DNA Replication

Lecture .3

By

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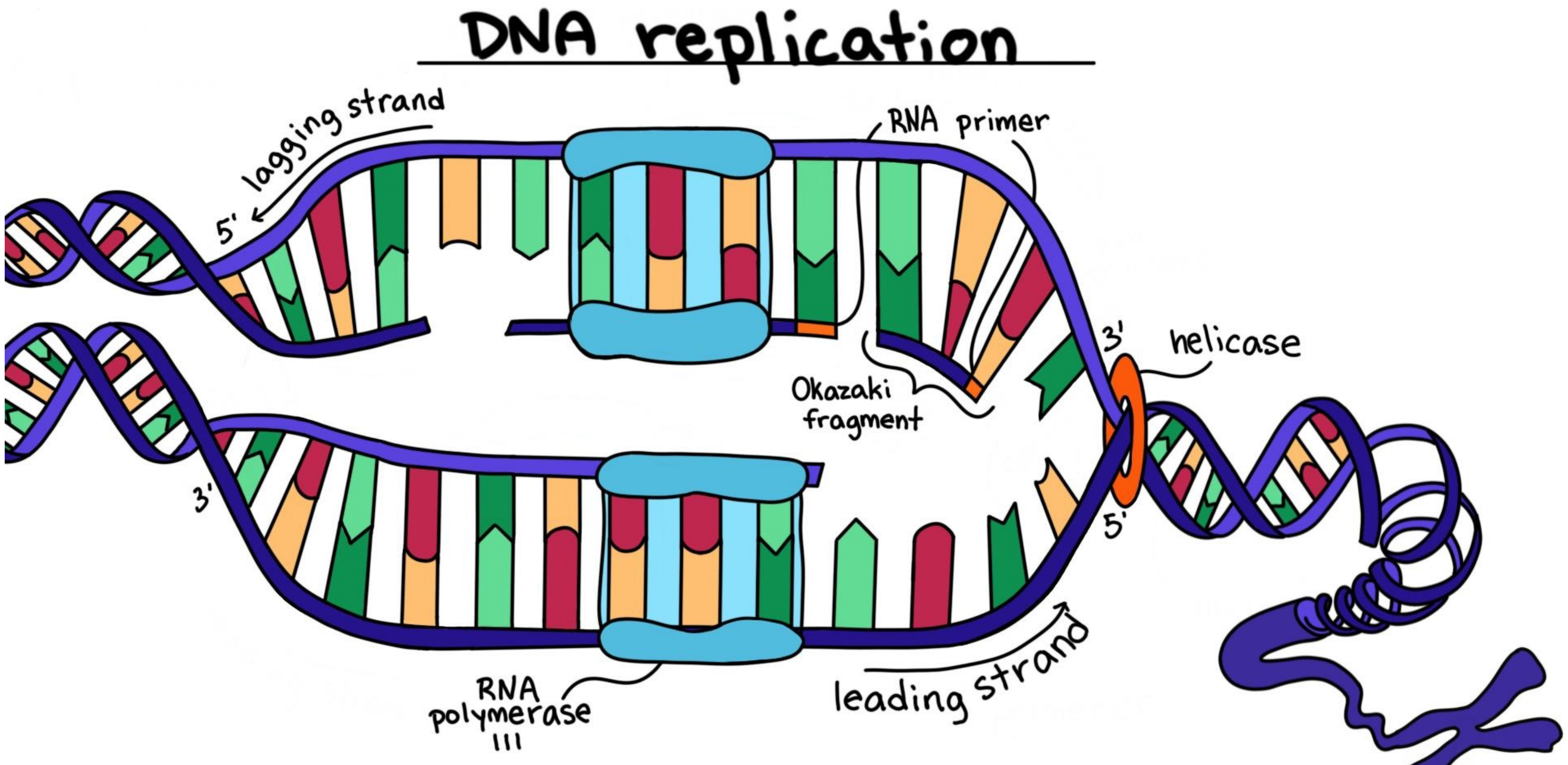
MSc. In Molecular Biology

Objectives:

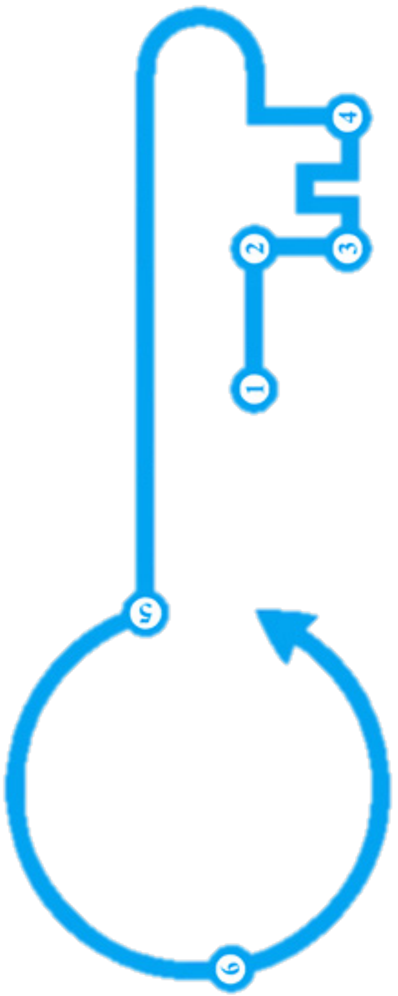
- To figure out DNA Replication Models
- To understand the DNA replication mechanism in eukaryotes and prokaryotes.
- Identifying the steps of DNA replication and DNA polymerases activities.

DNA Replication

- The basis process for biological inheritance, is a fundamental process occurring in all living organisms to copy their DNA.

