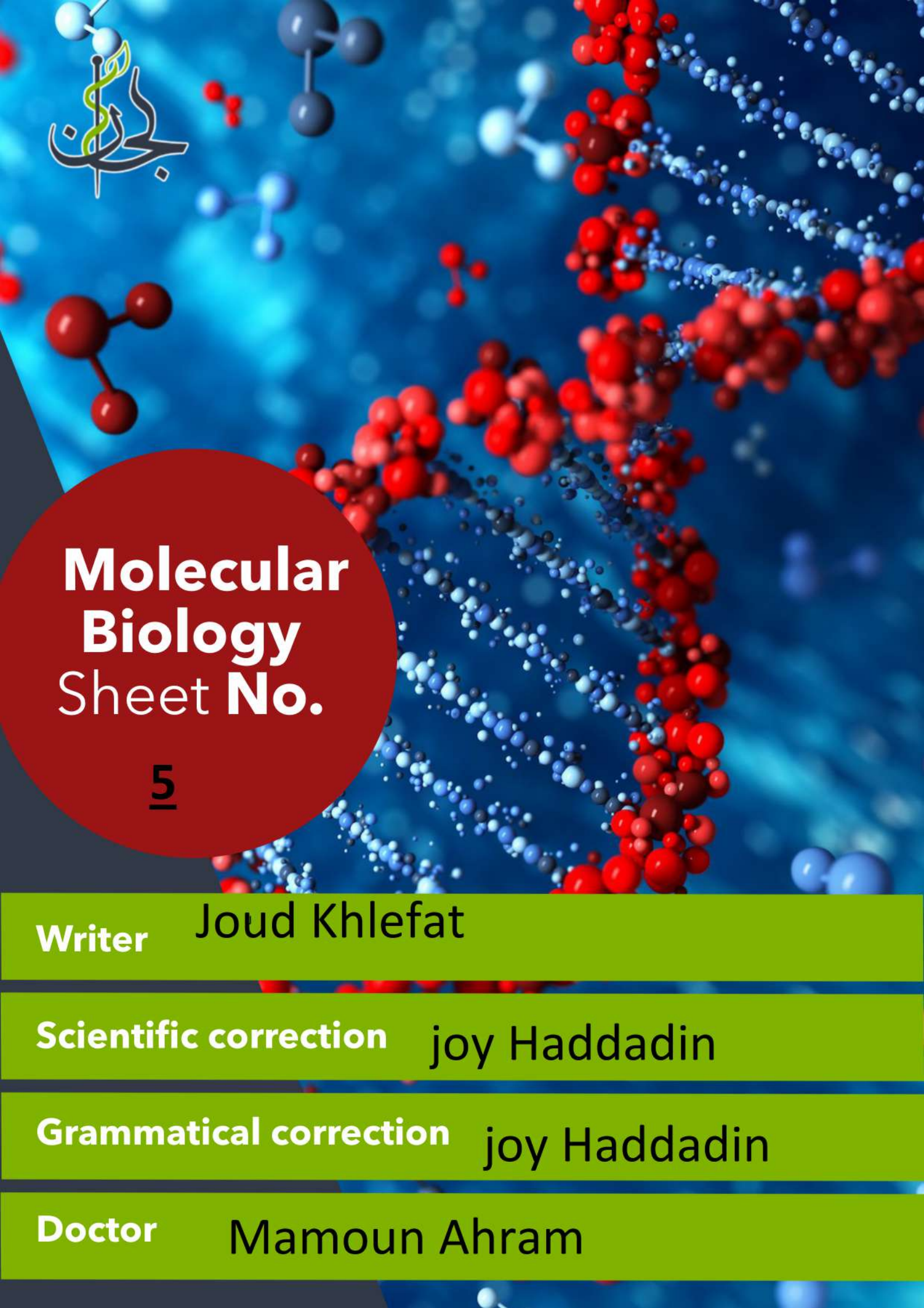


Molecular Biology Sheet No. 5

Writer Joud Khlefat

Scientific correction joy Haddadin

Grammatical correction joy Haddadin

Doctor Mamoun Ahram

SOME BASIC INFORMATION

=The entire DNA content of the cell (or an organism) is known as genome

=Whether you extract DNA from skin, liver or nerve cells it should be the same =DNA is organized into chromosomes.

=Bacterial genome: usually one and circular chromosome.

=Eukaryotic genome: multiple, linear chromosomes complexed with proteins known histones.

They are packaged Into nuclei

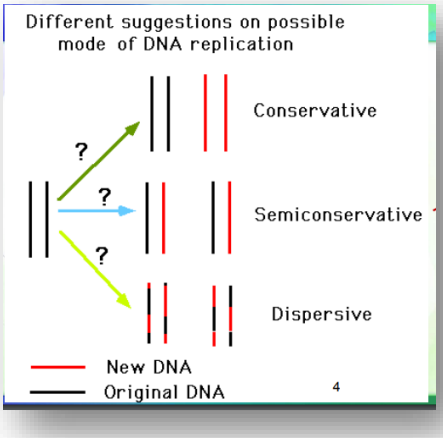
=DNA must be accurately copied (replicated), but variation is important.

#when cells divide it must be copied (duplicated) so there should not be any variation or codon code mutation in the DNA , it might resolved disease or certain pathological condition

#YET variation is important because that what makes us different overall, this is the mechanism of human evolution

=DNA synthesis is carried out by DNA polymerases.

=The substrates are deoxyribonucleotides.



IN DNA REPLICATION THERE ARE THREE ALTERNATIVE MODELS

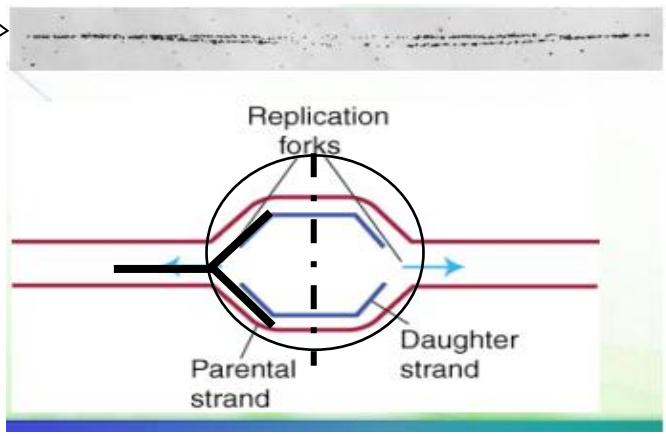
CONSERVATIVE The two parental (original) chromosomes reassociate in one daughter cell, and another daughter cell contains two new chromosome

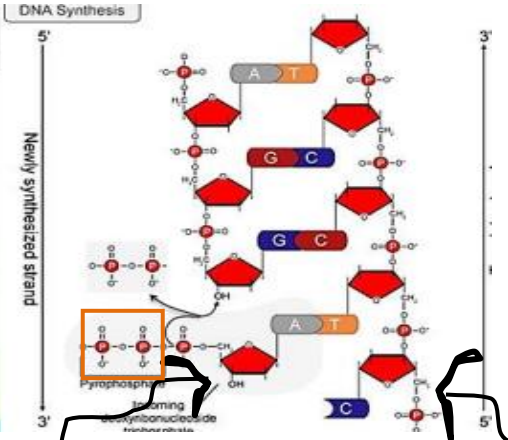
SEMICONSERVATIVE The two parental (original) chromosomes separates so each one of the daughter cells contains one original chromosome and one new chromosome

DISPERSIVE each chromosome in the daughter cells contains a mixture of old (from the original) and new synthesized DNA

BUT the model which has been adopted after experiments is semiconseravative .

