7

DNA: Structure and Replication

WORKING WITH THE FIGURES

1. In Table 7-1, why are there no entries for the first four tissue sources? For the last three entries, what is the most likely explanation for the slight differences in the composition of human DNA from the three tissue sources?

Answer: The first four are single-celled organisms; therefore, a tissue category does not apply. The differences in the values are most likely attributable to experimental error.

2. In Figure 7-7, do you recognize any of the components used to make Watson and Crick's DNA model? Where have you seen them before?
Answer: Watson and Crick made use of molecular models, ring stands, and clamps, typically found in a chemistry lab. The vertically oriented pentagons are the deoxyribose components of DNA. These are components of the two sugar-phosphate backbones of the DNA structure, one behind James Watson and the other just to the left of Francis Crick. Nitrogenous bases oriented horizontally are held in place in between the two backbones by clamps.

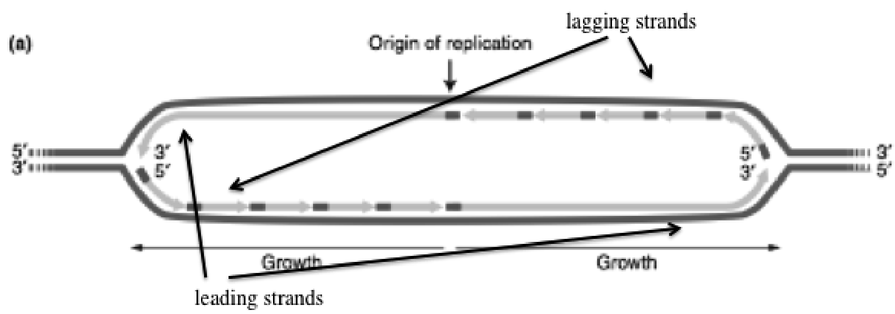
3. Referring to Figure 7-20, answer the following questions:
a. What is the DNA polymerase I enzyme doing?
b. What other proteins are required for the DNA polymerase III on the left to continue synthesizing DNA?
c. What other proteins are required for the DNA polymerase III on the right to continue synthesizing DNA?

Answer:
a. PolI is removing ribonucleotide primers and filling gaps between Okazaki fragments.
b. β-clamp and helicase
c. β-clamp, helicase, primase, and ssb

4. What is different about the reaction catalyzed by the green helicase in Figure 7-20 and the yellow gyrase in Figure 7-21?
Answer: Gyrase breaks the phosphodiester linkages in the DNA backbone, while helicase disrupts the hydrogen bonds between the paired bases of antiparallel strands.

5. In Figure 7-24(a), label all the leading and lagging strands.

Answer: Each fork has both a leading and a lagging strand, as labeled below:



BASIC PROBLEMS

6. Describe the types of chemical bonds in the DNA double helix.

Answer: The DNA double helix is held together by two types of bonds, covalent and hydrogen. Covalent bonds occur within each linear strand and strongly bond the bases, sugars, and phosphate groups (both within each component and between components). Hydrogen bonds occur between the two strands and involve a base from one strand with a base from the second in complementary pairing. These hydrogen bonds are individually weak but collectively quite strong.

7. Explain what is meant by the terms *conservative* and *semiconservative replication.*

Answer: Conservative replication is a hypothetical form of DNA synthesis in which the two template strands remain together but dictate the synthesis of two new DNA strands, which then form a second DNA helix. The end point is two double helices, one containing only old DNA and one containing only new DNA. This hypothesis was found to be not correct. Semiconservative replication is a form of DNA synthesis in which the two template strands separate and each dictates the synthesis of a new strand. The end point is two double helices, both containing one new and one old strand of DNA. This hypothesis was found to be correct.

8. What is meant by a *primer,* and why are primers necessary for DNA replication?
Answer: A primer is a short segment of RNA that is synthesized by primase using DNA as a template during DNA replication. Once the primer is synthesized, DNA polymerase then adds DNA to the 3´ end of the RNA. Primers are required because the major DNA polymerase involved with DNA replication is unable to initiate DNA synthesis and, rather, requires a 3´ end. (It is the 3´ -OH group that is required to create the next phosphodiester bond.) The RNA is subsequently removed and replaced with DNA so that no gaps exist in the final product.

