Molecular Biology (3)

DNA replication

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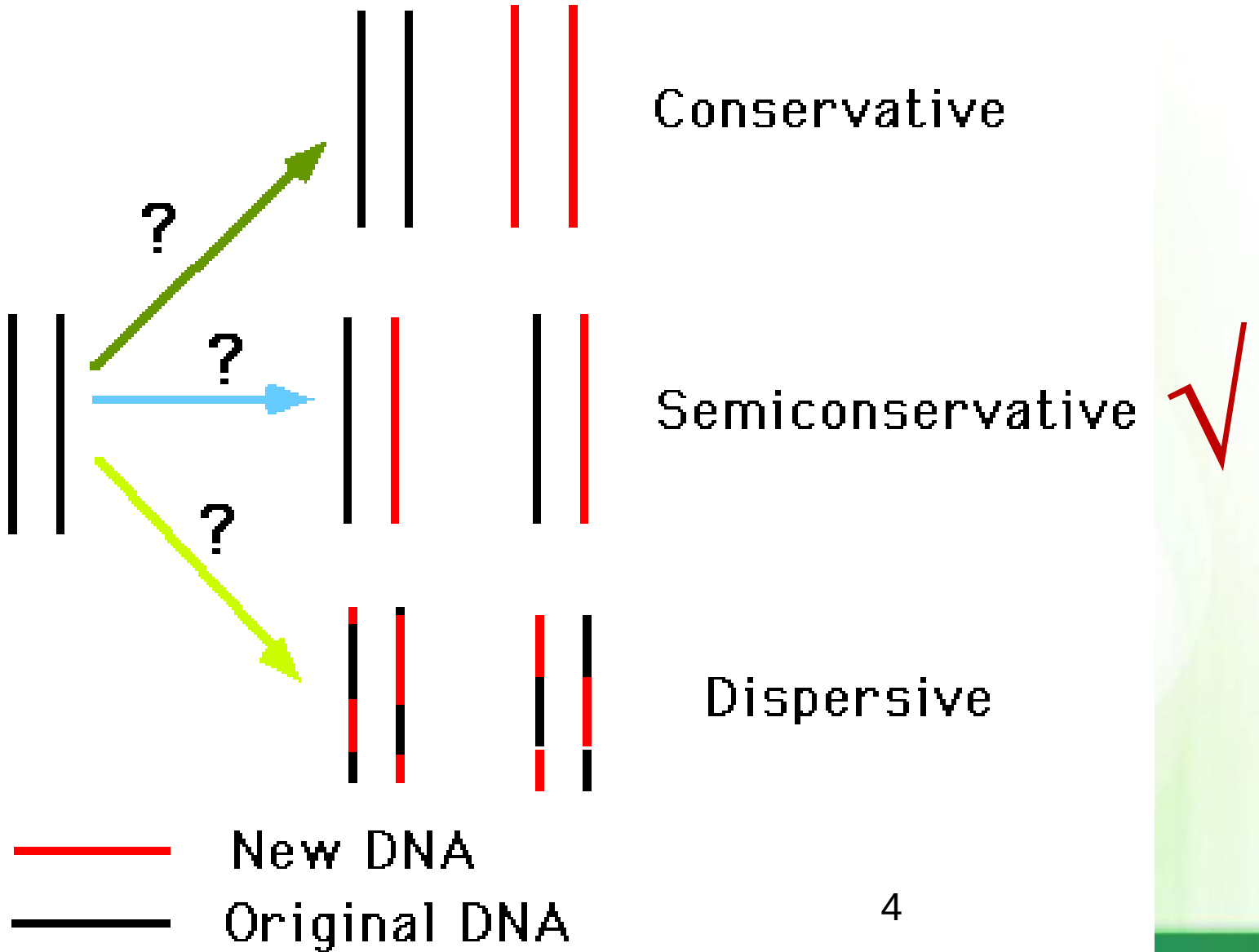
Resources

- This lecture
- Cooper, pp. 215-232

Some basic information

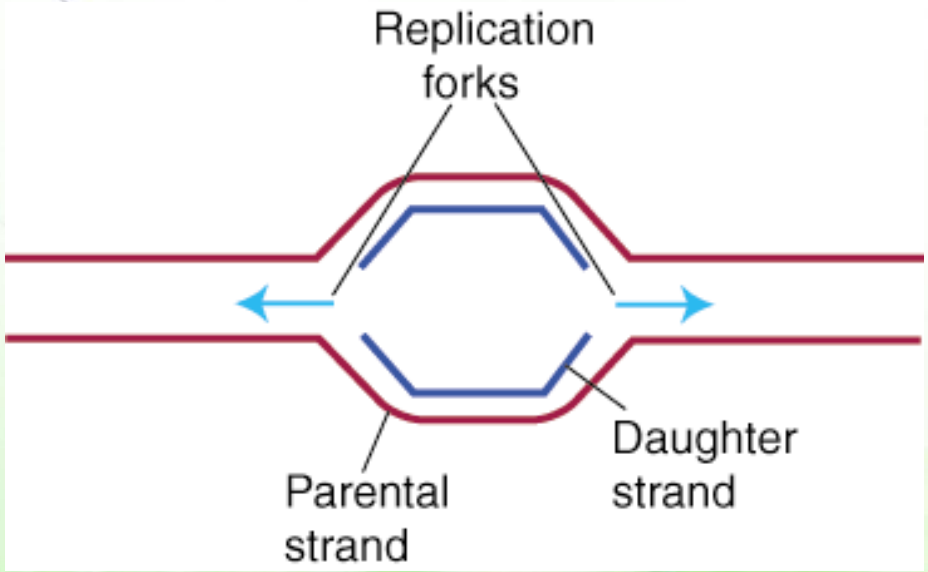
- The entire DNA content of the cell (or an organism) is known as genome.
- DNA is organized into chromosomes.
- Bacterial genome: usually one and circular chromosome.
- Eukaryotic genome: multiple, linear chromosomes complexed with proteins known as histones.
- DNA must be accurately copied (replicated), but variation is important.
- DNA synthesis is carried out by DNA polymerases.
- The substrates are deoxyribonucleotides.

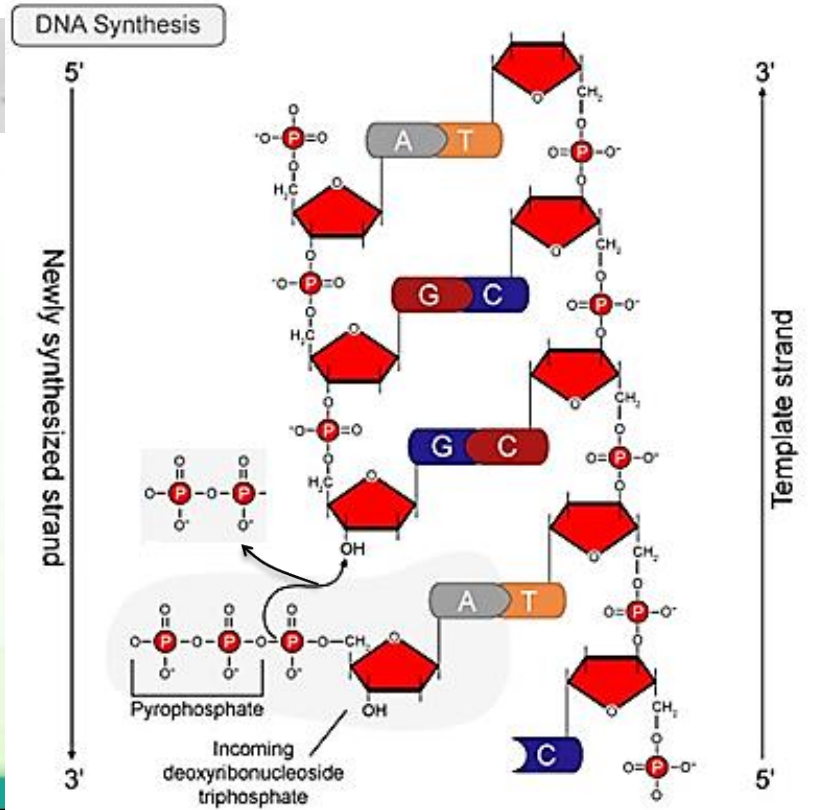
Different suggestions on possible mode of DNA replication



Bidirectionally…speaking

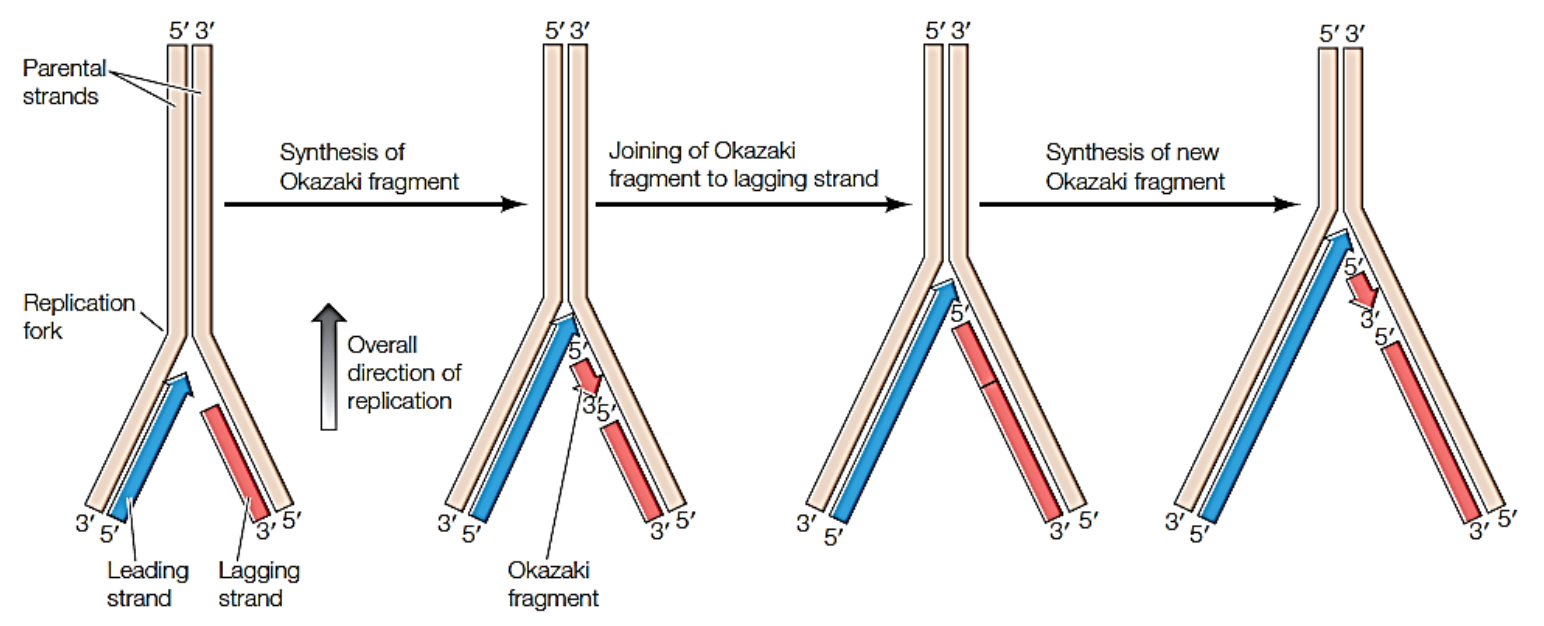
- Replication moves progressively along the parental DNA double helix bidirectionally.
- Because of its Y-shaped structure, this active region is called a replication fork.





Continuity of DNA synthesis

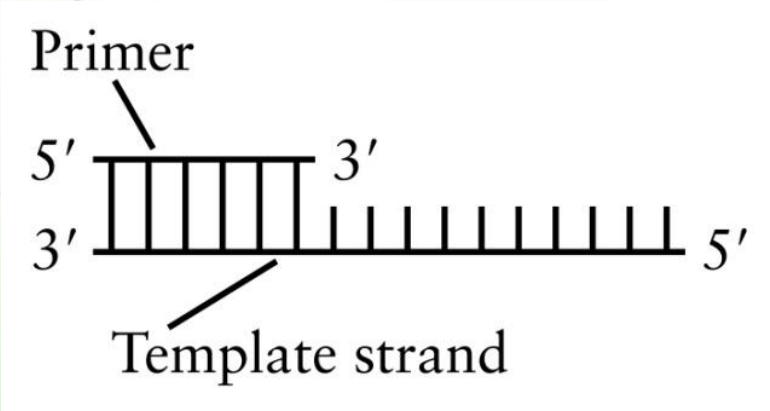
- DNA synthesis proceeds ONLY from the 5’-end to the 3’-end.
- A long strand of DNA (continuous) forms the leading strand and shorter pieces known as Okazaki fragments (discontinuous) form the lagging strand; both are present at the growing replication fork.