This is an image of an experiment was done on bacterial cell , replication was initiated and radioactive substrate was added, so the newly added substrate would be integrated into replicating DNA





***Replication moves progressively along the parental DNA double helix bidirectionally. starts at central point and go both opposite directions**

***it looks like a bubble , and it can be divided into two forks**

***Because of its Y-shaped structure, this active region is called a replication fork**

Look at the shapes drawn in the picture

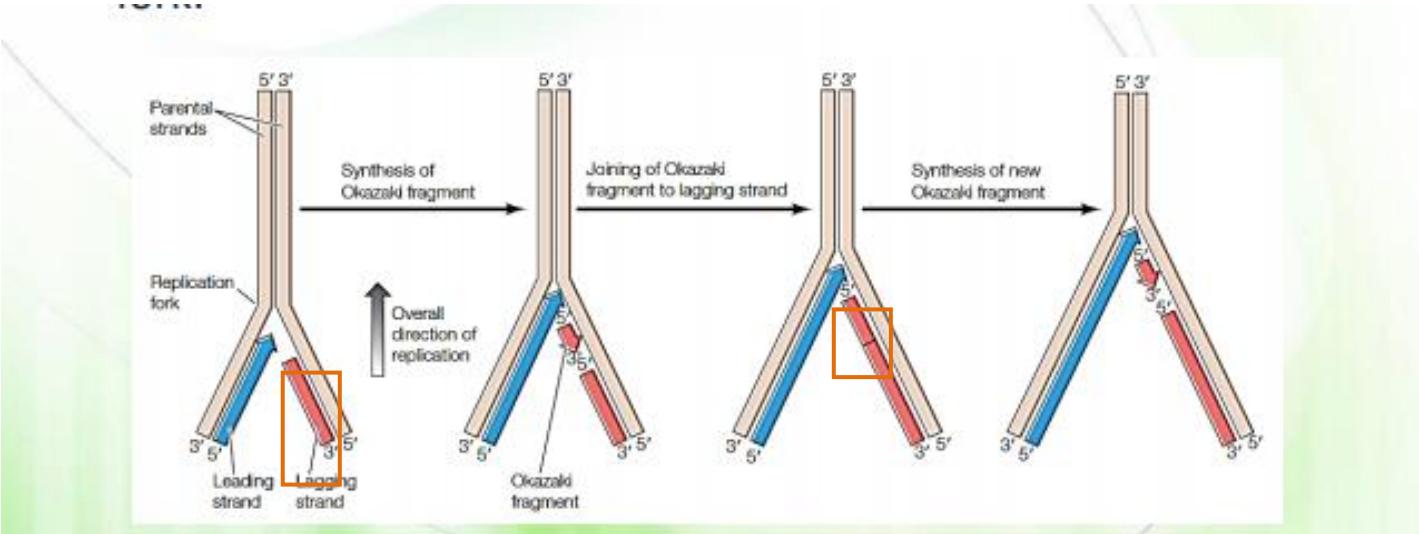
***This is parental strands , they also known as templates**

***This is the newly synthesized DNA , where newly nucleotides are added to the 3 prime end - 5 prime are not touched**

*** Energy comes from the substrate it self [the substrate contains triphosphate like ATP GTP CTP TTP all of them are deoxynucleotide**

*** Whenever the enzyme add the substrate to the 3' end a Pyrophosphate Two Phosphate Group \orange box\ is removed , and the third Phosphate is the one that forms phospdiester bond .**

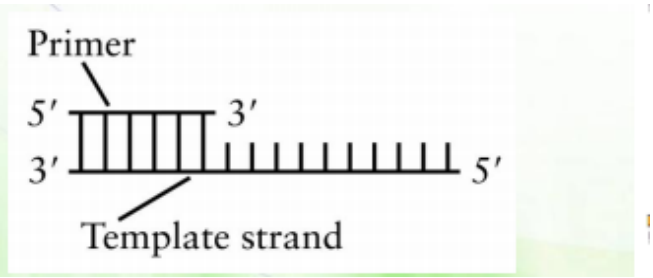
*** elongation of DNA takes place through the 3' end , and the template is anti parallel to the newly synthesized DNA**

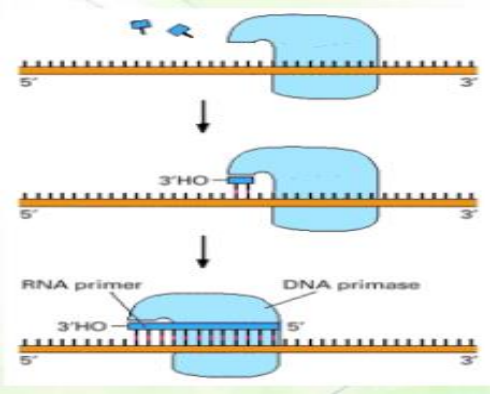


- The synthesis of the other strand happen in the same direction .
- The DNA in blue is synthesized from 5' to 3' , this DNA polymerase on the opposite strand synthesizing DNA from the 5' to 3' .
- As this strand keeps going on, it opens up fork and this allows another fragment of DNA to be synthesized -the red one- which connects to the others and so on.
- The blue one is called the leading strand because it is leading the way, and the other one the red is called the lagging strand (lagging mean it is late) because it waits for the leading strand to open up the fork .
- The leading strand synthesized continuously but the lagging strand discontinuously .
- The scientist who discovered this mechanism was known as Okazaki, and that is why these short fragments- orange boxes- that form the lagging strand called Okazaki fragment both are present at the growing replication fork.

