الوراثة البشرية \ المرحلة الثالثة

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المحاضرة الثالثة \ تصنيع الدنا

المحاضر الاستاذ الدكتور اياد احمد الطويل

كلية الاسراء الجامعة \ قسم تقنيات المختبرات الطبية

وزارة التعليم العالي و البحث العلمي

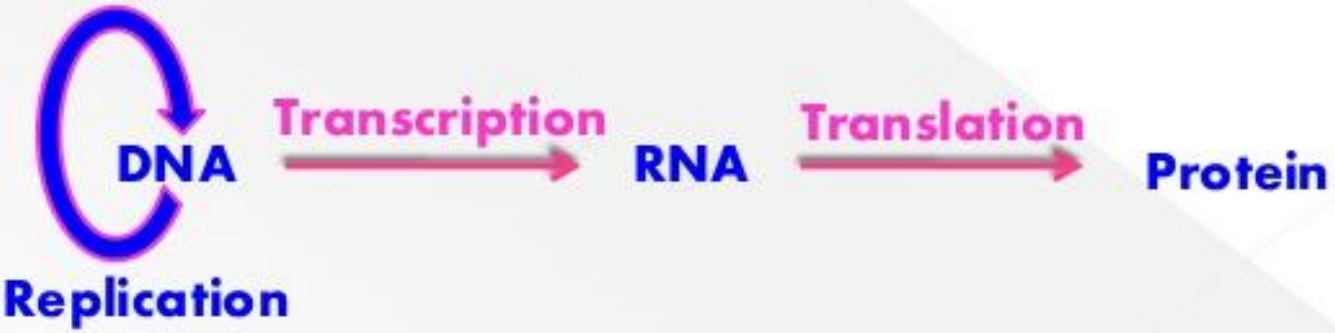
The Central Dogma of Molecular Biology and DNA Replication / Lecture Three

The central dogma of molecular biology is an explanation of **the flow of genetic information within a biological system.** It was first stated by Francis Crick in 1956 and re-stated in a Nature paper published in 1971.

The central dogma has also been described as DNA makes DNA (replication), DNA makes RNA (transcription), and RNA makes protein (translation).

Central Dogma of Life

- The biological information flows from DNA to RNA, & from there to proteins.
- This is central dogma of life.
- DNA in a cell must be duplicated (replicated), maintained & passed down accurately to the daughter cells.



