CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

6

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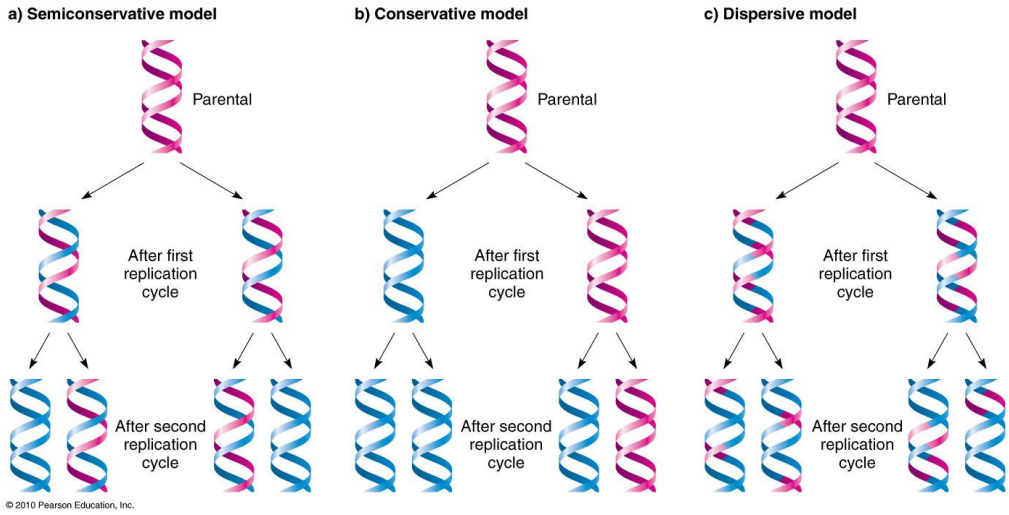
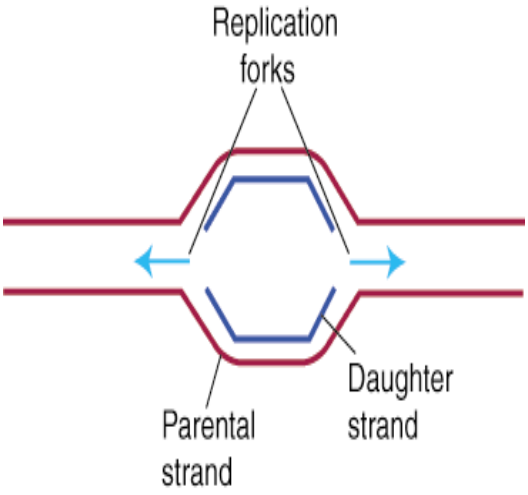
- In order for life to continue cells must divide and in order for cells to divide they need to copy their DNA (genetic material) this process is called DNA replication , whereby you end up with two new cells each with its own DNA .

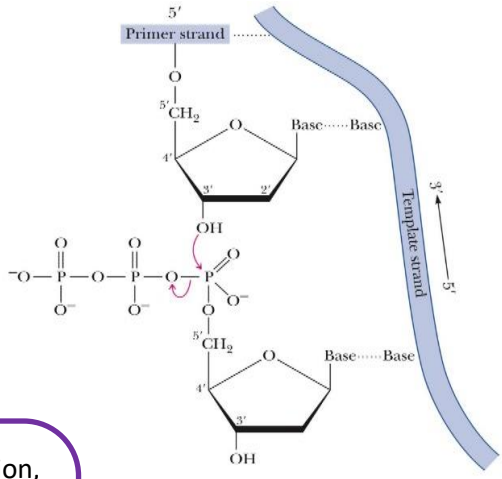
- years ago scientists were wondering how DNA is copied , they came up with three hypothesis ***(Conservative, Dispersive ,& Semi-Conservative models of DNA replication)***
- In the ***conservative model***, the two parental strands reassociate after acting as templates for new strands, thus restoring the parental double helix.
- In the ***dispersive model***, each strand in both daughter cells contain a **mixture** of old and newly synthesized DNA.
- In the ***Semiconservative model*** the two strands of parental molecule separate, and each functions as a template for synthesis of a new complementary strand.
- A brilliant experiment distinguished between the three models, and the results supported the **semiconservative model**



NOTE: details are not required, all you need to know is that DNA follows the semiconservative model of replication (one strand of parental DNA is conserved in each round of DNA replication and the other strand is completely new).

