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كلية العلوم

قـســـــــــم التقنيات الاحيائية الطبية

Molecular Biology

Lec. 3

DNA REPLICATION

by

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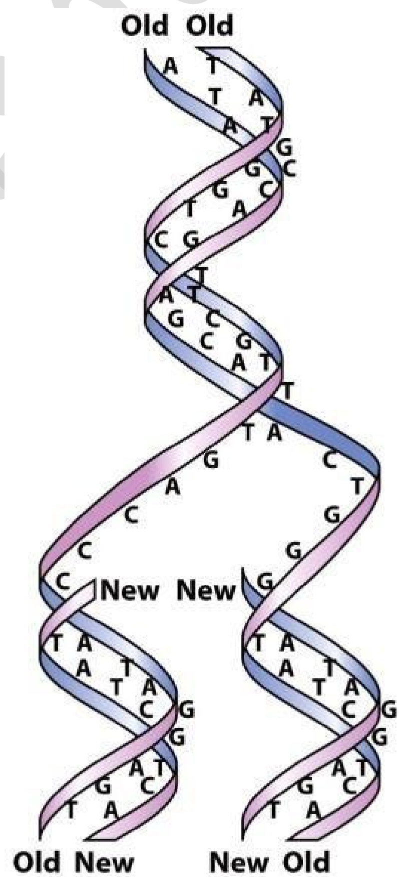
DNA Replication

- Reproduction is a property of all organisms.
- DNA duplicates by a process called **DNA replication**.
- The DNA replication machinery is also used for DNA repair.

DNA Replication

- DNA replication takes place by separation of the strands of the double helix, and synthesis of two daughter strands complementary to the two parental templates.



