بسم الله الرحمن الرحيم

مادة البيو من أسهل وامتع المواد في هذا العالم

لذلك قررنا ان نجعلها أسهل للجميع

شعارنا :دعونا سويا نفك السوبر كويل

* تعريف بالمذكرة :

- شاملة لجميع المواضيع . (كاملة)
- تركيز على النقاط الهامة .
- جداول تسهل عملية الحفظ .
- أسئلة عامة .

(الحلم أصبح حقيقة)

Team leader : مجهول

Team group :

أبو يسرا

Blue eye

Dr.noop

Ocean

والشكر للجنود المجهولين الذين فضلوا عدم ذكر أسمائهم

D)- RNA Primer Synthesis : (we will mention a lot of details)

- DNA polymerase **can not** initiate synthesis of a complementary strand of DNA on a totally single stranded template Rather, they require an **RNA primer** .

◙ RNA primer :
- a short , double stranded (**Not single**) fragment of (RNA paired to the single stranded DNA template) . (very important)
- has a free –OH group on the 3`-end of the RNA that serves as an acceptor of the first nucleotide by action of DNA polymerase .

◙ This note always come :
* in de novo DNA synthesis , the free 3 – OH is provided by(**short stretch)** of :
a) RNA (T)
b) DNA (F)
the answer is **short strech of RNA .**

1) primase :

- It is a specific RNA polymerase . (its one of the question in exam) .

Q , primase is DNA polymerase . (F) (RNA polymerase) .

- it is DNA dependent (T) – its RNA dependent (F) (very important)
- It synthesizes **short** (not long) (MCQ) fragments of RNA (~ 10 nucleotides), complementary & antiparallel to DNA template.

- RNA primers hybrid with DNA template (U in RNA pairs with A in DNA) (**Hybrid complex**)

* **In the replication fork**

- In the **leading strand**: only one RNA primer is synthesized.
- In the **lagging strand**, many RNA primers are constantly synthesized along the strand .

◙ RNA primase building block :
a) 5 **– rib**onucleoside triphosphate . (NTP) (be careful not deoxyribo)
(ATP , GTP , CTP , UTP) (NO TTP) (MCQ)
b) pyrophosphate is released so the reaction is **irreversible** .

2) Primosome :

- (Primase + Prepriming complex)
- So primosome consist of 4 componenet : (MCQ) :
  a)primase .
  b)DnaA protein .
  c) Single – stranded DNA – binding (SSB) protein .
  d)Helicases .

- This complex initiates Okazaki fragment formation by moving along the template for the lagging strand in **the 5`-3direction**

-**periodically recognizing specific sequences of nucleotides that direct it to create an RNA primer that is synthesized in the 5`-3` direction.**

revision :
a) 3 – end OH is the first acceptor nucleotide by the action of DNA polymerase .
b) okazaki fragment in **the 5`-3direction** .
c) RNA primer is synthesized in the **5`-3` direction.**

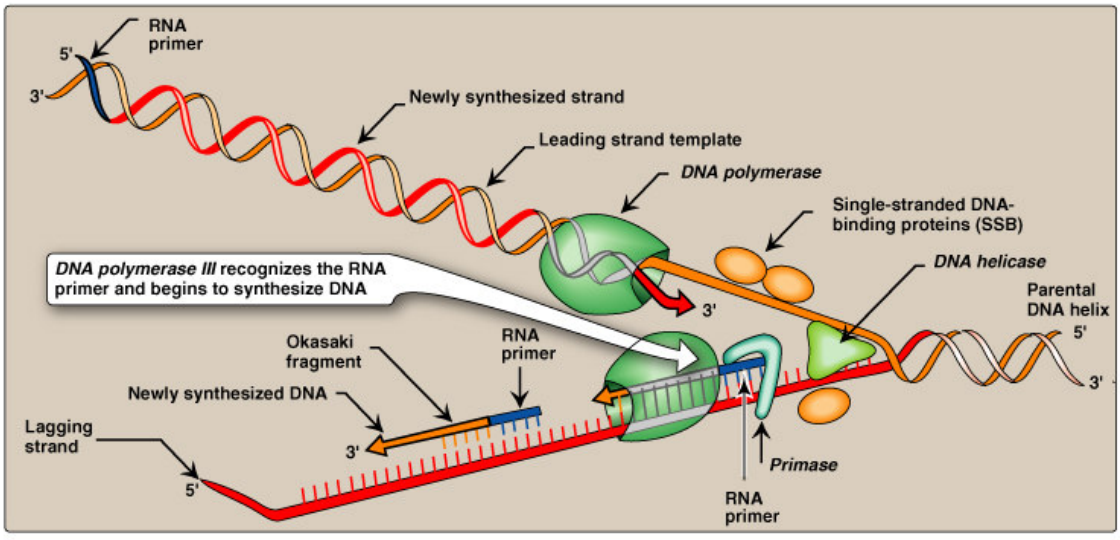

Figure 29.16
Elongation of the leading and lagging strands.

