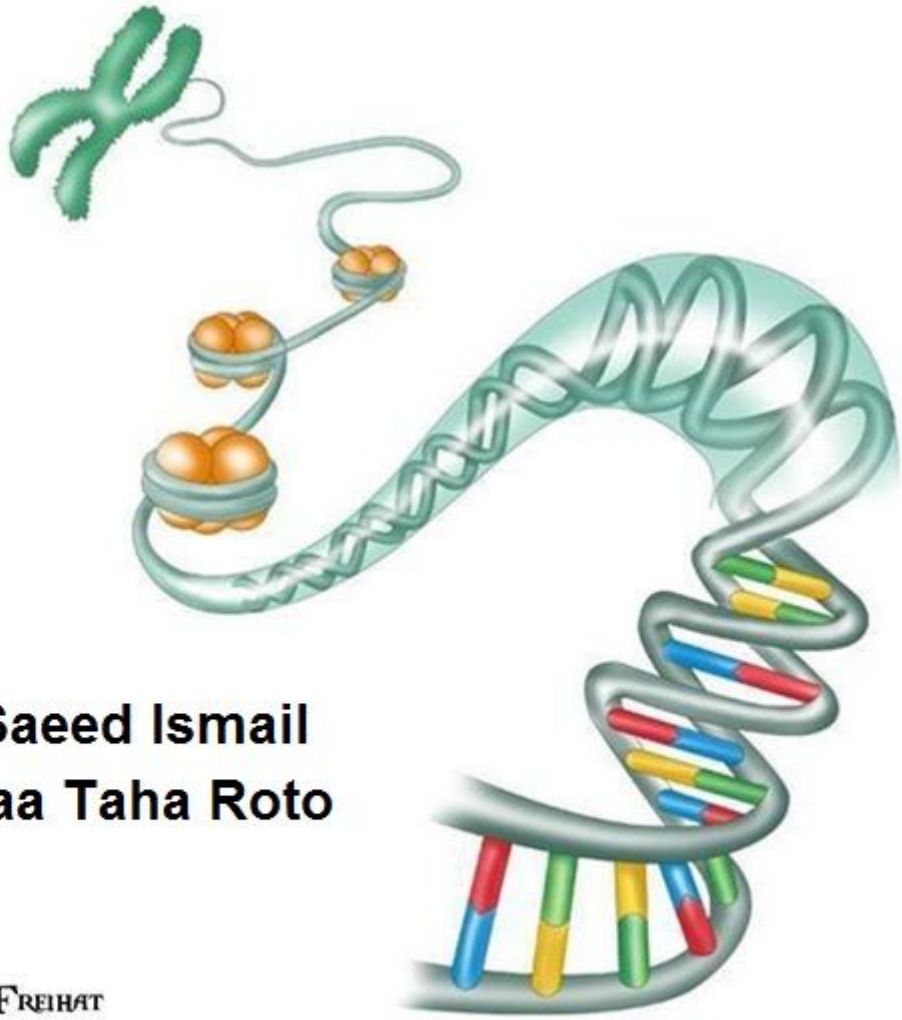
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ⊗ Sheet ○ Handout ○ other.....



Sheet#: 15

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Chapter 13

Replication : DNA synthesis

Once the cell wants to divide, it copies its DNA to guarantee passing its genetic material to its 2 daughter cells, and the enzyme responsible for copying the DNA is the **DNA polymerase.**

The process of DNA replication is very similar in all living organisms, starting from simple bacterial cells going up the ladder to the full human being, all have similar DNA replication mechanism,**which is** : you have to split the two strands , and then prime the place you want to start synthesis from (by a primer) , and then comes DNA polymerase to add nucleotides one by one in a ***5' to 3' direction .***

So the major process is the same, but of course there are differences. We will address the basic issues that are common in all living organisms and then mention the unique features of DNA replication in eukaryotes and prokaryotes.

Through the lecture, we will switch between prokaryotes and eukaryotes, so pay attention to the differences between them.

So what is the major difference between prokaryotes and eukaryotes related to the DNA replication process?

It is the presence of the nucleus. *Eu means true*, so eukaryotes possess *true nucleus (*meaning that the DNA is segregated by the nuclear envelope from the cytoplasm in a closed compartment named the nucleus), while prokaryotes have no true nucleus.

