



Molecular Biology (4)

DNA replication

Prof. Mamoun Ahram

School of Medicine

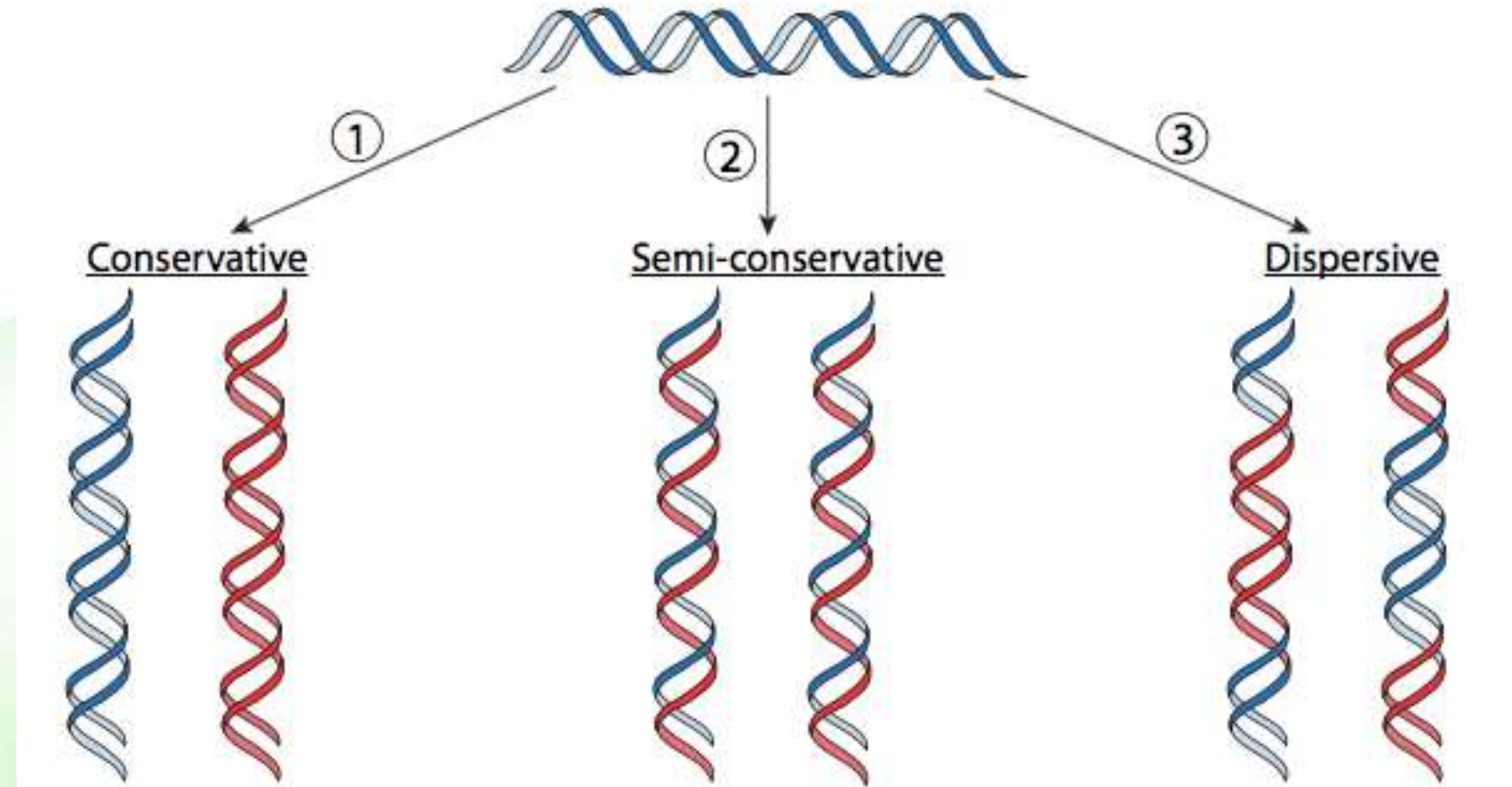
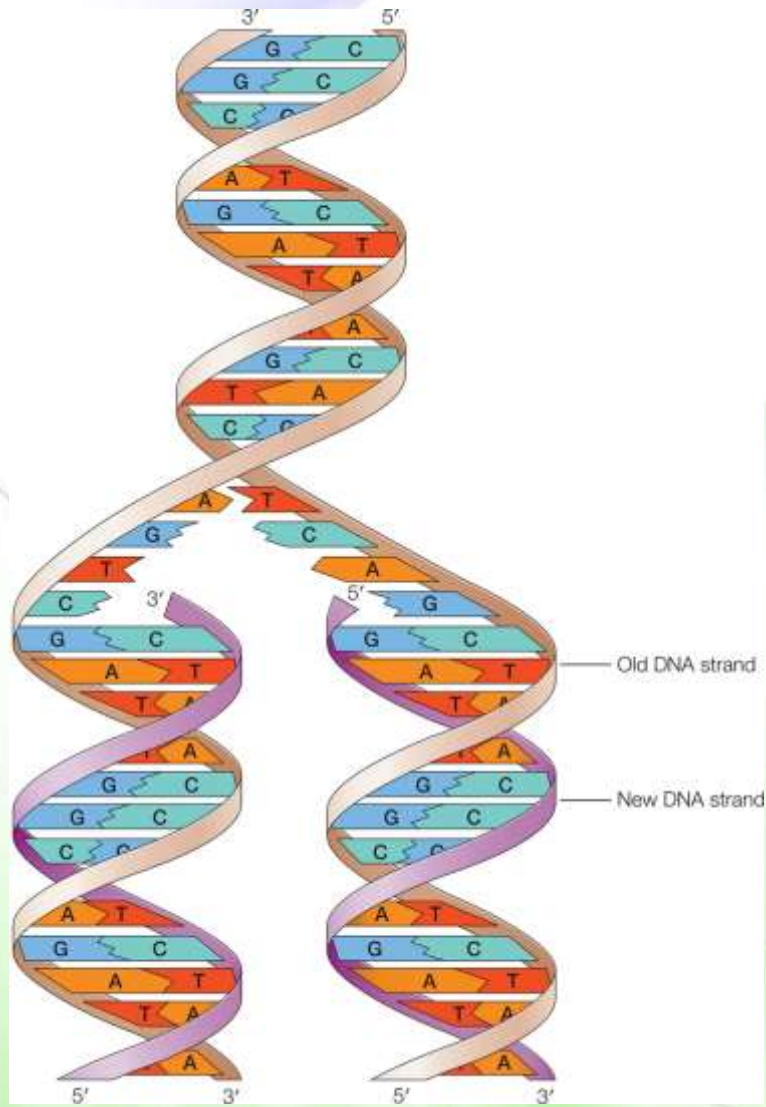
Second year, Second semester, 2024-2025

Some basic information



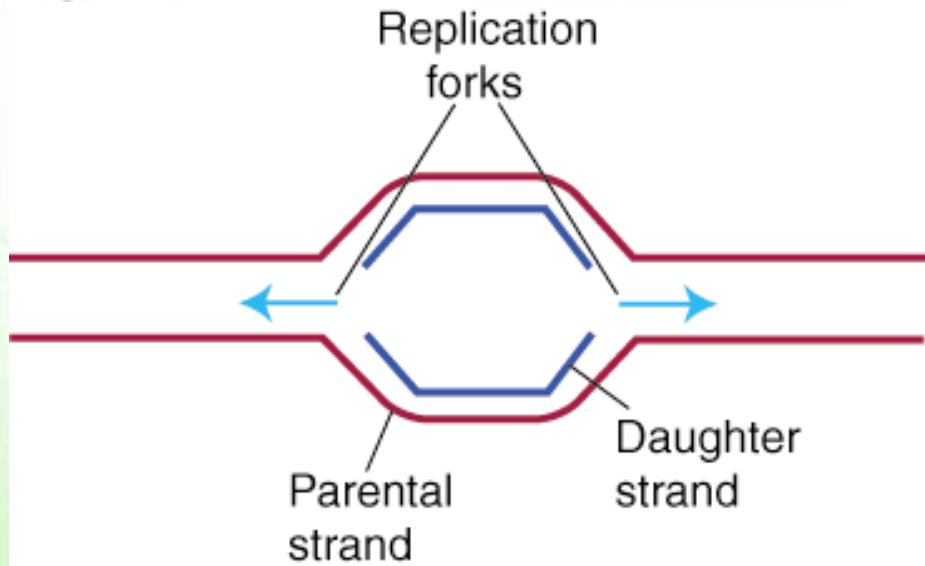
- The entire DNA content of the cell (or an organism) is known as a “genome”.
- DNA is organized into chromosomes.
 - Bacterial genome: usually one, circular chromosome.
 - Eukaryotic genome: multiple, linear chromosomes complexed with proteins known as histones, and the complex is known as chromatin.
- DNA must be accurately copied (replicated).
- DNA synthesis is carried out by DNA polymerases.
 - In bacteria (E. coli: DNA polymerases I, II, and III)
 - In Humans (DNA polymerases α , δ , and ϵ)
- The substrates are deoxyribonucleotides.

The hypotheses and fact

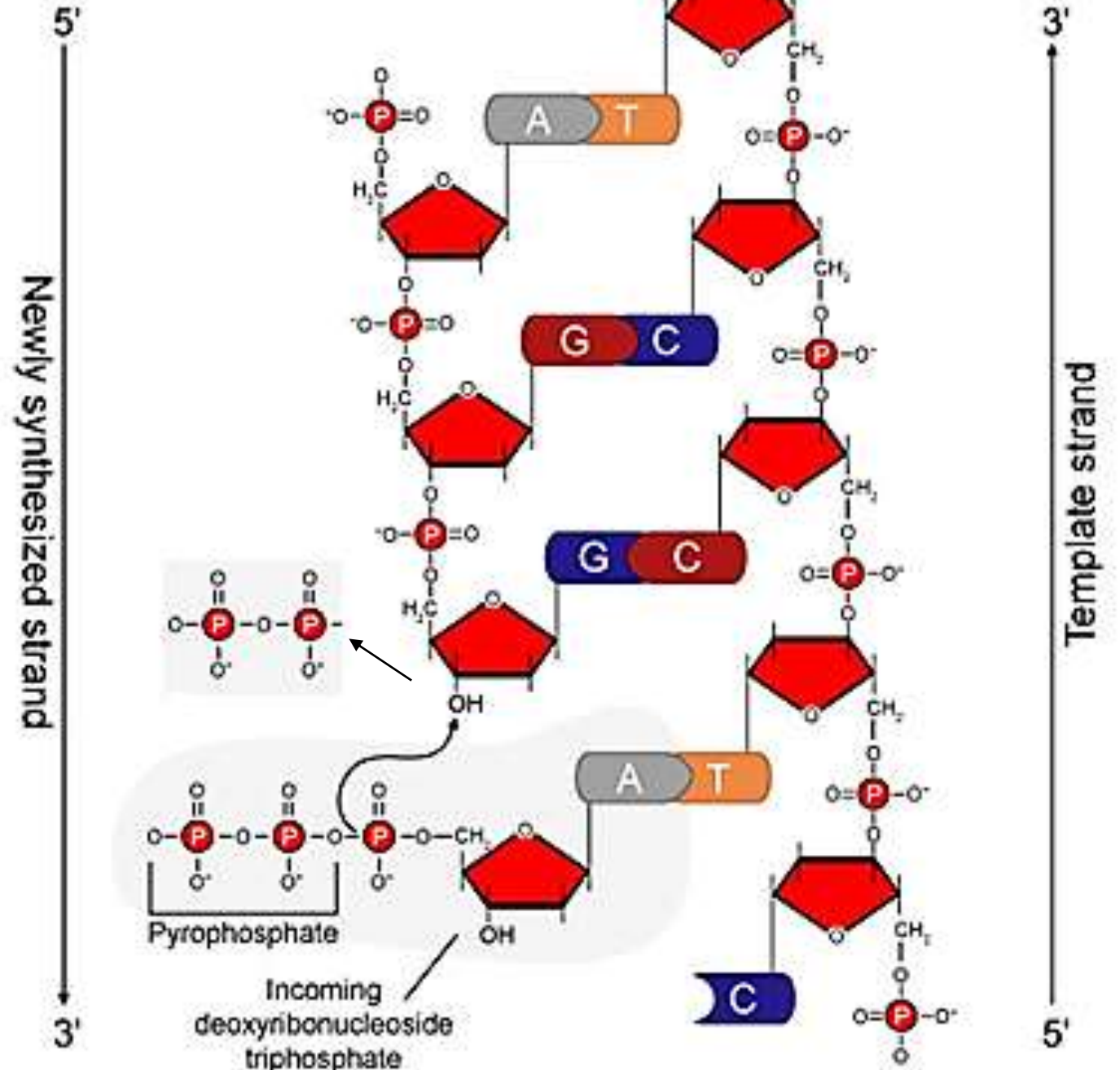


Bidirectional

- Replication is bidirectional.
- This replicative region is called a replication fork.



