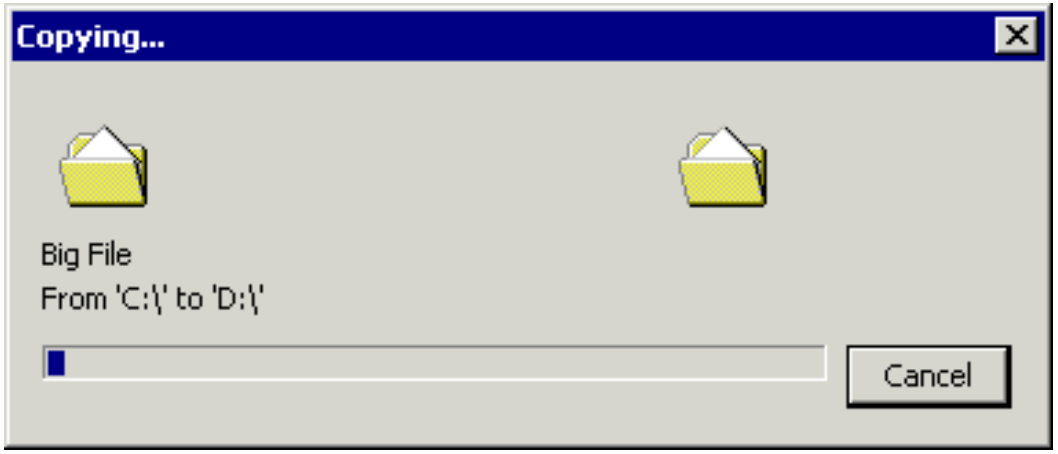
REPLICATION



Replication: Copying to molecule of life

The requirements for DNA to be 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

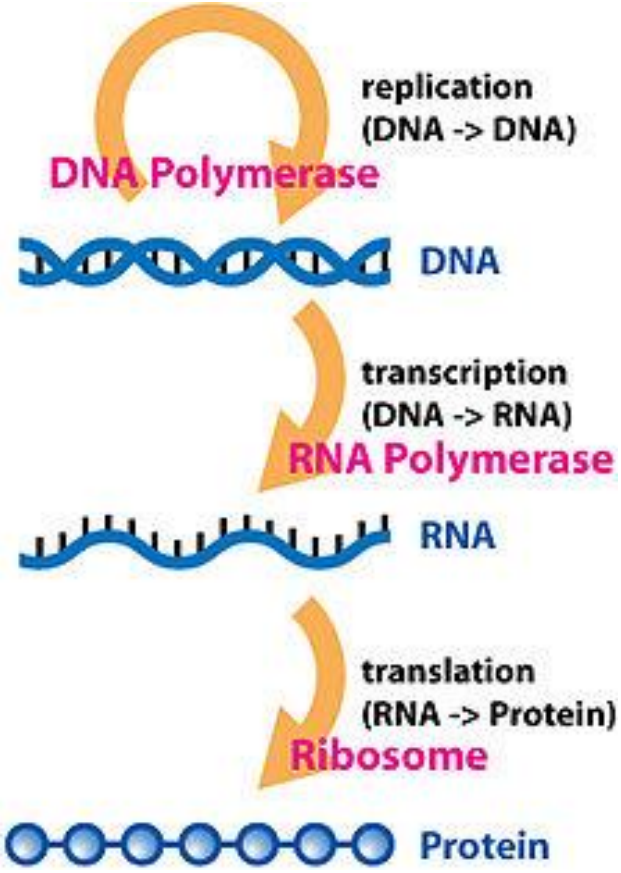
- The most important feature of DNA is its ability to replicate itself.

  self-duplication

- ***Goal;*** is the formation of an exact copy of the DNA molecule to transfer the genetic information to subsequent generations.

CENTRAL DOGMA

- All of the information transfer processes in the cell are called **central dogma**.



When and where DNA replication take place?

- The DNA molecule is replicated once per cell cycle.
- If there is a problem with DNA replication, cell division will stop.
- DNA synthesis occurs at the **S phase of the cell cycle in the nucleus**.

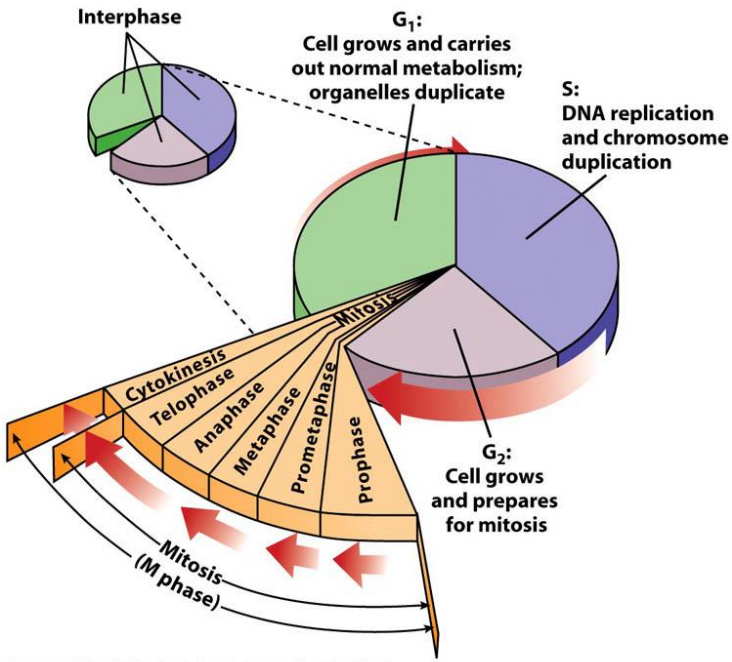


Figure 14-1 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

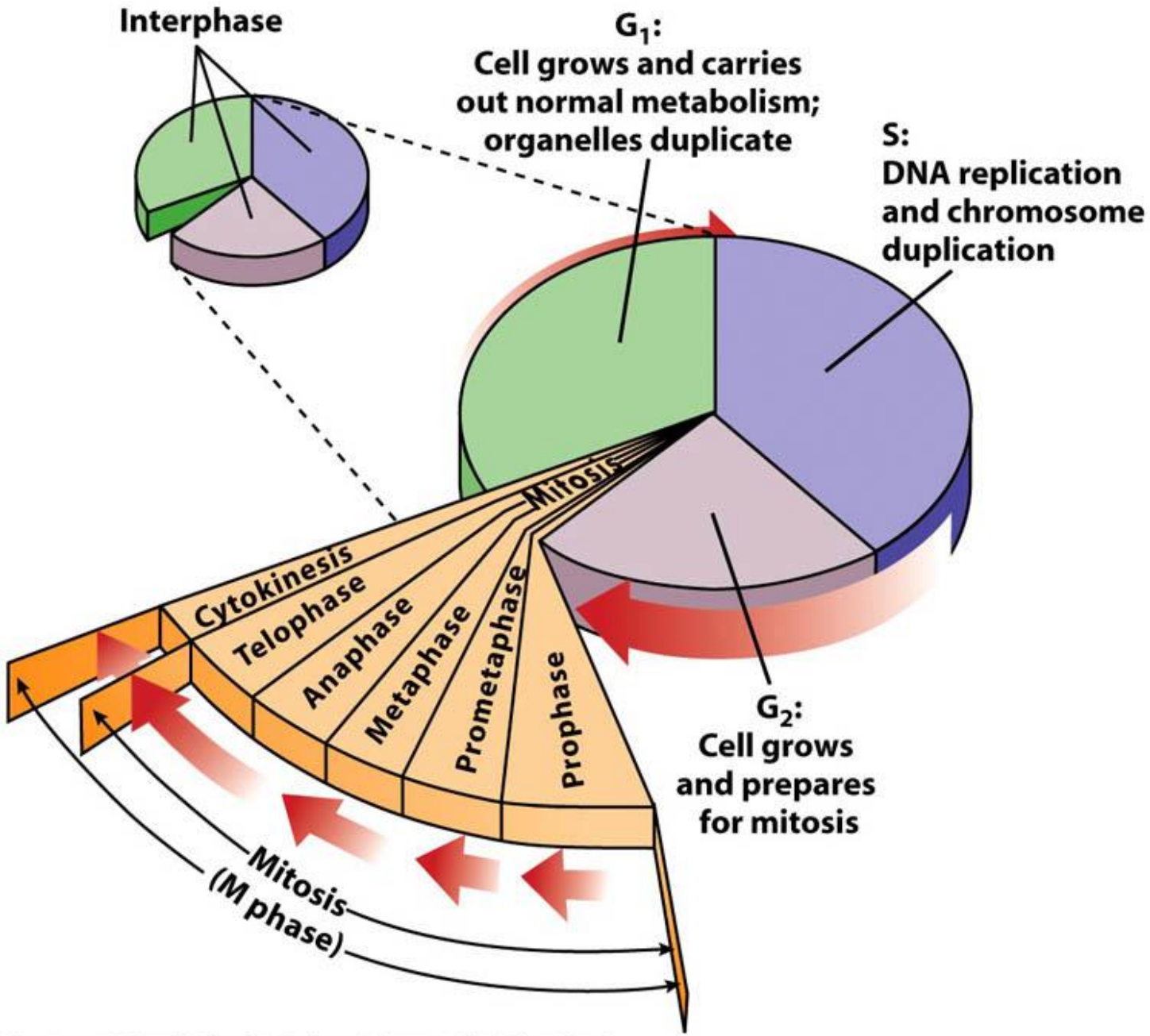
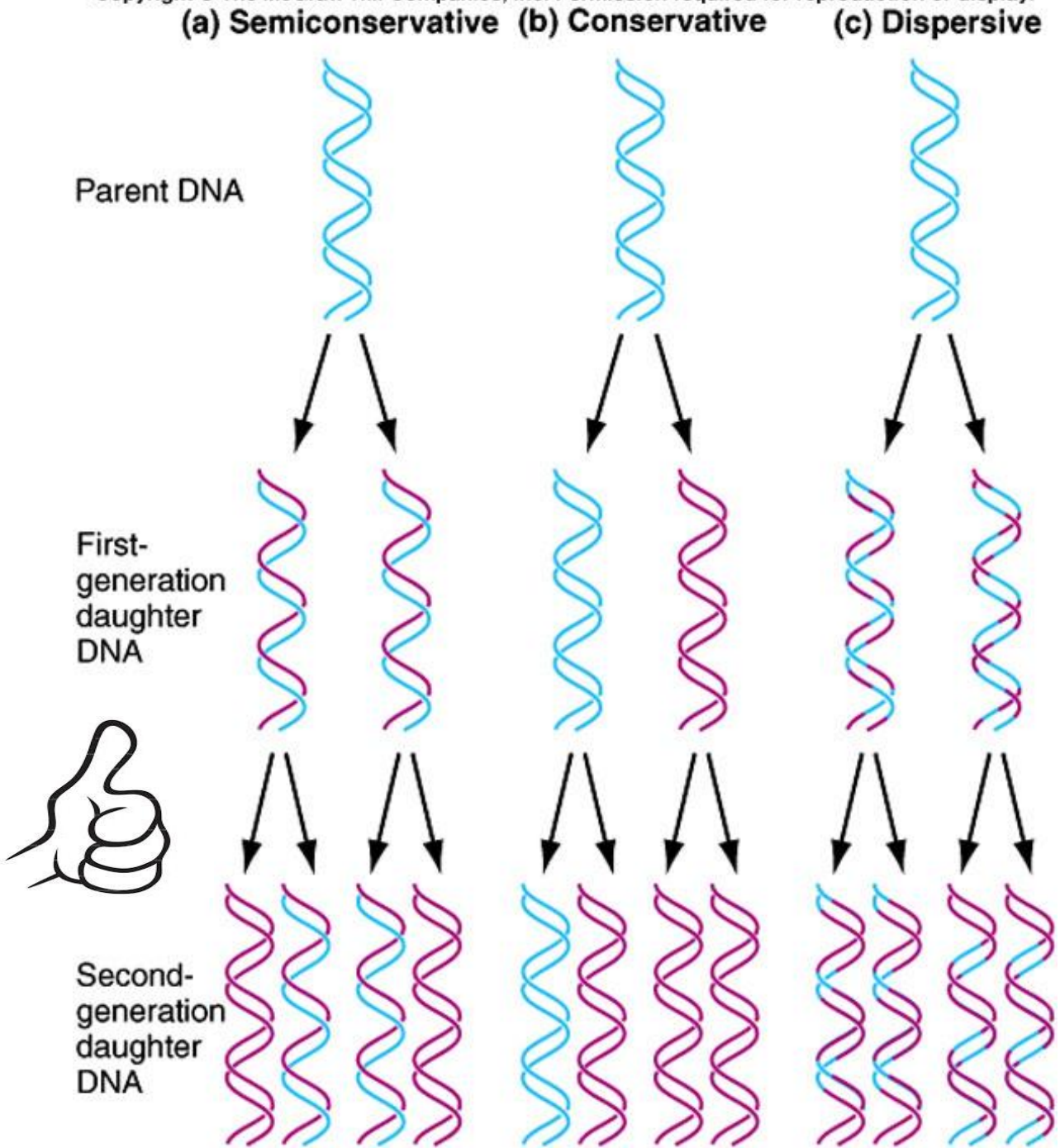


