DNA replication in Eukaryotes.

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DNA replication is the most fundamental action.

It is a biological process and occurs in all living beings.

It is basis of biological heritance.

Process : producing two identical replicas from one original DNA strand.

The process takes place in semi-conservative method.

DNA replication like all other biological process proceed in 3 co-ordinated process:

1. Initiation

2. Elongation

3. Termination

Initiation :

DNA replication initiate from specific sequences Origin of replication (ORI) called Replisoms.

Eukaryotic cells have multiple replication sites.

To initiate replication process, multiple replicative proteins must assemble on these replication sites.

It leads to formation of Pre-replication complex (pre-RC).

Pre-replication complex has steps :

First - association of Origin recognizing complex (ORC) with replication origin.

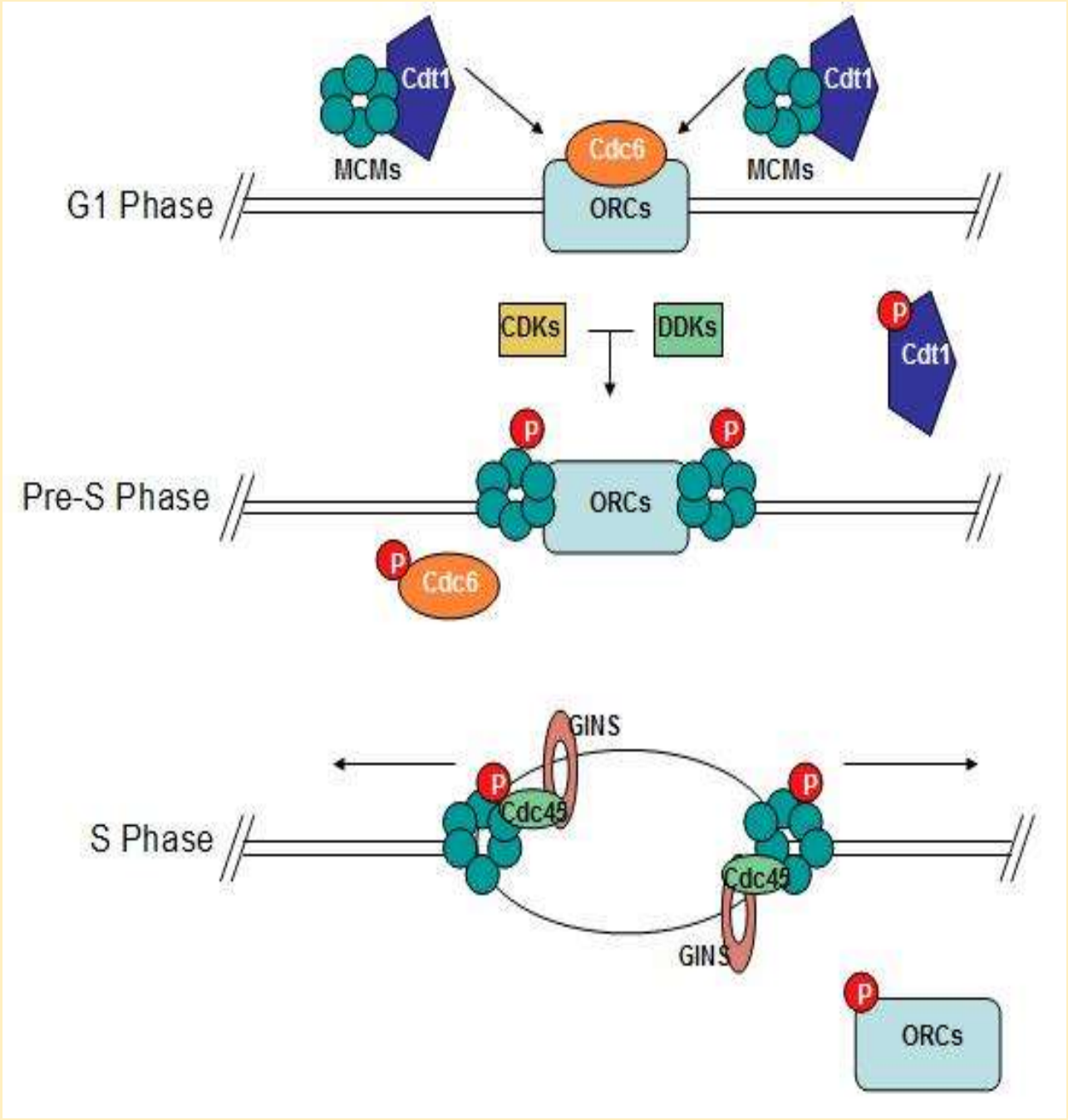
Second - binding of Cdc6 protein to ORC

Third - binding of Cdt1 and minichromosome maintenance protein.

This replicative complex assembly occurs during G1 phase prior to S phase.

During the transition between G1 phase to S phase, CDK proteins and DDK proteins get attached to the Pre-replication complex.

