RIZVI COLLEGE OF ARTS SCIENCE AND COMMERCE

Chap 4 – Molecular Basis of Inheritance

Class : SYJC Science

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Chapter – 4

MOLECULAR BASIS OF INHERITANCE

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Marks – 06 (04)

THE DISCOVERY OF DNA

- Friedrich Miescher → working on **white blood cell** → which are major component of **pus** from infection.
- Collected lots of pus from the bandages at hospital.
- Used **salt solution** to wash pus off bandages.
- Added **weak alkaline solution** to the cells, the cells **lysed** and **nuclei precipitated** out of the solution.
- From cell nuclei, he isolated a **unique chemical substance** to which he called **nuclein**.
- Chemically nuclein has **high phosphorus content**.
- Moreover it showed **acidic properties**.
- Hence it was named as **NUCLEIC ACID**.
- Two types → **DNA** and **RNA**

Structure of Eukaryotic DNA

Friedrich Miescher (1869)

↓

Cellular substance

↓

Nuclei of pus cells

↓

Nuclein *(Acidic properties)*

↓

Nucleic Acid

↓

**Deoxyribose Nucleic Acid*

**Ribose Nucleic Acid*

THE GENETIC MATERIAL IS A DNA

- **1900s** → Geneticist knew → *genes control inheritance of traits, genes are located on chromosomes and are mainly made of DNA and Proteins.*
- Initially thought → **Protein** are **large, complex** molecules and store information needed to **govern cell metabolism** and cause **variation**.
- On the other hand DNA thought to be **small, simple** molecule whose composition varied little among species.
- Over a period of roughly 25 years (**1928-1952**), geneticists became convinced that **DNA** and not protein was the **genetic material.**

GRIFFITH'S EXPERIMENT

British physician F. Griffith(1928)

↓

Bacterium- STREPTOCOCCUS PNEUMONIAE

↓

1. S-type → Capsulated ,Smooth &Virulent
2. R-type → Non-capsulated ,Rough & Non-virulent

i) Mice + S-type → Mice dead (Virulent or pathogenic)

ii) Mice + R-Type → Mice alive (avirulent or non-pathogenic)

iii) Mice + Heat killed S-type → Mice alive, healthy.

iv) Mice + Heat killed S-type + Living R-type → Mice dead

Blood sample (iv – Dead mice)

↓

Living S-type

↓

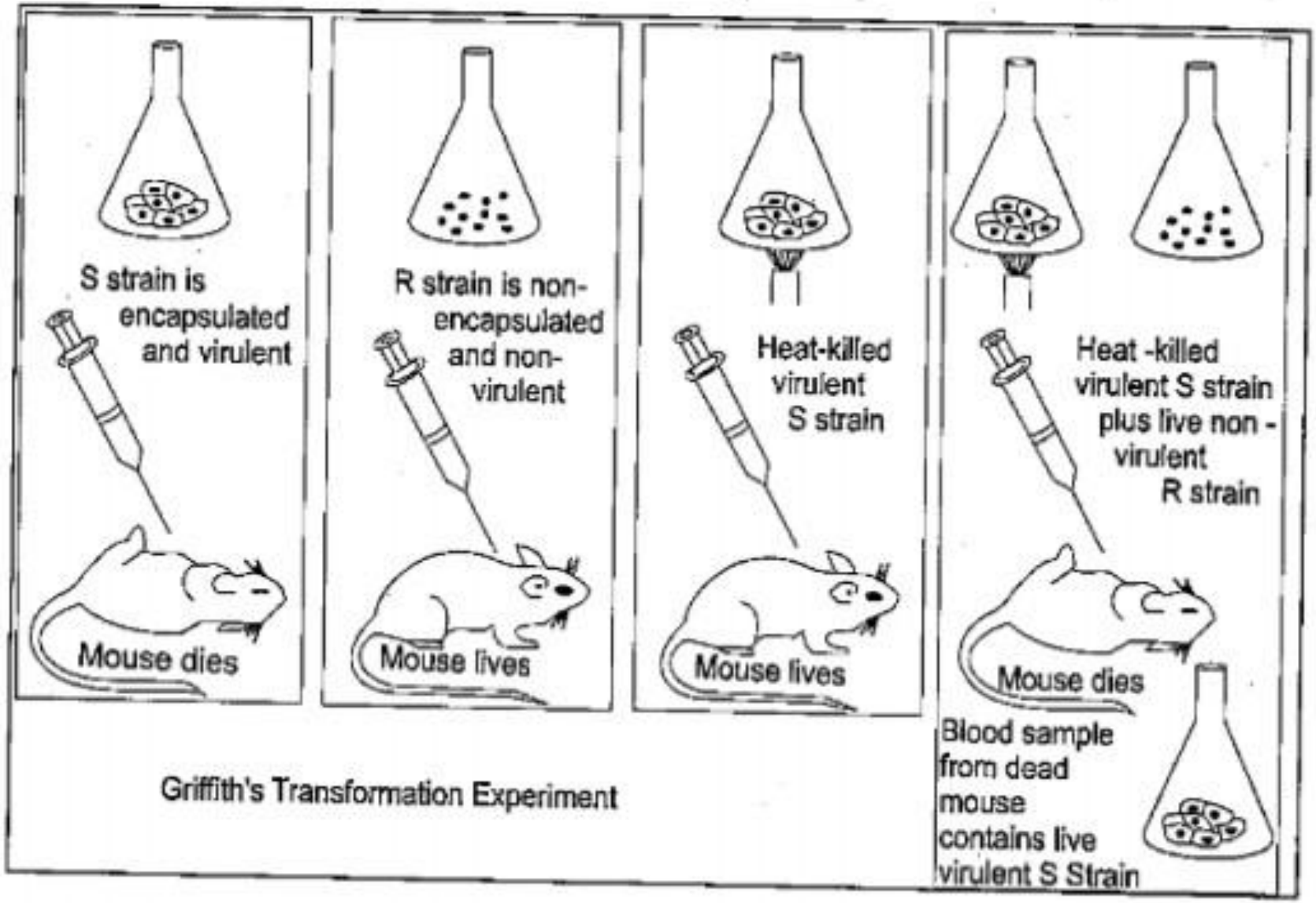
Conclution → **Living R-type** of bacteria must have picked up something from the surrounding medium that contains heat **killed S-type**.

↓

Transformation

↓

Transforming principle (DNA)



Griffith's Transformation Experiment

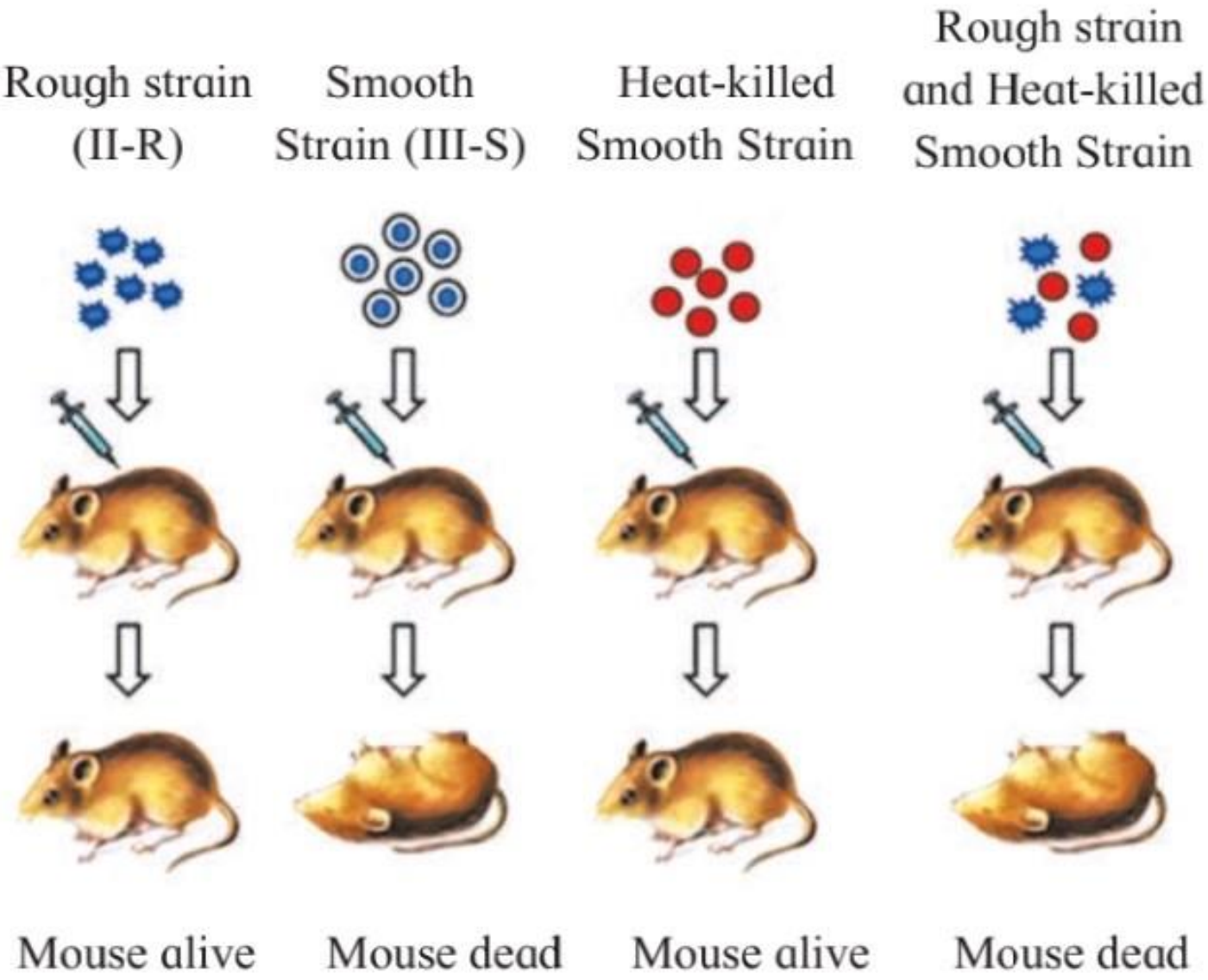


Fig. 4.1 : Griffith's experiment

Avery,Macleod and MaCarty Exp

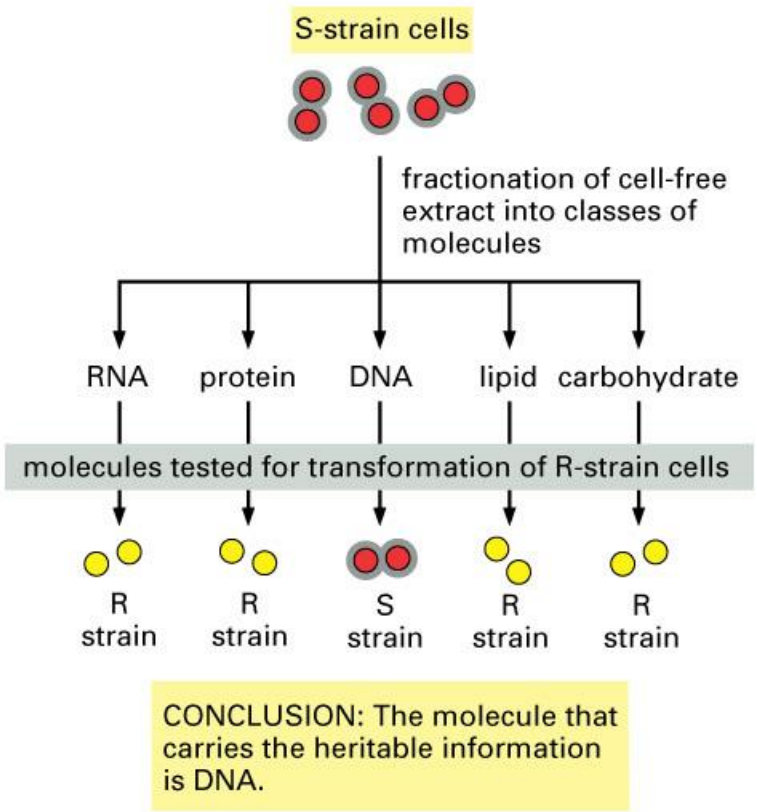


Figure 5-4 Essential Cell Biology, 2/e. (© 2004 Garland Science)

→ *1944, after some 10 years.*

→ ***Avery, McCarty and MacLeod's*** – *Purified **DNA, RNA** and **Proteins** and other materials from **heat killed S strain** and added to **living R strain**.*

- → *To **confirm** which one would transform **living R strain in S strain**.*
- → *Only **DNA was able to transform** harmless R strain into deadly S strain.*
- → *Addition of **Protein-digesting enzymes** (proteases) & **RNA-digesting enzymes** (RNAases) did not affect the transformation.*
- → *So the transforming substance was neither a protein nor RNA.*
- → ***DNA digested** with **DNAse** did inhibit the transformation, suggesting DNA caused the transformation.*
- → *All the biologist were not convinced.*

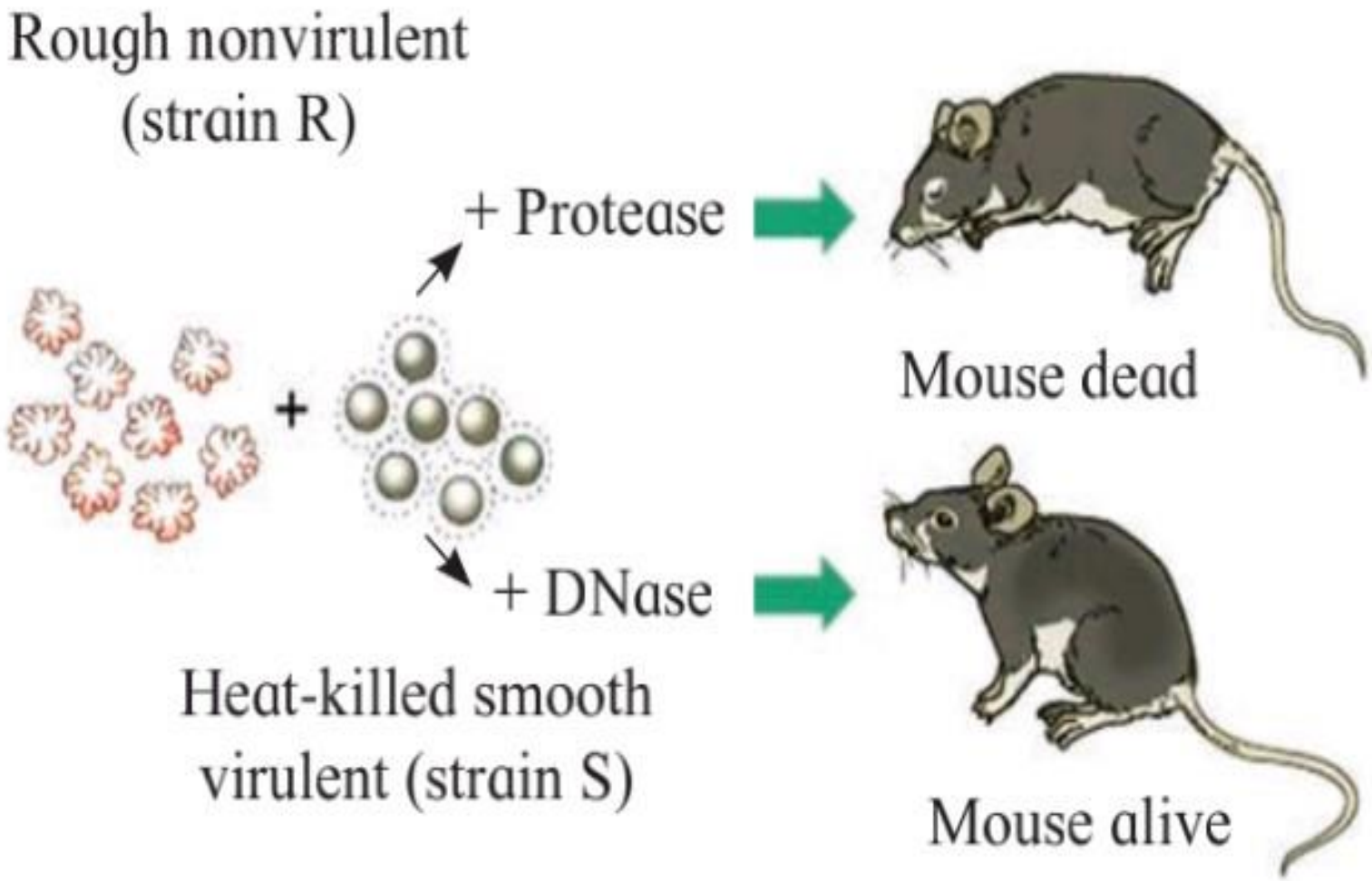
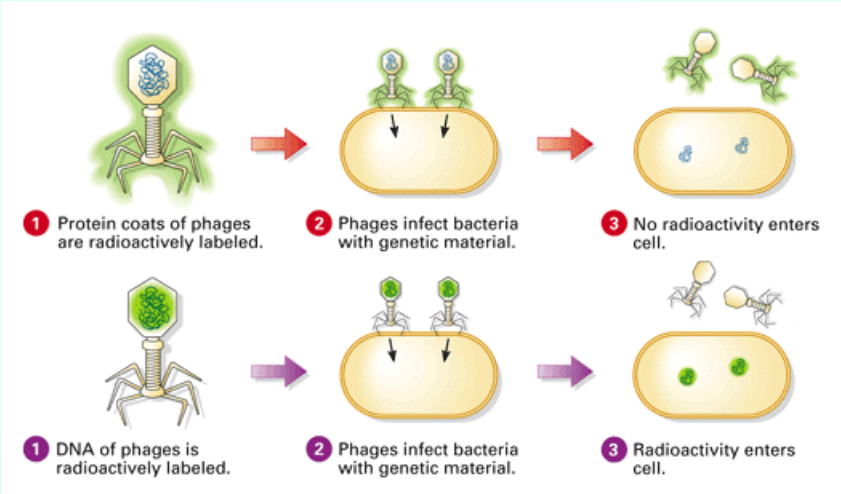


Fig. 4.2 : DNA transforms bacteria

Hershey-Chase experiments: S^{35}(Protein) and P^{32} (DNA)



Hershey-Chase Experiment

→Hershey and Chase worked with **viruses** that infect bacteria i.e **bacteriophages**, which are composed of **DNA** and **protein.**

→They used **radioactive phosphorus P^{32}** in the medium for some viruses and **radioactive sulphur S^{35}** for some others.

→ Viruses grown in the presence of **radioactive phosphorus** contained **radioactive DNA** (labelled DNA), but not radioactive proteins because DNA contains phosphorus but proteins do not.

- Similarly viruses grown on **radioactive sulphur** contained **radioactive protein** but not radioacitve DNA because DNA does not contain sulphur.

- **Viruses** were allowed to **infect bacteria**.

- **Bacteria** which were **infected by viruses with radioactive DNA** were radioactive, indicating that DNA was the material that passed from the virus to bacteria.

- Only radioactive P was found inside the bacterial cell, indicating that **DNA** is the **genetic materail**.

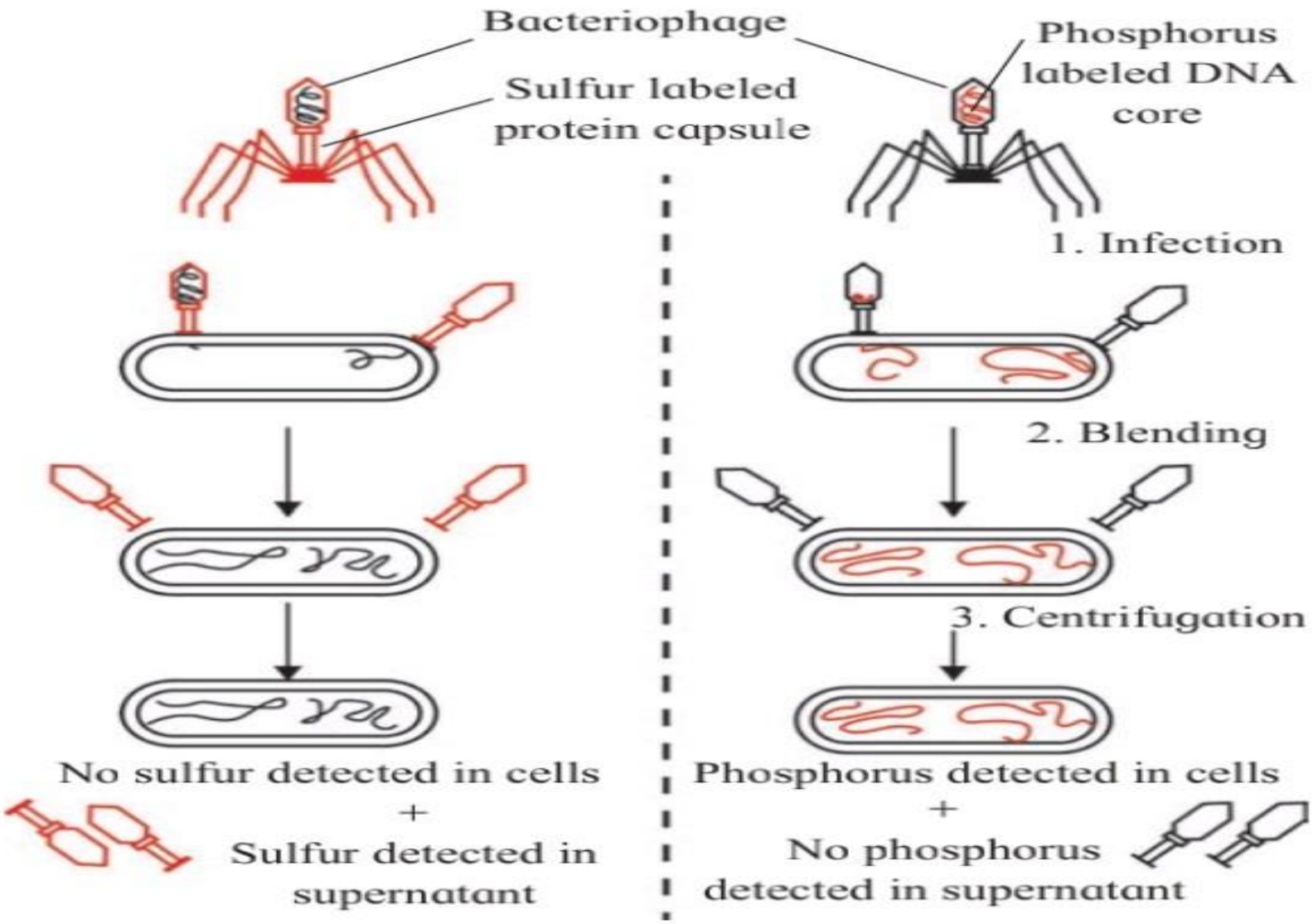


Fig. 4.3 : Hershey - Chase Experiment

DNA PACKAGING

1. Length of DNA appx. → 2.2 meters
2. Appx. Size of Nucleus → 10^{-6} m
3. DNA condensed , coiled & super coiled to fit in nucleus.
4. Complex process

→ Packaging in Prokaryotes

→ Packaging in Eukaryotes

Packaging in Prokaryotes

1. In **prokaryotes** like **E.coli**, cell size is almost 2- 3µ long.
2. They do **not** have **well organized nucleus**.
3. It is **without nuclear membrane** and **nucleolus**.
4. The **nucleoid** is **small, circular, highly folded naked ring of DNA** which is **1100µ long** in perimeter, containing about **4.6 million** base pairs.
5 The 1100µ long nucleoid is to be fitted or packaged into a cell which is **hardly 2-3µ long**. Hence the negatively charged DNA becomes **circular**, **reducing** the size to **350µm** in **diameter.**

6) This is further reduced to **30μm in diameter** because of **folding/looping**.

