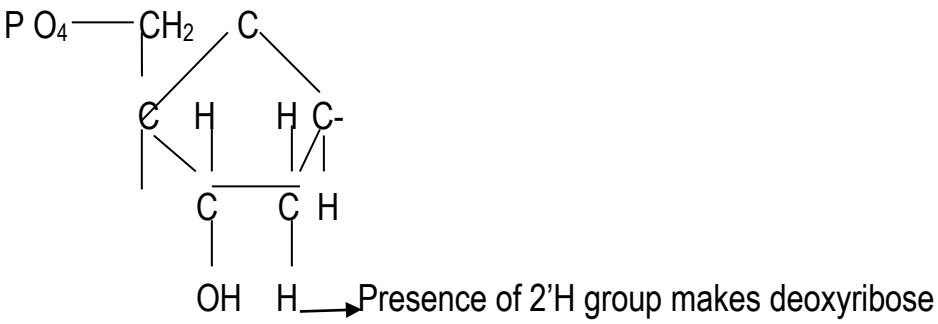
CHAPTER NO. 6

MOLECULAR BASIS OF INHERITANCE

- The cell is the fundamental unit of the living organisms has a complex network of many structures being coordinated by the large molecule-DNA
- DNA is a long polymer of nucleotides.
- A nucleotide has three parts – deoxyribose sugar, nitrogenous base and phosphate group.
- There are two types of nitrogenous bases namely two Purines(double ring structure)-Adenine (A) and Guanine(G) and two pyrimidines (single ring structure)-Thymine (T) and Cytosine (C)



DEOXYRIBOSE SUGAR MOEITY IN DNA showing PO_4^-

- The molecule consists of large number of nucleotides joined together between the sugars and the phosphates by 3'-5' phosphodiester bonds.
- The sugar-phosphate linked structure forms the backbone of the molecule
- We can see the presence of OH^- group at the 3'C and PO_4^- group at the 5'C.
- The molecule is double helical right handed structure (B-DNA) consisting of two antiparallel polynucleotide strands and held by hydrogen bonds.
- Two strands are complementary to each other ie., adenine(A) in strand with pair with thymine (T) in the opposite strand and vice versa. Like wise, Cytosine (C) will pair

with Guanine (G) . We can understand that the base sequence in of the strand is known, the base sequence of the complementary strand can be deduced.

- One strand shows the polarity 5'⟶3' and the other will show 3'⟶5'
- Adenine pairs with Thymine by two hydrogen bonds and guanine pairs with Cytosine by three hydrogen bonds.

A = T OR T = A

C ≡ G OR G ≡ C

- The double strand molecule has alternate major groove and minor groove held by proteins called histones (in the case of eukaryotes).
- The biochemical work of Erwin Chargaff reveals that A+T/C+G= 1 and so A+G = T+C. Therefore, purines are always equal to pyrimidines (1:1). We must keep In mind that A+T= C+G.
- The purine and pyrimidine bases are spaced by 0.34nm(3.4A^{o}) apart which gives 10 base pairs in one complete turn of the backbone. It results in 3.4nm (34A^{o}) per each complete turn of the helix.

PACKAGING OF DNA

- The nucleoplasm of the nucleus (Eukaryotes) has the mesh of chromatin. The chromatin fibre appears to a long string like structure having beads. The beads represents nucleosomes.
- Interestingly, it appears like a rugby ball when viewed the three dimensional shape.
- Nucleosome cores consist of an octamer of two molecules each of 4 histones namely H2A. H2B, H3, and H4.A fifth kind histone(H1) is located on the linker DNA between the nucleosome particles. The core is wrapped by superhelical strand of DNA having 165bp in two turns.
- Histones has rich of basic amino acids lysines and arginines , it is positively charged and you are aware that DNA is negatively charged particle.
- Further packaging is done by Non-histone chromosomal proteins(NHC).

- DNA is revealed as purple colour by the feulgen stain. It shows light regions (Euchromatin) and dark regions (heterochromatin). Remember, euchromatin is the regions of active DNA.