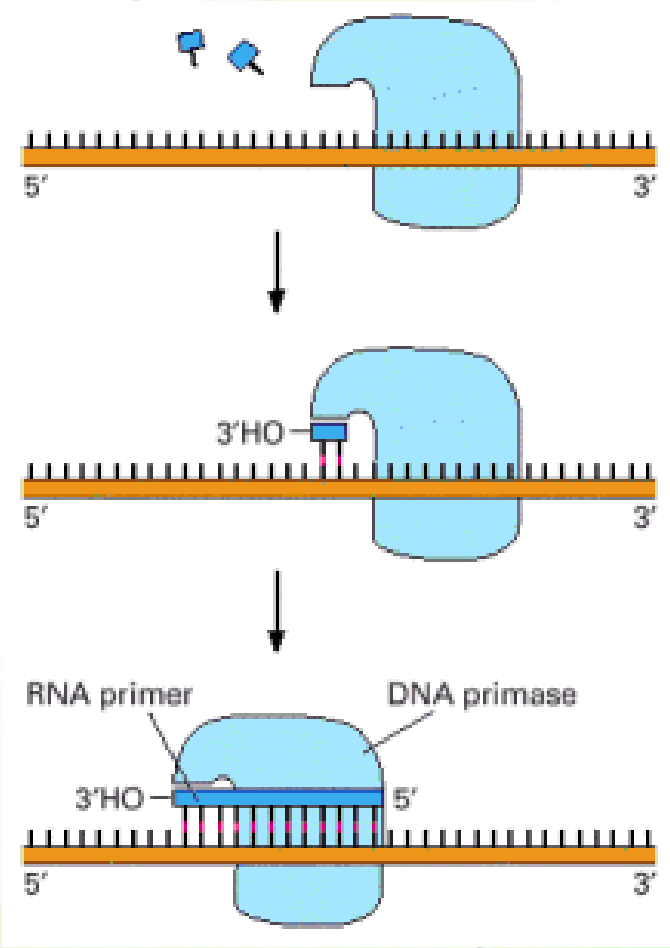


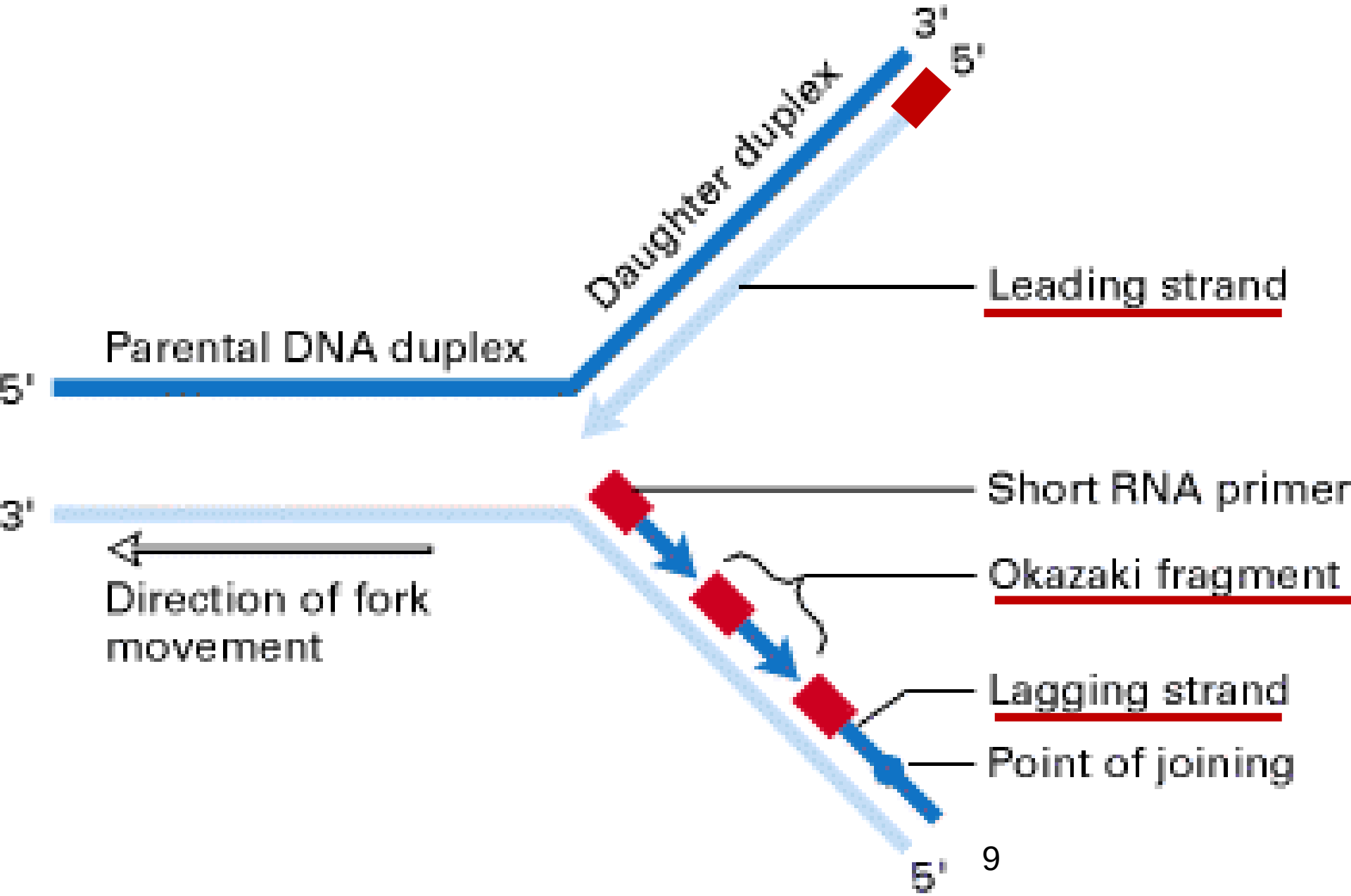
Components of DNA replication

RNA primer

- DNA polymerases cannot initiate replication *de novo*. So, 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.

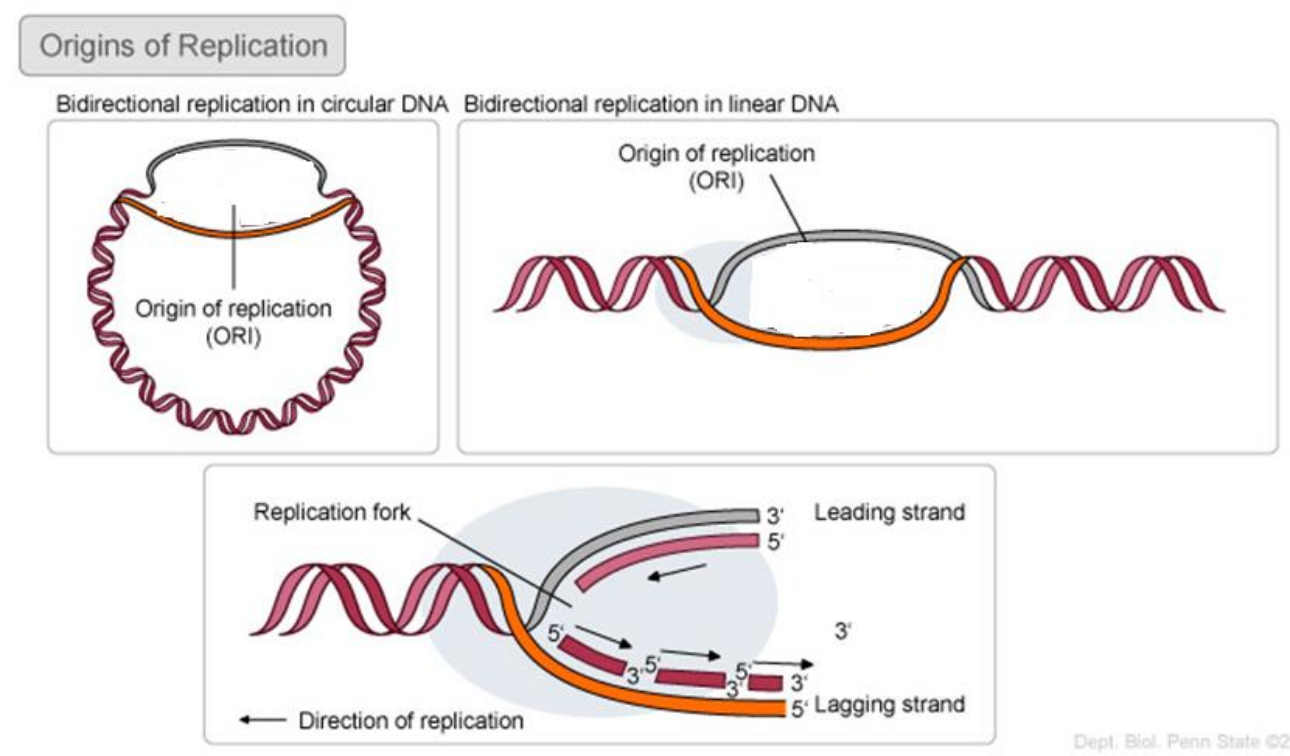





3'
5'
Daughter duplex
Leading strand
Parental DNA duplex
5'
3'
Short RNA primer
Okazaki fragment
Direction of fork
movement
Lagging strand
Point of joining
5'

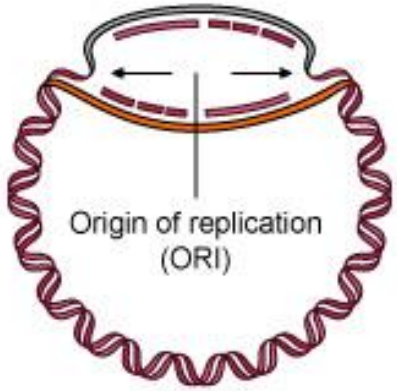
Exercise

- I have shown you how DNA synthesis proceeds in one half of the replication bubble, that is the DNA replication fork, draw how DNA replication proceeds in the whole bubble.

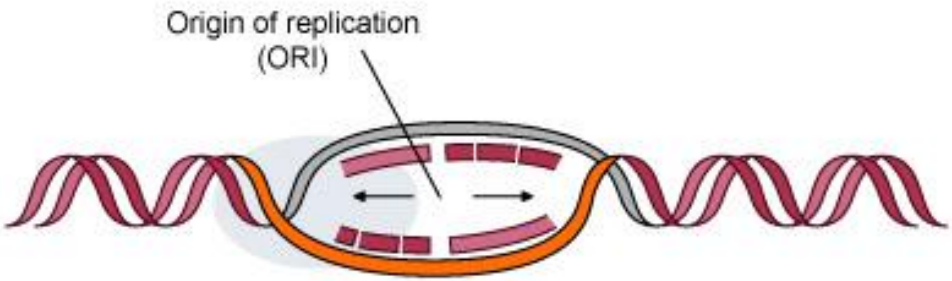


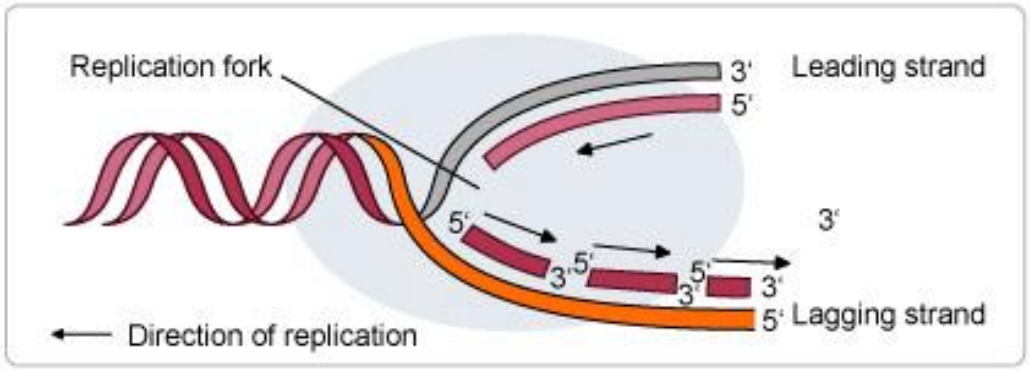
Origins of Replication

Bidirectional replication in circular DNA



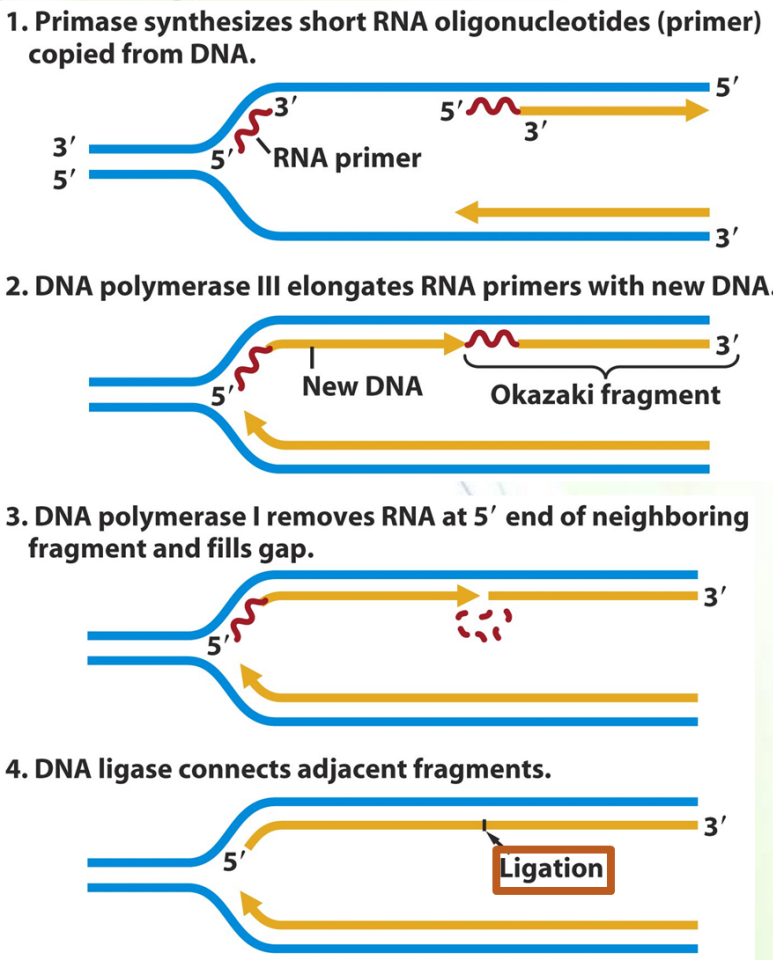
Bidirectional replication in linear DNA





The process in summary

- Primers are added by a primase.
- DNA polymerase synthesizes the DNA
- Primers are removed by a 5’-3’ exonuclease activity.
  - By a DNA polymerase in prokaryotes
  - By RNase H in eukaryotes
- DNA fragments are connected by a DNA ligase.
- Gaps are filled by DNA polymerase.