Biological sequence information

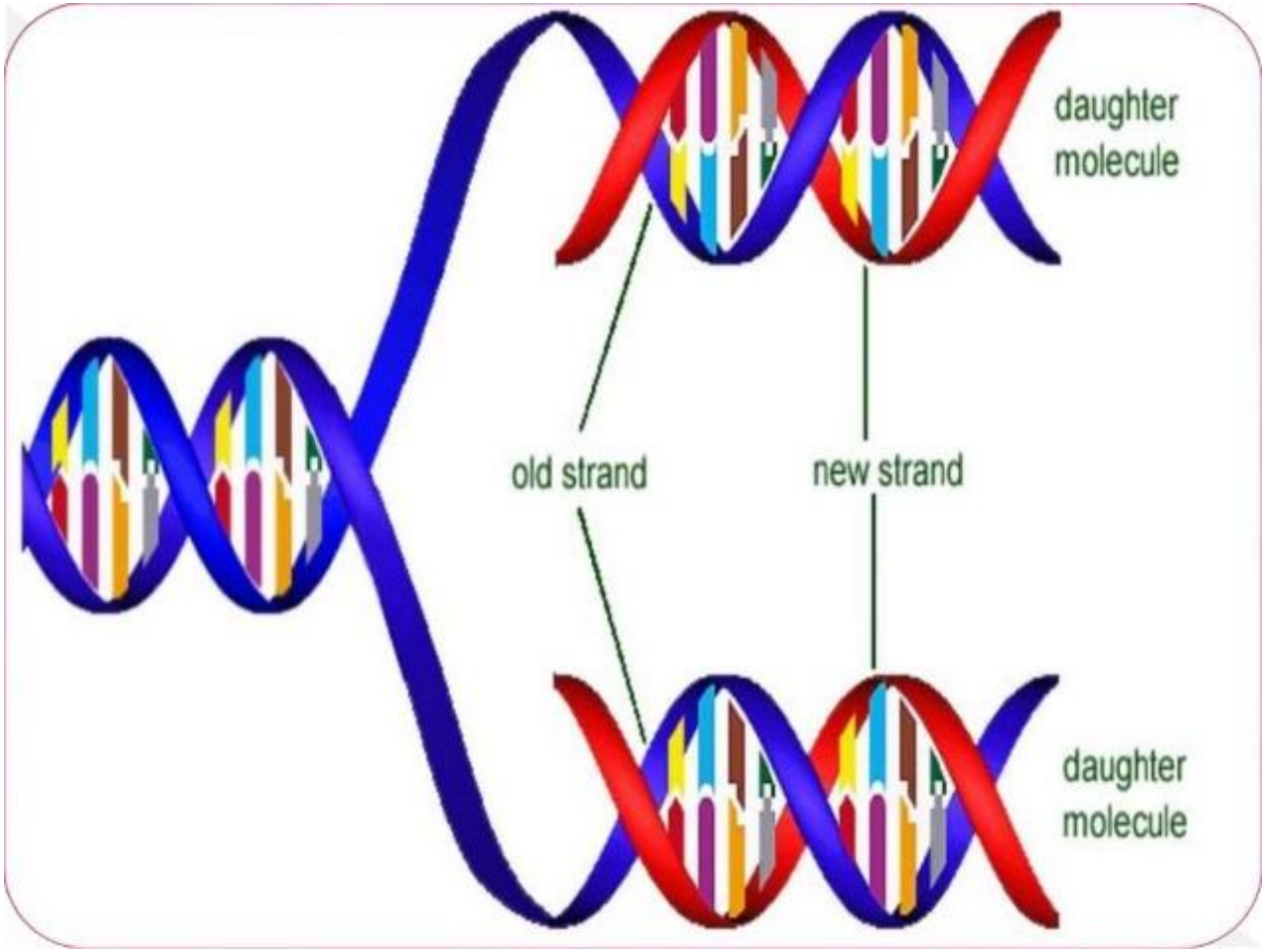
DNA, RNA and protein are linear polymers. One polymers sequence is used as a template for the contraction of another for the construction of another polymer.

Genetic materials have four important characteristic which are :

1) **Replication (mitosis and meiosis)**
2) **Storage of the information (though base pairs)**
3) **Expression of the information (through transcription and translation)**
4) **Variation (through mutation)**

In general, DNA is replicated by :

- **Uncoiling of the helix.**
- **Each individual strand of the original (parent) duplex separate.**
- **Each individual strand serves as a template for the synthesis of a new strand.**
- **New nucleotides will add to each new strand.**
- **More than 200 enzymes and proteins are involved in DNA replication.**
- **The mechanism is simple in principle, but a complex process that requires a variety of enzymes and other proteins.**
- **The end result of replication is the presence of two copies with identical sequences.**
- **DNA replication is bidirectional from the origin of replication.**


daughter
molecule
old strand
new strand
daughter
molecule

Possible Models for DNA Replication

There are three possible ways in which DNA can replicate :

