DNA REPLICATION

I MSC ZOOLOGY
CELL AND MOLECULAR BIOLOGY
UNIT IV

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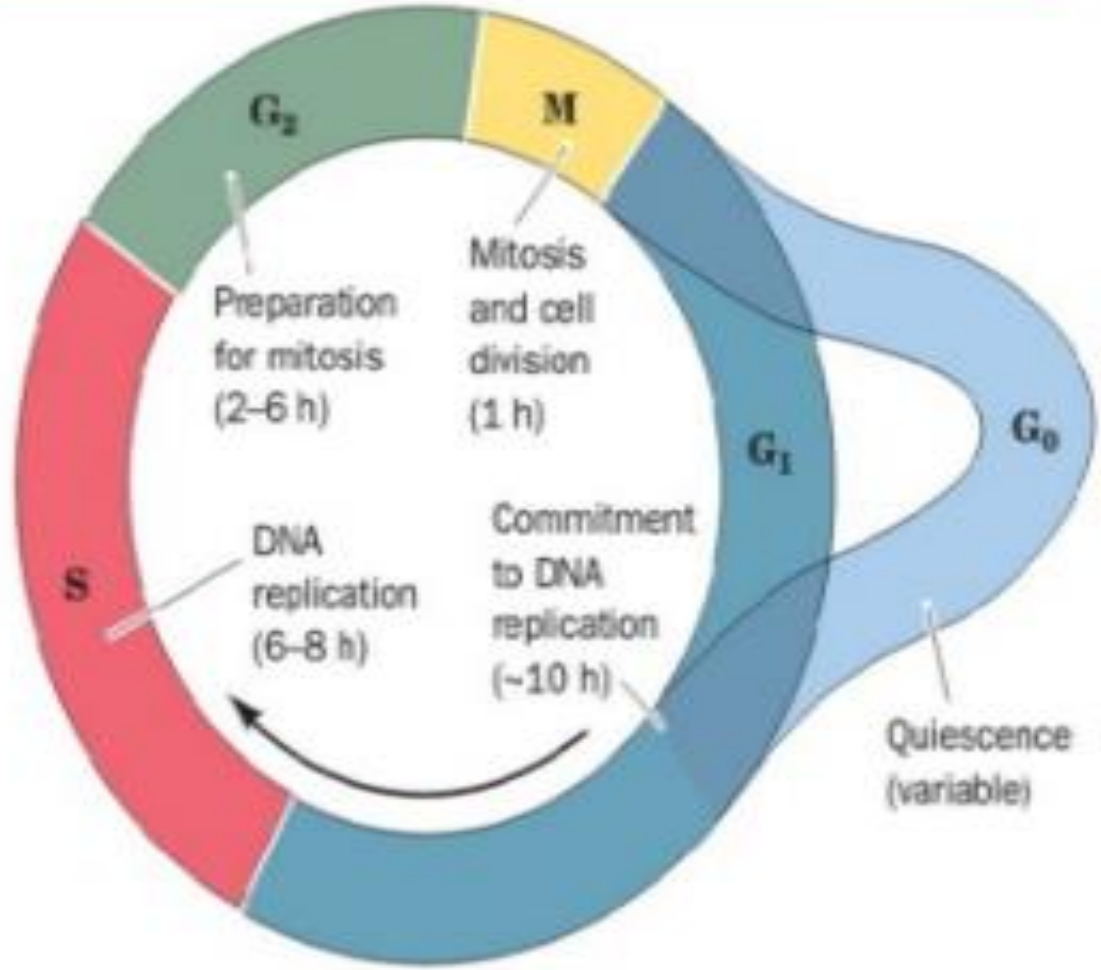
DNA Replication-Introduction

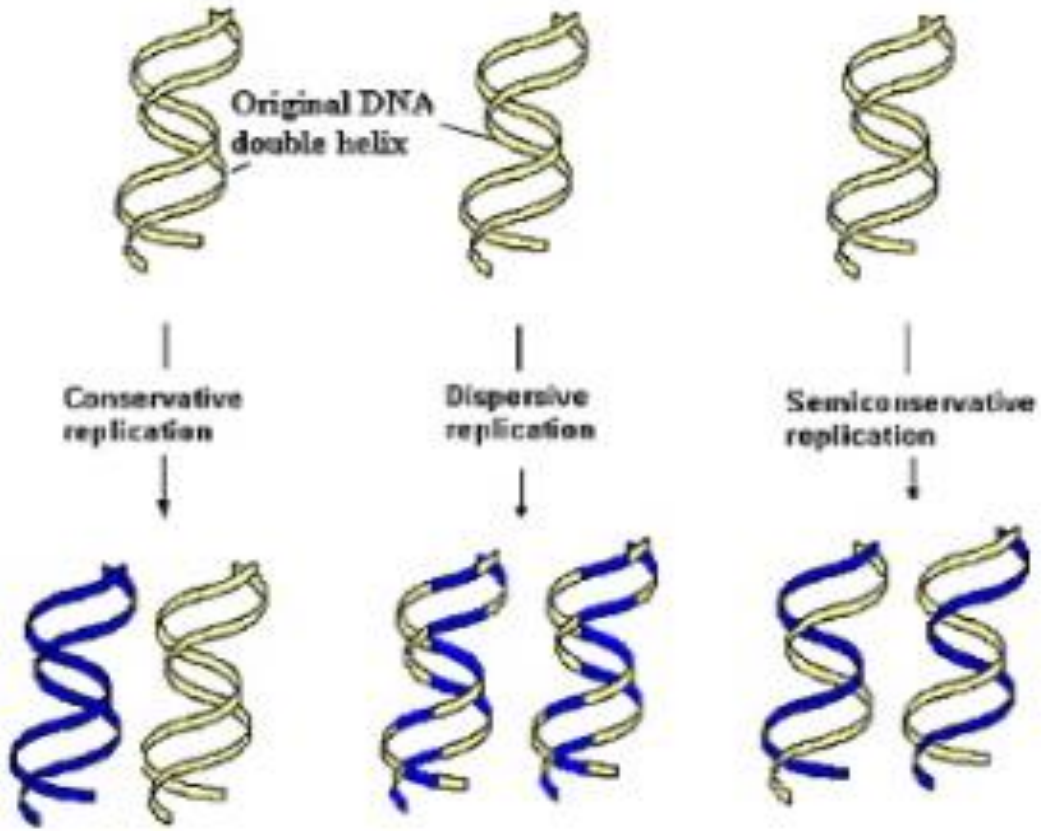
- Basis for inheritance
- Fundamental process occurring in all cells for transfer of genetic information to daughter cells
- Each cell must replicate its DNA before division.
- DNA is copied during the S or synthesis phase of interphase
- New cells will need identical DNA strands



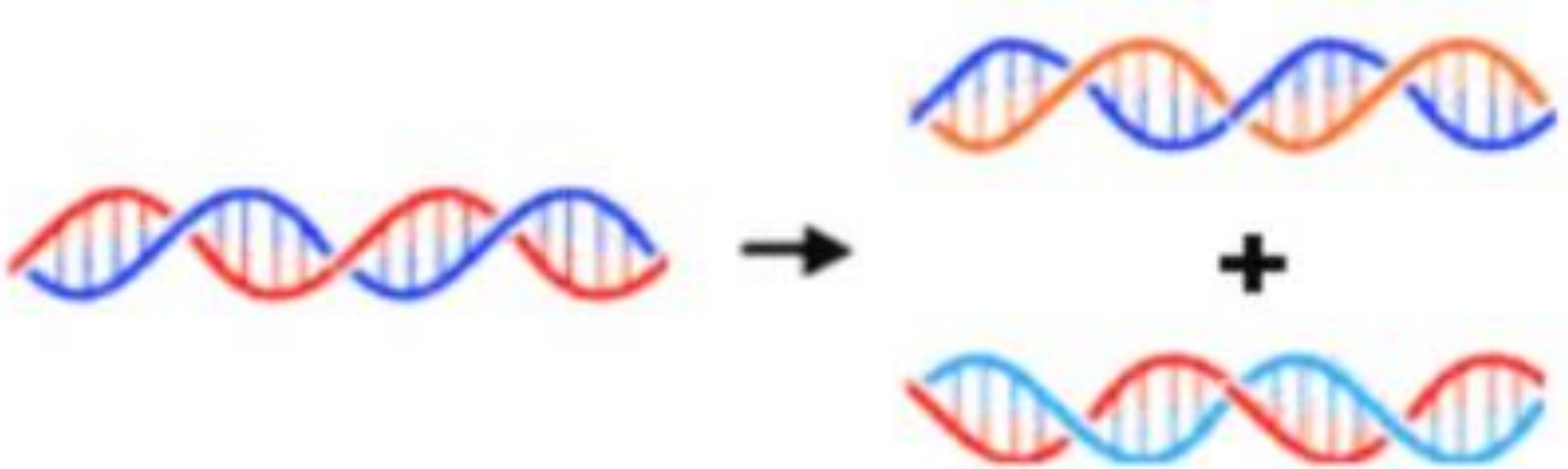
Cell cycle

Proposed Models of DNA Replication

- In the late 1950s, three different mechanisms were proposed for the replication of DNA
  - **Conservative model**
    - Both parental strands stay together after DNA replication
  - **Semi-conservative model**
    - The double-stranded DNA contains one parental and one daughter strand following replication
  - **Dispersive model**
    - Parental and daughter DNA are interspersed in both strands following replication



Possible Models of DNA Replication

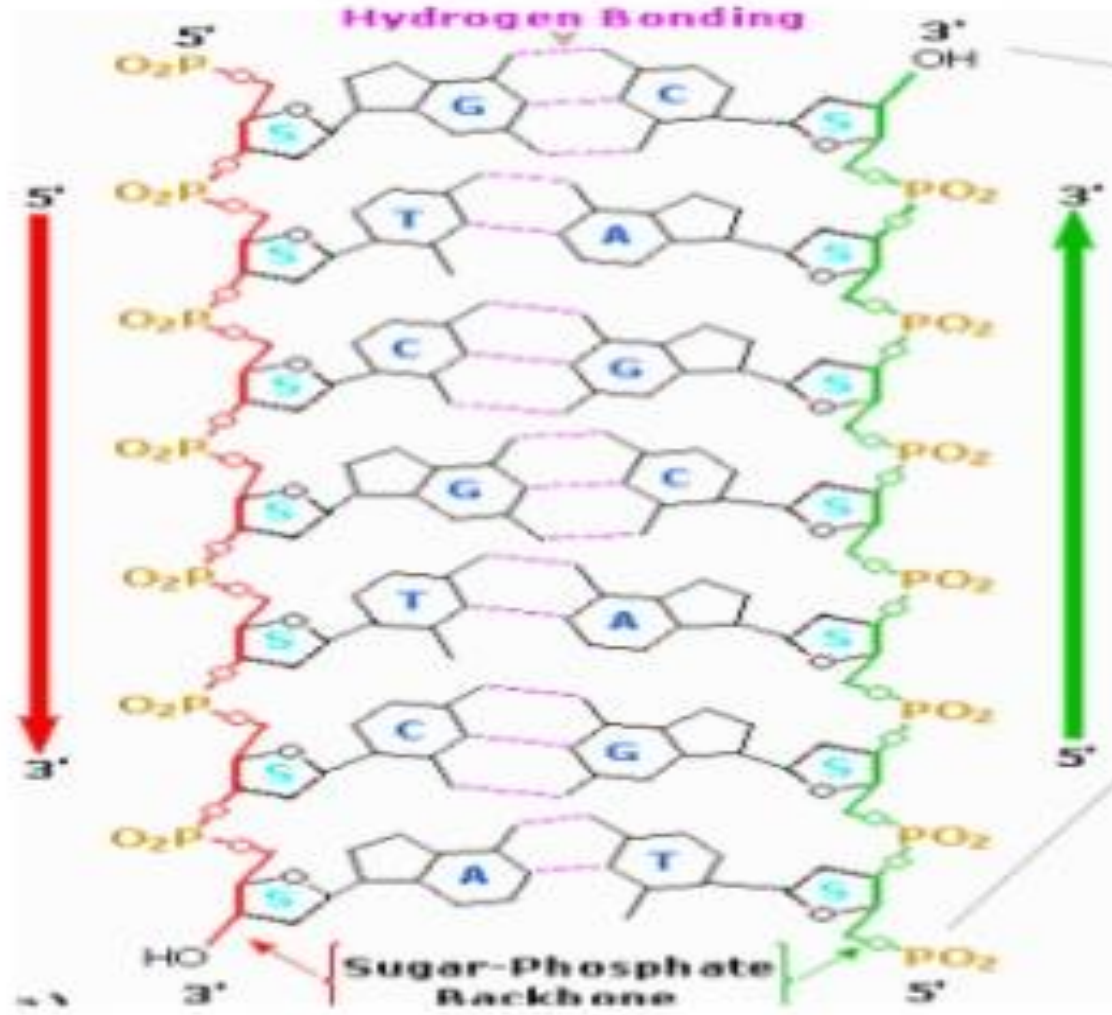


Semi-conservative Replication

DNA goes through a type of replication called semi-conservative replication, which means one strand on the DNA helix is new and the other strand is old

