

SOS in Biochemistry, Jiwaji University, Gwalior

M.Sc. II Semester (2019-20)

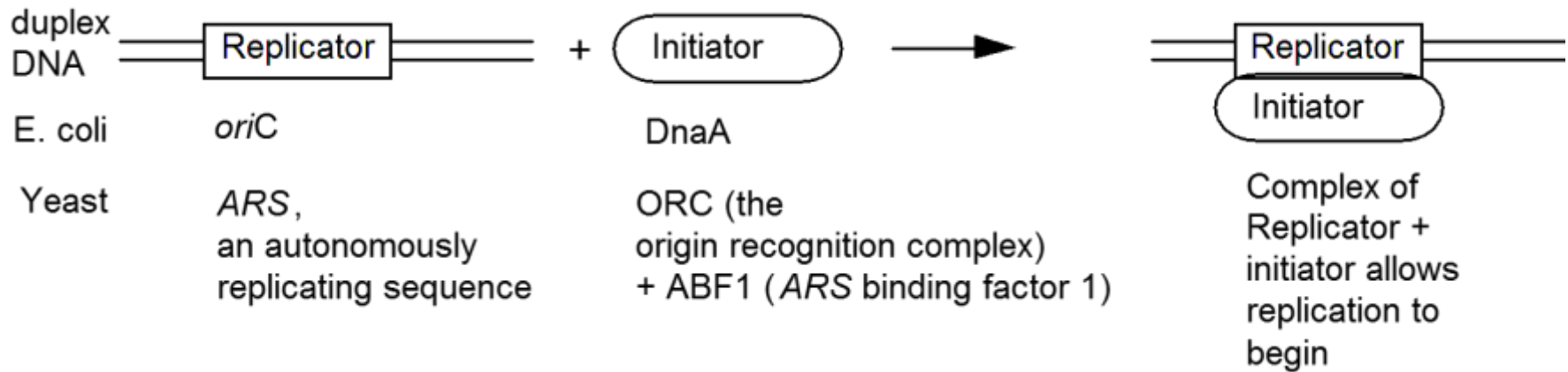
Paper BCH 201: Fundamentals of Molecular Biology (Unit 1)

REGULATION OF DNA REPLICATION

Replication Occurs in 3 Stages

- **Initiation:** begin at a specific site, e.g. *oriC* for *E. coli*.
- **Elongation:** movement of the replication fork
- **Termination:** at *ter* sites for *E. coli*

Replicon = unit that controls replication



Replicator: *cis*-acting DNA sequence required for initiation; defined genetically

Origin: site at which DNA replication initiates; defined biochemically

Initiator: protein needed for initiation, acts in *trans*

Regulation of Replication of Bacterial Plasmid DNA

✓ Occurs at Initiation of Plasmid DNA Replication

Two major mechanisms of control of initiation have been recognized:

1. Regulation by antisense RNA

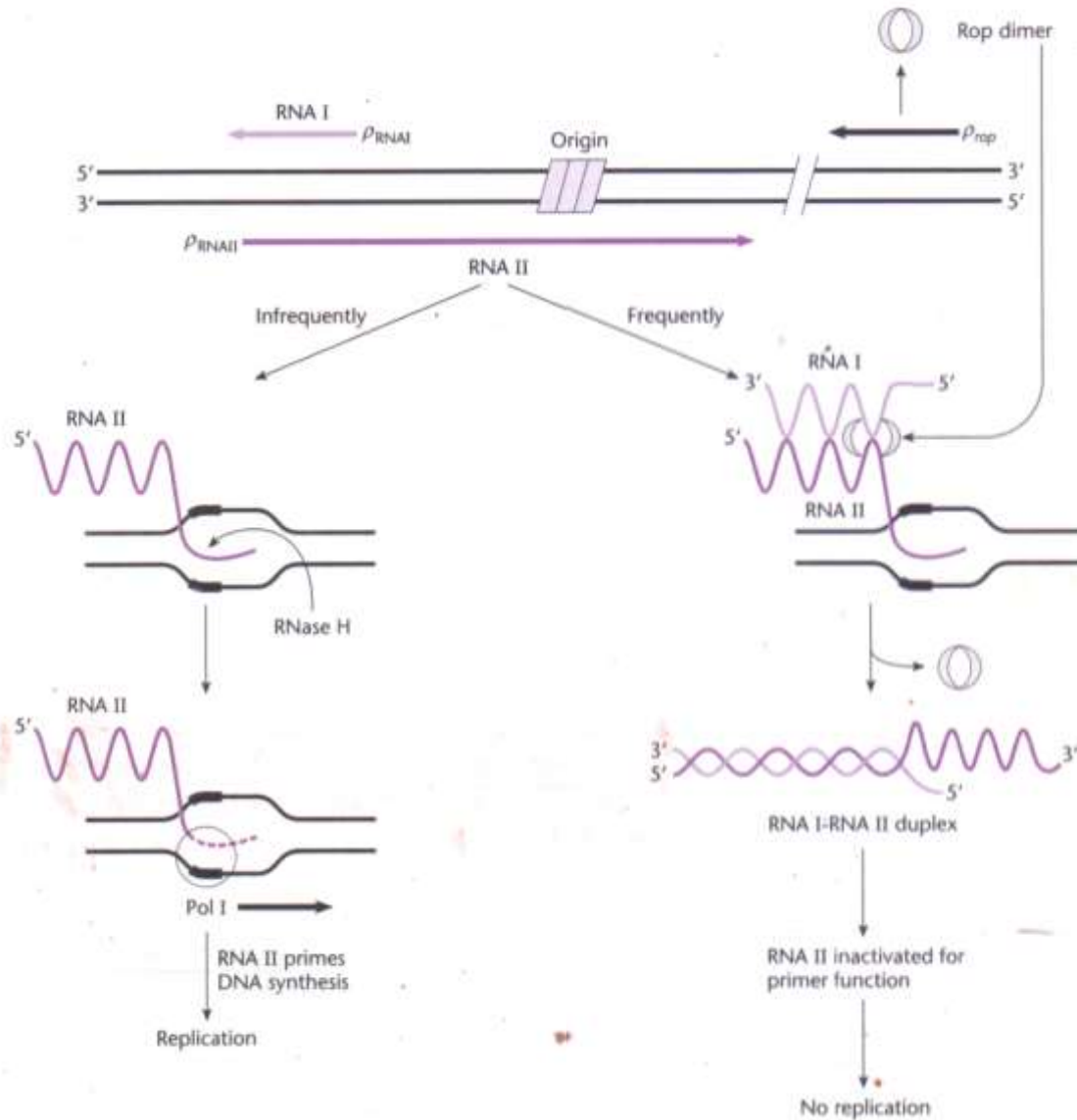
(e.g., ColE1-derived plasmids, **restricted host range**)

