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المرحلة الثالثة

الفصل الدراسي الثاني

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Under Graduated Students
Microbial Genetics
Third Stage

Lecture: 1 Introduction to Genetics of Microorganisms

Introduction to Genetics of Microorganisms

The study of molecular genetics initially focused almost entirely on their genes; today, bacteria and viruses are still essential tools for probing the nature of genes in more-complex organisms, in part because they possess a number of characteristics that make them suitable for genetic studies (Table -1).
The genetic systems of bacteria and viruses are studied because these organisms play important roles in human society.

Table 8.1 Advantages of using bacteria and viruses for genetic studies

1. Reproduction is rapid.
2. Many progeny are produced.
3. Haploid genome allows all mutations to be expressed directly.
4. Asexual reproduction simplifies the isolation of genetically pure strains.
5. Growth in the laboratory is easy and requires little space.
6. Genomes are small.
7. Techniques are available for isolating and manipulating their genes.
8. They have medical importance.
9. They can be genetically engineered to produce substances of commercial value.

- Genetics :The science of heredity ,the study of biological information of all livening organisms from single cell to multicellular cells must store replicate transmit to the next generation and use vast quantity of information to developed grow reproduce and survive in their environments. the study of what genes are, how they carry information, how information is expressed, and how genes are replicated.

- *Genetic information* Biological information is carried by the nucleic acid molecules, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

- Genes ,the units of genetic information .Genes specific sequences of nucleotides in the DNA molecule that encode for a functional product, a protein or enzyme.
- Genome:the total genetic information possessed by an organism .
- Codon: the basic unit of biological information a sequence of three adjacent nucleotides in DNA or mRNA that code for one amino acids.

Classification of Organisms

The highest level of classification is the **domain**. There are considered to be three domains:

1. *Eubacteria* :These are prokaryotic cells (traditional bacteria).

2. *Archaebacteria:* these are prokaryotes like eubacteriain that they lack a nucleus. slightly more closely relatedgenetically to eukaryotes than to eubacteria.

3. *Eukaryotes:* Higher organisms whose DNA is carried on several chromosomes which are found inside the nucleus.
Their cells are divided into separate compartments and usually contain other organelles in addition to the nucleus.

Eukaryotes are divided into four **kingdoms**:
Protoctista ,plant, animal and fungi

Characteristic	Bacteria	Archaea	Eukarya
Number of Chromosomes	One or rarely two	One or rarely two	Two or more
Type of Nucleic Acid	Circular or linear dsDNA	Circular or linear dsDNA	Linear DNA in nucleus; circular in mitochondria and chloroplasts
Location of DNA	Nucleoid and plasmids	Nucleoid and plasmids	Nuclei and in mitochondria, chloroplasts in cytosol
Histones Present	No	No	Yes

The Bacterial Genome

Bacteria are less structurally complex than animals and plants, they are often referred to as “lower organisms.” However, it is important to remember that present day bacteria are at least as well adapted to modern conditions as animals and plants, and are just as highly evolved as so-called “higher organisms.” In many ways, bacteria are not so much “primitive” as specialized for growing more efficiently in many environments than larger and more complex organisms.

Bacteria(unicellular organisms)are the simplest living cells and are classified as prokaryotes. By definition, prokaryotes lack a nucleus and their DNA is therefore in the same compartment as the cytoplasm(nucleoid region). Bacterial cells are always surrounded by a membrane (the cell or cytoplasm membrane) and usually also by a cell wall. Like all cells, they contain all the essential chemical and structural components necessary for life.

Most bacterial genomes consist of a circular chromosome that contains a single DNA molecule several million base pairs in length. carrying a full set of genes providing it with the genetic information necessary to operate as a living organism.

Some bacteria contain multiple chromosomes such as

Vibrio.cholera(2*chromosome *)

Paracoccus denitrificans(3* chromosome*), and a few even have linear chromosomes such as **Streptomyces spp and Borrelia spp.**

Typically,bacteria have (3,000–4,000) genes, although parasitic bacteria may have less than 1.000 genes ,most free living bacteria have(2000-4000) genes .somebacteria have as few as 500 genes. The ***Mycoplasma genitalium*** **consisting of 580.000base pairs** (bp)of DNA have approximately 500 genes which considered as a parasitic bacterium(300 genes are essential for the growth and reproduction) . The smallest prokaryotic genome belongs to ***Nanoarchaeum equitans*** a marine archebacterium .*N equitans* has about 15% less DNA than *M. genitalium* may also a parasitic it cannot grow unless attached to the surface of other microorganisms. Other bacteria with complex life cycles such as ***myxococcus*** have 9000 genes.

Plasmids

In addition to having a chromosome, many bacteria possess **plasmids,** small, circular extra chromosomal DNA molecules **.**Some plasmids are present in many copies per cell, whereas others are present in only one or two copies. In general, plasmids carry genes that are not essential to bacterial function but that may play an important role in the life cycle and growth of their bacterial hosts. Some plasmids promote mating between bacteria; others contain genes that kill other bacteria. plasmids are used in genetic engineering and some of them play arole in the spread of antibiotic resistance among bacteria.

Most plasmids are circular and several thousand base pairs in length. **Episomes** are plasmids that are capable of either freely replicating or integrating into the bacterial chromosomes.The **F** (fertility) **factor** of *E. coli* is an episome that controls mating and gene exchange between*E. coli* cells.

Where Are Bacteria Found in Nature?

Bacteria are found almost everywhere. Bacteria have been found 40 miles high in the atmosphere and seven miles deep beneath the ocean floor. Some bacteria live in the sea, others live in fresh water, and others are found growing happily in sewage. Some bacteria live in the soil, some are found living in the roots of plants, and some live inside animals. Most of the bacteria that live inside animals are harmless, and some are even of positive value in aiding digestion or synthesizing vitamins that are absorbed by their host animal. Over 90% are in the soil and subsurface layers below the oceans. Probably over half of the living matter on Earth is microbial.

In addition to the "normal" habitats, some bacteria live in extreme environments . Some bacteria can live in very concentrated salt solutions, such as the Dead Sea. Other bacteria inhabit hot sulfur springs, where temperatures approach boiling point and the pH is close to 1. Bacteria from these habitats may provide products that are useful because of their resistance to extreme conditions.***Thermus aquaticus***, a bacterium from hot springs, has provided the heat stable **DNApolymerase** needed for the polymerase chain reaction (PCR), a widely used technique.

Some bacterial strains secrete toxic chemicals in order to kill off others that are competing for the same resources. Certain bacteria synthesize toxic proteins, known as bacteriocins. These proteins are designed to kill closely related bacterial strains, yet are harmless to the producer strain. **Nisin**, a bacteriocin produced by some strains of ***Lactococcus lactis*** acts as a food preservative and kills food-borne pathogens including ***Listeria monocytogenes*** **and** ***Staphylococcus aureus***. **Antibiotics** are produced by bacteria, especially those of the ***Streptomyces*** group (naturally isolated from soil) to kill competing bacteria in the soil environment.

Bacteria Were Used for Fundamental Studies of Cell Function

Most of the early experiments providing the basis for modern day molecular biology were performed using bacteria such as *Escherichia coli* because they are relatively simple to analyze. Some advantages of using bacteria to study cell function are:

1. Bacteria are single-celled microorganisms. Furthermore, a bacterial culture consists of many identical cells due to lack of sexual recombination during cell division.In contrast, in multi-cellular organisms, even an individual tissue or organcontains many different cell types.

2. The most commonly used bacteria have about 4,000 genes as opposed to higher organisms, which have up to 50,000.

3. Bacteria are **haploid**, having only a single copy of most genes, whereas higher organisms are **diploid**, possessing at least two copies of each gene.

4. Bacteria can be grown under strictly controlled conditions and many will grow in a chemically defined culture medium containing mineral salts and a simple organic nutrient such as glucose.

5. Bacteria grow fast and may divide in as little as 20 minutes, whereas higher organisms often take days or years for each generation .

6. A bacterial culture contains around 10^9 cells per ml. Consequently genetic experiments that need to analyze large numbers of cells can be done conveniently.

7. Bacteria can be conveniently stored for short periods (a couple of weeks) by placing them in the refrigerator and for longer periods (years) in low temperature freezers at -70°C.

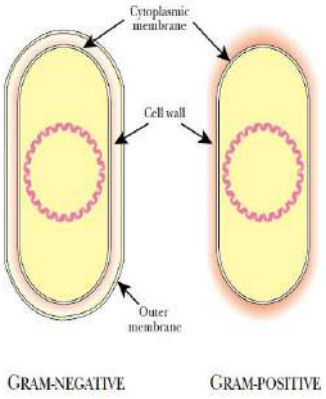
In practice, bacteria are usually cultured by growing them as a suspension in liquid inside tubes, flasks or bottles.They can also be grown as colonies (visible clusters of cells)on the surface of an agar layer in flat dishes, known as Petri dishes . Agar is acarbohydrate polymer extracted from seaweed that sets, or solidifies, like gelatin.It should be noted that the convenient properties noted above apply to commonly grown laboratory bacteria. In contrast, many bacterial species found in the wild are difficult or, by present techniques impossible, to culture in the laboratory. Many othershave specialized growth requirements and most rarely grow .

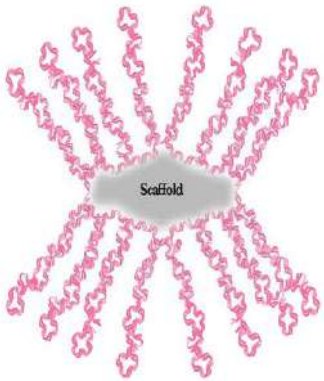
Escherichia coli (E. coli) Is a Model Bacterium

Although many different types of bacteria are used in laboratory investigations, the bacterium used most often in molecular biology research is *Escherichia coli*. is rod shaped and about two micrometers long and a micrometer wide. A micrometer (μm), also known as a micron, is a millionth of a meter (i.e., 10^{-6} meter). Bacteria are not limited to a rod shape; spherical, filamentous or spirally twisted bacteria are also found.*E. coli* is found inthe lower part of the large intestine of mammals, including humans in a symbiosis relationship, pathogenic strains of *E.coli* causes gastrointestinal, urinary, pulmonary and others.*E. coli* is a **gram-negative bacterium**, which means that it possesses two membranes.Outside the cytoplasmic membrane possessed by all cells are the cell wall anda second, outer membrane .The presence of an outermembrane provides an extra layer of protection to the bacteria.

the genome of *E. coli* has approximately 4.6 million base pairs of DNA However,is organized into 50-100 large loops or domains of 50-100kb in length .

The *E.coli* genome has 4639221bp , a total of 2657 protein coding genes with known function (62% of all genes)and 1632 genes (38%)without known function have been identified . the bacterial can double their proteins content every 20 minutes during cell division .





Viruses Are Not Living Cells

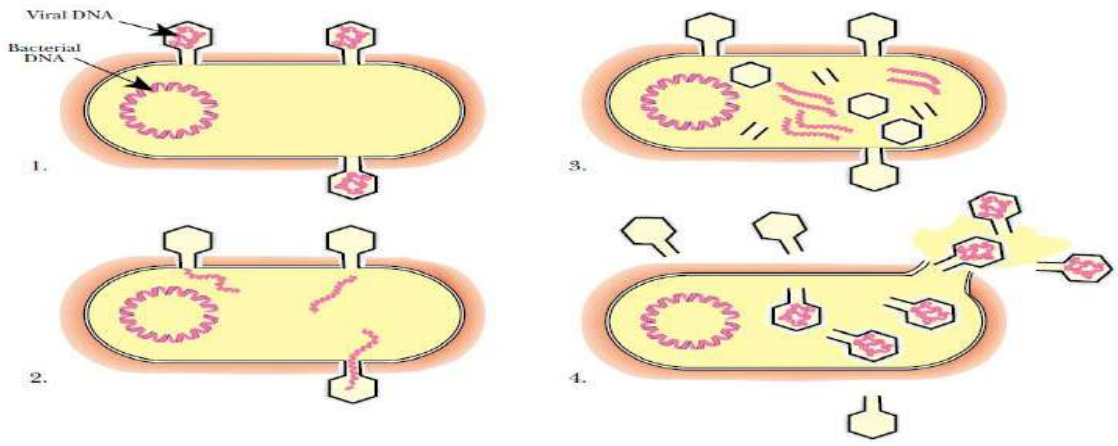
Viruses are obligate **parasites** that must infect a host cell in order to replicate themselves.Viruses are packages of genes in protein coats and are much smaller than bacteria.. Virus particles (**virions**) do contain genetic information in the form of DNA

orRNA, but are incapable of growth or division by themselves (viruses are certainly not living cells).
Viruses lack the machinery to generate their own energy or to synthesize protein.
After invading a host cell, the virus does not grow and divide like a cell itself. The virion disassembles and the virus genes are expressed using the machinery of the hostcell. In particular, viral proteins are made by the host cell ribosomes, using virus genetic information. In many cases, only the virus DNA or RNA enters the host cell and the other components are abandoned outside. Usually the host cell is killed and disintegrates. Typically, several hundred viruses may be released from a single infected cell.The viruses then abandonthe cell and look for another host.

Viruses are very important from a practical viewpoint:
Firstly, many serious diseases are due to virus infection.
Secondly, many genetic manipulations that are now used in genetic engineering are carried out using viruses.
viral diseases can often be prevented by **immunization**,if a potential victim is **vaccinated** before catching the virus. In this case, the invading virus will be killed by the immune system, which has been put on alert by the vaccine, and the disease will be prevented.
Antibiotics are of no use against viruses; they only kill bacteria. There are two mainreasons for not use antibiotics: The valid reason is that giving antibiotics may help combat secondary oropportunistic infections caused by bacteria, especially in virally-infected patients whoare in poor healthin addition contribution to the spread of antibiotic resistance among many infectious bacteria thus creating a major health problem

Bacterial Viruses Infect Bacteria

the bacteria infected by a virus are sometimes referred to as **bacteriophages**, or phages for short. Phage comes froma Greek word meaning to eat.When bacteria catch a virus, they do not merely get a mild infection, like a cold, as humans usually do. The bacteriophage takes over the bacterial cell and fills it up by manufacturing more bacteriophages .Then the bacterial cell bursts and liberates the new crop of bacteriophages to infect more bacteria. This takes only about an hour or so. Bacterial viruses infect only bacteria. Some have relatively broad host ranges,where as others infect only a single species or even just a few particular strains of bacteria.



Lecture(2):

Replication process in prokaryotic cells :

In molecular biology, DNA **replication** is the biological process of producing two identical replicas of DNA from one original DNA molecule. DNA **replication** occurs in all living organisms acting as the most essential part for biological inheritance. Following Meselson and Stahl's work, investigators confirmed that other organisms also use semiconservative replication. There are, however, several different ways that semiconservative replication can take place, differing principally in the nature of the template DNA—whether it is linear or circular—and in the number of replication forks(table 1).

Table (1): These models may differ with respect to the initiation and progress of replication, but all produce new DNA molecules by semi-conservative replication.

Replication Model	DNA Template	Breakage of Nucleotide Strand	Number of Replicons	Unidirectional or Bidirectional	Products
Theta	Circular	No	1	Unidirectional or bidirectional	Two circular molecules
Rolling circle	Circular	Yes	1	Unidirectional	One circular molecule and one linear molecule that may circularize
Linear eukaryotic	Linear	No	Many	Bidirectional	Two linear molecules

Replicon and Origin of Replication (ORI)

Individual units of replication are called replicons, each of which contains a replication origin. Replication starts at the origin and continues until the entire

replicon has been replicated. Bacterial chromosomes have a single replication origin, whereas eukaryotic chromosomes contain many. The points where DNA is currently unwinding are called replication forks.(figure 1).

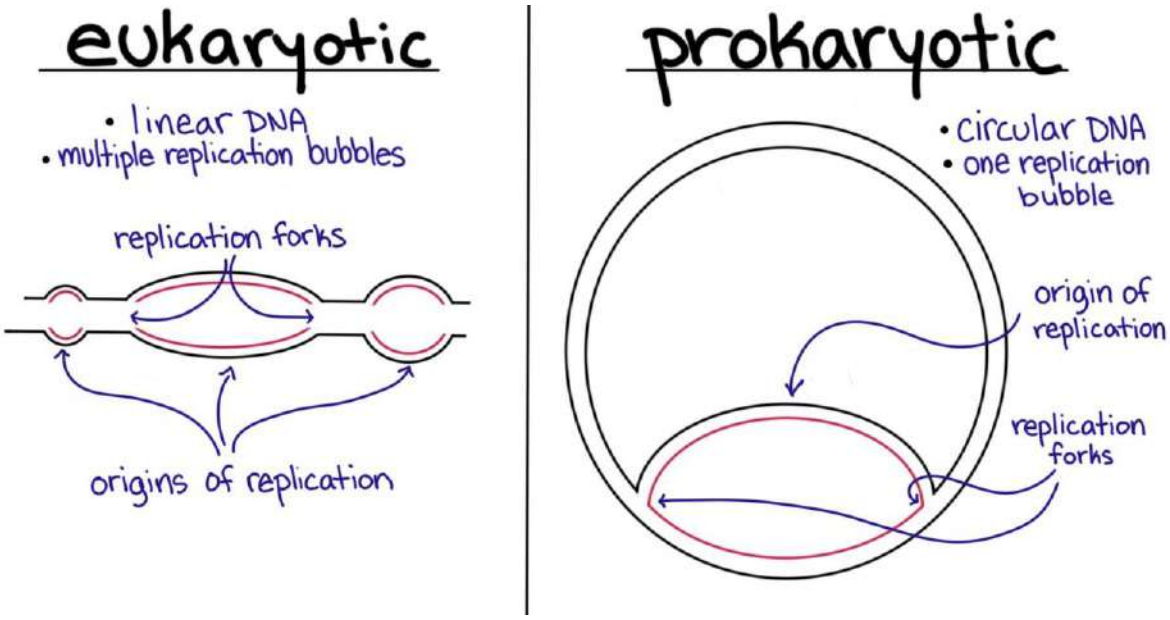


Figure (1): Origin of Replication

Models of Replication

1-Theta Model

A common type of replication that takes place in circular DNA, such as that found in *E. coli* and other bacteria, is called theta replication because it generates a structure that resembles the Greek letter theta (θ).In theta replication, double-stranded DNA begins to unwind at the replication origin, producing single-stranded nucleotide strands that then serve as templates on which new DNA can be synthesized. The unwinding of the double helix generates a loop, termed

a **replication bubble**. Unwinding may be at one or both ends of the bubble, making it progressively larger. DNA replication on both of the template strands is simultaneous with unwinding (figure 2).

