

"Extra Credit" Homework Key

1. Why is it beneficial for the 3' end of mRNAs to be protected from degradation by the hairpin formed by the factor-independent terminator? Another way of thinking about it – why is it better to degrade mRNA from the 5' end?

If degradation proceeded from the 3' end, with translation starting at the 5' end, proteins would be made but not completed (the stop codon would be deleted causing release problems (see below)) or truncated proteins would be made. With polycistronic messages, the 3' genes might not even be there, and incomplete pathways would be expressed.

2. A. What is the "logic"/purpose behind Rho-dependent termination? Why might rRNAs be susceptible to Rho-dependent termination?

If a complex pathway is encoded on a polycistronic message and the 5' genes are not being translated because of a mutation, there is no point in wasting energy making the terminal proteins. Rho is an example of translation affecting transcription. Note that there is also translational coupling where translation of a 5' gene affects translation of the next downstream gene. The latter has nothing to do with Rho. rRNAs might be susceptible to Rho because they are never translated – by design.

3. What is tmRNA? What is its function? What does Clp have to do with it?

tmRNA is a combination of tRNA and mRNA that enables proteins to be released from ribosomes if the stop codon is missing (see question 1). If it weren't for tmRNA, ribosomes would be stuck on defective messages, and this would adversely affect overall translation and health of the cell. Clp protease recognizes tmRNA-labeled proteins for degradation since they obviously had problems with being fully translated.

4. How does the GroEL chaperonin function?

It forms a two part barrel. When a misfolded protein (hydrophobic on the outside instead of inside) enters the barrel, the interior of the barrel is hydrophobic. The conformation of the barrel reverses so that hydrophilic residues line the inside, forcing the protein to refold and put the hydrophobic residues on the inside of the protein, where they belong.

5. What is a nonsense mutation? What is a suppressor mutation? How does a suppressor mutation for a nonsense mutation work? What would the consequences be if suppressor mutations were 100% effective?

A mutation that generates a stop codon. A suppressor mutation, in general, is a mutation that reverses the phenotype of another mutation without changing the DNA sequence of the initial mutation. There are intragenic and extragenic suppressors. Intragenic mutations of missense mutations (change in amino acid sequence) usually work by changing the conformation of a protein so that a functional conformation is restored. An intragenic suppressor of a frameshift mutation would create an additional frameshift change that restores the overall reading frame (note that there would still be a segment of the protein that has an altered amino acid sequence). An extragenic suppressor of a nonsense mutation is a mutation in the tRNA that normally adds an amino acid in translation. However, the anti-codon gets mutated so that with some frequency (about 10%), it will recognize the stop codon and put in the amino acid. If the suppression were 100%, all of the relevant stop codons would fail to function, and proteins would run on until one of the other two stop codons occurred. This would be very detrimental to the cell.

6. What is the difference between transcriptional and translational fusions? What are alkaline phosphatase fusions most widely used for. Would they be transcriptional or translational fusions? Why?

Make sure that you read this in the book and understand it. A transcriptional fusion (also called an operon fusion) places a gene (the reporter gene) under the control of another gene's promoter. The reporter gene must provide its own ribosome binding site and start codon. The report gene is made as an independent protein. With a translational fusion (also called a gene fusion) the reading frame of the reporter gene is fused in frame with the other gene's reading frame. A hybrid/fusion protein is made. The reporter gene then relies on the transcriptional and translational regulation of the other gene. There are instances of post-transcriptional regulation in bacteria, so gene fusions have their use. Plus, if you want to use PhoA alkaline phosphatase fusions to examine export and membrane topology, you must use translational fusions since you have to fuse the PhoA segment to the secretion signals of the other protein. Note that eukaryotic fusions are almost always gene fusions since eukaryotes do not handle polycistronic messages very well. There are examples of transcriptional fusions in eukaryotic genetics. These usually use translation initiation regions (TIRs) from viruses to accomplish this.