Course title: Introductory Molecular Biology (3 UNITS)

Course code: **BCH 202**

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COURSE CONTENT

- Nucleic acid : DNA, RNA
- Biochemistry of heredity
- Discovery and properties of genetic materials
- DNA replication

Week 1: Nucleic Acid

- Nucleic acids are essential biomacromolecules that contains genetic information
- Nucleic acids are required for the storage, expression and transmission of genetic information
- They can be separated into two chemically distinct group:

❖Deoxyribonucleic acid (DNA), and

❖Ribonucleic acid (RNA)

- Nucleic acids (DNA and RNA) are composed of build blocks called **Nucleotides**
- Each nucleotide consists of

❖ a heterocyclic nitrogenous base,

❖a sugar, and

❖one, two or three phosphate

Nucleic Acids: Nucleotides

Nucleotides

- A nucleotide is a nucleoside with an inorganic phosphate attached to a 5-hydroxyl group of the sugar in ester linkage
- The nitrogenous base is linked by an N-glycosidic bond to the anomeric carbon of the sugar, either ribose or deoxyribose
- The names and abbreviations of nucleotides specify the base, the sugar, and the number of phosphates attached

In deoxynucleotides, the prefix “d” precedes the abbreviation

For example, ADP is Adenosine diphosphate (the base Adenine attached to a ribose that has two phosphate groups), and

dATP is deoxyadenosine triphosphate (the base adenine attached to a deoxyribose with three phosphate groups)

Nucleic Acids: Nucleotides

DNA is a polymer of deoxyribonucleotide

- Deoxyadenosine triphosphate (dATP)
- Deoxyguanosine triphosphate (dGTP)
- Deoxycytidine triphosphate (dCTP)
- Deoxythymidine triphosphate (dTTP)

RNA is a polymer of ribonucleotide

Adenosine triphosphate (ATP)

Guanosine triphosphate (GTP)

Cytidine triphosphate (CTP)

Uridine triphosphate (UTP)

Nucleic acid contd’.

- Nucleotide triphosphates are the building blocks of DNA and RNA
- Polynucleotides such as DNA and RNA are linear sequences of nucleotides linked by 3- to 5- phosphodiester bonds between the sugars

- All cells require energy to ensure their survival and reproduction
- The cells get the energy by converting nutrients into a chemically useful form of energy
- The most important form of chemical energy is adenosine triphosphate (ATP), important components of coenzymes FAD, NAD and Coenzyme A

- **Nucleosides:** result from linking one of the sugars with a purine or pyrimidine base through an **N-glycosidic bond**

Nucleic acid contd’.

Purines are bonded to C1 of the sugar at their N9 atoms

Pyrimidines are bonded to C1 of the sugar at their N1 atoms

If the sugar is D-ribose, a **ribonucleoside** is produced , if the sugar is 2-deoxyribose a **deoxyribonucleoside** is produced

In DNA: Deoxyadenosine, Deoxyguanosine, Deoxycytidine, and Deoxythymidine

In RNA: Adenosine, Guanosine, Cytidine, and Uridine

Phosphate Group

- Phosphates can be bonded to either C3 or C5 atoms of the sugar
- The phosphate can be either mono-, di- or tri-phosphates

Nucleic Acids contd.': DNA

- **DNA:** Is the storehouse of genetic information.
- DNA is not only present in the nucleus of eukaryotic organisms, but also in mitochondria and the chloroplasts of the plants.
- The genetic information found in DNA is copied and transmitted to daughter cells through DNA replication

- DNA contains two nitrogenous bases namely

❖The Purine --- which is made up of Adenine (A) and Guanine (G)

❖And the Pyrimidine --- which is made up of Cytosine (C) and Thytmine (T)

- The DNA also contains a sugar known as **deoxyribose**
-

Nucleic Acids: DNA Structure

- DNA is a poly deoxyribonucleotide that contain many monodeoxyribonucleotide covalently linked by phosphodiester bonds.
- Phosphodiester bonds join the 3´-OH group of one nucleotide to the 5´-OH group of the next nucleotide
- DNA exist as a double stranded molecules in which the two strands wind around each other forming a double helix
- As proposed by Watson and Crick, each DNA molecule consists of two polynucleotide chains joined by hydrogen bonds between the bases
- In each base pair, a purine on one strand forms hydrogen bonds with a pyrimidine on the other strand
- In one type of base pair, adenine on one strand pairs with thymine on the other strand

Nucleic Acids: DNA Base Pairing

- This base pair is stabilized by two hydrogen bonds
- The other base pair, formed between guanine and cytosine, is stabilized by three hydrogen bonds
- As a consequence of base-pairing, the two strands of DNA are complementary, that is, adenine on one strand corresponds to thymine on the other strand, and guanine corresponds to cytosine
- The concept of base-pairing proved to be essential for determining the mechanism of DNA replication (in which the copies of DNA are produced that are distributed to daughter cells),
- and the mechanisms of transcription and translation (in which mRNA is produced from genes and used to direct the process of protein synthesis).