7) **40-50 domains(loops)** are formed.

8) Each **domain** if further **coiled and supercoiled** thereby reducing the size down **to 2μ in diameter.**

9) This coiling is assisted by positively charged **HU protein** (*Histone like DNA binding proteins*) and enzymes like **DNA Gyrase** and **Topoisomerase I**, for maintaining super coiled state.

PACKAGING IN PROKARYOTES

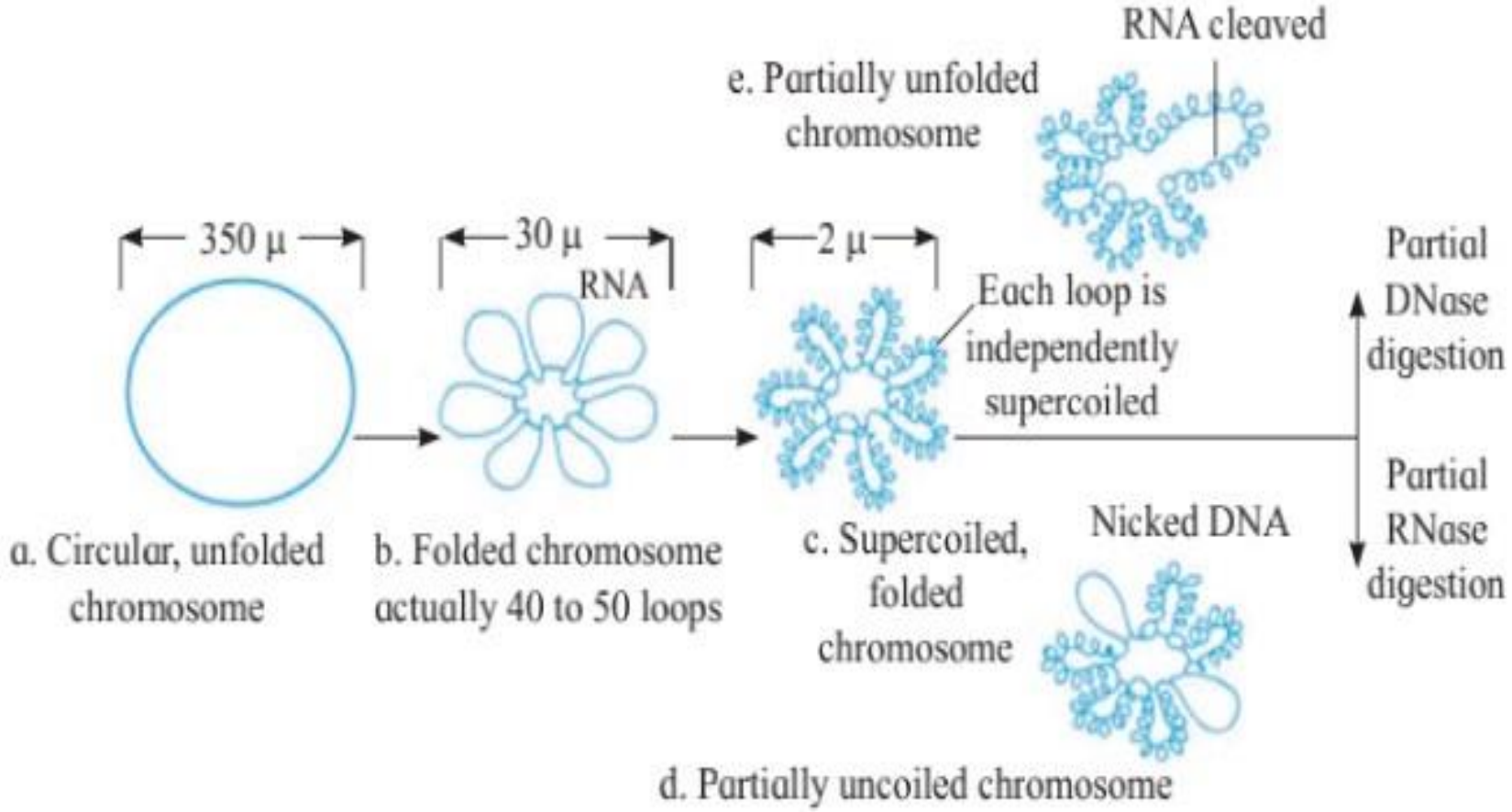
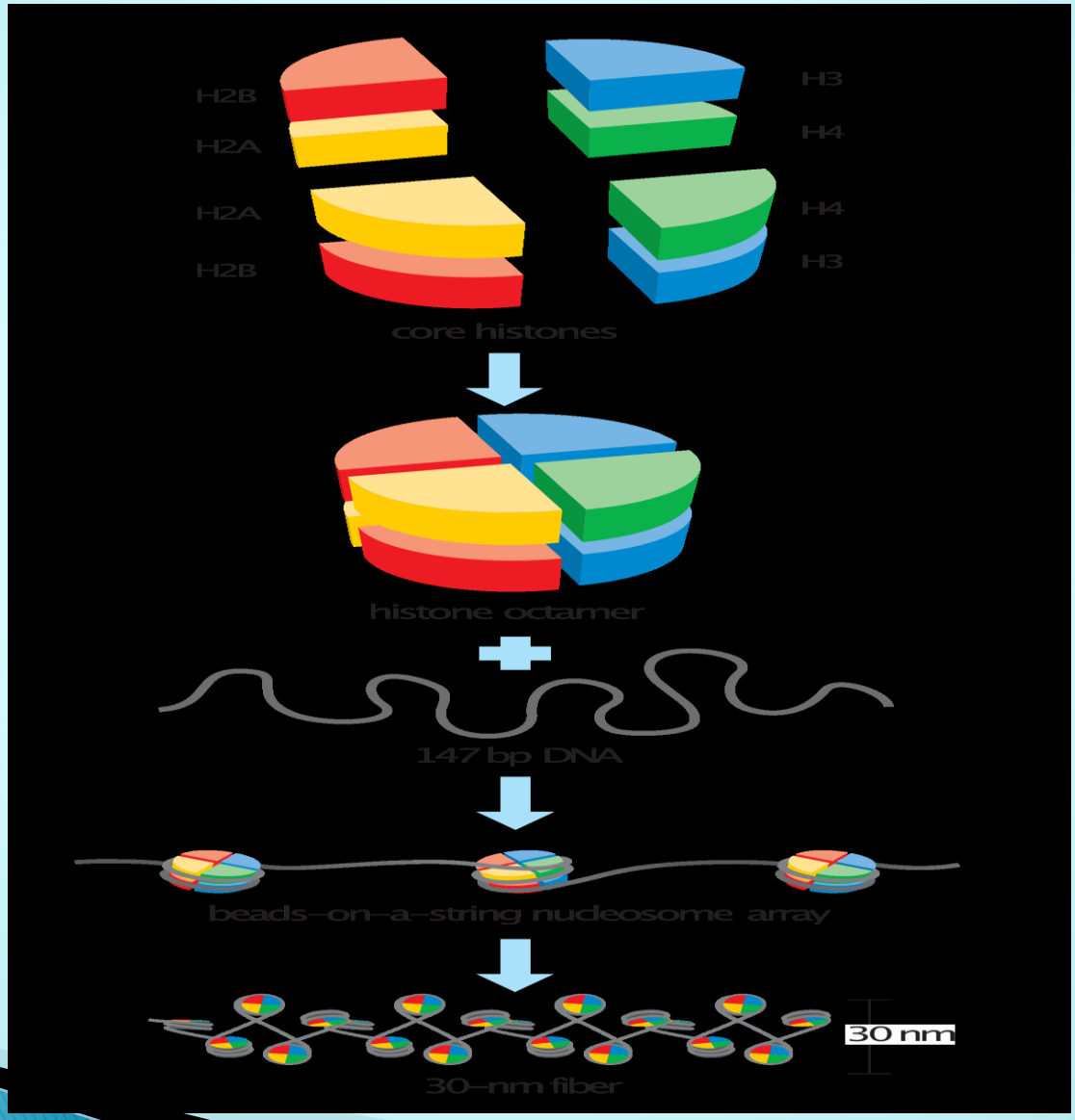


Fig. 4.4 : DNA Packaging in Prokaryotes

PACKAGING IN EUKARYOTES

1. The **organization** of DNA is much more **complex** in eukaryotes.
2. **Histones** are required for the **packaging of DNA**.
3. Histones are proteins that are rich in the basic amino acid residues **lysine** and **arginines** which carry **positive charge** in their side chain.
4. **Eight molecule** of histones (**two each of H2A, H2B, H3 and H4**) get organized to form histone **octamer**.
5. **DNA is negatively charged** and it is **wrapped** around the positively charged **histone octamer** forming a structure known as **Nucleosome**.

NUCLEOSOME


H2B
H2A
H2A
H2B
H3
H4
H4
H3
core histones
histone octamer
147 bp DNA
beads-on-a-string nucleosome array
30 nm
30-nm fiber

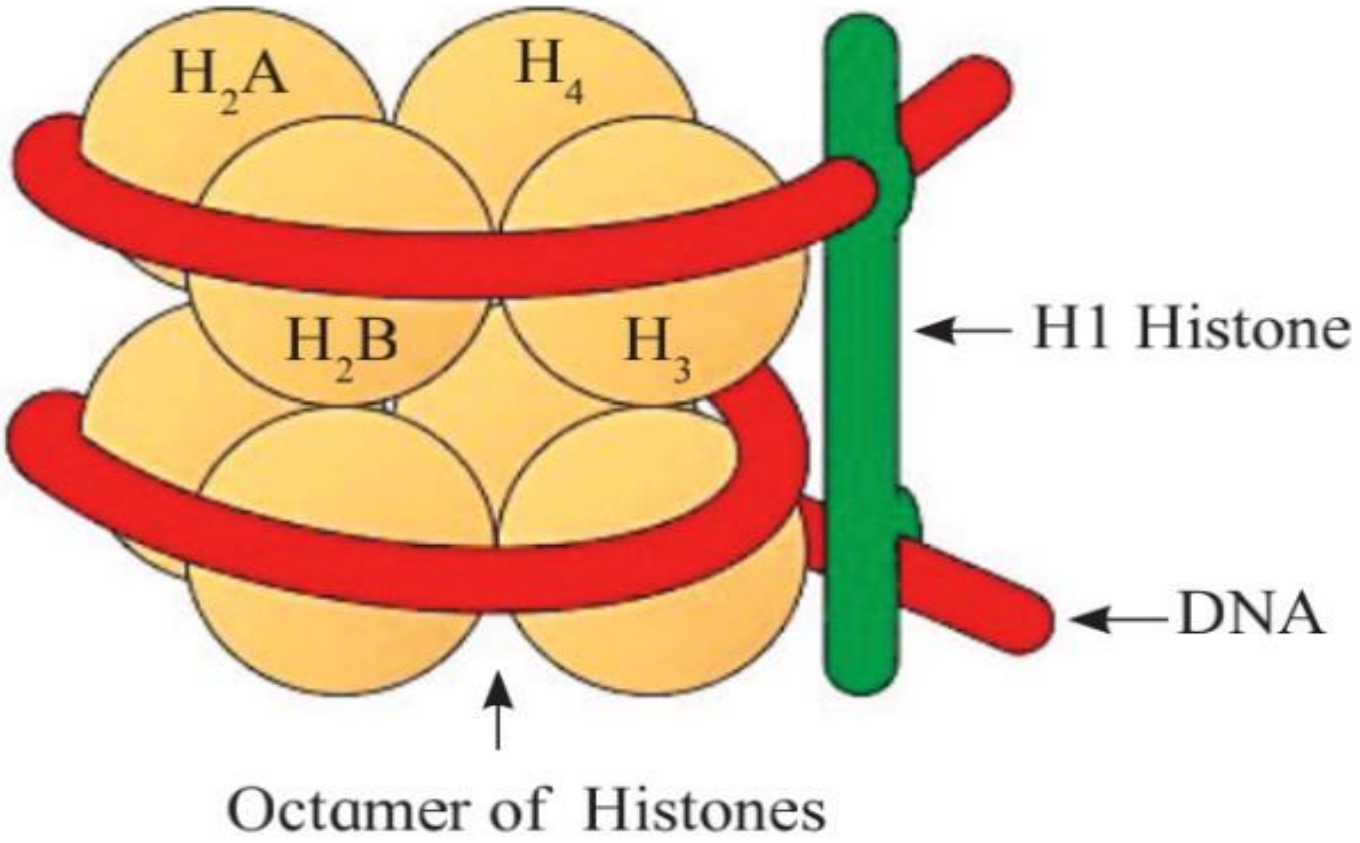


Fig. 4.5 : Nucleosome

6. **H1 protein** binds the DNA thread where it **enters and leaves the Nucleosome**.
7. Under the electron microscope, nucleus shows **Chromatin network**, the nucleosomes in Chromatin are seen as ‘ **Beads- on- string**’.

8. Around the octamer, DNA molecule is wrapped as **1 and 3/4th turn**. This DNA is called **Core DNA** and it consists of about **146bp** (base pairs).
9. **Adjacent Nucleosomes** are **linked** with small segment of DNA called **Linker DNA**; of about **54bp**.
10. This ‘**beads-on-string**’ structure gets condensed into **nucleosome fiber** which is coiled like a telephone wire to make **Solenoid fib**er with diameter **30nm or 300Å**.

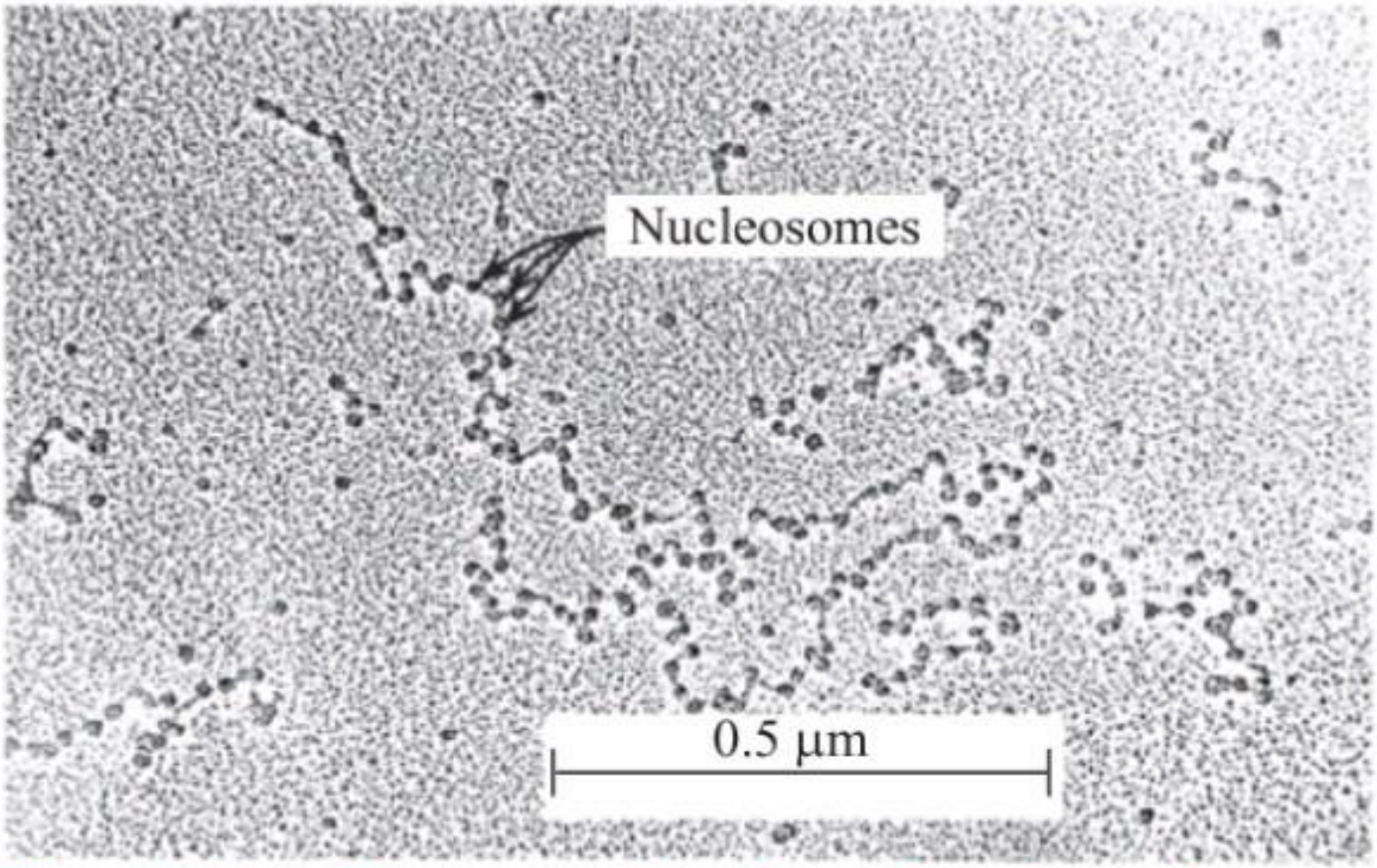
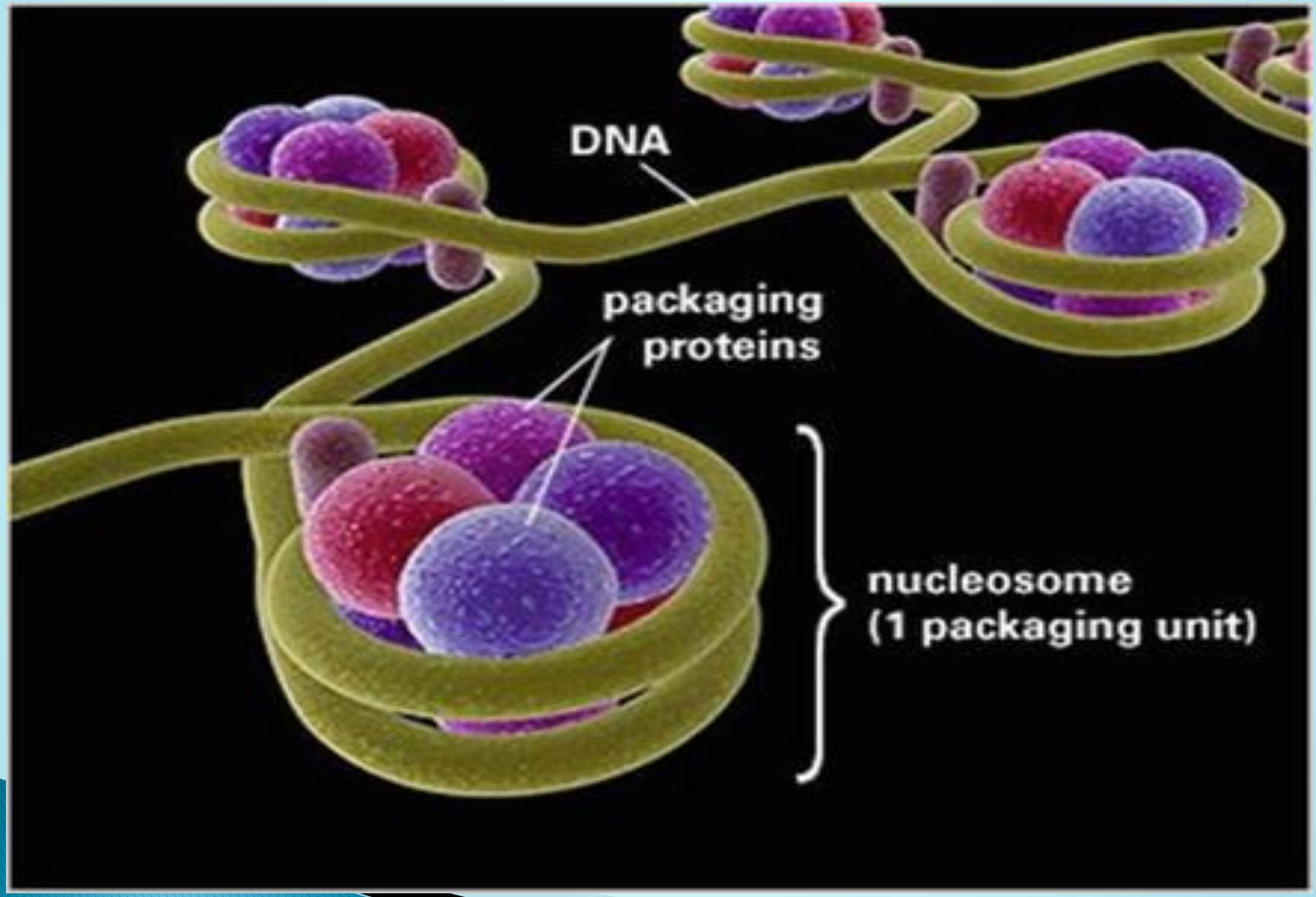
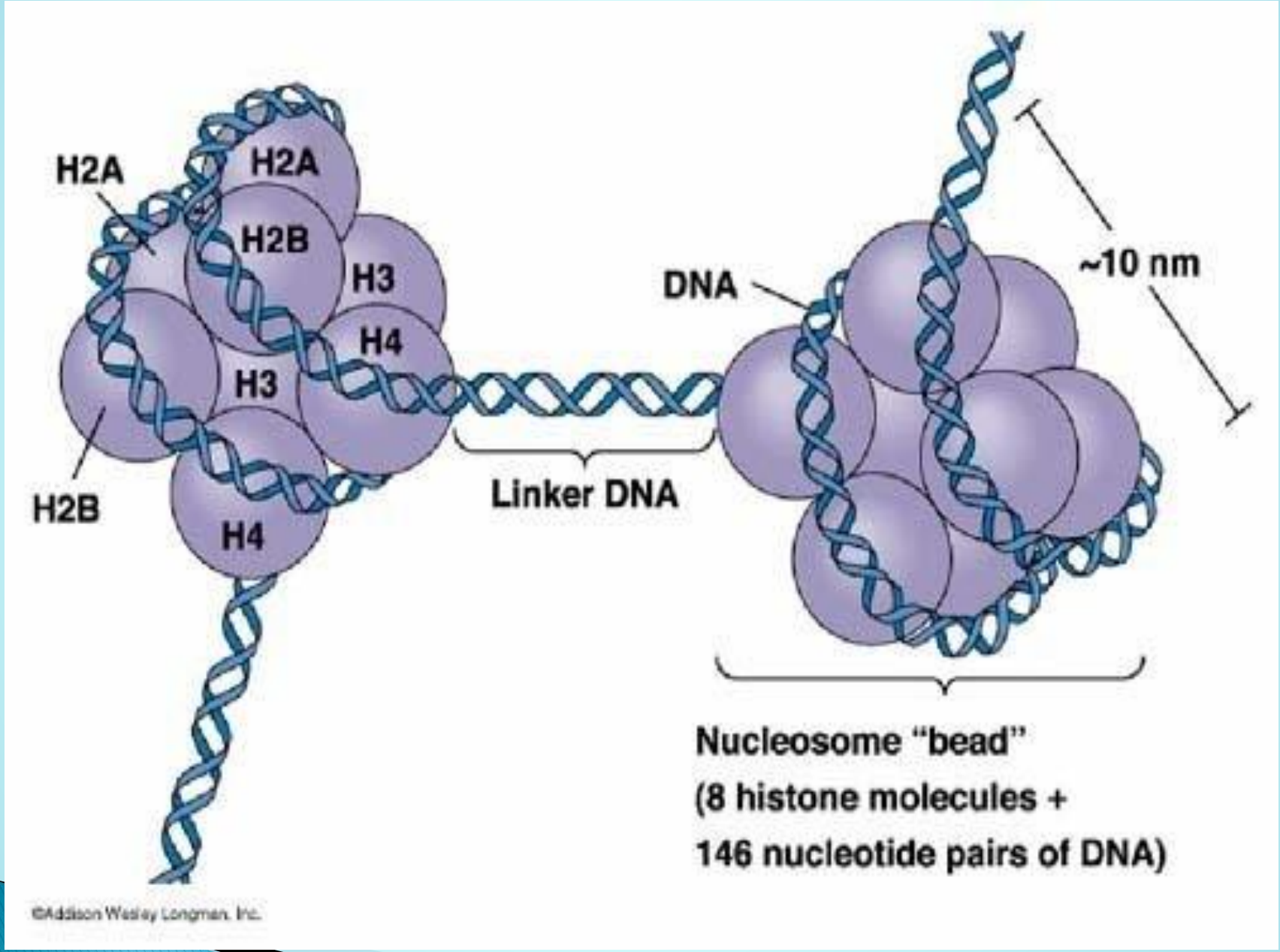
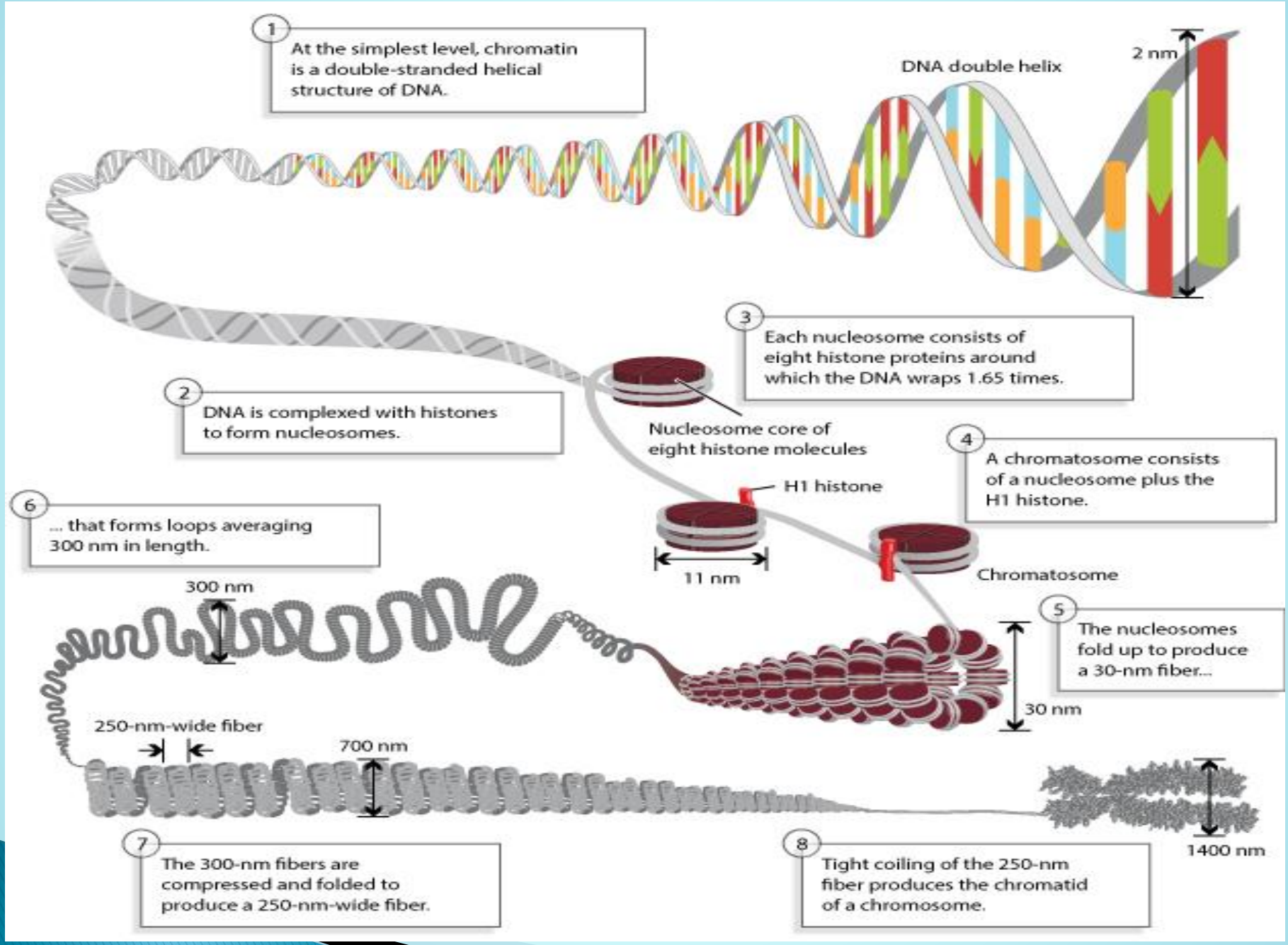


