Molecular Biology, Lecture 3 – DNA Replication

We will continue talking about DNA replication. We have previously t discussed the structure of DNA. DNA replication is the copying of the whole DNA content of the cell (genomic DNA) as the cell divides.

In which phase of eukaryotes' cell cycle does DNA synthesis occurs? In the "S" phase.**

- **Origin of replication**: a specific sequence on DNA, and this sequence will be recognized by "*Helicase*" enzyme… this enzyme **denaturates** the two strands at that point … making two "**forks**" for the replication which will be in two directions… this creates a "**Bubble**" or a "**Replicon**"… this bubble will expand to fuse with other bubbles that start at different origins of replication.

- DNA replication is **semi-conservative**… this means that each strand of the parental strands will be used as a template strand, to synthesize a new one.. So each daughter cell will have half of the original's cell DNA.. So it is "**semi conservative"**

- Synthesis occurs at each fork of replication… Both strands should be made in the **5' to 3' direction**; There are two strands:

1) **Continuous** if the 5' to 3' direction of synthesis (synthesis of the leading strand) is going in the same direction as the growing replication fork. (Or 3' to 5' direction on the leading *template* strand).

2) **Intermittent** if the 5' to 3' direction of synthesis (synthesis of the lagging strand) is going in an opposite direction of the growing replication fork. (Or the 3' to 5' direction on the lagging *template* strand)

- Note: direction of the growing replication fork is the direction of unwinding of the two strands by "*Helicase*" enzyme.

- The intermittent synthesis of the new strand produces: **a lagging strand** or "**okazaki fragments**" These strands or fragments are joined together by "*Ligase*" enzyme.

- So we have many enzymes:

- DNA "*Polymerase*": produces the new strand of DNA.
- DNA " *Ligase*": joining **Okazaki fragments** by **phosphodiester bonds**.
- DNA "*Helicase*": unwinds the two strands.
- SSB "Single Strand Binding Proteins": prevent "**renaturation**" or rewinding of the DNA

- *"Topoisomerase":* works upstream of "*helicase*" enzyme to identify the stress points which occur when DNA is overwound ahead of the fork, they relief the tension by cutting and rejoining the DNA and allow the DNA to be untangled.
- *"Primase* ": creates an RNA **primer** to allow DNA "*polymerase*" enzyme to start replication
- *"RNase*" (especially RNase **H**): removes the primer.

○ **DNA "*Polymerase*"**

There are many types of this enzyme:

- In **eukaryotes** we have up to **9** DNA polymerases. in **prokaryotes** we have **3-5** polymerases. but there is always a main enzyme out of these types either in eukaryotes or prokaryotes. This main enzyme does the major function while the other polymerases have their minor functions which are complementary to the function of the main DNA polymerase.

In prokaryotes: The polymerases are given Roman numbers. In E.coli the main polymerase (also called the *replicative enzyme*) is polymerase (**III**), it copies the DNA during cell division, while types (**I** and **II**) are mainly for repair.

In eukaryotes: they are given Greek litters (**β,α,ε**…etc). The replicative (main) polymerase in eukaryotes is the (**δ**) polymerase. The other polymerases' functions are for repair (if there is a small mutation in the sequence) or filling a small gap (for example when we remove the primer after beginning of replication we need to fill in with DNA nucleotides)

- Note: the replicative enzyme (the main) polymerase has the highest "processivity" and "fidelity":
  - **Processeivity**: how fast the enzyme is; how many nucleotides are added before it gets exhausted :P (number of base pairs/ time unit that it can add before it dissociates and must bind again)
  - **Fidelity**: the accuracy of the enzyme. Example: in eukaryotes → (**δ**) Polymerase chance of error (to add nucleotides in wrong sequence) is **1/10x10^9 to 1/100x10^9** while Polymerase type (**III**) in prokaryotes → chance of error to is **1/ 10^4**

Note: the low fidelity of bacterial polymerase may produce good mutations. Example: to be resistant against an antibiotic. Therefore the high chance of error is not always a disadvantage.

- The **main** (replicative) enzyme got "**3' to 5' Exonuclease Activity**": which is "**proof reading**" → Removes base-pairing errors, when polymerase adds a nucleotide in a wrong sequence, it goes back in the 3' to 5' direction (5' to 3' on the *template* strand) to remove that nucleotide then add the correct one in 5' to 3' direction. This proof reading causes high fidelity. {But according to Wikipedia: the polymerases that have this activity are only (ε and γ) in eukaryotes and polymerases type (I, II and III) in prokaryotes}.

- In eukaryotic cells, some of the polymerases have "**5' to 3' exonuclease activity**": Sometimes we may have spontaneous incorporation: free monophosphates which bind without being added by a polymerase (before it reaches that point). Here, polymerase can remove them when it reaches that point. This will be in 5' to 3' direction. {But according to Wikipedia, this activity is found in some polymerases of eukaryotic and prokaryotic cells}.

- **Note**: polymerase adds the nucleotides as **trinucleotides**. It takes **triphosphates** then adds them as **monophosphates**; a **biphosphate** is broken down and used as an energy source for the synthesis.

