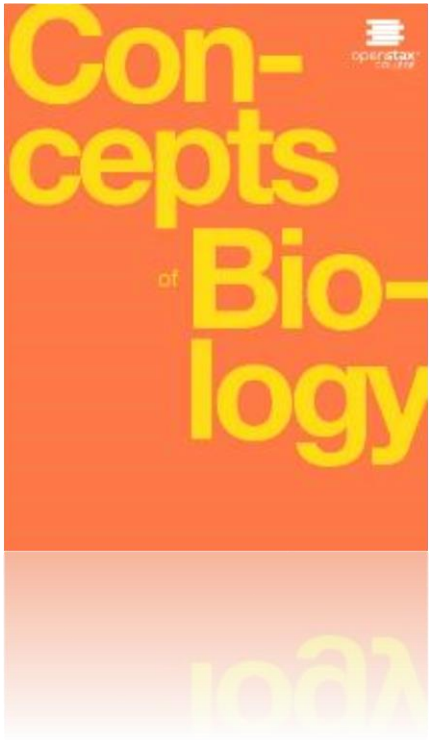
CONCEPTS OF BIOLOGY

Chapter 9 MOLECULAR BIOLOGY

PowerPoint Image Slideshow



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Introduction (1 of 2)

- The three letters “DNA” have become associated with crime solving, paternity testing, human identification, genetic testing & diagnostics, genealogy, identifying pathogens, vaccine development and cancer therapy
- DNA can be retrieved from hair, blood, or saliva
- With the exception of identical twins, each person’s DNA is unique and it is possible to detect differences between human beings on the basis of their unique DNA

Introduction (2 of 2)

- **DNA** is the genetic material passed from parent to offspring for all life on Earth
- The latest technology enables us to see deep into the history of life to deduce the relationships between living things in ways never thought possible
- Over a thousand species have had their entire genome sequenced (including humans)
- These sequences will allow us to understand human disease and the relationship of humans to the rest of the tree of life

FIGURE 9.1

Dolly the sheep was the first cloned mammal.

9.1 THE STRUCTURE OF DNA (1 of 8)

- In the 1950s, Francis Crick and James Watson worked together to determine the structure of DNA
- Other scientists, such as Linus Pauling, Maurice Wilkins, and Rosalind Franklin were also actively exploring this field
- In Wilkins' lab, researcher Rosalind Franklin was using X-ray crystallography to understand the structure of DNA

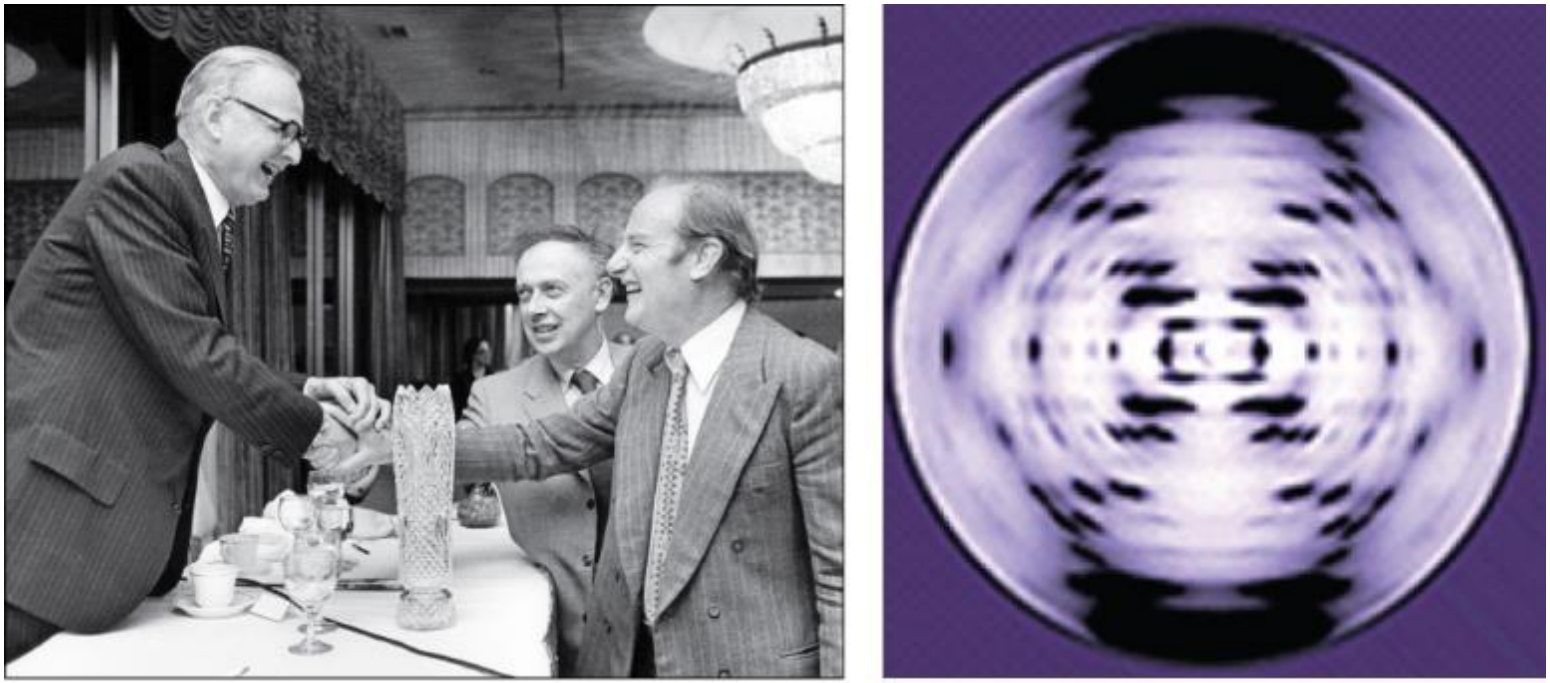
The Structure of DNA (2 of 8)

- Watson and Crick were able to piece together the puzzle of the DNA molecule using Franklin's data and key pieces of information available from other researchers such as Chargaff’s rules
- Chargaff had shown that of the four kinds of monomers (nucleotides) present in a DNA molecule, two types were always present in equal amounts and the remaining two types were also always present in equal amounts; this meant they were always paired in some way

The Structure of DNA (3 of 8)

- In 1962, James Watson, Francis Crick, and Maurice Wilkins were awarded the Nobel Prize in Medicine for their work in determining the structure of DNA

FIGURE 9.2



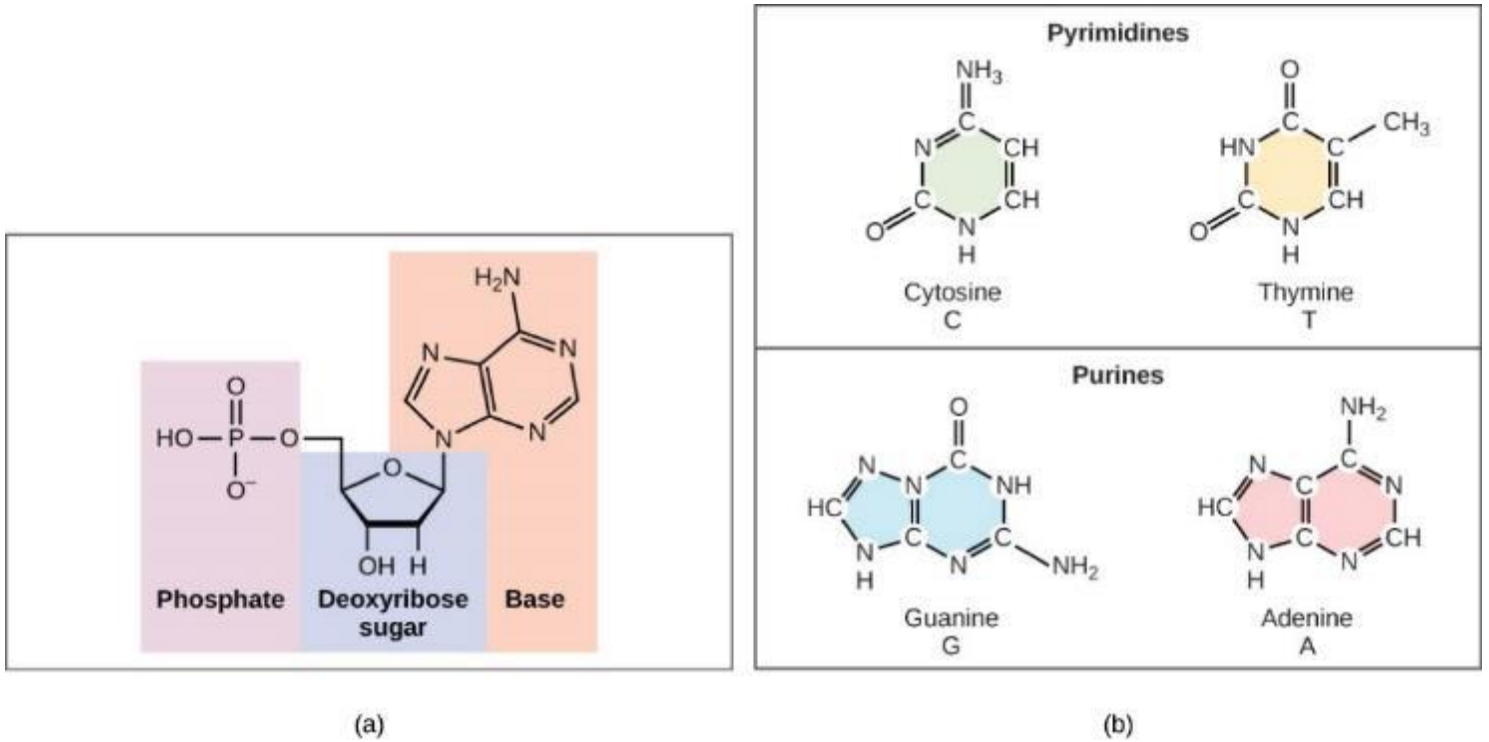
(a) (b)

Pioneering scientists (a) James Watson and Francis Crick are pictured here with American geneticist Maclyn McCarty. Scientist Rosalind Franklin discovered (b) the X-ray diffraction pattern of DNA, which helped to elucidate its double helix structure. (credit a: modification of work by Marjorie McCarty; b: modification of work by NIH)

The Structure of DNA (4 of 8)

- Now let's consider the structure of the two types of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).
- The building blocks of DNA are nucleotides, which are made up of three parts: a **deoxyribose** (5-carbon sugar), a **phosphate group**, and a **nitrogenous base** (Figure 9.3)
- There are four types of nitrogenous bases in DNA: Adenine (A) and guanine (G) are double-ringed purines, and cytosine (C) and thymine (T) are smaller, single-ringed pyrimidines

FIGURE 9.3



(a) Each DNA nucleotide is made up of a sugar, a phosphate group, and a base.

