Assignment Solutions

DNA Replication in Eukaryotes

DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule. This process is essential for cell division and occurs during the S phase of the cell cycle. Eukaryotic DNA replication is more complex than prokaryotic replication due to larger genomes, linear chromosomes, multiple origins of replication, and the presence of histones.

1. Steps Involved in Eukaryotic DNA Replication

1. **Initiation:**
    - **Origin Recognition:** Eukaryotic chromosomes have multiple origins of replication (Ori). The Origin Recognition Complex (ORC) binds to these specific DNA sequences.
    - **Pre-Replication Complex (pre-RC) Formation:** In the G1 phase, ORC recruits Cdc6 (Cell division cycle 6) and Cdt1 (Chromatin licensing and DNA replication factor 1), which then load the Mini Chromosome Maintenance (MCM) complex (a helicase) onto the DNA. This forms the pre-RC, licensing the origin for replication.
    - **Origin Firing (Activation):** In the S phase, cyclin-dependent kinases (CDKs) and Dbf4-dependent kinase (DDK) phosphorylate components of the pre-RC (ORC, Cdc6, MCM). This phosphorylation activates the MCM helicase, unwinding the DNA at the origin.
    - **Loading of Replication Proteins:** Activated origins recruit other replication factors, including RPA (Replication Protein A), DNA polymerase α-primase, and other accessory proteins.
2. **Elongation:**
    - **Primer Synthesis:** DNA polymerase α-primase synthesizes short RNA primers (10-12 nucleotides) on both leading and lagging strands. The α subunit synthesizes a short DNA extension after the RNA primer.
    - **Leading Strand Synthesis:** DNA polymerase ε (epsilon) takes over from polymerase α and synthesizes the leading strand continuously in the 5' to 3' direction, moving towards the replication fork. PCNA (Proliferating Cell Nuclear Antigen), a sliding clamp, encircles the DNA and helps tether DNA polymerase to the template, increasing its processivity.
    - **Lagging Strand Synthesis (Okazaki Fragments):** DNA polymerase δ (delta) takes over from polymerase α on the lagging strand. It synthesizes short DNA fragments (Okazaki fragments, typically 100-200 nucleotides long in eukaryotes) discontinuously in the 5' to 3' direction, moving away from the replication fork. Each fragment requires a new RNA primer. PCNA also assists DNA polymerase δ.
    - **Primer Removal and Gap Filling:** When DNA polymerase δ encounters the RNA primer of the preceding Okazaki fragment, the primer is removed by RNase H1 and FEN1 (Flap Endonuclease 1). RNase H1 removes most of the RNA, and FEN1 removes the last

ribonucleotide and any associated DNA flap. The resulting gap is then filled by DNA polymerase δ.

- **Ligation:** After the gap is filled, DNA ligase seals the nicks between adjacent Okazaki fragments, forming a continuous strand.
- **Chromatin Assembly:** As new DNA is synthesized, it is immediately re-packaged into chromatin by the assembly of new histones and the redistribution of parental histones. CAF-1 (Chromatin Assembly Factor 1) is involved in loading new histones onto the newly synthesized DNA.

3. **Termination:**
    - **Replication Fork Collision:** Replication forks originating from adjacent origins eventually meet and merge.
    - **Decatenation:** When two replication forks meet, the newly synthesized DNA molecules are still intertwined (catenanes). Topoisomerase II separates these intertwined DNA molecules.
    - **Telomere Replication:** Due to the linear nature of eukaryotic chromosomes and the inability of DNA polymerase to synthesize to the very end of the lagging strand template, telomeres (repetitive sequences at chromosome ends) shorten with each replication cycle. This shortening is counteracted by the enzyme telomerase, a reverse transcriptase that adds telomeric repeats to the 3' ends of chromosomes, preventing gene loss.