IN SEARCH OF GENETIC MATERIAL

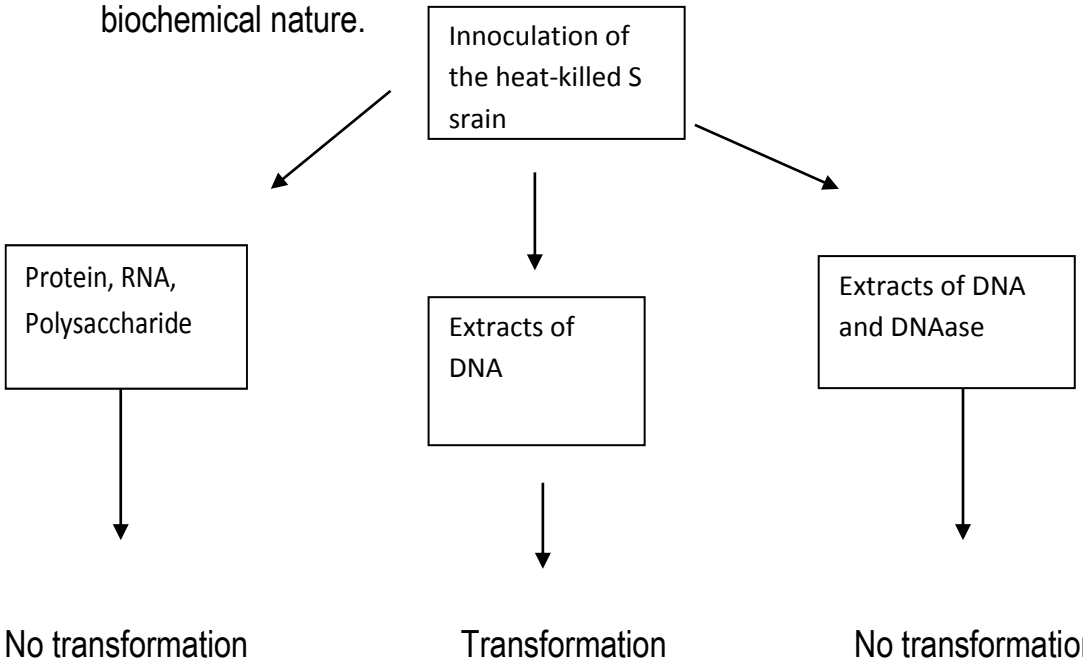
TRANSFORMING PRINCIPLE

- Fredrick Griffith proposed this principle of Transformation by conducting the experiment on mice by injecting two strains namely R (rough) and S(smooth having capsule made of polysaccharide) . R strain is non-virulent and S is virulent.
- Live R strain ⟶ Mice ⟶ Alive
- Live S strain ⟶ Mice ⟶ Die
- Heat-killed S strain ⟶ Mice ⟶ Alive
- Heat killed S strain + live R strain ⟶ Mice ⟶ Mice die

Griffith concluded that live R strain became transformed as virulent due to heat-killed S strain.

IDENTIFICATION OF THE TRANSFORMING PRINCIPLE

- It was tested by Oswald Avery, Colin Mcleod and Maclyn McCarty to determine the biochemical nature.



- It is concluded that the transforming principle is DNA

Further proof by Hershey and Chase experiment

- They did the experiment by using phage having radioactive labelled S^{35} and other set of the phage having P^{32}.

The flow chart is shown WITH STEPS INVOLVED

PHAGE WITH S35 — PHAGE WITH P32

GETS ATTACHES TO E,coli — GETS ATTACHES TO E,coli

Step 1 --- **I** = Infection

Step 2--- **B**= Blending means outer cell material will be removed

Step 3----**C**= Centrifugation will retain the genetic material only

CONCLUSION

E.coli has P32 and not S35 indicating the DNA .

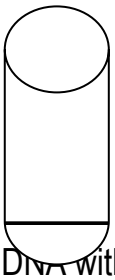
DNA REPLICATION

- DNA replication takes place during the `S' phase of the interphase in the cell-cycle(glance class XI-NCERT text Book)
- *I*t is semi-conservative in nature ie., in each DNA molecule, one strand is old and the other newly formed strand.

EXPERIMENTAL PROOF

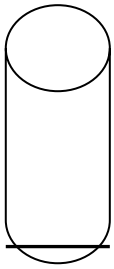
- The semi-conservative type of replication was confirmed by Meselson and Stahl in 1958.

