Name_____KEY_____ UT EID_____

BIO 330/391P
Exam 2
April 1, 2014

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

1. The hantaviruses are segmented, single-stranded, enveloped RNA viruses. They have three segments named S, M, and L (from smallest to largest). Based on what you know about other negative-sense viruses, predict the type of proteins encoded by each segment. (3 points) Give your reasoning. (3 points)

The information about the hantaviruses indicates that they are enveloped negative-sense RNA viruses that would share some characteristics with the rhabdoviruses. In general, we know that capsid proteins are the smallest and the polymerases are the largest proteins. The envelope proteins usually have a size intermediate between the capsid and polymerase proteins. The smallest (S) segment encodes the N (nucleocapsid) proteins. The M segment encodes the envelope proteins, and the L segment encodes the RNA-dependent RNA-polymerase.

Explain how RNA-containing viruses that we have studied that replicate exclusively in the cytoplasm make polyadenylated mRNAs. (4 points) How do such viruses make capped mRNAs? (4 points)

The togaviruses and rhabdoviruses have RNA-dependent RNA polymerases that reiteratively copy short U (uridine) sequences on the negative strand template RNAs. These polymerases also have guanyltransferase and methyltransferase activity to give capped mRNAs that can bind to the ribosomes. Some of the flaviruses also make capped mRNAs in the same way.

2. What is the function of a signal sequence? (3 points)

The function of a signal sequence is to direct a protein to cross a membrane. Typically, viruses make glycoproteins that have a signal peptide that binds to the signal recognition particle (SRP). Binding of SRP stops translation and allows docking onto the SRP receptor associated with the translocon in the ER membrane. Translation then resumes and allows co-translational transfer of the protein across the membrane. Signal sequences also can be present on proteins that cross organelle membranes, such as chloroplasts or mitochondria. In this case, the signal-containing protein is synthesized in its entirety before docking to a receptor on the organelle and transfer across the membrane.

Name at least three virus families that encode proteins with signal sequences. (6 points)

Rhabdoviridae, Flaviviridae, Bornaviridae, Filoviridae, Paramyxoviridae, Orthomyxoviridae, Togaviridae, Herpesviridae, and others

3. You have identified a new virus, TEX14. You determine the sequence of one of the viral proteins, and it has the following characteristics.

N-terminus—AUG --signal---coding sequence---stop codon--C-terminus

What will be the final destination of this protein in the cell during viral replication? (2 points) Why? (2 points) (Note: this protein will NOT go to the mitochondria.)

This protein will be secreted from the cell since the signal sequence will direct translation to the ER for co-translational transfer across the membrane and no halt/stop-transfer sequence is present.

4. You want to further characterize the V2009 virus and, therefore, you develop a series of temperature-sensitive mutants. 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	5.5×10^2	5.8×10^7	1.4×10^6	4.9×10^5	1.9×10^6
Ts2	--	3.4×10^3	5.4×10^3	9.3×10^5	4.3×10^4
Ts3	--	--	6.7×10^2	2.3×10^4	6.5×10^3
Ts4	--	--	--	4.5×10^3	8.2×10^6
Ts5	--	--	--	--	7.7×10^2

How many genes are represented by these mutants? (2 points) Justify your answer and show your calculations. (6 points)

To calculate the complementation index (CI):
Ts1 X ts2 = $5.8 \times 10^7/5.5 \times 10^2 + 3.4 \times 10^3 = 14{,}684$
Ts1 X ts3 = $1.4 \times 10^6/5.5 \times 10^2 + 6.7 \times 10^2 = 1{,}148$
Ts1 X ts4 = $4.9 \times 10^5/5.5 \times 10^2 + 4.5 \times 10^3 = 97$
Ts1 X ts5 = $1.9 \times 10^6/5.5 \times 10^2 + 7.7 \times 10^2 = 1{,}439$
Ts2 X ts3 = $5.4 \times 10^3/3.4 \times 10^3 + 6.7 \times 10^2 = 1.3$
Ts2 X ts4 = $9.3 \times 10^5/3.4 \times 10^3 + 4.5 \times 10^3 = 118$
Ts2 X ts5 = $4.3 \times 10^4/3.4 \times 10^3 + 7.7 \times 10^2 = 10.3$
Ts3 X ts4 = $2.3 \times 10^4/6.7 \times 10^2 + 4.5 \times 10^3 = 4.4$
Ts3 X ts5 = $6.5 \times 10^3/6.7 \times 10^2 + 7.7 \times 10^2 = 4.5$
Ts4 X ts5 = $8.2 \times 10^6/4.5 \times 10^3 + 7.7 \times 10^2 = 1{,}556$

All of the pairwise crosses grow more than 2-fold better than the sum of the single infections at the non-permissive temperature, except for the ts2 X ts3 cross. Only ts2 and ts3 would be defective in the same gene product and would be unable to complement. This suggests that there are 4 complementation groups (4 genes); one gene represented by ts1, one gene represented by ts2 and ts3, one gene represented by ts4 and one gene represented by ts5.

Explain why these temperature-sensitive mutants are unlikely to be in a cis-acting sequence. (4 points)

Cis-acting sequences must act on the genome that encodes them, for example, the 5' untranslated region or a promoter in a viral RNA. Ts mutants are generally defective in a protein that will lose its function by unfolding at the non-permissive temperature. RNAs are unlikely to change conformation and function between the permissive and non-permissive temperatures used for screening mutants.