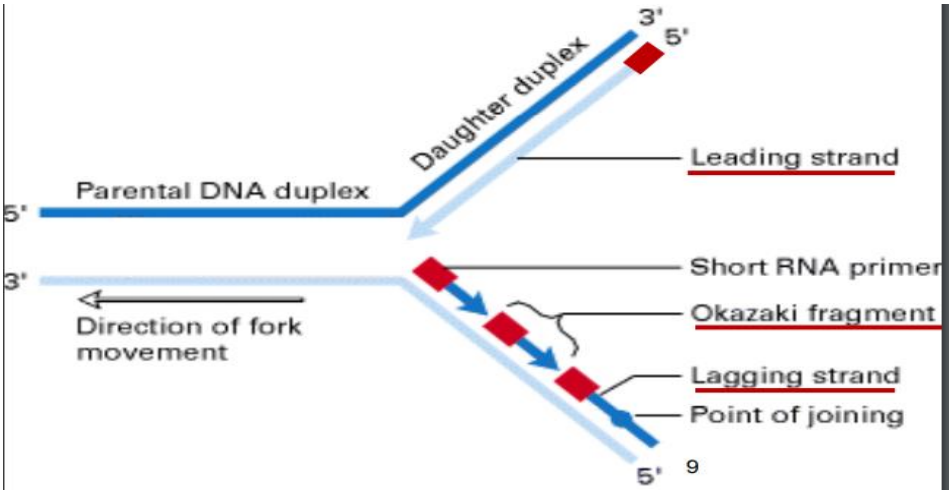
DNA replication is a complex process and it requires a number of molecules to complete it.

- **DNA polymerases cannot initiate replication de novo *it cannot start replication by itself ,it needs help which comes from primer RNA molecule ribonucleotide* . So, they require a RNA primer (3-10 nucleotides long) that is complementary to the DNA template and antiparallel to be added first.**
- **It is synthesized by a primase.**
- **After that DNA polymerase ``grap hand`` with the primer it starts synthesizing DNA.**

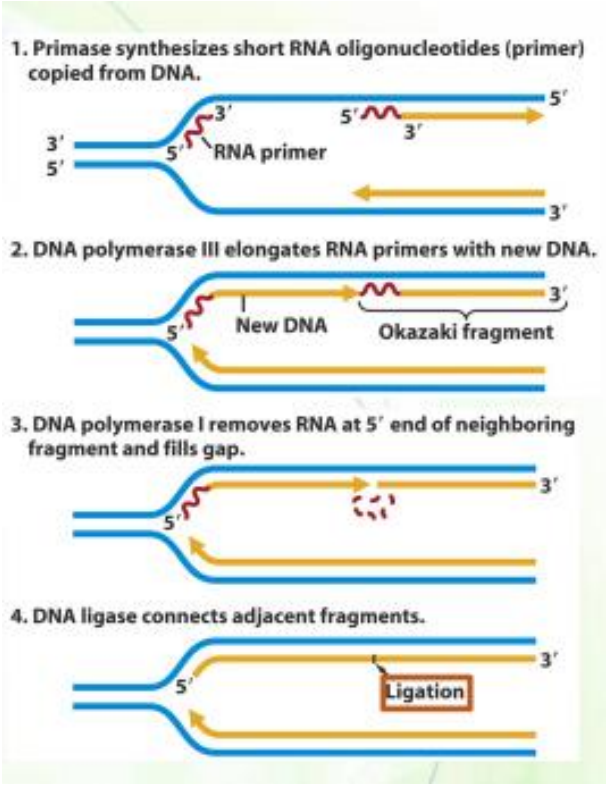




***Each leading strand required one primer . But each Okazaki fragment requires a primer of its own.**



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



<u>For DNA synthesis to proceed, the DNA double helix must be opened up ahead of the replication fork.</u>

Opening up the DNA is done by two types of protein that contribute to this process :

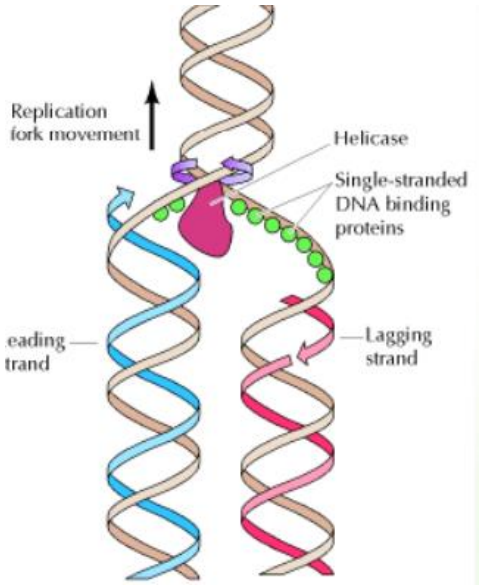
- **DNA helicases** ***(DNA helicase are important for separating the two strands from each other to allow DNA polymerase to do synthesizing)***

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

- **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**

- **This is something that is important for proteins and enzymes that tend to form complexes in order to process any mechanism really fast .**



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.**
  the DNA polymerase trying to read the template and if there are complementary strands ,you will have the formation of hairpin
  So the DNA polymerase stop working, it doesn't work anymore because it thinks it is double stranded DNA and it cannot move above it.

- **Protect single-stranded DNA from being degraded.**
  when cells see single stranded DNA they immediately think this could be violent DNA so they jump on top of DNA and degrade it

- **Aid helicases by stabilizing the unwound, single-stranded conformation.**
  It prevents the parental (template) strands from collapsing together because they are complementary and there is a big chance for forming double stranded again.

DNA POLYMERASES IN PROKARYOTES

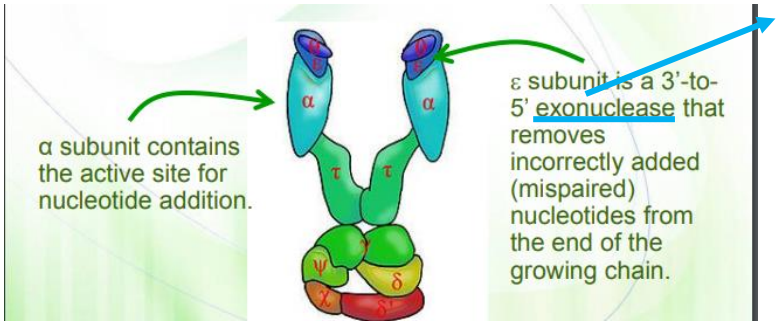
DNA polymerase III: DNA polymerization at the growing fork in E. coli. The complex of primosome and polymerase is known as replisome.

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.



