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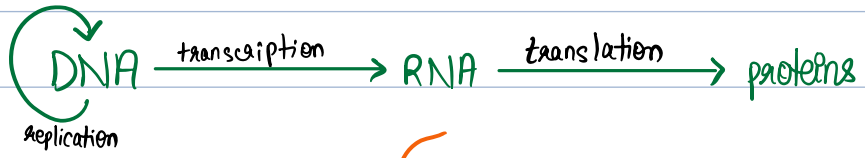
Molecular biology

- **Central Dogma**



- **DNA Replication:**
  - ori (1/many)
  - replication bubble, fork
  - DnaA, ORC
  - new strand synthesis in 5' → 3'
  - Leading strand (3' → 5')
  - Lagging strand (5' → 3') ⇒ Semi-discontinuous process
  - Okazaki fragments (150-200 nucleotides)
  - RNA primers (produced by RNA primase)
  - DNA polymerase
  - Ligase
  - DNA helicase/unwindase/swivelase
  - Helix destabilizing proteins (SSB)
  - DNA topoisomerase (DNA gyrase in prokaryotes)
  - Proof reading by DNA polymerases

  - Semi-conservative
  - Bidirectional
  - Semi discontinuous

  - deoxyribonucleotides [dATP, dGTP, dCTP, dTTP]

Functions of DNA polymerases:
- polymerase activity 5' → 3'
- proof-reading 3' → 5' exonuclease
- repair 3' → 5' exonuclease

INHIBITORS:
- Nucleotide analogues → arabinosyl cytosine → arabinosyl adenine } Humans
- Antibiotics → Ciprofloxacin → novobiocin ⇒ inhibit bacterial DNA gyrase
- Andriamycin, Doxorubicin ⇒ inhibits human topoisomerase
- 6-mercaptopurine ⇒ inhibits human DNA polymerase

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PROKARYOTES:

DNA pol I – main DNA repair

DNA pol II – least reactive

DNA pol III – main DNA polymerase

EUKARYOTES:

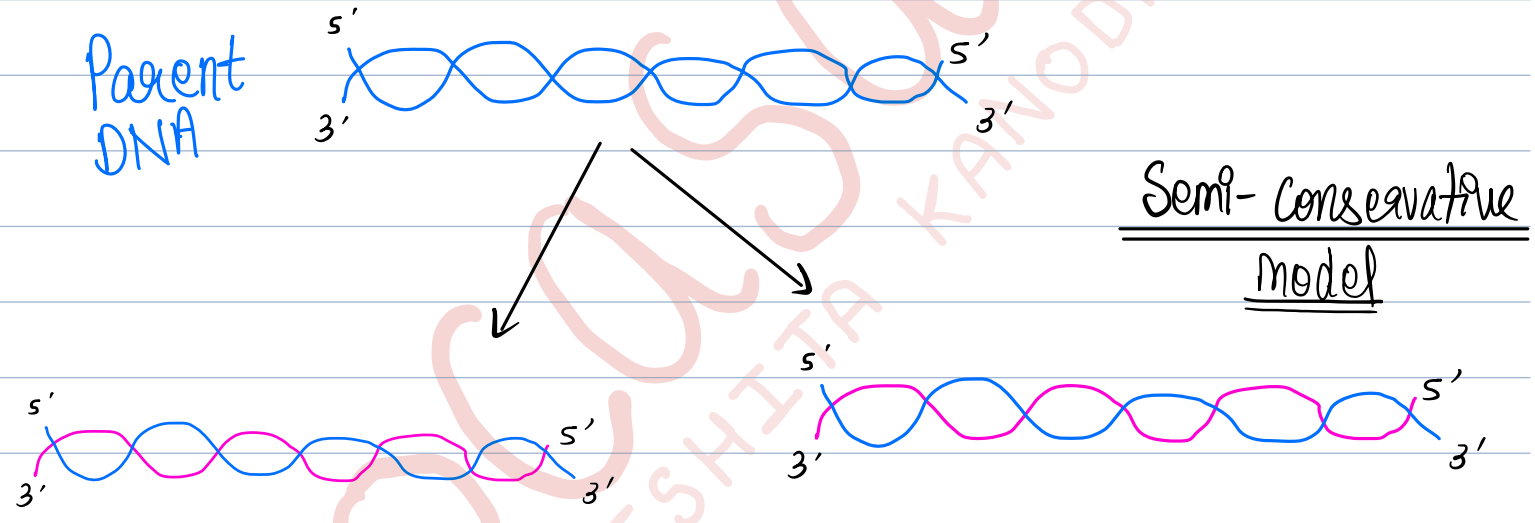
α – synthesis in lagging strand

β – DNA repair

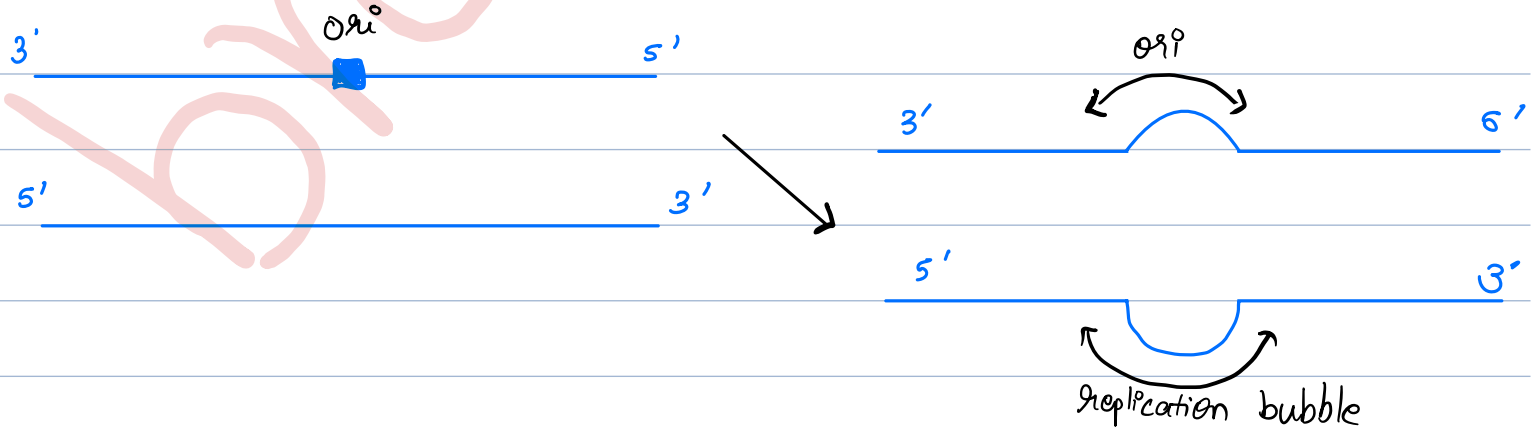
γ – synthesis of cytoplasmic/mitochondrial DNA