E) Chain elongation :

- DNA polymerase add deoxyribonucleotide one at a time .
_(not like RNA primase which add ribonucleotide) (MCQ)

*** DNA polymerase III :**

- elongates a new DNA strand by adding deoxyribonucleotides *dNTP* (dATP, dGTP, dCTP, TTP)
(NOT UTP) to the 3`-end of the growing chain (one at a time) starting from the RNA primer.

- The *sequence* of the nucleotides that are added is dictated by the base sequence of the template strand.

- The new strand grows in the 5`- 3` direction, antiparallel to the parental strand. (MCQ)

- DNA polymerase **III** is a highly processive enzyme .
{ processive enzyme means : remains bound to the template strand }

- Energy required:
  - Pyrophosphate (Ppi) is released when each new nucleotide is added to the growing chain
  - Further hydrolysis of Ppi to two Pi
  - Accordingly, a total of two high-energy bonds are used to drive the addition of each dNTP

- **Rivision :**

Location of work: Leading & lagging DNA strands starting from the RNA primer(s).
Enzyme : DNA polymerase III
Materials: dNTPs (dATP, dGTP, dCTP, dTTP)
Direction of elongation: 5`-3` direction antiparallel to parental strand
Energy required: two high-energy bonds
example: *dATP => d AMP + Ppi (Pi + Pi)*

2) Proofreading of newly synthesized DNA :

- Misreading of the template sequence could result in mutations. (may be lethal) .

=> very important (question in the exam)

- To ensure replication correctness of sequence, DNA polymerase III has a proofreading activity (3`- 5` exonuclease activity) (MCQ) **(3`to 5` be careful)**

- **Steps** :

1- DNA polymerase III checks for the correctness of matching of added nucleotide to its complementary base on the template.

2- If a wrong nucleotide is added, 3`-5` exonuclease activity edits the mistake

- Why 3`- 5` exonuclease does not degrade correctly paired nucleotide sequence ?

Answer :
Because the improperly base pairing 3- hydroxyl terminus .

Conclusion : **(the excision must be done in the reverse Direction from that of the synthesis)**

F . Excision of RNA primers and their replacement by DNA :

- **On the lagging strand** :
(DNA polymerase III continues to synthesize DNA until it is blocked by an RNA primer.)

◙ At that stage, (these notes I see it in all exams) (MCQ)(important)

1- RNA primer is removed (excised) by DNA polymerase I
(by 5`-3` exonuclease activity) (be careful not endonuclease)
SO (the DNA polymerase I locates the space " nick " between the 3-end
Of the DNA newly synthesized by DNA polymerase III & the 5 – end
Of the adjacent RNA primer .

2- Then, the gap is filled by DNA polymerase I
(by 5`-3` polymerase activity)

3- DNA polymerase I proofreads the filled gap DNA
(by 3`- 5` exonuclease activity)

◙ *The final result :*

* This removal / synthesis / proofreading continues (**one nucleotide at time**) , until The RNA is totally degraded & the gap is filled with DNA .

* Differences Between 5`-3` exonuclease & 3`- 5` exonuclease :

5`-3` exonuclease	3`- 5` exonuclease
Remove one nucleotide (**deoxy or ribo nucleotide**) from **at A time** from a region of DNA that is **properly** base –paired . (MCQ)	Not
Can also remove **altered** nucleotide (**one to ten at a time**) (**important in repairing DNA**)	