- E.coli was grown in N15 medium (heavy density non-radioactive isotope) for many generations. And centrifuged with $CsCl_2$ density gradient and found as-



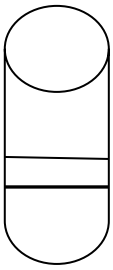
$N^{15}N1^{5}$

- Later, DNA with N^{15} were transferred to a N^{14} medium and allowed to replicate.(Replication duration is 20 min).
- After 20 minutes, it is shown as-



$N1^{5}N^{14}$ (Hybrid)

- After 40 minutes, it reveals-



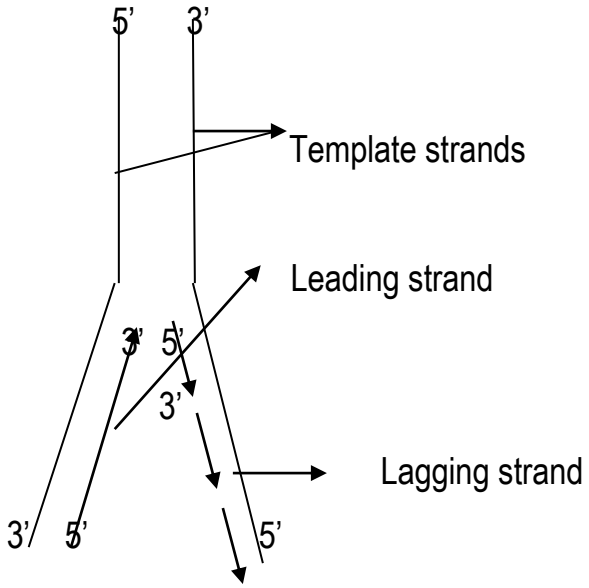
$N^{14}N^{14}$(light)

$N^{15}N^{14}$(intermediate)

- The various steps involved are as follows:

- The process starts at a specific point called origin of replication(ori). The strand breaksfollowed by cleaving of hydrogen bonds.
- The two strands starts un winding by the enzyme helicase.
- It appears like `Y; shaped and it refers as replication fork.

- The DNA polymerases which is responsible for synthesizing new DNA to the original strand (template strand) cannot function without the 3'OH group. Therefore, it is provided by short segment of RNA (primer) catalysed by the RNA polymerase.
- The synthesis of the new DNA takes place in the 5'⟶3'. The two template strands show 3' ⟶5' and the other one has 5'⟶3' polarity. The replication begins at .3'⟶5' template first and the new DNA strand is called leading strand. In the opposite strand, it progresses from back towards the previously transcribed segment. This is the lagging strand.
- The lagging strand consists of small fragments called Okazaki fragments. These are connected by the enzyme ligase.



RNA WORLD

- RNA serves as non-genetic material in eukaryotes /prokaryotes but it can behave as genetic material in few viruses referred as plant viruses and retroviruses.
- Having the presence of the 2'OH group in the ribose sugar makes it highly unstable, reactive and so it shows catalytic role.

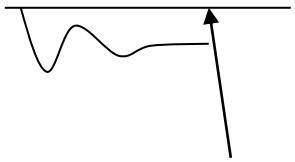
TYPES OF RNA

- mRNA- Messenger RNA is transcribed from DNA by the RNA polymerase II (Eukaryotes) and by single large holoenzyme (prokaryotes). mRNA carries the information of the DNA in the form of triplet bases called codons to the ribosome for protein synthesis. . It has a start codon as AUG (it is an amino acid –methionine) and ends with any stop codon (UGA, UAG, UAA). It is unstable and comprises less than 5% of the total RNA.
- tRNA- transfer RNA- It appears like a clover leaf or hair-pin pattern. It is the smallest among the RNA and comprises 10-20% of the total RNA. It carries amino acids to the mRNA during the protein synthesis. It may be less than 61 amino acids because tRNA shows wobble hypothesis. (According to Crick, the third base of the codon wobbles with the first base of the anti-codon of the tRNA.). RNA polymerase III is needed for the synthesis of tRNA.(eukaryotes)
- rRNA – ribosomal RNA- it comprise 80 % of the total RNA. It is found in the ribosome and provides proper binding site for the mRNA of the ribosome. It is produced by the RNA polymerase I .(eukaryotes).

