Genetics

Subject : Genetics

Lec no : 5 (part 2)

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B-Synthesis of the two DNA strands:

- **DNA polymerase III** enzyme is responsible for the synthesis of both new DNA strands. The enzyme synthesizes the new DNA strands **only in the 5'→3' direction**, and it cannot start DNA synthesis without the presence of **RNA primers**.

- DNA polymerase III يبدأ synthesis of new strand من 3' → 5'
(إذن فإن الطرف الذي سيكون واقف عنده (يبدأ البناء من عنده 5'
وبالتالي فإن الطرف الذي امامه في old strand سيكون 3'
- DNA polymerase III لا يمكنه البداية بالبناء من العدم (يحتاج RNA primer يكمل بناء عليه)

Synthesis of RNA primers:

- Primers are short RNA molecules about 5-10 nucleotides in length and are complementary to a segment of the DNA strand. Primers are synthesized in the direction of 5\→3\ direction by **primase** (**RNA polymerase**) enzyme using ribonucleotide triphosphate (ATP, GTP, CTP, **UTP**).

Synthesis of the DNA strand:

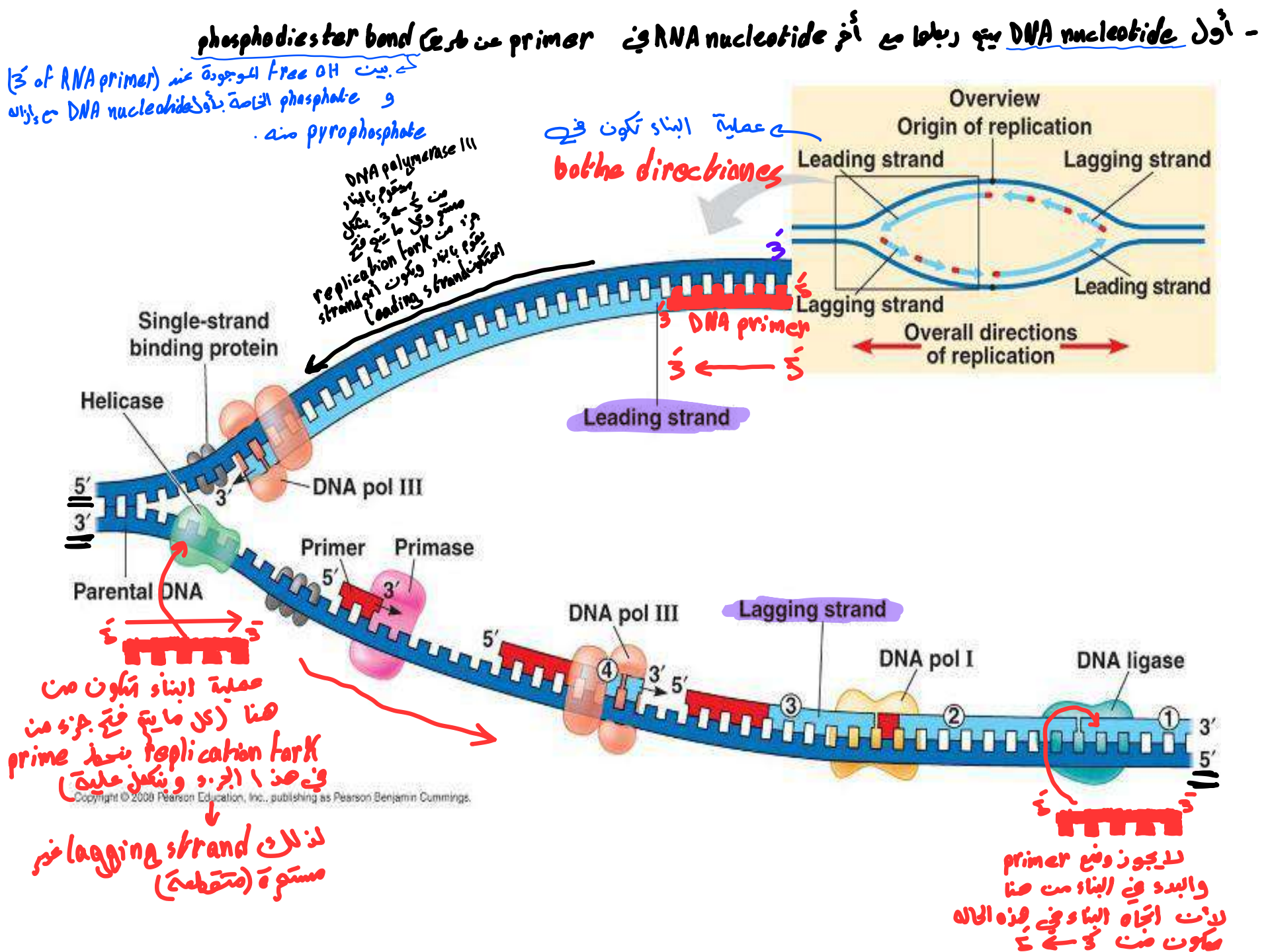
- **DNA polymerase III** synthesizes DNA in the 5\→3\ direction by using deoxyribonucleotide triophosphate (d ATP, d GTP ,d CTP & d ATP) to form the new strands in a **complementary sequence to that of the parent DNA according to base paring rule**. The first added deoxyribonucleotide triphosphate will form **phosphate diester bond with the OH at the 3\ end of the RNA primer with removal of pyrophosphate** .The next added deoxyribonucleotide triphosphate form phosphodiester bond with the previously added one with hydrolysis of pyrophosphate **to provide the energy** for the reaction.

