Module: Molecule, Gene and Disease

Semester: 2

Session: 4

Lecture: 8

Duration: 1 hr

Lecture Title:

DNA, Chromosomes and DNA Replication

Module staff:

- Dr. Farqad M. Al-Hamdani
- Dr. Wameedh Hashim Alqatrani
- Dr. Hamed Jaddoa
- Dr. Hussein K. Abdul-Sada
- Dr. Hameed Abbas
- Dr. Amani Niama
- Dr. Ilham Mohamed Jawad
- Dr. Ban M. Saleh
- Dr. Shant Sunbat
- Dr. Zainab Ahmad
- Dr. Myada Abd Allah
- Dr. Abeer Laili

This Lecture was loaded in blackboard and you can find the material in:
(Lippincott's Illustrated Reviews: Cell and Molecular Biology Chapter 5)
For more detailed instructions, any question, or you have a case you need help in, please post to the group of session

Lecture 8 Learning Outcomes:

1. Explain how eukaryotic DNA is condensed in nucleosomes and relate this to the structure of chromosomes. **(LO.1)**
2. Describe the process and role of DNA replication. **(LO.2)**
3. Explain the role of DNA polymerase and other enzymes in DNA replication. **(LO.3)**
4. Show an appreciation of the vast amount of DNA present in a cell and explain how even single base changes in this vast amount of DNA can cause disease. **(LO.4)**
5. Describe the process and the role of the cell cycle. **(LO.5)**

DNA Condensation

LO.1

Q: Why DNA needs to be condensed?

A:

A cell's genetic information, in the form of DNA, is stored in the nucleus.

The space inside the nucleus is limited and has to contain billions of nucleotides that compose the cell's DNA.

Therefore, the DNA has to be highly packaged or condensed.

The basic units of eukaryotic chromosome structure:

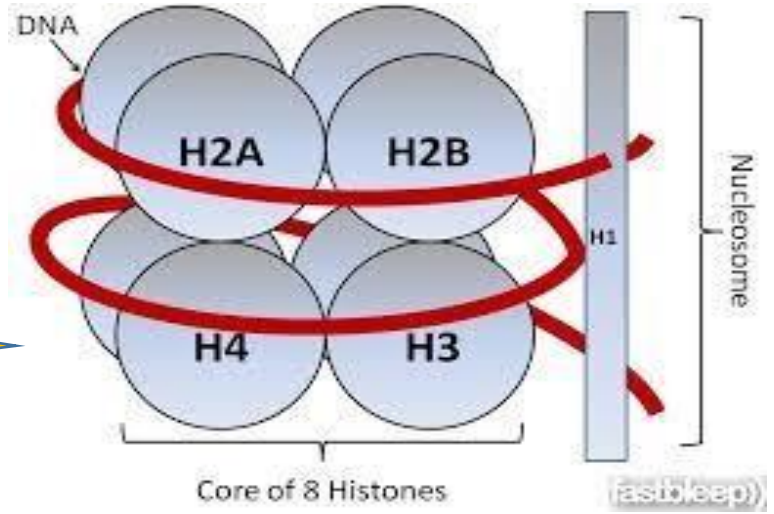
(LO. 1)

The proteins that bind to the DNA to form eukaryotic chromosomes are traditionally divided into two general classes:

Histones

Non-histone chromosomal proteins ??

The chromatin is a complex of DNA & protein



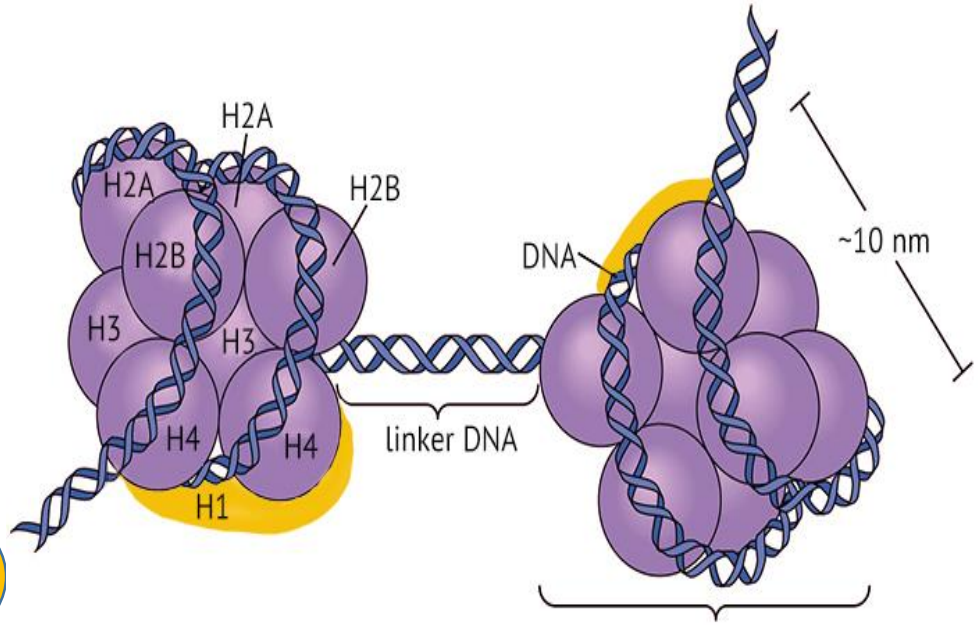
DNA Condensation LO.1

The DNA coils around **histones,** forming a beads-on-a-string-like structure.

The bead part is called a **nucleosome** ; **composed of eight histone proteins (a pair of each of four types).**

A fifth type of histone protein anchors nucleosomes to short "linker" regions of DNA.

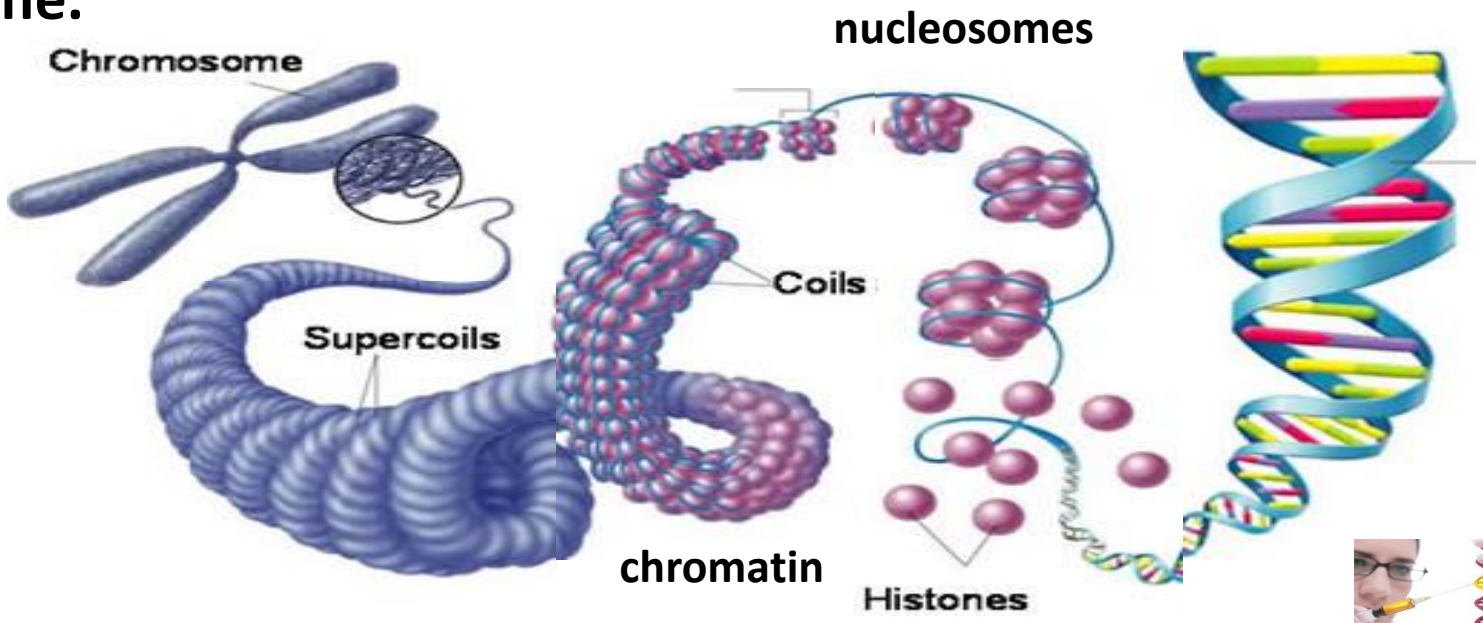
Q/what can the negative charge that coated the surface of DNA helix facilitate?



DNA coiling to visible structure of the chromosome

LO. 1

1. Primary coiling: DNA double helix
2. Secondary coiling: (wrapped around histones)--- nucleosomes
3. Tertiary coiling: Nucleosomes can also tightly pack into solenoid structures, forming 30 nm fibers (chromatin fibers).
4. Chromatin fibers form long loop on non-histone proteins.
5. Tighter coils.
6. Chromosome.