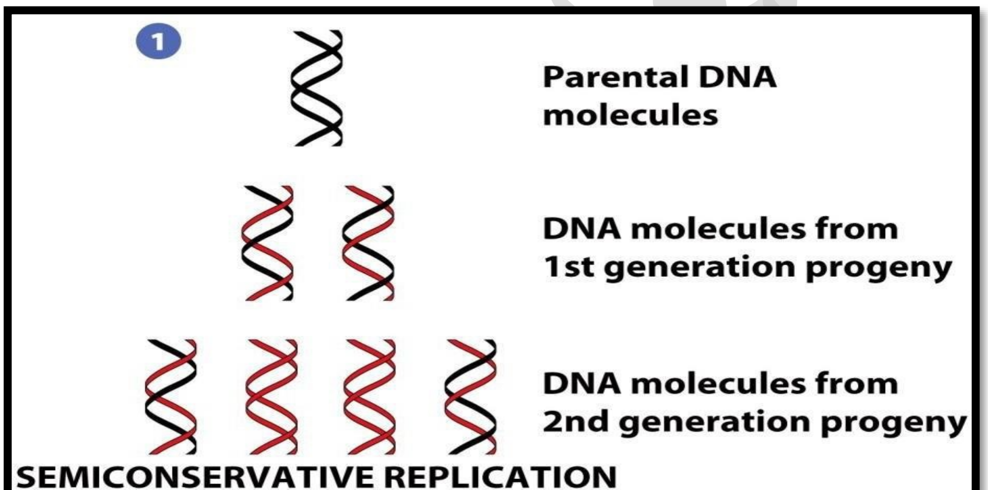
The models of DNA Replication

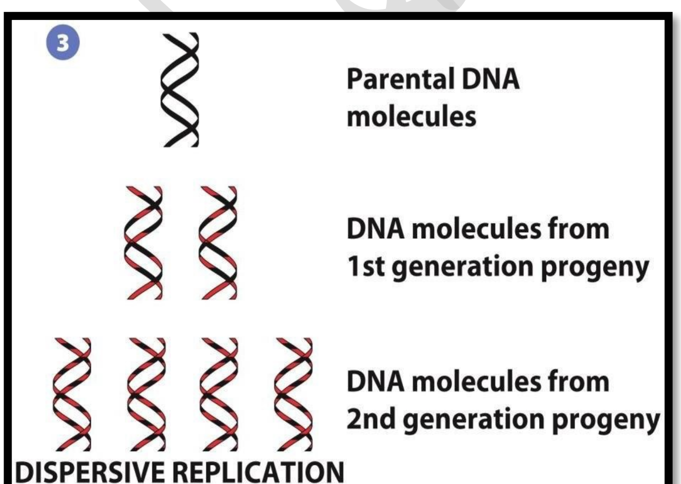
- **Semiconservative Replication**

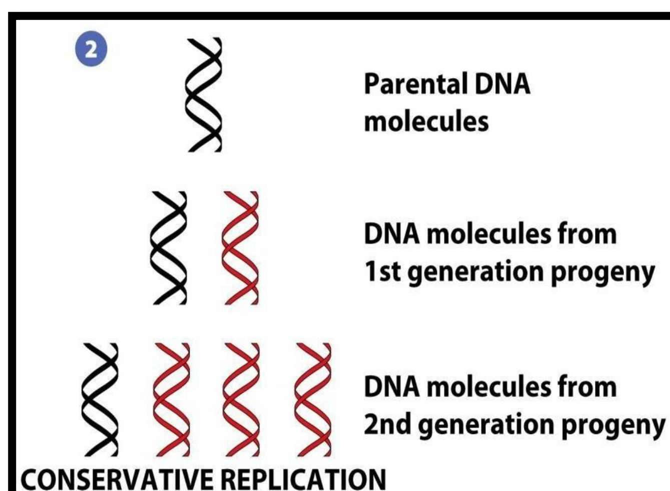
– DNA replication is called **semiconservative** because half of the parent structure is retained in each of the daughter duplexes.

– This model of DNA replication took over the other two models previously considered: *conservative* and *dispersive*.

Three alternate schemes of replication







Procaryote DNA Replication

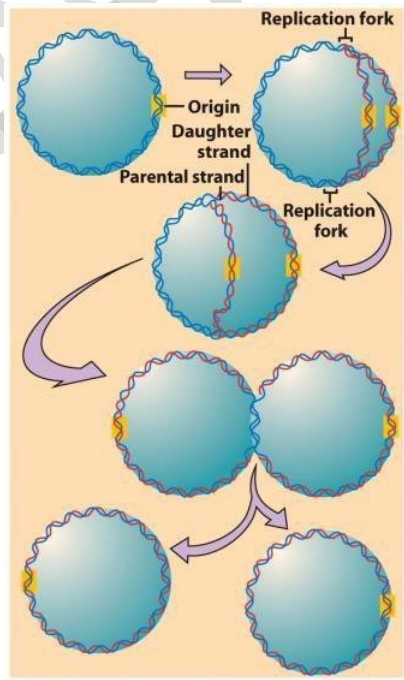
- Replication Forks and Bidirectional Replication

– Replication starts at the **origin** site, where a number of proteins bind to **initiate** replication.

– Replication proceeds *bidirectionally*.

– **Replication forks** are points where a pair of replicating segments come together and join the nonreplicated segments.

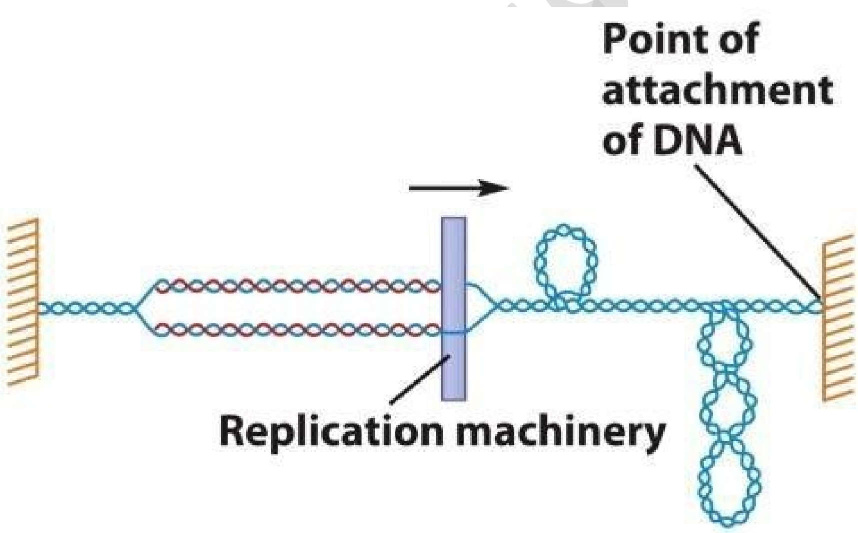
Model of a bacterial chromosome undergoing bidirectional replication



- Unwinding the Duplex and Separating the Strands

– Tension is built up as DNA begins the *unwinding* process and becomes positively supercoiled.

– **DNA gyrase** (topoisomerase II) relieves the tension by changing the DNA into negatively supercoiled DNA.



