DNA REPLICATION

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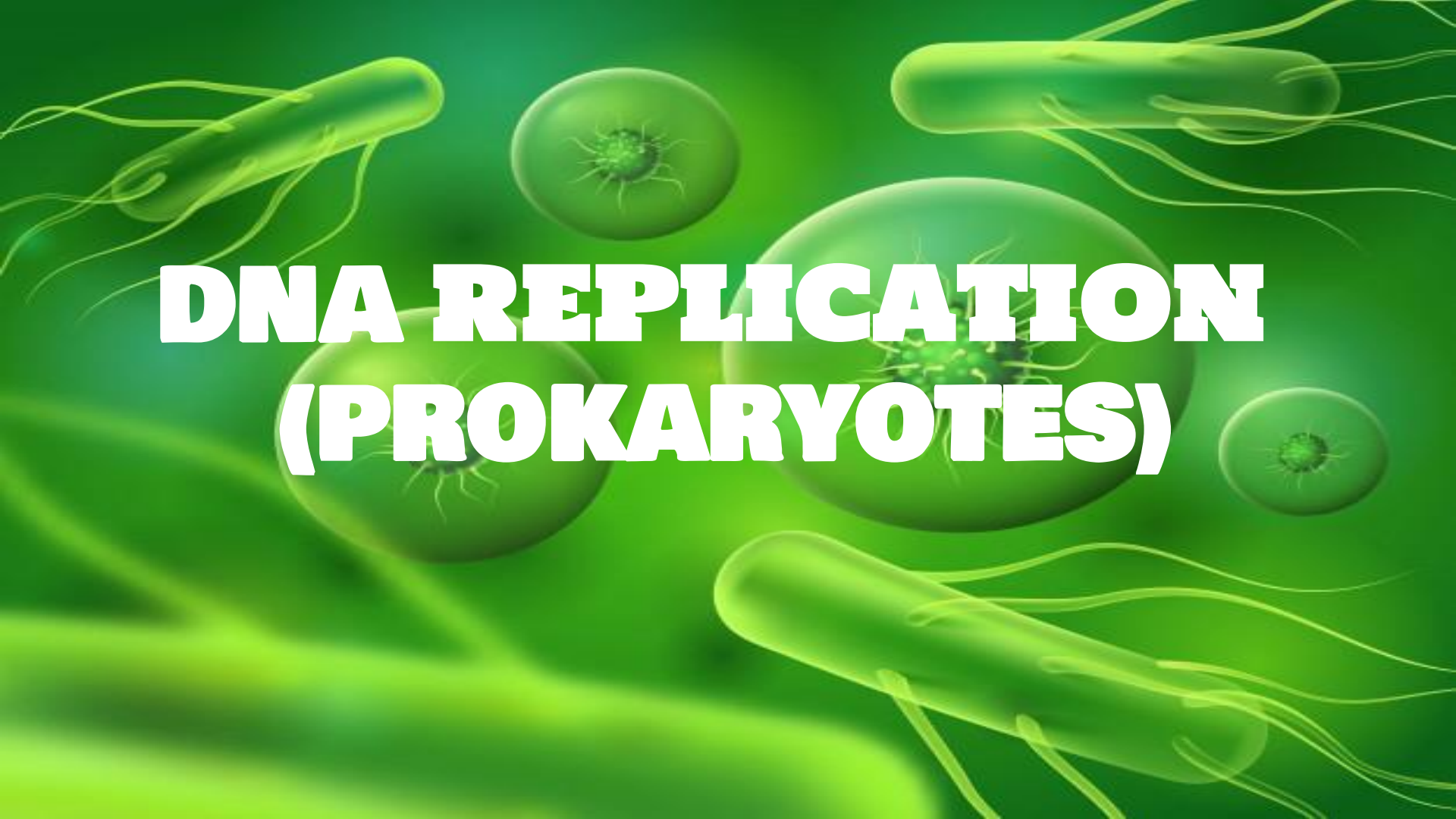
CONTENTS

WHAT IS DNA REPLICATION?

- ★ **DNA replication** is the biological process of producing two identical replicas of DNA from one original DNA molecule. DNA replication occurs in all living organisms acting as the most essential part for biological inheritance.
- ★ It occurs in both prokaryotes and eukaryotes.
- ★ The process is similar in both but differs at some site only.
- ★ This process start in S-PHASE of INTERPHASE in CELL CYCLE


DNA REPLICATION
(PROKARYOTES)

FEATURES OF DNA REPLICATION (PROKARYOTES)

- Replication is bi-directional and originates at a single origin of replication (OriC).
- Takes place in the cell cytoplasm.
- Synthesis occurs only in the 5′to 3′direction.
- Individual strands of DNA are manufactured in different directions, producing a leading and a lagging strand.
- Lagging strands are created by the production of small DNA fragments called Okazaki fragments that are eventually joined together.

1. WHAT DOES BIDIRECTIONAL MEANS?

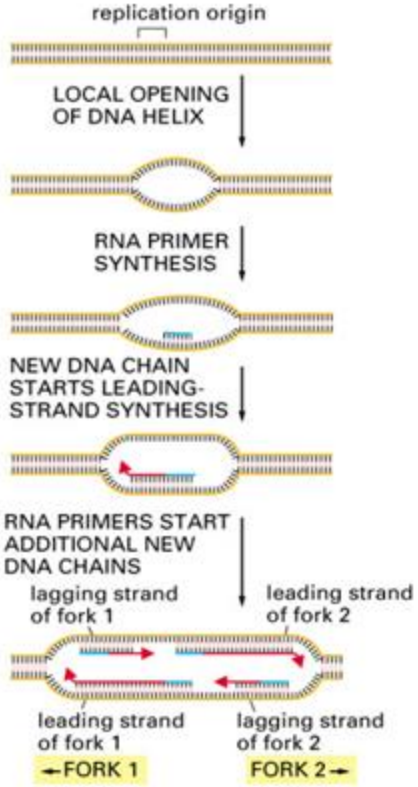
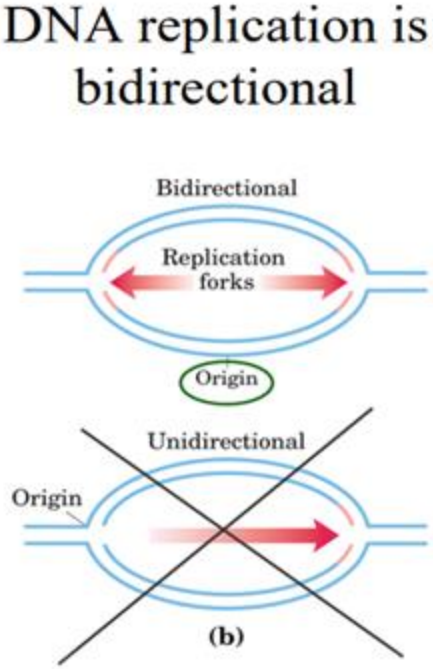
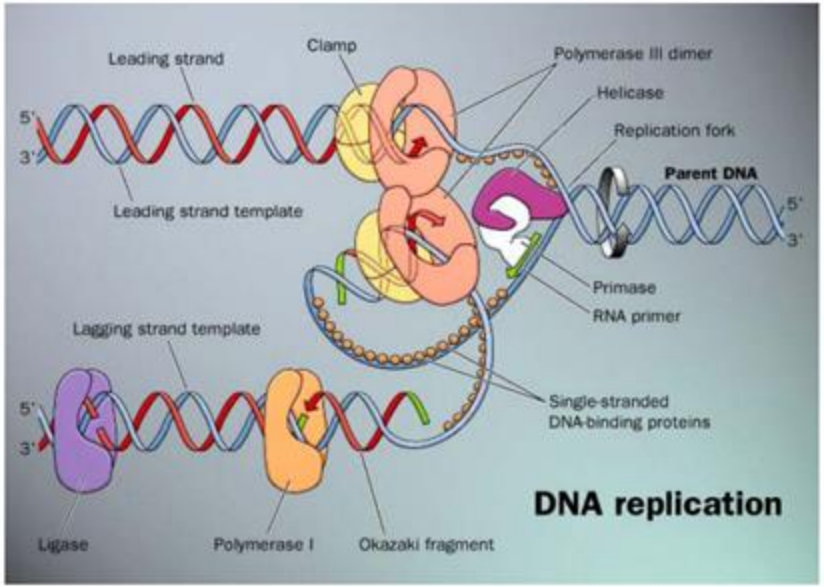


Figure 5–29. Molecular Biology of the Cell, 4th Edition.