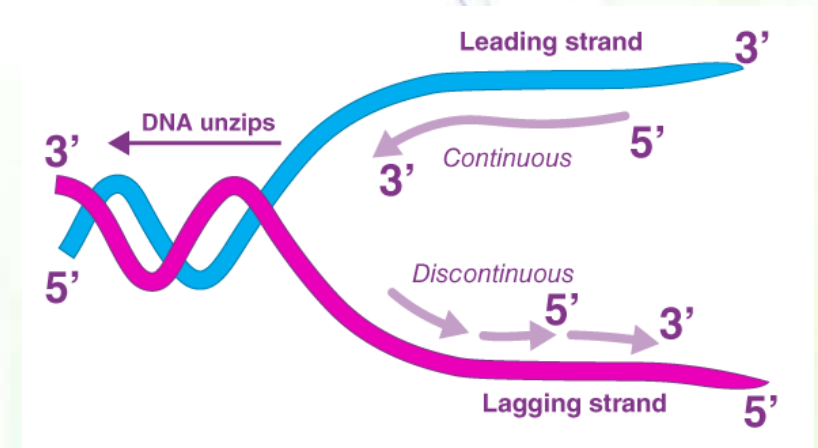
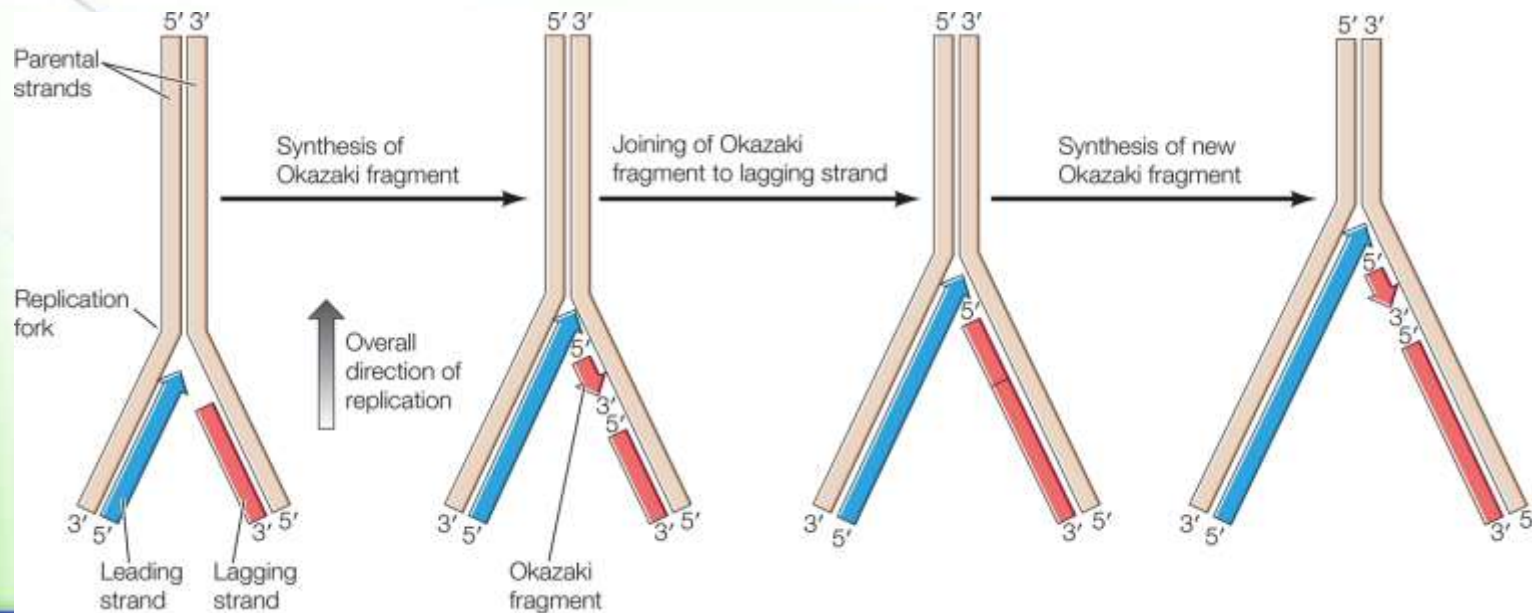
DNA Synthesis



Continuity of DNA synthesis



- The parental DNA is called a template.
- The new DNA is synthesized ONLY from the 5'-end to the 3'-end.
- One strand of DNA is continuously synthesized and called the leading strand.
- The other strand is synthesized discontinuously as shorter pieces known as Okazaki fragments and is called the lagging strand.



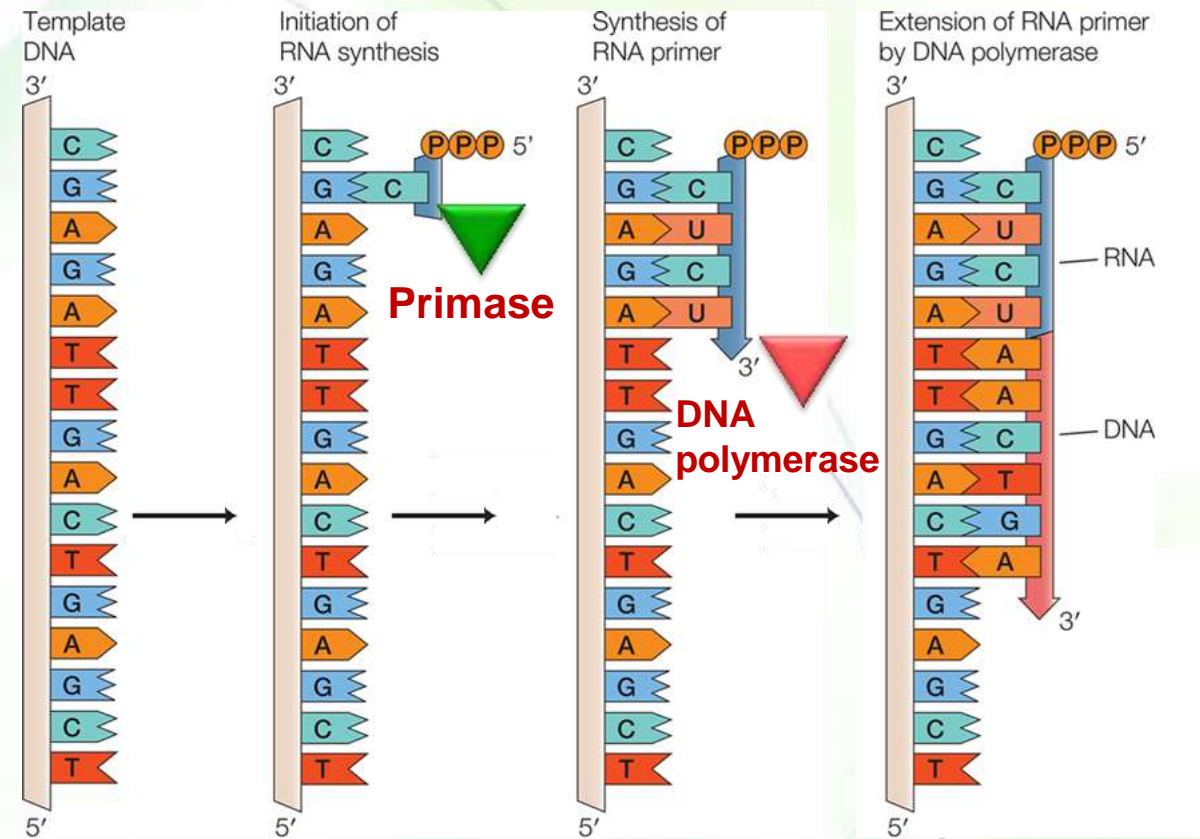


Components of DNA replication

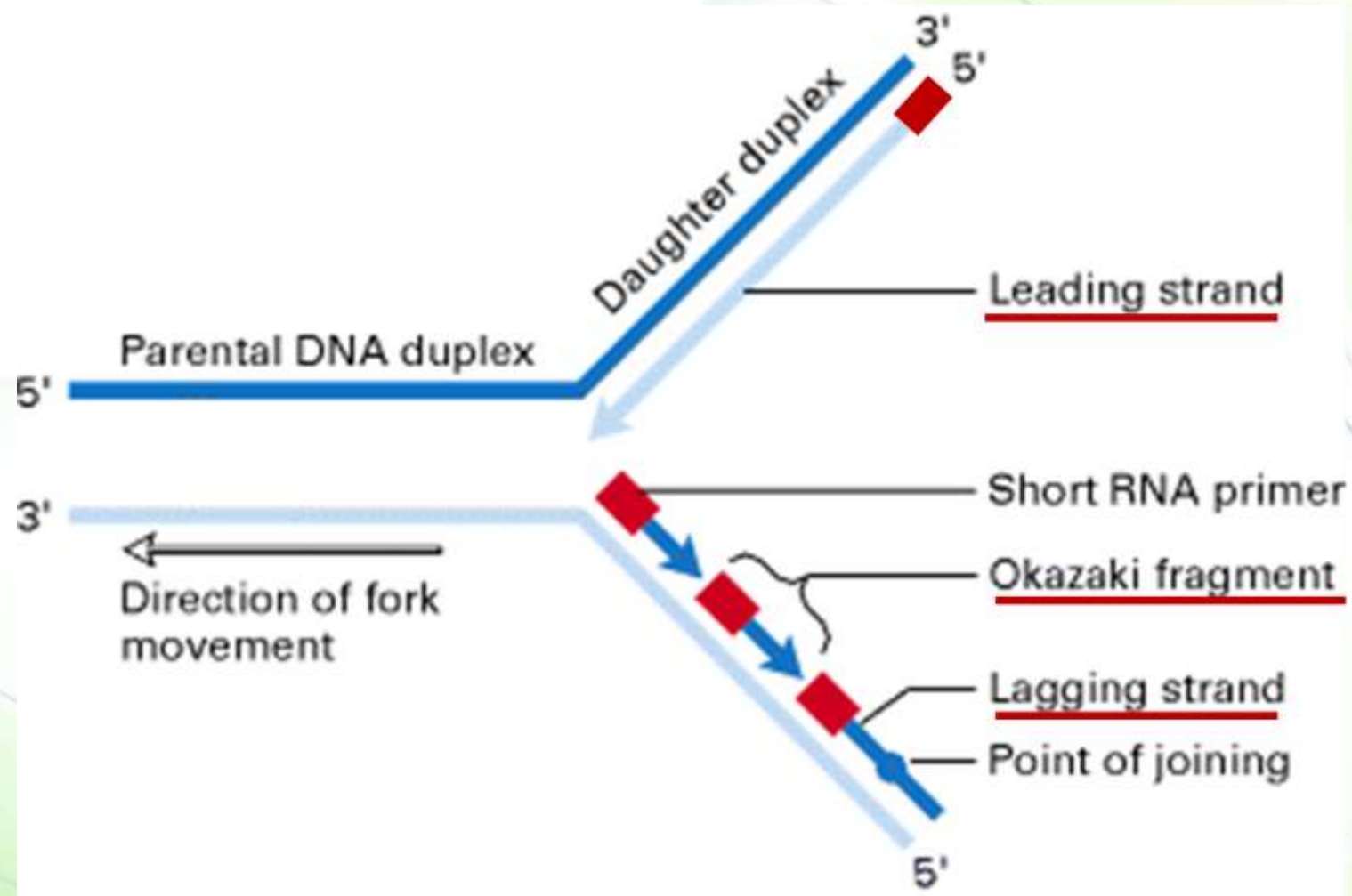
Primase and RNA primer



- DNA polymerases cannot initiate replication *de novo* (from scratch).
- They require a RNA primer (3-10 nucleotides long) that is complementary to the DNA template to be added first.
- It is synthesized by a primase.



