TRASK Zool 3200: Cell Biology
Exam 2
2/20/15

Answer each of the following short and longer answer questions in the space provided; circle the BEST answer or answers for each multiple choice question and circle either ‘True’ or ‘False’ to indicate whether each true/false statement is correct or not. Explain your answers when requested to do so. And hey, have fun and good luck! (© 70 points total)

Stepwise condensation of linear DNA happens in five different packing processes. Which of the following four processes has a direct requirement for histone H4? (1 point)

a.) formation of “beads-on-a-string”
b.) formation of the 30 nm fiber
c.) looping of the 30 nm fiber
d.) packing of loops to form interphase chromosomes

Interestingly, it is possible to transfer intact frog chromosomes from one frog cell to another. Upon transferring an intact chromosome that has been fluorescently labeled (so it can be differentiated from the cell’s endogenous chromosomes), it is noted that the fluorescent transferred chromosome is able to be compacted during metaphase of the cell division cycle. If the same experiment is attempted using an intact, labeled human chromosome (into a frog cell), the fluorescent transferred chromosome can form a 30 nm structure but is unable to be compacted into a ‘lamp brush’ configuration, or further into a metaphase chromosome. How might these results be explained? Be sure to address both how the 30 nm fiber is able to form and how the 300 nm conformation is unable to form. (4 points)

True / False. If an origin of replication is deleted from a eukaryotic chromosome, the DNA on either side will ultimately be lost as well, because it cannot be replicated. (1 point)

True / False: When bidirectional replication forks from adjacent origins meet, a leading strand always runs into a lagging strand. (1 point)

True / False. When read in the same direction (5’→ 3’), the sequence of the nucleotides in a newly-synthesized DNA strand is the same as in the parental template strand. (1 point)
True / False. The newly synthesized strand of a human chromosome was synthesized from multiple origins by either continuous or discontinuous DNA synthesis, depending on which specific daughter chromosome is being examined. (1 point)

True / False. Each time the genome is replicated, half of the newly-synthesized DNA is stitched together from Okazaki fragments. (1 point)

A born skeptic, you plan to confirm for yourself the results of a classic experiment originally performed in the 1960s by Meselson and Stahl who demonstrated, once and for all, that DNA replication is a semi-conservative process. To verify their results, you ‘synchronize’ a culture of growing cells so that virtually all cells in the culture begin and complete DNA replication at the exact same time. You first grow the cells in a medium that contains nutrients highly enriched in ‘heavy’ isotopes of nitrogen and carbon (¹⁵N and ¹³C in place of naturally-abundant ¹⁴N and ¹²C). Cells growing in this ‘heavy’ medium use the heavy isotopes to build all of their macromolecules, including nucleotides and nucleic acids. You then transfer the cells to a normal, ‘light’ medium containing the typical ¹⁴N and ¹²C nutrients. Finally, you isolate DNA from cells that have grown for different numbers of generations in the light medium and determine the density of their DNA by density-gradient centrifugation. You collect data, and plot the amount of DNA isolated versus its density. Complete the graphs for the first and second generations in light medium below. (4 points)
Look carefully at the structures of the two modified cytosine nucleotides shown below. One or the other of the two compounds is added to an *in vitro* DNA replication reaction. What product(s) would you expect if compound A were added to the reaction in large excess over the concentration of the available deoxycytosine triphosphate (dCTP)? What effects would you expect if compound B were added to the reaction under the same conditions? Explain your answers. (4 points)

![Diagram of compounds A and B]

Consider the copy of chromosome 3 that you received from your mother. Is it 100% identical to the same chromosome 3 in a neighboring cell? If it is not completely identical, how and why might the two chromosome 3s differ? (2 points)

Name 2 ways in which transcription is similar to replication and two ways in which the two processes differ. (4 points)

How long would it take to transcribe a prokaryotic gene containing $2.3 \times 10^4$ nucleotides? How many errors would this transcript be expected to contain? (4 points)
Although our cells have different enzymes for the process of transcription and replication, is it possible that the RNA polymerase used for transcription of mRNAs might be used as the polymerase that makes the RNA primer required for replication? Why or why not? (3 points)

Which of the following might decrease the global production of mRNA in a prokaryotic cell? (1 point).
   a.) A decrease in the amount of sigma (σ) factor.
   b.) A decrease in the amount of RNA polymerase II.
   c.) A mutation that introduces a stop codon in the 5’ UTR.
   d.) A mutation that introduced extensive sequence changes into the DNA preceding the transcription start site of the gene.
   e.) A mutation that moves the transcription termination signal for the gene farther away from the transcription start site.

