Almaaqal University
College of Health and Medical Techniques
Department of Medical Laboratory Techniques

Lectures in

Molecular Biology

by

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Lecture 1

Molecular biology: is the field of biology that studies the composition, structure and interactions of cellular molecules – such as nucleic acids and proteins – that carry out the biological processes essential for the cell's functions and maintenance.

Introduction to Genetics

Genetics is the science of heredity, i.e.: the science that studies the inheritance of biological characteristics by living organisms. This subject examines:

1- The transmission of biological properties (traits) from parents to offspring.
2- The expression and variation of those traits.
3- The structure and function of genetic material.
4- How this material changes and evolve.

DNA as Genetic Material

Although it is now hard to imagine, it was once thought that DNA was too simple molecule to store genetic information. The early work of Fred Griffith in 1928 on the transfer of virulence in the pathogen *Streptococcus pneumoniae*, commonly called pneumococcus (figure 1), set the stage for research showing that DNA was indeed the genetic material. Griffith found that if he boiled virulent bacteria and injected them into mice, the mice were not affected and no pneumococci could be recovered from the animals. When he injected a combination of killed virulent bacteria and a living nonvirulent strain, the mice died; moreover, he could recover living virulent bacteria from the dead mice. Griffith called this change of nonvirulent bacteria into virulent pathogens **transformation**.

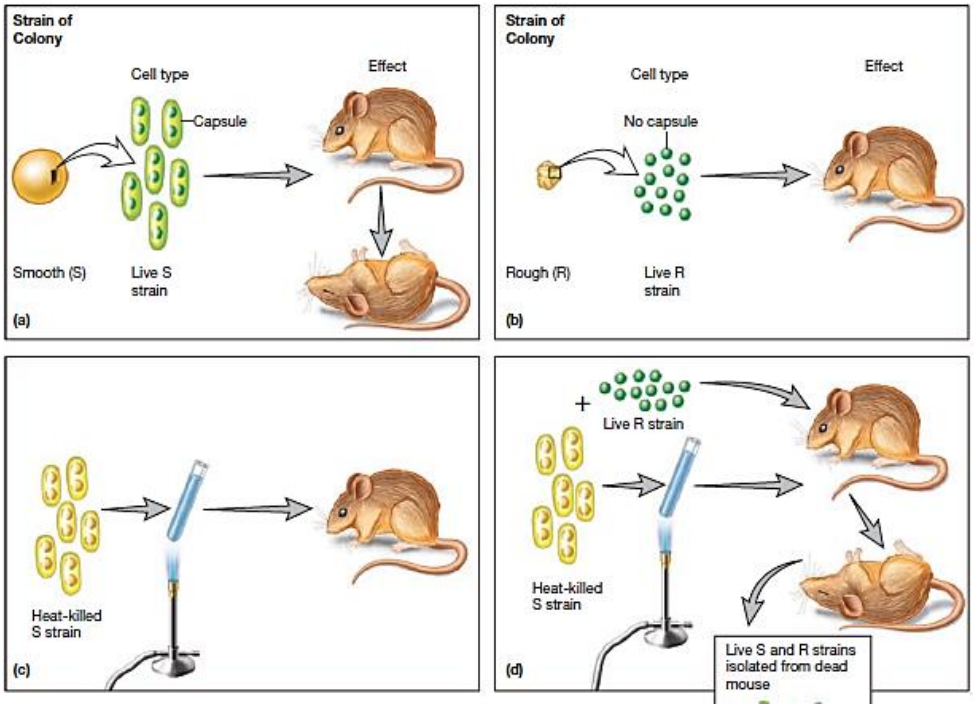

Figure 1: Griffith's Transformation Experiments

Oswald Avery and his colleagues then set out to discover which constituent in the heat-killed virulent pneumococci was responsible for Griffith's transformation. These investigators selectively destroyed constituents in purified extracts of virulent pneumococci (S cells), using enzymes that would hydrolyze DNA, RNA, or protein. They then exposed nonvirulent pneumococcal strains (R strains) to the treated extracts. Transformation of the nonvirulent bacteria was blocked only if the DNA was destroyed, suggesting that DNA was carrying the information required for transformation (figure 2). The publication of these studies by Avery in 1944 provided the first evidence that Griffith's transforming principle was DNA and therefore that DNA carried genetic information.

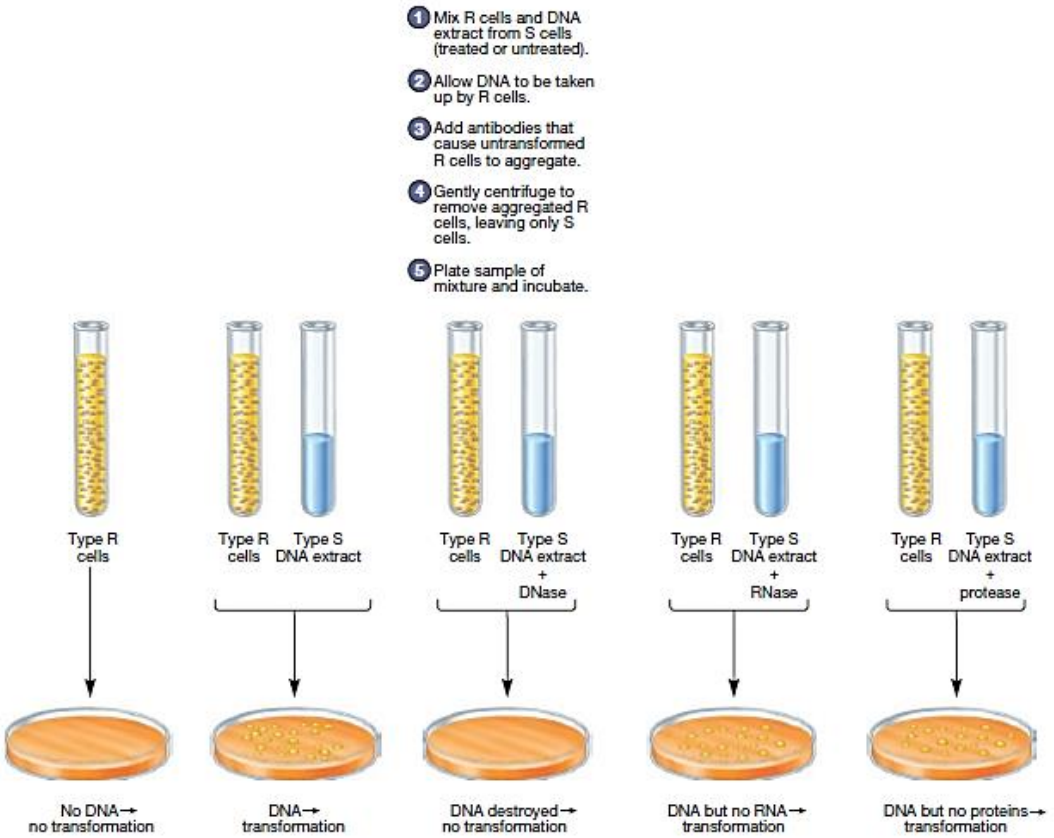

Figure 2: Avery Experiments on the Transforming Principle

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Lecture 2

Structure and Function of the Genetic Material

† The term **genome** refers to all the DNA present in a cell. A cell's genome includes its chromosomes and non-chromosomal sites, for example, bacteria and some fungi contain extra tiny pieces of DNA called plasmids and the mitochondria of eukaryotes are equipped with their own functional DNA.