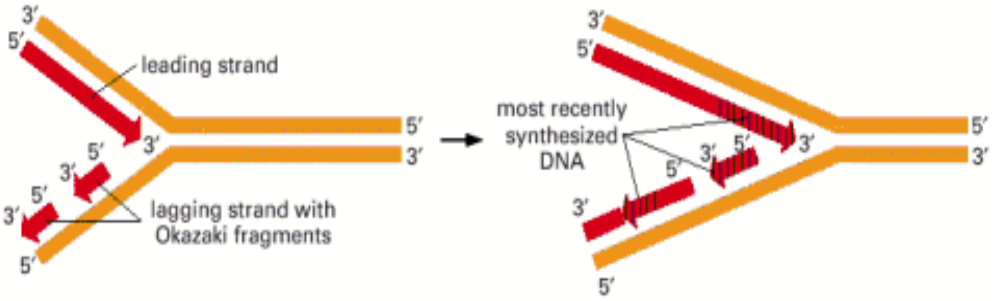
- The replication of chromosomal DNA begins at particular sites called **origins of replication** (short stretches of DNA that have specific sequence of nucleotides).
- The two strands are separated (by specific proteins will be talking about them later on) and a **replication "bubble"** is opened.
- At each end of a replication bubble is a **replication fork** (a Y-shaped region where the parental strands of DNA are being unwound)
- DNA polymerases can add nucleotides ONLY to the free 3' end of a primer or to a growing DNA strand, NEVER to the 5' end, thus a new DNA strand can elongate only in 5'⟶ 3' direction.
- (It doesn't go in the opposite direction (3'⟶ 5') simply because there is no enzyme (polymerase) that has this activity) .



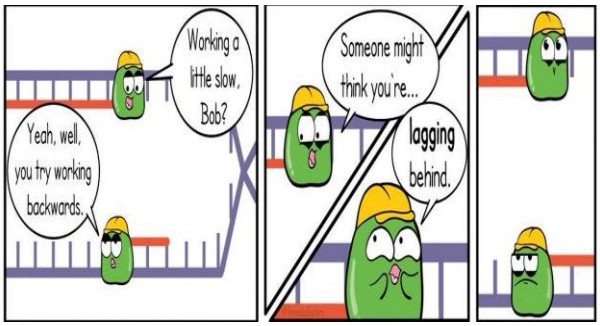


When the two parent strands of DNA are separated to begin replication, one strand is oriented in the 5' to 3' direction while the other strand is oriented in the 3' to 5' direction. However, DNA replication is inflexible, REMEMBER DNA polymerase only functions in the 5' to 3' direction. This characteristic of DNA polymerase means that the daughter strands are synthesized through **TWO** different methods

- Along one template strand DNA polymerase can **continuously** synthesize a complementary strand by elongating the new DNA in the 5' ⟶3' direction, it remains in the replication fork on that template strand and continuously adds nucleotides to the new complementary strand as the fork progresses, the DNA strand made by this mechanism is called the **Leading strand**
- To elongate the other new strand of DNA in the 5'⟶ 3' direction ,DNA polymerase must work along the other template strand in the direction *away* from the replication fork ,the DNA strand elongating in this direction is called the **lagging strand** ,in contrast to the leading strand the lagging strand is synthesized **discontinuously** (because it waits for the leading strand (the continuous one) to open the fork further to allow for its synthesis) as a series of segments which are called **OKAZAKI fragments** .

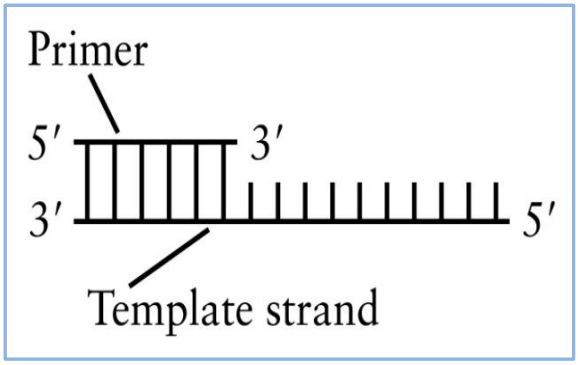


NOTE: *the strand that is synthesized continuously is known as the leading strand because it's leading the way and it's opening the fork further, while the other strand is known as the lagging strand because completion of its synthesis is delayed (it waits for the leading strand to open up the fork and it waits for the Okazaki fragments to be connected to each other forming phosphodiester bonds).*

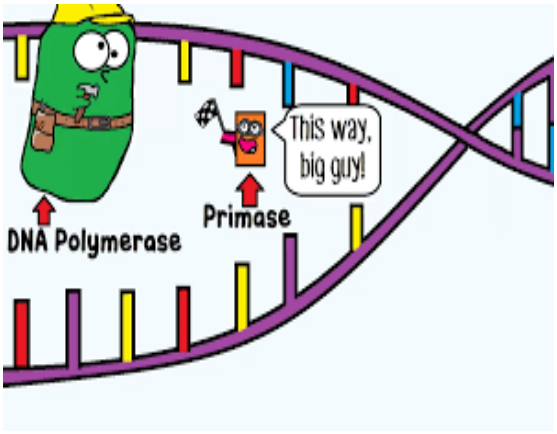


Molecular components: the molecules that are responsible for DNA synthesis:

The unwound sections of parental DNA strands are now available to serve as templates for the synthesis of new complementary DNA strands .However DNA polymerases cannot initiate the synthesis of replication de novo (from scratch) ,they can only add DNA nucleotides to the end of an already existing chain that is base-paired with the template strand.

