Overview of Lecture: Prokaryotes & viruses

Read: Text ch 19 & 27

Bullet Points:
- begin “tiptoe through the taxa:” the three domains
- prokaryotes: (eu)bacteria & archaea
- cells: prokaryotes vs eukaryotes
- survey of bacteria
- cell walls: peptidoglycan, gram+, gram-
- antibiotics and antibiotic resistance
- flagella
- quorum sensing {social behavior} & disease
- viruses: surface receptors; herpes
- phages - “Would you like mustard and phages on that?”
- Influenza: antigenic drift and shift

(a) Gram-positive. Gram-positive bacteria have a thick cell wall made of peptidoglycan that traps the crystal violet in the cytoplasm. The alcohol rinse does not remove the crystal violet, which masks the added red safranin dye.

(b) Gram-negative. Gram-negative bacteria have a thinner layer of peptidoglycan, and it is located in a layer between the plasma membrane and an outer membrane. The crystal violet is easily rinsed from the cytoplasm, and the cell appears pink or red.
The scientific community was shocked in the late 1970s by the discovery of an entirely new group of organisms -- the *Archaea*.

Carl Woese proposed that life be divided into **three domains**: Eukaryota, Eubacteria, and Archaebacteria.

**Carl R. Woese** [http://www.life.uiuc.edu/Micro/woese.html](http://www.life.uiuc.edu/Micro/woese.html)

*Using ribosomal RNA sequence as an evolutionary measure, my laboratory has reconstructed the phylogeny of the bacteria and the archea, and provided a phylogenetically valid system of classification for prokaryotes.*
The basic structural & functional unit of every organism is one of two types of cells – **prokaryotic** or **eukaryotic**. **Bacteria** and **Archaea** consist of **prokaryotic** cells. **Protists, fungi, animals, & plants** consist of **eukaryotic** cells.

Modern molecular biology depends on amplifying DNA samples with the Polymerase Chain Reaction (PCR), using a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the Archaean *Thermus aquaticus*, collected from Yellowstone hot springs.
All **prokaryotic & eukaryotic** cells have several basic features in common:

- **plasma membrane**: a phospholipid bilayer with proteins
- **cytosol**, in which organelles are found.
- **DNA chromosomes**, coding for **genes** & ‘junk’
  {RNA viruses aren’t cells; ‘junk’ influences genes}
- **ribosomes**: organelles made of ribosomal RNA and protein that make proteins according to instructions from the genes. {see ch 6}

The word prokaryotic is from the Greek pro, meaning “before,” and karyon, meaning “kernel,” referring here to the nucleus.

In a **prokaryotic cell**, the DNA is concentrated in a region called the **nucleoid**, but no membrane separates this region from the rest of the cell.

In contrast, the **eukaryotic cell** (Greek eu, true, and karyon) has a **true nucleus**, bounded by a membranous **nuclear envelope**.

The entire region between the nucleus and the plasma membrane is called the **cytoplasm**, a term also used for the interior of a prokaryotic cell. Within the cytoplasm of a eukaryotic cell are a variety of membrane-bounded organelles of specialized form and function. These are absent in prokaryotic cells.
The genome of a prokaryote is structurally very different from a eukaryotic genome and has on average only about one-thousandth as much DNA. **In most prokaryotes the genome consists of a ring of DNA that has relatively few proteins associated with it.**

In addition to its single chromosome, a prokaryotic cell may have smaller rings of DNA called **plasmids**, consisting of only a few genes. The plasmid genes provide resistance to antibiotics, direct the metabolism of rarely encountered nutrients, or have other such “contingency” functions. **Plasmids replicate independently of the main chromosome, and many can be readily transferred between partners when prokaryotes conjugate** {used in ‘genetic engineering’}

DNA replication, transcription, and translation are fundamentally similar in prokaryotes and eukaryotes. **However, prokaryotic ribosomes are slightly smaller than eukaryotic ribosomes and differ in their protein and rRNA content.** These differences allow antibiotics, such as erythromycin and tetracycline, to selectively bind prokaryote ribosomes and block protein synthesis ... kill bacteria without harming our {eukaryotic}selves.
Who are we?
The human gut contains at least a kilogram of bacteria alone. They contribute so much to human biology that it is difficult to say where the body ends and the microbes begin. Several massive projects have now started up to characterize the human microbiota in its entirety.

