

FINAL – Lecture #5

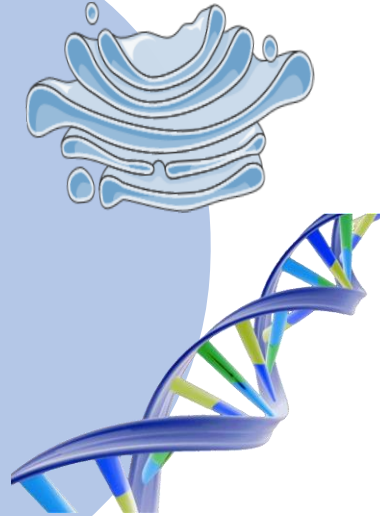
# DNA Replication

﴿ وَإِن تَتَوَلَّوْا يَسْتَبَدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَلَكُمْ ﴾

اللهم استعملنا ولا تستبدلنا

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# Human Genome Quiz !!

# Molecular Biology (4)

## DNA replication

Prof. Mamoun Ahram

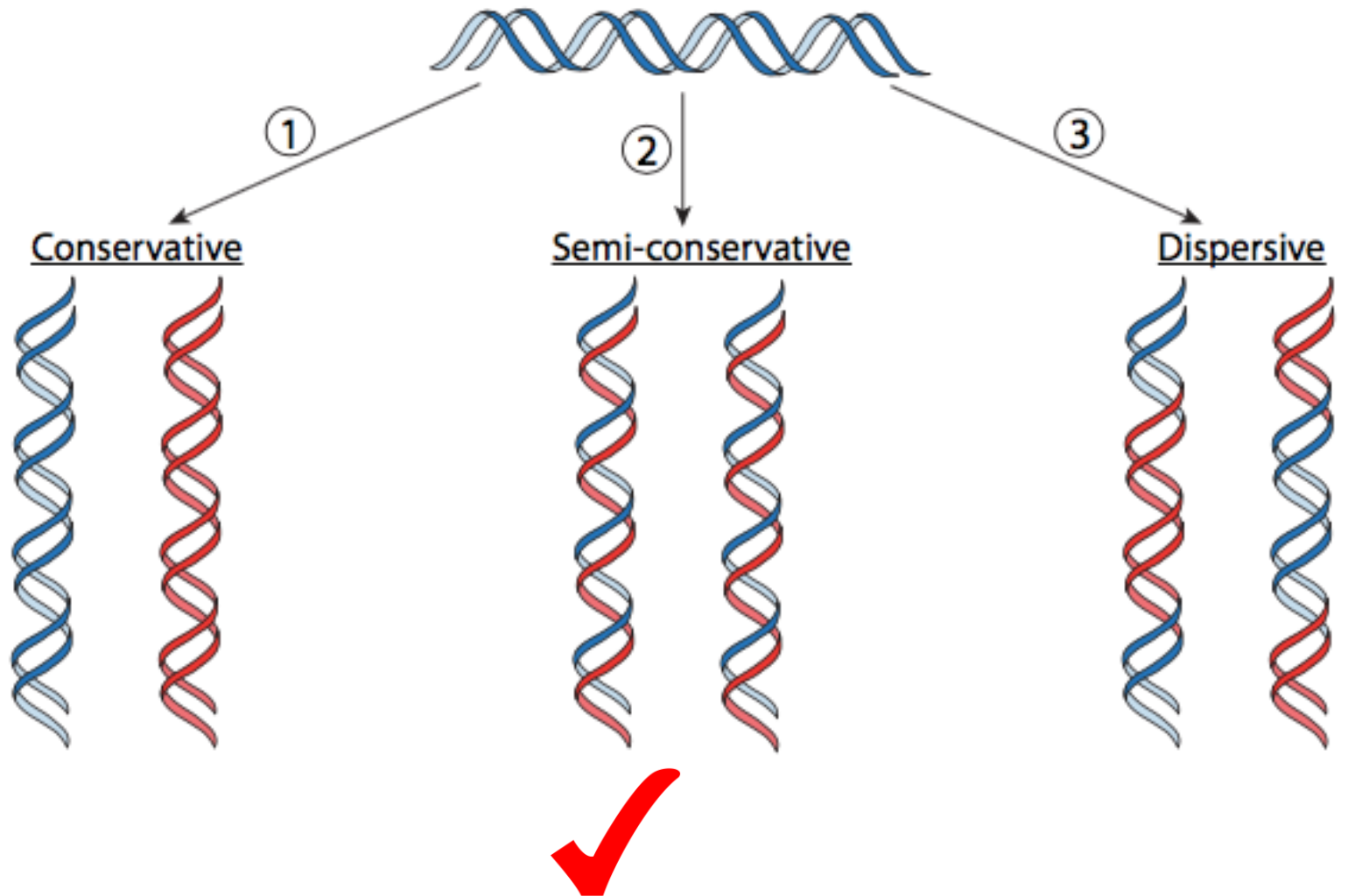
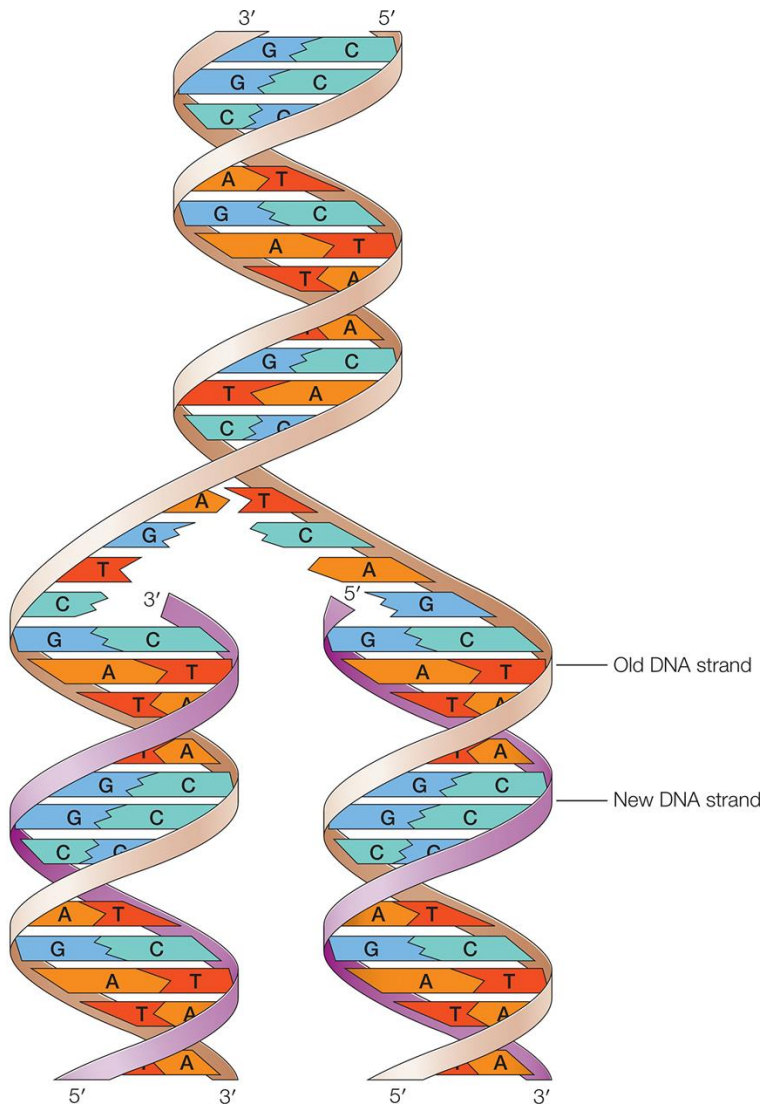
School of Medicine

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  $\alpha$ ,  $\delta$ , and  $\epsilon$ )
- The substrates are deoxyribonucleotides. *Specifically: deoxyribonucleoside triphosphate*

# The hypotheses and fact



# explanation for the previous slide :

- In the past scientists didn't know how DNA is replicated so they came up with this 3 hypothesis:
  1. conservative: when DNA is replicated the old DNA is conserved (that's why we call it conservative) and the new one contains two new strands
  2. Dispersive: the new DNA is composed from random fragments from the new and the old DNA strands
  3. Semi conservative (the true one): each daughter cell will have the DNA composed from one strand from the new DNA and the other strand from the old one as during DNA replication the parental strands are separated and the new ones are copied from each one of those strands (get back to the picture on the left in the previous slide to understand =)

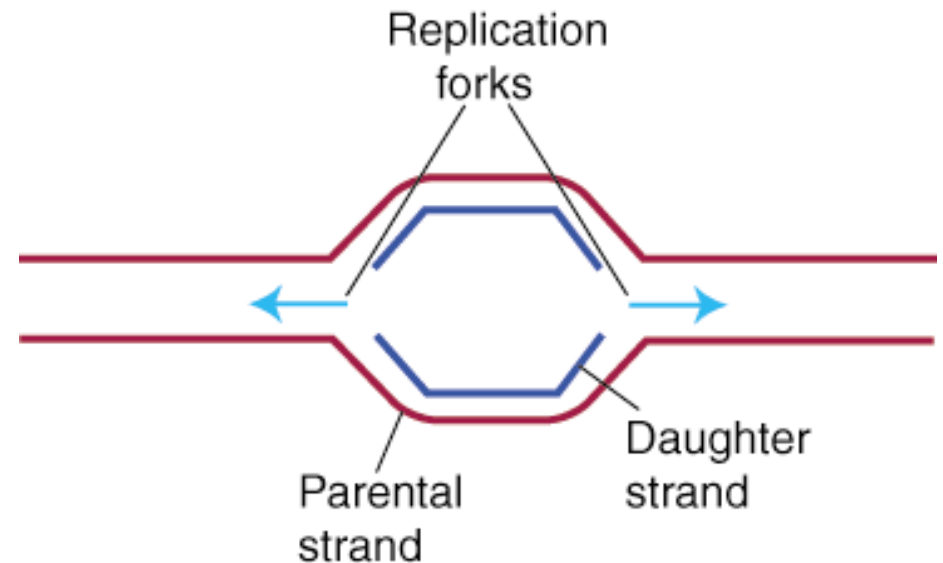
# Bidirectional

- Replication is bidirectional. Meaning that it goes in both directions
- This replicative region is called a replication fork.

- This is an EM image that shows the replication bubble that consists of two forks

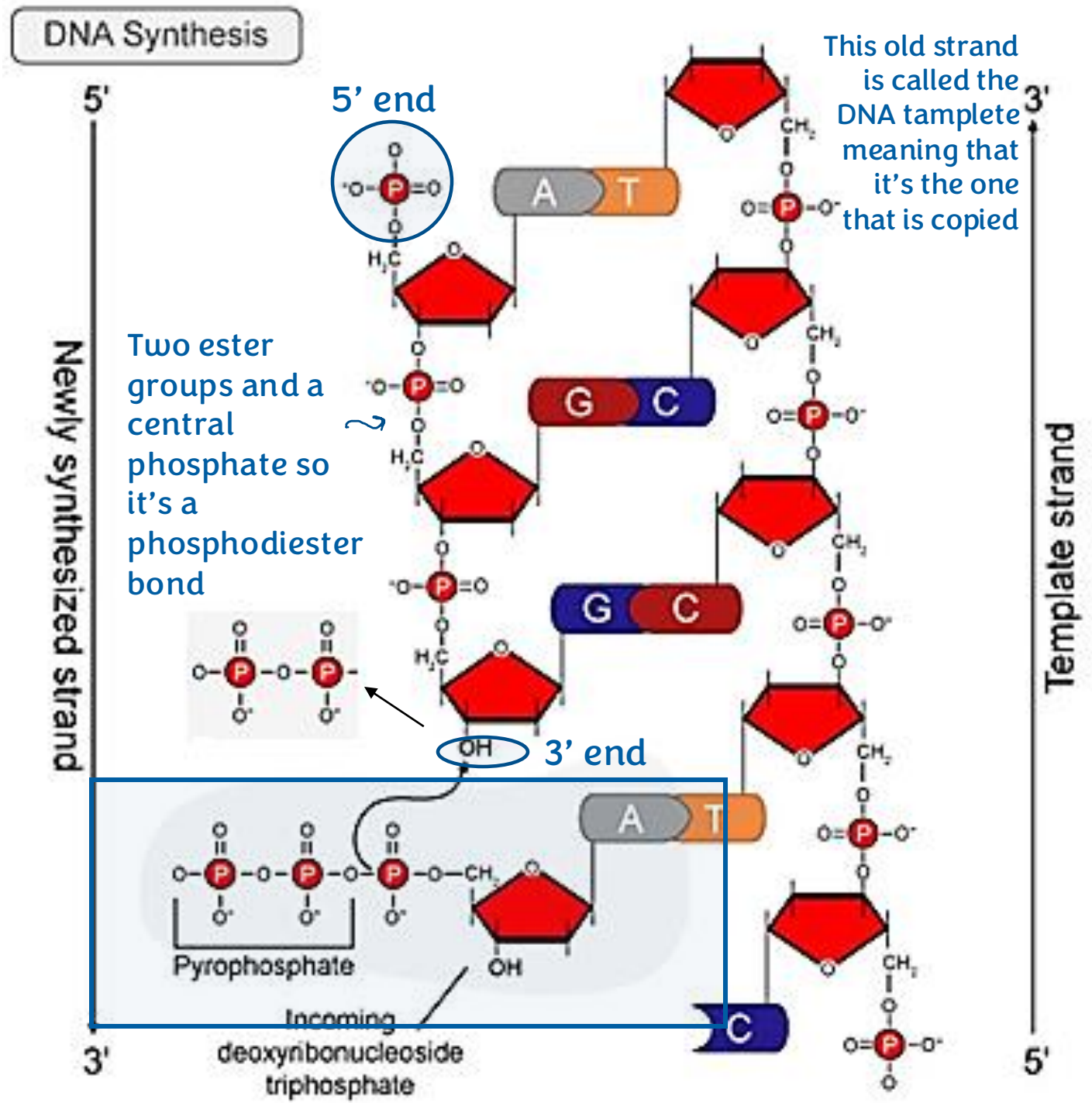


- If we divided this replication bubble we will have one fork to the left and the other is to the right



