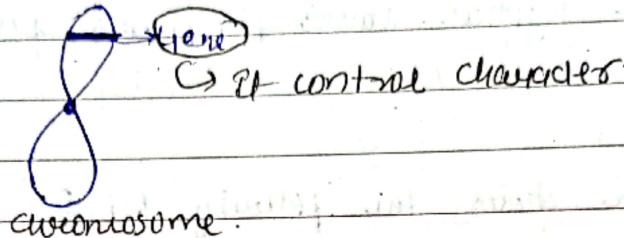


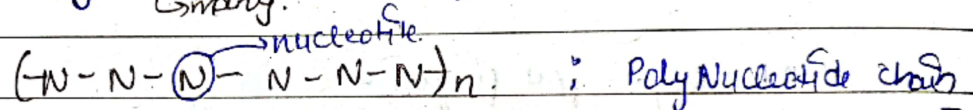
Molecular Basis of Inheritance

↓
"It is the transfer of characters [Features]"

- Unit of Inheritance: "Gene"

* THE DNA

- DNA is a long polymer of deoxyribonucleotides.

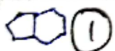


- length of DNA is usually defined as no. of nucleotides (a pair of nucleotide referred to as base pairs).

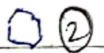
⇒ BASIC unit of DNA is a NUCLEOTIDE which has three comp

a) Nitrogenous Base

Two types:



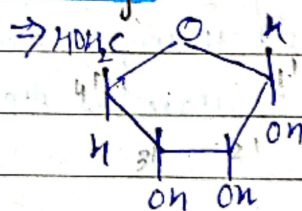
Purines: Adenine & Guanine



Pyrimidines: Cytosine, Uracil & Thymine.

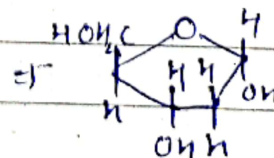
In DNA: A, G, C, T
is present.

In RNA: A, G, C, U is
present.

b) Pentose sugar

- Ribose sugar is present in case of RNA

- Ribose is nucleotide of RNA



- Deoxyribose sugar is present in DNA
- Deoxyribose is nucleotide of RNA

Date:

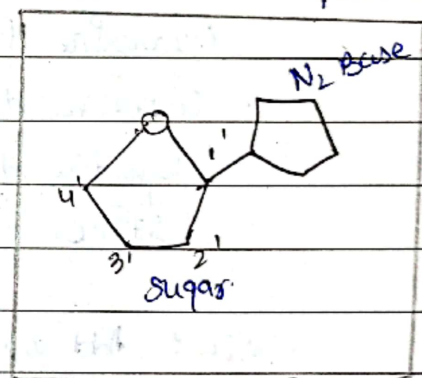
()

-

2)

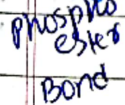
۲)

३



2) Pentose sugar +
N₂ Base

Nucleotide



phosphoester bond

POLYNUCLEOTIDE CHAIN.

Nucleoside :

Pentose sugar + N₂ Base = Nucleoside

- ① P.S + A = Adenosine
- ② P.S + G = Guanosine
- ③ P.S + C = Cytidine
- ④ P.S + T = Thymidine DNA ✓
RNA X
- ⑤ P.S + U = Uracine RNA ✓
DNA X

NOTE: For nucleosides of DNA add word deoxy in Prefix
e.g. P.S + A = deoxy Adenosine

Nucleotide

Nucleoside + P = Nucleotide

Adenoside + P = Adenosine mono phosphate (AMP)

Guanosine + P = Guanosine " " (GMP)

Cytidine + P = Cytidine " " (CMP)

Thymidine + P = Thymidine " " (TMP) DNA ✓
RNA X

Uridine + P = Uridine " " (UMP) RNA ✓
DNA X

NOTE: Add word deoxy for Nucleotides of DNA

e.g. deoxy Adenosine + P → deoxyadenosine mono phosphate (dAMP)

* Derivatives of DNA structure :

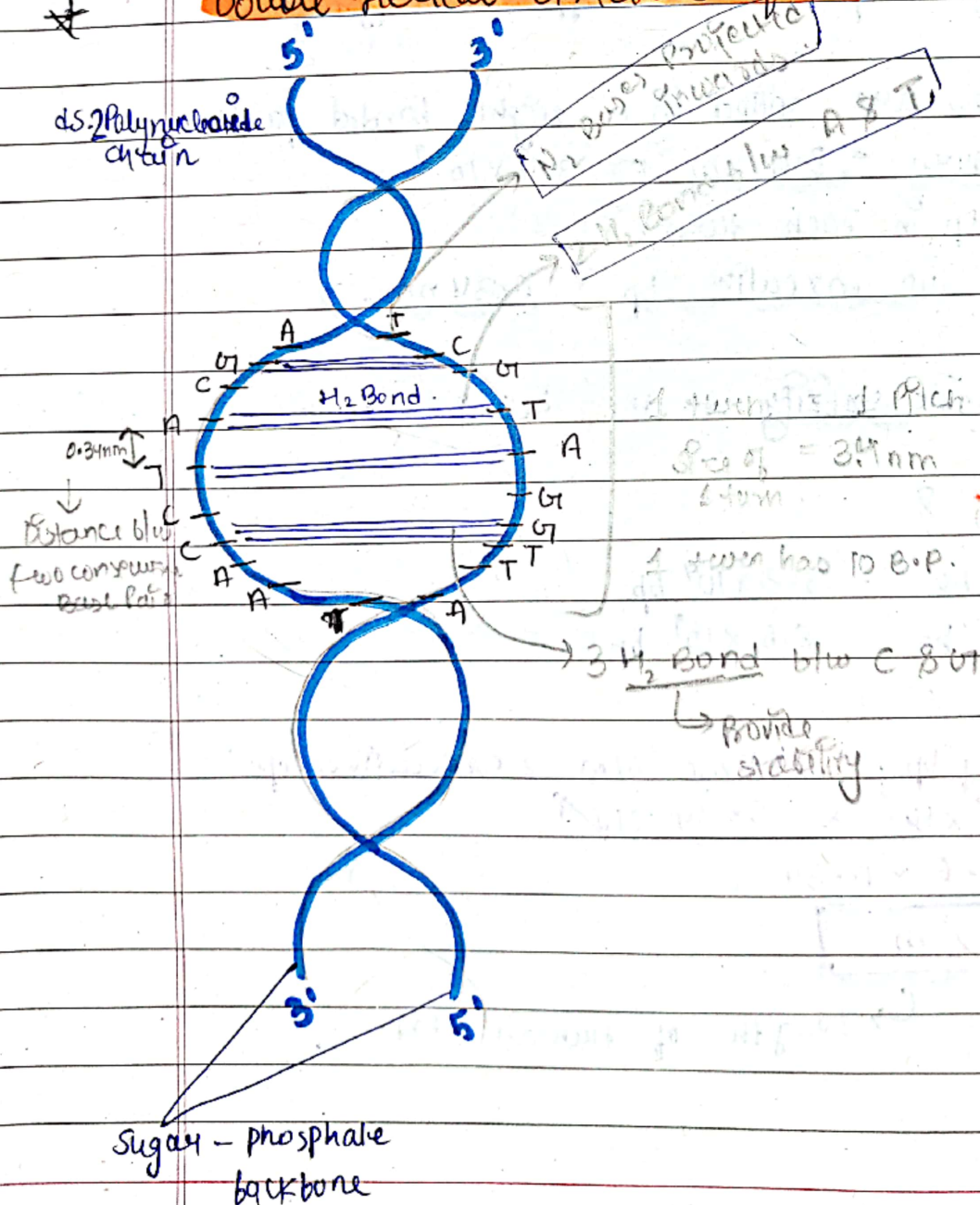
a) X-Ray Crystallography :

- given by Maurice Wilkins & Rosalind Franklin
- obtained very ^{small} x-ray diffraction pictures of DNA
- It was suggested that structure of DNA was sort of helix with 3.4 nm periodicity
- But they had not proposed a definitive model for DNA.

b) Erwin Chargaff's Rules :

- His rule was only applicable for double stranded DNA.
- Purines & pyrimidines occur in equal amounts.
 $(A, G) = (C, T)$
- $A + G = T + C$
- $\frac{A+G}{T+C} = 1$ this value is constant for all species.
- $A = T$, $G = C$
- Sugar deoxyribose & phosphate residues occur in equal no.

Double Helical Structure of DNA.



Salient Features of the Double Helix structure of DNA:

- * It is made up of 2 polynucleotide chains, where the backbone is constituted by sugar-phosphate, & the ^{base} 1 pair projected inside.
- The two chains have anti-parallel polarity. It means, if one chain has the polarity 5' → 3', the other has 3' → 5'.
- The bases in two strands are paired through H Bond, forming B.p.
 - ⇒ Adenine forms two hydrogen bond with Thymine [A = T]
 - ⇒ Guanine is bonded with cytosine with three Hydrogen bond [G ≡ C]
 - ⇒ Therefore, always a purine comes opp. to a pyrimidine.
- The two chains are coiled in a right handed fashion.
 - ⇒ Size of 1 turn = 3.4 nm or 3.4×10^{-9} m
 - ⇒ There is 10 bp in each turn.
 - ⇒ Distance b/w two consecutive bp = 0.34 nm.
- The H Bond provide stability to the helical structure.

Length of DNA?

1 strand has = 3.3×10^9 bp
2 strand has = 6.6×10^9 bp

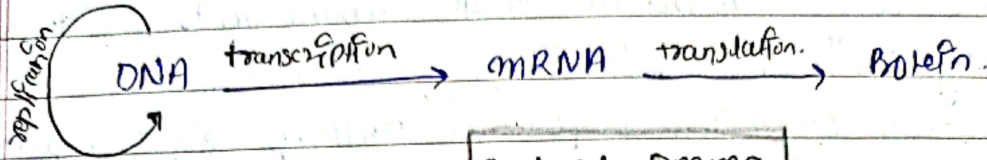
$$\begin{aligned} \text{Length} &= \text{No. of bp} \times \text{Distance b/w 2 consecutive bp.} \\ &= 6.6 \times 10^9 \times 0.34 \times 10^{-9} \\ &= 6.6 \times 0.34 \end{aligned}$$

$\text{Length} = 2.2 \text{ m.}$

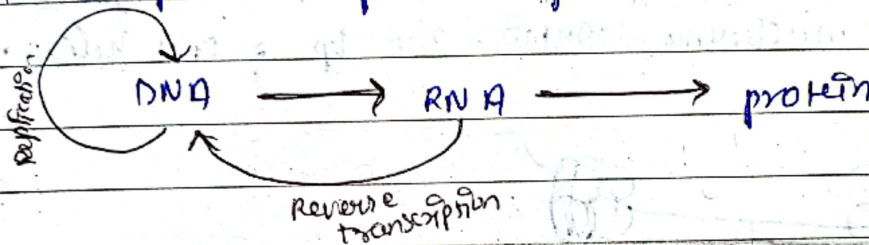
↪ Length of human DNA.