Gut microorganisms, mammalian metabolism and personalized health care.

The mammalian gut microbiota interact extensively with the host through metabolic exchange and co-metabolism of substrates. Such ... interactions are poorly understood, but might be implicated in the aetiology of many human diseases. In this paper, we assess the importance of the gut microbiota in influencing the disposition, fate and toxicity of drugs in the host, {find that metabolites of foods and drugs in blood and urine vary w/ composition of gut microbiome community} ... consideration of individual human gut microbial activities will be a necessary part of future personalized health-care paradigms

Symbiotic gut microbes modulate human metabolic phenotypes

The host metabolic phenotype is thus strongly influenced by the gut microbiome.
One of the useful roles performed by the human gut microbiota is to supply digestive enzymes missing from the human genome. For instance, polysaccharides from the terrestrial plants that have been part of the human diet throughout evolution are broken down in the gut by carbohydrate active enzymes, or CAZymes, many of them highly specific enzymes from Bacteroides spp. bacteria. Little is known about the gut enzymes acting on edible marine algae such as nori, sea lettuce and wakame, common in Japanese cuisine.

Now CAZymes able to digest sulphated polysaccharides from Porphyra sp. marine red algae have been identified in marine Bacteroides isolates. And surprisingly, genome data mining reveals that this enzyme is present in gut bacteria from Japanese — but not American — individuals. This demonstrates that the gene transfer has taken place — recently in evolutionary terms — from a marine environmental bacterium to the Japanese gut bacterium Bacteroides plebeius. Porphyra are otherwise known as nori and used traditionally in sushi, so it seems probable that contact with non-sterile food may be a general factor in stocking gut microbes with a varied arsenal of CAZymes.
Advances in microbiologic analysis and systems biology are now beginning to implicate the gut microbiome in the etiology of localized intestinal diseases such as the irritable bowel syndrome, inflammatory bowel disease (IBD), and colon cancer.

Fig 4. Bacterial species abundance differentiates IBD patients and healthy individuals. Principal component analysis* with health status as instrumental variables, based on the abundance of 155 species (microbes) ... carried out with 14 healthy individuals and 25 IBD patients (21 ulcerative colitis and 4 Crohn’s disease).

From the following article:

*PC analysis collapses many correlated variables into one PC axis; like combining length and width into PC axis 1 = “size”
Normal gut microbiota modulates brain development and behavior \{in mice\}

Heijtz, RD et al. 2011 PNAS 108: 3047-3052

Immediately after birth, the newborn organism is rapidly and densely populated with complex forms of indigenous microbes. This process has been shown to contribute to developmental programming …

The functional development of the mammalian brain is of particular interest because it has been shown to be susceptible to both internal and external environmental cues during perinatal life.

We tested the hypothesis that the “normal” gut microbiota is an integral part of the external environmental signals that modulate brain development and function.

Here, we report that colonization by gut microbiota impacts mammalian brain development and subsequent adult behavior.

Using measures of motor activity and anxiety-like behavior, we demonstrate that germ free (GF) mice display increased motor activity and reduced anxiety, compared with … SPF mice with a normal gut microbiota.

This behavioral phenotype is associated with altered expression of genes … in brain regions implicated in motor control and anxiety-like behavior.

Our results suggest that the microbial colonization process initiates signaling mechanisms that affect neuronal circuits involved in motor control and anxiety behavior.
**Domain Bacteria (Bacteria)** *(see text Fig 27.18)*

- Bacteria are the most abundant organisms on earth, and they carry out much of the earth's photosynthesis.
- Most taxonomists recognize 12-15 major groups of bacteria.

**Gram-positive bacteria.** *(explained soon)*

Largely solitary; many form spores. Responsible for many significant human diseases, including *Bacillus anthracis* (anthrax), *Clostridium botulinum* (botulism - botox), *Staphylococcus, Streptococcus*.  