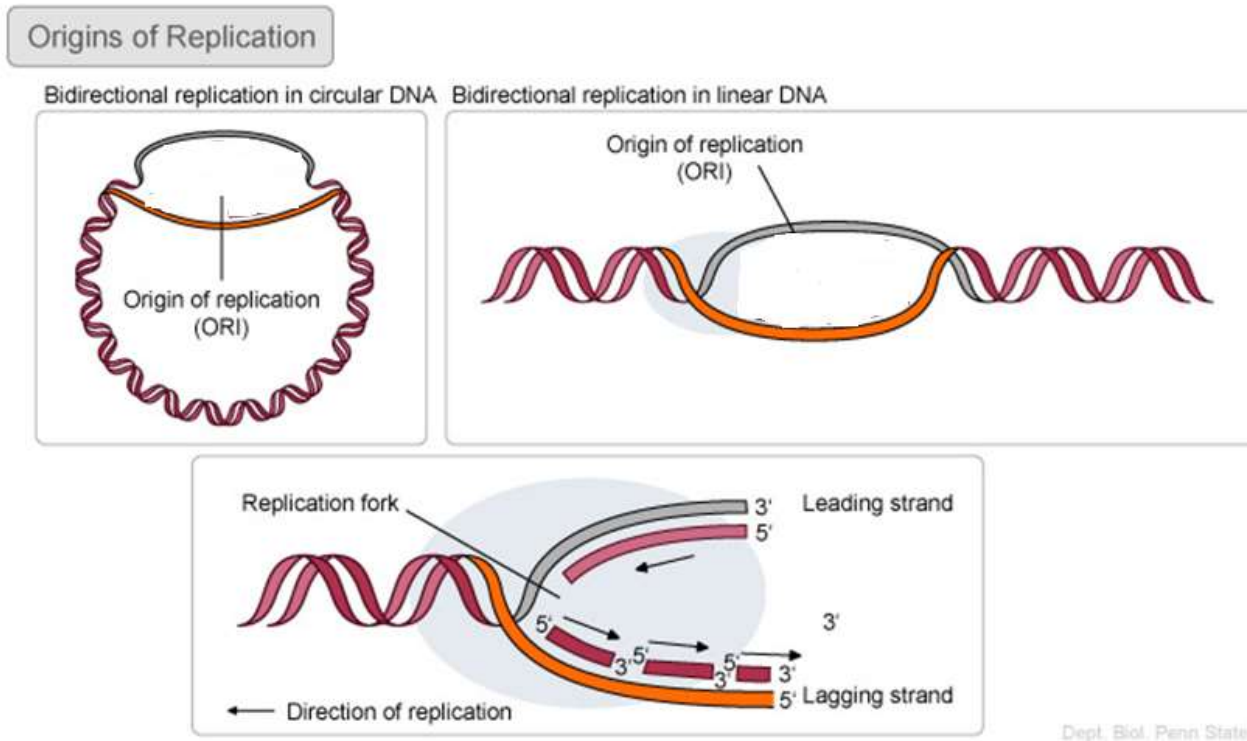
The need of primers



Exercise



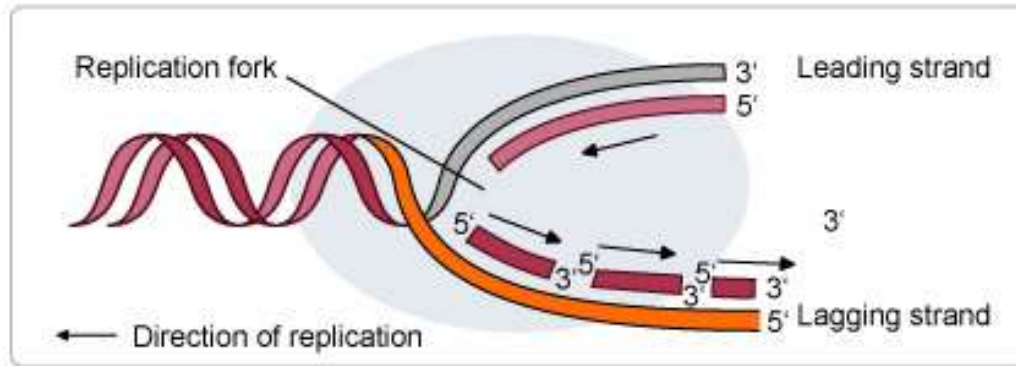
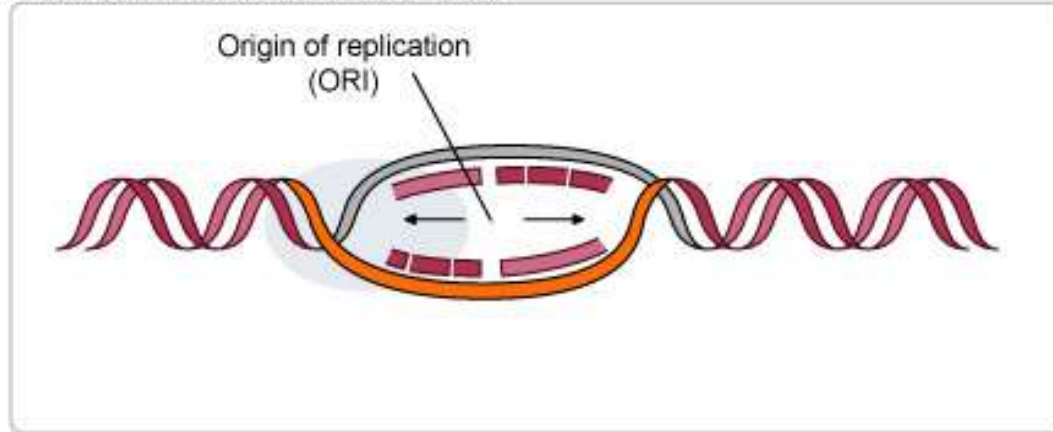
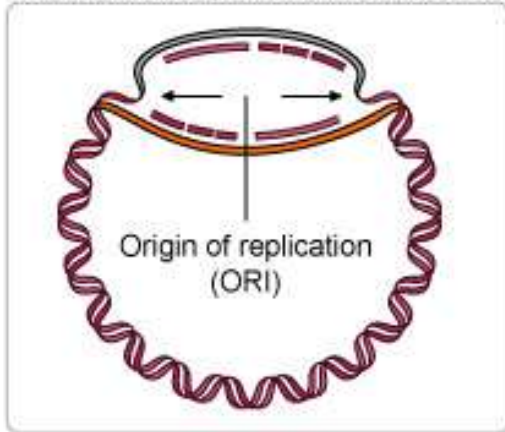
- I have shown you how DNA synthesis proceeds in the replication fork. Draw how DNA replication proceeds in the whole bubble.



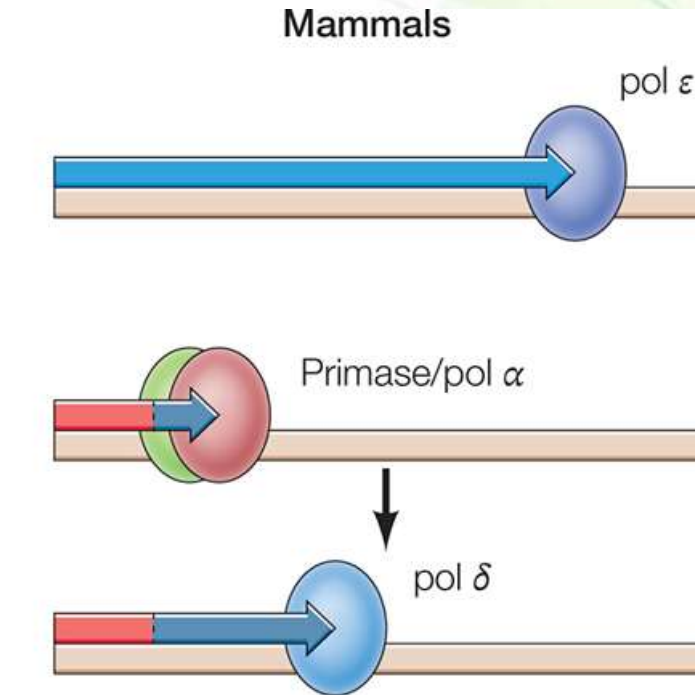
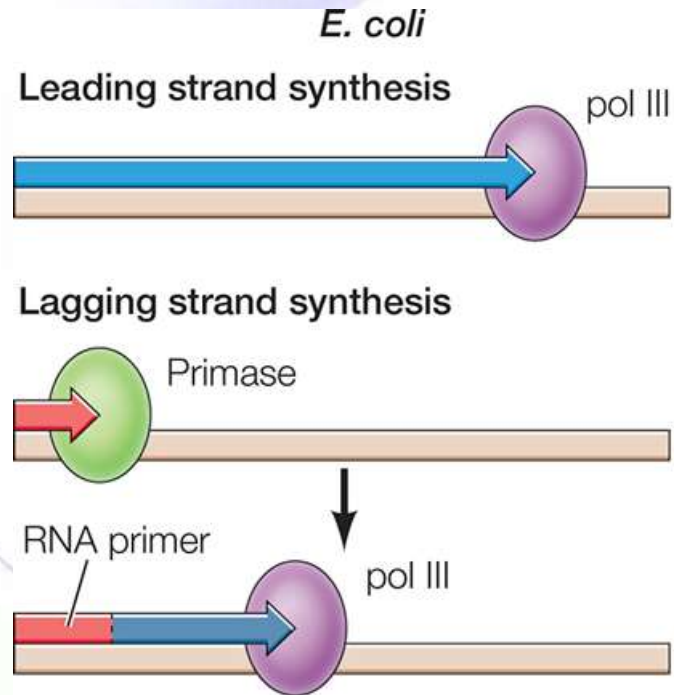


Origins of Replication

Bidirectional replication in circular DNA Bidirectional replication in linear DNA



