) *Sci. Am.* **269** (September).

A special issue on the immune system.

Marrack, P. & Kappler, J. (1987) The T cell receptor. *Science* **238,** 1073–1079.

Müller-Eberhard, H.J. (1988) Molecular organization and function of the complement system. *Annu. Rev. Biochem.* **57,** 321–337.

Parham, P. & Ohta, T. (1996) Population biology of antigen presentation by MHC class I molecules. *Science* **272,** 67–74.

Ploegh, H.L. (1998) Viral strategies of immune evasion. *Science* **280,** 248–253.

Thomsen, A.R., Nansen, A., & Christensen, J.P. (1998) Virus-induced T cell activation and the inflammatory response. *Curr. Top. Microbiol. Immunol.* **231,** 99–123.

Van Parjis, L. & Abbas, A.K. (1998) Homeostasis and self-tolerance in the immune system: turning lymphocytes off. *Science* **280,** 243–248.

York, I.A. & Rock, K.L. (1996) Antigen processing and presentation by the class-I major histocompatibility complex. *Annu. Rev. Immunol.* **14,** 369–396.

Molecular Motors

Finer, J.T., Simmons, R.M., & Spudich, J.A. (1994) Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature* **368,** 113–119.

Modern techniques reveal the forces affecting individual motor proteins.

Geeves, M.A. & Holmes, K.C. (1999) Structural mechanism of muscle contraction. *Annu. Rev. Biochem.* **68,** 687–728.

Goldman, Y.E. (1998) Wag the tail: structural dynamics of actomyosin. *Cell* **93,** 1–4.

Huxley, H.E. (1998) Getting to grips with contraction: the interplay of structure and biochemistry. *Trends Biochem. Sci.* **23,** 84–87.

An interesting historical perspective on deciphering the mechanism of muscle contraction.

Labeit, S. & Kolmerer, B. (1995) Titins: giant proteins in charge of muscle ultrastructure and elasticity. *Science* **270,** 293–296.

A structural and functional description of some of the largest known proteins.

Rayment, I. (1996) The structural basis of the myosin ATPase activity. *J. Biol. Chem.* **271,** 15,850–15,853.

Examining mechanism from a structural perspective.

Rayment, I. & Holden, H.M. (1994) The three-dimensional structure of a molecular motor. *Trends Biochem. Sci.* **19,** 129–134.

Spudich, J.A. (1994) How molecular motors work. *Nature* **372,** 515–518.

problems

1. Relationship between Affinity and Dissociation Constant Protein A has a binding site for ligand X with a K_d of 10^{-6} M. Protein B has a binding site for ligand X with a K_d of 10^{-9} M. Which protein has a higher affinity for ligand X? Explain your reasoning. Convert the K_d to K_a for both proteins.

2. Negative Cooperativity Which of the following situations would produce a Hill plot with $n_H < 1.0$? Explain your reasoning in each case.

(a) The protein has multiple subunits, each with a single ligand-binding site. Binding of ligand to one site decreases the binding affinity of other sites for the ligand.

(b) The protein is a single polypeptide with two ligand-binding sites, each having a different affinity for the ligand.

(c) The protein is a single polypeptide with a single ligand-binding site. As purified, the protein preparation is heterogeneous, containing some protein molecules that are partially denatured and thus have a lower binding affinity for the ligand.

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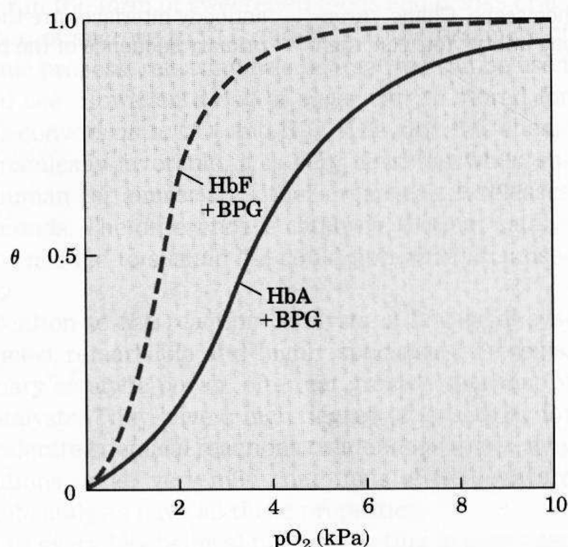
3. Affinity for Oxygen in Myoglobin and Hemoglobin What is the effect of the following changes on the O_2 affinity of myoglobin and hemoglobin? (a) A drop in the pH of blood plasma from 7.4 to 7.2. (b) A decrease in the partial pressure of CO_2 in the lungs from 6 kPa (holding one's breath) to 2 kPa (normal). (c) An increase in the BPG level from 5 mM (normal altitudes) to 8 mM (high altitudes).

4. Cooperativity in Hemoglobin Under appropriate conditions, hemoglobin dissociates into its four subunits. The isolated α subunit binds oxygen, but the O_2-saturation curve is hyperbolic rather than sigmoid. In addition, the binding of oxygen to the isolated α subunit is not affected by the presence of H^+, CO_2, or BPG. What do these observations indicate about the source of the cooperativity in hemoglobin?