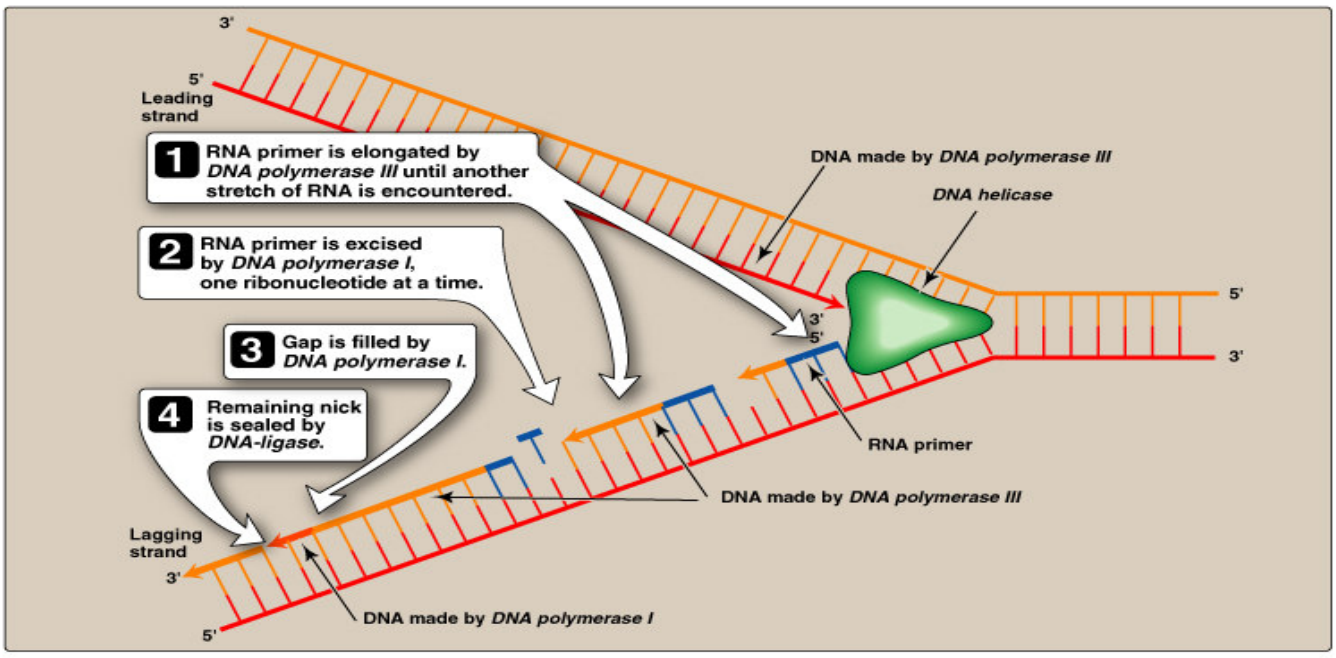

Figure 29.19
Removal of RNA primer and filling of the resulting "gaps" by *DNA polymerase I*.

DNA Polymerase III	DNA Polymerase I
5`-3`polymerase activity synthesis of new DNA strand	5`-3`polymerase activity synthesis (filling) of gap of removed RNA primer Step 2
3`-5` exonuclease activity proof reading of new strand	3`-5` exonuclease activity proof reading of the DNA filling the gap of removed RNA primer(سؤال مؤكد) Step 3
----------	5`-3` exonuclease activity removal (excision) of RNA primers. Step 1
Function Synthesis ---- Proofread	**Function** Removal ----synthesis ----- Proofread

* DNA ligase :

- The final phosphodiester linkage between the 5`-phosphate group on the DNA chain synthesized by DNA polymerase III and the 3`-OH group on the chain made by DNA polymerase I is catalyzed by DNA Ligase.
- This process requires energy derived from the cleavage of **ATP TO AMP + PPi** (MCQ)

* Eukaryotic DNA Replication :

- The process of eukaryotic DNA replication closely follows that of prokaryotic DNA synthesis.

Eukaryotes (MCQ)	prokaryotes
Multiple origins of replication	Single origin of replication
RNA primers are removed by RNase H (MCQ)	RNA primers are removed by DNA polymerase 1
Single – stranded DNA binding protein & ATP dependent DNA helicase	Single – stranded DNA binding protein & ATP dependent DNA helicase (so it's the same)

* Eukaryotic Cell Cycle :

- Cell cycle: the events surrounded DNA replication and cell division (mitosis)
- G1 phase (Gap1):the period before replication
- S (synthesis) phase: DNA replication occur (MCQ)
- G2 phase (Gap2):the period after replication and before mitosis
- M phase (mitosis):cell division occur
- G0 phase :

- cell that reactive and enter the cycle (through Gap1)

-cell ceased division exit the cycle (through Gap1) e.g. mature neurons

- (some cells leave the G0 phase and reenter G1pase to resume division)

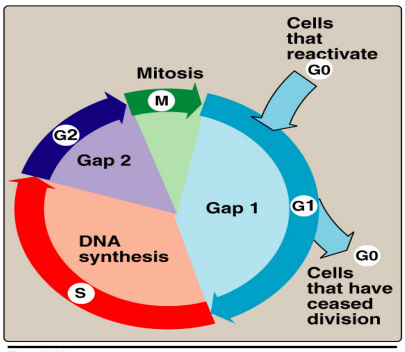

Figure 29.21
The eukaryotic cell cycle.

