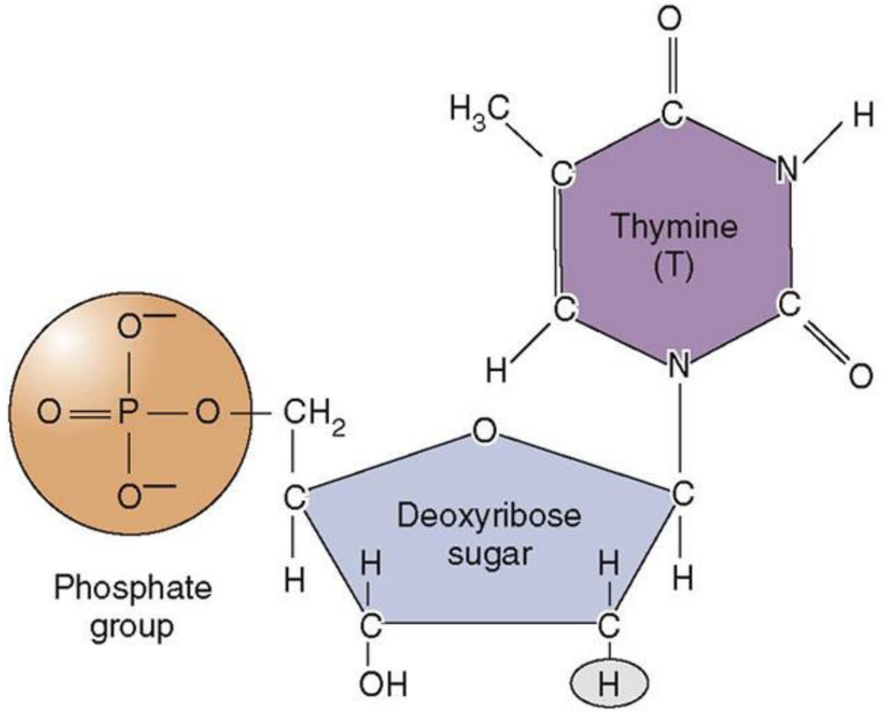
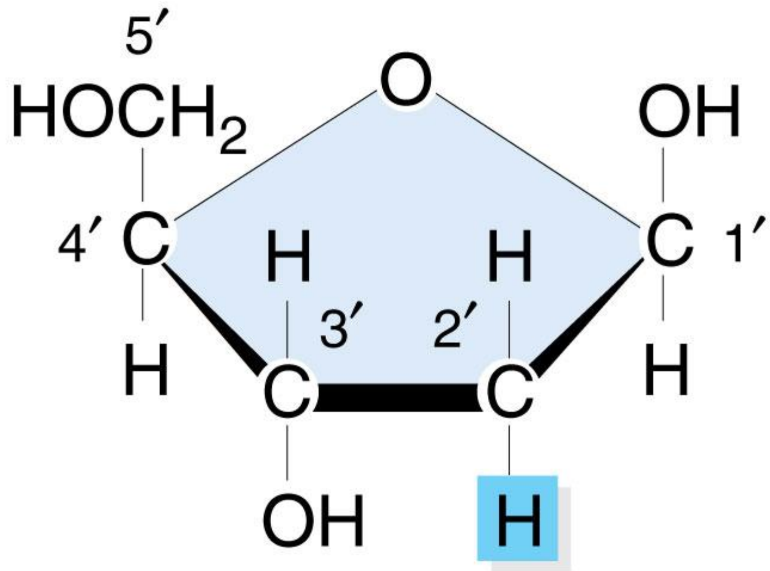
Nucleic Acids

- The **largest biological molecules**
- Store and transfer information within a cell
- Include **DNA and RNA**
- Are made of **nucleotides**
  - 5-carbon sugar
  - Phosphate group
  - Nitrogenous base

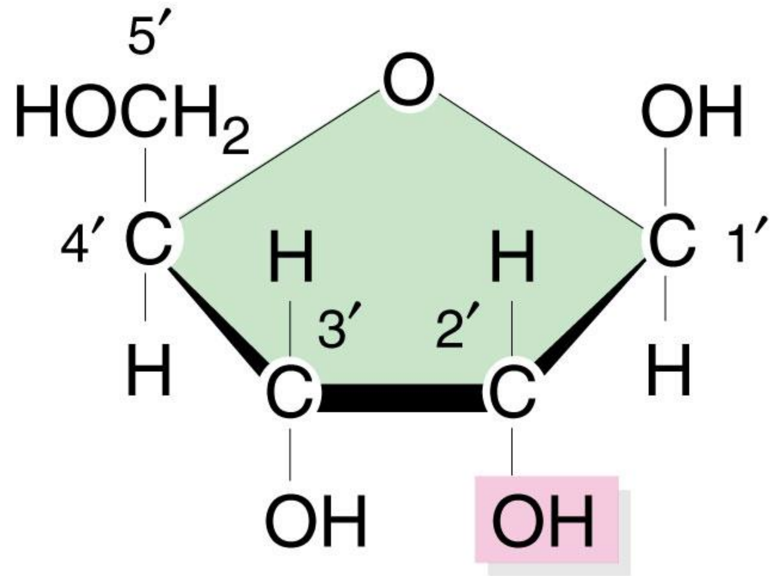


(a) Nucleotide

5-carbon sugar



Deoxyribose

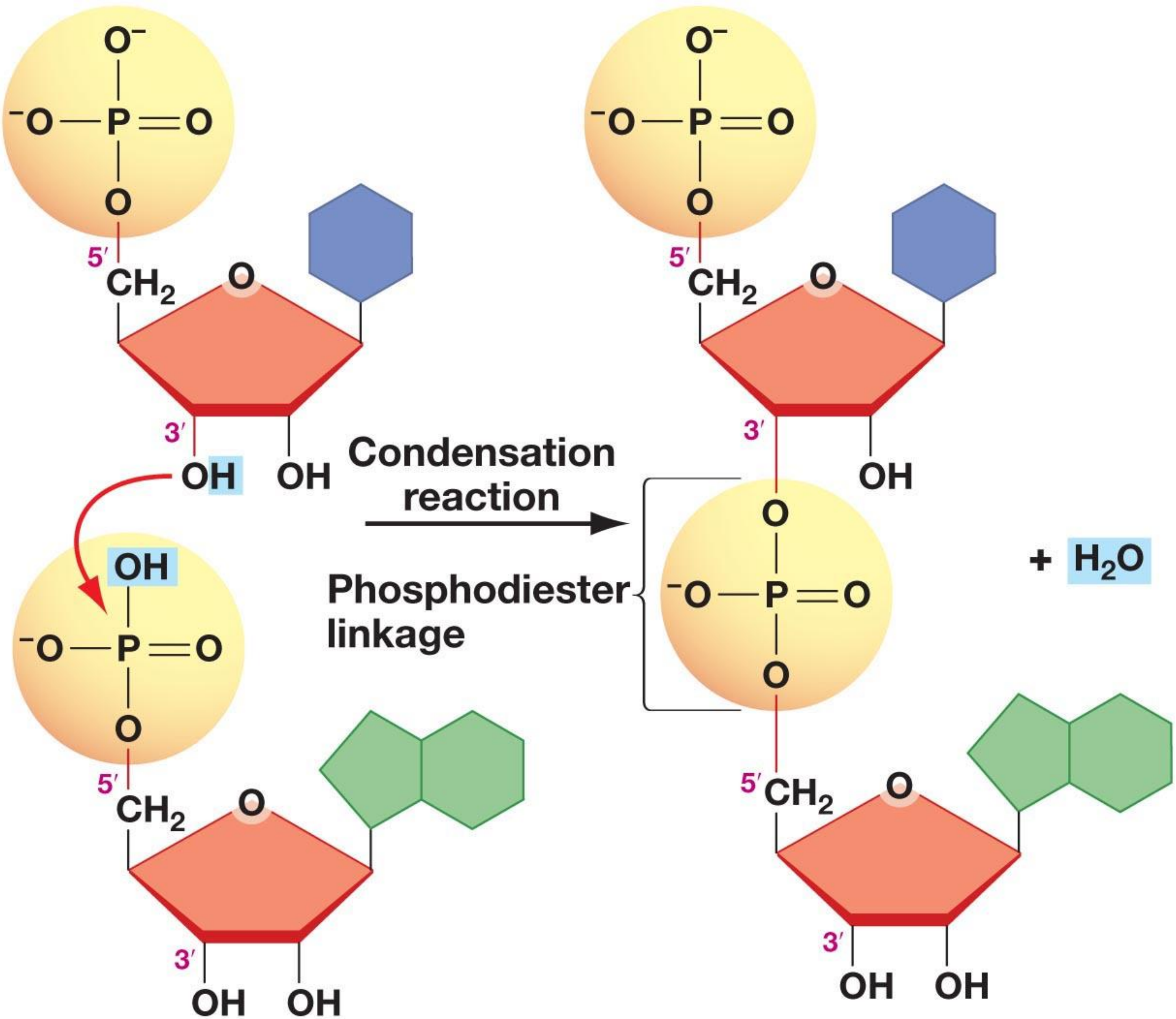


Ribose

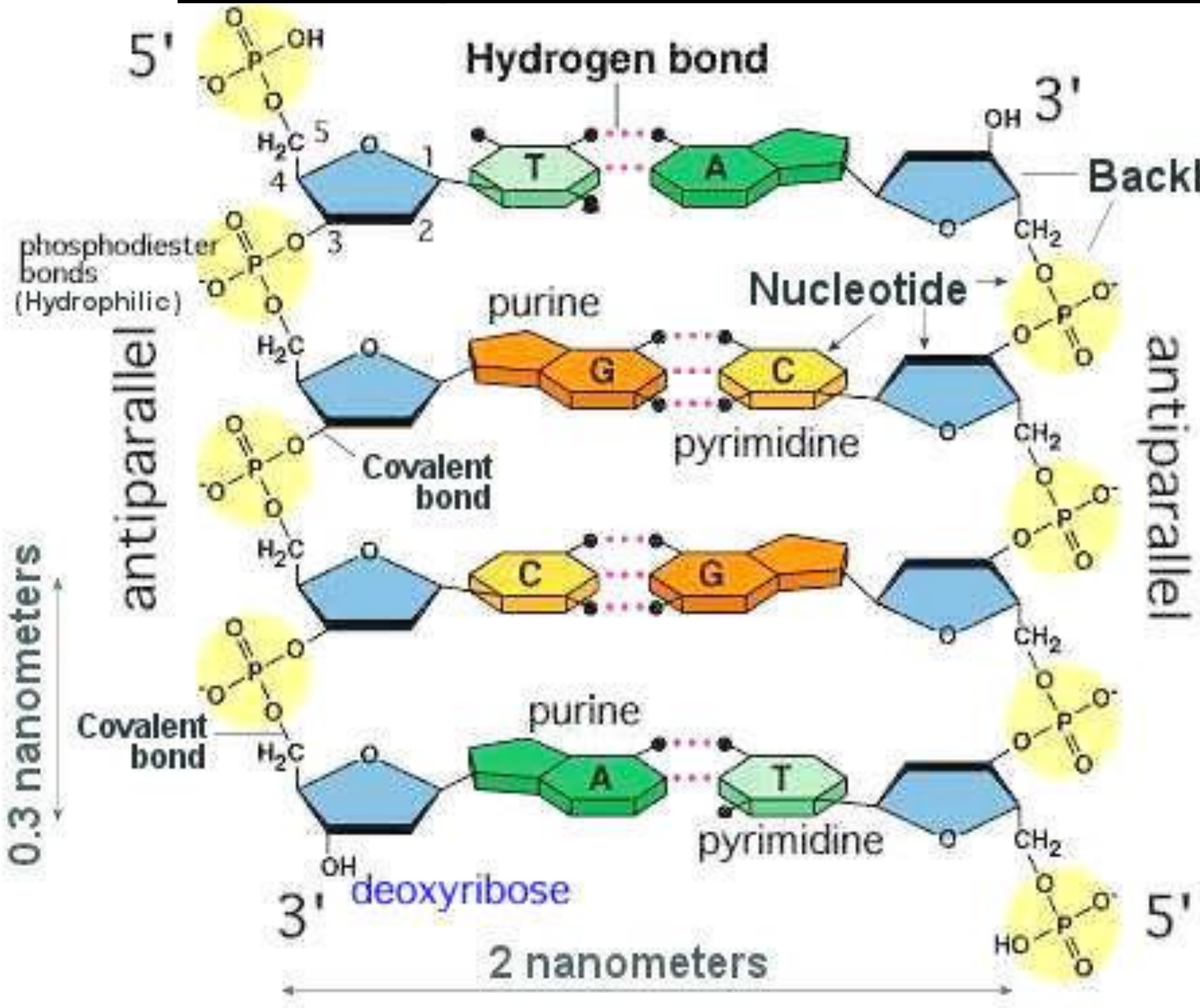
↓ **DNA**

↓ **RNA**

Sugar-phosphate backbone in Nucleic Acids



The Components and Structure of DNA



DNA Structure and Function

DNA accomplishes the following:

-Passes genetic information to the next generation

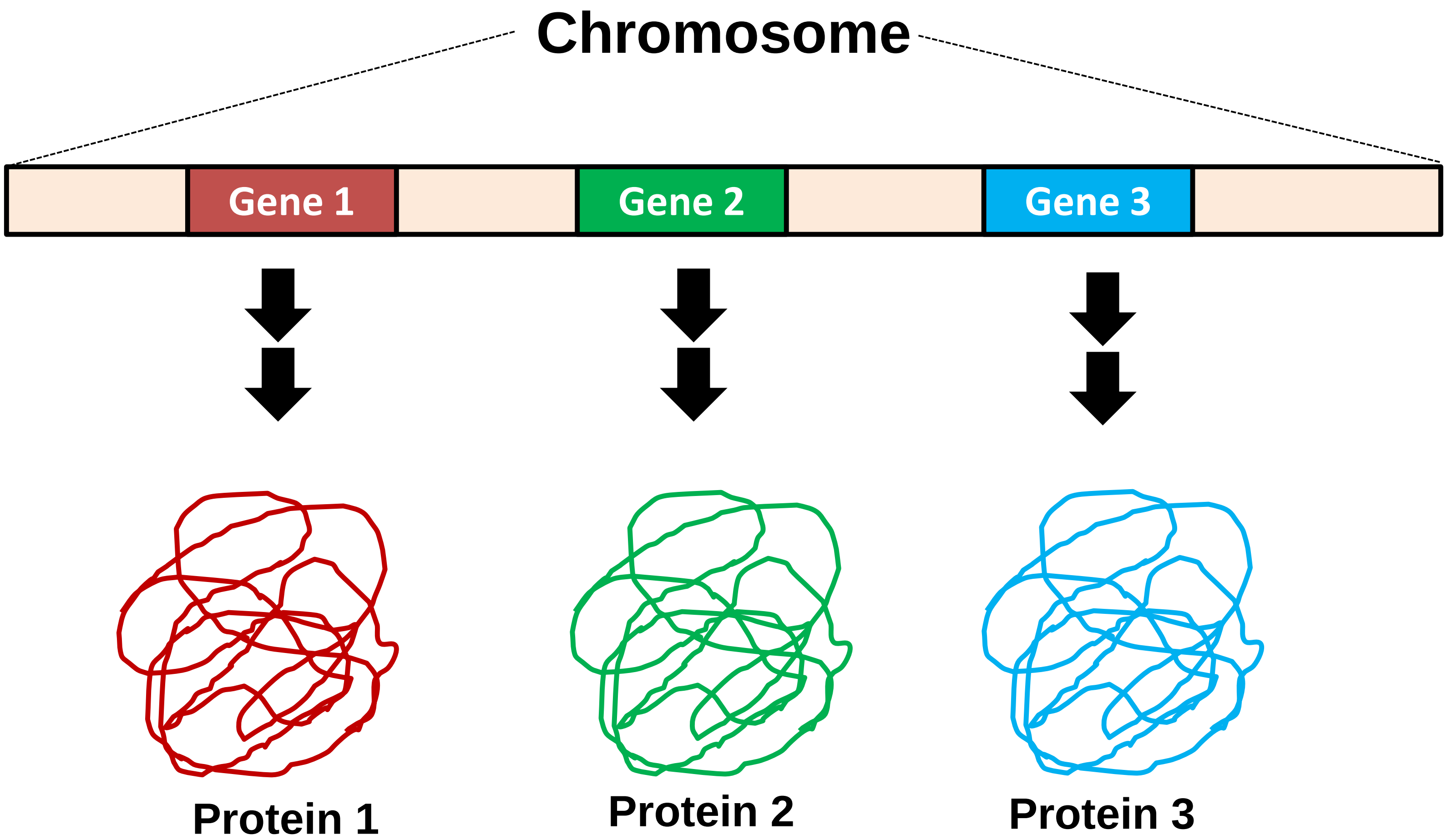
-Puts those information to work by controlling the synthesis of proteins

- Is copied easily so that cell's genetic information is replicated every time a cell divides.

DNA is able to accomplish these things because of its unique structure.

DNA and Chromosomes

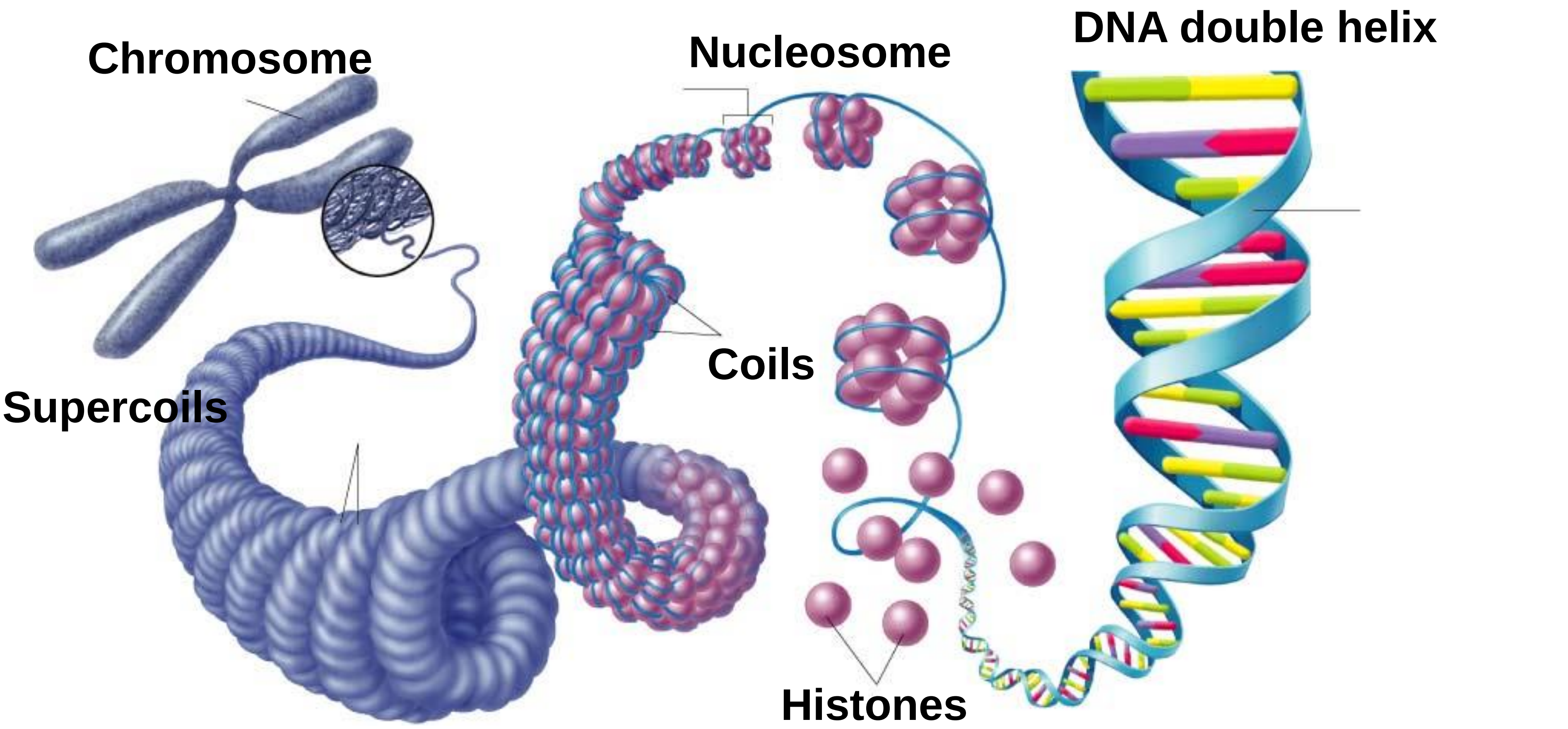
- **Each DNA strand is divided into segments.**
  - **Each segment forms a *gene*.**
  - **Genes are the recipes for proteins.**
    - ***The sequence of nucleotides in a gene dictate the order of amino acids in a polypeptide.***
- **Each DNA strand has many genes.**
- **Each DNA strand is called a *chromosome.***



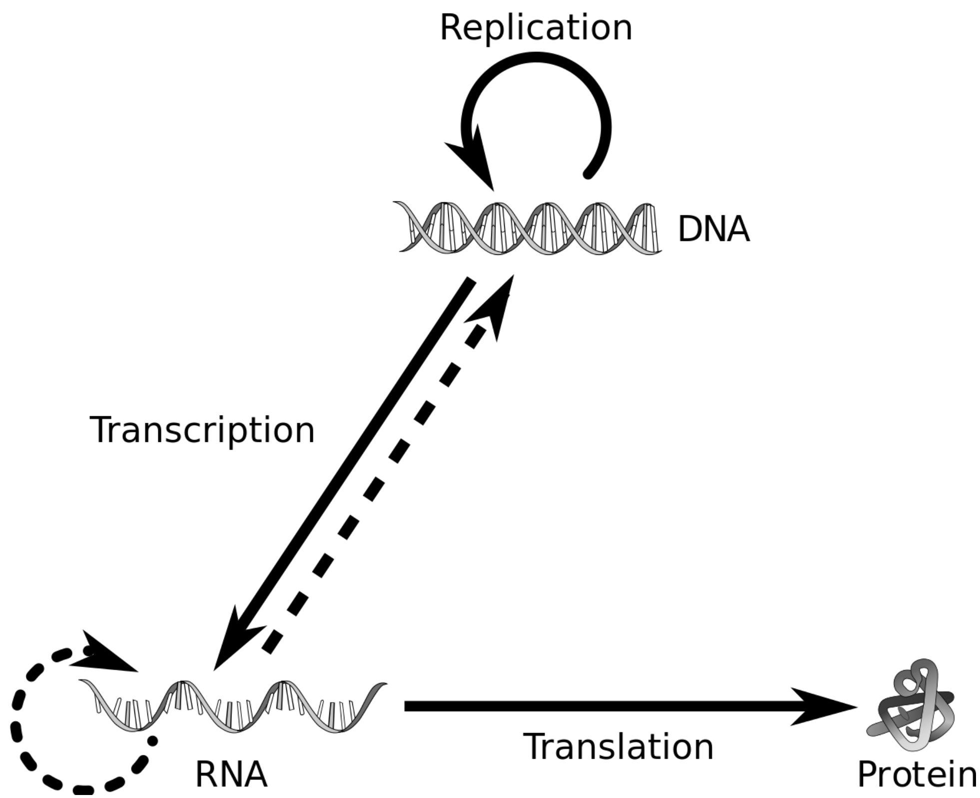
Human cells have 46 chromosomes in each cell.

Each cell copies all of these chromosomes before it divides to pass along to daughter cells.