9. What are helicases and topoisomerases?

Answer: Helicases are enzymes that disrupt the hydrogen bonds that hold the two DNA strands together in a double helix. This breakage is required for both RNA and DNA synthesis. Topoisomerases are enzymes that create and relax supercoiling in the DNA double helix. The supercoiling itself is a result of the twisting of the DNA helix that occurs when the two strands separate.

10. Why is DNA synthesis continuous on one strand and discontinuous on the opposite strand?

Answer: Because the DNA polymerase is capable of adding new nucleotides only at the 3´ end of a DNA strand, and because the two strands are antiparallel, at least two molecules of DNA polymerase must be involved in the replication of any specific region of DNA. When a region becomes single-stranded, the two strands have an opposite orientation. Imagine a single-stranded region that runs from right to left. The 5´ end is at the right, with the 3´ end pointing to the left; synthesis can initiate and continue uninterrupted toward the right end of this strand. Remember: new nucleotides are added in a 5´→ 3´ direction, so the template must be copied from its 3´ end. The other strand has a 5´ end at the left with the 3´ end pointing right. Thus, the two strands are oriented in opposite directions (antiparallel), and synthesis (which is 5´→ 3´) must proceed in opposite directions. For the leading strand (say, the top strand), replication is to the right, following the replication fork. It is continuous and may be thought of as moving “downstream.” Replication on the bottom strand cannot move in the direction of the fork (to the right) because, for this strand, that would mean adding nucleotides to its 5´ end. Therefore, this strand must replicate discontinuously: as the fork creates a new single-stranded stretch of DNA, this is replicated to the left (away from the direction of fork movement). For this lagging strand, the replication fork is always opening new single-stranded DNA for replication upstream of the previously replicated stretch, and a new fragment of DNA is replicated back to the previously created fragment. Thus, one

(Okazaki) fragment follows the other in the direction of the replication fork, but each fragment is created in the opposite direction.

11. If the four deoxynucleotides showed nonspecific base pairing (A to C, A to G, T to G, and so on), would the unique information contained in a gene be maintained through round after round of replication? Explain.
Answer: No. The information of DNA is dependent on a faithful copying mechanism. The strict rules of complementarity ensure that replication and transcription are reproducible.

12. If the helicases were missing during replication, what would happen to the replication process?

Answer: Helicases are enzymes that disrupt the hydrogen bonds that hold the two DNA strands together in a double helix. This breakage exposes lengths of single-stranded DNA that will act as the template and are required for DNA replication. Therefore, the absence of helicases would prevent the replication process.

13. Both strands of a DNA molecule are replicated simultaneously in a continuous fashion on one strand and a discontinuous one on the other. Why can't one strand be replicated in its entirety (from end to end) before replication of the other is initiated?

Answer: Theoretically, DNA could be replicated this way but not with the replisome, which is organized to replicate both stands simultaneously. Further, this would leave one strand of the DNA single-stranded where mutagenic events would be more likely, and it would certainly take longer.

14. What would happen if, in the course of replication, the topoisomerases were unable to reattach the DNA fragments of each strand after unwinding (relaxing) the DNA molecule?

Answer: The chromosome would become hopelessly fragmented.

15. Which of the following would happen if DNA synthesis were discontinuous on both strands?
a. The DNA fragments from the two new strands could become mixed, producing possible mutations.
b. DNA synthesis would not take place, because the appropriate enzymes to carry out discontinuous replication on both strands would not be present.

c. DNA synthesis might take longer, but otherwise there would be no noticeable difference.
d. DNA synthesis would not take place, because the entire length of the chromosome would have to be unwound before both strands could be replicated in a discontinuous fashion.

Answer: **c.** DNA synthesis might take longer.

