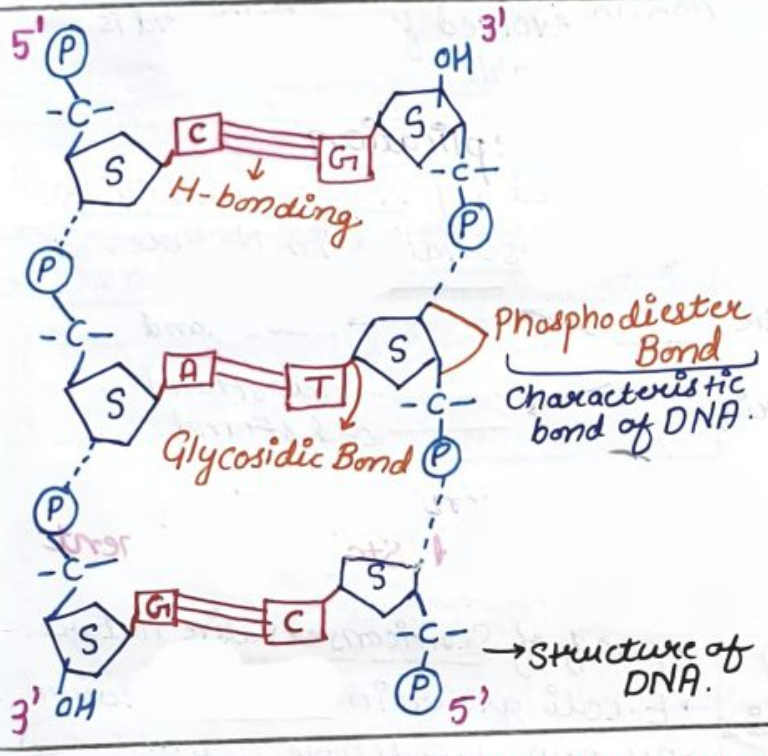
Molecular basis of Inheritance

① Molecular Basis of DNA →

→ Structure of DNA → Polynucleotide.
→ Double stranded → Antiparallel strands
→ Nitrogenous Basis

Purines — Pyrimidines
A = T
G ≡ C
U → only found in RNA.

→ Nucleotide
P) S N
↓
Nucleoside



→ Structure of DNA.

② DNA Double Helix (Right Handed Helix)

→ proposed in 1953 by Watson and Crick.
→ Based on X-diffraction data of Maurice Wilkins and Rosalind Franklin ⊛

1 turn = 3.4nm (34Å)
(Pitch of DNA)

20NB → 10 Base Pairs

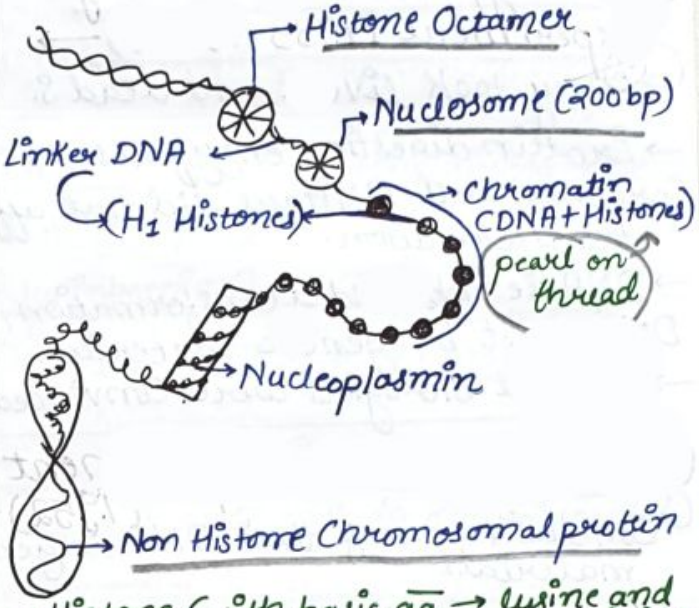
③ Chargoff's Rule (for ds DNA)

① Amount of Purine = Amount of Pyrimidine
Amount of Adenine = Amount of Thymine
Amount of Guanine = Amount of Cytosinine

② $\frac{A+T}{G+C} \neq 1$ (but Constant for Species)

④ Packaging of DNA Helix.

