Name:

1 point

Chem 465
Biochemistry II Test 3

Multiple choice (4 points apiece):

1. A certain bacterial mRNA is known to represent only one gene and to contain about 800 nucleotides. If you assume that the average amino acid residue contributes 110 to the peptide molecular weight, the largest polypeptide that this mRNA could code for would have a molecular weight of about:

A) 800.
B) 5,000.
C) 30,000.
D) 80,000.
E) An upper limit cannot be determined from the data given.

2. Which one of the following statements about ribosomes is true?

A) The large subunit contains rRNA molecules, the small subunit does not.
B) The RNA in ribosomes plays a structural, not catalytic, role.
C) There are about 25 of them in an E. coli cell.
D) There are two major subunits, each with multiple proteins.
E) They are relatively small, with molecular weights less than 10,000.

3. In the "activation" of an amino acid for protein synthesis:

A) leucine can be attached to tRNAPhe, by the aminoacyl-tRNA synthetase specific for leucine.
B) methionine is first formylated, then attached to a specific tRNA.
C) the amino acid is attached to the 5' end of the tRNA through a phosphodiester bond.
D) there is at least one specific activating enzyme and one specific tRNA for each amino acid.
E) two separate enzymes are required, one to form the aminoacyl adenylate, the other to attach the amino acid to the tRNA.

4. Which one of the following antibiotics does not function by interfering with the translational process?

A) Chloramphenicol
B) Cycloheximide
C) Penicillin
D) Puromycin
E) Streptomycin

5. "Housekeeping genes" in bacteria are commonly expressed constitutively, but not all of these genes are expressed at the same level (the same number of molecules per cell). The primary mechanism responsible for variations in the level of constitutive enzymes from different genes is that:

A) all constitutive enzymes are synthesized at the same rate, but are not degraded equally.
B) their promoters have different affinities for RNA polymerase holoenzyme.
C) some constitutively expressed genes are more inducible than others.
D) some constitutively expressed genes are more repressible than others.
E) the same number of mRNA copies are made from each gene, but are translated at different rates.

Short answer questions (5 points each) You may skip ONE

7. You have isolated a fragment of DNA that totally encodes at least two proteins, 120 and 80 amino acids long. The DNA fragment is 400 base pairs long. (A) Why might you consider this unusual? (B) You sequence th two proteins and find no homology. Propose a model to account for these findings

Two protein @ 120 and 80 AA (total of 200 AA) should require a minimum of 600 base pairs to encode, thus coding on a shorter piece of DNA is unusual. Since 400 base pairs codes for about 130 amino acids, the first possibility is that the sequence codes for one protein that is clipped down to make the second protein. However, since there is no homology between these two proteins that cannot be what is happening. The only possibility that remains is that there is some frame shift in how the mRNA is interpreted to you get two entirely different proteins from two different reading frames.

8. The addition of an amino acid to its correct t-RNA is said to involve the 'second genetic code' What is meant by this?

The first genetic code was find which nucleotide triplets coded for which amino acids. The 'second' genetic code is uncovering what are the structural clues on the tRNA that an amino acid synthetase uses to 'decide' if it has the correct t-RNA to attach a specific amino acid to.

9. How does a bacterial ribosome distinguish between an initiation AUG and an AUG for a methione in the middle of a protein, and does it make a difference?

A region at the end of the 16S rRNA of a prokariotic ribosome binds to the Shine and Delgano sequence of the mRNA. This sequence is just upstream of the initiation AUG and is used to first bind the $tRNA^{F\text{-}Met}$, before the ribosome is completely assembled. Once the ribosome is assembled and making a protein all other AUG sequences bind $tRNA^{Met}$. Ths matters because the formyl group on the amino terminus of the methionine prevents it from being incorporated into any internal position in a protein.

10.What is the difference between a promoter, an activator, and a repressor

A promoter is a sequence that all operons share that is used to bind an RNA polymerase to start making mRNA near that sequence.

An activator is an additional protein that usually binds to DNA near the promoter site and helps to increase the affinity of the RNA polymerase for the DNA and helps to increase the expression of the gene.

A repressor is protein that binds also bind to the DNA near the promoter site, but in this case the bnding of the protein blocks the binding of the RNA polymerase in soem way and decreases the expression of the gene.

11. Define polycistronic mRNA, an operon and a regulon.

Polycistronic is when a single mRNA codes for two or more proteins.

An operon term that describes the gene or polycistronic set of genes, and all promoters, repressors or activators for those genes.

A regulon as a set of several different genes at different locations in a chromosome that are all controlled by the same set of promoters or repressors.