Handwritten: General flow of information

- Prepared by Francis Gick.



- Central Dogma
- eg: HIV virus $\rightarrow$ RNA as genetic material
- In some viruses the flow of information is reversed, from RNA to DNA in the presence of an enzyme "Reverse transcriptase"



Reverse central Dogma or Transcription

Consideration flow of information

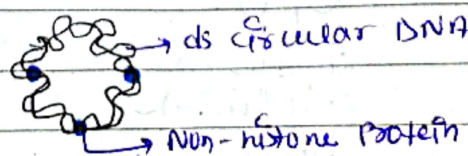
* Packaging of DNA Helix

① In: Prokaryotes

- length of *E. coli*^c DNA = 1.36 mm

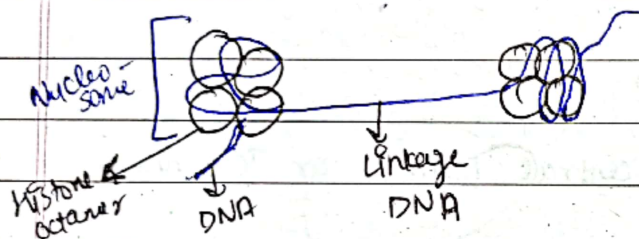
- DNA is packaged by non-histone basic proteins [Polyamines]
↓
+ve charged -ve charged.

- This DNA is present in a region termed as "nucleoid"

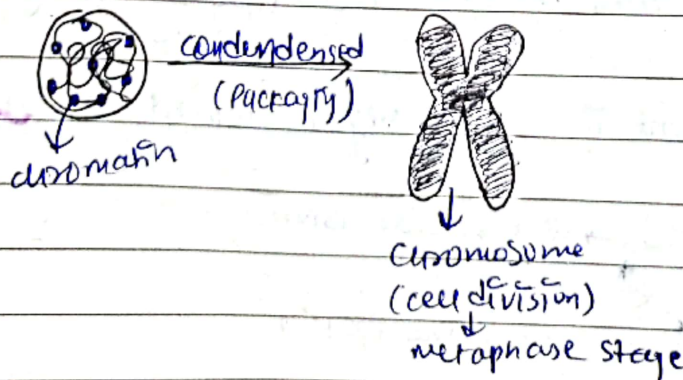


2) In Eukaryotes

- length of DNA = 2.2m [Human DNA]
- Nucleus size = small.
- packaged by histone protein.
- Histones are rich in the basic amino acids residues "lysines" & "arginines".
- Histones are organised to form a unit of eight molecules called histone octamer.
- The negative charged DNA is wrapped around the +ve charged histone octamer to form a structure called "Nucleosome".
- A typical nucleosome contains 200 bp of DNA helix.



- Nucleosomes constitute the repeating unit of structure in nucleus called ~~nucleosome~~ "chromatin". It is a thread like stained bodies seen in nucleus.
- The nucleosomes in chromatin are seen as "beads on string" structure when viewed under microscope.
- The chromatin is further coiled & condensed at metaphase stage of cell division to form "chromosomes".



- The packaging of chromatin at higher level requires a specific set

of protein $\rightarrow$ "Non-histone chromosomal [NHC] proteins"

There are two types of chromatin in a nucleus:

Euchromatin

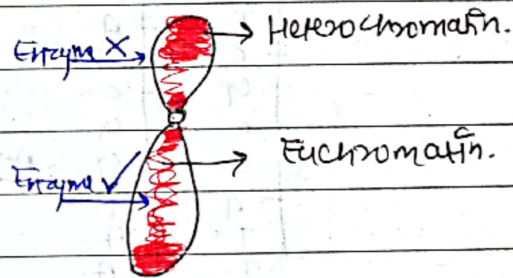
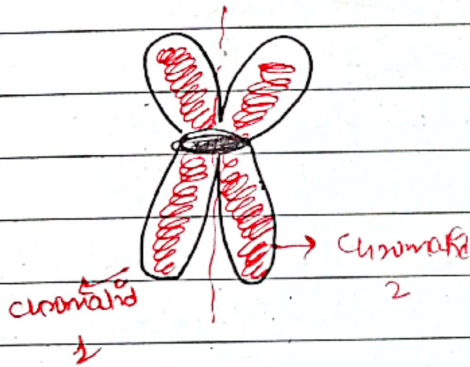
Heterochromatin

$\Rightarrow$ Some region of chromatin are loosely packed [stains light] k/a "Euchromatin"

$\Rightarrow$ The chromatin that is more densely packed & stains dark k/a Heterochromatin.

$\Rightarrow$ It is transcriptionally active ^{undergo process of converting into RNA}

$\Rightarrow$ Inactive



Chargaff's question:

In DNA (ds), $A = 28\%$ $\&$ $C = ?$
In ds DNA, $A = T$ $\&$ $C = G$

If $A = 28\%$, then $T = 28\%$

$28 + 28\% = 56\% = A + T$

$100 - 56\% = 44\% = G + C$

$44\% = G + C$

$22\% = G$
 $22\% = C$

Q DNA molecule has 2 bp. then calculate: ^{means 4 bases.} Sugar, Phosphate, Glycosidic bond & Phosphodiester bond.

Ans No. of Bases = No. of nucleotide = Sugar = No. of Phosphate
= No. of Glycosidic Bonds = No. of phosphodiester bonds
DNA is Circular

* If DNA is linear, then for calculating P.D.E.B.,
 $P.D.E.B = B - 2$
 $= 4 - 2 = 2$ [Linear DNA]

Q In a strand, find corresponding strand?

+	A	+	T
+	T	+	A
+	C	+	G
+	G	+	C
+	C	+	G
+	C	+	G
+	A	+	T
+	T	+	A
+	T	+	A
+	A	+	T

- AC

* The search for genetic material:

① Transforming Principle:

- Conducted by Frederick Griffith in 1928.
- Clear evidence in establishing the nature of genetic material.
- He performed series of experiments by selecting two strains of bacterium "Streptococcus pneumoniae".

a) S-III
Smooth strain

b) R-II
Rough strain.

⇒ S-strain^{bacteria} is smooth because it has mucous [polysaccharide] coat.
→ Glycocalyx (mucous + polysaccharide)
→ Cell wall

⇒ R-strain bacteria is rough because it has not the coat.

- S-strain / smooth or capsulated type have a mucous [polysaccharide] coat & produce smooth shiny colonies in culture plate. These are ~~virulent~~ ^{pathogenic} & cause pneumonia.
- R-strain / rough or non-capsulated type have no mucous coat & produce rough colonies. These are non-virulent & not cause pneumonia.

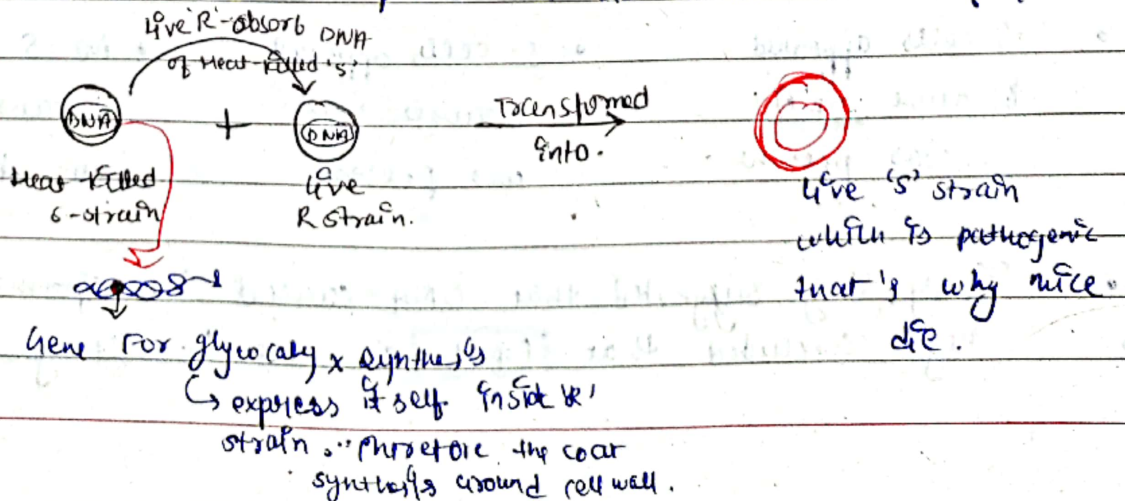
The experiment can be describe as follows:

S strain → Infected into mice → mice die
R strain → Infected into mice → mice live

S strain → " → mice live
[heat-killed]

S strain + R strain → Infected → mice die
[heat-killed] [live]

- He concluded that the R-strain bacteria had somehow been "transformed" by the heat-killed S strain bacteria.
- Some "transforming principle / substance", transferred from the heat-killed S-strain.
- The transforming substance had enabled the R-strain to synthesise a smooth polysaccharide coat & become virulent.
- This must be due to transfer of genetic material.
- He was not able to prove the biochemical nature of genetic material.



