MOLECULAR BIOLOGY

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LECTURE 1

Each species of living organism has a unique set of inherited characteristics that makes it different from other species. Each species has its own developmental plan—often described as a sort of "blueprint" for building the organism— which is encoded in the DNA molecules present in its cells.

This developmental plan determines the characteristics that are inherited. Because organisms in the same species share the same developmental plan, organisms that are members of the same species usually resemble one another, although some notable exceptions usually are differences between males and females. For example, it is easy to distinguish a human being from a chimpanzee or a gorilla.

Genetics is the study of biologically inherited traits, including traits that are influenced in part by the environment. The fundamental concept of genetics is that: *Inherited traits are determined by the elements of heredity that are transmitted from parents to offspring in reproduction; these elements of heredity are called genes*.

The Genetic Material

Experimental Proof of the Genetic Function of DNA

An important first step was taken by **Frederick Griffith in 1928** when he demonstrated that a physical trait can be passed from one cell to another. He was working with two strains of the bacterium *Streptococcus pneumoniae* identified as **S** and **R**. When a bacterial cell is grown on solid medium, it undergoes repeated cell divisions to form a visible clump of cells called a colony. The S type of S. pneumoniae synthesizes a gelatinous capsule composed of complex carbohydrate (polysaccharide). The enveloping capsule makes each colony large and gives it a glistening or smooth (S) appearance; **this capsule also enables the bacterium to cause pneumonia** by protecting it from the defense mechanisms of an infected animal.

The **R** strains of S. pneumonia are unable to synthesize the capsular polysaccharide; they form small colonies that have a rough (R) surface; this strain of the bacterium does not cause pneumonia, because without the capsule the bacteria are inactivated by the immune system of the host.

Both types of bacteria that formed by cell division have the capsular type of the parent, either S or R.

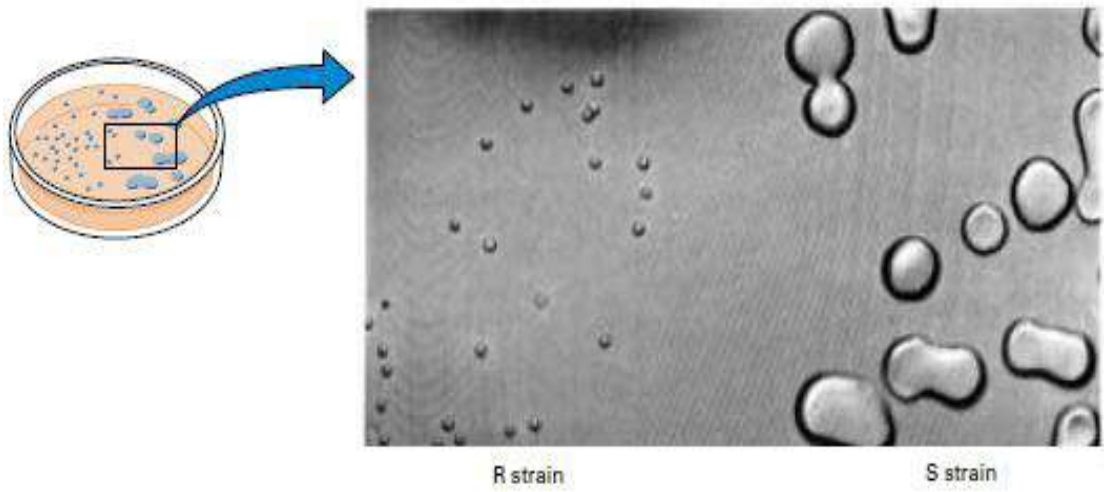


Figure Colonies of rough (R, the small colonies) and smooth (S, the large colonies) strains of Streptococcus pneumoniae. The S colonies are larger because of the gelatinous capsule on the S cells.

Mice injected with **living S cells** get pneumonia. Mice injected either with **living R cells** or with **heat-killed S cells** remain healthy. Here is Griffith's critical finding*:* mice injected with a mixture of **living R cells and heat-killed S** cells contract the disease they often die of pneumonia.

Bacteria isolated from blood samples of these dead mice produce S cultures with a capsule typical of the injected S cells, even though the injected S cells had been killed by heat. Evidently, the injected materials from the dead S cells includes a substance that can be transferred to living R cells and confer the ability to resist the immunological system of the mouse and cause pneumonia.

In other words, the R bacteria can be changed or undergo transformation into S bacteria. Furthermore, the new characteristics are inherited by descendants of the transformed bacteria.

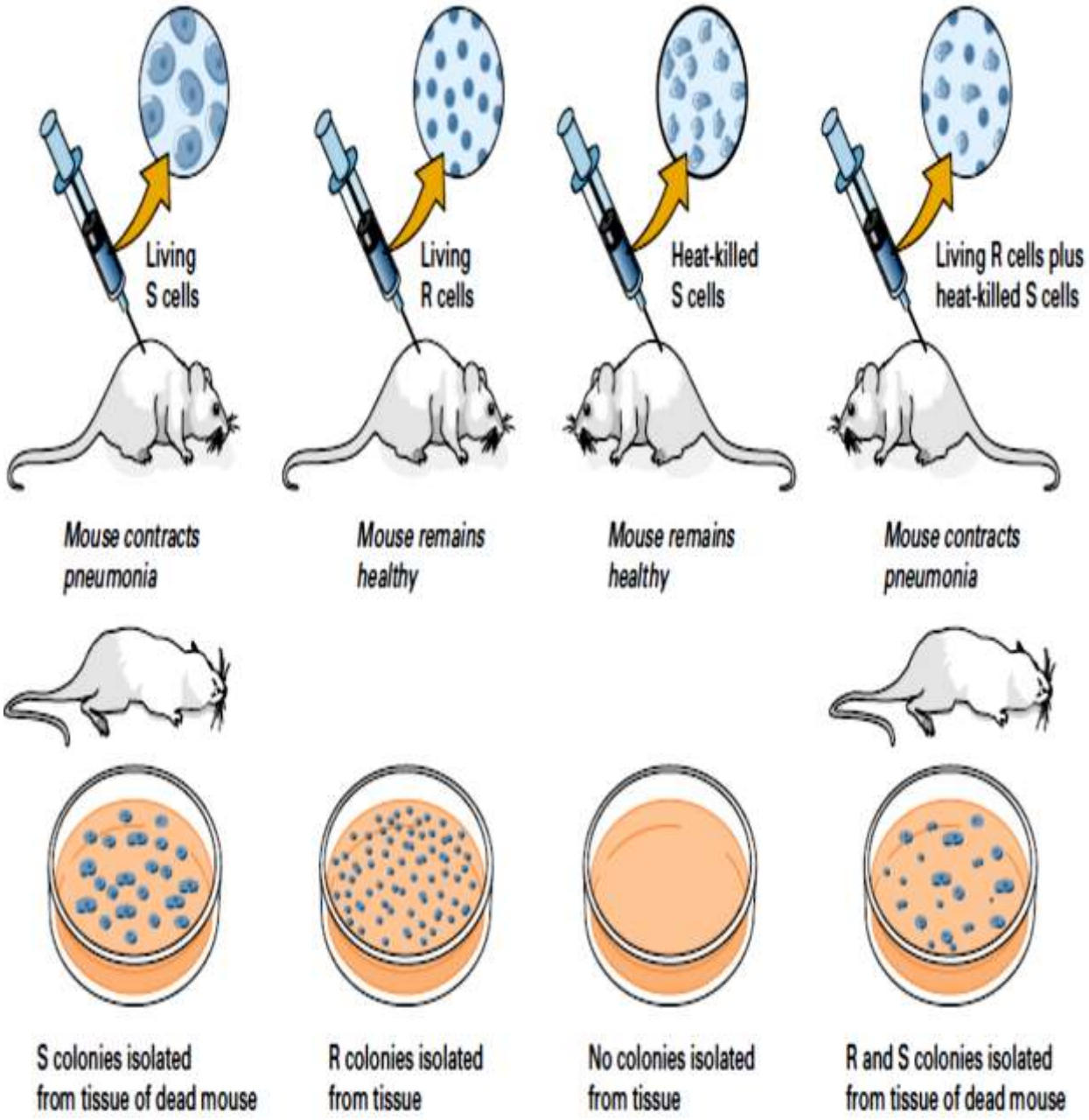


Figure 1.2 The Griffith's experiment demonstrating bacterial transformation. A mouse remains healthy if injected with either the nonvirulent R strain of *S. pneumoniae* or heat-killed cell fragments of the usually virulent S strain. R cells in the presence of heat-killed S cells are transformed into the virulent S strain, causing pneumonia in the mouse.

In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty showed that the substance causing the transformation of R cells into S cells was DNA.

They performed their experiments by:

1- Isolating almost pure DNA from S cells, which had never been done before.

2- Added DNA isolated from S cells to growing cultures of R cells.

3- They observed transformation: A few cells of type S cells were produced.

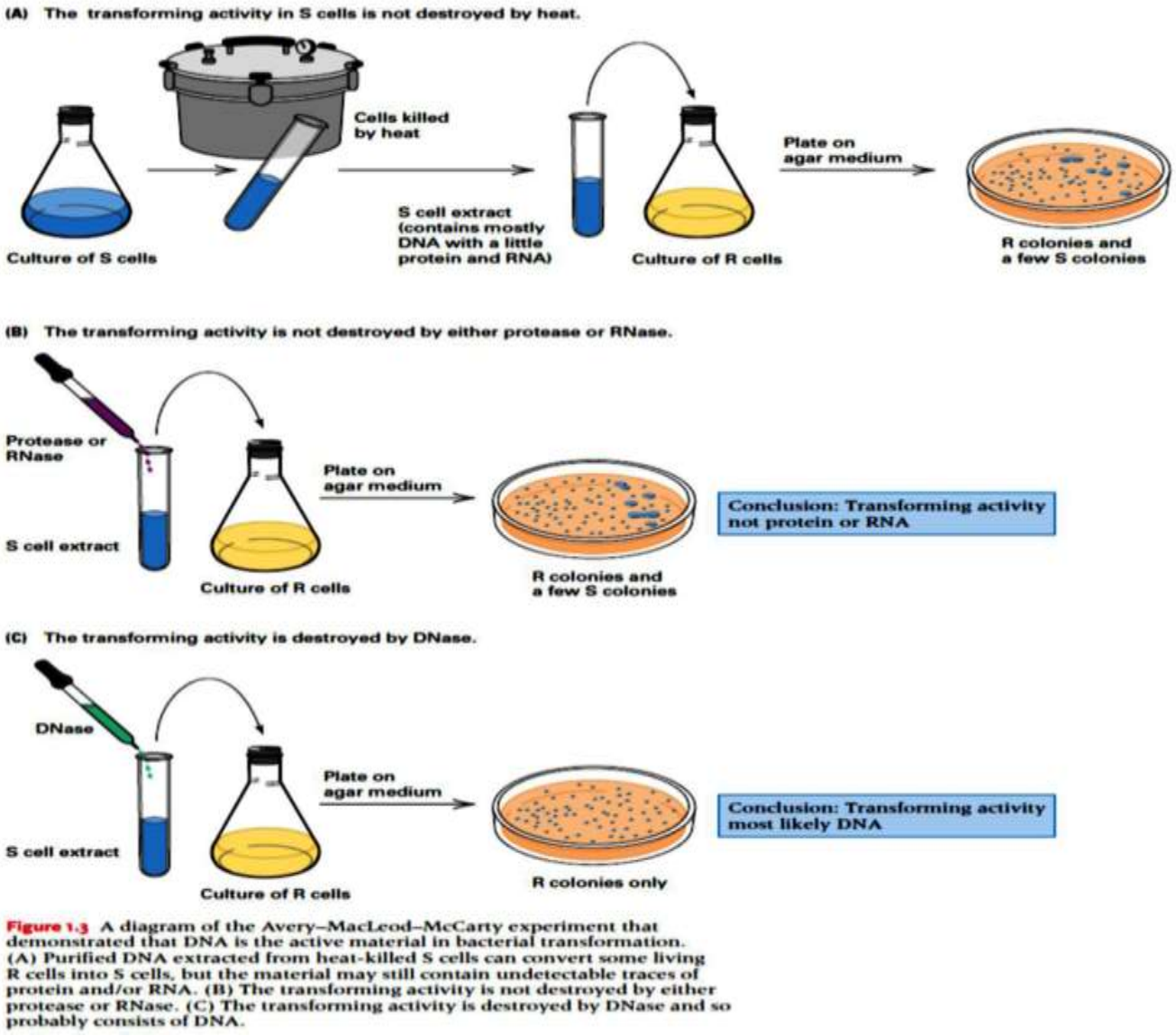


Figure 1.3 A diagram of the Avery–MacLeod–McCarty experiment that demonstrated that DNA is the active material in bacterial transformation. (A) Purified DNA extracted from heat-killed S cells can convert some living R cells into S cells, but the material may still contain undetectable traces of protein and/or RNA. (B) The transforming activity is not destroyed by either protease or RNase. (C) The transforming activity is destroyed by DNase and so probably consists of DNA.

They noted that:

Although the DNA preparations contained traces of **protein** and **RNA** (ribonucleic acid, an abundant cellular macromolecule chemically related to DNA), the transforming activity was **not altered** by treatments with heat, protease and RNAse which destroy either protein or RNA. However, treatments with DNAse that destroy DNA eliminate the transforming activity.

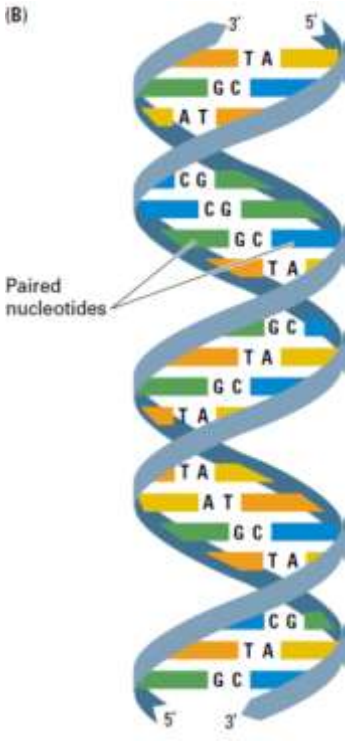
In the early 1950s, a second pivotal finding was reported by **Alfred Hershey** and **Martha Chase**. They studied cells of the intestinal bacterium *Escherichia coli* after infection by the virus T2. A virus that attacks bacterial cells is called a bacteriophage, a term often shortened to phage. Bacteriophage means "bacteria-eater".

Because DNA contains phosphorus but not sulphur, P^{32} effectively labels DNA, and because proteins contain sulphur but not phosphorus, S^{35} labels protein. If E. coli cells are grown in the presence of P^{32} or S^{35} and then infected with T2 virus, the newly synthesized phages will have either a radioactively labeled DNA core or a radioactively labeled protein coat, respectively. These labeled phages can be isolated from the medium of infected cultures and used to infect other unlabeled bacteria. They found that ~80% of the S^{35} label was removed from the bacteria whereas only ~20°/o of the P^{32} label was removed. They concluded that most of the phage DNA enters the cell, and a residue containing at least 80% of the sulphur-containing protein of the phage remains at the cell surface.

This work, together with that of Avery, MacLeod and McCarty, provided overwhelming evidence that DNA was the molecule responsible for heredity.

Structure of DNA: The Double Helix

In 1953, James Watson and **Francis Crick** at Cambridge University proposed the first essentially correct three-dimensional structure of the DNA molecule. In the Watson–Crick structure, DNA consists of two long chains of subunits, each twisted around the other to form a **double stranded helix**. The double helix is right handed, which means that as one looks along the barrel, each chain follows a clockwise path as it progresses.



The subunits of each strand called **nucleotides.** Each nucleotide has three parts: **a nitrogenous base**, **five-carbon sugar** and **a phosphate group**. The combination of base and sugar is termed a **nucleoside**, while the base-sugar-phosphate is called a **nucleotide**. The four nitrogenous bases in DNA are: **• Adenine (A) • Guanine (G) • Thymine (T) • Cytosine (C)**

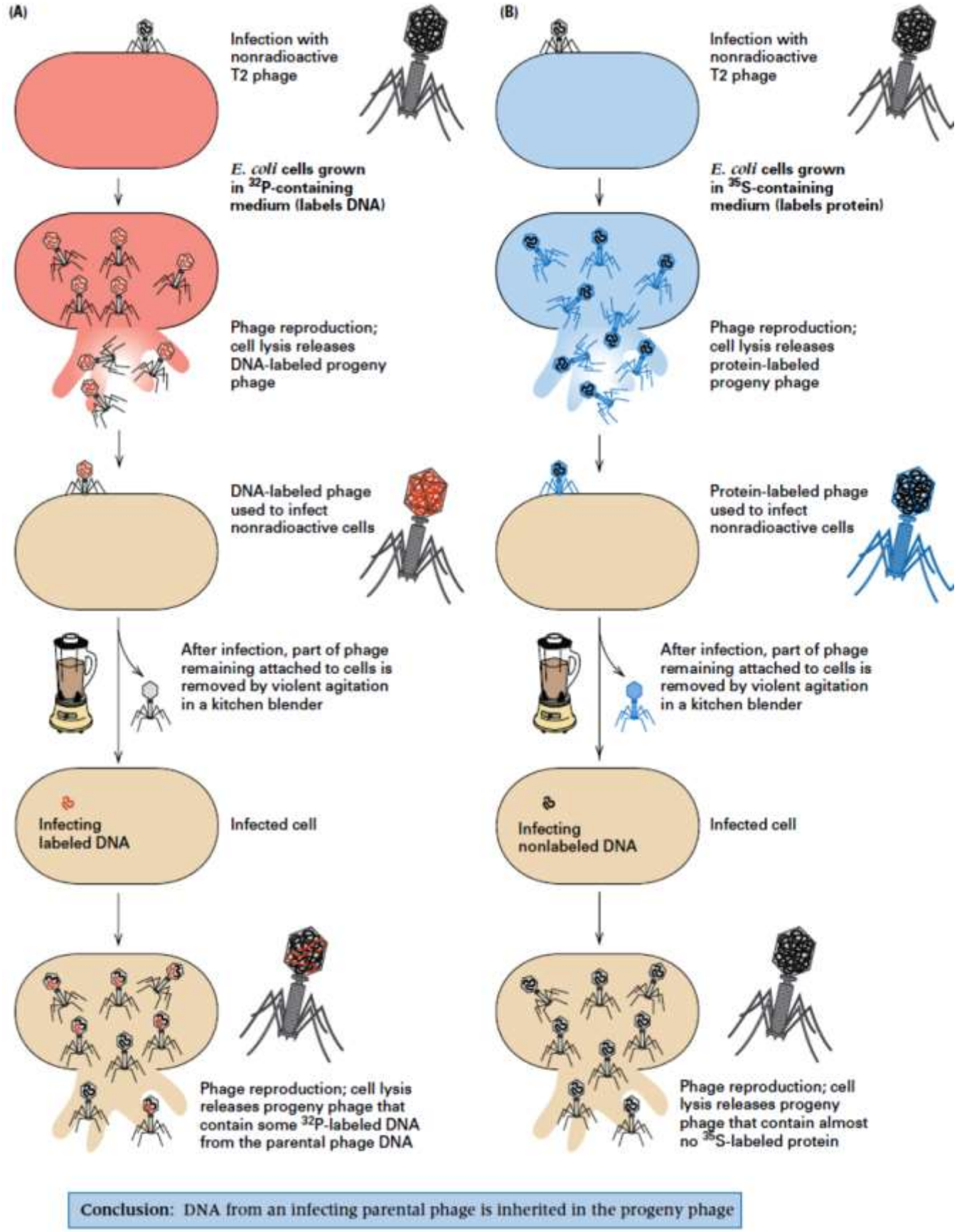


Figure The Hershey–Chase ("blender") experiment demonstrating that DNA, not protein, is responsible for directing the reproduction of phage T2 in infected E. coli cells. (A) Radioactive DNA is transmitted to progeny phage in substantial amounts. (B) Radioactive protein is transmitted to progeny phage in negligible amounts.

The bases in the double helix are paired **complementary**. At any position on the paired strands of a DNA molecule, the complement of **A is T**, and the complement of **G is C.** The complementary pairing means that each base along one strand of the DNA is matched with a base in the opposite position on the other strand. The complementary pairing is also called Watson–Crick pairing.

A = T **G ≡ C**

Each DNA strand has a **polarity or directionality**. The polarity is determined by the direction in which the nucleotides are pointing. The "trunk" end of the strand is called the **3' end of the strand**, and the "tail" end is called the **5' end of the strand**. In double-stranded DNA, the paired strands are oriented in **opposite directions**, the 5' end of one strand aligned with the 3' end of the other.

Polynucleotide Chains

In terms of biochemistry, a DNA strand is a polymer—a large molecule built from repeating units. The units in DNA are composed of:

2'-deoxyribose (a five-carbon sugar), phosphoric acid, and the four nitrogen bases A, T, G, and C.

The chemical structures of the bases are note that two of the bases have a double-ring structure; these are called **purines**. The other two bases have a single-ring structure; these are called **pyrimidines.**

- The purine bases are adenine (**A**) and guanine (**G**).
- The pyrimidine bases are thymine (**T**) and cytosine (**C**).

DNA and RNA are both built up from two purine containing nucleotides and two pyrimidine containing nucleotides. The purines of both DNA and RNA are the same - Adenine (**A**) and Guanine (**G**). The pyrimidine Cytosine (**C**) is also found in both nucleic acids, while the pyrimidine Thymine (**T**) is limited to DNA, being replaced by Uracil (**U**) in RNA.

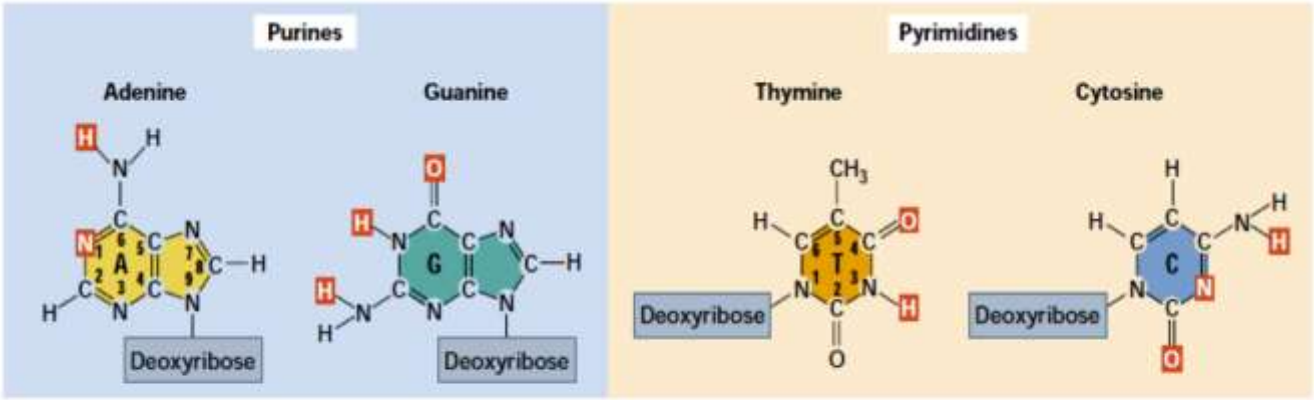


Figure 2.3 Chemical structures of the four nitrogen-containing bases in DNA: adenine, thymine, guanine, and cytosine. The nitrogen atom linked to the deoxyribose sugar is indicated. The atoms shown in red participate in hydrogen bonding between the DNA base pairs.

In DNA, each base is chemically linked to one molecule of the sugar deoxyribose, forming a compound called a nucleoside. When a phosphate group is also attached to the sugar, the nucleoside becomes a nucleotide, thus a **nucleotide is a nucleoside + phosphate**. The carbon atom of sugar to which the base is attached is the 1' carbon.

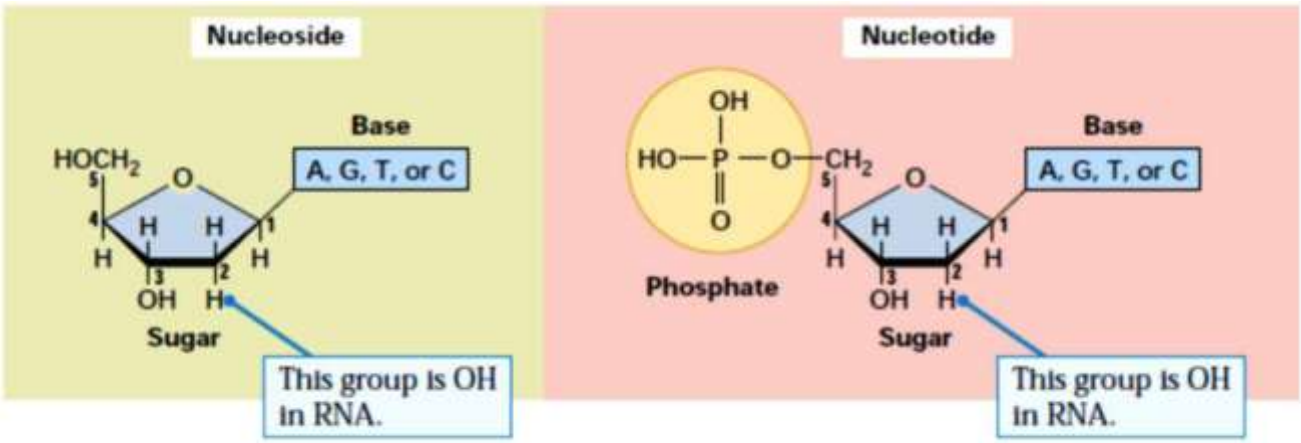


Figure 2.4 A typical nucleotide, showing the three major components (phosphate, sugar, and base), the difference between DNA and RNA, and the distinction between a nucleoside (no phosphate group) and a nucleotide (with phosphate). Nucleotides are monophosphates (with one phosphate group). Nucleoside diphosphates contain two phosphate groups, and nucleoside triphosphates contain three.

In nucleic acids, such as DNA and RNA, the nucleotides are joined to form a polynucleotide chain, in which the **phosphate attached to the 5' carbon of one sugar** is linked to the hydroxyl group attached to the 3' carbon of the next sugar in line.

The chemical bonds by which the sugar components of adjacent nucleotides are linked through the phosphate groups are called **phosphodiester bonds**. The 5'–3'–5'–3' orientation of these linkages continues throughout the chain, which typically consists of millions of nucleotides. Note that the terminal groups of each polynucleotide chain are a **5'-phosphate (5'-P) group** at one end and a **3'-hydroxyl (3'-OH) group** at the other. The asymmetry of the ends of a DNA strand implies that each strand has a polarity determined by which end bears the 5' phosphate and which end bears the 3' hydroxyl.

