

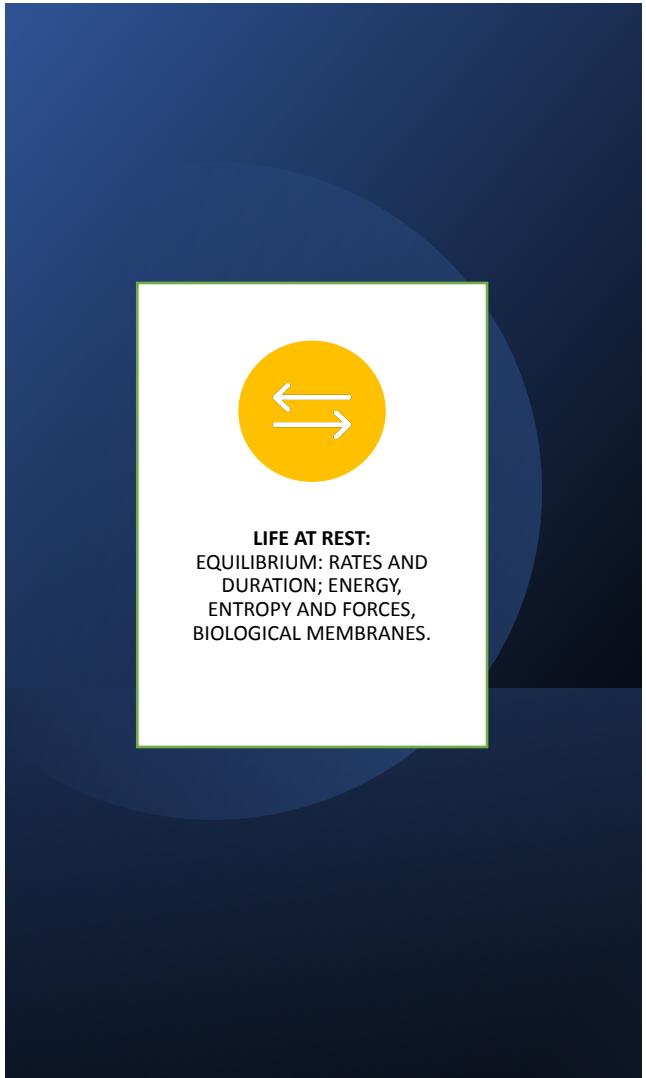


Biology for Engineers

FY-BTech

Unit 2- Life at Rest

Module-2: Life at rest



Thermodynamics and Static Properties of cells

Equilibrium: Mechanical and Chemical Equilibrium in the Living Cell; Cells as Chemical Factories; Chemical equilibrium, rate of reaction. The concept of steady state equilibrium.

Rates and duration: Time scales of small molecules; central dogma, Life cycle of cells.

Energy, Entropy and Forces: Thermal energy, photons and photosynthesis; energy currencies and budget.

Electrostatics

Biological Membranes: membrane permeability: pumps and channels, action potential.

An equation is worth a thousand words.

A physical biologist

How cells manage energy and how scientists compute energy transformations ?

Chemical transformations that consume and liberate energy are one of the hallmarks of living systems.

Living cells follow the same principles of conservation of matter and energy as do all other physical systems

How cells manipulate and store chemical energy in ways that can be used to perform material transformations, such as macromolecular synthesis, mechanical work, such as muscle contraction, or even production of light energy, as in a firefly's abdomen?

Thermodynamics is the study of energy transformations

Thermodynamics studies the energy flow, heat and movement, in structures among the universe.

A living system, i.e. a cell, it is usual to refer to **open system thermodynamics** or **nonequilibrium thermodynamics**.

First law of thermodynamics

Energy cannot be created or destroyed

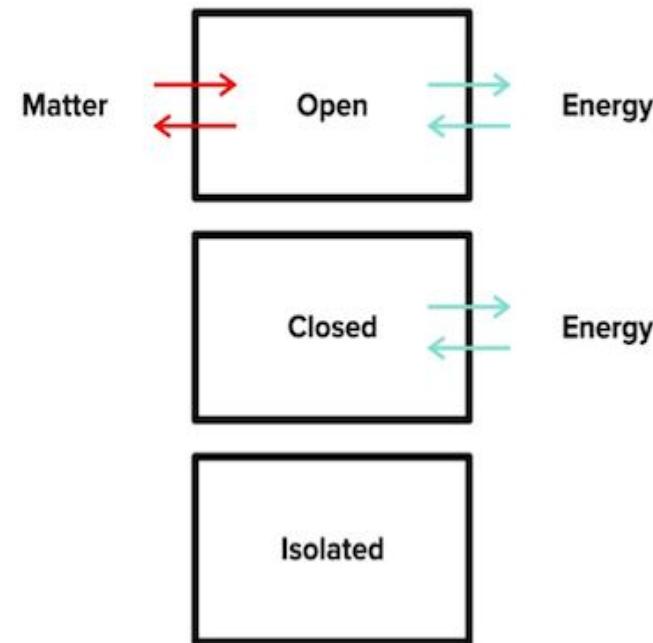
Second law of thermodynamics

Every energy transfer or transformation increases the entropy (disorder) of the universe

To measure “disorder” in the system we use a term “entropy”

Entropy is measure of disorder of a system
Or energy unavailable to do work

System Types



<https://www.shemmassianconsulting.com/blog/thermodynamics-mcat>

First law of thermodynamics

Energy cannot be created or destroyed

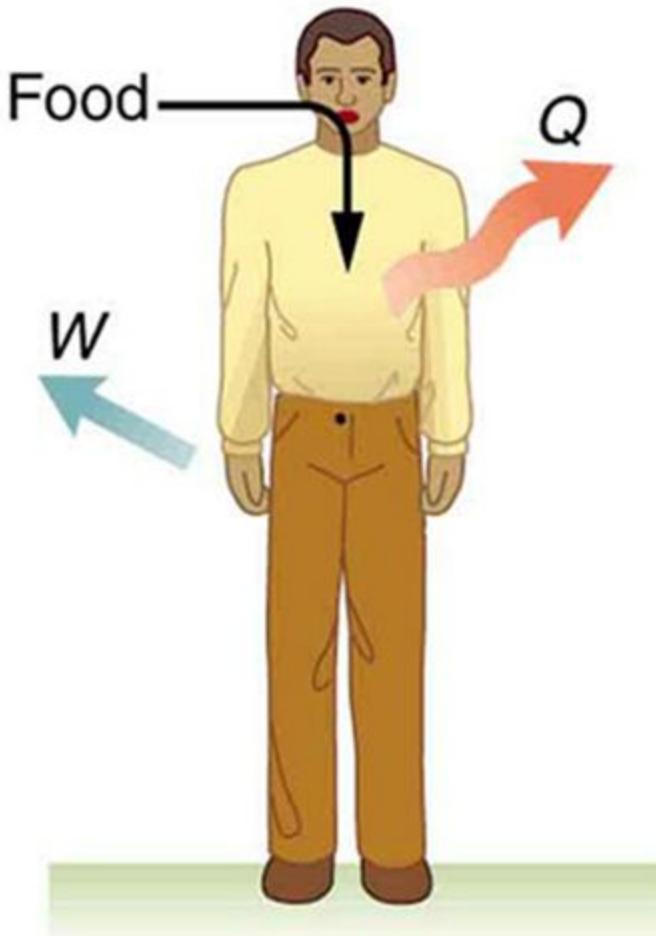


(a) First law of thermodynamics

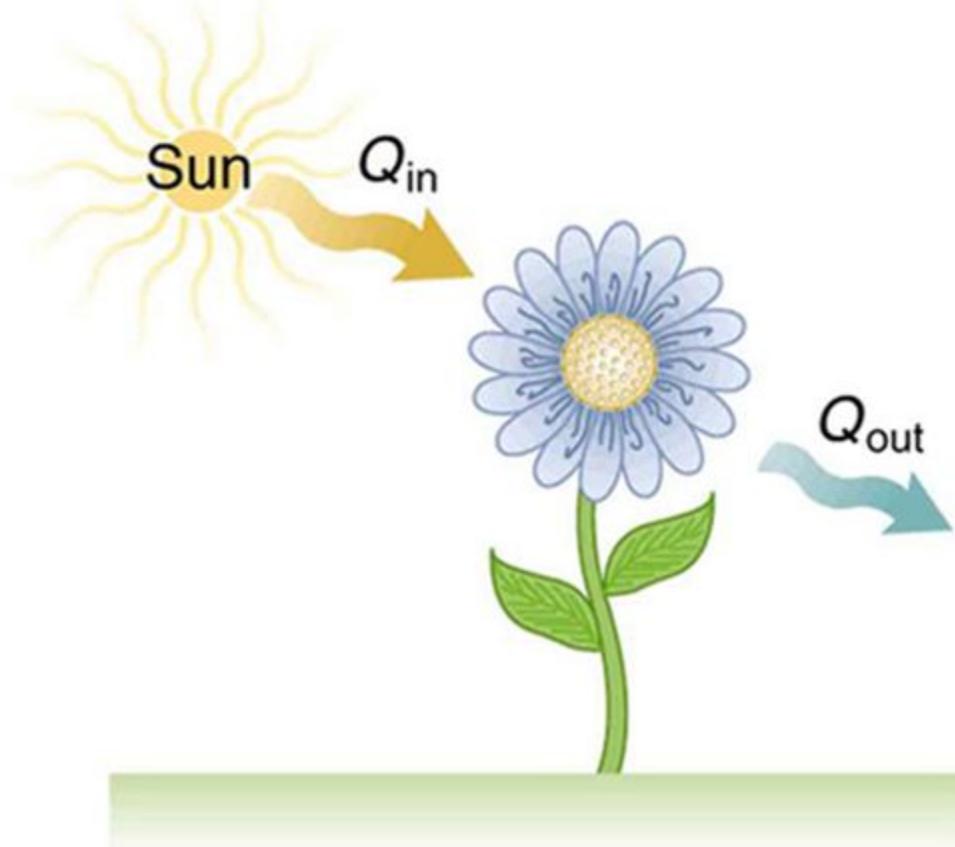
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First Law of Thermodynamics

$$\Delta U = -Q - W + \text{food energy} \quad \Delta U = \text{stored food energy}$$



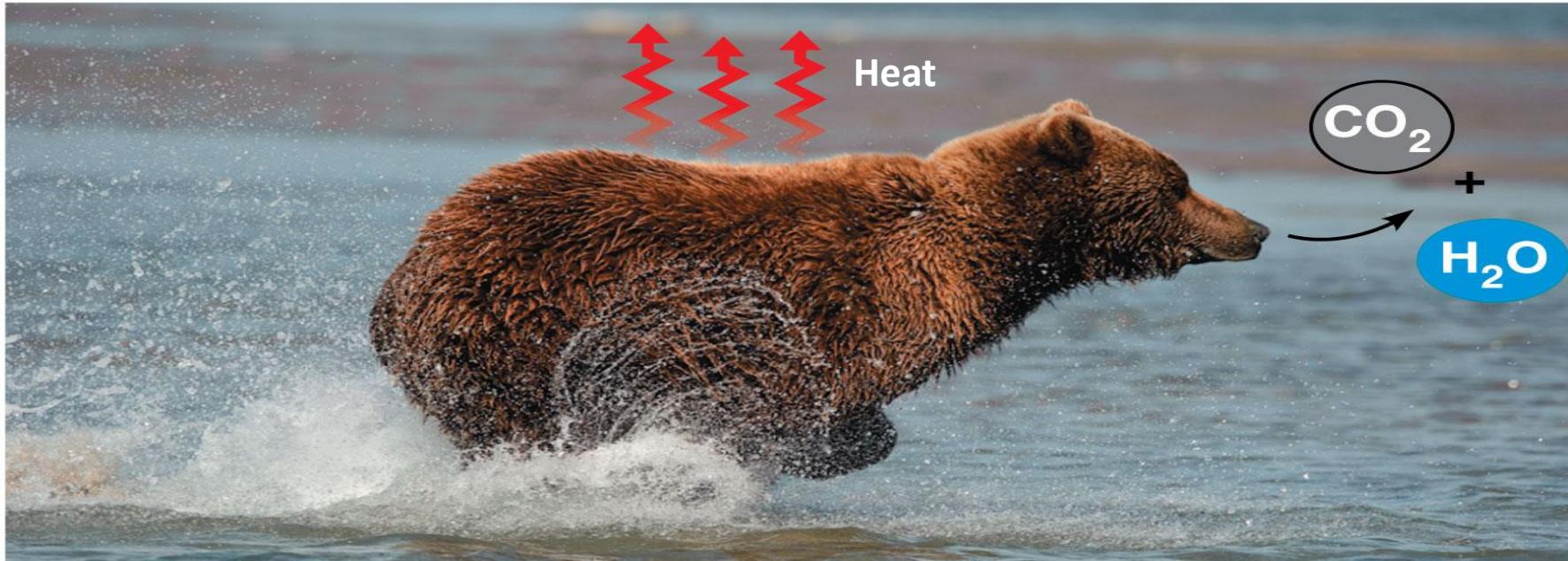
(a)



(b)

Second law of thermodynamics

Every energy transfer or transformation increases the entropy (disorder) of the universe

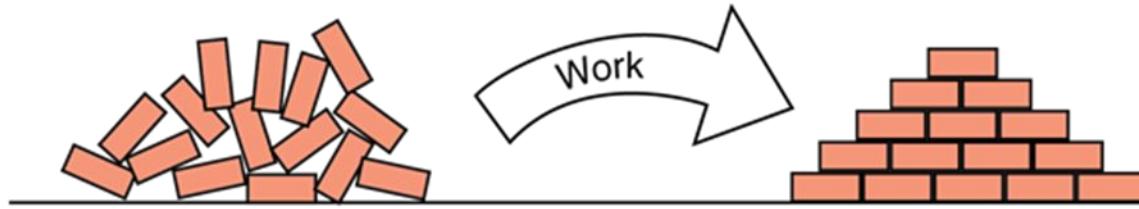


(b) Second law of thermodynamics

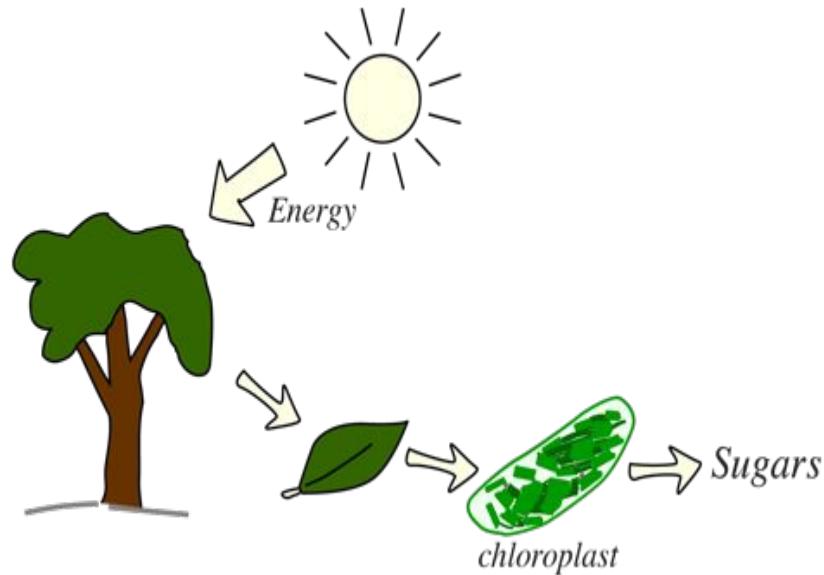
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- Living cells unavoidably convert organized forms of energy to heat
- **Spontaneous processes** occur without energy input; they can happen quickly or slowly
- For a process to occur without energy input, it must increase the entropy of the universe
- Here, the word *spontaneous* does not imply that such a process would occur quickly; rather, the word signifies that the process is **energetically favorable.**

Work is generally required to produce order out of disorder, so energy must be used to produce a highly ordered state.



Order can be produced with an expenditure of energy, and the order associated with life on the earth is produced with the aid of energy from the sun.



Plants use energy from the sun in tiny energy factories called chloroplasts.

Using chlorophyll in the process called photosynthesis, they convert the sun's energy into storable form in ordered sugar molecules. In this way, **carbon and water in a more disordered state are combined to form the more ordered sugar molecules.**

In animal systems there are also small structures within the cells called mitochondria which use the energy stored in sugar molecules from food to form more highly ordered structures.

Biological Order and Disorder

- Cells create ordered structures from less ordered materials
- Organisms also replace ordered forms of matter and energy with less ordered forms
- Energy flows into an ecosystem in the form of light and exits in the form of heat
- The evolution of more complex organisms does not violate the second law of thermodynamics
- Entropy (disorder) may decrease in an organism, but the universe's total entropy increases

Chemical equilibrium

Condition in the course of a reversible chemical reaction in which no net change in the amounts of reactants and products occurs.

A **reversible chemical reaction** is one in which the products, as soon as they are formed, react to produce the original reactants.

At equilibrium, the two opposing reactions go on at equal rates, or velocities, and hence there is no net change in the amounts of substances involved. At this point the reaction may be considered to be completed; i.e., for some specified reaction condition, the maximum conversion of reactants to products has been attained.

A reversible reaction at equilibrium is **not static**—reactants and products continue to interconvert at equilibrium, but the rates of the forward and reverse reactions are the same.

Mechanical equilibrium

Static equilibrium, also known as mechanical equilibrium, means the reaction has stopped. In other words, the system is at rest. In biology, the equilibrium of a system is called homeostasis.

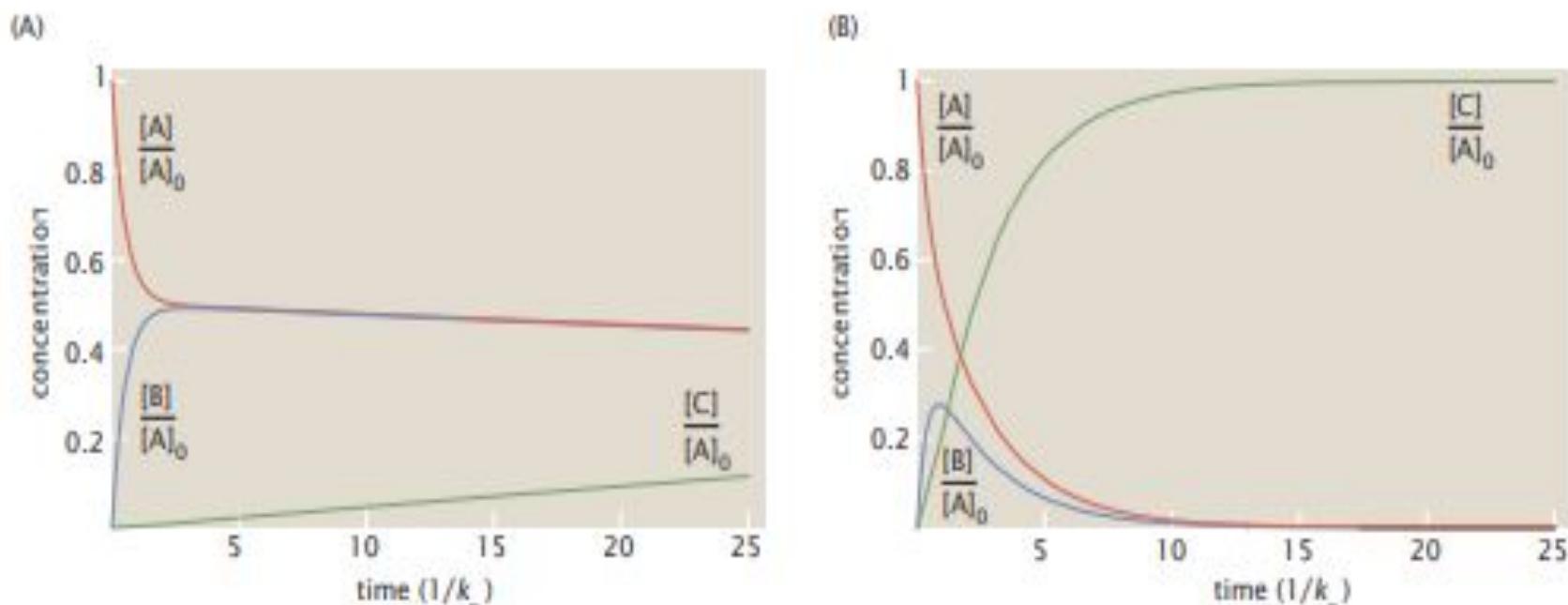
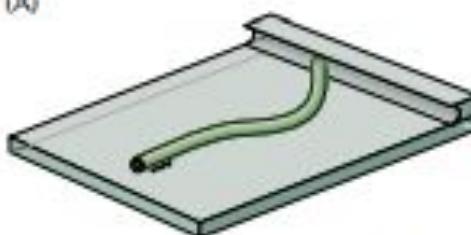


Figure 5.6: Rapid approach to equilibrium of a subprocess. Plot of the time-dependence of the concentrations in the reaction $A \rightleftharpoons B \rightarrow C$ of $A(t)$, $B(t)$ and $C(t)$ described by Equation 5.3. (A) For the case in which the rate for converting B to C is slow in comparison with the rates for the reaction between A and B, after an initial transient period, A and B reach their equilibrium values relative to each other for the remainder of the process. (B) Plot showing the case in which there is no rapid preequilibrium. Time is shown in nondimensional units by expressing it in units of the inverse of the rate k_- .

MECHANICAL EQUILIBRIUM

(A)



microtubule growing against a barrier

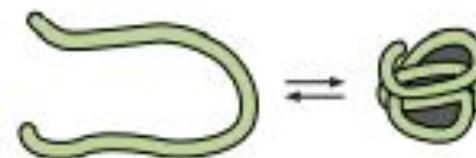
(B)



proteins partitioning in a density gradient

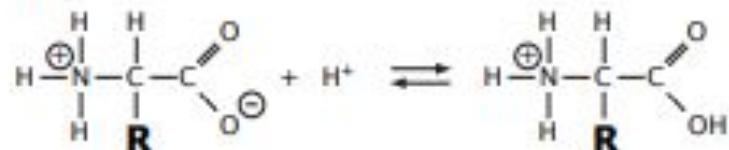
CHEMICAL EQUILIBRIUM

(C)



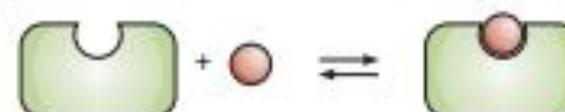
protein folding and unfolding

(D)



carboxylic acid group becoming
protonated and deprotonated

(E)



ligand binding and unbinding to receptor

(F)



ion channel opening and closing

Free Energy, Stability, and Equilibrium

- Free energy is a measure of a system's instability, its tendency to change to a more stable state
- During a spontaneous change, free energy decreases and the stability of a system increases
- Equilibrium is a state of maximum stability
- A process is spontaneous and can perform work only when it is moving toward equilibrium

Free-Energy Change, ΔG

- A living system's **free energy** is energy that can do work when temperature and pressure are uniform, as in a living cell
- The change in free energy (ΔG) during a process is related to the change in enthalpy, or change in total energy (ΔH), change in entropy (ΔS), and temperature in Kelvin (T)

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

- Only processes with a negative ΔG are spontaneous
- Spontaneous processes can be harnessed to perform work

- The standard transformed free-energy change, ΔG° is a physical constant that is characteristic for a given reaction and be calculated from the equilibrium constant for the reaction: $\Delta G^{\circ} = -RT \ln K'$ eq.
- The actual free-energy change, ΔG , is a variable that depends on ΔG° and on the concentrations of reactants and products: $\Delta G = \Delta G^{\circ} + RT \ln K'$ eq.
- When ΔG is large and negative, the reaction tends to go in the forward direction; when ΔG is large and positive, the reactions tends to go in reverse direction and when $\Delta G=0$ the system is in equilibrium.
- The free-energy change for a reaction is independent of the pathway by which the reactions occurs. Free-energy changes are additive; the net chemical reaction that results from successive reactions sharing a common intermediate has an overall free-energy change that is the sum of the ΔG values for the individual reactions

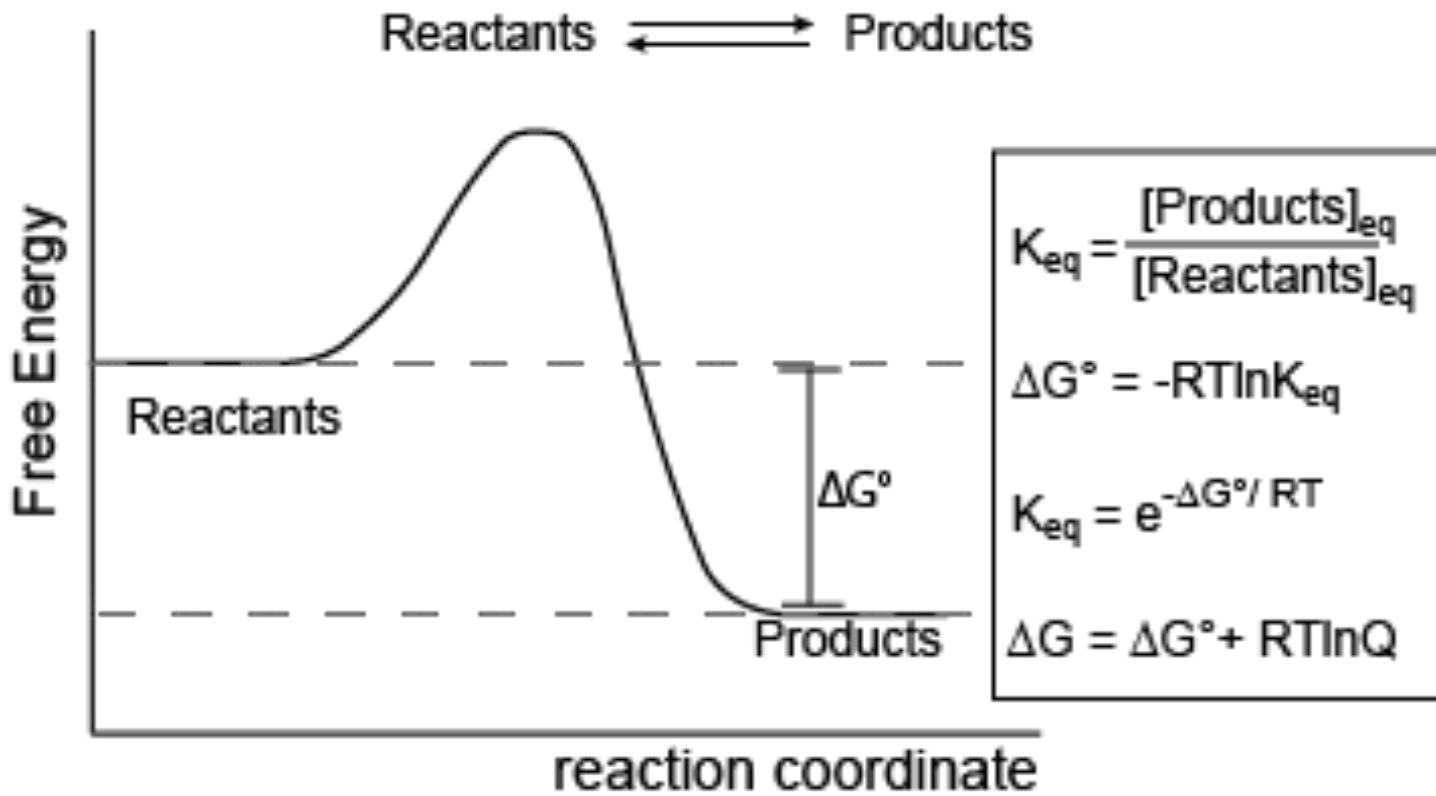


Figure 1. Reaction coordinate diagram for a generic exergonic reversible reaction. Equations relating Gibbs energy and the equilibrium constant: $R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ or $0.008314 \text{ kJ mol}^{-1} \text{ K}^{-1}$; T is temperature in Kelvin. Attribution: Marc T. Facciotti (original work)

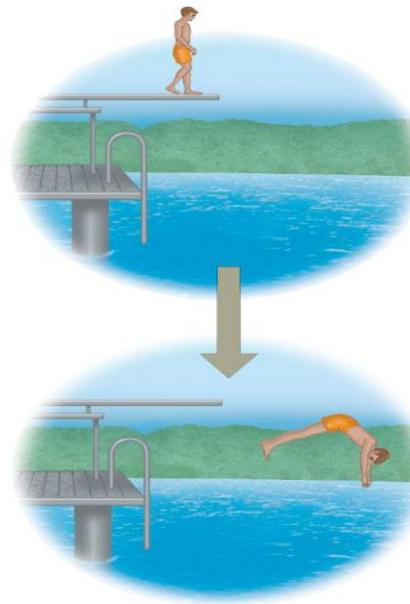
- More free energy (higher G)
- Less stable
- Greater work capacity

In a spontaneous change

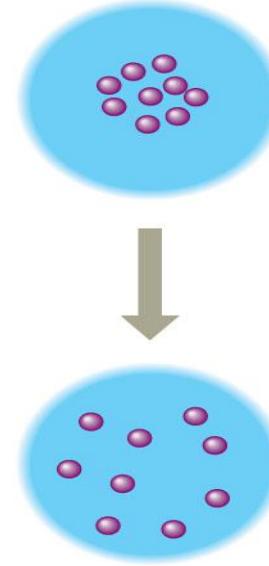
- The free energy of the system decreases ($\Delta G < 0$)
- The system becomes more stable
- The released free energy can be harnessed to do work

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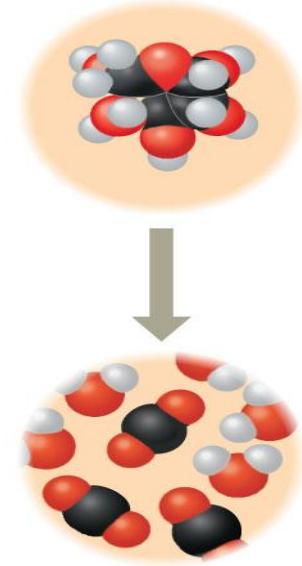
- Less free energy (lower G)
- More stable
- Less work capacity



(a) Gravitational motion



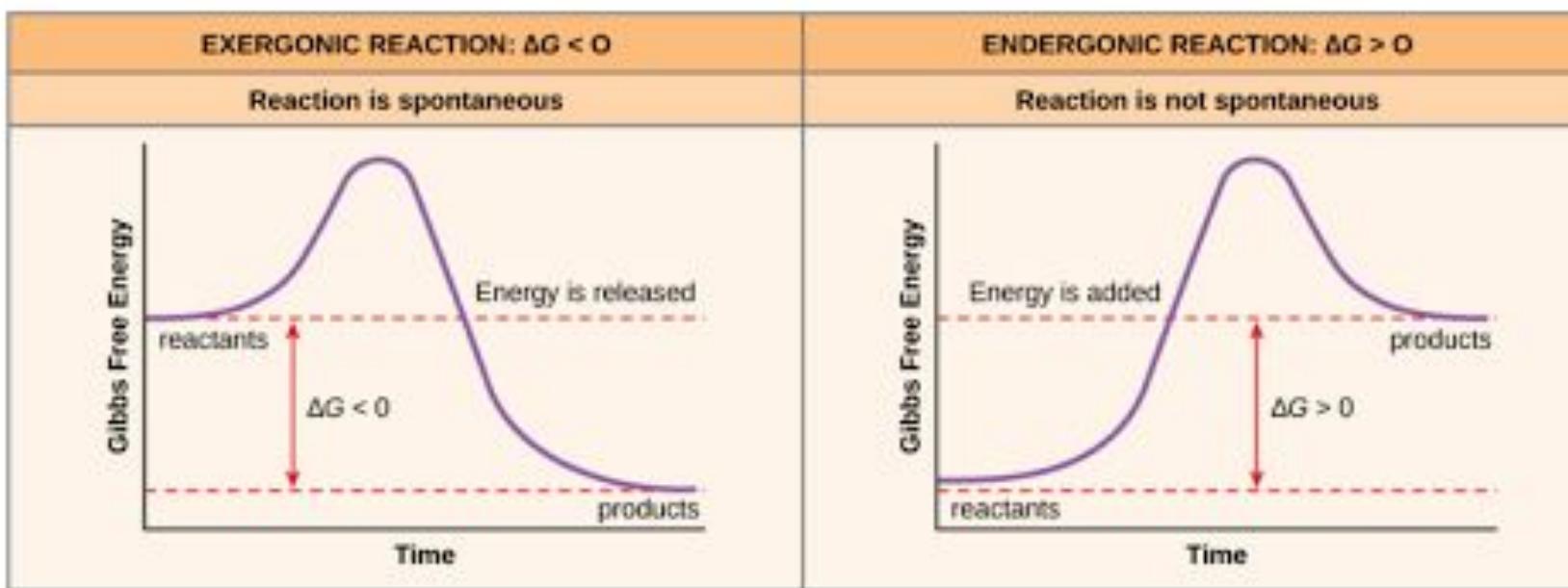
(b) Diffusion



(c) Chemical reaction

Gibbs free energy – G

The amount of energy capable of doing work during a reaction at constant temperature and pressure. When a reaction proceeds with release of free energy, the free energy change is ΔG has a negative value and the reaction is exergonic reaction. In the endergonic reactions the system gains free energy and ΔG is positive



The free energy is stored in the bonds present in the reactants and products of a reaction. In a thermodynamic reaction, the change in Gibbs free energy (ΔG) is represented as:

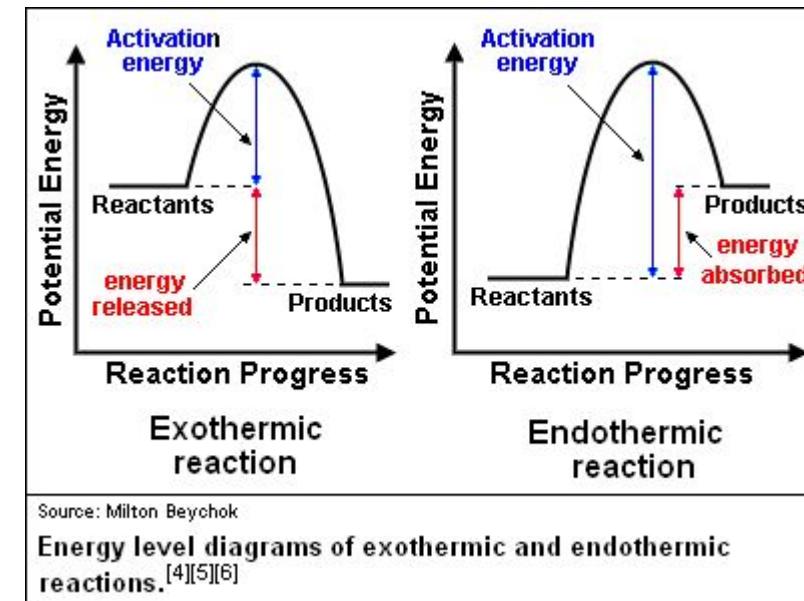
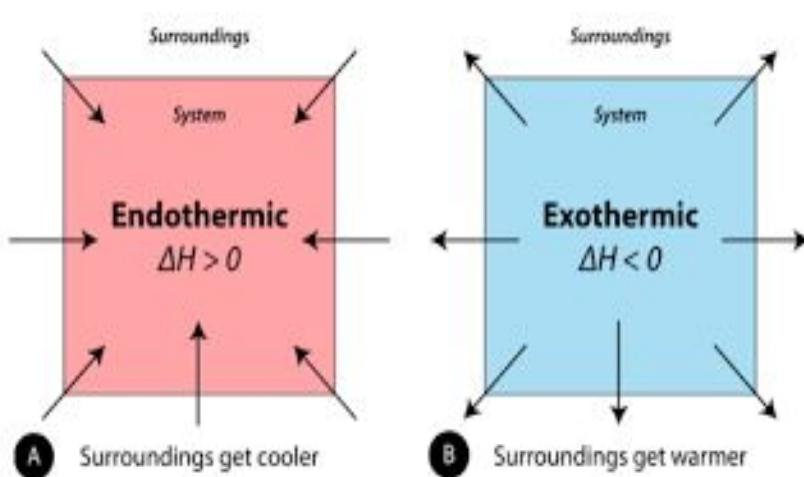
$$\Delta G = \text{total free energy of products} - \text{total free energy of reactants}$$

When $\Delta G = 0$, the reaction will be in equilibrium, which means the concentration of products and reactants does not change. $\Delta G < 0$, or a decrease in free energy, means energy is released during the reaction, and when $\Delta G > 0$, or an increase in free energy, it means energy is used up in the reaction.