2. MULTIENZYME COMPLEX

DNA replication is perfomed by a multienzyme complex >1 MDa

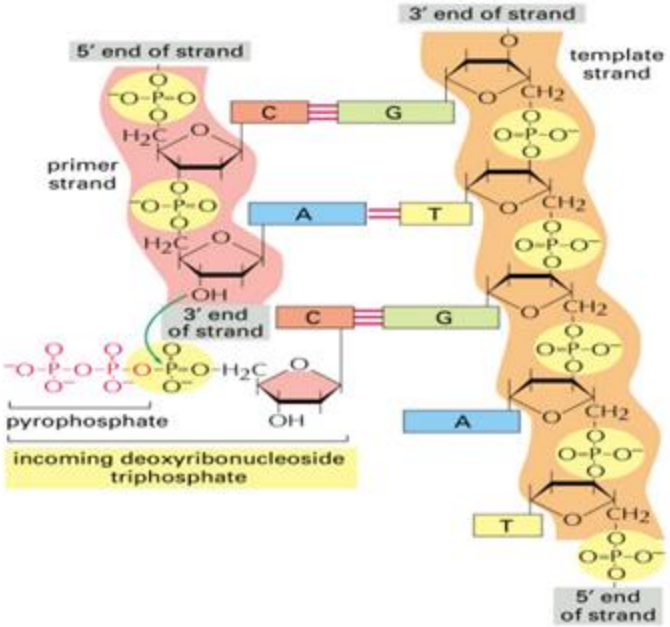


DNA
Nucleotides

Replisome:
DNA polymerases
Helicase
Primase
SSBs
DNA ligase
Clamps
(Topoisomerases)

3. REPLICATION IS SEMICONSERVATIVE

Replication is semiconservative,
accurrate and fast

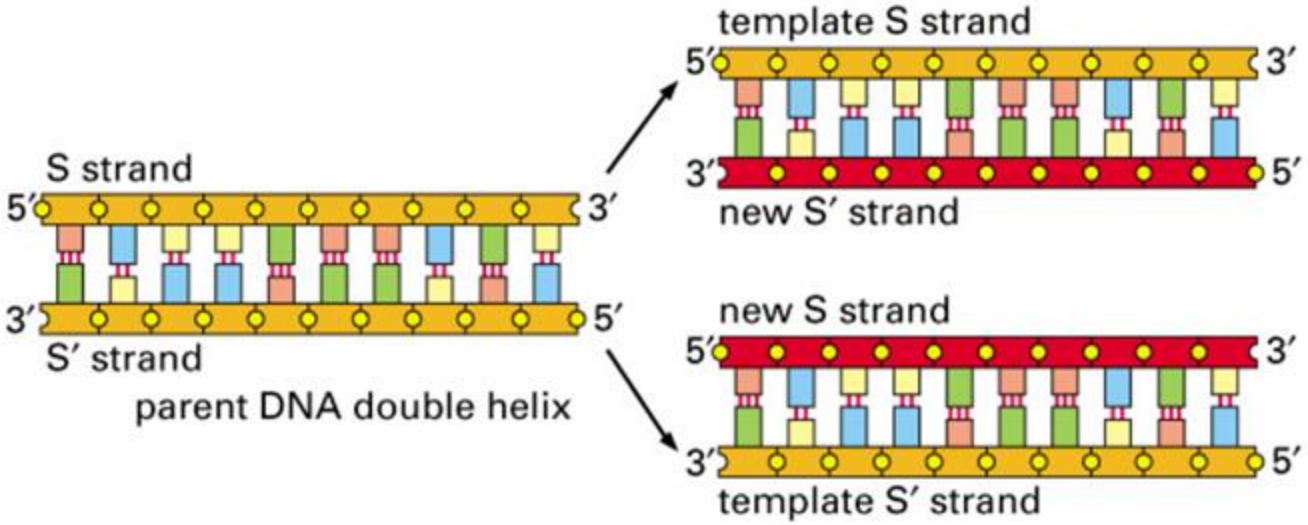


Accuracy
1 error in 1 billion bases

Speed
500 nt/s in bacteria
50 nt/s in mammals

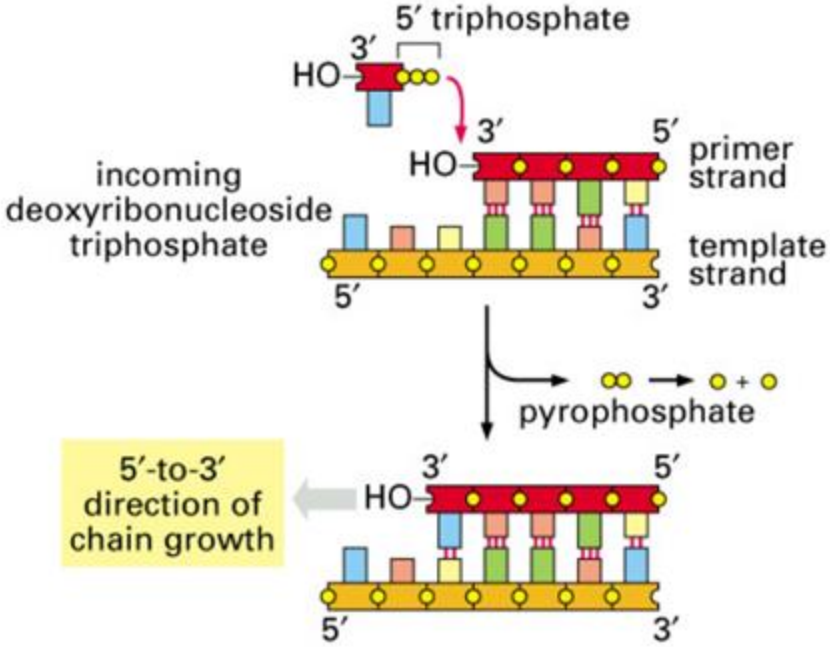
4. TEMPLATE

Each original strand functions as template for DNA synthesis



5. SYNTHESIS DIRECTION 5'-3'

DNA is synthesized in 5′to 3′direction



ENZYMES FOR DNA REPLICATION (PROKARYOTES)

Prokaryotic DNA Replication: Enzymes and Their Function

Enzyme/protein	Specific Function
DNA pol I	Exonuclease activity removes RNA primer and replaces with newly synthesized DNA
DNA pol II	Repair function
DNA pol III	Main enzyme that adds nucleotides in the 5'-3' direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Sliding Clamp	Helps to hold the DNA polymerase in place when nucleotides are being added
Topoisomerase	Helps relieve the stress on DNA when unwinding by causing breaks and then resealing the DNA
Single-strand binding proteins (SSB)	Binds to single-stranded DNA to avoid DNA rewinding back.

DNA REPLICATION

➢ The process of replication occur in the sequential form ie:

1. INITIATION

(a) INITIATION COMPLEX FORMATION
(b) CLOSED COMPLEX FORMATION
(c) OPEN COMPLEX FORMATION

2. ELONGATION

(a) LEADING STRAND SYNTHESIS
(b) LAGGING STRAND SYNTHESIS

3. TERMINATION

DUE TO Ter- Tus complex

1. INITIATION

- DNA replication begins from origin. In E coli, replication origin is called OriC which consists of 245 base pair and contains DNA sequences that are highly conserved among bacterial replication origin. Two types of conserved sequences are found at OriC, three repeats of 13 bp (GATRCTNTTNTTTT) and four/five repeats of 9 bp (TTATCCACA) called 13 mer and 9 mer respectively.