2. Regulation by binding of essential proteins to repeated sequences called iterons

(e.g., pSC 101-derived plasmids, **broad host range**)

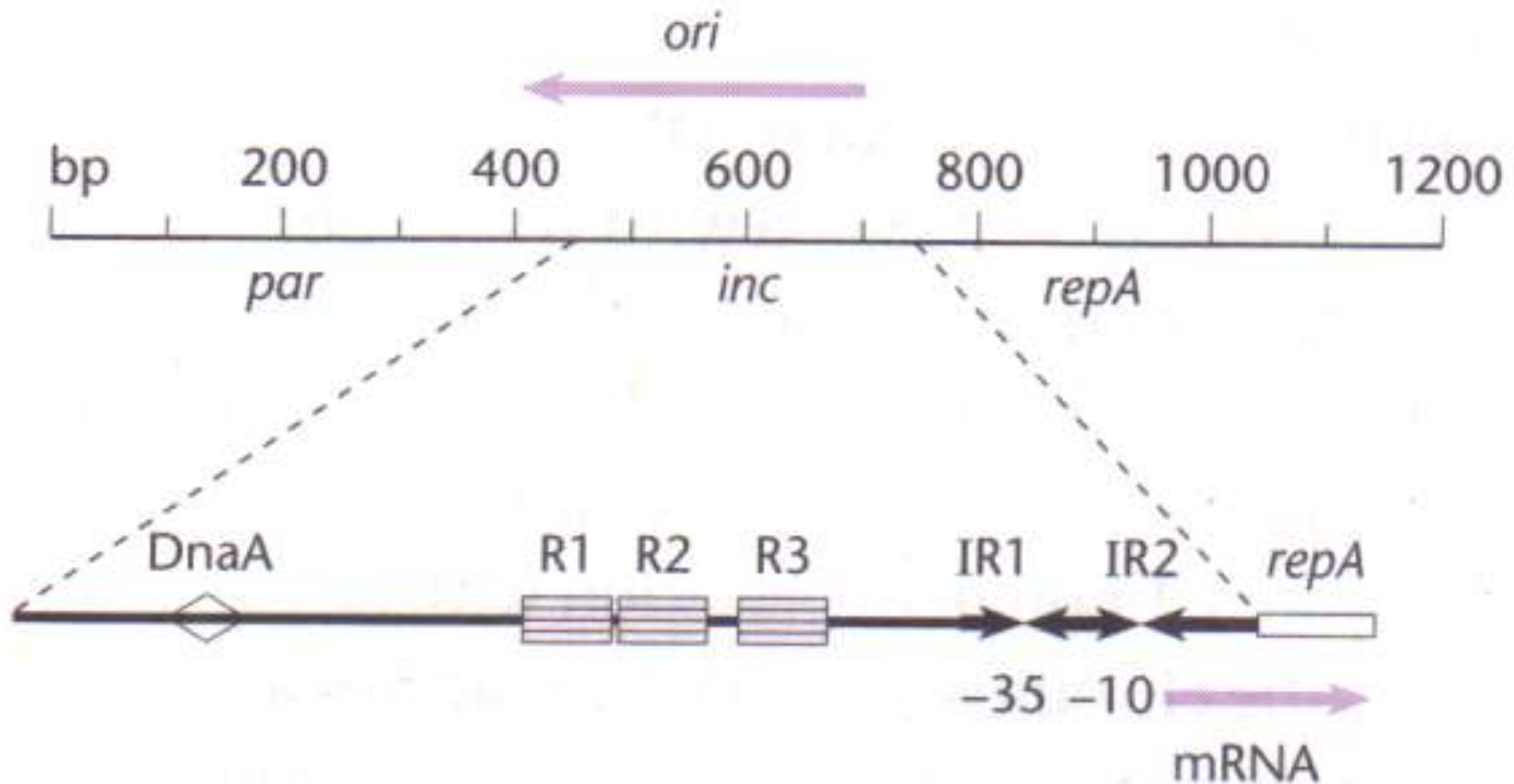
Regulation by Antisense RNA

Regulation of Replication of Col E1-derived Plasmids



Regulation by Iteron Sequences

Origin Region of pSC 101



✓ R1, R2 and R3 are the three iteron sequences.