TRANSCRIPTION

- The process of synthesis of mRNA from the DNA by the help of DNA dependent RNA polymerase.Let's see the process in prokaryotes and in eukaryotes

TRANSCRIPTION IN PROKARYOTES	TRANSCRIPTION IN EUKARYOTES
It occurs in the cytoplasm	It occurs in the nucleus
Single large RNA polymerase can transcribe.	It is transcribed by RNA polymerase II.
The DNA transcript acts as template	The DNA transcript acts as template

consists of three regions- Promotor, structural, terminator.	consists of three regions- Promotor, structural, terminator.
5’ ——————— 3’ 3’ 0 5’ DNA SEGMENT ∞sigma factor binds to the PROMOTOR starts 1st step=INTIATION It is the RNA POLYMERASE carries the  rho factor mRNA synthesis 2nd step= ELONGATION. → 3rd step- TERMINATION binds —————— mRNA	All the 3 steps occurs in this cell also.but results in hnRNA (primary transcript). 5’------- - - -------- - - ---------- - - --------3’ EXONS INTRONS STEP1= CAPPING- Adding of Methylguanosine tri phosphate to the 5’ end (MeG is required to form the mRNA-ribosome complex in the cytoplam) STEP2= SPLICING Removal of introns or intervening sequences 5’MeG——————————— 3’ STEP 3= TAILING Adding of 200-300 adenine residues to the 3’ end . It

	facilitates post-transcriptional processing.  5'MeG_____3'AAAAA IT IS THE mRNA.
No mRNA processing occurs	mRNA processing occurs
The DNA segment depicts polycistronic arising to polysome formation. Many genes are transcribed at the same time.	The structural DNA segment is monocistronic consisting of split gene arrangement.
Interestingly, Translation begins prior to the completion of the transcription as it takes in the cytosol Therefore, coupling of transcription-translation occurs.	Translation will begin only when the complete mRNA is available as protein synthesis occurs in cytoplasm and is separated by nuclear membrane.

GENETIC CODE

- Salient feature

TERMS	EXPLANATION
Triplet codon	It has three bases, so 61 codons code for 20 amino acids and 3 are stop codons
Unambiguous and apecific	There is only one codon specific for one amino acid. ie., no two amino acids will have same codon.
Degenerate	some amino acids can have many codons. For eg., Serine, Valine
Universal	mRNA having UUU as one of the codon in both bacteria and in Human will code for phenylalanine only. It underlies the phenomena of GENE MANIPULATION leading to TRANSGENICS.

TRANSLATION

The story of protein synthesis has come to the climax stage, till now it can be show as-

DNA - Has the language of nucleotides

↓

mRNA - Has the language of nucleotides

↓

Polypeptide- Has the language of amino acids.

STEPS INVOLVED

- CHARGING OF AMINO ACID

The activation of the amino acid is done by the enzyme aminoacylsynthetase with the help of ATP

Amino-acyl synthetase

AA+ ATP ⟶ AA ~AMP + PPi

- CHARGING OF tRNA (aminoacylation of tRNA)

It involves the transfer of activated amino acid to tRNA

AA ~AMP + tRNA $\xrightarrow{\text{Amino-acyl synthetase}}$ AA~tRNA + AMP

There are three steps involved:

- **INITIATION-** Small subunit of the ribosome 40s (eukaryotes) and 30s (prokaryotes)

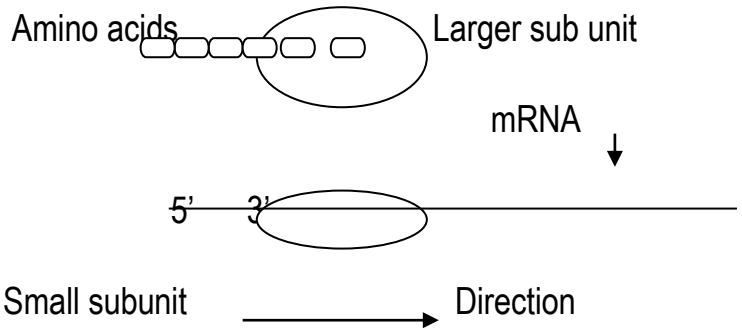
Consists of two sites- P and A, tRNA rests in P site.

