# Identification of DNA as The Genetic Material

Learning Outcomes, the student able to:

1. Describe the four criteria of the genetic material.

2. Analyze the results of (1) Griffith, (2) Avery, MacLeod, and McCarty, and (3) Hershey and Chase, and explain how they indicate that DNA is the genetic material.

3- understand DNA and RNA structure.

This lecture will focus on the relationship between the inheritance of genes and chromosomes and the outcome of an organism's traits. molecular genetics is the studies of DNA structure and function at the molecular level. The understanding of genetic material structure helps us in understanding its functions in the cells.

Also, this lecture will explain the classic experiments that showed that DNA is the genetic material and the molecular structure of DNA and RNA.

## The genetic material must meet four criteria.

**1. Information:** The genetic material must contain or storage the information necessary to produce an entire organism. In other words, it must provide a copy of information for determining the inherited traits of an organism.

**2. Transmission:** During reproduction, the genetic material must be passed from parents to offspring.

**3. Replication:** the genetic material must be copied so it can passe from parents to offspring, and from mother cell to daughter cells during cell division.

**4. Variation:** the genetic material must also vary to produce different phenotypes within each species.

## The story of discovery of the genetic material

In the 1880s, August Weismann and Carl Nägeli supported the idea that a chemical substance within living cells is responsible for the transmission of traits from parents to offspring. The chromosome theory of inheritance was developed, and experimentation demonstrated that the chromosomes are the carriers of the genetic material. Nevertheless, the story was not complete

because chromosomes contain both DNA and proteins. Also, RNA is found in nearness of chromosomes. Therefore, further research was needed to precisely identify the genetic material.

## Griffith's Transformation Experiment

Frederick Griffith studied a type of bacterium known then as pneumococci and now classified as ***Streptococcus pneumoniae.*** Certain strains of *S. pneumoniae* secrete a **polysaccharide capsule**, whereas other strains do not. When streaked onto petri plates containing a solid growth medium, capsule-secreting strains have a **smooth (S)** colony morphology when streaked onto petri plates containing a solid growth medium, whereas those strains unable to secrete a capsule produce colony with a **rough (R)** appearance. The different forms of *S. pneumoniae* also affect their **virulence,** or ability to cause disease. When smooth strains of *S. pneumoniae* infect a mouse, the capsule allows the bacteria to escape attack by the mouse's immune system. As a result, the bacteria can grow and eventually kill the mouse. In contrast, the nonencapsulated (**rough**) bacteria are destroyed by the animal's immune system.

The experiments conducted by in 1928, stated by injection of live and / or heat-killed bacteria into mice. He then observed whether or not the bacteria caused a lethal infection. Griffith was working with two strains of *S. pneumoniae*, **a type S (for smooth) and a type R (for rough)**.

1- When injected a **type S** into a live mouse, the bacteria proliferated within the mouse's bloodstream and killed the mouse. Then Griffith was able to re-isolated the type S bacteria from the mouse's blood.
2- In contrast, when **type R** bacteria were injected into a mouse, the mouse lived.
3- To verify that the proliferation of the smooth bacteria was causing the death of the mouse, Griffith killed the smooth bacteria with heat treatment before injecting them into a mouse. In this case, the mouse also survived.
4- In this experiment, **live type R bacteria were mixed with heat-killed type S bacteria.** the mouse died. Furthermore, extracts from tissues of the dead mouse were found to contain living type S bacteria! What happened!!!
Because living type R bacteria alone could not proliferate and kill the mouse, the interpretation of the result is t**hat something from the dead type S bacteria was transforming the type R bacteria into type S bacteria. Griffith called this process transformation,** and the unidentified substance causing this to occur was termed the **transforming principle.** The steps of bacterial transformation are shown in figure 1.

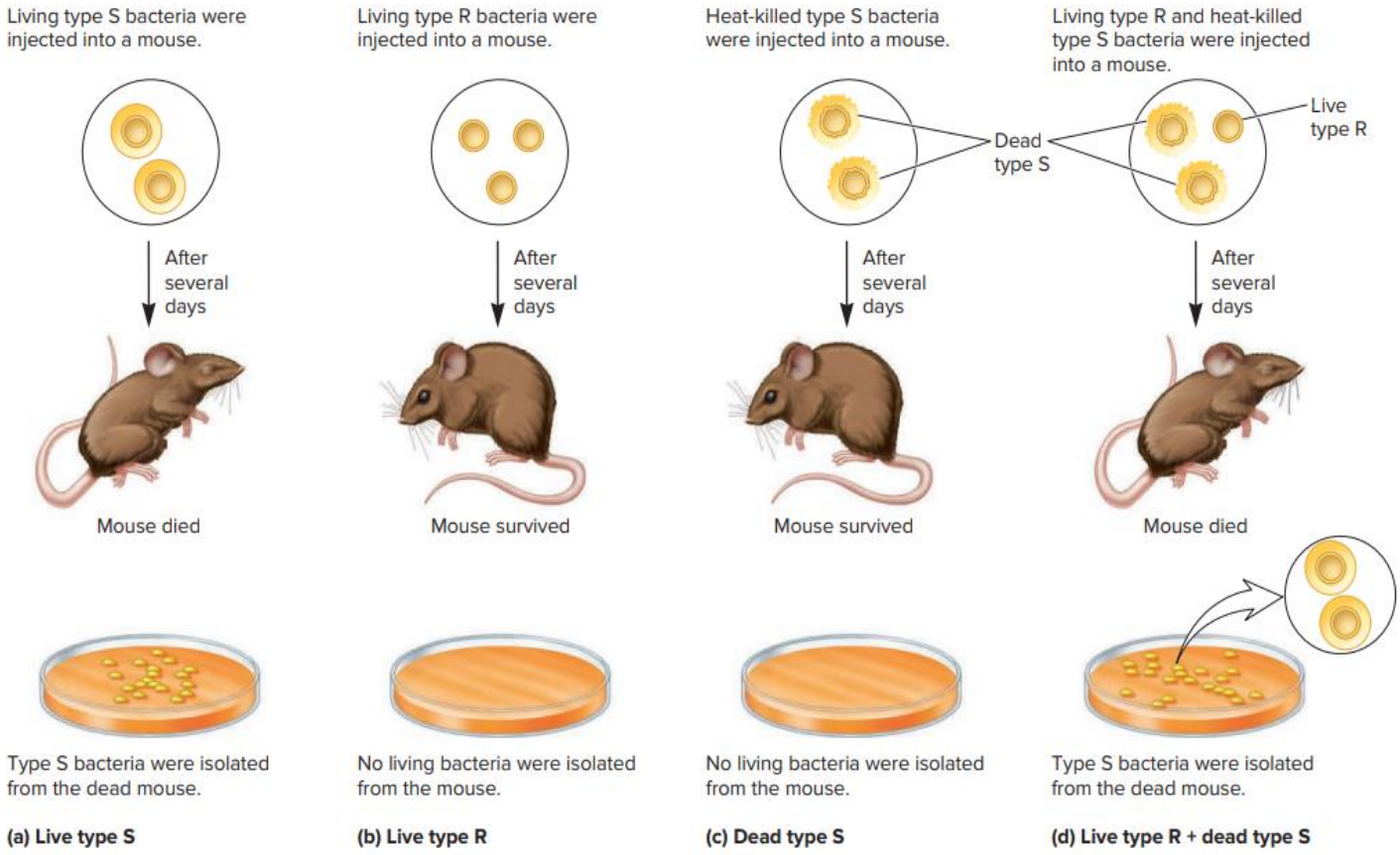



Figure 1: Griffith experiments the bacterial transformation. (Brooker, RJ, 2018, Genetics: Analysis & Principles, 6th Edi. McGraw-Hill Education, US).



## Avery, MacLeod and McCarty Experiment

At the time of these experiments in the 1940s, researchers already knew that DNA, RNA, proteins, and carbohydrates are major constituents of living cells.