Nucleic Acids: DNA Base Pairing contd.’

- Base-pairing allows one strand of DNA to serve as a template for the synthesis of the other strand.
- Base pairing also allows a strand of DNA to serve as a template for the synthesis of a complementary strand of RNA
- The DNA double helical chains are in antiparallel manner, with the 5’-end of one strand pairing with the 3′- end of the other strand
- The specific base pairing in DNA leads to Chargaff's rules: In any samples of double–strand DNA, the amount of Adenine equals the amount of Thymine, the amount of Guanine equals the amount of Cytosine and the total amount of purines (A + G) equals the total amount of pyrimidines (T + C)
- The base pairs are held together by hydrogen bonds: two between A and T and three between G and C
- These hydrogen bonds plus the van der Waals forces between the stacked bases stabilize the structure of the double helix.

Different forms of DNA

- DNA can occur in different three-Dimensional (3D) Forms
- Because DNA is a flexible molecule, considerable rotations are possible around a number of bonds in the sugar–phosphate (phospho-deoxyribose) backbone
- These structural variations generally do not affect the key properties of DNA defined by Watson and Crick: which is strand complementarity, antiparallel strands, and the requirement for A=T and G ≡ C base pairs

- These DNA forms are

❖B-form

❖A-form, and

❖Z-form

Different forms of DNA Contd.’

- **Characteristics of B-form of DNA**

❖Two helical polynucleotide chains, coiled about a common axis

❖Right-handed

❖Chains are anti-parallel

❖Sugar-phosphates on the outside; bases on the inside

❖The distance between base pairs is 0.34 nm with approx. 10 bases per helix turn

❖Rise and turn of helix is 3.57 nm

- **Characteristics of A-form of DNA**
- **A-Form of DNA** is caused by the dehydration of DNA

❖Although similar to the B form, but is more compact (The distance between base pairs is 0.26 nm with 11 base pairs per turn

❖Rise and turn of helix is 2.86 nm

DNA Supercoiling

- **Characteristics of Z-form of DNA**

❖The bases of the two DNA strands are the left-handed helix

❖It has a distance of 0.37 nm between base pairs with 12 base pairs per helix turn

❖Rise and turn of helix is 4.56 nm

- Two structural variants that have been well characterized in crystal structures are the A and Z forms.
- These structural changes deepen the major groove while making the minor groove shallower
- **DNA Supercoiling**
- Supercoiling means the coiling of a coil
- DNA is coiled in the form of a double helix, with both strands of the DNA coiling around an axis
- The further coiling of that axis upon itself produces DNA supercoiling
- When there is no net bending of the DNA axis upon itself, the DNA is said to be in a relaxed state.

Alternative DNA structures

- They are triplex and tetraplex (Quadruplex)
- **Triplex DNA**
- According to Watson-Crick, DNA base pair, can form a number of additional hydrogen bonds
- Example,
- For purines, the atoms that participate in the hydrogen bonding of triplex DNA, are often referred to as Hoogsteen positions, also called Hoogsteen pairing.
- Hoogsteen pairing allows the formation of triplex DNAs.
- **tetraplex (Quadruplex) DNA**
- Four DNA strands can also pair to form a tetraplex (quadruplex), but this occurs only for DNA sequences with Guanosine.
- The guanosine tetraplex, is stable over a wide range of conditions.

Nucleic Acid contd'.: RNA Structure

- RNA plays a vital role in the synthesis of proteins that mainly involves decoding and translation of genetic code and transcription to produce proteins
- RNA molecules are composed of phosphoric acid, a pentose sugar and some cyclic bases containing nitrogen
- It has D-ribose in it as a sugar moiety
- The heterocyclic nitrogenous bases present in RNA are Adenine (A), Guanine (G), Cytosine (C), and Uracil (U)
- Generally, RNA consists of a single strand, which sometimes folds back

Nucleic acid: Types of RNA and their functions

- There are several different types of RNA and each has a specific function
- **Ribosomal RNA:** It is one of the components of ribosomes that are involved in protein synthesis
- **Transfer RNA:** It is essential for the translation of mRNA in protein synthesis
- **Micro RNA:** It is the smallest among all RNA that helps in regulating gene expression
- **Messenger RNA:** It is the RNA transcript that is produced during DNA transcription

Functions of Nucleic Acid

- Nucleic acid is responsible for synthesis of protein in our body
- RNA is a vital component of protein synthesis
- Loss of DNA content is linked to many diseases
- All the information of a cell is stored in DNA
- DNA fingerprinting is a method used by forensic experts to determine paternity
- It is also used for the identification of criminals
- It has also played a major role in studies regarding biological evolution and genetics

Biochemistry of heredity

- Biochemistry of heredity is also known as the biochemistry of genetics
- The biochemistry of heredity centers on how genetic information encoded in DNA is transmitted and expressed, which eventually influences an organism's traits
- It investigates how DNA, the molecule of heredity, is transcribed and translated into functional proteins, and how these proteins influence various biological processes and traits.
- Heredity: Is the transmission of characters or traits in organisms from parents to offspring.
- It is observed that the offspring of a man usually resemble their parents and also resemble one another.
- This is because the offspring inherit characters or traits from their parents.