✓ IR1 & IR2 are inverted terminal repeats → RepA binds and autoregulates its own synthesis

Regulation of Replication of Bacterial Genomic DNA

✓ Occurs at **Initiation** of Replication of Bacterial Genome

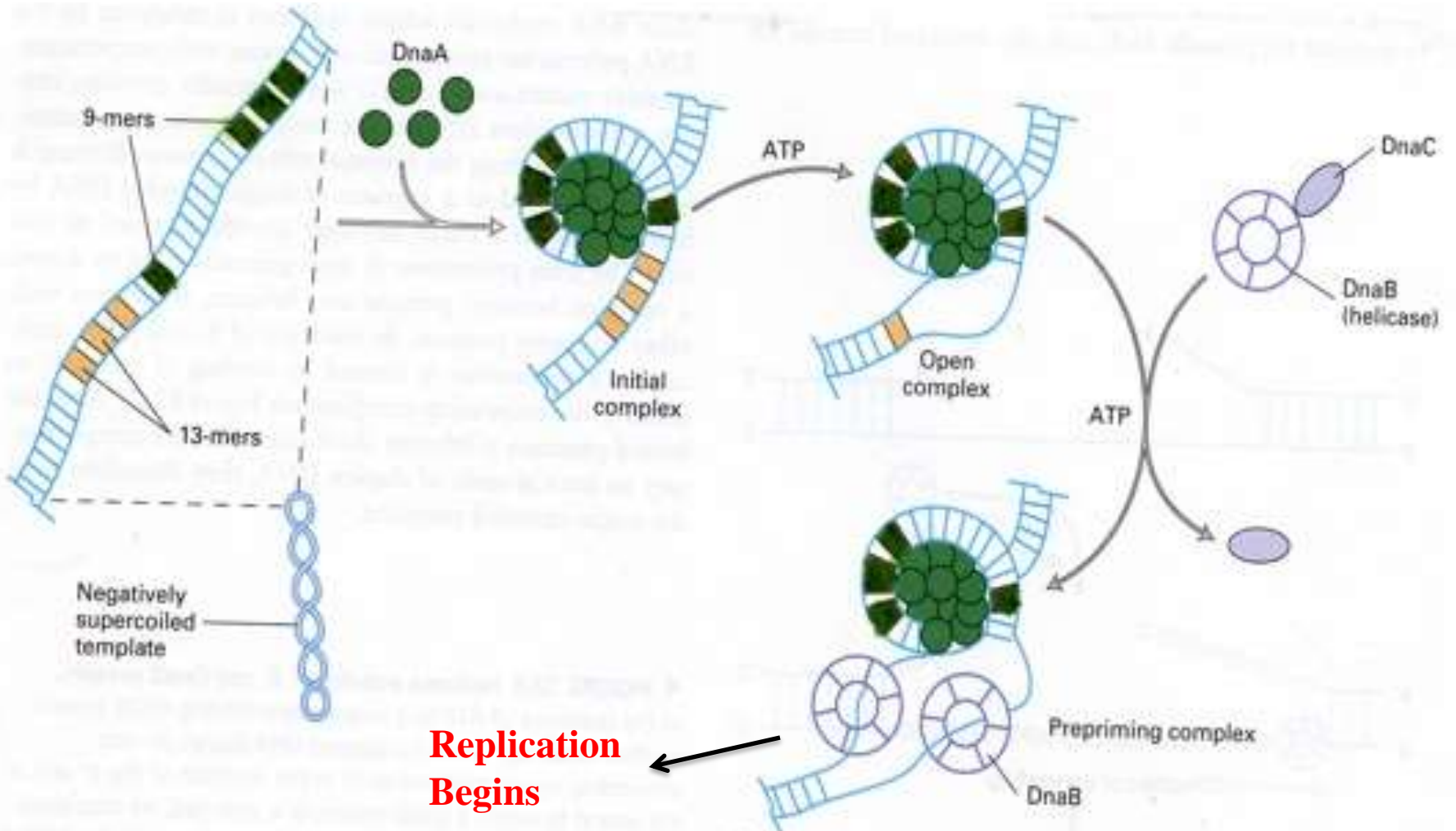
REGULATION OF DNA REPLICATION

1. Replication in bacteria and in eukaryotes is licensed and permitted to occur only once per cell cycle.
2. Each replicon is allowed to fire only once per cell cycle
3. What mechanisms are in place to ensure re-initiation **does not** occur in the same cell cycle?
4. Because it is critical to maintain genomic integrity, multiple mechanisms exist to ensure that each replicon fires once and only once during each cell cycle.
5. Three mechanisms are worth emphasizing over here:
 - a) **Regulatory inactivation of DnaA (RIDA)**
 - b) **DnaA titration**
 - c) **Sequestration**

Proteins needed for initiation at *oriC*

DnaA

- Only used at initiation
- It plays important role in regulation
- It is sometimes called as licensing factor in bacteria.
- Mutations cause a slow-stop phenotype
- Binds to the 4 copies of 9 bp repeats
- Further cooperative binding brings in 20 to 40 DnaA monomers
- Melts the DNA at the 3- 13 bp repeats



Replication Begins

-GATC T N T T N T T T T -
 -T T A T N C A N A -

13 bp repeat
 9 bp repeat

In Bacteria

S' - A | T - T - T - T - A - T - A | G - T - T - T - A | T - 3'] In Yeast

DnaA

1. ~1000 DnaA molecules are present in each *E. coli* are stable.
2. DnaA·ATP → active
3. DnaA·ADP → inactive
4. Intracellular DnaA·ATP levels rise and fall during the cell cycle
5. ~80% of the DnaA is present in the active DnaA·ATP level just prior to the initiation but falls to about 20% after replication is initiated.
6. There is no cyclic variation in the overall concentration or expression of DnaA.

Q/- How that happens?

Ans: Via different mechanisms.