In molecular biology, we take a representative organism from each one of these two domains.

So, what **are prokaryotes ?!** Basically bacteria, and especially E.coli is the representative one.

Eukaryotes ?!! Simply represented by yeast (unicellular organism) and also Humans (the ones that we are really interested in)

**You may wonder why do we study prokaryotes first and don't jump to eukaryotes directly.

Actually the major part of molecular biology and genetics was built on studies performed on prokaryotes. *The reason is that they have so many features that ease our studies*, **for example :**

1-If you want to study replication, it will be easier to study it in prokaryotes than eukaryotes, why?

As the replication time for prokaryotes is about 30 min , while the average replication time for a *human cell* is about *12-24 hours* .so it if faster to study it in prokaryotes .Also , the genome of bacteria is smaller than our genome , making the study of the basic concepts of replication much easier.

2-Moreover , *culturing* bacteria outside is easier as they can grow in test tubes or on petry dishes ,while it is difficult to grow human cells outside the body.

So we take the basic understanding from prokaryotes then we jump to eukaryotes and human cells to figure out the major differences.

DNA replication :

First, we have to define some terms, these terms are true for all organisms :

1- Origin of replication :

You have a double stranded molecule , and as we said in the sequence of DNA replication process we have to split the two strands from each other . Splitting is actually denaturation.

Let's return back to the previous lecture , we said that we have two ways to denature the DNA :

1- Raising the temperature
2- Raising PH

The two previous ways will kill the cell (excessive heat or PH will denature all the proteins and enzymes needed for cell survival), so we need another way to split the DNA without killing the cell, Enzymes will be the solution.

If we want to denature the DNA in a test tube by heating, the 2 strands will split **at once** directly, but this is not the case with enzymes. Enzymes don't split the two strands by simply breaking the H Bonds between nitrogenous bases from the beginning of the molecule till the end of it **at once** , in other words , they are not like a scissor that split the double stranded DNA by simply cutting the H bonds . Rather, *they scan the DNA searching for a certain sequences that tell these enzymes to start splitting here at a certain point.* **These certain sequences are called "Origins Of replication " وأخيرا**

Now, The enzyme that recognizes the origin of replication is **Helicase** . It is the denaturing enzyme that unwinds and splits the two strands at a certain point, which is ??! The Origin of Replication .

After helicase recognizes the origin of replication, it unwinds and splits the two strands at that point, making a bubble or a replicon. So each replicon will have two forks , and at each fork we have group of enzymes working there and copying the

DNA , the two forks will continue opening until they fuse and we will eventually end up with two new ds DNA molecules .

2-DNA replication is <u>bidirectional :</u>

As we have two forks , replication will proceed in both opposite directions, that's why it is bidirectional.

<u>3-Semiconservative model :</u>

DNA replication is semi conservative. *Semi means half* ,so when we split the two original strands ,each strand works as a template that DNA polymerase can use to synthesize the new strands . At the end we will have two new molecules that are double stranded , one of these two strands is an old one , and the other is a new one (half new and half old). So it is semi-conservative; each cell has conserved half of its maternal DNA.

Notice here that in replication , both strands are used as templates , while in transcription, when we make the RNA , just on strand works as a template , and this is an important difference between replication and transcription.

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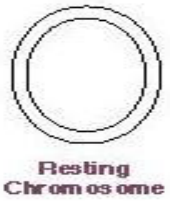
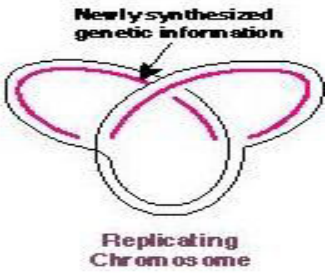
Let's look to the process in prokaryotes first :

<u>Prokaryotes :</u>

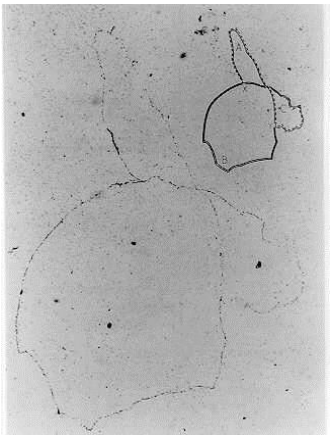
1- They have circular DNA

2- *One origin of replication* only. Helicase also recognizes that point , unwind and split the two strands making the bubble and the two forks ,then polymerases with other helper enzymes will work at the forks in a bidirectional





fashion and then the forks will continue opening up and eventually fuse giving us two circular new DNA molecules .