- Living cell constantly perform work. They require energy for maintaining their highly organized structures, synthesizing cellular components, generating currents etc.
- Bioenergetics is quantitative study of energy conversions in biological systems. Biological energy transformations obey thermodynamics laws.
- All chemical reactions are influenced by two forces: tendency to achieve most stable bonding state (enthalpy H) and tendency to achieve the highest randomness (entropy S). The net driving force is ΔG , the free energy change.
- $\Delta G = \Delta H - T \Delta S$

Enthalpy H

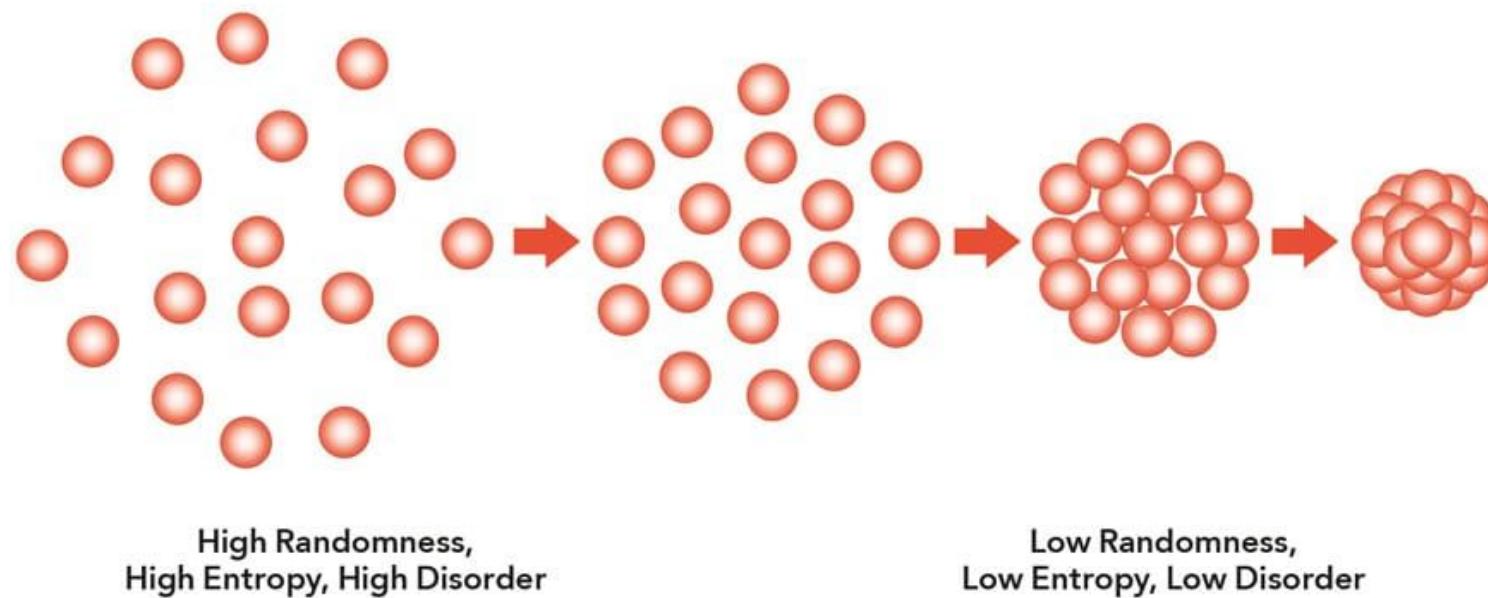
The heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and products. When a chemical reaction releases heat it is said to be exothermic reaction; the heat content of products is less than that of reactants and ΔH has a negative value. Reacting systems that take up heat from surroundings are endothermic and have positive ΔH



Entropy – S

Quantitative expression for the randomness or disorder in a system. When products of a reaction are less complex and more disordered than reactants, the reaction is said to proceed with a gain in entropy

Energy, Entropy, the 2nd law of Thermodynamics



Examples for understanding changes in enthalpy, entropy and free energy
with respect to each other



(a)



(b)



(c)

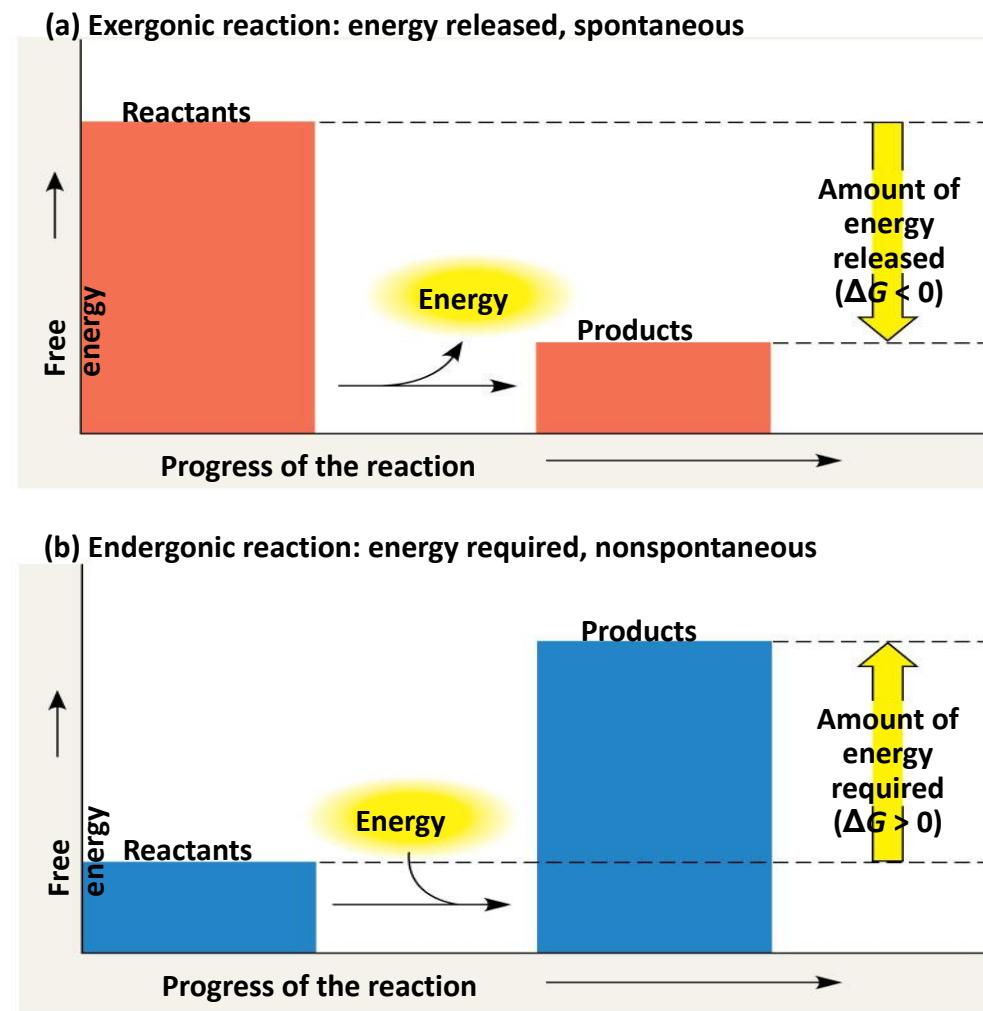


(d)

Exergonic and Endergonic Reactions in Metabolism

- An **exergonic reaction** proceeds with a net release of free energy and is spontaneous
- An **endergonic reaction** absorbs free energy from its surroundings and is nonspontaneous

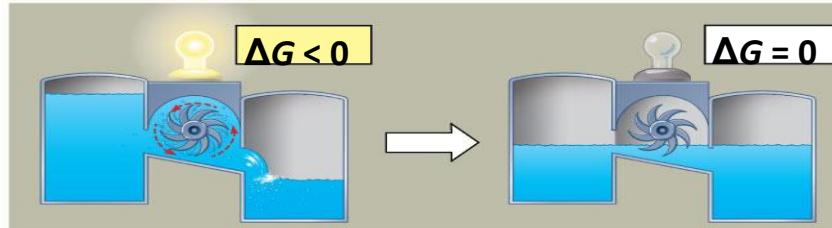
Figure 8.6



Equilibrium and Metabolism

- Reactions in a closed system eventually reach equilibrium and then do no work
- Cells are not in equilibrium; they are open systems experiencing a constant flow of materials
- A defining feature of life is that metabolism is never at equilibrium
- A catabolic pathway in a cell releases free energy in a series of reactions
- Closed and open hydroelectric systems can serve as analogies

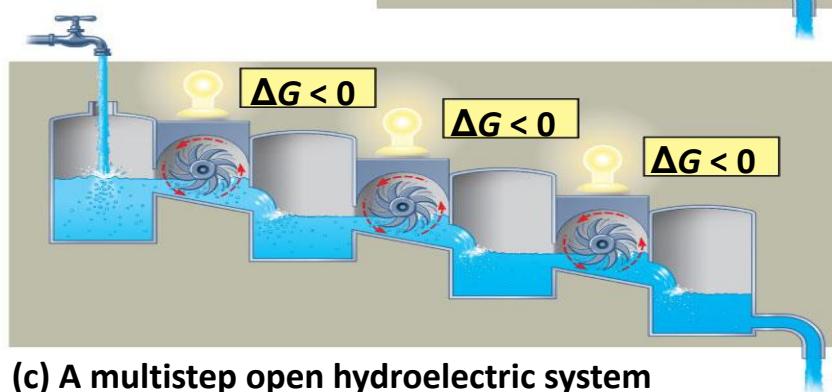
Figure 8.7



(a) An isolated hydroelectric system



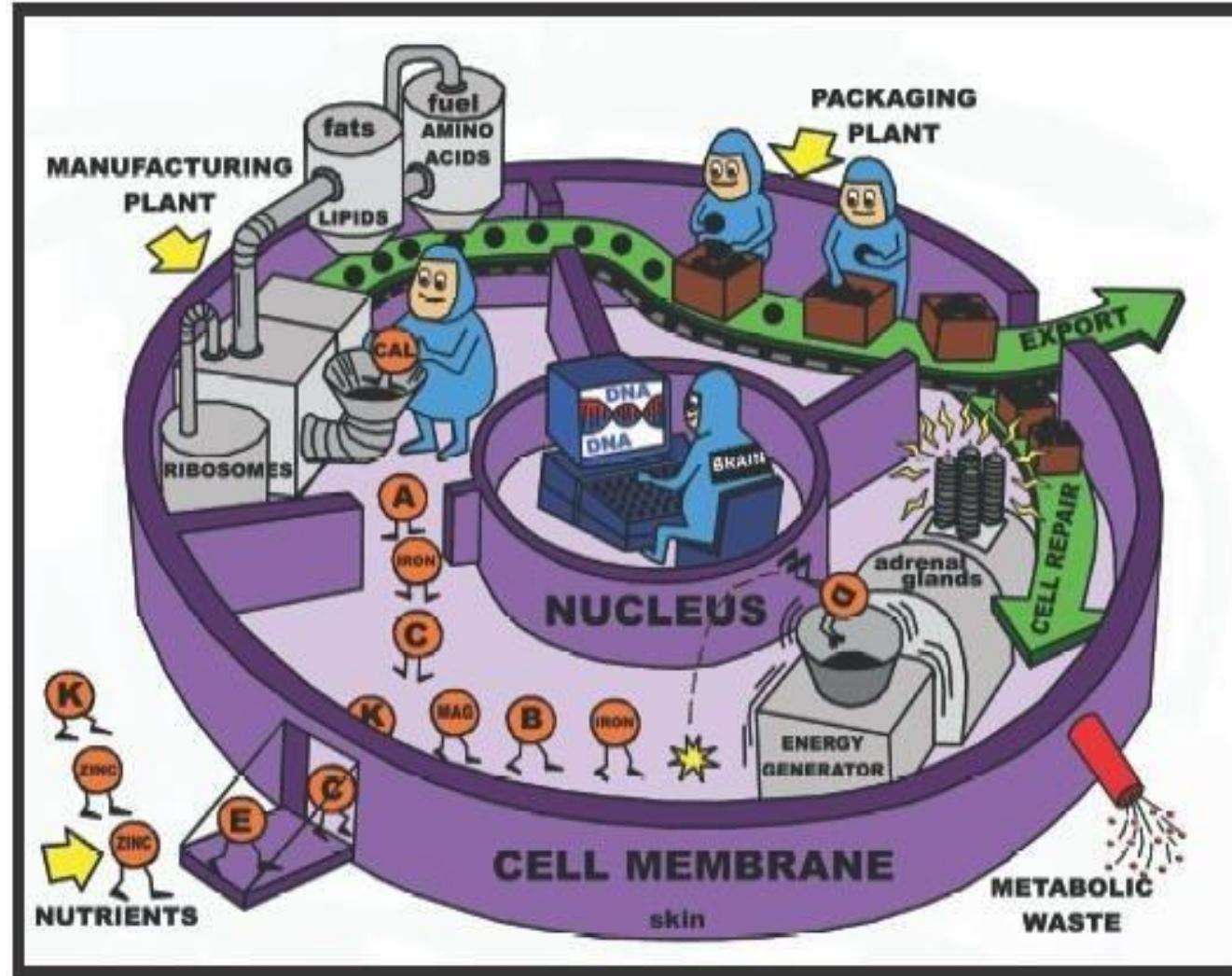
(b) An open hydroelectric system



(c) A multistep open hydroelectric system

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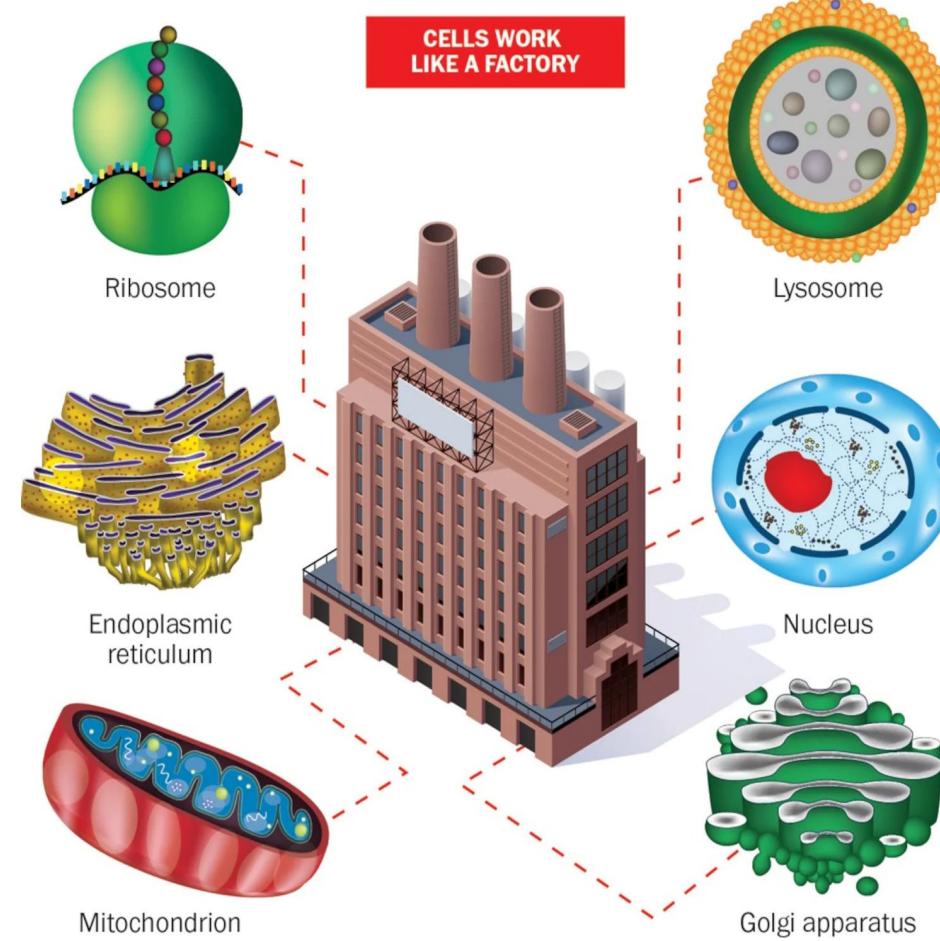
Cells as Chemical Factories



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Cells get raw materials — including water, oxygen, minerals and other nutrients — from the foods. Nutrients are transported through the cell membrane: the thin, elastic structure that forms the border of each cell.

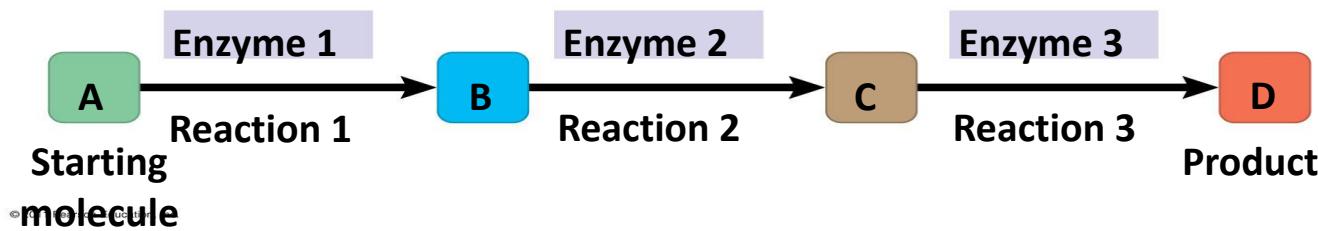
- Nucleus - it controls cell function. It contains DNA (deoxyribonucleic acid), the master organizer for how cells work.
- Mitochondria are the “batteries” in your cells. Chemical reactions within the mitochondria create the energy that powers cell functions.
- Lysosomes are fluid-filled vesicles, or sacs, that act as a waste-disposal system for cells.
- Ribosomes are the cell’s molecule makers. They assemble proteins from amino acids.
- The endoplasmic reticulum is a system of tubelike structures that’s essential for the production of proteins and lipids (fats).
- The Golgi apparatus is like a conveyor belt that “wraps” proteins inside vesicles so they can be “shipped” out of the cell.



(iStock images/The Washington Post illustration)

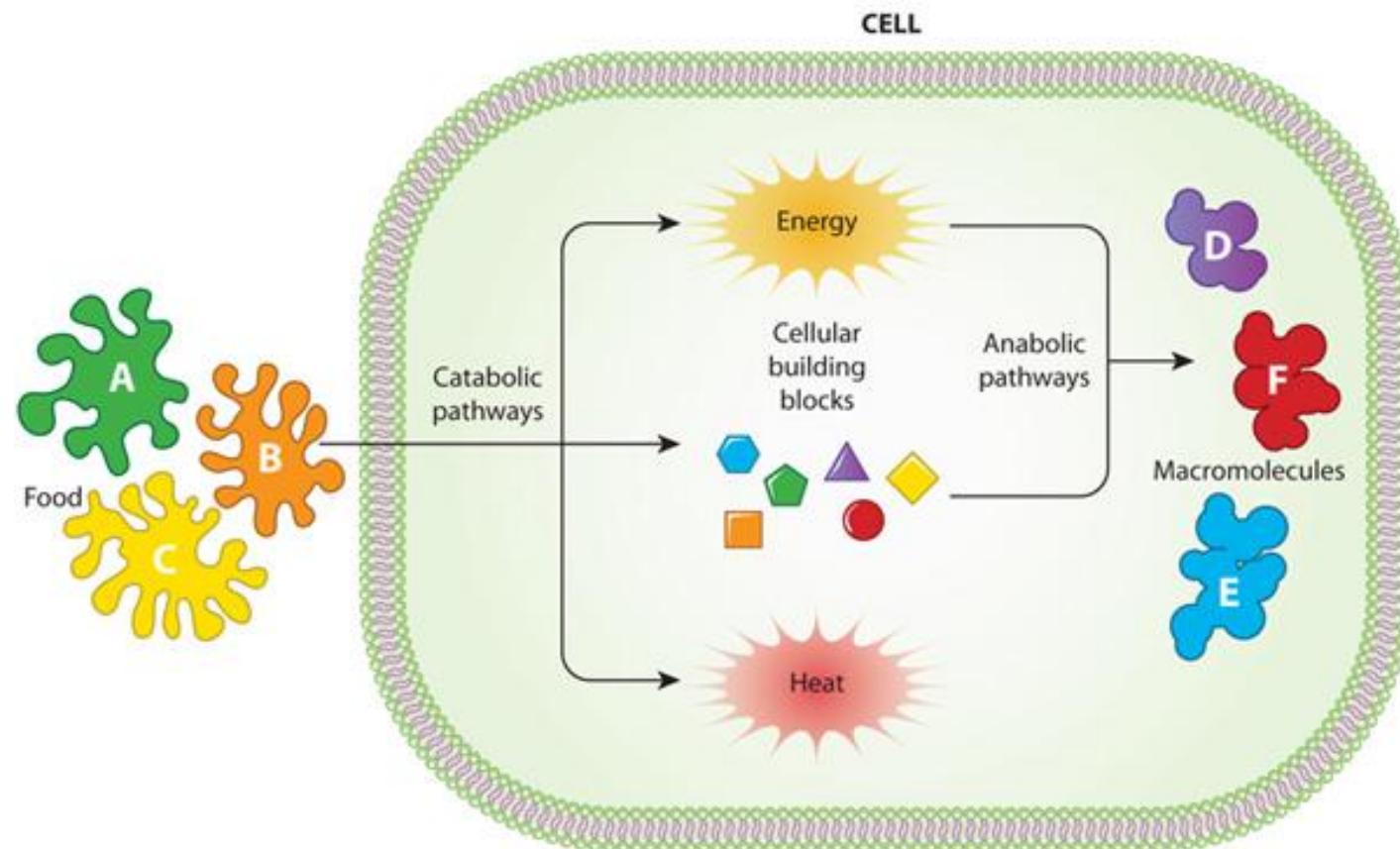
Metabolism

- **Metabolism** is the totality of an organism's chemical reactions
- Metabolism is an emergent property of life that arises from interactions between molecules within the cell
- An organism's metabolism transforms matter and energy, subject to the laws of thermodynamics



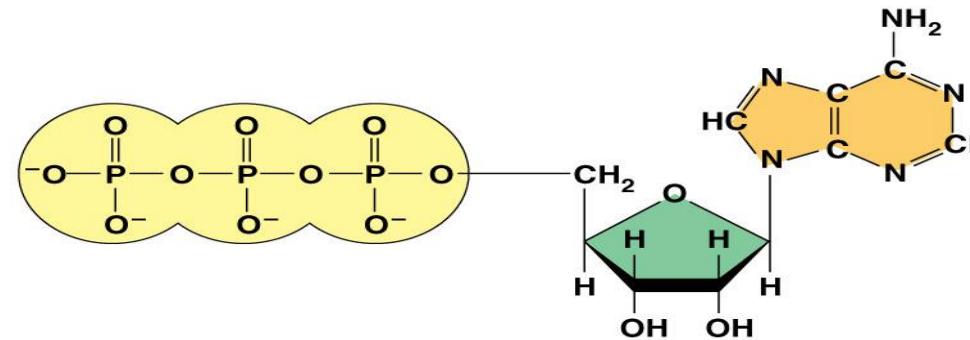
- A **metabolic pathway** begins with a specific molecule and ends with a product
- Each step is catalyzed by a specific enzyme

Catabolism and Anabolism

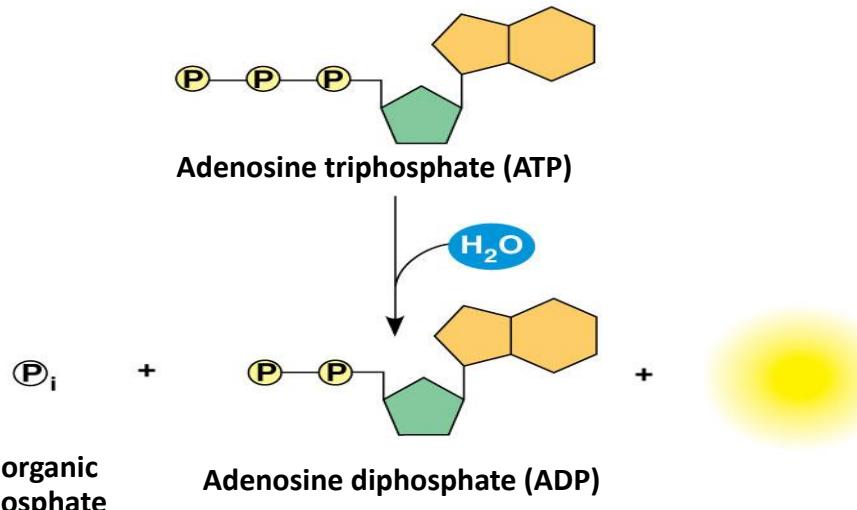


In living cells, energy is stored and transferred in several forms, most commonly in the form of a high-energy chemical bond on the molecule adenosine triphosphate (ATP).

The ultimate source of the energy used to synthesize ATP in fact comes from metabolic breakdown of glucose in a pathway known as glycolysis



(a) The structure of ATP



(b) The hydrolysis of ATP

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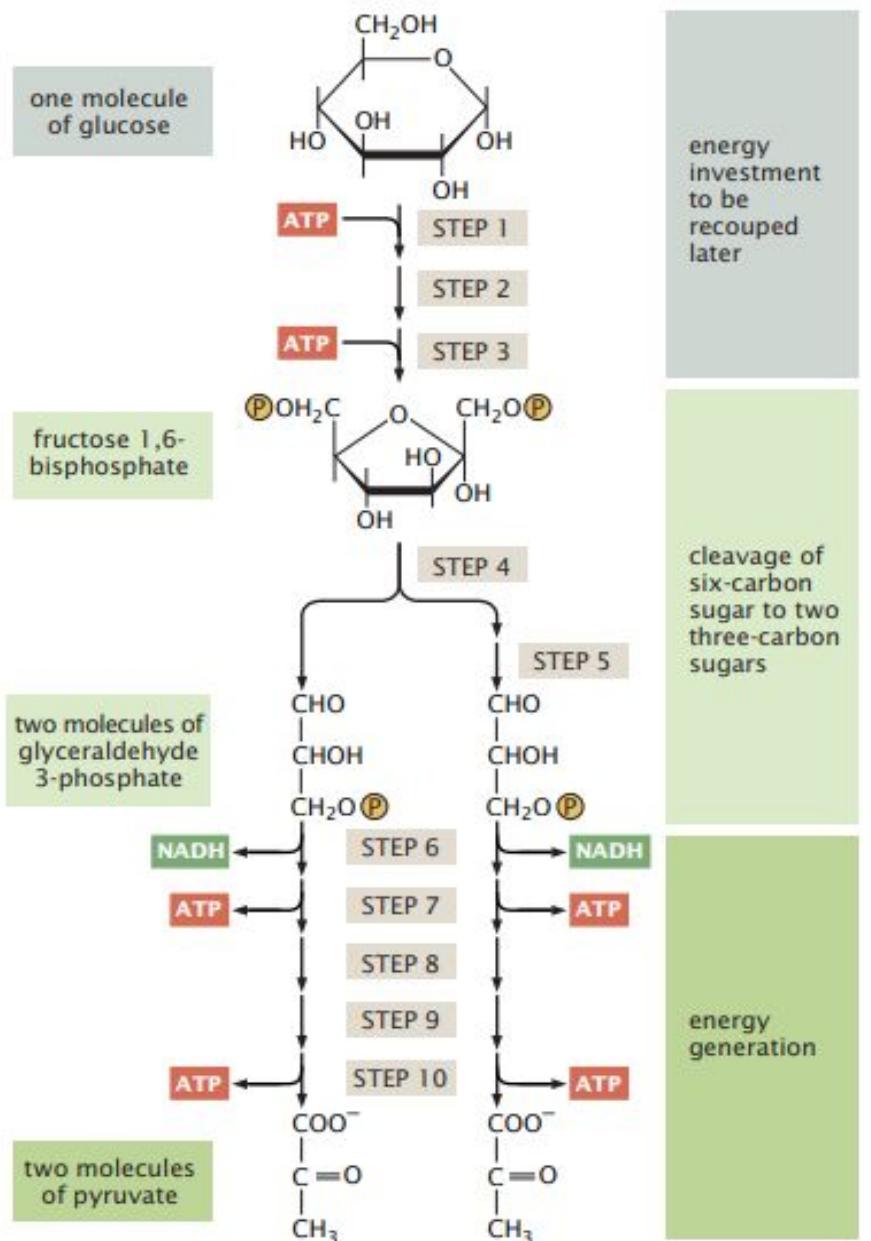


Figure 5.2: A schematic outlining the overall organization of the glycolytic pathway. The outcome of the 10 steps of glycolysis is the conversion of a single molecule of glucose into two molecules of pyruvate and the concomitant net production of two molecules of ATP and two of NADH. (Adapted from B. Alberts et al., Molecular Biology of the Cell, 5th ed. Garland Science, 2008.)