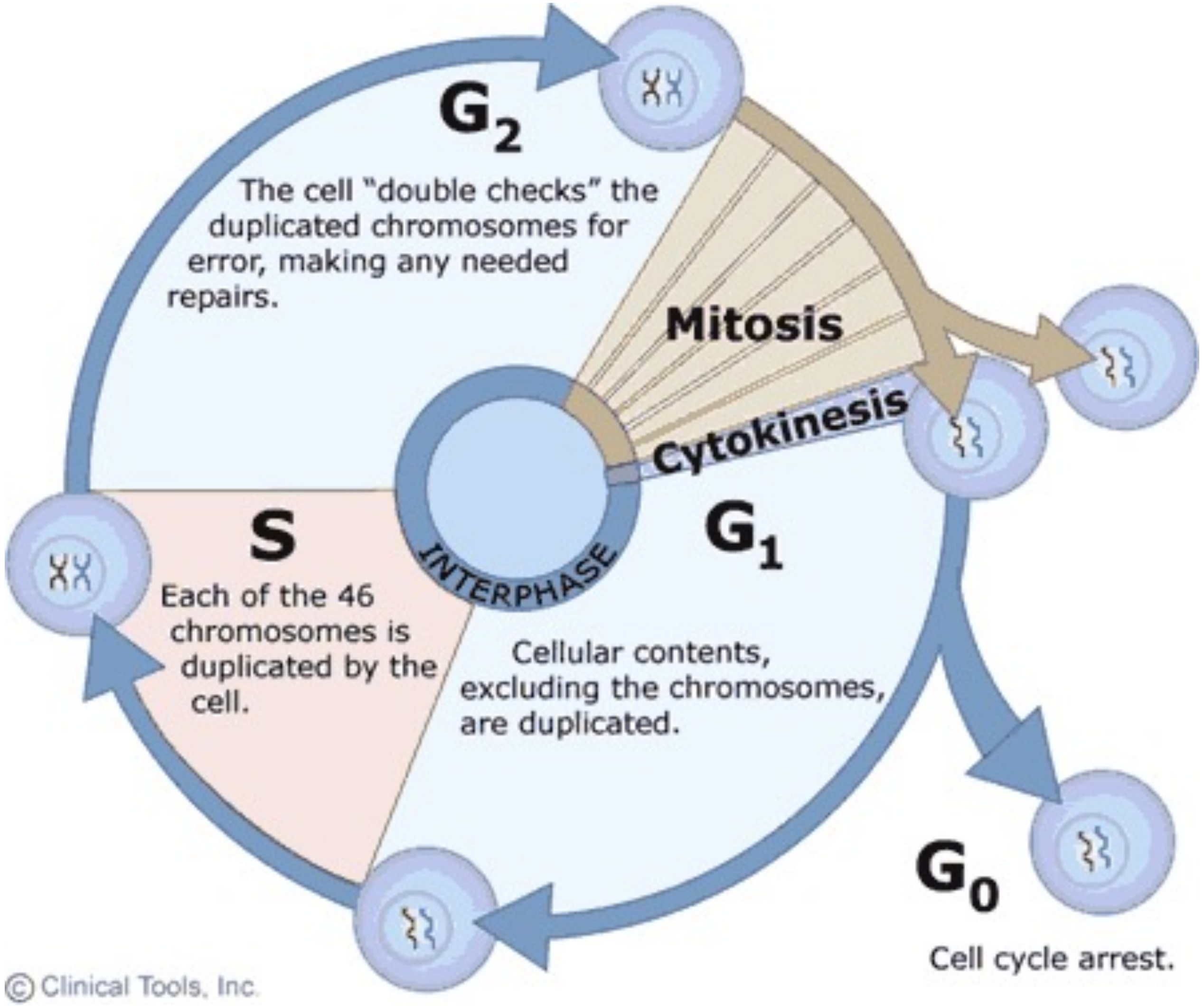


The Fundamentals of DNA Replication!



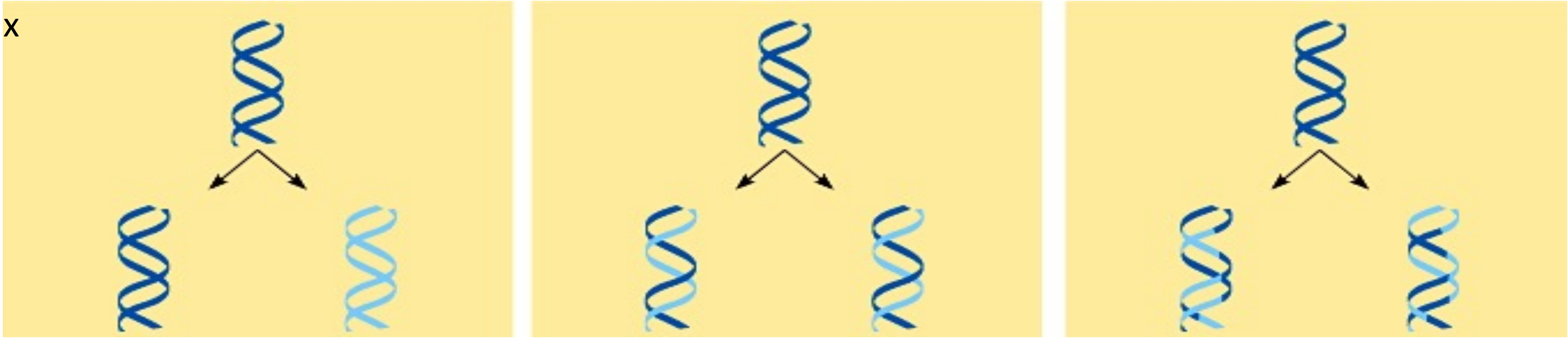
- WHY?
- HOW?
- DIRECTIONALITY
  - ➢FROM WHER TO WHERE?
  - ➢HOW?

Cell Cycle



http://scientopia.org/img-archive/scicurious/img_862.png

Models of DNA replication



Conservative model	Semiconservative model	Dispersive model
Daughter duplex made of 2 newly synthesized strands. Parent duplex conserved.	Daughter duplexes are made up of one parental strand and one newly synthesized strand	Daughter duplexes are made up of segments of parental DNA and newly synthesized DNA

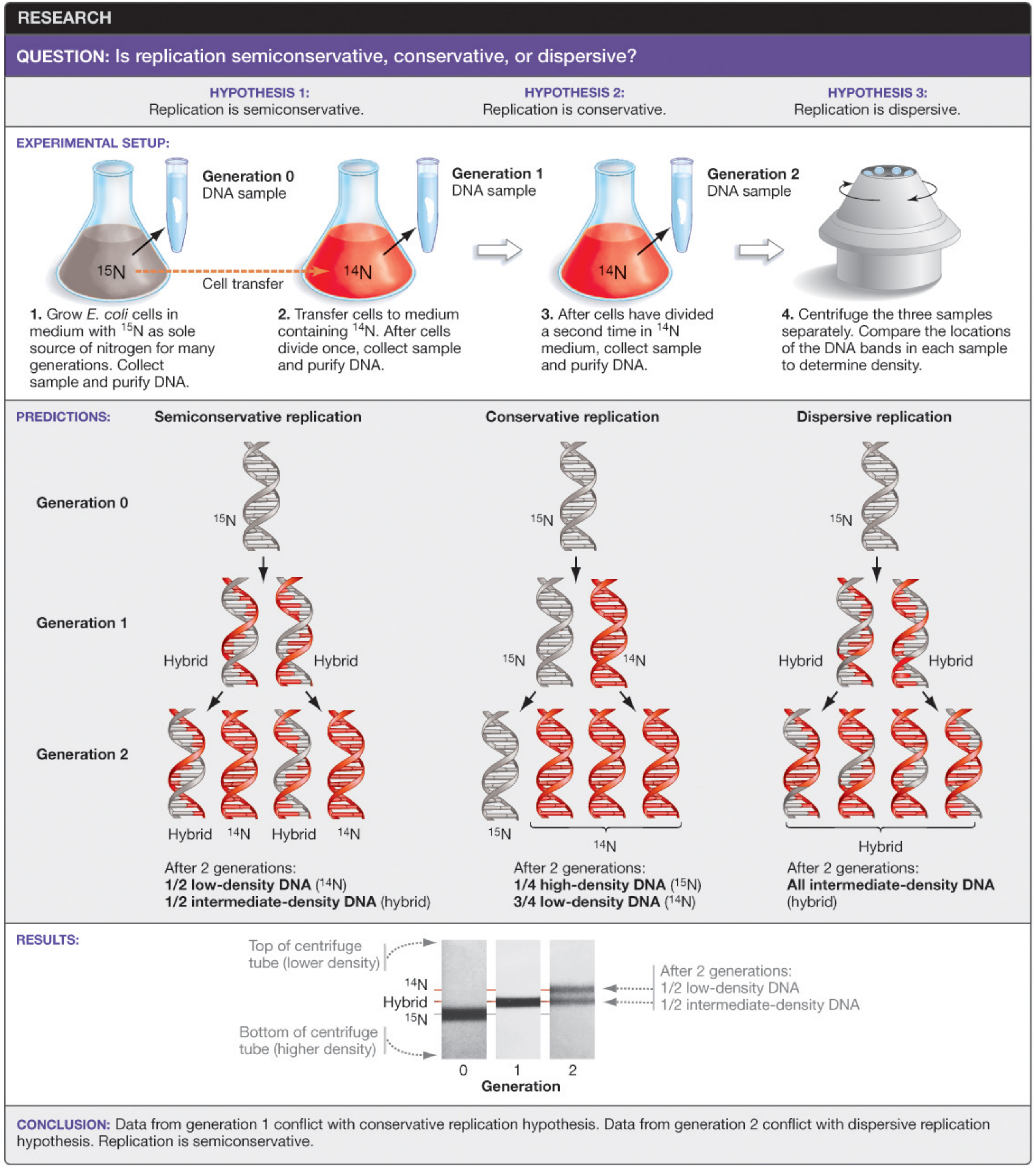
Matthew Meselson & Franklin Stahl (1958)