The replication origin OriC in *E.coli*

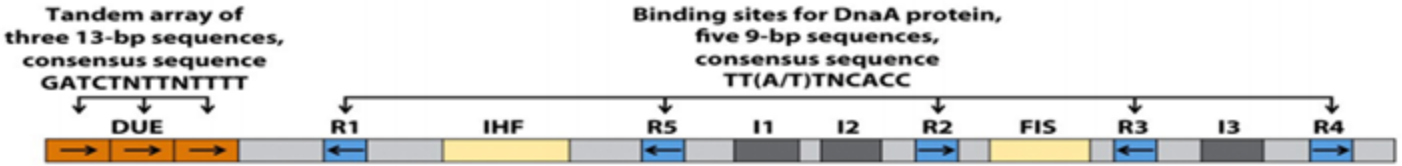
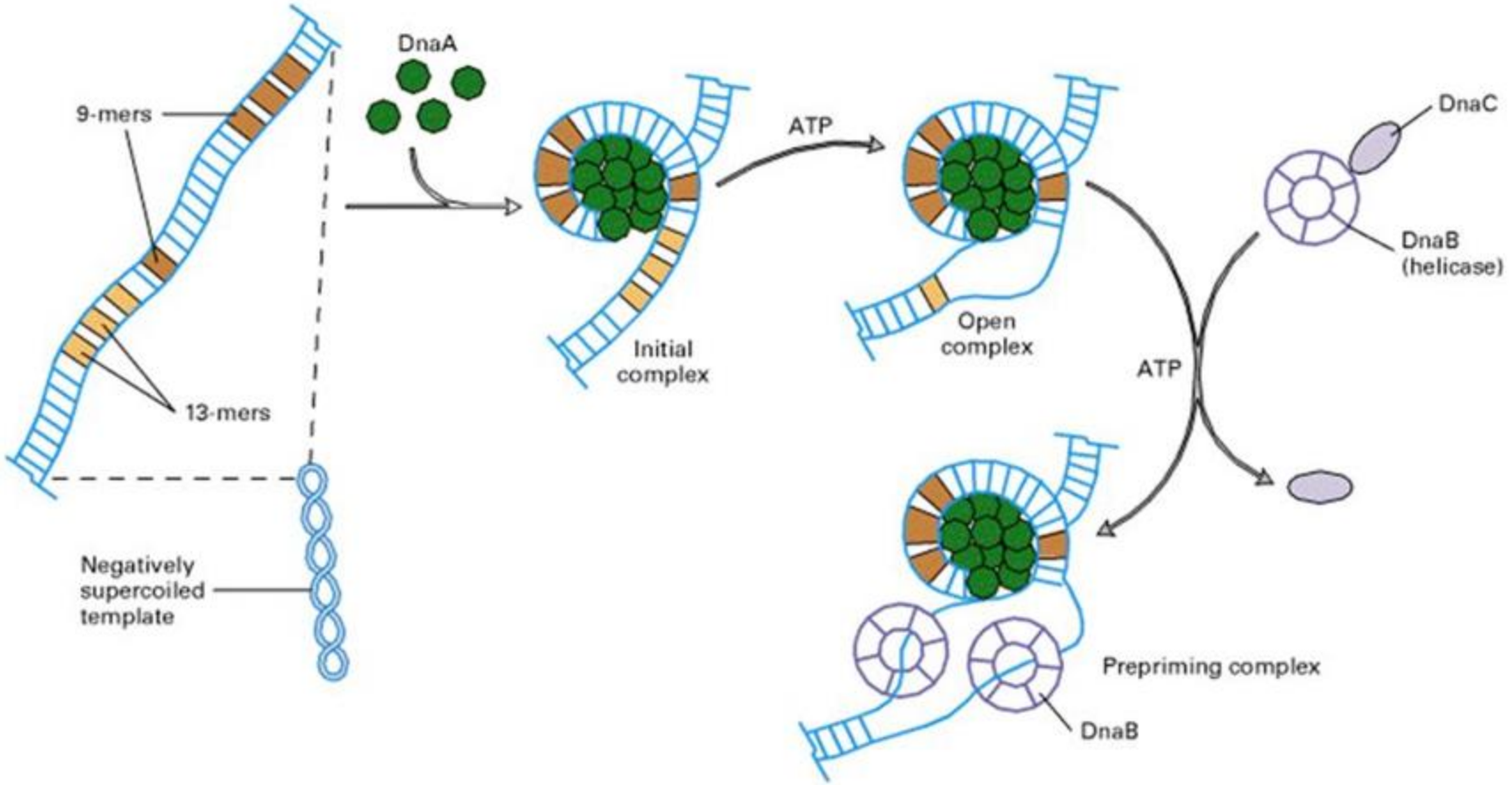
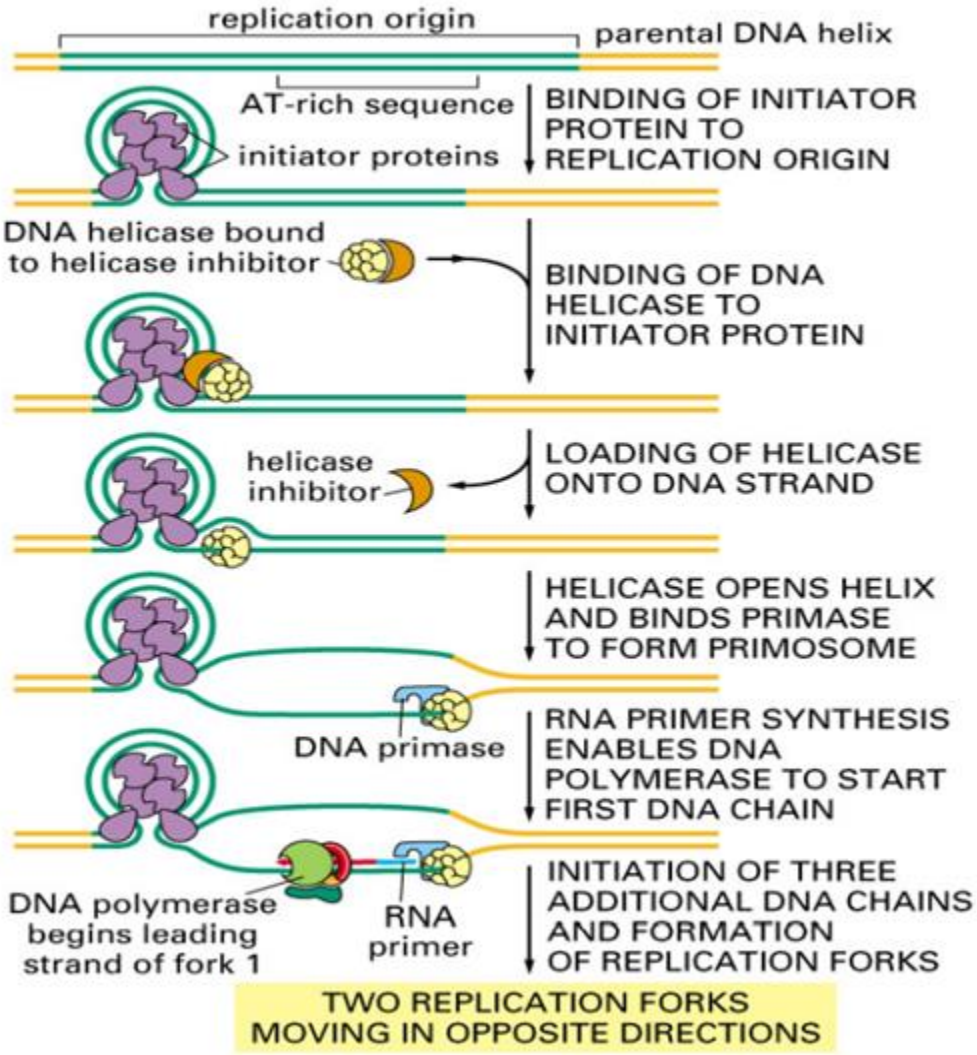


Figure 25-11
Lehninger Principles of Biochemistry, Fifth Edition

245 base pairs
AT-rich
Initiation proteins bind to 9 bp consensus sequence

- About 20 molecules of Dna A proteins binds with 9 mer repeats along with ATP which causes DNA to wraps around dnaA protein forming initial complex. The dna A protein and ATP trigger the opening of 13 mer repeats forming open complex.
- Two copies of dnaB proteins (helicase) binds to 13 mer repeats. This binding is facilitated by another molecule called dnaC. The dnaB - dnaC interaction causes dnaB ring to open which binds with each of the DNA strand. The hydrolysis of bound ATP release dnaC leaving the dnaB bound to the DNA strand.
- The binding of helicase is key step in replication initiation. dnaB migrates along the single stranded DNA in 5'-3' direction causing unwinding of the DNA.
- The activity of helicase causes the topological stress to the unwinded strand forming supercoiled DNA. This stress is relieved by the DNA topoisomerase (DNA gyrase) by negative supercoiling. Similarly, single stranded binding protein(SSB) binds to the separated strand and prevents reannealing of separated strand and stabilize the strand. This leads to formation of REPLICATION BUBBLE.
- The DNA polymerase cannot initiate DNA replication. So, at first primase synthesize 10±1 nucleotide (RNA in nature) along the 5'-3' direction. In case of E.coli primer synthesized by RNA primase starts with ppp-AG-nucleotide. Primer is closely associated with dnaB helicase so that it is positioned to make RNA primer as ssDNA of lagging strand.

