No .5

Molecular Biology

Doctor 2019 | Medicine | JU

Sheet Slides

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For the DNA replication and synthesis of DNA we need double helix separated into single strands of DNA. Which allows proteins and enzymes to bind and synthesize new DNA.

Origin of replication (OriC) in bacteria

- Actually, there is a starting point for DNA replication where the double helix separates into single stranded DNA, and this point usually is named "Origin of replication"
- Actually, starting the replication at the origin isn't a random mechanism. These sites are recognized and such recognition should be mediated by an organized mechanism

So, let's start talking about origin of replication in bacteria:

- There are some differences between prokaryotes and eukaryotes when we talk about the origin of replication, but both of them should have the origin of replication.
- In bacteria, the place where the replication starts is called *(OriC)
- oriC regions contain **repetitive 9-bp and AT-rich 13-bp sequences** (These are known as consensus sequences)

so, what do we mean by consensus sequences?

1-they are kind of sequences found in DNA and RNA

2-in different places

3-and do similar jobs (known job)

(basically a sequence of nucleotides that have a universal function in different organisms)

Mers means part

Like dimer(2 subunits)

Monomer(1 su)

Poly-mer(several subunits

Like DNA polymeraze: poly=many ase usually refers to an enzyme that do dna synthesiz

Type of consensus sequences, so we have different 2 types:

9-mers: binding sites for the DnaA protein**(plays an important**

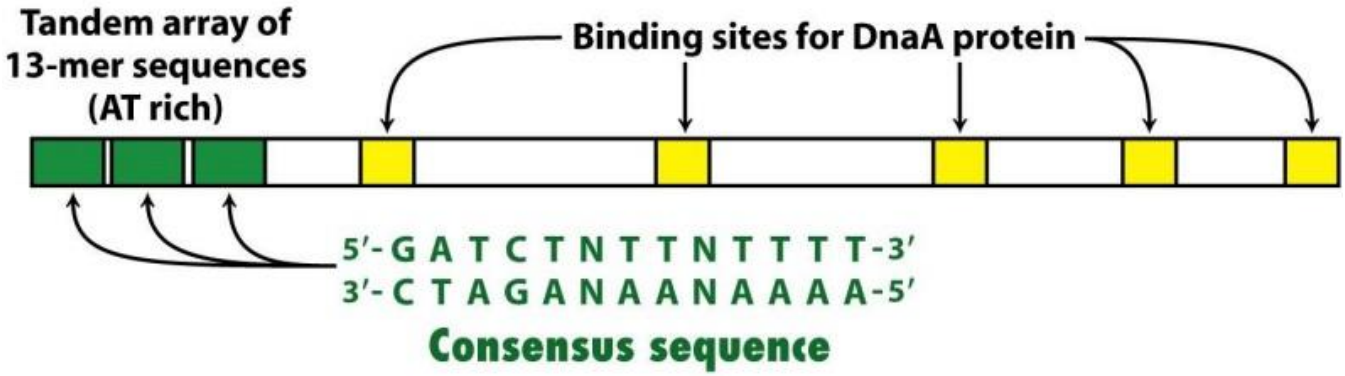
Role in the binding to DNA and trigging the separation between the double stranded DNA).

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

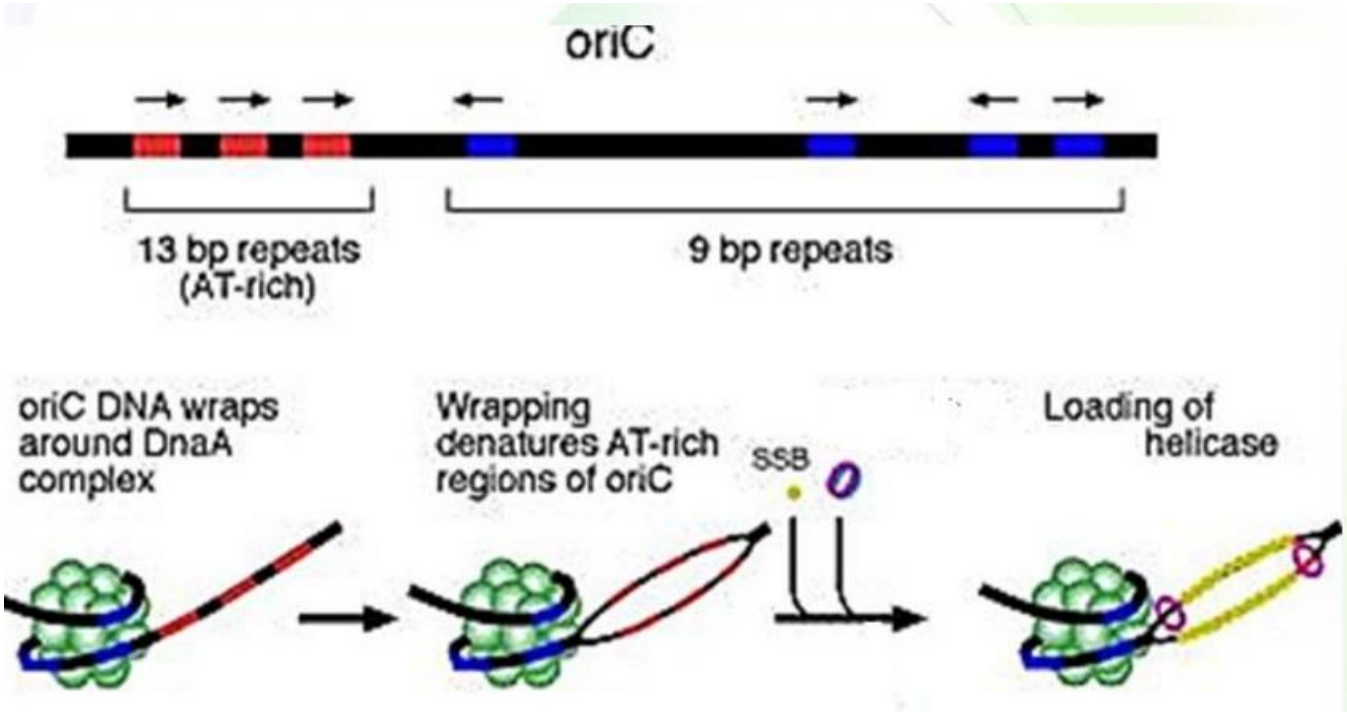
In the figure below: regarding to 13-mers and 9-mers, the yellow boxes contain 9-mers and the green boxes represents the tandem array of 13-mer sequences