Performed an experiment to determine which model of DNA replication was true

See first half of video up to CsCl centrifugation: http://highered.mcgraw-hill.com/olc/dl/120076/bio22.swf

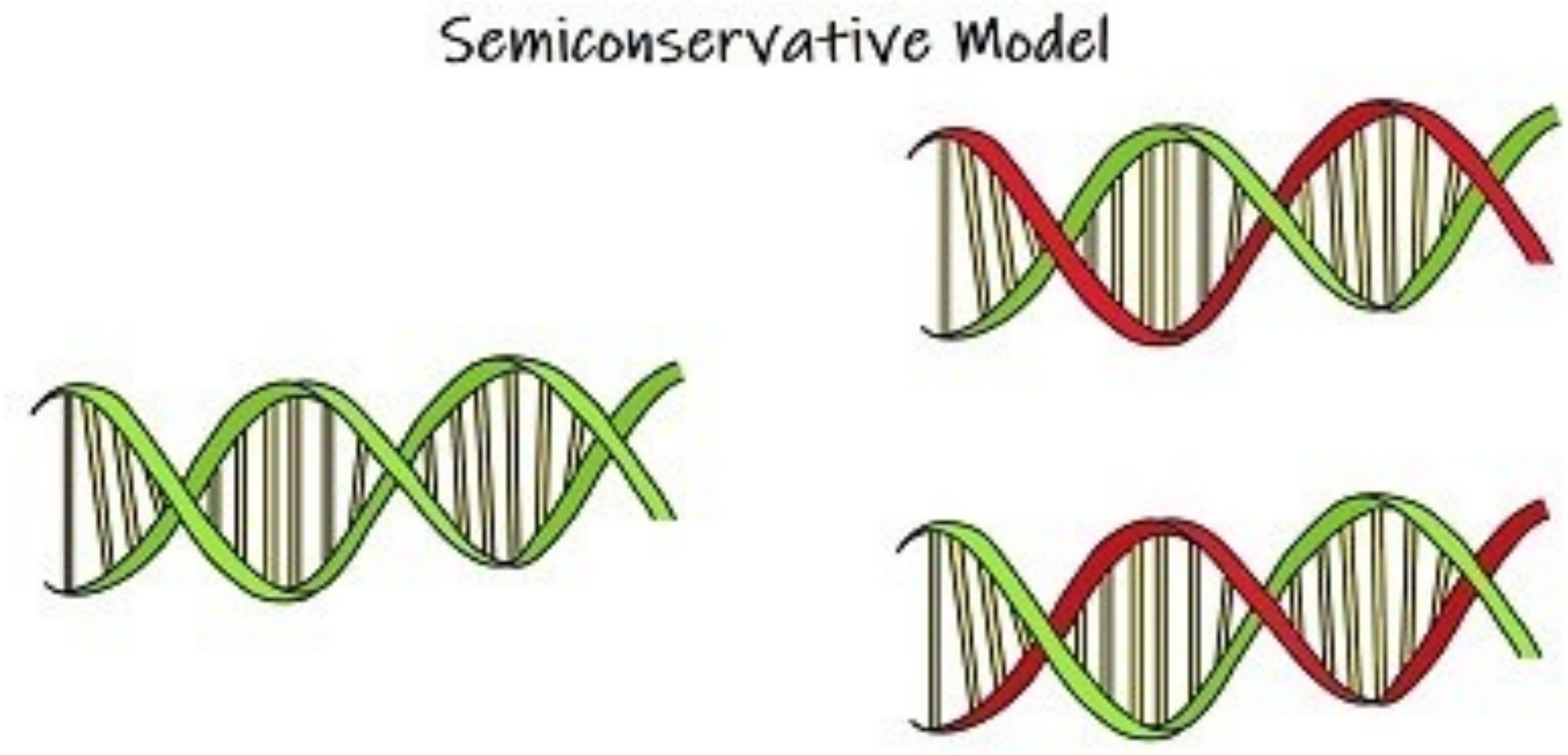
http://www.pnas.org/content/101/52/17889/F1.medium.gif

Matthew Meselson & Franklin Stahl experiment



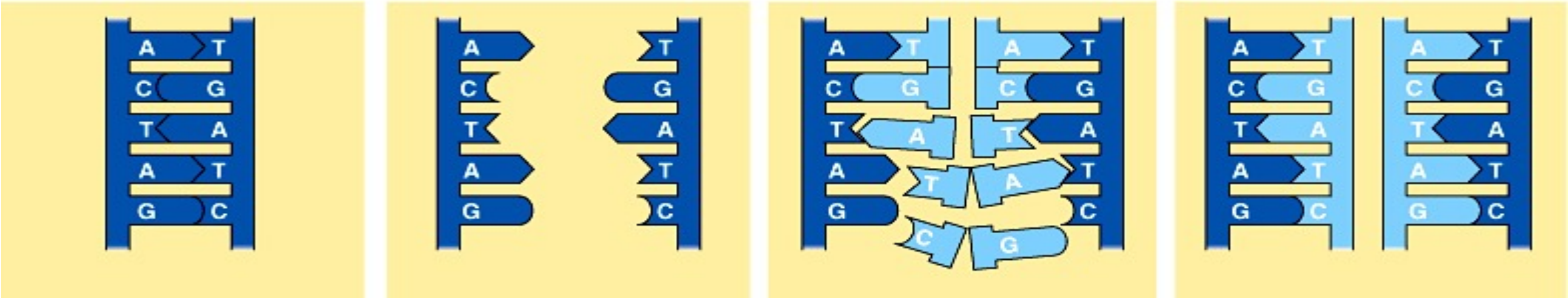
Semiconservative

- Each strand of the original double-stranded DNA molecule serves as template for the reproduction of the complementary strand.



Basic concept of DNA replication

During DNA replication, base pairing enables existing DNA strands to serve as templates for new complimentary strands



(a) The parent molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, A with T and G with C.

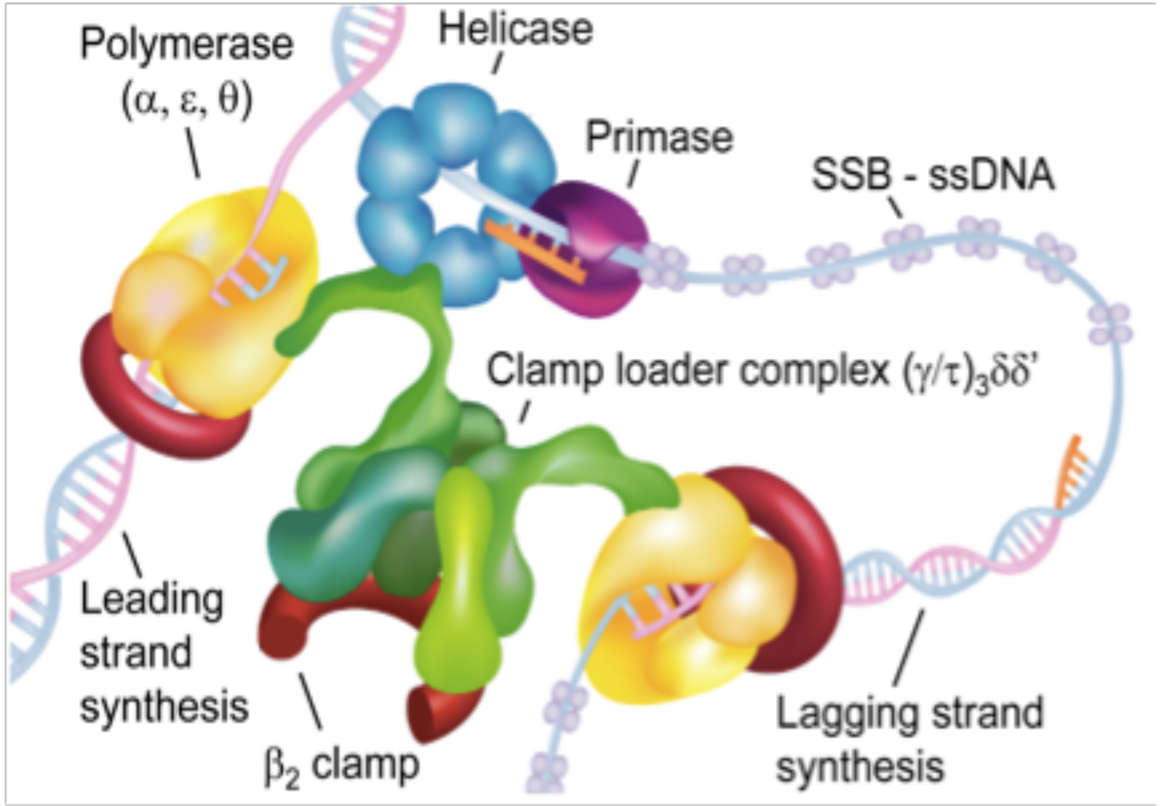
(b) The first step in replication is separation of the two DNA strands.