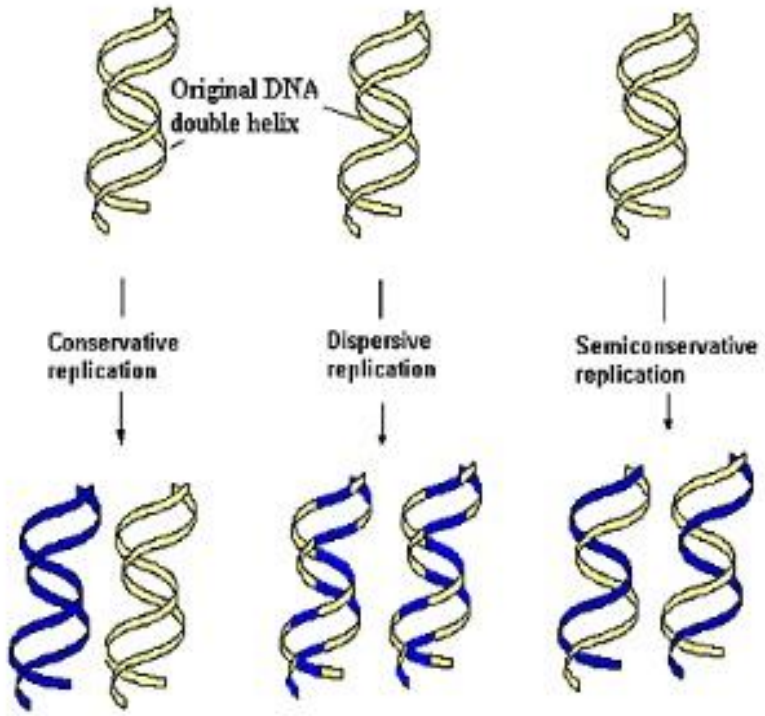
1) **Conservative replication**

2) **Dispersive replication**

3) **Semiconservative replication**

Proposed Models of DNA Replication

- In the late 1950s, three different mechanisms were proposed for the replication of DNA
  - **Conservative model**
    - Both parental strands stay together after DNA replication
  - **Semi-conservative model**
    - The double-stranded DNA contains one parental and one daughter strand following replication
  - **Dispersive model**
    - Parental and daughter DNA are interspersed in both strands following replication



Possible Models of DNA Replication

DNA replication has requirements that must be met:

1. **DNA template**
2. **Free 3'-OH group**
3. **The presence of enzymes and proteins**

Enzymes

DNA Helicase (Also known as helix destabilizing protein bind to the double standard DNA and stimulate the separation of the two strands and formation of the Replication Fork).

Single Stranded Binding(SSB) Proteins (Known also as " Helix destabilizing proteins ". They bind to ssDNA and prevent the DNA double helix from re-annealing after DNA helicase unwinds it, thus maintaining the strand separation. Replication is 100 times faster when these proteins are attached to the single-stranded DNA).

- **Topoisomerase (They produce breaks in the DNA and then rejoin in order to relieve the stress in the helical molecule during replication. There are many topoisomerases, but DNA gyrase is the most known).**

- **DNA Gyrase (A specific type of topoisomerase. This enzyme catalyzes the formation of negative supercoils that is thought to aid with the unwinding process. Un Relieves strain of unwinding by DNA helicase).**

- **DNA polymerases (Prokaryotic polymerases are three : DNA polymerase I "Pol I" has three activities 1) 5' to 3' elongation (polymerase activity), 2) 5' to 3' exonuclease (proof-reading activity), 3) 5' to 3' exonuclease (repair activity). The function of DNA polymerase I is to remove the DNA primers and replaces them with ds nucleotides. DNA polymerase II its function is not well known. Finally DNA polymerase III (Pol III) is the enzyme that perform the 5' – 3' polymerase function. It reads the existing template chain from its 3' end to its 5' end and adds new complementary nucleotides from 5' end to the 3' end of the daughter chain. There have been 5 distinct eukaryotic DNA polymerases identified as ALPHA, BETA, GAMMA, DELTA and EPSILON**

- **DNA clamp (A DNA clamp, also known as a sliding clamp, is a protein that binds DNA polymerase and prevents this enzyme from dissociating from the template DNA strand).**

- **DNA Ligase (DNA ligase forms a covalent phosphodiester linkage between 3'-hydroxyl and 5'-phosphate group to produce a continuous chain.**

- **Primase (RNA primase adds a complementary RNA primer to each template strand as a starting point for replication in order for polymerase to begin synthesis of the new DNA strand. The requirement for a free 3' hydroxyl group is fulfilled by the RNA primers that are synthesized at the initiation sites by these enzymes.**

