DNA Replication in Eukaryotes

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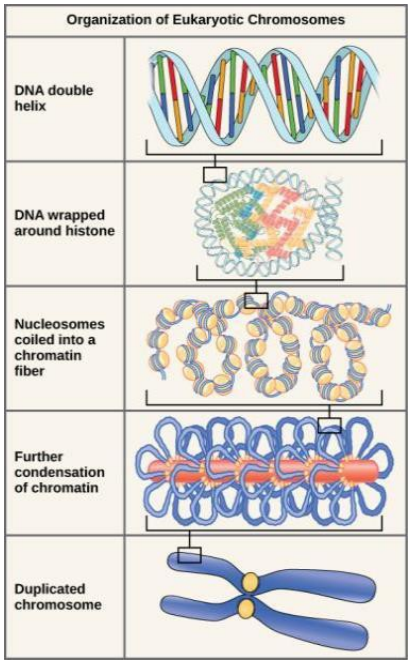


- These figures illustrate the compaction of the eukaryotic chromosome.

SEQUENTIAL STEPS IN EUKARYOTIC DNA REPLICATION

DNA replication is a very complicated process that involves several enzymes and other proteins. It occurs in following stages

- Pre-initiation
- Initiation
- Elongation
- Termination
- Telomerase function

PRE-INITIATION

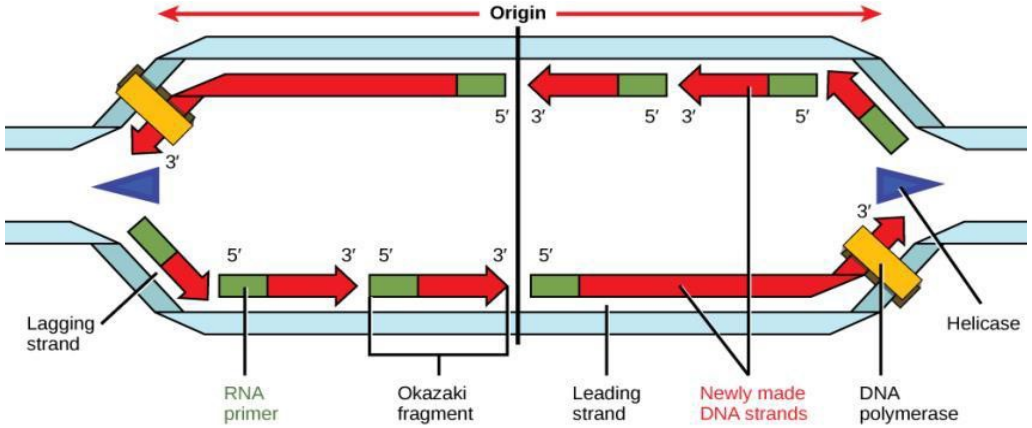
Actually during pre-initiation stage, replicator selection occurs. Replicator selection is the process of identifying the sequences that will direct the initiation of replication and occur in G1 phase (prior to S phase). This process leads to the assembly of a multiprotein complex at each replicator in the genome. Origin activation only occurs after cells enter S phase and triggers the Replicator – associated protein complex to initiate DNA unwinding and DNA polymerase recruitment.

Initiation of Replication

- Replication begins with assembly of the prereplication complex (preRC)

  Consist of 14 different proteins. 6 of them called **origin replication complex**.
- An important part of this is the Origin recognition complex (ORC)
  - A six-subunit complex that acts as the initiator of eukaryotic DNA replication
  - DNA replication at the origin begins with the binding of ORC, which usually occurs during G1 phase.
  - Other preRC proteins bind including MCM helicase

Elongation



- A replication fork is formed by the opening of the origin of replication, and helicase separates the DNA strands. An RNA primer is synthesized and elongated by the DNA polymerase. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches. The DNA fragments are joined by DNA ligase (not shown).