Biochemistry of heredity contd.’

- However, no offspring is exactly like their parents, and no two children of the same parents are exactly alike. The differences in characters that exist between parents and offspring are known as variation
- **Variation**: The morphological and physiological differences that occur within a species, which could be hereditary or acquired from the environment
- Characters that can be transmitted are those controlled by genes, and the total of the genes that an offspring inherits from its parents is known as its genotype or genetic make–up.
- It usually sets the limits within which characters can vary.

- **Some common terms used in genetics**
- DNA: Is the molecule of heredity, containing the genetic code, or is the primary molecule that stores and transmits genetic information
- Genes: They are segments of DNA that contain the instructions for building proteins.

Biochemistry of heredity contd.’

- **Genotype**: The genetic makeup of an organism, often represented by the combination of alleles for a particular gene
- **Phenotype**: is the observable physical or biochemical characteristics of an organism, which result from the interaction of its genotype with the environment
- Chromosomes: Thread-like structures in the cell nucleus that carry genetic information in the form of genes. Humans have 46 chromosomes (23 pairs).
- **Mutation**: A permanent change in the DNA sequence of an organism. Mutations can be beneficial, harmful, or do not affect the organism.
- **Genetic Disorder**: A condition caused by abnormal or mutated genes, leading to health problems or abnormal traits. Examples include cystic fibrosis, Down syndrome, and sickle cell anaemia
- **Allele**: Different forms or variants of a gene. Alleles can be dominant or recessive.

Biochemistry of heredity contd.’

Biochemical Processes in Heredity:

- **DNA Replication:**
- The process by which DNA is duplicated before cell division, ensuring each daughter cell receives a complete set of genetic instructions.
- **Transcription:**
- The process of copying the genetic information from DNA into RNA.
- **Translation:**
- The process where RNA molecules guide the synthesis of proteins, based on the genetic code.

Week 2: Introduction: Discovery of genetic material (DNA)

- Cells contain material that controls inheritance of traits from one generation to the next.
- It is also able to convey its effect through the formation and functioning of the traits
- This material is referred as genetic material
- In genetic material, the hereditary information is present in coded form in genes
- Thus, the genetic material of an individual consists of several thousands of genes

- In most of the living organisms, the genetic information is stored in the nucleotide sequences of DNA molecules
- Both DNA and RNA carry the genetic information in living organisms
- In most of the organisms, DNA is the genetic material, however in some viruses, bacteriophages, RNA has been found as the genetic material
- It has been noted that in organisms where DNA is absent, RNA functions as genetic material

Discovery of genetic material (DNA):

➢ TRANSFORMATION EXPERIMENTS FOR CONFIRMATION OF DNA AS GENETIC MATERIAL

- By the early twentieth century, scientists knew that **chromosomes** contain genes, as the bearers of hereditary information
- They also knew that chromosomes are made of DNA and proteins
- But it took the first fifty years of the twentieth century and many scientists to discover that DNA is the genetic material
- A series of experiments were conducted in the 1920s which finally established that DNA is the genetic material
- These experiments are describe below:

Discovery of DNA: Transformation Experiments for Confirmation of DNA as Genetic Material CONTD.'

- **Frederick Griffith's Experiment**

• In 1928, British physician Frederick Griffith while studying the pathogenic strain of bacterium called *Streptococcus pneumoniae* (causes pneumonia) discovered that the bacterium (*Streptococcus pneumoniae*) exists in two forms called **S strain** and the **R strain**

- The S strain produces smooth and shiny colonies when grown on solid agar medium, hence virulent (pathogenic)
- This is because of mucous, polysaccharide coat secreted by the cell
- The R strain lacks the ability to produce mucous coat, therefore produces colonies which are rough
- **Experiment**

➢ When the S strain is injected into mice, the bacterium triggered a fatal pneumonia and the mice dies

➢ The disease caused because S strain had a polysaccharide coat which protects the bacterial cell from attack by the mouse's immune system

➢ When R strain is injected into mice, the bacterium is not able to cause pneumonia. He suggested that since R stain lacks polysaccharide coat hence bacterial cell gets attacked by mouse's immune system