† **Chromosomes** are structures containing DNA that physically carry hereditary information; the chromosomes contain the genes.

† **Genes** are segments of DNA (except in some viruses, in which they are made of RNA) that code for functional products. Genes fall into three basic categories: structural genes that code for protein, genes that code for RNA and regulatory genes that control gene expression.

Genotype and Phenotype

The regulation of gene expression is important because it links the **genotype** of an organism— the specific set of genes it possesses—to the **phenotype** of an organism—the collection of characteristics that are observable.

DNA Structure

The nucleic acids, DNA and RNA, are polymers of nucleotides linked together by phosphodiester bonds. However, DNA and RNA differ in terms of

1- the nitrogenous bases they contain.

2- the sugar component of their nucleotides.

3- and whether they are double or single stranded.

• Deoxyribonucleic acid (DNA) contains the bases adenine, guanine, cytosine, and thymine. The sugar found in the nucleotides is deoxyribose, and DNA molecules are usually double stranded.

• James Watson and Francis Crick put the pieces of the molecular puzzle together in 1953. They discovered that DNA is a giant molecule, a type of nucleic acid, with two strands forming a double helix. The general structure of DNA is universal, except in some viruses that contain single-stranded DNA.

The basic unit of DNA structure is **a nucleotide**, composed of:

1- **Phosphate**.
2- **deoxyribose sugar**.
3- **a nitrogen base**.

As shown in simplified model in figure (3 and 4).

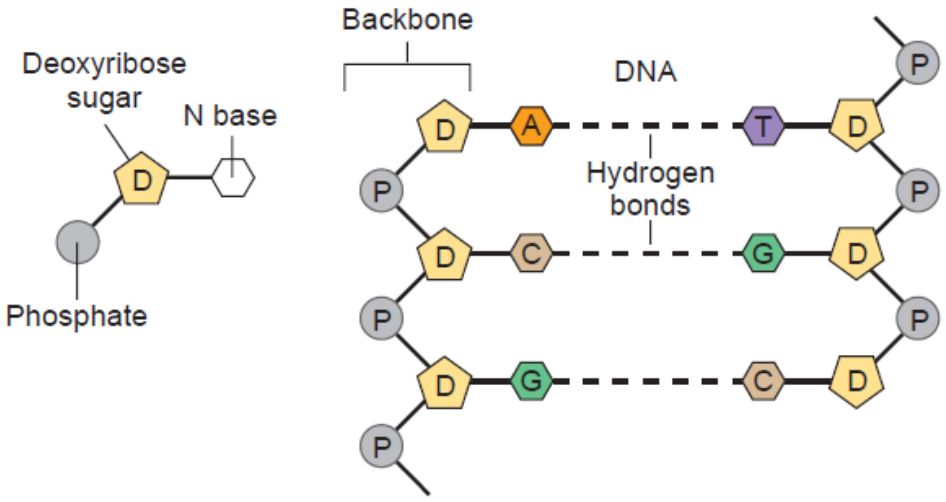


Figure 3: simple DNA structure

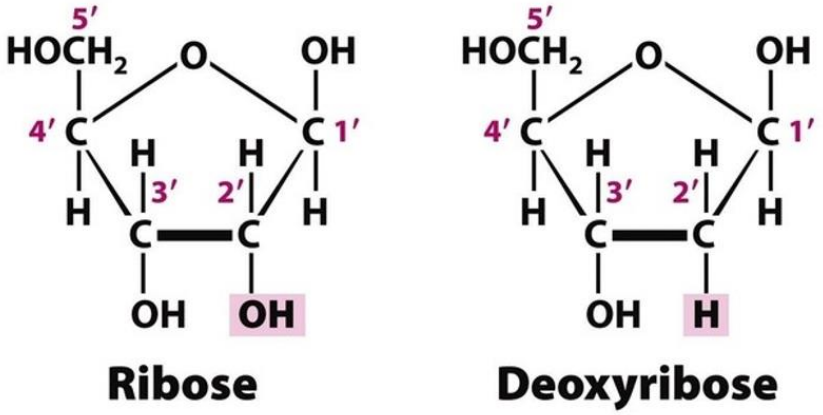


Figure 4: the chemical structure of ribose and deoxy ribose

- Each chain contains purine and pyrimidine deoxyribonucleosides joined by phosphodiester linkages. That is, a phosphoric acid molecule forms a bridge between a 3′-hydroxyl of one sugar and a 5′-hydroxyl of an adjacent sugar, which specifies the order and direction of each strand. (figure 5,6 and 7)

