Genetics

Subject : Replication initiation in eukaryotes

Lec no : 4

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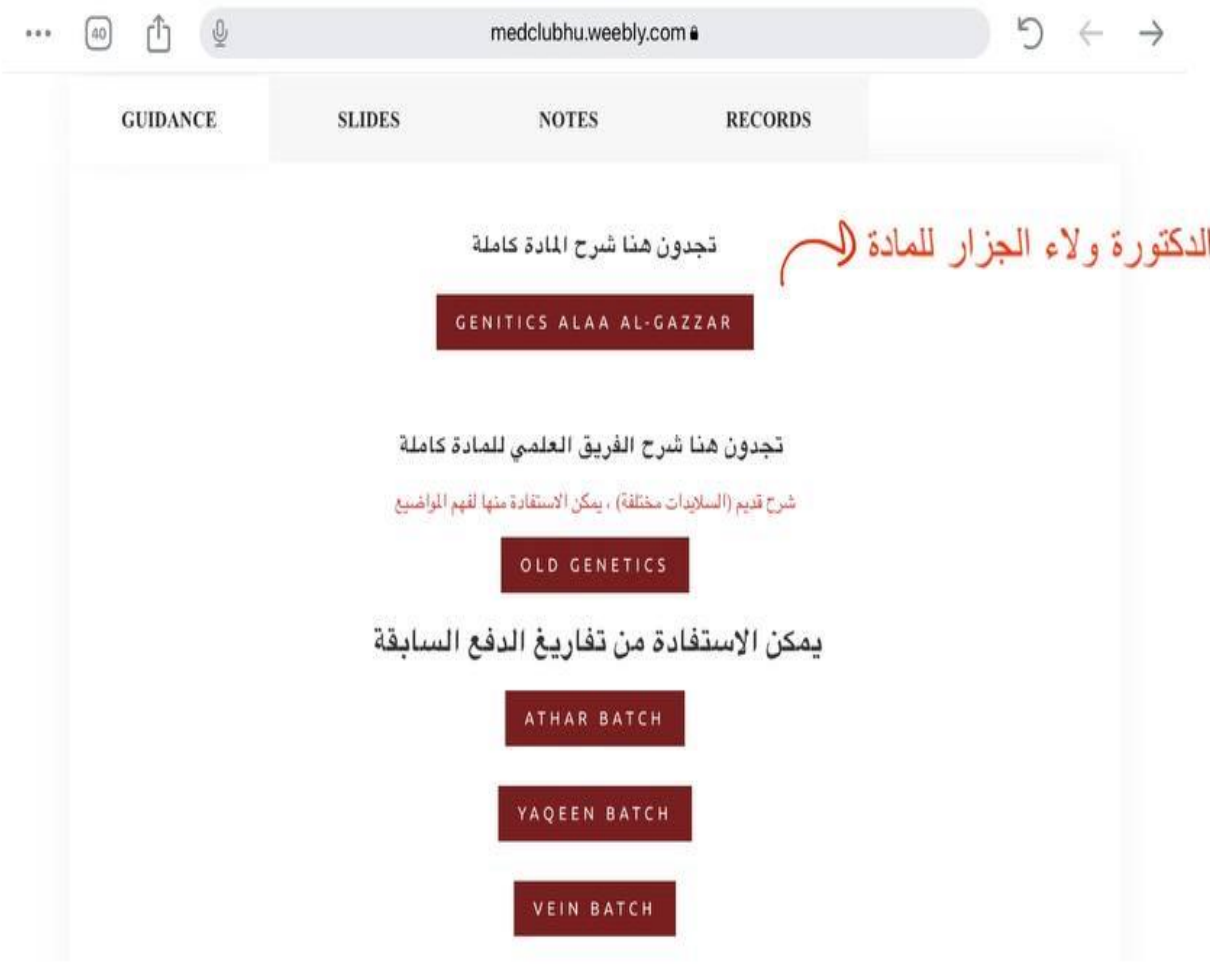
وقل رب زدني علماً

