SHRIMATI INDIRA GANDHI COLLEGE

DEPARTMENT OF BIOCHEMISTRY

III B.Sc BIOCHEMISTRY
Subject: Cell and Molecular Biology
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DNA STRUCTURE

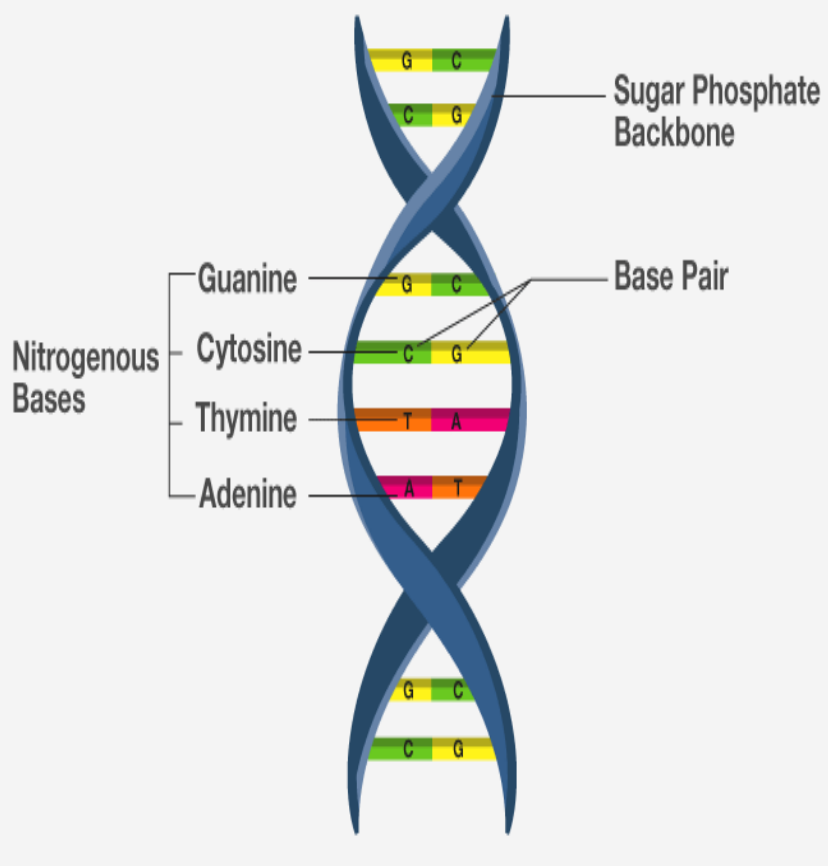
&

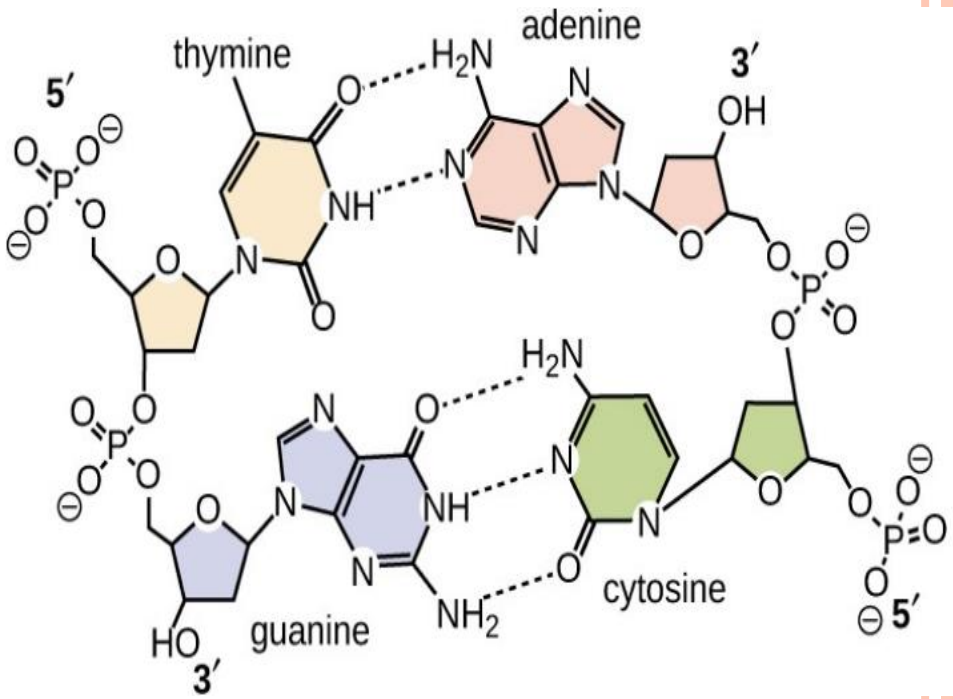
DNA REPLICATION IN PROKARYOTES

SYNOPSIS

- STRUCTURE OF DNA
- DNA IS THE GENETIC MATERIAL
- MODELS OF DNA REPLICATION
- STAGES OF REPLICATION

STRUCTURE OF DNA





Watson and Crick model of DNA

- DNA is a double helix structure because it looks like a twisted ladder.
- DNA molecule is comprised of two biopolymer strands coiling around each other.
- Each strand has a 5′end (with a phosphate group) and a 3′end (with a hydroxyl group).
- The strands are antiparallel, meaning that one strand runs in a 5′to 3′direction, while the other strand runs in a 3′to 5′direction.
- The diameter of the double helix is 2nm and the double helical structure repeats at an interval of 3.4nm which corresponds to ten base pairs.
- DNA is composed of deoxyribonucleotides (Nitrogenous base+2-deoxy ribose +Phosphate)
- The nitrogenous bases present are Purines (Adenine, Guanine) and Pyrimidines (Cytosine, Thymine)
- The two strands are held together by hydrogen bonds between the bases which are complementary to each other.
- Adenine always pair with Thymine and Guanine always pair with Cytosine
- There are two hydrogen bond between A=T base pair. Three hydrogen bond between G≡ C base pair.
- The deoxyribonucleotides are linked together by 3′- 5′phosphodiester bonds.
- The sides of the ladder are made of alternating sugar (deoxyribose) and phosphate molecules while the steps of the ladder are made up of a pair of nitrogen bases.
- As a result of the double helical nature of DNA, the molecule has two asymmetric grooves - Major groove and Minor groove .

Characters make DNA as genetic material

Must carry information

- Cracking the genetic code

Must replicate

- DNA replication

Must allow for information to change

- Mutation

Must govern the expression of the phenotype

- Gene function

HENCE DNA IS THE GENETIC MATERIAL AND UNDER GO REPLICATION PROCESS

DNA stores information in the sequence of its bases

✓**Much of DNA's sequence-specific information is accessible only when the double helix is unwound**

✓**Proteins read the DNA sequence of nucleotides as the DNA helix unwinds.**

✓**Proteins can either bind to a DNA sequence, or initiate the copying of it.**

✓ **Some genetic information is accessible even in intact, double-stranded DNA molecules**

✓ **Some proteins recognize the base sequence of DNA without unwinding it (One example is a restriction enzyme).**

Replication of DNA in Prokaryotes

- Understand the basic rules governing DNA replication
- Role Enzymes/proteins that are typically involved in DNA replication

