



# ***Genetics***

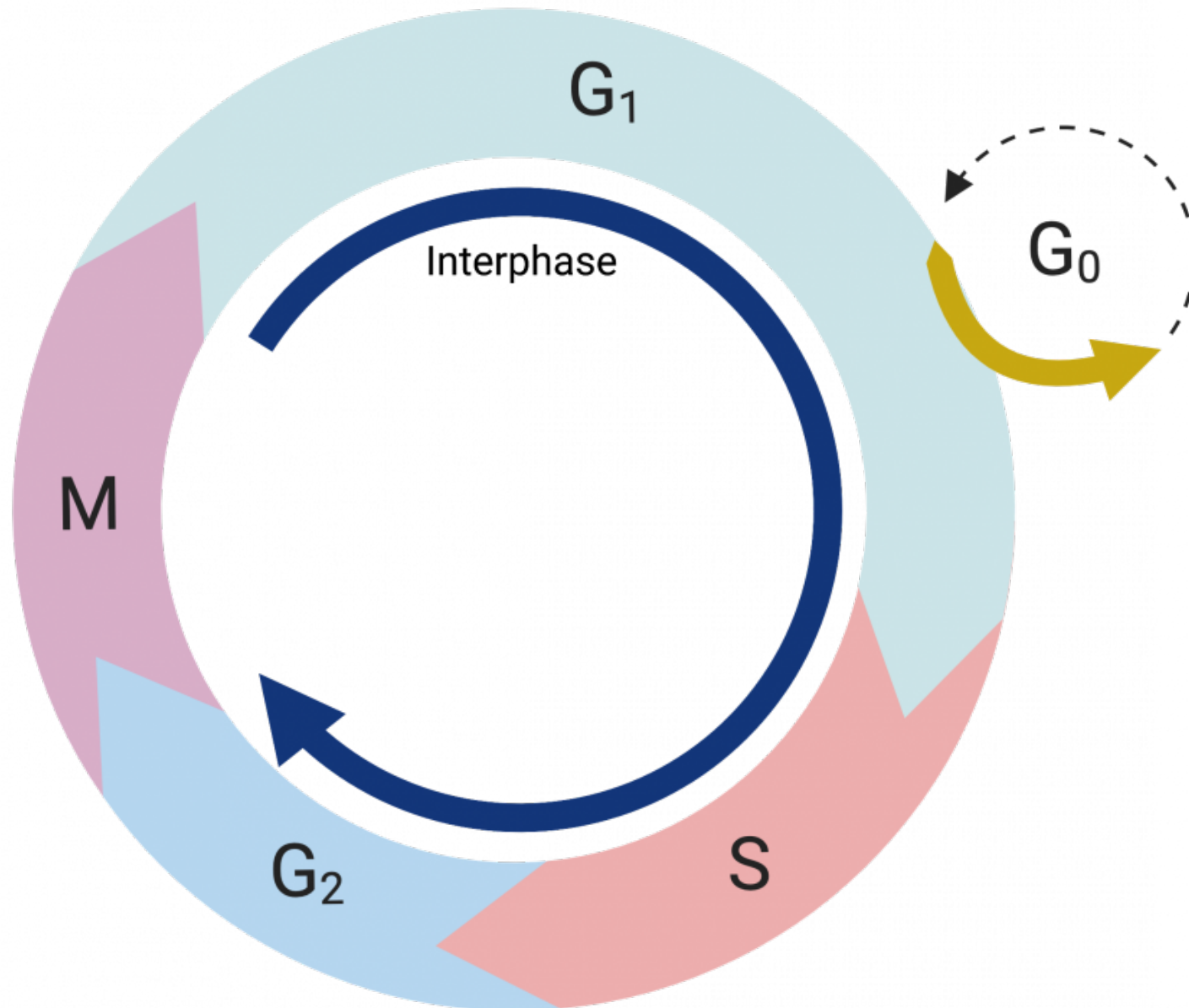
***Subject : Genetics***

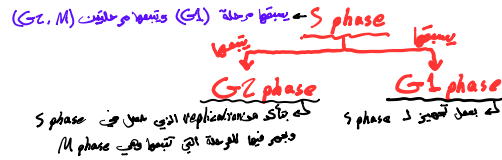
***Lec no : 5 (part 1)***

***Done By : Mahmoud Al Qusaíri***

وَقُلْ رَبِّ زِدْنِي عِلْمًا

# The Cell Cycle





initiation of replication in prokaryotes الحاضرة للحاضبة  
 \* عن هذا الجزء من الحاضرة متشابهة في Eukaryotes  
 initiation of replication في مرحلة (S phase)  
 ويستمره المرحلة (G<sub>1</sub> phase) ← وهي مرحلة تحضيرية لـ replication الذي يحدث في (S phase)