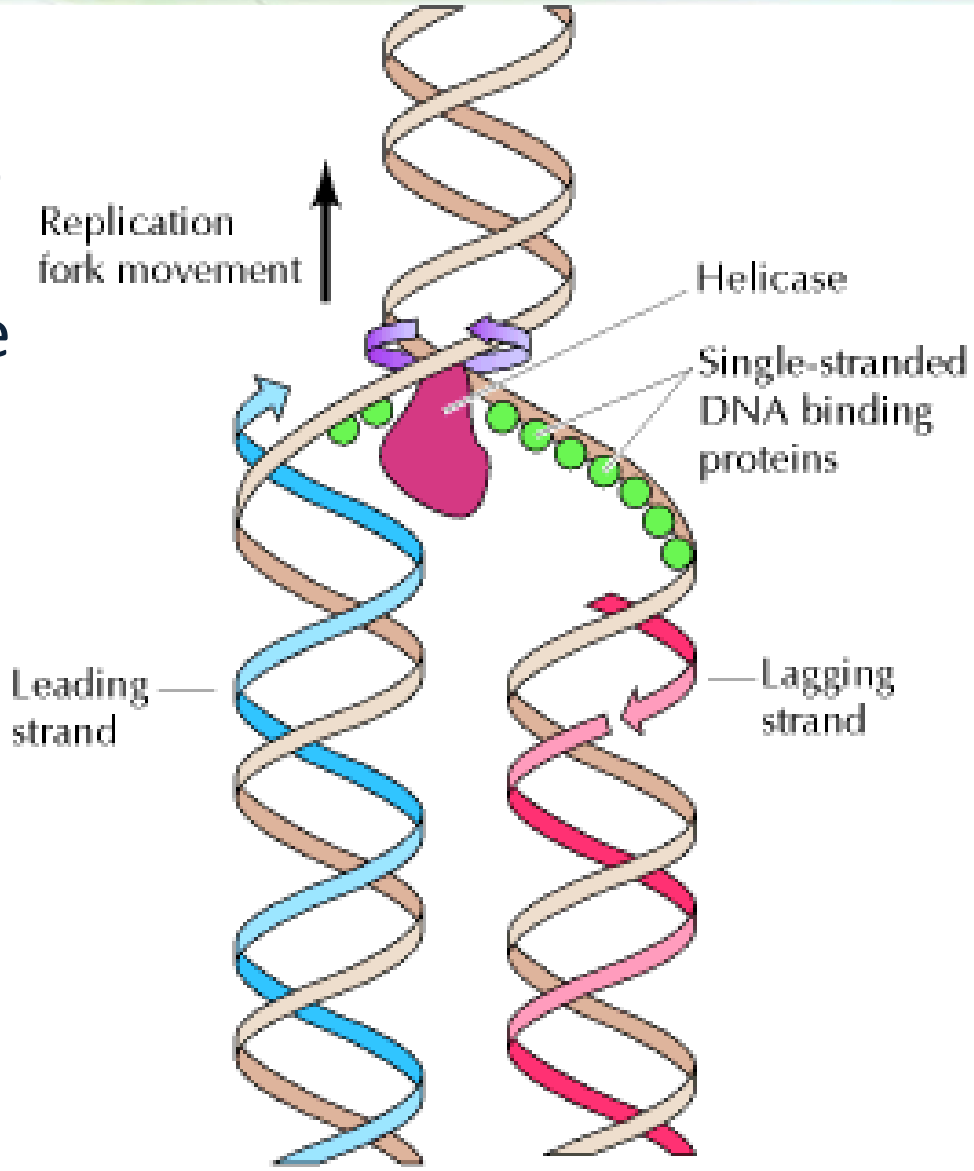
DNA helicases and SSB proteins

- For DNA synthesis to proceed, the DNA double helix must be opened up ahead of the replication fork.

- Opening up the DNA is done by two types of protein that contribute to this process
  - DNA helicases
  - single-strand DNA-binding proteins called **replication protein A (RPA)**.

DNA helicases

- DNA helicases use ATP to open up the double helical DNA as they move along the strands.
- In bacteria, helicases form a complex with the primase called primosome.



Single-strand DNA-binding (SSB) proteins

- Single-strand DNA-binding (SSB) proteins bind tightly to exposed single-stranded DNA strands without covering the bases, which remain available for templating.

- These proteins:
  - prevent the formation of the short hairpin structures
  - protect single-stranded DNA from being degraded
  - aid helicases by stabilizing the unwound, single-stranded conformation

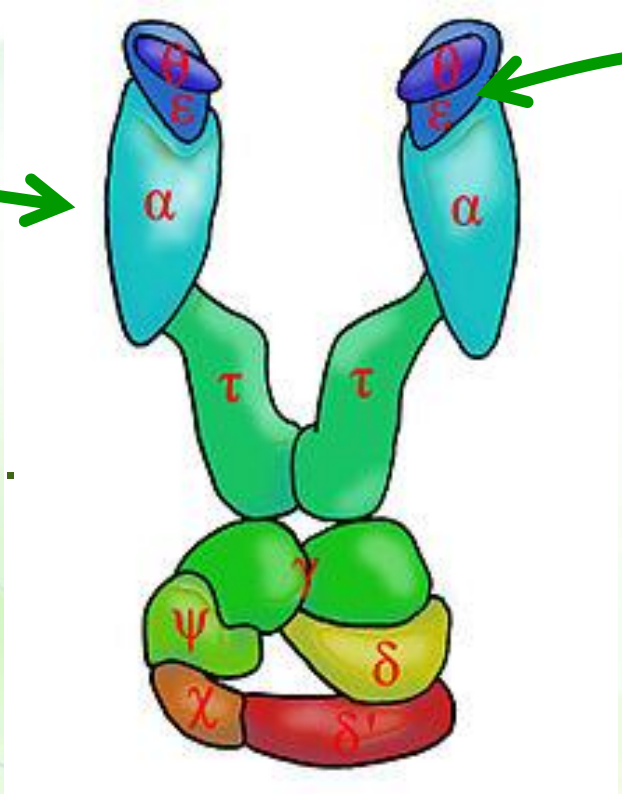
DNA polymerases in prokaryotes

- DNA polymerase III: DNA polymerization at the growing fork in E. coli.
  - The complex of primosome and polymerase is known as replisome.
- DNA polymerase I:
  - 5‘-to-3' exonuclease activity (removal of RNA primer) of each Okazaki fragment.
  - Fills in the gaps between the lagging-strand fragments.
  - DNA repair.
- DNA polymerase II, IV, and V : DNA repair

DNA polymerase III

- The DNA polymerase III is a very large protein composed of 10 different polypeptides with different functions.

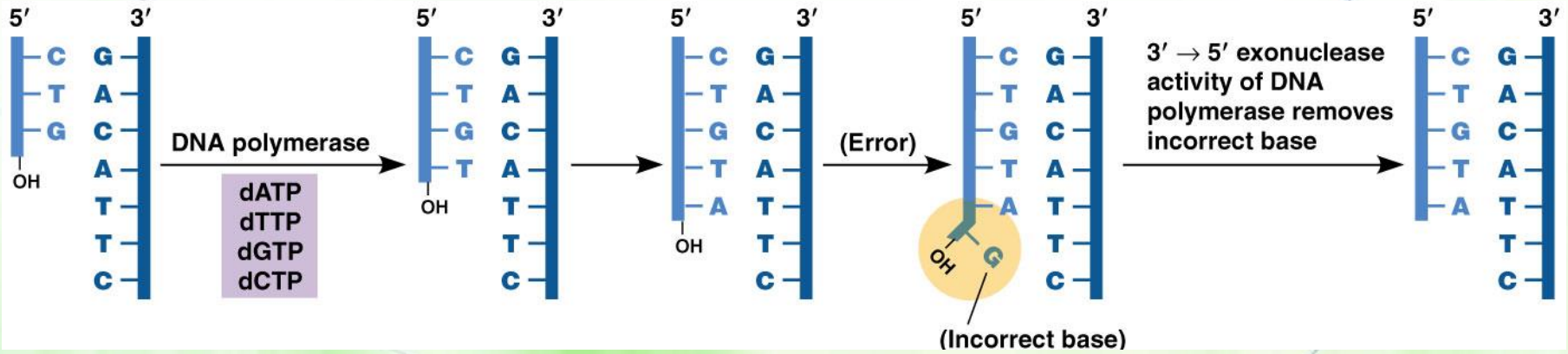
α subunit contains the active site for nucleotide addition.

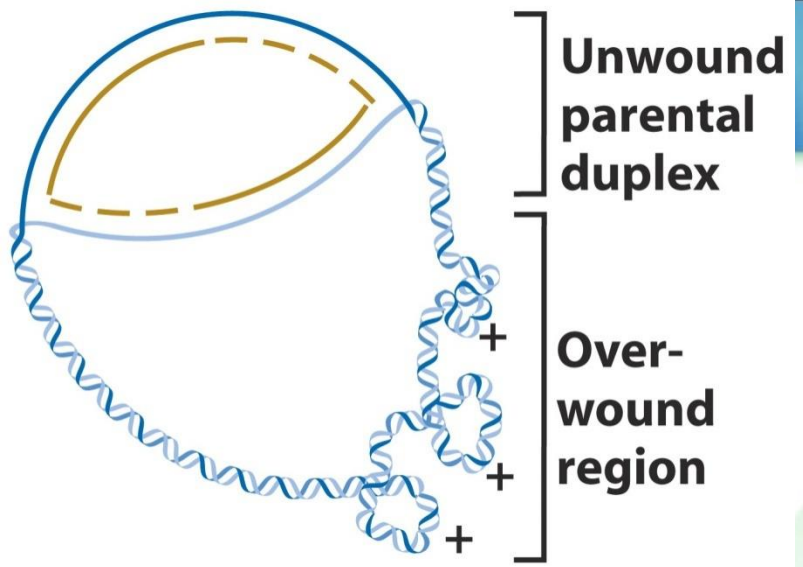


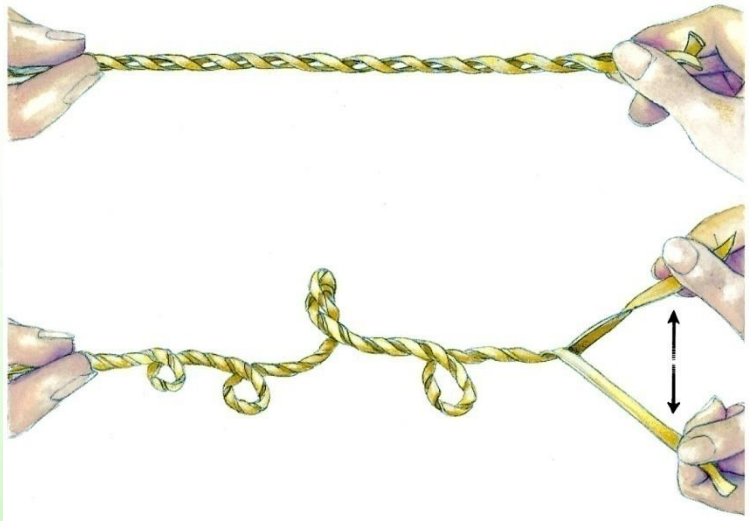
ε subunit is a 3'-to-5' exonuclease that removes incorrectly added (mispaired) nucleotides from the end of the growing chain.

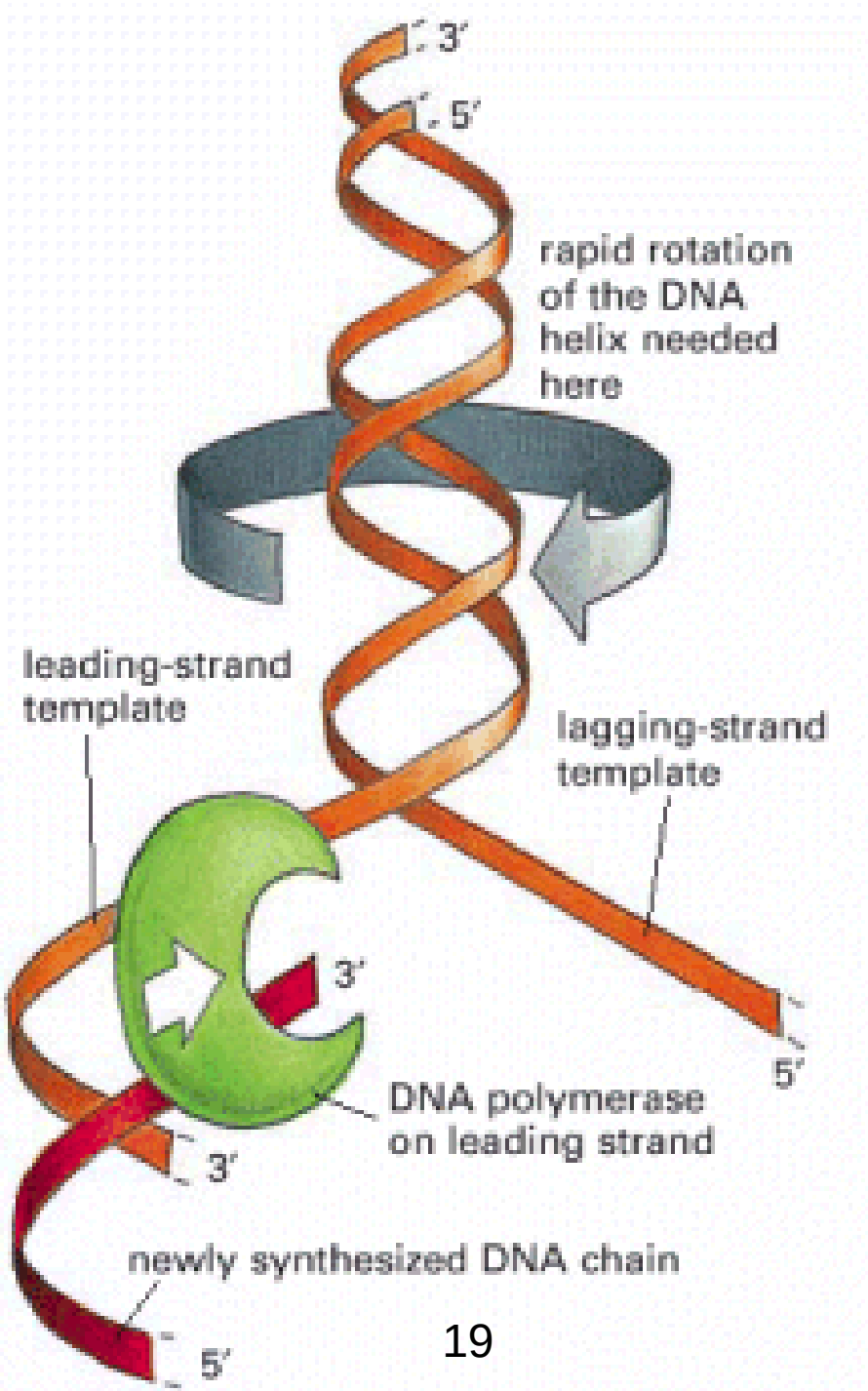
How accurate is DNA replication?