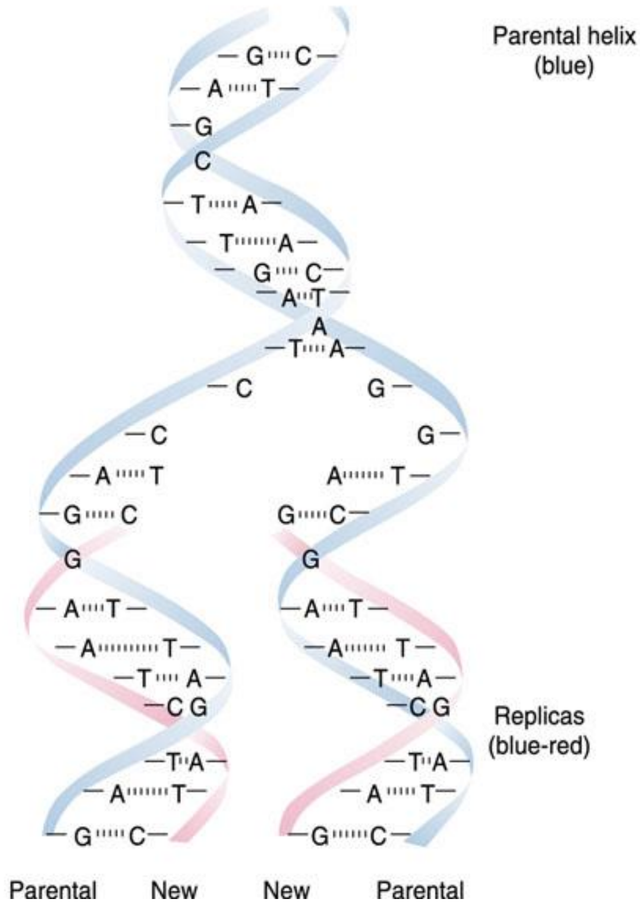
Chromosome Structure of Eukaryotes

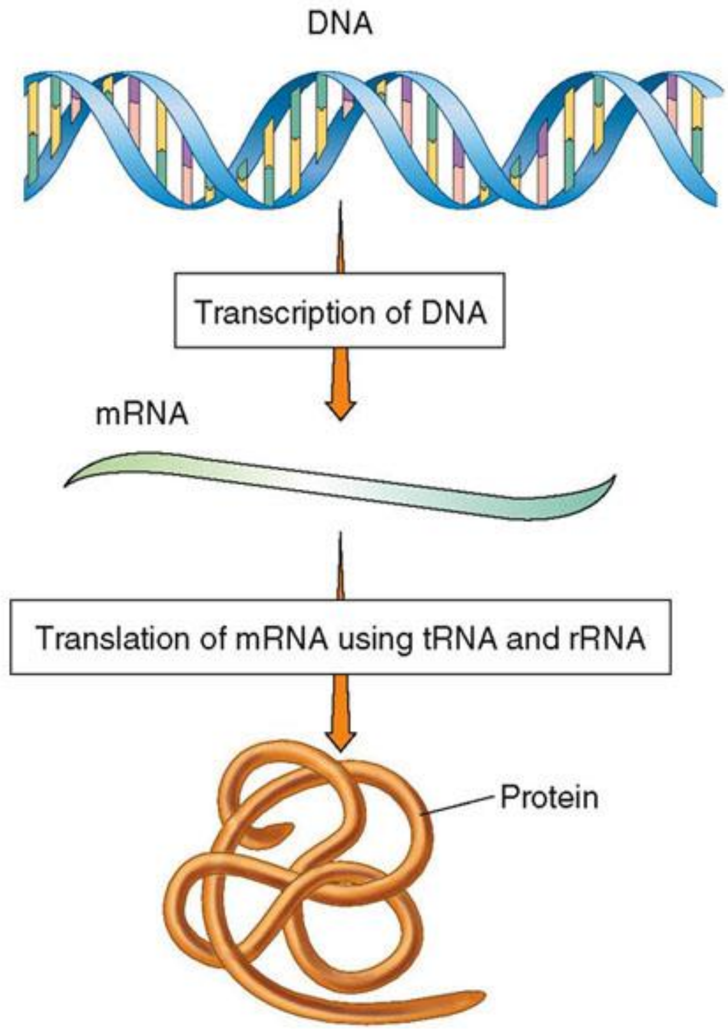


Central Dogma of Molecular Biology



The Functions of DNA





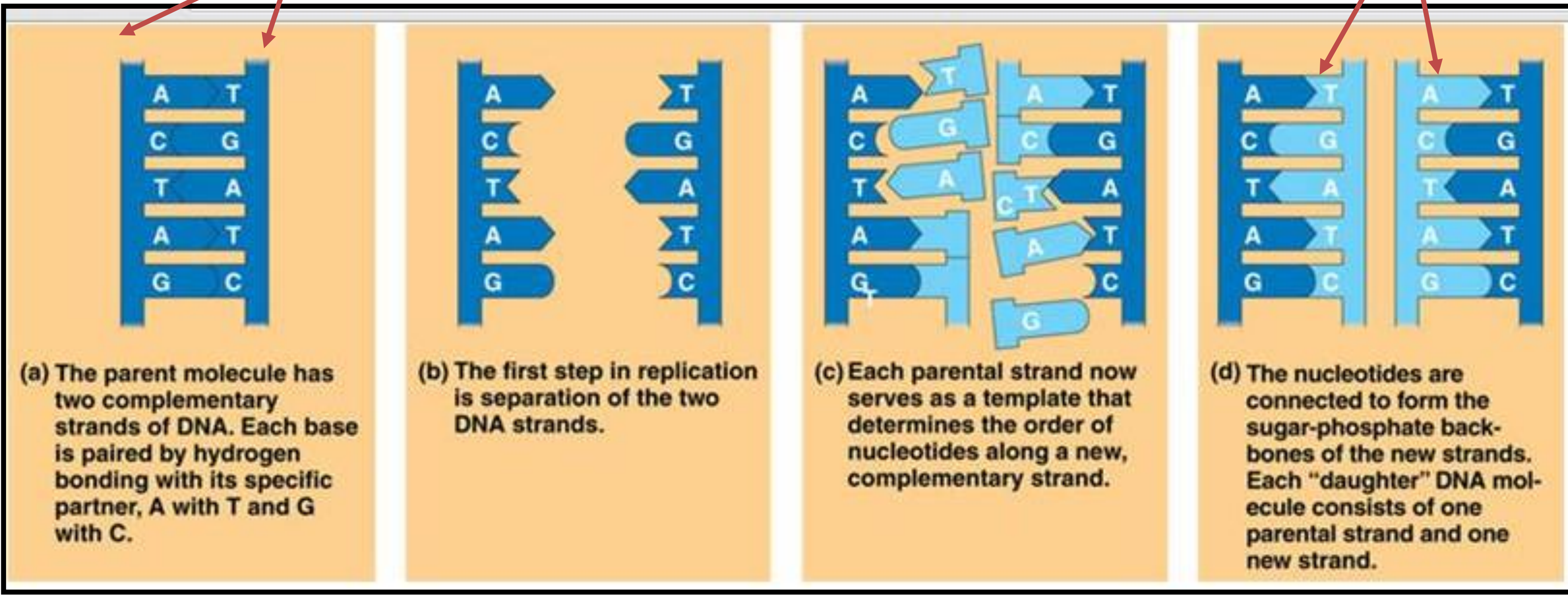
Base Pairing Aids DNA Replication

- **DNA replication**
  - **Is the process by which DNA is copied**
    - **This is done before cell division.**
    - **Provides the new cells with a copy of the genetic information**
  - **Relies on the base-pairing rules**

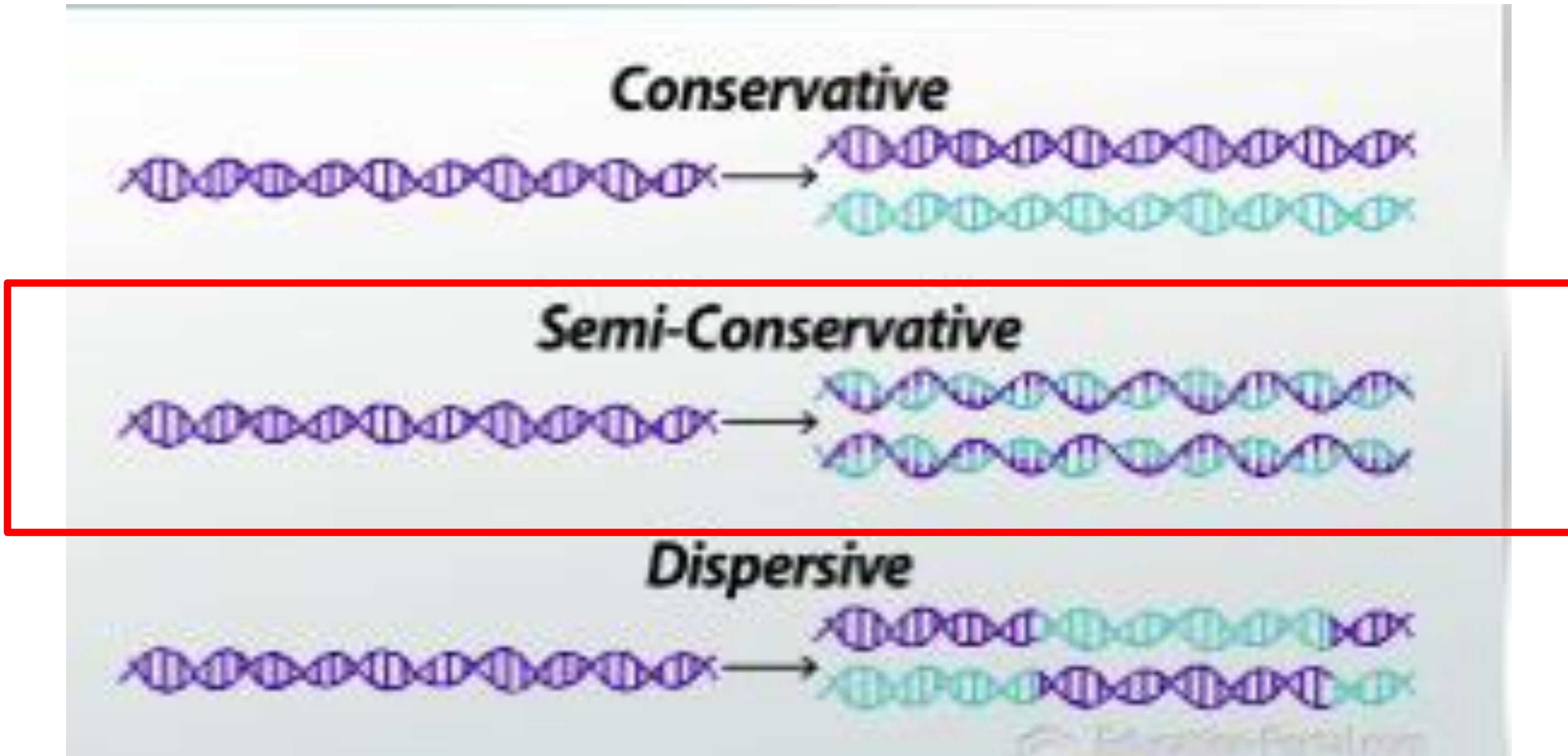
Base Pairing Aids DNA Replication

Parental (Template) Strands

Daughter Strands



Conservative, Semi conservative or Dispersive mode of DNA replication?



DNA Replication could be divided into three phases:

- Initiation
- Elongation
- Termination

Initiation of DNA replication starts at Origin of Replication.

Enzyme *helicase* unwinds and breaks H-bonds of the double stranded DNA

The *single-stranded binding proteins* stabilize the separated strands

Initiation stars at Origin of Replication

-Circular DNA

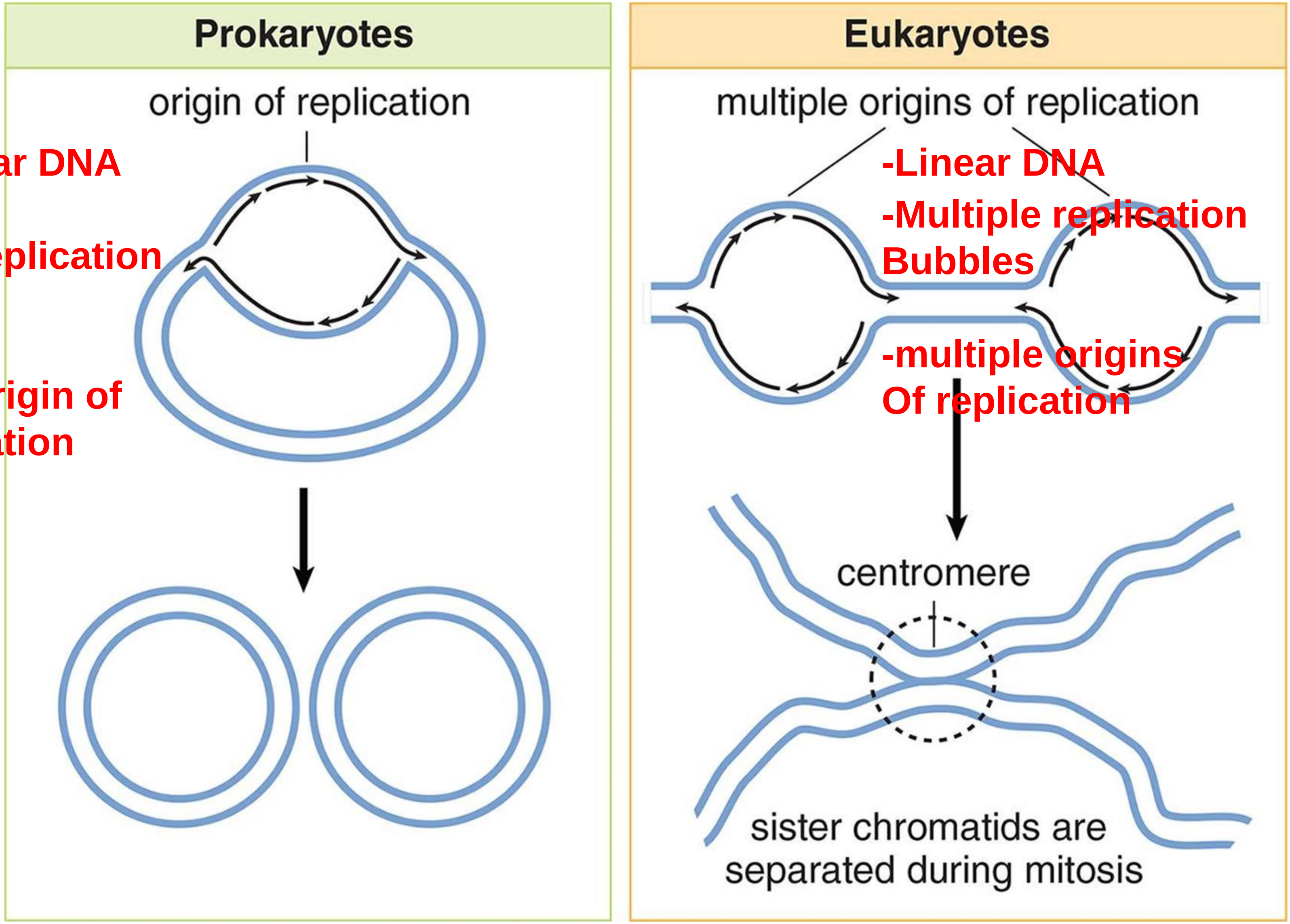
-One replication Bubble

-One origin of replication

-Linear DNA

-Multiple replication Bubbles

-multiple origins Of replication



Properties of DNA polymerases

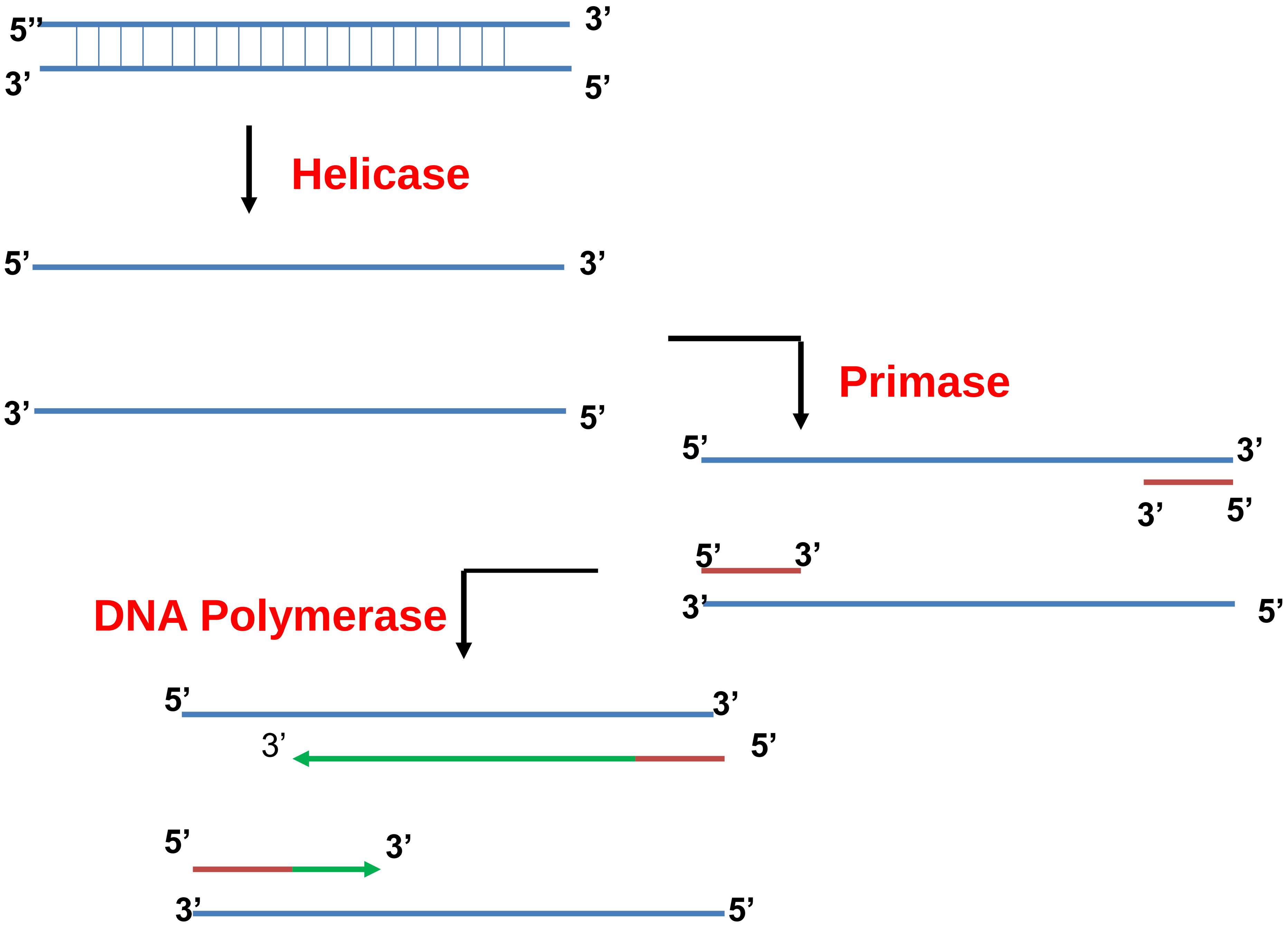
Enzyme ***DNA polymerase III*** builds new DNA strands that will pair with each old DNA strand by A-T, G-C manner

