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)

Packaging to a 30nm fiber requires histone proteins whereas further packaging to the lamp brush configuration or a metaphase chromosome depends upon DNA binding to non-histone proteins. Human DNA can apparently interact with the histone proteins in frog cells because human DNA is able to be packaged into the 30nm, histone-dependent conformation. Thus, frog and human histone proteins share similarity. Conversely, it appears that the non-histone proteins upon which further DNA packaging depends do not have sufficient similarity between frog and human cells. This dissimilarity explains why human DNA cannot interact with non-histone DNA packaging proteins in frog cells.

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 exclusively 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 ($^{15}$N and $^{13}$C in place of naturally-abundant $^{14}$N and $^{12}$C). 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 $^{14}$N and $^{12}$C 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)

If nucleotide A were added in excess to a replication reaction, no replication would be able to occur because the nucleotide shown is missing a 3’–OH group. Without the –OH group to chemically bond to the next ‘incoming’ nucleotide, no polymerization could occur. If the nucleotide shown in B were included in large excess, the same would occur. While the 2’–OH would not interfere with the reaction, the absence of a 5’ tri-phosphate would prevent this nucleotide from being added onto the 3’ end of a growing chain. Thus, if either of the nucleotides shown were added into a replication reaction, the reaction would not proceed—no double stranded products (or perhaps VERY short ones) would be produced.

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 3's differ? (2 points)

Probably suggests that they are likely 100% identical, but it is possible that they are not given that the rate of replication-induced error (mutation) is 1 nucleotide in every 10^9 (following repair) is wrong. With the size of the human genome, a single round of DNA replication therefore results in the production of approximately 6 errors in the genome. There are 46 chromosomes (23 from mom and 23 from dad) in the human genome, and it’s likely that these 6 replication-induced errors are spread across these 46 chromosomes. Thus, it is likely that these two neighboring cells do not have a replication-induced DNA alteration in the maternal copy of chromosome 3—but it is possible.

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

Multiple possible answers, including:
Similarities: 5’ to 3’, uses DNA template to pair nucleotides in a complementary manner, requires an enzyme, nucleotide triphosphates are used, starts at a defined site, etc.
Differences: Replication polymerizes dNTPs and transcription polymerizes NTPs, different enzymes are used, Replication product remains bonded to the template whereas transcription product is released, both strands are ‘read’ during replication but only one strand is ‘read’ during transcription, replication copies the whole genome whereas transcription is the copying of only part of the genome, replication starts at an origin whereas transcription starts at a promoter, etc.

How long would it take to transcribe a prokaryotic gene containing 2.3 x 10^6 nucleotides? How many errors would this transcript be expected to contain? (4 points)

Prokaryotic RNA polymerase works at a rate of 100 nt/sec with an error rate of 1 mistake/10^4 nts.
2.3 x 10^6 nucleotides / 100 nt/sec = 2.3 x 10^2 seconds (230 seconds or 3.8 minutes. 2.3 x 10^4 nucleotides / 1 error / 104 nucleotides = 2.3 mistakes (so roughly 2 mis-paired nucleotides in the transcript).
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)

It is not possible that the enzymes are interchangeable due to their different mechanisms of initiation and to their different levels of processivity. While both will polymerize ribonucleotides using DNA as a template, the RNA polymerases that are used during transcription must start at a promoter; those used to make the primers during DNA replication must associate with DNA polymerase at the replication fork. In addition, the polymerases used during replication have very low processivity, linking only a dozen or so nucleotides to produce a primer. This is significantly different than the processivity of RNA Polymerase II, which is responsible for transcribing several thousands of nucleotides in a single transcript.

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)

Both proteins recognize the promoter site immediate upstream of a transcription start site—both bind to the ‘TATA box’. They also both function as general transcription factors that do not remain associated with an RNA polymerase after transcription has initiated. These two proteins differ in that sigma factor is a single protein whereas TFIID is a complex containing the TBP. In addition, σ factor binds to a 2-part promoter that has recognition sequences located at the -10 and -35 regions upstream of the transcription start site whereas TFIID/TBP recognizes a single sequence located approximately 25 nucleotides upstream of the transcription start site.

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 during transcription? (1 point)

The coding strand.

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 TFIH.

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)

5’-GTAACGGATG-3’
3’-CAT TGCCTAC-5’

5’-GUAACGGAUG-3’

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)

mRNAs have a 5’ methyl-G cap and a 3’ poly-A tail.

