

7.013 Spring 2005 Problem Set 7

FRIDAY May 6th, 2005

Question 1

Bovine spongiform encephalopathy (BSE, also called mad cow disease) and other similar diseases are caused by infectious particles called prions.

a) Prions are made of (circle one.)

Viruses bacteria DNA RNA **protein** lipid carbohydrate

b) True or false, if false correct the sentence:

T	F	Several new mutations arise in the brains of cows that become affected by BSE. Affected and unaffected cells are genetically identical.
T	F	In BSE, viral factors convert proteins in the brain from one form to another. Protein conformational changes occur without contribution from an external pathogen.
T	F	BSE cannot be transmitted from one cow to another. BSE can be transmitted between cows by injection or ingestion of affected CNS material.
T	F	Spongiform encephalopathies cannot be transmitted from one species to another. These diseases are not spread as easily between species as among animals of the same species, but at low frequency they can jump the species barrier.

In yeast, there have been found prions that behave similarly to those that cause BSE. In the case of one yeast prion, one protein conformation causes the cells to be white and another conformation causes them to be red. This confers to colonies being red or white. Assume that the protein state analogous to the infectious form of BSE causes the yeast to be red.

You grow these yeast and you notice that individual colonies are mostly of a single color, but small white sectors arise within red colonies, and small red sectors arise within white colonies.

c) What causes red sectors to arise within white colonies?

Some protein spontaneously converts to the prion form.

d) If you took yeast from a red sector and replated them what color colonies would form and why?

Red. Once some of the prion form has been generated, it will mostly be stably maintained in the population. So there will be red colonies with some occasional white sectors.

e) What causes white sectors to arise within red colonies?

**Some cell division events result in cells that did not inherit any of the prion form of the protein. .
The prion is inherited as a cytoplasmic factor.**

f) If you took yeast cells from a white sector and replated them, what color colonies would form and why?

**White. Once the prion form is gone, it will only be spontaneously regenerated at very low frequency.
This will cause occasional red sectors in white colonies.**

g) Heat shock proteins help prevent the proteins present in cells from becoming aggregated and then degraded when cells are exposed to high temperature. They do this by unfolding and then refolding these proteins.

You find that when you express high levels of these heat shock proteins in red yeast cells (from above) and replate the yeast, now they grow into white colonies. Provide a possible explanation for this.

These heat shock proteins unfold and refold the prion proteins, when they refold they do not take on the prion conformation so the colonies are white.

Question 2

AIDS is an immunodeficiency disease caused by infection by a retrovirus called HIV.

a) What is the central dogma?

DNA to RNA to protein

b) How do retroviruses violate this principle?

RNA genome, reverse transcribed into DNA

Viruses require host cells for propagation. Each virus has a specific host cell type that it infects.

c) How are viruses able to specifically infect only one cell type?

General, proteins on virus capsid recognize and bind to specific receptors on host cell. Specifically for Aids, proteins on the virus bind to the CD4protein on the cell membrane

d) What is the role of helper T_H cells in the immune system?

Control B cells, regulate clonal expansion and antibody production

e) What is the effect of loss of helper T_H cells?

Weakens immune response, leads to susceptibility to all viruses

Question 3

You have a new position at the Center for Disease Control. You have been assigned to characterize a new epidemic that has spread rapidly among workers at a chicken farm in California. All affected have high fever and a severe cough. A co-worker has given you a purified vial of the infectious material.

a) You add some of the infectious material to an agar plate containing all necessary nutrients, but nothing grows. Why is this?

The infectious material could be a virus and therefore must have host cells to replicate.

b) You then add infectious material to a human cell line and see some cells begin to round up and die.

i) What is the likely infectious material and why didn't it grow on the agar plate?

The infectious material is a virus and it didn't grown on the agar plate because virus need host cells to replicate.

ii) If the cells were put into a tube with the media they grew in and spun down, Where would you find the material that could infect new cells?

In the media/supernatant

In the pelleted cells

(iii) Why?

The virus that hasn't exited the cells isn't fully formed.

c) You isolate the genetic material if the virus and send a portion off for sequencing. Your sequence contains Uracil instead of Thymadine and it seems it is a single stranded genome.

i) How could you tell if this was a (+) or (-) sense RNA?

Transform only the RNA into human cells and see if protein can be made directly from the genome.

ii) You determined it was a (-) ssRNA. List the steps this virus must go through to replicate.

Question 4

I. Order the steps below in the life cycle of AIDS

- a) Viral RNA degrades
- b) cDNA integrated into host genome
- c) New virus buds from cell
- d) Viral proviral DNA becomes double stranded
- e) Viral RNA translated into protein
- f) Viral envelope fuses with host cell plasma membrane
- g) viral RNA produced from host genome
- h) Reverse transcriptase makes cDNA
- i) cDNA enters the nucleus
- j) New viruses are assembled
- k) HIV binds to CD4 proteins