# Bidirectional

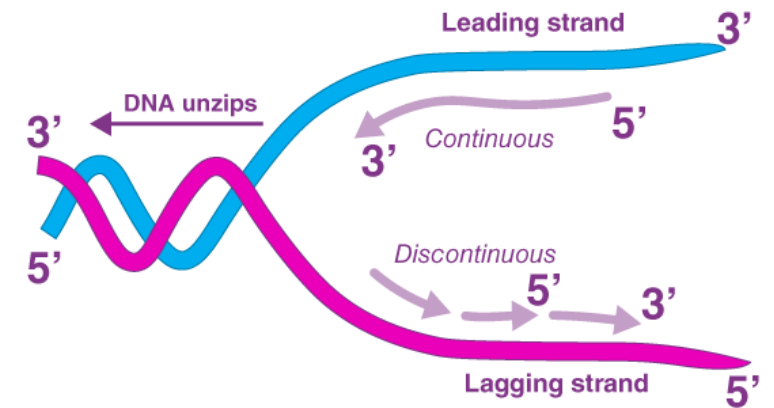
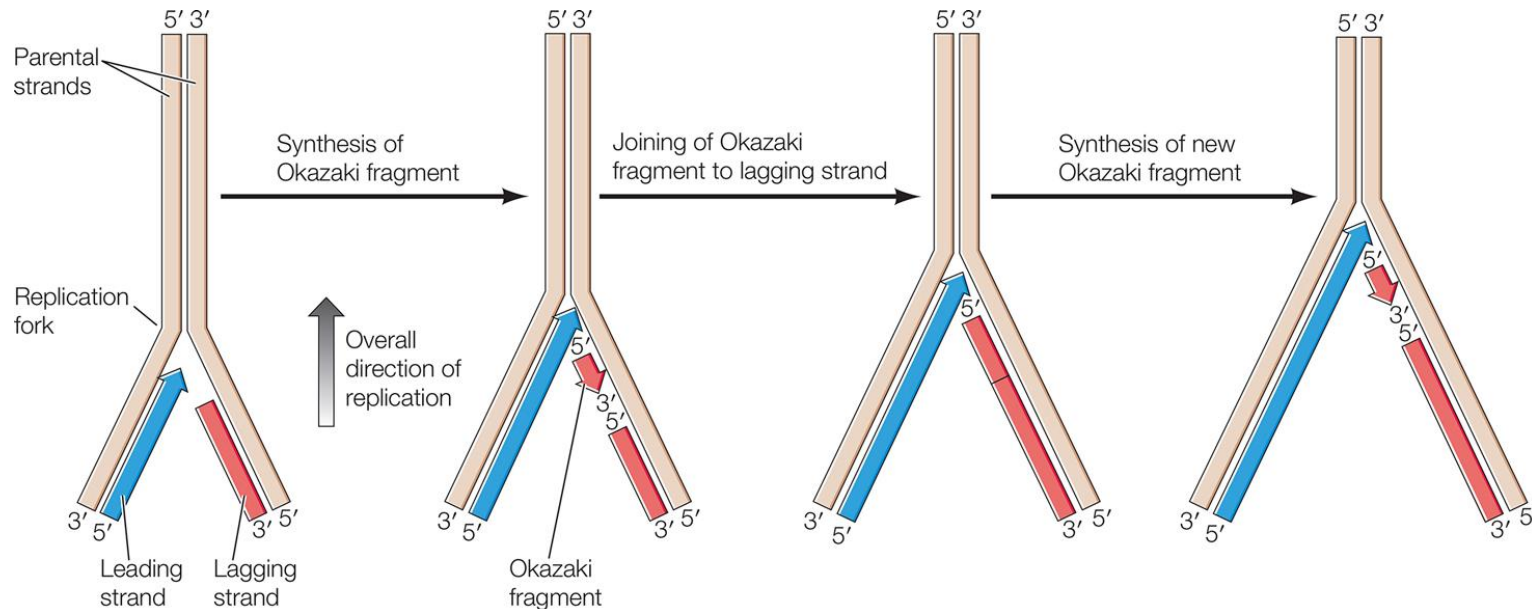
- When we add the nucleotides we need energy so the energy here is coming from the nucleotide itself
- the substrate or the incoming nucleotide is added to the 3' end of the newly synthesized strand by the enzyme DNA polymerase (notice the blue box)
- two phosphate groups are released generating energy when the new nucleotide is added so we end up with one phosphate group for each nucleotide and it's what connects them together by phosphodiester bonds
- Notice that we always add nucleotides from the 5' end to the 3' end and the new added nucleotide is the host for the next one
- The DNA strand on the right is anti parallel running from the 3' end to the 5' end and it's complementary to the strand on the left





# Continuity of DNA synthesis

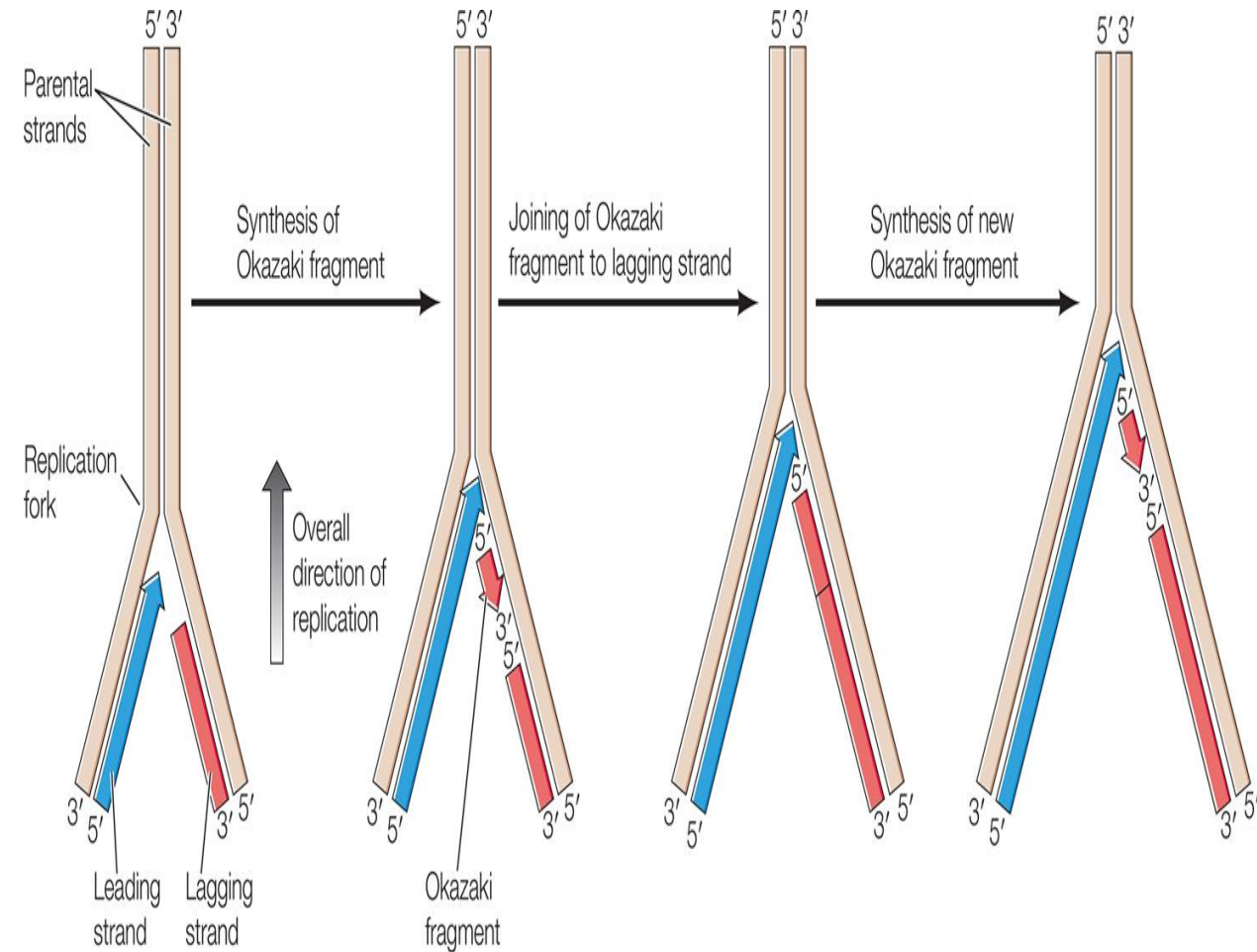
- The parental DNA is called a template.
- The new DNA is synthesized ONLY from the 5'-end to the 3'-end.
- One strand of DNA is continuously synthesized and called the leading strand.
- The other strand is synthesized discontinuously as shorter pieces known as Okazaki fragments and is called the lagging strand. *We call it this way as it's lagging behind and till the leading strand allows it to be synthesized*



Go to the next slide for explanation 9

# Continuity of DNA synthesis

- ❑ So what happens is that the leading strand is synthesized continuously and it opens the fork further allowing for the lagging strand to be synthesized
- ❑ The lagging strand is synthesized as fragments that are called Okazaki fragments once the leading strand opens up the fork for them and then those fragments are connected to each other by an enzyme called ligase to make up the lagging strand
- ❑ Extra note: the lagging strand is the strand that is running from the 3' end to 5' end as we said the double strands are anti parallel so one is 5' to 3' and the other is 3' to 5' and we said that the replication only happens from 5' to 3' that's what we form Okazaki fragments





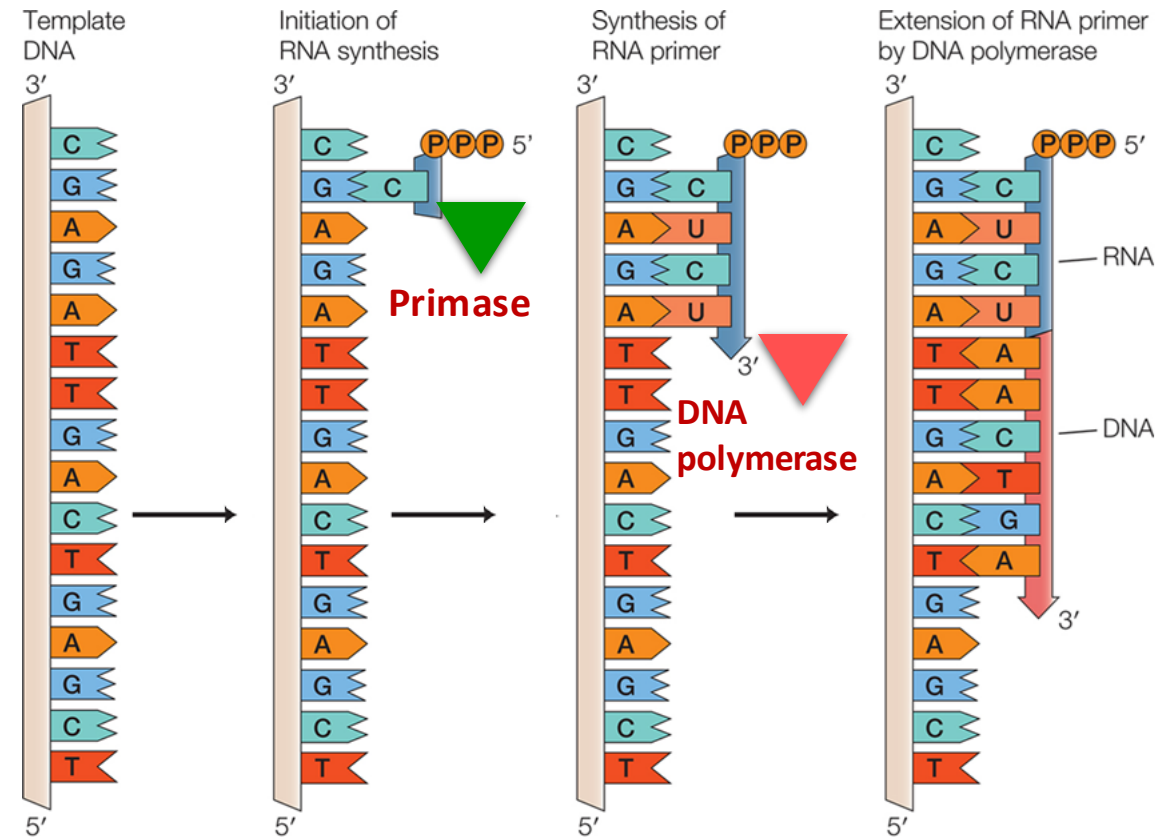
# Components of DNA replication

Take a deep breath =)

