DNA REPLICATION.

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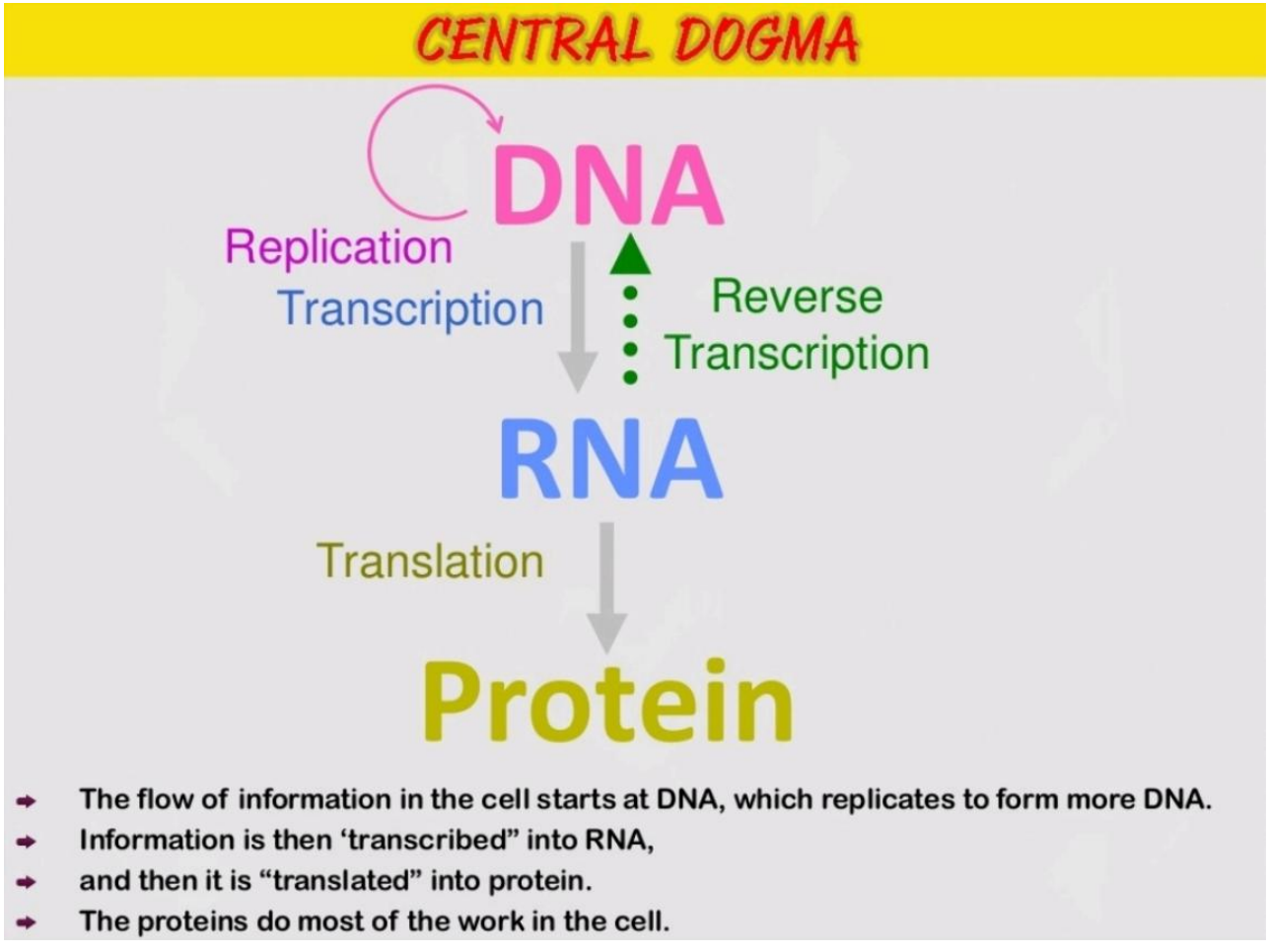
DNA replication is the process of synthesis of an identical duplicate copy of DNA from an existing DNA molecule.

CENTRAL DOGMA.

- REPLICATION- synthesis of daughter DNA from parental DNA.
- Transcription—synthesis of RNA using DNA as the template.
- Translation---protein synthesis using mRNA molecules as the template.
- Reverse transcription—synthesis of DNA using RNA as the template.

CENTRAL DOGMA



DNA REPLICATION.

- A reaction in which daughter DNAs are synthesized using the parental DNAs as the template.
- Transferring the genetic information to the descendant generation with a high fidelity.
- DNA replication is a series of 3’-5’ phosphodiester bond formation catalysed by a group of enzymes.

DNA replication system.

- Template—double stranded DNA.
- Substrate—dNTP.
- Primer------short DNA fragment with a free –OH end.
- Enzymes---DNA dependent poymerase(DDDP) and protein factor.

CHARACTERISTICS OF REPLICATION.

- Semi-conservative replication.
- Bidirectional replication.
- Semi continuous replication.
- High fidelity.

