Solutions to 7.014 Problem Set 5

Question 1

a) Which of the following molecules functions directly to transfer information from the nucleus to the cytoplasm? Circle all that apply.

DNA   mRNA   tRNA:   transporter vesicles   proteins

b) *Translation* is the synthesis of proteins on ribosomes, where the information for an amino acid sequence is encoded in *the mRNA*. This information is originally encoded by *DNA*, transcribed by a protein complex called *RNA polymerase* that binds to the *promoter* region of the gene.

c) Why are retroviruses considered unusual with respect to the central dogma?
*In general, the idea of the central dogma is that information flow in the cell is DNA → RNA → protein. Retroviruses carry their genetic material as RNA that when inserted into the cell is copied into DNA. In effect, in retroviruses, RNA → DNA*

d) From first principles, why do you need at least three RNA bases (a codon) to code for 1 amino acid?
*DNA is composed of 4 different nucleotides but the proteins encoded by DNA are composed of 20 different amino acids. $4^2 = 16$, not sufficient to give 20 different amino acids, whereas $4^3 = 64$.*
Question 2

After the double stranded model for DNA structure was proposed, two possible mechanisms for DNA replication were envisioned:

Semi-conservative

![Diagram of semi-conservative replication]

Conservative

![Diagram of conservative replication]

Each of these possibilities has distinct predictions with respect to the daughter molecules. Notice that in the semi-conservative case, each daughter duplex in the first generation will have one strand from the original and one new strand. In the conservative case, each daughter duplex will only have new strands. To distinguish between the two possibilities, the following experiment was carried out. Cells were grown in a medium containing “heavy” nitrogen (\(^{15}\text{N}\)) [REMEMBER: nucleic acids contain nitrogen in their rings!]. When all the DNA in the cells contained the “heavy” nitrogen, they were transferred to a medium containing the normal, “light” nitrogen (\(^{14}\text{N}\)) isotope. Samples of cells were removed at different times after addition of “light” nitrogen. The DNA was then analyzed by density-gradient centrifugation, which can separate heavy-heavy, heavy-light, and light-light chains of DNA.

This is what you would observe if you grow cells in either “heavy” or “light” nitrogen:

![Diagram of centrifugation bands]

a) Given what you know about DNA replication, where and how many bands would you expect to find in your tube after the cells had undergone exactly one round of DNA replication growing in “light” nitrogen? Draw the DNA bands in the empty tube taking into account the position for the “heavy” and “light” bands.

![Diagram of one round of replication bands]
Question 2, continued

b) If you let the cells undergo exactly two rounds of DNA replication, how many bands would you find? Where would you find them with respect to the “light” or heavy” bands? Draw your prediction in the empty tube.

Frustrated with your career as a biologist, you become an astronaut and travel to space. On a distant planet you are surprised to find life, in the form of chewing-gum like creatures. Curious to see if this form of life replicates DNA the same way as life on Earth does, you send a sample to your 7.014 TA’s. After some time, they send you an email saying that their experiments suggest that the new life form uses “random dispersive” replication. In random dispersive replication the DNA is copied so that each daughter duplex will end up with roughly 50% of the original DNA and 50% new DNA (See below). They do not tell you what experiments they did to come up with this model.

Random dispersive

---

c) If your TA’s did the “light”, “heavy” nitrogen experiment, what would they have observed? Draw your predictions in the empty tubes label below.

---

d) Why would your TA’s need to examine the cells after two rounds of replication to propose a model like this?

*After one round of DNA replication, the results for semi-conservative and random dispersive replication are the same. The difference only appears after two rounds.*
Question 3

_E.coli_ DNA polymerase I is an enzyme that has three activities:

1. 5’-to-3’ DNA Polymerase activity
2. 3’-to-5’ exonuclease activity
3. 5’-to-3’ exonuclease activity

a) What would be the effect of removing the 3’ to 5’ exonuclease activity on DNA replication? Removing this activity would affect the proofreading and fidelity of DNA replication.

b) In 1969, John Cairns isolated a viable _E. coli_ mutant that lacked DNA polymerase I (Pol I) activity.

i) Do you think that an organism that cannot replicate its DNA would be viable? No, any organism that cannot replicate DNA would die.

ii) What was the conclusion from Cairns’ experiment? From the viable _E. coli_ mutant, one must conclude that DNA polymerase I is not essential for DNA replication. There must be another DNA polymerase in _E. Coli_ cells.

