BASIC VIROLOGY, Third Edition by Wagner, Hewlett, Bloom and Camerini

Answers to Questions by Chapter and Part

Chapter 1

1. a. The genomes of viruses encode proteins which are essential for their structural components and replication of their genomes.

   b. Viruses do not produce energy through metabolism of small oxidizable molecules.

2. Viral genomes do not encode genes for ribosomal proteins and don't demonstrate evidence that they ever did. In addition, genes for enzymes involved in energy metabolism are completely absent from viral genomes.

3. Viroids and prions

4. Like other parasites, viruses need their hosts for replication. A deadly virus may destroy its host before it can be spread, thus losing the opportunity to survive.

5. Viruses can be a selective pressure for adaptation in cells and organisms that make them resistant to infection by viruses. Moreover, viruses can be vectors of gene transfer from cell to cell or organism to organism.

6. Size is not a universal distinguishing feature of viruses since mimivirus is as big as some bacteria and both viroids and prions are smaller than viruses. A better, but not perfect, definition of viuses is that they are self replicating, obligate parasites that contain their own protein-encoding genes, but depend on host cells for energy and other processes.

Chapter 2

1. The ability to replicate and maintain genetic information as RNA via and RNA-dependent RNA polymerase.

2. A vector serves to transmit a virus from one susceptible host to another. It may or may not allow viral replication or suffer pathogenic effects.

3. The eclipse period when virus particles are not present and the viral genome is being replicated exponentially.

4. Translation of mRNA.

5.

Feature	Cells	Viruses
The genetic information may be RNA rather than DNA.	No	Yes
New individuals arise by binary fission of the parents.	Yes	No

Proteins are translated from messenger RNAs.	Yes	Yes
New individuals assemble by spontaneous association of subunit structures.	No	Yes

6. Adaptation.

Chapter 3

1. Infection of a human with rabies virus in essentially a dead end for the virus since an infected human rarely spreads the virus to another human or animal.

2. All infect human liver cells (hepatocytes) and cause inflammation and destruction of the liver.

3. a. Western equine encephalitis virus, La Crosse encephalitis virus, Yellow fever virus, Dengue fever virus, Myxoma virus, Tomato spotted wilt virus, and plant rhabdoviruses

   b. Measles virus, varicella-zoster, influenza virus, small pox (variola) virus, rhinovirus, coronavirus, rubella, and adenovirus

   c. HIV, hepatitis B virus, hepatitis C virus, hepatitis delta, and HTLV

   d. Rabies virus, poliovirus, Western equine encephalitis virus, La Crosse encephalitis virus, measles virus, herpes simplex virus, varicella zoster virus, HIV and rubella

4. The team of scientists would be a source of new respiratory viruses for the naïve islanders. A local epidemic might occur, but then as the viruses passed through the population, resistance would build and infections would decline to a low level. This would only occur if the island's residents lacked effective immunity to the new respiratory viruses introduced by the arrival of the scientists.

5. Mutants 1 and 2 are complementary strains of virus. Each can compensate for the defective gene in the other. No recombination occurs between their genomes.

Chapter 4

1. Measles virus infects neurons, but viral maturation is blocked. Viral antigens are present, however, on the cell surface. Neurons bearing viral antigens are destroyed by the immune system causing SSPE.

2. All cause skin lesions.

3. a. Long incubation period that facilitates viral spread.
   b. Induces a change in behavior in the host, which favors viral spread.

4. **Acute**: The viral infection and disease follow a relatively short time course that is followed by death or viral clearance and recovery. **Persistent**: The virus remains in the individual after the acute phase as a chronic or persistent infection. The virus is not cleared because of deficiency in the immune response or because the infection is maintained where immunity is not complete. There may be chronic low-level replication or latency.

5. Herpes virus encephalitis is fatal unless treated early. In contrast, cases of encephalitis caused by arboviruses, are usually resolved with viral clearance and recovery of the host, unless the host is immunocompromised.

Part I

1. All viral infections start with the virus initiating a productive infection somewhere in the body. During this time, the virus must multiply to a level high enough to spread to the target tissue. Following this the patterns of infection diverge:

   acute infection - the virus replicates to high level in the target tissue (and perhaps elsewhere in the host). As this proceeds, host responses are elicited and virus is either cleared or the host succumbs.

   persistent infection - after a period of acute infection, the virus is able to replicate at reduced levels in some tissue or organ system in the host for a long period.

   latent infection - in a latent infection (i.e. by herpes viruses), the viral genome is maintained in the latently infected tissue, but only a limited number of viral genes are expressed (if any). During latent infection there is no evidence of infectious virus at all, but this may be interspersed with periods of reactivation where active virus replication occurs in some tissue.

2. a. Modes of transmission: HAV (fecal-oral),HBV (blood), HCV (blood), HDV (blood), HEV (fecal–oral).
   b. The liver filters the blood and is therefore accessible to any virus that circulates in the blood.

3. Virus 1--human host range, tropism cannot be determined.
   Virus 2--host range covers at least humans and mice, virus is tropic for muscle tissue.
   Virus 3--mouse host range, tropic for both muscle and nerve tissue.
   Virus 4--cannot determine either host range or tissue tropism.
   Virus 5--human host range, neurotropic.