- Why can't the polymerase join the **lagging strands** or **okazaki fragments** together?!**

Because its function is to add nucleotides in (5' to 3' direction), which means that the polymerase enzyme can add the **phosphodiester bond** in that direction only: each primer is extended from its 3′ end by DNA polymerase to form an Okazaki fragment. If there is a phosphodiester bond at the 3' end it adds to the 5' end. It runs in one direction only. When we talk about joining the **Okazaki fragments** it would require it to run in the opposite direction therefor DNA polymerase cannot do it. This is the function of the "*ligase*" enzyme!

- Note: in eukaryotes there are many origins of replication because the eukaryotic DNA is too long so we need more than one origin of replication in order to end the synthesis quickly, eventually all the replicons merge to form the ds DNA molecule. In prokaryotes there is only one origin of replication.

Note: The (**δ**) polymerase (main) in humans is associated with "*Helicase"* activity.

(α) DNA Polymerase

The (**α**) polymerase in eukaryotes has low processeivity: 200 to 250 base pairs; this is the same number of base pairs as in the **Okazaki fragments.** Also, we find this polymerase (**α**) at the beginning of each **Okazaki fragment** → so in *the past* they thought that this polymerase is responsible of making the **lagging strands** or **Okazaki fragments**, and that the (**δ**) polymerase is responsible for making the **leading strand**. But recently, they discovered that polymerase (**α**)'s function is to place the "*Primase*" enzyme (Primase adds the Primer), to initiate producing each **Okazaki fragment,** and after that it actually adds 19(+-3) base pairs (according to Wikipedia) {and this is one of the reasons why they thought that (α) polymerase is responsible of making the whole Okazaki fragment}. Then (**δ**) polymerase comes to remove them and continues producing the **Okazaki fragment**.

Functions of polymerases other than (**δ**) polymerase are mainly for repair. But (**γ**) polymerase is found in the mitochondria. Read them from the slides.

- **Mitochondria**

Mitochondria have their own DNA and replicate independently of the cell. Mitochondria are very similar to bacteria: similar size, it has circular DNA, no introns (non-coding regions). So it is believed that the relation between mitochondria and the human cell is symbiotic. And remember → **it has its own DNA polymerase which is: polymerase (γ)!**

- **Telomeres:** repetitive sequences at the ends of chromosomes

Most of our DNA is non-coding. There are non-coding regions inside genes (introns) and there are non-coding regions outside the genes (junk DNA). Each DNA in our bodies is divided into 2 regions:

- First region is for genes, which contain Introns and Exons.

- Second region is "**junk DNA**": accounts for 70% of the DNA and most of them are repeated sequences "**tandem repeats**" and they are non-coding. They are present in hundreds of thousands of locations but we only understand their function at one site: at the ends of each chromosome → and they are called "**telomeres**". They contain the repeated sequence: "**TTAGGG**" repeated a few thousand times at the end of each chromosome.

(Wikipedia: Telomere length varies greatly between species, from approximately 300 base pairs in yeast to many kilobases in humans)

- In the past, they thought that the only function of telomeres is to protect the genes at the ends of the chromosomes, since telomeres are single stranded and close on in themselves as if to protect gene at the edges from being degraded. This is actually true. To understand better, think of them as those plastic pieces at the end of a shoelace to protect the thread.

- Recently, they discovered another function of telomeres: telomeres work as **biological clock** of the cell. Each time a cell divides → **Telomeres** get shorter deliberately (the number of repeats decreases by a certain amount). It's as if the cell counts its own number of divisions or how much time is left before it dies. After a cell consumes the amount of divisions the length of the telomere allows, they reach a specific stage which is "**cell senescence**": a cell is alive, performs most of its functions but doesn't divide. After that point, apoptosis of the cell is induced!

- **In the zygote and embryonic cells**, there is "***Telomerase***" enzyme which is responsible of elongation of **telomeres** each time it is shortened. As we develop, cells seem to "forget" this enzyme! Possibly they stop the expression of this enzyme after they undergo differentiation. This is true for all cells except cells they don't differentiate which are the "**stem cells**". They (stem cells) keep on expressing the "*Telomerase*" enzyme so they are immortal cells, unlike the differentiating cells.

- What if we switched the "*Telomerase*" enzyme gene on in the differentiating cells?

- Theoretically, if cells can retrieve the Telomerase activity they may become immortal just like stem cells, they would be able to undergo division without being under the pressure of the biological clock, without senescence or apoptosis, so we could live more.

- Actually they did an experiment on a group of mice. They attempted by IVF to keep the telomerase gene active. The results were that some of the mice lived 3 times more than their usual age. But the others developed cancers because the scientists didn't know exactly how to activate "*Telomerase*" in a controlled fashion.

    - Actually, this the way some cancers work: They activate the "*Telomerase*" enzyme gene in a random way which ruins cell function and leads to uncontrolled growth. Whereas stem cells maintain the integrity of the cells once they activate the Telomerase gene.

At the end, we don't know if we can make our cells immortal or not, but we shouldn't stop thinking about it.

- Btw I think the sheet is not enough. the Dr asked us to read the slides