- **DNA replication has three stages: Initiation, Elongation and Termination**

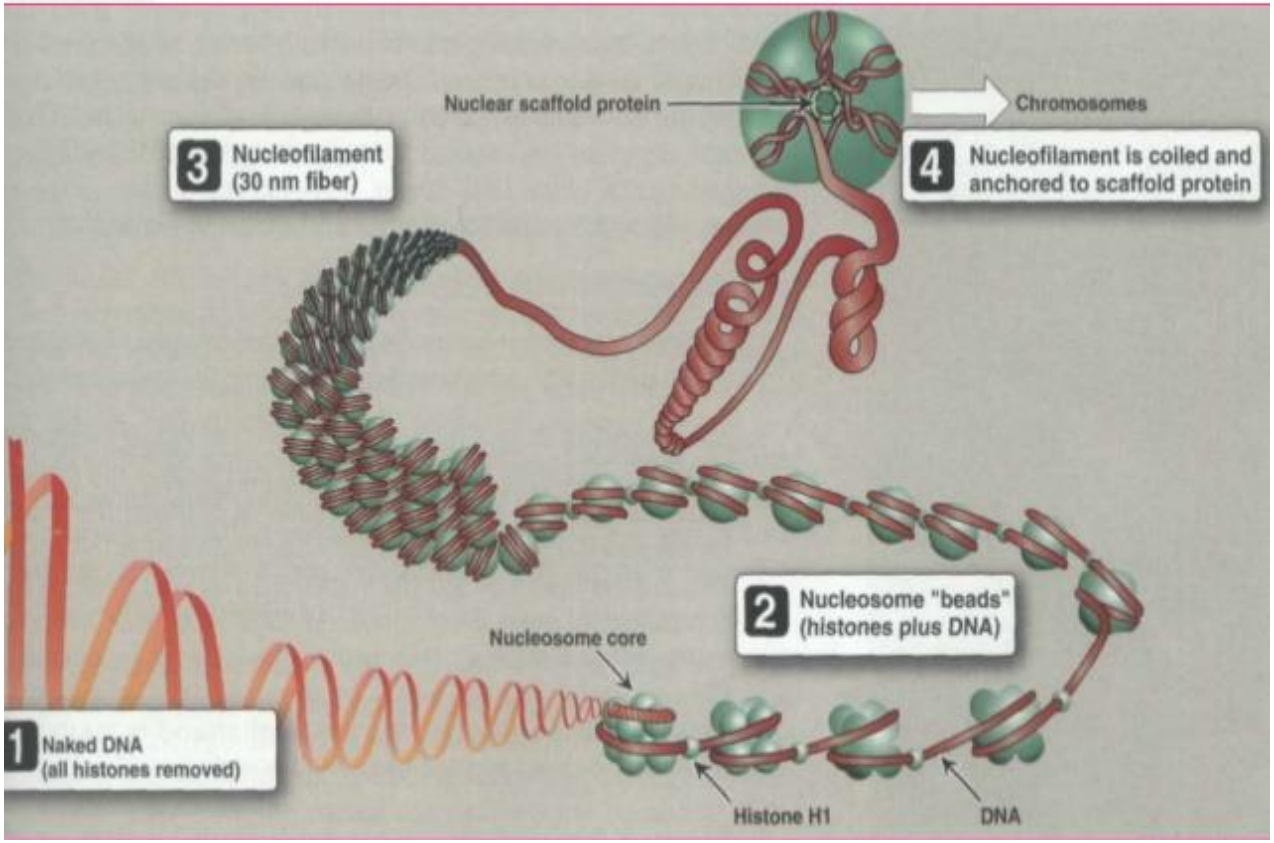
- **Initiation**
  - **Initiation in prokaryotes**
  - **Initiation in eukaryotes**
- **Elongation**
  - **The Leading Strand**
  - **The Lagging Strand**
- **Termination of Replication**