Chapter 5

1. Organization of the simplest icosohedral virus capsid is demonstrated by desmodium yellow mottle virus. The icosahedron is a regular solid shell with 12 vertices arranged in a simple pattern at centers of five-fold axes of symmetry. Each edge of the solid contains a two-fold axis of symmetry and the center of each of the 20 faces of the solid defines a three-fold axis of symmetry. Since there are 12 vertices and 20 faces, there are

180 capsomers making up the entire structure. (**Note**: this doesn't match fig 5.3b, which shows 12 capsomers/face)

2. Viruses with negative-sense RNA genomes have an RNA-dependent RNA polymerase activity found as part of the virion. Since host cells do not have need for such a polymerase, the virus must encode this enzyme. The first step in expression of the viral genome is the synthesis of positive-sense genomic-length RNA, a replicative intermediate that is the template for the generation of negative-sense genomic strands. The positive sense RNA can also serve as an mRNA for the synthesis of viral proteins.

3. Three properties of a virus that are used as criteria for classification include:

    a. type of genome (ss DNA, ds DNA, ss RNA, or ds RNA; circular or linear; single piece or multipartite, positive or negative sense)

    b. type of capsid (dimensions and symmetry)

    c. other components of the virion (envelope, viral enzymes necessary for replication)

4. The Baltimore scheme of virus classification is based on the manner in which a virus produces messenger RNA (mRNA) during an infection.

5. a. Examples of structural proteins include capsid proteins, fiber proteins and envelope glycoproteins

    b. Examples of proteins that have enzymatic activity and are included as part of the virion structure include: RNA-dependent RNA polymerases carried in negative-sense RNA viruses reverse transcriptase/RNAse H, protease, and integrase carried in retroviruses

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Chapter 6

1. a. Receptor mediated fusion with the plasma membrane; viral glycoproteins interact with cellular receptors and induce fusion of the viral lipid envelope with the cell's outer membrane. After fusion, the nucleocapsid is release into the cytoplasm. b. Receptor mediated endocytosis: viral attachment induces formation of an endocytic vesicle. The acidic pH, or other property of the endocytic vesicle leads to a modification of the viral envelope inducing its fusion with the membrane vesicle. After fusion, the nucleocapsid is released into the cytoplasm.

2. Nonenveloped viruses enter cells by translocation in which interaction of the virus with the cell surface induces endocytosis. In the case of rhinovirus, the acidic environment of the endocytic vesicle causes changes in the capsid proteins that allow release of the viral genome from the vesicle into the cytoplasm. The rhinovirus life cycle can then continue in the cytoplasm.

3. The entry of plant viruses requires a break in the cell wall. Where such breaks occur, the virus can get in contact with the cell membrane and enter the cell apparently without interaction with specific receptors.

4. The initial attachment of T4 bacteriophage is between its fibers and the ompC lipopolysaccharide receptor on the bacterial cell wall. Next, binding of the protein pins on the

base plate of T4 to the cell wall leads to contraction of the T4 tail sheath. This leads to the insertion of the tail tube through the cell wall. The pilot protein allows the attached phage DNA to enter the cell.

5. The capsids of helical viruses must assemble around the genome. Capsomers self-assemble to form disks. These disks initially interact with specific sequences in the genome called *pac*. Interactions with the genome convert the disk into a “lock washer” conformation. Subsequent capsomers assemble into a growing helical array to form the complete capsid.

For icosahedral capsids, the individual capsomer subunits pre-assemble into a procapsid, sometimes around scaffolding proteins. The scaffolding proteins if used are removed (recycled or cleaved) and the genome is introduced into the capsid. The procapsid may be modified by proteolytic cleavage to corm the mature capsid.

6. The lipid bilayer of the viral envelope is derived from the infected cell. Viral glycoproteins are synthesized in the endoplasmic reticulum and glycosylated in the Golgi. Viral glycoproteins are transported to the cell membrane where they are inserted. Simultaneously viral capsids assemble and associate with virus-modified cellular membranes. The virus then buds through the membrane, taking a coating of the cellular membrane as its lipid bilayer.

Chapter 7

1. a. False. T cells circulate throughout the body and can migrate into tissues by squeezing between cellular junctions.

   b. True. Proteins expressed on the surface of virions are more exposed for immune detection by B cells than proteins inside the particle.

   c. False. Proteins or peptides must be digested to small oligopeptides (~ 8 -18 amino acids) and reassembled at the surface bound to histocompatibility antigens in order to provoke immunity.

2. Soluble antibodies are good anti-viral agents because they circulate in the blood where they can bind to viral antigens on the virion surface and cause neutralization, lysis or clearance of viral particles.

3. a. B cells secrete antibodies which are soluble proteins that combine with native antigenic determinants on viral proteins.

   b. Helper T cells (CD4+) mediate the maturation of B cells and cytotoxic T cells during an immune response to antigen. They recognize processed foreign antigen bound to MHC molecules.

   c. Cytotoxic T cells (CD8+) attack and destroy cells expressing processed foreign antigen bound to MHC molecules, such as virus-infected cells.

4. B cell epitopes, are usually composed of hydrophilic amino acids. A conformational epitope is formed by amino acids brought close together by folding or conformation of the protein. An epitope composed of a specific linear sequence of amino acids is termed a sequential epitope. These determinants are present when a protein is either in its native or denatured

state and are therefore conformation-independent. In contrast, T cell epitopes are short peptides derived from foreign proteins by cellular processing and binding to MHC molecules.

5. As soon as a virus initiates an infection, innate immune reactions occur at its point of entry. The local immune response, mediated in part by toll-like receptors, defensins and interferon, is the first line of defense and limits the spread of the virus. Viral antigens are processed in infected and antigen presenting cells by partial proteolytic digestion. The processed viral peptides are then presented on the cell bound to major histocompatibility complex (MHC) proteins. T cells with specific receptors that recognize the viral antigen/MHC protein complex will be activated by the specific interaction. Helper T cells are stimulated to secrete cytokines that aid the maturation and proliferation of B cells and cytotoxic T cells (CTL). B cells secrete antibodies that bind viral particles and neutralize, lyse or clear them. CTL encounter infected cells bearing viral antigen and lyse them.

