KEY ***DNA Replication and Mutation***
September 19, 2011

1. Below is a short DNA sequence located on the *E. coli* chromosome. In class we talked about how during the process of DNA replication, an enzyme adds methyl (–CH3) groups to the newly synthesized strand of DNA after a short delay. The delay in methylation provides the cell the opportunity to distinguish between the old and new strands of DNA. The enzyme (comically named the dam methylase!) adds methyl groups specifically to the adenine base when it is located within a particular sequence: GATC. In the portion of the DNA molecule below, the adenine bases located within the GATC sequence would be "marked" by methyl groups.

5' **GATC**ATCCG**G**TAAGCTTAACCG 3'
3' **CTAG**TAGGCCATTCGAATTGGC 5'

a. Assume that this *E. coli* cell undergoes a round of DNA replication. **Draw** the resulting two DNA molecules in the space provided below. Assume that there has ***not*** yet been enough time for methylation to have occurred in this stretch of newly synthesized DNA. **Label** all of the DNA strands with 5' and 3' labels, and clearly show which strand(s) contain methyl groups.

CH3
|
5'GATCATCCGGTAAGCTTAACCG3' (-CH_3 on A in GATC of old DNA strand)
3'CTAGTAGGCCATTCGAATTGGC5' (green is newly replicated DNA strand)

5'GATCATCCGGTAAGCTTAACCG3'
3'CTAGTAGGCCATTCGAATTGGC5' (-CH_3 on A in GATC of old DNA strand)
|
CH3

b. Now imagine that when the cell is undergoing the next round of DNA replication, DNA Pol III makes a mistake and inserts an "A" instead of a "C" at the 10th base from the left when using the 5' to the 3' (top) strand as a template. (This position is marked in blue on the original DNA sequence). The 3' to 5' template strand, however, is copied correctly.

Draw the DNA molecule that contains the point mutation, showing the methylation pattern prior to the activity of the dam methylase. Will the repair system replace the G or the A? **Explain** your answer.

In this problem we are assuming that the DNA pol III proofreading enzyme did not fix the mistake and continued on with replication - the cell is now dependent on the excision repair systems, such as the methyl mismatch repair system, to fix this mistake. Excision repair enzymes will remove the DNA around the mistake and then DNA pol will fill in the missing chunk with newly synthesized DNA.

CH3
|
5'GATCATCCGGTAAGCTTAACCG3' (old strand is methylated)

3'CTAGTAGGCAATTCGAATTGGC5' (new strand NOT METHYLATED YET)

The repair system will recognize the G-A base pair as being incorrect. The repair system will remove the A because it is located on the unmethylated strand of DNA. The lack of methylation signals a newly synthesized strand of DNA and, therefore, the strand more likely to contain the mistake.

Explain whether or not the daughter cells will each receive an identical copy of the chromosome:

If the repair system is operating correctly, the "A" will be replaced with a "C" and both daughter cells will receive an identical copy of the chromosome.

c. Think about what would happen if this same mutation had occurred in a cell with a defective repair system, in other words, in a cell where the genes that code for the proteins involved in the excision repair process are mutated and the repair proteins no longer function properly. **Explain** whether or not the daughter cells will each receive an identical copy of the chromosome in this situation:

If the repair systems are not working, the incorrect deoxynucleotide will remain in the strand of DNA. One daughter cell receives a chromosome containing the mismatched base pair and the other receives a chromosome with an identical sequence to the original. Each of the two daughter cells contains a different chromosome with a slightly different sequence of DNA.

5'GATCATCCGGTAAGCTTAACCG3'
3'CTAGTAGGCAATTCGAATTGGC5'

5'GATCATCCGGTAAGCTTAACCG3'
3'CTAGTAGGCCATTCGAATTGGC5'

Now think about what will happen in the subsequent round of cell division. **Draw** the DNA sequences that will be found in the chromosomes of each of the grand-daughter cells following the next round of DNA replication.

DNA sequence found in each chromosome of each of the 4 granddaughter cells:

5'GATCATCCGGTAAGCTTAACCG3'
3'CTAGTAGGCCATTCGAATTGGC5'

5'GATCATCCGTTAAGCTTAACCG3'
3'CTAGTAGGCAATTCGAATTGGC5'

5'GATCATCCGGTAAGCTTAACCG3'
3'CTAGTAGGCCATTCGAATTGGC5'