c) You find an _E. coli_ temperature-sensitive (conditional lethal) mutant lacking only the 5’-to-3’ exonuclease activity of DNA polymerase I. This mutant is not viable at non-permissive temperatures. This is interesting because you know from part b above that DNA polymerase I activity is dispensable.

i) What can you say about the 5’ to 3’ exonuclease activity of Pol I? You can conclude that, if Pol I is present in the cells, then the 5’ to 3’ exonuclease activity is essential. If Pol I is completely absent, some other DNA polymerase can take over the job of removing and replacing the RNA primers. If a defective Pol I is present, it likely prevents the other DNA polymerase from taking over the job.

ii) Given what you know about the steps involved in DNA replication, what do you think the function of the 5’ to 3’ exonuclease activity is during this process? _E.coli_ DNA polymerase I is primarily responsible for removing the RNA primer of the newly made Okazaki fragments, and replacing it with DNA. Without this activity, the completion of the lagging strand would be inhibited.
Question 4

Shown here is the double-stranded DNA sequence coding for human hemoglobin. Below the 2 strands is the one letter abbreviation for the amino acids of the hemoglobin protein.

20                  40                  60
5’ ACACTCGCTCTTGGGAACGCTGAGATTATCTAAATAGCTCTTAGCCAGACGCCATGGGTCT
3’ TGTGAGCGAAGACCTTGGAGACCTCTAAATAGCTCTTAGCCAGACGCCATGGGTCT
M G

80                  100                 120
ATTTCAACAGAGGAGGACAAAGCTACTATCAACAAAGCTGGGGAAGGTGAATGTGGGAG
TAAATGCTGATCGCTCTCCGATGATAGTGTGTGGAGACCCCTTCCTTACCTAGGAG
H F T E D K A T I T S L W G K V N E

140                 160                 180
ATTTCACAGAGGAGGACAAAGCTACTATCAACAAAGCTGGGGAAGGTGAATGTGGGAG
TAAATGCTGATCGCTCTCCGATGATAGTGTGTGGAGACCCCTTCCTTACCTAGGAG
D A G G E T L G R L L V V Y P W T Q R F

200                 220                 240
TTGACAGCTTTTGGGACACTGCTTGCTGCTGCTCTCCGATGATAGTGTGTGGAGACCCCTTCCTTACCTAGGAG
F D S F G N L S S A S A I M G N P K V K

260                 280                 300
CACATGGCAAGAGGGTGTCAGCTCTCTCGGAGATGCTCTGCTCAAGCTGAG
H G K K V L T S L G D A I K H L D D L

320                 340                 360
AGGGCACTTTGCGACAGCTGCTGCTGCTGCTCTCCGATGATAGTGTGTGGAGACCCCTTCCTTACCTAGGAG
K G T F A Q L S E L H C D K L H V D P E

380                 400                 420
ACTTCAAGCTCTCTGGGAAATGTGCTGTGATCCGTTTGGGATTCATCATGGAC
A H G K K V L T S L G D A I K H L D D L

440                 460                 480
TCACCCCTGAGGTGACAGCTGCATCCCCCGAGAGATGCTGACTCGGAGGAGCAAGCTGCTGACAGCTGACTCGGAGGAGCA
F T P E V Q A S W Q K M V T A V A S A L

500                 520                 540
CCTCAGATACACTGACAGCTGCTCTGCTGCTGCTCTCCGATGATAGTGTGTGGAGACCCCTTCCTTACCTAGGAG
S S R Y H

560                 580
CTGCAAGCAATACAAATAATAAATCTAATTCTGCTGAGGATACAC
GACGTTGTTATATTTATTTGATAGAAGGAGACCTCTCTAGG
3’

a) Which strand is the template for transcription, the top or the bottom strand?
The bottom strand is the template strand. The resulting mRNA will resemble the top strand.

b) What three nucleotide sequences are used as stop codons? What is the stop codon above?
The three stop codons are UAA, UAG, and UGA. The stop codon in the sequence above is UGA.
c) The following sequence is part of the hemoglobin mRNA.

\[
5' \ldots \text{GAC AAG CAG} \ldots 3'
\]

i) Which of the following tRNA molecule would pair with codon a?  

ii) Which of the following tRNA molecule would pair with codon b?  

iii) Which of the following tRNA molecule would pair with codon c?  