- In G<sub>1</sub> phase of the cell cycle, many of the DNA replication regulatory processes are initiated.

initiation of replication للحمض النووي الذي يبدأ في عملية  
 Origin recognition complex ← هو بروتين يتكون من 6 وحدات من 6 subunits  
 multisubunit proteins ← يتكون من 6 وحدات من 6 subunits ويحتوي على بعض الوحدات (ORC)  
 (ORC1, ORC2, ORC3, ORC4, ORC5, ORC6) 6 genes  
 كما يتكونها very active في G<sub>1</sub> مثلان يعملان كـ subunits

- Initiation of DNA replication in eukaryotes begins with the binding of the **origin recognition complex (ORC)** to origins of replication during the G<sub>1</sub> phase of the cell cycle.
- 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.

- The ORC complex then serves as a platform for forming much more complicated **pre-replicative complexes (pre-RCs)**.

عند التعرف على ORC على origin of replication (نقطة البدء) - يمتد 5 platform to other proteins وقت في ordered assembly (تتابع معيّن) ← يكون مكوّن من Cdc 6 (47P) (2 copies) ← يكون مكوّن من Cdt 1 (Mcm2-7) ← يكون مكوّن من Mcm2-7 (hetero hexamer) مكوّن من 6 subunits (Mcm 2,3,4,5,6,7) مع ATP

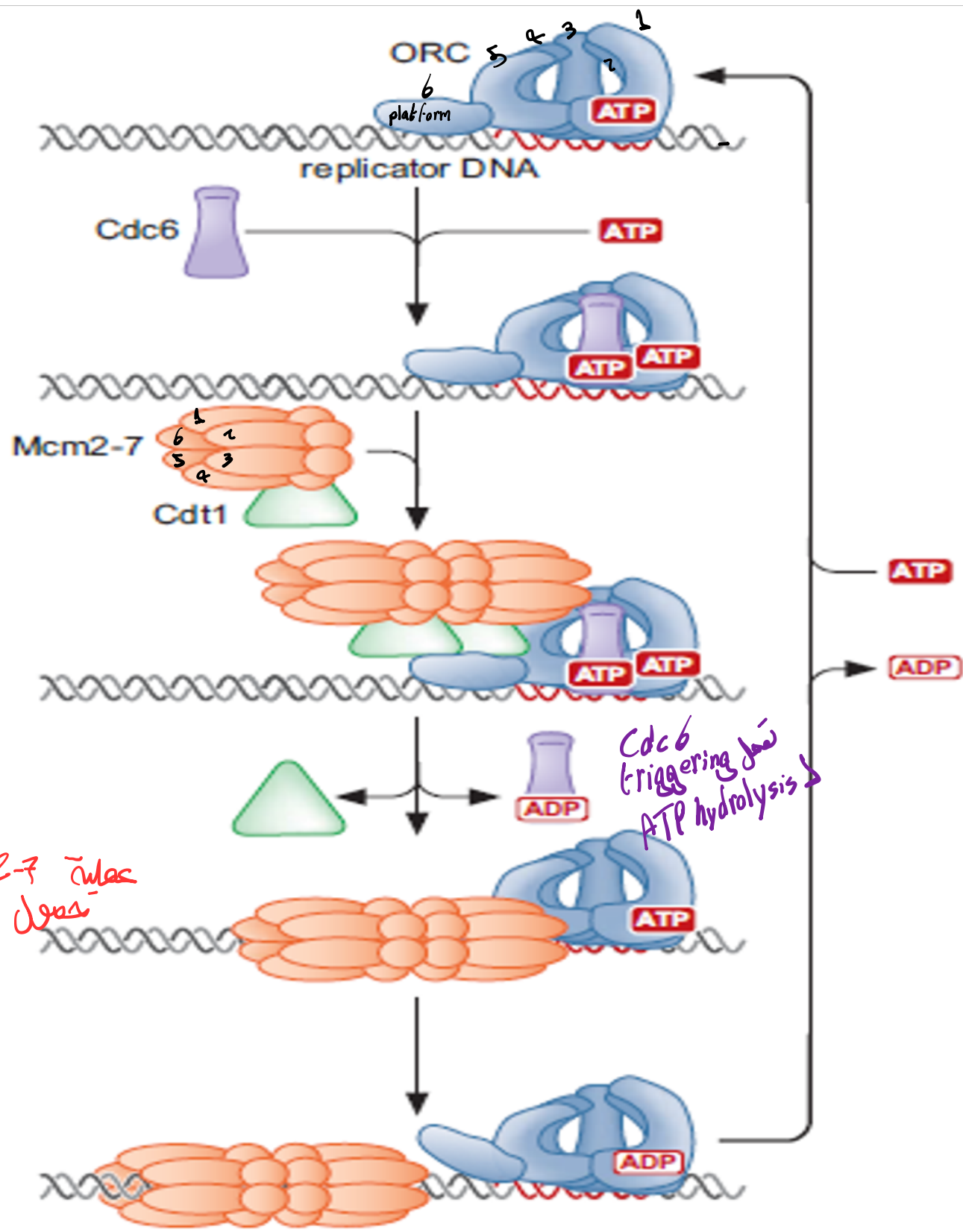
- The pre-RC formation involves the ordered assembly of many replication factors including: \* التّمام غير مطلوبة (فقط الاختصاصات)