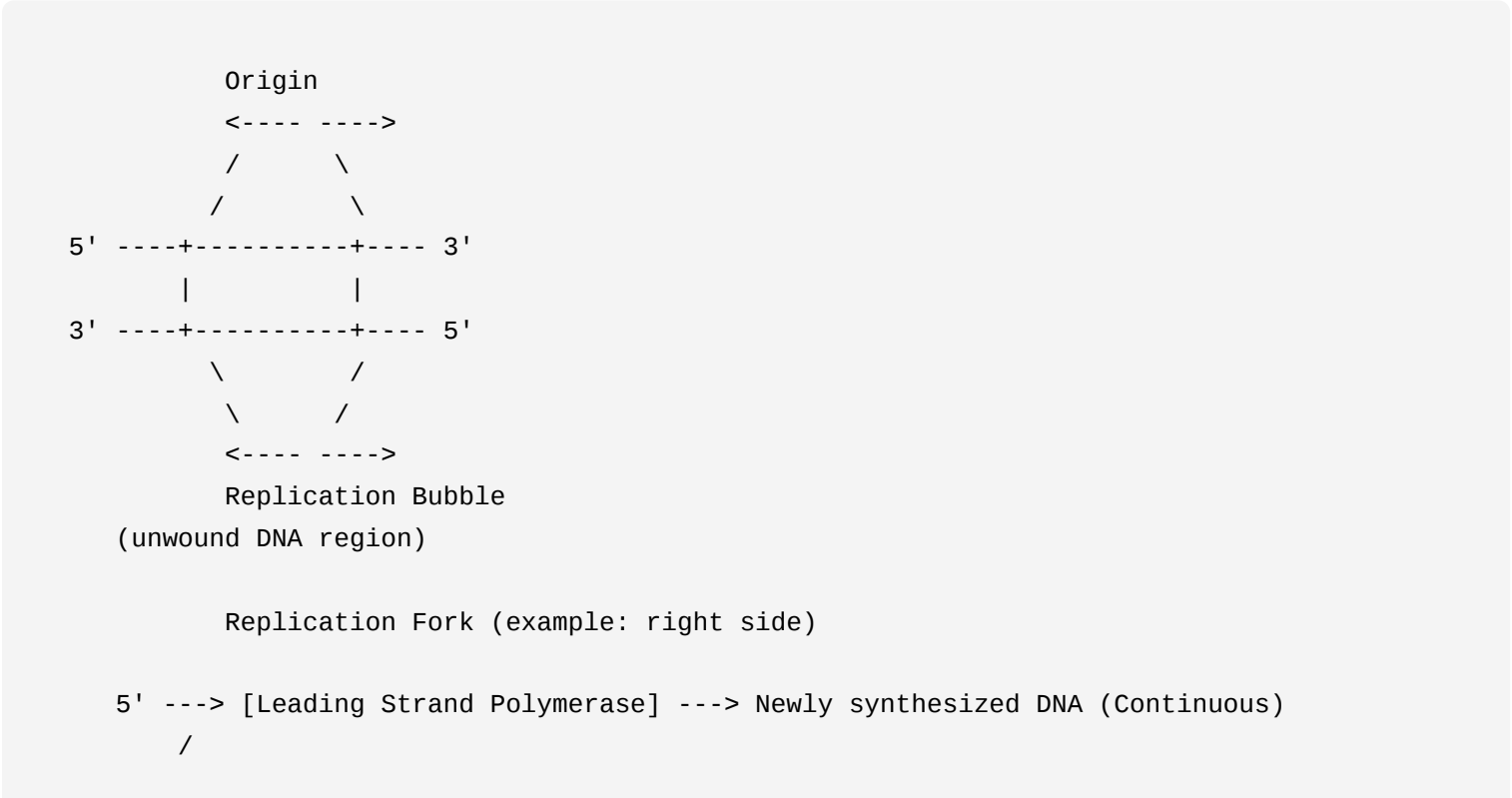
2. Enzymes and Proteins Involved in Eukaryotic DNA Replication

Enzyme/Protein	Function
Origin Recognition Complex (ORC)	Binds to origins of replication to initiate DNA replication.
Cdc6, Cdt1	Recruit and load the MCM helicase onto the origin, forming the pre-RC.
MCM Complex (Helicase)	Unwinds the DNA double helix at the replication fork, separating the two strands.
Topoisomerases (e.g., Topoisomerase I, II)	Relieve supercoiling tension ahead of the replication fork by making transient cuts in the DNA. Topoisomerase II also decatenates daughter chromosomes.
Replication Protein A (RPA)	Single-stranded DNA-binding proteins (SSBs) that bind to the separated DNA strands, preventing them from re-annealing and protecting them from degradation.
DNA Polymerase α-primase	Synthesizes short RNA primers and a short DNA extension to initiate DNA synthesis on both leading and lagging strands. Has low processivity.