- The frequency of errors during replication is only one incorrect base per 10^8 nucleotides incorporated
- How is fidelity high?
  - The DNA polymerase can catalyze the formation of phosphodiester bonds when the right hydrogen bonding takes place between the bases (accuracy=1/100).
  - Proofreading mechanism (a 3'→5' exonuclease activity)- Remember ε subunit of DNA pol III ($1/10^{7-8}$).



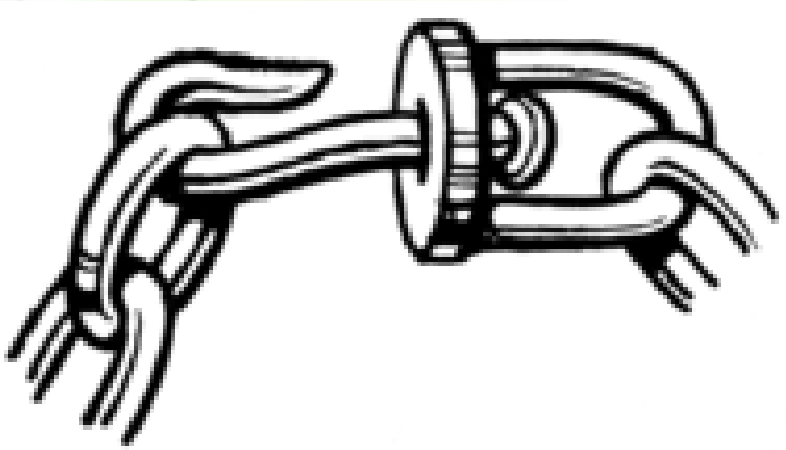

Unwound parental duplex
Over-wound region
+
+
+

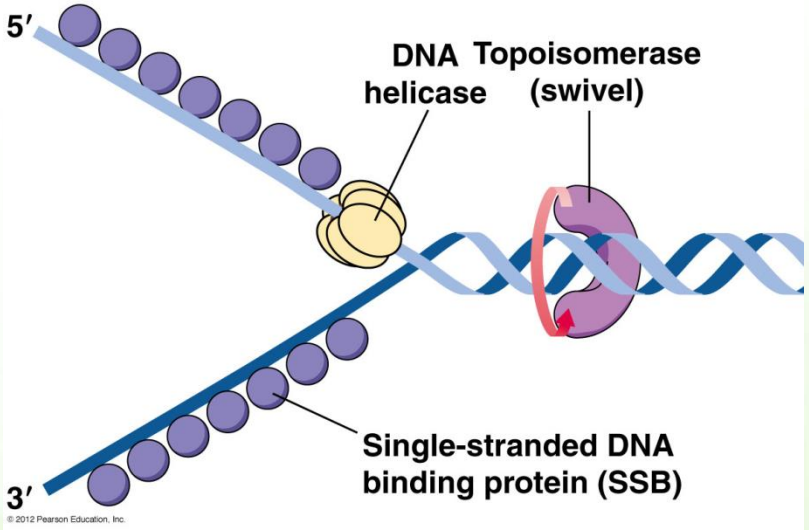



3′
5′
rapid rotation of the DNA helix needed here
leading-strand template
lagging-strand template
3′
5′
DNA polymerase on leading strand
3′
newly synthesized DNA chain
5′

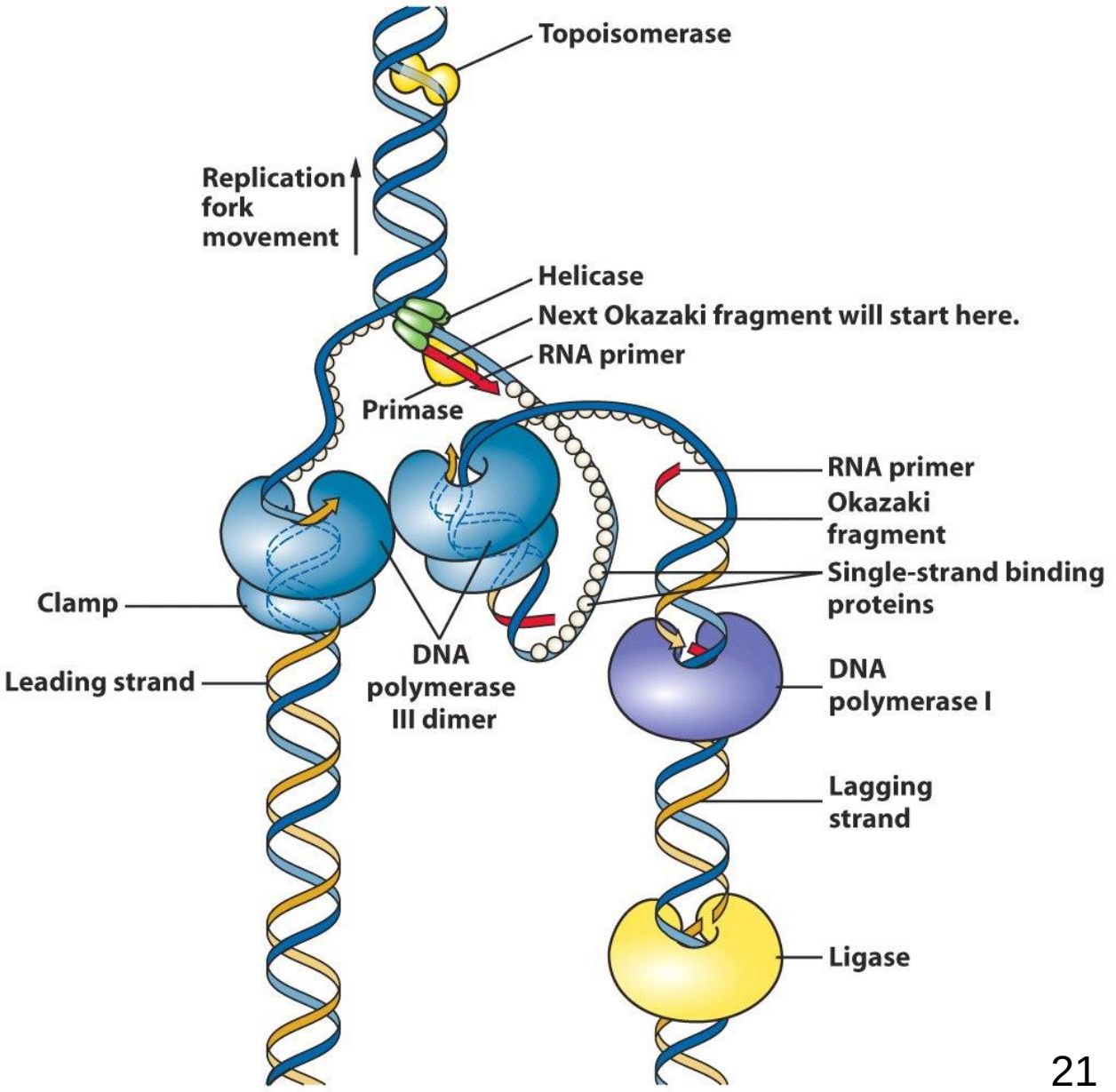
DNA topoisomerases

- A swivel is formed in the DNA helix by proteins known as DNA topoisomerases.
- A DNA topoisomerase breaks then re-forms phosphodiester bonds in a DNA strand.
- Topoisomerase I produces a transient single-strand break (or nick).
  - ATP-independent



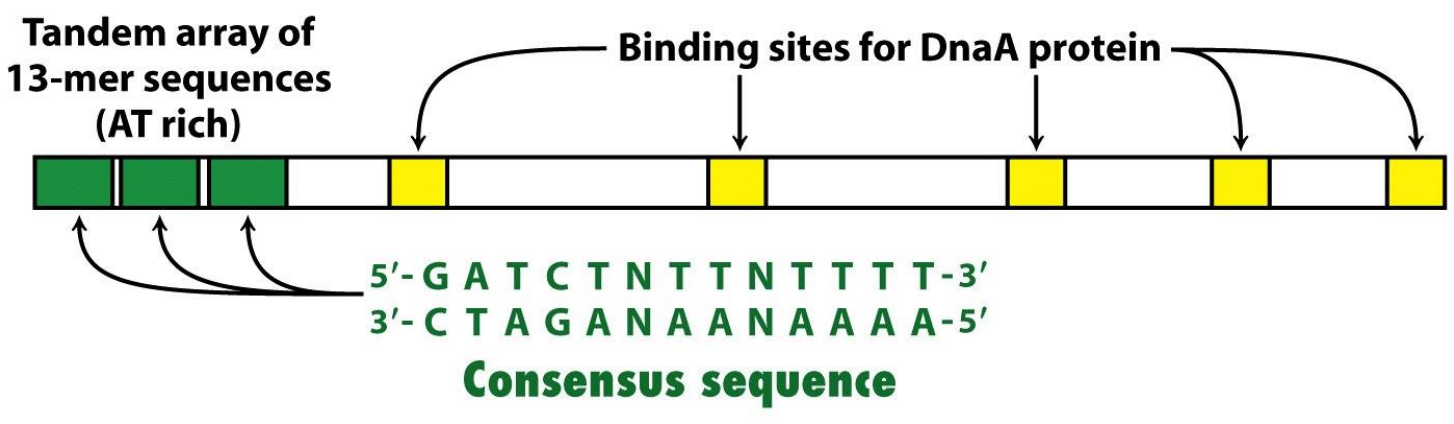


DNA replication machinery is coordinated



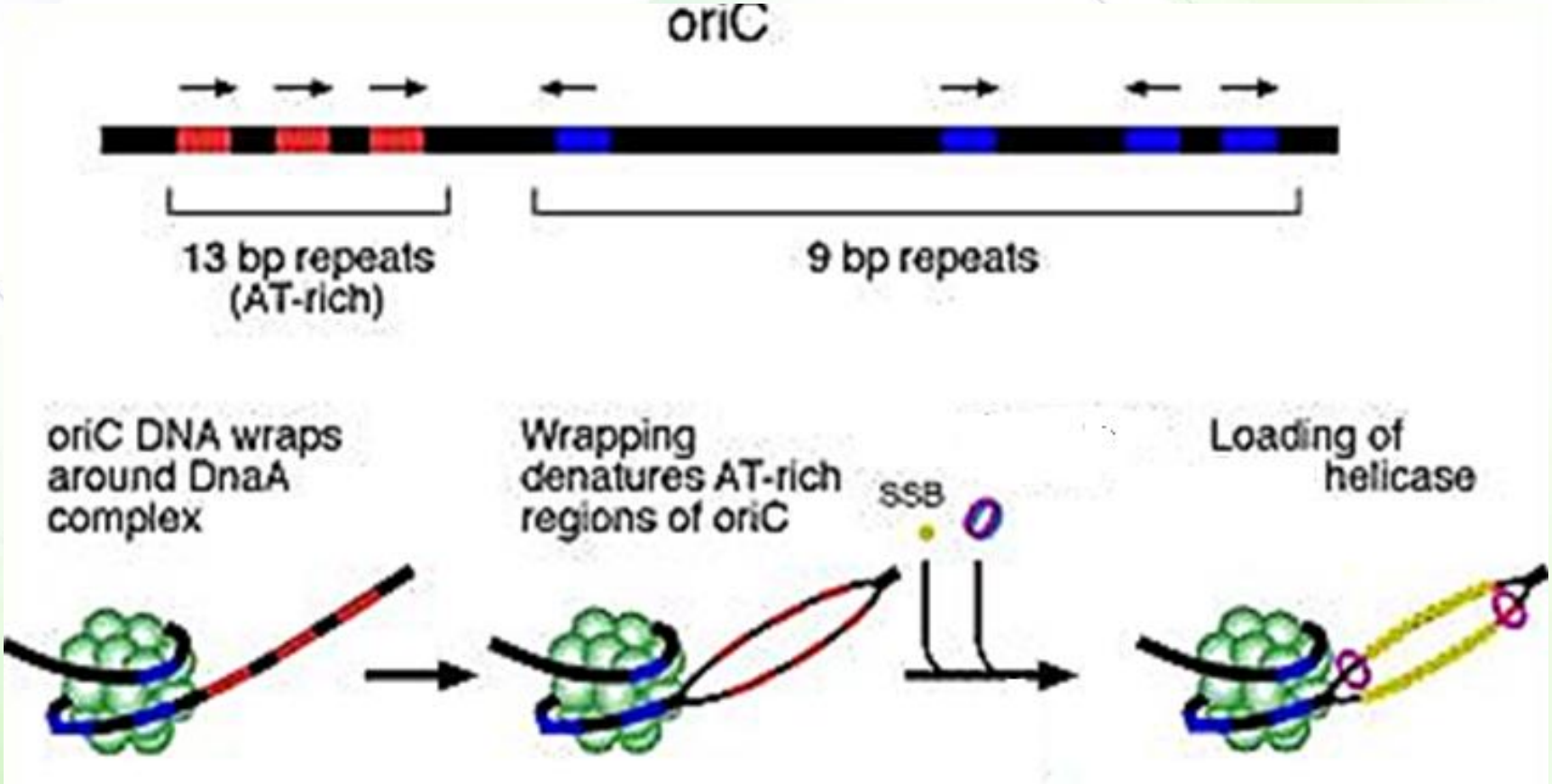
Origin of replication (OriC) in bacteria

- Bacterial replication starts at a origin known as origin of replication (OriC).
- oriC regions contain repetitive 9-bp and AT-rich 13-bp sequences (These are known as **consensus sequences**).
  - 9-mer: binding sites for the an “initiator” protein called DnaA.
  - 13-mers: AT-rich region - it facilitates separation of the double strand DNA.