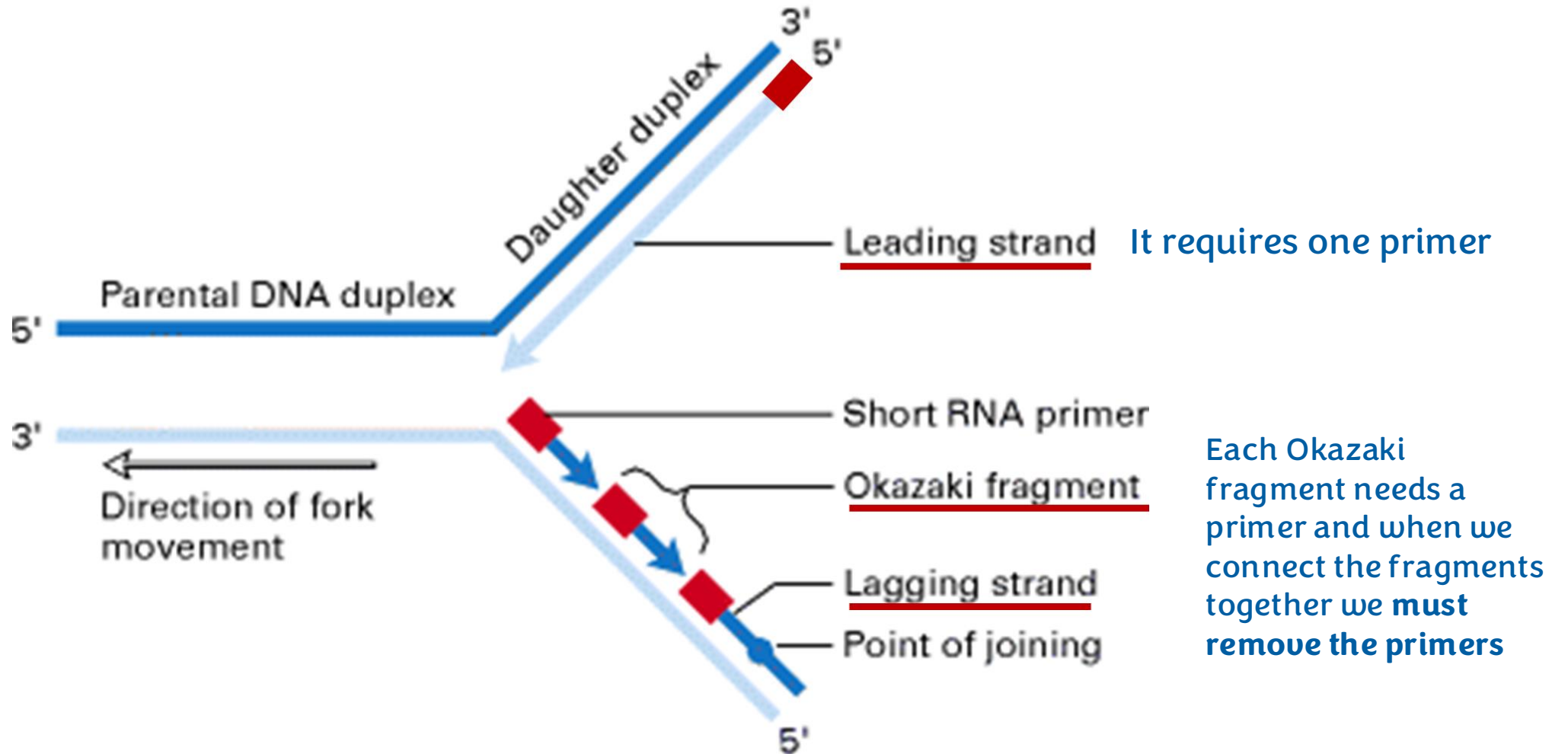
Now let's talk about the important molecules in DNA replication

# 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.
- To initiate DNA synthesis a RNA primer is first synthesized this primer is complementary to the DNA template but contains Uracil instead of Thiamine and uses ribonucleosides instead of the deoxyribonucleosides as substrates. Once the RNA primer is in place DNA polymerase binds to it and begins synthesizing the DNA strand

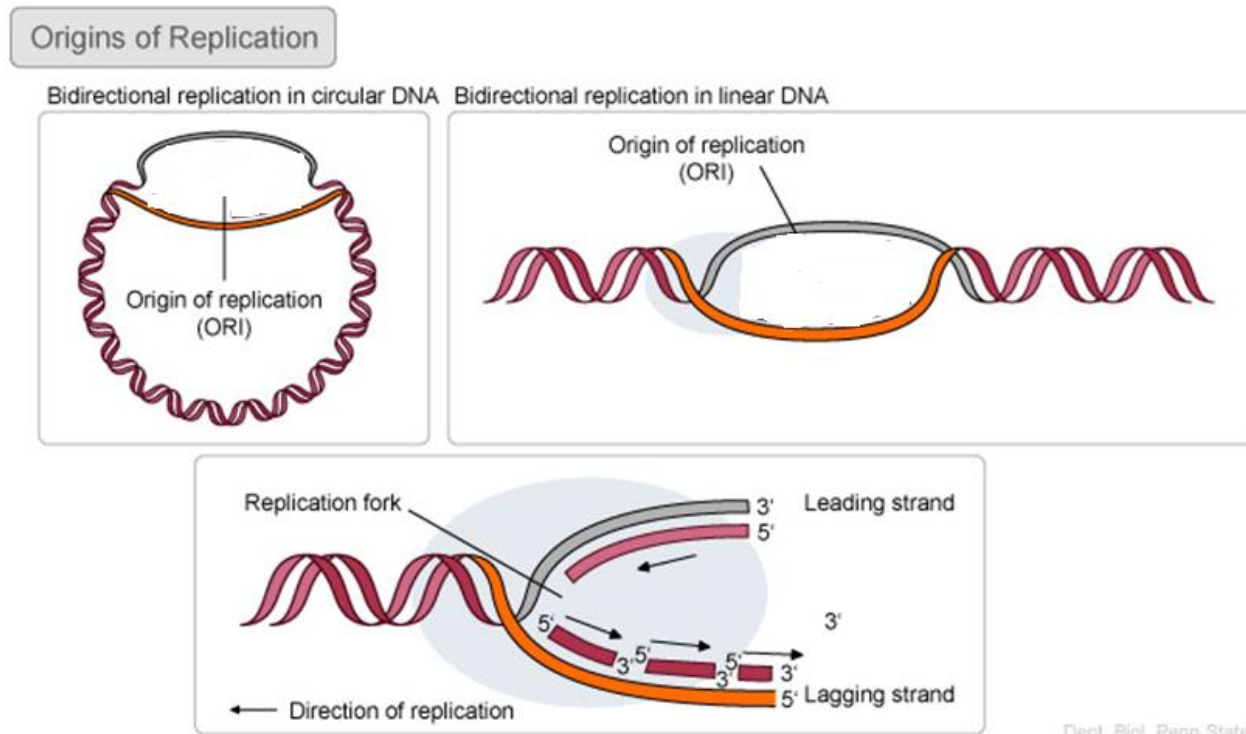


# The need of primers



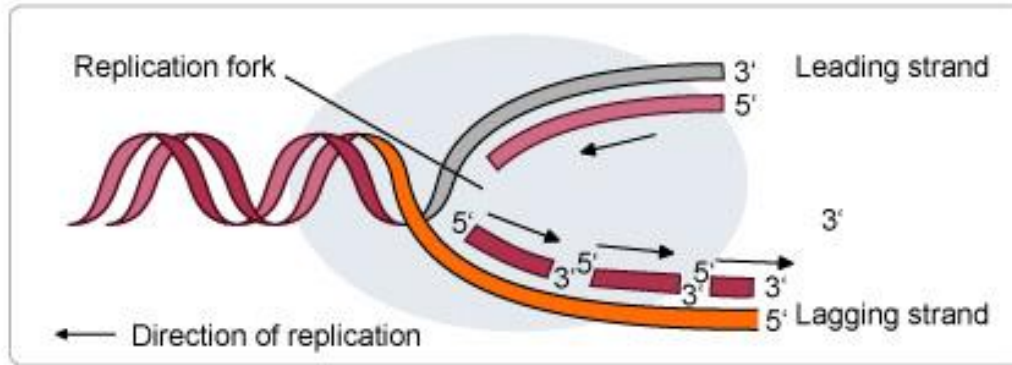
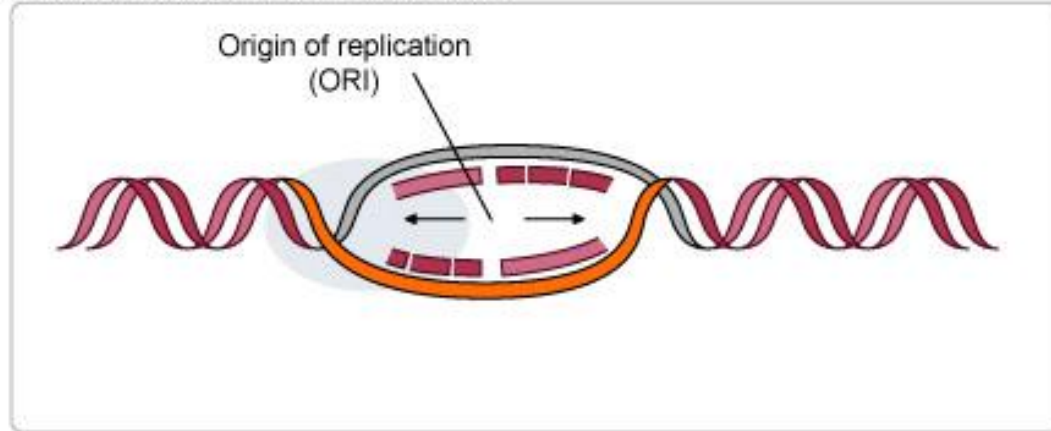
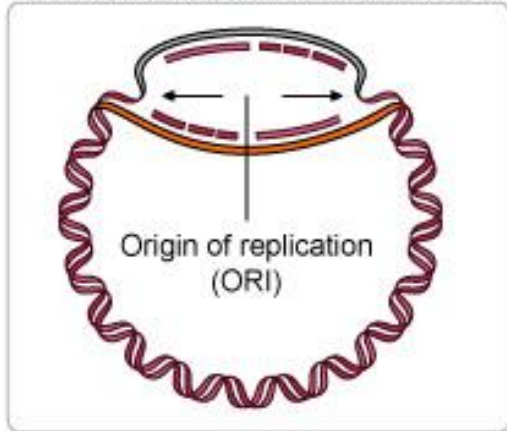
# Exercise

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

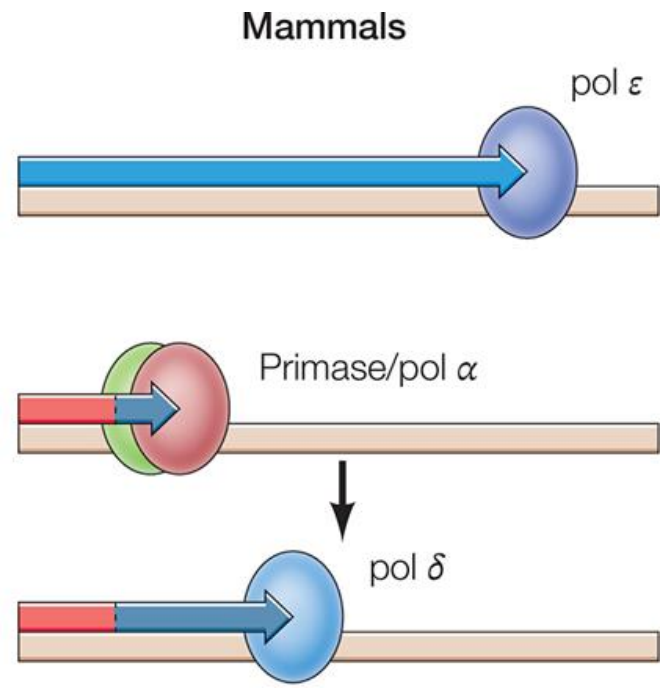
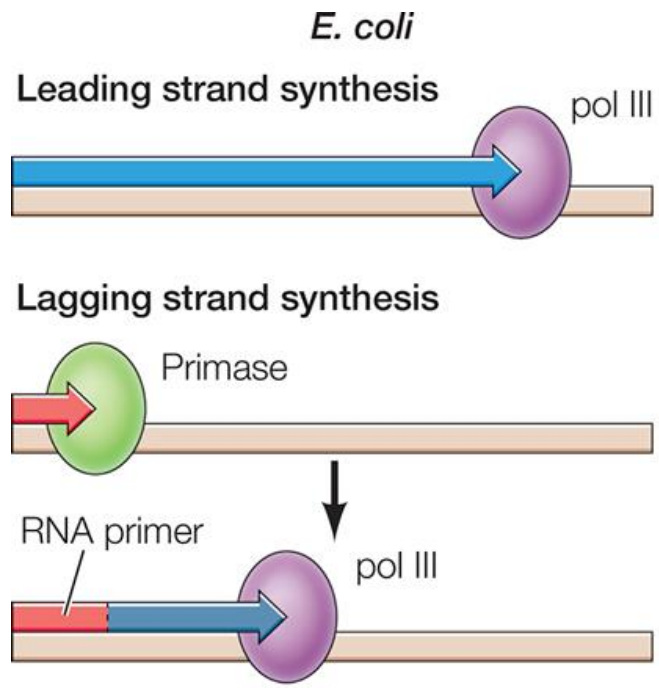


## Origins of Replication

Bidirectional replication in circular DNA    Bidirectional replication in linear DNA



# The replicative process



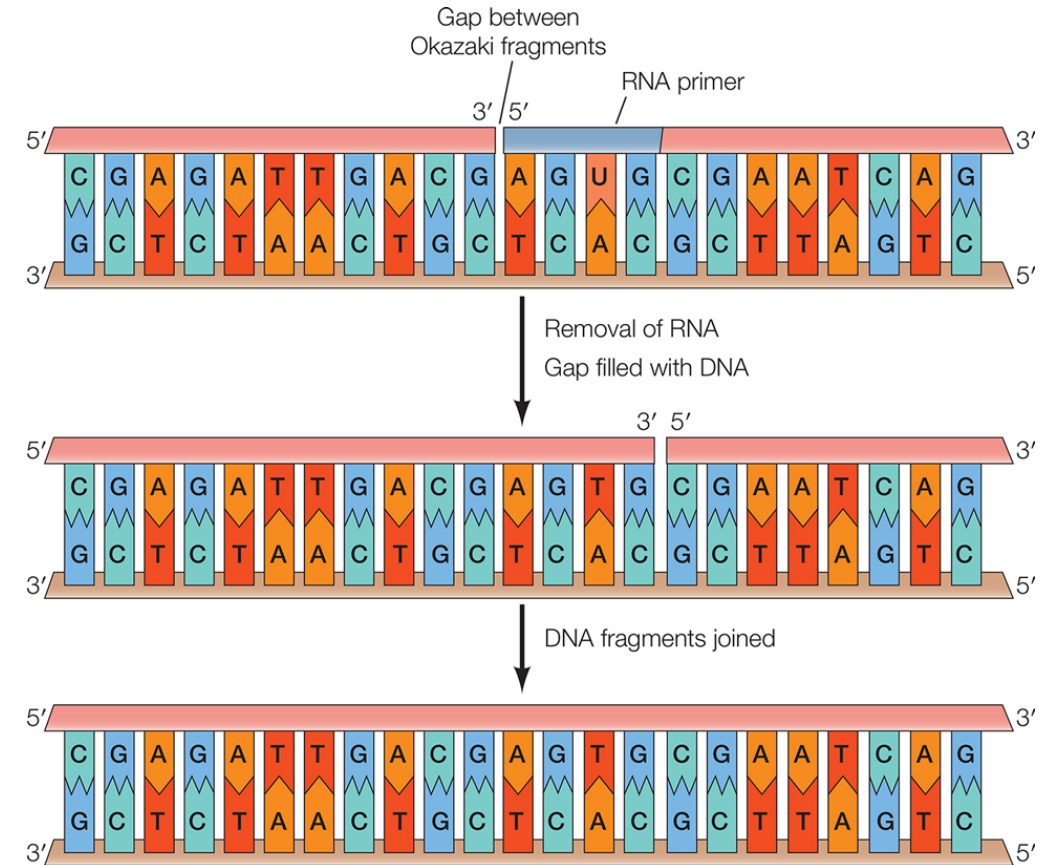
- the primase binds to the DNA polymerase alpha and they set on the DNA then the primase synthesize the primer so the DNA polymerase alpha can synthesize the first portion of DNA then they dissociate so the DNA polymerase delta comes and form the Okazaki fragment in the lagging strand and in the leading strand the DNA polymerase epsilon takes over

- In bacteria, DNA polymerase III is the major replicative enzyme
- In human cells:
- DNA polymerase  $\alpha$  is complexed with primase initiating the synthesis of DNA, and then
- DNA polymerase  $\epsilon$  synthesizes the leading strand.
- DNA polymerase  $\delta$  synthesizes the lagging strand.



