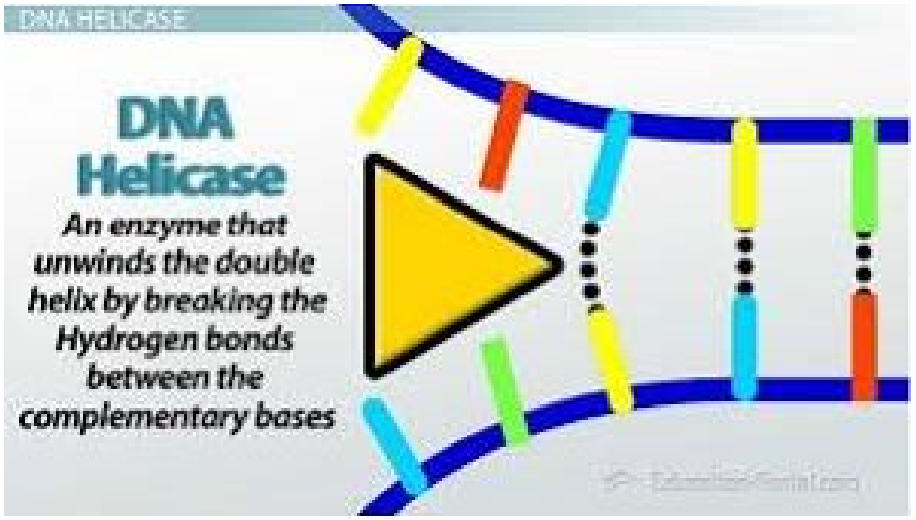
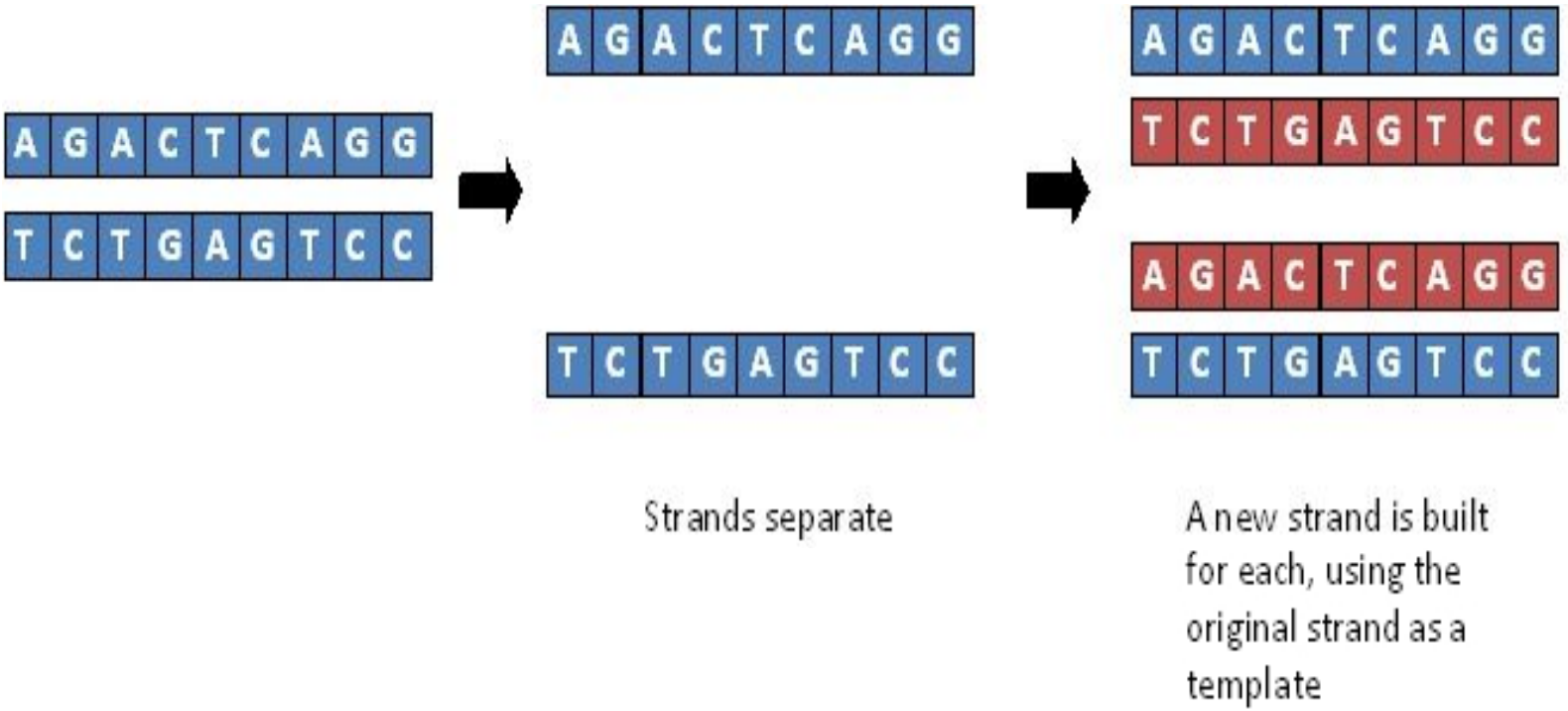
DNA REPLICATION

It is the process in which DNA makes exact copies of itself

The weak hydrogen bonds between the nitrogenous bases break and the two strands separate and uncoil.