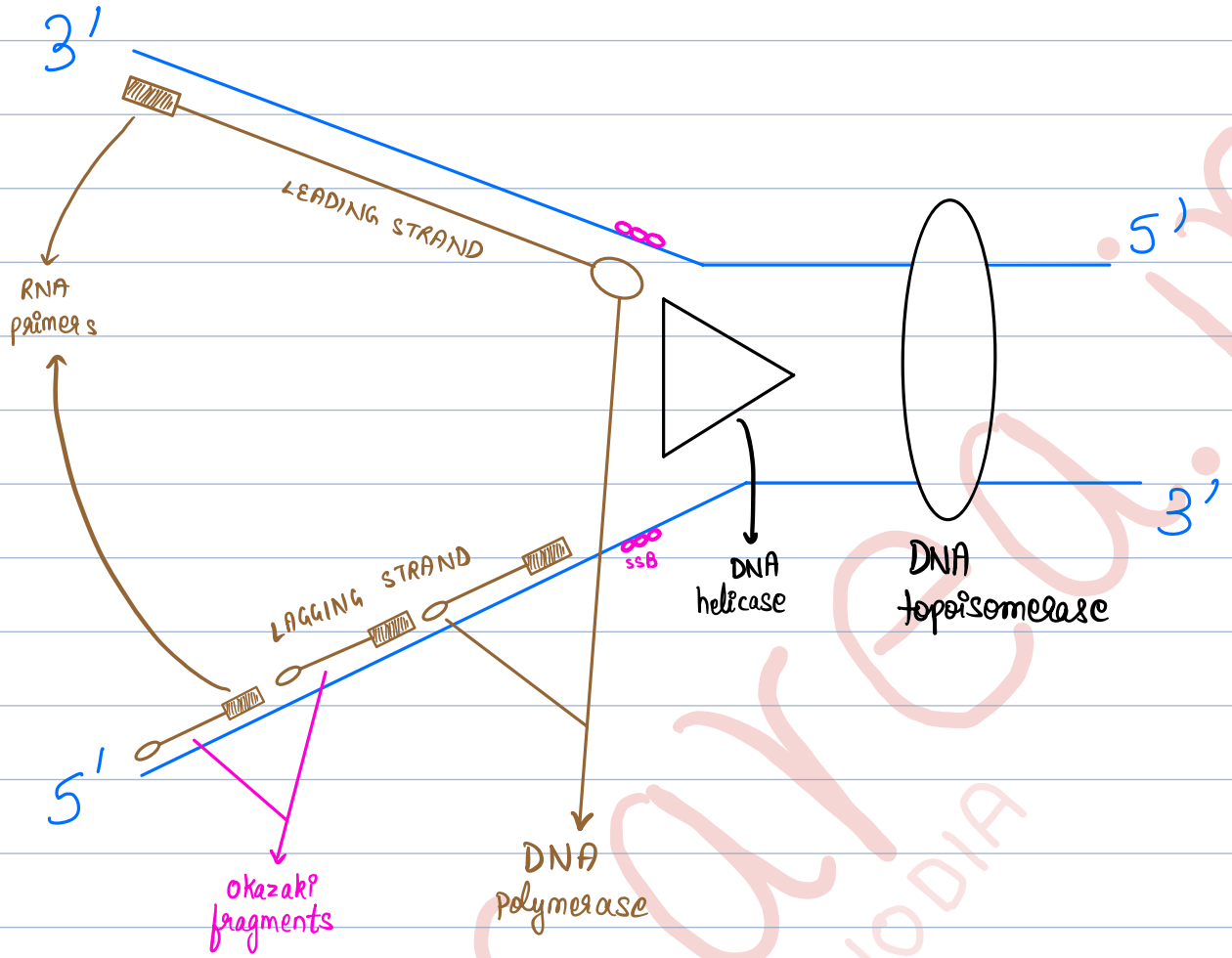
δ – synthesis of leading strand

ε – proof reading



Semi-conservative model





a segment of DNA unwinds at ORI

↓

2 replication forks run in opposite direction

↓

DNA helicase binds to DNA near replication fork

↓

SSB proteins bind with single-stranded DNA
& maintain separation of strands

↓

DNA topoisomerase removes supercoiling & produces negative supercoiling

↓

- Single RNA primer attaches to leading strand at 3' end
- many RNA primers attach to lagging strand

↓

DNA polymerases read parent strand in 3' → 5'
& synthesize new strand in 5' → 3'

↓

Leading strand is synthesized continuously
Lagging strand is synthesized discontinuously as okazaki fragments

↓

- RNA primers are removed & gaps are filled by DNA polymerases
- 2 pieces are joined by ligase

- **Telomere**: ends of each chromosome
  - → repeated telomeric sequence in humans ⇒ 'GGGTTA'
  - → telomeres prevent loss of DNA from chromosomal ends during replication by forming a cap
  - → everytime, during replication, telomere is lost & DNA remains undamaged.
  - → telomeres prevent chromosomes from fusing with each other

  Telomerase delays cell ageing.

• Transcription:

- RNA polymerases (DNA dependant RNA polymerases)
- Template Strand (3' → 5') [Non-coding / Antisense strand]
- Coding / Non-template / Sense strand (5' → 3')

→ (recognized by σ factor in prokaryotes ; RNA pol itself recognizes in eukaryotes)

Promoter: binding site for RNA polymerase on DNA

Prokaryotes: pribnow box ⇒ −10 bps ⇒ TATAAT

⇒ −35 bps ⇒ TTGACA

Eukaryotes: Hogness box ⇒ −20 bps ⇒ TATA AAA or TATA TAT
(TATA box)

⇒ −70 to −80 bps ⇒ CAAT box.

RNA polymerases:

RNA pol I ⇒ rRNA

RNA pol II ⇒ mRNA

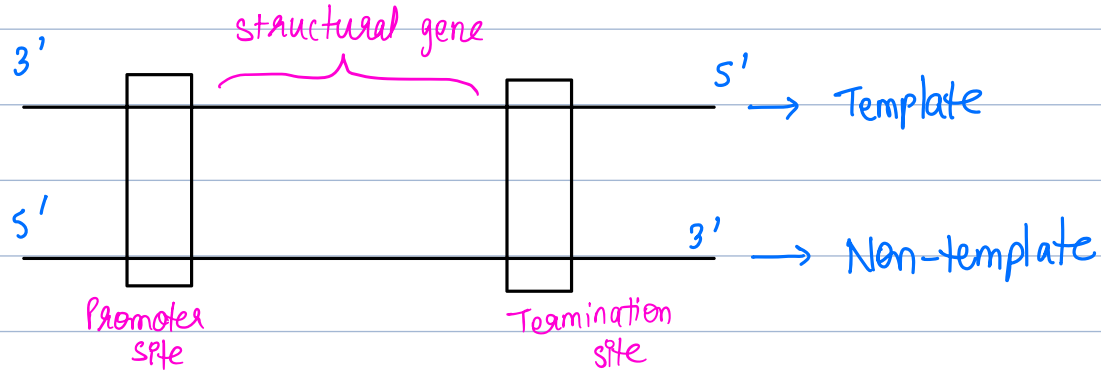
RNA pol III ⇒ tRNA

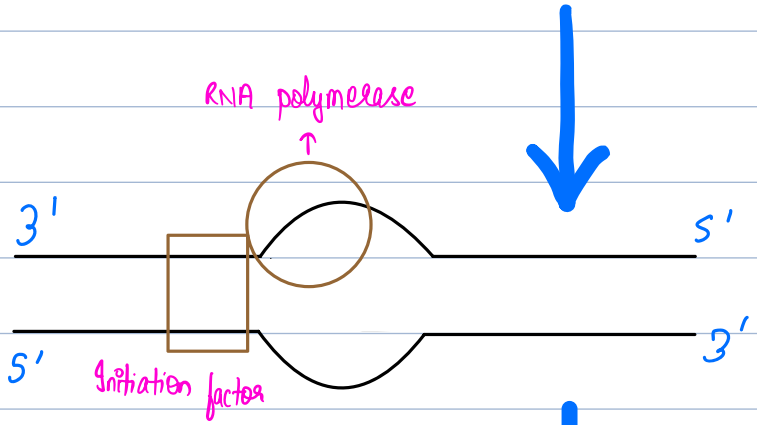
→ (recognized by ρ factor in prokaryotes ; RNA pol itself recognizes in eukaryotes)

Terminator:

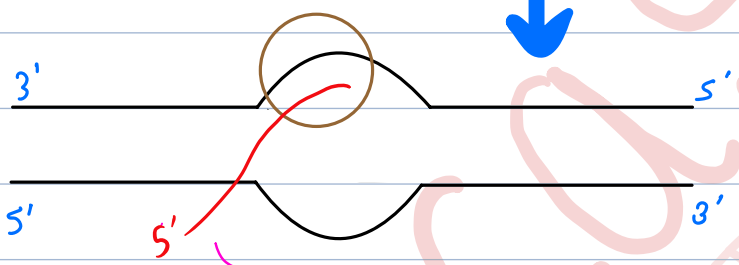
↳ site on DNA where transcription ends

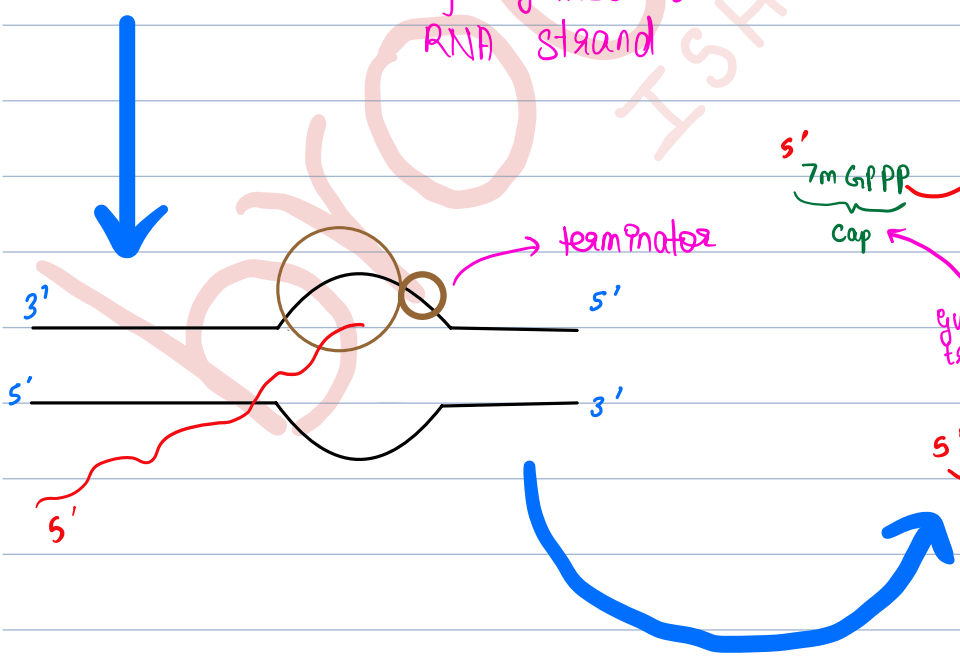
3 steps in transcription → initiation (I)
→ elongation (E)
→ termination (T)

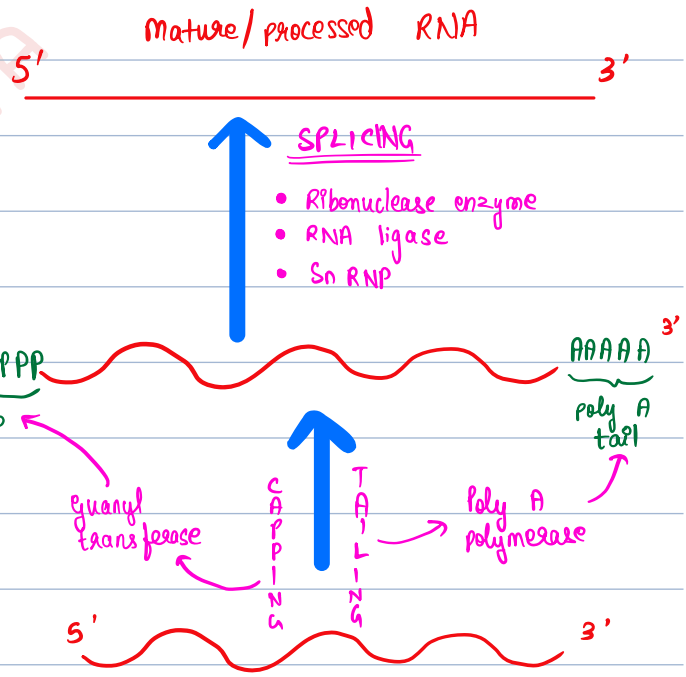




Splicing: removal of introns & joining of exons.







Newly synthesized strand of RNA (hnRNA) (primary transcript)

binding of RNA polymerase & transcription factors to promoter

↓

Unwinding of small DNA segment ⇒ transcription bubble

↓

(I) Attachment of 1st ribonucleotide to RNA polymerase

↓

Attachment of 2nd ribonucleotide to RNA polymerase

↓

Formation of phosphodiester bond between 1st & 2nd ribonucleotide

↓

(E) Elongation continues until teaminator site is reached

↓

(T) Recognition of termination factor & termination

INHIBITORS:

- Actinomycin-D, Mitomycin ⇒ binds with DNA template & interferes with movement of RNA polymerase
- Rifampicin, Rifamycin ⇒ bind with prokaryotic RNA polymerase.
- α-Amanitin (in poisonous mushrooms) ⇒ inhibit eukaryotic RNA polymerase

Post-transcriptional Modifications:

Splicing

5' capping

Poly A tailing

Methylation (of some internal nucleotides)

Endonuclease cleavage (from 3' end)

Transcription factors:

Prokaryotes ⇒ σ factors

Eukaryotes ⇒ GTF's ; TF II A, B, D, E, F, H.