تجدون في guidance مادة الجينتكس على موقع النادي :

للوصول الى guidance الجينتكس و تفاريغ المادة كاملة :



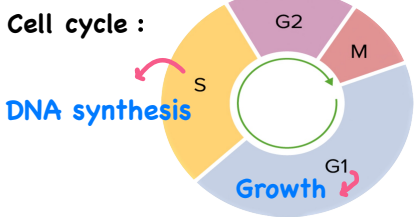
شرح الدكتورة ولاء الجزار للمادة

كل اعمال الفريق العلمي تنشر على قناة التيليغرام

يعطيكم العافية ، بدي احكي كم شغلة عن المحاضرة

- بدايةً المحاضرة فيها معلومات كتيرة بس مش صعبة اهم شي تفهموها منيح
- مهم تميزو بينها و بين المحاضرة الي قبل (Replication initiation in prokaryotes)
- بالنسبة للاختصارات الموجودة ، الدكتورة حكت احفظو الاختصار بدون الاسم كامل (و انا جمعتهم كلهم)
- رح اغيّر شوي بترتيب السلايدات عشان توصل المعلومة بشكل افضل

موفقين ❤️



* ال s-phase تسبقها مرحلة ال G1 و بعدها بيجي مرحلتين G2 , M

بتبلش عملية ال replication هون

- In G_1 phase of the cell cycle, many of the DNA replication regulatory processes are initiated.

بعمل recognize للمكان الي بدنا نعمله replication

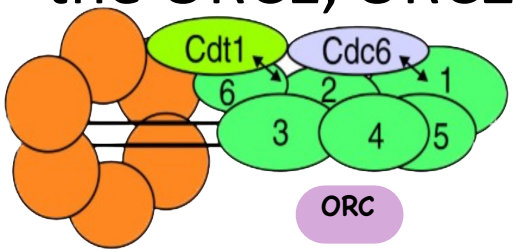
بما انه complex يعني هو multisubunit proteins

- Initiation of DNA replication in eukaryotes begins with the binding of the **origin recognition complex (ORC)** to origins of replication during the G_1 phase of the cell cycle.

* في ال eukaryotic cells عنا multiple origin of replication

بتتذكرو كنا نحكي عنه OriC بال prokaryotes

- **Origin recognition complex (ORC)** is a multi-subunit DNA binding complex (6 subunits) that binds in all eukaryotes in an ATP-dependent manner to origins of replication.
- The subunits of this complex are encoded by the ORC1, ORC2, ORC3, ORC4, ORC5 and ORC6 genes.



* ال ORC بتصير active في ال G1 phase

* ال ORC بتشكل platform عشان نعمل مركب معقد اكتر الي هو (pre-RCs)

- The ORC complex then serves as a platform for forming much more complicated **pre-replicative complexes (pre-RCs).**
- The pre-RC formation involves the ordered assembly of many replication factors including:

طيب من شو بتكون هاد ال pre-RCs :

✓ **the origin recognition complex (ORC),**

* هلأ لما تشوفو الصورة بتوضح الامور اكتر

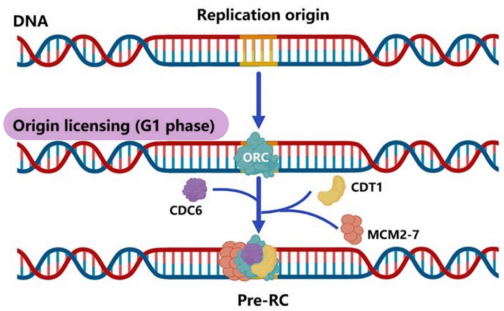
شابك ✓ **Cdc6 protein** (**cell division cycle 6**), معه ATP

✓ **Cdt1 protein** (**Chromatin licensing and DNA replication factor 1**), and

هاد كتير مهم ✓ **minichromosome maintenance proteins (Mcm2-7)** (**heterohexamer of the six MCM proteins (MCM2-7)**.