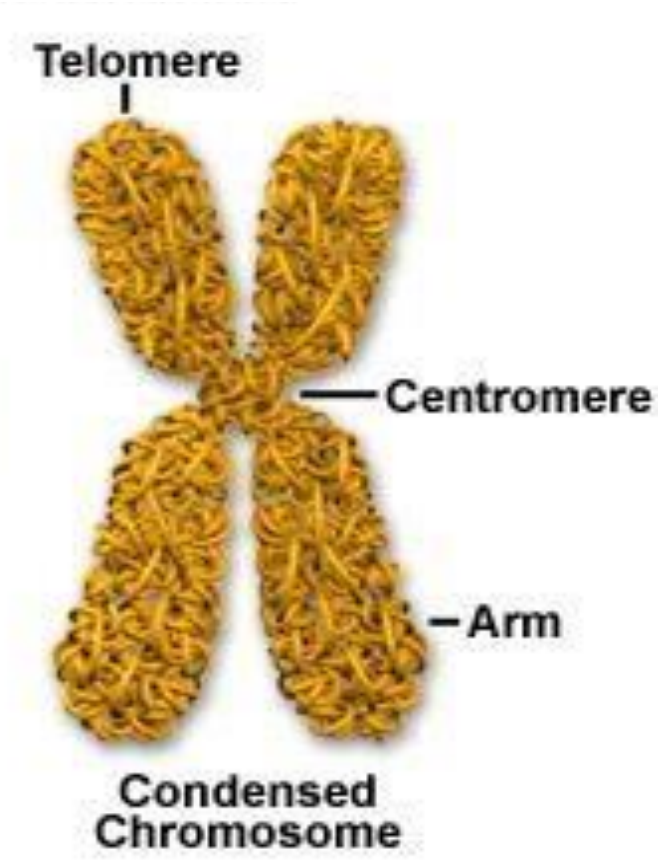
The structure of chromosome

LO. 1

Chromosome:

-A compact structure of nucleic acid and protein found in the nucleus of most living cells, carrying genetic information in the form of genes.

-Chromosomes are observable during M-phase or nuclear division.



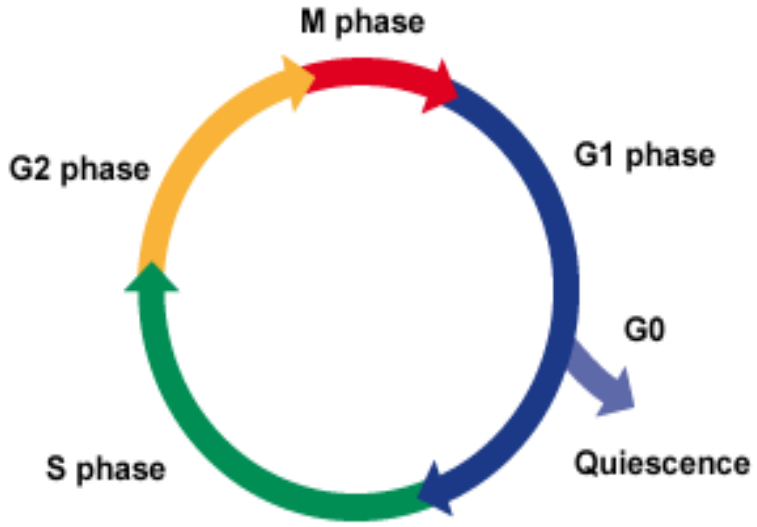
LO. 2

DNA replication

DNA replication is a biological process that occurs in all living organisms and copies their DNA, it is the basis for biological inheritance.

The process starts when one double-stranded DNA molecule produces two identical copies.

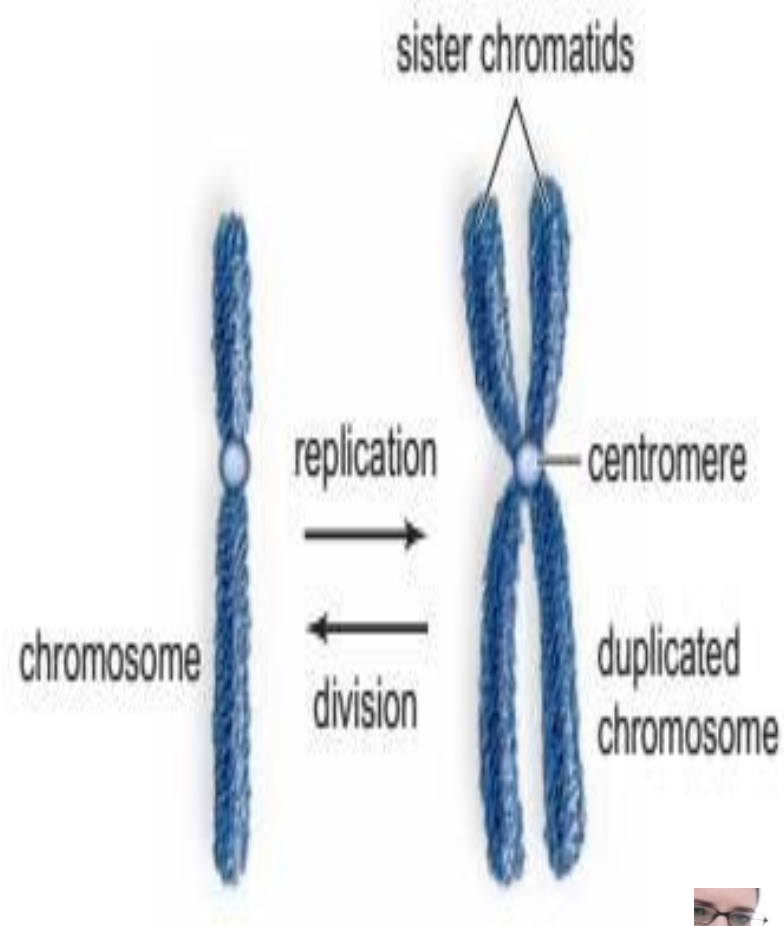
☺ DNA replication occurs during **(S phase)** of the cell cycle



LO. 2

DNA Replication

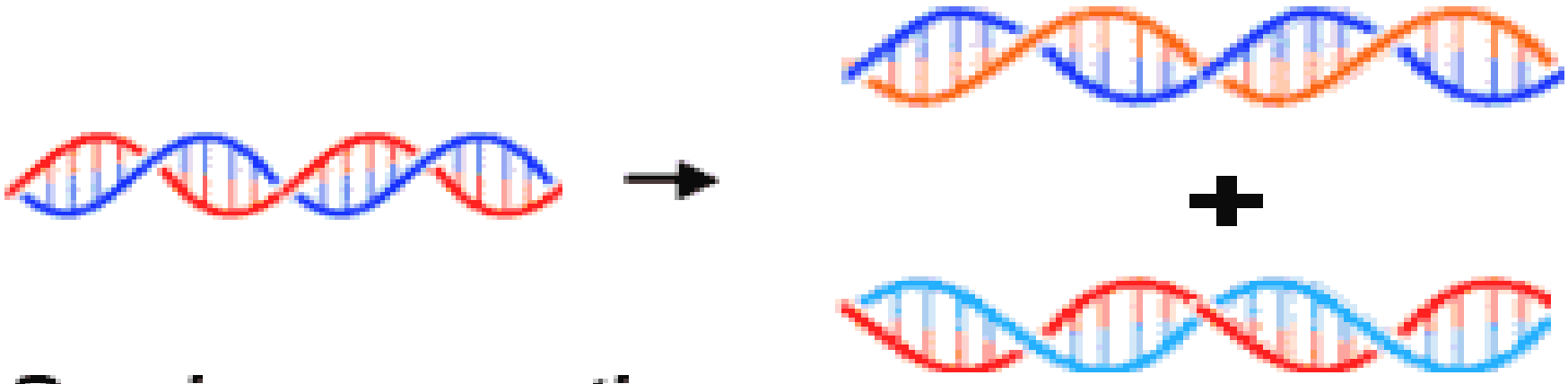
In a cell in which DNA synthesis (DNA Replication) has occurred all the chromosomes are duplicated and thus each consists of two identical sister chromatids.



LO. 2

DNA replication

Semiconservative replication would produce two copies that each contained one of the original strands and one new strand .