6. The product of gene C, the capsid protein, would most likely generate a neutralizing antibody as it would be exposed for antibody recognition on the virion surface.

7. An ideal vaccine would include material that is immunogenic, that will generate life-long protective immunity to a pathogen, and that is not pathogenic or toxic.

Chapter 8

1. Bacterial restriction enzymes cleave bacteriophage DNA at specific unmodified DNA sequences usually from 4 to 8 nucleotides long. These sequences are modified by methylation in the bacterial DNA and are therefore protected from cleavage.

2. a. Translation.
   b. 2', 5'-oligo A activates RNase L which degrades mRNA.
   c. PKR phosphorylates and inactivates eIF2, which blocks protein synthesis.
   d. It will not produce IFN because the synthesis of proteins is inhibited.

3.

Activity	Uninfected Normal Cells	Uninfected IFN-Treated cells	Virus Infected Normal Cells	Virus Infected IFN-Treated Cells
mRNA for IFN found in the cells	-	-	+	-
mRNA for 2', 5'-oligoA synthase found in cells	-	+	-	-
2', 5'-oligo A found in cells	-	-	-	+
Inactive protein kinase R found in cells	-	+	-	-
Receptor for IFN found on the surface of the cells	+	+	+	+

Part II

1. Virus 1. Positive strand RNA virus, since it has no polymerase it cannot be a retrovirus. Thus, it must start infection with translation of genome.
   Virus 2 Negative strand RNA virus, the virion makes RNA from an RNA template
   Virus 3 Must be a retrovirus since it has a positive strand RNA genome and reverse transcriptase (makes DNA from RNA template)

2. Viral infection of normal cell will result in normal protein synthesis
- Viral infection of IFN treated cell will show a significant decrease in protein synthesis.
- Insertion of dsRNA into a normal cell will decrease protein synthesis.
- Insertion of dsRNA into an IFN treated cell will result in a reduction of protein synthesis.

3. The viral capsomer protein would be a logical candidate if the virus is not enveloped. In this case, the capsomer protein would be on the virion surface and could therefore be a target of neutralizing antibodies, unlike the internal RNA-binding virion protein or viral RNA polymerase.

4. a. Since the virus is enveloped, treatment with lipid solvent would destroy viral infectivity.
b. It is a negative strand RNA virus based on RNase and alkaline sensitivity and the fact that the genome can't be translated *in vitro*.
c. Since the virus will not replicate in simian AGMK cells, but does so in human HeLa cells, it must be a human virus.

5. There will be a virion-associated RNA-dependent RNA polymerase that will transcribe mRNA from the genomic RNA template during the first step of the viral life cycle after entry.

6. Neuraminidase will inhibit attachment of the virus and uncoating.
- NH_4Cl would alter the virus ability to be uncoated and released from the endocytotic vesicle; it would have no effect on attachment.
- actinomycin D will have no effect on either process.

7. Viral mRNA of poliovirus is the genomic RNA. This is produced through the two replicative intermediates, RI-1 and RI-2. RI-1 functions to generate antisense templates for genomic RNA and mRNA, while RI-2 is the source of new virion genomic RNA and mRNA. Both processes utilize a viral-encoded polymerase that is expressed following translation of the virion RNA.
- Viral mRNAs of VSV are produced by transcription of the (-) sense genomic RNA template by virion polymerase. Under conditions of high levels of N-protein, the (+) strand produced from the RI-1 complex is full length instead of mRNA length.
- Viral RNA of RSV is expressed from proviral cDNA integrated into the host chromosomal DNA. It is expressed by cellular RNA polymerase II.

8. Degradation of mRNA is a result of the IFN response, immunity will have no such effect.
- MHC-I presentation of antigenic fragments is a function of the immune response and MHC-I expression is increased in response to IFN.
- The death of a virus-infected cell can be a result of both responses.
- Inhibition of the translation of capped mRNA is a consequence of the IFN response.

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 6×10^9 latex beads in two ml. Therefore, there are 3×10^9 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 **$3 \times 3 \times 10^9$ = $\underline{9 \times 10^9}$ 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→ 10-fold.
   Second dilution: 10 ul to 1 ml → 100-fold dilution,
   Total dilution: 10 x1 00 = 1000.

(29 + 25) / 2 = 27 27 pfu in 100 ul = 270 pfu/ml, $270 \times 1000 = 2.7 \times 10^5$ pfu/ml in original stock.

2. 5×10^8 cells / ml and 10^9 phages → MOI = 2

   $Po = (2^0\ e^{-2}) / 0\ ! = 0.135$
   P1 or more = 1 – Po = 1 - 0.135 = 0.865
   200 x 0.865 = 173.

3.
A No
B Yes
C Yes
D No

4. 400 pfu in 0.1 ml of a 10^6 dilution.

   a) (400 / 0.1) x 10^6 = 4 x 10^9

   b) 10 ml x 4 x 10^6 cells / ml = 4x10^7

      4 x 10^7 cells, MOI = 10 → 4 x 10^8 pfu

      4 x 10^8 pfu / 4 x 10^9 pfu / ml = 0.1 ml.

5.
   a) (200 pfu / 0.1 ml) x 10^5 dilution = 2 x 10^8 pfu/ml.
   b) 0.1 ml x 2 x 10^8 pfu / ml = 2 x 10^7 pfu
   - 10 ml / 10^5 cells / ml = 10^6 cells
   - MOI = (2 x 10^7 pfu) / 10^6 cells = 20 pfu/cell.

