Molecular Basis of Inheritance

Molecular Basis of Inheritance

- As soon as the inheritance pattern and genetic basis of such patterns were identified, the scientists started working on the nature of the factors regulating patterns of inheritance.
- Research over many years brought forward the biomolecules responsible for the inheritance of genetic information.
- It was discovered that nucleic acids (**DNA** and **RNA**) may act as the genetic material.

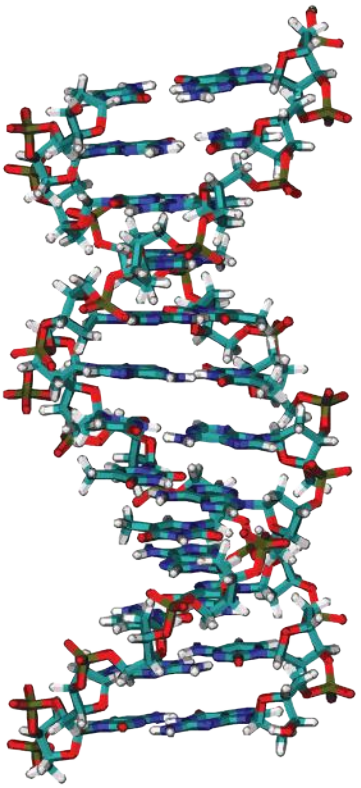
Definition

Inheritance: The process by which characters or traits pass from one generation (parents) to the next (offsprings).

The DNA

- DNA (Deoxyribonucleic acid) consists of a helix of two polynucleotide chains attached to each other by hydrogen bonds.
- The length of DNA is usually determined by the number of nucleotides (or a pair of nucleotides called base pairs) present in it.

Note:
Bacteriophage ϕ ×174 has 5386 nucleotides.
Bacteriophage lambda has 48502 base pairs (bp). *Escherichia coli* has 4.6×10^6 bp.
Haploid content of human DNA is 3.3×10^9 bp.



Structure of Polynucleotide Chain

- A polynucleotide chain is made of many monomers called **nucleotides**.
- A nucleotide consists of a **nitrogenous base**, a **pentose sugar** (ribose in RNA, and deoxyribose in DNA), and a **phosphate group.**
- **Purines** (Adenine and Guanine), and **pyrimidines** (Cytosine, Uracil and Thymine) are the two types of nitrogenous bases.
- A nitrogenous base is linked to the hydroxyl group (-OH) of 1' C (carbon) of pentose sugar through a **N-glycosidic** linkage to form a nucleoside.
- A phosphate group is linked to -OH of 5' C of a nucleoside through phosphoester linkage to form a nucleotide.

Gray Matter Alert!!!

The term 'DNA' (Deoxyribonucleic acid) was given by Zacharias.

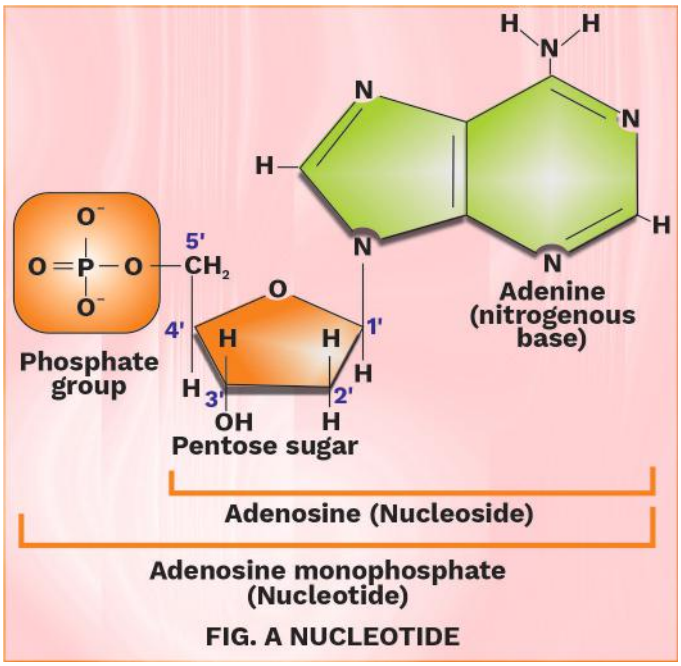


FIG. A NUCLEOTIDE

- The nucleotides are linked through 3'-5' **phosphodiester** linkage to form a polynucleotide chain which has 5'-end formed by a free phosphate moiety at **5' -end** of sugar and a **3' -end** formed by a free -OH of 3' C group of the sugar.

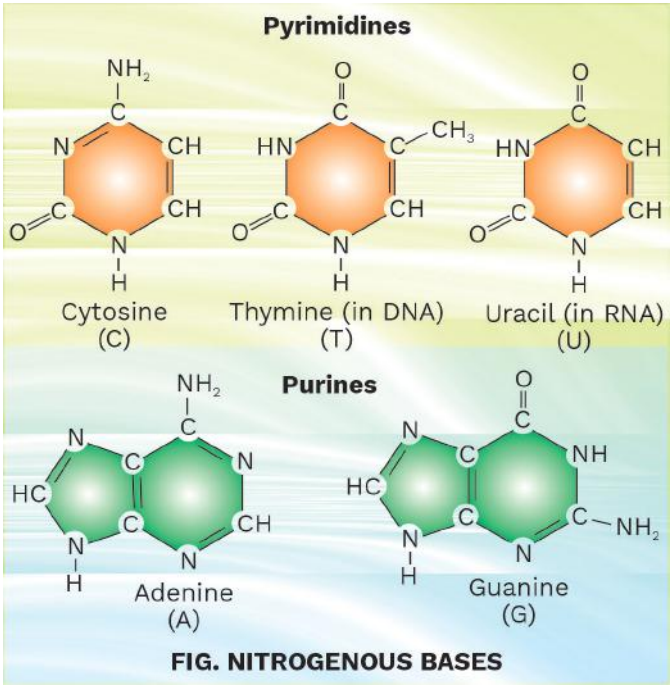


FIG. NITROGENOUS BASES

Previous Year's Question

Purines found both in DNA and RNA are
(1) Cytosine and thymine
(2) Adenine and thymine
(3) Adenine and guanine
(4) Guanine and cytosine

Previous Year's Question

In the polynucleotide chain of DNA, a nitrogenous base is linked to the -OH of
(1) 2'C pentose sugar
(2) 3'C pentose sugar
(3) 5'C pentose sugar
(4) 1'C pentose sugar

Previous Year's Question

Which of the following statements is correct?
(1) Adenine pairs with thymine through one H-bond.
(2) Adenine pairs with thymine through three H-bonds.
(3) Adenine does not pair with thymine.
(4) Adenine pairs with thymine through two H-bonds.

NITROGENOUS BASE, NUCLEOSIDE AND NUCLEOTIDE NOMENCLATURE				
Nitrogenous Base	**Nucleoside (Nitrogenous Base + Pentose Sugar)**		**Nucleotide (Nitrogenous Base + Pentose Sugar + Phosphate)**	
Purines	**DNA**	**RNA**	**DNA**	**RNA**
	(Deoxyribonucleoside)	(Ribonucleoside)	(Deoxyribonucleotide)	(Ribonucleotide)
Adenine (A)	Deoxyadenosine	Adenosine	Deoxyadenosine-5'-monophosphate (dAMP)	Adenosine-5'-monophosphate (AMP)
Guanine (G)	Deoxyguanosine	Guanosine	Deoxyguanylic acid (Deoxyguanylate) or Deoxyguanosine-5'-monophosphate (dGMP)	Guanylic acid (Guanylate) or Guanosine-5'-monophosphate (GMP)
Pyrimidines	**DNA**	**RNA**	**DNA**	**RNA**
Cytosine (C)	Deoxycytidine	Cytidine	Deoxycytidylic acid (Deoxycytidylate) or Deoxycytidine-5'-monophosphate (dCMP)	Cytidylic acid (cytidylate) or Cytidine-5'-monophosphate (CMP)
Thymine (T)	Thymidine or Deoxythymidine	———	Deoxythymidylic acid (Deoxythymidylate) or Deoxythymidine-5-monophosphate (dTMP)	———
Uracil (U)	———	Uridine	———	Uridylic acid (Uridylate) or Uridine-5'-monophosphate (UMP)

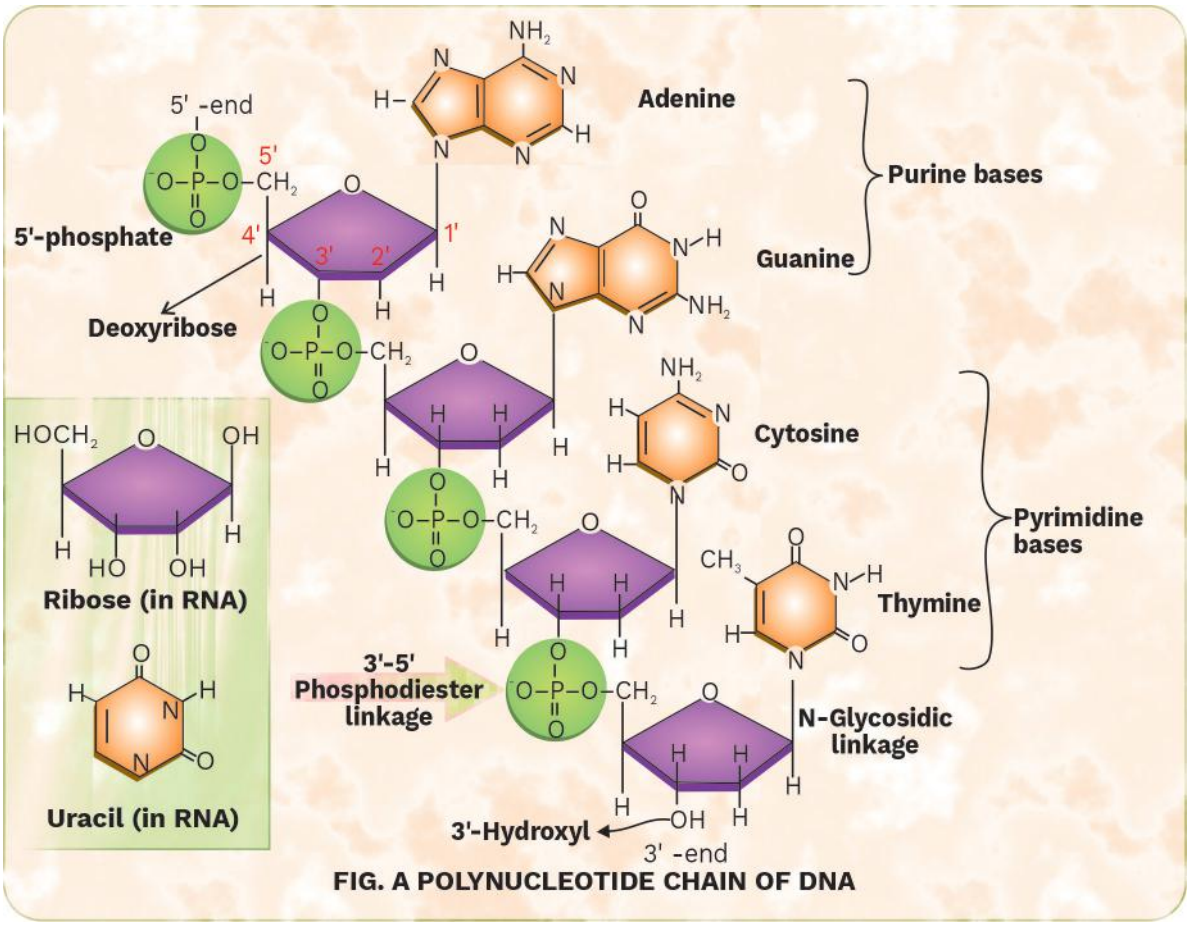


FIG. A POLYNUCLEOTIDE CHAIN OF DNA

- The pentose sugar and phosphate group (linked together by phosphodiester linkage) form the **backbone** of a polynucleotide chain, from which nitrogenous bases project out.
- DNA was first identified by **Friedrich Miescher** in 1869 in the nucleus of pus cells. He named it as '**Nuclein**'.
- **Rosalind Franklin**, along with **Maurice Wilkins**, used X-ray diffraction method to investigate the structure of DNA.
- The fibres of purified DNA were targeted by X-ray beam. The scattering pattern produced by DNA molecules revealed its nature (double stranded DNA), shape (double helix), and orientation of nitrogenous bases.

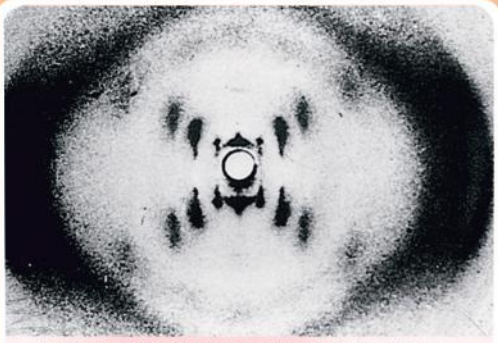


FIG. X-RAY DIFFRACTION PATTERNS PRODUCED BY DNA FIBRES-BY ROSALIND FRANKLIN AND MAURICE WILKINS

- Wilkins shared this data with Watson and Crick who used this information and proposed a molecular model of the basic structure of DNA in 1953.
- **Watson** and **Crick** along with **Wilkins** got the **Nobel prize in 1962** for finding out the double helical structure of DNA.
- **Erwin Chargaff** (1950) observed the nitrogenous bases of DNA and generalised his observations as the Chargaff's base equivalence rule.
- According to **Chargaff's rule**, for a double stranded DNA, the ratios between Adenine and Thymine, and Guanine and Cytosine are constant and equal to one.

Previous Year's Question

The term 'Nuclein' for the genetic material was used by
(1) Franklin
(2) Meischer
(3) Chargaff
(4) Mendel

Note : Chargaff's rule-

- Adenine + Guanine = Thymine + Cytosine ([A + G] = [T + C], i.e., $\frac{[A + G]}{[T + C]} = 1$)
  (Purines) (Pyrimidines)
- Molar concentration of Adenine is always equal to the molar concentration of Thymine. Similarly, molar concentration of Guanine is equal to the molar concentration of Cytosine.
  $[A] = [T]$, i.e., $\frac{[A]}{[T]} = 1$; $[G] = [C]$, i.e., $\frac{[G]}{[C]} = 1$
- Deoxyribose sugar and phosphate occur in equimolar proportions.
- A-T base pairs are rarely equal to C-G base pairs.
- The ratio of $\frac{[A + T]}{[G + C]}$ is variable but constant for a species.

- The base pairing pattern between the two polynucleotide chains showed that they are **complementary** to each other. Thus, if the sequence of bases in one strand is known then one can predict the sequence in another strand.
- It was also inferred that if each strand from a DNA (parent DNA) acts as a template for synthesis of a new daughter strand, the two double stranded DNA (daughter DNA), thus produced, would be identical to the parental DNA molecule.

Previous Year's Question

If one strand of DNA has the nitrogenous base sequence as ATCTG, what would be the complementary RNA strand sequence?
(1) TTAGU
(2) UAGAC
(3) AACTG
(4) ATCGU

The Salient Features of the Double-Helix Structure of DNA are as follows:

- DNA is made of **two polynucleotide chains**, where the backbone is made by pentose sugar-

phosphate group, and the nitrogenous bases project inside.

- DNA is **negatively charged** due to the presence of phosphate groups in its backbone.
- The nitrogenous bases in the two polynucleotide strands are paired through hydrogen bonds (H-bonds) forming base pairs (bp).
- Adenine of one strand forms two hydrogen bonds with Thymine of the opposite strand and vice-versa **(A=T)**. Guanine of one strand forms three hydrogen bonds with Cytosine of the opposite strand **(G≡C)**.
- As a result of this complementary base pairing, always a purine comes opposite to a pyrimidine. This generates approximately **uniform distance** between the two strands of the DNA double helix (DNA has a **diameter of 2 nm** or 20 Å or 2 x 10^{-9} m).

Previous Year's Question

DNA fragments are
(1) Positively charged
(2) Negatively charged
(3) Neutral
(4) Either positively or negatively charged depending on their size

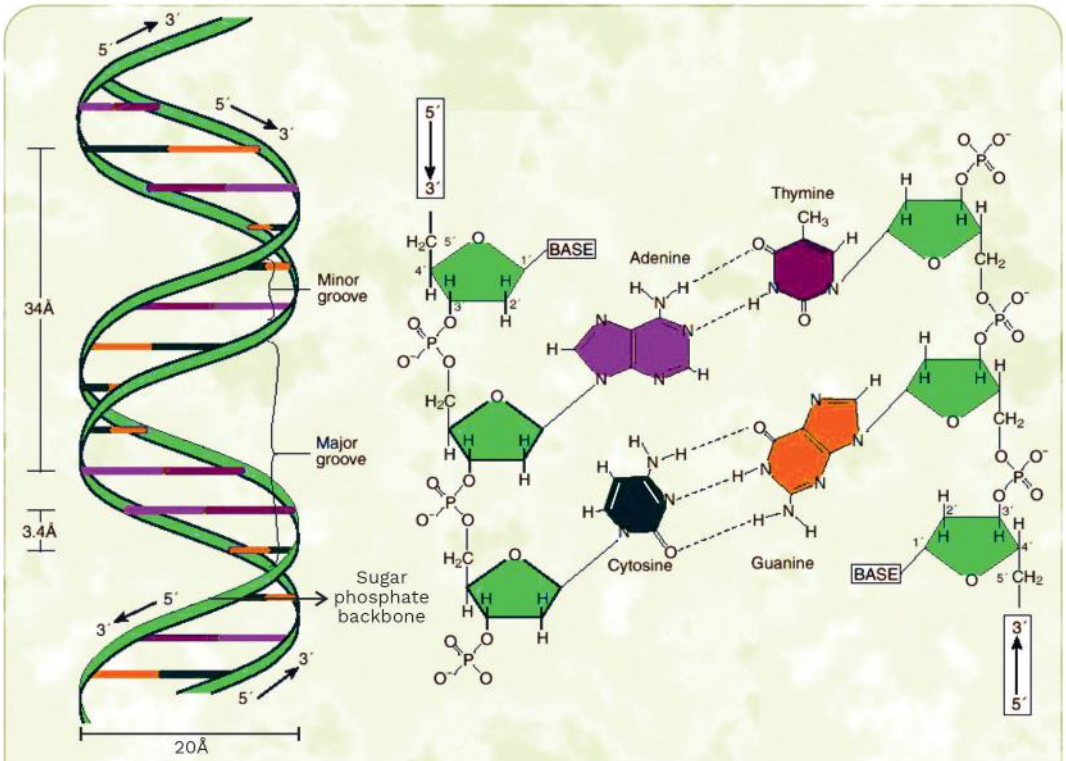


FIG. A PART OF DOUBLE STRANDED POLYNUCLEOTIDE CHAIN OF DNA SHOWING HYDROGEN BONDING BETWEEN NITROGENOUS BASES OF THE TWO STRANDS

- The two chains have anti-parallel polarity because of the pairing between purines and pyrimidines, i.e., one chain has 5'→3' polarity and the other has 3'→5' polarity.
- The two chains are coiled in a **right-handed helical** fashion. This coiling produces minor and major grooves alternately.
- The **pitch** of the DNA helix is **3.4 nm** (34 Å or 3.4 x 10^{-9} m).
- There are roughly **10 bp** in each turn.
- The distance between two adjacent base pairs in a helix is **0.34 nm** (3.4 Å or 0.34 x 10^{-9} m).
- The **plane** of one nitrogenous base pair **stacks** over the other in double helix. This, in addition to H-bonds, confers stability of the helical structure.
- Very soon, **Francis Crick** proposed the **Central dogma** in molecular biology, which states that the genetic information flows from DNA to RNA and from RNA to protein.
- The flow of information may reverse in direction, i.e., from RNA to DNA in some viruses.