*Eukaryotic DNA polymerase: (MCQ)

- At least 5 classes have been identified. Categorize on the basis of MW, cellular location, sensitivity to inhibitors, and templates or substrates on which they act.
- They are designated by Greek letters (α alpha, β beta, δ delta, γ gamma, ε epsilon)
- **(Note that all polymerase have specific function + proofreading except α and β)** (it very important always come in exams)

- *** Pol α :** (multisubunit enzyme) (MCQ)

- one subunit has(1) **primase activity:**

which **initiate** (only) synthesis on leading strand (one primer) and on lagging stands (beginning of each Okazaki fragment)

- (2) polymerase activity:

Which extend RNA primer by add short (not long) DNA pecis from 5` _ 3`

- **pol δ** : (MCQ)

-complete **(NOT initiate)** DNA synthesis on leading strand and elongate each Ocasaki fragment

-proofread using 3`_ 5` exonuclease

***pol β and pol ε**

- For repair .

-Polε also to proofread

POLYM-ERASE	FUNCTION	PROOF-READING
Pol α	• Contains primase • Initiates DNA synthesis	—
Pol β	• Repair	—
Pol γ	• Replicates mitochondrial DNA	+
Pol δ	• Elongates leading strands and Okazai fragments	+
Pol ε	• Repair	+

Figure 29.22
Activities of eukaryotic DNA polymerases (pols).

***pol γ**

Mitochondria DNA(which is circular)

-circular DNA occur in :

Mitochondria, plants, and prokaryotic

Reverse Transcriptase : (MCQ لا يخلو منه اختبار)

- A retrovirus as HIV, carries the genome in the form of **single-stranded RNA** molecule. (this sentence you I see it in all exams)

- Following infection of a host cell, the viral enzyme, **reverse transcriptase**, **uses the RNA as a template for the synthesis of viral DNA**

- Then, DNA becomes integrated into host chromosomes.

- Reverse transcriptase moves along the RNA template in the *3`-5`* direction,
- synthesizing new DNA in the *5`-3`* direction (MCQ)

No proofreading in reverse transcriptase high mutation rate of such viruses (MCQ)

- As an attempt to prevent HIV infection from progressing to AIDS, patients are treated by inhibitors of reverse transcriptase (nucleosides or non-nucleosides inhib.), in addition to protease inhibitors (which targets another HIV maturation enzyme) , thus produce a mixture " cocktail " that requires the virus to develop multiple resistance in order to continue to replicate .

دعائكم هذا ما نطلب

* Inhibitors of DNA synthesis by nucleoside analogs :

* DNA chain growth can be blocked by the incorporation of certain nucleoside analogs that have been modified in the **sugar portion** (MCQ) of the Nucleoside (as the 3`- end is not present for DNA chain elongation)

NUCLEOSIDE ANALOGS

1- Removal of (OH) from 3`-carbon of deoxyribose

{ 2`, 3` dideoxyinosine (didanosine) }

2- Conversion of deoxyribose to another sugar (arabinose)

*** slow the division of rapidly growing cell & viruses**
Cytosine arabinoside (cytarabine, araC) :cancer therapy
Adenine arabinoside (vidarabine, araA) : antiviral agent

3- Chemically modification of the sugar moiety

Zidovudine (AZT)

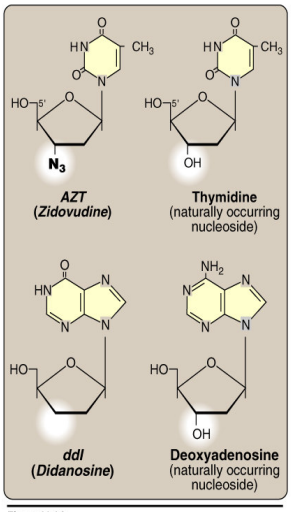

Figure 29.24
Examples of nucleoside analogs that lack a 3'-hydroxyl group.

- **These sugars are generally supplied as nucleosides which are then to nucleotides by cellular salvage enzyme converted (MCQ)**
- **These nucleotides blocks DNA replication by preventing further chain elongation**
- **So, these compounds slow the division of rapidly growing cells (cancer) and viruses.**

* Organization of the eukaryotic DNA :

- Typical human DNA contains 46 chromosomes
- Total DNA is ~ one meter long

- It is important to effectively packaging such large amount of genetic material into a volume of the size of a cell nucleus for effective replication & gene expression.

- To fulfill so, DNA interacts with large number of proteins
  - Eukaryotic DNA is associated with basic proteins called **histones.**
  - These serve to order the DNA into **basic structural** units called **nucleossomes** (resemble beads on a string)

- Nucleosomes are further arranged into more complex structures that organize and condense the long DNA molecules into chromosomes that can be segregated during cell division .

