Sheet no. 6

Molecular biology

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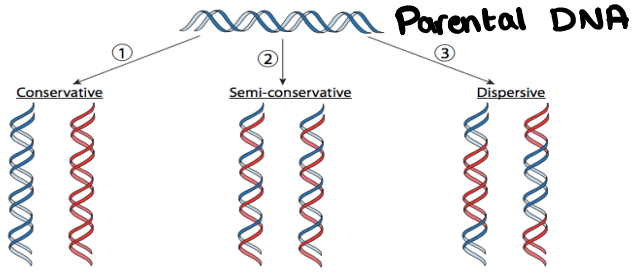
*The entire DNA content of the cell (or an organism) is known as genome.
DNA is organized into chromosomes.

- **Bacterial genome**: usually one and circular chromosome.
- **Eukaryotic genome**: multiple, linear chromosomes complexed with proteins known as histones.(and that's how the DNA is packaged inside the nuclei)

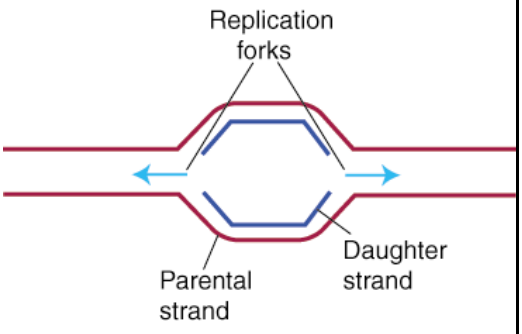
DNA must be accurately copied (replicated) in order to divide but this is all catalyzed by enzymes. DNA polymerase (the enzyme)
DNA synthesis(replication) is carried out by DNA polymerases (collection of enzymes each performing a certain function)
The substrates "in which the enzymes act on" are deoxyribonucleotides. (deoxy TTP, ATP, GTP, CTP)

- Scientists came up with three different models as they didn't know the process of replication.
  *__conservative model__: the parental chromosome is conserved, when replicating it produces two daughter DNA molecules one contains the both original strands and the other has the newly synthesized ones.
  *__semi-conservative model__ __(actual DNA)__: two strands are separated each one is copied separately so the newly synthesized DNA contains one new strand and an old one.
  *__dispersive model__:newly synthesized DNA has bits and pieces of old and new strands



*DNA is replicated in a bi-directional manner, meaning that it starts at a single point and continues in two different direction, also mentioning that the replication starts from 5' end continuing to the 3' end so any addition is on the 3' end.

*this is called a replication bubble. *Y-shaped structure, active region is called a replication fork.

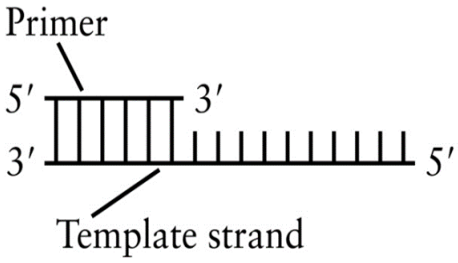


*each strand is synthesized in a specific way.
--In one strand the new strand is synthesized continuously from (5'-3') and in the other strand the DNA is synthesized in a discontinuous manner as short fragment (Okazaki fragments) so it can be synthesized in the direction of 5'-3' and the eventually merge with each other forming the

LAGGING STRAND, the continuously synthesized strand is called the leading strand as it leads the way and opens up the fork allowing a new sort fragment to be formed.
(the lagging strand waits until the leading strand opens up the way)
(this allows the synthesis of both of the strands in the 5’ – 3’ direction)

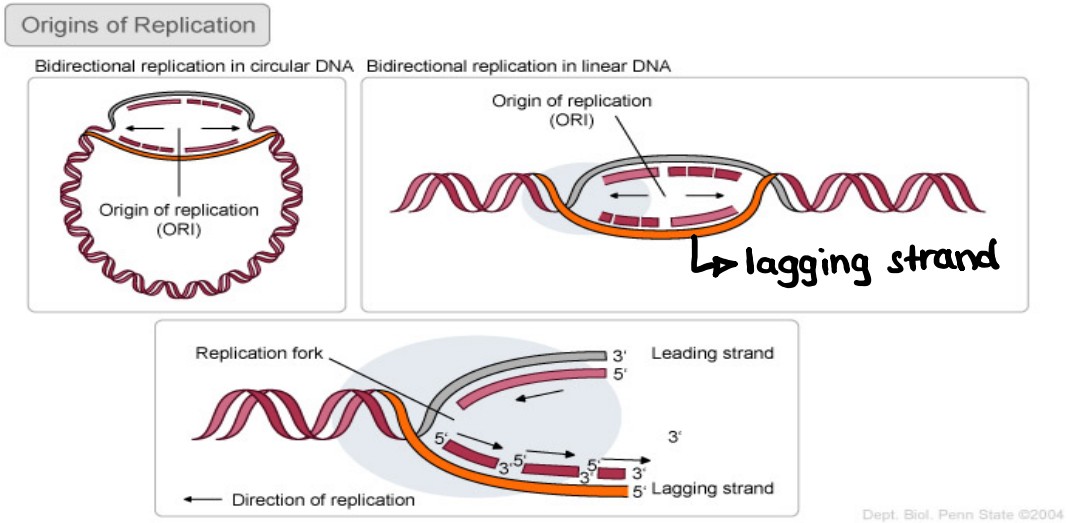
The synthesis reaction needs energy, this energy comes from the substrate itself,deoxyribo nucleoside triphosphate, when the new nucleotide is added by the polymerase the energy is released as the bonds faces cleavage. (Two phosphate are released “pyrophosphate”)

- Components of DNA replication
  1- RNA PRIMER (3-10 nucleotides long); to start the replication as the DNA polymerase cannot initiate the replication process from scratch (de novo).



