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Unit II

Eukaryotic Replication

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- Eukaryotic DNA is a double-stranded, linear molecule containing 6.4 billion base pairs. The entire genome can replicate in about 1 to 2 hours.

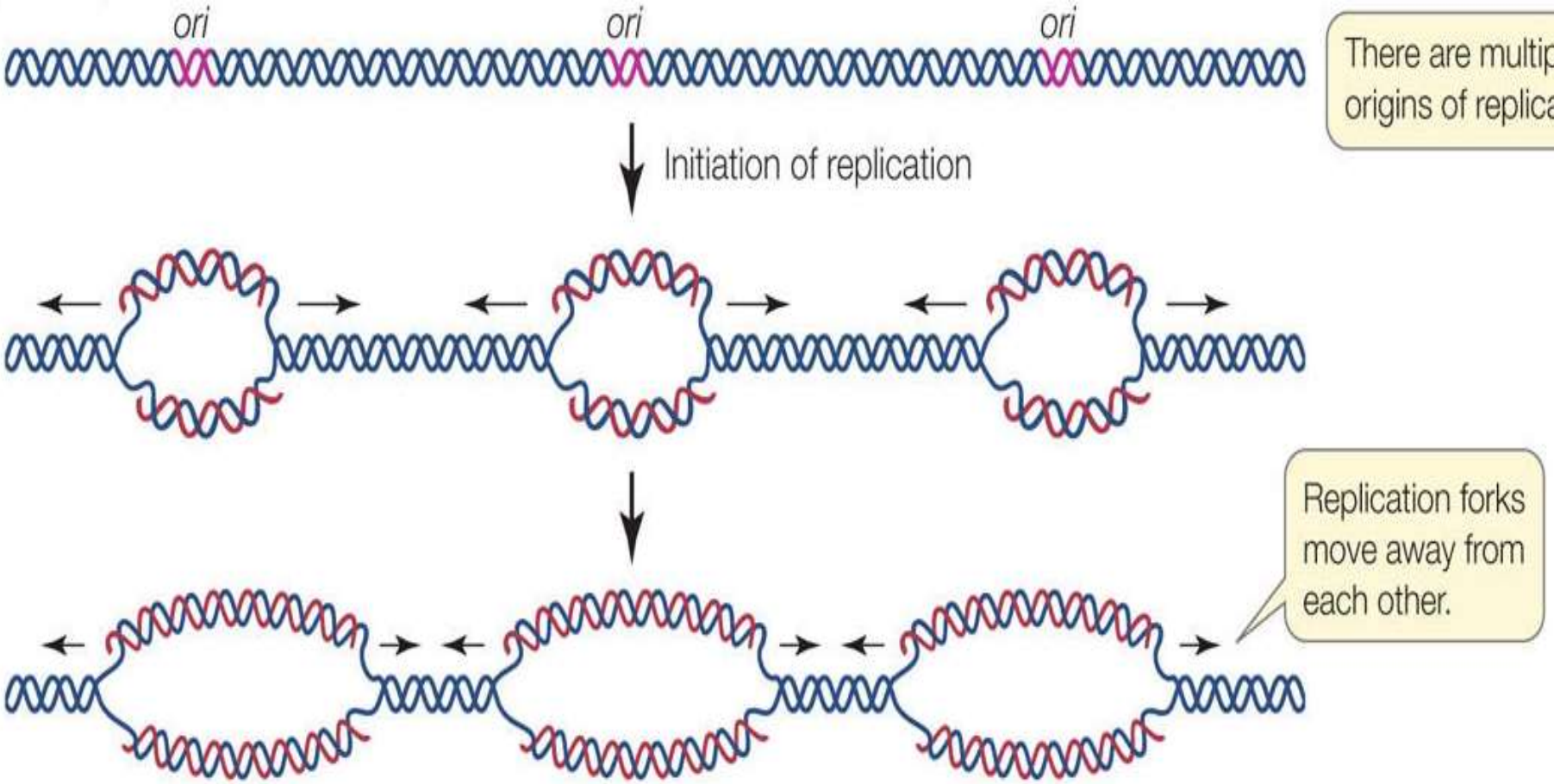
- There are five distinct types of DNA polymerases in eukaryotic cells:
- **DNA polymerase α**: This enzyme synthesizes the RNA primer for both the leading and lagging strands during DNA replication.
- **DNA polymerase β**: Primarily involved in DNA repair, similar to DNA polymerase II in prokaryotes.