- All DNA polymerases **require a primer**
  - RNA primer, synthesized by **Primase (**Enzyme ***Primase*** adds a small piece of RNA called a primer. DNA nucleotides can only be added to the **3' end of an existing chain**)

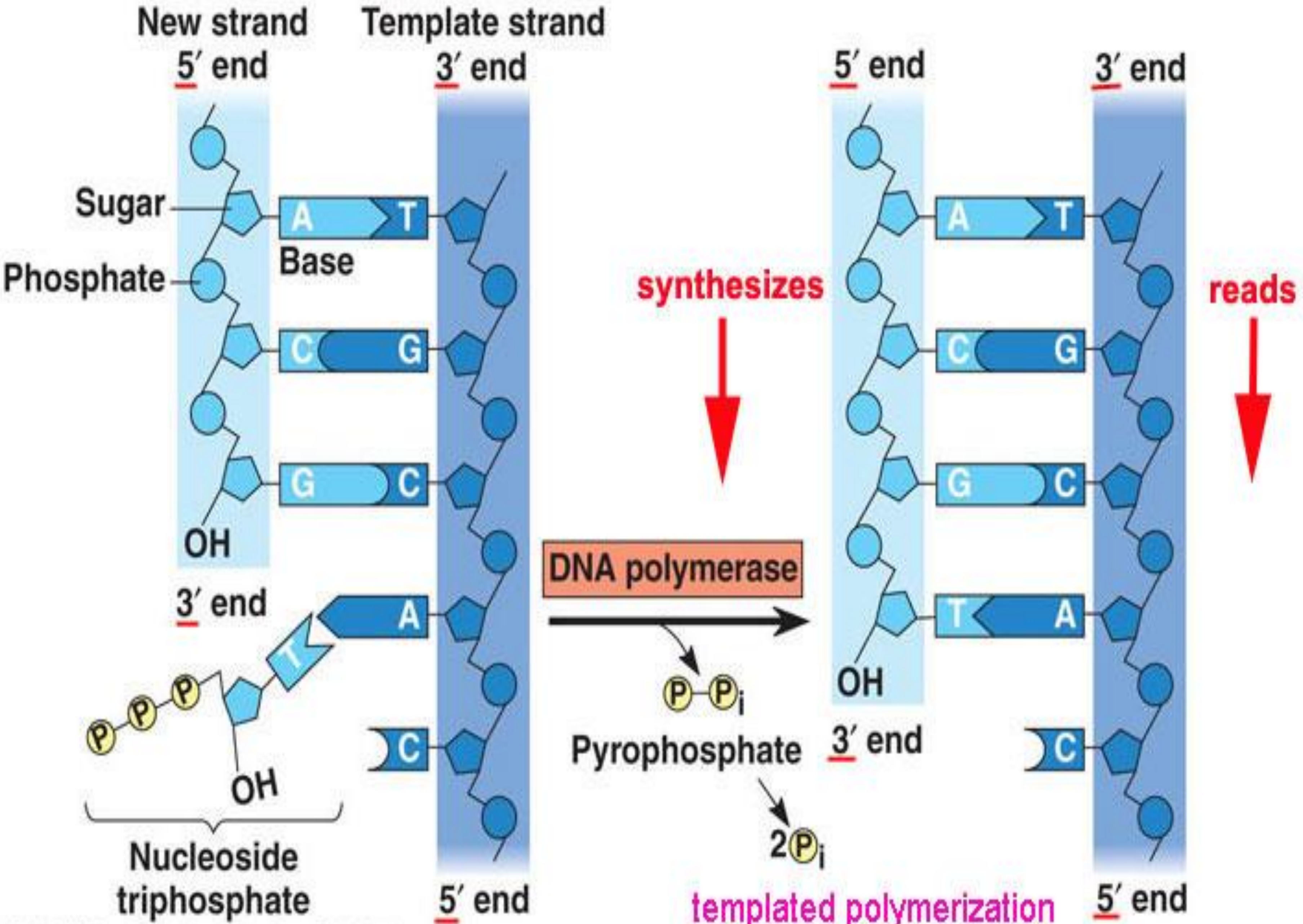
- DNA polymerases add basses only from the **5'-3' direction**

- All DNA polymerases have 3'-5' exonuclease activity

(proofreading ability)



5’’
3’
3’
5’
Helicase
5’
3’
3’
5’
Primase
5’
3’
3’
5’
5’
3’
3’
5’
DNA Polymerase
5’
3’
3’
5’
5’
3’
3’
5’


New strand
5′ end
Template strand
3′ end
Sugar
Phosphate
Base
A
T
C
G
G
C
OH
3′ end
A
T
P
P
P
OH
C
Nucleoside triphosphate
5′ end
synthesizes
reads
DNA polymerase
P
P i
Pyrophosphate
2 P i
5′ end
3′ end
A
T
C
G
G
C
T
A
OH
3′ end
C
5′ end
templated polymerization

5’ ATGGCCTAATGCAATCTGATGGCCTTAAGCGT 3’

Primer sequences (10 bases): **5’ ACGCUUAAGG 3’**

Enzyme that makes the primer: **Primase**

On which base, at the 3’OH, DNA Polymerase will add its first base? **G**

The First base that will be added by the DNA Pol III: **C**

Sequence that the DNA polymersase III will incorporate:

5’ CCATCAGATTGCATTAGGCCAT 3’

5’ ATGGCCTAATGCAATCTGATGGCCTTAAGCGT 3’

Primer sequences (10 bases): **5’ ACGCUUAAGG 3’**

5’ ATGGCCTAATGCAATCTGATGGCCTTAAGCGT 3’

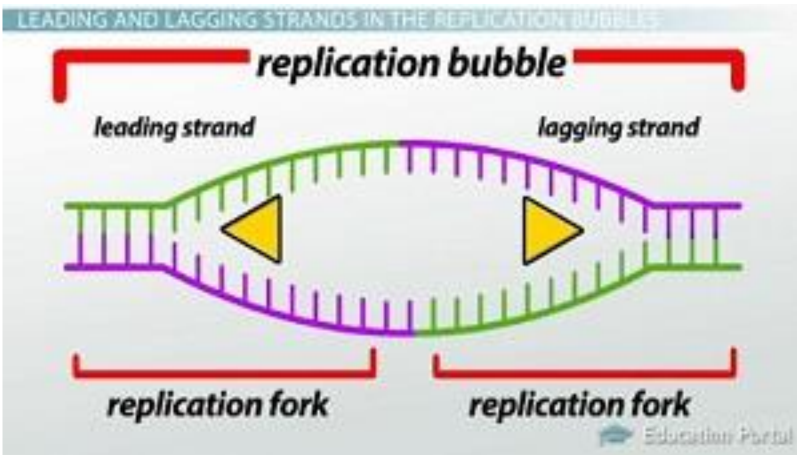
3’GGAAUUCGCA 5’

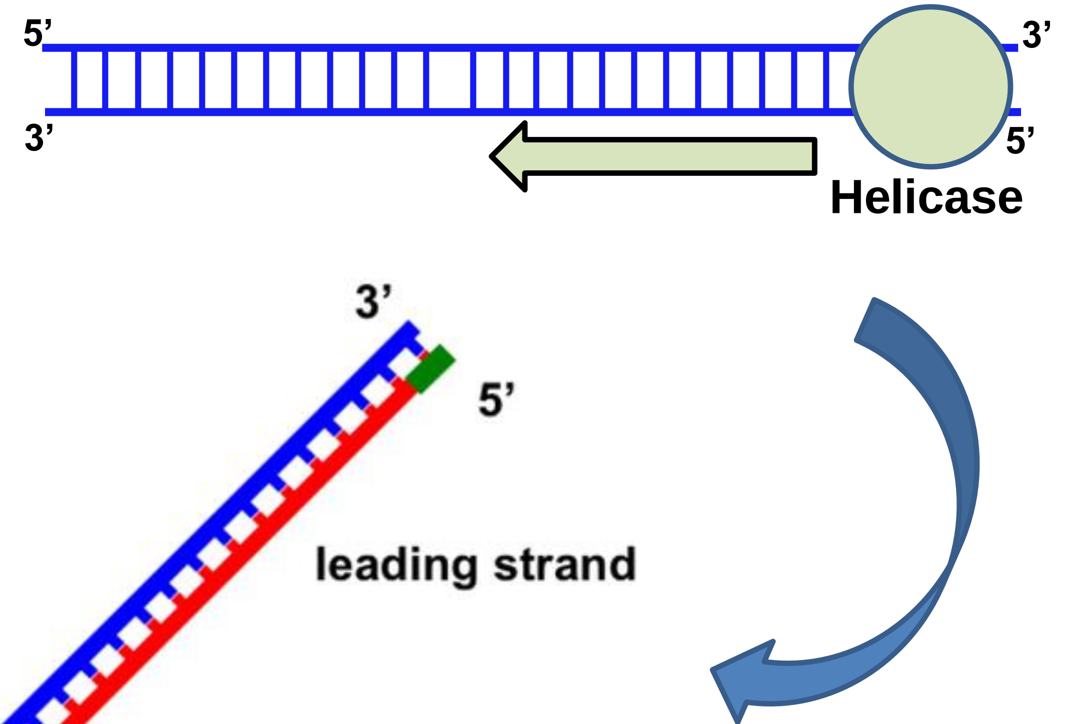
OH

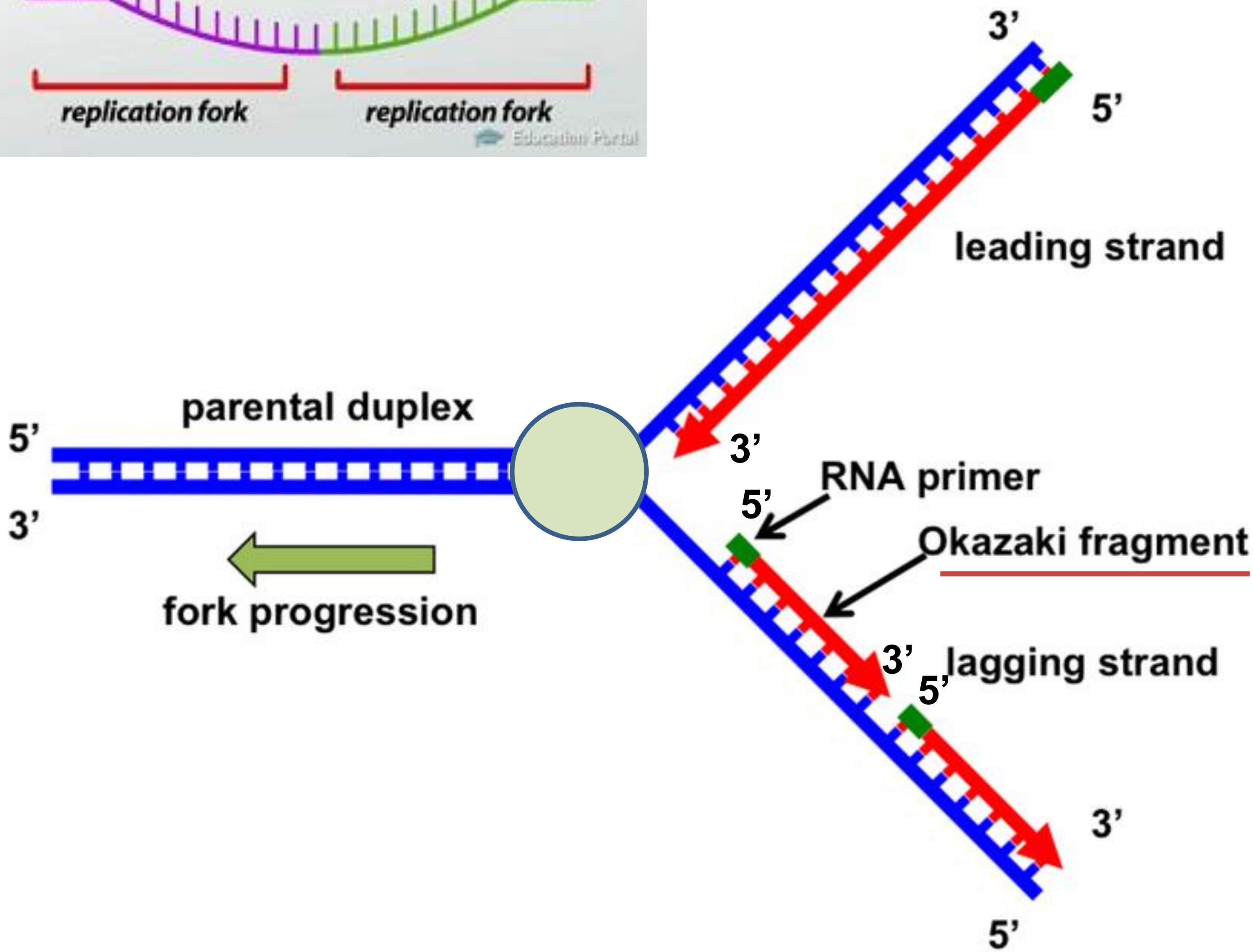
3’CGGAATTCGCA 5’