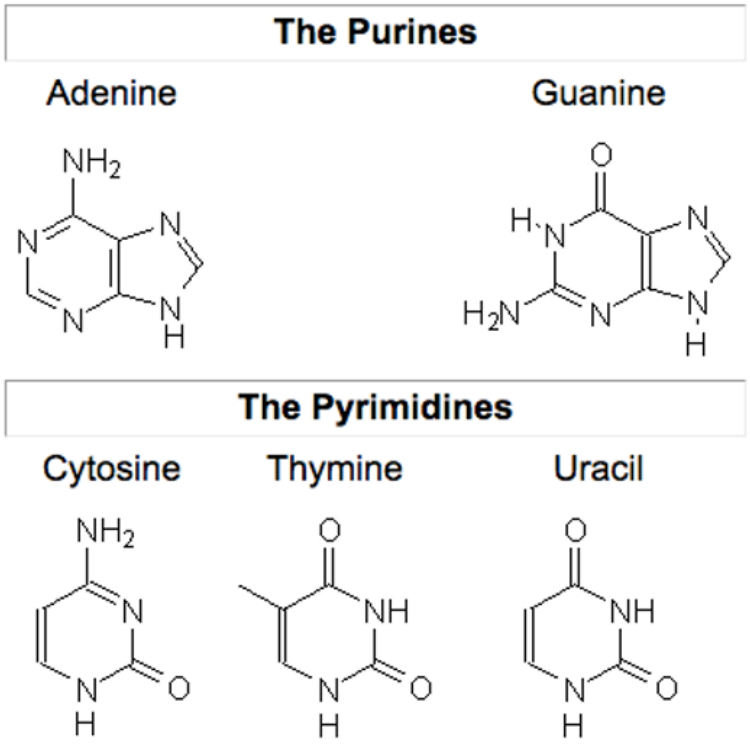


Figure 5: purines and pyrimidines nitrogen bases

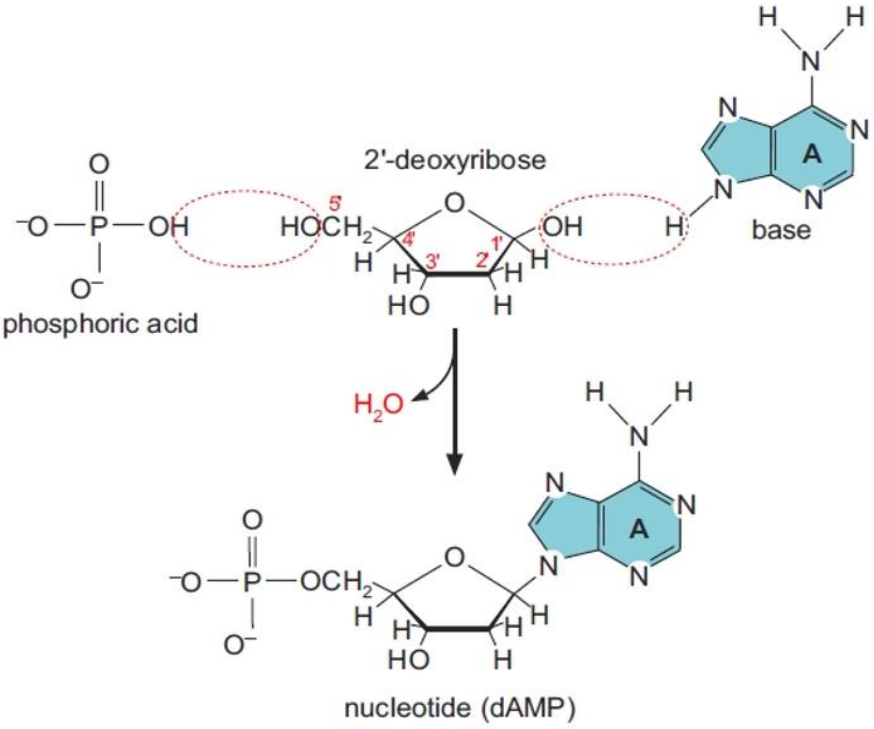


Figure 6: the formation of ester bond between the phosphate group and deoxyribose sugar within the same nucleotide

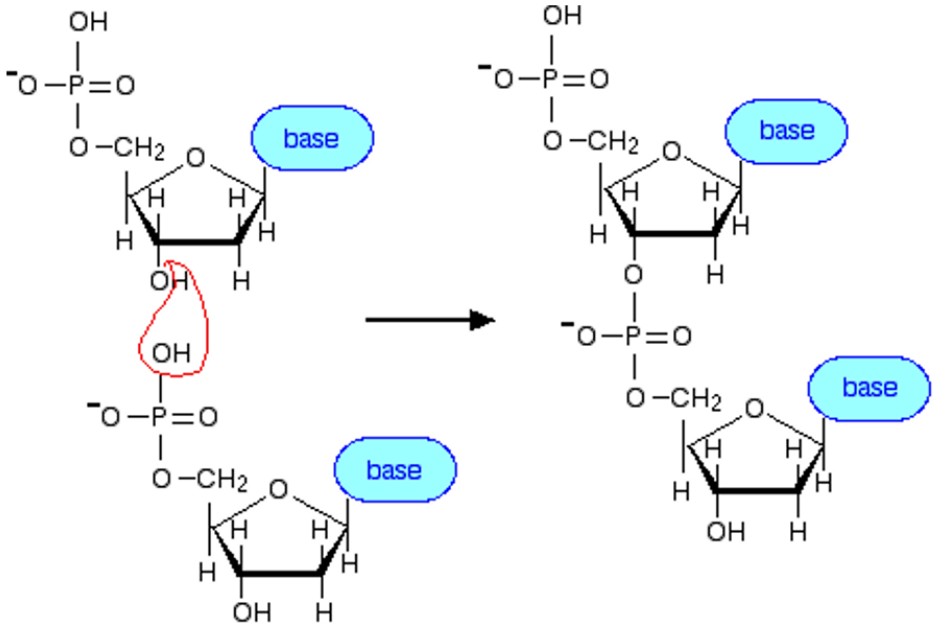
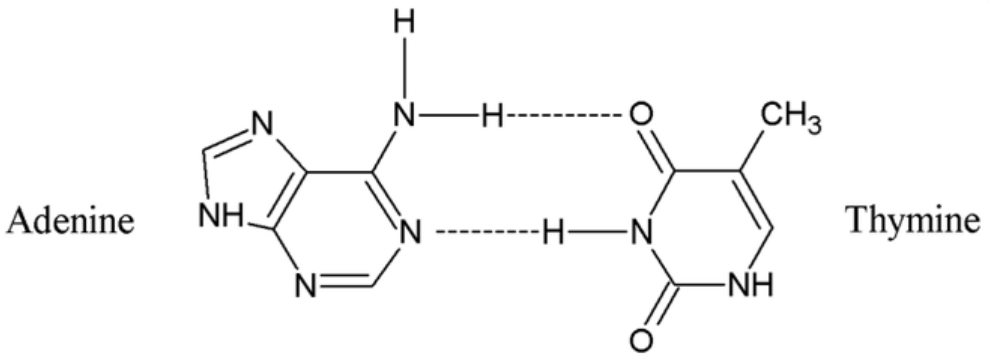
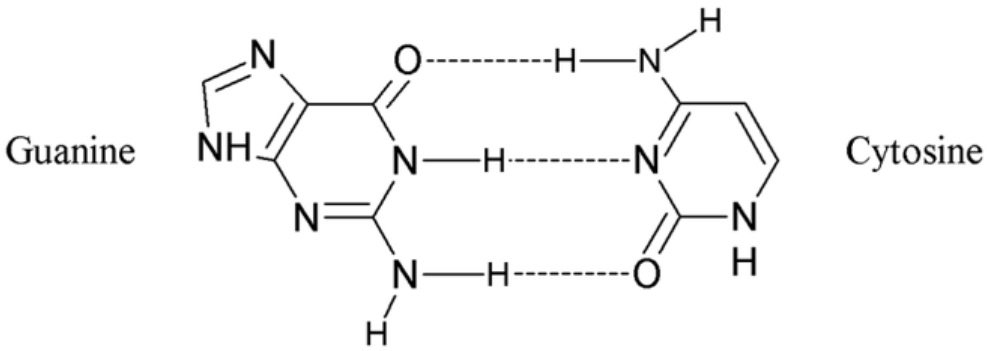


Figure 7: the formation of ester bond between the phosphate group and deoxyribose sugar between two nucleotides

• The paired bases are so aligned as to be joined by hydrogen bonds. Such weak bonds are easily broken, allowing the molecule to be “unzipped” into its complementary strands. This feature is of great importance in gaining access to the information encoded in the nitrogen base sequence.

• Pairing of purines and pyrimidines is not random; the purine adenine (A) of one strand is always paired with the pyrimidine thymine (T) of the opposite strand by two hydrogen bonds. The purine guanine (G) pairs with cytosine (C) by three hydrogen bonds.





• This AT and GC base pairing means that the two strands in a DNA double helix are complementary. The helix is righthanded— that is, the chains turn counterclockwise as they approach a viewer looking down the longitudinal axis.

• Other important considerations of DNA structure concern the nature of the double helix itself. The two strands are not oriented in the same direction. One side of the helix runs in the opposite direction of the other, in what is called an **antiparallel arrangement**, the order of the bond between the carbon on deoxyribose and the phosphates is used to keep track of the direction of the two sides of the helix. Thus, one helix runs from the 5′ to 3′ direction, and the other runs from the 3′ to 5′ direction. If one end of a double helix is examined, the 5′ end of one strand and the 3′ end of the other are visible. This characteristic is a significant factor in DNA synthesis and translation.

• As apparently perfect and regular as the DNA molecule may seem, it is not exactly symmetrical. The torsion in the helix and the stepwise stacking of the nitrogen bases produce two different-size surface features, the major and minor grooves (figure 8).