- **DNA polymerase γ**: Responsible for the replication of mitochondrial DNA.
- **DNA polymerase δ**: Comparable to DNA polymerase III in prokaryotes, this enzyme synthesizes both the leading and lagging strands, with built-in proofreading ability. It can add about 100 bases per second.
- **DNA polymerase ε**: Functioning similarly to DNA polymerase I in prokaryotes, it removes RNA primers from Okazaki fragments and synthesizes the DNA strand on the lagging strand, also with proofreading activity.

- DNA replication in eukaryotes occurs in three main stages:
- 1) Initiation,
- 2) Elongation, and
- 3) Termination.

Initiation

- Eukaryotic DNA replication begins at multiple origins of replication, which are spaced between 30 and 300 kilobase pairs (kbp) apart.
- In humans, approximately 30,000 replication origins are needed, with each chromosome containing several hundred origins that replicate DNA bidirectionally.
- Each origin of replication is considered a replicon, a unit of replication.



Eukaryotic Replication

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- To ensure that each sequence is replicated only once, complex mechanisms are in place. DNA replication in eukaryotes is tightly coordinated with the cell cycle, specifically with DNA synthesis and cell division (mitosis).
- The cell cycle includes several checkpoints that regulate the progression through the cycle, ensuring that DNA replication is fully completed before the cell moves into the next phase.

- The origins of replication are not fully understood in higher eukaryotes, but in yeast, the DNA sequence known as an **autonomously replicating sequence (ARS)** consists of an AT-rich region with specific sites.
- The ARS functions as a docking site for the **origin of replication complex (ORC)**, which is made up of six proteins with a total mass of about 400 kDa.

- The ORC recruits additional proteins to form the **prereplication complex**.
- Some of these recruited proteins are termed **licensing factors** because they enable the formation of the initiation complex, ensuring that each replicon is replicated only once during the cell cycle.

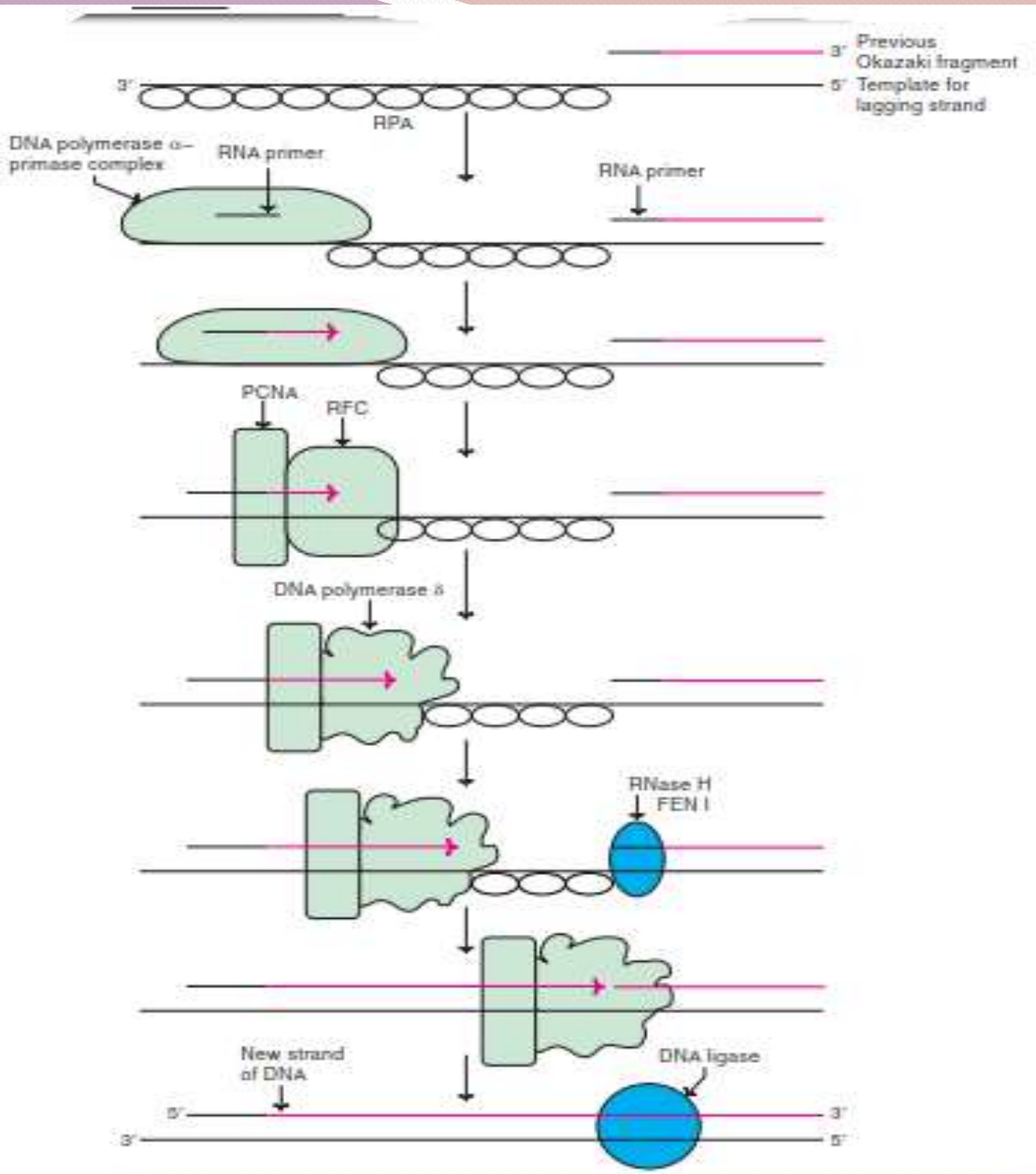
- After the licensing factors have formed the initiation complex, they are tagged for destruction with ubiquitin and subsequently degraded by the proteasome.
- DNA helicases then unwind the parental DNA strands, and the single strands are stabilized by **replication protein A (RPA)**, a single-stranded DNA-binding protein.
- Replication begins when **DNA polymerase α**, the initiator polymerase, binds to the DNA. This enzyme has primase activity, which synthesizes RNA primers, and DNA polymerase activity, though it lacks exonuclease activity. Once about 20 deoxynucleotides are added to the primer.

