Highlights DNA Replication II

1. 1.DNA polymerase I has three enzymatic activities - a 5' to 3' DNA polymerase activity, a 3' to 5' exonuclease activity (also called proofreading), and a 5' to 3' exonuclease activity.

2. All DNA polymerases require a primer to start DNA synthesis. The primer is formed inside of cells by a special RNA polymerase known as primase. (RNA polymerase does not require a primer)

3. Removal of RNA primers in E. coli requires DNA Polymerase I's 5' to 3' activity. Without it, the cell will die.

4. The linking number (L) of a DNA is the sum of the number of twists (T) of a DNA plus the number of writhes (W). Thus, L = T + W. The twists are the number of times two the two helices cross each other. The writhe is the number of superhelical turns found in a DNA. Writhes can be positive or negative and in either case, when the W is a non-zero value, the molecule is said to be superhelical = to have superhelicity.

5. Writhing of DNA occurs in an attempt by a DNA molecule to "relax." A DNA molecule is relaxed when its number of base pairs (bp) per twist (T) is that of B-DNA (10.4-10.5 bp per turn). Thus, if one takes a circular DNA, opens it and removes two twists from it and then closes it, the number of twists will decrease, but the number of base pairs remains the same. In this case, the numbers of bp per twist will INCREASE. This causes a tension that is relieved by the DNA TWISTING two turns. This will cause the writhe to compensate by forming two negative superhelical turns, giving W a value of negative two. Note that the linking number remains the same.

6. On the other hand if one takes a circular DNA, opens it and adds twists to it and then closes it, the number of twists will increase, but the number of base pairs remains the same. In this case, the numbers of bp per twist will DECREASE. The DNA will relax by UNTWISTING two turns, which will cause the writhe increase to a value of positive two.

7. Initiation of replication in *E. coli* occurs at a specific site on the *E. coli* genomic DNA, known as OriC, in the cell's circular chromosome. The OriC site contains three repeats of an AT rich sequence near some sequences bound by the DNA A protein.

8. Replication initiation begins with binding of the several copies of the dnaA protein to the OriC site. Bending and wrapping of the DNA around dnaA proteins causes the AT-rich sequences noted above to become single-stranded.

9. Next, the dnaBC complex binds the dnaB protein (helicase) to each of the single strands in opposite orientations. The dnaC protein is released in the process. Next, SSB and primase bind the exposed single-stranded regions and cause DNA A protein to be released. The primases begin synthesizing RNA primers (remember - 5' to 3' RNA synthesis only also) in opposite directions on each strand. The primases DO NOT require a pre-existing primer to function.