→ Length of DNA in Cell → 2.2 met.
→ Size of Nucleus ≈ 10^{-6} m.



→ Non Histone Chromosomal protein
→ Histone (with basic aa → lysine and arginine. ⊛)

H_1 H_2A H_2B H_3 H_4

→ Euchromatin → Losely packed (Transcriptionally active)
→ Heterochromatin → Densely packed (Transcriptionally inactive) ✗

The Search For Genetic Material →

① Griffith Experiment (1928)
(Transforming Principle)

→ Streptococcus pneumoniae

Smooth Strain	Rough Strain
(has mucus/ polysaccharide coat)	does not cause pneumonia.
Cause pneumonia	

S strain → inject in → Die.
R strain → inject in → Live.
S strain → inject in → Live
(Heat Killed)

S strain (Killed) + R strain (live) ⊛ → inject in → die

→ Live R somehow got transformed into S strain by transforming principle from heat killed S strain.
→ Transforming principle is genetic material.

② Avery, Macleod, Mc Carty Experiment (1933-1944) →
↳ They took live R and dead S.
→ Protein digesting enzymes (protease) and RNAses did not affect transformation.
→ DNAase inhibited transformation, So DNA must be genetic material.
→ Not all biologists were convinced.

③ Hershey-Chase Experiment (1952) →
↳ Conclusive proof that DNA is genetic material!
→ A bacteriophage attaches to bacteria, and it's genetic material enters the bacterial cell.

Group A	Group B
Viruses grown on radioactive P^{32}	Viruses grown on radioactive S^{35}
Radioactive DNA	Radioactive proteins
↓ attack E-coli	↓ attack e-coli
E-Coli becomes Radioactive	E-coli doesn't become radioactive

Properties Of Genetic Material →

RNA v/s DNA →

① It should be able to generate its replica (both DNA and RNA can do it)
② Chemically and Structurally Stable (RNA is less stable dete to 2'-OH group, and Uracil)
③ Should provide scope for slow changes (Mutation) (Both RNA and DNA mutate, but RNA mutates very fast)
④ Should be able to express itself in form of Mendelian Characters.

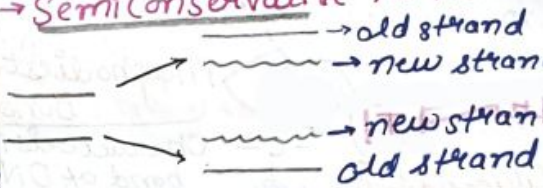
DNA being more stable, is better for storage of information.
RNA is better for transmission of information.

← RNA World →

① RNA was first genetic material.
② RNA used to act as genetic mate as well as catalyst enzyme.
→ Catalyst Enzyme = Ribozyme, Ribonuclease
③ DNA evolved from RNA and is more stable

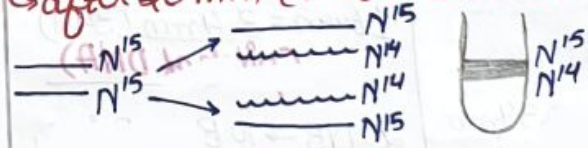
DNA Replication →

→ proposed by Watson and Crick
→ Semi Conservative in Nature.

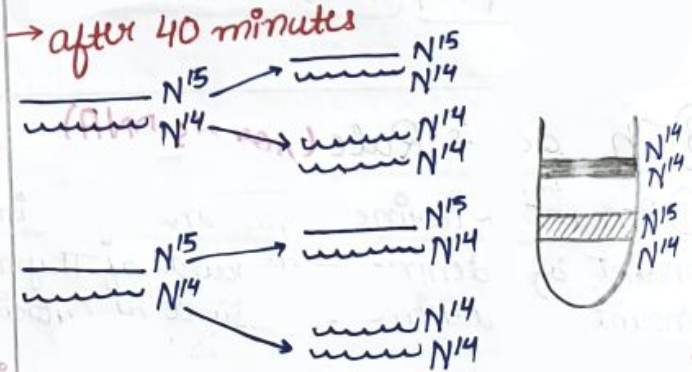


① Experimental Proof →
Meselson and Stahl Experiment (1958)
→ proof of Semiconservative nature.
→ E-coli grown in $^{15}NH_4Cl$ medium
→ All DNA strands have heavy nitrogen (N^{15})