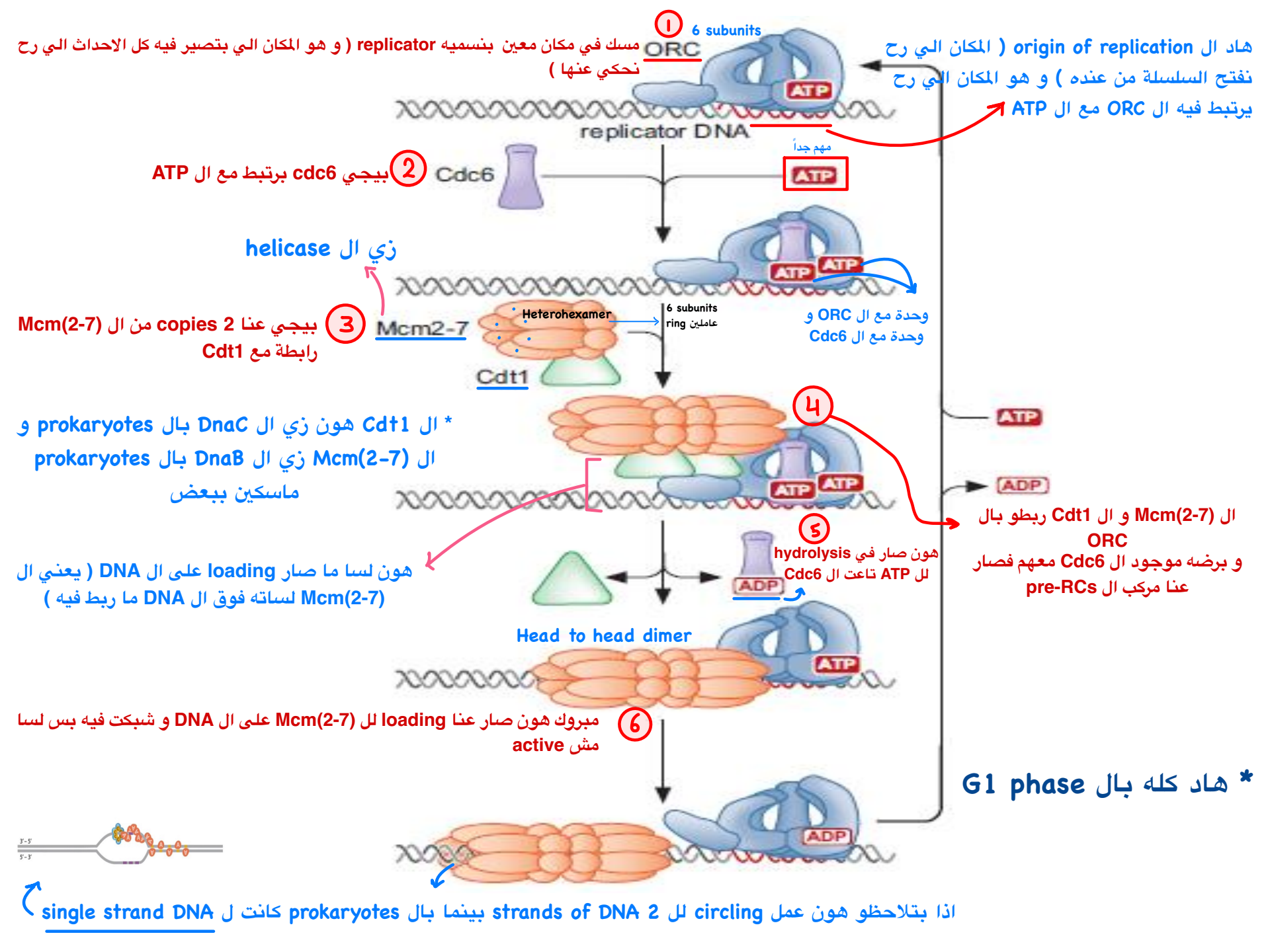
مكون من 6 polypeptide chains مختلفين

- Pre-RC assembly during G1 is required for **replication licensing** of chromosomes prior to DNA synthesis during S phase.