• The reason why DNA is negatively charged is the phosphate group that makes up every nucleotide. When forming part of the phosphodiester bond, they retai1 of 2 negative charges (the other being lost to form the other ester bond to a new pentose, that's why the bond is called "phospho-di-ester. (figure 9)

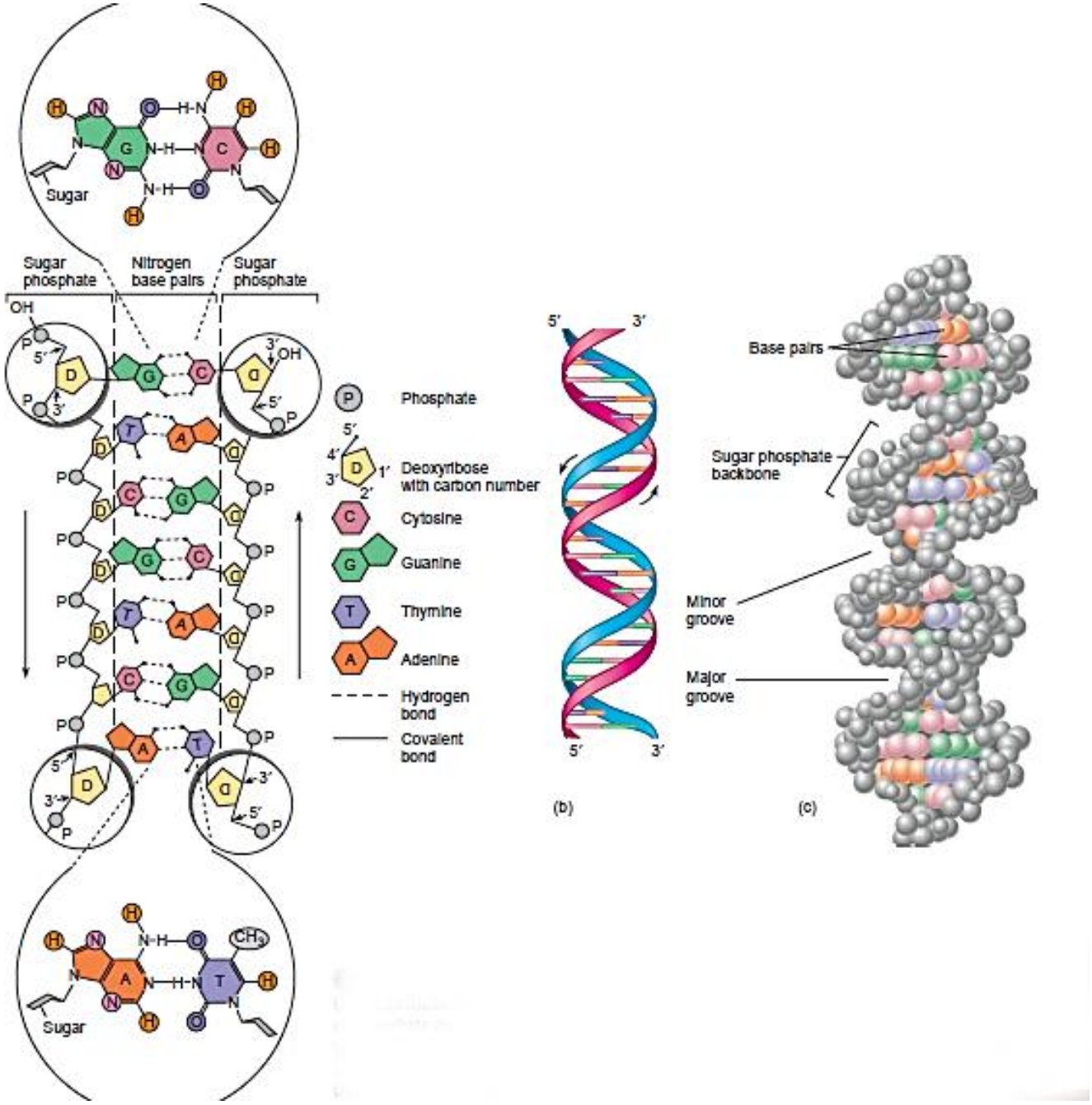


Figure 8: DNA structure

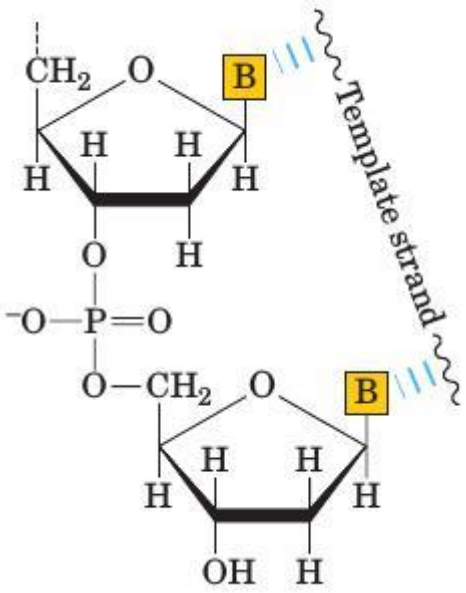


Figure 9: the DNA negative charge

The Significance of DNA Structure

The arrangement of nitrogen bases in DNA has two essential effects.

1. Maintenance of the code during reproduction. The constancy of base-pairing guarantees that the code will be retained during cell growth and division. When the two strands are separated, each one provides a template for the replication (exact copying) of a new molecule, Because the sequence of one strand automatically gives the sequence of its partner, the code can be duplicated with fidelity.

2. Providing variety. The order of bases along the length of the DNA strand provides the information needed to produce RNA and protein molecules, which in turn are responsible for the phenotype of the cell. changing the identity of the

bases or their order in the DNA molecule can have a dramatic effect on the phenotype of the organism.

The Organization of DNA in Cells

Although DNA exists as a double helix in all cells, its organization differs among cells in the three domains of life.

• DNA is organized in the form of a closed circle in all Archaea and most bacteria. This circular double helix is further twisted into supercoiled DNA (figure 10).

• In Bacteria, DNA is associated with basic proteins that appear to help organize it into a coiled, chromatin like-structure.

• The system in eukaryotes is more complex, with three or more levels of coiling. First, the DNA molecule of a chromosome, which is linear, is wound twice around the histone proteins, creating a chain of nucleosomes **(This combination of histones plus DNA, or nucleoprotein complex, is called a nucleosome).**

• The nucleosomes fold in a spiral formation upon one another.

• An even greater supercoiling occurs when this spiral arrangement further twists into a giant spiral. This extreme degree of compactness is what makes the eukaryotic chromosome visible during mitosis. (figure 11).