Gram-positive bacteria.  
Form branching filaments and produce spores; **produce many commonly used antibiotics**, including streptomycin and tetracycline.  
One of the most common types of soil bacteria; also common in dental plaque.  
*Streptomyces, Actinomyces.*

**The prokaryotes that are not bacteria.**

**Cell walls lack peptidoglycan:** plasma membranes made of different kinds of lipids than bacterial plasma membranes;  
*rRNA and ribosomal proteins more like eukaryotes than bacteria.* Mostly anaerobic. *(hence methane, not CO₂)*

**The Aquificae represent the deepest or oldest branch of bacteria.**

*Aquifex pyrophilus* ...is a hyperthermophile with a temperature optimum at 85°C; a chemoautotroph, it oxidizes hydrogen or sulfur.

High G/C Low G/C  
Gram–positive bacteria
A form of photosynthetic bacteria called “blue-green algae,” common in both marine and freshwater environments often responsible for “blooms” in polluted waters. Both colonial and solitary forms are common. Some filamentous forms have anaerobic cells specialized for nitrogen fixation. Anabaena, Chlamydia.

Gammas include photosynthetic sulfur bacteria, pathogens, like Legionella, and the enteric bacteria that inhabit animal intestines. Enterics include Escherichia coli, Salmonella (food poisoning), and Vibrio cholerae (cholera). Pseudomonas are responsible for many plant diseases.

The cells of myxobacteria exhibit gliding motility; when the soil dries out, cells aggregate to form upright multicellular colonies called fruiting bodies, carrying spores. Other delta bacteria are solitary predators that attack other bacteria. Chondromyces, Bdellovibrio.
The cell walls of prokaryotes differ from those of eukaryotes {ch 6}. Eukaryotic cell walls are made of cellulose {plants} or chitin {fungi}. {Animals lack cell walls, but arthropods extrude extracellular chitin}

**Bacterial cell walls** contain peptidoglycan, a network of modified-sugar polymers cross-linked by short polypeptides.

**Archaean cell walls** contain a variety of polysaccharides and proteins but lack peptidoglycan.

**Gram-positive bacteria** have simpler walls with a large amount of exposed peptidoglycan.

**Gram-negative bacteria** have less peptidoglycan, and an outer membrane with lipopolysaccharides that shield peptidoglycan from gram stain and antibiotics that target peptidoglycan synthesis.
We have been emphasizing the **Unity of Life**, but the **Diversity of Life** is essential to antibiotic strategies.
... natural penicillins obtained from culture filtrates of fungus *Penicillium spp.* are active only against Gram-positive bacteria (competitors on rotting food) (which have a thick layer of peptidoglycan on the surface of the wall)

**Mode of action**

β-lactam antibiotics (Penicillin) binds to the enzyme (transpeptidase) that links the peptidoglycan molecules in bacteria, inhibiting the formation of peptidoglycan cross links in the bacterial cell wall.

**Modes of resistance**

There are 2 main modes of bacterial resistance to β-lactams:

1. Attack: the bacteria produces lactamase enzymes that render the antibiotic ineffective.
   - β-lactam antibiotics may be co-administered with a β-lactamase inhibitor. The genes encoding these enzymes may be acquired via plasmid transfer,

2. Hide: altered transpeptidase does not bind β-lactams
Farmers and ranchers will for the first time need a prescription from a veterinarian before using antibiotics in farm animals, in hopes that more judicious use of the drugs will reduce the tens of thousands of human deaths that result each year from the drugs’ overuse.

http://www.nearingzero.net/screen_res/nz149.jpg

It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

… about 80% of all antibiotics sold are used by the meat and poultry industry to make animals grow faster … The declining effectiveness of antibiotics has become a public-health crisis, leading doctors and scientists to call for much more careful use of antibiotics so that disease-causing organisms don't become immune to them.
About half of all prokaryotes are capable of directional movement—up to 50 times their body length per second. The most common structures that enable prokaryotes to move are **flagella**.

**The flagella of prokaryotes differ from those of eukaryotes** in both structure and mechanism of propulsion. Prokaryotic flagella are one-tenth the width of eukaryotic flagella and are not covered by an extension of the plasma membrane.