Basic rules of DNA replication

- Specificity of base pairing
- Synthesis direction 5'-3' (C at 3' position in 3' end has free –OH)
- Nucleotides added at 3' of growing strand – *DNA polymerase*



Components of DNA Replication

- DNA polymerases- Deoxynucleotide polymerization
- Helicase -Processive unwinding of DNA
- Topoisomerases Relieve torsional strain that results from helicase-induced unwinding
- RNA primase Initiates synthesis of RNA primers
- Single-strand binding proteins Prevent premature reannealing of dsDNA
- DNA ligase Seals the single strand nick between the nascent chain and Okazaki fragments on lagging strand

Steps In DNA Replication

- Identification of the origins of replication
- Unwinding (denaturation) of dsDNA to provide an ssDNA template
- Formation of the replication fork
- Initiation of DNA synthesis and elongation - Synthesis of leading and lagging strands(okazaki fragment)
- Primer removal and ligation of the newly synthesized DNA segments
- Reconstitution of chromatin structure

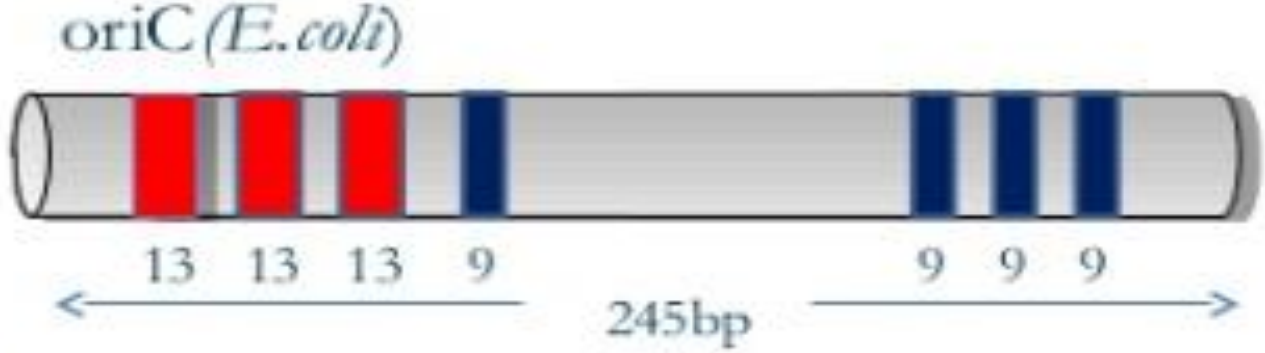
PROKARYOTIC DNA POLYMERASE

Property	Polymerase I	Polymerase II	Polymerase III
Mass (kDa)	103	90	830
Turnover no/ min	600	30	1200
No. of subunits	One	>4	>10
Structural gene	*pol*A	*pol*B	*pol*C
Polymerization 5'-3'	Yes	Yes	Yes
Exonuclease 5'-3'	Yes	No	No
Exonuclease 3'-5'	Yes	Yes	Yes

DNA Replication in Prokaryotes

Origin of Replication(oriC)

- 245 bp long
- contains several conserved sequences:



✓ Four 9-bp repeats

5' - TTATCCACA – 3' (DnaA box)

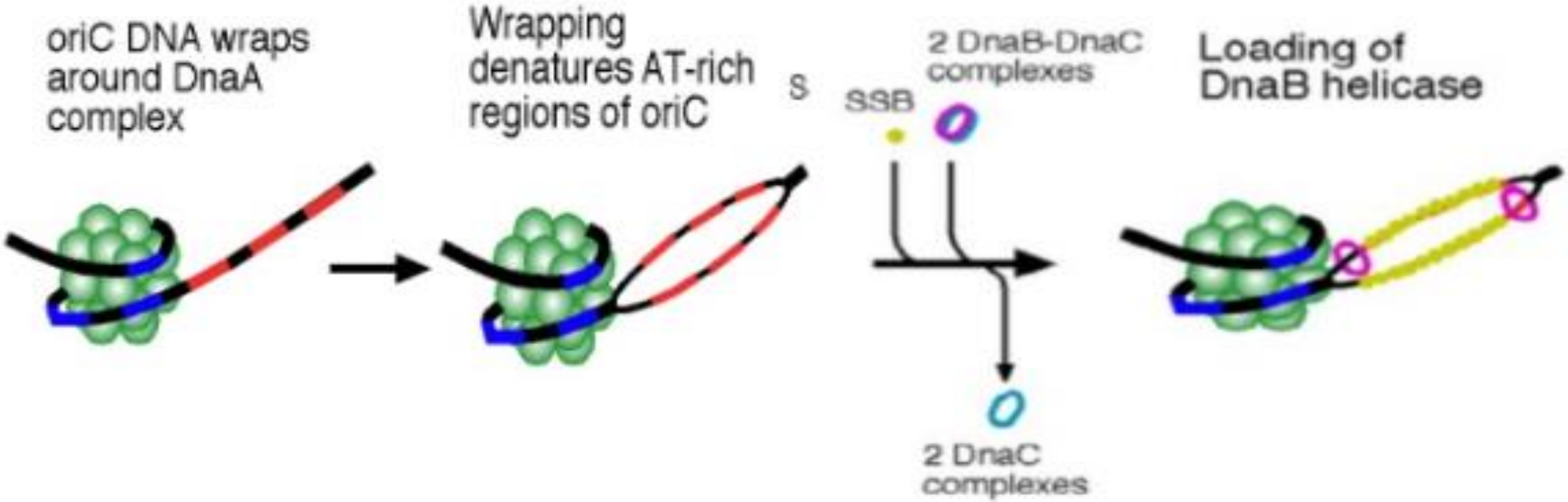
Orientation, spacing, and sequences of the 9-bp repeats are critical for function of oriC

✓ Three 13-bp repeats

5' - GATCTNTTNTTTT – 3' (DnaB box)