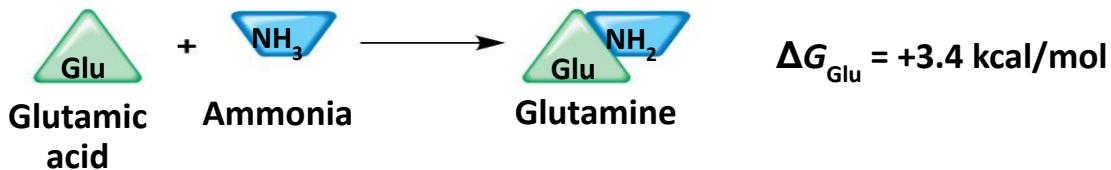
How the Hydrolysis of ATP Performs Work

- The three types of cellular work (mechanical, transport, and chemical) are powered by the hydrolysis of ATP
- In the cell, the energy from the exergonic reaction of ATP hydrolysis can be used to drive an endergonic reaction
- Overall, the coupled reactions are exergonic

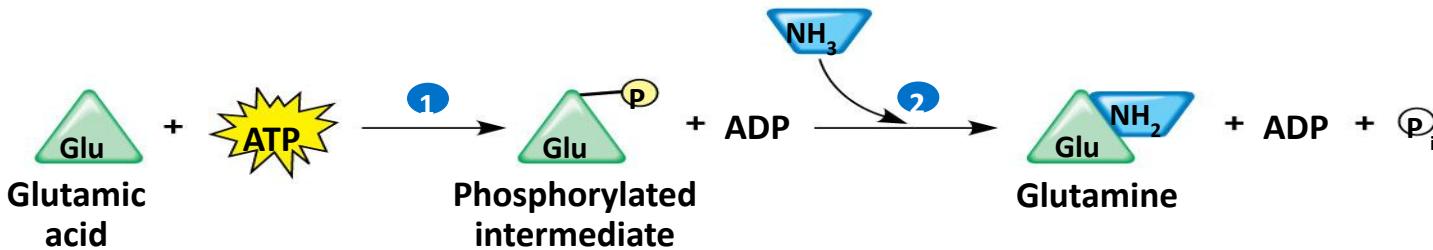
- ATP drives endergonic reactions by phosphorylation, transferring a phosphate group to some other molecule, such as a reactant
- The recipient molecule is now called a **phosphorylated intermediate**

Figure 8.9

(a) Glutamic acid conversion to glutamine



(b) Conversion reaction coupled with ATP hydrolysis



(c) Free-energy change for coupled reaction

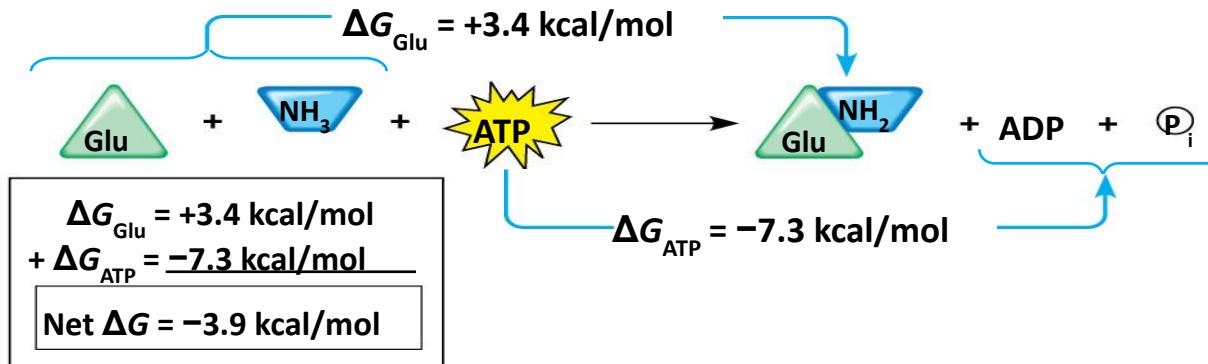
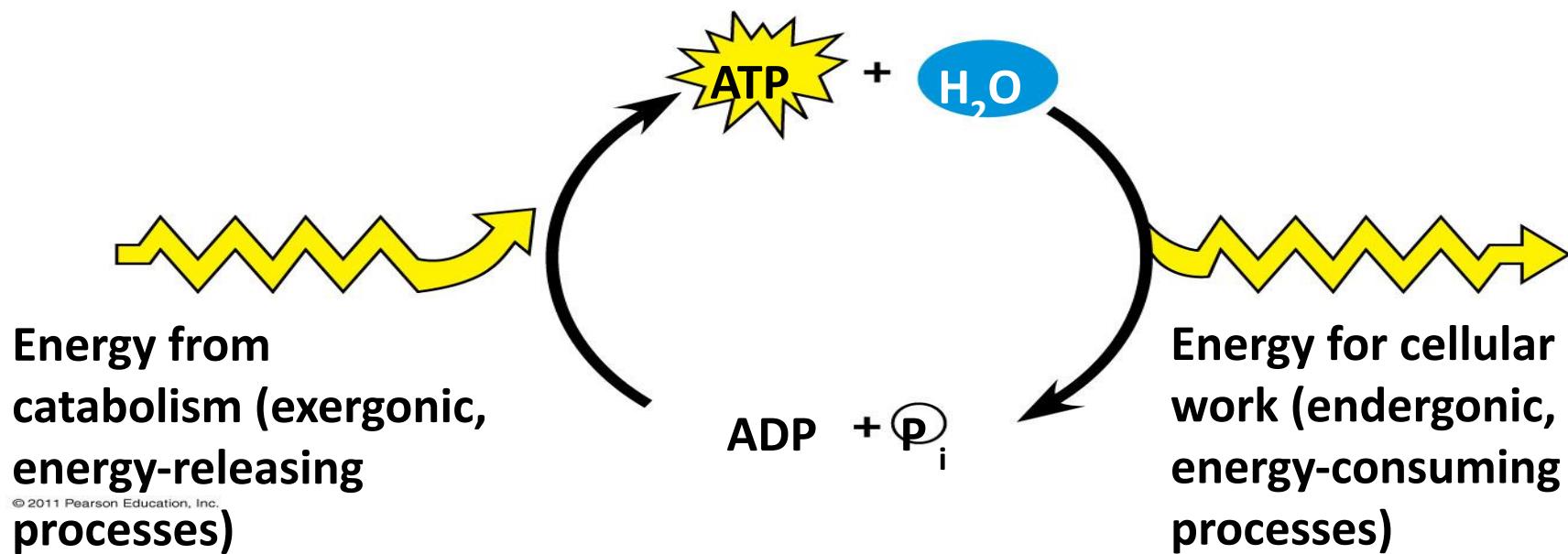


Figure 8.11

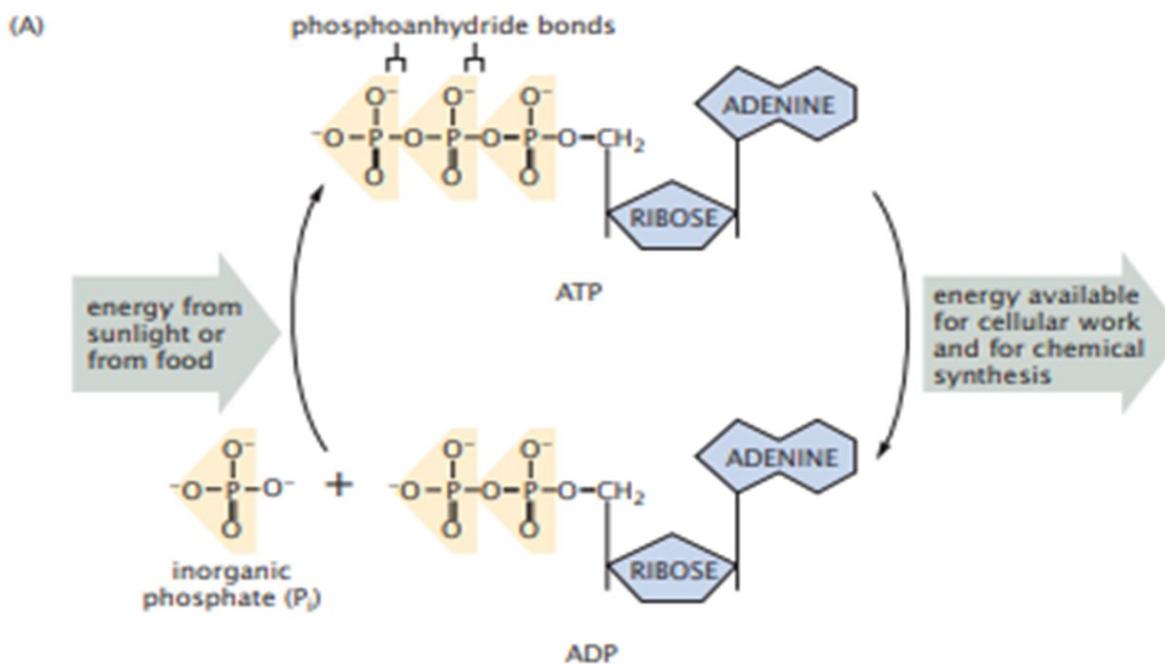


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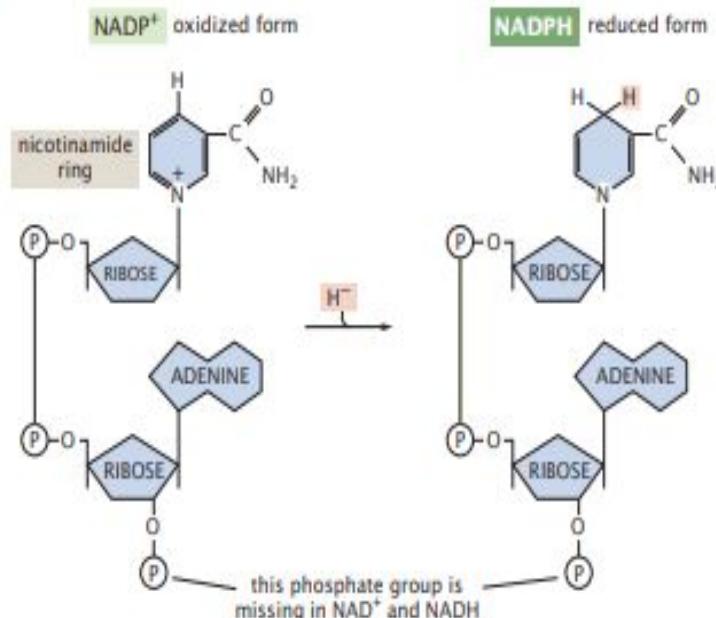
Three important forms of biological energy.

(A) Energy for chemical synthesis and for force generation is stored in the form of ATP, which can be converted to ADP + Pi releasing roughly 20 kBT . kT (energy) kT (also written as kBT) is the product of the Boltzmann constant, k (or k_B), and the temperature, T , of useful energy. ADP + Pi can then be converted back to ATP.

While many enzymes use ATP itself, others use guanosine triphosphate (GTP), uridine triphosphate (UTP), or cytosine triphosphate (CTP), but the energies are equivalent.



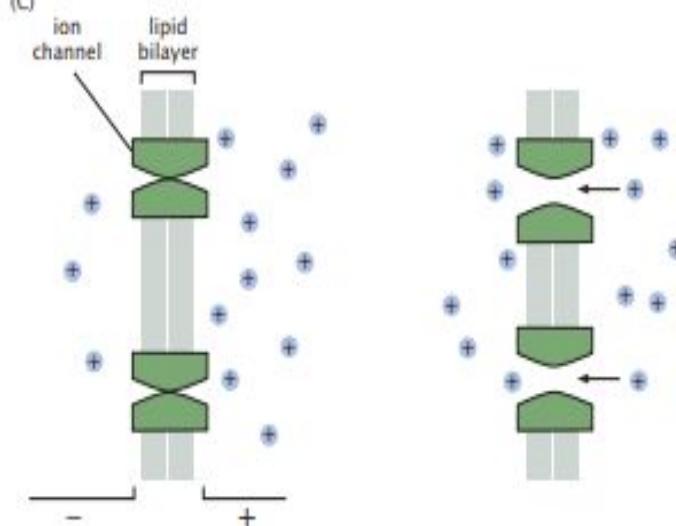
(B)



(B) Reducing potential is carried in the form of transferrable high-energy electrons on NADH (or the very similar molecule NADPH). Two electrons can be transferred from NADPH to reduce an oxidized organic compound, liberating one hydrogen ion (H^+) and the oxidized form of the carrier molecule NADP⁺.

In this case, the energy liberated by oxidation of one mole of NADH can be used to synthesize roughly two or three moles of ATP.

(C)



(C) Transmembrane ion gradients, particularly in the form of H^+ gradients, are also used to store energy. The H^+ gradient across the membrane yields a negative potential on the left and a positive potential on the right. When ion channels open, the ions can flow down their electrochemical gradient.

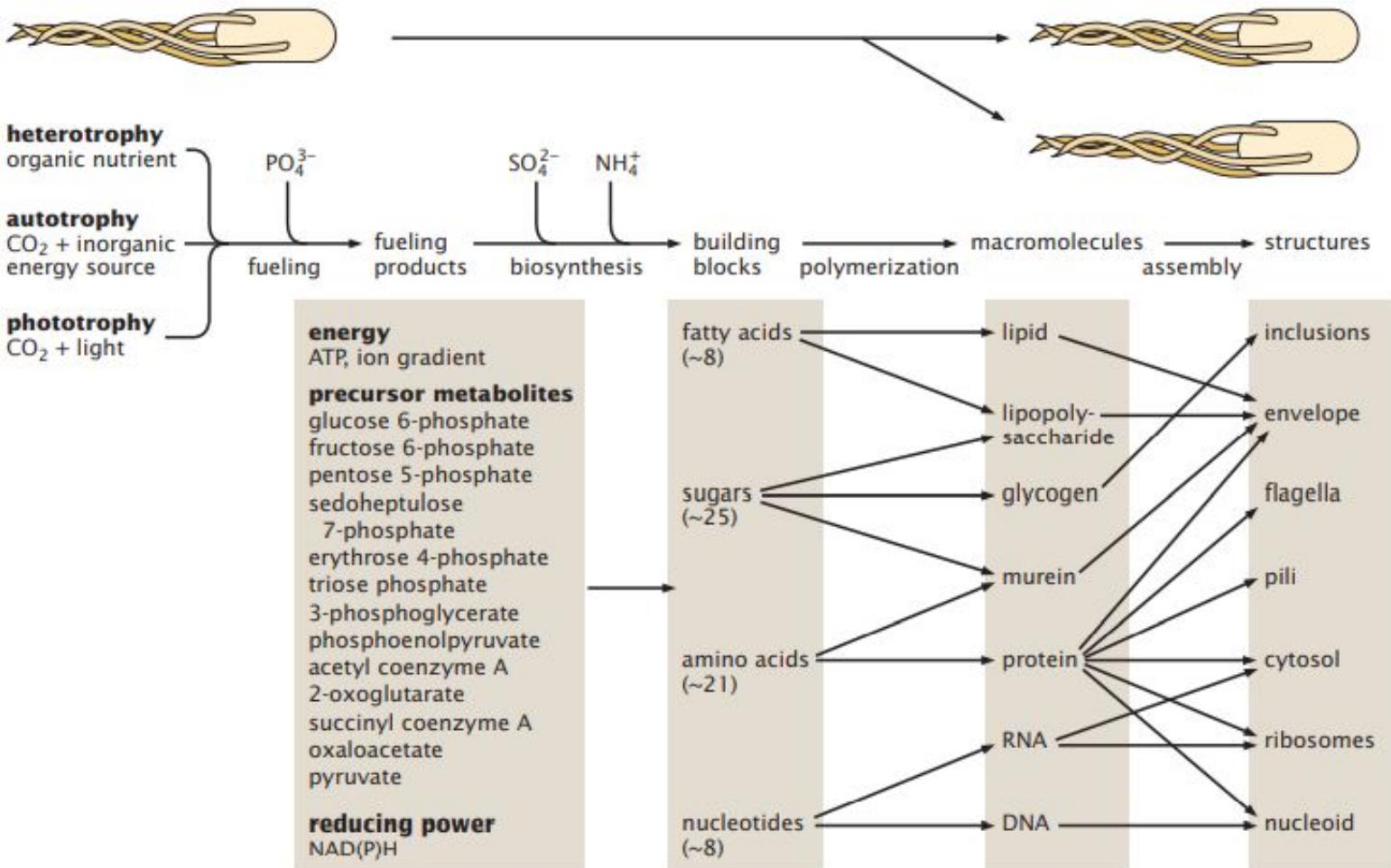


Figure 5.4: Energy and mass costs to make a new bacterial cell. This diagram illustrates the flow of materials and energy required for bacterial duplication. Nutrients are taken from the environment, either organic molecules provided by other organisms or carbon dioxide and light in the case of photosynthetic bacteria. Together with a few inorganic ions such as phosphate, sulfate, and ammonium, the carbon sources consumed by the bacterium are converted into precursor metabolites and then into the fatty acids, sugars, amino acids, and nucleotides that are used to build macromolecules. The macromolecules are further assembled into large-scale structures of the cell. The numbers shown in the "building blocks" column correspond to the rough number of molecular building blocks of each type. (Adapted from M. Schaechter et al., *Microbe*. ASM Press, 2006.)

The Molecular Basis of Inheritance

Figure 16.1

- Overview: Life's Operating Instructions
- In 1953, James Watson and Francis Crick shook the world
 - With an elegant double-helical model for the structure of deoxyribonucleic acid, or DNA

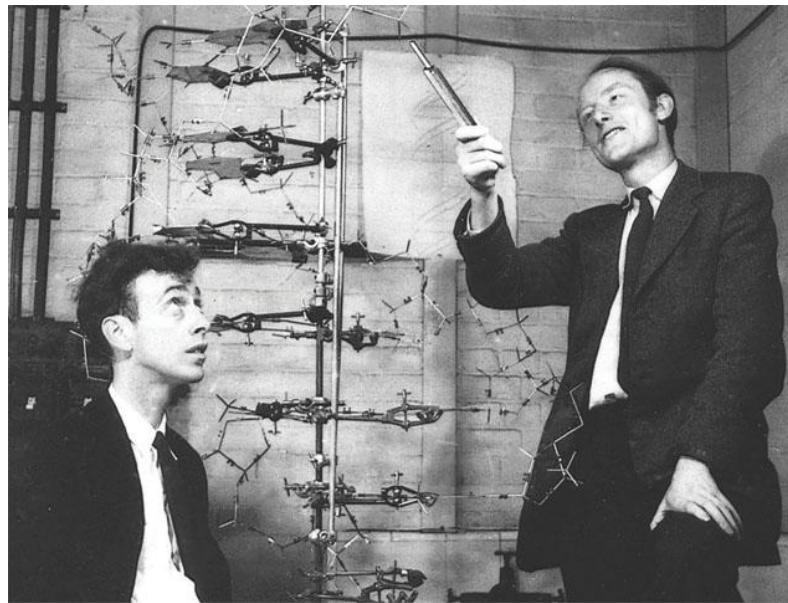


Figure
16.1

- DNA, the substance of inheritance
 - Is the most celebrated molecule of our time
- Hereditary information
 - Is encoded in the chemical language of DNA and reproduced in all the cells of your body
- It is the DNA program
 - That directs the development of many different types of traits

- Concept 16.1: DNA is the genetic material
- Early in the 20th century
 - The identification of the molecules of inheritance loomed as a major challenge to biologists

The Search for the Genetic Material: *Scientific Inquiry*

- The role of DNA in heredity
 - Was first worked out by studying bacteria and the viruses that infect them

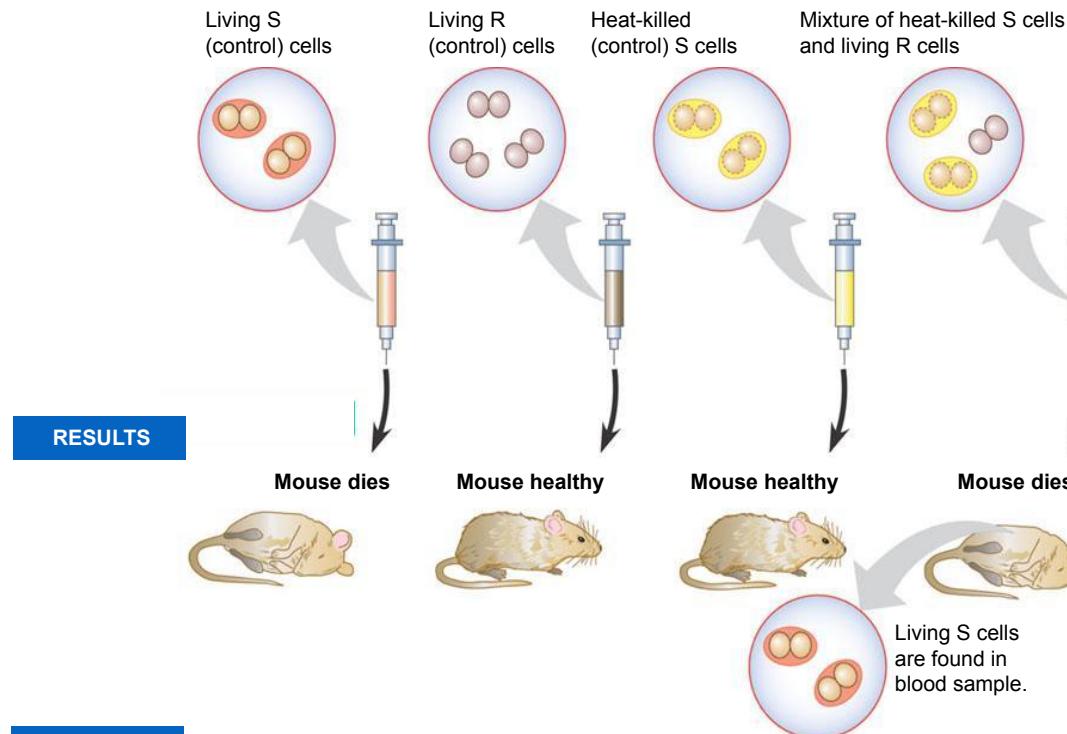
Evidence That DNA Can Transform Bacteria

- Frederick Griffith was studying *Streptococcus pneumoniae*
 - A bacterium that causes pneumonia in mammals
- He worked with two strains of the bacterium
 - A pathogenic strain and a nonpathogenic strain

- Griffith found that when he mixed heat-killed remains of the pathogenic strain
 - With living cells of the nonpathogenic strain, some of these living cells became pathogenic

EXPERIMENT

Bacteria of the "S" (smooth) strain of *Streptococcus pneumoniae* are pathogenic because they have a capsule that protects them from an animal's defense system. Bacteria of the "R" (rough) strain lack a capsule and are nonpathogenic. Frederick Griffith injected mice with the two strains as shown below:



RESULTS

Figure 16.2

CONCLUSION

Griffith concluded that the living R bacteria had been transformed into pathogenic S bacteria by an unknown, heritable substance from the dead S cells.

- Griffith called the phenomenon transformation
 - Now defined as a change in genotype and phenotype due to the assimilation of external DNA by a cell

Evidence That Viral DNA Can Program Cells

- Additional evidence for DNA as the genetic material
 - Came from studies of a virus that infects bacteria

Figure 16.3

- Viruses that infect bacteria, bacteriophages
 - Are widely used as tools by researchers in molecular genetics

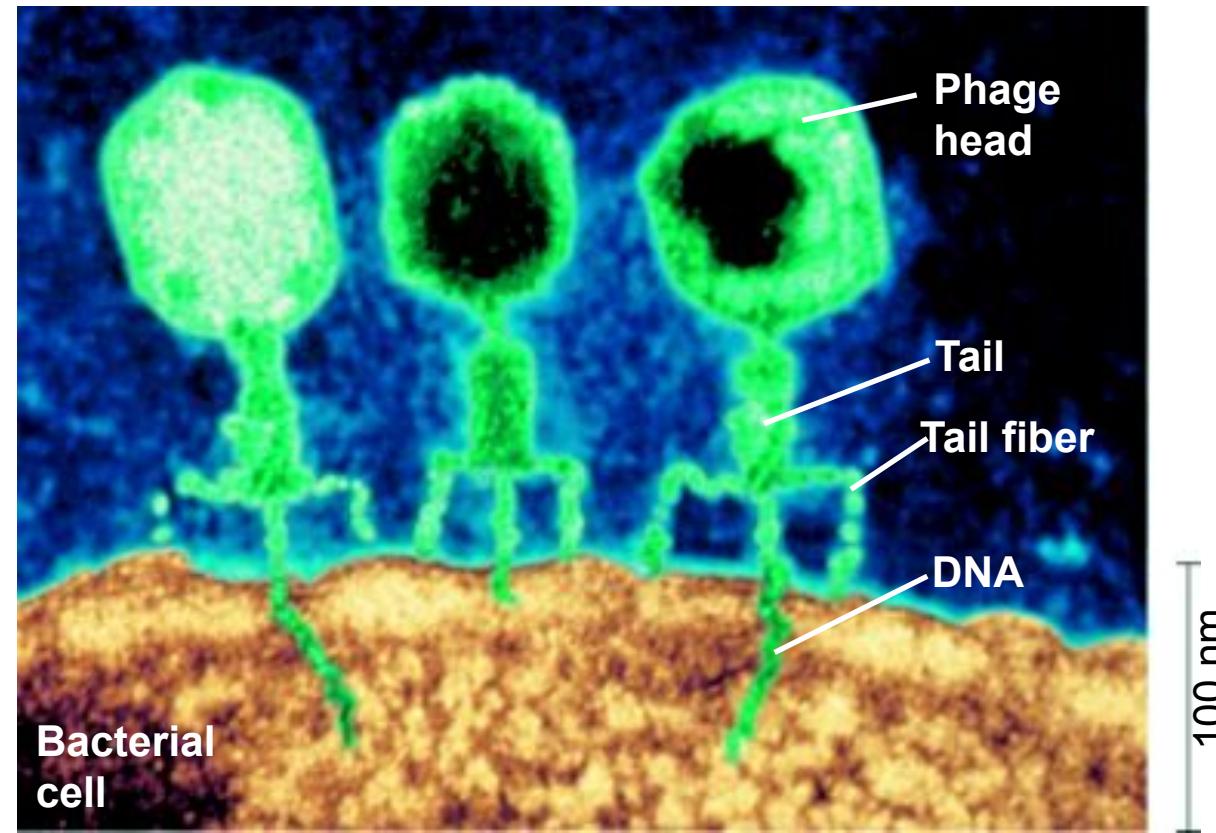


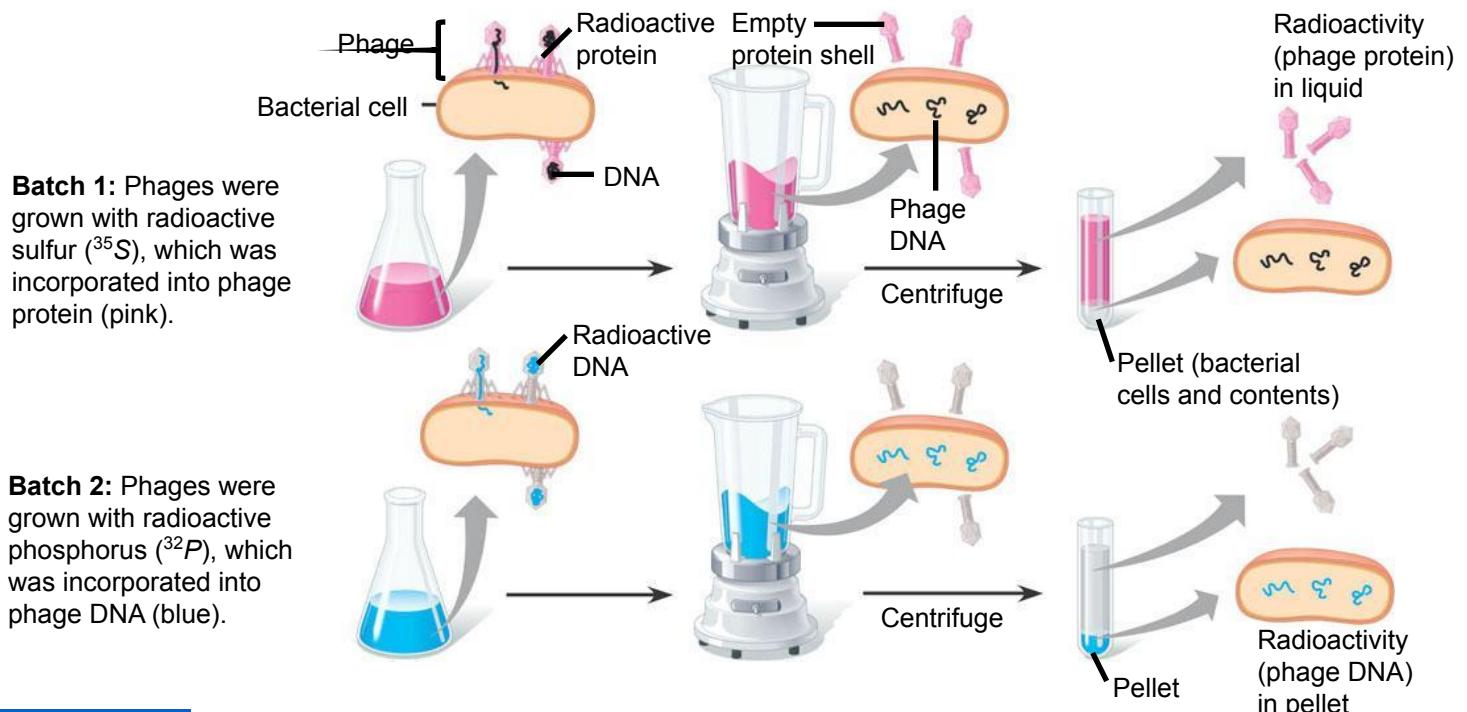
Figure 16.3

- Alfred Hershey and Martha Chase
 - Performed experiments showing that DNA is the genetic material of a phage known as T2

The Hershey and Chase experiment

EXPERIMENT In their famous 1952 experiment, Alfred Hershey and Martha Chase used radioactive sulfur and phosphorus to trace the fates of the protein and DNA, respectively, of T2 phages that infected bacterial cells.

- 1 Mixed radioactively labeled phages with bacteria. The phages infected the bacterial cells.
- 2 Agitated in a blender to separate phages outside the bacteria from the bacterial cells.
- 3 Centrifuged the mixture so that bacteria formed a pellet at the bottom of the test tube.
- 4 Measured the radioactivity in the pellet and the liquid



RESULTS Phage proteins remained outside the bacterial cells during infection, while phage DNA entered the cells. When cultured, bacterial cells with radioactive phage DNA released new phages with some radioactive phosphorus.

CONCLUSION Hershey and Chase concluded that DNA, not protein, functions as the T2 phage's genetic material.