It transforms the Pre-replication complex into active replication fork.


Cdt1
MCMs
Cdc6
ORCs
Cdt1
MCMs
G1 Phase
CDKs
DDKs
P
Cdt1
P
P
ORCs
Pre-S Phase
P
Cdc6
GINS
P
Cdc45
P
Cdc45
GINS
S Phase
P
ORCs

Elongation :

Once the complex forms and cell pass into S phase, then unwinding of DNA strand takes place.

Unwinding takes place by enzyme Helicases and it leads of exposure of 2 DNA templates.

After unwinding, polymerization of the daughter strands takes place.
It occurs with help of DNA polymerase enzymes.

There are total 15 DNA polymerase enzymes indentified till now but only 3 are involved in replication process.

They are :

1. DNA polymerase alpha
2. DNA polymerase epsilon
3. DNA polymerase delta

DNA polymerase alpha :
DNA polymerase α associated with enzyme Primase, forms RNA primer which are 8-10 nucleotide long.

Later DNA polymerase α elongates this RNA primer by more 20 nucleotides and then leaves the place.

DNA polymerase ε :
DNA polymerase ε synthesis nucleotides on the leading strand.

It will continuously add nucleotides leading to continous process of replication.

Thus it will require only one RNA primer at the beginning.

DNA polymerase δ :

DNA polymerase δ helps the synthesis of DNA on lagging strand.

