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GENETICS & Molecular Biology

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Last time we started talking about DNA replication

Scientists noticed that when DNA is replicating in bacteria:

First: the double strand opens up (unwinds).

Second: the replication is bidirectional, which means that the nucleotides will be added in the two opposite strands. (but in both ways it will be added 5'to3').

We remember that the bacterial DNA is circular, it opens in a certain area called **replication bubble,** and each half of the bubble is called **replication fork.**

In both of the forks we have a new strand being synthesized.

Remember: DNA synthesis is always 5' to 3' and the old DNA **"parental**" DNA is read 3' to 5'.

So the DNA strands open up, the two strands are antiparallel, so we have two templates, one of them running 5' to 3', and the other running 3' to 5'→ this template is used to make the "**the leading strand".**

What about the strand going from 5' to 3'? The new strand will also be synthesized 5' to 3', by being synthesized into short fragments, it's called the lagging strand "because of the delayed synthesis "

So the new lagging strand is composed of short fragments known as **"okazaki fragments** "

But why is it delayed??

Because it waits for the leading strand to open up the fork and also because these okazaki fragments must be ligated, so the lagging strand synthesis will be delayed as a result of that too.

Components of DNA replication:

First you have to keep in your mind the following information:

DNA polymerase cant synthesize DNA DE NOVO “from scratch “,it needs to build on an existing prime.

It waits for the synthesis of a primer, this primer in cells is usually RNA molecules not DNA "so the substrate for the primer are riboneuclutides not deoxyriboneucleotides "

So first we need a "primase" (for the synthesis of the primer).

An enzyme that puts in short RNA primer and then the DNA polymerase 3 come and extends the primer, and start DNA synthesis putting in deoxyriboneucleotides

*the previous mechanism happens in both eukaryotic and prokaryotic cells.

In the leading strand one primer is necessary but in the lagging strand for each Okazaki fragment we have a primer. Because

Once the DNA polymerase 3 hits the primer, it will fall off then comes the action of DNA polymerase 1 that removes the primer and replace it with Deoxyribonucleotides then each Okazaki fragment is joined by the action of ligase enzyme.

Remember that the DNA is double stranded, we need an enzyme to unwind and separate the two original strand..This enzyme is called helicase.

The DNA helicase in bacteria is part of the primase, so we have a complex of two enzymes together one of them is the DNA helicase separating the two strands and the primase puts in the primer

This complex is known as a **primosome**

Cells also need single stranded DNA binding protein (SSB)

They are very important for:

1- They prevent the single strands from binding to each other (rewinding)

2- They protect single stranded DNA from being degraded →

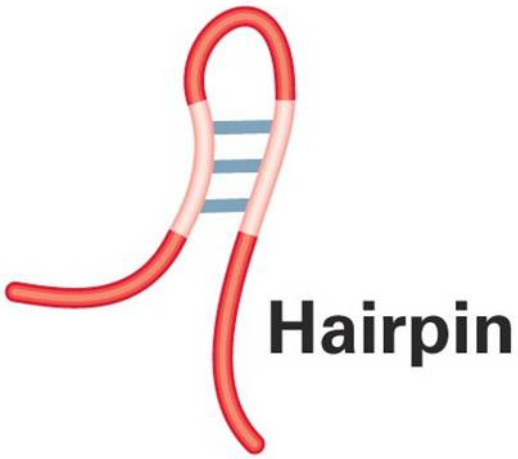
Because it's Unusual for bacterial cells to have single stranded DNA, whenever there's a single stranded DNA what happens is we have **DNAase** enzymes that it.

3- Sometimes we have the formation of what's called "stem loop structures" or hairpin they tend to happen when we have two complementary parts on the same single strand DNA they may bind together forming the Hairpin structure.