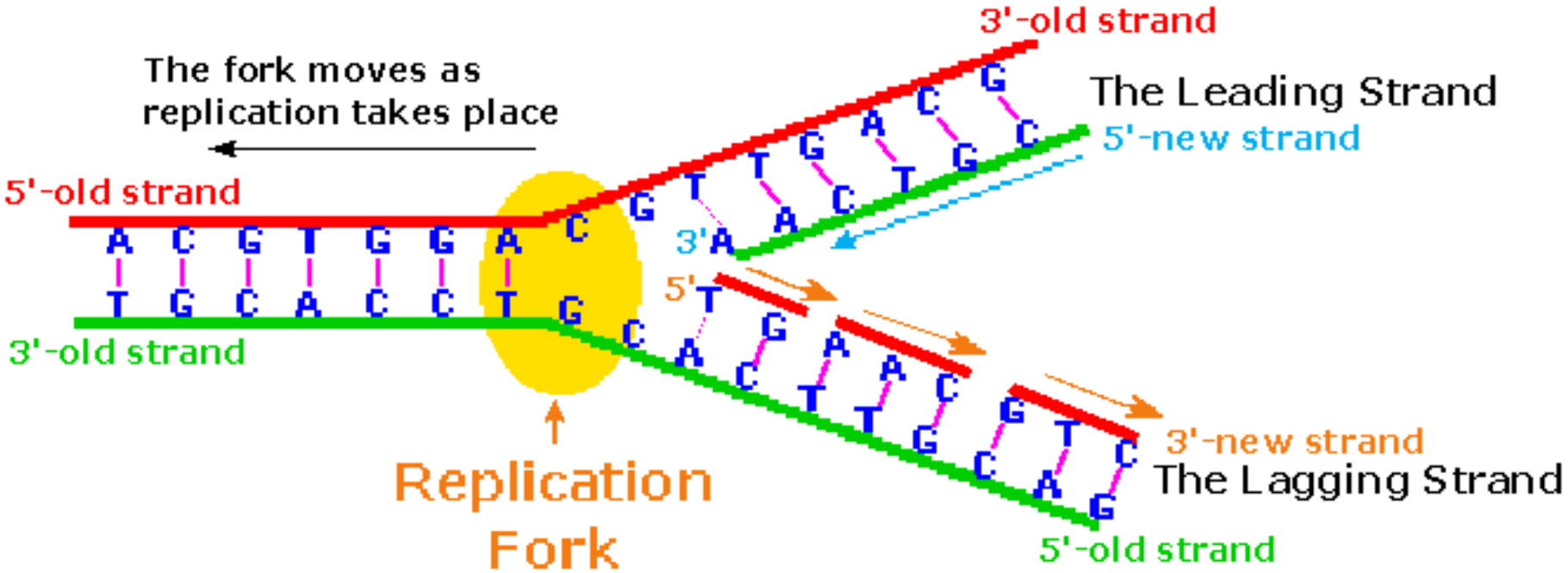

9-mers
13-mers
Negatively supercoiled template
DnaA
Initial complex
ATP
Open complex
DnaC
DnaB (helicase)
ATP
Prepriming complex
DnaB



INITIATION PROCESS IN PROKARYOTES

2. ELONGATION

- **i. LEADING STRAND SYNTHESIS:**
- Leading strand synthesis is more a straightforward process which begins with the synthesis of RNA primer by primase at replication origin.
- DNA polymerase III then adds the nucleotides at 3′end. The leading strand synthesis then proceed continuously keeping pace with unwinding of replication fork until it encounter the termination sequences.

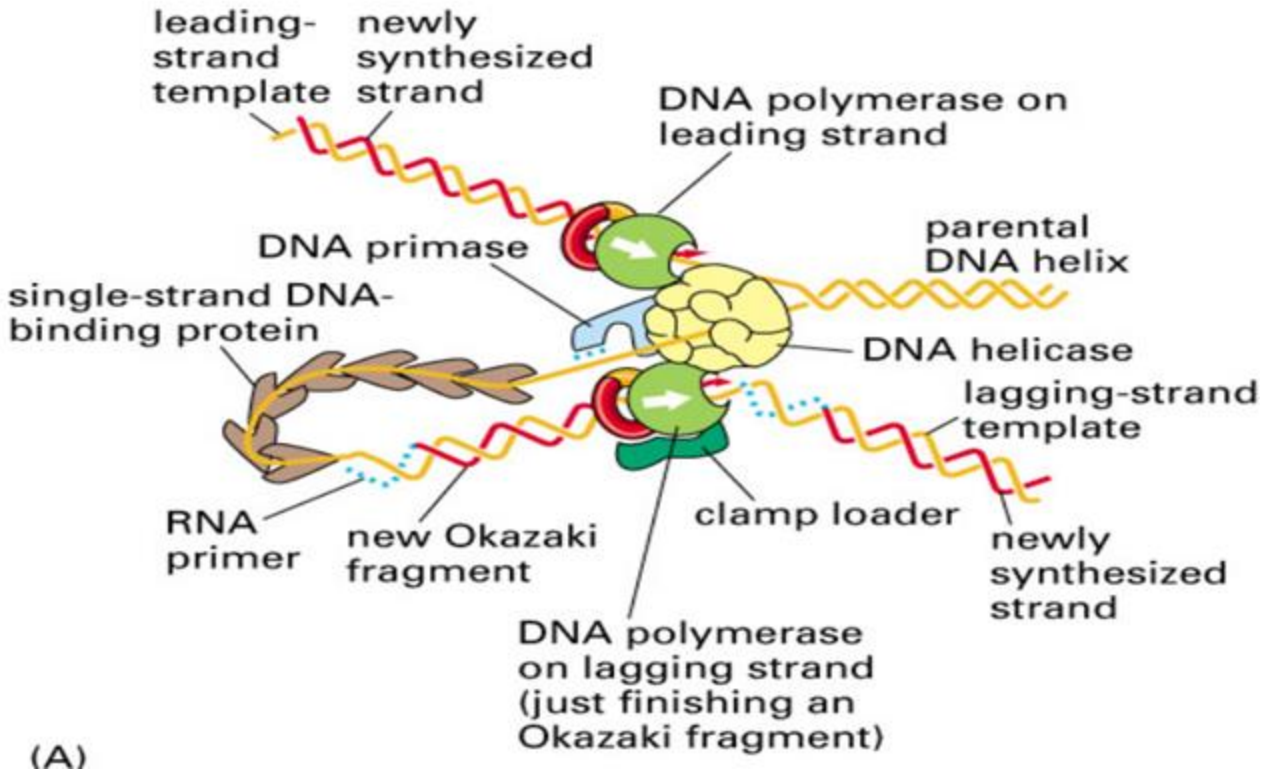

3'-old strand
The fork moves as replication takes place
The Leading Strand
5'-new strand
5'-old strand
3'-old strand
3'-new strand
The Lagging Strand
5'-old strand
Replication Fork

(ii) LAGGING STRAND SYNTHESIS

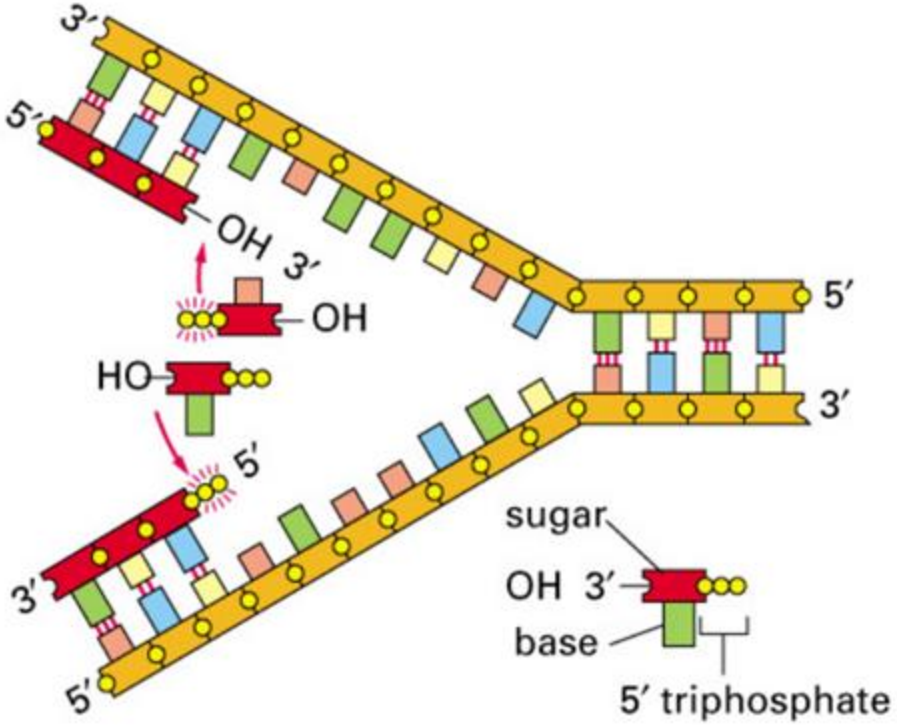
- The lagging strand synthesized in short fragments called Okazaki fragments. At first RNA primer is synthesized by primase and as in leading strand DNA polymerase III binds to RNA primer and adds dNTPS.
- On this level the synthesis of each okazaki fragments seems straight forward but the reality is quite complex.
- **MECHANISM OF SYNTHESIS OF LAGGING STRAND**
- The complexity lies in the coordination of leading and lagging strand synthesis. Both the strand are synthesized by a single DNA polymerase III dimer which accomplished the looping of template DNA of lagging strand synthesizing Okazaki fragments.
- Helicase (dnaB) and primase (dnaG) constitute a functional unit within replication complex called **primosome.**
- DNA pol III use one set of core sub unit (Core polymerase) to synthesize leading strand and other set of core subunit to synthesize lagging strand.