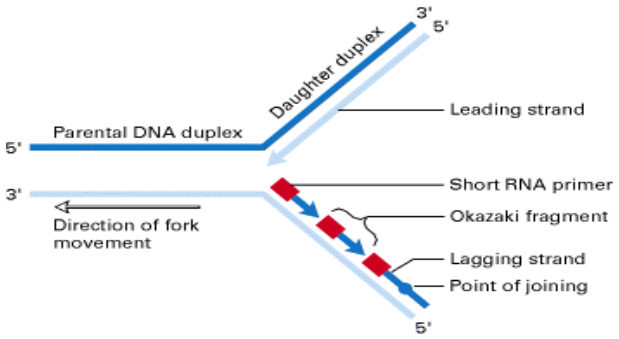


- The initial nucleotide chain that is produced during DNA synthesis is a short stretch of **RNA**, NOT DNA .This RNA chain is called a **Primer** and is synthesized by an enzyme called **Primase**.

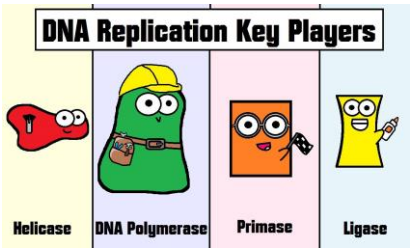


- A primer is a short stretch of RNA, it is made up of **ribonucleotides** not **deoxyribonucleotides.**
- So, the DNA primase synthesizes the primer and then the DNA polymerase can catch up at the end of the primer and it can continues the DNA synthesis.
- The leading strand needs only one primer, but each Okazaki fragment must be primed first (requires its own primer).

- The figure shows that after the primer is made, DNA polymerase start to synthesize the strand continuously in the 5'⟶ 3' direction as the fork progress, on the other hand the lagging strand is synthesized discontinuously . After DNA polymerase forms an Okazaki fragment and as it hits the second primer it detaches, it keeps adding nucleotides to form each Okazaki fragment and detaching once hitting the next primer.

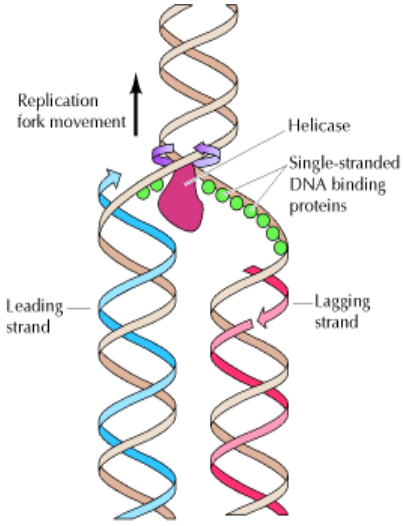


- Okazaki fragments are joined by an enzyme called **DNA ligase** (it joins the sugar-phosphate backbones of all Okazaki fragments into continuous DNA strand by **phosphodiester bonds**).
- **DNA ligase** also joins the 3' end of DNA that replaces primer to rest of leading strand DNA.



Other components are required for replication:

- DNA is double-stranded and helical, so the two strands must be separated from each other, and this is accomplished by an enzyme known as **DNA helicase**
- **Helicase** is an enzyme that removes or disrupts the helical structure of DNA (what helicase does is unwinding or separating the parental DNA strands).
- Helicase is **ATP-dependent** (it requires ATP to open up the double helical DNA as it moves along the strand).



- Helicase doesn't work by itself, in Bacteria DNA helicase is complexed with **DNA primase**, so that helicase opens the fork and then primase adds a primer so primase and helicase in bacteria **only** form a complex known as ***primosome.*** This strategy (***complexing enzymes, proteins to each other***) is used by cells in different organisms including humans) making it easier to speed up chemical reactions, but why do we need this method particularly?

