

Molecular Basis of Inheritance

DNA - most organism
RNA - some virus (TMV)



- Bacteriophage - ϕ X174 - 5386 ntde - bp
- Bacteriophage λ - 48502 bp $\rightarrow$ ∞
- E. coli - 4.6×10^6 bp
- Human (n) - 3.3×10^9 bp hap Genome

RNA/DNA - Polynntd (ntd) - (ntd) - (ntd)

DNA - most ds, RNA - ss

Structure of DNA

DNA - polymer
monomer: ntde
length $\sim$ no ntde joined in space

Nuclein - Meischer

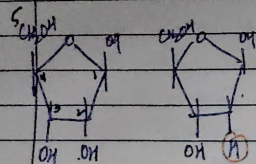
Nucleic acid $\rightarrow$ Altman

Nucleotide

Sugar Pentose

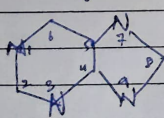
Nitrogen base

Phosphate



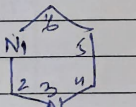
(Ribose sugar) (2-deoxyribose sugar)
 $\downarrow$
RNA
 $\downarrow$
DNA

Purines $\rightarrow$ (A, G)
• 2 rings



(1, 3, 7, 9) (5 methyl) $\rightarrow$ Thymine only in DNA
Uracil - RNA

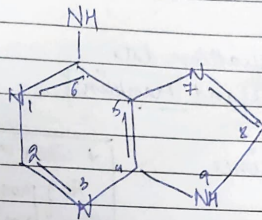
Pyrimidines



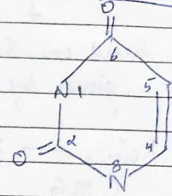
(C-bom DNA, RNA)

Thymine only in DNA
Uracil - RNA

Adenine



Cytosine
Uracil

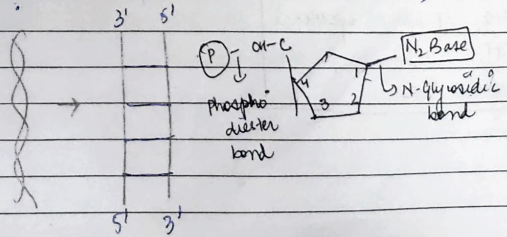


Nucleotide $\rightarrow$ Nucleoside + Phosphate
 $\downarrow$
sugar + base + Phosphate

Nucleoside: A + sugar $\rightarrow$ Adenosine + P $\rightarrow$ Adenylic acid
G + sugar $\rightarrow$ Guanosine + P $\rightarrow$ Guanylic acid
C + sugar $\rightarrow$ Cytidine + P $\rightarrow$ Cytidylic acid
T + sugar $\rightarrow$ Thymidine + P $\rightarrow$ Thymidylic acid
U + sugar $\rightarrow$ Uridine + P $\rightarrow$

Structure of DNA

called as "Nuclein" - Friedrich Meischer - (1869)
DNA - double helix (Watson & Crick)



— / — / —

Based on X ray diffraction data
given by Wilkins and Franklin

$$\begin{array}{r} C \equiv G \\ A = T \end{array}$$

No of phosphodiester
 $\rightarrow n-1$
 $= 5-1 = 4$

Amount of A = Amt of T
Amount of C = Amount of G

$$A + G = C + T$$

* No of Glycosidic bond = $n-5$

linear DNA (ds)
 ↳ 5' end = 5' phosphate
 diester bond =
 5' glycosidic

9) If T is 20%, C = ?, G = ?, A = ?

$$20A + G = C + 20\% \dots A < T \dots$$

20. 20

$$A = T$$

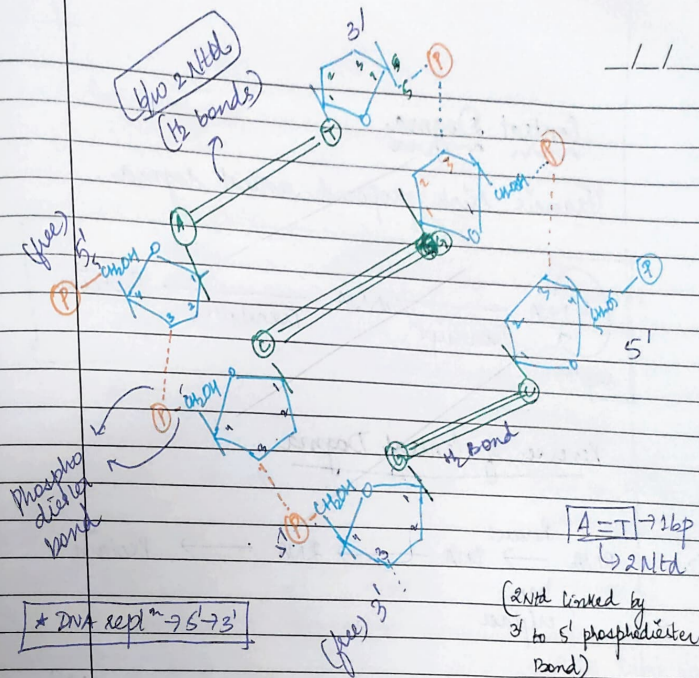
$q \geq c$

① $P_{\text{prime}} = P_{\text{pyrimidine}}$

② $A+G = C+T$

③ $\frac{A+G}{C+T} = 1$ (for all species)