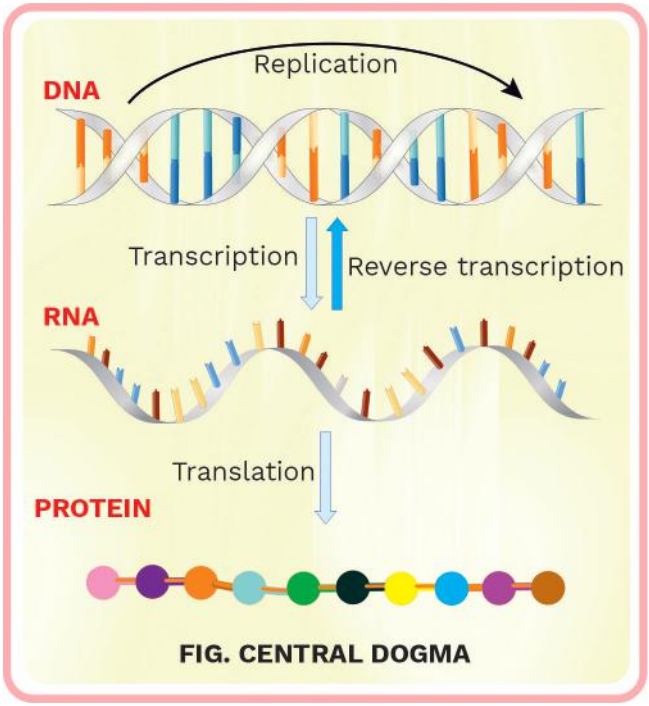


FIG. CENTRAL DOGMA

Previous Year's Question

In the double helix model of DNA, how far is each base pair from the next base pair.
(1) 3.4 nm
(2) 0.34 nm
(3) 2.0 nm
(4) 34 nm

Previous Year's Question

DNA is double helix and
(1) Right-handed complementary and parallel
(2) Right-handed complementary and antiparallel
(3) Without supercoils
(4) Always circular

Previous Year's Question

If the distance between two consecutive base pairs is 0.34 nm and the total number of base pairs of a DNA double helix in a typical mammalian cell is 6.6 x 10^9 bp, then the length of the DNA is approximately
(1) 2.5 meters
(2) 2.2 meters
(3) 2.7 meters
(4) 2.0 meters

Packaging of DNA Helix

- The length of DNA double helix in a typical mammalian cell is approximately **2.2 metres** (the total number of bp, i.e., 6.6×10^9 bp multiplied by the distance between two consecutive bp, i.e., 0.34×10^{-9} m/bp).
- **DNA packaging in Prokaryotes:**
  - In prokaryotes, due to the absence of a defined nucleus, the DNA is present in the cytoplasm.
  - The prokaryotic DNA is not scattered throughout the cytoplasm, but it is supercoiled with the help of RNAs and some positively charged proteins (non-histone proteins like polyamines) to form a compact mass called **nucleoid**.
- **DNA packaging in Eukaryotes:**
  - In eukaryotes, the negativity charged DNA is coiled by a set of positively charged basic proteins called **histones**.
  - Histones are rich in the basic amino acid residues **lysine** and **arginine**.
  - Eight histone proteins (two H2A, two H2B, two H3, and two H4) are organised to form a unit called **histone octamer**.
  - About **200 bp** of the negatively charged DNA is wrapped around the positively charged histone octamer to form a structure called **nucleosome** (about 11 x 6 nm in size).
  - The DNA connecting two adjacent nucleosomes is called the **linker DNA** (about 145 Å with 70 bp). It bears H1 histone protein.
  - Nucleosome chain is seen as **'beads-on-string'** structure when viewed under the electron microscope (EM).

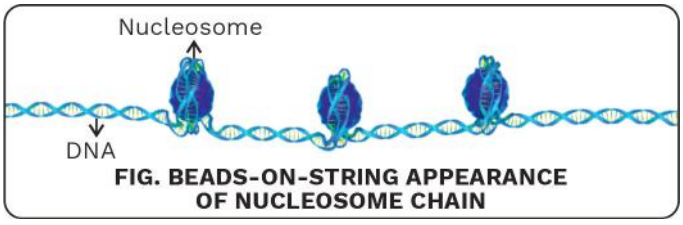


FIG. BEADS-ON-STRING APPEARANCE OF NUCLEOSOME CHAIN

Previous Year's Question

What are those structures that appear as 'beads-on-string' in the chromosomes when viewed under the electron microscope?

(1) Nucleotides
(2) Nucleosomes
(3) Base pairs
(4) Genes

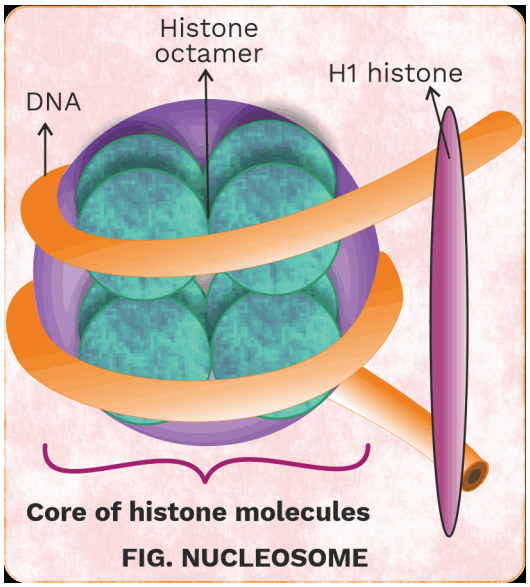


FIG. NUCLEOSOME

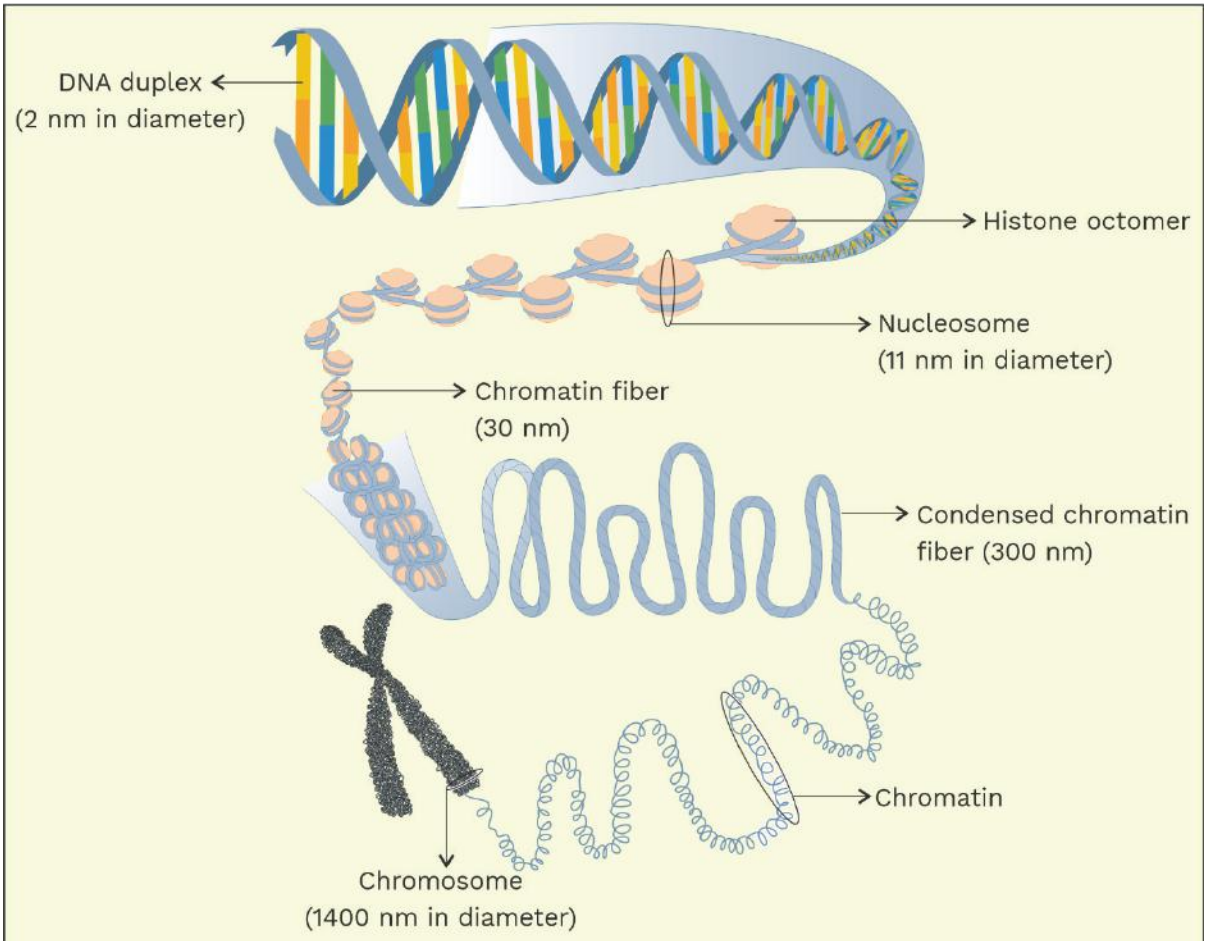


FIG. PACKAGING OF DNA HELIX

- The nucleosome chain in the nucleus is further coiled by many **Non-Histone Chromosomal proteins** (NHC proteins) to form condensed, thread-like, stained (coloured) body called **chromatin**.
- The chromatin is packaged or condensed to form **chromatin fibers** that are further coiled and condensed at metaphase stage of cell division to form **chromosomes**.
- At some regions, chromatin is loosely packed and are called **euchromatin**. Because of the loose packaging, euchromatin is lightly stained. It is transcriptionally active chromatin.

Previous Year's Question

The association of histone H1 with a nucleosome indicates
(1) Transcription is occuring
(2) DNA replication is occuring
(3) The DNA is condensed into chromatin fiber
(4) The DNA double helix is exposed

- The densely packed chromatin is called **heterochromatin** and it stains dark. It is transcriptionally inactive and late replicating due to its dense packaging.

THE SEARCH FOR GENETIC MATERIAL

- Previous discoveries by Gregor Mendel, Walter Sutton, Thomas Hunt Morgan, and numerous other scientists had narrowed the search for the genetic material to the chromosomes located in the nucleus of most cells.
- Chromosomes, which are responsible for heredity, are chemically made of proteins and nucleic acids.
- Earlier, proteins were considered as the most probable genetic material. But various experiments gave evidences in favour of DNA as the genetic material.

Transforming Principle
Transformation (Griffith Effect)

- In 1928, **Frederick Griffith**, worked on different strains of *Streptococcus pneumoniae*, also known as *Diplococcus pneumoniae* or *Pneumococcus pneumoniae*.
- One of the bacterial strains produced **smooth shiny** colonies when grown on a culture plate (**S strain** bacteria) and were **encapsulated** by a **mucous** (polysaccharide) **coat**. This mucilaginous polysaccharide sheath made them **virulent** (pneumonia causing) and protected the bacteria from phagocytes of the host.
- The other bacterial strain produces **rough** colonies when grown on a culture plate (**R strain** bacteria) as they are **devoid** of the **mucous coat**. They are **non-virulent** (do not cause pneumonia).
- Griffith tested the virulence of the two strains of *Streptococcus* by injecting live R strain and live S strain bacteria separately into mice.
- Mice infected with the S strain (virulent) die from pneumonia infection, but mice infected with the R strain do not develop pneumonia.

Previous Year's Question

The length of DNA molecule greatly exceeds the dimensions of the nucleus in eukaryotic cells. How is the DNA accommodated?

(1) Deletion of non-essential genes
(2) Supercoiling in nucleosomes
(3) DNAase digestion
(4) Through elimination of repetitive DNA

Previous Year's Question

Transformation was discovered by

(1) Meselson and Stahl
(2) Hershey and Chase
(3) Griffith
(4) Watson and Crick

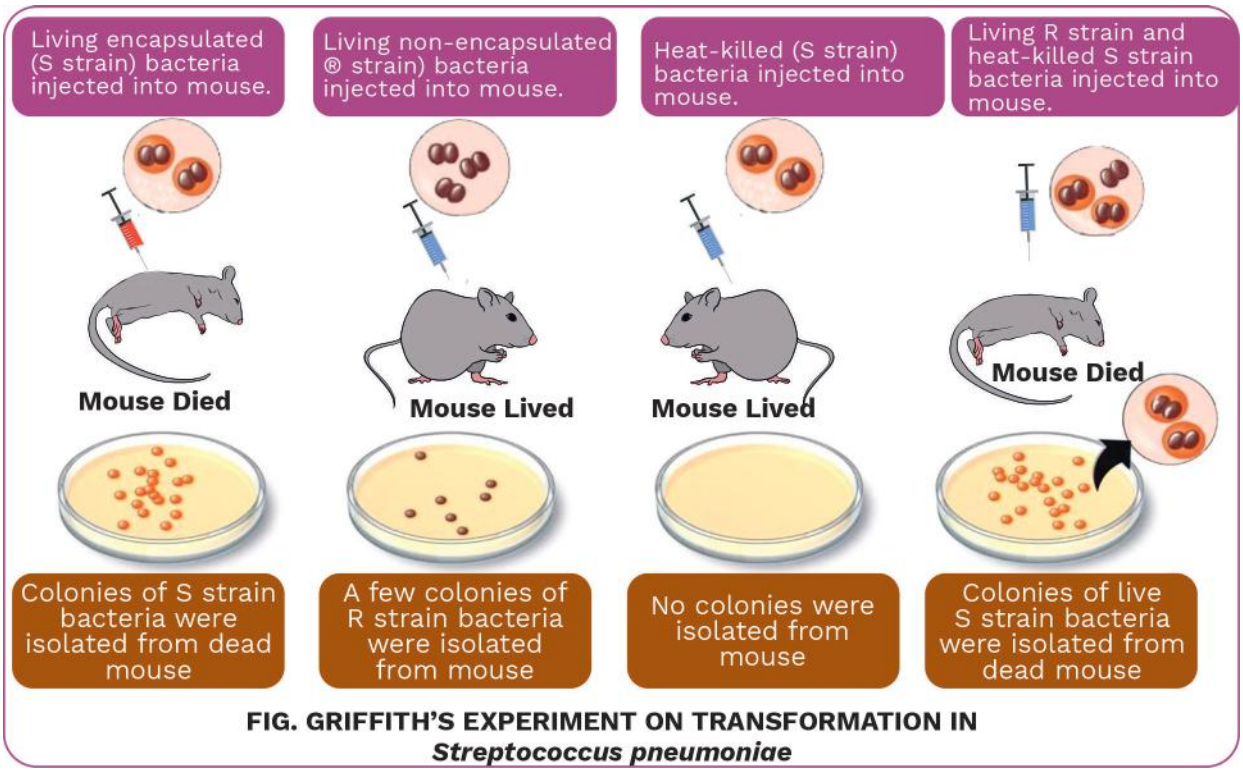


FIG. GRIFFITH'S EXPERIMENT ON TRANSFORMATION IN *Streptococcus pneumoniae*

- When Griffith injected heat-killed S strain bacteria into the mice, they did not produce any symptoms of pneumonia and **remained alive**.
- When he injected a mixture of heat-killed S strain bacteria and live R strain bacteria, the **mice died**.
- The autopsy of these dead mice showed the presence of live S strain bacteria and live R strain bacteria.
- He concluded that the presence of live S strain virulent bacteria is possible only by their formation from live R strain (non-virulent) bacteria which, somehow, picked up a transforming material having virulence trait from the dead S strain bacteria.
- This process is called **transformation** or **Griffith effect**. According to Griffith, some '**transforming principle'**, released from the heat-killed S strain bacteria, had enabled the R strain bacteria to synthesise a smooth polysaccharide mucous coat and become virulent.

Rack your Brain

Why the Griffith's transformation experiment work was not fully accepted by other scientists?

- Since this was a permanent genetic change, the most possible inference was the transfer of the genetic material occurred from the dead S strain bacteria to live R strain bacteria.
- However, it was not proven whether mice were essential for transformation and which biochemical component of S strain bacteria (carbohydrates, proteins, or nucleic acids) behaves as the genetic material.

Biochemical Characterisation of Transforming Principle

- **Oswald Avery**, **Colin MacLeod** and **Maclyn McCarty** (1933-44) worked to determine the biochemical nature of the 'transforming principle' in Griffith's experiment.
- They purified biochemicals (proteins, DNA, RNA, etc.) from the heat-killed S strain bacteria (*Streptococcus pneumoniae*).
- They added enzymes like protein-digesting enzymes (**proteases**), DNA-digesting enzymes (**DNases**), RNA-digesting enzymes (**RNases**), etc., to cultures of heat killed S strain bacteria kept in separate test tubes.
- Live R strain bacteria were then added to all the test tubes.
- They discovered that addition of proteases and RNases did not affect the transformation as live S strain bacteria were found. This proved that the transforming substance was neither a protein nor RNA.
- Addition of DNases showed no live S strain bacteria in the culture, thus inhibiting transformation. This suggested that **DNA** carries the character of virulence and causes the transformation.
- They concluded that DNA is the hereditary material, but not all biologists were convinced.

Definition

Transformation: The process of uptake and incorporation of exogenous DNA by a cell from its environment causing change in its phenotype.

Rack your Brain

Name the experimental organism used in determining the biochemical characterisation of Griffith's transforming principle.

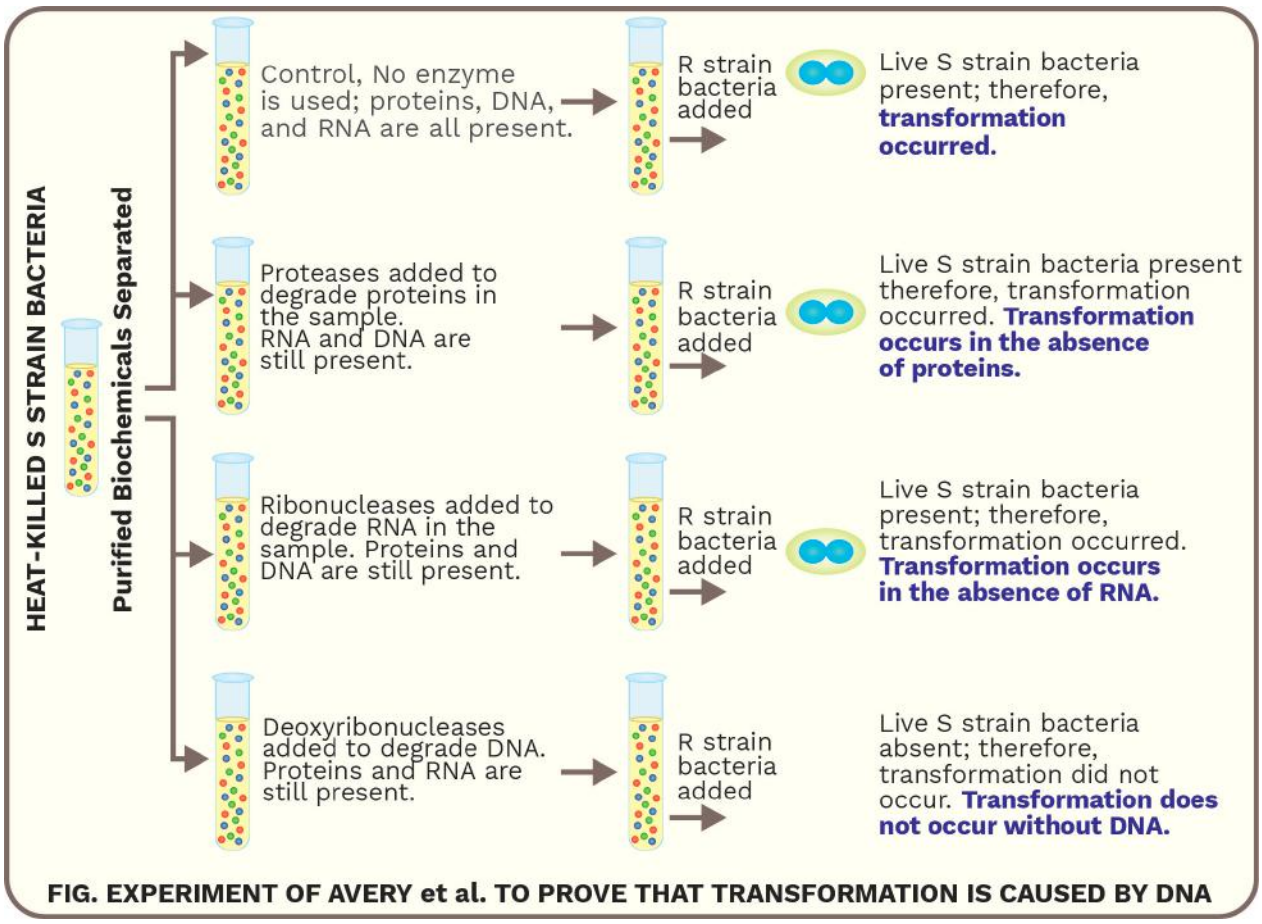


FIG. EXPERIMENT OF AVERY et al. TO PROVE THAT TRANSFORMATION IS CAUSED BY DNA

DNA — The Genetic Material

- **Alfred Hershey** and **Martha Chase** (1952) worked with **T_2 bacteriophage** viruses that infect *Escherichia coli* bacteria to prove that DNA is the genetic material.
- It was already known that the bacteriophage attaches to the bacteria to deliver its genetic material into the bacterial cell.
- The bacteria treat the viral genetic material as their own and thus, produce more virus particles.
- Hershey and Chase worked to discover whether it was the protein or DNA from the viruses that behaved as the genetic material and entered the bacteria.