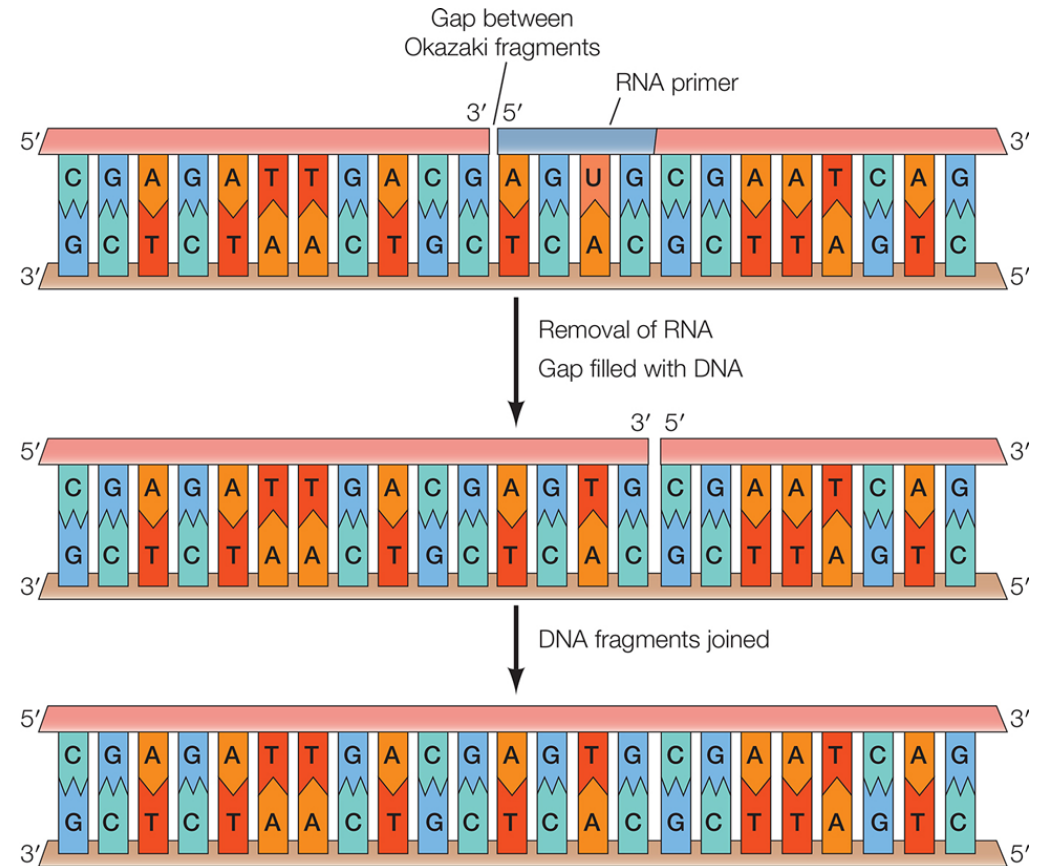
# Removal of primers

- 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
  - **Meaning that when DNA polymerase comes in it moves along the strand removing the primers from 5' to 3' and replacing deoxyribonucleoside instead of ribonucleoside**
  - A DNA polymerase activity where it fills in the gap.
  - **Then a ligase enzyme links them together**

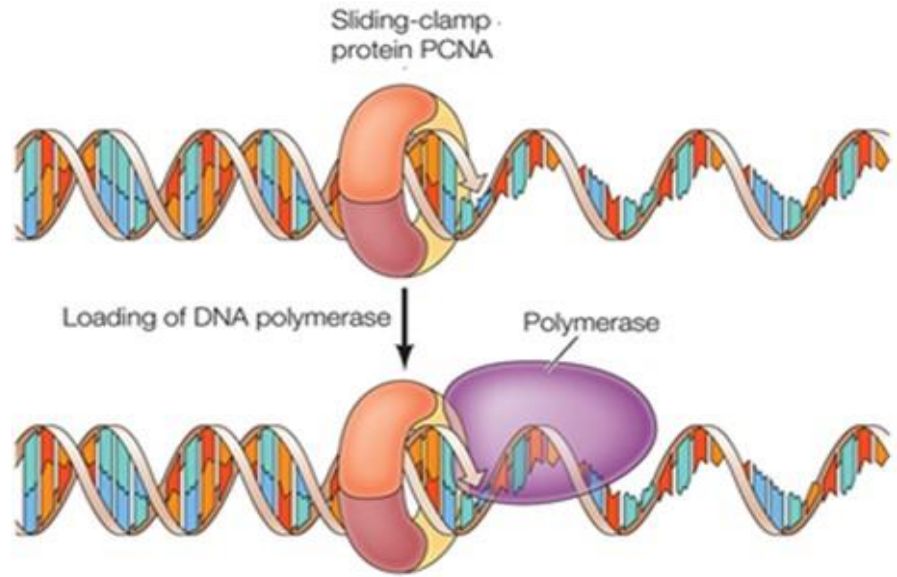


# Removal of primers

- In human cells, 3 enzymes are involved:
  - RNase H, a 5' to 3' exonuclease that removes the primers.
  - polymerase  $\delta$  that fills in the gaps
  - DNA ligase that joins the fragments.
- **Notice that the ligase is important in both humans and bacteria**



# Clamping and sliding

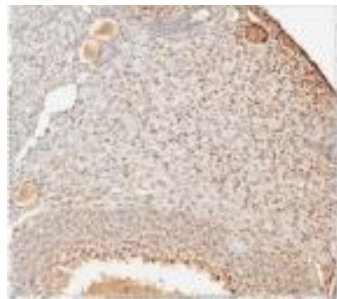


- The sliding-clamp protein, which is called proliferating cell nuclear antigen [PCNA] in human cells is associated with the major replicative polymerases loading them onto the primer and stabilizing their association with the DNA template.
- What it first do that it guides the DNA polymerase to where the primer is and it binds to the DNA polymerase as it's synthesizing the DNA so it strengthen the interaction between it and the DNA
- Note: PCNA is a diagnostic marker of proliferating cancer cells. And tells if the cancer cells are aggressive or not

Normal cells



Cancer cells



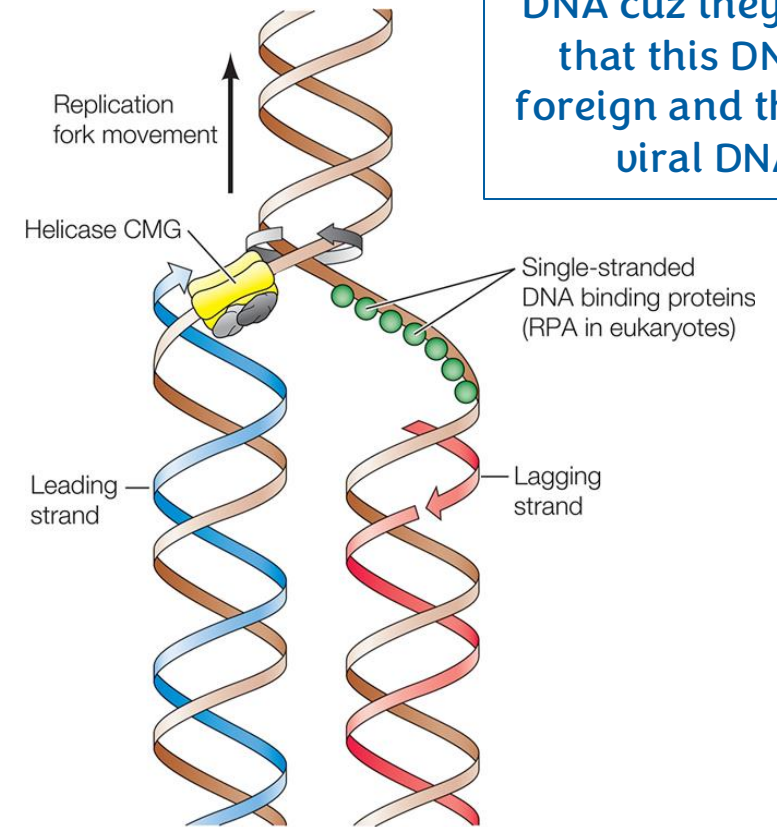
The brown color indicates the expression of a protein and as you can see in cancer cells there is more brown color so more protein is abundant mainly PCNA

# DNA helicases and SSB proteins

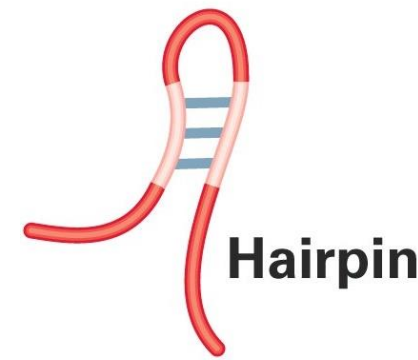
- The double-stranded DNA is opened up by DNA helicases

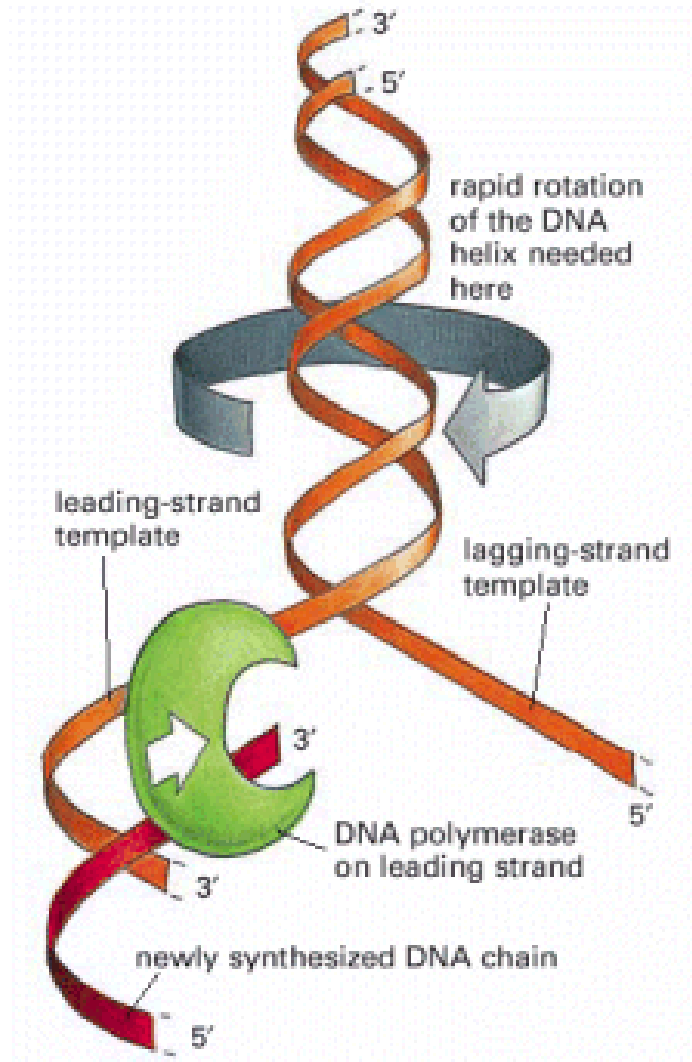
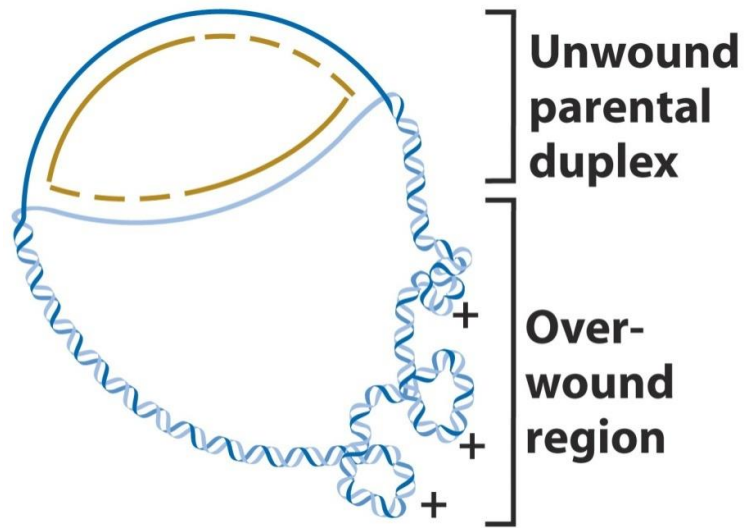
Allowing the DNA polymerase to read each strand

- single-stranded DNA-binding proteins called replication protein A (RPA) do these:
  - Prevent the formation of short hairpin structures,
  - DNA & RNA are dynamic molecules so if a part of the DNA was complementary with another one in the same strand it will form a hairpin shape which will block the DNA polymerase from moving forward as it thinks that this is a dsDNA and it can't synthesize DNA in this case
- Protect single-stranded DNA from being degraded, and
- Prevent the renaturation of DNA.
- Remember that the two strands are complementary to each other so they can come back and renature



Cells doesn't like to see a single stranded DNA cuz they think that this DNA is foreign and that it's viral DNA

