Question 1

Shown below are two sequential close-up views of a replication fork as replication proceeds from left to right.

![Diagram of replication fork](image)

"old" (template) DNA
DNA synthesized before time = 1
DNA synthesized between time = 1 and time = 2

a) Redraw both figures (time = 1 and 2), labeling the 5' and 3' ends of all DNA strands. Also label which strand is the "leading" strand and which is the "lagging" strand.
b) DNA synthesis requires the presence of some DNA ahead of the sequence to be copied, that can serve as the template for an RNA primer. This requirement has an interesting effect on replication. Imagine a linear piece of DNA that is being replicated. The final end of the 3’ strand cannot be replicated, because there is no DNA left from which to make the RNA primer.

Therefore, this results in a short single-stranded region at the end. As single-stranded DNA is less stable it will be degraded leaving only double-stranded DNA.

i) What will happen to the length of a linear chromosome after one round of replication? Draw a picture of a linear chromosome before replication and the products after one round of replication. Be sure to state the overall result. Use a different color pen for the newly synthesized DNA.

Since each strand of the starting DNA is used as a template for one copy of the replicated DNA (semi-conservative replication) the DNA length will be shorter after every round of replication.
ii) What would then happen after many successive rounds of replication?

*If this problem were left uncorrected, linear chromosomes would shrink with each successive replication. You might guess correctly that this would have a detrimental effect on an organism. Therefore, organisms have evolved ways to combat this problem.*

iii) Some organisms such as bacteria and viruses have circular, not linear, chromosomes. Explain how having a circular chromosome could solve the problem explained above.

*With a circular chromosome, the DNA is continuous - it has no "end". This means that there will always be DNA from which to make the RNA primer for the lagging strand.*

iv) Another strategy organisms evolved is to have linear chromosomes with telomeres at either end. (See pages 226-227 in *Purves et al.*) A telomere is simply a long stretch of repeated nucleotides that are not important for coding information. For example, in yeast (*S. cerevisiae*), there is a telomere composed of many *(TGTGTGTG)*\(_n\) repeats, \((ACACAC)\_n\) on the complementary strand, at the end of each chromosome where \(n\) can equal several hundred. (In humans, it's \((5'-TTAGGG-3')\_n\).) A special enzyme called telomerase periodically extends the length of this repeat sequence without requiring a template! How could having a telomere solve the above problem?

*A small piece of the telomeric DNA will be lost during replication. The absence of this DNA does not cause harm to the organism, because the telomeric DNA does not encode any genes. Its function is to protect the rest of the chromosome from being slowly lost (from the ends inward) during successive rounds of replication. The shrinkage of the telomere is later compensated by the action of telomerase.*

c) Given what is known about DNA structure, which of the following general shapes might be found? (Horizontal lines represent bonding between complementary bases.)

![Diagram](image)

i) A only

ii) B only

iii) A and B, both

iv) Neither A or B

d) Explain your answer in c). *DNA doublestrand are antiparallel. ALWAYS. 5'-------3' Only B satisfies this. 3'-------5'*
Question 2

Meselson and Stahl demonstrated that *E. coli* replicates DNA in a semiconservative manner. This was shown by first growing *E. coli* in a medium containing $^{15}$N for the first several generations, shifting the culture to one containing $^{14}$N and then examining the density of the next generation of DNA. Their results are shown below and discussed on pages 220-222 in *Purves et al.*

![Diagram of DNA replication](image)

a) On the diagram below, indicate what the results would be if a Martian organism replicates DNA in a conservative manner. Use a similar format as shown above.

![Diagram of conservative DNA replication](image)

b) On the diagram below, indicate what the results would be if the Martian organism replicates DNA in a dispersive manner. Use a similar format as shown above.

![Diagram of dispersive DNA replication](image)
c) An alien bacterium has been recovered from the atmosphere of Ganymede, a moon (the size of Mars!) of planet Jupiter. You isolate its genetic material, and find it to be similar to DNA in composition, except that it contains 6 types of "bases", that are of three different types: jovines - J and K, zeusines - Y and Z, and herenes - V and W. You determine the "base" composition of the alien genetic material to be approximately 13% J, 20% K, 20% Y, 13% Z, 20% V and 13% W. Propose a structure for the alien genetic material.