Previous Year's Question

The final proof for DNA as the genetic material come from the experiments of

(1) Griffith
(2) Hershey and Chase
(3) Avery, MacLeod and McCarty
(4) Hargobind Khorana

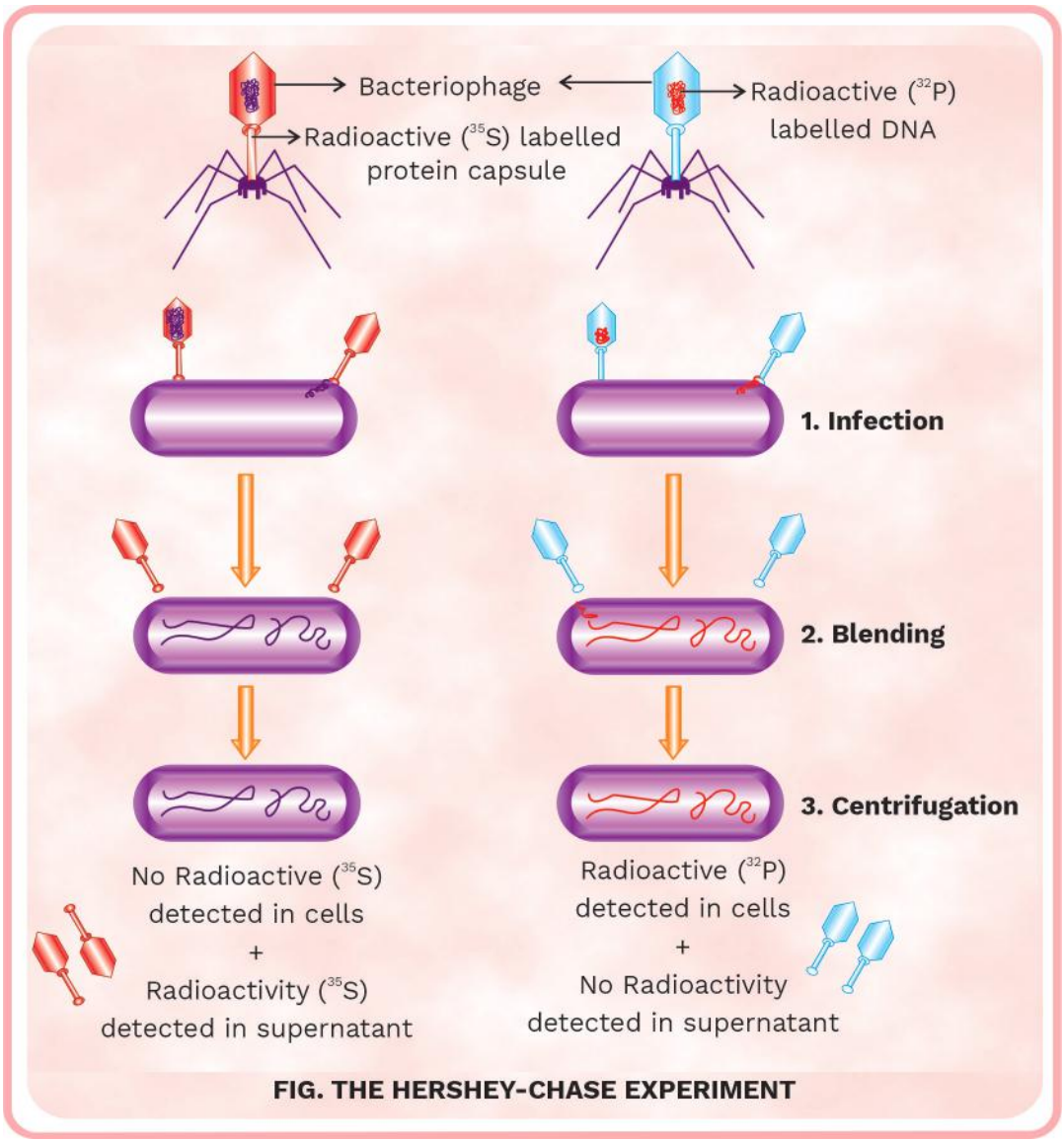


FIG. THE HERSHEY-CHASE EXPERIMENT

- They grew two cultures of *E. coli*. In one culture, radioactive sulphur, **^{35}S**, was added, whereas the other culture was supplied with radioactive phosphorus, **^{32}P**.
- ^{35}S got incorporated into the sulphur containing **amino acids**, and thus, became a part of bacterial proteins.
- ^{32}P got incorporated into **nucleotides** of DNA and RNA of the bacteria in the culture.

Gray Matter Alert!!!

Cysteine and Methionine are sulphur-containing amino acids.

- Thus, bacteria of both the cultures became radioactively labelled.
- Hershey and Chase then introduced T_2 bacteriophages to both the bacterial cultures, which was followed by multiplication of the viruses in the bacteria.
- Virus progeny from both the cultures were tested.
- The viruses were found to be radioactive. Viruses grown in the presence of ^{32}P contained radioactive DNA but not radioactive protein (as DNA contains phosphorus but protein does not).
- Viruses grown on ^{35}S contained radioactive protein but not radioactive DNA (as DNA does not contain sulphur).
- These radioactive T_2 bacteriophages were then introduced in two separate cultures of normal or unlabelled *E.coli*.
- Just after the viral infection, the two cultures were agitated in a blender to remove the virus coats from the bacteria.
- The cultures were then centrifuged which separated the virus particles from the bacteria.
- The heavier bacteria (infected with the bacteriophage) settled down as pellet, whereas the lighter viral particles formed the supernatant.
- On analysis of the pellet and the supernatant, it was observed that bacteria which was infected with bacteriophages that had radioactive DNA were radioactive, whereas bacteria that were infected with bacteriophages that had radioactive proteins were not radioactive.
- This indicated that DNA was passed from the virus to the bacteria and thus, **DNA** is the **genetic material** and not protein.

Properties of Genetic Material (DNA versus RNA)

- A biomolecule must show some specific characteristics to become a genetic material.
- Both the nucleic acids (DNA and RNA) can direct their replication unlike other biomolecules in a cell.

Previous Year's Question

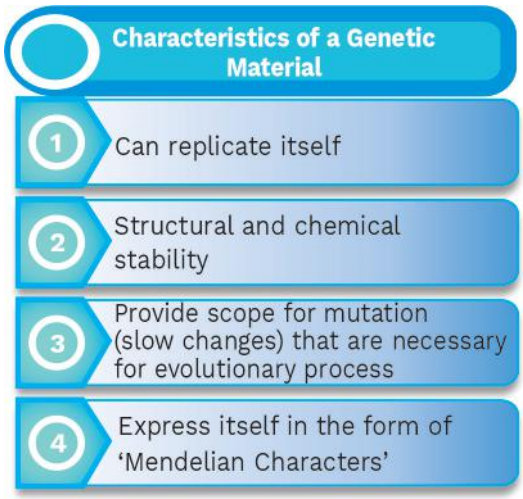
Which scientist experimentally proved that DNA is the sole genetic material in bacteriophage?

(1) Beadle and Tatum
(2) Meselson and Stahl
(3) Hershey and Chase
(4) Jacob and Monod

Previous Year's Question

Which one of the following is not applicable to RNA?

(1) 5' phosphoryl and 3' hydroxyl ends
(2) Heterocyclic nitrogenous bases
(3) Chargaff's rule
(4) Complementary base pairing



- **RNA is not a very suitable genetic material because:**
  - The ribose sugar of every nucleotide in RNA consists of a reactive **2'-OH** (hydroxyl group). This makes RNA highly reactive, labile, and easily degradable.
  - RNA also functions as an **enzyme** in ribosomes, thus making it unstable and reactive. This shows that the protein synthesising machinery has evolved around RNA.
  - **Uracil** present in RNA is less stable than thymine of DNA.
  - Being **single stranded,** the nitrogenous bases in RNA can easily bind with other molecules by forming H-bonds, again showing unstable and reactive nature.
  - Being unstable, RNA mutates at a faster rate. Such fast changes are harmful in higher life forms.
- **DNA is the genetic material in most of the organisms because:**
  - DNA is structurally more stable and chemically less reactive.
  - DNA does not have a reactive hydroxyl group at its 2' carbon (**2'-OH absent**).
  - H-bonding between purines of one strand of DNA and the pyrimidines of the other strand

Previous Year's Question

A molecule that can act as a genetic material must fulfill the traits given below, except

(1) It should be able to express itself in the form of 'Mendelian Characters'

(2) It should be able to generate its replica

(3) It should be unstable structurally and chemically

(4) It should provide the scope for slow changes that are required for evolution

Previous Year's Question

Which one of the following is not applicable to RNA?

(1) Complementary base pairing

(2) 5' phosphoryl and 3' hydroxyl ends

(3) Heterocyclic nitrogenous bases

(4) Chargaff's rule

of DNA and their stacking makes DNA a more stable molecule.

- Presence of **thymine** (**5-methyl uracil**) in DNA instead of uracil provides further stability to it.
- The two strands of DNA being complementary, if separated by heating (**denaturation**), rejoin (**renaturation**), when appropriate conditions are provided.
- DNA can undergo **slow mutations**.
- DNA has the ability to **repair** itself.

- Therefore, DNA is chemically less reactive and structurally more stable as compared to RNA. Hence, among the two nucleic acids, the DNA is preferred as a genetic material, whereas for the transmission of genetic information, RNA is better.

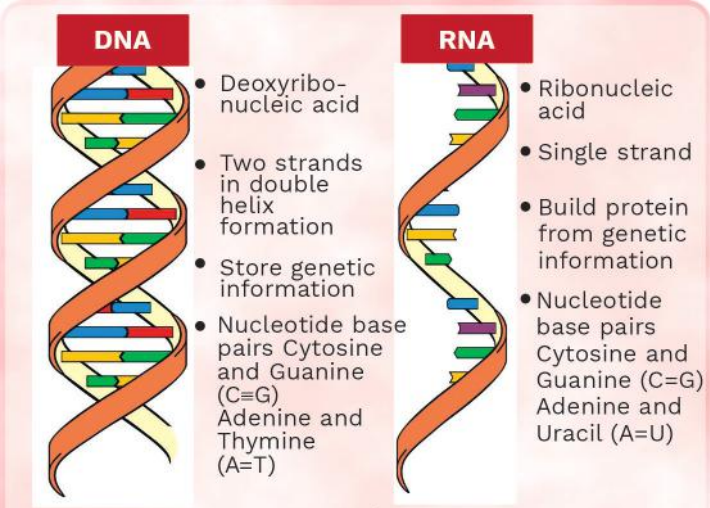


FIG. DNA v/s RNA

RNA WORLD

- RNA was the first genetic material. Metabolism, translation, splicing, etc.), evolved around RNA.
- RNA used to act as a biocatalyst. Ribozymes are enzymes made of RNAs and protein which are used in protein synthesis (translation).
- As the Earth's environment became stable, the reactive and unstable RNA was replaced by a

Previous Year's Question

DNA is methylated at
(1) A-residue
(2) G-residue
(3) T-residue
(4) C-residue

Previous Year's Question

Purines of DNA are represented by
(1) Uracil and Thymine
(2) Guanine and Adenine
(3) Uracil and Cytosine
(4) Thymine and Cytosine

more stable DNA. Chemical modifications in RNA like double stranded nature, replacing 2' –OH with 2' -H, replacing uracil with thymine made it more stable. Therefore, DNA has evolved from RNA.

REPLICATION

- Watson and Crick proposed that the DNA replication is **semiconservative** in nature.
- According to the semiconservative scheme of DNA replication, one strand of the daughter DNA duplex is derived from the parent DNA while the other strand is newly formed.
- This occurs as the two parent DNA strands separate and act as a template for the synthesis of the two new complementary strands.
- Each newly formed DNA molecule has one parental and one newly synthesised strand.

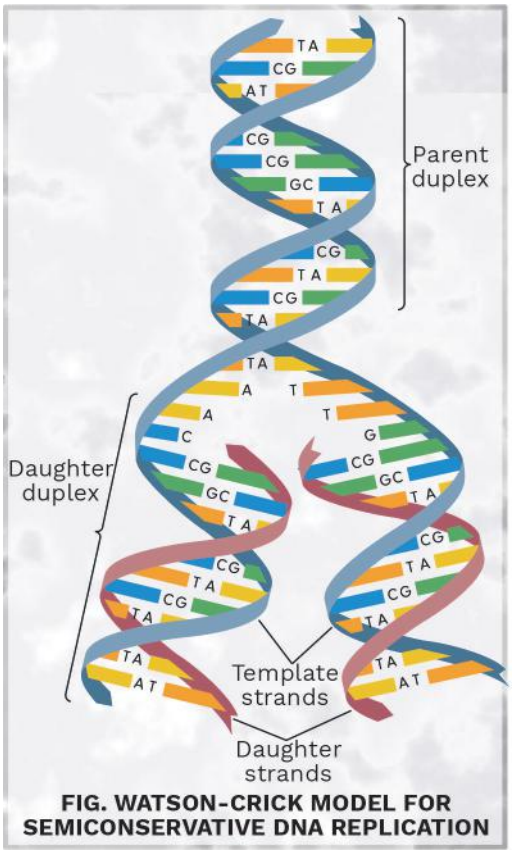


FIG. WATSON-CRICK MODEL FOR SEMICONSERVATIVE DNA REPLICATION

Previous Year's Question

True replication of DNA is possible due to

(1) Hydrogen bonding
(2) Phosphate backbone
(3) Complementary base pairing rule
(4) None of the above

Previous Year's Question

Semiconservative model of DNA replication was proposed by which workers in eukaryotes?

(1) Taylor, Woods and Hughes, 1957
(2) Meselson and Stahl, 1957
(3) Nirenberg and Khorana, 1967
(4) Watson and Crick, 1952

The Experimental Proof of Semiconservative Replication of DNA

- **Matthew Meselson** and **Franklin Stahl** proved the semiconservative replication of DNA in **1958**.
- They grew *Escherichia coli* for many generations in a medium containing **$^{15}NH_4Cl$** (^{15}N is the heavy isotope of nitrogen) to completely label the bacterial DNA with the heavy isotope.
- A heavy **$^{15}N^{15}N$** band of bacteria was obtained by centrifugation in a **cesium chloride** (CsCl) density gradient centrifugation.

Previous Year's Question

The experimental proof for semiconservative replication of DNA was first shown in a
(1) Fungus
(2) Bacterium
(3) Plant
(4) Virus

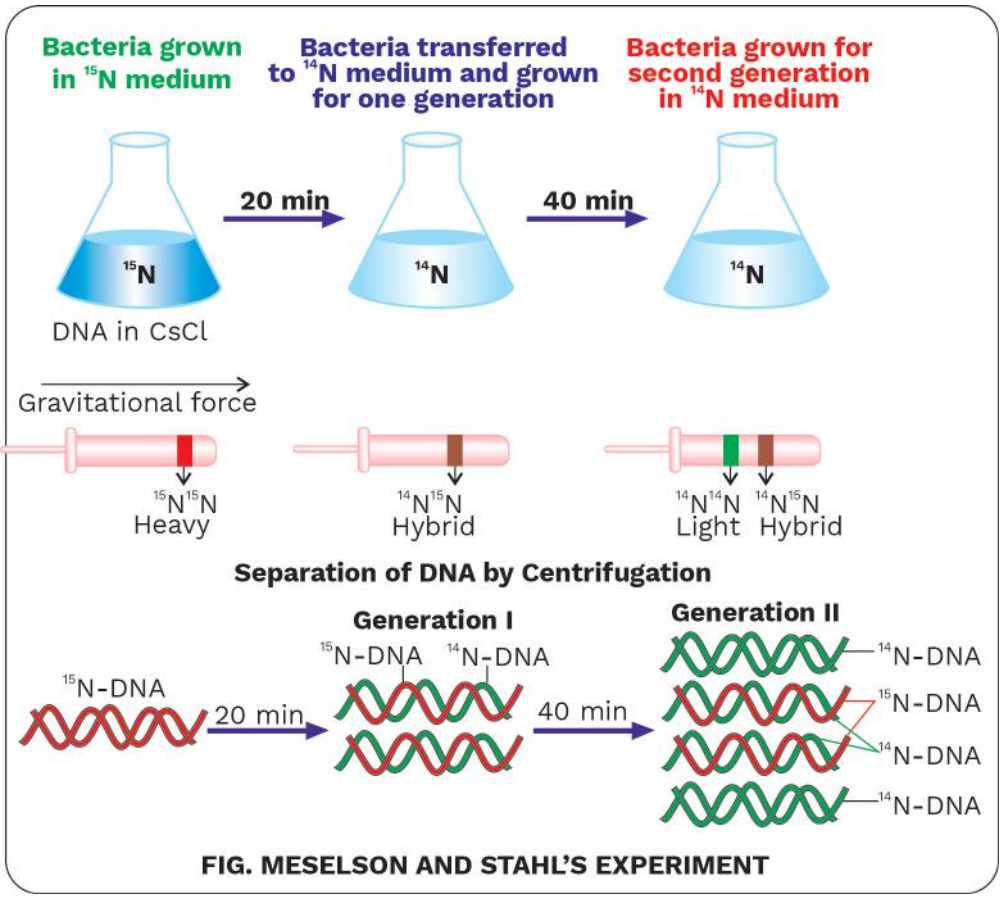


FIG. MESELSON AND STAHL'S EXPERIMENT

- The labelled bacteria were then transferred to a new medium having normal **$^{14}NH_4Cl$** (^{14}N is the normal nitrogen).
- Samples were taken after each generation, i.e., after every 20 minutes (*E.coli* divides in

20 minutes) and the DNA was again tested for the heavy isotope of nitrogen by centrifugation in a cesium chloride (CsCl) density gradient.

- It was observed that DNA of the first generation was hybrid or intermediate (**$^{14}N^{15}N$**), i.e., the daughter DNA had one strand having ^{14}N and the other strand having ^{15}N because the band settled in cesium chloride solution above the fully labelled DNA of the parent bacteria ($^{15}N^{15}N$).
- The DNA extracted from the culture after 40 minutes, i.e., second generation had two types of DNA, **50% light** (**$^{14}N^{14}N$**) and **50%** intermediate or **hybrid** ($^{14}N^{15}N$).
- This was possible only when the two strands of parental DNA duplex with ^{15}N separate at the time of replication and behave as a template for the synthesis of new complementary strands of DNA with normal nitrogen (^{14}N).
- It was hence proved that at each replication, one strand of parent DNA is conserved in the daughter DNA while the second is freshly synthesised (semiconservative replication).
- **Taylor** and colleagues (1958) grew cells of the root tips of *Vicia faba* (faba beans) in **radioactive ^{3}H** containing thymine medium. The radioactive thymine got incorporated in the DNA of the cells.
- It was found that all the chromosomes in the cells showed radioactivity.
- When the labelled thymine was replaced by normal thymine in the medium, the next generation cells showed radioactivity in one of the two chromatids of each chromosome while in the next generation, the radioactivity was observed only in 50% of the chromosomes.
- This experiment also proved that out of the two strands of a chromosome, one is formed afresh and the other is conserved during replication, i.e., the DNA in chromosomes also replicate semiconservatively.

Previous Year's Question

Semiconservative replication of DNA was first demonstrated in
(1) *Drosophila melanogaster*
(2) *Escherichia coli*
(3) *Streptococcus pneumoniae*
(4) *Salmonella typhimurium*

Previous Year's Question

Taylor conducted the experiments to prove semiconservative mode of chromosome replication on
(1) *Vinca rosea*
(2) *Vicia faba*
(3) *Drosophila melanogaster*
(4) *E.coli*

Previous Year's Question

E.coli has only 4.6 x 10^6 base pairs and completes the process of replication within 18 minutes; then the average rate of polymerisation is approximately
(1) 2000 base pairs/second
(2) 3000 base pairs/second
(3) 4000 base pair/second
(4) 1000 base pairs/second

The Machinery and the Enzymes of DNA Replication

- **Origin of Replication (ori):**
  - It is the site on DNA where the replication starts. Prokaryotic and viral DNA has a single ori which functions as a single replicating unit (replicon).
  - Eukaryotic linear DNA has many ori sites (multirepliconic) to facilitate opening of such long DNA at multiple sites. Replication will not occur in the absence of ori.
- **DNA dependent DNA polymerase:**
  - It catalyses the polymerisation of deoxyribonucleotides to form the daughter strands.
  - It is mostly very accurate and rapid. For example, *E. coli* having 4.6 $\times 10^6$ bp completes the process of replication within 18 minutes, i.e., approximately 2000 bp per second.

NOTE: Prokaryotic DNA Polymerases add deoxyribonucleotides in 5'→3' direction on 3'→5' DNA template strand. They also show 3'→5' exonuclease activity. They are of three types:

- DNA Polymerase I – Major repair enzyme. It also has 5'→3' exonuclease activity.
- DNA Polymerase II – Minor repair enzyme.
- DNA Polymerase III – Main enzyme involved in polymerisation of deoxyribonucleotides

Eukaryotic cell consists of five types of DNA Polymerases, i.e., α, β, γ, δ and ε.