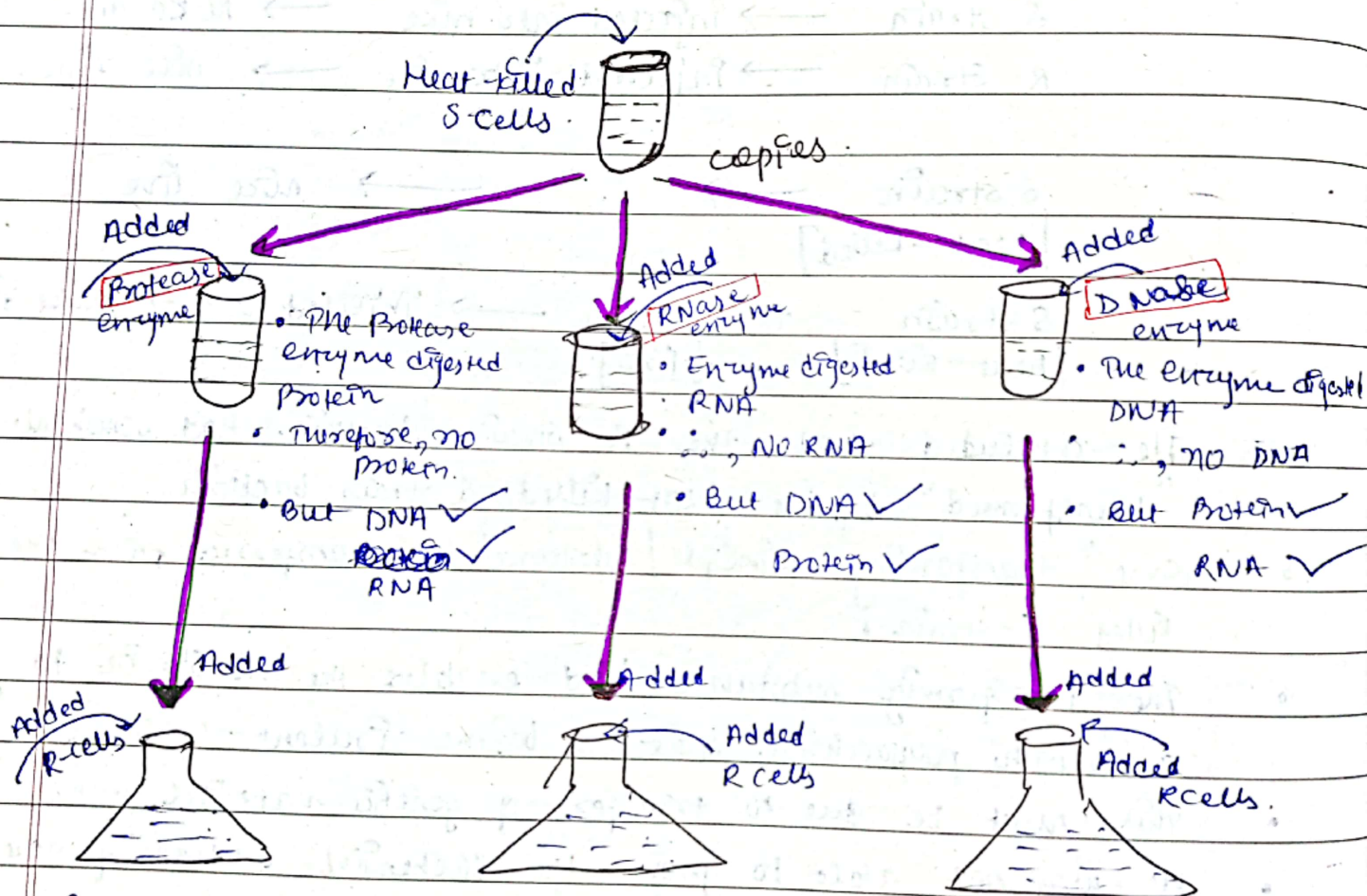
②

Biochemical characterisation of Transforming Principle

Oswald Avery, Colin MacLeod & Maclyn McCarty [1944] repeated the experiment in vitro to identify the biochemical nature of transforming substance.

The transforming substance is DNA, they proved.

Their experiment



S cells appeared because DNA was present.
Die

S-cells appeared because DNA was present.
Die

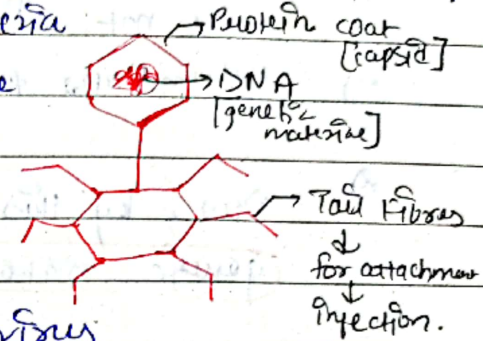
No S cells appeared because DNA was not present.
Mice lives

Finally, they suggested that DNA caused transformation.
They concluded that DNA is the hereditary material.

The process of transfer of genetic material of bacteriophage from one bacteria to several other bacteria is k/a "transduction".

* The genetic material is DNA.

- The unequivocal proof that DNA is the genetic material came from the experiment of Alfred Hershey & Martha Chase in [1952].
→ k/a Transduction experiment/principle
- They worked with viruses that infect bacteria called "bacteriophage".
- The bacteriophage attaches to the bacteria & its genetic material then enters the bacterial cell (injection).
- Bacterial cell treats the viral genetic material as if it was its own.
- And subsequently manufactures more virus particles.
- Hershey & Chase discovered whether it was protein or DNA from virus that enters bacteria.



Experiment

- 1) Hershey & Chase grew some viruses on a medium that contained radioactive phosphorus [^{32}P] & some others on the medium that contained radioactive sulphur.
- 2) ~~radioactive~~ ~~phosphorus~~ ~~viruses~~ ~~grown~~ ~~in~~ ~~presence~~ ~~of~~ ~~radioactive~~ ~~phosphorus~~ contained radioactive DNA but not radioactive protein, because DNA contains phosphorus.
- 3) Viruses grown in presence of radioactive sulphur contained radioactive protein but not radioactive DNA, because DNA ~~contains~~ does not contain sulphur but proteins contain sulphur.
- 4) Radioactive phages were allowed to infect E. coli Bacteria.

- 5) As the injection proceeded, the viral coats were removed from the bacteria by agitating them in a blender.
- 6) Bacteria injected with viruses containing radioactive DNA were radioactive, whereas bacteria injected with viruses containing radioactive proteins were not radioactive.
- 7) This indicates that DNA enters the bacterial cell & not protein.
- 8) Thus, by this experiment, Hershey & Chase proved that DNA is genetic material.

* Properties of genetic material [DNA versus RNA]

Imp A molecule that can act as genetic material must fulfil the following criteria:

- 1) It should chemically & structurally be stable.
- 2) It should be able to generate its replica [replication]
- 3) It should provide the scope for slow changes [mutation] that are required for evolution.
- 4) It should be able to express itself in the form of "hereditary characters".

RNA → Genetic material [eg: Tobacco mosaic virus, ϕ 3 bacteriophage etc.]
Also → As messenger & adapter etc.

DNA → Predominant genetic material.

DNA more stable store of

RNA better transmission of

Genetic material.

Genetic material must fulfill	DNA	RNA
Replication	Yes	Yes
Stable chemically & structurally	• Structurally more stable • Because thymine provide more stability • Chemically less reactive	• Structurally less stable • Uracil provide less stability • Chemically more reactive because 2'-OH group.
Slow mutation per evolution	• Slower rate	• Faster rate.
Express in form of Mendelian characters	DNA → RNA → Protein Depends on RNA for synthesis of protein	• RNA → Protein - RNA can directly synthesise protein hence easily express character

* RNA world.

- RNA was first genetic material.
- Evidence shows that essential life processes [such as metabolism, translation, splicing etc] evolved around RNA.
- RNA used to act as a genetic as well as catalyst.

- RNA being a catalyst was reactive & hence unstable.
- Then, DNA has evolved from RNA with chemical modifications that make it more stable.
- DNA ~~is~~ being ds & having complementary strand further resist changes by evolving a process of repair.

* Replication mode

⇒ DNA replication is the biological process where a cell duplicates DNA molecules from one original strand.

(1953)

Watson-Crick model of semiconservative DNA replication

Scheme of Replication by Watson & Crick :

During the process of replication.



2 strands of DNA separate & act as template strand

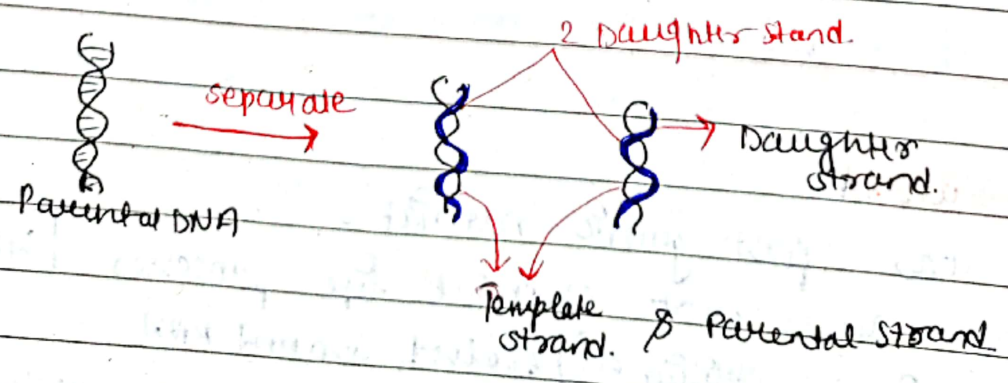


with the help of template strand.

2 new daughter strands are formed



After the completion of replication, Each DNA will have one parental strands & one newly synthesised strand.



* The experimental Proof

Meselson & Stahl Experiment

Taylor et al. Experiment

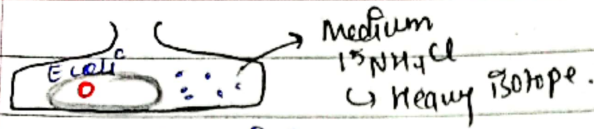
- ⇒ used prokaryotic DNA to proof semiconservative mode of replication
- ⇒ used heavy isotope of nitrogen $[^{15}\text{N}]$

- ⇒ used *vicia faba* beans [eukaryotic DNA]

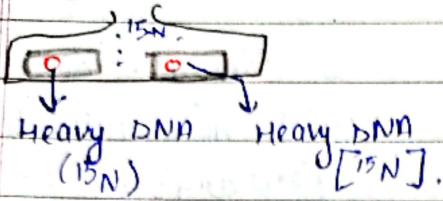
- ⇒ Not use heavy isotope, used radioactive isotope.
- ⇒ The experiment showed that DNA in chromosomes also replicate semiconservatively.

Meselson & Stahl Experiment [1958]

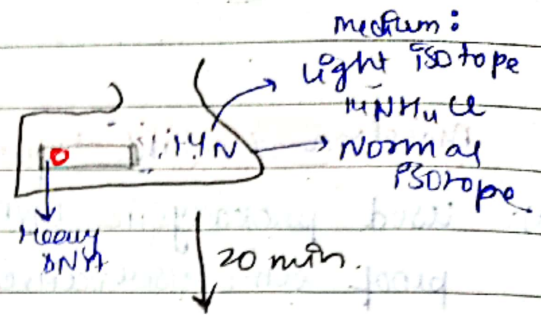
- They grew *E. coli* in a medium containing $^{15}\text{NH}_4\text{Cl}$ [^{15}N is the heavy isotope of nitrogen].
 - They grow *E. coli* in ^{15}N isotope of nitrogen for several generation.
 - The result was that ^{15}N was incorporated into newly synthesised DNA.
 - This heavy DNA molecule could be distinguished from the normal DNA by centrifugation in cesium chloride [CsCl] density gradient.
- Note: ^{15}N is not radioactive isotope, & it can be separated from ^{14}N only based on densities.
- Then they transferred the cells into a medium with normal $^{14}\text{NH}_4\text{Cl}$ & took samples at various definite time intervals as the cells multiply.
 - And then extracted the DNA that remained as double-stranded helices.
 - The various samples were separated independently on CsCl gradients to measure the densities of DNA.