Fig. 4.6 : Chromatin showing beads-on-string

NUCLEOSOME

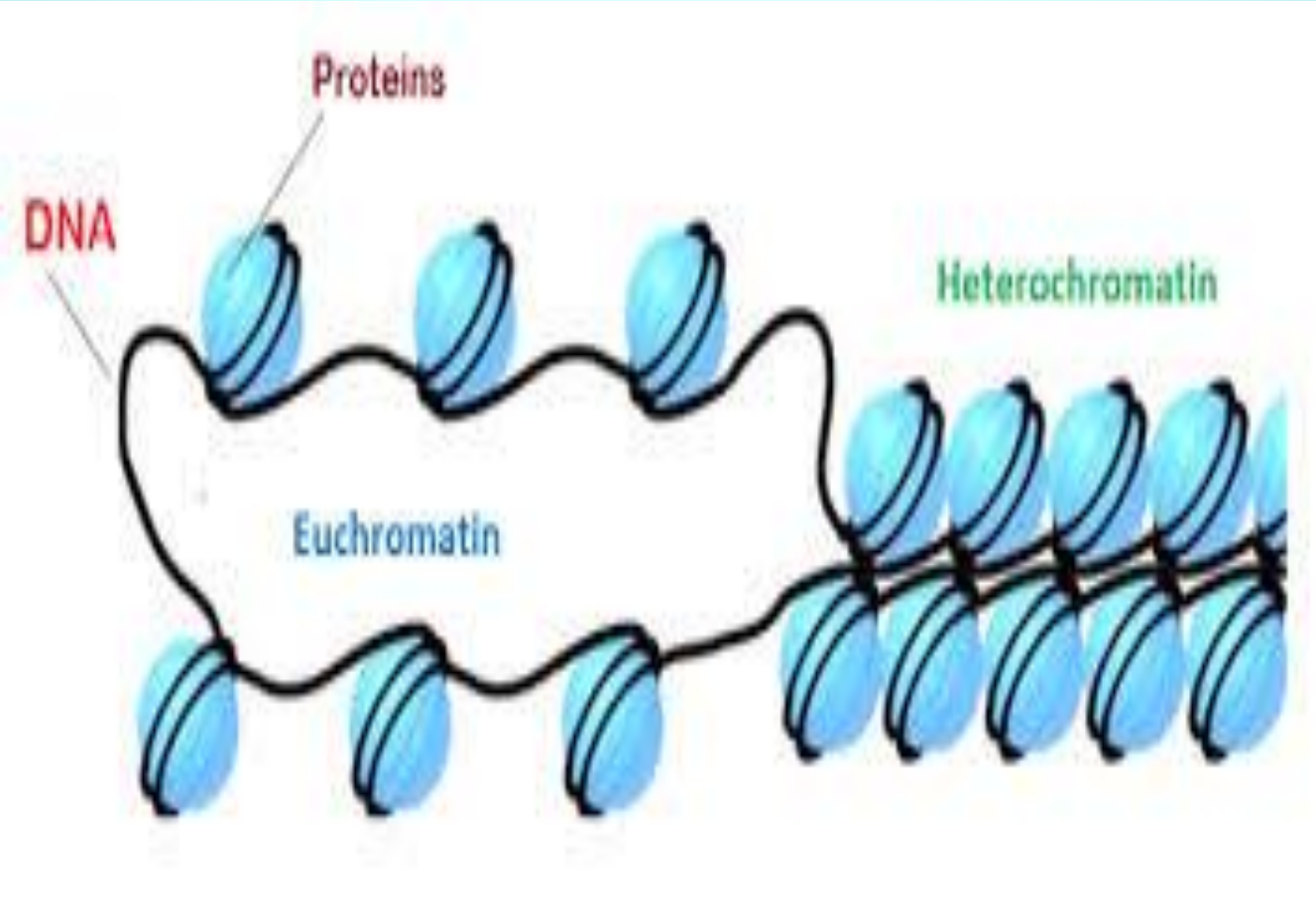


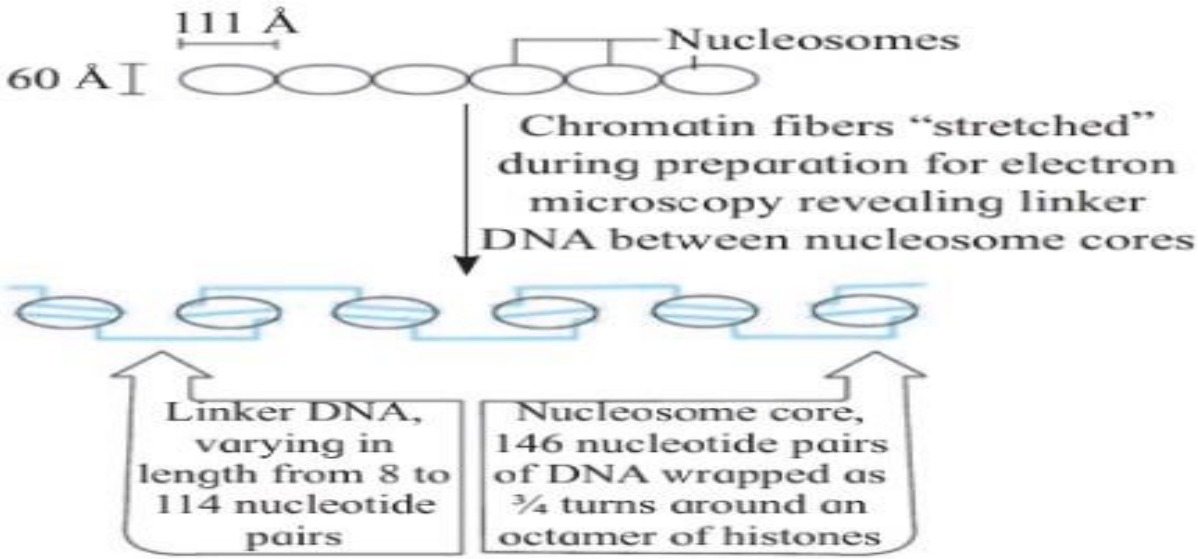

H2A
H2A
H2B
H3
H4
H3
H2B
H4
DNA
~10 nm
Linker DNA
Nucleosome "bead"
(8 histone molecules +
146 nucleotide pairs of DNA)
©Addison Wesley Longman, Inc.


1
At the simplest level, chromatin is a double-stranded helical structure of DNA.
DNA double helix
2 nm
3
Each nucleosome consists of eight histone proteins around which the DNA wraps 1.65 times.
2
DNA is complexed with histones to form nucleosomes.
Nucleosome core of eight histone molecules
4
A chromatosome consists of a nucleosome plus the H1 histone.
H1 histone
6
... that forms loops averaging 300 nm in length.
11 nm
Chromatosome
300 nm
5
The nucleosomes fold up to produce a 30-nm fiber...
30 nm
250-nm-wide fiber
700 nm
1400 nm
7
The 300-nm fibers are compressed and folded to produce a 250-nm-wide fiber.
8
Tight coiling of the 250-nm fiber produces the chromatid of a chromosome.

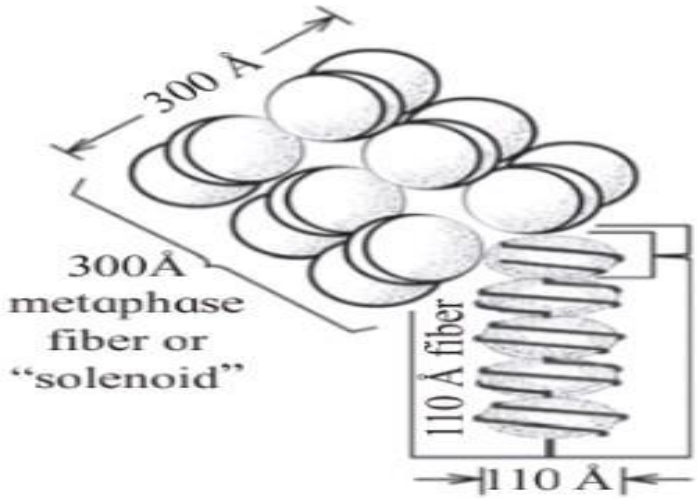
- The packaging of chromatin at higher levels need additional set of proteins that are collectively called **Non-Histone Chromosomal** (NHC) **proteins**.
- A loosely packed region of chromatin that **stains light**, is called **Euchromatin** and densely packed region that **stains dark** is called **Heterochromatin.**
- **Euchromatin** is considered as transcriptionally **active chromatin**, while **Heterochromatin** is **inactive**.
- Heterochromatin is 2 to 3 times **more rich in DNA** than in the Euchromatin.

HETEROCHROMATIN AND EUCHROMATIN





B : Chain of nucleosomes forming 10 to 11 nm thick fibre



C : Solenoid forming 30 nm thick fibre

Fig. 4.7 : DNA packaging

DNA REPLICATION

- *The DNA molecule regulates and controls all the activities of the cell. As a carrier of genetic information, DNA has to perform two important functions:-*

A. **Heterocatalytic function**:- when DNA directs the synthesis of chemical molecules other than itself, then such functions of DNA are called heterocatalytic *functions.*

Eg. **Synthesis of RNA(Transcription), synthesis of protein (Translation),** etc.

B. **Autocatalytic function**:- when DNA directs the synthesis of DNA itself, then such function of DNA is called autocatalytic function.

function.→ EG **Replicaition**

Semiconservative mode of Replication

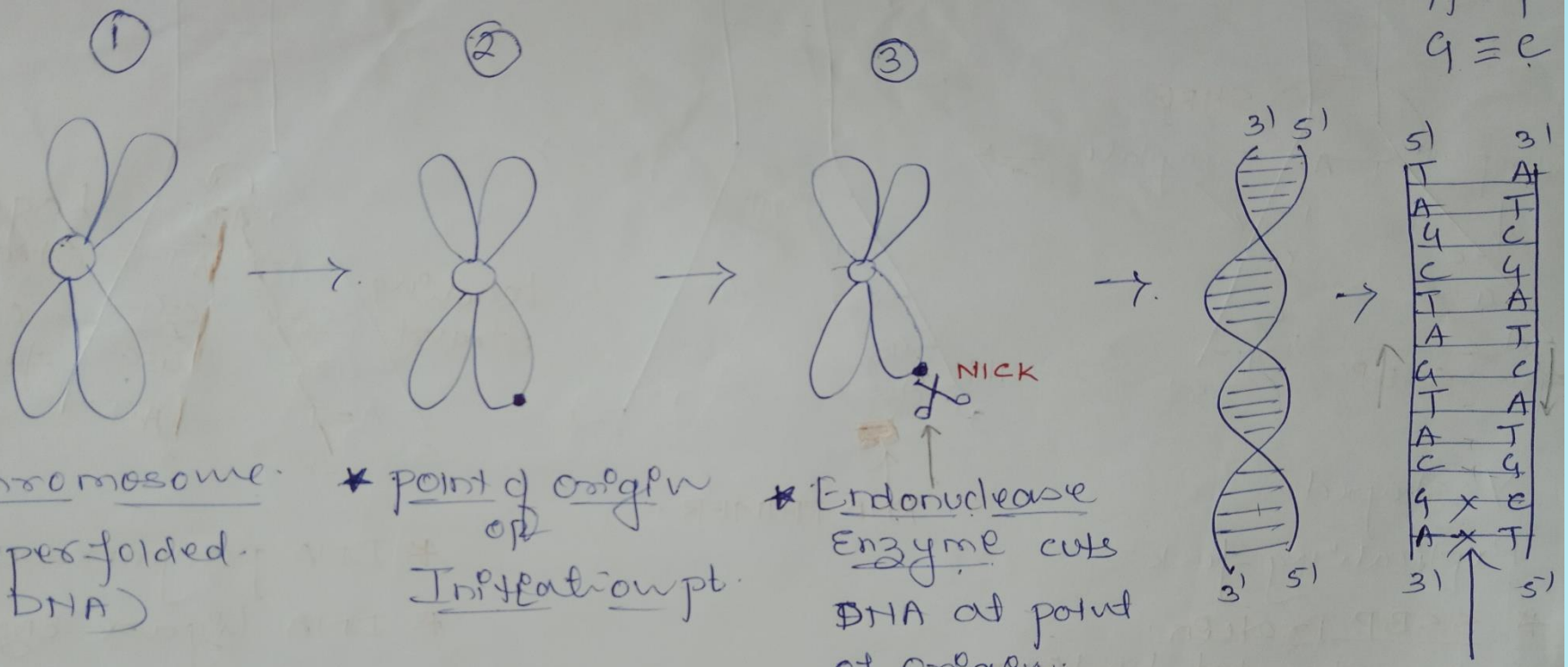
- The process by which DNA duplicates itself is called replication.
- Two copies are form that are identical.
- **Eukaryotic organisms** – Replication of DNA takes place only once in the cell cycle.
- Occurs in **S-Phase** of **interphase** in the cell cycle.
- **Watson and Crick** proposed **semi-conservative** mode of replication, on the basis of **antiparallel** and **complementary** nature of DNA strands.

Semi-conservative Replication of DNA

A. Activation of Nucleotide
B. Origin or Initiation point
C. Un-winding of DNA strand
D. Replicating fork
E. Synthesis of new strand
F. Leading and lagging strand
G. Formation of daughter DNA molecules

DNA REPLICATION.

A = T
G ≡ C



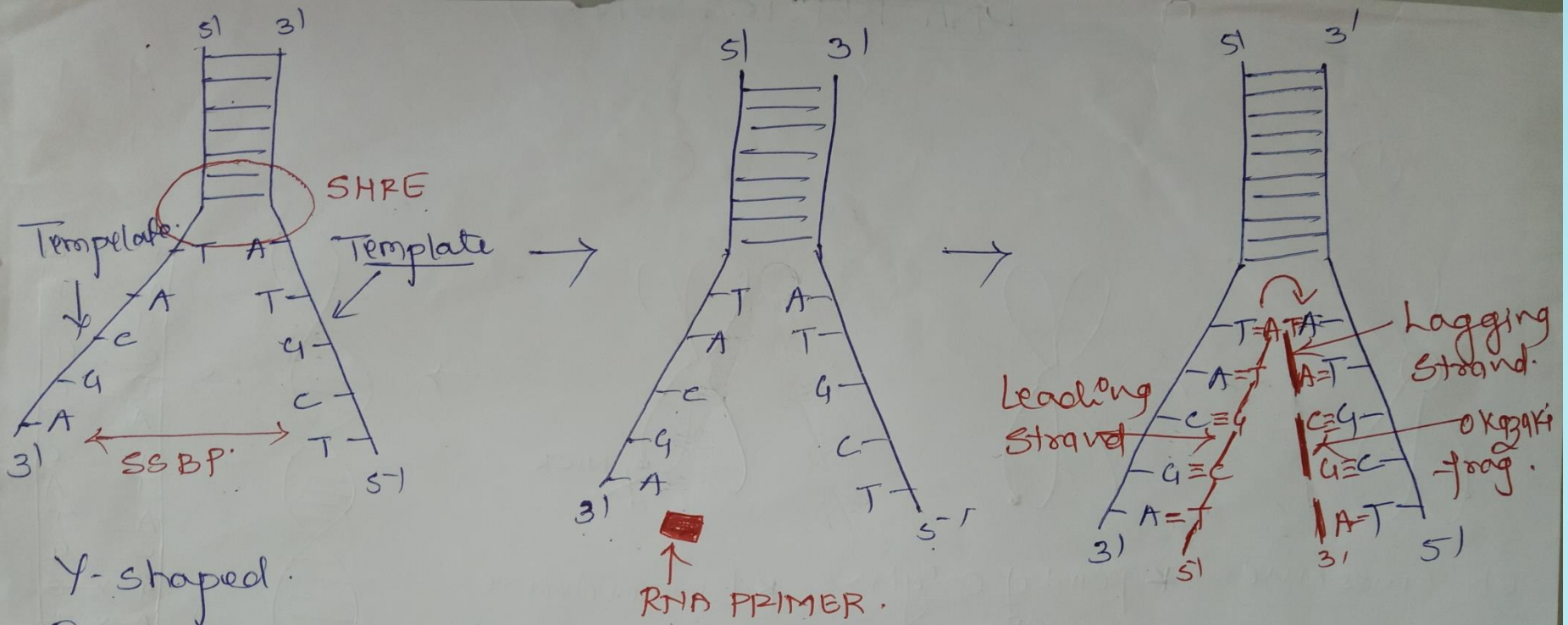
Chromosome.
(Super-folded DNA)

* point of origin or Initiation pt.

* Endonuclease Enzyme cuts DNA at point of origin.

Hydrogen Bonds Broken with

* Enz. Helicase



Y-shaped Replicating fork.

* SSBP Protein: Single Stranded binding protein.

* Super Helix relaxing enzyme

* DNA polymerase Enz.

* DNA ligase Enz.