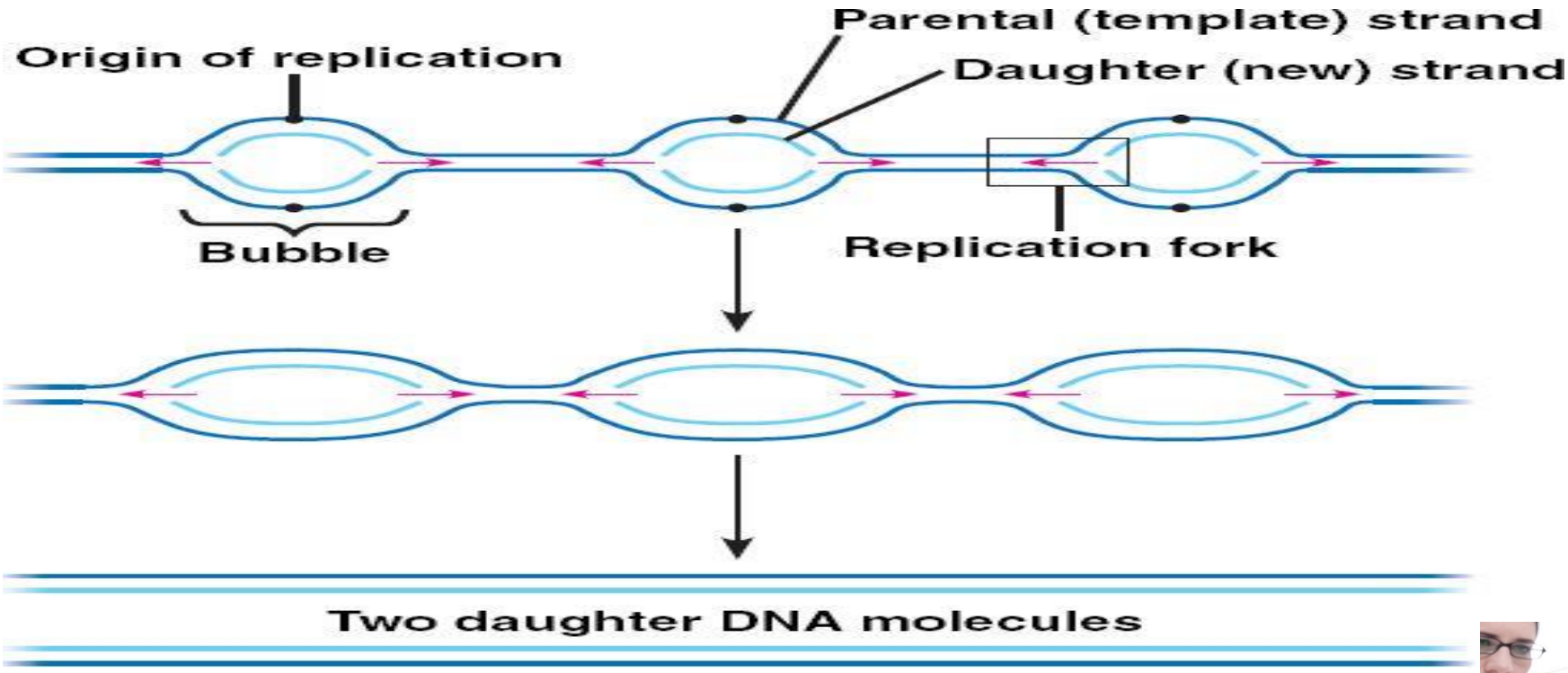
Semi-conservative Replication

DNA replication

LO. 2

Replication begins at specific sites on DNA molecule called "origins of replication

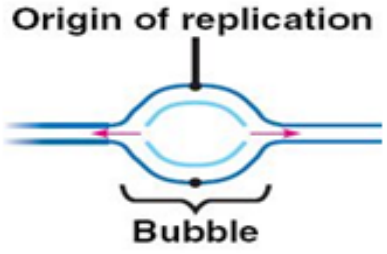
- origins are specific sequence of bases
- mammalian DNA have many origins



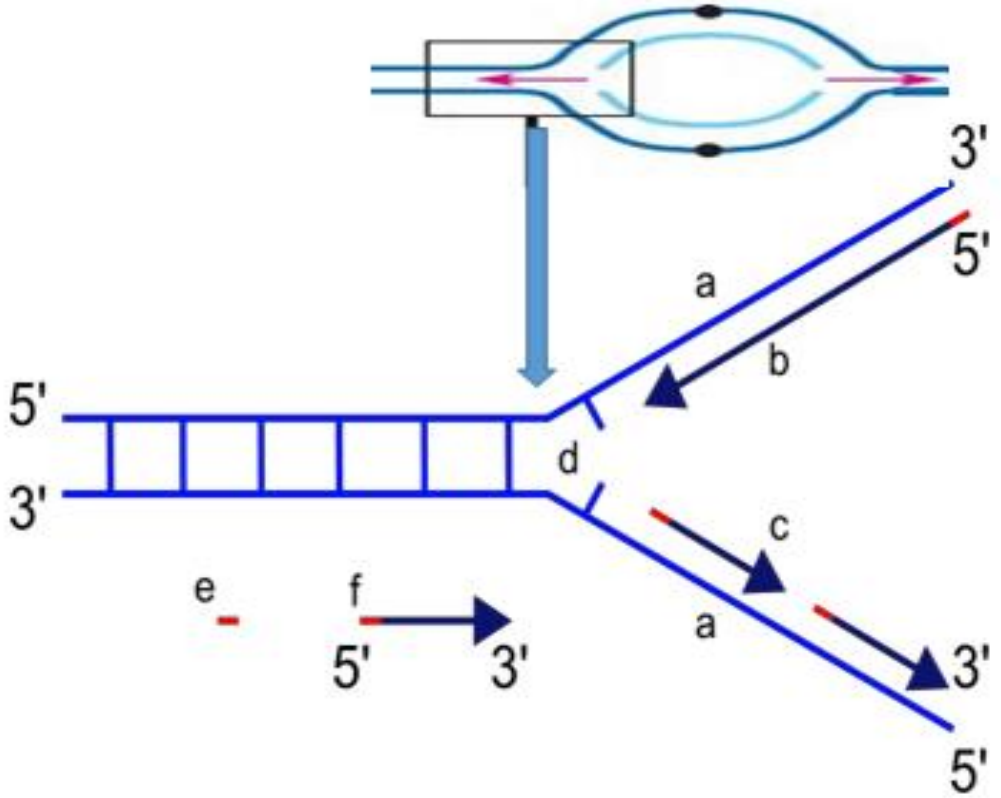
DNA replication

LO. 2

The replication fork :is a structure that forms within the nucleus during DNA replication. It is created by helicases, which break the hydrogen bonds holding the two DNA strands together.



Replication bubbles allow DNA replication to speed up therefore the untwisted DNA would not be attacked by enzymes while replicating



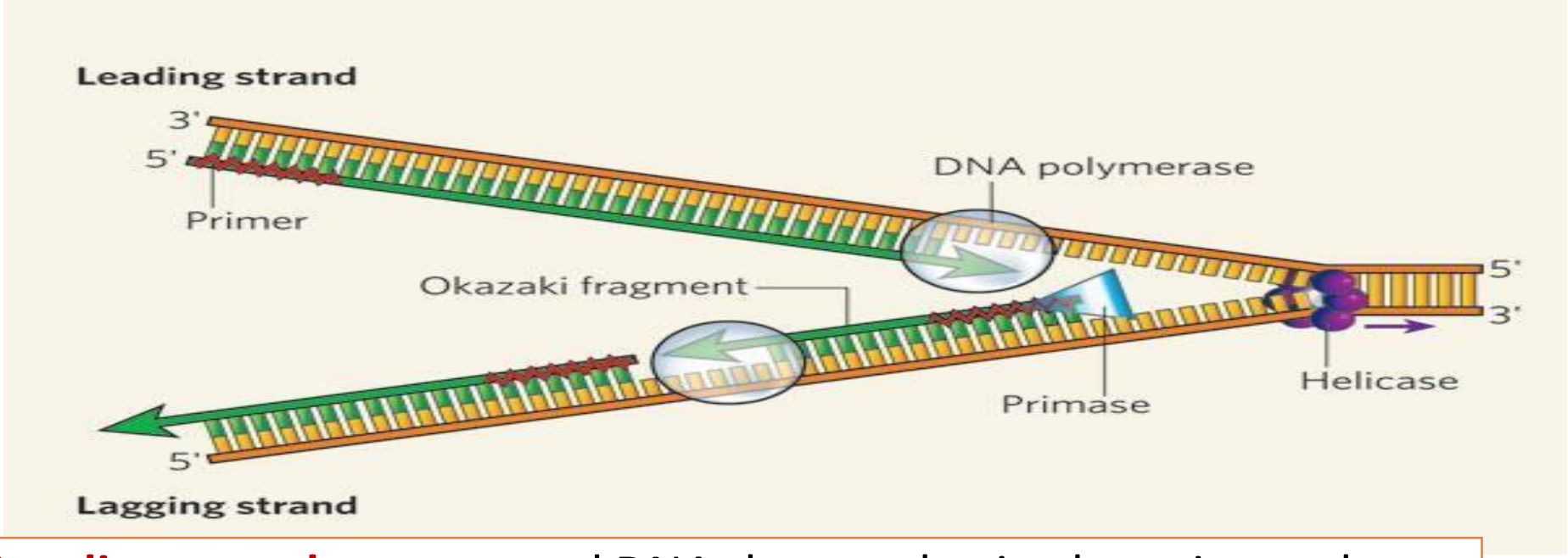
DNA replication

LO. 2&3

1. The helicase unwinds the double-stranded DNA for replication, making a forked structure.

2. The primase generates short strands of RNA that bind to the single-stranded DNA to initiate DNA synthesis by the DNA polymerase.

3. DNA polymerase III can work only in the 5' to 3' direction, so it replicates the leading strand continuously. Lagging-strand replication is discontinuous, with short **Okazaki fragments** being formed and later linked together.