DNA Polymerase ε (epsilon)	The primary enzyme for leading strand synthesis. High processivity.
DNA Polymerase δ (delta)	The primary enzyme for lagging strand synthesis and fills gaps after primer removal. High processivity.
PCNA (Proliferating Cell Nuclear Antigen)	A sliding clamp that encircles the DNA and increases the processivity of DNA polymerases δ and ε.
RFC (Replication Factor C)	A clamp loader that loads PCNA onto the DNA.
RNase H1	Removes most of the RNA primer nucleotides.
FEN1 (Flap Endonuclease 1)	Removes the last ribonucleotide of the primer and any displaced DNA flaps.
DNA Ligase	Seals nicks in the phosphodiester backbone of DNA, joining Okazaki fragments.
Telomerase	A specialized reverse transcriptase that extends telomeres at chromosome ends by synthesizing new telomeric repeats.
CAF-1 (Chromatin Assembly Factor 1)	Delivers new histones to the replication fork for nucleosome assembly on newly synthesized DNA.

3. Diagrammatic Representation (Conceptual)

Imagine a "replication bubble" forming at an origin of replication, expanding outwards in two directions, forming two "replication forks."



```
      /  [MCM Helicase]
  Template DNA --->
      \  [SSBs/RPA]
       \
    3' ---> [Lagging Strand Template]
          <--- [Okazaki Fragment 3] <--- [Pol δ]
               <--- [Okazaki Fragment 2] <--- [Pol δ]
                    <--- [Okazaki Fragment 1] <--- [Pol δ]
                    (Discontinuous synthesis away from fork)

Key:
-   Long lines represent parental DNA strands.
-   Arrows indicate the direction of DNA synthesis or enzyme movement.
-   Newly synthesized DNA is shown by shorter lines or brackets.
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4. Regulatory Mechanisms

Eukaryotic DNA replication is tightly regulated to ensure that the genome is replicated only once per cell cycle, preventing re-replication and maintaining genome stability.

- **Cell Cycle Control:**
    - **Licensing (G1 Phase):** Origins are "licensed" for replication during G1 by the formation of the pre-RC (ORC + Cdc6 + Cdt1 + MCM). This process makes origins competent to initiate replication.
    - **Firing (S Phase):** The licensed origins "fire" during S phase, driven by the activity of CDKs (Cyclin-Dependent Kinases) and DDKs (Dbf4-Dependent Kinases). These kinases activate the MCM helicase and recruit other replication factors.
    - **Prevention of Re-replication:** CDKs also prevent re-replication by phosphorylating ORC, Cdc6, and Cdt1. Phosphorylation of Cdc6 and Cdt1 leads to their degradation or nuclear export, preventing re-loading of MCM onto origins until the next G1 phase. ORC phosphorylation inhibits its activity.
- **DNA Damage Checkpoints:**
    - If DNA damage is detected during replication, cell cycle checkpoints (e.g., S-phase checkpoint) are activated. These checkpoints, primarily involving ATM and ATR kinases, halt DNA synthesis, allowing time for repair mechanisms to fix the damage. This prevents replication of damaged DNA, which could lead to mutations or chromosomal aberrations.
- **Chromatin Structure:**
    - Eukaryotic DNA is packaged into chromatin. The accessibility of origins of replication is influenced by chromatin structure (histone modifications, nucleosome positioning). Euchromatin (less condensed) regions tend to replicate earlier than heterochromatin (more condensed) regions.

5. Etc. (Additional Considerations)

- **Multiple Origins:** Eukaryotic chromosomes are much larger than prokaryotic ones, so replication starts at multiple origins simultaneously to complete DNA synthesis within a reasonable timeframe.
- **Fidelity of Replication:** DNA replication is highly accurate due to:
    - **Proofreading:** DNA polymerases have 3' to 5' exonuclease activity, allowing them to remove incorrectly incorporated nucleotides.
    - **Mismatch Repair:** A post-replication repair system that corrects errors that escape proofreading.
- **Telomere Replication and End-Replication Problem:** As mentioned in termination, telomeres address the "end-replication problem" unique to linear chromosomes. Without telomerase, chromosomes would progressively shorten with each division, leading to loss of genetic information and cellular senescence. Telomerase activity is often repressed in somatic cells but active in germ cells, stem cells, and cancer cells.
- **Histone Management:** During replication, nucleosomes must be disassembled ahead of the replication fork and reassembled immediately behind it on the newly synthesized DNA. Parental histones are distributed to both daughter strands, and new histones are synthesized and incorporated.