CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

7

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Note: This sheet represents the last lecture of this week (week 2 after midterm exams). We are going to discuss some enzymes which are involved in the replication of DNA.

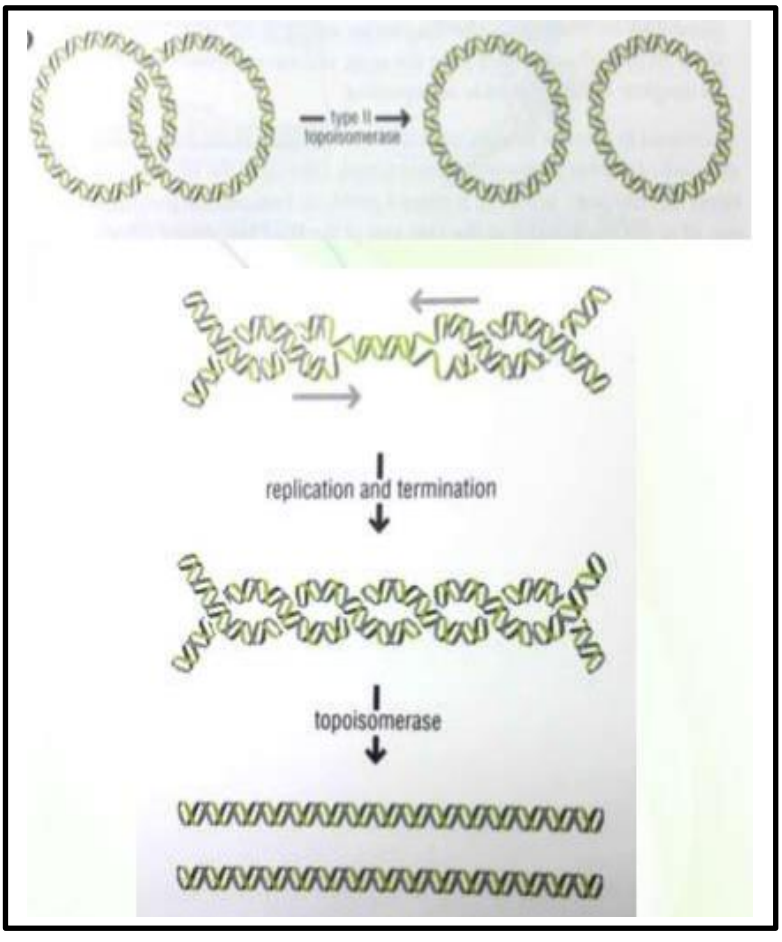
- **Additional note before starting:** No one knows what the origins of replication in eukaryotes (e.g. human genome) are, they think that they know but they actually do not. Nevertheless, the general concept is the same for eukaryotes and prokaryotes.

❖ Beginning:

- ✓ **In prokaryotic cells**, replication goes bidirectionally (in two opposite directions simultaneously) and they meet halfway, and then the two daughter DNA molecules get separated. (there is only one origin of replication in bacteria)
- ✓ **In eukaryotic cells**, the DNA is linear and helical, and there are multiple origins of replication. In each origin of replication, replication also goes bidirectionally and the mechanism is analogous to that of prokaryotes but it's not as simple as it looks.

❖ Role of topoisomerase II:

- ✓ By the end of replication, the two DNA daughter molecules are **entangled**.
- ✓ Notice how in **prokaryotes**, the resulting rings are entangled. Similarly, notice how in **eukaryotes**, the two resulting molecules are entangled.
- ✓ Entanglement is the reason why we need **topoisomerase II (also known as gyrase in bacteria).**
- ✓ **Topoisomerase II** is responsible for **untangling DNA molecules** or chromosomes by making a **transient "double-strand break"**.