Leading strand : new strand DNA that synthesized continuously

Lagging strand : new strand DNA that synthesized dis-continuously

LO. 2

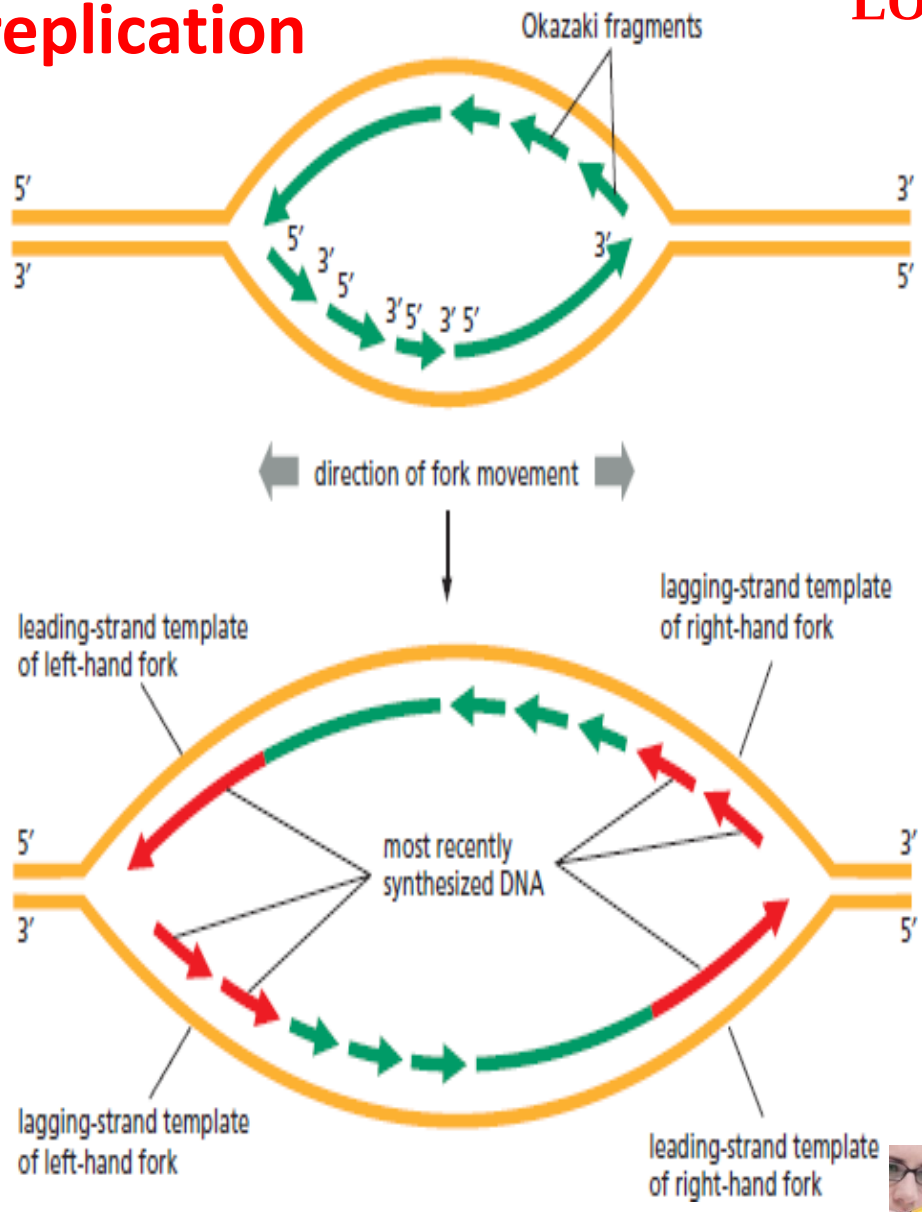
DNA replication

DNA replication forks are asymmetrical because both of the new strands are synthesized in the 5’-to-3’ direction

The lagging strand of DNA must be made initially as a series of short DNA strands that are later joined together.

To synthesize the lagging strand, DNA polymerase must “backstitch”

Backstitch: synthesizes short pieces of DNA (called Okazaki fragments) in the 5’-to-3’ direction, and then must move in the opposite direction along the template strand (toward the fork) before synthesizing the next fragment.

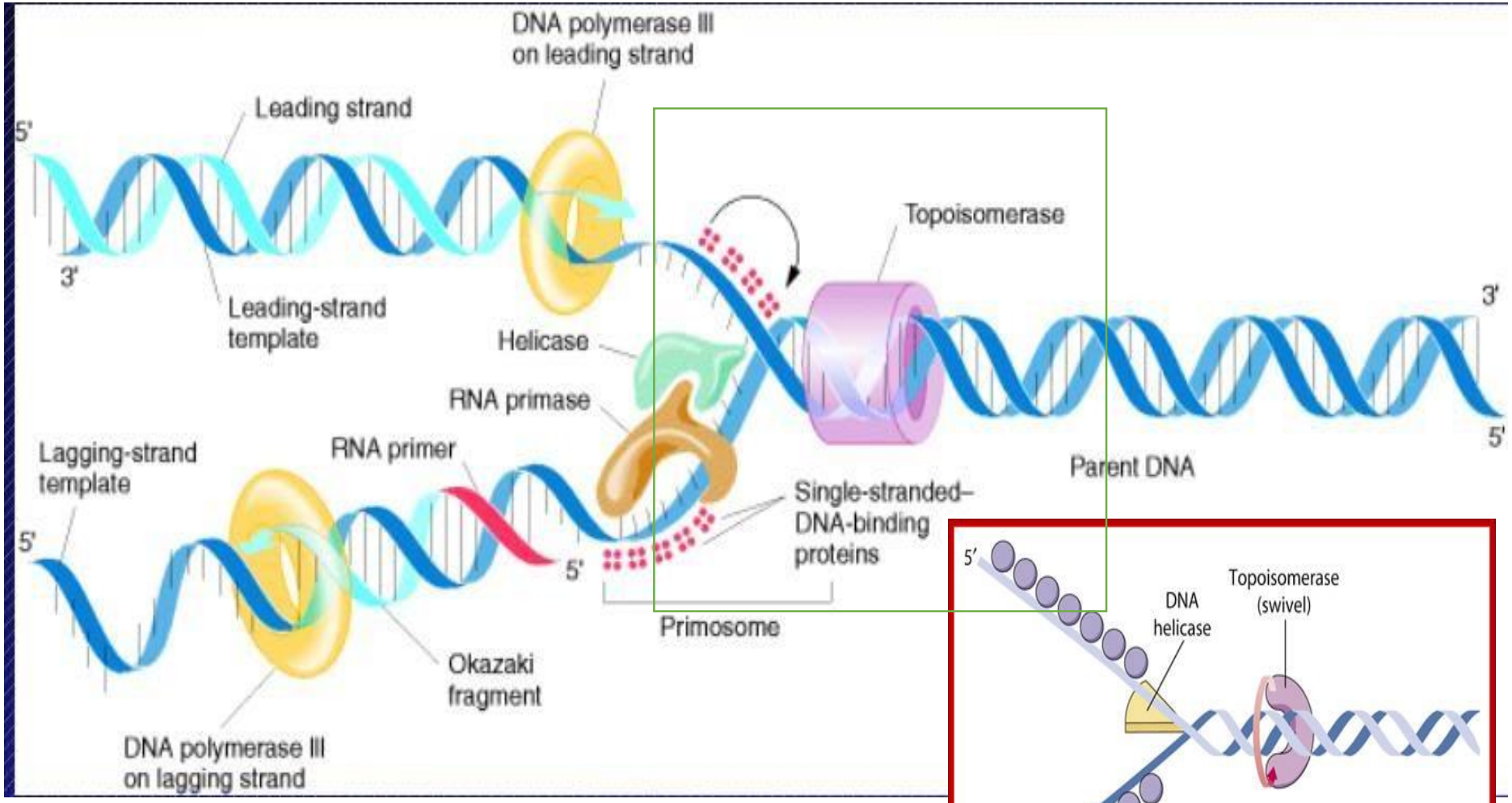


DNA polymerase and other important enzymes participating in DNA Replication

LO.3

Enzyme	Function in DNA replication
DNA Helicase	Also known as helix destabilizing enzyme. Unwinds the DNA double helix at the Replication Fork.
DNA Polymerase	Builds a new duplex DNA strand by adding nucleotides in the 5' to 3' direction. Also performs proof-reading and error correction.
Single-Strand Binding (SSB) Proteins	Bind to ssDNA and prevent the DNA double helix from re-annealing after DNA helicase unwinds it thus maintaining the strand separation.
Topoisomerase	Relaxes the DNA from its super-coiled nature.
DNA Ligase	Re-anneals the semi-conservative strands and joins Okazaki Fragments of the lagging strand.
Primase	Provides a starting point of RNA (or DNA) for DNA polymerase to begin synthesis of the new DNA strand.

LO.3


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

5'
DNA helicase
Topoisomerase (swivel)
Single-stranded DNA binding protein (SSB)
3'
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DNA polymerase and other important enzymes participating in DNA Replication

LO.3

Three DNA polymerases (I, II, and III) have been purified from *E. coli.*

- **DNA Polymerase I:** Fill the gaps between Okazaki fragments.