- ✓ **the origin recognition complex (ORC)**,
- ✓ **Cdc6 protein (cell division cycle 6)**,
- ✓ **Cdt1 protein (Chromatin licensing and DNA replication factor 1)**, and
- ✓ **minichromosome maintenance proteins (Mcm2-7) (heterohexamer of the six MCM proteins (MCM2-7))**.
- Pre-RC assembly during G1 is required for **replication licensing** of chromosomes prior to DNA synthesis during S phase.

# Eukaryotic helicase loading

replicator مكان د ORC  
مكان على DNA  
replicator: المنطقة كلها التي يحدث فيها opening  
physical site: origin of replication  
يعني من على proteins لـ د يكون او origin of replication  
يعني وي لـ هو fraction من replicator

- ❖ 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.



double strands of DNA molecule

Loading of Msm 2-7  
hydrolysis of ATP

Cdc 6 triggering ATP hydrolysis

متى 2 copies of MCM و ينفخوا في DNA double strands ويدخلوا فيها ويسكروا عليها؟ عند حدوث hydrolysis ATP في الوجود في Cdc6 فيحدث loading لـ 2 copies MCM 2-7 (head to head) ثم Cdc6 و Cdt1. يروحوا بعد ما أنفخوا وظيفتهم في S phase تكون active و يبدأ عمل في phase S

