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) ________________ is the synthesis of proteins on ribosomes, where the information for an amino acid sequence is encoded in ___________. This information is originally encoded by __________, transcribed by a protein complex called ________________, which initially binds to the _________________ region of the gene.

c) Why are retroviruses considered unusual with respect to the central dogma?

d) From first principles, why do you need at least three RNA bases (a codon) to code for 1 amino acid?
Question 2

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

![Diagram](Image)

Semi-conservative

Conservative

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 (\(^{15}\)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 (\(^{14}\)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](Image)

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.
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

\[
\begin{align*}
\text{Light} & \quad \text{Heavy} \\
\text{After 1 round} & \quad + \\
\text{After 2 rounds} & 
\end{align*}
\]

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.

\[
\begin{align*}
\text{Light} & \quad \text{Heavy} \\
\text{After 1 round} & \\
\text{After 2 rounds} 
\end{align*}
\]

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

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?
   
ii) What was the conclusion from Cairns’ experiment?

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?

   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?
a) Which strand is the template for transcription, the top or the bottom strand?

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

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

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

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

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

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

**WILD-TYPE**

```
      5'       3'
G   A   C

      5'       3'
D   A   C
```

**YOUR MUTANT**

```
      5'       3'
U   C   A

      5'       3'
D   A   C
```

\[ \text{GUC CUG UUCCUU} \]

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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.

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

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

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

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?
## The Genetic Code:

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<td>UCU ser (S)</td>
<td>UAU tyr (Y)</td>
<td>UGU cys (C)</td>
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<td>UCC ser</td>
<td>UAC tyr</td>
<td>UGC cys</td>
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<tr>
<td></td>
<td>UUA leu (L)</td>
<td>UCA ser</td>
<td>UAA STOP</td>
<td>UGA STOP</td>
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<tr>
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<td>UUG leu</td>
<td>UCG ser</td>
<td>UAG STOP</td>
<td>UGG trp (W)</td>
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<tr>
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<td>CUU leu</td>
<td>CCU pro (P)</td>
<td>CAU his (H)</td>
<td>CGU arg (R)</td>
</tr>
<tr>
<td></td>
<td>CUC leu</td>
<td>CCC pro</td>
<td>CAC his</td>
<td>CGC arg</td>
</tr>
<tr>
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<td>CCA pro</td>
<td>CAA gln (Q)</td>
<td>CGA arg</td>
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<td>CGG arg</td>
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<td>ACU thr (T)</td>
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<td>ACG thr</td>
<td>AAG lys</td>
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