Each separated strand acts as a template for the synthesis of new strand.



Basic rules for replication

- ✓ There is specificity of base pairing during replication.
- ✓ Nitrogenous bases are added one by one to the 3’ end by the enzyme DNA polymerase.
- ✓ The direction of replication is 5’ → 3’.
- ✓ The sequence of Nitrogenous bases in the daughter (NEW) strand is complementary to the template (OLD) strand.

REQUIREMENTS

1. **Precursor nucleotide molecules - All the four deoxyribonucleotide tri phosphates (dNTPs)**
   - **dATP, dGTP, dTTP, dCTP**
2. **Template DNA**
3. **RNA primer**
4. **Enzymes – DNA polymerase (add nucleotides to the growing strand), DNA ligase (join two fragments of DNA by forming phosphodiester bonds).**
5. **Proteins – 20 different proteins (ex) helicase – for unwinding DNA strands**

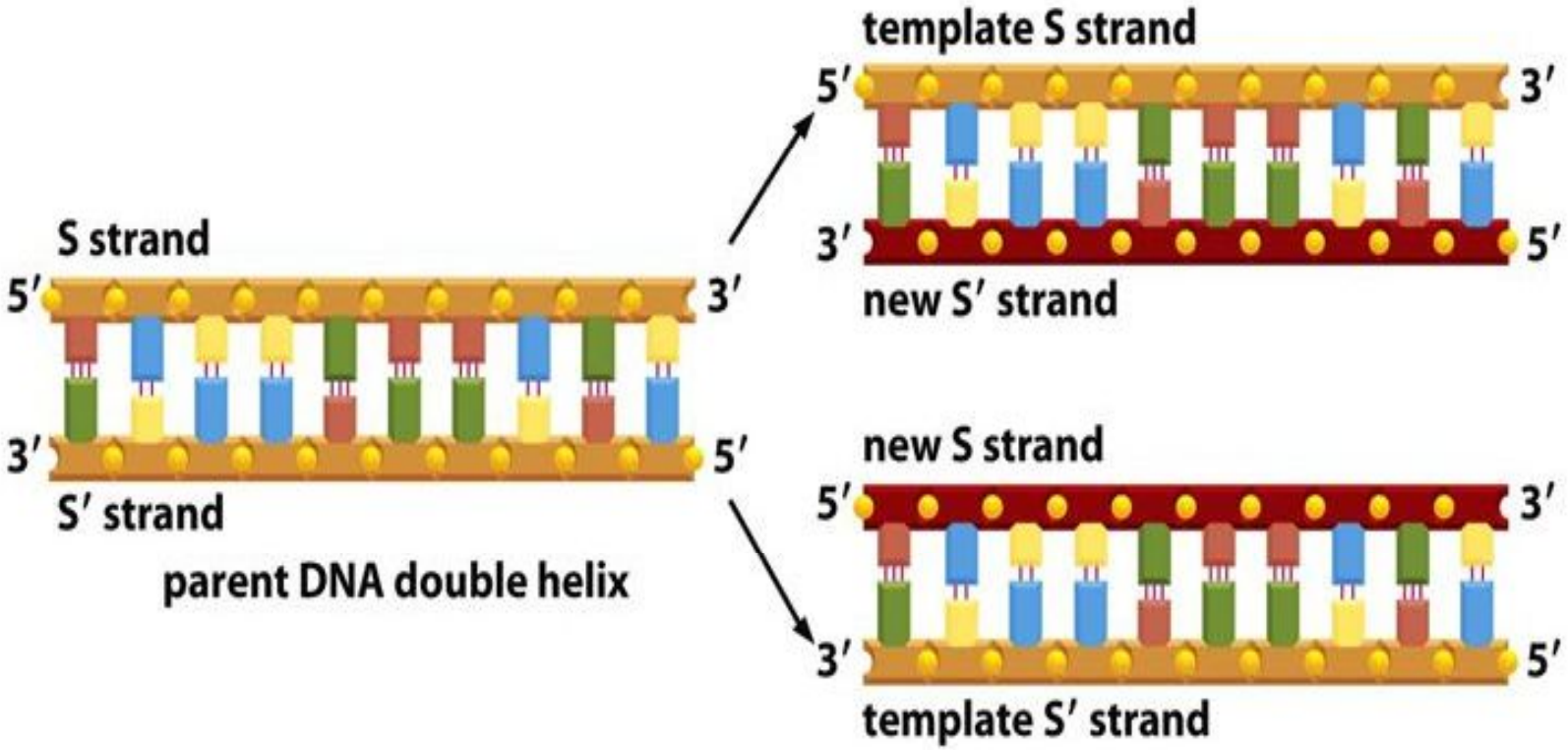
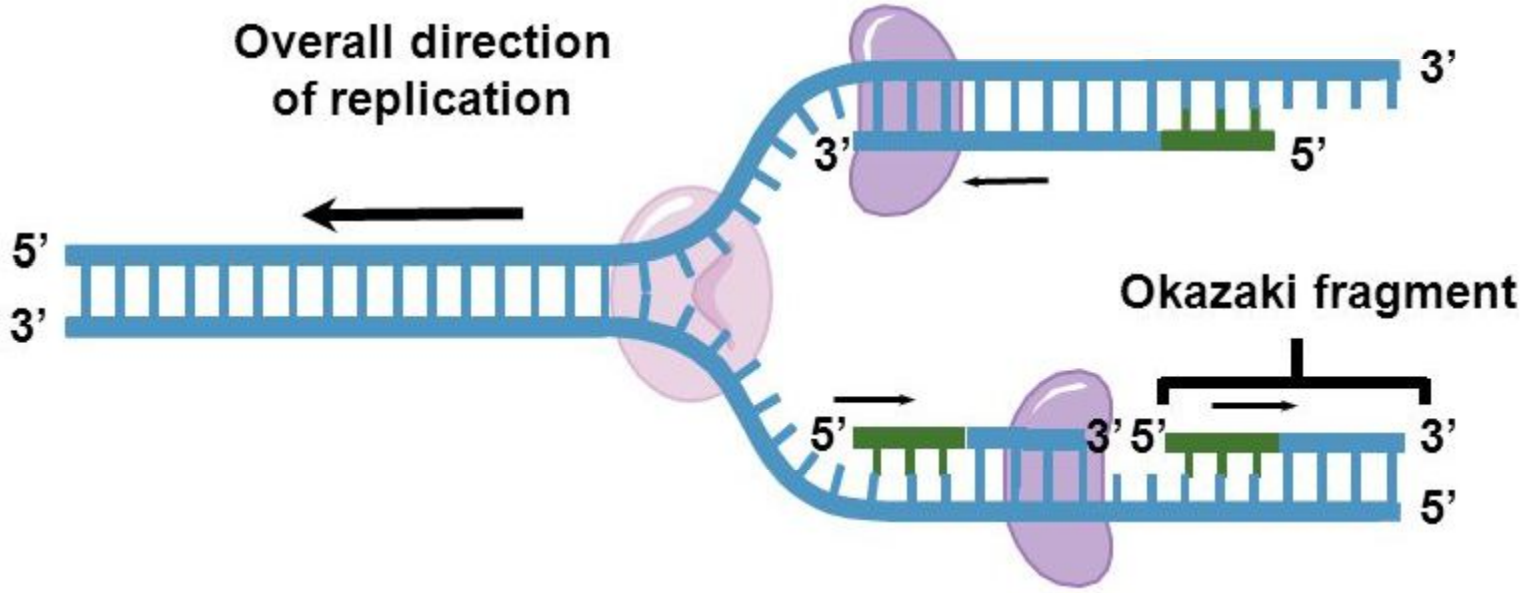
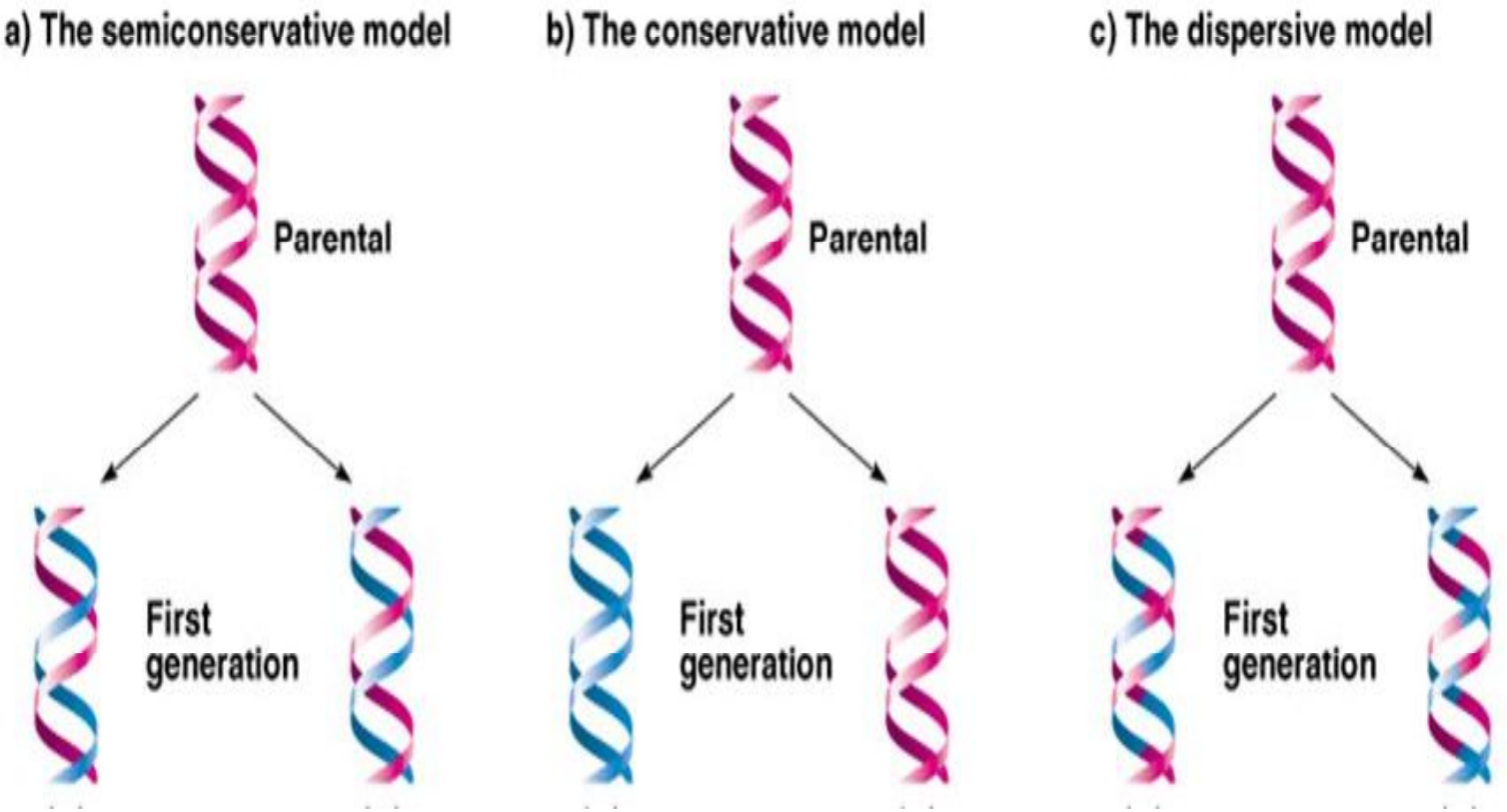