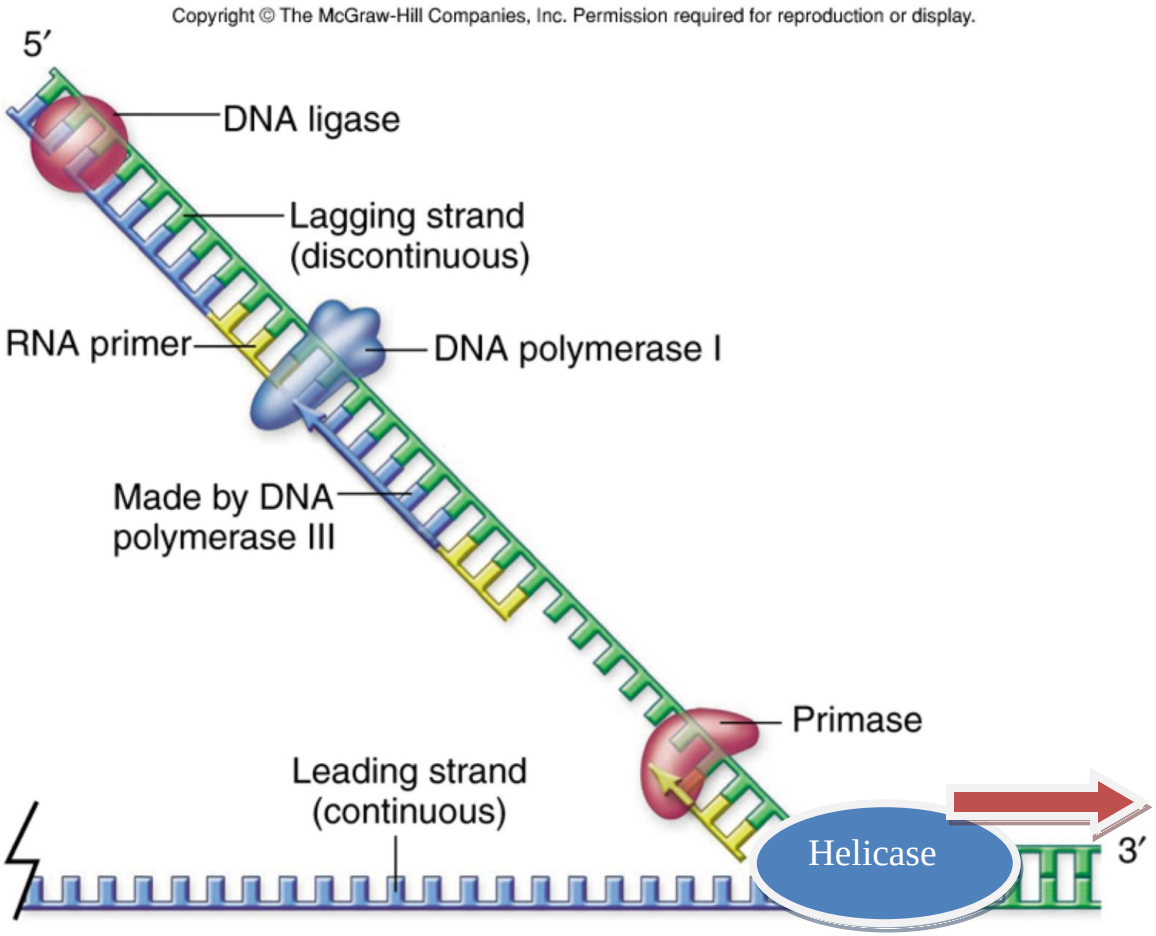
5'GATCATCCGGTAAGCTTAACCG3'
3'CATGTAGGCCATTCGAATTGGC5'

Use your textbook to answer the following questions about DNA structure and DNA replication

1. Use Figure 3.13 and the figure legend (p. 41) in your text to answer the following question. Why do we say and write 3'- OH and 5'- PO_4 group (the ' is read prime, so 3 prime or 5 prime) instead of 3 - OH and 5 - PO_4 when we refer to a chain of nucleotides?

In a nucleotide, the carbons in the sugar ring are identified by numbers followed by a prime symbol, and so are referred to as 1 prime or 1′. The carbons in the base are given a number without the prime symbol. In the DNA molecule, the OH and PO_4 groups are covalently bound to the sugar ring, not the base, and so we use the prime following the number.

2. Redraw figure 14.18 (p. 268). Where must the helicase be located? Draw it on your figure. Add an arrow to show the direction the helicase is moving.

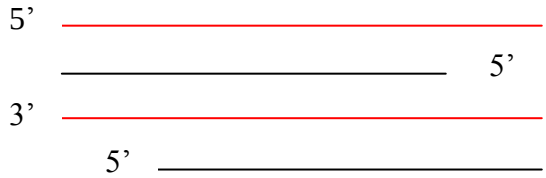


3.a) In the legend to Figure 14.23 (p. 271), what are the authors referring to when they say "Only one end is shown for simplicity....?" b) Draw and label the "other end," and clearly indicate how that end would look after replication, but prior to telomerase activity. c) Is the chromosome shown in figure 14.23 more likely to be from an E. coli (bacterium) or from a cat?

a) The authors are referring to one end of a long, linear chromosome. The newly synthesized lagging strand will be shortened.

b) The other end of this newly replicated chromosome should be fine.

Original DNA molecule



Black line represents newly replicated DNA; the 5' end of the newly synthesized strand is shorter; telomerase will extend this end.

c) The chromosome is more likely to be from a cat than a prokaryotic cell, such as E. coli. Prokaryotes have a circular genome and therefore don't have to deal with the end problem; eukaryotic organisms, such as cats, have linear chromosomes.