7.016 Recitation 21 – Fall 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*

Summary of Lectures 32 (12/3) & 33 (12/5):

Viruses: Viruses are obligate intracellular parasites. They consist of a protein coat (capsid) surrounding a genome, which encodes for proteins. These may include structural or coat proteins, other proteins necessary to get inside the host cell and make copies of the viral genome and the proteins that provide the virus with the anti–host defense system. **Non-enveloped/ naked viruses** do not have a lipid bilayer surrounding their capsid. They typically dock onto a protein on the surface of the target cells and inject their genomes into the host. **Enveloped viruses** have a lipid bilayer. They fuse their own membrane with the host membrane, such that the entire viral particle is endocytosed into the host cell. Once a virus is inside its host, it takes over the host machinery and uses it to make coat proteins and viral genomes. The genomes are then packaged into the coats and the new viral particles escape from the host cell either by lysing the cell or budding off from the cell.

Viral genomes can be composed of either DNA or RNA. They can be single-stranded or double-stranded.

There are two types of single stranded RNA viruses: **plus (+) stranded or minus (-) stranded**. The plus (+) stranded RNA genome can be directly read and translated by the host cell translation machinery. So these viruses bring only their genome, with no additional proteins, into the host cells at the time of infection.

Minus (-) stranded RNA is complementary to the viral mRNA that codes for viral proteins and can therefore NOT be directly read and translated by the host cell machinery. At the time of infection, minus (-) stranded RNA viruses bring both their genome and an RNA depended RNA polymerase into the host cells.

Retroviruses have RNA genomes that are reverse transcribed into DNA in the host cells. Retroviral genomes contain a gene that encodes the Reverse Transcriptase (RT) enzyme. RT is a polymerase that reads a strand of RNA as a template, and synthesizes the complementary strand of DNA. Retroviruses use RT to convert their RNA genomes into DNA such that these pieces of DNA can randomly integrate into the host cell's genome. In this way, the viral genome is replicated along with the host genome and is passed on to all daughter cells of the original cell it infected.

Viral genomes may be **segmented** or **non-segmented**. For example, the genome of the influenza virus is composed of 8 gene segments. If different strains of the influenza virus simultaneously infect a cell, then their genome segments can reshuffle and combine resulting in new viral strains that have different cell surface antigens. This new viral strains have the potential of causing global pandemic since no one or very few people may have immunity against them.

Human immunodeficiency virus (HIV): is a retrovirus that infects the T_H cells of our immune system. HIV gets into our T_H cells by docking onto the CD4 protein on the surface of T_H cells. CD4 protein normally helps T_H cells recognize the MHC class II molecules on the surface of antigen presenting cells (macrophages, dendritic cells and B cells). However, the HIV virus has evolved to have a glycoprotein on its surface that binds to CD4, thus targeting HIV to T_H cells. This glycoprotein also has the ability to fuse the lipid bilayer of HIV to the cell membrane of our T_H cells, thus releasing the contents of the HIV virus into our T_H cells. The HIV virus harms the T_H cells, thereby depleting our immune system and our ability to fight the virus. HIV mutates rapidly due to it having a reverse transcriptase that is error prone. This allows HIV to be constantly changing the amino acid makeup and the shape of its viral proteins so that our immune system cannot gain immunity to the HIV.

Discovery of antibiotics: Alexander Fleming, a well establishes scientist in 1928 was studying the properties of Staphylococcus bacteria, the causative agent of Staph infections. One night, he left a petridish of bacteria uncovered, next to a window. When he returned a few days later, he found that the bacterial petridish had been contaminated with a mold and most interestingly the bacteria in close proximity to the mold were dead!

It was well established that fungi such as mold, digest their food outside their body. They secrete digestive enzymes to break down large food molecules in the environment then absorb the breakdown product through the plasma membranes of their cells in a process called absorptive heterotrophy.