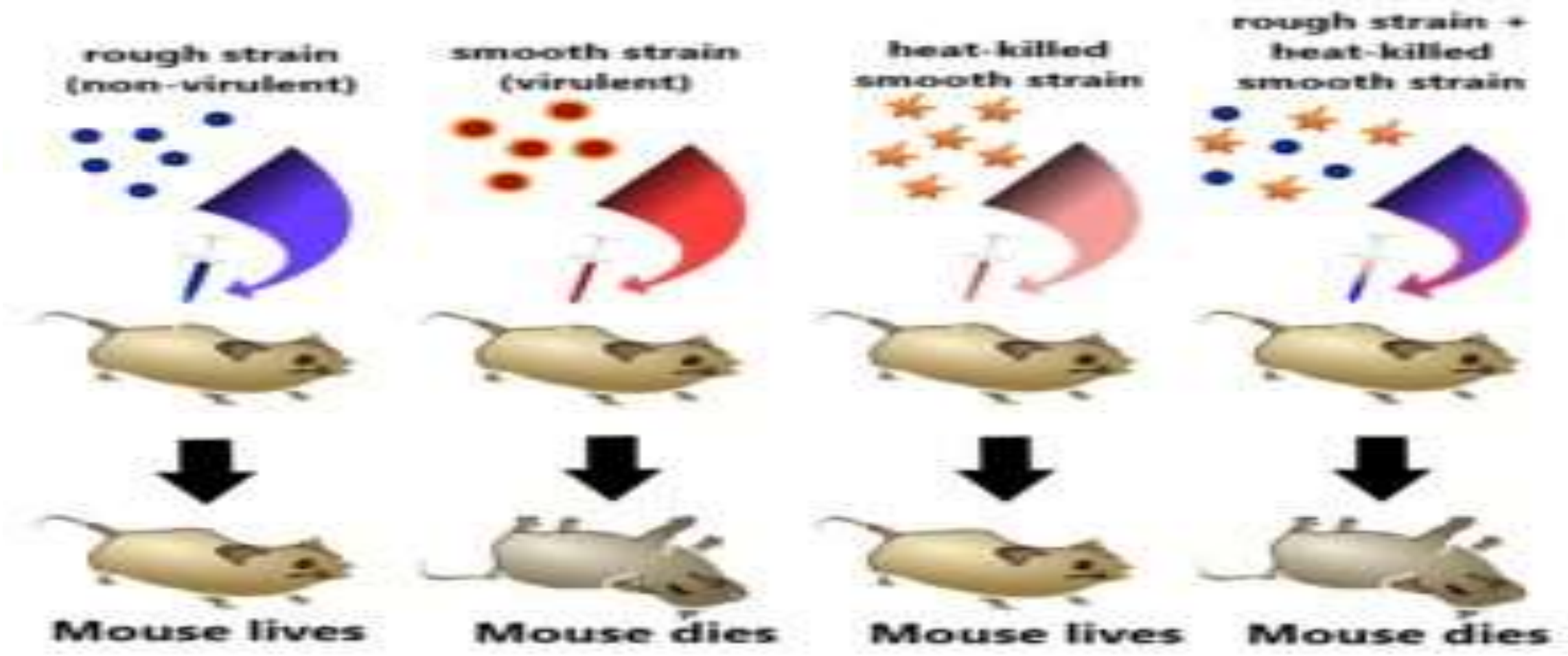
➢ The injection of heat killed S strain alone did not induce disease

Discovery of DNA: Transformation Experiments for Confirmation of DNA as Genetic Material CONTD.'

• Frederick Griffith's Experiment contd.'

- The injection of heat killed S strain (that is, dead S-strain) did not induce disease
- When mice was injected with a mixture of live R strain and dead S strain, the bacterium triggered a fatal pneumonia and the mice dies
- **Observation**
- Griffith observed that the injecting of live R- strain or dead S-strain alone did not caused disease in mice.
- He also recovered live S strain bacteria from these dead mice when autopsied animals that were injected with the mixture of live R and dead S strain
- **Conclusion**
- He concluded that non pathogenic strain somehow converted into pathogenic S strain by a substance present in the heat killed S strain (dead S strain)
- He called the phenomenon **genetic transformation**
- The active substance responsible for inducing pathogenicity was termed '**transforming principle'**
- The chemical factor from heat killed S strain was able to cause heritable change of non-pathogenic R stain into pathogenic S strain.
- Although significant, his observations did not identify the biochemical nature of the transforming principle

Discovery of DNA: Transformation Experiments for Confirmation of DNA as Genetic Material CONTD.'



- Figure 1: *Frederick Griffith's experiment [Source: Wikimedia Commons]*

Discovery of DNA: Transformation Experiments for Confirmation of DNA as Genetic Material CONTD.'

