6.4 Replication :→ (Duplication)

2C amt ⟶ 4C amt

✓ Replication is a duplication process

* Watson & crick had immediately proposed a scheme of DNA replication while proposing the double helical structure of DNA (1953)

To back their original statement that is as follows:

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for genetic material" (watson and crick, 1953).

* The scheme suggested that the two strands would separate and act as a templat for synthesis of new complementary strands. After completion of replication, each DNA molecule would have one parental and one newly synthesized strand. This scheme was termed as semiconservative DNA replication.

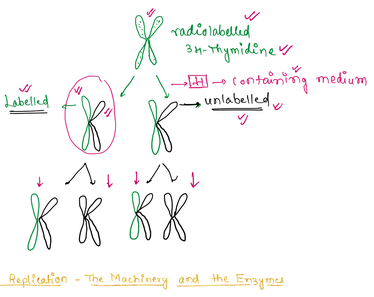
⇒ The Experimental Proof :→ It is now proven that DNA replicates semiconservatively.

Experiment 1 :-

* It was first shown in E.Coli (Bacteria) and subsequently in higher organism such as plant and human cells. (1958)
* Matthew Meselson and franklin Stahl performed experiment using heavy nitrogen (^{15}N) in E.Coli.
* They grew E. coli in a medium containing $^{15}NH_4Cl$ as the only source of nitrogen for many generations.
* the result was that ^{15}N was incorporated into newly synthesized DNA (as well as other nitrogen containing compounds)
* This heavy DNA molecule could be distinguished from the normal DNA by centrifugation in a CsCl density gradient. (we use gravitational force)
* Then they transferred the cells into a medium with normal $^{14}NH_4Cl$ and took sample at various definite time intervals as the cells multiplied, and extract the DNA that remained as double stranded helices. The various samples were separated independently on CsCl gradients to measure the densities of DNA.
* Thus, the DNA that was extracted from culture one generation after the transfer from ^{15}N to ^{14}N medium [that is after 20min: E.Coli divide in 20min] had a hybrid or intermediate density.
* DNA extracted from the culture after another generation [i.e. after 40 min, II generation] was composed of equal amounts of this hybrid DNA and of light DNA.
* Increase in the amount of light DNA and less in hybrid DNA amount can be possible due to semiconservative mode of replication.

If E.Coli was allowed to grow for 80min. then proportions of light and hybrid densities DNA molecule are 7:1 (IV gen; Light:Hybrid 14:2)

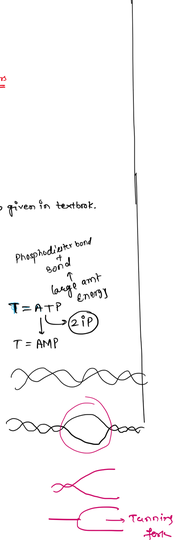
Experiment 2 : chromosome Replication is semiconservative "Taylor" (1958), have proved semiconservative mode of chromosome replication in eukaryotes using radioactive tritiated thymidine (^3H-thymidine) in Vicia faba (faba beans)



DNA Replication - The Machinery and the Enzymes

* Large number of enzymes are required for DNA replication.
* The main enzyme is DNA dependent DNA polymerase, since it uses a DNA templat to catalyse the polymerization of deoxynucleotides.
* DNA polymerase was discovered by Kornberg (1959)
* Prokaryotes (eg E.coli) have 3 main DNA polymerases
  - → DNA polymerase I, II and III
  - → Polymerase III is main enzyme responsible for DNA replication in E.coli.
  - → Polymerase I (via kornberg enzyme) & polymeraseII function primarily in DNA repair
* Eukaryotes have 5 types of DNA polymerases
  - α → initiation of replication
  - β → DNA repair
  - γ → mitochondrial DNA repair
  - δ → replication activity
  - ε → polymerase activity of the La cells.
* DNA ligase :- Joins newly synthesized fragments of DNA. Discovered by Khorana (1967)
* Other enzyme required are Primase, Topoisomerase and Helicase, etc.
* These enzymes are highly efficient enzymes as they have to catalyse polymerization of a large number of nucleotides in a very short time. [Speed + Accuracy]
* E.coli that has 4.6×10^6 B.P. completes replication process with in 18 minutes; that means the average rate of polymerization has to be approximately 4500 bp per second. (2000 given in textbook.)
* Not only they polymerise fast, but they also have to catalyse the reaction with high degree of accuracy.
* Any mistake during replication results into mutation.
* Furthermore, energetically replication is very expensive process. (Large amt energy is required) (monomers)
* Deoxyribonucleoside triphosphates serves dual purposes. In addition to acting as substrates, they provide energy for polymerization reactions (the two terminal phosphates in deoxyribonucleoside triphosphates are high energy phosphate)
* DNA cannot be separated in its entire length (due to very high energy requirement), the replication occur within a small opening of DNA helix, refred to as replication fork. (Y-shape)
* Replication in eukaryote occurs in the nucleus during S-phase of cell cycle.
* In prokaryotes, replication takes place in cytoplasm and occur prior to binary fission.
* DNA replication is a complex multistep process (E.coli)