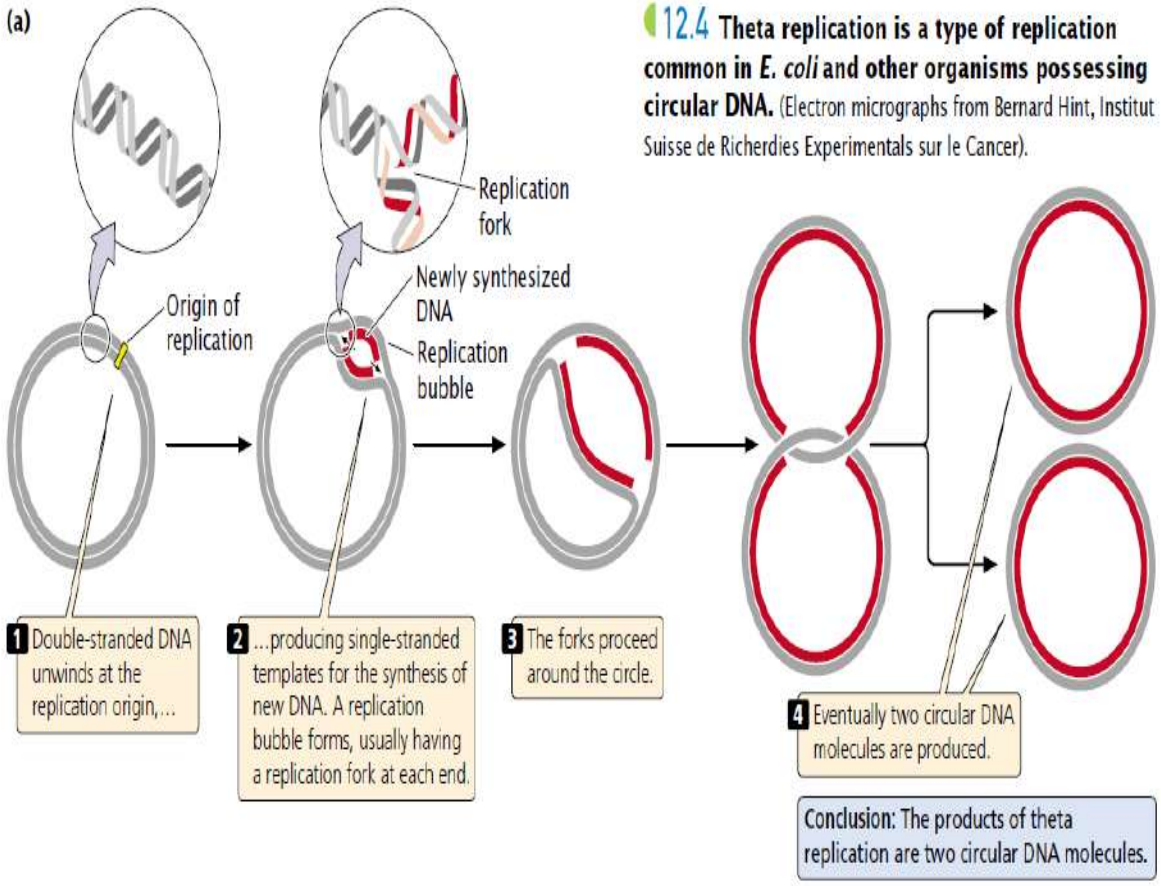


Figure (2): Theta replication

The point of unwinding, where the two single nucleotide strands separate from the double-stranded DNA helix, is called a replication fork. If there are two replication forks, one at each end of the replication bubble, the forks proceed outward in both directions in a process called bidirectional replication, simultaneously unwinding and replicating the DNA until they eventually meet. If a single replication fork is present, it proceeds around the entire circle to produce two complete circular DNA molecules, each consisting of one old and one new nucleotide strand.

2- Rolling Circle Replication

Whereas many bacterial replicate by a process similar to that used to copy the bacterial chromosome, other plasmids, several **bacteriophages**, and some **viruses** of eukaryotes use **rolling circle replication** (Figure 3). The circular nature of plasmids and the circularization of some viral genomes on infection make this possible. Rolling circle replication begins with the enzymatic nicking of one strand of the double-stranded circular molecule at the **double-stranded origin (dso) site**. In bacteria, DNA polymerase III binds to the 3′-OH group of the nicked strand and begins to unidirectionally replicate the DNA using the un-nicked strand as a template, displacing the nicked strand as it does so. Completion of DNA replication at the site of the original nick results in full displacement of the nicked strand, which may then recircularize into a single-stranded DNA molecule. RNA primase then synthesizes a primer to initiate DNA replication at the **single-stranded origin (sso) site** of the single-stranded DNA (ssDNA) molecule, resulting in a double-stranded DNA (dsDNA) molecule identical to the other circular DNA molecule.

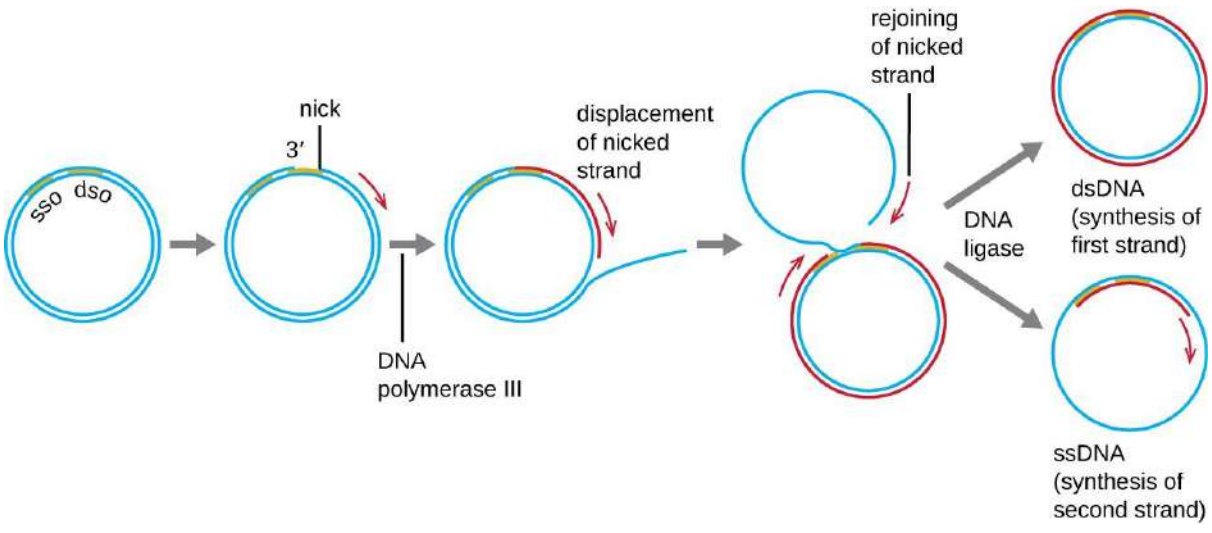
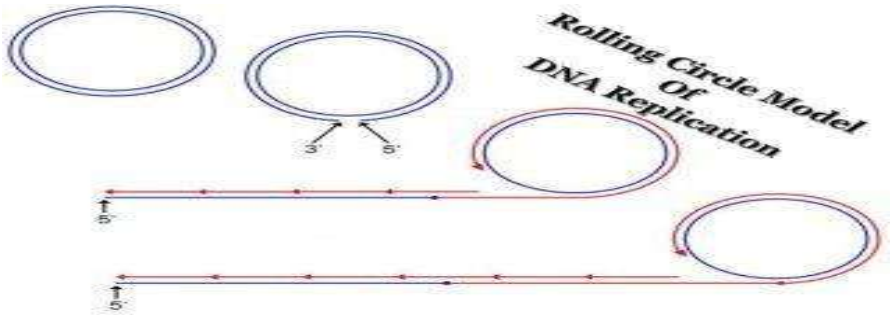


Figure (3): The process of rolling circle replication results in the synthesis of a single new copy of the circular DNA molecule, as shown here.

Rolling circle replication or covalent extension process, describes a process of unidirectional nucleic acid replication that can rapidly synthesize multiple copies of circular molecules of DNA or RNA, such as plasmids, the genomes of bacteriophages, and the circular RNA genome of viroids.

Theta replication is what naturally our DNA undergoes during replication process.actually when replication fork is moved bidirectional it looks like an eye or the Greek letter there thats why we call it like that.



Lecture (3):

DNA Replication in Bacteria

DNA replication has been well studied in bacteria primarily because of the small size of the genome and the mutants that are available. ***E. coli*** has 4.6 million base pairs (Mbp) in a single circular chromosome and all of it is replicated in approximately 42 minutes, starting from a single origin of replication and proceeding around the circle bidirectionally (i.e., in both directions). This means that approximately 1000 nucleotides are added per second. The process is quite rapid and occurs with few errors.DNA replication uses a large number of proteins and enzymes (Table 2).

Table (2): The Molecular Machinery Involved in Bacterial DNA Replication.

DNA pol I	Exonuclease activity removes RNA primer and replaces it with newly synthesized DNA
DNA pol III	Main enzyme that adds nucleotides in the 5′ to 3′ direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments on the lagging strand to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Single-stranded binding proteins	Bind to single-stranded DNA to prevent hydrogen bonding between DNA strands, reforming double-stranded DNA
Sliding clamp	Helps hold DNA pol III in place when nucleotides are being added
Topoisomerase II (DNA gyrase)	Relaxes supercoiled chromosome to make DNA more accessible for the initiation of replication; helps relieve the stress on DNA when unwinding, by causing breaks and then resealing the DNA
Topoisomerase IV	Introduces single-stranded break into concatenated chromosomes to release them from each other, and then reseals the DNA

One of the key players is the enzyme **DNA polymerase**, also known as DNA pol. In bacteria, three main types of DNA polymerases are known: DNA pol I, DNA pol II, and DNA pol III. It is now known that DNA pol III is the enzyme required for DNA synthesis; DNA pol I and DNA pol II are primarily required for repair. DNA pol III adds deoxyribonucleotides each complementary to a nucleotide on the template strand, one by one to the 3′-OH group of the growing DNA chain. The addition of these nucleotides requires energy. This energy is present in the bonds of three phosphate groups attached to each nucleotide (a triphosphate

nucleotide), similar to how energy is stored in the phosphate bonds of adenosine triphosphate (ATP). When the bond between the phosphates is broken and diphosphate is released, the energy released allows for the formation of a covalent phosphodiester bond by dehydration synthesis between the incoming nucleotide and the free 3′-OH group on the growing DNA strand.

Initiation

The **initiation of replication** occurs at specific nucleotide sequence called the **origin of replication**, where various proteins bind to begin the replication process. ***E. coli*** has a single origin of replication (as do most prokaryotes), called *oriC*, on its one chromosome. The origin of replication is approximately 245 base pairs long and is rich in adenine-thymine (AT) sequences.

Some of the proteins that bind to the origin of replication are important in making single-stranded regions of DNA accessible for replication. Chromosomal DNA is typically wrapped around **histones** (in eukaryotes and archaea) or histone-like proteins (in bacteria), and is **supercoiled**, or extensively wrapped and twisted on itself. This packaging makes the information in the DNA molecule inaccessible. However, enzymes called topoisomerases change the shape and supercoiling of the chromosome. For bacterial DNA replication to begin, the supercoiled chromosome is relaxed by **topoisomerase II**, also called **DNA gyrase**. An enzyme called **helicase** then separates the DNA strands by breaking the hydrogen bonds between the nitrogenous base pairs. Recall that AT sequences have fewer hydrogen bonds and, hence, have weaker interactions than guanine-cytosine (GC) sequences. These enzymes require ATP hydrolysis. As the DNA opens up, Y-shaped structures called **replication forks** are formed. Two replication forks are formed at the origin of replication, allowing for bidirectional replication and formation of a structure that looks like a bubble when viewed with a transmission electron microscope; as a result, this structure is called

a **replication bubble**. The DNA near each replication fork is coated with **single-stranded binding proteins** to prevent the single-stranded DNA from rewinding into a double helix.

Once single-stranded DNA is accessible at the origin of replication, DNA replication can begin. However, DNA pol III is able to add nucleotides only in the 5′ to 3′ direction (a new DNA strand can be only extended in this direction). This is because DNA polymerase requires a free 3′-OH group to which it can add nucleotides by forming a covalent phosphodiester bond between the 3′-OH end and the 5′ phosphate of the next nucleotide. This also means that it cannot add nucleotides if a free 3′-OH group is not available, which is the case for a single strand of DNA. The problem is solved with the help of an RNA sequence that provides the free 3′-OH end. Because this sequence allows the start of DNA synthesis, it is appropriately called the **primer**. The primer is five to 10 nucleotides long and complementary to the parental or template DNA. It is synthesized by RNA **primase**, which is an **RNA polymerase**. Unlike DNA polymerases, RNA polymerases do not need a free 3′-OH group to synthesize an RNA molecule. Now that the primer provides the free 3′-OH group, DNA polymerase III can now extend this RNA primer, adding DNA nucleotides one by one that are complementary to the template strand.

Elongation

During **elongation in DNA replication**, the addition of nucleotides occurs at its maximal rate of about 1000 nucleotides per second. DNA polymerase III can only extend in the 5′ to 3′ direction, which poses a problem at the replication fork. The DNA double helix is antiparallel; that is, one strand is oriented in the 5′ to 3′ direction and the other is oriented in the 3′ to 5′ direction (see **Structure and Function of DNA**). During replication, one strand, which is complementary to the 3′ to 5′ parental DNA strand, is synthesized continuously toward the replication fork because polymerase can add nucleotides in this direction. This continuously synthesized strand is known as the **leading strand**. The other strand,

complementary to the 5′ to 3′ parental DNA, grows away from the replication fork, so the polymerase must move back toward the replication fork to begin adding bases to a new primer, again in the direction away from the replication fork. It does so until it bumps into the previously synthesized strand and then it moves back again (figure 4). These steps produce small DNA sequence fragments known as **Okazaki fragments**, each separated by RNA primer. Okazaki fragments are named after the Japanese research team and married couple Reiji and Tsuneko **Okazaki**, who first discovered them in 1966. The strand with the Okazaki fragments is known as the **lagging strand**, and its synthesis is said to be discontinuous.

The leading strand can be extended from one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3′ to 5′, and that of the leading strand 5′ to 3′. A protein called the **sliding clamp** holds the DNA polymerase in place as it continues to add nucleotides. The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place. Beyond its role in initiation, **topoisomerase** also prevents the overwinding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it. As synthesis proceeds, the RNA primers are replaced by DNA. The primers are removed by the **exonuclease** activity of DNA polymerase I, and the gaps are filled in. The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme **DNA ligase** that catalyzes the formation of covalent phosphodiester linkage between the 3′-OH end of one DNA fragment and the 5′ phosphate end of the other fragment, stabilizing the sugar-phosphate backbone of the DNA molecule.

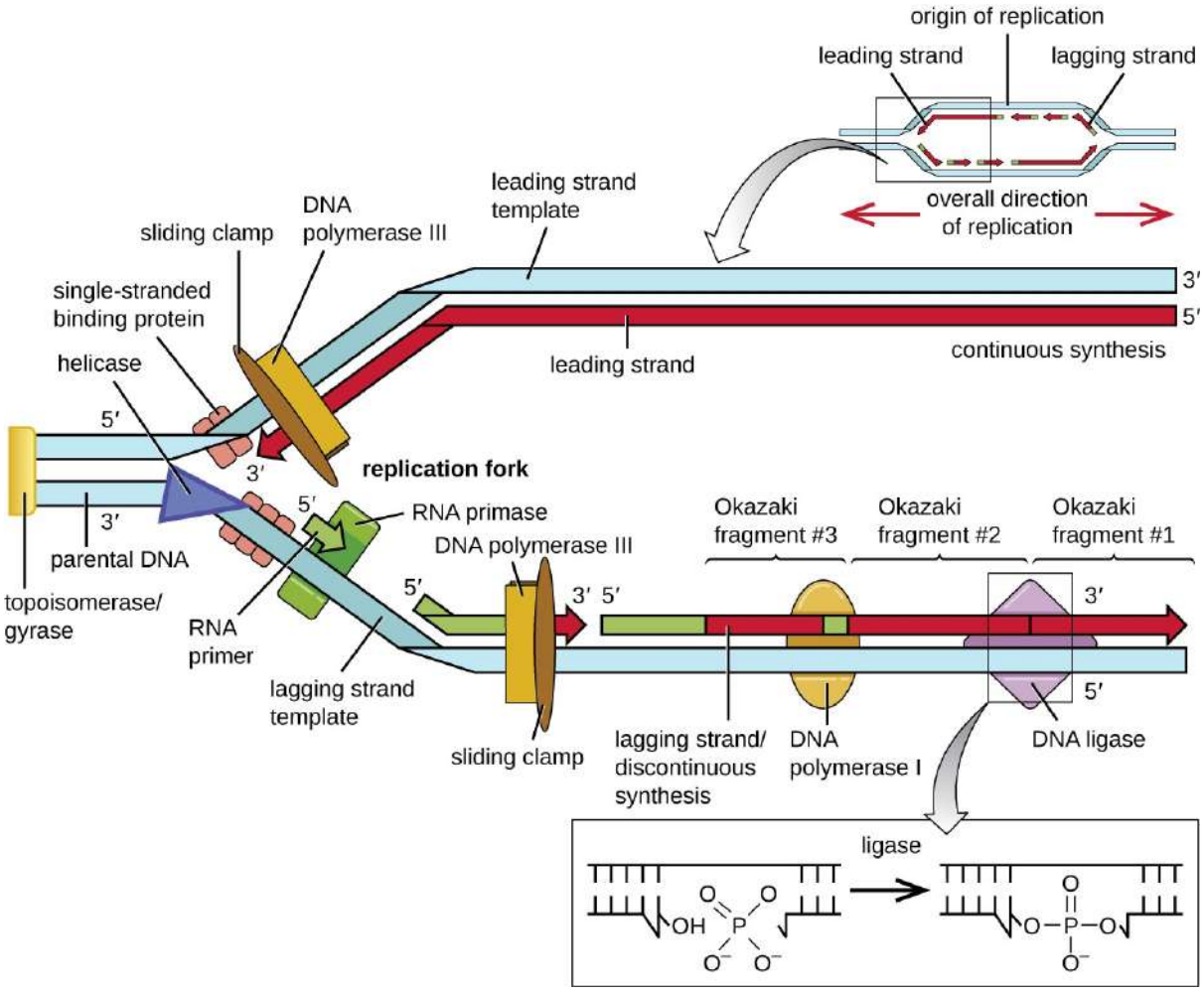


Figure (4): Click for a larger image. At the origin of replication, topoisomerase II relaxes the supercoiled chromosome. Two replication forks are formed by the opening of the double-stranded DNA at the origin, and helicase separates the DNA strands, which are coated by single-stranded binding proteins to keep the strands separated. DNA replication occurs in both directions. An RNA primer complementary to the parental strand is synthesized by RNA primase and is elongated by DNA polymerase III through the addition of nucleotides to the 3′-OH end. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches called Okazaki fragments. RNA primers within the lagging strand are removed by the exonuclease activity of DNA polymerase I, and the Okazaki fragments are joined by DNA ligase.