- **Deoxyribonucleoside triphosphates (dNTPs):**
  - dNTPs are added to the daughter strands based on the complementary sequence on the parent template DNA strand.
  - Since DNA replication is energetically very expensive, the dNTPs provide the energy for replication by breakdown of two terminal phosphates (pyrophosphates) from them.
- **Helicase:**
  - It unwinds the two strands of parent DNA. For this, it destroys the H-bonds between the base pairs.

Previous Year's Question

Name the enzyme that facilitates opening of DNA helix during replication.

(1) DNA helicase
(2) DNA polymerase
(3) RNA polymerase
(4) DNA ligase

Previous Year's Question

DNA replication is aided by

(1) DNA polymerase only
(2) DNA ligase only
(3) Both DNA polymerase and ligase
(4) RNA polymerase

- **Single Stranded Binding Proteins (SSBPs):**
  - They stabilise the separated parent DNA strands now called the template strands.
- **Topoisomerases:**
  - They release the tension in the parent DNA created due to the uncoiling of DNA at the ori by nicking off and resealing the DNA strand.
- **Primase:**
  - Primase is a DNA-dependent RNA polymerase enzyme which synthesises a small strand of RNA called RNA primer at the 5' -end of the new DNA strand.
  - DNA polymerase can add the dNTPs only in the presence of an RNA primer.
- **DNA ligase:**
  - DNA ligase join the Okazaki fragments of the discontinuous DNA strand.

Previous Year's Question

DNA replication in bacteria occurs
(1) Within nucleolus
(2) Prior to fission
(3) Just before transcription
(4) During S phase

Mechanism of DNA Replication

- DNA replication occurs during the S-phase of the cell cycle and consists of the following steps:
- **Activation of deoxyribonucleotides:**
  - Firstly, the deoxyribonucleoside monophosphates (dAMP, dGMP, dTMP, and dCMP) occurring in the cell are phosphorylated by the enzyme **phosphorylase**, converting them into their activated form (dATP, dGTP, dTTP, and dCTP).
- **Unwinding of the DNA:**
  - The enzyme helicase unwinds the parent DNA duplex at the site of ori.
  - The two separated parental DNA strands tend to join again by forming H-bonds. This is avoided by Single Stranded Binding Proteins (SSBPs) which attach to the two parent template strands.
  - Topoisomerases release any tension created in the parent DNA due to its uncoiling.
  - The point of separation of parent DNA slowly proceeds towards opposite directions (**bidirectional replication**), thus giving rise to

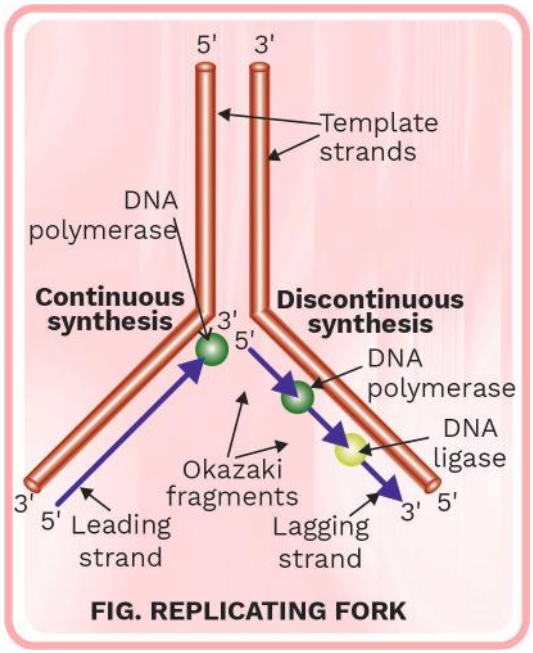


FIG. REPLICATING FORK

Y-shaped structure called the replication fork.

- Since the two DNA strands are antiparallel, one DNA template strand has 5'→3' polarity, while the other strand has 3'→5' polarity.

- **Formation of RNA primer:**
  - RNA primers are formed by the enzyme primase at the 5' -end of the new daughter DNA strand formed.
- **Polymerisation of the daughter DNA strands and base pairing:**
  - The DNA-dependent DNA polymerases start polymerisation of the dNTPs on the primers in 5'→3' direction.

Previous Year's Question

During DNA replication, the addition of nucleotides on the lagging strand occurs

(1) Towards the replication fork
(2) At a faster rate than leading strand
(3) Continuously
(4) Discontinuously

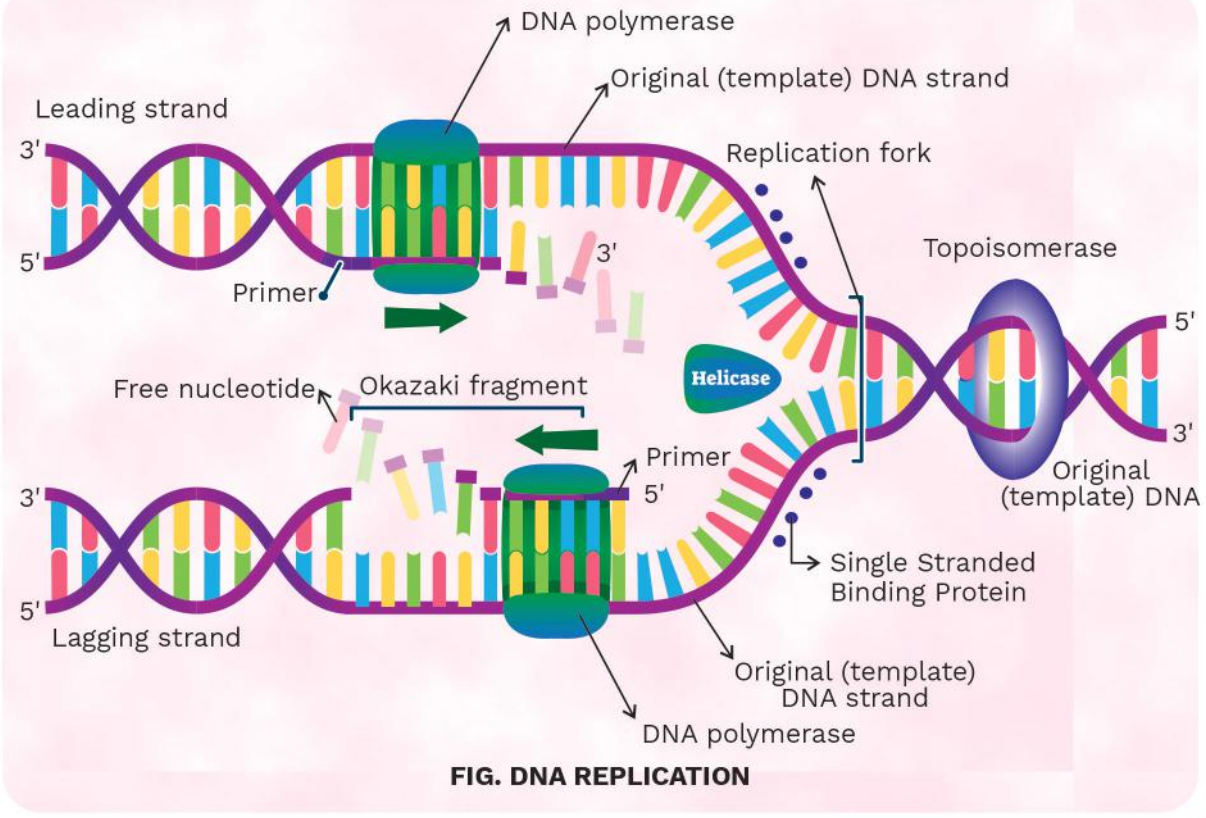


FIG. DNA REPLICATION

- The dNTPs come to lie opposite the nitrogenous bases exposed on the template strands according to complementary base pairing rule (dATP opposite Thymine, dTTP opposite adenine, dGTP opposite cytosine and dCTP opposite guanine).
- The terminal two phosphates (pyrophosphates, PPi) separate from the dNTPs followed by the hydrolysis of pyrophosphates to release energy.
- This energy is utilised for establishing H-bonds between the nitrogenous bases of the template strand and the daughter strand.
- Simultaneously, the deoxyribonucleoside monophosphates of the daughter strand establish phosphodiester linkages to form the replicated (daughter) DNA strand.

• **Daughter DNA strand Elongation:**
- Replication over the two antiparallel parent or template DNA strands proceed in opposite directions because DNA polymerase can polymerise nucleotides only in 5'→3' direction on 3'→5' strand as it adds them at the 3' -end.
- On one parent strand with 3'→5' polarity, the new complementary strand is formed continuously in 5'→3' direction towards the replication fork as its 3' end is always open for elongation. This is called leading strand.
- On the other parent strand with 5'→3' polarity, the new complementary strand is initially formed as short segments of DNA strand in 5'→3' direction away from the replication fork due to exposure of a small stretch of the template strand at a time. These short segments, each having an RNA primer at their 5' -end, are called **Okazaki fragments** (**discontinuous synthesis**).
- The primers are later removed, and the gap is filled with complementary nucleotides by **DNA polymerase I**.

Previous Year's Question

The Okazaki fragments in DNA chain growth
(1) Result in transcription
(2) Polymerize in the 3'→5' direction and forms replication fork
(3) Prove semiconservative nature of DNA replication
(4) Polymerize in the 5'→3' direction and explain 3'→5' DNA replication

Gray Matter Alert!!!

Each Okazaki fragment has about 1000-2000 bp in prokaryotes and about 100-200 bp in eukaryotes.

Previous Year's Question

During DNA replication, Okazaki fragments are used to elongate
(1) The leading strand towards replication fork
(2) The lagging strand towards replication fork
(3) The leading strand away from replication fork
(4) The lagging strand away from replication fork

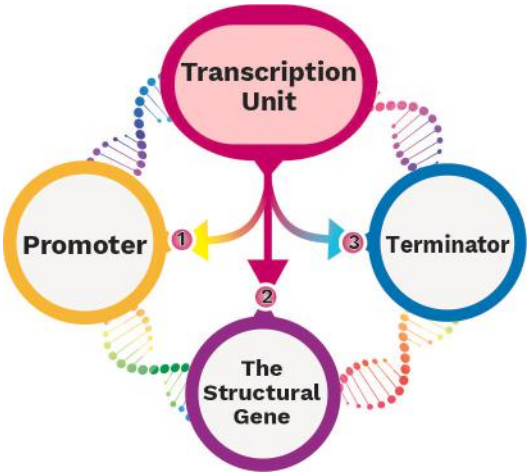
- The Okazaki fragments are joined together by **DNA ligase** to form the daughter DNA strand, also called the **lagging strand**.

TRANSCRIPTION

- In transcription, the coded information on a DNA segment (gene) is copied to mRNA by following the principle of complementarity, except the **adenosine** of the DNA template forms base pair with **uracil** instead of thymine and **only one** strand (**template**) of the DNA segment is transcribed (unlike in replication).
- If both strands of DNA act as a template, they would code for RNA molecule with different sequences, and in turn, if they code for proteins, the sequence of amino acids in the proteins would be different.
- Also, the two RNA molecules if produced simultaneously would be complementary to each other, hence would form a double stranded RNA which would prevent RNA from being translated into protein.

Transcription Unit

- The segment of DNA that takes part in transcription is called transcription unit.
- A transcription unit has three regions: a promoter, the structural gene, a terminator.



Definition

Transcription: The process of copying genetic information from one strand of the DNA into RNA is termed as transcription.

Rack your Brain

Why both strands of a DNA segment are not transcribed into an mRNA?

Previous Year's Question

Which one of the following is not a part of a transcription unit in DNA?

(1) The inducer
(2) A terminator
(3) A promoter
(4) The structural gene

Previous Year's Question

Choose the correct pair from the following.

(1) Polymerases-Break the DNA into fragments
(2) Nucleases-Separate the two strands of DNA
(3) Exonucleases-Make cuts at specific positions within DNA
(4) Ligases-Join the two DNA molecules

- The two strands of the DNA in the structural gene of a transcription unit are treated differently.
- Since, DNA-dependent RNA polymerase catalyses the polymerisation of ribonucleotides (NTPs) in 5'→3' direction, the DNA strand having polarity 3'→5' acts as a template and therefore, it is called the **template strand.**
- The DNA strand having polarity 5'→3' has similar nucleotide sequence as the RNA transcript (except thymine at the place of uracil) is called the coding strand but is not involved in transcription.
- All the reference point while defining a transcription unit is made with **coding strand**.

Previous Year's Question

DNA-dependent RNA polymerase catalyses transcription on one strand of the DNA which is called the
(1) Template strand
(2) Coding strand
(3) alpha strand
(4) anti strand

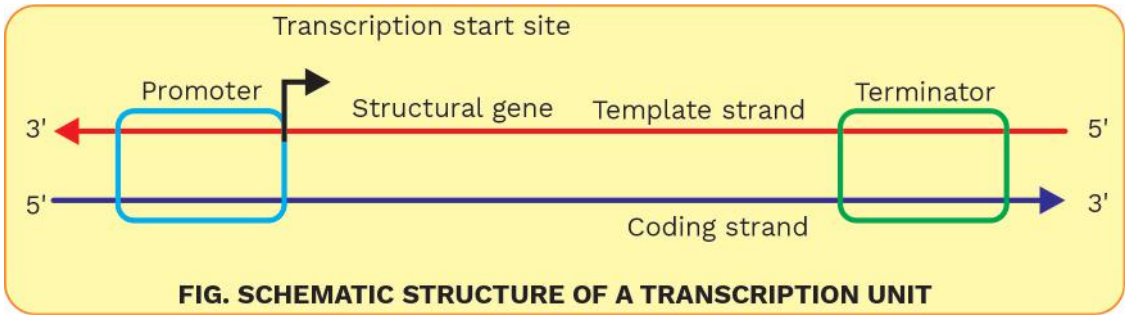


FIG. SCHEMATIC STRUCTURE OF A TRANSCRIPTION UNIT

- The promoter and terminator flank the ends of the structural gene in a transcription unit.
- The promoter is located towards 5' -end (upstream) of the structural gene (the reference is made with respect to the polarity of coding strand).
- It provides binding site for RNA polymerase, and its presence defines the template and coding strands.
- The terminator is present towards 3' -end (downstream) of the coding strand and it usually defines the end of the process of transcription.

Transcription Unit and the Gene

- Johannsen, in 1909, defined a gene as an elementary unit of inheritance which can be assigned a particular character.
- A functional segment of DNA is called as a gene. Such a functional DNA segment may carry

Previous Year's Question

What will be the sequence of mRNA produced by the following stretch of DNA?
3' ATGCATGCATGCATG 5'
TEMPLATE STRAND
5' TACGTACGTACGTAC 3' CODING STRAND
(1) 3' AUGCAUGCAUGCAUG 5'
(2) 5' UACGUACGUACGUAC 3'
(3) 3' UACGUACGUACGUAC 5'
(4) 5' AUGCAUGCAUGCAUG 3'

information to form an mRNA, tRNA, rRNA or a protein.

- A **cistron** is a segment of DNA that codes for one polypeptide chain and consists of both the structural (coding) sequences and regulatory sequences (promoter and terminator).
- Mostly in **eukaryotes**, the structural gene in a transcription unit is **monocistronic**, i.e., carrying information for the formation of one polypeptide chain only.
- In **prokaryotes**, the structural gene is **polycistronic** as it consists of a nucleotide sequence carrying information to form more than one polypeptide chain required for a common metabolic reaction in the cell.
- In eukaryotes, the monocistronic structural genes have interrupted coding sequences, i.e., the genes in eukaryotes are split.
- The coding sequences or expressed sequences which are present in mature RNA are called **exons**.
- The exons are interrupted by **introns** or intervening sequences which do not appear in mature or processed RNA.

Previous Year's Question

The equivalent of a structural gene is
(1) Muton
(2) Cistron
(3) Operon
(4) Recon

Mechanism of Transcription

- In prokaryotes, the site of transcription is the cytoplasm as it is where the bacterial DNA is present.
- **Activation of ribonucleoside triphosphates (NTPs):**
  - Just before transcription, the four types of ribonucleotides (AMP, GMP, CMP and UMP), which are freely present in the bacterial cytoplasm, are phosphorylated by the enzyme **phosphorylase** to form activated or phosphorylated ribonucleoside triphosphates (NTPs), i.e., ATP, GTP, CTP and UTP.
- **Initiation:**
  - The main enzymes responsible for transcription is **DNA-dependent**

Rack your Brain

The transcription and translation can be coupled in bacteria. Give reason?

RNA polymerase. Only one type of RNA polymerase catalyses transcription of all types of RNA in bacteria.

- RNA polymerase of *E. coli* consists of five polypeptide chains – β, β', α, α' and σ (sigma factor). The sigma factor recognises the promoter region of DNA and allows the RNA polymerase to bind to promoter to initiate transcription.
- The RNA polymerase uncoils the two DNA strands progressively. The DNA strand with 3'→5' polarity functions as the **template** or **antisense** or **non-coding** strand, on which the transcript is formed in the 5'→3' direction.
- The NTPs lie opposite to the nitrogenous bases of the template DNA strand according to the complementary base pairing rule. The pyrophosphate is released from each ribonucleotide, which is further **hydrolysed** to release energy.
- RNA polymerase joins the adjacent ribonucleotides (substrates) to form a small RNA chain.

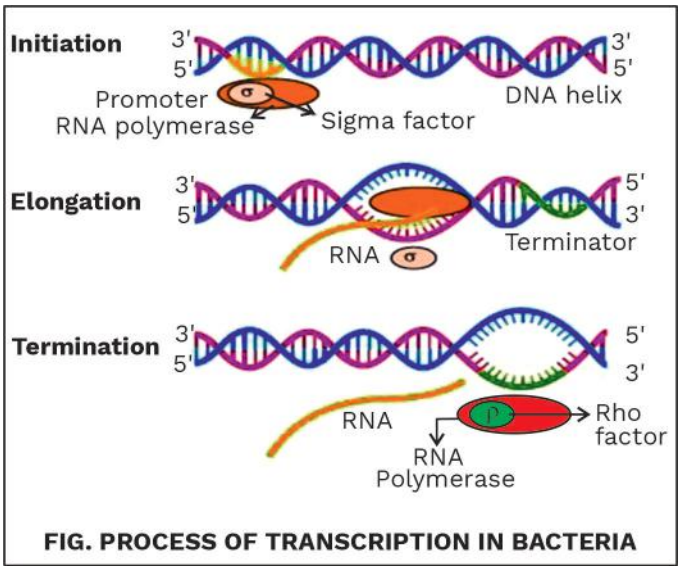


FIG. PROCESS OF TRANSCRIPTION IN BACTERIA

- **Elongation:**
  - As the RNA chain formation initiates, the **initiation-factor** (σ) or the **sigma factor**

Previous Year's Question

AGGTATCGCAT is a sequence from the coding strand of a gene. What will be the corresponding sequence of the transcribed mRNA?

(1) AGGUAUCGCAU
(2) UGGTUTCGCAT
(3) ACCUAUGCGAU
(4) UCCAUAGCGUA

Previous Year's Question

In the process of transcription in Eukaryotes, RNA polymerase I transcribes

(1) mRNA with additional processing, capping and tailing
(2) tRNA, 5S rRNA and snRNAs
(3) rRNAs – 28S, 18S and 5.8S
(4) Precursor of mRNA, hnRNA

separates which allows the RNA polymerase to move along the DNA template causing elongation of the RNA transcript.

- **Termination:**
  - Once the RNA polymerase reaches the terminator region, the **termination-factor** (ρ) or **rho factor** helps it to release the completed RNA transcript. This is followed by the release of RNA polymerase to terminate transcription.
- In bacteria, many times the transcription is coupled with translation much before the mRNA is fully transcribed.
- In **eukaryotes**, there are **at least three** RNA polymerases in the nucleus (in addition to the RNA polymerase found in the organelles).
- The **RNA polymerase I** transcribes rRNAs (28S, 18S, and 5.8S). **RNA polymerase III** transcribes tRNA, 5srRNA, and snRNAs (small nuclear RNAs) and **RNA polymerase II** transcribes precursor of mRNA, the heterogeneous nuclear RNA (hnRNA).