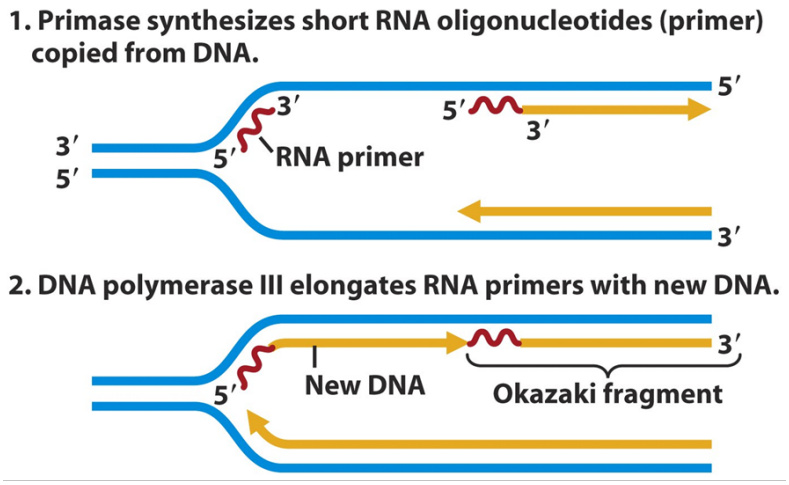
-It is synthesized by a primase, in the direction of 5’ to 3’.
- The idea is that the primer attaches at the first part of the template strand to start the replication then the DNA polymerase attaches and continues DNA synthesis.

Each leading strand needs one primer, but each okazaki fragment needs a primer of it own.



you should be able to know how to draw the leading and the lagging strands and the okazaki fragments in the right direction

- Lagging strand formation:
  After RNA primer is synthesized by the primase it attaches to the 5’ end allowing DNA to be synthesized and a new okazaki fragment to be formed and what actually elongates the RNA primer is **DNA polymerase III** (it synthesizes each okazaki fragment) and the elongation continues until it reaches the following RNA primer, so now we have many okazaki fragments but they need to be linked by removing the RNA primer at the 5’ end and continues to synthesize DNA in its place.
  DNA polymerase I is responsible for removing the RNA primer.
  DNA ligase is responsible for connecting the adjacent okazaki fragments together.



- Prokaryotic enz. → DNA polymerase → Protein → DNA helicase

- Eukaryotic enz. → RNAH

*removal of the primer requires an enzymatic activity which is the exonuclease(nuclease is an enzyme that degrades the nucleic acid "DNA) SO what DNA POLYMERASE I does is filling the gap caused by the removal of primer.

*the direction of the exonuclease activity is from 5' – 3'

2-DNA helicases and SSB (single strand DNA binding) proteins :

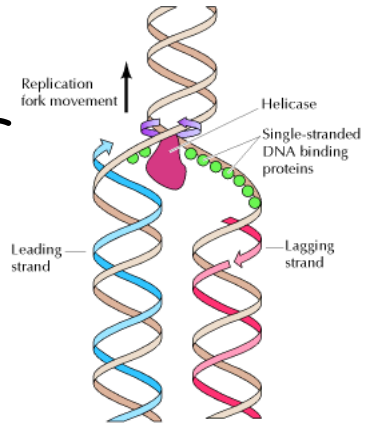
-**Helicase** – to separate the two DNA strands, For DNA synthesis to proceed, the DNA double helix must be opened up ahead of the replication fork.

*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.

(enzymes and proteins tend to form complexes in order to process any mechanism in a fast manner)

work together at the same time.



-**single-strand DNA-binding proteins called replication protein A (RPA)**

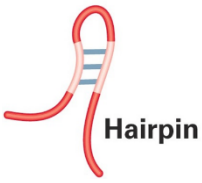
Single-strand DNA-binding (SSB) proteins bind tightly to exposed single-stranded DNA strands without covering the bases, which remain available for templating.

These proteins:

-prevent the formation of the short hairpin* structures

-protect single-stranded DNA from being degraded (as having a single stranded DNA isn't preferable in cells so they tend to degrade it)
-aid helicases by stabilizing the unwound, single-stranded conformation (by preventing the reformation of double stranded DNA molecule)

*Hairpin structures: they are complementary parts found within the DNA molecule and are bound to each other, their effect is that they block the movement of DNA polymerase during synthesis.



- DNA POLYMERASES:

E.coli has 5 types DNA polymerases with differenet functions

*DNA polymerase III (major one, responsible for synthesizing the whole genome): DNA polymerization at the growing fork in E. coli.

-The complex of primosome(helicase and primase) and polymerase is known as *replisome.*

*DNA polymerase I:

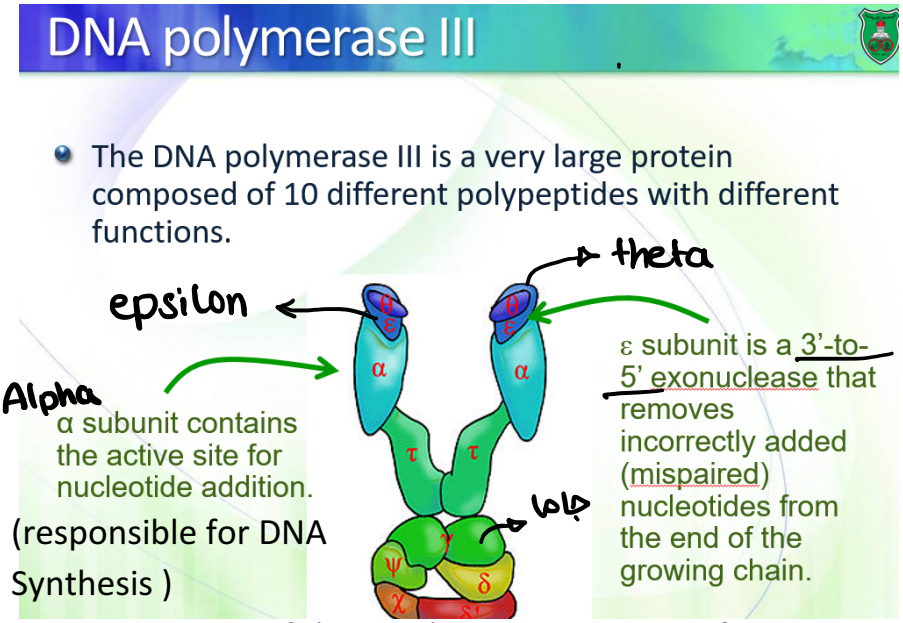
-5'-to-3' exonuclease(degrades nucleic acids from the end) activity (removal of RNA primer) of each Okazaki fragment.

{so what happens is that when DNA polymerase III is done synthesizing the okazaki fragment and hits the RNA primer occurring ahead of it the DNA polymerase I comes in and removes the RNA primer and places deoxyribonucleotides instead of ribonucleosides of the primer }

-Fills in the gaps between the lagging Okazaki fragments.

-DNA repair.