16. Which of the following is *not* a key property of hereditary material?
a. It must be capable of being copied accurately.
b. It must encode the information necessary to form proteins and complex structures.
c. It must occasionally mutate.
d. It must be able to adapt itself to each of the body's tissues.

Answer: **d.** It does not have to adapt to each of the body's tissues.

17. It is essential that RNA primers at the ends of Okazaki fragments be removed and replaced by DNA because otherwise which of the following events would result?
a. The RNA might not be accurately read during transcription, thus interfering with protein synthesis.
b. The RNA would be more likely to contain errors because primase lacks a proofreading function.
c. The stretches of RNA would destabilize and begin to break up into ribonucleotides, thus creating gaps in the sequence.
d. The RNA primers would be likely to hydrogen bond to each other, forming complex structures that might interfere with the proper formation of the DNA helix.

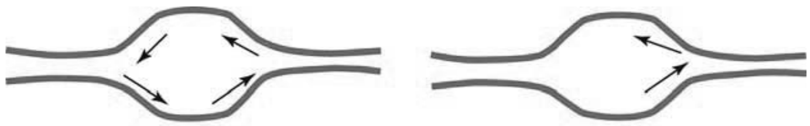
Answer: **b.** The RNA would be more likely to contain errors.

18. Polymerases usually add only about 10 nucleotides to a DNA strand before dissociating. However, during replication, DNA pol III can add tens of thousands of nucleotides at a moving fork. How is this addition accomplished?

Answer: Part of the replisome is the sliding clamp, which encircles the DNA and keeps pol III attached to the DNA molecule. Thus, pol III is transformed into a processive enzyme capable of adding tens of thousands of nucleotides.

19. At each origin of replication, DNA synthesis proceeds bidirectionally from two replication forks. Which of the following would happen if a mutant arose having only one functional fork per replication bubble? (See diagram.)
a. No change at all in replication.

b. Replication would take place only on one half of the chromosome.
c. Replication would be complete only on the leading strand.
d. Replication would take twice as long.



Answer: **d.** Replication would take twice as long.

20. In a diploid cell in which 2*n* = 14, how many telomeres are there in each of the following phases of the cell cycle?
(a) G1 **(c)** mitotic prophase
(b) G2 **(d)** mitotic telophase

Answer:

a. Prior to the S phase, each chromosome has two telomeres, so in the case of 2n = 14, there are 14 chromosomes and 28 telomeres.

b. After S, each chromosome consists of two chromatids, each with two telomeres, for a total of four telomeres per chromosome. So, for 14 chromosomes, there would be 14 × 4 = 56 telomeres.

c. At prophase, the chromosomes still consist of two chromatids each, so there would be 14 × 4 = 56 telomeres.

d. At telophase, there would be 28 telomeres in each of the soon-to-be daughter cells.

21. If thymine makes up 15 percent of the bases in a specific DNA molecule, what percentage of the bases is cytosine?

Answer: If the DNA is double stranded, A = T and G = C and A + T + C + G = 100%. If T = 15%, then C = [100 – 15(2)]/2 = 35%.

22. If the GC content of a DNA molecule is 48 percent, what are the percentages of the four bases (A, T, G, and C) in this molecule?

Answer: If the DNA is double stranded, G = C = 24% and A = T = 26%.

23. Bacteria called extremophiles are able to grow in hot springs such as Old Faithful at Yellowstone National Park in Wyoming. Do you think that the DNA of extremophiles would have a higher content of GC or AT base pairs? Justify your answer.

Answer: Higher GC content. The increased number of hydrogen bonds would help to counteract the destabilizing effect of increased heat.

24. Assume that a certain bacterial chromosome has one origin of replication. Under some conditions of rapid cell division, replication could start from the origin before the preceding replication cycle is complete. How many replication forks would be present under these conditions?
Answer: Six. The first replication start would have two replication forks proceeding to completion, and the now replicated origins would each start replication again. Each would have two more replication forks, for a total of six.