Elongation

- Elongation starts with the binding of another protein, **replication factor C (RFC)**, displaces DNA polymerase α and recruits **proliferating cell nuclear antigen (PCNA)**.
- PCNA, which is homologous to the β2 subunit of **E. coli polymerase III**, then binds to **DNA polymerase δ**. This interaction enhances polymerase δ's processivity, enabling it to efficiently replicate long stretches of DNA. This transition from DNA polymerase α to DNA polymerase δ is known as **polymerase switching**.
- Polymerase δ possesses 3' to 5' exonuclease activity, allowing it to proofread and correct errors in the newly synthesized DNA. Replication proceeds bidirectionally from the origin of replication until adjacent replicons converge and fuse.

- Okazaki fragments in eukaryotes are 150 – 250 bp in length. After the synthesis of Okazaki fragments , RNA primer hybrid is removed by 2 enzymes, Rnase H and FEN I (flap endonuclease I).
- Rnase H hydrolysis RNA – DNA hybrid.
- FEN I cuts the bend where RNA peeled from the template.

- The RNA primers are then removed, and the gap is filled by DNA polymerase ε.
- The gap between the DNA synthesised by DNA polymerase δ and DNA polymerase ε (short DNA fragments) are ligated by **DNA ligase**.



. An outline of DNA replication on the lagging strand in eukaryotes (RPA–Replication protein A; PCNA–Proliferating cell nuclear antigen; RFC–Replication factor C; RNase H–Ribonuclease H; FEN I–Flap endonuclease I; **Note :** Leading strand not shown).