Solitary E. coli and other pathogenic bacteria exhibit **positive chemotaxis** toward other members of their species, enabling the formation of colonies—**biofilms**—that block access by antibiotics. Clustering allows bacteria to perform a coordinated activity called **quorum sensing** in which they turn on certain genes only when they sense that they are part of a dense population. **{cooperative social behavior}**

Some disease-causing bacteria are believed to rely on quorum sensing in mounting a successful infection.

Quorum sensing is the regulation of gene expression in response to fluctuations in cell-population density.

Bonnie L. Bassler,
Bacteria assess their population density by detecting the concentration of a particular autoinducer. This “census-taking” enables the group to express specific genes only at particular population densities. Processes controlled by quorum sensing are unproductive when undertaken by an individual bacterium but become effective when undertaken by the group. For example, quorum sensing controls bioluminescence, secretion of virulence factors, sporulation, and conjugation.

Quorum sensing allows bacteria to function as multi-cellular organisms.

Infection Control by Antibody Disruption of Bacterial Quorum Sensing Signaling {“quorum quenching”}  
... report a new type of antibiotic: an antibody that binds to a signalling molecule S. aureus use to communicate with each other. This communication, known as quorum sensing, regulates the production of some proteins associated with virulence. The antibody ... fully protected mice against lethal doses of the bacterium.
Viruses are obligate intracellular parasites. Viruses code their genes on either DNA or RNA, but viruses lack ribosomes and the enzymes necessary for protein synthesis. Viruses are able to reproduce because their genes are translated into proteins by the cell's genetic machinery.

Viruses identify their host cells by a “lock-and-key” fit between proteins on the outside of the virus (ex H5N1 on influenza virus) & specific molecules on the surface of cells. {HIV targets CD4 receptor on helper T cells}

Some viruses have broad host ranges: West Nile virus can infect mosquitoes, birds, and humans ...

Other viruses infect only a single species: Measles virus and poliovirus, for instance, can infect only humans. Human cold viruses infect only the cells lining the upper respiratory tract.

A herpes simplex lesion, also known as a cold sore ... caused by herpes simplex virus (HSV-1) {emerging}, The HSV-1 virus remains in the body throughout an exposed person’s entire life. {more later on latent viruses in nervous system}
**Bacteriophages** are viruses that infect bacteria.

Infection of an E. coli cell by phage $\lambda$ begins when the phage binds to the surface of the cell and injects its DNA.

What happens next depends on the reproductive mode:

- **lytic cycle** or **lysogenic cycle**.

During a **lytic cycle** the viral genes immediately turn the host cell into a $\lambda$-producing factory; the cell soon lyses and releases its viral products.

During a **lysogenic cycle** the $\lambda$ DNA is incorporated into the host cell’s chromosome. When integrated into the bacterial chromosome in this way, the viral DNA is known as a **prophage**. {recall Human Endogenous RetroViruses}

Research now shows that a **bacteriophage that infects V. cholerae** introduces into the host bacterial cell a gene that codes for the cholera toxin. This gene becomes incorporated into the bacterial chromosome, where it is translated along with - the other host genes, hereby converting the benign bacterium to a disease-causing agent.

When phage DNA successfully enters a bacterium, the DNA often is recognized as foreign and cut up by cellular enzymes called restriction endonucleases, or simply **restriction enzymes**. {“innate” immunity} {important in molecular genetics and genetic engineering – cut at specific sites}
The latest US-approved additive to ready-to-eat lunchmeat and poultry products is a combination of six bacteriophages -- parasitic viruses that destroy the Listeria monocytogenes bacterium, which sickens thousands and kills hundreds of people each year.

The Food and Drug Administration on Friday declared the virus mix safe to spray on such foods as cold cuts, hot dogs, sausages, sliced ham and turkey prior to packaging.

... these are vulnerable to Listeria because they are often not reheated prior to consumption,...

