What constitutes a bacterial promoter? What recognizes and binds to it? (2 points)
The promoter is a segment of DNA lying immediately ‘upstream’ but adjacent to the transcription start site. It contains two elements, a TATA (TA-rich) “box” that is located approximately 10 nucleotides upstream (-10 site) and a second GC-rich site approximately 35 nucleotides upstream (-35). These sequences are bound/recognized by the prokaryotic polymerase’s sigma factor.

Answer each of the following True / False questions by circling the correct answer (1 point each; 15 points total)

True / False. The sigma factor of RNA polymerase is responsible for catalyzing condensation reactions between ribonucleotides during prokaryotic transcription.

True / False. The process of prokaryotic transcription is terminated when sigma factor disengages from the polymerase complex.

True / False: The process of prokaryotic transcription must be terminated before translation can occur due to compartmentalization.

True / False: The process of eukaryotic transcription termination depends upon the 3’ untranslated region’s hairpin structure.

True / False: The process of eukaryotic transcription initiates at a start codon.

True / False. In any one region of a eukaryotic chromosome, it is typical for only one DNA strand to be used as a template for transcription.

True / False: Eukaryotic RNA polymerase associates with several RNA modifying enzymes throughout the transcription process.

True / False: Modification of an immature RNA product occurs simultaneously with the process of eukaryotic transcription.

True / False: Initiation of prokaryotic translation depends upon the mRNA’s 5’ cap.
True / False: All proteins regardless of the cell type that has produced them are made with some sort of methionine amino acid at their amino terminal ends.

True / False: All proteins, regardless of the cell type that has produced them, are made with a stop codon at their 3’ ends.

True / False. Wobble base-pairing occurs between the 3’ position in the codon and the 5’ position in the anti-codon.

True / False. All charged tRNAs must first bind to a ribosome’s A site prior to moving to the ribosome’s P site.

True / False. Stop codons are those for which no reverse-complementary anticodons exist.

True / False. Prior to initiating eukaryotic translation, large ribosomal subunits carrying a charged tRNA scan mRNAs from the 5’ end until they stall at the start codon.

The 3’ end of a prokaryotic gene is shown below. What are the implications of a DNA mutation at the circled base pair from its normal C-G to an T-A? (3 points)

5’-CCC ACAGCCGCCAGITTCGC CTGGCGGCATT TA ACTTT CT TTAATGA-3’
3’-GGGT GTCCGCGGTC AAGGCGCCGTAAAAT TGAAAGAAATTACT-5’

The boxed sequences, if at the 3’ end of a prokaryotic gene, are those that will form the hairpin structure (the boxed sequences are complementary to one another) in the RNA that is transcribed from it. This hairpin at the 3’ end is responsible for putting strain on the polymerase such that transcription is terminated. If the C-G base pair is mutated to a T-A base pair, the hairpin will likely be unstable (it won’t be 100% complementary) and may not form. Thus, transcription will continue, likely for hundreds of nucleotides if not more. While this will waste energy and create a longer RNA product, it won’t have an effect on the product of translation (assuming this gene will produce an mRNA) because a stop codon would definitely have been coded upstream of the transcription termination signal.

Complete the table below by providing the names of appropriate polymerases and/or transcription products for a-c. (3 points)

<table>
<thead>
<tr>
<th>Eukaryotic Polymerase</th>
<th>Transcription product</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Polymerase II</td>
<td>Micro RNAs</td>
</tr>
<tr>
<td>Polymerase I</td>
<td>b. most rRNAs</td>
</tr>
<tr>
<td>c. Polymerase III</td>
<td>tRNAs</td>
</tr>
</tbody>
</table>
Once it is determined that a bacterium needs to transcribe an operon, hundreds (if not thousands) of copies of polycistronic mRNAs are generated, as is shown in the image. Further, each mRNA is translated multiple times to produce an explosive increase in the concentration of each encoded protein inside the cell. How is the bacterium able to rapidly produce so many copies of each mRNA? Similarly, how is the process of translation made more efficient? (4 points)