Figure 14-1 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

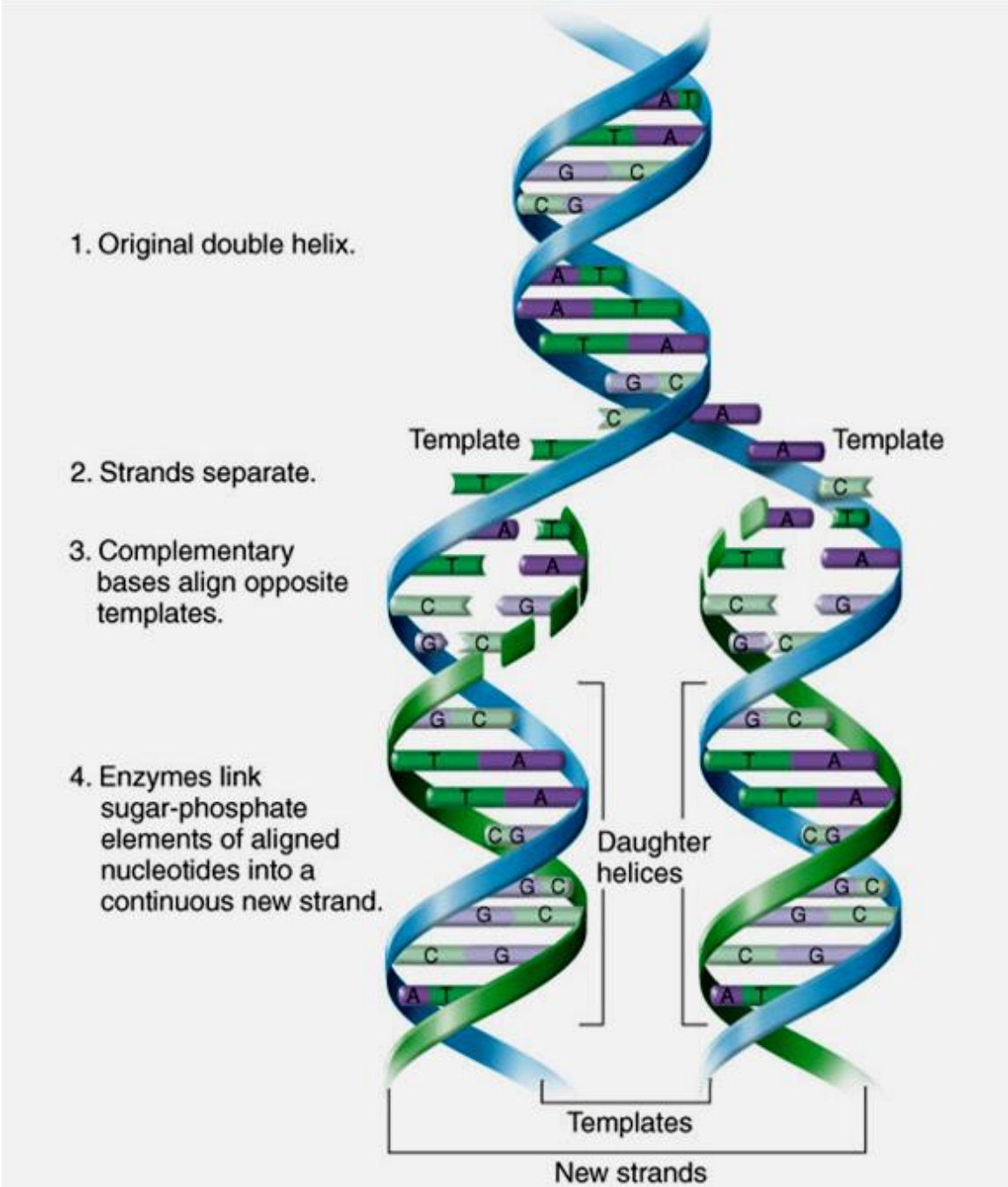
Basic rules of replication

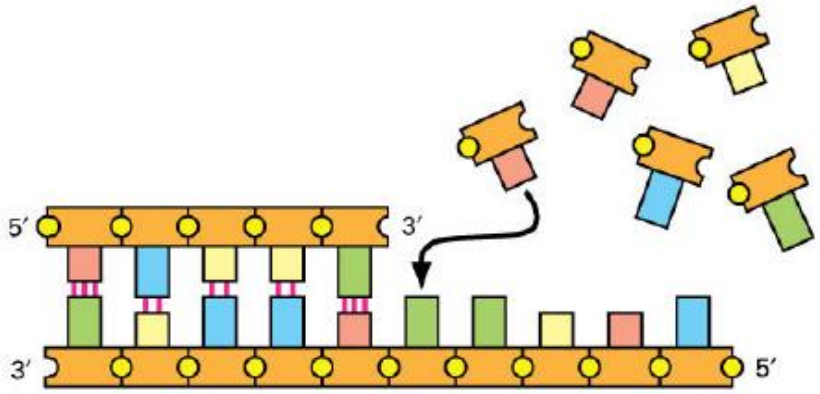
A. Semi-conservative
B. Starts at the ‘origin’
C. Synthesis always in the 5-3’ direction
D. Can be uni or bidirectional
E. Semi-discontinuous
F. RNA primers required

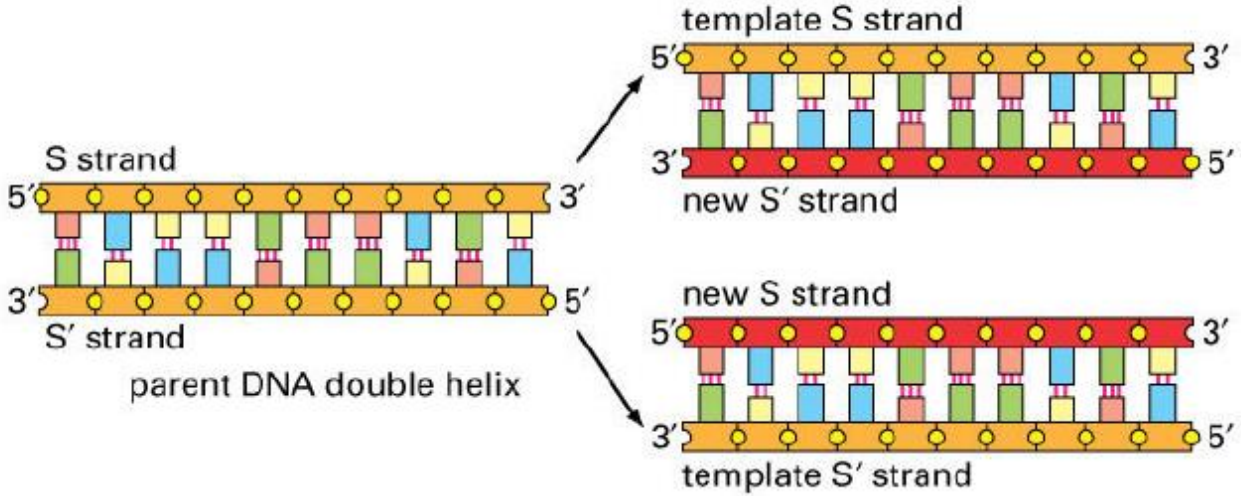


Semi-conservative replication

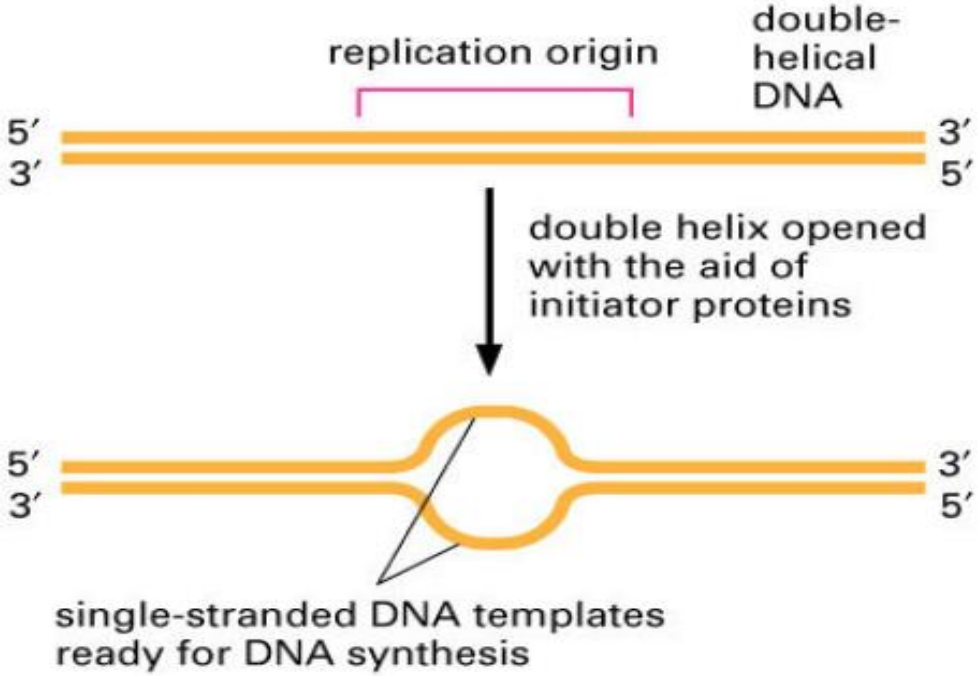
each one of the **parent DNA** strands is passed to the daugher DNA + **one new strand for each**



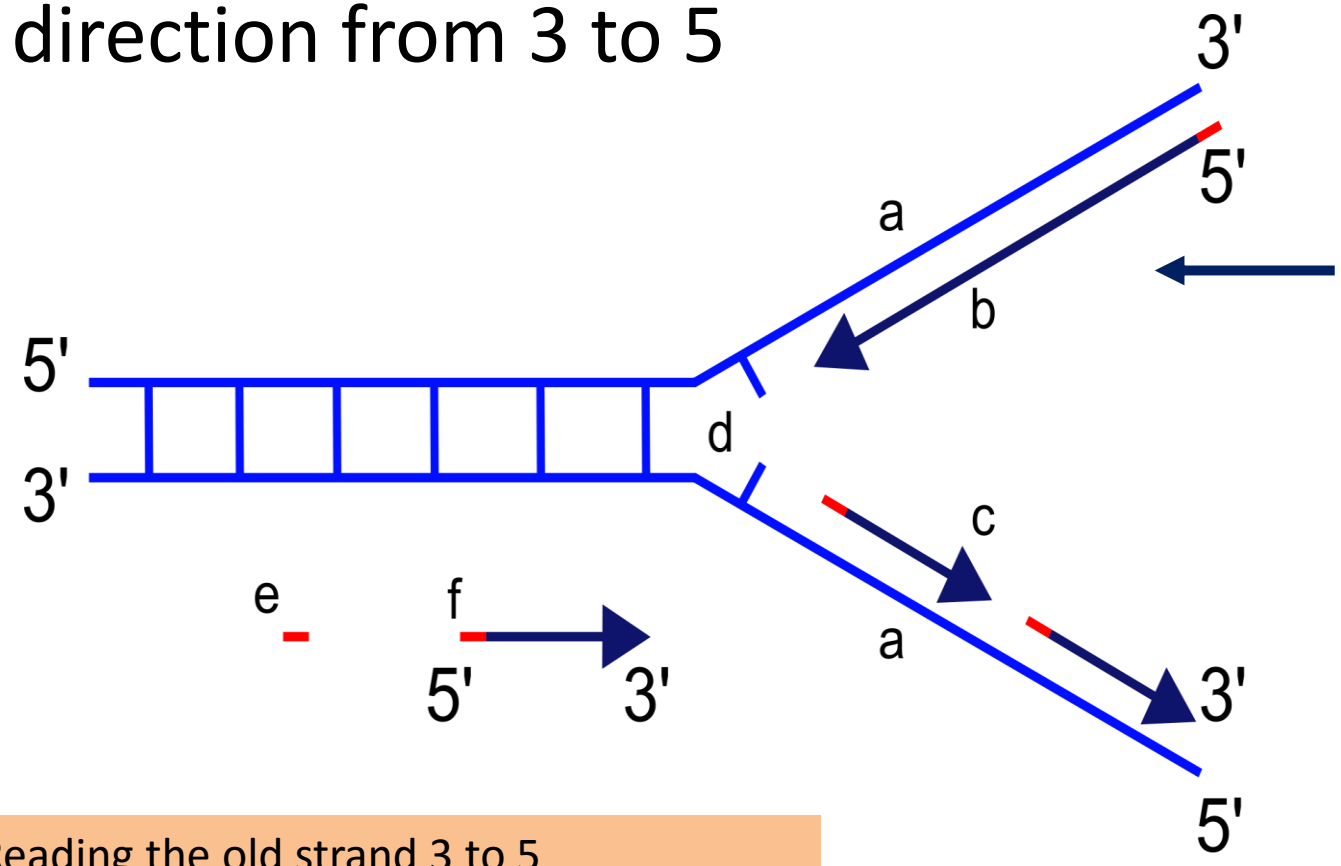

5′
3′
3′
5′


S strand
5′
3′
3′
5′
S′ strand
parent DNA double helix
template S strand
5′
3′
3′
5′
new S′ strand
new S strand
5′
3′
3′
5′
template S′ strand

- DNA replication does not start at any point in the random DNA chain. Initiator proteins identify specific base sequences on DNA called sites of origin
- Starts in regions called "REPLICATION ORIGIN"
- This point is one in prokaryotic cell, whereas it is much higher in eukaryotic cells (›1000).