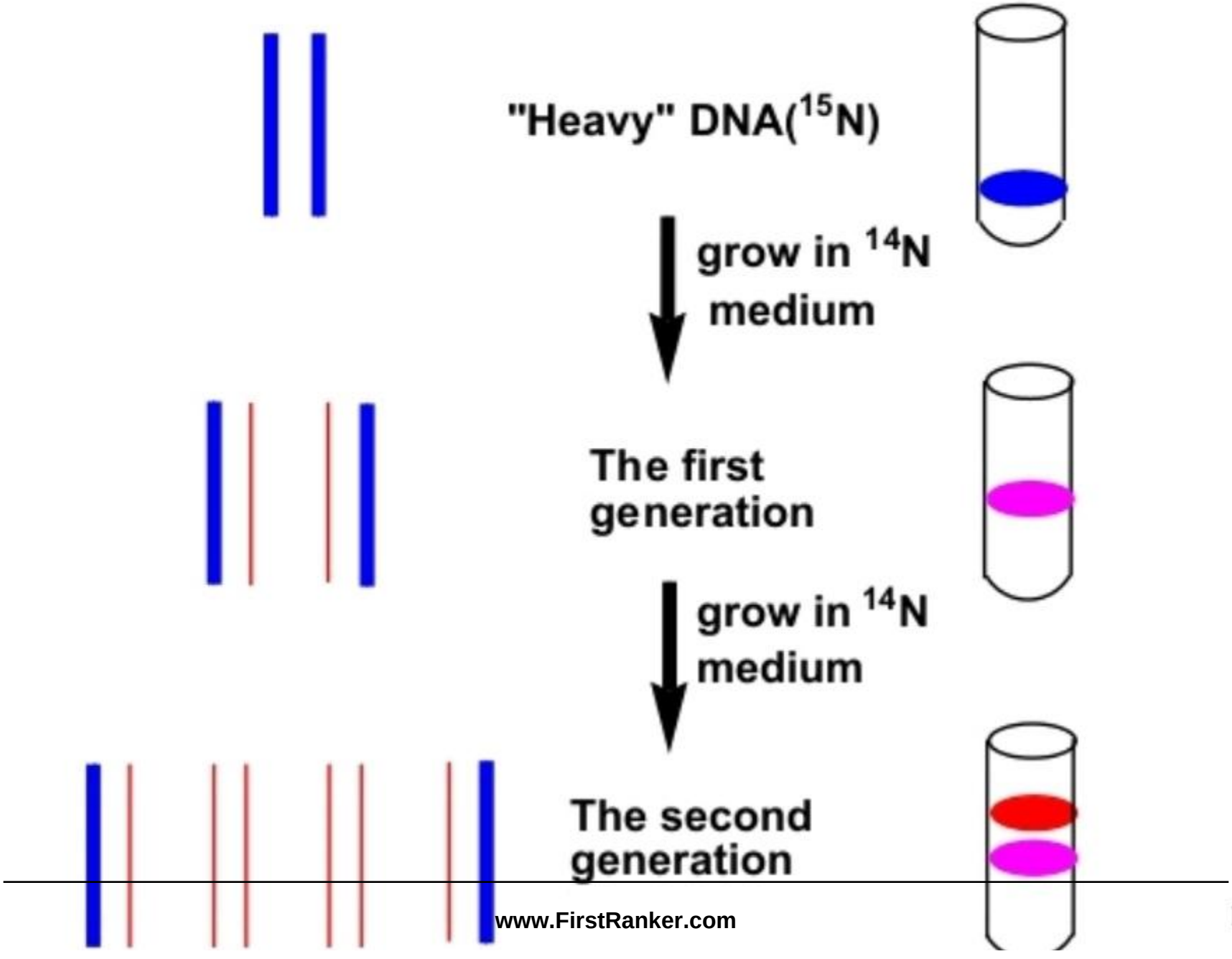
Semi -conservative replication.

- *Half of the DNA molecule is conserved in each newly double helix, paired with a newly synthesized complementary strand.This is called semi conservative replication.*
- *SIGNIFICANCE—The genetic information is ensured to be transferred from one generation to the next generation with a high fidelity.*
- **MESELSON AND STAHL EXPERIMENT-**
- *semi -conservative replication was proposed by Watson and Crick.*
- *This experiment provided the proof of semi-conservative replication of DNA.*

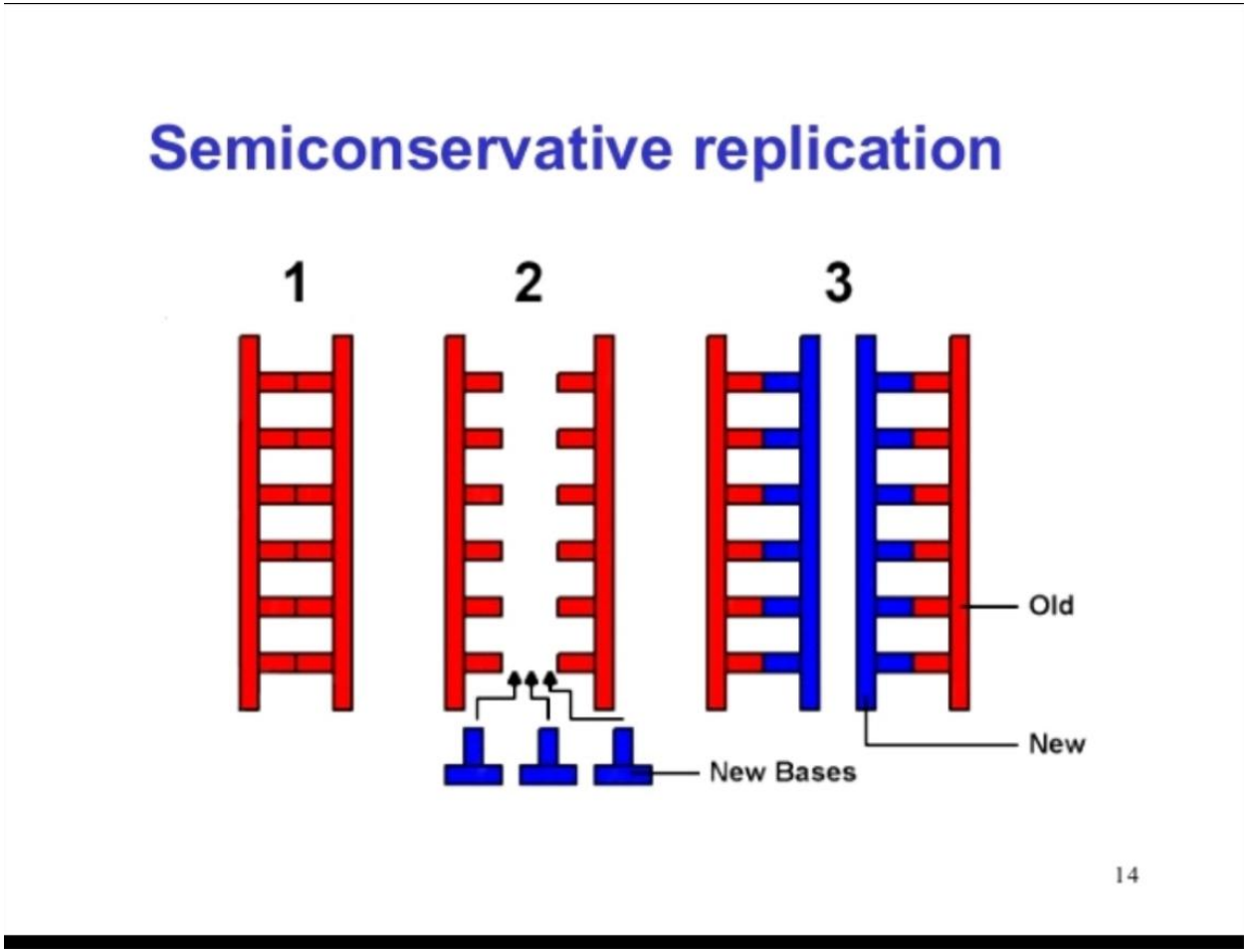
Meselson – Stahl Experiment.