- **Avery's Experiments**

- In 1944, Avery Oswald, MacLeod Collin, and McCarty Maclyn, together set out to determine the biochemical nature of the 'transforming principle' identified by Griffith.
- These researches, purified DNA, RNA, and proteins from the heat-killed S strain (dead S strain) and determined which macromolecule converted the R strain into the S strain

- **Experiment** –
  - They first treated the heat-killed S strain with proteases to break down proteins.
  - Subsequently, they treated it with RNAses and then DNAses to break down RNA and DNA, respectively
- **Observations** –
  - Both protease and RNAse treatments did not affect the transformation of the R strain into the virulent one
  - Finally, treatment with DNAses inhibited the transformation of the R strain.
- **Conclusions** – They concluded that the genetic material is not protein or RNA, but it is **DNA**
  - However, this discovery was not accepted by all biologists.

Discovery of DNA: Transformation Experiments for Confirmation of DNA as Genetic Material CONTD.’

- **Alfred Hershey & Martha Chase Bacteriophage Experiment**

- Bacteriophages are the viruses that infect bacteria
- Bacteriophage T2 infects bacterium ***Escherichia coli*** (*E. coli*)
- During infection, the virus attaches to the bacterial cell surface and injects material into the cell
- The bacterial cell begins to produce thousands of new copies of the virus
- The material injected into the bacterial cell carries the genetic information that guides the production of the virus.
- In 1952, Alfred Hershey and Martha Chase designed an experiment to prove that T2 virus is made up of two kinds of molecules, DNA and proteins

Alfred Hershey & Martha Chase Bacteriophage Experiment contd.'

- The proteins of the T2 virus contain elemental sulfur (present in amino acids methionine and cysteine) and not phosphorus
- While the DNA contains phosphorus (present as sugar phosphate backbone) but not sulfur

➢ **Experiment**

- **Labelling –**
- They prepared two batches of T2 phage particles with different radioactive labelling
- Some viruses were grown on a medium containing radioactive phosphorous and some on a medium with radioactive sulfur
- In one batch the phage proteins were labelled with the radioactive isotope 35S
- And in the other batch the phage DNA was labelled with radioactive isotope 32P
- By radioactive labelling Hershey and Chase were able to trace the fate of protein and DNA during infection process

- **Infection-**
- They started the experiment by mixing (infecting) the radioactive phage with intact bacterial cells- *E. coli*.
- They allowed phage particles to attach to the bacterial cell surface and let them inject their genetic material into the cells.

Alfred Hershey & Martha Chase Bacteriophage Experiment contd.'

- **Blending and centrifugation**
- Then they removed protein coats from the surface of the bacterial cells by agitating the suspension in a blender and recovered the bacterial cells by centrifugation
- They measured radioactivity in the supernatant liquid and in the pellet of bacteria at the bottom of the tube

➢ **Observation**

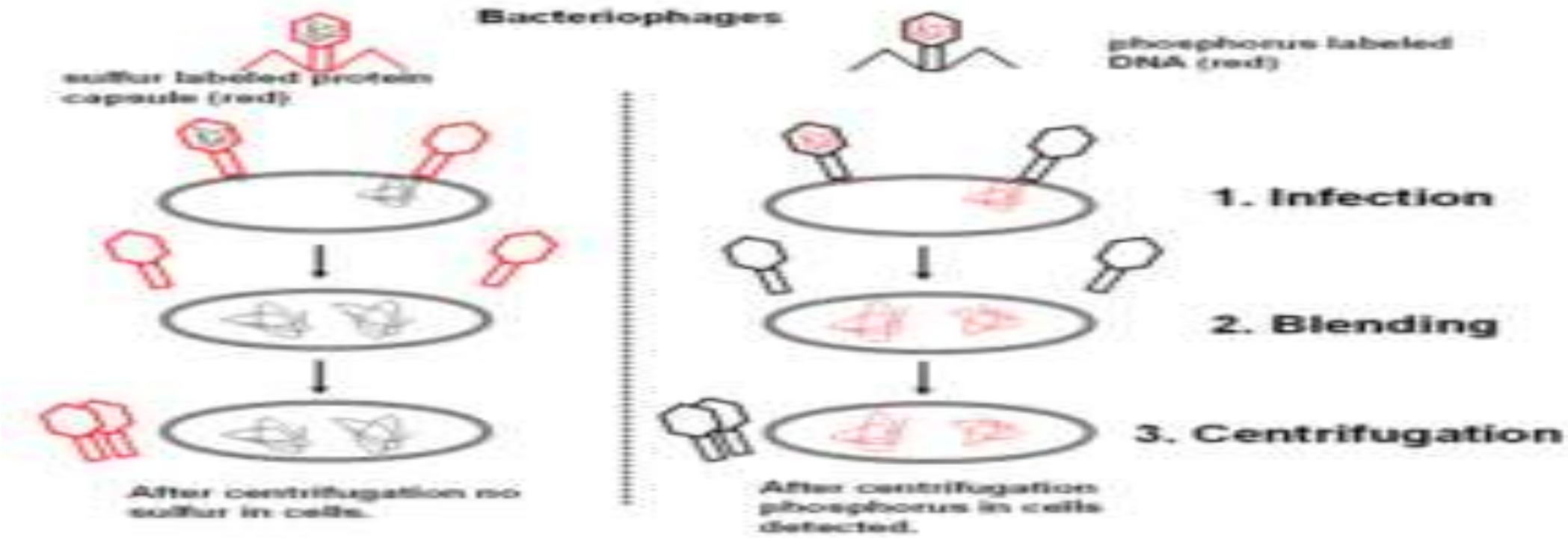
- The studies showed that most of the (65%) 32P remained in the bacterial cells while the bulk of the 35S was released in the surrounding medium.