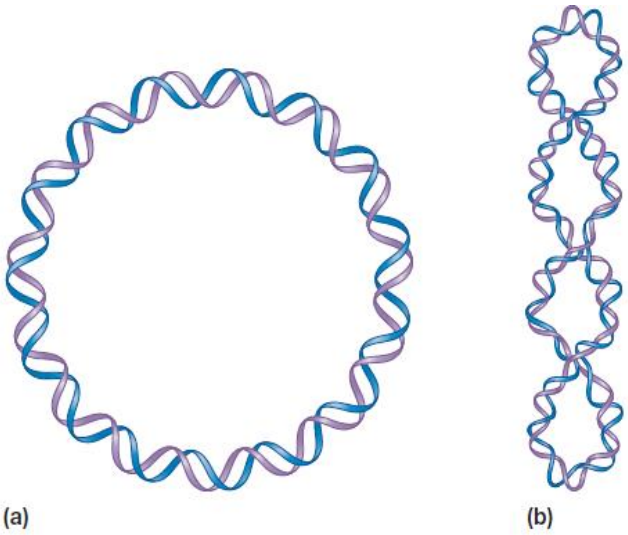


Figure 10: DNA Forms. (a) The DNA double helix of most prokaryotes is in the shape of a closed circle. (b) The circular DNA strands, already coiled in a double helix, are twisted a second time to produce supercoils

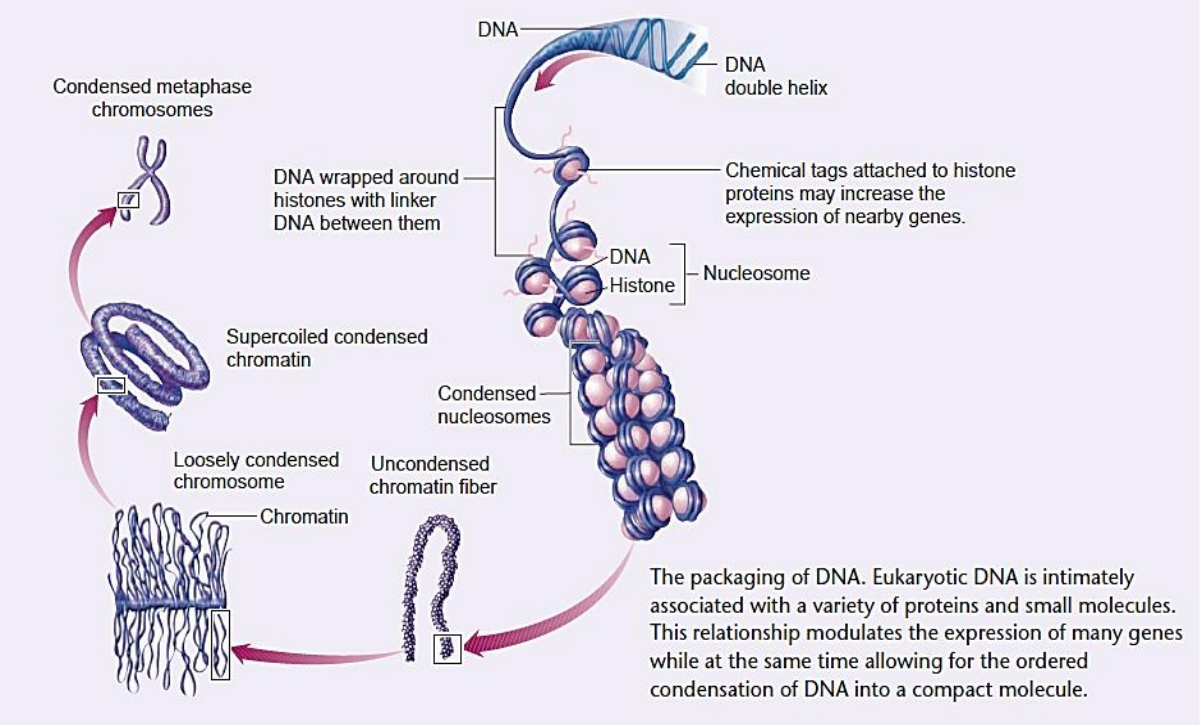


Figure 11: An illustration of how a string of nucleosomes, each associated with a histone, might be organized to form a highly supercoiled chromatin fiber.

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RNA Structure

• Ribonucleic acid (RNA): is a polymer of ribonucleotides contains the bases adenine, guanine, cytosine, and uracil (instead of thymine) . Its sugar is ribose, and most RNA molecules are single stranded.

• nucleotides are joined by a phosphodiester bond, just as they are in DNA.

• RNA strand can coil back on itself to form a hairpin-shaped structure with complementary base pairing and helical organization. The formation of double-stranded regions in RNA is often critical to its function. Regulation of transcription elongation.

• The three different types of RNA—messenger RNA, ribosomal RNA, and transfer RNA—differ from one another in function, site of synthesis in eukaryotic cells, and structure.

Protein Structure

• Proteins are polymers of amino acids linked by peptide bonds; thus, they are also called polypeptides.

• Twenty amino acids are normally used to form proteins. However, two unusual amino acids have recently been discovered in some proteins.

• The peptide bonds linking the amino acids together are formed by a reaction between the carboxyl group of one amino acid and the amino group of the next amino acid in the protein.

• Proteins do not typically exist as extended chains of amino acids. Rather, they fold back on themselves to form three dimensional structures.

• The final shape is determined to a large extent by the sequence of amino acids in the polypeptide. This sequence is called the primary structure. Secondary and tertiary structures result from the folding of the chain. Finally, two or more polypeptide strands can interact to form the final, functional protein. This level of structure is called quaternary structure. These higher levels of structure are stabilized by intra- (and inter-) chain bonds.

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Lecture 3

DNA Replication

For a species to survive, it must reproduce. this involves division of the cell, but it also involves the accurate duplication and separation of the genetic material into each daughter cell to ensure normal function. This process of duplication is called **DNA replication.**

*During DNA replication, the two strands of the double helix are separated; each then serves as a template for the synthesis of a complementary strand according to the base pairing rules. Each of the two progeny DNA molecules consists of one new strand and one old strand. Thus, DNA replication is **semiconservative.** (figure1).

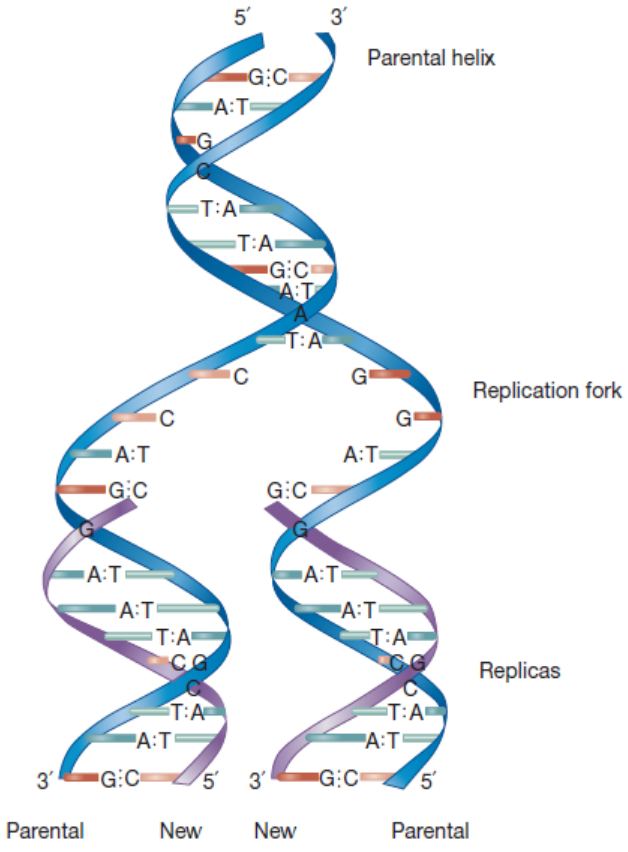

Figure 1: DNA Replication

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Events in Replication in Eukaryotic Cell

1- Before replication can start, the DNA has to be made available as a template. In Eukaryotic cells, histones must be removed and the chromatin may undergo some chemical modifications, so that the DNA be able to slide off the proteins or be accessible to the enzymes of the DNA replication machinery.