- Bacteria were grown in medium containing the heavy isotopes of nitrogen N15 when all the DNA was labeled with heavy nitrogen. These cells were allowed to divide in a medium containing normal nitrogen N14. In the first generation , all DNA molecule were half labeled.
- In the second generation half labeled and completely unlabeled molecule were present in equal numbers.
- From this experiment , it was prooved that DNA replication is semiconservative.

Experiment of DNA semiconservative replication



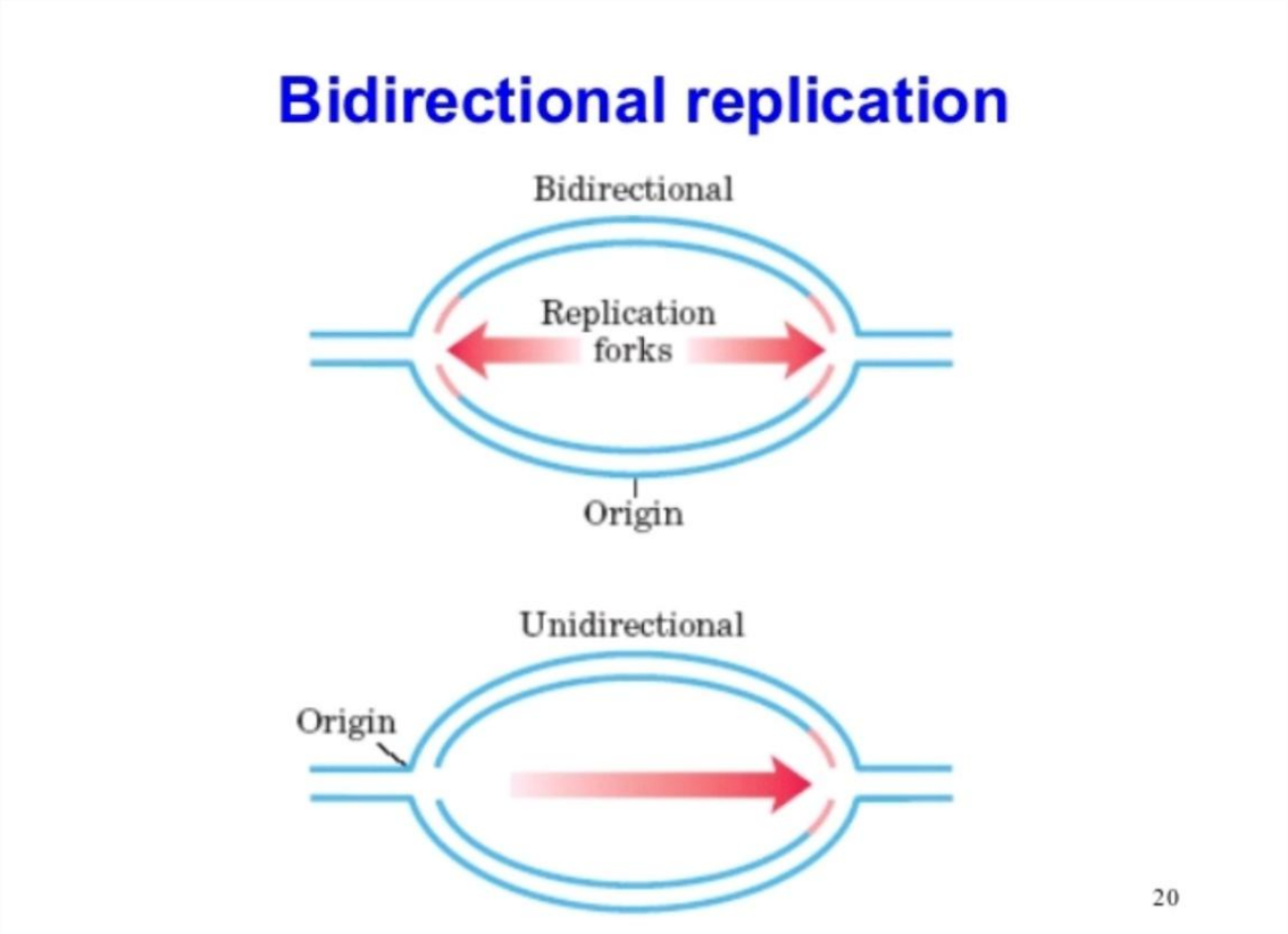
SEMICONSERVATIVE REPLICATION.