- Since 32P labelled the viral DNA and 35S labelled the viral protein, this indicated that DNA had been injected into the bacterial cells and functions as the genetic material of phage T2.
- It is important to know that when the 32P-labelled phages were used in the experiment, the majority of the radioactivity was found inside the bacterial cells, which indicates that the phage DNA has entered the cells
- But when the 35S-labelled phages were used in the experiment, most of the radioactive material was found in the phage ghosts, thus it indicates that the phage protein had never entered the bacterial cell

Alfred Hershey & Martha Chase Bacteriophage Experiment contd.'

➢ **Conclusion**

- This experiment conclusively showed that DNA is the genetic material transferred from virus to bacteria, and not protein.
- This 32P can also be recovered from phage progeny
- When infected radioactively labelled bacteria were suspended in fresh liquid and incubated for longer time, the 32P was transferred to some of the offspring phage particles
- This gave the view that genes are made up of DNA
- Fig. 2: *Hershey and Chase experiment [Source: Wikimedia Commons]*



Biochemical evidence that confirms that DNA is the genetic material

- **Apart from experimental evidence, biochemical evidence due to certain facts such as those given below confirmed that DNA is the genetic material**

- i) Amount of DNA in every cell of an organism is the same.
- ii) Amount of DNA in gametes is half that of DNA present in somatic cells.
- iii) Amount of DNA is the same in all members of same species.
- iv) Amount of DNA in a cell is constant and does not change by internal or external environment.
- v) DNA or amount of genetic information is proportional to evolutionary complexity.

Properties Of Genetic Material

- For a molecule to act as the genetic material, it should have the following characteristics:

- (i) It must be capable of accurate replication, that is, create its own replica
- (ii) It should be stable, structurally and chemically
- (iii) It must be capable of being transmitted from parent to progeny without change (mutation)
- (iv) It must be capable of being expressed when needed
- (v) The replicated genetic material must be transferred from a cell to its daughter cells and from one generation to the next

- Although, DNA is the genetic material in most organisms, in some viruses, RNA is the genetic material
- In fact, according to studies, RNA was the first genetic material
- But, since RNA is unstable, DNA evolved from RNA with chemical modifications, making it more stable and more fit to carry genetic information.

Week 3: DNA Replication and cell division

➢DNA Replication

- It is has been an established fact that the sequence of bases along the length of a gene constitutes the language of DNA
- For this language to be preserved for hundreds of generations, it is necessary for the genetic program to be duplicated and passed on to each offspring.
- This process of duplication is called DNA replication
- Therefore, DNA replication involves use of an existing strand of DNA (parent DNA) as a template for the synthesis of a new, identical strand
- Hence, we can state that DNA replication is a process of passing down genetic code or material to the next generation for onward preservation

➢ **Steps involved in DNA replication includes the following;**

- Initiation of replication at the origin of replication
- Uncoiling/unwinding of the parent DNA molecule
- Unzipping of the hydrogen bond between the base pairs, thus separating the two strands and exposing the nucleotides sequence of the helix to serves as the 'templates'
- Addition of the primers by RNA primase
- Elongation
- Termination

DNA Replication contd’.

