

Chapter 8

An Introduction to Metabolism

Overview: The Energy of Life

- Thousands of reactions occur within the microscopic space of a living cell.
 - Small molecules are assembled into polymers, which are later hydrolyzed as the needs of the cell change.
 - Cells within a multicellular organism may export chemical products.
 - Cellular respiration extracts energy from sugar and other fuels, using the energy to perform various types of work.
- The set of reactions that make up cell metabolism are complex, efficient, coordinated, and highly responsive to subtle changes.

Concept 8.1 An organism's metabolism transforms matter and energy, subject to the laws of thermodynamics.

- The sum total of an organism's chemical reactions is called **metabolism**.
- Metabolism is an emergent property of life that arises from interactions between molecules within the orderly environment of the cell.

The chemistry of life is organized into metabolic pathways.

- A **metabolic pathway** begins with a specific molecule, which is then altered in a series of defined steps to form a specific product.
- A specific enzyme catalyzes each step of the pathway.
- **Catabolic pathways** release energy by breaking down complex molecules to simpler compounds.
 - A major pathway of catabolism is cellular respiration, in which the sugar glucose is broken down in the presence of oxygen to carbon dioxide and water.
 - The energy released by catabolic pathways becomes available to do the work of the cell, such as ciliary beating or membrane transport.
- **Anabolic pathways**, also called biosynthetic pathways, consume energy to build complicated molecules from simpler compounds.
 - The synthesis of amino acids from simpler molecules and the synthesis of a protein from amino acids are both examples of anabolism.
- Energy released from the “downhill” reactions of catabolic pathways can be stored and then used to drive the “uphill” reactions of anabolic pathways.
- Energy is fundamental to all metabolic processes, and therefore an understanding of energy is key to understanding how the living cell works.
- **Bioenergetics** is the study of how energy flows through living organisms.

Organisms transform energy.

- **Energy** is the capacity to cause change.

- In everyday life, some forms of energy can be used to do work—that is, to move matter against opposing forces, such as gravity and friction.
- Energy exists in various forms, and cells transform energy from one type to another.
- **Kinetic energy** is the energy associated with the relative motion of objects.
 - Objects in motion can perform work by imparting motion to other matter.
 - **Heat or thermal energy** is kinetic energy associated with the random movement of atoms or molecules.
 - The energy of light can be harnessed to power photosynthesis in green plants.
- **Potential energy** is the energy that matter possesses because of its location or structure.
 - Water behind a dam possesses energy because of its altitude above sea level.
 - Molecules possess energy because of the arrangement of electrons in the bonds between their atoms.
 - **Chemical energy** is a term used by biologists to refer to the potential energy available for release in a chemical reaction.
 - During a catabolic reaction, some bonds are broken and others are formed, releasing energy and producing lower-energy breakdown products.
- Energy can be converted from one form to another.
 - For example, as a woman climbs stairs to a diving platform, she is releasing chemical energy stored in her cells from the food she ate for lunch.
 - The kinetic energy of her muscle movement is converted to potential energy as she climbs.
 - As a person dives, the potential energy is converted back to kinetic energy, which is transferred to the water as the diver enters it.
 - Some energy is converted to heat due to friction.

The energy transformations of life are subject to two laws of thermodynamics.

- **Thermodynamics** is the study of energy transformations that occur in a collection of matter.
- In this field, the term *system* refers to the matter under study, and the *surroundings* include the rest of the universe—everything outside the system.
- An *isolated system*, approximated by liquid in a thermos, is unable to exchange either energy or matter with its surroundings.
- In an *open system*, energy and matter can be transferred between the system and its surroundings.
- Organisms are open systems: They absorb energy—light or chemical energy in the form of organic molecules—and release heat and metabolic waste products, such as urea or CO₂, to their surroundings.
- Two laws of thermodynamics govern energy transformations in organisms and all other collections of matter.
- The **first law of thermodynamics** states that the energy of the universe is constant: *Energy can be transferred and transformed, but it cannot be created or destroyed.*
 - The first law is also known as the *principle of conservation of energy*.
 - Plants do not produce energy; they transform light energy to chemical energy.
- During every transfer or transformation of energy, some energy is converted to heat, which is the energy associated with the random movement of atoms and molecules.
- A system can use heat to do work only when there is a temperature difference that results in heat flowing from a warmer location to a cooler one.
 - If temperature is uniform, as in a living cell, heat can be used only to warm the organism.

