Genetics
& Cell biology

◉ Sheet ○ Slides

Number

4

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** In this lecture we will talk about DNA replication (cover slides 32-59 (from slide #2))

So let's begin….

In order for life to continue DNA must makes a copy of itself, and in order to do that it must have a specific mechanism of replication.

The following terms and information you must be familiar with it:-

1- Genome: It's the total content of the DNA of the cell.

2- DNA is organized into chromosomes in the cell.

3- Bacterial chromosomes are circular and usually have one chromosome inside them.
4- Eukaryotic chromosomes are multiple, linear pieces of DNA which composed of nucleic acids with proteins such as histones (this complex known as **chromatin**).

70 years ago the scientists didn't know how DNA makes a copy of itself so they came with some theories explaining the models of replication of DNA.

Conservative, Semi-Conservative & Dispersive models of DNA replication

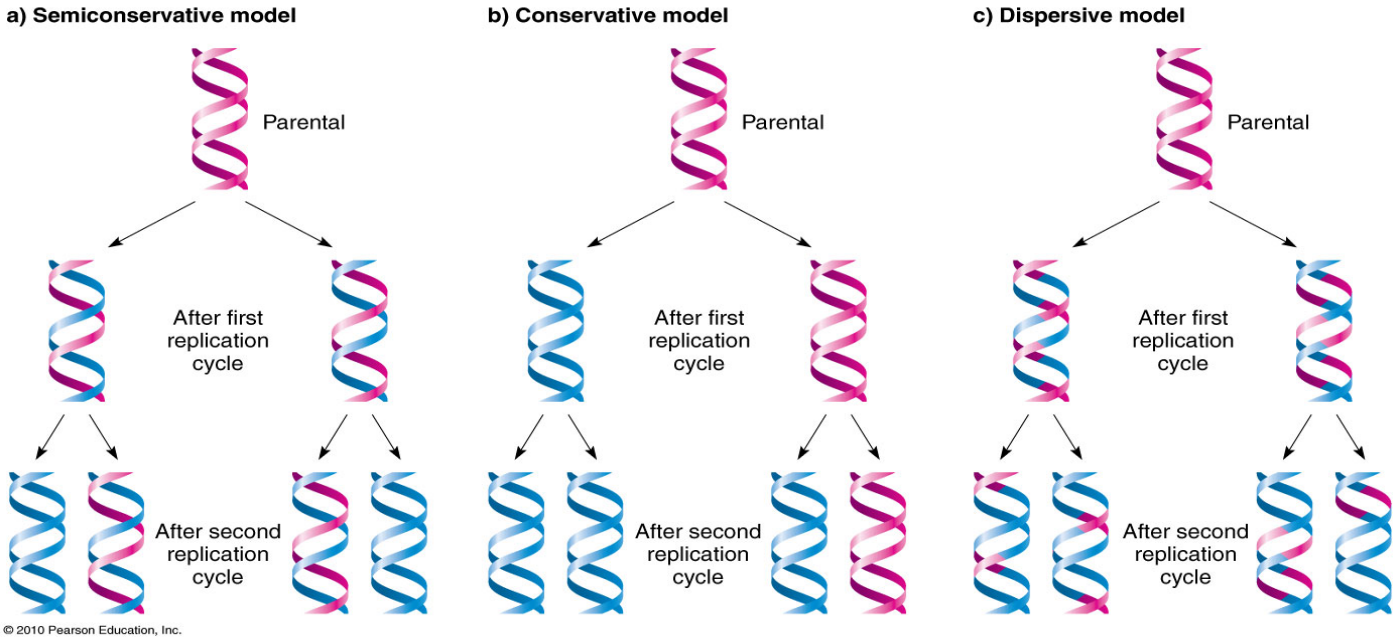
-In the **conservative model**, the mother cell turn into two daughter cells, one has the old strands (came from the mother), and the other one has a new strand. And that's WRONG because in each cell's DNA we must have pieces from the old strand and pieces from the new strand.

-In the **semi-conservative model**, the two old strands separates and each makes a copy of itself. After one round of replication,

the two daughters DNA each has one old strand and one new strand and **this is what actually occurs in our DNA**.
-so what's special in this model?
After two rounds of replication, two of the **DNA** molecules consist only of new material, while the other two contain one old and one new strand.

-In the **dispersive model**, material in the two old strands is distributed more or less randomly between the two daughter cells. In the model, the old DNA is distributed between the two daughter cells.

Figure showing the models of replication:-



After that scientist made further experiments to determine the direction of replication and they found that DNA replication occurs Bidirectional (nucleotides will be added in the two opposite strands) although it occurs in only one direction in terms of 5`end→ 3` end.