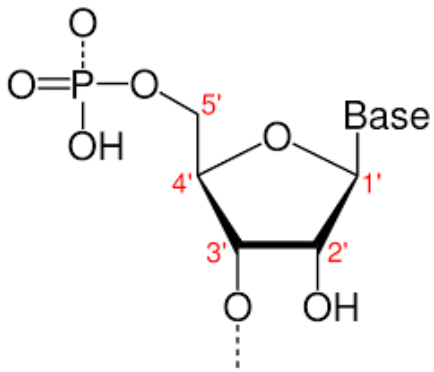
- Generally, replication in the replication forks proceeds from 5 to 3;
- The strand acting as a template is read in the direction from 3 to 5

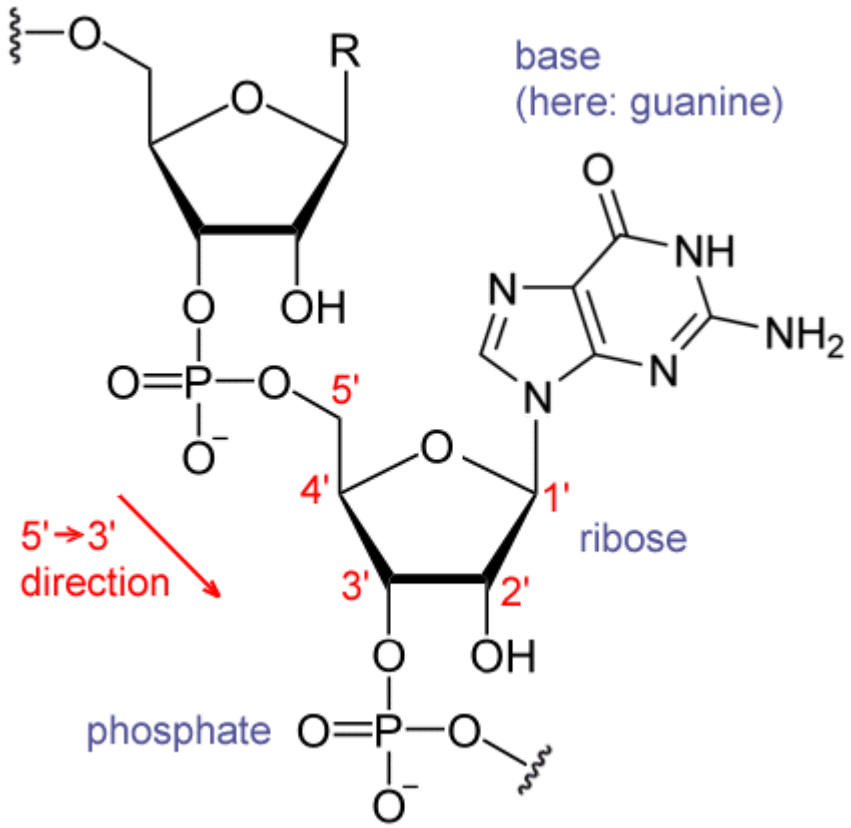


Reading the old strand 3 to 5
Synthesis a new strand (replication) 5 to 3

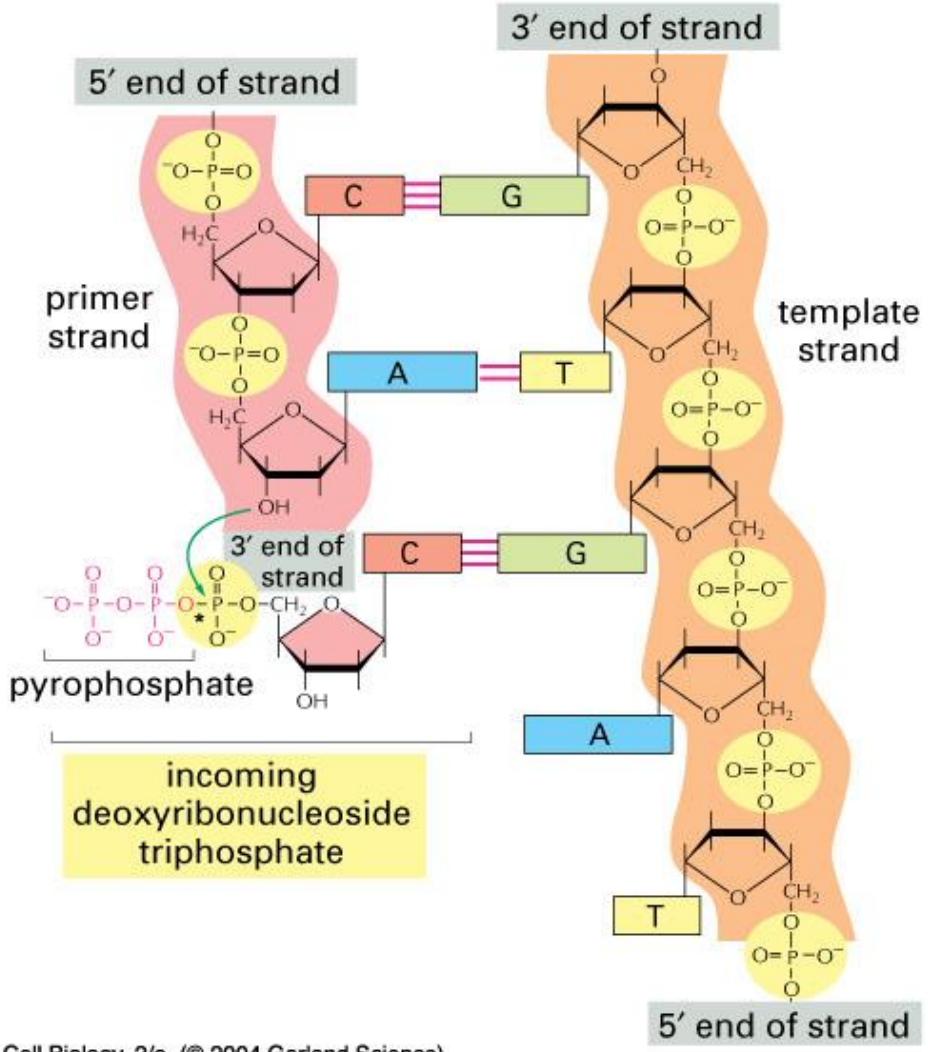
Why does DNA replication only occur in the 5’ to 3’ direction?

When a 3 'hydroxyl group of another nucleotide is attached to the phosphate group at the 5' end of one nucleotide, a phosphodiester bond is formed.
therefore the synthesis direction of DNA is from 5 to 3.





<u>Anti-parallel</u> strand builds in the opposite direction (always in 5’ to 3’ direction)



Building blocks for replication:

1. Four types of dNTP (dATP, dGTP, dTTP, dCTP) (substrates)
   and Mg^{+2} ion.

2. A primer having a free 3'-OH group

(Without the primer, the DNA polymerase enzyme cannot initiate a new synthesis !!!)

3. A DNA template (DNA strand)

4. Single strand binding proteins (SSBs)

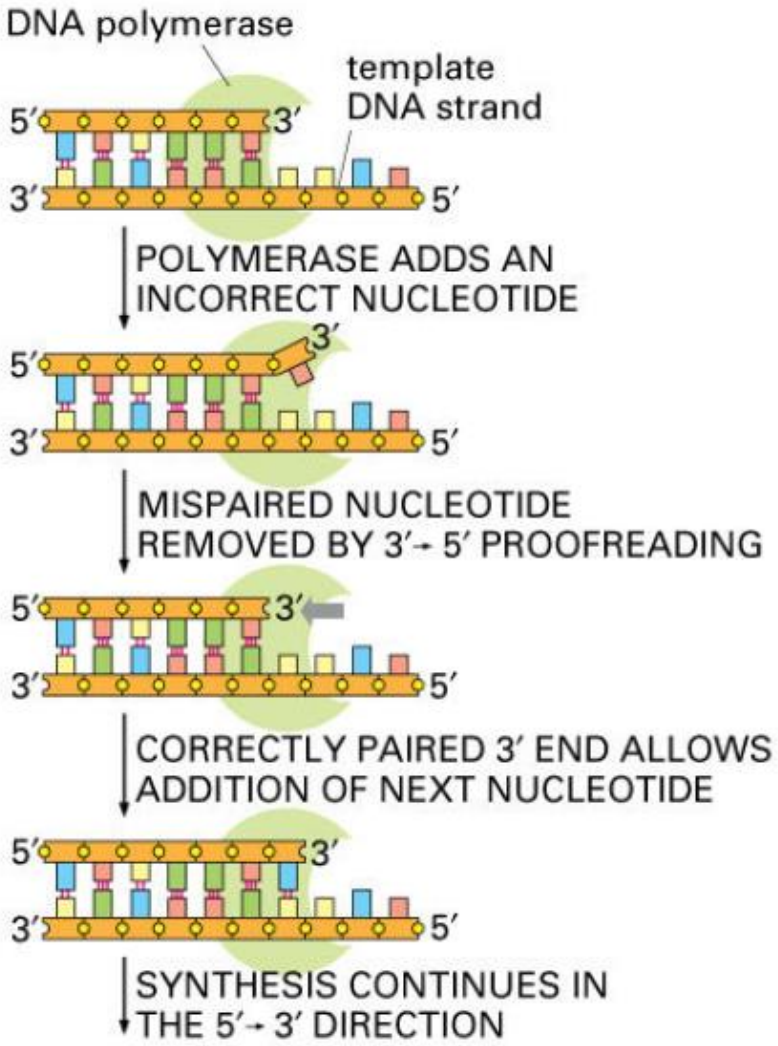
5. Enzymes !!!!!

Enzymes!!!

- Topoisomerase also called DNA gyrase------ unwinds double helix
- Helicase------ separates double helix at the replication fork (breaking H-bonds between the complementary base pairs (A-T, G-C))
- Primase------ makes RNA nucleotides into a primer (Nucleotides for the starting point for DNA replication)
- DNA ligase------ links the strands
- DNA polymerases ------- synthesise new strand

- DNA Polymerase I
- Cuts off RNA primers and fills in with DNA (between Okazaki fragments) –lagging strand
- Can proofread
- DNA Polymerase III
- Elongates the strand by adding DNA nucleotides on leading strand
- Also proofreads and corrects the DNA strand

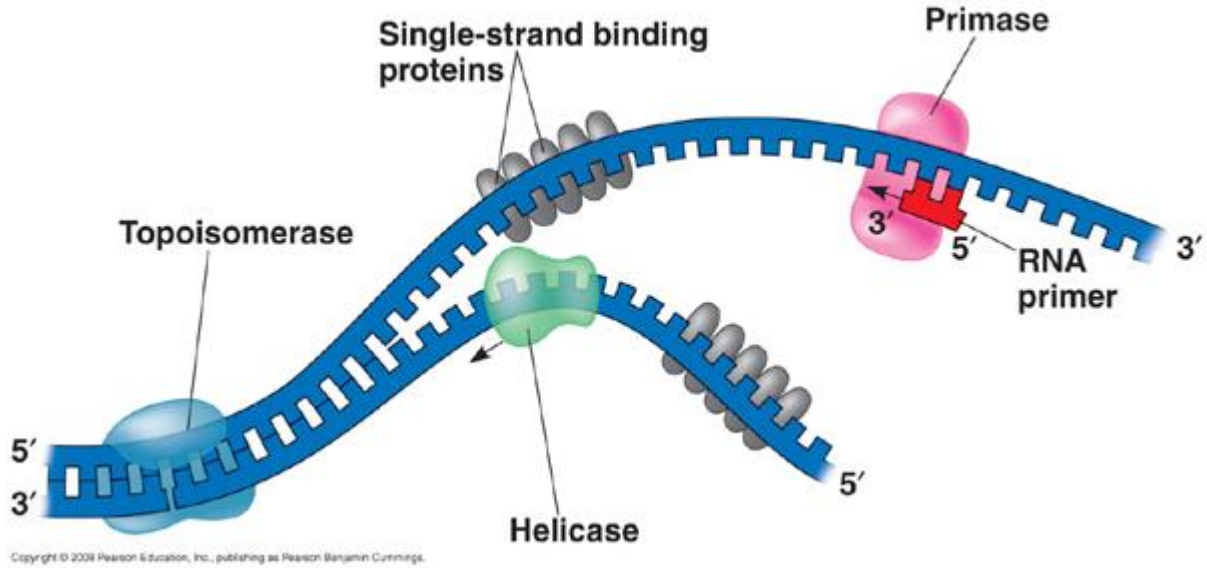
- Errors in newly synthesized DNA are corrected by 3’-5 ’exonuclease activity