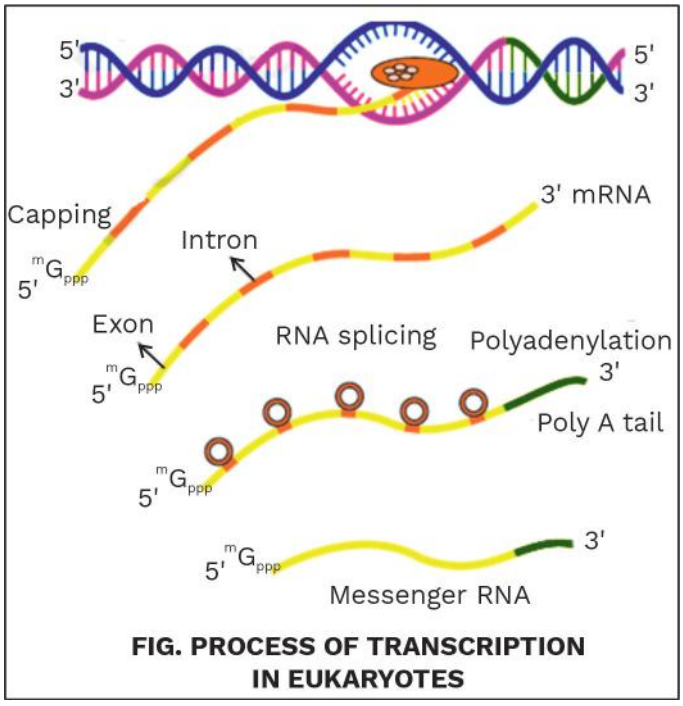


FIG. PROCESS OF TRANSCRIPTION IN EUKARYOTES

- Also, the primary transcript prepared in the nucleus of a eukaryotic cell (heterogenous RNA,

Previous Year's Question

Removal of RNA polymerase III from nucleoplasm will affect the synthesis of
(1) tRNA
(2) hnRNA
(3) mRNA
(4) rRNA

Previous Year's Question

What initiation and termination factors are involved in transcription in prokaryotes?
(1) sigma and rho, respectively
(2) alpha and beta, respectively
(3) beta and gamma, respectively
(4) alpha and sigma, respectively

hnRNA) contains both the exons and the introns and are non-functional.

- To make it functional, it is subjected to post **transcription modifications** or processing such as splicing, capping and tailing.
- **Splicing** is the removal of introns and fusion of exons in a defined order to form a functional RNA.
- In **capping,** methyl guanosine triphosphate is added to the 5' -end of hnRNA.
- In **tailing**, adenylate residues or poly-A segments (200-300) are added at 3' -end in a template independent manner.
- A fully processed hnRNA, now called mRNA, moves out of the nucleus for translation.

GENETIC CODE

- According to the central dogma of molecular biology, the information to form a protein is present in a DNA segment which is carried to a ribosome by an mRNA.
- DNA is made of nucleotides, whereas a protein is a polymer of amino acids. Thus, they show no complementarity. But it is seen that any change in the genetic material (nucleotide sequence of DNA) results in altering the sequence of amino acids in a protein.
- This led to the proposition of a genetic code that could direct the sequence of amino acids during synthesis of proteins.
- **George Gamow** hypothesised that since there are only 4 nitrogenous bases and if they must code for 20 amino acids, the code should constitute a combination of bases. To code for all the 20 amino acids, the code should be made up of three nucleotides (triplet code, $4^3 = 64$ codes).
- Francis Crick, Severo Ochoa, Marshall Nirenberg, Har Gobind Khorana and J.H. Mathhaei were among the scientists who contributed to deciphering the genetic code in 1960s.
- **Har Gobind Khorana** developed a chemical method to synthesise RNA molecules with

Previous Year's Question

Removal of the introns and joining of exons in a defined order during transcription is called
(1) Looping
(2) Inducing
(3) Capping
(4) Splicing

Definition

Genetic code: The relationship between the sequence of amino acids in a polypeptide chain and nucleotide sequence of DNA or mRNA is called genetic code.

Previous Year's Question

From the following, identify the correct combination of salient features of Genetic Code
(1) Universal, non-ambiguous, overlapping
(2) Degenerate, overlapping, commaless less
(3) Universal, ambiguous, degenerate
(4) Degenerate, non-overlapping, non-ambiguous

defined combinations of bases (homopolymers and copolymers).

- **Marshall Nirenberg's** cell-free system for protein synthesis finally helped the code to be deciphered.
- **Severo Ochoa** discovered polynucleotide phosphorylase enzyme which polymerise ribonucleotide to form RNA without any DNA template, i.e., enzymatic synthesis of RNA in a template-independent manner.
- Slowly all the codes (codons) were deciphered and a checker-board for genetic code was prepared.

Previous Year's Question

Which one of the following is the starter codon?

(1) UGA
(2) UAA
(3) UAG
(4) AUG

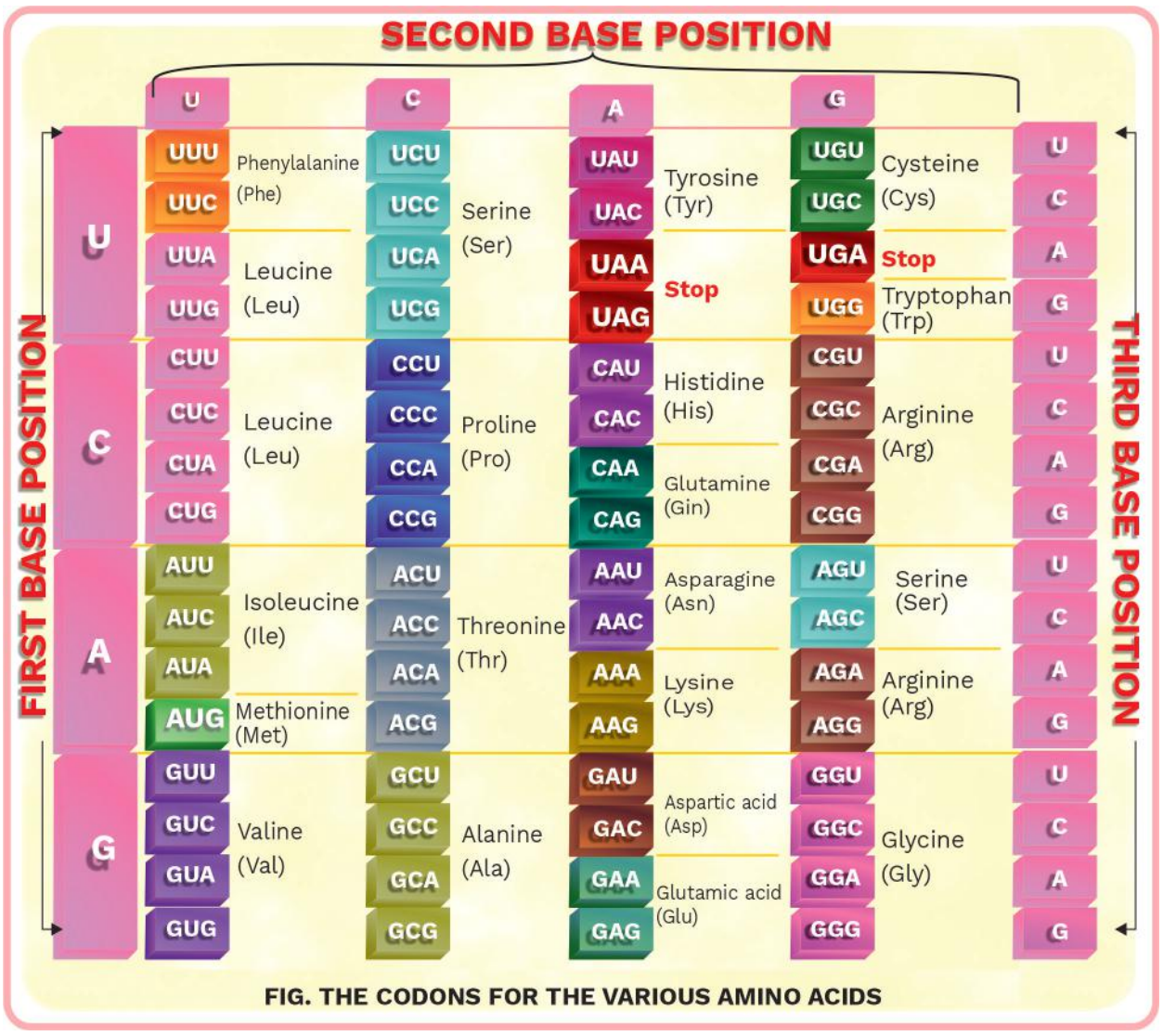
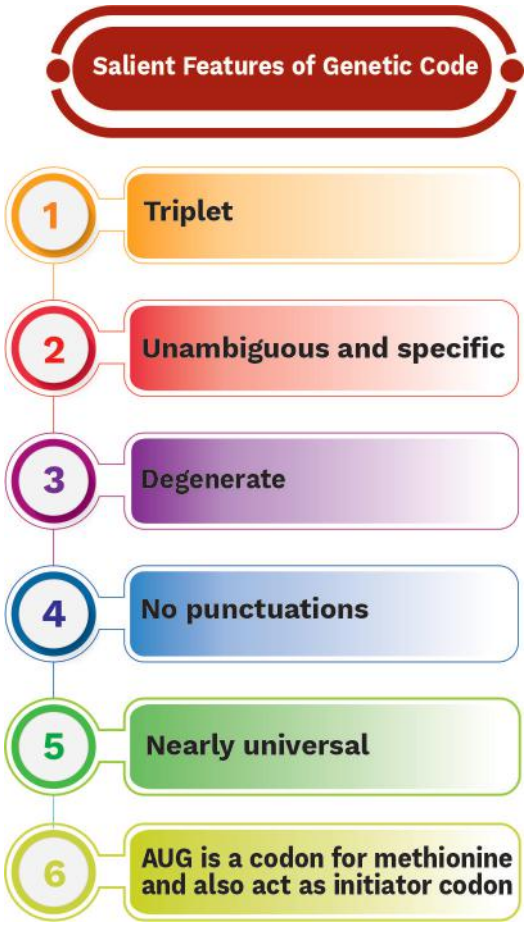


FIG. THE CODONS FOR THE VARIOUS AMINO ACIDS

- **The salient features of genetic code are:**
  - The codon is triplet, i.e., made of three adjacent nitrogenous bases. A triplet codon specifies the placement of one amino acid in a polypeptide chain. **61 codons** code for **20 amino acids. 3 codons** do not code for any amino acids. They are called **stop codons**.
  - The genetic code is unambiguous (specific), i.e., one codon specifies only one amino acid.
  - The genetic code is degenerate. All amino acids, except tryptophan and methionine, are coded by more than one codon.
  - The genetic code in the mRNA is read continuously as there is no comma, pause or overlapping in between the triplet codons.
  - The genetic code is nearly universal, i.e., a codon specifies the same amino acid from a virus to a plant or human beings. Some exceptions to this rule are present in the mitochondrial codons and in some protozoans.
  - Codon AUG has dual functions. It codes for methionine amino acid and also acts as initiator codon. Rarely GUG or valine codon acts as the initiator codon.
- Insertion or deletion of one or two bases changes the reading frame on mRNA from the point of insertion or deletion (frameshift insertion or deletion mutations).
- Insertion or deletion of three or its multiple nitrogenous bases, insert or delete one or multiple codons, hence one or multiple amino acids. This causes the reading frame to remain unaltered from that point onwards.



Gray Matter Alert!!!

In degenerate codons, generally the first two nitrogenous bases are similar while the third one is different. As the third nitrogenous base does not show any effect on coding, the same is called wobble position (Wobble hypothesis by Crick in 1966).

TRANSLATION

- Translation is the process of converting or translating the sequence of an mRNA to a sequence of amino acids joined by peptide bonds to form a polypeptide chain.
- Translation requires ribosomes, amino acids, mRNA, tRNAs and aminoacyl tRNA synthetases.

Machinery for Translation

- **Ribosomes:**
  - Ribosomes are the site for protein formation (translation). They are, therefore, called the **protein factories**.
  - A ribosome is made of two unequal parts – a smaller subunit and a larger subunit. The two subunits attach only at the time of polypeptide synthesis in the presence of Mg^{2+}.
  - A complete ribosome consists of a tunnel for mRNA between the two subunits, a groove in the larger subunit for the exit of the polypeptide formed, a peptidyl transfer site (**P-site**), an aminoacyl site or acceptor site (**A-site**), an exit site (**E-site**), an enzyme peptidyl transferase which is a component of larger subunit (23S rRNA in prokaryotes).

Previous Year's Question

Which of the following rRNA act as structural RNA as well as ribozyme in bacteria?
(1) 5S rRNA
(2) 18S rRNA
(3) 23S rRNA
(4) 5.8S rRNA

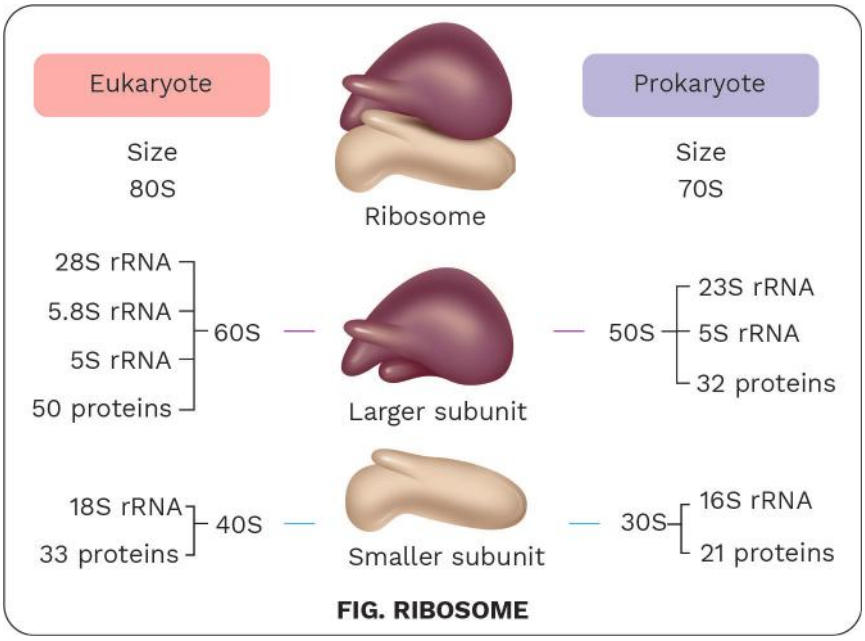


FIG. RIBOSOME

- **Amino acids:**
  - 20 different types of amino acids occur in the cellular pool which are arranged in a specific sequence in a protein to impart it a specific nature.

Rack your Brain

Whose experiments cracked the DNA and discovered unequivocally that a genetic code is a triplet?

- **mRNA:**
  - The **messenger RNA** (mRNA) constitutes about **2-5%** of the total RNA and consists of the coded information from the DNA and takes part in its translation by binding amino acids in a particular sequence to synthesis a protein.
  - The codons of mRNA are recognised by the anticodons of their adaptor molecules (aminoacylated or charged tRNA).
- **tRNA– the adapter molecule:**
  - Since the codons of mRNA are not recognised by the amino acids due to their lack of structural specialities to read the code uniquely, **Francis Crick** postulated the presence of an adapter molecule that would on one hand read the code and on other hand would bind to specific amino acids. This adapter molecule is called the tRNA (**transfer RNA**) or sRNA (**soluble RNA**).
  - tRNA constitutes about **15%** of the total RNA. It has a two-dimensional **clover leaf-like** structure and a three-dimensional **L-shaped** structure.
  - A tRNA binds with a particular amino acid at their amino acid acceptor end (CCA 3' -end) in a process called **charging** of **tRNA**. The charged tRNA takes its amino acid to the mRNA over its specific codon where H-bonds are formed between its anticodon (present on the **anticodon loop** of tRNA) and the corresponding codon on the mRNA.
  - A tRNA molecule also has an area for coming in contact with the ribosome (**TΨC loop**) and the enzyme aminoacyl tRNA synthetase (**DHU loop**) and a **variable arm**.

Previous Year's Question

Which of the following RNAs should be most abundant in animal cell?

(1) rRNA (2) tRNA
(3) mRNA (4) miRNA

Gray Matter Alert!!!

rRNA or ribosomal RNA (70-80% of the total RNA): It is a constituent of ribosomes. There are four types of rRNA in eukaryotes, namely, 28S rRNA, 18S rRNA, 5.8S rRNA and 5S rRNA. The prokaryotes have three types of rRNA, namely, 23S rRNA, 16S rRNA, and 5S rRNA. They form ribosomes along with various proteins and also acts as enzymes in translation.

Previous Year's Question

Amino acid sequence, in protein synthesis is decided by the sequence of

(1) tRNA
(2) mRNA
(3) cDNA
(4) rRNA

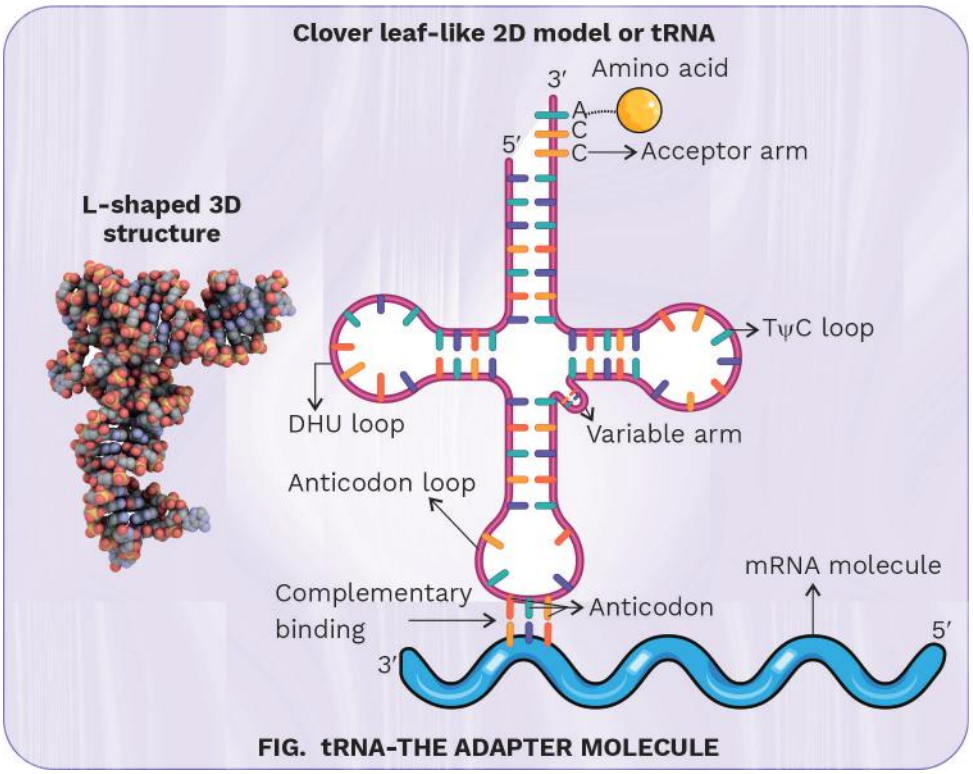


FIG. tRNA-THE ADAPTER MOLECULE

- **Aminoacyl tRNA synthetase:**
  - The enzyme aminoacyl tRNA synthetase combines an amino acid to its tRNA. There is a specific enzyme for each amino acid.

Mechanism of Translation

- **Activation of amino acids:**
  - In the presence of ATP and Mg^{2+}, an amino acid combines with its specific aminoacyl tRNA synthetase to form aminoacyl adenylate enzyme complex.
- **Charging or aminoacylation of tRNA:**
  - The aminoacyl adenylate enzyme complex reacts with tRNA specific for the amino acid and produces aminoacyl-tRNA-complex (charged tRNA). The enzyme and the AMP are released.
- **Initiation:**
  - The mRNA attaches itself to the smaller subunit of ribosome. The attachment is in such a way that the initiation codon of mRNA

Previous Year's Question

The first phase of translation is
(1) Recognition of DNA molecule
(2) Aminoacylation of tRNA
(3) Recognition of an anticodon
(4) Binding of mRNA to ribosome

(AUG) comes to lie at the P-site. It requires GTP and **initiation factors**.

- The methionine-containing charged tRNA (**tRNA^{Met}**) attaches with the start codon on mRNA with its anticodon at the P-site.
- In the presence of Mg^{2+}, the larger subunit of the ribosome, now binds the small subunit-mRNA-tRNA^{Met}-complex to form the intact ribosome.
- An mRNA also has some additional sequences that are not translated and are referred to as untranslated regions (**UTR**).