*DNA polymerase II, IV, and V : DNA repair

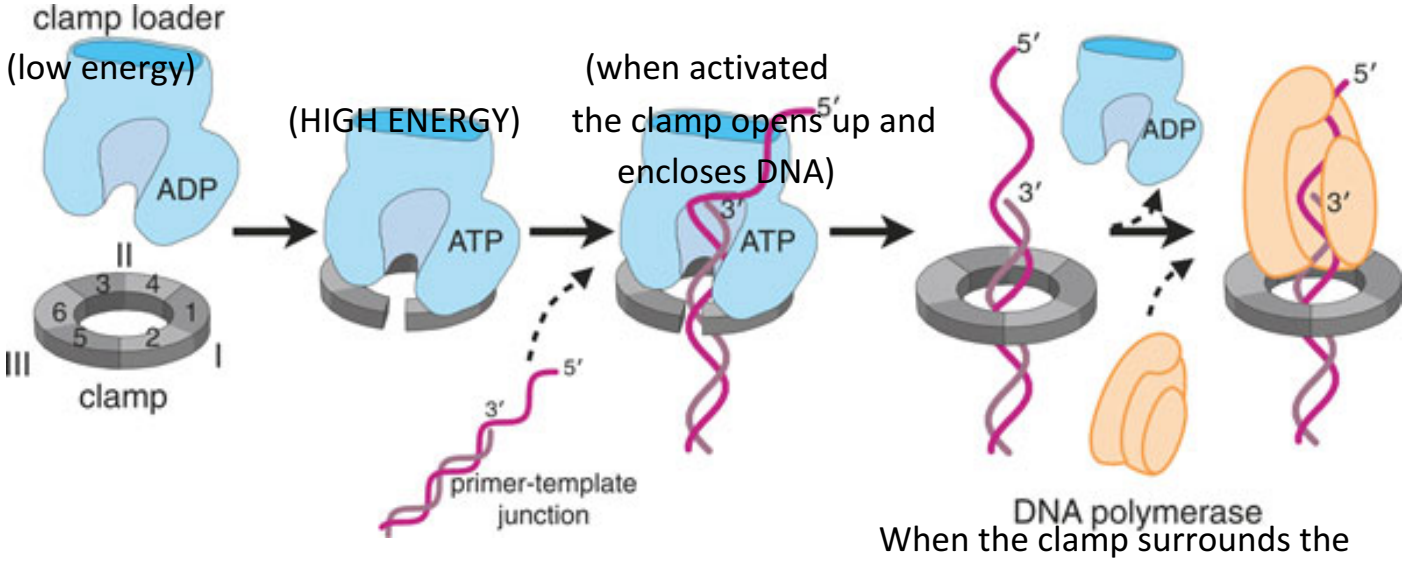


(has an exonuclease acivity but In the direction of 3'—5')
– the directions are important.

(responsible for DNA Synthesis)

Functions of these subunits: -enzymatic functions – maintaining enzyme structure
-making interactions

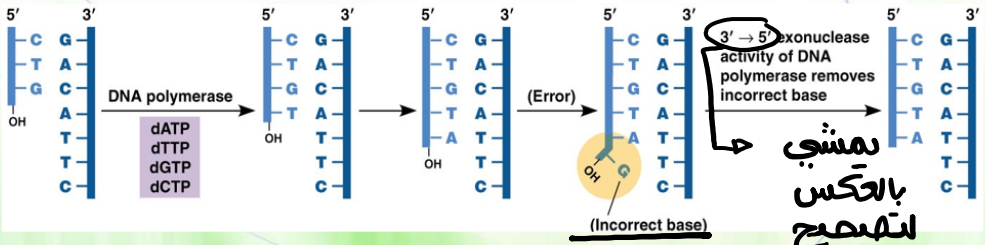
- Note that when DNA polyemerase III synthesizes the DNA it creates non-covalent bonds between proteins and DNA and this reaction is weak requiring something to stabilize it otherwise DNA will keep on falling off as it moves ahead.
- What assists in stabilizing (efficiency of the replication process)
  The sliding-clamp protein (β-clamp) is loaded by ATP-bound clamp-loading protein to the primed DNA.
  ATP is hydrolyzed to ADP driving the release of the loader that loads DNA polymerase III onto the primer and stabilizes its association with the DNA template while the polymerase "slides" along the DNA



When the clamp surrounds the DNA the loader is hydrolyzed and become bounded to ADP allowing for DNA polymerase to come in and binds to the beta clamp.

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? fast enzyme
  - 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 ($1/10^{7-8}$). very accurate