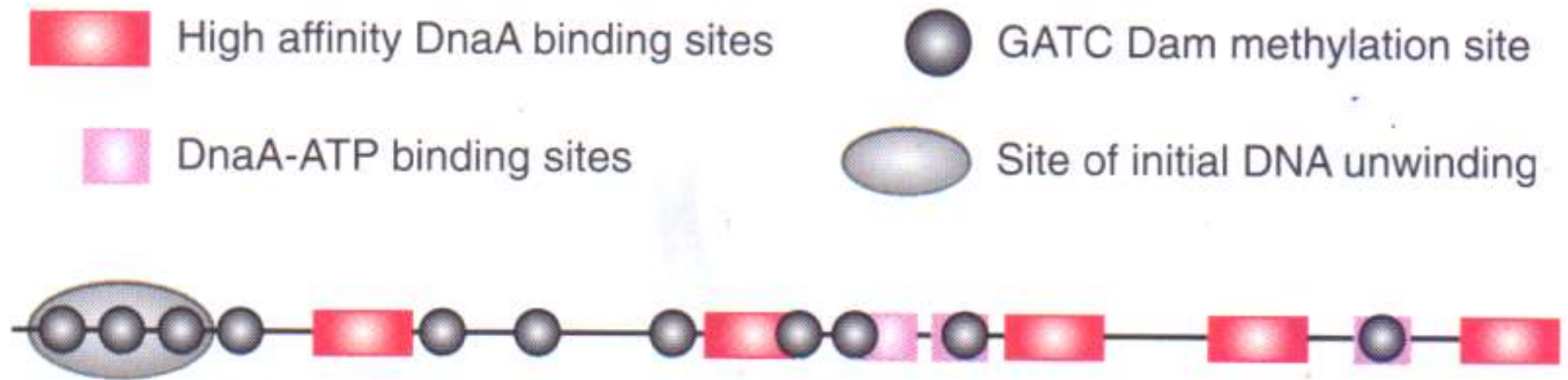
Three mechanisms are worth emphasizing over here:

- a) Regulatory inactivation of DnaA (RIDA)**
- b) DnaA titration**
- c) Sequestration**

**Methylation of Bacterial Origin
Regulates Replication during
Initiation**

Functions of DNA methylation in bacteria are dual

- 1. To regulate DNA replication at the initiation level**
- 2. To distinguish between old template strand from newly synthesized strand during DNA mismatch repair**



Architecture of *E. coli oriC*

FIGURE 11.10 The *E. coli* origin of replication, *oriC*, contains multiple binding sites for the DnaA initiator protein. In a number of cases these sites overlap Dam methylation sites.