SSB's

single strand binding proteins

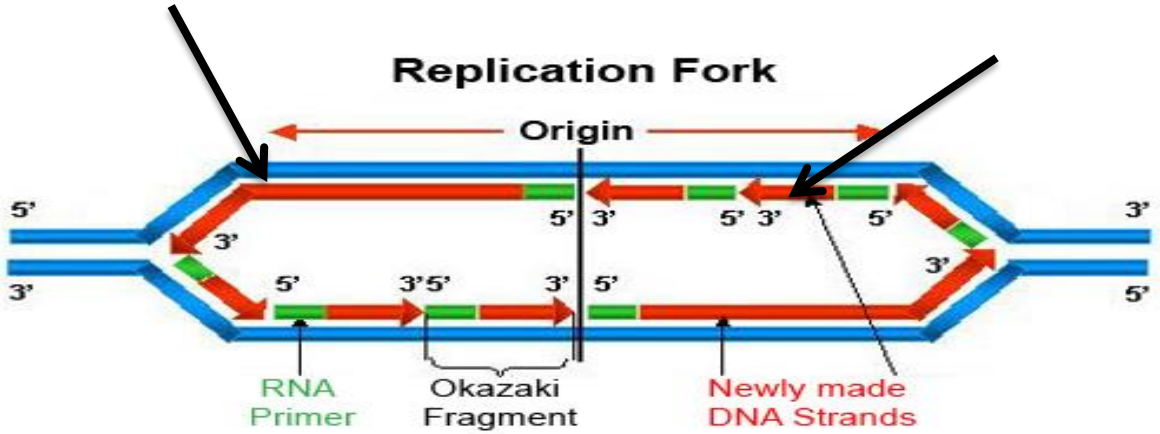
- Stabilize the DNA strands as they are being replicated
- Prevents rejoining of DNA strands



Leading Strand Lagging Strand

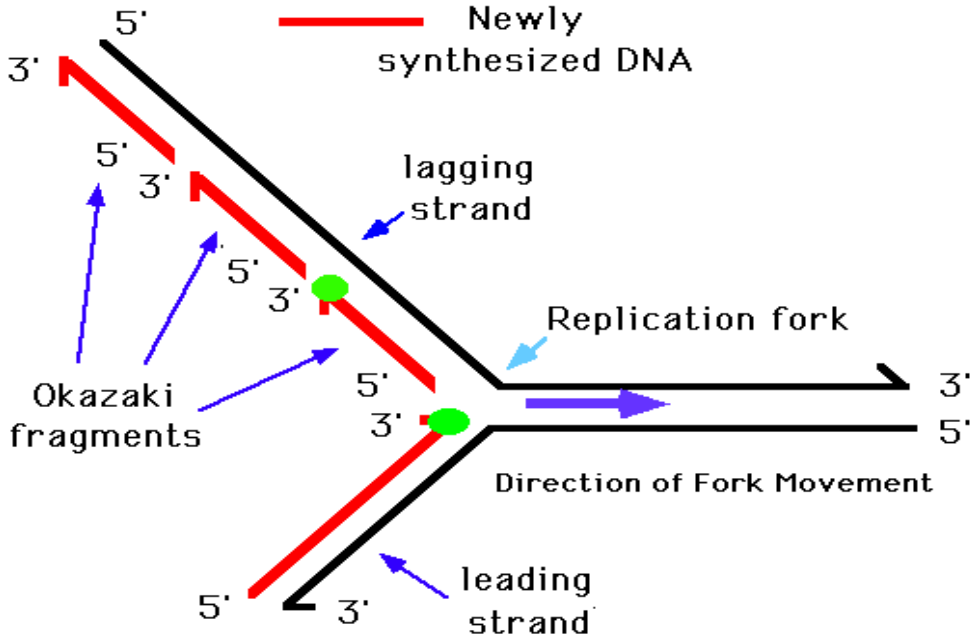
- **Template strand of DNA**
- Continuous addition of nitrogenous bases
- in 5’ to 3’ direction
- McGraw-Hill Replication Fork

- **Other DNA strand**
- Forms short strands of Okazaki fragments (that will be joined later)
- in the 5’ to 3’ direction
- DNA Replication You Tube (1:35)



• OKAZAKI FRAGMENTS

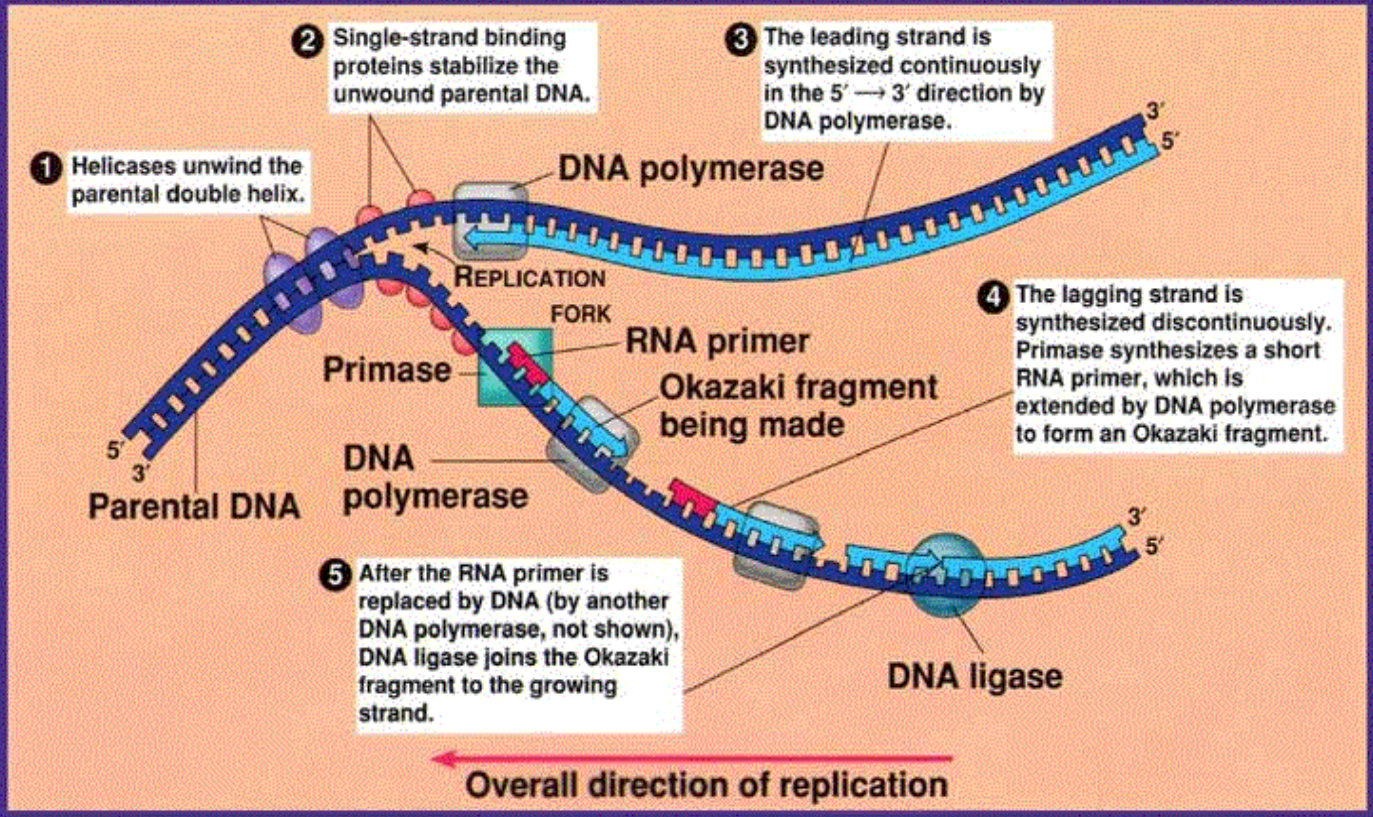
- The short strands of newly made DNA fragments on the <u>lagging strand</u> are called <u>Okazaki fragments</u> after the Japanese Biochemist Reiji Okazaki.