→ CsCl density gradient centrifugation.
→ This E-coli was put on normal N^{14} media.
↳ after 20 min (E-coli divides)



→ after 40 minutes



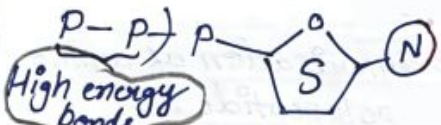
NEET SLAYER

② The Machinary and Enzymes of DNA Replication →

→ Enzyme = DNA dependent DNA polymerase. (Very fast = 2000 bp/sec)

→ Deoxyribonucleoside triphosphates

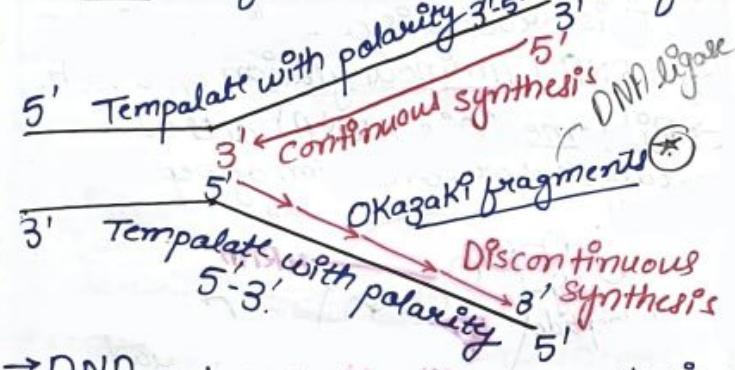
provide nucleotides for polymerisation — Provide energy for polymerisation.



NEET SLAYER

→ Replication starts at Origin of Replication (ori)

→ 2 complimentary strands are separated at ORI to form the replication fork.



→ DNA polymerase always synthesises new strand in 5'→3'.

→ Primase, SSB proteins, DNA A proteins are also used in replication.

→ Okazaki fragments are joined by DNA ligase.

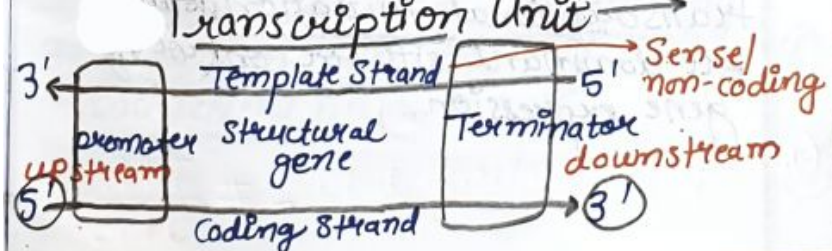
Transcription →

DNA → RNA

→ Only One DNA strand acts as template for RNA production. Why?

↳ Because, if both DNA strands act as template, 2 different but complementary RNAs will be produced which will form a dsRNA.

Transcription Unit →



→ promoter is located at 5' end of structural gene (take reference of coding strand only) and terminator is located at 3' end of structural gene.

Transcription Unit and Gene →

Gene → Segment of DNA giving rise to complete RNA.

Cistron → part of gene giving rise to single polypeptide chain.

Polycistronic Gene (in proKaryotes) → One RNA gives rise to many polypeptide chain.

Monocistronic Gene (euKaryotes) → One RNA gives rise to one polypeptide chain.

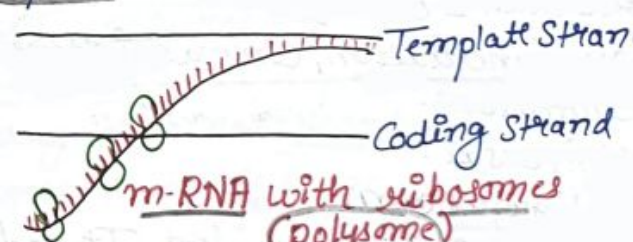
Bacterial Transcription →

→ Only one type of DNA dependent, RNA polymerase to synthesize all types of RNA (m-RNA, r-RNA, t-RNA)

→ mRNA does not require any processing

→ Transcription and translation occur in same compartment.

→ Transcription and translation coupled.