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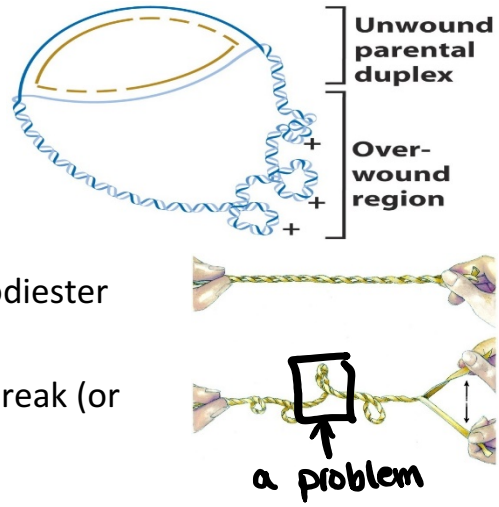
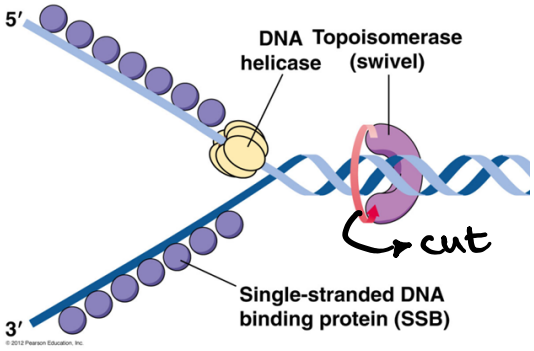
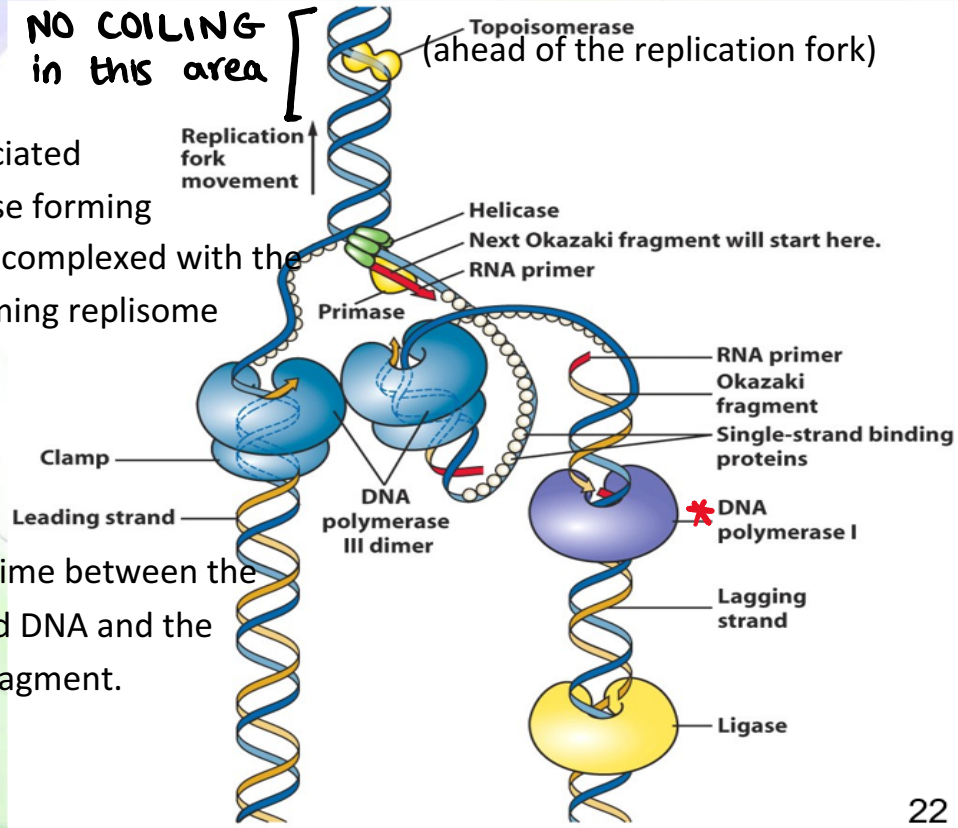
- DNA replication is accurate.
-DNA polymerase III can make mistakes placing the right nucleotide (doesn't follow the base-pairing rules)
- So it uses the exonuclease activity to go backwards(3'-5') to remove the wrong nucleotide and places the right one making sure that the replication is with high fidality.

- The DNA helicase separates the two strands but ahead of the DNA polymerase the DNA becomes over-wound. This makes it impossible to continue with the synthesis.
- Solution : DNA topoisomerases (I and II)
  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)(prevents the formation of over-wound)
  <u>ATP-independent (doesn't Need energy)</u>





DNA replication machinery is coordinated



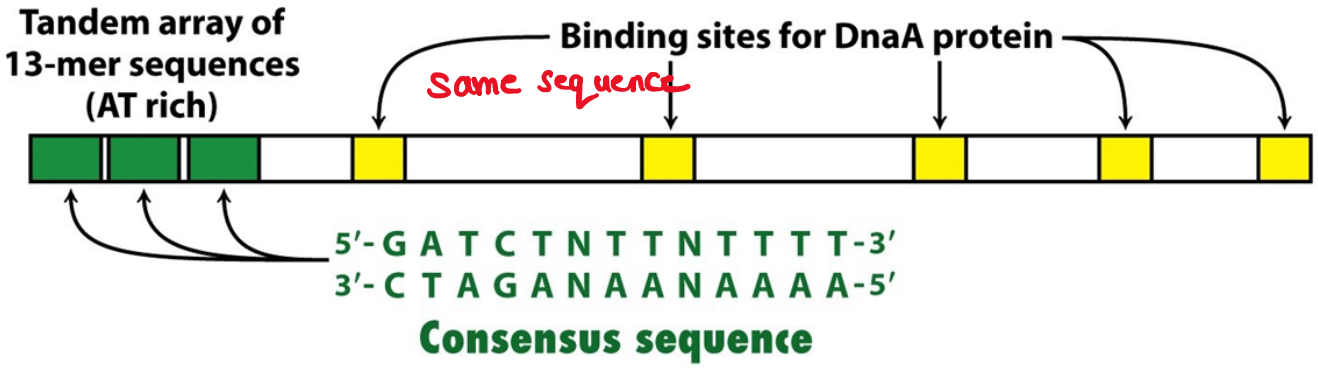
Primase is associated With the helicase forming the primosome complexed with the DNA pol. III forming replisome

*<u>Dna pol. I;</u>
Removes the prime between the new synthesized DNA and the other okazaki fragment.

<u>Ligase</u> (connects the Okazaki fragments)

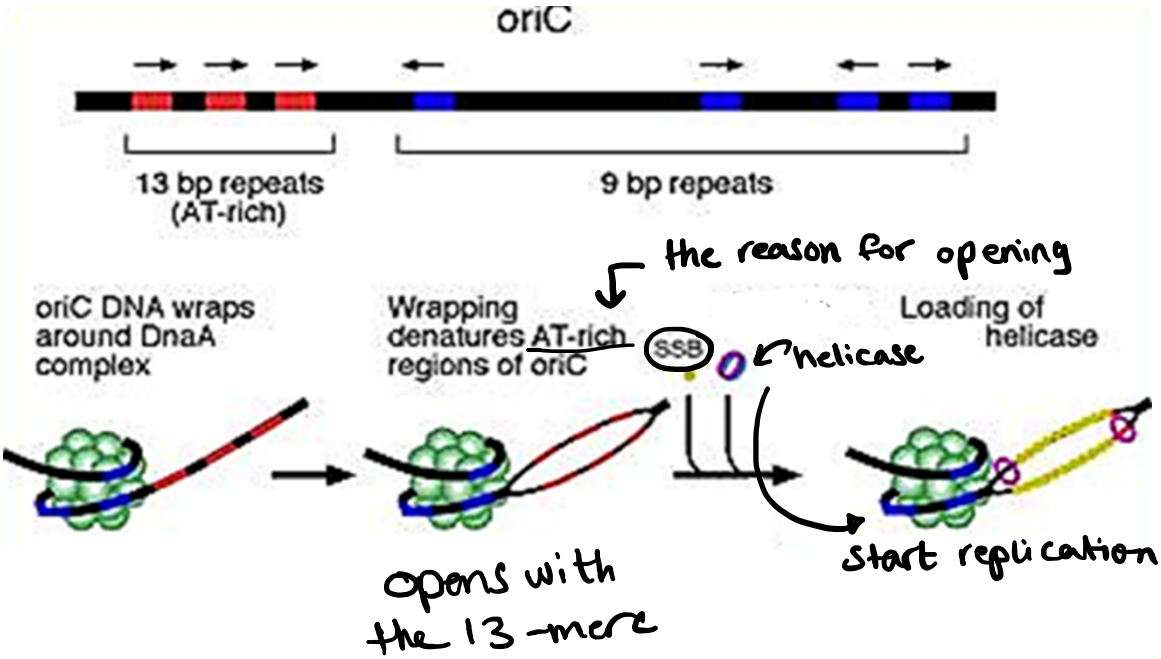
Origin of replication (OriC) in bacteria (special region where there replication initiates)