Termination

Once the complete chromosome has been replicated, **termination of DNA replication** must occur. Although much is known about initiation of replication, less is known about the termination process. Following replication, the resulting complete circular genomes of prokaryotes are concatenated, meaning that the circular DNA chromosomes are interlocked and must be separated from each other. This is accomplished through the activity of bacterial topoisomerase IV, which introduces double-stranded breaks into DNA molecules, allowing them to separate from each other; the enzyme then reseals the circular chromosomes. The resolution of concatemers is an issue unique to prokaryotic DNA replication because of their circular chromosomes. Because both bacterial **DNA gyrase** and **topoisomerase IV** are distinct from their eukaryotic counterparts, these enzymes serve as targets for a class of antimicrobial drugs called **quinolones**.

Lecture (4):

Gene expression

The expression of the genetic material occurs for the most part through the production of proteins, involving two consecutive steps in which the information is converted from one form to another: transcription and translation.

First Process ;

Transcription

Transcription is the synthesis of RNA molecules, with DNA as a template, and it is the first step in the transfer of genetic information from genotype to phenotype.

Classes of RNA

RNA molecules perform a variety of functions in the cell .

1-Ribosomal RNA (rRNA): along with ribosomal protein subunits, makes up the ribosome, the site of protein assembly.

2-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. Bacterial cells do not possess pre mRNA; in these cells, transcription takes place concurrently with translation.

3-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 .

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, it would be highly inefficient for a cell to constantly transcribe all of its genes 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 prope time and place.

1-The first step is the conversion of the information into messenger RNA (mRNA).

2- This process (transcription) is carried out by RNA polymerase.

3-The RNA strand is made in the 5′ to 3′ direction.

The start signals (promoters) also convey the information as to the direction in which transcription should proceed.

In E. coli, depending on growth conditions, (2000–5000) copies of RNA polymerase may be engaged on mRNA synthesis at any time.

❖ Transcription requires three major components:

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

❖ **Transcribe: to make an RNA that is complementary to a region within a strand of DNA.**

❖ **The transcription unit**

A **transcription unit** is a stretch of DNA that codes for an RNA molecule and the sequences necessary for its transcription (Figure – 2).

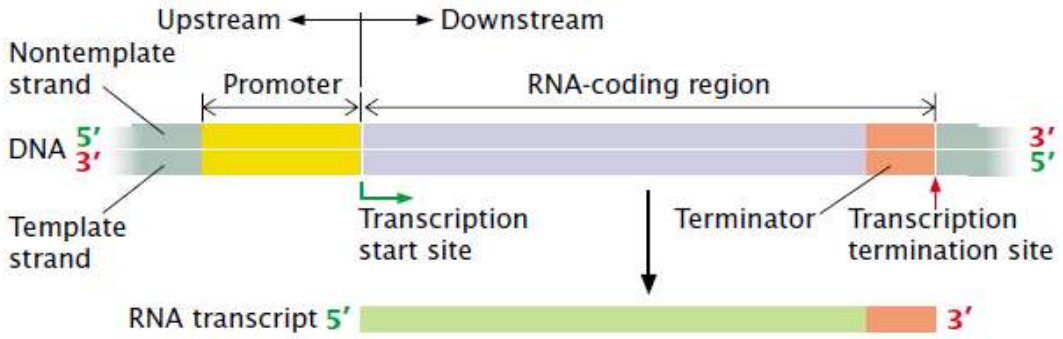
Included within a transcription unit are three critical regions:,

1-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.

2-The RNA-coding region, a sequence of DNA nucleotides that is copied into an RNA molecule.

3-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.

The terms ***upstream*** **a**nd ***downstream*** to refer to the direction of transcription and the location of nucleotide sequences surrounding the RNA coding sequence.



Figure(2):A transcription unit includes a promoter, an RNA-coding and a terminator.

The Substrate for 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. Nucleotides are always added to the 3′end of the RNA molecule, and the direction of transcription is therefore 5′−3′, the same as the direction of DNA synthesis during replication. RNA is made complementary and antiparallel to one of the DNA strands (the template strand).

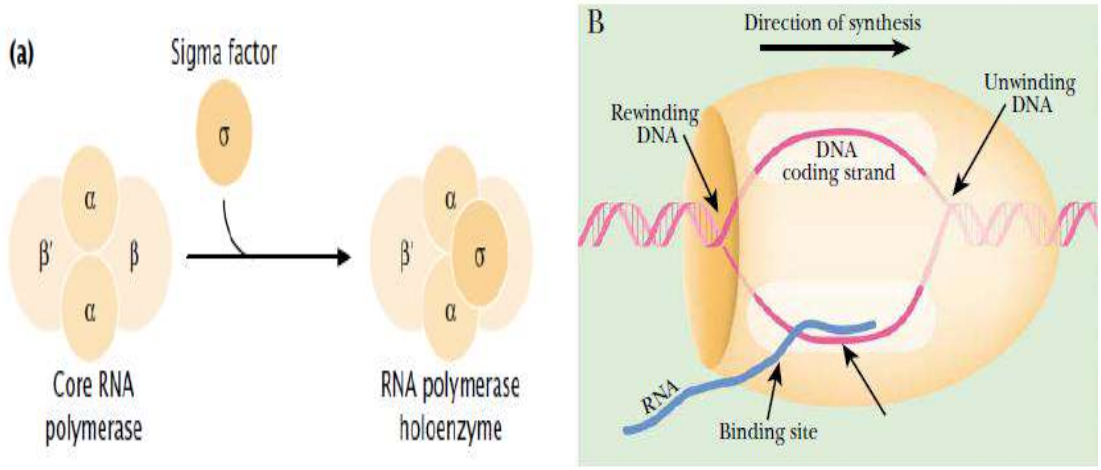
$$RNA_n + rNTP \longrightarrow RNA_{n+1} + PP_i$$

The Transcription Apparatus

A single enzyme—**RNA polymerase**— carries out all the required steps of transcription. The action of RNA polymerase is enhanced by a number of accessory proteins that join and leave the polymerase at different stages of the process. Each accessory protein is responsible for providing or regulating a special function.

- Bacterial RNA polymerase Bacterial cells typically possess only 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, multimeric enzyme (meaning that it consists of several polypeptide chains).
- Bacterial RNA polymerase are four subunits (individual polypeptide chains) that make up the **Core Enzyme: (α2β β'):** two copies of a subunit called alpha ,single copy of beta and single copy of beta prime .
- The core enzyme catalyzes the elongation of the RNA molecule by the addition of RNA nucleotides. Other functional subunits join and leave the core enzyme at particular stages of the transcription process.
- The **sigma 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: α2ββ'σ**.



Lecture (5):

The Process of Bacterial Transcription

Transcription can be conveniently divided into three stages:

1. Initiation, in which the transcription apparatus assembles on the promoter and begins the synthesis of RNA.

2.Elongation, in which RNA polymerase moves along the DNA, unwinding it and adding new nucleotides, one at a time, to the 3′ end of the growing RNA strand.

3. Termination, the recognition of the end of the transcription unit and the separation of the RNA molecule from the DNA template.

1. Initiation

Initiation includes all the steps necessary to begin RNA synthesis, including; (1) promoter recognition, (2) formation of the transcription bubble, (3) creation of the first bonds between rNTPs, and (4) escape of the transcription apparatus from the promoter.

Bacterial promoters Essential information for the transcription Unit where it will start transcribing, which strand is to be read, and in what direction the RNA polymerase will move is imbedded in the nucleotide sequence of the promoter. **Promoters are sequences in the DNA that are recognized by the transcription apparatus and are required for transcription to take place.** In bacterial cells, promoters are usually adjacent to an RNA coding sequence (is located just upstream of the start site)(figure-3) .

a) Promoter recognition: closed complex

- **Promoters possess a consensus sequence**

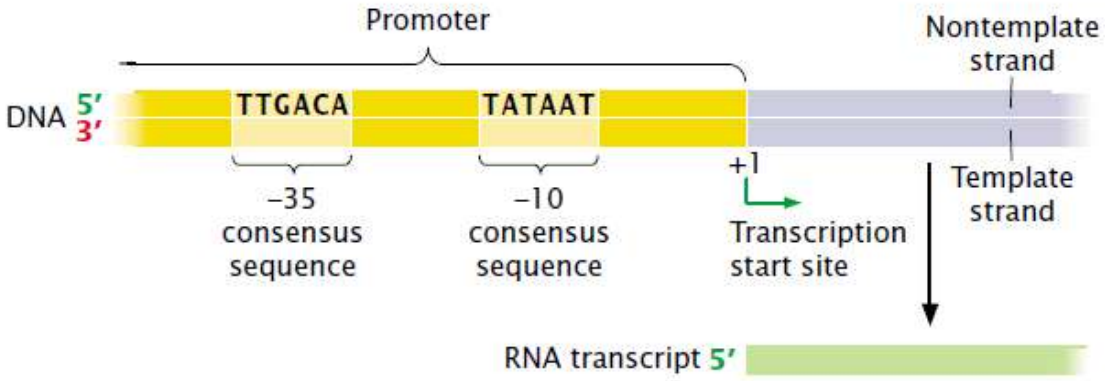
• **Typical *E. coli* promoter sequence ,** sometimes we call it the **Pribnow box**

-10 box: TATAAT
-35 box: TTGACA

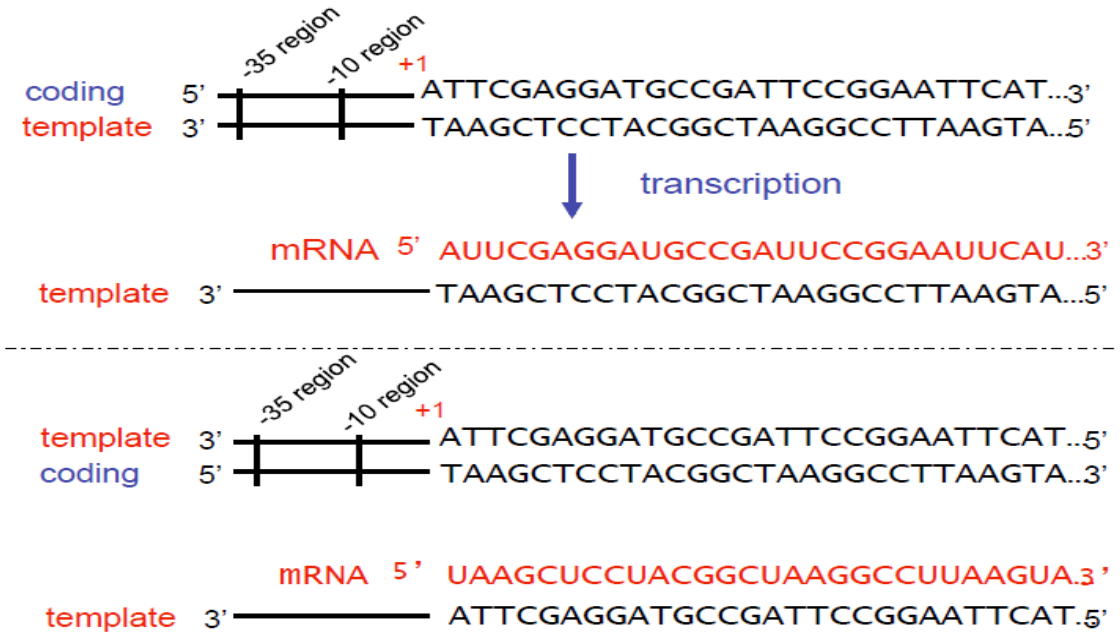
• **σ subunit**

b) Unwinding of promoter region: open complex
begins at the +1 site
• **σ subunit I released once transcript synthesis is initiated**

To begin the synthesis of an RNA molecule, RNA polymerase pairs the base on a ribonucleoside triphosphate with its complementary base at the start site on the DNA template strand .No primer is required to initiate the synthesis of the 5′end of the RNA molecule. Two of the three phosphates are cleaved from the ribonucleoside triphosphate as the nucleotide is added to the 3′ end of the growing RNA molecule. However, because the 5′end of the first ribonucleoside triphosphate does not take part in the formation of a phosphodiester bond, all three of its phosphates remain. An RNA molecule therefore possesses, at least initially, three phosphates at its 5′end (Figure -4).



Figure(3): In bacterial promoters, consensus sequences are found upstream of the start site, approximately at positions _10 and _35.



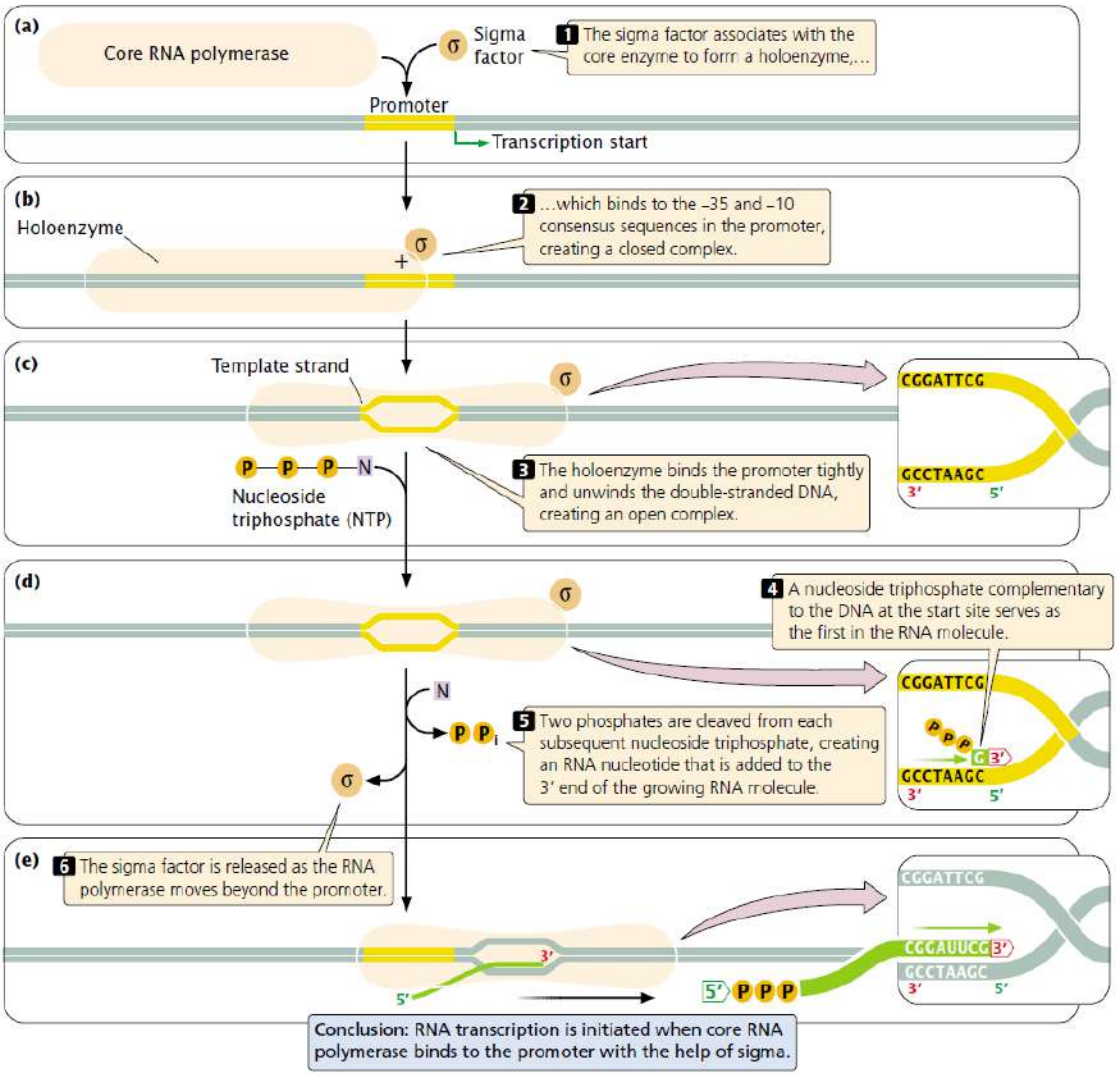


Figure-4: Transcription in bacteria is carried out by RNA polymerase, which must bind to the sigma factor to initiate transcription.