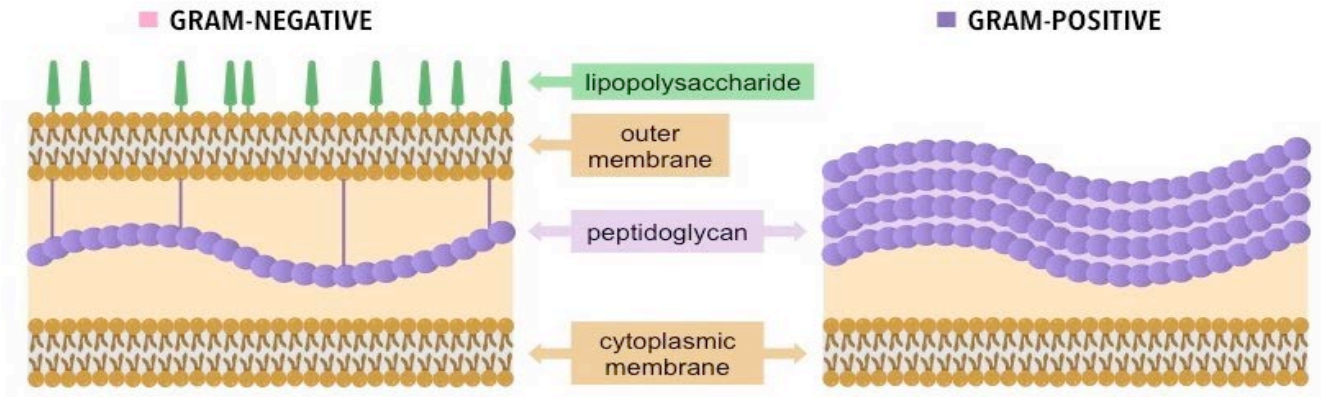
He therefore hypothesized that the bacteria-free rings around the fungal colonies resulted from a substance excreted from the mold, which he initially called “mould juice”. He then identified the growing mold as a member of penicillium family and eventually named this anti-bacterial substance “penicillin”.

By 1945, penicillin was being produced and distributed as an antibiotic on a large scale. Fleming along with Howard Florey and Ernst Chain received the Nobel Prize for their contribution.

This was one of the most important achievements of Modern science as it provided cure to diseases like gangrene, tuberculosis, syphilis, which were otherwise fatal. Soon other antibiotics were isolated from fungi or synthesized in the laboratory thus leading to the golden era of Human heath.

Definition of Antibiotics: Antibiotics, also known as anti- bacterial agents, are compounds that destroy (**bactericidal**) or slow down the growth (**bacteriostatic**) of bacteria. Antibiotics function by targeting the bacterial cell structures or molecules that the host cell does not have such as bacterial cell wall, bacterial cell membrane, bacterial replication, transcription and translation machinery.

Bacteria (their classifications) and mycoplasmas: Bacteri can be classified based on their staining pattern as Gram- positive or Gram- negative. The gram-positive bacteria have no outer membrane and they stain blue or violet by Gram staining dye. This is due to their thick peptidoglycan layer, which retains the crystal violet stain. In comparison, Gram-negative bacteria cannot retain the crystal violet stain, due to a thin peptidoglycan layer that is located between the two membranes. Instead they retain the counterstain (safranin or fuchsine) and appear red or pink.



Mycobacteria have no outer wall like Gram + bacteria. But they stain poorly with crystal violet dye due to their waxy, hydrophobic, thick mycolic acid coat. They are resistant to many antibiotics.

Antibiotic resistance: There is concern worldwide that antibiotics are being overused. Antibiotic overuse is one of the factors that contribute towards the growing number of bacterial infections, which are becoming resistant to antibacterial medications. According to the ECDC (European Centre for Disease Prevention and Control), antibiotic resistance continues to be a serious public health threat worldwide.