- ✓ In other words, **topoisomerase ll** makes **two cuts** (one in each strand of a DNA molecule) and untangles it from the other DNA molecule (allows rotation), then it rejoins the broken strands together.
- ✓ Topoisomerase ll has an important role in replication and in cell proliferation, as it plays a major role in separating the two daughter DNA molecules by breaking and rejoining strands. Depending on that, **topoisomerase inhibitors** are commonly used in **treatment of cancer** (because if the daughter DNA molecules remain entangled, the cell cannot survive).
- ✓ **Topoisomerase ll is also responsible for chromosome condensation during the cell cycle. How?**

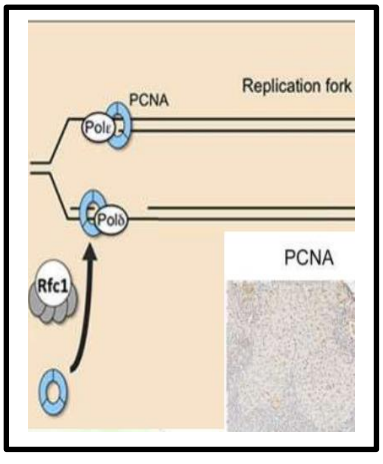
  Normally, when the cell is not dividing, chromosomes are present in the form of chromatin (like spaghetti), and so each DNA molecule (chromosome) cannot be seen individually because different DNA molecules are actually entangled. But at some point during the cell cycle, chromosomes need to be condensed, but this condensation cannot occur if different chromosomes are entangled with each other in chromatin. So, topoisomerase ll untangles the DNA molecules to free them and allow their condensation into chromosomes (X-shape).

Extra: What is the difference between topoisomerase I and topoisomerase ll ?

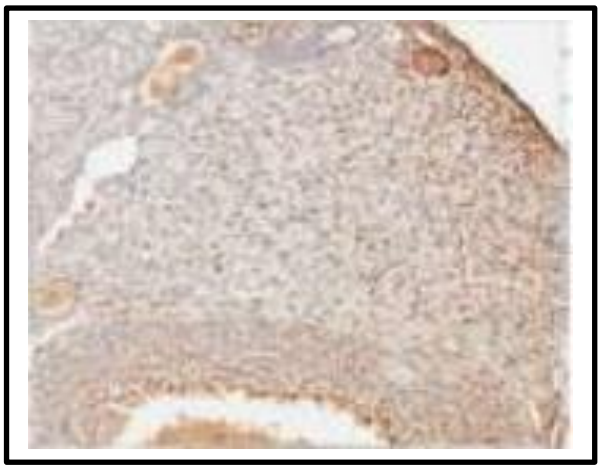
- ✓ **Topoisomerase l** : it releases strain within one DNA molecule during replication by making a **single-strand break**. (ATP-independent).
- ✓ **Topoisomerase ll** : it untangles the two daughter DNA molecules from each other by making a **double-strand break**. (ATP-dependent).

❖ Role of PCNA proteins:

- ✓ **PCNA (proliferating cell nuclear antigen)**: a protein that guides DNA polymerases to where the primers are.
- ✓ They are abundant in proliferating cells to speed up DNA replication. So, in order to differentiate between a proliferating cell and a non-proliferating cell, we stain PCNA in tissues.
- ✓ Proliferating cells have higher levels of PCNA, and so they are extensively stained (the color of the stain is brown).



Not stained a lot – Not a lot of PCNA – non-proliferating cell



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

- ✓ 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.

❖ DNA polymerases in Eukaryotes;

- Eukaryotic cells contain 9 DNA polymerases, most of them are for DNA repair.