BIDIRECTIONAL REPLICATION.

- Replication starts from unbinding the dsDNA at a particular point (called origin), followed by synthesis on each strand .
- The parental dsDNA and two newly formed dsDNA form a Y – shaped strucure called replication fork.
- Once the dsDNA is opened at the origin , two replication forks move in opposite direction as the synthesis continue.

BIDIRECTIONAL REPLICATION.



SEMICONTINUOUS REPLICATION

- The daughter strands on two template strands are synthesized differently since the replication process obeys the principle that DNA is synthesized from that 5’ end to the 3’ end.

LEADIND STRAND.

- On the template having the 3’ – end , the daughter strand is synthesized continuously in the 5’- 3’ direction. This strand is referred to as the leading strand.

Okazaki Fragments.

- Many DNA fragments are synthesized sequentially on the DNA template strand having the 5’- end. These DNA fragments are called okazaki fragments.
- The daughter strand consisting of okazaki fragment is called lagging strand.

SEMI-CONTINUOUS REPLICATION.

- Continuous synthesis of the leading strand and discontinuous synthesis of the lagging strand represent a unique feature of DNA replication . It is referred to as the semi – continuous replication.

ENZYMES AND PROTEIN FACTOR.

Dna A protein	Recognise origin.
DnaB protein	Open dsDNA
DnaC protein	Assist DnaB binding.
DNA pol	Elongate the DNA strand.
Dna G protein	Synthesized RNA primer.
SSB/DNA topoisomerase	Single strand binding/release supercoil constrant.

DNA topoisomerase. (prokaryotes)

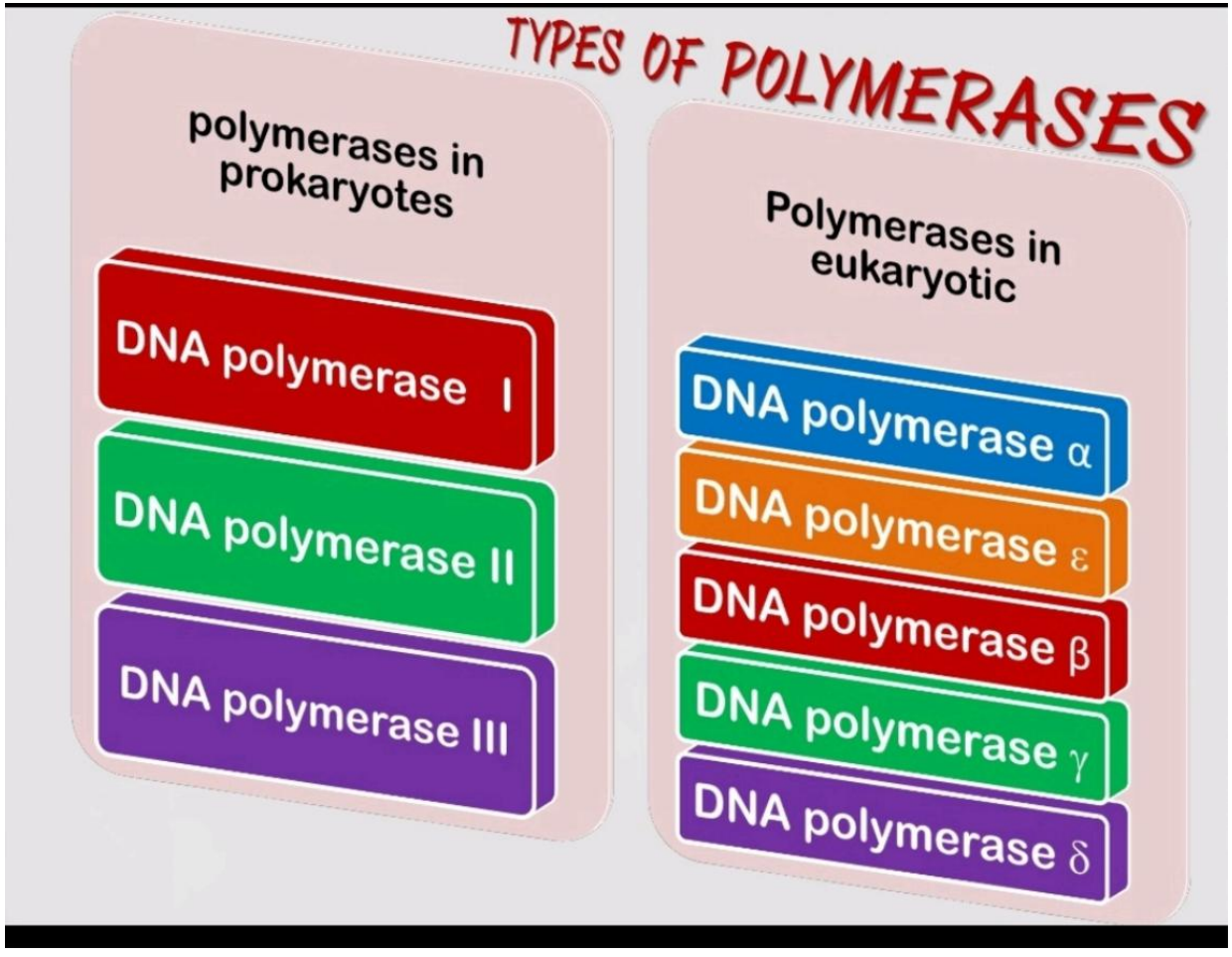
- DNA- pol I, DNA pol II, DNA pol III were identified in experiment using mutated E. Coli cell line.