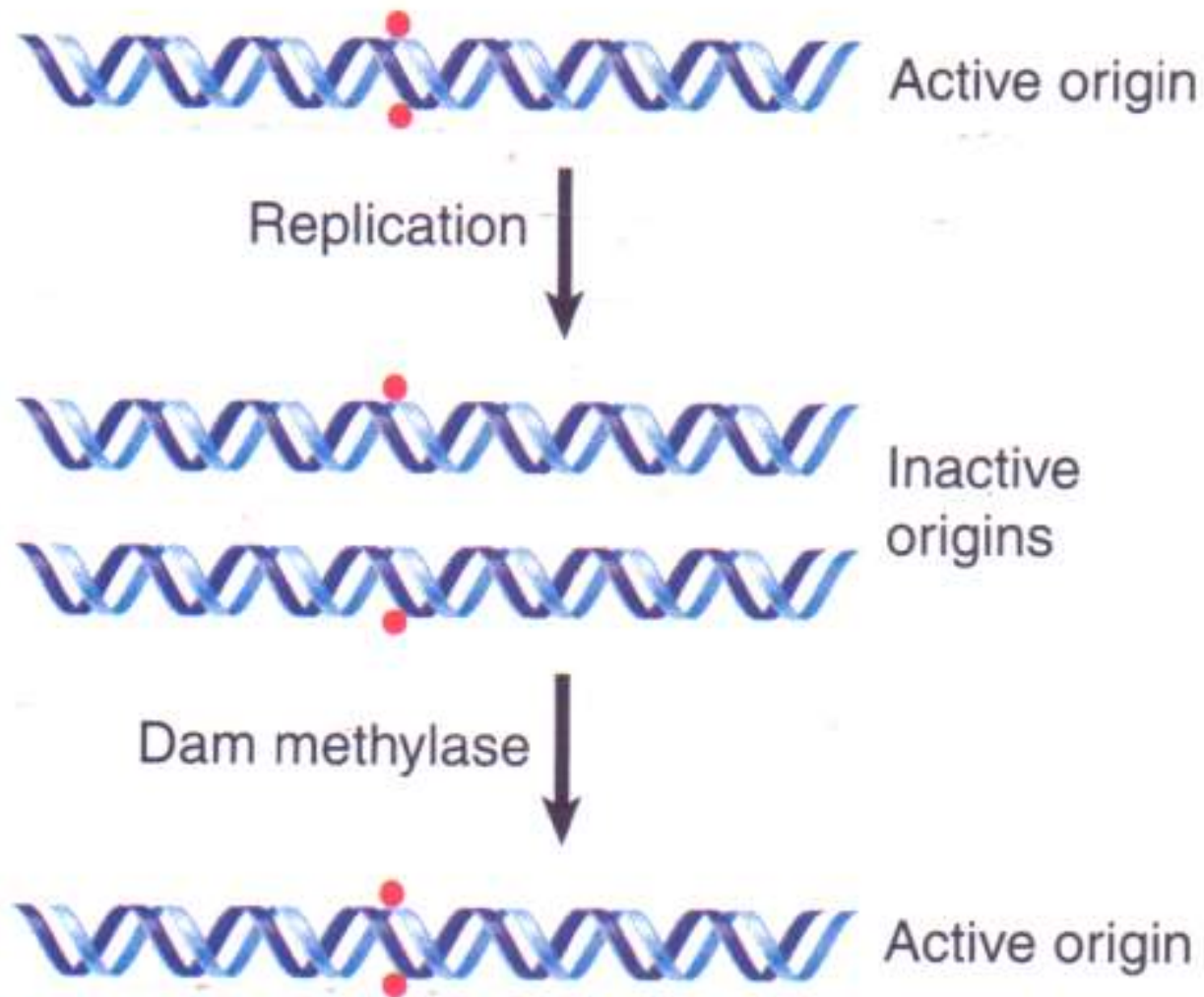
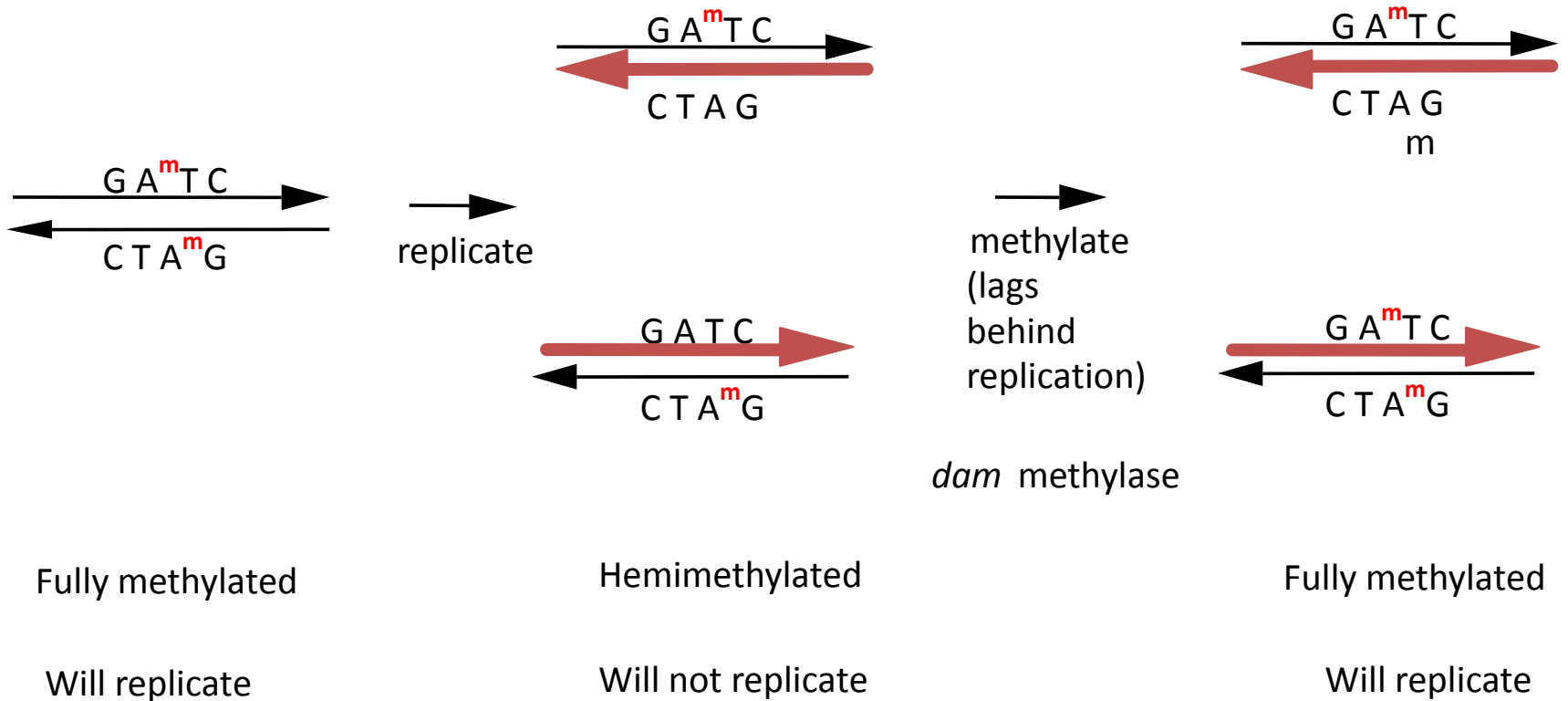


FIGURE 11.11 Only fully methylated origins can initiate replication; hemimethylated daughter origins cannot be used again until they have been restored to the fully methylated state.

Regulation of replication by methylation



CONTROL BY METHYLATION

- GATC motifs are substrates for methylation by *dam* methylase.
- Methylase transfers a methyl group from S-adenosylmethionine to N-6 of adenine in GATC.
- Methylated GATC on BOTH strands: *oriC* will serve as an origin
- Methylated GATC on ONLY one strand (hemimethylated): *oriC* is not active
- Re-methylation is slow, delays use of *oriC* to start another round of replication.

SUMMARY

1. The full scope of the system used to control re-initiation of replication in the same cell cycle is **not** clear.
2. But several mechanisms may be involved:
 - a) Physical sequestration of the origin
 - b) delay in remethylation of origin
 - c) Inhibition of DnaA binding
 - d) Repression of *dnaA* transcription
3. It is not immediately obvious which of these events cause the others, and whether their effects on initiation are direct or indirect.