The replicative process



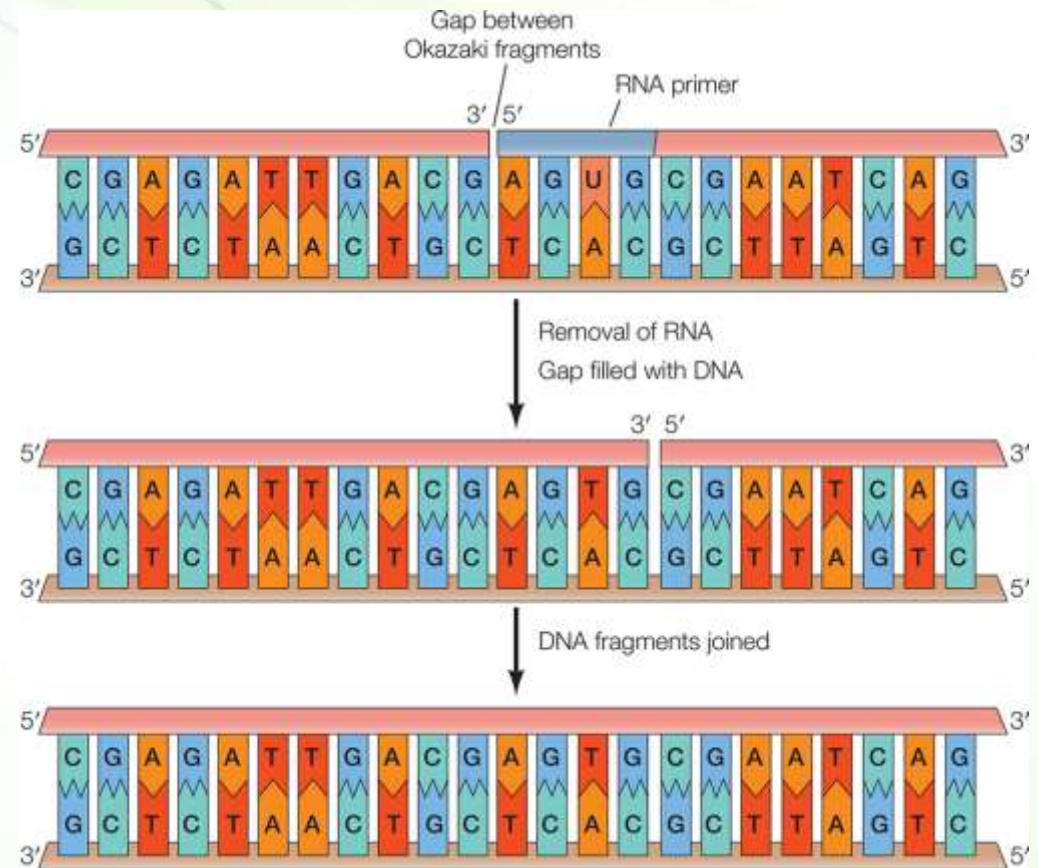
- In bacteria, DNA polymerase III is the major replicative enzyme

- In human cells:
- DNA polymerase α is complexed with primase initiating the synthesis of DNA, and then
- DNA polymerase ϵ synthesizes the leading strand.
- DNA polymerase δ synthesizes the lagging strand.

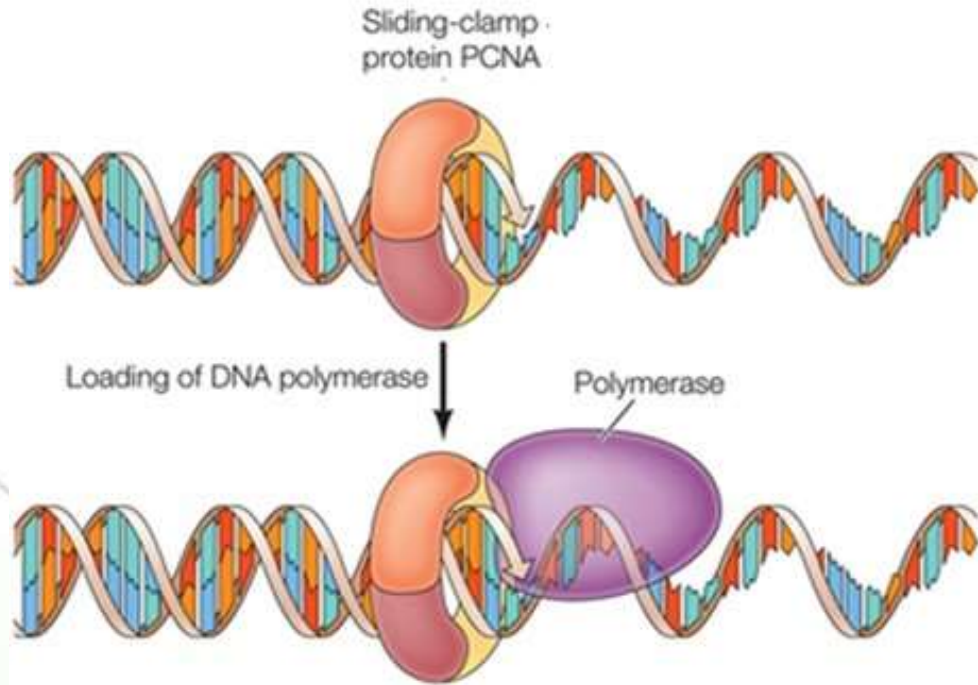
Removal of primers



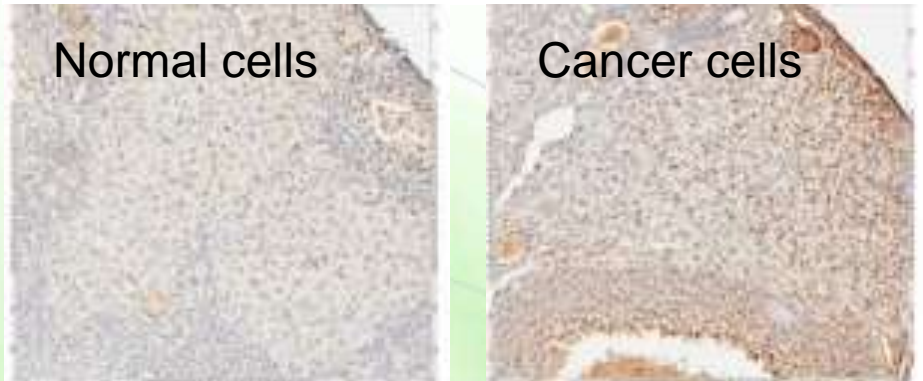
- In bacteria, RNA primers are removed by DNA polymerase I, which has two activities:
 - a 5' to 3' exonuclease activity hydrolyzing the primer in the 5' to 3' direction
 - A DNA polymerase activity where it fills in the gap.
- In human cells, 3 enzymes are involved:
 - RNase H, a 5' to 3' exonuclease that removes the primers.
 - polymerase δ that fills in the gaps
 - DNA ligase that joins the fragments.



Clamping and sliding



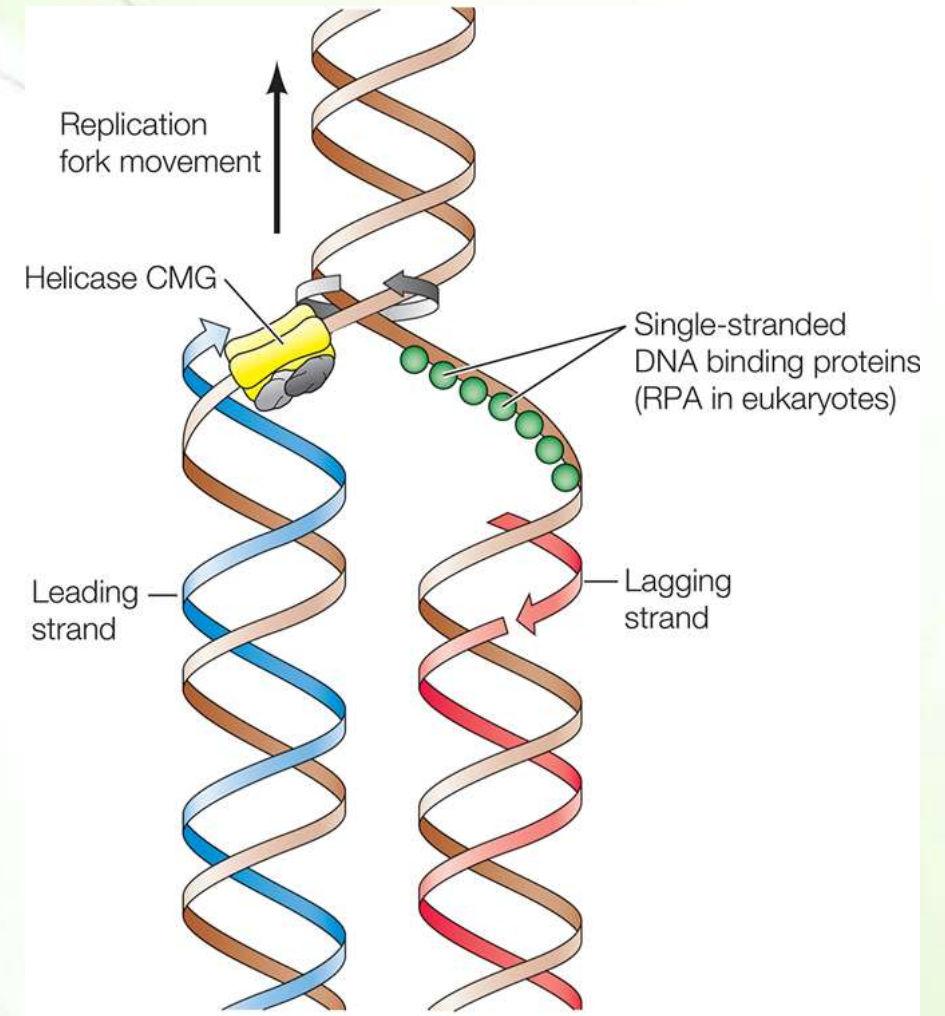
- The sliding-clamp protein, which is called proliferating cell nuclear antigen [PCNA] in human cells is associated with the major replicative polymerases loading them onto the primer and stabilizing their association with the DNA template.
- Note: PCNA is a diagnostic marker of proliferating cancer cells.