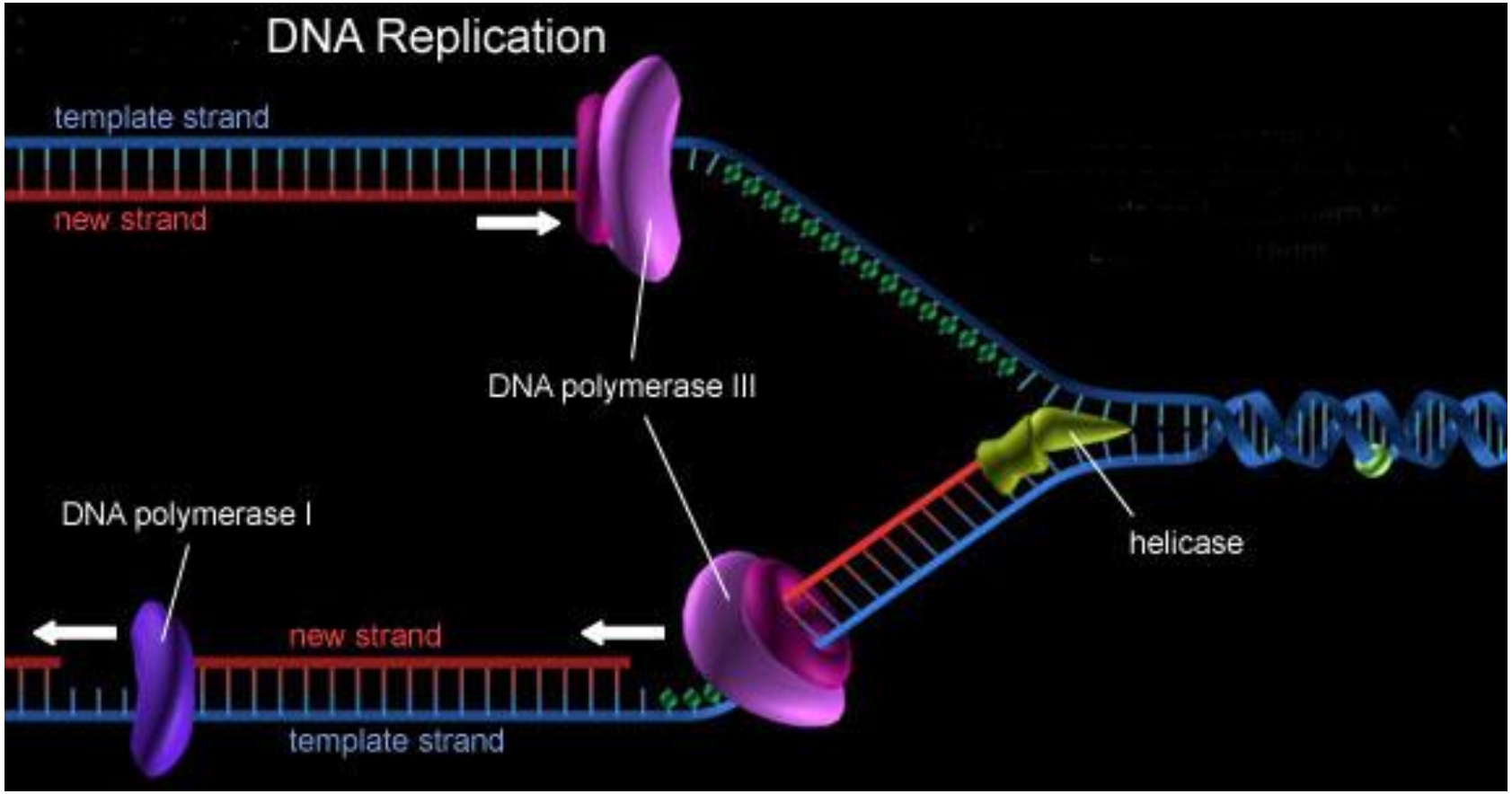


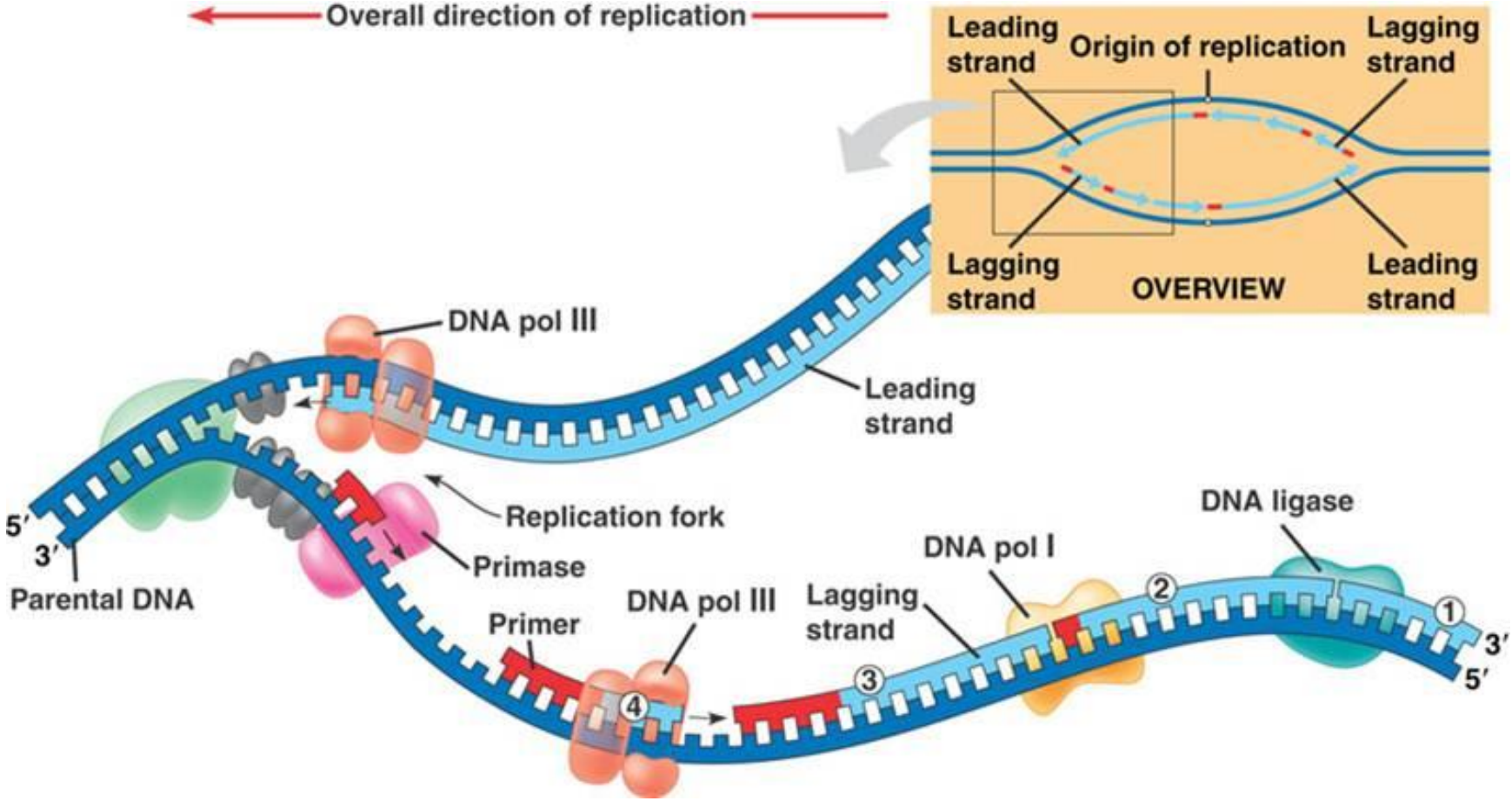
DNA Replication Activity
Work in Groups

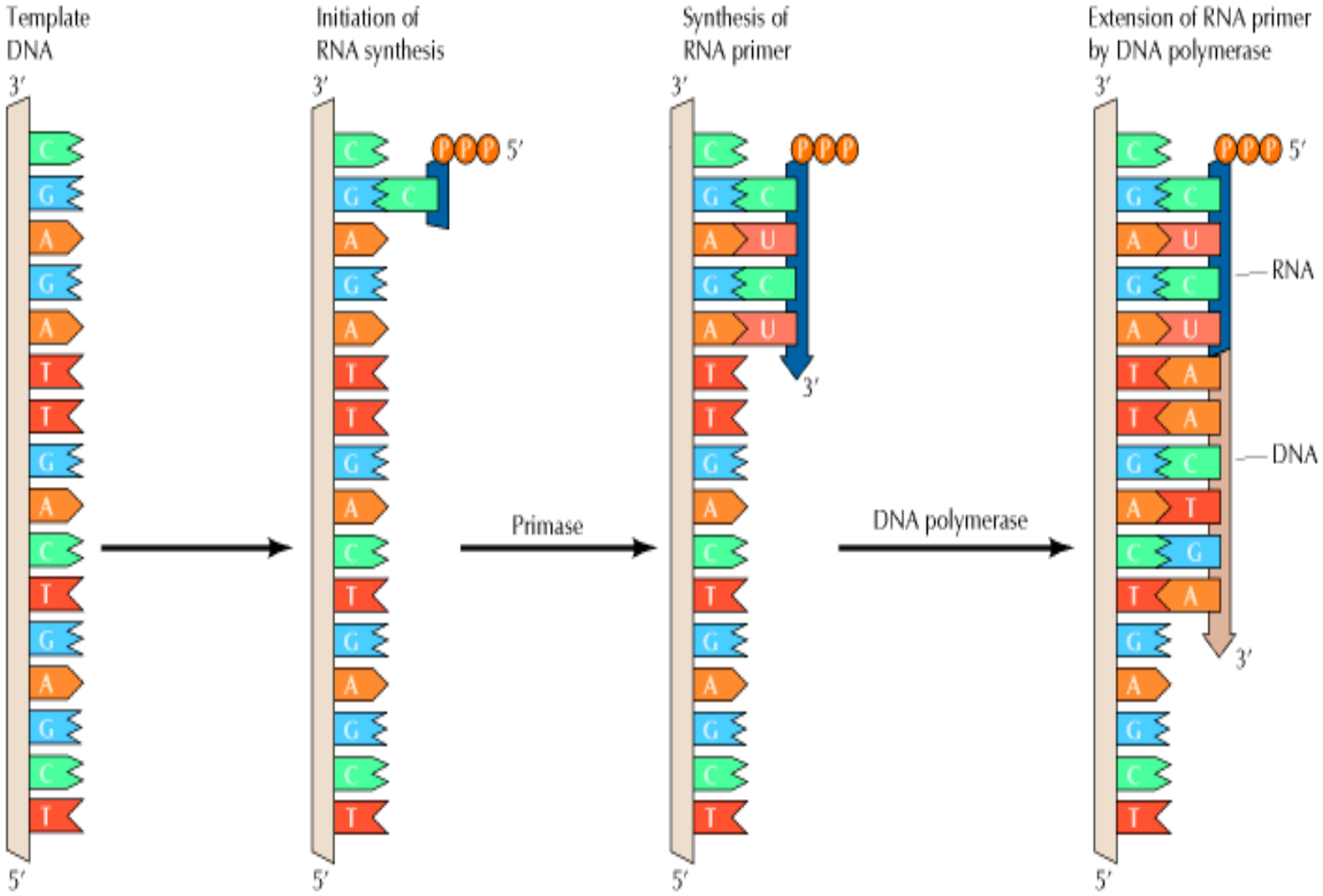
- Card stock papers are original DNA strands
- Colored paper are new DNA strands
- MAKE OWN ENZYMES- construction paper

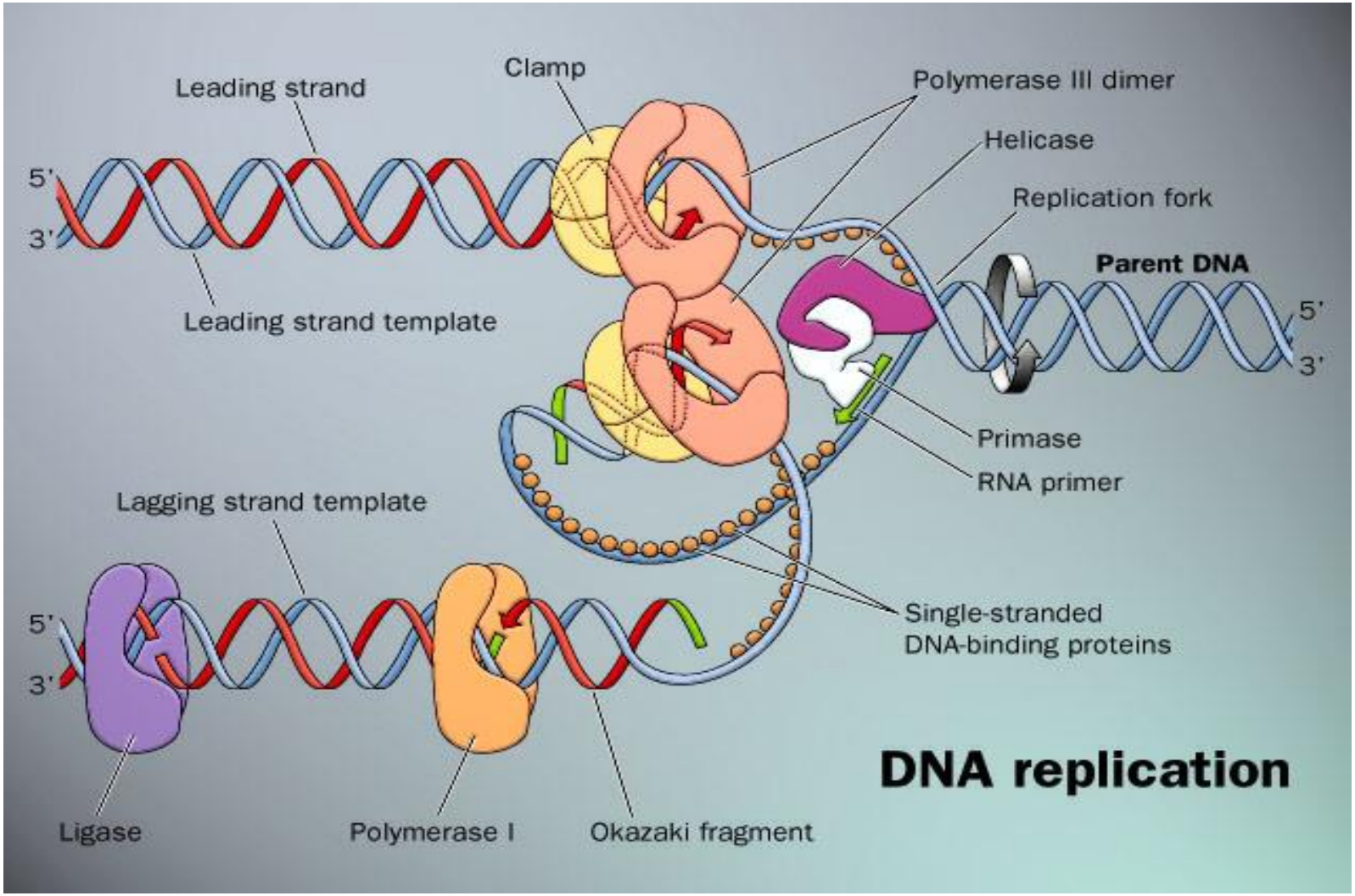
A SUMMARY OF DNA REPLICATION



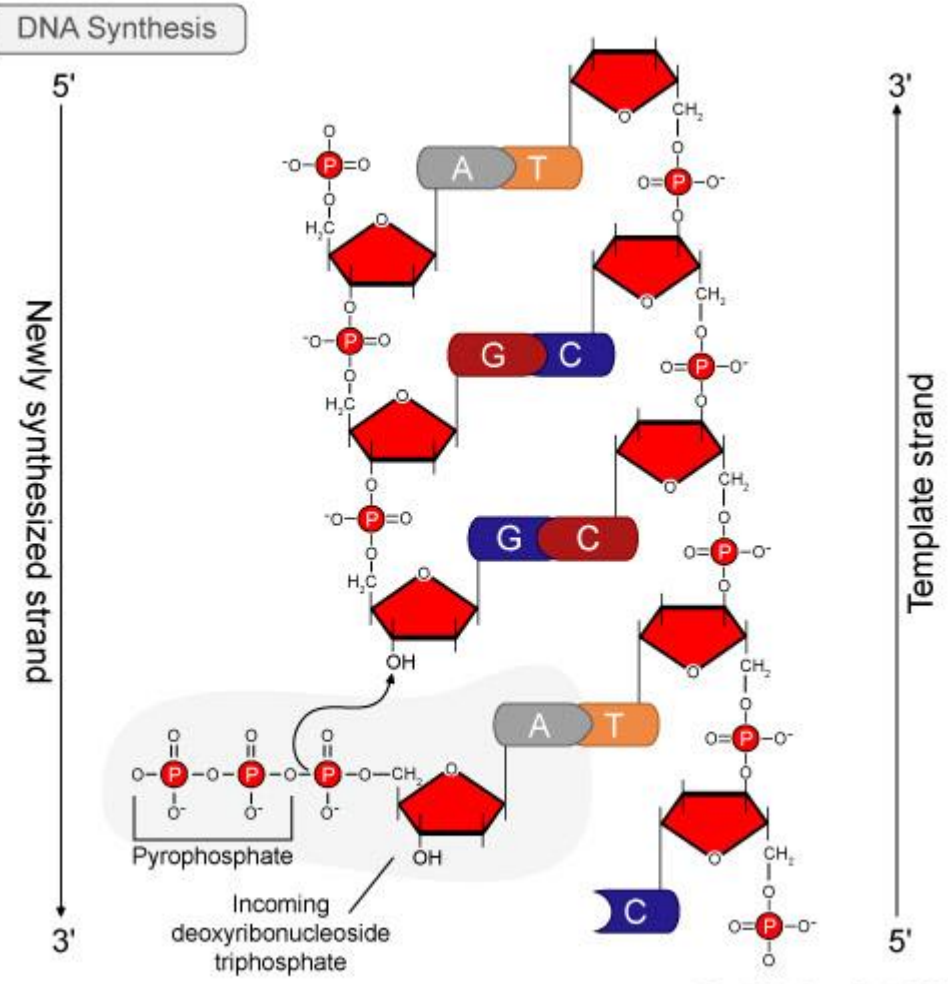

DNA Replication
template strand
new strand
DNA polymerase III
DNA polymerase I
helicase
new strand
template strand