Figure 16.4

Additional Evidence That DNA Is the Genetic Material

- Prior to the 1950s, it was already known that DNA
 - Is a polymer of nucleotides, each consisting of three components: a nitrogenous base, a sugar, and a phosphate group

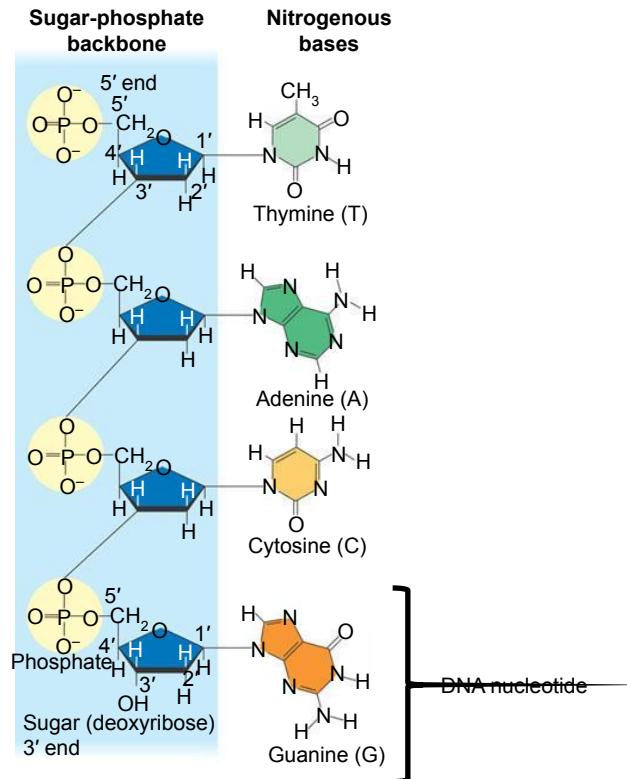
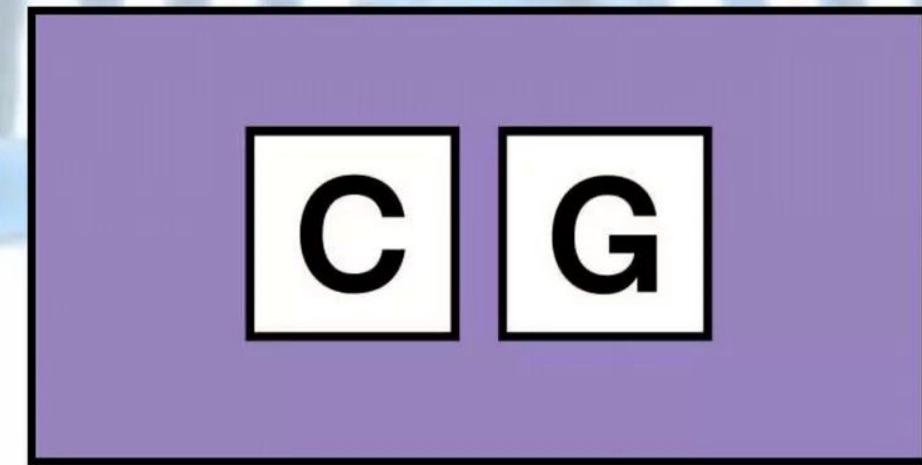
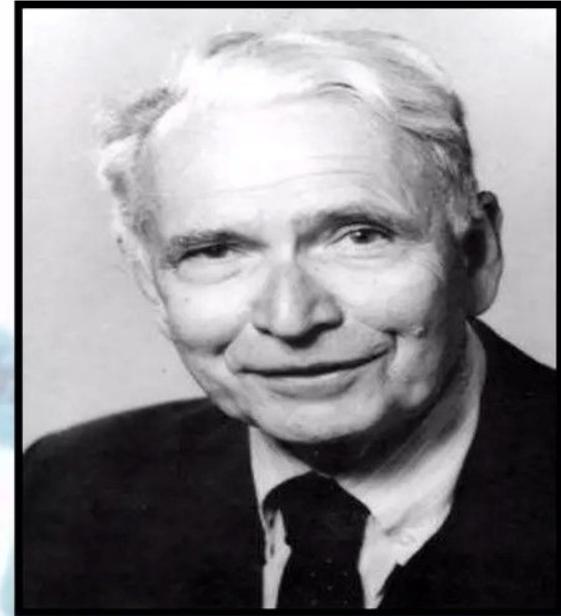


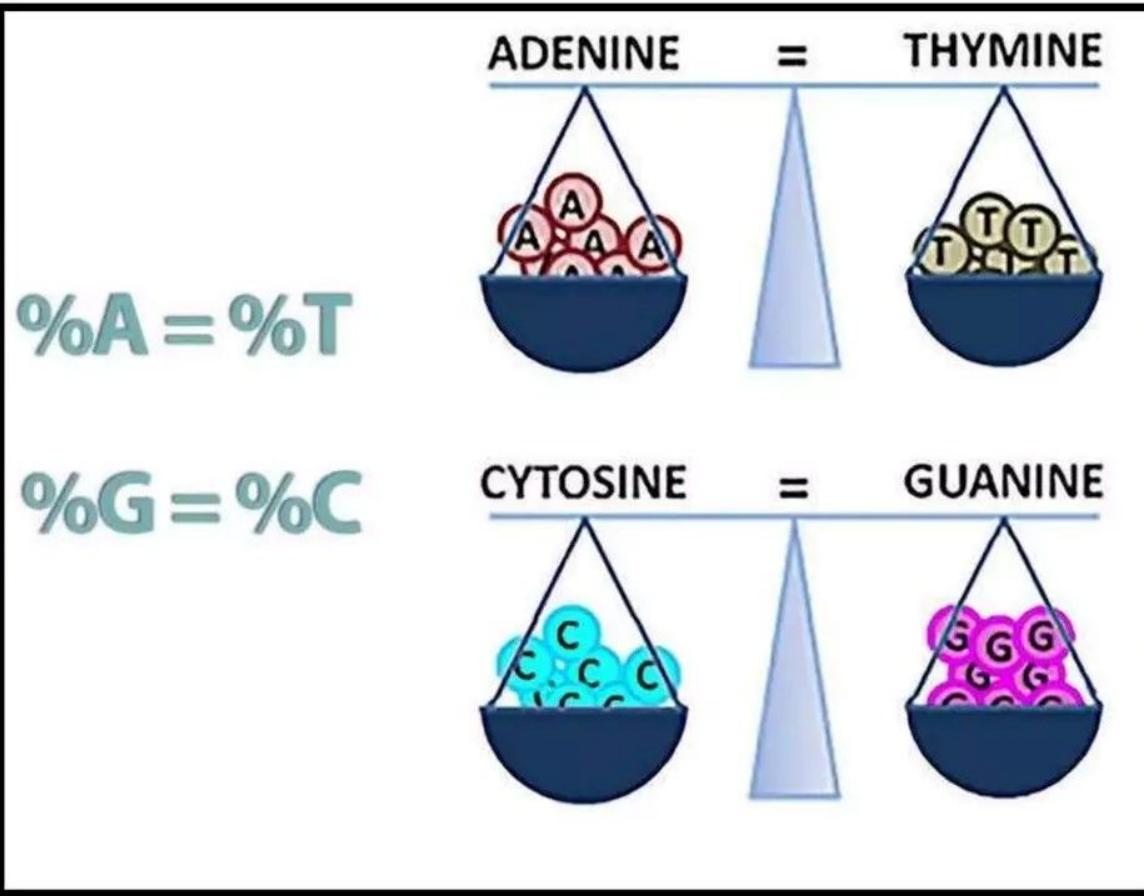
Figure 16.5

- Erwin Chargaff analyzed the base composition of DNA
 - From a number of different organisms
- In 1947, Chargaff reported
 - That DNA composition varies from one species to the next
- This evidence of molecular diversity among species
 - Made DNA a more credible candidate for the genetic material

Chargaff's rule:

- The composition of DNA from many different organisms was analyzed by E.Chargaff and his colleagues.
- It was observed that concentration of thymine was always equal .to the concentration of adenine ($A = T$). And the concentration of cytosine was equal to the concentration of guanine ($G=C$).
- This strongly suggest that thymine and adenine as well as cytosine and guanine were present in DNA with fixed interrelationship.
- Also the total concentration of purines ($A + G$) always equal to the total concentration of pyrimidine ($T + C$). All DNA possess purine and pyrimidine in equal proportions (1:1 ratio).
- However, the $(T+A)/ (G+C)$ ratio was found to vary widely in DNAs of different species.





$$\frac{A + G}{T + C} = 1$$

- If $(A + T) > (G + C)$ then DNA is referred as **(AT- type)**.
- If $(G + C) > (A + T)$ then DNA is referred as **(GC- type)**.

Building a Structural Model of DNA: *Scientific Inquiry*

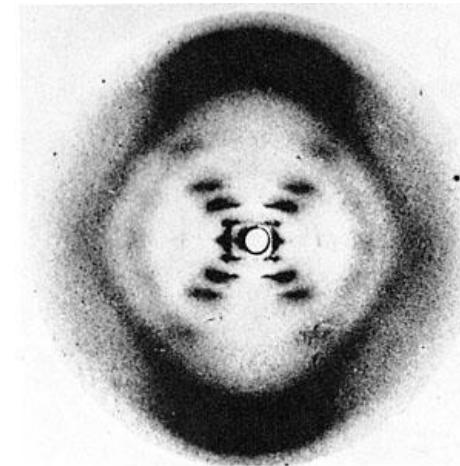
- Once most biologists were convinced that DNA was the genetic material
 - The challenge was to determine how the structure of DNA could account for its role in inheritance

(a) Rosalind Franklin

- Maurice Wilkins and Rosalind Franklin
 - Were using a technique called X-ray crystallography to study molecular structure
- Rosalind Franklin
 - Produced a picture of the DNA molecule using this technique



(a) Rosalind Franklin

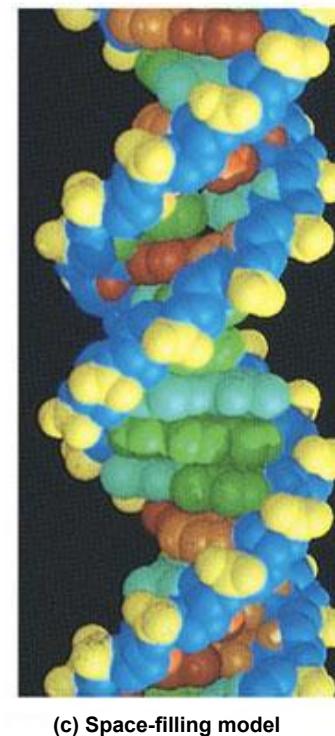
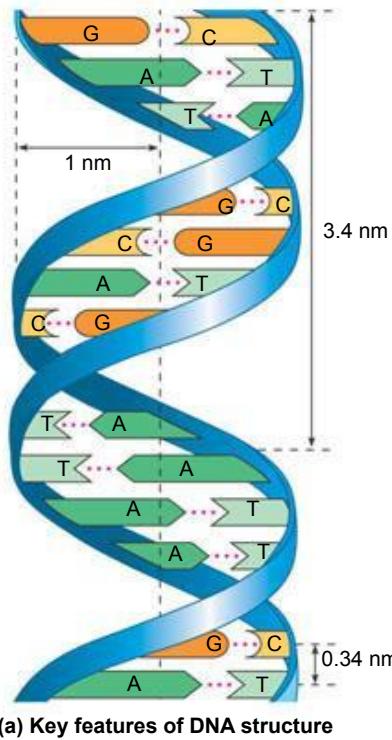


(b) Franklin's X-ray diffraction Photograph of DNA

Figure 16.6 a, b

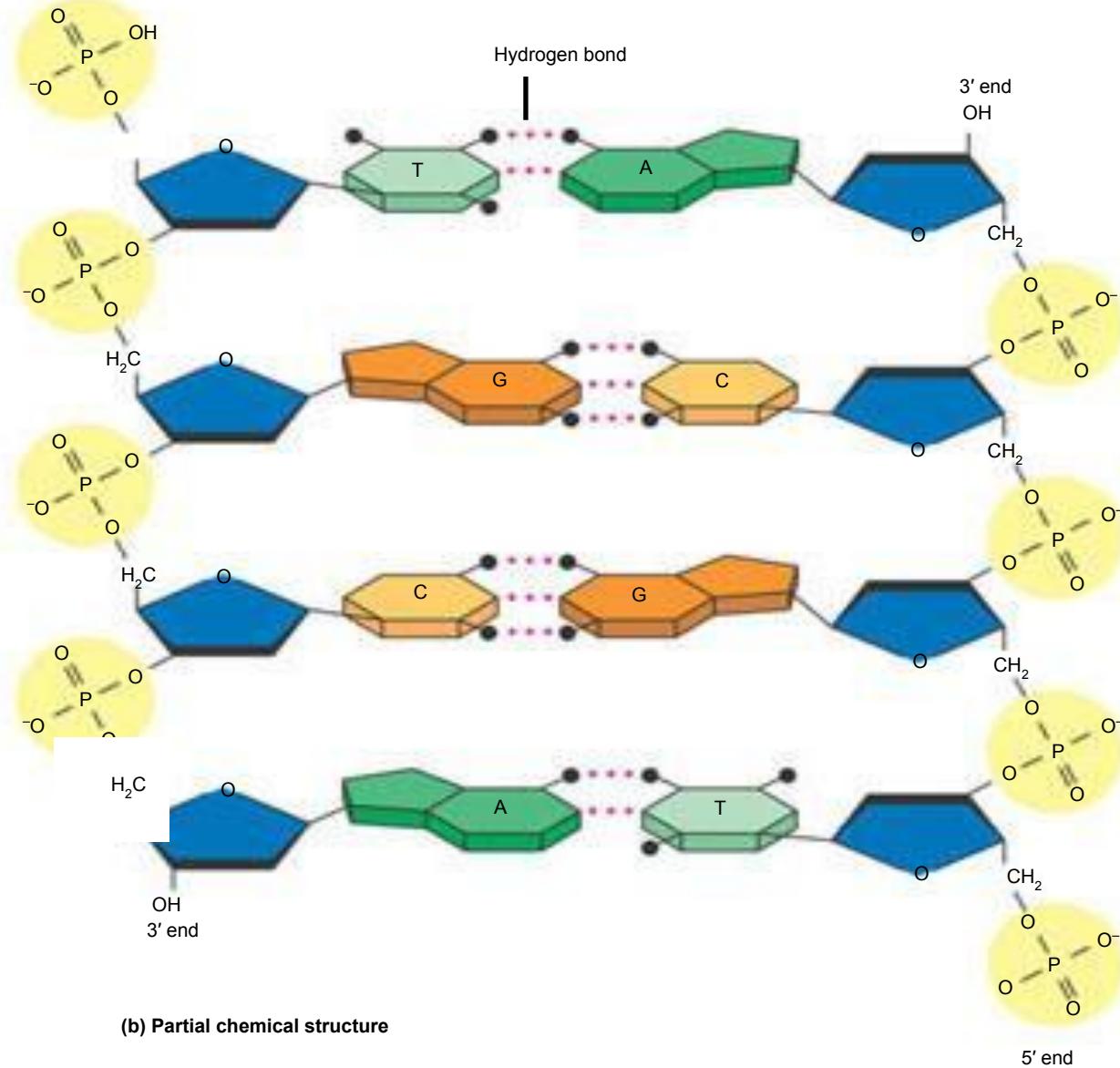
Figure 16.7a, c

- Watson and Crick deduced that DNA was a double helix
 - Through observations of the X-ray crystallographic images of DNA



- Franklin had concluded that DNA
 - Was composed of two antiparallel sugar-phosphate backbones, with the nitrogenous bases paired in the molecule's interior
- The nitrogenous bases
 - Are paired in specific combinations: adenine with thymine, and cytosine with guanine

5' end



- Watson and Crick reasoned that there must be additional specificity of pairing
 - Dictated by the structure of the bases
- Each base pair forms a different number of hydrogen bonds
 - Adenine and thymine form two bonds, cytosine and guanine form three bonds

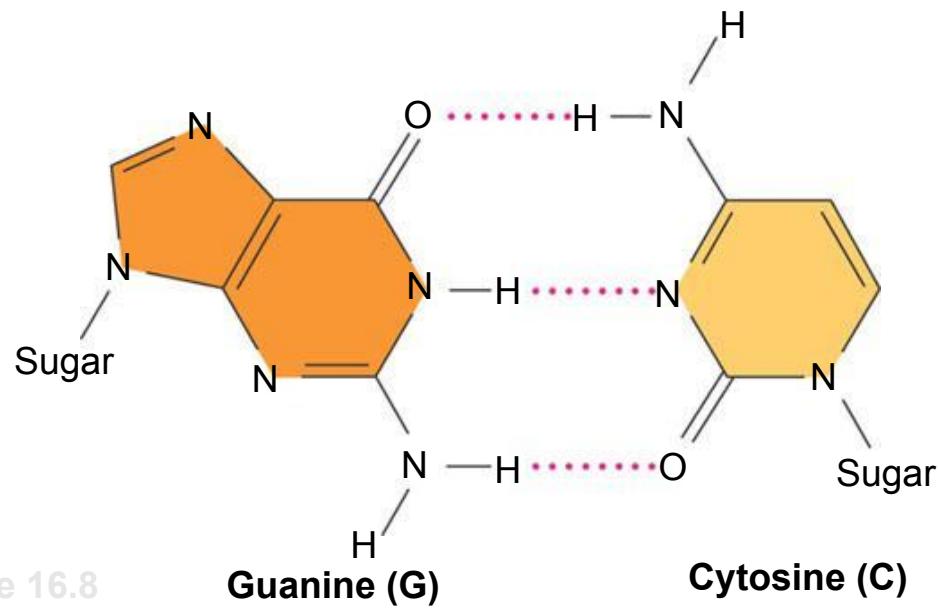
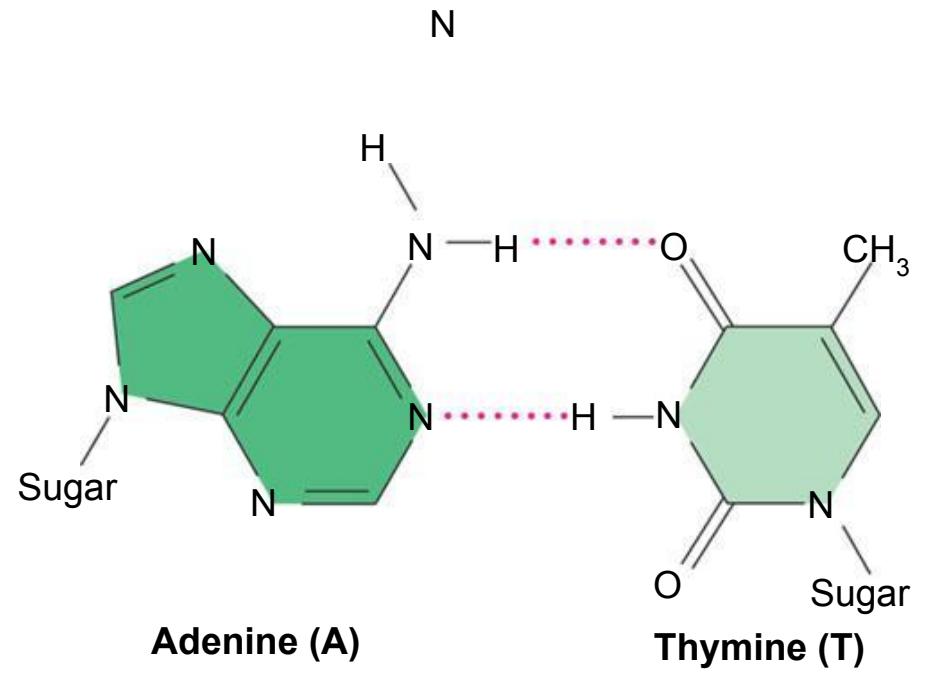


Figure 16.8

- Concept 16.2: Many proteins work together in DNA replication and repair
- The relationship between structure and function
 - Is manifest in the double helix

The Basic Principle: Base Pairing to a Template Strand

- Since the two strands of DNA are complementary
 - Each strand acts as a template for building a new strand in replication

- In DNA replication
 - The parent molecule unwinds, and two new daughter strands are built based on base-pairing rules

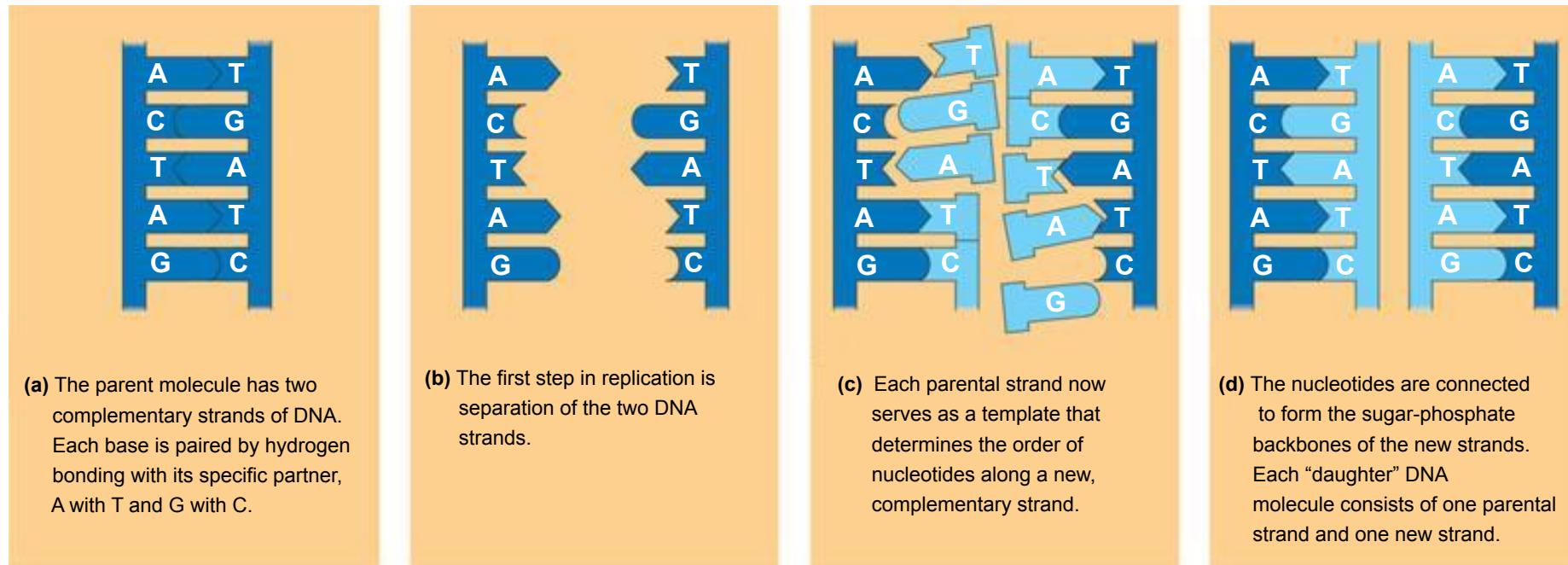


Figure 16.9 a-d

• DNA replication is semiconservative

- Each of the two new daughter molecules will have one old strand, derived from the parent molecule, and one newly made strand

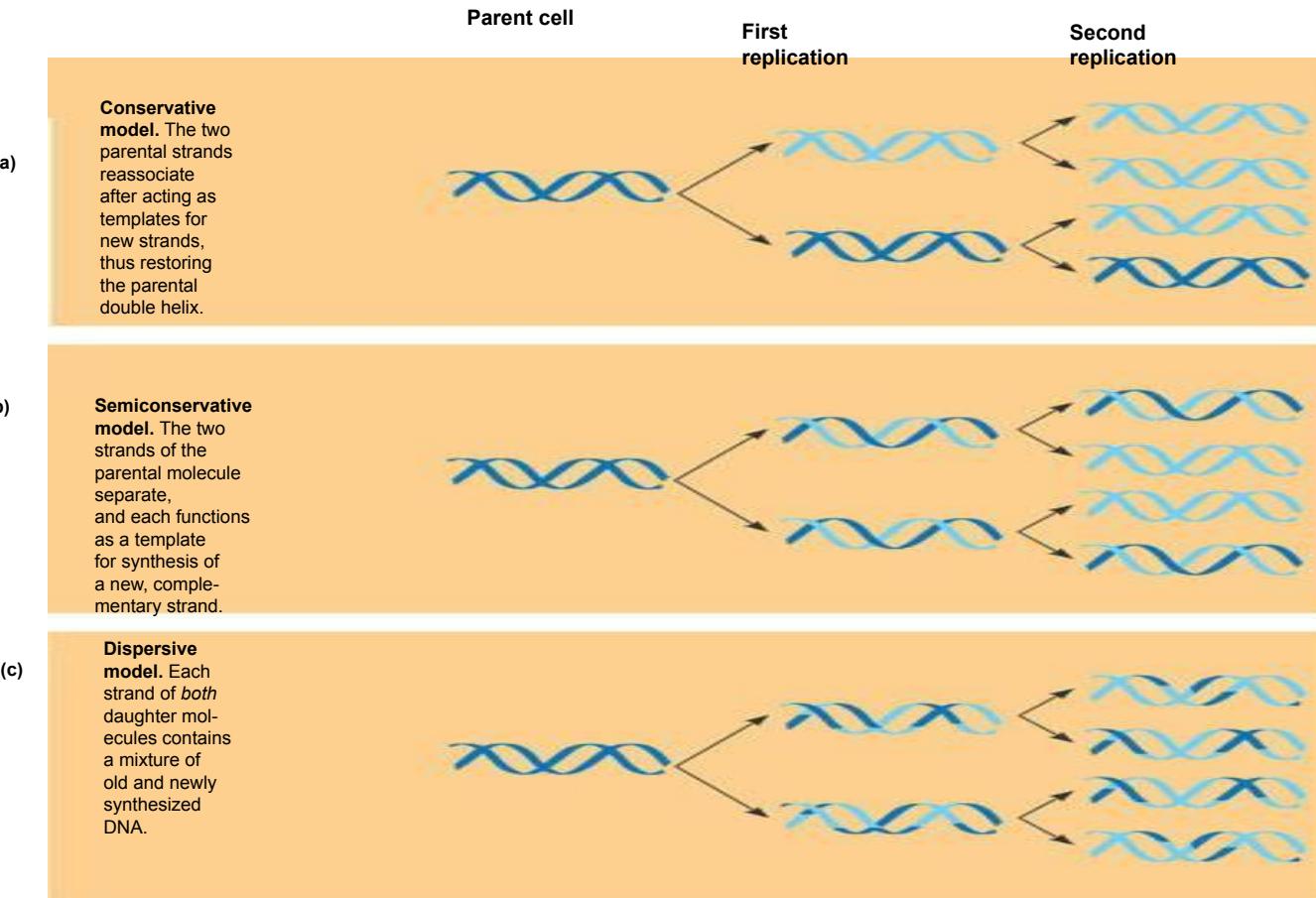
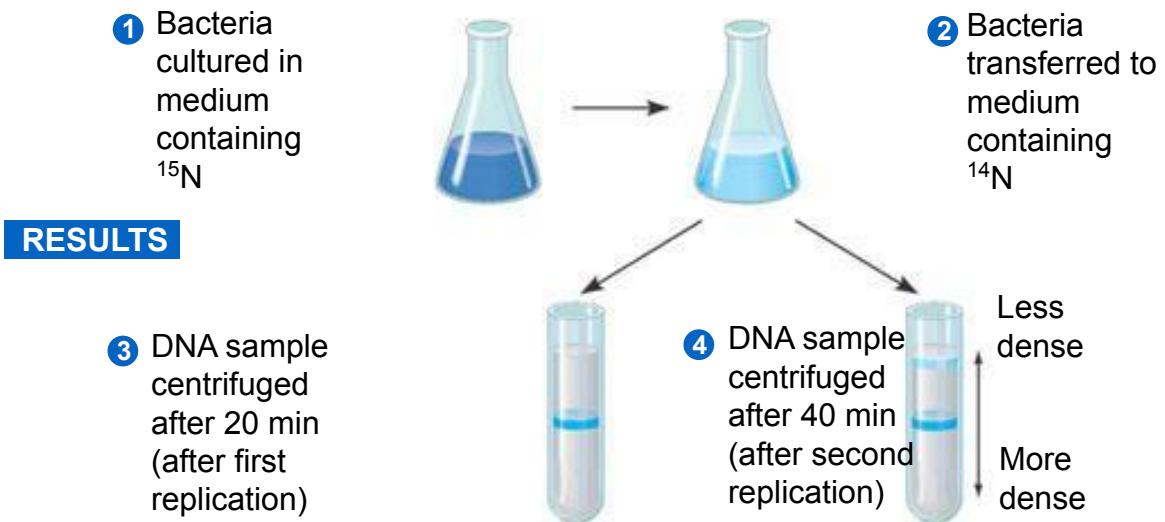


Figure 16.11

- Experiments performed by Meselson and Stahl
 - Supported the semiconservative model of DNA replication

EXPERIMENT Matthew Meselson and Franklin Stahl cultured *E. coli* bacteria for several generations on a medium containing nucleotide precursors labeled with a heavy isotope of nitrogen, ^{15}N . The bacteria incorporated the heavy nitrogen into their DNA. The scientists then transferred the bacteria to a medium with only ^{14}N , the lighter, more common isotope of nitrogen. Any new DNA that the bacteria synthesized would be lighter than the parental DNA made in the ^{15}N medium. Meselson and Stahl could distinguish DNA of different densities by centrifuging DNA extracted from the bacteria.

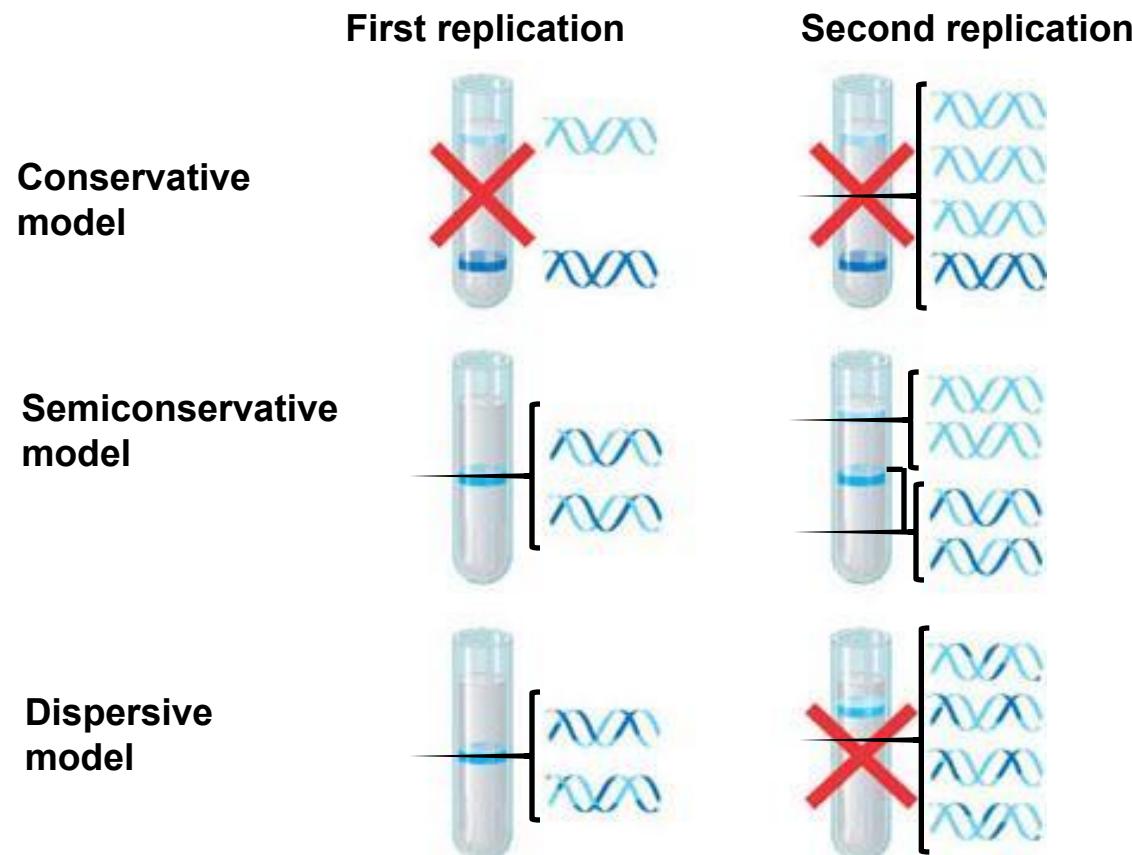


The bands in these two centrifuge tubes represent the results of centrifuging two DNA samples from the flask in step 2, one sample taken after 20 minutes and one after 40 minutes.

Figure 16.11

CONCLUSION

Meselson and Stahl concluded that DNA replication follows the semiconservative model by comparing their result to the results predicted by each of the three models in Figure 16.10. The first replication in the ^{14}N medium produced a band of hybrid (^{15}N – ^{14}N) DNA. This result eliminated the conservative model. A second replication produced both light and hybrid DNA, a result that eliminated the dispersive model and supported the semiconservative model.