- All of them possess following biological activity..
- 5’-3 polymerizing.
- Exonuclease.

DNA POLYMERASE.

DNA-pol I	**Proofreading, filling the gaps, repairing DNA damaze.**
DNA-pol II	DNA repairing
DNA pol III	Elongation process.

POLYMERASES.



DNA –pol of eukaryotes

- DNA – pol alpha—initiate replication and synthesize primers.---DnaG, primase.
- DNA – pol beta—replication with low fidelity.-repairing.
- DNA-pol gamma—polymerization in mitochondria.
- DNA –pol delta—elongation—DNA-pol III.
- DNA-pol epsilon– proofreading and filling gap.

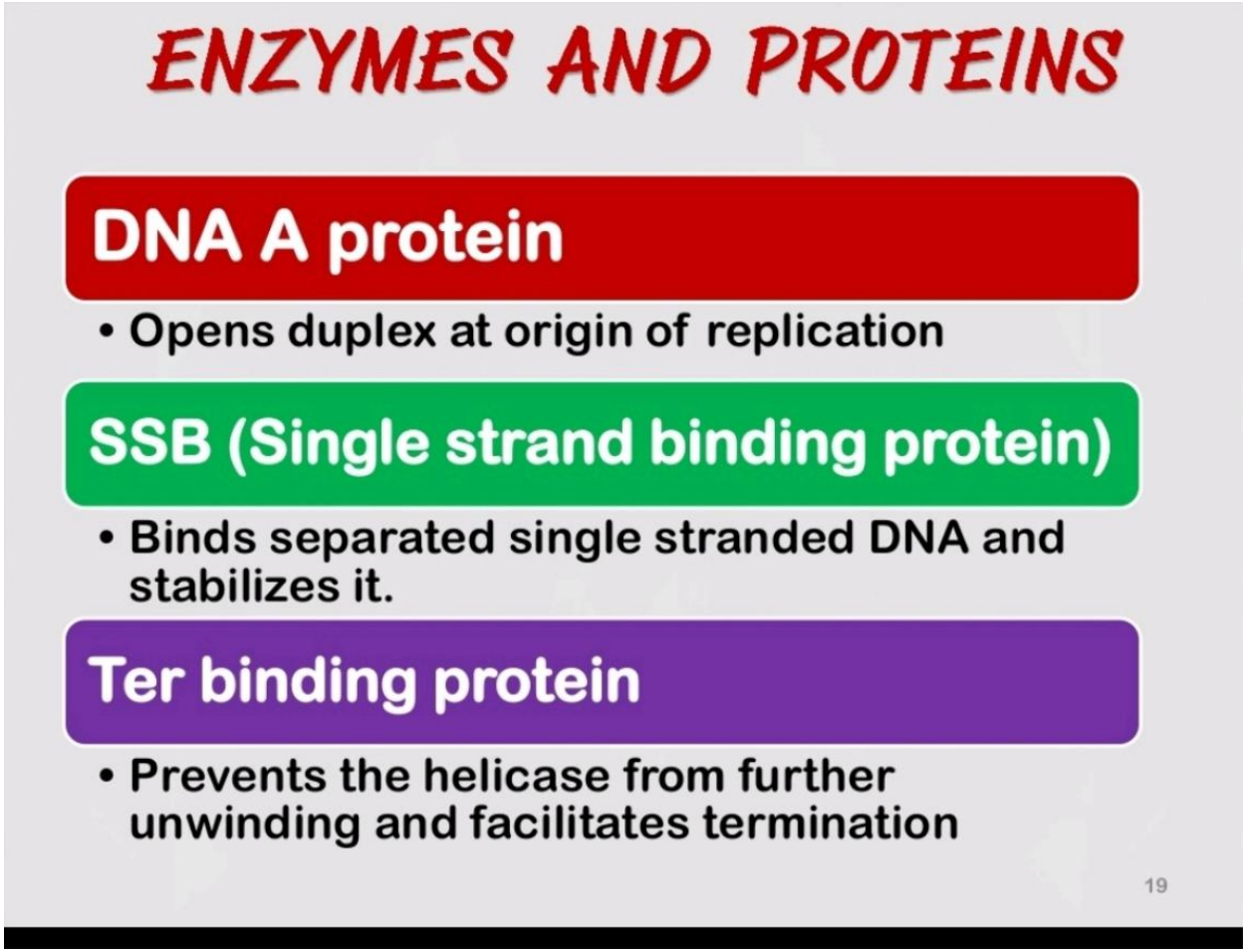
PRIMASE

- Also called DnaG.
- Primase is the able to synthesize primers using free NTPs as the substrate and the ssDNA as the template.
- Primers are short RNA fragments of a several decade of nucleotides long.
  primase privide free 3’-OH groups to react with the alpha –P atom of the dNTP to form phosphodiester bond.

PRIMOSOME COMPLEX.

- Primase , DnaB, DnaC and origin form a primosome complex at the initiation phase.
- HELICASE—
- Also referred as Dna B.
- Opens the double stranded DNA.
- SSB protein---
- maintain the DNA template in the single strand form in order to prevent ds DNA formation.

ENZYMES AND PROTEIN.



TOPOISOMERASE

- Opening the dsDNA will create supercoil ahead of replicate fork.
- Supercoil constraint needs to be released by topoisomerases.
- The interconversion of topoisomers of dsDNA is catalysed by a topoisomerase in a three step process-
- Cleavage of one or both strand of DNA.
- Passage of a segment of DNA through the break.
- Resealing of the DNA break.