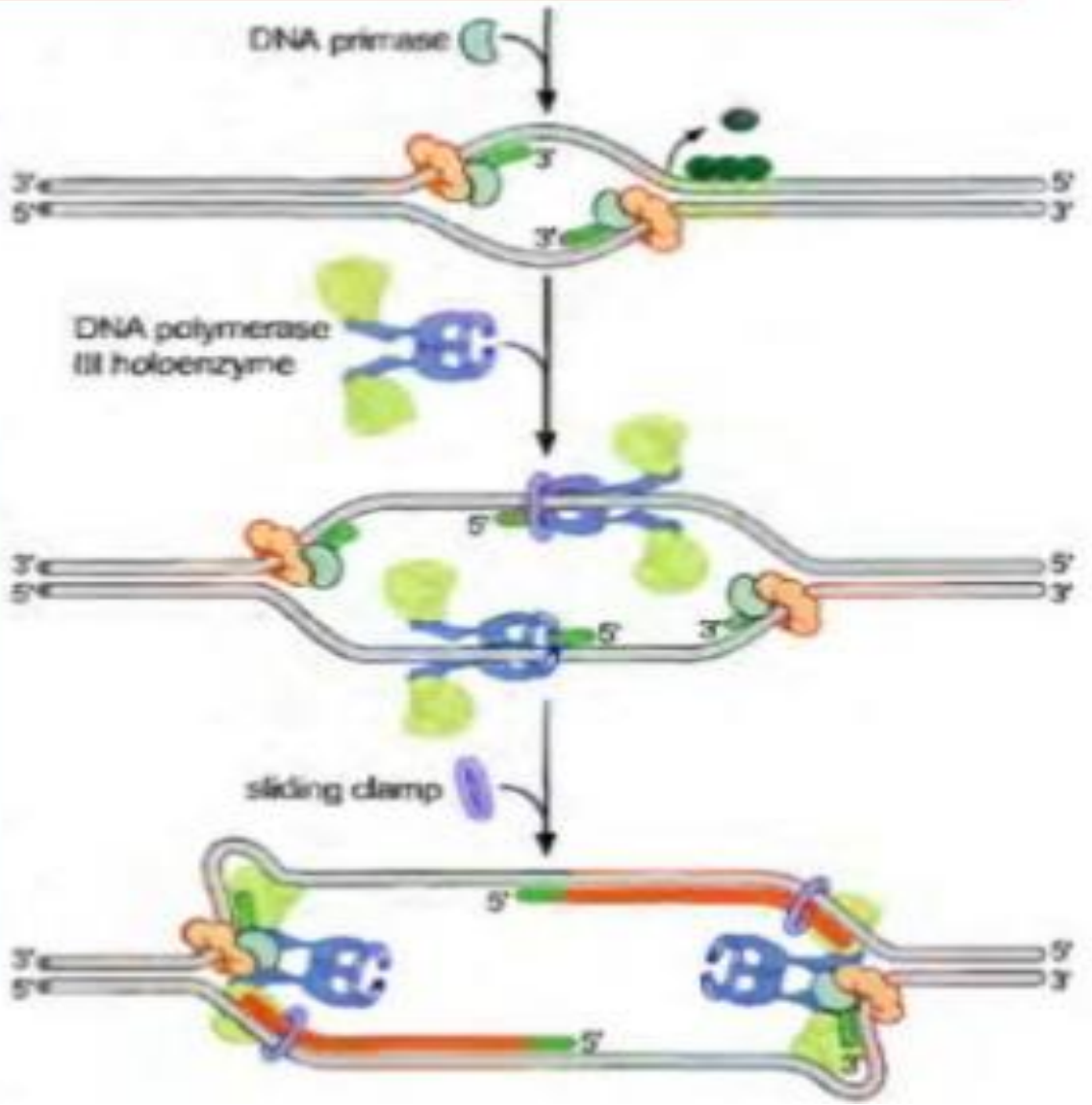
AT-richness of 13-bp repeats crucial

Initiation

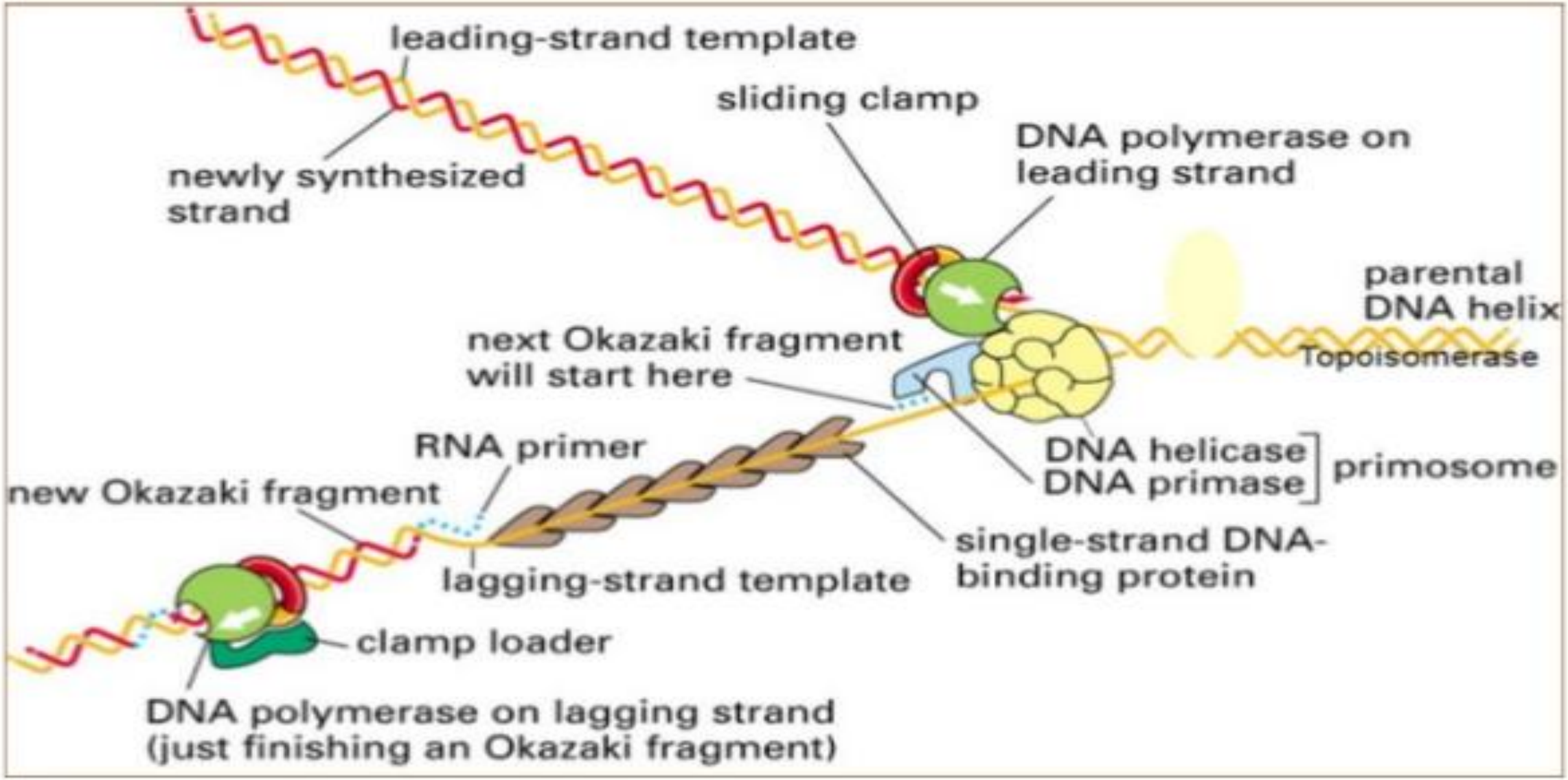

oriC DNA wraps around DnaA complex
Wrapping denatures AT-rich regions of oriC
S
SSB
2 DnaB-DnaC complexes
2 DnaC complexes
Loading of DnaB helicase

Elongation

- Once priming is complete DNA pol III is loaded into the DNA and replication begins
- Nucleophilic attack by the 3' OH on the alpha phosphate releases pyrophosphate
- Subsequently hydrolyzed (by inorganic phosphatase) into two phosphates
- This hydrolysis drives DNA synthesis to completion

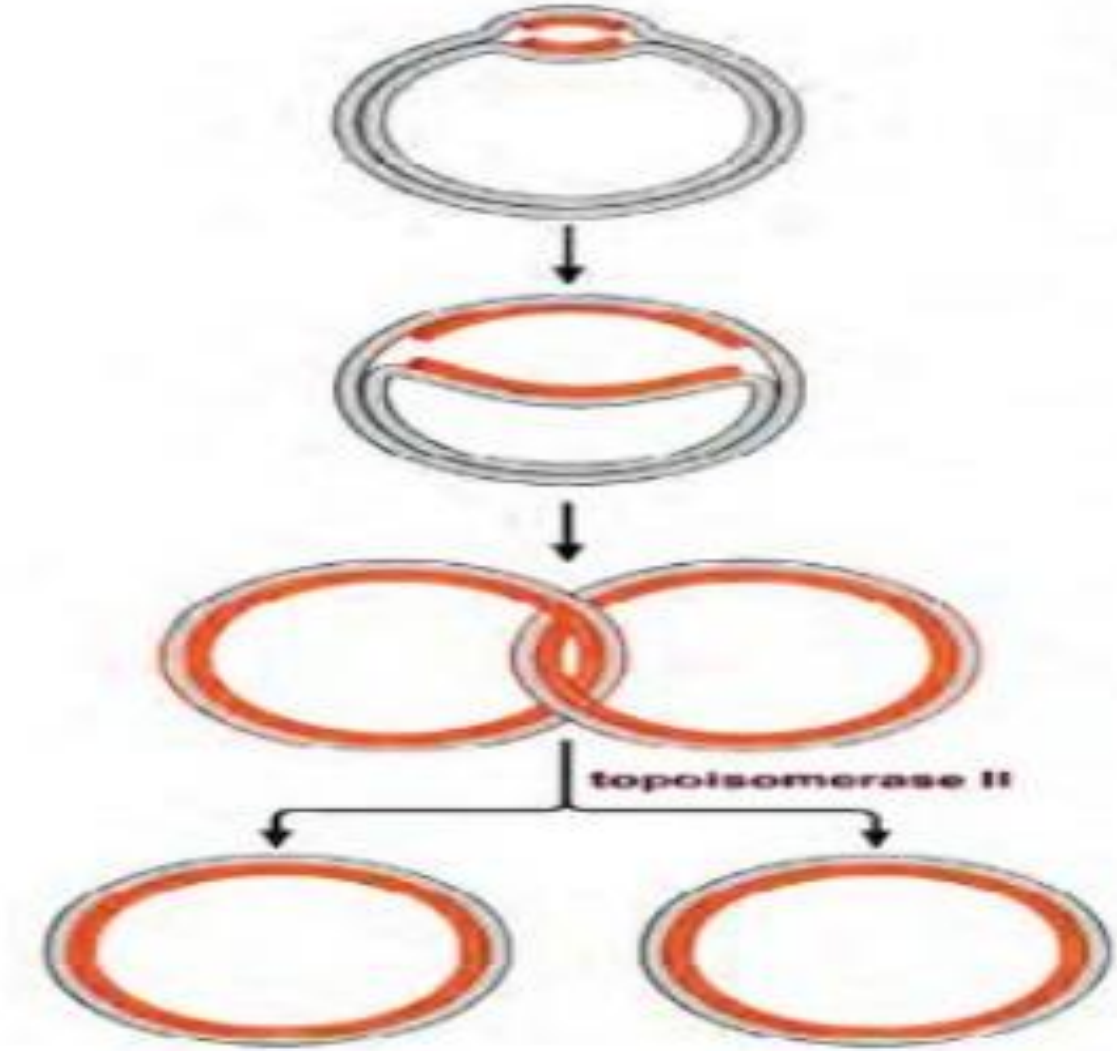


Protein complexes of the replication fork



Termination

- *E. coli* is completed through use of termination sequences and the Tus protein
- After replication, daughter DNAs remain linked as catenanes (two linked circles)
- Topoisomerase IV catalyzes the decatenation
- A break is made in one daughter molecule and second molecule pass through the break