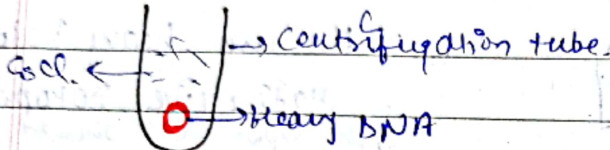
grown for several generations in ^{15}N



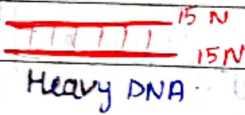
transferred
the DNA into.



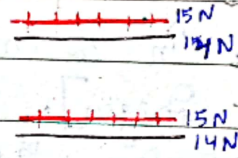
20 min.



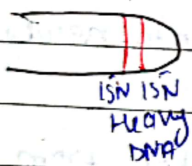
1st generation.



transferred into $^{14}\text{NH}_4\text{Cl}$ (20 min)

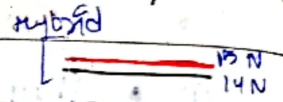
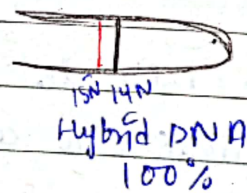


centrifugation by CsCl gradient density.

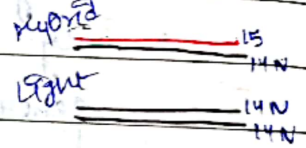


Hybrid generation.

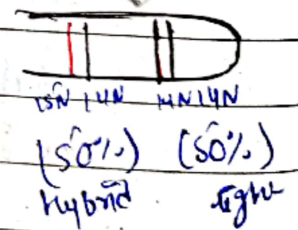
centrifugation



40 min in $^{14}\text{NH}_4\text{Cl}$



centrifugation



- (iii) • Thus, the DNA that was extracted from the culture one generation after the transfer from ^{15}N to ^{14}N medium [that is after 20 min because E. coli divides in 20 min] had a hybrid or intermediate density.
- DNA extracted from the culture after another generation [that is after 40 min, 2 generations] was composed of equal amounts of this hybrid DNA of 'light DNA'.

4 Enzymes involved in DNA replication:

① Helicase: Unzipping enzyme

③ RNA Primase

④ Ligase: Gluing enzyme

② DNA dependent DNA polymerase: Builder enzyme

Page No.
Date:

* The Machinery & the Enzymes / DNA replication mechanism.

- The process of replication in living cell such as *E. coli* requires a set of enzymes.
- The main enzyme is referred to as DNA-dependent DNA polymerase-III, since it uses DNA template to catalyse the polymerisation of deoxynucleotides.
- These enzymes are highly efficient with the ability to polymerise some 2000 bp per second.
- Not only these polymerase have to be fast, but they also have to catalyse the reaction with high degree of accuracy.
- Any ^{during replication} mistake will result in mutation.
- The whole genome of *E. coli* having 4.6×10^6 bp is replicated within 18 minutes.
- Energetically replication is a very expensive process.
- Deoxynucleoside triphosphates serve dual purposes:
 - ① In addition to acting as substrate
 - ② Provide energy for polymerisation reaction [the two terminal phosphates in a deoxynucleoside triphosphate are high-energy phosphates, same as in case of ATP]
- In addition to DNA-dependent DNA polymerases many additional enzymes are required to complete the process.
- Since, the two strands can't be separated in its entire length [due to high energy requirement].
- The replication occurs within a small opening of DNA helix k/a "Replication Fork".
- One strand [the template with polarity $3' \rightarrow 5'$], the replication is continuous.
- Another strand [polarity $5' \rightarrow 3'$], the replication is discontinuous.
- Later the fragments are joined by "DNA ligase".

- DNA polymerases on their own can't initiate the process
- There is a definite region where the replication ~~is~~ originates k/a origin of replication.

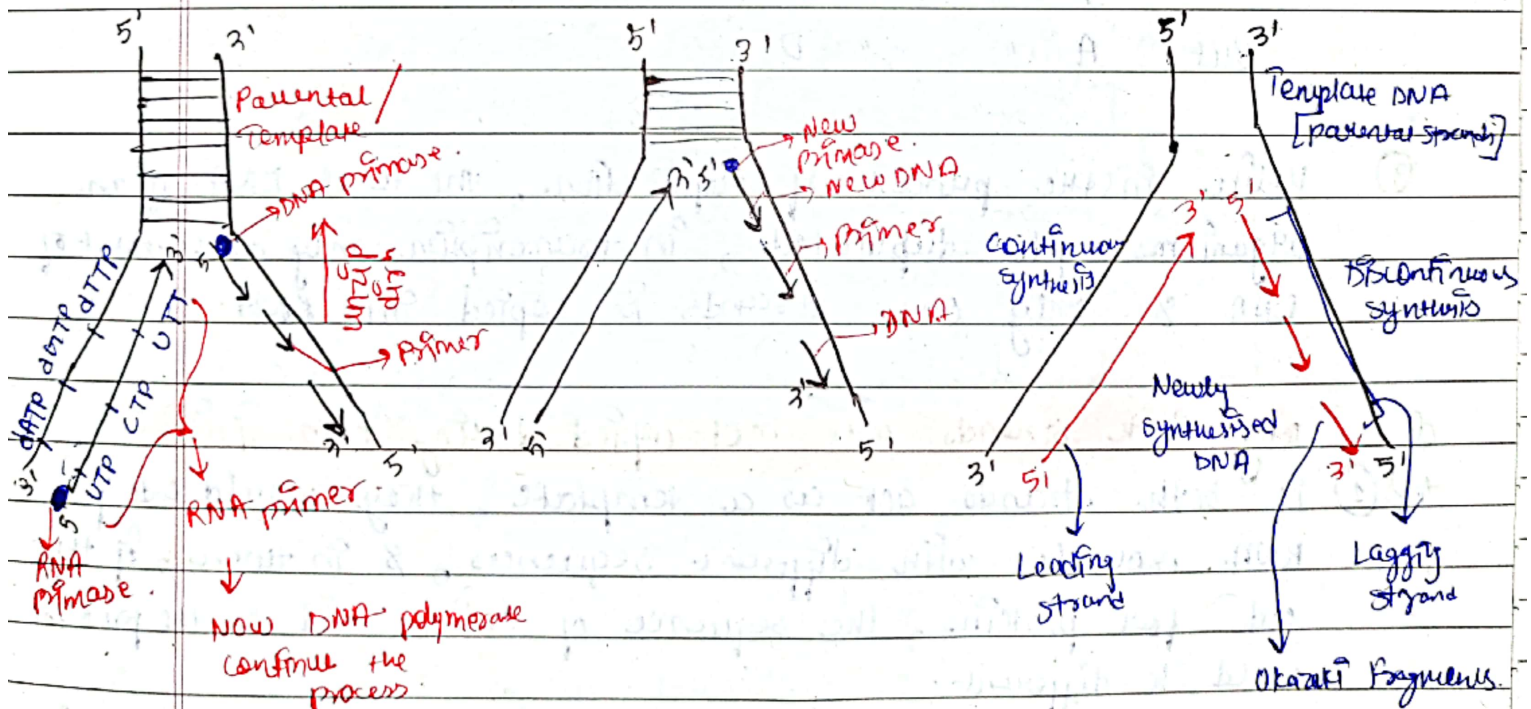
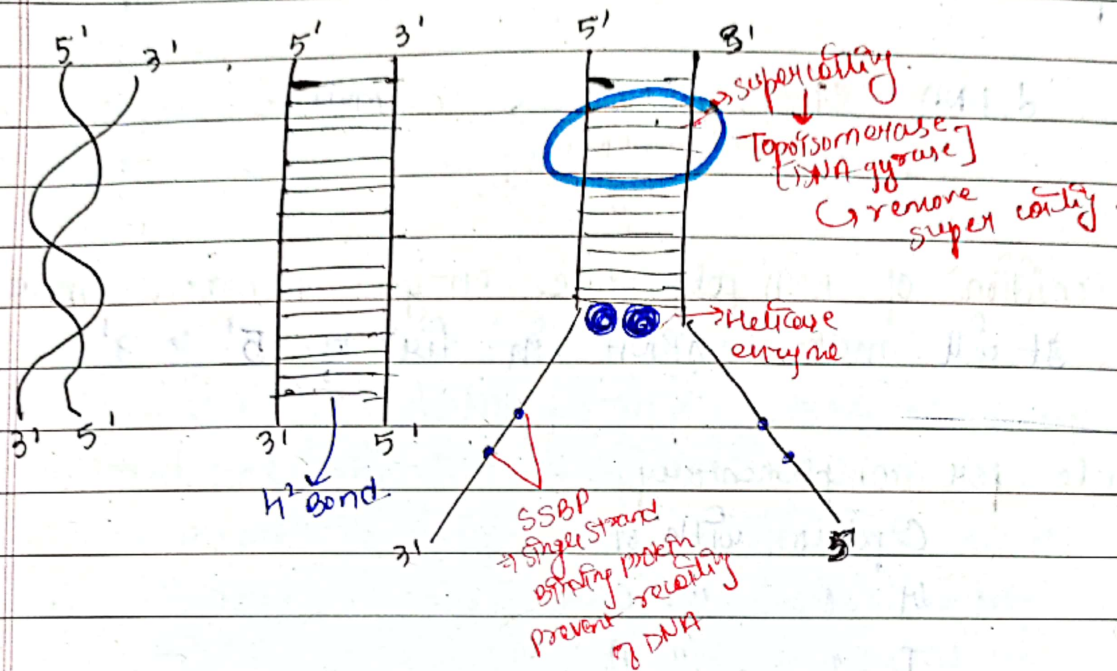
Q (i) Explain the process of DNA replication with the help of diagram.

Q (ii) In which phase of the cell cycle does replication occur in eukaryotes? what would happen if cell-division is not followed after DNA replication?