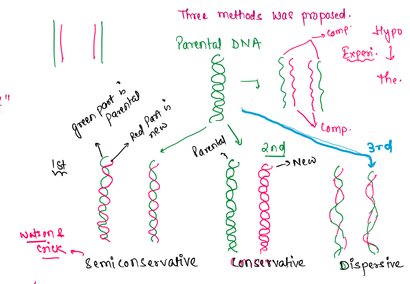
1. ori → origin of Replication
   - Replication begins at a particular spot called ori.
   - Bacteria & viral DNA has single ori
   - In Eukaryote multiple ori.
   - In Absence of it there is no replication
2. Activation of deoxyribonucleotides.
   - Deoxyribonucleotides occur freely in nucleoplasm. (at cytoplasm: dATP, dTTP, dGTP, dCTP)
   - all monophosphate + $H_4P_2O_7$ (Phosphorylase) → Pyrophosphate
   - They Provide Energy as well as act as substrate.
3. Exposure of parent DNA strands (unwinding of ds DNA)
   - Helicase (unwindase) unwind DNA at ori by breaking of H-bond. It finally forms Y-shape replication fork.
   - SSB → Stabilize single strand and Topoisomerase relax tension. (In Prokaryote DNA gyrase has topoisomerase activity)
4. Formation of Primer (RNA primer)
   - at free 3' end small strand of RNA is synthesized by using Primase enzyme. RNA Primer 4-12 nucleotide long. It provide 3-OH grp. for joining of DNA nucleotide.
5. Base Pairing
   - Enzyme Pyrophosphatase removes two phosphate.
   - released energy used to form covalent bond & H-bond
6. New Strand Formation.
   - Leading → (continuous) leading strand
   - Lagging → Okazaki fragments (DNA fragments) → lagging strand 1000-2000 bp in prokaryote; 100-200 bp in Eukaryotes.
   - DNA formation in semi discontinuous manner. as well as bidirectional
   - DNA ligase join okazaki fragments (discovered by Khorana)
   - RNA primers are removed by DNA poly I
7. Proof reading → DNA polymerase I



** Process of Transcription & Replication → "Complementary base pairing"

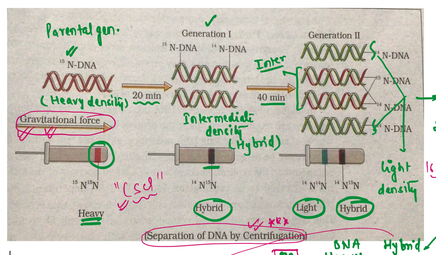
** Three Proposed Mechanism for DNA Replication **

* (1) conservative
* (2) semi conservative → "exp. proof"
* (3) Dispersive



- Meselson & Stahl
- first prokaryotes, Bacteria, E.coli
- NH_4Cl (source of nitrogen)
- use heavy & light isotopes of N (Not radioactive)

(Heavy) N^{15} N^{14} (Light)



8 ds DNA → Gen III → 2 band → Ids DNA Gen IV → 2 bands

no.	Heavy DNA	Hybrid DNA	Light DNA
	0	2	14
ratio	0	1	7

N^{14} N^{15}

First Exp ⟶ E.coli (Bacteria) = Prokaryote

Meselson & Stahl

Heavy = N^{15}; Light = N^{14} → They are not radioactive

$^{15}NH_4Cl$ as source of nitrogen

E.coli ⟶ compound which contain nitrogen → Incorporated with ^{15}N including nucleotides.