REGULATION OF REPLICATION OF EUKARYOTIC DNA

Each Eukaryotic Chromosome Contains Many Replicons

1. A chromosome is divided into many replicons
2. The progression into S phase is tightly controlled
3. Each replicons are ~40 (yeast/fly) to 100 kb (animal) in length
4. The origin in each replicon is activated once and only once in a single division cycle
5. Individual replicons are activated at characteristic timing during S phase
6. Regional activation patterns suggest that replicons near one another are activated at the same time
7. Available evidences suggest that most chromosomal replicons do not have a termination region like bacteria
8. Replication fork continues from its origin until it meets a fork proceeding towards it from the adjacent replicon

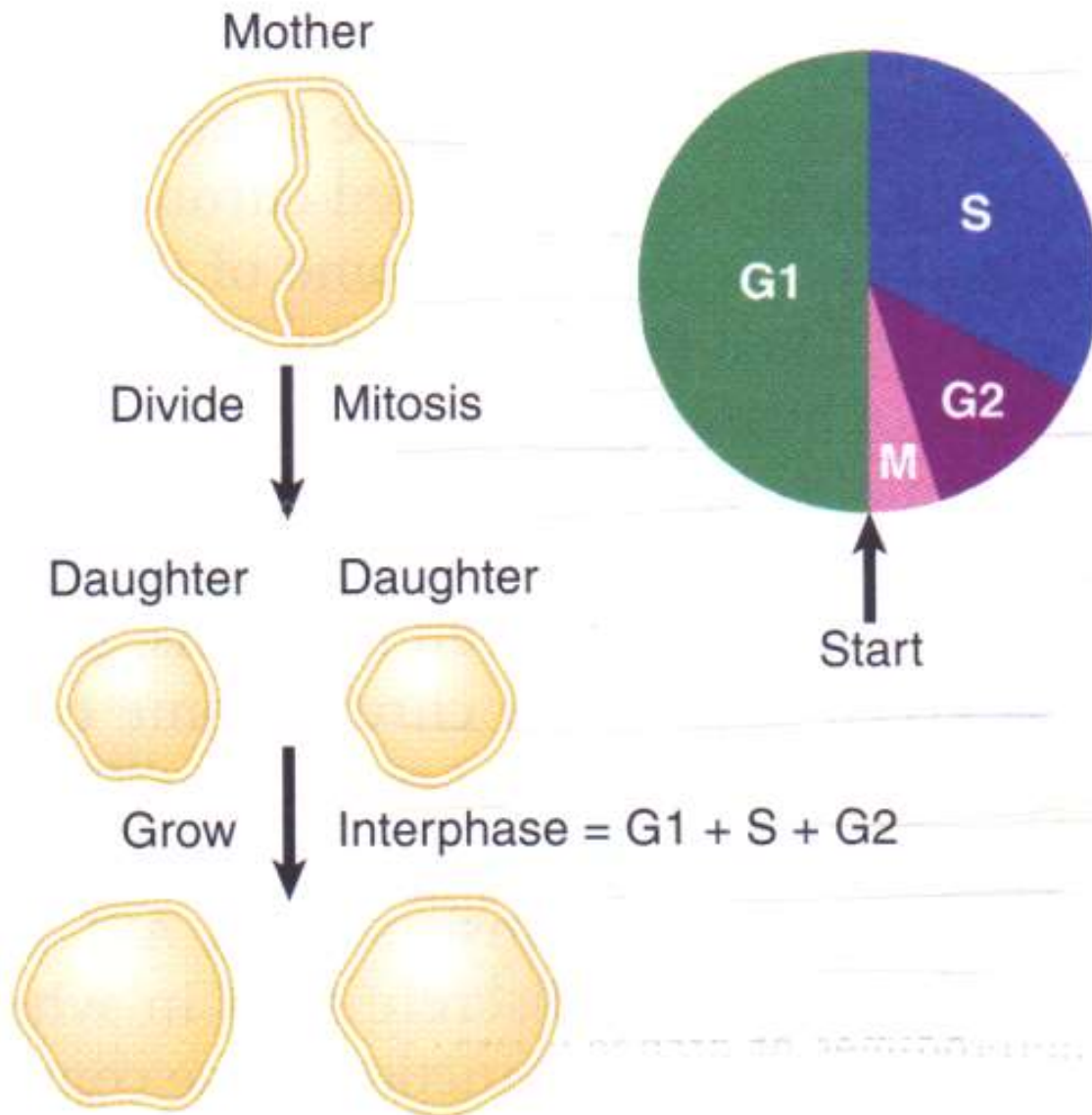


FIGURE 11.13 A growing cell alternates between cell division of a mother cell into two daughter cells and growth back to the original size.