25. A molecule of composition

5´-AAAAAAAAAAAA-3´
3´-TTTTTTTTTTTTT-5´

is replicated in a solution of adenine nucleoside triphosphate with all its phosphorus atoms in the form of the radioactive isotope 32P. Will both daughter molecules be radioactive? Explain. Then repeat the question for the molecule

5´-ATATATATATATAT-3´
3´-TATATATATATATA-5´

Answer: Only the DNA molecule that used the poly-T strand as a template would be radioactive. The other daughter molecule would not be radioactive because it would not have required any dATP for its replication.
Because each strand of the second molecule contains T, both daughter molecules would require dATP for replication, so each would be radioactive.

26. Would the Meselson and Stahl experiment have worked if diploid eukaryotic cells had been used instead?

Answer: Yes. DNA replication is also semi-conservative in diploid eukaryotes.

27. Consider the following segment of DNA, which is part of a much longer molecule constituting a chromosome:

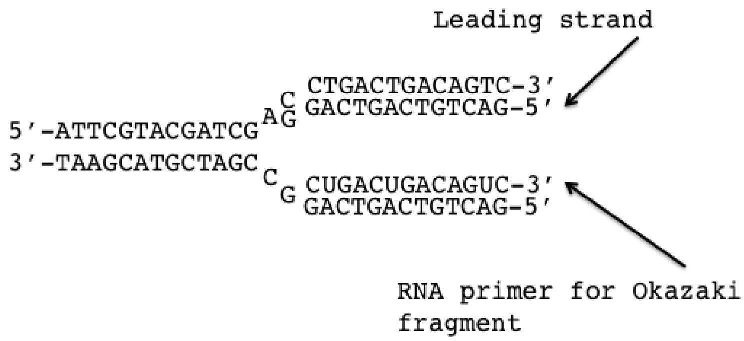
5´….ATTCGTACGATCGACTGACTGACAGTC….3´

3´….TAAGCATGCTAGCTGACTGACTGTCAG….5´

If the DNA polymerase starts replicating this segment from the right,

a. which will be the template for the leading strand?
b. Draw the molecule when the DNA polymerase is halfway along this segment.
c. Draw the two complete daughter molecules.
d. Is your diagram in part *b* compatible with bidirectional replication from a single origin, the usual mode of replication?

Answer:
a. If replication is proceeding such that the DNA on the right is replicated first, then the top strand is the template for the leading strand.
b.



c.
....5′-ATTCGTACGATCGACTGACTGACAGTC-3′....
....3′-TAAGCATGCTAGCTGACTGACTGTCAG-5′....

....5′-ATTCGTACGATCGACTGACTGACAGTC-3′....
....3′-TAAGCATGCTAGCTGACTGACTGTCAG-5′....

d. Yes, it simply represents replication at one of the forks.

28. The DNA polymerases are positioned over the following DNA segment (which is part of a much larger molecule) and moving from right to left. If we assume that an Okazaki fragment is made from this segment, what will be the fragment’s sequence? Label its 5´ and 3´ ends.

5´….CCTTAAGACTAACTACTTACTGGGATC….3´

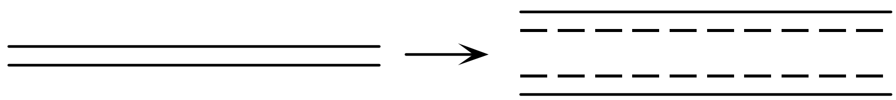
3´….GGAATTCTGATTGATGAATGACCCTAG….5´

Answer: The bottom strand will serve as the template for the Okazaki fragment so its sequence will be:

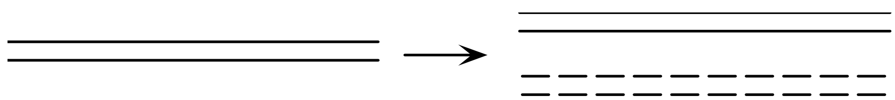
5´ ....CCTTAAGACTAACTACTTACTGGGATC....3´