Larger sub unit binds to mRNA-tRNA complex.

- **ELONGATION**-Other charged tRNA occupies the A site .

Peptide bond occurs between initiator codon(met)and 2nd amino acid

By the help of peptidyltransferase.

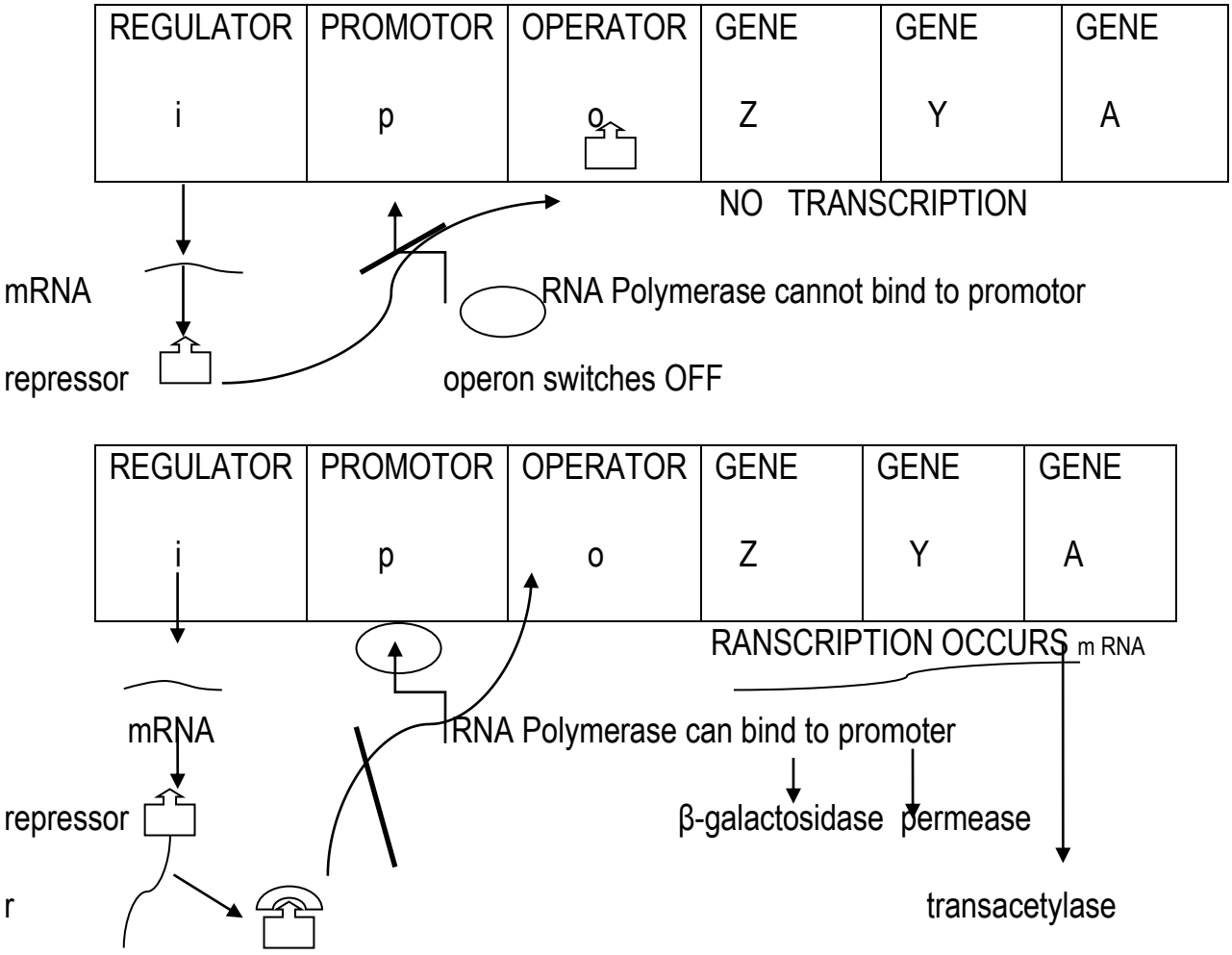


- **TERMINATION**- It stops at the site of stop codons as no tRNA arrives.