- The reason is that enzymes are not smart ,they are blind , so it's not like an enzyme sees a substrate and says : That's my substrate let's go and catalyze the reaction . This is not the case, the truth is that molecules hit each other and collide with each other randomly, once they hit each other in the **proper angle**, then the reaction occurs, but if they hit each other in another angle NO reaction can take place.
- EXPLANATION : imagine that we have two reactions : A is converted to B and B is converted to C , now you have an enzyme for the first reaction which converts A to B and you need another enzyme for the second reaction to convert B to C , so let's say that you have the first enzyme converting A to B , B is released and swimming in the cell , the second enzyme is blind (it waits for the right moment to collide with B (it takes time because they swim blindly) , so what cells do is that they complex enzymes , so the first enzyme catalyzes the first reaction and the product which is the substrate for the second enzyme doesn't leave that enzyme , actually it jumps to the active site of the second enzyme **reducing the rate of diffusion , thereby speeding up and facilitating the reaction.**
- when DNA becomes single stranded, we face three problems:

1. Nothing prevents the two single strands from reforming a double helix since they're complementary to each other.
2. Cells don't like single stranded DNA .they think it is a viral DNA and immediately degrade it.
3. We can observe the formation of structures called **Hairpins** (when single-stranded DNA contains complementary sequences within the same strand[there is a sequence that is complementary to another sequence in the same strand]so hydrogen bonds can be formed).

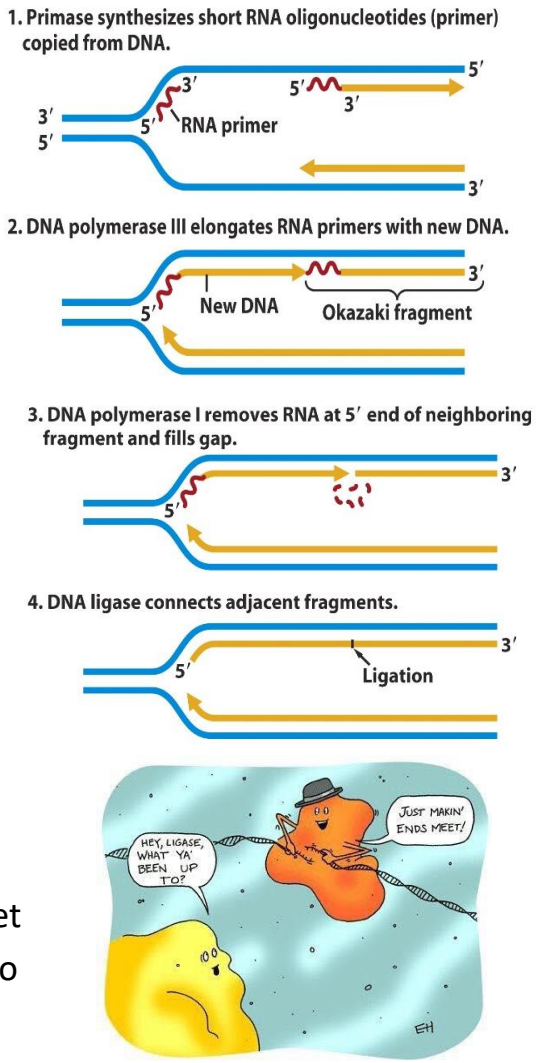
- If DNA polymerase hits a hairpin it would stop, it thinks that it's a double-stranded DNA, **(DNA polymerase cannot read double-stranded DNA, so it stops working**).
- In order to prevent: reformation of double-stranded DNA, Degradation of single-stranded DNA ,formation of hairpin structures, we have proteins known as ***single-strand DNA binding proteins(SSB)*** , also called ***Replication protein A(RPA)***.
- What they do is that they coat the DNA protecting it from (1) and (2) an (3) process mentioned above.
- **SSB** proteins bind tightly to exposed single-stranded DNA strands without covering the bases, which remain available for templating, aiding helicases by stabilizing the unwound **(single-stranded conformation).**

- In bacteria, there are five DNA polymerases, each one of them has its own function.
- DNA polymerase **III** is the enzyme responsible for DNA polymerization at the growing fork (the major synthesis of the DNA) .
  The complex of prismosome (Helicase+Primase) and polymerase is known as **replisome**.

DNA polymerase I has two functions: it synthesizes DNA (a **polymerase activity**) and it also has an **exonuclease activity**.