⑨ Sugar = PO_4



(2nd linked by
3' to 5' phosphodiester
bond)

Salient feature

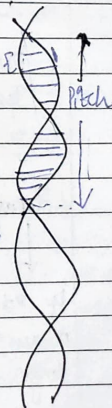
$$\frac{3.4 \text{ mo}}{10}$$

- Antiparallel polynucleotide chain
- H₂ bonds b/w polynucleotide
- sugar-phosphate backbone, H₂ bases inside

Pitch - $34 \text{ \AA}$ or 3.4 nm

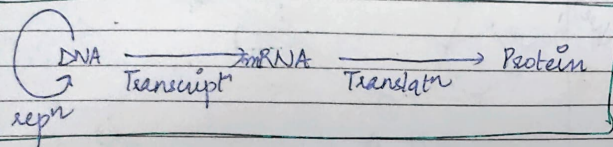
1 Pitch - 1 complete turn - 10 base pairs
1 plane of base stacks over other

Stability $\rightarrow$ H₂ Bond
 $\hookrightarrow$ Base one upon other.

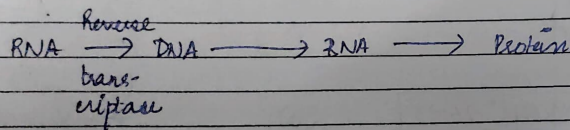


Central Dogma

Francis Crick prepared central dogma.



Reverse of Central Dogma



Packaging of DNA

Genome = 3.3×10^9

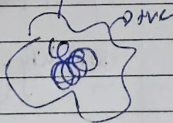
DNA human = 6.6×10^9 bp
length = $6.6 \times 10^9 \times 0.34 \times 10^{-9}$
= 2.2 m

Prokaryotes - -ve charged DNA

+ some +ve protein

Nucleoid

Non Histone

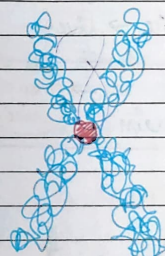
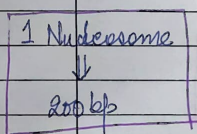
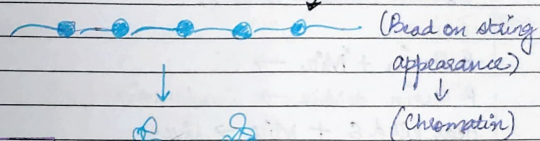
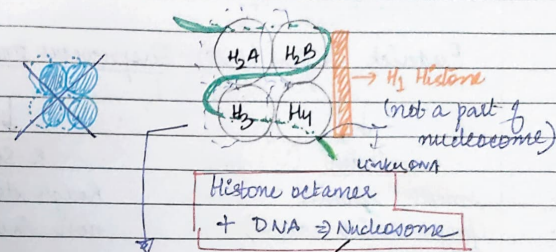


bp = 4.6×10^6 bp

Distance b/w bp = 0.34 nm

length = No of bp $\times$ Distance
= $4.6 \times 10^6 \times 0.34 \times 10^{-9}$
= 1.56 nm

Prot Eukaryotes



Histones are basic aa
↳ lysine, Arginine

for further packaging
Non histone ch. protein

Heterochromatin
dark stained
tightly packed
transcript inactive

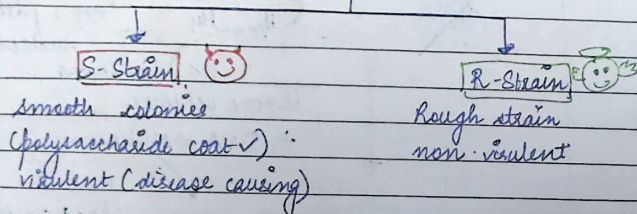
Euchromatin
light stained
loosely packed
transcript active

Search for Genetic Material

1/1

① Transforming Principle

Frederick Griffith (1928) - *Streptococcus pneumoniae*



S Strain + Mice → Die

R Strain + Mice → Live/survive

Heat killed S + Mice → Live

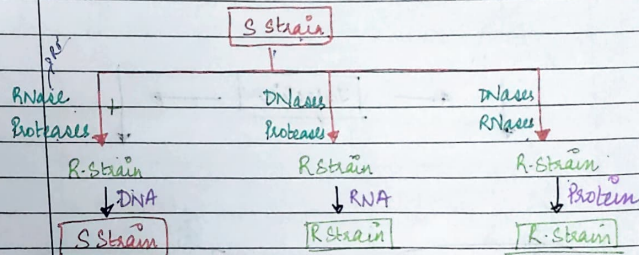
* Heat killed S Strain + R Strain → Mouse dies.

transformed
R into S

* Biochemical Nature of Genetic Material
was not defined....