29. *E. coli* chromosomes in which every nitrogen atom is labeled (that is, every nitrogen atom is the heavy isotope 15N instead of the normal isotope 14N) are allowed to replicate in an environment in which all the nitrogen is 14N. Using a solid line to represent a heavy polynucleotide chain and a dashed line for a light chain, sketch each of the following descriptions:

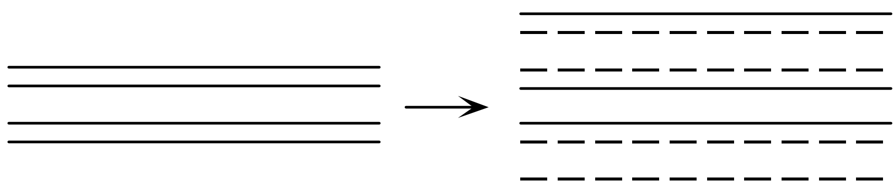
a. The heavy parental chromosome and the products of the first replication after transfer to a 14N medium, assuming that the chromosome is one DNA double helix and that replication is semiconservative.



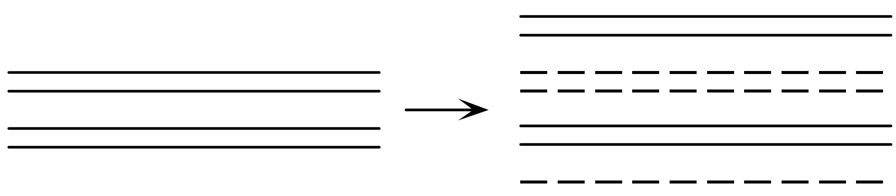
b. Repeat part *a,* but now assume that replication is conservative.



c. Repeat part *a,* but assume that the chromosome is in fact two side-by-side double helices, each of which replicates semiconservatively.



d. Repeat part *c,* but assume that each side-by-side double helix replicates conservatively and that the overall *chromosome* replication is semiconservative.



e. If the daughter chromosomes from the first division in 14N are spun in a cesium chloride density gradient and a single band is obtained, which of the possibilities in parts *a* through *d* can be ruled out? Reconsider the Meselson and Stahl experiment: What does it *prove*?

Answer: Model (b) is ruled out by the experiment. The results are compatible with semiconservative replication, but the exact structure could not be predicted. The other models would all give one band of intermediate density.

CHALLENGING PROBLEMS

30. If a mutation that inactivated telomerase occurred in a cell (telomerase activity in the cell = zero), what do you expect the outcome to be?
Answer: Without functional telomerase, the telomeres would shorten at each replication cycle, leading to eventual loss of essential coding information and death. In fact, there are some current observations that decline or loss of telomerase activity plays a role in the mechanism of aging in humans.

31. On the planet Rama, the DNA is of six nucleotide types: A, B, C, D, E, and F. Types A and B are called *marzines,* C and D are *orsines,* and E and F are *pirines.* The following rules are valid in all Raman DNAs:

Total marzines = total orsines = total pirines
A = C = E
B = D = F

a. Prepare a model for the structure of Raman DNA.
b. On Rama, mitosis produces three daughter cells. Bearing this fact in mind, propose a replication pattern for your DNA model.
c. Consider the process of meiosis on Rama. What comments or conclusions can you suggest?

Answer:
a. A very plausible model is of a triple helix, which would look like a braid, with each strand interacting by hydrogen bonding to the other two.
b. Replication would have to be terti-conservative. The three strands would separate, and each strand would dictate the synthesis of the other two strands.
c. The reductional division would have to result in three daughter cells, and the equational would have to result in two daughter cells, in either order. Thus, meiosis would yield six gametes

32. If you extract the DNA of the coliphage *φ*X174, you will find that its composition is 25 percent A, 33 percent T, 24 percent G, and 18 percent C. Does this composition make sense in regard to Chargaff's rules? How would you interpret this result? How might such a phage replicate its DNA?

Answer: Chargaff's rules are that A = T and G = C. Because this is not observed, the most likely interpretation is that the DNA is single-stranded. The phage would first have to synthesize a complementary strand before it could begin to make multiple copies of itself.