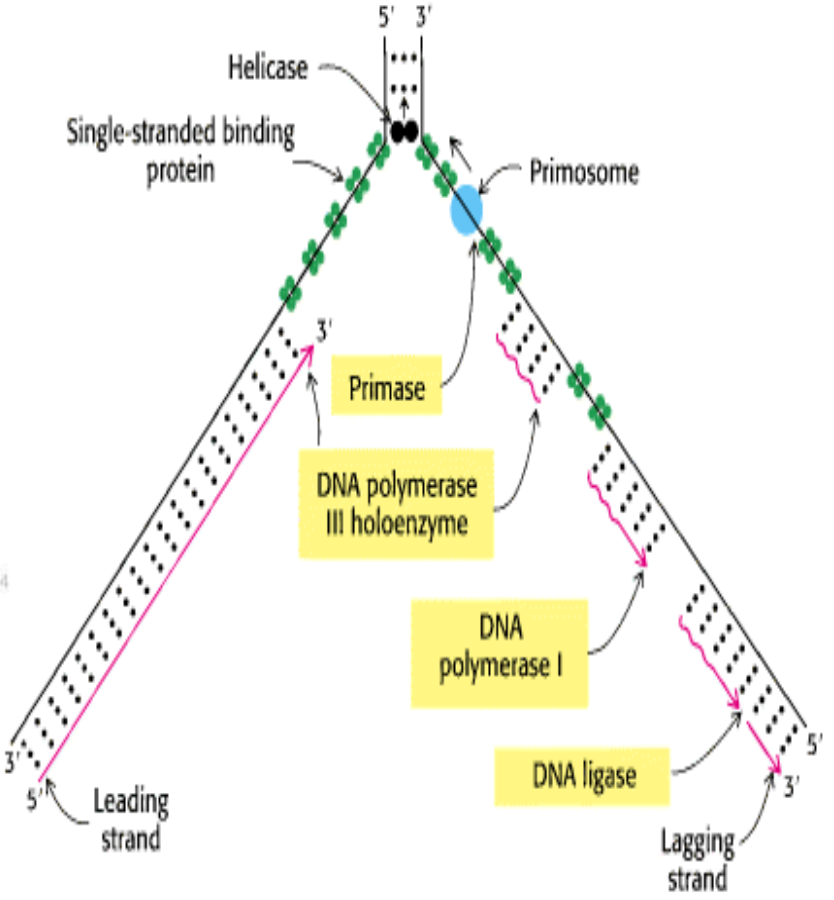

Overall direction of replication
Leading strand
Origin of replication
Lagging strand
Lagging strand
OVERVIEW
Leading strand
DNA pol III
Leading strand
Replication fork
5′
3′
Parental DNA
Primase
Primer
DNA pol III
Lagging strand
DNA pol I
DNA ligase
1
2
3
4
3′
5′

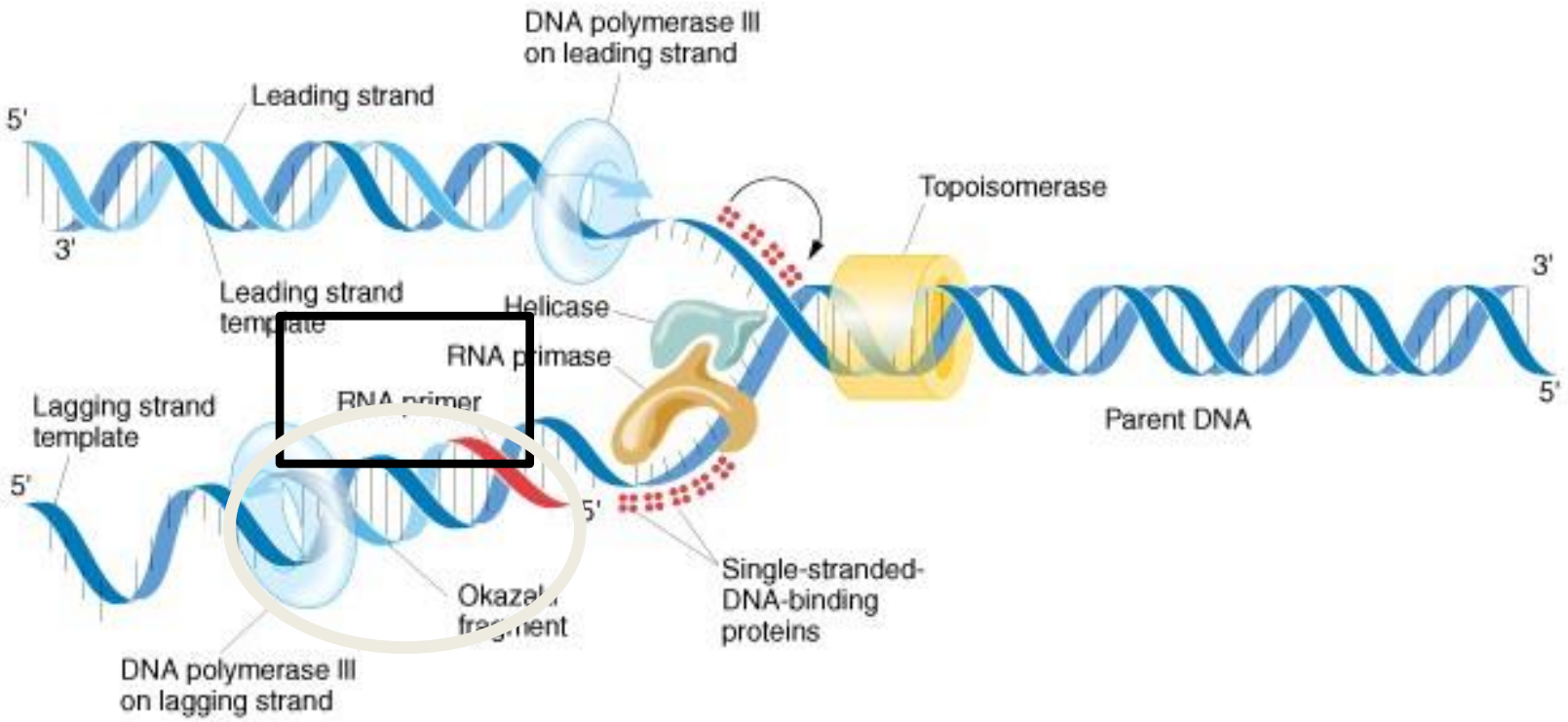

Template DNA
Initiation of RNA synthesis
Synthesis of RNA primer
Extension of RNA primer by DNA polymerase
Primase
DNA polymerase
RNA
DNA
3′
5′
P P P

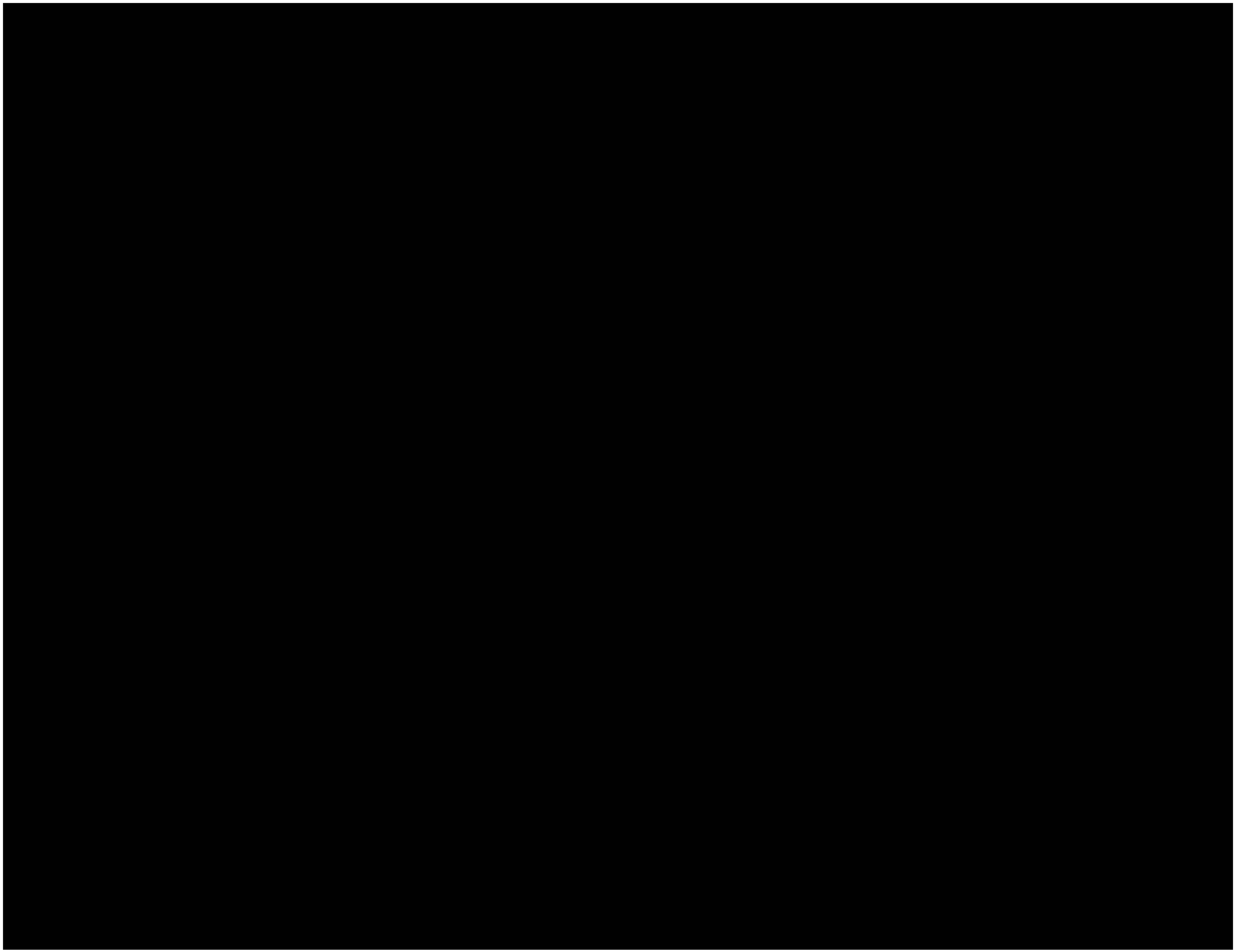

Leading strand
Clamp
Polymerase III dimer
Helicase
Replication fork
5'
3'
Parent DNA
Leading strand template
5'
3'
Primase
RNA primer
Lagging strand template
Single-stranded
DNA-binding proteins
5'
3'
DNA replication
Ligase
Polymerase I
Okazaki fragment

