Genetics and Molecular Biology: Lecture 4

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- DNA Polymerase can add new nucleotides to the 3' end of a DNA strand (removes triphosphate and adds new nucleotide which moving towards the 5' end of the template strand - 5' to 3' synthesis)
- Limitations of DNA Polymerase:
  - Cannot unwind double-standed DNA
  - Cannot start a chain, only adds nucleotides to existing chains
  - Can only add nucleotides to 3' ends
  - Cannot link existing DNA chains together
- DNA Helicase
  - Unwinds double-stranded DNA
  - Overwinds DNA in front of it (postive supercoiling: requires topoisomerase to reduce strain)
- Primase (a type of RNA polymerase)
  - Builds short RNA strands called primers that DNA polermase can work from to build a DNA strand
  - Binds to template strand and synthesizes an RNA primer
  - When primer is complete, primase is released and DNA polymerase continues synthesis
- Leading Stand vs. Lagging Strand
  - Leading strand: continuous
  - Lagging strand: discontinuous (Okazaki fragments)
  - Fork advancement direction opposite of one strand (lagging strand syntesized in the 3' to 5' direction at a high level - still synthesized 5' to 3' inside fragments)
  - Leading strand syntehsis occurs continuously - synthesized towards the replication Fork
  - Sliding clmap increases "processivity" of DNA polymerase (the ability to keep going without falling off)
  - Prokaryotes: DNA polymerase III beta subunit (sliding clamp), Eukaryotes: Proliferating Cell Nuclear Antigen (PCNA)
  - Lagging strand synthesis occurs discontinuously - synthesized away from the replication Fork
  - Series of segements called Okazaki fragments
  - DNA polymerases replaces RNA primers with DNA

  - Synthesis of individual segements on lagging strand goes away from fork, but fragments closer to the origin are built first
  - Single stranded binding proteins (SSBPs) prevent lagging strand from folding on itelf and blocking replication
  - Single stranded DNA tends to fold back on itself and form base-paired hairpins

- Multiple DNA Polermerases are used in DNA replication
  - DNA polymerase III: main DNA polymerase - 5' to 3' synthesis polymerase, 3' to 5' exonuclease for proofreading (removes incorrect nucleotides)
  - DNA polyermase I: specialzied DNA polymerase - 5' to 3' synthesis polymerase, 5' to 3' exonuclease for replacing RNA primers with DNA, 3' to 5' exonuclease for proofreading
  - "Exonuclease": cuts nucleotides of nucleic acid strands

- DNA Ligase
  - Connects adjacent strands of DNA together to combine Okazaki fragments to form one continuous new strand

- New nucleotides come in as nucleotide triphosphates (dNTPs, like ATP). Los of two phosphate groups releases energy to connect to nucleotide to the growing DNA strand

- Only adding new nucleotides to the 3' end of a strand allows for proofreading and removal of incorrect nucleotides

- Phosphate group is on the 5' end of a nucleotide, hydroxyl group is on the 3' end

- Telomeres and Telomerase
  - End replication problem: Eukaryotes linear choromosomes can never be fully replication due to lagging strand dynamics
  - Chromosomes shorter during each replicative cycle
  - Telomers act as a buffer that postpones erosion of genes
    * Non-protein coding reptitive sequences found at the end of chromosomes
    * Act as a buffer to prevent protein coding genes from being lost
    * Shorter with each replication
    * Limit replicative potential of cells, prevents cancer, but may contribute to aging
  - Telomerase Maintains Telomere Length in Gamete Producing Cells
    * Telomerase: protein-RNA complex, RNA directed, DNA synthesis
    * Telomerase is inappropriately activated in as many as 90% of human cancers