7.013 Recitation 6 – Spring 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*

Summary of Lectures 9 (2/26) and 10 (3/2):

DNA replication: This occurs during the "S phase" (S = synthesis) of cell division when two double-stranded DNA molecules (dsDNA) are made from one dsDNA molecule. 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 ("lagging strand"). The lagging strand is made continuous by the action of the ligase enzyme.

DNA polymerase catalyzes the addition of complementary deoxyribonucleotides (dNTPs) to the 3' OH end of the newly synthesized strand. Replication starts at the ori site, which is usually an AT rich sequence. DNA polymerase is a primer dependent enzyme i.e. it adds complementary bases only to the 3'OH end of small RNA primer that are made by the primase enzyme in the cell. These RNA primers are later degraded by RNAses 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) 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 to the chromosomal ends.

Processes that repair mutations in DNA: DNA polymerase enzyme has a 3->5' exonuclease activity due to which it proofreads each dNTP 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 DNA strand thus lowering the error rate to 1 in 10^9.

Transcription: A gene is a sequence of bases that encodes a final product (mostly a protein but sometimes an RNA). It is defined as a promoter (a regulatory sequence to which the RNA polymerase and transcription factors bind) followed by the sequence of bases that are transcribed to RNA. Since the promoter is located prior to the transcribed region and the DNA strand that serves as the template for transcription is always read in a 3'->5' direction to make an mRNA transcript in a 5'->3' direction, it is the location of the promoter for a gene which determines which DNA strand (top or the bottom) serves as the template strand to make an RNA transcript. Each gene also has a transcription start site and a transcription stop site.

The transcribed RNA can be one of three types: mRNA, tRNA or rRNA. All these types are identical in their chemical composition, but they differ in function. The mRNAs are transcribed from genes that encode proteins; these mRNAs get translated to proteins by ribosomes. The rRNAs and tRNAs participate in the process of translating mRNAs.

Errors during transcription are far higher than errors during replication since the RNA polymerase unlike the DNA polymerase lacks the proofreading ability. But the cell tolerates transcription errors much better than errors during replication. It is also to be noted that RNA polymerase unlike DNA polymerase does not require a primer to make an RNA transcript.

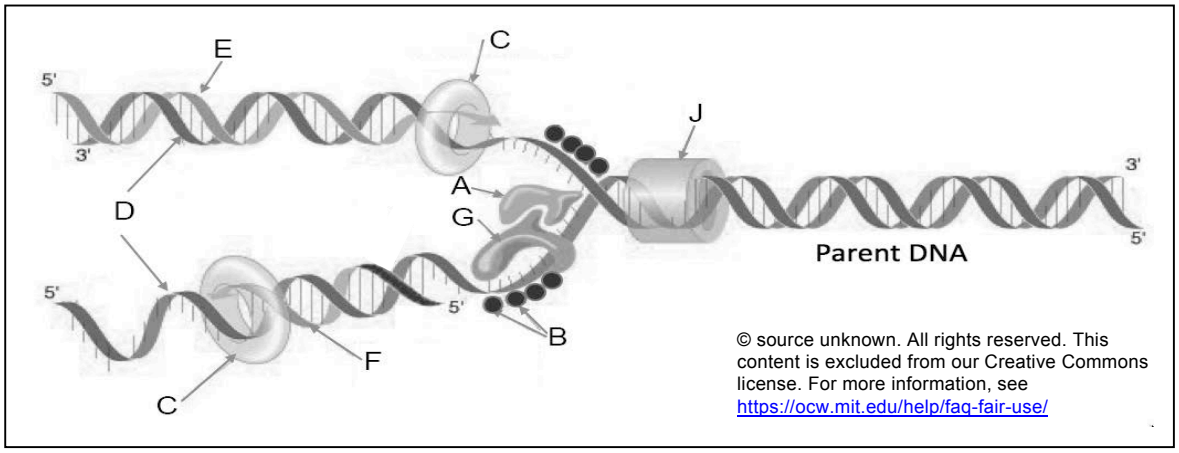
The rate of transcription is regulated at many levels: chromatin modifications, how strong the promoter is, the presence or absence of transcription factors and distal regulatory base sequences such as enhancers.