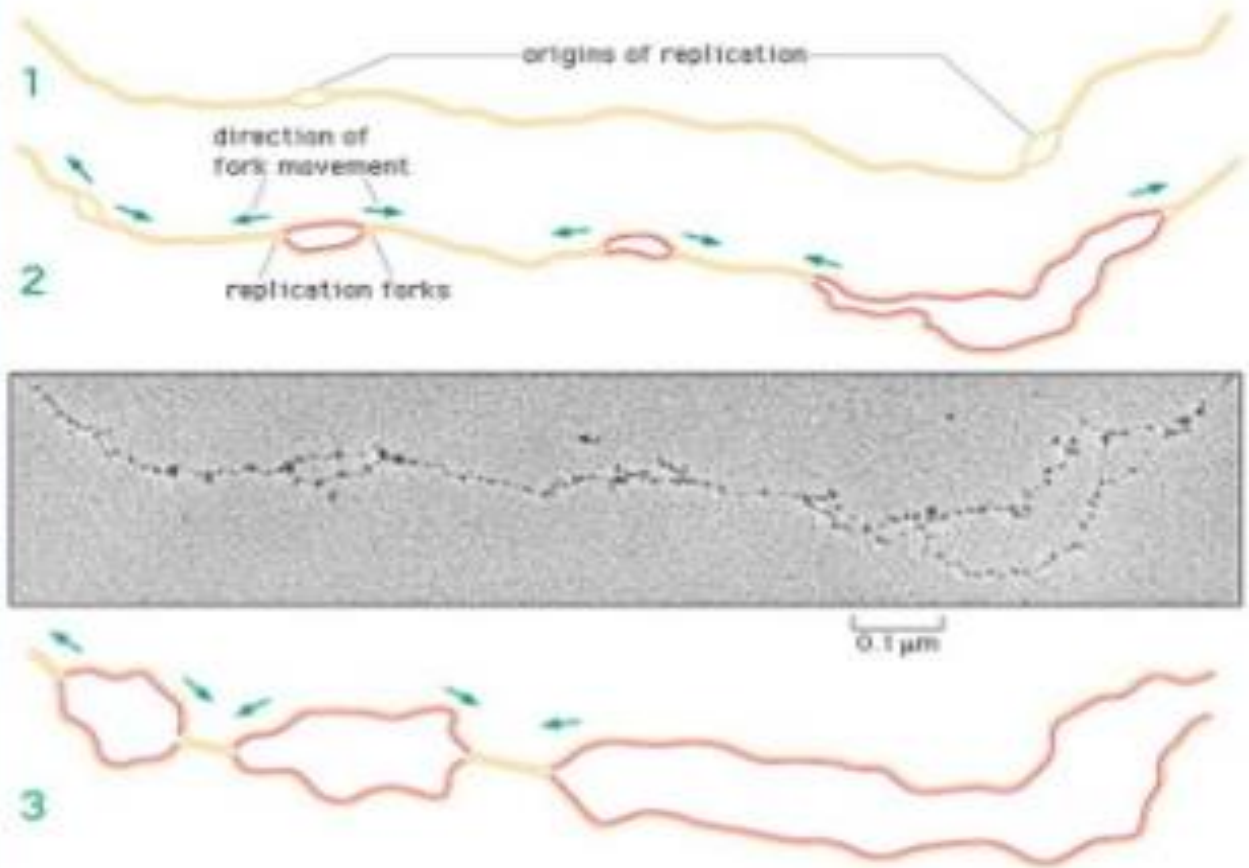
DNA Replication in Eukaryotes

- Multiple replication origins

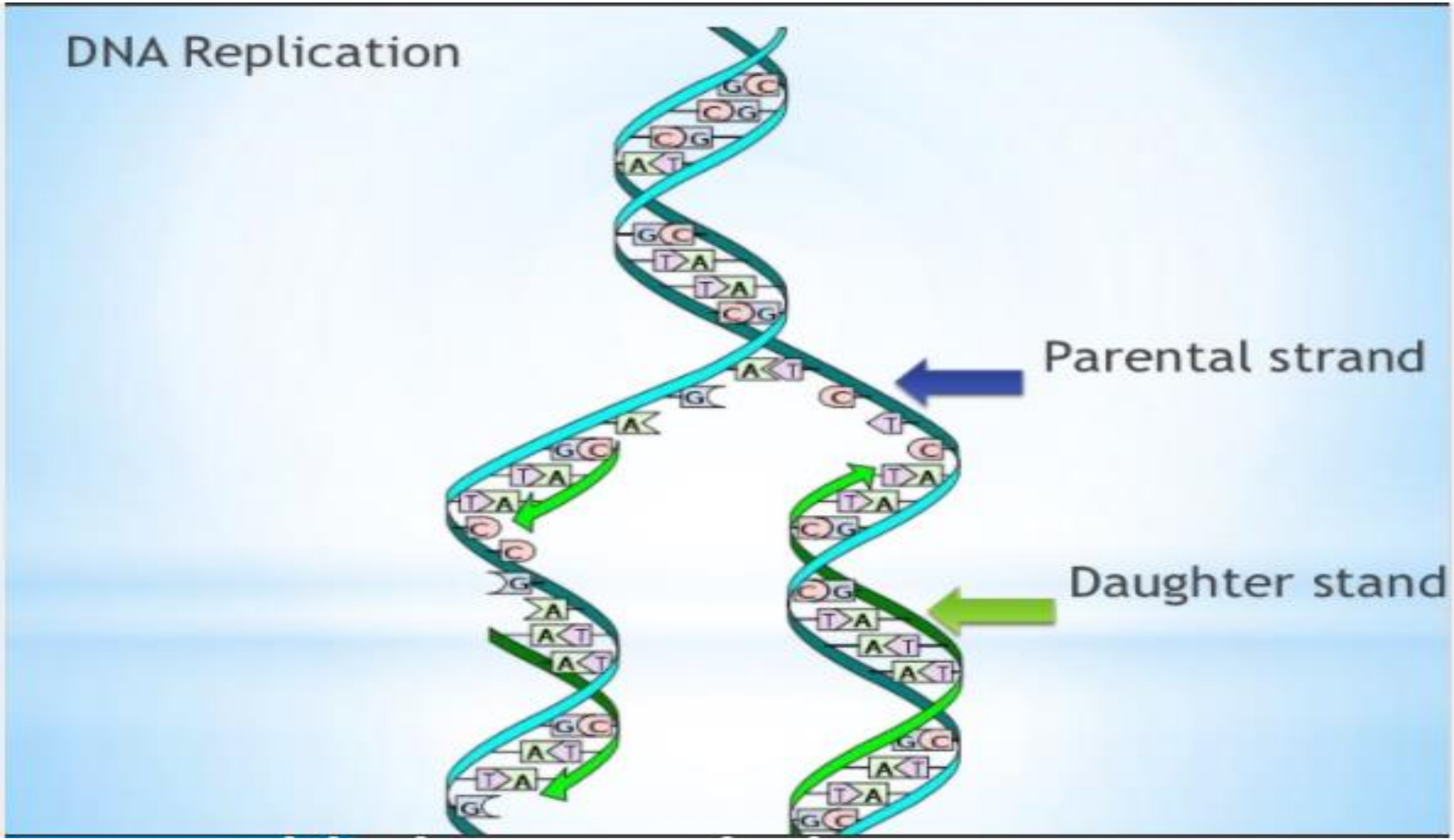
✓ Eukaryotic chromosomes have multiple replication origins

✓ One origin for 3-300kb

✓ Clusters of 20-80 adjacent replicons (DNA segments that are served by a origin) are activated simultaneously



Bidirectional movement of the Eukaryotic DNA replication machinery


DNA Replication
Parental strand
Daughter stand

Eukaryotic DNA Polymerase

DNA polymerase	Activities	Role
α	Polymerase Primase 3′ → 5′ Exonuclease[a]	Primer synthesis Repair
β	Polymerase	Repair
γ	Polymerase 3′ → 5′ Exonuclease	Mitochondrial DNA replication
δ	Polymerase 3′ → 5′ Exonuclease	lagging-strand synthesis Repair
ε	Polymerase 3′ → 5′ Exonuclease 5′ → 3′ Exonuclease	Leading-strand synthesis Gap filling on lagging strand

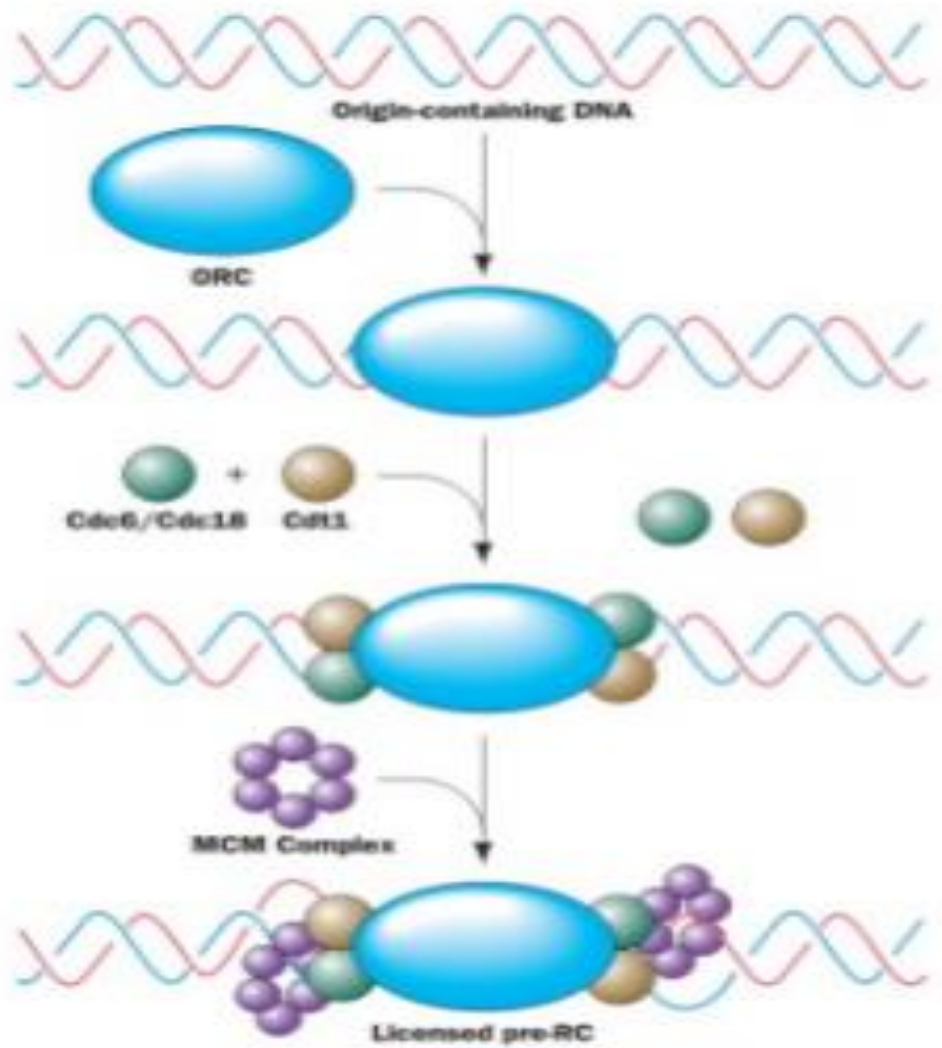
Eukaryotic Initiation Complex

Pre-replicative complex (pre-RC)

- Assembled at each ori. during the **G1 phase**
- **Licensing** – This is the only phase when pre-RC assembles – ensuring single DNA replication/cell cycle
- Pre-RC will be activated only during **S phase** to start replication
- This temporal separation of pre-RC assembly and origin activation ensures that a new pre-RC cannot assemble on an origin that is already "fired"

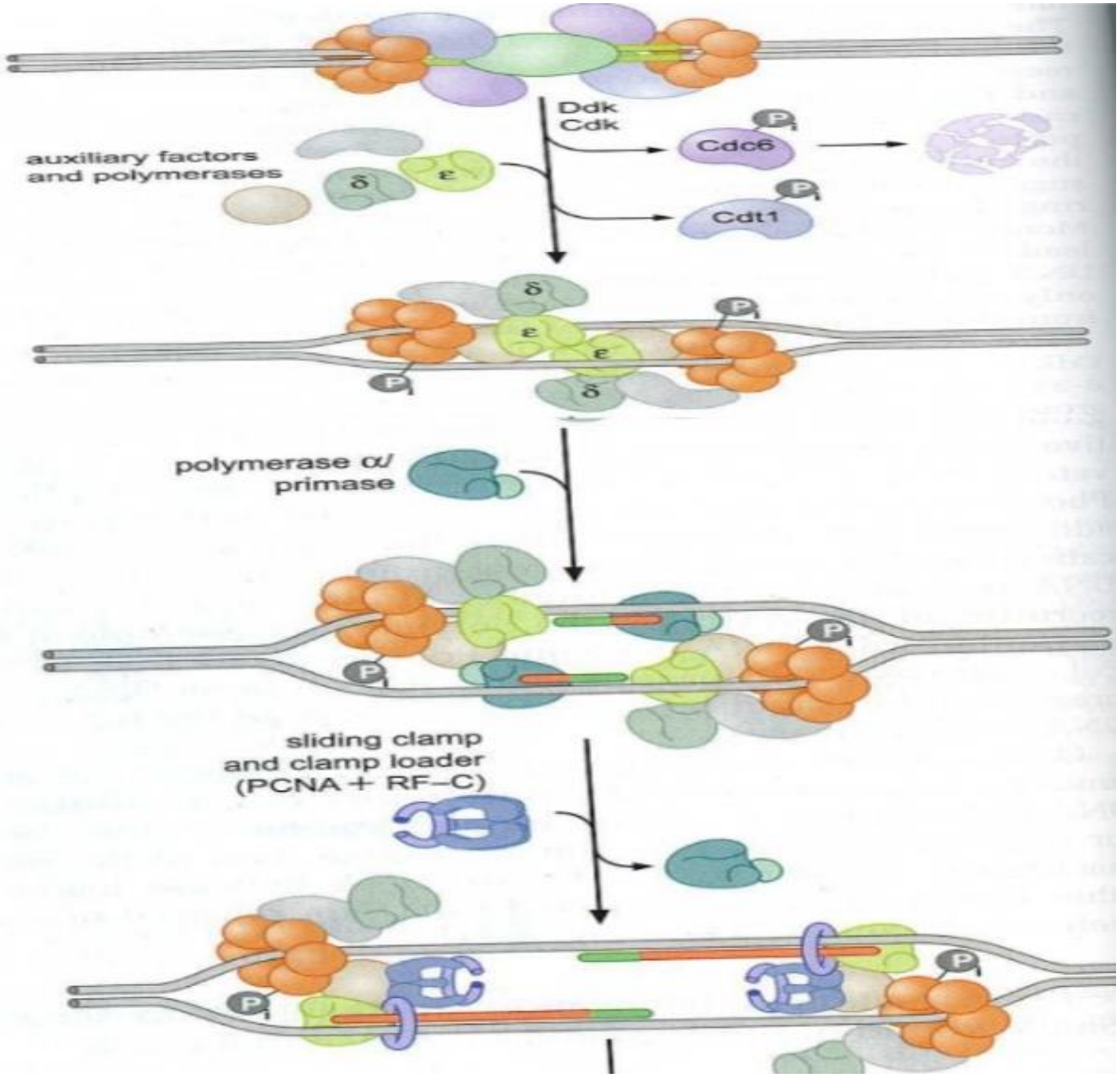
Assembly of licensed pre-RC

- Begins with binding of the origin recognition complex (ORC **hexamer Orc 1-6**) proteins to the origin
- ORC - functional analog of DnaA proteins in prokaryotes
- ORC recruits two proteins, **Cdc6 and Cdt1**
- These proteins then cooperate with the ORC to load the **MCM** complex (hexamer of Mcm2-7, does MiniChromosome Maintenance function)
- Cdc6/Cdc18 + Cdt1 - analog of prokaryote DnaC - facilitates DnaB loading
- MCM complex - **ATP-driven helicase** - analog of prokaryote DnaB helicase