② Biochemical Nature of Transforming Principle

Avery, MacLeod, McCarty - 1944



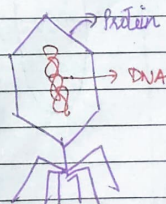
* Only DNA could transform.

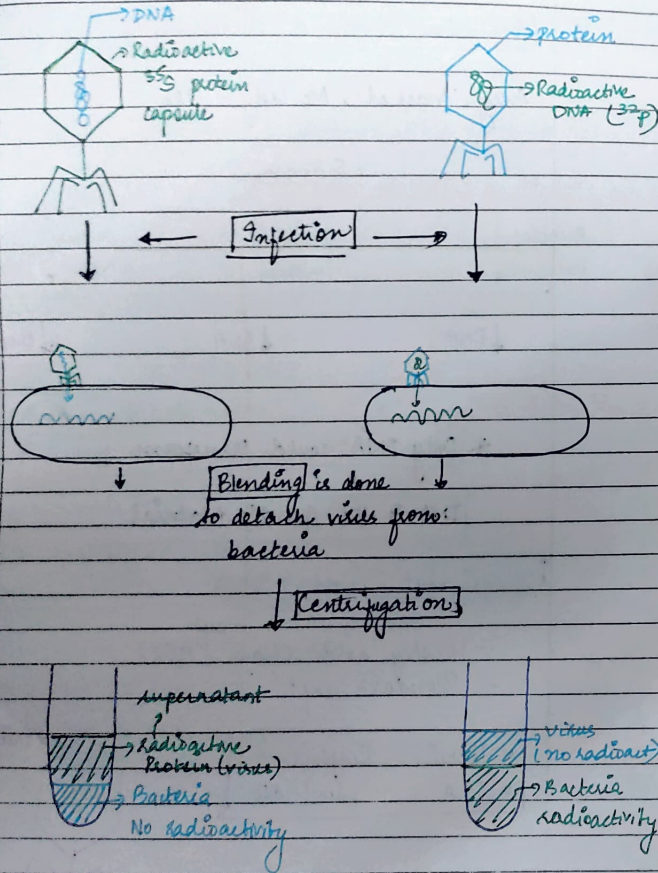
DNA is the Genetic Material

③ Unequivocal Proof of DNA

- Hershey and Chase ^{expt} (1952)
- Blumberg expt.

- Protein - Sulfur
- DNA - phosphate





*** DNA is the Genetic Material**

* Properties of Genetic Material

- should replicate $\xrightarrow{\text{DNA}} \text{RNA} \rightarrow \text{protein}$
- chemically and structurally stable
- scope for slow change (mutation) for evolution
- express itself as mendelian character.

$\hookrightarrow \text{DNA} \rightarrow \text{DNA} \rightarrow \text{protein}$

RNA
 2' C - OH (Reactive)
 catalyst (Reactive)
 unstable (less stable)

RNA mutates fast
 ↳ short lifespan

RNA	DNA
1) OH group @ 2nd position.	1) No OH group @ 2nd pos ⁿ
2) reactive (more)/unstable.	2) stable.
3) No Thymine	3) Thymine provides extra stability.
4) shows catalytic activity	4) No No catalytic activity.
5) Mutation faster	5) Mutation slow
6) RNA $\rightarrow$ protein (express faster)	6) DNA $\rightarrow$ RNA $\rightarrow$ Protein. (Regulation is good)

RNA world - 1st Genetic material

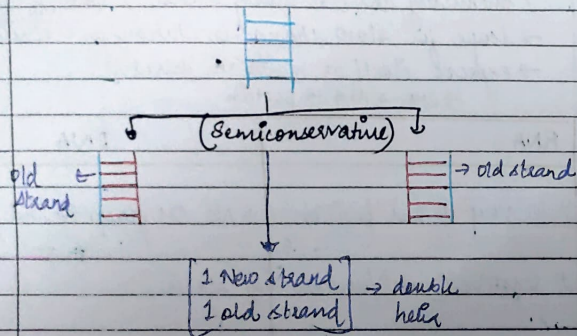
- ↳ catalyst
- ↳ metabolism
- ↳ splicing
- ↳ translation