What do sigma (σ) factor and TFIID have in common? Other than being in different cell types, how do these two proteins differ? (2 points)

Indicate with an arrow (e.g., ↑ or ↓) the approximate transcription start site. Be sure to place the arrow on the strand that will be READ. (2 points)
What term is used to describe the strand of DNA that is NOT read? (1 point)

Which of the following will prevent the activation of RNA polymerase, but not necessarily the assembly of eukaryotic general transcription factors at the promoter? (1 point)
   a.) Mutation of the TATA box.
   b.) Absence of the TBP element of TFIID.
   c.) Absence of TFIIH.
   d.) Absence a distant enhancer element.

Imagine that RNA polymerase II is transcribing the sequence below—in the middle of an mRNA, so transcription has already initiated—from left to right. What will the sequence of the mRNA be? (2 points)

\[
\begin{align*}
5' & - \text{GTAACGGATG} - 3' \\
3' & - \text{CAT TGCCTAC} - 5'
\end{align*}
\]

In the cytoplasm, how is it possible to distinguish an mRNA from any other type of RNA such as a tRNA or an rRNA? (2 points)

In eukaryotic cells, RNA polymerase _____ is responsible for transcribing tRNAs while RNA polymerase _____ is responsible for the transcription of microRNAs. (Fill in the blanks with appropriate Roman numerals; 2 points)
You have identified a mutant mouse that has an unusual capacity to metabolize alcohol, apparently because of a new enzyme in the liver. Using various molecular biological techniques, you successfully clone the entire gene for this enzyme and name it drunk’n. You would like to perform some experiments on the drunk’n protein to determine its alcohol-metabolizing activity in a test tube (in vitro). You decide to clone the mouse gene into bacteria and insert it adjacent to a 70 promoter (i.e., ~10 nts from the TATA box) to ensure that the gene is transcribed nearly all the time. To your dismay, bacteria into which the drunk’n gene has been cloned do not synthesize the expected drunk’n protein. Hypothesize a reason for the bacteria’s inability to produce the expected drunk’n protein from the mouse gene. Explain. (3 points)

What is the consensus recognition sequence for poly-A addition in eukaryotic organisms? Describe the steps required for the addition of multiple ‘A’ nucleotides? (4 points)

Regarding a prokaryotic translation product, specifically what amino acid would be at the extreme amino terminus of the polypeptide chain that translation produced? (1 point)
A cell has acquired a spontaneous mutation in a gene encoding for a tRNA that is meant to carry an asparagine amino acid. The mutation converts the penultimate (next-to-last) nucleotide to a G. Typically, what are the three terminal nucleotides of this tRNA supposed to be? What is one potential sequence of the anticodon of this mutant tRNA? (2 points)

As a result of this mutation, when the asparaginyl tRNA synthetase enzyme attempts to charge these mutant tRNA with an asparagine amino acid, it is unable to do so. When the ribosome encounters an AAC codon, what happens to the polypeptide that is being synthesized? (1 point)

a.) Because an amino acid is not attached to the tRNA, the ribosome ‘skips over’ the AAC codon and simply creates a peptide bond between the amino acid before the AAC codon and the amino acid after it.
b.) Because an amino acid is not attached to the tRNA, no peptide bond can be formed so polypeptide synthesis is terminated, similar to what would occur if a stop codon were encountered.
c.) Because an amino acid is not attached to the tRNA, when it enters the ‘A’ site, it will be rejected by the ribosome until a charged tRNA enters. Thus, protein synthesis will be slowed, but an asparagine amino acid will be incorporated at the appropriate site.
d.) Because the polypeptide cannot be synthesized without this tRNA, the mRNA encoding it will be degraded.