Figure 5-2 Molecular Biology of the Cell 5/e (© Garland Science 2008)


Overall direction
of replication
5’
3’
3’
5’
3’
Okazaki fragment
5’
3’
5’
3’
5’

MODES OF REPLICATION

- **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

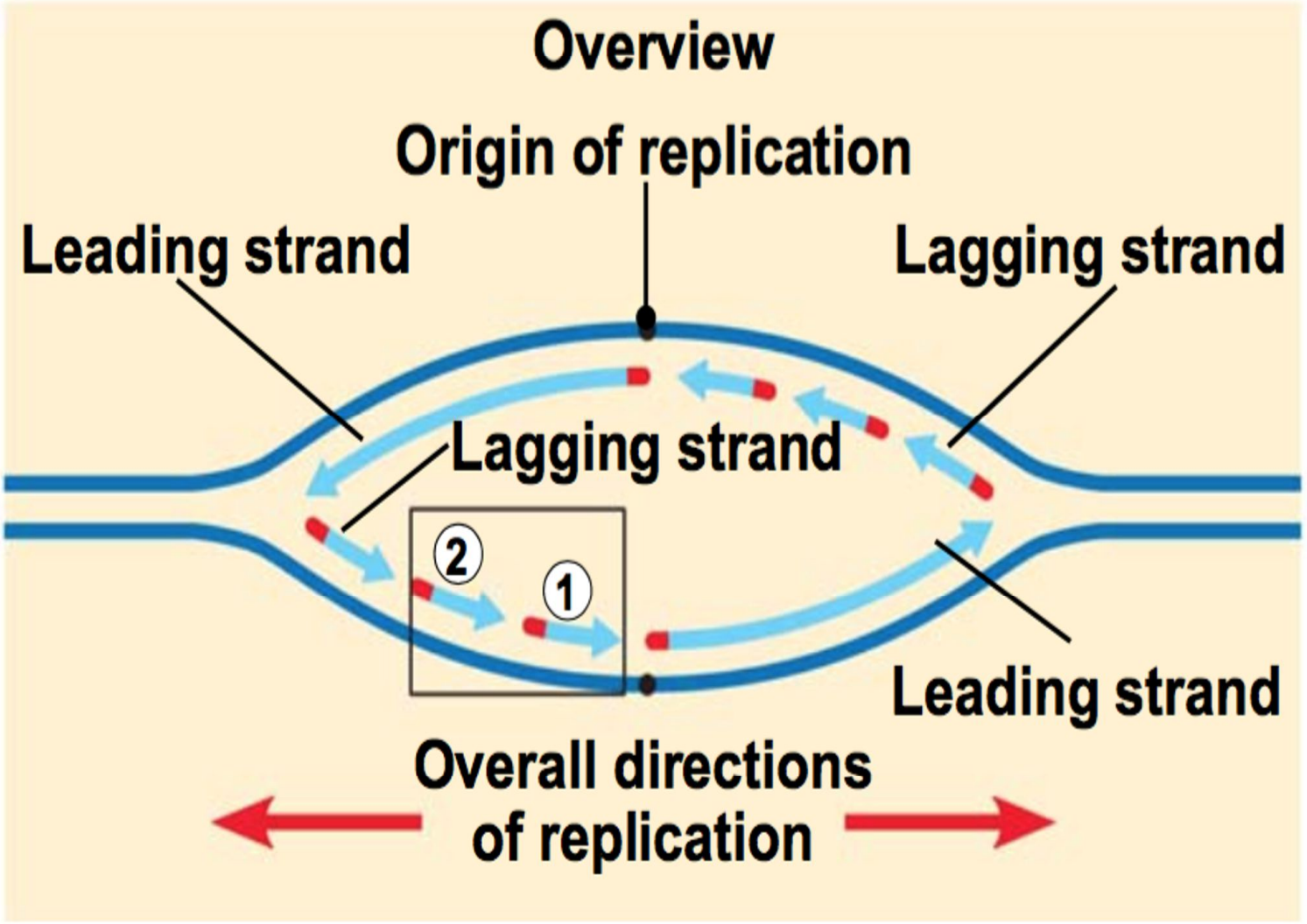

a) The semiconservative model
Parental
First generation
b) The conservative model
Parental
First generation
c) The dispersive model
Parental
First generation

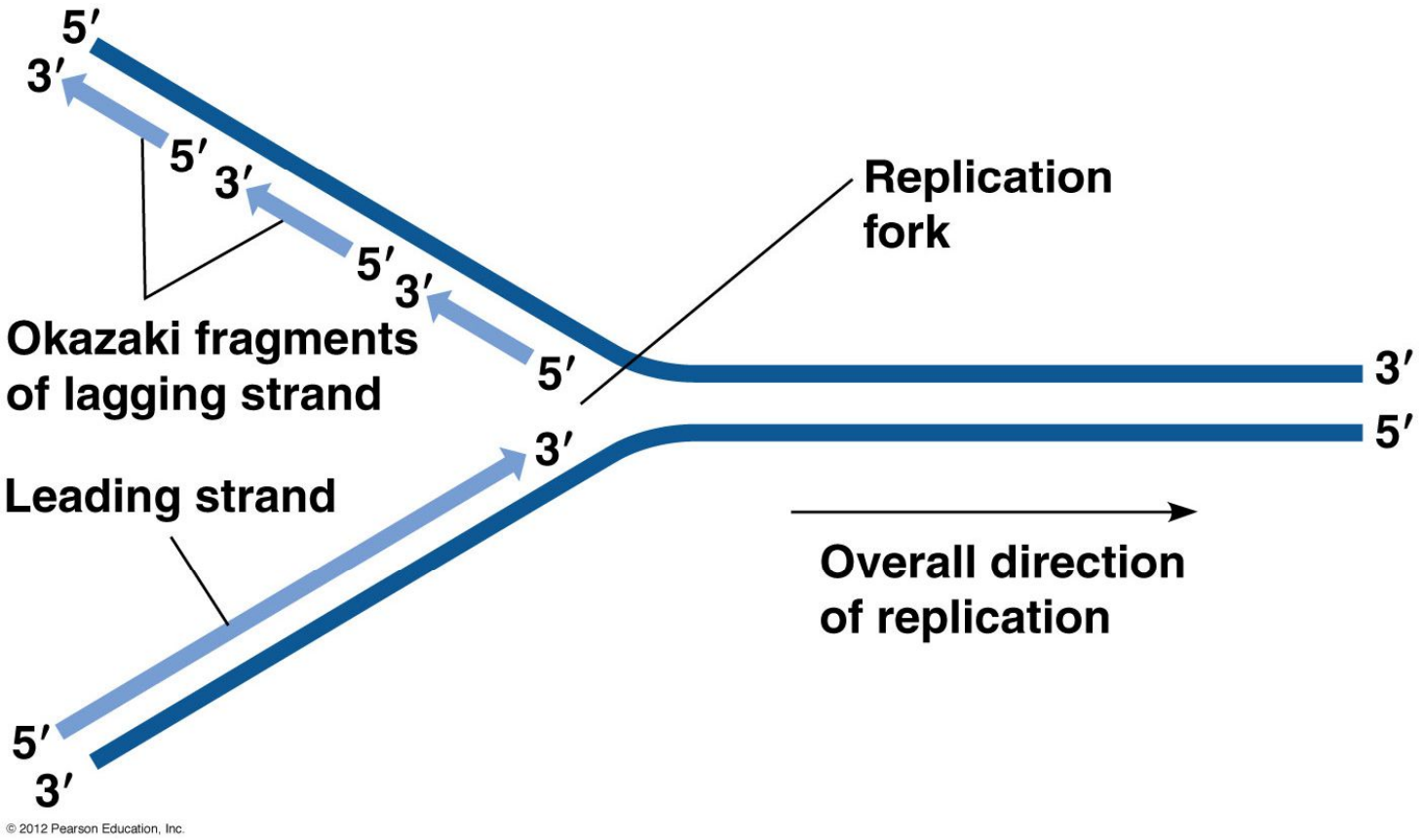
Process of Replication

- **Replication starts at a specific or unique sequence of nitrogenous bases in the DNA molecule called as ORIGIN OF REPLICATION.**
- **Anti parallel strands of DNA exhibit continuous and discontinuous DNA synthesis. This is called semi – discontinuous replication.**

Since the two strands are anti parallel, the new strands at the replication fork are oriented in opposite directions along the anti parallel parent templates.