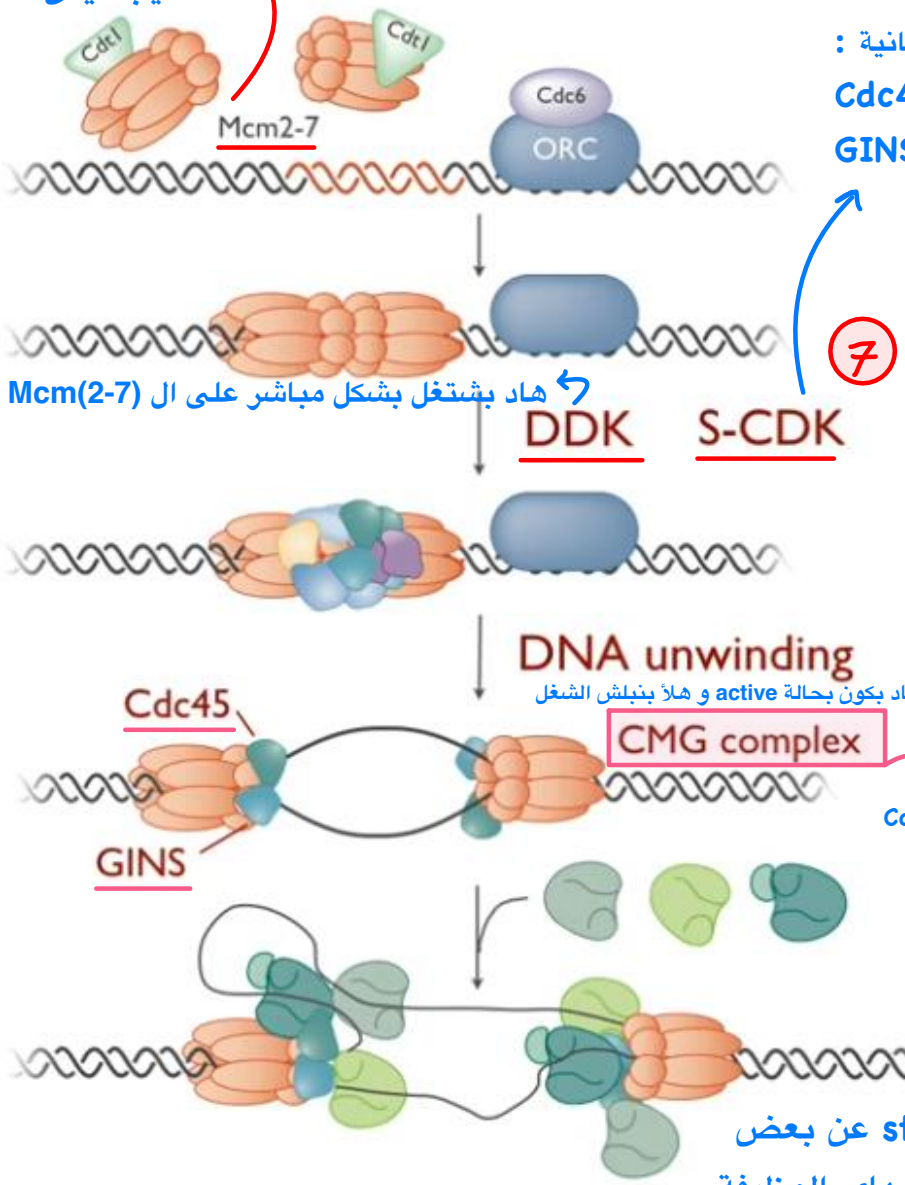


(2-7) : 2,3,4,5,6,7 عددهم ٦

Pre-replicative complex (pre-RC) :
(ORC + Cdc6 + Cdt1 + Mcm2-7)