Translation:

- Degeneracy of codons ⇒ one amino acid ($\bar{a}\cdot\bar{a}$) is coded by ≥1 codon(s).
- Stop Codons ⇒ UAA
  UAG
  UGA
- Polarity ⇒ genetic code is read in 5' ⟶ 3' direction
- Universality ⇒ universally applicable genetic code (except mitochondrial & protozoan DNA)
- Non-ambiguous ⇒ specific
- Non-overlapping Code
- Commaless Code
- Initiation codon ⇒ AUG (methionine)

Steps in Translation →
- activation of $\bar{a}\cdot\bar{a}$
- charging of t RNA
- protein synthesis →
  - Initiation
  - Elongation
  - Termination

Activation of $\bar{a}\cdot\bar{a}$

$$\bar{a}\cdot\bar{a} + ATP \xrightarrow[\text{synthetase}]{\text{Amino-acyl tRNA}} \underset{\text{enzyme-complex}}{\text{Amino acyl AMP}} + PP_i$$

Charging of t RNA

$$\underset{\text{enzyme-complex}}{\text{Amino acyl AMP}} + tRNA \longrightarrow \underset{\text{complex}}{\text{Amino acyl tRNA}} + \text{enzyme} + AMP$$

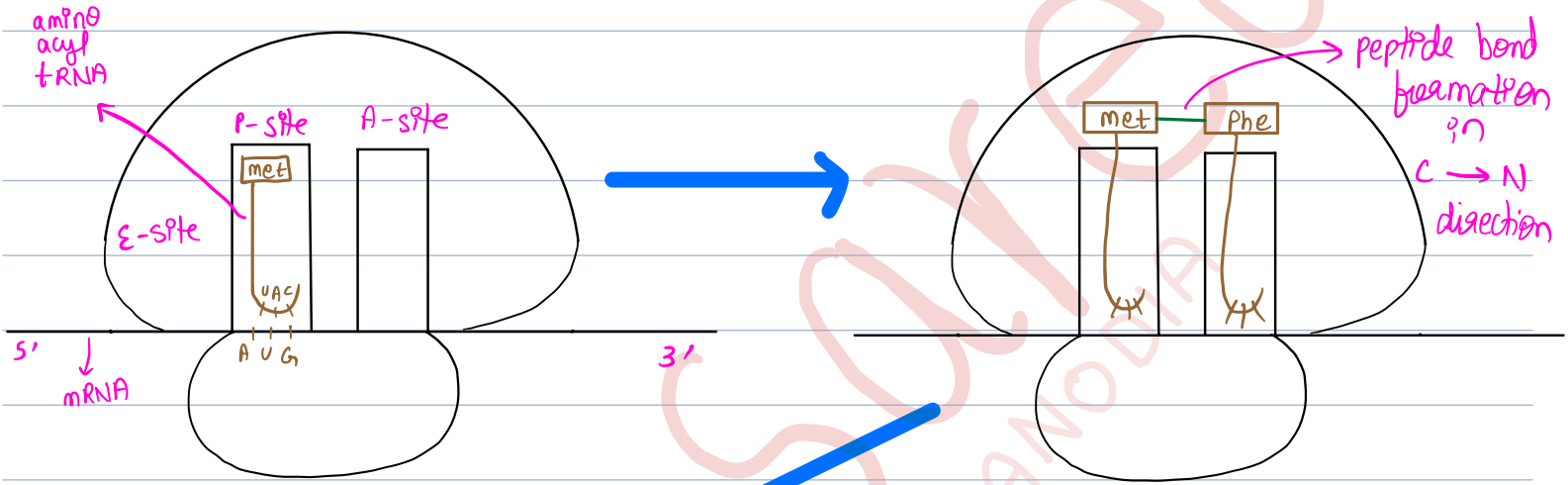
Initiation

40s + mRNA $\xrightarrow[Mg^{+2}]{eIF}$ 40s mRNA complex

40s mRNA complex + Met tRNA $\xrightarrow[GTP]{Mg^{+2},\ eIF}$ 40s mRNA formyl met tRNA

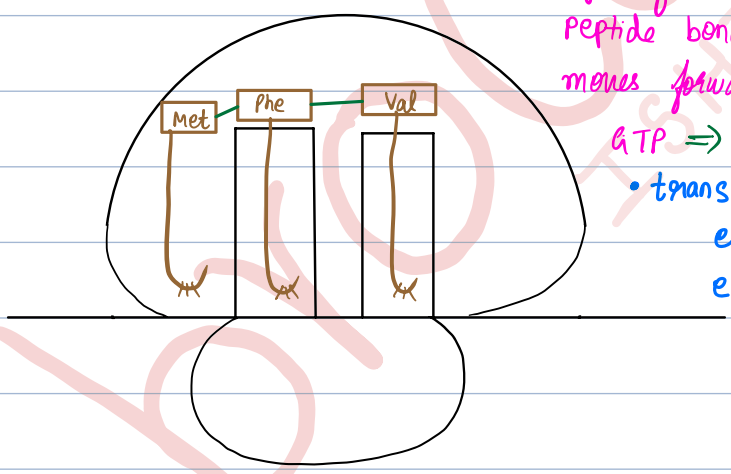
40s mRNA formyl met tRNA $\xrightarrow{Mg^{+2},\ eIF\ (2,5),\ GTP}$ 60s

40s mRNA met tRNA 60s complex



After formation of peptide bond, ribosome moves forward by using GTP ⇒ translocation

- translocase enzyme
  - eEF_1
  - eEF_2



once stop codon is encountered, release factors cause termination (eRF)

* 7m G PPP cap, Poly A Tail } Untranslated Regions

INHIBITORS:

Prokaryotes

- Tetracycline → inhibits binding of amino acyl tRNA to ribosomal complex
- Chloramphenicol → inhibits peptidyl transferase activity
- Erythromycin → prevents translocation process
- Streptomycin → binds to smaller ribosomal subunit of prokaryotes & causes misreading of genetic code

Eukaryotes

- Cycloheximide / Puromycin → inhibits peptidyl transferase
- Diptheria toxin → prevents translocation
- Ricin → (from castor bean)
  - ↳ inactivates eukaryotic 28S rRNA.

Post - translational Modification:

- Glycosylation → Immunoglobulin formation
- Proteolytic degradation (trimming) → removal of a.a residues from N-terminal
  - pepsinogen → pepsin ; preproinsulin → insulin
- Phosphorylation of hydroxyl groups of serine, threonine, tyrosine
- γ-carboxylation of glutamic acid of prothrombin converts it to active form.
- Hydroxylation of proline & lysine residues converts protocollagen → active collagen.