Longer questions (12.5 points each) - You make skip ONE

12. Define the following

A. Shine-Dalgarno sequence A sequence on a prokariotic mRNA just a few bases before the initiation AUG that is used to align the mRNA and bind $tRNA^{Fmet}$ in the initiation of protein synthesis in prokariots.

B. SRP Signal Recognition Particle. A protein that recognizes a signal peptide as it emerges from a ribosome. It binds to the ribosome, and shuts down protein synthesis. The SRP arrested ribosome is then recognized by a receptor on the surface of the ER. Once the arrested ribosome is bound to the receptor on the ER, the SRP is released, and protein synthesis continues, but the protein is pushed into the interior of the ER.

C. NLS Nuclear localization sequence A sequence of 4-8 residues that includes several consecutive Lys and Arg amino acids in the interior of a protein that is the signal that this protein should be transported into the nucleus of the cell

D. Ubiquitin a highly conserved peptide of 76 residues that is attached to lysines of proteins that are to be degraded by the 26S proteosome.

E. Specificity factor or σ factor. A protein that is part of the prokariotic RNA polyerase that targets the polymerase to bind to specific promtors.

F. CRP cAMP receptor protein or CAP catabolite gene activator protein. A protein that binds to several operons in the presence of cAMP to enhance the binding of RNA polymerase to these operons to increase expression of the operons when an e coli has low levels of glucose (that stimulates the production of cAMP)

13. How is the charging of an $tRNA^{fMet}$ the same and different than the charging of a $tRNA^{Met}$

They are the same in that the same tRNA synthetase charges both $tRNA^{FMet}$ and $rRNA^{Met}$. The difference lies in the next step where transformylase specifically recognizes the $tRNA^{FMet}$ and only formylates the Met on that tRNA.

14. Compare and contrast to process of translation of mRNA into a protein on a ribosome between prokariots and eukariots. Focus on the phases of initiation, elongation, and termination, and the different factors that were involved in each of these steps.

Initiation

Prokariots

30 S ribosome binds IF1 & IF3
mRNA uses Shine and Delgarno sequence get initiation AUG lined up with P site
GTP bound IF-2 and $tRNA^{Fmet}$ bind
50S part of ribosome now binds, GTP is hydrolyzed to GDP and all initiation factors are released

Eukariots

12 initiation factors instead of 3
eIF-1A and eIF-3 function in a manner similar to IF-1 and IF-3
eIF-2 binds to GTP and $tRNA^{Met}$
These plus other factors join to make the 43S preinitiation complex
No Shine and Delgarno sequence is used to align the mRNA, instead a complex of factors bind to the mRNA, and to the 43S preinitiation complex and work down from the 5' end of the mRNA until they find the first AUG in the message.
At this poin the 60S ribosomal subunit unit bind and the initiation factors are released.

Elongation

Prokariots

The appropriate charged tRNA is bound to EF-Tu and this complex binds to the A site of the ribosome
GTP is hydrolyzed and EF-Tu with bound GDP is released
(EF-Tu re recharged using EF-Ts)
A peptide bond in now formed using a peptidyl transferse activity in the 23S rRNA
EF-G GTP now binds with the tRNA bound peptide at the A site
the GTP is hydrolyzed to GDP and the t-RNA-peptide is moved from te A site to the P site

Eukariots
Very similar
eEF1α instead of EF-Tu
eEF1βγ instead of EF-Ts
eF2 instead of EF-G

Termination
Prokariots
RF-1 recognizes UAG and UAA
RF-2 recognizes UGA and UAA
Bind at termination codon and hydrolyzes peptide from tRNA
EF-G ·GTP, IF3, RRF bind
GTP hydrolyzed
all components are released

Eukariots
eRF instead of RF-1, RF-2 the rest of the steps should be similar
eF2·GTP instead of EF-G

15. Describe the process by which proteins are deliberately destroyed by the eukariotic cells

Ubiquitin activating enzyme + ATP attaches ubiquitin to an S of E1
E2 conjugating enzyme then takes the ubiquitin from E1 and places it on an S of E2
E3 ligases then attach the ubiquitin to a lysine of the target protein.

This can be repeated multiple times until the protein has many ubiquitins attached to it.

The ubiquinated protein is then placed in a 26S proteosome where it is degraded.

16. Create your own operon. There are at least four genes in this operon that are involved in the conversion of zephulose to glucose-6-phosphate. This operon is normally turned off in the presence of glucose, but is slightly turned on in the absence of glucose and zephulose, and completely turned on the absence of glucose and the presence of zephulose. In this operon make sure you include the promoter and the sequence for the four enzyme genes, but also the promoters and structural genes for any regulating proteins. Once you have diagramed your complete operon, please explain how it works.

What I expected to see was an operon much like the lac operon with diagrams like figure 28-17 to explain how it works.