- **The Properties of DNA Polymerases**

– **DNA polymerase** is responsible for synthesizing new DNA strands from a DNA **template**

– DNA polymerase requires a **primer** that provides the 3' hydroxyl terminus to add new nucleotides.

– Polymerization occurs in the 5'-to-3' direction.

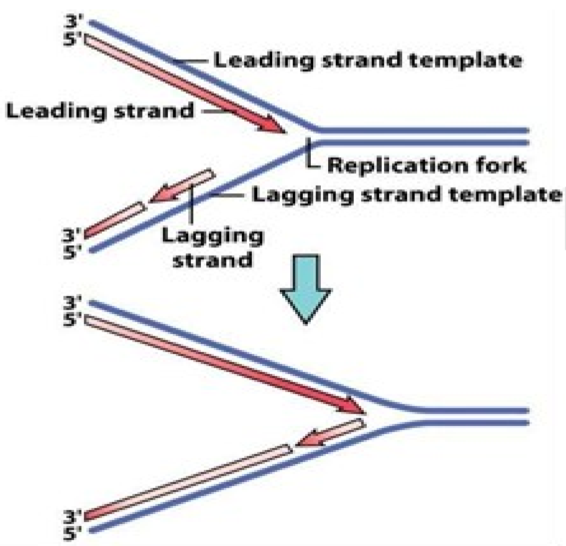
- **Semidiscontinuous Replication**

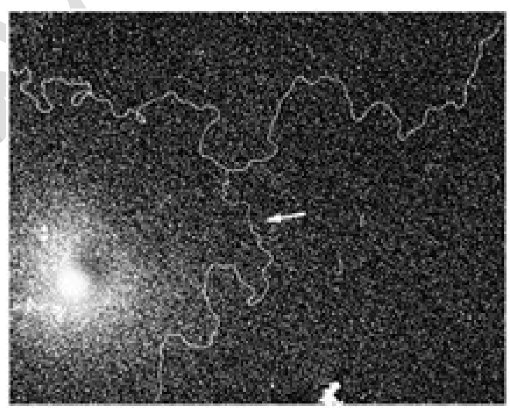
– Both daughter strands are synthesized simultaneously.

– The **leading strand** (in the direction of the replication fork movement) is synthesized *continuously*.

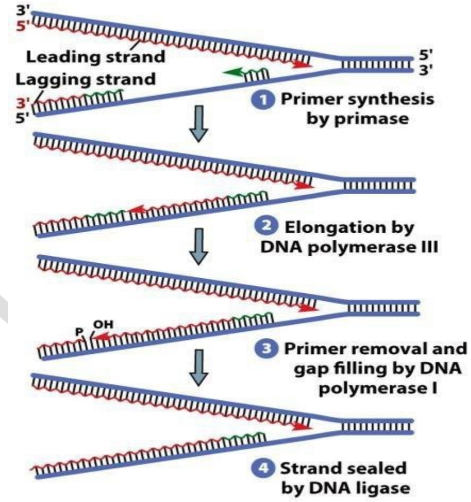
– The **lagging strand** (in the opposite direction of the replication fork movement) is synthesized *discontinuously*.

– The lagging strand is constructed of small **Okazaki fragments**, which are joined by **DNA ligase**.





The two strands of a double helix are synthesized by a different sequence of events



- **Primase** is an RNA polymerase that assembles short RNA primers.
- These primers are later removed and the gaps are sealed.

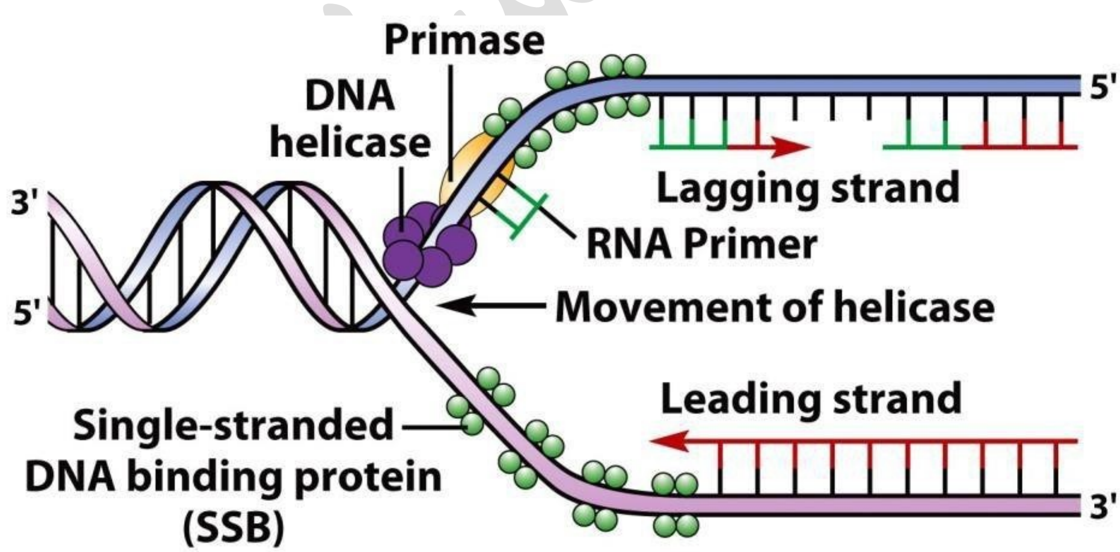
- **The Machinery Operating at the Replication Fork**