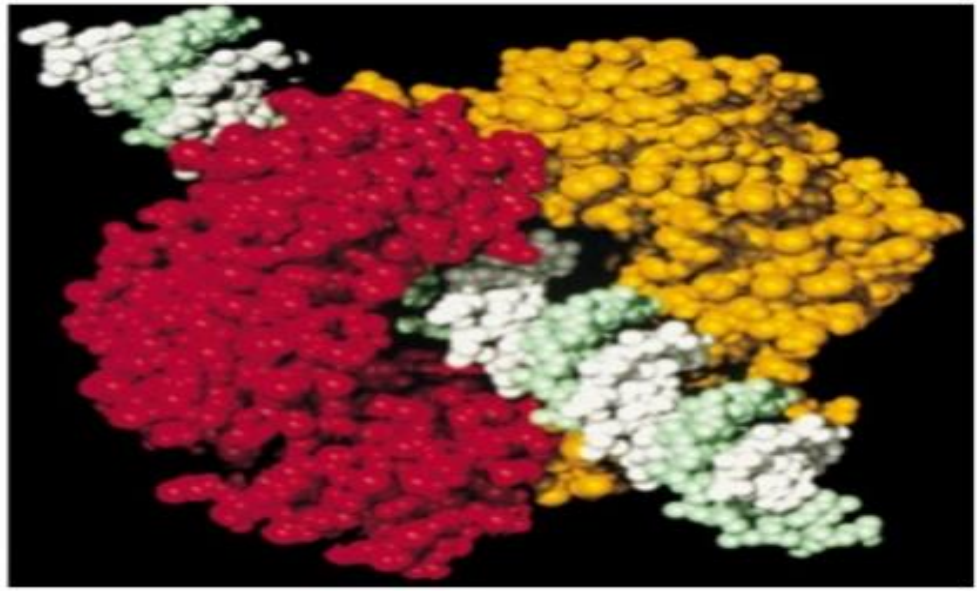
Activation of licensed pre-RC to initiation complex

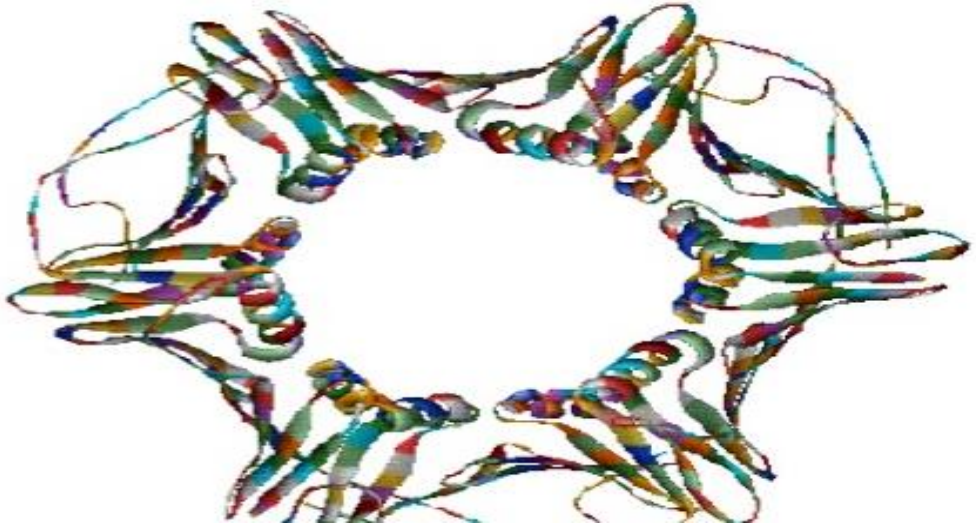
- Needs the addition of pol α/primase, pol ε, and several accessory proteins
- Process begins with addition of **Mcm10** protein to pre-RC
- Mcm10 displaces Cdt1
- Addition of at least two protein kinases, a **Cdk and Ddk** happens
- Ddk phosphorylates five of the six MCM subunits (except Mcm2) - activate the MCM complex as a **Helicase**
- Cdc6/Cdc18 and Cdt1are phosphorylated by Cdks(Removal of Cdc6 and Cdt1 molecules)
- Ddk together with a Cdk also recruits **Cdc45**
- Cdc45 – assembles the initiating synthetic machinery at the replication fork (**pol α/primase + pol ε + PCNA + replication protein A** (RPA which is the heterotrimeric eukaryotic counterpart of SSB)


Ddk
Cdk
auxiliary factors
and polymerases
δ
ε
Cdc6
Cdt1
P
δ
ε
ε
δ
P
P
polymerase α/
primase
P
P
sliding clamp
and clamp loader
(PCNA + RF–C)
P
P

Proliferating Cell Nucleus Antigen(PCNA)

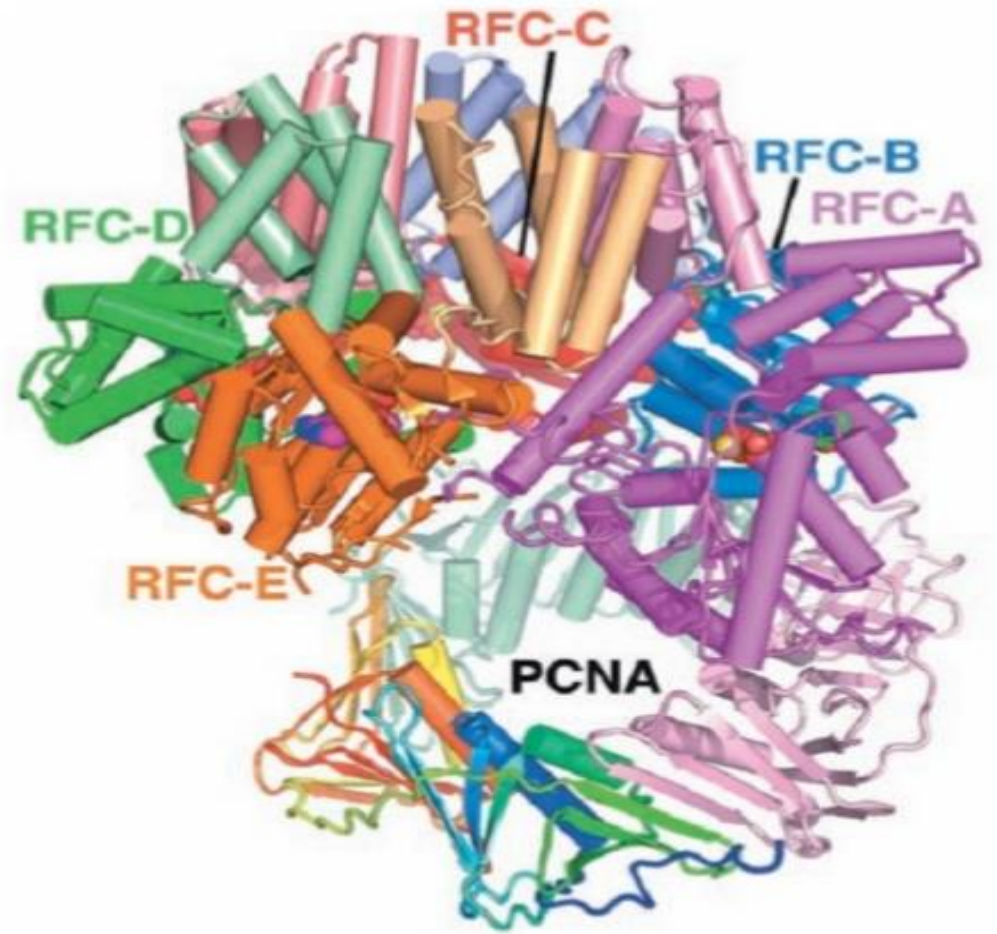
- Encircles DNA
- Acts as DNA clamp same as β-clump in prokaryotes
- DNA Pol is not very processive (falls off the DNA easily)
- It keeps DNA Pol. on and help to slide along strand





Replication Factor-C(RF-C)

- Eukaryotic clamp loader function same as DnaC in prokaryotes
- Heteropentamer – A-E subunits
- C-terminals of each subunits A-E associate to form a ring-shaped collar (as the C-terminal domains of the *E. coli* clamp loader)



Lagging strand synthesis

- Pol α/primase generates RNA primers and extends it by ~20 nt
- Pol α lacks proofreading (this primer extension will have more errors than the DNA synthesized by pol δ)
- When Pol. δ reaches the previously synthesized Okazaki fragment, it partially displaces RNA primer through DNA synthesis
- This generates an RNA flap

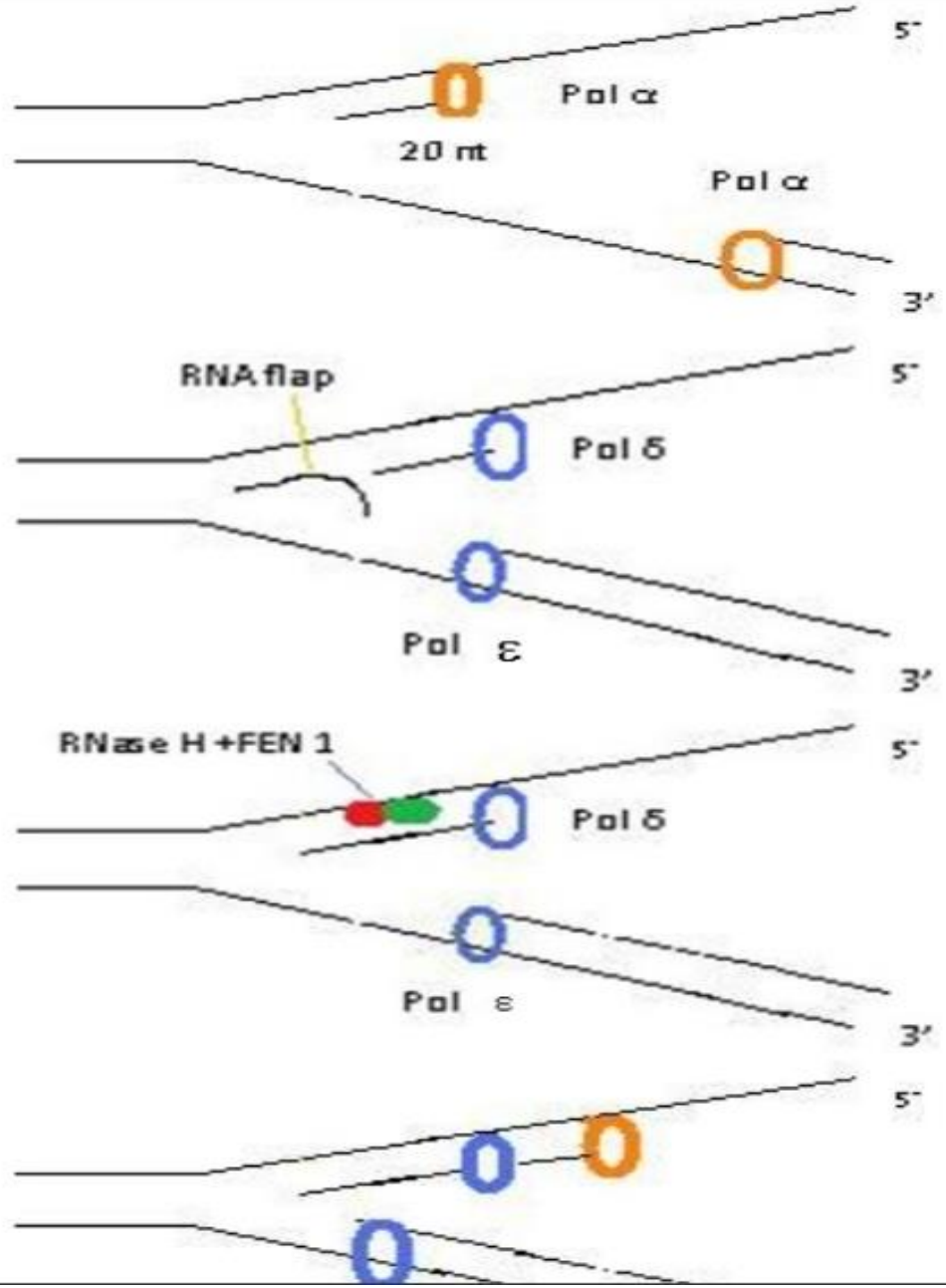
- The primer is then removed by two enzymes

