7.016 Recitation 5 – Fall 2017

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*
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Summary of Lecture 7 (9/19):

Historical experiments: In 1952, Alfred Hershey and Martha Chase did an experiment using T2 bacteriophage that confirmed that DNA and NOT the protein s was the genetic material. Messelson and Stahl's experiment elegantly proved the semiconservative mode of DNA replication.

DNA replication: This occurs during the "S phase" (S = synthesis) of cell division when two double-stranded DNA molecules are made from one double-stranded DNA (dsDNA) molecule Each daughter cell then receives one copy of newly synthesized dsDNA, which is identical that in parental cell. DNA replication is semi-conservative and the newly synthesized strand is antiparallel and complementary to the template strand. Replication is continuous for one strand ("leading strand") and discontinuous for the other strand ("lagging strand"). DNA polymerase catalyzes the addition of complementary bases to the 3' OH end of the newly synthesized strand. Replication starts at the ori site. DNA polymerase adds complementary bases only to the 3'OH end of small RNA primer that are made by the primase. These RNA primers are degraded later on by RNAses after replication and the gaps sealed using the dNTPs and DNA polymerase.

During replication, Helicase unwinds the two strands of DNA such that they can be replicated. The single stranded DNA binding proteins (SSDBP) then bind to and stabilize the unwound/separated strands of DNA. Topoisomerases remove the extra coils that form as the new DNA is being synthesized and allow the DNA to unwind. Each replication cycle results in the shortening of chromosomal ends. This process is slowed down by the action of telomerase enzyme that adds telomere repeats (5'TTAGGG3') to the chromosomal ends. The telomerases are functional only in embryonic stage but do become activated in diseases like cancer.

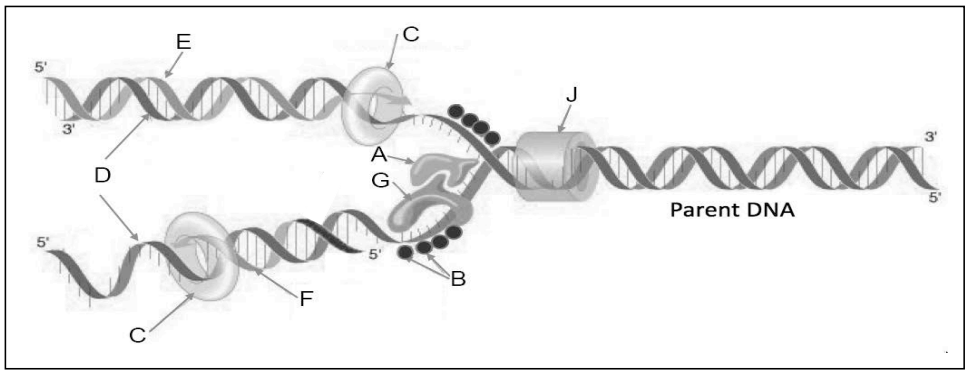
Processes that repair mutations in DNA: DNA polymerase enzyme has a 3->5' exonuclease activity due to which it proofreads the each base that it adds to increase the fidelity of replication. The fidelity of replication is further enhanced by mismatch repair and base - excision repair mechanism. These enzymes recognize the DNA strand with incorrect base(s) based on the difference in the methylation pattern of bases in the parental and newly synthesized strand. Lowering the error rate to 1 in 10^9.

Questions:

1. Complete the table below.

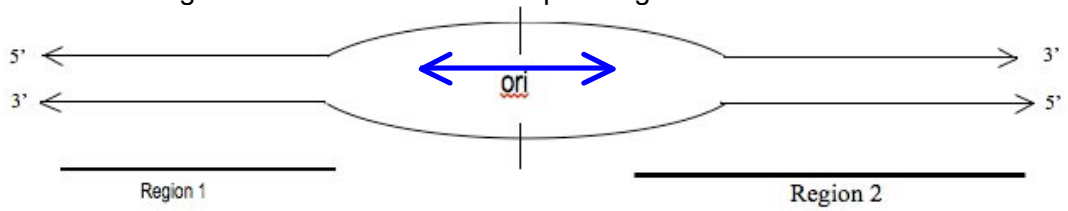
	Replication
Subcellular organelle (s) in eukaryotic cell where replication occurs is…	
Monomer used to form DNA polymer	
Rule for adding the incoming monomer?	
Covalent bond formed between two adjacent monomers in a DNA strand?	
Number of template strands needed to make DNA duplex	
Direction is the template DNA strand read?	
Direction is the new DNA template made?	

2. The following is the schematic of replicating genomic DNA in the nucleus of a eukaryotic cell. ***Note:*** letters A-J represents different components of replication.



On the schematic, neatly write the **CORRECT** component of replication, next to each letter by choosing from: *DNA polymerase, primer, helicase, single-strand DNA binding protein (SSDBP), Leading strand, Lagging strand, template strand, primase, topoisomerase*. Also, on the schematic, show the **movement of replication fork** by drawing an arrow.

3. Consider the following schematic that shows a replicating DNA.



a) Use an arrow(s) to show the direction of movement of replication forks.

b) Select the best option and provide a brief explanation for the option that you selected. The schematic represents a replicating ***prokaryotic/ eukaryotic*** DNA.

c) In Region 1, which strand (top/bottom) is the template for leading strand synthesis?

d) In Region 2, which strand will require a functional ligase?