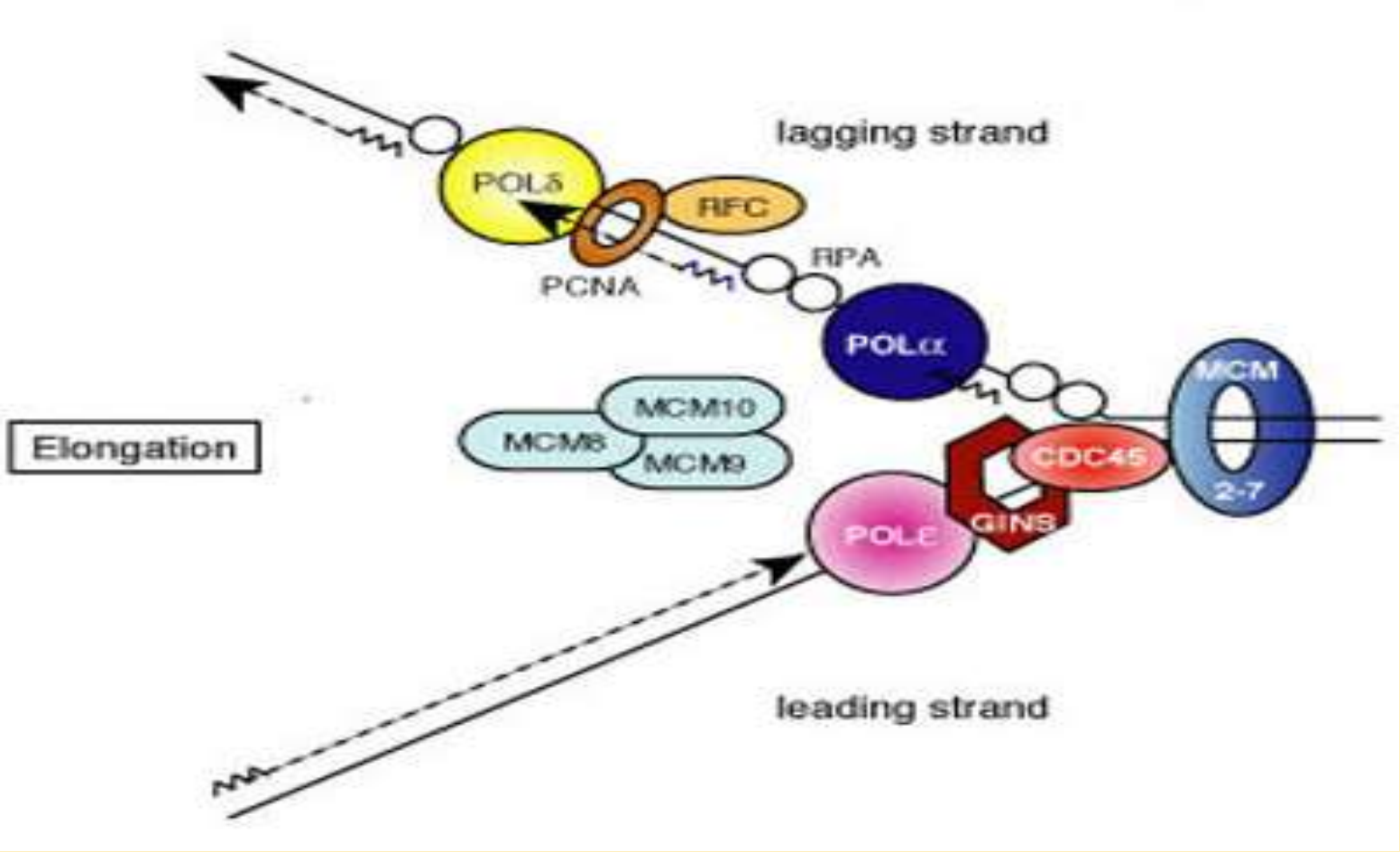
On the lagging strand multiple RNA primers are required.

On the lagging strand, DNA polymerase δ synthesize small fragments of DNA called Okazaki fragments.

At the end of each Okazaki fragment, DNA polymerase δ runs to previous Okazaki fragment and replaces the RNA primer nucleotides with Dna nucleotides.

this leads to flap formation which is removed and the nick between is replaced by enzyme DNA ligases .

This process is known as Okazaki fragments maturation.


lagging strand
POLδ
RFC
PCNA
RPA
POLα
MCM
MCM10
MCM8
MCM9
Elongation
CDC45
2-7
GINS
POLε
leading strand

Termination :

This process is very complex in eukaryotes.

End replication problem occurs in eukaryotes as the DNA polymerase is only able to add nucleotides from 3’ end.

This problem is solved by enzyme Telomerase.

The end regions where primers are left are known as telomers.