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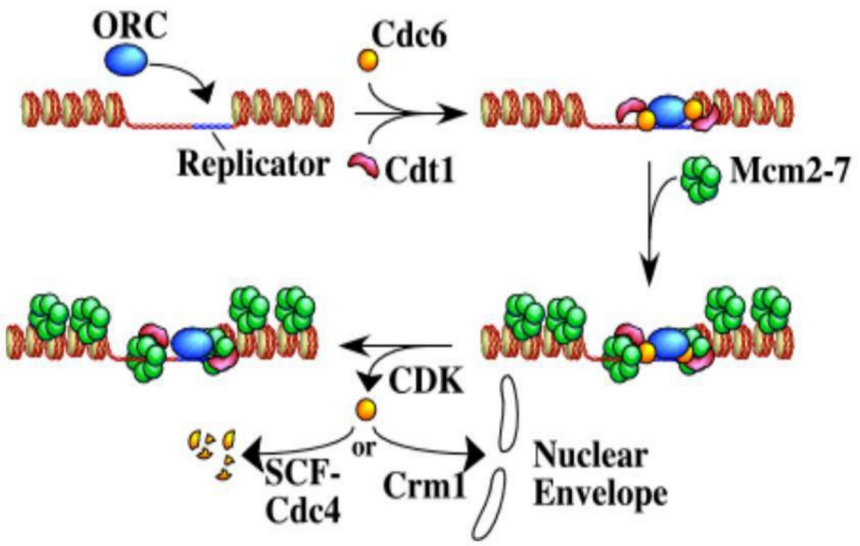
Formation of pre-Replicative complex



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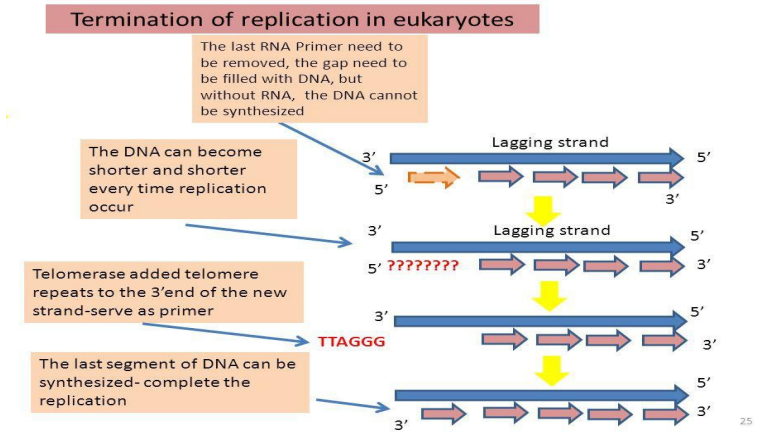
Termination of replication in eukaryotes



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In the leading strand, synthesis continues until the end of the chromosome is reached. On the lagging strand, DNA is synthesized in short stretches, each of which is initiated by a separate primer. When the replication fork reaches the end of the linear chromosome, there is no place for a primer to be made for the DNA fragment to be copied at the end of the chromosome. These ends thus remain unpaired, and over time these ends may get progressively shorter as cells continue to divide. This is known as **end replication problem.**

The telomerase enzyme contains a catalytic part and a built in RNA template. It attaches to the end of the chromosome, and complementary bases to the RNA template are added on the 3′ end of the DNA strand. Once the 3′ end of the lagging strand template is sufficiently elongated, DNA polymerase can add the nucleotides complementary to the ends of the chromosomes. Thus, the ends of the chromosomes are replicated.

