Bi 1 Problem Set Answer Key

Refer to the Meselson and Stahl paper to complete this set.

Part A

Replication of DNA has been a topic of debate when it became clear that DNA molecules contain genes. There have been numerous hypotheses for the mechanism of DNA replication. In the paper, *The Replication of DNA in Escherichia Coli*, we learn that most of these hypotheses differ in their predictions concerning the distribution among progeny molecules of atoms derived from parental molecules.

1. i. In no more than 3 sentences and using your own words: What were the key observations in Meselson & Stahl's paper? What's the broader perspective for this result? (3 points)

   They should mention these 3 points:

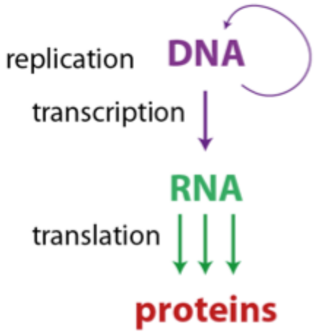
   1. The nitrogen of a DNA molecule is spilt up equally between two subunits/strands which remain intact through many generations.
   2. After replication, each daughter molecule has received one parental strand.
   3. The replicative act results in a molecular doubling.

   ii. One alternative hypothesis for DNA replication has been that the parental double helix remains associated after cell division. What distribution of DNA molecules would be expected in the centrifugation experiments after the first and second generations if this 'conservative' mechanism were indeed the case? (3 points)

   During conservative replication, after the first generation, half of the molecules would be 15N-15N, and the other have would be 14N-14N. After the second generation, one-quarter of the molecules will be 15N-15N, the other three-quarters will be 14N-14N. Note: There would be no 14N-15N molecules observed in conservative replication.

2. i. Draw a diagram of the Central dogma of molecular biology, and indicate the key enzymes and the names of processes involved.

   The student should draw a diagram of the central dogma like so:



DNA polymerase

RNA polymerase

Ribosome, mRNA, tRNA

Note: When grading, the students should get full credit even if they leave out mRNA and tRNA because they are not enzymes.

3. Which technique did Meselson & Stahl use to separate isotope labeled DNA? Which forces and properties determine the location of a compound in this experiment?

   Density-gradient centrifugation. Heavier molecules end up on the bottom whereas lighter one end up on top. Centrifugal force, density (of solution gradient and compound), diffusion.

5. The chemical structure of proteins. The ribosome translated the genetic code into a chain of amino acids that are connected by peptide bonds. (10 points)

a) Draw a peptide bond and indicate bonds that are free to rotate.

b) The secondary structure of a protein mainly contains the elements alpha helix, parallel and anti-parallel beta sheet, and loop regions. Illustrate the critical role of hydrogen bonds for the formation of secondary structure by a schematic drawing of a anti-parallel beta sheet.

c) In the cell almost all proteins fold into a specific three dimensional conformation (tertiary structure). Three intramolecular "forces", namely hydrogen bonds, ionic bonds and the hydrophobic effect, lead to the minimisation of the free energy of a polypeptide chain during protein folding.

i. Explain in one or two sentences what is meant by hydrophobic effect?

The tendency of nonpolar molecules to accumulate with each other in an aqueous solution.

ii. Where do you expect most hydrophobic amino acids to be located in a protein?

Side-chain.

iii. Which of the three forces is the strongest?

Ionic bonds.

iv. Which force do you think makes overall the biggest contribution to the stability of proteins? Why?

The biggest contribution is via H bonds, especially because there are so many of them. If someone puts Hydrophobic effect as the answer, then they should explain the effect and why it is better than the other forces.

d) Levinthal's paradox states that a polypeptide chain with a length of 150 amino acid size should need ~10E24 years to adopt its correct three-dimensional structure, yet in the cell this happens usually on the order of seconds. Name two mechanisms that ensure proteins are folding rapidly and correctly in the cell.

- sequential folding of domain
- molecular chaperons
- selective slow translation speed
- hydrophobic effect

(- protein degradation)

e) Find out which of the following diseases can be directly linked to misfolded protein:

- Alzheimer's disease X
- Parkinson X
- Huntington's disease X
- Sickle cell anemia
- Prion caused diseases X
- Malaria
- Tuberculosis
- Cystic fibrosis X

6. What was one problem or fault you found in Meselson & Stahl's paper, either in their experimental technique or how they ended up with their conclusion?

Anything reasonable should be accepted.

Part B

1. The ribosome translates the genetic information encoded in mRNA into protein. Can your first name be spelt using the single letter abbreviation for the 20 standard amino acids? Write a DNA sequence that encodes your name as a peptide. (**If no**, then use the peptide sequence: CALTECH). Please also label the amino acid corresponding to each part of your sequence.

   For Caltech, this would be:

   C = UGU, UGC

   A = GCU, GCC, GCA, GCG

   L = UUA, UUG, CUU, CUC, CUA, CUG

   T = ACU, ACC, ACA, ACG

   E = GAA, GAG

   C = UGU, UGC

   H = CAU, CAC

   Their answer should be in the form of a DNA sequence with each amino acid labeled under the sequence!

2. Is the sequence CALTECHBIONE part of a known protein? (Hint: Google "protein blast" and submit the online form to find out. Make sure the coverage is 100%, and that the protein is known). http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome.

3. One constraint for protein size is the error rate of transcription and translation. Given that, in bacteria, transcription and translation have an error rate of approximately 10^{-4} each, what is the length of amino acid when 50% of the protein would still be made without mistake? How does this

compare to the largest proteins in E. coli (~2,300 aa) or humans (~34,000 aa).

[(1-10-4)^4]^x=0.5; x=~1700; is about the size of the biggest E.coli protein. Eukaryotes should have other mechanisms to decrease the fidelity of gene expression (transcription and translation are 'decoupled').

4. In recent decades, technological advancements have allowed the development of efficient experimental techniques to replicate a piece of DNA. Please describe one of the modern techniques used to replicate DNA in 5 sentences or less.

   PCR, etc. A description of the procedure should also be included!