To separate these components and to determine if any of them was the genetic material, **Avery, MacLeod, and McCarty** prepared extracts from type S bacterial strains that contained DNA extract, RNA extract or protein extract. they discovered that only one of the extracts, namely, the one that contained purified DNA from type S bacteria, was able to convert type R bacteria into type S. when this extract was mixed with type R bacteria, some of the bacteria were **converted to type S.** However, if no DNA extract was added, no type S bacterial colonies were observed on the petri plates.

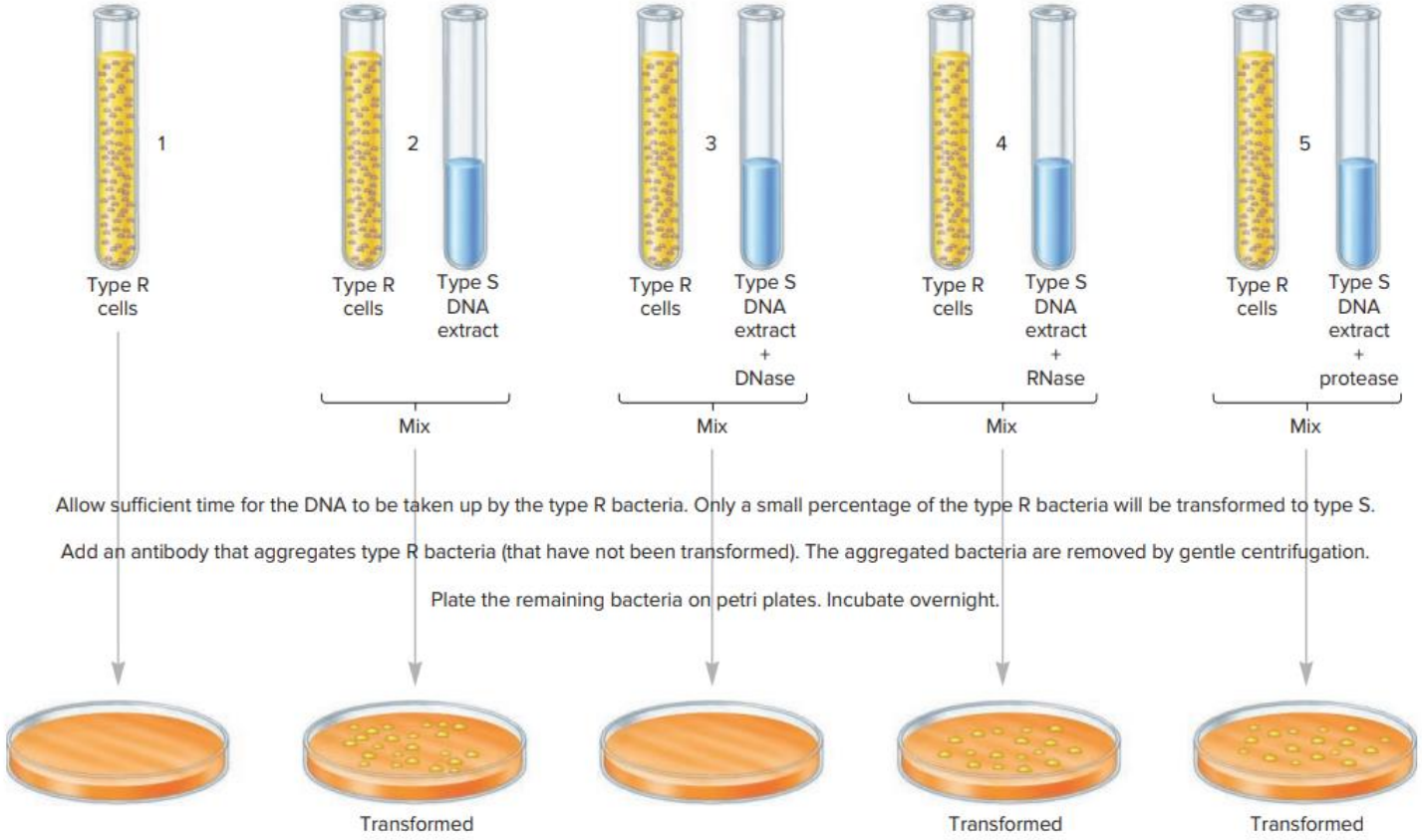



**Figure 2: Experiments of Avery, MacLeod, and McCarty to identify the transforming principle. 1-** Samples of ***S. pneumoniae*** **R** cells were not exposed to a type S DNA extract .
**2-**5 Samples of ***S. pneumoniae*** **R** exposed to a type S DNA extract. Extracts used in experiments 3, 4, and 5 also contained DNase, RNase, or protease, respectively. After incubation, the cells were exposed to antibodies, which are molecules that can specifically recognize the molecular structure of macromolecules. In this experiment, the antibodies recognized the cell surface of type R bacteria and caused the bacteria to clump together. The clumped bacteria were removed by a gentle centrifugation step. Only the bacteria that were not recognized by the antibody (namely, **the type S bacteria**) remained in the supernatant. The cells in the supernatant were plated on solid growth media. After overnight incubation, visible colonies may be observed. (Brooker, RJ, 2018, Genetics: Analysis & Principles, 6th Edi. McGraw-Hill Education, US).

These experiments faced a criticism because the DNA extract may not be 100% pure. It was thought that a small amount of contaminating material in the DNA extract might actually be the genetic material. The most likely contaminating substance in this case would be RNA or protein.

## The Hershey-Chase Bacteriophage Experiment:

More evidence for DNA as the genetic material came in 1953 with Alfred Hershey and Martha Chase's work on ***E. coli*** infected with **bacteriophage T2**. The structure of the T2 phage consists of genetic material that is packaged inside a phage coat, simply it is composed only from DNA and proteins.

During infection, the phage coat remains attached on the outside of the bacterium and does not enter the cell. Only the genetic material of the phage enters the bacterial cell.