2-Elongation

After initiation, RNA polymerase moves downstream along the template, progressively unwinding the DNA at the leading (downstream) edge of the transcription bubble, joining nucleotides to the RNA molecule according to

the sequence on the template, and rewinding the DNA at the trailing (upstream) edge of the bubble. In bacterial cells at 37°C, about 40 nucleotides are added per second. This rate of RNA synthesis is much lower than that of DNA synthesis, which is more than 1500 nucleotides per

second in bacterial cells Transcription takes place within a short stretch of about 18 nucleotides of unwound DNA—the transcription bubble. Within this region, RNA is continuously synthesized with single-stranded DNA used as a template. About 8 nucleotides of newly synthesized RNA are paired with the DNA-template nucleotides at any one time. As the transcription apparatus moves down the DNA template, it generates positive supercoiling ahead of the transcription bubble and negative supercoiling behind it. Topoisomerase enzymes probably relieve the stress associated with the unwinding and rewinding of DNA in transcription, as they do in DNA replication.

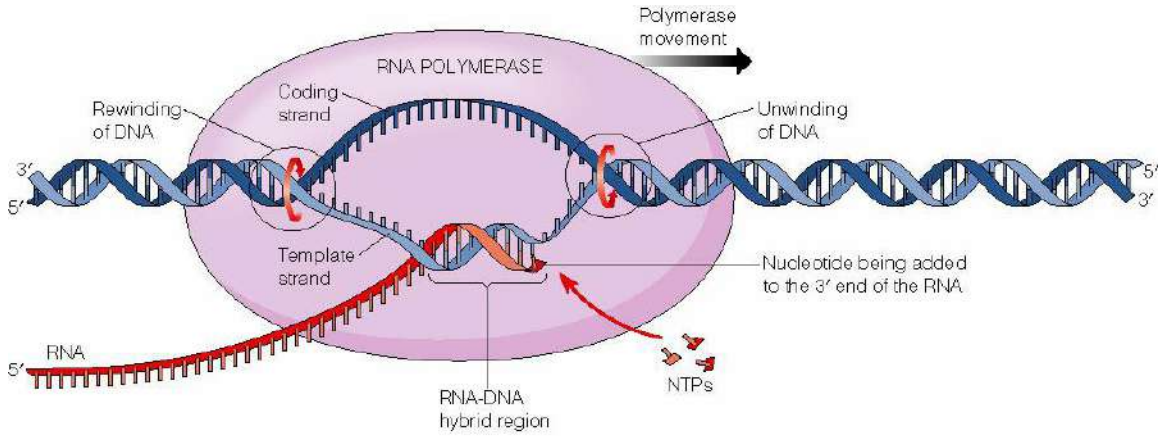


Figure-5: Elongation stage

3-Termination

RNA polymerase moves along the template, adding nucleotides to the 3′ end of the growing RNA molecule until it transcribes a terminator. Most terminators are found upstream of the point of termination. Rather, transcription ends after the terminator has been transcribed. At the terminator, RNA polymerase must stop synthesizing RNA, the RNA molecule must be released from RNA polymerase, the newly made RNA molecule must dissociate fully from the DNA, and RNA polymerase must detach from the DNA template.

Bacterial cells possess two major types of terminators:

A-Rho-independent terminators have two common features.

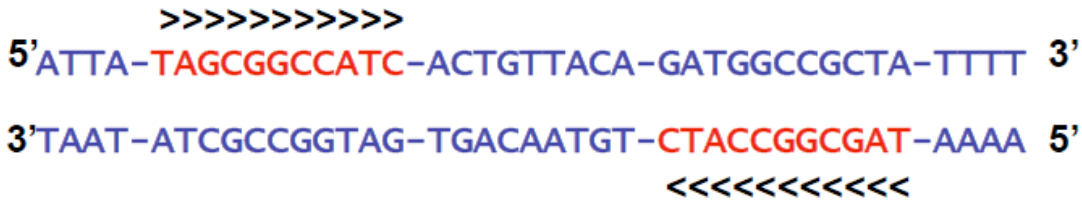
1-First, they contain **inverted repeats** (sequences of nucleotides on one strand that are inverted and complementary).When inverted repeats have been transcribed into RNA, a hairpin secondary structure forms .

2-Second, in rho-independent terminators, a string of approximately six adenine nucleotides follows the second inverted repeat in the template DNA. Their transcription produces a string of uracil nucleotides after the hairpin in the transcribed RNA.

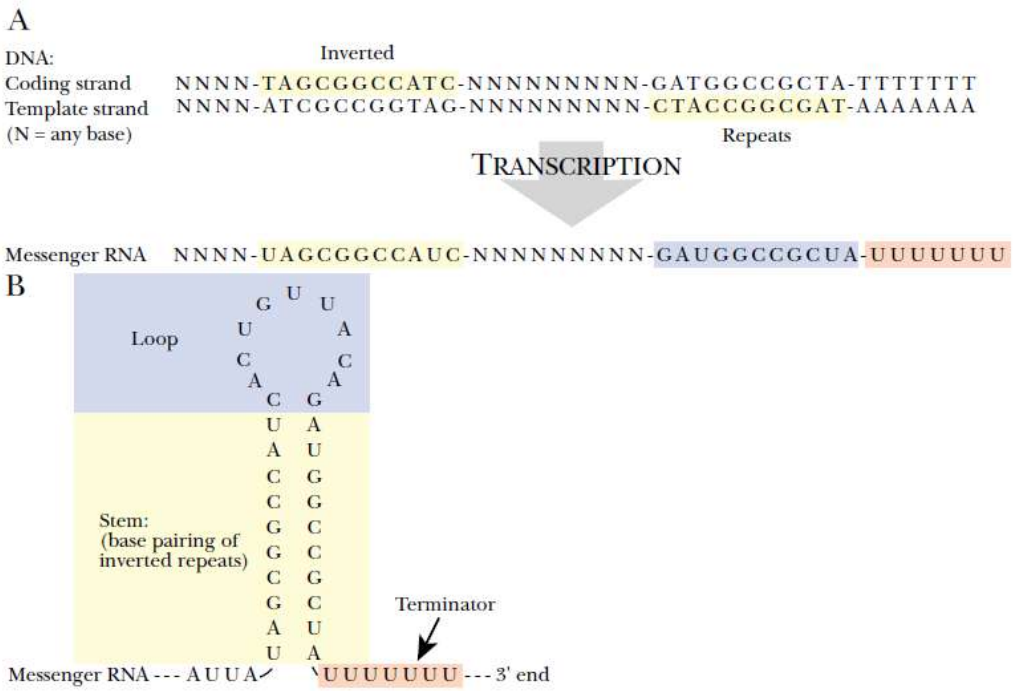
So Transcription stops at terminator sequences

• Inverted repeats:

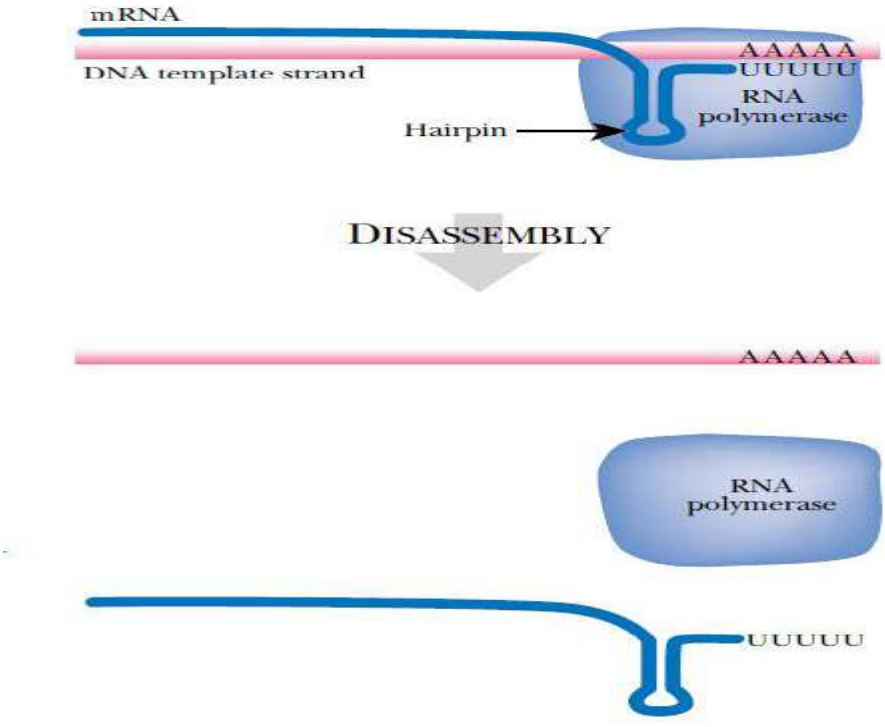
•Stretch of AAAAAAA…. on template strand



The presence of a hairpin in an RNA transcript causes RNA polymerase to slow down or pause, which creates an opportunity for termination. The adenine–uracil base pairings downstream of the hairpin are relatively unstable compared with other base pairings, and the formation of the hairpin may itself destablize the DNA–RNA pairing, causing the RNA molecule to separate from its DNA template. When the RNA transcript has separated from the template, RNA synthesis can no longer continue (Figure -6).



Figure(6): Termination by bacterial rho-independent terminators is a multistep process.



B- Rho-dependent terminators have two features:

(1) DNA sequences that produce a pause in transcription;

(2) A DNA sequence that encodes a stretch of RNA upstream of the terminator that is devoid of any secondary structures. This unstructured RNA serves as binding site for the rho protein, which binds the RNA and moves toward its 3_ end, following the RNA polymerase (Figure-7) .When RNA polymerase encounters the terminator, it pauses, allowing rho to catch up. The rho protein has helicase activity, which it uses to unwind the RNA–DNA hybrid in the transcription bubble, bringing an end to transcription.

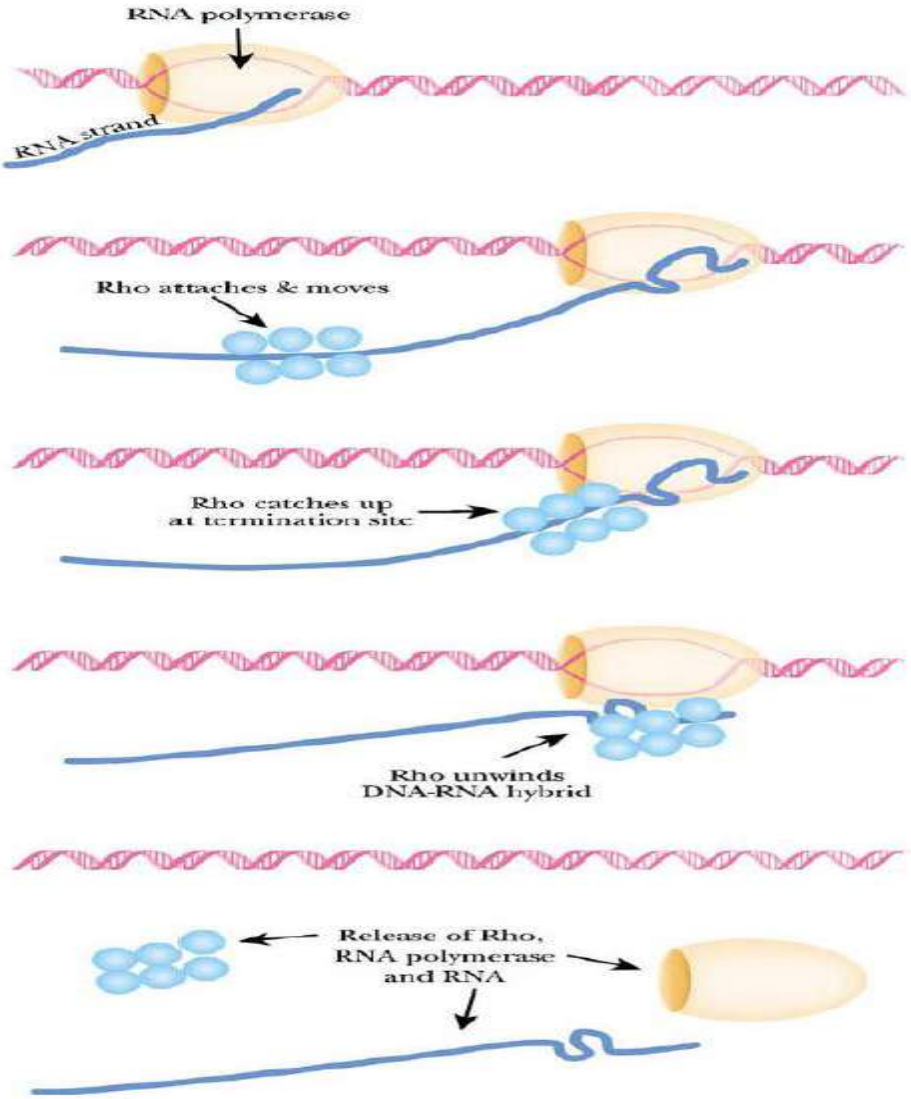


Figure-7: The termination of transcription in some bacterial genes requires the presence of the rho protein.

Lecture (6):

Translation of DNA

Translation is a process by which the genetic code contained within an mRNA molecule is decoded to produce the specific sequence of amino acids in a polypeptide chain (figure 1). Amino acids are covalently strung together by interlinking peptide bonds. Each individual amino acid has an amino group (NH_2) and a carboxyl (COOH) group. Polypeptides are formed when the amino group of one amino acid forms an amide (i.e., peptide) bond with the carboxyl group of another amino acid (Figure 2). This reaction is catalyzed by ribosomes and generates one water molecule.

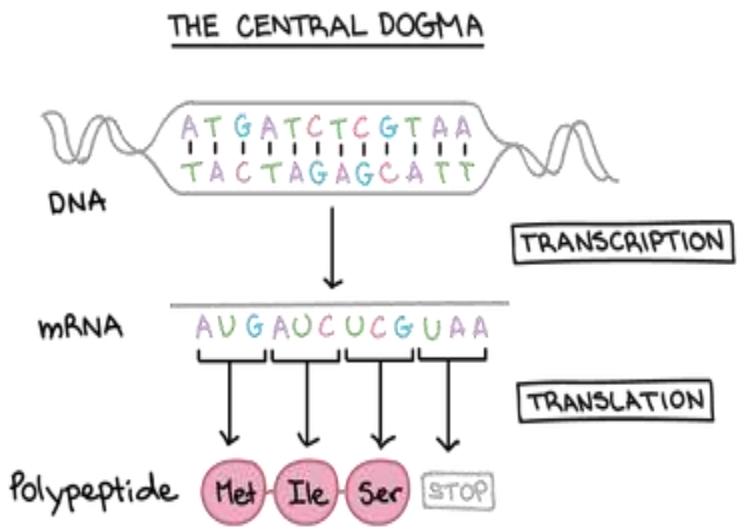


Figure 1: Central dogma of gene expression.

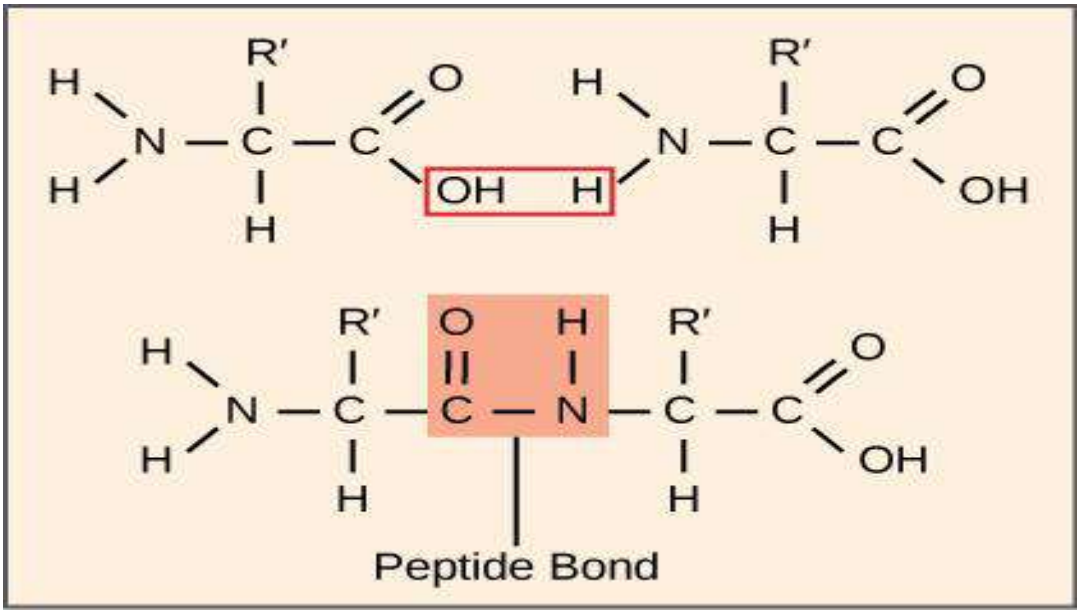


Figure 2: A peptide bond links the carboxyl end of one amino acid with the amino end of another, expelling one water molecule. For simplicity in this image, only the functional groups involved in the peptide bond are shown. The R and R′ designations refer to the rest of each amino acid structure.

It occurs in the **cytoplasm** following transcription and, like transcription, has three stages: initiation, elongation and termination.

Requirements for Translation

The key components required for translation are mRNA:

1- The ribosomes

- A ribosome is a complex macromolecule composed of structural and catalytic rRNAs, and many distinct polypeptides. Ribosomes exist in the cytoplasm in prokaryotes as separate subunits. In *E. coli*, the small subunit is described as 30S, and the large subunit is 50S, for a total of

70S. The small subunit is responsible for binding the mRNA template, whereas the large subunit sequentially binds tRNAs.

- The subunits come together to form a ribosome when they bind to an mRNA, near its 5' end.
- On binding to an mRNA, the ribosome reads the nucleotide sequence from the 5' to 3' direction, synthesizing the corresponding protein from amino acids in an N-terminal (amino-terminal) to C-terminal (carboxyl terminal) direction.