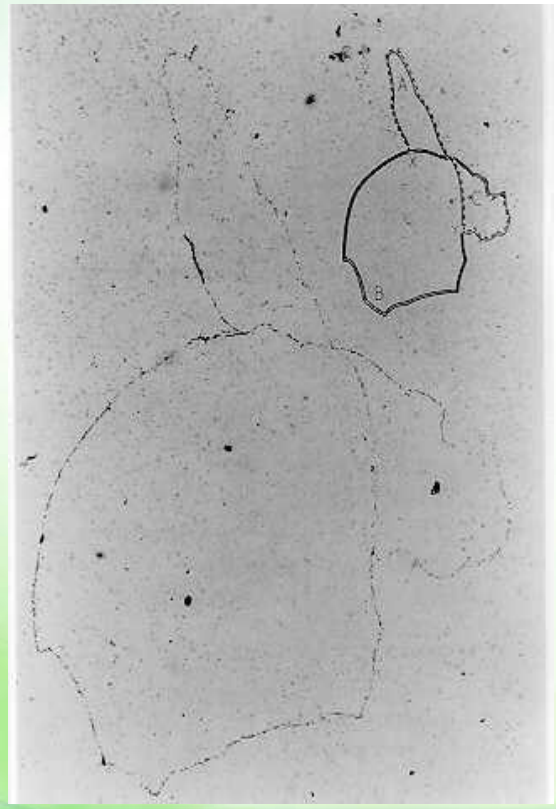
Possible mechanism

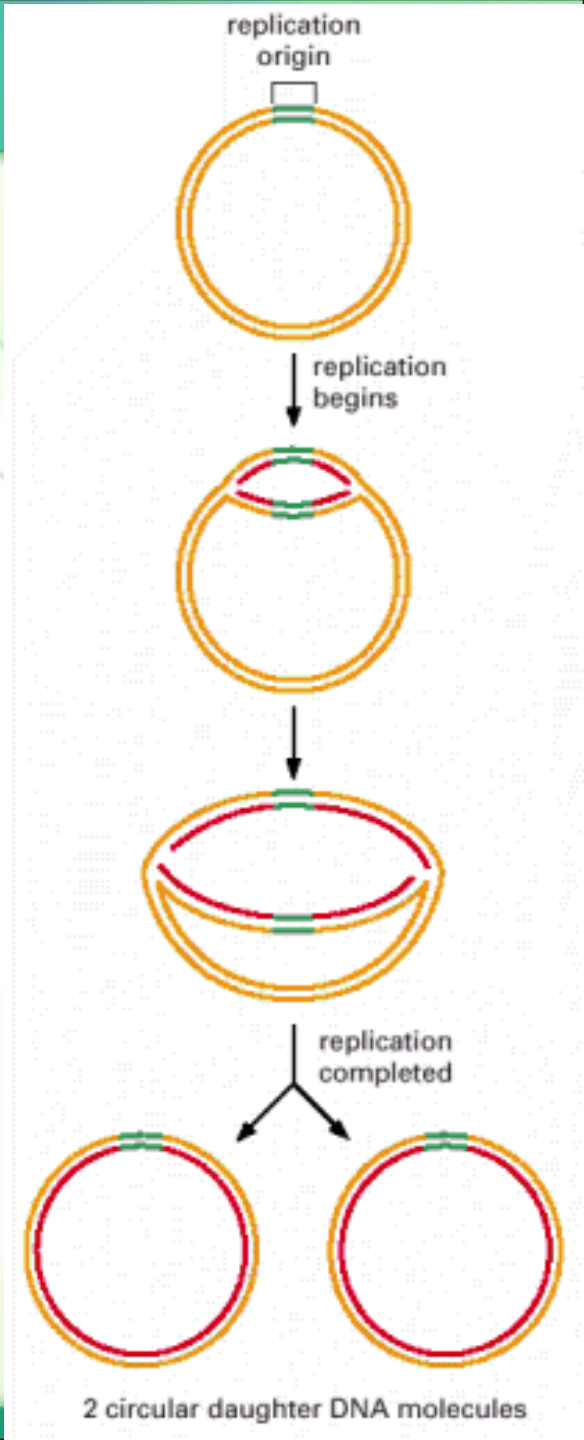
- When DnaA protein binds to 9-mers, it applies stress on the AT-rich region resulting in DNA "melting“.



Two replication forks (bacteria)

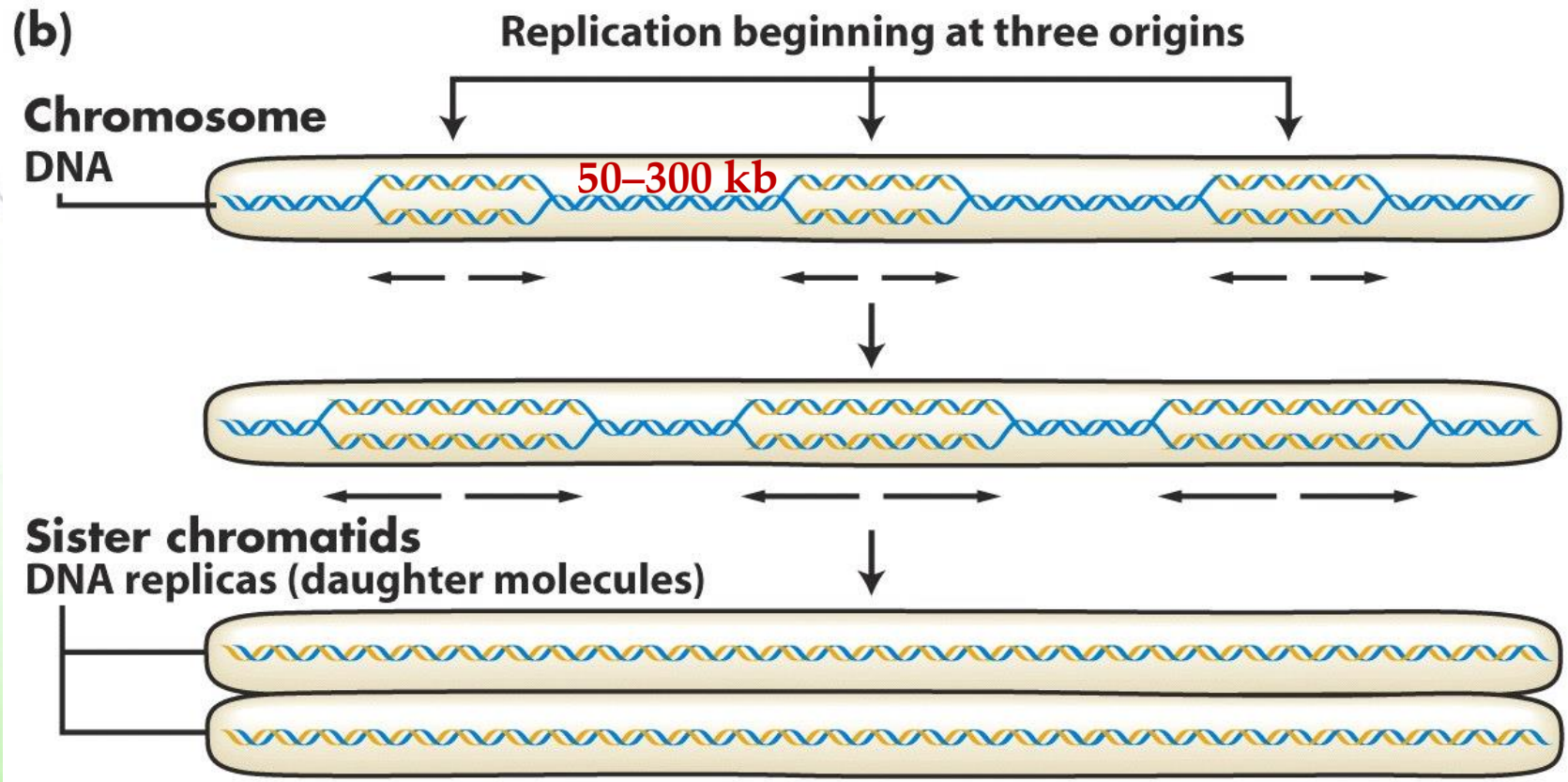
- The two replication forks proceed in opposite directions until they meet up roughly halfway around the chromosome.





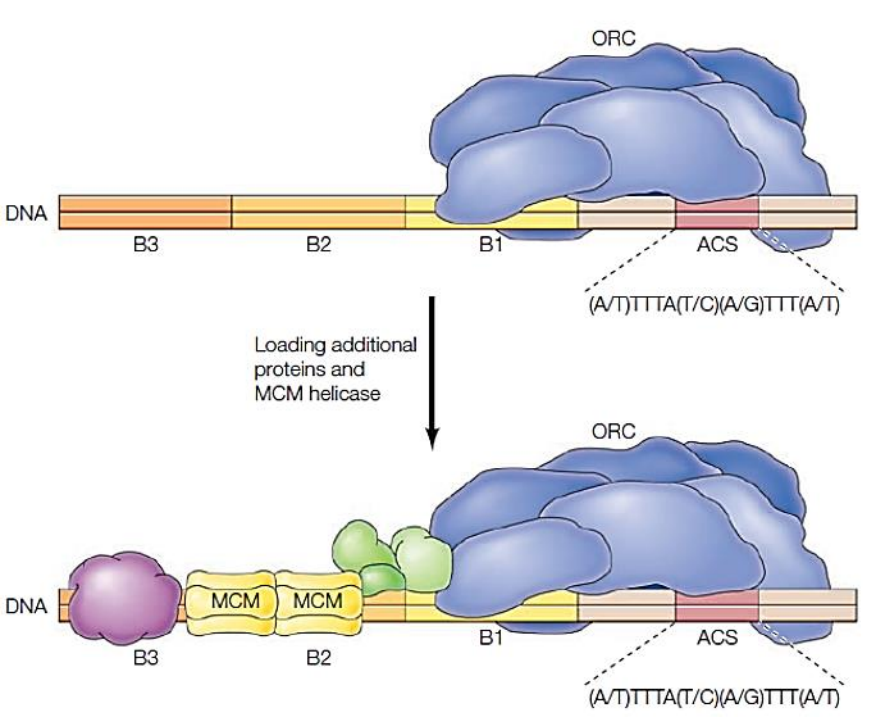
Origins of replication in human genome

- The human genome contains ~30,000 origins of replication.



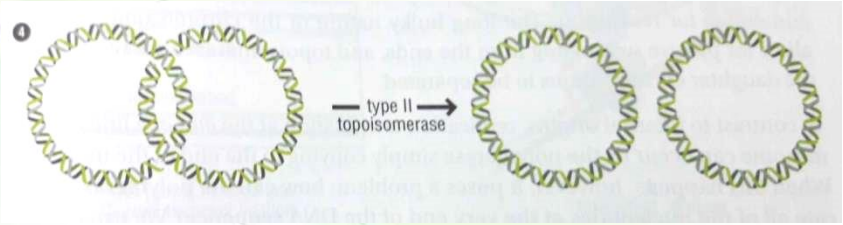
Oric in eukaryotes (yeast vs. humans)

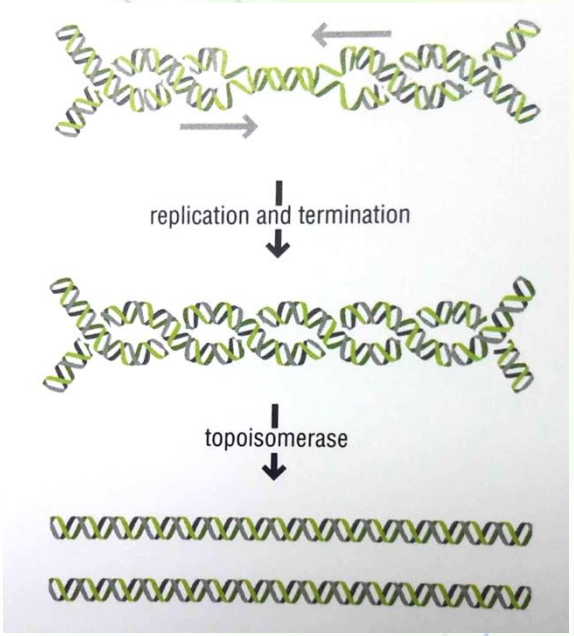
- Several autonomously replicating sequences, or ARSs, each containing an 11-base-pair ARS consensus sequence (ACS) and three additional elements (B1, B2, and B3).
- The origin recognition complex (ORC) binds to the ACS and B1 and recruits additional proteins, including the MCM DNA helicase, to the origin.
- In higher eukaryotes, the ORC proteins appear to recognize ORC based on chromatin structure, rather than specific DNA sequences.