Previous Year's Question

PCR and Restriction Fragment Length Polymorphism are the methods for
(1) Study of enzymes
(2) Genetic transformation
(3) DNA sequencing
(4) Genetic fingerprinting

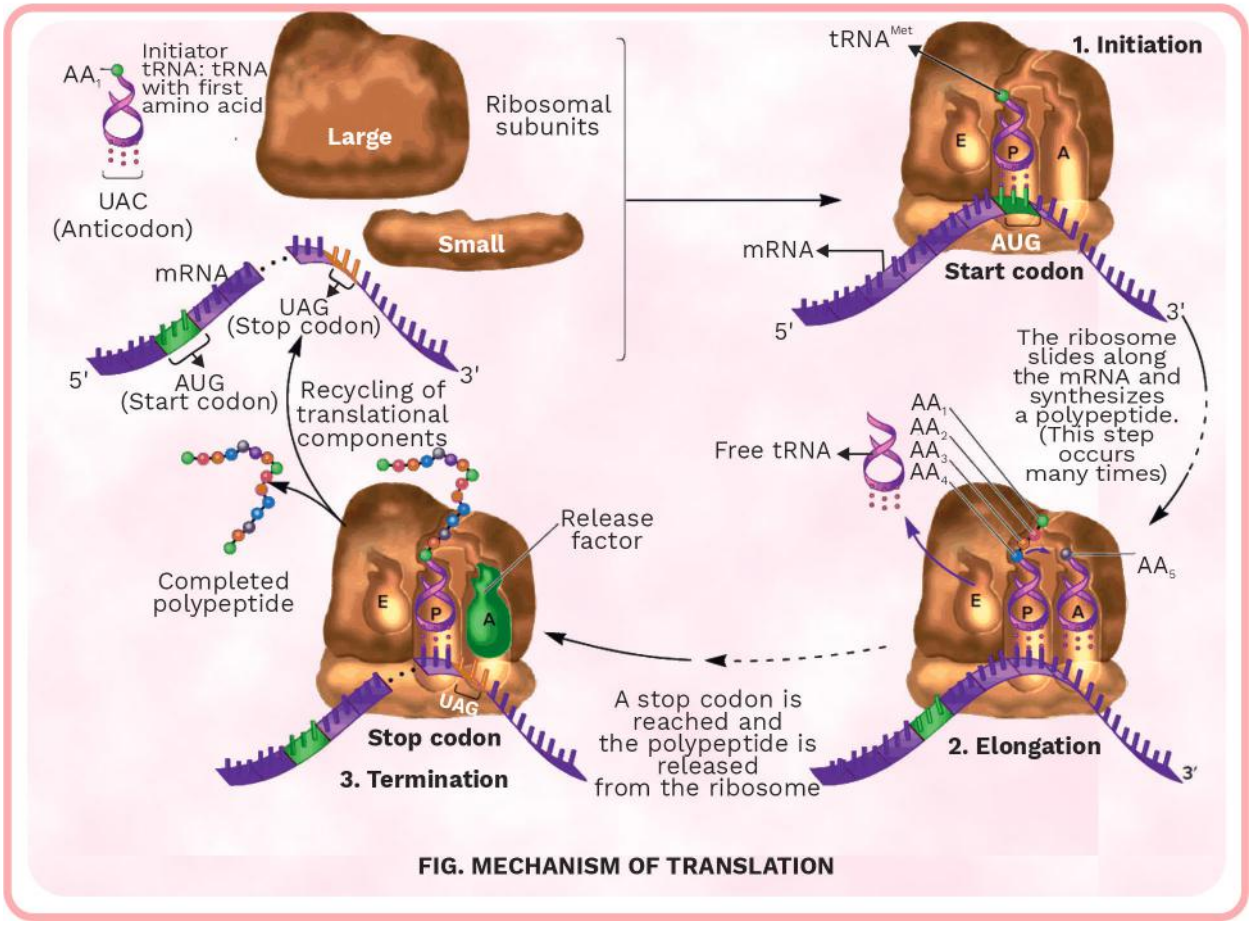


FIG. MECHANISM OF TRANSLATION

- The UTRs are present at both 5' -end (before start codon) and at 3' -end (after stop codon). They are required for efficient translation process.

- **Elongation (Polypeptide chain formation):**
  - A charged tRNA reaches A-site and attaches to the mRNA codon next to the initiation codon with the help of its anticodon. It requires GTP and elongation factors.
  - A **peptide bond** is established between the carboxyl group (-COOH) amino acid attached to tRNA at P-site and amino group ($-NH_2$) of amino acid attached to tRNA at A-site. The reaction is catalysed by the enzyme **peptidyl transferase**.
  - In this process, the connection between the tRNA and the amino acid at the P-site breaks.
  - The ribosome, then translocates along the mRNA in 5'→3' direction which results in the translocation of the A-site mRNA codon along with the peptidyl-tRNA-complex from A-site to the P-site and the free tRNA at the P-site to slip to the E-site. This translocation requires **elongation factors**.
  - Also, a new codon is exposed on A-site to which a new aminoacyl-tRNA-complex will attach.
  - The process of peptide bond formation and translocation continues to elongate the polypeptide chain.
- **Termination**
  - Protein synthesis stops when a stop codon (UAA, UGA, UAG) of mRNA reaches the A-site. These stop codons are not recognised by any tRNAs.
  - The P-site is hydrolysed, and the completed polypeptide chain is released with the help of a **releasing factor**.
  - The ribosome slips off the mRNA chain and dissociates into smaller and larger subunits.

REGULATION OF GENE EXPRESSION

- A gene expresses itself when it is transcribed into an RNA to finally form a protein which is observed as a phenotype of an individual.

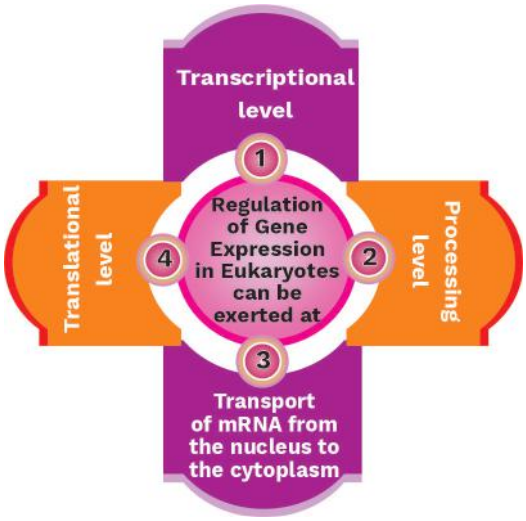
Gray Matter Alert!!!

In 1977, Frederick Sanger and his colleagues developed the Sanger sequencing method for determining the nucleotide sequence of DNA. Automated DNA sequencers worked on the principle of this method. Sanger also developed a method for determining the amino acid sequences in proteins.

Previous Year's Question

Which of the following pairs of codons is correctly matched with their function or the signal for the particular amino acid?

(1) GUU, GCU - Alanine
(2) UAG, UGA - Stop
(3) AUG, ACG – Start/methionine
(4) UUA, UCA - Leucine



- Regulation of gene expression refers to controlling the functioning of genes which may occur at various molecular levels due to different metabolic, physiological, or environmental conditions.
- In prokaryotes, control of the rate of transcriptional initiation is the predominant site for control of gene expression.
- In a transcription unit, the activity of RNA polymerase at a given promoter is in turn regulated by interaction with accessory regulatory proteins, which may act both positively (**activators**) and negatively (**repressors**).
- The accessibility of promoter regions of prokaryotic DNA is in many cases regulated by the interaction of regulatory proteins with sequences termed **operators** present adjacent to the promoter elements in most operons and in most cases the sequences of the operator bind a repressor protein.
- Each operon has its specific operator and specific repressor, e.g., *lac* operator is present only in the *lac* operon and it interacts specifically with *lac* repressor only.

The *Lac* operon

- Francois Jacob and Jacque Monod were the first to elucidate a transcriptionally regulated system called the *lac* operon in *Escherichia coli*.
- In the *lac* operon, a polycistronic structural gene is regulated by a common promoter and regulatory genes.
- It consists of one regulatory gene (*i* gene) and three structural genes (*z*, *y*, and *a*).
- The *i* gene codes for the repressor of the *lac* operon. It is a regulator protein which is synthesised all the time by the *i* gene. It blocks the operator gene so that RNA polymerase is unable to transcribe the structural genes.

Definition

Operon: An operon is segment of DNA which acts as a single regulated unit having one or more structural genes, an operator gene, a promoter gene, a regulator gene, a repressor, and an inducer (from outside), e.g., *lac* operon, *trp* operon, *ara* operon, *his* operon, *val* operon, etc.

Previous Year's Question

Select the correct match.

(1) Alec Jeffreys – *Streptococcus pneumoniae*

(2) Alfred Hershey and Martha Chase - TMV

(3) Matthew Meselson and F. Stahl – *Pisum sativum*

(4) Francois Jacob and Jacques Monod – *Lac* operon

- The *z* gene codes for beta-galactosidase (β-gal), which hydrolyse the disaccharide lactose into galactose and glucose.
- The *y* gene codes for lactose or galactoside permease, which increases permeability of the cell to β-galactosides.
- The *a* gene encodes an enzyme called transacetylase to metabolise the toxic thiogalactosides which are also allowed to enter the cell by lactose permease.

Previous Year's Question

Which of the following is required as inducer (s) for the expression of *lac* operon?
(1) Galactose
(2) Lactose
(3) Lactose and galactose
(4) Glucose

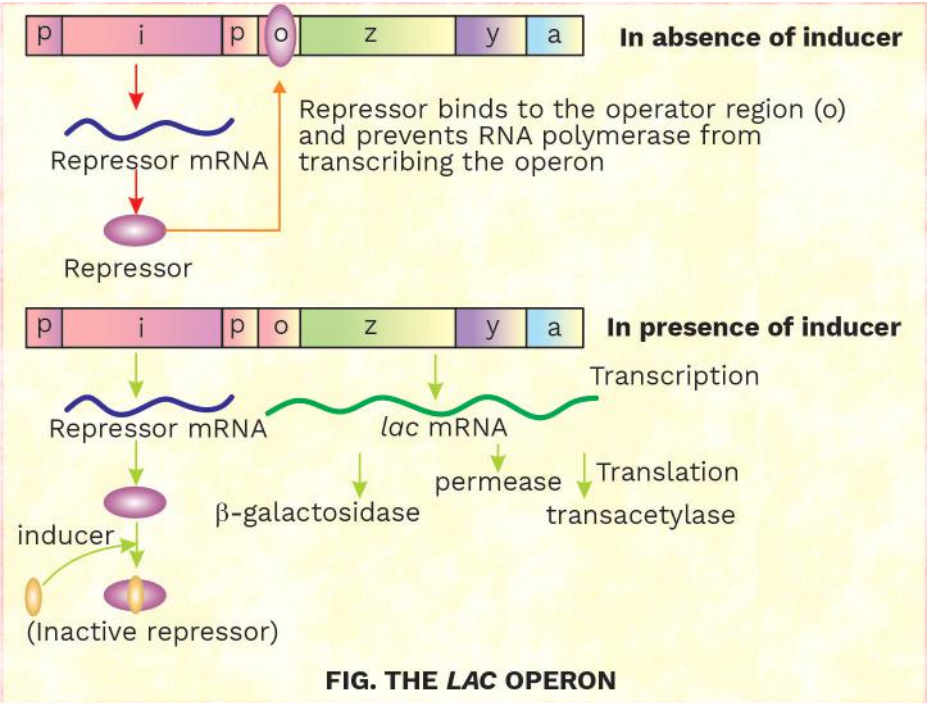


FIG. THE *LAC* OPERON

- Hence, all the three gene products in *lac* operon are required for metabolism of lactose.
- Lactose acts as the substrate for the enzyme beta-galactosidase and it regulates switching on and off of the operon. Hence, it is inducer of the operon.
- In the absence of a preferred carbon source such as glucose, if lactose is provided in the culture medium of the bacteria, the lactose is allowed to enter the bacterial cells through the action of permease as a very low level of expression of *lac* operon is always present in the cell.

Previous Year's Question

Which enzyme/s will be produced in a cell in which there is a non-sense mutation in the *lac y* gene?
(1) Beta- galactosidase
(2) Lactose permease
(3) Transacetylase
(4) Lactose permease and transacetylase

- In the presence of an inducer, such as lactose or allolactose, the repressor undergoes conformational change such that it is unable to combine with the operator.
- This allows RNA polymerase to bind to the promoter and proceed with the transcription.
- Regulation of *lac* operon by repressor is called negative regulation as the product of the regulatory gene, i.e., the repressor, shuts off the expression of the structural genes.

HUMAN GENOME PROJECT

- Although being individuals of the same species, every human being has a different identity due to the difference in their genetic make-up, which is a result of the different nucleotide sequences at some places in their DNA.
- It was, thus, an ambition of scientists from all over the world to sequence the human genome.
- In 1990, a mega project called Human Genome Project (HGP) was launched by the U.S. Department of Energy and the National Institute of Health.
- The Wellcome Trust (U.K.) joined the project and became a major partner. Other countries like Japan, France, Germany, China, and others also joined later.
- The Human Genome Project was a **13-year** project which was completed in 2003.
- Two different approaches were taken for analysing the human genome:
  - Identifying all the genes (only the coding regions of the DNA) that are expressed as RNA, referred to as **Expressed Sequence Tags** or **ESTs**.
  - Sequencing the whole set of genome that contained all the coding and non-coding sequences and later assigning different regions in the sequence with functions (**Sequence Annotation**).

Previous Year's Question

Gene regulation governing lactose operon of *E.coli* that involves the *lac i* gene products is

(1) Positive and inducible because it can be induced by lactose
(2) Negative and inducible because repressor protein prevents transcription
(3) Negative and repressible because repressor protein prevents transcription
(4) Feedback inhibition because excess of beta-galactosidase can switch off transcription

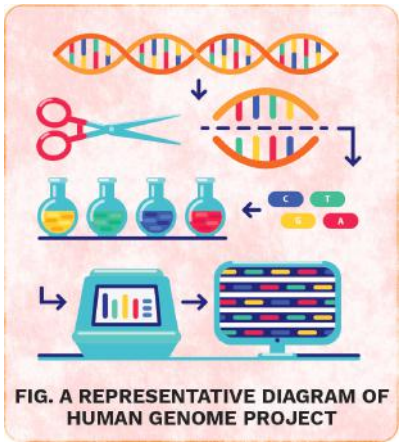
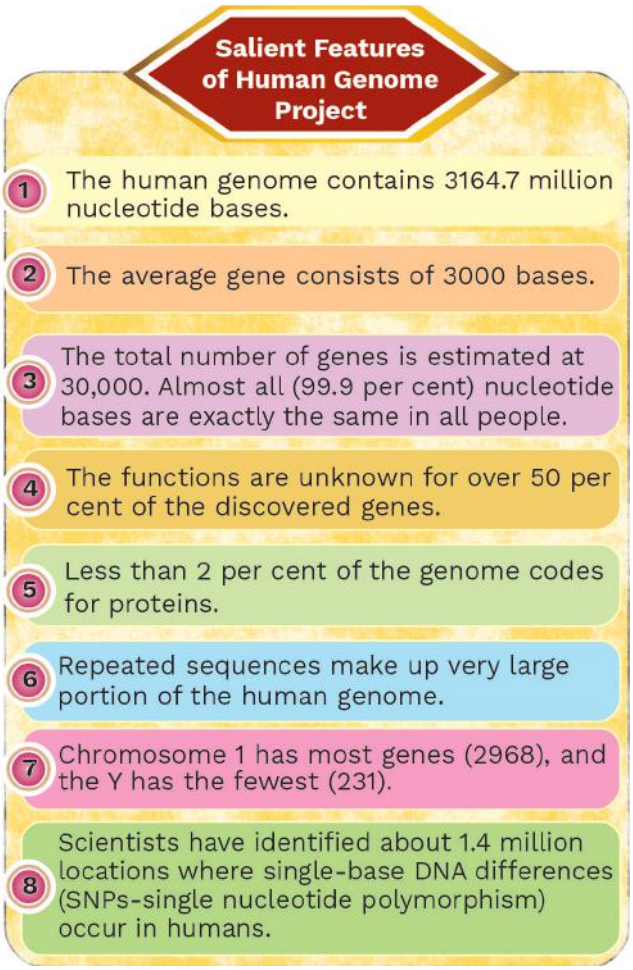


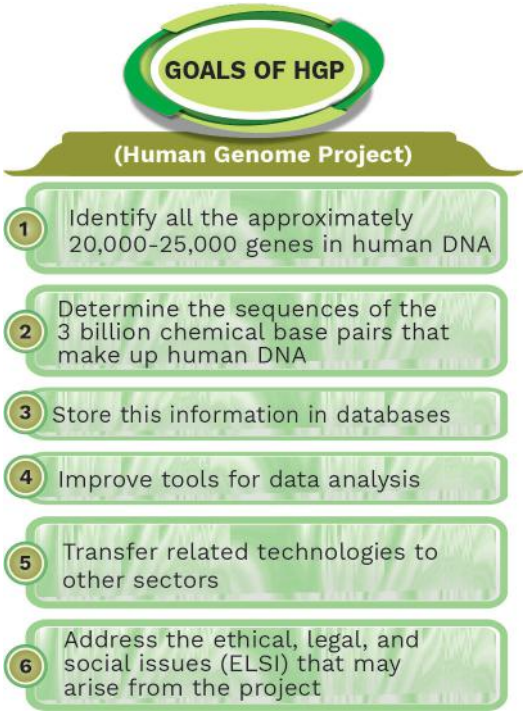
FIG. A REPRESENTATIVE DIAGRAM OF HUMAN GENOME PROJECT

Previous Year's Question

Expressed Sequence Tags (ESTs) refers to

(1) Novel DNA sequence
(2) Genes expressed as RNA
(3) Polypeptide expression
(4) DNA polymorphism





- **Steps of Sequence Annotation**
  - Isolation of DNA from a human cell.
  - Isolated DNA broken randomly into smaller sized fragments.
  - DNA fragments inserted into specialised vectors like **BAC** (bacterial artificial chromosome) and **YAC** (yeast artificial chromosome).
  - DNA fragments cloned in hosts like bacteria and yeast.
  - The fragments were sequenced as annotated DNA sequences using automated DNA sequencers.
  - Sequences were arranged based on some overlapping regions present in them using

Previous Year's Question

Commonly used vectors for human sequencing are
(1) T-DNA
(2) BAC and YAC
(3) Expression vectors
(4) T/A cloning vectors

specialised computer-based programs.
- Sequences were annotated and assigned to each chromosome. Chromosome 1 was the last to be sequenced in May 2006.

- The genetic and physical maps of the genome have now been generated using information on polymorphism in restriction endonuclease recognition sites and microsatellites (repetitive DNA sequences).

Note:

- Total number of genes estimated before HGP was 80,000 to 1,40,000.
- The largest known human gene is Duchenne Muscular Dystrophy (DMD), which is present on the X-chromosome and contains information to make dystrophin protein. It consists of 2.4 million bases.

Previous Year's Question

The main aim of the human genome project is
(1) To introduce new genes into humans
(2) To identify and sequence all the genes present in human DNA
(3) To develop better technique for comparing two different human
(4) To remove disease-causing genes from human DNA

Applications and Future Challenges

- **Molecular interactions:**
  - All the genes in a genome can be studied to understand how they work together in interconnected networks.
- **Disorders:**
  - Knowledge about the effects of DNA variations among individuals may help in better diagnosis, treatment and hopefuly prevention of many disorders that affect human beings.
- **Non-human organisms:**
  - By understanding DNA sequences of non-human organisms, information about their natural capabilities can be applied toward solving challenges in health care, agriculture, energy production, and environmental remediation.
  - For this purpose, many non-human model organisms like bacteria, yeast, *Caenorhabditis elegans*, *Drosophila melanogaster*, plants (rice and *Arabidopsis*), etc., have been sequenced.

Previous Year's Question

The Human Genome Project (HGP) was initiated in
(1) 1988
(2) 1990
(3) 1992
(4) 1994

DNA FINGERPRINTING

- DNA fingerprinting is a technique of determining nucleotide sequences of some specific regions of DNA which are unique to each human being.
- These specific regions are called **repetitive DNA**, because in these sequences, a small stretch of DNA is repeated several times tandemly, i.e., these short nucleotide repeats in the DNA vary in number in different individuals of the human population.
- They occur near telomeres, centromeres, Y chromosomes and heterochromatic regions.
- These repetitive DNA sequences are separated as **satellite DNA** from bulk genomic DNA as different peaks during density gradient centrifugation (major peak representing bulk DNA, whereas other small peaks showing satellite DNA).
- The satellite DNA can be classified as **micro-satellites** or **mini-satellites** depending on base composition (A:T rich or G:C rich), length of segment and number of tandemly repetitive units.
- Repetitive DNA sequences show high degree of **polymorphism**.
- Polymorphism of a DNA sequence represents the occurrence of more than one variant of that sequence in the human population with a high frequency of about 1% (**0.01**) or greater.
- These variations are introduced in the specific repetitive DNA sequence by mutations and can be inherited if they occur in the germ cells without impairing the sexual reproductive ability of the individual.
- A higher probability of DNA polymorphism (variation at genetic level) is observed in noncoding DNA sequences (heterochromatin) as mutations in these sequences generally do not have any immediate impact on an individual's reproductive ability.
- The polymorphisms may range from single nucleotide change to very large-scale changes in the repetitive DNA sequence.

Previous Year's Question

Satellite DNA is important because it

(1) Codes for protein needed in cell cycle

(2) Shows high degree of polymorphism in population and also the same degree of polymorphism in an individual, which is heritable from parents to children

(3) Does not code for proteins and is same in all members of the population

(4) Codes for enzymes needed for DNA replication

Previous Year's Question

Which is the basis of genetic mapping of the human genome as well as DNA fingerprinting?