(c) Each parental strand now serves as a template that determines the order of nucleotides along a new complementary strand.

(d) The nucleotides are connected to form the sugar-phosphate backbones of the new strands. Each "daughter" DNA molecule consists of one parental strand and one new strand.

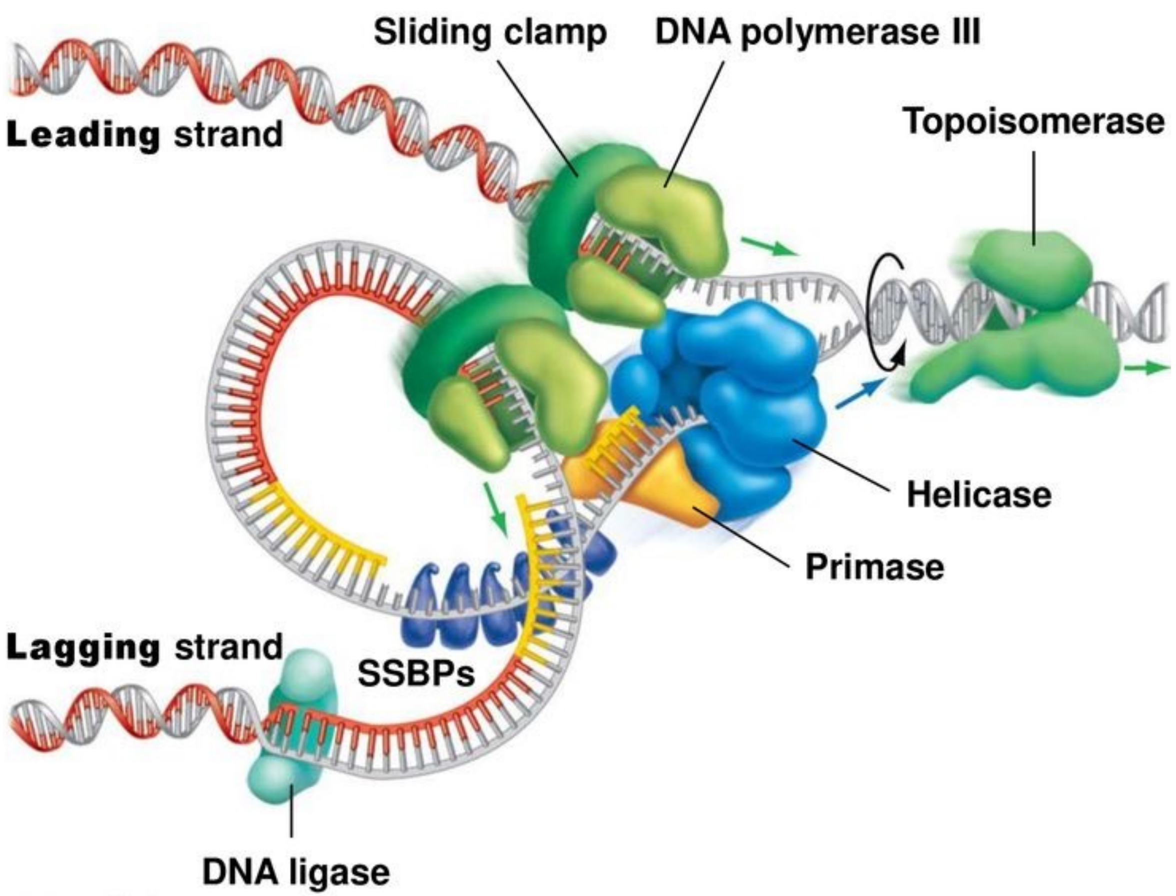
Replisome

- The replisome is a complex molecular machine that carries out replication of DNA. It is made up of a number of subcomponents that each provides a specific function during the process of replication.



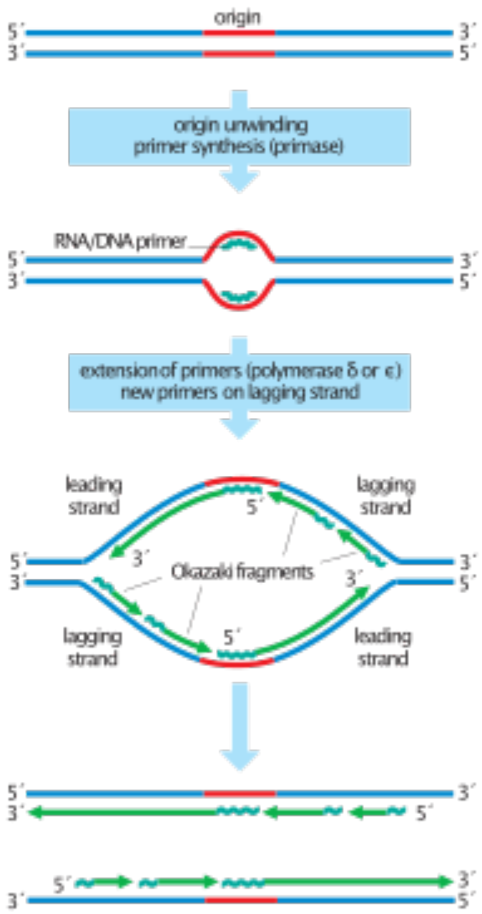
Major Components of Replisome

- Helicase
- Gyrase (Topoisomerases)
- Primase
- DNA pol. III
- DNA pol. I
- Ligase
- SSB (Single strand binding protein).
- Exonuclease