- DNA polymerase III يستخدم في بناء new strand ← deoxyribonucleotide triphosphate
- ملاحظة مهمة: RNA polymerase يستخدم في بناء primer ← ribonucleotide triphosphate ← primer ولذلك primer عبارة عن RNA
- بينما DNA polymerase يستخدم في بناء new strand ← deoxyribonucleotide triphosphate ← strand ولذلك في DNA

- أول DNA nucleotide بيتم ربطها مع RNA nucleotide أخر في primer عن طريق phosphodiester bond

(3' of RNA primer) عند الموجودة Free OH بين
و phosphate الخاصة بأول DNA nucleotide بيزاله
منه pyrophosphate .

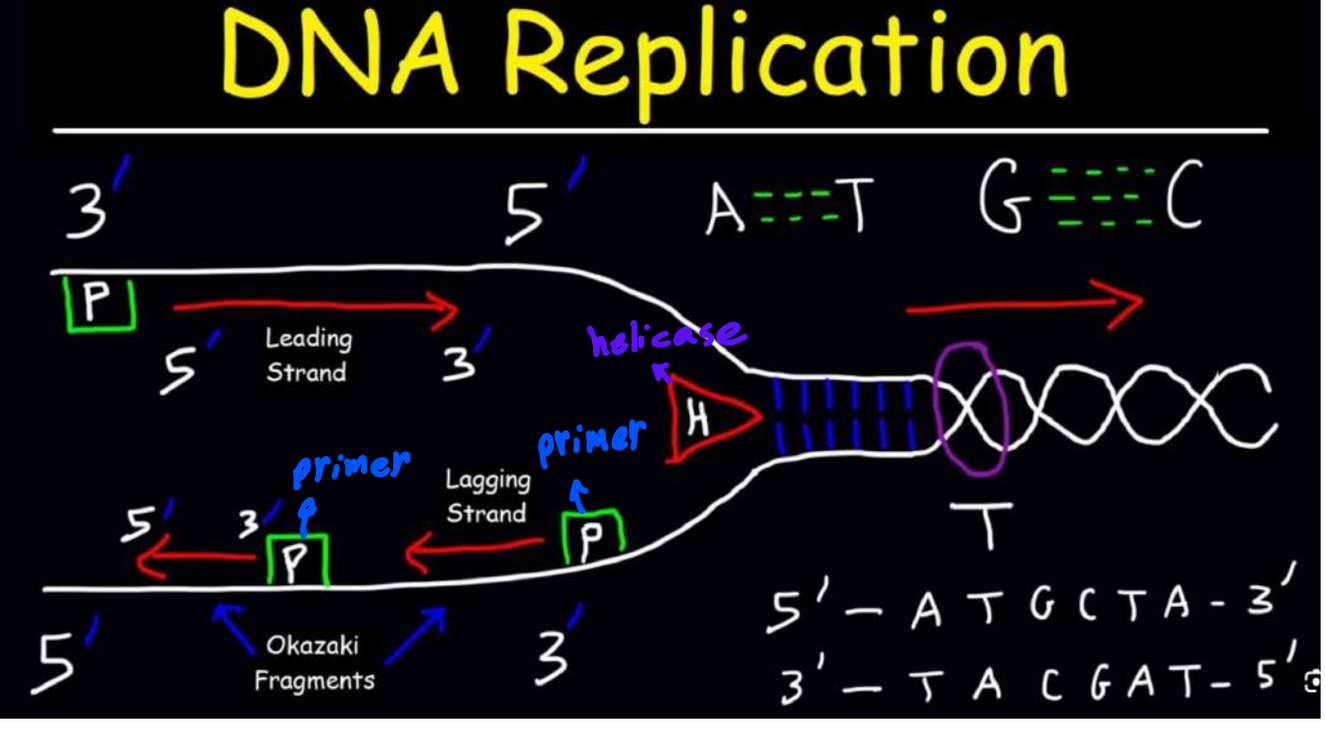
عملية البناء تكون في both the directions