The large number of bacterial cells, combined with their rapid generation cycle (one cell division every almost every 20 minutes) facilitates the development of antibiotic resistant bacterial mutants. In a typical bacterial population of 10^{11} cells in an infected patient there can be 1000 mutants. If a mutant confers a selective advantage over the bacteria (i.e. the ability to live in the presence of an antibiotic) then that resistant bacterium will be selected and continue to grow while its neighbors perish. This progressively gives rise to the antibiotic resistant strains and shows the need to continue developing the next generation antibiotics.

Questions

1. The following sequence is a short viral gene from a double-stranded DNA virus that encodes three different proteins. ***Note:*** *use the upper strand as a template in transcription and answer the questions based on the number of 5'AUG3' start codons. The stop codons are 5'UAA3', 5'UAG3' and 5'UGA3'.*

```
5′TACTCTATCGCTTTAGCGGTATGCTATCAGAGCCATGCATGCATC 3′
3′ATGAGATAGCGAAATCGCCATACGATAGTCTCGGTACGTACGTAG 5′
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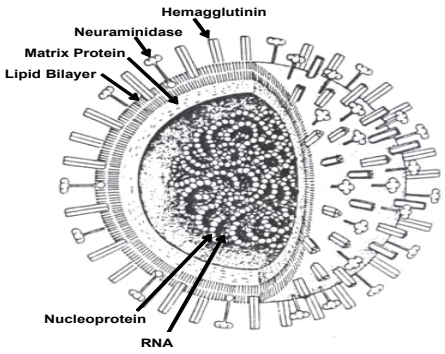
a) How many amino acids long would each of the three proteins be that are produced from this gene?

b) What is a major advantage of overlapping genes?

c) What is a major disadvantage of overlapping genes?

2. Influenza virus is a **minus-stranded, segmented, enveloped RNA virus**. The following is a diagram representing an influenza virus. This RNA virus does not replicate via a DNA intermediate. The virus typically infects vertebrate epithelial cells.



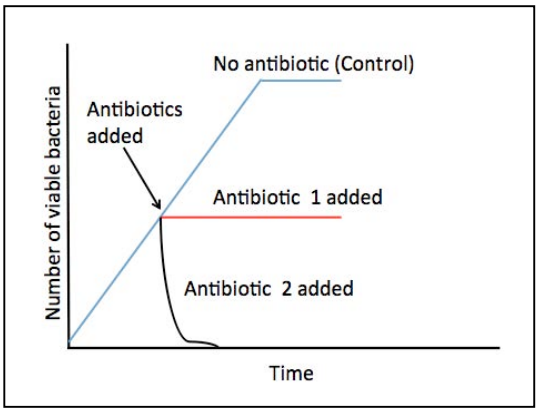
a) Once an individual has been exposed to a particular strain of influenza virus, a humoral immune response develops, causing the secretion of antibodies in the mucosal lining of the epithelium that render the host "immune" to further infection from subsequent exposure to that viral strain. Which of the structures labeled in the diagram to the left (hemaglutinin/ neuraminidase/ matrix protein/ nucleoprotein/ lipid bilayer/ RNA genome) may serve as an antigen for these antibodies?

b) Even if a host develops immunity to one strain of influenza, the host may still be infected by other strains. **Why?**

c) Pigs can be infected both by the avian and human forms of the Influenza virus. This allows them to act as virus mixing vessels in which the two viral strains can readily exchange RNA. This leads to the generation of new viral strains, as the virus that emerges from such doubly infected species can represent a unique assortment of genes. The H1N1 virus that causes Swine Flu is one such example. Why is a newly emerged virus from the pig considered a greater threat than a seasonal viral strain?

e) Each year, the world health organization (WHO) recommends development of a new flu vaccine based on the analysis of the newly emerged strains. Why does the influenza virus mutate so rapidly?

3. The following schematic represents the effect of two different antibiotics on the same strain of bacteria. Propose a mechanism that explains the action of Antibiotic 1 and Antibiotic 2.



4. Penicillin targets a bacterial enzyme that catalyzes peptide cross-linking to form the peptidoglycan cell wall.

a) Briefly **explain** why penicillin causes bacterial cell death.

b) Overuse of penicillin results in the evolution of penicillin-resistant bacterial strains. List **two mechanisms** by which the bacterial strains may acquire penicillin resistance.