- In elongation steps, helicase in front of primase and pol III, unwind the DNA at the replication fork and travel along lagging strand template along 5'-3' direction.
- DnaG primase occasionally associated with dnaB helicase synthesizes short RNA primer. A new B-sliding clamp is then positioned at the primer by B-clamp loading complex of DNA pol III.
- When the Okazaki fragments synthesis is completed, the replication halted and the core subunit dissociates from their sliding clamps and associates with new clamp. This initiates the synthesis of new Okazaki fragments.
- Both leading and lagging strand are synthesized co-ordinately and simultaneously by a complex protein moving in 5'-3' direction. In this way both leading and lagging strand can be replicated at same time by a complex protein that move in same direction.
- Every so often the lagging strands must dissociates from the replicosome and reposition itself so that replication can continue.
- Lagging strand synthesis is not completed until the RNA primer has been removed and the gap between adjacent Okazaki fragments are sealed. The RNA primer are removed by exonuclease activity (5'-3') of DNA pol-I and replaced by DNA. The gap is then sealed by DNA ligase using NAD as cofactor.

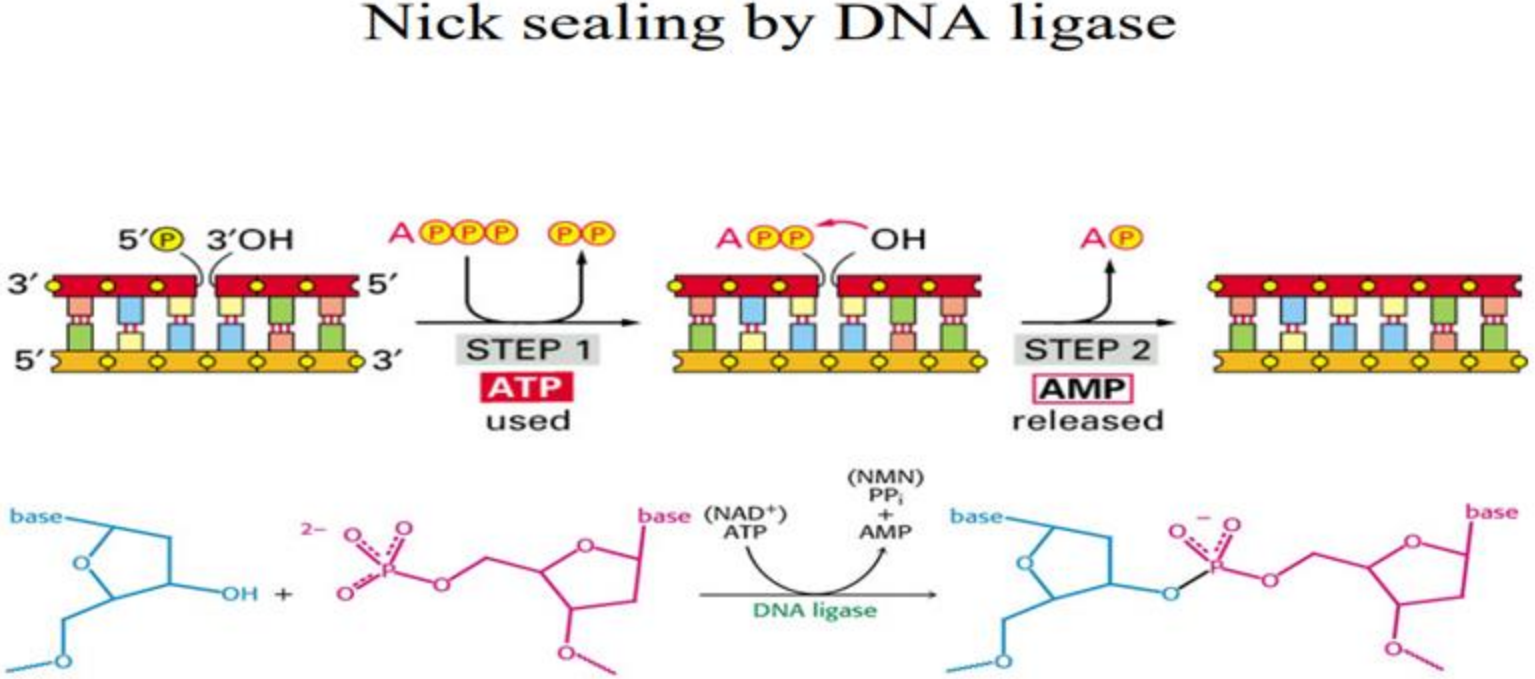
DNA is bent duing replication process



DNA is synthesized in the replication fork in 5’ to 3’ direction

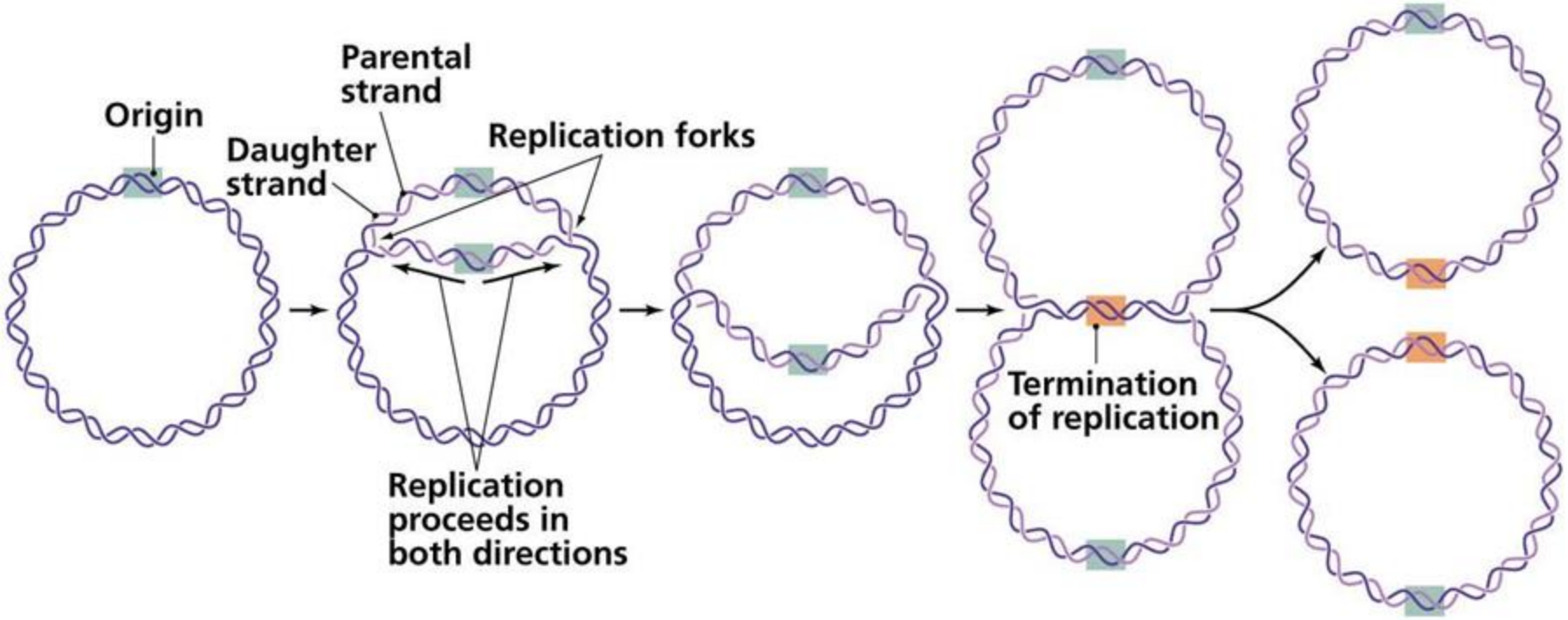


DNA LIGASE ACTIVITY

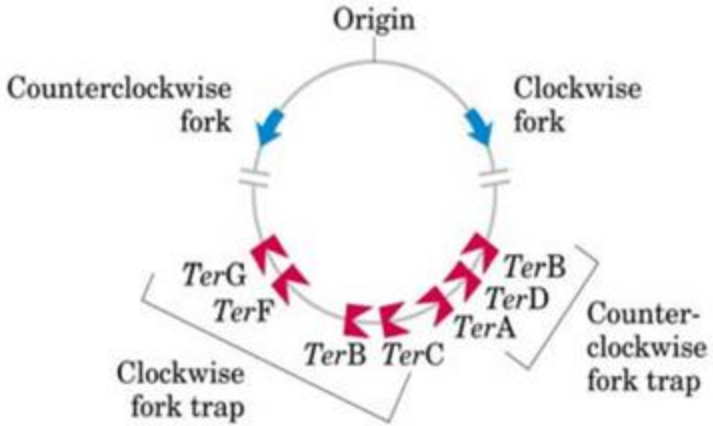


3. TERMINATION

- Eventually the two replication fork of circular E. coli chromosome meet at termination recognizing sequences (ter).