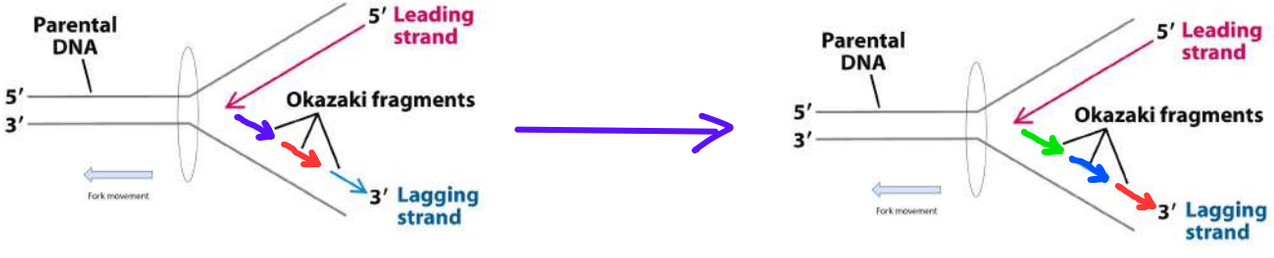


عملية البناء تكون من هنا (كل ما يتم فتح جزء من prime بنحط replication fork في هذا الجزء وبتكمل عملية)

لذلك الـ lagging strand غير مستمرة (متقطعة)

لا يجوز وضع primer والبدء في البناء من هنا لأن اتجاه البناء في هذه الحالة سيكون من 5' إلى 3'