→ Initiation factor (σ Sigma) for initiation of transcription

→ Termination factor (ρ rho) for termination of transcription

Eukaryotic Transcription →

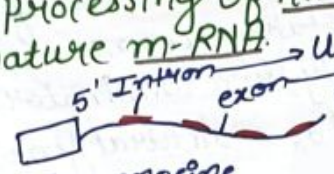
① 3 RNA Polymerases

RNA Polymerase I → r RNA (28s, 18s, 5.8s)

RNA Polymerase II → hn RNA (precursor of m-RNA)

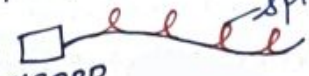
RNA Polymerase III → t RNA, 5s rRNA, snRNAs (small nuclear RNA's)

② processing of hnRNA to form mature m-RNA



① capping

methyl guanosine triphosphate ($^{m}GPPP$)



② Splicing of Introns

③ pony Adenylate tale (200-300)

Tailing

Genetic Code →

↳ How are sequence of Nitrogenous bases, related with amino acids of protein?

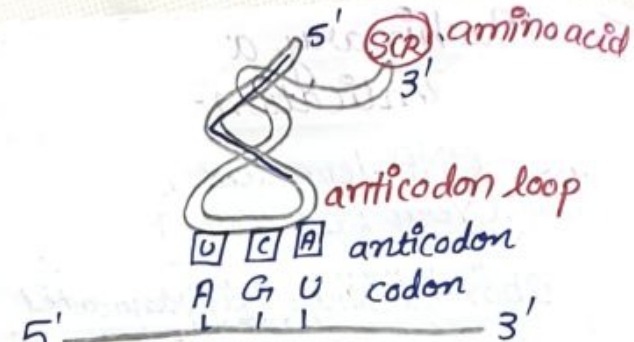
→ George Gamao (physicist), suggest that, since there are only 4 N Bases, to code for 20 aa, then codon should be triplet. (made of 3 nucleotides)

→ Salient features of Genetic Code →

① Code is triplet.
② 61 codons for 20 aa, 3 are stop Codons. (UAA, UAG, UGA)
③ Degenerate (some aa are coded by more than 1 codon)
④ No punctuation, commas, in genetic code
→ Continuous.
⑤ Universal.
⑥ Ambiguous (non).
⑦ AUG is initiater codon, It codes for Methionine.

t-RNA (the adapter Molecule)

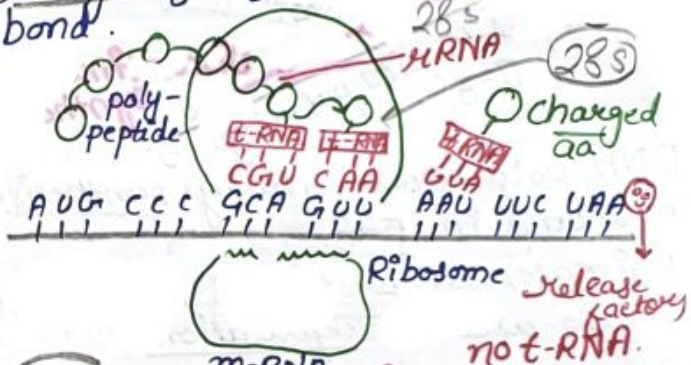
→ Reads code and binds to specific aa.
→ Also called soluble RNA.
→ Secondary structure of t-RNA (2-D)
↳ Clover leaf
→ Actual structure (3-D)
↳ Inverted L shaped.



Translation →

RNA → Protein

→ process of polymerisation of amino acids to form a polypeptide
→ formation of peptide bond requires energy
→ So, aa are activated, in presence of ATP and linked to t-RNAs.
→ This process is called charging of t-RNA / Aminoacylation of t-RNA.
→ Ribsome (28s rRNA) acts as catalyst for formation of peptide bond.



Mg^{2+} helps in binding.