* Histones :

- 5 classes of histones
  (H1, H2A, H2B, H3 & H4)

- Histones are positively charged at physiologic pH (high contents of lysine & argenine)

- They can form ionic bonds(MCQ) هام with negatively charged DNA

(in addition with positively charged ion such as Mg2+ can neutralize –ve charged DNA phosphate groups)

1- Formation of the nucleosome :

- Two molecules each of H2A, H2B, H3 and H4 form the structural core of the **individual nucleosome** (bead).(MCQ)

- Around this core, a segment of DNA double helix is wound twice (MCQ) forming a **negatively** (MCQ) supertwisted helix.

- Neighboring nucleosomes are joined by **linker DNA** ~ 50 base pairs long.
- **H1** binds to the linker DNA chain between the nucleosome beads.

H1 is the most tissue-specific and species-specific of the histone , it facilitate the packing of nucleosomes into the more compact structure . (MCQ) (always come in exams)

-- (The N-terminal ends of these histone can be acetylated , methylated , or phosphorylated . these reversible (MCQ) modification can influence :

a) how tightly the histone bind to DNA .
b) expression of specific genes .

2- Higher levels of organization :

- **Nucleosomes** can be packed more tightly to form **polyuncleosome** (**nucleofilament** or 30-nm fiber)

- The fiber is organized into loops that are anchored a nuclear **scafold proteins**.

- Additional levels of organization lead to the final chromosomal structure.

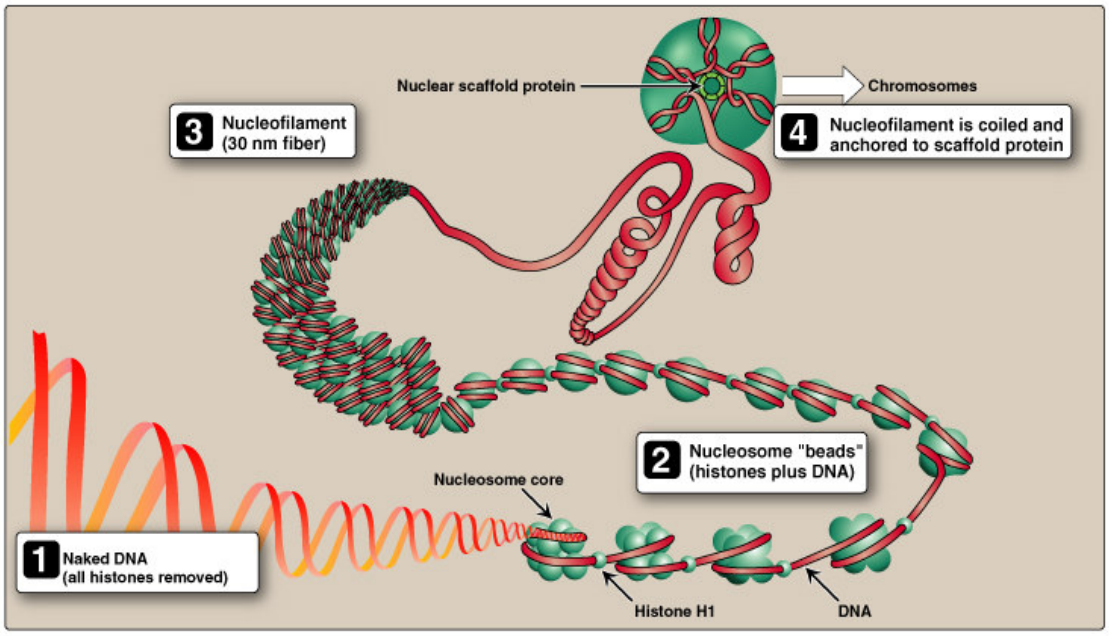

Figure 29.26
Structural organization of eukaryotic DNA.

B – Fate of nucleosomes during DNA replication :

- **highly structure chromatin must be relaxed .**

- nucleosome displaced ,dissociation of the nucleosome core from the DNA is **incomplete** . (MCQ)

- **All parental histones accociated with (only one) of the parental DNA strand . (remember only one) (very important)**
- Synthesis of new histones occurs **simultaneously** with DNA replication
  And nucleosome containing the newly synthesized histones associate with
  Only one of the new daughter helices .
- **The parental histones octamer are conserved (NOT SEMICONSEVE) (MCQ)**

أرجو أن نكون قد فككنا السوبر كويل

Team leader : مجهول

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والشكر للجنود المجهولين الذين فضلو عدم ذكر أسمائهم