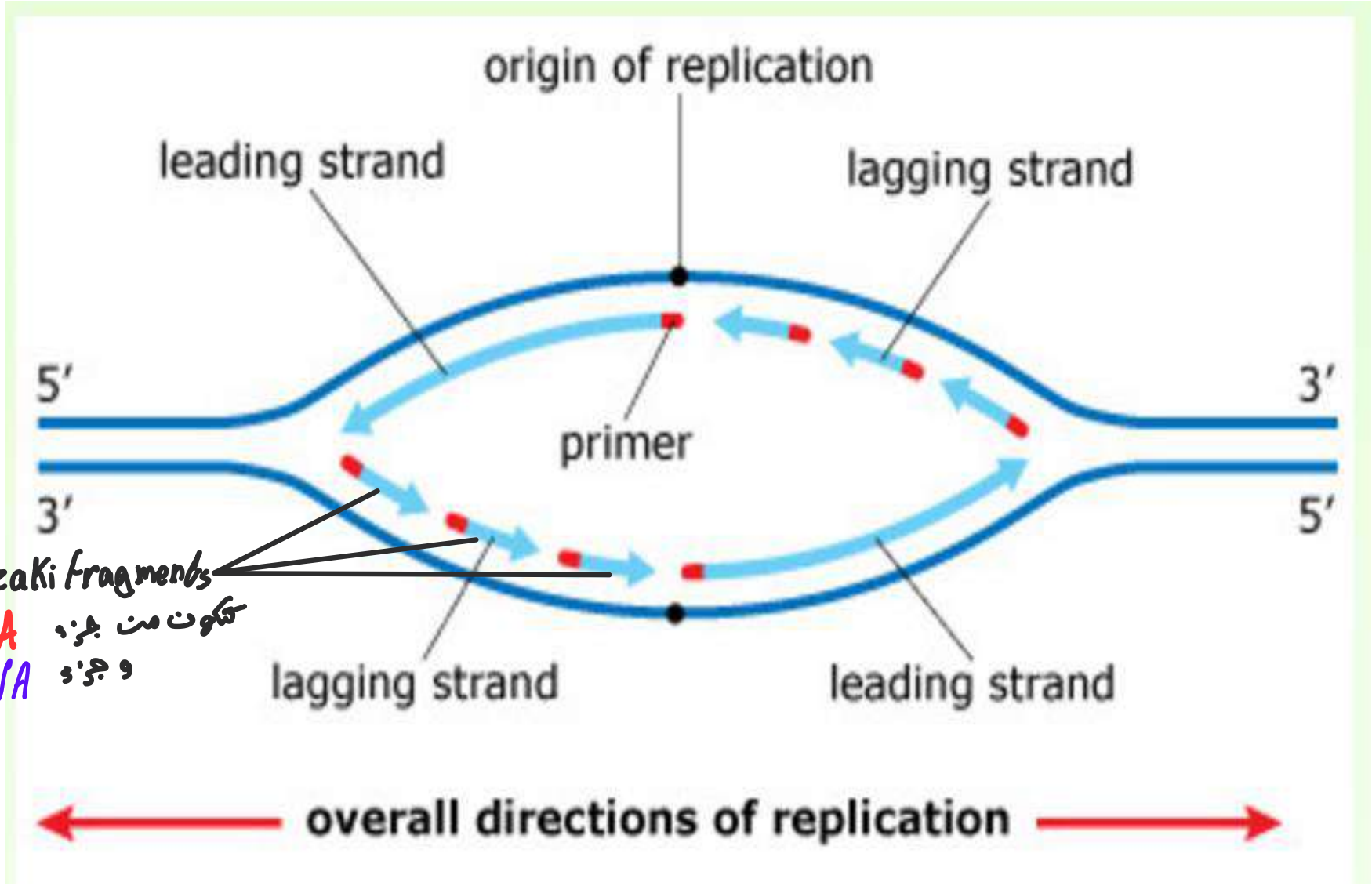




بعد فتح جزء من replication fork يتم إضافة primer جديد وتكملة البناء عليه ويتوقف البناء عند الوصول إلى الـ primer الذي أمامه.

DNA Replication

1. DNA replication usually proceeds bidirectionally.
2. Helicase separates the DNA strands
3. Topoisomerase reduces torsional strain & positive supercoils.
4. SSB proteins stabilizes the isolated strands.
5. DNA replication requires a RNA primer to begin.
6. DNA Pol. III adds nucleotides in the 5' to 3' direction.
7. DNA replication is semidiscontinuous
8. DNA Pol. I removes the RNA primer & replaces it with DNA.
9. DNA ligase seals the nick after the primer is replaced with DNA.
10. DNA Pol. I & III have 3' to 5' exonuclease activity. (Proofreading)
11. DNA Pol. I has 5' to 3' exonuclease activity. (DNA repair)

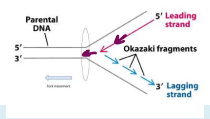

origin of replication
leading strand
lagging strand
5′
3′
primer
3′
5′
okazaki fragments
RNA
DNA
تتكون من جزء
و جزء
lagging strand
leading strand
overall directions of replication

DNA synthesis in two different directions:

Leading strand

Lagging strand

بنائها يكون في نفس اتجاه فتح replication fork



تحتاج لبنائها 1 RNA primer

- **Leading strand:** The strand that is being copied **in the direction of the advancing replication fork** is called the leading strand and is synthesized continuously. It is **complementary to the parent strand that has the direction of 3\→5**

قطعة واحدة مستمرة

old strand 3′ → 5′ leading strand

بناتها يكون عكس اتجاه فتح replication for K

يتم بناتها discontinuously تتكون من small fragments (stretches) of discountinuous DNA

- **Lagging strand:** The strand that is being copied **in the direction away from the replication fork** is synthesized discontinuously, with small fragments of DNA being copied near the replication fork. It is **complementary to the parent strand that has the direction of 5'→3'** These short stretches of discontinuous DNA, termed **Okazaki fragments**, are eventually joined (ligated) to become a single, continuous strand. The new strand of DNA produced by this mechanism is termed the lagging strand.**(Many RNA primers are needed for synthesis of the lagging strand)**

تتمت قطعة مستوة بك اكثر من قطعة

Excision of RNA primers and their replacement by DNA

DNA polymerase I has a 5` exonuclease activity
يعني يشيل nucleotides of RNA primers من الاتجاهين (5` و 3`) فيشيل من عندها

- DNA polymerase III continues to synthesize DNA on the lagging strand until it is becomes very close to the next RNA primer. When this occurs, **DNA polymerase I** excise the RNA primer (has a 5` exonuclease activity)and the gap filled by DNA nucleotides. **DNA ligase** enzyme connects the DNA fragments.