6.

MOI	0 PFU	1 PFU	≥2 PFU
0.01	0.99	0.0099	0.0001
0.1	0.90	0.09	0.01
1	0.37	0.37	0.26
10	$5x10^{-5}$	$5x10^{-4}$	0.999

7.
   a. Stock #1 has the highest; Stock #2 the lowest
   b. 1/16

8. Po = e^{-7} = 0.001

   P1 or more = 1-Po = 1-e^{-7} = 0.999 or 99.9%

9. MOI = (3 x 10^6 particles)/(3 x 10^5 cells)
   MOI = 10.

Chapter 11

1. To solve this problem, divide the radioactivity by the molecular weight for each protein.

   For protein E: **29,348 ÷ 5,280 = 5.6**
   For protein K: **101,185 ÷ 18,795 = 5.4**

For protein W: **122,674 ÷ 10,776 =** **11.4**

Therefore, the ratio of the proteins **E:K:W** is **1:1:2**.

2. a. Isolate C as it has identically sized capsid proteins.

   b. Isolate A has the same number of capsid proteins with sizes very close to those of PV1.

   c. Isolate B has a total of six proteins of fairly high molecular weight. Use this information to begin your search.

3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is used to fractionate proteins and estimate their size. When exposed to the detergent, SDS, proteins are denatured and given a net negative charge with the charge:mass ratio for all proteins being fairly constant. The proteins can then be fractionated by size in the polyacrylamide gel based on migration rates in an electric field.

4. You would need to know the total protein in your sample and the total number of virus particles in your sample.

5. The two bands are of equal intensity and migrate at 30,000 daltons and 60,000 daltons. Therefore, you could conclude that the molar ratios of the two proteins represented by the bands would be **2:1 (30,000:60,000)**. In order for the 30,000 dalton band to be as intense as the 60,000 dalton band, there would have to be twice as many copies of that protein present.

Chapter 12

1. Since HSV-1 enters the cell by receptor mediated cell fusion, the label will be observed on the cell membrane.

2. a, b, e.

3. Radiolabeled amino acid can be used to determine the pattern of viral protein synthesis by giving pulses of amino acid at different times post infection and carrying out electrophoresis and autoradiography. For instance, in the case of poliovirus, administering 2-hours pulses with ^{35}S-methionin at different times post-infection, results in different labeled proteins depending on the time of the administration of the pulse.

4. A monoclonal antibody (or a specific polyclonal) can be used to follow the pattern of a viral protein synthesis during productive infection. For this, proteins are extracted from infected cultures at different times post infection and the level of the protein can be evaluated by Western blots. The same analysis may show changes in the relative molecular weight of the proteins. This will suggest modifications of the protein during the course of the infection. Antibodies can also be used to purify the protein for further analysis. The antibody can also be used to determine the location of the protein in virally infected cells by immunohistochemical staining of infected cells or sectioned tissues.

Chapter 13

1. The AUG would code for the initial methionine residue and then the following **2097** bases would code for **699** amino acids. Therefore the maximum number of amino acids that the final protein product could contain would be **700**.

2. Deletion of the fourth A would cause a shift in the reading frame after the deletion.

   AUG...(300 bases)...CGC AAU CAU GCC CUA CCA **UGA** AUA AUA CCU AAg guaaaug...

   This would generate a premature stop codon (bold) and a shorter protein would be produced. The original UAA stop codon would be abolished.

3.

Feature	Eukaryotic mRNA	Prokaryotic mRNA
The small ribosomal subunit is correctly oriented to begin translation by association with the Shine-Dalgarno sequence	No	Yes
Open reading frames generally begin with an AUG codon	Yes	Yes
The 5' end of the mRNA has a methylated cap structure covalently attached after transcription	Yes	No
During protein synthesis, an open reading frame can be translated by more than one ribosome, forming a polyribosome	No	Yes
Termination of transcription may occur at a site characterized by the formation of a GC-rich stem loop structure just upstream from a U-rich sequence	No	Yes

4. a. True

   b. True

   c. True

5. Polycistronic translation (b) is characteristic of prokaryotes not eukaryotes.

6. In the rough endoplasmic reticulum (RER) since these proteins would need modification in the ER and Golgi apparatus.

7. Assuming that the methionine encoded by the initial AUG is included, the minimum size of a viral mRNA encoding a structural protein of 1100 amino acids would be 3303 nucleotides. Each amino acid codon is composed of three nucleotides and there would also be a three-nucleotide stop codon.

Part III

1. a. The drug lacks any homologue to the 3'-OH of a deoxyribose portion of a deoxynucleoside triphosphate. This means it will terminate chain growth when incorporated into a DNA replication fork.

b. The drug must be phosphorylated by the HSV thymidine kinase to a monophosphate, and then phoshorylated to a triphosphate by cellular enzymes.

2. a. There are two ways to do this.

Both require you to calculate the total dilution of 10^8.

Since the OD of stock A is 0.1 or 10^{12} virions per ml (OD is always per ml), then the final dilution has 10^4 virions of which 200 are infectious. PFU/particle is thus, 0.02.

Stock B has an OD of 0.5 or 5×10^{12} particles per ml. Then the final dilution is 5×10^4 virions of which 50 are infectious. PFU/particle is thus, 0.001.

You can also do this by just calculating that stock A has 2×10^{10} PFU while stock B has 5×10^9 PFU.

b. If you assume that both stocks are grown to the same cell density, then AGMK cells produce more particles. While I gave credit for this, you might note that you really have not been told that, and thus a better answer is that you really can't be sure.

c. Here, again if you assume that both stocks are prepared from equivalent amounts of cells then HeLa cells are better, but, again, you really don't know for sure. I gave credit for either.