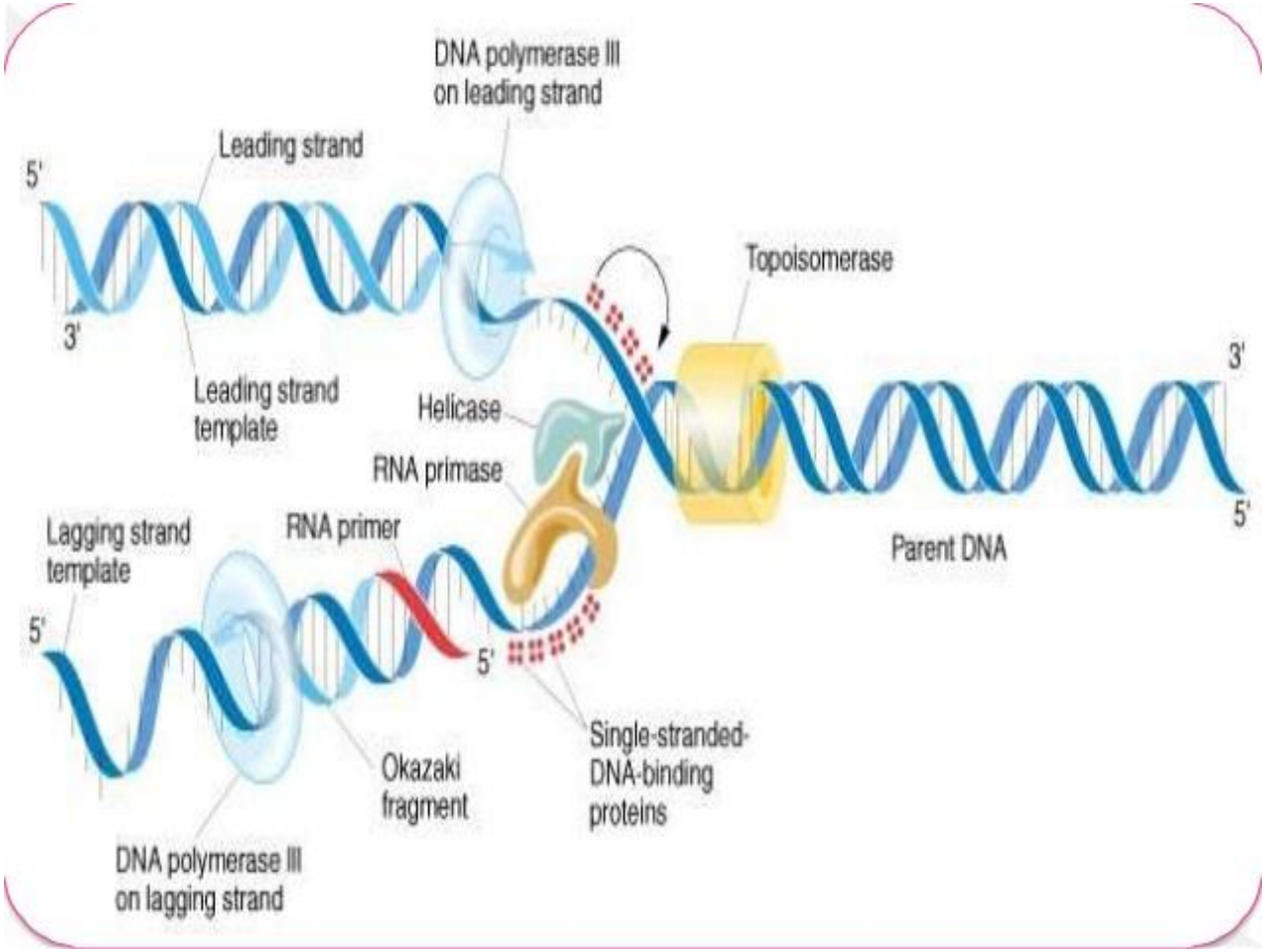
- **Importance of Proof Reading DNA and Repair of Damaged DNA.**