– **Helicase** and **single-stranded DNA-binding** (**SSB**) **proteins** unwind the parental duplex and separate the two strands.

– ***Replisome*** is **a large protein complex that carries out DNA replication, starting at the replication origin**. It contains several enzymatic activities, such as helicase, primase and DNA polymerase and creates a replication fork to duplicate both the leading and lagging strands.

– ***primosome*** is a protein complex responsible for creating RNA primers on single stands DNA during DNA replication.

– The role of DNA helicase, SSB proteins, and primase at the replication fork



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- **The Functions of DNA Polymerases types**

– DNA polymerase I is involved in DNA repair and also removes RNA primers and replaces them with DNA.
– DNA polymerase II is involved with DNA replication in some capacity, synthesizing chains of nucleic acids and repair of DNA damaged by UV irradiation.
– DNA polymerase III is the primary replication enzyme.

Replication in Eukaryotic Cells

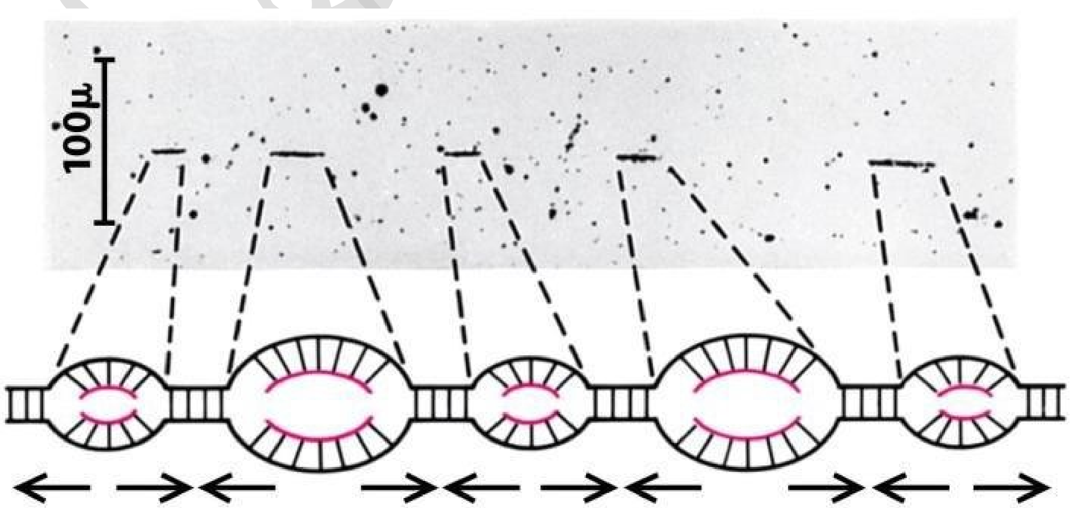
–Eukaryotes are not as well understood as replication in bacteria. Some advances include:

- Using mutant yeast cells unable to produce specific gene products for replication.
- Development of in vitro systems where replication can occur in cellular extracts or mixtures of purified proteins.

–Initiation of Replication in Eukaryotic Cells

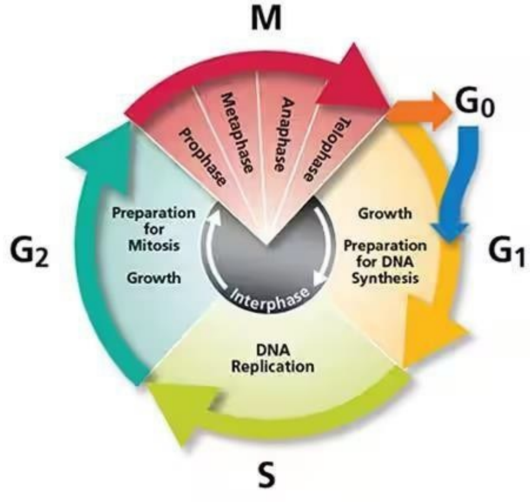
- Eukaryotes replicate their genome in small portions (replicons).
- Initiation of DNA synthesis in a replicon is regulated.