- **DNA polymerase II:** functioning in the DNA repair.

- **DNA polymerase III:** catalyzes chain elongation at the growing fork in leading and lagging strands.

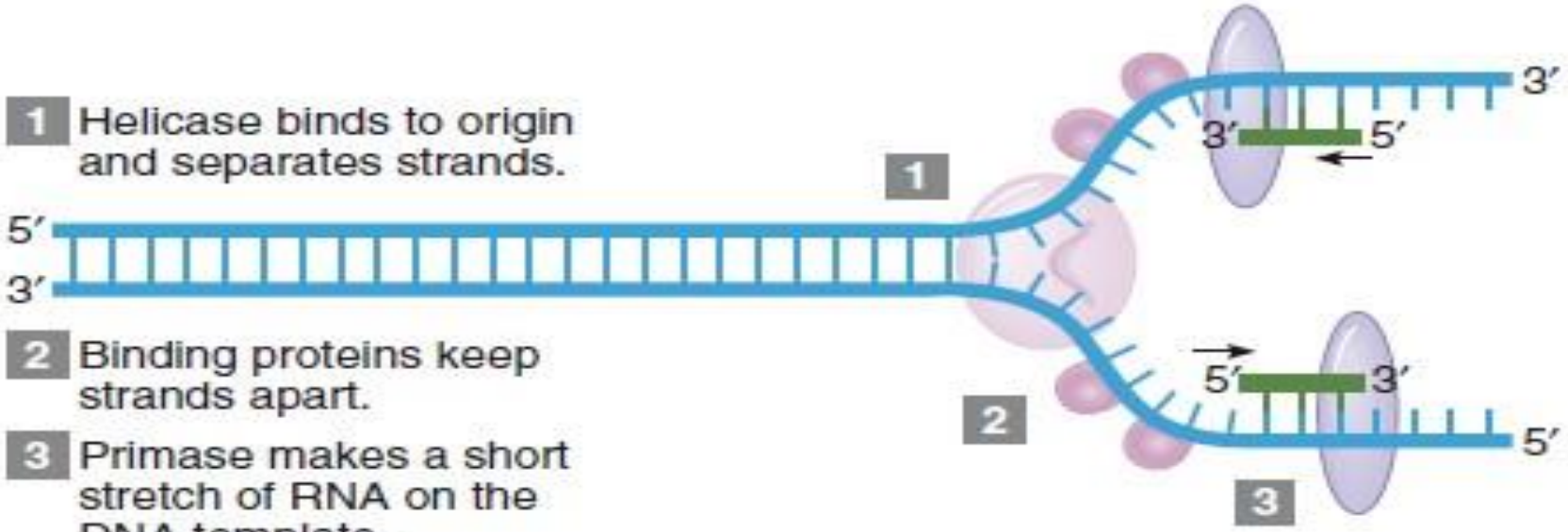
☺ The rate nucleotide additions in mammals add about 50 bases/second while in bacteria add about 500 bases/second.

LO.2&3

1 Helicase binds to origin and separates strands.

2 Binding proteins keep strands apart.

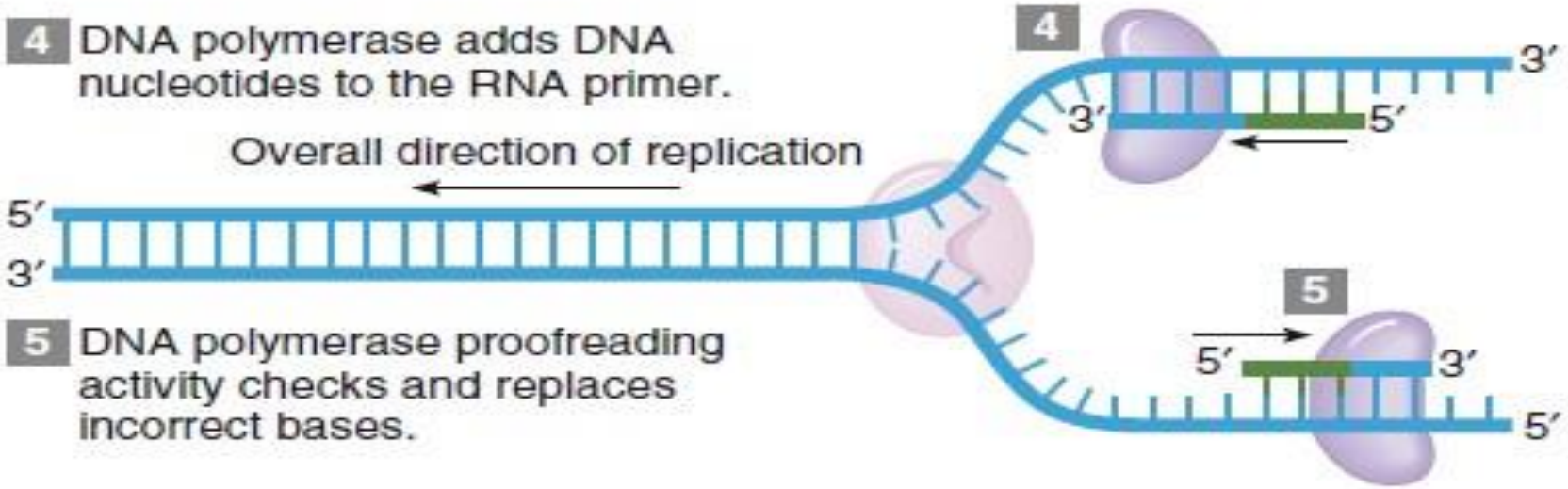
3 Primase makes a short stretch of RNA on the DNA template.



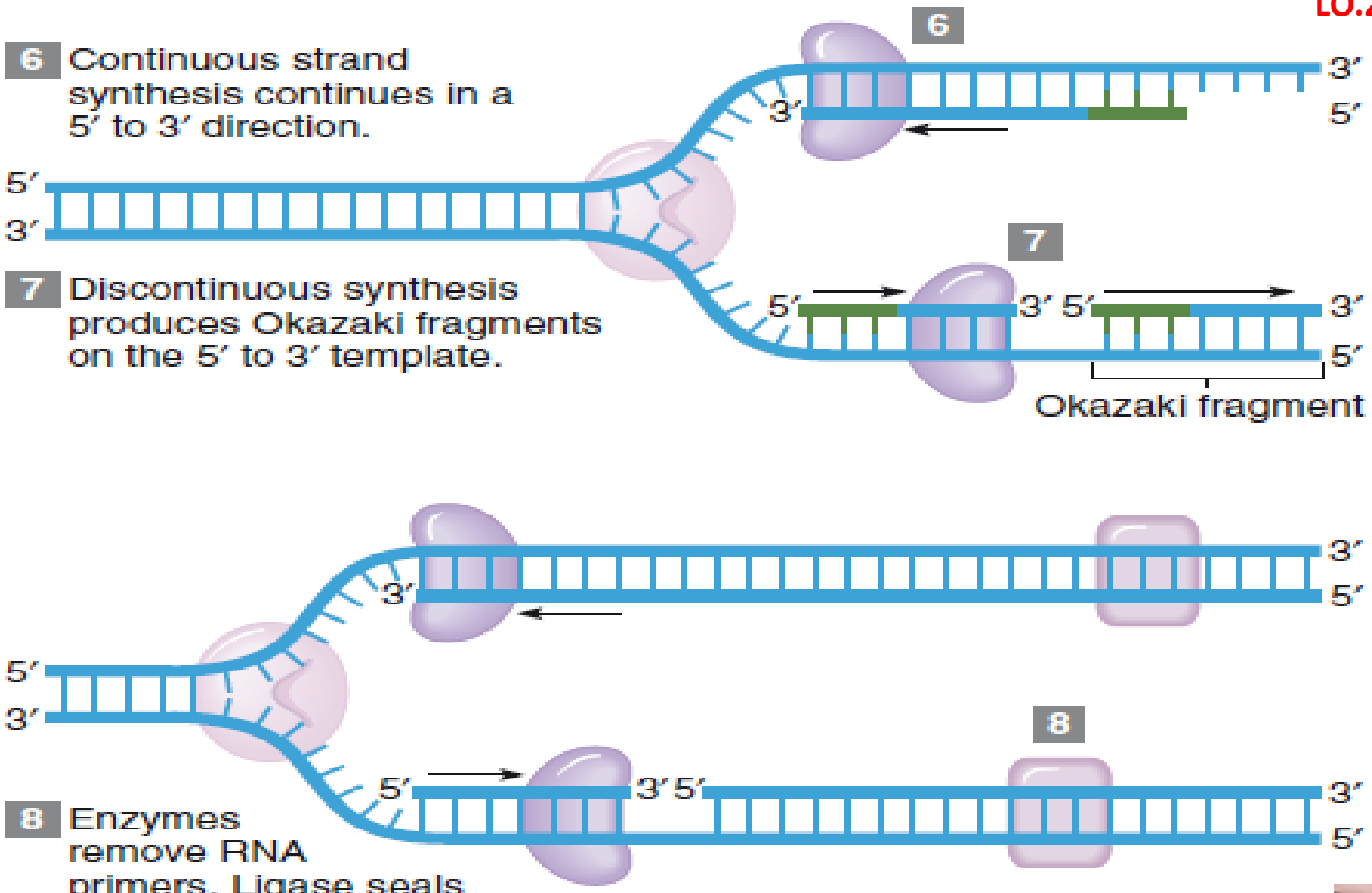
4 DNA polymerase adds DNA nucleotides to the RNA primer.