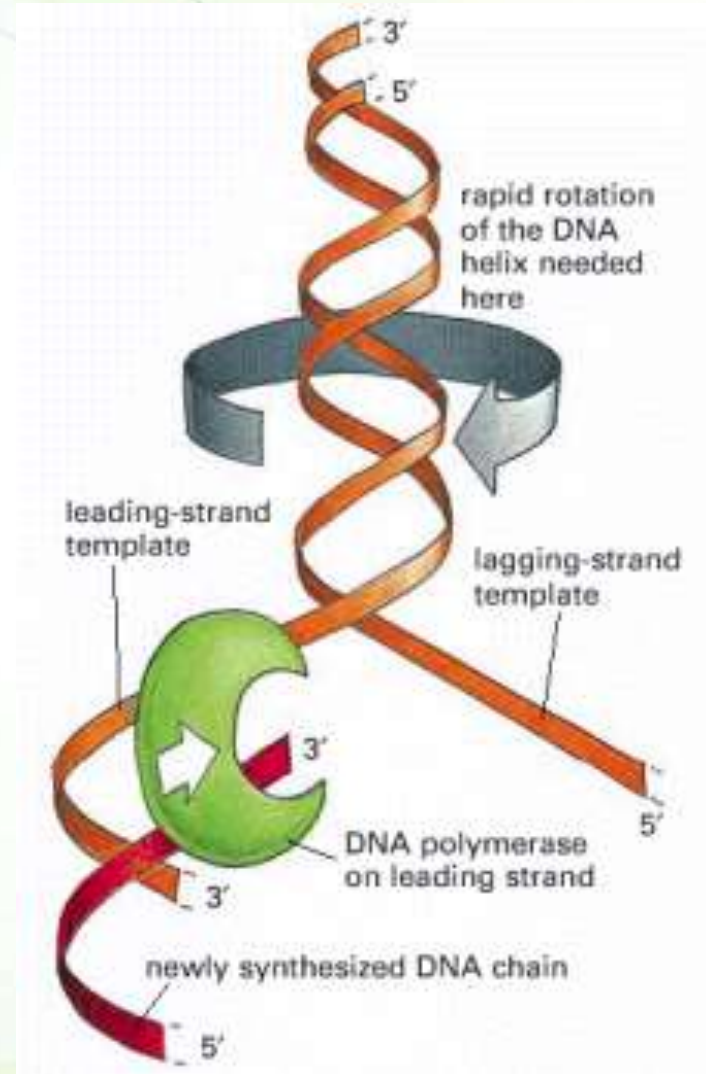
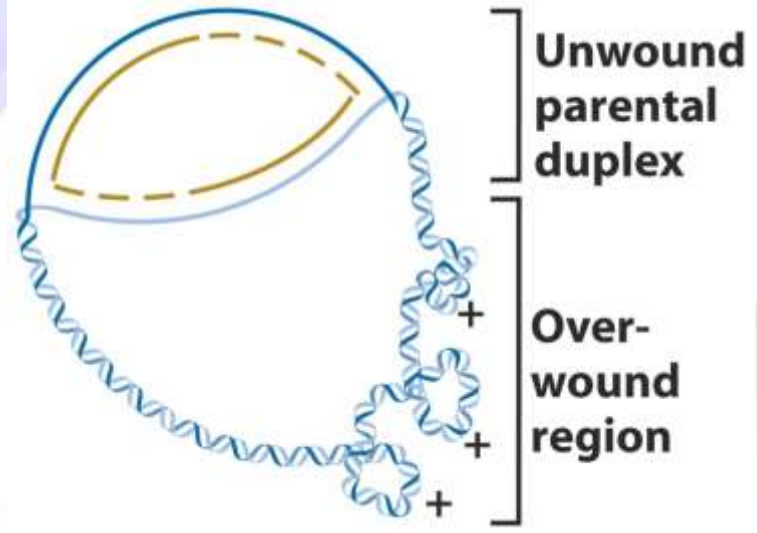


DNA helicases and SSB proteins



- The double-stranded DNA is opened up by DNA helicases.
- single-stranded DNA-binding proteins called replication protein A (RPA) do these:
 - Prevent the formation of short hairpin structures,
 - Protect single-stranded DNA from being degraded, and
 - Prevent the renaturation of DNA.

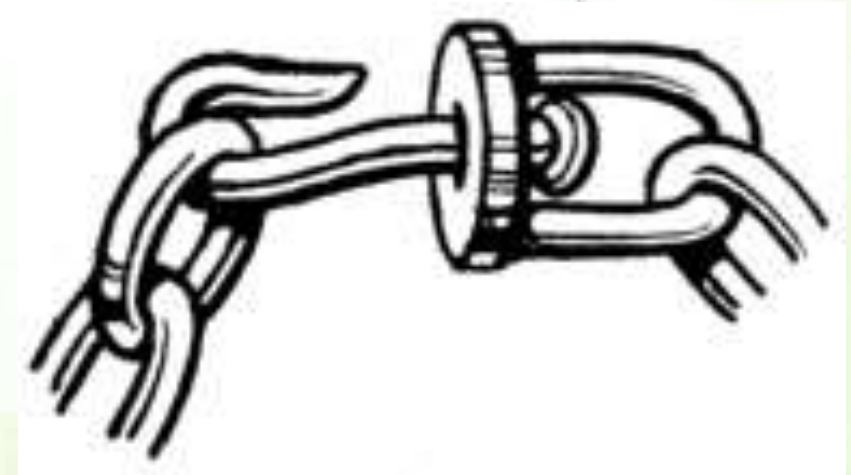
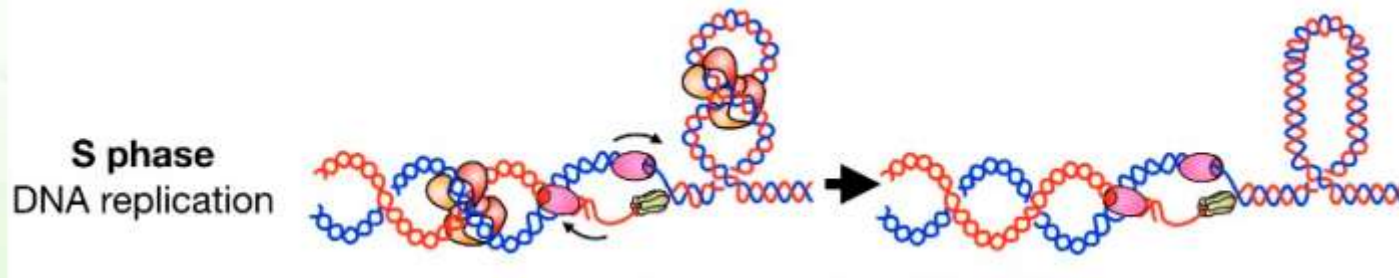
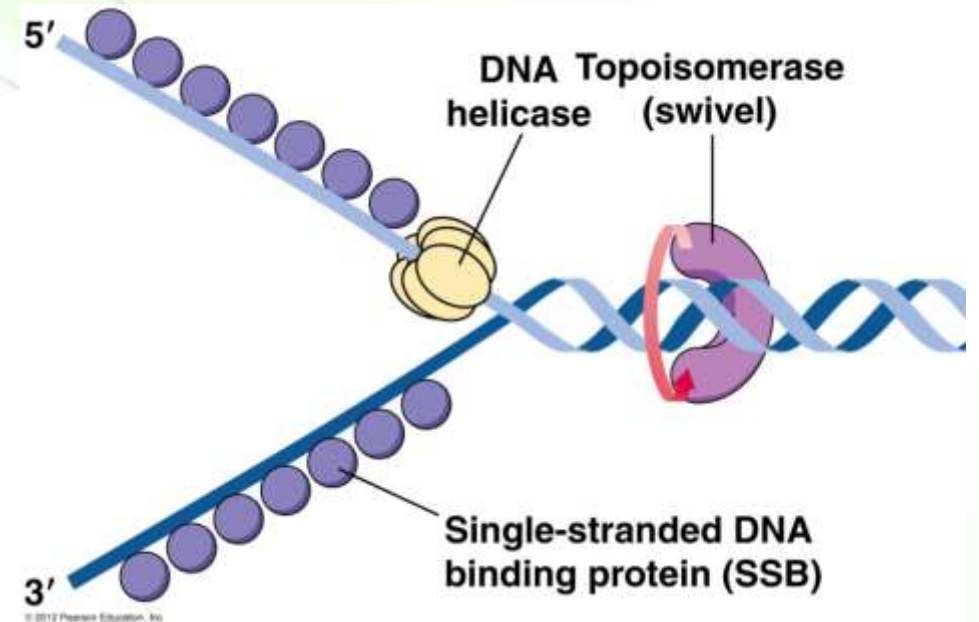




DNA topoisomerases



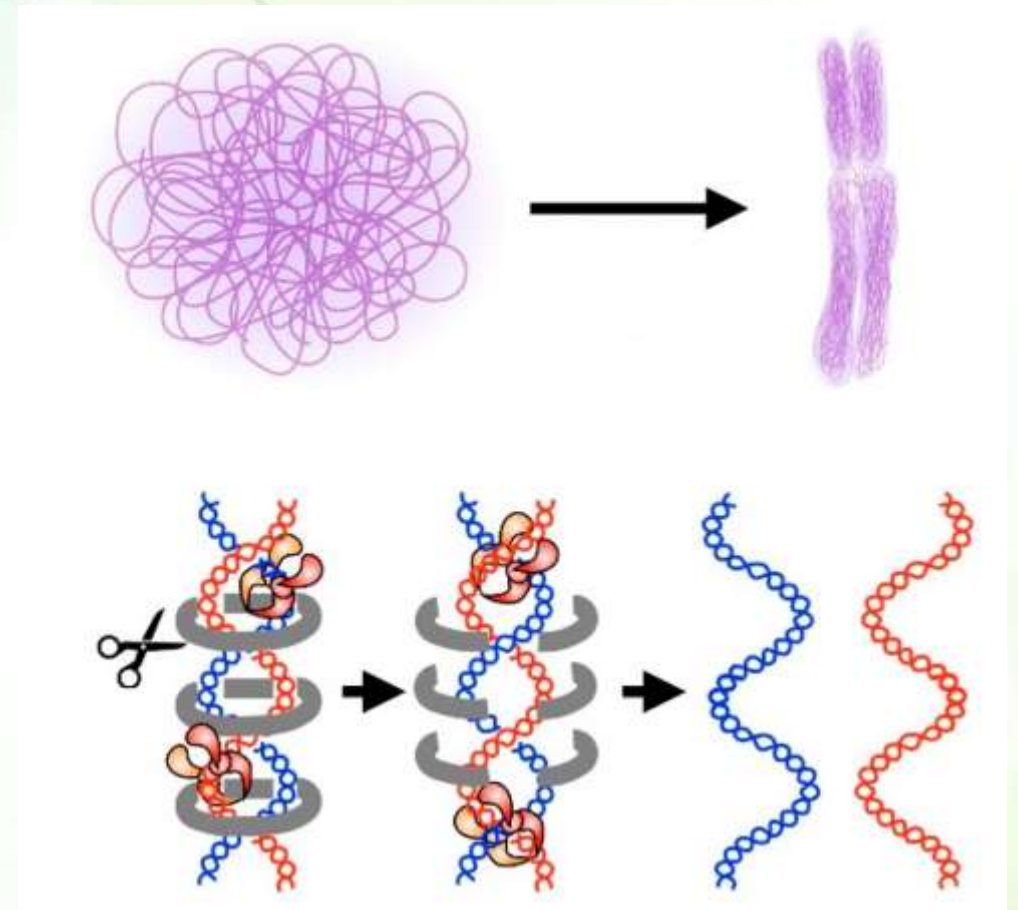
- A swivel is formed in the DNA helix by DNA topoisomerases.
- A DNA topoisomerase breaks then re-forms phosphodiester bonds in a DNA strand.
- Type I topoisomerases break just one strand of DNA
- Type II topoisomerases introduce two breaks: one break on each strand.