- Origins of replication identified in yeast cells are called **autonomous replicating sequences (ARS).**
- A multiprotein **origin recognition complex (ORC)** is assembled at the ARS.
- Replication in mammalian cells has been more difficult to study.



Restricting Replication to Once Per Cell Cycle

- Replication origins pass through different states so that they only replicate their DNA once during a cell cycle.



The Eukaryotic Replication Fork

- Replication activities are similar in eukaryotes and prokaryotes.
- There are several DNA polymerases in eukaryotes.
- Eukaryotic DNA polymerases elongate in the 5'-to- 3' direction and require a primer.

major components of the eukaryotic replication fork

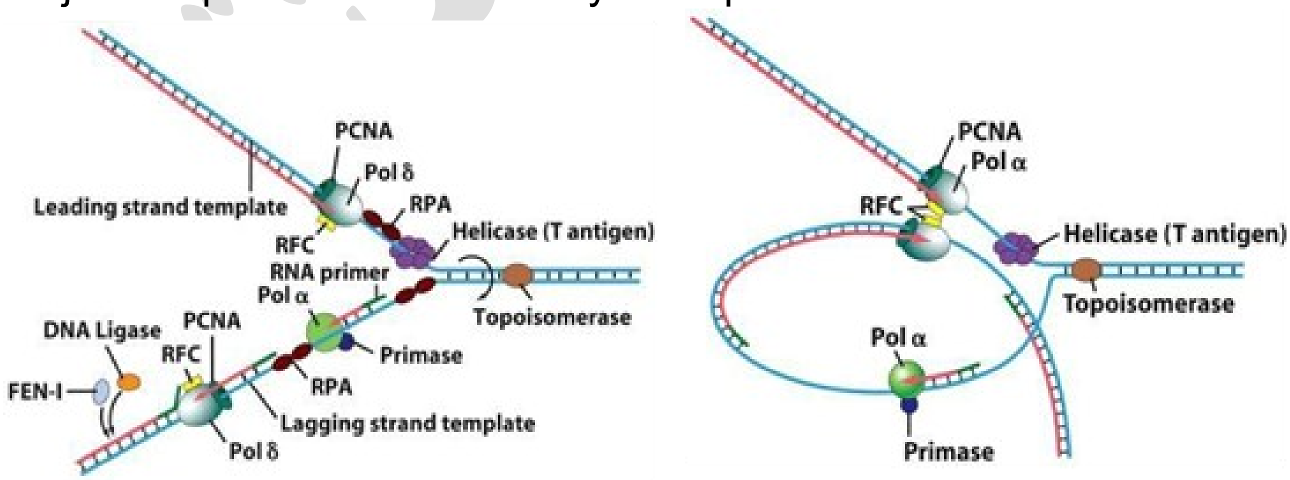


TABLE 13.1 Some of the Proteins Required for Replication

E. coli protein	Eukaryotic protein	Function
DnaA	ORC proteins	Recognition of origin of replication
Gyrase	Topoisomerase I/II	Relieves positive supercoils ahead of replication fork
DnaB	Mcm	DNA helicase that unwinds parental duplex
DnaC	Cdc6, Cdt1	Loads helicase onto DNA
SSB	RPA	Maintains DNA in single-stranded state
γ-complex	RFC	Subunits of the DNA polymerase holoenzyme that load the clamp onto the DNA
pol III core	pol δ/ε	Primary replicating enzymes; synthesize entire leading strand and Okazaki fragments; have proofreading capability
β clamp	PCNA	Ring-shaped subunit of DNA polymerase holoenzyme that clamps replicating polymerase to DNA; works with pol III in *E. coli* and pol δ or ε in eukaryotes
Primase	Primase	Synthesizes RNA primers
———	pol α	Synthesizes short DNA oligonucleotides as part of RNA-DNA primer
DNA ligase	DNA ligase	Seals Okazaki fragments into continuous strand
pol I	FEN-1	Removes RNA primers; pol I of also fills gap with DNA