Overall direction of replication

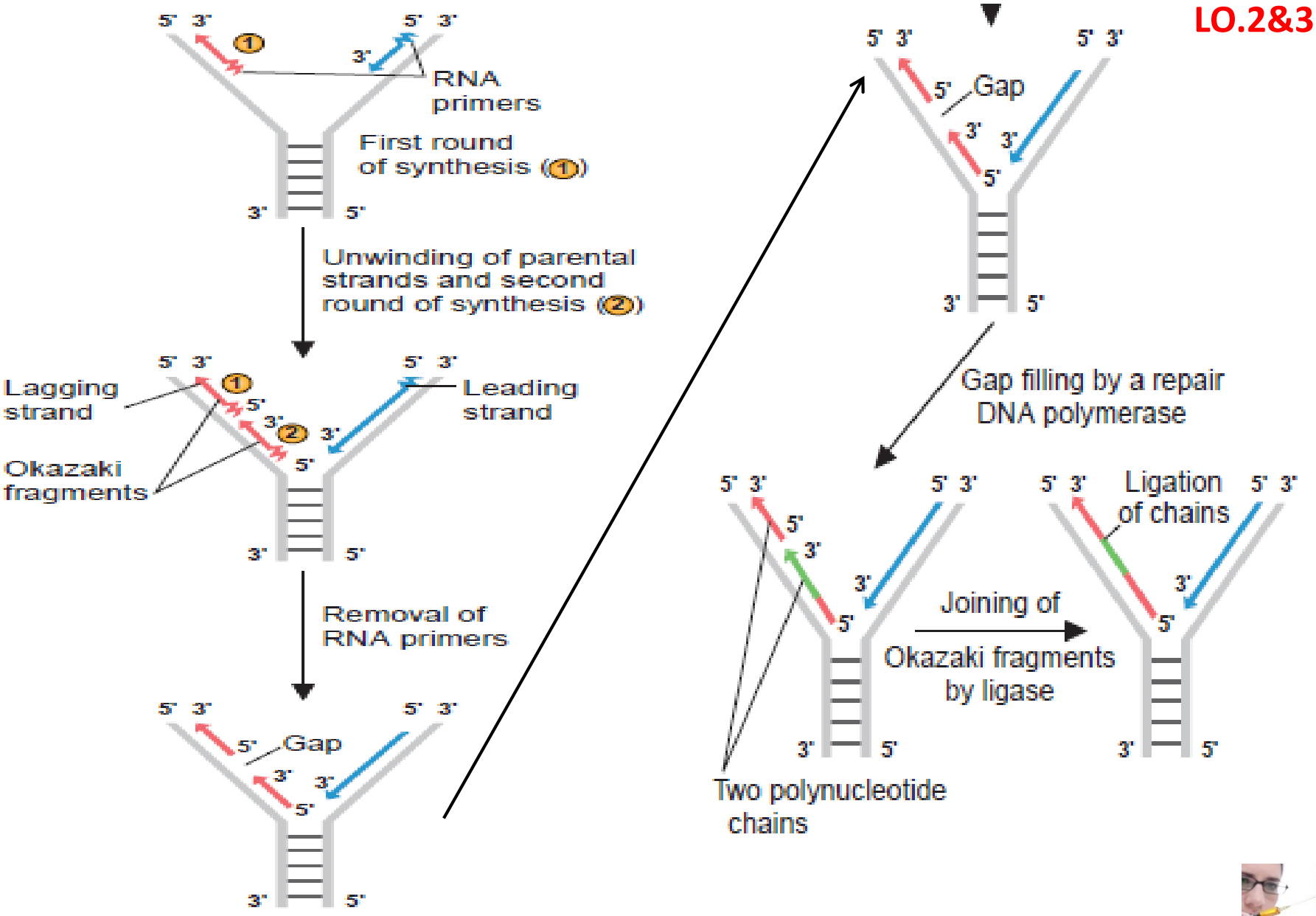
5 DNA polymerase proofreading activity checks and replaces incorrect bases.



LO.2&3


6 Continuous strand synthesis continues in a 5′ to 3′ direction.
7 Discontinuous synthesis produces Okazaki fragments on the 5′ to 3′ template.
8 Enzymes remove RNA primers. Ligase seals sugar-phosphate backbone.
5′
3′
6
7
8
Okazaki fragment

LO.2&3


RNA primers
First round of synthesis (1)
Unwinding of parental strands and second round of synthesis (2)
Lagging strand
Leading strand
Okazaki fragments
Removal of RNA primers
Gap
Gap filling by a repair DNA polymerase
Joining of Okazaki fragments by ligase
Ligation of chains
Two polynucleotide chains

You are going to find details of DNA replication in this video :
https://www.youtube.com/watch?v=TEQMeP9GG6M

LO. 4

Amount of DNA present in a cell

In eukaryotes, each chromosome contains one continuous, linear DNA helix. The DNA of the longest human chromosome is 7 cm in length.

In fact, if the DNA from all 46 chromosomes in a diploid human cell were placed end to end, our total DNA would span a distance of about 2 m (6 ft.). Our total DNA contains about 6 x10^9 base pairs.

