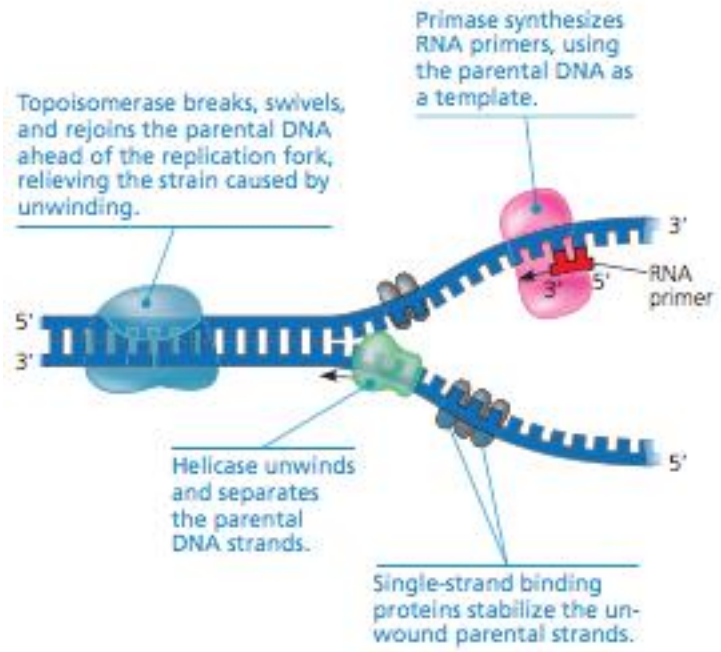
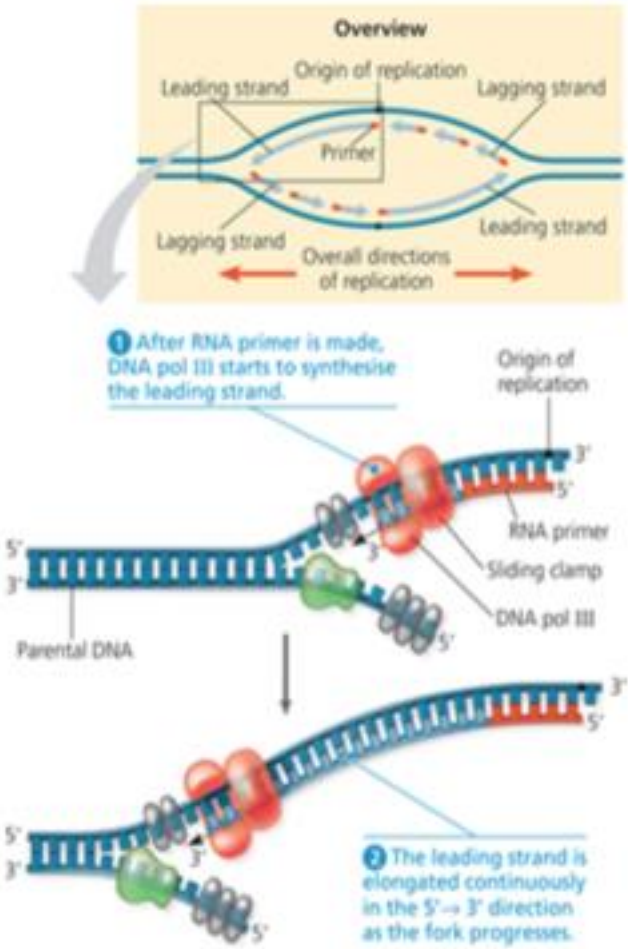
Lectures 10/11, Module 1 - DNA, Protein and the Central Dogma:

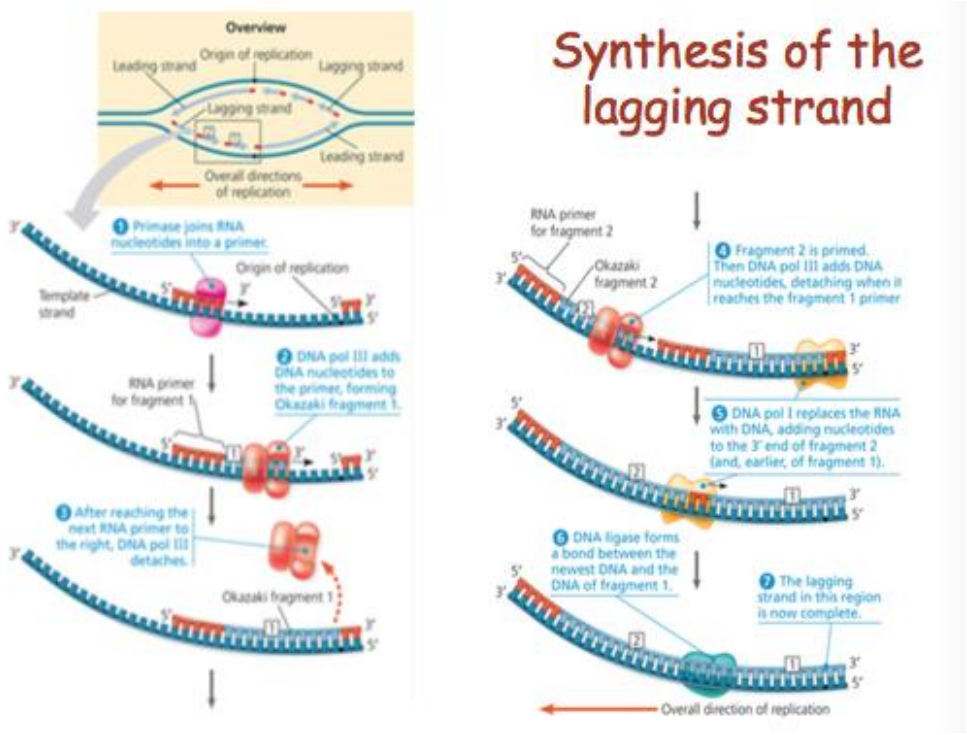
- DNA
  - Genetic material
  - Polymer consisting of nucleotides
    - Nucleotides consist of a nitrogenous base, a pentose sugar (deoxyribose) and a phosphate group
    - Bases can be
      - Adenine (A)
      - Thymine (T)
      - Guanine (G)
      - Cytosine (C)
  - Arranged in a double helix
    - Sugar phosphate backbones covalently bonded to base pairs
      - A-T (joined by 2 hydrogen bonds) and C-G (joined by 3 hydrogen bonds)
      - A and G are purines (two organic rings) and T and C are Pyrimidines (one organic ring)
      - Backbones run antiparallel (in opposite directions)
- DNA Replication
  - Semi-conservative
    - Parental molecules have two complementary strands of DNA, each base pair is joined by hydrogen bonding
    - The strands separate and each parental strand serves as a template for a new strand
    - Complementary nucleotides line up and are connected to form two new double helixes (each consisting of an old parental strand and a new strand)
    - New strand=limited methylation, old strand=fully methylated
  - A closer look at replication
    - Replication begins at particular sites with a specific sequence of nucleotides called origins of replication
    - Proteins attach to this sequence and separate the two strands forming a replication bubble
    - Replication proceeds in both directions
    - Multiple bubbles form and eventually fuse
    - At the ends of each bubble replication forks form
      - Y-shaped regions where the parental DNA is being unwound
    - Helicases are enzymes that untwist the double helix at the replication forks
    - After the strands are separated, single stranded binding proteins bind to the DNA strands and keep them from repairing (keeps them stable)
    - Untwisting of the double helix causes tighter twisting and strain ahead of the replication fork
      - Topoisomerase relieves this strain by breaking, swivelling and re-joining the strands
    - The enzymes that synthesise DNA cannot initiate the synthesis of a polynucleotide - they can only add base pairs to an already existing chain that is base-paired with the parent strand
      - The enzyme primase synthesises RNA primers using the DNA strand as a template
      - RNA nucleotides are added one at a time
      - The completed primer is generally about 5-10 nucleotides long
      - The new DNA strand will start from the 3' end of the RNA primer