Topo I

- It cuts a phosphoester bond on one DNA strand, rotate the broken DNA freely around the other strand to relax the constraint ,and release the cut.

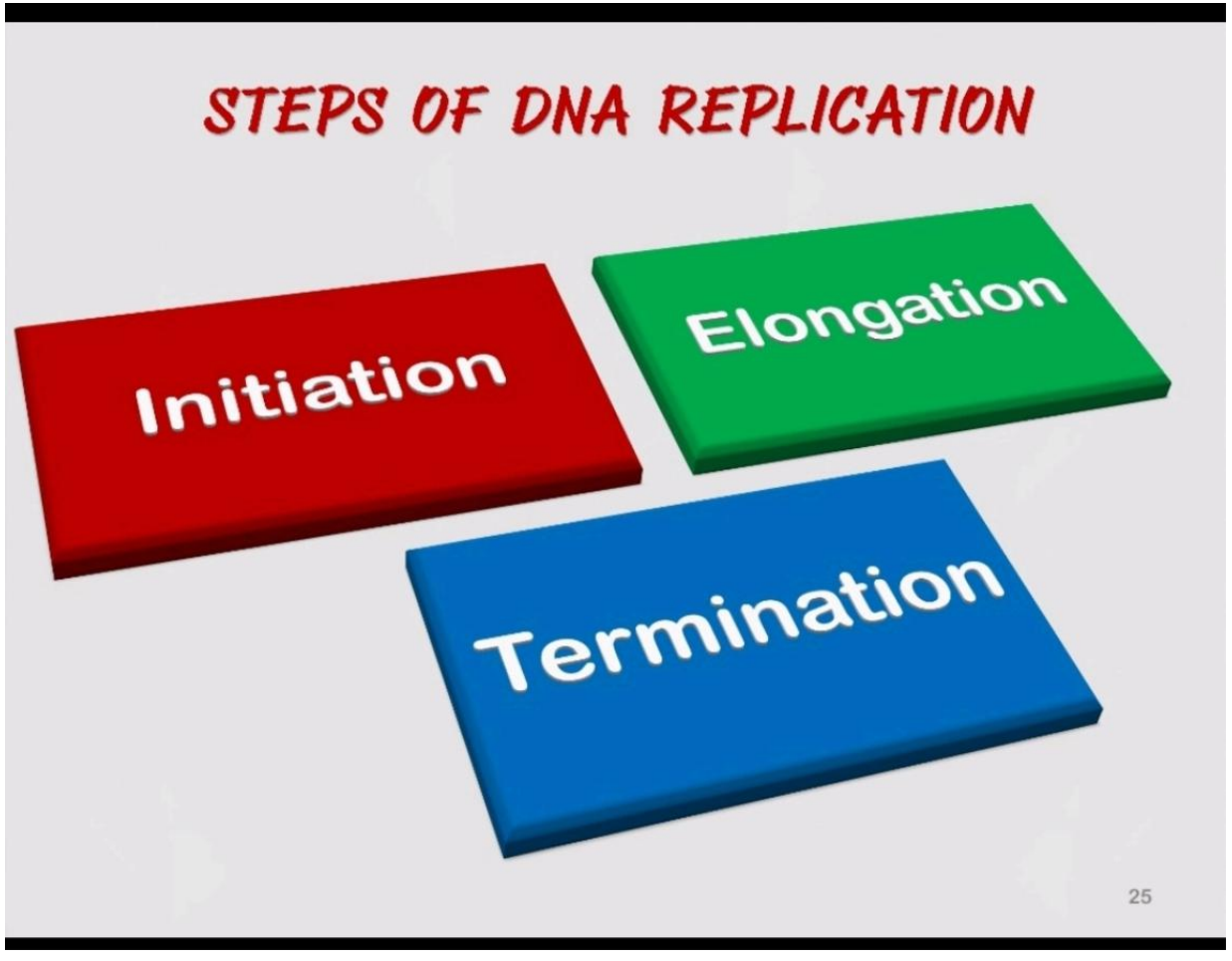
Topoisomerase II

- It is named gyrase in prokaryotes.
- It cuts phosphodiester bonds on both strands of dsDNA ,release the supercoil constrant, and reforms the phosphoester bonds.
- DNA Ligase----
- Sealing the nick in the process of replication, repairing, recombination and splicing.

DNA REPLICATION.

- INITIATION-
- recognise the starting point , separate dsDNA, primer synthesis.
- ELONGATION–
- add dNTP to the existing strand, form phosphodiester bond, correct mismatch bases, extending DNA strand,
- TERMINATION—stop the replication.

DNA REPLICATION.