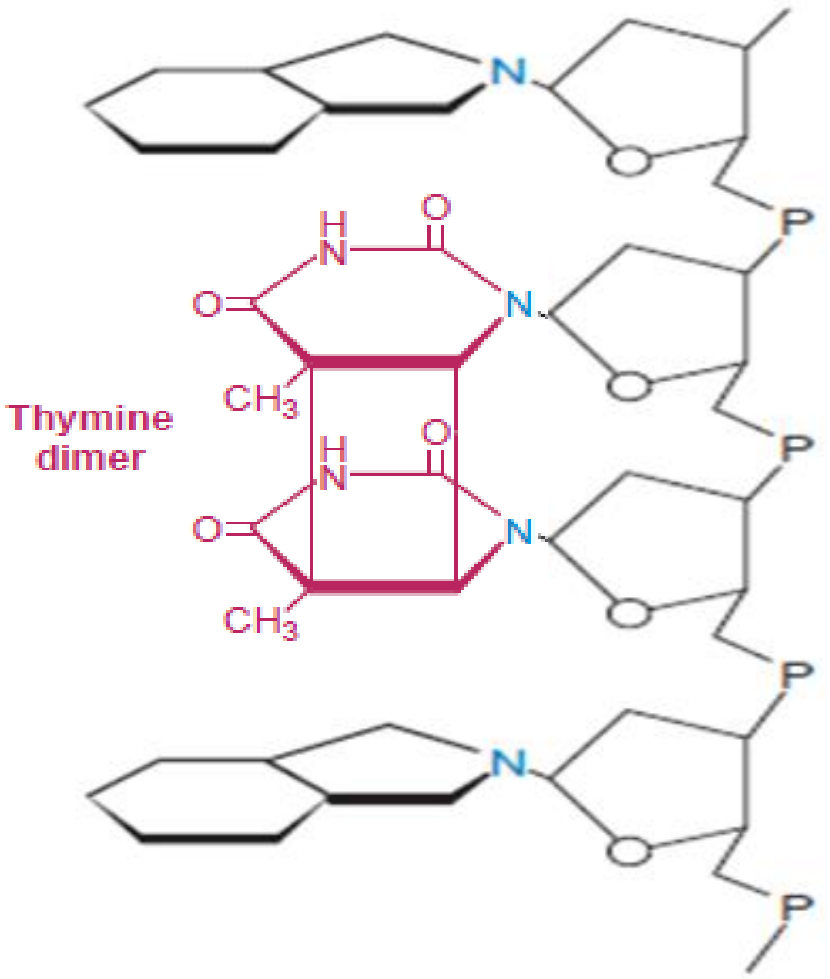
LO. 4

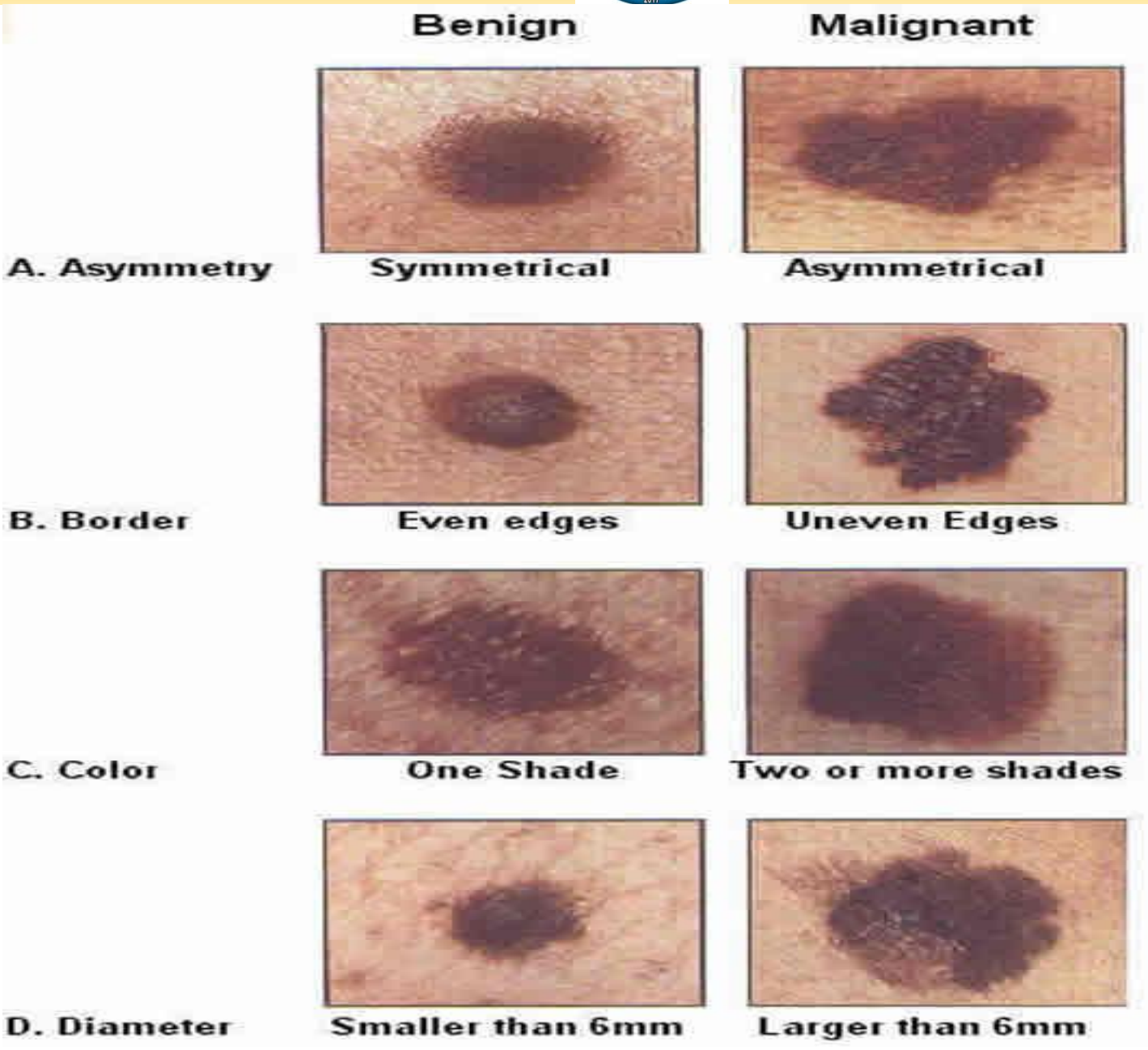
How even single base changes in DNA can cause disease

Melanomas develop from exposure of the skin to the UV rays of the sun.

The UV radiation causes **pyrimidine dimers** to form in DNA. Mutations may result from non-repair of the dimers that produce melanomas,

appearing as dark brown growths on the skin.

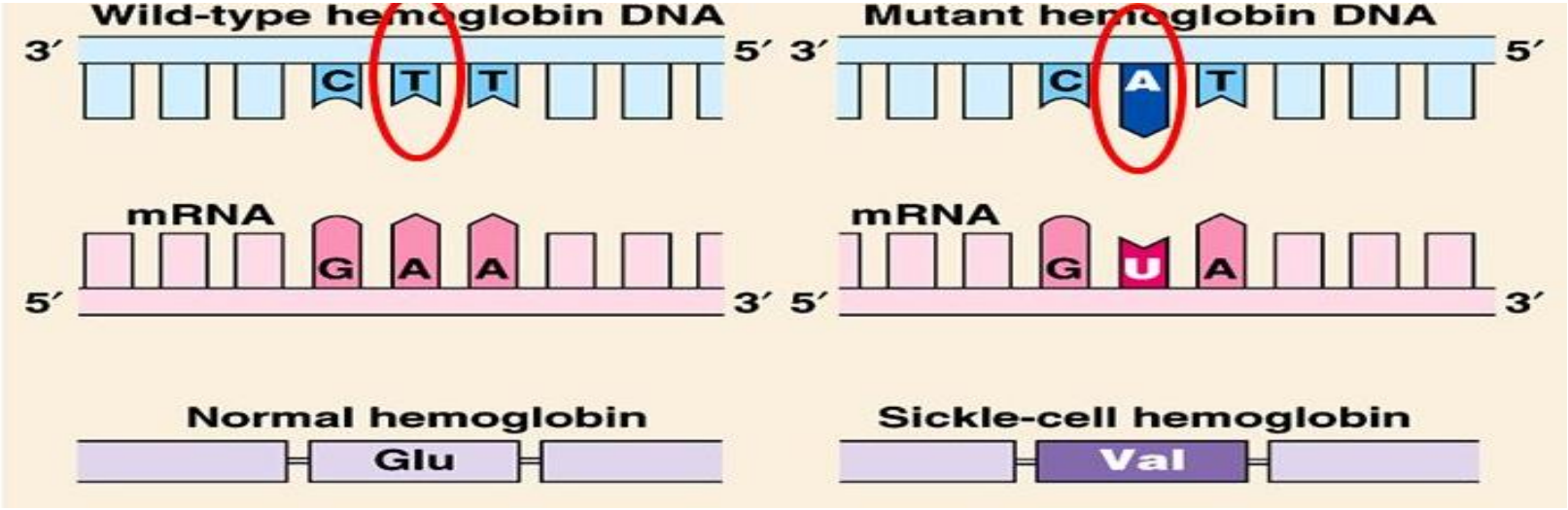



Benign
Malignant
A. Asymmetry
Symmetrical
Asymmetrical
B. Border
Even edges
Uneven Edges
C. Color
One Shade
Two or more shades
D. Diameter
Smaller than 6mm
Larger than 6mm

LO. 4

Substitution: For example, sickle-cell disease is caused by a mutation of a single base pair in the gene that codes for one of the polypeptides of hemoglobin.

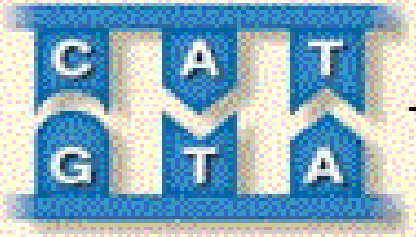
- A change in a T to A (substitution) in the DNA template leads to an abnormal protein.

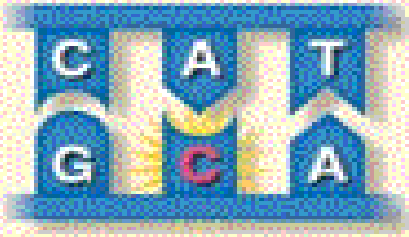


Effect of Base-Pair substitution

LO. 4

original base triplet in a DNA strand





a base substitution within the triplet (red)

As DNA is replicated, proofreading enzymes detect the mistake and make a substitution for it

POSSIBLE OUTCOMES:

OR

One DNA molecule carries the original, unmutated sequence

The other DNA molecule carries a gene mutation

The cell cycle

LO.5

The cell cycle or **cell-division cycle** is the series of events that take place in a cell leading to its division and duplication of its DNA (DNA replication) to produce two daughter cells .

Phases of cell cycle:-

1- Interphase _ G1(Gap1), S (Synthesis) and G2.

