

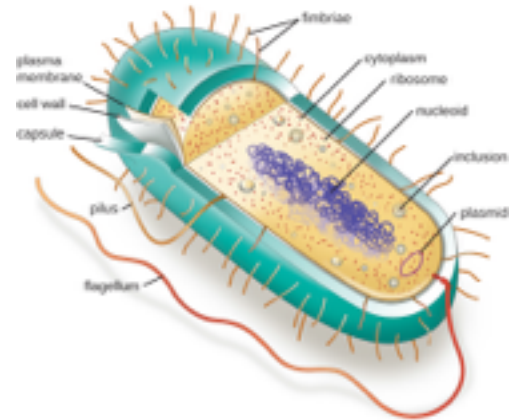
BIO3124 Final Exam

Module 2: Microbial Cell Structure and Metabolism

Describe internal and external structures of prokaryotic (bacteria and archaea) and eukaryotic cells in terms of their physical structure, chemical structure, and function.

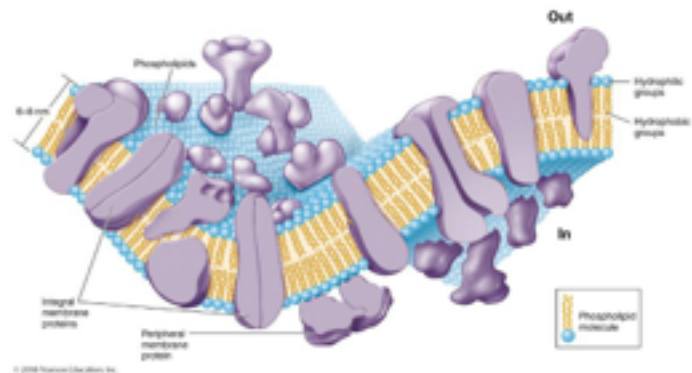
(A) Prokaryotic cells

- A typical prokaryotic cell contains (1) a cell membrane, (2) chromosomal DNA that is concentrated in a nucleoid and extrachromosomal DNA called plasmids, (3) ribosomes, and (4) a cell wall.
- Some prokaryotic cells may also possess flagella, pili, fimbriae, and capsules.
- Cells of *Bacteria* and *Archaea* vary in size. For a cell, there are advantages to being small:
 - More surface area relative to cell volume than large cells (i.e., higher S/V ratio).
 - Support greater nutrient and waste product exchange per unit cell volume.
 - Smaller cells tend to grow faster than larger cells (partly because cell division rate is higher due to smaller proteins being easier to replicate, and for given amount of resources, larger population of small cells can be supported).
 - Mutations lead to faster evolution: prokaryotic cells are genetically haploid (typically only have one copy of each gene so mutations are expressed immediately).
 - Eukaryotic cells adapt slower because their diploid character allows for a mutation in one gene to be masked by a second, unmutated gene copy.



Cell membrane

- Bacterial cytoplasmic membrane:
 - Cell membrane of all cells is a phospholipid bilayer containing embedded proteins. Phospholipids are composed of both hydrophobic and hydrophilic components.
 - In *Bacteria* and *Eukarya*, hydrophobic component consists of fatty acids, hydrophilic component consists of a glycerol molecule containing phosphate and one of several other functional groups (e.g. sugars, ethanolamine, choline) bonded to the



