Chapter 9

1. a. HeLa and BHK-21 are both susceptible to infection since you see virus in endosomes in both types. This indicates that virus gained entry to the cells.

b. Only the BHK-21 cells are permissive for LAC infection. BHK-21 cells have all the necessary machinery to support replication of LAC virus. This is indicated by 200 progeny virions produced per cell. With a virus yield of only 5 virions per cell, it is not clear that there is any replication of virus in HeLa cells.

c. Perhaps BHK-21 cells have a specific receptor expressed on the surface which is recognized by LAC virus. When virus attaches to the cell receptors, receptor mediated endocytosis is initiated. Uncoating of the virus can then occur which liberates the viral genome for replication. Entry of virus into HeLa cells may have occurred in a more accidental way and proper uncoating may not follow. Therefore, the virus could not replicate. To test this hypothesis, you might use a drug to inhibit uncoating and determine how this would affect the virus yield in each cell type.

2. Your buffered solution contains a total of $6 \times 10^9$ latex beads in two ml. Therefore, there are $3 \times 10^9$ beads per ml. You find an average of 9 virions for every 3 beads. So the number of virus particles per ml would be 3 times the number of latex beads per ml or $3 \times 3 \times 10^9 = 9 \times 10^9$ virions per ml.

3. The electron microscope (EM) can visualize virus particles and even large molecules such as DNA, RNA, and large proteins by virtue of the short wavelengths, which have high resolving power, produced by accelerated electrons. Rich detail can be revealed with the use of different staining procedures. Also, virus particles can be enumerated by use of the EM.

4. To determine the number of virions that are infectious you would plaque out your dilutions of the virus stock onto monolayers of a permissive cell line. After plaques have developed, you would count the plaques and calculate how many infectious particles (each producing 1 plaque) there were per ml of the original suspension. You would then divide this number by the number of particles to determine the particle: PFU ratio.

Chapter 10

1. First dilution: 100 ul stock + 0.9 ml buffer $\rightarrow$ 10-fold.
   Second dilution: 10 ul to 1 ml $\rightarrow$ 100-fold dilution,
   Total dilution: $10 \times 100 = 1000$.

   \[
   (29 + 25) / 2 = 27 \quad 27 \text{ pfu in } 100 \text{ ul} = 270 \text{ pfu/ml}, \quad 270 \times 1000 = 2.7 \times 10^5 \text{ pfu/ml in original stock}.
   \]

   2. $5 \times 10^8$ cells / ml and $10^9$ phages $\rightarrow$ MOI = 2

   \[
   \text{Po} = (2^0 \times e^{-2}) / 0! = 0.135
   \]
   \[
   P1 \text{ or more} = 1 - \text{Po} = 1 - 0.135 = 0.865
   \]
   \[
   200 \times 0.865 = 173.
   \]
Chapter 19

1. a. RNA-directed transcriptase, DNA-directed transcriptase and RNase H.
   b. Specific tRNAs from the cell.

2. Passage from cell-to-cell, reverse transcription, integration of proviral DNA into the host chromosome, proteolytic processing of Gag:Prot:Pol during maturation.

3. a. 
   
   **Ts Mutation in:**
   
<table>
<thead>
<tr>
<th>Protein</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pol</td>
<td>Stop: No reverse transcription.</td>
</tr>
<tr>
<td>Gag</td>
<td>Stop: No coat protein synthesis</td>
</tr>
<tr>
<td>Src</td>
<td>No effect on the replicative cycle, but cannot transform cell.</td>
</tr>
</tbody>
</table>

   b. PBS: tRNA primer binding site for reverse transcription.

4. All of them are true (a, b, c, and d).

5. Growth hormones, Receptors for cellular growth signals, G-proteins (GTPases encoding receptor signaling), Protein kinases regulating the action of other enzymes, Transcription factors.