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

➢ **From the previous table:**

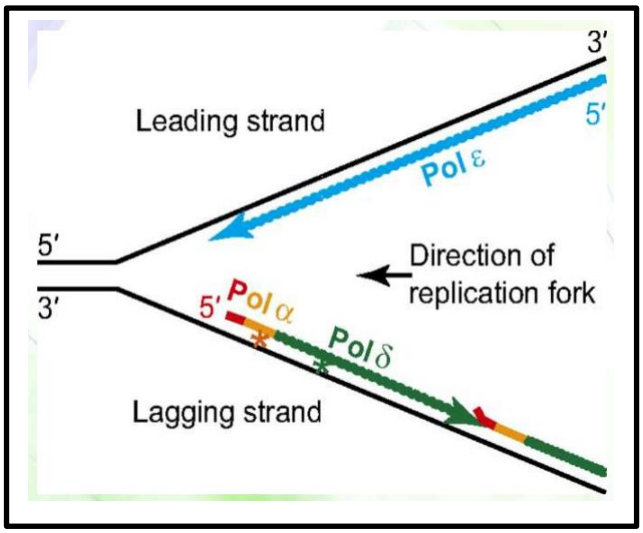
- ✓ There are at least nine DNA polymerases, we are going to focus on four of them.
- ✓ **DNA polymerase γ:** it is found in mitochondria
  - Mitochondria have their own chromosomes
  - Mitochondrial chromosomes are circular (like bacterial chromosomes)
  - This polymerase is responsible for mitochondrial DNA replication.
- ✓ **DNA polymerase α:** primase is part of this polymerase.
  - Primase adds the primer.
  - DNA polymerase α extends the DNA, so it **initiates DNA replication** (starts synthesizing).
  - Its **processivity (how fast it replicates DNA)** is **low (slow)**.
  - Its **fidelity (accuracy or "loyalty" in preserving the DNA sequence during replication) is high.**

- ✓ **DNA polymerase δ** - they do **NOT** have a primase associated with them.
- ✓ **DNA polymerase ε** - they do **NOT** initiate DNA replication.
  - their **processivity** is really **high** compared to α.
  - their **fidelity** is also **high**.

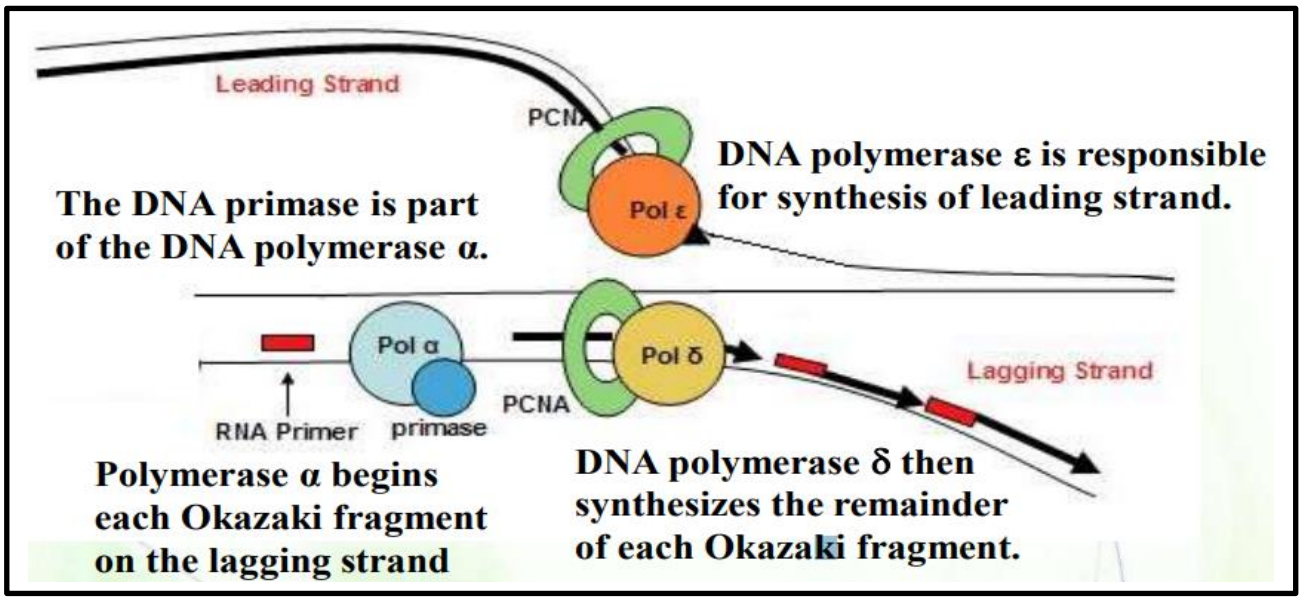
➢ **How do the different polymerases work together?**