Protein Targeting / Protein Sorting:

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→ for transport of newly synthesized proteins to their target sites
→ golgi apparatus plays a major role

Newly Synthesized Proteins

Proteins for external secretion: - plasma membrane integral proteins, lysosomal enzymes
- presence of signal sequence that targets them to correct destination
- synthesized in ribosomes of rough ER

Proteins for internal part of cell: - cytosolic, mitochondrial, nuclear, peroxisomal proteins
- synthesized in free ribosomes
- do not have signal sequences

Zellweger Syndrome, Cystic fibrosis ⟹ diseases associated with defect in protein targeting

Base Excision Repair:

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↳ to repair DNA damages like deamination of cystine to uracil / adenine to hypo-xanthine

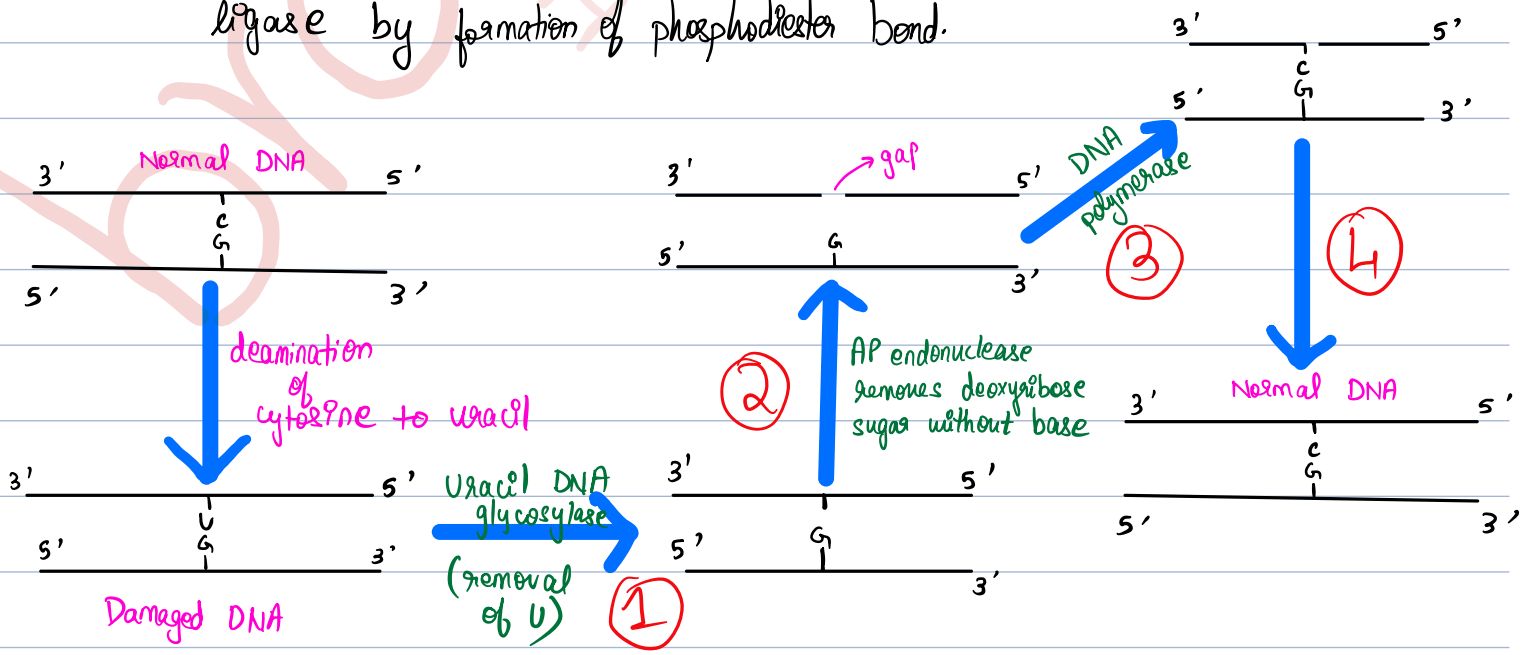
4 steps → Incision, Excision, Resynthesis, Ligation

(1) **Incision:** DNA damage is recognised
↳ Uracil DNA glycosylase enzyme removes uracil in DNA (by hydrolyzing β-N-glycosidic linkage between base & deoxyribose)
→ This leaves an apyrimidinic / apurinic site ⇒ AP sites.

(2) **Excision:** Specific AP endonuclease enzymes remove deoxyribose with missing base from DNA
→ this leaves a gap in DNA

(3) **Resynthesis:** DNA polymerase synthesizes the gap stretch

(4) **Ligation:** gap b/w resynthesized nucleotide & existing nucleotide is joined by DNA ligase by formation of phosphodiester bond.



Xeroderma Pigmentosum:

→ genetic defect

→ due to defect in nucleotide excision repair mechanism of DNA required for UV-damage repair

→ Exposure of skin to UV rays can cause joining of 2 adjacent thymines to form thymine dimer which prevents action of DNA polymerase.

→ defect in gene required for nucleotide-excision repair

Clinical manifestation – marked sensitivity to sunlight

– consequent formation of multiple skin cancers & premature death.

Other diseases associated with DNA Repair – Fanconis anemia, Bloom's disease, Ataxia telangiectasia.

Mutations

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- Point mutation
  - Substitution
    - Transition
    - Transversion
  - Frame Shift Mutation
    - Addition (Insertion)
    - Deletion
- Chromosomal mutation
  - Chromosomal Aberration
    - Deletion
    - Inversion
    - Duplication
    - Translocation
  - Numerical Variation
    - Euploidy
    - Aneuploidy

Substitution: change of nitrogenous base by another one

Ex: Sickle Cell Anemia

Transition ⟹ purine – purine / pyrimidine – pyrimidine substitution

Transversion ⟹ purine – pyrimidine substitution

Frame-Shift: Addition / Deletion of one or more bases

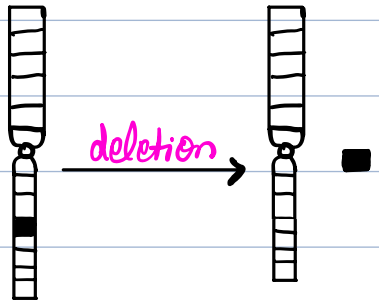
Ex: Thalassemia

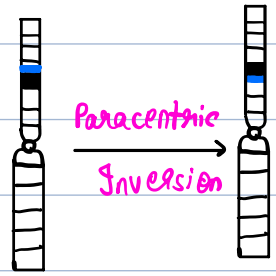
Consequences of point mutation

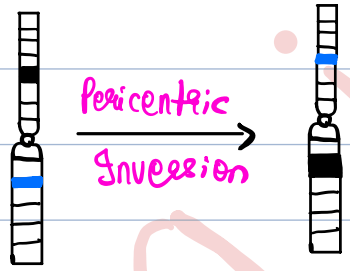
- Silent mutation: change in nitrogenous base does not cause change in a.a coded
- Missense ": changed codon codes for a different a.a
- Nonsense ": changed Nitrogenous base codes for termination codon resulting in premature termination of translation.