Other functions of topoisomerase II

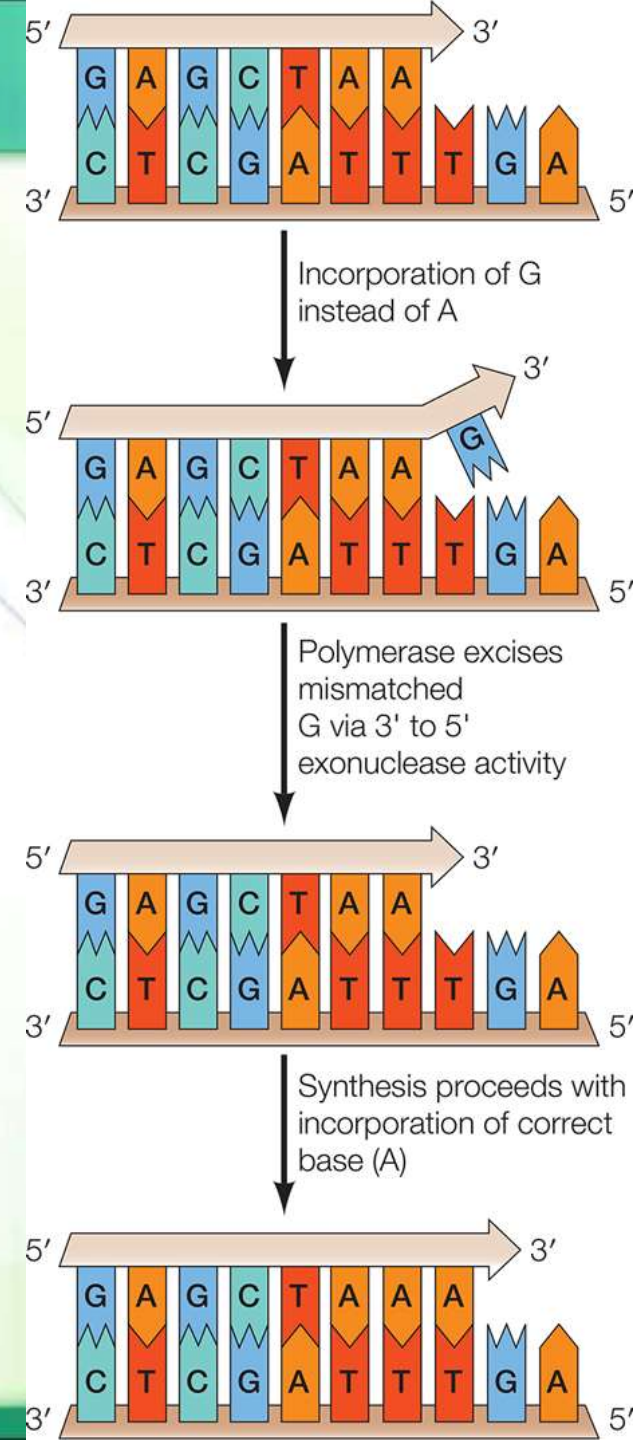


- Note: topoisomerase II is also required for
 - mitotic chromosome condensation
 - the separation of daughter chromatids at mitosis.
- Antineoplastic anti-topoisomerase II inhibitors include:
 - Anthracyclines
 - Doxorubicin
 - Mitoxantrone



How accurate is DNA replication?

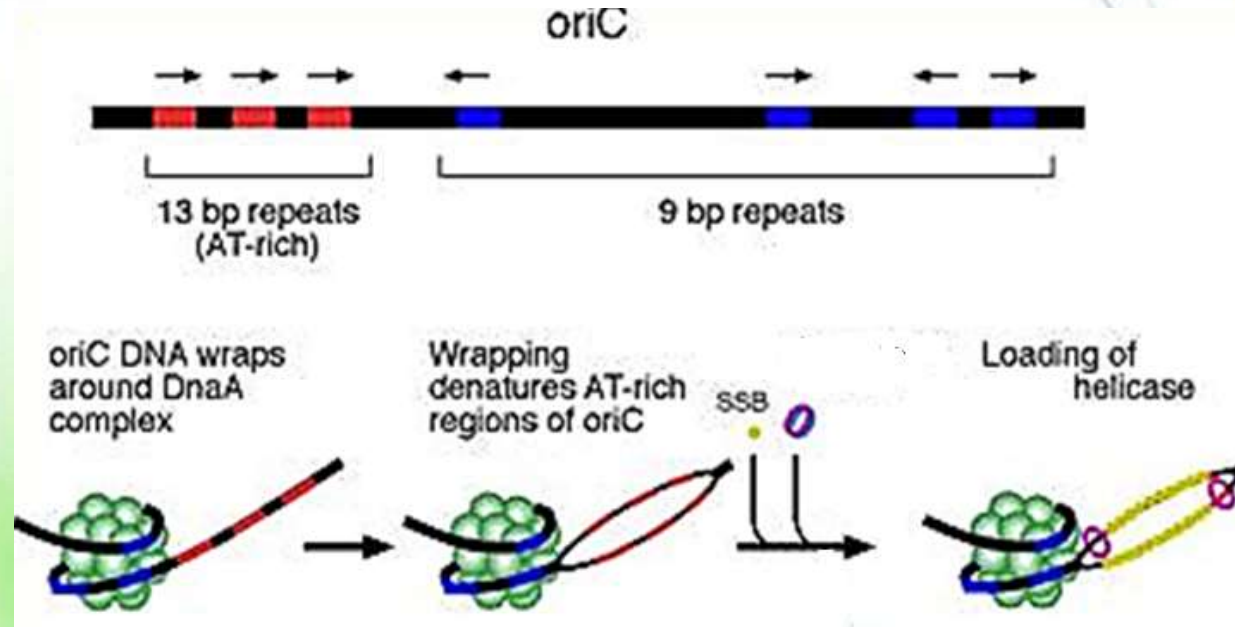
- The frequency of errors during replication is only one incorrect base per 10^9 nucleotides incorporated.
- How is accuracy high?
 - The DNA polymerase can catalyze the formation of the right phosphodiester bonds between the complementary bases with the proper hydrogen bonding (accuracy= 10^{-5}).
 - Proofreading mechanism (a $3' \rightarrow 5'$ exonuclease activity) increasing the accuracy to 10^{-8} .
 - Repair mechanisms (to be discussed later)



Origin of replication (OriC) in bacteria



- Bacterial replication starts at an origin of replication (OriC).
- oriC regions contain repetitive 9-bp and AT-rich 13-bp sequences (These are **consensus sequences**).
 - 9-mer: binding sites for DnaA protein.
 - 13-mers: AT-rich region - it facilitates separation of the double-stranded DNA.
- DnaA protein binds to 9-mers, applies stress on the AT-rich region, and OriC opens up.
- The helicase and SSB proteins jump on, followed by the replication machinery.

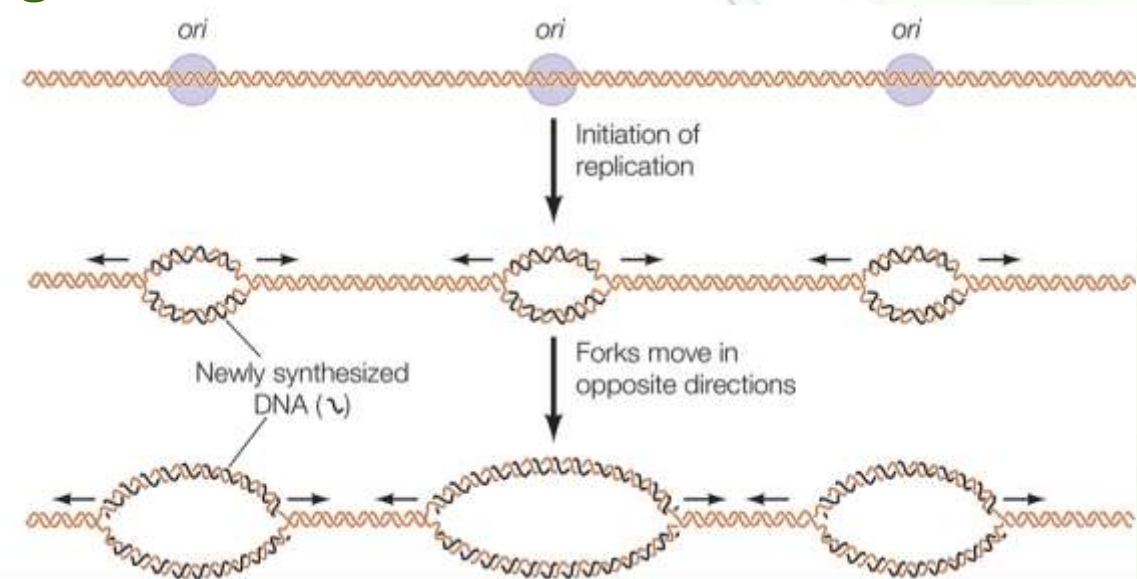
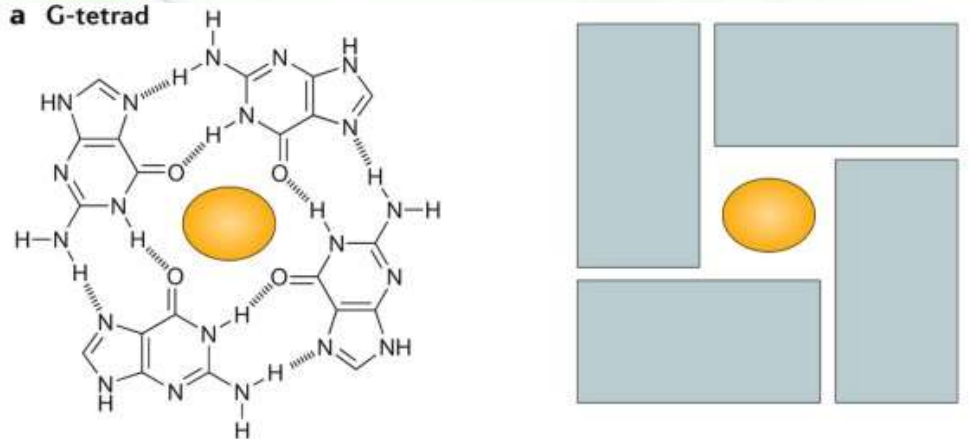


Origins of replication in the human genome



- The human genome has about 30,000 origins of replication with the following characteristics:
 - G-rich sequences that can form G-quadruplex secondary structures.
 - Modified histones that promote chromatin decondensation and activation of gene expression.
 - Close proximity to actively transcribed genes.
 - Cell-specific