- ✓ **DNA polymerase α** ,which has primase as part of it, adds the primer and extends the DNA (it starts DNA synthesis). It does not have to be really fast, because it merely starts synthesis, and so it is not needed to synthesize the whole DNA, but only the initial part of DNA. Hence, it has low processivity.
- ✓ After this, DNA polymerase α falls off, and **DNA polymerase ε** comes to the **leading strand** and extends it.
- ✓ **DNA polymerase δ** comes to the **Okazaki fragments**.
- ✓ Both DNA polymerase ε and δ work fast (high processivity) because they are responsible for synthesis of the bulk (the majority) of DNA. At the same time, their fidelity is high.
- ✓ **DNA polymerase ε** synthesizes the **leading strand**.
- ✓ **DNA polymerase δ** synthesizes the **lagging strand.**

- ✓ **To clarify: DNA polymerase α initiates the replication in both lagging and leading strands**. (It initially extends the DNA in the leading strand, and each Okazaki fragment in the lagging strand and then falls off).
- ✓ **In leading strands**, DNA polymerase **ε** jumps in and binds to complete the mission.
- ✓ **In lagging strands**, DNA polymerase **δ** continues the work for each Okazaki fragment.

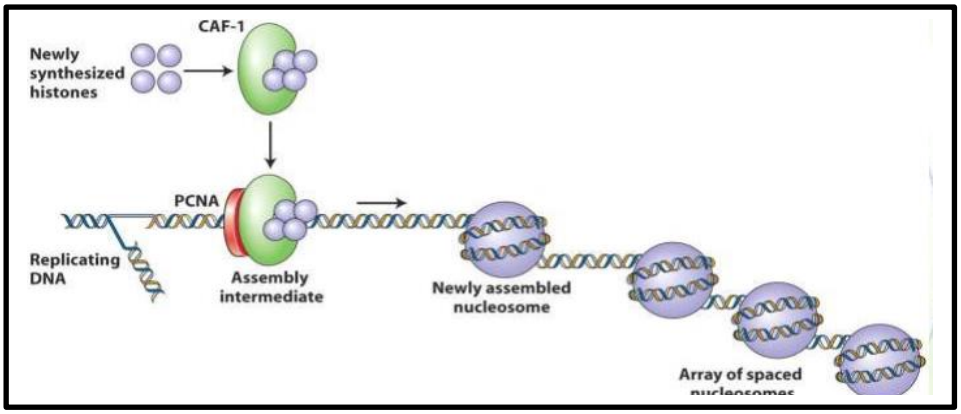


- ✓ Once the polymerase hits the next primer, it falls off. Other **special enzymes** remove primers, and then DNA polymerase δ comes to fill the gaps after primer removal.
  - ➢ **Notice here**: the polymerases in eukaryotes do not have 5'-3' exonuclease activity (unlike DNA polymerase 1 in bacteria) and so special enzymes are needed to remove the primers.



- **Note:** Do not confuse names of eukaryotic polymerases with the names of subunits of DNA polymerase 3 in bacteria. For instance, DNA polymerase α is a eukaryotic polymerase that consists of multiple polypeptides (subunits). On the other hand, DNA polymerase 3 in bacteria, has many subunits designated the symbols α,ε..etc.