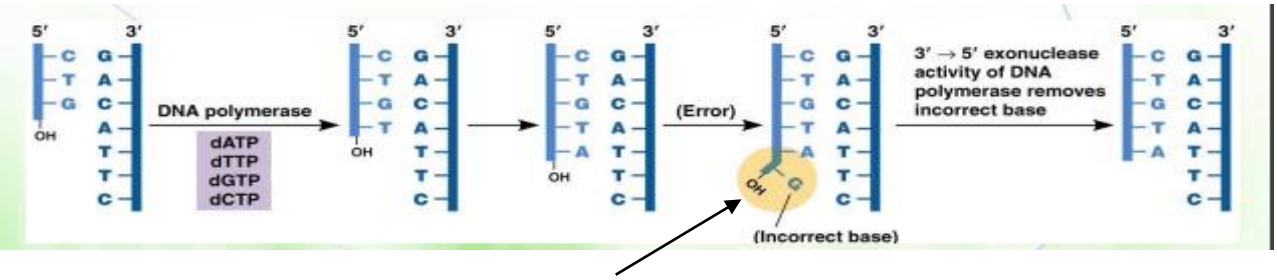
This exonuclease activity is different than the polymerase one have, because it goes in the opposite direction from 3' to 5' end

The frequency of errors during replication is only one incorrect base per 108 nucleotides incorporated .

Our genome is composed of 3 billion nucleotide so just three mistakes are made.

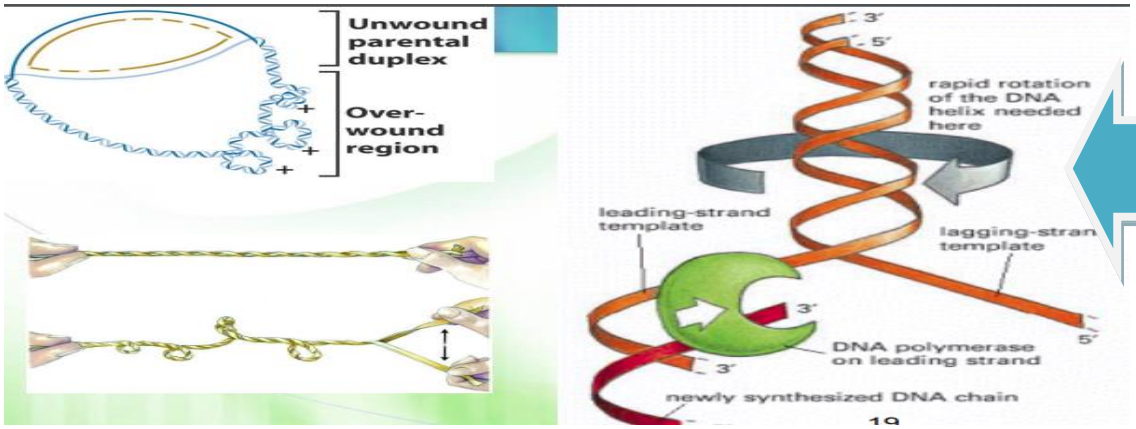
How is fidelity high?

- **The DNA polymerase can catalyze the formation of phosphodiester bonds when the right hydrogen bonding takes place between the bases (accuracy=1/100 one mistake per hundred to thousand nucleotide synthesis).**
  (whenever you have G - for example - and the polymerase attempts to add T , we have the () formation of hydrogen bonds between the two and the substrate doesn't fit into the enzyme).
- **Proofreading mechanism (a 3'→5' exonuclease activity)- Remember ε subunit of DNA pol III ($1/10^{7-8}$).**
  SO the polymerase has two enzymatic activity first one is DNA synthesizing activity and the second activity is the opposite exonuclease activity { cleaves DNA \ opposite of polymerisation } It acts at the end of the DNA
  {exo the opposite of endo } so the direction is important, this mechanism go from the opposite direction from 3' end backward to 5' end.



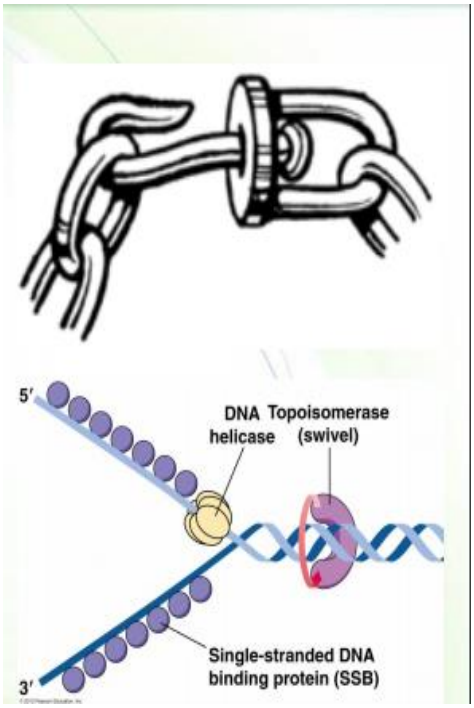
This is wrong it doesn't fit and the DNA polymerase synthesis , so it goes backward and removes this G using its exonuclease activity and it adds the right complementary nucleotide instead of it .

- There is a problem that the DNA helicase separate each strand from other each but the DNA above the DNA polymerase becomes overwound .



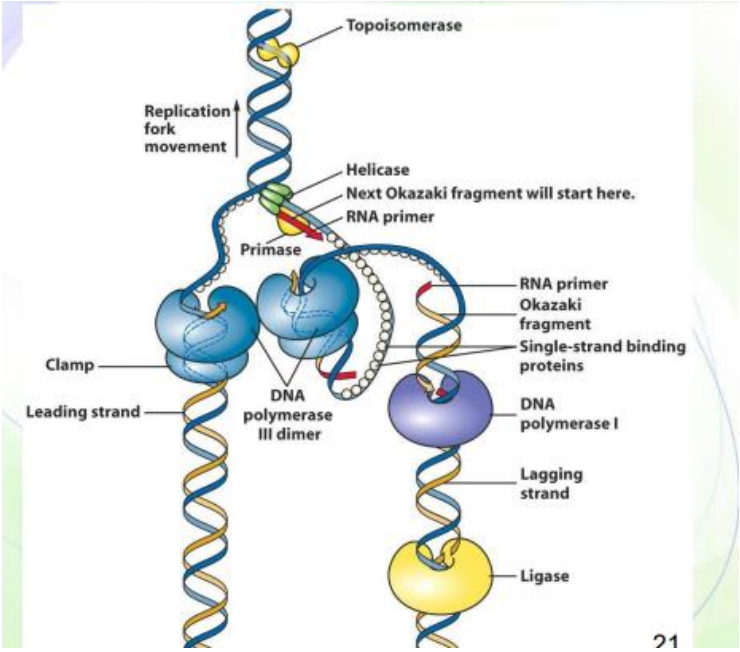
- (the solve of the previous problem)

SWIVEL meaning that it separates the two chains from each other ,so when DNA is rotated only one portion is rotated and the other is not rotated.