2- **tRNAs**

The tRNAs are structural RNA molecules that were transcribed from genes by RNA polymerase III. Serving as adaptors, specific tRNAs bind to sequences on the mRNA template and add the corresponding amino acid to the polypeptide chain. Therefore, tRNAs are the molecules that actually "translate" the language of RNA into the language of proteins.

Of the 64 possible mRNA codons—or triplet combinations of A, U, G, and C—three specify the termination of protein synthesis and 61 specify the addition of amino acids to the polypeptide chain. Of these 61, one codon (AUG) also known as the "start codon" encodes the initiation of translation. Each tRNA anticodon can base pair with one of the mRNA codons and add an amino acid or terminate translation, according to the genetic code. For instance, if the sequence CUA occurred on an mRNA template in the proper reading frame, it would bind a tRNA expressing the complementary sequence, GAU, which would be linked to the amino acid leucine.

Through intramolecular hydrogen bonds, the tRNA, bind to the amino acid at one end (**amino acid site**) and to the codon at the other end (**anticodon site**) (Figure 3).The anticodon is a three-nucleotide sequence

in a tRNA that interacts with an mRNA codon through complementary base pairing.

tRNAs need to interact with three factors:

1. They must be recognized by the correct aminoacyl synthetase.
2. They must be recognized by ribosomes.
3. They must bind to the correct sequence in mRNA.

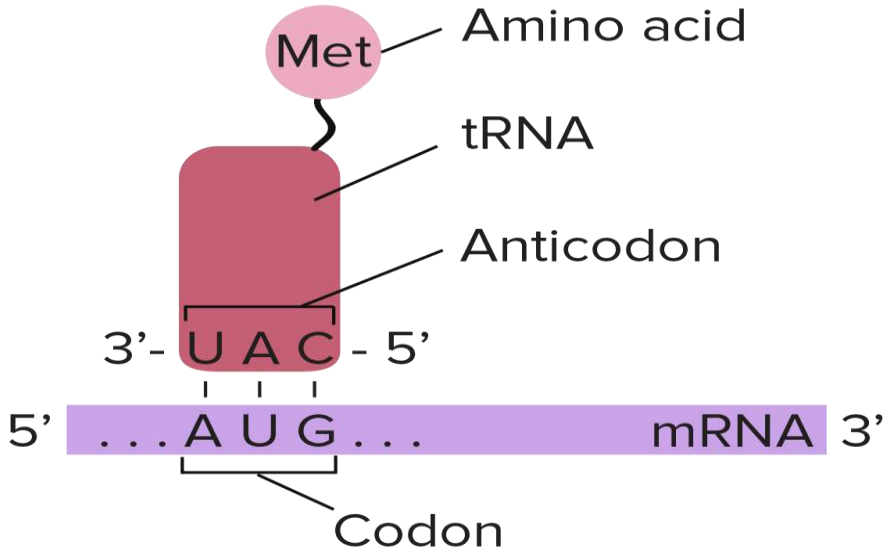


Figure 3: Amino acid – tRNA.

3- **Aminoacyl tRNA Synthetases**

Through the process of tRNA "charging," each tRNA molecule is linked to its correct amino acid by a group of enzymes called **aminoacyl tRNA synthetases**. At least one type of **aminoacyl tRNA synthetase** exists for each of the 20 amino acids.

Ribosomal Sites for Protein Translation

Each prokaryotic ribosome, shown schematically, has three binding sites for tRNAs (figure 4).

1. **The aminoacyl-tRNA binding site** (or A site) is where, during elongation, the incoming aminoacyl-tRNA binds.
2. **The peptidyl-tRNA binding site** (or P site) is where the tRNA linked to the growing polypeptide chain is bound.
3. **The exit site** (or E site) is a binding site for tRNA following its role in translation and prior to its release from the ribosome.

All three sites (A, P and E) are formed by the rRNA molecules in the ribosome.

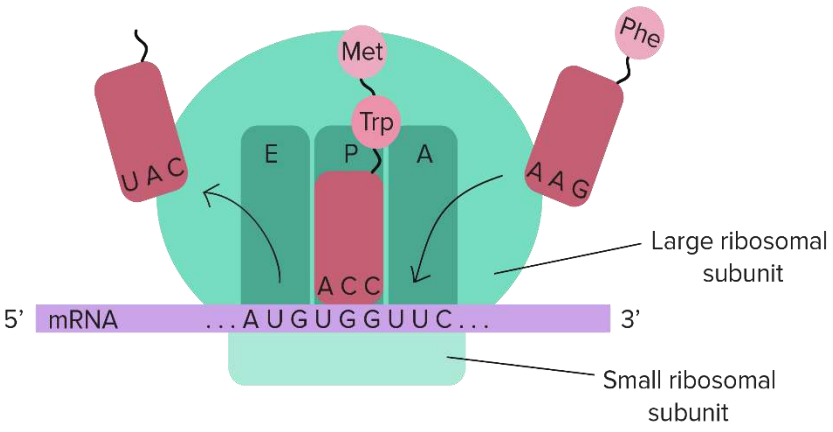


Figure 4: Ribosomal Sites for Protein Translation

Lecture (7):

The process of translation

Protein synthesis (or translation) takes place in three stages (figure 5):

1. Initiation
2. Elongation and
3. Termination.

- During initiation, the mRNA–ribosome complex is formed and the first codon (always AUG) binds the first aminoacyltRNA (called initiator tRNA).
- During the elongation phase, the other codons are read sequentially and the polypeptide grows by addition of amino acids to its C-terminal end.
- This process continues until a termination codon (Stop codon), which does not have a corresponding aminoacyl-tRNA with which to base pair, is reached.
- At this point, protein synthesis ceases (termination phase) and the finished polypeptide is released from the ribosome.

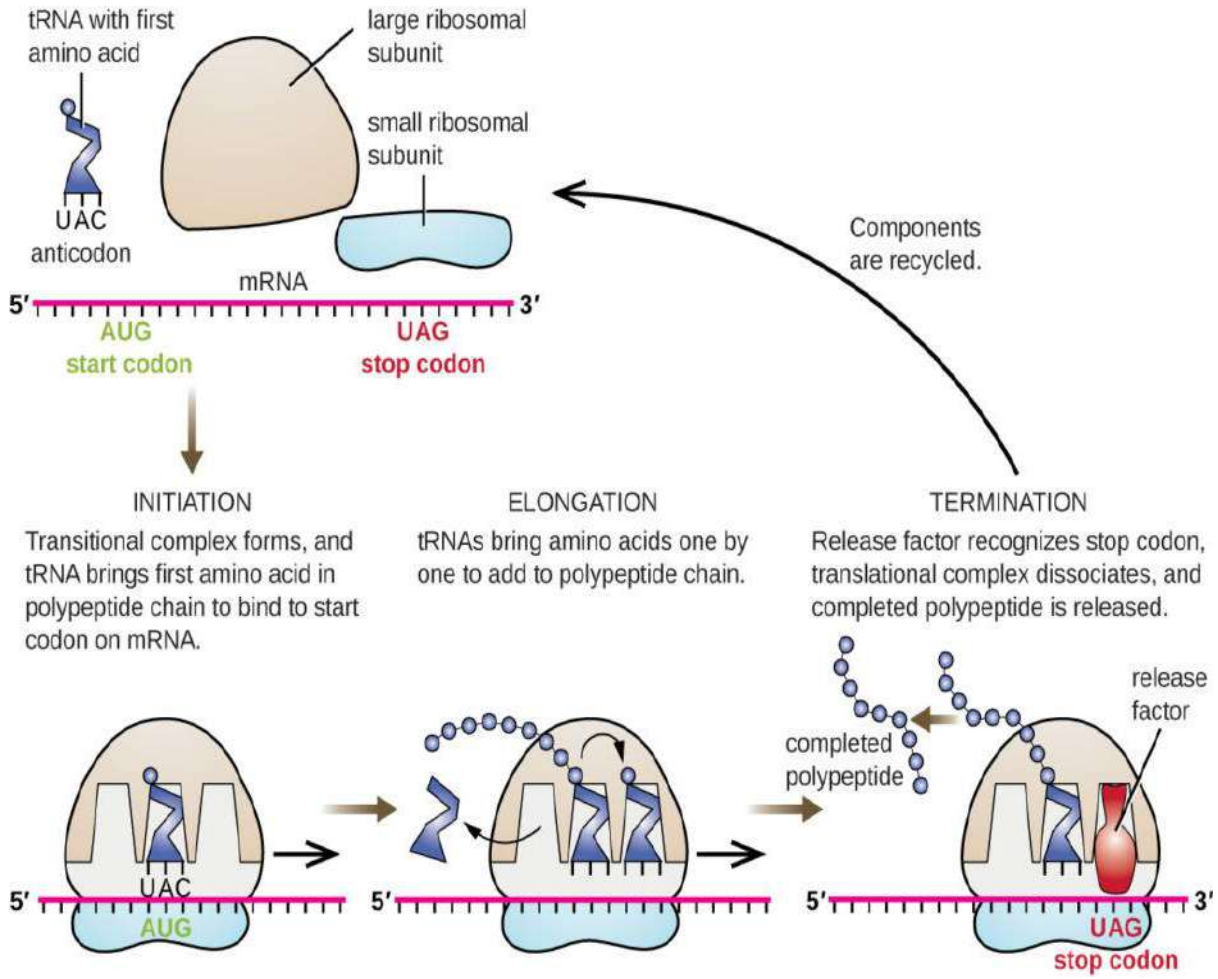


Figure 5: The process of translation

Synthesis of aminoacyl-tRNA

- Synthesis of aminoacyl-tRNAs is crucially important for two reasons:

1. Each amino acid must be covalently linked to a tRNA molecule in order to take part in protein synthesis, which depends upon the ‘adaptor’ function of tRNA to ensure that the correct amino acids are incorporated.
2. The covalent bond that is formed between the amino acid and the tRNA is a high energy bond that enables the amino acid to react with the end of the growing polypeptide chain to form a new peptide bond.

For this reason, the synthesis of aminoacyl-tRNA is also referred to as **amino acid activation.**

- Each tRNA molecule has a cloverleaf secondary structure with the anticodon accessible at the end of the anticodon stem loop.
- During synthesis of the aminoacyl-tRNA, the amino acid is covalently bound to the A residue of the CCA sequence at the 3’ end (figure 6).
- Each tRNA molecule carries only a single amino acid.
- The attachment of an amino acid to a tRNA is catalyzed by an enzyme called **aminoacyl-tRNA synthetase.**
- A separate aminoacyl-tRNA synthetase exists for every amino acid, making 20 synthetases in total.

The synthesis reaction occurs in two steps.

1. The first step is the reaction of an amino acid and ATP to form an aminoacyl-adenylate (also known as aminoacyl-AMP).
2. In the second step, without leaving the enzyme, the aminoacyl group of aminoacyl-AMP is transferred to the 3’ end of the tRNA molecule to form aminoacyl-tRNA

The overall reaction is:

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

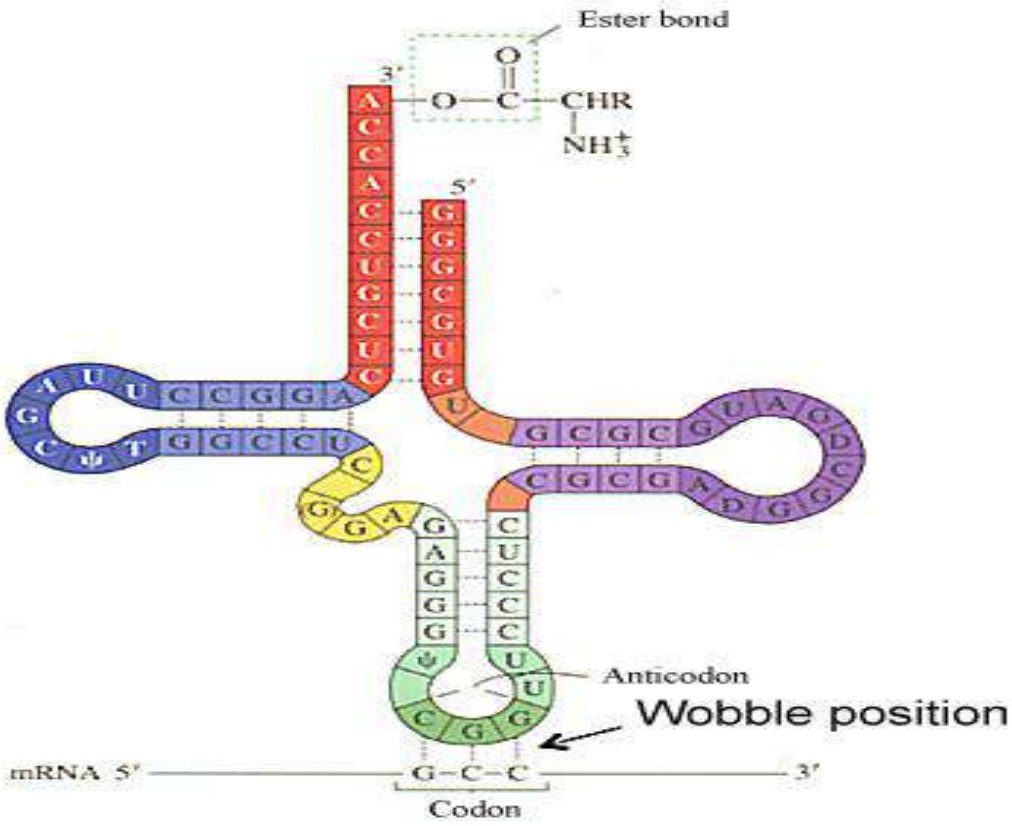


Figure 6: The amino acid is covalently bound to the A residue of the CCA sequence at the 3' end.

Initiation of Protein Synthesis

- The first codon translated in all mRNAs is the start codon or initiation codon, AUG which codes for methionine.
- Two different tRNAs are used for the two types of AUG codon; $tRNA_f^{Met}$ is used for the initiation codon and is called the initiator tRNA whereas $tRNA_m^{Met}$ is used for internal AUG codons.
- In prokaryotes the first amino acid of a new protein is N-formylmethionine (abbreviated fMet). Hence the aminoacyl-tRNA used in initiation is fMet-$tRNA_f^{Met}$.

- A short sequence rich in purines (5'-AGGAGGU-3'), called the **Shine–Dalgarno sequence**(figure 7), lies 5' to the AUG initiation codon and is complementary to part of the 16S rRNA in the small ribosomal subunit.
- Therefore, this is the binding site for the 30S ribosomal subunit which then migrates in a 3' direction along the mRNA until it encounters the AUG initiation codon.
- Initiation of protein synthesis requires proteins called initiation factors (IFs).
- In prokaryotes, three initiation factors (IF-1, IF-2 and IF-3) are essential.
- Because of the complexity of the process, the exact order of binding of IF-1, IF-2, IF-3, fMet-tRNAf is controversial.

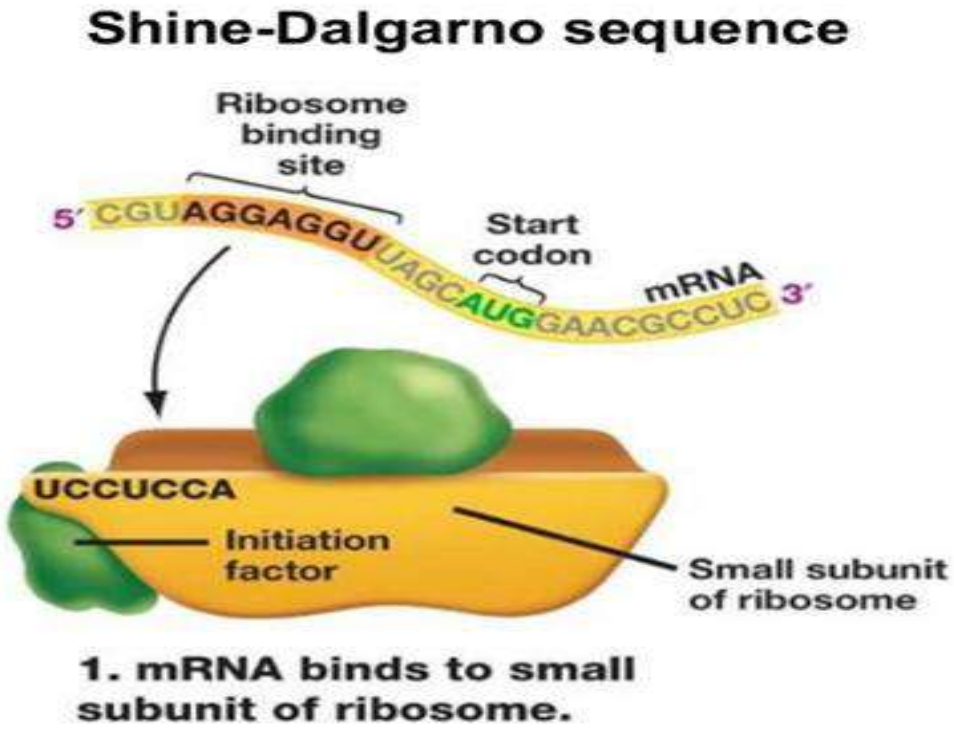


Figure 7: Shine-Dalgarno sequence.

Steps Involved for Intiation

1. Initiation begins with the binding of IF-1 and IF-3 to the small (30S) ribosomal subunit.

Their role is to stop the 30S subunit binding to the 50S subunit in the absence of mRNA and fMet-$tRNA_f^{Met}$ which would result in a nonfunctional ribosome.

2. The small subunit then binds to the mRNA via the Shine–Dalgarno sequence and moves 3' along the mRNA until it locates the AUG initiation codon.
3. The initiator tRNA charged with N-formylmethionine and in a complex with IF-2 and GTP (fMet-$tRNA^{fMet}$/IF-2/GTP) now binds.
4. IF-3 is released.
5. The complex of mRNA, fMet-$tRNA^{fMet}$-, IF-1, IF-2 and the 30S ribosomal subunit is called the 30S initiation complex.
6. The large (50S) ribosomal subunit now binds, with the release of IF-1 and IF-2 and hydrolysis of GTP, to form a 70S initiation complex.