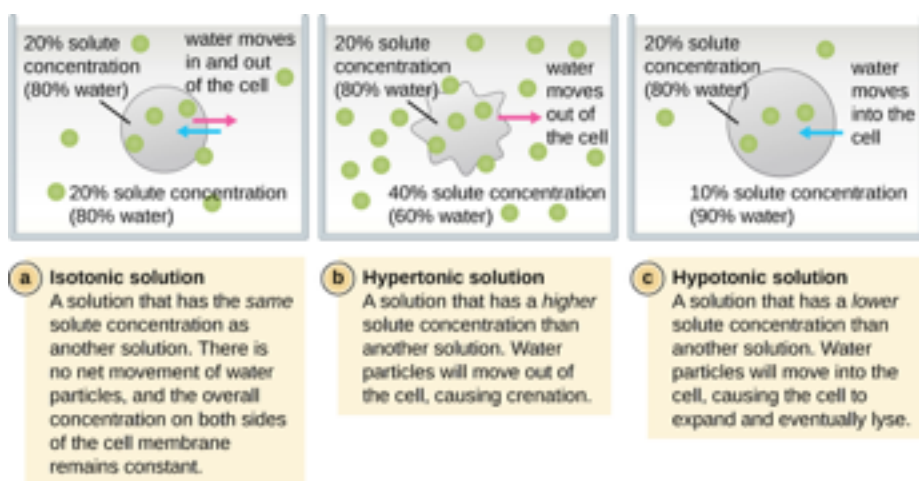
- phosphate.
 - Fatty acids point inward toward each other to form a hydrophobic region, hydrophilic portion remains exposed to either the environment or the cytoplasm.
 - A variety of proteins are attached to or integrated into cytoplasmic membrane; membrane proteins typically have hydrophobic domains that span the membrane and hydrophilic domains that contact the environment or cytoplasm. Proteins significantly embedded in the membrane are called *integral* membrane proteins, and *peripheral* membrane proteins are more loosely attached.
 - *Ester* linkages bond *fatty acids* to glycerol.
- Archaeal cytoplasmic membrane:
 - Has different lipid constituents and chemistry, but structurally similar.
 - Unlike *Bacteria* and *Eukarya*, in which ester linkages bond fatty acids to glycerol, the lipids of *Archaea* contain *ether* linkages between glycerol and a hydrophobic side chain that is not a fatty acid, but rather repeating units of the five-carbon hydrocarbon *isoprene*.
 - *Archaea* can form membranes with lipid monolayers or bilayers. In a monolayer, one isoprene chain has two polar heads instead of just one.
- Cytoplasmic membrane function:

Has at least three major functions:

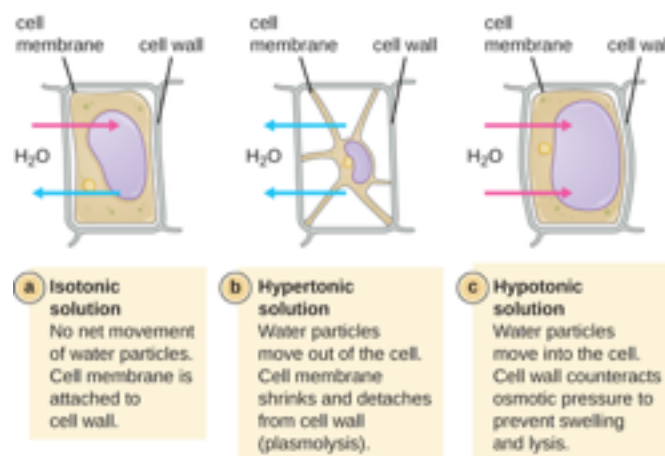
 - Permeability barrier: prevents passive leakage of solutes into or out of the cell, functions as a gateway for transport of nutrients into, and wastes out of, the cell. The cell membrane is a barrier to diffusion of most substances, especially polar or charged molecules. Thus, most substances that enter or leave the cell must be transported. Transport proteins function to accumulate solutes against the concentration gradient.
 - Protein anchor: site of proteins that participate in transport, bioenergetics, and chemotaxis. Also holds transport proteins in place.
 - Energy conservation: site of generation and dissipation of the proton motor force. Membrane is energized when protons (H^+) are separated from hydroxyl ions (OH^-) across membrane surface. Charge separation creates energized state of membrane called *proton motive force*.

Cell wall

- All cells have a cytoplasmic membrane, but not all have a cell wall. However, only a few prokaryotes lack cell walls:
 - Mycoplasmas (group of pathogenic bacteria related to gram-positive *Bacteria*)
 - Thermoplasma (*Archaea*)
 - These cells have tough cytoplasmic membranes (e.g., sterols or lipoglycans).



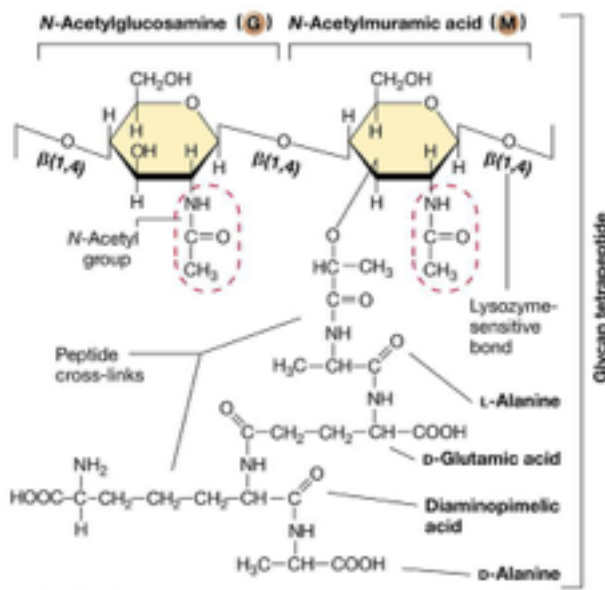
- Function:
 - In cells that lack a cell wall, changes in osmotic pressure can lead to crenation in hypertonic environments or cell lysis in hypotonic environments.
 - In prokaryotic cells, the cell wall provides some protection against changes in osmotic pressure, allowing it to maintain its shape longer. The cell membrane is typically attached to the cell wall in an isotonic medium (left). In a hypertonic medium, the cell membrane detaches from the cell wall and contracts (plasmolysis) as water leaves the cell. In a hypotonic medium (right), the cell wall prevents the cell membrane from expanding to the point of bursting, although lysis will eventually occur if too much water is absorbed.