DNA Synthesis

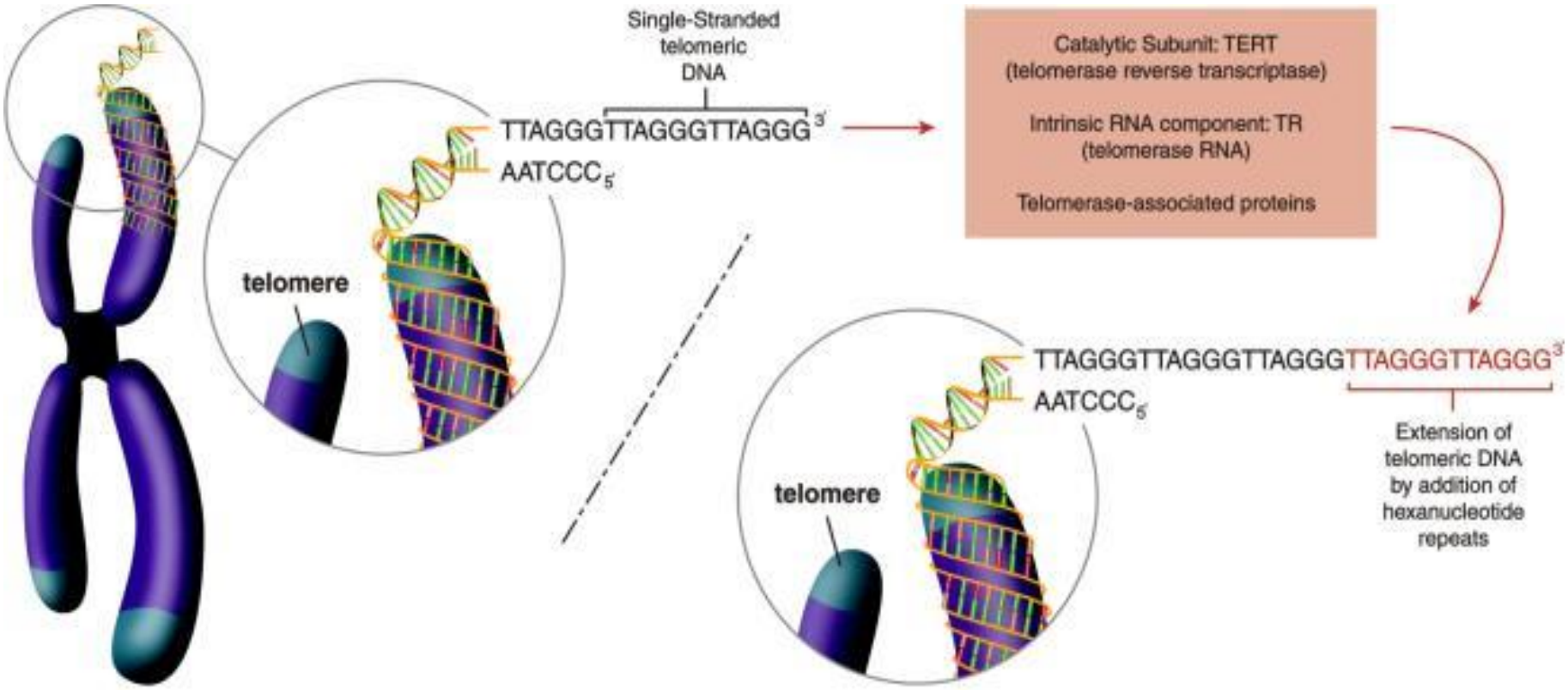


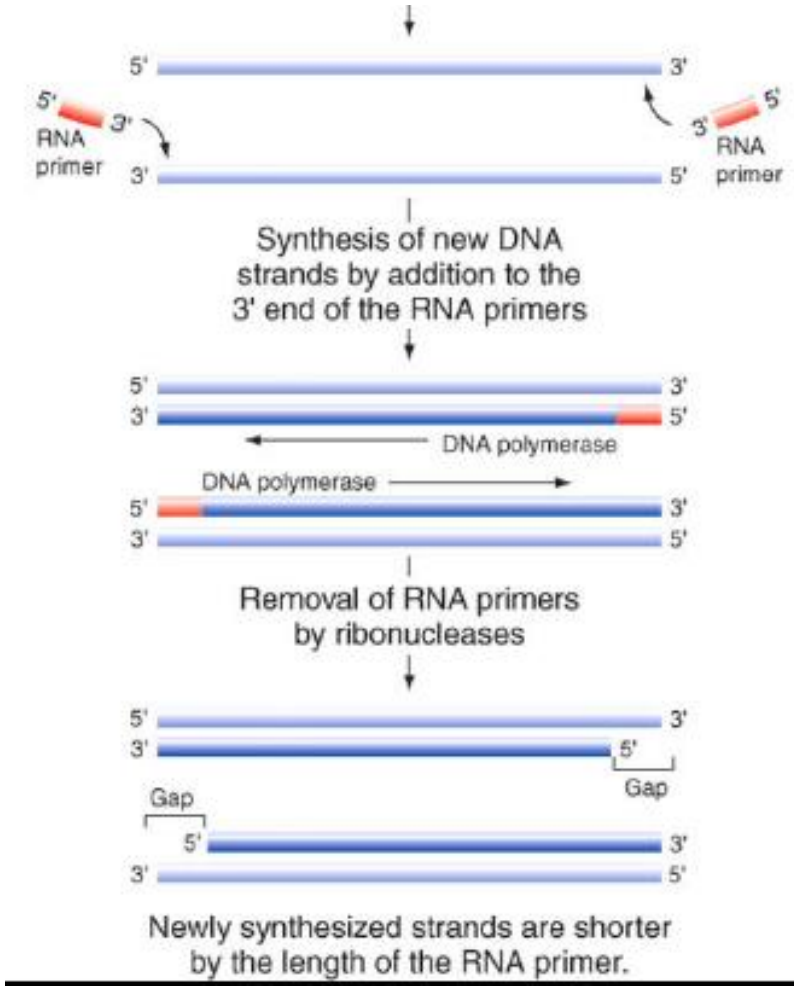


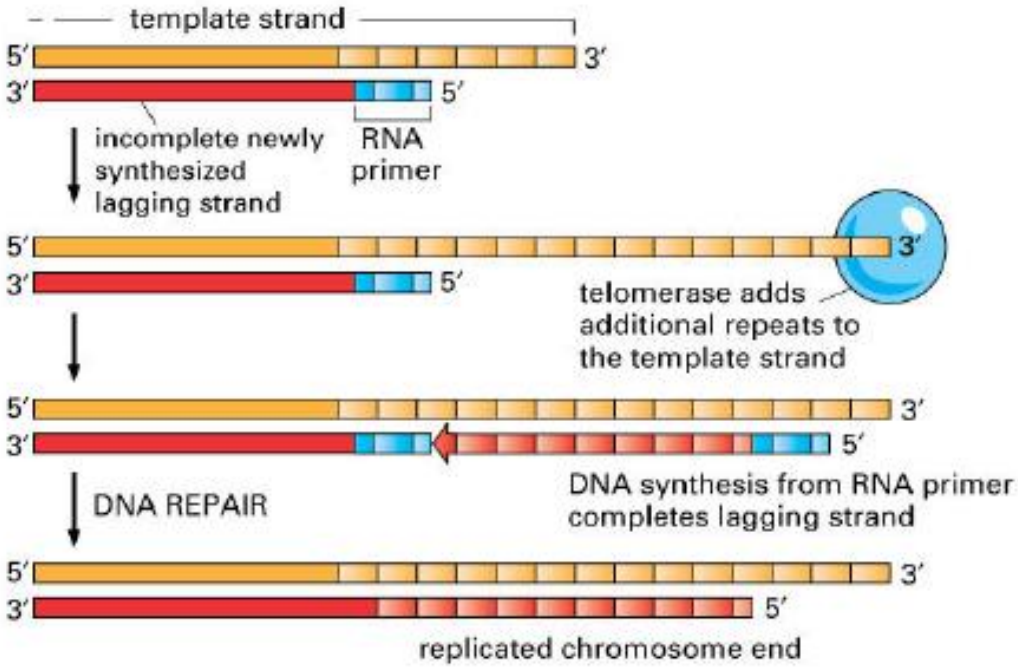

DNA polymerase III
on leading strand
Leading strand
5'
3'
Topoisomerase
3'
5'
Parent DNA
Leading strand
template
Helicase
RNA primase
RNA primer
Lagging strand
template
5'
5'
Single-stranded-
DNA-binding
proteins
Okazaki
fragment
DNA polymerase III
on lagging strand