- 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

- **Primase associated with helicase forming the structure of primosome and this structure can also be complex with DNA polymerase ||| and forming the replisome , we have also DNA POLYMERASE | that removes the primer from the newly synthesized DNA and the other DNA okazaki and fills in the gap and the DNA ligase then comes in and connects the two strands with each other , the head of the replication fork the topoisomerase |.**



<u>WHERE DOES DNA REPLICATION STARTS AT ?!</u>

Bacterial replication starts at an origin known as origin of replication (OriC).

There is only ONE oriC in the bacterial genome.

oriC regions contain repetitive 9-bp and AT-rich 13-bp sequences (These are known as consensus sequences).

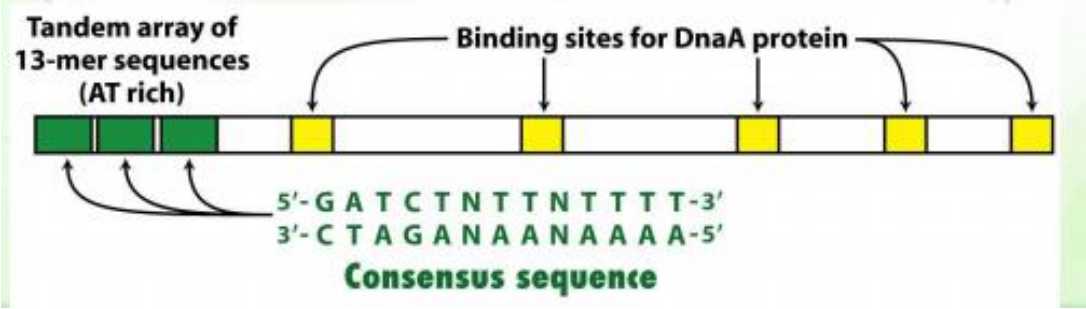
Consensus means that there is an agreement happen to a certain region that is almost the same in different bacterial cells whether from same species or different species they share the same consequence and that is why it is known consensus

So if you LOOK at DNA of different bacterial genomes you may find similar sequences it means that this sequence is important and that is why evolutionary it has stayed in placed it hasn't changed because otherwise this peaces may be disappeared }

1) 9-mer: binding sites for an "initiator" protein called DnaA.

2) 13-mers: AT-rich region - it facilitates separation of the double strand DNA.

they are rich with A and T

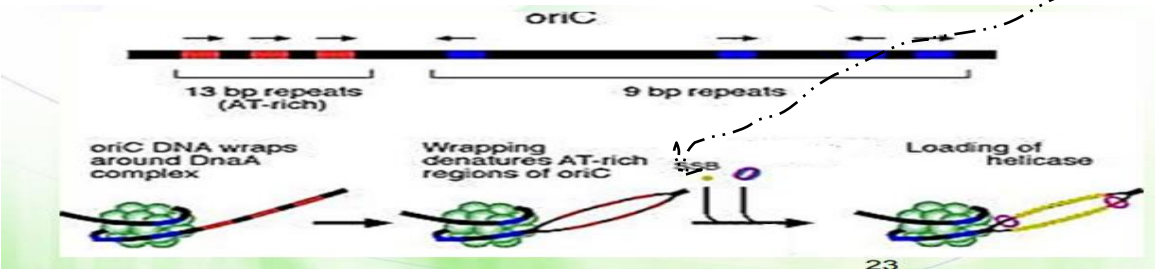


Possible mechanism

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

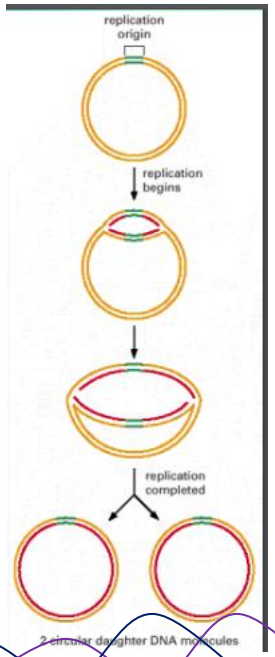
DnaA binds to the 9mers form a structure like here and it squeezes on DNA opening up the ATrich sequences

The interaction between two strands becomes weak because they are ATrich and when cells see a bubble they know that it is time to replicate so DNA the helicase and SSD they start opening DNA further ,,,.

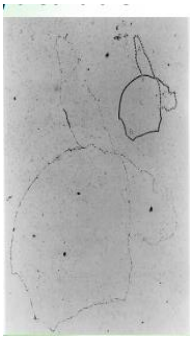


Two replication forks (bacteria)

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



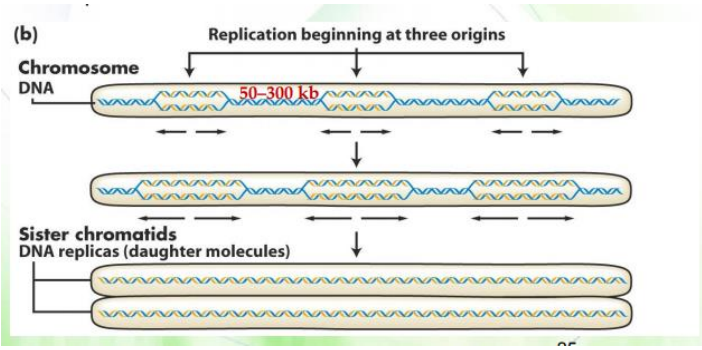
(What happens in bacteria that synthesis happens in both direction and until it hits the other side of DNA and then the two strands separated from each other)



Origins of replication in human genome

The human genome contains

~30,000 origins of replication.



- **The human genome is huge so one origin of replication is not enough and as a result there is multiple of them , the synthesis is also bidirectional and eventually these replication bubbles meet halfway and the two strands separated from each other .**
- **THE RESULT IS ALWAYS ONE OLD STRAND AND ONE NEW STRAND**
- **DNA replication in eukaryotes and human can be finished in a short time.**

Oric in eukaryotes (yeast vs. humans)

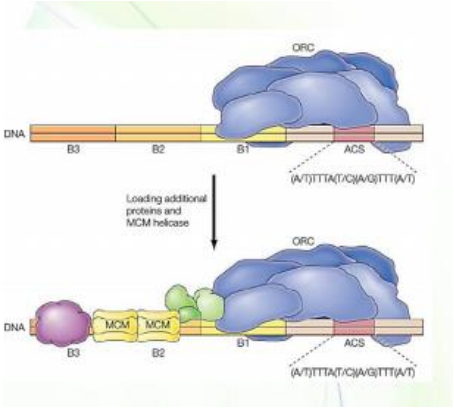
- **Eukaryotes DNA replication is more complex .**
- **A lot of studies have been done on yeast cells .**

WHY? They are among the simplest eukaryotic systems .