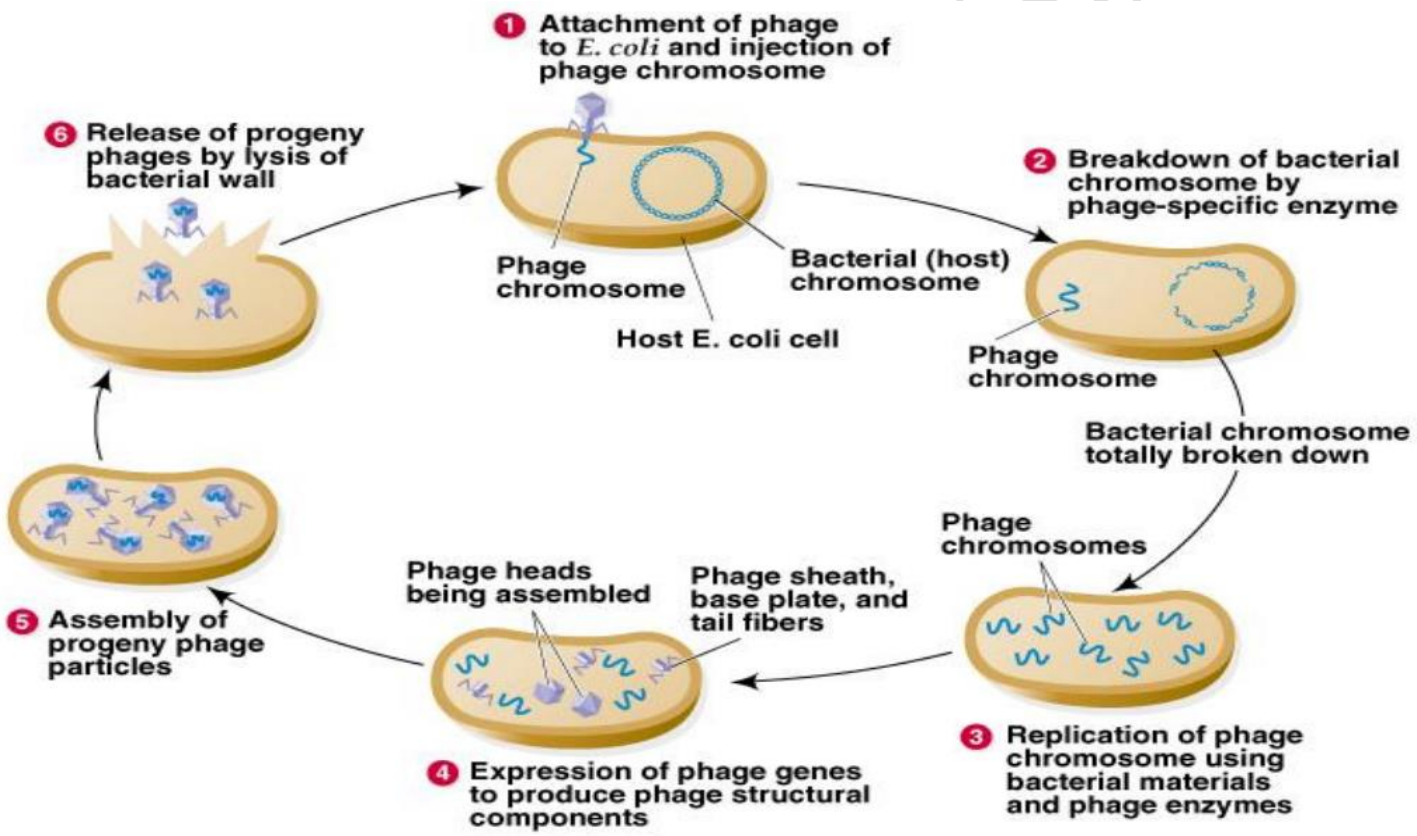



Figure 3: Lytic life cycle of a virulent phage, such as T2, (Russell, P, I Genetics, A molecular approach, 3rd edition , 2009, edited by Yue-Wen Wang. Pearsons,USA.)

The researchers used radioisotopes to distinguish proteins from DNA. **Sulfur atoms are found in proteins but not in DNA**, whereas **phosphorus atoms are found in DNA but not in phage proteins.** Therefore, $^{35}S$ (a radioisotope of sulfur) and $^{32}P$ (a radioisotope of phosphorus) were used to specifically **label phage proteins and DNA**, respectively.

Then each group of labeled viruses was mixed separately with the *E. coli* host. After a short time, phage attachment was disrupted with a kitchen blender, and the location of the label determined.

The $^{35}S$-labeled protein was found outside the infected cells, while the $^{32}P$-labeled DNA was inside the *E. coli,* indicating that DNA carried the information needed for viral infection. This provided additional support for the idea that genetic inheritance occurs via DNA.

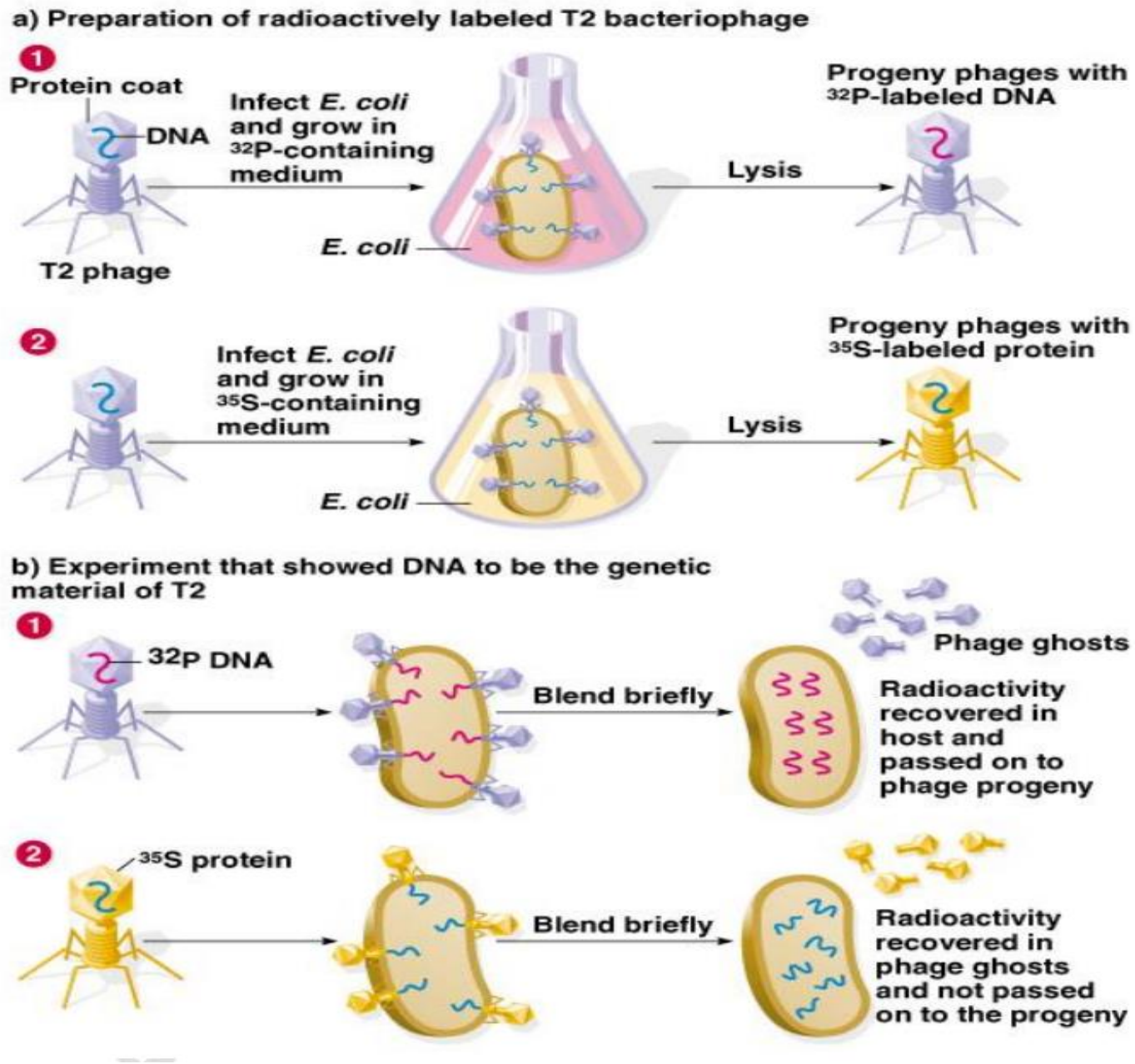



Figure4: Hershey-Chase experiment demonstrating DNA is genetic material. (Russell, P., I Genetics, a molecular approach, 3rd edition, 2009, edited by Yue-Wen Wang. Pearson's, USA).

## Viral Genome

The viral genomes vary among different types of viruses. The nucleic acid of some viruses is **DNA, so they are referred to as DNA** viruses, whereas in others it is **RNA; and referred as RNA viruses**. In some viruses, the nucleic acid is **single-stranded**, whereas in others, it is **double-stranded.** ( dsDNA, ssDNA, dsRNA, ssRNA) The genome can **be linear or circular**, depending on the type of virus. Some viruses **have more than one copy of the genome**. Also, viral genomes vary in size, ranging from a **few thousand to more than a hundred thousand** nucleotides or nucleotide pairs in length.

## Discovery that RNA is the genetic material of the viruses

In 1956, Alfred Gierer and Gerhard Schramm isolated RNA from tobacco mosaic virus (TMV), which infects plant cells. When this purified RNA was applied to plant tissue, the plants developed the same types of lesions that occurred when they were exposed to intact TMVs. Gierer and Schramm correctly concluded that the viral genome of TMV is composed of RNA.