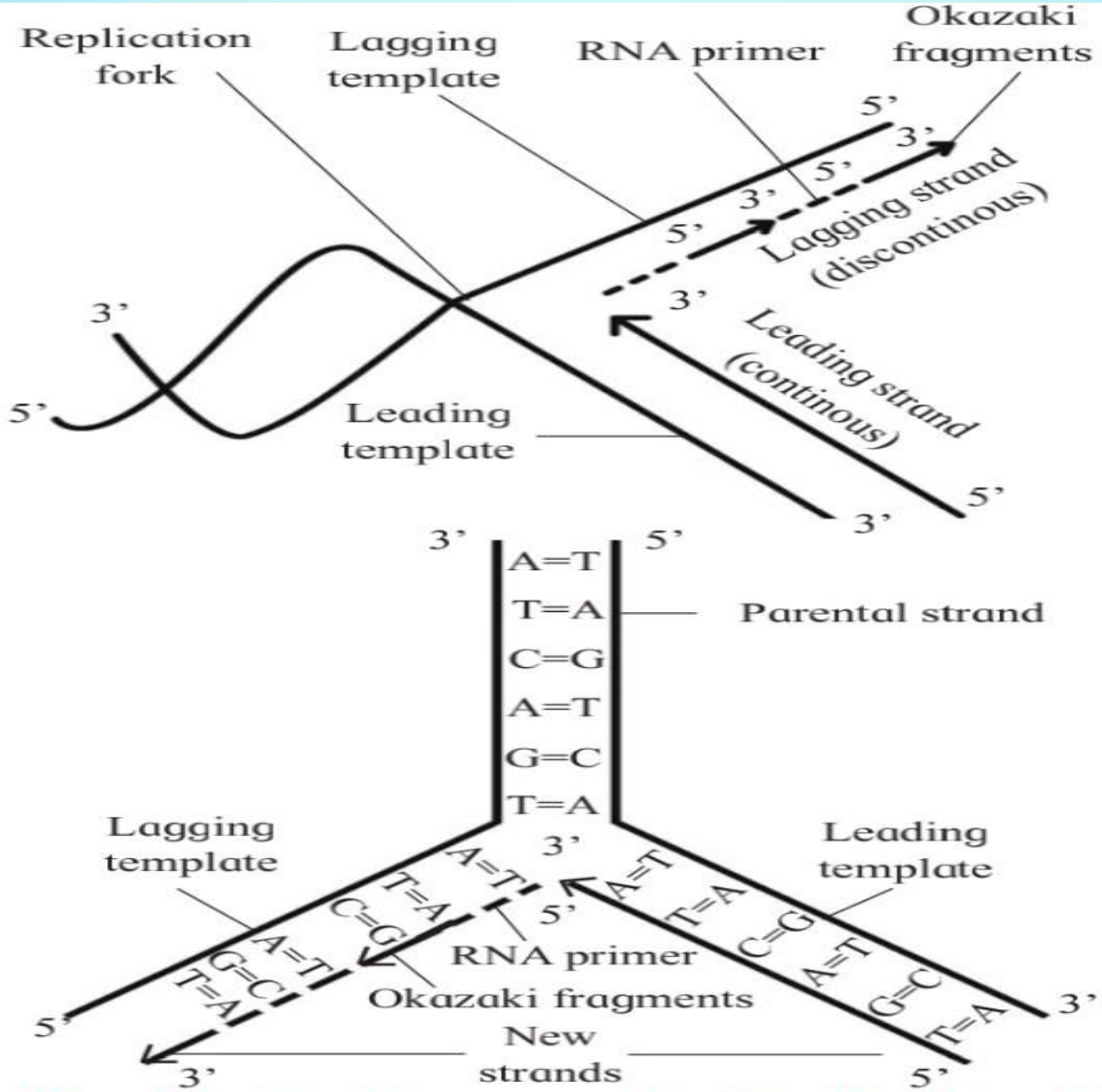


Fig. 4.8 : Semiconservative Replication of DNA

1. Activation of Nucleotide.

Nucleoplasm

↓

4 DNA nucleotide

↓

Monophosphate form (**dAMP, dGMP, dCMP, dTMP**)

↓

Triphosphate form (**dATP, dGTP, dCTP, dTTP**)

↓

In presence of **ATP & enz.Phosphorylase**

↓

Process – Activation of Nucleotide/Phosphorylation

2. Origin or Initiation point.

Replication starts – *Specific point on DNA*

↓

Origin or Initiation point & termiantes at point T

↓

Origin is flanked by 'T' sites

↓

Prokaryotes – *only one origin*

↓

Eucaryotes – *Several origin points*

↓

Enzyme Endonuclease → *Incision* (nick)→Phosphodiester bond

↓

To uncoil DNA

3. Unwinding of DNA strands.

DNA strands free but not separated

⇓

Enzyme Helicase → *Breaking of hydrogen bonds* (Unzipping)

↓

To split DNA strands

↓

Y shaped *Replication fork*

↓

Coiling prevented by SSBP protein

↓

Single stranded binding protein

↓

Helix destabilizing protein

4. Replicating fork

Point form- *Unwinding and separation of two strands*

↓

Y- Shaped fork

↓

Unwinding of strands impose strain

↓

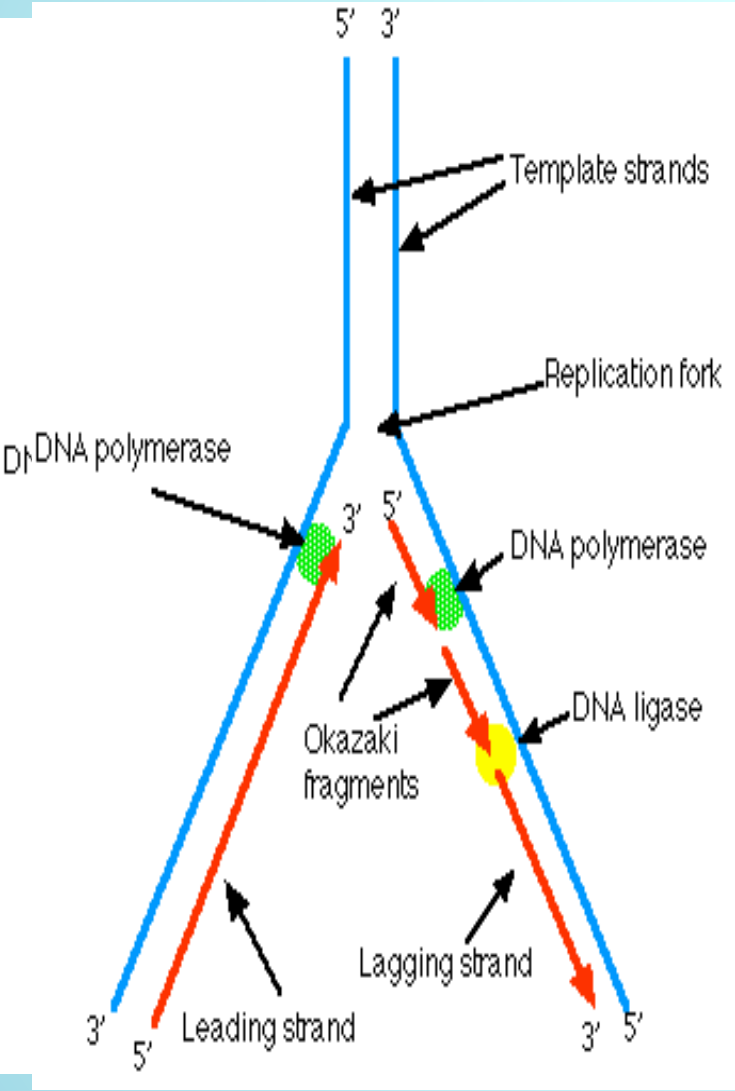
Relieved by

↓

Super-helix relaxing enzyme

↓

5.Synthesis of new strand



Each strand act as template

↓

For synthesis of complementary strand

↓

RNA Primer → Small RNA molecule

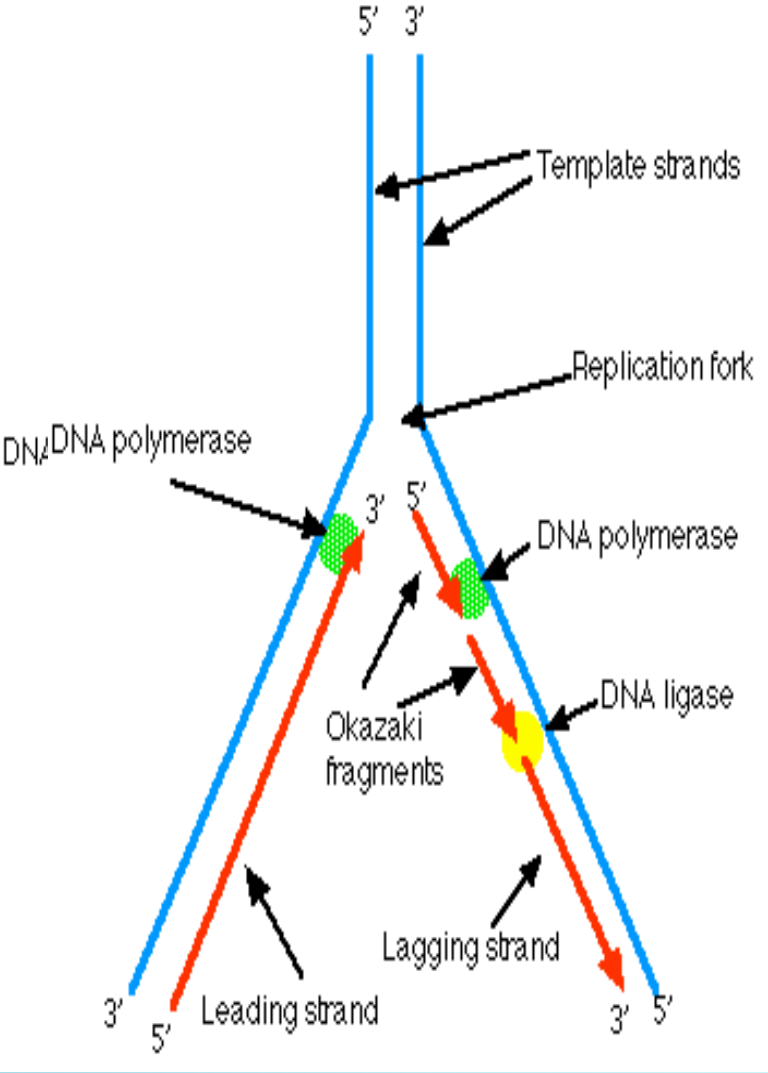
↓

RNA primer attach to 3' end of template

↓

Attracts complementary nucleotide from nucleoplasm

5.Synthesis of new strand



Bind to template by Hydrogen bond

↓

A=T and G=C

↓

Newly bond nucleotide join by Phosphodiester bond

↓

New strand catalyze by DNA Polymerase enzyme

↓

New complementary strand form – 5’→3’

6. Leading and Lagging strands.

DNA polymerase catalyses polymerisation

↓

One direction i.e. 5^{I} ----→ 3^{I}

↓

On one strand replication is continous & faster

↓

LEADING STRAND /TEMPLATE

↓

Starts from 3^{-} of parent strand

On other strand replication is discontinous & slower

↓

LAGGING STRAND

↓

Constructed in the form of short fragments

↓

Okazaki fragments

↓

Each fragments need one RNA primer

↓

DNA Ligase– Joins all fragments

↓

Ends at 5I

↓

RNA primer removed by Enzyme DNA Polymerase α

7. Formation of Daughter DNA

For each old strand , a new complementary strand is constructed

↓

*Both strand (**Old & new**) undergo coiling*

↓

Two daughter DNA molecules are formed

↓

Each daughter DNA (One old & one new strand)

↓

50% of mother molecule is conserved

↓

SEMI-CONSERVATIVE REPLICATION.

EXPERIMENTAL CONFIRMATION

1. **Meselson and Stahl** (1958)– Performed exp. To prove **semiconservative** nature of replication.
2. Cultured bacteria **E.coli** in the medium containing ^{14}N (light nitrogen) and obtained equilibrium density gradient band by using 6M $CsCL_2$
3. Position of band is recorded.
4. **E.Coli cells** --- Transferred to ^{15}N medium (heavy isotope nitrogen) and allowed to replicate for several generation.
5. Again the position of band is recorded.
6. Heavy DNA (15N) molecule can be distinguished from normal DNA by **centrifugation** in **6M Cesium chloride** ($CsCL_2$) density gradient.

7. Density gradient value of **6M CsCL2** and **^{15}N DNA** is almost **same**.
8. At the equilibrium point **^{15}N DNA** will form a band
9. Both strands are labelled with 15N.
10. Such **E.Coli** cells were then transferred to another medium containing **^{14}N**.
11. After **first generation** the density gradient band for **$^{14}N^{15}N$** was obtained.
12. After **second generation** two density gradient bands were obtained one at **$^{14}N^{15}N$** and other at **^{14}N** position.
13. Position of band after two generation clearly proved that **DNA replication is semi-conservative**.

Meselson's and Stahl Experiment

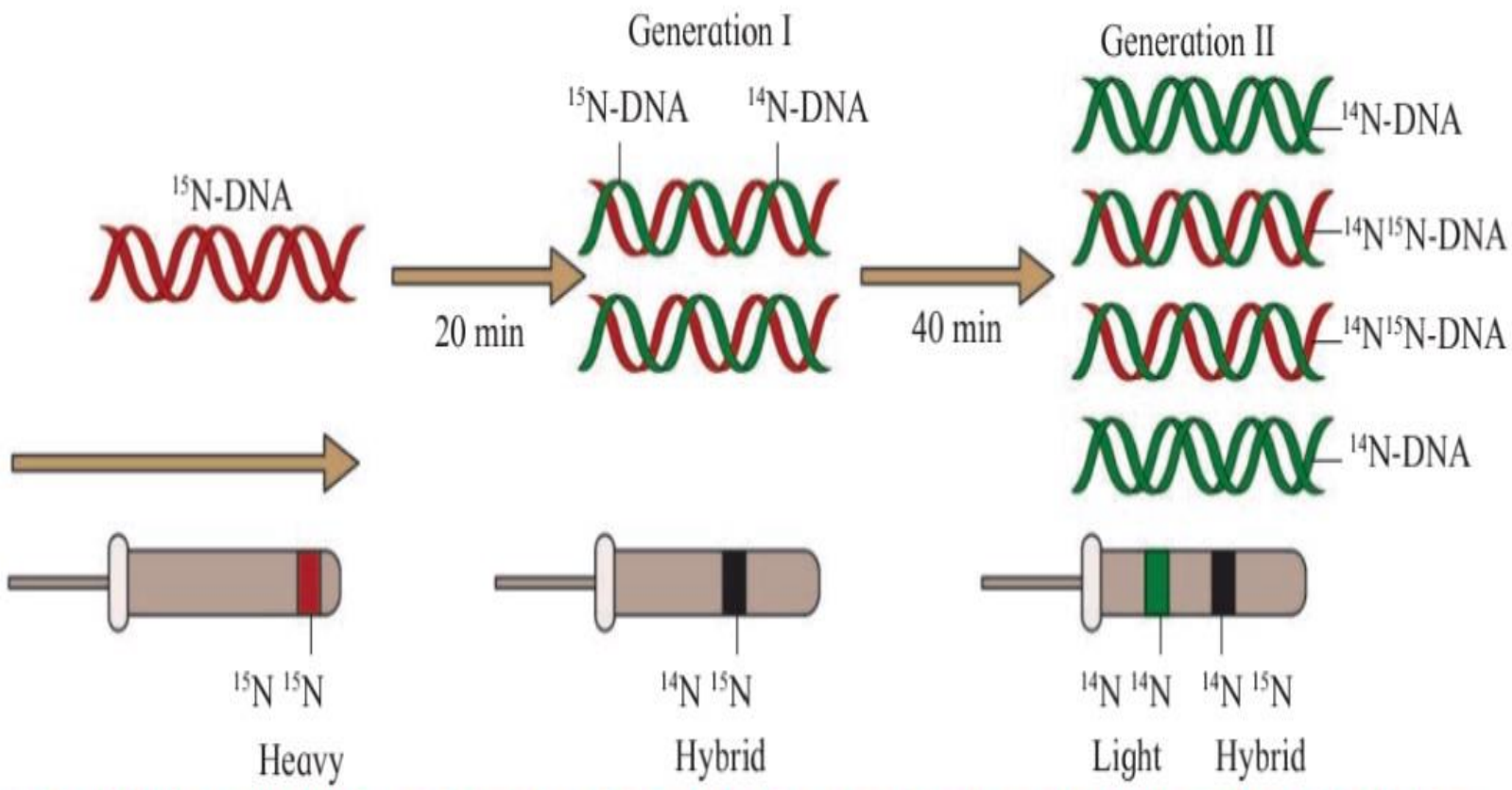
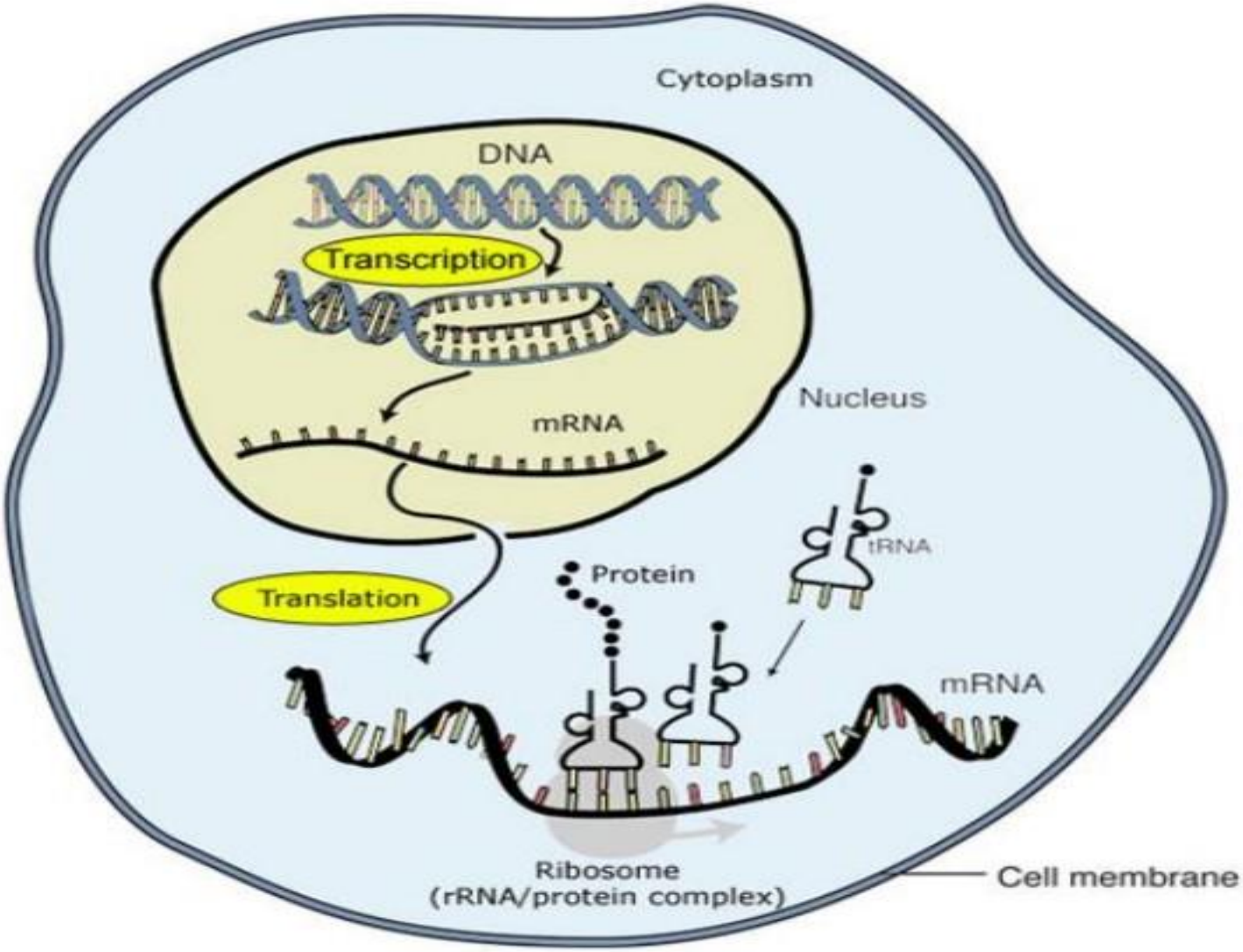


Fig. 4.9 : Interpretation of results of Meselson's experiment on the separation of DNA by equilibrium density gradient centrifugation

– protein synthesis



PROTEIN SYNTHESIS

→ *Important biomolecules.*

→ *Serve as structural components, enzymes and hormnes.*

→ *Cells needs to synthesize new protein molecules.*

Process of protein synthesis includes:-

- Transcription
- Translation

Process of copying of genetic information from one strand of DNA into a single stranded RNA transcript, is termed as transcription. (complementary RNA strand is form, where thymine is replaced by uracil)

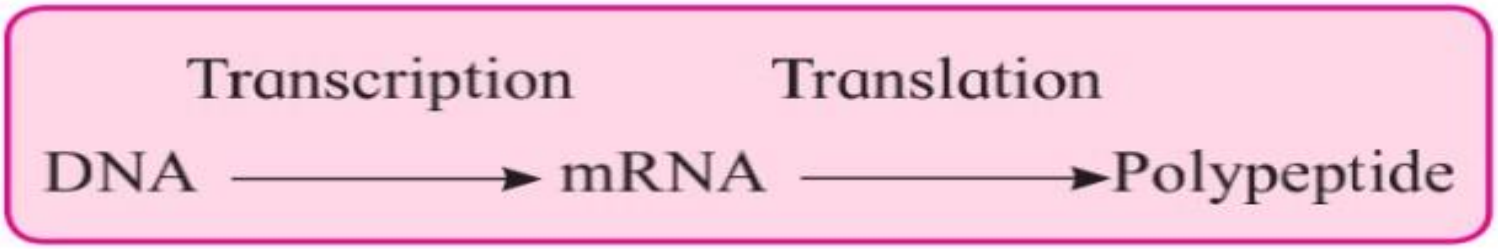
Not A=T .....but A=U

G=C G=C

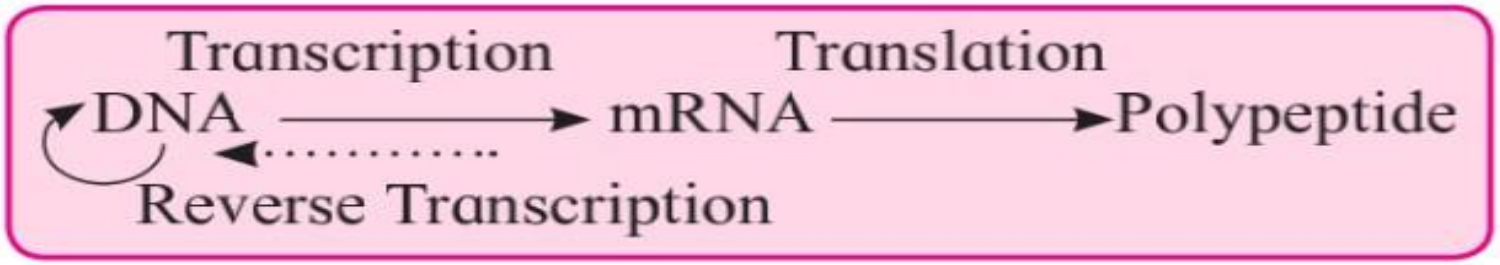
CENTRAL DOGMA

Unidirectional flow of information from DNA to RNA to Protein is referred as Central dogma.