Transcription efficiency is enhanced by sigma factor disengaging from the polymerase/DNA template just a few nucleotides from the transcription start site. Its “falling off” occurs in close proximity to the promoter so that it can more easily re-bind to the promoter sequences and initiate another polymerase’s assembly at the promoter so it can start transcribing immediately ‘behind’ another polymerase. In this way, sigma factor can facilitate the assembly of many, many polymerases, one starting immediately after another has left the promoter. To enhance the efficiency of translation, the small ribosomal subunit can essentially do the same thing as sigma factor—not disengage shortly after starting—but disengaging shortly after finishing translation. This is because mRNAs that are being actively translated form “polyribosomes” that are circular in the cytoplasm. The 3’ ends of actively-translated mRNAs are brought in close proximity to the 5’ ends where translation begins (by tail- and cap-binding proteins that associate with one another) so that as the ribosome falls apart at a stop codon, the small ribosomal subunit can pick up another initiator tRNA to start translation of the same mRNA again.

Describe the chemical composition of the 5’ end of mature mRNAs. What function(s) does this chemistry serve? (4 points)

mRNAs are “capped” with a methylated guanosine base that is part of a ribonucleotide (methyl-G nucleotide), attached via a tri-phosphate bridge to the 5’ end (a 5’-to-5’ linkage). The functions of this “capping” include: facilitation of nuclear export to the cytoplasm, stabilization of the mRNA (protecting it from degradation), and facilitating the process of translation by association with translation-initiation factors in the cytoplasm.

The total nucleic acids are extracted from a culture of yeast cells are mixed with beads to which the polynucleotide 5’-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3’ has been covalently attached. When you analyze the cellular nucleic acids that are stuck to the beads, what would you find? (2 points)

I would find only mRNAs because these are RNAs containing long stretches of A nucleotides as part of their 3’ “poly-A tails.”
You had a scare last weekend. Before heading to your cabin in the woods so you could get a lot of Cell Biology studying done over the Presidents’ Day weekend, you printed out a whole set of DNA sequences that encode for the same protein in different species. You took the thick file of sequences with you over the long weekend. When you looked at the printout, you discovered that, much to your chagrin, you forgot to indicate where in the gene these sequences are from!

Knowing that the sequences in the figure contain an exon/intron boundary, you may be able to save your weekend! All you need to do is determine where the boundary is, and which side of the boundary is intronic and which is exonic. In desperation, you consider the problem from an evolutionary perspective. Because exons are ultimately used to encode a protein whereas introns are not, and because you know that there is more selective pressure to conserve similarity between species, thank goodness you were able to figure this out! Identify the exon/intron boundary, and label each side of the boundary line as either intronic or exonic. Finally, what ribonuclear particle will recognize and bind to this border? (3 points)

Human: UAAAGCUGCACCUGACAACGUGCUCAAUCGAUCAGAUCGGA
Mouse: UAAAGCUCCAACCACAAGGUGCGAGGGCTTGTTCAGUGGCGGAU
Cow: UAAACCACCACCAACAGBUUGAAACUACCAGGAUCCUUAAC
Fish: UAAACCACCACCAACAGGUUUGACUAGCGUAUAAGACUGG
Bird: UUAACCACCCACCAAGGUAAAGUCCAGUAAACUGGACGU
Serpent: UUAACCACCCACCAACAGGUUCUAAGUCCAGUACUGGACU

This side is exonic   This side is intronic.

This is able to be determined because the sequences to the left of the line are more similar to one another than the sequences to the right of the line—they are more similar because there is more evolutionary pressure to keep the “coding” regions of DNA the same whereas there is less pressure to keep intronic sequences similar. As long as the border sequences and the ‘branch point A’ sequences are maintained, the resultant protein product will be maintained.

Bacterial genes are arranged much closer together on prokaryotic chromosomes when compared to genes on human chromosomes. Provide one advantage and one disadvantage for the extra “space” that is contained within human chromosomes. (2 points)

An advantage of having extra space is that there is more opportunity to have regulatory sequences that can be used to more finely control gene expression. Another would be that mutations may potentially be less deleterious because they are more likely to occur in ‘non-important’ regions. An obvious disadvantage is that having more DNA requires more time and energy to copy (so division must be slower) and it is also more cumbersome and unwieldy to store.