To further confirm that TMV uses RNA as its genetic material, Heinz Fraenkel-Conrat and Beatrice Singer did an experiment by using the **wild-type strain** and a **mutant strain** called the Holmes ribgrass (HR) strain. The two strains differ in two ways.

**First,** they cause different symptoms when they infect plants. the wild-type strain produces a mottled area with yellow and green irregularly shaped lesions on infected leaves, whereas the HR strain often produces streaks along the veins and ringlike markings on other parts of the leaves.

**Second**, the capsid protein in the **mutant HR strain** has two amino acids (histidine and methionine), **which are not found in the wild-type capsid protein**.

The experiments included

1- Fraenkel-Conrat and Singer mixed purified wild-type RNA with HR proteins or purified HR RNA with wild-type proteins and then used the reconstituted viruses to infect tobacco leaves.
2- Following infection, they observed the symptoms caused by the viruses and analyzed the amino acid composition of the proteins of viruses produced after the infection.
3- they concluded that RNA is the genetic material of TMV, because of the type on symptoms on the leaves as well as the analysis of the amino acids extracted from the infected leaves.

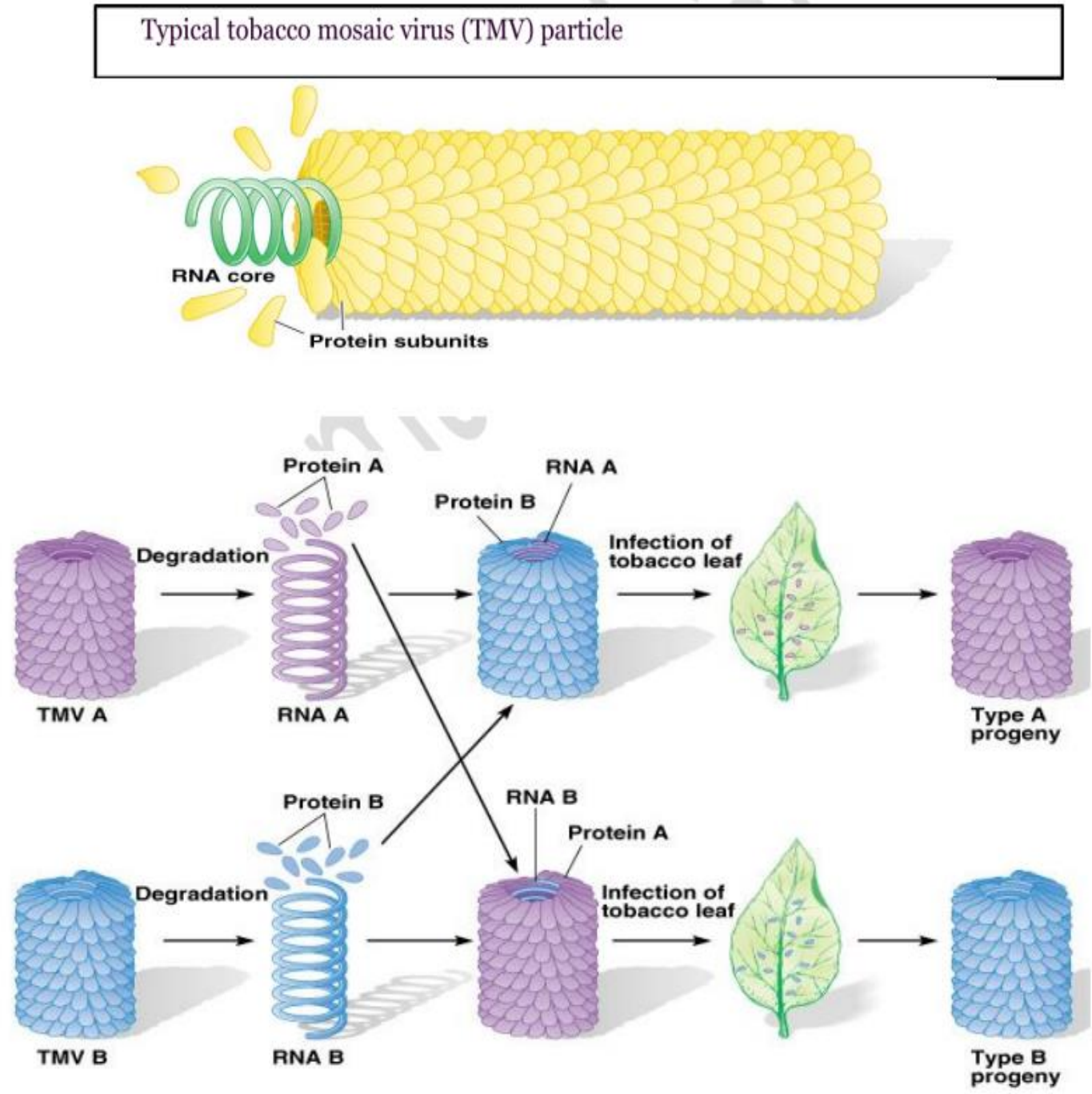



Figure 5: Demonstration thet the RNA is the genetic material in TMV. (Russell, P., I Genetics, a molecular approach, 3rd edition, 2009, edited by Yue-Wen Wang. Pearson's, USA).

## Nucleic acid structure:

Nucleic acids composed of the repeating structural unit of nucleic acids called nucleotides.

- Each nucleotide is composed of at least one phosphate group, a pentose sugar, and a nitrogenous base.
- The sugar in DNA is always **deoxyribose** and in RNA is always **ribose**.
- The five different bases are subdivided into two categories: **the purines and the pyrimidines.** The purine bases, **adenine (A) and guanine (G), contain a double-ring structure; the pyrimidine** bases, **thymine (T), cytosine (C), and uracil (U), contain a single-ring structure.**
- Also, the base thymine is not found in RNA, which contains the base uracil instead of thymine.
- Nucleotides are linked together in a linear manner to form a strand of DNA or RNA.