1
6 subunits
ORC
مسك في مكان معين بنسميه replicator (و هو المكان الي بتصير فيه كل الاحداث الي رح نحكي عنها)
هاد ال origin of replication (المكان الي رح نفتح السلسلة من عنده) و هو المكان الي رح يرتبط فيه ال ORC مع ال ATP
ATP
replicator DNA
مهم جداً
2
بيجي cdc6 برتبط مع ال ATP
Cdc6
زي ال helicase
3
بيجي عنا 2 copies من ال Mcm(2-7) رابطة مع Cdt1
Mcm2-7
Heterohexamer
6 subunits
ring عاملين
Cdt1
وحدة مع ال ORC و وحدة مع ال Cdc6
4
* ال Cdt1 هون زي ال DnaC بال prokaryotes و ال Mcm(2-7) زي ال DnaB بال prokaryotes ماسكين ببعض
ADP
ال Mcm(2-7) و ال Cdt1 ربطو بال ORC و برضه موجود ال Cdc6 معهم فصار عنا مركب ال pre-RCs
5
هون لسا ما صار loading على ال DNA (يعني ال Mcm(2-7) لساته فوق ال DNA ما ربط فيه)
هون صار في hydrolysis لل ATP تاعت ال Cdc6
Head to head dimer
6
مبروك هون صار عنا loading لل Mcm(2-7) على ال DNA و شبكت فيه بس لسا مش active
* هاد كله بال G1 phase
اذا بتلاحظو هون عمل circling لل strands of DNA 2 بينما بال prokaryotes كانت ل single strand DNA

Events of Eukaryotic DNA Replication Initiation



لما يروح عال S-phase بصير active

طيب ليش ؟ لانه هناك في 2 kinases بخلوه active

هاد بشتغل على بروتينات تانية :

الاول Cdc45

و التاني GINS

helicase loading

هاد بشتغل بشكل مباشر على ال Mcm(2-7)

هدول الانزيمين بعملو activation عن طريق ال phosphorylation (من اسمهم kinases)

هاد بكون بحالة active و هلأ بنبلش الشغل

helicase activation

C M G

Cdc45 Mcm(2-7) GINS

replisome assembly

CMG complex الله وظيفتين :

١. helicase ← فصل ال 2 strands عن بعض

٢. ATPase ← عشان نقدر نقوم بهاي الوظيفة

* هون كله شرح زي الي عالرسمة

- ORC, Cdc6, and Cdt1 are all required to load the six protein minichromosome maintenance (Mcm 2-7) complex onto the DNA. (It is thought that the Cdc6p-Cdt1 complex uses ATP hydrolysis to thread DNA through the central hole of the MCM doughnut).

- Pre-RCs formed during the G_1 phase are converted to the **initiation complex** during cell cycle transition from G_1 to S by the action of two kinases: **cyclin-dependent kinase (CDK)** and **Dbf4-dependent kinase (DDK)**. i.e. Once the pre-RC is formed, activation of the complex is triggered by two kinases, cyclin-dependent kinase (CDK) and Dbf4-dependent kinase (DDK) that help transition the pre-RC to the initiation complex prior to the initiation of DNA replication.

- Formation of an initiation complex, which includes helicase activity, unwinds the DNA double helix at the origin site.

Eukaryotic helicase loading

- Loading of the eukaryotic replicative DNA helicase is an ordered process that is initiated by the association of the **ATP-bound origin recognition complex (ORC)** with the replicator.(The initiation of DNA replication is directed by specific DNA sequences called replicators).
- Once bound to the replicator, ORC recruits **ATP-bound Cdc6** and **two copies of the Mcm2-7 helicase** bound to a second **helicase loading protein, Cdt1**.
- This assembly of proteins triggers ATP hydrolysis by Cdc6, resulting in the loading of a head-to-head dimer of the Mcm2-7 complex encircling double-stranded origin DNA and the release of Cdc6 and Cdt1 from the origin.

- Eukaryotic helicase loading does not lead to the immediate unwinding of origin DNA. Instead, helicases that are loaded during G1 are **only** activated to unwind DNA and initiate replication **after cells pass from the G1 to the S phase** of the cell cycle. حكينا لما يروح عال s-phase بصير active
- Loaded helicases are activated by two protein kinases: CDK (cyclin dependent kinase) and DDK (Dbf4-dependent kinase). These kinases are activated when cells enter S phase. Once activated, DDK targets the loaded helicase, and CDK targets two other replication proteins. Phosphorylation of these proteins results in the **Cdc45 and GINS proteins** binding to the Mcm2-7 helicase.