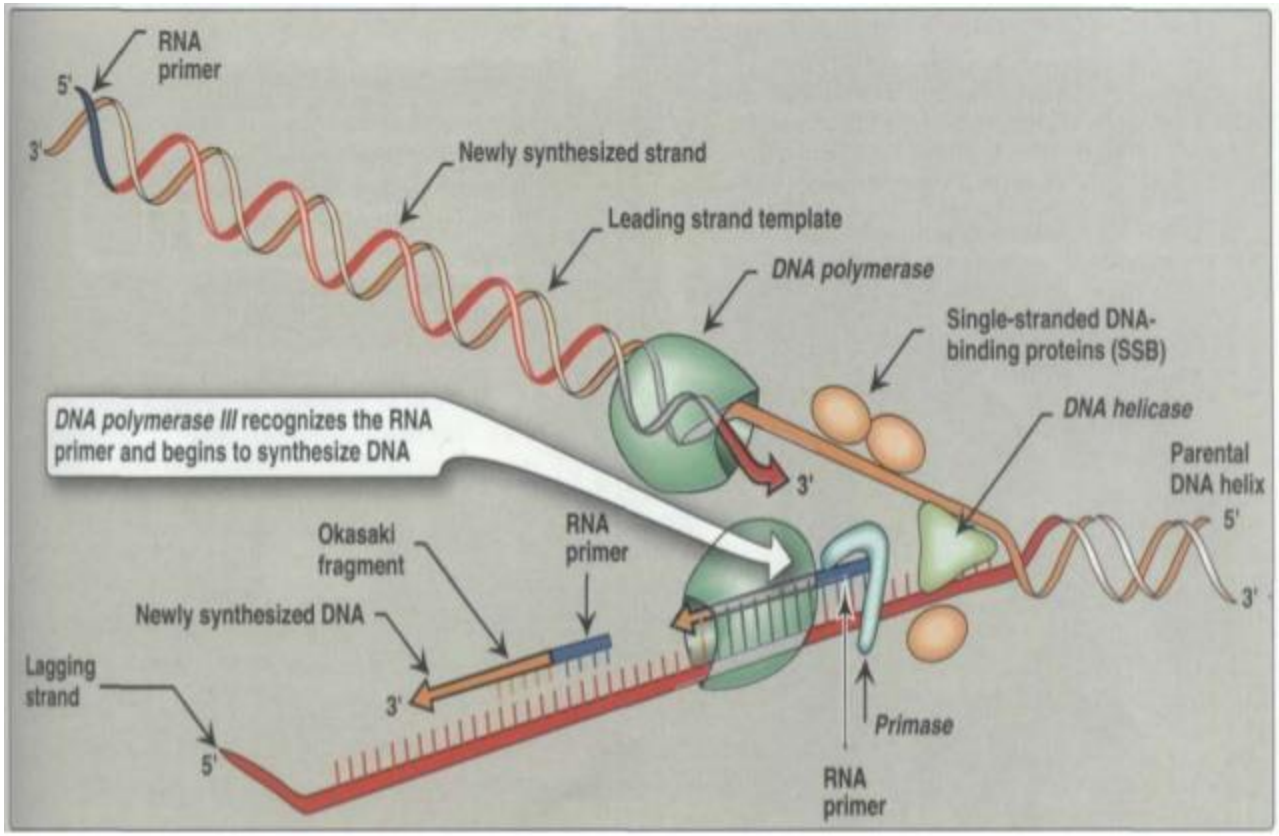
- **Differences between Prokaryotes and Eukaryotes Replication.**

- **Differences between formation of Leading Strand and Lagging Strand.**

Structural Organization of eukaryotic DNA




DNA polymerase III
on leading strand
Leading strand
5'
3'
Leading strand
template
Topoisomerase
3'
Helicase
RNA primase
5'
Parent DNA
Lagging strand
template
RNA primer
5'
5'
Single-stranded-
DNA-binding
proteins
Okazaki
fragment
DNA polymerase III
on lagging strand



Elongation of leading & lagging strands

One of two strands of DNA found at the replication fork, being replicated continuously	The other strand fund at the replication fork, replicating discontinuously from the 5' to the 3' direction
Grows continuously	Grows discontinuously by forming Okazaki fragments
Opens up in the 3’ to 5’ direction	Opens up in the 5’ to 3’ direction
Grows in the 5’ to 3’ direction	Grows in the 3’ to 5’ direction
Requires a single primer for the synthesis	Requires a new primer to start each Okazaki fragment
Starts to grow at the beginning of replication	Starts to replicate a bit later
Grows towards the replication fork	The Okazaki fragments grow away from the replication fork
Forms at high speed	Forms slwoly
Does not require DNA ligase	Requires DNA ligase to ligate Okazaki fragments together

Thanks for your listening

Dr. Ayad