- Energy transfers and transformations make the universe more disordered due to the loss of usable energy.
- **Entropy** is a measure of disorder or randomness: The more random a collection of matter, the greater its entropy.
- The **second law of thermodynamics** states: *Every energy transfer or transformation increases the entropy of the universe.*
- Although order can increase locally, there is an unstoppable trend toward randomization of the universe.
- Much of the increased entropy of the universe takes the form of increasing heat, which is the energy of random molecular motion.
- In most energy transformations, ordered forms of energy are converted at least partly to heat.
 - Automobiles convert only 25% of the energy in gasoline to motion; the rest is lost as heat.
 - Living cells unavoidably convert organized forms of energy to heat.
- For a process to occur on its own, without outside help in the form of energy input, it must increase the entropy of the universe.
- **Spontaneous processes** can occur without an input of energy, although they need not occur quickly.
 - Some spontaneous processes, such as an explosion, are instantaneous. Some, such as the rusting of an old car, are very slow.
- A *spontaneous* process is one that is energetically favorable. A process that cannot occur on its own is said to be nonspontaneous: it will happen only if energy is added to the system.
 - Water flows downhill spontaneously but moves uphill only with an input of energy, such as when a machine pumps the water against gravity.
- Here is another way to state the second law of thermodynamics: *For a process to occur spontaneously, it must increase the entropy of the universe.*
- Living systems increase the entropy of their surroundings, even though they create ordered structures from less ordered starting materials.
 - For example, amino acids are ordered into polypeptide chains.
- The structure of a multicellular body is organized and complex.
- However, an organism also takes in organized forms of matter and energy from its surroundings and replaces them with less ordered forms.
 - For example, an animal consumes organic molecules as food and catabolizes them to low-energy carbon dioxide and water.
- Over evolutionary time, complex organisms have evolved from simpler ones.
- This increase in organization does not violate the second law of thermodynamics.
- The entropy of a particular system, such as an organism, may decrease as long as the total entropy of the *universe*—the system plus its surroundings—increases.
- Organisms are islands of low entropy in an increasingly random universe.

Concept 8.2 The free-energy change of a reaction tells us whether or not the reaction occurs spontaneously.

- How can we determine which reactions occur spontaneously and which ones require an input of energy?

- The concept of free energy (symbolized by the letter G) is useful for measuring the spontaneity of a system.
- **Free energy** is the portion of a system's energy that can perform work when temperature and pressure are uniform throughout the system, as in a living cell.
- The change in free energy, ΔG , can be calculated for any specific chemical reaction by applying the following equation:

$$\Delta G = \Delta H - T\Delta S$$

- In this equation, ΔH symbolizes the change in the system's *enthalpy* (in biological systems, equivalent to total energy); ΔS is the change in the system's entropy; and T is the absolute temperature in Kelvin (K) units ($K = ^\circ\text{C} + 273$).
- For a process to occur spontaneously, the system must give up enthalpy (H must decrease), give up order ($T\Delta S$ must increase), or both.
- ΔG must have a negative value ($\Delta G < 0$) in order for a process to be spontaneous.
 - In other words, every spontaneous process decreases the system's free energy, and processes that have positive or zero ΔG are never spontaneous.
- Knowing the value of ΔG gives biologists the power to predict which kinds of change can happen without help.
 - Such spontaneous changes can be harnessed to perform work.
 - This information is important in the study of metabolism, where a major goal is to determine which reactions can supply energy to do work in the living cell.
- In any spontaneous process, the free energy of a system decreases (ΔG is negative).
- We can represent the change in free energy from the start to the finish of a process by

$$\Delta G = G_{\text{final state}} - G_{\text{starting state}}$$

- ΔG can be negative only when the process involves a loss of free energy during the change from initial state to final state.
- Because it has less free energy, the system in its final state is less likely to change and is therefore more stable than it was previously.
- A system at equilibrium is at maximum stability.
 - Unstable systems (higher G) tend to change in such a way that they become more stable (lower G).
- Another term for a state of maximum stability is *equilibrium*.
 - In a chemical reaction at equilibrium, the rates of forward and backward reactions are equal, and there is no change in the relative concentrations of products or reactants.
 - At equilibrium, $\Delta G = 0$, and the system can do no work.
- *A process is spontaneous and can perform work only when it is moving toward equilibrium.*
- Movements away from equilibrium are nonspontaneous and will have a positive ΔG .