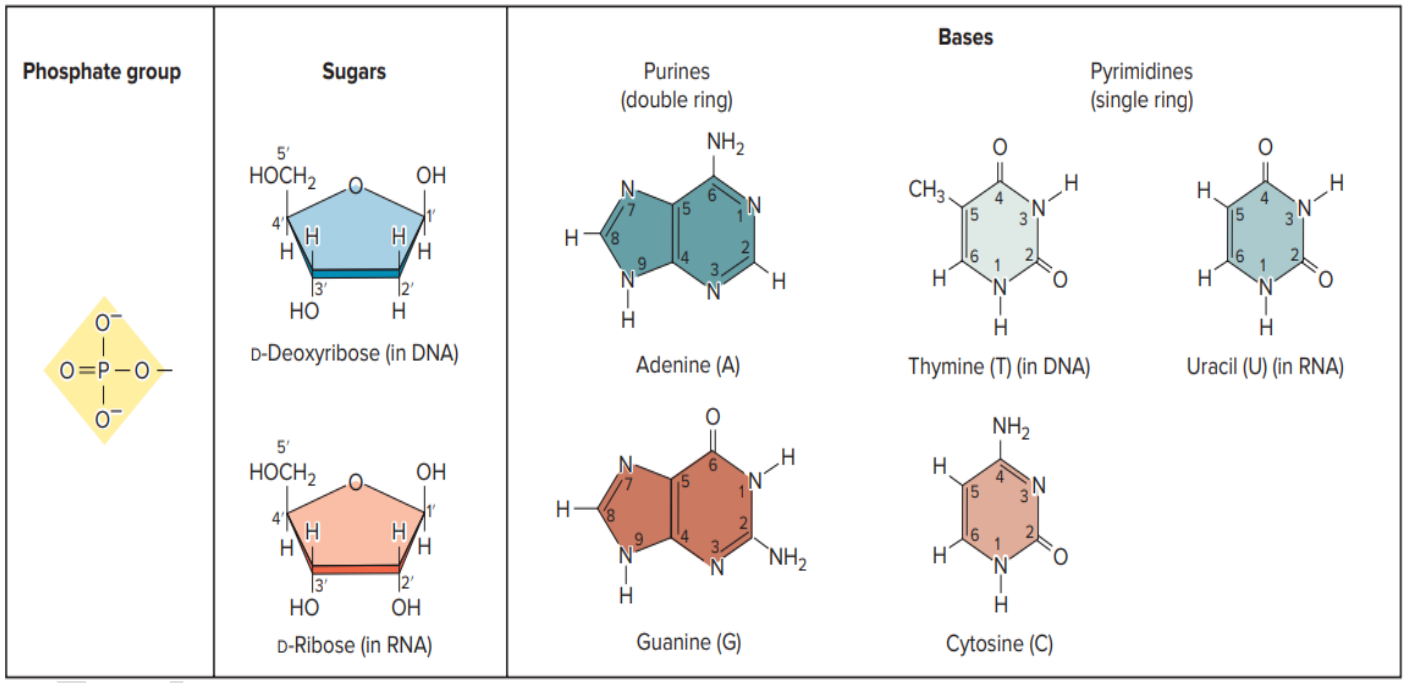



Figure (6): the components of the nucleotides . (Brooker, RJ, 2018, Genetics: Analysis & Principles, 6th Edi. McGraw-Hill Education, US).

- The locations of the attachment sites of the base and phosphate to the sugar molecule are important to the nucleotide's function.
- In the sugar ring, carbon atoms are numbered in a **clockwise direction**.
- The 1st a carbon atom adjacent to the **ring oxygen atom**. The **5th carbon is outside the ring structure**.
- In a single nucleotide, the **base is always attached to the 1′ carbon atom**, and **one or more phosphate groups are attached at the 5′ position** and OH group attached to the 3′ carbon is important in allowing nucleotides to form covalent linkages with each other.

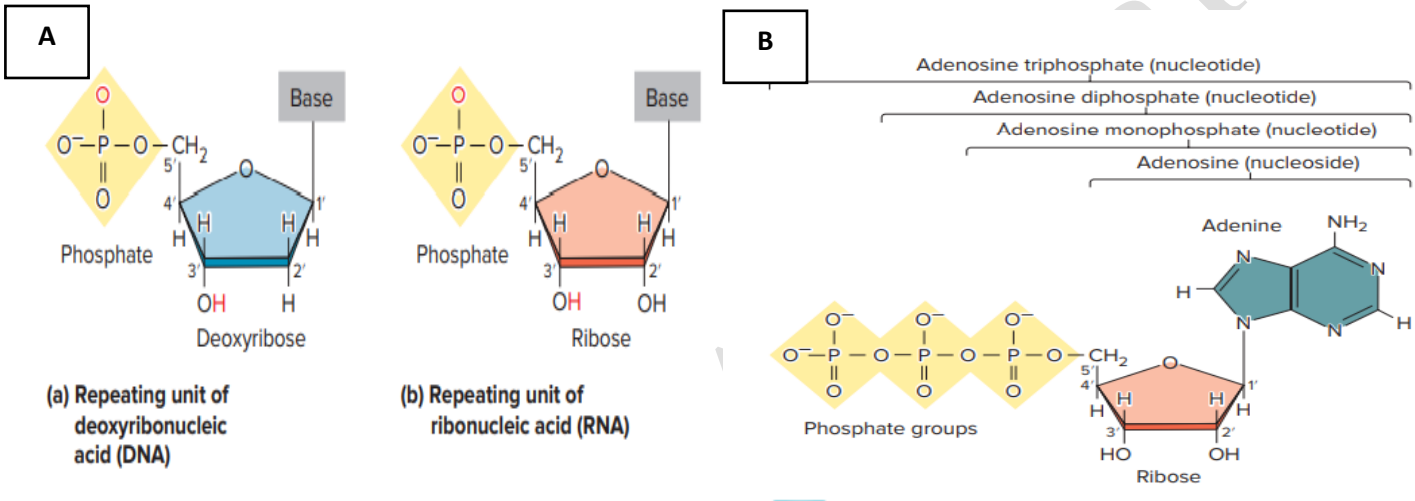



Figure (7): A - Nucleotide composition in DNA and RNA. B- The adenine - containing Nucleoside and nucleotide. (Brooker, RJ, 2018, Genetics: Analysis & Principles, 6th Edi. McGraw-Hill Education, US).

- When a sugar is attached to only a base, this pair is a **nucleoside**. If ribose is attached to adenine, this nucleoside is called **adenosine** (Figure 7B). Nucleosides composed of ribose and guanine, cytosine, or uracil are named **guanosine, cytidine, and uridine,** respectively.
- Nucleosides made of deoxyribose and adenine, guanine, thymine or cytosine are called **deoxyadenosine, deoxyguanosine, deoxythymidine, and deoxycytidine**, respectively. By comparison, a nucleotide made of deoxyribose, adenine, and three phosphate groups is referred to as **deoxyadenosine triphosphate (dATP)**
- A strand of DNA (or RNA) has nucleotides that are linked to each other in a **linear fashion**
- **A phosphate group connects two sugar molecules via ester bonds**. For this reason, the linkage in DNA (or RNA) strands is called **a phosphodiester linkage**.
- The phosphates and sugar molecules form the **backbone of the strand**.
- The backbone is negatively charged due to a negative charge on each phosphate.
- The strand has a **directionality** because all sugar molecules have the same orientation. The direction of the strand is 5′ to 3′ when going from top to bottom

## The Double Structure of DNA Strands

the DNA double helix model was based on three previous discoveries.

1- Early 1950s, Linus Pauling proposed that regions of proteins can fold into a secondary structure known as an **α helix**. Pauling built large models by linking together simple ball-and-stick units. He could visualize whether atoms fit together properly in a complicated three-dimensional structure of the protein.

2- A second important development was the use of **X-ray diffraction data. Rosalind Franklin** isolated purified DNA fibers and subjected the DNA to X-rays, it produces a well-defined diffraction pattern. Rosalind Franklin's X ray diffraction images of DNA showed a helical structure with regularities at 0.34 nm and 3.4 nm along the axis of the molecule

3- The third finding was the **Chargaff rules**.
During the late 1940s and early 1950s, Erwin Chargaff developed a chemical technique to measure the A, C, G, T indifferent DNA molecules. The compelling observation was that the amount of adenine was similar to that of thymine, and the amount of guanine was similar to cytosine. The idea that the amount of A in DNA equals the amount of T, and the amount of G equals C. and He found that the percent G + C, differs among species but is constant in all cells of an organism and within a species.