- The Ter sequence of 23 bp are arranged on the chromosome to create trap that the replication fork can enter but cannot leave. Ter sequences function as binding site for TUS protein.
- Ter-TUS complex can arrest the replication fork from only one direction. Ter-TUS complex encounter first with either of the replication fork and halt it. The other opposing replication fork halted when it collide with the first one. This seems the Ter-TUS sequences is not essential for termination but it may prevents over replication by one fork if other is delayed or halted by a damage or some obstacle.
- When either of the fork encounter Ter-TUS complex, replication halted.
- Final few hundred bases of DNA between these large protein complexes are replicated by not yet known mechanism forming two interlinked (catenated) chromosome.
- In E. coli DNA topoisomerase IV (type II) cut the two strand of one circular DNA and segrate each of the circular DNA and finally join the strand. The DNA finally transfer to two daughter cell.



Termination of replication



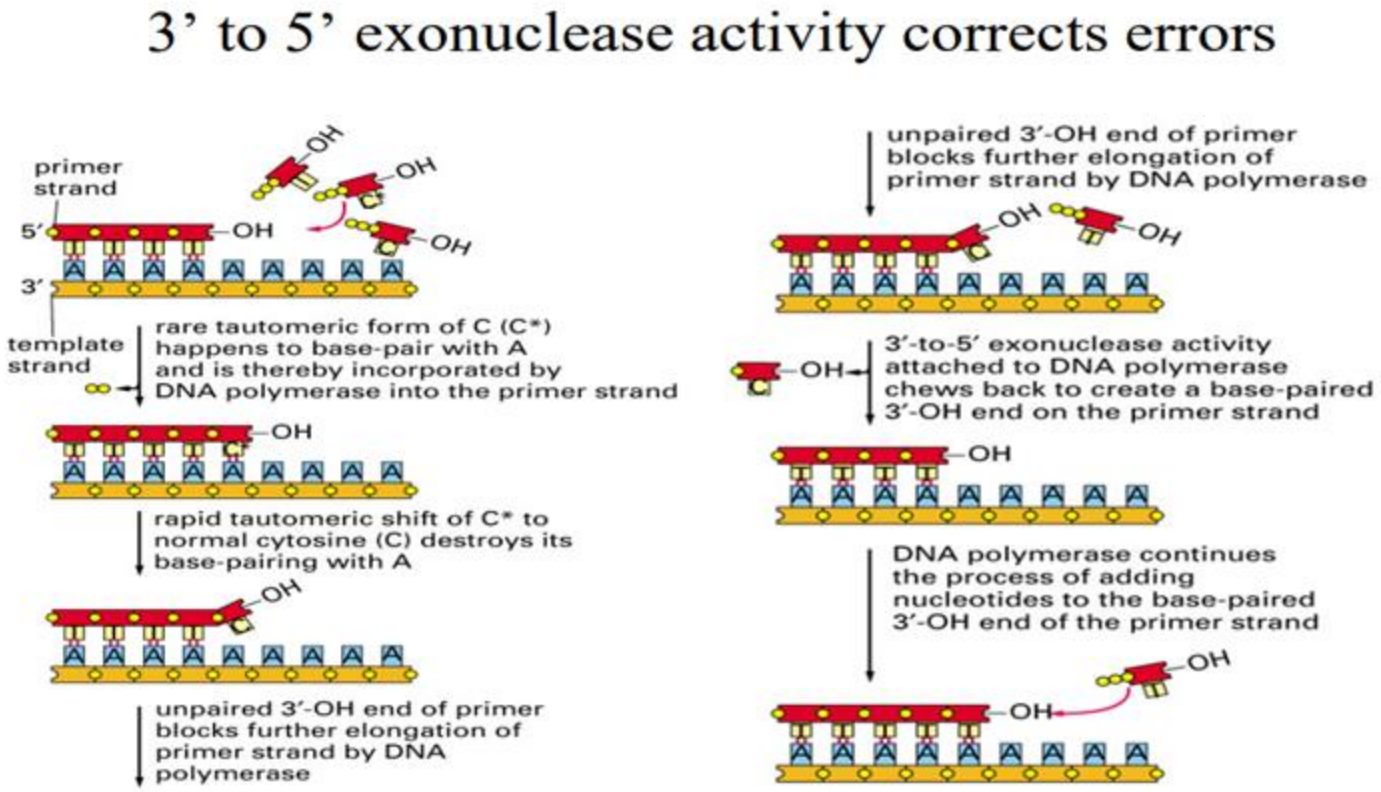
The two replication forks are synchronized by 10 23 bp Ter sequences that bind Tus proteins

Tus proteins can only be displaced by replisomes coming from one direction

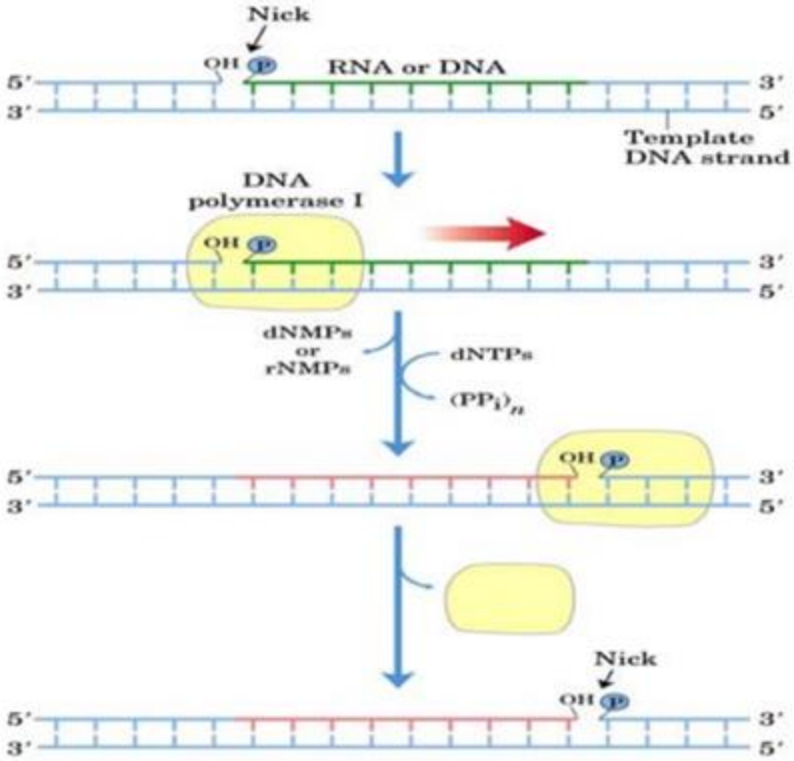
PROOFREADING

- In bacteria, all three DNA polymerases (I, II and III) have the ability to proofread, using 3’ → 5’ exonuclease activity.
- When an incorrect base pair is recognized, DNA polymerase reverses its direction by one base pair of DNA and excises the mismatched base.
- Following base excision, the polymerase can re-insert the correct base and replication can continue.