DNA REPLICATION

Process of duplication of the entire genome prior to cell division

Biological significance

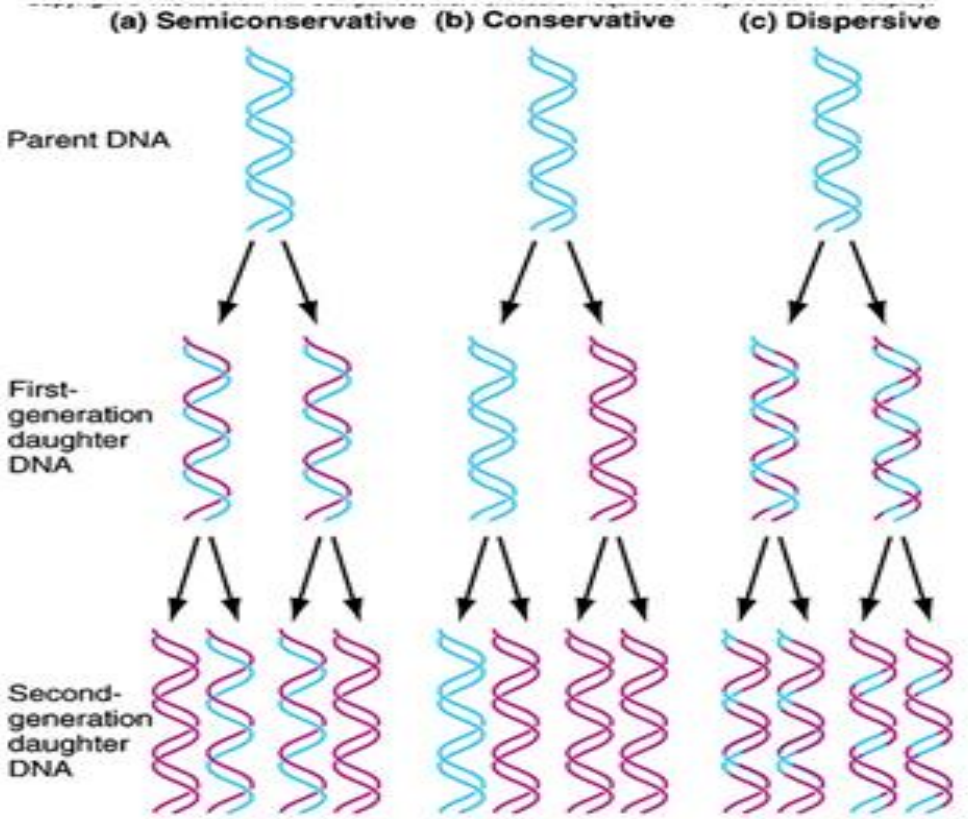
- extreme accuracy of DNA replication is necessary in order to preserve the integrity of the genome in successive generations
- In eukaryotes , replication only occurs during the S phase of the cell cycle.
- Replication rate in eukaryotes is slower resulting in a higher fidelity/accuracy of replication in eukaryotes

Basic rules of replication

A. Semi-conservative
B. Starts at the ‘origin’
C. Synthesis always in the 5-3’ direction
D. Can be uni or bidirectional
E. Semi-discontinuous
F. RNA primers required