3. First, a higher S value means that all things being equal, a molecule will sediment faster in a centrifugal field and migrate more slowly in a viscous medium in a potential field. The frictional coefficient of linear DNA molecules in a sucrose gradient is not particularly sensitive to size. THIS IS NOT THE CASE FOR MIGRATION THRU A GEL. Therefore, linear molecules will separate by size alone. Same for circular.

Thus, T4 faster than T7.

PhiX174 RF DNA faster than pBR322.

Linear pBR322 (cut at the one EcoR-I site) will sediment more slowly than the more compact circular form (especially if it is supercoiled)

Finally, T7 DNA is much bigger than linear pBR322 DNA, so will go faster in the gradient.

Chapter 14

1. The virion attaches to the host cell receptor which leads to the formation of a clathrin coated pit into which the particle is engulfed. The acidic environment within the endocytotic vesicle (within the cytoplasm) induces a conformational change in the capsid proteins leading to insertion of the RNA genome into the cytoplasm through the vesicle's membrane.

2. a. The smaller proteins of poliovirus are cleaved from the precursor polyprotein by the two proteases 2A and 3C. Autocleavage into P1, P2, and P3 occurs by the activity of these internal proteases. Most of the subsequent proteolytic steps are accomplished by the protease 3C, either by itself or in association with the protein 3D.

   b. The cell ribosomes initiate translation of the message through a cap-independent mechanism involving recognition of an internal ribosome entry site (IRES).

3.

Characteristic	Present or Absent for FMDV
5' methylated cap	absent
Subgenomic RNAs	absent
3' polyadenylation	present
Single-stranded positive-sense genome	present
Expression of genome as a polyprotein	present

4. a. If VPg was left out of the mixture and it is required for RNA synthesis, then there would be no synthesis of the negative-sense replicative intermediate using the positive-sense genomic strand as template.

   b. If VPg acts as an endonuclease and endonucleolytic activity was inhibited in your reaction, then VPg would most likely prime the RNA synthesis but would not self-cleave or process the RNA fully. Therefore, the replication products (negative-sense and positive-sense strands) would be different than usual.

5. RI-1 generates RNA complementary to the virion genomic RNA. The positive-sense genomic strand is used as a template to synthesize the negative-sense replicative intermediate. This negative-sense strand serves as the template for new virion genomic RNA in RI-2. Please see Figure 15.1 on page 251.

6. a. True. The repeating adenines are part of the genome.

   b. False. It is approximately 7500 bases long.

   c. False. VPg is bound to the 5' end of the genome prior to packaging.

   d. False. The single precursor protein is cleaved by viral enzymes, both internal and cleaved.

7. A subgenomic 26 S RNA is generated by transcription initiated at an internal site for the viral replicase and is translated into structural proteins. The open reading frame (ORF) in the 26 S RNA is cryptic in the 49 S positive-sense genomic RNA.

Chapter 15

1. Viral proteins mediate the synthesis of full-length (+) RNA and then full-length (-) RNA.

2. When purified genomes of (-) RNA viruses are introduced into cells, there is no infection because virus transcriptase is not present.

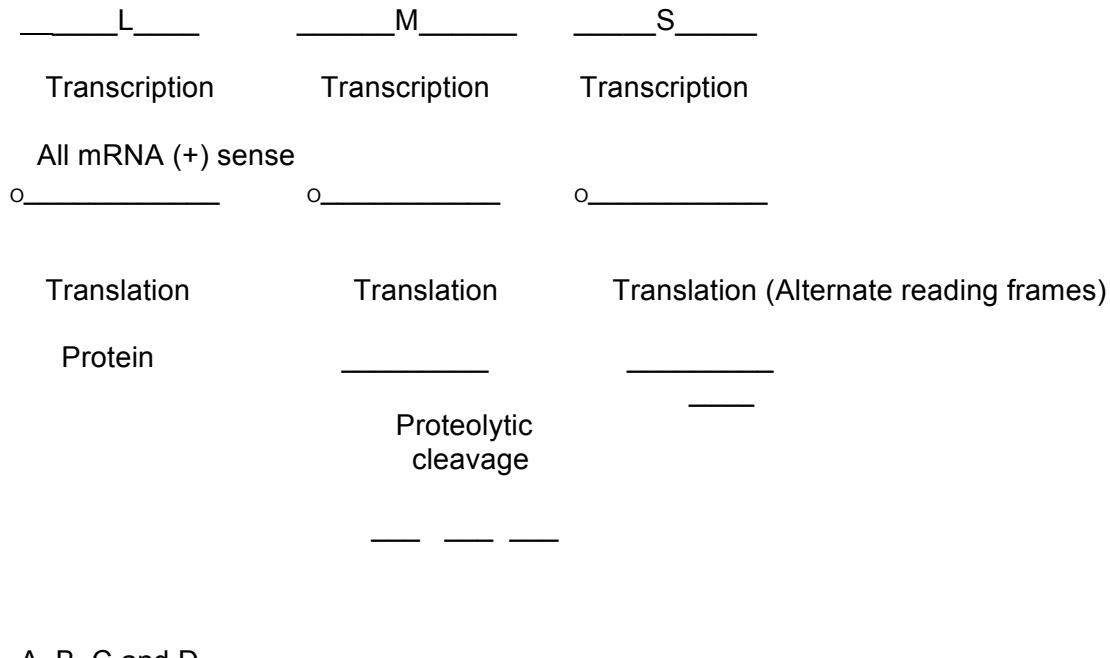
3. The virus genome of rhabdoviridae is initially transcribed to mRNA. Transcription begins at the 3' end with RNA capping and proceeds until it encounters an intrinsic pause. The enzyme pauses and adds a number of "A" residues and the mRNA is released. The transcriptase then either dissociates the template or continues to synthesize the next transcript. At the end of this gene, the same process occurs.