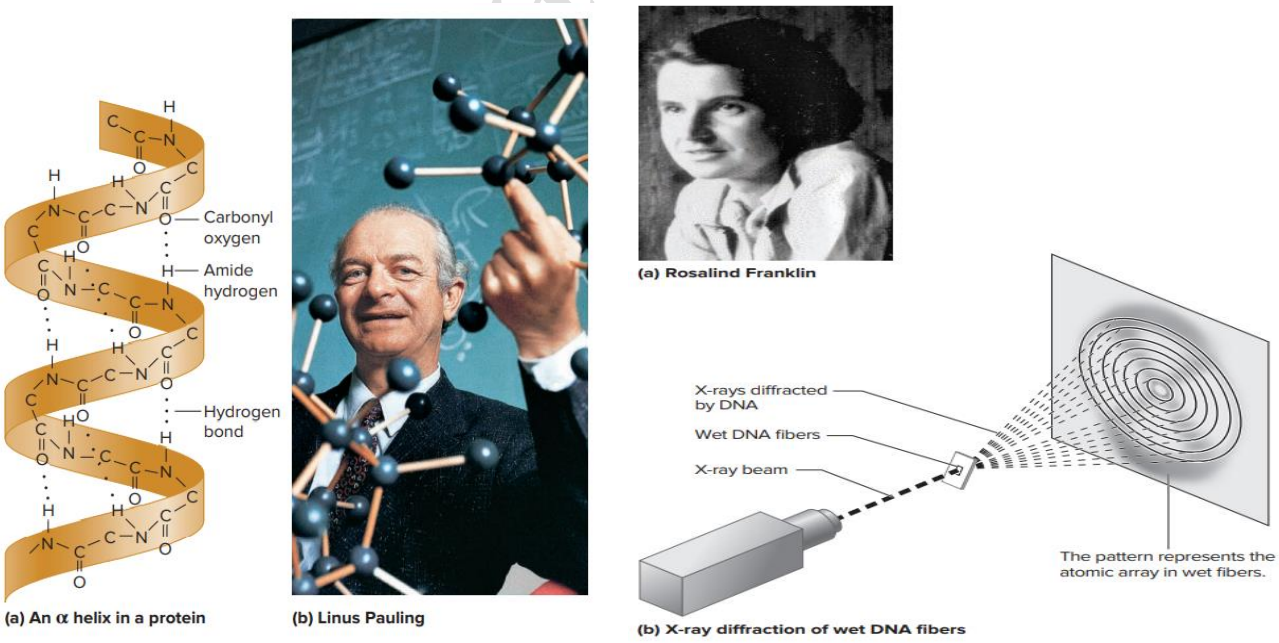



Figure (8): Linus Pauling and his α helix model for the protein and Rosalind Franklin and her DNA image by X ray diffraction. (Brooker, RJ, 2018, Genetics: Analysis & Principles, 6th Edi. McGraw-Hill Education, US).

## Watson and Crick Double helix model

Watson and Crick assumed DNA is composed of nucleotides that are linked together in a linear fashion.

- In a DNA double helix, two DNA strands are twisted together around a common axis to form a structure that resembles a **spiral staircase**. This double-stranded structure is stabilized by **base pairs (bp)**.

- pairs of bases in opposite strands bond together by hydrogen bonds. An **adenine** base in one strand is bonded by 2 hydrogen bonds with a thymine base in the opposite strand , or a guanine is bonded by 3 hydrogen bonds with a cytosine.

- For this reason, DNA sequences with a high proportion of G and C tend to form more **stable double-stranded structures.**

- This AT/GC rule explained the earlier data of Chargaff. The AT/GC rule indicates that purines (A and G) always bond with pyrimidines (T and C). This keeps the width of the double helix relatively **constant**.

- The AT/GC rule allows us to **predict** the sequence in one DNA strand if the sequence in the opposite strand is known. For example, let's consider a DNA strand with the sequence 5′–ATGGCGGATTT–3′. The opposite strand has to be 3′–TACCGCCTAAA–5′, these two sequences are **complementary to each other**, so DNA strands show **complementarity**.

- Each 10 bp, it gone 360° around the backbone. The linear distance of a **complete turn is 3.4 nm;** each **base pair traverse 0.34 nm.**,

- In addition, the nucleotide sequences are labeled with 5′ and 3′ ends. These numbers designate the direction of the DNA backbones.

- This opposite orientation of the two DNA strands is referred to as an **antiparallel** arrangement.

- Within DNA, the bases are oriented so the flattened regions are facing each other, an arrangement referred to as base stacking. stabilizes the double helix by excluding water

molecules. **The helical structure of the DNA backbone depends on the hydrogen bonding between base pairs and also on base stacking.**

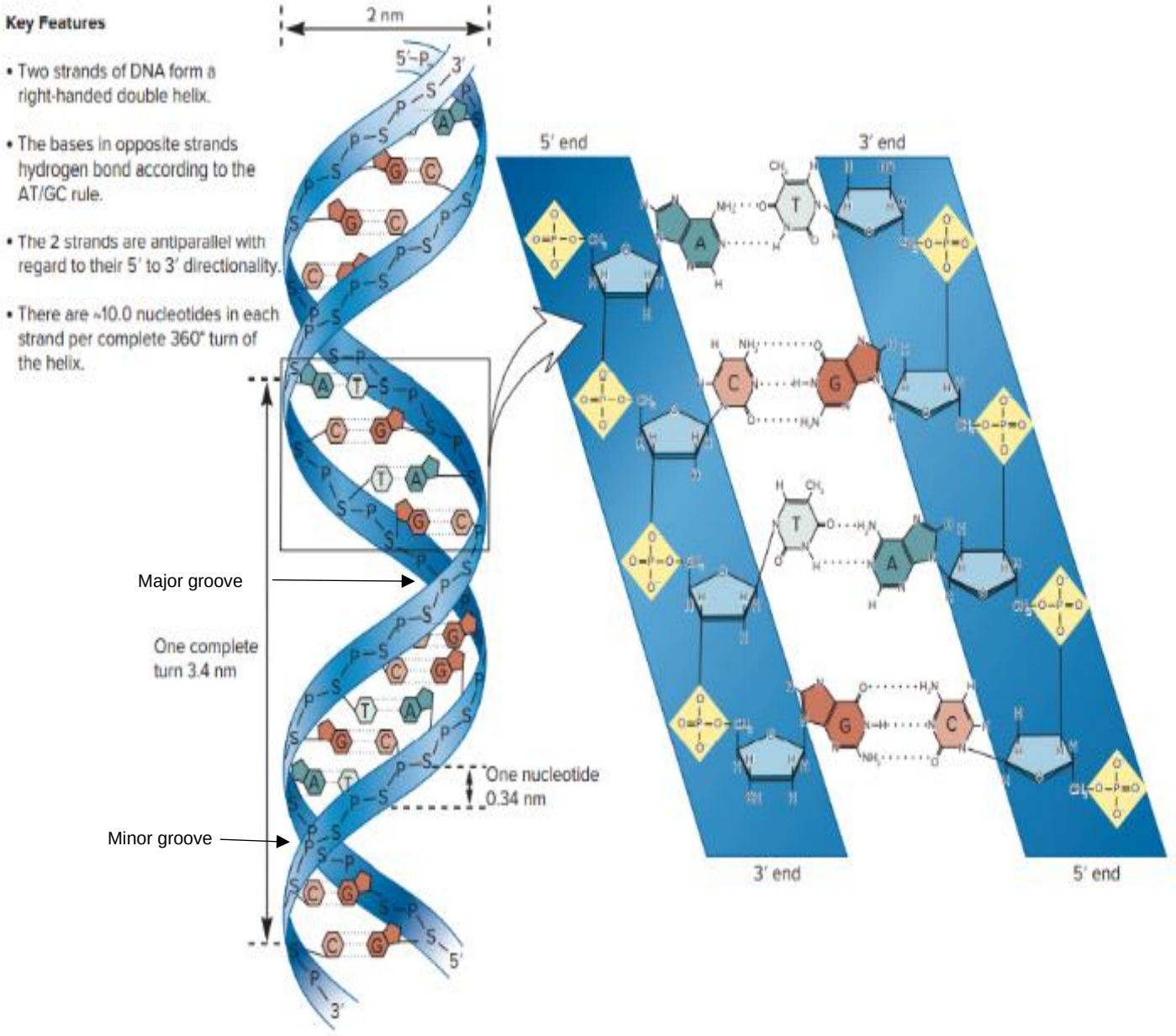



Figure (9): The double helix structure of DNA proposed by Watson and Crick in 1953. (Brooker, RJ, 2018, Genetics: Analysis & Principles, 6th Edi. McGraw-Hill Education, US).

## Alternative types of Double helix

The DNA double helix can form different types of structures. Known as B DNA and Z DNA.

- A-DNA is the dehydrated form, and so it is not usually found in cells. It is a right-handed helix with 10.9 bp/turn, with the bases inclined 13° from the helix axis. A-DNA has a deep and narrow major groove, and a wide and shallow minor groove.