(1) Polymorphism in the DNA sequence

(2) Single nucleotide polymorphism

(3) Polymorphism in hnRNA sequence

(4) Polymorphism in the RNA sequence

- **Alec Jeffreys** (1984) developed the DNA fingerprinting technique at Leicester University, UK.
- **Steps involved in DNA fingerprinting:**
  - DNA from a suitable human cell, e.g., a hair follicle cell or a sperm is first isolated.
  - The DNA is broken down into small fragments by the enzyme restriction endonuclease. These DNA fragments contain satellite DNA showing high degree of polymorphism (small DNA sequence arranged tandemly in many copy numbers varying from chromosome to chromosome in an individual) called the Variable Number of Tandem Repeats (**VNTRs**) or **minisatellites**. The size of a VNTR may vary from 0.1 to 20 kb.
  - The DNA fragments are separated according to their size by **gel electrophoresis** technique.
  - Fragments of a particular size having VNTRs are multiplied by Polymerase Chain Reaction (**PCR**) technique, thereby further increasing the sensitivity of the DNA fingerprinting technique.
  - The DNA fragments are treated with alkaline chemicals to cause their denaturation to form **single stranded** DNA fragments.
  - The single stranded DNA fragments are transferred onto synthetic membranes, such as **nitrocellulose** or **nylon** membrane by **Southern Blotting** technique.
  - Radioactive DNA **probes** having repeated base sequences complementary to possible VNTRs are introduced over the nylon membrane with an assumption of their binding to the possible complementary VNTRs present on the single stranded DNA fragments.

Definition

Probe: It is a labelled single stranded DNA or RNA sequence used to find its complementary sequence in a sample genome.

Previous Year's Question

DNA fingerprinting refers to

(1) Techniques used for molecular analysis of different specimens of DNA

(2) Techniques used for identification of fingerprints of individuals

(3) Molecular analysis of profiles of DNA samples

(4) Analysis of DNA samples using imprinting devices

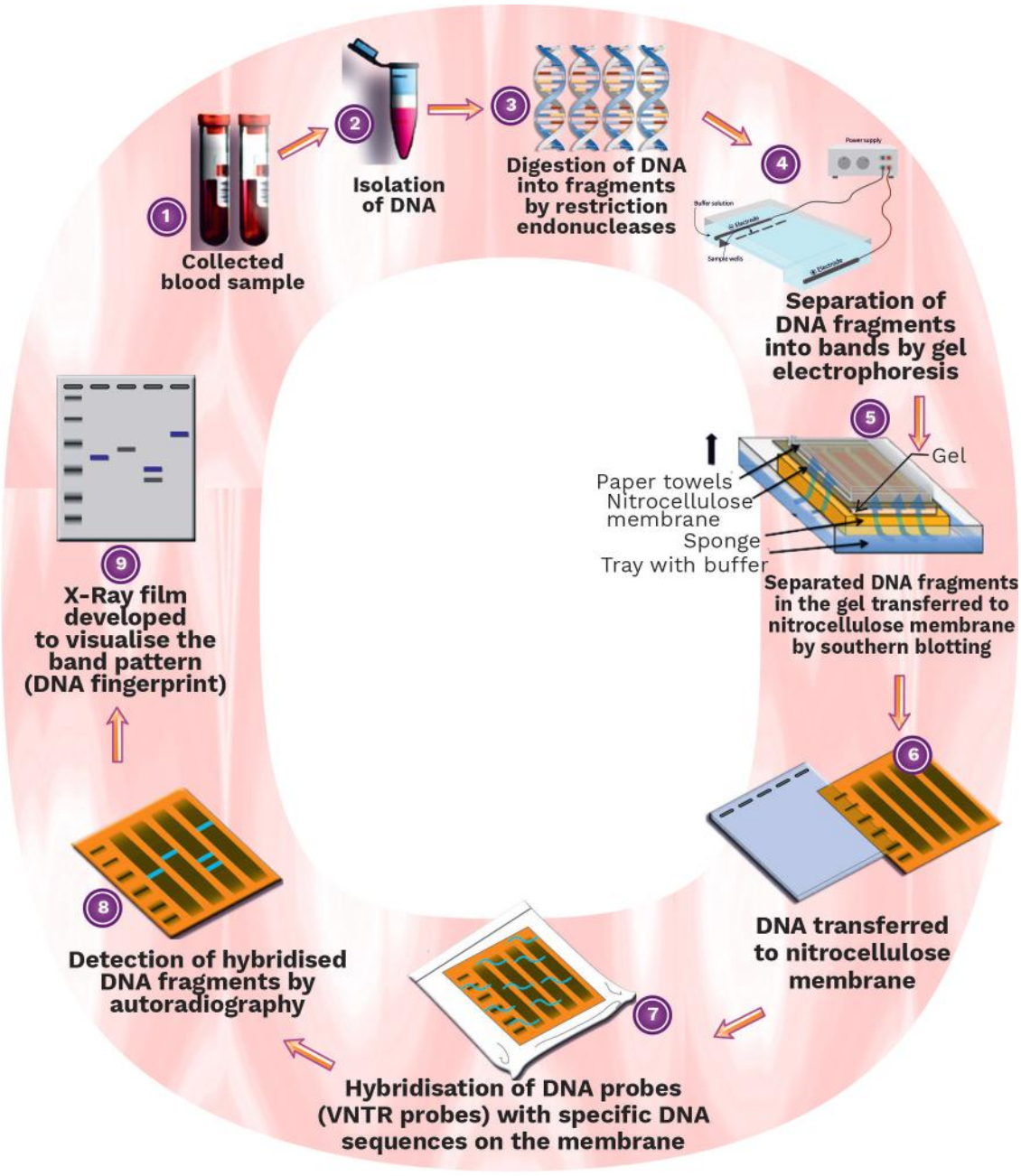


FIG. THE DNA FINGERPRINTING PROCESS

- After hybridisation of single stranded DNA fragments with labelled (radioactive) DNA probes, the nylon membrane is washed to remove extra probes.

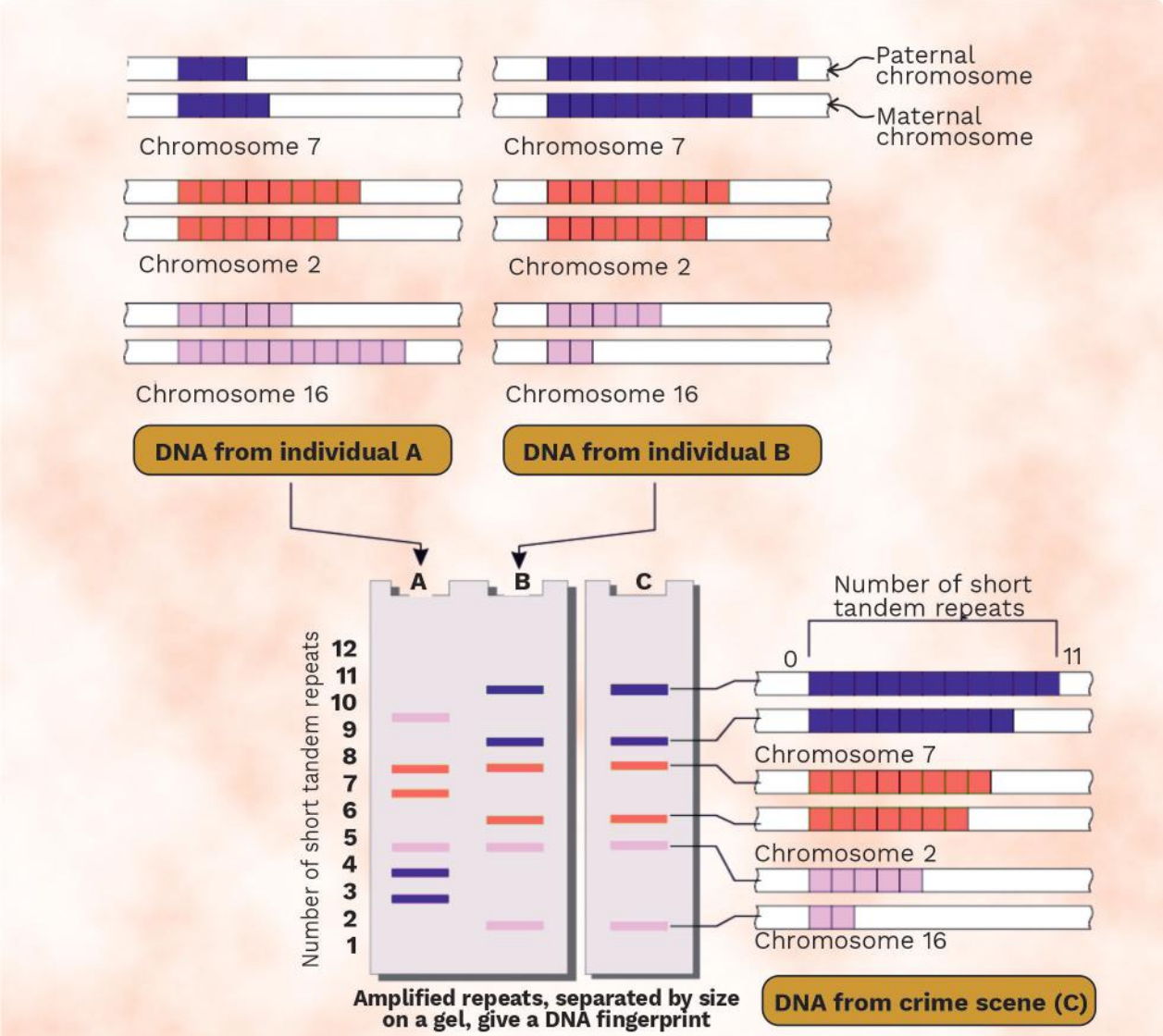


FIG. SCHEMATIC REPRESENTATION OF DNA FINGERPRINTING: FEW REPRESENTATIVE CHROMOSOMES HAVE BEEN SHOWN TO CONTAIN DIFFERENT COPY NUMBER OF VNTR.

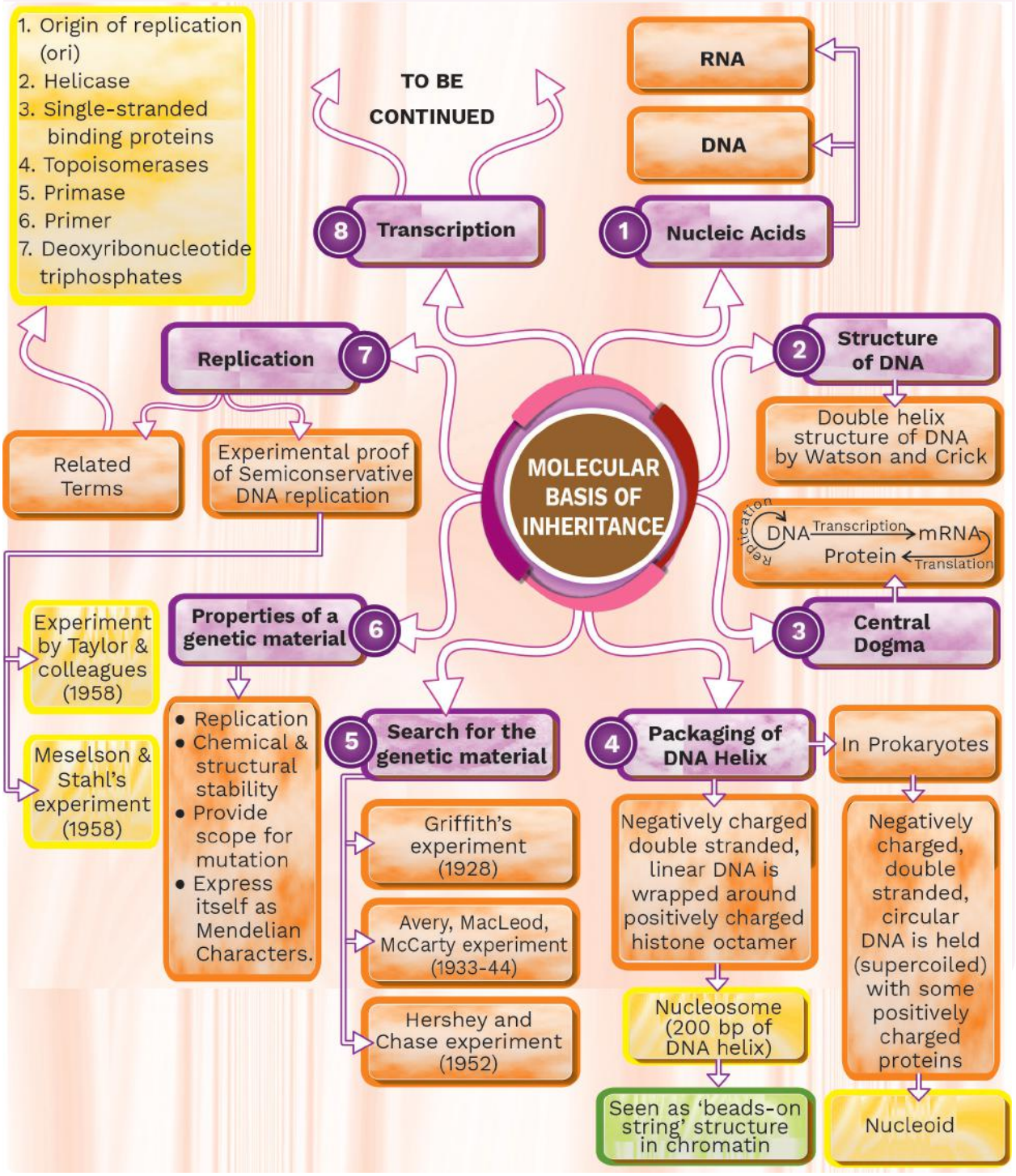
- An X-ray film is placed next to the nylon membrane to detect the areas of hybridisation of radioactive DNA probes and the single stranded DNA fragments having VNTRs by **autoradiography** technique, which appear as dark bands of differing sizes in the developed X-ray film (autoradiogram).

- These bands give a characteristic pattern for an individual DNA sequence (VNTR) which differs from individual to individual in a population (DNA fingerprints) except in the case of monozygotic (identical) twins.
- DNA fingerprinting has its application in **forensic science**. It is used in detecting a crime and settling a **legal** paternity/maternity **dispute** (as the polymorphisms are inheritable from parents to children).
- DNA fingerprinting is also used in determining population and genetic diversities, i.e., identifying racial groups, their origin, historical migration, and invasions.
- Further, this technique is being used to identify genes connected with hereditary diseases.

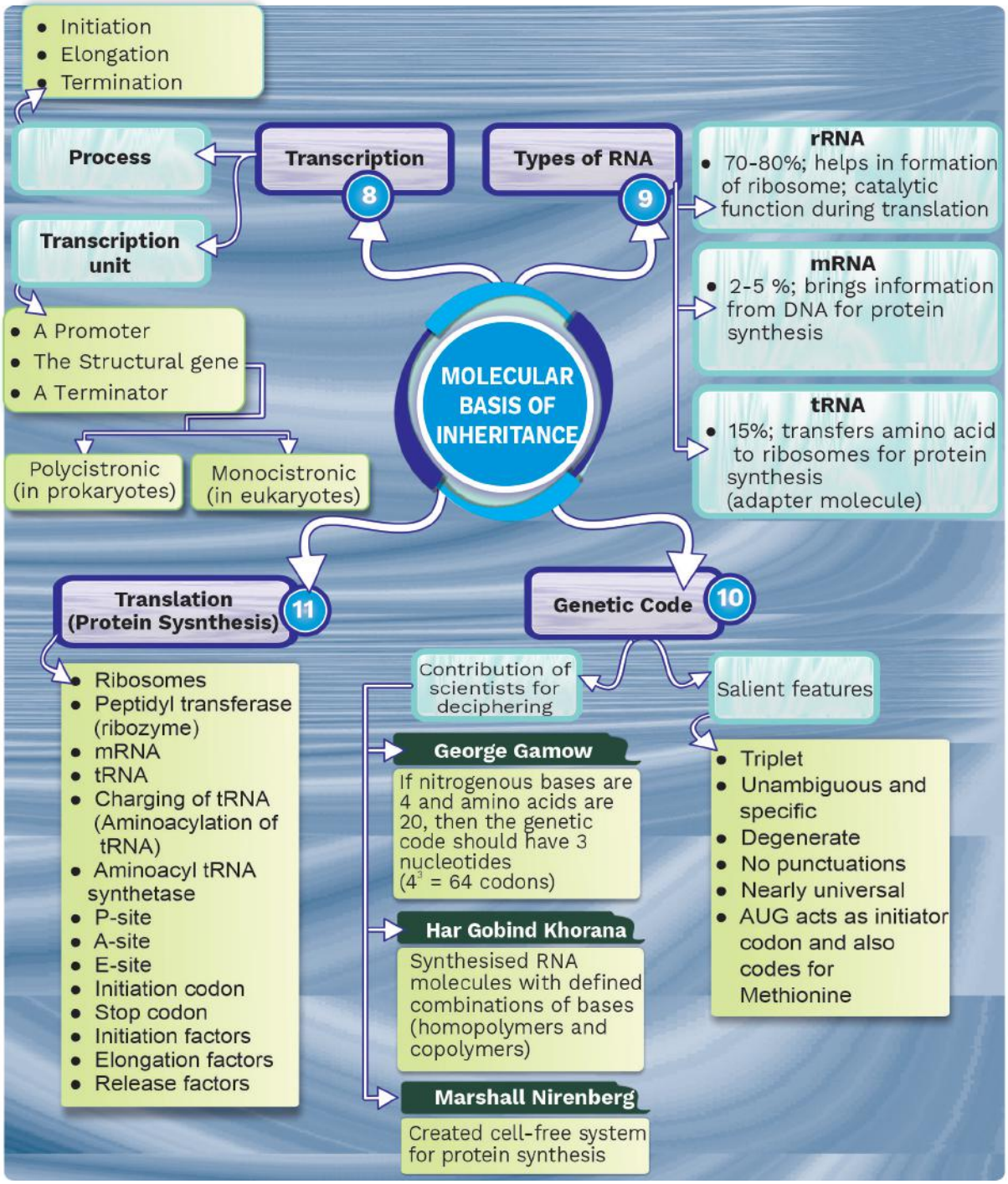
Previous Year's Question

Satellite DNA is useful tool in
(1) Organ transplantation
(2) Sex determination
(3) Forensic science
(4) Genetic engineering