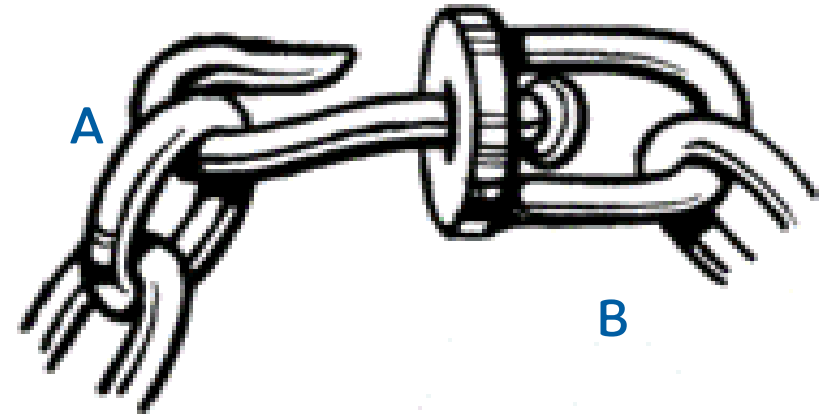
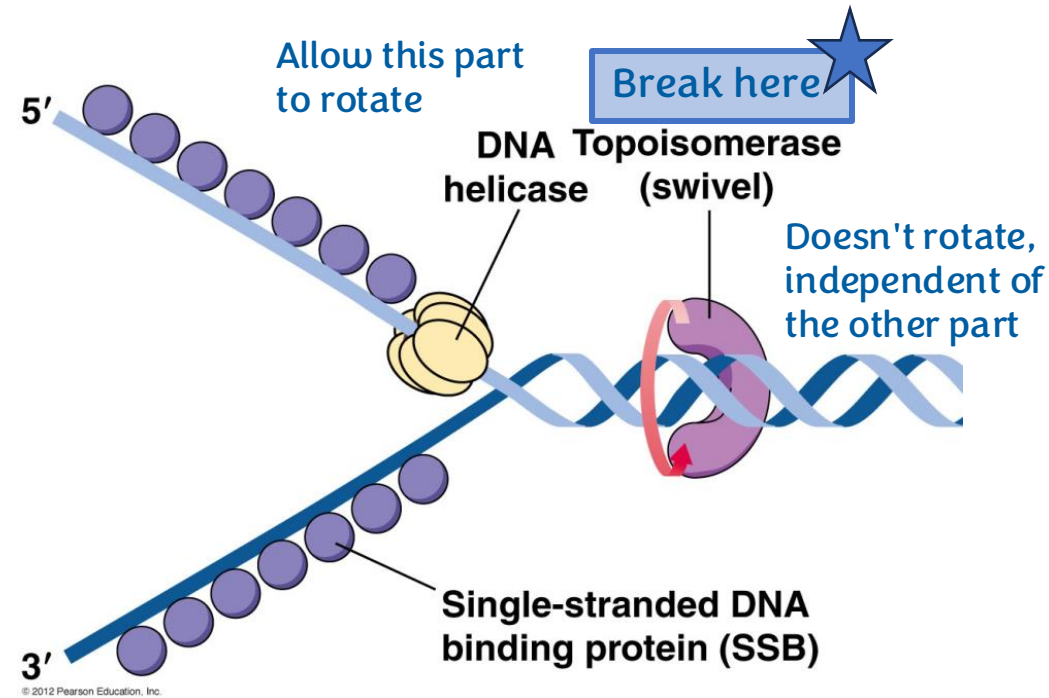
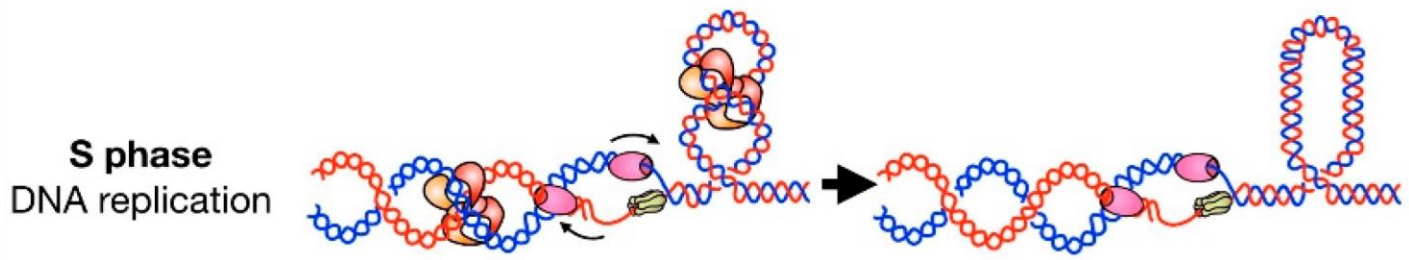




- We have another challenge which is when helicase separate the dsDNA it will cause a rotation in the regions that it didn't separate yet causing the formation of over wound regions whether it's a linear or circular DNA preventing the movement of DNA polymerase

# DNA topoisomerases

- It's an enzyme that changes the structure of a molecule. It doesn't add or remove groups, rather than that, it changes the position and structure of a molecule and it helps in removing the over wound regions
- They are found right ahead of the replication fork.
- 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.

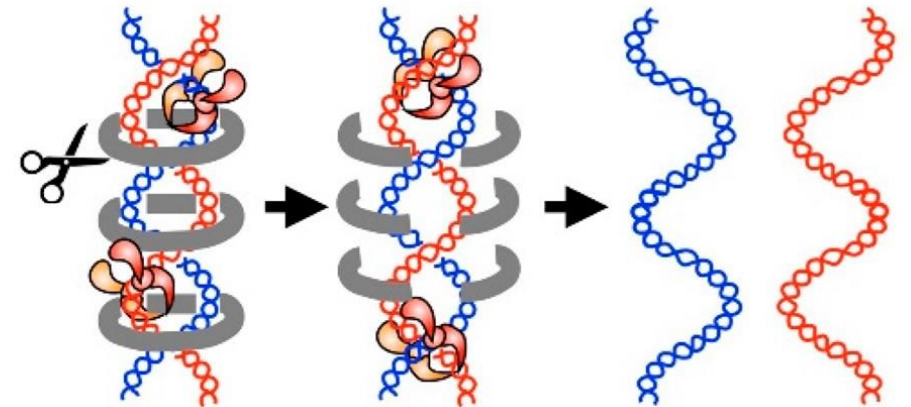
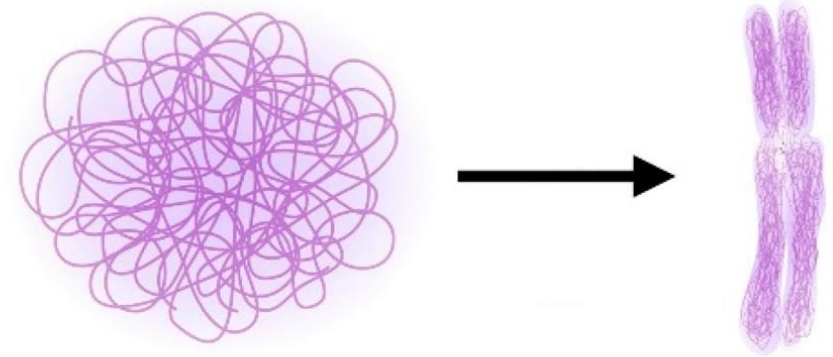


This is a swivel, where one half "A" could be rotated without affecting the other half "B". Topoisomerase functions similarly by rotating only a small portion of the DNA to remove overwound regions while maintaining the replication fork and the DNA ahead intact.

# Other functions of topoisomerase II

- Note: topoisomerase II is also required for
  - mitotic chromosome condensation
  - Right before cells divide, their DNA must be replicated, and the chromosomes must be condensed, this process is facilitated by topoisomerase II
  - the separation of daughter (**sister**) chromatids at mitosis.
- Since this enzyme has a role in DNA replication and in cell division, it's targeted by inhibitors to treat cancer by preventing cancer cells from dividing:
- Antineoplastic (**Anti-tumor**) anti-topoisomerase II inhibitors include:
  - Anthracyclines
  - Doxorubicin
  - Mitoxantrone

Chromatin converted into condensed chromosomes that are separated from each other (X shape)

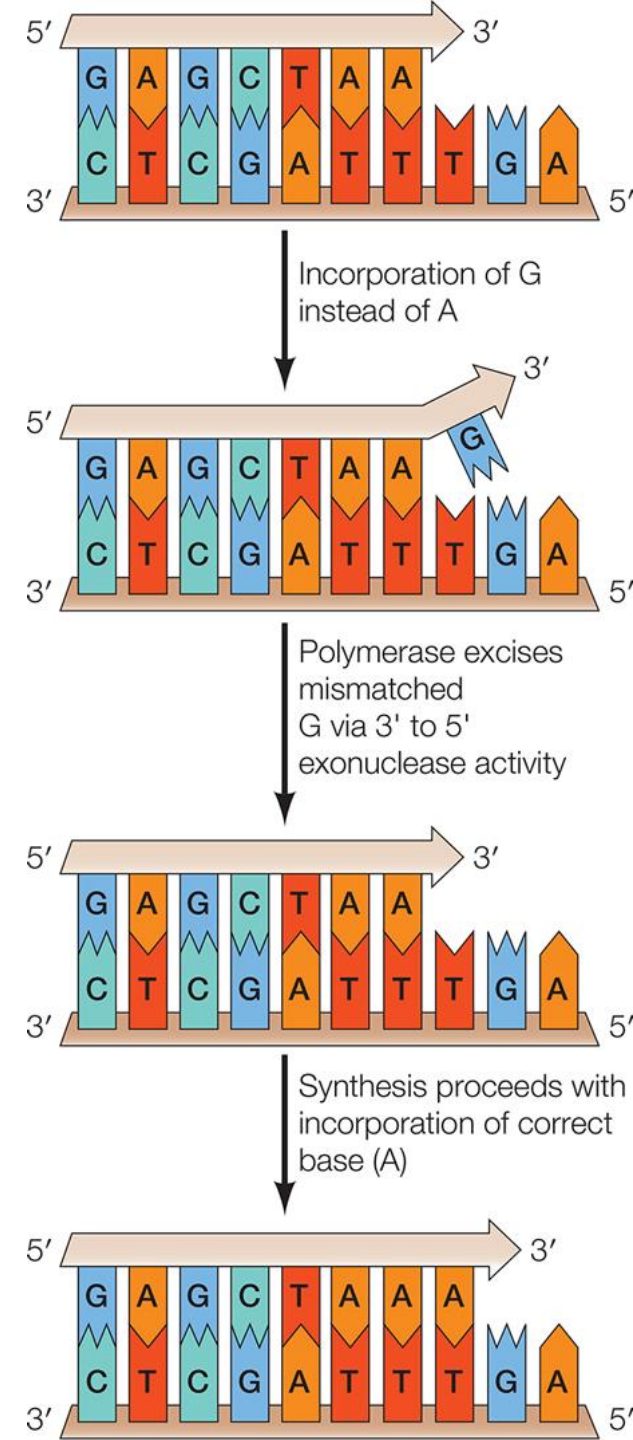


Separation of sister chromatids, ensuring that each chromatid moves to opposite poles of the cell during mitosis.

# How accurate is DNA replication?

سبحان الله وبحمده،  
سبحان الله العظيم

- DNA replication **MUST** be accurate, mistakes are not tolerated, as it is the basis of life.
- The frequency of errors during replication is only one incorrect base per  $10^9$  nucleotides incorporated.
- Since the human genome consists of 3 billion nucleotides, at the end of replication there are 3 mistakes only.
- How is accuracy high? **How is DNA's fidelity maintained?**
  - The DNA polymerase can catalyze the formation of the right phosphodiester bonds between the complementary bases with the proper hydrogen bonding (accuracy= $10^{-5}$ ).
    - **Meaning: 1 error per 10 thousand nucleotides added.**
    - **The enzyme itself senses if the two bases are complementary to each other or not. If not, it doesn't add the wrong nucleotide.**
  - Proofreading mechanism (a  $3' \rightarrow 5'$  exonuclease activity) increasing the accuracy to  $10^{-8}$ . **Done by the enzyme to correct itself by removing any misplaced nucleotides, it is in the backward direction.**
  - Repair mechanisms (to be discussed later)



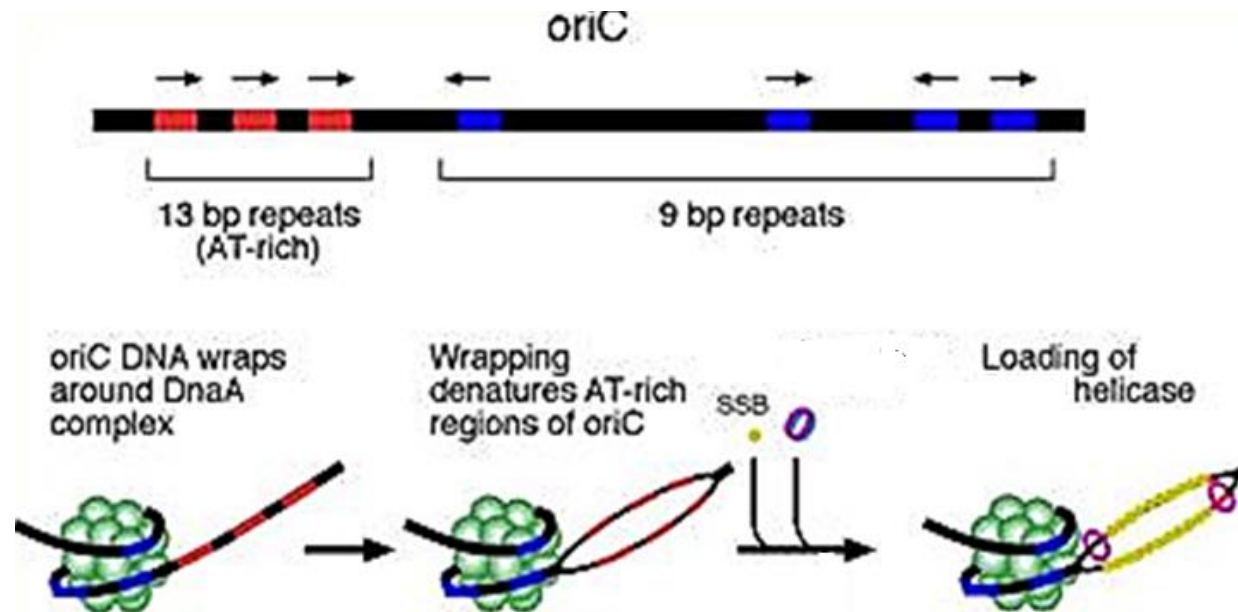


# Origin of replication (OriC) in bacteria

- 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** which are preserved DNA sequences, found in different DNA molecules of different species. A consensus sequence of genes means the same sequence is found in different genes These sequences are important, and they have different functions).
  - 9-mer: binding sites for DnaA protein.
  - 13-mers: AT-rich region - it facilitates separation of the double-stranded DNA.