REMEMBER: exonucleases degrade nucleic acids at **either end**, this exonuclease activity is responsible for removing primers of Okazaki fragments.

so what happens is that DNA polymerase III (starting at the primer end) synthesizes Okazaki fragment and then it hits the primer right in front of it , it falls off and dissociate from the DNA ,DNA polymerase I jumps in and uses both of its enzymatic activities, the ***exonuclease activity*** removes the ribonucleotide of the primer , and the ***polymerase activity*** then places a deoxyribonucleotide , so it does two thing at the same time completing the synthesis of the DNA by filling in the gap .Okazaki fragments must be connected to each other , they get connected by an enzyme known as **DNA ligase** (it also requires energy ⟶ **ATP-dependent**)



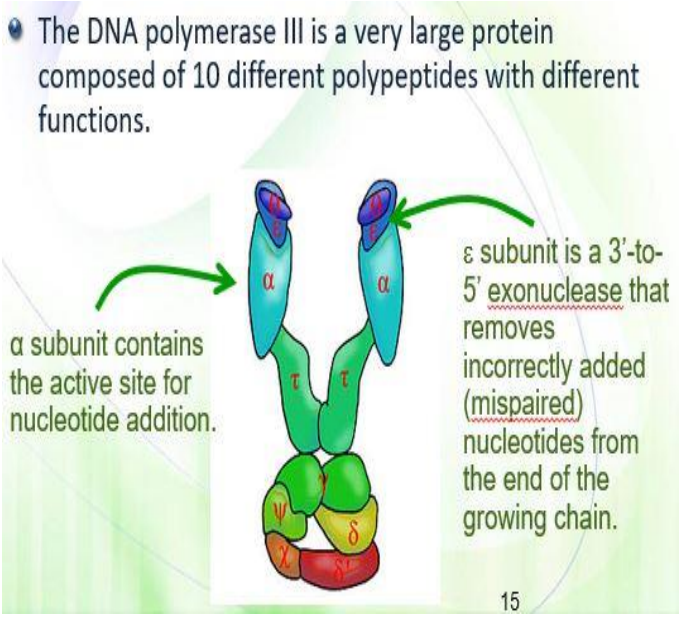
DNA **polymerase I**, is responsible for

- **DNA synthesis:** having polymerase activity for filling the gaps between the lagging-strand fragments.
- **5' ⟶ 3' exonuclease activity** (removal of RNA primer) of each Okazaki fragment.
- **DNA repair.**

- DNA polymerases II, IV and V : DNA repair.

- ⇨ DNA polymerase 3 is a large enzyme that is made up of multiple subunit (multiple polypeptides), each one of them has its own function and they are given designations, α, β, γ, and so on.

- ⇨ We will focus on two of these subunits, the first one is the **α** subunit, which is responsible for the polymerization (DNA synthesis).



The second is the ε subunit, which also has an exonuclease activity, this exonuclease activity is for a totally different purpose, the purpose is to ensure the **fidelity** of DNA (to make sure that the synthesis is accurate, which make sense because the DNA is the genetic material, if there is any change in the DNA, this will affect all incoming generations).

- There are two mechanisms by which DNA polymerase III can ensure the accuracy of DNA synthesis , the first mechanism is that it is an enzyme (it has an **active site** and only specific substrates can fit in this active site), it forms the correct hydrogen bonds, if it puts in the wrong substrate and forms wrong hydrogen bonds, the enzyme would know that and it wouldn't accept this substrate, it only accepts the proper (the right substrate), so if it puts in the active site let's say a C from the template (the original strand that is to be copied) ,only G can fit into the active site in order to form hydrogen bonds
- the second mechanism is that it has an **exonuclease activity,** if it places the wrong nucleotide, moving one step forward, it feels that there's something wrong, it goes back and removes the wrong nucleotide, so it uses an exonuclease activity in the opposite direction because it moves backward in the direction of **3 ⟶ 5'**, (so it's different from polymerase I which uses 5' to 3' exonuclease activity) , this mechanism is known as **proofreading mechanism .**