-- the first time viruses have been approved for use as a food additive --

According to Intralytix,

- typical phages have hollow heads that store their viral DNA and tunnel tails that bind to specific molecules on their target bacteria.
- The viral DNA is injected through the tail into the host cell, where it directs the production of progeny phages.
- These "young" phages burst from the host cell, thereby destroying it, and go on to infect more bacteria.
- The viruses will not kill any organism other than their target bacteria.
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REVIEW Phage therapy {for humans}: Facts and fiction
Recent examples of the use of bacteriophages in controlling bacterial infections are presented, some of which show therapeutic promise. ... future research on more phage–bacterium systems has to be undertaken ...
Influenza A viruses are found in many different animals, including ducks, chickens, pigs, whales, horses, & seals. Influenza B viruses circulate widely only among humans.

**Influenza A viruses are divided into subtypes based on two proteins** on the surface of the virus: **hemagglutinin (H)** \{the ‘key’ to entry into the host cell\} and **neuraminidase (N)** \{the ‘key’ to exit from the host cell\}

There are 15 different hemagglutinin subtypes and 9 different neuraminidase subtypes, all of which have been found among influenza A viruses in wild birds.

**Wild birds are the primary natural reservoir for all subtypes of influenza A viruses** and are thought to be the source of influenza A viruses in all other animals.

**Pigs can be infected with both human and avian influenza viruses.** ... **the viruses can mix (reassort)** and produce a new virus that had most of the genes from the human virus, but H & N surface proteins from the avian virus ... **Antigenic shift** results when a new influenza A subtype to which most people have little or no immune protection infects humans ... a pandemic can occur.
Like all living things, influenza makes small errors—mutations—when it copies its genetic code during reproduction. But influenza lacks the ability to repair those errors, because it is an RNA virus; RNA, unlike DNA, lacks a self-correcting mechanism. As a result, influenza is not genetically stable. Every generation is slightly different, and those differences accumulate as time passes.

That slow deviation is called **antigenic drift**, and it is the reason why it is necessary to **reformulate flu vaccines every year**. Every flu season, the genetic make-up of the dominant strains from the prior year will have drifted, changing the surface structure of those strains just enough to diminish, or even destroy, the effectiveness of the previous year’s vaccine. Antigenic drift is slow and moderately predictable. Each winter, health authorities must make an educated guess which strains are likely to dominate in the next flu season. It takes six more months to develop and manufacture vaccines for chosen strains. But in some years, genetic drift during just those six months can render the newly formulated vaccine ineffective, leaving populations more vulnerable to the newly evolved virus.
Rapid change in circulating flu viruses is known as **antigenic shift**.

Influenza’s genome is made up of **eight loosely linked RNA segments**, each of which harbors at least one important gene. Those genes direct the expression of influenza’s major viral proteins, including **hemagglutinin** and **neuraminidase**, the antigenic proteins on the viral surface that foster infection and stimulate immune reaction.

When flu viruses infect cells, they use the cell’s molecular machinery to manufacture individual virus components and package them into new viruses that bud off of the cell. If the virus has infected a cell that is simultaneously infected by another strain, the RNA segments can reassort, creating new viruses with different properties from the original infecting strains.

The new virus could possess an avian hemagglutinin (H) protein against which humans have little or no immunity plus human influenza genes that are more likely to cause sustained human-to-human transmission. The net result could be a **new strain that has pandemic potential**.
Resurrected **{H1N1} Influenza Virus**
Yields Secrets of Deadly 1918 Pandemic

Researchers have figured out the traits that made the 1918 influenza virus, which killed 20 - 50 million people, so virulent.

The researchers reconstructed the complete virus from preserved tissue from **{snippets of lung tissue from two soldiers and an Alaskan woman who died in the 1918 pandemic}** ...

... the structure of **the 1918 hemagglutinin (HA)**, the crucial surface protein that flu viruses use to latch onto host cells was unusually potent.

The 1918 flu had a couple of other tricks up its sleeve as well. One is that **the virus doesn't need to rely on its host cells for the protease trypsin to cleave and activate the HA protein**; instead, another viral surface protein, neuraminidase (NA), appears to help activate the HA.

That suggests the 1918 virus, like some **highly virulent bird flu strains**, can grow in any cell type, not just trypsin-laden lung cells.

**The resulting immune hyper-reaction (“cytokine storm”) was most deadly for young young adults with vigorous immunity.**