You can also see the consensus sequence containing several DNA bases and **rich in A&T**

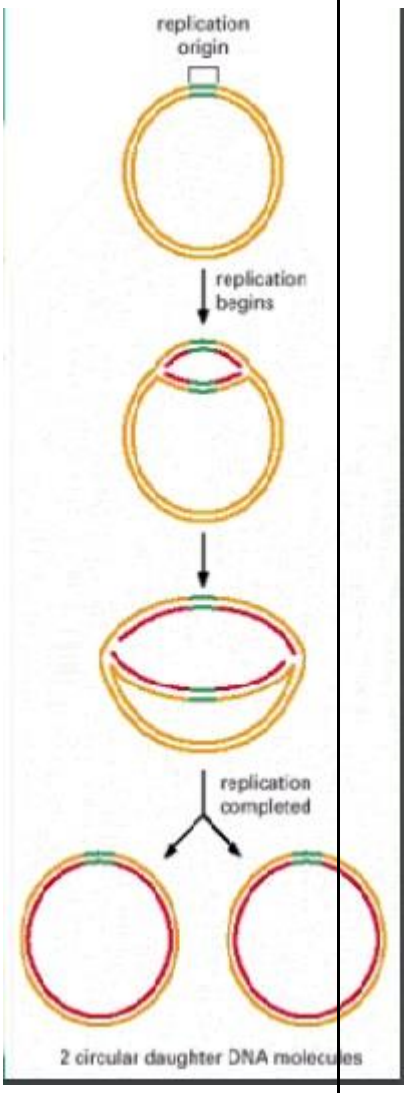
So, why A&T? because the interaction between A&T, remember we have 3 hydrogen bonds between the G&C and 2 bonds between A&T so it's easier to separate the strands rich in A&T, because its much weaker than G&C.



Again, this figure below maybe explains more, we have several 9 bp consensus sequence that binds and recognized by DnaA protein, after recognizing the 9bp repeats wrapping happens around the dnaA protein, so after wrapping DNA around DnaA protein at the OriC, It triggers and activate the separation of double strands at the AT-rich region and this separation allow other proteins such as SSB and helicase to start or initiate DNA replication

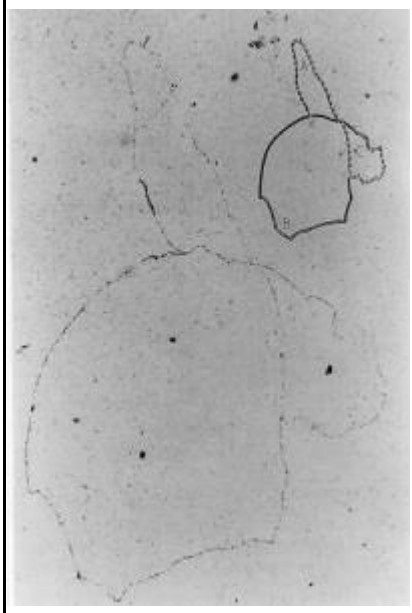


- So in bacteria we have 2 replication forks, you know that in bacteria we have a circular chromosome and to initiate replication we will have only <u>single</u> origin of replication.
- The two replication forks proceed in opposite directions until they meet up roughly halfway around the chromosome.



  - You see that we have two opposite directions for the replication and the replication is continuous in opposite directions to produce two daughter DNA molecules. (right figure)

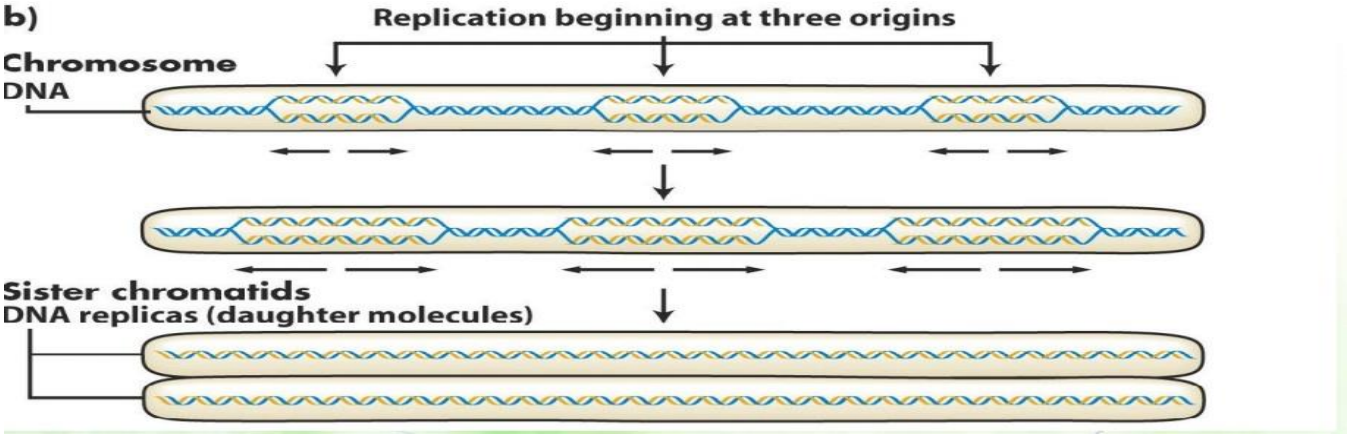
حتى ابسطلكم إياها تخيل تجيب ورقتين دائريات حطهم فوق بعض الان بعد ما تلصقهم بلش بفصلهم اول ما تمسكهم عشان تفصلهم هذا هو الاوريجين اوف ريبليشكشن,اول ما تبدا عن فصلهم شوي شوي هذا اسمه دي ان أ ريبليكاشن واستمر بفصلهم عن بعض عبين ما توصل لاخر اشي انه عندك ورقتين منفصلات