Answer: k, f, h, a, d, i, b, g, e, j, c

II. The figure below shows the structure of the AIDS virus and the genome of the virus illustrating the essential genes.

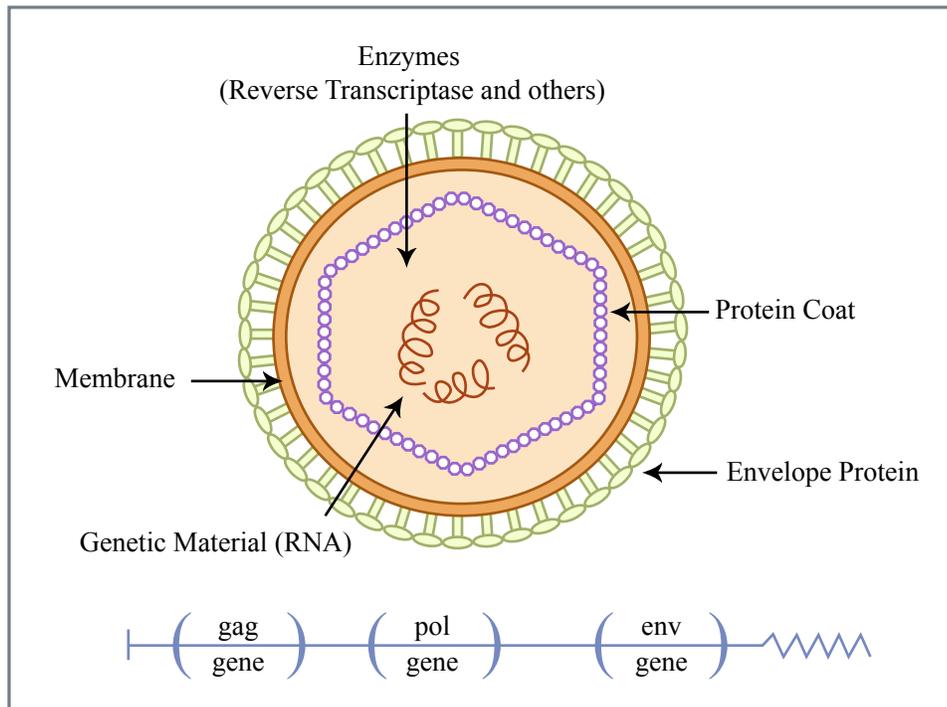


Figure by MIT OCW.

For each of the genes listed below explain what the function of the protein it encodes is:

***gag* gene encodes structural proteins of the viral core and matrix**

***pol* gene -encodes proteins involved in viral replication and integration (RT, protease, integrase)**

***env* gene -encodes envelope proteins (glycoproteins, gp120 (binds to CD4). gp 41 (required for fusion) and gp5**

III. Which steps of the AIDS life cycle would make good drug targets?

- (1) Attachment and penetration of virus**
- (2) Reverse transcription**
- (3) Transcription and translation**
- (4) Viral Maturation, Transport & Packaging**
- (5) Assembly and release**

IV. How could you make a drug targeting the host cell recognition step of the virus life cycle?

Soluble CD4

Antibody to CD4 to block binding of virus

Antibody against viral glycoprotein

V. Why is reverse transcriptase a good drug target?

Reverse transcriptase is specifically needed by the virus to convert its RNA genome into DNA. It has no function in human cells.

VI. How do inhibitors of reverse transcriptase such as AZT work?

Nucleoside analogs-replace 3'OH

AZT binds to reverse transcriptase at a site that normally binds to physiological nucleoside tri-PO₄

Reverse transcriptase selectively utilizes AZT-5'-triphosphate compared to 2'-deoxythymidine-5'-triphosphate whereas DNA polymerase does not

Question 5

Avian influenza virus is a flu virus that infects many species of birds, including both livestock and migratory birds. The virus is highly mutable, giving rise to strains of high or low pathogenicity. The more pathogenic strains of the virus can approach 100% mortality in a very short period of time.

Is a more pathogenic form of this virus more likely appear on a commercial poultry farm, or in a wild population of ducks?

Poultry farm. In ducks, they are not in as close contact, and if they die, or are sick, they will most likely fall into the ocean or something, rather than being around all of the other birds, as a sick chicken would.

Is the virus more likely to be spread worldwide by livestock or migratory birds?

Migratory birds. They migrate.

Avian virus was identified in Italy, over 100 years ago. Human infection did not occur until 1997. At a molecular level, why could it be difficult for avian virus to infect humans?

Wrong cell proteins on the surface of the viral capsid or cell surface.

While studying avian flu in the lab, you notice that replication of the viral genome happens using a very low fidelity polymerase. Could this confer any benefit to the virus? What?

High frequency of mutation

Given that there are indeed documented cases of human infection, what do you imagine occurred to facilitate this?

Mutation of capsid proteins

Are humans more or less likely to contract avian flu from infected humans, or from infected poultry?

From humans - the virus is likely already mutated to be able to infect humans.

What modern farm practices could lead to the prevalence of avian virus that can infect humans?

Overcrowding in factory farms allows for easy transmission from bird to bird. Not allowing poultry to roam about makes it harder to detect sick birds. Grinding up dead birds and feeding them to live birds allows for transmission of disease.

All of these allow highly virulent forms of the protein to spread in the population.

Also, selling the meat from sick or dead birds could pass infection to humans.

Why don't the high levels of antibiotics pumped into commercial poultry reduce the incidence of avian flu?

Antibiotics don't affect viruses.