They are single cell organism they grow like bacteria and have similar characteristics like them yet they are eukaryotic systems (they have linear chromosome , chromatins in their DNA , shared DNA sequences with human cells , similar mechanisms to the way cells grow and divide and die as well)

- **Several autonomously replicating sequences, or ARSs, each containing an 11-base-pair ARS consensus sequence (ACS) and three additional elements (B1, B2, and B3).**

**** whenever you hear the world element in molecular biology it means it is a specific sequence of DNA ^elements ^origin of replication***



- **The origin recognition complex (ORC) binds to the ACS and B1 and recruits additional proteins, including the MCM DNA helicase, to the origin**

they open up the DNA allowing for other protiens to come on DNA and starts DNA replication .

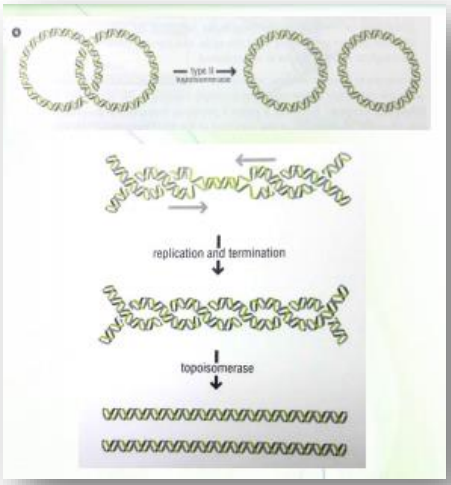
- **In higher eukaryotes, the ORC proteins appear to recognize ORC based on chromatin structure, rather than specific DNA sequences.**

This is a very similar system to bacterial replication, but things are complex in humans we don't know yet how DNA replicates, we know that it starts at certain reasons but we don't know why, because there is no elements AND no consensus sequence . It is though so far that proteins recognize origin replications according to chromatin structure(the way the DNA is Complex with proteins and the way it looks like in three dimensional space).

Role of topoisomerase II

Whether Bacterial DNA or human DNA chromosomes become tangled up and they must separated from each other and that is why we need the other topoisomerase which is know as DNA topoisomerase ||

- **Topoisomerase II is responsible for untangling chromosomes by making a transient double-strand break. also**
  - ***Known as gyrase in bacteria***
  - ***ATP-dependent***



So the differences between DNA topoisomerase | and DNA topoisomerase||

1)the second one requires DNA which is different than the first one that doesn't require DNA .

2) DNA topoisomerase| creates a single cut in one of the strands not both of them on the other hand DNA topoisomerase || it craves two cuts at the same strand but eventually what it does it separates the two strands from each other .

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

BECAUSE DNA topoisomerase || is important for DNA replication and for DNA division topoisomerases whether | or || can prevent cell from dividing as a result of block DNA replication and as a result

>>Topoisomerase inhibitors are commonly used in treatment of cancer.

Role of PCNA proteins

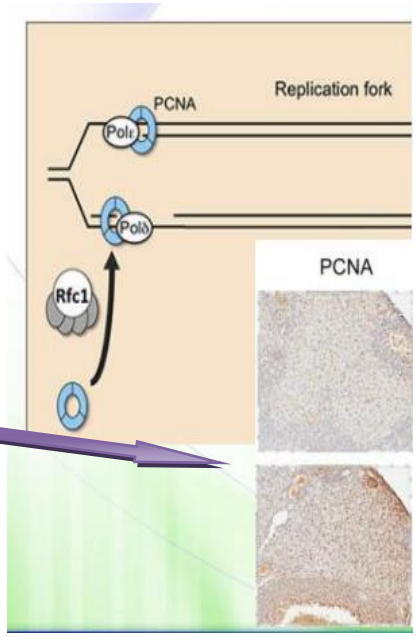
- **DNA polymerases are guided to the primers by a protein called PCNA (proliferating cell nuclear antigen).**
- **PCNA is a diagnostic marker of cancer.**

Because cancer cells tend to do a lot of replication and cell division SO as a result they increase the synthesis of PCNA .

 # This is shown by a technique known as immunhistochemistry (in tissues sections)

A STAIN FOR PCNA WAS USED

A IS NORMAL || **B** IS CANCEROUS



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

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ACCORDING TO THE PREVIOUS TABLE ;

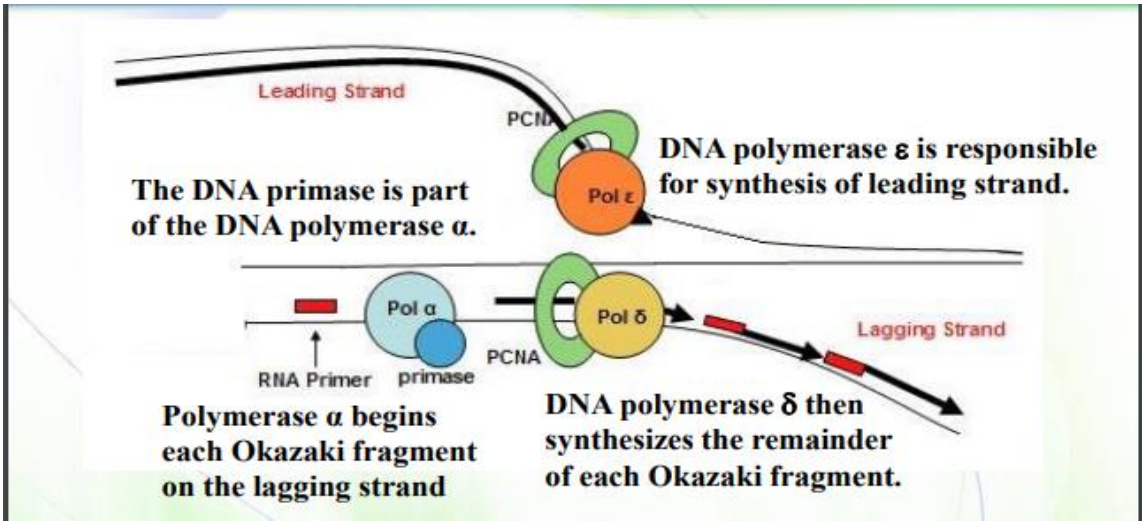
- **Eukaryotic cells contain 9 DNA polymerases; most of them for DNA repair.**

DNA polymerases in eukaryotes are multiple like in bacteria but there are more of them HERE.