DNA is more stable

↳ prepared for storage

REPLICATION

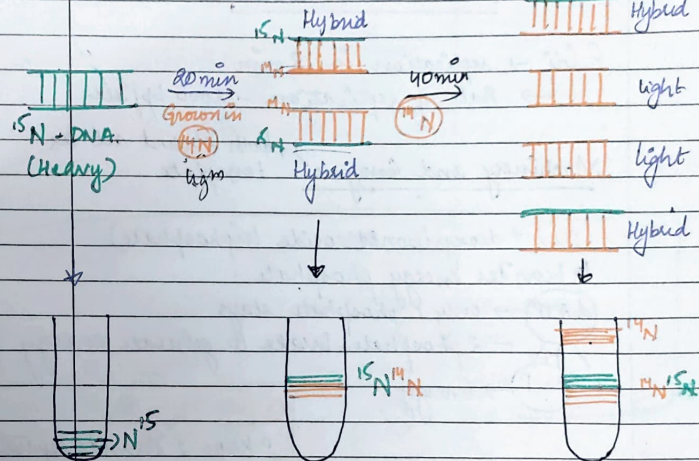
* Watson and Crick proposed replication is semiconservative



Experimental Proof (semiconservative)

- Meselson and Stahl (1958)
- E. coli → ^{14}N isotope (light) } source of N
 ↓ Grow → ^{15}N isotope (dark) } $^{15}\text{NH}_4\text{Cl}$
- centrifugation in CsCl

^{14}N
 ^{15}N



* Taylor and Colleagues
 Used radioactive Thymidine
 → Vicia faba (faba beans)
 → proved DNA replication is semiconservative in plants

DNA REPLICATION

E. coli $\rightarrow$ replication in 18 min

$\rightarrow$ Rate of replication - 2000 bp/sec.

Machinery and Enzyme $\rightarrow$ Both strand act as template

① dNTPs $\rightarrow$ deoxyribonucleoside triphosphate

$\rightarrow$ provides energy phosphate.

② dATP $\rightarrow$ only 1 phosphate stays

- 2 phosphate breaks to generate energy

P-P-P $\rightarrow$ Energy

④ SSB Protein

- keeps 2 strands separated
- No more complementation
- H₂ bond formation

⑤ Topoisomerase

- To release supercoiling

⑥ Ligase

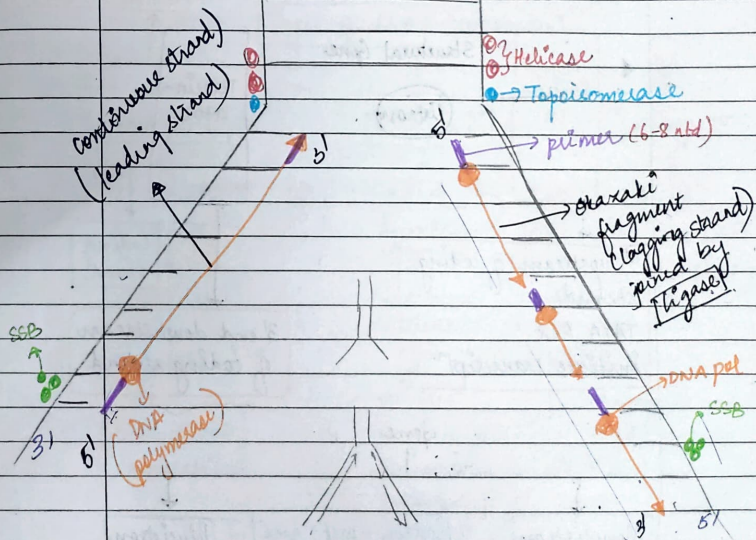
join fragments of DNA

③ Helicase

- unwinding 2 strands of DNA
- breaks H₂ bond

② DNA polymerase

- $\rightarrow$ DNA dependent
- $\rightarrow$ polymerisation 5' $\rightarrow$ 3'
- $\rightarrow$ needs Mg²⁺ for activity
- $\rightarrow$ needs primer (small segment of RNA made by primase)



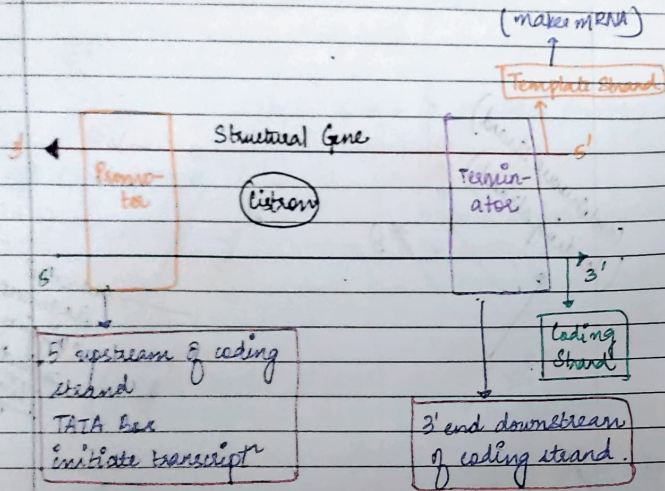
$3' \rightarrow 5'$ ATG CAA TTA CAG T $5'$ (T.S.)
 $5'$ TAC GTT AAT GTC A $3'$ coding /
 UAC G UUA A UG UGA mRNA