1. **Leading strand or Continuous strand synthesis**
   This occurs in 5'-3' direction using 3'-5' parental strand as template. It is synthesized as one piece adding nucleotides to its 3' end.
2. **Lagging strand synthesis**
   It is synthesized on 5'-3' direction in short segments of 1000 -2000 nucleotides. These segments are called OKAZAKI FRAGMENTS. Synthesis of Okazaki fragments need a RNA primer and is discontinous.


Overview
Origin of replication
Leading strand
Lagging strand
Lagging strand
2
1
Leading strand
Overall directions
of replication



MOLECULAR MECHANISM

DNA replication involves four steps and each step is governed by specific enzymes.

1. **ACTIVATION OF DEOXYRIBONUCLEOTIDES**
2. **INITIATION**
3. **SYNTHESIS OF NEW STRAND**
4. **TERMINATION**

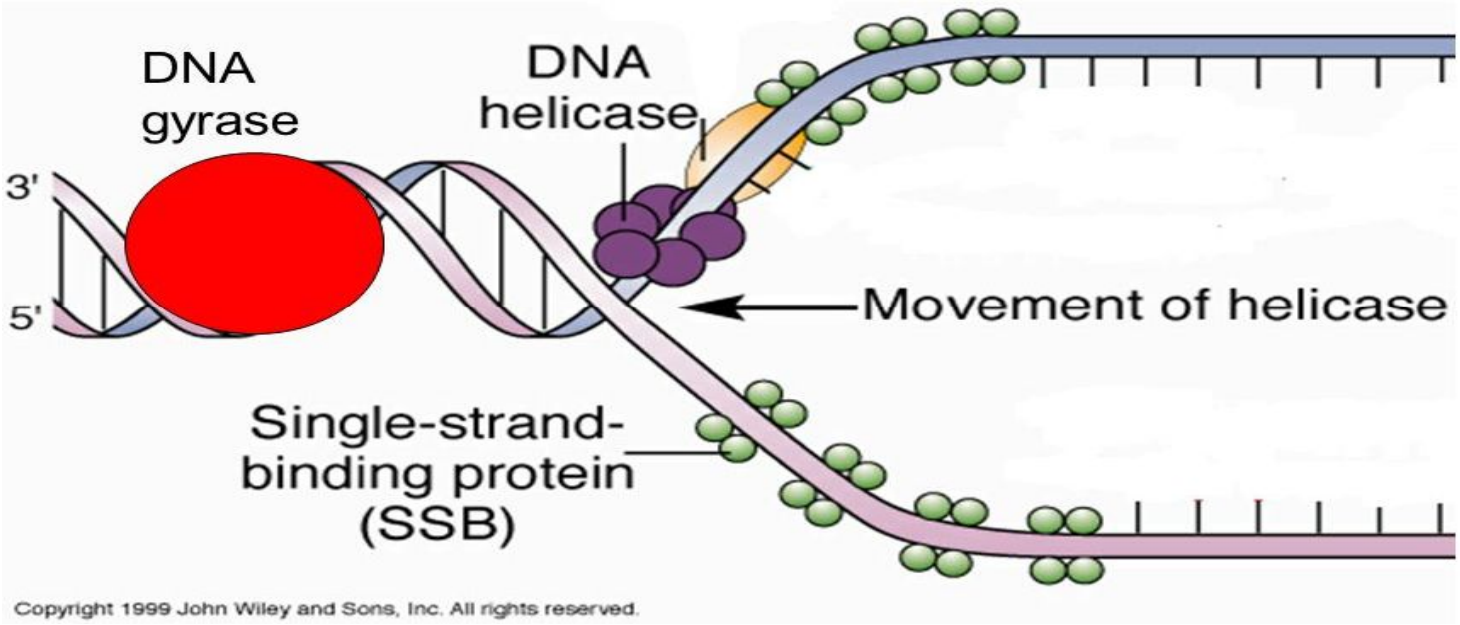
I. ACTIVATION OF DEOXYRIBONUCLEOTIDES

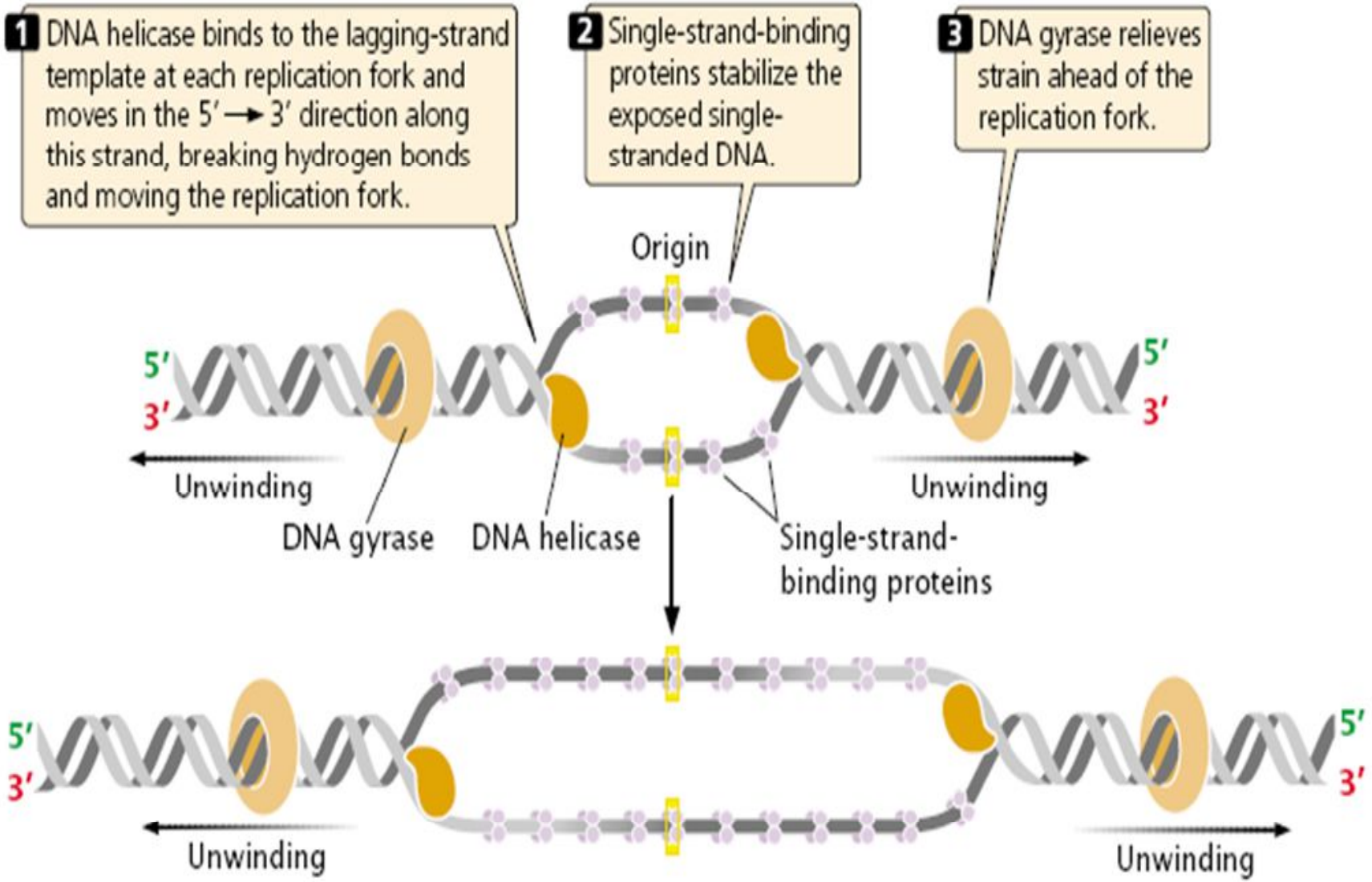
- The deoxyribonucleoside monophosphates (AMP, GMP, TMP. CMP) present in the nucleoplasm are activated into triphosphates (ATP, GTP, TTP,CTP) by ATP.
- This process is known as **PHOSPHORYLATION** and catalysed by the enzyme **phosphorylase.**

II. INITIATION

- DNA replication is initiated at a definite sequence of nucleotides (100-200 or more bases), called as INITIATION POINT or ORIGIN OF REPLICATION (ori C).
- Each origin of replication along with the DNA to be replicated forms one REPLICON.
- In E.coli, oriC consists of 245 bp with 3 repeats of 13 bp (13mers) and 4 repeats of 9 bp(9mers).
- Specific roles for different proteins/enzymes.

1. Recognisition of initiation point–Dna A
