Unit 1 - Concept of Central Dogma

* Central Dogma of Molecular Biology -

- 1958 → Francis Crick

Replication ↻ DNA $\xrightarrow{\text{Transcription}}$ RNA $\xrightarrow{\text{Translation}}$ Protein

RNA → DNA : Reverse transcription

Defⁿ - Theory stating that genetic information flows only in one direction - from DNA to RNA to protein or RNA directly to protein.

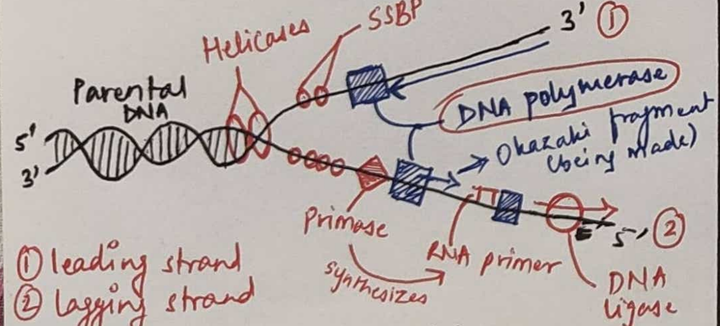
Genomic process = conversion of DNA information into a functional product.

DNA → provides instructions for making proteins

RNA → messenger to carry information through ribosomes to proteins.
precise determination of sequence
{bases in NA / a.a. residues in protein}.

I] DNA Replication -

- DNA = self-replicating structure → New DNA molecule = 1 new strand + 1 original strand
- Replication = semi-conservative
  - ↳ enzyme-catalyzed reaction
  - ↳ Biological polymerization (makes multiple copies of itself)



Helicases - unwinds parental double helix

SSBP - stabilize unwound parental DNA

DNA polymerase - synthesizes leading strand continuously (5' → 3')

lagging strand - synthesized discontinuously
↳ Primases - synthesizes short RNA primes - further extended by DNA polymerase to form Okazaki fragment → joined to growing strand by DNA ligase

* Steps of DNA replication -

① Initiation

- Replication begins at "origin of replication" (particular point where replication originates - any other random point - would cause mutations).

+

- Unwinding and unzipping of DNA strands → formation of replication fork. (ENZYME → HELICASE)
  Y-shaped region in a chromosome that serves as growing site for DNA replication.
- To remain separated → ENZYME SSBP (single stranded binding proteins) attach to the strands

↓

② Elongation

ENZYME - DNA POLYMERASE - starts synthesizing complementary seq. in each strand.
↳ Parental strand = template for synthesizing new daughter strands.

- Elongation is unidirectional (5' → 3')
- Leading strand (3'-5') → continuous
  Lagging strand (5'-3') → discontinuous replication.
  occur as "Okazaki fragments"
  (later joined by ENZYME - DNA LIGASE)

(★) Purines (A, G). pair with pyrimidines. (C, T) A = T C ≡ G

③ Termination

- occurs in diff. ways in diff. organisms
eg - E. coli - chromosomes are circular
∴ termination occurs when 2 replication forks b/w 2 terminals meet each other.

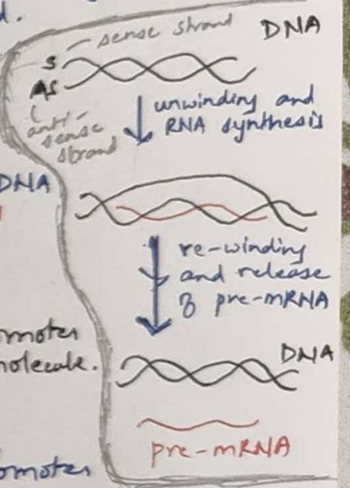
II] Transcription -

- process in which a gene's DNA sequence is copied (transcribed) - to make an RNA molecule.
- 1st step of gene expression.

(★) Only one strand of DNA is copied during transcription
↳ "template strand"
↳ RNA synthesized → mRNA. ⇒ carries info. used to encode a protein.

(★) Enzyme - DNA-dependent RNA polymerase
↳ uses single-stranded DNA to synthesize complementary RNA strand.

* Steps of Transcription -



① Initiation

- ENZYME - RNA POLUMERASE attaches to & moves along the DNA molecule until it - recognizes a promoter sequence
(indicates the starting point of transcription
\# there may be multiple promoter sequences within a DNA molecule.

(★) Transcription factors → proteins that control rate of transcription - bind to promoter sequences with RNA polymerase.