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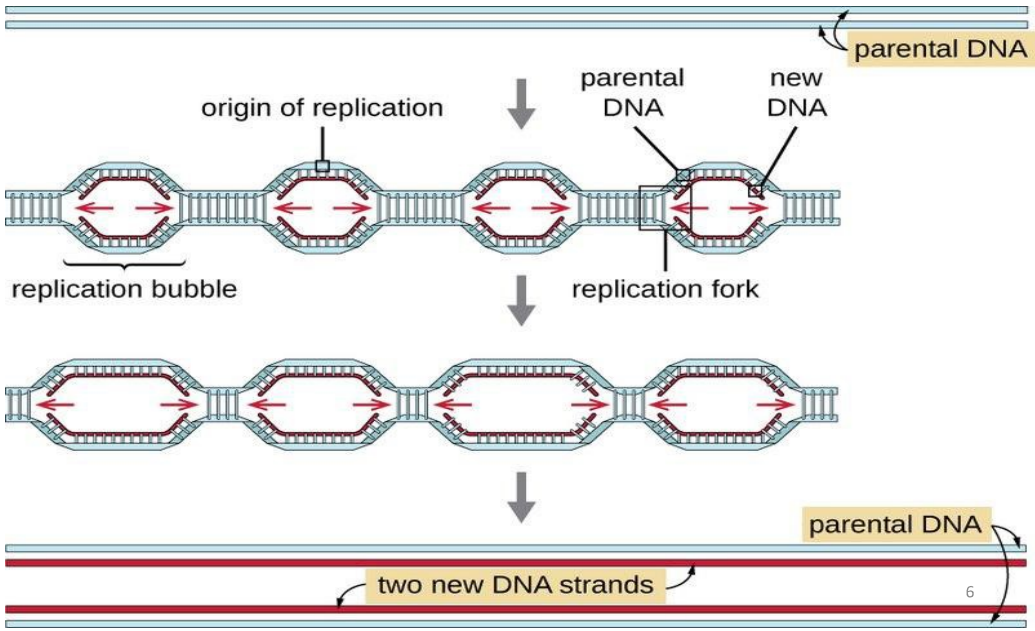
Multiple Origins of Replication



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DNA Replication Through Histones

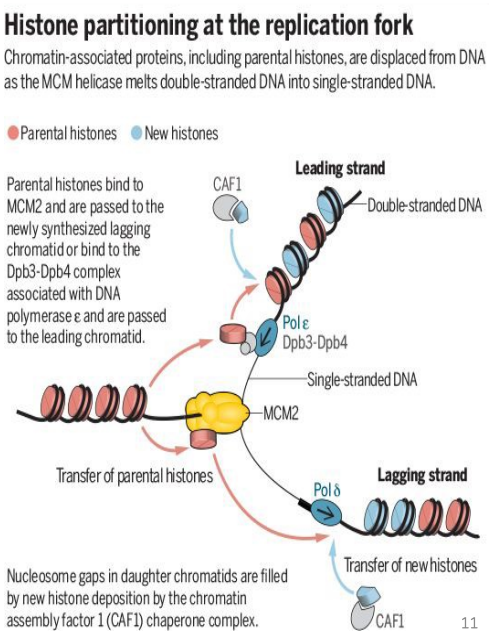
Histone Dissociation and Association

Since DNA is present in packaged form as chromatin, DNA replication is sandwiched between two additional steps in eukaryotes.

1. Carefully ordered and in complete dissociation of the chromatin.
2. Re-association of DNA with the histone octomers to form nucleosome.

Dissociation of histone: methylation at the fifth position of cytosine residues by a DNA methyl transferase appears to functioning by loosening up the chromatin structure. This allows DNA access to proteins and enzymes needed for DNA replication.

Synthesis of histone: the synthesis of new histone occurs simultaneously with DNA replication.

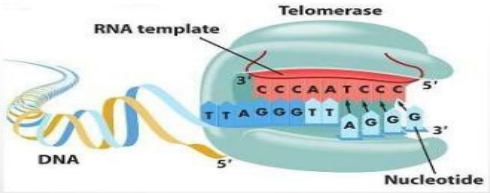


- DNA replication-coupled (RC) nucleosomeDNA replication-coupled (RC) nucleosome assembly is mediated by histoneDNA replication-coupled (RC) nucleosome assembly is mediated by histone chaperones and is fundamental for epigenetic inheritance and maintenance of genomic integrity. The mechanisms that promote this process are only partially understood.
- The histone chaperone FACT (facilitates chromatin transactions), promotes newly synthesized histone H3-H4 deposition. It collaborates with
- CAF-1 and Rtt106 in RC nucleosome assembly.

