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**BIO 330/391P**

**Exam 2**

**April 2, 2013**

Please read each question carefully before answering. Many questions have multiple parts. There are 9 questions on 4 pages.

1. Name the RNAs found in rabies virus-infected cells that are not polyadenylated. (4 points)

Explain how RNA-containing viruses that replicate exclusively in the cytoplasm can make polyadenylated mRNAs. (3 points)

What is the function of the leader RNA of rhabdoviruses? (2 points)

1. You have discovered a togavirus temperature-sensitive mutant. At the non-permissive temperature, all of the viral nonstructural proteins are cleaved rapidly to the individual proteins. How would you predict that this mutation will affect the replication cycle of the virus at the non-permissive temperature? Explain. (4 points)

3. You have identified a new virus, V2004. You determine the sequence of one of the viral proteins, which is not part of a polyprotein. The protein has the following characteristics.

N-terminus--signal-----------C-terminus

Based on the virus families that we have studied, what will be the final destination of this protein in the cell during viral replication and why? (4 points)

Will the mRNA for this protein be localized on membrane-bound or free polysomes? (2 points) Justify your answer. (3 points)

4. You want to further characterize the V2004 virus and, therefore, you develop a series of temperature-sensitive mutants. Describe why you want to avoid mutants that are leaky. (2 points). How do you determine leakiness of mutants? (2 points)

You subject your mutants to complementation tests, and you obtain the following data.

|  |  |
| --- | --- |
|  | **Yield at non-permissive temperature (PFU/ml)** |
| **Mutant** | Ts1 | Ts2 | Ts3 | Ts4 | Ts5 |
| Ts1 | 5.5 X 102 | 5.8 X 104 | 1.4 X 104 | 4.9 X 104 | 1.9 X 104 |
| Ts2 | -- | 3.4 X 103 | 5.4 X 103 | 9.3 X 104 | 4.3 X 104 |
| Ts3 | -- | -- | 6.7 X 102 | 2.3 X 104 | 6.5 X 103 |
| Ts4 | -- | -- | -- | 4.5 X 103 | 8.2 X 104 |
| Ts5 | -- | -- | -- | -- | 7.7 X 102 |

 How many genes are represented by these mutants? Justify your answer and show your calculations. (4 points)

What is happening inside an infected cell when two mutants complement each other at the non-permissive temperature? (6 points)

How could you determine the order of these mutants on a linear map of the virus? (6 points)

5. The V2004 virus causes neurological symptoms in humans, and you think that it may be either a flavivirus or a rhabdovirus. You extract the RNA from purified virions so that it is free from any proteins. The naked RNA then is introduced into cells that are susceptible to both viruses. After incubation of the cells at 37oC for approximately 48 hours, you do not observe plaque formation. Do you have a flavivirus or a rhabdovirus? (2 points) Why do you think this? (4 points)

Could you also discriminate between these two types of viruses by electron microscopy? Why or why not? (6 points)

6. Name two virus families in the viral order Mononegavirales. (4 points)

Name two shared features of the viral genomes of these families. (4 points)

7. How are different amounts of togavirus nsp1 proteins synthesized relative to envelope proteins? (4 points)

Why are different amounts of N protein synthesized relative to L protein in rhabdovirus-infected cells? (6 points)

What is the function of rabies virus M protein? (6 points)

8. Name at least two functions of viral proteases. (6 points)

Name a function of a host protease that is required in a viral replication cycle. (5 points)

Why doesn’t rabies virus encode a viral protease? (5 points)

9. West Nile virus is a flavivirus. Name at least two similarities between the replication phase (not the structure of the genome or morphology) of the togaviruses and flaviviruses. (6 points)