When the level of N protein increase, it associates with nascent positive-sense RNA strands blocking the polyadenylation and cleavage of individual mRNAs. This allows the positive-sense strand to become a full-length positive sense strand complementary to the viral genome that serves as a template for negative-sense RNA synthesis.

4.

a. Sin Nombre is a Bunyavirus (Hantavirus).
b. Rodents.
c. The vectors are rodents instead of insects. In addition, the virus causes respiratory rather than renal symptoms.

5.



6.

a) A, B, C and D.
b) F.

c) Membrane glycoproteins.
d) Antigenic shift.

7. 1. The vector is a rodent instead of an insect. 2. It is transmitted by aerosols from fecal pellets.

8. a. Cap snatching and splicing.
   b. The physical presence of the nucleus is required for splicing.

9. a. Virus 1=H1N1, Virus 2=H2N3→Virus 3=H2N1.

   b. Because the genome fragment corresponding to M1 and M2 are of the same size in 1 and 3 and different in 2. M2 is an ion channel that is involved in preventing the acidification of the endocytotic vesicle. Amantadine blocks M2 (p. 133).
   c. Reassorting of genome fragments when a cell is infected by two viruses.

10. They have double strand RNA. Transcription takes place inside a modified capsid. The capped (+) RNA is encapsidated (instead of the genomic RNA) and the second strand synthesis takes place inside the capsid.

11. The delta virus genome is transcribed and replicated by host RNA polymerase II.

12. Viroids spread from plant to plant through mechanical damage of plants. Thy do not encode any gene products and the replication of the genome is carried out by the plant polymerase in the cell nucleus. Large multimeric structures are formed and monomers are generated by self-cleavage.

13. A prion is considered a self-replicating entity because the presence of a prion protein can induce its normal counterpart to adopt the prion conformation.

Chapter 16

1. a) Structure 1 and 4 would not be possible; structures 2, e, and 5 would be possible. Adenovirus replication includes all three of these as possible, since they have the terminal genome-linked primer protein.

Possible for Adenovirus
No
Yes
Yes
No
Yes

b)

Modification	**Type I**	**Type II**
Control (no treatment)	+	+
Removal of the terminal protein from both 5' ends of the DNA	+	+
Removal of the terminal complementary sequences from one	+	-

Prevention of maturation of terminal protein from 80-kd to 55-kd	+	+

2.

Chemical	Effect of Cell	Reason for Ad2 inhibition
NH_4Cl	Blocks acidification of secondary lysosomes and	Prevents uncoating
Vinblastine	Disrupts the microtubular cell cytoskeleton	Inhibits migration of DNA to the nucleus
Emetine	Inhibits protein synthesis	Inhibits viral protein synthesis

3. This is an example of a persistent infection.
4. The large T antigen of SV40 has the following functions:
    i. Activation of cellular DNA and RNA synthesis mediated by binding and inactivating the cellular growth control gene products Rb and p53
    ii. Blockage of apoptosis that normally occurs in cells where p53 in inactivated at inappropriate times in the cell cycle
    iii. Initiation of viral DNA replication by binding the SV40 ori
    iv. Shutting off early viral transcription by binding to the early promoter area
    v. Activation of transcription
5. Choice "a" is false. SV40 expresses two late transcripts encoding three capsid proteins.

Chapter 17

1. Viral protein ICP47 prevents the presentation of viral antigens on the cell surface by MHAI.
2. Because the HSV genome contains genes encoding the enzymes necessary for DNA replication, therefore host gene transcription is not necessary.
3. All of the methods would work except (a), which would work for adenovirus-infected cells.
4. a.) Alpha-TIF is the virion associated transcriptional activator. It enhances immediate-early transcription through cellular Oct-1 and CTF binding to TATGARAT sites. b.) At earlier times a ts-mutation will have no effect. At late times, it will slow secondary infections (no effect will be observed is the infection is done at high MOI).

5. It is required for early infection, and synthesized late in infection to be stored in the virion.
6. Viral thymidine kinase and viral DNA polymerase.
7.

HSV-1 Feature	Epithelial Cells	Sensory Ganglia Cells
Viral DNA in the nucleus	–	+
Expression of alpha class of viral transcripts.	–	–
Expression of LAT1 RNA	–	+
Production of viral capsid proteins	–	–

Chapter 18

1. a. The cI repressor protein.

b. Induction of the lytic pathway will occur. Production of progeny phage and lysis of the cell will follow.

2. a. In the 10-ml culture of cells, there is a total of 10 ml x 3 x 10^8 cells/ml or 3 x 10^9 cells. To start the infection, you use 0.3 ml of a stock containing 1 x 10^{10} PFU/ml. Therefore, the total input virus is 0.3 ml x 1 x 10^{10} PFU/ml or 3 x 10^9 PFU. Therefore the multiplicity of infection is 3 x 10^9 PFU/ 3 x 10^9 cells = 1.

b. First, you need to calculate the probability of a cell being uninfected, and subsequently, the probability of a cell being infected at this MOI. Using the equation for the Poisson distribution, $P_0 = e^{-m}$ (where P_0 = the probability that a cell will be uninfected, or infected with 0 virus particles, and m = the MOI), your answer is 0.37. Therefore, the probability that a cell will be infected is 0.63. To continue, you multiply the total number of cells times the probability for a cell to be infected times the number of virus particles produced per infected cell or 3 x 10^9 cells x 0.63 x 200 particles/infected cell = 3.78 x 10^{11} particles at the end of one cycle of virus growth.

c.