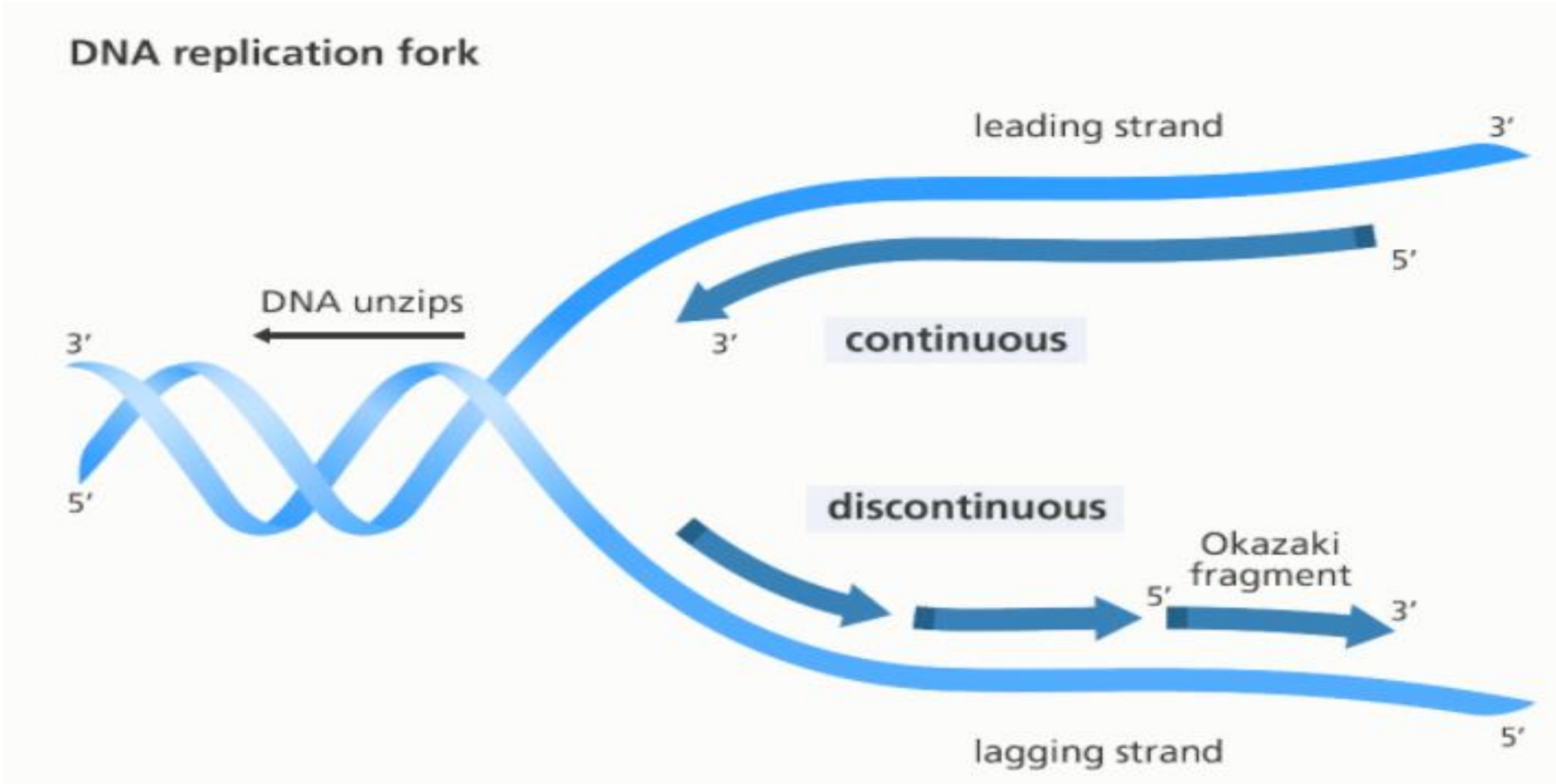
  (i) **RNase H1** - removes most of the RNA, leaving only a 5' ribonucleotide adjacent to the DNA

  (ii) **Flap endonuclease-1 (FEN1)** – removes 5' ribonucleotide and effects 5'-3' proof reading

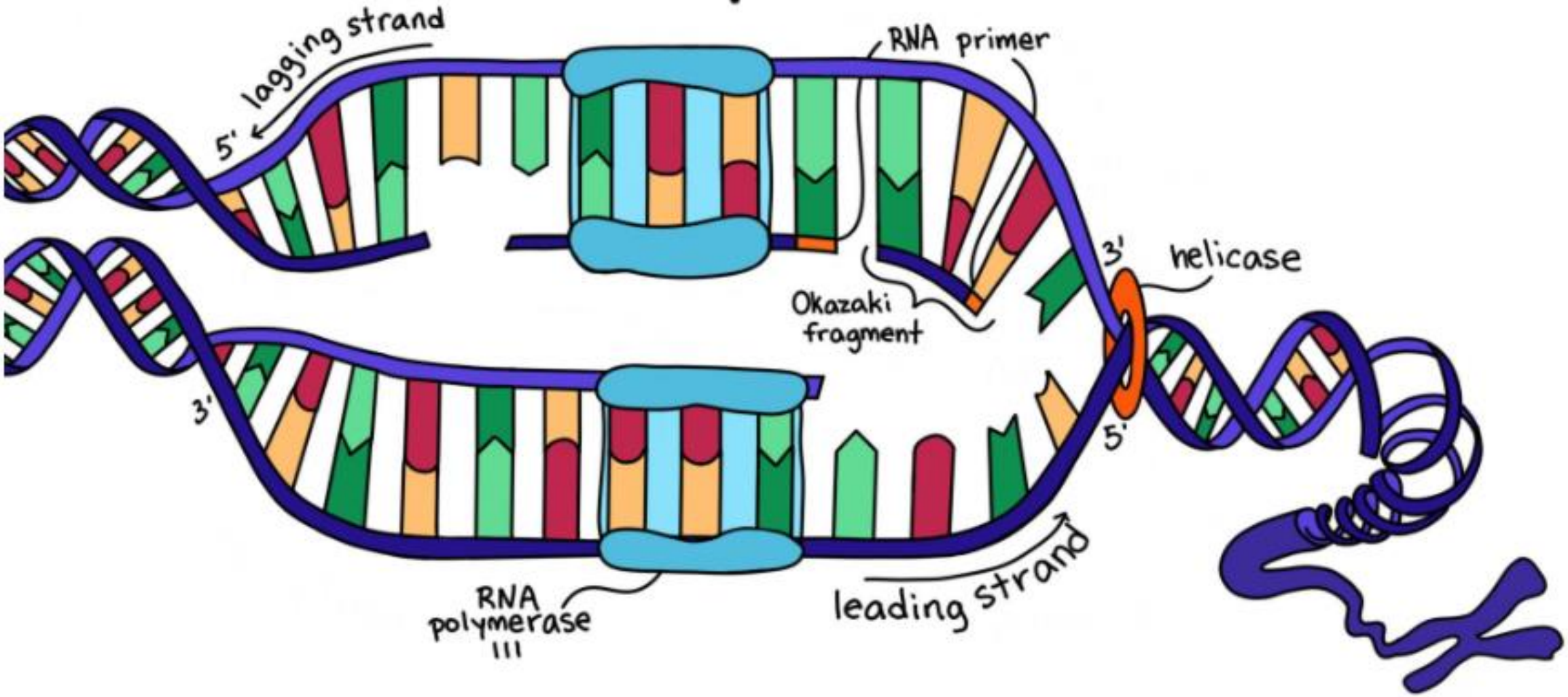
- Any excision during the FEN1 proofreading is later replaced by pol δ, during succeeding Okazaki fragment synthesis

- Eukaryotes lack termination sequences and proteins analogous to *Ter* sites and Tus protein in prokaryotes

