Name_____KEY_____ UT EID_____

BIO 330
Quiz 4
March 20, 2014

1. Briefly describe the function of one viral protease and one cellular protease in the togavirus life cycle. (5 points)

The togaviruses have two different virally encoded proteases, one for the non-structural protein precursors (nsp123 and nsp1234). These precursors are cleaved by the nsp2 protease. The capsid protein (C) also acts as a protease for the structural protein precursor. Cellular proteases involved in the togavirus life cycle include: (1) the signal peptidase, which cleaves the signal peptides in the structural polyprotein precursor, (2) furin, which cleaves the PE2 precursor into E2 and E3 envelope proteins (maturational cleavage), and (3) the cellular caspases, which are involved in cellular apoptosis during cell lysis.

2. What is a halt-transfer signal? (2 points) Why is this signal important for the replication of togaviruses and flaviviruses? (3 points)

A halt-transfer is a hydrophobic amino acid sequence within a protein that has been directed for translation to the membrane (often ER membrane for viruses). The halt-transfer sequence does not halt translation, but prevents further transfer of the protein across the membrane. A combined signal-halt-transfer specifies membrane insertion without further transfer of sequences into the lumen of the organelle. The halt-transfer sequence anchors or tethers a protein in the membrane, which is required for the envelope transmembrane glycoproteins of both viruses.

3. You have isolated a temperature-sensitive togavirus mutant that only produces capsid at the permissive temperature. Would you expect that this mutant will synthesize RNA at the non-permissive temperature? (2 points) Give an explanation. (3 points)

Temperature-sensitive mutants will be isolated under conditions where a single mutation leads to the unfolded state and defective function at the non-permissive temperature. Since the capsid is only produced at the permissive temperature, then the mutation (defect) is in capsid. The capsid can only be produced from the 26S subgenomic RNA after viral RNA synthesis occurs. The RNA dependent RNA polymerases also must be translated from the incoming genomic RNA prior to RNA replication and transcription since togaviruses are positive-sense RNA viruses that do not package their polymerases. Therefore, this capsid mutant would be expected to make viral RNA at the non-permissive temperature using the non-defective RNA polymerase.

4. What is the relationship between complementation groups and the number of viral genes? Explain. (5 points)

The number of complementation groups equals the number of viral genes if a large number of mutants are examined in pairwise assays. A complementation group is defined as a group of mutants that cannot provide a full set of functional gene products in mixed infections at the non-permissive temperature. All mutants in the group are defective in the same gene. On the other hand, mutants in different complementation groups can provide a full set of functional gene products during a mixed infection, leading to increased virus production. Functional gene products are exchanged by diffusion between mutants at the non-permissive temperature because they are defective in different genes.