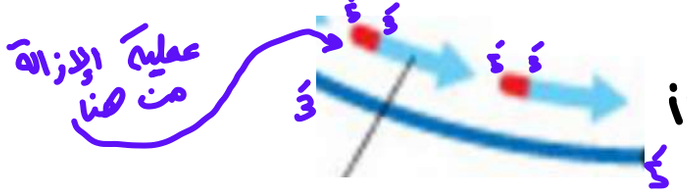
RNA primers يبني ← RNA polymerase
DNA يبني جزء (primers يكمل) ← DNA polymerase III
DNA nucleotides ووضع مكانها primers يقوم بإزالة ← DNA polymerase I
segments يقوم بتوصيل ← DNA ligase
يشترك في بنائها lagging strand ←

- جزء DNA المتكون لـ Okazaki fragment يتم بناؤه عن طريق DNA polymerase III وعند وصول جزء DNA إلى primer المجاور يتوقف ويبدأ عمل DNA polymerase I .

- DNA polymerase I يقوم بإزالة RNA primers ويضع مكانها DNA nucleotides

RNA primers ← يبني RNA polymerase
DNA ← يبني جزء DNA polymerase III (يكمل primers)
DNA nucleotides ← يقوم DNA polymerase I بإزالة primers ووضع مكانها
→ يشترك في بناء lagging strand

DNA polymerase has a 5' exonuclease activity
(يعني يشيل من عندها nucleotides of RNA primers (لما تكون فاضية فيشيل



in lagging strand

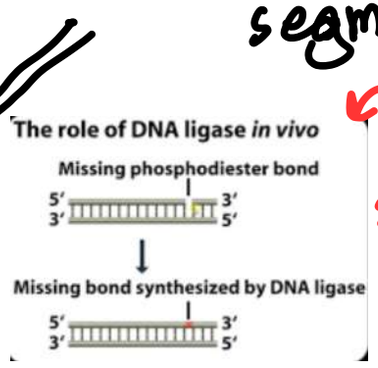
in leading strand

← حتى بعد إزالة primers وبناء DNA nucleotides مكانها

لا زال يوجد space صغيرة بين segments

DNA polymerase III ← ما بيعرف يشيل DNA الذي يبنيه الذي يليه .

DNA polymerase I ← ما بيقدر يشيل DNA الذي بناه segments primer مكان



DNA ligase ← هو من يحل هذه المشكلة
يربط بين آخر nucleotide في segment وأول nucleotide في segment التي تليها عن طريق phosphodiester bond

التي سبقه

Proofreading of newly synthesized DNA:

- To ensure replication fidelity, DNA polymerase III has, in addition to its 5\→3\ polymerase activity, a "**proofreading**" activity (**3`→5` exonuclease**). As each nucleotide is added to the chain, DNA polymerase III checks to make certain the added nucleotide is, in fact, correctly matched to its complementary base on the template. If it is not, the 3\→5\ exonuclease activity corrects the mistake.

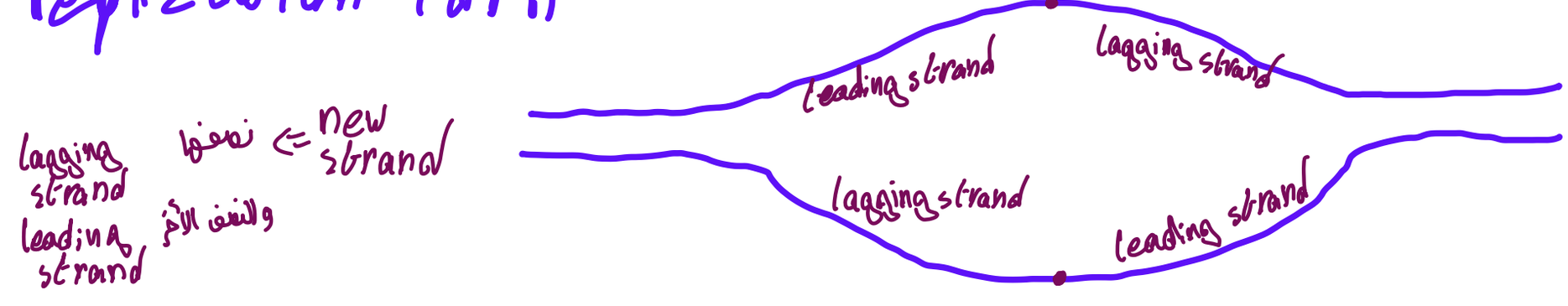
DNA polymerase III

- 5'→3' polymerase activity
- proofreading activity (3'→5' exonuclease)

