7.016 Recitation 6 – Fall 2018

(**_Note:_** The recitation summary should NOT be regarded as the substitute for lectures)
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Summary of Lecture 8 (9/24):

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 base - excision repair and nucleotide excision repair mechanisms. 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.

Transcription: A gene is a sequence of bases that encodes a protein. It is defined as a promoter (a regulatory sequence to which the RNA polymerase and transcription factors bind to start the process followed by the sequence of bases that are transcribed to make the RNA. Since the promoter is always located prior to the region that is transcribed 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 (the 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 DNA is unwound both during the processes of replication and transcription.

Errors during transcription are far higher compared to the errors during replication. But these errors during transcription are much better tolerated by the cell since mRNA has a shorter half-life compared to DNA and also because multiple copies of the an mRNA transcript are made during the transcription of a gene of which only some have errors. The rate of transcription of a gene is regulated at many levels: chromatin modifications, how strong the promoter is, the presence or absence of the transcription factors and distal regulatory base sequences such as enhancers.

Questions:

1. A small region of **replicated DNA** with a mismatched base (bold and underlined) is shown.

```
5'_____T T AAGCG_____3'  (Top strand)
3'_____ATTTCGC_____5'  (Bottom strand)
```

a) Which process will repair the mismatch: **DNA Proofreading/ Base excision repair (BER)/ Nucleotide excision repair**?

b) Which activity of DNA polymerase will add the correct base: **3'→ 5' exonuclease / 5'→ 3' exonuclease / 3'→ 5' polymerization / 5'→ 3' polymerization?**

2. 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 RNA strand?	
Number of template strands needed to make an mRNA transcript	
In what direction is the mRNA transcribed?	

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In what direction is the DNA template read?	
Types of RNA produced	
Type of RNA that is translated to proteins	
DNA being transcribed is methylated/ demethylated?	
Chromatin acetylation promotes transcription?	

3. 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.*

```
     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' GTCCATAGCCAGACTAGATCGATCGAAGAGAAGAGAGAGAGGGGGCGCCCCCGACATGATAGTACGCAGC
           RBS

             150       160       170       180       190       200       210
     ---------I---------I---------I---------I---------I---------I---------I
  5' TCTCGGCTACTACGTAAACGCGCGCATATATCGATATCTAGCTAGCTATCGGTCTCGGCTACTACGTAAA
  3' AGAGCCGATGATGCATTTGCGCGCGTATATAGCTATAGATCGATCGATAGCCAGAGCCGATGATGCATTT
```

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?

4. The following is a partial sequence from the hypothetical gene, gene X. The boxed region is the promoter, and the arrow indicates the direction of transcription. Transcription begins at and includes the first G/C base pair after the box.

```
  5' ATCAGACAACGTCTCATGGGAGTACTTGGATGGAAGAGTAGAAGGTCATGACCAACCTCTTCCAATCCAACCACAAACAG
     +---------+---------+---------+----+   ←   +------+---------+---------+---------
  3' TAGTCTGTTGCAGAGTACCCTCATGAACCTACCTTCTCATCTTCCAGTACTGGTTGGAGAAGGTTAGGTTGGTGTTTGTC

  5' AAAATCAGCCAATATGTCCGACTTCGAGAACAAGAACCCCAACAACGTCCTTGGCGGACACAAGGCCACCCTTCACAACC
     +---------+---------+---------+---------+---------+---------+---------+---------
  3' TTTTAGTCGGTTATACAGGCTGAAGCTCTTGTTCTTGGGGTTGTTGCAGGAACCGCCTGTGTTCCGGTGGGAAGTGTTGG
```

Give the first 10 nucleotides of the mRNA produced from Gene X.

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Solutions to Questions:

1. A small region of **replicated DNA** with a mismatched base (bold and underlined) is shown.

```
5'_____TTAAGCG_____3' (Top strand)
3'_____ATTTCGC_____5' (Bottom strand)
```

a) Which process will repair the mismatch: *DNA Proofreading/ Base excision repair (BER)/ Nucleotide excision repair*?

b) Which activity of DNA polymerase will add the correct base: *3'→ 5' exonuclease / 5'→ 3' exonuclease / 3'→ 5' polymerization / 5'→ 3' polymerization*?

2. Complete the table below.

	Transcription
Subcellular organelle (s) in eukaryotic cell where transcription occurs is…	*Nucleus*
Monomer used to form RNA polymer	*Ribonucleotide (NTPs)*
Rule for adding the incoming monomer?	*Gets added to the 3' end of the growing chain*
Covalent bond formed between two adjacent monomers in a growing RNA strand?	*3'->5' phosphodieester bond*
Number of template strands needed to make an mRNA transcript	*One, selection based on the orientation of the promoter*
In what direction is the mRNA transcribed?	*5'->3'*
In what direction is the DNA template read?	*3'->5'*
Types of RNA produced	*mRNA, tRNA, rRNA*
Type of RNA that is translated to proteins	*mRNA*
DNA being transcribed is methylated/ demethylated?	*Demethylated*
Chromatin acetylation promotes transcription?	*Yes*

3. 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.*

```
      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' GTCCATAGCCAGACTAGATCGATCGAAGAGAAGAGAGAGAGGGGGCGCCCCCGACATGATAGTACGCAGC
      RBS

      150       160       170       180       190       200       210
      ---------I---------I---------I---------I---------I---------I---------I
   5' TCTCGGCTACTACGTAAACGCGCGCATATATCGATATCTAGCTAGCTATCGGTCTCGGCTACTACGTAAA
```

a) Which strand (*Top/ bottom*) is the template strand for transcription of Gene 1? *Bottom*

b) What are the first 6 nucleotides of the mRNA transcribed from Gene 1?
 5' UAGGCU3'

4. The following is a partial sequence from the hypothetical gene, gene X. The boxed region is the promoter, and the arrow indicates the direction of transcription. Transcription begins at and includes the first G/C base pair after the box.

```
5' ATCAGACAACGTCTCATGGGAGTACTTGGATGGAACAGTAGAAGGTCATGACCAACCTCTTCCAATCCAACCACAAACAG
   +---------+---------+---------+----←------+---------+---------+---------+---------
3' TAGTCTGTTGCAGAGTACCCTCATGAACCTACCTTGTCATCTTCCAGTACTGGTTGGAGAAGGTTAGGTTGGTGTTTGTC

5' AAAATCAGCCAATATGTCCGACTTCGAGAACAAGAACCCCAACAACGTCCTTGGCGGACACAAGGCCACCCTTCACAACC
   +---------+---------+---------+---------+---------+---------+---------+---------+---------
3' TTTTAGTCGGTTATACAGGCTGAAGCTCTTGTTCTTGGGGTTGTTGCAGGAACCGCCTGTGTTCCGGTGGGAAGTGTTGG
```

Give the first 10 nucleotides of the mRNA produced from Gene X.
5'CUUCCAUCCA3'

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