DNA Replication Mechanism

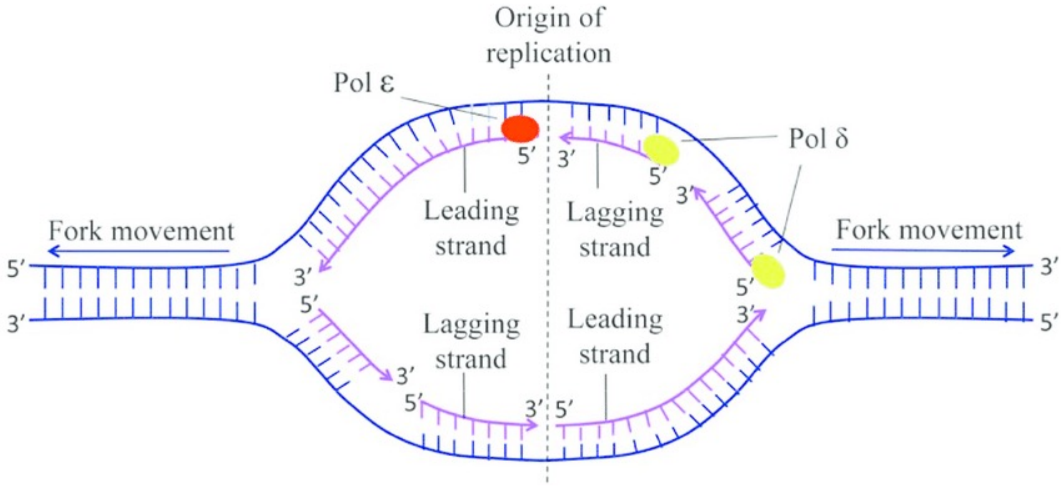
- **Initiation**
- Elongation
- Termination



DNA replication: Initiation

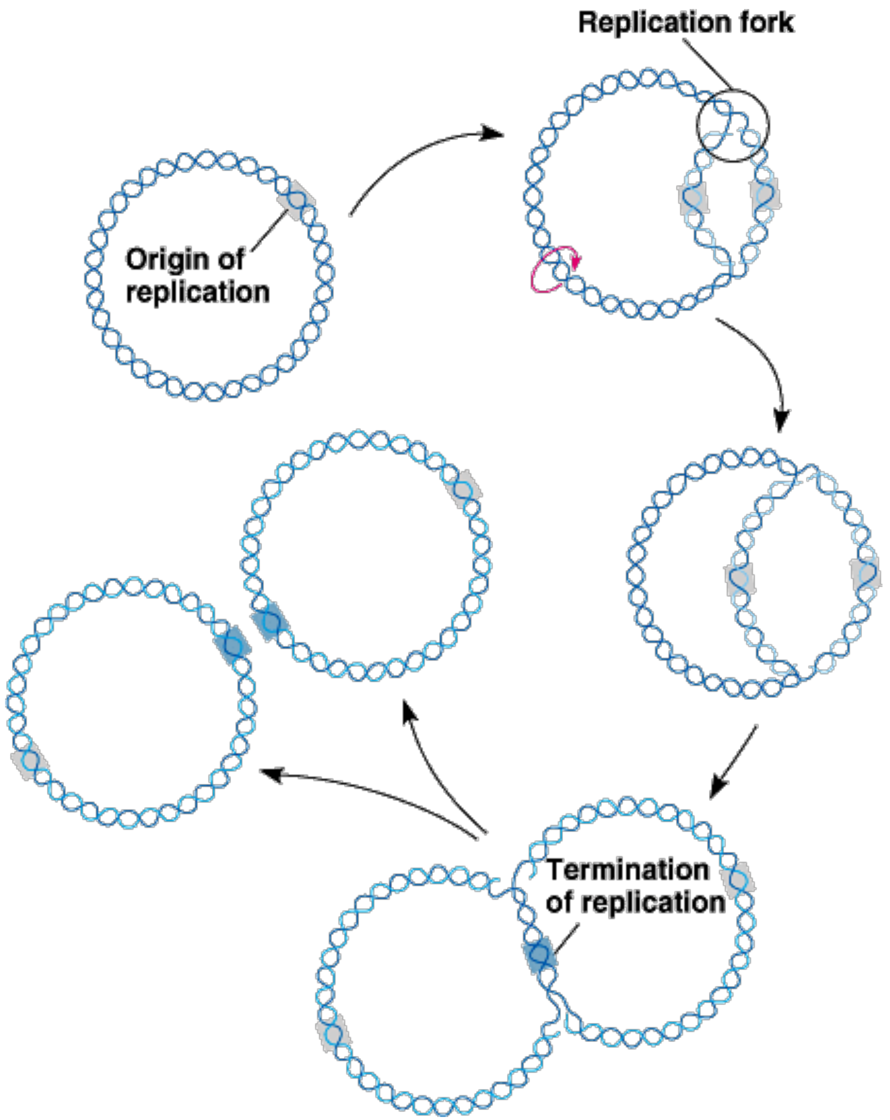
- **Origins of replication (ori)**: Special sites on DNA where replication begins.

- Origins tend to be "**AT-rich**" (rich in adenine and thymine bases) to assist this process, because A-T base pairs have two hydrogen bonds (rather than the three formed in a C-G pair)—strands rich in these nucleotides are generally easier to separate because a greater number of hydrogen bonds requires more energy to break them.



Replication Initiation: Prokaryote

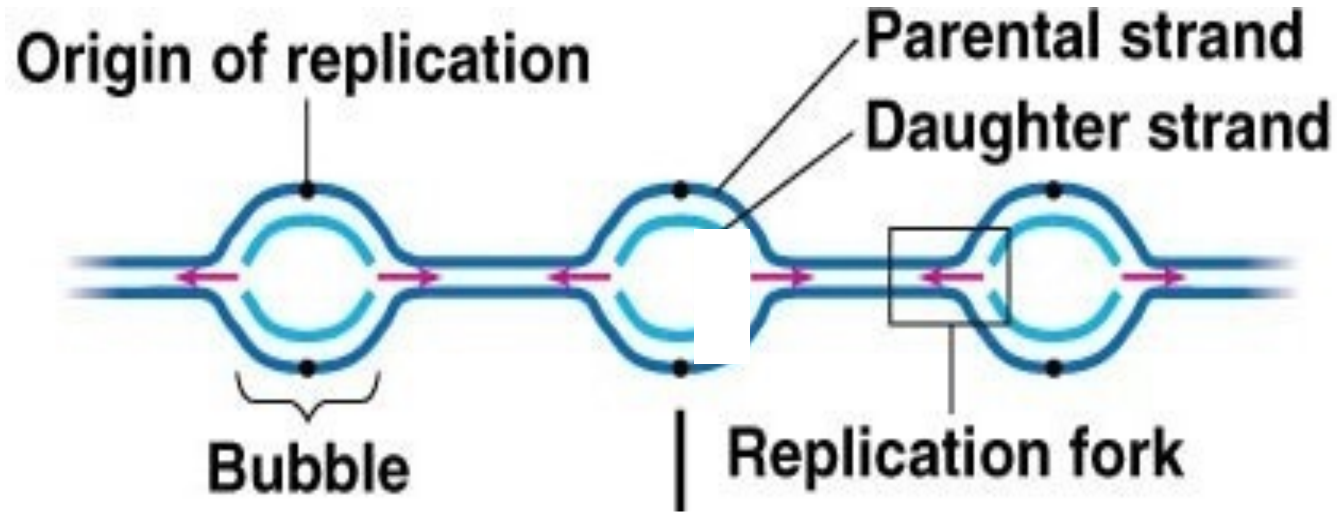
- Replication begins at one fixed origin (only 1 ori)
- Replication proceeds bidirectionally until the DNA is replicated