(Crick -- 1958)



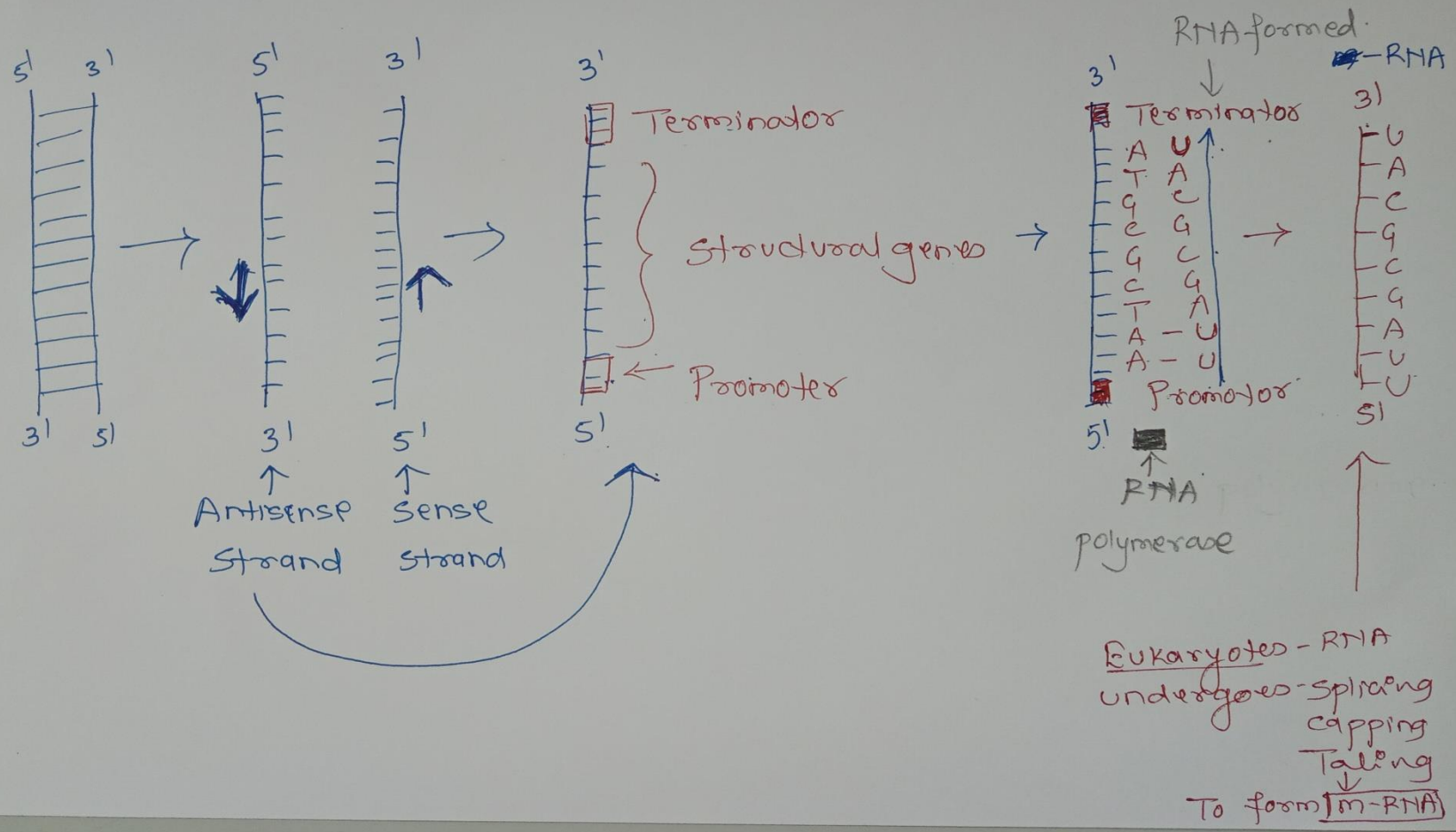
The present concept of central dogma in retroviruses or riboviruses is given by Temin (1970) and Baltimore (1970):



TRANSCRIPTION

- During transcription, information of **only one strand** of **DNA** is copied into **RNA.**
- DNA strand act as **template.**
- Enzyme **RNA polymerase** catalyzes **RNA transcript**.
- Location – DNA → **Prokaryotic nucleoid**
  DNA → **Eukaryotic nucleus**
- DNA transcription – **Nucleus**
- DNA translation – **Cytoplasm**
- **DNA** transfers information to **m-RNA** which then moves to **ribosomes**.
- Transcription takes place → **G1 and G2** phase of cell cycle

TRANSCRIPTION



- DNA has **promotor** and **terminator** sites.

- Transcription three stages:
  - →Initiation
  - →Elongation
  - →Termination

TRASCRIPTION UNIT

Transcription Unit → Each transcribed segment of DNA.

Consist of → Promotor

→ The Structural gene

→ A Terminator

Two strands of DNA shows following features:

1. Small DNA sequence– *binding site for RNA polymerase* →Promoter(5')

Small DNA sequence– *which terminates transcription*

→Terminator(3')

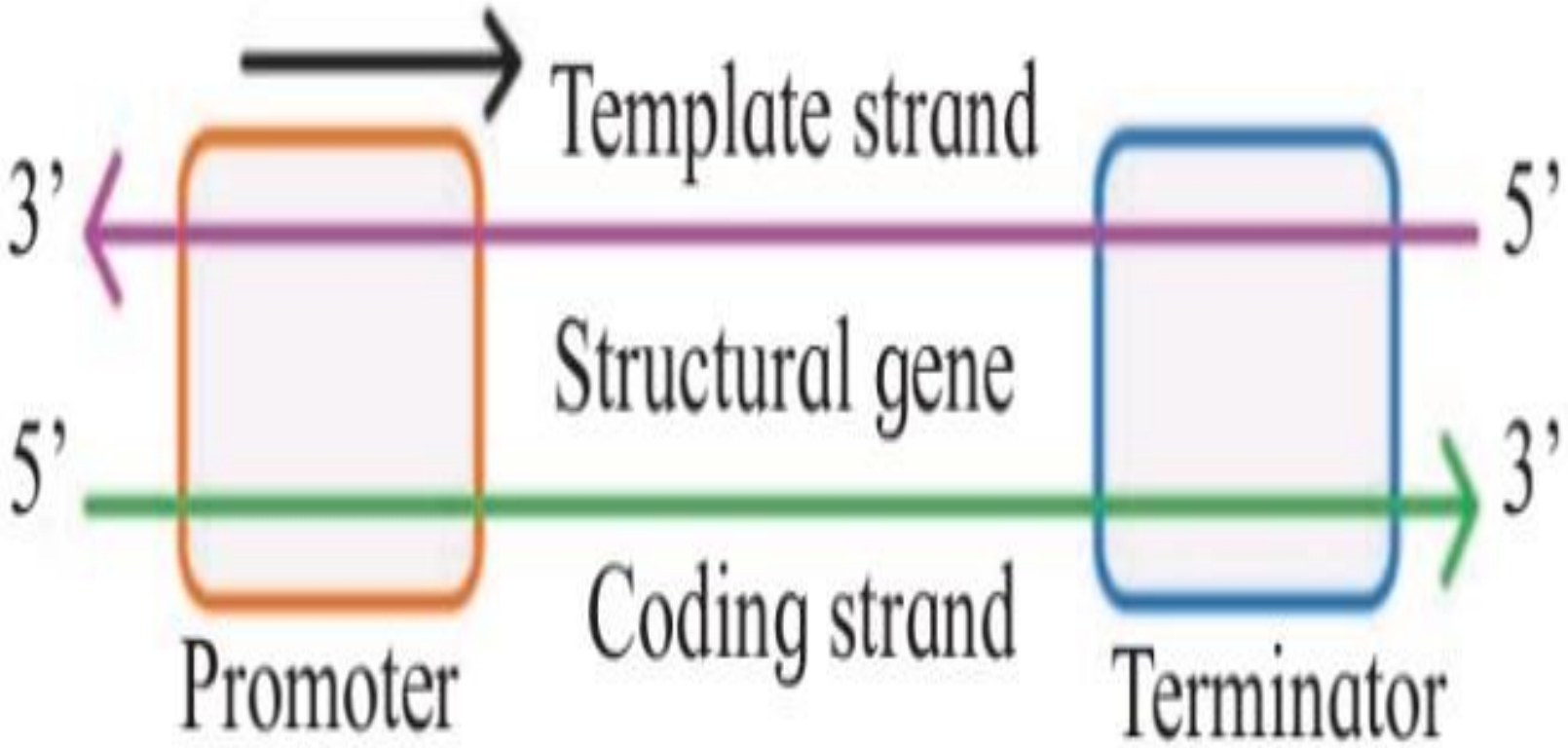


Fig. 4.10 : Transcription unit

STRUCTURAL GENES

2. DNA dependent RNA polymerase catalyses polymerisation in 5' →3' direction.

DNA strand →*3'-5'direction*→**Antisense strand** is used for synthesis of RNA

DNA strand→*5'-3'direction*→**Sense strand** is not used for synthesis of RNA.

The base sequence in this strand is same as in RNA.

(where Thymine is replaced by Uracil)

*The information on this strand of DNA is copied on mRNA. This is called **sense strand**.*

- After binding to **promoter**, **RNA polymerase** moves along the DNA and causes local **unwinding of DNA** duplex into **two chains**.
- Exposed **ATCG bases** project into **nucleoplasm**.
- Only **one strand** functions as template (**antisense strand**).
- Other strand is complementary which is actually a coding strand (sense strand).
- **Ribonucleoside tri phosphate** join to bases of DNA **template chain**.
- As transcription proceeds the **hybrid DNA-RNA** molecule dissociates and makes **mRNA** molecule **free**.
- **RNA polymerase** reaches the **terminator signal** on the **DNA**, it leaves DNA and fully formed mRNA is released.
- **DNA molecules** becomes **spirally coiled** and attains double helical form.

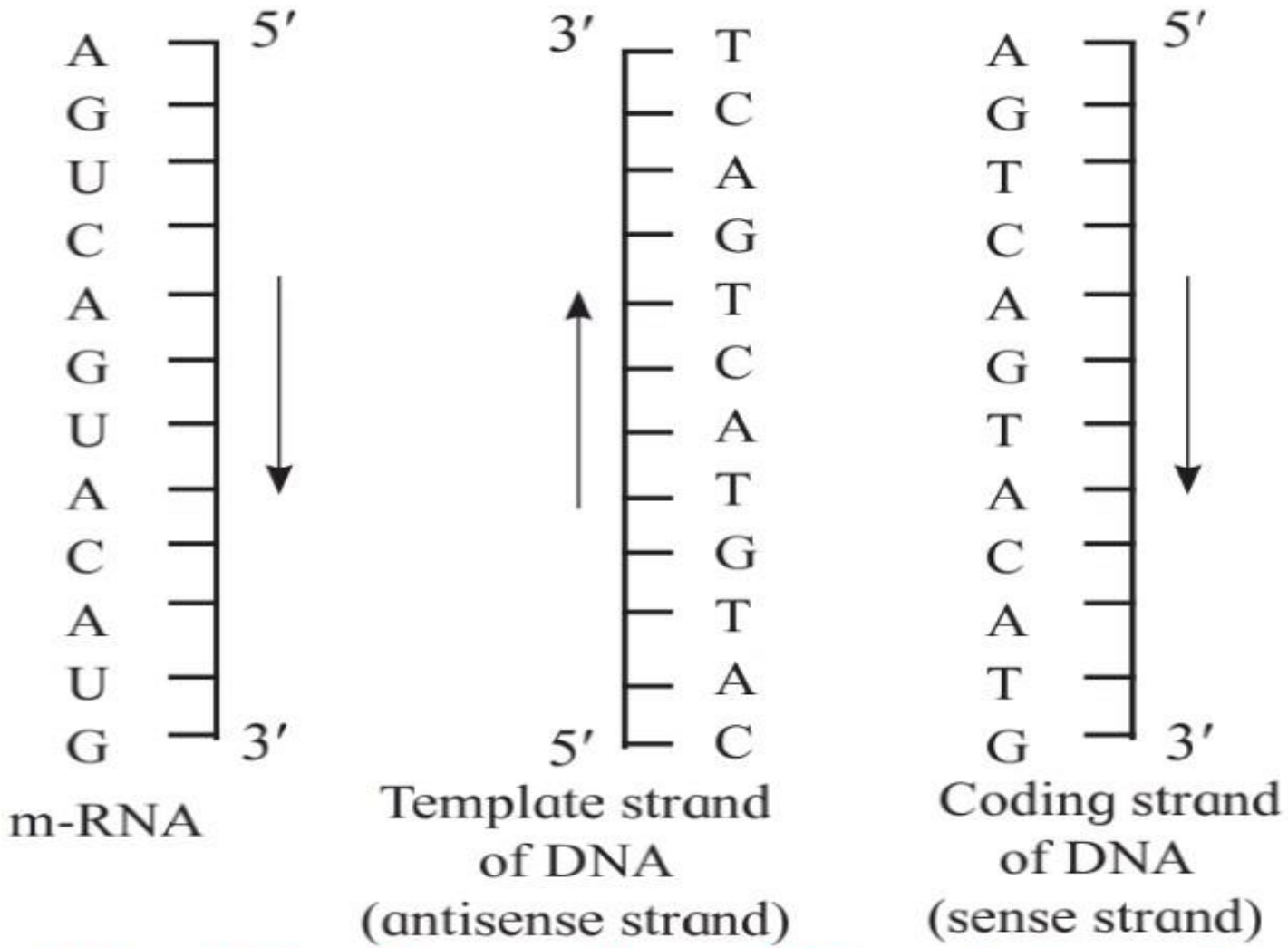
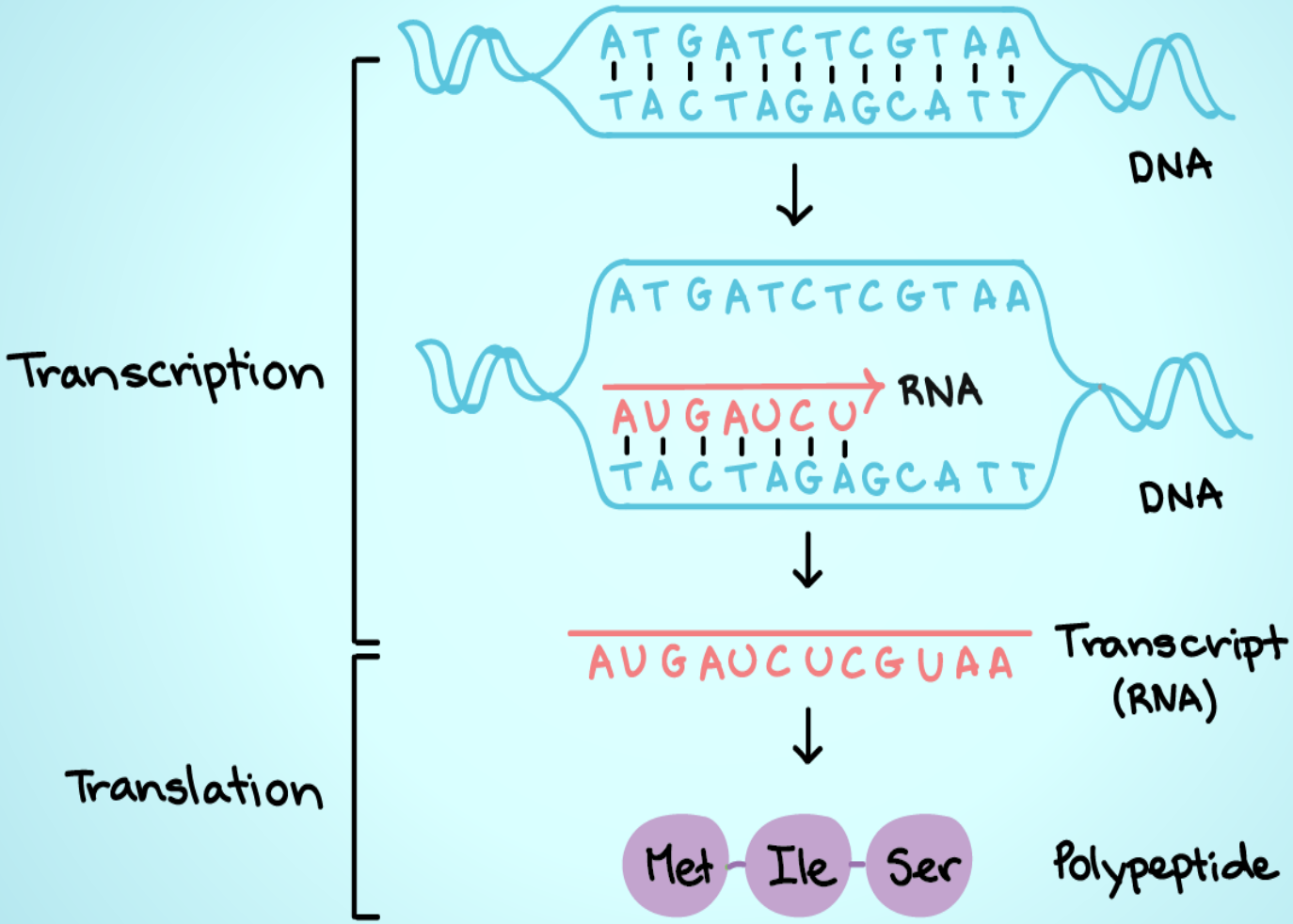


Fig. 4.11 : Formation of Template and Coding strand of DNA

Formation of Template and Coding strand



- In **bacteria**, **m-RNA** does not require any processing because it has **no introns**.

- **Prokaryotes** posses only **one** type of **RNA polymerase**.
- **Eukaryotes** posses **three type** of **RNA polymerases**:

→RNA polymerase I -- **Transcribes r-RNA**

→RNA polymerase II -- **Transcribes m-RNA** and **hnRNA** (Heterogeneous nuclear RNA)

→RNA polymerase III -- **Transcribes t-RNA** and **snRNA** (small nuclear RNA)

TRANSCRIPTION UNIT AND THE GENE

- *Gene -- DNA sequence coding for m-RNA, t-RNA or r-RNA.*
- *Mono-cistronic -- single structural gene in transcription unit.*
- *Poly-cistronic -- Long segment of DNA having set of various structural genes in one transcription unit.*

- *Introns -- Structural genes in eukaryotes have interrupted non-coding sequences .*
- *Exons -- Coding sequences or express sequences .*

Only exons appear in processed **mRNA** in **eukaryotes**.

Processing of hnRNA

- Eukaryotes, **RNA transcribed** from DNA are **primary transcribes.**
- **Primary transcript** is **non-functional.**
- It undergo changes called **processing** or maturation before becoming **functional.**
- It consist of both **exons** and **introns**.
  - **RNA Undergoes three process**.

* ***Splicing*** -- *Only introns are removed from m-RNA. Exons are joined by DNA ligase enzymes.*
* ***Capping*** -- *Methylated guanosine tri phosphate is added to 5' of hnRNA.*
* ***Tailing*** -- *Polyadenylation take place at 3' end.*

- **Now this fully processed hnRNA is called m-RNA**.
- *For translation m-RNA is transported out of the **nucleus** through the **nuclear pore** to **cytoplasm***

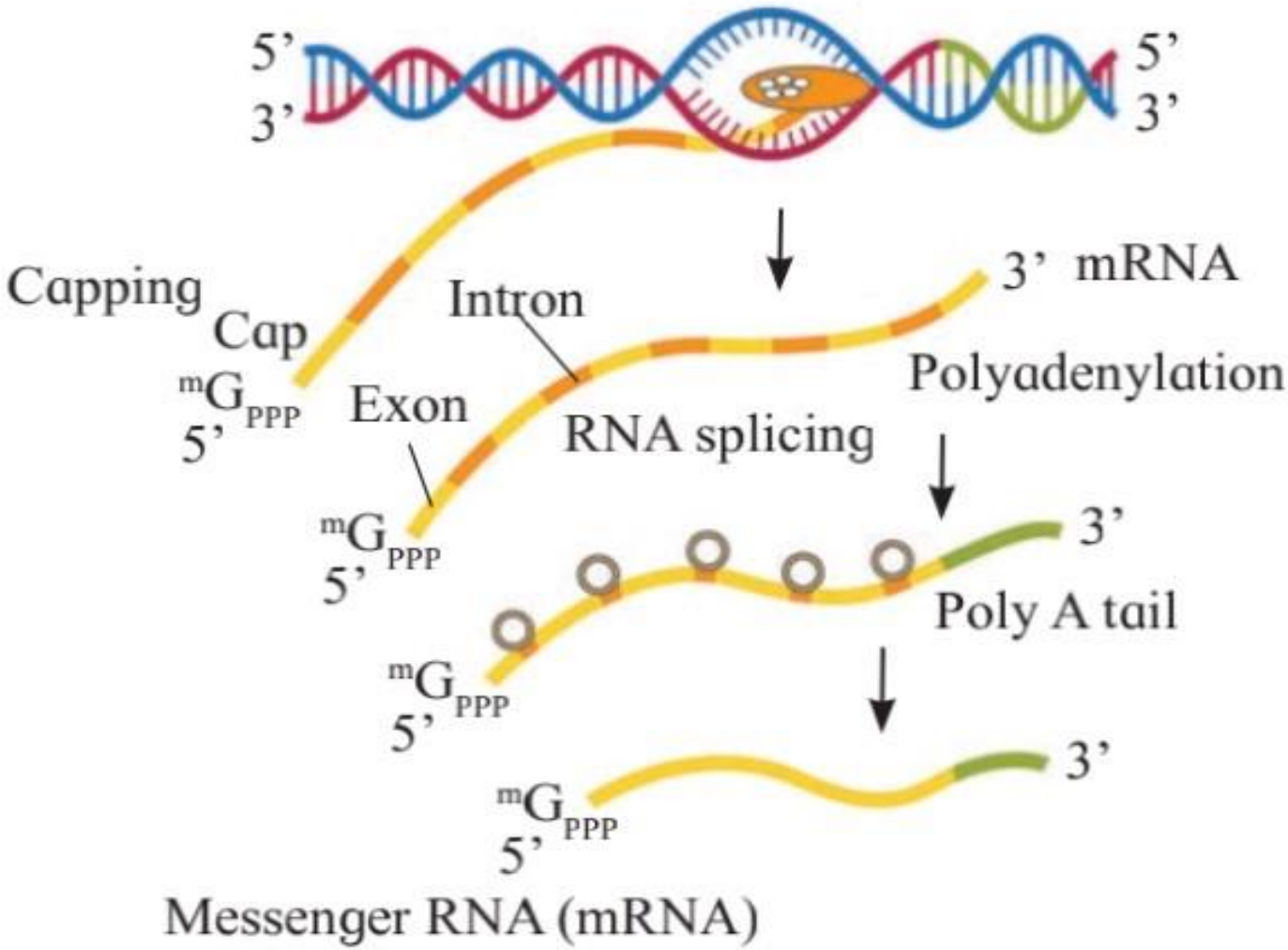


Fig. 4.12 : Transcription and Processing of hnRNA to mRNA in Eukaryotes

GENETIC CODE

- *DNA is a master molecule that guides, regulates and controls process of protein synthesis.*
- *Site for storing this information lies in the sequence of nucleotides (i.e. nitrogen bases).*
- *20 different types of amino acids are involved in the process synthesis of proteins.*
- *DNA molecule has 4 types of nitrogen bases to identity 20 amino acid.*

- Information for protein synthesis is stored in the form of coded language (cryptogram) called genetic code.
- Genetic code contains code words (codons) each one specifying specific amino acid.
- Genetic code -- Collection of base sequence that correspond to each amino acid.

->*Single nitrogen base* -- *codon* -- *Encode only 4 amino acid*

->*Two nitrogen bases* -- *codon* -- *Encode only 16 amino acid*

->*Three nitrogen bases* -- *codon* -- *Encode 64 amino acid*

- Every three consecutive nucleotides in DNA will constitute a triplet codon.

Dr. Har Gobind Khorana

→ **Artificially synthesized m-RNA** with **repeated sequence** of known nucleotides.

- → By using synthetic DNA, Dr. Khorana prepared chains of polyribonucleotide's with known repeated sequence of **two or three** nucleotides.
- → Eg. **CUC, UCU, CUC, UCU**. (Leucine, serine)

→ Resulted in formation of polypeptide chain having two different amino acids **placed alternately**.

→ Similarly, polynucleotide chain with **three-nitrogen base repeats** gave polypeptide chain with only **one amino acids.**

- → Eg. **CUA, CUA, CUA, CUA.** (Leucine)

Second Letter

First Letter	U	C	A	G	Third Letter
U	UUU, UUC } Phe UUA, UUG } Leu	UCU, UCC, UCA, UCG } Ser	UAU, UAC } Tyr UAA Stop UAG Stop	UGU, UGC } Cys UGA Stop UGG Trp	U C A G
C	CUU, CUC, CUA, CUG } Leu	CCU, CCC, CCA, CCG } Pro	CAU, CAC } His CAA, CAG } Gln	CGU, CGC, CGA, CGG } Arg	U C A G
A	AUU, AUC, AUA } Ile AUG Met	ACU, ACC, ACA, ACG } Thr	AAU, AAC } Asn AAA, AAG } Lys	AGU, AGC } Ser AGA, AGG } Arg	U C A G
G	GUU, GUC, GUA, GUG } Val	GCU, GCC, GCA, GCG } Ala	GAU, GAC } Asp GAA, GAG } Glu	GGU, GGC, GGA, GGG } Gly	U C A G

Fig. 4.13 : Dictionary of genetic code

Replication and transcription

- *Nucleic acid is copied to form another nucleic acid.*
- *These two processes are based on complementarity principle.*

Translation

- *Genetic information is transferred from a polymer of nucleotides to a polymer of amino acids.*
- *Here, complementarity principle does not exit.*
- *This clearly explains that genetic code directs the sequence of amino acids during synthesis of proteins.*

What does the DNA of all these organisms have in common?

