

1. Explain in as much detail as possible how the translation of *E. coli* RF-2 is regulated. A one word answer will not be sufficient for full credit. **(3 points)**

2. Detail the events associated with prokaryotic translation termination and depict the mechanism involved in polypeptide release. **(3 points)**

3. Recently, you purified a human protein and sent it to the Molecular Genetics Instrumentation Facility (MGIF) for N-terminal sequencing. There were no problems with the purification and you were careful to keep the sample from undergoing non-specific degradation or denaturation. The results of the N-terminal sequencing indicate that the protein does NOT have an N-terminal methionine. This question has two parts. A correct answer for Part A is not necessarily required to answer Part B.

Part A. Using information from your course readings or lecture, explain two possible reasons for the results described above. There are more than two answers (**2 points**)

Part B. Last week you isolated the gene for your protein. Upon sequencing of the gene, you find that a mutation exists at the second codon of the open reading frame. You have recently evaluated the mutant protein and found that the protein is cytosolic (like the normal protein), does not exhibit aberrant mobility by SDS-PAGE, and appears to have normal activity. However, you have determined that the cellular levels of the protein are significantly reduced. Please explain a possible reason for this observation given the topics discussed in class (a correct answer for Part A is not necessarily required to answer this question). (**3 points**)

4. Describe the process by which an improperly folded cytosolic protein is degraded to amino acids in eukaryotic systems. Begin your description at the time of polypeptide emergence from the ribosome. Assume that the cytosolic protein normally undergoes assisted protein folding, but is not dependent on chaperonins (Hsp60/GroEL-like chaperones). Identify and describe the roles of all relevant enzymatic and protein components/complexes related to the process. (**6 points**; if necessary use the back of the page for additional answer space)

5. Describe the events associated with the first 3 stages of prokaryotic translation (as defined in lecture) AND detail how nucleotide triphosphate (NTP) hydrolysis is utilized in these stages (*e.g.*, the effects mediated by hydrolysis; the enzymes utilizing the energy, *etc.*). (**8 points**; if necessary use the back of the page for additional answer space).

6. You are studying the process of translation scanning. You and a labmate are independently following a protocol to make ribosomal preparations that should yield a purified 48S preinitiation complex. Your classmate mistakenly added too much salt (~0.5 M final) to a wash buffer required in one of the final stages of the ribosome isolation procedure. Your preparation was made according to protocol. The two ribosome preparations are independently used in separate toe-print assays similar to that described in class and in Pestova *et al.* (this was required reading), otherwise the assay components are identical. This question has two parts. A correct answer for Part A is not required to answer Part B. (5 points total)

Part A. Other than the 48S preparations, what are the key components typically added to the assay AND why are they needed? (2 points)

Part B. The results of the assays using your and your labmate's ribosome preps are disturbing in that the results are not identical. Diagram the results that you believe might have been observed and explain your reasoning. Make sure to include the expected results for a control and indicate the orientation of the gel (top, bottom, lane assignments, *etc.*). (3 points)

Bonus Question for Extra Points: There was a discrepancy between the lecture notes on the stages of translation and your assigned textbook readings. What specifically was this discrepancy? (2 points)