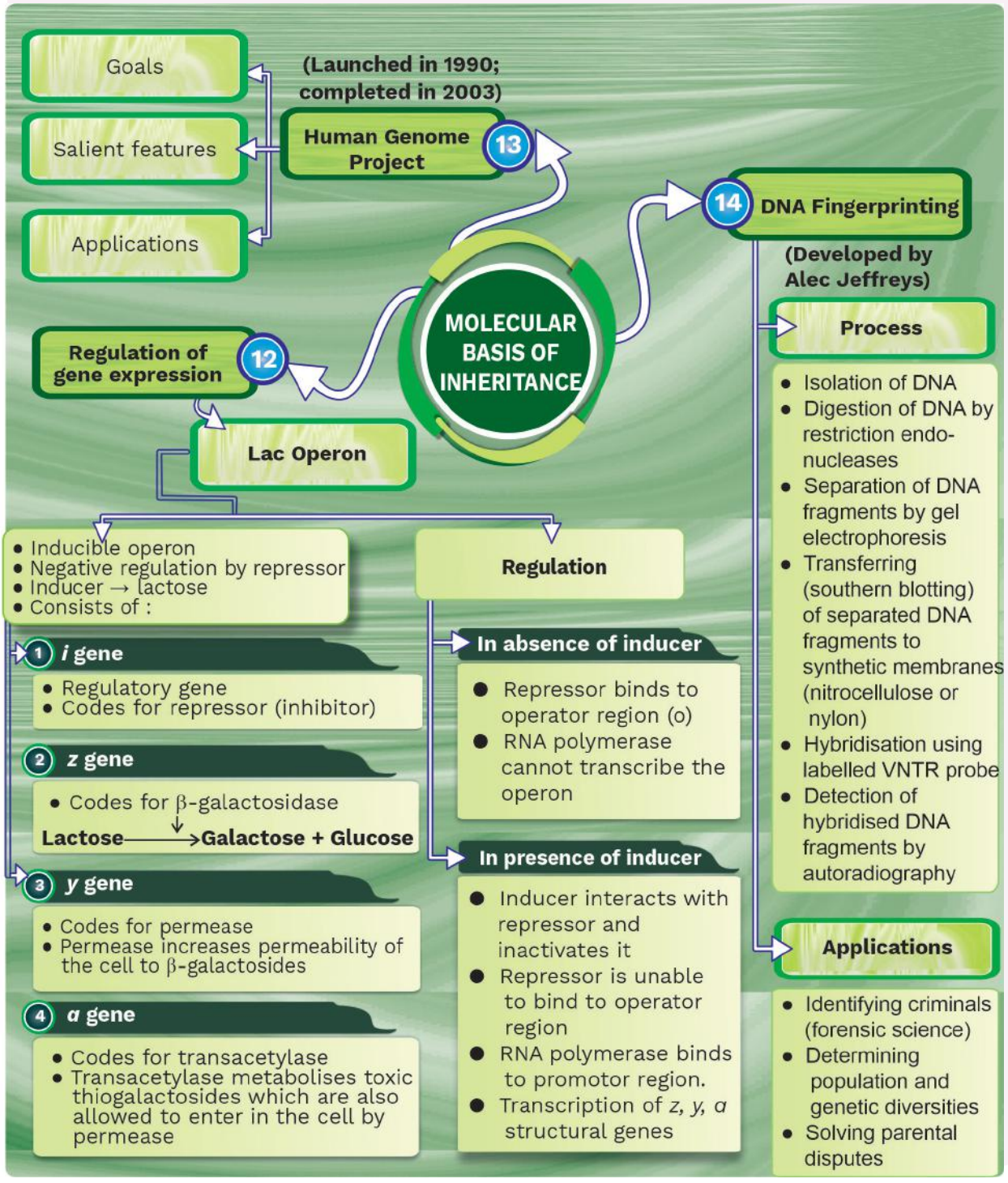
Summary


MOLECULAR BASIS OF INHERITANCE
1. Origin of replication (ori)
2. Helicase
3. Single-stranded binding proteins
4. Topoisomerases
5. Primase
6. Primer
7. Deoxyribonucleotide triphosphates
TO BE CONTINUED
RNA
DNA
1 Nucleic Acids
8 Transcription
Replication 7
Related Terms
Experimental proof of Semiconservative DNA replication
2 Structure of DNA
Double helix structure of DNA by Watson and Crick
Replication
DNA
Transcription
mRNA
Protein
Translation
3 Central Dogma
Properties of a genetic material 6
Experiment by Taylor & colleagues (1958)
Meselson & Stahl's experiment (1958)
• Replication
• Chemical & structural stability
• Provide scope for mutation
• Express itself as Mendelian Characters.
5 Search for the genetic material
Griffith's experiment (1928)
Avery, MacLeod, McCarty experiment (1933-44)
Hershey and Chase experiment (1952)
4 Packaging of DNA Helix
Negatively charged double stranded, linear DNA is wrapped around positively charged histone octamer
Nucleosome (200 bp of DNA helix)
Seen as 'beads-on string' structure in chromatin
In Prokaryotes
Negatively charged, double stranded, circular DNA is held (supercoiled) with some positively charged proteins
Nucleoid

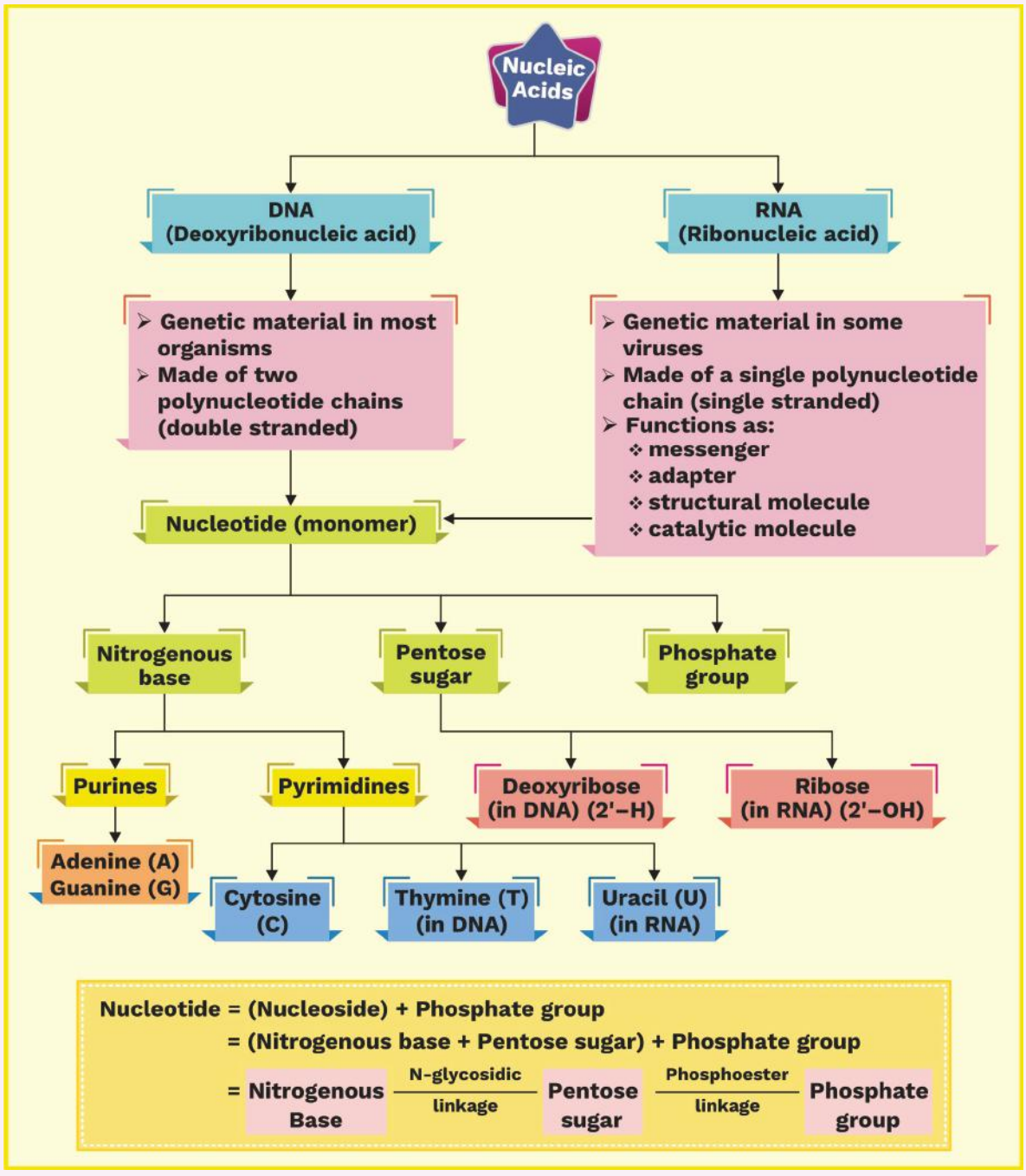
Summary


MOLECULAR BASIS OF INHERITANCE
Transcription
8
Process
Initiation
Elongation
Termination
Transcription unit
A Promoter
The Structural gene
A Terminator
Polycistronic (in prokaryotes)
Monocistronic (in eukaryotes)
Types of RNA
9
rRNA
70-80%; helps in formation of ribosome; catalytic function during translation
mRNA
2-5 %; brings information from DNA for protein synthesis
tRNA
15%; transfers amino acid to ribosomes for protein synthesis (adapter molecule)
Translation (Protein Sysnthesis)
11
Ribosomes
Peptidyl transferase (ribozyme)
mRNA
tRNA
Charging of tRNA (Aminoacylation of tRNA)
Aminoacyl tRNA synthetase
P-site
A-site
E-site
Initiation codon
Stop codon
Initiation factors
Elongation factors
Release factors
Genetic Code
10
Contribution of scientists for deciphering
George Gamow
If nitrogenous bases are 4 and amino acids are 20, then the genetic code should have 3 nucleotides (4^3 = 64 codons)
Har Gobind Khorana
Synthesised RNA molecules with defined combinations of bases (homopolymers and copolymers)
Marshall Nirenberg
Created cell-free system for protein synthesis
Salient features
Triplet
Unambiguous and specific
Degenerate
No punctuations
Nearly universal
AUG acts as initiator codon and also codes for Methionine

Summary


MOLECULAR BASIS OF INHERITANCE
Goals
Salient features
Applications
(Launched in 1990; completed in 2003)
Human Genome Project
13
14
DNA Fingerprinting
(Developed by Alec Jeffreys)
Process
Isolation of DNA
Digestion of DNA by restriction endo-nucleases
Separation of DNA fragments by gel electrophoresis
Transferring (southern blotting) of separated DNA fragments to synthetic membranes (nitrocellulose or nylon)
Hybridisation using labelled VNTR probe
Detection of hybridised DNA fragments by autoradiography
Applications
Identifying criminals (forensic science)
Determining population and genetic diversities
Solving parental disputes
Regulation of gene expression
12
Lac Operon
Inducible operon
Negative regulation by repressor
Inducer → lactose
Consists of :
Regulation
1 i gene
Regulatory gene
Codes for repressor (inhibitor)
2 z gene
Codes for β-galactosidase
Lactose → Galactose + Glucose
3 y gene
Codes for permease
Permease increases permeability of the cell to β-galactosides
4 a gene
Codes for transacetylase
Transacetylase metabolises toxic thiogalactosides which are also allowed to enter in the cell by permease
In absence of inducer
Repressor binds to operator region (o)
RNA polymerase cannot transcribe the operon
In presence of inducer
Inducer interacts with repressor and inactivates it
Repressor is unable to bind to operator region
RNA polymerase binds to promotor region.
Transcription of z, y, a structural genes

Summary


Nucleic Acids
DNA (Deoxyribonucleic acid)
RNA (Ribonucleic acid)
Genetic material in most organisms
Made of two polynucleotide chains (double stranded)
Genetic material in some viruses
Made of a single polynucleotide chain (single stranded)
Functions as:
messenger
adapter
structural molecule
catalytic molecule
Nucleotide (monomer)
Nitrogenous base
Pentose sugar
Phosphate group
Purines
Pyrimidines
Deoxyribose (in DNA) (2'–H)
Ribose (in RNA) (2'–OH)
Adenine (A)
Guanine (G)
Cytosine (C)
Thymine (T) (in DNA)
Uracil (U) (in RNA)
Nucleotide = (Nucleoside) + Phosphate group
= (Nitrogenous base + Pentose sugar) + Phosphate group
= Nitrogenous Base — N-glycosidic linkage — Pentose sugar — Phosphoester linkage — Phosphate group

Summary

SEARCH FOR THE GENETIC MATERIAL

	Griffith's Experiment (Transforming Principle)
Scientist	Frederick Griffith
Year	1928
Experimental Organisms used	*Streptococcus pneumoniae* (bacteria) **S strain** ❖ Have mucous (polysaccharide) coat ❖ Shining colonies ❖ Virulent **R strain** ❖ Mucous coat absent ❖ Rough colonies
Experiment	❖ S strain → inject into mice → Mice die ❖ R strain → inject into mice → Mice live ❖ S strain (heat-killed) → inject into mice → Mice live ❖ S strain (heat-killed) + R strain → inject into mice → Mice die
Conclusion	Some transforming principle, transferred from heat-killed S strain to R strain which made the latter virulent.

Summary

SEARCH FOR THE GENETIC MATERIAL

	Hershey and Chase Experiment
Scientists	❖ Alfred Hershey ❖ Martha Chase
Year	1952
Experimental Organisms used	❖ *Escherichia coli* ❖ T_2 Bacteriophage

Experiment

1. Labelling of bacteria with radioactive sulphur (^{35}S) and phosphorus (^{32}P)

Culture 1

- ❖ *E. coli* supplied with ^{35}S
- ❖ ^{35}S become part of bacterial proteins (because of ^{35}S containing cysteine and methionine amino acids).

Culture 2

- ❖ *E. coli* supplied with ^{32}P
- ❖ ^{32}P gets incorporated into nucleotides of bacterial DNA.

2. Introduction of bacteriophage T_2 to the radioactive bacterial cultures and production of radioactive bacteriophages.

Culture 3

- ❖ Progeny bacteriophage with radioactive protein coat were formed.

Culture 4

- ❖ Progeny bacteriophage with radioactive DNA were formed.

3. **Infection** - Introduction of radioactive bacteriophages in separate cultures with normal *E.coli.*

Culture 5

- ❖ Normal *E. coli* infected by bacteriophage with radioactive protein coat

Culture 6

- ❖ Normal *E. coli* infected by bacteriophage with radioactive DNA

4. **Blending** - Viral coats removed from the bacteria by agitating culture 5 and 6 separately in a blender.
5. **Centrifugation** - The two cultures (5, 6) were separately centrifuged to separate virus particles or viral protein coat from bacteria.

	Culture 5	Culture 6
Lighter supernatant	Radioactive viral coat (^{35}S, in the amino acids of the protein coat)	Viral particles not radioactive
Heavier pellet	Bacteria do not show any radioactivity	The infected bacteria show radioactivity (DNA with ^{32}P)

Conclusion DNA passes from the virus to bacteria. Thus, DNA is the genetic material.

Summary

REPLICATION		
Experimental Proof of Semiconservative DNA Replication		
EXPERIMENT	**YEAR**	**CONCLUSION**
Meselson and Stahl's Experiment ❖ Worked on *E. coli.* ➢ Grew *E. coli* in a medium having $^{15}NH_4Cl$. ➢ Transferred these *E. coli* into $^{14}NH_4Cl$ medium. ➢ Extracted DNA samples after 20 minutes and 40 minutes by centrifugation in a CsCl density gradient.	1958	Each newly synthesised DNA molecule has one parental and one newly synthesised strand (Semiconservative replication)
EXPERIMENT	**YEAR**	**CONCLUSION**
Taylor and colleagues ❖ Worked on *Vicia faba* (faba beans) ➢ Detected the distribution of newly synthesised DNA in the chromosomes using radioactive thymidine.	1958	DNA in chromosomes also replicate semiconservatively.

PROCESS OF REPLICATION

- Activation of deoxyribonucleotides by phosphorylase.
- Unwinding of DNA by helicase at 'ori' site.
- Stabilising the separated DNA strands by single stranded binding proteins.
- Releasing of tension in the uncoiled DNA by topoisomerase.
- Formation of Y-shaped replication fork.
- Formation of RNA primer at 5' end of new DNA strand by primase.
- DNA polymerase polymerises dNTPs in 5'→3' direction on the primer on both new DNA strands.
- On parent strand with 3'→5' polarity, its complementary new strand is continuously formed (leading strand). Replication is continous.
- On parent strand with 5'→3' polarity, replication is discontinuous. Okazaki fragments are formed, later joined by DNA ligase (lagging strand).

Summary

TRANSCRIPTION

(Copying of genetic information from one DNA strand into RNA)

In Prokaryotes (occurs in the cytoplasm)

Process

- Activation of ribonucleotides by phosphorylase.
- Binding of RNA polymerase to the promoter and unwinding of DNA to expose the template strand.
- Base pairing between NTPs and nucleotides of template strand.
- Separation of sigma factor (σ, initiation factor).
- Elongation or polymerisation of mRNA.
- Completed mRNA chain (primary transcript) separates from DNA template at terminator region by the action of rho factor (ρ, termination factor).

In Eukaryotes (occurs in the nucleus)

3 different RNA polymerases

- RNA polymerase I $\rightarrow$ r RNAs (28S, 18S, 5.8S)
- RNA polymerase II $\rightarrow$ hnRNA $\rightarrow$ mRNA
- RNA polymerase III $\rightarrow$ tRNA, 5S rRNA, snRNAs

Post Transcriptional Modification

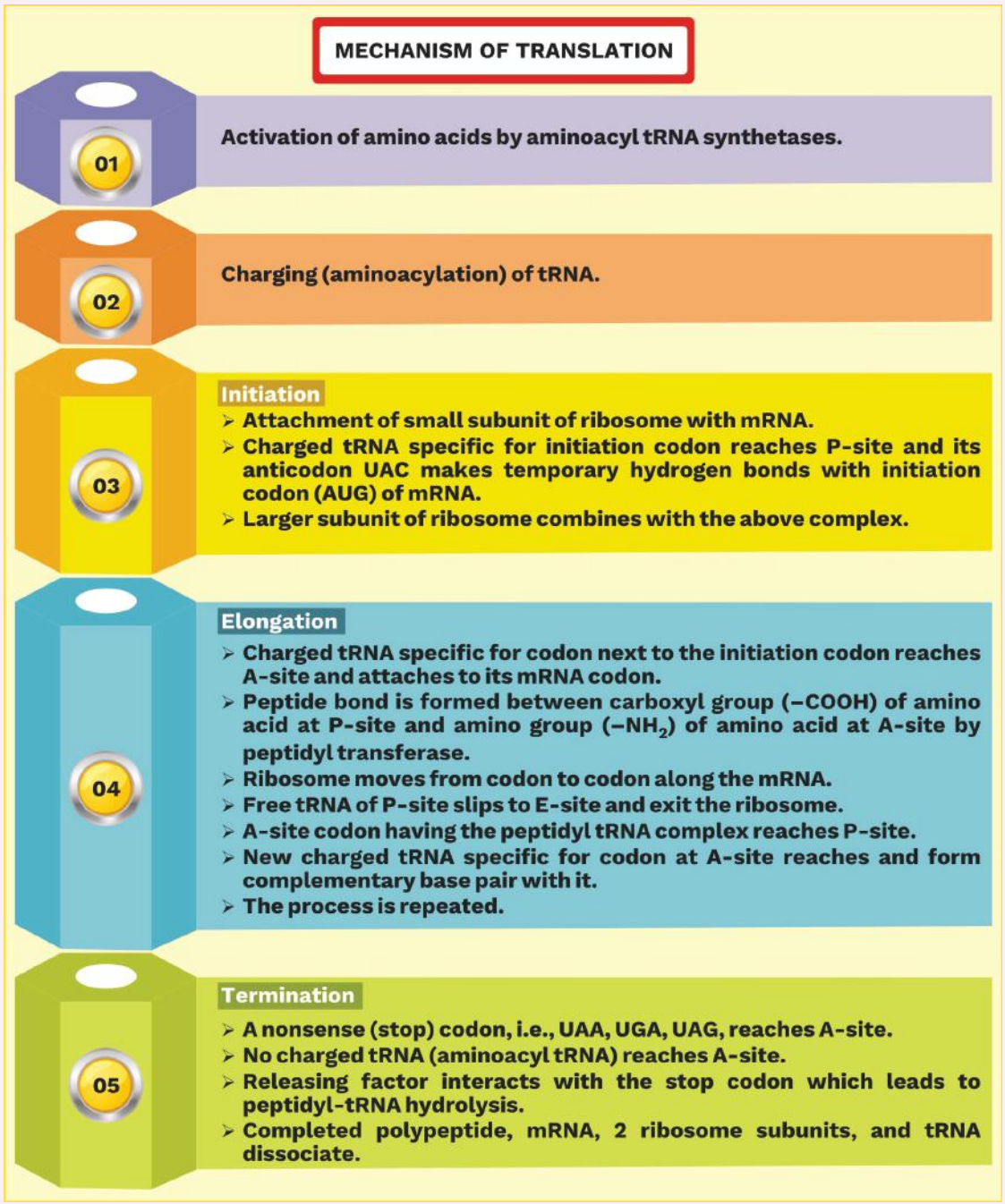
- Primary transcript (non-functional)
- Capping $\rightarrow$ Methyl guanosine triphosphate is added to 5' – end of hn RNA
- Tailing $\rightarrow$ 200 – 300 adenylate residues (poly - A segments) are added to 3'-end of hnRNA in a template independent manner

Primary transcript

Exons (coding sequences) $\rightarrow$ Joined by RNA ligase

Introns (non-coding sequences) $\rightarrow$ Removed by splicing

Summary


MECHANISM OF TRANSLATION
01
Activation of amino acids by aminoacyl tRNA synthetases.
02
Charging (aminoacylation) of tRNA.
03
Initiation
➢ Attachment of small subunit of ribosome with mRNA.
➢ Charged tRNA specific for initiation codon reaches P-site and its anticodon UAC makes temporary hydrogen bonds with initiation codon (AUG) of mRNA.
➢ Larger subunit of ribosome combines with the above complex.
04
Elongation
➢ Charged tRNA specific for codon next to the initiation codon reaches A-site and attaches to its mRNA codon.
➢ Peptide bond is formed between carboxyl group (–COOH) of amino acid at P-site and amino group (–NH2) of amino acid at A-site by peptidyl transferase.
➢ Ribosome moves from codon to codon along the mRNA.
➢ Free tRNA of P-site slips to E-site and exit the ribosome.
➢ A-site codon having the peptidyl tRNA complex reaches P-site.
➢ New charged tRNA specific for codon at A-site reaches and form complementary base pair with it.
➢ The process is repeated.
05
Termination
➢ A nonsense (stop) codon, i.e., UAA, UGA, UAG, reaches A-site.
➢ No charged tRNA (aminoacyl tRNA) reaches A-site.
➢ Releasing factor interacts with the stop codon which leads to peptidyl-tRNA hydrolysis.
➢ Completed polypeptide, mRNA, 2 ribosome subunits, and tRNA dissociate.