(b) Cytosine and thymine are pyrimidines. Guanine and adenine are purines.

The Structure of DNA (5 of 8)

- The phosphate group of one nucleotide bonds covalently with the sugar molecule of the next nucleotide, and so on, forming a long polymer of nucleotide monomers
- The sugar–phosphate groups line up in a “backbone” for each single strand of DNA, and the nucleotide bases stick out from this backbone

The Structure of DNA (6 of 8)

- The carbon atoms of the five-carbon sugar are numbered clockwise from the oxygen as 1', 2', 3', 4', and 5' (1' is read as “one prime”)
- The phosphate group is attached to the 5' carbon of one nucleotide and the 3' carbon of the next nucleotide
- In its natural state, each DNA molecule is actually composed of two single strands held together along their length with hydrogen bonds between the bases

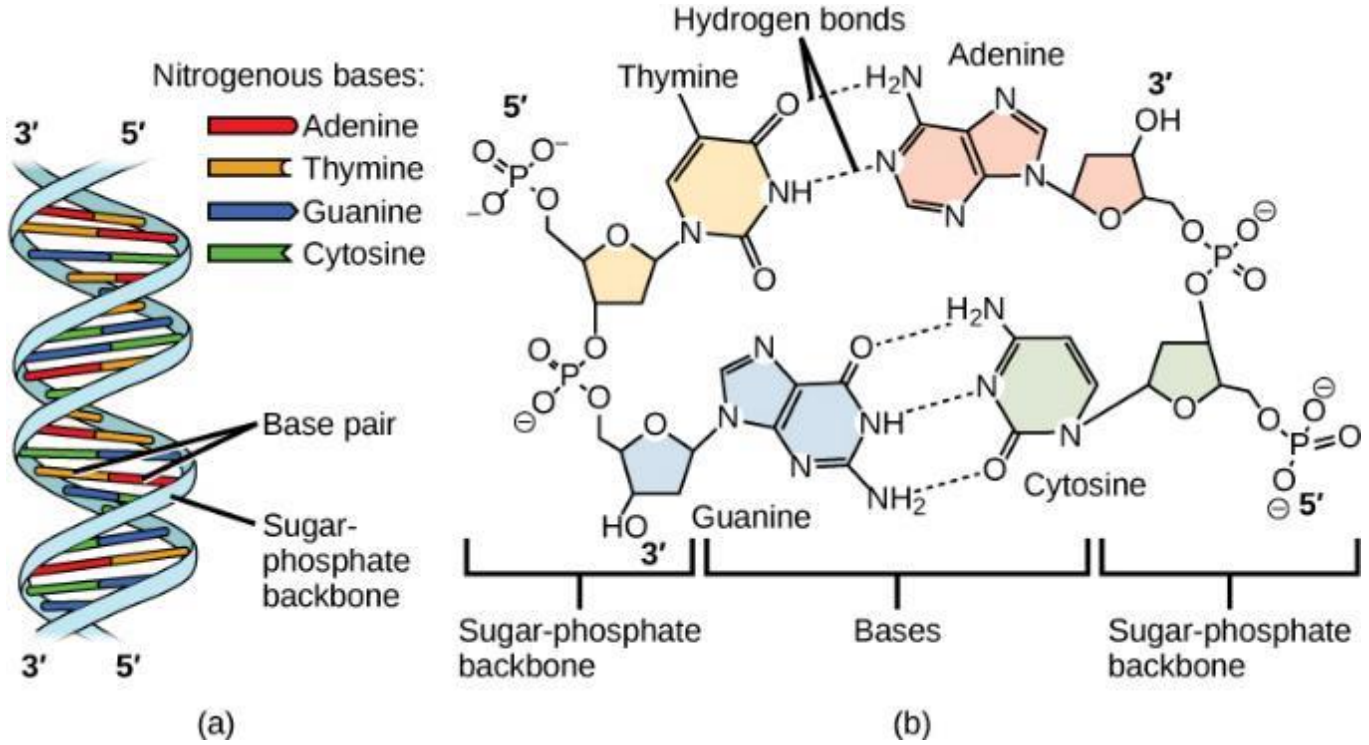
The Structure of DNA (7 of 8)

- Watson and Crick proposed that the DNA is made up of two strands that are twisted around each other to form a right-handed helix, called a **double helix**
- Base-pairing takes place between a purine and pyrimidine: A pairs with T, and G pairs with C
- This is the basis for Chargaff's rule; because of their complementarity, there is as much adenine as thymine in a DNA molecule and as much guanine as cytosine

The Structure of DNA (8 of 8)

- Adenine and thymine are connected by two hydrogen bonds, and cytosine and guanine are connected by three hydrogen bonds
- The two strands are anti-parallel in nature; that is, one strand will have the 3' carbon of the sugar in the “upward” position, whereas the other strand will have the 5' carbon in the upward position
- The diameter of the DNA double helix is uniform throughout because a **purine** (two rings) always pairs with a **pyrimidine** (one ring) and their combined lengths are always equal (Figure 9.4)

FIGURE 9.4

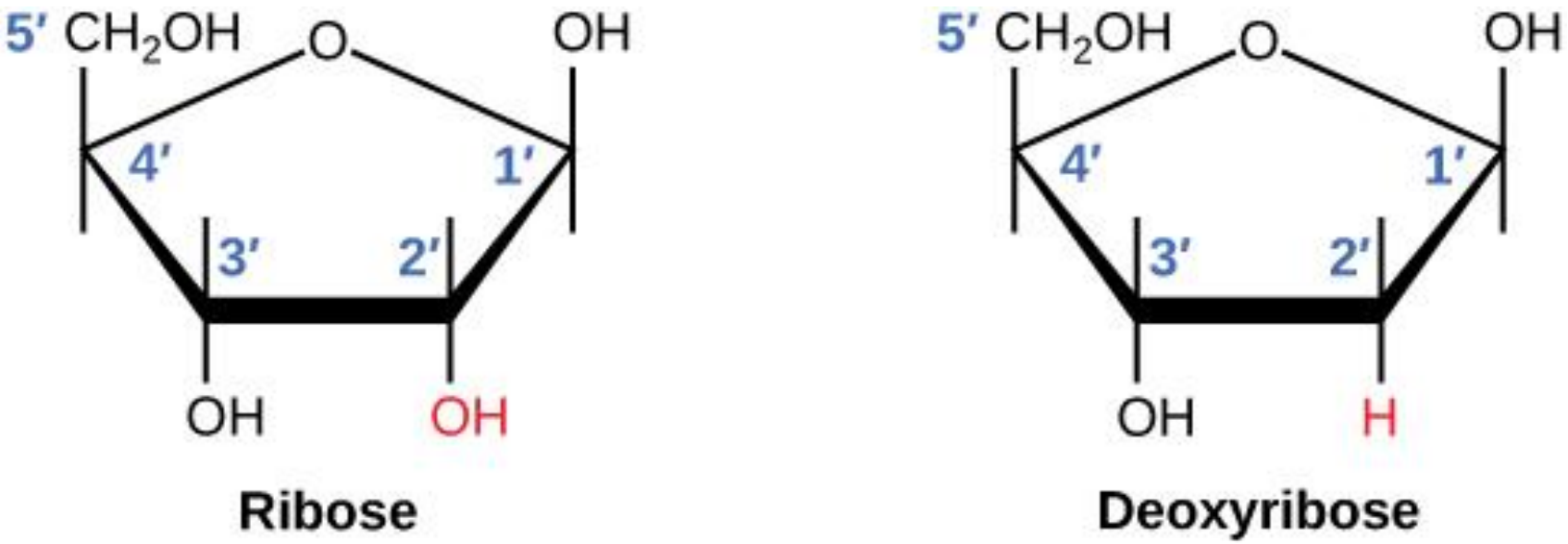


DNA (a) forms a double stranded helix, and (b) adenine pairs with thymine and cytosine pairs with guanine. (credit a: modification of work by Jerome Walker, Dennis Myts)

The Structure of RNA (1 of 2)

- There is a second nucleic acid in all cells called **ribonucleic acid**, or **RNA**
- Like DNA, RNA is a polymer of nucleotides
- Each of the nucleotides in RNA is made up of a nitrogenous base, a five-carbon sugar, and a phosphate group
- In the case of RNA, the five-carbon sugar is ribose, not deoxyribose
- Ribose has a hydroxyl group at the 2' carbon, unlike deoxyribose, which has only a hydrogen atom (Figure 9.5)

FIGURE 9.5



The difference between the ribose found in RNA and the deoxyribose found in DNA is that ribose has a hydroxyl group at the 2' carbon.

The Structure of RNA (2 of 2)

- RNA nucleotides contain the nitrogenous bases adenine, cytosine, and guanine; however, they do not contain thymine, which is instead replaced by uracil, symbolized by a “U”
- RNA exists as a single-stranded molecule rather than a double-stranded helix
- Molecular biologists have named several kinds of RNA on the basis of their function: messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA)
- RNA molecules that are involved in the production of proteins from the DNA code

How DNA is Arranged in the Cell (1 of 6)

- DNA is a working molecule; it must be replicated when a cell is ready to divide, and it must be “read” to produce the molecules, such as proteins, to carry out the functions of the cell
- For this reason, the DNA is protected and packaged in very specific ways