- B DNA is the predominant form of DNA in living cells, B DNA is a right-handed helix, contains a 10.0 base pair / turn. In B DNA, the bases tend to be centrally located. B-DNA has a wide major groove and a narrow minor groove, and its major and minor grooves are of about the same depth.
- a Z DNA conformation is found in some cells. It is left-handed. In addition, the helical backbone in Z DNA appears to zigzag shape, it contains12.0 bp/turn, its inclined 8.8° from the helix axis. Z DNA may play a role in chromosome structure and regulate transcription of particular genes.
  Sometimes B DNA converted into a Z-DNA conformation depends on various factors. Such as high ionic strength (i.e., high salt concentration) and high GC rich sequences such as is 5′–GCGCGCGCG–3′ 3′–CGCGCGCGC–5′. Z-DNA has a deep minor groove, and a very shallow major groove. Its existence in living cells has not been proven

## Triplex DNA

A surprising discovery made in 1957 by Alexander Rich, David Davies, and Gary Felsenfeld was that DNA can form a triple-helical structure called **triplex DNA**. This triplex was formed **in vitro** using pieces of DNA that were made synthetically. Its formed in vitro when natural double-stranded DNA and a third short strand that is synthetically made are mixed. The synthetic strand binds into the major groove of the naturally occurring double-stranded DNA. The pairing rules are that a thymine in the synthetic DNA hydrogen bonds at an AT pair in the biological DNA and a cytosine in the synthetic DNA hydrogen bonds at a GC pair.

## RNA structure

- The structure of an RNA strand is similar to that of the DNA.
- Strands of RNA are linear and composed of a few hundred to several thousand nucleotides in length which are much shorter than chromosomal DNA, which is typically millions of base pairs long.

- When RNA is made by transcription, from DNA template. In most cases, only one of the two DNA strands is used as a template for RNA synthesis. Therefore, only one complementary strand of RNA is usually made.
- Short segments of RNA to form a double-stranded region that is helical as in the t RNA. Base pairing between A and U and between G and C may occur within one RNA molecule or between two separate RNA molecules. the complementary regions are held together by hydrogen bonds between base pairs, whereas the noncomplementary regions have their bases projecting away from the double-stranded region.

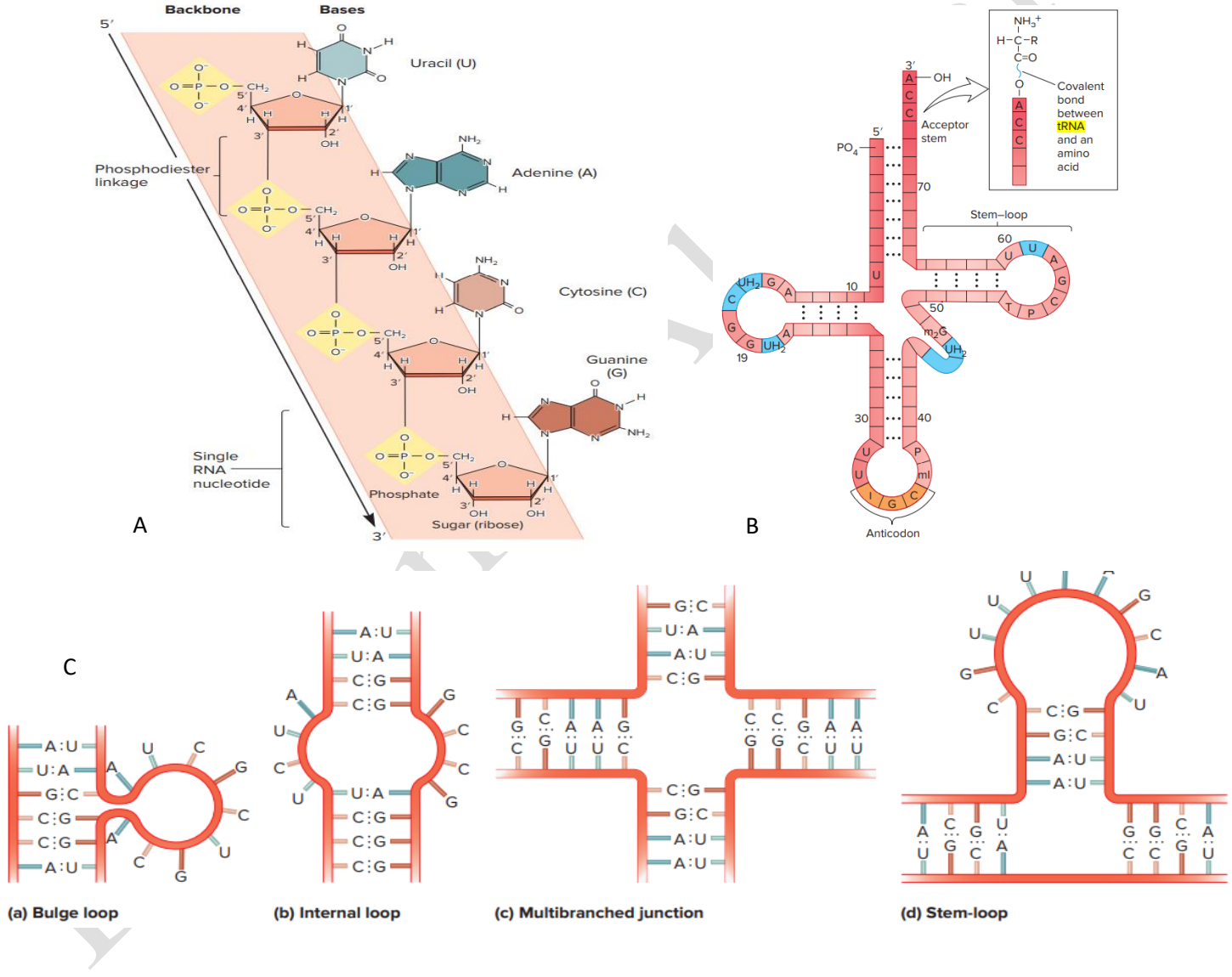


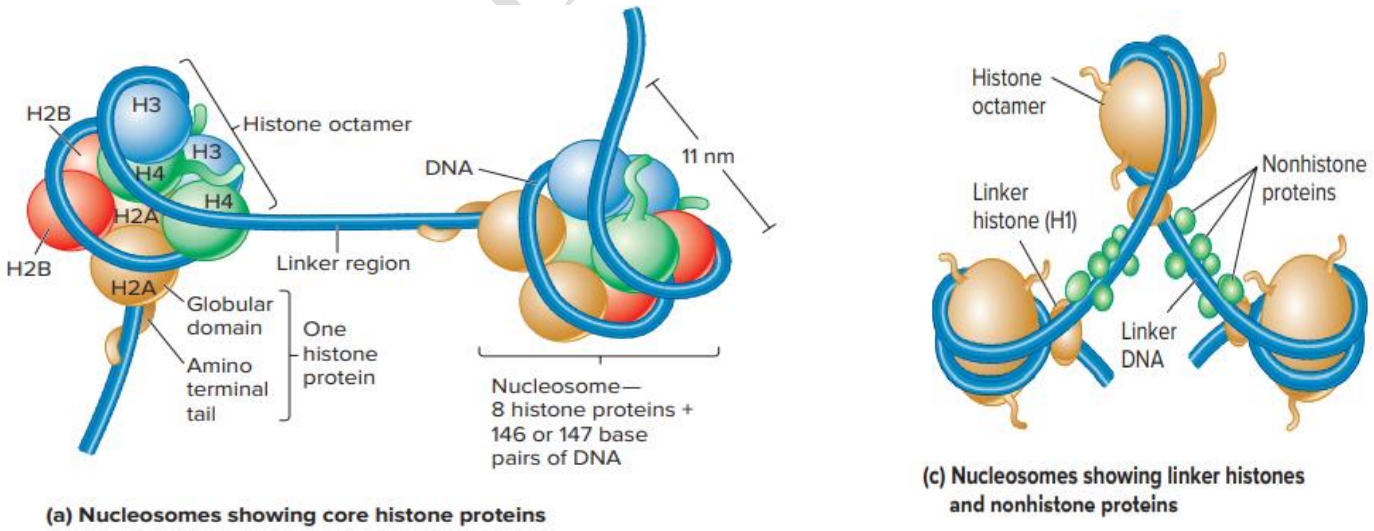

Figure (10): A- The structure of RNA, B- t RNA structure. C- double helix regions in the RNA. (Brooker, RJ, 2018, Genetics: Analysis & Principles, 6th Edi. McGraw-Hill Education, US).