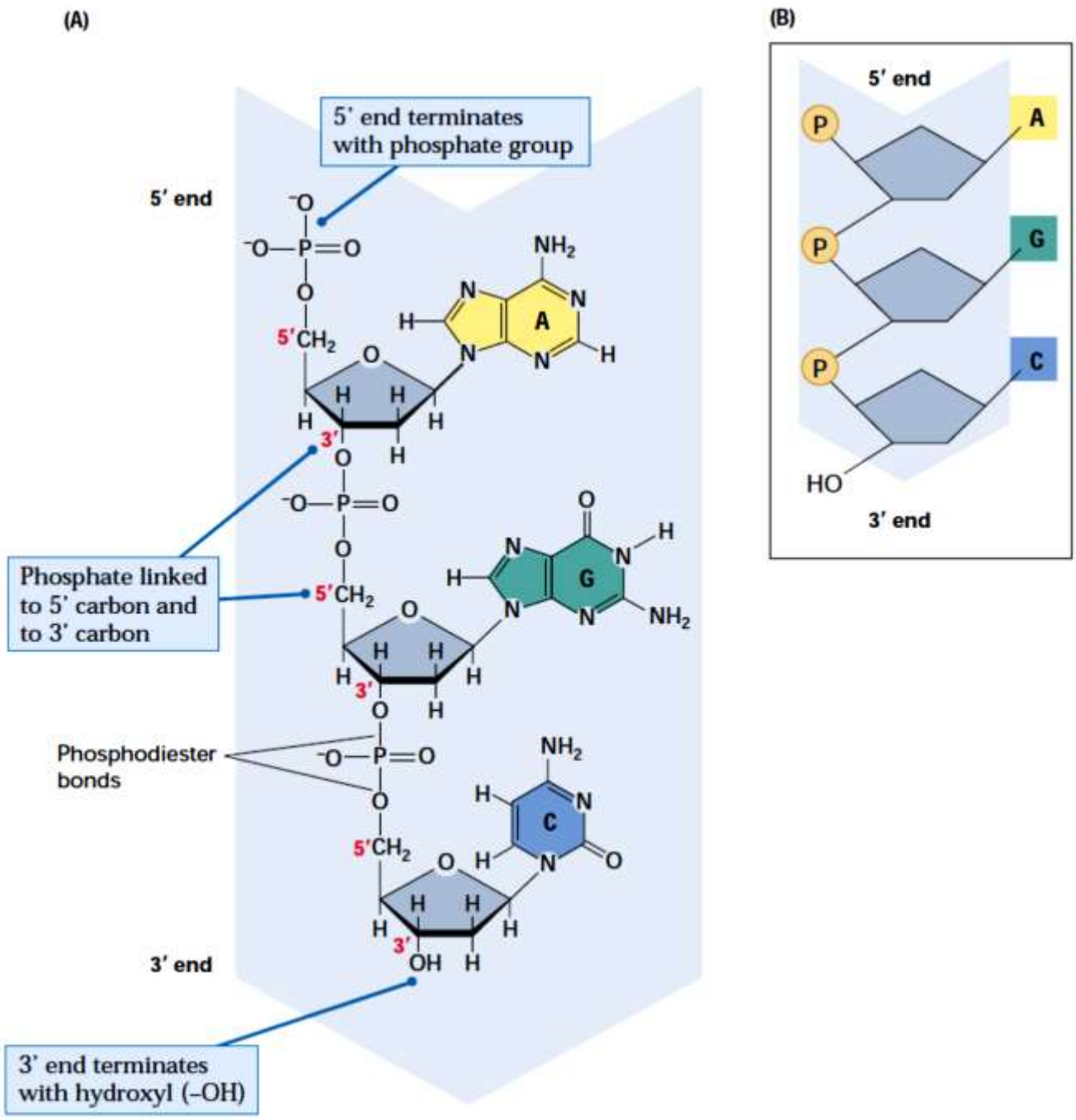


Figure 2.5 Three nucleotides at the 5' end of a single polynucleotide strand. (A) The chemical structure of the sugar–phosphate linkages, showing the 5'-to-3' orientation of the strand (the red numbers are those assigned to the carbon atoms). (B) A common schematic way to depict a polynucleotide strand.

The nomenclature of the nucleoside and nucleotide derivatives of the DNA

The nomenclature of the nucleoside and nucleotide derivatives of the DNA bases is summarized in following table.

Table 2.1 DNA nomenclature

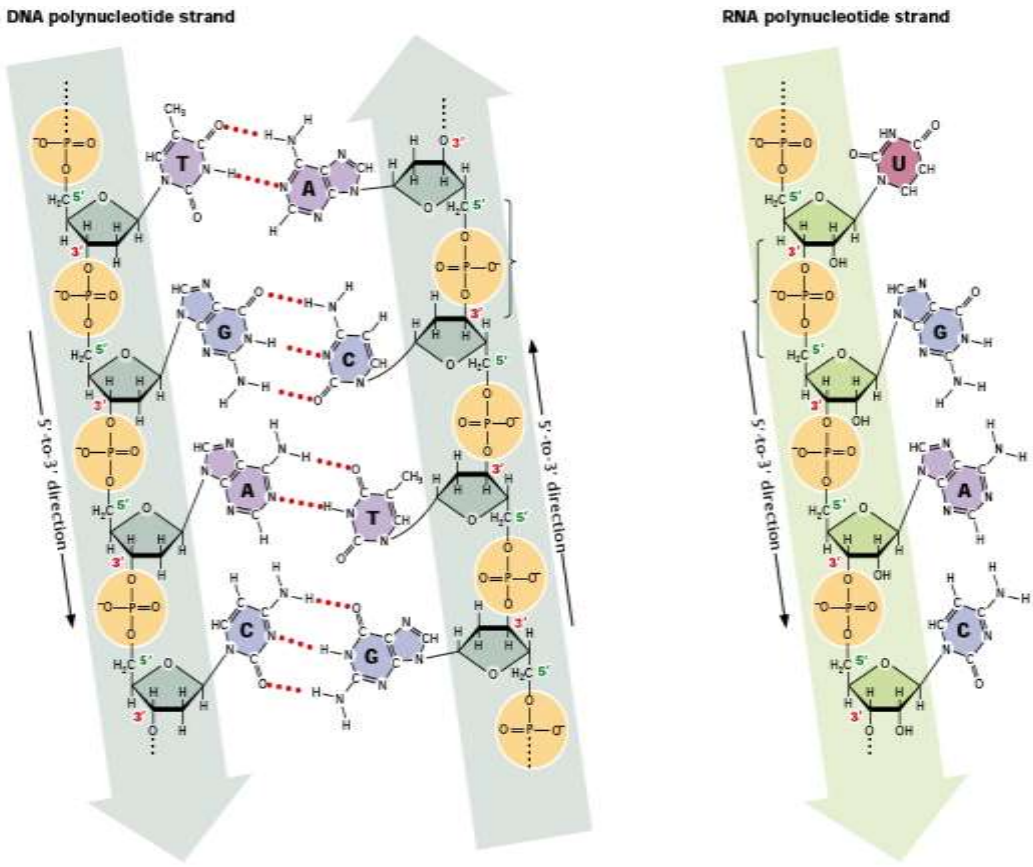
Base	Nucleoside	Nucleotide
Adenine (A)	Deoxyadenosine	Deoxyadenosine-5′ monophosphate (dAMP) diphosphate (dADP) triphosphate (dATP)
Guanine (G)	Deoxyguanosine	Deoxyguanosine-5′ monophosphate (dGMP) diphosphate (dGDP) triphosphate (dGTP)
Thymine (T)	Deoxythymidine	Deoxythymidine-5′ monophosphate (dTMP) diphosphate (dTDP) triphosphate (dTTP)
Cytosine (C)	Deoxycytidine	Deoxycytidine-5′ monophosphate (dCMP) diphosphate (dCDP) triphosphate (dCTP)

Secondary Structures of DNA

The double helix, a fundamental characteristic of DNA's secondary structure is that it consists of two polynucleotide strands wound around each other—it's a double helix. The sugar–phosphate linkages are on the outside of the helix, and the bases are stacked in the interior of the molecule (see Figure below). The two polynucleotide strands run in opposite directions—they are **antiparallel**, which means that the 5' end of one strand is opposite the 3' end of the second.

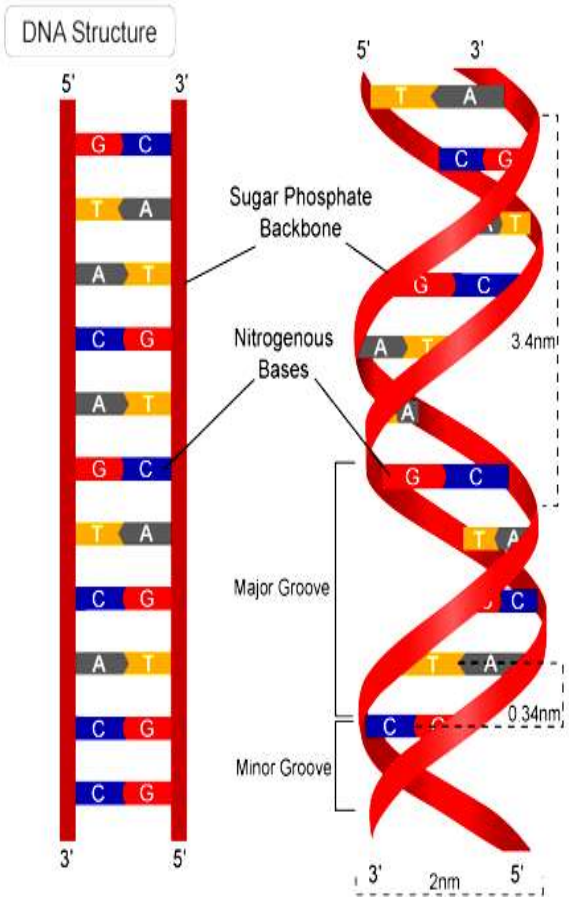
The strands are held together by two types of molecular forces. **Hydrogen bonds** link the bases on opposite strands. These bonds are relatively weak compared with the covalent **phosphodiester bonds** that connect the sugar and phosphate groups of adjoining nucleotides.

As we will see, several important functions of DNA require the separation of its two nucleotide strands, and this separation can be readily accomplished because of the relative ease of breaking and reestablishing the hydrogen bonds.



10.13 **DNA consists of two polynucleotide chains that are antiparallel and complementary, and RNA consists of a single nucleotide chain.**

Different secondary structures, as we have seen, DNA normally consists of two polynucleotide strands that are **antiparallel and complementary** (exceptions are single stranded DNA molecules in a few viruses). The precise three-dimensional shape of the molecule can vary, however, depending on the conditions in which the DNA is placed and, in some cases, on the base sequence itself.



In the standard structure (Watson and Crick), which is called the **B form of DNA**, each chain makes one complete turn every 34 Å. The helix is right-handed. The bases are spaced at 3.4 Å, so there are (10) bases per helical turn in each strand and (10) base pairs per turn of the double helix. The diameter of the helix is 2 nm; Spiraling of the nucleotide strands creates major and minor grooves in the helix, features that are important for the binding of some DNA-binding proteins that regulate the expression of genetic information.

The two grooves spiraling along outside of the double helix are not symmetrical; one groove, called the **major**

groove, is larger than the other, which is called the **minor groove**. Proteins that interact with double-stranded DNA often have regions that make contact with the base pairs by fitting into the major groove, into the minor groove, or into both grooves.

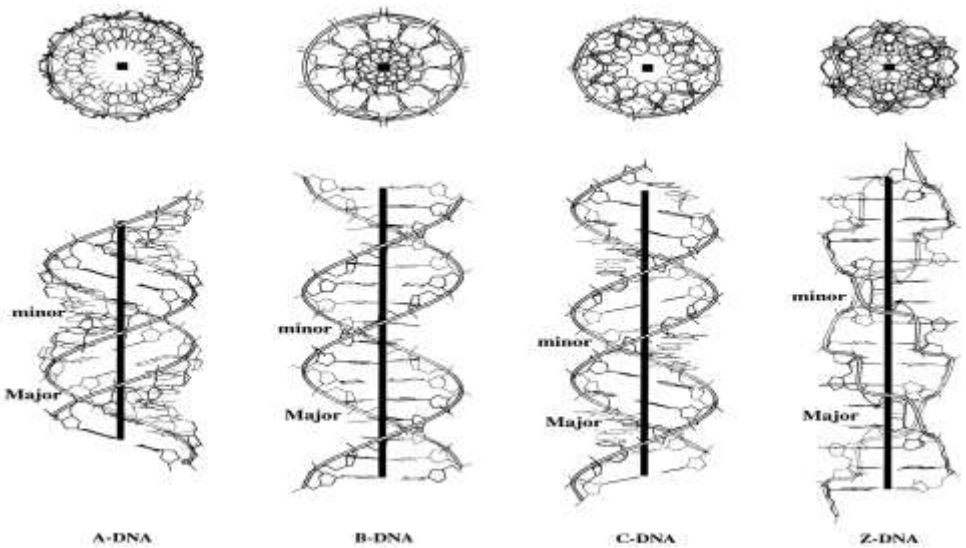
Another secondary structure that DNA can assume is the **A-DNA structure**. Like B-DNA, A-DNA is an alpha (right-handed) helix, but it is shorter and wider than B-DNA and its bases are tilted away from the main axis of the molecule.

A radically different secondary structure called **Z-DNA** forms a left-handed helix. In this form, the sugar–phosphate backbones zigzag back and forth, giving rise to the name Z-DNA (for zigzag). some regions can even form helices in which the strands twist to the left. Z-DNA structures can arise under physiological conditions when particular base sequences are present, such as stretches of alternating C and G sequences. Parts of some active genes form Z-DNA, suggesting that Z-DNA may play a role in regulating gene transcription.

Table 10.2 Characteristics of DNA secondary structures

Characteristic	A-DNA	B-DNA	Z-DNA
Conditions required to produce structure	75% H_2O	92% H_2O	Alternating purine and pyrimidine bases
Helix direction	Right-handed	Right-handed	Left-handed
Average base pairs per turn	11	10	12
Rotation per base pair	32.7°	36°–30°	
Distance between adjacent bases	0.26 nm	0.34 nm	0.37 nm
Diameter	2.3 nm	1.9 nm	1.8 nm
Overall shape	Short and wide	Long and narrow	Elongated and narrow

Note: Within each structure, the parameters may vary somewhat owing to local variation and method of analysis.



Chargaff's rules:

A few years before Watson and Crick proposed their essentially correct three dimensional structure of DNA as a double helix; Erwin Chargaff developed a chemical technique to measure the amount of each base present in DNA. Chargaff used his technique to measure the [A], [T], [G], and [C] content of the DNA from a variety of sources.

He found that **the base composition of the DNA, defined as the percent G + C, differs among species but is constant in all cells of an organism and within a species.**

Chargaff also observed certain regular relationships among the molar concentrations of the different bases. These relationships are now called Chargaff's rules:

• The amount of adenine equals that of thymine: [A] = [T].

• The amount of guanine equals that of cytosine: [G] = [C].

• The amount of the purine bases equals that of the pyrimidine bases: [A] + [G] = [T] + [C].

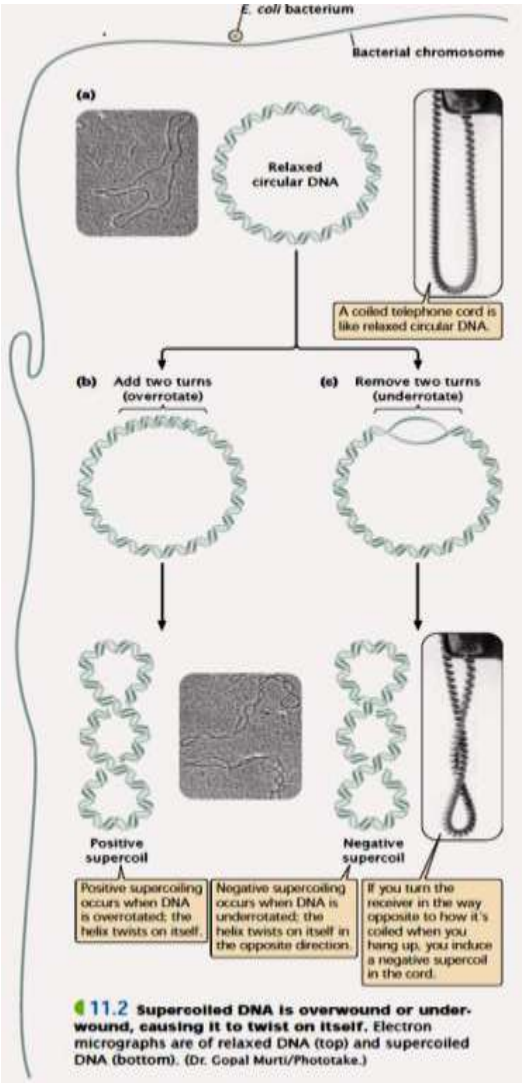
DNA Methylation

The primary structure of DNA can be modified in various ways. These modifications are important in the expression of the genetic material, as we will see in the chapters to come. One such modification is DNA methylation, in which methyl groups (–CH3) are added (by specific enzymes) to certain positions on the nucleotide bases.

- In bacteria, adenine and cytosine are commonly methylated, whereas, in eukaryotes, cytosine is the most commonly methylated base. Bacterial DNA is frequently methylated to distinguish it from foreign, unmethylated DNA that may be introduced by viruses; bacteria use proteins called restriction enzymes to cut up any unmethylated.
- In eukaryotic DNA, cytosine bases are often methylated to form 5-methylcytosine. Methylation is often related to gene expression. Sequences that are methylated typically show low levels of transcription while sequences lacking methylation are actively being transcribed. Methylation can also affect the three-dimensional structure of the DNA molecule.

Supercoiling of DNA

The packaging of tremendous amounts of genetic information into the small volume of a cell has been called the ultimate storage problem. Consider the chromosome of the bacterium E. coli, a single molecule of DNA with approximately 4.64 million base pairs. Stretched out straight, this DNA would be about 1000 times as long as the cell within which it resides Human cells contains 6 billion base pairs of DNA, which would measure some 1.8 meters stretched end to end. Even DNA in the smallest human chromosome would stretch 14,000 times the length of the nucleus. Clearly, DNA molecules must be tightly packed to fit into such small spaces.



11.2 **Supercoiled DNA is overwound or underwound, causing it to twist on itself.** Electron micrographs are of relaxed DNA (top) and supercoiled DNA (bottom). (Dr. Gopal Murti/Phototake.)

One type of DNA tertiary structure is supercoiling, **Supercoiling** is a natural consequence of the overrotating or underrotating of the helix; it occurs only when the molecule is placed under strain. Molecules that are overrotated exhibit positive supercoiling. Underrotated molecules exhibit negative supercoiling, in which the direction of the supercoil is opposite that of the right-handed coil of the DNA helix.

A twist in the direction in which the strands are already entwined about each other, the rope becomes overwound and is thrown into **a positive supercoil**. Conversely, if the rope is given a left-handed twist before sealing (that is, twisted in the direction opposite to that in which it is wound), it becomes under wound and is thrown into a **negative supercoil**. So, a relaxed DNA molecule can be converted into a negative supercoil by a left-handed twist and into a positive supercoil by a right-handed twist.

Molecules that differ only in their state of supercoiling are called topological isomers of one another. Correspondingly, **the enzymes that carry out the inter-conversion of relaxed and supercoiled forms of DNA are called topoisomerases**. Most topoisomerases are classified as **type I** or **type II**.
Both types catalyze the stepwise relaxation of supercoiled DNA.

- Type I enzymes relax one coil at a time,
- Type II enzymes relax two coils at a time.

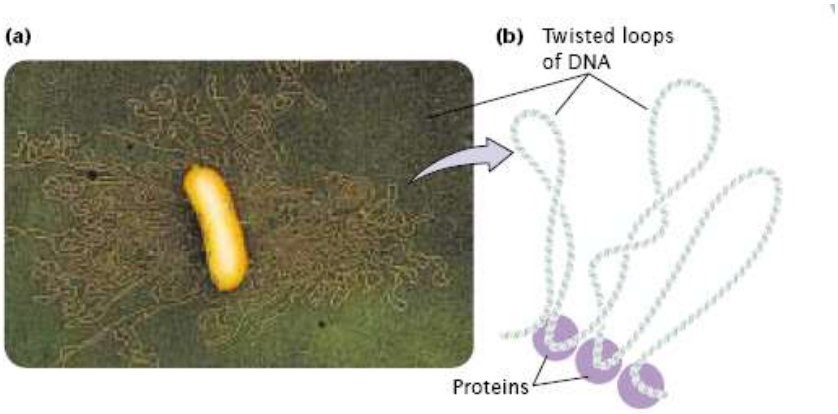
Topoisomerases function by attaching to a supercoiled duplex and producing a transient break, or nick, in either one (type I) or both (type II) of the strands. The two strands can then rotate around each

other, followed by resealing of the nick. This relaxation reaction requires no energy source, suggesting that the phosphoester bond energy that is liberated when a strand is nicked must somehow be conserved and used to form the new bond.

DNA gyrase is a type II topoisomerase that can induce relaxation of supercoiling. DNA gyrase is one of several enzymes required for DNA replication, it can relax the positive supercoiling that results from partial unwinding of the double helix, or it can actively introduce negative supercoils that then promote strand separation, allowing access of the other enzymes involved in the replication process. It is not surprising that DNA gyrase requires ATP to generate supercoiling but not to relax an already-supercoiled molecule

- **Bacterial Chromosome**

Most bacterial genomes consist of **a single, circular DNA molecule**, although **linear DNA molecules** have been found in a few species. In circular bacterial chromosomes, the DNA does not exist in an open, relaxed circle; the 3 million to 4 million base pairs of DNA found in a typical bacterial genome would be much too large to fit into a bacterial cell When a bacterial cell is viewed with the electron microscope, its DNA frequently appears as a distinct clump, the nucleoid, which is confined to a definite region of the cytoplasm. If a bacterial cell is broken open gently, its DNA spills out in a series of **twisted loops** .The ends of the loops are most likely held in place by **proteins**. Many bacteria contain additional DNA in the form of small circular molecules called **plasmids**, which replicate independently of the chromosome



11.3 **Bacterial DNA is highly folded into a series of twisted loops.**
(Part a, Dr. Gopal Murti/Photo Researchers.)

- **The Eukaryotic Chromosome**

Each eukaryotic chromosome consists of a single, extremely long molecule of DNA. For this entire DNA to fit into the nucleus, tremendous packing and folding are required, the extent of which must change through time. In the course of the cell cycle, the level of DNA packaging changes—chromosomes progress from a highly packed state to a state of extreme condensation. DNA packaging also changes locally in replication and transcription, when the two nucleotide strands must unwind so

that particular base sequences are exposed. Thus, **the packaging of eukaryotic DNA (its tertiary, chromosomal structure) is not static but changes regularly in response to cellular processes**.

Chromatin Structure

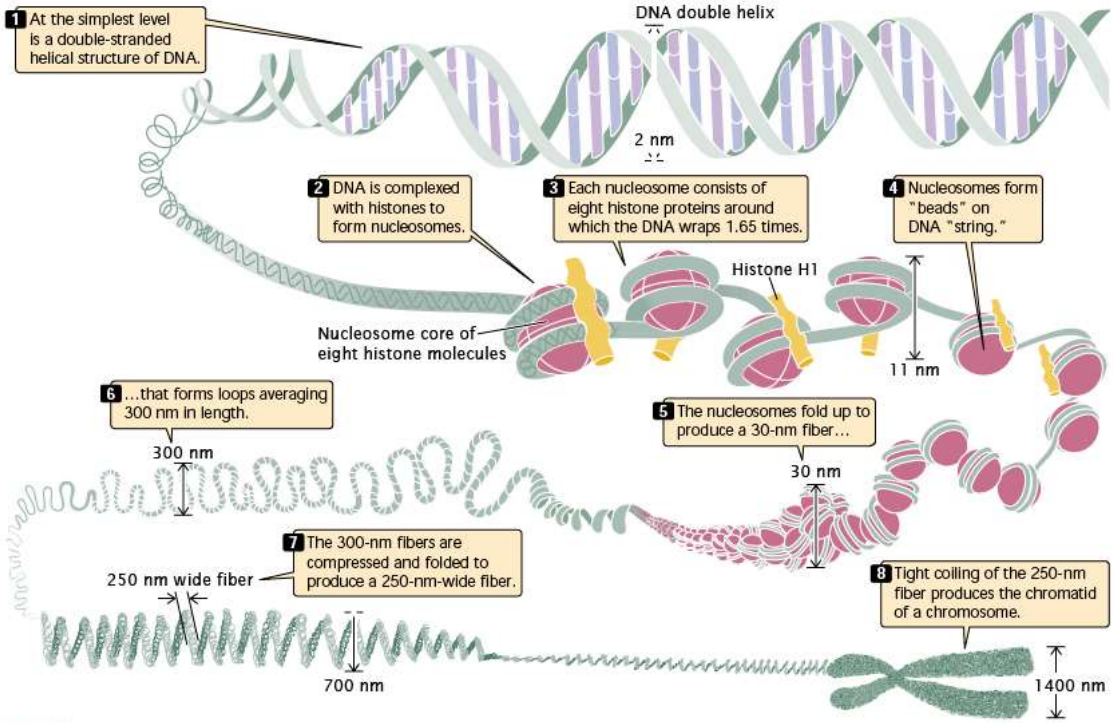
DNA is closely associated with proteins, creating chromatin. The two basic types of chromatin are: **euchromatin**, which undergoes the normal process of condensation and de-condensation in the cell cycle, and **heterochromatin**, which remains in a highly condensed state throughout the cell cycle, The most abundant proteins in chromatin are the **histones**, which are relatively **small, positively charged proteins of five major types: H1, H2A, H2B, H3, and H4. All histones have a high percentage of arginine and lysine, positively charged amino acids that give them a net positive charge**. The positive charges attract the negative charges on the phosphates of DNA and hold the DNA in contact with the histones. Properties of these classes of histones:

- Histone HI is especially rich in lysine,
- H2A and H2B are slightly lysine-rich,
- H3 and H4 are rich in arginine instead.

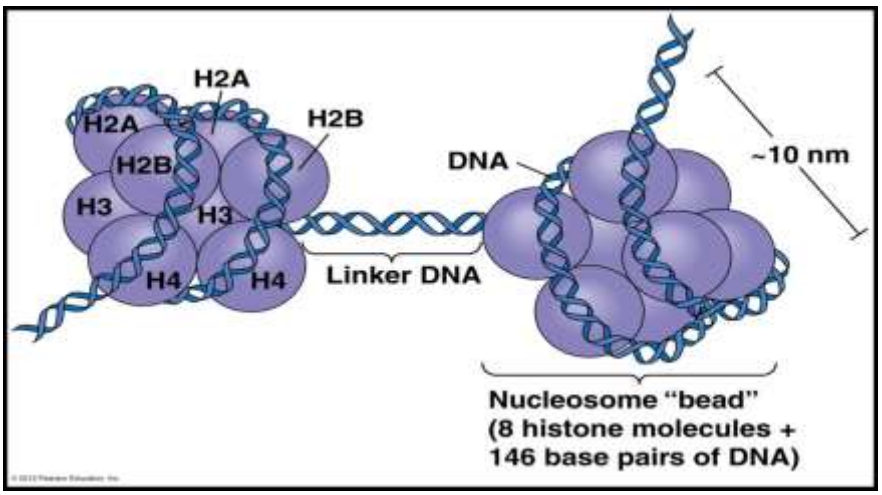
Chromatin has a highly complex structure with several levels of organization. When chromatin is isolated from the nucleus of a cell and viewed with an electron microscope, it frequently looks like beads on a string (nucleosome). **The nucleosome is a core particle consisting of DNA wrapped about two times around an octamer of eight histone proteins** (**two copies each of H2A, H2B, H3, and H4**), The DNA in direct contact with the histone octamer is between **145 and 147 bp in length**, coils around the histones in **a left-handed direction**, and supercoiled.

Histones H3 and H4 are believed to be in the **center of the core** and therefore associated with the main loop of DNA, while dimers of H2A and H2B bind to the DNA at **either end of the loop**.

Nucleosomes are separated from each other by a length of **spacer or linker DNA**, at least isolated chromatin. Histone HI is not a part of the nucleosomal structure but associates instead with the spacer DNA between nucleosomes. HI is thought to bring adjacent nucleosomes into close juxtaposition, thereby forming chromatin fibers. These fibers are regarded as the basic structural element of eukaryotic chromosomes.