2- At the origin of replication (a particular sequence in a genome at which replication is initiated), A helicase opens up the DNA helix. Replication forks (the place at which the DNA helix is unwound and individual strands are replicated) are formed at each replication origin as the DNA unwinds.

3- The opening of the double helix causes over-winding, or supercoiling, in the DNA ahead of the replication fork. These are resolved with the action of topoisomerases.

4- Primers are formed by the enzyme primase, and using the primer, DNA polymerases can start synthesis.

5- DNA polymerase is then involved which adds a short (20 to 30 nucleotides) DNA fragment to the RNA primer on both strands, while, the leading strand is continuously synthesized by the enzyme, the lagging strand is synthesized discontinuously.

6-The primer RNA is then removed by RNase H (endonuclease) and replaced with DNA nucleotides.

7- The Okazaki fragments in the lagging strand are joined after the replacement of the RNA primers with DNA. The gaps that remain are sealed by DNA ligase, which forms the phosphodiester bond.

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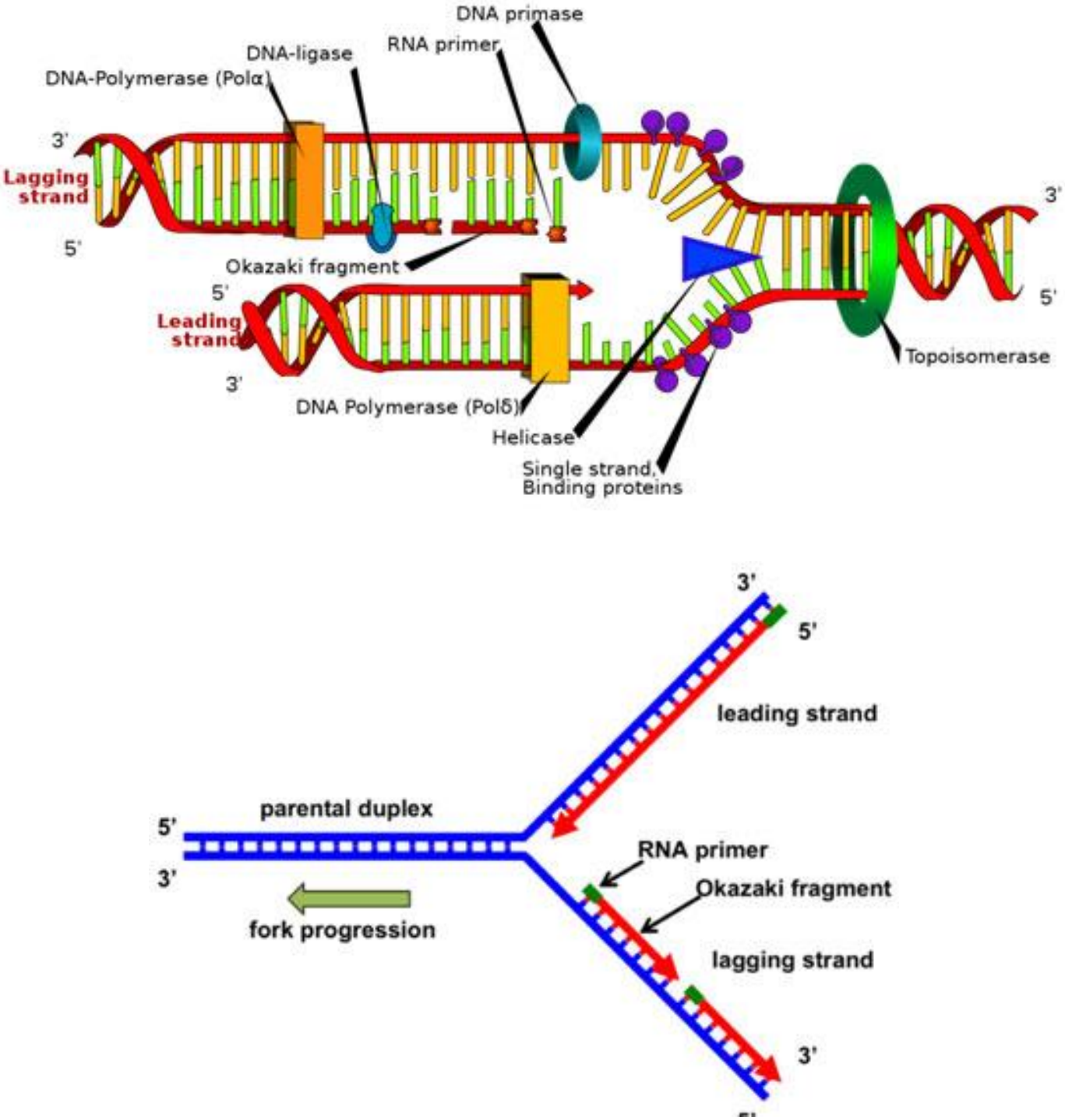
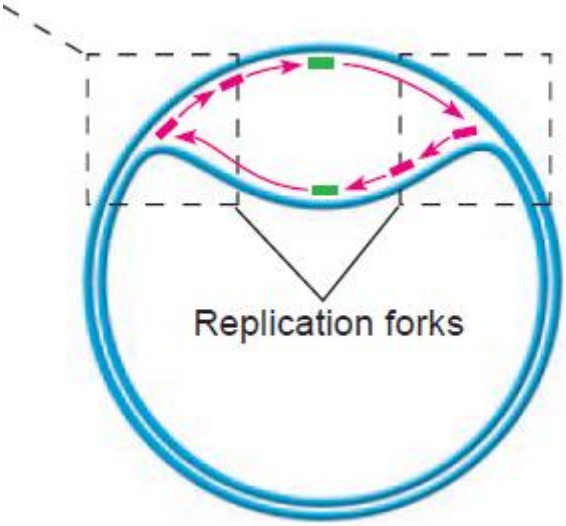


Figure 2: replication steps

Note: DNA replication is an amazingly accurate process. Typically, mistakes are made at a rate of only 1 in every 10^{10} bases incorporated. Such accuracy is largely due to the proofreading capability of DNA polymerase. As each new base is added, the enzyme evaluates whether it forms the proper complementary base-pairing structure. If not, the enzyme excises the improper base and replaces it with the correct one. In this way, DNA can be replicated very accurately, allowing each daughter chromosome to be virtually identical to the parental DNA.

