S1.Describe three ways to account for the high fidelity of DNA replication. Discuss the quantitative contributions of each of the three ways.

Answer:

First: AT and GC pairs are preferred in the double helix structure. This provides fidelity to around one mistake per 1,000.

Second: Induced fit by DNA polymerase prevents covalent bond formation unless the proper nucleotides are in place. This increases fidelity another 100- to 1,000-fold, to about one error in 100,000 to 1 million.

Third: Exonuclease proofreading increases fidelity another 100- to 1,000-fold, to about one error per 100 million nucleotides added.

S2. What do you think would happen if the *ter* sequences were deleted from the bacterial DNA?

Answer: Instead of meeting at the *ter* sequences, the two replication forks would meet somewhere else. This would depend on how fast they were moving. For example, if the counterclockwise-moving fork was advancing faster than the clockwise-moving fork, they would meet closer to where the clockwise-moving fork started. In fact, researchers have actually conducted this experiment. Interestingly, *E. coli* without the *ter* sequences seemed to survive just fine.

S3. Summarize the steps that occur in the process of chromosomal DNA replication in *E. coli*.

Answer:

Step 1. DnaA proteins bind to the origin of replication. The AT-rich region is denatured after binding.

Step 2. DNA helicase breaks the hydrogen bonds between the DNA strands, topoisomerases alleviate positive supercoiling, and single-strand binding proteins hold the parental strands apart.

Step 3. Primase synthesizes one RNA primer in the leading strand and many primers in the lagging strand. DNA polymerase III then synthesizes the daughter strands of DNA. DNA polymerase I excises the RNA primers and fills in with DNA. DNA ligase covalently links the DNA fragments together.

Step 4. The processes described in steps 2 and 3 continue until the two forks reach the *ter* sequences on the other side of the circular bacterial chromosome.

Step 5. Topoisomerases unravel the intertwined chromosomes, if necessary.

S4. If a strain of *E. coli* overproduced the methylase enzyme, how would that affect the DNA replication process? Would you expect such a strain to have more or less chromosomes per cell, compared to a normal strain of *E. coli*? Explain why.

Answer: If a strain overproduced the methylase enzyme, it would be easier to replicate the DNA. The GATC sites in the origin of replication have to be fully methylated for DNA replication to occur. Immediately after DNA replication, there is a delay in the next round of DNA replication because the two copies of newly replicated DNA are hemimethylated. A strain that overproduces methylase would rapidly convert the hemimethylated DNA into fully methylated DNA and more quickly allow the next round of DNA replication to occur. For this reason, the overproducing strain might have more copies of the *E. coli* chromosome because it would not have a long delay in DNA replication.