5. Comparison of Fetal and Maternal Hemoglobins Studies of oxygen transport in pregnant mammals have shown that the O_2-saturation curves of fetal and maternal blood are markedly different when measured under the same conditions. Fetal erythrocytes contain a structural variant of hemoglobin, HbF, consisting of two α and two γ subunits ($\alpha_2\gamma_2$), whereas maternal erythrocytes contain HbA ($\alpha_2\beta_2$).



(a) Which hemoglobin has a higher affinity for oxygen under physiological conditions, HbA or HbF? Explain.

(b) What is the physiological significance of the different O_2 affinities?

(c) When all the BPG is carefully removed from samples of HbA and HbF, the measured O_2-saturation curves (and consequently the O_2 affinities) are displaced to the left. However, HbA now has a greater affinity for oxygen than does HbF. When BPG is reintroduced, the O_2-saturation curves return to normal, as shown in the graph. What is the effect of BPG on the O_2 affinity of hemoglobin? How can the above information be used to explain the different O_2 affinities of fetal and maternal hemoglobin?

6. Hemoglobin Variants There are almost 500 naturally occurring variants of hemoglobin. Most are the result of a single amino acid substitution in a globin polypeptide chain. Some variants produce clinical illness, though not all variants have deleterious effects. A brief sample is presented below:

HbS (sickle-cell Hb): substitutes a Val for a Glu on the surface

Hb Cowtown: eliminates an ion pair involved in T-state stabilization

Hb Memphis: substitutes one uncharged polar residue for another of similar size on the surface

Hb Bibba: substitutes a Pro for a Leu involved in an α helix

Hb Milwaukee: substitutes a Glu for a Val

Hb Providence: substitutes an Asn for a Lys that normally projects into the central cavity of the tetramer

Hb Philly: substitutes a Phe for a Tyr, disrupting hydrogen bonding at the $\alpha_1\beta_1$ interface

Explain your choices for each of the following:

(a) The Hb variant *least* likely to cause pathological symptoms.

(b) The variant(s) most likely to show pI values different from that of HbA when run on an isoelectric focusing gel.

(c) The variant(s) most likely to show a decrease in BPG binding and an increase in the overall affinity of the hemoglobin for oxygen.

7. Reversible (but Tight) Binding to an Antibody An antibody binds to an antigen with a K_d of 5×10^{-8} M. At what concentration of antigen will θ be (a) 0.2, (b) 0.5, (c) 0.6, (d) 0.8?

8. Using Antibodies to Probe Structure-Function Relationships in Proteins A monoclonal antibody binds to G-actin but not to F-actin. What does this tell you about the epitope recognized by the antibody?

9. The Immune System and Vaccines A host organism needs time, often days, to mount an immune response against a new antigen, but memory cells permit a rapid response to pathogens previously encountered. A vaccine to protect against a particular viral infection often consists of weakened or killed virus or isolated proteins from a viral protein coat. When injected into a human patient, the vaccine generally does not cause an infection and illness, but it effectively "teaches" the immune system what the viral particles look like, stimulating the production of memory cells. On subsequent infection, these cells can bind to the virus and trigger a rapid immune response. Some pathogens, including HIV, have developed mechanisms to evade the immune system, making it difficult or impossible to develop effective vaccines against them. What strategy could a pathogen use to evade the immune system? Assume that antibodies and/or T-cell receptors are available to bind to any structure that might appear on the surface of a pathogen and that, once bound, the pathogen is destroyed.

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10. How We Become a "Stiff" When a higher vertebrate dies, its muscles stiffen as they are deprived of ATP, a state called rigor mortis. Explain the molecular basis of the rigor state.

11. Sarcomeres from Another Point of View The symmetry of thick and thin filaments in a sarcomere is such that six thin filaments ordinarily surround each thick filament in a hexagonal array. Draw a cross section (transverse cut) of a myofibril at the following points: (a) at the M line; (b) through the I band; (c) through the dense region of the A band; (d) through the less dense region of the A band, adjacent to the M line (see Fig. 7–31b).

Biochemistry on the Internet

12. Lysozyme and Antibodies To fully appreciate how proteins function in a cell, it is helpful to have a three-dimensional view of how proteins interact with other cellular components. Fortunately, this is possible using the Internet and on-line protein databases. Go to the biochemistry site at

http://www.worthpublishers.com/lehninger

to learn how to use the Chemscape Chime three-dimensional molecular viewing utility. You can then use the Protein Data Bank and Chemscape Chime to investigate the interactions between antibodies and antigens in more detail.

To examine the interactions between the enzyme lysozyme (Chapter 6) and the Fab portion of the anti-lysozyme antibody, go to the Protein Data Bank Website. Use the PDB identifier 1FDL to retrieve the data page for the IgG1 Fab Fragment-Lysozyme Complex (antibody-antigen complex). Open the structure using Chemscape Chime, and use the different viewing options to answer the following questions:

(a) Which chains in the three-dimensional model correspond to the antibody fragment and which correspond to the antigen, lysozyme?

(b) What secondary structure predominates in this Fab fragment?

(c) How many amino acid residues are in the heavy and light chains of the Fab fragment and in lysozyme? Estimate the percentage of the lysozyme that interacts with the antigen-binding site of the antibody fragment.

(d) Identify the specific amino acid residues in lysozyme and in the variable regions of the heavy and light chains that appear to be situated at the antigen-antibody interface. Are the residues contiguous in the primary sequence of the polypeptide chain?

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