11.5 Chromatin has a highly complex structure with several levels of organization.



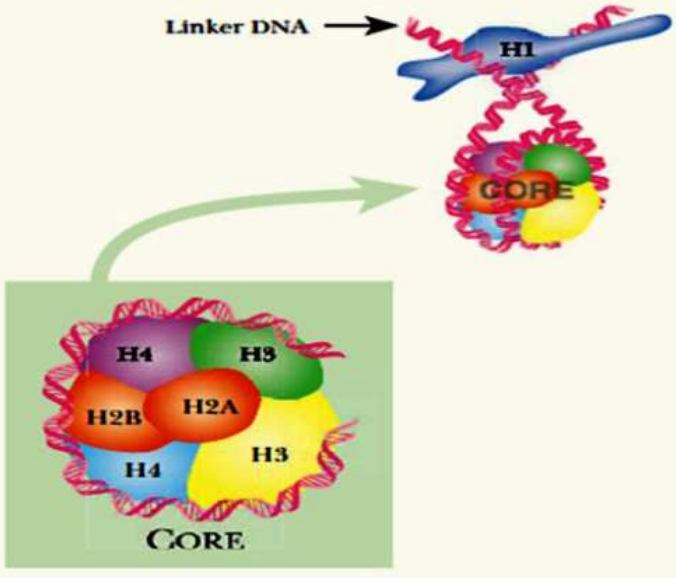


FIGURE 4.23 *Nucleosomes and Histones*

The basic unit in the folding of eukaryotic DNA is the nucleosome as shown here. A nucleosome is composed of eight histones comprising a core and one separate histone (H1) at the site where the wrapped DNA diverges. The enlarged region shows the packing of histones in the core. The H3-H4 tetramer dictates the shape of the core. Only one of the H2A and H2B dimers is shown; the other is on the other side, hidden from view.

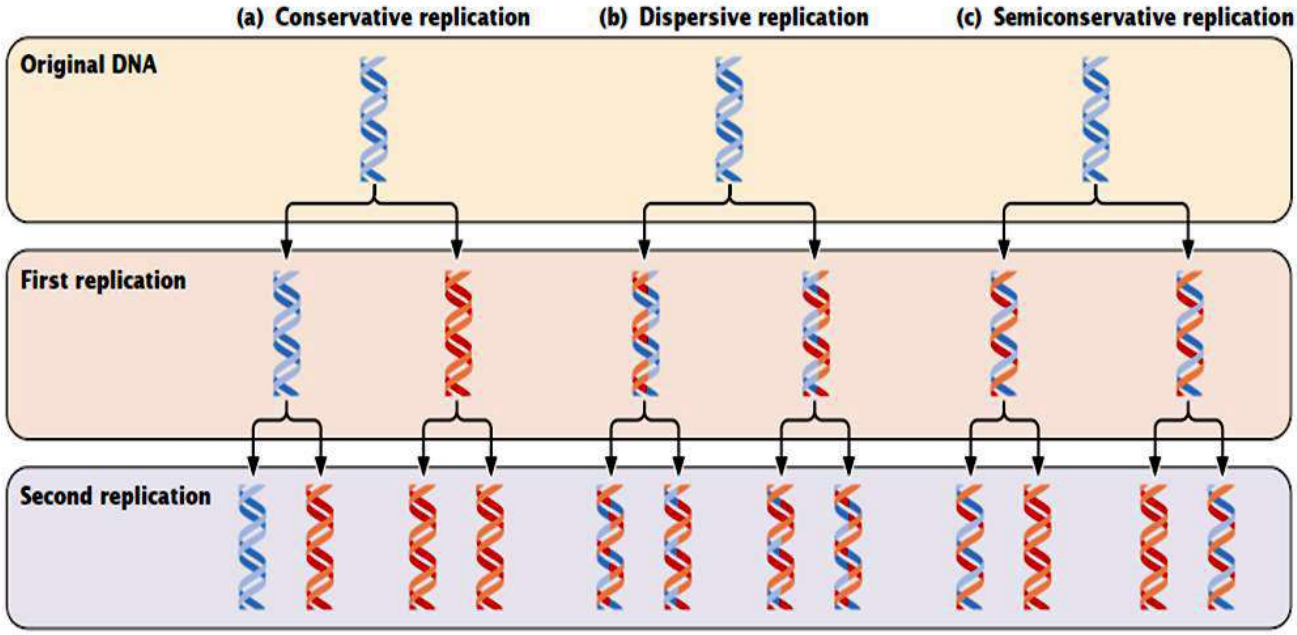
DNA Replication

Proposed models of replication

Initially, three alternative models were proposed for DNA replication.

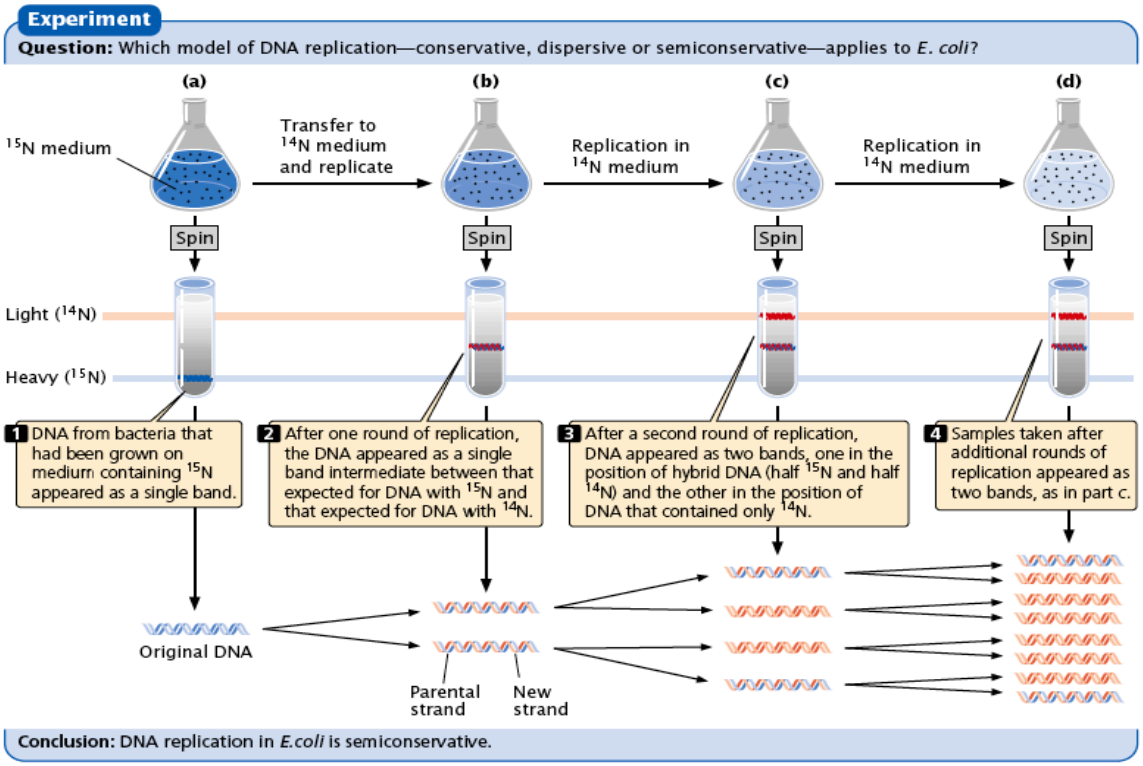
1. **In conservative replication**, the entire double-stranded DNA molecule serves as a template for a whole new molecule of DNA, and the original DNA molecule is fully conserved during replication.
2. **In dispersive replication**, both nucleotide strands break down (disperse) into fragments, which serve as templates for the synthesis of new DNA fragments, and then somehow reassemble into two complete DNA molecules. In this model, each resulting DNA molecule is interspersed with fragments of old and new DNA; none of the original molecule is conserved.
3. **Semi-conservative replication** is intermediate between these two models; the two nucleotide strands unwind and each serves as a template for a new DNA molecule.

The complementary nature of the two nucleotide strands in a DNA molecule suggested that, during replication, each strand can serve as a template for the synthesis of a new strand. The specificity of base pairing (adenine with thymine; guanine with cytosine) implied that only one sequence of bases can be specified by each template, and so two DNA molecules built on the pair of templates will be identical with the original. This process is called **semiconservative replication**, because each of the original nucleotide strands remains intact (conserved), despite no longer being combined in the same molecule; the original DNA molecule is half (semi) conserved during replication.



12.1 Three proposed models of replication are conservative replication, dispersive replication, and semiconservative replication.

Meselson and Stahl's Experiment



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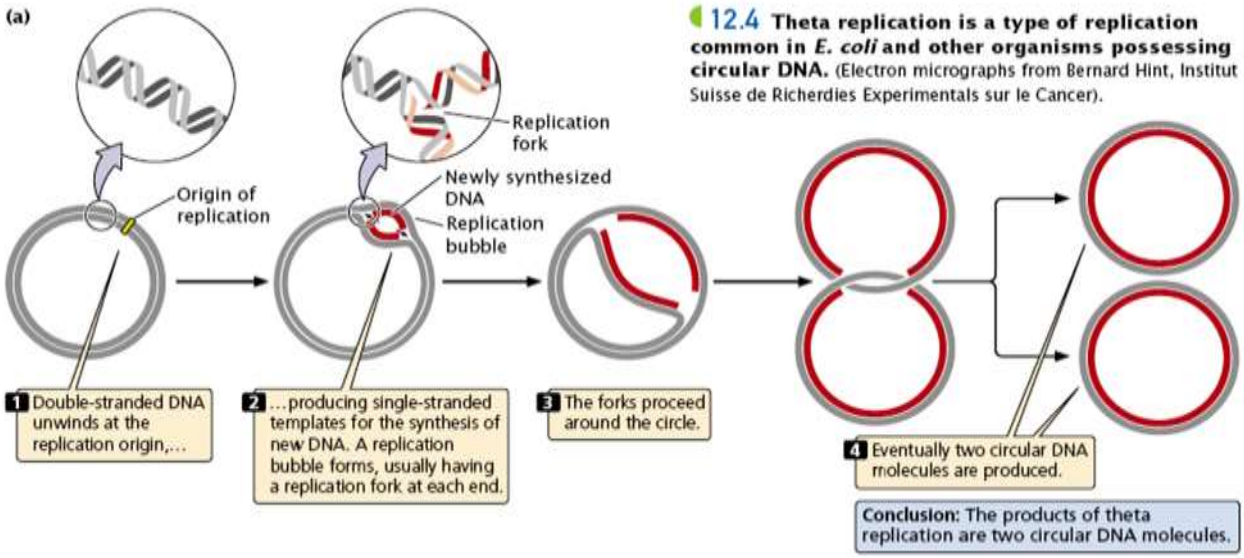

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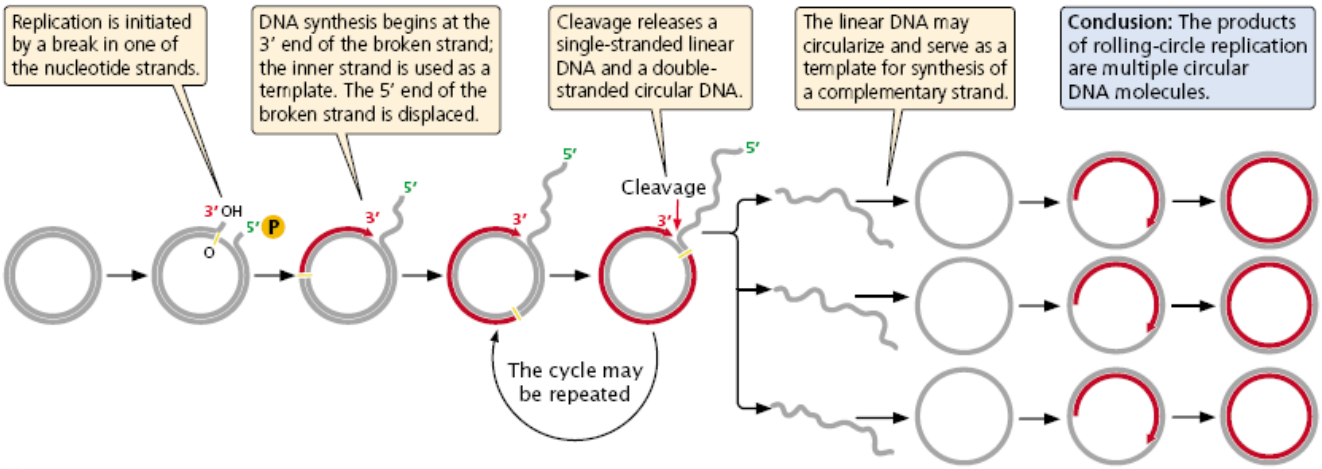

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The linear DNA molecules of some viruses and of all eukaryotic chromosomes are also replicated bidirectional from initiation points. Small viral DNAs have a single such point, but the large DNA molecules present in eukaryotic chromosomes may have hundreds or even thousands of initiation point.

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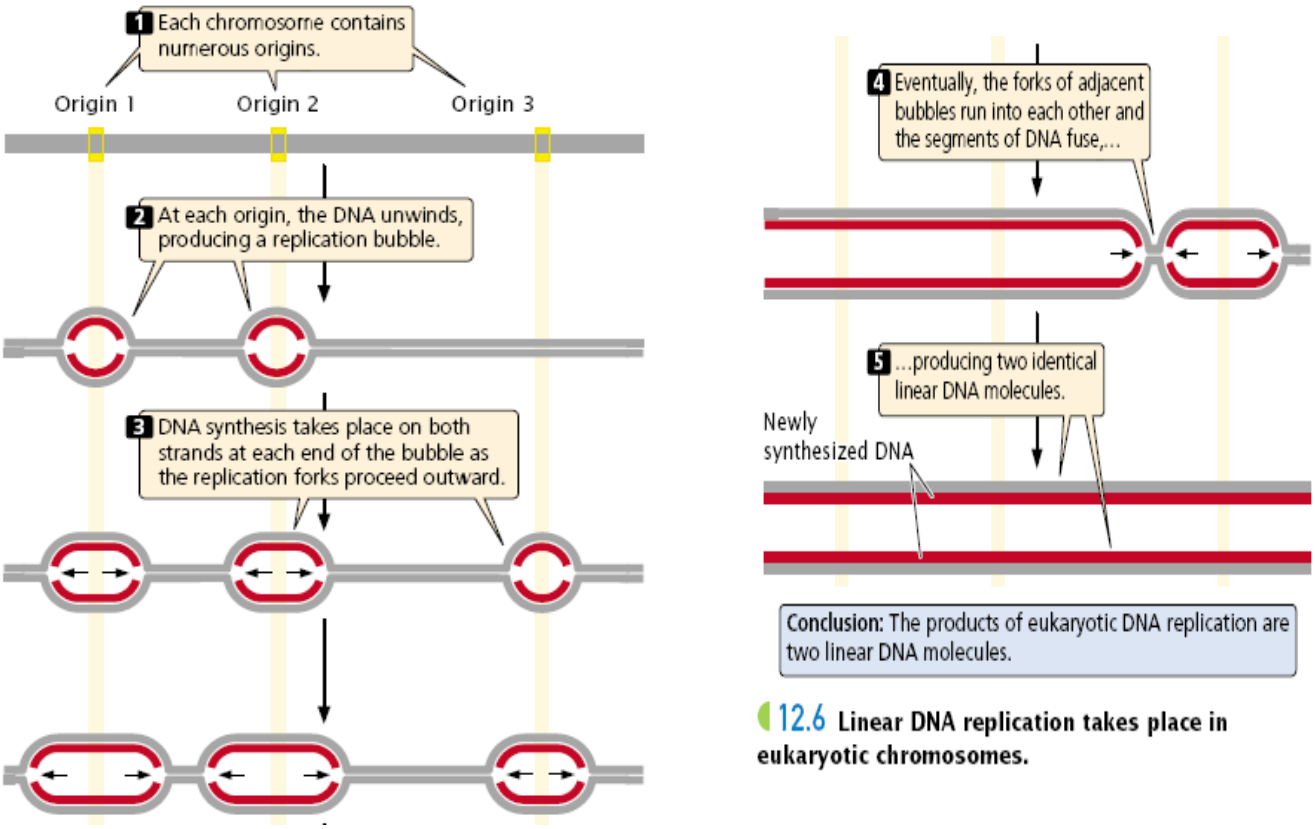

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DNA Replication Process

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Although the process of replication includes many components, they can be combined into three major groups:

1. **A template** consisting of single-stranded DNA.
2. 12Raw materials (substrates) to be assembled into a new nucleotide strand **(deoxyribonucleoside triphosphates (dNTPs)**
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Because of the semiconservative nature of DNA replication, a double-stranded DNA molecule must **unwind** to expose the bases that act as a template for the assembly of new polynucleotide strands, which are made complementary and antiparallel to the template strands.

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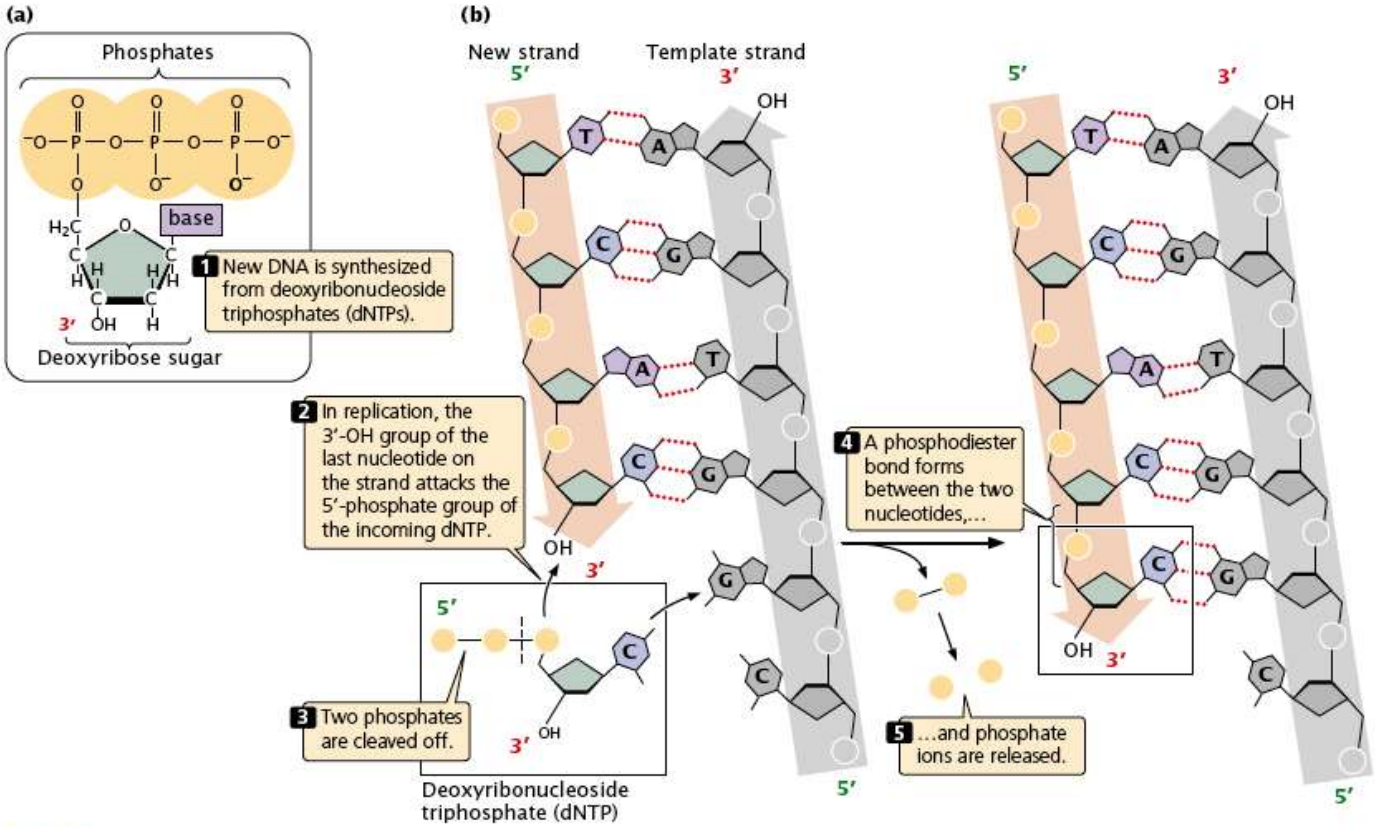

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The antiparallel nature of the two DNA strands means that one template will be exposed in the **5'→3'** direction and the other template will be exposed in the **3'→5'** direction.

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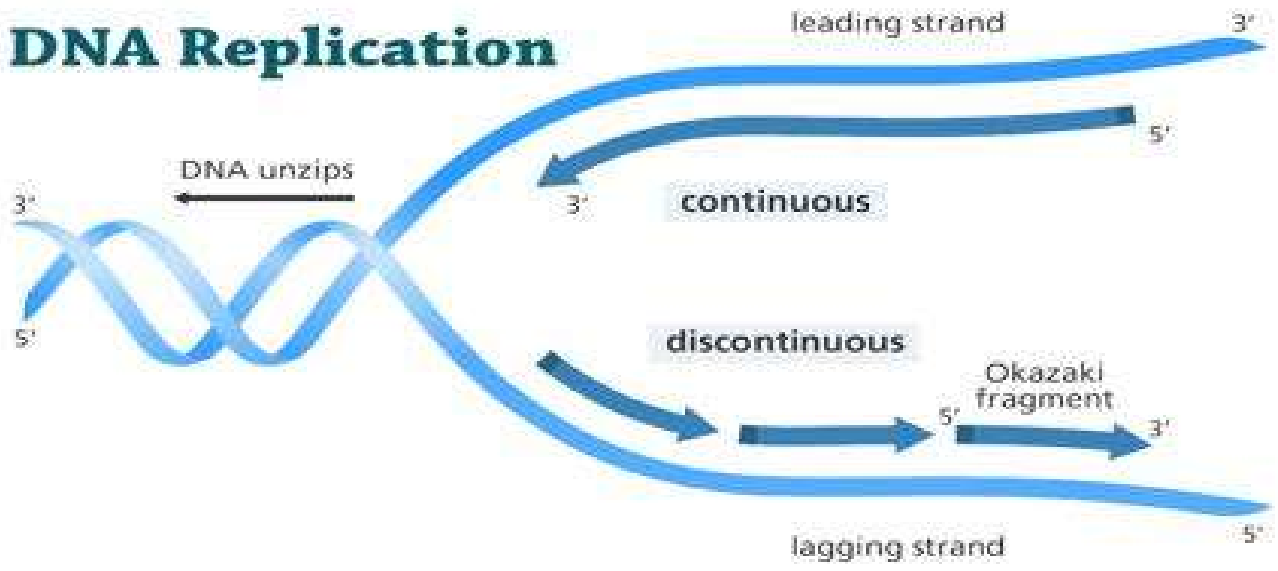

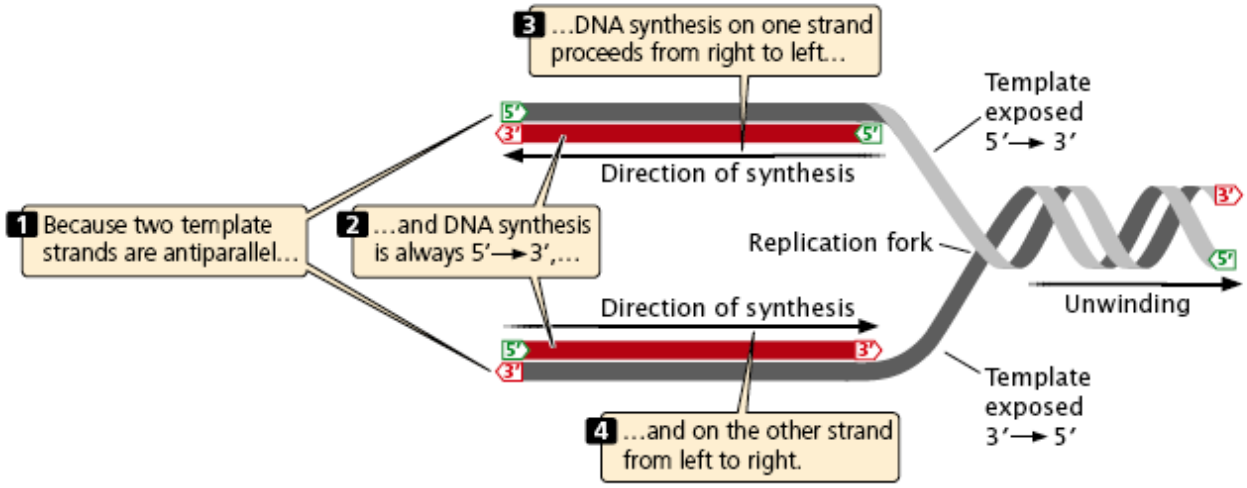

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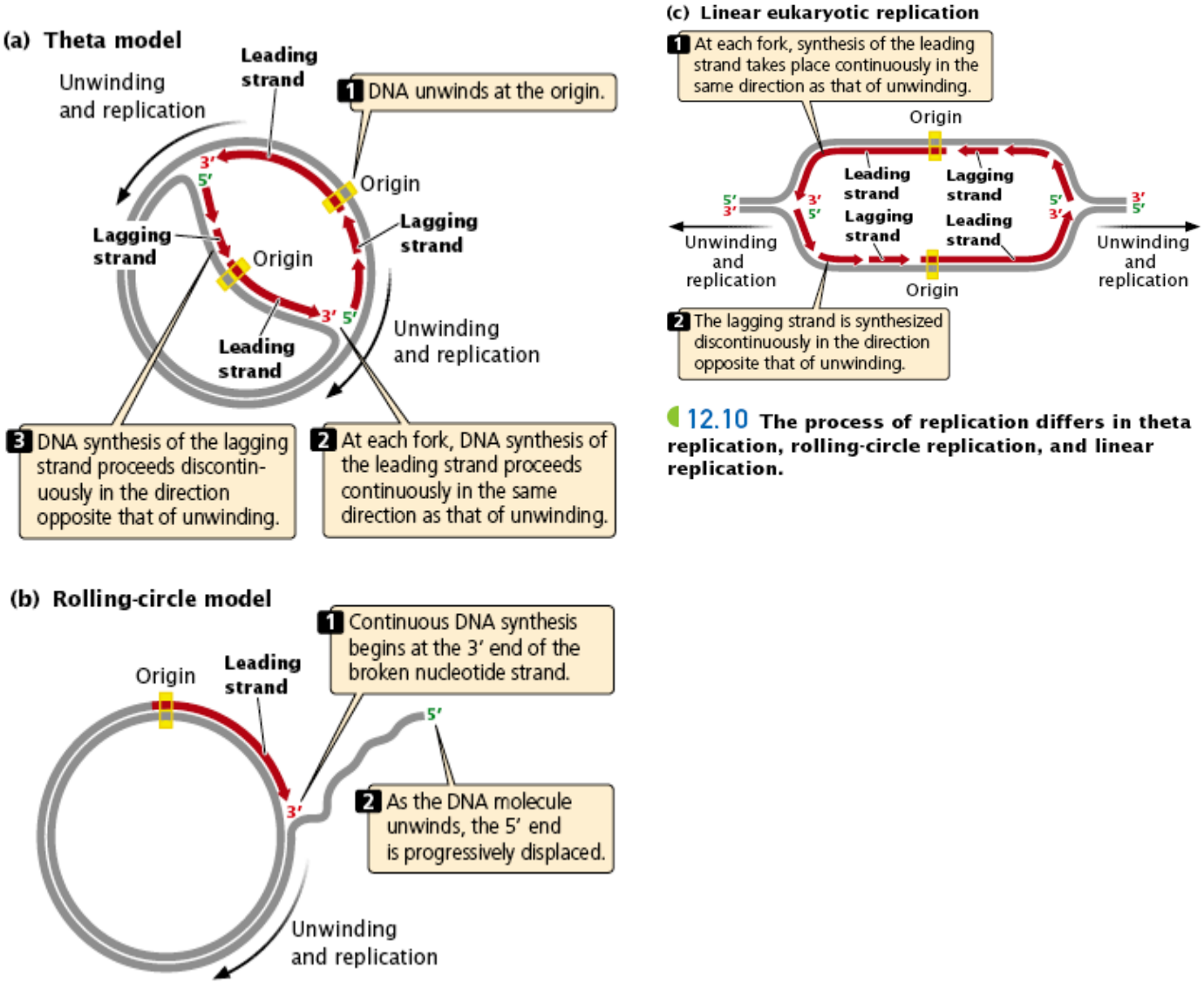

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The replication of linear molecules of DNA (eukaryotic cells) produces a series of replication bubbles. DNA synthesis in these bubbles is the same as that in the single replication bubble of the theta model; it begins at the center of each replication bubble and proceeds at two forks, one at each end of the bubble. At both forks, synthesis of the leading strand proceeds in the **same direction** as that of unwinding, whereas synthesis of the lagging strand proceeds in the **direction opposite** that of unwinding.