Look to the picture to the right , it is an electron micrograph showing bacterial chromosome replication.

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Eukaryotes : (human for example)

1- They have linear DNA that is much larger than the bacterial one.

2- There are Multiple origins of replication . **Now why do we need many of them?**

That's because our DNA is very huge, we have 46 chromosomes , each single chromosome is larger than the bacterial chromosome .So with only one origin of replication , it will take the cell forever to copy its DNA and then divide.

Now notice that although we have multiple origins of replication to speed up the process , **bacteria replicates faster than us , why ?**

-Firstly , they have smaller genome (one chromosome containing 3 million nucleotides) compared to us (we have 6 billion nucleotides arranged in 46 chromosomes)

-Secondly, their DNA polymerase enzyme is much faster than ours (notice : faster doesn't mean more efficient) .

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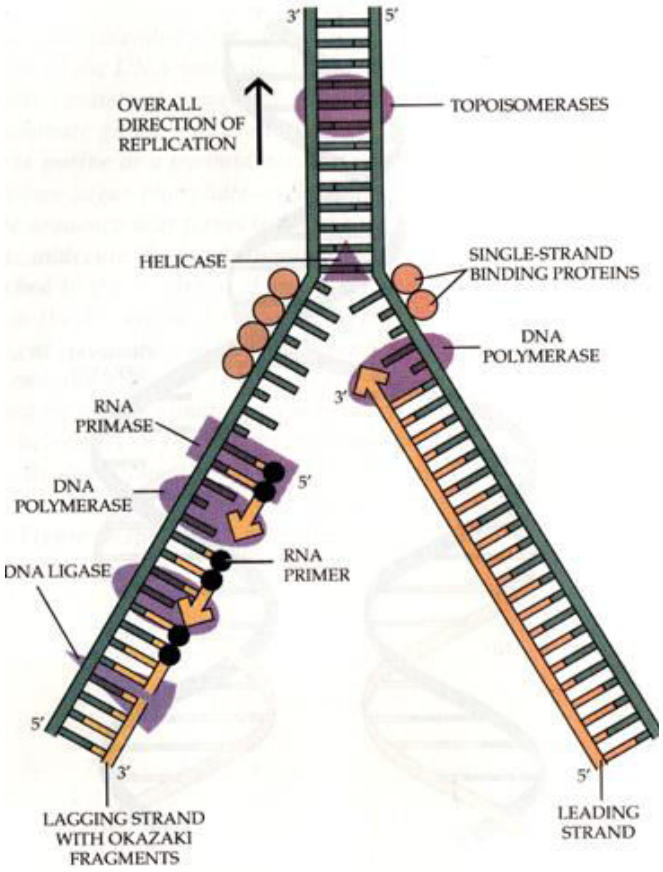
Let's now go to the details of DNA replication process in **eukaryotes:**

We said we have a bubble with two forks, now the picture to the right is showing just one of them , the other is not shown.



- First, helicase unwinds and denatures the DNA at the origin of replication, making the replicon and opening the two forks.

Upstream the fork , where the two strands are not separated yet , there will be a stress caused by the unwinding action of helicase. Helicase actually unwinds the DNA against its normal turn .

Imagine you have a spring and you wind it with its turn , it will just get smaller and shorter . But when you unwind it against its turn , it will be stressed and then it will break . Same thing happens here ,upstream the fork ,the DNA will be stressed , so we need someone to relieve the stress and prevent this breakage , this *noble person is the Enzyme Topoisomerase.* Topoisomerase will recognize the stress points , cut ,relieve the stress and rejoin again.

Imagine there is no topoisomerase , after finishing DNA replication , we will end up with 100 thousand pieces of broken DNA due the stress generated by helicase unwinding action.

We said previously that DNA splitting occurs by enzymes under cellular conditions , *(PH=7 and T= 37 °C)* ,so there is nothing to prevent the renaturation of the unwound and splitted DNA , sometimes we call renaturation "snap back " which is the rejoining of the two strands again .