- **Role of chromatin:**



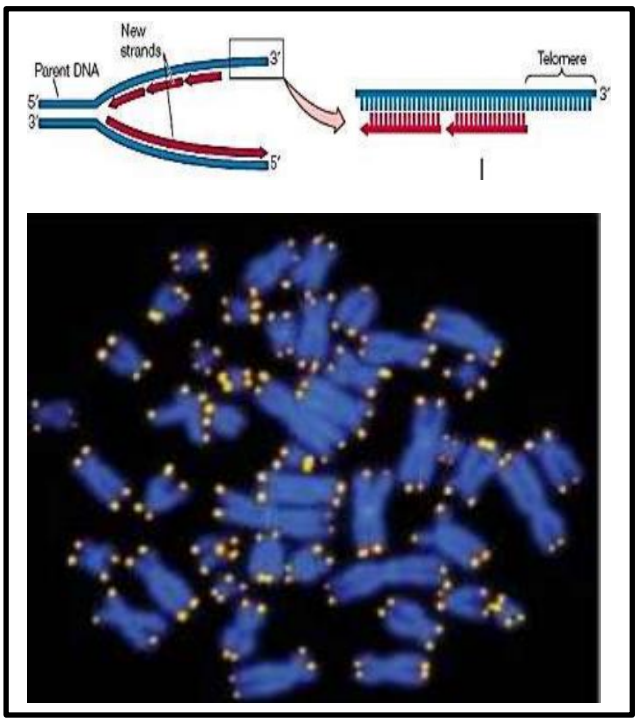
- ✓ In eukaryotic cells, DNA is in the form of chromatin (DNA with histones).
- ✓ DNA polymerases cannot synthesize DNA until histones are removed.
- ✓ DNA is freed from histones by **chromatin remodeling proteins**, they remove the histones ahead of polymerases, to allow enzymes to move along the DNA.
- ✓ We also need to have new histones produced because we have new DNA that has to be wrapped.
- ✓ So we have **chromatin assembly factors (CAFs),** as they form (assemble) chromatin again.
  - ➢ **Chromatin remodeling proteins** are **ahead (in front) of** polymerases
  - ➢ **Chromatin assembly proteins** are **behind** the polymerase.(behind each replication fork).

❖ A problem at the ends of chromosomes:

- ✓ Since the chromosomes are linear, they end with regions called **telomeres**. (telo: end, mere: sequence of nucleotides). Telomeres are made of special repeated sequences.

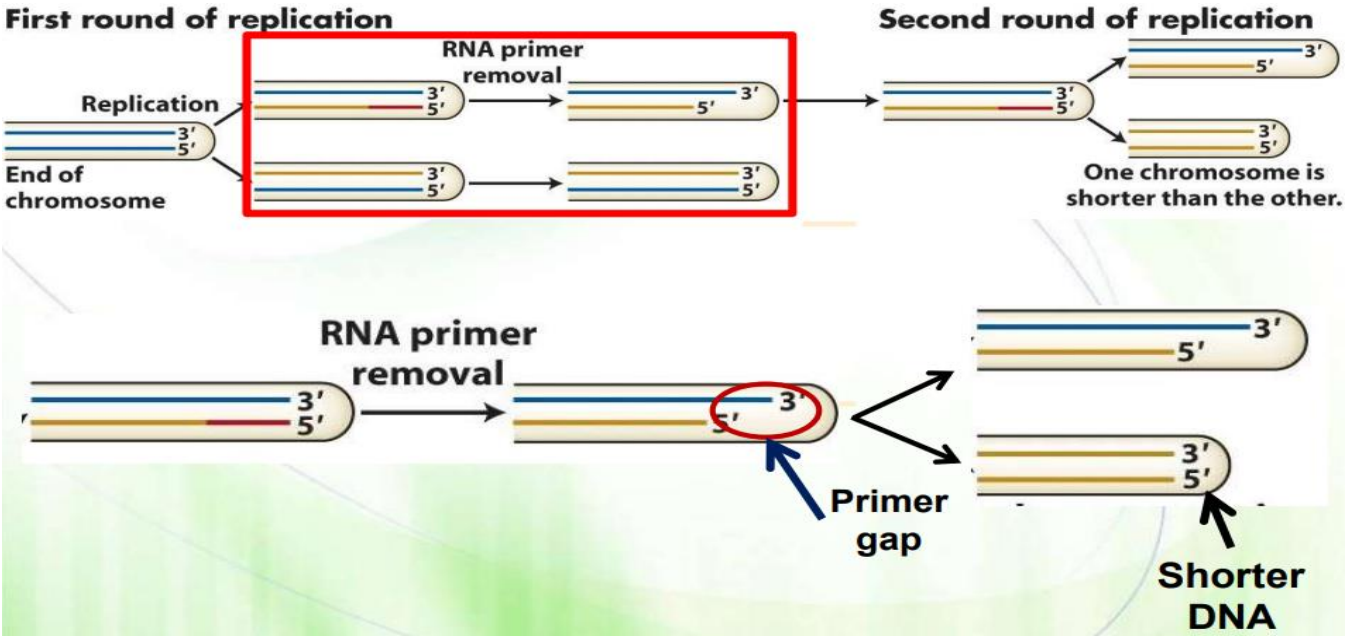
- ✓ Telomere DNA sequences consist of many GGGTTA repeats extending about 10,000 nucleotides.