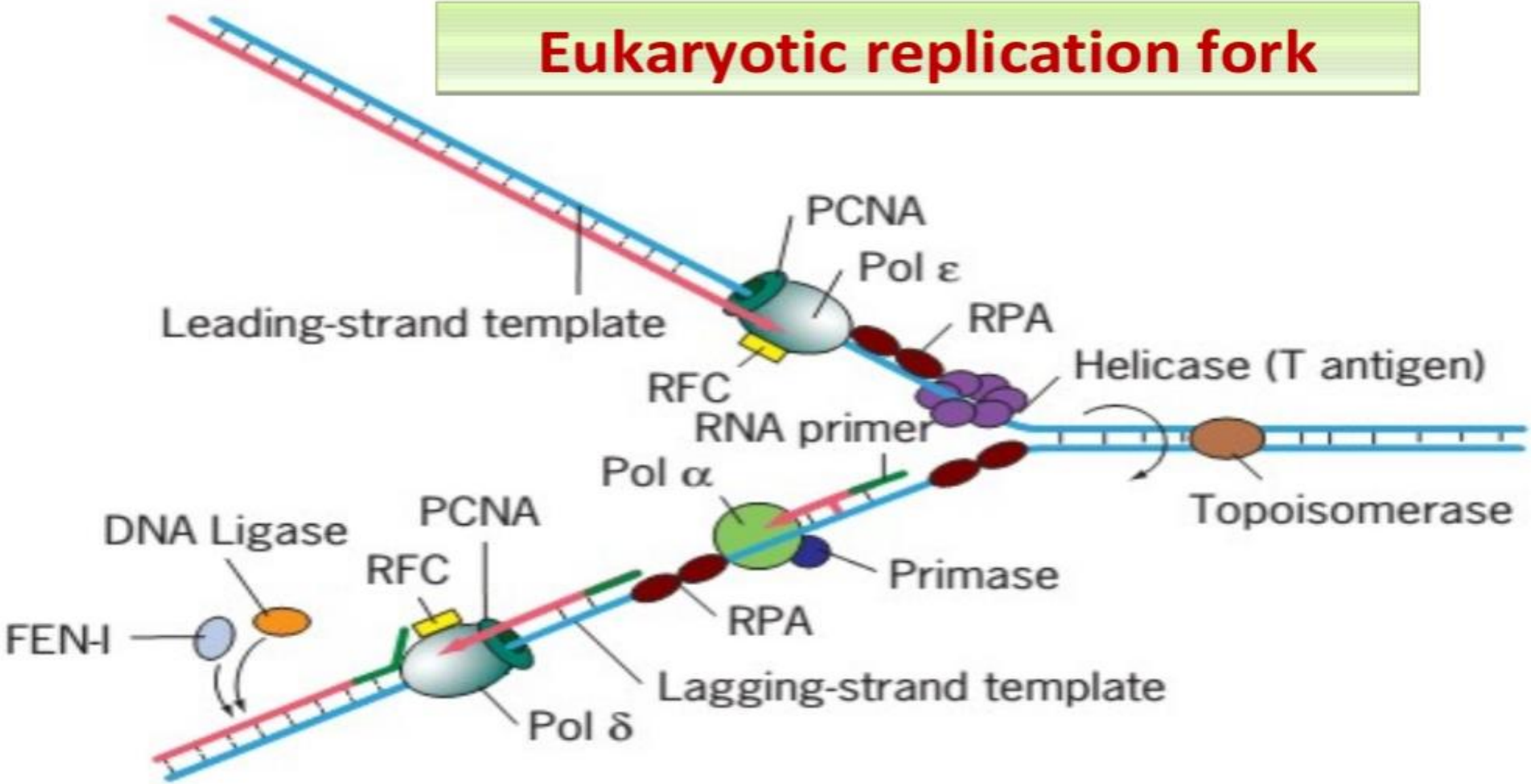


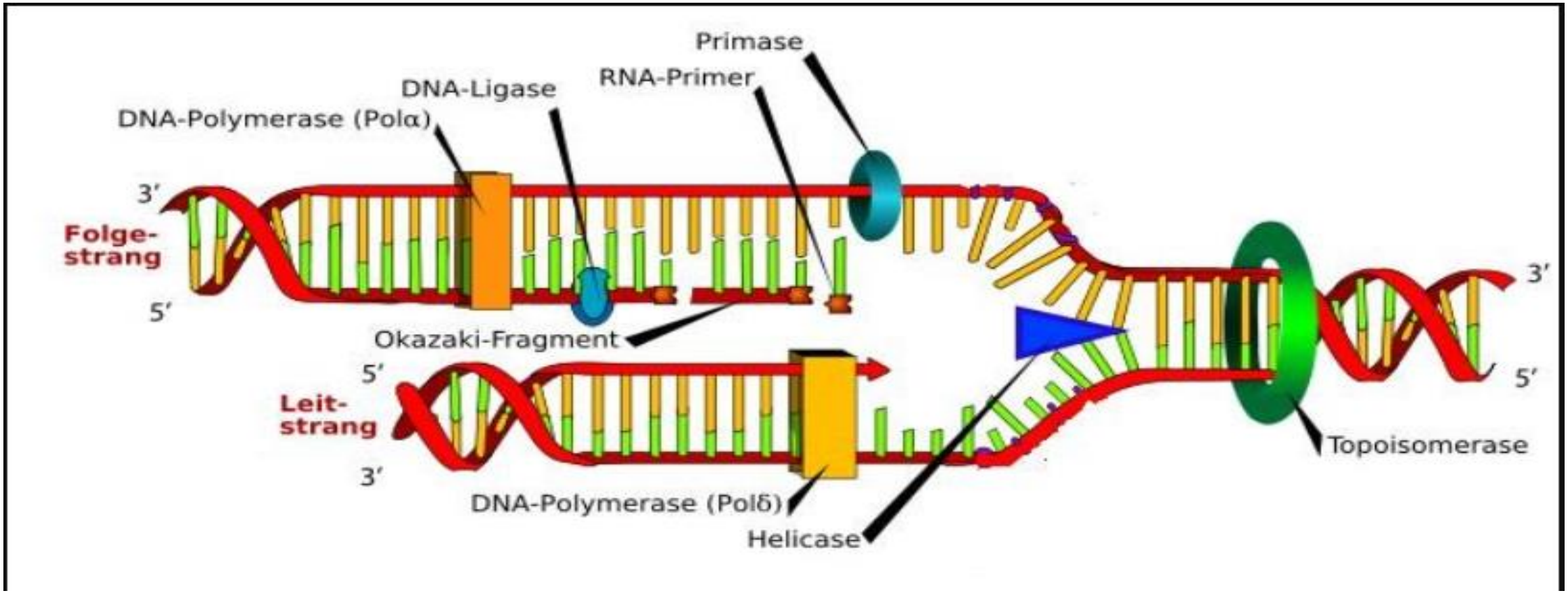

DNA replication fork
leading strand
3′
5′
DNA unzips
3′
3′
continuous
5′
discontinuous
Okazaki
fragment
5′
3′
5′
lagging strand

DNA replication



Eukaryotic replication fork





The replication fork,

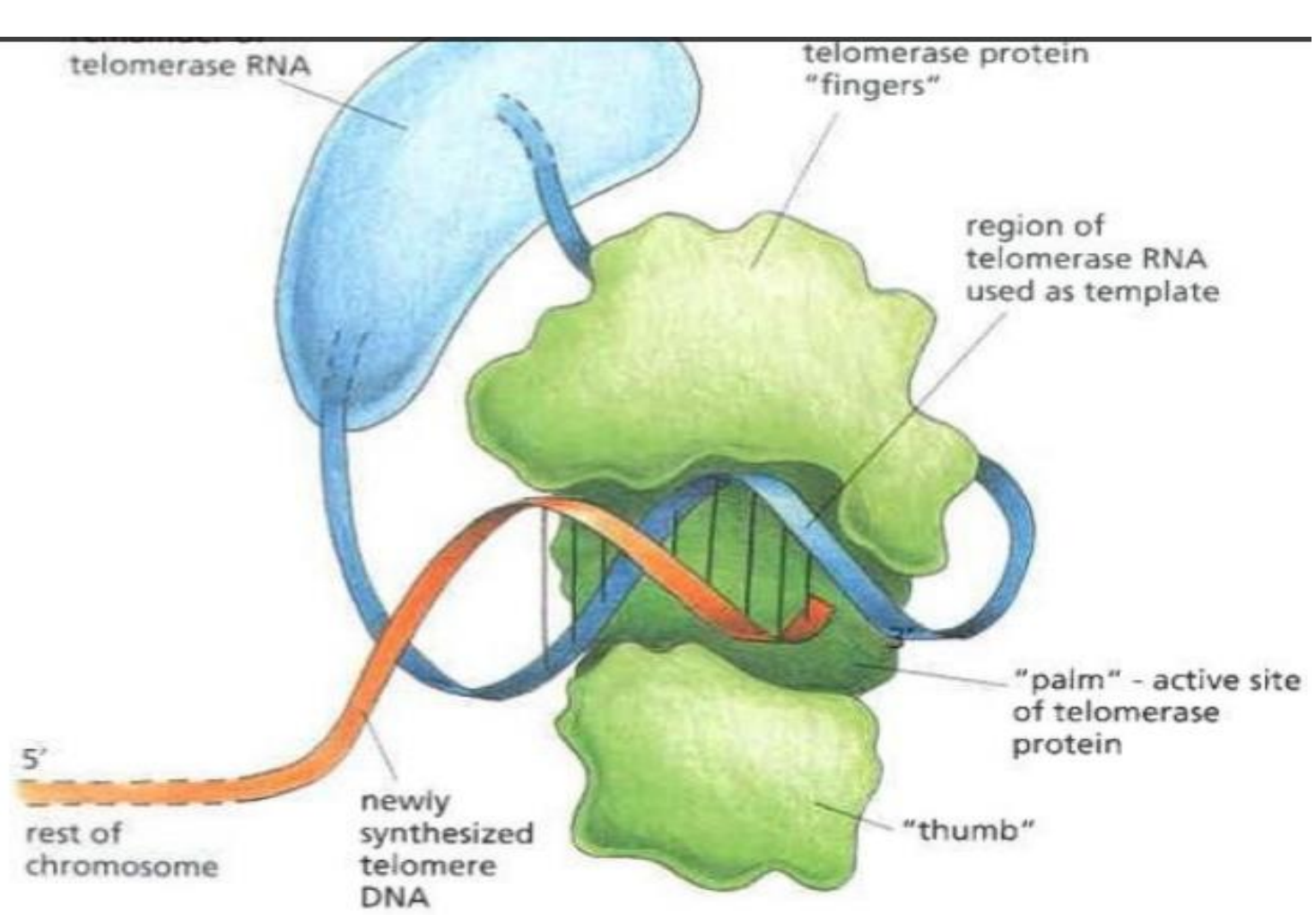
Telomeric Replication

Telomeres of linear chromosomes

- Repair polymerases & ligase cannot fill gap at end of chromosome after RNA primer is removed
- If this gap is not filled, chromosomes would become shorter each round of replication

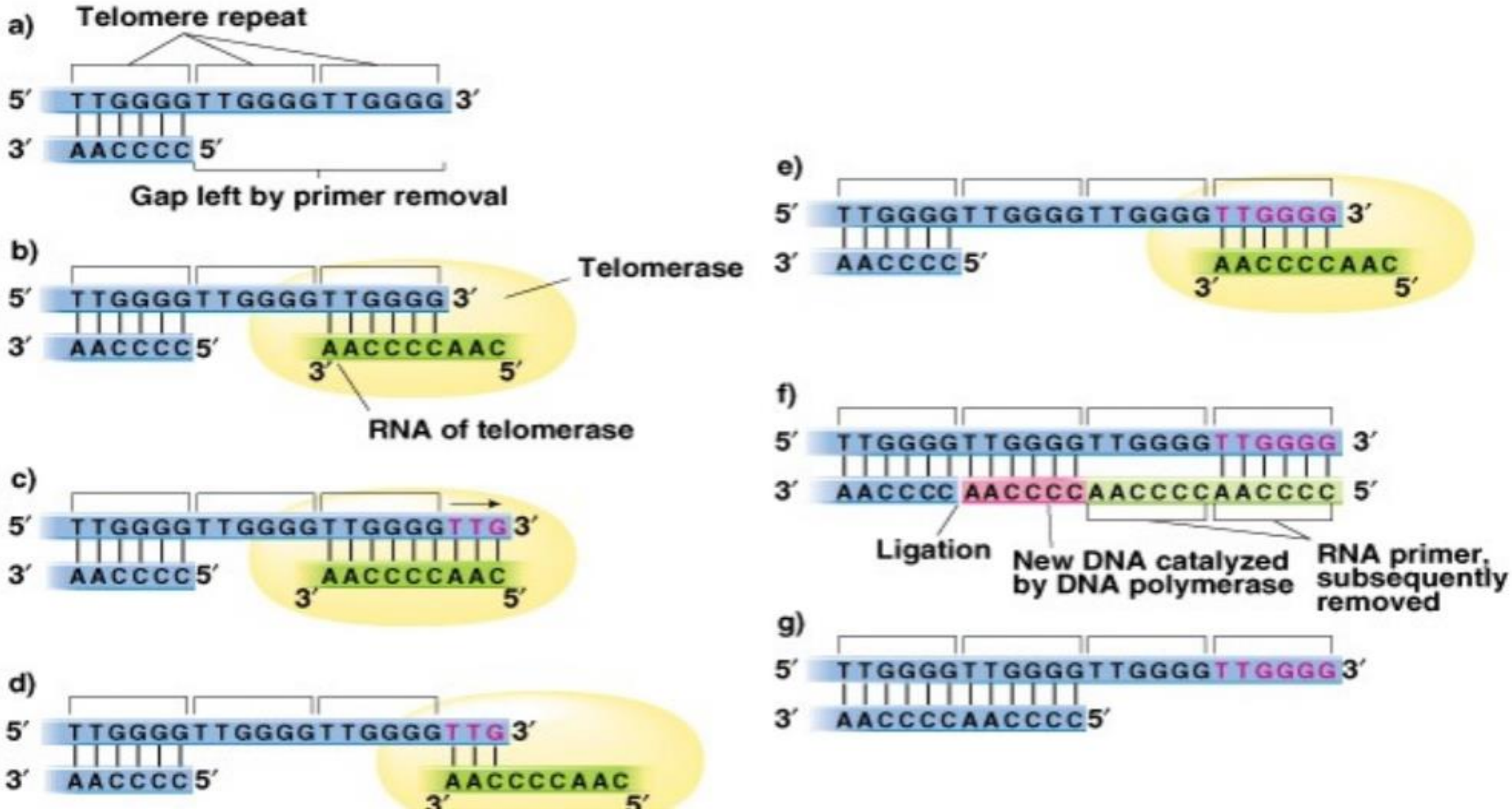
Telomerase

- A large protein-RNA complex
- The RNA (blue) contains a templating sequence for synthesizing new DNA telomere repeats.
- The synthesis reaction is done by the **reverse transcriptase domain** of the protein (green)