DNA Replication: *A Closer Look*

- The copying of DNA
 - Is remarkable in its speed and accuracy
- More than a dozen enzymes and other proteins
 - Participate in DNA replication

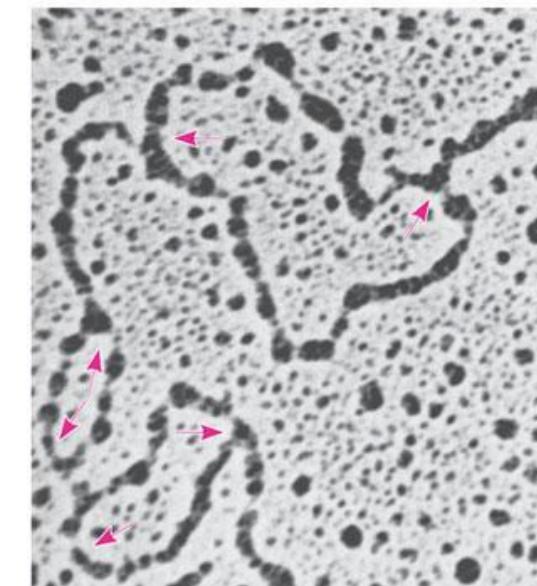
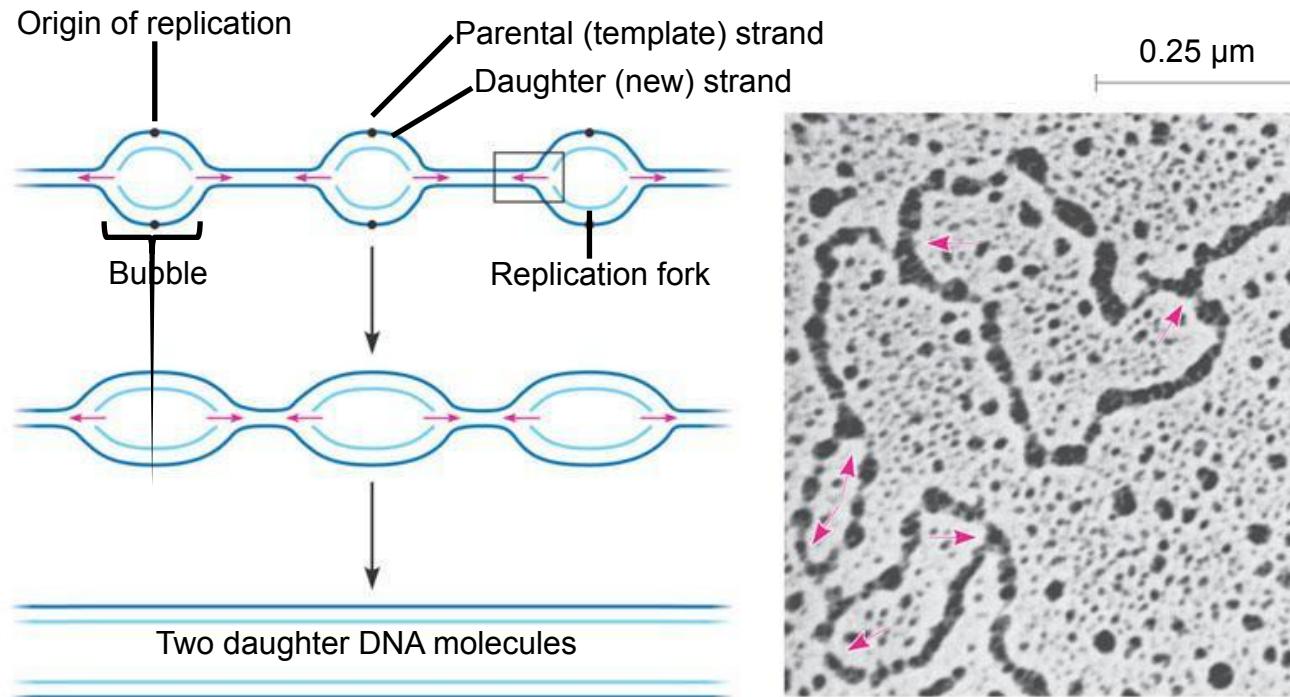
Getting Started: Origins of Replication

- The replication of a DNA molecule
 - Begins at special sites called origins of replication, where the two strands are separated

A eukaryotic chromosome

- May have hundreds or even thousands of replication origins

- 1 Replication begins at specific sites where the two parental strands separate and form replication bubbles.
- 2 The bubbles expand laterally, as DNA replication proceeds in both directions.
- 3 Eventually, the replication bubbles fuse, and synthesis of the daughter strands is complete.



(b) In this micrograph, three replication bubbles are visible along the DNA of a cultured Chinese hamster cell (TEM).

Figure 16.12 a, b

Elongating a New DNA Strand

- Elongation of new DNA at a replication fork
 - Is catalyzed by enzymes called DNA polymerases, which add nucleotides to the 3' end of a growing strand

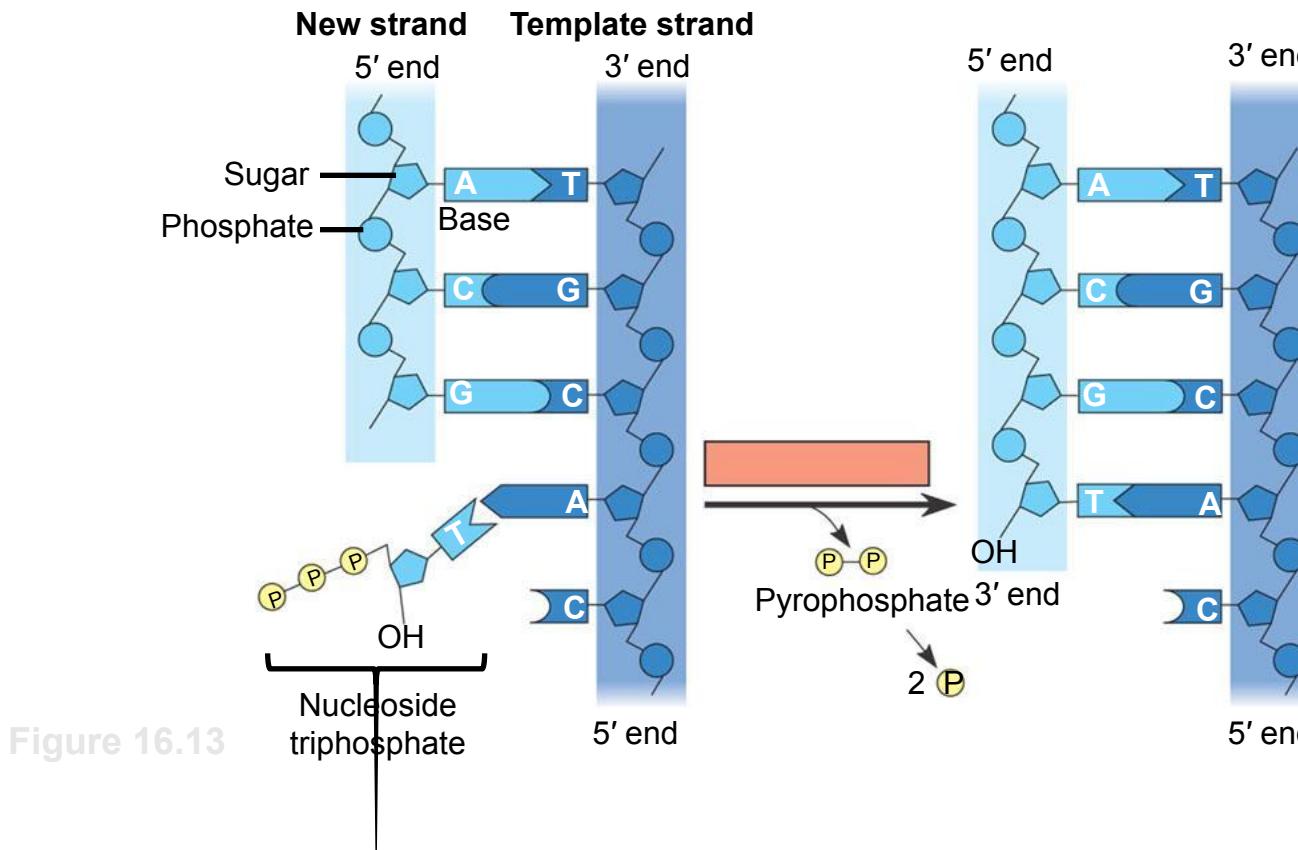


Figure 16.13

Antiparallel Elongation

- How does the antiparallel structure of the double helix affect replication?

- DNA polymerases add nucleotides
 - Only to the free 3' end of a growing strand
- Along one template strand of DNA, the leading strand
 - DNA polymerase III can synthesize a complementary strand continuously, moving toward the replication fork

- To elongate the other new strand of DNA, the lagging strand
 - DNA polymerase III must work in the direction away from the replication fork
- The lagging strand
 - Is synthesized as a series of segments called Okazaki fragments, which are then joined together by DNA ligase

- Synthesis of leading and lagging strands during DNA replication

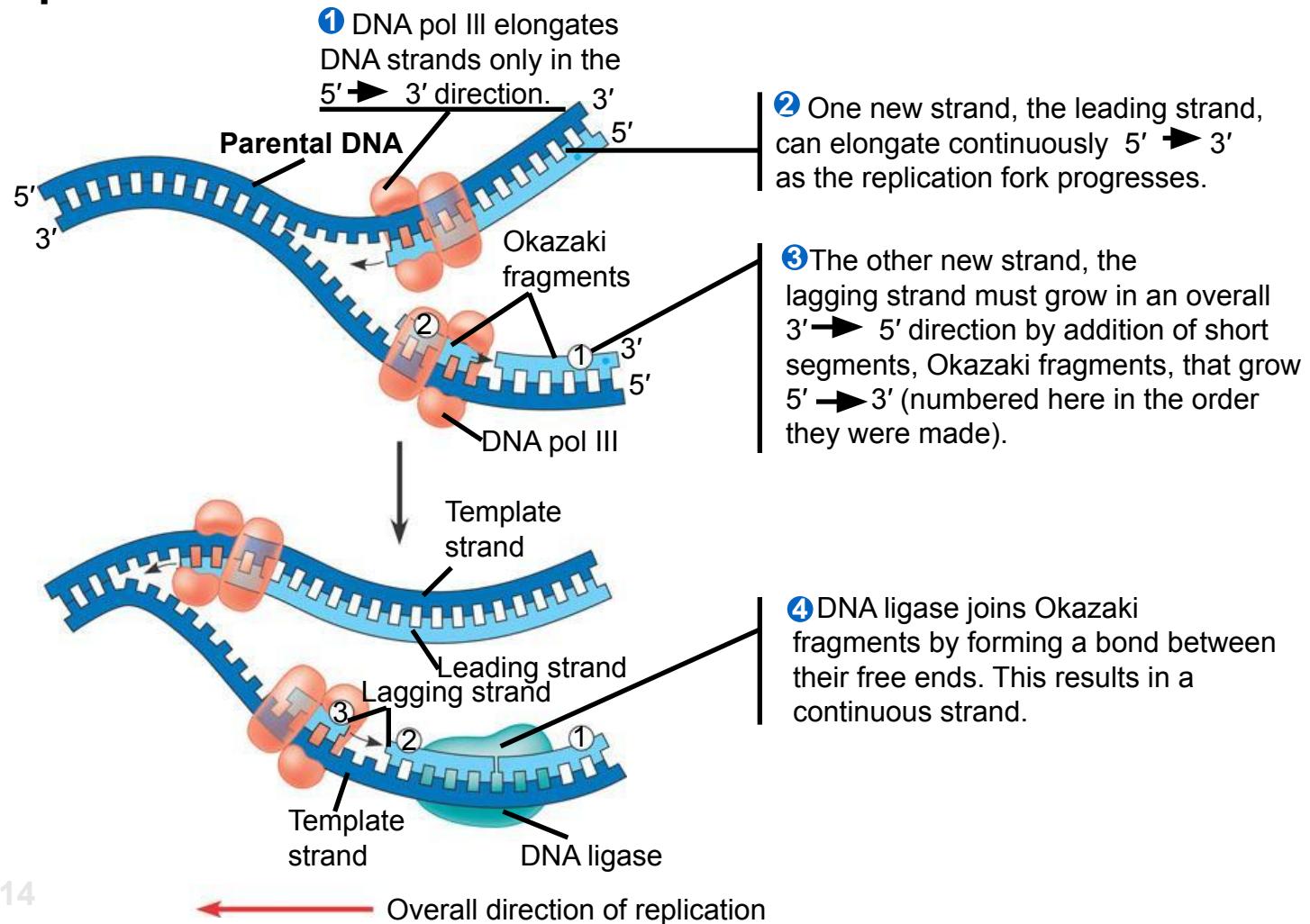


Figure 16.14

Priming DNA Synthesis

- DNA polymerases cannot initiate the synthesis of a polynucleotide
 - They can only add nucleotides to the 3' end
- The initial nucleotide strand
 - Is an RNA or DNA primer

- Only one primer is needed for synthesis of the leading strand
 - But for synthesis of the lagging strand, each Okazaki fragment must be primed separately

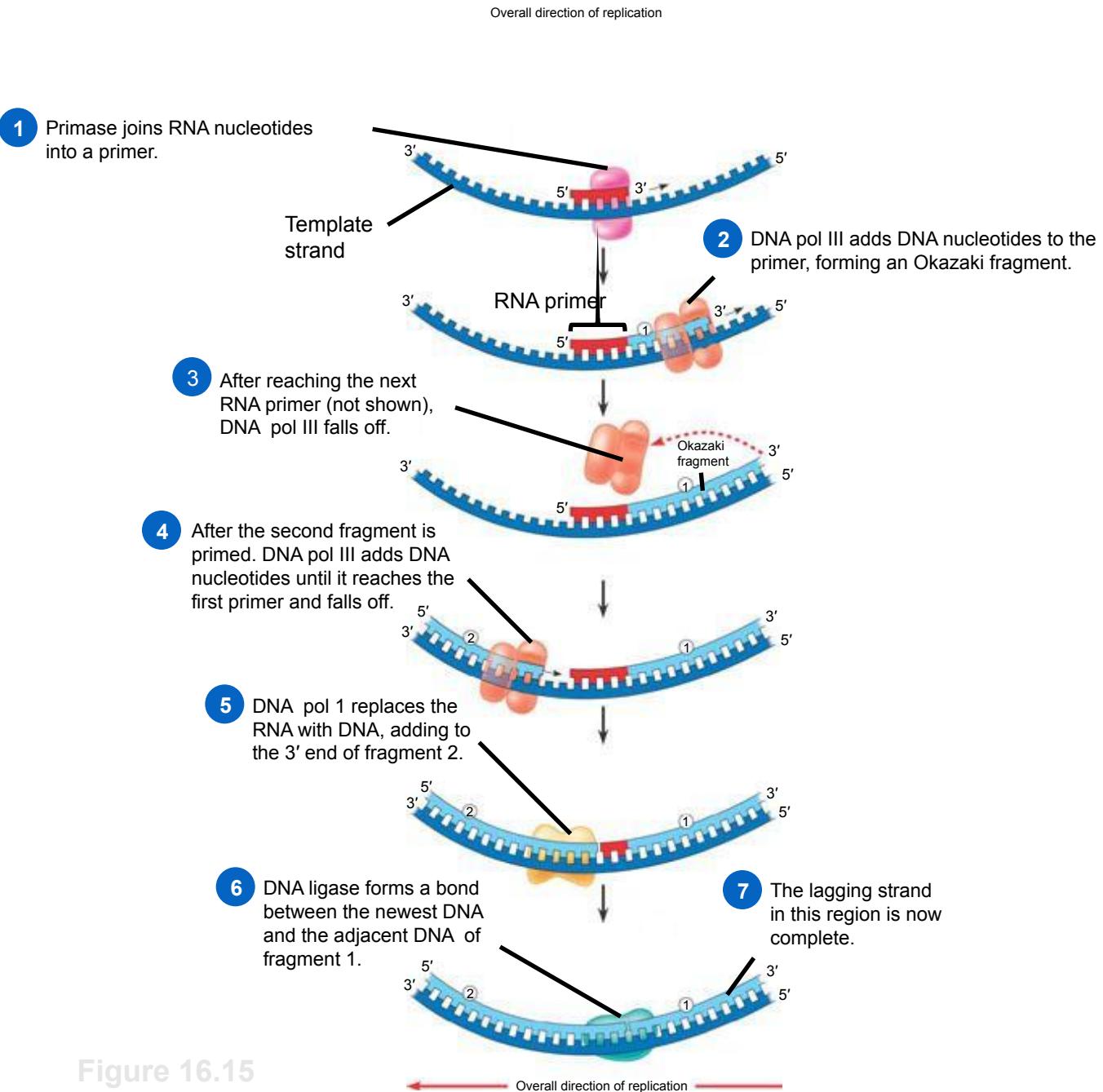


Figure 16.15

Other Proteins That Assist DNA Replication

- Helicase, topoisomerase, single-strand binding protein
 - Are all proteins that assist DNA replication

Table 16.1 Bacterial DNA replication proteins and their functions

Protein	Function for Leading and Lagging Strands	
Helicase	Unwinds parental double helix at replication forks	
Single-strand binding protein	Binds to and stabilizes single-stranded DNA until it can be used as a template	
Topoisomerase	Corrects "overwinding" ahead of replication forks by breaking, swiveling, and rejoining DNA strands	
	Function for Leading Strand	Function for Lagging Strand
Primase	Synthesizes a single RNA primer at the 5' end of the leading strand	Synthesizes an RNA primer at the 5' end of each Okazaki fragment
DNA pol III	Continuously synthesizes the leading strand, adding on to the primer	Elongates each Okazaki fragment, adding on to its primer
DNA pol I	Removes primer from the 5' end of leading strand and replaces it with DNA, adding on to the adjacent 3' end	Removes the primer from the 5' end of each fragment and replaces it with DNA, adding on to the 3' end of the adjacent fragment
DNA Ligase	Joins the 3' end of the DNA that replaces the primer to the rest of the leading strand	Joins the Okazaki fragments

Table 16.1

Figure 16.16

• A summary of DNA replication

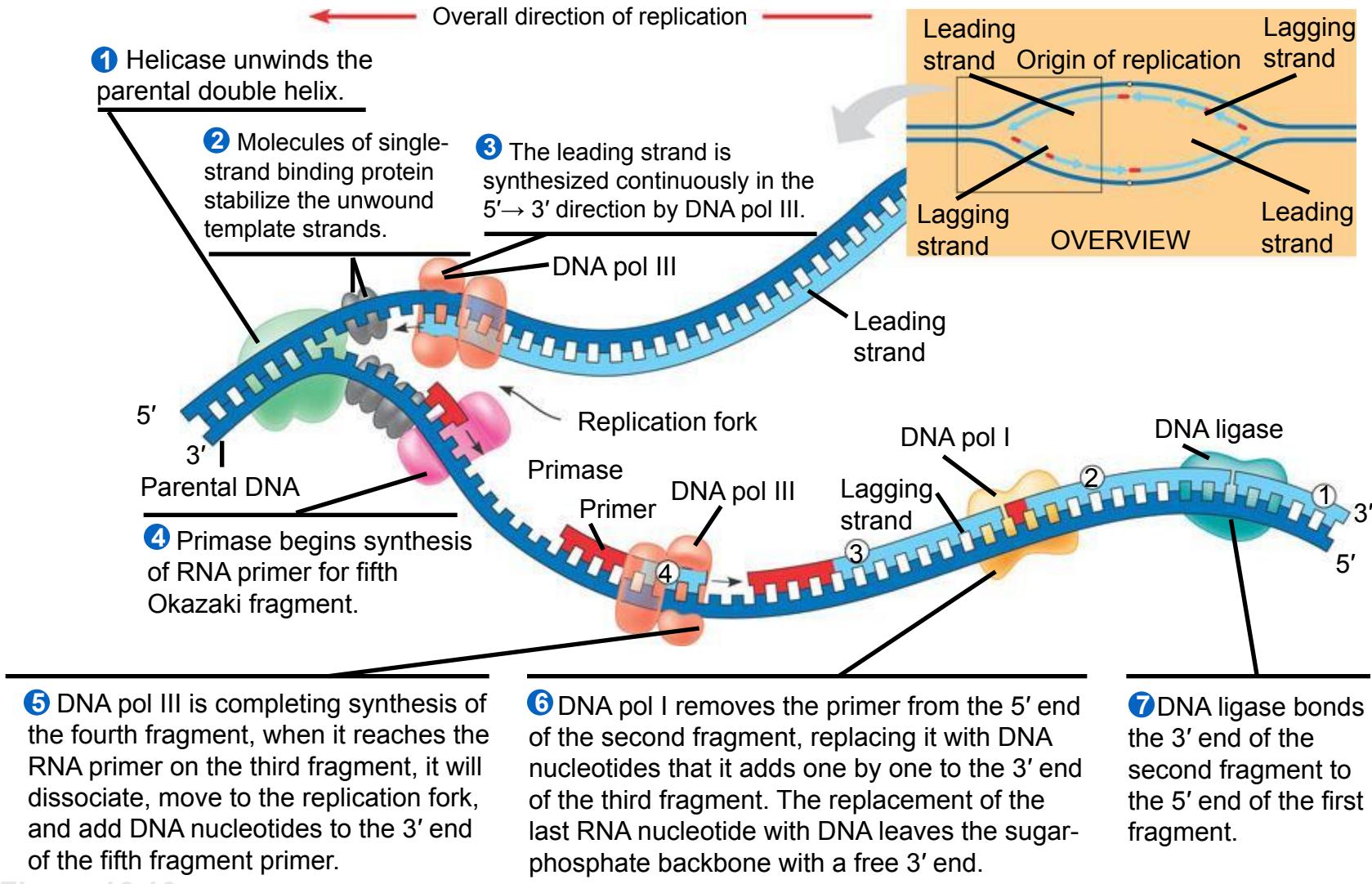


Figure 16.16

The DNA Replication Machine as a Stationary Complex

- The various proteins that participate in DNA replication
 - Form a single large complex, a DNA replication “machine”
- The DNA replication machine
 - Is probably stationary during the replication process

Proofreading and Repairing DNA

- DNA polymerases proofread newly made DNA
 - Replacing any incorrect nucleotides
- In mismatch repair of DNA
 - Repair enzymes correct errors in base pairing

Figure 16.17

- In nucleotide excision repair
 - Enzymes cut out and replace damaged stretches of DNA

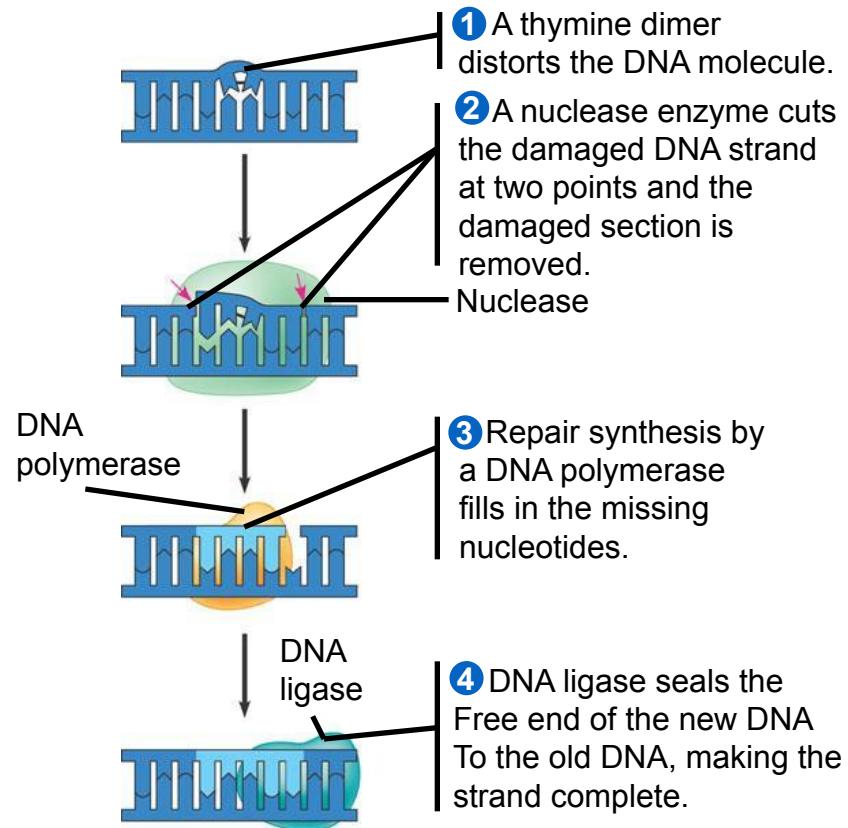


Figure 16.17

Replicating the Ends of DNA Molecules

- The ends of eukaryotic chromosomal DNA
 - Get shorter with each round of replication

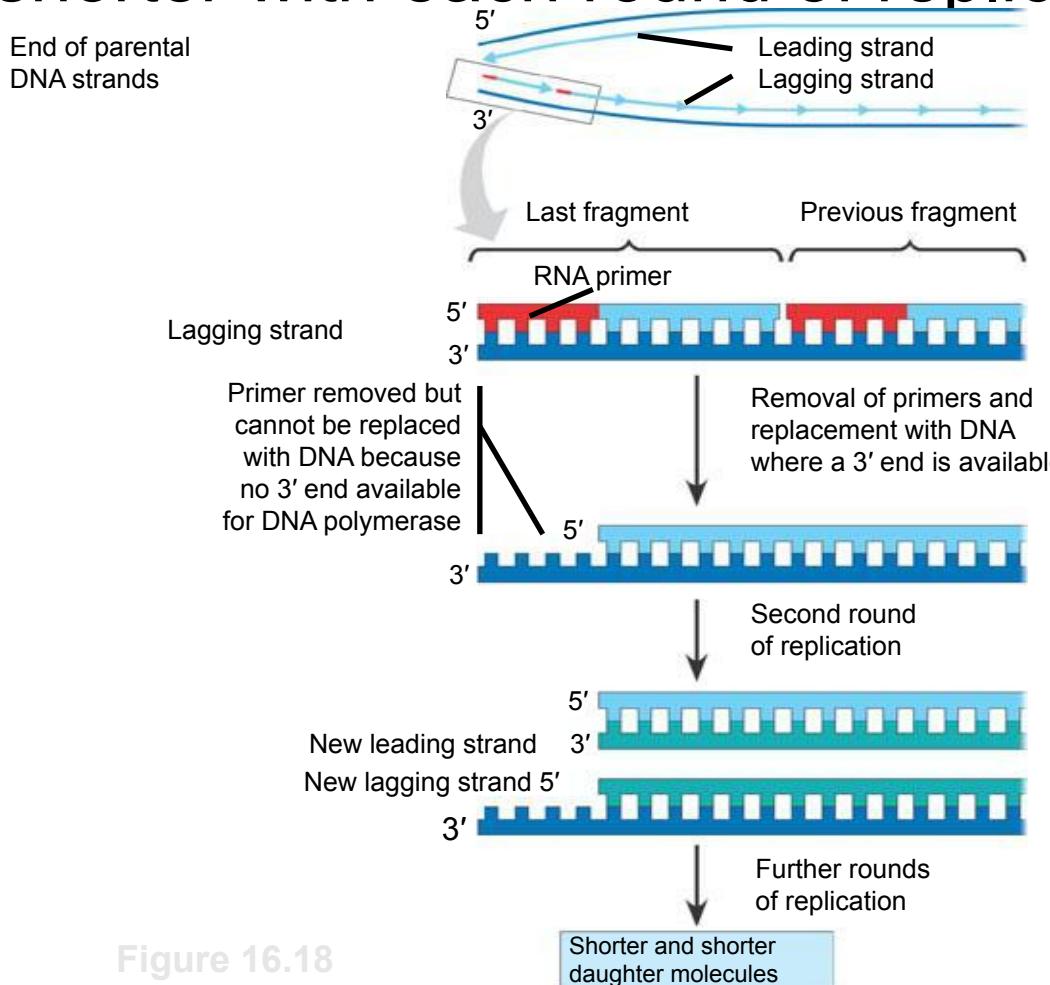


Figure 16.18

Figure 16.19

- Eukaryotic chromosomal DNA molecules
 - Have at their ends nucleotide sequences, called telomeres, that postpone the erosion of genes near the ends of DNA molecules

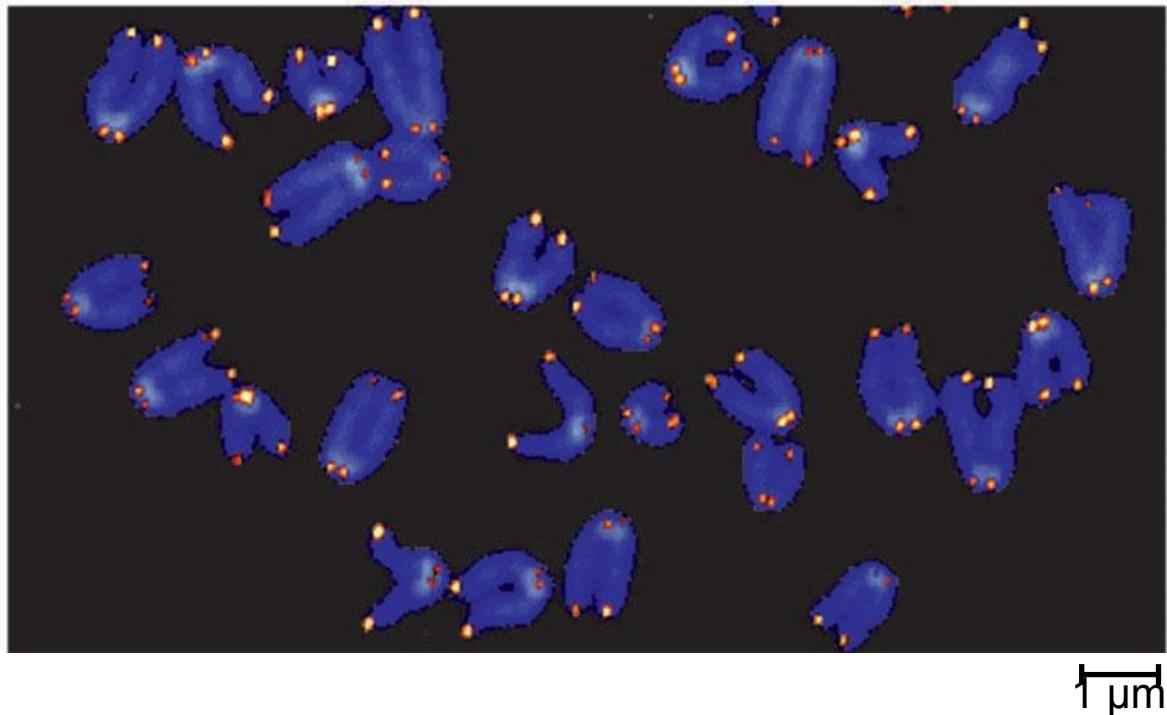


Figure 16.19

- If the chromosomes of germ cells became shorter in every cell cycle
 - Essential genes would eventually be missing from the gametes they produce
- An enzyme called telomerase
 - Catalyzes the lengthening of telomeres in germ cells

From Gene to Protein

- Overview: The Flow of Genetic Information
- The information content of DNA
 - Is in the form of specific sequences of nucleotides along the DNA strands

- The DNA inherited by an organism
 - Leads to specific traits by dictating the synthesis of proteins
- The process by which DNA directs protein synthesis, gene expression
 - Includes two stages, called transcription and translation

Figure 17.1

- The ribosome
 - Is part of the cellular machinery for translation, polypeptide synthesis

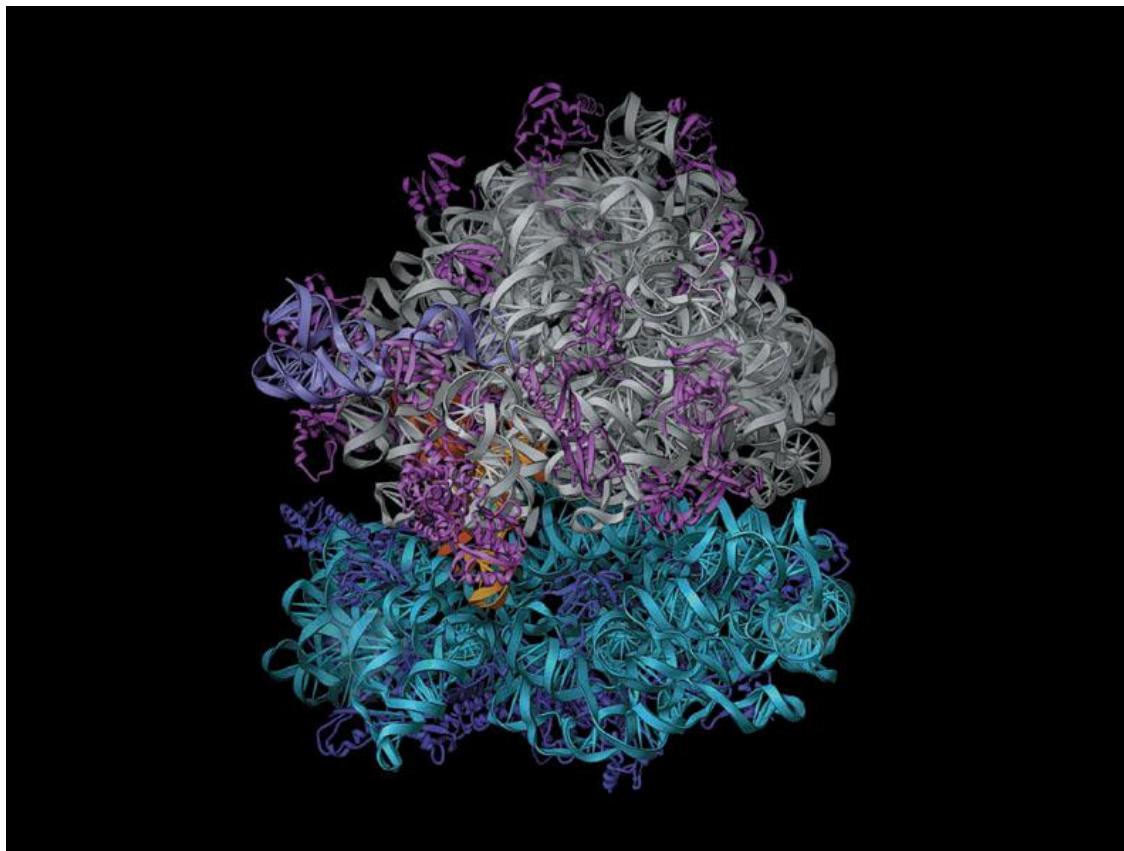


Figure 17.1

- Concept 17.1: Genes specify proteins via transcription and translation

Evidence from the Study of Metabolic Defects

- In 1909, British physician Archibald Garrod
 - Was the first to suggest that genes dictate phenotypes through enzymes that catalyze specific chemical reactions in the cell

Nutritional Mutants in Neurospora: Scientific Inquiry

- Beadle and Tatum causes bread mold to mutate with X-rays
 - Creating mutants that could not survive on minimal medium

Figure 17.2

- Using genetic crosses
 - They determined that their mutants fell into three classes, each mutated in a different gene

EXPERIMENT

Working with the mold *Neurospora crassa*, George Beadle and Edward Tatum had isolated mutants requiring arginine in their growth medium and had shown genetically that these mutants fell into three classes, each defective in a different gene. From other considerations, they suspected that the metabolic pathway of arginine biosynthesis included the precursors ornithine and citrulline. Their most famous experiment, shown here, tested both their **one gene—one enzyme hypothesis** and their postulated **arginine pathway**. In this experiment, they grew their three classes of mutants under the four different conditions shown in the Results section below.