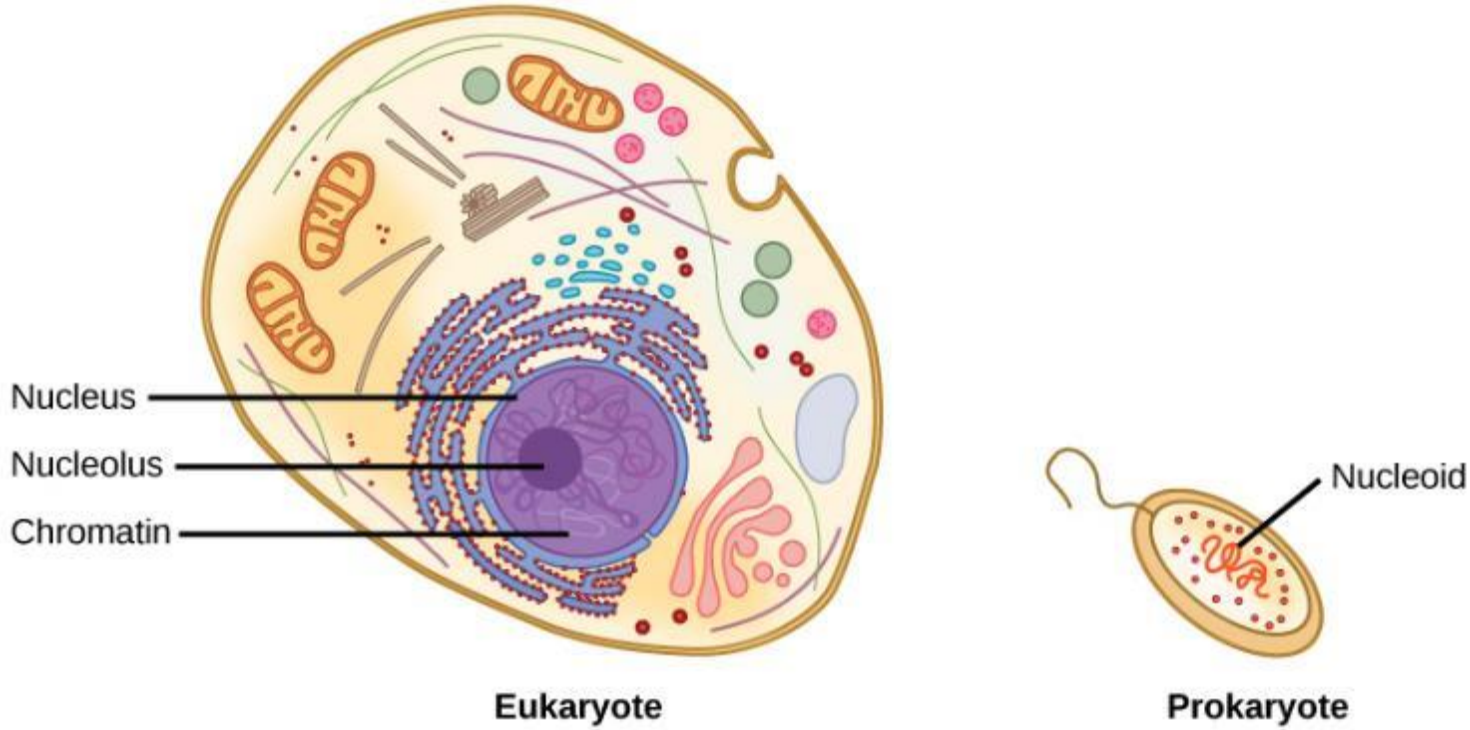
How DNA is Arranged in the Cell (2 of 6)

- In addition, DNA molecules can be very long. Stretched end-to-end, the DNA molecules in a single human cell would come to a length of about 2 meters
- Thus, the DNA for a cell must be packaged in a very ordered way to fit and function within a structure (the cell) that is not visible to the naked eye

How DNA is Arranged in the Cell (3 of 6)

- The chromosomes of prokaryotes are much simpler than those of eukaryotes in many of their features (Figure 9.6)
- Most prokaryotes contain a single, circular chromosome that is found in an area in the cytoplasm called the **nucleoid**

FIGURE 9.6



A eukaryote contains a well-defined nucleus, whereas in prokaryotes, the chromosome lies in the cytoplasm in an area called the nucleoid.

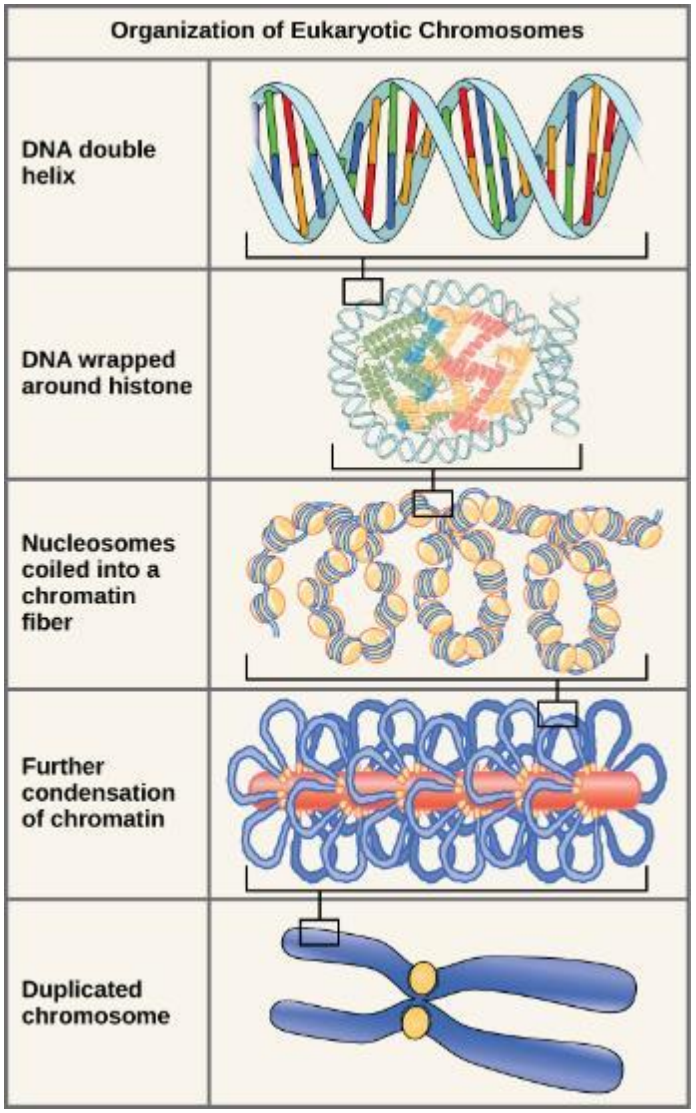
How DNA is Arranged in the Cell (4 of 6)

- The size of the genome in one of the most well-studied prokaryotes, *Escherichia coli,* is 4.6 million base pairs, which would extend a distance of about 1.6 mm if stretched out; so how does this fit inside a small bacterial cell?
- The DNA is twisted beyond the double helix in what is known as **supercoiling**

How DNA is Arranged in the Cell (5 of 6)

- Eukaryotes, whose chromosomes each consist of a linear DNA molecule, employ a different type of packing strategy to fit their DNA inside the nucleus (Figure 9.7)
- At the most basic level, DNA is wrapped tightly around proteins known as **histones** to form structures called **nucleosomes**
- This nucleosome is linked to the next one by a short strand of DNA that is free of histones
- This is also known as the “beads on a string” structure; the nucleosomes are the “beads” and the DNA between them are the “string”

FIGURE 9.7



These figures illustrate the compaction of the eukaryotic chromosome.

How DNA is Arranged in the Cell (6 of 6)

- At the metaphase stage of mitosis, when the chromosomes are lined up in the center of the cell, the chromosomes are at their most compacted; they are approximately 700 nm in width, and are found in association with scaffold proteins.
- In interphase, the phase of the cell cycle between mitoses at which the chromosomes are decondensed

9.2 DNA REPLICATION (1 of 4)

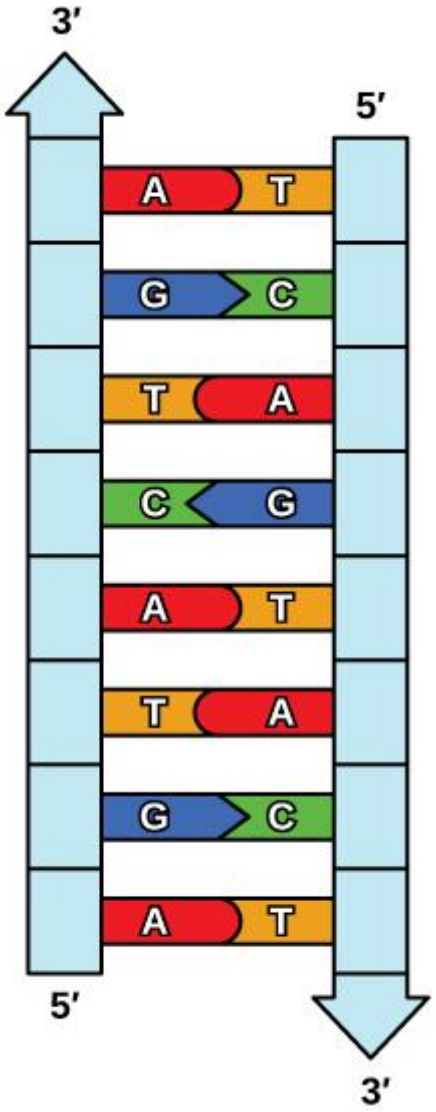
- When a cell divides, it is important that each daughter cell receives an identical copy of the DNA
- This is accomplished by the process of **DNA replication**
- The replication of DNA occurs during the synthesis phase, or S phase, of the cell cycle, before the cell enters mitosis or meiosis

DNA Replication (2 of 4)

- The elucidation of the structure of the double helix provided a hint as to how DNA is copied
- Recall that adenine pairs with thymine nucleotides, and cytosine with guanine
- This means that the two strands are complementary to each other
- For example, a strand of DNA with a nucleotide sequence of AGTCATGA will have a complementary strand with the sequence TCAGTACT (Figure 9.8)

FIGURE 9.8

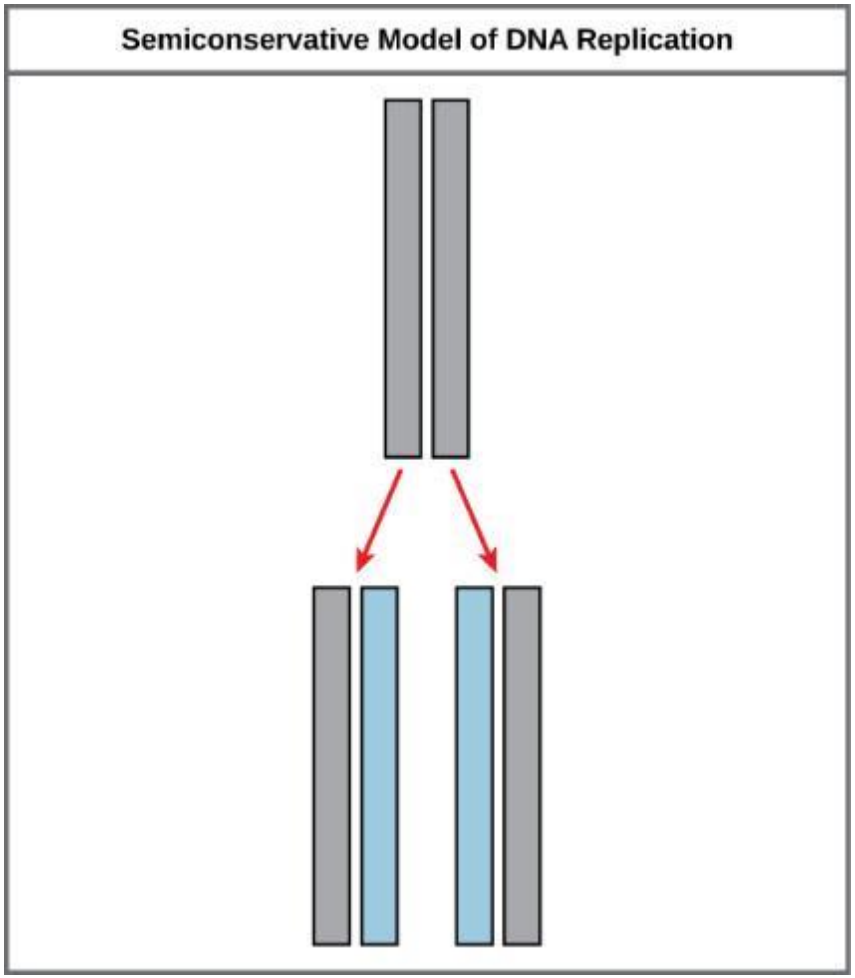
The two strands of DNA are complementary, meaning the sequence of bases in one strand can be used to create the correct sequence of bases in the other strand.



