lecture 2

Comprehensive DNA Replication Notes
Initiation of Replication

- **Origins of Replication**: DNA replication starts at designated origins. Prokaryotes typically have a single origin (e.g., oriC in *E. coli*), while eukaryotes possess multiple origins along their larger genomes.
- **Initiator Proteins**: In *E. coli*, the protein DnaA recognizes oriC, while in eukaryotes, the process involves the ORC and additional factors like Cdc6 and Cdt1 to prepare for helicase loading.
- **ATP Consumption in Initiation**: The initiator protein DnaA in prokaryotes requires ATP binding and hydrolysis to facilitate the unwinding of the oriC region. In eukaryotes, ATP is also essential for the activity of ORC, Cdc6, and Cdt1 during the loading of the MCM helicase complex.

Helicase and Unwinding

- **Prokaryotes**: DnaC assists DnaB helicase to bind and unwind the DNA at oriC.
- **Eukaryotes**: The MCM helicase complex unwinds the DNA at origins activated by the initiation factors.
- **SSBs/RPA**: In prokaryotes, SSB proteins coat ssDNA; in eukaryotes, RPA serves a similar protective role.
- **Helicase Activation**: Both DnaB in prokaryotes and the MCM complex in eukaryotes hydrolyze ATP to drive the unwinding of DNA. This ATPase activity is crucial for the movement of helicase along the DNA, separating the double helix into single strands.

Elongation and Replisome Function

- The replisome is a complex of enzymes and proteins that work together to synthesize DNA.
- **DNA Polymerase III Holoenzyme**: In prokaryotes, this complex contains the core DNA Polymerase III, the β-clamp, clamp loader (γ-complex), and τ subunits, along with the helicase, making up the replisome.**DNA Pol III** is the subunit with the highest processivity (consecutive reactions without releasing its substrate), and exists as part of the Pol III holoenzyme.
- **Clamp Loader Dynamics**: The clamp loader, especially the γ-complex in prokaryotes and RFC in eukaryotes, uses ATP to open the sliding clamp and load it onto DNA. This ATP-dependent mechanism ensures that the clamp is dynamically placed at the primer-template junction for high processivity of DNA polymerase.
- **DNA Polymerases in Eukaryotes**: DNA Pol ε synthesizes the leading strand, and DNA Pol δ is responsible for lagging strand synthesis. DNA Pol α lays down the initial RNA-DNA primer.
- **Polymerase Structure**: DNA polymerases feature a thumb, finger, and palm domain. The palm domain is essential for the catalytic function and checks base pairing, fingers are involved in dNTP selection, and the thumb helps to maintain the DNA-polymerase complex stability.
- **Trombone model** of DNA replication: there are three clamps on the DNA polymerase holoenzyme: In the "trombone" model of DNA replication, DNA Polymerase III on the lagging strand completes the synthesis of an Okazaki fragment and then disengages from the sliding clamp. This sliding clamp, positioned by the clamp loader at the RNA primer, remains affixed to the DNA. Subsequently, another free DNA Polymerase III molecule rapidly recognizes and binds to this clamp, initiating the synthesis of the next Okazaki fragment. This system allows for swift transitions between the synthesis of multiple Okazaki fragments without the polymerase completely dissociating from the DNA template, thereby enhancing replication efficiency. The process is repeated along the lagging strand, enabling continuous and seamless synthesis of new Okazaki fragments. The inherent structure of DNA Polymerase III, with its

finger, palm, and thumb domains, facilitates this process; the palm domain ensures catalytic activity and fidelity, the fingers domain secures the incoming dNTPs, and the thumb domain maintains the enzyme's stability on the DNA.

Leading and Lagging Strand Synthesis

- The leading strand is synthesized continuously by DNA Pol III (prokaryotes) or DNA Pol ε (eukaryotes).
- The lagging strand is synthesized discontinuously in Okazaki fragments, initiated by primers added by DNA primase and extended by DNA Pol III in prokaryotes and by DNA Pol δ in eukaryotes.

RNA Primer Removal and Okazaki Fragment Processing

- **DNA Polymerase I and FEN1**: Prokaryotic DNA Pol I uses its 5'→3' exonuclease activity to remove RNA primers, which does not directly consume ATP. However, eukaryotic FEN1, which helps remove RNA primers, may require ATP for structural changes or coordination with other proteins.
- **Ligation by DNA Ligase**: DNA ligase seals the nicks between Okazaki fragments through an ATP-dependent mechanism. In prokaryotes, ligase uses NAD+ as a cofactor, while eukaryotic DNA ligase uses ATP, catalyzing the formation of a phosphodiester bond and ensuring a continuous DNA strand.

Termination and Proofreading

- Both prokaryotic and eukaryotic DNA polymerases have 3'→5' exonuclease activity for proofreading.
- Termination occurs when converging replication forks meet and DNA synthesis is completed.

Replisome Dynamics

- The replisome coordinates the activities of all these enzymes to ensure efficient replication.
- In the "trombone" model of lagging strand synthesis, the continuous loop formation allows the lagging strand to be synthesized in the same direction as the movement of the replication fork.