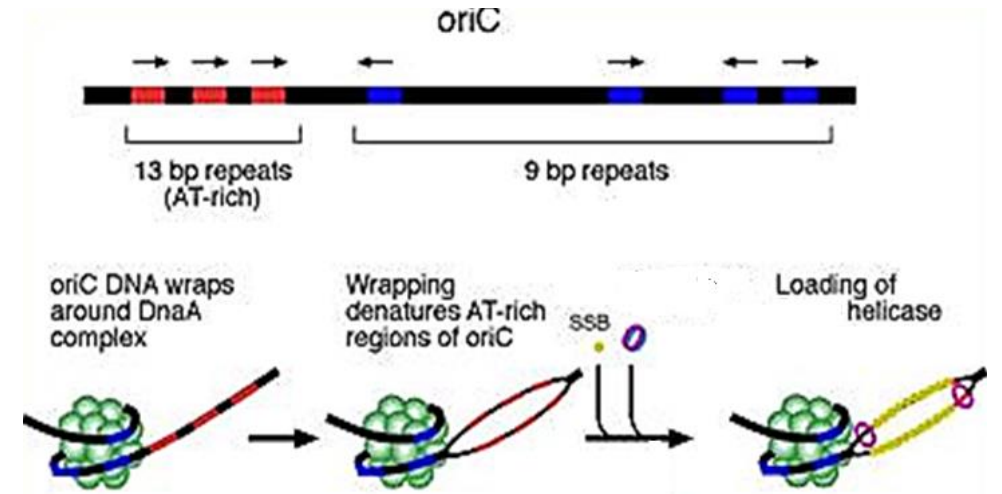
Mer = unit,  
Just like octamer, dimer etc.

- DnaA protein binds to 9-mers, applies stress on the AT-rich region, and OriC opens up.
- The helicase, SSB proteins and primase etc jump on, followed by the replication machinery.



# Origin of replication (OriC) in bacteria, Further explanation

- To explain the repetition pattern:
- Look at this diagram, it shows that inside of the OriC region there 2 consensus sequences that are repeated & function as follows:



- 9mer sequence aka 9 base pairs:

- This consensus sequence is repeated several times non-tandemly in different orientations within the short OriC region.
- It functions as binding sites for the protein DnaA.
- After binding to the 9mers, DnaA wraps the DNA around it, then it exerts pressure on 13mers (which have weak interactions between dsDNA) and so, this pressure will cause the AT-rich 13mer region to open up for replication to start.
- **Analogy: Think of a balloon:** if you press on one end of the balloon, the other end will inflate.

- 13mer sequence aka 13 base pairs:

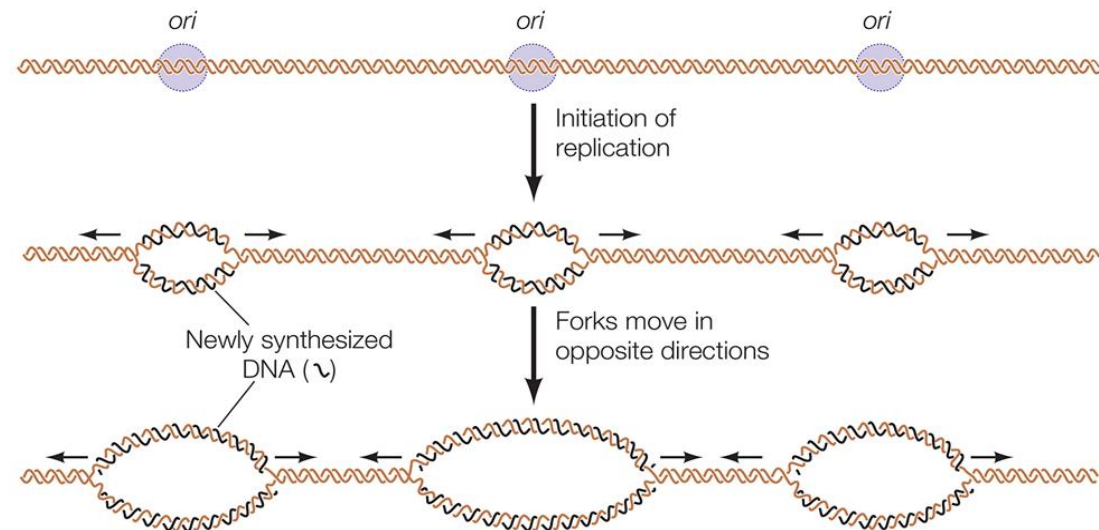
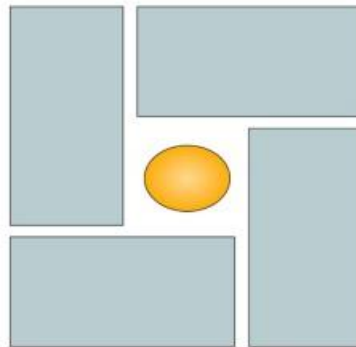
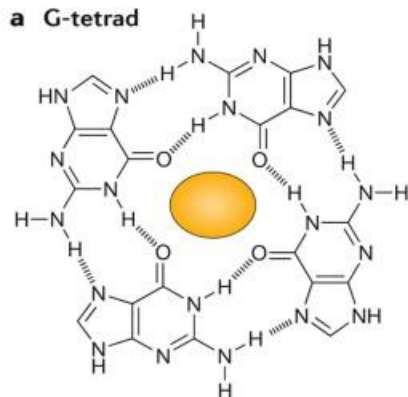
- This consensus sequence is repeated **tandemly** in one direction within OriC.
- **Tandemly** repeated means repeated one after the other with no interruptions.
- These sequences are AT rich, with 2 hydrogen bonds between A's & T's, so the interaction between the two strands isn't very strong.
- This weak interaction functions in facilitating the separation of dsDNA.
- After the opening of OriC, other molecules will jump on the open DNA to create the replication bubble

# Origins of replication in the human genome

- Unlike bacteria, which has 1 OriC that is sufficient for its replication:
- The human genome has about 30,000 origins of replication with the following characteristics/features:
  - G-rich sequences that can form G-quadruplex secondary structures. (4 G nucleotides form hydrogen bonds with each other.)
  - **Modified** histones that promote chromatin **decondensation** and activation of gene expression. The modification compromises the interaction between histones and DNA and weakens it, thus DNA opens up more easily.
  - Close proximity to actively transcribed gene.
  - Cell-specific

All of them work at a reasonably similar time. And since DNA replication is bidirectional, eventually they will meet each other, and fusion of newly-synthesized DNA will take place.

This is a G-quadruplex

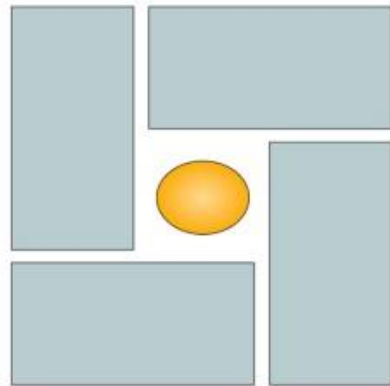
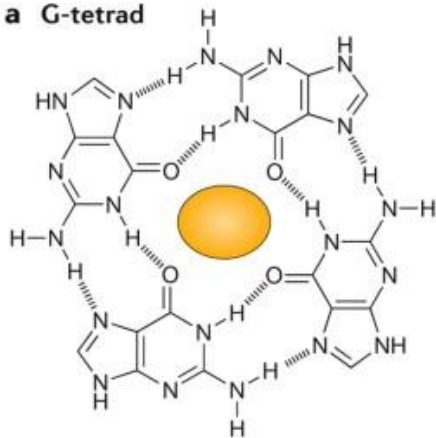


# Origins of replication in the human genome

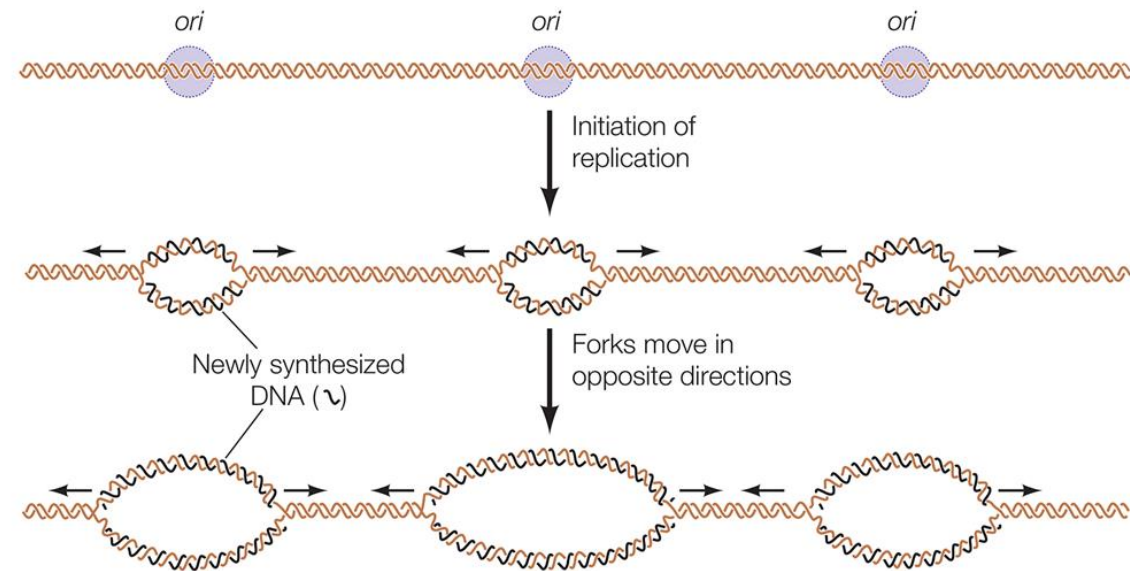
- The human genome has about 30,000 origins of replication with the following characteristics
  - G-rich sequences that can form G-quadruplex secondary structures.
  - **Modified** histones that promote chromatin **decondensation** and activation of gene expression.
  - Close proximity to actively transcribed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

This is important. Genes are not similarly active in all cells; some are cell-specific, like pancreatic or brain-specific genes, while others are commonly expressed in all cells. Similarly, the active origins of replication differ among cells, aligning with their specific actively expressed and common genes.

a G-tetrad



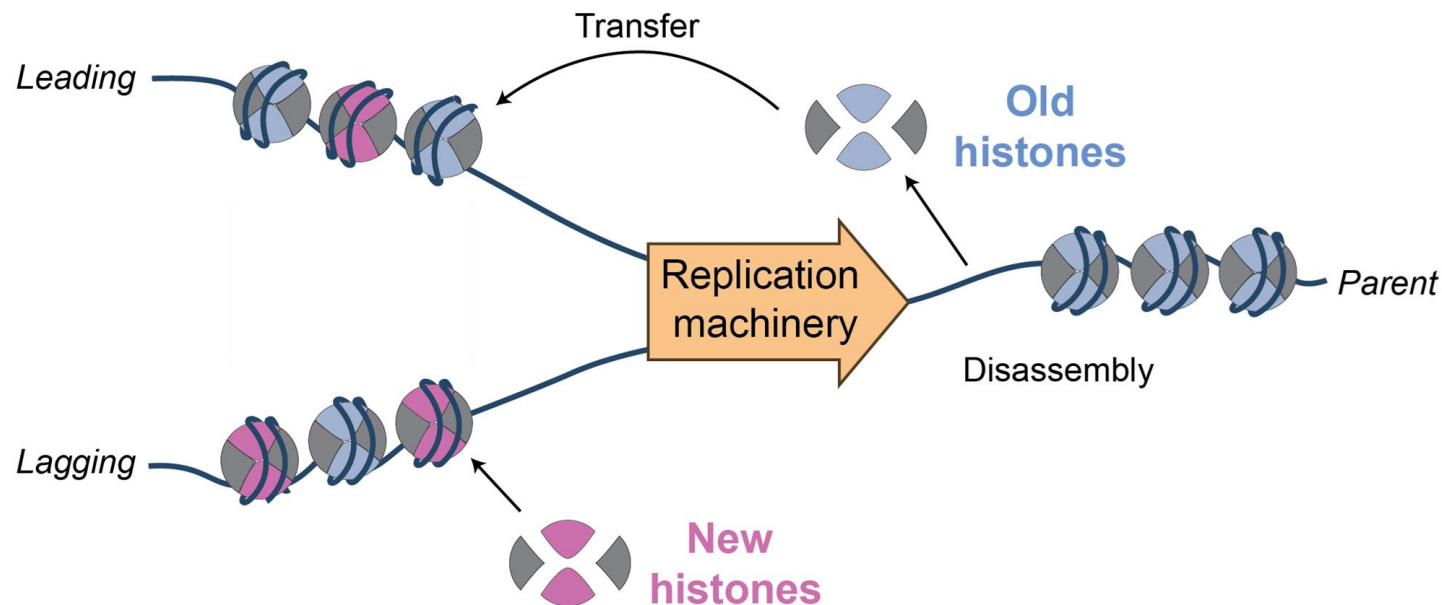
This is a G-quadruplex



# The formation of nucleosomes

## Problem #1

- 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.