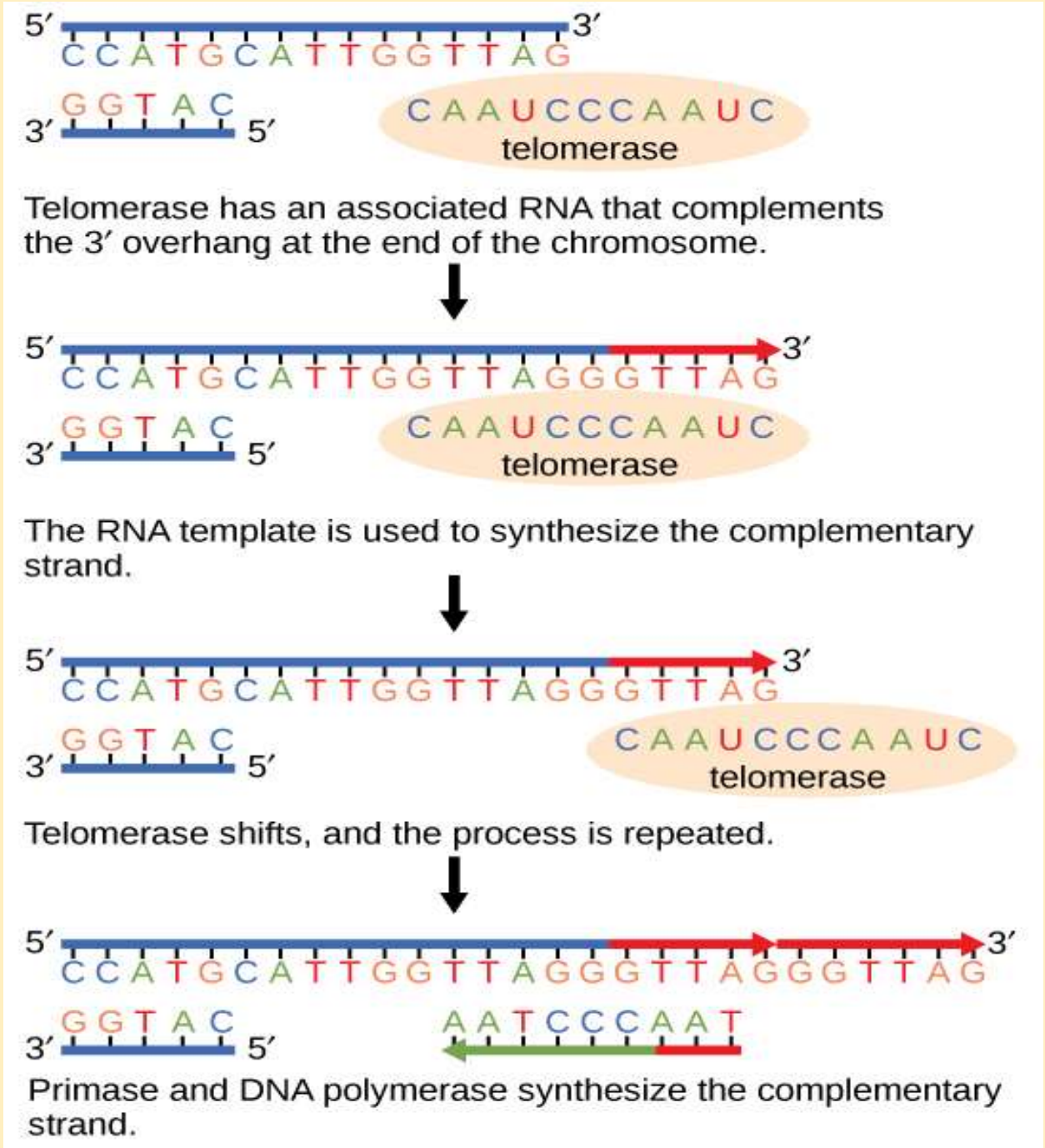
Telomers extend the 3’ end of parental strand beyond the 5’ end of daughter strand.

It then behaves as ORI region and recruit telomerase.

Telomerase forms RNA complementary basepairs.

The process continues.

Then Primase and DNA polymerase δ helps in further replication.


5′
3′
C C A T G C A T T G G T T A G
G G T A C
3′
5′
C A A U C C C A A U C
telomerase
Telomerase has an associated RNA that complements the 3′ overhang at the end of the chromosome.
5′
3′
C C A T G C A T T G G T T A G G G T T A G
G G T A C
3′
5′
C A A U C C C A A U C
telomerase
The RNA template is used to synthesize the complementary strand.
5′
3′
C C A T G C A T T G G T T A G G G T T A G
G G T A C
3′
5′
C A A U C C C A A U C
telomerase
Telomerase shifts, and the process is repeated.
5′
3′
C C A T G C A T T G G T T A G G G T T A G G G T T A G
G G T A C
3′
5′
A A T C C C A A T
Primase and DNA polymerase synthesize the complementary strand.