That's why we need other guys to hold each single strand by binding to it and preventing it from base pairing with the other complementary strand . Our guys here are the ***single strand DNA binding proteins*** (by the way , they are not enzymes, just proteins)

** **Now , let's continue our story with the main enzyme of DNA replication process (DNA polymerase).**

First , I want you to remember that DNA strands are Anti-parallel. So we will have one running from 3' to 5' , and another one running from 5' to 3' , and put in your mind that DNA polymerase works from 5' to 3'.

1- Strand 3' to 5' → this strand is a template for the 5' to 3' new strand , so notice that this new strand runs *in the same direction* that DNA polymerase works in , so there will be no problems in synthesizing this strand .it will be made continuously , that's why we call it the *leading strand* , and it will grow at the same direction of the replication fork (away from the origin of replication and towards the fork opening direction).
2- Strand 5' to 3' → this strand is a template for the 3' to 5' new strand , so notice that this new strand runs in the *opposite direction* DNA polymerase works in , so there will be a problem . This strand will wait for the fork to enlarge to make many fragments ,at the end , these fragments will be joined together. So this strand is

synthesized discontinuously and away from the fork, that's why we call it the *lagging strand* . The fragments are called ***Okazaki fragments*** (it is the name of the Japanese scientist who first described them), and their length is about 200-250 nucleotides.

Another point to understand is that DNA polymerase has a weakness point . It can add nucleotides on a ss DNA but it can't add nucleotides without the presence of preexisting double stranded DNA piece , even a very small piece of ds DNA will be enough. So DNA polymerase can't make the full double stranded DNA from null , *there must be a starting ds piece to build on it* .Theoretically , give it one nucleotide that is linked to the template strand and it will add the rest 1000 or 10000 nucleotides to make the full new strand for example .

W*hat DNA polymerase actually needs is the 3' free OH group* of the existing nucleotide to add on it the 5' phosphate group of the next added nucleotide and *make the phosphodiester bond* .

Note that making the new strand starts by forming phosphodiester bond , not the hydrogen bonds , and that's why we need the primer , the primer will enable the DNA polymerase to make a phosphodiester bond.

Who is going to give us that primer ?

RNA primase , it is the enzyme that adds ***RNA nucleotides*** (about 6 to 10 nucleotide) on a specific place on the DNA template strand and creates the stating double stranded piece that polymerase needs to build on .

Now eventually , the RNA nucleotides must be removed and replaced by DNA nucleotides . **RNase enzyme** removes the primer , and then comes a specific type of DNA polymerase to

fill the gap and add DNA nucleotides (you will understand this sentence as you proceed).

But what is really weird about it that RNA nucleotides are added not DNA , we don't know why but there must be a good reason as our cells are really clever and economic. Some of you may think that the **DNA polymerase** requires an RNA primer and can't work if the primer was a DNA piece. This is wrong , in genetic engineering , if you put a ***ss DNA , a DNA primer and DNA polymerase*** in a test tube ,polymerase will be able to copy that ss DNA using the DNA primer , but this doesn't happen in our cells , we don't know why but there must be a good reason.

What RNA nucleotides mean to you ?

Ribose sugar rather than deoxy ribose , and uracil base rather than thymine.

Another enzyme that is really important in **joining okazaki fragments** is **DNA ligase**. DNA polymerase as we said works from 5' to 3' , adding 5' phosphate group on 3' OH group .

Imagine now there is a primer and a DNA polymerase making an okazaki fragment , and upstream there is another primer and another DNA polymerase doing the same thing . this DNA polymerase will move close to the primer , now the last nucleotide added by the polymerase has an 3'OH and the nucleotide in the primer in front of it has a free 5' phosphate . so if the polymerase wants to make a phosphodiester bond between these two nucleotides , it will make it 3' to 5' , IT CAN'T ☹. So ligase is there to solve the problem , it can form that 3' to 5' phosphodiester bond.

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Make sure you know each enzyme and what is its function , a question in the exam will come on them.