اثناء عمله في البناء من 5'→3' يرجع من 3'→5' للتأكد من عمله
إذا كان في خطأ في nucleotide بيزيلها.

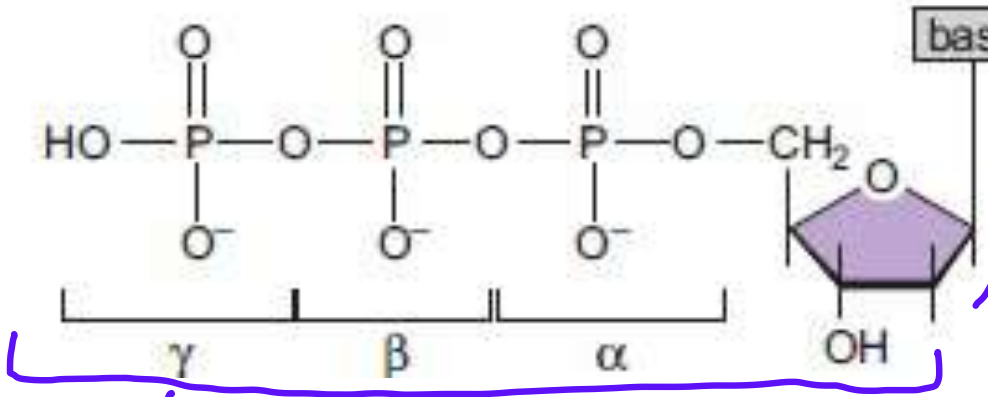
* كل الكلام مني ما سبق كان على النصف الأيسر.
النصف الأيمن فقط عليك فهم direction وكل شيء راح يكون بسيط.

← أول شيء انظر إلى direction الخاص بـ old strand
ثم طبق ما تعرف وهو أن البناء يكون من 5' → 3'
Leading strand تبدأ من origin of replication (مع اتجاه فتح replication fork)
lagging strand تبدأ من مكان الفتح (اتجاهها عكس اتجاه فتح replication fork)



Notes

- Nucleoside triphosphates have three phosphoryl groups that are attached via the 5`-hydroxyl of the 2`-deoxyribose. The phosphoryl group proximal to the deoxyribose is called the α-phosphate, whereas the middle and distal groups are called the β-phosphate and the γ-phosphate, respectively.



nucleoside triphosphate

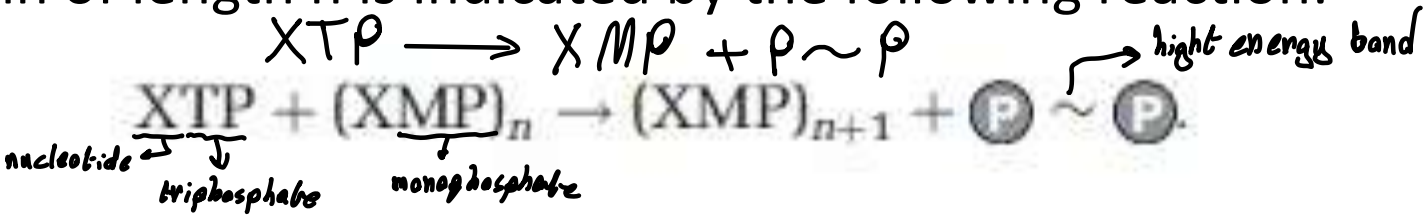
لما عنه الأدينوسين ثلاثي الفوسفات بـ nucleotide
الي فوقيها (اللي يسبقها) فإن الإرتباط
يكون مع α-phosphate حيث أن
release بيصير لهم pyrophosphate

release of pyrophosphate ← لأنه يعطي طاقة
polymerisation لإكمال

- **DNA Is Synthesized by Extending the 3` End of the Primer**: the hydroxyl group at the 3` end of the primer strand attacks the α-phosphoryl group of the incoming nucleoside triphosphate. The leaving group for the reaction is pyrophosphate, which is composed of the β-phosphate and γ-phosphate of the nucleotide substrate.