2. Unwinding – helicase, Dna B, Dna C,
3. Strand separation – DNA gyrase or topoisomerase enzyme
4. Maintenance of separated strands – SSB (single strand binding proteins)




1 DNA helicase binds to the lagging-strand template at each replication fork and moves in the 5′ → 3′ direction along this strand, breaking hydrogen bonds and moving the replication fork.
2 Single-strand-binding proteins stabilize the exposed single-stranded DNA.
3 DNA gyrase relieves strain ahead of the replication fork.
Origin
5′
3′
5′
3′
Unwinding
Unwinding
DNA gyrase
DNA helicase
Single-strand-binding proteins
5′
3′
5′
3′
Unwinding
Unwinding

III. SYNTHESIS OF NEW STRAND

This involves SIX steps

1) **Formation of RNA primer:** The PRIMASE enzyme binds to priming sequence on leading strand and synthesizes a short primer RNA segment of 10-60bp at the origin of replication.
2) **Assembling of complementary strands:** Deoxyribonucleotide triphosphates pair with appropriate nitrogenous bases according to base pairing rule.
3) **Conversion of deoxyribonucleotide triphosphates to monophosphates:** On pairing, deoxyribonucleotide triphosphates release pyrophosphate molecules and change to deoxyribonucleotides

4. Formation of new DNA chains

❖ The addition of adjacent nucletides is catalysed by DNA polymerase enzyme.

❖ The new strand is synthesised in 5'-3' direction continously, keeping in pace with the unwinding of DNA .

❖Since the two strands are anti parallel, the new strands at the replication fork are oriented in opposite directions along the anti parallel parent templates.