- ✓ In the leading strand, the polymerase synthesizes this strand continuously until the end. So there is no problem here.



- ✓ **The problem is at the lagging strand:**
  - We have Okazaki fragments one after the other.
  - When we reach the edge, however, there is not much space to add another primer which prevents replication of the ends of chromosomes resulting in shortening of the lagging strands.
  - Even if we were able to add a primer at the edge, once the primer is removed, the gap cannot be filled because there are no nucleotides behind it to which we can add other nucleotides after primer removal (because polymerases only add nucleotides to a pre-existing 3' end).

- ✓ The problem is that the resulting DNA is shorter.
- ✓ This problem becomes more significant when we have a second round of replication, the DNA produced will be shorter than normal because the DNA template used was already shorter than normal from the previous round.
- ✓ With each round of replication, the DNA becomes shorter and shorter.



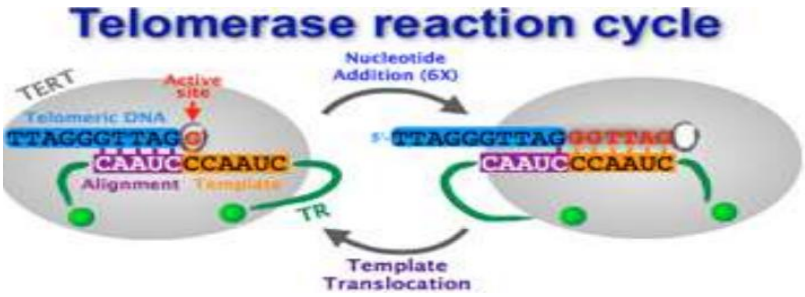
- ✓ The function of telomeres is not only marking the ends of chromosomes, they are also responsible for **stabilizing** the chromosomes. Therefore, if they get shorter the chromosome becomes unstable as a molecule, what does that mean?