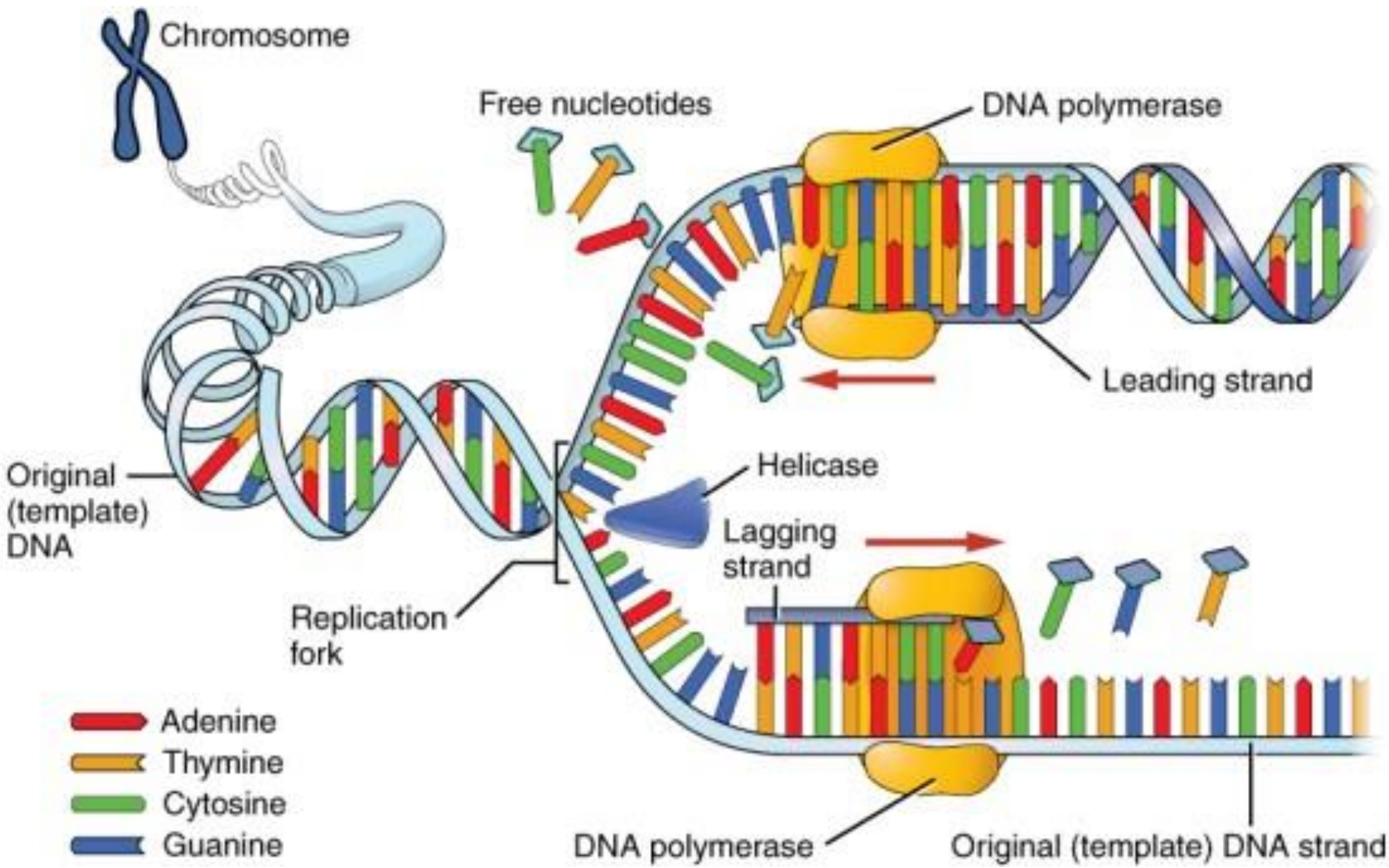
OH

DNA Polymerase III starts adding bases

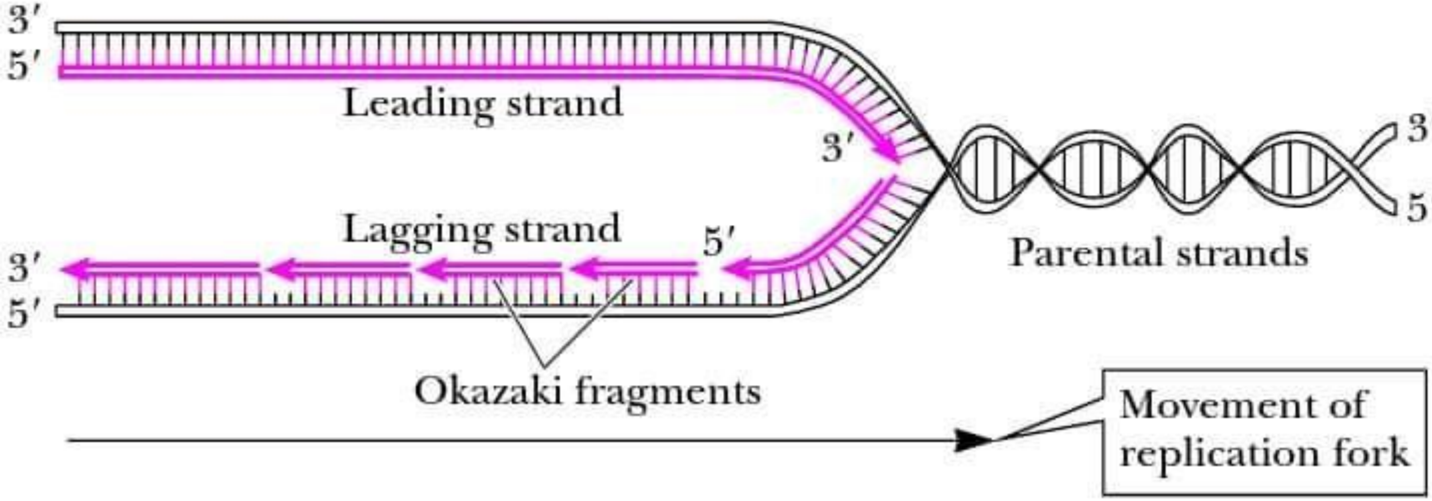

LEADING AND LAGGING STRANDS IN THE REPLICATION BUBBLES
replication bubble
leading strand
lagging strand
replication fork
replication fork
Education Portal

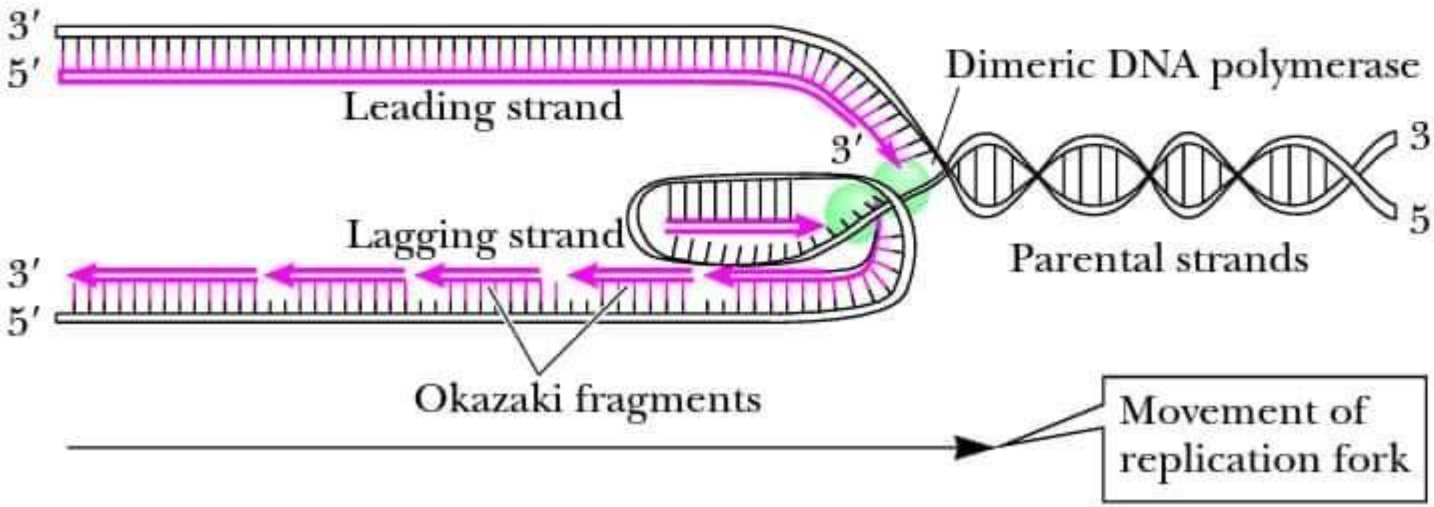

5'
3'
3'
5'
Helicase

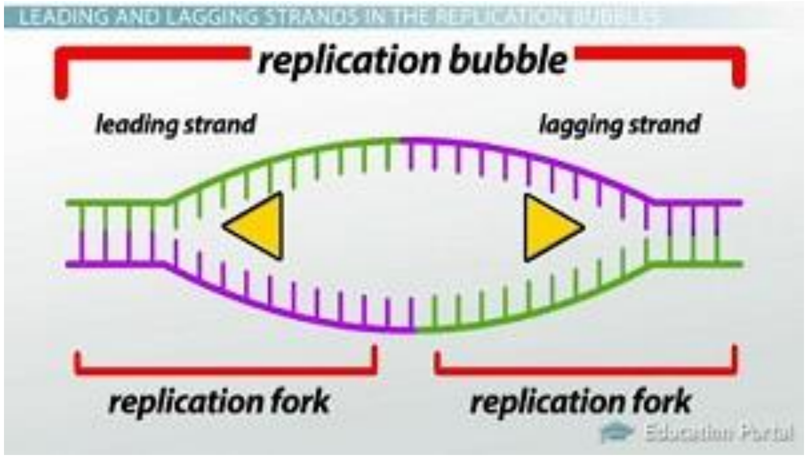

3'
5'
leading strand
parental duplex
5'
3'
3'
fork progression
RNA primer
5'
Okazaki fragment
3'
lagging strand
5'
3'
5'