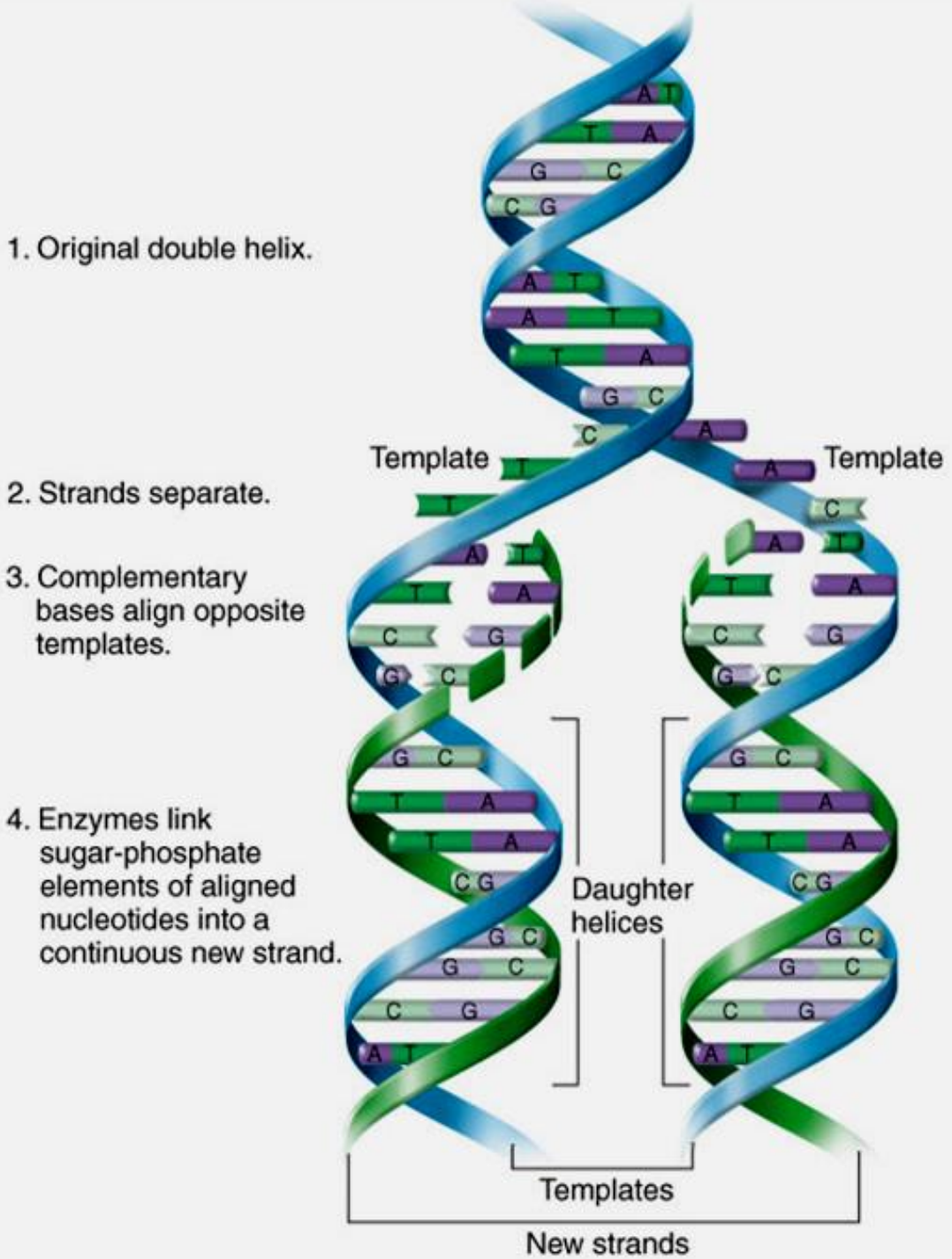
TYPES OF DNA REPLICATION

DNA REPLICATION
3 POSSIBLE MODELS



Semi-conservative replication:

One strand of duplex passed on unchanged to each of the daughter cells. This 'conserved' strand acts as a template for the synthesis of a new complementary strand by the enzyme DNA polymerase



The mechanism of DNA replication

- Initiation
  - Proteins bind to DNA and open up double helix
  - Prepare DNA for complementary base pairing
- Elongation
  - Proteins connect the correct sequences of nucleotides into a continuous new strand of DNA
- Termination
  - Proteins release the replication complex

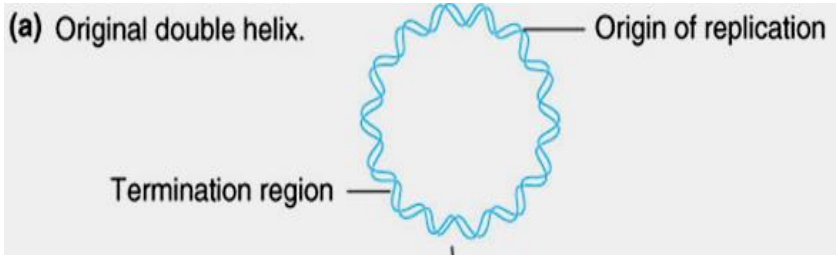
STARTS AT ORIGIN

Initiator proteins identify specific base sequences on DNA called sites of origin

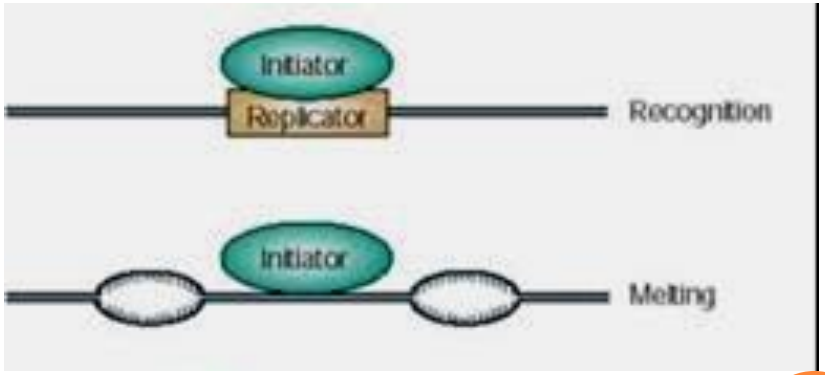
Prokaryotes – single origin site E.g *E.coli* - *oriC*