Chemical reactions can be classified as either exergonic or endergonic.

- An **exergonic reaction** proceeds with a net release of free energy; ΔG is negative.
 - The magnitude of ΔG for an exergonic reaction is the maximum amount of work the reaction can perform.
 - The greater the decrease in free energy, the greater the amount of work that can be done.
- For the overall reaction of cellular respiration, $\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$, $\Delta G = -686 \text{ kcal/mol}$.

- For each mole (180 g) of glucose broken down by respiration under standard conditions (1 M of each reactant and product, 25°C, pH 7), 686 kcal of energy are made available to do work in the cell.
- The products have 686 kcal less free energy per mole than the reactants.
- An **endergonic reaction** is one that absorbs free energy from its surroundings.
 - Endergonic reactions *store* energy in molecules; ΔG is positive.
 - Endergonic reactions are nonspontaneous, and the magnitude of ΔG is the quantity of energy required to drive the reaction.
- If cellular respiration releases 686 kcal/mol, then photosynthesis, the reverse reaction, must require an equivalent investment of energy.
 - For the conversion of carbon dioxide and water to sugar, $\Delta G = +686$ kcal/mol.
 - Photosynthesis is strongly endergonic, powered by the absorption of light energy.
- Reactions in an isolated system eventually reach equilibrium and can do no work.
 - A cell that has reached metabolic equilibrium has a $\Delta G = 0$ and is dead!
- Metabolic disequilibrium is one of the defining features of life.
- Cells maintain disequilibrium because they are open systems. The constant flow of materials into and out of the cell keeps metabolic pathways from ever reaching equilibrium.
 - A cell continues to do work throughout its life.
- A catabolic process in a cell releases free energy in a series of reactions, not in a single step.
- Some reversible reactions of respiration are constantly “pulled” in one direction, as the product of one reaction does not accumulate but becomes the reactant in the next step.
- The overall sequence of reactions is kept going by the huge free-energy difference between glucose and oxygen at the top of the energy “hill” and carbon dioxide and water at the “downhill” end.
- As long as cells have a steady supply of glucose or other fuels and oxygen and can expel waste products to the surroundings, their metabolic pathways never reach equilibrium and can continue to do the work of life.
- Sunlight provides a daily source of free energy for photosynthetic organisms.
- Nonphotosynthetic organisms depend on a transfer of free energy from photosynthetic organisms in the form of organic molecules.

Concept 8.3 ATP powers cellular work by coupling exergonic reactions to endergonic reactions.

- A cell does three main kinds of work:
 1. *Chemical work*, pushing endergonic reactions such as the synthesis of polymers from monomers;
 2. *Transport work*, pumping substances across membranes against the direction of spontaneous movement;
 3. *Mechanical work*, such as the beating of cilia, contraction of muscle cells, and movement of chromosomes during cellular reproduction;
- Cells manage their energy resources to do this work by **energy coupling**, using an exergonic process to drive an endergonic one.
- ATP is responsible for mediating most energy coupling in cells, and

ATP mediates most energy coupling in cells.

- **ATP (adenosine triphosphate)** is a nucleotide triphosphate consisting of the sugar ribose, the nitrogenous base adenine, and a chain of three phosphate groups.
- In most cases, ATP acts as the immediate source of energy that powers cellular work.
- The bonds between the phosphate groups on ATP can be broken by hydrolysis.
- When the terminal phosphate bond is broken, a molecule of inorganic phosphate (HOPO_3^{2-} , abbreviated P_i here) leaves ATP, which then becomes adenosine diphosphate, or ADP:

$$\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i$$
- Under standard conditions, $\Delta G = -7.3 \text{ kcal/mol}$ (-30.5 kJ/mol).
- In the cell, ΔG for the hydrolysis of ATP is about -13 kcal/mol because reactant and product concentrations differ from 1 M .
- Although the phosphate bonds of ATP are sometimes referred to as high-energy phosphate bonds, these are actually fairly weak covalent bonds.
 - The reactants (ATP and water) themselves have high energy relative to the energy of the products (ADP and P_i).
- The release of energy during the hydrolysis of ATP comes from the chemical change to a state of lower free energy, not from the phosphate bonds themselves.
- Why does the hydrolysis of ATP yield so much energy?
 - Each of the three phosphate groups has a negative charge.
 - These three like charges are crowded together, and their mutual repulsion contributes to the instability of this region of the ATP molecule.
 - The triphosphate tail of ATP is the chemical equivalent of a compressed spring.
- In the cell, the energy from the hydrolysis of ATP is directly coupled to endergonic processes by the transfer of the phosphate group to another molecule.
- This recipient molecule with a phosphate group covalently bonded to it is called a **phosphorylated intermediate**. It is more reactive (less stable) than the original unphosphorylated molecule.
- Mechanical, transport, and chemical work in the cell are nearly always powered by the hydrolysis of ATP.
- In each case, ATP hydrolysis leads to a change in a protein's shape and often its ability to bind another molecule.
 - This change may occur via a phosphorylated intermediate.
- In most examples of mechanical work involving motor proteins "walking" on cytoskeletal elements, a cycle occurs in which ATP is bound noncovalently to the motor protein and hydrolyzed, then ADP and P_i are released, followed by binding of another ATP molecule.
 - In each state, the motor protein exhibits a different shape and ability to bind the cytoskeleton, resulting in movement of the protein along the cytoskeletal track.

ATP is a renewable resource.

- Although organisms use ATP continuously, ATP is a renewable resource that can be regenerated by the addition of a phosphate group to ADP.
- The free energy to phosphorylate ADP comes from exergonic (catabolic) reactions in the cell.
- The ATP cycle, the shuttling of inorganic phosphate and energy, couples the cell's energy-yielding (exergonic) processes to the energy-consuming (endergonic) ones.
- A working muscle cell recycles its entire pool of ATP once each minute.
 - More than 10 million ATP molecules are consumed and regenerated per second per cell.

- Regeneration of ATP is an endergonic process, requiring an investment of energy:
 $ADP + P_i \rightarrow ATP + H_2O$ where $\Delta G = +7.3$ kcal/mol, under standard conditions.
- Catabolic (exergonic) pathways, especially cellular respiration, provide the energy for the endergonic regeneration of ATP.
- Plants also use light energy to produce ATP.
- The chemical potential energy temporarily stored in ATP drives most cellular work.

Concept 8.4 Enzymes speed up metabolic reactions by lowering energy barriers.

- Spontaneous chemical reactions may occur so slowly as to be imperceptible.
 - The hydrolysis of table sugar (sucrose) to glucose and fructose is exergonic, with $\Delta G = -7$ kcal/mol.
 - Despite this, a solution of sucrose dissolved in sterile water may sit for years at room temperature with no appreciable hydrolysis.
 - If a small amount of the enzyme sucrase is added to a solution of sugar, all the sucrose is hydrolyzed within seconds.
- An **enzyme** is a macromolecule that acts as a **catalyst**, a chemical agent that speeds up the rate of a reaction without being consumed by the reaction.
 - Here, we will focus on protein enzymes.
- Every chemical reaction involves bond breaking and bond forming.
 - To hydrolyze sucrose, the bond between glucose and fructose must be broken and new bonds must form with hydrogen and hydroxyl ions from water.
- To reach a state at which bonds can break and reform, reactant molecules must absorb energy from their surroundings. When the new bonds of the product molecules form, energy is released as heat as the molecules assume stable shapes with lower energy.
- The initial investment of energy for starting a reaction is the *free energy of activation*, or **activation energy** (E_A).
- Activation energy is the amount of energy necessary to push the reactants over an energy barrier so that the “downhill” part of the reaction can begin.
- Activation energy is often supplied in the form of thermal energy that the reactant molecules absorb from the surroundings.
 - The absorption of thermal energy accelerates the reactant molecules, which then collide more often with more force.
 - It also agitates the atoms within the molecules, making the breakage of bonds more likely.
 - When the molecules have absorbed enough energy for the bonds to break, the reactants are in an unstable condition called the *transition state*.
- Consider a hypothetical exergonic reaction that swaps portions of two reactant molecules:

$$AB + CD \rightarrow AC + BD$$
- The energizing, or activation, of the reactants is represented by the uphill portion of the graph, with the free-energy content of the reactant molecules increasing.
- At the summit, when the energy equivalent to E_A has been absorbed, the reactants are in the transition state. They are activated and their bonds can be broken.