Imagine the following sequence is one template of DNA:

CCCTGAAGCTCAGGGAC

NOTICE that in the beginning of the sequence we have "CCCTG" and in the end we have "GGGAC" these two are within the same strand ,and they are complementary, so when we have the single strand without the binding protein we could have these two sequences bind together forming a loop



The problem with the formation of these stem loop structures that if they are present they force the DNA polymerase to stop replication.

Let’s talk about DNA polymerases in bacteria:

Bacterial cells have 3 main types of polymerases and each one of them serve certain functions:

1-DNA polymerases revolve in DNA repair: these are DNA polymerase 2, 4, 5.

2- DNA polymerase 3: is the one responsible for the DNA synthesis

3- DNA polymerase 1: Have two important functions, one of them is exoneuclease activities that cuts off the RNA primer at 5’to3’ and the other function is polymerase that add deoxyribonucleotides that fill in the gaps in Okazaki fragments.

These are two opposite action that are complementary to each other.

What really happens??

DNA polymerase 3 synthesize the DNA in okazaki fragment, it hits a primer, this primer must be removed and replaced by DNA , so when polymerase 3 hit the primer it falls off the strand and DNA polymerase 1 comes in ,using its exoneuclease activity , it degrades nucleic acids , it removes one nucleotide at a time of a primer "the substrate here is riboneuclutides " and add "deoxyriboneuclutide" .

Seems as It replaces one RNA nucleotide by DNA .until it hits the other okazaki fragment and it falls off and DNA ligase comes in and it connects the okazaki fragments with each other.

Note: the exoneuclease activity of polymerase 1 is 5'to 3'

DNA polymerase 3:

Is a complex of subunits and each one of them has a certain function:

Alpha subunit: responsible for the polymerization

Beta: responsible for the attachment of the polymerase to the DNA so it stabilizes the binding between them

Epsilon subunit: has exoneuclease activity , but this time it's different from the exoneuclease activity of the polymerase 1, as it's in 3' to 5'→ this helps in **proof reading**.

DNA replication is really accurate!

The fidelity is high, so the DNA polymerase puts in the right bases accurately, with little mistakes

And that’s made possible through:

1- The hydrogen bonds that are formed between the new nucleotide added and the old one "the complementary one" this makes is like Lego, you can't put the wrong pieces together,

And if the new nucleotide added is not complementary(G to T for example) it won't fit it feels like there's something wrong so it drops it away and tries another one.

2- The other reason is the magical 3' to 5' exoneuclease activity→ proof reading:

it uses this activity to proof read the added nucleotide and make sure that everything is right .

So it synthesizes 5' to 3' and when it put the wrong base, it goes back 3' to 5' to remove the wrong bases and then continues on.

So the differences between the exoneuclease activity of polymerase 1 and 3:

1- Polymerase 1 removes primers while polymerase 3 removes mismatched nucleotides

2- Polymerase 1 cut 5' to 3' while polymerase 3 cut 3' to 5'

We know that the DNA is super coiled, so the action of the helicase could create tension ahead to the helicase activity and the DNA become tangled up so this tension must be released.

The tension happens in both linear and circular DNAs.

That's why we have DNA topoisomerase,

We have two types:

Topoisomerase 1: make a single cut (at one strand).

Topoisomerase 2: makes two cuts one in each strand

What we need for DNA replication is topoisomerase 1, it makes a cut in one of the strands and makes it easier for the strands to be separated from each other because one of them keeps on rotating easy.

Cells don't have to rotate the whole thing, it only need to rotate short single strand so topoisomerase 1 .

The topoisomerase acts ahead of the DNA polymerase.

How does the cell know that it's time for DNA replication and synthesis??

In bacterial cells they found out that the synthesis starts at a certain point which is called "origin of replication" ORIC.

And this origin of replication made of consciences sequences, sequences agreed upon, this means that these sequences are common among different bacterial species, so they are preserved throughout evolution.