- Acceptable Missense: Hb Hikari
- Partially acceptable Missense: HbS
- Unacceptable Missense: HbM

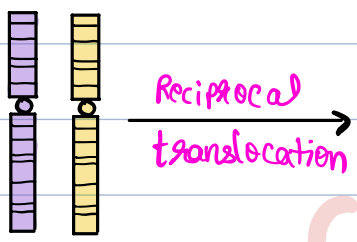
Structural Aberrations

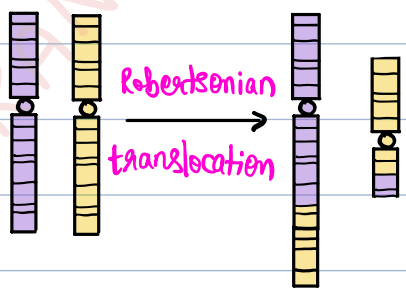


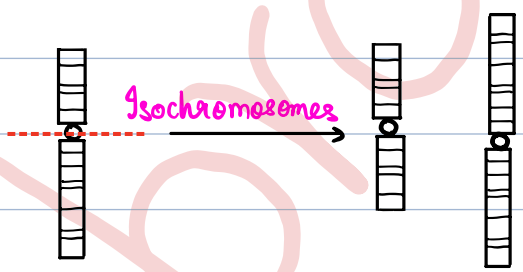


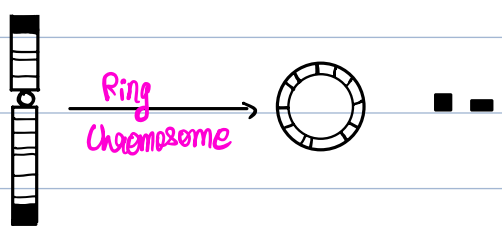


Translocation









Mutagens:

Physical
Chemical
Biological

→ Mutation is any damage to DNA that is passed on to subsequent generations

→ agents that trigger DNA damage = mutagens

Physical Agents: UV rays, X-rays, γ-rays, visible rays, excessive heat

Chemical Agents: Nitrous acid, aflatoxin B, arsenic, asbestos, 5-bromouracil

Biological Agents:

DNA viruses: Epstein Barr virus, Hepatitis B virus, Human papilloma virus

RNA viruses: HIV, Rous Sarcoma virus

↳ make DNA copy of viral genome

↓

Insert newly formed viral DNA into host DNA
(they have powerful promoter sequence)

↓

∴ elevated expression of gene

↓

proto-oncogenes are converted to oncogenes

} Insertion mutagenesis

Oncogens / Carcinogens: agents that are capable of causing cancer in humans by causing damage to genetic material

(All oncogens = mutagens
All mutagens ≠ oncogens)

Cancer:

malignant growth / tumor caused by uncontrolled cell division

↓

multifactorial in nature

- **Oncogens:** agents causing cancer
- **Oncogenes:** genes causing cancer (represented in lower case)
- **Anti-oncogenes:** genes that suppress cancer (represented in upper case)
- **Proto-oncogenes:** (c-oncogenes) genes that are potentially capable of becoming oncogenes by mutation.

Oncogenes:

src: rous sarcoma virus oncogene that causes sarcoma

↳ produces a protein having tyrosine kinase activity that causes transformation of normal cell into cancer cell.

Proto-oncogene in human — c-src

Conversion of proto-oncogenes to oncogenes: (Mechanisms)

- **Point Mutation:** most common
- **Promoter Insertion** ⇒ viral DNA with powerful promotor is inserted in the vicinity of proto-oncogene
- **Chromosomal Translocation** ⇒ exchange of one part of a chromose with another chromosome.
- **Amplification of Proto-oncogene** ⇒ multiplication of a segment of DNA producing multiple copies of gene in the cell.

Tumor Suppressor Genes / Anti-oncogenes:

→ provide protection against cancer

Ex: p53 gene & RB gene (retino blastoma).

p53 gene: "Guardian of genome"

↳ in chromosome 17

→ produces protein of 53 kD

→ protein is expressed during cell damage & inhibits cell division until damage is repaired

→ if damage is severe, it directs the cell to apoptosis

RB gene: first tumor suppressor gene to be discovered

↳ chromosome 13

→ produces a protein ⇒ p105

→ protein binds & inactivates transcription factor & thus suppresses cell division.

BRCA gene: chromosome 3

↳ prevents breast cancer.

[failure of apoptosis may lead to cancer]

Oncogenic Viruses:

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Epstein – Barr virus ⇒ Burkitt's lymphoma
Human Papilloma virus ⇒ Hepatoma
Hepatitis B virus ⇒ uterine cervical cancer

} DNA viruses

Cancer Treatment:

- Radiotherapy ⇒ uses radiations (X-rays, protons, etc) to kill cancer cells
- Chemotherapy ⇒ actinomycin D, 5-fluorouracil, vincristine & vinblastin, etoposide, cyclophosphamide, 6-mercaptopurine, methotrexates.
- Surgical removal of tumor
- Hormonal Therapy (in prostate & breast cancer)
- Combined Therapy
- Modifiers (interleukins, monoclonal antibodies)
- Bone Marrow Transplantation (in leukemia)

Tumour Markers:

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↳ biological indicators employed to detect the presence of cancer
→ abnormally synthesized & released specifically by cancer cells.
→ also used in localization, differentiation & prognosis of different cancers
→ not specific only for cancers
→ significant elevations of these biomarkers is also seen in non-cancerous conditions (Ex: elevation of carcinoembryonic antigen (CEA) occurs in variety of cancers, heavy smokers, people with ulcerative colitis & cirrhosis)

- **AFP (α-feto Protein)**: oncofetal antigen
  ↳ synthesized by yolk sac in fetal life
  → elevated in – liver cancer
  – germ cell tumor
  – teratoma of ovary

- **CEA**: oncofetal antigen
  ↳ synthesized by embryonic tissues
  → elevated in – colorectal cancer
  – lung "
  – breast "
  – ovary "
  – pancreatic "
  – alcoholic liver cirrhosis
  – smokers
  – ulcerative colitis
  – diabetes mellitus

- **PSA (Prostate Specific Antigen)**: for early detection of prostate cancer

→ Normal Serum level: 1 – 4 ng/dL
→ > 10 ng/dL ⇒ prostate cancer
→ more reliable marker than acid phosphatase in detection of prostate cancer
→ also elevated in – prostatitis
– benign prostatic hypertrophy (BPH)

- Prostatic Acid Phosphatase (normal level < 3 KA unit/dL) ⇒ prostate cancer
- Calcitonin ⇒ medullary carcinoma of thyroid
- β-hCG ⇒ choriocarcinoma, germ cells cancer, trophoblastic disease
- CA-125 (secreted cancer antigens) ⇒ ovarian cancer
- Anti-thyroid peroxidase (tpo) antibodies ⇒ Grave's disease
- Estrogen, Progesterone ⇒ prognostic value in breast cancer

Growth Factors: stimulate cell proliferation by stimulating mitosis & differentiation of target cells

Ex:
- Epidermal growth factor [EGF] — growth of epidermal cells
- Nerve growth factor [NGF] — growth of sensory & sympathetic neurons
- Platelet derived growth Factor [PDGF] — Wound-healing
- Insulin-like growth factor [ILF-I, ILF-II] — Sulfation of cartilage
- Tumor necrosis factor (TNF) — stimulates necrosis of tumor cell
- Erythropoetin (EP) — Stimulate erythropoiesis
- Fibroblast growth factor (FGF)

Action:

GF bind with receptors on cell surface / inside the cell

↓

activates tyrosine kinase activity inside the cell

↓

phosphorylation of target proteins at tyrosine kinase

↓

phosphorylated proteins promote growth & proliferation

PCR: → in vitro method of DNA amplification

↳ much faster & more sensitive than cloning

- **Taq polymerase**: high temperature (94°c) denaturates DNA polymerase
  - ↳ thermostable
  - → Thermus aquaticus (hot springs)
- **Primers**: two synthetic DNA primers complementary to the ends of each strand

3 steps ← Denaturation, Annealing, Extension.