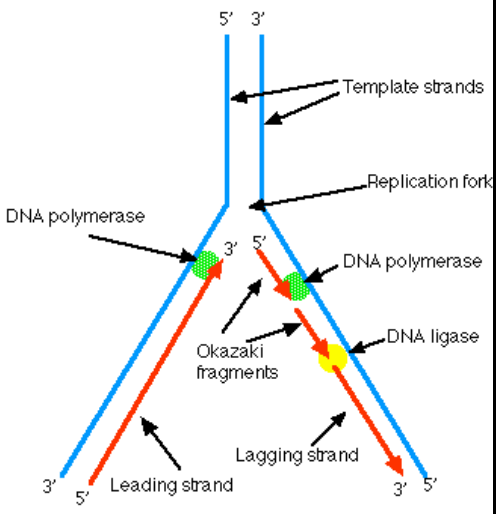
We remember when the DNA is starting replication the first thing that needs to occur is the unwinding of the two double strands by a

helicase enzyme. So, it opens in a certain area to form **replication bubble**. And each half of the bubble is called **replication fork.** So we have **two templates**, one of them running from 5' to 3', and the other one running from 3' to 5' (Because the two strands of DNA are antiparallel).

The 1st one (5`→3`) will make **lagging strand** (lagging means there is delay in synthesis and this delay occurs because the replication here occurs in short and discontinuous fragments those fragments known **as okazaki fragments**).

The 2nd one (3`→5`) will make **leading strand**.

**The 2nd one is the responsible for the unwinding of the two strands and allowing the formation of the replication fork allowing the 1st one (5`→3`) to start the replication and that's why the 1st one is slower than 2nd (3`→5`).



*** DNA helicases**: is an enzyme that unwinds the two strands of DNA. In bacteria, helicases form a complex with the primase called **primosome.**

-Components of DNA replication:

First of all DNA polymerase can't synthesize DNA *DE NOVO* (from scratch). It starts when a primer binds to a certain DNA fragment. This primer is a short RNA sequence added by an enzyme called **primase**.

*for leading strand we need only one primer (3`→5`).

*for okazaki fragments we need a primer for each single fragment (5`→3`).

So, we need a primer (which is added by an enzyme called **primase) to start DNA synthesize, then another enzyme called **DNA Polymerase 3** comes and extends from the primer (starting point), and starts DNA elongation by putting in **deoxyriboneucleotides**.
*In the lagging strand each fragment requires a primer and DNA elongation occurs by the enzyme polymerase as usual. Now when the polymerase hits the primer of the following fragment it removes the primer (of the following fragment) and adds a deoxyriboneucleotides, and so one. When the polymerase finishes the synthesis of the Okazaki fragments they get connected to each other to form a strand by an enzyme called **ligase**.

***Single-strand DNA-binding (SSB) proteins**:-
They bind to the single stranded DNA, and they have three functions:-

1- Protect the single stranded DNA from degradation by enzymes like **DNAase**(Use in drugs to kill bacteria)
2- Preventing the single strands from binding together again (rewinding).
3- Preventing formation of **hairpin** "stem loop structures"

* happens when we have two complementary parts on the same single strand DNA they may bind together. If this thing happens the polymerase will be forced to stop replication.

**3:00-20:00(rec#4)

- DNA polymerases in prokaryotes

Bacterial cells have 3 main types of polymerases and each one of them serves a certain function:-
1- DNA polymerase **3**: is the one responsible for the DNA synthesis.
2- DNA polymerase **2**, 4, 5:- are responsible for the DNA repair. (The doctor mentioned in the lecture only polymerase 2).

3-DNA polymerase **1**: have two important functions, one of them is **exoneuclease** and it's responsible for the removal of the RNA primer at 5'to 3'. The other function is a polymerase function that adds deoxyribonucleotides that fill in the gaps in Okazaki fragments.
Now let's explain what exactly happens …

DNA polymerase 3 synthesize the DNA in okazaki fragment, it hits a primer, this primer must be removed and replaced by a DNA sequence, so when polymerase 3 hits the primer it falls off the strand and DNA polymerase 1 comes in, using its exoneuclease activity, it removes one nucleotide at a time of a primer " riboneuclutides" and adds "deoxyriboneuclutide". Until it hits the other okazaki fragment and it falls off and DNA ligase comes in and connects the okazaki fragments with each other.