Time of Addition of Nalidixic Acid	Phage DNA Synthesis	Immediate-Early Gene Expression	Yield of Progeny per Cell
Control (no inhibitor)	+	+	+
0 minutes	–	+	–
5 minutes	–	+	–
18 minutes	+	+	+

3.

Treatment	Immediate-Early mRNA Expression
Rifampicin (an inhibitor of host RNA polymerase)	–
Nalidixic acid (an inhibitor of DNA replication)	+
A mutation that inactivates the phage DNA polymerase	+
A mutation that inactivates the phage lytic enzyme	+
Chloramphenicol (an inhibitor of protein synthesis)	+

4.

Phage Mutant	Immediate-Early	Delayed-Early	Quasi-Late	Late
Control (wild type)	+	+	+	+
A phage mutant that cannot catalyze ADP-ribosylation	+	–	–	–
A nonfunctional mutant of the phage DNA polymerase	+	+	+(reduced amount)	-
A nonfunctional mutant of the phage protein gp55	+	+	+	–
A nonfunctional mutant of the phage receptor-binding proteins on the tail fibers (infected under permissive conditions and then changed to non-permissive conditions)	+	+	+	+

5.

T7 Phage	Early (Class I)	Delayed Early (Class II)	Late (Class III)
Control (wild type)	+	+	+
T7 RNA polymerase mutant	+	–	–
T7 DNA polymerase mutant	+	+	–

6. a.

T7 Function	Class
Head capsid protein	III
RNA polymerase	I
DNA polymerase	II

b. This treatment will have little effect on the expression of bacteriophage T7 genes in the cell since the product of an immediate early gene, a protein kinase, inactivates the host polymerase. The delayed-early and late genes are transcribed by the viral RNA polymerase.

7.

Mutant	Lytic?	Lysogenic?
Wild type	Yes	Yes
N-minus	No	Yes
CII-minus	Yes	No
Deletion of the P_{RM} region	Yes	No
CIII-minus	Yes	No
Mutated O_R3 that fails to bind cro	No	Yes

Deletion of the cro gene	No	Yes

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:	
Pol	Stop: No reverse transcription.
Gag	Stop: No coat protein synthesis
Src	No effect on the replicative cycle, but cannot transform cell.

   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, but this 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 in since MHC down regulation is less needed and greater CD4 down regulation leads to higher viral titers.

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 should 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.

c. AZT would block viral reverse transcriptase, and thus block formation of cDNA.
d. A Rev inhibitor might (this is speculative) block the ability of the virus to maintain low level persistence following the initial acute phase of infection. This could lead to efficient viral clearance.

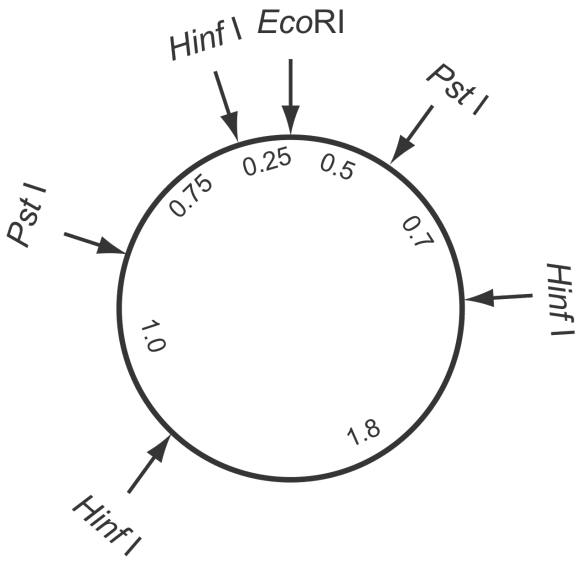
13. a. Features A, B, C, and E are consistent with Bunyaviridae. Feature D is unusual since they steal caps in the cytoplasm.
b. As noted, feature D.
c. The fact that the virus causes encephalitis is not typical of a hantavirus.
14. Poliovirus--monocistronic to polyprotein; Sindbis virus--monocistronic to polyprotein (26s); VSV monocistronic to single proteins; LaCrosse encephalitis- overlapping reading frames, monocistronic to polyprotein, monocistronic to single proteins.
15. a.) In a mixed infection poliovirus translation would predominate over adenovirus because of polio's inhibition of capped mRNA translation and its rapid replication cycle; b.) in the case of VSV and LaCrosse encephalitis, the former should predominate because LaCrosse virus must steal cellular mRNA caps as they are transported from the nucleus, and VSV blocks that transport. There is, however, the possibility that LaCrosse virus could steal VSV caps; c.) poliovirus will predominate in a mixed infection with influenza for the same reasons as discussed above; d.) herpesvirus will predominate in a mixed infection with adenovirus both because of its rapid replication cycle and because it inhibits splicing.
16. Procapsid assembly is diagnostic of icosahedral viruses, thus you would see it with T4 and poliovirus; VSV has helical capsid symmetry; proteolytic processing as the final step in capsid maturation would apply to poliovirus; budding of virus particles is a feature of VSV infection.
17. RSV requires the use of tyrosine tRNA as a primer; viral protein (VpG) primes poliovirus replication; RSV replication involves reverse transcription of ssRNA to dsDNA; both viruses yield (+) (mRNA) genomes.
18. See Table 19.1 for specific examples in each of these cases. Also remember that papovaviruses and adenoviruses interact with tumor suppressor genes to result in transformation of the host cell.