They all share a universal genetic code.

CHARACTERISTIC OF GENETIC CODE

1. Genetic code is a triplet code

   Sequence of three consecutive bases form codon, which specifies one particular amino acid. It is universal and read in 5' → 3' direction.

2. Genetic code has distinct polarity.

   Always read in 5' → 3' direction and not in 3' → 5' direction. Otherwise message will change eg. 5' AUG 3'.

3. Genetic code is non-overlapping.

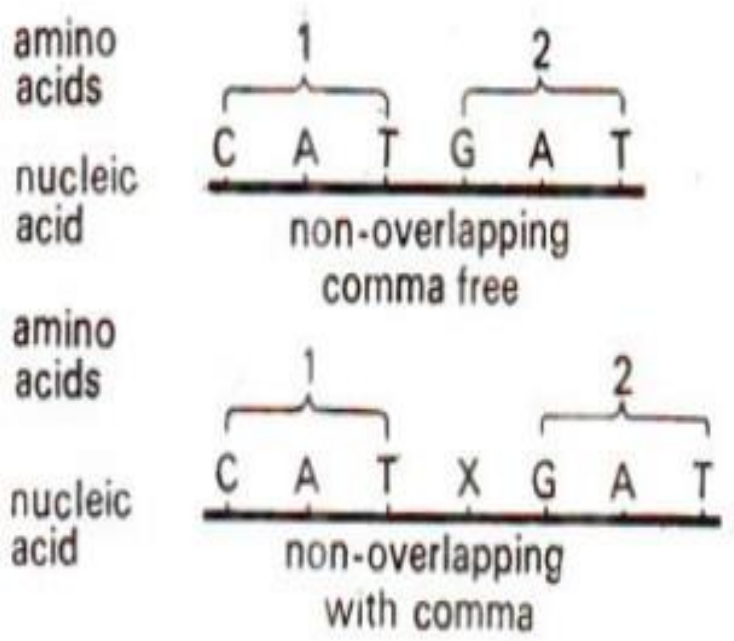
   Each single base is a part of only one codon.

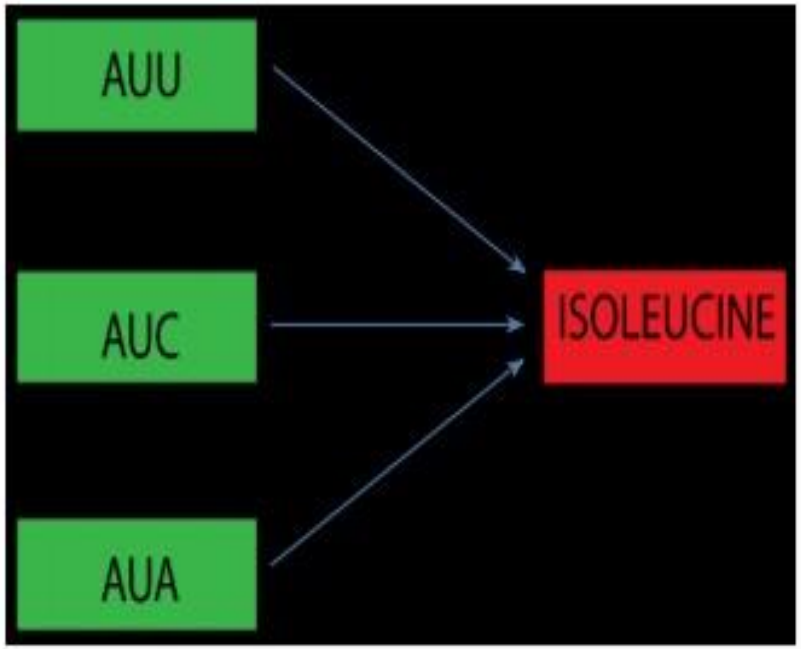
4. Genetic code is commaless.

   These is no gap or punctuation mark between consecutive codons.

.

Non-Overlapping codon





5. Genetic code has degeneracy.

Usually single amino acid is encoded by single codon, however some amino acids are encoded by more than one codons.

eg. **cysteine has two codons**

Isoleucine has three codons

This is called degeneracy of code.

6. Genetic code is universal.

All the living organisms the specific codon specifies same amino acid.

eg. codon AUG always specifies amino acid methionine in all the organisms from bacteria to human.

7. Genetic code is non-ambiguous.

Two different amino acid will never be encoded by the same codon

8. Initiation codon and termination codon.

→ *AUG is always an **initiation** codon in any and every mRNA.*

→ *Out of 64 codons , three codons, **UAA, UAG** and **UGA** are **termination** codons which stop the process of elongation of polypeptide as they **do not code** for any amino acid.*

9. Universal

*In all organisms the **specific codon** specifies **same amino acid.***

10. Codon and anticodon.

→ *Codon is a part of **DNA**. Eg. AUG is codon. It is always represented as **5' AUG 3'**.*

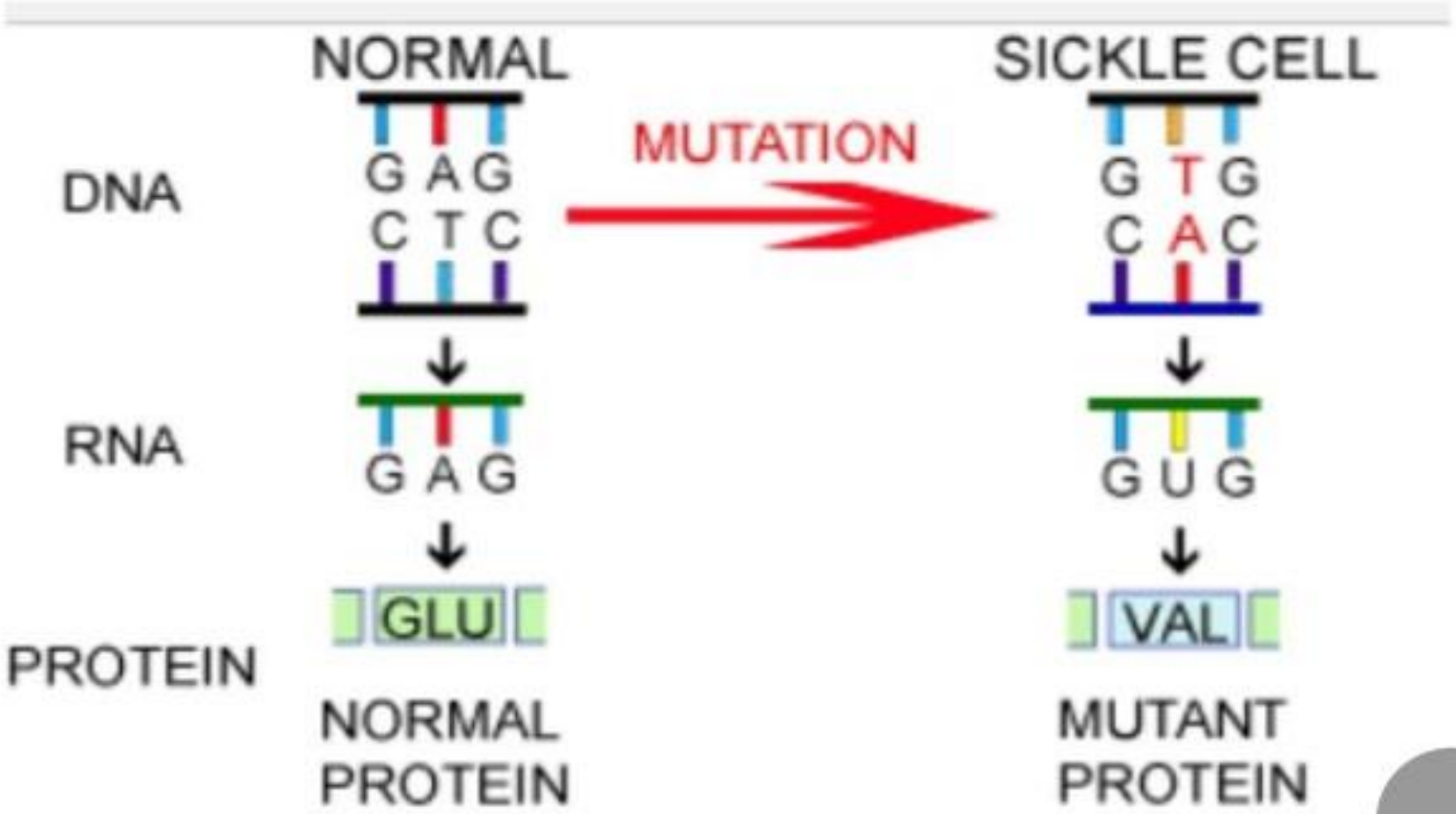
→ *Anticodon is a part of **t-RNA**. It is always represented as **3' UAC 5'**.*

MUTATION AND GENETIC CODE

→**Mutation** – Sudden **change** in the **DNA sequence.**

- → Results in change of **genotype**.
- → **Recombination and mutation** – Raw material for **evolution** → results in **variations.**
- → **Possibility of loss** (deletion) or **gain** (insertion/duplication) of a **segment of DNA.**
- → Results in **alternation** in **chromosomes.**
- → **Point Mutation** -- Occur due to change in a **single base pair** of **DNA.**
- → Eg. Sickle cell anaemia

Point Mutation in Sickle cell anaemia



→ *Deletion* or *insertion* of base pair of *DNA* causes *frame – shift mutations* or *deletion mutation*.

→ *Insertion* or *deletion* of one or *two bases* → *Changes* the *reading frame* from the point of deletion or insertion.

→ *Deletion* or *insertion* of *three* or *multiples* of *three bases* results in insertion or deletion of *amino acid* and the *reading frame* remains *unaltered* from that point altered.

T-RNA THE ADAPTER MOLECULE

→Scientists considered – mechanism – t-RNA read codon and bind with the amino acid as amino acid does not have any special capacity to read the codon.

→so t-RNA is considered as an adapter molecule.

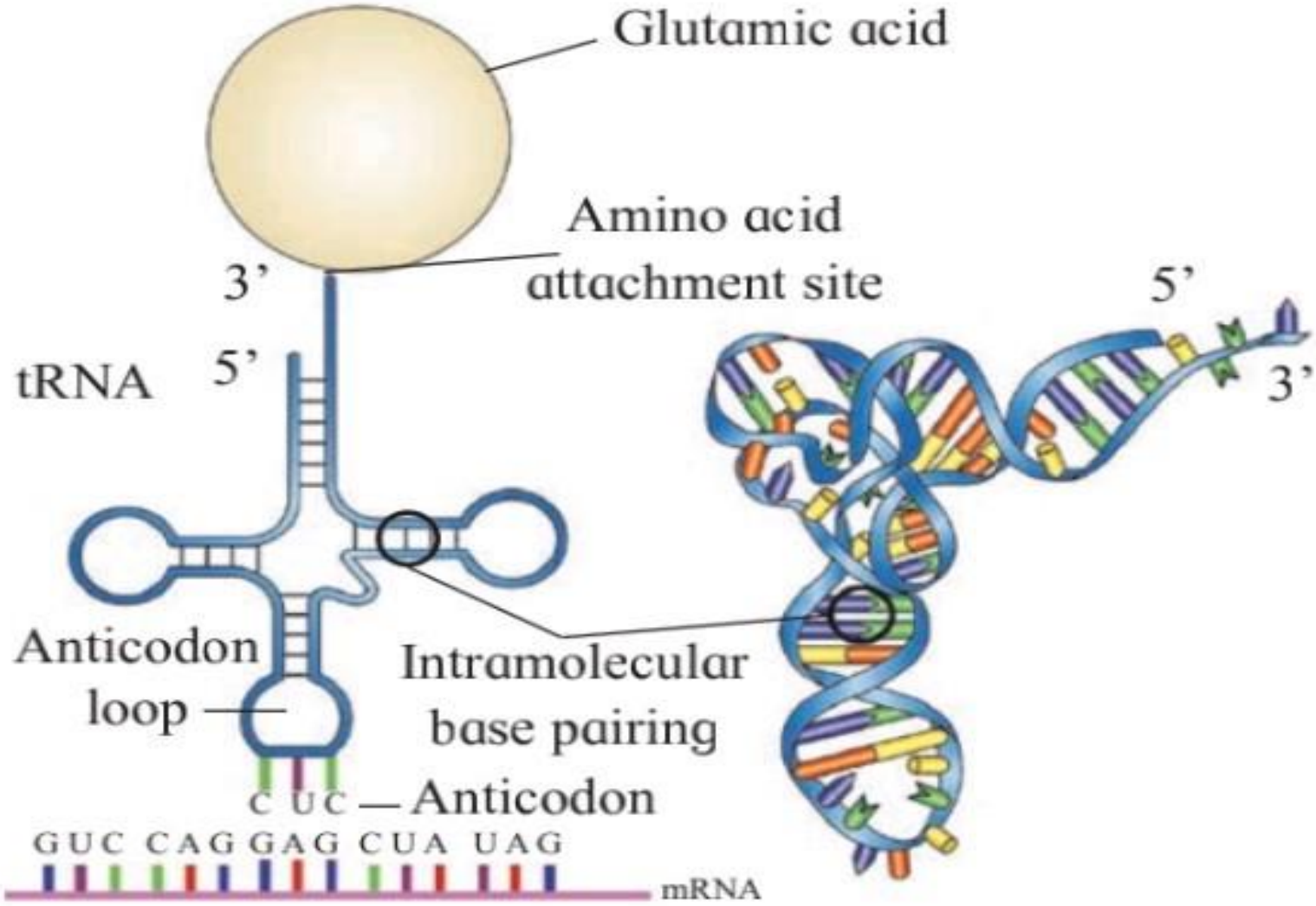


Fig. 4.14: t-RNA - the adapter molecule

Clover leaf structure of t-RNA

→**Clover leaf** structure of (2 dimensional) of **t-RNA** possess an **anticodon loop** that has **bases complementary** to the **codon.**

→It is called **anticodon**.

→It shows **amino acid acceptor end** (3'end) having **unpaired CCA bases** to which **amino acid binds**.

→For every amino acid there is **specific t-RNA**.

→**Initiator t-RNA** is specific for **methionine**.

→there are **no t-RNA's** for **stop codons**.

→Actual structure, t-RNA look like **inverted L**. (3 dimensional structure)

TRANSLATION – PROTEIN SYNTHESIS

Translation

Mechanism in which codons of mRNA are translated and specific amino acids in a sequence form a polypeptide on ribosomes.

All types of proteins are synthesized by the cell, within itself.(intracellularly)

Process of translation requires…

1. Amino acid

→Form raw material for protein synthesis.

→About 20 different amino acids are known to form protein and are found in cytoplasm.

2. DNA

→Controls synthesis of proteins having amino acid in specific sequence.

→Possible through transcription of m-RNA. Genetic code is specific for particular amino acid.

3. RNAs

→Serve as intermediate molecules between DNA and protein.

4. Ribosomes

→Serve as the site for **protein synthesis**.

→Ribosome consists of **large** and **small subunits**.

→These subunits occur separately in **cytoplasm**.

→Only during protein synthesis, these two subunits get associated together due to **Mg++ ions**.

Ribosome has one binding site for m-RNA and 3 binding site for t-RNA.

→**P site** – Peptidyl t-RNA site

→**A site** – Aminoacyl t-RNA site

→**E site** – Exit site

- Only **first t-RNA amino acid complex**, directly enters **P site** of ribosome.
- In Eukaryotes a **groove** is present between **two subunits** of ribosomes, it **protects** the polypeptide chain from the action of **cellular enzymes** and also **protects m-RNA** from the action of **nucleases.**

MECHANISM OF TRANSLATION

ie. Synthesis of polypeptide chain

Translation :-

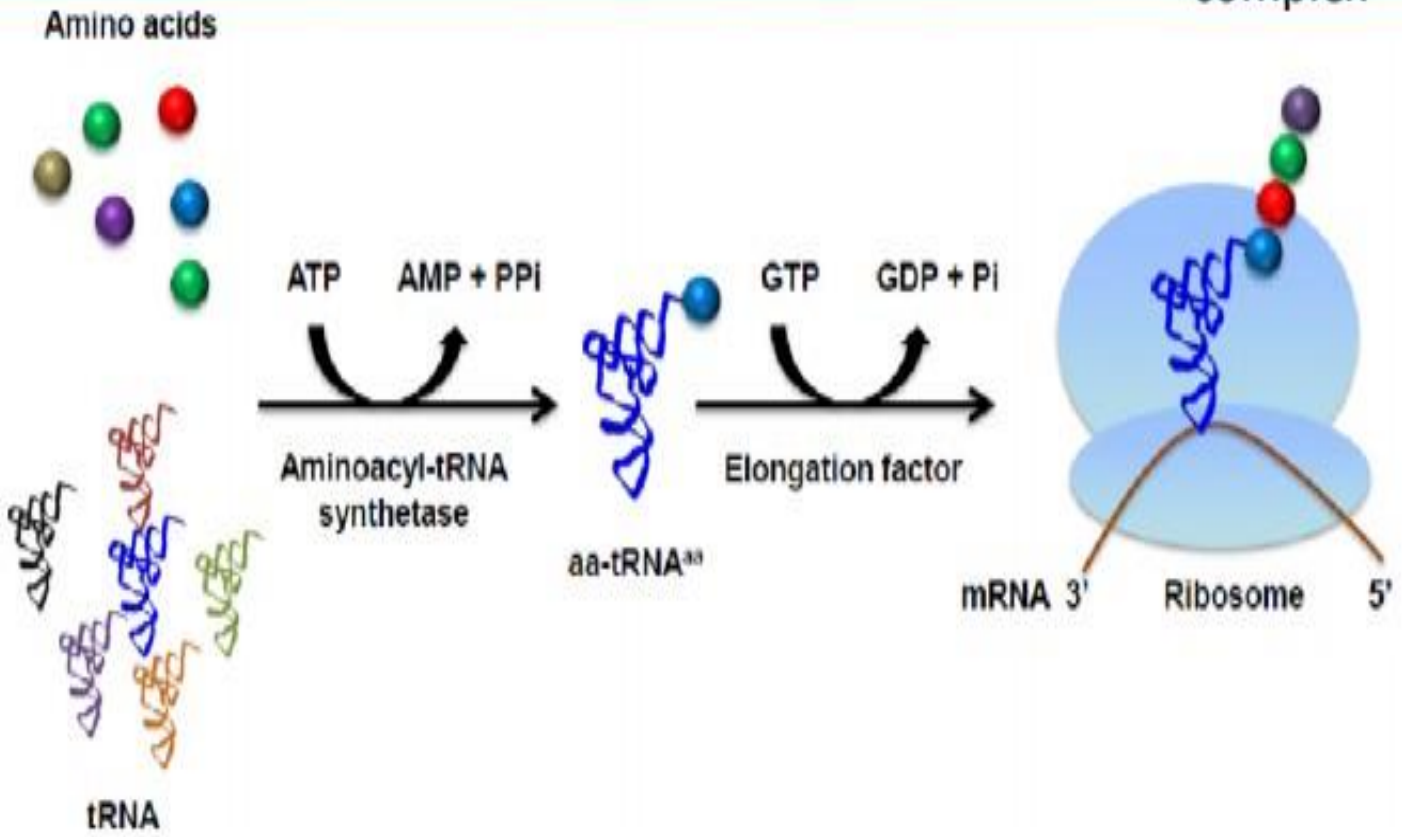
It is the process in which the **sequence** of **codons** on the **mRNA strand** is **read/ decoded** and accordingly the **amino acids** are joined to each other to form a **polypeptide chain** that makes .

Process of Translation

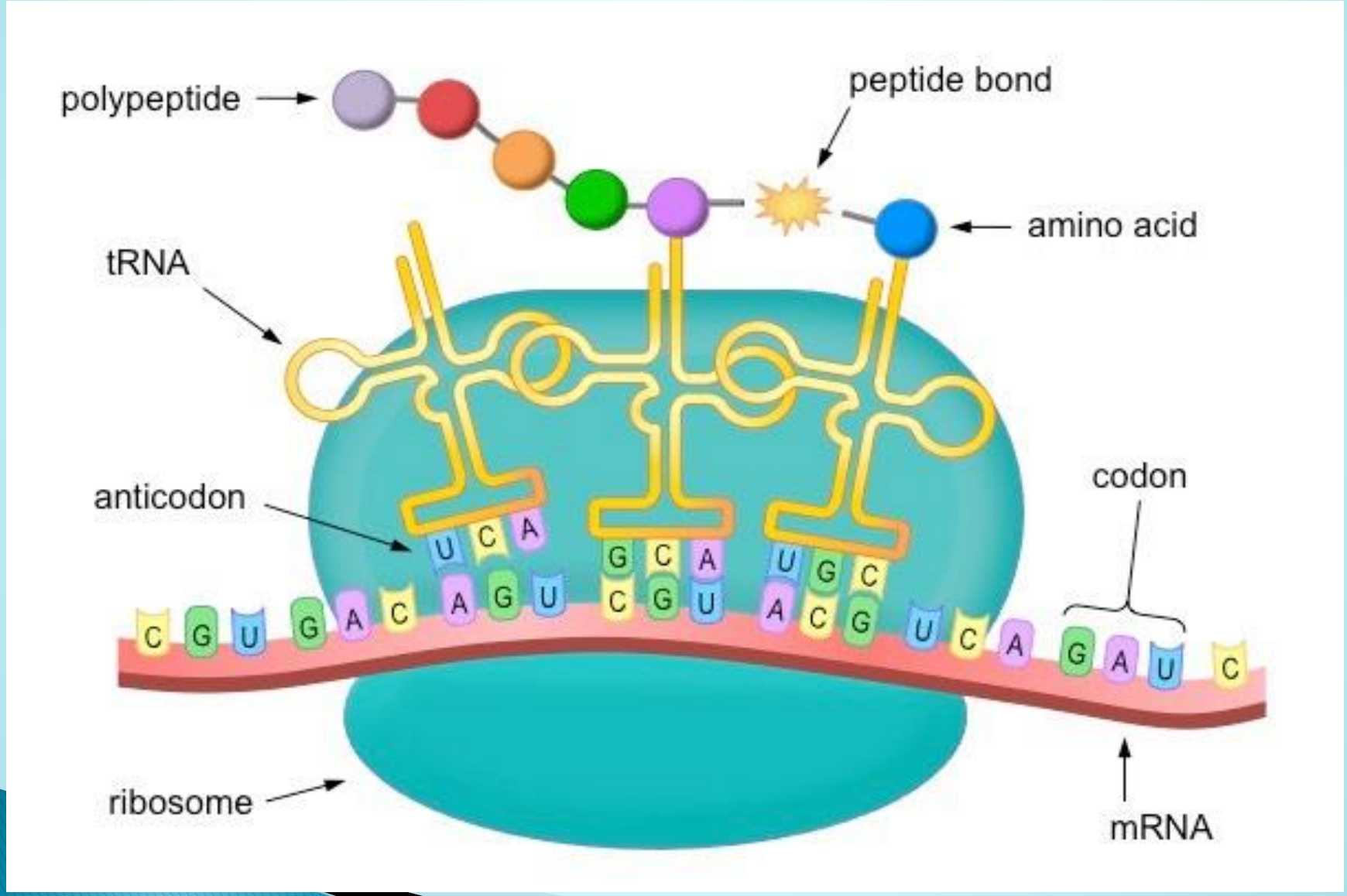
(A) Activation of amino acids and formation of AA–tRNA complex :–

- In presence of an enzyme amino acyl t-RNA synthetase the amino acid (AA) molecule is activated and then each amino acid is attached to specific t-RNA molecule at 3'/CCA end to form amino acyl t-RNA complex. The reaction needs ATP. This process is called charging of t-RNA or amino acylation of t-RNA.