Ans (i) Process of DNA replication:

- The process of DNA replication begins at a point called the origin of replication [ori], to form a replication fork.
- Activation of deoxyribonucleotides:
 - ⇒ Deoxyribonucleotide serve dual purposes:
 - ① In addition to acting as substrates
 - ② Provide energy for replication reaction.
- The Helicase enzyme bind to DNA strand & breaks the H₂ bond.
- DNA can't be separated fully, so the replication occur within a small opening of DNA helix k/a replication fork.
- Primase enzyme synthesizes RNA primers to start new strand synthesis.
- Now DNA polymerase will come & continue the process.
- DNA polymerase adds nucleotides in the 5' to 3' dirⁿ.
- On one strand [polarity 3' → 5'], the replication is continuous so the newly synthesised DNA is k/a leading strand.
- The other strand [polarity 5' → 3'], the replication is discontinuous so the discontinuous synthesised DNA fragments is k/a Okazaki fragment & the strand is k/a lagging strand.
- DNA polymerase checks & corrects wrongly paired nucleotides
- DNA ligase enzyme joins the Okazaki fragments with the help of phosphodiester bond.

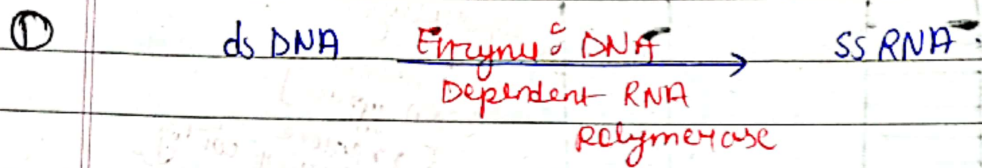
- Topoisomerase prevents tangling ahead of the fork
- After the entire DNA is copied, the two double helices separate



(ii) DNA replication takes place in S-phase [Synthetic phase] of cell cycle in eukaryotes. If this replication of DNA is not followed by cell division the DNA contents of the cell will get doubled i.e., duplicate set of DNA will be formed. This will result in aneuploidy.

* Transcription

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



② ⇒ Condition of RNA polymerase enzyme to make mRNA is that, it will make mRNA in dirⁿ of 5' to 3'.

③ Rule for complementarity

C pairs with G

G " " C

T " " A

But A " " U

④ Unlike in the process of replication, the total DNA of an organism gets duplicated, in transcription only a segment of DNA & only one strand is copied into RNA.

Q. Why both strands are not copied during transcription?

Ans ① If both strands act as a template, they would code for RNA molecule with different sequences, & in turn, if they code for proteins, the sequence of amino acids in the proteins would be different.

Hence, one segment of the DNA would be coding for two different proteins & this would complicate the genetic information transfer machinery.

② The two RNA molecules formed would be complementary to each other, & hence would form a ds RNA, which can't be translated into protein.

③ Therefore, only one strand [template strand] is transcribed to ensure correct & useful RNA synthesis.

* Transcription unit

• specific!

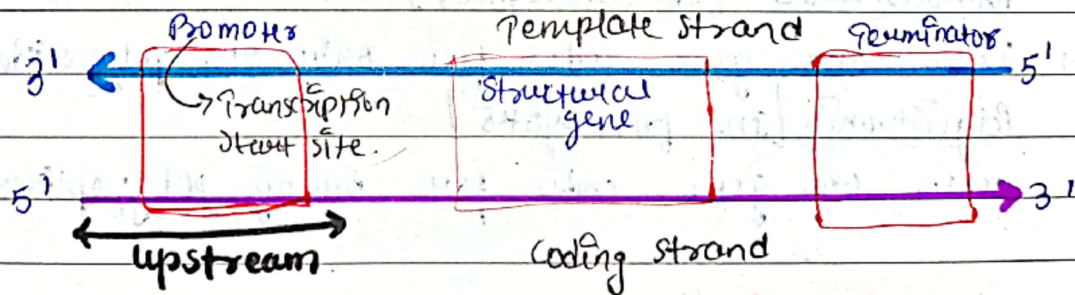
↳ initiates transcrip.

① A promoter: A DNA sequence where RNA polymerase binds

② The structural gene: The sequence of DNA which forms mRNA

③ A Terminator: A specific DNA sequence in a transcription unit that signals RNA polymerase to stop transcription.

• The strand which is used for synthesising RNA has polarity in $3' \rightarrow 5'$ dirⁿ [template strand].



• Structure of a transcription unit

• The presence of a promoter in a transcription unit that defines the template & coding strands.

• The promoter is located towards 5' end of structural gene. [The reference is made with respect to the polarity of coding strand].

- The terminator is located towards 3'-end [downstream] of the coding strand.

* Transcription unit of the gene.

- A gene is defined as the functional unit of inheritance.
- Genes are present on DNA.
- They carry information to make proteins or RNA molecules [like tRNA, rRNA]

• Defining a gene in terms of DNA

- ⇒ DNA sequence coding for rRNA or tRNA is also called a gene.
- ⇒ Gene can be defined as a Genon [a segment of DNA which code for polypeptide/protein].

• Types of genes [Based on Genon]

① Monocistronic [In eukaryotes]

- ⇒ when one gene codes for only one polypeptide

② Polycistronic [In prokaryotes]

- ⇒ when one gene codes for many polypeptides

• Gene structure in Eukaryotes.

- ⇒ ^{structural} Genes in eukaryotes are "split genes"

- ⇒ Two types of sequences:

• Exons: Exons are said to be those sequence ~~expressed~~ that appears in mature or processed RNA.

• Introns: This is non-coding sequence which do not appear in mature or processed RNA. [removed during RNA process]

• Complication in defining gene

- ⇒ "Because of exons & introns, it is hard to simply call a gene a "DNA segment"."

- Role of Regulatory sequences :
- Gene expression is also controlled by Promoter & regulatory sequences of a structural genes.
- These sequences do not code for protein or RNA but still affect inheritance.

- * Transcription in Prokaryotes. [Also in Prokaryotes site of translation is also cytoplasm]
- Occurs in "cytoplasm" with the help of transcribing enzyme.
- Transcribing enzyme that is, DNA-dependent RNA polymerase is only of one type that transcribe all of type of RNAs : mRNA, tRNA & rRNA.

Steps :

① Initiation

- RNA polymerase catalysed by sigma (σ) factor or initiation factor.
- Sigma factor along with a RNA polymerase binds to the promoter site of DNA.
- The RNA core polymerase [minus sigma factor] moves down DNA at a faster pace and this continues to synthesise a new RNA chain.

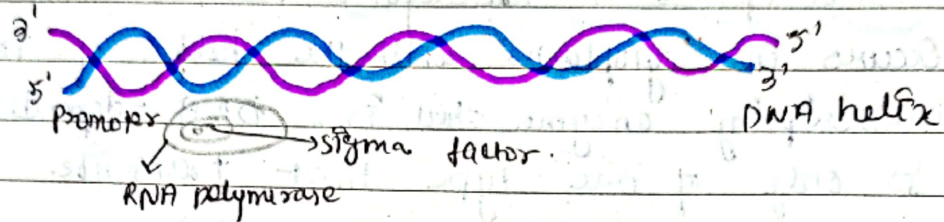
② Elongation

- Ribonucleoside triphosphates help in the polymerisation on a DNA template.
- The RNA polymerase [core enzyme] is only capable of catalysing the process of elongation.
- The enzyme facilitates the opening of DNA helix & continues elongation.

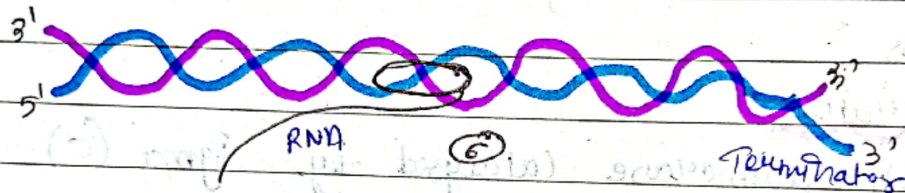
③ Termination:

- When RNA polymerase reaches the terminator region, another protein particle the rho (ρ) factor, forms a complex with RNA polymerase.
- This causes the enzyme to go off the DNA track, & thus new m-RNA is released.

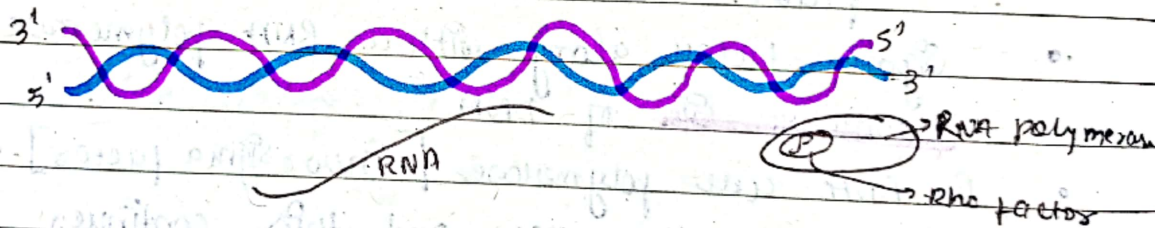
Initiation:



Elongation:



Termination:



* Transcription in Eukaryotes:

There are three types of transcriptional enzymes i.e., RNA polymerase

- 1) RNA polymerase I: Synthesis 5.8S, 18S, 28S rRNA
- 2) RNA polymerase II: hnRNA [heterogeneous nuclear RNA]
- 3) RNA polymerase III: tRNA, 5S rRNA & snRNAs [small nuclear RNA]

① Initiation

- Takes place in "nucleus".
- Eukaryotes have three types of RNA polymerase:
 - i) RNA polymerase I $\rightarrow$ rRNA [except 5S rRNA]
 - ii) RNA polymerase II $\rightarrow$ mRNA [code for proteins]
 - iii) RNA polymerase III $\rightarrow$ tRNA & 5S rRNA

② Elongation

- RNA polymerase moves along the DNA template strand & adds complementary ribonucleotides [A, U, G, C]
- The RNA strand grows in the $5' \rightarrow 3'$ dirⁿ
- Only a short stretch of DNA is open at a time

③ Termination

- When RNA polymerase reaches a terminator sequence, transcription stops.
- The newly formed RNA molecule is released.

* Post-Transcriptional Processing [unique to eukaryotes]

The primary transcript [hnRNA] made by RNA polymerase II is not functional because it contains the functional exons & non-functional introns. It must be processed:

- ① Capping: At the 5' end, a methyl guanosine triphosphate is added.

Function: Protects mRNA from degradation, helps in ribosome binding.

- ② Tailing: At the 3' end, a stretch of adenylate residues [200-300] [poly-A-tail] is added.

Function: stability of mRNA, transport from nucleus to cytoplasm.

- ③ Splicing: Non-coding sequences [introns] are removed & coding sequences [exons] are joined.

Function: Produce a continuous coding sequence.

⇒ The final product is functional mRNA, which leaves the nucleus for translation.