double strands → encircling على MCM 2-7 \* في prokariotes

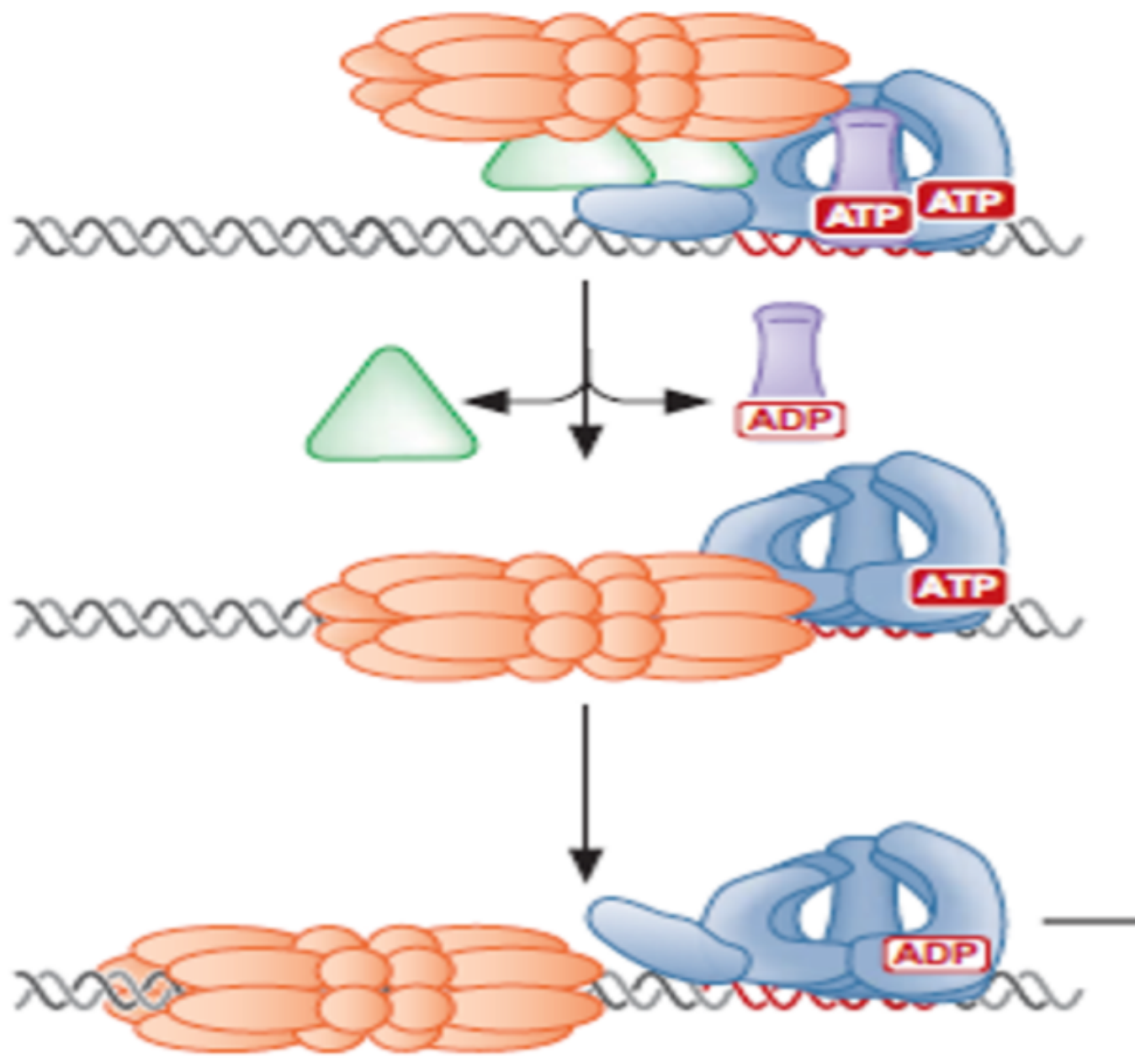
دائري helicase dnaC مع بروتين من كماله  
 دائري helicase loader  
 دائري وثنائي encircling  
 دائري وثنائي single strand

• 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).

الذات في S phase الهدف هو جعل Msm 2-7 active (عشان يفضل الـ 2 strands حتى تبدأ عملية replication) مع DDK و مع Msm 2-7 و مع phosphorylation و مع Cdk و مع phosphorylation لبروتينات اخرى كتحسين نوعيت اخف من البروتينات Cdc45 GINS مسم 2-7 ما يكونوا ما كين بـ

• Pre-RCs formed during the G<sub>1</sub> phase are converted to the **initiation complex** during cell cycle transition from G<sub>1</sub> 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

replicator مكان ب ORC  
مكان على DNA  
replicator : المنطقة التي يحدث فيها GL  
physical site : origin of replication الذي يحدث فيه بداية opening  
origin of replication - يعني مكان يكونوا يعنيه  
proteins ما في ده يكونوا  
origin of replication هو fraction من replicator  
يعني وينه

- ❖ 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.

صوبلور فوق

- 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.
- 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.

activation of  
Mcm2-7  
عن طريق  
phosphorylation

activation  
of Cdc45  
and GINS  
proteins  
عن طريق

- 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 Mcm2-7 DNA helicase.**

Cdc45 Mcm2-7 GINS protein

↔ strands دو سب سے

ATPase activity helicase activity

hydrogen bonds  
Nitrogen basis

## B-Synthesis of the two DNA strands:

enzyme دمج يبدأ عملية البناء ← A يحدد اتجاهها  
T  
C " " G

- **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.

لما يعرف يبدأ بناء لوحده  
يحتاج primer وهو يملك على  
(لازم يشوف في nucleotide فيه free OH  
لما يوصل phosphodiester bond بيننا وبينه another nucleotide)