❖ the figure on the left shows the replication of the circular DNA

❖ **long arrows:** are the replication forks and they are in opposite directions

We said that in bacteria we have one origin of replication, so is it the same in the human genome? Actually no



An average **human chromosome** may have **<u>several hundred</u> origins of replication**.

so why do we need multiple origin of replications in human genome? actually you know that the human chromosome is long and the presence of multiple origin of replications may help for rapid replication of the human chromosomes that's because we have many chromosomes and we need many origin of replications in order to synthesize all of them.

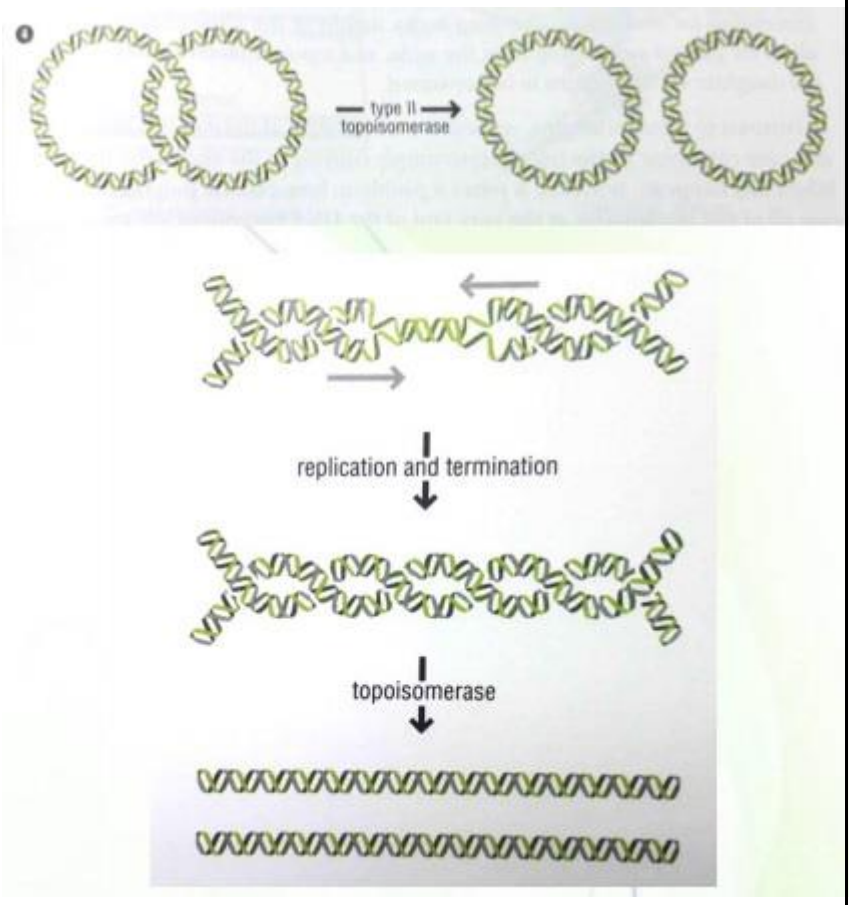
Note: check the figure above

Role of topoisomerase II

- In the previous lecture we talked about topoisomerase and topoisomerase I that removes supercoils and twists in the DNA so allowing DNA to continue without contraption.
- So now we will talk about topoisomerase II AND its function:

- Topoisomerase II is responsible for <u>untangling</u> chromosomes by making a transient double-strand break.(remember that topoisomerase I do a single strand break)
- **So what do we mean by untangling**? Mean بالعربي فك الاشتباك بين الكروموسومات

- also known as gyrase in bacteria
- ATP-dependent(uses atp as a source of energy)



It is also responsible for chromosome condensation during the cell cycle.
Maybe this figure explains better what we talked about we see here a bacterial replicated chromoses and for eukaryoutes here you see that the resulted 2 chromosomes are tangled together so topoisomerase 2 will cut double strand of 1 of them and join again allowing the separation of the 2 chromosomes alone as you can see here, in eukayotes we have also similar function, also we have double stranded DNA and these double stranded dna supercoiled and alligned togther So the topoisomerase help to remove and untangle these 2 chromoses or dna structures as a separated chromosomes

Extra: What is the difference between topoisomerase I and topoisomerase II ?
✓ **Topoisomerase I : it releases strain within one DNA molecule during replication by making a single-strand break. (ATP-independent).** ✓

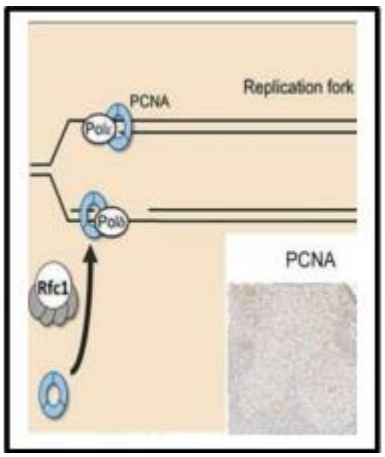
Topoisomerase II : it untangles the two daughter DNA molecules from each other by making a double-strand break. (ATP-dependent).