Capping	Splicing	Poly
① Addition of methyl Guanosine triphosphate	① Removal of Introns	① Addition of Adenylate
② At 5' end	② By snurps [snRNA + Protein]	② Also k/a Polyadenylation
③ Enzyme: Guanylate transferase	③ Represents donithance of RNA word	③ At 3' end
④ Provides binding site for ribosome		④ Enzyme: Poly A polymerase

Prokaryotic Transcription

- ① Cytoplasm
- ② RNA polymerase
- ③ σ factor & β factor require

Eukaryotic Transcription

- ① Nucleus (28S, 18S, 5.8S)
- ② RNA polymerase I: rRNA
II: mRNA
III: tRNA (5S, 5.8S, 5.4S, 22S, 23S, 28S, 29S, 30S, 31S, 32S, 33S, 34S, 35S, 36S, 37S, 38S, 39S, 40S, 41S, 42S, 43S, 44S, 45S, 46S, 47S, 48S, 49S, 50S, 51S, 52S, 53S, 54S, 55S, 56S, 57S, 58S, 59S, 60S, 61S, 62S, 63S, 64S, 65S, 66S, 67S, 68S, 69S, 70S, 71S, 72S, 73S, 74S, 75S, 76S, 77S, 78S, 79S, 80S, 81S, 82S, 83S, 84S, 85S, 86S, 87S, 88S, 89S, 90S, 91S, 92S, 93S, 94S, 95S, 96S, 97S, 98S, 99S, 100S)
- ③ No need.

* Genetic Code

- Genetic code is the sequence of nucleotides [Nitrogenous bases] in the mRNA that contains information for protein synthesis [Translation].
- The code is made up of 4 nucleotide eg: A, G, C, T
- Total 64 genetic code eg: AUU, AGA, UAA

U
C
A
G

$4 \times 1 = 4$ codes

1 Singlet code

	U	C	A	G
U	UU	UC	UA	UG
C	CU	CC	CA	CG
A	AU	AC	AA	AG
G	GU	GC	GA	GG

$4 \times 4 = 16$ codes

2 Doublet code

Not enough
for 20
amino acids

- But for 20 amino acids, the code should be made up of three nucleotides. $4^3 = 4 \times 4 \times 4$ would generate 64 codons.

CRICK, NIRENBERG, KORNBERG & LEADER: Tested on different kinds of errors by making of artificial mRNA & Triplet codes

* Salient features of genetic code

- The codon is triplet.
- 61 codons code for amino acids & 3 codons do not code for any amino acids, hence they function as stop codons.
- Some amino acids are coded by more than one codon, hence the code is degenerate. [except: AUG, UGA $\rightarrow$ Non Degenerate codon]
- The codon is read in mRNA in a contiguous fashion. There are no punctuations.
- The code is nearly universal: for eg, from bacteria to human UUU would code for Phenylalanine [Phe]
- Some exceptions to this rule have been found in mitochondrial codons & in some ~~pro~~ protozoans.

vi) AUG has dual functions: (1) It codes for methionine [Met]
(2) It also act as initiator codon

vii) UAA, UAG, UGA are stop terminator codons.

How many amino acids will code for amino acids

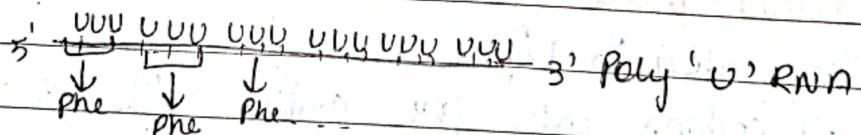
Ans: ^{These process with stop} AUG, GUA, UUU, UAG, UUG, GUA, UAA, UGA
3 codons will code for AA. because there is one UAG [stop codon].

* Scientists theories.

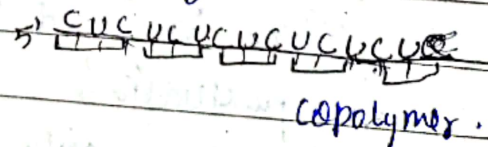
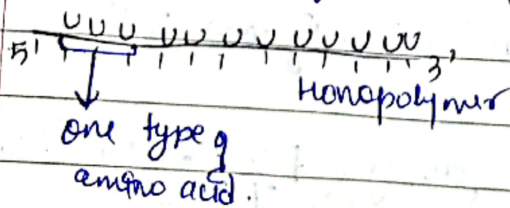
The idea that the genetic code must be triplet was first proposed by "Francis Crick" in 1961

The important discovery was the result of experiments by Marshall Nirenberg & J. Heinrich Matthaei & later by U. Khorana

Nirenberg & Matthaei used a synthetic poly U RNA & deciphered the code by translating this as "polyphenylalanine"



The chemical methods developed by Hae Gobind Khorana was instrumental in synthesizing RNA molecules with defined combinations of bases [homopolymers & copolymers].



"severo ocha" enzyme is "polynucleotide phosphorylase", it is helpful in polymerizing RNA with defined sequences in

a template-independent manner i.e., enzymatic synthesis of RNA.

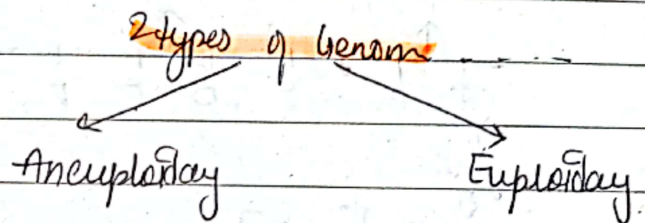
* Mutations & Genetic Code

- Sudden, discontinuous variation in Genotype & Phenotype of organism due to change in genetic material.

→ Types

① Chromosomal mutation [Numerical mutation]

→ change in no. of chromosome -

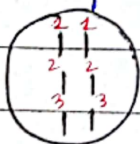


• Aneuploidy

- change in 1/few chromosomes.
- Gain or loss of chromosomes.

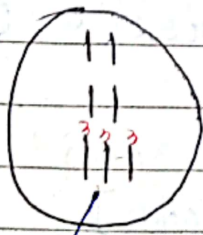
"Hyperploidy"

→ Gain of chrom.



$2n = 6$
[3 pairs of chrom.]

$+1^3$



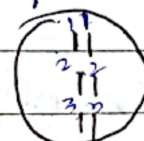
$n+1$

trisomy of 3rd chromosome

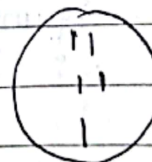
eg: Down Syndrome
Klinefelter syndrome

Hypoploidy

- loss of chromo...



-1^3



$n-1$

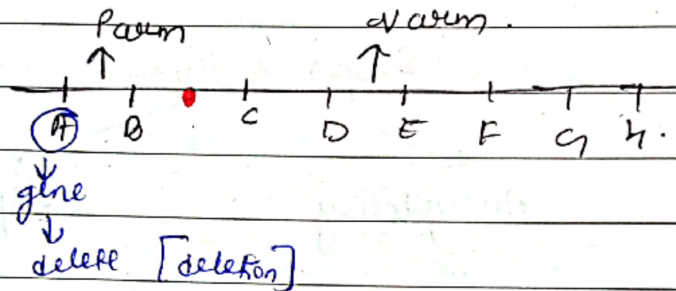
- Cause: Non-Disjunction of chromosome during cell division
separation

Euploidy

- change in set of chromosome.
- Addition or Deletion of complete set of chromosome
→ "Polyploidy" → Haploidy.

② Chromosomal Abberation [cancer cell due to two mutations]

- Change in "structure" of chromosome.



③ Gene mutation: Sudden change in nucleotide sequence of gene

① Point mutation:

- change in single base pair.
- eg: Sickle cell Anaemia [GAG → GTG, glutamic acid replaced by valine].

② Frameshift mutation

- caused by insertion or deletion of one or two bases
- changes the reading frames of codons → alters entire protein sequence.
- eg: Insertion / deletion experiment in NCERT book Pg no. 94

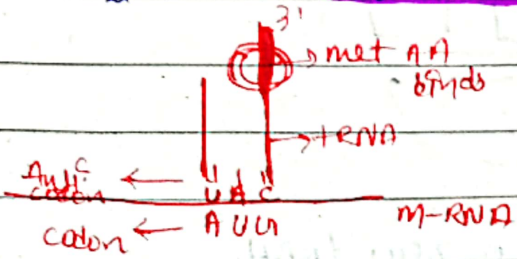
③ Insertion / deletion of 3 or multiples of 3 bases.

- Adds or removes whole codons [amino acids]
- Does not shift the reading frame, but protein structure is altered.

→ Transfer.

* t-RNA - The Adapter molecule.

- From the very beginning of the proposition of code.
- It was clear to Francis Crick that there has to be a mechanism to read the code & also to link it to AA.
- Amino acids can't directly read the genetic code on mRNA [because they don't have the "structural ability" to recognize codons].
- Therefore, an intermediate "adapter" was required to read the mRNA code & bring the correct amino acids.
- Earlier the tRNA, called SRNA [soluble RNA]
- Later recognized as transfer RNA [tRNA] because it transfers amino acids to the ribosome during protein synthesis.
- Act as an "adapter molecule" b/w codon [mRNA] & AA.
- t-RNA has an anticodon loop that has a triplet bases [anticodon] complementary to codon on mRNA.
eg: Codon = AGU [on mRNA], Anticodon = UCA [on tRNA].
- tRNA also has an amino acid accepter end [3' end].
- Each tRNA with anticodon UCA carries amino acid Serine [Ser].
- tRNA with anticodon AUG carries AA Tyrosine [Tyr].
- A special type of tRNA brings the first aa [Methionine in eukaryotes, formylmethionine in prokaryotes].
- There are no tRNA for stop codons.
- tRNA was shaped like a clover-leaf in 2D.
- looks like an inverted L in actual 3D.