4. If you wanted to replicate the following piece of DNA:

5'-GTACGTTTACGCCGTATATATCGTCGTAATGCTACGTAGCTCTACGAACA-3'
3'-CATGCAAATGCGGCATATATAGCAGCATTACGATGCATCGAGATGCTTGT-5'

a) Design a 10-bp-long primer you would use to generate a new copy of the entire bottom strand of DNA. Label the 5' and 3' ends of the primer.

b) In what direction does the **new strand** of DNA get synthesized?

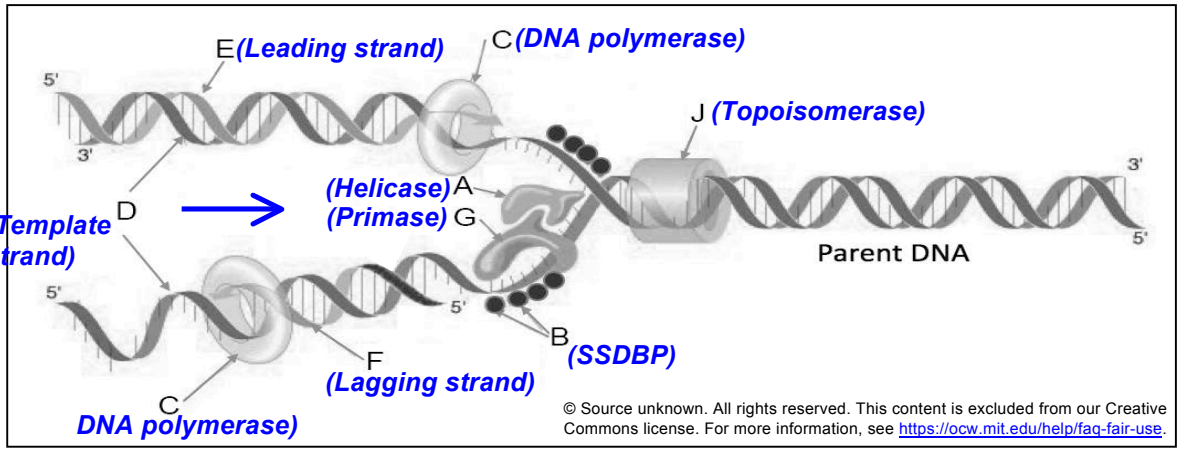
c) In what direction is the **template strand** for DNA synthesis read?

Solutions to Questions:

1. Complete the table below.

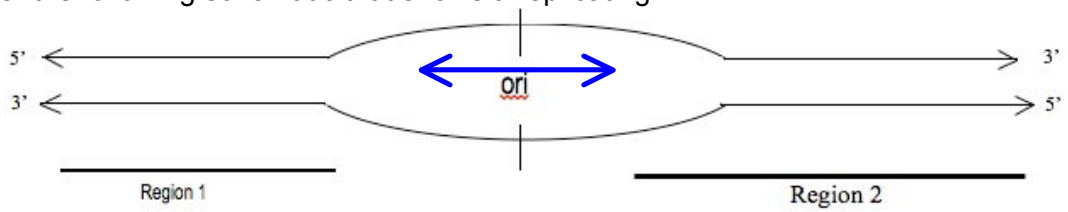
	Replication
Subcellular organelle (s) in eukaryotic cell where replication occurs is…	*Nucleus*
Monomer used to form DNA polymer	*Deoxyribonucleotide (dNTPs)*
Rule for adding the incoming monomer?	*3' end of the growing chain*
Covalent bond formed between two adjacent monomers in a DNA strand?	*3'->5' phosphodiester bond*
Number of template strands needed to make DNA duplex	*Two*
Direction is the template DNA strand read?	*3'->5'*
Direction is the new DNA template made?	*5'->3'*

2. The following is the schematic of replicating genomic DNA in the nucleus of a eukaryotic cell.
Note: letters A-J represents different components of replication.



On the schematic, neatly write the **CORRECT** component of replication, next to each letter by choosing from: *DNA polymerase, primer, helicase, single-strand DNA binding protein (SSDBP), Leading strand, Lagging strand, template strand, primase, topoisomerase*. Also, on the schematic, show the **movement of replication fork** by drawing an arrow.

3. Consider the following schematic that shows a replicating DNA.



a) Use an arrow(s) to show the direction of movement of replication forks.

b) Select the best option and provide a brief explanation for the option that you selected. The schematic represents a replicating ***prokaryotic***/eukaryotic DNA.
Considering there is only ori shown you may say it is a prokaryotic DNA. But you may also argue that the DNA shown is not circular and hence may be just a small segment of a big piece of replicating eukaryotic DNA.

c) In Region 1, which strand (top/bottom) is the template for leading strand synthesis? ***TOP STRAND***

d) In Region 2, which strand will require a functional ligase? ***TOP STRAND***

4. If you wanted to replicate the following piece of DNA:

5'-GTACGTTTACGCCGTATATATCGTCGTAATGCTACGTAGCTCTACGAACA-3'
3'-CATGCAAATGCGGCATATATAGCAGCATTACGATGCATCGAGATGCTTGT-5'

a) Design a 10-bp-long primer you would use to generate a new copy of the entire bottom strand of DNA. Label the 5' and 3' ends of the primer. *5'UGUUCGUAGA3'*

b) In what direction does the **new strand** of DNA get synthesized? ***5'->3'***

c) In what direction is the **template strand** for DNA synthesis read? ***3'->5'***

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