

7.013 Section Problem Viruses Solutions

Poliovirus is a type of picornavirus. Its genome consists of a single (+) strand of RNA. The protein coat of poliovirus is composed of four viral proteins, VP1-VP4, each protein present in 60 copies per protein coat. The poliovirus infects eukaryotic cells, specifically the epithelial cells, fibroblasts, and nerve cells of primates, including humans.

Upon infection, the RNA genome faces three possible fates:

- a) the RNA genome can be used as the mRNA template for protein synthesis. In addition to coat proteins, proteins required for other processes are also synthesized.
 - b) the RNA genome can be used for replication of more RNA genomes.
 - c) the RNA genome can be packaged into new virions.
- 1) The poliovirus genome consists of only one transcription unit; however, the virus is capable of making many different proteins.
 - i) If the poliovirus genome were a polycistronic mRNA, explain how the poliovirus could produce the many proteins it requires.

If the genome were polycistronic, then the RNA genome would act as a single, long mRNA template with multiple translation initiation and termination sites. From this polycistronic message, multiple proteins could be synthesized.

- ii) If the poliovirus genome were a monocistronic mRNA, explain how the poliovirus could produce the many proteins it requires. *If the genome were monocistronic, then the RNA genome would act as a single, long mRNA template for the synthesis of a single, long polypeptide. The polypeptide could then undergo proteolytic cleavage to yield separate, distinct peptides.*

2) Production of poliovirus proteins is sensitive to protease inhibitors. In other words, if you block proteolytic cleavage from occurring, no functional poliovirus proteins are made. Given this information, is the poliovirus mRNA likely to be polycistronic or monocistronic? Explain your answer.

Poliovirus is likely to be monocistronic. The mRNA produces a long polypeptide that must undergo proteolytic cleavage to produce its many proteins.

3) Since the genome of poliovirus is a single (+) strand of RNA, replication of its genome means the production of more single (+) strands of RNA. The enzyme responsible for poliovirus replication is an RNA-dependent RNA polymerase.

What strand does the poliovirus use as the template to make more (+) strand RNA? Explain your answer.

The (-) strand must be used as the template to make more (+) strand RNA, because only replication of the (-) strand will produce (+) RNA strands.

How does the poliovirus generate this template that it uses to make more (+) strand RNA?

The (+) strand RNA must have been used as the template to generate the (-) strand.

Then the (-) strand is used to make more (+) strands.

4) The RNA of the poliovirus genome contains a 3' poly (A) tail. However, unlike in eukaryotic transcription where the poly (A) tail is added on post-transcriptionally, the poly (A) tail of poliovirus is replicated along with the rest of the genome. What nucleotides must be present in the template (-) strand to ensure that the poly (A) tail is replicated? At which end of the template strand are these nucleotides located?

The template (-) strand contains a sequence of (U) nucleotides at its 5' end.

5) Of the three possible fates that the poliovirus genome faces (listed as (a), (b) and (c) on the previous page), which are likely to occur at the following times: soon after infection; at an intermediate time after infection; and late in infection? Explain why each fate occurs at its respective time.

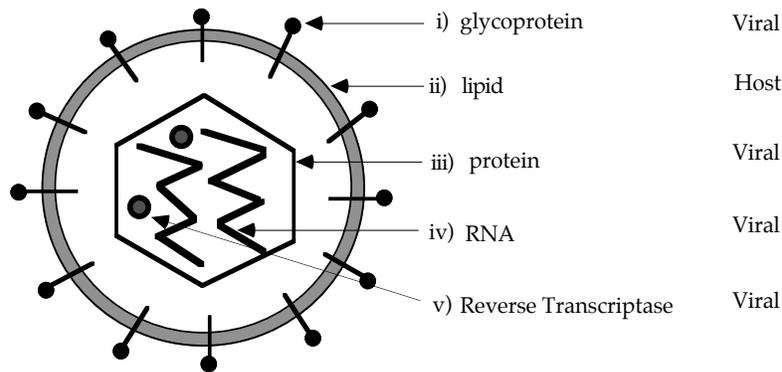
Early on in infection, the RNA must serve as template for protein synthesis, because the proteins that are required for survival of the virus are all encoded in the viral genome.

At an intermediate time after infection, after the RNA-dependent RNA polymerase is made, the RNA serves as the template to make (-) strands for the replication process.

Late in infection, when viral proteins are abundant (including coat proteins), the RNA genomes can be packaged into new protein coats.

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a)



b) The gp120 protein on the surface of the HIV envelope binds to the CD4 receptor, and this ligand-receptor binding event is the first step in infection.

c) i)

1. Synthesis of single (-) strand of DNA using the single (+) strand RNA genome as the template.
2. Degradation of the single (+) strand RNA template.
3. Synthesis of the complementary (+) strand of DNA using the single (-) strand of DNA as the template to form double stranded DNA.

- ii)
1. RNA-directed DNA polymerase activity.
 2. RNase activity.
 3. DNA-directed DNA polymerase activity.

d) While AZT will inhibit DNA synthesis mediated by reverse transcriptase, it will also inhibit nucleic acid synthesis in other cells of the body, mediated by DNA polymerases and thus kill cells which are dividing. All bodily cells that are actively replicating (e.g.: red blood cell precursors, skin cells, etc.) can be affected by these nucleotide analogs. However, HIV reverse transcriptase will use AZT and thymidine interchangeably while host cell DNA polymerase prefers thymidine over AZT. Therefore, AZT may be used at concentrations that inhibit HIV replication but are not yet toxic to host cell polymerases.

e) It would be effective to target the integration of the double-stranded DNA into the host cell genome. Not only is the integrase enzyme a retroviral-specific enzyme, but developing therapies to disrupt this enzyme's activity won't likely affect other cellular properties, because we do not possess any enzymes which have this type of activity. Additionally, viral protease inhibitors will affect the processing of the viral polyprotein into active proteins. The host proteins are not synthesized as polyproteins.

f) The mutations are most likely to be in the viral gene coding for reverse transcriptase (the pol gene) because mutations in this gene may directly cause the mutated reverse transcriptase not to prefer AZT during replication and thus become resistant to AZT.

g) Order of steps: B D C A F E