Heat Denaturation: (94°c)

↳ separation of ds DNA into 2 ss DNA

Annealing: of primers to flanking regions of ss DNA.
(2 primers – one for each strand)

Extension: with Taq polymerase & deoxyribonucleotides

↳ new nucleotides are added to 3' end of primer

- Two identical copies of original DNA are formed.

{If no. of cycles = n
Copies at end of 'n' cycles = 2^n}

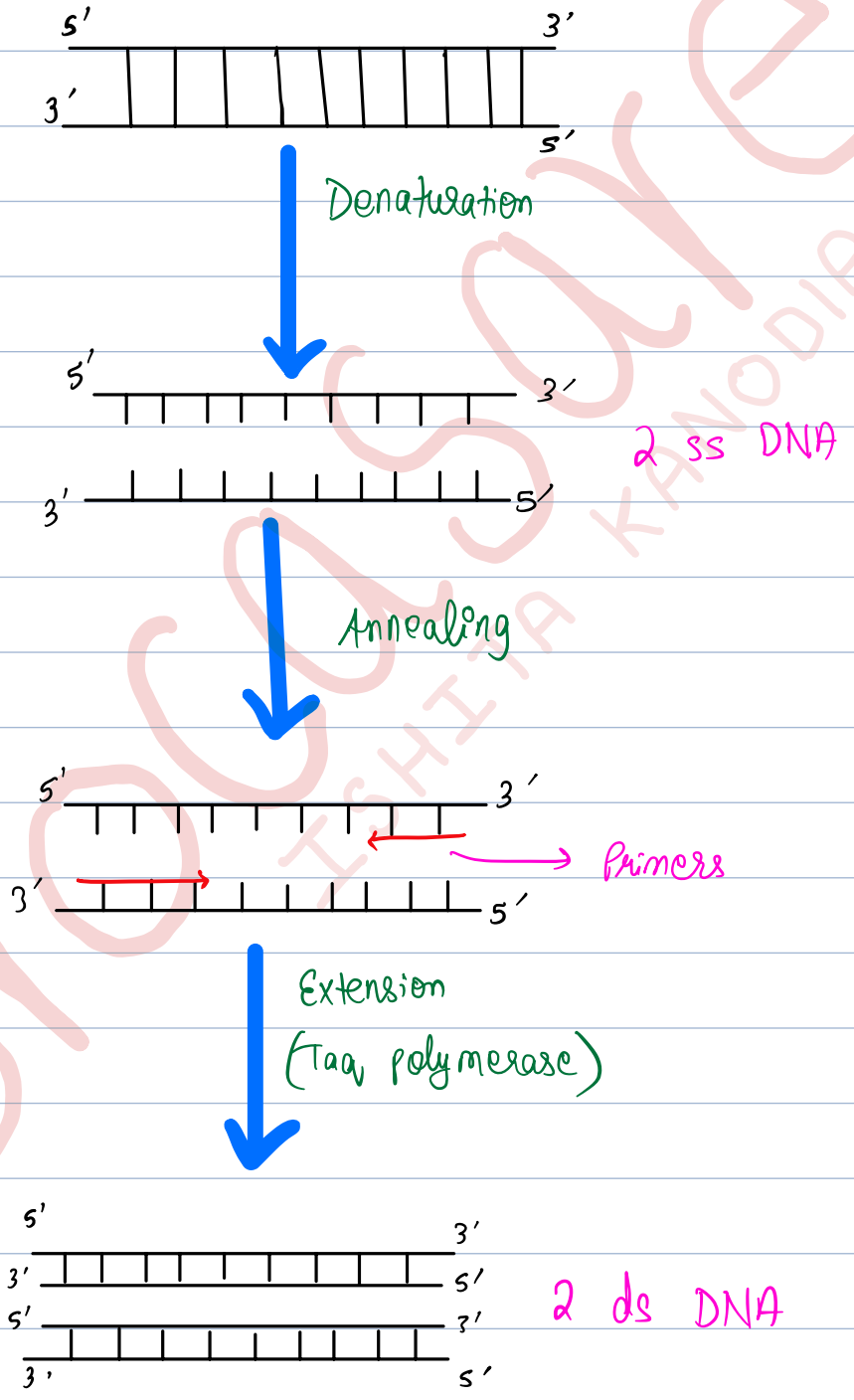
Applications of PCR:

Forensic tests – DNA fingerprinting, southern blotting, etc.

Prenatal diagnosis – from amniotic fluid for diagnosis of diseases

Diagnostic Uses – to detect microbial infections like AIDS, tuberculosis

Study of mutations – study of mutagenesis.



Probes: → tools to locate DNA or RNA of interest

↳ labeled probe is made to base pair with target nucleic acid sequence

→ ss DNA or RNA which is labeled (mostly radio-labeled) & has base sequence complementary to target DNA or RNA fragment.

Principle: → labeled probe is allowed to move freely with mixture of DNA/RNA fragments

→ if target sequence is present in mixture

↓

probe will detect & bind tightly with target fragment.

→ Labeling of probes helps them to be detected even in small quantities

→ use of radioactive ^{32}P

→ radiolabeled probes are detected by X-ray films.

Application: used in southern & nothern blotting

→ used for in-situ hybridisation

Southern Blotting:

→ to detect & characterize specific segments of DNA.

→ employs restriction endonucleases (RE) to cut DNA fragments

STEPS:

[AGE = agarose gel electrophoresis]

Digestion with RE: fragments of different lengths are produced

Separation of DNA fragments by AGE: sorts out DNA fragments according to length [shorter ones move faster than longer fragments]

Transfer of DNA from agarose gel to nitrocellulose membrane:

(∵ agarose is fragile & cannot be used for further procedures)

→ Agarose gel is treated with dilute NaOH to convert ds DNA into ss DNA [Nitrocellulose paper binds to ss DNA]

→ Nitrocellulose paper is layered over agarose & pressed with absorbent paper on top.

→ separated DNA fragments are transfered without disturbing their position

→ DNA is fixed to nitrocellulose paper by baking at 80°c or using UV light.

Hybridization & Detection:

→ specific DNA probes are used to detect target DNA fragments.

→ nitrocellulose membrane is immersed in a buffer containing P^{32} labeled DNA probes.

→ excess probe is washed

→ X-ray film is placed over membrane

→ location of DNA fragment bound to probe is seen as dark line on X-ray film ⟹ Autoradiography.