DNA Replication (3 of 4)

- Because of the complementarity of the two strands, having one strand means that it is possible to recreate the other strand
- This model for replication suggests that the two strands of the double helix separate during replication, and each strand serves as a template from which the new complementary strand is copied (Figure 9.9)

FIGURE 9.9



The semiconservative model of DNA replication is shown. Gray indicates the original DNA strands, and blue indicates newly synthesized DNA..

DNA Replication (4 of 4)

- During DNA replication, each of the two strands that make up the double helix serves as a template from which new strands are copied
- The new strand will be complementary to the parental or “old” strand
- Each new double strand consists of one parental strand and one new daughter strand
- This is known as **semiconservative replication**

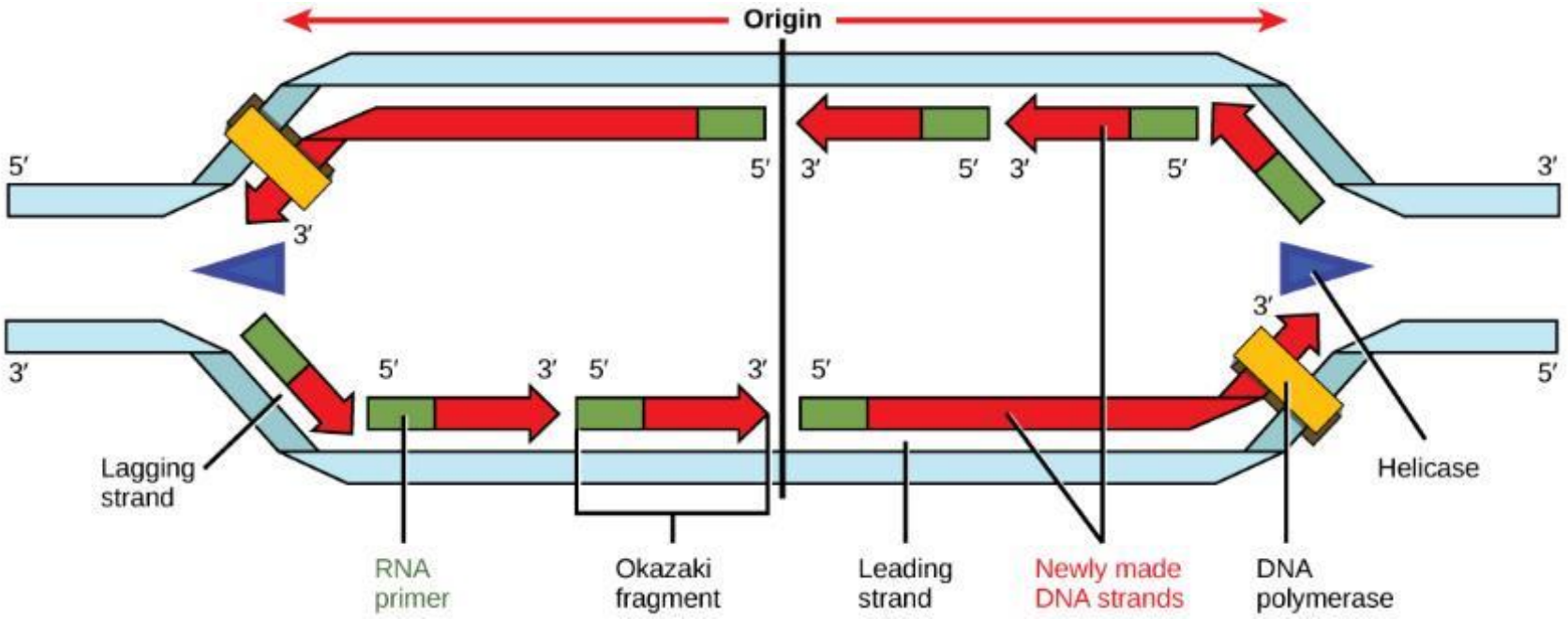
DNA Replication in Eukaryotes (1 of 7)

- Because eukaryotic genomes are very complex, DNA replication is a very complicated process that involves several enzymes and other proteins
- It occurs in three main stages:
  - ✓ initiation
  - ✓ elongation
  - ✓ termination

DNA Replication in Eukaryotes (2 of 7)

- During initiation, the DNA is made accessible to the proteins and enzymes involved in the replication process
- There are specific nucleotide sequences called **origins of replication** at which replication begins
- Certain proteins bind to the origin of replication while an enzyme called **helicase** unwinds and opens up the DNA helix
- As the DNA opens up, Y-shaped structures called **replication forks** are formed (Figure 9.10)

FIGURE 9.10



A replication fork is formed by the opening of the origin of replication, and helicase separates the DNA strands. An RNA primer is synthesized, and is elongated by the DNA polymerase. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches. The DNA fragments are joined by DNA ligase (not shown).

DNA Replication in Eukaryotes (3 of 7)

- Two replication forks are formed at the origin of replication, and these get extended in both directions as replication proceeds
- There are multiple origins of replication on the eukaryotic chromosome, such that replication can occur simultaneously from several places in the genome

DNA Replication in Eukaryotes (4 of 7)

- During elongation, an enzyme called **DNA polymerase** adds DNA nucleotides to the 3' end of the template
- Because DNA polymerase can only add new nucleotides at the end of a backbone, a **primer** sequence, which provides this starting point, is added with complementary RNA nucleotides (this primer is removed later, replaced with DNA nucleotides)

DNA Replication in Eukaryotes (5 of 7)

- One strand, which is complementary to the parental DNA strand, is synthesized continuously toward the replication fork so the polymerase can add nucleotides in this direction
- This continuously synthesized strand is known as the **leading strand**

DNA Replication in Eukaryotes (6 of 7)

- Because DNA polymerase can only synthesize DNA in a 5' to 3' direction, the other new strand is put together in short pieces called **Okazaki fragments**
- The Okazaki fragments each require a primer made of RNA to start the synthesis
- The strand with the Okazaki fragments is known as the **lagging strand**
- As synthesis proceeds, an enzyme removes the RNA primer, which is then replaced with DNA nucleotides, and the gaps between fragments are sealed by an enzyme called **DNA ligase**

DNA Replication in Eukaryotes (7 of 7)

The process of DNA replication can be summarized as follows:

1. DNA unwinds at the origin of replication
2. New bases are added to the complementary parental strands (one new strand is made continuously, while the other strand is made in pieces)
3. Primers are removed, new DNA nucleotides are put in place of the primers and the backbone is sealed by DNA ligase

Telomere Replication (1 of 7)

- Because eukaryotic chromosomes are linear, DNA replication comes to the end of a line in eukaryotic chromosomes
- In the leading strand, synthesis continues until the end of the chromosome is reached; however, on the lagging strand there is no place for a primer to be made for the DNA fragment to be copied at the end of the chromosome

Telomere Replication (2 of 7)

- This presents a problem for the cell because the ends remain unpaired, and over time these ends get progressively shorter as cells continue to divide
- The ends of the linear chromosomes are known as **telomeres**, which have repetitive sequences that do not code for a particular gene
- As a consequence, it is telomeres that are shortened with each round of DNA replication instead of genes

Telomere Replication (3 of 7)

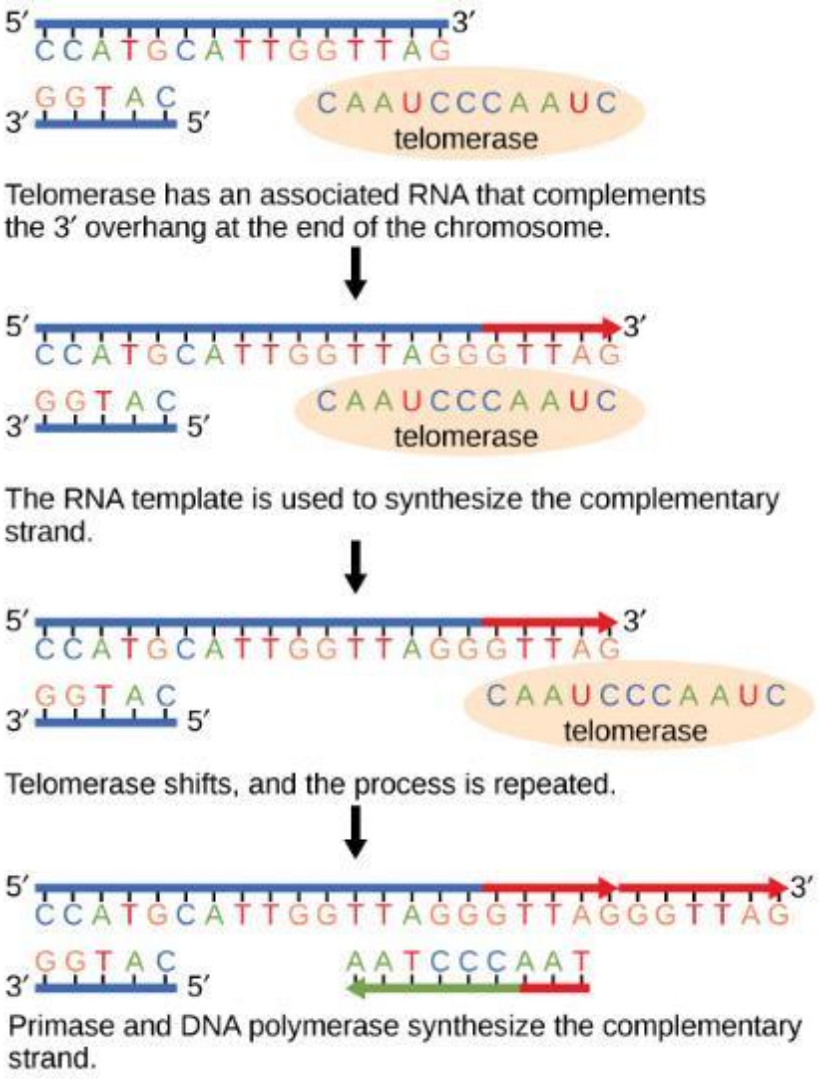
- For example, in humans, a six base-pair sequence, TTAGGG, is repeated 100 to 1000 times
- The discovery of the enzyme **telomerase** (Figure 9.11) helped in the understanding of how chromosome ends are maintained

Telomere Replication (4 of 7)

- The telomerase attaches to the end of the chromosome, and complementary bases to the RNA template are added on the end of the DNA strand
- Once the lagging strand template is sufficiently elongated, DNA polymerase can now add nucleotides that are complementary to the ends of the chromosomes
- Thus, the ends of the chromosomes are replicated

FIGURE 9.11

The ends of linear chromosomes are maintained by the action of the telomerase enzyme.