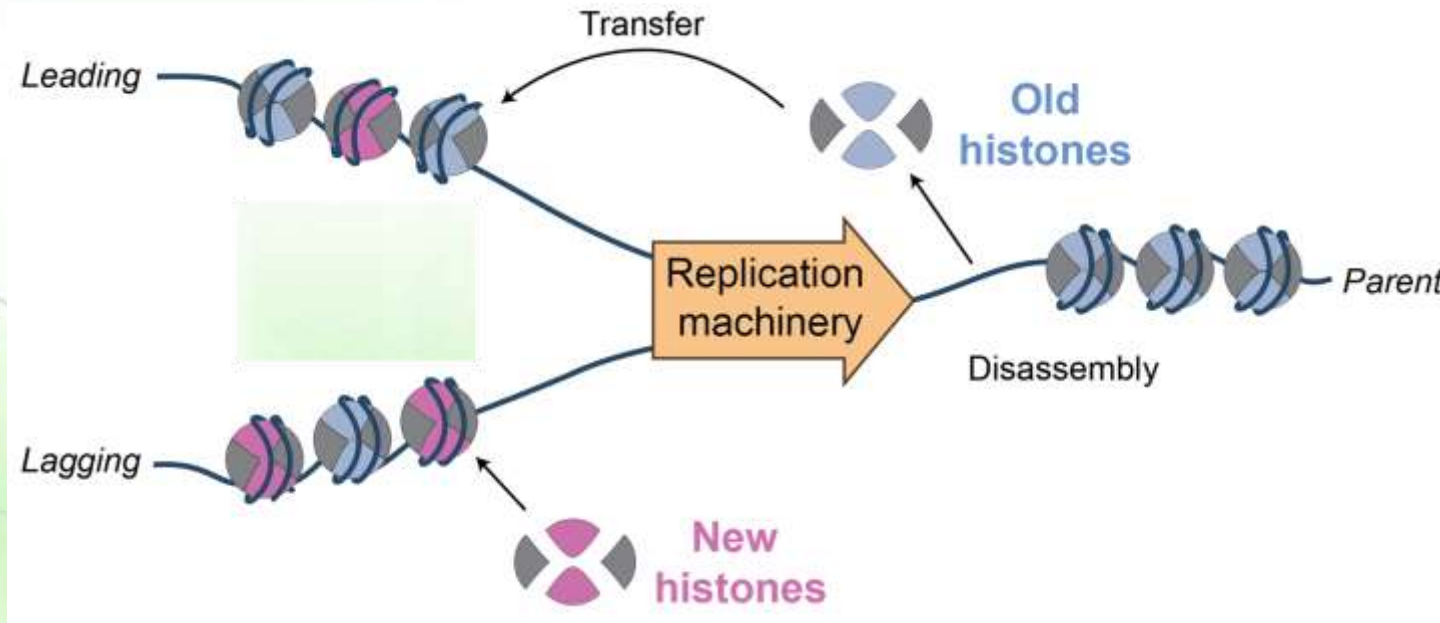
a G-tetrad



The formation of nucleosomes



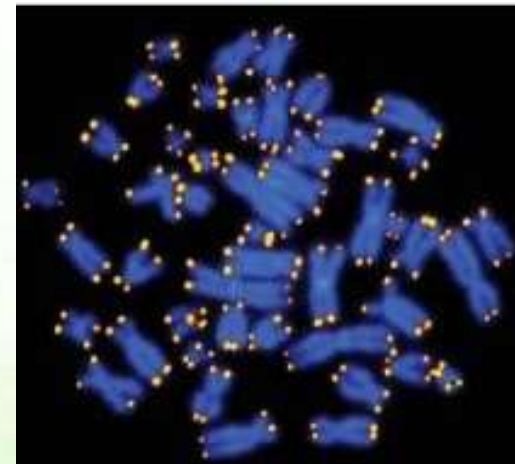
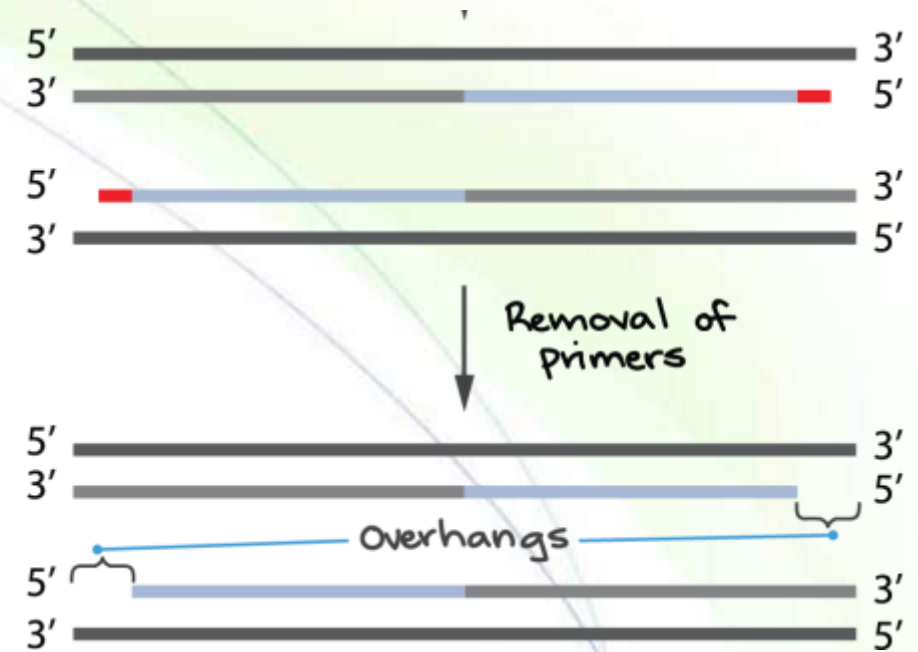
- In the human genome, there are 4 genetic clusters containing a total of 65 histone-coding genes.
- Nucleosomes are disassembled and reassembled during DNA replication by histone chaperones, which use recycled and newly synthesized histones.



A problem in the lagging strand

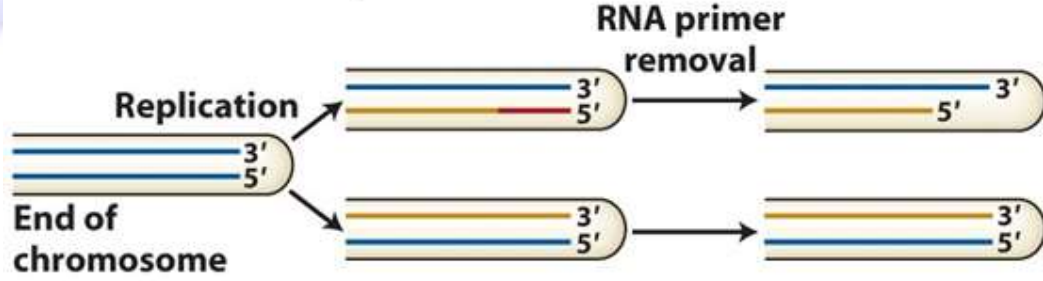


- As the growing fork approaches the end of a linear chromosome, the lagging strand is not completely replicated. Why?
- When the final RNA primer is removed, there is no place onto which the DNA polymerase can fill the resulting gap leading to the shortening of the lagging strand.

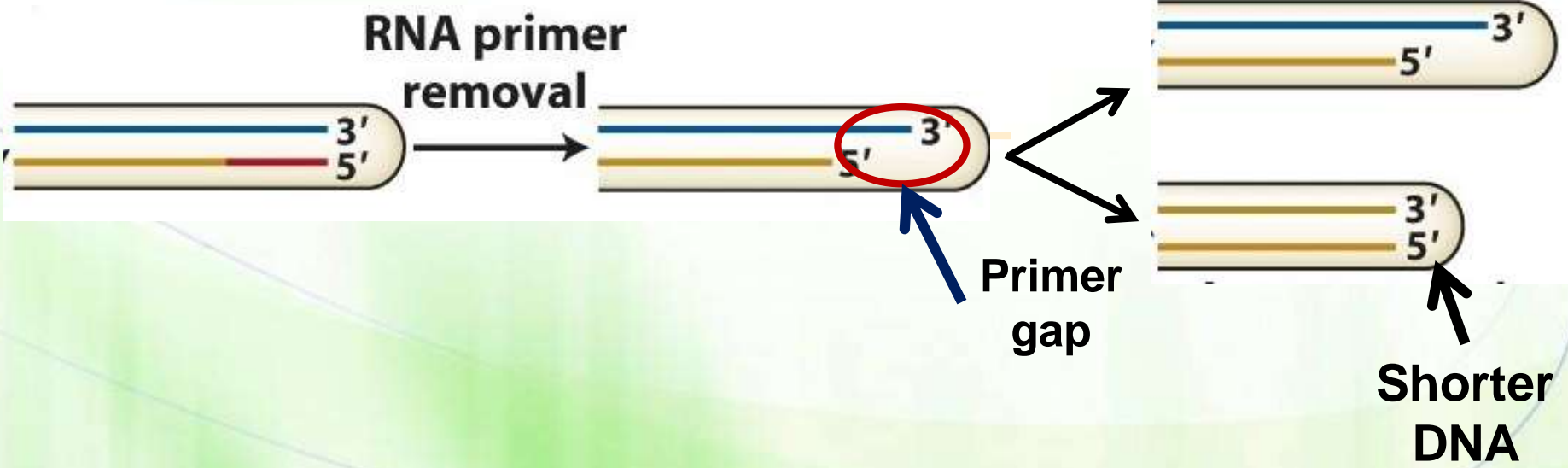
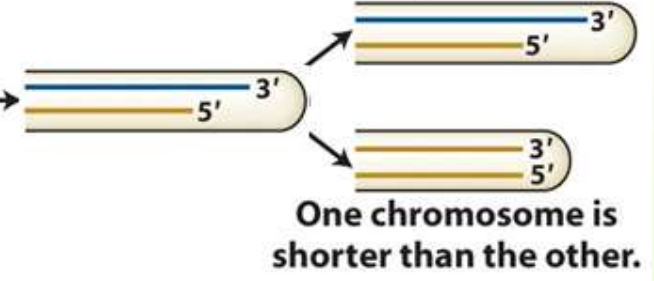




First round of replication



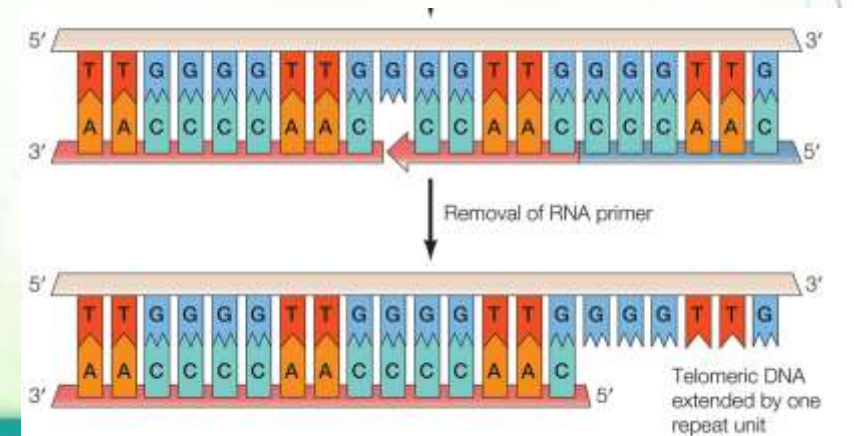
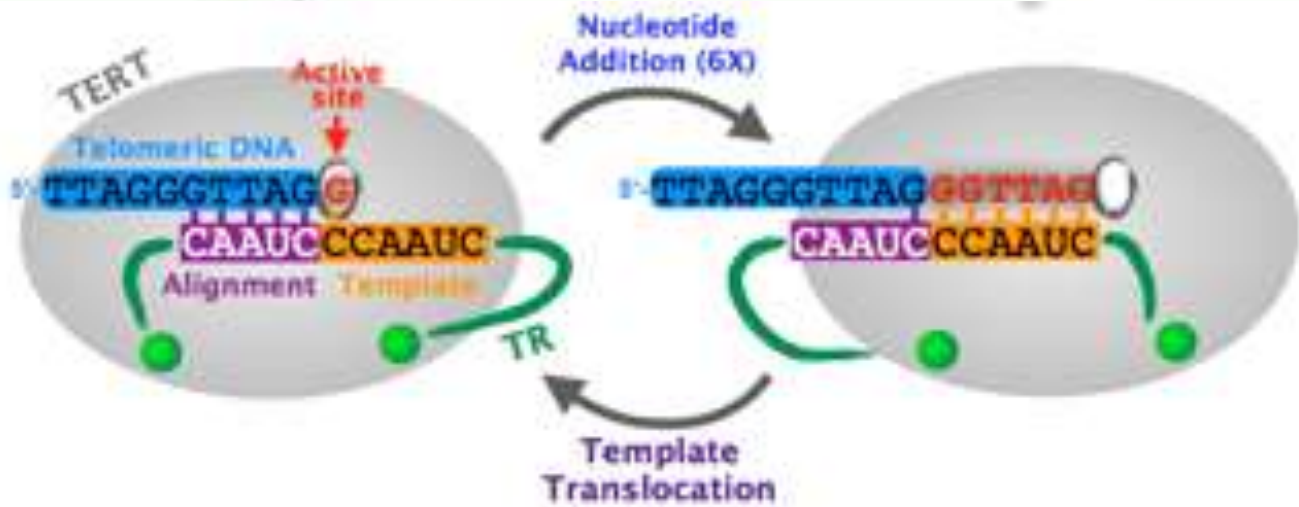
Second round of replication