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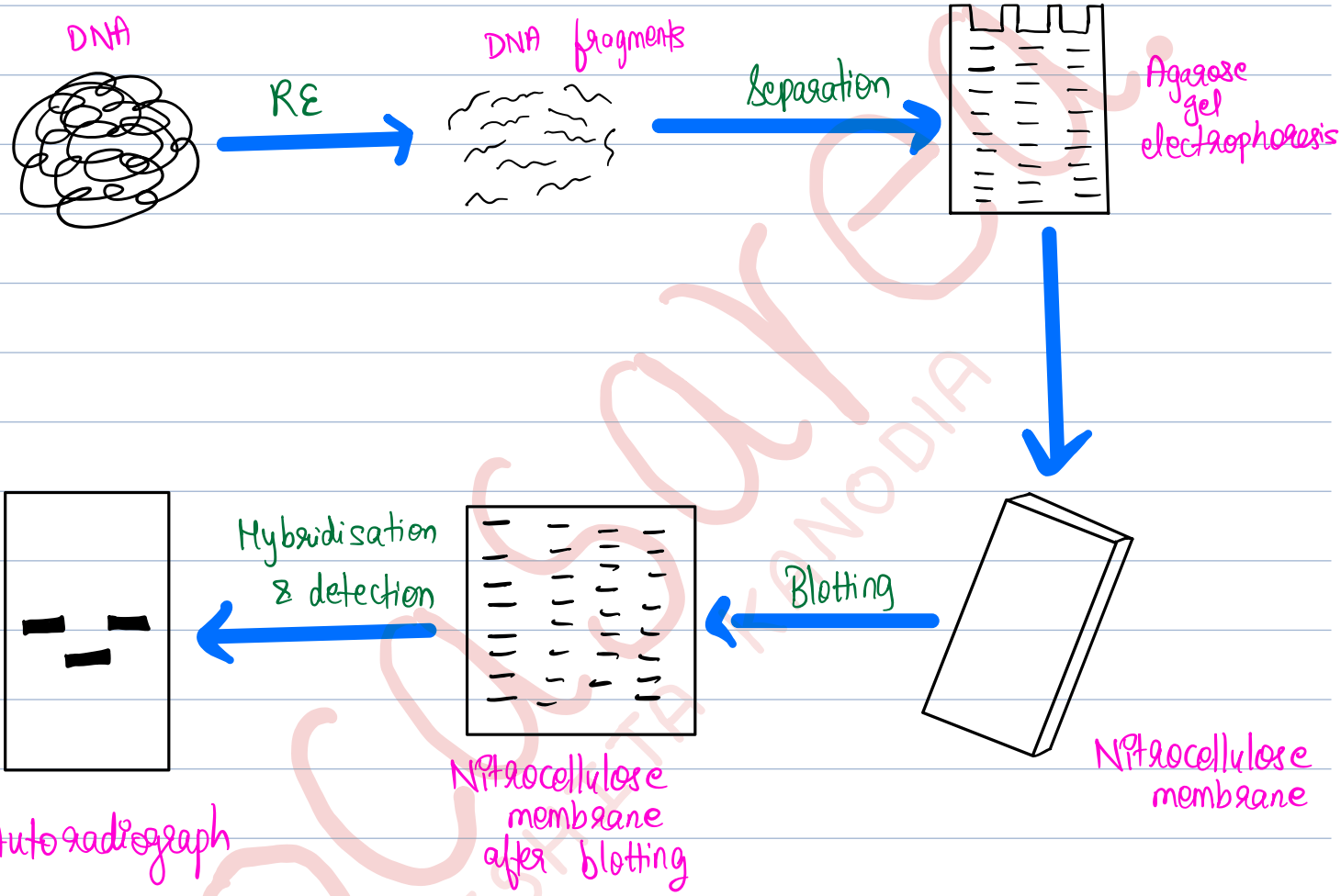
Applications:

→ detection of particular gene in thousands of genes

→ locating gene on chromosome (gene mapping)

→ to study mutations

→ Used in RFLP & DNA fingerprinting.



Gene Regulation:

- Constitutive / Housekeeping genes — not regulated
  - → always expressed
- Regulated / Inducible genes — regulated
  - → expressed only under certain conditions

Promoter (P gene): RNA polymerase attaches here for initiation of transcription

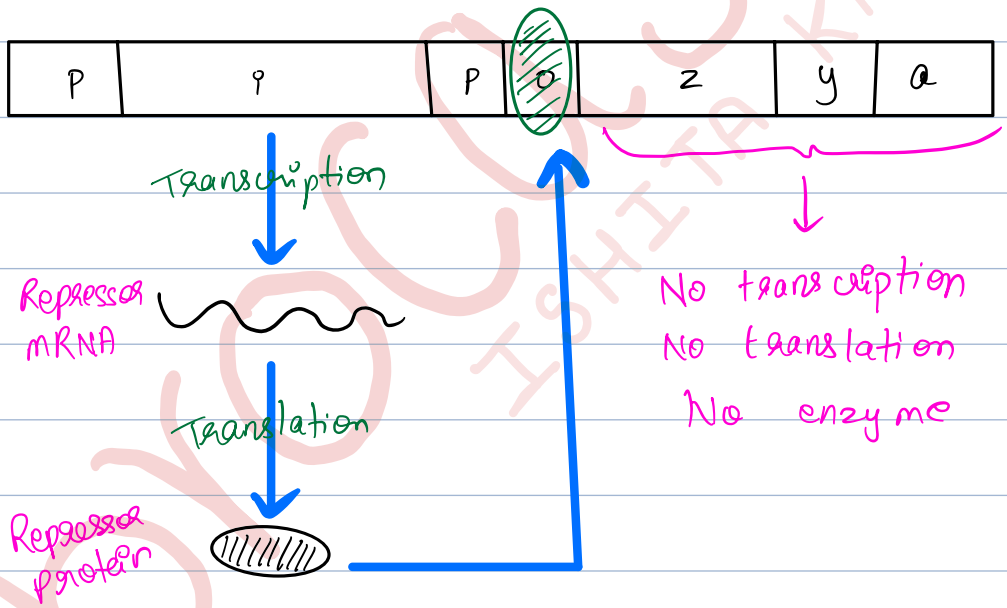
Regulator (i gene): synthesizes repressor

Operator (o gene): binding site for repressor

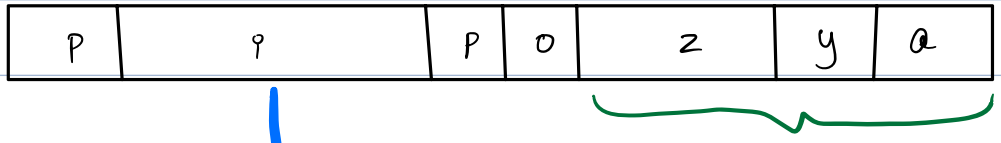
Structural genes (z, y, a): genes that encode for enzymes/proteins.

Lac Operon

Absence of Inducer (Allolactose)



Presence of Inducer (Lactose):



Transcription, Translation

β-galocidase, lac permease, transacetylase

Allolactose

Inactive repressor