Name two other types of information that you can obtain with these same mutants. (6 points)

Recombination tests can provide a map order of these mutants (although these mutants are all in the same gene and would be expected to cluster on the map). You could also get information about the function of the gene or the stage of the life cycle affected by the defective gene product.

5. The TEX14 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 37^{o}C for approximately 48 hours, you observe plaque formation. Do you have a flavivirus or a rhabdovirus? (2 points) Justify your answer. (4 points)

The virus must be a flavivirus because the flaviviruses have an infectious RNA genome. This means that introduction of the RNA (without associated proteins) will allow the entire replication cycle of the virus to occur, resulting in release of infectious virus (and therefore, plaques). The rhabdoviruses have a negative-stranded, non-infectious genome, which requires an associated RNA-dependent RNA polymerase to initiate infection inside a susceptible cell.

Could you also discriminate between these two types of viruses by electron microscopy? (2 points) Why or why not? (4 points)

Yes, you should be able to discriminate the viruses by EM because the flaviviruses have an icosahedral capsid and the rhabdoviruses have a helical nucleocapsid. Since both of the viruses are enveloped, you might have to remove the envelope to see the capsid clearly. However, the rhabdoviruses will tend to look bullet-shaped even with the envelope.

6. The rhabdovirus genome has leader and trailer sequences. What purpose do these sequences have in common? (3 points) What function is different between these sequences? (3 points)

The leader and trailer sequences have the encapsidation site for N protein. If N protein is abundant and the N + L + P replicase is formed, then N will bind first in a sequence-specific manner and then cooperatively to the leader RNA or trailer RNA. In each case, there is no pausing at the intergenic regions and the full-length encapsidated RNA is made (replication). However, if N protein is not abundant, there is no binding to the leader or trailer RNA and pausing and termination of synthesis occurs at the first intergenic region. Subsequently, transcription of mRNAs (not encapsidated) occurs by a start-stop mechanism. Thus, the encapsidation of the leader and trailer sequences by N protein partially determine whether replication or transcription will occur in infected cells.

These sequences differ because they are recognized for either (+) strand or (-) strand synthesis, and they have different affinities for the RNA-dependent RNA polymerase, leading to different levels inside infected cells.

What is the mechanism for synthesis of different amounts of N protein synthesized relative to L protein in rhabdovirus-infected cells? (6 points)

In the start-stop mechanism of transcription, initiation of RNA synthesis by the RNA-dependent RNA polymerase only occurs at the 3' end of the template. At each intergenic region, the polymerase pauses and terminates in some cases. Polymerization can only resume on the immediately 3' gene or the polymerase must start again at the 3' end of template. Initiation of transcription cannot occur internally. Therefore, genes at the 3' end of the minus-strand template are transcribed more frequently than genes at the 5' end of the template, leading to higher synthesis of N (nucleocapsid) compared to L (polymerase).

7. The bornaviruses are members of the Mononegavirales like the rhabdoviruses. Will transcription or replication of viral RNA be affected the most if you inhibit translation? (3 points) Give your reasoning. (3 points)

Transcription of bornaviruses and rhabdoviruses does not require new protein synthesis, whereas replication requires continuous synthesis of the N proteins to encapsidate the (+) and (-) strands.

8. A togavirus temperature-sensitive mutant synthesizes RNA at the non-permissive temperature (41^{o}C). However, you determine that this mutant makes full-length plus and minus RNA at 41^{o}C, but it does not make subgenomic RNA. What might be the defect in this mutant? (3 points) Give your rationale. (4 points)

The ts mutant is likely to be defective in a protein product since it must be able to have a conformational change and loss of function between the permissive and non-permissive temperatures. Since the mutant is only defective in subgenomic mRNA synthesis but not replication, it seems likely that one of the nonstructural proteins (involved in both replication and transcription) would have a defect. The mutant polymerase may be defective in recognition of the subgenomic RNA promoter.

What is the maturation cleavage of the togaviruses? (3 points) Why is this cleavage necessary for the infectivity of the virus? (3 points)

The maturational cleavage of the togaviruses is cleavage of PE2 precursor to E2 and E3. The cleavage causes a change in the conformation of the glycoprotein allowing it to be “primed” and ready on the newly budded virus for attachment to a receptor on a susceptible cell.

What is the maturation cleavage of picornaviruses? (3 points)

The maturational cleavage of picornaviruses is cleavage of the VP0 precursor (1AB) to VP4 + VP2 (1A + 1B).

9. West Nile virus is a flavivirus. Name at least two similarities between the replication phase of the infectious cycles of the togaviruses and flaviviruses. (6 points)

Both families have members that translate capped mRNAs, although many flaviviruses use an IRES to translate their RNA genome (HCV). Both families encode infectious positive-stranded RNAs that start their replication cycle with translation of a multi-subunit RNA-dependent RNA polymerase. Both families appear to replicate entirely in the cytoplasm of susceptible cells. Both families make polyproteins, which are cleaved by virally encoded proteases. Both families make glycoproteins.

If the natural reservoir of these viruses is birds, how are these viruses transmitted to humans? (4 points)

These viruses depend on an insect vector (mosquitoes). Mosquitoes feed on birds, and the virus replicates in these insects. The insects transmit the virus to humans through biting.