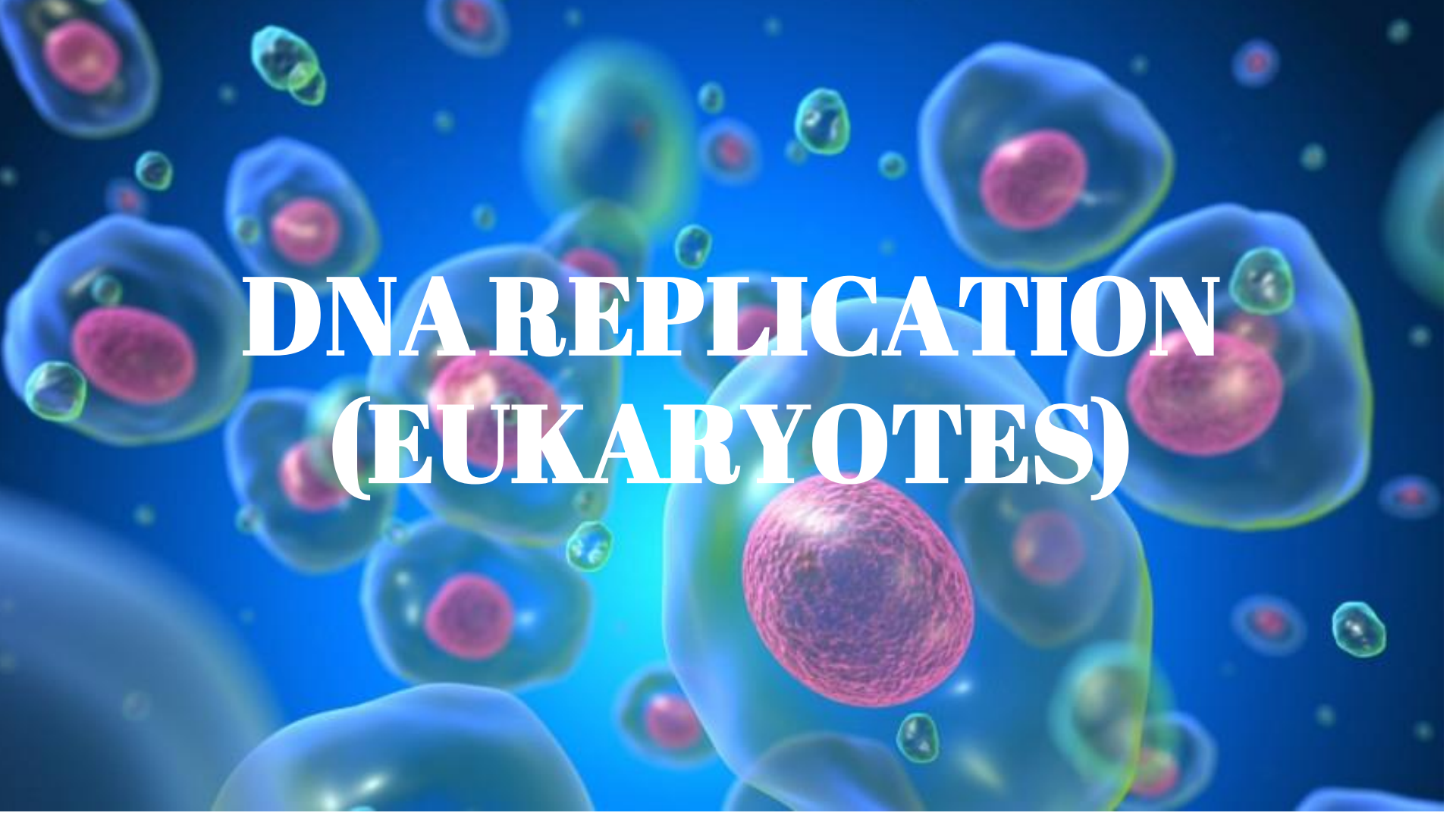
FOR ANY ERROR WHILE REPLICATION



5’ to 3’ exonuclease activity causes strand displacement/nick translation



No net synthesis


DNA REPLICATION
(EUKARYOTES)

ENZYMES INVOLVED IN DNA REPLICATION

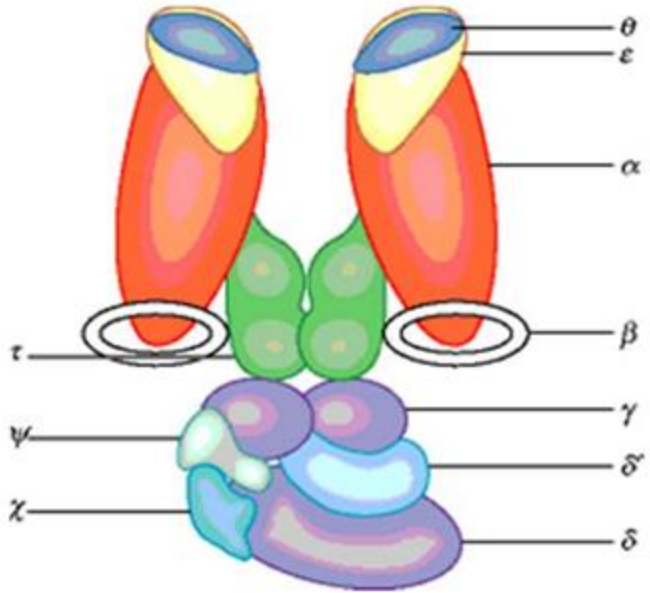
Difference between Prokaryotic and Eukaryotic Replication

Property	Prokaryotes	Eukaryotes
Origin of replication	Single	Multiple
Rate of replication	1000 nucleotides/s	50 to 100 nucleotides/s
DNA polymerase types	5	14
Telomerase	Not present	Present
RNA primer removal	DNA pol I	RNase H
Strand elongation	DNA pol III	Pol δ, pol ε
Sliding clamp	Sliding clamp	PCNA

DNA POLYMERASE ENZYME IN EUKARYOTES

Eukaryotic DNA Polymerase

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



Subunit	function
θ	not known
ε	3’ exonuclease
α	polymerase
β	clamp
τ	dimerisation
γ	clamp loader
δ	
ψ	
χ	

DNA POLYMERASE SUBUNITS

STAGES OF DNA REPLICATION

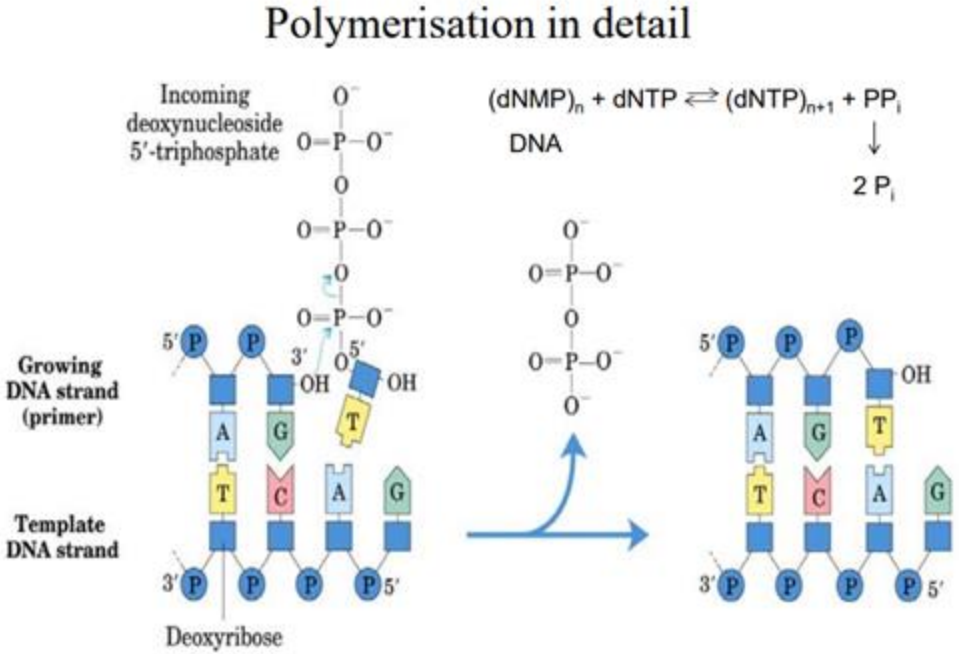
- DNA replication in eukaryotes occur only in S-phase of cell cycle. However pre-initiation occur in G1 phase. Due to sheer size of chromosome in eukaryotes, chromosome chromosome contains multiple origin of replication.
- ARS (autonomously replicating sequence) in case of yeast is origin for replication.
- A replication bubble is formed at each ORI, forks moving in both direction.