Topoisomerase inhibitors are commonly used in treatment of cancer. such as doxorubicin and etoposide both of them are topoisomerase inhibitors and probably used in treatment of cancer

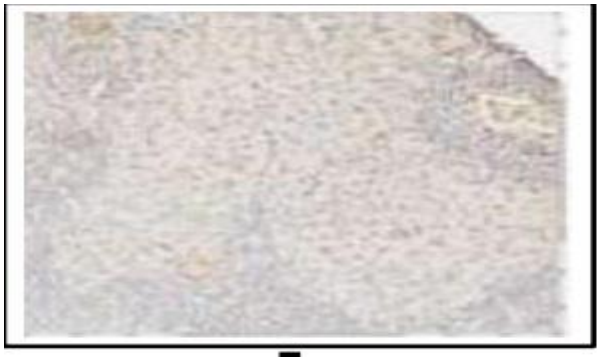
Role of PCNA proteins

➢ Let's talk about another important protein involved in DNA replications in humans

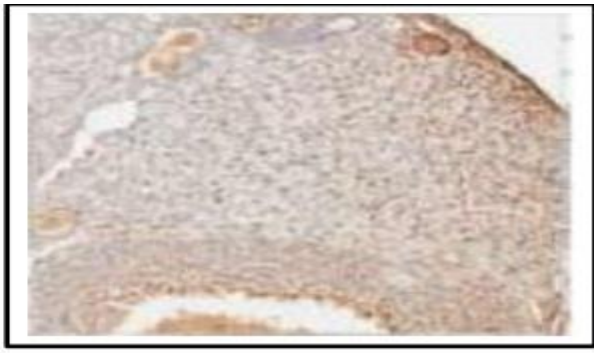
- DNA polymerases are guided to the primers by a protein called PCNA (proliferating cell nuclear antigen).actually these proteins involved in DNA replication and repair and help in guiding the DNA polymerize to the primers in the replication forks (remember that the function of primer to provide the 3 prime OH for DNA polymerase to work)



- These DNA evolve in the cell prefoliation, **so Proliferating cells have higher levels of PCNA, and so they are extensively stained (the color of the stain is brown)**
  - As we know cancer cells profilate faster so we need PCNA and it can be considered as a marker for cancer



Not stained a lot – Not a lot of PCNA – non-proliferating cell(normal cell)



More stained (more brown) – a lot of PCNA – proliferating cell(cancer cells)

In addition, this staining mechanism can be used to diagnose cancer; how severe it is, and how aggressive it is. If there are high levels of PCNA, the cells are likely to be proliferating, we use it also in histology in determining special antigen or antibody(you will know more about it in the future)

DNA polymerase in eukaryotes

You remember that in the previous lecture we talked about DNA polymerases in eukaryotes DNA replications named DNA polymerase 1 2 3 4 5 until 9 , then most of the DNA polymerase involved in the DNA repair

Actually there is 5 types of DNA polymerase you should know summarized in the table below

TABLE 10.4

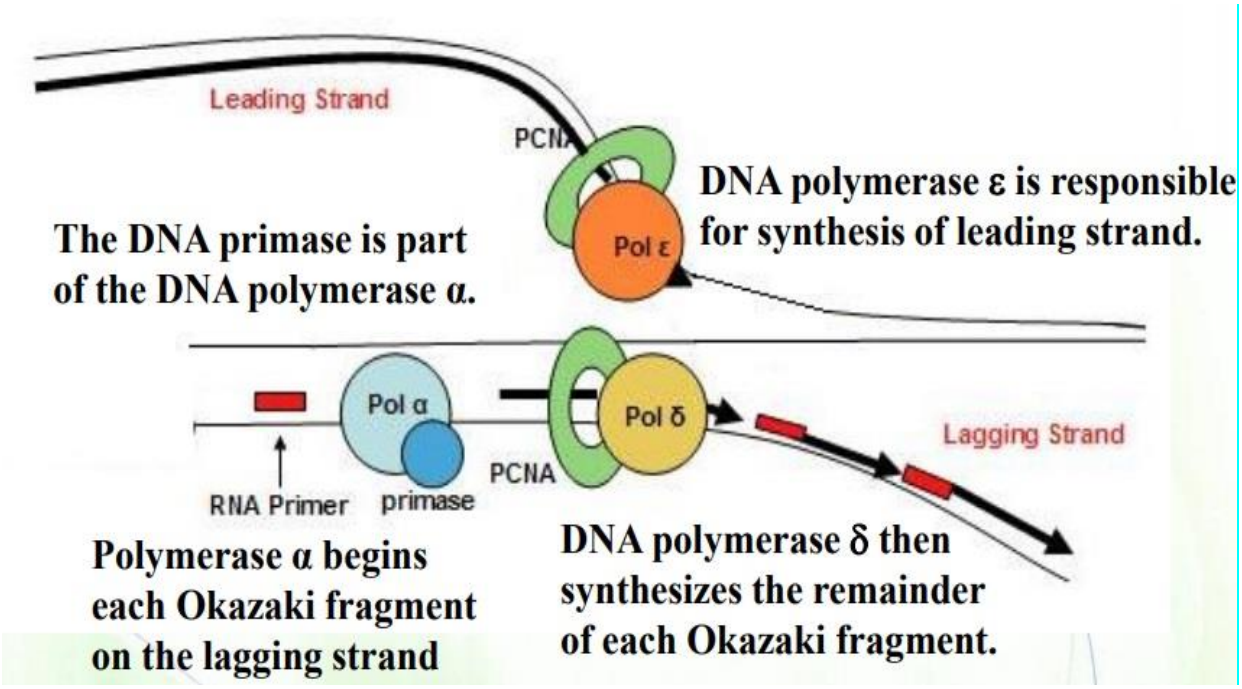
The Biochemical Properties of Eukaryotic DNA Polymerases