Regulation of Gene Expression

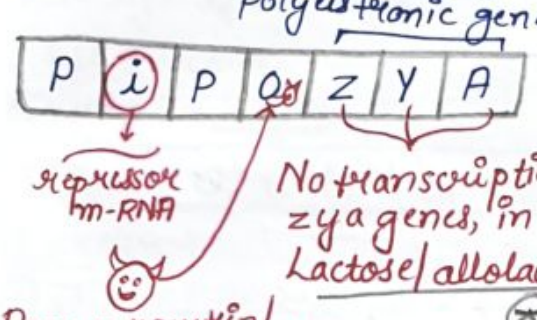
In eukaryotes, gene regulation could be exerted at →
① Transcriptional level.
② Processing level (splicing)
③ Transport of m-RNA from nucleus to cytoplasm.
④ Translation level.

In prokaryotes, control of rate of transcriptional initiation is the pre-dominant site for control of gene expression ✱

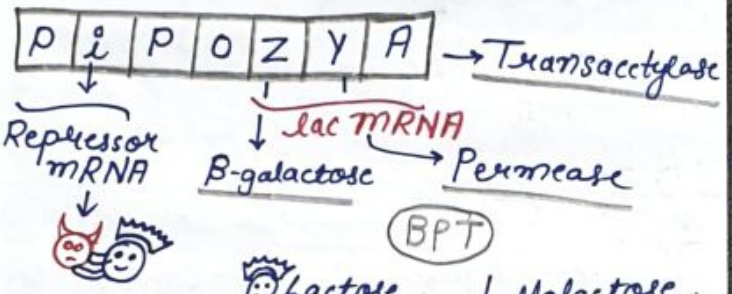
The Lac Operon

→ By Francis Jacob (geneticist), and Jacque Monod (biochemist)

Operon → Structural gene along with promoter and regulatory genes.



In presence of Lactose →



→ A very low level of expression of Lac-operon is present in cell at all times

→ This kind of regulation is referred to as negative Regulation.

Human Genome Project →

→ To find out complete DNA sequence of Human Genome.

→ 1990-2003 (13 Years)

→ Sequencing of Chromosome 1 finished in 2006

→ Mega Project.

→ approx 3×10^9 b.p. in human genome.

→ Cost US $3/bp (estimated)

(*) 9 Billion US Dollars

→ Obtained sequence would occupy 3300 books of 1000 pages each with 1000 letters on each page.

Goals Of HGP →

1. Identify approx 20,000-25,000 genes
2. Determine sequence of 3 million bases.
3. Store this information
4. Improved tools for data analysis.
5. Transfer related technologies to other sectors.
6. Adress ethical, legal and social issues (ELSI)

→ Project co-ordinated by U.S. Department of Energy and National Institute of Health.

→ Welcomm trust - Major Partner

Methodology

- Expressed Sequence Tag (To sequence only genes expressed as RNA) (*)
- Sequence Annotation (To sequence entire DNA) (actually used)

Host → Bacteria, Yeast

Vectors → BAC (Bacterial artificial chromosome), YAC (Yeast artificial chromosome)

→ Fragments were sequenced using method developed by Fredrick Sanger

Salient Features →

1. 3164.7 million (base pairs) ≈ 3 million bp
2. Average gene has 3000 bases
3. Largest Gene → dystrophin (2.4 million bases) (*)
4. 30,000 total genes
5. 99.9% nucleotides are same in all people.
6. Function unknown for over 50% of discovered genes.
7. Less than 2% of genome codes for proteins.
8. Repeated sequences form a large portion.
9. Chromosome 1 = 2968 genes
   Chromosome Y = 231 genes

⑩ 1.4 million SNPs.
(single nucleotide polymorphism)

DNA Fingerprinting →

→ For comparing DNA sequences of different individuals.
→ Developed by Alec Jeffery
→ Only 0.1% of DNA is different in humans.
→ DNA separated by density gradient.

Density Gradient Centrifugation →

Bulk DNA → Satellite DNA (Repetitive DNA)

Satellite DNA:
- Minisattelite (VNTR) — Variable number Tandem Repeats
- Microsattelite

On the basis of →
① A:T / C:G
② Length of segment
③ Number of Repetitive Unit.

Method

① Isolation of DNA
② Digestion with restriction endonuclease.
③ Gel Electrophoresis.
④ Transfer on Nitrocellulose membrane (Blotting)
⑤ Hybridisation with radio labelled VNTR.
⑥ Autoradiography.

DNA Polymorphism →

An inheritable mutation occuring in population at high frequency (> 0.01)

Applications →
① Solve paternity issue.
② Solve murder mystery