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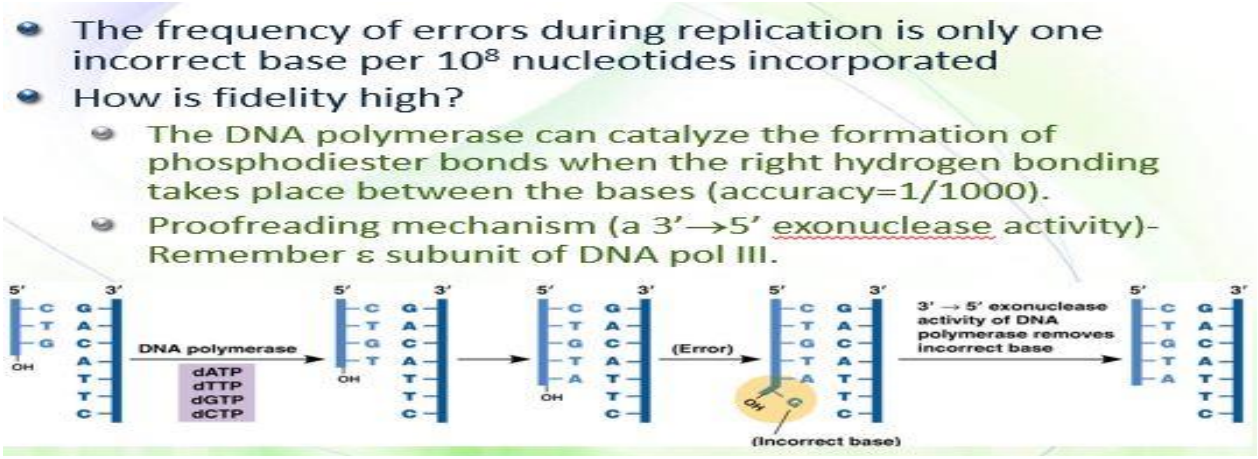
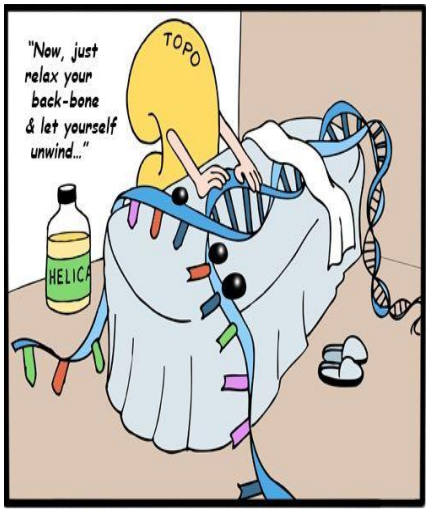
- **Proofreading** means looking for mistakes and correcting them.
- So, the enzymatic property of DNA polymerase III makes one mistake per 1000 nucleotides added, which is in terms of molecular biology and genetics is too much , but because of the proofreading mechanism, DNA **polymerase III** makes only one mistake per **10^8** (hundred millions) bases added, which is really accurate.



Remember: endonucleases cut the nucleic acid within the chain (in the middle of a polynucleotide chain). While exonucleases degrade nucleic acid from either ends of a polynucleotide chain.

- After DNA helix is unwounded, another problem arises, the untwisting of the double helix causes tighter twisting and strain ahead of replication fork. This leads to **<u>overcoiling of the DNA into tight knots</u>**, with the two strands wrapped too tightly around each other, DNA becomes tangled and really mixed.

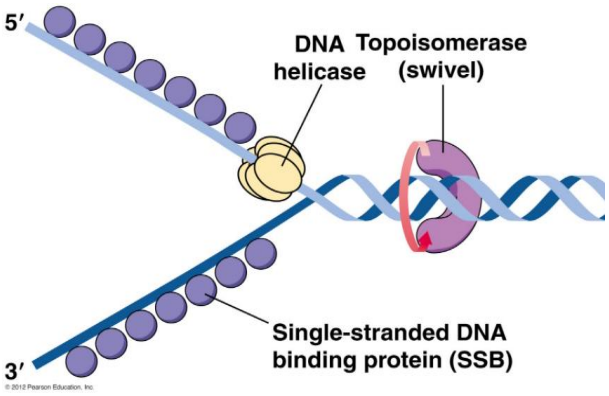


- DNA must be relaxed and this achieved by an enzyme known as Topoisomerase (Topoisomerases are isomerase enzymes that act on the topology of DNA).

- Isomerase is an enzyme that converts isomers, which are molecules that have the same molecular component (atoms) but different structures like

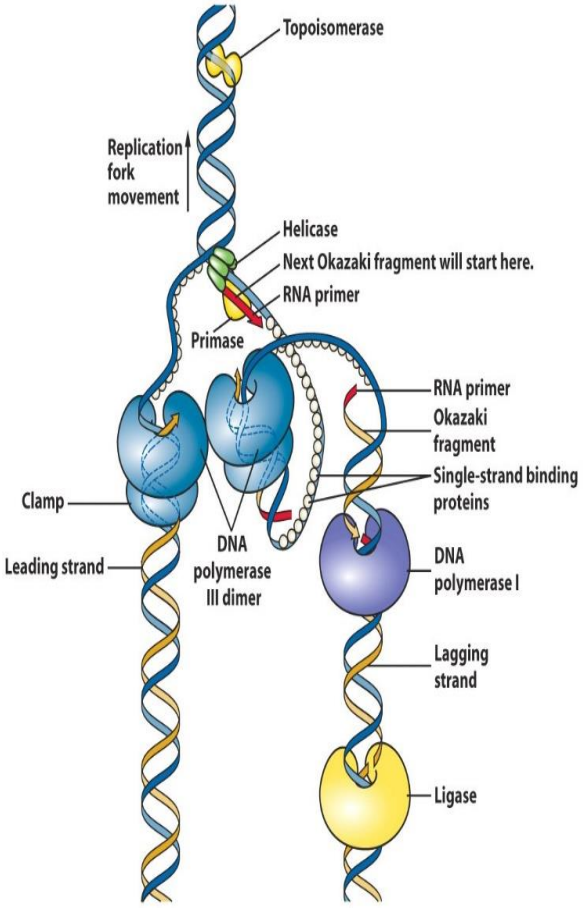
glucose and fructose, so there is an isomerase that converts glucose to fructose.