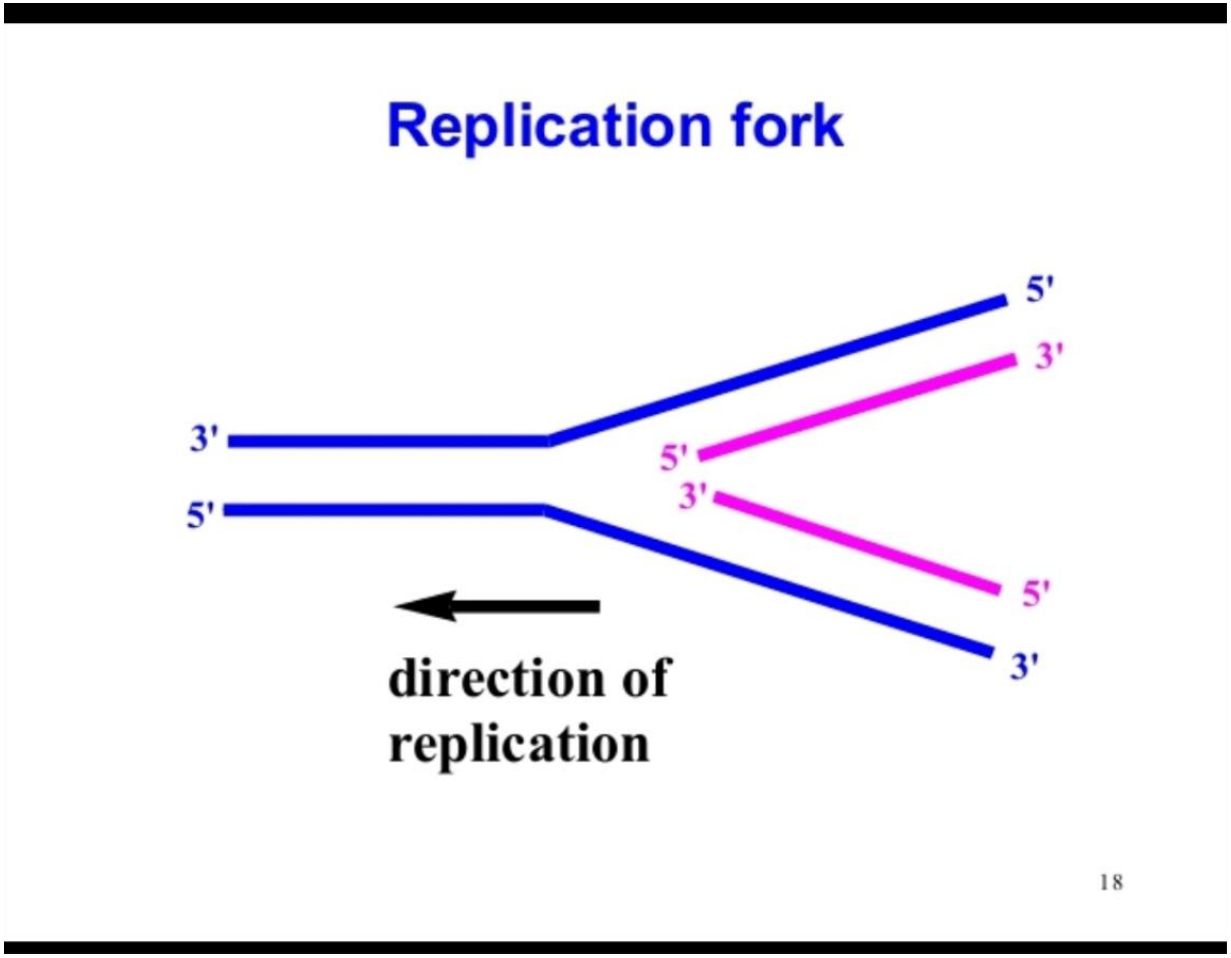
Replication of prokaryotes.

- INITIATION--
- The replication starts at a particular point called origin.
- The structure of the origin is 248 bp long and AT-rich.

Formation of replication fork.

- DnaA recognise ori C.
- DnaB and DnaC join the DNA-DnaA complex , open the local AT-rich region, andmove on the template downstream further to separate enough space.
- DnaA is replaced gradually.
- SSB protein binds the complex to stabilise ssDNA.

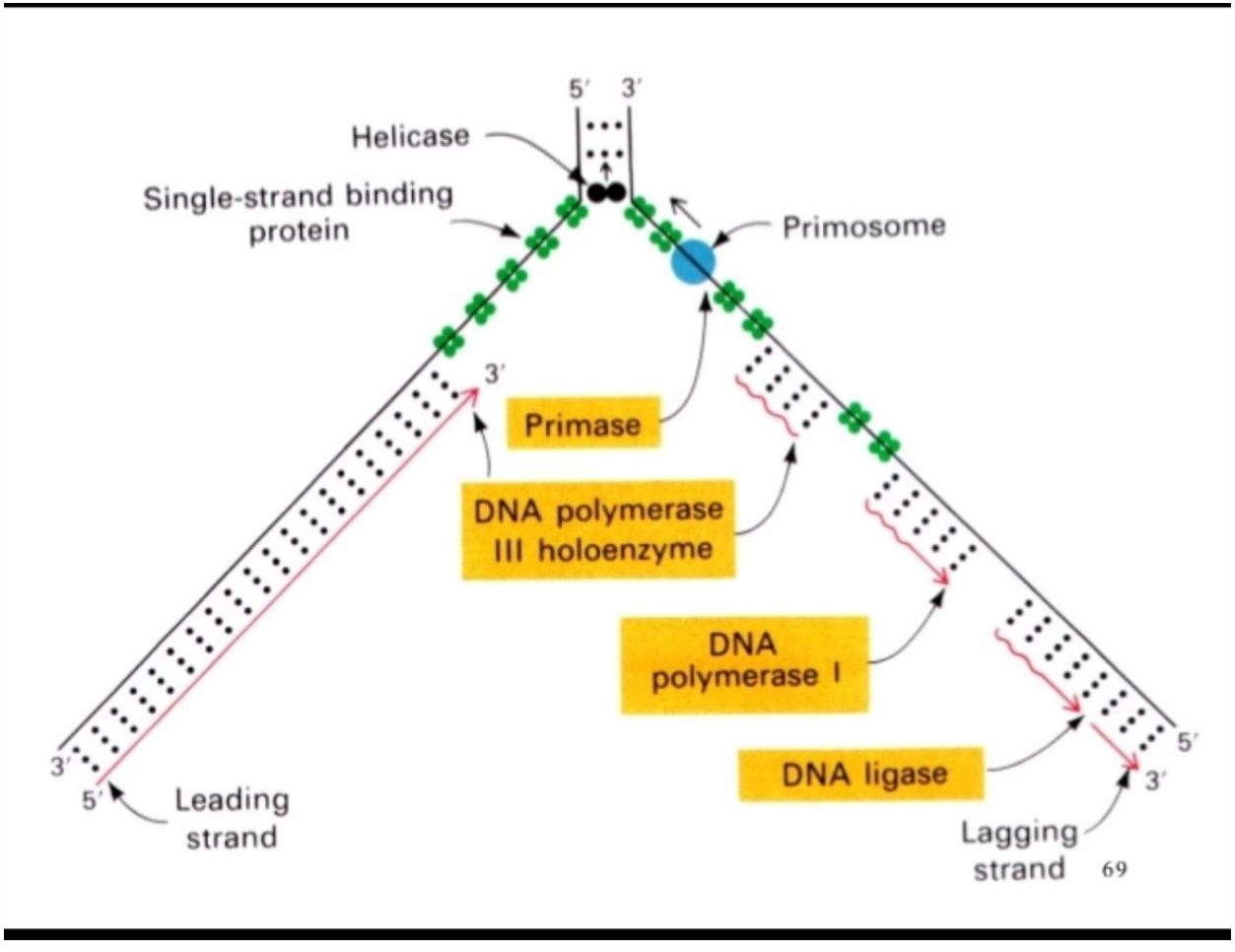
Replication fork.