Enzyme	Function
Helicase	denaturates the DNA by splitting the two strands and unwinding the DNA at the origin of replication , creating a replicon that has two forks.
Topoisomerase	it works upstream to helicase to relieve the stress that helicase creates by (cut –relieve – rejoin) cycle
RNA primase	providing a primer (nucleotide stretch measuring 6-10 RNA nucleotides) for DNA polymerase to add on.
RNase	removes the primer added by primase.
DNA polymerase	copying a new DNA strand using the old one as a template, but has a weakness that it needs a primer that provides 3' OH to be able to form the phosphodiester bond.
Ligase	joins Okazaki fragments , as it can work 3' to 5'

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Now we will focus on the main enzyme that copies the DNA , which is DNA polymerase :

DNA polymerase is the *main enzyme* that build the new strands of DNA by adding nucleotides 5' to 3',and other enzymes are just accessory ones (they are helpers) ,so the main guy is the DNA polymerase . We will highlight the differences between DNA polymerase of the prokaryotes and eukaryotes .

What do we know till now about this DNA polymerase molecule ?

This is an area of research where you can still see how molecular biology is evolving. If you remember we have divided molecular biology into applied (molecular engineering) and basic , most of the new work and updates are in the applied part , but still we have changes in the basic part , the information that scientists have settled hundred years ago are changing every day.

Considering DNA polymerase updates :

Scientists were interested in discovering how many types of DNA polymerase we have, what the function of each one is and which one of them is the main one. Our book was updating its information in each addition , for example : 1st edition said eukaryotes have 3 types of DNA polymerase , 2nd said they are 5 , then they said that there are AT LEAST 9 types , and the last edition says they are 15 , who knows how many they will be in the upcoming years ☺. Why is all that?

Because it is really difficult to watch these too similar guys working at that micro scale and determine the function of each

one , the experiments are really genius and complicated ,so they may miss something , and then discover it later on.

Now let's talk about **DNA polymerase of E.coli** which is the representative organism of prokaryotes :

-They gave them roman numbers (I,II,III,IV,V) .The main one is **DNA polymerase III** and others are the minor ones. **What do we mean by the main enzyme ?**

The mean enzyme, which is the DNA polymerase III, will do 99% of the job , others will do minor jobs (repair jobs) , and we mean by minor jobs that if we have small gaps created due to the following reasons , these small gaps will be filled by the helpers not the main one.

For example :

1- when RNase removes RNA primer(which is about 6-10 nucleotides), the Helper polymerases come and fill this small gap.

2- when there is a break in the DNA due to radiation or a DNA damage involving small number of nucleotides, the gap that is generated will be filled by the helpers not DNA polymerase III .

All polymerases have the 5' to 3' activity , but the main ones have a unique feature ,which is 3' to 5' exonuclease activity. So what is 3' to 5' exonuclease ?

When the polymerase adds nucleotides 5' to 3' and incorporate one of them in the wrong place , like base pairing C with T instead of A with T , it will return back (in 3' to 5' direction) , remove that wrong piece (and this is the exonuclease activity) , and then add the correct one.

So it returns in 3' to 5' direction , but it doesn't incorporate a nucleotide in this direction, instead DNA polymerase removes it.(don't confuse yourself why it is exo not endo , it depends on the place from where the enzyme has interned , anyway, forget about it) .

Importance of 3' to 5' exonuclease activity :

This feature is really important, this is the proof reading. Imagine you are writing notes at a very high speed and your eye is immediately behind your pencil , if you make a mistake you return back and erase it , then you continue writing in the forward direction , you can't write while you are returning back , you just erase what you have written .

So , why it is important ?

DNA polymerase works at a very high speed, it is adding a huge number of nucleaotides each second , so there is a probability to make mistakes . That's why we need 3' to 5' exonuclease activity , to erase the mistakes . imagine there is no proof reading , by the end of copying the DNA we will have thousands of mistakes and the subsequent disastrous mutations.

DNA polymerase *Error rate* in prokaryotes and eukaryotes :

The error rate *depends on* ***the size of the whole genome*** *that is being copied, and* ***number of origins of replication*** *we have* .

So let's compare now ?

1- error rate of human DNA polymerase is ***1/10-100 billion*** nucleotides added . So if you know that our whole genome is ***6 billion nucleotides*** , and this genome is fragmented to 46 chromosomes, each containing different number of nucleotides , and each chromosome has ***multiple origins of replication*** ,so the DNA polymerase that is working at each origin of

replication (we have many of them there) will add 1000-2000 nucleotides , so the error rate is almost zero , meaning that our DNA polymerases don't make mistakes.

