Highlights DNA Replication II

1. Prokaryotic replication forks are bidirectional, having started from a single replication origin (the origin is where replication starts) in opposite directions. The major players in *E. coli* replication are 1) DNA polymerase III; 2) beta clamp (holds polymerase to DNA); 3) Single strand binding protein - protects single strand DNA; 4) helicase - unwraps DNA duplex ahead of replication fork; 5) primase - makes RNA primer necessary to start DNA replication; 6) DNA gyrase - topoisomerase that relieves superhelical tension created by helicase; 7) DNA ligase - joins pieces of DNA, such as Okazaki fragments together; 8) DNA polymerase I - removes RNA primers and replaces with DNA.

2. Leading strand replication occurs as a single continuous piece of DNA. Lagging strand DNA synthesis is made in pieces called Okazaki fragments that are later joined together (using DNA polymerase I to remove the RNA and DNA ligase to physically link the pieces). Note also that DNA polymerase I uses a 5' to 3' exonuclease activity to remove RNA primers and a 5' to 3' DNA polymerase activity to put new DNA in place of the RNA it removes. Note that both leading AND lagging strand synthesis are both occurring at the same replication fork AND that both leading and lagging strand synthesis are occurring exclusively in the 5' to 3' direction.

3. Proofreading is a phenomenon that occurs as a result of a 3' to 5' exonuclease activity on many DNA polymerases (including DNA Polymerases I and III). Proofreading improves the accuracy of DNA replication about a hundred fold. Note that the accuracy of incorporating the correct nucleotide into DNA using simple hydrogen bonds gives about one error per ten million or so. With proofreading the error rate drops to 1 per billion or so. Cells that have mutations that destroy the proofreading of the DNA polymerases form mutations at a much higher rate than normal cells.

4. The most prominent DNA polymerases that lack proofreading are those of reverse transcriptases of retroviruses. HIV's reverse transcriptase is VERY error prone and is the primary reason that drugs lose the effectiveness on the disease over time.

5. DNA Polymerase III is a multi-subunit enzyme that gets on a DNA stays on it for a long time (highly processive). DNA Polymerase I is not so highly processive. The difference in their processivity is the fact that Polymerase III has the beta clamp, which is a ring that helps hold it tightly to DNA during replication.

6. Ultraviolet light can act as a mutagen (an agent that causes mutations). It does this by creating cross-links between adjacent thymines in a strand of DNA. If these are not properly repaired, a mutation can result. It is for this reason that people who tan/burn in tanning booths often develop skin cancer later in life. Repairing this damage is performed by the system known as nucleotide excision repair. In this system, an excinuclease excises a portion of the strand with the damage, DNA polymerase fills in the gap, and DNA ligase seals everything up.

7. Thymine dimers are probably the most common damage that occurs to DNA. They arise from exposure to UV light (tanning booths, excessive sun tanning) and result in a covalent bond formed between adjacent thymine bases in DNA. If they are not repaired, thymine dimers can result in mutation. The more exposure you have to UV light, the more likely you will develop skin cancer. Stay out of tanning booths.

8. DNA can be damaged by other means. Oxidation is a common one. When oxidation of guanine occurs, for example, 8-oxo-guanine is created. This base can form stable base pairs with adenine. If DNA containing 8-oxo-guanine is allowed to replicate, adenine will be inserted in place of cytosine, causing a mutation. A

repair mechanism called base excision repair helps to fix this.

9. Base excision repair is a cellular process for removing damaged bases in a DNA strand - such as 8-oxoguanine. This occurs as a result of action by an enzyme which catalyzes the removal of the damaged base followed by an exonuclease action to remove a few more bases and then filling in of the gap by DNA polymerase(s) and sealing of the last bond by DNA ligase.

10. Another repair mechanism in *E. coli* is that of the Mut S / Mut H / Mut L system, which recognizes a mismatch that occurs in DNA as a result of an error in polymerization and proofreading. I incorrectly implied in class that Mut H is the first protein that binds. In fact, Mut S is the first protein to bind.