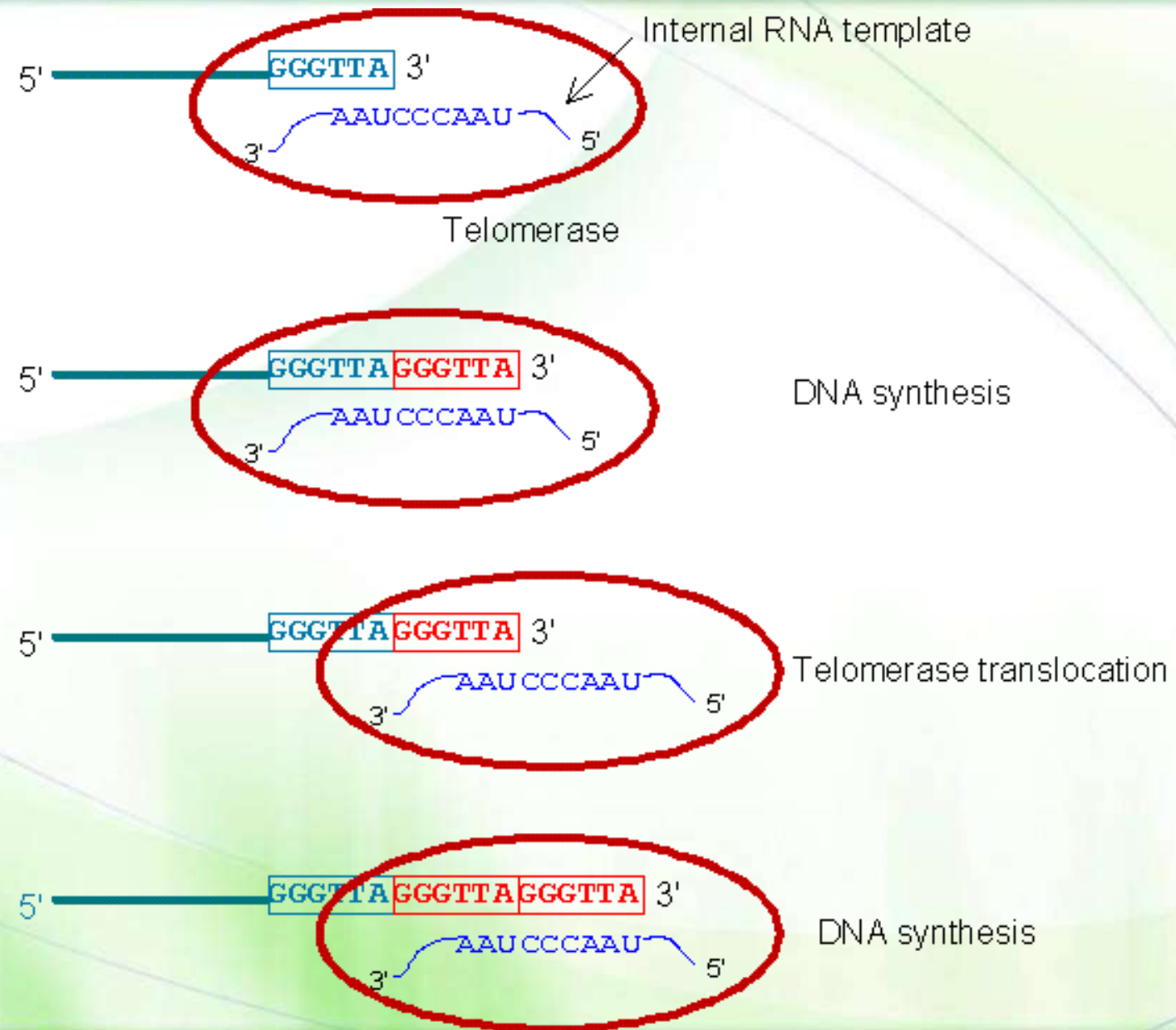


Telomerase comes to the rescue



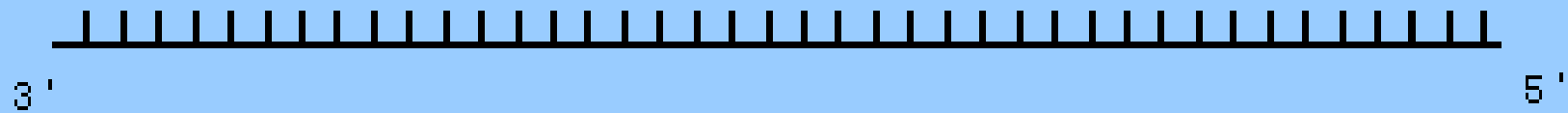
- Telomere DNA sequences consist of many GGGTTA repeats extending about 10,000 nucleotides.
- Telomerase (a reverse transcriptase) prevents the progressive shortening of the lagging strand. How?
- Telomerase elongates it in the 5'-to-3' direction using a RNA template that is a component of the enzyme itself.
- When the last primer is removed, a 3'-overhang is left.







Replication of the lagging strand of a linear chromosome encounters a problem at the 3' end

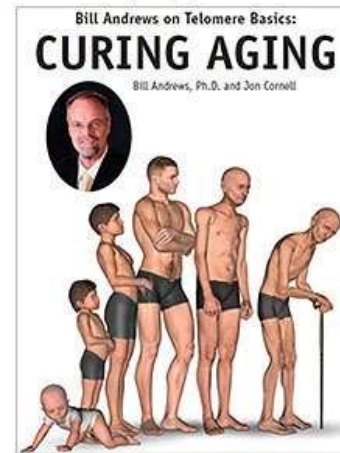
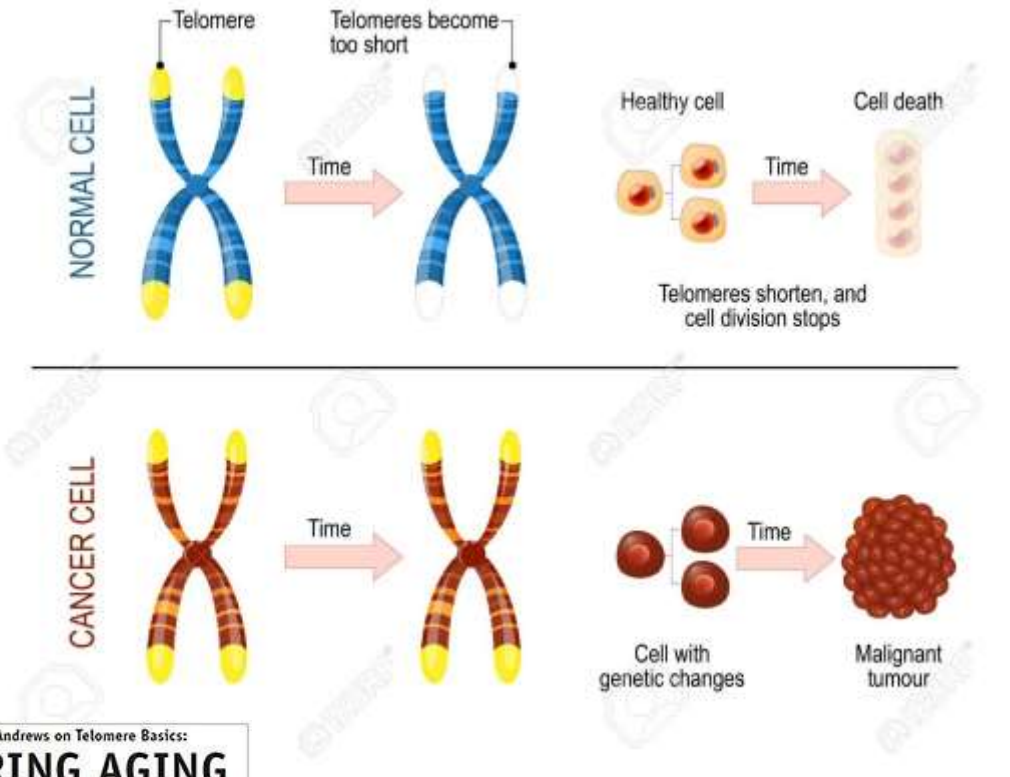


Note: Although this animation is good, there are wrong pieces of information within it. Find them.

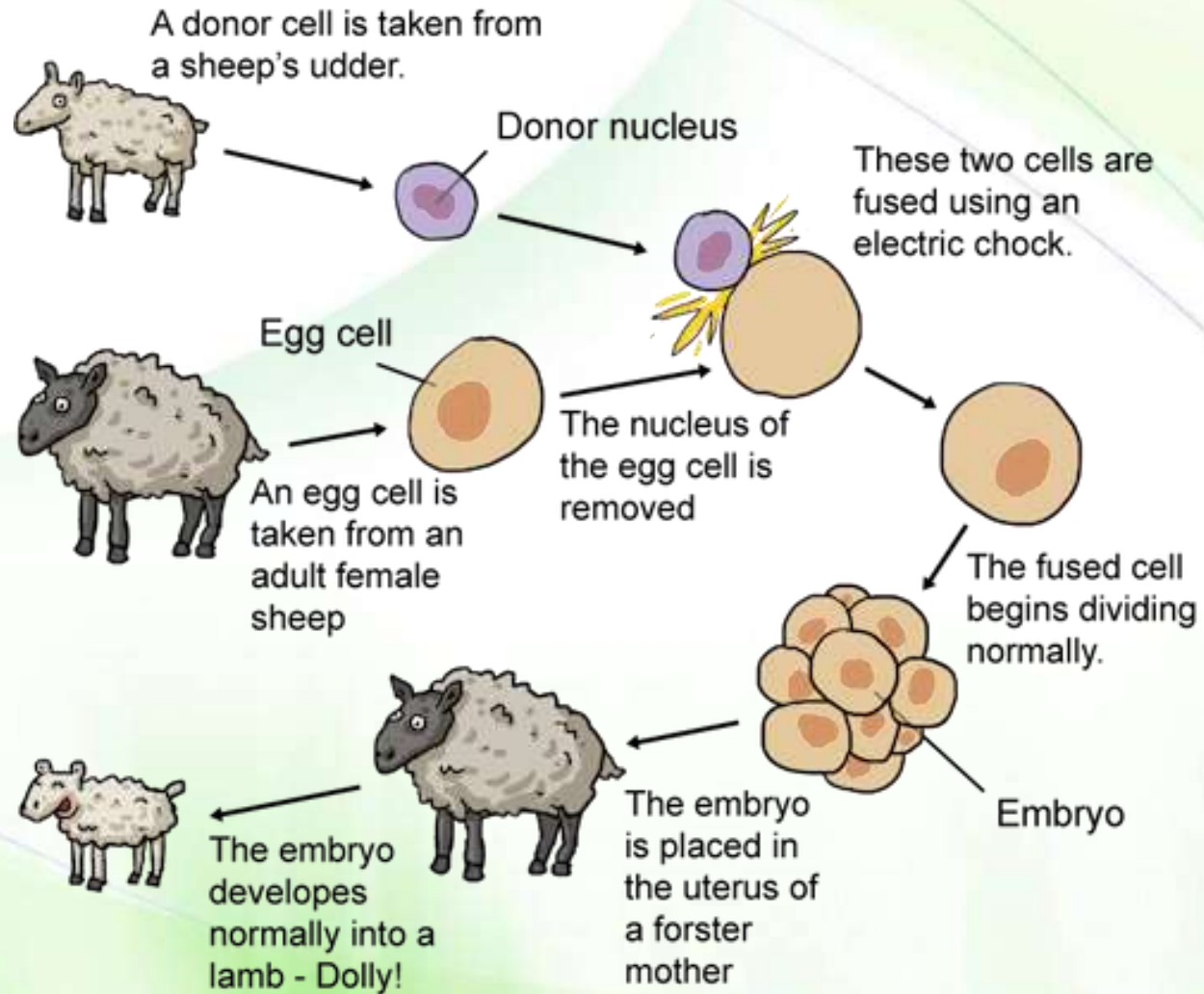
Facts of life about telomerases



- Most somatic cells do not have high levels of telomerase and, hence, have a finite number of cell divisions.
- As we grow older, the levels and activity of telomerase are reduced.
- The gradual shortening of the chromosome ends leads to senescence and cell death.
- Germline and cancer cells express high levels of telomerase.



Dolly, the sheep



Dolly lived for 6.5 years instead of the normal **11-12 years**.