	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

You should know that the **molecular weights** are measured in Da unit and they are **different** between proteins

- Most of the DNA polymerase found in the nucleus except for gamma polymerase which is found in mitochondria.
- Remember that we also have in the mitochondria the mitochondrial DNA which is also synthesized gamma polymerase
  - Now what about exonuclease 3' → 5' activity the proofreading you remember that there is mismatched and such mismatched bases should be corrected by the exonuclease(**we have the exonuclease only in delta,epsilon and gamma polymerases)**
  - You remember the primase the enzyme adding the primer actually alpha DNA polymerize the only dna polymerase that has the function of primase or adding primer to DNA strands
  - Regarding the processivity or rate of synthesis actually delta ,epsilon and gamma can synthesize the DNA very fast while alpha and beta have slow rate of synthesis.
  - Regarding the replication capacity actually all these DNA polymerases can replicate and do DNA synthesis except beta that has no replication activity
  - Regarding repair or involving the enzyme in DNA repair we have it only for the epsilon and beta DNA polymerizes

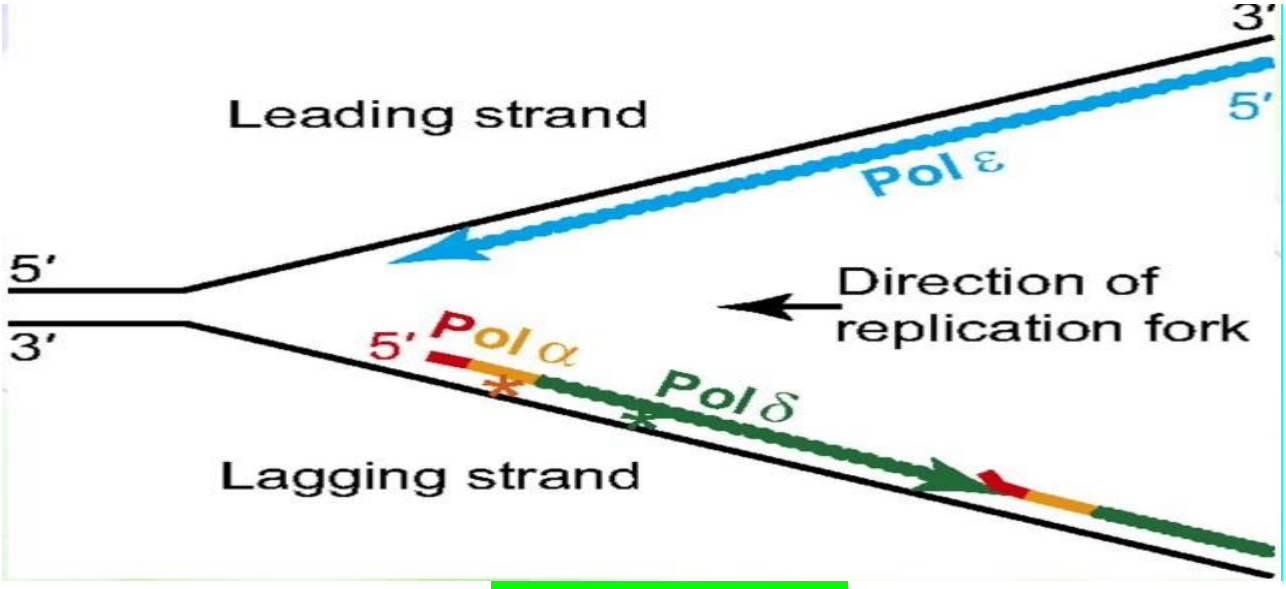
The mechanism of replication



Here this slide summarizes the mechanism of replication and the function of DNA polymerases in the synthesis

- In the leading strand DNA polymerase epsilon synthesizes the strand
-
- And in the synthesis of lagging strand we have 2 DNA polymerases (gamma and alpha) you remember that alpha it has the primase activity so it adds theRNA primer and start the synthesis of each of okazaki fragment, later DNA polymerase delta come and complete the synthesis of the lagging strand and fill the gaps between okazaki fragment to synthesis the lagging strands
- The polymerases do not have a 5' 3' exonuclease. Actually the dna polymerase epsilon and delta have the 3' 5' exonuclease which means the opposite direction to the DNA synthesis
- **Remember: DNA synthesis occurs from 5' to 3' but the removal of mismatched occurs in 3' to 5' direction**

- This figure explains the mechanism in an easier way as you can see here DNA polymerase epsilon synthesize the leading strand from 5' to 3' in a continuous way
- While in the lagging strand DNA polymerase alpha synthesizes each okazaki fragment then dna polymerase delta completes the synthesis and fills the gaps and joins each okazaki fragment to form the lagging strand