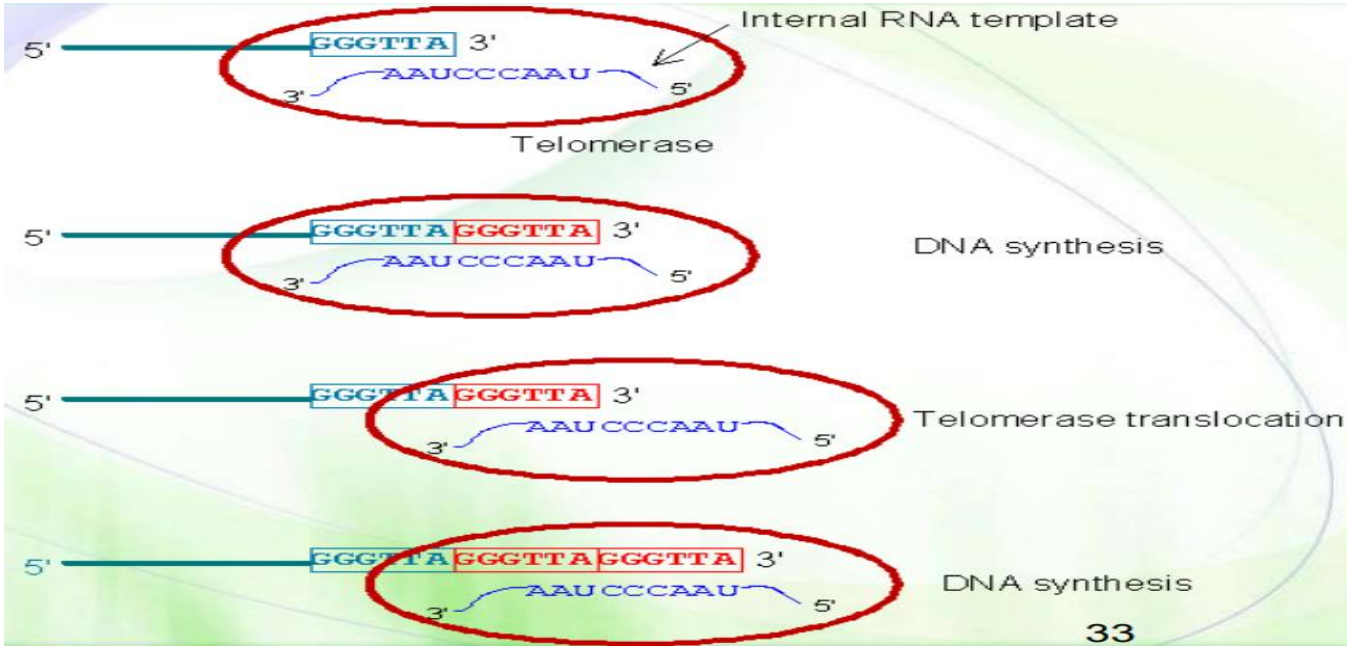
- If the chromosome is not stable, it can be fragmented (broken up) and lot of mutations could occur (either during replication or even without replication), so the cells become unstable because they try to fix their chromosomes and they fail. After that, the cells kill themselves.

- ✓ A lot of cell divisions take place starting from the fertilized egg reaching a mature adult. Imagine that with every cell division, the chromosomes become shorter and shorter, this would mean that we wouldn't survive at all. So there must be a solution.

- ➢ **This problem is solved by telomerase (a reverse transcriptase) which prevents the progressive shortening of the lagging strand … How?**

- Telomerase elongates the chromosome (in the 5' to 3' direction using an RNA template that is a component of the enzyme itself), and makes the **template for the lagging strand longer.**
- This enzyme is large and contains an RNA molecule (that's why it is known as a ribonucleoprotein-a protein with some RNA molecules).
- Telomerase has an RNA sequence complementary to the special repeated sequence (GGGTTA) that exists in telomeres.
- The RNA acts as a template itself to extend the DNA template, elongating it so that it creates space for primase to come and add primer to synthesize another Okazaki fragment and thus extend the lagging strand.





- **Note:** check the animation that the doctor provided in the slides for better understanding. (slide 34)
- Cancer cells have high levels of telomerase, so they keep on dividing and their chromosomes never get shorter. That is why these cells are "immortal".

- **How do we age?**

✓ When we are young, we have high levels of telomerases and their activity is also very high.

✓ As we age, the level and activity of telomerases in our bodies decrease, and that is why with every cell division, our chromosomes get shorter and shorter and that's how we age.

- An inverse relationship between age and telomeric length has been observed. (e.g. if a person's DNA consists of short telomeres, that indicates that he/she is old).

✓ The gradual shortening of the chromosome ends leads to cell death, and it has even been suggested that life span is determined by the length of telomeres.

- Average age of sheep is 12 years.
- Dolly, a sheep that is shown in the adjacent image, lived only for 6 years…**why?**

✓ Because Dolly was cloned **using DNA that was 6 years old.** (at birth, Dolly was actually sick, and had aging problems like wrinkles on the face and organ failure).

✓ So the DNA was already old, and this shortened Dolly's age. (when she was born (at day 1), it was as if she was actually 6 years old already).