TRANSCRIPTION

DNA $\rightarrow$ RNA

only 1 template strand is used.

Transcription unit



Gene

Monocistron
 One cistron -
 one protein
 eukaryotes

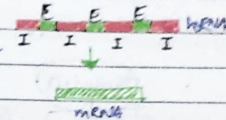
Cistron - part of gene
 which codes for
 protein

Polycistron
 (1 cistron - many
 protein)
 Prokaryotes

Split Gene Concept

Exons
(coding)

Introns
(non coding)



Types of RNA (eukaryotes)

RNA pol I

rRNA (28S, 18S, 5.8S)

synthesis

Nucleolus

Structural
 +
 (catalytic role)
 translation

RNA pol II

mRNA

hnRNA (pre-mRNA)

Nucleolus
 Nucleus

provides
 template for
 translation
 protein

RNA pol III

tRNA

(5S rRNA), 5S rRNA

Nucleus
 Nucleolus

brings aa and
 reads genetic
 code.

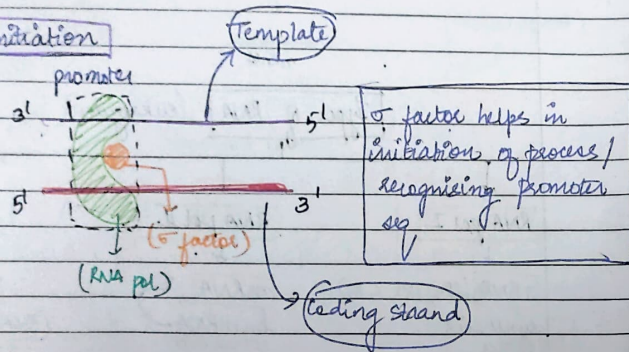
* In prokaryotes - only 1 RNA pol is present.

* TRANSCRIPTION IN PROKARYOTES

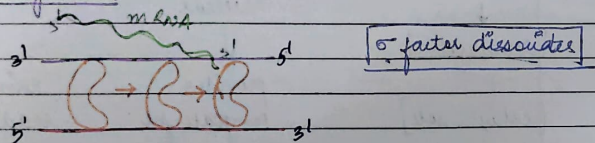
- Euchromatin should be taken
- open double helix - RNA pol

Steps

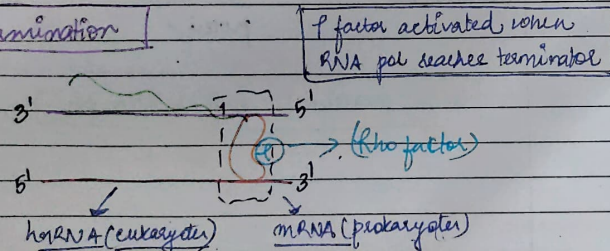
① Initiation



② Elongation



③ Termination



Transcription in Eukaryotes

Initiation

elongation

Termination

→ same as prokaryotes but processing is required

(Post Transcription Modification)

(Spliceosome)

* Splicing



hnRNA

① Splicing → Snpase

② capping

③ Tailing

5' cap

mRNA

poly A tail

AAAAA

(200-300 A)

7-methyl guanosine triphosphate

(Gppp)

7mGppp

protection to reach cytoplasm

GENETIC CODE

- George Gamow - Genetic code (proposed)
- Har Gobind Khorana - chemical synthesis of RNA
- Marshall Nirenberg - synthesised protein
- Severo Ochoa - synthesis of mRNA

↓
Severo Ochoa enzyme
(polynucleotide phosphorylase)

* Total 20 64 codons - 20 aa → Non sense

- UAA, UAG, UGA - Stop codon
- AUG - Methionine - Start codon
- UGG - Tryptophan (only 1 codon)

* Salient feature of Genetic Codon

- ① Triplet codon: 61 codon - Aa
3 codon - stop codon (No Aa)
- ② Degenerate: ABC, ABA, BAC → 1 Aa → Leucine
CUU, CUC, CUA, CUG
- ③ Read in contiguous
- ④ Universal (UV) - true in bacteria - except - Mt codon, protozoans

⑤ AUG - Start codon / Initiator codon

⑥ UAG, UGA, UAA - Stop codon

⑦ Non Ambiguous: 1 codon : 1 aa

GUU - Valine, GUC - Valine, GUA - Valine, GUG - Valine
CUU - Leu, CUC, CUA, CUG

GUU
GUC
GUA
GUG

⇒ Valine

CUU
CUG
CUA
CUG

⇒ Leucine

Q. An organism has 5 types of N₂ bases and the body consist of 30 types of AA. Calculate No of N₂ bases present in codon?