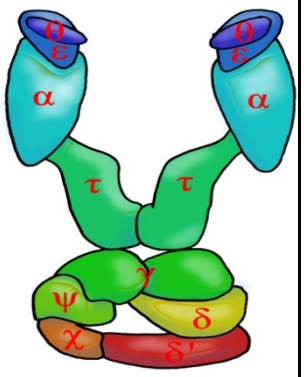
*its direction from 5`→3`

-DNA polymerase III

It's a lager protein consist of many subunits and composed of 10 different polypeptides.

The core polymerase is composed of three subunits:-

1- Alpha subunit (α): responsible for the polymerization.
2- Epsilon subunit (ε): 3'-to-5' exonuclease that removes incorrectly added (mispaired) nucleotides from the end of the growing chain.
3- Theta subunit (θ): regulation and activation of exonuclease (Epsilon subunit (ε)).



*** DNA replication is so accurate! It makes only 1 error per 1 billion nucleotides added!!! And that's related to the fidelity of the enzymes, so the DNA polymerase puts in the right bases accurately, with little mistakes.

Q: How can the polymerase 3 be in this high rate of fidelity??!

A/ 1- The hydrogen bonds that are formed between the new nucleotide and the old one "the complementary one" this makes it like Lego, you can't put the wrong pieces together, and if the new nucleotides added are not complementary (G with T for example) it won't fit, it senses that there's something wrong so it drops it away and tries another one.

2- The other reason is the magical 3' to 5' exoneuclease (ε) activity **(proof reading)** it uses this activity to proof read the added nucleotide and make sure that everything is right.
PROOF READING mechanism: synthesizes occurs from 5' to 3' and when it puts the wrong base, it goes back 3' to 5' to remove the wrong base and then continue on.
Q: So what's the different between poly.1 and poly.3??!
A/

Polymerase 1	Removes primers	cut 5' to 3'
Polymerase 3	Removes mismatched nucleotides	cut 3' to 5'

*There is something called **DNA tension**. This tension happens in both linear and circular DNAs, and happens because of the helicase activity. The DNA is super coiled so the action of the helicase could create tension ahead to the helicase activity and the DNA becomes tangled up so this tension must be released.
Releasing of this tension happens by **DNA topoisomerase** (2 types)

1- **Topoisomerase 1**: produce single strand breaks (nick), and it's ATP independent.
2- **Topoisomerase 2**: produce double strand breaks, and it's ATP dependent.

Q: Which type do we need in DNA synthesis??!
A/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.

Q: So what about the other type??!
A/It can participate in DNA replication but it's not its main function. It's important in cell division specifically, in chromosomal condensation.

***for your information
Targeting the Tpoisomerase2 is used in chemotherapy with cancer.

**20:00-36:00

Q: How does the cell know that it's time for DNA replication and synthesis??!
A/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 a certain sequence, these sequences are common among different bacterial species (consciences sequence), so they are preserved throughout evolution.

We divide these sequences into 2 types depending on the number of their bases:-
-Ninemers "9 units": a region that's made of 9 bases, which are a binding site for a protein called **DNA A**.
- Thriteenmers "13 units": made of 13 bases, these thriteenmers are made of repeated units, which are rich with (Adenine) and (Thiamin).

Q: What's the DNA A and how it works??!
A/**DNA A** is a protein that activates initiation of DNA replication in bacteria. DNA A it binds to the ninemers then the strand of DNA twist on it(forming a bubble) and because of the pressure that result

from twisting, the thriteenmers will separate because they are rich with A and T(weak hydrogen bonds).
Q: What about eukaryotic cells (e.g. Human)??!
A/An average human chromosome may have several hundred replicators (origins of replication and multiple polymerases).

** Eukaryotic cells contain 9 DNA polymerases; most of them for DNA repair. (We won't talk about them and about their functions in details)

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

**memorize only the properties that mark with ★ for each type.

Further notes about the table you must know

1- The exonuclease activity of the delta and epsilon is the responsible of **proof reading**.
2- The high processivity of delta and epsilon (in comparison with alpha processivity) makes them responsible for synthesis of bulk DNA.
3- Leading strand is under control by epsilon polymerase.
4- Lagging strand is under control by delta polymerase.

5- Alpha polymerase is bond with primase.
6- The primer is removed by two special enzymes.
7- DNA polymerase delta fills the gap.

Q: Why the synthesis of DNA in eukaryotic cells takes time??!
A/1-We have histones that pack DNA together.
2-DNA is freed from histones by **chromatin-remodeling proteins** in order for enzymes to move along the DNA.
3-New histones are assembled onto the DNA behind each replication fork **by chromatin-assembly factors (CAFs)**.
All these steps take time to be done.

The End

"What we can or cannot do, what we consider possible or impossible, is rarely a function of our true capability. It is more

likely a function of our beliefs about who we are."

-Tony Robbins