Elongation of Protein Synthesis

- At the start of the first round of elongation, the initiation codon (AUG) is positioned in the P site with fMet-$tRNA_f^{Met}$ bound to it via codon–anticodon base pairing.
- The next codon in the mRNA is positioned in the A site.
- Elongation of the polypeptide chain occurs in three steps called the elongation cycle, namely aminoacyl-tRNA binding, peptide bond formation and translocation:

Aminoacyl-tRNA binding

- The corresponding aminoacyl-tRNA for the second codon binds to the A site via codon–anticodon interaction.
- Binding of the aminoacyl-tRNA requires elongation factor EF-Tu and GTP which bind as an aminoacyl-tRNA/EF-Tu/GTP complex.
- Following binding, the GTP is hydrolyzed and the EF-Tu is released, now bound to GDP.
- Before the EF-Tu molecule can catalyze the binding of another charged tRNA to the ribosome, it must be regenerated by a process involving another elongation factor, EF-Ts.

This regeneration is called the EF-Tu–EF-Ts exchange cycle.

- First, EF-Ts binds to EF-Tu and displaces the GDP. Then GTP binds to the EF-Tu and displaces EF-Ts. The EF-Tu-GTP is now ready to take part in another round of elongation.

Peptide bond formation

- The second step, peptide bond formation, is catalyzed by peptidyl transferase.
- In this reaction the carboxyl end of the amino acid bound to the tRNA in the P site is uncoupled from the tRNA and becomes joined by a peptide bond to the amino group of the amino acid linked to the tRNA in the A site.

Translocation

- In the third step, a complex of elongation factor EF-G (also called translocase) and GTP (i.e. EF-G/GTP) binds to the ribosome.
- Three concerted movements now occur, collectively called translocation:

1. the deacylated tRNA moves from the P site to the E site
2. the dipeptidyl-tRNA in the A site moves to the P site, and
3. the ribosome moves along the mRNA (5’ to 3’) by three nucleotides to place the next codon in the A site.

- During the translocation events, GTP is hydrolyzed to GDP and inorganic phosphate, and EF-G is released ready to bind more GTP for another round of elongation.
- After translocation, the A site is empty and ready to receive the next aminoacyltRNA.
- The A site and the E site cannot be occupied simultaneously. Thus the deacylated tRNA is released from the E site before the next aminoacyl-tRNA binds to the A site to start a new round of elongation.
- Elongation continues, adding one amino acid to the C-terminal end of the growing polypeptide for each codon that is read, with the peptidyl-tRNA moving back and forth from the P site to the A site as it grows.

Termination of Protein Synthesis

- Eventually, one of three termination codons (also called Stop codons) becomes positioned in the A site. These are UAG, UAA and UGA.
- Unlike other codons, prokaryotic cells do not contain aminoacyl-tRNAs complementary toStop codons. Instead, one of two release factors (RF-1 and RF-2) binds instead.
- RF-1 recognizes UAA and UAG whereas RF-2 recognizes UAA and UGA. A third release factor, RF-3, is also needed to assist RF-1 or

RF-2 interaction with the ribosome. Thus either RF-1 + RF-3 or RF-2 + RF-3 bind depending on the exact termination codon in the A site.

- RF-1 (or RF-2) binds at or near the A site whereas RF-3/GTP binds elsewhere on the ribosome.
- The release factors cause the peptidyl transferase activity to transfer the polypeptide to a water molecule instead of to aminoacyl-tRNA, effectively cleaving the bond between the polypeptide and tRNA in the P site.

The free polypeptide now leaves the ribosome, followed by the mRNA and free tRNA, and the ribosome dissociates into 30S and 50S subunits ready to start translation again.

Lecture: 8

The Plasmids

A **plasmid** is an extra chromosomal DNA molecule occurring free in the cytoplasm or is a small DNA molecule that is physically separate from and capable of autonomous replication (independently of the chromosomal DNA). Most commonly found as small circular, double-stranded DNA molecules in bacteria, archae, It usually occurs in bacteria, sometimes in eukaryotic organisms (e.g .,the *2–micron–ring* in *Saccharomyces cerevisiae*).

In nature, plasmids carry genes that may benefit survival of the organism (e.g. antibiotic resistance), and can frequently be transmitted from one bacterium to another (even of another species) via horizontal gene transfer. Artificial plasmids are widely used as vectors in molecular cloning, serving

to drive the replication of recombinant DNA sequences within host organisms.

The size of plasmids varies from 1 to over 400 kilobase pairs (kbp). Depending on the size of the plasmids, the number of copies of the same plasmid in a single cell varies from one to several hundreds. The larger the plasmid, the fewer is the number of plasmids. The number may even reach thousands for certain artificial plasmids (such as the **pUC** series of plasmids) selected for high copy number.

Plasmids are considered *replicons*, capable of replicating autonomously within a suitable host. However, plasmids, like viruses, are not considered by some to be a form of life. Plasmids can be transferred between bacterial hosts through a process known as bacterial conjugation. Because conjugation is a mechanism of horizontal gene

transfer, plasmids can be considered part of the mobilome. Unlike viruses (which encase their genetic material in a protective protein coat called a capsid), plasmids are "naked" DNA and do not encode genes necessary to encase the genetic material for transfer to a new host. However, some classes of plasmids encode the conjugative "sex" pilus necessary for their own transfer.

The Characteristic features of Plasmid in points:

- A plasmid typically is a circular and double–stranded DNA molecule; but linear plasmids have also been reported (*Streptomyces spp*, *Borrelia spp*).
- It is smaller than and independent of chromosome.
- It is capable of self–replication using already existing cellular enzymes. But the initiation of replication and the distribution of plasmid copies to the daughter cells is controlled by plasmid genes. Thus, every plasmid contains at least one DNA sequence that serves as an *origin of replication*, or *ori* (a starting point for DNA replication), which enables the plasmid DNA to be duplicated independently from the chromosomal DNA (Figure - 1) and to be distributed to the daughter cells.
- Many plasmids also have genes responsible for phenotypic expression of the organism.
- Plasmids that exist only as one or a few copies in each bacterium are, upon cell division, in danger of being lost in one of the segregating bacteria. Such single–copy plasmids have systems that are oriented toward actively distributing a copy to both daughter cells.

1-**Fertility F-plasmids**, which contain *tra* genes. They are capable of conjugation and result in the expression of sex pilli.

2-**Resistance (R)plasmids**, which contain genes that provide resistance against antibioticsor poisons. Historically known as R-factors, before the nature of plasmids was understood.

3-**Col plasmids**, which contain genes that code for bacteriocins, proteins that can kill other bacteria.

4-**Degradative plasmids**, which enable the digestion of unusual substances, e.g. tolueneand salicylic acid.

5-**Virulence plasmids**, which turn the bacterium into a pathogen.

Fertility or F–plasmids are capable of carrying out bacterial conjugation, a plasmid regulated complex process of sexual transfer of F–plasmid to another bacterium (Figure 3). Among the two conjugating strains of bacteria, the one with F–factor is known as male, donor, or F+ strain and the one without F–factor is known as female, One of the early detected R–plasmids is the penicillinase–plasmid of *Staphylococcus aureus. S. aureus* is a Gram positive bacterial pathogen causing infection of skin and wound of humans. After treatment with penicillin antibiotic, several penicillin–resistant staphylococci developed throughout the world by 1950. High level resistance to penicillin was due to secretion of an enzyme, penicillinase, that degrades penicillin by hydrolysis **Heavy–metal resistance plasmid**

There are several bacterial strains that contain genetic determinants of resistance to heavy metals, such as Hg++, Ag+, Cd++, CrO4, Cu++, Ni++, Pb+++, Zn++, and so forth. These determinants for resistance are often found on plasmids and transposons. Bacteria that have been found resistant to heavy metals are *E. coli, Pseudomonas aeruginosa, P. florenscens, P. syringae,* and so forth.

Col–plasmid

There are many bacterial strains that produce proteinaceous toxins known as *bacteriocin,* which are lethal to other strains of the same genus. The bacteriocin producing genes are present not in chromosomes, but in special plasmids known as bacteriocin factor. Bacteriocins are already isolated from *Escherichia coli* (Colicin),*Pseudomonas aeruginosa* (Pyocin), *Bacillus megaterium* (Megacine), and others. Toxins secreted by the strains of *E. coli* are called colicins and the plasmid with responsible gene is known as colicinogeny or Col–factor. Several Col–plasmids like Col B, Col E, Col I, Col V have been recognized and they produce different types of colicins. Some of the Col–plasmids are *conjugative* (e.g., Col B, Col V) while others are *non–conjugative* (e.g., Col E) and are non–transmissible by their own means. receptor, or F- strain. F–factor consists of genes responsible for self–replication, for the formation of *sex–pili* or *f–pili* to establish contact between the cells of two strains, and for the formation of cytoplasmic bridge and *tra–genes* responsible for the transfer of the plasmid. Sometimes, the F–factor can also transfer chromosomal DNA to the receptor bacterium if the factor happens to be integrated to the chromosomal DNA. Such strains have genetic recombination rate 103 times greater than that of F+ and F- strains; so, they are termed *high frequency recombinants*(Hfr). Thus, plasmids can be part of the mobilome, since they are often associated with conjugation, a mechanism of horizontal gene transfer.

 Hence, another way of grouping plasmids is by their ability to transfer genetic material to other bacteria. *Conjugative* plasmids contain so–called *tra–genes,* which perform the complex process of *conjugation. Non–conjugative* plasmids are incapable of initiating conjugation; they can only be transferred with the assistance of conjugative plasmids, by "accident." An intermediate class of plasmids carry only a subset of the genes required for

transfer and they can "parasitize" a conjugative plasmid, thus transferring at high frequency only in its presence F–plasmid.

Resistance or R–plasmids contain genes or gene cassettes that confer a selective advantage to the bacterium harboring them, such as the ability to make the bacterium antibiotic resistant, i.e., resistant against antibiotics or poisons. Historically they were known as R–factors.

Such a plasmid consists of two segments of DNA, one the resistance transfer factor (RTF) responsible for replication as well as transfer of R–plasmid, and the second resistant determinants (r–determinants), which are the genes producing substances neutralizing the action of one or another antibiotics or other drugs. These plasmids are *conjugative* and spread among the bacteria through conjugation. The antibiotic resistance observed in *Shigella* and *Salmonella* were due to R–factors.

Degradative plasmid

Degradative plasmids consist of genes that equip the bacteria (e.g., *Pseudomonas sps.*) with special enzymes or enzyme system to enable them to digest unusual substances (**Xenobiotics**) like chlorinated aromatic or hydrocarbon compounds. For example, the camphor (CAM) plasmid of *P. putida* encodes enzymes for degradation of camphor, octane (OCT) plasmid helps it degrade octane, XYL–plasmid helps degrade xylene and toluene, NAH–plasmid helps degrade naphthalene, and SAL–plasmid helps it degrade salicilate. These plasmids are *conjugative.*

Virulence plasmid

In the race between immunity of the host and virulence of the pathogen, the latter evolves new virulence factors to keep it safely nourished from the host. Much of this virulence is found to be due to the genes in the plasmid known as *virulence plasmids.* Formation of invasin due to its virulence plasmid

makes *Shigella flexneri* (a human intestinal pathogen) able to penetrate intestinal mucosa. Production of **enterotoxin** and the cause of diarrhea are also associated with virulence plasmids present in the pathogen. The lysis of R.B.C. (erythrocyte) of blood caused by some of the pathogenic strains of *E. coli* is because of the production of haemolysin from a specific plasmid. Another example of virulence plasmid is **Ti–Plasmid** (tumor inducing plasmid), which is found in *Agrobacterium tumefaciens.* It is a Gram–negative soil bacterium that infects over 300 different dicots, causing crown gall disease at collar region. Ti–plasmid consists of, besides other genes, T–DNA, which encodes enzymes for the synthesis of auxin and cytosine. These plant hormones affect plant metabolism, develop tumors, and enable plants to produce compounds called opines, which is used by the pathogen as sources of carbon and nitrogen. Closely related to Ti–plasmid is the **Ri–plasmid** (root inducing plasmid). It is found in *A. rhizogenes* and causes hairy root disease in the plants

Cryptic plasmid

During isolation of plasmid DNA from a large number of bacteria, every bacterium was found to contain a low molecular weight DNA as a plasmid. Thus, it seems that the presence of plasmids is a general rule rather than exception. However, not all plasmids consist of genes having any phenotypic significance. The plasmids without any functional genes are termed as cryptic plasmid.

Conformations

Plasmid DNA may appear in one of five conformations, which (for a given size) run at different speeds in a gel during electrophoresis. The conformations are listed below in order of electrophoretic mobility (speed for a given applied voltage) from slowest to fastest:

1-*Nicked open-circular* DNA has one strand cut.

2-*Relaxed circular* DNA is fully intact with both strands uncut, but has been enzymatically *relaxed* (supercoils removed). This can be modeled by letting a twisted extension cord unwind and relax and then plugging it into itself. 3-*Linear* DNA has free ends, either because both strands have been cut or because the DNA was linear *in vivo*. This can be modeled with an electrical extension cord that is not plugged into itself.

4-*Supercoiled* (or *covalently closed-circular*) DNA is fully intact with both strands uncut, and with an integral twist, resulting in a compact form. This can be modeled by twisting an extension cord and then plugging it into itself.

5-*Supercoiled denatured* DNA is like *supercoiled DNA*, but has unpaired regions that make it slightly less compact; this can result from excessive alkalinity during plasmid preparation.

Lecture (9):

Bacterial Mutation

A mutation is any heritable change in DNA sequence.This may, or may not, affect the phenotype of the organism. The term "mutation" derived from Latin word meaning "to change". The process of mutation is called **mutagenesis** and the agent inducing mutations is called **mutagen**. (Organisms selected as reference strains are called **wild type**, and theirprogeny with mutations are called **mutants**). Changes in the sequence of template DNA (**mutations**) can drastically affect the type of protein end product produced.

Mechanisms of mutation

1- Substitution of a nucleotide: Base substitution, also called point mutation, involves the changing of single base in the DNA sequence.

a) This mistake is copied during replication to produce a permanent change. If one purine [A or G] or pyrimidine [C or T] is replaced by the other, the substitution is called a transition.

b) If a purine is replaced by a pyrimidine or viceversa, the substitution is called a transversion. This is the most common mechanism of mutation.

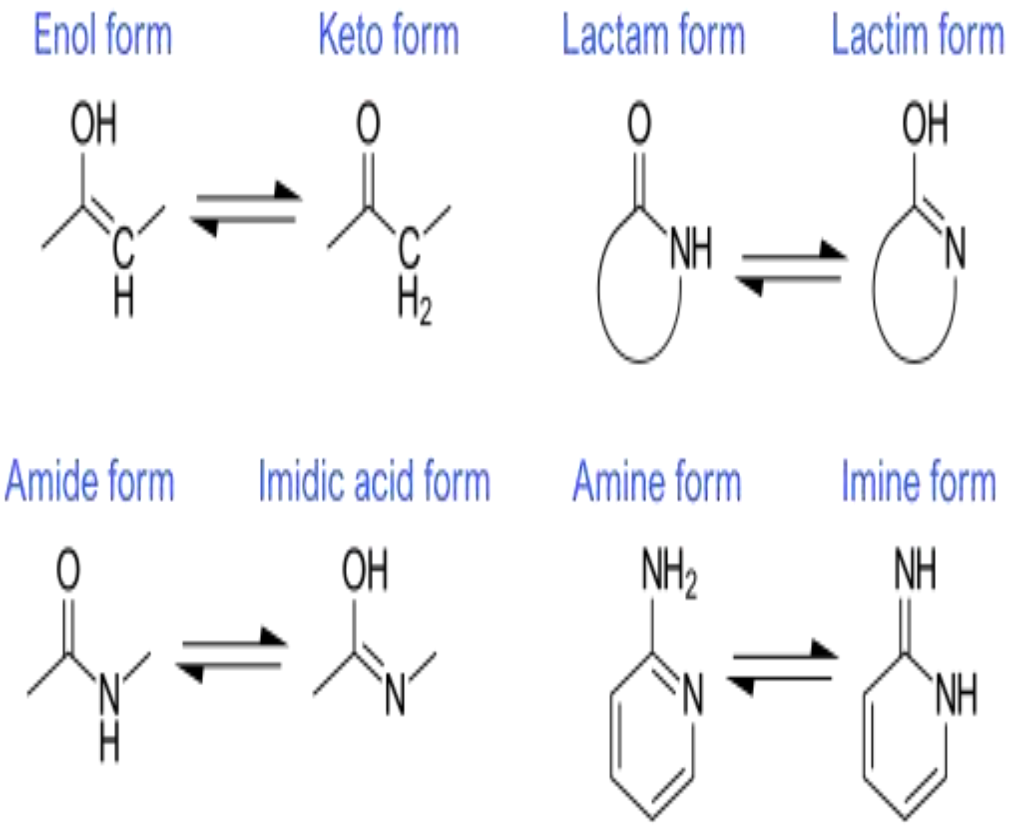
2- Deletion or addition of a nucleotide: deletion or addition of a nucleotide during DNA replication. When a transposon (jumping gene) inserts itself into a gene, it leads to disruption of gene and is called insertional mutation.

Causes of mutations

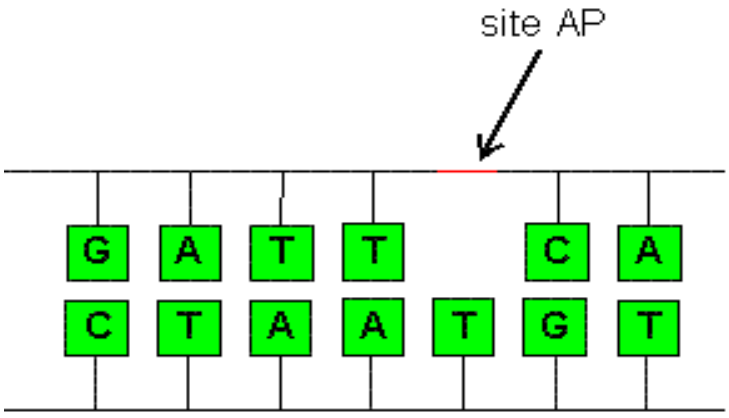
Mutations can occur spontaneously or be caused by exposure to mutation-inducing agents.