(Types of N₂ base)^{No of N₂ bases present in each codon}
codons ×

$$(5)^2 = \frac{25}{1} \rightarrow \text{cannot give 20}$$

$$(5)^3 = 125 \rightarrow 30$$

No of N₂ bases in codon → 3

Mutations and Genetic Code

① Mis-Sense Mutation

AGC → AGA
Serine → Arginine

UGA → UGG
Stop codon → Tryptophan

② Silent Mutation

UGU → UGC
Cysteine → Cysteine

AGU → AGC
Serine → Serine

change in codon
does not change
Aa

CAT BAT RAT SAT
1 2 3 4 4 Aa

Non overlapping

③ Frameshift Mutation

CAT AATG THE RAT
CAT SAT AATG THE RAT

Addition

CAT SAT TRA ISA T

Deletion

CAT AT BAT RAT SAT

Point Mutation

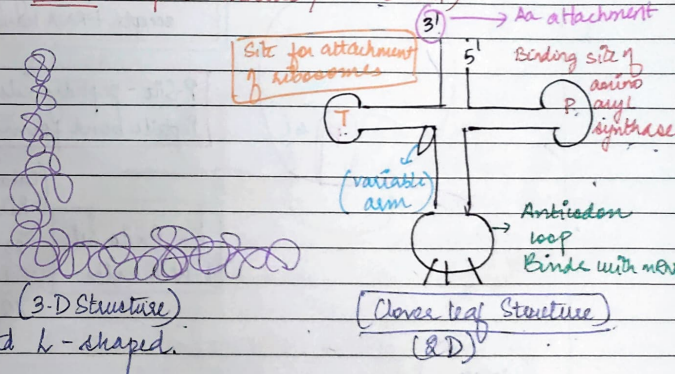
Eg: sickle cell anaemia

Glutamate → Valine
GAG → GUG
(Normal) (Disease)

Addition of 3 ntd - least effect

*

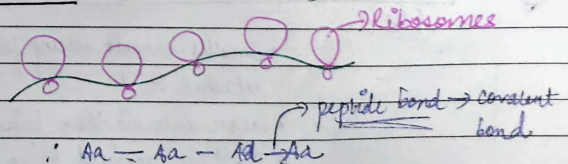
t-RNA (Adaptor molecule / soluble RNA)



*

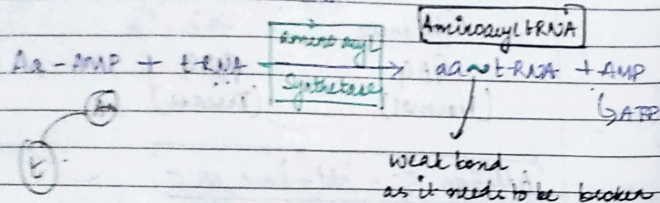
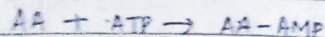
Translation (mRNA → Proteins)

(Polysome)

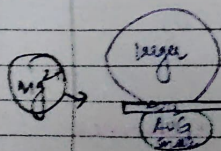
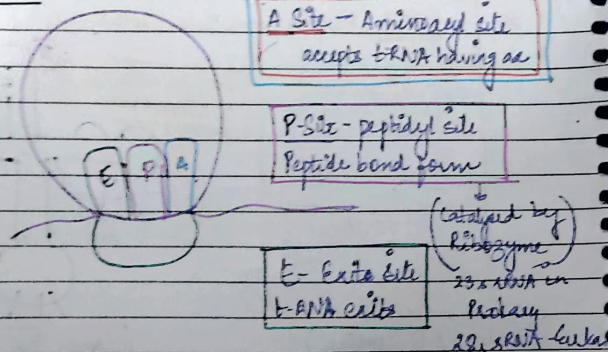