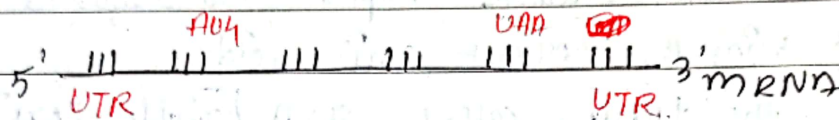
Translation

- The Process of synthesis of protein from mRNA with the help of Ribosome. → Protein factory of cell.
- Translation occurs in cytoplasm

⇒ Translation unit:

mRNA [5' → 3']

- Start codon [AUG]
- Region coding for a polypeptide
- Stop codon [UAA, UAG, UGA]
- Untranslated regions [UTRs] [5' & 3']



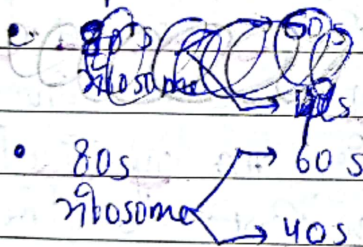
⇒ Ribosome [consist about 80 protein & structural tRNA]

smaller subunit

larger subunit

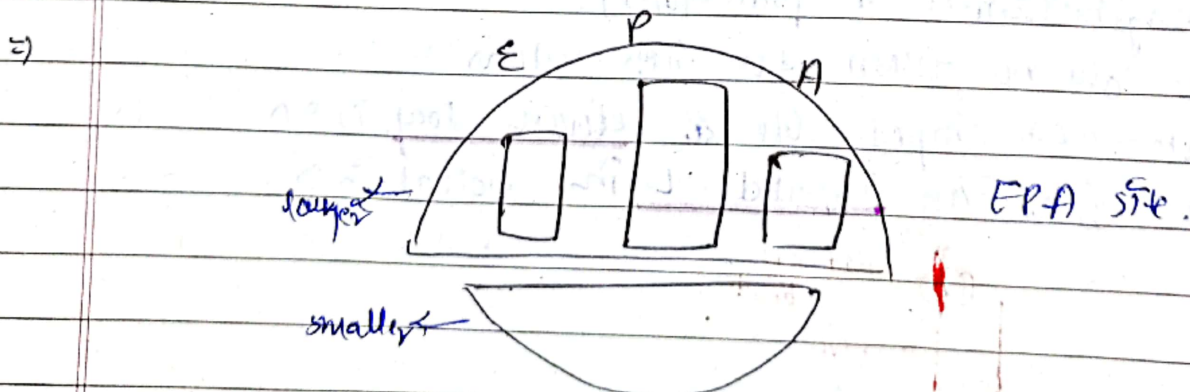
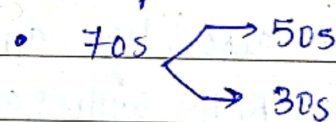
Eukaryotes

- mRNA contains cap & tail



Prokaryotes

- mRNA doesn't contain cap & tail

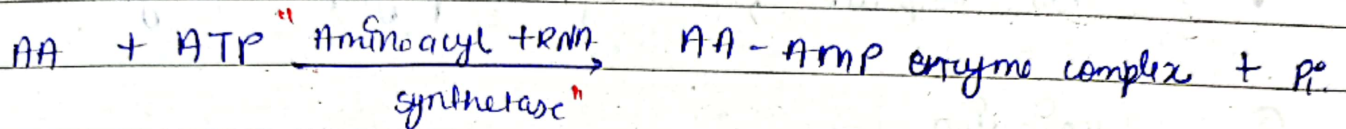


- A = Site for entry of new tRNA
- P = has tRNA containing the growing polypeptide chain
- E = through which a tRNA molecule after delivery of amino acid exits the Ribosome

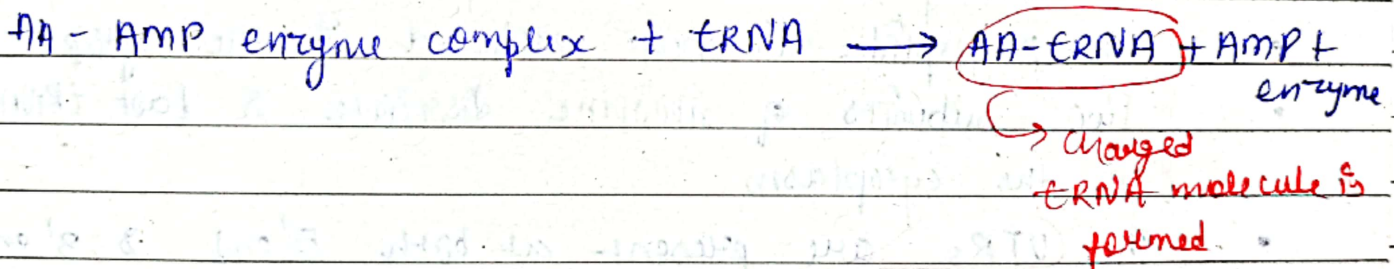
Mechanism

① Initiation

- Activation of Amino Acid.
- Amino acid binds with an enzyme named "aminoacyl tRNA synthetase".
- In the presence of ATP, 2 phosphate group leaves & now there is only one present that is "AMP" now.
- Amino acid & AMP enzyme complex is formed.



- Transfer of AA to tRNA / charging of tRNA / aminoacylation of tRNA.
- AA-AMP enzyme complex get attached to tRNA molecule, AA & tRNA binds together & AMP enzyme complex leaves.



- A small subunit of ribosome attaches to the mRNA at 5' end.
- The initiator codon, AUG is present on mRNA with initiates the process of protein synthesis.
- Initiator charged tRNA [with activated AA methionine] binds with the initiation codon [AUG] by its anticodon [UAC] through H-bonds.
- Then larger ribosome subunit joins, forming the complete ribosome.
- Ribosome has 3 sites: A [aminoacyl], P [peptidyl], E [exit].
- Initiator tRNA is placed at the P-site.

② Elongation

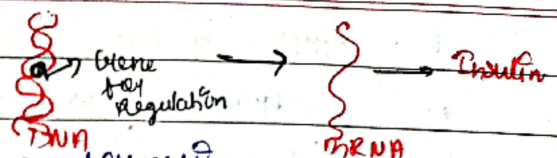
- A new charged tRNA carrying the next AA enters the A-Site
- "Peptidyl transferase" enzyme forms a peptide bond b/w the amino acids.
- Ribosome shifts [translocation] $\rightarrow$ tRNA moves :
 $\Rightarrow A \rightarrow P, P \rightarrow E, E \text{ exits}$
- This repeats, making the polypeptide longer.

③ Termination

- Towards the 3' end of mRNA, there is a stop codon [UAA / UAG / UGA]. It is exposed at the A-Site.
- It is not read & paired by the anticodon of any tRNA.
- The release factor binds to the stop codon, thereby terminating the translation process.
- The polypeptide is now released in the cytoplasm.
- Two subunits of ribosome dissociate & last tRNA is set free in the cytoplasm.
- The UTRs are present at both 5' end & 3' end. They are required for an efficient translation process.
- Finally mRNA is also released in the cytoplasm & get denatured by nuclease immediately.
- Hence mRNA is short-lived.

NOTE :

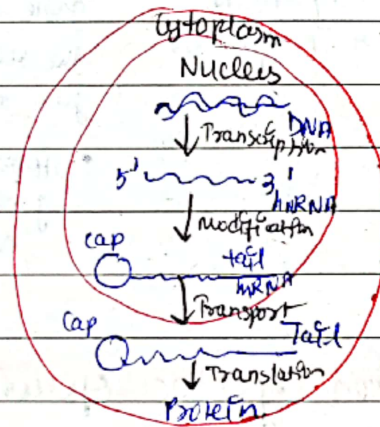
23S tRNA in Prokaryotes
28S tRNA in Eukaryotes.
forms Peptide bonds.

* Regulation of gene expression. eg: 

⇒ Controlling the amount & time of formation of gene products according to the requirements of the cell.

⇒ In eukaryotes, the regulation could be exerted at different levels:

- 1) Transcriptional level [formation of primary transcript]
- 2) Processing level [regulation of splicing]
- 3) transport of mRNA from nucleus to cytoplasm
- 4) Translational level.



* The Lac operon: [only for Prokaryotes].

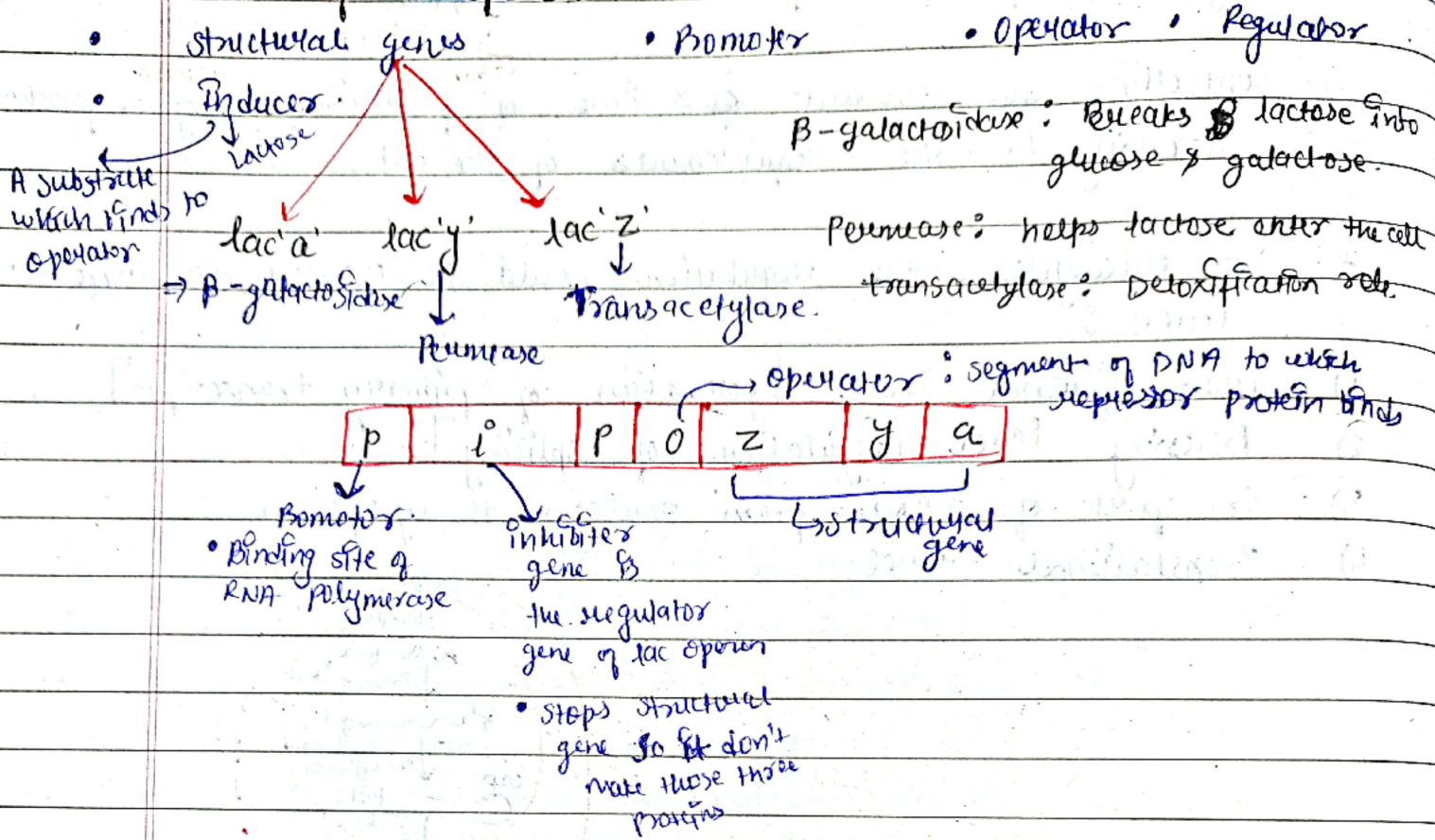
• By François Jacob [a geneticist] & Jacques Monod [Biochemist].