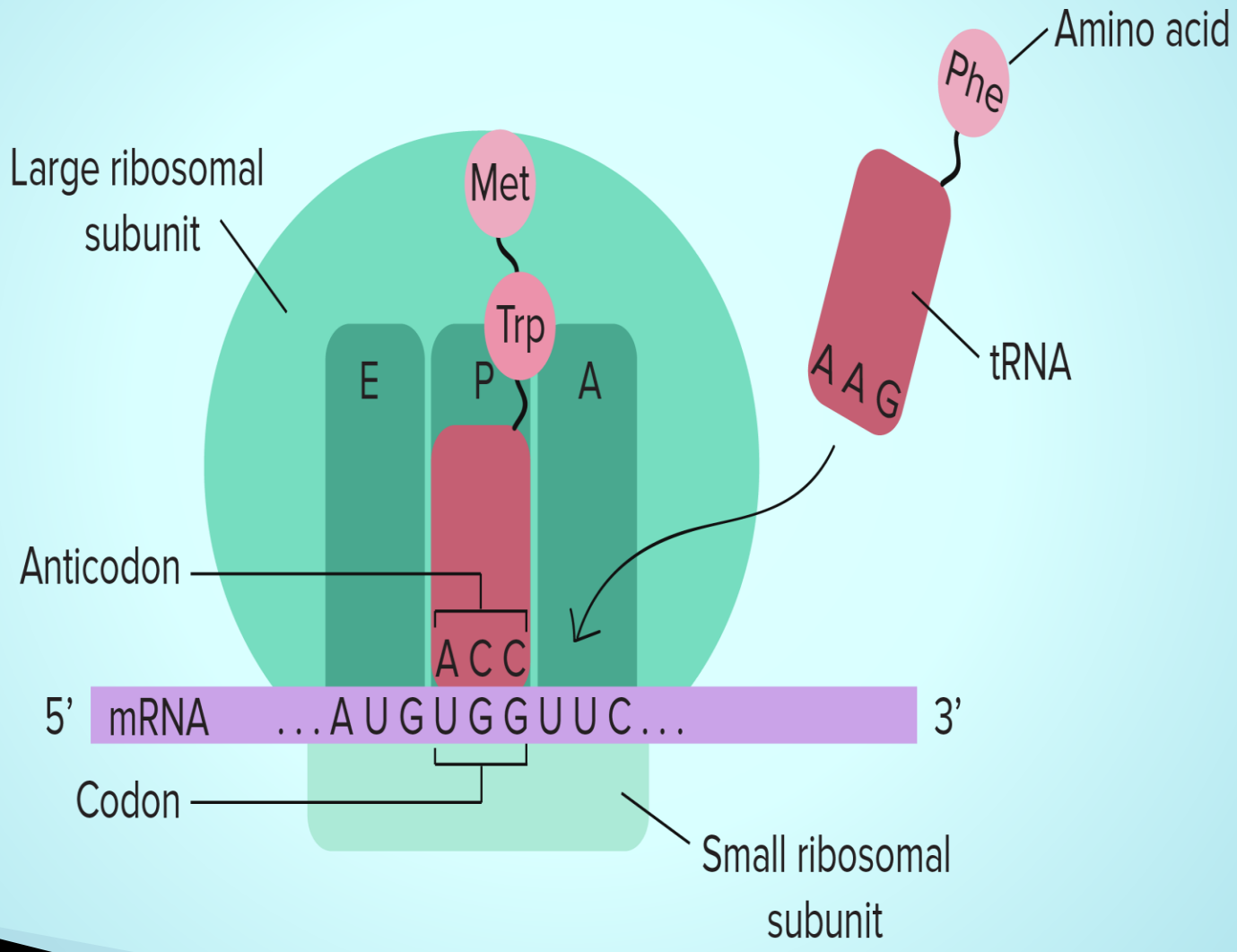
Amino acid(AA)+ATP+tRNA $\xrightarrow{\text{Aminoacyl tRNA synthetase}}$ aminoacyl-tRNA complex



Translation



Translation



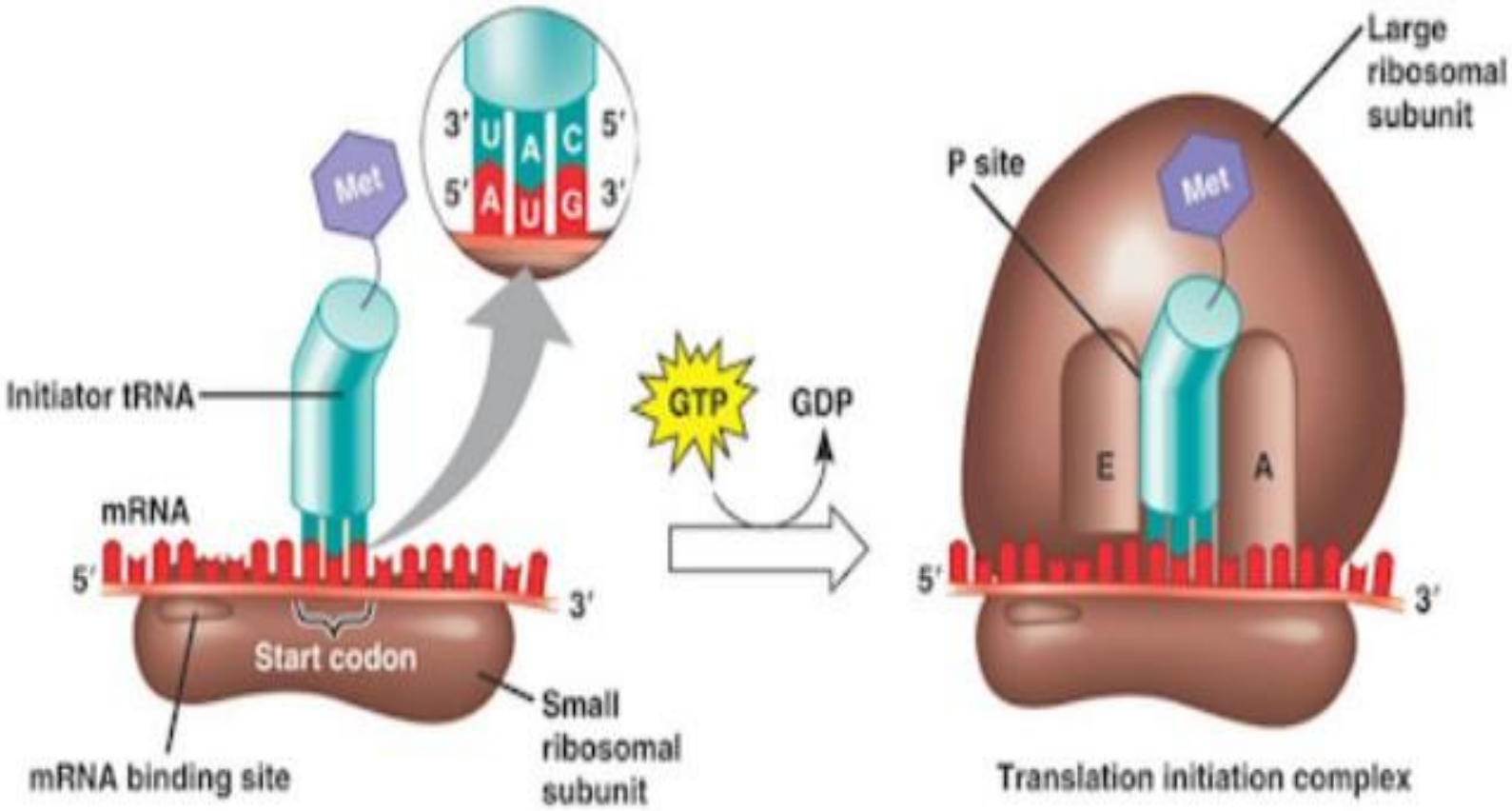
Initiation of polypeptide chain

- It begins with the formation of initiation complex which requires the mRNA having codons for a polypeptide, the smaller (30s) and larger (50s) sub-units of ribosomes, the initial AA1 -tRNA complex

  and ATP and GTP as source of energy.
- The process starts with the binding of mRNA on the smaller 30s sub-unit of ribosome.
- AUG is present on mRNA which initiates the process of protein synthesis.
- Initiator t-RNA binds with initiation codon (AUG) by its anticodon (UAC) through hydrogen bonds. It carries activated amino acid methionine (met) in Eukaryotes or formyl methionine (f-met) in prokaryotes.

- Now the large subunit of ribosome joins with the smaller subunits, that requires Mg ++ ions.
- The ribosome has three sites namely : aminoacyl (A) site, peptidyl site (P) and exit (E) site.
- The empty t-RNA leaves from E site. Only the AA1-t-RNA complex binds at P site directly while all the other incoming t-RNA complexes get attached first at A site and then are shifted to P site.

Translation: Initiation



ELONGATION OF POLYPEPTIDE CHAIN

→During this process, **activated amino acids** are added **one by one** to first **amino acid** (methionine).

→This amino acid binds with **amino acid binding site** of t-RNA. This result in formation of **t-RNA amino acid complex.**

Addition of amino acid occurs in 3 steps cycle-

a) Codon recognition:-Amino acyl t-RNA molecule enters the ribosome at A-site. Anticodon binds with the codon by hydrogen bonds.

b) Amino acid on the first initiator t-RNA at P-site and amino acid on t-RNA at A-site join by peptide bond. At this time first t-RNA at 'P' site is kicked off.

c) Translocation :-

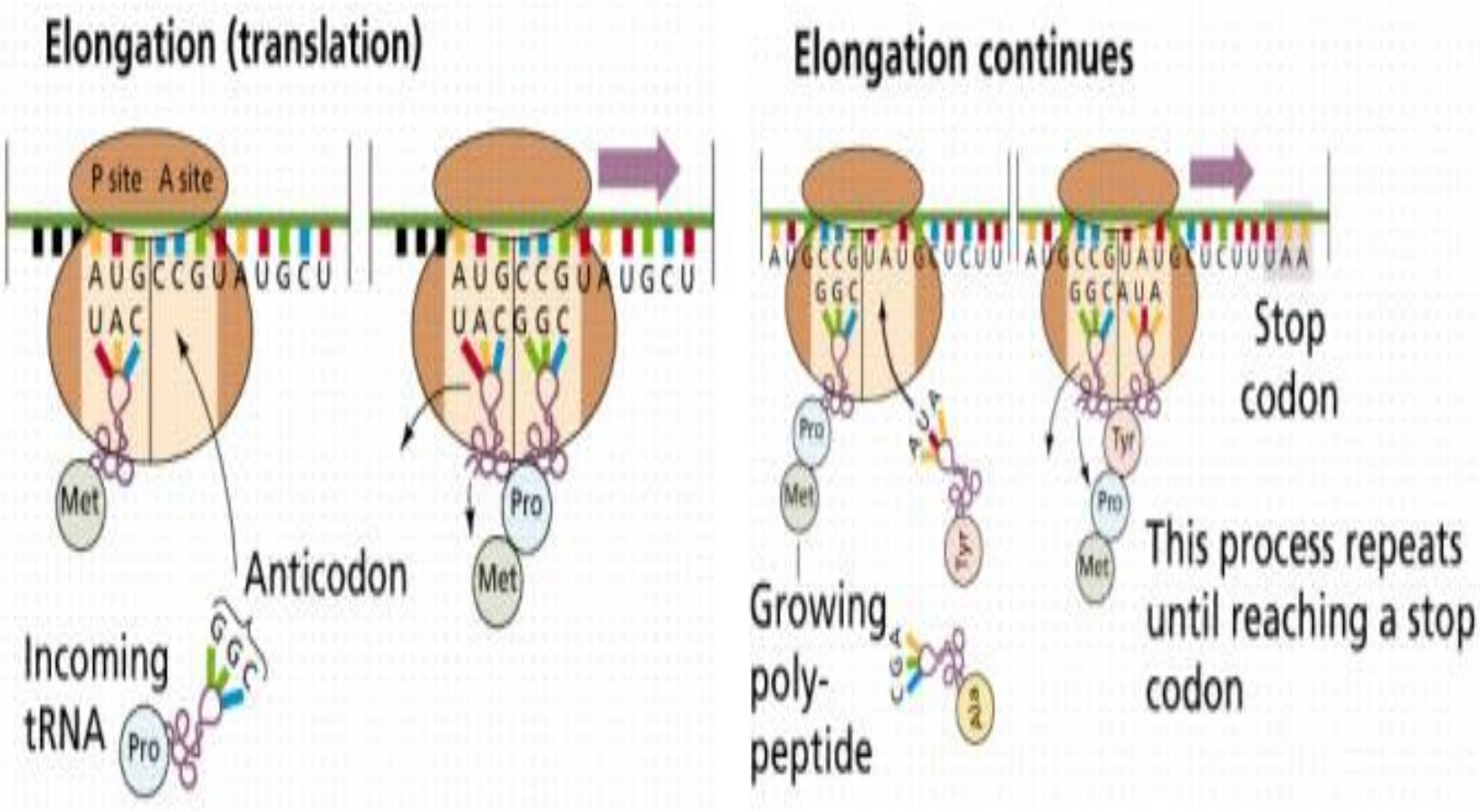
→The t-RNA at A-site carrying a dipeptide at A-site moves to the P-site. This process is called translocation.

→ In translocation, both the subunits of ribosome move along in relation to t-RNA and m-RNA.

→Hence, t-RNA carrying dipeptide now gets positioned at 'P' site of ribosome, making 'A' site vacant.

→At this site, then next charged t-RNA molecule carrying amino acid will be received. During this process, first uncharged t-RNA is discharged from E-site.

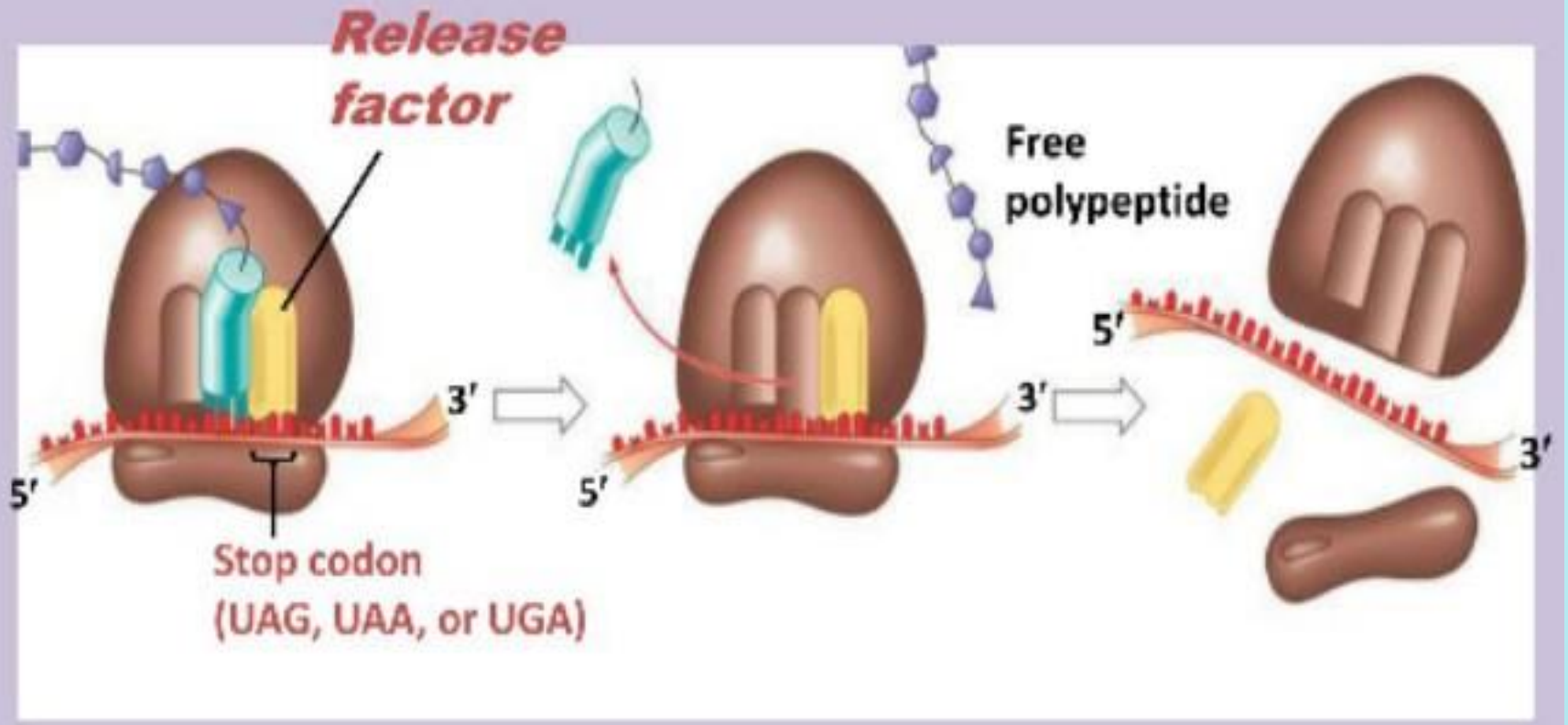
→This process is repeated as amino acids are added to polypeptide. It takes less than 0.1 second for formation of peptide bond.


Elongation (translation)
P site
A site
AUGCCGUAUGCU
UAC
Met
Anticodon
Incoming
tRNA
Pro
AUGCCGUAUGCU
UACGGC
Pro
Met
Elongation continues
GGC
Pro
Met
Growing
poly-
peptide
Tyr
GGCAUA
Tyr
Pro
Met
Stop
codon
This process repeats
until reaching a stop
codon

Termination and release of polypeptide

- At the end of mRNA, there is a **stop codon** (**UAA/UAG/UGA**).
- It is exposed at the **A-site**. It is **not read** and joined by anticodon of **any t-RNA**.
- The **release factor binds** to the **stop codon**, thereby **terminating** the translation process. The **polypeptide** is now **released** in the **cytoplasm**.
- **Two subunits** of ribosome **dissociate** and last **t-RNA** is set **free** in the **cytoplasm.**
- Finally **m-RNA** is also **released** in the **cytoplasm.** It get **denatured** by **nucleases** immediately. Hence **mRNA** is **short lived**.

Termination of Translation



Regulation of gene expression

- Multistep process by which a gene is regulated and its product is synthesized.
- Gene expression results in the formation of a polypeptide.
- Gene expression process is regulated at different levels. (Eukaryotes)

- ✓ Transcriptional level. (formation of primary transcript)
- ✓ Processing level.(regulation of splicing)
- ✓ Transport of m-RNA from nucleus to the cytoplasm.
- ✓ Translational level.

Genes of the cell are expressed to perform different functions.

For eg.

→An enzyme beta galactosidase is synthesized by E.coli.

→This enzyme is used for hydrolysis of **lactose** into galactose and glucose.

(B-galactosidase)

Lactose ----------→ Galactose + Glucose

→If E.coli do not have lactose in the surrounding medium as a source of energy, then enzyme B-galactosidase is not synthesized.

→So it is metabolic or physiological or environmental conditions that regulate expression of genes.

Development and differentiation of embryo into an adult organism, is also a result of the co-ordinated regulation or expression of several sets of genes.

- **E.coli** adapt to their **chemical environment** by synthesizing certain **enzymes** depending upon the **substrate present**.
- Such an **adaptive enzyme** is called **Inducible enzymes.**
- A **set gene** will be **switched on** when there is necessity to **metabolize** a **new substrate**.
- This phenomenon is called **induction**.
- Small molecule responsible for this is known as **inducer.**
- This is **positive control.**

Operon concept

- It is the **transcriptional control** mechanism of gene regualtion.
- **Eg**. In E.coli, when **lactose sugar** is provided to the **culture medium**, cell induces production of three enzymes necessary for **digestion** of lactose.

The enzymes are :

i. B-galactosidase – Digests lactose into galactose and glucose.

ii. B-galactoside permease – Permits lactose molecules to enter into the cell.

iii. Transacetylase -- Transfers an acetyl COoA to galactoside.

→ Synthesis of these three enzymes is controlled by a long segment of DNA known as **Operon**.

*Structure of the Operon

Structural genes &

Control elements(Promotors and regulators)

i- Structural genes – code for protein

ii. Promotor – Signal sequences in DNA that start RNA synthesis and the site to bound RNA polymerase.

iii. Operators – present between the promotors and structural genes.

iv. Repressor protein – Binds to operator region of the operon

v. Regulatory genes – Formation of repressor which interact with operators.

The gene expression depends on whether operator is switched on or switched off.

→Operator switched on – Three genes z, y, and a are transcribed by RNA polymerase into a single m-RNA.

- Each structural gene is known as cistron.
- Transcribed long m-RNA covering various cistrons – Polycistron.
- **Repressor** – *Switching on* or *switching off* of the operator is accomplished by a *protein* called *repressor.*

LAC OPERON

→ Lactose or Lac operon of E.coli is inducible operon .

→ Operon is switched on when a chemical inducer–lactose is present in the medium.

Lac operon consists of following components:

1. Regulator gene (repressor gene)
2. Promoter gene
3. Operator gene
4. Structural genes
5. Inducer- It is not a component of operon.

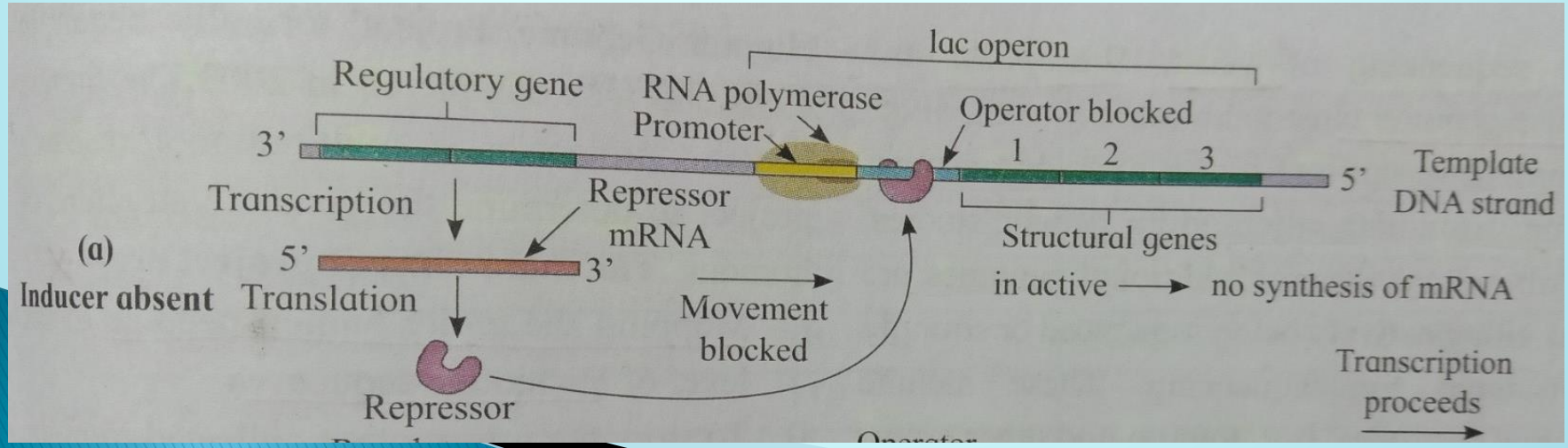
1. **Regulator gene**

→Controls the **operator gene** in cooperation with an **inducer** present in the cytoplasm.

→It **preceeds** the **promoter gene**.

→Regulatory gene produces **protein** called **repressor protein/regulatory protein**.