2- Error rate of prokaryotic DNA polymerase is 1/10,000 nucleotides added , and you know that bacteria have a single chromosome containing ***3 million nucleotides*** and just ***one origin of replication*** , doing a simple calculation will reveal that the error rate and the mutation rate as well are really high ($3x10^6$ /10000=300 mistake) . Remember that bacterial DNA has no introns , all the genes are coding for polypeptides , so you would expect that these error are going to change gene expression of the bacteria , they can mess up things, the mutation may create a non functional protein , or the opposite thing could happen , it may give the bacteria new traits that make it more resistant to antibiotics , by making it produce enzymes that degrade penicillin for example ,so if you give it penicillin , it will just chew it and throw it away . That's how bacteria evolve.

when Darwin put his theory about evolution, he didn't know anything about the DNA polymerase and the mutations it could create. Part of his theory can be proven by the evolution of bacteria that happens with time. Before 50 or 60 years ago , penicillins were effective against a large spectrum of bacteria , and with time this spectrum was getting narrower and narrower due to bacterial evolution. Now , pencillins are not that effective and most of bacteria are resistant to them ,**<u>why ?</u>**

This is due to *natural selection* , the environment selects the type of bacteria that is able to cope with the harsh condition ;the type that has the advantage of mutations caused by errors of DNA polymerase ,the mutations that make them resistant to antibiotics and able to still live in the presence of these toxins .

Note : the cell cycle slides were discussed with Dr. Mamoun , Dr said will not ask about them in the exam.

Now let's talk in more details about the DNA polymerase in eukaryotes , basically human polymerases:

- They are 9 at least in the 3rd edition , but now we are talking about 15 different types (in 4th edition) .

-Here ,we give them Greek letters not roman numbers as in prokaryotes. (**α, β, γ, δ, ε, ζ, κ, η, ι).**

-in their way to discover the function of each polymerase , here you have a short story about what happened:

In the 1st edition , they thought that delta makes the Leading strand and alpha makes the lagging strand. In the 3rd edition, they claim that delta makes both , leading and lagging . In the 4th , they say that:

1- ε (epsilon) makes the leading strand
2- δ (delta) makes the lagging strand

We will focus on the previous two main polymerases (**ε** & **δ**) .

Why do we have these misinterpretations ?

Firstly , they thought that alpha makes the lagging strand because they looked at its processivity.

Processivity ?!

What is the processivity of polymerase ?

The ability of the polymerase to stick with the replication job and not fall off the template DNA. It referrers to how many nucleotides the polymerase is able to add , or what is the length of the DNA strand that the polymerase is able to synthesize , is it able to add 10 nucleotides , 10000 or million??.

Returning back to alpha polymerase , when they looked to its ***processivity*** ,they found it 200-250 nucleotides , which is equal to the length of Okazaki fragments. So they thought that alpha is the one that makes the lagging strand , however this claim was proven to be wrong .

Another reason for thinking that alpha makes the lagging strand is that *alpha was very abundant in the region where the lagging strand was being synthesized*, but later on they explained its presence in that region; *alpha polymerase's job was to bring the* ***RNA primase*** to put the primer on the *5' to 3' parent strand* so **delta polymerase** can now continue the Okazaki fragment .And as we need many primers because it is a lagging strand , there will be of course so many alpha polymerases as well as RNA primases.

Other polymerases are mainly repair polymerases , as we said previously when we have a damage involving small piece of DNA , β polymerase or κ polymerase will repair it.

γ Gama Is interesting because it is the mitochondrial DNA polymerase. As we know that mitochondria is the only place where you can find DNA outside the nucleus in HUMAN cells. Also, the DNA of the mitochondria is circular like bacteria and even the polymerase of mitochondria is very similar to the polymerase of the bacteria. The theory's name that claims that mitochondrial origin is an engulfed bacteria is the ***symbiosis theory***.

The bacteria benefits from the nutrients in the cell and the cell benefits from the energy provided by the bacteria , which is the mitochondria here.