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Helicases bind to the lagging-strand template at each replication fork to break the hydrogen bonds that exist between the bases of the two nucleotide strands of a DNA molecule and move in the 5'→ 3' direction along this strand, thus also moving the replication fork.

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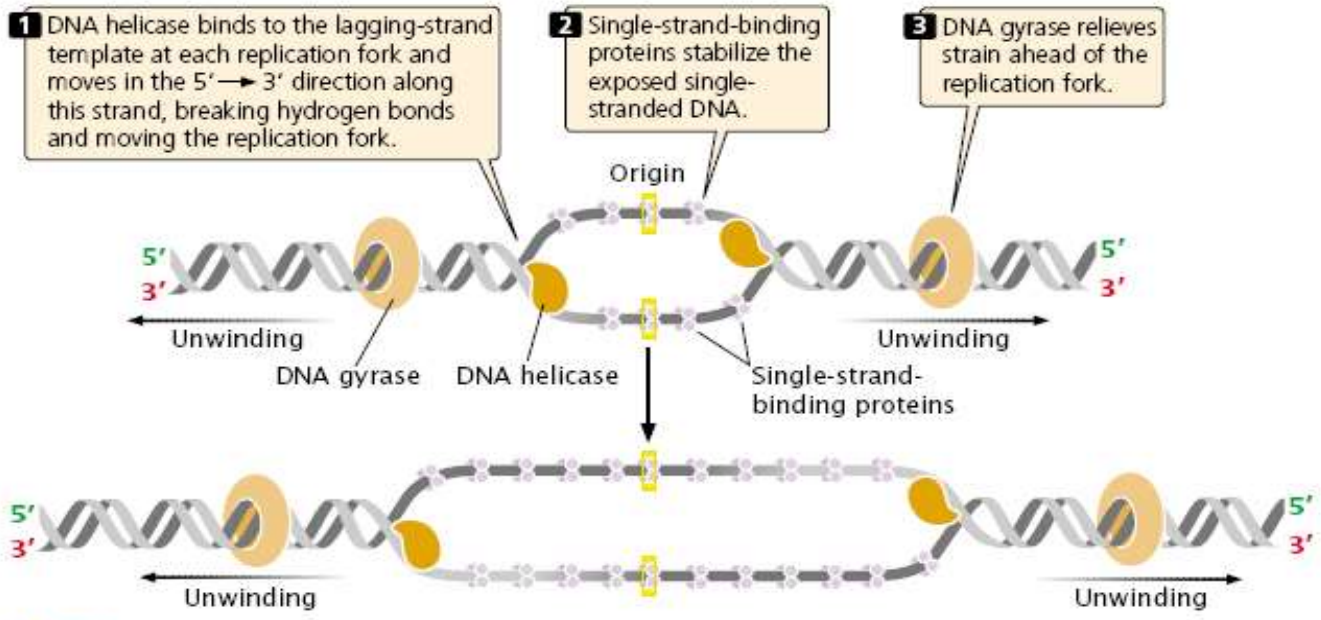

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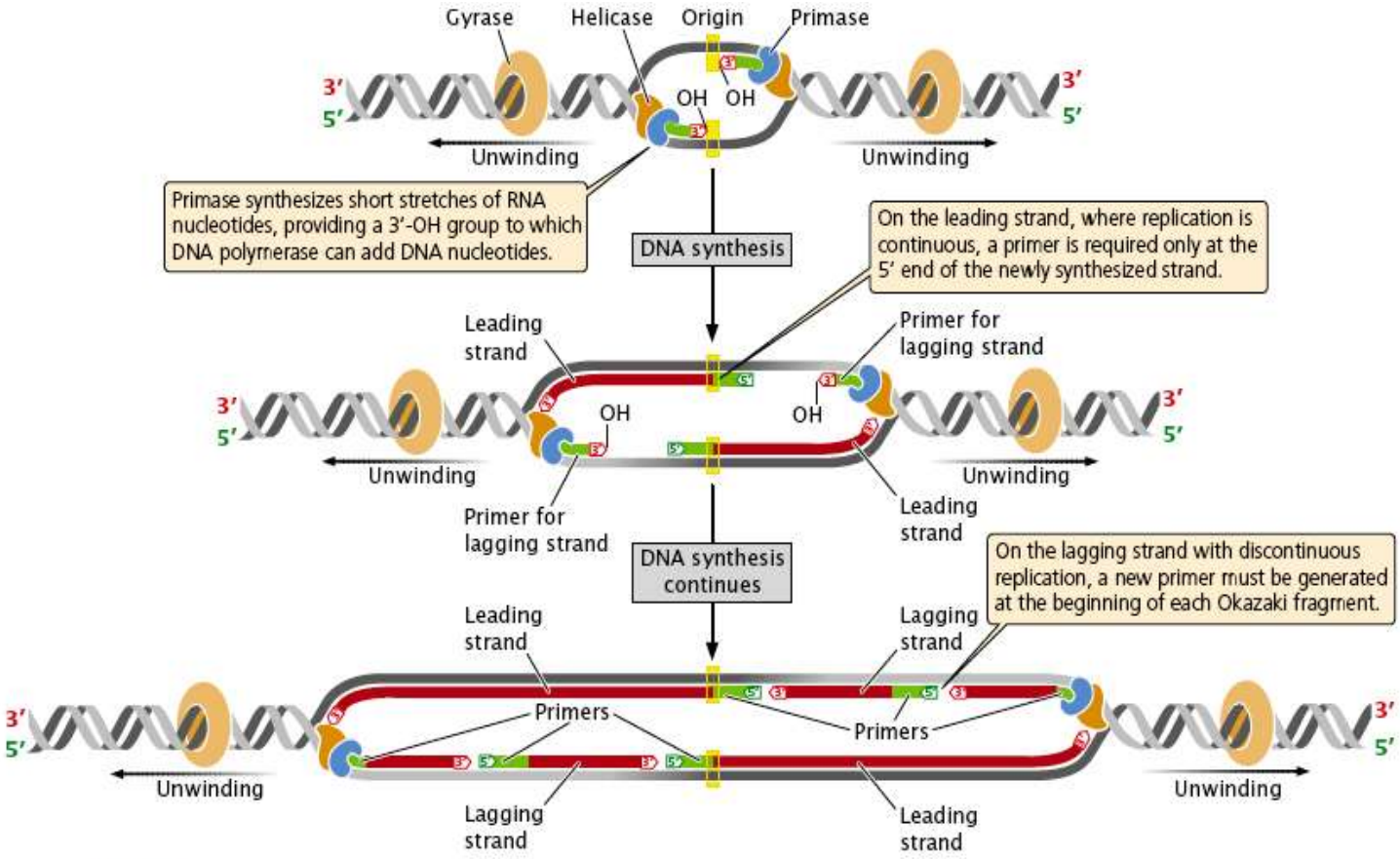

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5- DNA ligase

After polymerase I have replaced the last nucleotide of the RNA primer with a DNA nucleotide, a nick remains in the sugar phosphate backbone of the new DNA strand. The 3' OH group of the last nucleotide to have been added by DNA polymerase I is not attached to the 5' phosphate group of the first nucleotide added by DNA polymerase III. This nick is sealed by the enzyme **DNA ligase**, which catalyzes the formation of a phosphodiester bond without adding another nucleotide to the strand.

6- Termination

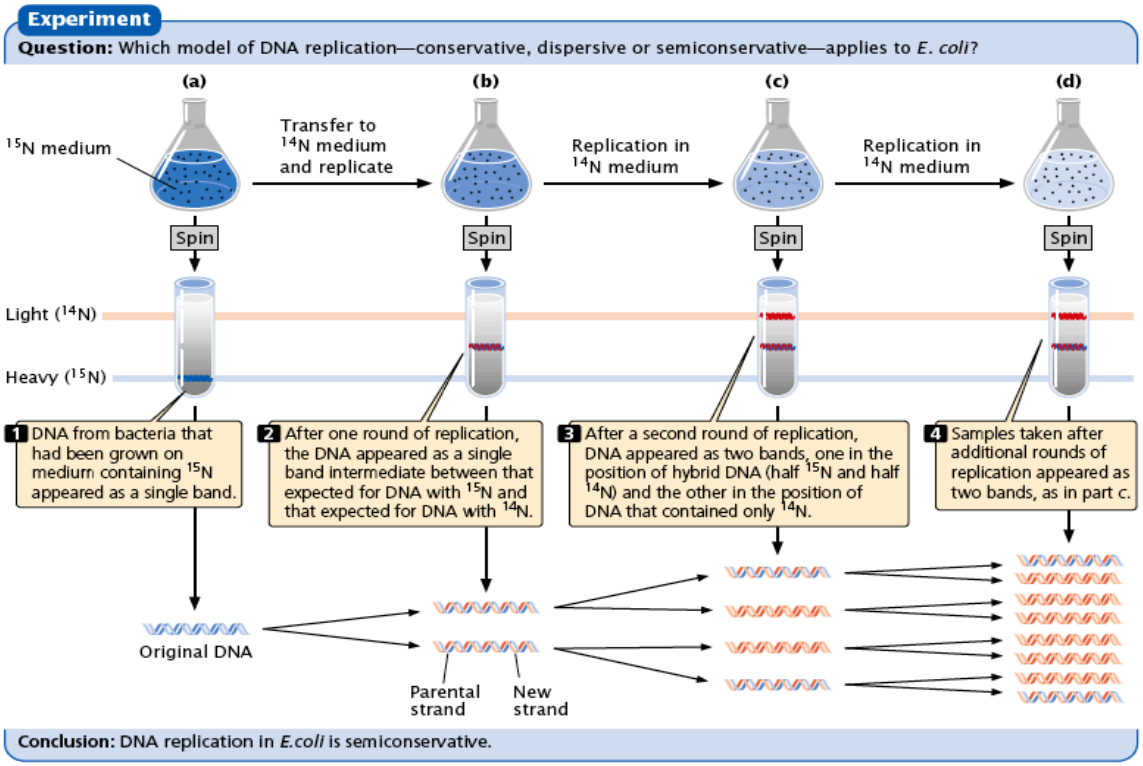
Replication is terminated whenever two replication forks meet. In others, specific **termination sequences** block further replication. A termination protein, called **Tus** in E. coli, binds to these sequences. Tus blocks the movement of helicase, thus stalling the replication fork and preventing further DNA replication.

Some of the major enzymes and proteins required for replication are summarized in the following table:

Table 12.4 Components required for replication in bacterial cells

Component	Function
Initiator protein	Binds to origin and separates strands of DNA to initiate replication
DNA helicase	Unwinds DNA at replication fork
Single-strand-binding proteins	Attach to single-stranded DNA and prevent reannealing
DNA gyrase	Moves ahead of the replication fork, making and resealing breaks in the double-helical DNA to release torque that builds up as a result of unwinding at the replication fork
DNA primase	Synthesizes short RNA primers to provide a 3′-OH group for attachment of DNA nucleotides
DNA polymerase III	Elongates a new nucleotide strand from the 3′-OH group provided by the primer
DNA polymerase I	Removes RNA primers and replaces them with DNA
DNA ligase	Joins Okazaki fragments by sealing nicks in the sugar–phosphate backbone of newly synthesized DNA

Meselson and Stahl's Experiment



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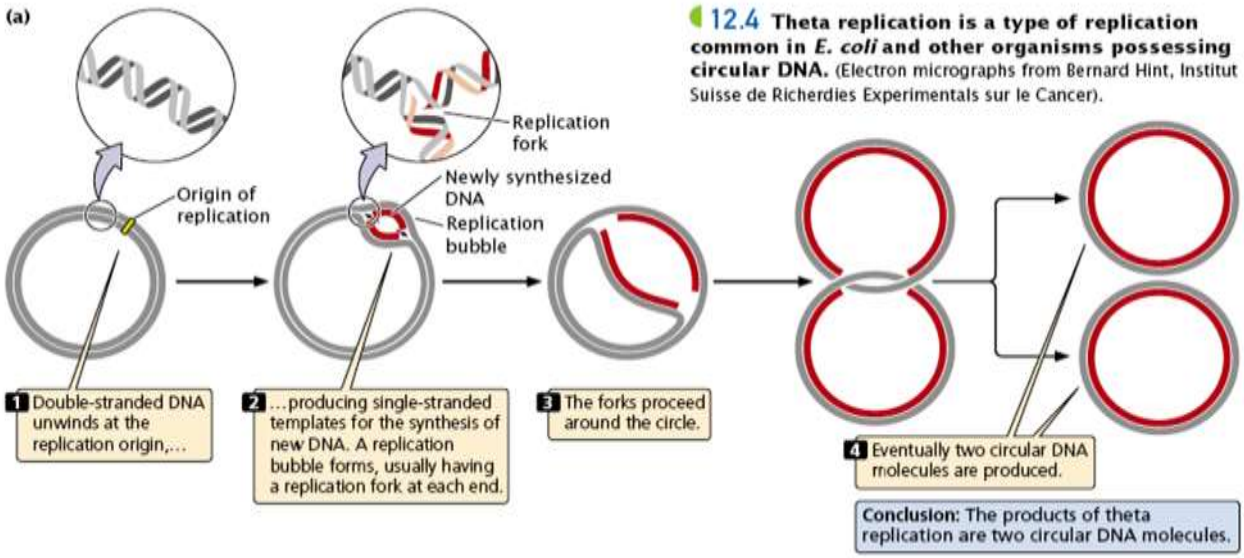

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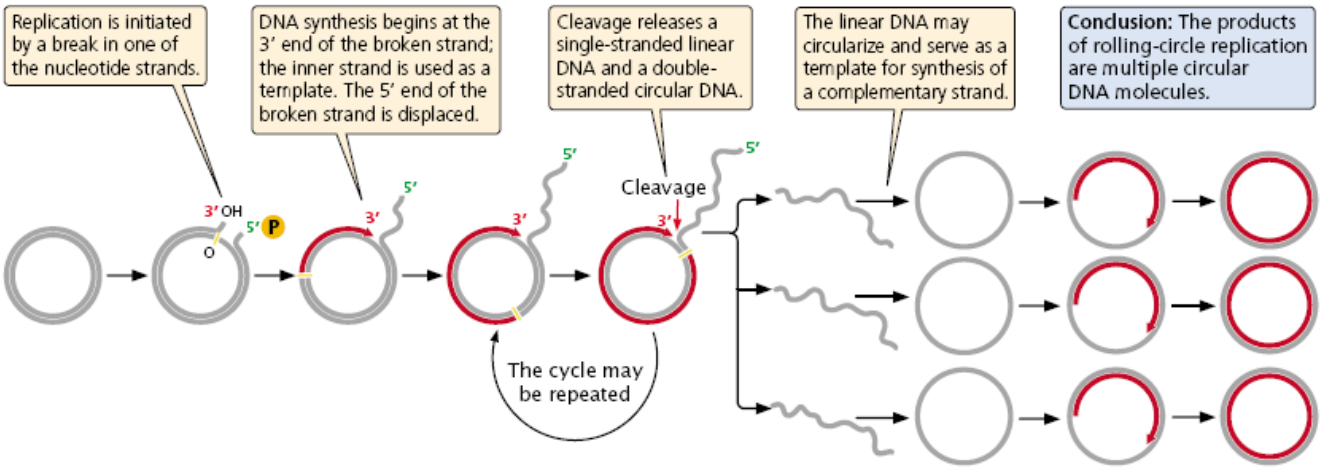

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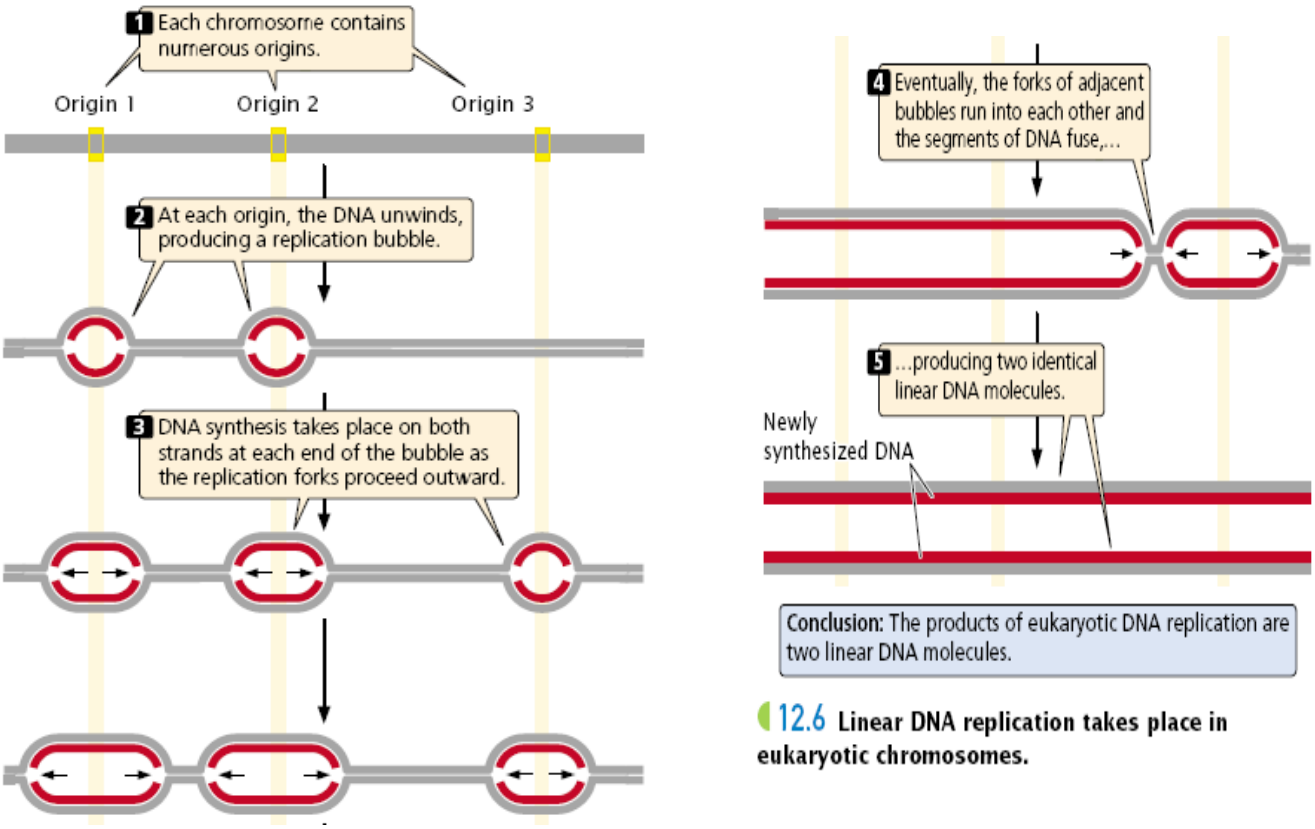

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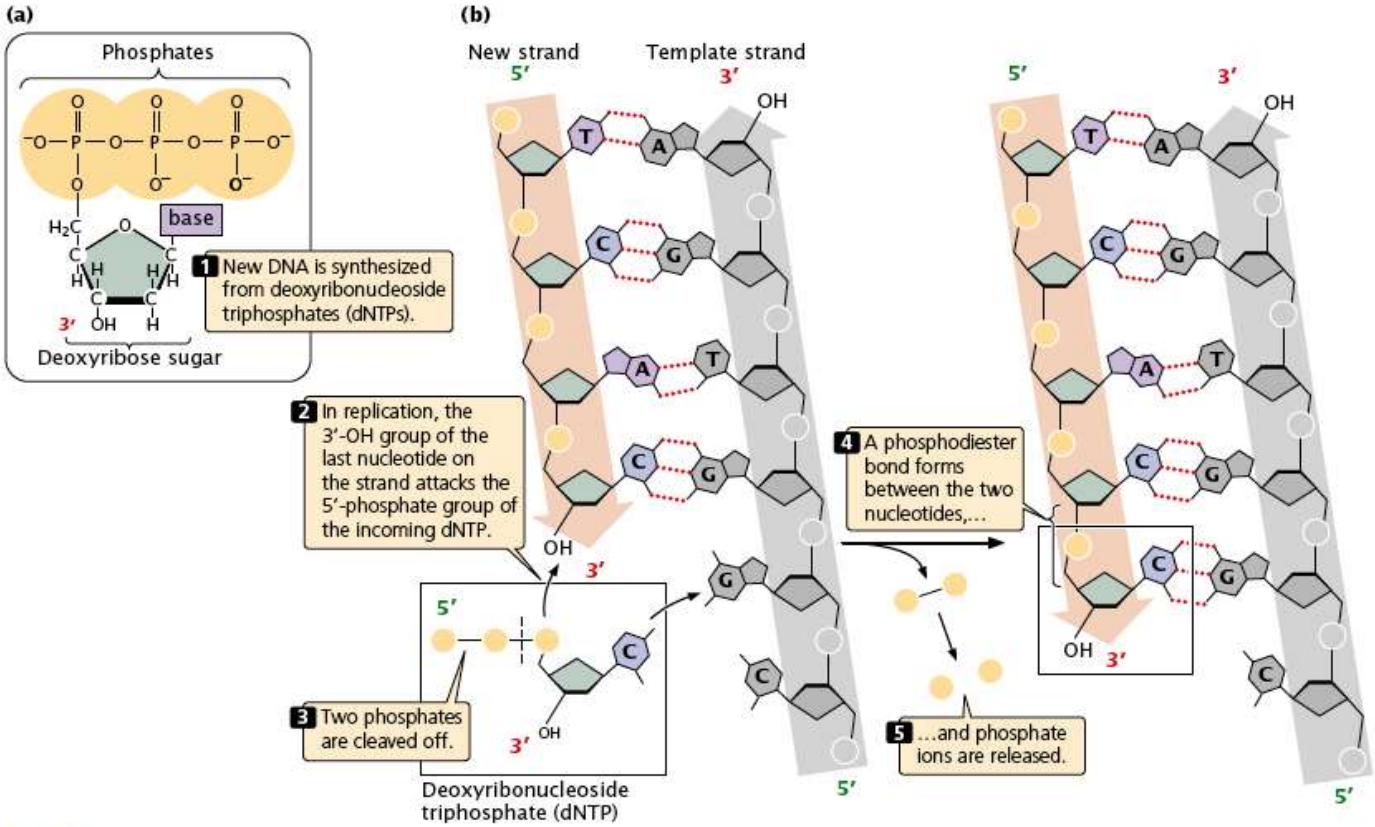

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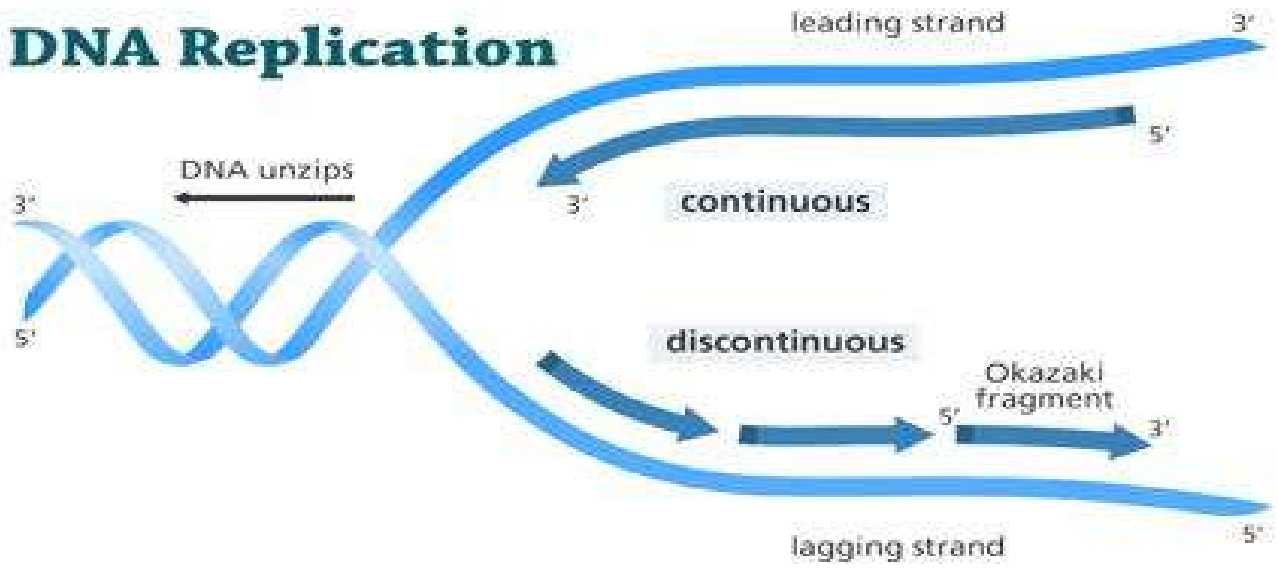