- Once bound to ~~RNA~~ promoter sequences, RNA polymerase - unwinds a portion of DNA double helix - exposing the bases on each of the two DNA strands.

↓

② Elongation - (elongates in 5' → 3' direction)

- RNA Polymerase - adds RNA nucleotides complementary to DNA strand.
- Template strand - read in 3' → 5' direction; provides template for new mRNA molecule.
  +
- Coding strand - other DNA strand - ∵ base seqs. are identical to synthesized mRNA (except for T and U).

↓

③ Termination -

- Elongation continues until RNA polymerase encounters a stop (termination) sequence. → ∴ mRNA building complete
- Transcription stops → RNA polymerase released from DNA template → crude mRNA processed - capping, tailing, splicing

III] Translation — (site - ribosomes)

- process by which mRNA codes for a particular protein
- **ribosome** — translates mRNA produced from DNA into a chain of specific a.a. which leads to protein synthesis.
- ATP required — given by charged tRNA.
- Translation = process of polymerization of a.a. to form a polypeptide.

- Genetic code contained within mRNA
  - ↳ decoded to produce a specific seq. of a.a. in a polypeptide chain.
  - ↳ mRNA nucleotide bases — read as codons of three bases

∴ Each "codon" codes for a particular a.a.

* RNA —

- Ribonucleic acid — helps in protein synthesis in our body.
  + responsible for production of new cells in the human body.
- single - stranded
- Consists of single ribose sugar molecule.
- also referred to as an enzyme
  - ↳ helps in process of chemical rxns in the body.
- Nitrogen bases — A, G, C, U

major building blocks of RNA

A = U
C ≡ G

- resembles a — "hairpin structure"
- Functions of RNA —

① Facilitate translation of DNA → proteins
② Adapter molecules in protein synthesis.
③ Serves as messenger b/w DNA and ribosomes.
④ Carrier of genetic information in living cells.

	mRNA	tRNA	rRNA
Also called	Messenger RNA	transfer RNA/ soluble RNA	ribosomal RNA/ catalytic RNA
Shape	Lineas	Clover - leaf shaped	Sphere shaped
Function	Carries message of transcript DNA codes of polypeptides from nucleus to ribosomes.	Carries specific a.a. to ribosome ∴ aids in translation	Associated with specific proteins to form ribosomes
Location	formed in nucleus; transported to cytoplasm	end points of each a.a.	component of ribosomes - within cytoplasm
	✱ vital role in transcription and translation	✱ forms link between mRNA and a.a.	✱ most pre-dominant ✱ fundamental role in translation

* Operon Model — (Jacob and Monod - 1961)

- Operon = cluster of genes that are transcribed together to give a single mRNA molecule which encodes multiple proteins.
  - ↳ group of structural genes whose expression is coordinated by an operator.
- Repressor = encoded by a regulatory gene binds to the operator and expresses the transcription of operon.
- Gene product = section of DNA that contains adjacent genes such as —

① structural genes + ② Operator genes + ③ Regulatory genes.

∴ Operon → "primary component of translation and genetic regulation"

3 components —

① Structural genes — encode enzymes/ proteins
② Regulatory genes — encode regulatory proteins (trans - acting molecules) which either promote / reduce structural gene expression.
③ Regulatory sites (cis - acting elements)
  ↳ where regulatory proteins ~~and~~ bind and control gene expression.

1. Lac operon -

① It is an operon or a group of genes with a single promoter that encode genes for the transport and metabolism of lactose.

② The genes are expressed only when lactose is present and glucose is absent.

③ It is an example of inducible operon. The lac operon encodes three structural genes necessary to acquire & process the disaccharide lactose from the env., breaking it down into the simple sugars glucose & galactose.

④ Lac operon has two independent genetic systems. One is promoter-operator - lacZ-lacY-lacA and another is promoter - lacI system.

⑤ Two regulators turn the operon "on" and "off" in responsible to lactose & glucose levels: the lac repressor & catabolite activator protein (CAP).