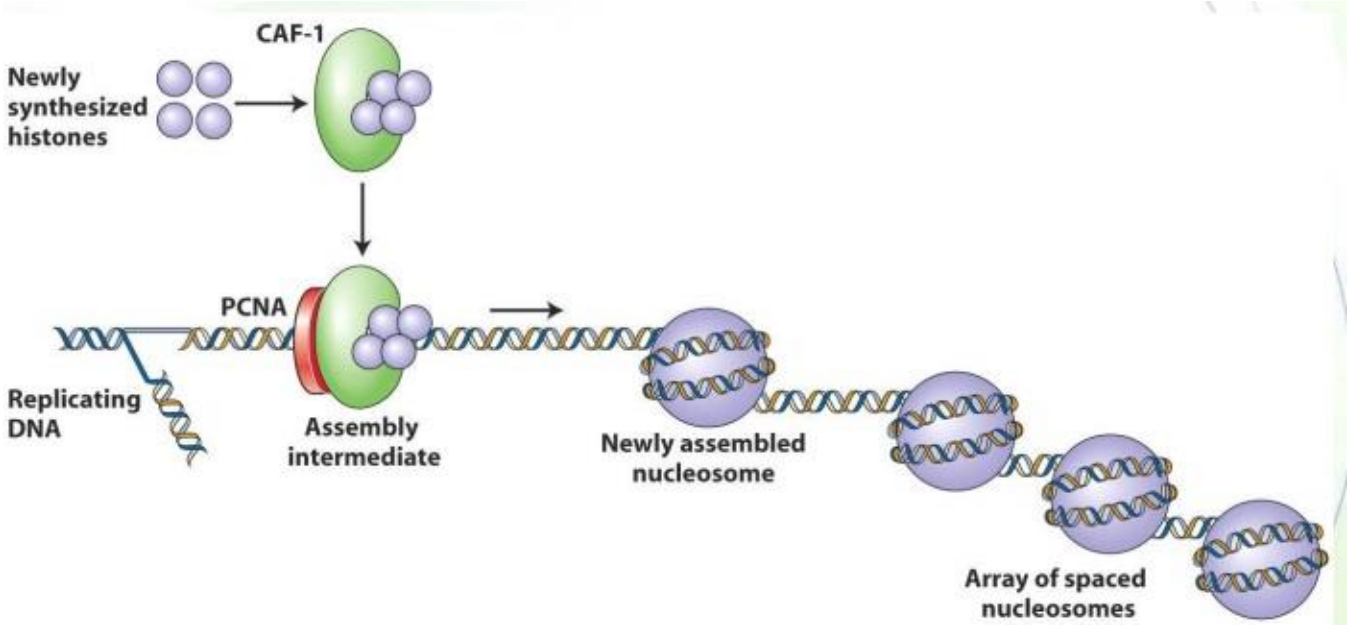


Role of chromatin

- So what is chromatin? Chromatin is the mass of genetic material composed of DNA and histones
- Definitely replication of DNA should be linked to DNA packing by histones, in order for DNA to replicate we need to unpackage the DNA from histones, to have the double strands free for enzymes to do their replicative functions
- 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)

In this figure below, we have histones that are positively charged proteins these

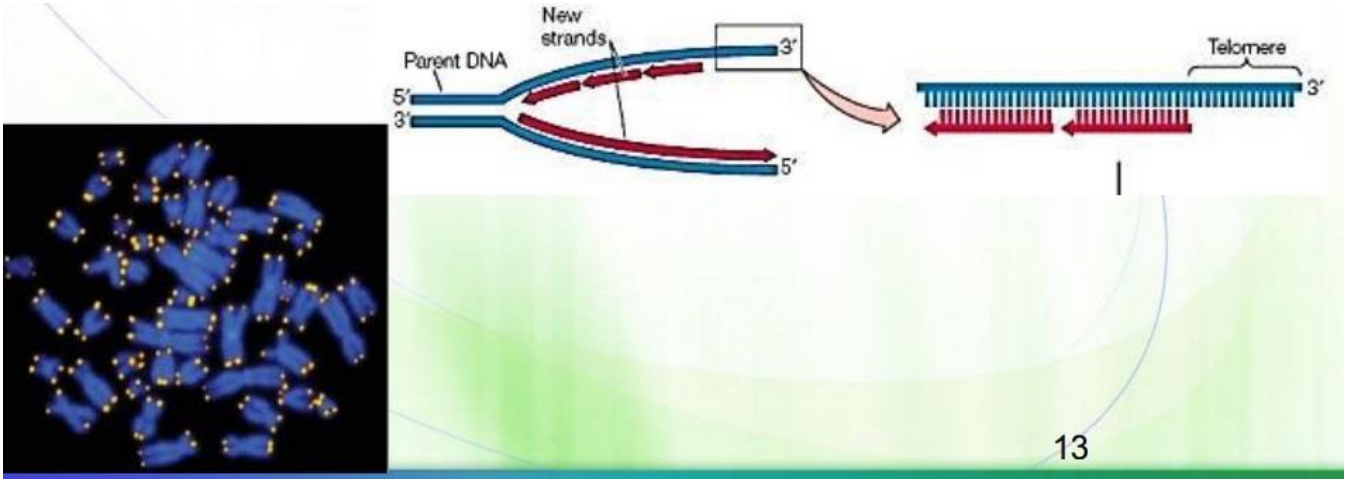
histones bind to chromatin assembly factor 1 these complex start to package and assemble the DNA into histones so we have what we call it newly assembled nucleosome with the progress we have more and more arrays of spaced nucleosome



خليني اشبهلكم إياها بطريقة ابسط تخيل الكروماتين كرة صوف لفوفة حولين بعضها البعض الخيط فيها هو الدي ان أ فتخيل انت انه هاي كرة الصوف مش ملفوفة بطريقة منظمة وعشوائية رح تاخ1 مساحة كبيرة وعشوائية جدا وبالتالي حتى تأخذ مساحة اقل لازم تكون منظمة وملفوفة بطريقة مرتبة حتى تصير عملية الريبليكيشن لازم نبلش نفك الخيط من الصوف شوي شوي عبين ما نصنع الدي ان أ الجديد ونعمل وحدة ملفوفة ومرتبة الهدف منها تقليل المساحة الي رح ياخذها