Telomerase:

- Telomerase is ribonucleoprotein. It contains a RNA component which has repeat of 9 to 30 nucleotides long. This RNA component serves as the template for the synthesis of telomeric repeats at the parental DNA ends.
- Telomerase is a RNA dependent DNA polymerase with a RNA component.

Telomerase uses the
- 3′ end of parental DNA strand as primer,
- RNA component of telomerase as template,
- adds successive telomeric repeats to the parental DNA strand at its 3′ end due to its 5′-3′ RNA dependent DNA polymerase activity.



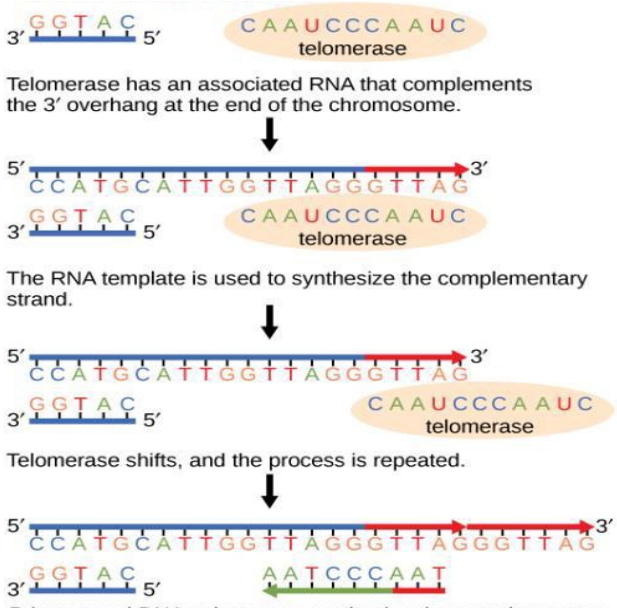
Eukaryotic enzymes:

Five common DNA polymerases from mammals.

1. **DNA Polymerase α (alpha): is responsible for synthesis of RNA primer for both leading and lagging strands of DNA**
2. **DNA Polymerase β (beta): nuclear, DNA repair.**
3. **DNA Polymerase γ (gamma): mitochondria, DNA repl., proofreading**
4. **DNA Polymerase δ (delta): nuclear, DNA replication on the leading strand, and proofreading function.**
5. **DNA Polymerase ε (epsilon): nuclear , DNA synthesis on the lagging strand and proofreading function**

- **Different polymerases for the nucleus and mt DNA**
- **Some polymerases proofread; others do not.**
- **Some polymerases used for replication; others for repair.**

Replication of Telomeres



The ends of linear chromosomes are maintained by the action of the telomerase enzyme.

Comparision of Replication

Step in Replication	Prokaryotic cells	Eukaryotic cells
Recognition of origin of replication	Dna A protein	Unknown
Unwinding of DNA double helix	Helicase (requires ATP)	Helicase (requires ATP)
Stabilization of unwound template strands	Single-stranded DNA-binding protein (SSB)	Single-stranded DNA-binding protein (SSB)
Synthesis of RNA primers	Primase	Primase
Synthesis of DNA Leading strand Lagging strand	 DNA polymerase III DNA polymerase III	 DNA polymerase δ DNA polymerase α
Removal of RNA primers	DNA polymerase I (5 →3' exonuclease)	Unknown
Replacement of RNA with DNA	DNA polymerase I	Unknown
Joining of Okazaki fragments	DNA ligase (requires NAD)	DNA ligase (requires ATP)
Removal of positive supercoils ahead of advancing replication forks	DNA topoisomerase II (DNA gyrase)	DNA topoisomerase II

Thank you