Telomere Replication (5 of 7)

- Telomerase is typically found to be active in germ cells, adult stem cells, and some cancer cells
- For her discovery of telomerase and its action, Elizabeth Blackburn (Figure 9.12) received the Nobel Prize for Medicine and Physiology in 2009.

FIGURE 9.12

Elizabeth Blackburn, 2009 Nobel Laureate, was the scientist who discovered how telomerase works. (credit: U.S. Embassy, Stockholm, Sweden)

Telomere Replication (6 of 7)

- Telomerase is not active in adult somatic cells
- Adult somatic cells that undergo cell division continue to have their telomeres shortened
- This essentially means that telomere shortening is associated with aging
- In 2010, scientists found that telomerase can reverse some age-related conditions in mice, and this may have potential in regenerative medicine

Telomere Replication (7 of 7)

- Telomerase-deficient mice were used in these studies; these mice have tissue atrophy, stem-cell depletion, organ system failure, and impaired tissue injury responses
- Telomerase reactivation in these mice caused extension of telomeres, reduced DNA damage, reversed neurodegeneration, and improved functioning of the testes, spleen, and intestines
- Thus, telomere reactivation may have potential for treating age-related diseases in humans

DNA Replication in Prokaryotes (1 of 2)

- Recall that the prokaryotic chromosome is a circular molecule with a less extensive coiling structure than eukaryotic chromosomes
- DNA replication has been extremely well-studied in prokaryotes, primarily because of the small size of the genome and large number of variants available

DNA Replication in Prokaryotes (2 of 2)

- *Escherichia coli* has 4.6 million base pairs in a single circular chromosome, and all of it gets replicated in approximately 42 minutes, starting from a single origin of replication and proceeding around the chromosome in both directions
- This means that approximately 1000 nucleotides are added per second; the process is much more rapid than in eukaryotes
- See Table 9.1

DNA Replication in Prokaryotes vs. Eukaryotes

Differences between Prokaryotic and Eukaryotic Replications

Property	Prokaryotes	Eukaryotes
Origin of replication	Single	Multiple
Rate of replication	1000 nucleotides/s	50 to 100 nucleotides/s
Chromosome structure	circular	linear
Telomerase	Not present	Present

Table 9.1

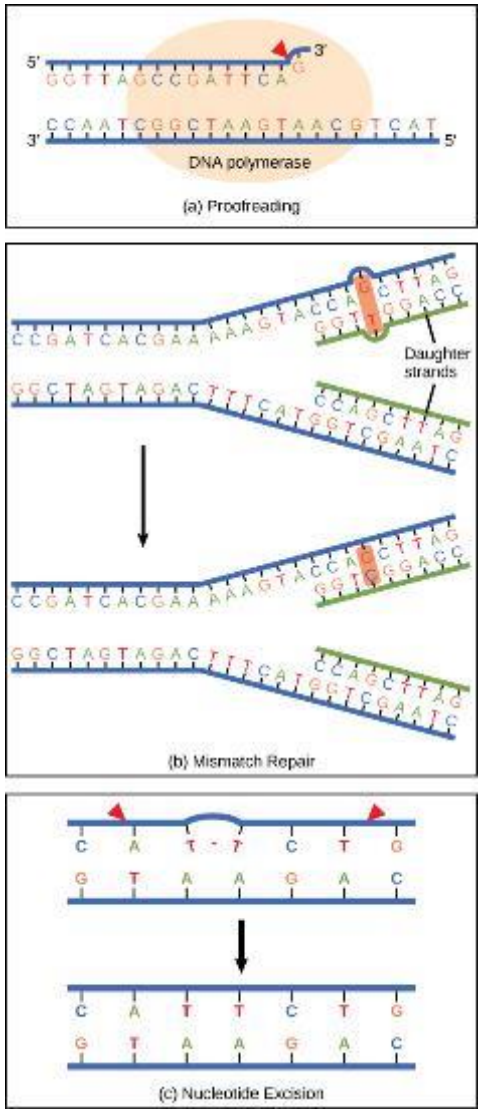
DNA Repair (1 of 4)

- DNA polymerase can make mistakes while adding nucleotides
- It edits the DNA by proofreading every newly added base
- Incorrect bases are removed and replaced by the correct base, and then polymerization continues (Figure 9.13a)
- Most mistakes are corrected during replication, although when this does not happen, the **mismatch repair** mechanism is employed

DNA Repair (2 of 4)

- Mismatch repair enzymes recognize the wrongly incorporated base and excise it from the DNA, replacing it with the correct base (Figure 9.13b)
- In yet another type of repair, **nucleotide excision repair,** the DNA double strand is unwound and separated, the incorrect bases are removed along with a few bases on the 5' and 3' end, and these are replaced by copying the template with the help of DNA polymerase

FIGURE 9.13



Proofreading by DNA polymerase (a) corrects errors during replication. In mismatch repair (b), the incorrectly added base is detected after replication. The mismatch repair proteins detect this base and remove it from the newly synthesized strand by nuclease action. The gap is now filled with the correctly paired base. Nucleotide excision (c) repairs thymine dimers. When exposed to UV, thymines lying adjacent to each other can form thymine dimers. In normal cells, they are excised and replaced.

DNA Repair (3 of 4)

- Nucleotide excision repair is important in correcting **thymine dimers**, which are primarily caused by ultraviolet light
- In a thymine dimer, two thymine nucleotides adjacent to each other on one strand are covalently bonded to each other rather than their complementary bases (Figure 9.13c)
- If the dimer is not removed and repaired it will lead to a mutation

DNA Repair (4 of 4)

- Individuals with flaws in their nucleotide excision repair genes show extreme sensitivity to sunlight and develop skin cancers early in life
- Most mistakes are corrected; if they are not, they may result in a **mutation**—defined as a permanent change in the DNA sequence
- Mutations in repair genes may lead to serious consequences like cancer

9.3 TRANSCRIPTION

- In both prokaryotes and eukaryotes, the second function of DNA (the first was replication) is to provide the information needed to construct the proteins necessary so that the cell can perform all of its functions
- Through the processes of transcription and translation, a protein is built with a specific sequence of amino acids that was originally encoded in the DNA

The Central Dogma: DNA Encodes RNA; RNA Encodes Protein

- The flow of genetic information in cells from DNA to mRNA to protein is described by the central dogma (Figure 9.14), which states that genes specify the sequences of mRNAs, which in turn specify the sequences of proteins
- The copying of DNA to mRNA is relatively straightforward, the translation to protein is more complex

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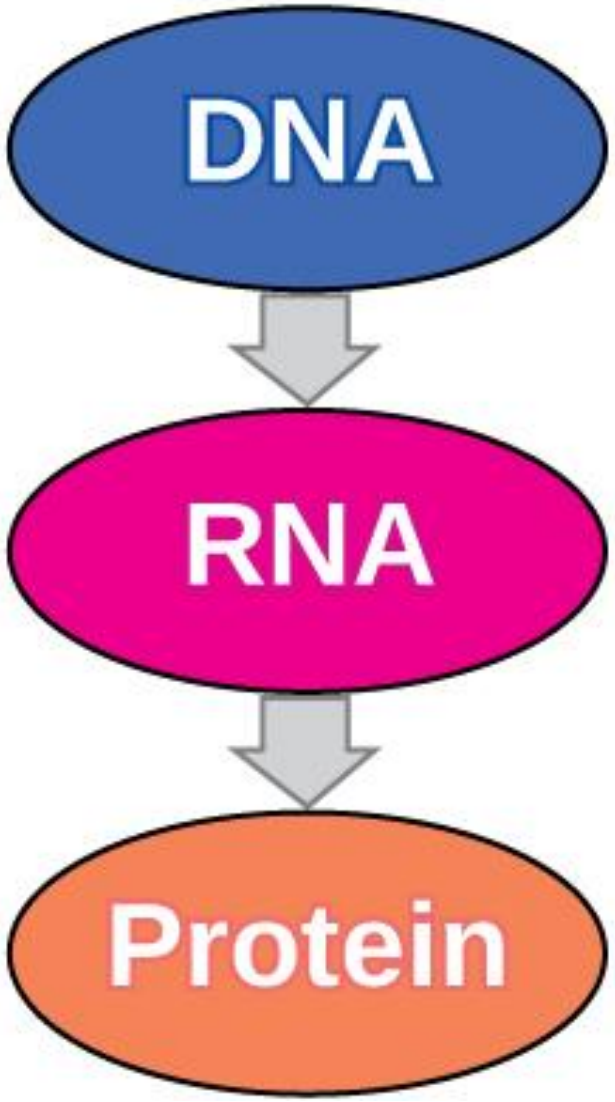
FIGURE 9.14

The central dogma states that DNA encodes RNA, which in turn encodes protein.