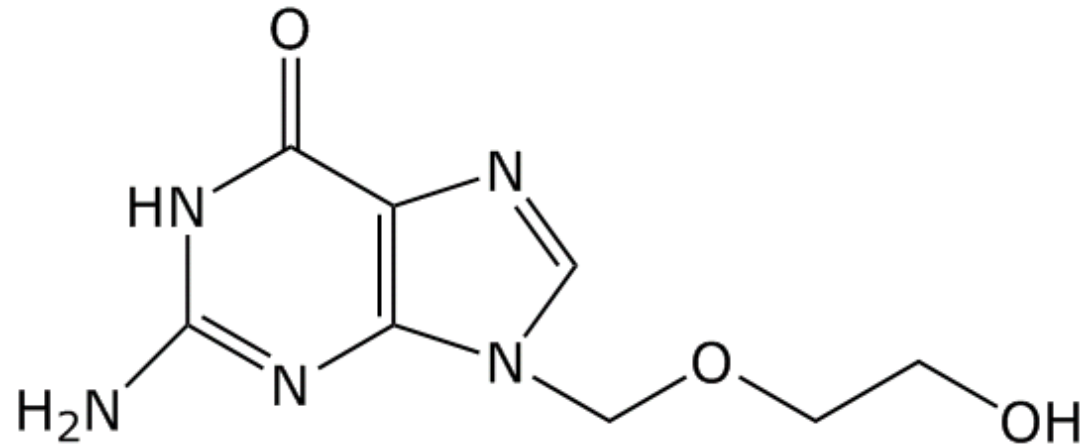
primase ← هو الانزيم الذي يقوم ببناء RNA primer. يحتاجه DNA polymerase III  
(RNA polymerase)

## ■ Synthesis of RNA primers:

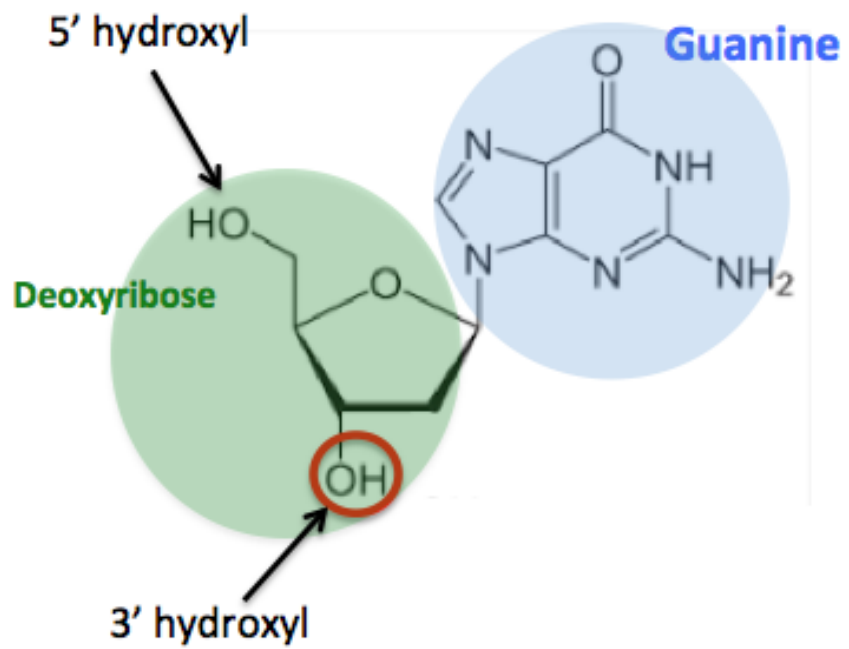
- Primers are short RNA molecules about 5-10 nucleotides in length and are complementary to a segment of the DNA strand. Primers are synthesized in the direction of 5'→3' direction by primase (RNA polymerase) enzyme using ribonucleotide triphosphate (ATP, GTP, CTP, UTP).



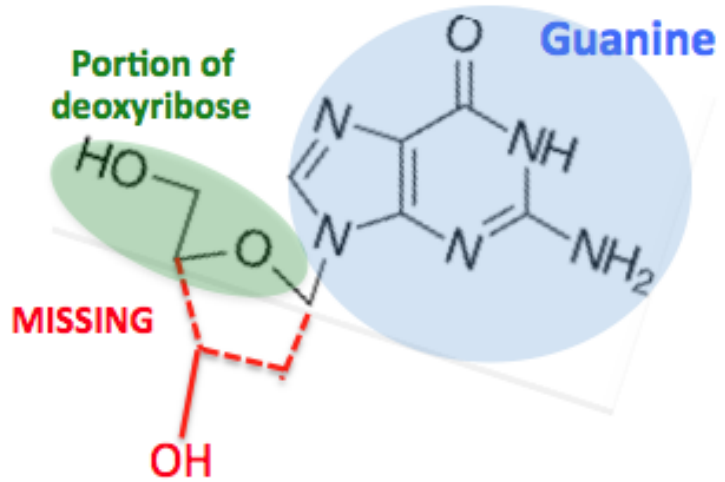
The antiviral drug Acyclovir (structure pictured below) is used to treat infections caused by double-stranded DNA viruses such as herpes simplex virus. Acyclovir acts at the level of DNA synthesis.



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