## Bacterial genome:

Bacterial genome is usually arranged in single, circular, and supercoiled chromosome, it must be compacted about 100-fold to fit within the bacterial cell. Most bacterial chromosome is circular molecule, while some bacteria have linear chromosomes. Some bacteria have more than one copy of its chromosome. A typical chromosome is composed of a few million base pairs (bp) in length. For example, the chromosome of *Escherichia coli* has approximately 4.6 million bp, and the *Haemophilus influenzae* chromosome has roughly 1.8 million bp. A bacterial chromosome contains a few thousand different genes, which are protein-encoding genes (also called structural genes) account for the majority of bacterial DNA. The non-transcribed regions of DNA located between adjacent genes are termed intergenic regions.

## Eukaryotic genome:

1- Cellular DNA is organized into chromosomes. A genome is a set of chromosomes that contains all the DNA of an organism.
Eukaryotic chromosomes are linear dsDNA, and by weight contain about twice as much protein as DNA. with equal amounts of DNA and histones.
2- The DNA-protein complex is called **chromatin formed by warping of the DNA around the histones and condenses the DNA so it will fit into the cell.**
3- Both histones and non-histones are involved in physical structure of the chromosome.
4- Histones are abundant, small proteins with a net (+) charge, composed of five main types are H1, H2A, H2B, H3 and H4. Histones are highly conserved between species (H1 less than the others).



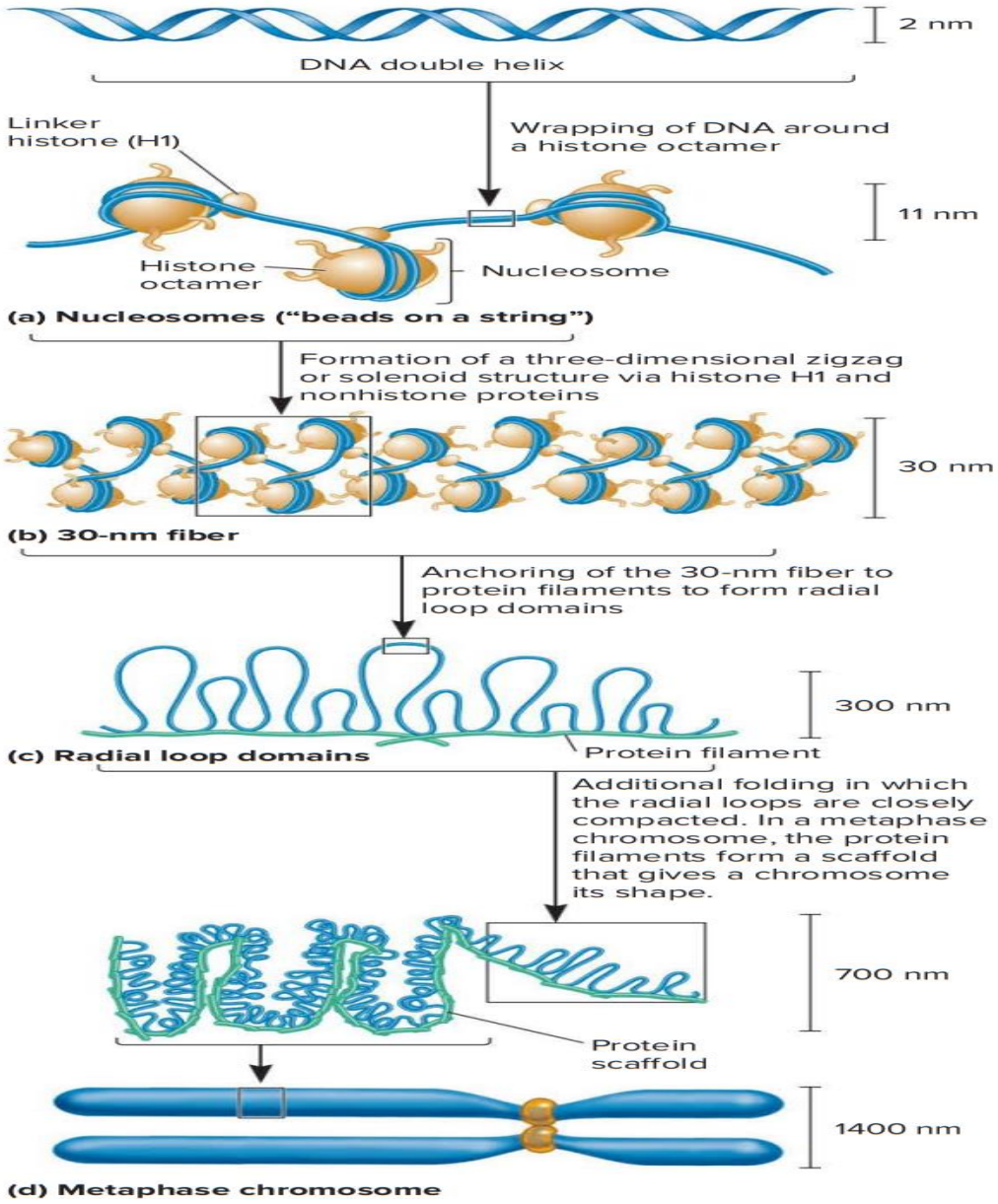

Figure(11) nucleosome composition and chromatin formation.

5- Chromatin is formed when:

a- two molecules each of histones H2A, H2B, H3 and H4 associate to form a **nucleosome core**, and DNA wraps around it 1 3⁄4 times for a 7- fold condensation factor. Nucleosome cores are about 11 nm in diameter.

b- H1 serves as the linker histone, connecting nucleosomes to create chromatin with a diameter of 30 nm, for an additional 6- fold condensation.

c- Non-histone is a general name for other proteins associated with DNA. This is a big group, with some structural proteins, and some that bind only transiently.




Figure(12): DNA packaging and eukaryotic chromosome formation.

## Euchromatin and Heterochromatin:

The cell cycle enforces the DNA packing into chromosomes, by DNA condensing for passing through mitosis and meiosis, and decondensing during interphase. Chromosomes are most condensed at metaphase, when the looped domains are further coiled and the chromatic has a diameter of about 700 nm. Non-histone proteins form the scaffold for this additional condensation.

2. Staining of chromatin reveals two forms:

a. **Euchromatin** condenses and decondenses with the cell cycle. It is actively transcribed, and lacks repetitive sequences. Euchromatin accounts for most of the genome in active cells.

b. **Heterochromatin** remains condensed throughout the cell cycle. It replicates later than euchromatin, and is transcriptionally inactive. There are two types based on activity:

**Centromeric and Telomeric DNA**:

1. Centromeres and telomeres are eukaryotic chromosomal regions with special functions.

2. Centromeres are the site of the kinetochore, where spindle fibers attach during mitosis and meiosis. They are required for accurate segregation of chromatids.

3. Telomeres are needed for chromosomal replication and stability. Generally composed of heterochromatin, they interact with both the nuclear envelope and each other. All telomeres in a species have the same sequence. Simple telomeric sequences are short, species-specific and tandemly repeated. (Examples: Tetrahymena is 5-TTGGGG-3, and human is 5-TTAGGG-3