Questions

1. Complete the table below.

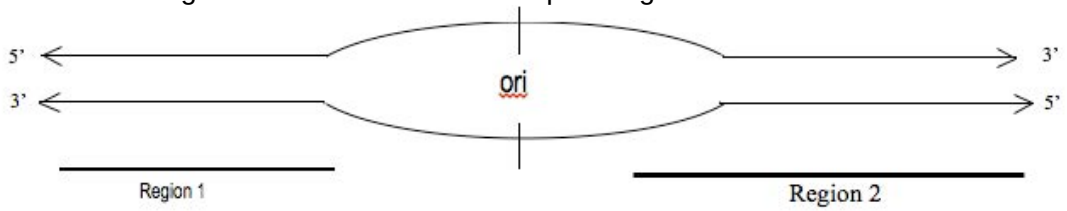
	Replication
Subcellular organelle (s) in eukaryotic cell where replication occurs is…	
Monomer used to form DNA polymer	
Rule for adding the incoming monomer?	
Covalent bond formed between two adjacent monomers in a growing DNA strand?	
Number of template strands needed to make DNA duplex	
In what direction is the template DNA strand read?	
In what 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: Different components of replication are represented by letters A-J .



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. Complete the table below.

	Transcription
Subcellular organelle (s) in eukaryotic cell where transcription occurs is…	
Monomer used to form RNA polymer	
Rule for adding the incoming monomer?	
Covalent bond formed between two adjacent monomers in a growing DNA strand?	
Number of template strands needed to make an mRNA transcript	
In what direction is the mRNA transcribed?	
In what direction is the DNA template read?	
Types of RNA produced	
Type of RNA that is translated to proteins	
Type(s) of RNA that are spliced	

5. The following is the partial DNA sequence of Gene 1 in a prokaryotic cell. ***Note:*** *The underlined sequence (from position 20-54) represents the promoter for Gene 1 and the underlined and italicized sequence (from position 71-90 represents its ribosomal binding (RBS) site. Transcription begins at and includes the bold T (Top)/A (bottom) base pair at position 60.*

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   1         10         20         30         40         50         60         70
   I---------I---------I---------I---------I---------I---------I---------I
5' ATCGGTCTCGGCTACTACATAAACGCGCGCATATATCGATATCTAGCTAGCTATCGGTCTAGGCTACTAC
3' TAGCCAGAGCCGATGATGTATTTGCGCGCGTATATAGCTATAGATCGATCGATAGCCAGATCCGATGATG
                                  Promoter

             80         90         100        110        120        130        140
   ---------I---------I---------I---------I---------I---------I---------I
5' CAGGTATCGGTCTGATCTAGCTAGCTTCTCTTCTCTCTCTCCCCCGCGGGGGCTGTACTATCATGCGTCG
3' GTCCATAGCCAGACTAGATCGATCGAAGAGAAGAGAGAGGGGGCGCCCCCGACATGATAGTACGCAGC
       RBS

             150        160        170        180        190        200        210
   ---------I---------I---------I---------I---------I---------I---------I
5' TCTCGGCTACTACGTAAACGCGCGCATATATCGATATCTAGCTAGCTATCGGTCTCGGCTACTACGTAAA
3' AGAGCCGATGATGCATTTGCGCGCGTATATAGCTATAGATCGATCGATAGCCAGAGCCGATGATGCATTT
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a) Which strand (*Top/ bottom*) is the template strand for transcription of Gene 1?

b) What are the first 6 nucleotides of the mRNA transcribed from Gene 1?

7.013 Introductory Biology

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