- **Initiation and Unwinding**

- During initiation, an initiator proteins called (the proteins that recognizes a specific DNA sequence within the origin of replication) bind to the origin of replication, which is a base-pair sequence of nucleotides known as oriC
- These specific DNA sequence within the origin of replication is areregions with high concentration of Adenine, Thymine example TATA or TATATA, this is because it is easier to break two hydrogen (requires less energy) than it will be to break three hydrogen as seen between Guanine and Cytosine

- This binding triggers events that unwind the DNA double helix into two single-stranded DNA molecules by an enzyme called Helicases
- These helicases attaches to the center of the replication fork at both ends of the double helix and starts to unwinding and unzipping the double helix by breaking the hydrogen bonds that join the complementary nucleotide bases to each other, creating what we a replication bubble, as the unwinding continuous the other ends of the helix begins to supercoil, thereby making it more difficult for the Helicase to continue to unwind

- The protein called topoisomerases, immediately binds to the double helix head of the replication fork and relieves the torsional strain placed on the double helix as it unravels, which was caused by the supercoiling of the helix during the unwinding of the double helix

- Because the newly unwound single strands have a tendency to rejoin, another group of proteins, the single-strand-binding proteins, is introduced to keep the single strands stable until elongation begins and prevent the re-annealing of the two strands of the DNA

DNA Replication contd’.

- **Primer Synthesis**
- Primer synthesis marks the beginning of the actual synthesis of the new DNA molecule
- Primers are short stretches of nucleotides (about 10 to 12 bases in length) synthesized by an RNA polymerase enzyme called primase
- Primers are required because DNA polymerases, the enzymes responsible for the actual addition of nucleotides to the new DNA strand, can only add deoxyribonucleotides to the 3'-OH group of an existing chain and cannot begin synthesis *de novo*
- Primase, on the other hand, can add ribonucleotides *de novo*
- Later, after elongation is complete, the primer is removed and replaced with DNA nucleotides by DNA polymerase III
-

DNA Replication contd’.

➢ **Elongation**

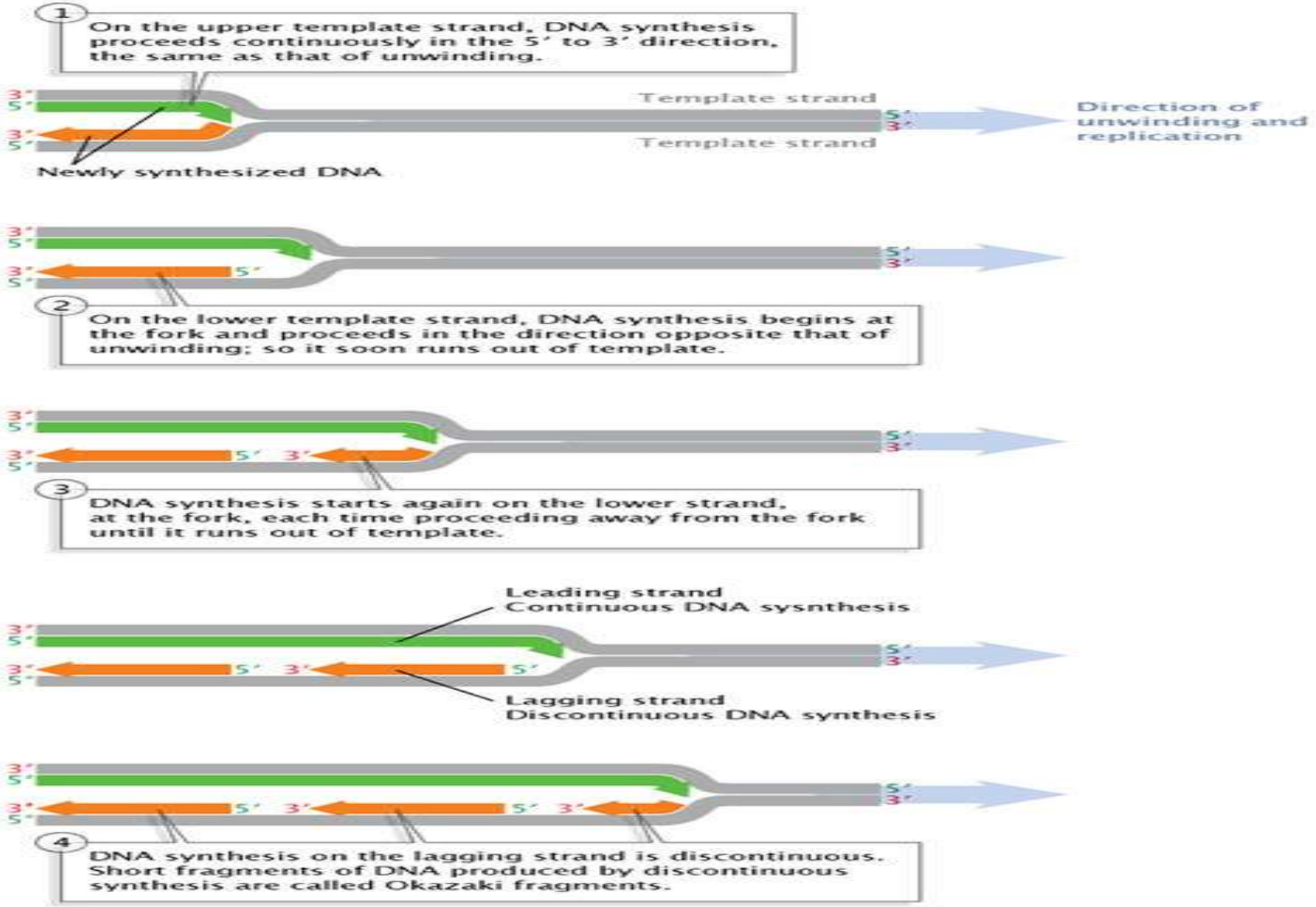
- Finally, elongation—which the addition of nucleotides to the new DNA strand, begins after the primer has been added.
- Synthesis of the growing strand involves adding nucleotides, one by one, in the exact order specified by the original (template) strand.
- Recall that in the DNA model **Adenine** is always paired with **Thymine** and **Cytosine** is always paired with **Guanine**
- So, for example, if the original strand reads A-G-C-T, the new strand will read T-C-G-A to complement each other
- DNA is always synthesized in the 5'-to-3' direction, meaning that nucleotides are added only to the 3' end of the growing strand