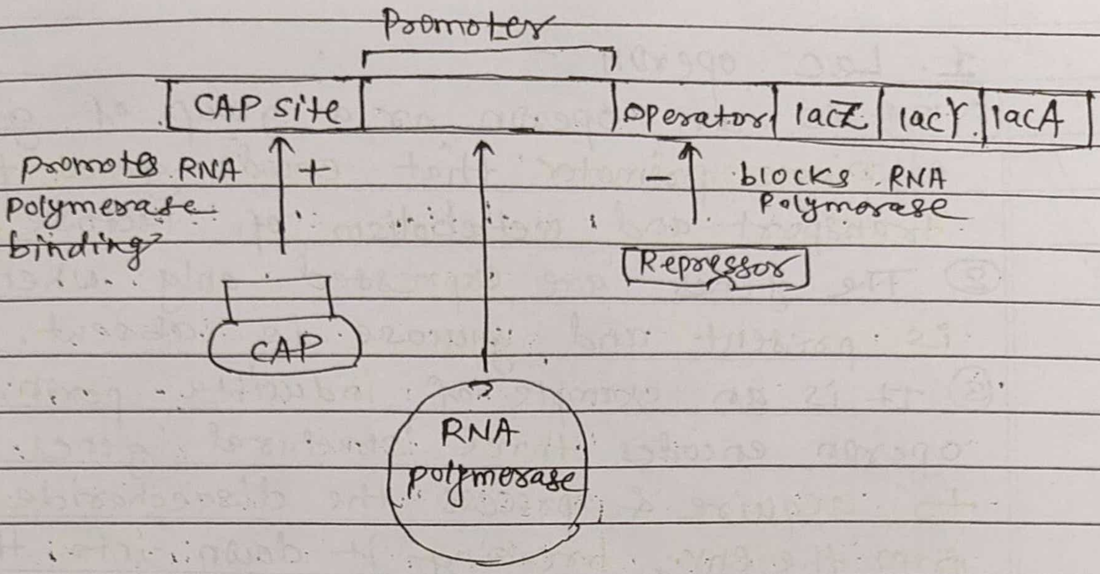
⑥ Lac repressor acts as lactose sensor. It normany blocks transcription of the operon, but stop acting as a repressor when lactose is present.

⑦ Catabolite activator protein acts as glucose sensor. Activates transcription of operon only when glucose level are low.

⑧ It is made up of promoter with operator, and three genes (lacZ, lacY, lacA) which encode β-galactosidase, permease & transacetylase.

⑨ Three genes involved in breakdown of lactose into its metabolites: β-galactosidase breaks lactose down into glucose and galactose while other two proteins aid in metabolic process.

⑩ Its expression controlled by regulatory gene lacI.



The lac operon

2. Trp operon –

① The Trp operon is responsible for synthesis of the aa- tryptophan when it is not available in the envi.

② It is made up of promoter with operator, and five genes that encode enzymes for tryptophan synthesis.

③ The Trp operon regulated by regulatory gene trpR, a gene that is located at a distance from the Trp operon.

④ It is example of repressible operon; it is on unless turned off by a repressor protein. The repressor protein is synthesized by trpR.

⑤ When corepressor is present, in this case tryptophan, it binds to the repressor protein in an allosteric site.

⑥ This changes the conformation of the protein such that it can bind to the operator & block transcription by preventing the binding of RNA Polymerase to the promoter.

⑦ In this way the cell saves energy by not producing tryptophan when it is already present.

* What is gene splicing? –

① Gene splicing is a post-~~translation~~ transcriptional modification in which a single gene can code for multiple proteins.

② Gene splicing is done in eukaryotes, prior to mRNA translation, by differential inclusion or exclusion of regions of pre-mRNA.

③ Introns (non-coding region), are excised out of the primary messenger RNA transcripts and the exons (coding regions) are joined together to generate mature messenger RNA which serves as the template for the synthesis of a specific protein.

④ Thousands of genes presents in eukaryotes produce millions of proteins through the gene splicing event which forms splice variants of pre mRNA.

⑤ Gene splicing event cuts the pre-mRNA to produce a variety of proteins that are a resultant of varied combination of exon/intron retention & removal both.

Uses of gene splicing technology:-

① for the production of vaccines.

② for the production of vitamin B.

* What is Post translation modification –

① Post-translational modifications involve modifications of the aa chain, terminal amino, or carboxyl group by enzymes.

② Due to these modifications, the structure, stability, or activity of the proteins get affected.

③ In post-translational modifications, the polypeptide chains are synthesized by going through translation inside the cell cytoplasm.

④ The processes which causes modifications are methylation, glyicosylation, nitrosylation, ubiquitination, proteolysis, etc.

⑤ post-translational modifications of proteins plays some significant functions in cell growth, cell processes, etc.

⑥ It is important for controlling the stability of proteins, localization and conformation.

⑦ By multiple post-translational modifications, genetic information imbibed in DNA is transcribed, translated, & increases its complexity.

⑧ It help in the mediation of stress perception, protein homeostasis, energy shift control, & defense by the immune system.