Primer synthesis.

- Primase joins and forms a complex called primosome.
- Primase starts the synthesis of primers on the ssDNA template using NTP as the substrate in the 5’-3’ direction at the expense of ATP.

DNA REPLICATION.



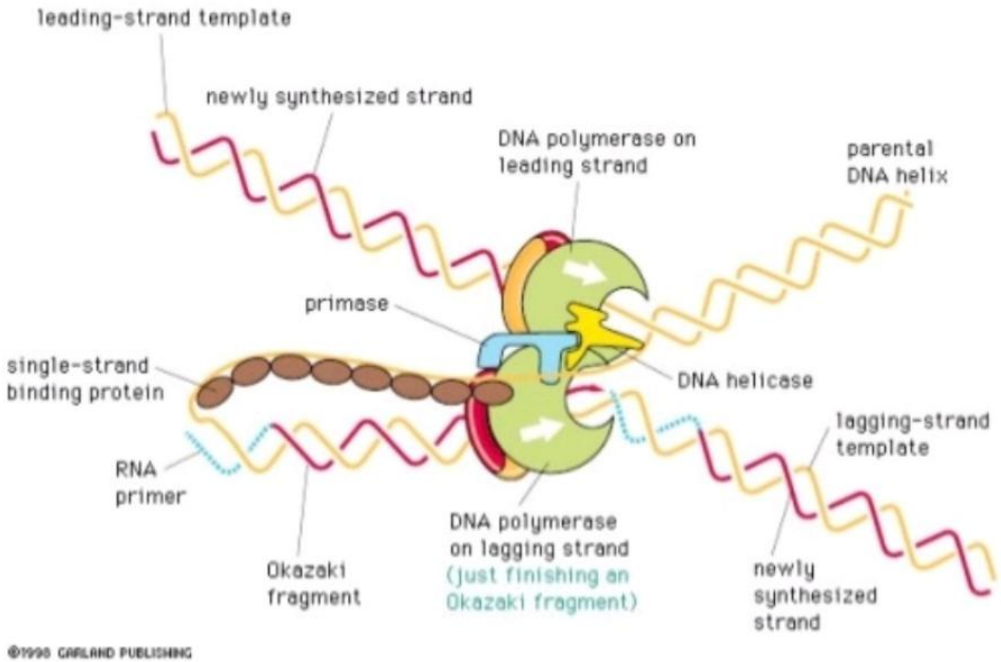
ELONGATION

- dNTP arev continuously connected to the primer or the nascent DNA chain by DNA –pol III.
- The core enzyme catalyse the synthesis of leading and lagging strand , respectively.
- Chain elongation is the series formation of the phosphodiester bonds.
- The synthesis direction of both leading strand and okazaki fragments are same as that of replication fork.

LAGGING STRAND SYNTHESIS.

- Primer on okazaki fragments are digested by Rnase.
- The gaps are filled by DNA –pol in the 5’-3’ direction.
- The nick between the 5’ and of one fragment and the 3’ end of the next fragment is sealed by LIGASE.

DNA REPLICATION.



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TERMINATION.

- The replication of E.coli is bidirectional from one origin and , and the two replication fork must meet at one point called ter at 32.
- All the primers will be removed , and all the fragments will be connected by DNA-pol I ligase.

REPLICATION IN EUKARYOTES.

- Basic principle of eukaryotic replication are same.
- Some major points of consideration are—
- There is relationship with cell cycle and replication. it occurs during synthetic phase of cell cycle and is regulated.
- There are multiple sites of origin proceeding bidirectionally.
- There are several DNA polymerases in eukaryote comparable to E.coli polymerases.

MAMMALIAN DNA POLYMERASES.

E.COLI CELLS	MAMMALIN	FUNCTION
I		Gap filling, repair
II		Proof reading
DnaG	alpha	primase
	beta	DNA repair
	gamma	Mitochondrial DNA synthesis
	delta	Lagging strand synthesis
III	epsilon	Leading strand synthesis.

DNA- PROOF READING MECHANISM.

- Replication is a high fidelity(accurate process).
- Chance of incorporation of a wrong nucleotide are very less.
- The DNA polymerase III is the main enzyme responsible for proof reading in E.coli.