INITIATION

- Certain proteins recognize and bind to the origin of replication and then allow the other proteins necessary for DNA replication to bind the same region.
- The first proteins to bind the DNA are said to “recruit” the other proteins. Two copies of an enzyme called helicase are among the proteins recruited to the origin.
- Each helicase unwinds and separates the DNA helix into single-stranded DNA. As the DNA opens up, Y-shaped structures called replication forks are formed. Because two helicases bind, two replication forks are formed at the origin of replication; these are extended in both directions as replication proceeds creating a replication bubble.
- There are multiple origins of replication on the eukaryotic chromosome which allow replication to occur simultaneously in hundreds to thousands of locations along each chromosome

ELONGATION

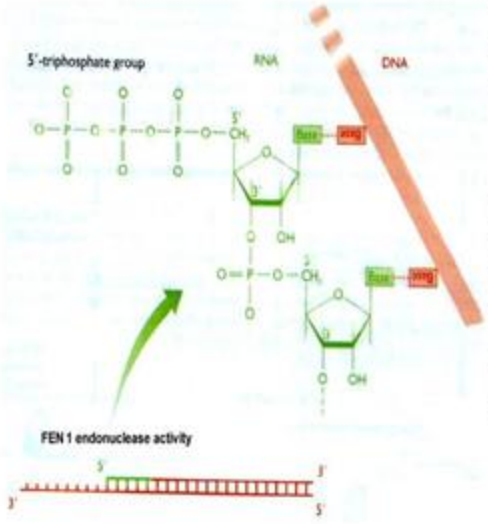
- DNA polymerase δ synthesizes and adds dNTPs at 3’ end of RNA primer.
- The leading and lagging strands are synthesized in the similar fashion as in prokaryotic DNA replication.



TERMINATION

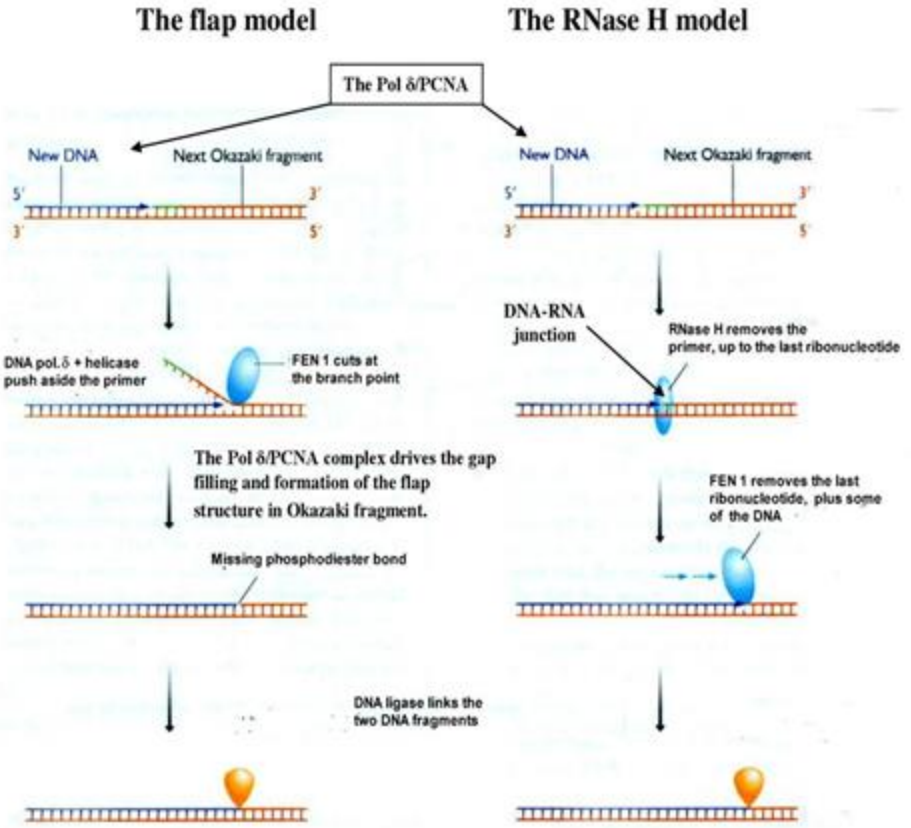
- At the end of DNA replication the RNA primer are replaced by DNA by 5’-3 exonuclease and polymerase activity of DNA polymerase ε.
- Exonuclease activity of DNA polymerase removes the RNA primer and polymerase activity adds dNTPs at 3’-OH end preceding the primer.
- Basically two types of models are there : RNASE H and FLAP ENDONUCLEASE MODEL
- RNASE H cannot remove the last nucleotide of primer that is attached to the DNA
- For this the Flap endonuclease work that cut it from within and help in whole removal of the primer from lagging strand.

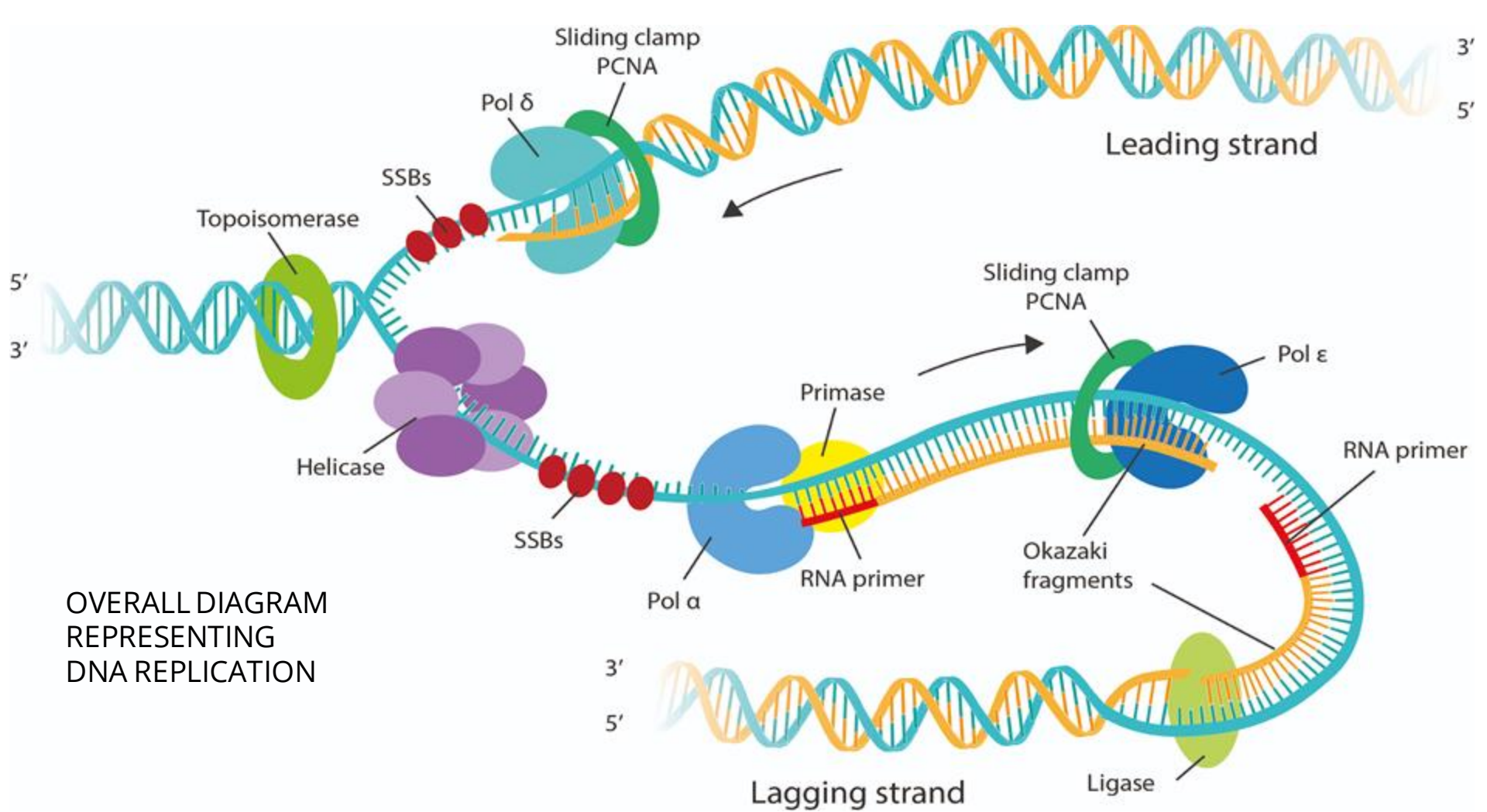
Completation of lagging strand synthesis in eukaryotes



None of the eukaryotic polymerase have a 5' →3' exonuclease activity.

FEN1 endonuclease cannot initiate primer degradation because its activity is blocked by the triphosphate group present at the 5'-end of the primer.





OVERALL DIAGRAM REPRESENTING DNA REPLICATION

THE END.....