https://www.youtube.com/watch?v=bm_BB0587gE

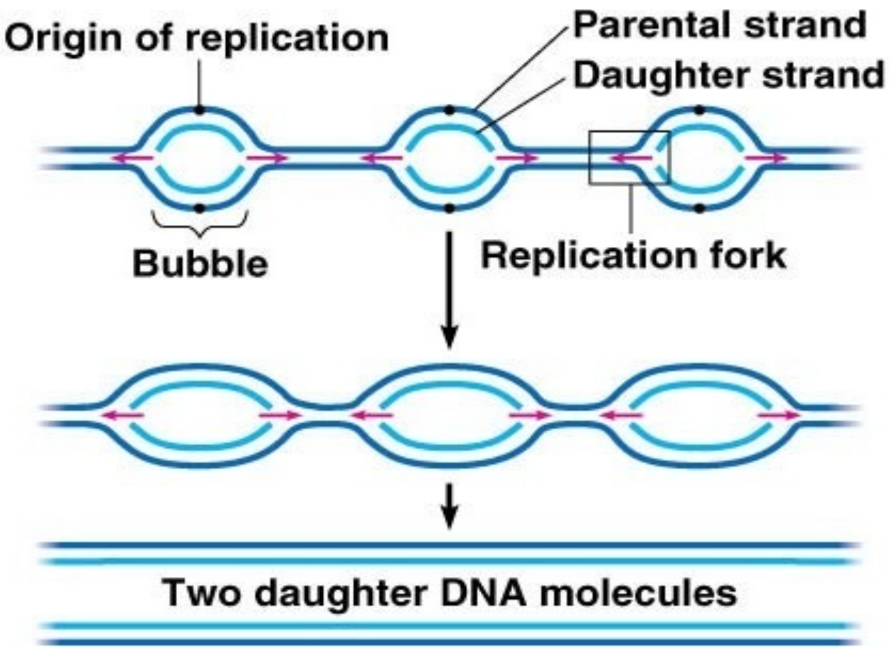
Replication Initation: Eukaryote

More than one origin of replication (thousands of origin sites perchromosome)



Replication forks and bubbles

- At the origin sites, the DNA strands separate forming a replication "bubble" with replication forks at each end.
- The replication bubbles elongate as the DNA is replicated and eventually fuses.



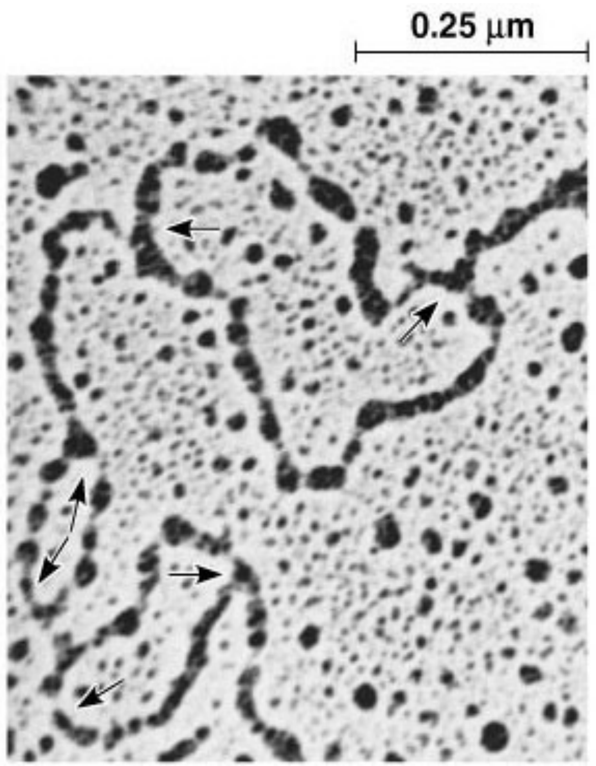
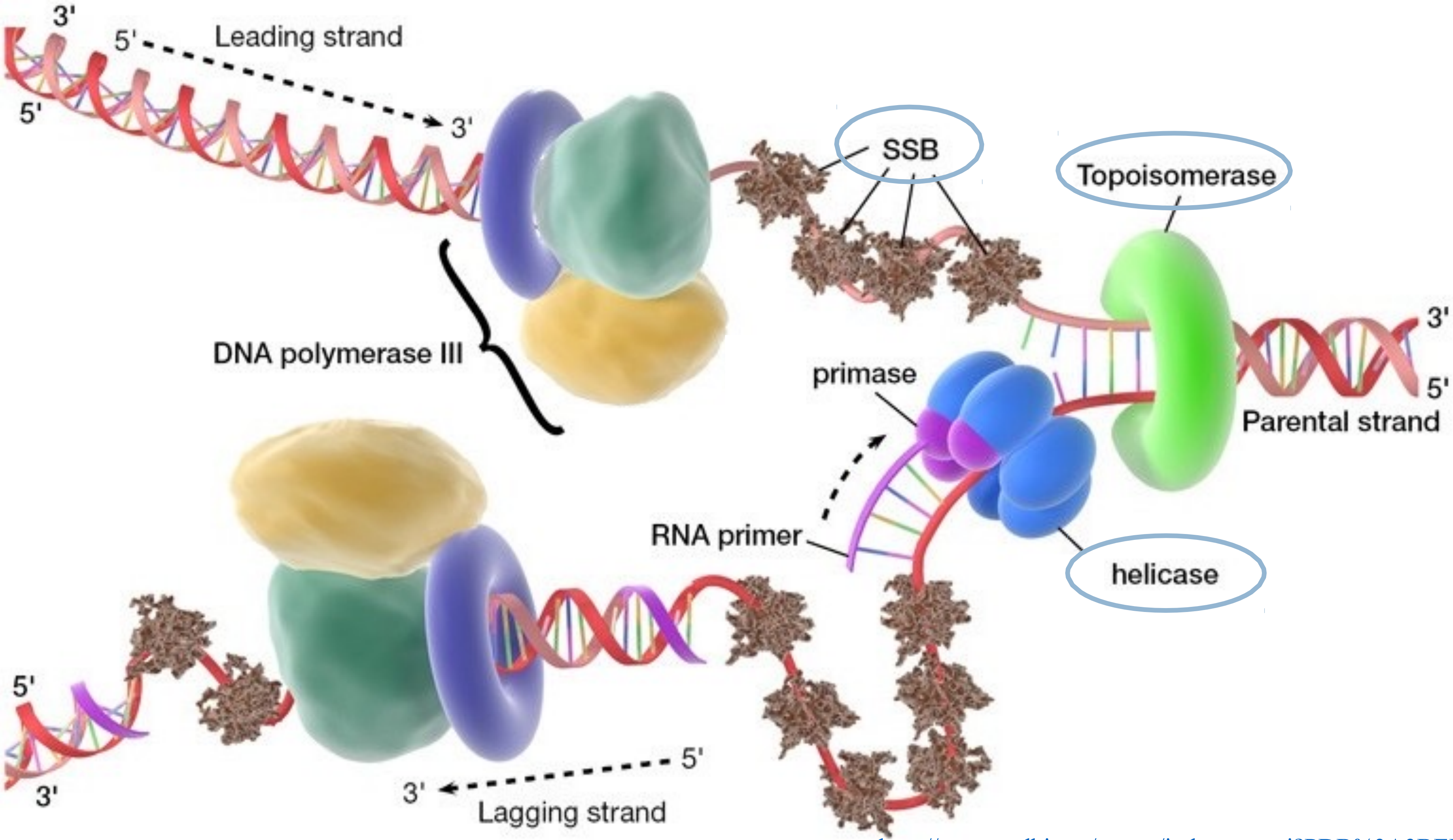