We have two types of sequences:.

ninemers "9 units " a region that's made of 9 bases "nucleotide "

Binding site for a protein called DNA A,

thriteenmers "13 units " made of 13 bases

These 13 mers are made of repeated units, that are rich with As and Ts

So when DNA A is activated, This is a signal that the replication must start thus DNA A binds to the 9mers and it squeeze on the DNA this leads to the expansion of the 13 mers "because they are rich with the T, and A so they have less hydrogen bonds " once you have a bubble that's it , the replication begins. And the

machinery is assembled by primers and polymerases and helicases and we have initiated synthesis

What about eukaryotic cells?

Their DNA is much larger than the bacterial DNA , it would take forever if they start by only one ORIC .Rather, these cells have multiple origins of replication, they also have consciences sequences, synthesis is also bidirectional, once the distant origins meat the strands would be separated.

That's why it takes less time to synthesize DNA .

Note: DNA polymerase is specific and binds to certain sites on the DNA .

In eukaryotic cells there are multiple polymerases just like the bacteria but we will focus on number of them

Remember that everything related to eukaryotic cells is more complex and because the DNA is larger it needs more enzymes

Examples:

DNA polymerase gamma: is mainly found in mitochondria

All the other ones are nuclear polymerases.

There are polymerases that have 3' to 5' exoneuclease hence Proofreading such as alpha ,delta, epsilon .

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

about the table: the doctor mentioned the info as comparison so you should know the highest .the slowst,and which have a exonuclease activity

Processivity: how fast the polymerase is.

Mechanism of DNA replication:

DNA primase is associated with DNA polymerase alpha, so polymerase alpha begins each replication, and then because its Processivity is low , it falls of the DNA and then polymerase delta comes in and continues synthesis because it's really fast relative to alpha , so it continues until it hits a primer then it falls of , epsilon is necessary for the synthesis of the leading strand ,so each one of them has its own function.

These polymerases mentioned don't have 5' to 3' exoneuclease activity to remove the primers but we have other proteins that do.

After those exoneuclease remove the primers polymerase delta fills the gaps.

Other polymerases are responsible for mainly DNA repair

Problem is that we have histones around DNA; DNA polymerases can't synthesize DNA in the presence of the histones! So they must be removed. They are removed by specific proteins known as "chromatin remodelling factors" .

They act ahead of the polymerases so they remove the histones and the polymerase thus can continue freely

Behind the polymerases we have chromatin assembly factor (CAF)"because we still want to maintain the original structure and packing of the DNA" they reform the chromatin

Still we have a prime major problem in synthesizing the linear DNA molecules; our problem is located at the ends of the DNA molecule

These ends are known as telomeres (Telo means end) they are regions located at the end of a linear DNA molecule.

With every single DNA replication, the DNA becomes shorter.

The main function of telomeres is to stabilizes the molecule, because they are specialized region of the molecule, the shorter the chromosome gets, the less stable the DNA becomes so the length of the chromosome must be maintained

It becomes shorter because In synthesizing the leading strand, synthesis continues all the way until the end, so in this strand the DNA is complete

While on the lagging strand we have the primer that is removed leaving an empty space that is not enough for another primer. Thus lagging strand is shorter

Well cells are smart! They can maintain the length of their chromosomes using an enzyme called "telomerase" This enzyme maintain the length of the chromosome

what's interesting about them is that they have their own primer , it's an enzyme with a primer attached inside

The primer has certain sequences and repeated sequences "GGGTTA" always the same, and this is why the ends of the chromosome is made of repeated sequences,

"There's animation in the slide"

First we have a primer that is removed normally, telomerase add their primer, and thus elongate the ends giving us a space for add a primer to complete replication.

We get older, because our telomerase activity gets less, and our cells die and organs get weak.

When they cloned the sheep dolly, even though its age was day one but Its DNA was 6 years old! That's why it looked older; it got many diseases that are not supposed to be in her age... And she died at the age of 6 "her DNA age was 12"

reactivate our telomerase→ Elixir of life? Think pathos!

refer to the animation for a better understanding

That's it :D always remember..that you own your future..just remodel it and be your life polymerase ^^