In another cell, the non-coding strand of DNA in a region for an Arginine tRNA gene has acquired a single nucleotide mutation that corresponds to the tRNA anticodon loop. The C→T mutation results in a tRNA with an anticodon that reads 5’-UCA-3’ rather than the un-mutated 5’-UCG-3’. Describe what protein products you would expect to observe in this cell. (2 points)
You are interested in examining the regulation of the gene that encodes an enzyme, Tre-ase, important in metabolizing trehalose into glucose in bacteria. Trehalose is a disaccharide formed of two glucose units. It is known that two DNA binding proteins, TreA and TreB, are important for binding to the promoter of the Tre-ase gene and are involved in regulating the transcription of the Tre-ase gene: TreA binds to the “A” site in the promoter region, and TreB binds to the “B” site. You make mutations in the TreA and TreB genes to create cells lacking these genes, observe what happens to transcription of the Tre-ase gene, and obtain the results in the table below. What is/are the roles for both TreA and for TreB proteins in controlling Tre-ase expression? Explain how you were able to discern this.

<table>
<thead>
<tr>
<th></th>
<th>Glucose Only</th>
<th>Trehalose Only</th>
<th>Glucose + Trehalose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>OFF</td>
<td>ON</td>
<td>ON (low level)</td>
</tr>
<tr>
<td>Cells lacking Tre A</td>
<td>ON (low level)</td>
<td>ON</td>
<td>ON (low level)</td>
</tr>
<tr>
<td>Cells lacking Tre B</td>
<td>OFF</td>
<td>ON (low level)</td>
<td>ON (low level)</td>
</tr>
<tr>
<td>Cells lacking both Tre A and Tre B</td>
<td>ON (low level)</td>
<td>ON (low level)</td>
<td>ON (low level)</td>
</tr>
</tbody>
</table>

From these data above, what do you predict will happen to Tre-ase transcription (compared with that in normal cells) in the presence of trehalose if you were to create a version of the TreA protein that will constitutively (i.e., always automatically) bind to the “A” element (the site to which A binds) in the Tre-ase promoter? Explain. (6 points)
You are interested in understanding the gene regulation of Lkp1, a protein that is normally produced in liver and kidney cells in mice. Interestingly, you find that the *LKP1* gene is not expressed in heart cells. You isolate the DNA upstream of the *LKP1* gene, and clone it upstream of the gene for green fluorescent protein (GFP). You then insert this entire piece of recombinant DNA into mice. You find GFP expressed in liver and kidney cells but not in heart cells, an expression pattern similar to the normal expression of the *LKP1* gene. Further experiments demonstrate that there are three regions in the promoter, labeled A, B, and C in the figure below, that contribute to this expression pattern. Assume that a single and unique transcription factor binds to each site such that protein X binds site A, protein Y binds site B, and protein Z binds site C. You want to determine which region is responsible for the observed tissue-specific expression, and create mutations in the promoter to determine the function of each of these regions. The data you obtain is shown in the figure below—if the site is missing, it is mutated such that it cannot bind its corresponding transcription factor. Which of the DNA binding proteins (X, Y and/or Z) are likely to act as gene repressors, if any? Do any function as a gene activator protein? If so, which one(s)? In what tissue, if any, is factor Z normally present and bound to the DNA? Explain your rationale. (6 points)

![Promoter Regions and Expression Patterns](image)

In the question above, Experiment 1 in the figure the positive control, demonstrating that the region of DNA upstream of the gene for GFP results in a pattern of expression that we normally find for the *LKP1* gene. Experiment 2 shows what happens when the sites for binding factors X, Y, and Z are removed. Which experiment above demonstrates that factor X alone is sufficient for expression of LPK1 in the kidney? (1 point)

a.) experiment 3  
b.) experiment 5  
c.) experiment 6  
d.) experiment 7