- The bond-forming phase of the reaction corresponds to the downhill part of the curve, which shows the loss of free energy by the molecules.
- As the molecules settle into new, stable bonding arrangements, energy is released to the surroundings.
 - The overall decrease in free energy means that E_A is repaid with interest.
- For some processes, E_A is not high, and the thermal energy provided by room temperature is sufficient for many reactants to reach the transition state.
- In most cases, E_A is high enough that the transition state is rarely reached and the reaction hardly proceeds at all. In these cases, the reaction will occur at a noticeable rate only if the reactants are heated.
 - A spark plug provides the activation energy to energize a gasoline-oxygen mixture and cause combustion.
 - Without a spark, the hydrocarbons of gasoline are too stable to react with oxygen.
- Proteins, DNA, and other complex organic molecules are rich in free energy. Their hydrolysis is spontaneous, with the release of large amounts of energy.
- However, there is not enough energy at the temperatures typical of the cell for the vast majority of organic molecules to make it over the hump of activation energy.
- How are the barriers for selected reactions surmounted to allow cells to carry out the processes of life?
 - Heat would speed up *all* reactions, not just those that are needed.
 - Heat also denatures proteins and kills cells.
- Enzymes speed reactions by lowering E_A . The transition state can then be reached even at moderate temperatures.
- Enzymes do not change ΔG ; they hasten reactions that would occur eventually.
- Because enzymes are so selective, they determine which chemical processes will occur at any time.

Enzymes are substrate specific.

- The reactant that an enzyme acts on is the **substrate**.
- The enzyme binds to a substrate, or substrates, forming an **enzyme-substrate complex**.
- While the enzyme and substrate are bound, the catalytic action of the enzyme converts the substrate to the product or products.
- The reaction catalyzed by each enzyme is very specific.
- What accounts for this molecular recognition? The specificity of an enzyme results from its three-dimensional shape, which is a consequence of its amino acid sequence.
- Only a restricted region of the enzyme binds to the substrate.
 - The **active site** of an enzyme is typically a pocket or groove on the surface of the protein where catalysis occurs.
 - The active site is usually formed by only a few amino acids. The rest of the protein molecule provides a framework that determines the configuration of the active site.
- The specificity of an enzyme is due to the fit between the active site and the substrate.
- The active site is not a rigid receptacle for the substrate.
- As the substrate enters the active site, interactions between the chemical groups on the substrate and those on the side chains of the amino acids that form the active site cause the enzyme to change shape slightly.

- This change leads to an **induced fit** that brings the chemical groups of the active site into position to catalyze the reaction.

The active site is an enzyme's catalytic center.

- In most cases, substrates are held in the active site by weak interactions, such as hydrogen bonds and ionic bonds.
- The R groups of a few amino acids on the active site catalyze the conversion of substrate to product.
- The product then leaves the active site.
- A single enzyme molecule can catalyze thousands of reactions a second.
- Enzymes are unaffected by the reaction and are reusable.
- Most metabolic enzymes can catalyze a reaction in both the forward and reverse directions.
- The actual direction depends on the relative concentrations of products and reactants.
- Enzymes catalyze reactions in the direction of equilibrium.
- Enzymes use a variety of mechanisms to lower the activation energy and speed up a reaction.
 - In reactions involving more than one reactant, the active site brings substrates together in the correct orientation for the reaction to proceed.
 - As the active site binds the substrate, the enzyme may stretch the substrate molecules toward their transition-state form, stressing and bending critical chemical bonds that must be broken during the reaction.
 - The R groups at the active site may create a microenvironment that is conducive to a specific reaction. For example, an active site may be a pocket of low pH, facilitating H⁺ transfer to the substrate as a key step in catalyzing the reaction.
 - Enzymes may briefly bind covalently to substrates. Subsequent steps of the reaction restore the side chains of amino acids within the active site to their original state.
- The rate at which a specific number of enzymes convert substrates to products depends in part on substrate concentrations.
- At low substrate concentrations, an increase in substrate concentration speeds binding to available active sites.
 - There is a limit to how fast a reaction can occur, however.
- At high substrate concentrations, the active sites on all enzymes are engaged.
 - The enzyme is *saturated*, and the rate of the reaction is determined by the speed at which the active site can convert substrate to product.
 - The only way to increase productivity at this point is to add more enzyme molecules.