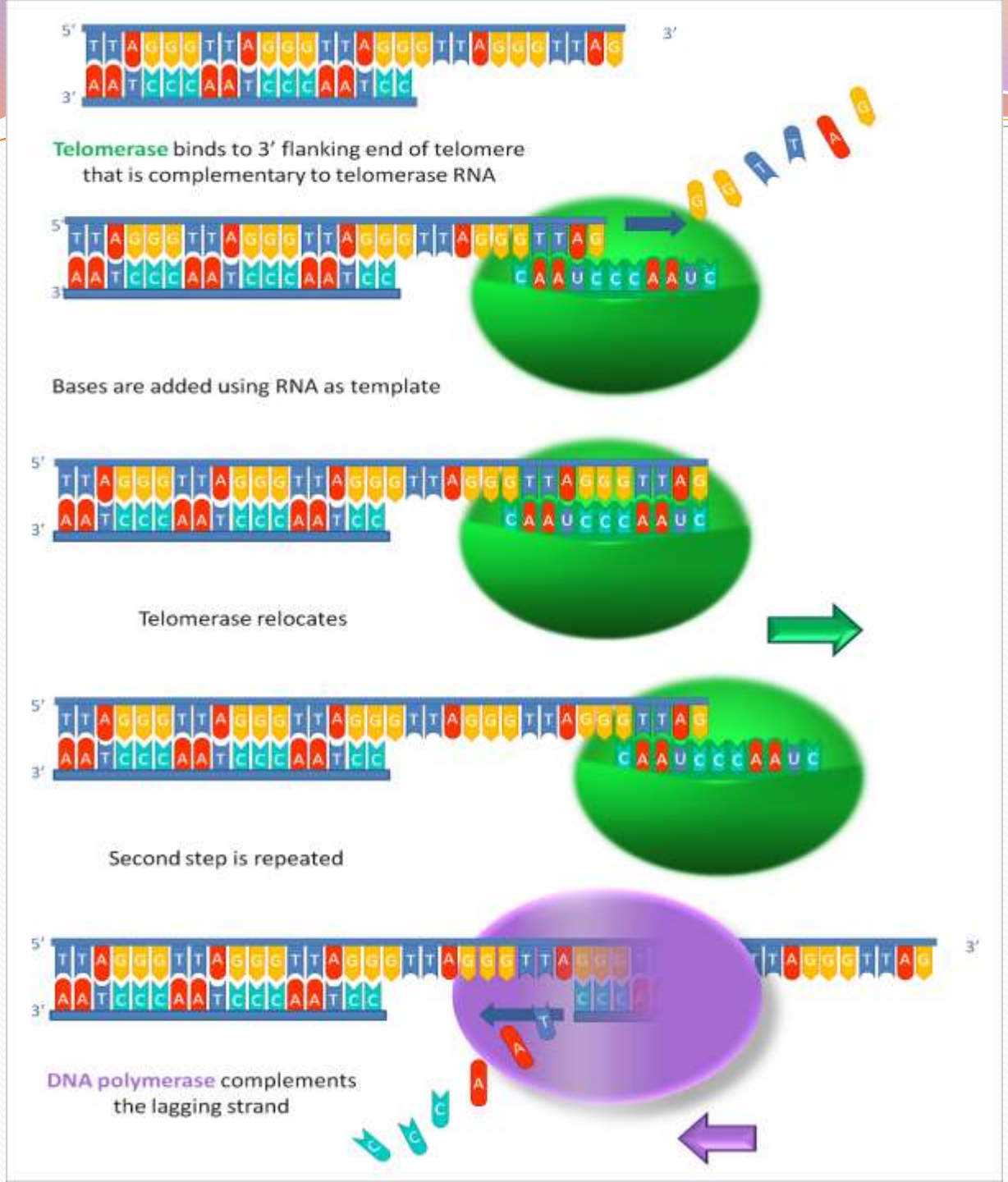
Termination

- Termination of eukaryotic DNA has some different process because of linear chromosome.
- The ends of linear chromosomes are called Telomeres.
- The leading strand is completely synthesized till the end of the template strand whereas the ends of lagging strand have some gap due to the removal of RNA primer, cannot be filled with deoxyribonucleotides. This will lead to shortened chromosomes in daughter cells.

- Telomeres are repetitive six nucleotide sequnces TTAGGG.
- Telomerase or Telomere terminal transferase enzymes that maintains the telomeres to keep the chromosome intact and functional.
- Telomerase is a special kind of reverse transcriptase that carries its own RNA template of about 450 nucleotide long with 3'- CUAACCCUAAC – 5' sequence complementary to the Telomere repeat sequence 5' – TTAGGG-3'.

- Telomerase extends DNA by adding back telomeric DNA at the end of chromosomes by compensating the loss of telomeres occurs as cell divide.
- The telomerase RNA base pairs with the telomeres of DNA molecule and extends to a small distance. Then telomerase translocates and fresh extension of DNA occurs.
- This process of DNA synthesis and translocation occurs several times until required extension of chromosome occurs.
- Then the elongated new strand is sealed by DNA ligase.

- Eukaryotic DNA after replication binds to a octomer core of histones (two each of four histone classes H2A, H2B, H3, H4) to form nucleosomes.
- The nucleosomes inturn organize into chromosomes.



Telomerase

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