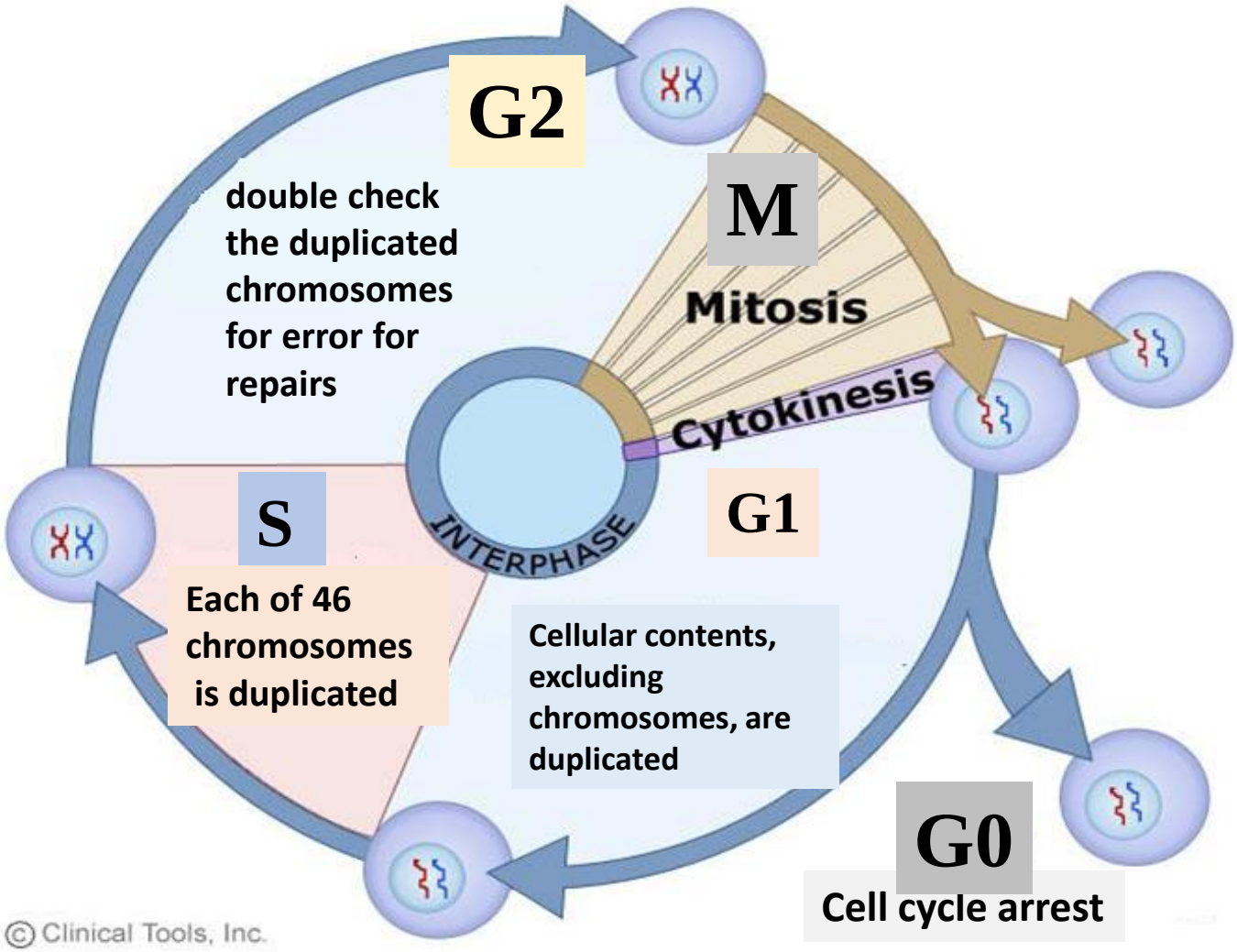
2- Mitosis.

3- Cytokinesis.

A human has 46 chromosomes (2n = 46) in diploid cells that involving all somatic cells, and 23 chromosome in haploid cell that involving the gametes (egg and sperm).

The cell cycle

LO.5

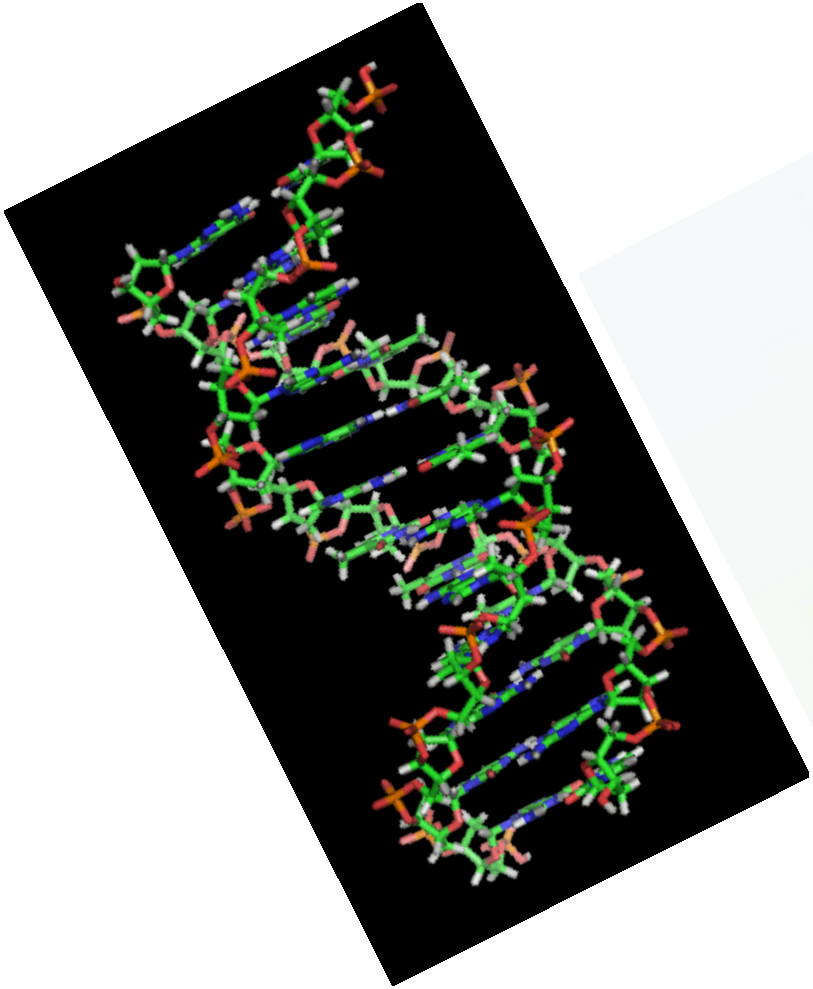


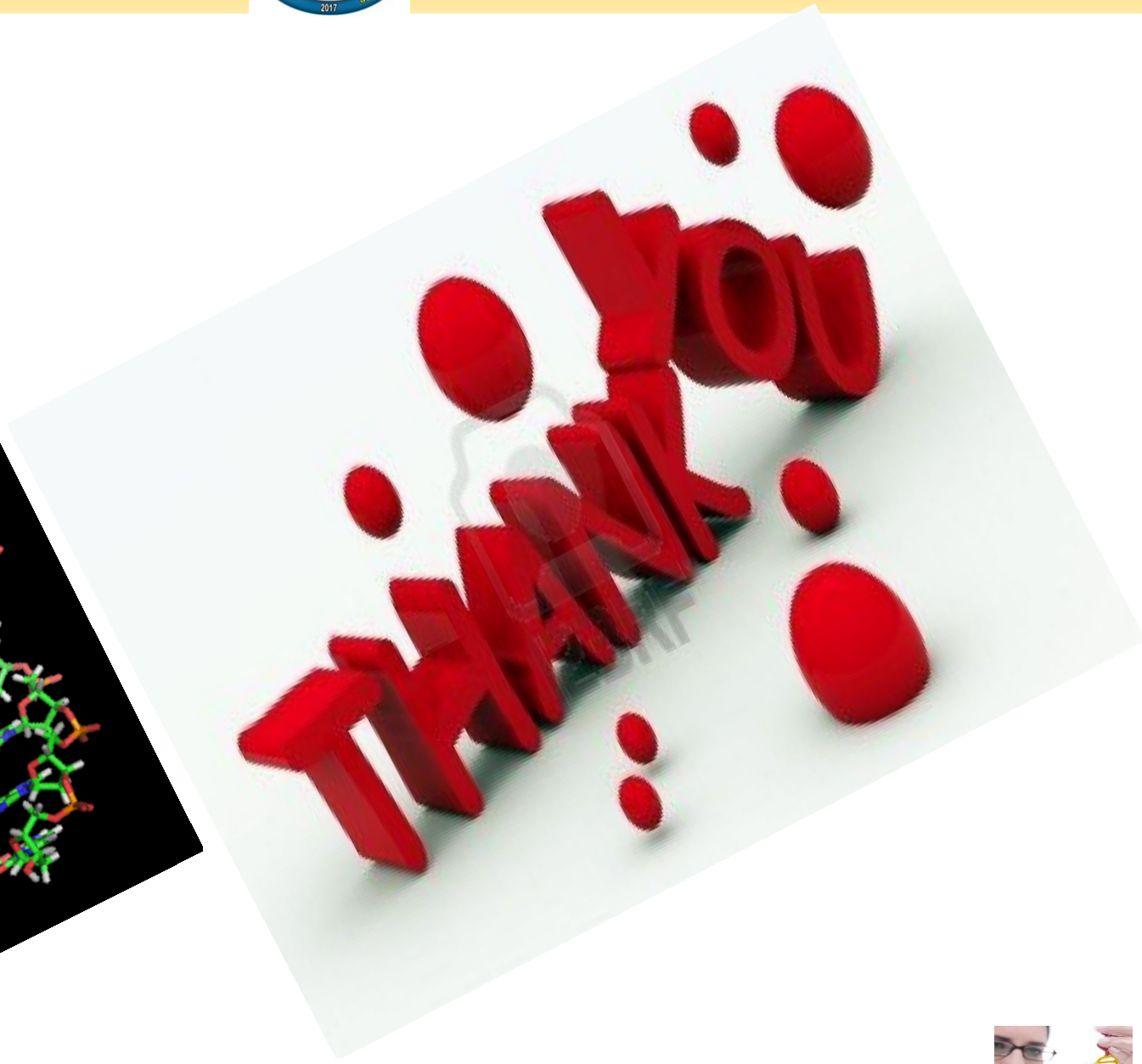
G1: the cell prepares for DNA replication (growth & metabolism) [6-12 h.]

S: DNA replication [6-8 h.]

G2: the cell prepares for cell division [3-4 h.]

M: cell division (mitosis) [1 h.]




THANK YOU