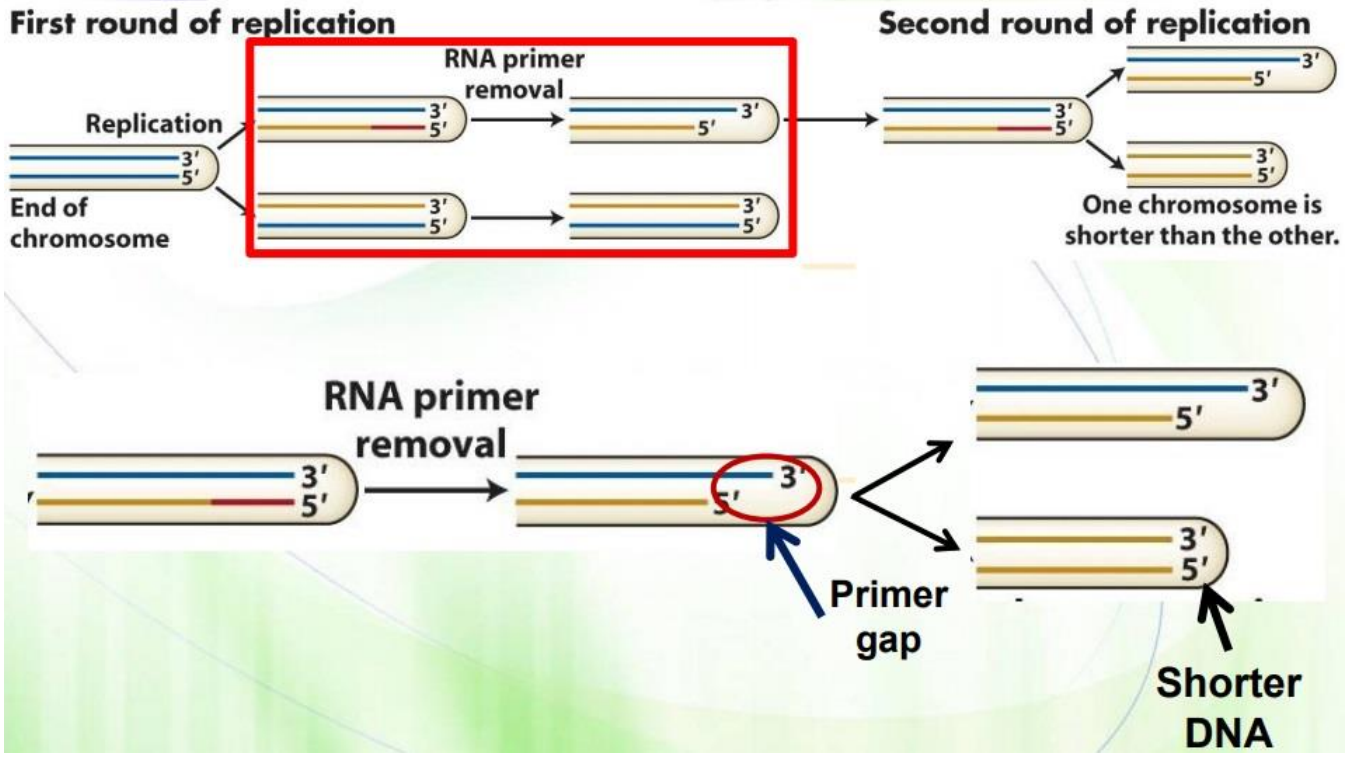
- Remember the DNA polymerase needs rna primer to complete the synthesis of DNA and we have primer for each okazaki fragment the primer later on will be removed and gaps will be filled with DNA

- So what about the final rna primer? There is no space for DNA polymerase to fill the gaps and that will lead to shortening of the lagging strand actually this is a very important issue



This figure explains better (في حال عدم الفهم يرجى الرجوع لفيديو الدكتور لانه كان ياشر بمواقع عالرسمة والموضوع صعب ينكتب)

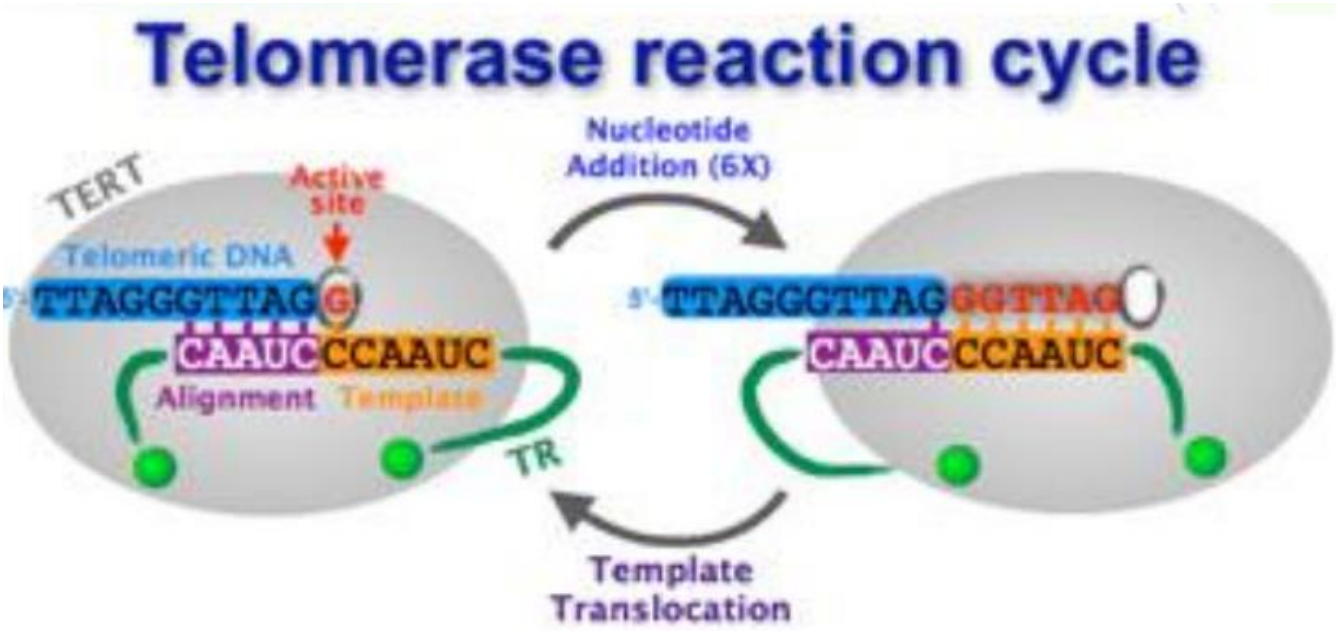
In the second round there will be shorter ends of chromosomes and this process continue and the shortening continues, and this is really a problem because the shortening of these pieces of DNA will reach the coding regions actually, you know we need the gene full for the expression for producing proteins that's doing its job.