The Difference Between Prokaryotic and Eukaryotic DNA Replication

Property	**Prokaryotic**	**eukaryotic**
place	In the cytoplasm	In the nucleus
Origen of replication	Single	Multiple
Rate of replication	2000 nucleotide/sec	100 nucleotide/sec
Strand elongation	DNA polymerase III	DNA pol α, δ and ε
RNA primer removal	DNA pol I	RNase
Direction	Bidirectional	unidirectional
Replication fork number	two replication forks are formed	Several replication forks are formed
duration	It is a continuous process.	DNA replication occurs in the S phase of interphase during the cell cycle



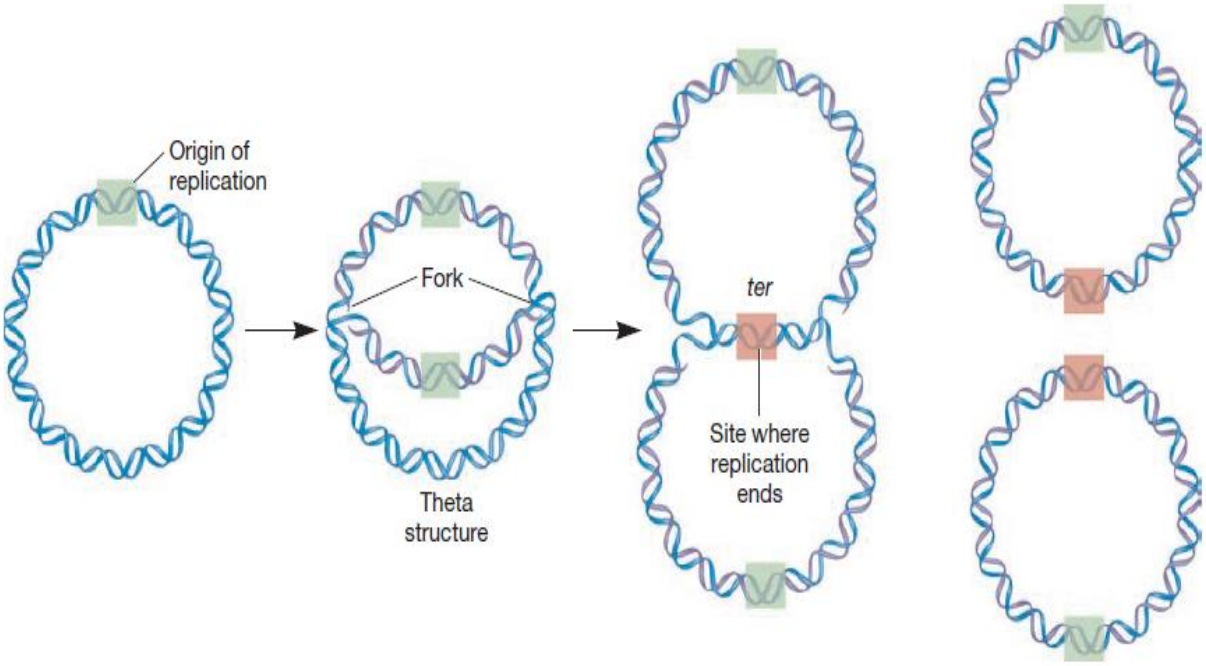


Figure 3: replication in prokaryotes

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Lecture 4

The Flow of Genetic Information

1. DNA replication makes possible the flow of genetic information from one generation to the next.

2. The DNA of a cell replicates before cell division so that each offspring cell receives a chromosome identical to the parents.

3. The genetic information contained in DNA also flows in another way: it is transcribed into mRNA and then translated into protein within a single cell, a process also called **gene expression**.

4. The pathway from DNA to RNA and RNA to protein is conserved in all forms of life and is often called **the central dogma (figure 1).**

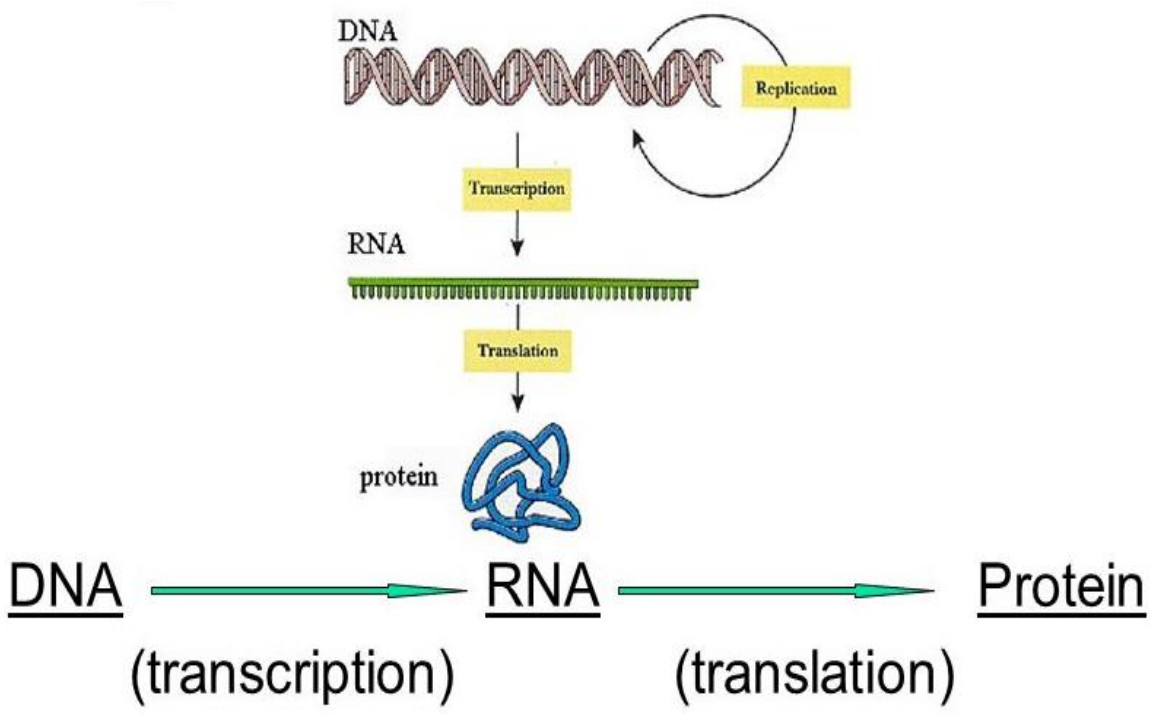

Figure 1: the central dogma

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Transcription: The First Stage of Gene Expression

- During transcription, an RNA molecule is synthesized using the codes on DNA as template.
- **RNA polymerase** is responsible for this process. This polymerase is more multipurpose than DNA polymerase, because it can bind to DNA and unwind it, as well as, synthesize RNA.
- Transcription proceeds in three stages: initiation, elongation and termination.
- RNA synthesis, like DNA synthesis, proceeds in a 5′ to 3′ direction.
- Transcription always proceeds from the same DNA strand for each gene, which is called the template strand or (-) antisense strand.
- The mRNA product is complementary to the template strand and is almost identical to the other DNA strand, called the (+) sense strand, or the coding strand.

Transcription in Prokaryotes

A. Initiation of Transcription in Prokaryotes

1. In bacteria, a single RNA polymerase transcribes all genes.

2. In Bacteria and Archaea, the mRNA often bears coding information transcribed from adjacent genes. Therefore, it is said to be **polycistronic** (is an mRNA that encodes several proteins) as shown in figure (2).