In eukaryotic cells, RNA polymerase ____ __ III ____ is responsible for transcribing tRNAs while RNA polymerase ______ __ II ____ 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 **TATA** 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)

The reason that expression of the *drunk’n* gene in bacteria does not produce a functional protein is because the mouse gene contains introns that bacteria do not have the cellular machinery to remove. Mouse genes like *drunk’n* are comprised of both exons that encode for the *drunk’n* protein and introns that should be spliced out. When the mouse gene is expressed in a mouse cell, the introns can be accurately removed by the splicing machinery. When the mouse gene is expressed in a bacterium, even when it is transcribed it is not properly spliced. Because the entire mouse gene sequence is transcribed when expressed in bacteria, a bacterium will translate the entire transcript. The entire transcript, including introns, is not likely going to produce a functional protein.

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)

The consensus sequence is AAUAAA and it can be found on the mRNA transcript some number of nucleotides after its stop codon. When the AAUAAA sequence is transcribed by RNA Polymerase II, two cleavage factors (CstF and CSF) cut the transcript a few nucleotides after it. The cleavage factors recruit a PAP (poly-A polymerase) enzyme that is able to add adenosine nucleotides onto the 3’ end of the transcript without the use of a template. Several hundred adenosine nucleotides are polymerized by the PAP to make up the 3’ ‘tail’ of every Polymerase II transcript.

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 formylated methionine (f-Met) amino acid makes up the N-terminus of all bacterially-produced proteins, at least initially.
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)

The last 3 nucleotides of every tRNA are supposed to be 5’-CCA-3’. With the mutation described in the question, this tRNA would have a terminal 5′-CGA-3 sequence—this mutation may result in failure of the AA tRNA synthetase enzyme to properly bind to and “charge” this mutant tRNA.

Because the codons for asparagine include: AAC and AAU, the anti-codon options are: 5’-GUU-3’ & 5’-AUU-3’ (respectively).

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’-UGC-3’. Describe what protein products you would expect to observe in this cell. (2 points)

The mutation produces a tRNA that will bind to a 5’-UGA-3’ codon; this is normally a ‘STOP’ codon that would terminate translation of the protein encoded by this mRNA. The reason a stop codon results in translation termination is because (normally), no tRNA binds to this codon. However, in this cell with the mutant tRNA, there is a tRNA that will bind to the UGA stop codon; the tRNA will be carrying an Arginine amino acid. Thus, the protein products that one would expect to observe in this cell would be extra-long—translation of every mRNA that was meant to stop via an mRNA’s UGA stop codon would continue into what should have been the 3’ UTR of the mRNA. This is likely to produce several non-functional proteins.
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>

Tre A is a repressor protein that, when absent, results in cells that have constitutive expression (i.e., “always on”) of the Trehalose operon (2nd line of the table), even in the absence of trehalose (glucose only). [2 points]

Tre B is an activator protein. When it is present, in the absence of glucose, cells actively transcribe the trehalose operon (trehalose only, normal cells). However, when environmental conditions are the same but Tre B is absent (trehalose only, lacking Tre B), transcription continues [because the repressor is unbound] but only at a low level—cells cannot be activated to transcribe the operon at a high level. [2 points]

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)

Since the data presented support that Tre A is a repressor protein, if a version of the protein were always bound to the A element of the promoter, transcription of the trehalose operon will never be able to proceed—even in the presence of the carbohydrate. [2 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 \textit{LKP1} gene is not expressed in heart cells. You isolate the DNA upstream of the \textit{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 \textit{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)

Repressors: Z is a repressor protein and represses transcription in the heart.

Activators: Factors X and Y are activators. Factor X binds to the “A” enhancer element to activate transcription in the kidney and factor Y binds to the “B” element to activate transcription in the liver. When the “A” element is present, X factor can activate transcription of the LKP in kidney. When it is absent (experiments 5 & 8), transcription in the kidney does not occur. This happens regardless of the presence of the “B” element (experiment 6) or the “C” element (experiment 7). When the “B” element is present, Y factor can activate transcription of the LKP in liver. When it is absent (experiments 3, 5 & 7), transcription in the liver does not occur. This happens regardless of the presence of the “A” element (experiment 6) or the “C” element (experiment 8).

Factor Z is normally present in heart tissue and bound to the “C” DNA element to repress transcription of the LKP gene in the heart. Whenever the “C” element is present (experiments 5, 7 & 8), transcription in the heart does not occur; when it is absent, transcription in the heart \textit{does} occur.

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 \textit{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 LKP1 in the kidney? (1 point)

\begin{itemize}
  \item [a.)] experiment 3
  \item [b.)] experiment 5
  \item [c.)] experiment 6
  \item [d.)] experiment 7
\end{itemize}