Eukaryotes – multiple sites of origin (replicator)

E.g. yeast - ARS (autonomously replicating sequences)



Prokaryotes



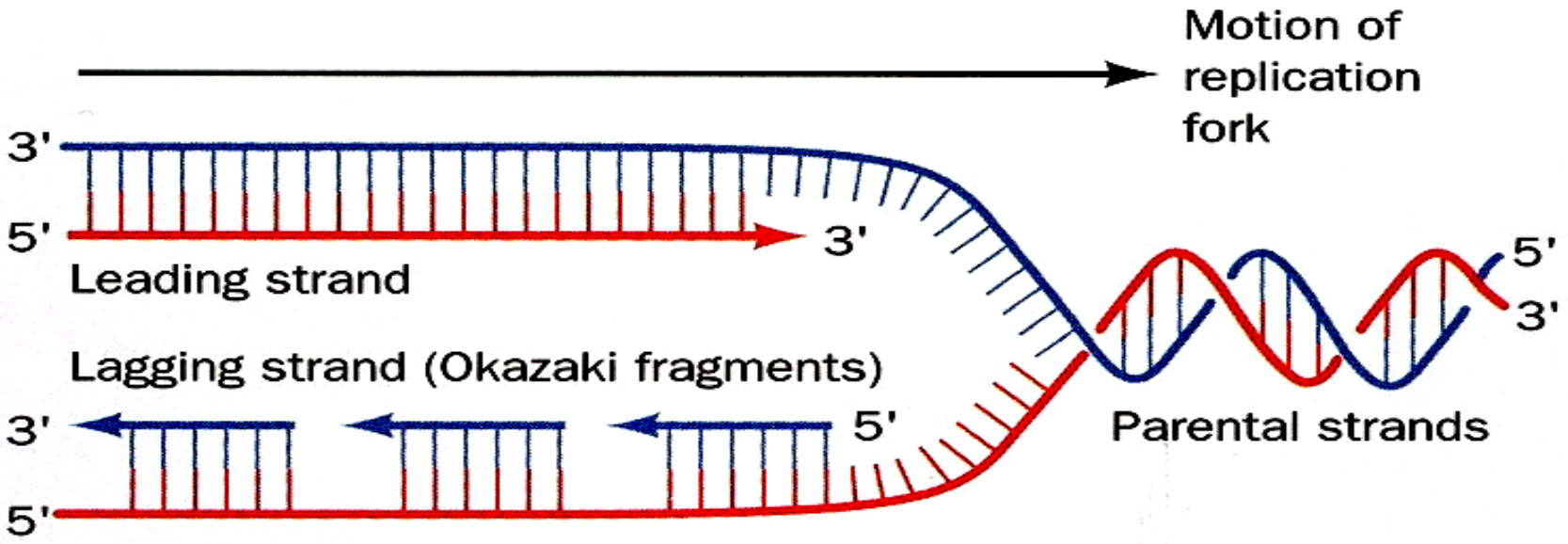
Eukaryotes

SEMI-DISCONTINUOUS REPLICATION

- DNA Helicase unwinds the strands.
- Single strand binding proteins maintain single strands.
- RNA polymerase synthesises RNA primer
- DNA polymerase III synthesises most of the DNA
- DNA polymerase I fills the gap between okazaki fragments.
- DNA ligase seals the nick between fragments