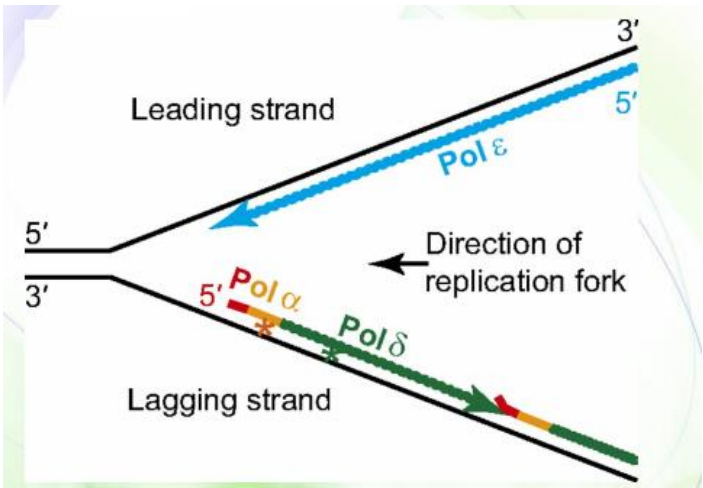
=BLUE accurate |proofreading mechanism|.

=ORANGE slow enzymes .

=PURPLE fast enzymes .



- **Specifically DNA polymerase £ is responsible for for synthesizing the leading strand Alpha associated with DNA primase which synthesis the RNA primer and the DNA polymerase beta starts synthesizing the begining of okazaki fragment but then it falls off and the DNA polymerase delta jumps on DNA and continues with DNA synthesis .**
- **Both DNA polymerases £ and Δ are associated with PCNA so they are guided by this proteins .**
- **DNA polymerase Δ as it synthesis the okazaki fragment it hits the primer in the next okazaki fragment and it needs special enzymes to remove this primer.**

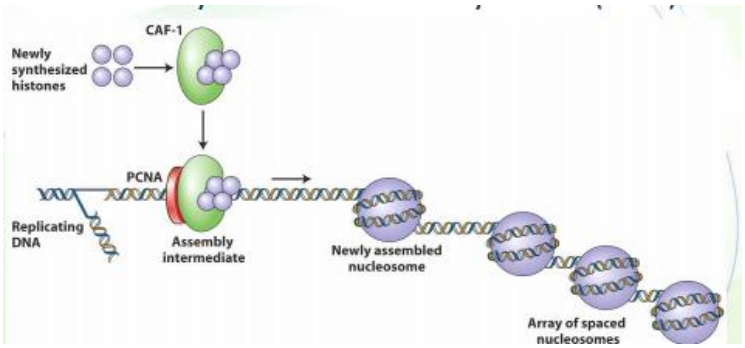


- **The polymerases do not have a 5'→3' exonuclease.**

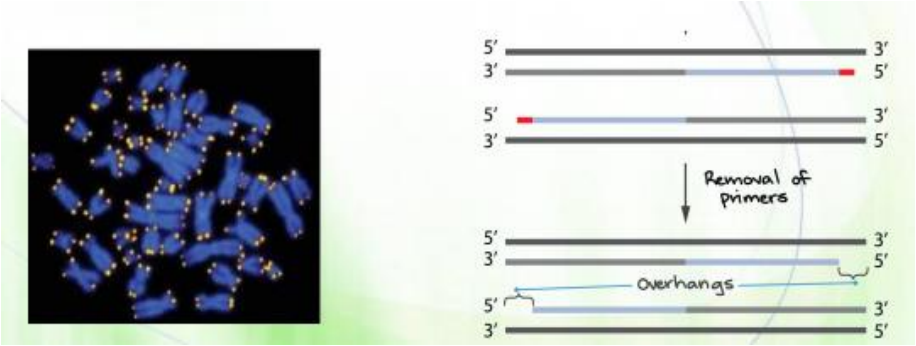
Role of chromatin

In eukaryotic DNA , DNA polymerase cannot synthesis DNA until DNA is free with histones which are removed by an enzyme .

The same old histones as well as new histones can be used to refold DNA.



- **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).**



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?

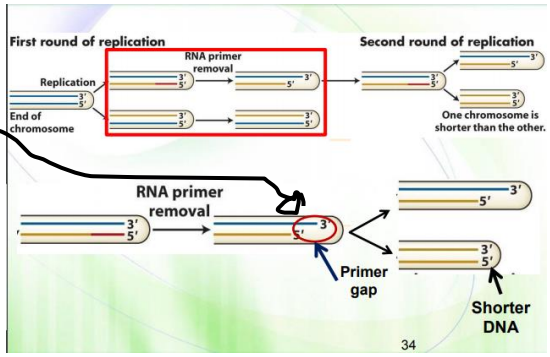
When we have this here and it is not completed and we have another round of DNA replication when this removed this becomes actually short so one chromosome is shorter here.

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.

There is a problem with the lagging strand this primer is removed and there is no place where a new RNA primer is added so the primase tries to set here and synthesis the primer but it cannot there is no space any more .

Which is called telomers (the ends of chromosomes) telom means end

When we have this here and it is not completed and we have another round of DNA replication when this removed this becomes actually short so one chromosome is shorter here.

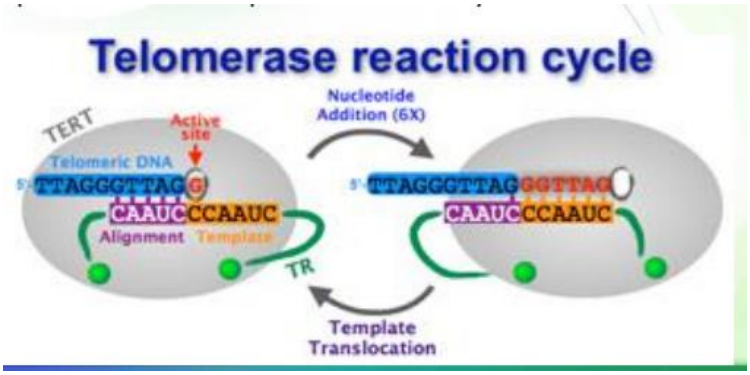


- Telomerase comes to the rescue Telomere DNA sequences consist of many GGGTTA repeats extending about 10,000 nucleotides.
- Telomerase (a reverse transcriptase) prevents the progressive shortening of the lagging strand. How?