You create a mutant that has the following altered tRNA for tryptophan:

WILD-TYPE

YOUR MUTANT

Spring 2002 7.014
Question 4, continued

The three shaded letters on the schematic above represent the anticodon. Your mutant cells carry both the wild-type version of the tRNA for tryptophan and the altered version of the tRNA for tryptophan.

d) By looking at the DNA sequence given for hemoglobin, indicate how the translation of hemoglobin in your mutant cells is different from the translation of hemoglobin in normal cells.

In the mutant cells, the mRNA codon that is UGA (which in non-mutant cells is seen as a stop codon) can have the amino acid trp inserted. For the sequence given above, this would result in a protein that is 6 amino acids longer than that translated in a normal cell.

e) Will the first 42 amino acids of the hemoglobin protein be the same or different in the mutant and wild-type cells?

They will be the same as shown in the figure.

f) Will all the hemoglobin molecules in the mutant cell look like this? Why or why not?

Not necessarily. The cell could still recognize the codon UGA as a stop, so in some cases the gene will be transcribed in the normal way.

g) Will any other proteins in the mutant cell be affected? If yes, what proteins?

Yes, the mutant tRNA can be used to insert a trp at any UGA stop codon of any protein. SO any protein ending with UGA is the stop codon could be affected.

A friend comes by and looks at your experiments. He mentions that he is doing similar experiments. Shown below is how he modified the tRNA for glycine. He added one C to the anticodon sequence. See the schematic below.

h) He makes mutant cells that carry both the wild-type version of the tRNA for glycine and the altered version of the tRNA for glycine. Assume that the mutant tRNA for glycine can be used by the cell. In general (not only for hemoglobin), what will be the effect of introducing this tRNA into your cells?

When this mutant tRNA is used, the translation of that protein is frame-shifted and results in a completely different protein.
## The Genetic Code:

<table>
<thead>
<tr>
<th>U</th>
<th>C</th>
<th>A</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>UU</td>
<td>CU</td>
<td>AU</td>
<td>GU</td>
</tr>
<tr>
<td>UC</td>
<td>CC</td>
<td>AC</td>
<td>GC</td>
</tr>
<tr>
<td>UA</td>
<td>CA</td>
<td>AA</td>
<td>GA</td>
</tr>
<tr>
<td>UG</td>
<td>CG</td>
<td>AA</td>
<td>GG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C</th>
<th>UU</th>
<th>CU</th>
<th>AU</th>
<th>GU</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC</td>
<td>CC</td>
<td>AC</td>
<td>AA</td>
<td>GA</td>
</tr>
<tr>
<td>UA</td>
<td>CA</td>
<td>AA</td>
<td>GA</td>
<td>GG</td>
</tr>
<tr>
<td>UG</td>
<td>CG</td>
<td>AA</td>
<td>GA</td>
<td>GG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A</th>
<th>UU</th>
<th>CU</th>
<th>AU</th>
<th>GU</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC</td>
<td>CC</td>
<td>AC</td>
<td>AA</td>
<td>GA</td>
</tr>
<tr>
<td>UA</td>
<td>CA</td>
<td>AA</td>
<td>GA</td>
<td>GG</td>
</tr>
<tr>
<td>UG</td>
<td>CG</td>
<td>AA</td>
<td>GA</td>
<td>GG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>G</th>
<th>UU</th>
<th>CU</th>
<th>AU</th>
<th>GU</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC</td>
<td>CC</td>
<td>AC</td>
<td>AA</td>
<td>GA</td>
</tr>
<tr>
<td>UA</td>
<td>CA</td>
<td>AA</td>
<td>GA</td>
<td>GG</td>
</tr>
<tr>
<td>UG</td>
<td>CG</td>
<td>AA</td>
<td>GA</td>
<td>GG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phe</th>
<th>Ser</th>
<th>Tyr</th>
<th>Cys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu</td>
<td>Leu</td>
<td>Leu</td>
<td>Leu</td>
</tr>
<tr>
<td>Ile</td>
<td>Ile</td>
<td>Ile</td>
<td>Ile</td>
</tr>
<tr>
<td>Val</td>
<td>Ala</td>
<td>Ala</td>
<td>Ala</td>
</tr>
<tr>
<td>Thr</td>
<td>Thr</td>
<td>Thr</td>
<td>Thr</td>
</tr>
<tr>
<td>Asp</td>
<td>Asp</td>
<td>Asp</td>
<td>Asp</td>
</tr>
<tr>
<td>Gln</td>
<td>Gln</td>
<td>Gln</td>
<td>Gln</td>
</tr>
<tr>
<td>Arg</td>
<td>Arg</td>
<td>Arg</td>
<td>Arg</td>
</tr>
</tbody>
</table>