- 

- New nucleotides are added by nucleoside triphosphates
- New strand can elongate only in 5' to 3' direction and nucleotides can only add to that 3' end of the RNA primer
  - DNA has "one direction"
- Leading strand
  - DNA pol III remains at the replication fork on the new complementary strand and continuously adds nucleotides as the fork progresses
  - Only one RNA primer is required
    - 

- Lagging strand
  - On the other strand, DNA pol III must work away from the replication fork in the mandatory 5' - 3' direction

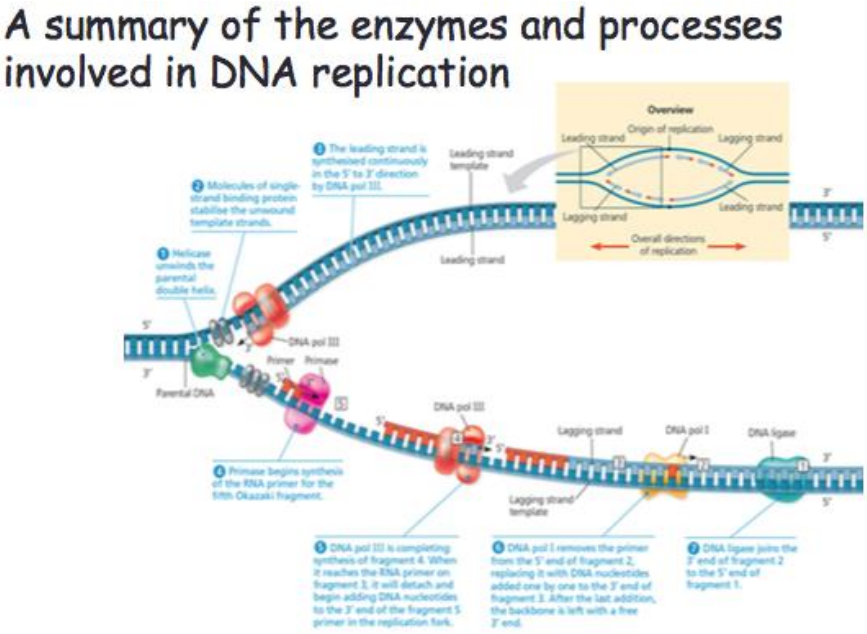
- This strand in synthesised in a series of segments (Okazaki fragments)
- Each fragment requires a separate primer



- Ends of DNA Molecules
  - The ends of eukaryotic chromosomal DNA consist of telomeres (multiple repetitions of one short nucleotide sequence) that do not contain genes
  - Buffer that protects the organism's genes by postponing the erosion of genes located near the ends of DNA molecules

○ Repairing DNA
- Incorrectly paired, or altered nucleotides are corrected by enzymes (approx. 130 in humans) before they become mutations
- Nuclease enzymes cut damaged DNA strands at two points so the segment can be removed
- Repair synthesis by DNA polymerase fills in the missing nucleotides
- DNA ligase seal the new DNA to the old DNA

○ A summary:



- Must be able to redraw this - know what all the enzymes do!!!!! Must understand DNA replicates in one direction (leading and lagging)