1- Spontaneous mutations (molecular decay): occur with non-zero probability even given a healthy, uncontaminated cell. They can be characterized by the specific change:

- **Tautomerism**: A base is changed by the repositioning of a hydrogen atom, altering the hydrogen bonding pattern of that base, resulting in incorrect base pairing during replication.

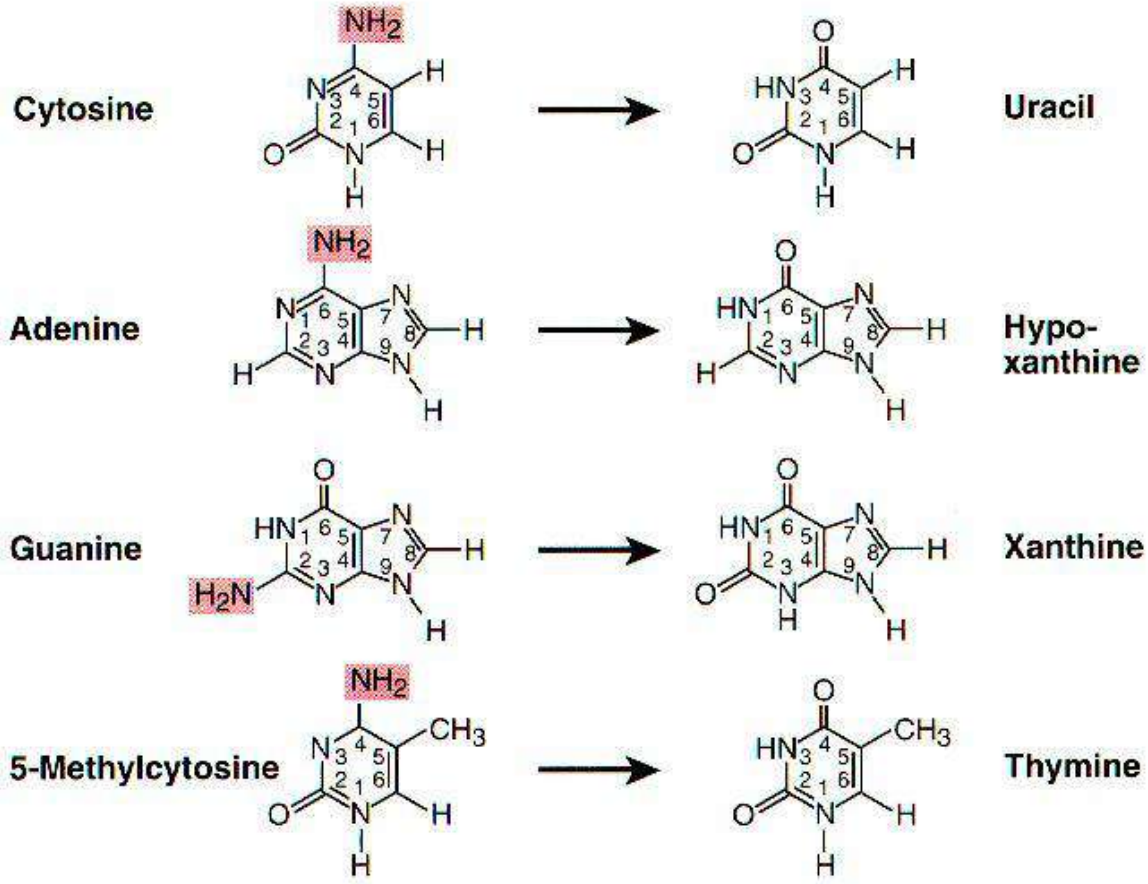


- **Depurination**: Loss of a purine base (A or G) to form an apurinic site (AP site).

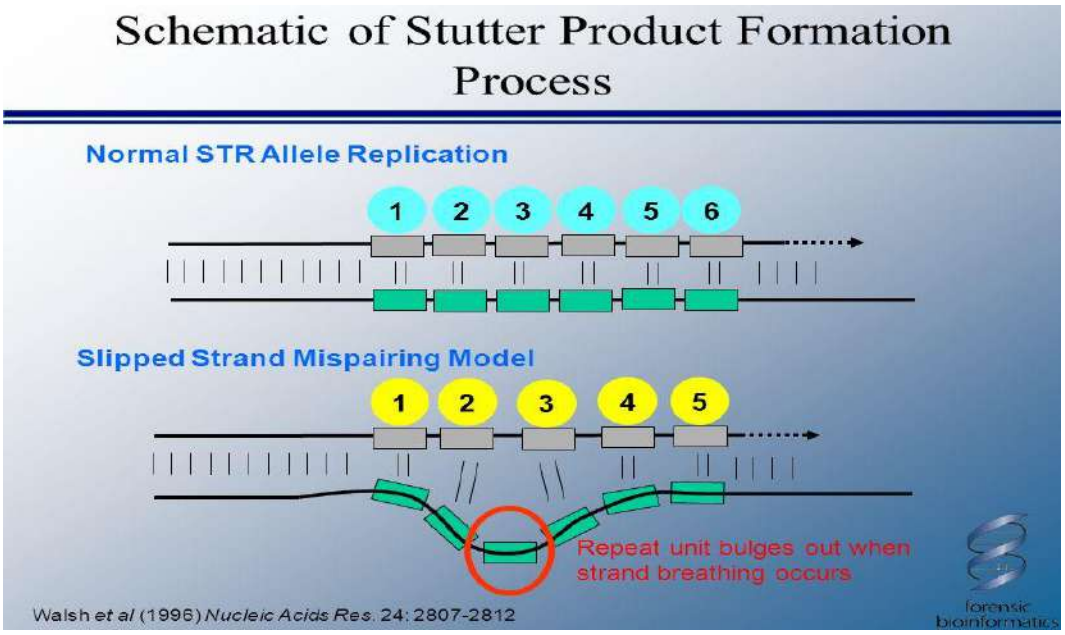


- **Deamination**: Hydrolysis changes a normal base to an atypical base containing a keto group in place of the original amine group. Examples include C → U and A → HX (hypoxanthine), which can be corrected by DNA repair mechanisms; and 5MeC (5-methylcytosine) → T, which is

less likely to be detected as a mutation because thymine is a normal DNA base.



- **Slipped strand mispairing: or called Replication slippage**:Denaturation of the new strand from the template during replication, followed by renaturation in a different spot ("slipping"). This can lead to insertions or deletions.

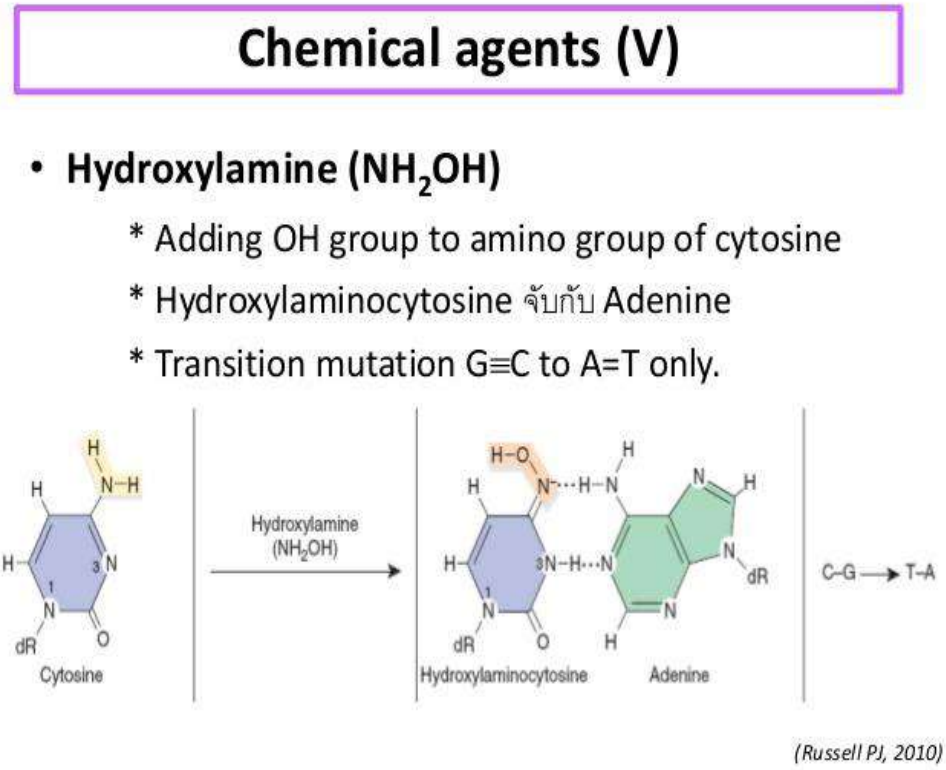


2- **Mutations due to error-prone replication bypass**: Naturally occurring DNA damage (also called error-prone translesion synthesis): There is increasing evidence that the majority of spontaneously arising mutations are due to error-prone replication (translesion synthesis) past DNA damage in the template strand. Naturally occurring oxidative DNA damages arise at least 10,000 times per cell per day in humans and 50,000 times or more per cell per day in rats. In mice, the majority of mutations are caused by translesion synthesis. Likewise, in yeast, more than 60% of the spontaneous single base pair substitutions and deletions were caused by translesion synthesis.

2-Errors introduced during DNA repair: Although naturally occurring double-strand breaks occur at a relatively low frequency in DNA, their repair often causes mutation. Non-homologous end joining (NHEJ) is a major pathway for repairing double-strand breaks. NHEJ involves removal

of a few nucleotides to allow somewhat inaccurate alignment of the two ends for rejoining followed by addition of nucleotides to fill in gaps. As a consequence, NHEJ often introduces mutations.

3- **Induced mutations caused by mutagens**:Induced mutations are alterations in the gene after it has come in contact with mutagens and environmental causes. *Induced mutations* on the molecular level can be caused by:



(Russell PJ, 2010)

- Base analogs (e.g., Bromodeoxyuridine (BrdU))
- Alkylating agents (e.g., *N*-ethyl-*N*-nitrosourea (ENU)). These agents can mutate both replicating and non-replicating DNA. In contrast, a base analog can mutate the DNA only when the analog is incorporated in replicating the

DNA. Each of these classes of chemical mutagens has certain effects that then lead to transitions, transversions, or deletions.

- Agents that form DNA adducts (e.g., ochratoxin A)
- DNA intercalating agents (e.g., ethidium bromide)
- DNA crosslinker.
- Oxidative damage.
- Nitrous acid converts amine groups on A and C to diazo groups, altering their hydrogen bonding patterns, which leads to incorrect base pairing during replication

Radiation

- **Non-ionizing radiation** (Ultraviolet light (UV).Cytosine and thymine—are most vulnerable to radiation that can change their properties. UV light can induce adjacent pyrimidine bases in a DNA strand to become covalently joined as a pyrimidine dimer. UV radiation, in particular longer-wave UVA, can also cause oxidative damage to DNA.
- **Ionizing radiation.** Exposure to ionizing radiation, such as gamma radiation, can result in mutation, possibly resulting in cancer or death.

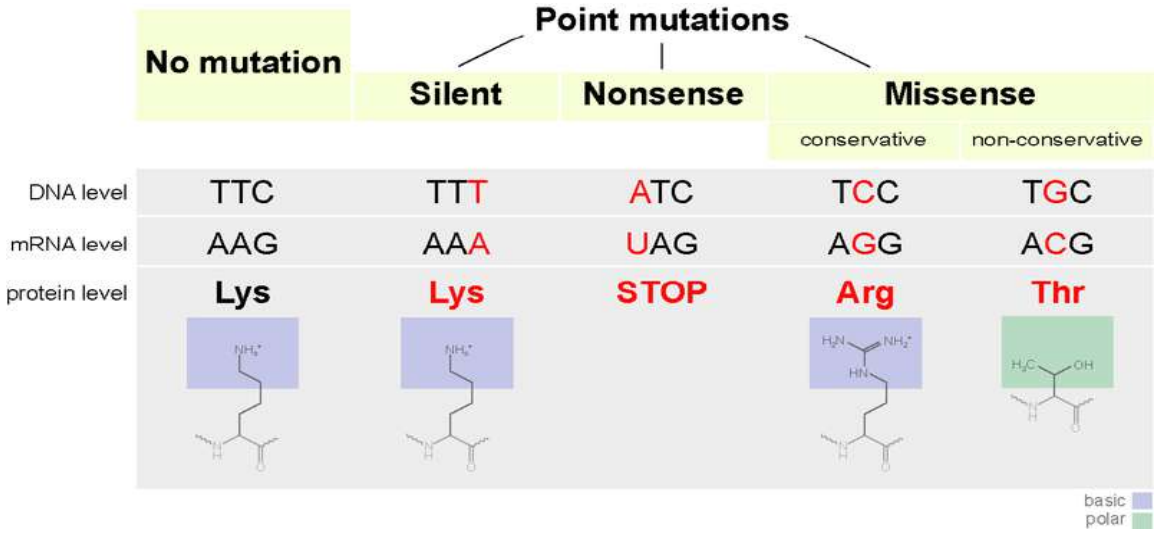
Effect of mutation

1) Point mutation

a)Silent mutation: Sometimes a single substitution mutation change in the DNA base sequence results in a new codon still coding for the same amino acid. Since there is no change in the product, such mutations are called silent.

b) Nonsense mutation: A mutation that leads to the formation of a stop codon is called a nonsense mutation. Since these codon cause the termination of protein synthesis, a nonsense mutation leads to incomplete protein products.

c) **Missense mutation**: Missense mutations are DNA mutations which lead to changes in the amino acid sequence (one wrong codon and one wrong amino acid) of the protein product. For example, if a missense mutation causes the substitution of a chemically similar amino acid, referred to as a synonymous substitution, then it is likely that the alteration will have a less-severe effect on the protein's structure and function. Alternatively, chemically different amino acid substitutions, called **nonsynonymous substitutions,** are more likely to produce severe changes in protein structure and function.



2- **Frameshift mutation**: Frameshift mutations involve the addition or deletion of base pairs causing a shift in the "reading frame" of the gene. This causes a reading frame shift and all of the codons and all of the amino acids after that mutation are usually wrong. Since the addition of amino acids to the protein chain is determined by the three base codons, when the overall sequence of the gene is altered, the amino acid sequence may be altered as well.

3- **Lethal mutation**: Sometimes some mutations affect vital functions and the bacterial cell become nonviable. Hence those mutations that can kill the cell are called lethal mutation.

4- **Suppressor mutation**: It is a reversal of a mutant phenotype by another mutation at a position on the DNA distinct from that of original mutation. True reversion or back mutation results in reversion of a mutant to original form, which occurs as a result of mutation occurring at the same spot once again.

5- **Conditional lethal mutation**: Sometimes a mutation may affect an organism in such a way that the mutant can survive only in certain environmental condition. Example; a temperature sensitive mutant can survive at permissive temperature of 35 C but not at restrictive temperature of 39 C.

Lecture 10 :

Movable Genes (Mobile Elements)

The term "Mobile DNA refers to segments of double-stranded DNA that move as discrete units from place to place within other DNA molecules. Segments of mobile DNA may move from one site to another on the same larger DNA molecule or from one host DNA molecule to another. Some insert more or less at random whereas others can insert only at specific sequences on the host DNA molecule.

Although the DNA of certain viruses can insert itself into the chromosomes of the host cell, most mobile DNA consists of genetic elements known as transposons or transposable elements. They are also sometimes called "**jumping genes**" because they may hop around from place to place among the chromosomes and plasmids.The process of jumping from one site to another is called **transposition**. Transposons are not merely dependent on a host cell like plasmids and viruses; they are dependent on a host DNA molecule! Transposons are always inserted into other DNA molecules so they are never free as separate molecules . Insertion of a DNA fragment into

a gene will usually result in the inactivation of that gene, and it is by the loss of that function. A number of genetic elements, including some phages and plasmids , can be inserted into the bacterial chromosome.

Terms included in this lecture:

- **Genetic element :** Any molecule or segment of DNA or RNA that carries genetic information and acts as a heritable unit.
- **Jumping gene :**Popular name for a transposable element
- **Mobile DNA:** Segment of DNA that moves from site to site within or between other molecules of DNA.
- **Transposable element:** A mobile segment of DNA that is always inserted in another, host molecule, of DNA. It has no origin of replication of its own and relies on the host DNA molecule for replication. Includes both DNA-based transposons and retrotransposons.
- **Transposition :**The process by which a transposon moves from one host DNA molecule to another
- **Transposon:** Same as transposable element, although the term is usually restricted to DNA-based elements that do not use reverse transcriptase.

The Nature of Transposable Elements

Transposable elements are mobile DNA sequences found in the genomes of all organisms. In many genomes, they are quite abundant.

Most transposable elements are able to insert at many different locations, relying on mechanisms that are distinct from homologous recombination. They often cause mutations, either by inserting into another gene and disrupting it or by promoting DNA rearrangements such as deletions, duplications, and inversions.

General Characteristics of Transposable Elements

There are many different types of transposable elements:

1- Simple structures, only those sequences necessary for their own transposition (movement).

2- Complex structures and encode a number of functions not directly related to transposition.

Despite this variation, many transposable elements have certain features in common included :

1-Short, **flanking direct repeats** of 3 to 12 base pairs are present on both sides of most transposable elements. They are not a part of a transposable element and do not travel with it. They are generated in the process oftransposition, at the point of insertion. The sequences of these repeats vary, but the length is constant for each type of transposable element.