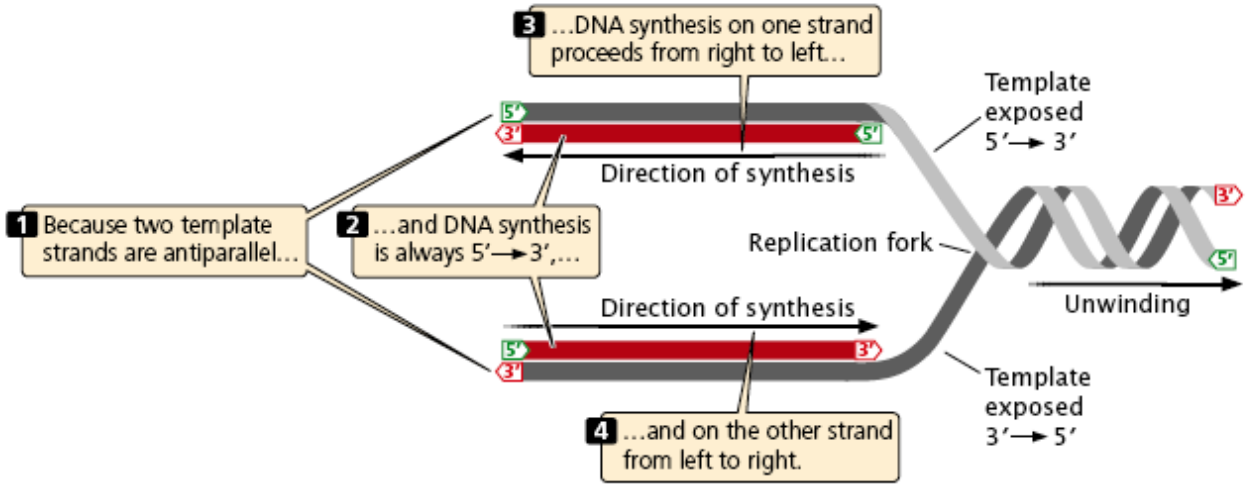

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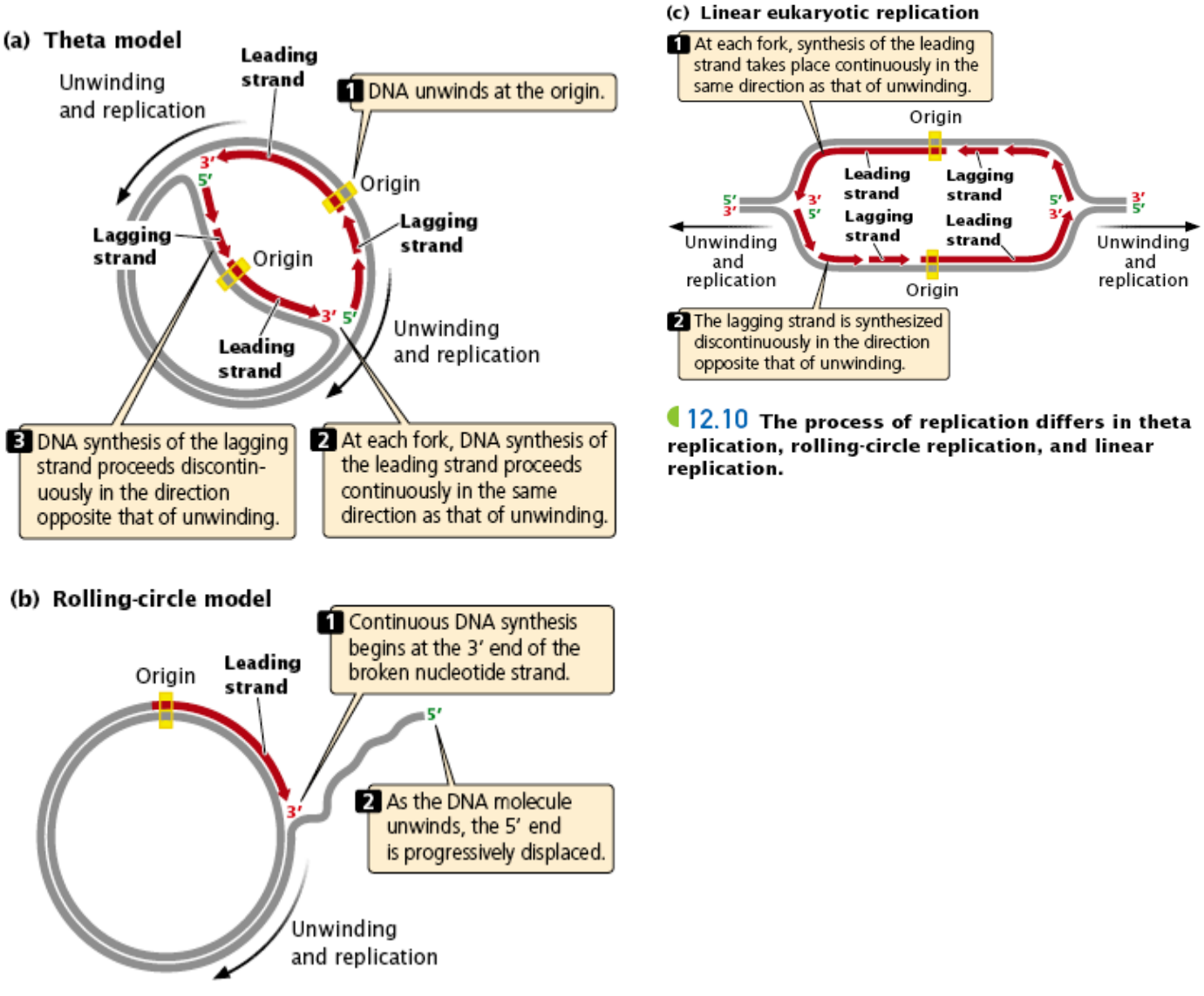

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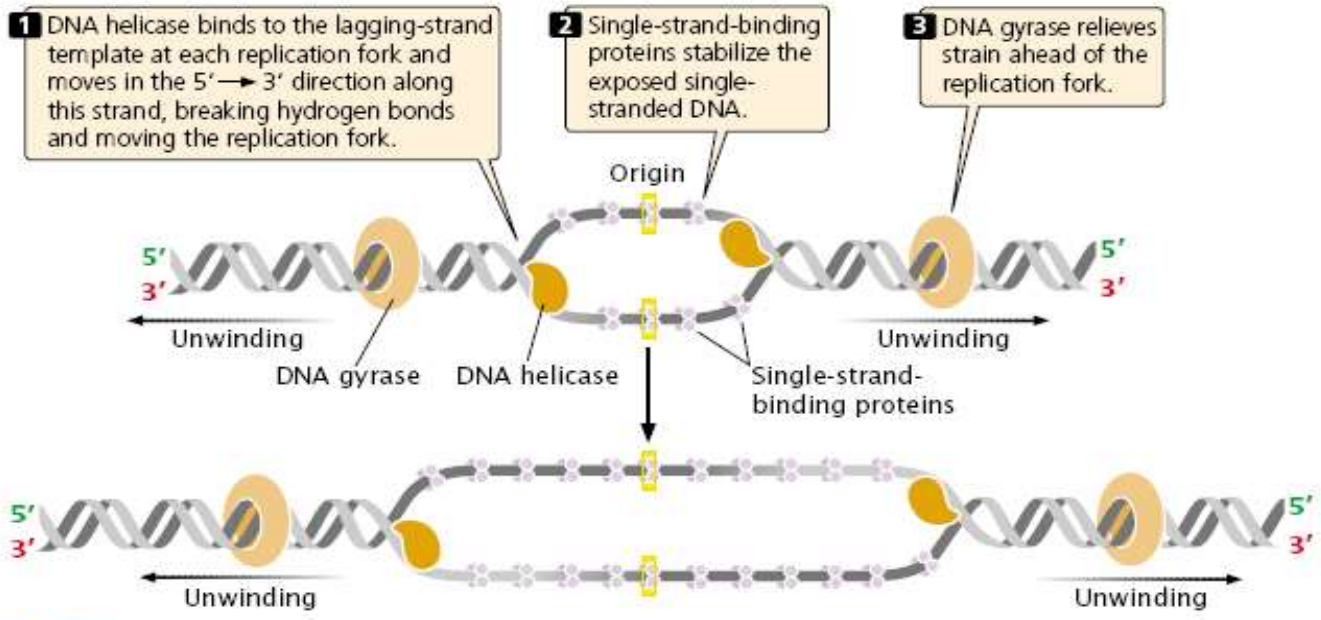

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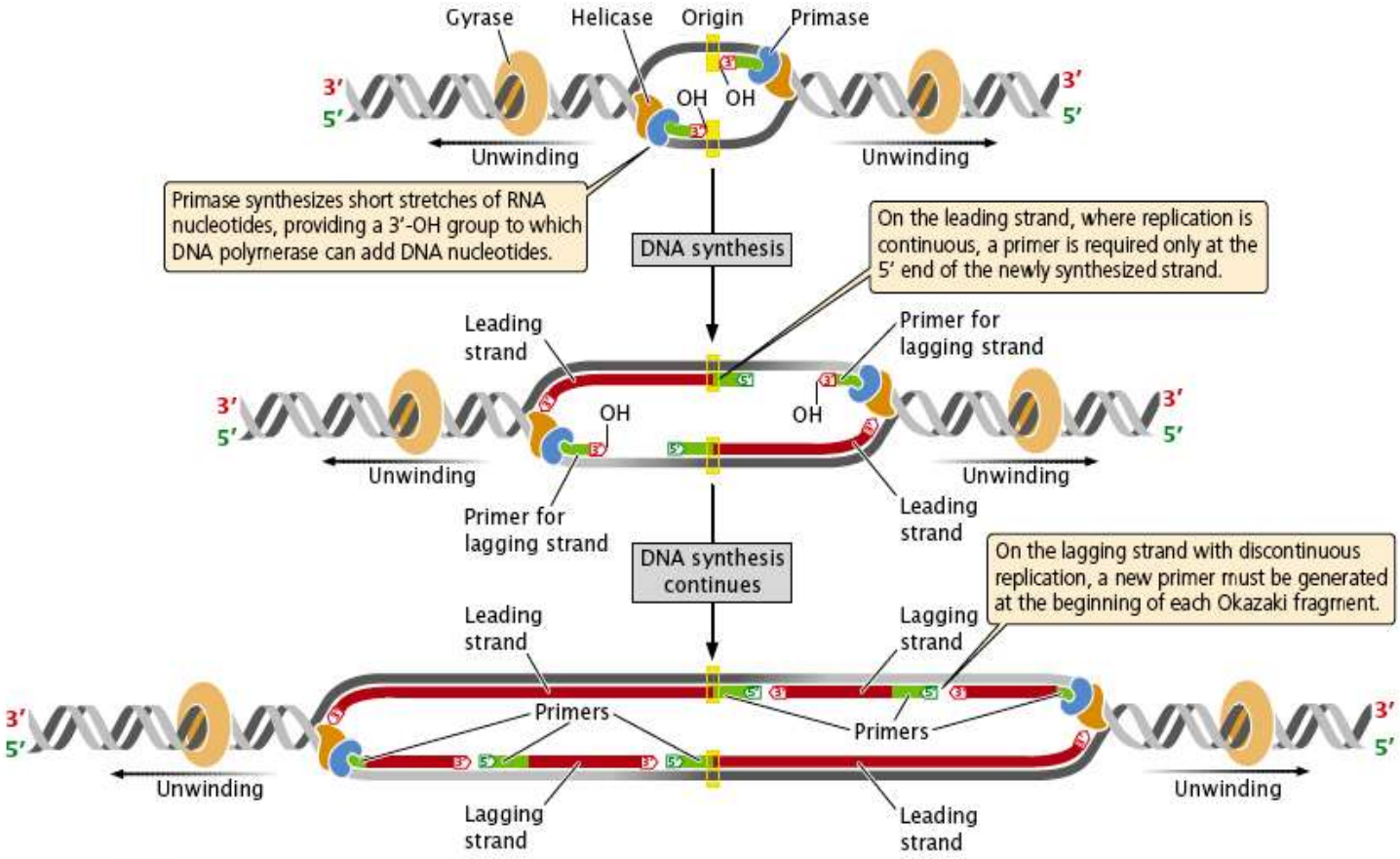
V	Yes	No	No	DNA repair; translesion DNA synthesis

DNA polymerases have been described; the main replication enzyme in E. coli cells is **DNA polymerase III.** DNA polymerase I functions primarily as an editing and repairing enzyme DNA polymerase I have a 5' →3' exonuclease activity that seems to the part of a repair process for removing and replacing damaged bases DNA. In addition, the 5'→3' exonuclease activity is involved in removing the small pieces of RNA that are needed as primers in DNA.



12.13 Primase synthesizes short stretches of RNA nucleotides, providing a 3′-OH group to which DNA polymerase can add DNA nucleotides.

5- DNA ligase

After polymerase I have replaced the last nucleotide of the RNA primer with a DNA nucleotide, a nick remains in the sugar phosphate backbone of the new DNA strand. The 3' OH group of the last nucleotide to have been added by DNA polymerase I is not attached to the 5' phosphate group of the first nucleotide added by DNA polymerase III. This nick is sealed by the enzyme **DNA ligase**, which catalyzes the formation of a phosphodiester bond without adding another nucleotide to the strand.

6- Termination

Replication is terminated whenever two replication forks meet. In others, specific **termination sequences** block further replication. A termination protein, called **Tus** in E. coli, binds to these sequences. Tus blocks the movement of helicase, thus stalling the replication fork and preventing further DNA replication.

Some of the major enzymes and proteins required for replication are summarized in the following table:

Table 12.4 Components required for replication in bacterial cells

Component	Function
Initiator protein	Binds to origin and separates strands of DNA to initiate replication
DNA helicase	Unwinds DNA at replication fork
Single-strand-binding proteins	Attach to single-stranded DNA and prevent reannealing
DNA gyrase	Moves ahead of the replication fork, making and resealing breaks in the double-helical DNA to release torque that builds up as a result of unwinding at the replication fork
DNA primase	Synthesizes short RNA primers to provide a 3′-OH group for attachment of DNA nucleotides
DNA polymerase III	Elongates a new nucleotide strand from the 3′-OH group provided by the primer
DNA polymerase I	Removes RNA primers and replaces them with DNA
DNA ligase	Joins Okazaki fragments by sealing nicks in the sugar–phosphate backbone of newly synthesized DNA

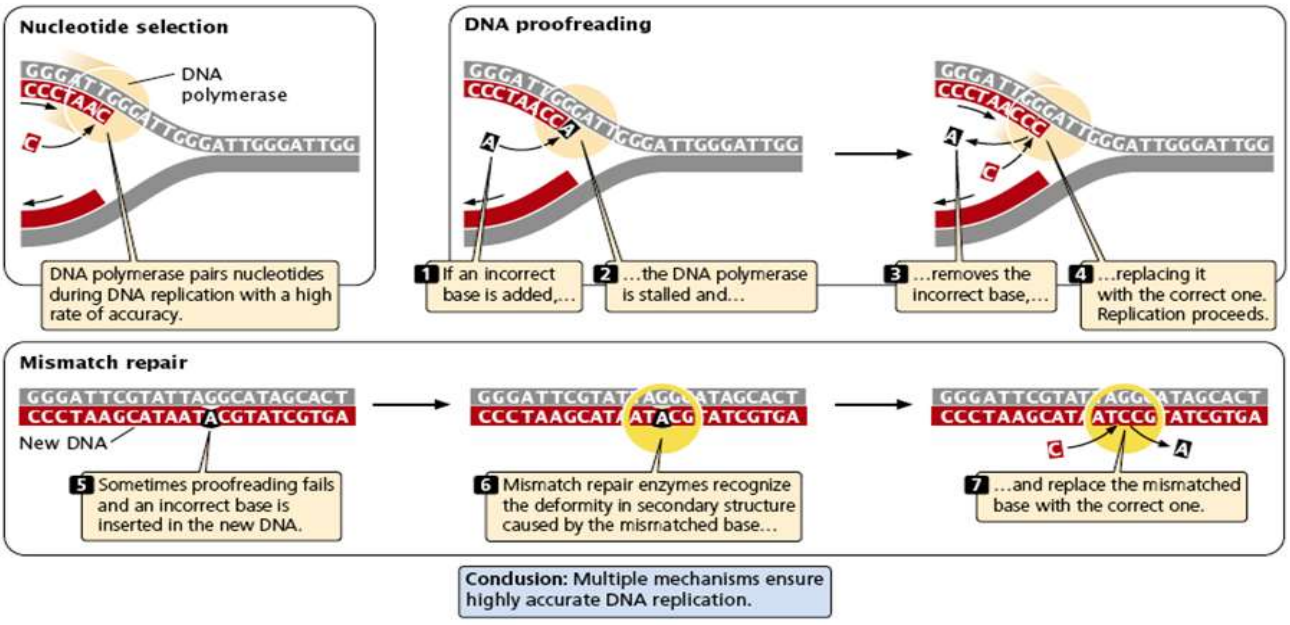
The Accuracy of DNA replication

DNA polymerases are very particular in pairing nucleotides with their complements on the template strand. Errors in nucleotide selection by DNA polymerase arise only about once per 100,000 nucleotides. Most of the errors that do arise in nucleotide selection are corrected in a second process called **proofreading.**

When a DNA polymerase inserts an incorrect nucleotide into the growing strand, the 3'-OH group of the mispaired nucleotide is not correctly positioned for accepting the next nucleotide. The incorrect positioning stalls the polymerization reaction and the 3' →5' exonuclease activity of **DNA polymerase removes the incorrectly paired nucleotide.** DNA polymerase then inserts the correct nucleotide. Together, proofreading and nucleotide selection result in an error rate of only one in 10 million nucleotides.

A third process, called **mismatch repair**, corrects errors after replication is complete. Any incorrectly paired nucleotides remaining after replication produce a deformity in the secondary structure of the DNA; the deformity is recognized by enzymes that excise an incorrectly paired nucleotide and use the original nucleotide strand as a template to replace the incorrect nucleotide.

Collectively, Replication is extremely accurate, with less than one error per billion nucleotides. This accuracy results from the processes of nucleotide selection, proofreading, and mismatch repair.



12.16 A series of processes are required to ensure the incredible accuracy of DNA replication. Among these processes are DNA selection, proofreading, and mismatch repair.

The Basic Rules of Replication

Bacterial replication requires a number of enzymes, proteins, and DNA sequences that function together to synthesize a new DNA molecule. These components are important, but it is critical that we not become so immersed in the details of the process that we lose sight of general principles of replication.

1. Replication is always semiconservative.
2. Replication begins at sequences called origins.
3. DNA synthesis is initiated by short segments of RNA called primers.
4. The elongation of DNA strands is always in the 5'→3' directions.
5. New DNA is synthesized from dNTPs; in the polymerization of DNA, two phosphates are cleaved from a dNTP and the resulting nucleotide is added to the 3'OH group of the growing nucleotide strand.
6. Replication is continuous on the leading strand and discontinuous on the lagging strand.
7. New nucleotide strands are made complementary and antiparallel to their template strands.
8. Replication takes place at very high rates and is astonishingly accurate, thanks to precise nucleotide selection, proofreading, and repair mechanisms.

Eukaryotic and Prokaryotic DNA Replication

Eukaryotic replication resembles bacterial replication in many respects. The most obvious differences are that eukaryotes have:

(1) Multiple replication origins in their chromosomes;

(2) More types of DNA polymerases, with different functions; and

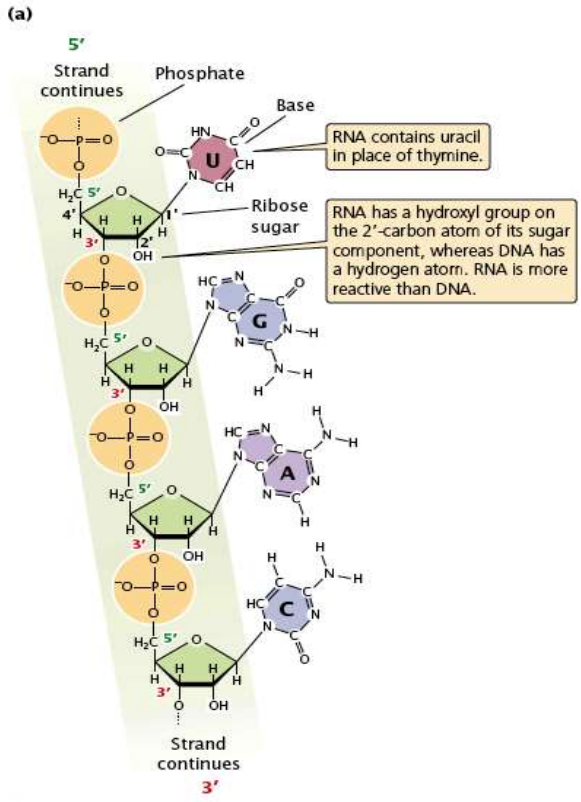
(3) Nucleosome assembly immediately following DNA replication.

Transcription

Before we begin our study of transcription, let's review the structure of RNA and consider the different types of RNA molecules.

The Structure of RNA

RNA, like DNA, is a polymer consisting of nucleotides joined together by phosphodiester bonds. However, there are several important differences in the structures of DNA and RNA. Whereas DNA nucleotides contain deoxyribose sugars, **RNA nucleotides have ribose sugars**. With a free hydroxyl group on the 2'-carbon atom of the ribose sugar, RNA is degraded rapidly under alkaline conditions. The deoxyribose sugar of DNA lacks this free hydroxyl group; so DNA is a more stable molecule. Another important difference is that **thymine, one of the two pyrimidines found in DNA, is replaced by uracil in RNA**. A final difference in the structures of DNA and RNA is that **RNA is usually single stranded**, consisting of a single polynucleotide strand, whereas DNA normally consists of two polynucleotide strands joined by hydrogen bonding between complementary bases.



13.1 **RNA has a primary and a secondary structure.**

Table 13.1 The structures of DNA and RNA compared

Characteristic	DNA	RNA
Composed of nucleotides	Yes	Yes
Type of sugar	Deoxyribose	Ribose
Presence of 2′-OH group	No	Yes
Bases	A, G, C, T	A, G, C, U
Nucleotides joined by phosphodiester bonds	Yes	Yes
Double or single stranded	Usually double	Usually single
Secondary structure	Double helix	Many types
Stability	Quite stable	Easily degraded

Classes of RNA

RNA molecules perform a variety of functions in the cell. **Ribosomal RNA (rRNA),** along with ribosomal protein subunits, makes up the ribosome, the site of protein assembly. **Messenger RNA (mRNA)** carries the coding instructions for polypeptide chains from DNA to the ribosome. After attaching to a ribosome, an mRNA molecule specifies the sequence of the amino acids in a polypeptide chain and provides a template for joining amino acids. Large precursor molecules, which are termed **pre-messenger RNAs** (pre-mRNAs), are the immediate products of transcription in eukaryotic cells. Pre-mRNAs are modified extensively before they exit the nucleus for translation into protein. Bacterial cells do not possess premRNA; in these cells, transcription takes place imediately with translation.

Transfer RNA (tRNA) serves as the link between the coding sequence of nucleotides in the mRNA and the amino acid sequence of a polypeptide chain. Each tRNA attaches to one particular type of amino acid and helps to incorporate that amino acid into a polypeptide chain. Additional classes of RNA molecules are found in the nuclei of eukaryotic cells. **Small nuclear RNAs** (snRNAs) combine with small nuclear protein subunits to form small nuclear ribonucleoproteins (snRNPs), affectionately known as “snurps”). The snRNPs are analogous to ribosomes in structure, only smaller, and they typically contain a single RNA molecule combined with approximately 10 small nuclear protein subunits. Some snRNAs participate in the processing of RNA, converting pre-mRNA into mRNA. Small nucleolar RNAs (snoRNAs) take part in the processing of rRNA. SmallRNA molecules also are found in the cytoplasm of eukaryotic cells; these molecules are called **small cytoplasmic RNAs** (scRNAs). The different classes of RNA molecules are summarized in Table.

Table 13.2 Locations and functions of different classes of RNA molecules

Class of RNA	Cell Type	Location of Function* in Eukaryotic Cells	Function
Ribosomal RNA (rRNA)	Bacterial and eukaryotic	Cytoplasm	Structural and functional components of the ribosome
Messenger RNA (mRNA)	Bacterial and eukaryotic	Nucleus and cytoplasm	Carries genetic code for proteins
Transfer RNA (tRNA)	Bacterial and eukaryotic	Cytoplasm	Helps incorporate amino acids into polypeptide chain
Small nuclear RNA (snRNA)	Eukaryotic	Nucleus	Processing of pre-mRNA
Small nucleolar RNA (snoRNA)	Eukaryotic	Nucleus	Processing and assembly of rRNA
Small cytoplasmic RNA (scRNA)	Eukaryotic	Cytoplasm	Variable

*All eukaryotic RNAs are transcribed in the nucleus.

RNA differs from DNA in that it possesses a hydroxyl group on the 2'-carbon atom of its sugar, contains uracil instead of thymine, and is normally single stranded. Several classes of RNA exist within bacterial and eukaryotic cells.

Transcription: Synthesizing RNA from a DNA Template

All cellular RNAs are synthesized from a DNA template through the process of transcription. Transcription is in many ways similar to the process of replication, but one fundamental difference relates to the length of the template used. During replication, all the nucleotides in the DNA template are copied, but, during transcription, only small parts of the DNA molecule— usually a single gene or, at most, a few genes—are transcribed into RNA because not all gene products are needed at the same time or in the same cell. Furthermore, much of the DNA does not code for a functional product, and transcription of such sequences would be pointless. Transcription is, in fact, a highly selective process—individual genes are transcribed only as their products are needed. But this selectivity imposes a fundamental problem on the cell—the problem of how to recognize individual genes and transcribe them at the proper time and place.

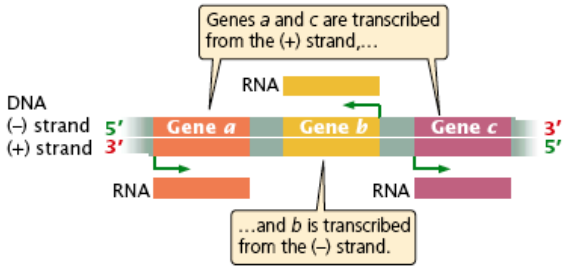
Like replication, transcription requires three major components:

1. A **DNA template**;
2. The **raw materials** (substrates) needed to build a new RNA molecule; and
3. The transcription apparatus, consisting of the **proteins** necessary to catalyze the synthesis of RNA.

➢ The Template

The transcribed strand is template for RNA synthesis, as for DNA synthesis, is a single strand of the DNA double helix. Unlike replication, however, transcription typically takes place on only one of the two nucleotide strands of DNA. The nucleotide strand used for transcription is termed the **template strand.** The other strand, called the **non-template strand**, is not ordinarily transcribed. Thus, in any one section of DNA, only one of the nucleotide strands normally carries the genetic information that is transcribed into RNA.

During transcription, an RNA molecule is synthesized that is complementary and antiparallel to the DNA template strand. The RNA transcript has the same polarity and base sequence as does the non-template strand, with the exception that U in RNA substitutes for T in DNA.



13.6 **RNA is transcribed from one DNA strand.** In most organisms, each gene is transcribed from a single DNA strand, but different genes may be transcribed from one or the other of the two DNA strands.

➢ The transcription unit

A transcription unit is a stretch of DNA that codes for an RNA molecule and the sequences necessary for its transcription. A transcription unit consists of three critical regions: **a promoter, an RNA coding sequence, and a terminator.**

The promoter is a DNA sequence that the transcription apparatus recognizes and binds. It indicates which of the two DNA strands is to be read as the template and the direction of transcription. The promoter also determines the transcription start site, the first nucleotide that will be transcribed into RNA. In most transcription units, the promoter is located next to the transcription start site but is not, itself, transcribed.

The second critical region of the transcription unit is **the RNA-coding region**, a sequence of DNA nucleotides that is copied into an RNA molecule.

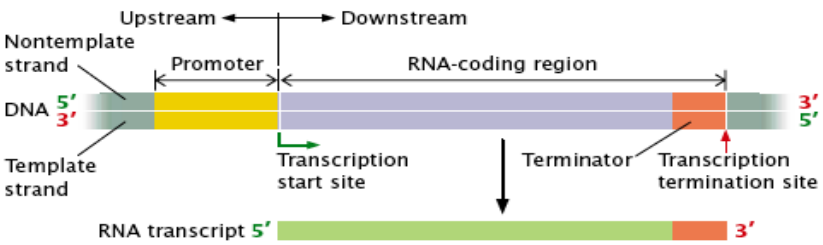
A third component of the transcription unit is **the terminator**, a sequence of nucleotides that signals where transcription is to end. Terminators are usually part of the coding sequence; that is, transcription stops only after the terminator has been copied into RNA.

A transcription unit is a piece of DNA that encodes an RNA molecule and the sequences necessary for its proper transcription. Each transcription unit includes a promoter, an RNA-coding region, and a terminator.