Complete polypeptide is released by the release factor.

REGULATION OF GENE EXPRESSION

The schematic representation of Lac operon – Three genes z y a , promoter and operator.



HUMAN GENOME PROJECT (HGP)

☺It is the entire sequencing of the Human Genome .

TECHNIQUES

- Expressed sequence tags
- Sequence annotation

TOOL

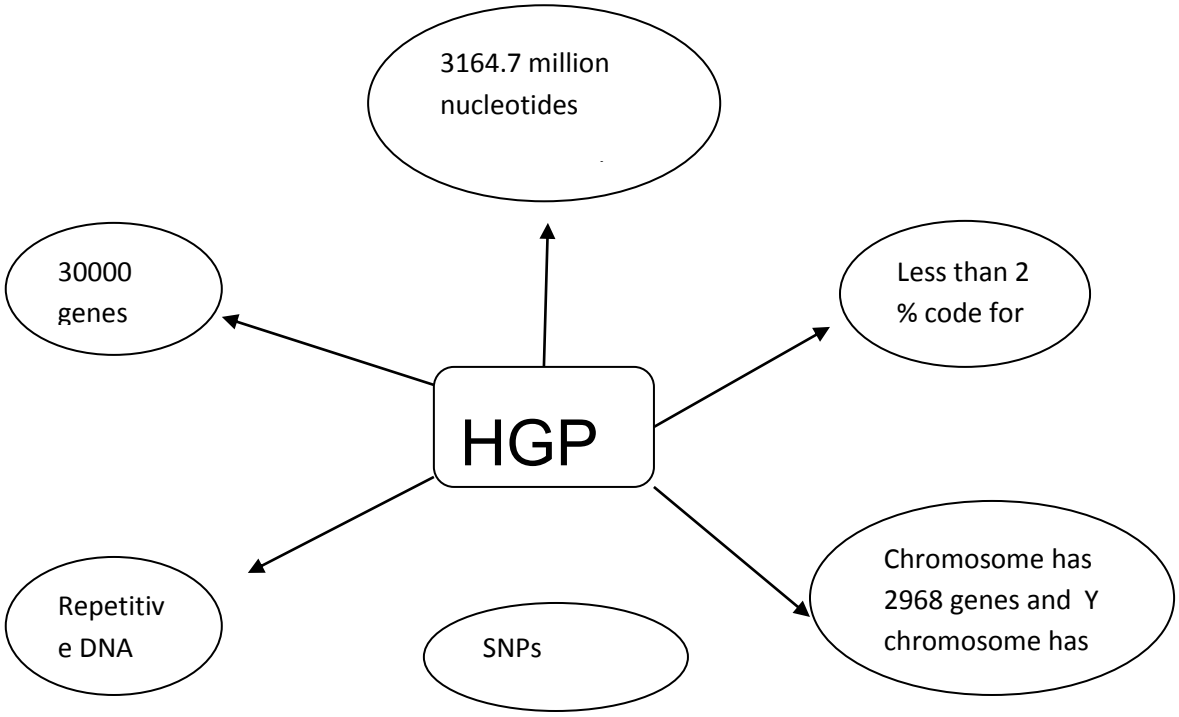
- Restriction enzymes
- Vectors- YAC, BAC

- Dideoxynucleotides (dATP , dTTP,dCTP, dGTP)
- Primers
- Polyacrylamide gel
- Nitrocellulose membrane
- DNA probes

METHOD

Sanger's dideoxy gel electrophoresis

FINDINGS



DNA FINGERPRINTING

It was developed by Alec Jeffres.

It is based on the principle of DNA polymorphism consisting of repetitive DNA including satellite DNA. It is two types STR and VNTR

VNTR (mini satellites) is significant in this method.

STEPS

1. EXTRACTION OF DNA .
2. AMPLICATION OF DNA USING PCR
3. DIGESTION OF DNA USING RESTRICTION ENZYMES
4. SEPARATION OF DNA FRAGMENTS BY GEL ELECTROPHORESIS.
5. HYBRIDISATION
6. AUTORADIOFRAPHY BY X-RAY DIFFRACTION