- Topo means 3D structure, so DNA topoisomerase works on DNA changing its structure, different topoisomerases are found, **topoisomerase I** specifically works on DNA replication, it creates a **swivel** that is a single cut in one of the phosphodiester bonds in one of the strands so when DNA rotates only a short region can rotate thereby preventing the formation of knots.
- Summary: Topoisomerase 1 functions ahead of DNA polymerase complex (replisome) it makes a single cut of a nucleotide**, breaking one phosphodiester bond in one of the strands *producing a transient single-strand break (or nick).***
  So, the DNA behind the topoisomerase doesn't get affected.



- Topoisomerase I is **ATP-independent.**

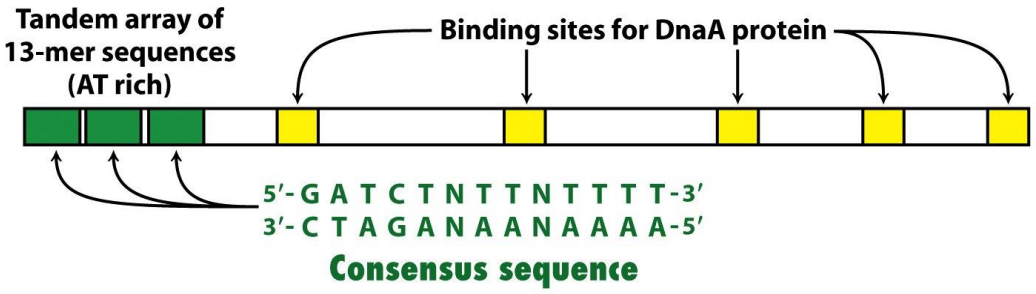
- The figure shows two DNA **polymerases** synthesizing two strands of DNA molecule, one of them works continuously forming the leading strand, the other one works discontinuously forming Okazaki fragments (lagging strand) that get connected to each other via **DNA ligase**, and this comes after the removal of the primer by **DNA polymerase I.**
- **Helicase and primase** (primosome complex) are working ahead of DNA polymerase, the helicase opens up DNA, and primase adds the primer.
- Ahead of them all is **topoisomerase** making a single break in one of the strands allowing the rotation of only a short region of DNA rather than rotating the whole molecule.

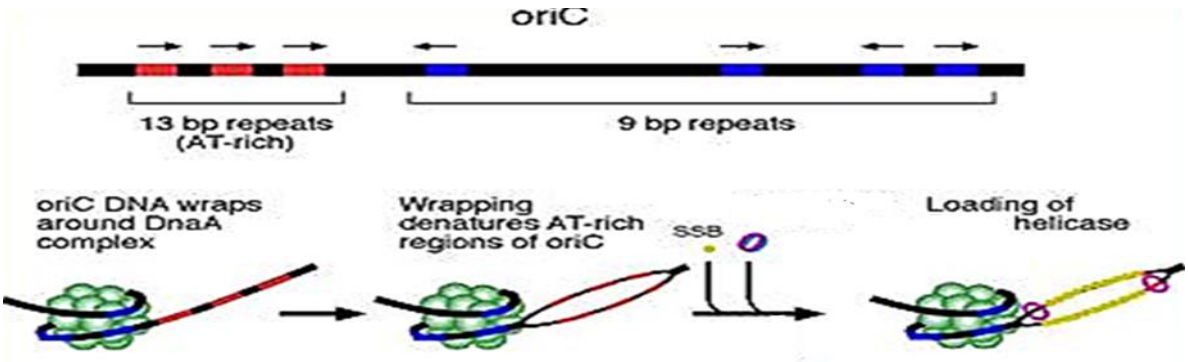


- DNA replication starts at a particular site known as the **origin of replication (OriC)**, and there's a single origin of replication in prokaryotic genomes **(only one starting point).**
- When scientists looked at the origin of replication in different bacterial species, they found out that this origin of replication is a region in the DNA that contains sequences that are similar between the bacterial species, and these sequences collectively known as **consensus sequences** these sequences are important because they have been preserved for millions of years of creation**.**

❖ In molecular biology the **consensus sequence** is a sequence that exists in different organisms, and there are two types of these sequences existing in origin of replication in bacterial genome :

1) **9-mers**: a region that contains 9 base pairs, these sequences are repeated serving as binding sites for the **DnaA** protein.
2) **13-mers**: **AT-rich region** - it facilitates separation of the double strand DNA.