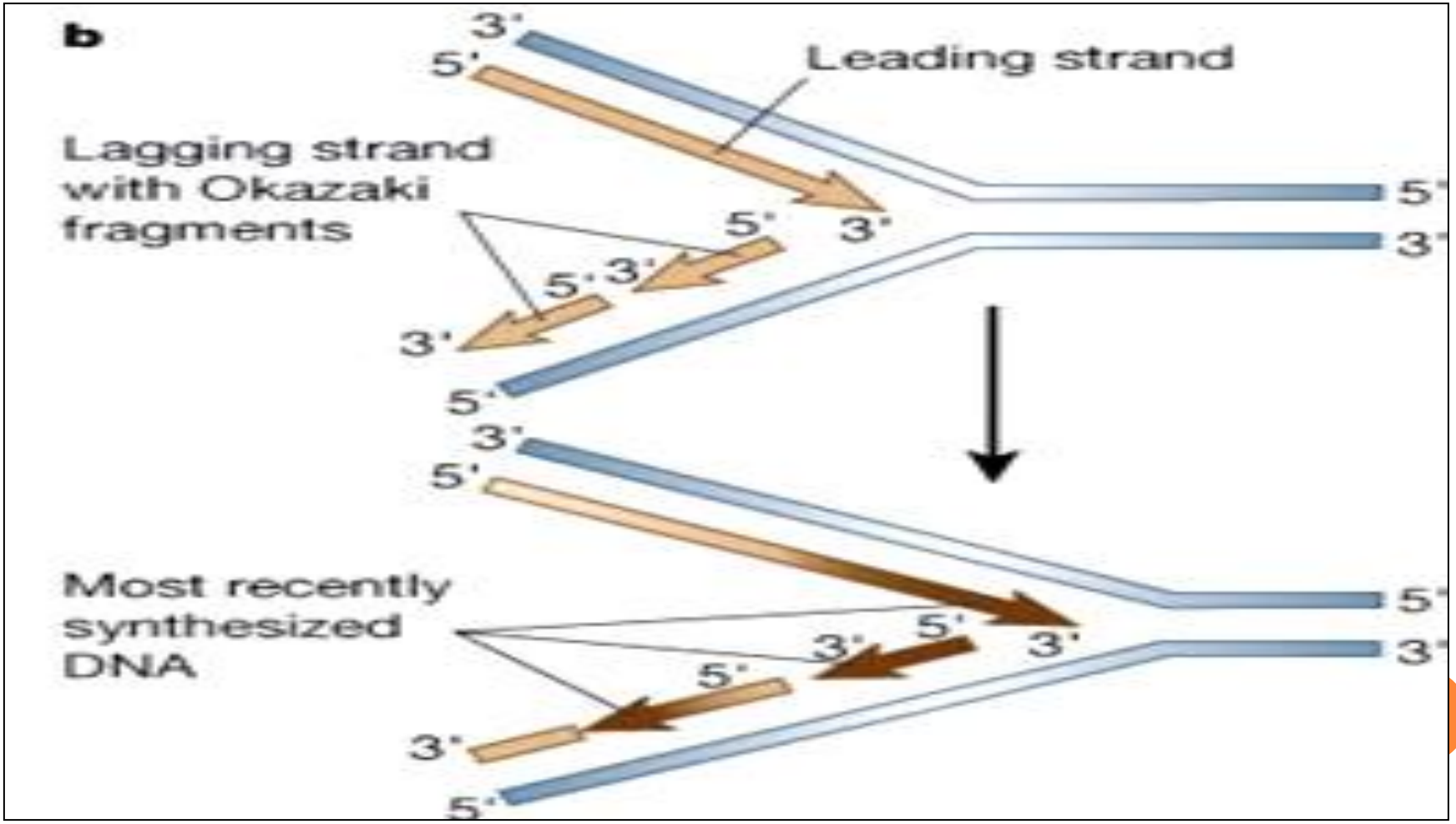
Anti parallel strands replicated simultaneously

- Leading strand synthesis **continuously** in 5'– 3'
- Lagging strand synthesis in **fragments** in 5'-3'