Chapter 22

1. For phage T7 the ds DNA is a linear molecule and, using restriction enzymes, can be mapped as such. In addition, the genetic arrangement of the T7 is co-linear with the ds DNA. A genetic map therefore reflects this same arrangement.
2. Complementation results when a host cell is infected with two different mutant viruses that each provides a function that the other is lacking. This results in growth of each mutant only when the other is present and replicating. Recombination, on the other hand, results in the physical exchange of DNA between the viral genomes, with the production of wild type virus as a consequence.
3. Let's use pUC19 as an example. First, obtain the SV40 fragment in question, using PstI as the restriction enzyme and gel electrophoresis to separate the pieces. Digest pUC19 with the same enzyme, opening the MCS. Insert and ligate the SV-40 fragment. Transform a host cell, select on ampicilin plates containing X-gal. White colonies growing under these conditions potentially contain the SV40 fragment inserted into the plasmid.
4. Treat a stock of phage T4 with an appropriate mutagen (e.g., 5-FU or 5-BrUdR), and grow on lawns of *E. coli* host cells at the permissive temperature (25˚C). A laborious method would be to identify plaques appearing at 25˚C and replica plate those at 39˚C (non-permissive). Characterizing these individually would be by brute force. A more elegant method would be to devise a screen that allows for identification of host attachment defects. For instance,

attachment and penetration can occur in the absence of cell metabolism. Selected ts phage can be added to host cells in the presence of cyanide at the non-permissive temperature. Cells are washed free of phage and cyanide is removed. Infection is then allow to proceed at the permissive temperature. Only those mutant in attachment will not have productive infections.

5.



Chapter 23

1. a. The LD_{50} of XC-50 is ~ 10^4 PFU. The LD_{50} of 17+ is ~ 1 PFU. It is attenuated 10^4 fold compared to strain 17+. XC-50 = ~8000 PFU; 17+ = ~2 PFU

   b) XC-50 is neurovirulent only at relatively high doses, and I attenuated in neurovirulence compared to 17+.

Chapter 24

1. Blocking the antiviral activity of the interferon induced protein kinase.
2. The following results were obtained by nucleotide BLAST search of the NCBI Chromosome database: Sequence #1 – human herpesvirus 6A; sequence #2 – bovine adeno-associated virus; sequence #3 – vaccinia virus; sequence #4 – influenza A Hong Kong (H2N2).
3. Set up a yeast two-hybrid system with all of the expressed sequences from H5N1 and avian cells. The interactome for these two sets can then be determined.

Chapter 25

1. a. The potential evolution of novel infectious strains of virus due to co-infection of the same individual .

b. Breakdown of public heath measures due to social and economic disruptions such as war of depression.
c. Concentration of people which share certain lifestyles.
d. The global economy.
e. Habitat disruptions.
f. Periodical disruptions in typical whether patterns.
g. Technology.
h. Invasive medical procedure.

2. a. Development of a vaccine is a preventive method. It must take into account the antigenicity of the viral proteins. For a significant reduction of the infection massive vaccinations are required. Therefore, the cost of producing and administering vaccines must also be considered.
   b. The development of an antiviral drug is a curative method. An antiviral is particularly interesting for viruses that can easily evade the immune response (by becoming latent, for instance). An important factor to consider is that toxic side effects that the antiviral can produce.
3. Two features of the virus that make them attractive to deliver a gene are: a) Their ability to enter cells and deliver nucleic acids, b) their tropism for tissues and cells. This tropism can be further engineer to make the tropism efficient and specific.
   The problems that virus present as vectors are: a) The possibility that an immune response is evoked, which can destroy the viral vector, b) the pathogenicity of the vector or its possible virus contaminants. If the pathogenicity of the virus has been eliminated, there is the risk of reversion.

Part V

1. One very high-tech solution would be to genetically engineer a mouse strain that has the capability for VZV replication. This would entail placing the necessary human genes into the mouse embryo during early development.
2. C57BL/6 likely resist mousepox virus infection due to an effective CD8+ CTL response following effective presentation of processed mousepox virus antigens on C57BL/6 MHC-I molecules. In contrast Balb/c mice, which express different MHC-I molecules, are likely susceptible to mousepox virus because they do not mount an effective CTL response due to poor or absent presentation of mousepox virus antigens on Balb/c MHC-I molecules. This hypothesis could be tested by transferring the MHC-I locus from C57BL/6 mice to Balb/c mice by cross breading the two strains and subsequent back-crossing with Balb/c mice with selection for C57BL/6 MHC-I alleles at every generation. Alternatively, C57BL/6 MHC-I alleles could be transferred to Balb/c blastocysts to create MHC-I transgenic mice.
3. It would be possible to infect test animals, sacrifice them at various times during the seven-day incubation period, and produce whole body thin sections of the animals. Once these have been constructed, the sections could then be probed with viral signals (nucleic acid or protein) to locate the viral replication sites and follow these through the animal during the course of the infection.
4. Mutagen A: A transition mutation, resulting in a CG to AT change. The result is a silent mutation, leaving the amino acid leucine unchanged. Mutagen B: A transition mutation, resulting in a CG to AT change. The result is a neutral mutation, changing the leucine to an isoleucine. Mutagen C: A transversion, resulting in a CG to GC change. The result is a nonsense mutation. Mutagen D: A deletion of a CG pair. The result is a frameshift mutation.

5. The following results were obtained by nucleotide BLAST search of the NCBI Chromosome database: Sequence #1 – human herpesvirus1; sequence #2– poliovrius; sequence #3 – influenza virus A (H9N2), SEGMENT 8; sequence #4 – simian immunodeficiency virus.