6. It is a good procedure because the relative abundance of RT will be higher in virions than in infected cells. An immunoaffinity step may separate it from the rest of the virion components.

7. They are different from minus-strand RNA viruses, because they are (+)RNA. They are different from plus-strand RNA viruses because their RNA is not translated immediately, but after it is reverse transcribed to DNA that is integrated in the host genome and then transcribed. Oncornaviruses contain v-onc genes that are genes (obtained originally from cells) that can induce tumors in the infected tissues, whereas lentiviruses do not transform cells.

8. The proviral DNA has the LTR at both ends. This combines U3 and U5. Furthermore, the proviral DNA has no poly-A region.

9. CD4+ helper T-cells are killed thereby destroying the adaptive immune response mediated by B cells and and cytotoxic T cells.

Chapter 20

1. The HIV protein Rev is responsible for this action. Rev binds to the Rev response element (RRE) sequence in the RNA and escorts the unspliced and singly spliced mRNA molecules out of the nucleus.

2. Unlike other retroviruses, the HIV pre-integration complex (PIC), consisting of two copies of the viral genome, the matrix protein, nucleocapsid, Vpr, RT, and integrase, is transported to the nucleus of the cell, providing access to the chromosomes.

3. Prior to the discovery of reverse transcriptase (RT), it was assumed that information flow in biological systems always moved from DNA to RNA to protein. RT reverses this flow,
copying RNA information into DNA. During the retrovirus life cycle, RT is responsible for producing the proviral DNA that integrates into the host genome.

4. Cytosine deaminases convert C residues into U residues in DNA, signaling the repair system to remove the U’s, creating an apyrimidinic site, which is a target for degradation. If left unrepaired, such U residues result in a GC to AT change in the DNA. APOBEC proteins are packaged into HIV particles, resulting in hypermutation and degradation of the HIV DNA.

5. The CD4 binding site is in a small cleft of the HIV surface glycoprotein, gp120, and is not accessible to antibodies. Subsequent to CD4 binding, gp120 undergoes a conformational change that exposes the coreceptor binding site. The coreceptor binding site is therefore not normally exposed for recognition by antibodies except during the brief period while the virus is bound to CD4 on a target cell and prior to coreceptor binding. This entry pathway minimizes the possibility of antibodies blocking viral entry into cells.

6. Tat binds to a region of HIV RNA transcript called the Tat activation region (TAR). Binding to this stem loop region recruits cellular kinases that phosphorylate RNA polymerase, increasing its processivity and allowing rapid elongation of transcripts.

7. Vpu degrades intracellular forms of the host CD4 protein, allowing the viral gp120 to migrate to the cell surface and become part of the maturing viral particles.

8. The evolution of gp120 towards higher affinity for CCR5 or binding to CXCR4 indicate that the availability of CCR5 or the ability of gp120 to bind it and enter cells is a rate-limiting step in viral replication. Viruses that can more efficiently bind to CCR5 or use CXCR4 instead for entry into cells are selected because they replicate better and are therefore present at higher levels in an infected person.

9. The answer to this is not known for certain, but a reasonable hypothesis is that if both phenotypes can not be optimized in the same protein, then Nef mediated MHC-I down regulation is selected early following infection since evasion of the CD8+ T cell response is needed in a healthy host that it is activity is not selected for later when the immune system is less active due to the loss of CD4+ helper T cells. CD4 down regulation conversely, is selected later it is needed. CD4 down regulation is less needed and greater CD4 down regulation leads to higher viral titers.

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Chapter 21

1. For hepadnaviruses, the largest mRNA (C-mRNA) transcribed from the episomal viral chromosome encodes the core protein and a core protein-polymerase precursor protein. In retroviruses gag is mostly expressed as a single protein, but at times a gag:prot:pol fusion protein is produced by suppression of the termination signal following gag and ribosomal skipping to a different downstream reading frame.