Hydrolysis of Pyrophosphate Is the Driving Force for DNA Synthesis

- The addition of a nucleotide to a growing polynucleotide chain of length n is indicated by the following reaction:



- But the free energy for this reaction is rather small. What, then, is the driving force for the polymerization of nucleotides into DNA? Additional free energy is provided by the rapid hydrolysis of the pyrophosphate into two phosphate groups by an enzyme known as **pyrophosphatase**.

pyrophosphate ← hydrolysis عملية
driving force ← تعطي high energy
polymerization عملية

hydrolysis of pyrophosphate عملية ← pyrophosphatase

DNA polymerase III ← ضروري لعمل primer ← نحتاج DNA ← لا نستطيع بناء

يعني لازم لحتى نبدأ بناء DNA

انه يكون موجود free OH في آخر nucleotide في primer (مثل كذا)

DNA polymerase III → ss-rand لا يستطيع عمل من الصدع

RNA polymerase → ss-rand يستطيع عمل من الصدع

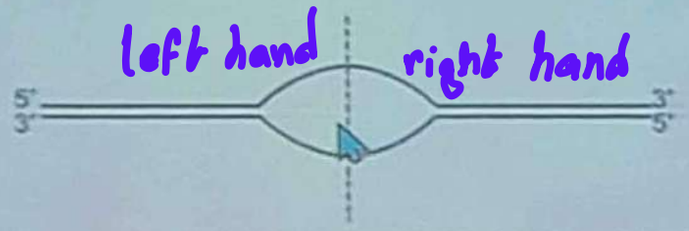
- All DNA polymerases require a primer with a free 3`-OH. **They cannot initiate a new DNA strand de novo**. **How, then, are new strands of DNA synthes is started?** To accomplish this, the cell takes advantage of the ability of RNA polymerases to do what DNA polymerases cannot: start new RNA chains de novo.

- Although the leading-strand DNA polymerase can replicate its template as soon as it is exposed, synthesis of the lagging strand must wait for movement of the replication fork to expose a substantial length of template before it can be replicated. Each time a substantial length of new lagging-strand template is exposed, DNA synthesis is initiated and continues until it reaches the 5` end of the previous newly synthesized stretch of lagging –strand DNA.

ـــ الفكرة من الشلايد انه عند بناء lagging strand (Okazaki fragment) يجب أن انتظر بعد فتح replication fork حتى تكشف substantial length كافية حتى تتسع لـ primer تقريباً" اكثر من ١٥ nucleotides لانه ممكن يكون ١٥ nucleotide وأنا بدي أضيف DNA part ايضاً".

- **Eukaryotic cells** also have multiple DNA polymerases. Of these, three are essential to duplicate the genome: **DNA Pol δ, DNA Pol ε, and DNA Pol α/primase.**
- Each of these eukaryotic DNA polymerases is composed of multiple subunits. DNA Pol α/primase is specifically involved in initiating new DNA strands. This four-subunit protein complex consists of a two-subunit DNA Pol α and a two-subunit primase.
- After the primase synthesizes an RNA primer, the resulting RNA primer:template junction is immediately handed off to the associated DNA Pol α to initiate DNA synthesis. **Because of its relatively low processivity, DNA Pol α/primase is rapidly replaced by the highly processive DNA Pol δ and Pol ε.** The process of replacing DNA Pol a/primase with DNA Pol δ or Pol ε is called **polymerase switching** and results in three different DNA polymerases functioning at the eukaryotic replication fork.
- DNA Pol δ and ε are specialized to synthesize different strands at the replication fork, with **DNA Pol ε synthesizing the leading strand** and **DNA Pol δ the lagging strand.**
- *Processivity, the average number of bases a pol will extend before falling off a template.*

Below is a picture of a single origin of replication in a eukaryotic cell.



A. With respect to the dotted line, in which direction(s)—right, left, or both—does total replication proceed?

B. On the right-hand side of the dotted line, the replication of which template strand (top or bottom) will be continuous by DNA polymerase?

C. On the left-hand side of the dotted line, the complete replication of which template strand (top or bottom) will be more affected by a mutation that causes DNA ligase to be partially functional?