Table 16.1 Bacterial DNA Replication Proteins and Their Functions

Protein	Function
Helicase 	Unwinds parental double helix at replication forks
Single-strand binding protein 	Binds to and stabilises single-stranded DNA until it is used as a template
Topoisomerase 	Relieves overwinding strain ahead of replication forks by breaking, swivel-ing, and rejoining DNA strands
Primase 	Synthesises an RNA primer at 5′ end of leading strand and at 5′ end of each Okazaki fragment of lagging strand
DNA pol III 	Using parental DNA as a template, synthesises new DNA strand by add-ing nucleotides to an RNA primer or a pre-existing DNA strand
DNA pol I 	Removes RNA nucleotides of primer from 5′ end and replaces them with DNA nucleotides
DNA ligase 	Joins Okazaki fragments of lagging strand; on leading strand, joins 3′ end of DNA that replaces primer to rest of leading strand DNA

- Chromosomes
  - Histones
    - Responsible for the first level of DNA packaging into chromatin
    - H2A, H2B, H3, and H4 are most common in chromatin
  - Nucleosomes (10nm fibre)
    - Consists of DNA wound twice around a protein core composed of two molecules each of the four main histone types
    - The amino end (N-terminus, or tail) of each histone extends outward from the nucleosome
  - 30nm fibre
    - Coiling due to interactions between the histone tails of one nucleosome and the linked DNA nucleosomes on either side
  - Looped domains (300nm fibre)
    - 30nm fibre forms loops attached to a chromosome scaffold made of proteins
  - Metaphase chromosome
    - Looped domains coil and fold to further compact the chromatin to produce a characteristic metaphase chromosome
- The Central Dogma
  - DNA -> RNA -> Proteins
  - Transcription:
    - Initiation
      - The DNA sequence where RNA polymerase attaches and initiates transcription is known as the promoter
      - The promoter includes the transcription start point (a single nucleotide)
      - In bacteria, the RNA polymerase recognises the start point
      - In eukaryotes, transcription factors mediate the binding of RNA polymerase and the initiation process
        - The whole complex of transcription factors and RNA polymerase II bound to the promoter is called a transcription initiation complex.

        - Once the RNA polymerase is attached in the correct orientation, it unwinds the DNA strands and starts to transcribe the template
- Elongation
    - RNA polymerase moves along the DNA and continues to unwind it and add nucleotides at the 3' end of the growing RNA strand
    - 5' - 3' direction
    - The RNA strand peels away and the DNA double helix reforms
    - A single gene can be transcribed simultaneously by several molecules of RNA polymerase following each other
- Termination
    - Bacteria
        - Transcription proceeds through a terminator DNA sequence
        - The transcribed RNA of the terminator sequence functions as the termination signal
        - The polymerase detaches from the DNA and releases the transcript which can be immediately translated
    - Eukaryotes
        - In eukaryotes, RNA polymerase II transcribes a sequence on the DNA called the polyadenylation signal sequence, which codes for a polyadenylation signal (AAUAAA) in the pre-mRNA.
        - Then, at a point about 10–35 nucleotides downstream from the AAUAAA signal, proteins associated with the growing RNA transcript cut it free from the polymerase, releasing the pre-mRNA.
- RNA Processing (Eukaryotes)
    - Alteration of pre-mRNA ends
        - The 5 end is synthesized first; it receives a 5 cap (modified form of G nucleotide) added onto the 5 end after transcription of the first 20–40 nucleotides.
        - An enzyme adds 50–250 more adenine (A) nucleotides, forming a poly-A tail at the 3' end.
        - The 5 cap and poly-A tail share several important functions.
            - Facilitate the export of the mature mRNA from the nucleus
            - Help protect the mRNA from degradation by hydrolytic enzymes.
            - Help ribosomes attach to the 5 end of the mRNA once the mRNA reaches the cytoplasm.
    - Split Genes and RNA Splicing
        - Eukaryotic Genes and their RNA transcripts have long non-coding stretches that are spliced out
            - Introns
                - Non-coding sequences (of DNA and RNA) that lie between coding sequences
            - Exons
                - The coding areas (of RNA and DNA)
                - Sequences of RNA that exit the nucleus
        - Introns are cut out from the molecule and the exons are joined together to form the mRNA involved in translation