RESULTS

The wild-type strain required only the minimal medium for growth. The three classes of mutants had different growth requirements

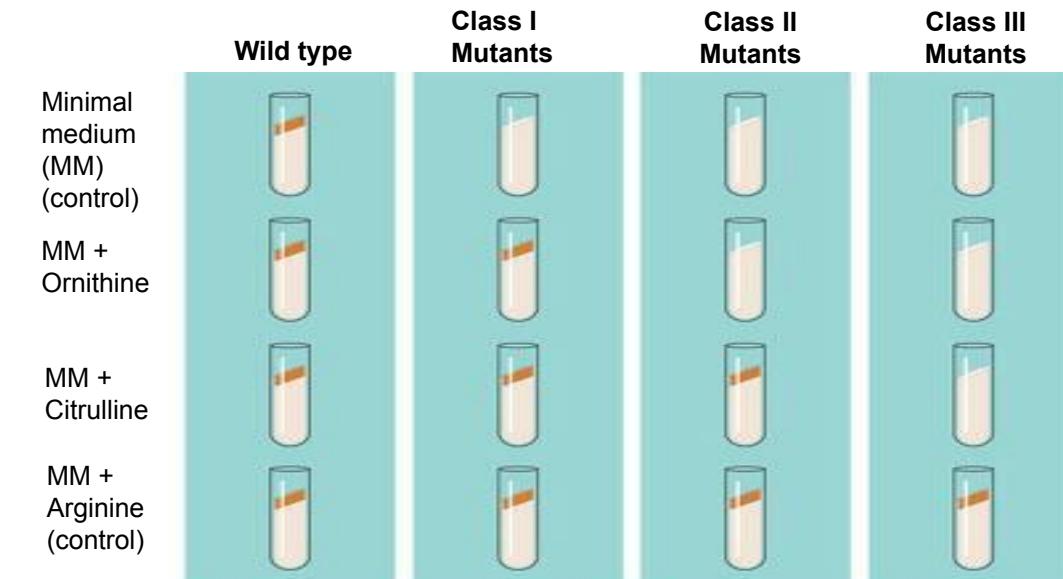
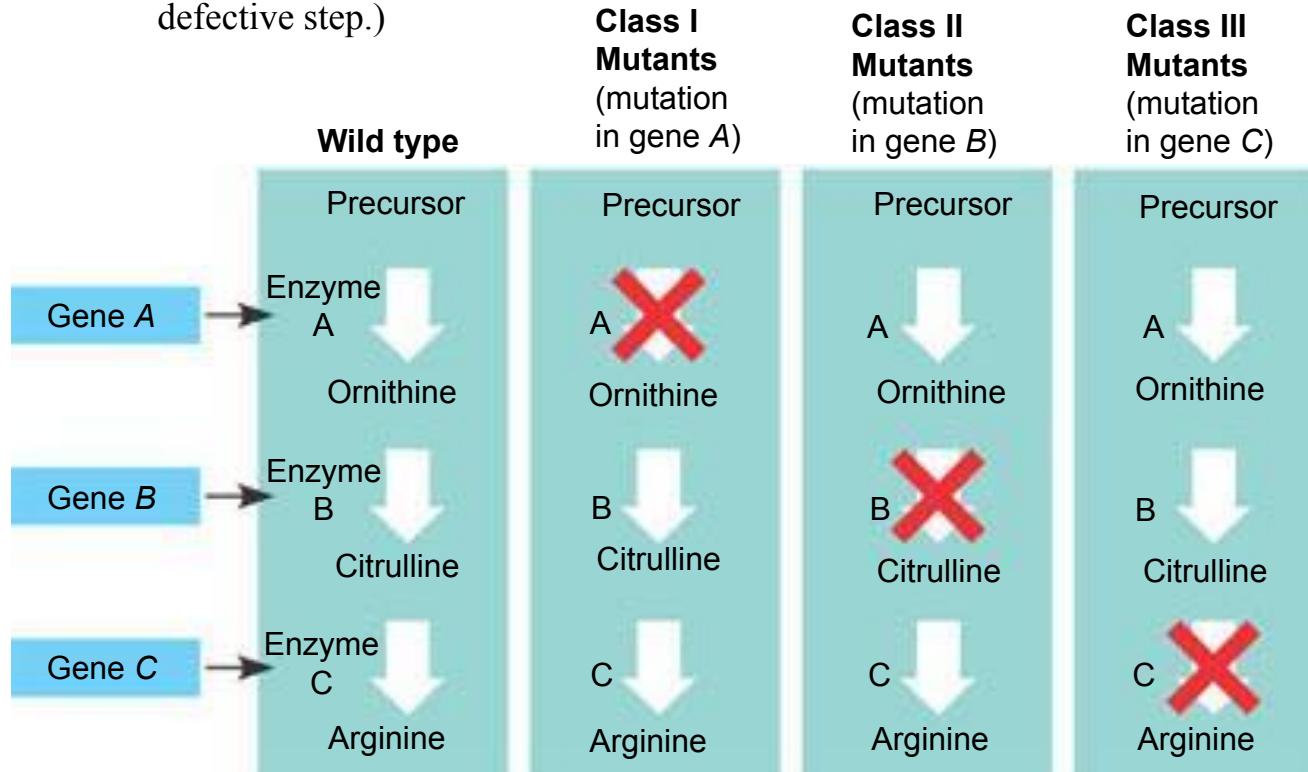


Figure 17.2

CONCLUSION

From the growth patterns of the mutants, Beadle and Tatum deduced that each mutant was unable to carry out one step in the pathway for synthesizing arginine, presumably because it lacked the necessary enzyme. Because each of their mutants was mutated in a single gene, they concluded that each mutated gene must normally dictate the production of one enzyme. Their results supported the one gene–one enzyme hypothesis and also confirmed the arginine pathway.

(Notice that a mutant can grow only if supplied with a compound made *after* the defective step.)



- Beadle and Tatum developed the “one gene—one enzyme hypothesis”
 - Which states that the function of a gene is to dictate the production of a specific enzyme

The Products of Gene Expression: A Developing Story

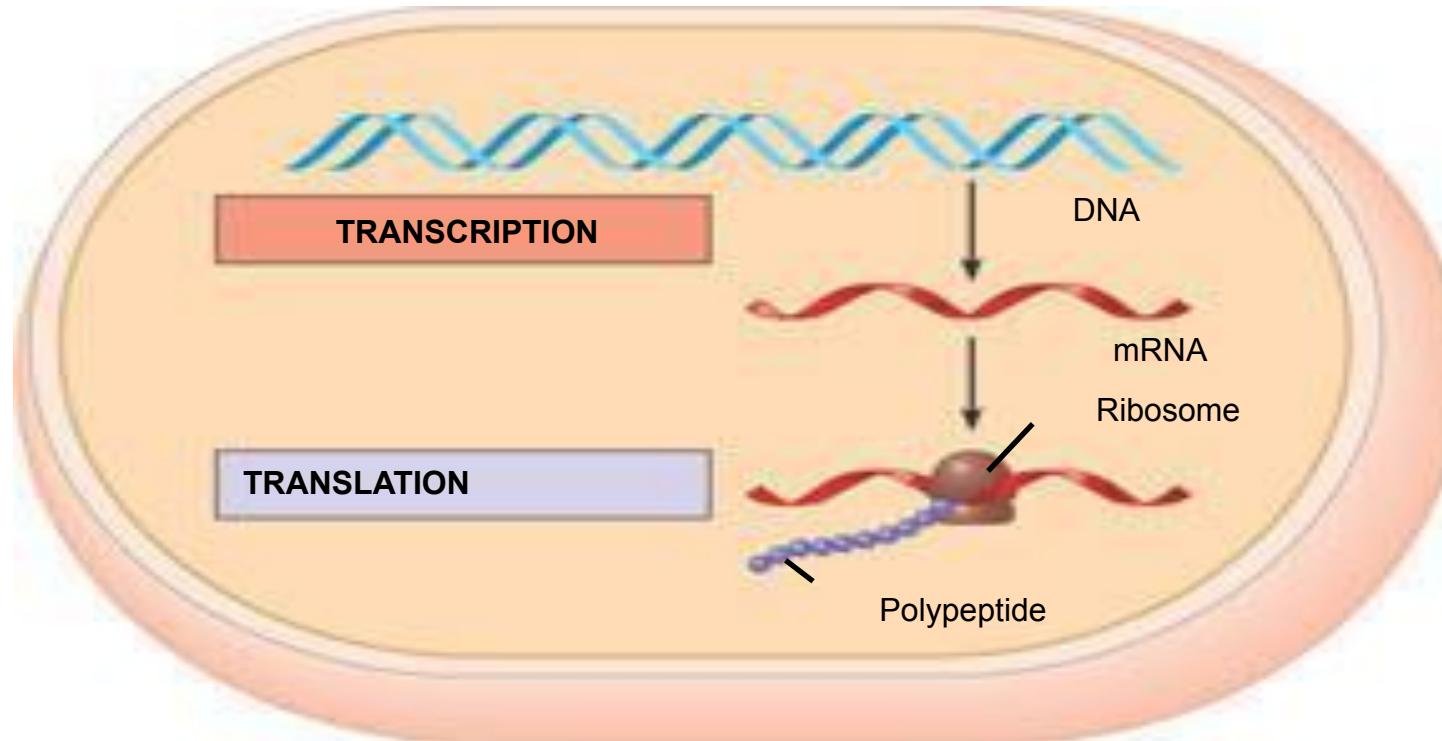
- As researchers learned more about proteins
 - They made minor revision to the one gene—one enzyme hypothesis
- Genes code for polypeptide chains or for RNA molecules

Basic Principles of Transcription and Translation

- Transcription
 - Is the synthesis of RNA under the direction of DNA
 - Produces messenger RNA (mRNA)
- Translation
 - Is the actual synthesis of a polypeptide, which occurs under the direction of mRNA
 - Occurs on ribosomes

Figure 17.3a

- In prokaryotes
 - Transcription and translation occur together



Prokaryotic cell. In a cell lacking a nucleus, mRNA produced by transcription is immediately translated without additional processing.

Figure 17.3a

Figure 17.3b

- In eukaryotes
 - RNA transcripts are modified before becoming true mRNA

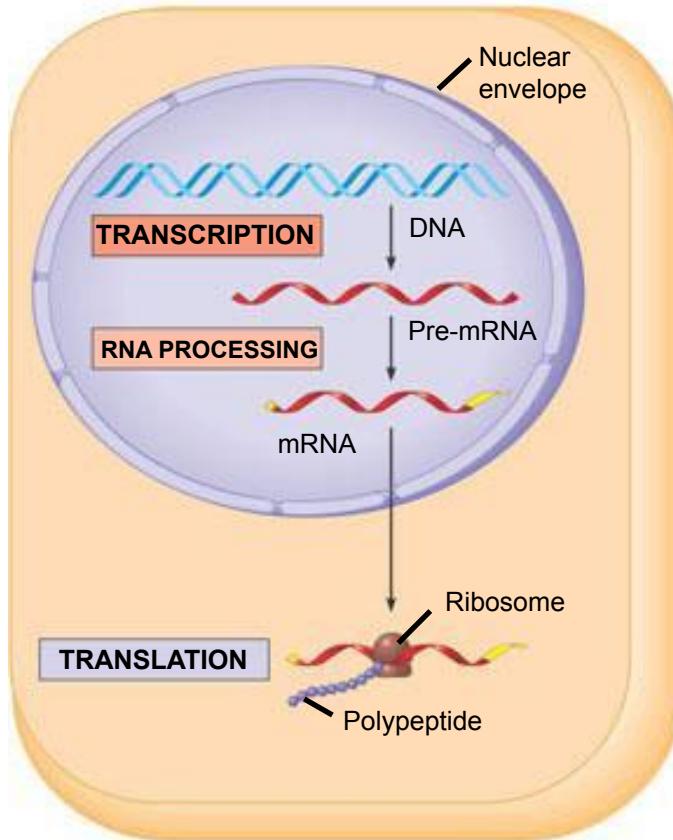


Figure 17.3b

(b) **Eukaryotic cell.** The nucleus provides a separate compartment for transcription. The original RNA transcript, called pre-mRNA, is processed in various ways before leaving the nucleus as mRNA.

- Cells are governed by a cellular chain of command
 - DNA → RNA → protein

The Genetic Code

- How many bases correspond to an amino acid?

Codons: Triplets of Bases

- Genetic information
 - Is encoded as a sequence of nonoverlapping base triplets, or codons

Figure 17.4

- During transcription
 - The gene determines the sequence of bases along the length of an mRNA molecule

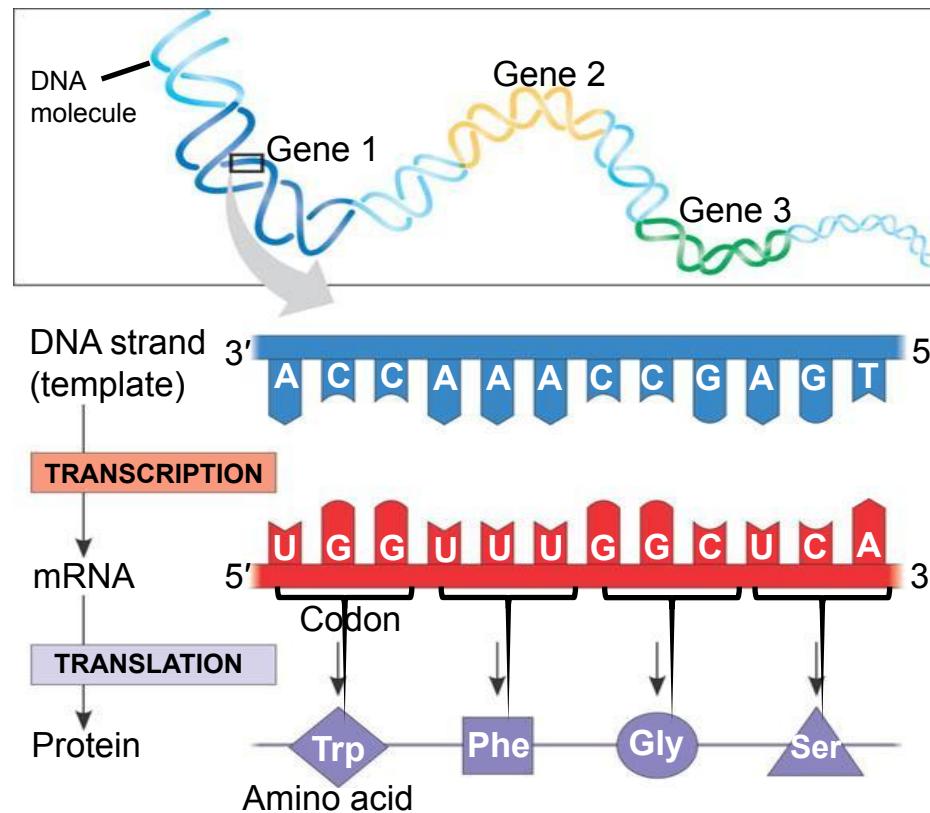


Figure 17.4

Cracking the Code

- A codon in messenger RNA
 - Is either translated into an amino acid or serves as a translational stop signal

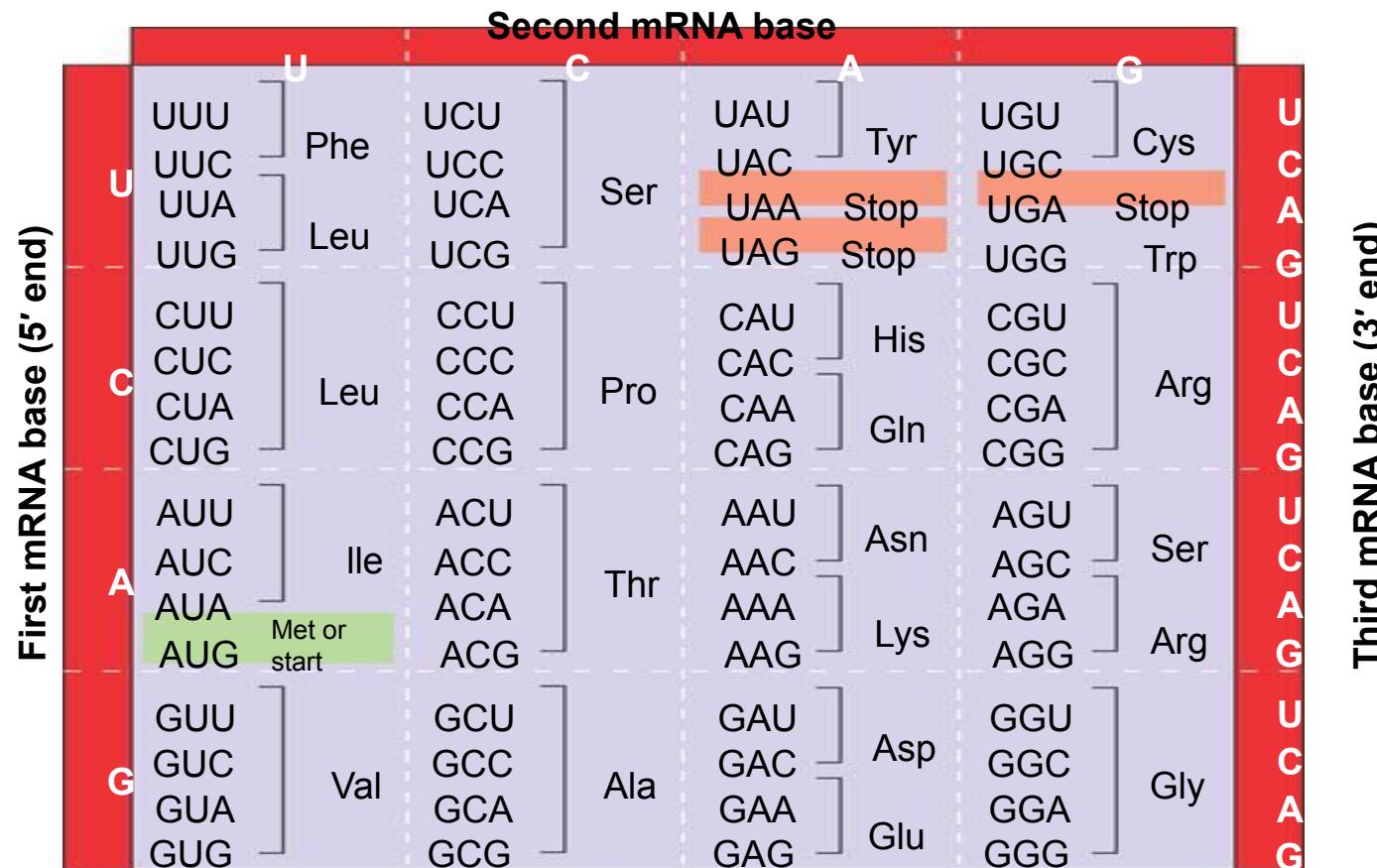


Figure 17.5

- Codons must be read in the correct reading frame
 - For the specified polypeptide to be produced

Evolution of the Genetic Code

- The genetic code is nearly universal
 - Shared by organisms from the simplest bacteria to the most complex animals

Figure 17.6

- In laboratory experiments
 - Genes can be transcribed and translated after being transplanted from one species to another

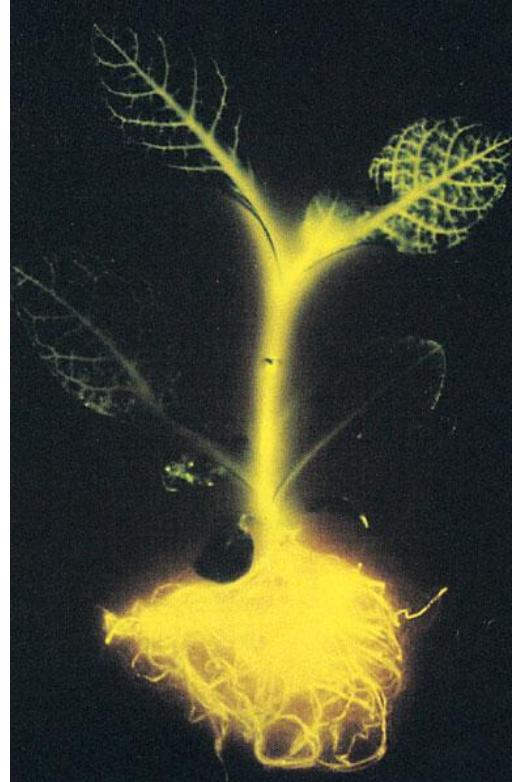


Figure 17.6

- Concept 17.2: Transcription is the DNA-directed synthesis of RNA: *a closer look*

Molecular Components of Transcription

- RNA synthesis
 - Is catalyzed by RNA polymerase, which pries the DNA strands apart and hooks together the RNA nucleotides
 - Follows the same base-pairing rules as DNA, except that in RNA, uracil substitutes for thymine

Synthesis of an RNA Transcript

- The stages of transcription are

- Initiation
- Elongation
- Termination

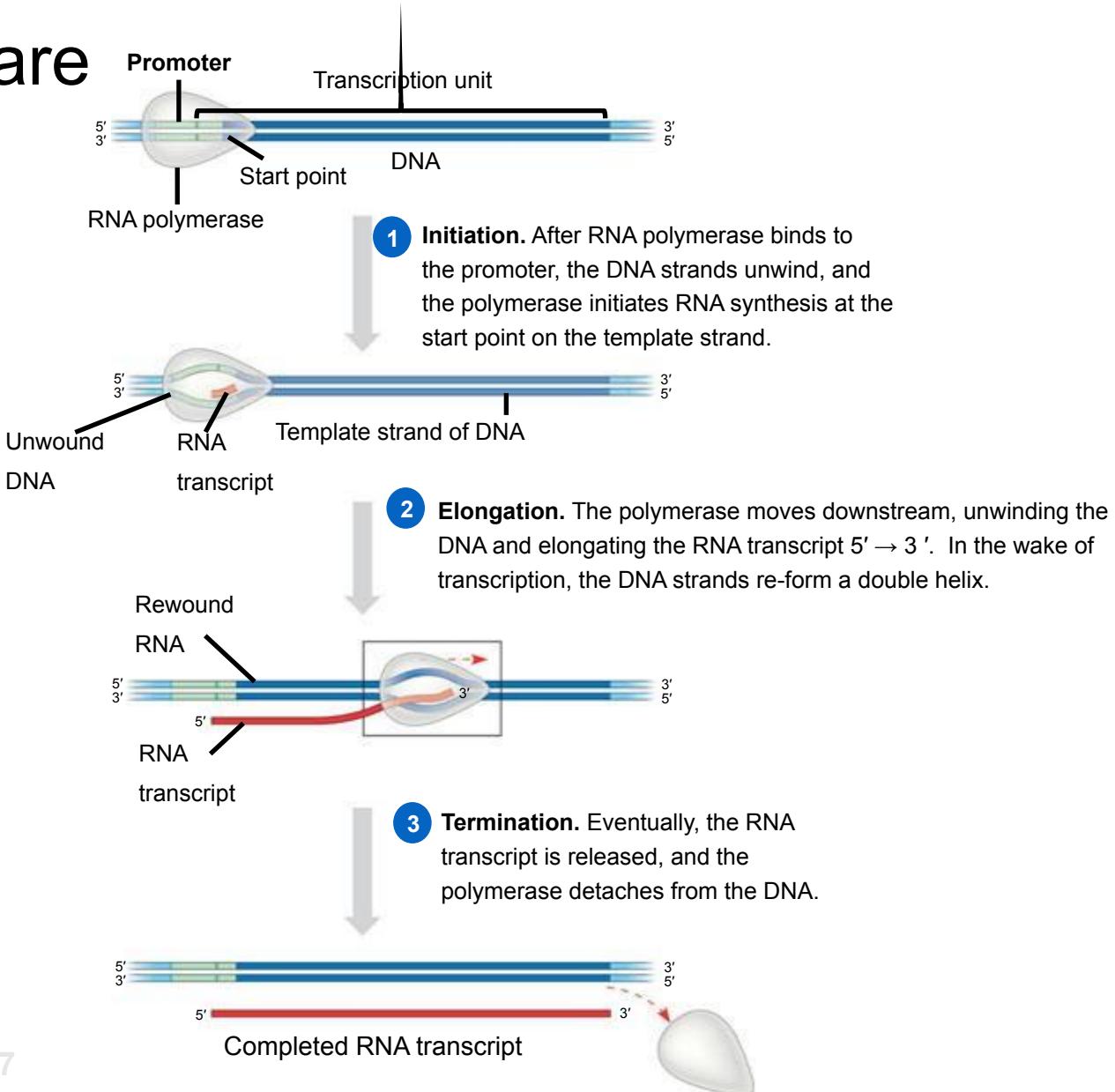
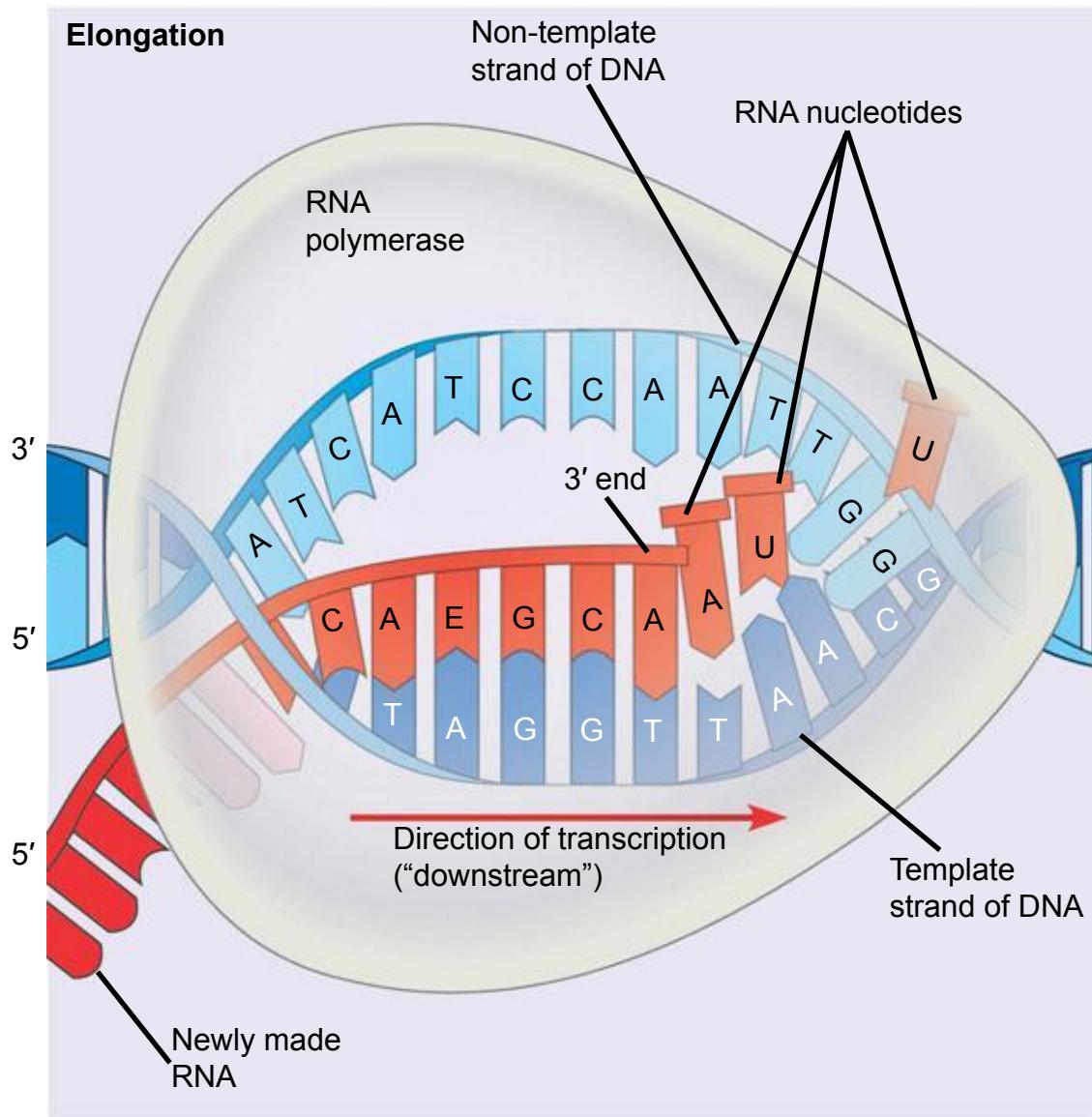


Figure 17.7

Elongation



RNA Polymerase Binding and Initiation of Transcription

- Promoters signal the initiation of RNA synthesis
- Transcription factors
 - Help eukaryotic RNA polymerase recognize promoter sequences

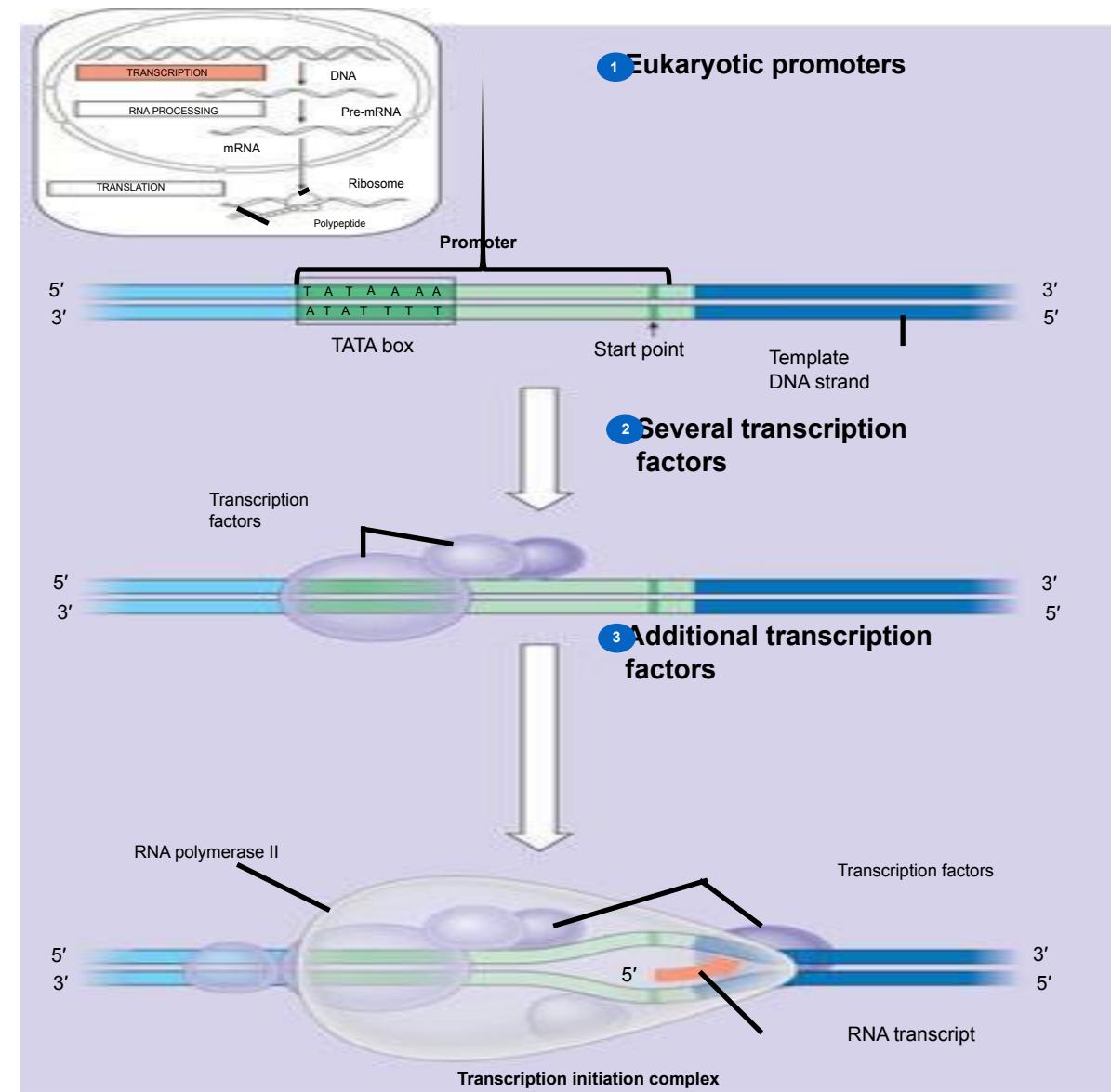


Figure 17.8
Figure 17.8

Elongation of the RNA Strand

- As RNA polymerase moves along the DNA
 - It continues to untwist the double helix, exposing about 10 to 20 DNA bases at a time for pairing with RNA nucleotides