One possibility is by analogy to DNA, the alien genetic material is composed of three strands, held together by base triplets. J, Z and W form triplets, as do K, Y and V. I’ve seen other creative answers that also are consistent with the data.

d) Curious as to how this alien organism replicates its genetic material, you grow it in heavy N\textsuperscript{15} for several generations (the alien bases contain nitrogen) until all the genetic material is labeled and sediments at a "heavy" position on a gradient. You then shift your culture to N\textsuperscript{14} containing medium, and examine the density of the next generations of genetic material. The results are shown below.

i) What can you say about how this alien genetic material replicates?

| Conservatively | Dispersively | Semiconservatively | None of These |

ii) Explain your answer in i).

This is the equivalent of a Meselson-Stahl experiment. Since all the new genetic material produced after shifting the culture sediments at a new and lighter position, all the new molecules must contain some light N\textsuperscript{14} material, so replication cannot be conservative. Since the new molecules all sediment at the same position, replication is not dispersive; therefore it is semiconservative.

You can also deduce that replication requires just one strand of the triple helix to generate a whole new molecule, as illustrated below: The molecules in the first generation after shift all consist of one heavy strand and two light ones, and are only 1/3 as heavy. In the second generation, 1/3 of the molecules consist of a heavy strand and two light ones, while the other 2/3 are all light strands.

```
1st gen. after shift  2nd gen. after shift

1 : 2
```
Question 3

Protein X, from the fungus *S. sevenowontoo* is encoded entirely by the fragment of DNA below.

```
1                  20                  40
5´...ACGTATAATGCACGTACATGCGATCTCAGTGCAGGGTGACAGTGACTGC
3´...TGCATATTACGTGCAGTGTACGCTAGTAGTCACGTCCACTGTCACTGACG
```

```
51                 70                  90
A
|ACACGAATTATTTCGTCACGTATACAGTCACGGTCGTCGATCGATCGAGT
TGGTGCTTAATAAGCGAGTGCATATGTCAGTGCGCAAGCATGCTAGCTCA
```

```
101               119
|   .    *    .   |
CGATCTAGCCTAATAAAC...3´
GCTAGATCGTGATTATTTG...5´
```

In *S. sevenowontoo*, the sequence 5´ TATAAT 3´ is a strong promoter, and transcription begins 4 nucleotides downstream (3´) of the final T.

a) Which of the following shows the beginning of the mRNA?

i) 5´-GCAGTGTACGCTAGAGTCACGTCC-3´

ii) 5´-GCAGUGUACGCUAGAGUACAGUCC-3´

iii) 5´-CGUCACAUUGCGAUCAGUCAGGAGG-3´

iv) 5´-CGTCACATGCGATCTCAGTCAGGG-3´

v) 5´-AUGCGAUCAGUCAGGUGACAG-3´

vi) 5´-UACGCUAGAGUACAGGUCACUCAGUC-3´

vii) none of the above.

b) If an mRNA is transcribed from the DNA fragment using the promoter mentioned above, what is the greatest possible length of protein X?

29 amino acids. This is from the only AUG start codon (19-21) to the UAG stop codon at 106-108.

c) You isolate Protein X from the cell and determine it is actually 17 amino acids long. Protein sequencing reveals the following sequence...

```
Met-Arg-Ser-Gln-Cys-Arg-Val-Thr-Ser-Arg-Val-Arg-Thr-Ile-Glu-Ser-Ile
```

single letter code→ MRSQRVTSVRTIESI

You wonder if the difference between the actual and estimated lengths of the protein may be due to the presence of an intron in the gene. If this is true, what would be the first and last bases corresponding to the intron? Box the coding sequence above encoding the intron.

