DNA Replication (Chapter 10)

- The Secret of Life- How does DNA replicate?
    - H-bonding between the bases would allow for "unzipping" of the DNA and access to the template by cellular replication machinery
    - There were 3 main possibilities for the mode of replication of the two strands namely
        - Conservative
        - Semi conservative
        - Dispersive
- 10.1 DNA is reproduced by semiconservative replication
    - Figure 10-1: Generalized model of semiconservative replication of DNA
    - New synthesis is shown in blue
    - Although this model of replication was suspected after the discovery of the structure of DNA- it required several years of work to prove this over alternative modes
    - 10-2: Results of one round of DNA replication for each of the three possible modes by which replication could be accomplished
- 10-3 Meselson and Stahl Experiment (1958)
    - Used different densities of old vs. newly replicated DNA to distinguish method of DNA replication
- Fractionation of cells for analysis of constituents
    - cells are homogenized by:
        - Ultrasonication
        - Disruption by mild detergent
        - Forced through a small gap (French Press)
        - Mortar and pestle (Dounce homogenizer)
    - Contents are separated by:
        - Centrifugation
        - Chromatography
        - Electrophoresis
- How Equilibrium Centrifugation Works
    - The sample is distributed throughout the sucrose density gradient
    - At equilibrium components have migrate to a region in the gradient that makes their own density
- Figure 10-4: The expected results of two generations of semiconservative replication in the Meselson-Stahl experiment
- Equilibrium sedimentation was used to follow the fate of parental and daughter DNA strands
    - After generation 1- semiconservative: mixed and conservative: light and heavy
    - After many generations- semiconservative: light and mixed, conservative: light and heavy
- Taylor-Woods - Hughes experiment showing semiconservative replication in eukaryotes

    - figure 10-5: unlabeled pea cells are allowed time to replicate once in presence of tritiated thymidine (3H- Thymedine) and both new chromatids become labeled
    - Cells are removed to grow with unlabeled thymidine and one of the new chromatids is not labeled
        - except...where a new chromatid has undergone crossing over (reciprocal exchange) - in which case segments of each chromatid will be labeled
- Bacteria have one origin of replication:
    - the 9bp sequence below is repeated 4 times
        - 5' TTAT (C/A) CA (C/A)A 3'
    - This is the binding site for a protein named DnaA protein replication is bidirectional from this point
- Eukaryotes have multiple origins of replications (Fig 10-14)
    - clearly required for the larger genomes Drosophila (fruit fly) Genome = 70,000,000 bp rate of DNA replication is 2600bp/minute - with 1 origin of replication it would take 2 weeks In reality it takes only 4 min.
    - about 7000 origins of replication (25000 in mammals)
- 10.2 DNA synthesis in bacteria
    - During each step a single nucleotide is added to the growing complement of the DNA template using a nucleoside triphosphate as the substrate
    - the release of inorganic pyrophosphate drives the reaction energetically
- DNA synthesis in microorganisms
    - DNA polymerase in presence of a template DNA and all 4 deoxynucleotide triphosphates and Mg ions will synthesize new DNA
    - New nucleotide are added only in a 5' to 3' direction by addition of one nucleotide at a time forming a phosphodiester bond with the loss of inorganic diP
- Other Polymerases
    - In 1969 it was found that a PolA1 mutant of E coli could still replicate
    - Now we know there are at least 5 polymerases
    - Of these the Polymerase III holoenzyme complex is the central player in replication with other polymerases playing roles in DNA repair and maintenance
    - Those with 3' to 5' exonuclease activity have proofreading roles
- Sections 10.3, .4, and .5 Complex issues in DNA synthesis and a coherent model for DNA synthesis
    - DnaA binds to a 9mer and 10mer repeats in oriC (needs ATP) helps inbinding of DnaB and DnaC helicases
    - Single stranded binding proteins (SSBPs) stabilize open DNA
    - Supercoiling ahead of the replication fork is relaxed by DNA gyrase (A DNA topoisomerase)
    - An RNS primer is generated by Primase (an RNA polymerase)(Later replaced by DNA)
    - Opposite polarity of strands results in Continuous DNA synthesis on Leading strand Discontinuous DNA synthesis on Lagging Strand

    - DNA pol I removes RNA primers and fills in DNA ligase joins the gap in phosphate backbone
    - DNA synthesis is continuous on both strands by virtue of the fact that the lagging strand is "looped" around to provide the same 5' to 3' orientation for replication. A new loop is formed at the end of each "Okazaki fragment". Beta subunit acts as a "Sliding clamp"
    - Proofreading of errors performed by DNA Pol I and III which have 3' - 5' exonuclease activity. Epsilon subunit of DNA Pol II is involved in this step
    - Since complete mutations of DNA polymerases would be lethal "conditional mutations" have been isolated
        - Ex) 'temp.-sensitive' mutations that stop replicating at a nonpermissive temp.
- Old figure 10-9
    - Helical unwinding of DNA at the origin of replication is accomplished by DnaA, DnaB, and DnaC proteins
        - Initial binding of may monomers of DnaA occurs at DNA sites containing repeating sequences of 9 nucleotides called 9mers
        - not illustrated are 13mers, which are also involved
        - Removal of tension ahead of the replication fork is performed by DNA gyrase - collection of enzymes known as DNA topoisomerases
    - The initiation of DNA synthesis
        - A complementary RNA primer is 1st synthesized to which DNA is added
        - All synthesis is the 5' to 3' direction, eventually the RNA is replaced with DNA under the direction DNA polymerase I
- Figure 10-10
    - opposite polarity of DNA synthesis along the two strands necessary because the two strands run antiparallel to one another and DNA polymerase III synthesizes only in one direction (5' to 3')
    - On the lagging strand synthesis must be discontinuous resulting in the production of Okazaki fragments
- Figure 10-11
    - Illustration of how concurrent DNA synthesis is achieved on both the leading and lagging strands at a single replication fork
    - The lagging template strand is "looped" in order to invert the physical direction of synthesis but not biochemical direction
    - the enzyme functions as a dimer with each core enzyme achieving synthesis on one or the other strand
- Figure 10-12
    - summary of DNA synthesis at a single replication fork
        - various enzymes and proteins essential to the processes are shown
- 10.6 DNA replication in Eukaryotes is similar to that in Prokaryotes but more complicated