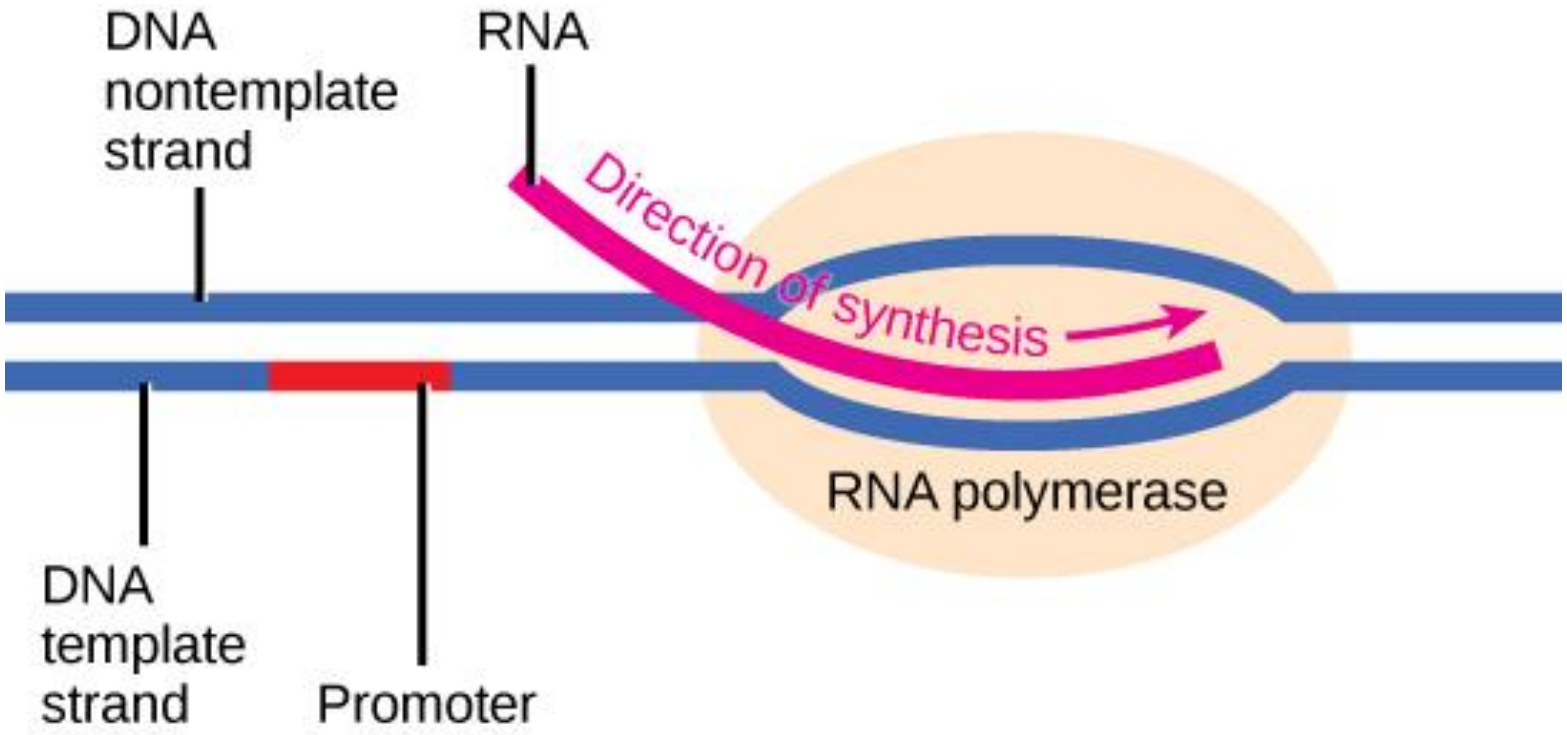
Transcription: from DNA to mRNA

- Both prokaryotes and eukaryotes perform fundamentally the same process of transcription having three main stages:
  - ✓ initiation
  - ✓ elongation
  - ✓ termination
- In eukaryotes, with the genes bound in the nucleus, transcription occurs in the nucleus of the cell and the mRNA transcript must be transported to the cytoplasm
- The prokaryotes, which include bacteria and archaea, lack membrane-bound nuclei and other organelles, and transcription occurs in the cytoplasm of the cell

Initiation

- Transcription requires the DNA double helix to partially unwind in the region of mRNA synthesis, this region is called a **transcription bubble**
- **Promoter** – the DNA sequences to which the involved proteins and enzymes bind to initiate the process of transcription
- Promoters are very important because they determine whether the corresponding gene is transcribed all of the time, some of the time, or hardly at all (Figure 9.15)

FIGURE 9.15



The initiation of transcription begins when DNA is unwound, forming a transcription bubble. Enzymes and other proteins involved in transcription bind at the promoter.

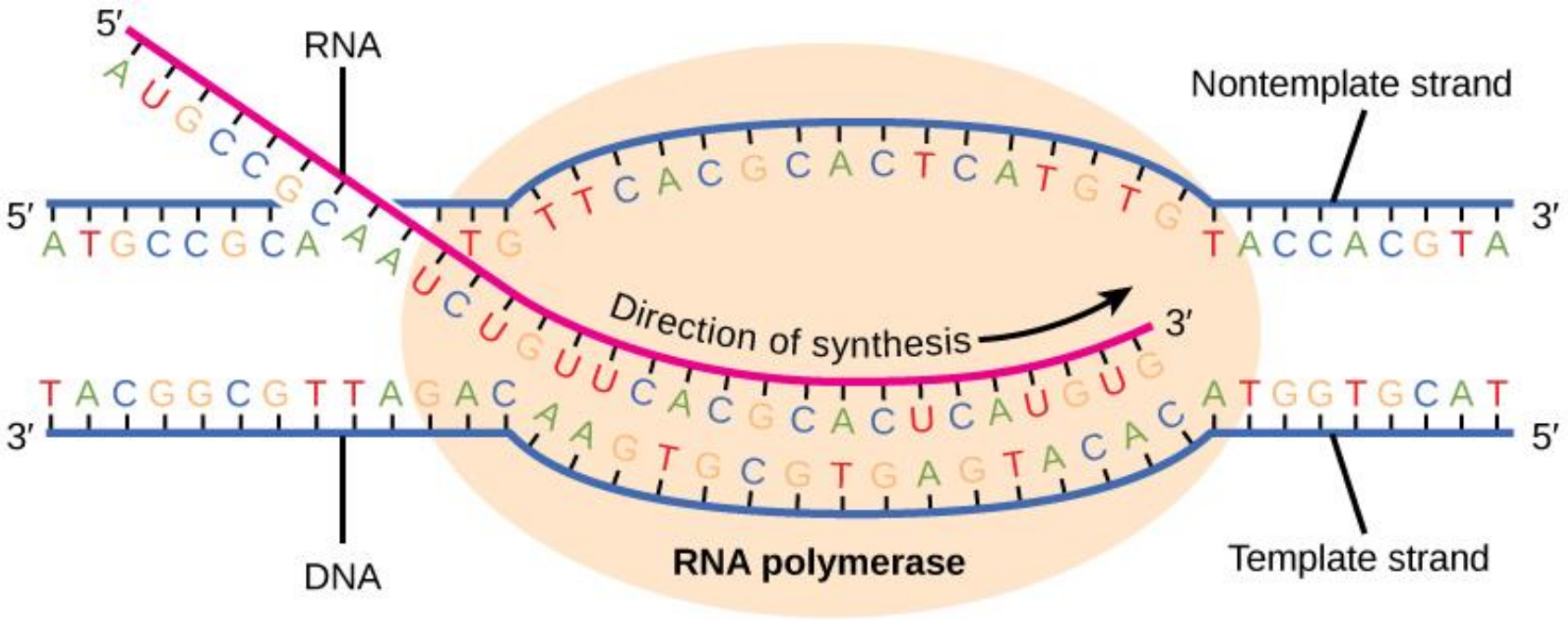
Elongation (1 of 2)

- Transcription always proceeds from one of the two DNA strands, which is called the **template strand**
- The mRNA product is complementary to the template strand and is almost identical to the other DNA strand, called the **nontemplate strand**, with the exception that RNA contains a uracil (U) in place of the thymine (T) found in DNA

Elongation (2 of 2)

- During elongation, an enzyme called **RNA polymerase** proceeds along the DNA template adding nucleotides by base pairing
- As elongation proceeds, the DNA is continuously unwound ahead of the core enzyme and rewound behind it (Figure 9.16)

FIGURE 9.16



During elongation, RNA polymerase tracks along the DNA template, synthesizes mRNA in the 5' to 3' direction, and unwinds then rewinds the DNA as it is read.

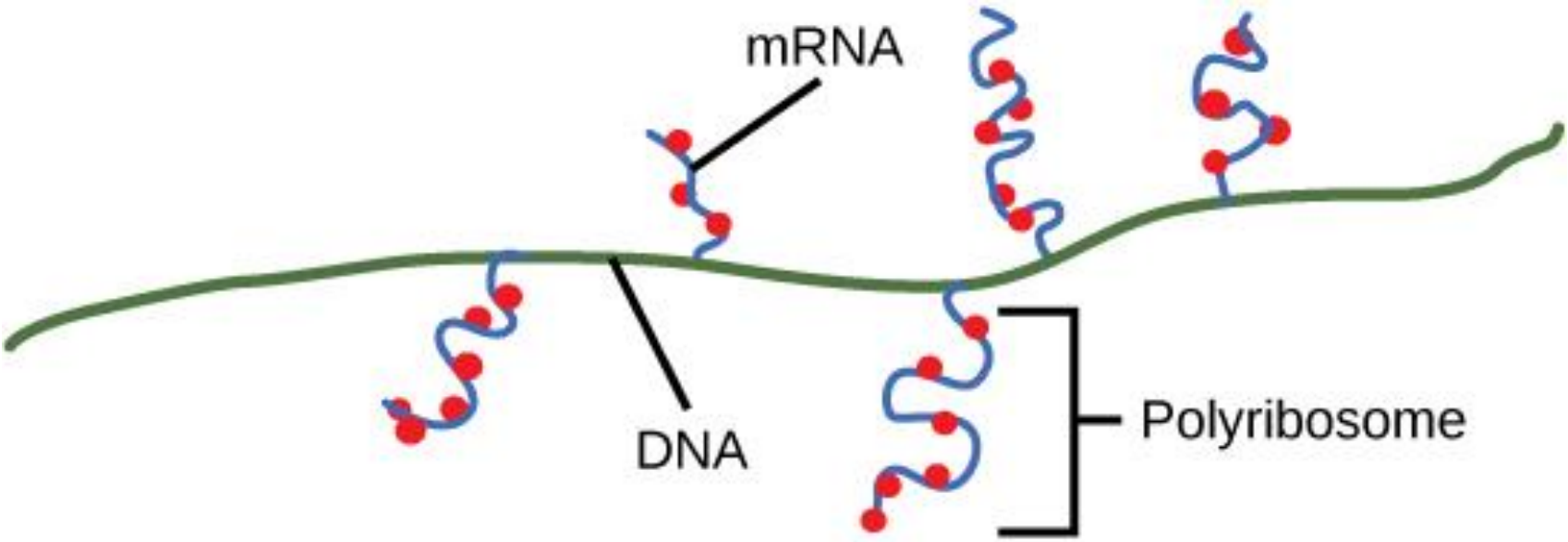
Termination (1 of 2)

- There are two kinds of termination signals, but both involve repeated nucleotide sequences in the DNA template that result in RNA polymerase stalling, leaving the DNA template, and freeing the mRNA transcript
- On termination, the process of transcription is complete

Termination (2 of 2)

- In a prokaryotic cell, by the time termination occurs, the transcript would already have been used to partially synthesize numerous copies of the encoded protein because these processes can occur concurrently using multiple ribosomes (polyribosomes) (Figure 9.17)
- In contrast, the presence of a nucleus in eukaryotic cells precludes simultaneous transcription and translation

FIGURE 9.17



Multiple polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

Eukaryotic RNA Processing (1 of 5)

- The newly transcribed eukaryotic mRNAs must undergo several processing steps before they can be transferred from the nucleus to the cytoplasm and translated into a protein
- The additional steps create a molecule that is much more stable than a prokaryotic mRNA
- For example, eukaryotic mRNAs last for several hours, whereas the typical prokaryotic mRNA lasts no more than five seconds.