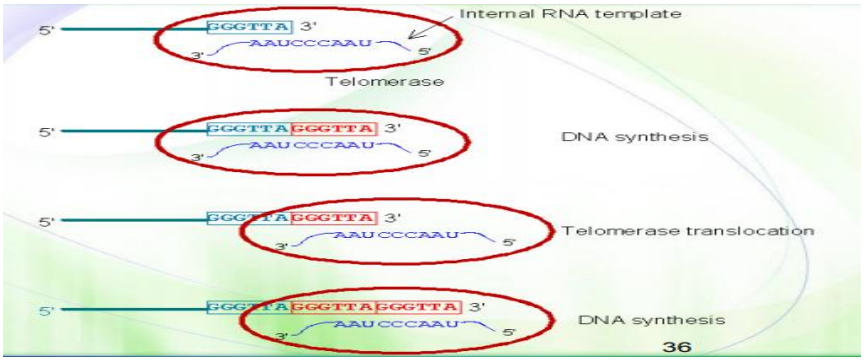
The end of the chromosome \telomere \ is very important for stabilizing DNA and this is completed by an enzyme called telomerase which is ribonucleo protein meaning that it is a protein that made of protein and RNA .

- Telomerase elongates it in the 5'-to-3' direction using a RNA template that is a component of the enzyme itself.

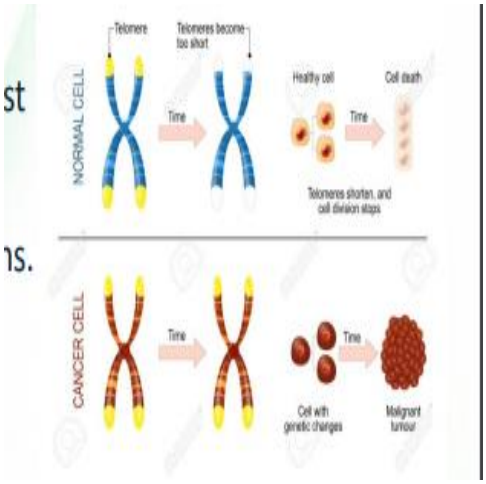
so what the telomerase does is that the RNA itself can hyperedized to the end of DNA and it elongates the template of the lagging strand it keeps adding the same repeat the same sequences and that is why telomers are composed of repetitive sequences of DNA



It uses the RNA that is associated with it to elongate the template of the strand over and over again



- **In contrast to germline cells**(*Egg and sperm cells \ stem cells \ replicating cells)* **most somatic cells***(Differentiated cells such like skin cells blood cells)* **do not have high levels of telomerase disabling indefinite number of cell divisions.***(They have large quantities of telomerase s and they are active but somatic cells don't have this luxury)*



- **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*1) express abnormally high levels of telomerase, allowing them to continue dividing indefinitely.**

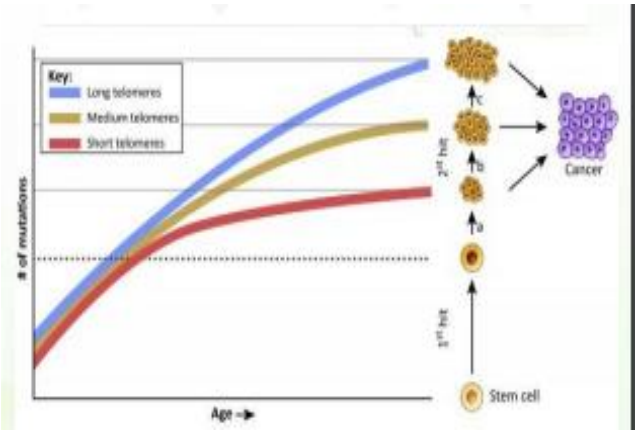
Somatic the telomers get shorter and shorter with every cell division and that is lead to what is known as senescence (they stop growing and reacting with they environment , they do nothing just with very low metabolism not divide no function or they eventually die)

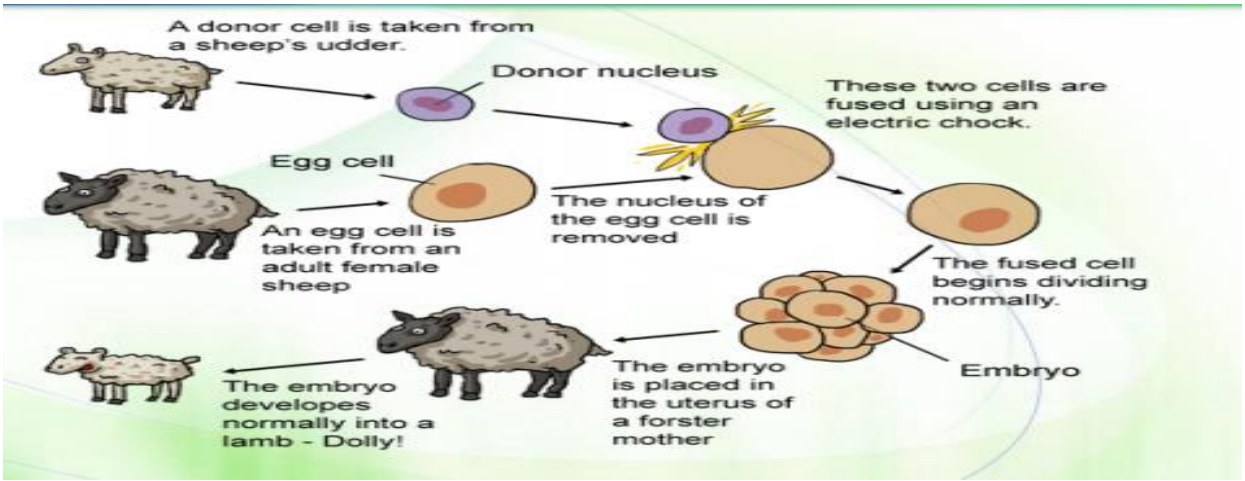
The chromosomes of the Stem cells are kept in same length so it is constant

***1 melanomes are skin cancers they do not die**

***There was a coloration that was found between the activity and amount of telomerases with formation of tumors which can lead to cancer cells .**

***The *higher* the activity of tolemerases the *higher* the quantity of telomerases the *more* aggressive cells the *larger* the tumors are and the *higher* probability this tumors would progress to cancer.**





Dolly was a cloned sheep and this Dolly was cloned from another sheep so DNA of another sheep is taken into an egg and this egg was fertilized.

This DNA was transferred which is already diploid transferred to another egg and the egg was stimulated as an embryo to become Dolly .

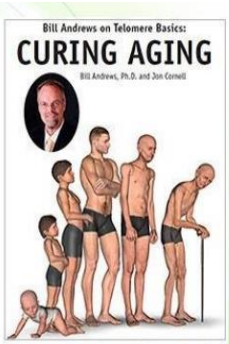
The cell was taken from the mammary gland (the gland that produces milk when the sheep gives birth to)

- ✓ **Dolly looked old early young**

- ✓ **She had certain diseases \ arthritis**

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

And the reason of that the DNA that was taken from the donor was actually six years old (6+6=12) when Dolli was born she was -moleculary- 6 years old and because the chromosome was six years old they were shorter SSthan normal.



GOOD LUCK EVERYONE ☺ !!!