The presence of flanking direct repeats indicates that staggered cuts are made in the target DNA when a transposable element inserts itself, The staggered cuts leave short, single-stranded pieces of DNA on either side of the transposable element. (**Figure -1).**Replication of the single-stranded DNA then creates the flanking direct repeats.

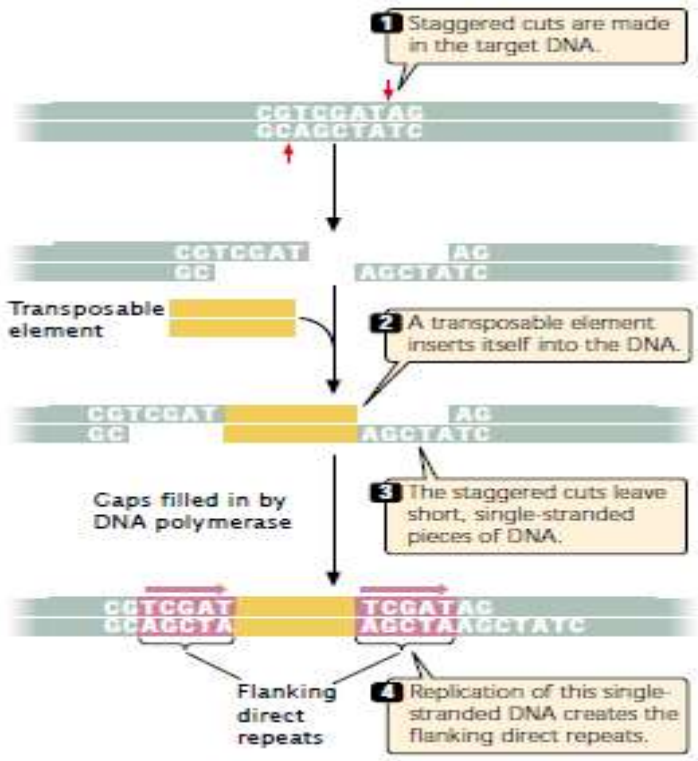


Figure -1: Flanking direct repeats are generated when a transposable element inserts into DNA.

2- At the ends of many, but not all, transposable elements are **terminal inverted repeats,** which are sequences from 9 to 40 bp in length that are inverted complements of one another. For example, the following sequences are inverted repeats:

5_–ACAGTTCAG . . . CTGAACTGT–3_

3_–TGTCAAGTC . . . GACTTGACA–5_

that the sequence from left to right in the top strand is the same as the sequence from right to left in the bottom strand. Terminal inverted repeats are recognized by enzymes that carry out transposition and are required for transposition to take place. (**Figure-2)**

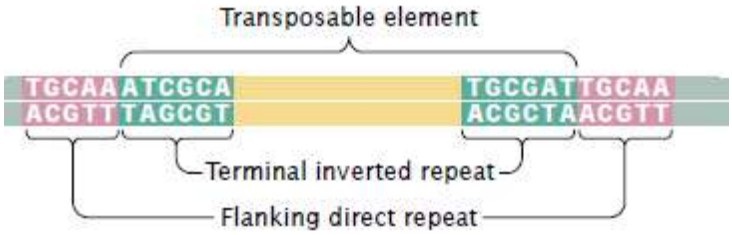


Figure-2: Many transposable elements have common characterFistics. Most transposable elements generate flanking direct repeats on each side of the point of insertion into target DNA. Many transposable elements also possess terminal inverted repeats.

Transposition

Transposition is the movement of a transposable element from one location to another. several different mechanisms are required for transposition in both prokaryotic and eukaryotic cells. all types of transposition have several features in common:

(1) staggered breaks are made in the target DNA (Figure -1).

(2) the transposable element is joined to single-stranded ends of the target DNA.

(3) DNA is replicated at the single strand gaps.

Mechanisms of Transposition

Some transposable elements transpose through DNA intermediates, whereas others use RNA intermediates. Among those that transpose through DNA, transposition may be replicative or nonreplicative.

A-In replicative transposition: a new copy of the transposable element is introduced at a new site while the old copy remains behind at the original site; the number of copies of the transposable element increases.

B- In nonreplicative transposition, the transposable element excises from the old site and inserts at a new site without any increase in the number of its copies. Nonreplicative transposition requires replication of only the few nucleotides that constitute the direct repeats.

Transposable Elements in Bacteria

The Structure of Transposable Elements in Bacteria possess a number of different types of transposable elements.

The two major groups of bacterial transposable elements are:

(1) Simple transposable elements that carry only the information required for movement

(2) more-complex transposable elements that contain DNA sequences not directly related to transposition.

1)- Insertion sequences : The simplest type of transposable element in bacterial chromosomes and plasmids is an **insertion sequence** (IS). This type of element carries only the genetic information necessary for its movement. Insertion sequences are common constituents of bacteria and plasmids. They are designated by IS, followed by an identifying number. For example, *IS1* is a common insertion sequence found in *E. coli*. Insertion sequences are typically from 800 to 2000 bp in length and possess the two hallmarks of transposable elements: terminal inverted repeats and the generation of flanking direct repeats at the site of insertion. Most insertion sequences contain one or two genes that code for transposase. *IS1*, a typical insertion sequence, is 768 nucleotide pairs long and has terminal inverted repeats of 23 bp at each end **(Figure-3)**. The flanking direct repeats created by *IS1* are each 9 bp long the most common length for flanking direct

repeats.(Table -1 summarizes these features for several bacterial insertion sequences).

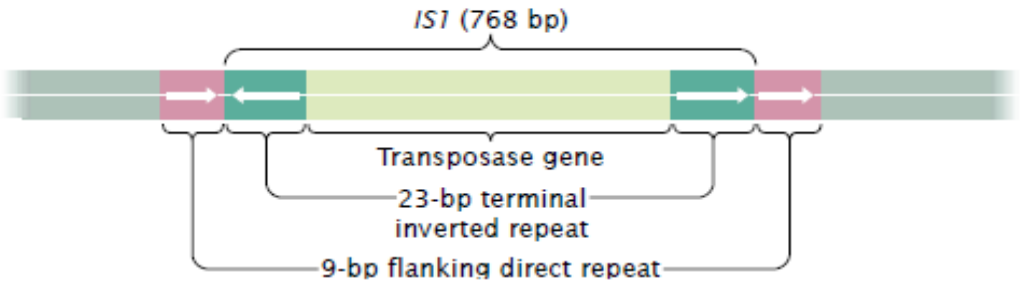


Table 11.4 Structures of some common insertion sequences

Insertion Sequence	Total Length (bp)	Length of Inverted Repeats (bp)	Length of Flanking Direct Repeats (bp)
IS1	768	23	9
IS2	1327	41	5
IS4	1428	18	11 or 12
IS5	1195	16	4

2- Complex transposable elements included two types ;

A-Composite transposons :

Composite transposons Any segment of DNA that becomes flanked by two copies of an insertion sequence may itself transpose and is called a **composite transposon.** So **a composite transposon is a more complex element that consists of two insertion sequences plus intervening DNA.**

Each type of composite transposon is designated by the abbreviation *Tn*, followed by a number. *Tn10* is a composite transposon of about 9300 bp that carries a gene (about 6500 bp) for tetracycline resistance between two *IS10* insertion sequences (Figure-4). The insertion sequences have terminal inverted repeats; so the composite transposon also ends in inverted repeats.

Composite transposons also generate flanking direct repeats at their sites of insertion (Figure-4). The insertion sequences at the ends of a compositetransposon may be in the same orientation or they may be inverted relative to one other (as in *Tn10*).

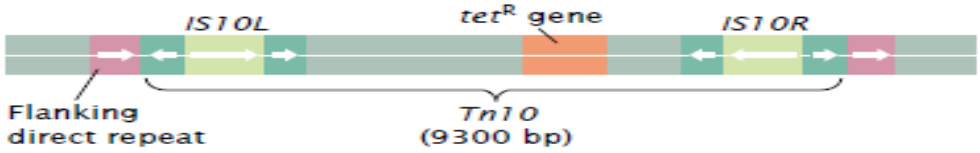


Figure - 4 : Tn10 is a composite transposon in bacteria

Table 11.5 Characteristics of several composite transposons

Composite Transposon	Total Length (bp)	Associated IS Elements	Other Genes Within the Transposon
Tn9	2500	*IS1*	Chloramphen-icol resistance
Tn10	9300	*IS10*	Tetracycline resistance
Tn5	5700	*IS50*	Kanamycin resistance
Tn903	3100	*IS903*	Kanamycin resistance

B-Non Composite transposons.

Some transposable elements in bacteria lack insertion sequences and are referred to as noncomposite transposons. For instance, *Tn3* is a non composite transposon that is about 5000 bp long, possesses terminal inverted repeats of 38 bp, and generates flanking direct repeats that are 5 bp in length. *Tn3* carries genes for transposase and resolvase plus a gene that codes for the enzyme _-lactamase, which provides resistance to ampicillin.

3- Mu transposone (Bacteriophage Mu): A bacterial virus that replicates by transposition and causes mutations by insertion within host cell bacterial chromosome in their lysogenic cycle; Although Mu does not possess terminal inverted repeats, it does generate short (5-bp) flanking direct

repeats when it inserts randomly into DNA. Mu replicates through transposition and causes mutations at the site of insertion(Figure-5).

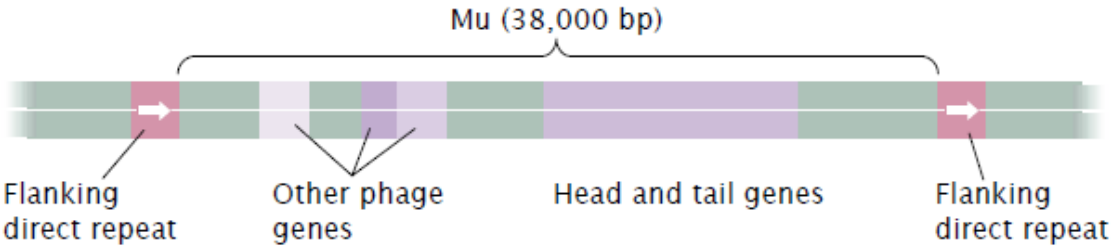


Figure -5 : Mu is a transposing bacteriophage.

4-Conjugative transposon: A transposon that is also capable of transferring itself from one bacterial cell to another by conjugation.

5-Retro transposon : Genetic element found in bacteria that encodes reverse transcriptase and uses it to make a bizarre RNA/DNA hybrid molecule.

Viral Genetics

All organisms—plants, animals, fungi, and bacteria—are infected by viruses. A **virus** is a simple replicating structure made up of nucleic acid by a protein or membrane coat (Capsid).
Viruses come in a great variety of shapes and sizes some have DNA as their genetic material, whereas others have RNA; the nucleic acid may be double stranded **dsDNA,**or single stranded **ssDNA**, linear or circular.

Bacteriophages: viruses that infect bacteria

Bacteriophages (phages) have played a central role in genetic research since the late 1940s. They are ideal for many types of genetic research because they have small and easily manageable genomes, reproduce rapidly, and produce large numbers of progeny. Bacteriophages have two alternative life cycles: the lytic and the lysogenic cycles. In the lytic cycle, a phage attaches to a receptor on the bacterial cell wall and injects its DNA into the cell (Figure-6)Once inside the cell, the phage DNA is replicated, transcribed, and translated, producing more phage DNA and phage proteins. New phage particles are assembled from these components. The phages then produce an enzyme that breaks open the cell, releasing the new phages.
Virulent phages reproduce strictly through the lytic cycle and always kill their host cells.

Temperate phage can utilize either the lytic or the lysogenic cycle.
The lysogenic cycle begins like the lytic cycle (Figure -6) but, inside the cell, the phage DNA integrates into the bacterial chromosome, where it remains as an inactive **prophage.** The prophage is replicated along with the bacterial DNA and is passed on when the bacterium divides. Certain stimuli cause the prophage to dissociate from the bacterial chromosome and enter into the lytic cycle, producing new phage particles and lysing the cell.

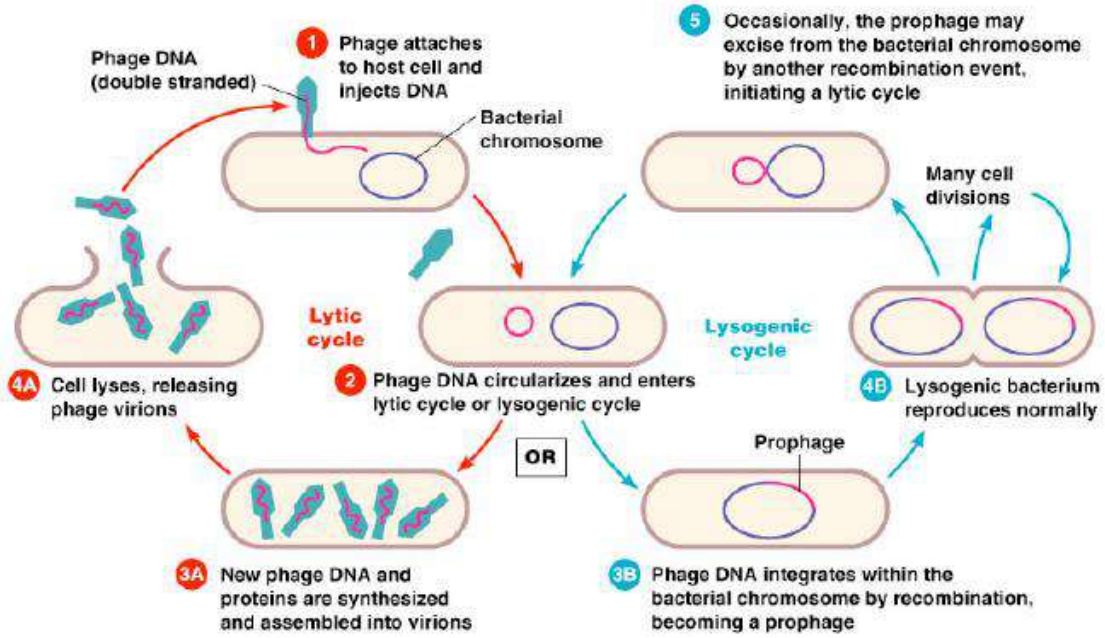
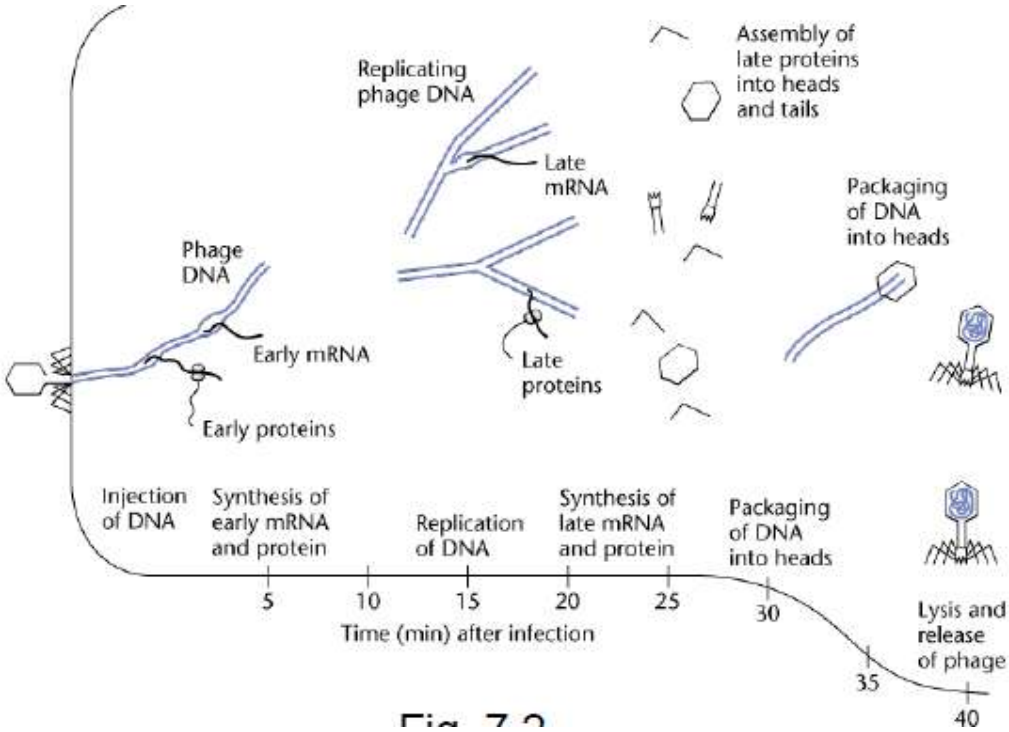


Figure-5 :Bacteriophages have two alternating lifecycles—lytic and lysogenic.



Techniques for the Study of Bacteriophages

Viruses reproduce only within host cells; so bacteriophages must be cultured in bacterial cells. To do so, phages and bacteria are mixed together and plated on solid medium in a petri plate. A high concentration of bacteria is used so that the colonies grow into one another and produce a continuous
layer of bacteria, or "lawn," on the agar. An individual phage infects a single bacterial cell and goes through its lytic cycle. Many new phages are released from the lysed cell and infect additional cells; the cycle is then repeated. The bacteria grow on solid medium; so the diffusion of the phages is restricted and only nearby cells are infected. After several rounds of phage reproduction, a clear patch of lysed cells (a **plaque**) appears on the plate (Figure – 7) **.** Each plaque represents a single phage that multiplied and lysed many cells. Plating a known volume of a dilute solution of phages on a bacterial lawn and counting the number of plaques that appear can be used to determine the original concentration of phage in the solution.

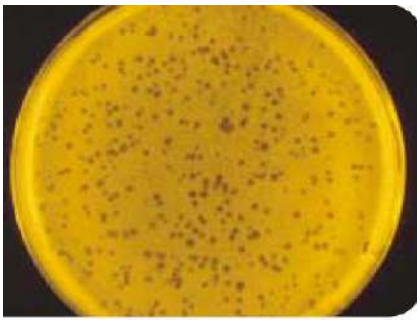


Figure -7 : plaques are clear patches of lysed cells on a lawn of bacteria