- Importantly, Cdc45 and GINS strongly stimulate the Mcm2-7 ATPase and helicase activities and together form the **Cdc45–Mcm2-7–GINS (CMG) complex**, **which is the active form of the Mcm2-7DNA helicase.**

الاختصارات :

(ORC): Origin recognition complex

(Pre-RC):pre-replication complex

(Cdc6):cell division cycle 6

(Cdt1):chromatin licensing and DNA replication factor 1

(Mcm2-7):minichromosome maintenance

(CDK):cyclin-dependent kinase

(DDK):Dbf4-dependent kinase

• A-Separation of the two DNA strands:

بالمحاضرة الماضية حكينا عن ال steps of DNA replication in prokaryotes و كانت اول نقطة :
و هلأ رح نكمل النقطة التانية

B-Synthesis of the two DNA strands:

* طبعًا ما رح نحكي كيف بصير بال eukaryotes لانه معقد

new 2 strands ببلش يبني

- **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.**

ال DNA polymerase III عنده مشكلتين :
١. ما بمشي غير باتجاه 5' ل 3' (رح نعرف ليش لقدام)
٢. ما بعرف يبلش بناء لوحده لازم نحطله primer

بيجي ال DNA Polymerase III بربط بال primer و

* بال parent strand لازم طرفه يكون 3'
عشان لما امشي يكون باتجاه 5' ل 3' لانه بنبني بعكس الاتجاه

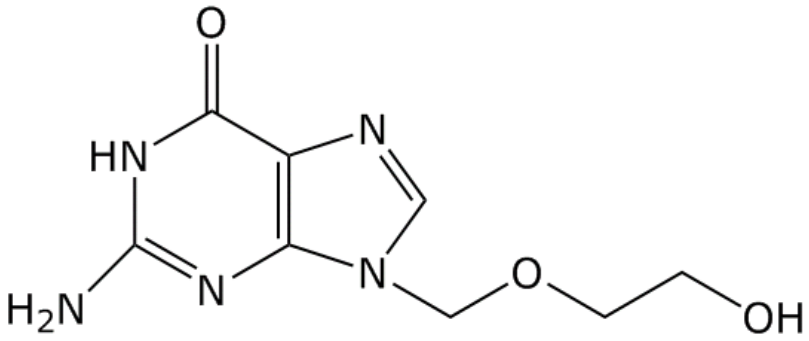
Synthesis of RNA primers:

ال RNA primase هي الي بتصنعلنا primer عشان ال DNA polymerase III يبلش شغله من عنده

- 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**).

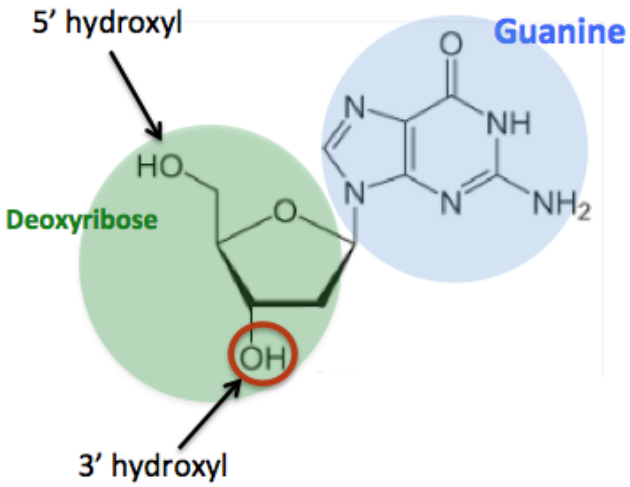
* هاد سؤال اعطتنا اياه الدكتورة
فكرته معتمدة انه تكونو عارفين شكل ال Guanine و كيف ال Nucleotides برتبطو مع بعض