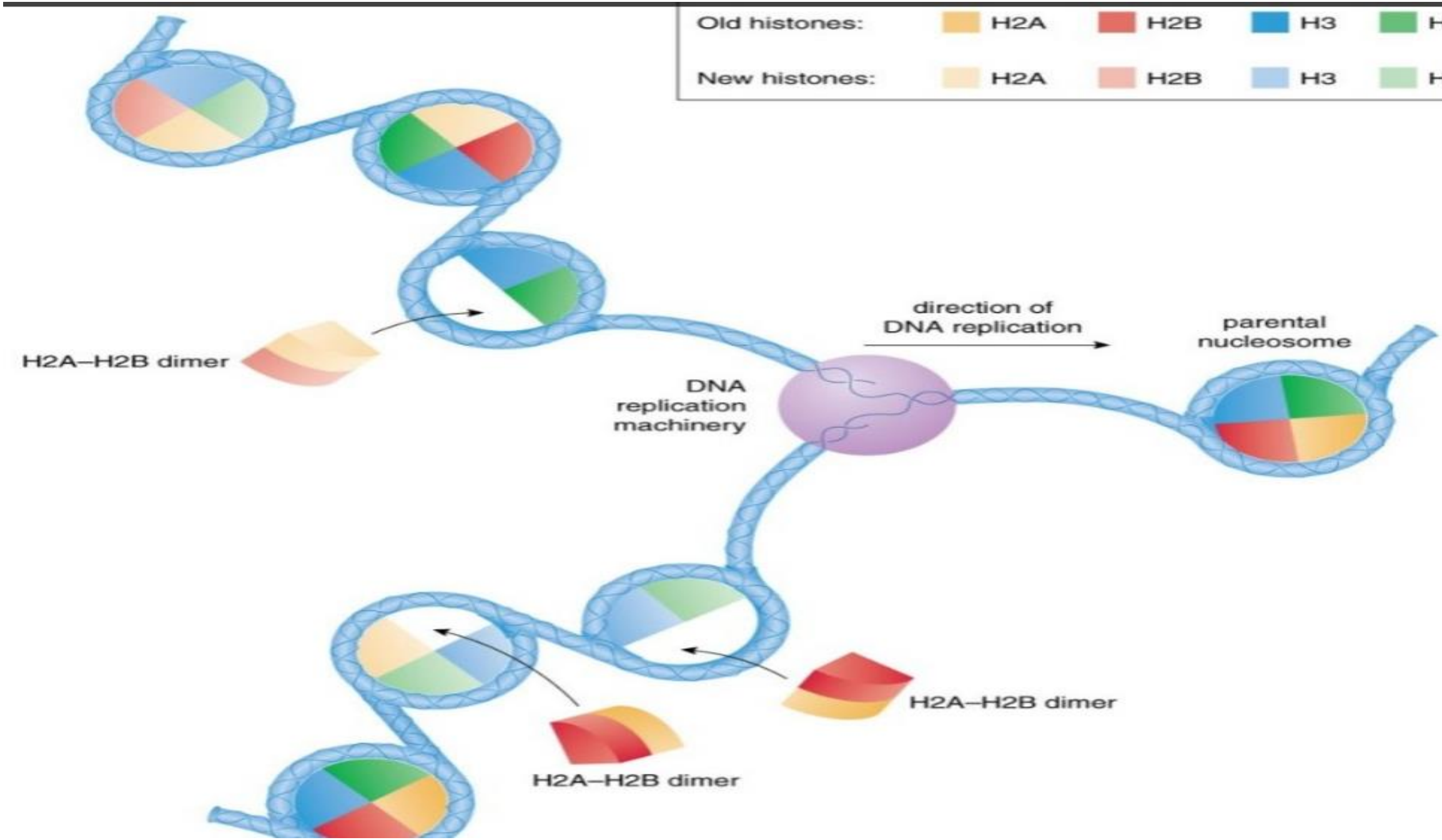
- Telomerase – carries its own RNA template with it at all times
- Telomerase also has several additional protein domains needed to assemble the enzyme at the ends of chromosomes properly

Synthesis of telomeric DNA by telomerase

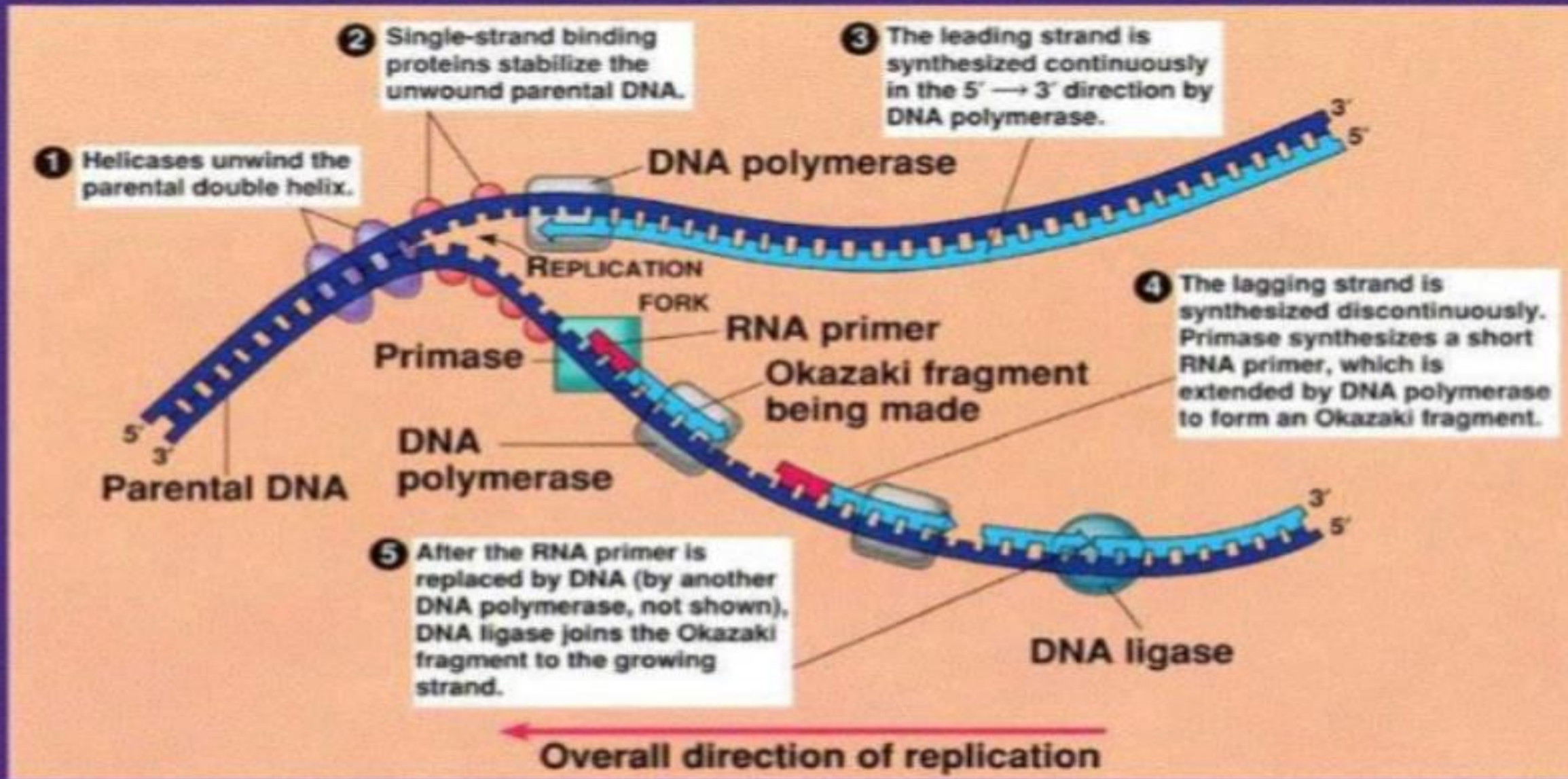


Final Step - Assembly into Nucleosomes

- As DNA unwinds, nucleosomes must disassemble
- Histones and the associated chromatin proteins must be duplicated by new protein synthesis
- Newly replicated DNA is assembled into nucleosomes almost immediately
- Histone chaperone proteins control the assembly


Old histones:
H2A
H2B
H3
H4
New histones:
H2A
H2B
H3
H4
direction of DNA replication
parental nucleosome
H2A–H2B dimer
DNA replication machinery
H2A–H2B dimer
H2A–H2B dimer

A SUMMARY OF DNA REPLICATION



Summary

E. coli protein	Eukaryotic protein	Function
DnaA	ORC proteins	Recognition of origin of replication
Gyrase	Topoisomerase I/II	Relieves positive supercoils ahead of replication fork
DnaB	Mcm	DNA helicase that unwinds parental duplex
DnaC	Cdc6, Cdt1	Loads helicase onto DNA
SSB	RPA	Maintains DNA in single-stranded state
γ-complex	RFC	Subunits of the DNA polymerase holoenzyme that load the clamp onto the DNA
pol III core	pol δ/ε	Primary replicating enzymes; synthesize entire leading strand and Okazaki fragments; have proofreading capability
β clamp	PCNA	Ring-shaped subunit of DNA polymerase holoenzyme that clamps replicating polymerase to DNA; works with pol III in *E. coli* and pol δ or ε in eukaryotes
Primase	Primase	Synthesizes RNA primers
———	pol α	Synthesizes short DNA oligonucleotides as part of RNA–DNA primer
DNA ligase	DNA ligase	Seals Okazaki fragments into continuous strand
pol I	FEN-1	Removes RNA primers; pol I of *E. coli* also fills gap with DNA

Replication fork:

- Where the DNA strands split off
- Moves with the path of the replication bubble

Primer:

Short sequence of RNA that binds to the lagging parental strand in order for DNA polymerase to copy the lagging strnad

Primase:

A protein that binds to the parental lagging strand to make a primer

Helicase:

A protein that unwinds DNA

Single strand binding protein:

A protein that binds each DNA strand to stabilize it and hold it apart

DNA polymerase:

A protein that adds free nucleotides from 5' to 3' to make a new strand of DNA. Also fixes mistakes in DNA

DNA ligase:

A protein that binds the phosphates of nucleic acids to the sugar group of adjacent nucleic acids

Reference

1. Life Science-Fundamental and Practices (part-II). Page no.-95-111

2. Molecular Biology of the Gene 212/755 p. 184

3. Instant notes on Biochemistry, thired edition,p-173

4. e-Sources

5. Classnote (Microbiology)