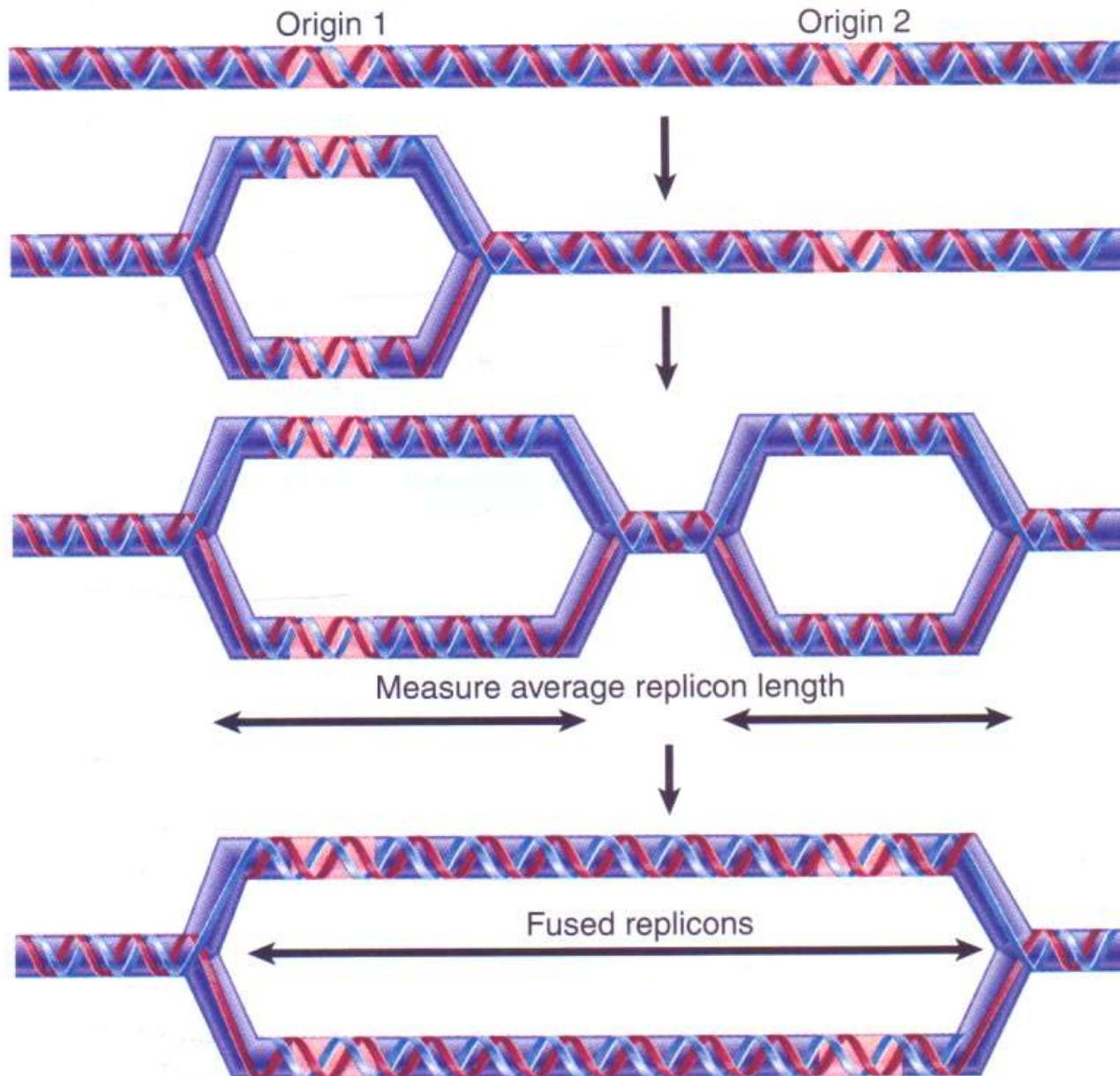


FIGURE 11.14 A eukaryotic chromosome contains multiple origins of replication that ultimately merge during replication.

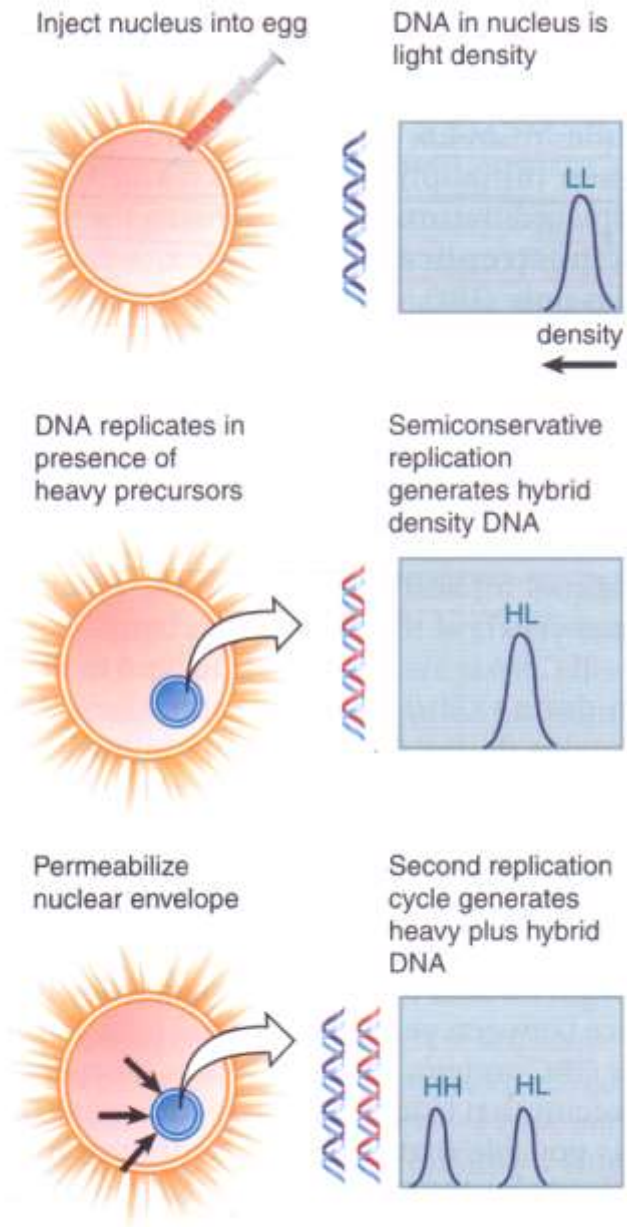
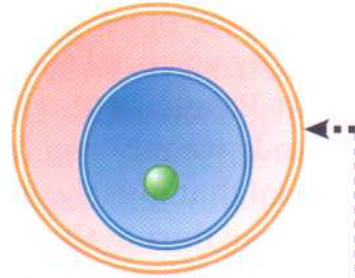
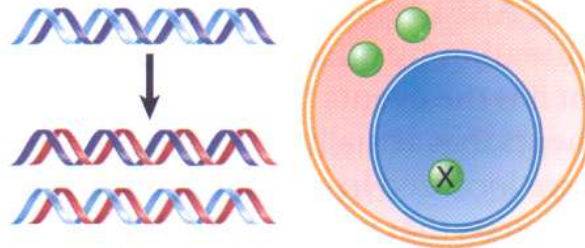


FIGURE 12.13 A nucleus injected into a *Xenopus* egg can replicate only once unless the nuclear membrane is permeabilized to allow subsequent replication cycles.