Eukaryotic RNA Processing (2 of 5)

- The mRNA transcript is first coated in RNA-stabilizing proteins to prevent it from degrading while it is processed and exported out of the nucleus
- This occurs while the pre-mRNA still is being synthesized by adding a special nucleotide “cap” to the 5' end of the growing transcript
- In addition to preventing degradation, factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes

Eukaryotic RNA Processing (3 of 5)

- Once elongation is complete, an enzyme then adds a string of approximately 200 adenine residues to the 3' end, called the **poly-A tail**
- This modification further protects the pre-mRNA from degradation and signals to cellular factors that the transcript needs to be exported to the cytoplasm

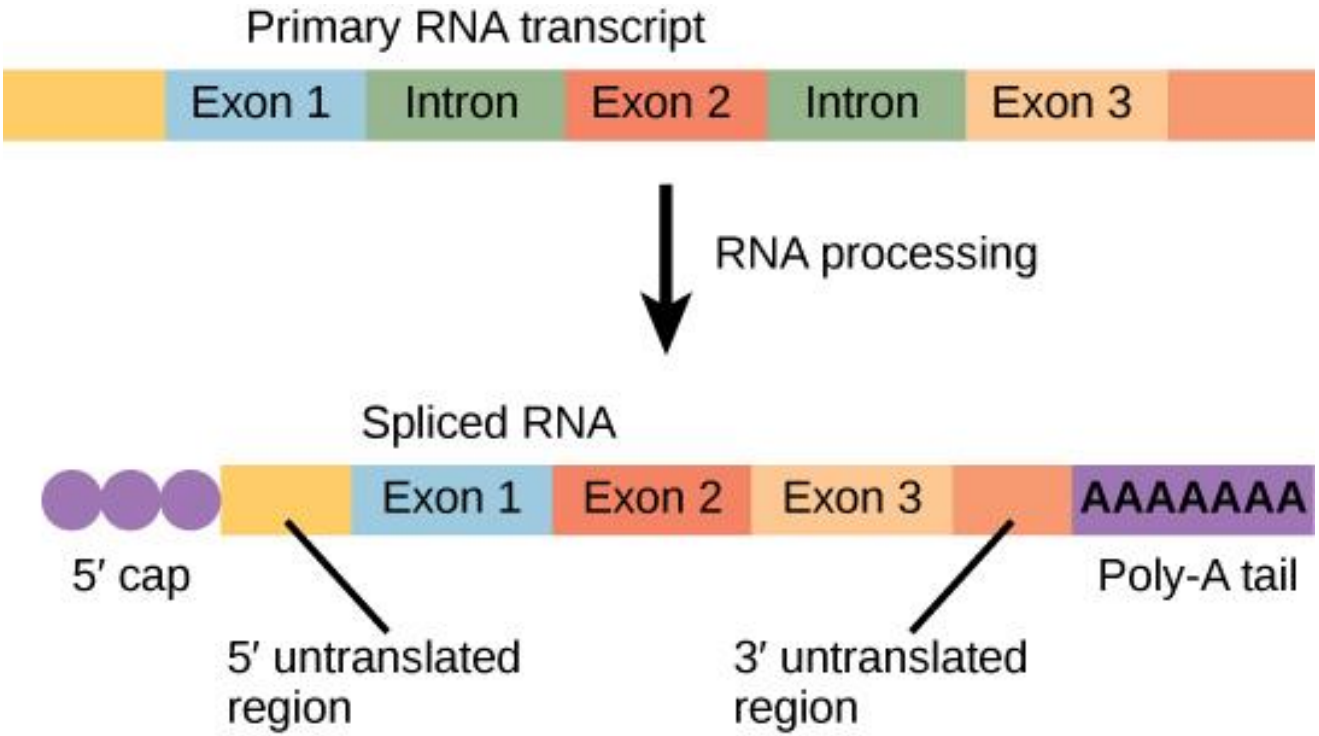
Eukaryotic RNA Processing (4 of 5)

- Eukaryotic genes are composed of protein-coding sequences called **exons** (*ex*-on signifies that they are *ex*pressed) and *int*ervening sequences called **introns** (*int*-ron denotes their *int*ervening role)
- Introns are removed from the pre-mRNA during processing
- Intron sequences in mRNA do not encode functional proteins

Eukaryotic RNA Processing (5 of 5)

- It is essential that all of a pre-mRNA's introns be completely and precisely removed before protein synthesis so that the exons join together to code for the correct amino acids
- If the process errs by even a single nucleotide, the sequence of the rejoined exons would be shifted, and the resulting protein would be nonfunctional
- The process of removing introns and reconnecting exons is called **splicing** (Figure 9.18)

FIGURE 9.18



Eukaryotic mRNA contains introns that must be spliced out. A 5' cap and 3' tail are also added.

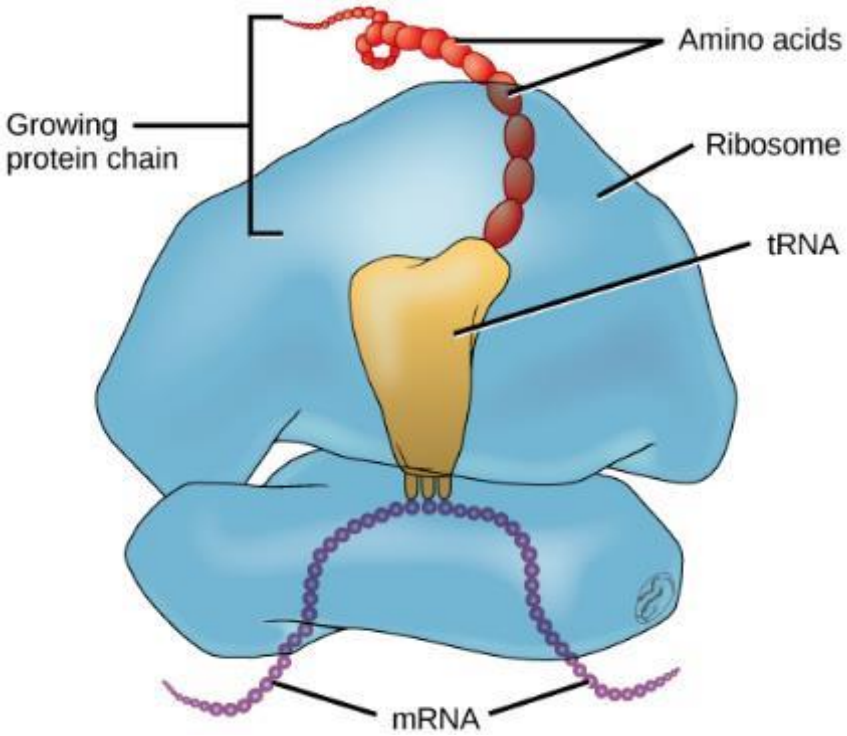
9.4 TRANSLATION

- The synthesis of proteins is one of a cell's most energy-consuming metabolic processes
- In turn, proteins account for more mass than any other component of living organisms (with the exception of water), and proteins perform a wide variety of the functions of a cell

The Protein Synthesis Machinery (1 of 3)

- The general structures and functions of the protein synthesis machinery are comparable from bacteria to human cells
- Translation requires the input of a mRNA template, ribosomes, tRNAs, and various enzymatic factors (Figure 9.19)

FIGURE 9.19



The protein synthesis machinery includes the large and small subunits of the ribosome, mRNA, and tRNA. (credit: modification of work by NIGMS, NIH)

The Protein Synthesis Machinery (2 of 3)

- **Ribosome** - a complex macromolecule composed of structural and catalytic rRNAs, and many distinct polypeptides
- Ribosomes are located in the cytoplasm in prokaryotes and in the cytoplasm and endoplasmic reticulum of eukaryotes
- Ribosomes are made up of a large and a small subunit that come together for translation

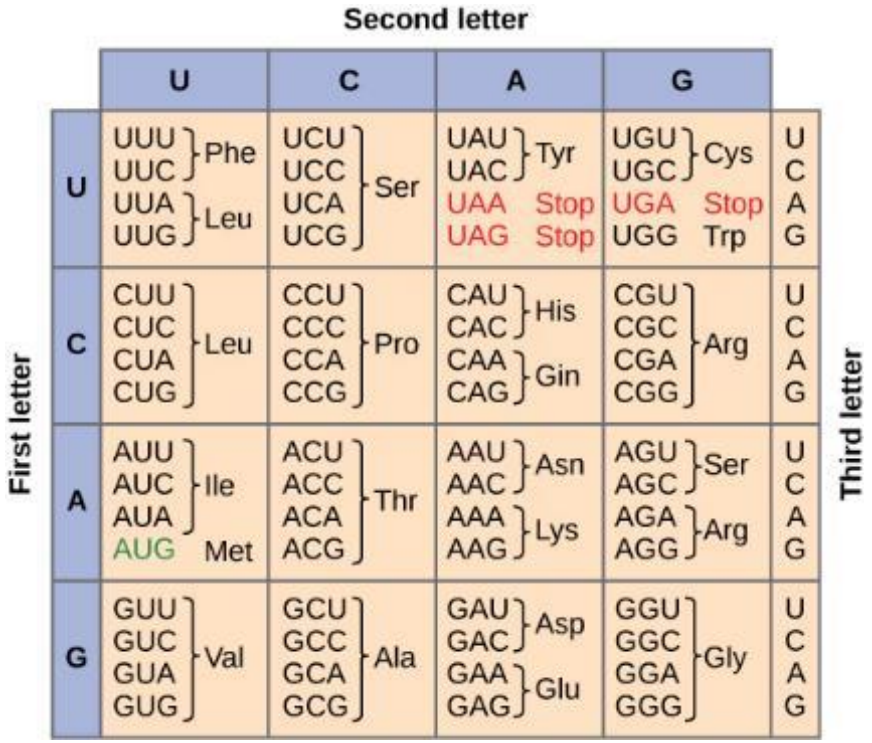
The Protein Synthesis Machinery (3 of 3)

- The small subunit is responsible for binding the mRNA template, whereas the large subunit sequentially binds **tRNAs**, a type of RNA molecule that brings amino acids to the growing chain of the polypeptide
- Each mRNA molecule is simultaneously translated by many ribosomes, all synthesizing protein in the same direction
- Serving as adaptors, specific tRNAs bind to sequences on the mRNA template and add the corresponding amino acid to the polypeptide chain

The Genetic Code (1 of 2)

- Protein sequences consist of 20 commonly occurring amino acids; therefore, it can be said that the protein alphabet consists of 20 letters
- Each amino acid is defined by a three-nucleotide sequence called the triplet **codon**
- The relationship between a nucleotide codon and its corresponding amino acid is called the **genetic code**
- There are 64 possible codons (Figure 9.20)

FIGURE 9.20



This figure shows the genetic code for translating each nucleotide triplet, or codon, in mRNA into an amino acid or a termination signal in a nascent protein. (credit: modification of work by NIH)

The Genetic Code (2 of 2)