Role of topoisomerase II

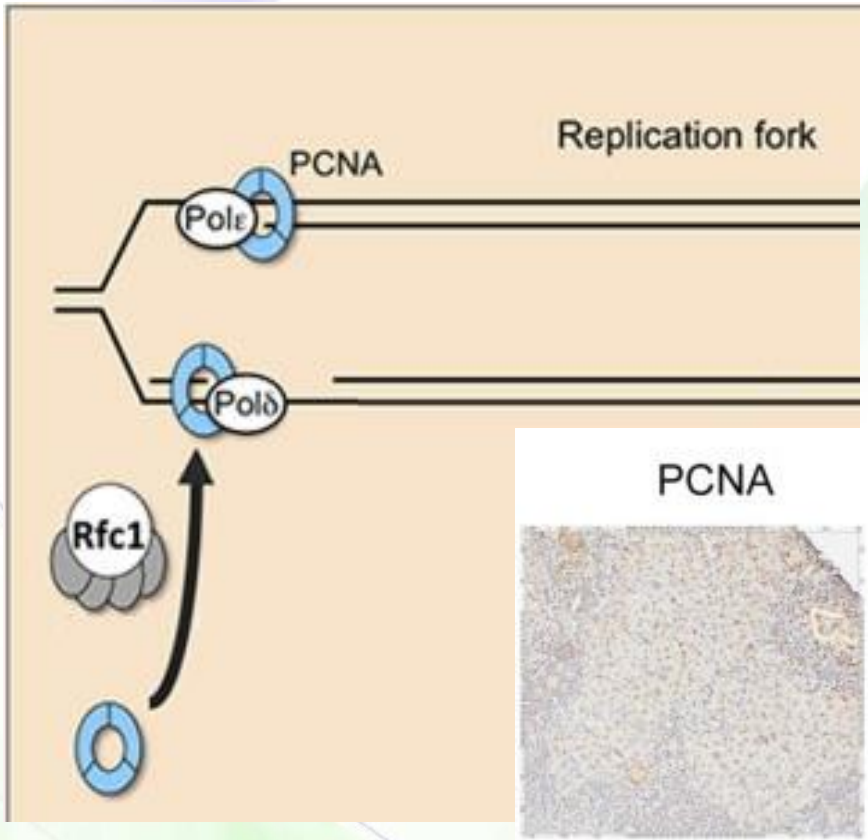
- Topoisomerase II is responsible for untangling chromosomes by making a transient double-strand break.
  - also known as gyrase in bacteria
  - ATP-dependent
- It is also responsible for chromosome condensation during the cell cycle.





Topoisomerase inhibitors are commonly used in treatment of cancer.

Role of PCNA proteins



- DNA polymerases are guided to the primers by a protein called PCNA (proliferating cell nuclear antigen).
- PCNA is a diagnostic marker of cancer.

DNA polymerase in eukaryotes

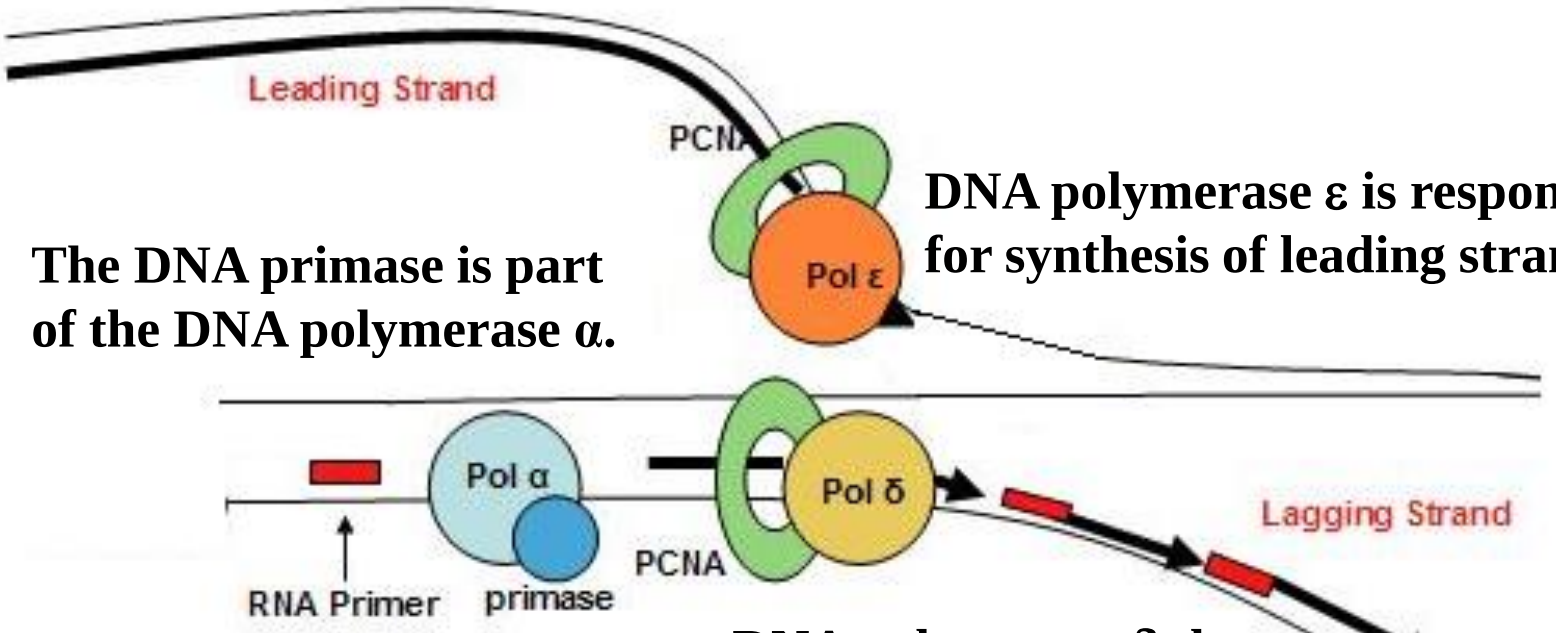
- Eukaryotic cells contain 9 DNA polymerases; most of them for DNA repair.

TABLE 10.4

The Biochemical Properties of Eukaryotic DNA Polymerases

	α	δ	ε	β	γ
Mass (kDa)					
Native	>250	170	256	36-38	160-300
Catalytic core	165-180	125	215	36-38	125
Other subunits	70, 50, 60	48	55	None	35, 47
Location	Nucleus	Nucleus	Nucleus	Nucleus	Mitochondria
Associated functions					
3' → 5' exonuclease	No	Yes	Yes	No	Yes
Primase	Yes	No	No	No	No
Properties					
Processivity	Low	High	High	Low	High
Fidelity	High	High	High	Low	High
Replication	Yes	Yes	Yes	No	Yes
Repair	No	?	Yes	Yes	No

The mechanism of replication



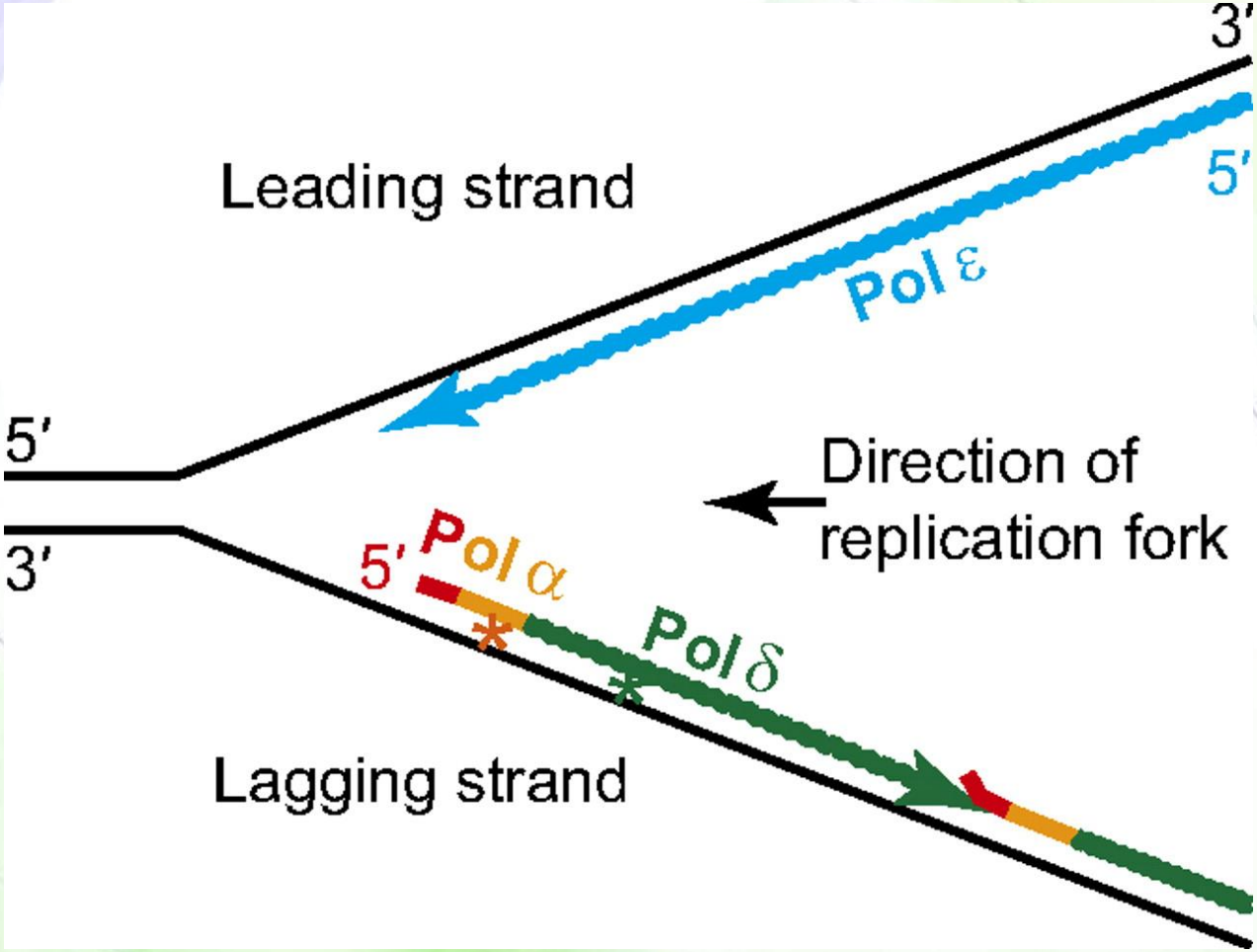
The DNA primase is part of the DNA polymerase α.

DNA polymerase ε is responsible for synthesis of leading strand.

Polymerase α begins each Okazaki fragment on the lagging strand

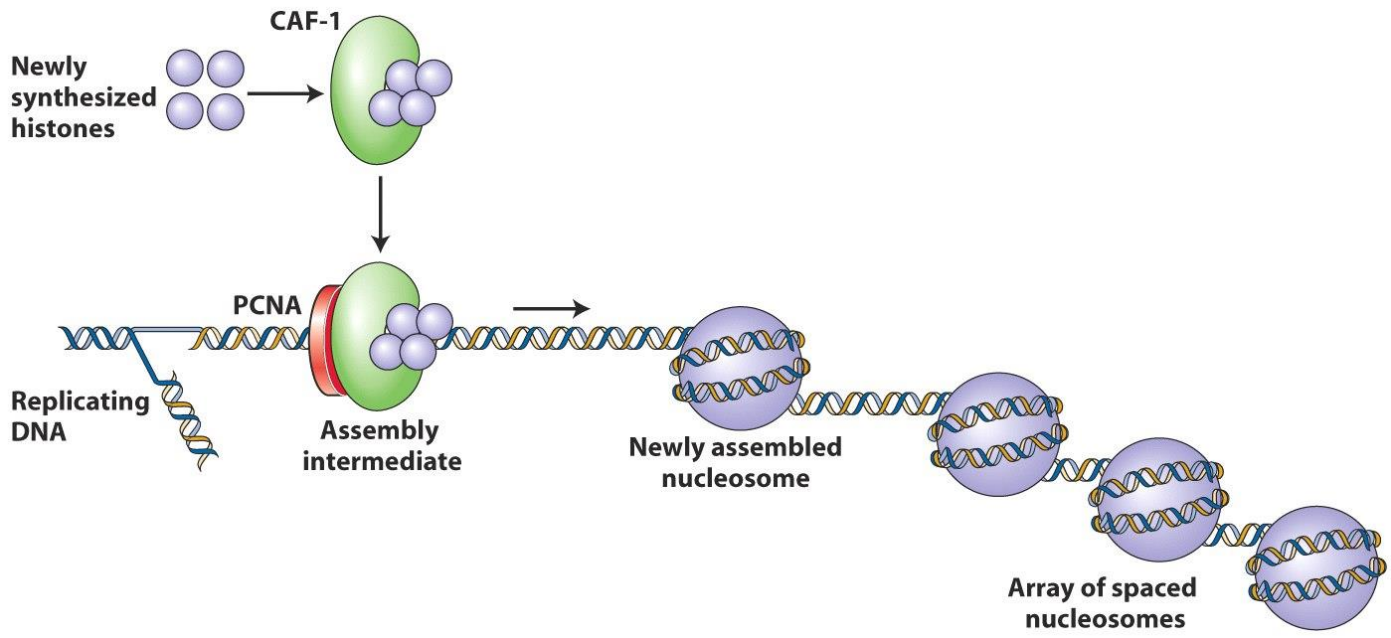
DNA polymerase δ then synthesizes the remainder of each Okazaki fragment.

- The polymerases do not have a 5'→3' exonuclease.
  - Primers are removed by special enzymes.
  - DNA polymerase δ then fills in the gap.