G43 and G78 are the boundaries of the intron.
d) State the type of mutation listed below and predict the effects on the resultant protein.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Describe the Type of mutation using these terms: point, deletion, insertion, nonsense, missense, silent, frameshift</th>
<th>Effect on Protein</th>
</tr>
</thead>
</table>
| i) C33 to A33     | Point mutation  
Nonsense Mutation                                                                                         | This changes the 5th codon to a stop codon, resulting in a truncated peptide of just 4 amino acids. |
| ii) C101 to G101  | Point mutation  
Missense mutation  
Changes the identity of the second to last amino acid from Ser to Trp.                      |                                                                                   |
| iii) G30 to A30   | Point mutation  
Silent mutation  
This mutation is in a wobble position and has no effect on the protein sequence.             |                                                                                   |
| iv) G49 to A49    | Point mutation  
Mutation occurs in the intron, and thus probably has no effect on the expression of Protein X.  
Note that some intronic mutations can affect splicing, and thus the amino acid sequence of the protein. |                                                                                   |
| v) A24 to nothing | deletion → frameshift  
This mutation causes a frame shift by which all downstream codons are misread. This will result in the sequence Met-Arg-Leu-Ser-Ala-Gly.  
The protein will end here. |                                                                                   |
| vi) A7 to G7      | point mutation  
This mutation occurs in the promoter, and could possibly diminish, increase, or even abolish transcription of the mRNA. |                                                                                   |

<table>
<thead>
<tr>
<th>U</th>
<th>C</th>
<th>A</th>
<th>G</th>
</tr>
</thead>
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<td>phe</td>
<td>UAU</td>
<td>cys</td>
</tr>
<tr>
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<td>phe</td>
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<tr>
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<td>gly</td>
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</table>
Question 4

The enzymes TrpA, TrpB, TrpC, TrpD, TrpE and AroH are all required for tryptophan synthesis in *E. coli*. In the presence of tryptophan, wild-type bacteria do not synthesize any of these enzymes; however, in the absence of tryptophan, all of these enzymes are synthesized at high levels.

a) Theoretically speaking, if the synthesis of the above enzymes is negatively regulated...

i) what change to the repressor protein would cause the enzymes to be synthesized even in the presence of tryptophan?
*Inactivation of the functional repressor so that it cannot bind the operator.*

ii) what change in the operator sequence would cause the enzymes to be synthesized even in the presence of tryptophan?
*A mutation in the sequence of the operator such that it no longer binds the repressor.*

iii) what change in the repressor protein would cause the inhibition of enzyme synthesis, even in the absence of tryptophan?
*A change that increases the affinity of the repressor for the operator, i.e., a constitutively active repressor.*

b) If the synthesis of the above enzymes is positively regulated...

i) what change in the activator protein would cause the enzymes to be synthesized even in the presence of tryptophan?
*A change that increases the affinity of the activator for the operator, i.e., a constitutively active activator. OR... A change in the activator such that tryptophan can no longer bind.*

ii) what change in the activator protein would prevent synthesis of the enzymes, even in the absence of tryptophan?
*A mutation in the sequence of the activator gene, such that a nonfunctional activator protein is produced.*

iii) what change in the operator sequence would prevent synthesis of the enzymes, even in the absence of tryptophan?
*A mutation in the operator sequence rendering it unable to bind activator.*
c) By mutational analysis you identify two regions of DNA that are important in the regulation of tryptophan synthesis. The first of these regions, called \textit{trpR}, is a gene that encodes a DNA-binding protein. The second region is a DNA sequence to which the \textit{trpR} gene product binds, called \textit{trpO}. Analysis of three bacterial strains with different genotypes at the \textit{trpR} and \textit{trpO} loci yields the following results.

<table>
<thead>
<tr>
<th>Strain</th>
<th>growth medium</th>
<th>\textit{trpA}, \textit{trpB}, \textit{trpC}, \textit{trpD}, and \textit{trpE}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{trpR}^+\textit{trpO}^+</td>
<td>with tryptophan</td>
<td>0.1%</td>
</tr>
<tr>
<td></td>
<td>without tryptophan</td>
<td>100%</td>
</tr>
<tr>
<td>\textit{trpR}^-\textit{trpO}^+</td>
<td>with tryptophan</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>without tryptophan</td>
<td>100%</td>
</tr>
<tr>
<td>\textit{trpR}^+\textit{trpO}^-</td>
<td>with tryptophan</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>without tryptophan</td>
<td>100%</td>
</tr>
</tbody>
</table>

i) Is the control of tryptophan synthesis likely an example of positive or negative regulation?

- negative
- positive
- can't tell

ii) Is the protein made from the \textit{trpR} gene an activator or a repressor?

- activator
- repressor