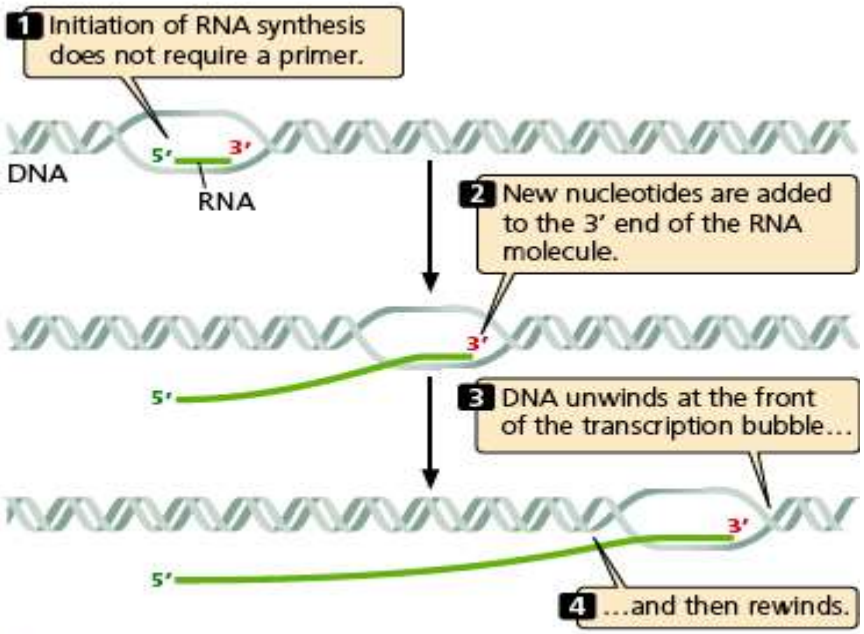
➢ The substrates of transcription

RNA is synthesized from **ribonucleoside triphosphosphates (rNTPs)**. In synthesis, nucleotides are added one at a time to the 3'-OH group of the growing RNA molecule. Two phosphates are cleaved from the incoming ribonucleoside triphosphate; the remaining phosphate participates in a phosphodiester bond that connects the nucleotide to the growing RNA molecule.

RNA is synthesized from ribonucleoside triphosphates. Transcription is 5'→3'. each new nucleotide is joined to the 3'-OH group of the last nucleotide added to the growing RNA molecule. RNA synthesis does not require a primer.



13.7 **A transcription unit includes a promoter, an RNA-coding region, and a terminator.**



13.9 In transcription, nucleotides are always added to the 3′ end of the RNA molecule.

RNA Polymerases

Bacterial RNA polymerase

- Bacterial cells typically possess one type of RNA polymerase, which catalyzes the synthesis of all classes of bacterial RNA: mRNA, tRNA, and rRNA. Bacterial RNA polymerase is a large enzyme consists of several polypeptide chains.
- Bacterial RNA polymerase composed of two unites Sigma factor and core enzyme. **the core enzyme** made up of four subunits: two copies of a subunit called alpha (α), a single copy of beta (ß), and single copy of beta prime (ß'). The core enzyme catalyzes the elongation of the RNA molecule by the addition of RNA nucleotides. **The sigma (δ) factor** controls the binding of the RNA polymerase to the promoter. Without sigma, RNA polymerase will initiate transcription at a random point along the DNA. After sigma has associated with the core enzyme (forming a holoenzyme), **RNA polymerase binds stably only to the promoter region** and initiates transcription at the proper start site. Sigma is required only for promoter binding and initiation; when a few RNA nucleotides have been joined together, sigma detaches from the core enzyme.

Eukaryotic RNA polymerases

- Eukaryotic cells possess three distinct types of RNA polymerase, each of which is responsible for transcribing a different class of RNA: **RNA polymerase I** transcribes rRNA; **RNA polymerase II** transcribes pre-mRNAs, snoRNAs, and some snRNAs; and **RNA**

polymerase III transcribes small RNA molecules—specifically tRNAs, small rRNA, and some snRNAs (Table 13.3).

- All three eukaryotic polymerases are large, typically consisting of more than a dozen subunits. Some subunits are common to all three RNA polymerases, whereas others are limited to one of the polymerases. As in bacterial cells, a number of accessory proteins bind to the core enzyme and affect its function.

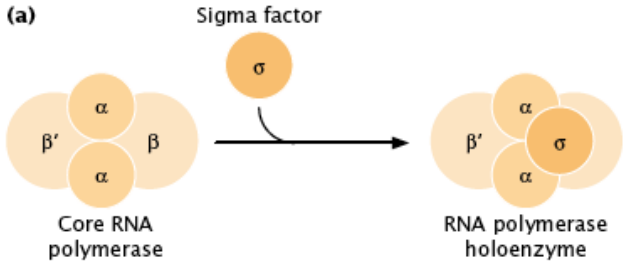


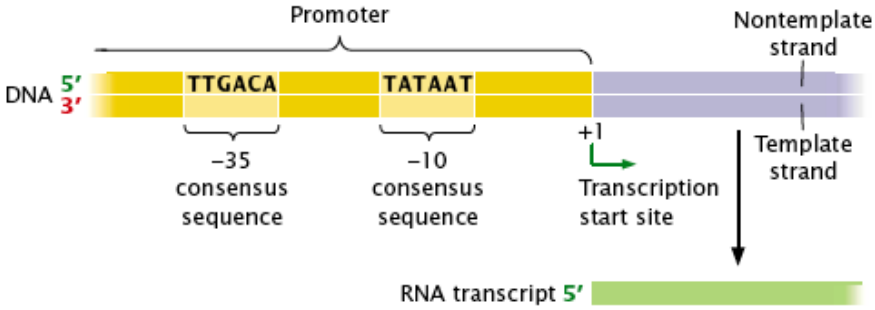
Table 13.3 Eukaryotic RNA polymerases

Type	Transcribes
RNA polymerase I	Large rRNAs
RNA polymerase II	Pre-mRNA, some snRNAs, snoRNAs
RNA polymerase III	tRNAs, small rRNA, snRNAs

The Process of Bacterial Transcription

1-Inititation

- Transcription initiation requires the polymerase recognize and bind to the promoter in DNA strand.
- Promoters are sequences in the DNA that are recognized by the transcription apparatus and are required for transcription to take place.
- The most commonly **consensus sequence**, found in almost all bacterial promoters, is located just upstream of the start site, centered on position -10. Called the **-10 consensus sequence** or, sometimes, the **Pribnow box**, its sequence **5' TATAAT 3'**
- Another consensus sequence common to most bacterial promoters is TTGACA, which lays approximately 35 nucleotides upstream of the start site and is termed the **-35 consensus sequence.**
- The sigma factor associates with the core enzyme to form a holoenzyme, which binds to the -35 and -10 consensus sequences in the DNA promoter that are important in the initiation of transcription.



13.11 In bacterial promoters, consensus sequences are found upstream of the start site, approximately at positions −10 and −35.

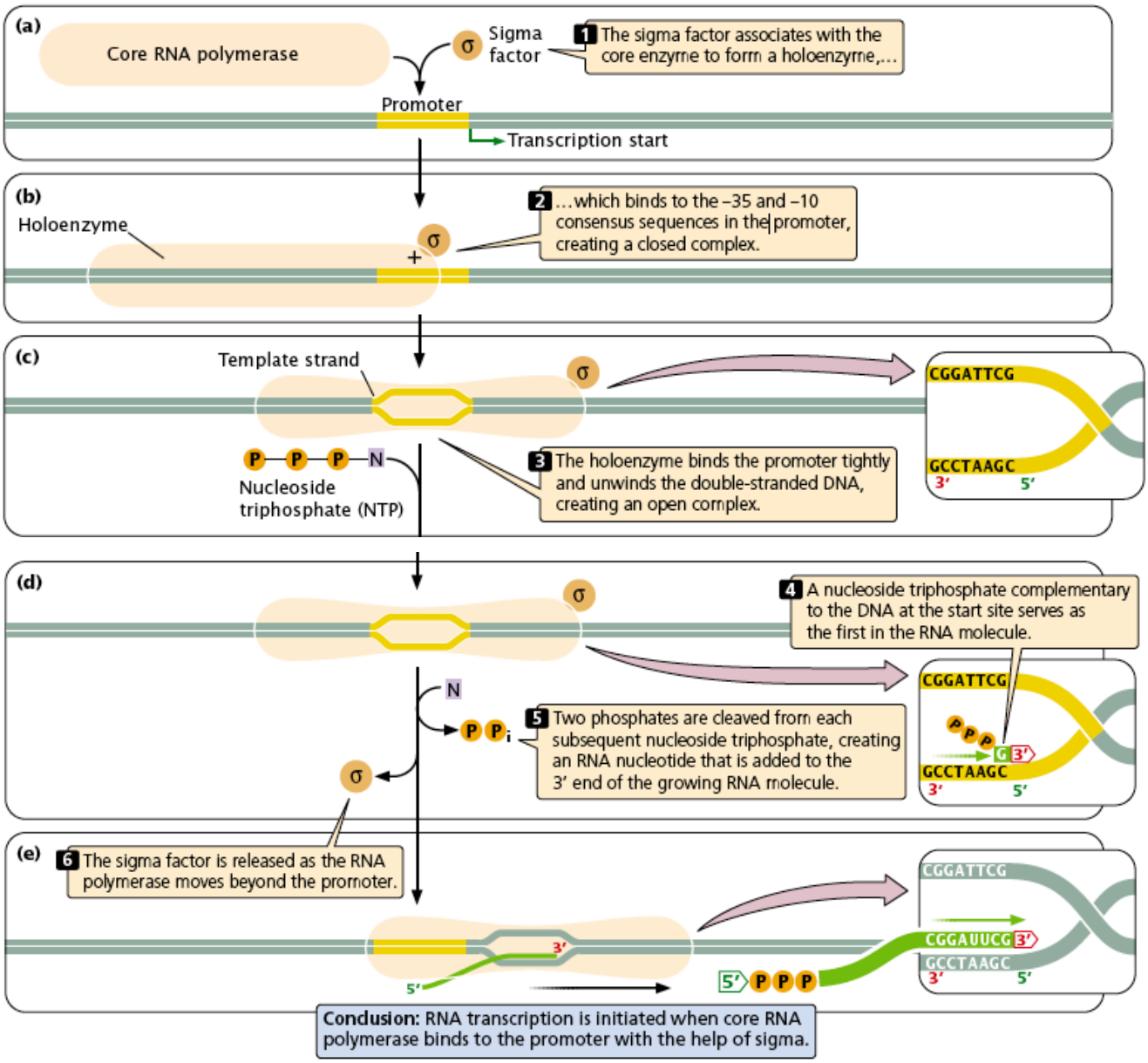
- After the holoenzyme has attached to the promoter, RNA polymerase is positioned over the start site for transcription (at position +1) and has unwound the DNA to produce a single-stranded template. RNA polymerase pairs the base on a ribonucleoside triphosphate with its complementary base at the start site on the DNA template strand.

2. Elongation

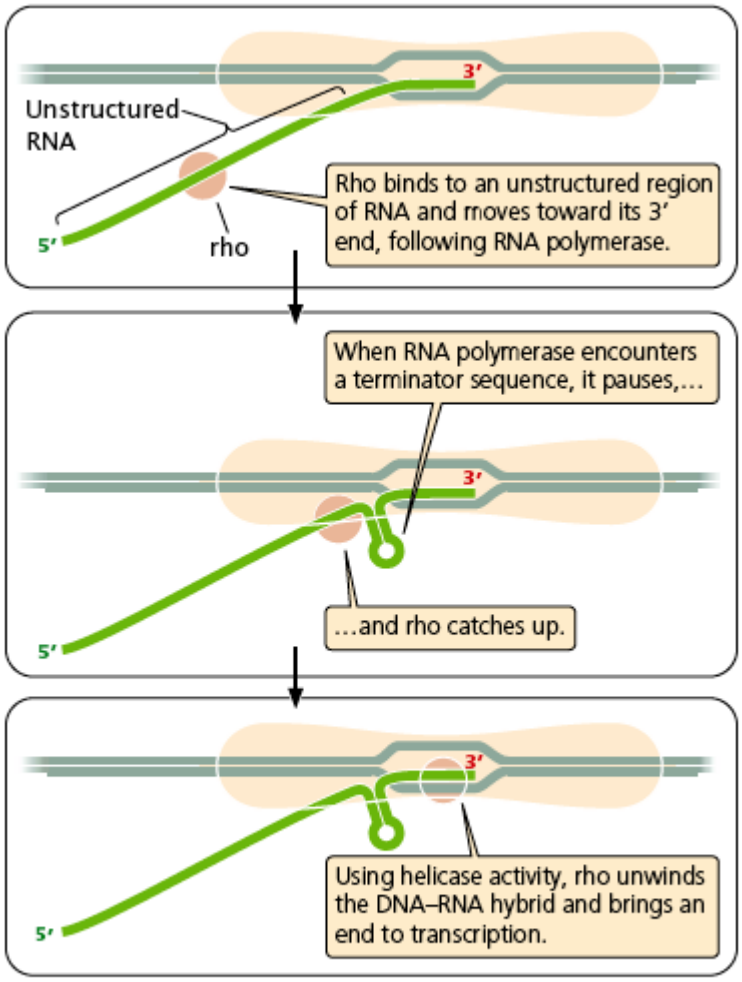
- After initiation, RNA polymerase moves downstream along the template. RNA is continuously synthesized, with single-stranded DNA used as a template.
- Elongation by the polymerase continues at a rate between 30 and 50 nucleotides per sec.

3. Termination

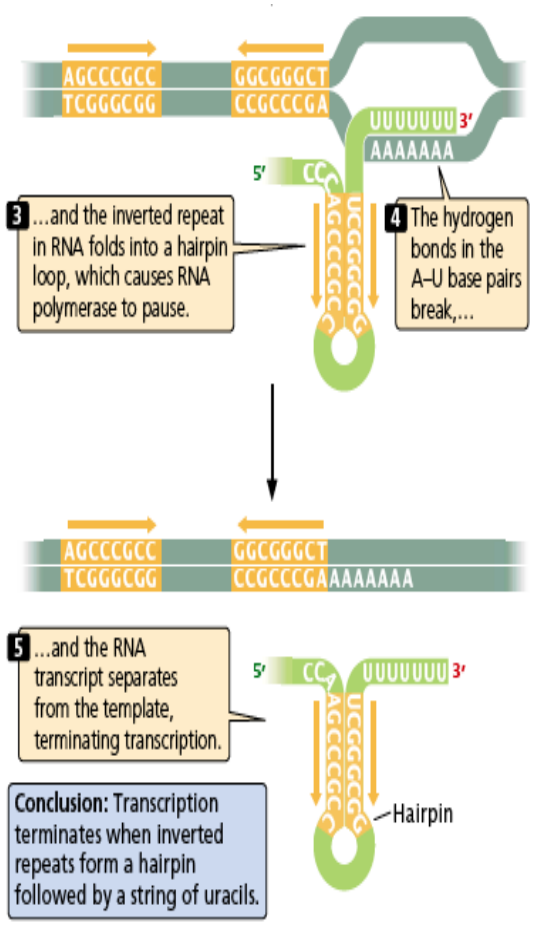
- RNA polymerase moves along the template, adding nucleotides to the 3' end of the growing RNA molecule until it transcribes a terminator.
- At the terminator, several overlapping events are needed to bring an end to transcription:
  1. RNA polymerase must stop synthesizing RNA,
  2. the RNA molecule must be released from RNA polymerase,
  3. The newly made RNA molecule must dissociate fully from the DNA, and
  4. RNA polymerase must detach from the DNA template.
- Bacterial cells possess two major types of terminators. **Rho-dependent terminators** are able to cause the termination of transcription only in the presence of an ancillary protein called the rho factor. **Rho-independent terminators** are able to cause the end of transcription in the absence of rho.
- Rho-independent terminators contain **inverted repeats** (sequences of nucleotides on one strand that are inverted and complementary).When inverted repeats have been transcribed into RNA, a **hairpin secondary structure** forms which causes RNA polymerase to pause.



13.13 Transcription in bacteria is carried out by RNA polymerase, which must bind to the sigma factor to initiate transcription.



13.15 The termination of transcription in some bacterial genes requires the presence of the rho protein.



13.14 Termination by bacterial rho-independent terminators is a multistep process.

The Basic Rules of Transcription

1. Transcription is a selective process; only certain parts of the DNA are transcribed.
2. RNA is transcribed from single-stranded DNA. Normally, only one of the two DNA strands—the template strand—is copied into RNA.
3. Ribonucleoside triphosphates are used as the substrates in RNA synthesis. Two phosphates are cleaved from a ribonucleoside triphosphate, and the resulting nucleotide is joined to the 3'-OH group of the growing RNA strand.
4. RNA molecules are antiparallel and complementary to the DNA template strand. Transcription is always in the 5'→3' direction, meaning that the RNA molecule grows at the 3' end.

5. Transcription depends on RNA polymerase—a complex multimeric enzyme. RNA polymerase consists of a core enzyme, which is capable of synthesizing RNA, and other subunits that may join transiently to perform additional functions.
6. The core enzyme of RNA polymerase requires a sigma factor in order to bind to a promoter and initiate transcription.
7. Promoters contain short sequences important in the binding of RNA polymerase to DNA; these consensus sequences are interspersed with nucleotides that play no known role in transcription.
8. RNA polymerase binds to DNA at a promoter, begins transcribing at the start site of the gene, and ends transcription after a terminator has been transcribed.

The Genetic Code and Translation

How many nucleotides are necessary to specify a single amino?

Three nucleotides per codon, there are 4*4*4=64 possible codon, which Therefore, ***a triplet requiring three nucleotides per codon*** is the most efficient way to encode all **20 amino acids.** Thus, 61 codons, called **sense codons**, code for amino acids and **three of these codons are called** *stop codons, termination* **codon**, or *nonsense codons* (**UAA, UAG, and UGA**) do not encode amino acids, specifying the end of translation. The code contains more information than is needed to specify the amino acids and is said to be a degenerate code. The degeneracy of the genetic code mean that **amino acids may be specified by more than one codon**.

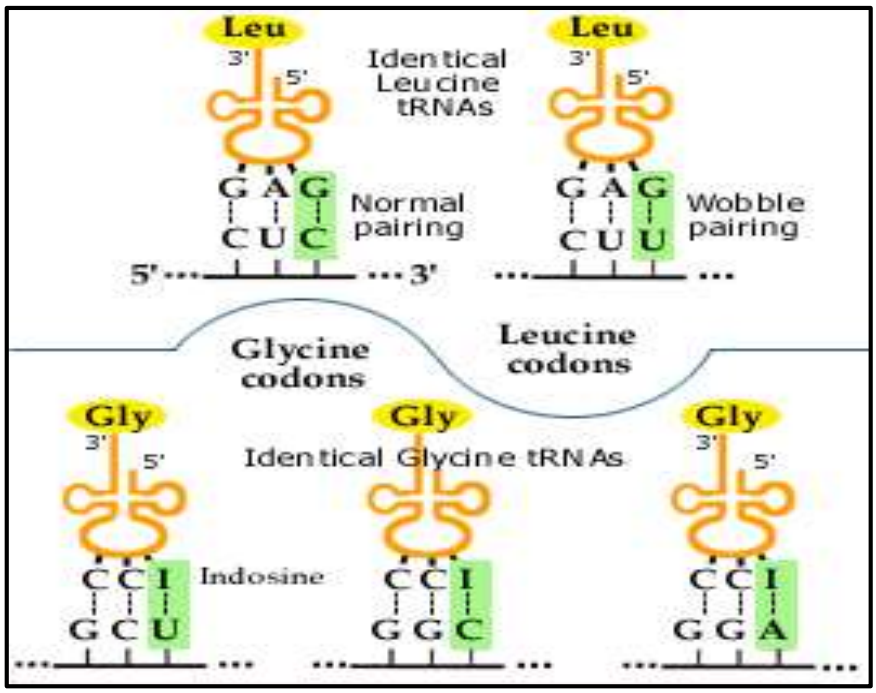
- Only tryptophan and methionine are encoded by a single codon,
- Others amino acids are specified by two codons,
- Some, such as leucine, are specified by six different codons.

Codons that specify the same amino acid are said to be **Synonymous**.

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phe UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr UAA: Stop UAG: Stop	UGU, UGC: Cys UGA: Stop UGG: Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile AUG: Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn AAA, AAG: Lys	AGU, AGC: Ser AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

Isoaccepting tRNAs:

Each tRNAs serve as adapter molecules, binding particular single amino acids and delivering them to a ribosome, where the amino acids are then assembled into polypeptide chains. The cells of most organisms possess from about 30 to 50 different tRNAs, Thus, some amino acids are carried by more than one tRNA. **Different tRNAs that accept the same amino acid but have diiferent anti-codons are called isoaccepting tRNAs**



Wobble: Many synonymous codons differ only in the third position. For example, alanine is encoded by codons GCU, GCC, GCA, and GCG, all begin GC. When the codon on the mRNA and the anticodon of the tRNA join.

- the first (5') base of the codon pairs with the third base (3') of the anticodon.
- Next, the middle bases of codon and anticodon pair,
- the third bases pair weakly there may be flexibility, or wobble, in their pairing.

The wobble hypothesis, which proposed that some **nonstandard pairings** of bases could occur at the third position of a codon. **For example**, a G in the anticodon may pair with either a C or U in the third position of the codon. **The important thing to remember about wobble is that it allows some tRNAs to pair with more than one codon on an mRNA; thus from 30 to 50 tRNAs can pair with 61 sense codons. Some codons are synonymous through wobble.**

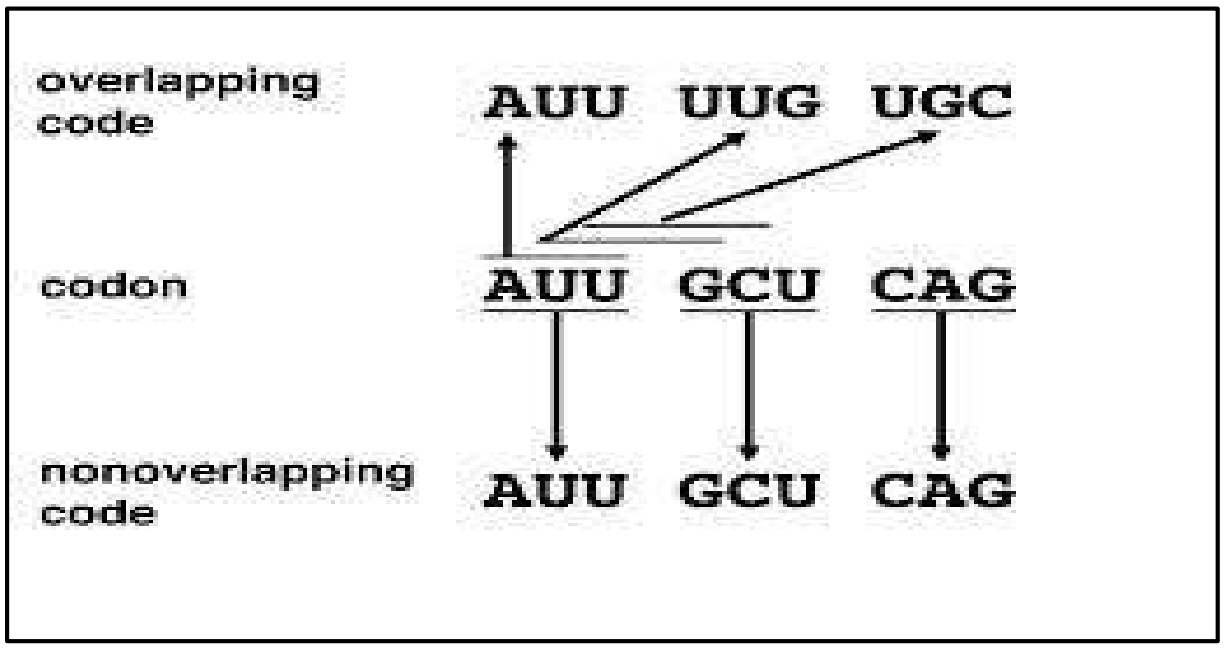
The Reading Frame and Initiation Codons

The genetic code indicated that it is generally non-overlapping. A few overlapping codes are found in viruses; in these cases, two different proteins may be encoded within the same sequence of mRNA. The translational machinery to use the correct reading frame.

How is the correct reading frame established?

The reading frame is set by the initiation codon, which is the first codon of the mRNA to specify an amino acid. After the initiation codon, the other codons are read as successive groups of three nucleotides. No bases codons; so there separate the codons. **The initiation codon is usually AUG. although GUG and UUG are used on rare occasions.**

The initiation codon is not just a punctuation mark; it specifies an amino acid. **In bacterial cells**, **AUG encodes a modified type of methionine**, **N-formylmethionine**; all proteins in bacteria begin with this amino acid, **but the formyl group may be nucleotide belongs to only one codon.** When the codon AUG is at an internal position in a gene, it **codes for un-formylated methionine.**



The Process of Translation

Translation takes place on ribosomes, **when attaches near the 5' end of an mRNA strand moves toward the 3' end**, translating the codons as it goes. Synthesis begins at the amino end of the protein, and the protein is elongated by the addition of **new amino acids to the carboxyl end.**

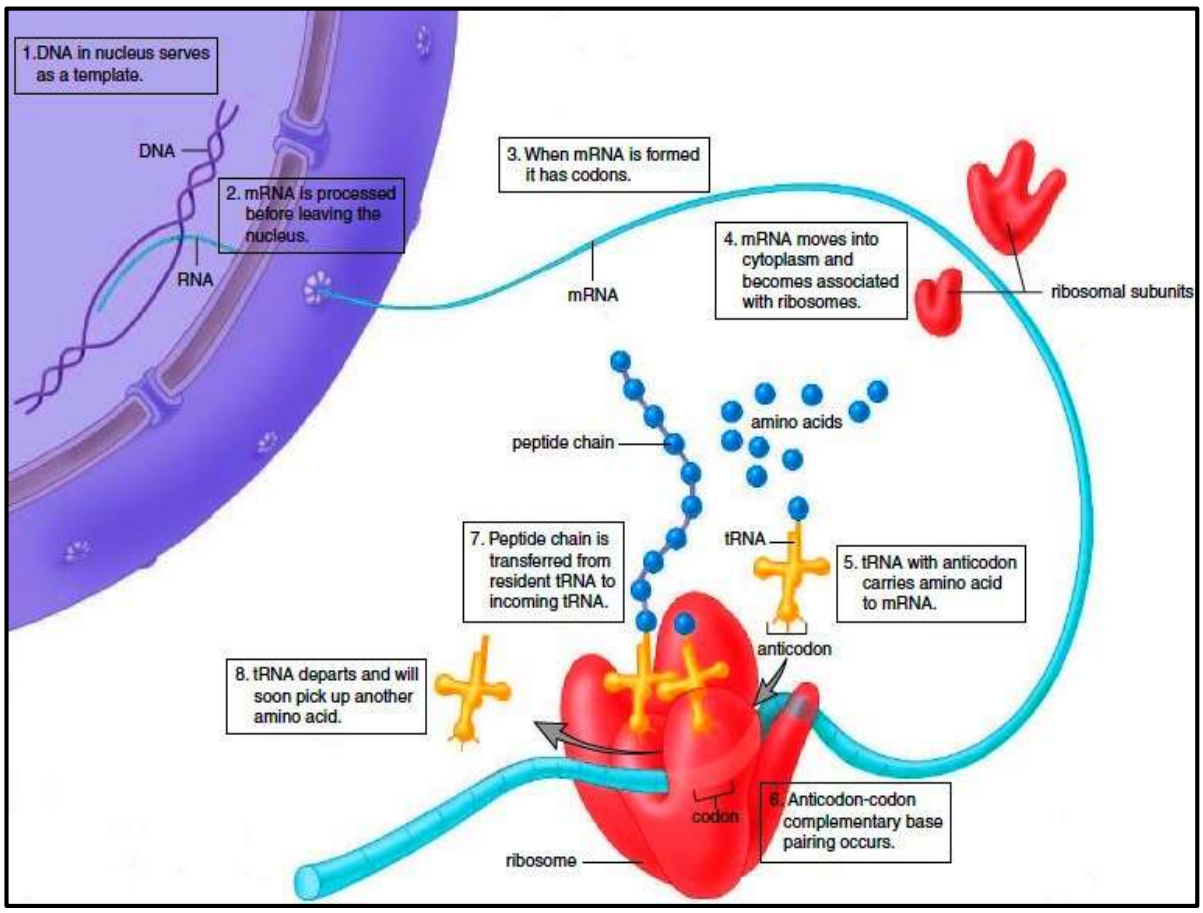
Protein synthesis can be conveniently divided into four stages:

1- The binding of amino acids to the tRNAs.