Bacterial replication starts at a origin known as origin of replication (OriC).
-oriC regions contain repetitive 9-bp region and AT-rich 13-bp region sequences (These are known as consensus sequences).”is a sequence that is agreed upon meaning that you can find the same sequence in different bacterial species”
9-mer: binding sites for the an “initiator” (starter) protein called **DnaA.**
13-mers: AT-rich region - it facilitates separation of the double strand DNA.

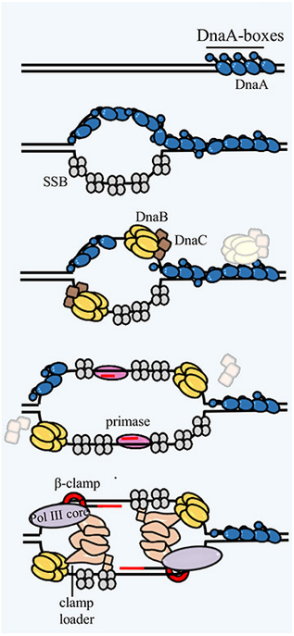


When **DnaA protein** binds to 9-mers, it applies stress on the AT-rich region resulting in DNA "melting“.

DNA melting.



As cells grow, DnaA protein level increases (growth and good nutrition state) and binds to (9-mer) OriC opening it forming a bubble.the ssDNA binding proteins (RPAs) bind to the ssDNA.

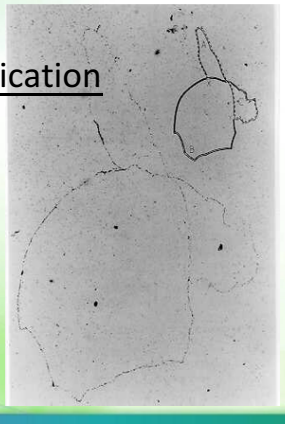


DnaB (a helicase), assisted by its loader (DnaC), binds to the OriC. *There is a formation of a complex between the helicase and its loader and the loader is then released as there is no need for it and the DNA becomes unwound allowing the primase to come in forming the primosome with the helicase.*

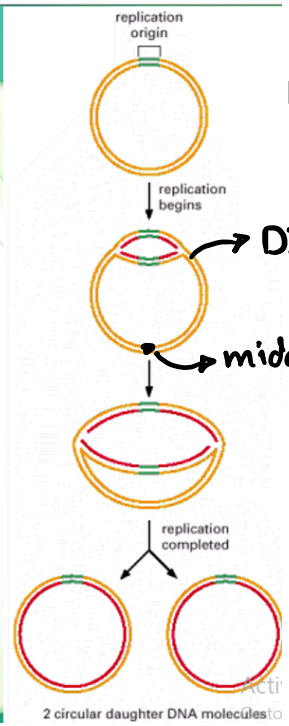
This is followed by binding of the primase, then the sliding clamp and the beta clamp protein , and finally DNA polymerase III forming the replisome.

Two replication forks (bacteria)

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



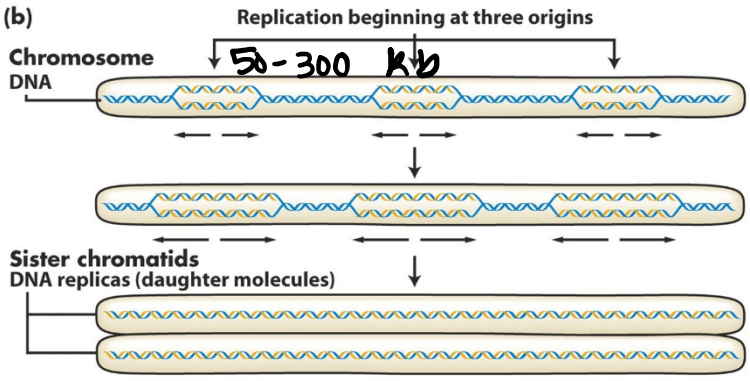
At the end of the replication we have one old strand and one new strand

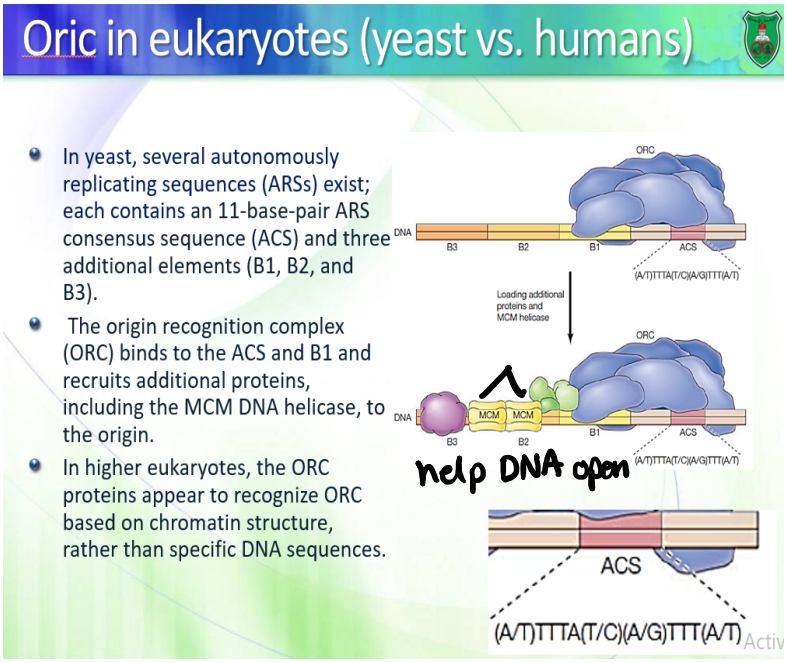


Replication is bi-drirectional

-The human genome contains ~30,000 origins of replication as it is very large and one origin isn't enough.