Fig. 16.10

Proteins in Replication Initiation

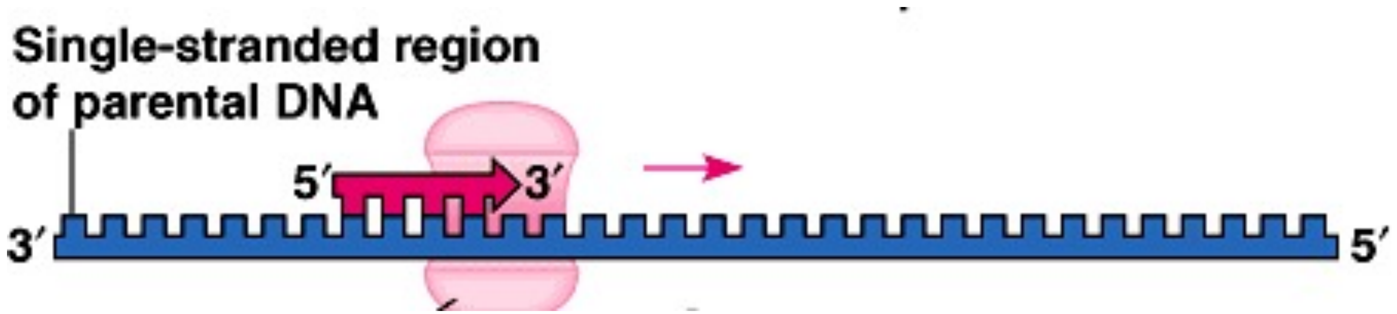


http://eprots.pdbj.org/eprots/index_en.cgi?PDB%3A3BEP

Proteins in Replication Initiation

- **Topoisomerases**: enzymes that can break bonds in DNA and then reforms the bonds
  - Purpose is to release the twists in DNA that are generated during DNA replication
  - Example of a topoisomerase: **DNA gyrase**
- **Helicase**: enzyme that disrupts H bonds between two strands of DNA to separate the template DNA strands at the replication fork.
- **Single-strand binding proteins (SSBPs)**: proteins that bind to unwound single-stranded regions of DNA to keep the template strands apart during replication

Priming DNA for replication



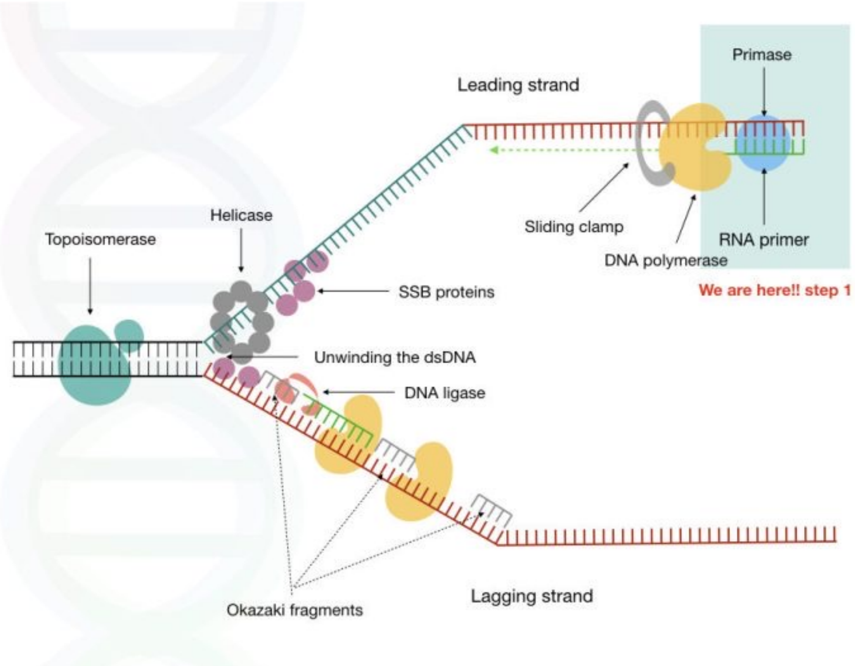
- **Blue line:** DNA to be copied
- **Pink line:** RNA nucleotides added = Primer
- **Light pink blob:** Enzyme that adds RNA primer = RNA polymerase

Priming DNA for replication

- **Primer**: a short segment of RNA needed to initiate DNA replication

Note: all nucleic acids are formed in the 5' to 3' direction, even RNA (thus primers)

- **Primase**: the RNA polymerase (RNAP) which synthesizes the primer by adding ribonucleotides that are complementary to the DNA template
- **Polymerase**: enzyme that makes polymers



Initiation of DNA replication by RNA primase

Why is priming required?

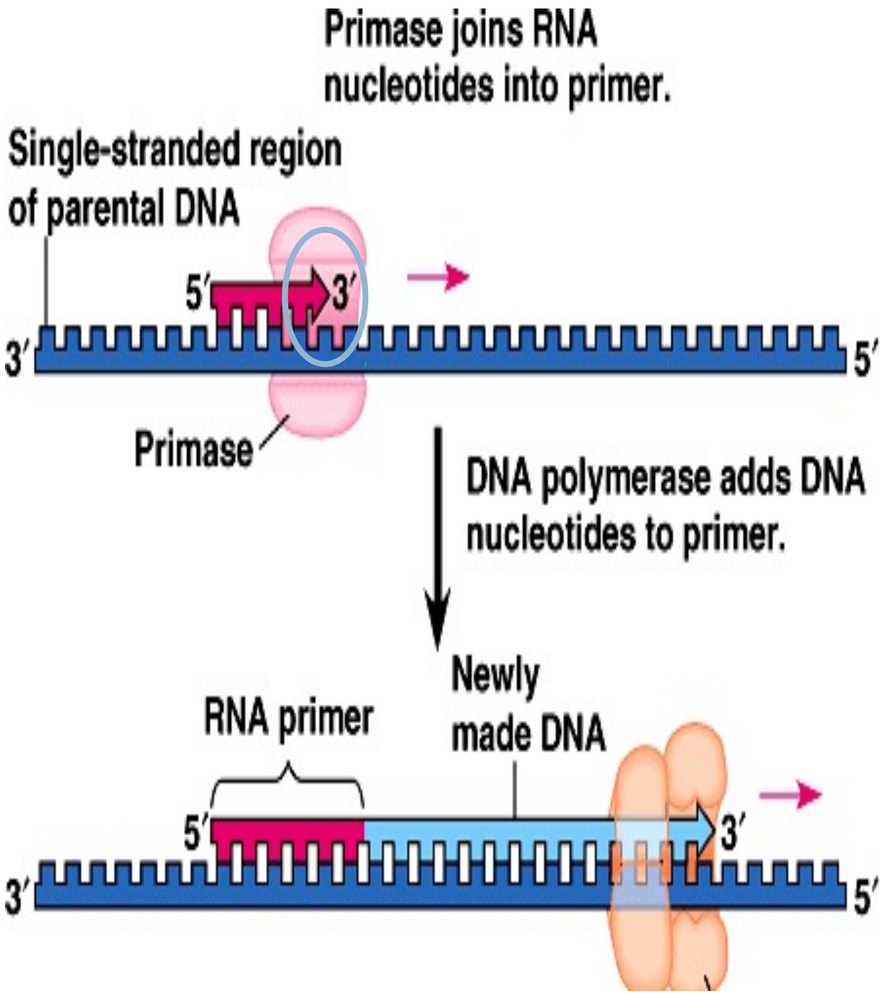
Due to the different abilities of RNA polymerase (RNAP) versus DNA polymerase (DNAP)

RNAP:

- can start a new chain without an existing end
- All it needs is a template
- E.g. primase

DNAP:

- can only add nucleotides to the end of an existing chain
- can **never** start a new chain because it needs the 3' OH



DNA Polymerase (DNAP)