2- Initiation, in which the components necessary for translation are assembled at the ribosome.

3- Elongation, in which amino acids are joined, one at a time, to the growing polypeptide chain.

4- Termination, in which protein synthesis halts at the termination codon and the translation components are released from the ribosome.

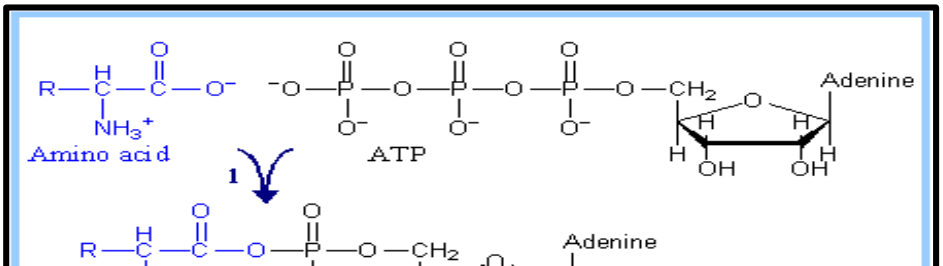


The Binding of Amino Acids to Transfer RNAs

The first stage of translation is the binding of tRNA molecules to delivers their appropriate amino acids to the ribosome, where the tRNA's anticodon pairs with a codon on mRNA. This process enables the amino acids to be joined in the order specified by the mRNA. Proper translation, then, **first requires the corcect binding of tRNA and amino acid.**

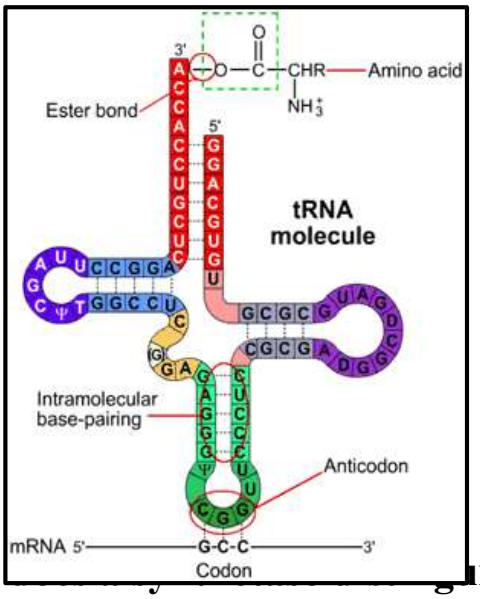
A cell typically possesses from 30 -50 different tRNAs, and these tRNAs are attached to the 20 different amino acids.

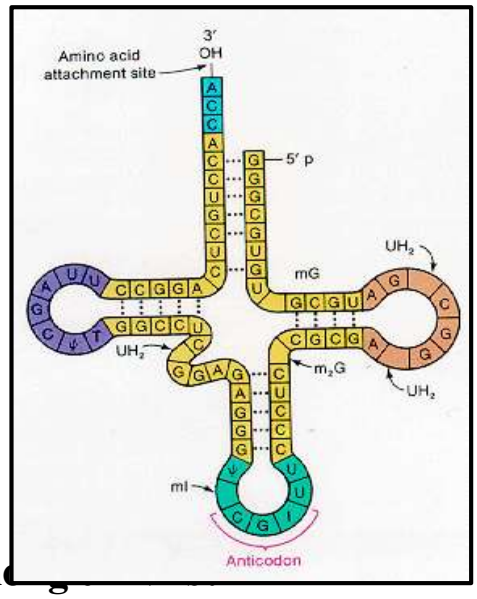
Each tRNA is specific for a particular kind of amino acid. **All tRNAs have the sequence CCA at the 3'end; and the carboxyl group (COO-) of the amino acid is attached to the 3- hydroxyl group of the adenine nucleotide at the end of the tRNA, all amino acids are attached to the same nucleotide (A) at the 3' end of a tRNA.**



How does a tRNA link up with its appropriate amino acid?

The key to specificity between an amino acid and its tRNA is a set of enzymes called **aminoacyl-tRNA synthetases.** A cell has 20 different aminoacyl-tRNA synthetases, one for each of the 20 amino acids. Each synthetase recognizes a particular amino acid, as well as all the tRNAs that accept that amino acid. **Recognition of the appropriate amino acid by a synthetase is based on the different sizes, charges, and R groups of the amino acids. The tRNAs, however, are all similar in tertiary structure.**





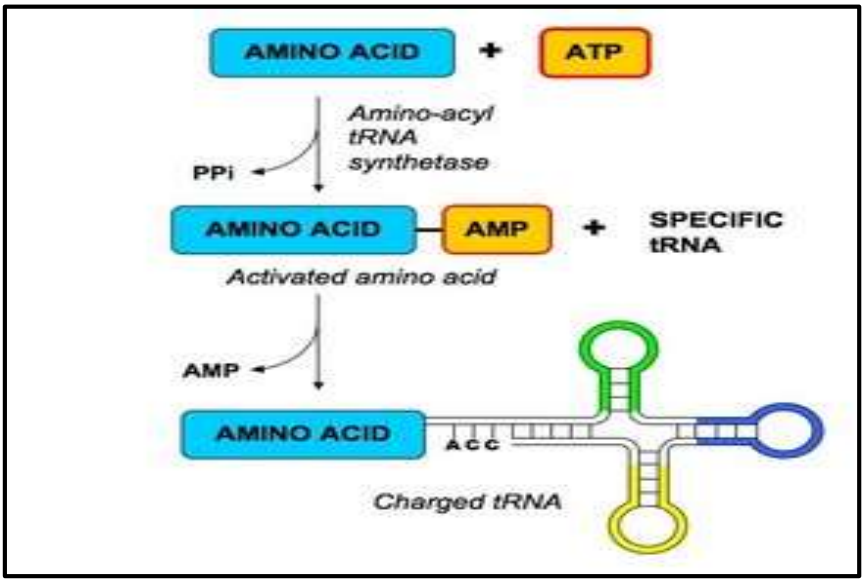
How ish am

The recognition of tRNAs by a synthetase depends **on the differing nucleotide sequences of tRNAs. the anticodon loop, the DHU-loop, and the acceptor stem are particularly critical for the identification of most tRNAs.**

The attachment of a tRNA to its appropriate amino acid (**termed tRNA charging) requires energy, which is supplied by adenosine triphosphate (ATP):** Two phosphates are cleaved from ATP, producing adenosine monophosphate (AMP) and pyrophosphate (PPi), as well as the aminoacylated tRNA (the tRNA with its attached amino acid).

Amino acid + tRNA + ATP → aminoacyl-tRNA + AMP + PP

To identify the resulting aminoacylated tRNA. we write the three-letter abbreviation for the amino acid in front of the tRNA; for example, the amino acid alanine (Ala) attaches to its tRNA (tRNAAla), giving rise to its aminoacyl-tRNA (Ala-tRNAAla).



Errors in tRNA charging are rare; they occur in only about **1 in 10,000 to 1 in 100,000** reactions. This fidelity is due to the presence of **proofreading activity** in the synthetases, which detects and removes incorrectly paired amino acids from the tRNAs.

The Initiation of Translation

The second stage in the process of protein synthesis is initiation. During initiation, all the components necessary for protein synthesis assemble:

- **mRNA**
- **The small and large subunits of the ribosome 30s and 50s**
- **A set of three proteins called initiation factors**
- **Initiator tRNA with N- formylmethionine attached (fMet- tRNAfMet)**
- **Guanosine triphosphate (GTP).**

Initiation comprises three major steps.

- mRNA binds to the small subunit of the ribosome.
- initiator tRNA binds to the mRNA through base pairing between the codon and anticodon.
- Third, the large ribosome joins the initiation complex.

An mRNA molecule can bind to the small ribosome subunit only when the subunits are separate. **Initiation factor 3 (IF-3)** binds to the small subunit of the ribosome and **prevents the large subunit from binding during initiation**.

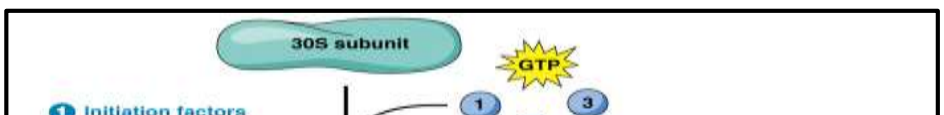
During initiation, the nucleotide sequence pair with their complementary nucleotides in the 16S rRNA, allowing the small subunit of the ribosome to attach to the mRNA and positioning the ribosome directly over the initiation codon.

Next, the initiator fMet-tRNAfMet attaches to the initiation codon. This step requires **initiation factor 2 (IF-2)**, which forms a complex with GTP.

A third factor, **initiation factor 1 (IF-1)**, enhances the dissociation of the large and small ribosomal subunits. At this point, **the initiation complex consists of:**

- the small subunit of the ribosome
- the mRNA
- the initiator tRNA with its amino acid (fMet-tRNAfMet);
- one mclecule of GTP
- IF-3, IF-2, and IF-1. These components are collectively known as **the 30S initiation complex.**

In the final step of initiation, **IF-3 dissociates from the small subunit, allowing the large subunit of the ribosome to join the initiation complex**. The molecule of GTP (provided by IF-2) is hydrolyzed to guanosine diphosphate (GDP), and IF-1 and



IF-2 depart. When the large subunit has joined the initiation complex, it is called **the 70S initiation complex.**

The Elongation of Translation

The next stage in protein synthesis is elongation. Which amino acids are joined to create a polypeptide chain.

Elongation requires:

1- the 70S complex just described
2- tRNAs charged with their amino acids
3- several elongation factors (EF-Ts, EF-Tu. and EF-G)
4- GTP.

A ribosome has three sites that can be occupied by tRNAs; **The aminoacyl, or A site, the peptidyl or P site,** and **the exit, or E site.**

The initiator tRNA immediately occupies the p site (the only site to which the fMet-tRNAfMet is capable of binding), but all other tRNAs first enter the A site. After initiation, the ribosome is attached to the mRNA, and fMet-tRNAfMet is positioned over the **AUG start codon** in the **P** site; the adjacent **A** site is unoccupied.

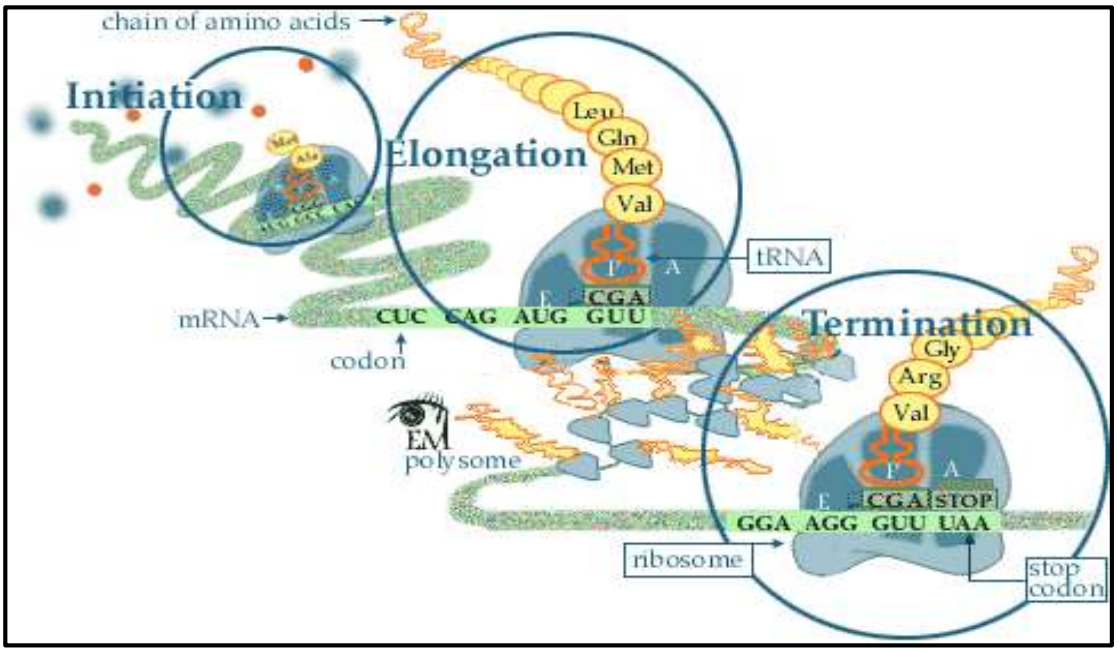
Elongation occurs in three steps. The first step is the delivery of a charged tRNA (tRNA with its amino acid attached) to the A site. This requires the presence of **elongation factor Tu (EF-Tu), elongation factor Ts (EF-Ts), and GTP. EF-Tu first joins with GTP and then binds to a charged tRNA to form a three-part complex**. This three-part complex enters the **A** site of the ribosome, where the anticodon on the tRNA pairs with the codon on the mRNA. After the charged tRNA is in the **A** site, GTP is cleaved to GDP, and the EF-Tu-GDP complex is released. Factor EP-Ts regenerates EF-Tu-GDP to EF-Tu-GTP.

The second step of elongation is the creation of a peptide bond between the amino acids that are attached to tRNAs in the **P** and **A** sites. The formation of this peptide bond releases the amino acid in the **P** site from its tRNA. The activity responsible for peptide bond formation in the ribosome is referred to **as peptidyl transferase.**

The third step in elongation is translocation, the movement of the ribosome down the mRNA in the 5' → 3' direction. This step positions the ribosome over the next codon and requires elongation factor G (EF-G) and the hydrolysis of GTP to GDP.

Because the tRNAs in the **P and A site** are still attached to the mRNA through codon- anticodon pairing, they do not move with the ribosome as it translocate. Consequently, the ribosome shifts so that the tRNA that previously occupied the **P site**

now occupies the **E site,** from which it moves into the cytoplasm where it may be recharged with another amino acid. Translocation also causes the tRNA that occupied the **A** site (which is attached to the growing polypeptide chain) to be in the **P** site, leaving the **A** site open, the initiator tRNA is an exception: it attaches directly to the p site and never occupies the **A** site.



The Termination of Translation

Protein synthesis terminates when the ribosome translocates to a **termination codon**. Because there are no trainees with anticodons complementary to the termination codons, no tRNA enters the A site of the ribosome when a termination codon is encountered. Instead, proteins called **Release factors (RF)** bind to the ribosome. *E.coli* has three release factors—**RF1, RF2 and RF3**.

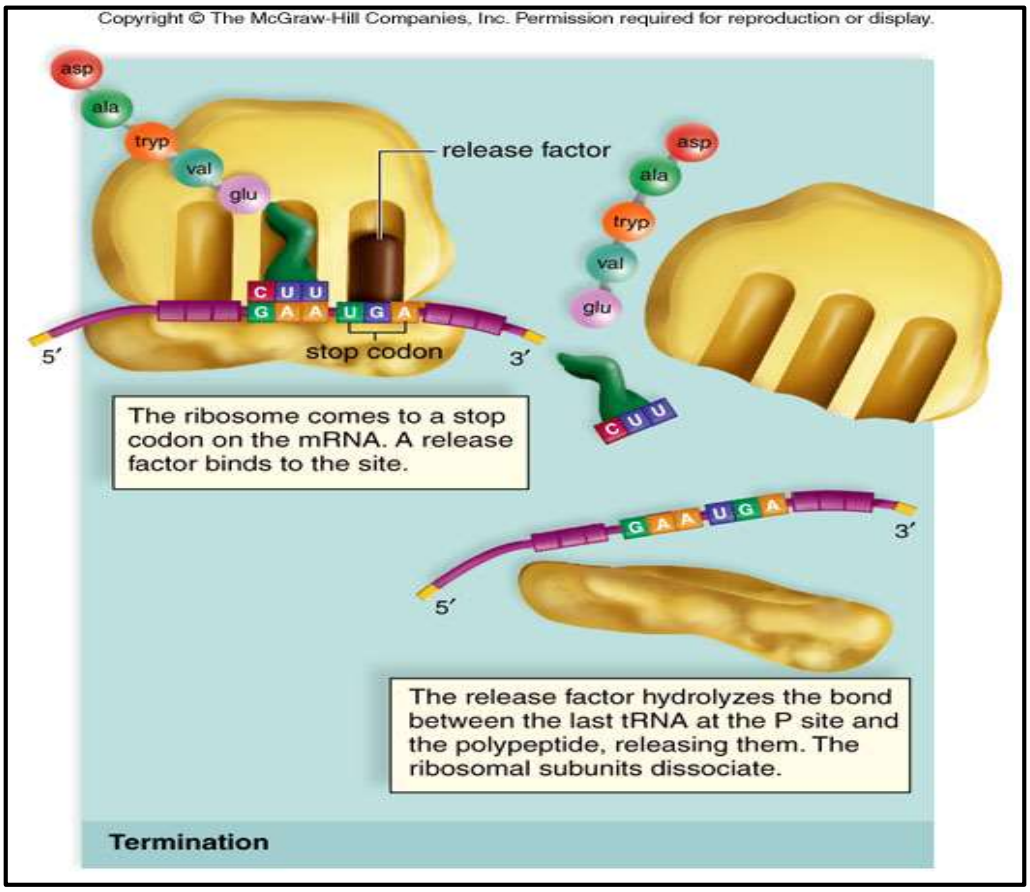
1-Release factor 1 recognizes tire termination codons UAA and UAG.

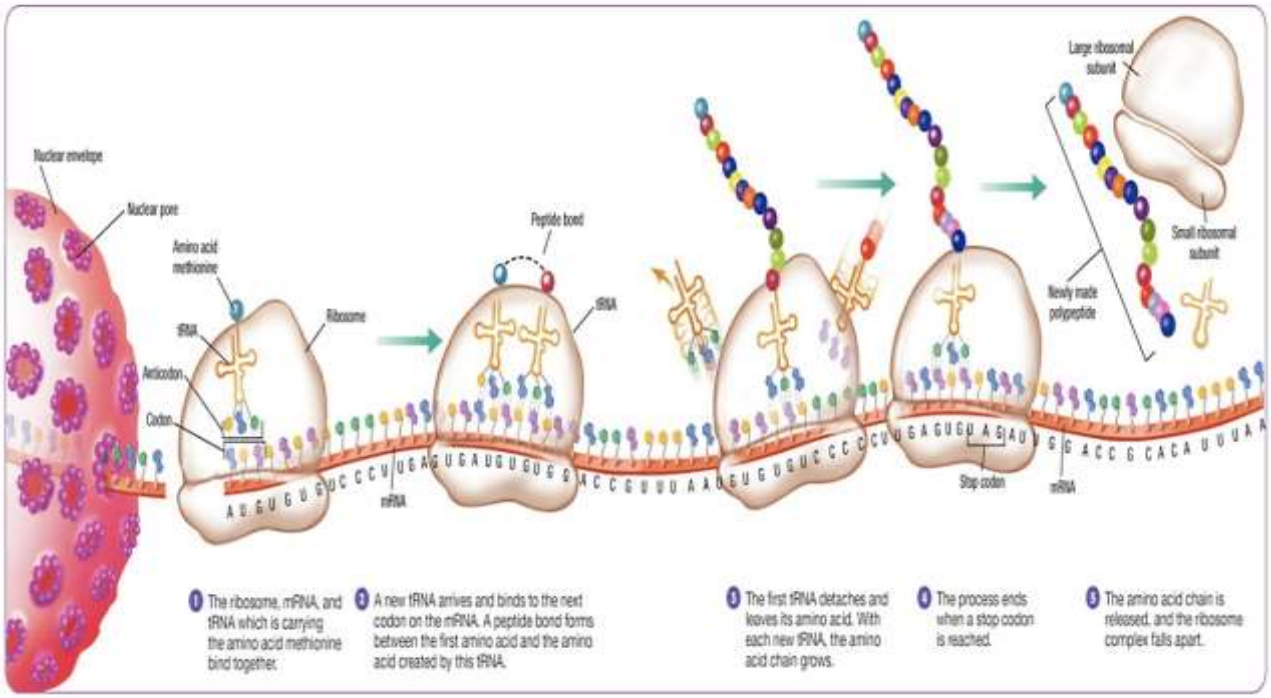
2-RF2 recognizes UGA and UAA.

3-RF 3 forms a complex with GTP and binds to the ribosome.

The release factors then promote the cleavage of the tRNA in the **P** site from the polypeptide chain; in the process, the GTP that is complex to RF3 is hydrolyzed to GDP.

Additional factors help bring about the release of the tRNA from the **P** site, the release of the mRNA from the ribosome, and the dissociation of the ribosome.



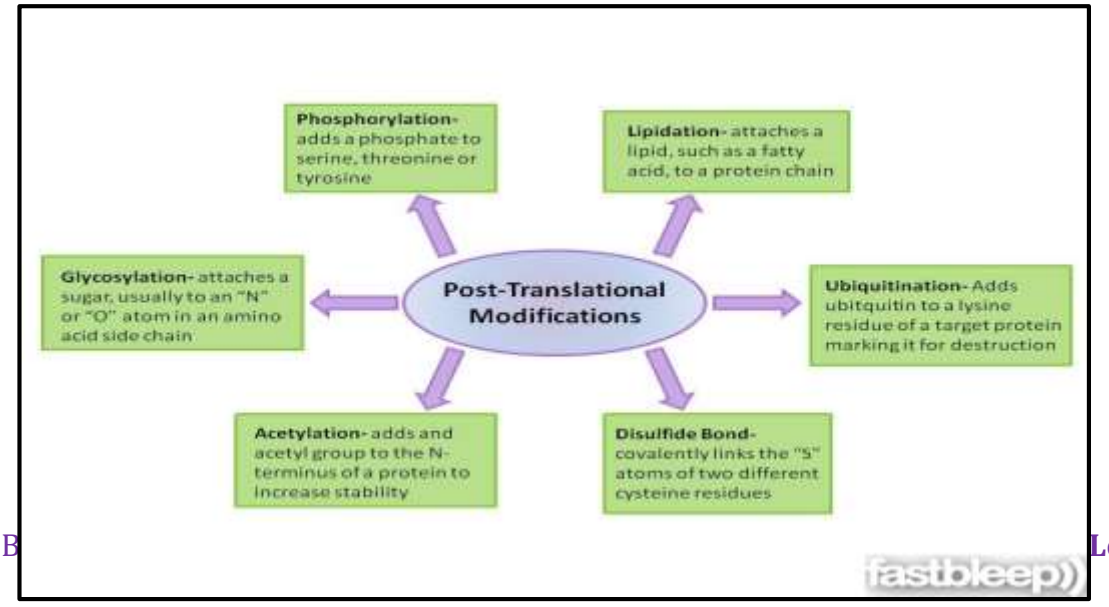


The Posttranslational Modifications of Proteins

After translation, proteins in both prokaryotic and eukaryotic cells may undergo alterations termed posttranslational modifications. A number of different types of modifications are possible. As mentioned earlier, the fomryl group or the entire methionine residue may be removed from the amino end of a protein. Some proteins are synthesized as larger precursor proteins and must be cleaved and trimmed by enzymes before the proteins can become functional. For others, the attachment of carbohydrates may be required for activation.

The functions of many proteins depend critically on the proper folding of the polypeptide chain; some proteins spontaneously fold into their correct shapes, but, for others, correct folding may initially require the participation of other molecules called molecular chaperones. In eukaryotic cells, the amino end of a protein is often acetylated after translation.

Another modification of some proteins is the removal of 15 to 30 amino acids, called the **signal sequence**, at the amino end of the protein. The signal sequence helps direct a protein to a specific location within the cell, after which the sequence is removed by special enzymes. Amino acids within a protein may also be modified: phosphates, carboxyl groups, and methyl groups are added to some amino acids.



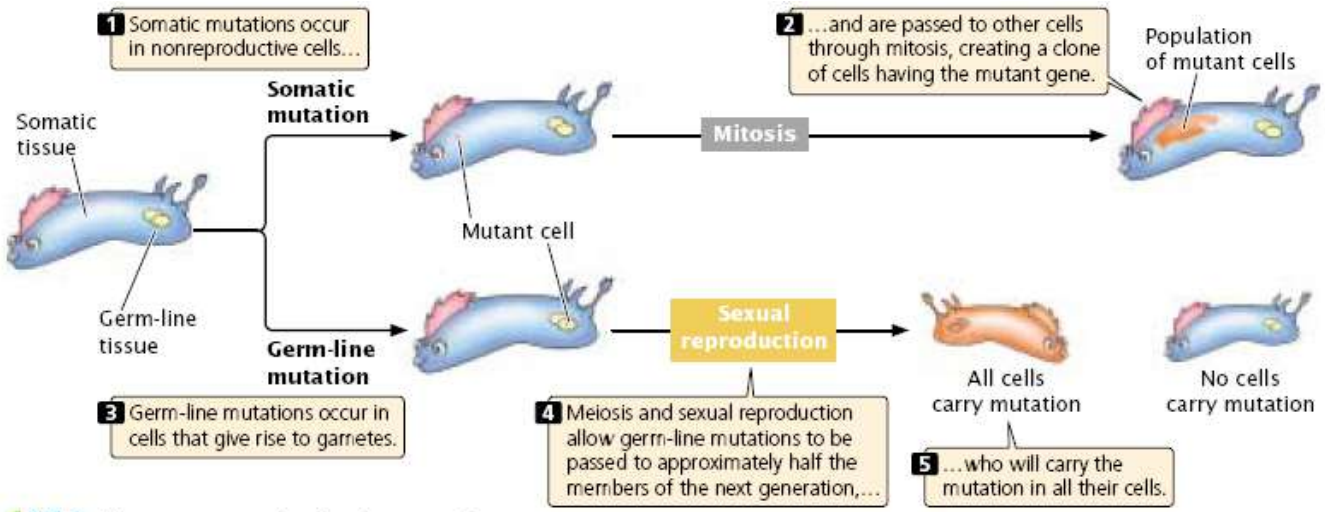
Mutations are heritable changes in the genetic coding instructions of DNA. They are essential to the study of genetics and are useful in many other biological fields.

Categories of Mutations

In multicellular organisms, we can distinguish between two broad categories of mutations: somatic mutations and germline mutations. **Somatic mutations** arise in somatic tissues, which do not produce gametes. These mutations are passed on to other cells through the process of mitosis, which leads to a population of genetically identical cells (a clone).

The effect of these mutations depends on many factors, including the type of cell in which they occur and the developmental stage at which they arise. Many somatic mutations have no obvious effect on the phenotype of the organism, because the function of the mutant cell (even the cell itself) is replaced by that of normal cells. However, cells with a somatic mutation that stimulates cell division can increase in number and spread; this type of mutation can give rise to cells with a selective advantage and is the basis for all cancers.