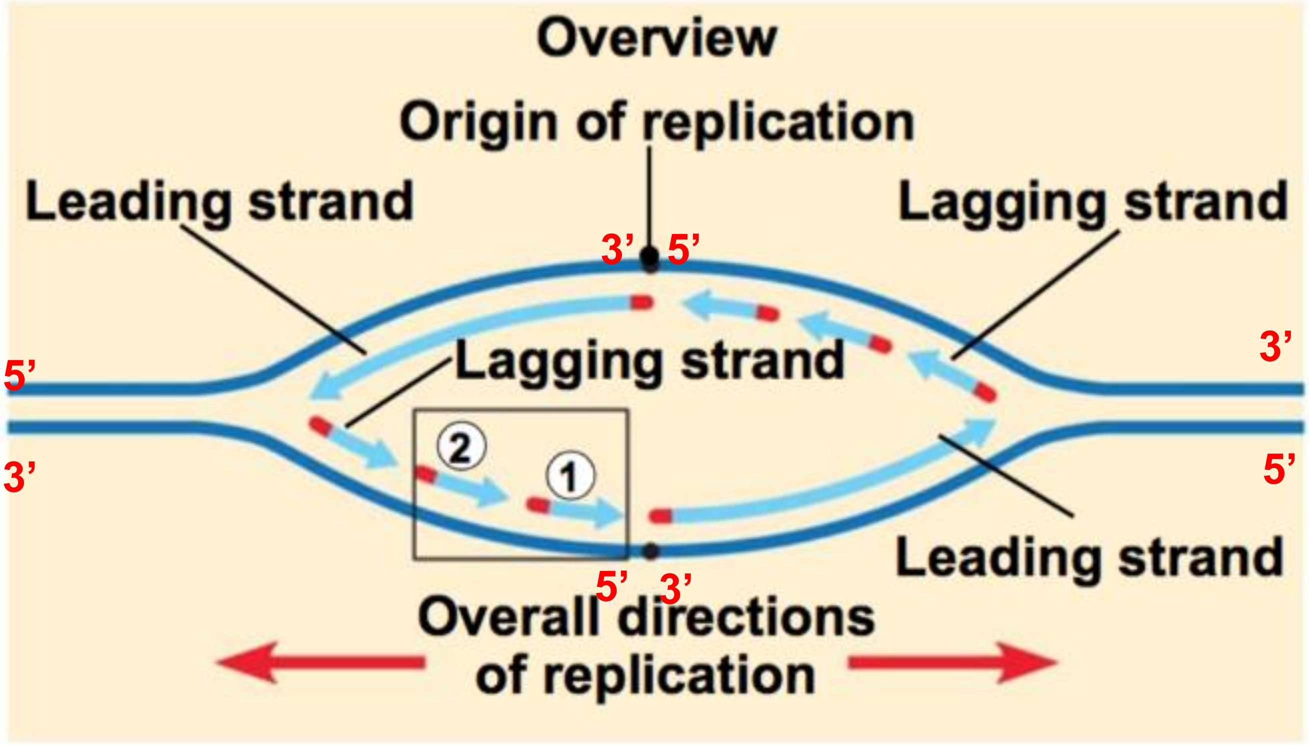


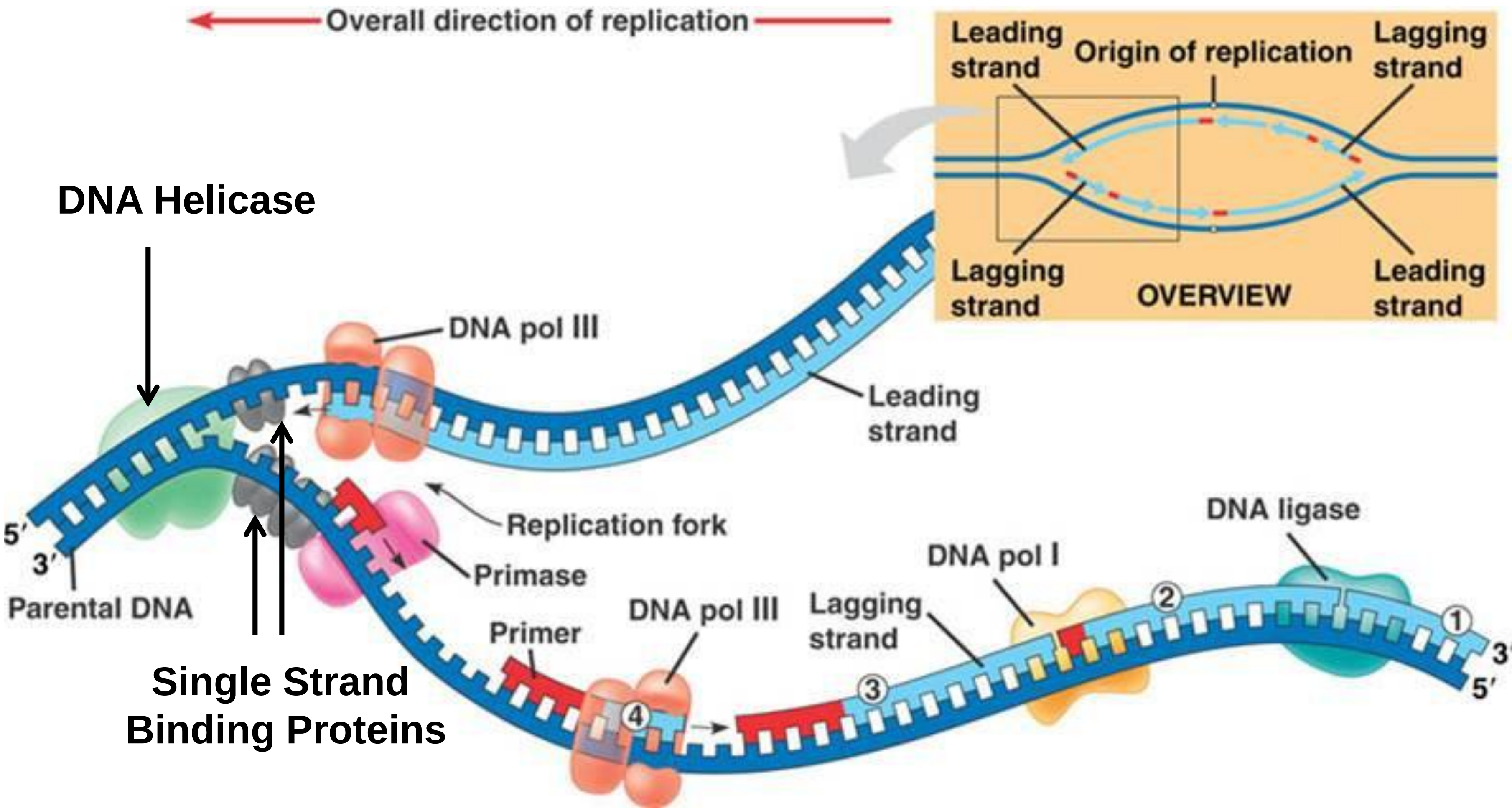
Chromosome
Free nucleotides
DNA polymerase
Leading strand
Original (template) DNA
Helicase
Lagging strand
Replication fork
Adenine
Thymine
Cytosine
Guanine
DNA polymerase
Original (template) DNA strand


3′
5′
Leading strand
3′
3′
5′
Lagging strand
5′
3′
5′
Parental strands
Okazaki fragments
Movement of
replication fork

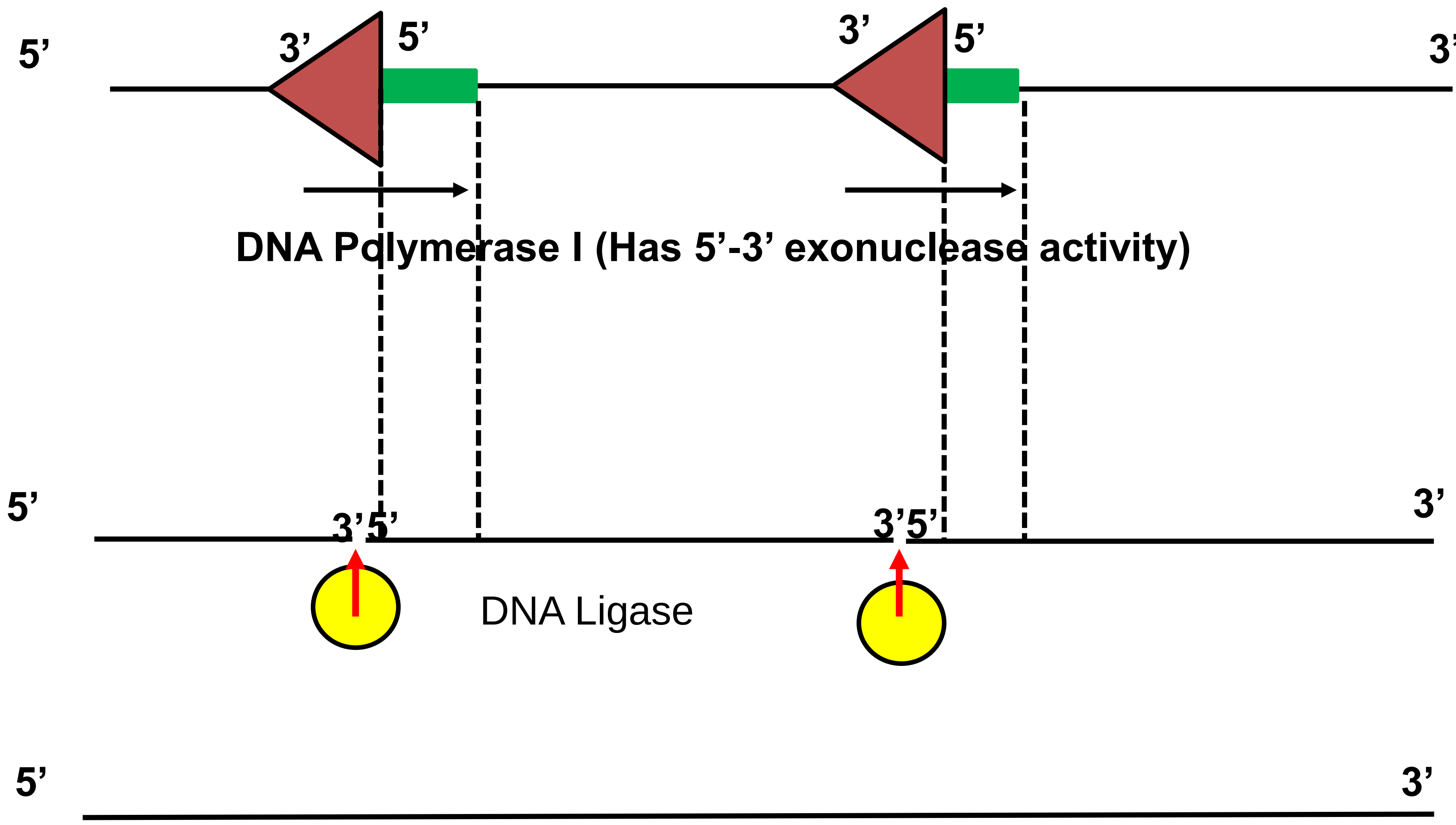

3′
5′
Leading strand
Dimeric DNA polymerase
3′
3′
5′
Lagging strand
3′
5′
Parental strands
Okazaki fragments
Movement of
replication fork


LEADING AND LAGGING STRANDS IN THE REPLICATION BUBBLES
replication bubble
leading strand
lagging strand
replication fork
replication fork
Education Portal


Overview
Origin of replication
Leading strand
Lagging strand
3’
5’
5’
3’
Lagging strand
3’
5’
2
1
Leading strand
5’
3’
Overall directions
of replication


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

OKAZAKI Fragments



DNA replication is accomplished by DNA polymerase and other enzymes:

A.Enz. ***helicase*** unwinds and breaks H-bonds

B. ***single-stranded binding proteins*** stabilize the molecule

C. Enz. ***primase*** adds a small piece of RNA (called a primer) to origin of replication (DNA nucleotides can only be added to the 3' end of an existing chain)

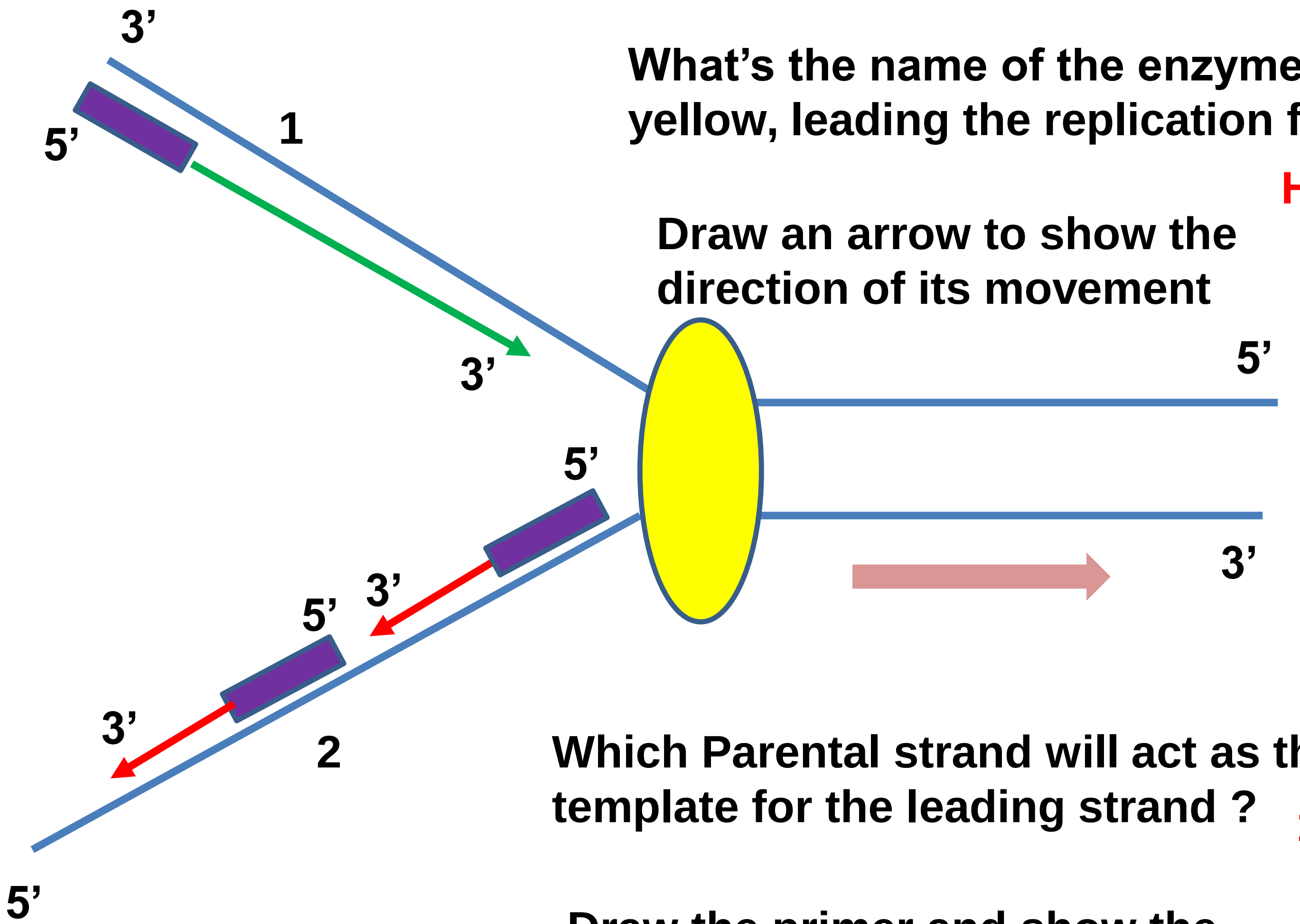
1. ***leading strand*** - one primer needed

2. ***lagging strand*** - **Okazaki fragments** - primer needed for each fragment

D. Enz. ***DNA polymerase III*** builds new DNA strands that will pair with each old DNA strand. It adds new dNTPs to the 3′end of the primer by A-T, G-C manner (**from the 5′ to 3′ direction only**).

E. ***DNA polymerase I*** replaces RNA primers w/ DNA

F. Enz. ***DNA ligase*** joins Okasaki fragments together



What’s the name of the enzyme in yellow, leading the replication fork ?