c) Explain why the treatment with penicillin and ciproflaxin, an antibiotic which inhibits bacterial topoisomerase enzyme as opposed to penicillin alone, can delay the emergence of penicillin resistant strains.

5. Crystal violet is a dye often used to distinguish Gram-positive bacteria and Gram-negative bacteria.

a) Explain, why mycobacteria tend to stain poorly with Crystal violet dye similar to the Gram-negative bacteria.

b) Explain why the mycobacterial infections are very hard to treat in contrast to the infections caused by Gram-positive bacteria.

Solution key

1. The following sequence is a short viral gene from a double-stranded DNA virus that encodes three different proteins. ***Note:*** *use the upper strand as a template in transcription and answer the questions based on the number of 5'AUG3' start codons. The stop codons are 5'UAA3', 5'UAG3' and 5'UGA3'.*

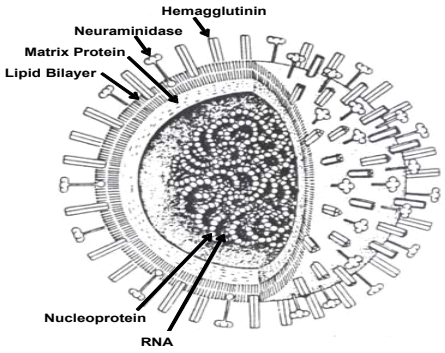
```
5′TACTCTATCGCTTTAGCGGTATGCTATCAGAGCCATGCATGCATC 3′
3′ATGAGATAGCGAAATCGCCATACGATAGTCTCG   C   CGTAG 5′
```

a) How many amino acids long would each of the three proteins be that are produced from this gene?
The peptides produced will be 5, 11 and 7 amino acids long using the 1st, 2nd and 3rd open reading frames from the 5' end of the transcript.

b) What is a major advantage of overlapping genes?
The virus can use the same transcript to produce different proteins. Additionally, overlapping genes allows the virus to use limited space more efficiently.

c) What is a major disadvantage of overlapping genes?
A single mutation in one transcript (or the genome itself) influences not just one viral protein, but may have an effect on all the viral proteins that are being made from the single transcript.

2. Influenza virus is a **minus-stranded, segmented, enveloped RNA virus**. The following is a diagram representing an influenza virus. This RNA virus does not replicate via a DNA intermediate. The virus typically infects vertebrate epithelial cells.



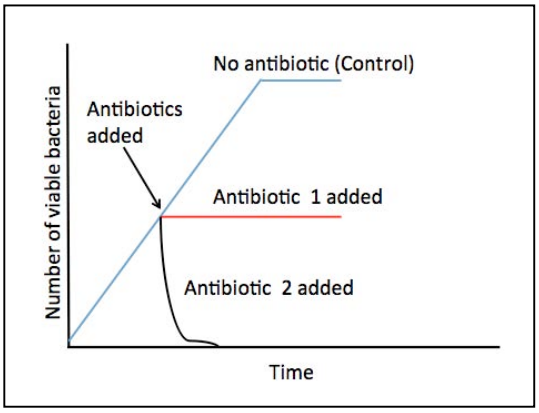
a) Once an individual has been exposed to a particular strain of influenza virus, a humoral immune response develops, causing the secretion of antibodies in the mucosal lining of the epithelium that render the host "immune" to further infection from subsequent exposure to that viral strain. Which of the structures labeled in the diagram to the left (hemaglutinin/ neuraminidase/ matrix protein/ nucleoprotein/ lipid bilayer/ RNA genome) may serve as an antigen for these antibodies?
Hemagglutinin (HA) and neuraminidase; these structures are located at the surface of the virus, so they can be recognized and bound by antibodies.

b) Even if a host develops immunity to one strain of influenza, the host may still be infected by other strains. **Why?** *The influenza virus contains RNA dependent RNA polymerase enzyme, which is involved in transcription of the viral genome. This enzyme does not have any proofreading activity and therefore the virus accumulates mutations at a very high frequency. These mutations can cause small changes in in the hemagglutinin and neuraminidase proteins / viral antigens on the surface of the virus leading to an antigenic drift which creates new strains over time against which people have no immunity.*