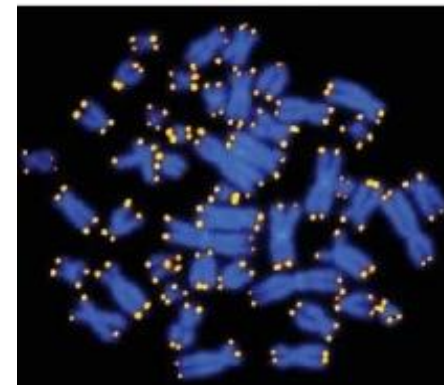
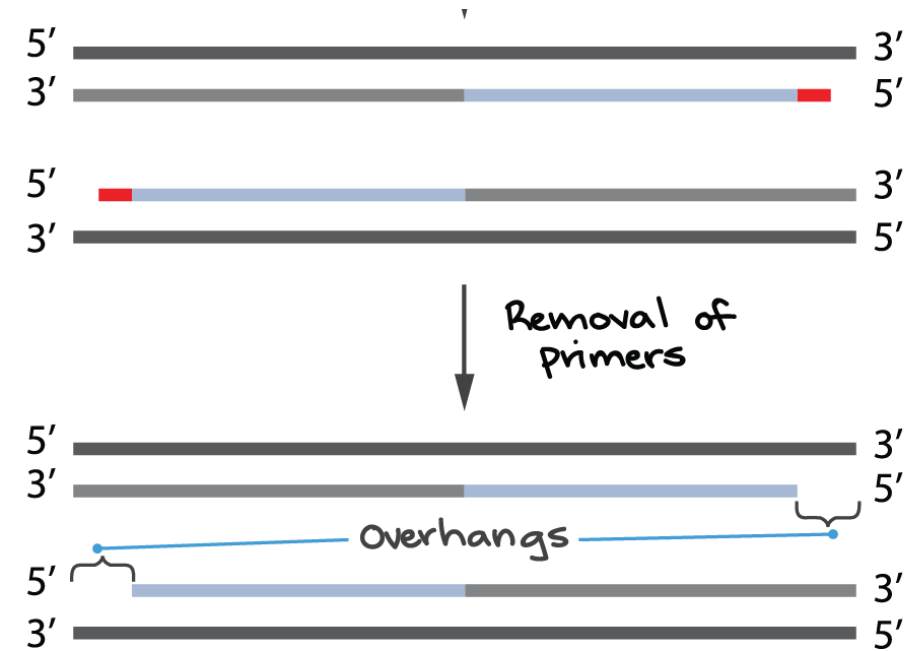
# The formation of nucleosomes

- This doesn't exist in bacteria because only eukaryotic DNA is organized as chromatin (DNA complexed with histones).
- As DNA doubles, **histones** must also double to maintain chromatin structure.
- To double histones, they are synthesized by transcription & translation, which can't take place simultaneously as DNA replication. So, to combat this, there are 4 **genetic clusters** that have the 65 histone encoding genes. Meaning that, DNA could be replicated at the same time as histone proteins are produced from a different DNA region (the clusters) that is not being replicated.
- There's continuous production of histones as the 65 histone encoding genes are actively transcribed.
- During DNA replication, histones are removed and then readded afterward. (assembly and disassembly of nucleosomes). This process is facilitated by **histone chaperons**.
- **Histone chaperones** can take old histones that were removed during the disassembly of nucleosomes and recycle them to assemble new nucleosomes. They can also take new histones and assemble them on the newly replicated DNA.

# A problem in the lagging strand

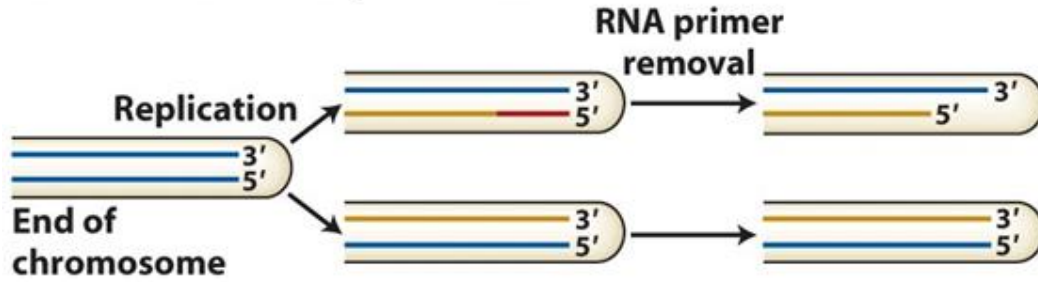
## Problem #2

- Since DNA is synthesized in an antiparallel manner, the lagging strand synthesis faces a challenge: after primer RNA is added, and okazaki fragments are synthesized and ligased, then:
- 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.

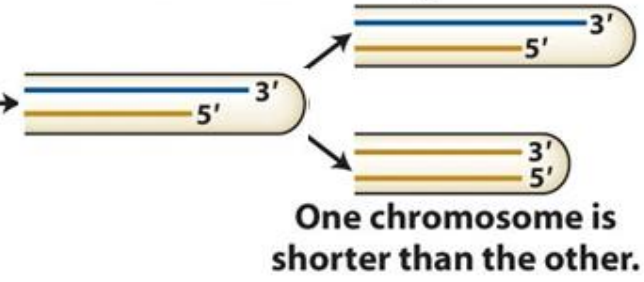


"no room" for DNA polymerase to fill the gap happens because there's No upstream 3' hydroxyl group available to replace the final RNA primer with DNA, as DNA polymerase can only add in 3' to 5' direction.

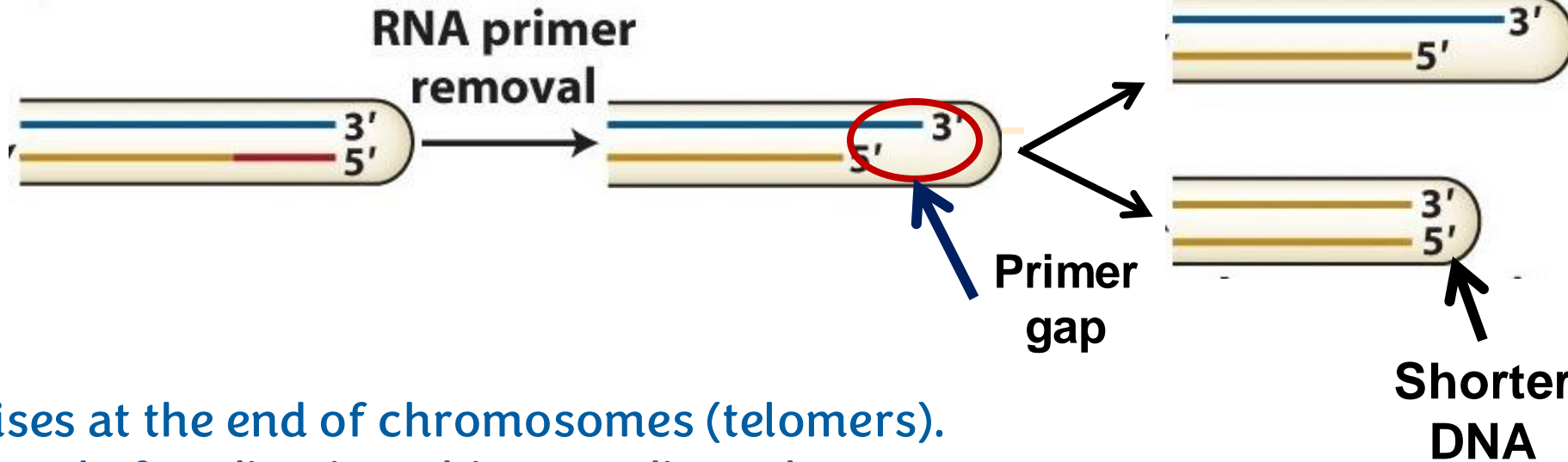
### First round of replication



### Second round of replication



Notice the 3' overhang, the end of DNA is a single-stranded 3' end, that loops around shelterin proteins.



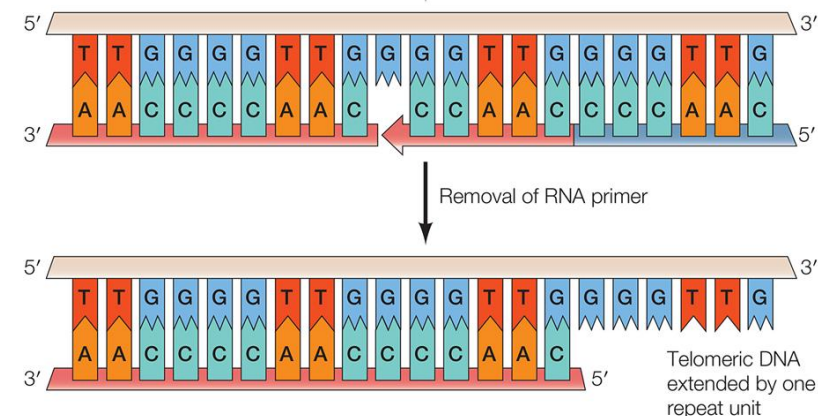
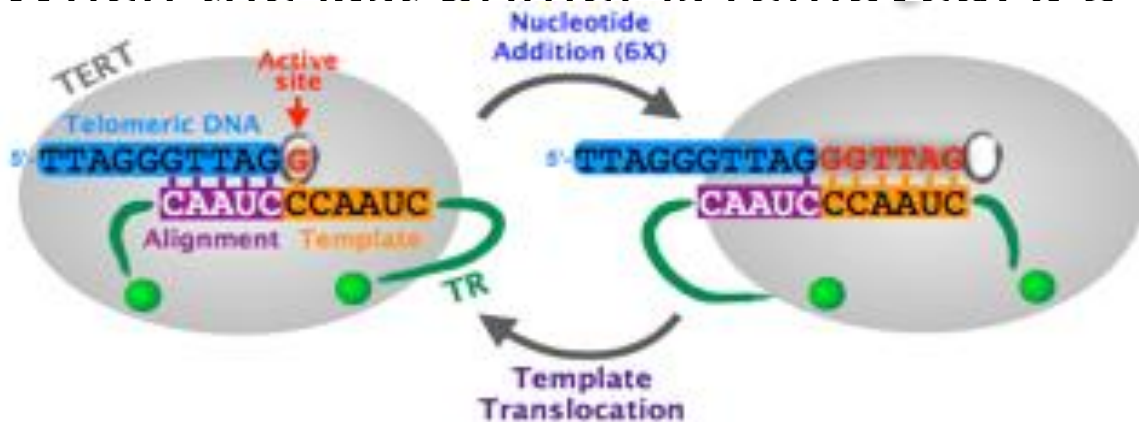
This issue arises at the end of chromosomes (telomers). With each round of replication, this unreplicated DNA leads to chromosome shortening. Over time, this affects genome stability, making chromosomes more prone to mutations, breaks, and damage. This process contributes to aging, as shorter chromosomes destabilize cells and lead to cell death.

Chromosome shortening is linked to aging. As we age, chromosomes get shorter and shorter, destabilizing cells. This process raises a question: why are we not dead before birth, given the extensive DNA replication during development? (next slide)



# Telomerase comes to the rescue The solution

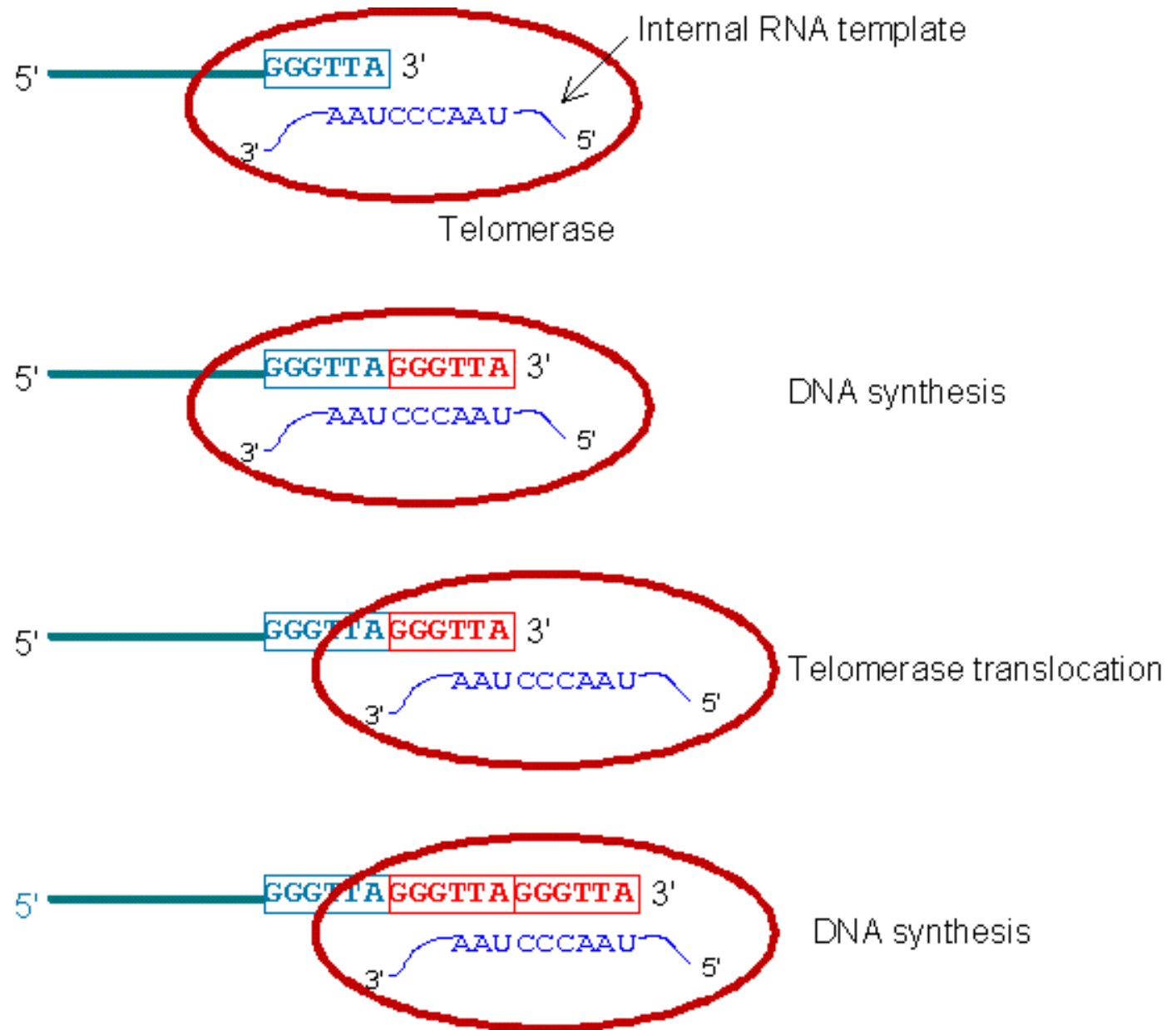
- An enzyme that elongates the lagging strand.
- Telomere DNA sequences consist of many GGGTTA repeats extending about 10,000 nucleotides.
- Telomerase (a **reverse transcriptase**) prevents the progressive shortening of the lagging strand. How?
- Telomerase elongates it in the 5'-to-3' direction using **an RNA template (primer)** that is a component of the enzyme itself. This makes it a ribonucleoprotein (a holoprotein, part of it composed of protein and the other part of RNA)
- When the last primer is removed, a 3'-overhang is left. (next slide for further explanation)