So how our body can deal with this issue and protecting our genes?

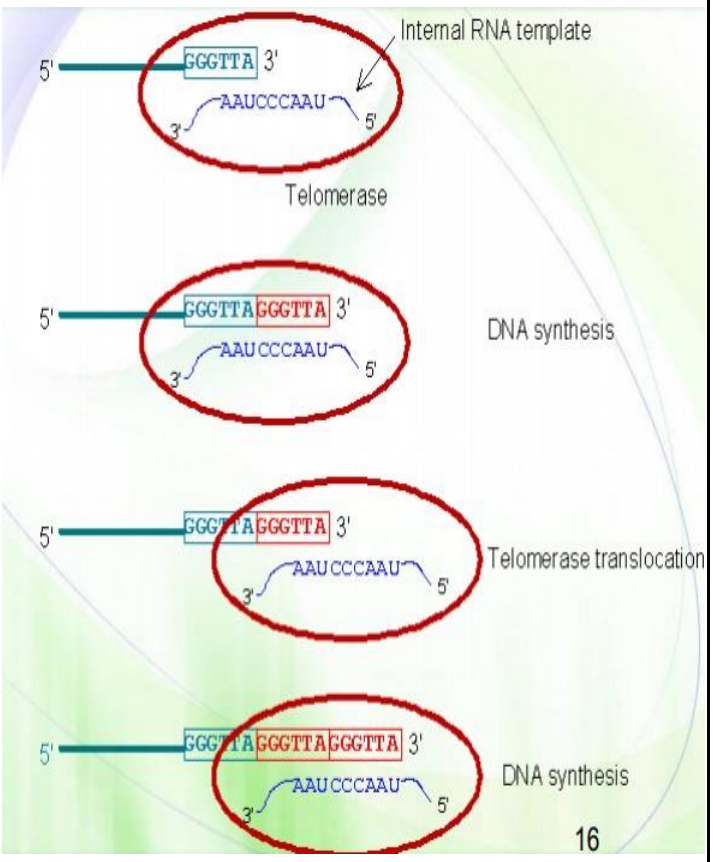
Telomerase comes to the rescue

- There is what we call it a telomerase, telomerase is responsible for adding repeating sequences of DNA and these repeats can reach to 10000 nucleotides
- Telomerase (a reverse transcriptase) prevents the progressive shortening of the lagging strand. How? Reverse transcriptase is a type of enzyme that can convert RNA into DNA so it read the RNA and synthesis a complementary DNA strand, it's important for you to know that this the mechanism of some viruses like the HIV virus
- There is a reverse transcriptase in virus article that convert RNA to DNA and later on is integrated into the chromosomes of hosting cells.
- So how can our cells can solve the problem of the shortening the ends of our chromosomes, in fact telomerase comes to the rescue. so at the ends of our chromosomes we have long DNA sequences called telomeres that consist of many GGGTTA repeats and extend about 10000 nucleotides
  So in the telomerase we have a template that is a component of the enzyme itself telomerase bind the end of the DNA and starts the synthesis of the 6 nucleotide which is the synthesis of the telomere (figure below) so this process continues to build a long sequence of DNA around 10000 nucleotides



a) This figure here explain more. You can see here at the first round the structure of the telomerase, there is a complementary between the end of DNA template and RNA template found in the telomerase,
b) and the telomerase here will start synthesizing the first “repeat” and newly synthesized repeats should be complementary to the 6 nucleotides found in the telomerase
c) so again this is the first cycle then the telomerase will start to translocate

and then DNA synthesis occurs and 6 nucleotide are added and the cycle is repeated several times to produce telomere ends.(up to 10000 nucleotides)



In slide 17 there is an animation you have to check in the PowerPoint in order to find out what is wrong with it

How do we age?

- The story of telomerases opens the question about the relationship between age and the telomerase activity and length of polymers so do we have any relationship? In fact, yes
- As we grow older, the activity of telomerase is reduced.
- An inverse relationship between age and telomeric length has been observed. (every cell division there is a decrease in length at the telomer end and because we don't have a telomerase active so we can't renew and synthesize new telomer ends
- In many of our somatic ends the telomerase is inactive, we have it active actually in some cells like stem cells maybe also you need to know that the telomerase is very active in most of the cancer , that's why cancer cells can proliferate for long time without death why? Because the normal cells every time they divide there is a shortening of the telomer , the telomer will be removed and there will be shorter DNA and the genes will be affected ,so the cells die, this is actually how the aging occurs
- اخر سلايد بده يحكي فيه قصة طريفة خلاااص متت مش كاتبها