E.coli (N^{15}) → ds DNA with N^{15} in nucleotide

$^{14}NH_4Cl$, medium, 20 min

E.coli (N^{15} N^{14}) (1 band) (1st gener.) we have to Extract ds DNA from E.coli

20min $^{14}NH_4Cl$ (2nd gen) → 2 intermediate, 2 Light

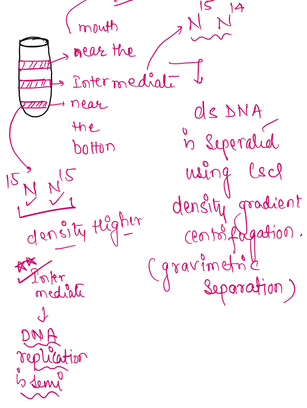
20min $^{14}NH_4Cl$ 3rd gen. → 4 intermediate, 6 Light

20min $^{14}NH_4Cl$ 4th gen. → 2 Light

allow settle down in CsCl density gradient centrifugation

2 bands

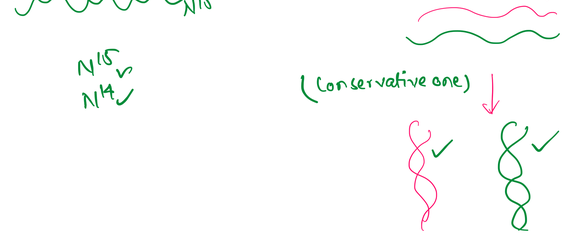
3rd gen → 2 band



If the DNA replication was conservative

How many bands appear in 1st gen → 2 band

1 Heavy 1 Light



Replication of DNA → Prokaryotes (E.Coli)

ds circular DNA

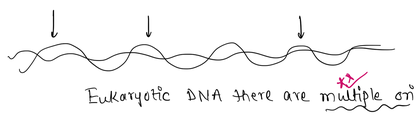
Portion from which Replication start. ds DNA 4.6×10^6 Pair

ORI

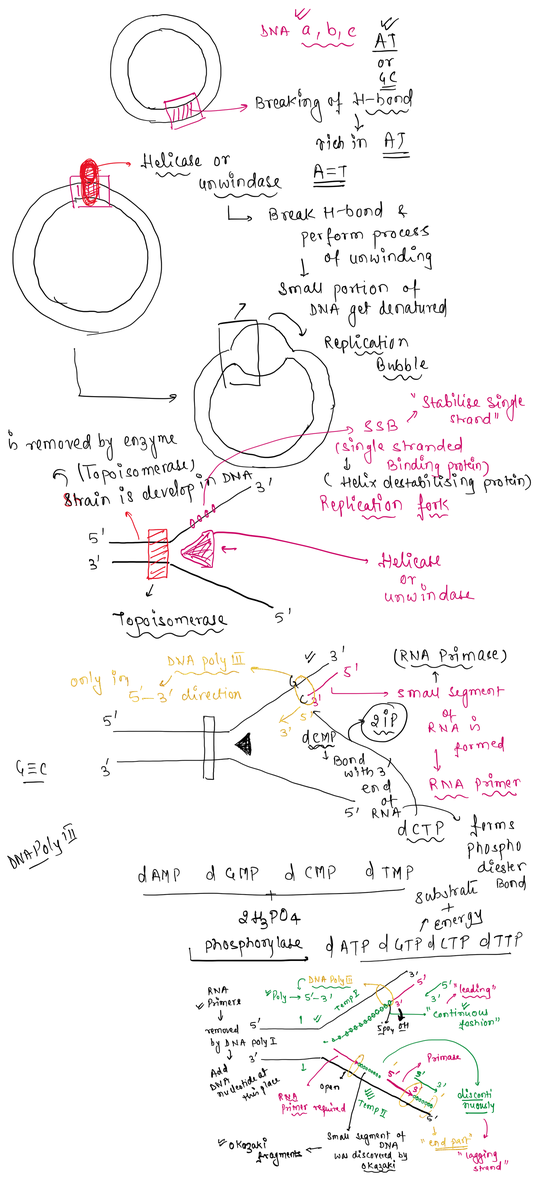
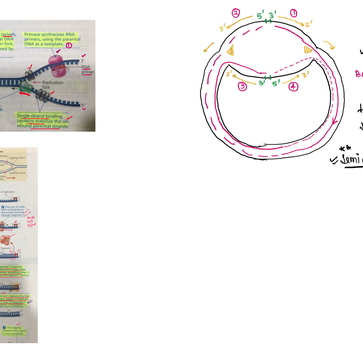
ORI → origin of Replication

ORI → If ori is absent from DNA → There will be no replication

* Bacterial & viral DNA → small in size → they posses single ori



Eukaryotic DNA there are multiple ori

* Plasmid (1 — 100) → ori → Self replicating unit
  - ori act as origin of replication
  - also decide the copy number of plasmid

In case of E.coli