Termination of Transcription

- The mechanisms of termination
 - Are different in prokaryotes and eukaryotes

- Concept 17.3: Eukaryotic cells modify RNA after transcription
- Enzymes in the eukaryotic nucleus
 - Modify pre-mRNA in specific ways before the genetic messages are dispatched to the cytoplasm

Alteration of mRNA Ends

- Each end of a pre-mRNA molecule is modified in a particular way
 - The 5' end receives a modified nucleotide cap
 - The 3' end gets a poly-A tail

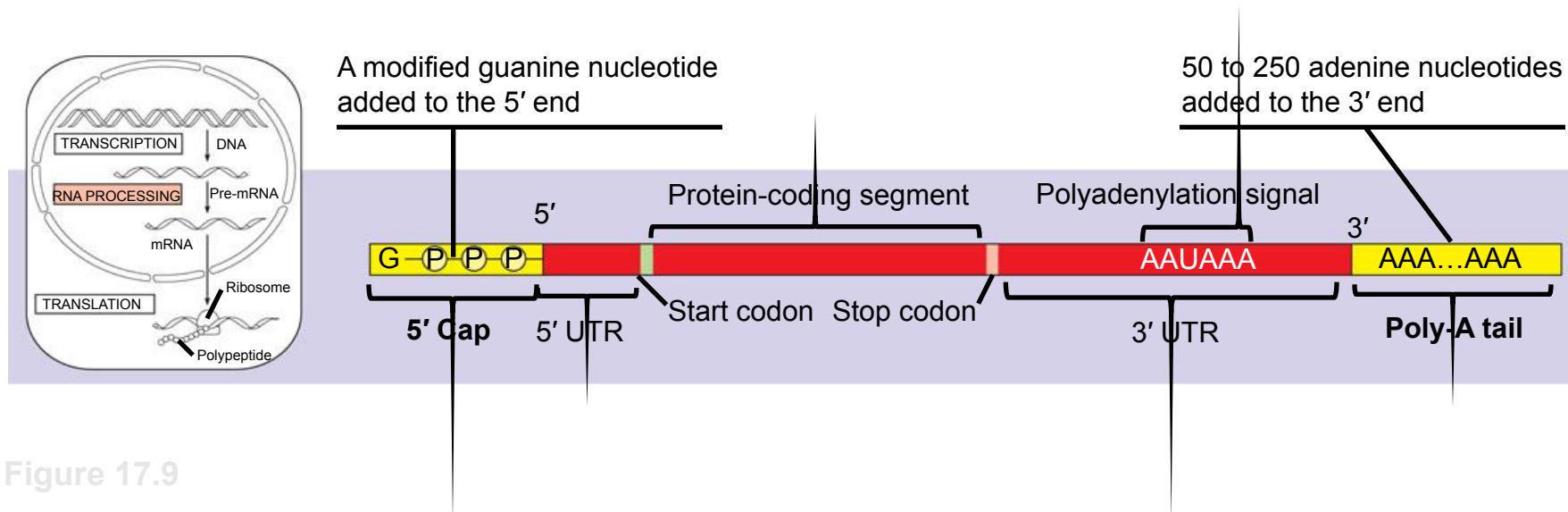


Figure 17.9

Split Genes and RNA Splicing

- RNA splicing
 - Removes introns and joins exons

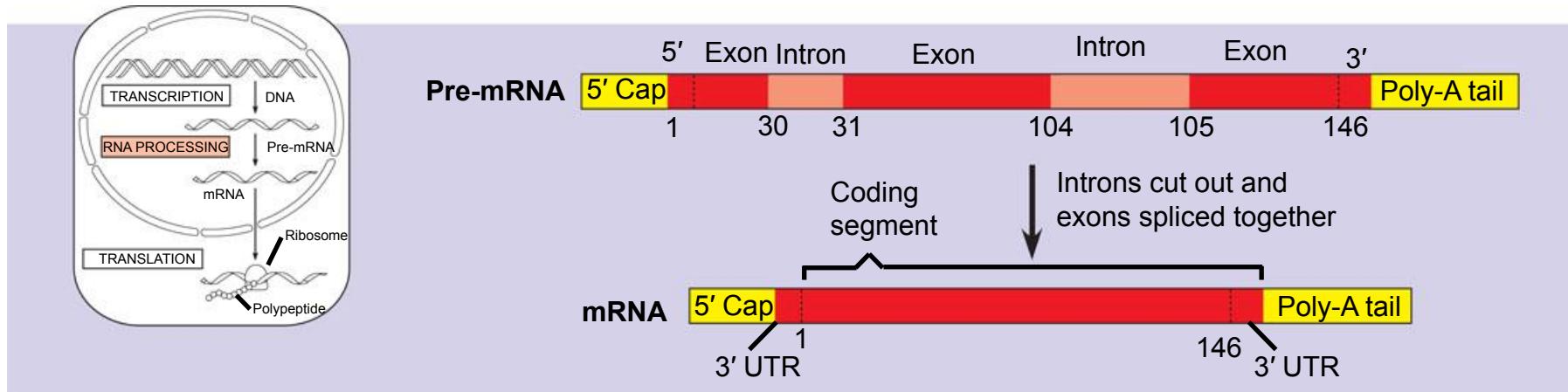


Figure 17.10

Figure 17.11

- Is carried out by spliceosomes in some cases

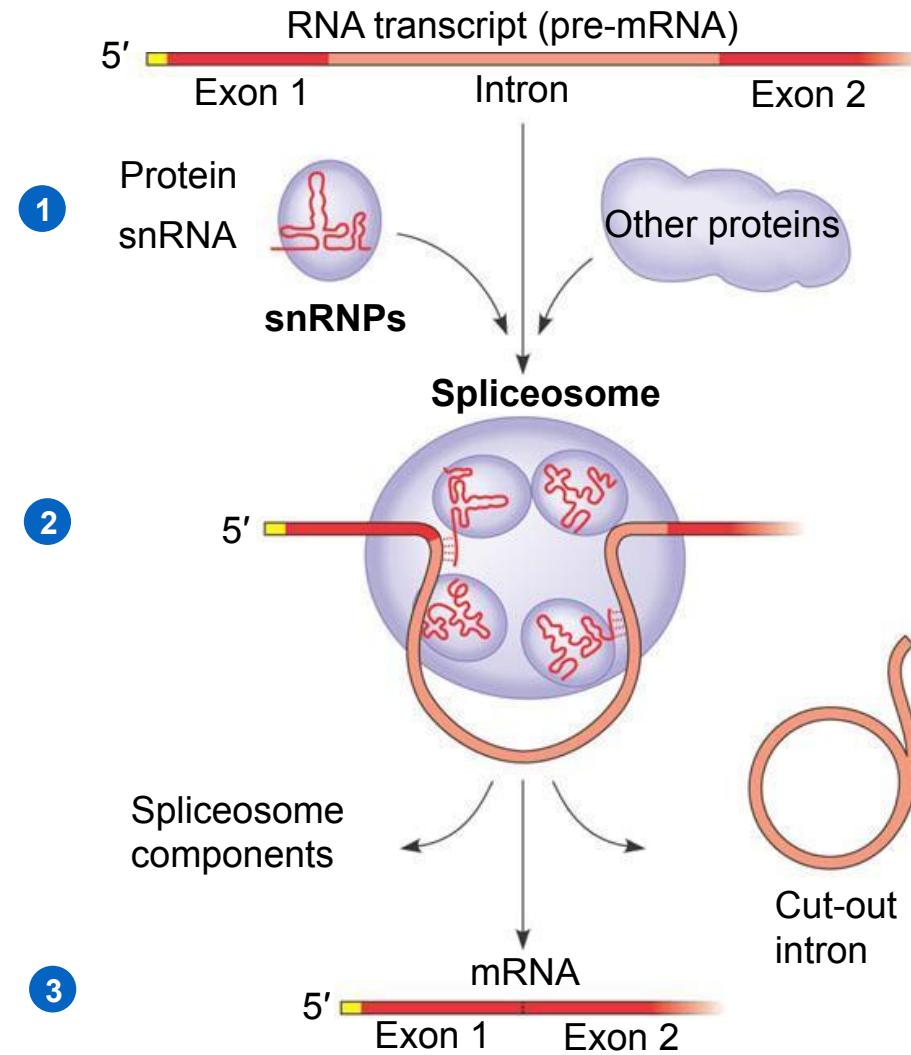


Figure 17.11

Ribozymes

- Ribozymes
 - Are catalytic RNA molecules that function as enzymes and can splice RNA

The Functional and Evolutionary Importance of Introns

- The presence of introns
 - Allows for alternative RNA splicing

Figure 17.12

- Proteins often have a modular architecture
 - Consisting of discrete structural and functional regions called domains
- In many cases
 - Different exons code for the different domains in a protein

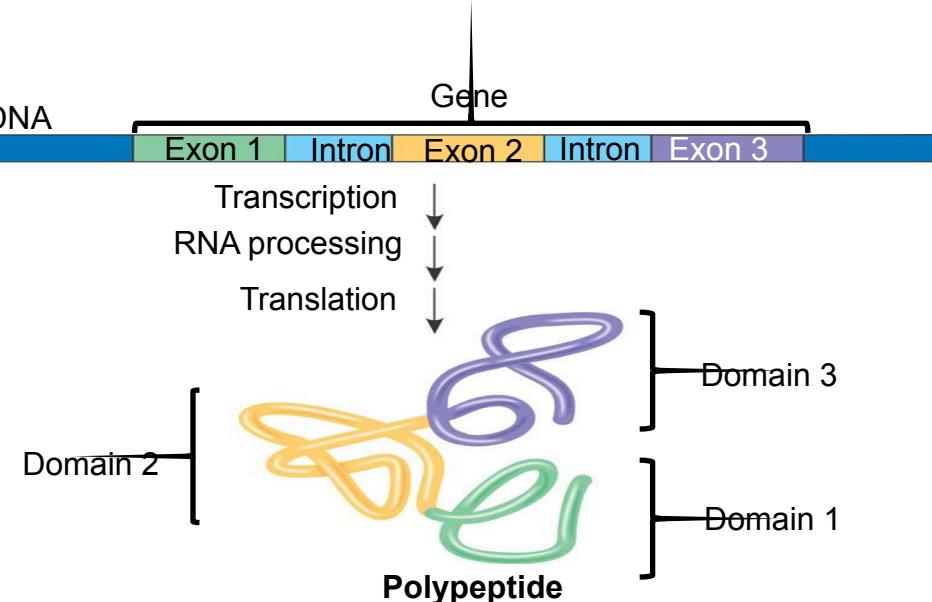


Figure 17.12

- Concept 17.4: Translation is the RNA-directed synthesis of a polypeptide: a *closer look*

Molecular Components of Translation

- A cell translates an mRNA message into protein
 - With the help of transfer RNA (tRNA)

Figure 17.13

- Translation: the basic concept

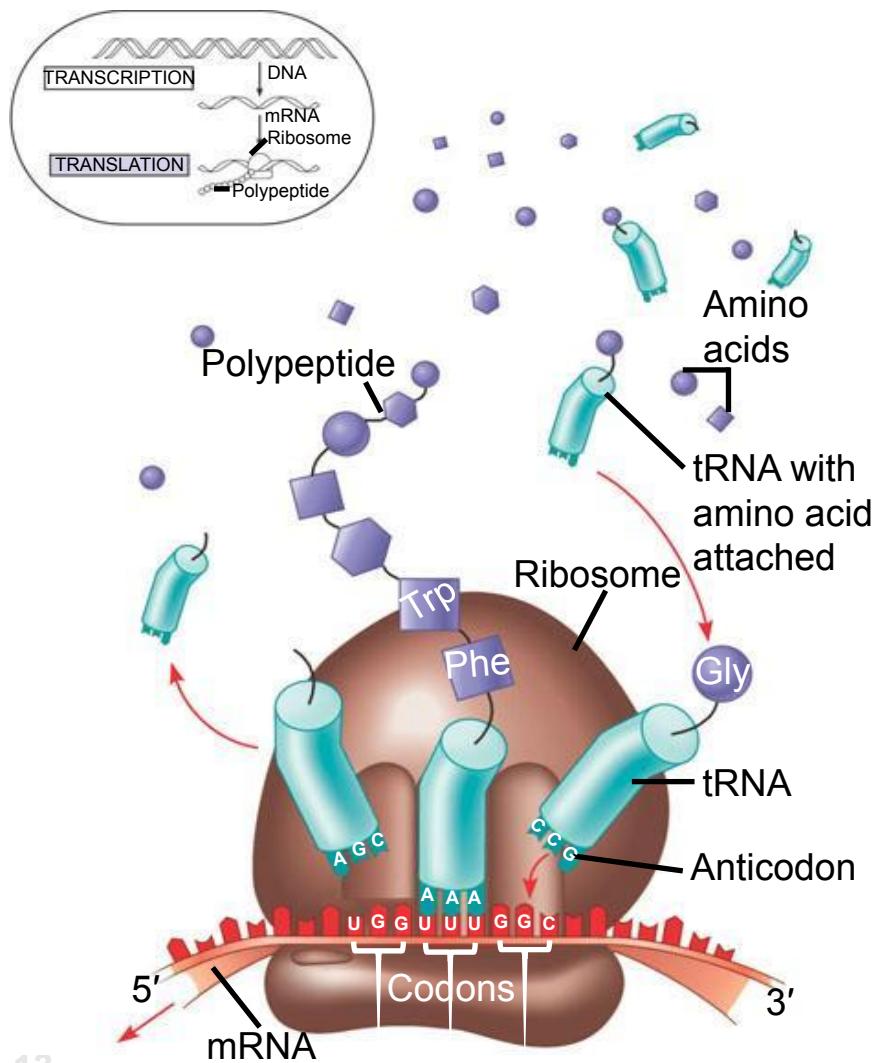
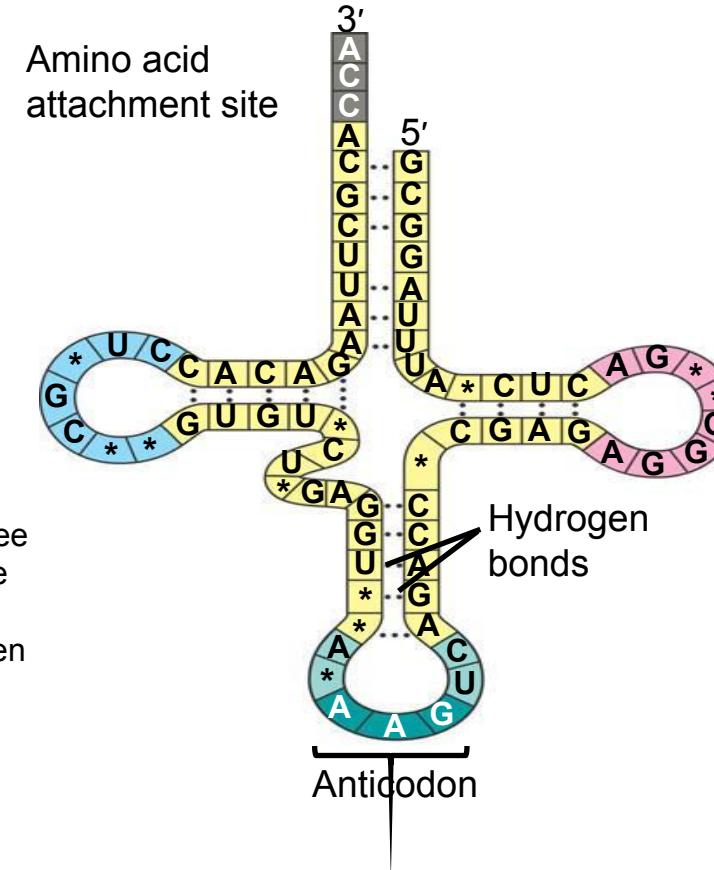


Figure 17.13

- Molecules of tRNA are not all identical
 - Each carries a specific amino acid on one end
 - Each has an anticodon on the other end

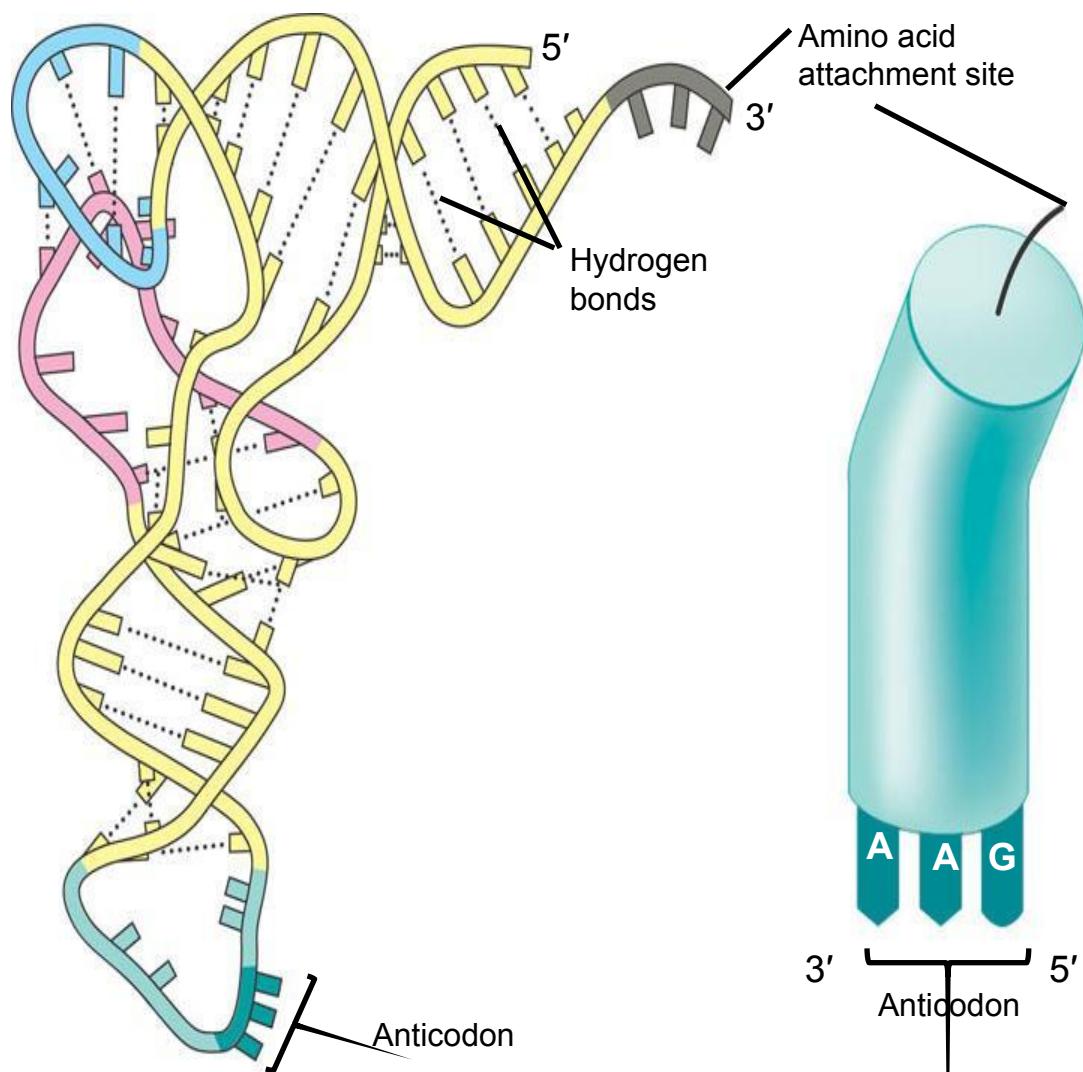
The Structure and Function of Transfer RNA

- A tRNA molecule
 - Consists of a single RNA strand that is only about 80 nucleotides long
 - Is roughly L-shaped



(a) **Two-dimensional structure.** The four base-paired regions and three loops are characteristic of all tRNAs, as is the base sequence of the amino acid attachment site at the 3' end. The anticodon triplet is unique to each tRNA type. (The asterisks mark bases that have been chemically modified, a characteristic of tRNA.)

Figure 17.14a



(b) Three-dimensional structure

(c) Symbol used
in this book



- A specific enzyme called an aminoacyl-tRNA synthetase
 - Joins each amino acid to the correct tRNA

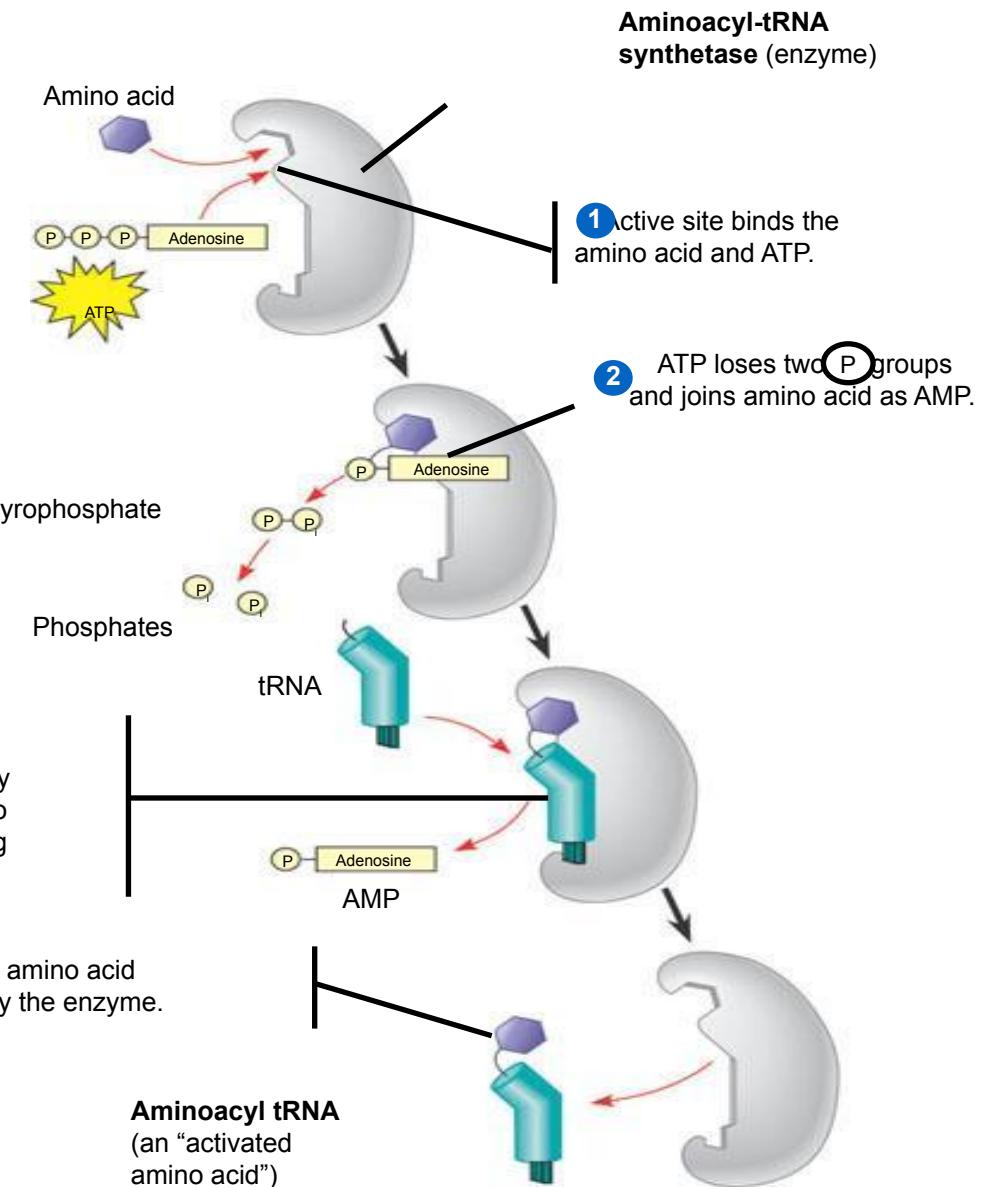


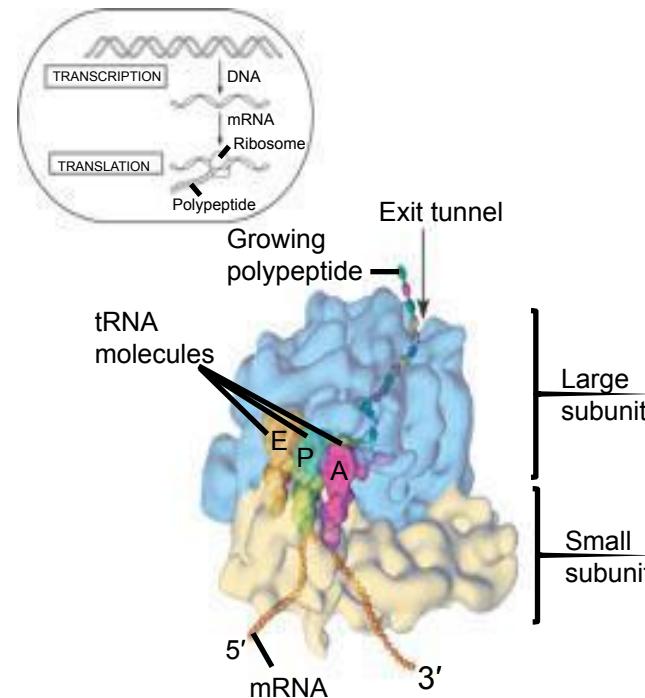
Figure 17.15

Ribosomes

- Ribosomes
 - Facilitate the specific coupling of tRNA anticodons with mRNA codons during protein synthesis

Figure 17.16a

- The ribosomal subunits
 - Are constructed of proteins and RNA molecules named ribosomal RNA or rRNA

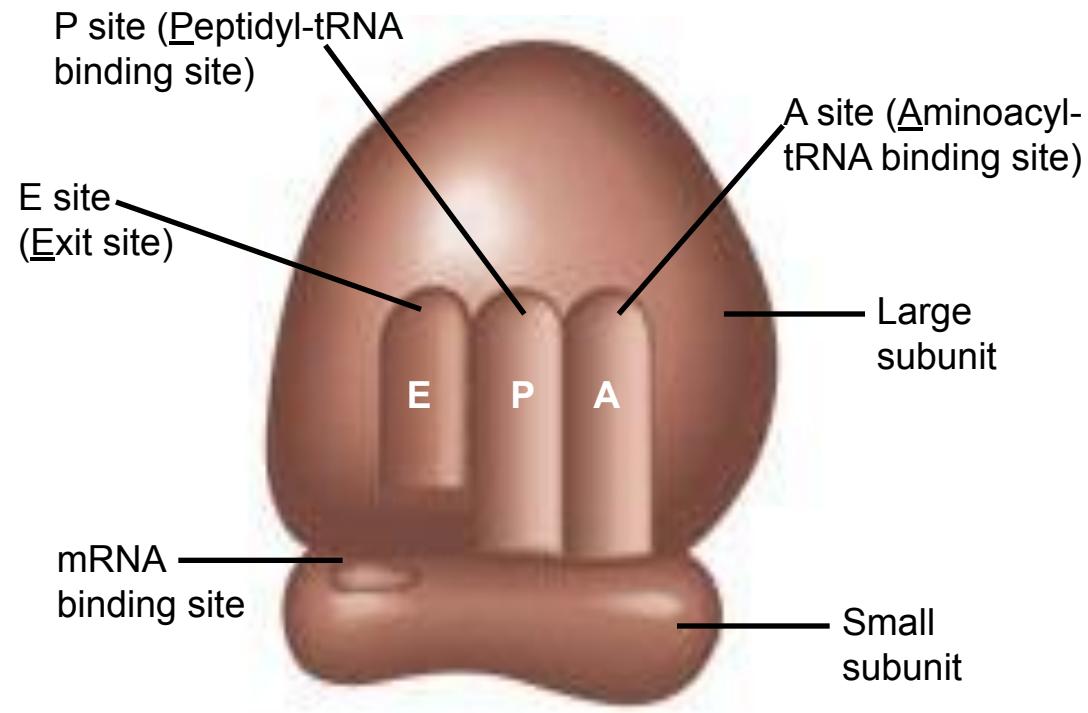


(a) Computer model of functioning ribosome. This is a model of a bacterial ribosome, showing its overall shape. The eukaryotic ribosome is roughly similar. A ribosomal subunit is an aggregate of ribosomal RNA molecules and proteins.

Figure 17.16a

Figure 17.16b

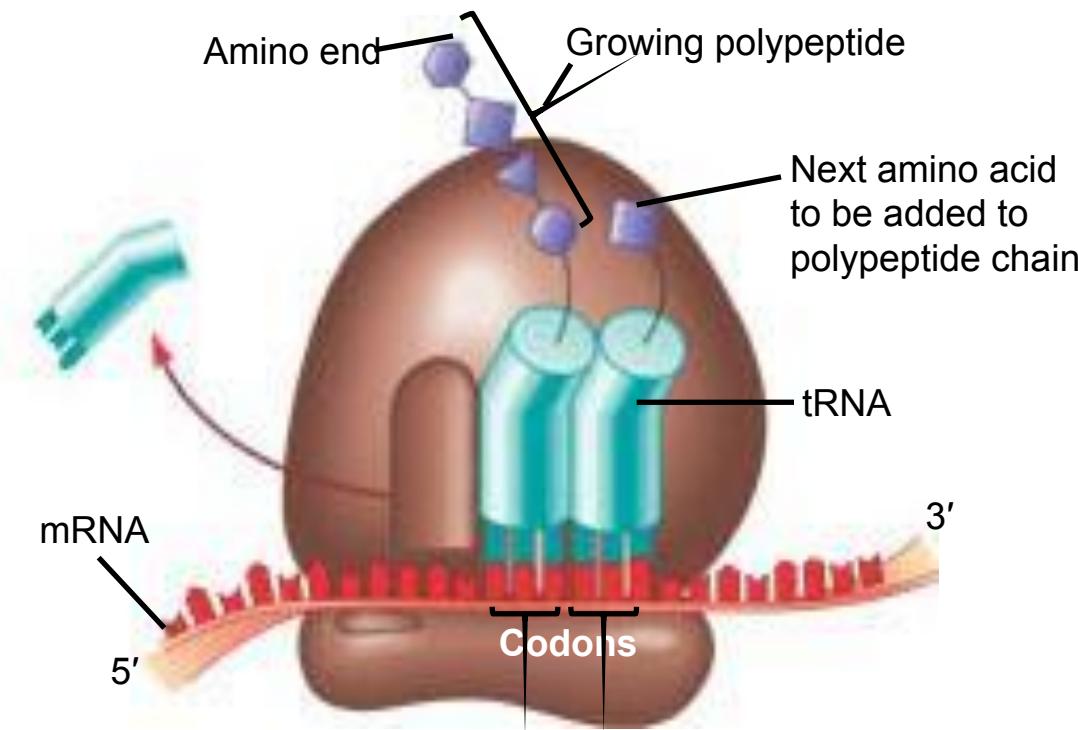
- The ribosome has three binding sites for tRNA
 - The P site
 - The A site
 - The E site



(b) **Schematic model showing binding sites.** A ribosome has an mRNA binding site and three tRNA binding sites, known as the A, P, and E sites. This schematic ribosome will appear in later diagrams.