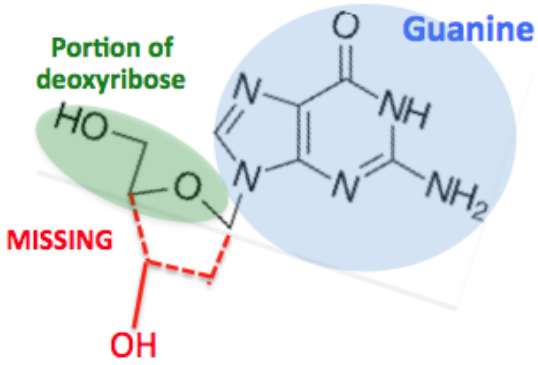
The antiviral drug Acyclovir (structure pictured below) is used to treat infections caused by double-stranded DNA viruses such as herpes simplex virus. Acyclovir acts at the level of DNA synthesis.



A. Acyclovir functions as the analog of what deoxynucleoside?

B. Acyclovir cannot be incorporated into the DNA unless it is modified by a virally encoded kinase. Explain why the activity of a kinase is required for Acyclovir to be incorporated during DNA synthesis.

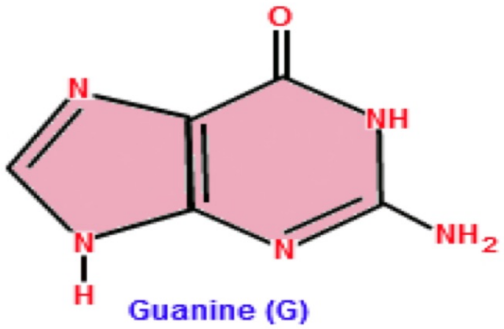




Natural substrate

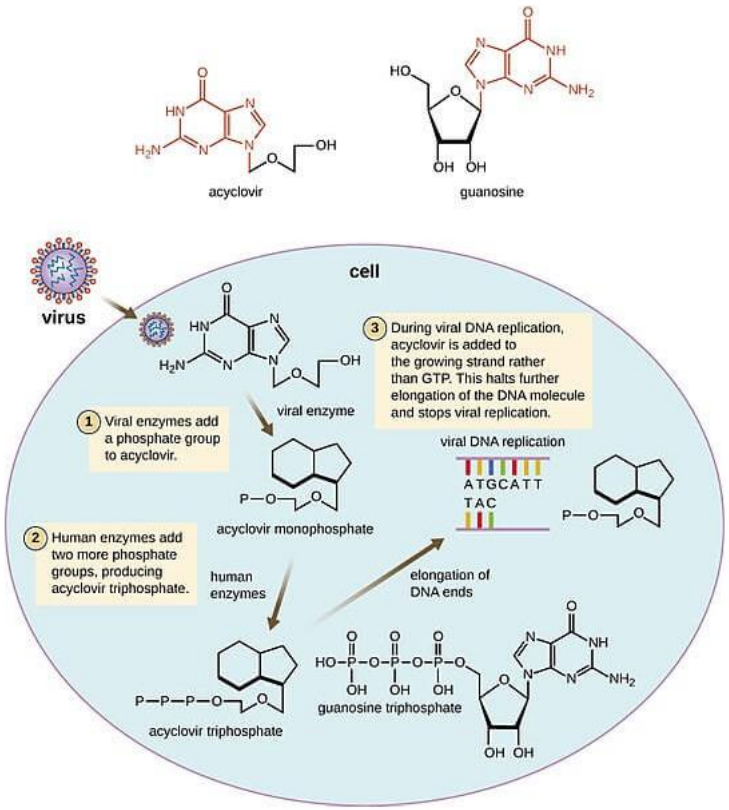
Acyclovir

- A. Deoxyguanosine.



Guanine (G)

- B. Without the triphosphate group, Acyclovir cannot incorporate (نربط / ندمج) into a growing strand of DNA. Kinases phosphorylate their substrate. The kinase adds the phosphate groups that Acyclovir is missing.



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