3′
Leading strand
5′
Pol ε
5′
Direction of
replication fork
3′
5′
Pol α
Pol δ
Lagging strand

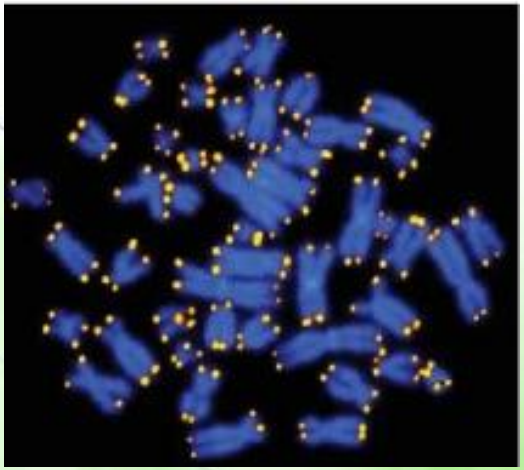
Role of chromatin

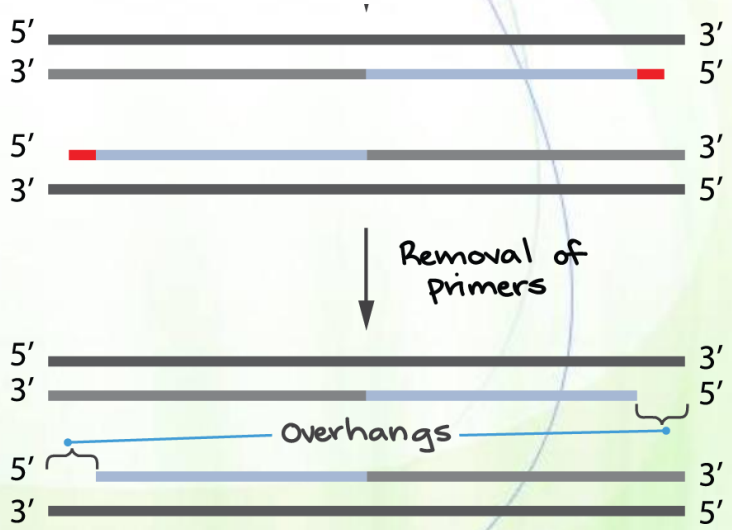
- Replication is linked to DNA packing by histones.
- DNA is freed from histones by chromatin-remodeling proteins in order for enzymes to move along the DNA.
- New histones are assembled onto the DNA behind each replication fork by chromatin assembly factors (CAFs).

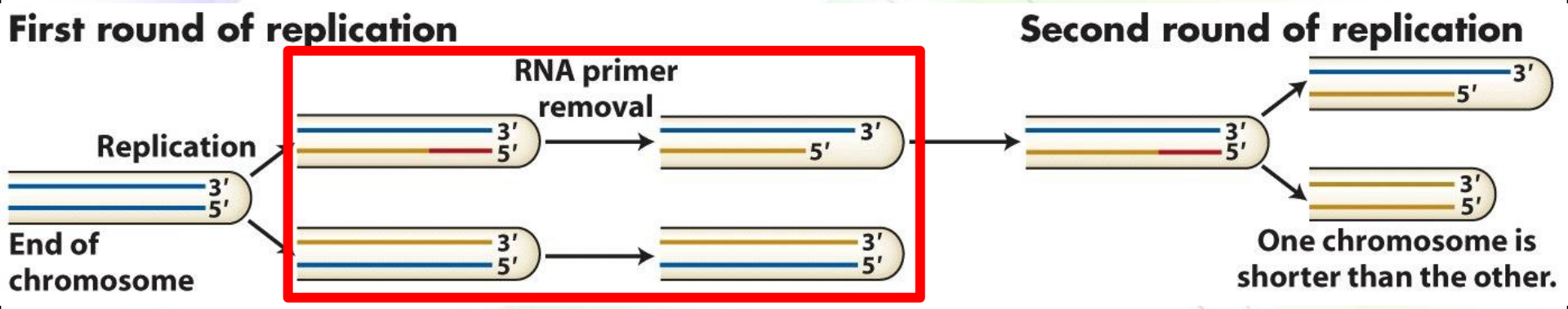


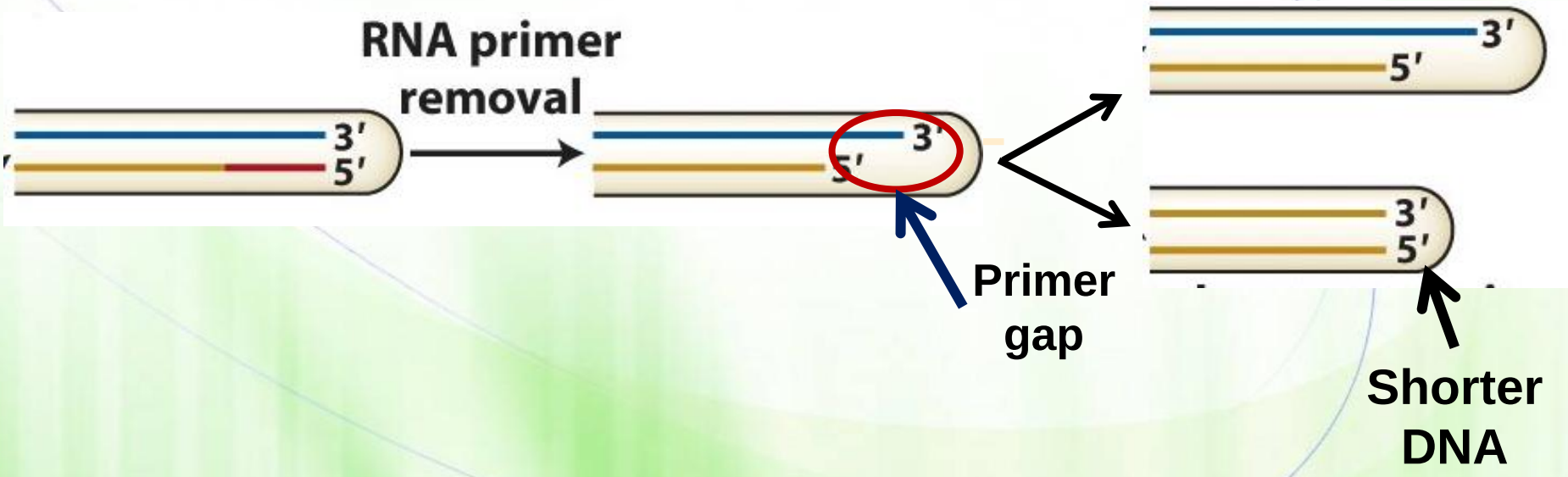
A problem in the lagging strand

- In human cells, for example, telomeres span approximately 10 kb.
- 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 DNA polymerase can build to fill the resulting gap leading to shortening of the lagging strand.



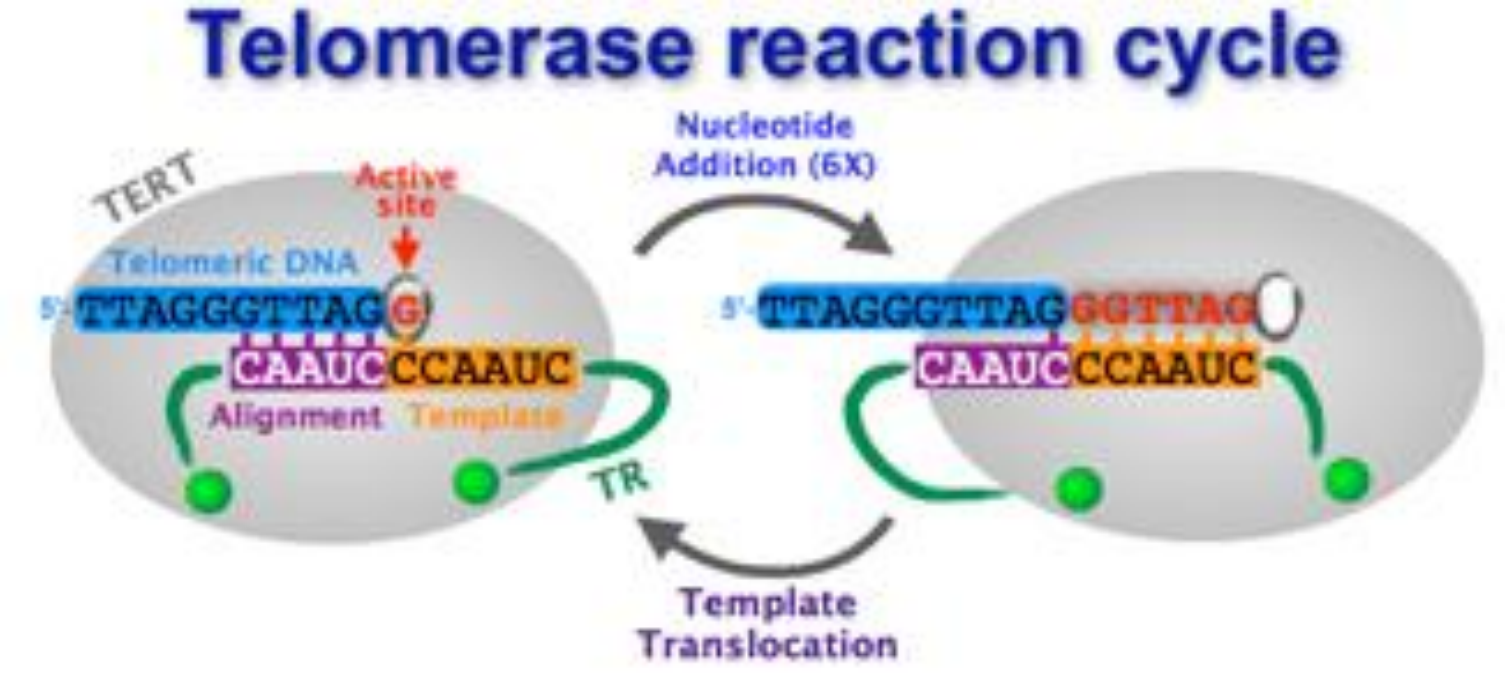


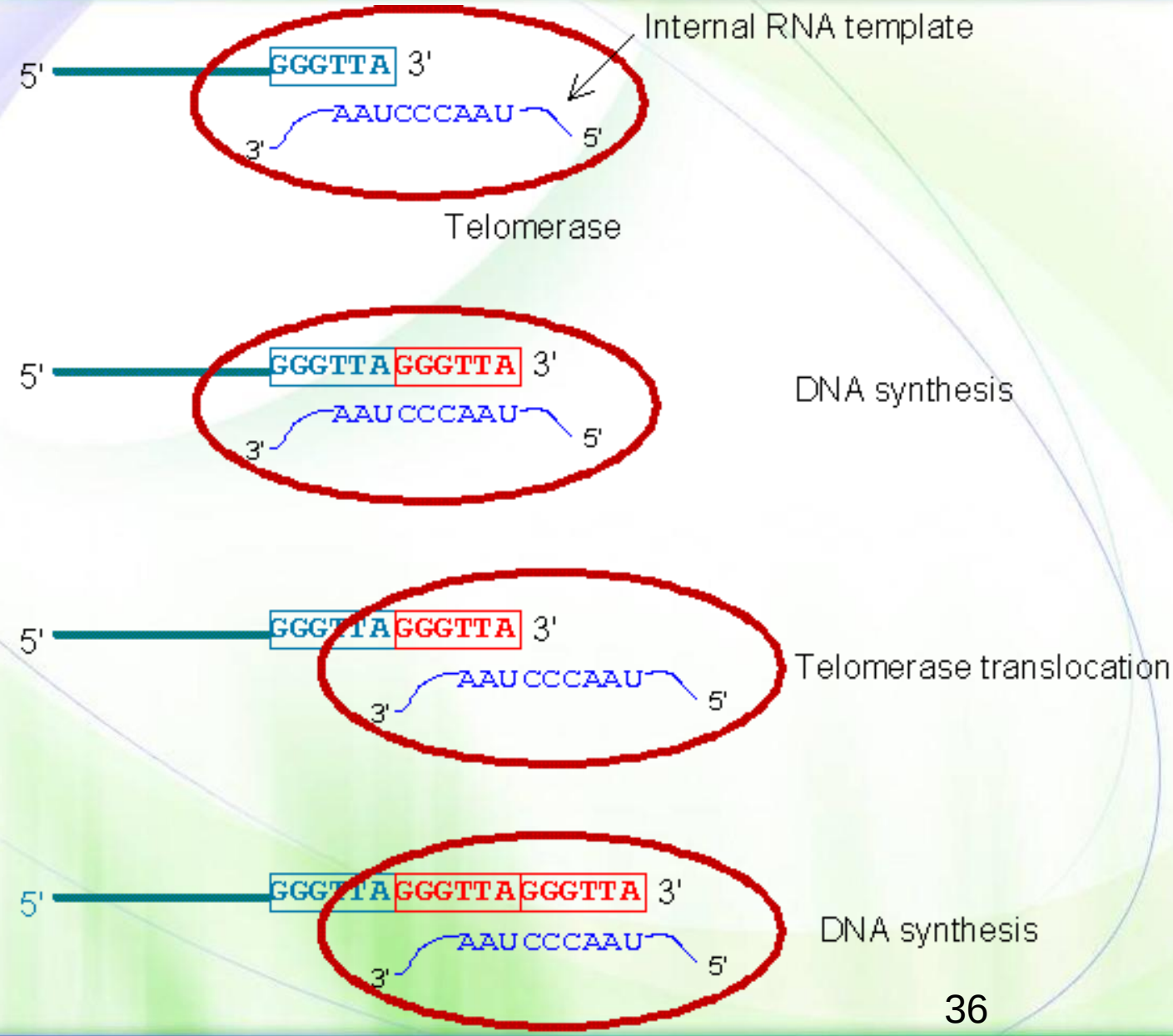

First round of replication
Second round of replication
RNA primer removal
Replication
End of chromosome
3′
5′
One chromosome is shorter than the other.