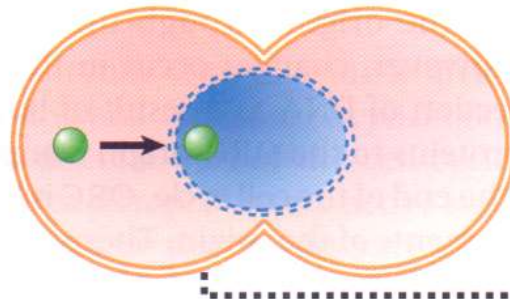
Prior to replication, nucleus contains active licensing factor



After replication, licensing factor in nucleus is inactive; licensing factor in cytoplasm cannot enter nucleus



Dissolution of nuclear membrane during mitosis allows licensing factor to associate with nuclear material



Cell division generates daughter nuclei competent to support replication

FIGURE 12.14 Licensing factor in the nucleus is inactivated after replication. A new supply of licensing factor can enter only when the nuclear membrane breaks down at mitosis.

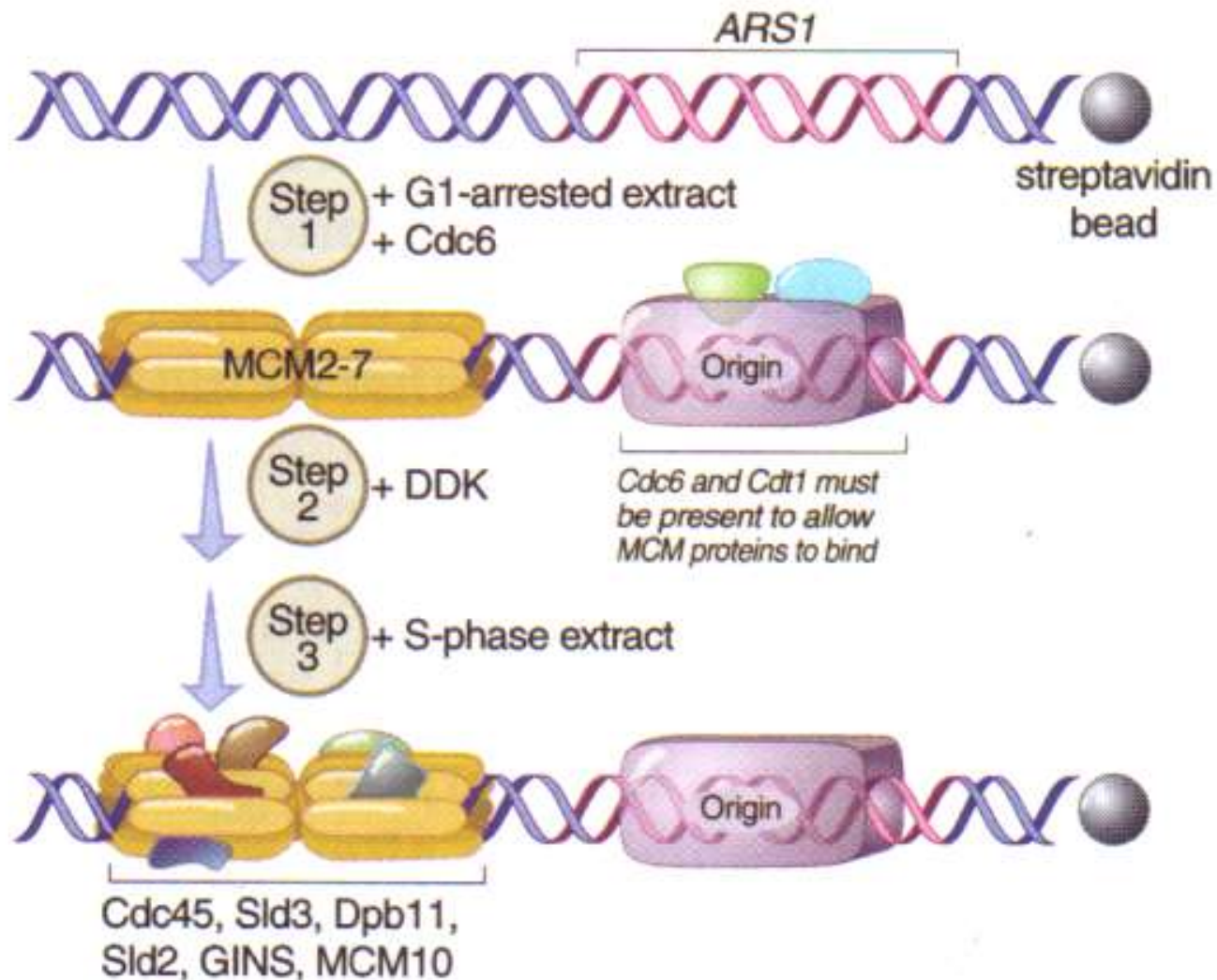


FIGURE 12.15 Proteins at the origin control susceptibility to initiation. Adapted from R. C. Heller, et al., *Cell* 146 (2011): 80-91.