• Operon is a co-ordinated group of genes such as structural gene, operator gene, promoter gene, regulator gene which function together & regulates a metabolic pathway as a single unit.

OR

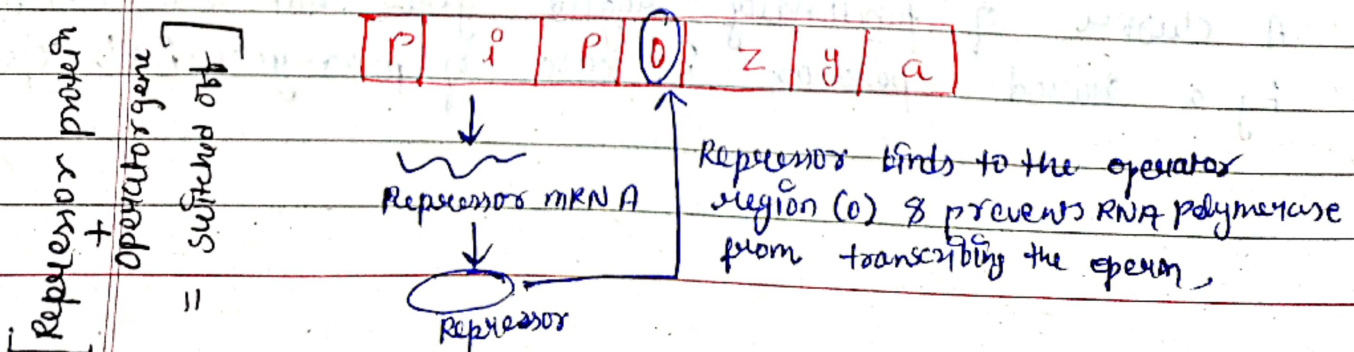
• A cluster of functionally-related genes that are controlled by a shared operator in case of prokaryotes is k/a "Operon".

Parts of lac operon.

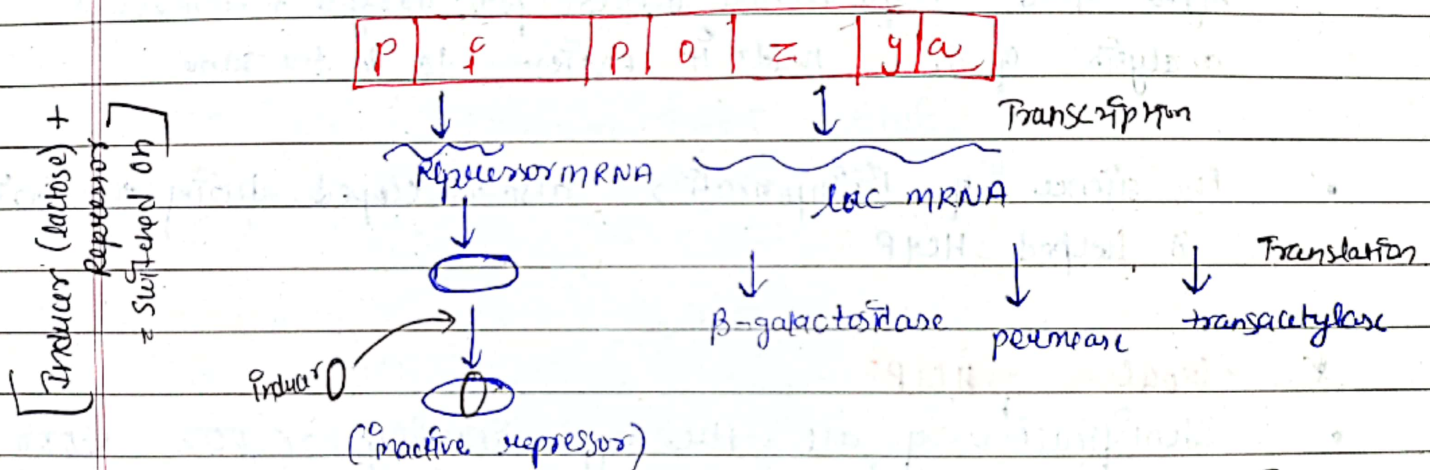


Function of lac operon:

- In the absence of inducer/lactose.
- When lactose is not present [absence of inducer].
- The regulatory gene produces the repressor protein.
- The repressor binds to the operator site.
- This binding prevents RNA polymerase from transcribing the structural gene.
- As a result, the enzymes needed for lactose metabolism are not produced.



- Functioning in the presence of lactose.
- =) when lactose is present
- =) lactose acts as an inducer by binding to the repressor protein
- =) this binding inactivates the repressor, preventing it from binding to the operator
- =) with the repressor inactive, RNA polymerase can bind to the promoter & transcribe the structural genes
- =) consequently, the enzymes [β -galactosidase, permease & transacetylase] are produced, allowing the cell to metabolise lactose



* Human Genome Project [HGP].
Launched in the year 1990
"Mega project" & also a "13 year-project"
Co-ordinated by the US department of Energy & the National Institute of Health.
soon welcome Trust (UK) joined the project as major partner additional contributions came from Japan, France, Germany, China & others.
The project was completed in 2003.

- Human genome is said to have approx. 3×10^9 bp.
- Huge cost estimated to be 9 billion US dollars, the cost of sequencing 1 bp is ~~1000~~ US\$3.
- Requires a large no. of technicians & supporting staff & scientists.
- Storage of data generated which requires some 3300 books, each with 1000 pages & each page having 1000 letters. However, high speed computational devices for storage, retrieval & analysis of data made it easier to do the same.
- The science of Bioinformatics also developed during this period & helped HGP.

* Goals of HGP:

- Identification of all the approximately 20,000 - 25,000 genes in human DNA.
- To determine the sequences of the 3 billion chemical base pairs that make up human DNA.
- To store this information in databases.
- Improve tools for data analysis.
- Transfer related technologies to other sectors, such as industries.
- Address the ethical, legal, & social issues [ELSI] that may arise from the project.

=> The knowledge about the effects of DNA variations, can lead to revolutionary new ways of diagnose, treat & prevent disorders that affect human beings.

=> Many non-human model organisms such as bacteria, yeast, *Caenorhabditis elegans* [a free living non-pathogenic nematode], *Drosophila* [fruit fly], plants [rice & *Arabidopsis*] etc. have also been sequenced.

* Methodologies:

The methods involved two major approaches:

- ESTs / Expressed Sequence Tags: Identifying all genes that are expressed as RNA.

- Sequence Annotation: Sequencing the whole set of genome that contained all the coding & non-coding sequences & later assigning different regions in the sequence with functions.

=> DNA sequencing was done using:
Automated DNA sequencing.

① DNA isolation & converted into random fragments of smaller sizes.

② Digestion with Enzyme.

③ => cloned in suitable host using specialised vectors. The cloning result into amplification of each piece of DNA fragments.

=> The commonly used hosts were bacteria & yeast, & the vectors were called as BAC [bacterial artificial chromosomes] & YAC [yeast artificial chromosomes].

Cut the DNA into overlapping fragments short enough for sequencing.

clone the fragments in plasmid or other vectors.

Order the sequences into one overall computer software. [Automated DNA sequencer]

Sequence each fragments

* Salient features of Human Genome.

- The human genome contains 3164.7 million bp
- The average gene consists of 3000 bases, but sizes vary greatly with the largest known human gene being dystrophin at 2.4 million bases.
- TDF gene is smallest gene with 14 bases.
- The total no. of gene is estimated at 30,000 - much lower than previous estimates of 80,000 to 1,40,000 genes. Almost all (99.9%) nucleotide bases are exactly the same in all people.
- The functions are unknown for over 50% of the discovered genes.
- Less than 2% of the genome codes for proteins.
- Repeated sequences makes up very large portion of the human genome.
- Repetitive sequences are stretches of DNA sequences that are repeated many times, sometimes hundred to thousand times.
- chromosome 1 has most gene [2968] & the X has the fewest [231]
- Scientists have identified about 101 million locations where single base DNA differences occur in humans. This is k/a SNPs - "Single nucleotide polymorphisms", pronounced as "snips"

* Applications of HGP

- Understanding genetic diseases → helps in diagnosis, prevention & treatment
- Development of personalised medicines
- Evolutionary studies by comparing genomes of different organisms
- Improvement in forensic science
- Gene therapy & biotechnology advancements.

* DNA fingerprinting

- Human DNA is 99.9% identical, but the remaining 0.1% differs b/w individuals. These differences are the basis of identification.
- Much of the variable part consists of repetitive DNA [non-coding]:
- The bulk DNA forms a major peak & the other small peaks are referred to as satellite DNA.

minisatellites /

VNTRs [Variable no. tandem repeats]

- ⇒ Repeats units are long
- ⇒ The total VNTR region can vary from 0.1 kb to 20 kb b/w chromosomes / individuals.

microsatellites /

STRs [Short tandem repeats]

- ⇒ very short repeat units.
- ⇒ commonly 1-6 bp repeated many times.

- Polymorphism: the no. of repeat units at a VNTR / STR locus differs b/w individuals [inherited from parents].
- Identical [monozygotic] twins have essentially the same DNA fingerprint; siblings / relatives share more bands than unrelated people.

sample for DNA fingerprinting can be taken from blood, hair follicle, saliva, semen, skin, bone, teeth etc.

Page No.

Date

* Method of DNA fingerprinting

- 1) Isolation & extraction of DNA
- 2) Digestion of DNA by restriction endonucleases
↳ Biological scissors which cut DNA at specific region.
- 3) Separation of DNA fragments by gel electrophoresis (Agarose gel used)
↳ Separating DNA on the basis of size.
- 4) Transferring [blotting] of separated DNA fragments to synthetic membrane such as nitrocellulose or nylon
- 5) Hybridisation using labelled VNTR probe
- 6) Detection of hybridised DNA fragments by autoradiography.

* Applications

- Forensic identification [crime scenes]
- Paternity / maternity testing & family relations
- Identification of dead bodies
- Human genetic mapping & population genetics