A cell's physical and chemical environment affects enzyme activity.

- The activity of an enzyme is affected by general environmental conditions, such as temperature and pH.
- Each enzyme works best at certain *optimal conditions*, which favor the most active conformation for the enzyme molecule.
- Temperature has a major impact on reaction rate.
 - As temperature increases, collisions between substrates and active sites occur more frequently as molecules move more rapidly.
 - As temperature increases further, thermal agitation begins to disrupt the weak bonds that stabilize the protein's active conformation, and the protein denatures.

- Each enzyme has an optimal temperature that allows the greatest number of molecular collisions and the fastest conversion of the reactants to product molecules.
 - Most human enzymes have optimal temperatures of about 35–40°C.
 - The thermophilic bacteria that live in hot springs contain enzymes with optimal temperatures of 70°C or higher.
- Each enzyme also has an optimal pH.
- Maintenance of the active conformation of the enzyme requires a particular pH.
 - This optimal pH falls between 6–8 for most enzymes.
 - However, digestive enzymes in the stomach are designed to work best at pH 2, whereas those in the intestine have an optimal pH of 8.
- Many enzymes require nonprotein helpers, called **cofactors**, for catalytic activity.
 - Cofactors bind permanently or reversibly to the enzyme.
 - Some inorganic cofactors are zinc, iron, and copper in ionic form.
 - Organic cofactors are called **coenzymes**.
 - Most vitamins are coenzymes or the raw materials from which coenzymes are made.

Binding by inhibitors prevents enzymes from catalyzing reactions.

- Certain chemicals selectively inhibit the action of specific enzymes.
- If inhibitors attach to the enzyme by covalent bonds, inhibition may be irreversible.
- If inhibitors bind by weak bonds, inhibition may be reversible.
- Some reversible inhibitors resemble the substrate and compete for binding to the active site. These molecules are called **competitive inhibitors**.
 - Competitive inhibition can be overcome by increasing the concentration of the substrate.
- **Noncompetitive inhibitors** impede enzymatic reactions by binding to another part of the molecule.
 - Binding by the inhibitor causes the enzyme to change shape, rendering the active site less effective at catalyzing the reaction.
- Toxins and poisons are often irreversible enzyme inhibitors.
 - Sarin, the nerve gas that was released by terrorists in the Tokyo subway in 1995, binds covalently to the R group on the amino acid serine.
 - Serine is found in the active site of acetylcholinesterase, an important nervous system enzyme.
 - DDT acts as a pesticide by inhibiting key enzymes in the nervous system of insects.
- Many antibiotics are inhibitors of specific enzymes in bacteria.
 - Penicillin blocks the active site of an enzyme that many bacteria use to make their cell walls.
- Not all enzyme inhibitors are metabolic poisons.
- Molecules naturally present in the cell often regulate enzyme activity by acting as inhibitors.
- Such regulation—selective inhibition—is essential to the control of cellular metabolism.

How did the great diversity of enzymes arise?

- So far, biochemists have discovered and named more than 4,000 different enzymes in various species; there are certainly many more.
- How did this diversity arise? Most enzymes are proteins, and proteins are encoded by genes.
 - A permanent change in a gene, known as a mutation, can result in a protein with one or more changed amino acids.

- In an enzyme, if the changed amino acids are in the active site or some other crucial region, the altered enzyme might have a novel activity or it might bind to a different substrate.
- Under environmental conditions where the new function benefits the organism, natural selection would tend to favor the mutated form of the gene, causing it to persist in the population.
- Researchers have used a lab procedure that mimics evolution in natural populations.
 - One group tested whether the function of an enzyme called β -galactosidase could change over time in populations of the bacterium *Escherichia coli* (*E. coli*).
 - β -galactosidase breaks down the disaccharide lactose into the simple sugars glucose and galactose.
- Using molecular techniques, the researchers introduced random mutations into *E. coli* genes and then tested the bacteria for their ability to break down a slightly different disaccharide.
 - They selected the mutant bacteria that could do this best and exposed them to another round of mutation and selection.
 - After seven rounds, the “evolved” enzyme bound the new substrate several hundred times more strongly, and broke it down 10 to 20 times more quickly than did the original enzyme.
- The researchers found that six amino acids had changed in the enzyme altered in this experiment.
 - Two of these changed amino acids were in the active site, two were nearby, and two were on the surface of the protein.
- This experiment and others like it support the idea that minor changes in enzyme structure can alter enzyme function.