Steps

1) Activation of AA / charging of t-RNA / Aminoacylation

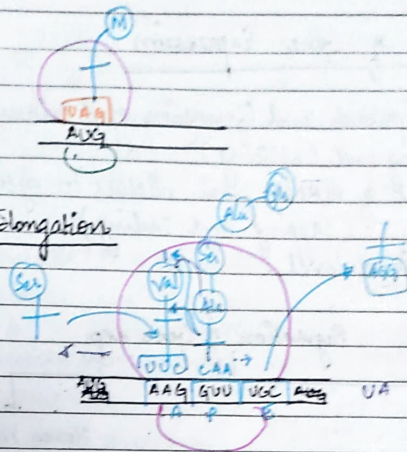


2) Initiation

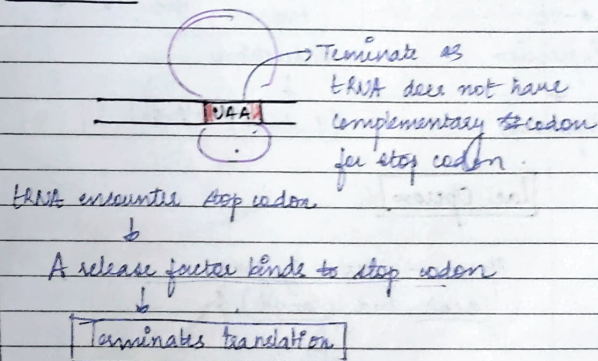


- Smaller subunit scans for AUG and attaches to it
- Larger subunit then binds to AUG.
- Mg^{2+} is required to join smaller and larger subunit of ribosome.

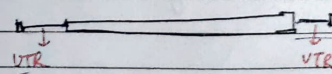
3) Elongation



4) Termination



5) Regulation of translation



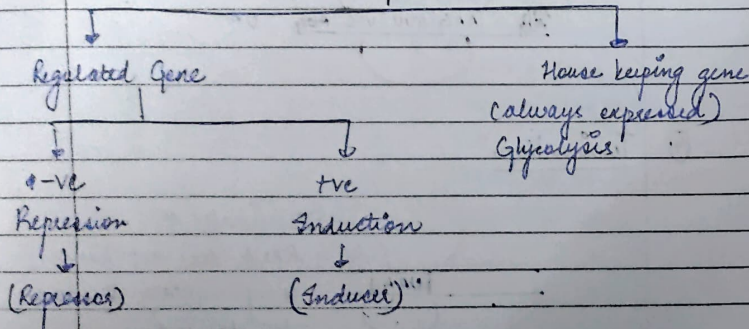
untranslated region

in prokaryotes - only transcriptional level.

* Regulation of Gene Expression

- transcriptional level (formation of P transcript)
- Processing level (splicing)
- transport of mRNA from nucleus to cytoplasm (capping and tailing)
- translational level.

Regulation of Gene exp



Lac Operon

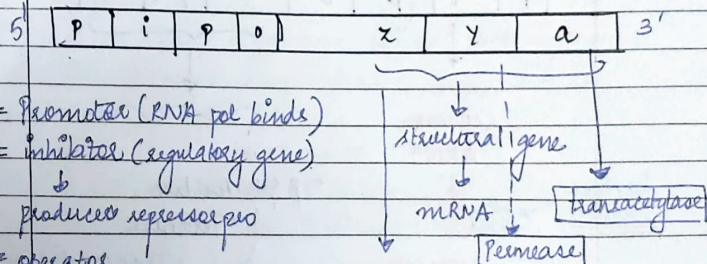
Operon - cluster of gene
(Jacob and Monod)

E. coli $\rightarrow$ utilised Glucose

$\rightarrow$ in the absence of Glucose $\rightarrow$ (lactose)

Lactose $\xrightarrow{\beta\text{-Galactosidase}}$ Glucose + Galactose

lac operon should be switched on



P = Promoter (RNA pol binds)

i = inhibitor (regulatory gene)

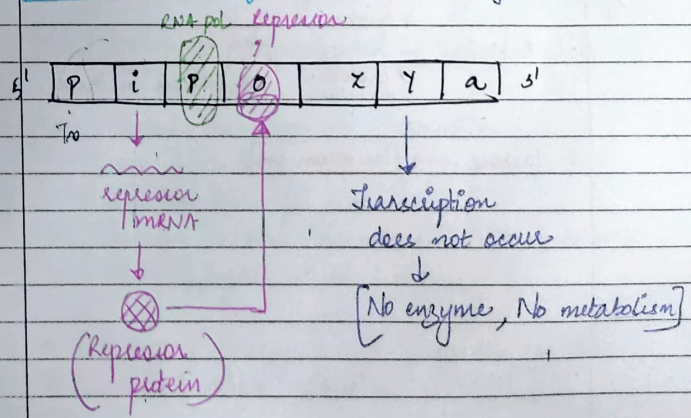
$\rightarrow$ produces repressor protein

O = operator

the repressor protein binds.

