

MCB 110 Problem set 2. DNA replication - **Answers**

1. Developing a useful assay is a key to success in biochemistry. Try your hand at assay development: You purchased an old, multi-story house, and you're fixing it up. You notice that there are three lights in the attic, but there are no switches to turn the lights on and off. In the basement, you notice three switches on a panel labeled "Attic Lights". Devise a method to determine which switch controls each light. One catch. It's a long way up the stairs to the attic, so you want to make only one trip up the stairs. How would you assign all three switches to their corresponding lights in one trip up the stairs?

Turn on two lights for a few minutes. Turn one off. Go upstairs. The light that's on corresponds to the switch that's still on. The light that's off and warm corresponds to the light that you turned off. The switch you didn't touch controls the light that's off and cold.

2. Which DNA polymerase polymerizes DNA in the 3'-5' direction? **None.**

3. If a DNA polymerase lacks a 3'-5' exonuclease activity, do you expect it to be more or less accurate than *E. coli* DNA polymerase I. Explain.

Less accurate. The 3'-5' exonuclease is the proofreading activity that increases fidelity.

4. Is the 3'-5' exonuclease activity of DNA Pol I simply the reverse of the 5'-3' polymerase activity? Explain. **No. The polymerase starts with dNTP, adds dNMP to the chain and produces PPI. The exonuclease simply removes dNMP without synthesizing dNTP.**

5. a) List three mechanisms by which DNA polymerase I achieves a high fidelity of replication.

1. H-bonding of the incoming base. 2. H-bonding and shape at the site of incorporation. 3. Proofreading up to 5 bases after the insertion site based on shape of the mismatch. 4. Chewing away the RNA primer with 5'->3' exo.

b) Assuming an error rate of 1/1000 in the primer, a primer length of 10 bps, and Okazaki fragments of 1000 bp, how many errors would you expect in the replication of the *E. coli* genome (4,000,000 bps) if the Okazaki fragments were primed with DNA (which is not removed from the daughter strand)?

**Assuming an error rate of ~0 (or 10^{-9}) outside the "primers",
1 error/1000 bp x 10 bp/primer x 1 primer/Okazaki fragment x 1 Okazaki fragment/1000 bp x**

4,000,000 bp/genome = (10 x 4,000,000)/(1000 x 1000) error/genome = 40 errors/genome

6. Why is DNA synthesis discontinuous on the lagging strand? How are the discontinuous fragments joined to make a continuous strand? **Without a 3'-5' polymerase, the lagging strand must be made in 5'-3' pieces as the ss template is produced by the helicase. The RNA primers are removed, replaced with DNA, and the resulting nick is closed by DNA ligase.**

7. What is the evidence that the sliding clamps in *E. coli* (β) and human (PCNA) evolved from a common ancestral protein? **The domains have a common fold.**

8. Why do the clamp loading complex and primase function mainly on the lagging strand? **The leading strand is primed once and the clamp/polymerase complex is stable on the DNA.**

9. What is the function of SSB in *E. coli* DNA replication? What experiment would you do to determine if SSB plays an essential role in DNA replication? **SSB stabilizes the ssDNA template and stimulates the activity of DNA polymease. Try to knock out the gene. If no knockout mutants can be isolated, mutagenize the gene and screen for a ts phenotype.**

10. Consider Fig. 5-28 in Alberts (pg. 254) showing a mammalian DNA replication fork. How does this eukaryotic fork differ from the fork in prokaryotes? Are these differences actual (real) or do they reflect gaps in knowledge or inaccuracies in the figure?

In the figure, the pol δ holoenzymes are not coupled at the replication fork. This is likely an oversimplification in the figure.

In eukaryotes, the replicative helicase is made up of six different homologous subunits (as shown), and there is a hand-off of the lagging strand from primase to pol α to pol δ .

11. What are two roles for topoisomerases in DNA replication? Could a type I topoisomerase perform both of these functions? **Relieve strain ahead of the replication fork and decatenate (separate) the completely replicated chromosomes. In theory, the right type I topoisomerase could relieve strain ahead of the fork, but only a type II topoisomerase can unlink the interlinked circular chromosomes. However, the type I topoisomerases in *E. coli* don't work in replication.**

12. Deletion of the telomerase RNA from the mouse genome was not immediately lethal. Surprisingly, it took several generations for the mutant mice to show decreased survival.

a) What is the function of the telomerase RNA? **Template for telomere synthesis.**

b) Why do you think several generations of the mutant mice survived? **It took many cell divisions for the telomeres to shorten so much that key cell types died and chromosomes became unstable. Perhaps telomeres were maintained by recombination.**

13. ATP or NAD⁺ play the same role in the reactions of different DNA ligases. What is this role? **Add an AMP leaving group to activate the 5' PO₄ for nucleophilic attack by the 3' OH.**

14. What is the function of the tau subunit of the DNA polymerase III holoenzyme? **Binds both polymerases of the replication fork to coordinate leading and lagging strand synthesis. Binds DnaB helicase to assemble the replisome. The two tau subunits contain 2 of the 3 catalytic sites in the clamp loader that hydrolyze ATP to send the signal to release the clamp.**

15. Bacterial chromosomes have a single origin of replication but eukaryotic chromosomes have many origins. Why are there so many origins in eukaryotic chromosomes? **The genomes are too big to be replicated efficiently from a single origin.**

16. Describe an assay that was used to identify origins of DNA replication. **Ligate random pieces of genomic DNA to a drug resistance gene, insert the DNA into a drug-sensitive organism, and select for growth in the presence of the drug.**

17. Why is a heat-stable DNA polymerase used for the polymerase chain reaction (PCR)? **Each PCR cycle requires boiling the DNA to separate the DNA strands. A heat-sensitive polymerase would be inactivated by boiling, and more enzyme would have to be added to the reaction in each cycle. In contrast, the heat-stable polymerases (like *Taq* polymerase) tolerate being boiled, so fresh polymerase need not be added in every cycle.**