Mutation of a Shine-Dalgarno sequence would: (1 point)

a) Prevent translation of a single mRNA
b) Interrupt translation elongation
c) Interfere with assembly of the 80S ribosome
d) Result in translation of a truncated protein
e) Negate the binding of translation initiation factors.
Which amino acid would you expect a tRNA with the anticodon 5’-CUU-3’ to carry? Refer to the genetic code table attached at the end of this exam. (1 point)
Codon = AAG; Lysine

A tRNA for the amino acid lysine is mutated such that the sequence of the anticodon is 5’-UAU-3’ instead of 5’UUU-3’. Use the genetic code to determine which of the following aberrations in protein synthesis might this tRNA mutation might cause. (1 point)
a) Read through of stop codons.
b) Substitution of lysine for isoleucine.
c) Substitution of lysine for tyrosine.
d) Substitution of lysine for internal methionine.
e) Substitution of lysine for amino-terminal methionine.

Assume that ‘your favorite protein’ (YFP) contains a Valine amino acid at position 124. After treating cells that carry a gene for YFP with a mutagen, you isolate two mutant forms of YFP, one with an Ala and the other with a Met at site 124. After treating each of these two mutant cells with the mutagen a second time, you isolate yet another version of the protein that now has a Thr at the site of the original Val. Assuming that all mutations caused by the mutagen involve single nucleotide changes, deduce the codons that are used for Val, Ala, Met and Thr at position. Use the figure below if you think it might help. After the first mutagenic treatment, are the resultant changes at position 124 likely to have an effect on the protein’s shape? Why or why not? After the second mutagenic treatment, are the resultant conversions from Ala to Thr and from Met to Thr likely to have an effect on the protein’s shape? Why or why not? (8 points)

Codon for Val: GUG
Codon for Ala: GCG
Codon for Met: AUG
Codon for Thr: ACG (C converted from U in the Met codon and A converted from the G in the Ala codon.

After the first mutagenic treatment, a Valine is converted to either an Alanine or a Methionine. All three amino acids are hydrophobic so it’s possible that neither mutation will have an effect on the shape of a protein (since all would likely be shielded inside of the protein after it’s folded). Of course, because all three of these amino acids do have different shapes, and because Met’s side chain contains a sulfur group, it is possible that even though they have the same chemical character, the protein’s shape would be altered. The second mutation, on the other hand, is much more likely to result in a shape change because both Alanine and Methionine are hydrophobic, and Threonine is hydrophilic/polar. Because the Thr amino acid would want to be on the outside of a folded protein, the protein would change is usual shape to accommodate it.
Refer to the diagram to answer the following questions about prokaryotic translation. Assume that the mRNA sequence shown is at the extreme 5’ end of the transcript. (6 points)

1) A tRNA carrying which amino acid would be located in the E site?
   None—assuming that this is the extreme 5’ end of an mRNA, the start codon is “downstream of the E site. The first site to have a tRNA bound is the P.

2) Which amino acid would be carried by the tRNA binding to the P site?
   Because this is prokaryotic translation, the first amino acid (the one bound to the tRNA in the P site) would be a formylated methionine (f-Met).

3) What is the sequence of the anticodon on the tRNA that would be bound in the A site?
   5’-GGG-3’

4) What is responsible for catalyzing the reaction that links the amino acids carried by tRNAs in the P and A sites?
   The “enzyme” that catalyzes formation of the peptide bond that links two amino acids is the large (23S) rRNA that is part of the large ribosomal subunit.

5) From what is the small ribosomal subunit constructed?
   Like the large subunit, the small ribosomal subunit is constructed of rRNA and proteins—however, the small subunit only has one rRNA (16S) and many proteins.

6) What is the size (in Svedberg units) of the small ribosomal subunit?
   30S
Imagine that you have created a fusion between the *trp* operon that encodes the enzymes for tryptophan biosynthesis and the *lac* operon that encodes the enzymes necessary for lactose metabolism (see the figure below). Under which of the following conditions will β-galactosidase (the *Z* gene product) be expressed by bacteria that carry the fused operons? (2 points)

![Figure depicting the fusion of *trp* and *lac* operons](image)

- a) Only when lactose and glucose are absent.
- b) Only when lactose and glucose are present.
- c) Only when lactose is absent and glucose is present.
- d) Only when lactose is present and glucose is absent.
- e) Only when tryptophan is absent.
- f) Only when tryptophan is present.