Good Luck

- **Resume to next page for extra clarification of topoisomerases's general mechasim of function**

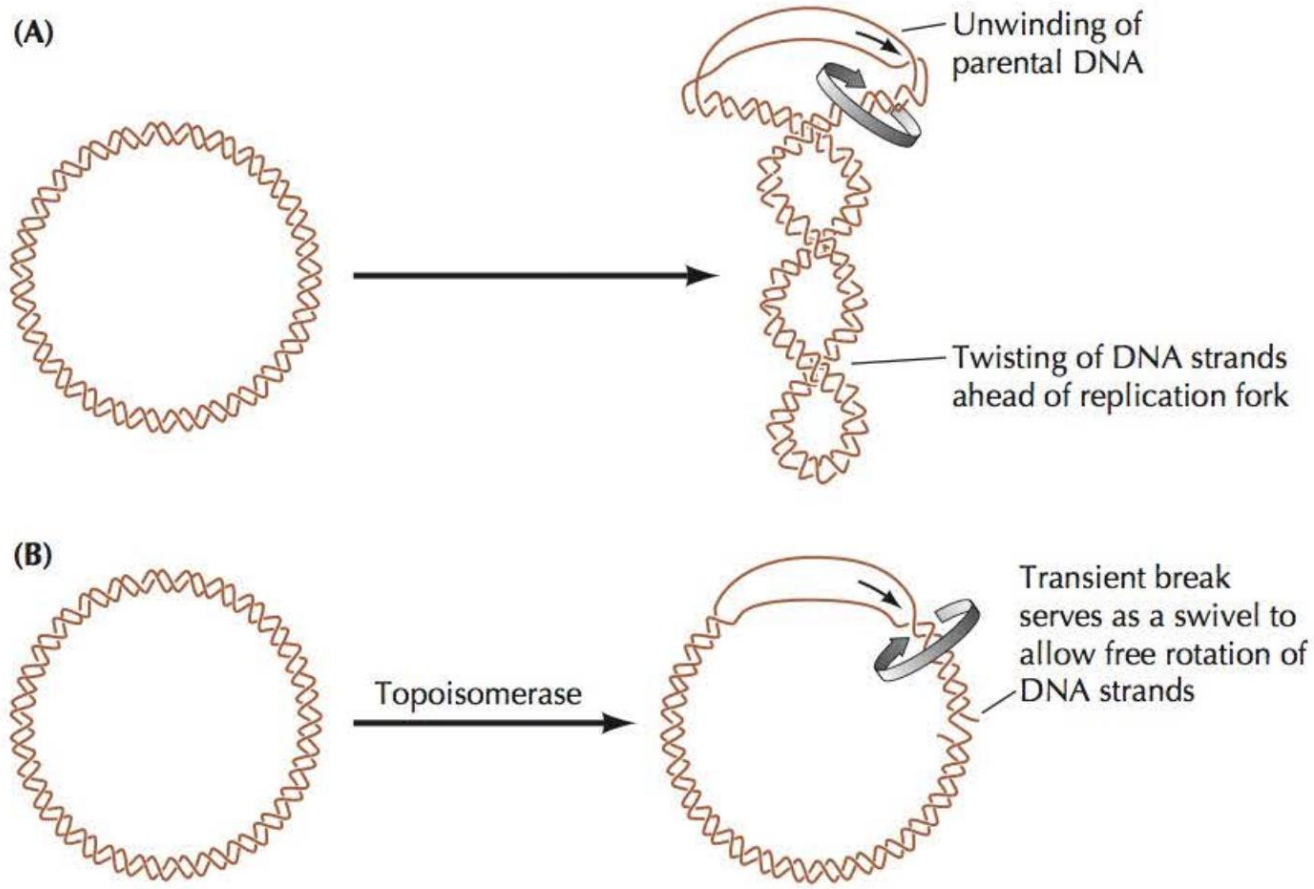


Figure 7.9 Action of topoisomerases during DNA replication (A) As the two strands of template DNA unwind, the DNA ahead of the replication fork is forced to rotate in the opposite direction, causing circular molecules to become twisted around themselves. (B) This problem is solved by topoisomerases, which catalyze the reversible breakage and rejoining of DNA strands. The transient breaks introduced by these enzymes serve as swivels that allow the two strands of DNA to rotate freely around each other.

The previous image was taken from the textbook, and it is merely for obtaining a better understanding of topoisomerase in general. The image is not included in the slides.