The replication is bi-directional and the replication bubbles eventually meet up half way and the two strands are separated.



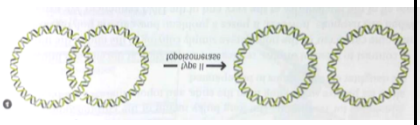


-ACS in the yeast is A/T rich just like 13-mere.

-In higher eukaryotes there is an origin recognition complex that recognizes the origin of replication but it is not that straightforward as yeast or bacteria, not a consensus sequence, but we have the 3D structure of chromatin that specifies the locations of origins of replication

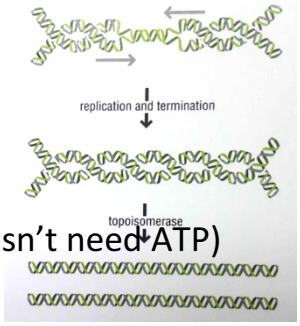
(MCM binds to B2 and portion of B3)

TOPOISOMERASE II



(chromosomes become tangled up and they need to be separated)

Topoisomerase II is responsible for untangling chromosomes by making a transient double-strand break. (creates two cuts on the same strand) (separates the two strands from each other)



{topoisomerase I creates one cut in one of the strands}

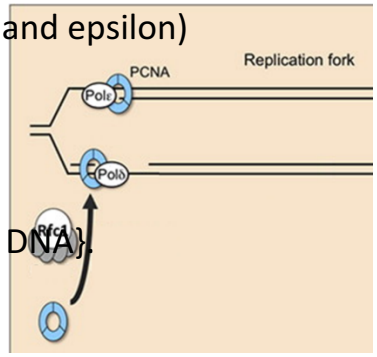
<u>also known as gyrase in bacteria</u>

ATP-dependent and double cutting(opposite to topoisomerase I that doesn't need ATP)

It is also responsible for chromosome condensation during the cell cycle.

- As topoisomerase II is important for DNA replication inhibiting it can prevent cells from dividing by blocking DNA from being synthesized so they are used in treatment of cancer and also cells in stomach, blood and immune cells.

DNA polymerases need guidance as just like the DNA polymerase III in bacterial cells, the guidance is **PCNA protein.** (it complexes with DNA polymerase delta and epsilon)



DNA polymerases are guided to the primers (at the leading and lagging strand) by a protein called PCNA (proliferating cell nuclear antigen) assisted by Replication factor C (RFC) {help when binding to DNA}

-PCNA is a diagnostic <u>marker</u> of cancer

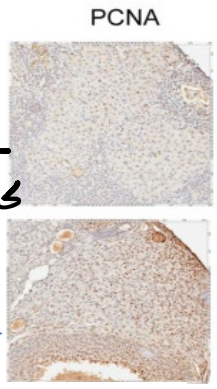
- gives an indication of the cancer whether it is aggressive or not.
- and if it is proliferating or not.

(if cells in a tissue look like cancer cells, to know how aggressively cancerous they are, the tissue is stained for PCNA, and they appear in brown)

(if the level of PCNA is low, cells are not actively proliferating which indicates a good sign)

there's is a tumor mass but it is not that vivid كمية تكاثر

(actively proliferating cells are aggressively cancerous thus needing an immediate treatment and they appear to be stained strongly and we notice high level of PCNA)



DNA polymerase in eukaryotes

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

TABLE 10.4

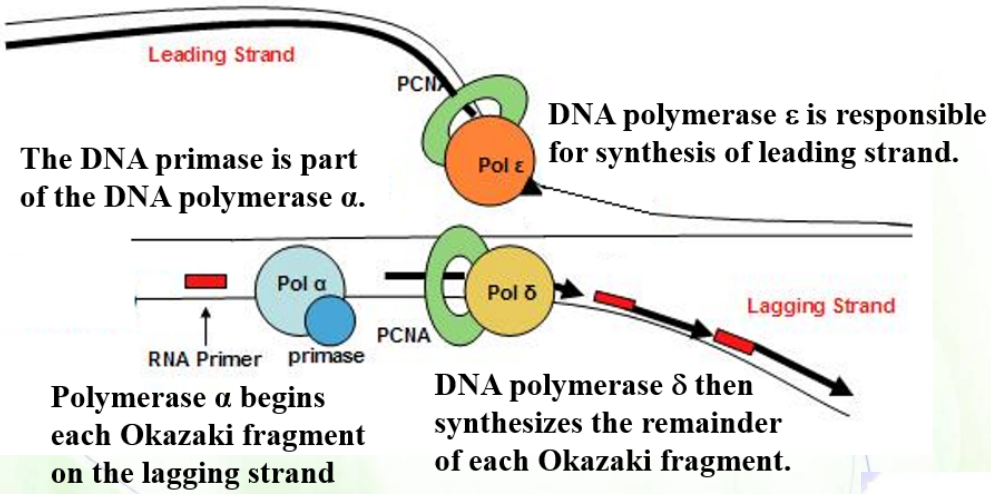
The Biochemical Properties of Eukaryotic DNA Polymerases

	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

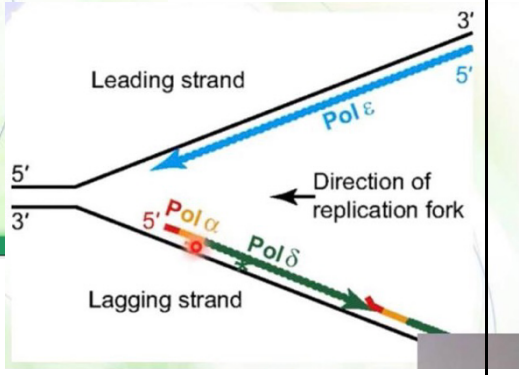
the doctor focused on the underlined stuff

- Alpha :high fidelity meaning it doesn't create that much mistakes and probably because of low processivity meaning it works slowly
- Delta and epsilon has high fidelity and high processivity and that because they have the proof reading mechanism (3'- 5' exonuclease activity)

The mechanism of replication



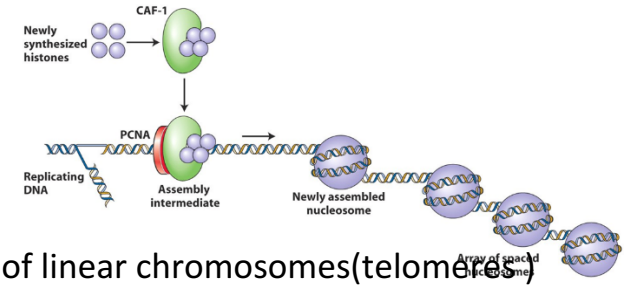
- The polymerases do not have a 5'→3' exonuclease.
  - Primers are removed by RNase H.
  - DNA polymerase δ then fills in the gap.