Upon infection of a bacterium, lambda (λ) bacteriophage continues to multiply by inducing transcription of ‘multiplication’ genes but represses genes that result in bacterial lysis (known as lytic genes). It does this using the transcriptional repressor shown in the figure below. The repressor binds to DNA as a dimer as indicated in the figure. Each molecule of the repressor consists of an N-terminal DNA binding domain and a C-terminal dimerization domain. Upon some sort of stimulus (such as irradiation with UV light), the lytic genes begin being expressed. Transcription of these genes is initiated by cleavage (cutting) the λ repressor at a site between the DNA-binding domain and the dimerization domain. In the absence of bound repressor, RNA polymerase can bind to lytic genes and transcribe them. Given that the concentration of DNA binding domains is unchanged by cleavage of the repressor, why do you suppose that its cleavage results in its removal from the DNA? (2 points)

![Figure showing the cleavage of the repressor](image)

When the two repressor proteins bind to make a dimer, the amino-terminal ends are in the right conformation to bind DNA. Stress-induced cleavage of the repressor proteins results in loss of the “molecular binding” domain (the dimerization domain); this causes a protein shape change (proteolytic cleavage as a means of changing protein activity) such that the DNA binding domain has decreased affinity for DNA and therefore cannot bind to the operator to repress transcription of lytic genes.
In a eukaryotic organism with 8 different transcription factors, how many different cell types are theoretically possible? (1 point)
Cells have the option of having or not having (2 choices) each of the 8 transcription factors. Thus, the answer is $2^8$, which equals 256 possible cell types (theoretically).

Translational regulation of the eukaryotic mRNA for ferritin is controlled by the interaction between a stem-loop structure in the mRNA termed the ‘iron-response element’ (IRE), and an iron-response protein (IRP) that binds to it. When the IRE is bound by IRP, translation is inhibited. The location of the IRE in the mRNA is critical for its function. To work properly, it must be positioned near the 5’ end of the mRNA. If it is moved more than 60 nucleotides downstream of the cap structure, its ability to regulate translation is dramatically reduced. Why do you suppose that there is such a critical position dependence for regulation of translation by IRE and IRP? (2 points)
This scenario is very similar to an example of how translation is regulated in prokaryotic cells that was provided in class. Basically, the IRE that is part of the mRNA forms a stem-loop structure to which a protein, IRP, binds to inhibit translation. It is most likely that this inhibition occurs in the area where translation is initiated because once translation starts, a ribosome can basically barrel its way through any 3D structure that the RNA might have. This is supported by the scenario; if the stem-loop is moved “downstream”, translation can occur—it starts and is able to continue. Thus, the most likely explanation for this is that the stem-loop either involves the start codon or is close enough to it such that the small ribosomal subunit can’t “see” it and therefore translation initiation is inhibited.

Describe how translation of mRNAs can be regulated by the RISC complex. (4 points)
miRNAs are synthesized in the nucleus as small hairpin RNAs. The ends of the miRNA hairpin are cut off by dicer to generate a small double-stranded RNA that is bound by the RISC complex. RISC hangs onto only one of the two strands. RISC, along with its single-stranded RNA, look for mRNAs in the cytoplasm that contain sequences complementary to the single-stranded RNA it’s holding. The complementary sequences are often found in the 3’ UTRs of mRNAs because a single mRNA is able to inhibit translation of multiple gene products. Once the RISC complex/ssRNA binds to a complementary mRNA, the complementary mRNA is degraded by nuclease enzymes that are part of RISC. Degradation of the mRNAs therefore effectively inhibits translation to decrease the amount of protein that is made.

Which of the following treatments might cause this protein to unfold? (1 point)
a) Heat.
b) Extremes of pH.
c) High salt concentrations.
d) Reducing agents.
e) All of the above.
f) None of the above.
Which of the eight amino acids listed below would be expected to be on the outside of a globular extracellular protein? (3 points)

1) Lysine
2) Alanine
3) Valine
4) Proline
5) Threonine
6) Tryptophan
7) Aspartic Acid
8) Phenylalanine

The three highlighted amino acids are all hydrophilic/polar so would be more likely exposed to the polar aqueous environment than non-polar/hydrophobic amino acids that would want to be “hidden” inside a globular protein.