→**Repressor binds** with **operator gene** and **represses** (stops) its **action.**



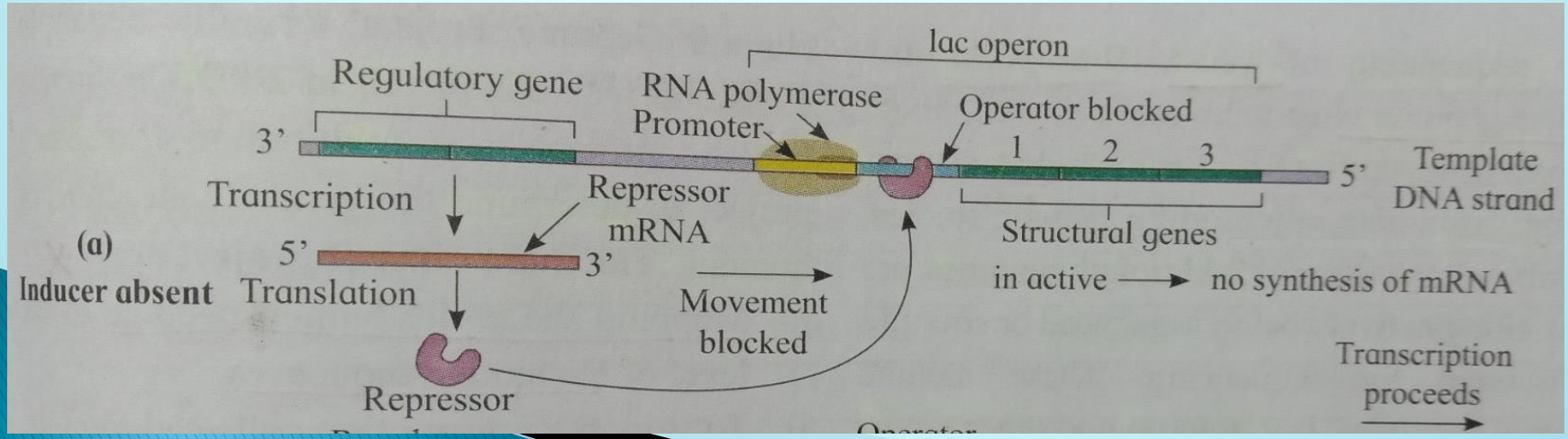
2. Promoter gene.

→Preceeds the **operator gene**.

→Promoter gene marks the **site** at which the **RNA polymerase** enzyme **binds**.

→When operator gets **turned on** the **enzyme moves** over the **operator gene** and **transcription** of **structural genes starts.**

→Promoter gene base sequence determines which strand of **DNA** acts a **template.**



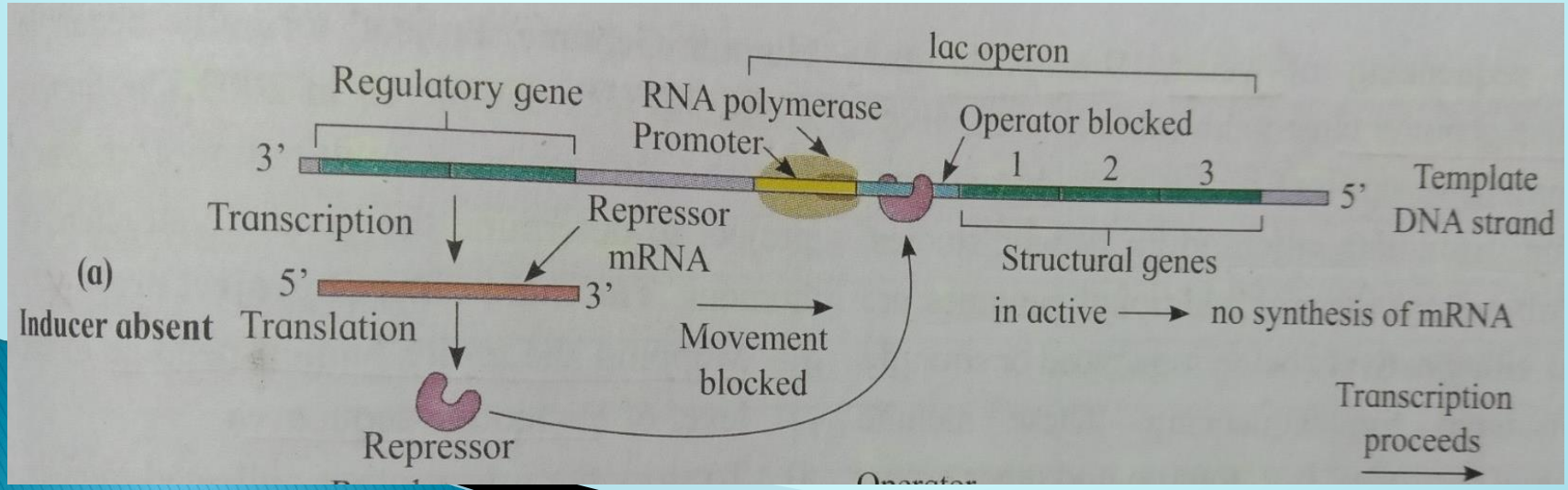
3. Operator gene

→Preceeds the structural gene.

→Controls the functioning of structural genes.

→When operator gene is turned on by an inducer, the structural genes produce m-RNA.

→Operator is turned off by a product of repressor gene.



4. Structural gene

→When **lactose** is added to the **E.coli culture**, the **structural genes** produce **m-RNA** which in turn produces **polypeptides**, on the ribosomes.

→**Polypeptide** formed, act as **enzymes** to **catalyze lactose** in the cell.

→There are structural genes in sequence **lac-Z, lac-Y** and **lac-A.**

→Enzymes produced are **B-galactosidase, B-galactoside permease** and **transacetylase** respectively.

5. Inducer

→It is the chemical in the cytoplasm (allolactose) which **inactivates** the repressor.

→When **lac operon switched on**, then **inducer** joins with **repressor protein** preventing the **binding** of **repressor** to the **operator gene**.

→So the **Operator gene is free** and now enzyme **RNA polymerase** can move from **promoter** to **structural genes** via **operator gene**.

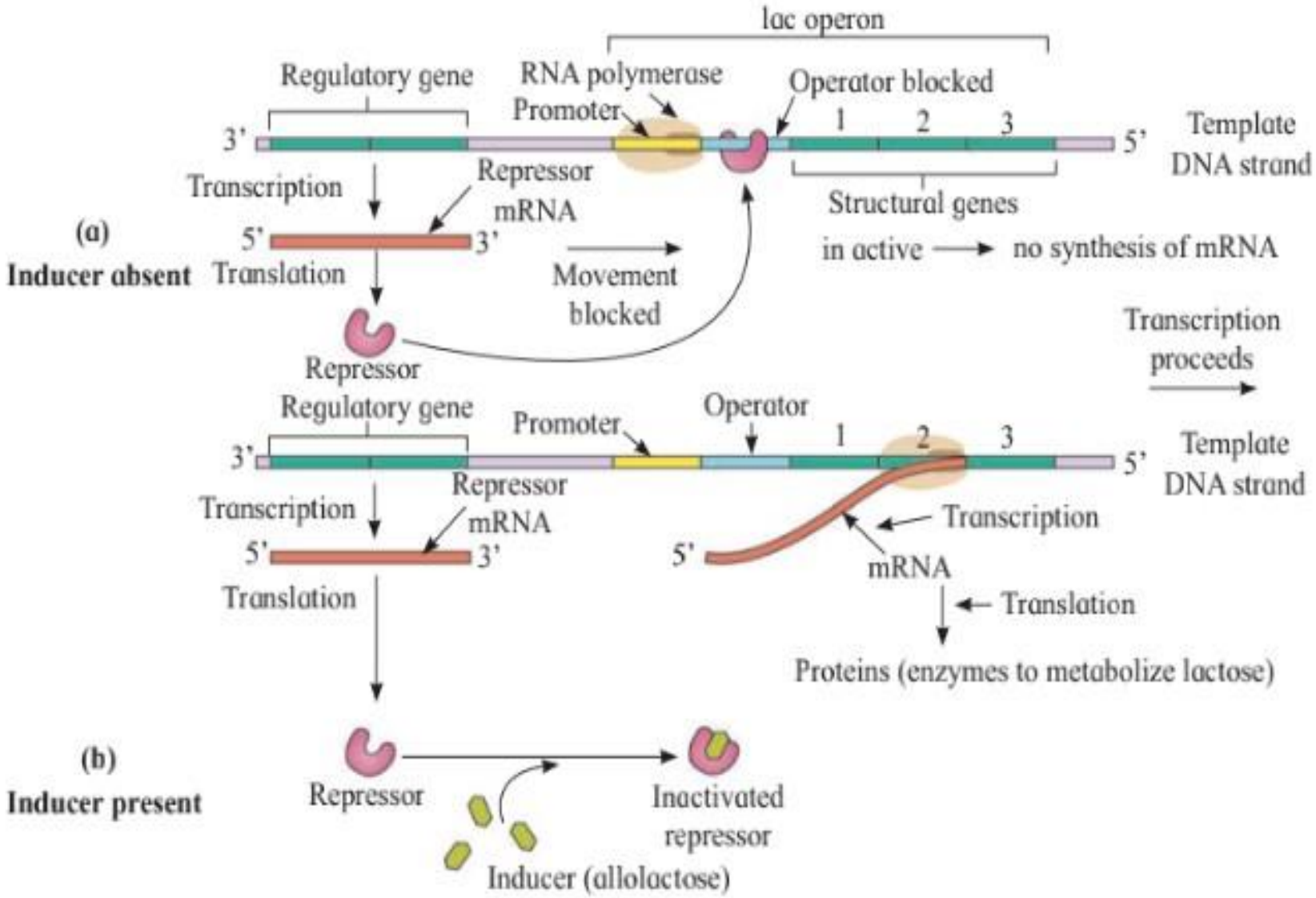


Fig. 4.16 : Working of Lac Operon

ROLE OF LACTOSE

1. Few molecules of **lactose** enter into the **cell** by an enzyme **permease**.
2. A small amount of this enzyme is present even when operon is **switched off.**
3. Few molecules of **lactose**, act as **inducer** and **bind to repressor.**
4. This **repressor-inducer complex** fails to join with the **operator gene**, which is then **turned on**.
5. **Structural gene** produces **all enzymes**.
6. **Lactose** act as the **inducer** of its **own breakdow**n.
7. When the **inducer level falls**, the **operator** is **blocked** again by **repressor**.
8. **Structural genes** are repressed/**inactivated** again. This is **negative feedback**.

GENOMICS

Genomics – By H.Winkler (1920)

Total genetic constitution of an organism.

It is a complete copy of genetic information(DNA) or one complete set of chromosomes of an organism.

Genomics – By T.H. Roderick (1986)

Study of genomics through analysis, sequencing and mapping of genes along with the study of their functions.

- Sequencing of **yeast, Drosophila** and **mouse** genome → facilitate → **comparative studies** → between and **human** and **other organisms**.
- Additional genomes are now either actively being **sequenced** or **strongly considered** for sequencing.
- Include several **microbes, bee, tomato** and other **crops.**

CLASSIFICATION OF GENOMICS

a) **Structural genomics**

It involves mapping, sequencing and analysis of genome.

b) **Functional genomics**

It deals with the study of functions of all gene sequences and their expression in organisms.

Application of Genomics

1. Improvement of **crop plant, human health** and **livestock**.
2. Application in sector like **medicine**, **biotechnology** and **social sciences**.
3. Helps in treatment of **genetic disorders** by **gene therapy.**
4. Used in **agriculture** to develop **transgenic crops** having **desirable characters**.
5. **Genetic markers** used in **forensic analysis**.
6. Introduction of **new gene** in **microbes** to produce **enzymes, therapeutic proteins** and even **biofuels**.

Human Genome Project

- Project initiated in 1990 and completed in 2003.
- Under administration of – HUMAN GENOME ORGANIZATION (HUGO)
- Project co-ordinated by – **US department of Energy and National institute of health.**
- **Multinational research project** to determine the **genomic structure** of **humans**.

The main aims of project are :

1. **Mapping** the entire **human genome** at the level of **nucleotide sequences**.
2. To store the information collected from the project in **database**.
3. To develop **tools** and **techniques** for analysis of the data.
4. Transfer of the related technologies to the **private sectors**. Such as **industries**.
5. Taking care of the **legal, ethical** and **social issues** which may arise from project.

- HGP–Human genome project associated with new area bioinformatics.
- Helps to understands the Blueprint in building and constructing the human genome.
- Study will help in field of medicine, Biotechnology and Life sciences.
- Therefore HGP is very important.
- Provides complete and accurate sequence of the 3 billion DNA base pairs that make up the human genome.
- Help us to estimate total number of human gene.
- About 33000 genes have been estimated to be present in humans.

The project also aimed to sequence the genome of

Table 4.17 : Comparative genome sizes of humans and other models organisms.

Organism	Chromosome number	Estimated gene number	Estimated size (base pairs)
Human (*Homo sapiens*)	46	33,000	3 billion
Mouse (*Mus musculus*)	40	25,000	2.9 billion
Fruit fly (*Drosophila melanogaster*)	8	13,000	165 million
Plant (*Arabidopsis thaliana*)	10	25,000	157 million
Roundworm (*Caenorhabditis elegans*)	12	19,000	97 million
Yeast (*Saccharomyces cerevisiae*)	32	6000	12 million
Bacteria (*Escherichia coli*)	1*	4400	4.6 million

DNA FINGERPRINTING

- **Genes** on **chromosomes** are responsible for determining **characters** of organisms and as well as **inheritance**.
- Due to **recombination** of paternal and maternal genes, we **differ** from our parents.
- Differences also arise due to **infrequent mutations** that occur during **gamete formation**.
- So, every individual has its own **unique genetic make-up** called **fingerprint.**
- *Technique developed to identify a person with the help of DNA restriction analysis is known as DNA profiling or DNA fingerprinting.*
- **Dr. Alec Jeffreys** -- 1984

- **DNA fingerprinting technique**– Based on identification of **nucleotide** present in **DNA**.
- **99.9%** of **nucleotide** sequence in all persons, is **same**.
- Only some **short sequences** of **nucleotides** differ from **person to person**.
- Every person shows unusual sequences of **20–100 base pairs** which are **repeated several times** and is termed as **Variable Number of Tandem Repeats** (VNTRs).
- **Length** of the regions having **VNTRs** is **different** in each individual and hence is the **key factor** in **DNA profiling.**

Steps Involved In DNA fingerprinting

1. Isolation of DNA

- DNA must be recovered from the cells or tissues of the body(host) .
- Only small amount of tissue.

2. Restriction digestion

→ Isolated DNA is treated with restriction enzymes.

- Enzymes cut the DNA into small fragments having variable lengths.
- Phenomenon is called Restriction Fragment Length Polymorphism.(RFLP)

3. Gel Electrophoresis

→DNA samples are loaded for agarose gel electrophoresis under an electric influence.

→DNA fragments which are negatively charged move to the positive pole.

→Movements of these fragments depends on length of the fragments.

- This results in formation of bands.

  dsDNA splits into ssDNA by alkali treatment.

4. Southern blotting

→Separated DNA fragments are transferred to a nylon membrane or a nitrocellulose filter paper by placing it over the gel and soaking them with filter paper overnight.

5. Selection of DNA probe

→A known sequence of **single-stranded DNA** is prepared.

→It is called **DNA probe** is obtained from **organisms** or prepared by **cDNA** preparation method.

→DNA probe is labelled with **radioactive isotopes.**

6. Hybridization

→**Probe DNA** is added to the **nitrocellulose filter paper** containing **host DNA**.

→Single stranded **DNA probe pairs** with the **host DNA**.

→As result **DNA-DNA hybrids** are formed on the nitrocellulose filter paper.

→**Remaining** single stranded DNA probe fragments are **washed off**.

7. Photography

→Nitrocellulose filter is photographed on an X-ray film by autoradiography.

→Film is analyzed to determine the presence of hybrid DNA.

Application of DNA fingerprinting

1. Forensic science – Solve problems of rape and some complicated murder cases.
2. To find out the biological father or mother or both, of the child, in case of disputed parentage.
3. Used in pedigree analysis in cats, dogs, horses and humans.

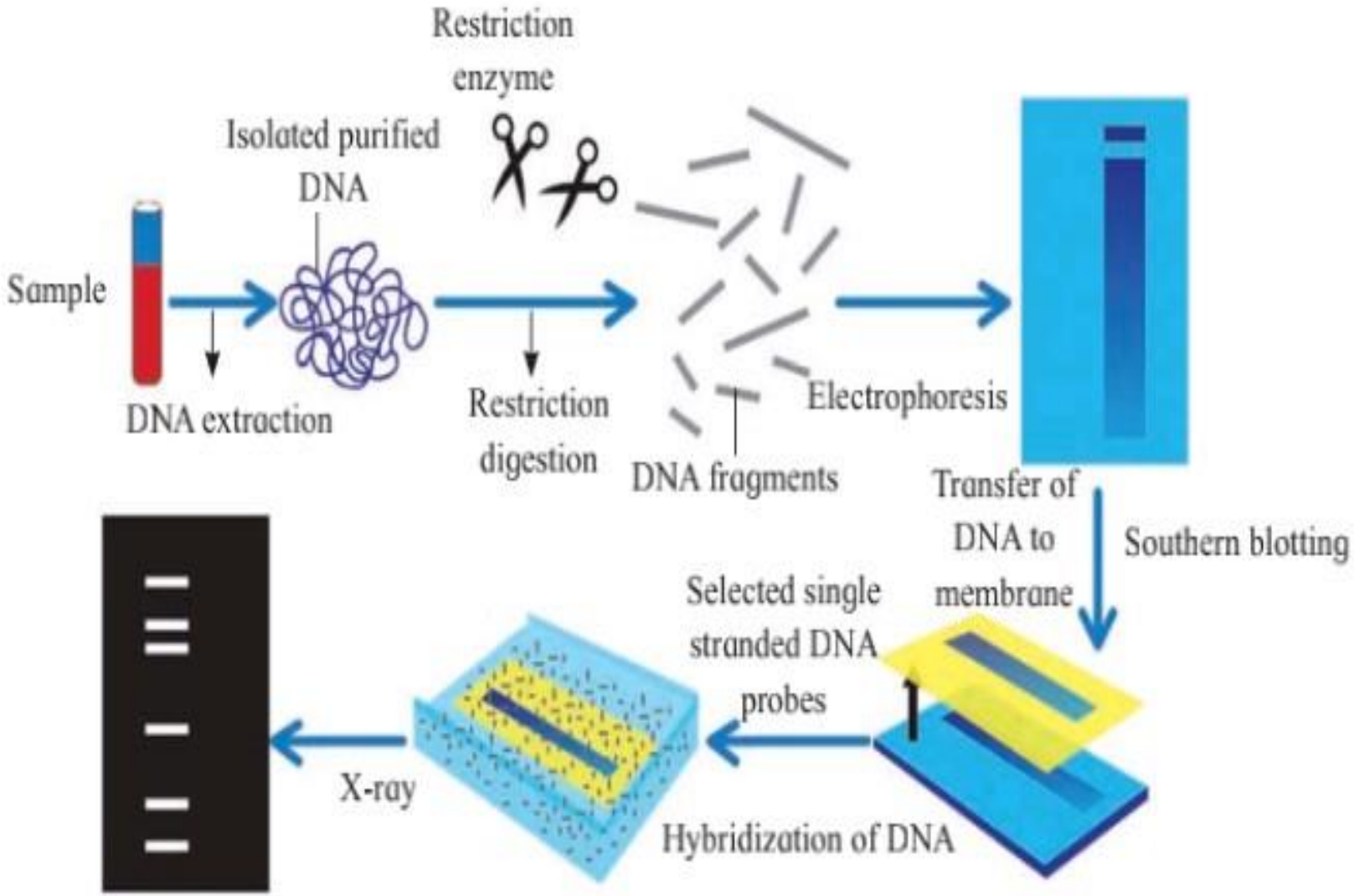
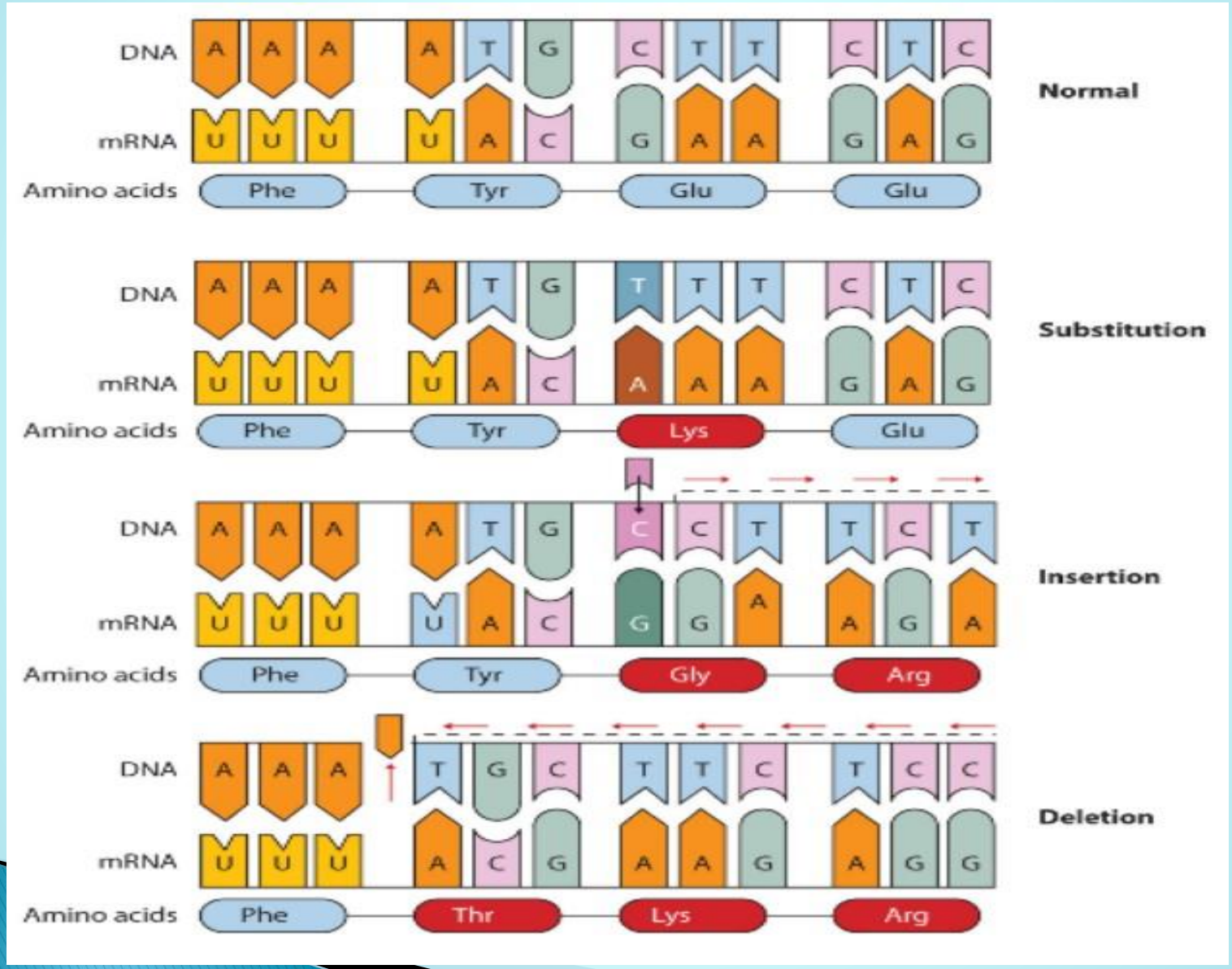


Fig. 4.18 : DNA Fingerprinting


DNA
mRNA
Amino acids
Normal
Phe
Tyr
Glu
Glu
Substitution
Lys
Insertion
Gly
Arg
Deletion
Thr

THANK YOU