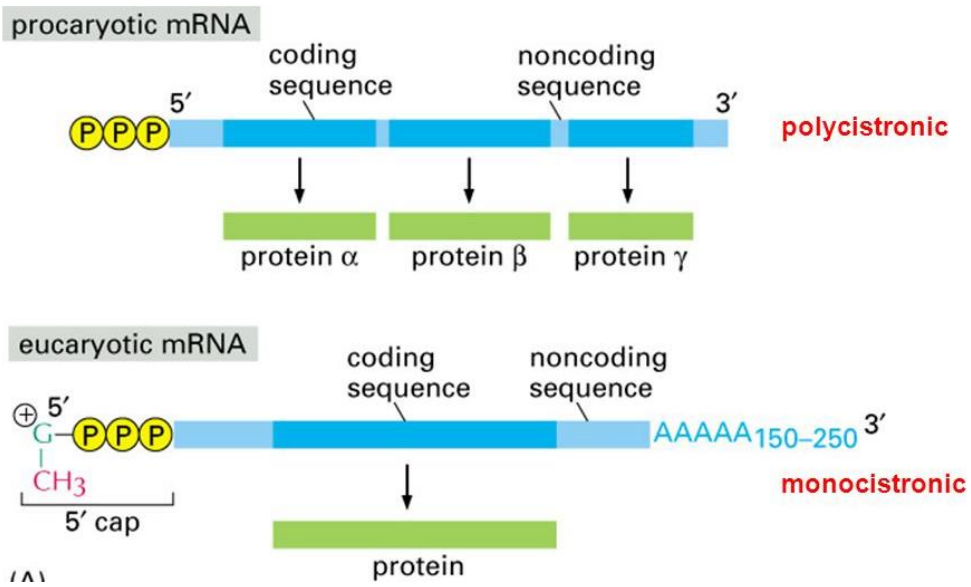

Figure 1. comparison between polycistronic and monocistronic mRNA

3. Prokaryotic RNA polymerase contain six types of polypeptide chains. the six-subunit complex is termed RNA polymerase holoenzyme and only holoenzyme can begin transcription (figure 3).

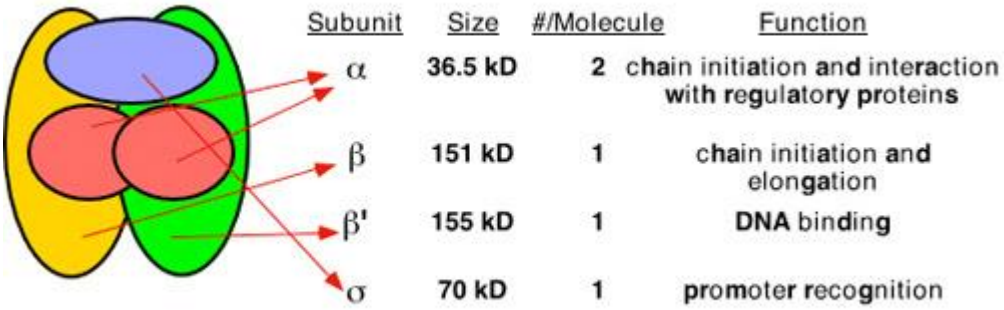

Figure 3: prokaryotic RNA polymerase holoenzyme

4. Initiation requires the RNA polymerase to recognize a region on a gene called the **promoter region**.

5. The promoter is neither transcribed nor translated; it functions strictly to provide a position for initial binding of the RNA polymerase.

6. Prokaryotic promoters have two characteristic features: a sequence of six bases (TTGACA) about **35** base pairs before (upstream) the transcription starting point and (TATAAT) sequence, or **Pribnow box**, usually about **10** base pairs upstream of the transcriptional start site. These regions are called the -35 and -10 sites, respectively (figure 4).

7. These sequences are the **consensus sequences** which are regions that are similar across all promoters and across various prokaryotic species.

8. Once bound to the promoter site, RNA polymerase is able to unwind the DNA without the aid of helicases. The -10 site is rich in adenines and thymine, making it easier to break the hydrogen bonds that keep the DNA double stranded.

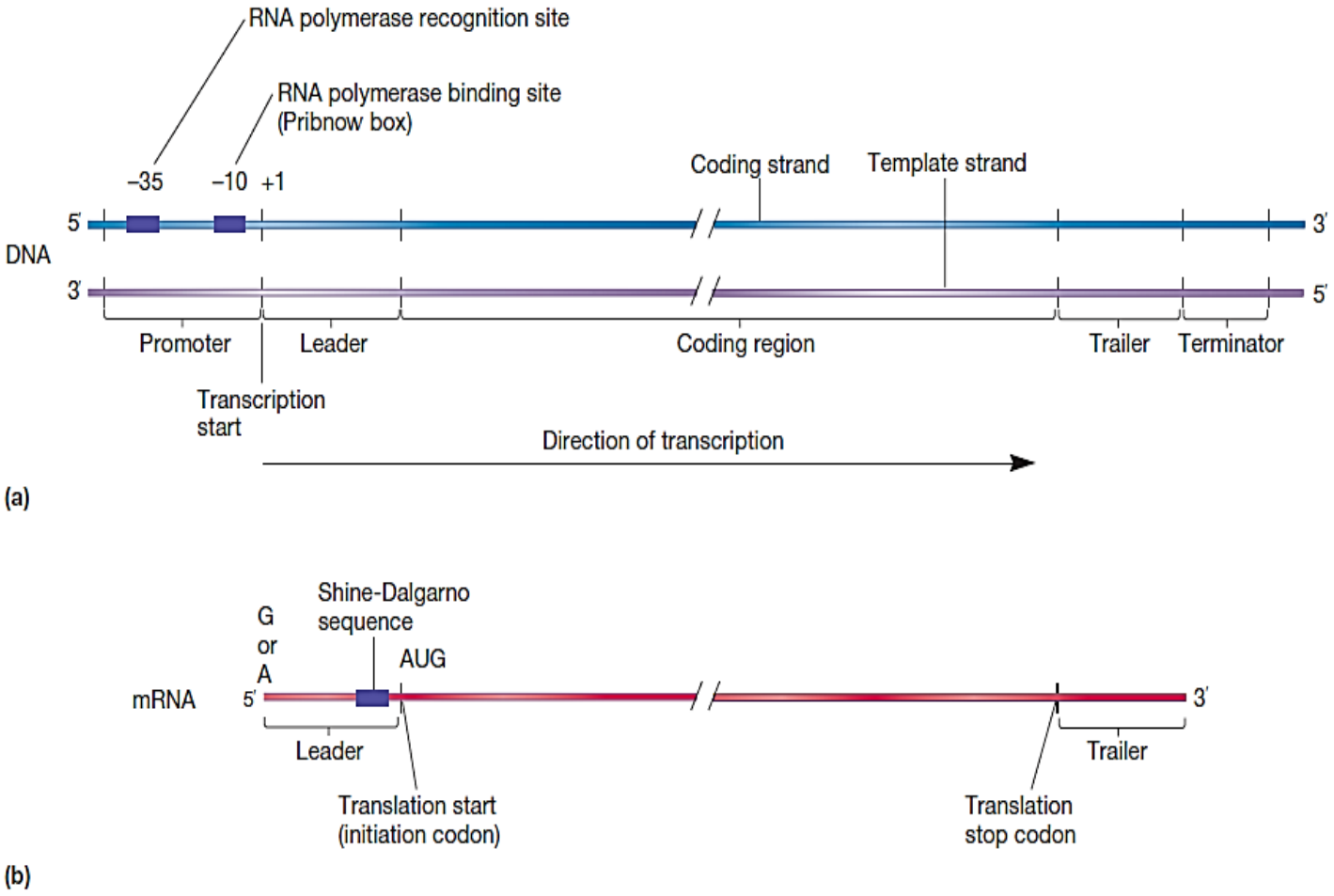

Figure 4: A Bacterial Structural Gene and Its mRNA Product