Figure 17.16b

Figure 17.16c



(c) Schematic model with mRNA and tRNA. A tRNA fits into a binding site when its anticodon base-pairs with an mRNA codon. The P site holds the tRNA attached to the growing polypeptide. The A site holds the tRNA carrying the next amino acid to be added to the polypeptide chain. Discharged tRNA leaves via the E site.

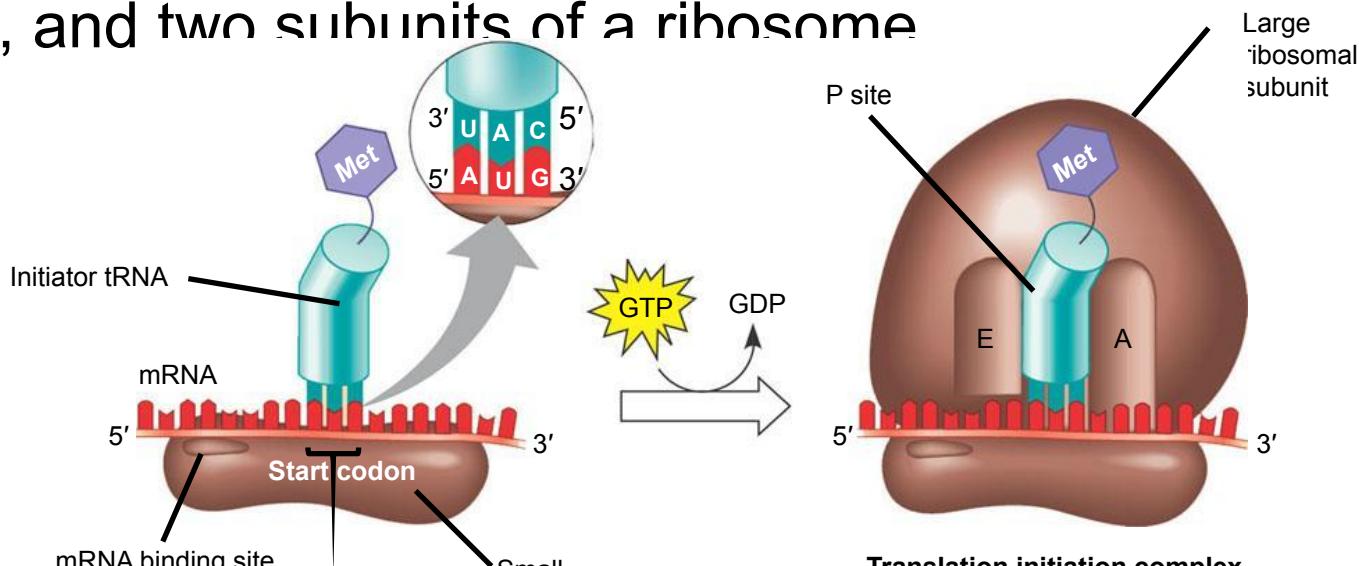
Building a Polypeptide

- We can divide translation into three stages
 - Initiation
 - Elongation
 - Termination

Ribosome Association and Initiation of Translation

- The initiation stage of translation

- Brings together mRNA, tRNA bearing the first amino acid of the polypeptide, and two subunits of a ribosome



1

A small ribosomal subunit binds to a molecule of mRNA. In a prokaryotic cell, the mRNA binding site on this subunit recognizes a specific nucleotide sequence on the mRNA just upstream of the start codon. An initiator tRNA, with the anticodon UAC, base-pairs with the start codon, AUG. This tRNA carries the amino acid methionine (Met).

2

The arrival of a large ribosomal subunit completes the initiation complex. Proteins called initiation factors (not shown) are required to bring all the translation components together. GTP provides the energy for the assembly. The initiator tRNA is in the P site; the A site is available to the tRNA bearing the next amino acid.

Figure 17.17

Elongation of the Polypeptide Chain

- In the elongation stage of translation
 - Amino acids are added one by one to the preceding amino acid

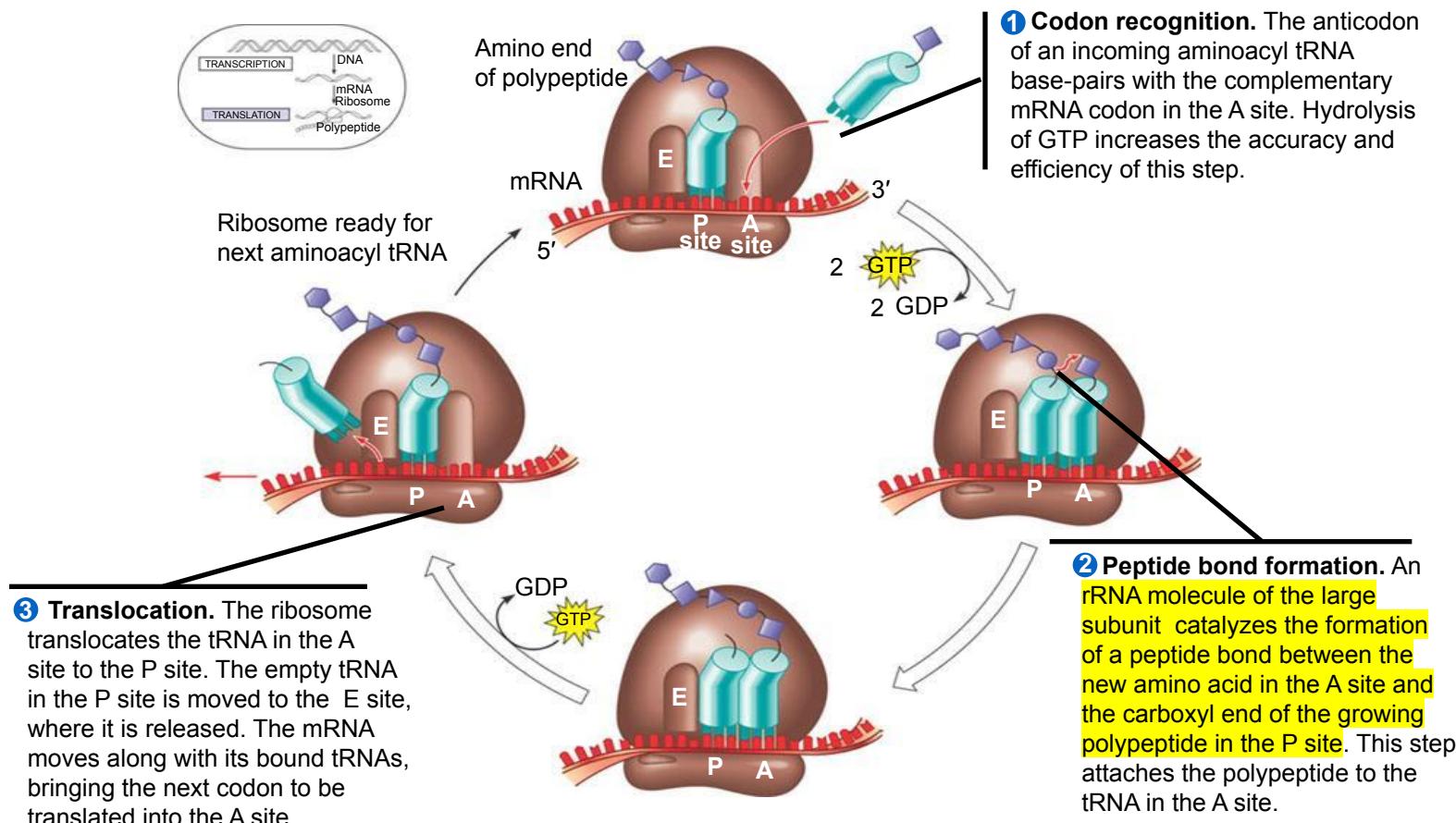
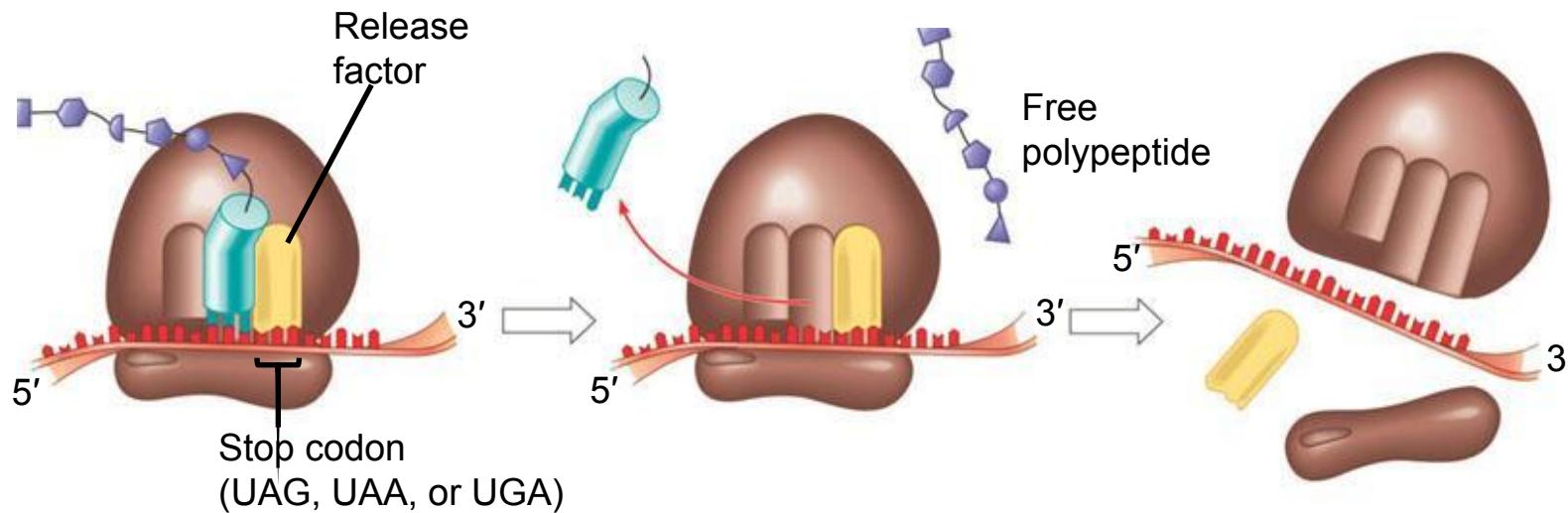


Figure 17.18

Termination of Translation

- The final stage of translation is termination
 - When the ribosome reaches a stop codon in the mRNA

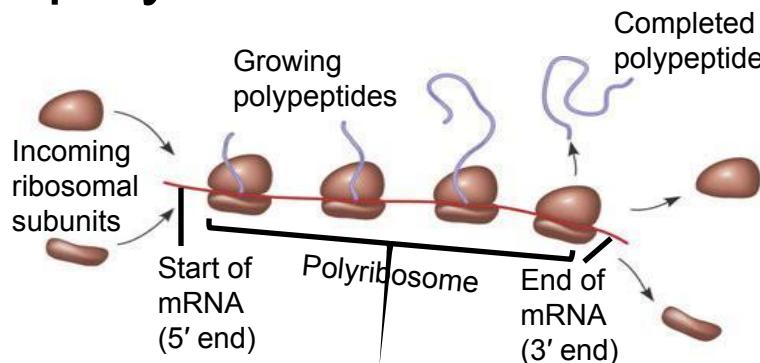


- ➊ When a ribosome reaches a stop codon on mRNA, the A site of the ribosome accepts a protein called a release factor instead of tRNA.
- ➋ The release factor hydrolyzes the bond between the tRNA in the P site and the last amino acid of the polypeptide chain. The polypeptide is thus freed from the ribosome.
- ➌ The two ribosomal subunits and the other components of the assembly dissociate.

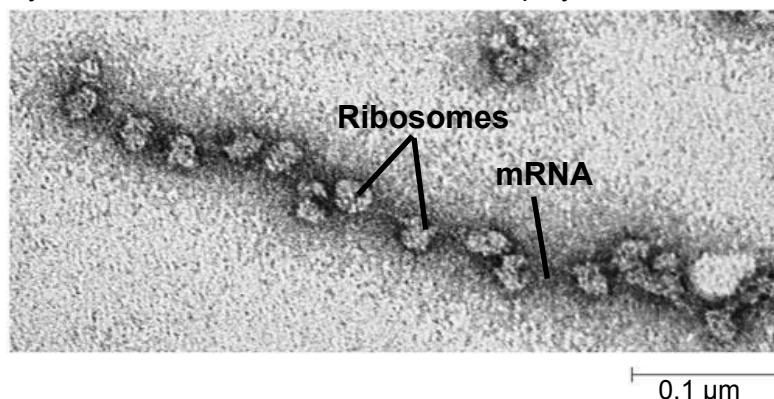
Figure 17.19

Polyribosomes

- A number of ribosomes can translate a single mRNA molecule simultaneously
 - Forming a polyribosome



(a) An mRNA molecule is generally translated simultaneously by several ribosomes in clusters called polyribosomes.



(b) This micrograph shows a large polyribosome in a prokaryotic cell (TEM).

Figure 17.20a, b

Completing and Targeting the Functional Protein

- Polypeptide chains
 - Undergo modifications after the translation process

Protein Folding and Post-Translational Modifications

- After translation
 - Proteins may be modified in ways that affect their three-dimensional shape

Targeting Polypeptides to Specific Locations

- Two populations of ribosomes are evident in cells
 - Free and bound
- Free ribosomes in the cytosol
 - Initiate the synthesis of all proteins

- Proteins destined for the endomembrane system or for secretion
 - Must be transported into the ER
 - Have signal peptides to which a signal-recognition particle (SRP) binds, enabling the translation ribosome to bind to the ER

Figure 17.21

- The signal mechanism for targeting proteins to the ER

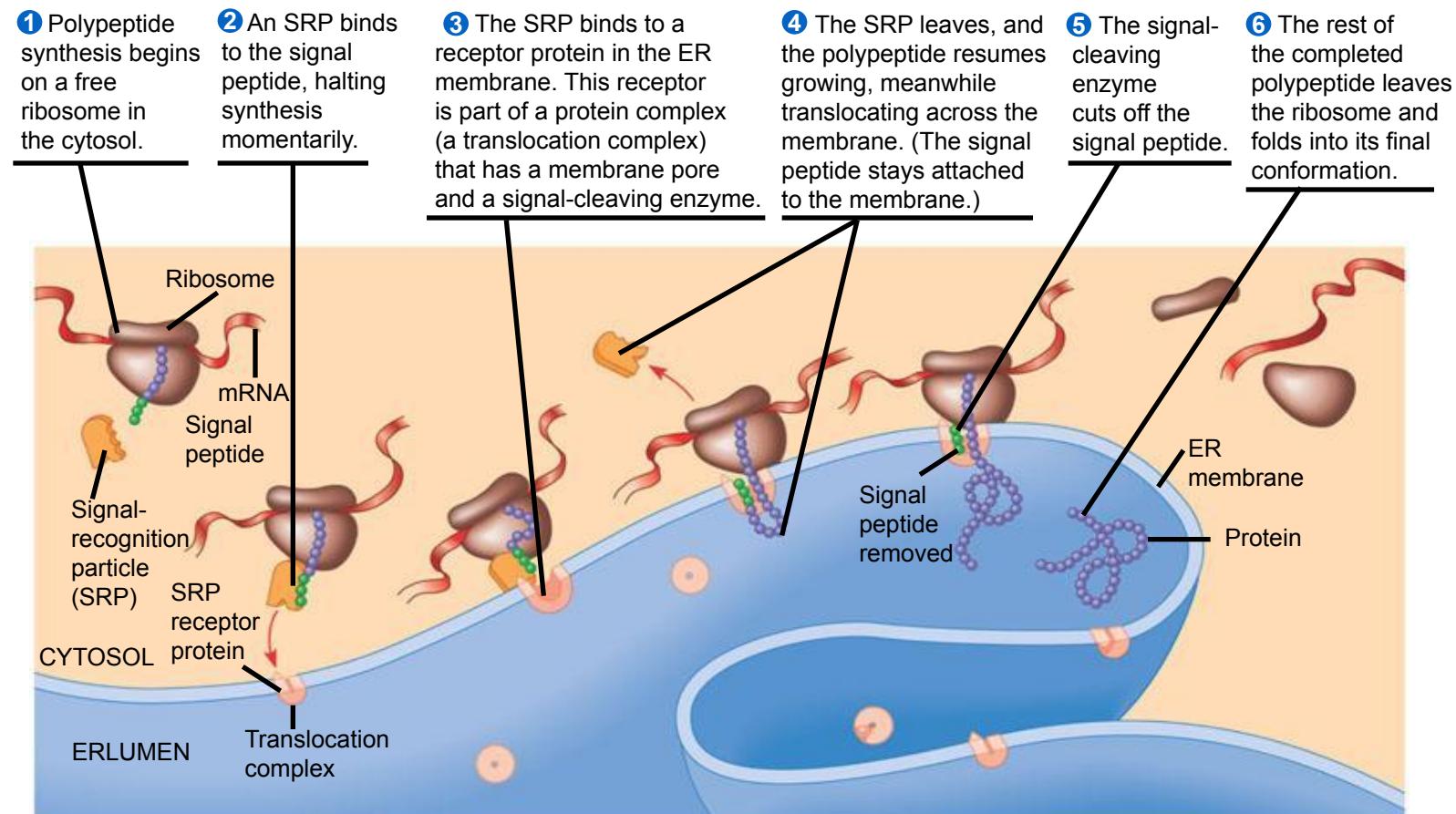


Figure 17.21

- Concept 17.5: RNA plays multiple roles in the cell: *a review*
- RNA
 - Can hydrogen-bond to other nucleic acid molecules
 - Can assume a specific three-dimensional shape
 - Has functional groups that allow it to act as a catalyst

Table 17.1

• Types of RNA in a Eukaryotic Cell

Type of RNA	Functions
Messenger RNA (mRNA)	Carries information specifying amino acid sequences of proteins from DNA to ribosomes.
Transfer RNA (tRNA)	Serves as adapter molecule in protein synthesis; translates mRNA codons into amino acids.
Ribosomal RNA (rRNA)	Plays catalytic (ribozyme) roles and structural roles in ribosomes.
Primary transcript	Serves as a precursor to mRNA, rRNA, or tRNA, before being processed by splicing or cleavage. Some intron RNA acts as a ribozyme, catalyzing its own splicing.
Small nuclear RNA (snRNA)	Plays structural and catalytic roles in spliceosomes, the complexes of protein and RNA that splice pre-mRNA.
SRP RNA	Is a component of the signal-recognition particle (SRP), the protein-RNA complex that recognizes the signal peptides of polypeptides targeted to the ER.
Small nucleolar RNA (snoRNA)	Aids in processing of pre-rRNA transcripts for ribosome subunit formation in the nucleolus.
Small interfering RNA (siRNA) and microRNA (miRNA)	Are involved in regulation of gene expression.

Table 17.1

Figure 17.22

- Concept 17.6: Comparing gene expression in prokaryotes and eukaryotes reveals key differences
- Prokaryotic cells lack a nuclear envelope
 - Allowing translation to begin while transcription is still in progress

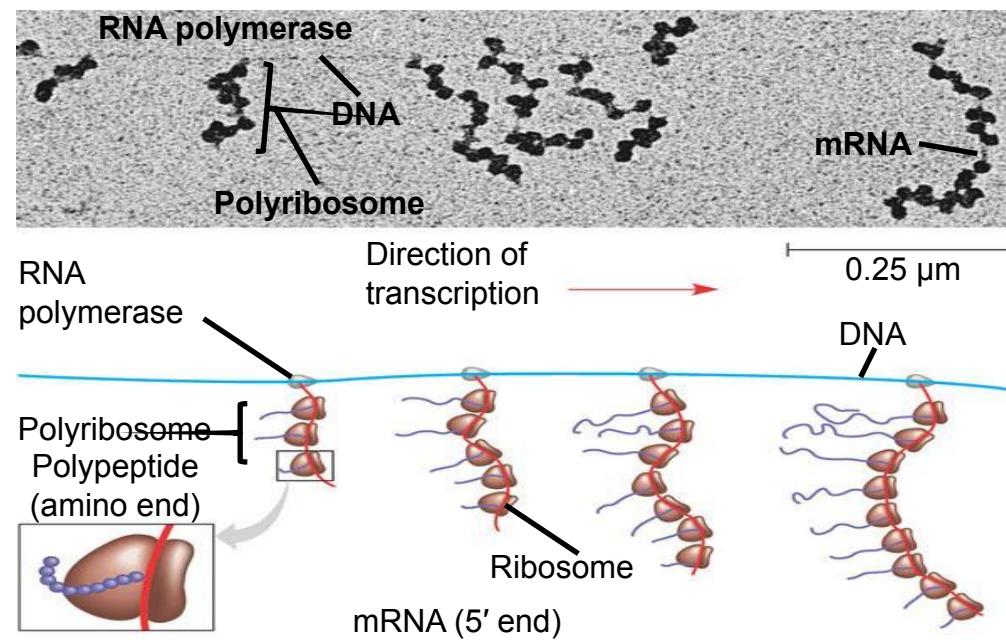


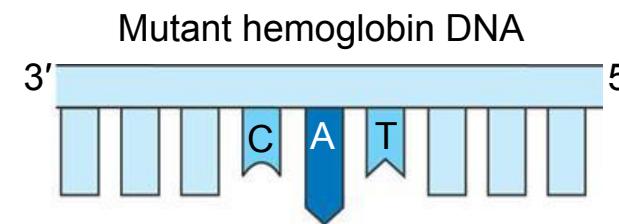
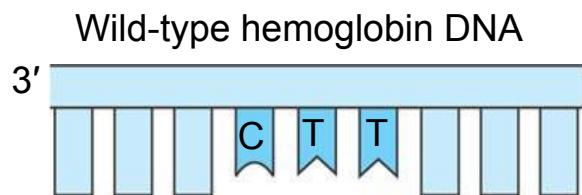
Figure 17.22

- In a eukaryotic cell
 - The nuclear envelope separates transcription from translation
 - Extensive RNA processing occurs in the nucleus

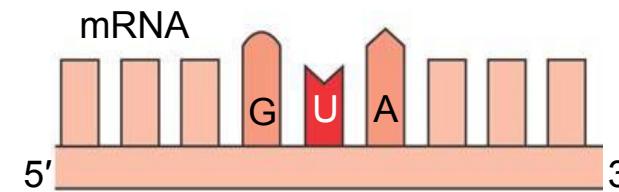
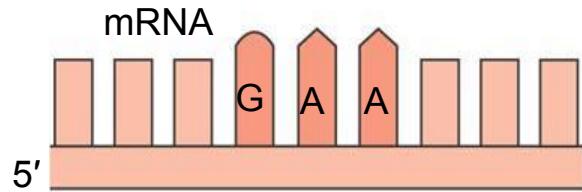
- Concept 17.7: Point mutations can affect protein structure and function
- Mutations
 - Are changes in the genetic material of a cell
- Point mutations
 - Are changes in just one base pair of a gene

Figure 17.23

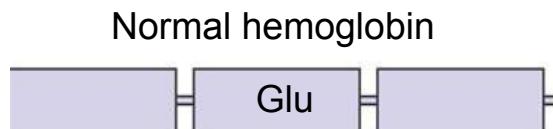
- The change of a single nucleotide in the DNA's template strand
 - Leads to the production of an abnormal protein



In the DNA, the mutant template strand has an A where the wild-type template has a T.



The mutant mRNA has a U instead of an A in one codon.



The mutant (sickle-cell) hemoglobin has a valine (Val) instead of a glutamic acid (Glu).

Figure 17.23

Types of Point Mutations

- Point mutations within a gene can be divided into two general categories
 - Base-pair substitutions
 - Base-pair insertions or deletions

Substitutions

- A base-pair substitution
 - Is the replacement of one nucleotide and its partner with another pair of nucleotides
 - Can cause missense or nonsense

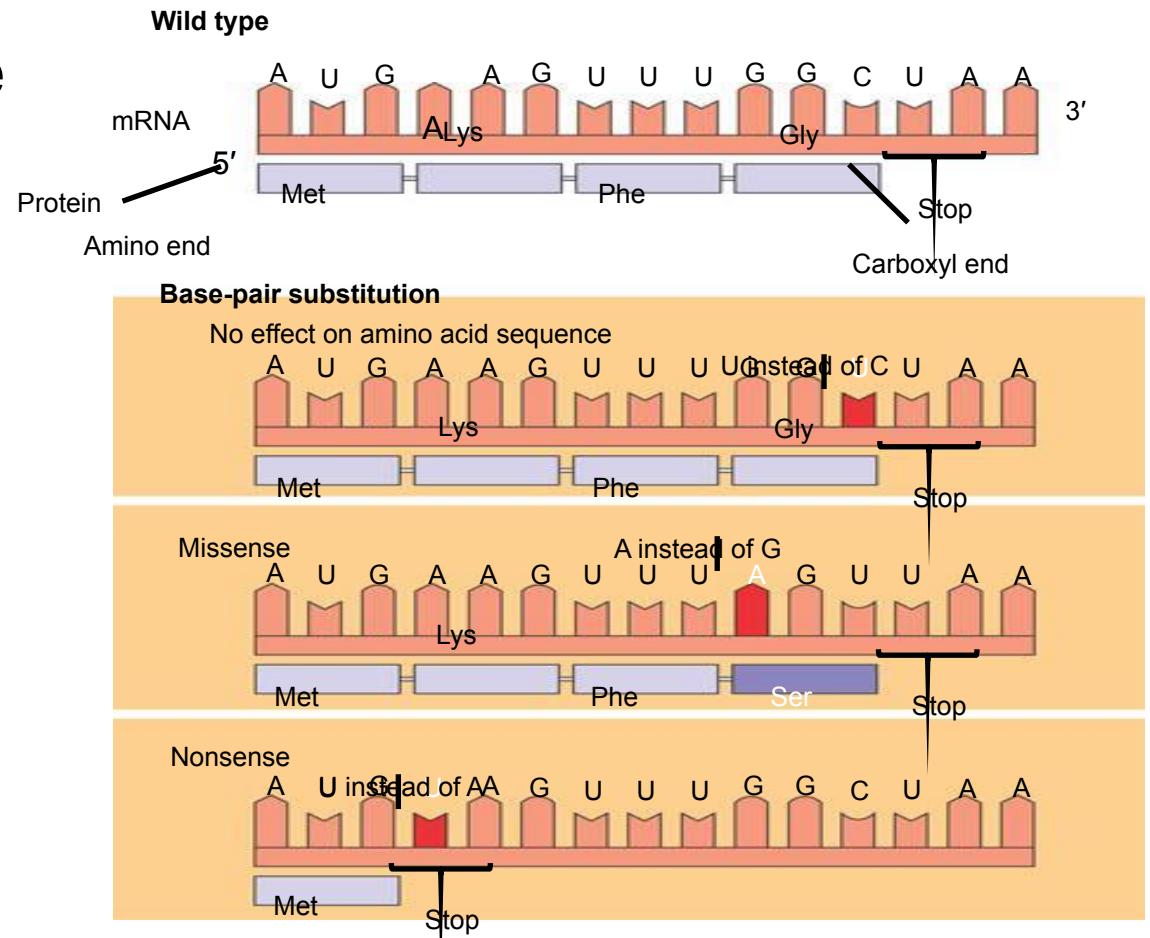


Figure 17.24

Insertions and Deletions

- Insertions and deletions
 - Are additions or losses of nucleotide pairs in a gene
 - May produce frameshift mutations

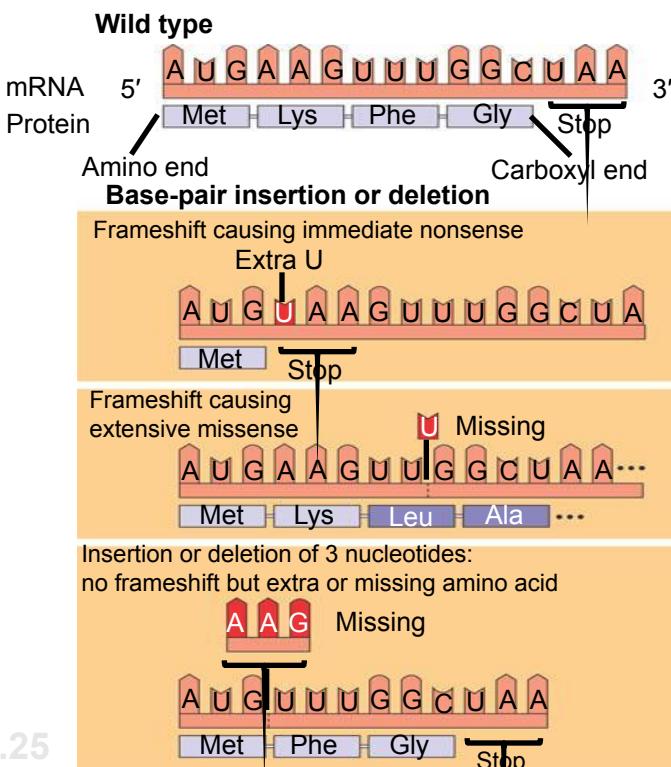


Figure 17.25

Mutagens

- Spontaneous mutations
 - Can occur during DNA replication, recombination, or repair

- Mutagens
 - Are physical or chemical agents that can cause mutations

What is a gene? *revisiting the question*

- A gene
 - Is a region of DNA whose final product is either a polypeptide or an RNA molecule

Figure 17.26

- A summary of transcription and translation in a eukaryotic cell

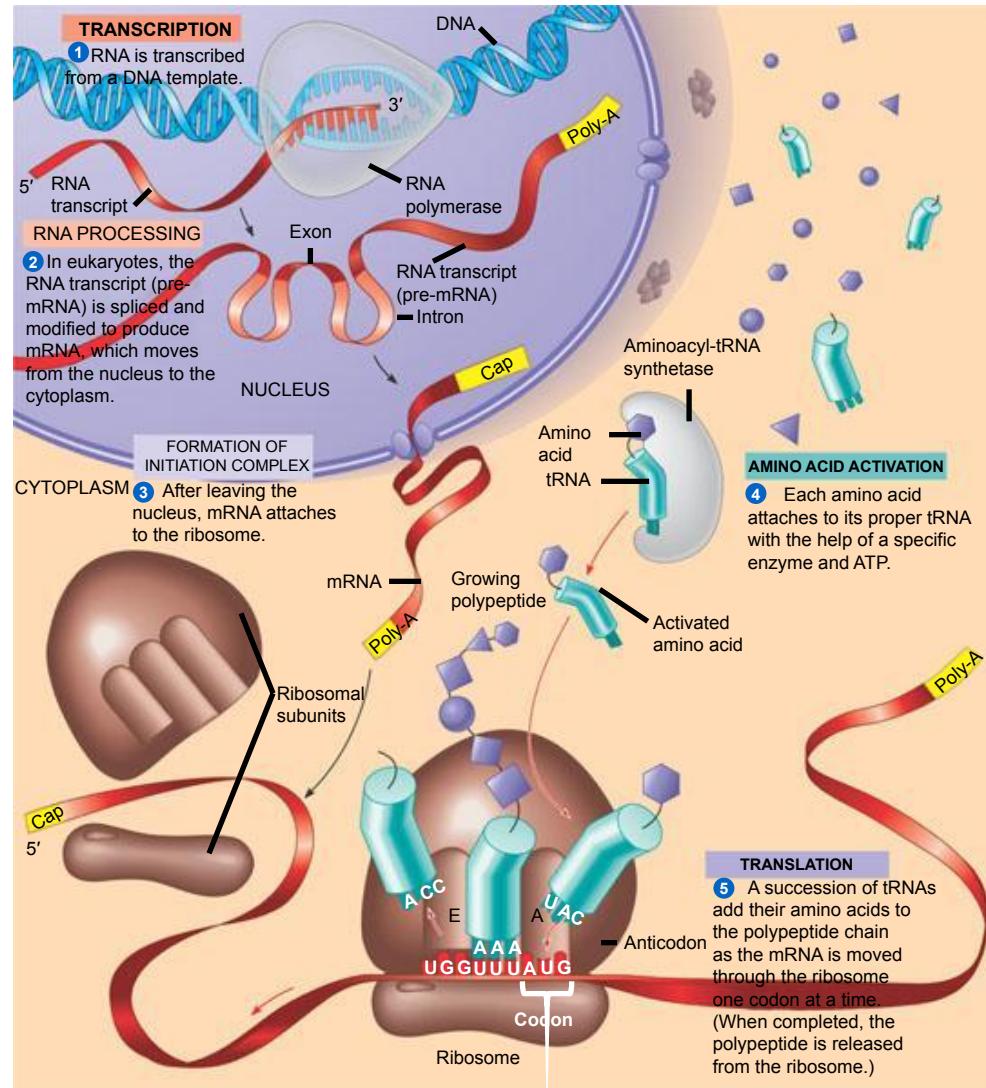


Figure 17.26