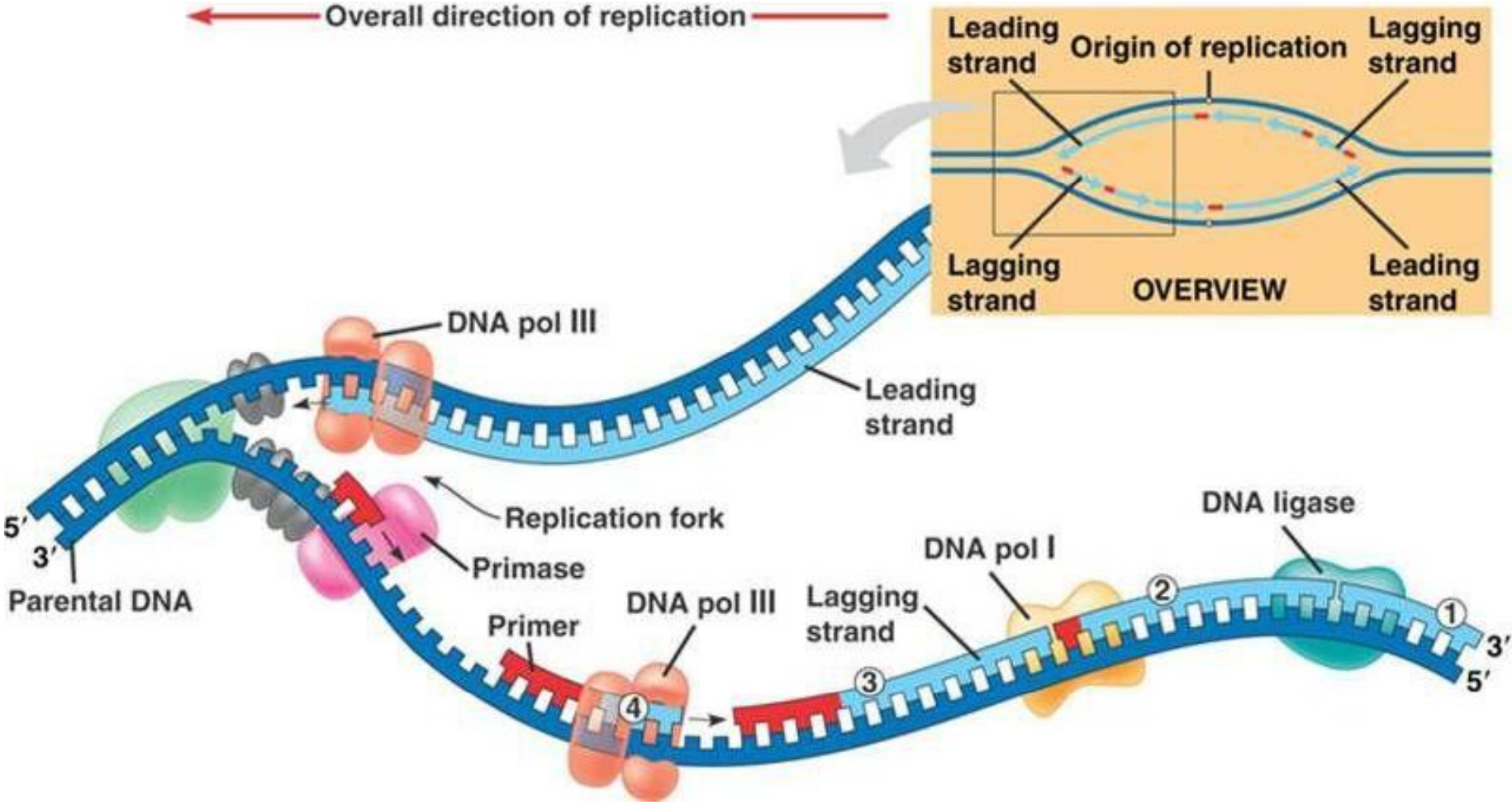
1. **Leading strand or Continuous strand synthesis**
   This occurs in 5'-3' direction using 3'-5' parental strand as template. It is synthesized as one piece adding nucleotides to its 3' end.
2. **Lagging strand synthesis**
   It is synthesized on 5'-3' direction in short segments of 1000 - 2000 nucleotides. These segments are called OKAZAKI FRAGMENTS.

5. **Excision of RNA primers :** The RNA primers are removed after the formation of okazaki fragments from 5’ end by the action of DNA polymerase I and replaced with DNA nucleotides

6. **Joining of Okazaki fragments:** the adjacent 5’ and 3’ ends of okazaki fragments are joined by DNA ligase.

IV. TERMINATION

- The replication completes at the Ter sites (a terminus region with 20 bp).
- This Ter region binds to tus protein and arrests trh replication fork.
- The new daughter DNA moleules formed become spirally coiled to get double helical structure.


Overall direction of replication
Leading strand
Origin of replication
Lagging strand
Lagging strand
OVERVIEW
Leading strand
DNA pol III
Leading strand
Replication fork
5′
3′
Parental DNA
Primase
Primer
DNA pol III
Lagging strand
DNA pol I
DNA ligase
1
2
3
4
3′
5′

DNA replication