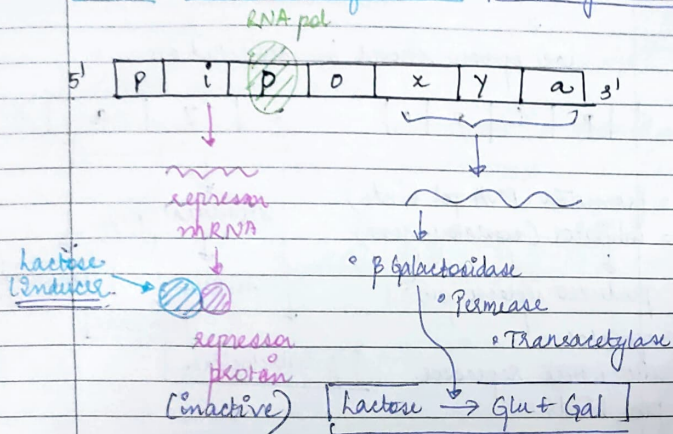
Case I: Absence of Lactose

-ve Regulation

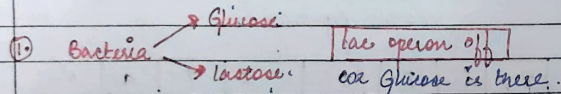


Case II: In Presence of lactose

↑ve regulation



Q.



Q. lactose → lac operon on

* HUMAN GENOME PROJECT (1990)

3300 book
1000 page in each book

(Haploid set of chromosome)

2 individual different → Different DNA sequence
Genetic engineering - isolate, clone, DNA seq.

HGP - 1990 - Mega project - 13 Year

3×10^9 bp → US \$3 per bp

→ Total 9 billion US dollars

Huge data - high speed computational data

↓
[Storage, retrieval, analysis]

[Bioinformatics - New field of biology]

Goals of HGP → completed in 2003

→ 20k-25k genes identify

→ 3 Billion chemical bp - determine

→ Store information

→ Improve tools for data analysis

→ Transfer related technologies to other sectors

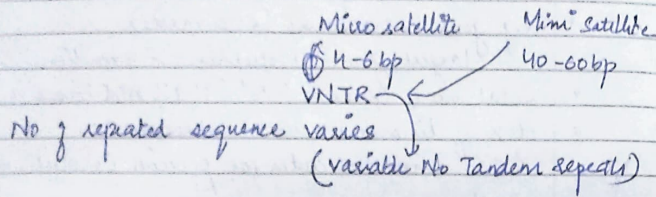
→ Ethical, legal, social issue

* U.S Dept of Energy

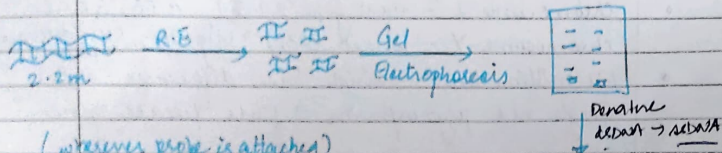
* National Institute of Health

} coordinated the project

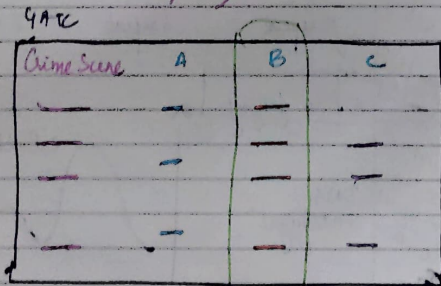
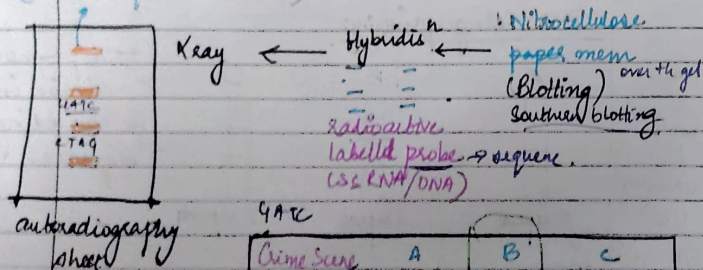
for DNA finger printing $\rightarrow$ repetitive DNA / satellite DNA



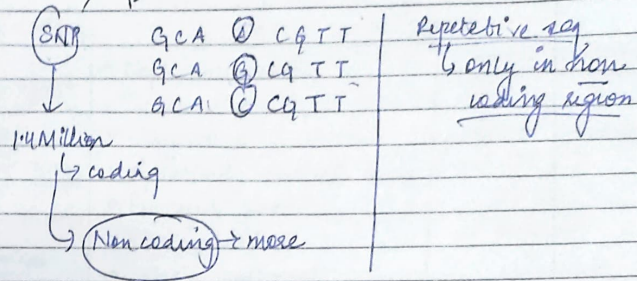
Procedure :



(wherever probe is attached)



Sickle cell anemia



Repetitive seq