- **Stop codons** – The three triplets of the 64 codons that terminate protein synthesis and release the polypeptide from the translation machinery
- Another codon, AUG, also has a special function… in addition to specifying the amino acid methionine, it also serves as the **start codon** to initiate translation
- The genetic code is universal; with a few exceptions, virtually all species use the same genetic code for protein synthesis, which is powerful evidence that all life on Earth shares a common origin

The Mechanism of Protein Synthesis (1 of 4)

- Just as with mRNA synthesis, protein synthesis can be divided into three phases:
  - ✓ initiation
  - ✓ elongation
  - ✓ termination
- Protein synthesis begins with the formation of an initiation complex which involves the small ribosome subunit, the mRNA template, three initiation factors, and a special initiator tRNA
- The initiator tRNA interacts with the AUG start codon, and links to a special form of the amino acid methionine that is typically removed from the polypeptide after translation is complete

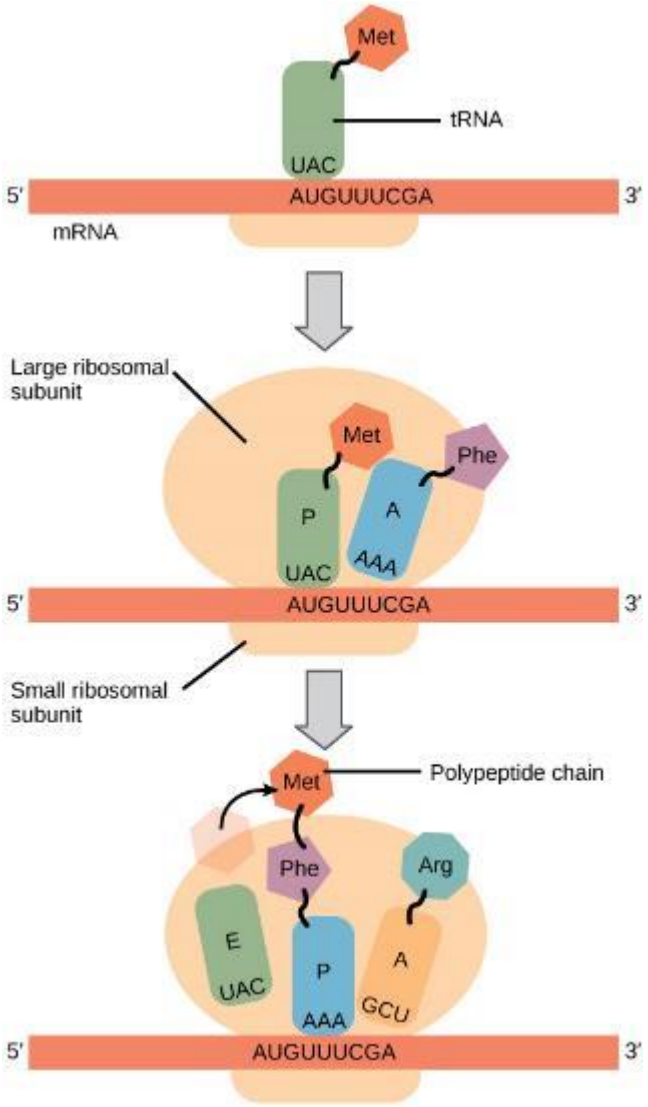
The Mechanism of Protein Synthesis (2 of 4)

- The large ribosomal subunit consists of three compartments:
  - ✓ The **A site** binds incoming charged tRNAs with their attached specific amino acids
  - ✓ The **P site** binds charged tRNAs carrying amino acids that have formed bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA
  - ✓ The **E site** releases dissociated tRNAs so they can be recharged with free amino acids

The Mechanism of Protein Synthesis (3 of 4)

- The ribosome shifts one codon at a time, catalyzing each process that occurs in the three sites
- With each step, a charged tRNA enters the complex, the polypeptide becomes one amino acid longer, and an uncharged tRNA departs
- The energy for each bond between amino acids is derived from GTP, a molecule similar to ATP (Figure 9.21)
- Amazingly, the *E. coli* translation can translate a 200-amino acid polypeptide in just 10 seconds

FIGURE 9.21



Translation begins when a tRNA anticodon recognizes a codon on the mRNA. The large ribosomal subunit joins the small subunit, and a second tRNA is recruited. As the mRNA moves relative to the ribosome, the polypeptide chain is formed. Entry of a release factor into the A site terminates translation and the components dissociate.

The Mechanism of Protein Synthesis (4 of 4)

- Termination of translation occurs when a stop codon (UAA, UAG, or UGA) is encountered
- When the ribosome encounters the stop codon, the growing polypeptide is released and the ribosome subunits dissociate and leave the mRNA
- After many ribosomes have completed translation, the mRNA is degraded so the nucleotides can be reused in another transcription reaction

9.5 HOW GENES ARE REGULATED (1 of 3)

- For a cell to function properly, necessary proteins must be synthesized at the proper time
- All organisms and cells control or regulate the transcription and translation of their DNA into protein
- The process of turning on a gene to produce RNA and protein is called **gene expression**

How Genes Are Regulated (2 of 3)

- Cells in multicellular organisms are specialized; for example, a muscle cell is very different from a liver cell, which is very different from a skin cell
- These differences are a consequence of the expression of different sets of genes in each of these cells
- Cells will turn on or off certain genes at different times in response to changes in the environment or at different times during the development of the organism

How Genes Are Regulated (3 of 3)

- The control of gene expression is extremely complex
- Malfunctions in this process are detrimental to the cell and can lead to the development of many diseases, including cancer

Prokaryotic versus Eukaryotic Gene Expression (1 of 6)

- To understand how gene expression is regulated, we must first understand how a gene becomes a functional protein in a cell
- The process occurs in both prokaryotic and eukaryotic cells, just in slightly different fashions

Prokaryotic versus Eukaryotic Gene Expression (2 of 6)

- Because prokaryotic organisms lack a cell nucleus, the processes of transcription and translation occur almost simultaneously
- When the protein is no longer needed, transcription stops, when more protein is required, more transcription occurs
- Therefore, in prokaryotic cells, the control of gene expression is almost entirely at the transcriptional level

Prokaryotic versus Eukaryotic Gene Expression (3 of 6)

- The first example of such control was discovered using *E. coli* in the 1950s
- Lactose is a food source for *E. coli*
- When lactose is not present in the bacterium's environment, the *lac* genes are transcribed in small amounts
- When lactose is present, the genes are transcribed and the bacterium is able to use the lactose as a food source

Prokaryotic versus Eukaryotic Gene Expression (4 of 6)

- When there is no lactose present, a protein known as a repressor binds to the operator and prevents RNA polymerase from binding to the promoter, thus very little of the protein products is made
- When lactose is present, an end product of lactose metabolism binds to the repressor protein and prevents it from binding to the operator
- This allows RNA polymerase to bind to the promoter and freely transcribe the three genes, producing lactase

Prokaryotic versus Eukaryotic Gene Expression (5 of 6)

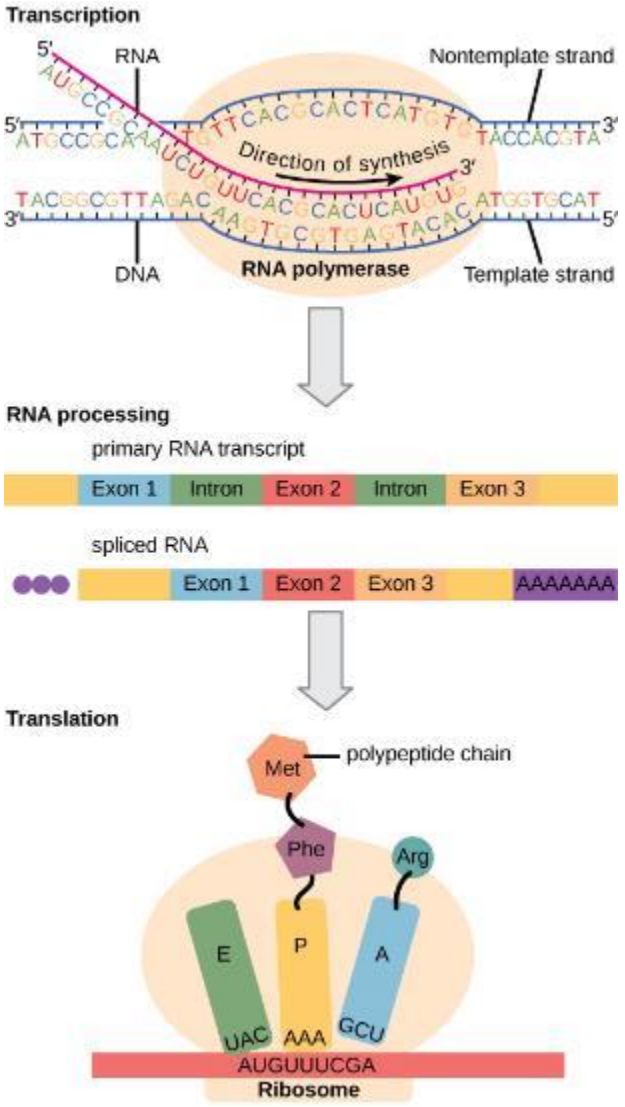
- Eukaryotic cells, in contrast, have intracellular organelles and are much more complex; recall, the DNA is contained inside the cell's nucleus and it is transcribed into mRNA there
- The newly synthesized mRNA is then transported out of the nucleus into the cytoplasm, where ribosomes translate the mRNA into protein
- Transcription occurs only within the nucleus, and translation only occurs outside the nucleus in the cytoplasm

Prokaryotic versus Eukaryotic Gene Expression (6 of 6)

- The regulation of gene expression can occur at all stages of the process (Figure 9.22)
- Regulation may occur:
  - when the DNA is uncoiled and loosened from nucleosomes to bind transcription factors (**epigenetic** level)
  - when the RNA is transcribed (transcriptional level)
  - when RNA is processed and exported to the cytoplasm after it is transcribed (**post-transcriptional** level)
  - when the RNA is translated into protein (translational level)
  - after the protein has been made (**post-translational** level)

FIGURE 9.22

Eukaryotic gene expression is regulated during transcription and RNA processing, which take place in the nucleus, as well as during protein translation, which takes place in the cytoplasm. Further regulation may occur through post-translational modifications of proteins.



Prokaryotic versus Eukaryotic Gene Expression

Differences in the Regulation of Gene Expression of Prokaryotic and Eukaryotic Organisms

Prokaryotic organisms	Eukaryotic organisms
RNA transcription and protein translation occur almost simultaneously	RNA transcription occurs prior to protein translation, and it takes place in the nucleus. RNA translation to protein occurs in the cytoplasm. RNA post-processing includes addition of a 5' cap, poly-A tail, and excision of introns and splicing of exons.
Gene expression is regulated primarily at the transcriptional level	Gene expression is regulated at many levels (epigenetic, transcriptional, post-transcriptional, translational, and post-translational)

Table 9.2