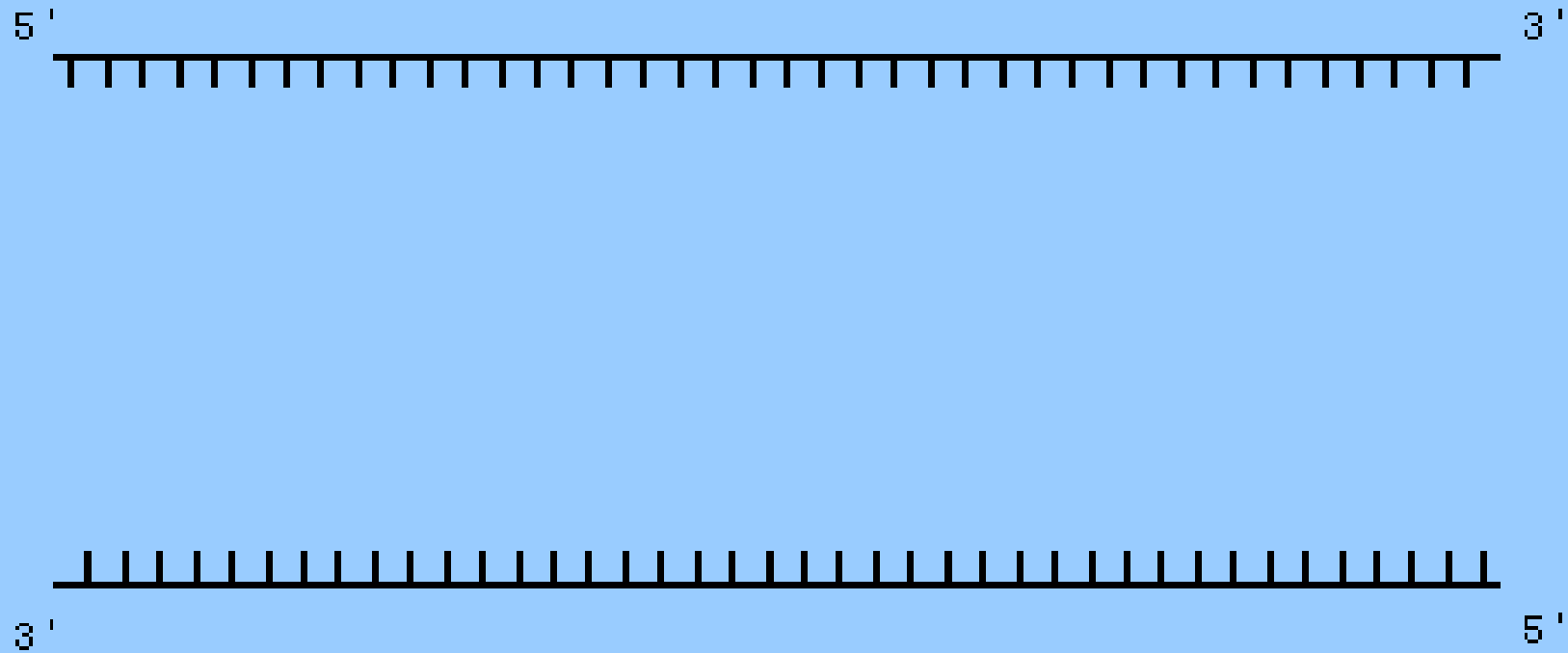
Telomerase enzyme uses its primer to elongate DNA. This is why it's a reverse transcriptase (uses an RNA template to synthesize DNA).

### Mechanism of Action:

- **Telomerase** binds to 3' overhang and **elongates** the DNA at the telomere by repeating the sequence GGGTTAG (which characterizes telomeres) multiple times.
- Once the DNA is elongated, **primase** has enough space to add a new primer, enabling **DNA polymerase** to synthesize another Okazaki fragment.
- This process keeps the DNA at telomeres long enough to stabilize the chromosome.
- **Telomeres** end with a **3' overhang**, which is single-stranded and loops around proteins like **shelterin** to form a protective cap.
- This looping protects chromosome ends from being recognized as DNA breaks.  
(discussed in the genome lecture)



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

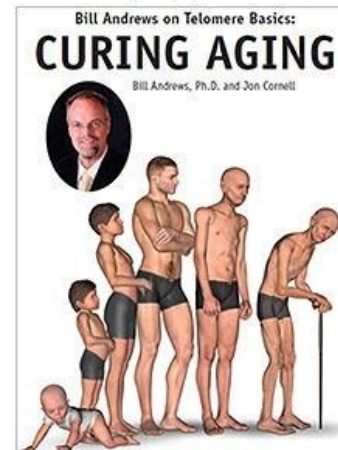
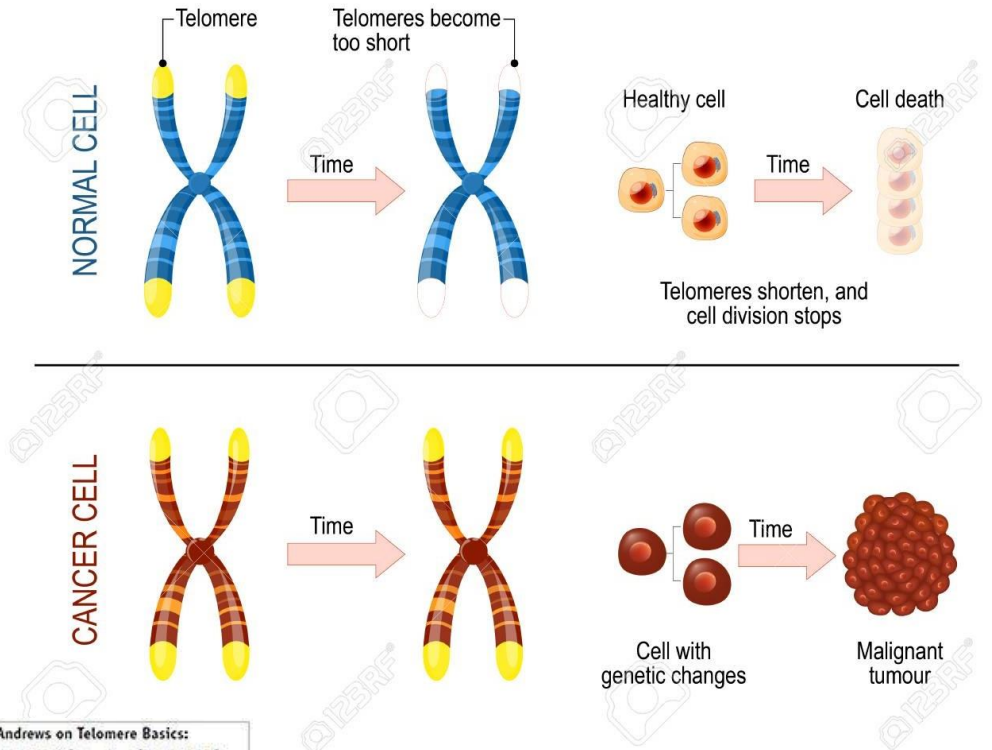


[Link to animation](#)  
:)

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

# Facts of life about telomerases

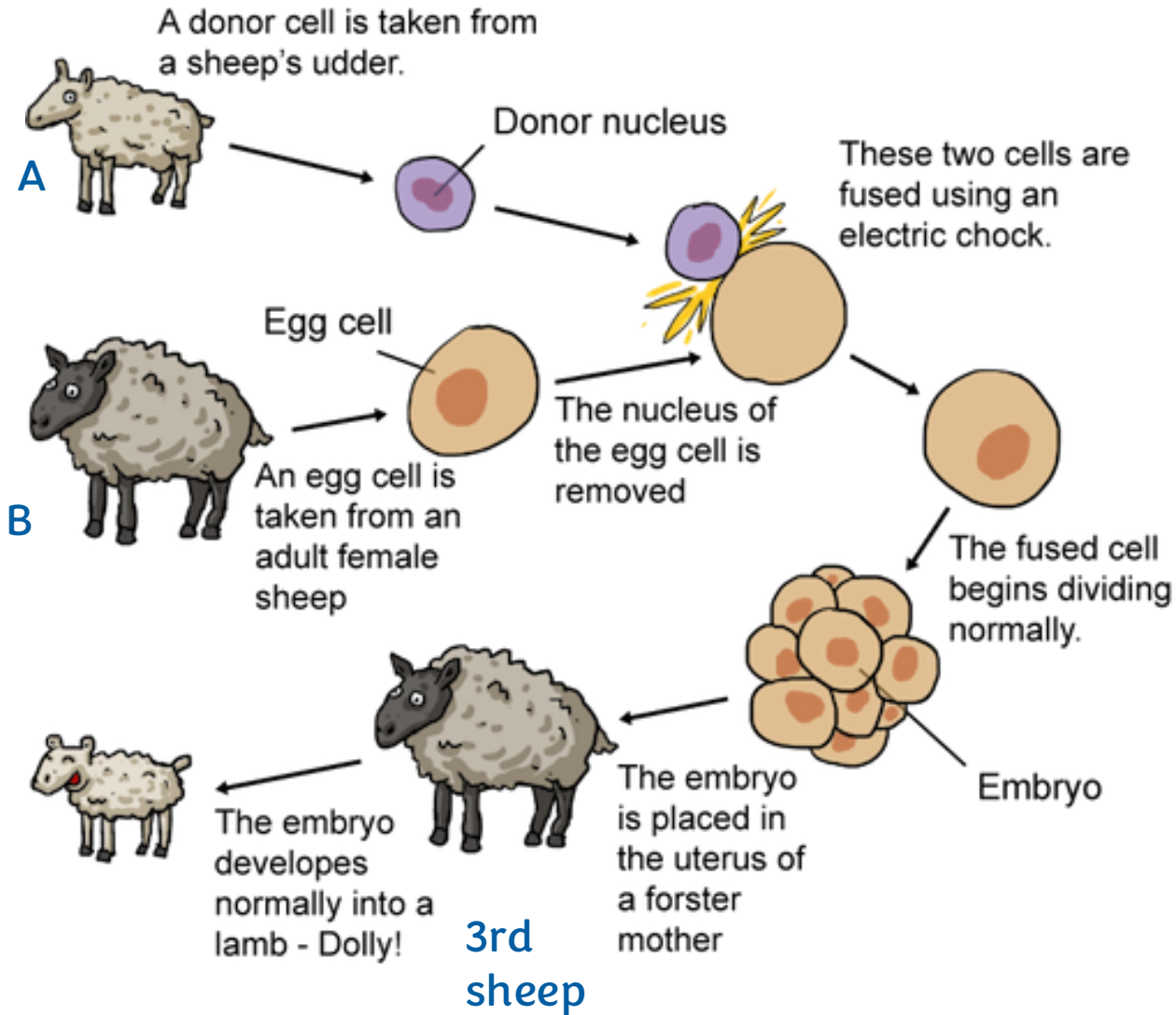
- Most somatic cells do not have high levels of telomerase and, hence, have a finite number of cell divisions.
- As we grow older, the **levels** and **activity** of telomerase are reduced.
- The gradual shortening of the chromosome ends leads to senescence and cell death.
- Germline (**stem cells**) and cancer cells express high levels **and activity** of telomerase. **This is what makes them immortal. As chromosomes do not get shorter as they divide**



# Dolly, the sheep



The first mammal ever to be cloned.



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

Scottish scientists took an egg from a donor "A" and stripped it from its nucleus.

Then a nucleus from a cell was taken from another donor "B".

The nucleus taken from donor "B" was inserted into the egg cell from donor "A" and the egg was fertilized.

The resulting zygote was implanted within a third sheep, and dolly came out to be a clone of donor "A" that gave up its genetic information to dolly.

Interesting to note that, the DNA taken from donor "A" was 6 years old, so the telomeres were already shortened, this was thought to be a contributing factor to why dolly lived shorter (her DNA was already 6yrs old and she lived for 6 additional years)

But it turns out this wasn't the case :) dolly died because of an infection

# # DNA Replication Overview



For any feedback, scan the code or click on it.



Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

# Additional Resources:

# رسالة من الفريق العلمي:

## Reference Used:

(numbered in order as cited in the text)

1. The cell book

## Extra References for the Reader to Use:

1. [Dolly, the Sheep](#) The NewYorkTimes
2. [DNA replication](#) Animation
3. [DNA replication](#) Ninja Nerd
4. [DNA replication](#) Prof. Dave
5. [Telomerase](#) YTV
6. [Telomerase](#) YTV
7. [Telomerase](#) Animation

بعد معركة الميد الكل تعبان ومنهك ويمكن بعضنا فقد الحماس  
بسبب درجة ما توقعها أو استنفذ كل طاقته في الامتحانات بس لا  
تنسوا هاد كان اقل من نصف الطريق، قوموا واستجمعوا حالكم!  
إذا درجتك كانت منخفضة خليها دافع للكفاح، إذا كانت عالية  
احسنت بس خليها فعلا حافظ للاستمرار بنفس القوة وتذكر دائما:  
إذا ما ضحيت بالأشياء الصغيرة الي بتحب تعملها كل يوم راح  
تضحى باشي بتحلم فيه طول عمرك  
شد حالك وادرس!



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

فَلَمَّا نَسُوا مَا ذُكِّرُوا بِهِ فَتَحْنَا عَلَيْهِمُ أَبْوَابَ كُلِّ شَيْءٍ

حَتَّى إِذَا فَرِحُوا بِمَا أُوتُوا أَخَذْنَاهُمْ بَغْتَةً فَإِذَا هُمْ

مُبْلِسُونَ ﴿٤٤﴾