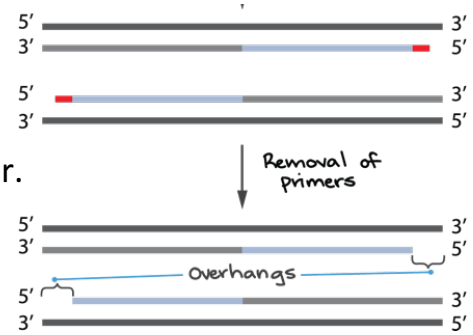
*DNA pol. Epsilon is responsible for the synthesis of leading strand and it binds to PCNA that stabilizes the interaction between DNA pol epsilon and DNA.
*DNA pol. Alpha that is associated with the primase, begins each Okazaki fragment on the lagging strand and then it falls off as it doesn't bind to PCNA here comes the Delta and interacts with the PCNA and is responsible for synthesizing the remainder of each Okazaki fragment.

- Role of chromatin :
- *DNA cannot be synthesized until its free from histones by the chromatin-remodeling proteins*

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).



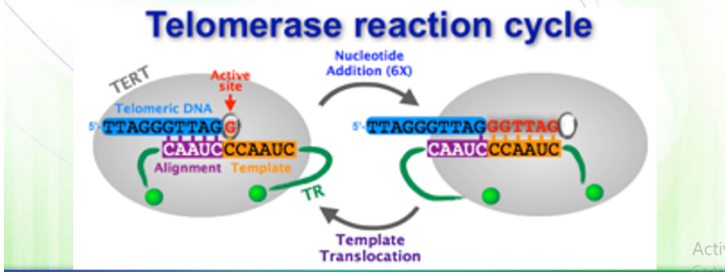
- We have a problem synthesizing the ends of linear chromosomes(telomeres)
- As the growing fork approaches the end of a linear chromosome, the lagging strand is not completely replicated. Why?
- When the final RNA primer is removed, there is no place onto which DNA polymerase can build to fill the resulting gap leading to shortening of the lagging strand.(*this gap isn't filled because there is no space to add another primer and fill in the gap)*
- There is no problem synthesizing the leading strand But in the lagging strand we notice that its being shorter as being replicated as there is no space to add a new primer so the chromosome becomes shorter.

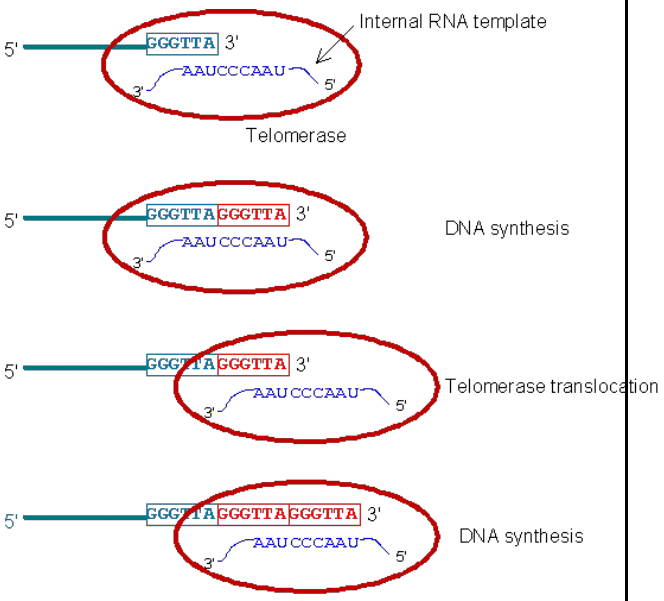


- Telomeres are important because they stabilize the chromosomes, so when they become shorter they make the chromosome less stable.
- So the lagging strand is completed by the enzyme telomerase (ribonucleoprotein)
- Telomerase : RNA(functions as a template, hybridize to the end of DNA) + PROTEIN
  So the telomerase elongate the lagging strand .
  And it adds the same sequences and that's why telomeres are composed of repetitive sequences of 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.

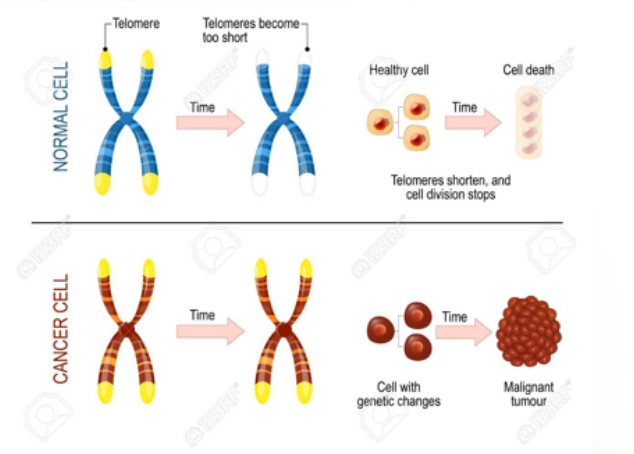


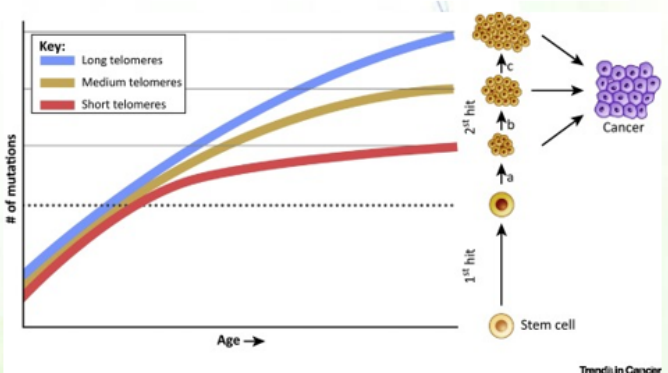


Telomerase uses the RNA associated with it in order to elongate the template of the lagging strand, keeps on adding the same exact sequence over and over again.

Facts of life about telomerases

- 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.