RNA primer removal
3′
5′
Primer gap
Shorter DNA

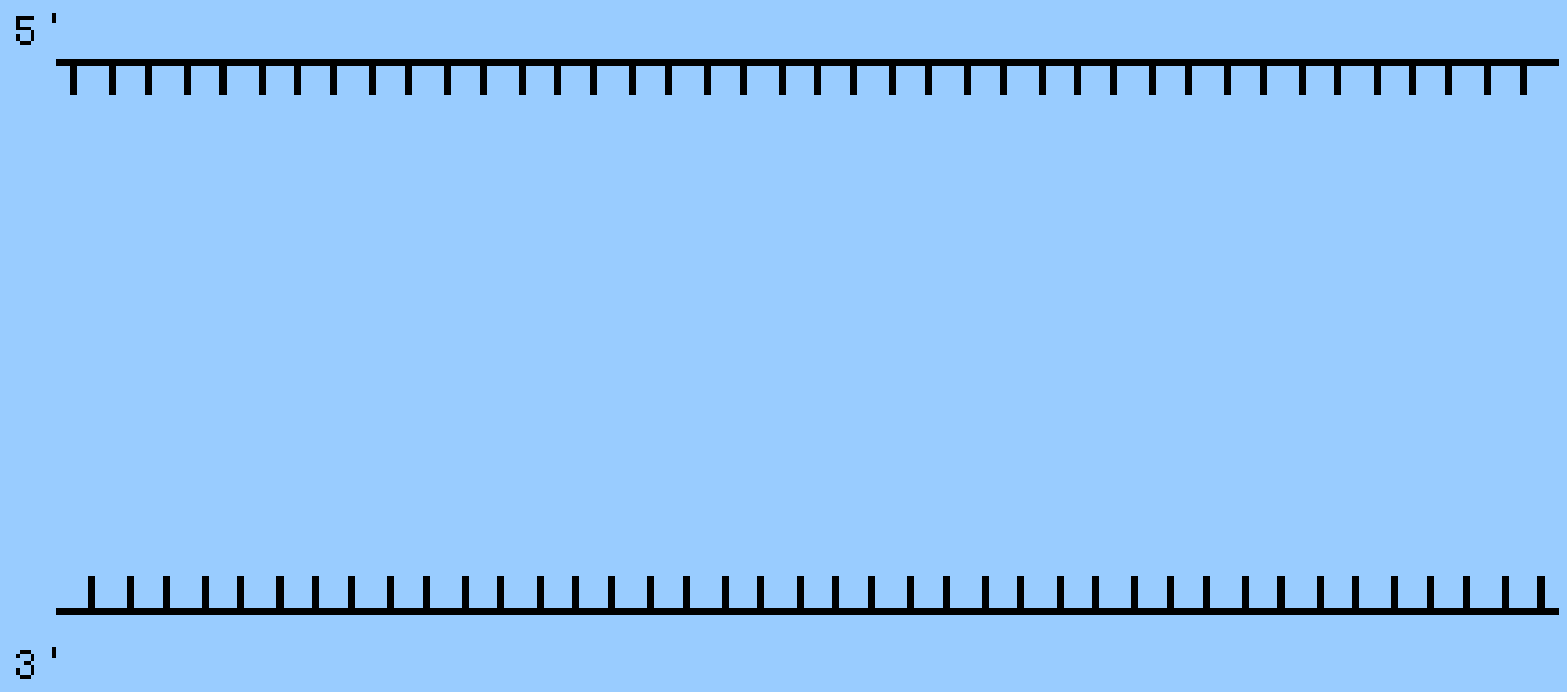
Telomerase comes to the rescue

- 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.




Internal RNA template
5'
GGGTTA 3'
AAUCCCAAU
3'
5'
Telomerase
5'
GGGTTA GGGTTA 3'
AAUCCCAAU
3'
5'
DNA synthesis
5'
GGGTTA GGGTTA 3'
AAUCCCAAU
3'
5'
Telomerase translocation
5'
GGGTTA GGGTTA GGGTTA 3'
AAUCCCAAU
3'
5'
DNA synthesis

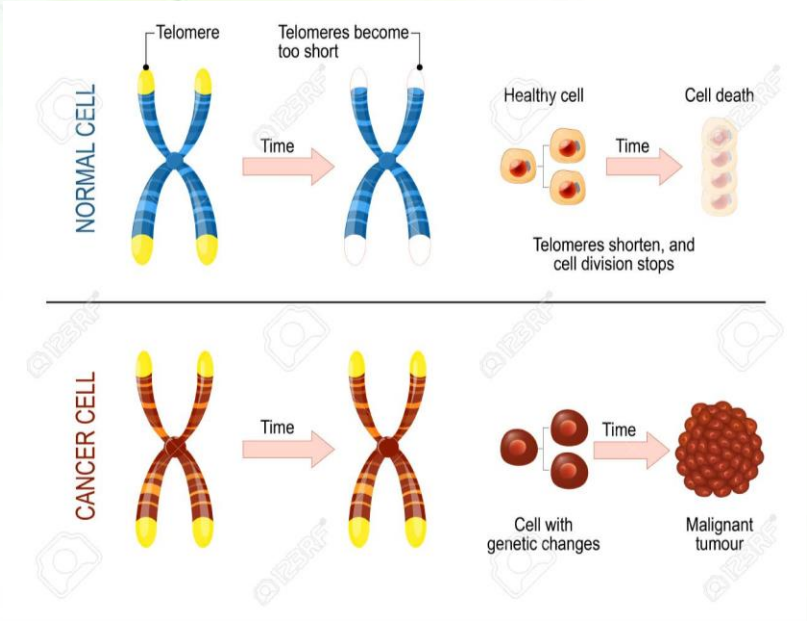
Replication of the lagging strand of a linear chromosome encounters a problem at the 3' end

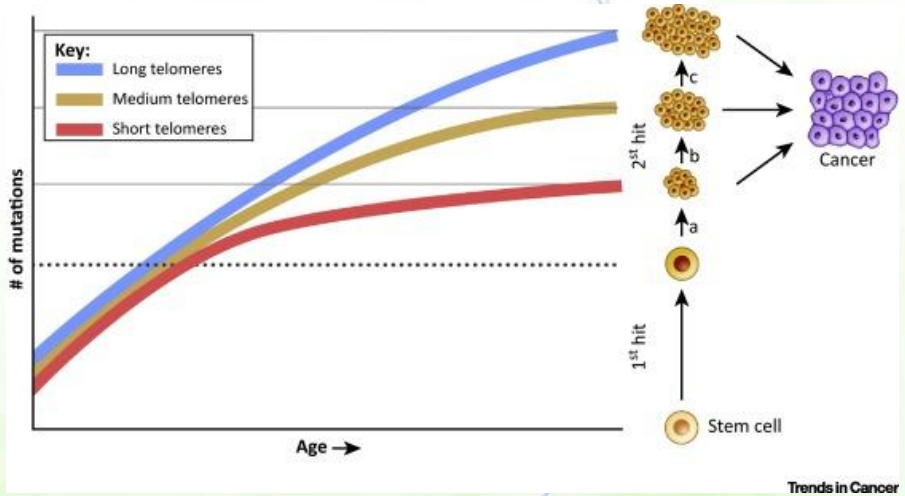


Note: Although this animation is good, there are wrong pieces of nformation within it. Find them.

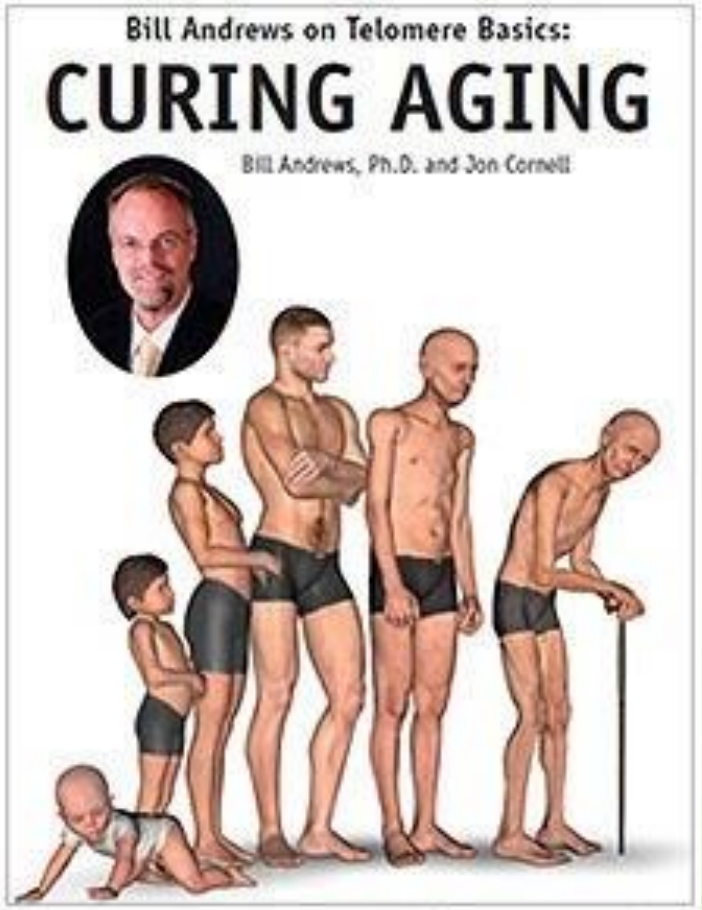
Facts of life about telomerasas

- In contrast to germline cells, most somatic cells do not have high levels of telomerase disabling indefinite number of cell divisions.
- As we grow older, the activity of telomerase is reduced.
- The gradual shortening of the chromosome ends leads to senescence and cell death.
- On the other hand, cancer cells (e.g. melanomas) express abnormally high levels of telomerase, allowing them to continue dividing indefinitely.

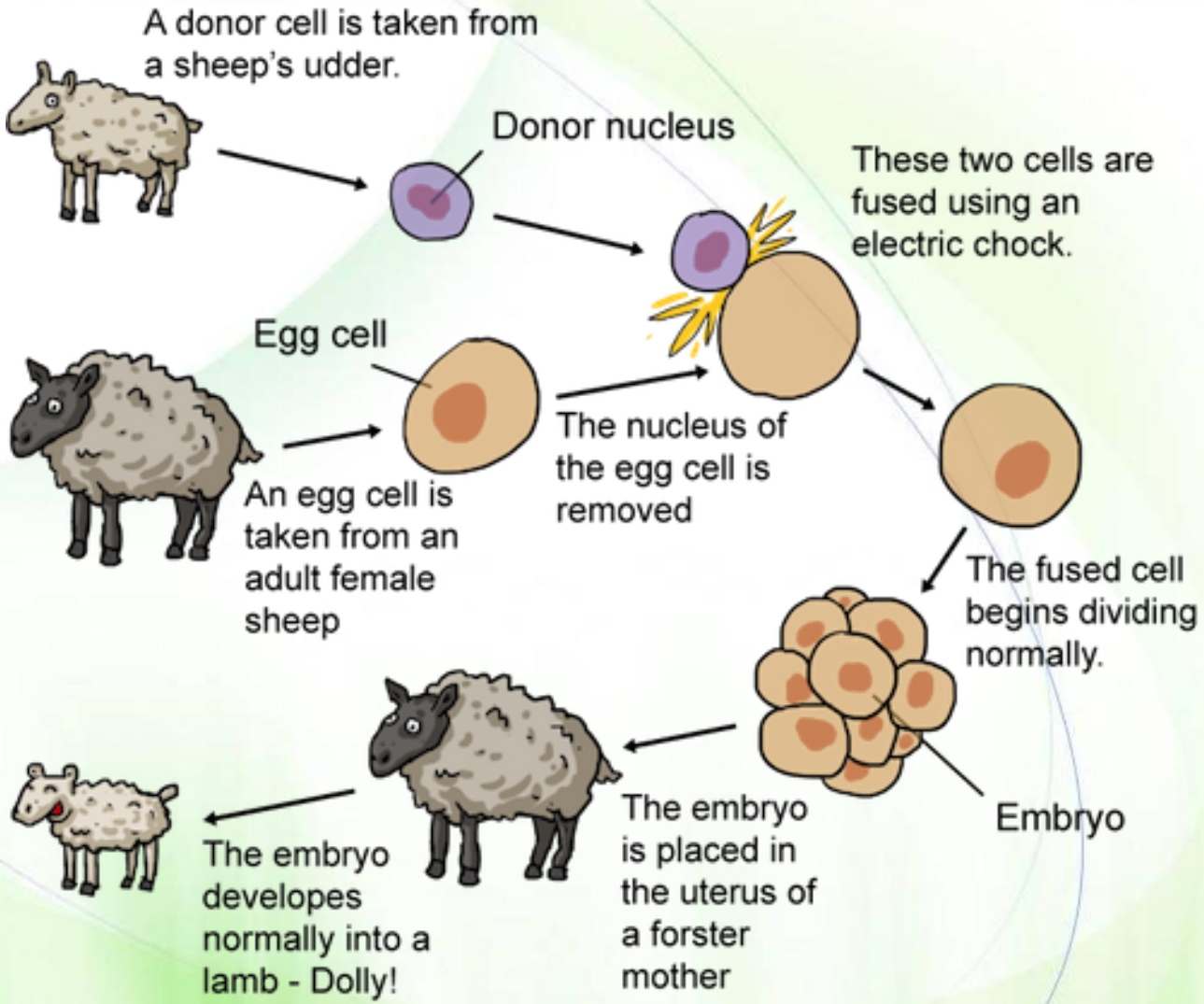




Elixir of youth



Dolly, the sheep



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