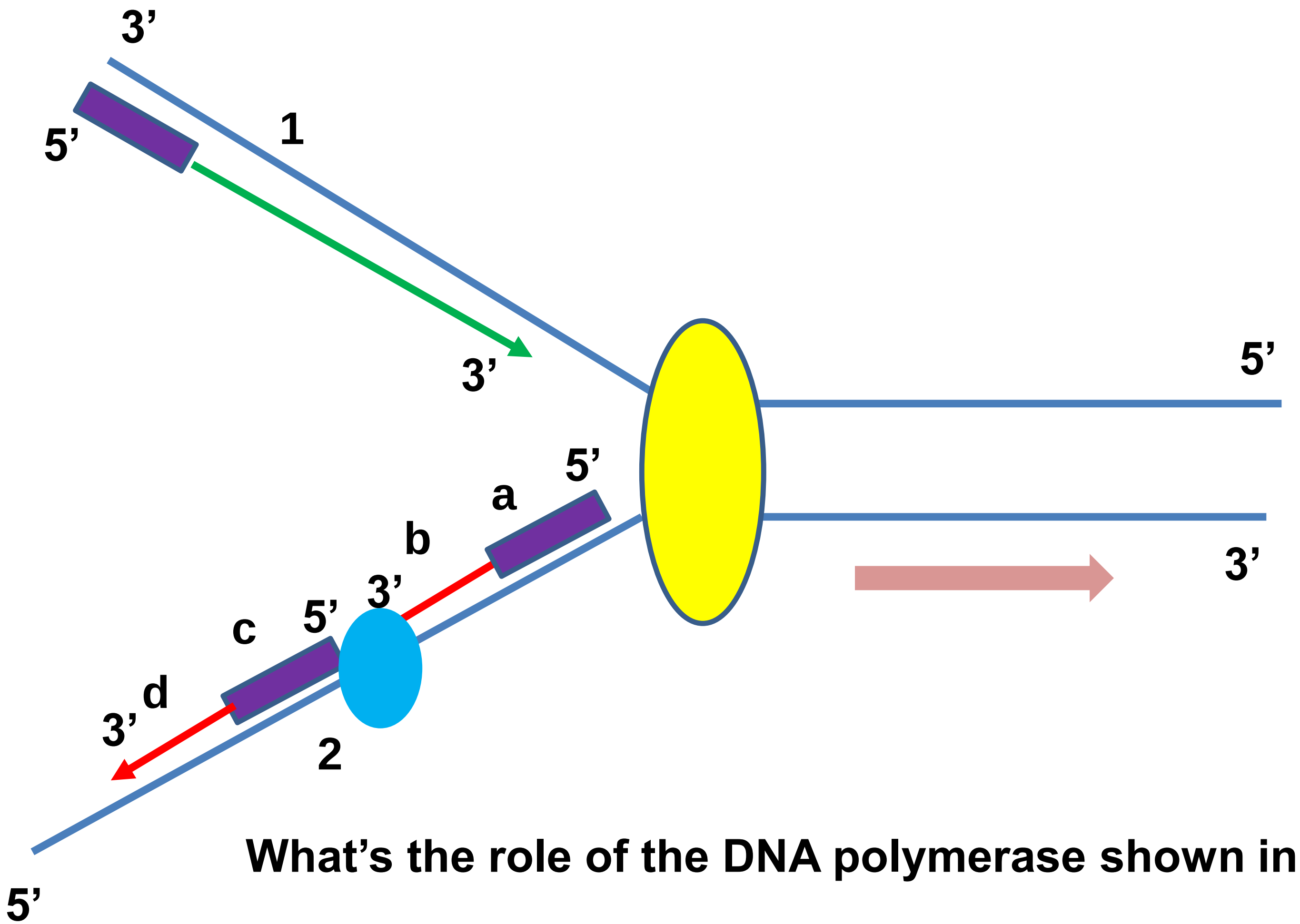
Helicase

Draw an arrow to show the direction of its movement

Which Parental strand will act as the template for the leading strand ? 1

Draw the primer and show the movement of the leading strand with proper orientation with an arrow

Draw the Okazaki fragments (at least 2) with Directions



What’s the role of the DNA polymerase shown in blue ?

Which fragment will it remove and replace ? (a or b or c or d) c

Which property of this DNA polymerase is unique ? 5’ to 3’ exonuclease activity

What’s the role of DNA ligase here ?

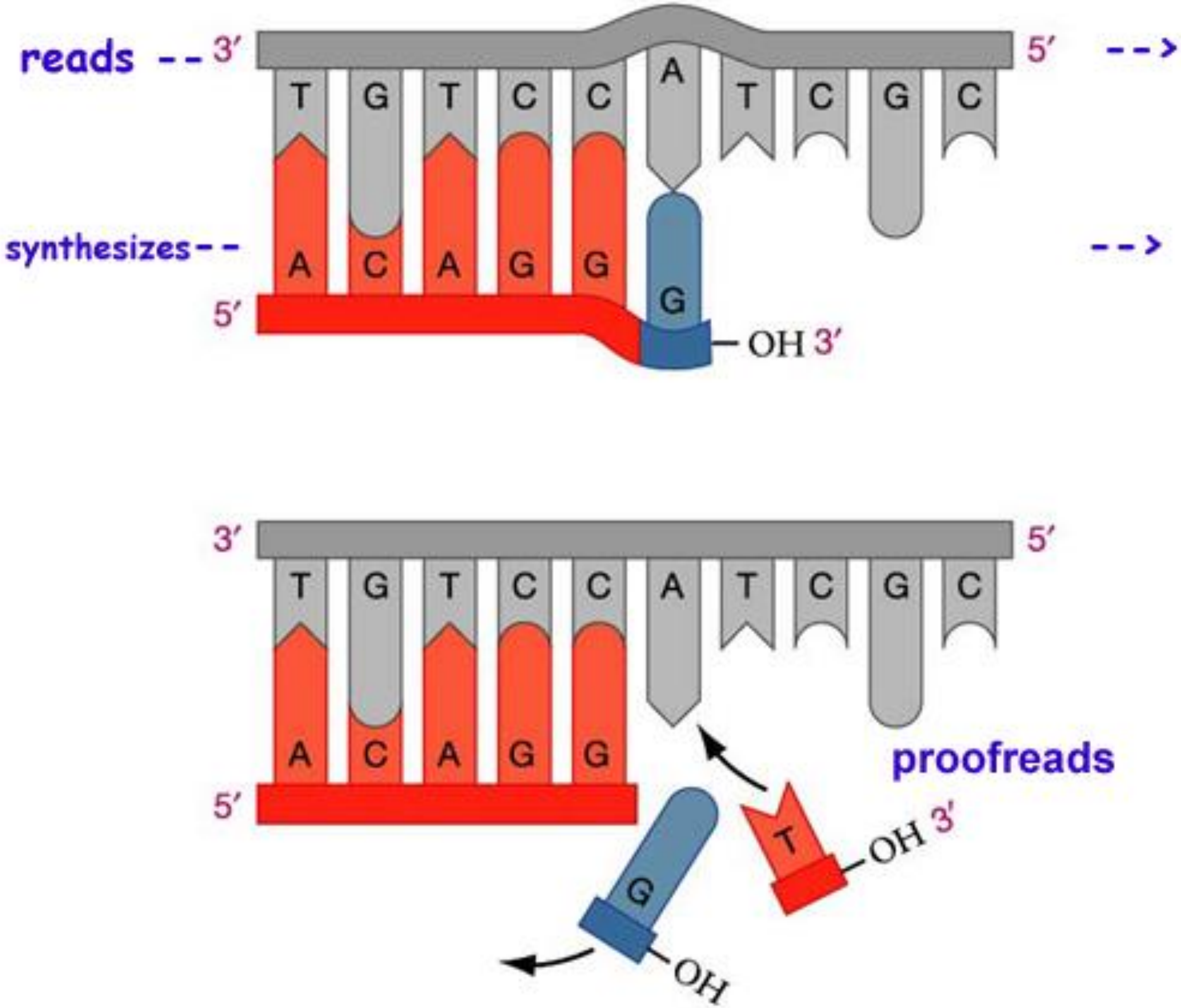
<u>DNA Proof Reading</u>

- It takes <u>*E. coli*</u> <1 hour to copy 5 million base pairs in its single chromosome
  - divide to form 2 identical daughter cells

- Human cell copies its 6 billion bases & divide into daughter cells in only few hours
  - remarkably accurate
  - **only ~1 error per 100 million bases**
  - ~30 errors per cell cycle

DNA Proof Reading

All DNA polymerases have 3’-5’ exonuclease activity (proofreading ability)



DNA Repair Systems

- One major concern for an organism's survival is genomic stability

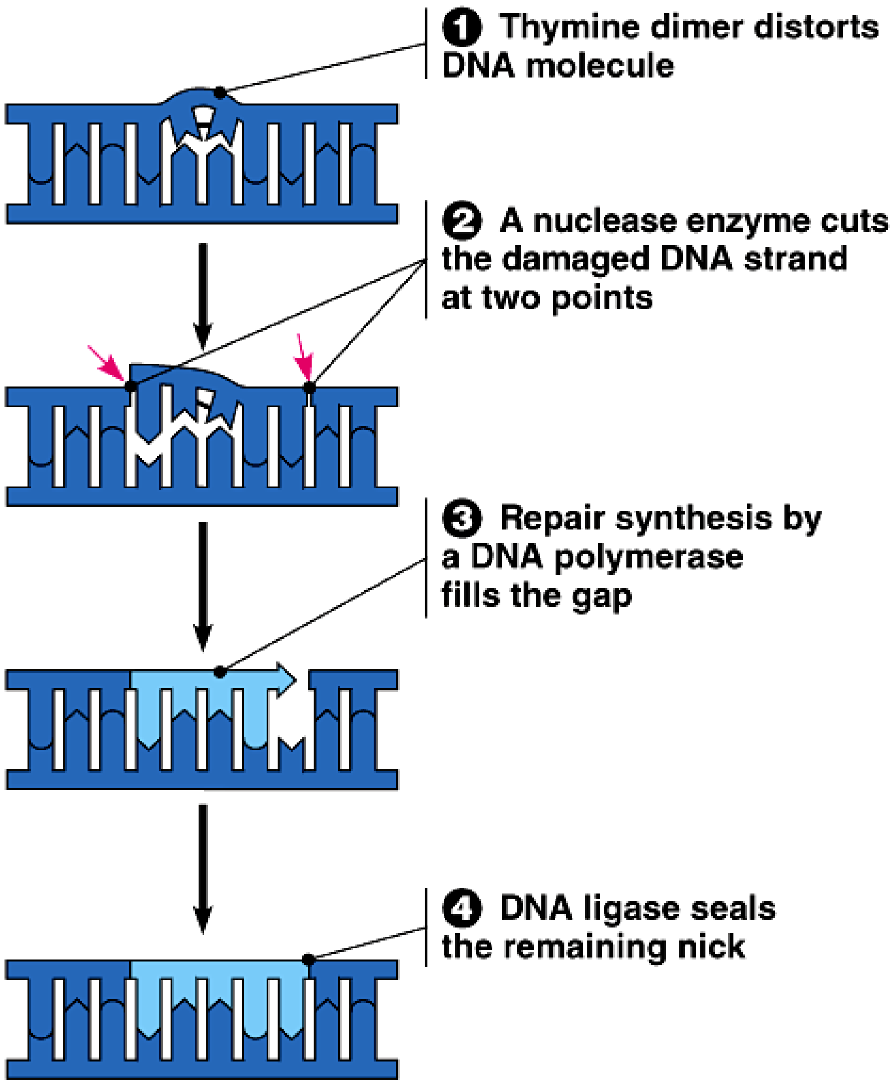
- Errors in DNA can occur during synthesis or post replication from environmental factors (i.e. UV radiation)

- Injury to DNA is minimized by systems that recognize and correct the damage. The repair systems are as complex as the replication apparatus itself, which indicates their importance for the survival of the cell.

- Errors in DNA may lead to mutations, which can cause a variety of problems including cancer

- Organisms have multiple repair systems
    - Base Excision
    - Nucleotide Excision
    - Mismatch Excision Repair (MMR)

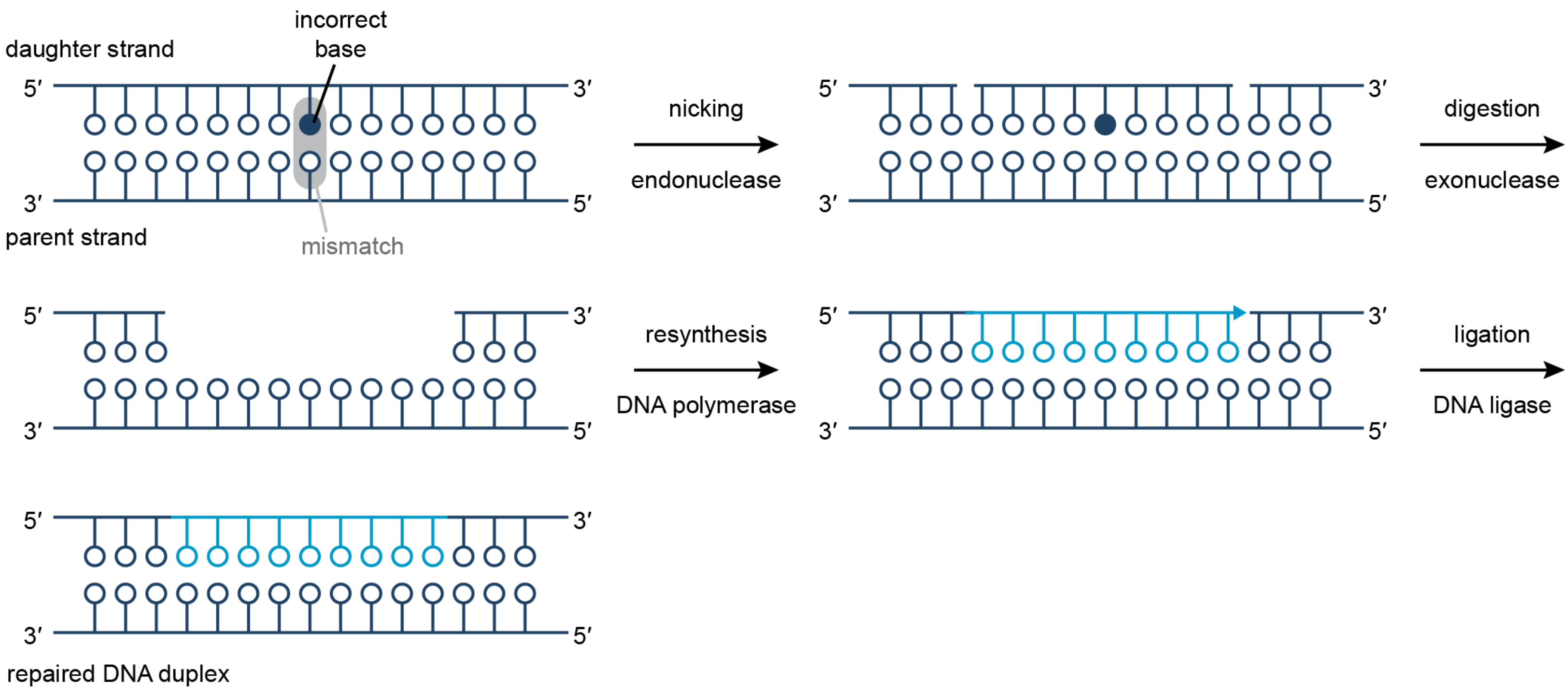
Excision-repair removes and replaces a stretch of DNA that includes the damaged base(s).