➢ When conditions are optimal (when there are enough nutrients and bacteria is happy ☺) it replicates its genetic material and starts division, the level of **DnaA** protein is increased during this period .

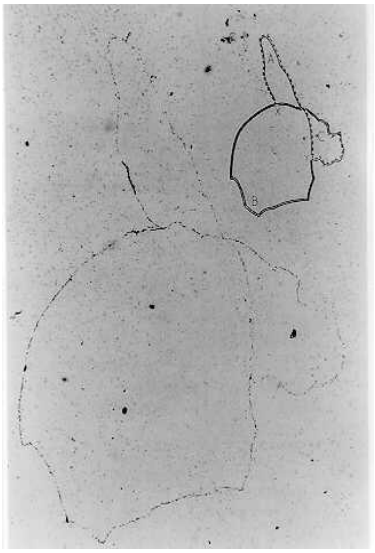
- **DnaA** binds to the **9-mers** wrapping DNA around it, squeezing the DNA and causing the **13-mers** to pop up (to open up).Now that is the signal for helicase to jump in, and replication starts.

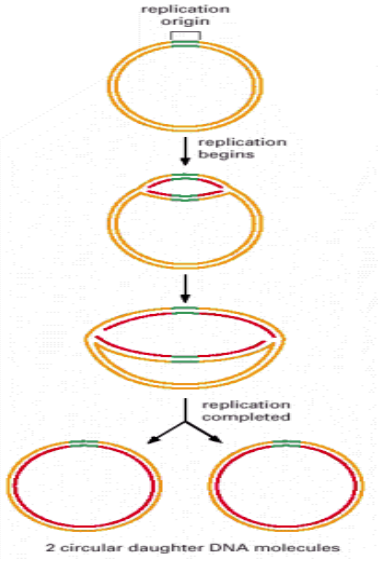
- What helped the 13-mer to pop up?
  REMEMBER it's an **AT-rich** region, the bonds between the two strands are weak since **A** and **T** forms only **two hydrogen** bonds when base paired, it's easier to separate these strands than if this region contained high GC-content (with three hydrogen bonds in between).
- **DnaA protein opens up the DNA ⟶ DNA helicase jumps in unwinding the helix ⟶ Primase add a primer ⟶ DNA polymerase III elongates the DNA 5' ⟶ 3' and other proteins join to complete the replication.**

The end of replication in bacteria:

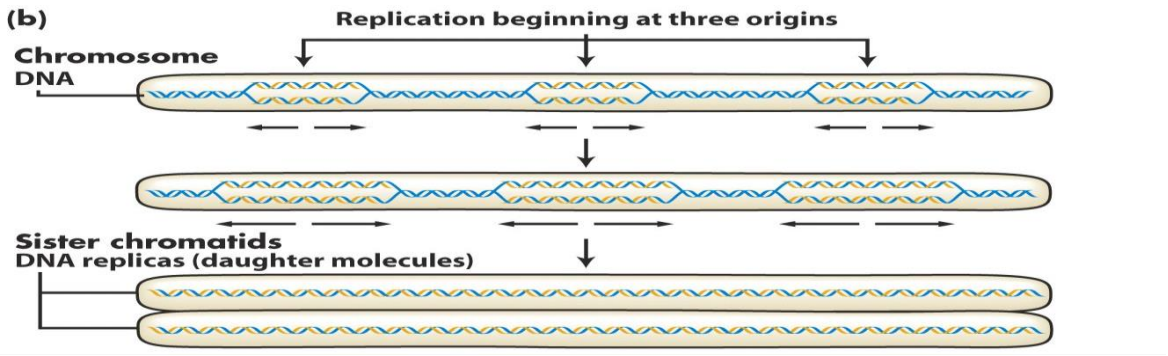
Bacteria has only one origin of replication, replication of DNA proceeds in both directions until they meet halfway around the chromosome, and the two strands are separated.

- **Termination of DNA replication** occurs when the two forks meet and fuse, creating two separated **double-stranded DNA** molecules.





- In contrast to a bacterial DNA, eukaryotic DNA may have hundreds or even thousands replication origins, multiple replication bubbles form and eventually fuse, thus speeding up the copying of the very long DNA molecules. Unlike prokaryotes which can double under optimal conditions in about 20 min, the eukaryotic cell cycle takes about 18 to 24 h to complete.



We are alphabetic beings and DNA is the language we are written in.

Friendly reminder that 'doing your best does not mean pushing yourself to the limits of endurance, but only doing the best you can without hurting yourself. Further, even friendlier reminder that it's completely fine if that means you don't do as much as someone else.

They're not you, and your contribution is just as valid as theirs

Best wishes ♥