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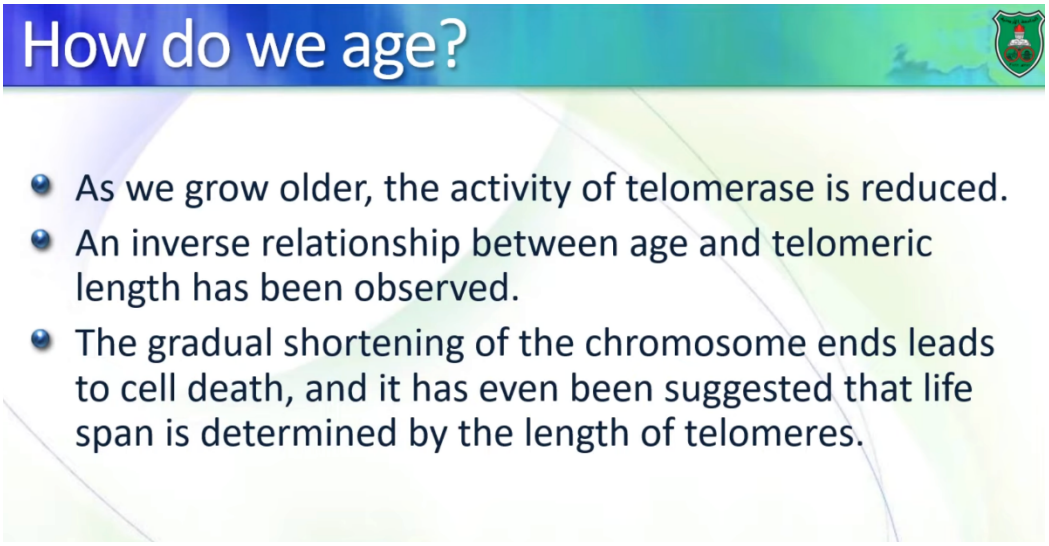
-with aging telomeres develop low levels of activity and telomere races and that results of theirshortening and less stabilization in chromosomes which causes mutations and breaking up of chromosomes in cells and eventually these cells would die with time.

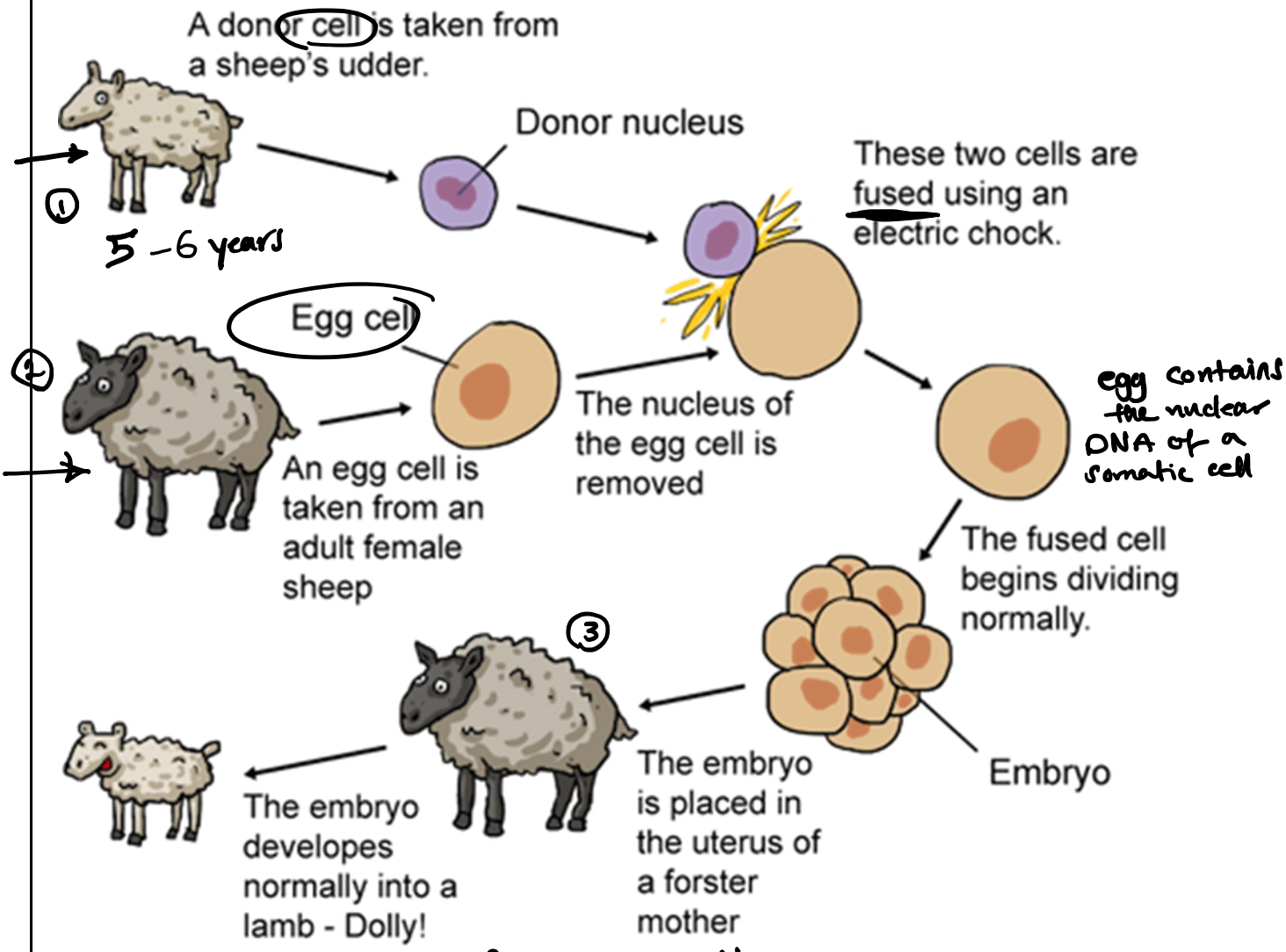
- in cancer cells, sperm and egg, germline, they have high telomere activity and chromosomes don't become shorter and that maintain the cells' life and keeps proliferating

-cells with short telomeres have lower tendency to become cancerous.



Elixir of youth (magical molecule that keeps us alive for longer time)

Scientists thought of the telomere to be that elixir of youth.



- doesn't live for a long time
- because the DNA taken was originally 5-6 years old even though the dolly is a new born
- Meaning that it has already had mutations accumulated and that resulted in looking older.

GOOD LUCK FOR YOU ALL !!!