Germ-line mutations arise in cells that ultimately produce gametes. These mutations can be passed to future generations, producing individual organisms that carry the mutation in all their somatic and germ-line cells.

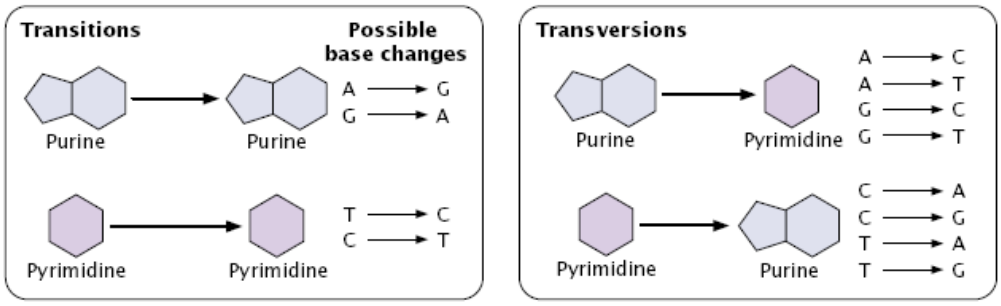


17.2 There are two basic classes of mutations: somatic mutations and germ-line mutations.

Types of Gene Mutations

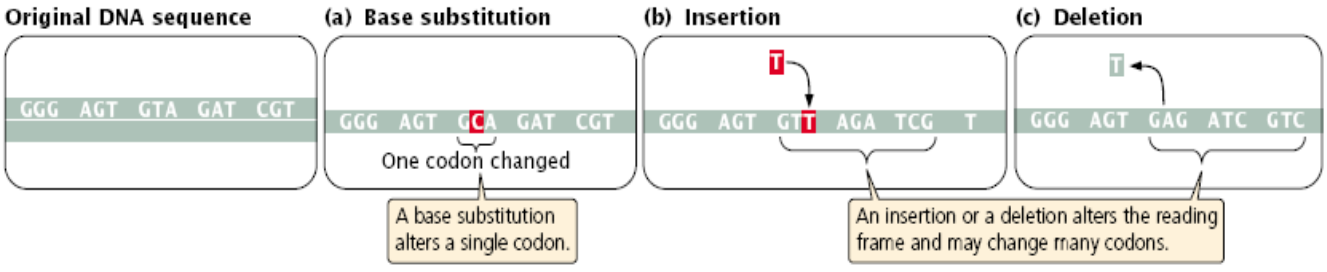
There are a number of ways to classify gene mutations. Some classification schemes are based on the nature of the phenotypic effect (mutation alters the amino acid sequence of the protein). Other schemes are based on the causative agent of the mutation, and still others focus on the molecular nature of the defect. The most appropriate scheme depends on the reason for studying the mutation. Here, we will categorize mutations primarily on the basis of their molecular nature, but we will also encounter some terms that relate the causes and the phenotypic effects of mutations.

a. **Base substitutions**: The simplest type of gene mutation is a base substitution, the alternation of a single nucleotide in the DNA. Base substitutions are of two types. In a **transition**, a purine is replaced by a different purine or, alternatively, a pyrimidine is replaced by a different pyrimidine. In a **transversion**, a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine.



17.4 A transition is the substitution of a purine for a purine or a pyrimidine for a pyrimidine; a transversion is the substitution of a pyrimidine for a purine or a purine for a pyrimidine.

b. **Insertions and deletions:** The second major class of gene mutations contains insertions and deletions—the addition or the removal, respectively, of one or more nucleotide pairs. Insertions and deletions within sequences that encode proteins may lead to **frame shift mutations**, changes in the reading frame of the gene.

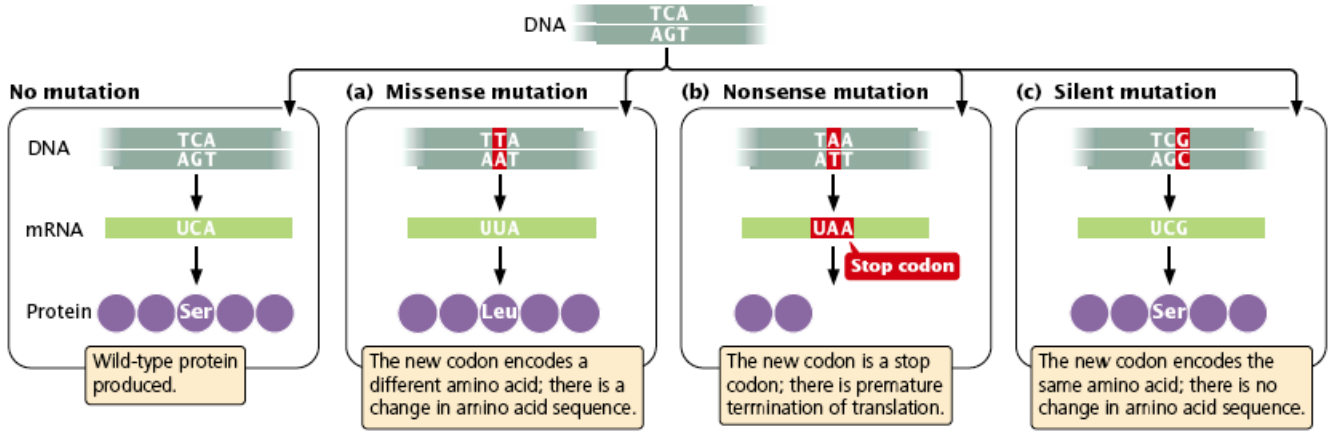


17.3 Three basic types of gene mutations are base substitutions, insertions, and deletions.

c. **Missense mutation** is a base substitution that alters a codon in the mRNA, resulting in a different amino acid in the protein.

d. **Nonsense mutation** changes a sense codon (one that specifies an amino acid) into a nonsense codon (one that terminates translation). If a nonsense mutation occurs early in the mRNA sequence, the protein will be greatly shortened and will usually be nonfunctional.

e. **Silent mutation** alters a codon but the codon still specifies the same amino acid.



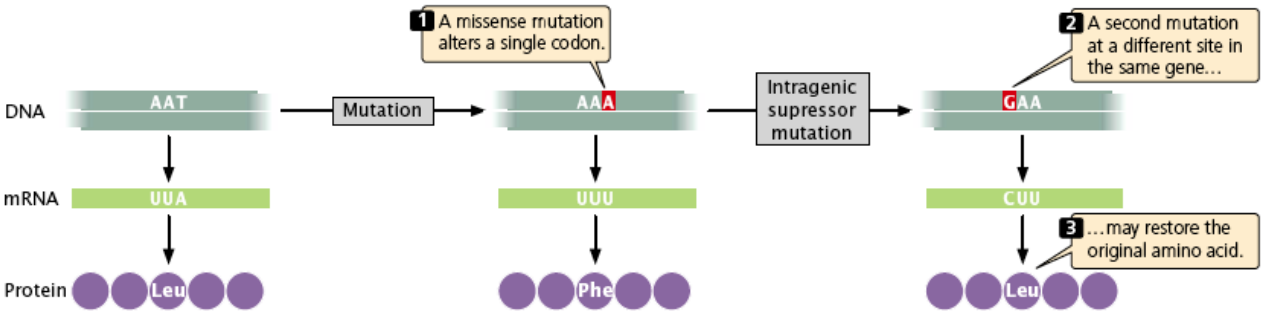
17.7 **Base substitutions can cause (a) missense, (b) nonsense, and (c) silent mutations.**

Suppressor mutation is a genetic change that hides or suppresses the effect of another mutation. A suppressor mutation occurs at a site that is distinct from the site of the original mutation; thus, an individual organism with a suppressor mutation is a double mutant, possessing both the original mutation and the suppressor mutation but exhibiting the phenotype of an unmutated wild type.

Geneticists distinguish between two classes of suppressor mutations: intragenic and intergenic.

1. Intragenic suppressor is in the same gene as that containing the mutation being suppressed.

2. Intergenic suppressor, in contrast, occurs in a gene that is different from the one bearing the original mutation.



17.9 **An intragenic suppressor mutation occurs in the same gene that contains the mutation being suppressed.**

Causes of Mutations

Mutations result from both **internal** and **external** factors. Those that are a result of natural changes in DNA structure are termed **spontaneous mutations,** whereas those that result from changes caused by environmental chemicals or radiation are **induced mutations**.

1. Spontaneous Chemical Changes

One such change is **depurination**, the loss of a purine base from a nucleotide. Depurination results when the covalent bond connecting the purine to the 1'-carbon atom of the deoxyribose sugar breaks. Another spontaneously occurring chemical change that takes place in DNA is **deamination**, the loss of an amino group (NH2) from a base. Deamination may occur spontaneously or be induced by mutagenic chemicals.

2. Chemically Induced Mutations

Although many mutations arise spontaneously, a number of environmental agents are capable of damaging DNA, including certain chemicals and radiation. Any environmental agent that significantly increases the rate of mutation above the spontaneous rate is called a **mutagen.**

1. Base analogs: One class of chemical mutagens consists of base analogs, chemicals with structures similar to that of any of the four standard bases of DNA. DNA polymerases cannot distinguish these analogs from the standard bases; so, if base analogs are present during replication, they may be incorporated into newly synthesized DNA molecules.

2. Alkylating agents Alkylating agents are chemicals that donate alkyl groups. These agents include methyl (CH3) and ethyl (CH3–CH2) groups, which are added to nucleotide bases by some chemicals.

3. Deamination In addition to its spontaneous occurrence, deamination can be induced by some chemicals.

4. Hydroxylamine .Hydroxylamine is a very specific base modifying mutagen that adds a hydroxyl group to cytosine, converting it into hydroxylaminocytosine.

5. Oxidative reactions Reactive forms of oxygen (including superoxide radicals, hydrogen peroxide, and hydroxyl radicals) are produced in the course of normal aerobic metabolism, as well as by radiation, ozone, peroxides, and certain drugs. These reactive forms of oxygen damage DNA and induce mutations by bringing about chemical changes to DNA.

6. Intercalating agents Intercalating agents, such as proflavin, acridine orange, ethidium bromide, and dioxin are about the same size as a nucleotide. They produce mutations by sandwiching themselves (intercalating) between adjacent bases in DNA.

3. Radiation

The high energies of X-rays, gamma rays, and cosmic rays are all capable of penetrating tissues and damaging DNA. Ultraviolet light causes mutations primarily by producing pyrimidine dimers that disrupt replication and transcription.

Transfer of Genetic Material

Plasmids

Plasmids are the extra chromosomal structures in the cells of bacteria which have the ability to self-replicate. They do not combine with the genetic material of the host cell but stay independent. They are genetically modified and are used in the recombinant DNA technology. Plasmids are usually made up of double stranded, non-chromosomal DNA, but in some cases they are circular. They make their structure circular by combining the two ends of the double stranded DNA together. These ends are combined through covalent bonds.

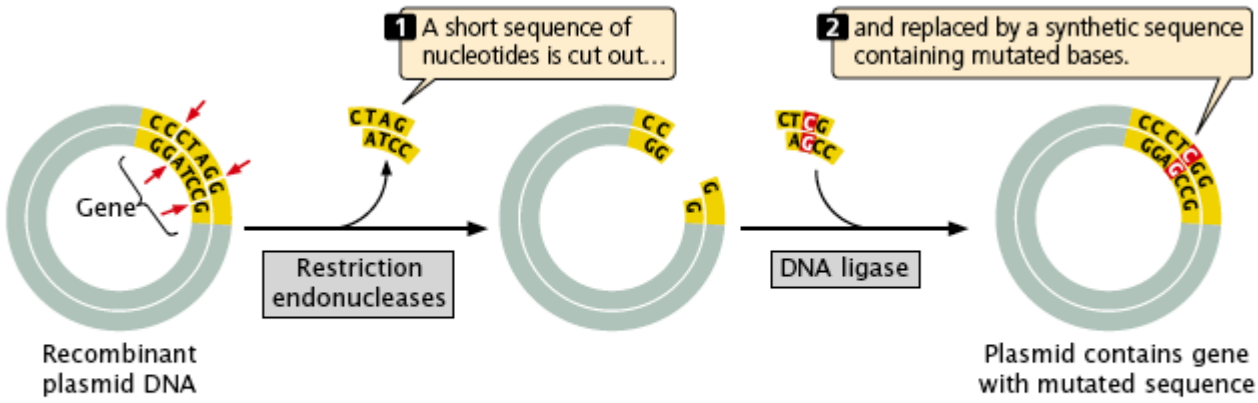
Plasmids Functions

1. The main function of plasmids is to carry antibiotic resistant genes and spread them in the whole human or animal body. In this way many diseases of humans and animals can be treated.

2. The other function of plasmids is to carry those genes which are involved in metabolic activities and are helpful in digesting the pollutants from the environment.

3. They are also capable of producing antibacterial proteins.

4. Plasmids also able to carry the genes which are concerned with increasing the pathogenicity of bacteria which cause diseases like anthrax and tetanus.



18.21 **In site-directed mutagenesis, restriction enzymes cut out a short sequence of nucleotides that is then replaced by a synthetic mutated DNA sequence.**

Types of plasmids

There are five types of plasmids which are used for different purposes.

1. Resistance Plasmids: This type of plasmids is involved in the bacterial conjugation. They usually carry those genes which code for the resistance of antibiotics or poisons. They also code for those genes which are responsible for the production of conjugation pili. The main role of conjugation pili is to transfer the R plasmid from a donor bacterium to the recipient bacterium. This is how the other bacteria also become antibiotic resistant.

2. Degradative plasmids: This type of plasmids is capable of degrading or digesting the dead organic matter from dead animals or plants. They use this organic matter in the process of biosynthesis and make energy and recycle them.

3. Fertility Plasmids: These plasmids carry the tra-genes which are used in the process of conjugation. They are helpful in transferring the genetic material between bacteria.

4. Col Plasmids: The plasmids of this type produce such antibiotics which are involved in killing the other harmful strains of bacteria by staying in the host bacterial cell. The antibiotics are also called as colicin.

5. Virulence Plasmids: As the name shows, these plasmids have the ability to transform bacteria into a pathogen. So they are responsible for carrying the genes which cause diseases.

Recombinant DNA technology:

Recombinant DNA technology makes use of plasmids for many purposes. For the drug delivery, this technology makes use of the plasmids to insert the desired drug into the body. They are also involved in causing antibiotic resistance and are used to kill harmful bacteria from the body. Recombinant DNA technology applied plasmids for the first time on the human body for the insertion of human insulin. It gave very efficient results. The other application of plasmids is the insertion of human growth hormone in the mammalian cells of animals**.**

Transformation

Transformation involves the uptake of free or naked DNA released by donor. It was the first example of genetic exchange in bacteria to have been discovered. This was first demonstrated in an experiment conducted by **Griffith in 1928**. The presence of a capsule around some strains of **pneumococci** gives the colonies a glistening**, smooth (S)** appearance while **pneumococci** lacking capsules have produce **rough (R)** colonies. Strains of **pneumococci** with a capsule (type I) are virulent and can kill a mouse whereas strains lacking it (type II) are harmless. Griffith found that mice died when they were injected with a mixture of live non capsulated (R, type II) strains and heat killed capsulated (S, type I) strains. Neither of these two when injected alone could kill the mice, only the mixture of two proved fatal. Live S strains with capsule were isolated from the blood of the animal suggesting that some factor from the dead S cells converted the R strains into S type. The factor that transformed the other strain was found to be DNA by Avery, McLeod and McCarty in 1944.

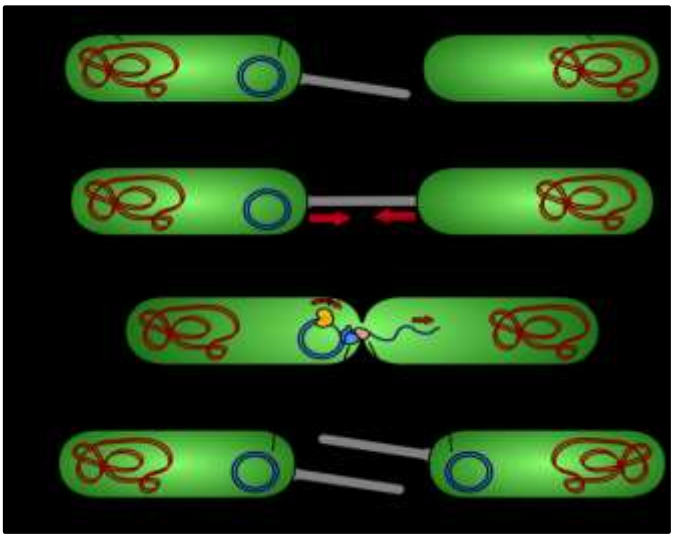
Transformation is gene transfer resulting from the uptake by a recipient cell of naked DNA from a donor cell. Certain bacteria (e.g. *Bacillus, Haemophilus, Neisseria, Pneumococcus*) can take up DNA from the environment and the DNA that is taken up can be incorporated into the recipient's chromosome.

Conjugation

Bacteria can transfer genetic information to other bacteria through a process known as conjugation. Bacterial conjugation is the transfer of DNA from a living donor bacterium to a recipient bacterium.

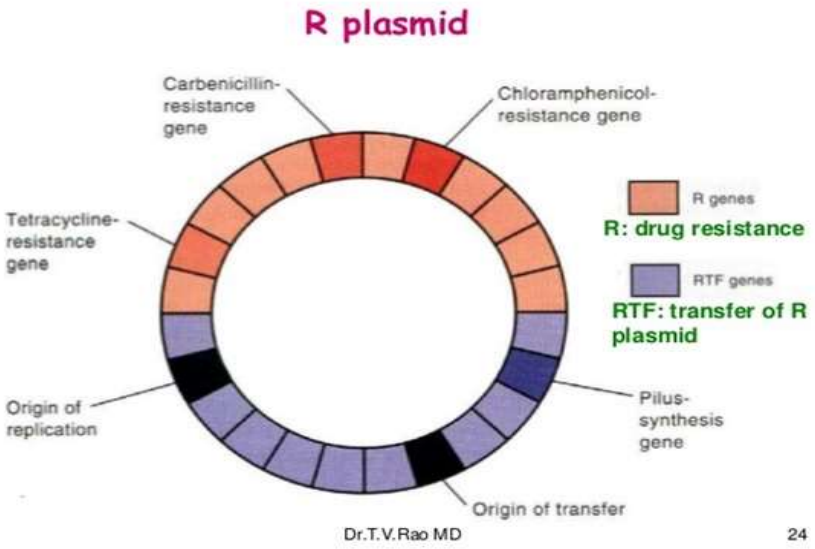
Conjugation involves the transfer of plasmids from donor bacterium to recipient bacterium. Plasmid transfer in Gram-negative bacteria occurs only between strains of the same species or closely related species. Some plasmids are designated as **F factor (F plasmid, fertility factor or sex factor) because they carry genes that mediate their own transfer**. The F factor can replicate autonomously in the cell. **These genes code for the production of the sex pilus and enzymes necessary for conjugation**. Cells possessing F plasmids are **F+ (male) and act as donors**. Those cells lacking this plasmid are **F- (female) and act as recipient**. All those plasmids, which confer on their host cells to act as donors in conjugation are called transfer factor.

Each Gram negative F+ bacterium has 1 to 3 sex pili that bind to a specific outer membrane protein on recipient bacteria to initiate mating. The sex pilus then retracts, bringing the two bacteria on contact and the two cells become bound together at a point of direct envelope-to-envelope contact. In Gram-positive bacteria sticky surface molecules are produced which bring the two bacteria into contact. Gram-positive donor bacteria produce adhesions that cause them to aggregate with recipient cells, but sex pili are not involved. DNA is then transferred from the donor to the recipient. Plasmid-mediated conjugation occurs in ***Bacillus subtilis***, ***Streptococcus lactis***, and ***Enterococcus faecalis*** but is not found as commonly in the Gram-positive bacteria as compared to the Gram-negative bacteria.



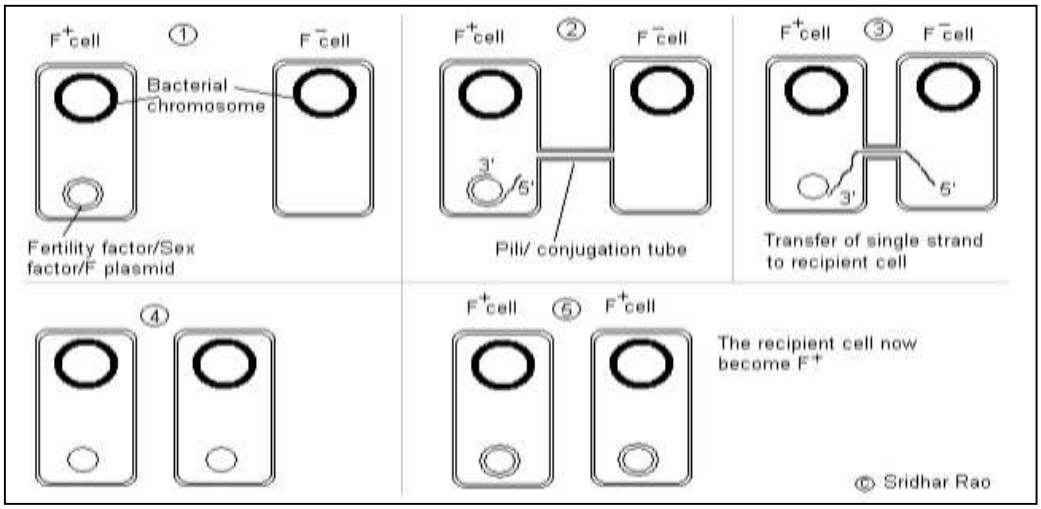
A. F+ conjugation:

This results in the transfer of an F+ plasmid (coding only for a sex pilus) but not the chromosomal DNA from a male donor bacterium to a female recipient bacterium. The two strands of the plasmid separate. One strand enters the recipient bacterium progressing on the 5' to 3' direction while one strand remains in the donor. The complementary strands are synthesized in both donor and recipient cells. The recipient then becomes an F+ male and can make a sex pilus. During conjugation, no cytoplasm or cell material except DNA passes from donor to recipient.



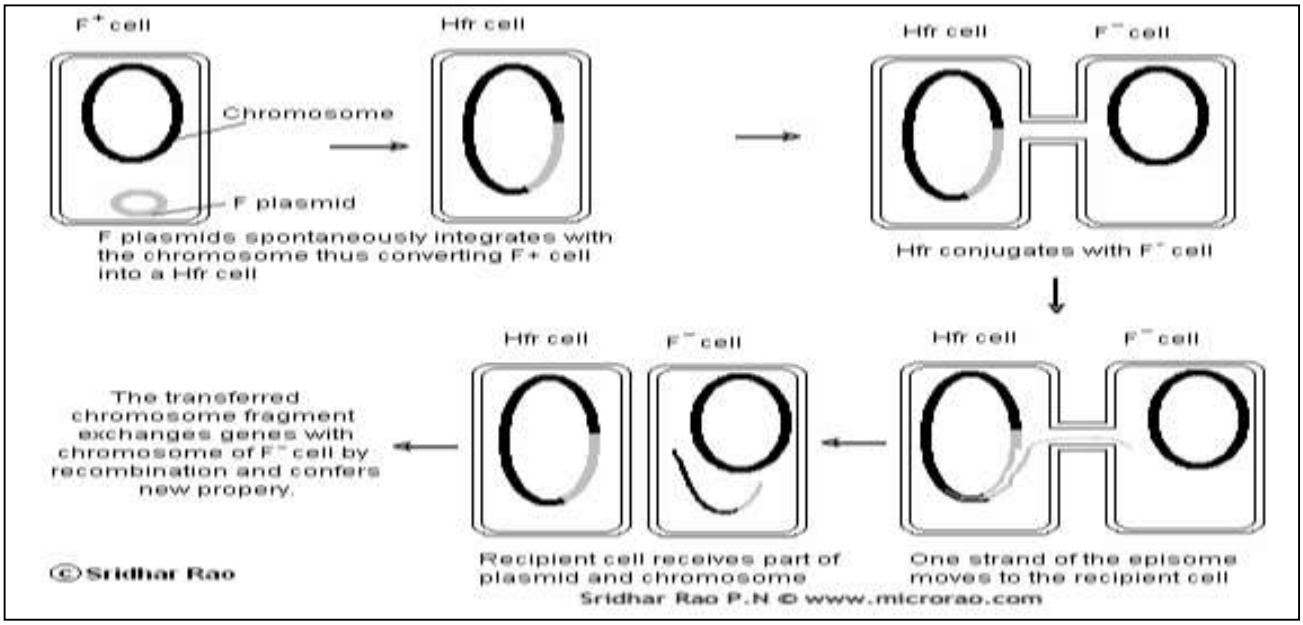
B. Resistance plasmid conjugation:

Some Gram-negative bacteria harbor plasmids that contain antibiotic resistance genes; such plasmids are called **R factors**. The R factor has two components, one that codes for self-transfer (like F factor) called RTF (resistance transfer factor) and the other R determinant that contains genes coding for antibiotic resistance. R plasmids may confer resistance to as many as five different antibiotics at once upon the cell and by conjugation; they can be rapidly disseminated through the bacterial population.



C. Hfr (high frequency recombinant) conjugation:

Plasmids may integrate into the bacterial chromosome by a recombination event depending upon the extent of DNA homology between the two. After integration, both plasmid and chromosome will replicate as a single unit. A plasmid that is capable of integrating into the chromosome is called an episome. If the F plasmid is integrated into the chromosome it is called an Hfr cell. After integration, both chromosome and plasmid can be conjugally transferred to a recipient cell. Hfr cells are called so because they are able to transfer chromosomal genes to recipient cells with high frequency.



Transduction

Bacteriophages are viruses that parasitic bacteria and use their machinery for their own replication. During the process of replication inside the host bacteria the bacterial chromosome or plasmid is erroneously packaged into the bacteriophage capsid. Thus newer progeny of the phages may contain fragments of the host chromosome along with their own DNA or entirely host chromosome. When such phage infects another bacterium, the bacterial chromosome in the phage also gets transferred to the new bacterium. This fragment may undergo recombination with the host chromosome and confer new property to the bacterium.

Life cycle of bacteriophage may either by lytic or lysogenic. In the former, the parasitized bacterial cell is killed with the release of mature phages while in the latter the phage DNA gets incorporated into the bacterial chromosome as a prophage.

Following are the stages of transduction involving a lytic phage:

1. A lytic bacteriophage adsorbs to a susceptible bacterium.

2. The bacteriophage genome enters the bacterium. The phage DNA directs the bacterium's metabolic machinery to manufacture bacteriophage components and enzymes.

3. Occasionally during maturation, a bacteriophage capsid incorporates a fragment of donor bacterium's chromosome or a plasmid instead of a phage genome by mistake.

4. The bacteriophages are released with the lysis of bacterium.

5. The bacteriophage carrying the donor bacterium's DNA adsorbs to another recipient bacterium.

6. The bacteriophage inserts the donor bacterium's DNA it is carried into the recipient bacterium.

7. The donor bacterium's DNA is exchanged by recombination for some of the recipient's DNA.

Two types of transduction are known; **restricted transduction** and **generalized transduction**. Generalized transduction can transfer any bacterial gene to the recipient. In restricted transduction only those chromosomal genes that lie adjacent to the prophage are transmitted. The *lambda phage* that infects *E.coli* always transfers gal+ gene (responsible for galactose fermentation). Specialized transduction is only effective in transducing a few special bacterial genes while generalized transduction can transduce any bacterial gene.