Concept 8.5 Regulation of enzyme activity helps control metabolism.

Metabolic control often depends on allosteric regulation.

- Many molecules that naturally regulate enzyme activity behave like reversible noncompetitive inhibitors.
- In **allosteric regulation**, a protein’s function at one site is affected by the binding of a regulatory molecule to a separate site.
 - This regulation may result in either inhibition or stimulation of an enzyme’s activity.
- Most allosterically regulated enzymes are constructed of two or more polypeptide chains.
 - The complex oscillates between two shapes, one catalytically active and the other inactive.
- In the simplest case of allosteric regulation, an activating or inhibiting regulatory molecule binds to a regulatory site (sometimes called an allosteric site), often located where subunits join.
- The binding of an *activator* stabilizes the conformation that has functional active sites, whereas the binding of an *inhibitor* stabilizes the inactive form of the enzyme.
 - The subunits of an allosteric enzyme fit together in such a way that a shape change in one subunit is transmitted to all others.
 - Through this interaction of subunits, a single activator or inhibitor molecule that binds to one regulatory site will affect the active sites of all subunits.
- As the chemical conditions in the cell shift, the pattern of allosteric regulation may shift as well.
- By binding to key enzymes, the reactants and products of ATP hydrolysis may play a major role in balancing the flow of traffic between anabolic and catabolic pathways.
 - For example, ATP binds to several catabolic enzymes allosterically, inhibiting their activity by lowering their affinity for substrate.
 - ADP functions as an activator of the same enzymes.

- ATP and ADP also affect key enzymes in anabolic pathways.
 - In this way, allosteric enzymes control the rates of key reactions in metabolic pathways.
- In enzymes with multiple catalytic subunits, binding by a *substrate* molecule to one active site in a multisubunit enzyme triggers a shape change in all the subunits.
 - This mechanism, called **cooperativity**, amplifies the response of enzymes to substrates, priming the enzyme to accept additional substrates.
 - Cooperativity is considered to be “allosteric” regulation because binding of the substrate to one active site affects catalysis in a *different* active site.
- The vertebrate oxygen-transport protein hemoglobin is a classic example of cooperativity.
 - Hemoglobin is made up of four subunits, each with an oxygen-binding site.
 - The binding of an oxygen molecule to each binding site increases the affinity for oxygen of the remaining binding sites.
 - Under conditions of low oxygen, as in oxygen-deprived tissues, hemoglobin is less likely to bind oxygen and releases it where it is needed.
 - When oxygen is at higher levels, as in the lungs or gills, the protein has a greater affinity for oxygen as more binding sites are filled.
- Although allosteric regulation is probably widespread, relatively few of the many known metabolic enzymes are known to be regulated in this way.
- Allosteric regulatory molecules tend to bind the enzyme at low affinity and are thus hard to isolate.
- Pharmaceutical companies are turning their attention to allosteric regulators, which exhibit higher specificity for particular enzymes than do inhibitors that bind to the active site.
 - An active site may be similar to that in another, related enzyme, whereas allosteric regulatory sites appear to be quite distinct between enzymes.
- A common method of metabolic control is **feedback inhibition**.
- An early step in a metabolic pathway is switched off by inhibitory binding of the pathway’s final product to an enzyme acting early in the pathway.
- Feedback inhibition prevents a cell from wasting chemical resources by synthesizing more product than is needed.

The localization of enzymes within a cell helps order metabolism.

- The cell is compartmentalized: The organization of cellular structures helps bring order to metabolic pathways.
- A team of enzymes for several steps of a metabolic pathway may be assembled as a multienzyme complex.
- The product from the first reaction becomes the substrate for an adjacent enzyme in the complex until the final product is released.
- Some enzymes and enzyme complexes have fixed locations within the cells as structural components of particular membranes.
 - Others are confined within membrane-enclosed eukaryotic organelles.
- Metabolism, the intersecting set of chemical pathways characteristic of life, is a choreographed interplay of thousands of different kinds of cellular molecules.