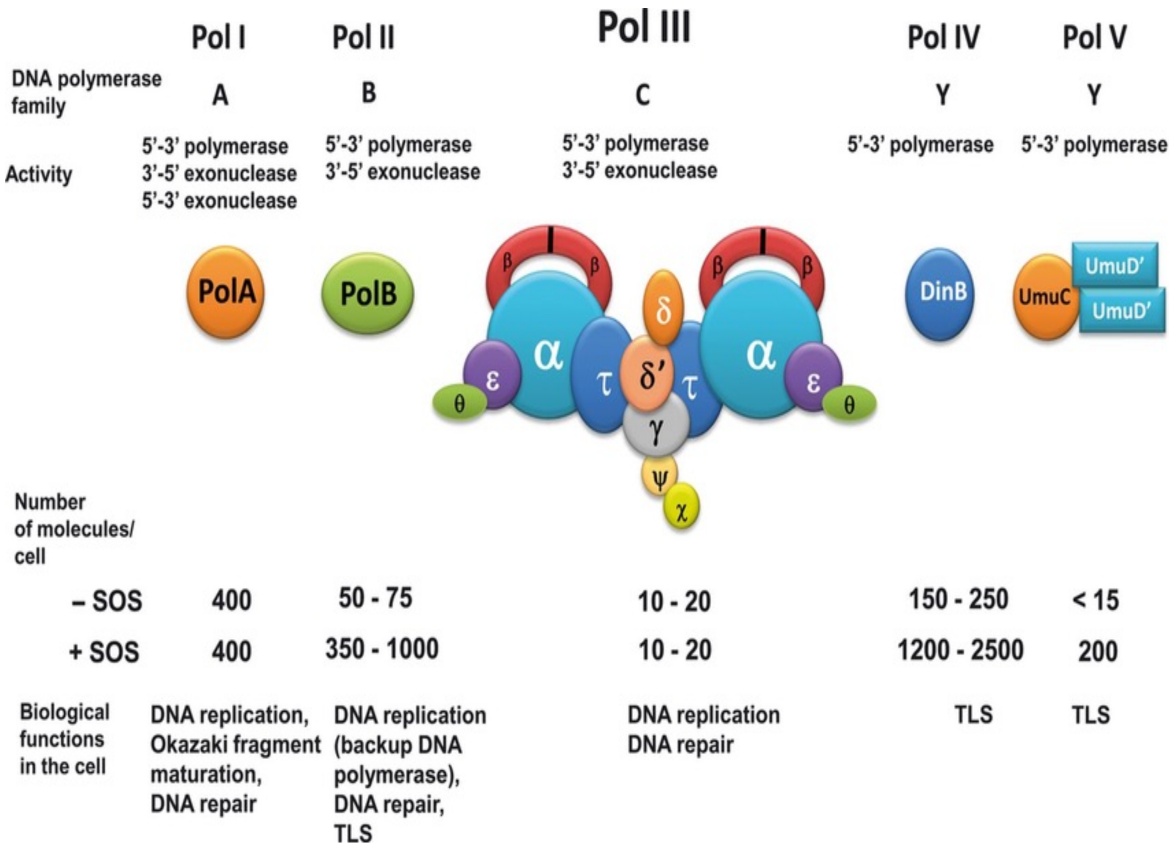
DNA polymerases are a family of enzymes that carry out all forms of DNA replication (Enzyme which synthesizes nucleotidechains)

Prokaryotic DNAP :

- DNA polymerase I, II, III, IV & V

Eukaryotic DNAP :

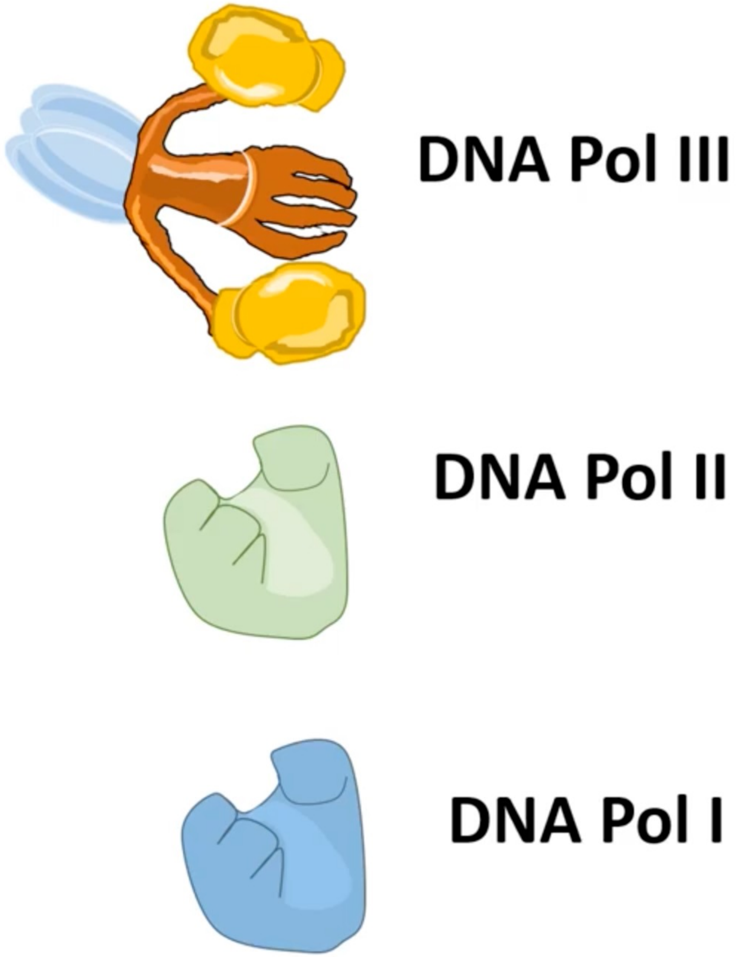
- Over 15 different types named with Greek letters (e.g. DNAP α)



amily[16]	Types of DNA polymerase	Taxa	Examples	Feature
A	Replicative and Repair Polymerases	Eukaryotic and Prokaryotic	T7 DNA polymerase, Pol I, Pol γ, θ, and ν	Two exonuclease domains (3'-5' and 5'-3')
B	Replicative and Repair Polymerases	Eukaryotic and Prokaryotic	Pol II, Pol B, Pol ζ, Pol α, δ, and ε	3'-5 exonuclease (proofreading); viral ones use protein primer
C	Replicative Polymerases	Prokaryotic	Pol III	3'-5 exonuclease (proofreading)
D	Replicative Polymerases	Euryarchaeota	PolD (DP1/DP2 heterodimer)[17]	No "hand" feature, RNA polymerase-like; 3'-5 exonuclease (proofreading)
X	Replicative and Repair Polymerases	Eukaryotic	Pol β, Pol σ, Pol λ, Pol μ, and Terminal deoxynucleotidyl transferase	template-independent; 5' phosphatase (only Pol β)
Y	Replicative and Repair Polymerases	Eukaryotic and Prokaryotic	Pol ι, Pol κ, Pol η,[18] Pol IV, and Pol V	Translesion synthesis[19]
RT	Replicative and Repair Polymerases	Viruses, Retroviruses, and Eukaryotic	Telomerase, Hepatitis B virus	RNA-dependent

Prokaryotic DNAP

- Three types of DNA polymerase classified in prokaryotes,
- Type I, used to fill the gap between DNA fragments of the lagging strand.
- Type II, involved in the SOS response to DNA damage.
- Type III, DNA replication is mainly carried out by the DNA polymerase III.



Eukaryotic DNAP

- There are five types of DNA polymerases in mammalian cells: a, b, g, d, and e.
- The (g) subunit is located in the mitochondria, responsible for the replication of mtDNA. Other subunits are located in the nucleus. Their major roles of each subunits are:
- a: synthesis of lagging strand.
- b: DNA repair.
- d: synthesis of leading strand.
- e: DNA repair.

References

- Cox, M. M., Doudna, J. A., & O'Donnell, M. (2012). *Molecular biology: principles and practice* (p. 809). New York, NY. USA:: WH Freeman and Company.
- Lodish, H., Berk, A., Kaiser, C. A., Krieger, M., Scott, M. P., Bretscher, A., ... & Matsudaira, P. (2008). *Molecular cell biology*. Macmillan.
- Salas, M. (1991). Protein-priming of DNA replication. *Annual review of biochemistry*, *60*(1), 39-71.