c) Pigs can be infected both by the avian and human forms of the Influenza virus. This allows them to act as virus mixing vessels in which the two viral strains can readily exchange RNA. This leads to the generation of new viral strains, as the virus that emerges from such doubly infected species can represent a unique assortment of genes. The H1N1 virus that causes Swine Flu is one such example. Why is a newly emerged virus from the pig considered a greater threat than a seasonal viral strain?
If two different viral strains (Strain A and Strain B) simultaneously infect a cell the progeny virus may have mixture of genome segments, some coming from strain A and others from strain B. Hence they may have new antigens against which almost no one would have immunity so causing a pandemic.

e) Each year, the world health organization (WHO) recommends development of a new flu vaccine based on the analysis of the newly emerged strains. Why does the influenza virus mutate so rapidly?
It is segmented and this allows the mixing of genomes of different viral strains that are co-infecting a cell resulting in the mergence of new strains. Additionally, the viral RNA dependent RNA polymerase lacks proofreading ability, which increases the mutation rate results in rapid emergence of new viral strains.

3. The following schematic represents the effect of two different antibiotics on the same strain of bacteria. Propose a mechanism that explains the action of Antibiotic 1 and Antibiotic 2.



Antibiotic 2 is killing the target bacterial cells (it is bacteriocidal) as shown by the decrease in the number of bacterial cells over time whereas Antibiotic 1 arrests bacterial cells growth and division (bacteriostatic) as shown by plateaued bacterial cell growth curve.

4. Penicillin targets a bacterial enzyme that catalyzes peptide cross-linking to form the peptidoglycan cell wall.

a) Briefly **explain** why penicillin causes bacterial cell death.
It is the peptidoglycan layer that provides rigidity and mechanical strength to the bacterial cell wall. If this is disrupted, the bacterial cells suffer osmotic shock, which results in their death.

b) Overuse of penicillin results in the evolution of penicillin-resistant bacterial strains. List **two mechanisms** by which the bacterial strains may acquire penicillin resistance.
-Horizontal transfer of bacterial resistance gene fom one bacterium to other
- Selection pressure imposed by penicillin may cause bacterium to acquire spontaneous mutations that may make it penicillin resistant
-Transformation of bacteria with plasmid contacting beta lactamase enzyme encoding gene
-Changes in protein channels that may either prevent penicillin entry or promote its rapid efflux
c) Explain why the treatment with penicillin and ciproflaxin, an antibiotic which inhibits bacterial topoisomerase enzyme as opposed to penicillin alone, can delay the emergence of penicillin resistant strains.
Combination therapy with multiple drugs makes it less likely for resistant bacteria to emerge i.e. simultaneous mutations in two or more protein targets is less likely

5. Crystal violet is a dye often used to distinguish Gram-positive bacteria and Gram-negative bacteria.

a) Explain, why mycobacteria tend to stain poorly with Crystal violet dye similar to the Gram-negative bacteria.
Their thick mycolic acid coat allows them to retain the dye and stain pink just like the Gram-negative bacteria

b) Explain why the mycobacterial infections are very hard to treat in contrast to the infections caused by Gram-positive bacteria.
Their thick mycolic acid coat likely prevents entry of any drug into them to mediate any action

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