2. Following entry into the cell and partial uncoating of an hepadnavirus, the partial positive-sense genomic strand is completed by virion reverse transcriptase. Following expression of viral proteins and genome replication, encapsidation of C-mRNA occurs followed by completion of the negative-sense cDNA copy of the RNA by encapsidated reverse transcriptase. Partial replication of the positive-sense DNA then occurs using the negative strand as a template.

3. The best current model for carcinogenesis by a hepatitis virus postulates that integration of viral DNA into an infected cell genome could result in interference with a cellular growth control pathway by a viral protein. The X protein of hepatitis C virus has been suggested as a possible suspect due to its regulatory and transcriptional stimulatory activities. Also, continued destruction of liver tissue due to chronic infection which leads to abnormal growth constitutes another model for oncogenesis.
4. Retroviruses seem to have emerged in the early biological world based on the fact that their sequences are found ubiquitously throughout the plant, animal, and bacterial kingdoms. The relationship between the hepadnaviruses and retroviruses in terms of replication strategy and the presence of reverse transcriptase is evidence that the former most likely derived from the latter.

Part IV

1. a.) Endonucleolytic cleavage of nuclear mRNA caps is a feature of influenza virus infection. 
   b.) RNAse L activation requires IFN induction of antiviral state. c.) Phosphorylation of eIF-2 requires IFN induction of the antiviral state. d.) Proteolytic cleavage of eIF-4 is a result of poliovirus infection.
2. a.) Vegetative replication of the viral genome is the major dividing line between early and late transcription phases. 
   b.) capsid protein: late; DNA pol: early; Inhibition of host transcription: (usually) early; a lytic enzyme: expected to be late.
3. It is a bunyavirus (a hantavirus). So genome is (–) - strand, has three (3) genomic segments, has a virion associated polymerase, and replicates in the cytoplasm.
4. a. Virus can enter, but gene expression does not occur. 
   b. Since many students take the bus and eat at Louie’s, and the disease is widespread one might start by looking at the biochemists. Do the students work in the same laboratory? Is it something in their work that confers the resistance? 
   c. Explore the common thread of the major. What common laboratory experiences do these resistant students have?
5. a. This is diagnostic of an endocytotic pathway.
   b. This suggests fusion as a route of entry.
6. a. (See table 15.3) Two membrane glycoproteins, cytoplasmic cap stealing, the S RNA genome may be ambisense; virus would mature in the cytoplasm. 
   b. Probably an aerosol since an insect vector would require a source of the virus.
   c. Arboviruses are not spread as aerosols.
7. a. Cro is expressed in this cell in the presence of IPTG. Therefore lysogeny cannot be established or maintained. In both cases the lytic response will result. 
   b. In this case, the cell produces the lambda repressor in the presence of IPTG. Therefore, lysogeny will be established and maintained. The infected cell will enter the lysogenic cycle and no virus replication at all will be seen since repressor level is high. Nothing will happen to the lambda lysogen since the c1 repressor will by very high and no virus can be released.
8. a.) Poliovirus—translation of the entire open reading frame and proteolytic processing of a large precursor polyprotein. b.) VSV—transcriptional generation of mono-cistronic mRNAs from a single virion template RNA.
9. Virus 1 is a Bunyavirus, thus LaCrosse encephalitis; Virus 2 is a type A myxovirus, influenza A, Virus 3 is VSV (a mononegavirus).
10. Polio and VSV will grow in eunucleated cells. Both HIV and influenza require the cell nucleus.
11. a. Acyclovir works against HSV because it is efficiently phosphorylated by HSV thymidine kinase and incorporated into viral DNA molecules being synthesized with HSV DNA pol. 
   b. The drug cannot eliminate latent virus because it is not actively replicating its genome; therefore, the target for the drug does not exist.
12. a. A protease inhibitor blocks virion maturation and, thus, production of infectious virus. 
   b. An integrase inhibitor would block the virus’ ability to initiate an infection in a new cell.