Mismatch Repair (MMR)

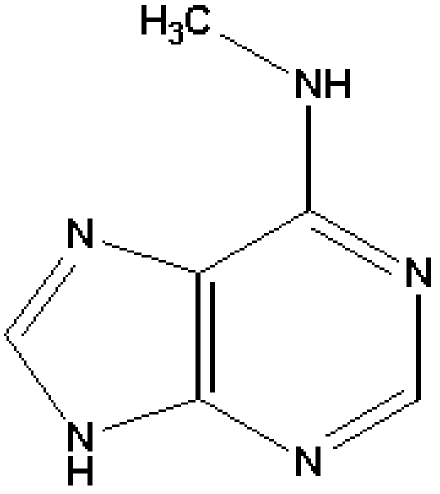
- MMR is responsible for locating and removing mismatched base pairs
- Highly conserved throughout evolution
- Increases genomic stability 100 to 1,000 fold
- Lack of MMR has been linked to several forms of cancer

Mismatch Repair (MMR)



Recognition of Daughter Strand

- Recognition of newly synthesized and damaged daughter stands is critical in repair
- In prokaryotes MMR machinery recognizes the difference between the two strands by methylation status
- A parental strand is methylated at A or C, thus the unmethylated strand is the daughter strand

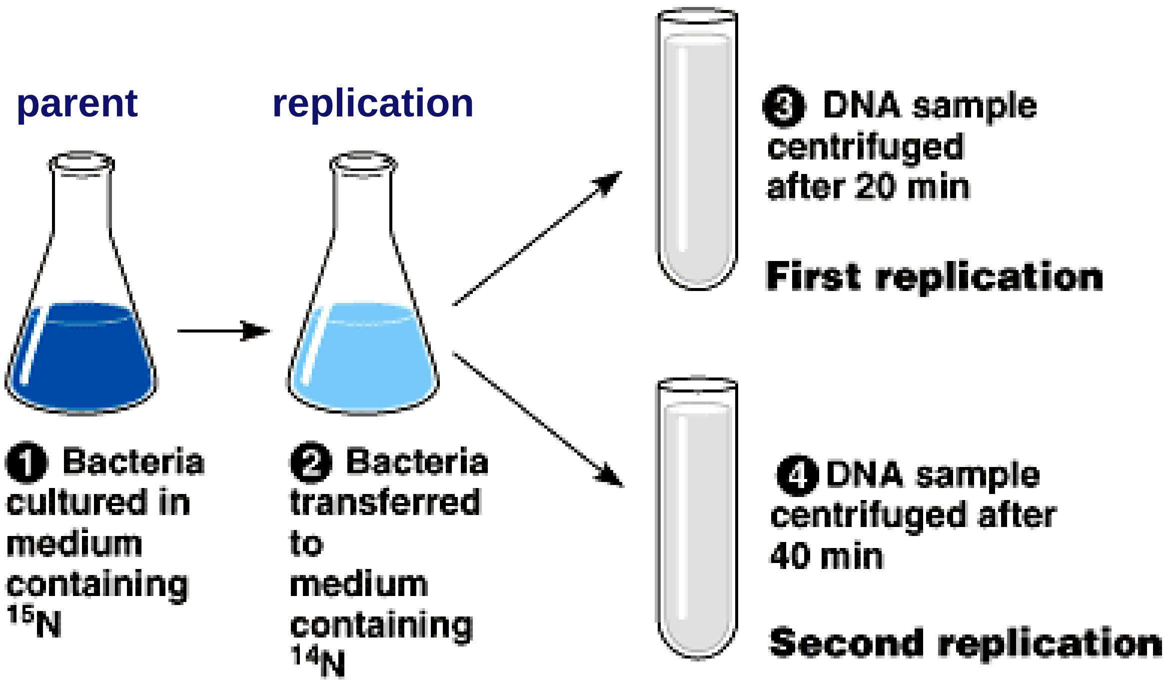


methyladenine

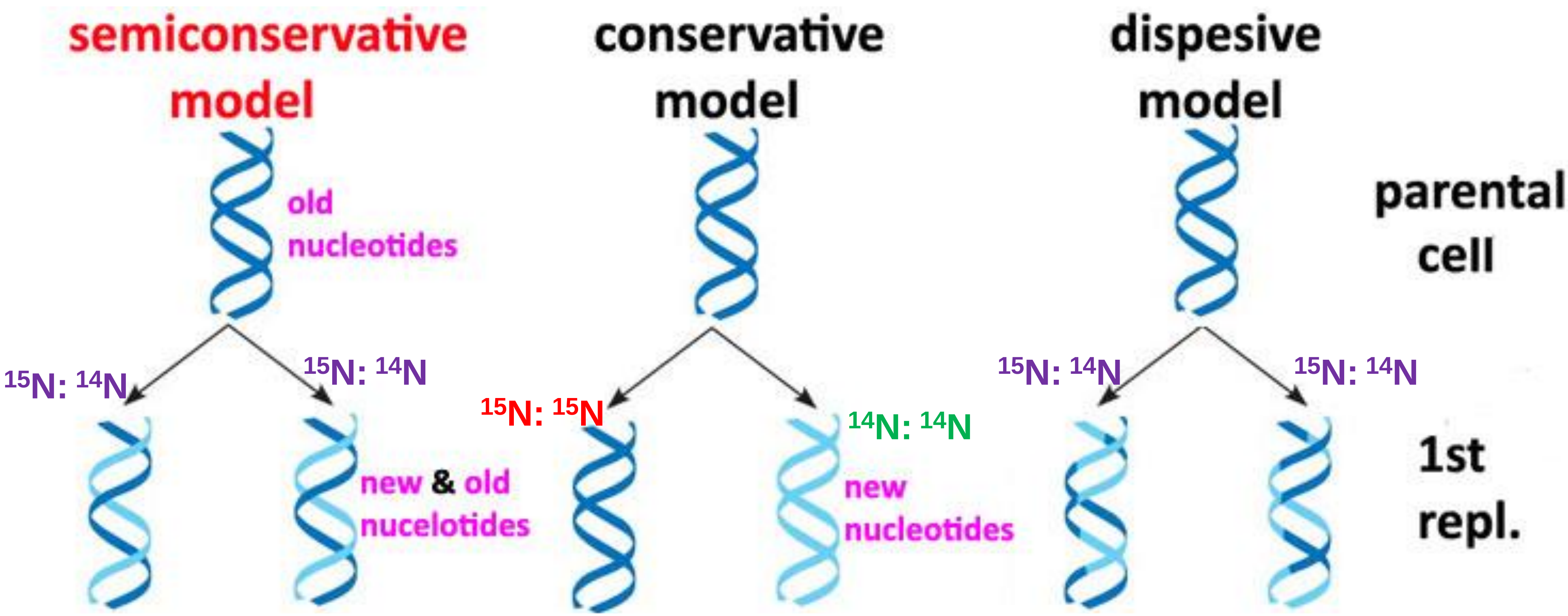
EXTRA SLIDES FOR ADDITIONAL READINGS
(Not part of evaluative components)

Semi-conservative replication

- Meselson & Stahl
  - label nucleotides of "parent" DNA strands with heavy nitrogen = ^{15}N
  - label new nucleotides with lighter isotope = ^{14}N

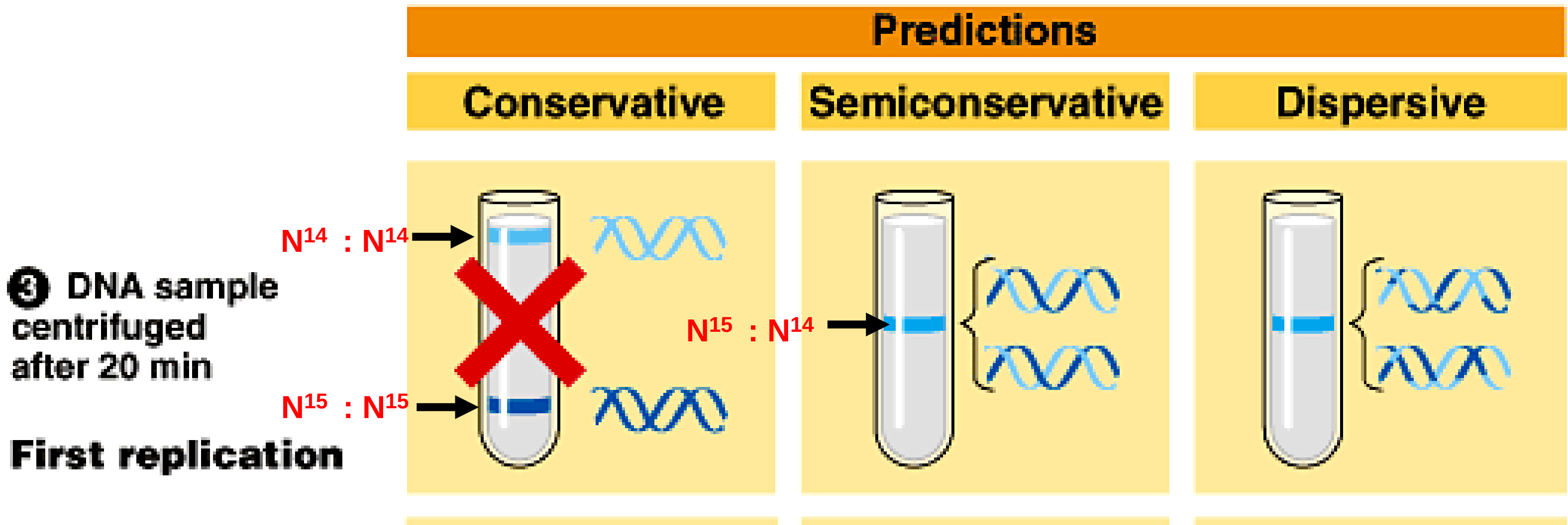


DNA Replication

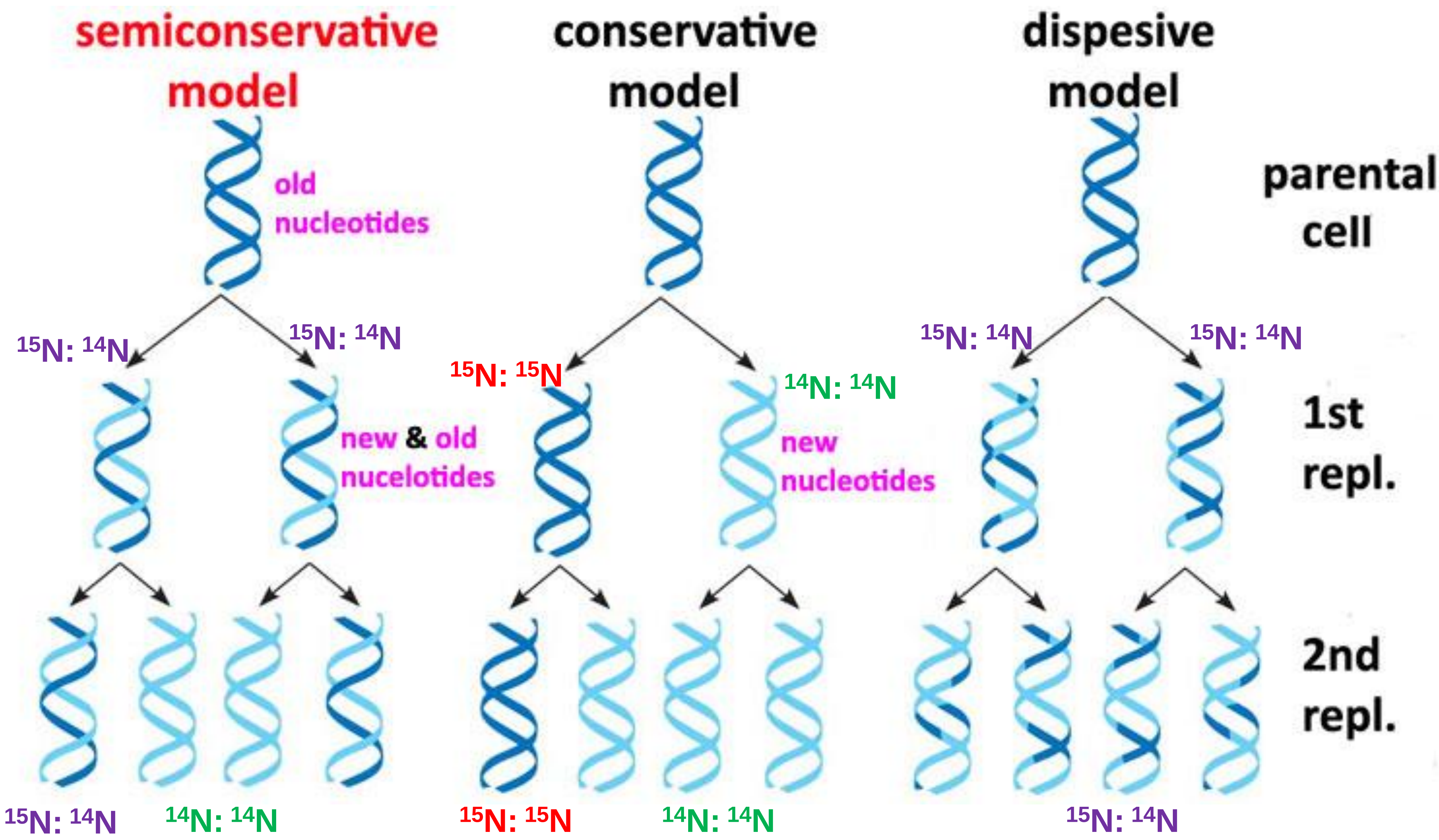


Semi-conservative replication

- Make predictions…
  - ^{15}N strands replicated in ^{14}N medium
  - 1st round of replication



DNA Replication



Semi-conservative replication

- Make predictions…
  - ^{15}N strands replicated in ^{14}N medium
  - 1st round of replication
  - 2nd round