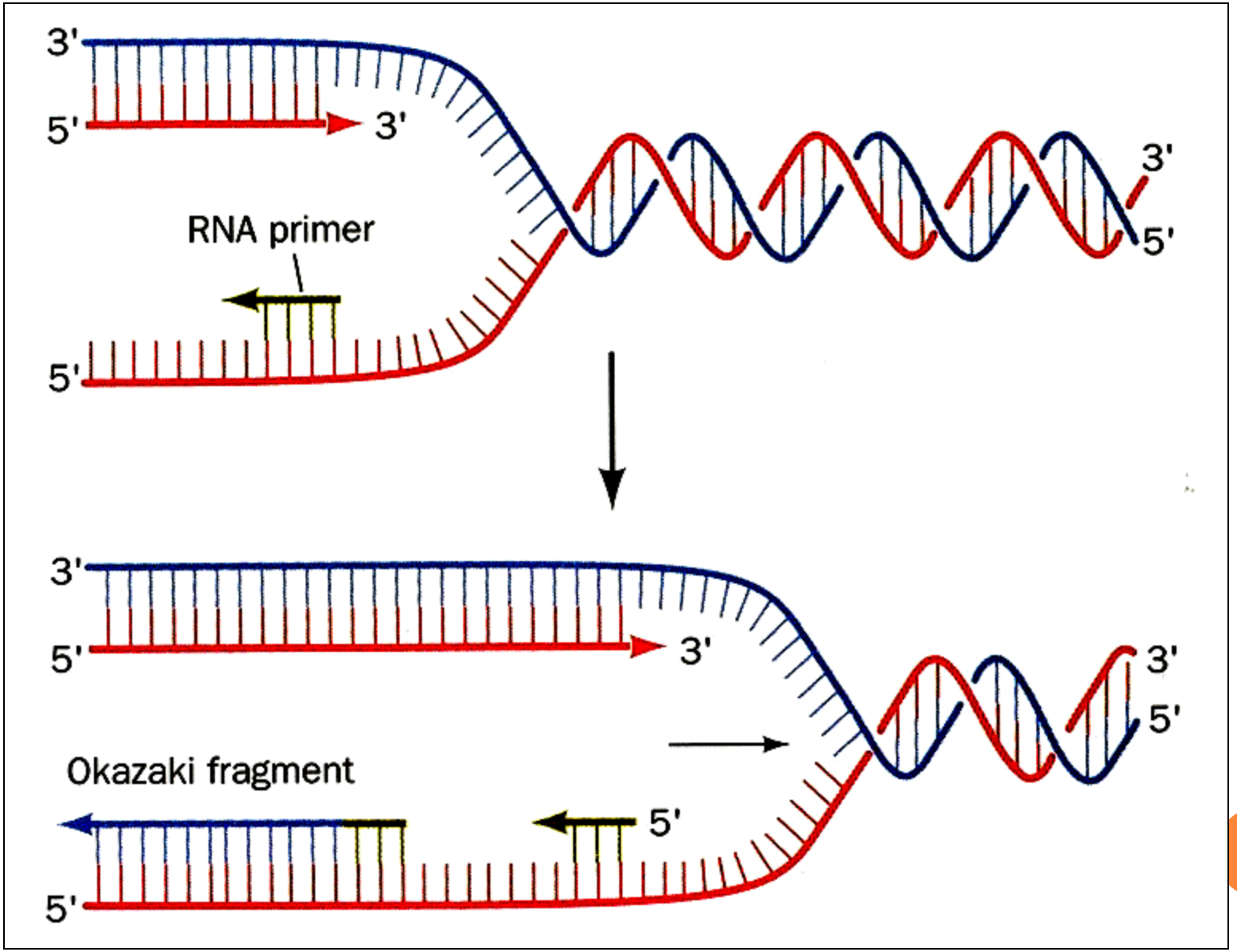


SEMI-DISCONTINUOUS REPLICATION

New strand synthesis always in the 5′-3′ direction



RNA PRIMERS REQUIRED



CORE PROTEINS AT THE REPLICATION FORK

Topoisomerases -Prevents torsion by DNA breaks

Helicases- Separates 2 strands

Primase- RNA primer synthesis

Single strand binding proteins -prevent reannealing of single strands

DNA polymerase I & III - synthesis of new strand

DNA ligase - seals nick via phosphodiester bond

TERMINATION OF DNA REPLICATION

- ✓ In prokaryotes (E.coli) termination is signalled by specific sequences called "Ter elements" which serve as a binding site for "Tus proteins"
- ✓ Tus protein binds to Ter element forms Tus-Ter complex and stops DNA from unwinding.
- ✓ This stops the movement of the replication fork. The leading strand is replicated up to the Ter element, while the lagging strand replication is stopped 50-100 bp before the Ter element

IMPORTANT QUESTIONS

1. What is the role of RNA primer?
2. List out the enzymes involved in DNA replication
3. Describe the mechanism of DNA replication
4. What are okazaki fragments?
5. Write a note on possible types of DNA replication

THANK YOU