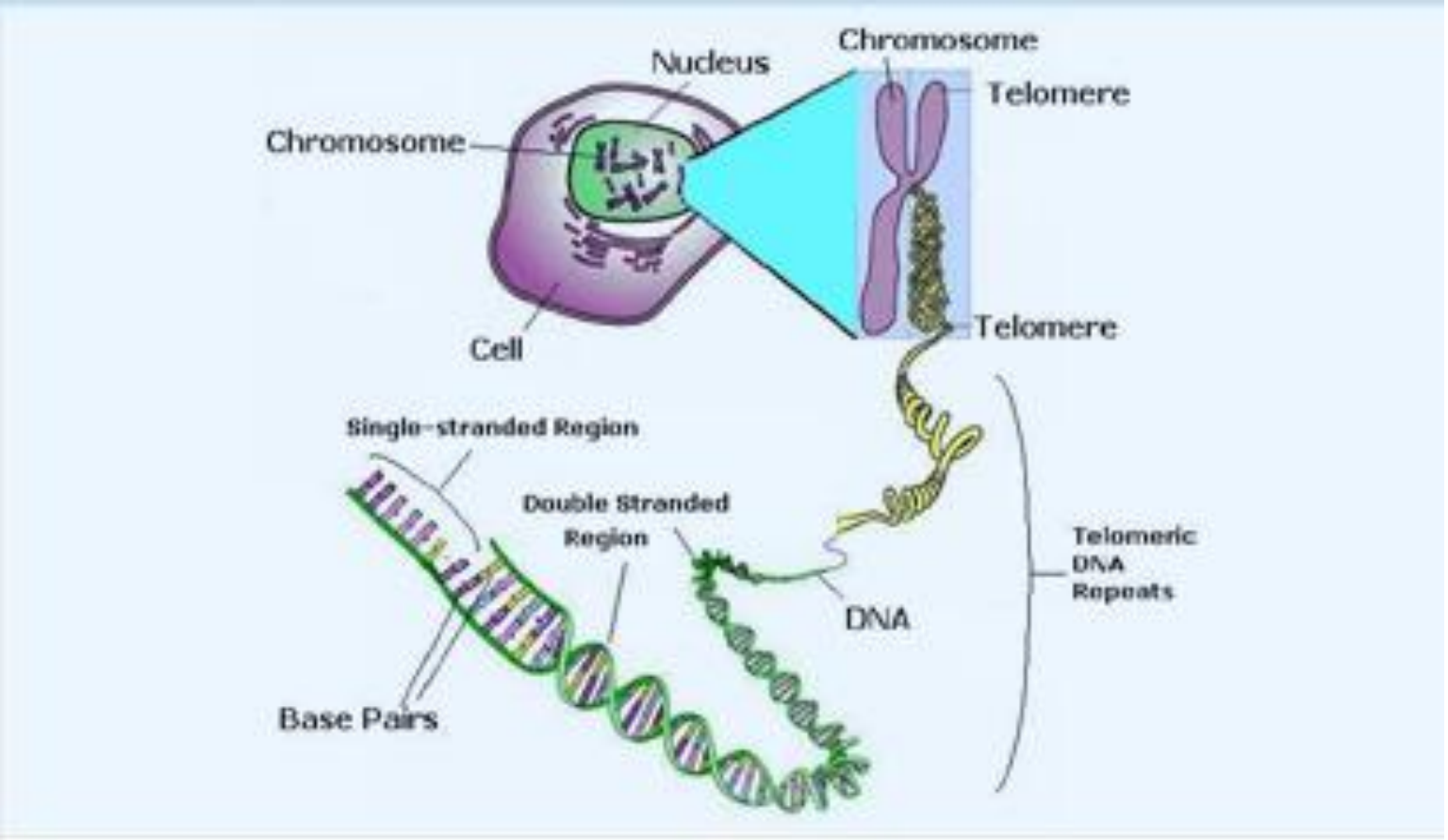
TELOMERE- TELOMERASE

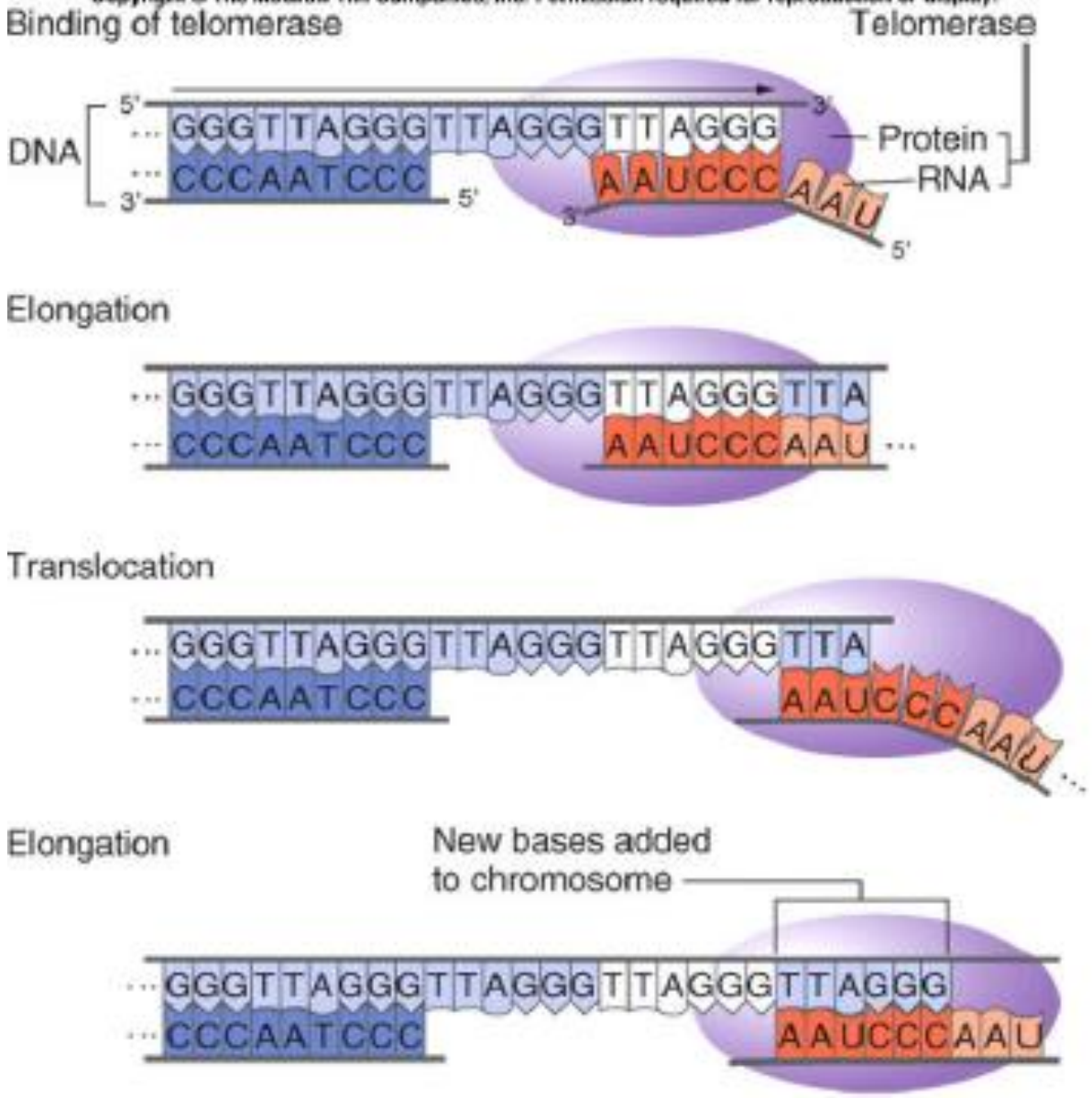


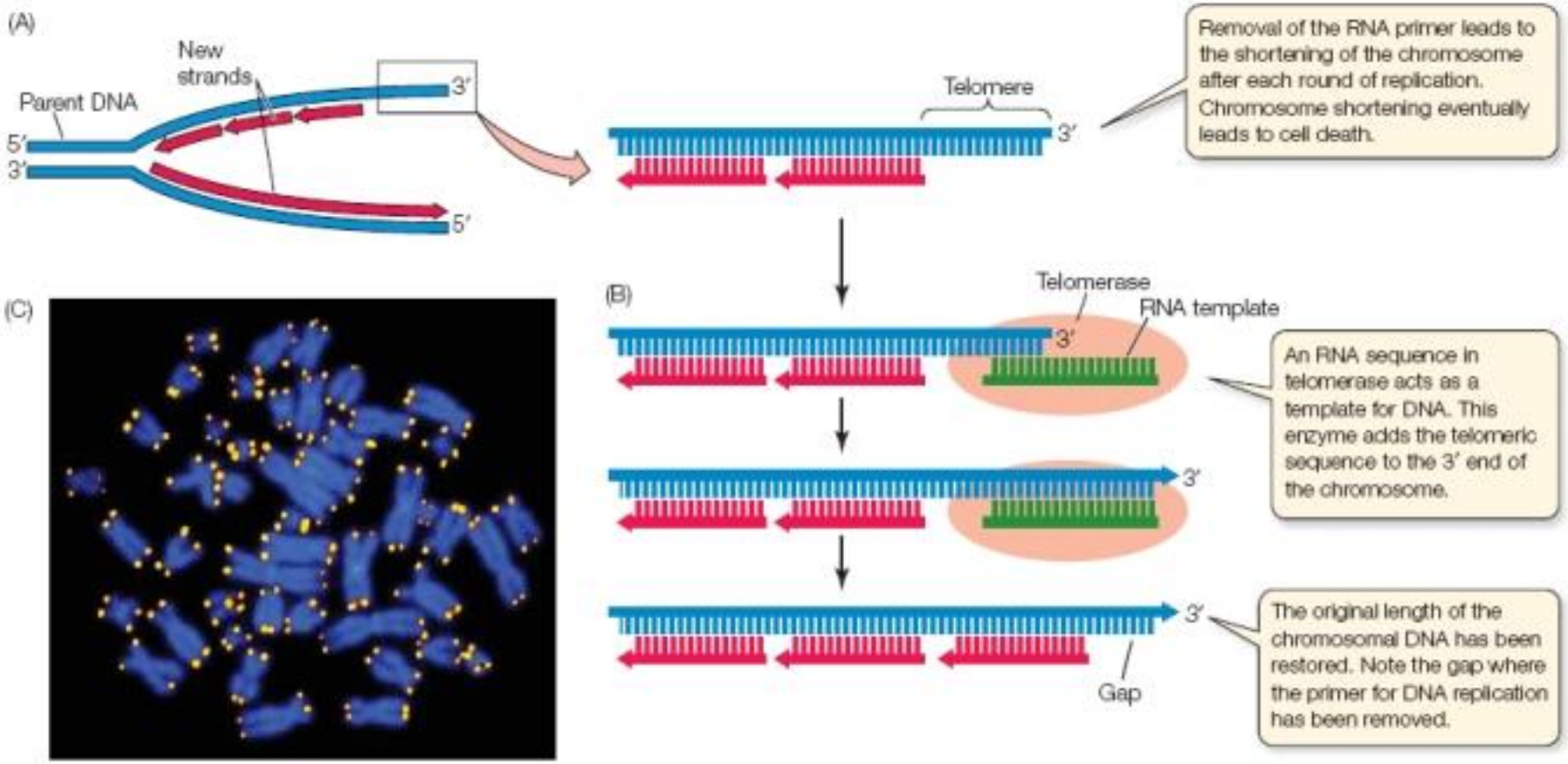

5′
3′
5′
3′
RNA primer
3′
5′
3′
5′
RNA primer
Synthesis of new DNA strands by addition to the 3′ end of the RNA primers
DNA polymerase
DNA polymerase
Removal of RNA primers by ribonucleases
Gap
Gap
Newly synthesized strands are shorter by the length of the RNA primer.


template strand
incomplete newly synthesized lagging strand
RNA primer
telomerase adds additional repeats to the template strand
DNA REPAIR
DNA synthesis from RNA primer completes lagging strand
replicated chromosome end

TELOMERAZ


Nucleus
Chromosome
Chromosome
Telomere
Cell
Telomere
Single-stranded Region
Double Stranded Region
DNA
Telomeric DNA Repeats
Base Pairs




(A)
New strands
Parent DNA
5′
3′
3′
5′
Telomere
3′
Removal of the RNA primer leads to the shortening of the chromosome after each round of replication. Chromosome shortening eventually leads to cell death.
(B)
Telomerase
RNA template
3′
An RNA sequence in telomerase acts as a template for DNA. This enzyme adds the telomeric sequence to the 3′ end of the chromosome.
3′
3′
Gap
The original length of the chromosomal DNA has been restored. Note the gap where the primer for DNA replication has been removed.
(C)

TELOMERASE ACTIVITY

Embryonic cells

Germ cells

Continuously proliferating cells (Hematopetic stem cells, active lymphocytes, intestinal cells)

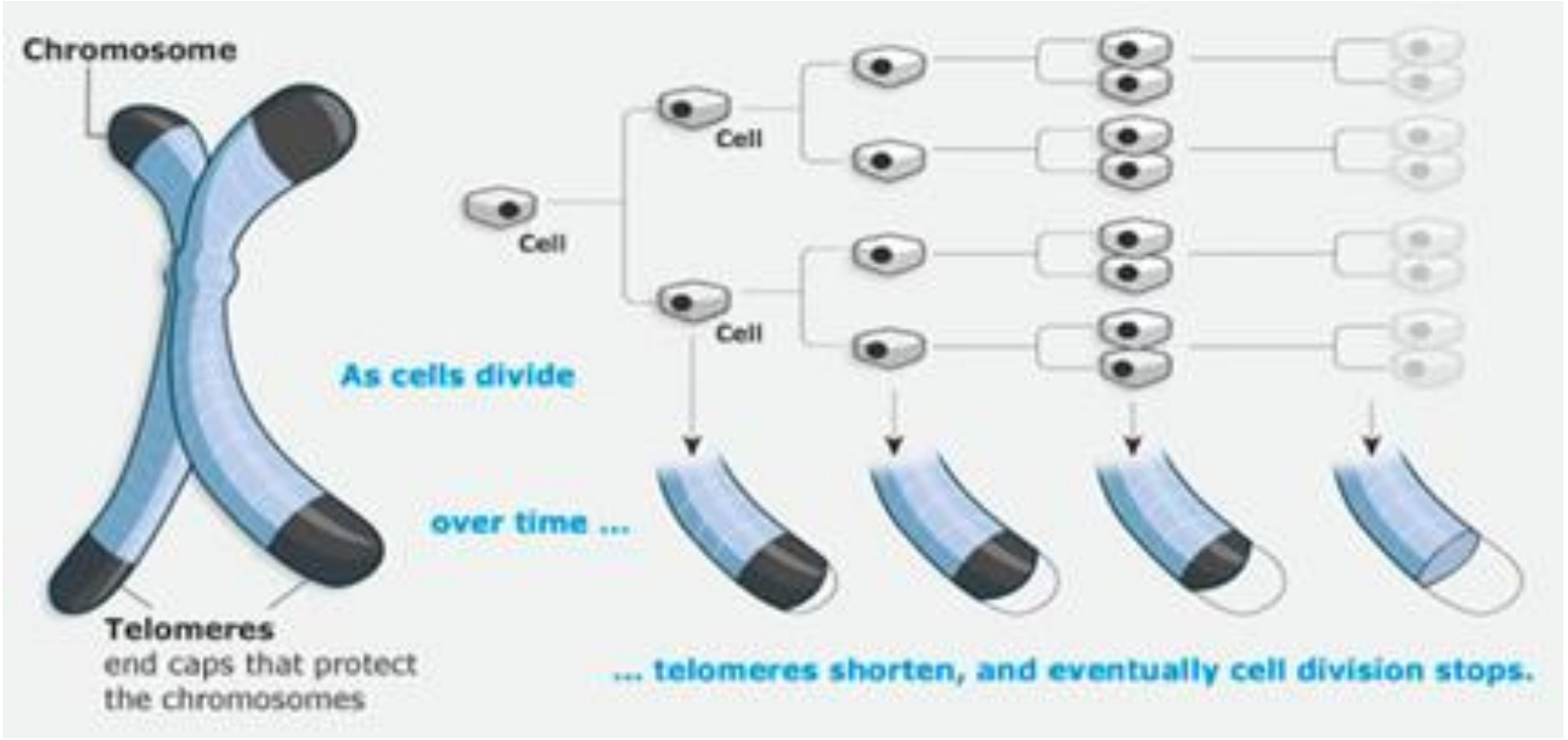
Cancer cells

Under normal conditions, somatic cells do not show telomerase activity.

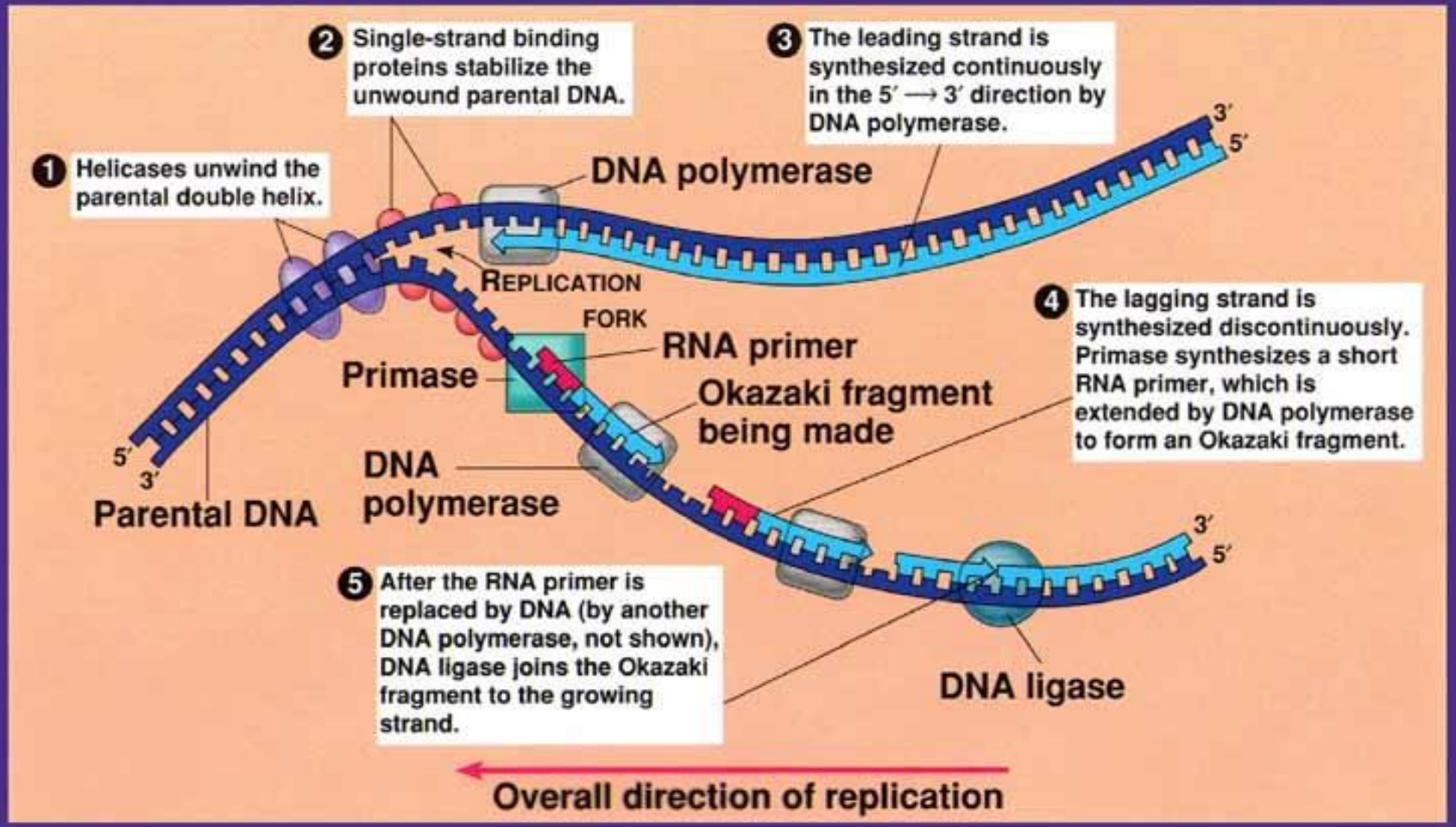
In somatic cells:

There is a close relationship between telomere loss and old age

PROGERIA (Rapid Aging Disease), severe telomere shortening and loss are observed.


Chromosome
Cell
Cell
Cell
As cells divide
over time ...
Telomeres
end caps that protect
the chromosomes
... telomeres shorten, and eventually cell division stops.

A SUMMARY OF DNA REPLICATION



- http://www.wiley.com/college/pratt/0471393878/student/animations/dna_replication/