To summarize polymerases :

Take a look on this table ☺

α polymerase	bring the RNA primase to the lagging strand synthesis area.
ε Polymerase	makes the leading strand
δ polymerase	makes the lagging strand
γ polymerase	mitochondrial DNA polymerase
Other polymerases	Repair the damaged DNA

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Replication of the ends of the chromosomes :

Now let's talk about telomeres and telomerase :

What are the telomeres ? they are the ends of the DNA molecule that get shorter and shorter as the cell divides , they contain a specific sequence that is rich in G and T bases (TTAGGG) , and this sequence is repeated 100 or 1000 times.

Please go and see the video to understand the process .

http://www.youtube.com/watch?v=AJNoTmWsE0s

When cell divides , it copies its DNA in the mechanism we were explaining throughout the lecture , which is simply:

- Helicase opens the origin of replication ,Then at the forks ,the polymerases and primases will work to copy the new strands using the parental strands as a template until reaching the ends of the chromosome , here at the ends , there will be a problem .

So , why do we have a problem in copying the last bits of DNA?

At each replication fork and while the ***lagging strand*** is synthesized , the primase comes first to add the primer and then polymerase continue the Okazaki fragment . As the fork opens more and more, the process of priming is repeated until we reach the last bit. *When the primase comes to add the primer at the end of the chromosome , it can't because there is not enough space for the primase to dock and add the primer* ,so that part that is not primed and copied will still be single stranded , and if you notice that the new strand is going to be shorter than the parental one. Now in the next round of replication , telomeres will get shorter and shorter on both ends of the chromosome . So the *shortening is due to the problem of priming the very last bit.*

Now ,Who can solve the problem ?

The solution would be the enzyme Telomerase. This enzyme has an RNA prime attached to it, it will use it as a template to add nucleotides to the 3' end of the DNA strand which is longer than the other . so will go to the ends of the chromosome and add new nucleotides containing G and T in a repeated fashion , so what it is actually doing is elongating the shortened telomeres and providing enough space for the RNA primase to dock and work as usual then, polymerase comes and complete the new strand . There will be a part that's longer than the other , they call it 3' overhang.

In the past , they thought that these 3' overhangs will protect the DNA from being degraded as these 3' overhangs will form loops , like the plastic part found at the end of the shoelace . it is just for protection . so telomerase protect the DNA from disintegrating .

But now ,we found out that telomerase is one of the biological clocks , it is one of the most important discoveries in history of humanity.

So as our cells grow and differentiate , telomeres will get shorter and shorter , and that's how senescence occur . when the telomeres get very short , they are recognized as DNA damage and the cell will eventually die (apoptosis).it is like a sand clock , as the time passes , more sand well flow , and the life of the cell will end and eventually die.

If there is a telomerase , then why do our cells undergo senescence and eventually die ?

Actually all our cells have telomerase , but as cells differentiate they forget about it (the reason may be the epigenetic changing that close or open certain genes) . only stem cells still have the activity of this enzyme , that's why they keep dividing. And as you know that cancer cells can retain the activity of telomerase and become immortal.

Recently , scientists were thinking of reactivating telomerases in all our cells !!! **what will happen ?**

Our cells will become immortal , and you also will be immortal IF you are living a healthy life and no accident happened or a plane has fallen and killed you (b3eed elshar 3annak). So if we are living a healthy life , we will die at the end because our telomeres are getting shorter and from oxidative stress that our cells are prone to , and which cause the cell to undergo apoptosis .

But if you could reverse that and make our cells like the stem cells not like cancer cells , you could actually prolong the life of humans .

This is not a dream , a lot of experiments have been done , either they add things that activate the telomerase and remind the cell of its presence , or they add telomerase itself in the drug.

- nothing is proven yet but some experiments could make mice live 3 times their original life expectancy ,if this was applied on us , we will live 200-250 years . Other experiments say that treating a mouse with such drugs for one month could prolong his life by 16 % , and if this was applied to humans , taking this drug for one month also will prolong your life by 10 years .

To conclude : Telomerase reverse or slow the process of senescence and death , stem cells have its activity , but terminally differentiated cells have lost it . If we could reactivate our telomerases in a controlled manner , we will be immortal or just prolong the life by 10 or more years .

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Written by :Alaa Taha Roto

لا تنسوني من صالح دعائكم ☺

إهداء إلى أحسن وأنبل فريق في العالم .. الكوركشن تيم ☺