DNA Replication contd’.

- **Elongation contd.’: DNA Polymerase Only Moves in One Direction**

- After a primer is synthesized on a strand of DNA and the DNA strands unwind, synthesis and elongation can proceed in only one direction
- DNA polymerase III can only add to the 3' end of the primer to the complementary 5’ end of the original DNA template, so the 5' end of the primer remains unaltered
- Consequently, synthesis proceeds immediately only along the leading strand.
- This immediate replication is known as continuous replication
- The other strand (in the 5' direction from the primer) is called the lagging strand, and replication along it is called discontinuous replication
- The double helix has to unwind a bit before the synthesis of another primer can be initiated further up on the lagging strand
- Synthesis can then occur from the 3' end of that new primer
- Next, the double helix unwinds a bit more, and another spurt of replication proceeds
- As a result, replication along the lagging strand can only proceed in short, discontinuous spurts (Figure 3).

DNA Replication contd'.



DNA Replication contd’.

- **Termination**
- The termination of the DNA replication occurs when two replication forks meet on the same stretch of DNA
- During termination

❖ The remaining gaps in the lagging strand are filled and ligated/sealed by DNA ligase

❖ The primers are removed and replaced with new DNA nucleotides

❖ Forks converge until all the intervening DNA is unwound

❖ Catenanes (double-stranded intertwines between two DNA molecules) are removed by Type II topoisomerase

❖ Replication proteins are unloaded

- In eukaryotes, most termination sites are determined by the location of replication initiation sites
- In prokaryotes, termination generally occurs at a specific locus

DNA Replication contd’.

- The fragments of newly synthesized DNA along the lagging strand are called **Okazaki fragments**
- It is important to note that the Okazaki fragments of bacterial DNA are typically between 1,000 and 2,000 nucleotides long, whereas in eukaryotic cells, they are only about 100 to 200 nucleotides long
- DNA replication occurs during the S phase of cell division
- In *E. coli*, this means that the entire genome is replicated in just 40 minutes, at a pace of approximately 1,000 nucleotides per second
- In eukaryotes, the pace is much slower: about 40 nucleotides per second
- The coordination of the protein complexes required for the steps of replication and the speed at which replication must occur in order for cells to divide are impressive, especially considering that enzymes are also proofreading, which leaves very few errors behind

DNA Repair

- Sometimes during replication, DNA usually encounters damage or errors that needs to be repaired
- There are two different ways that DNA can be repaired
- They are

❖ **Repair during replication**

- During the DNA replication, every once in a while, it is possible the incorrect base to be placed in the new strand
- Therefore, when this kind of error occurs, it can be repaired by the use of DNA polymerase III
- The DNA polymerase III, has an exonucleases which proofreads the attachment of the new bases/nucleotides to the new strand, once it detects any error, it removes the erroneous sequence and replaces it with the correct one

- **Repair of mutations**
- Mutation can be caused by water—which can lead to lose of bases (this really occur but it can happen spontaneously)
- This is because, for bases to be joined together, a condensation reaction is often carried out which releases water
- Also, hydrolysis can also occur and in this case, water can be used to break those bonds that formed
- Mutation can be caused by chemical damage ---which leads to permanent structural changes in the DNA
- Mutation can also be caused by radiation---- which cuts the phosphate sugar backbone of the DNA

DNA Repair Contd.’

- So, in other to repair the DNA damage done by mutation
- This can be done in either of the ways:

❖ (i) Base pair excision– which is done by removing single base and replace a new correct base using DNA polymerase and DNA ligase, to seal-up the gap

❖ (ii) short patch excision --- this is where the damaged strand (if its erroneous) is cut off

---- and the helicase, unwinds the strand again and DNA polymerase comes and replaces the segment with the correct bases.