- The cytoplasm of prokaryotic cells maintains a high concentration of dissolved solutes that creates significant osmotic pressure. To withstand these pressures and prevent bursting—a process called *cell lysis*—most cells of *Bacteria* and *Archaea* have a layer outside the cytoplasmic membrane called the cell wall. Besides protecting against osmotic lysis, cell walls also confer shape and rigidity on the cell.
- Cells of *Bacteria* can be divided into two major groups: Gram-positive and Gram-negative: distinction based on the Gram stain reaction.
 - Gram stain: Most well established method for distinguishing bacterial morphology and cell wall composition.
 - After Gram staining, Gram + retain the crystal violet dye but Gram – do not. A counterstain is added (fuchsine or safranin) and whilst Gram + stay violet-blue, Gram - retain the red of the safranin. Colour difference due to differences in the cell wall structure: Gram-negative cell wall (or cell envelope) consists of at least two layers, whereas Gram-positive cell wall is typically thicker and consists primarily of a single type of molecule.
- Cell walls of *Bacteria* contain a rigid polysaccharide called peptidoglycan: rigid layer that provides strength. Found in all *Bacteria* that contain a cell wall, but not present in the cell walls of *Archaea* or *Eukarya*.

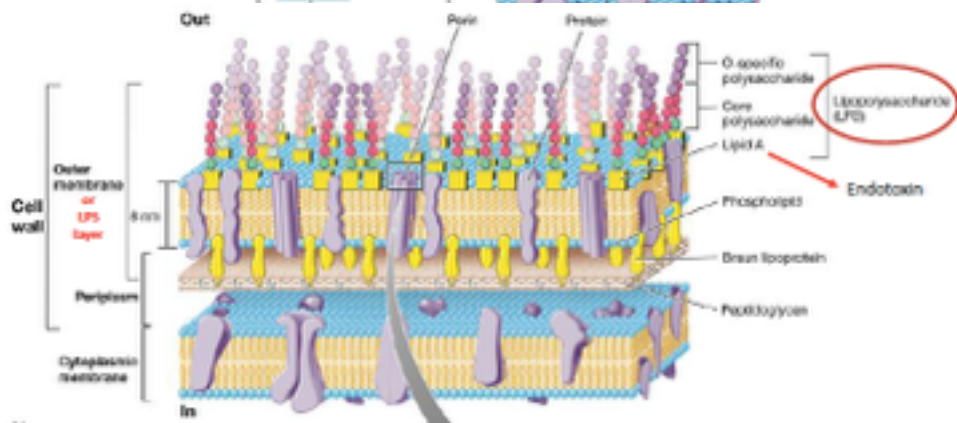
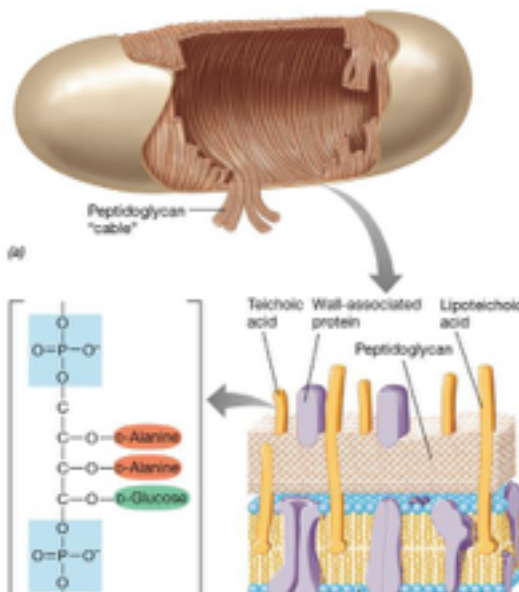
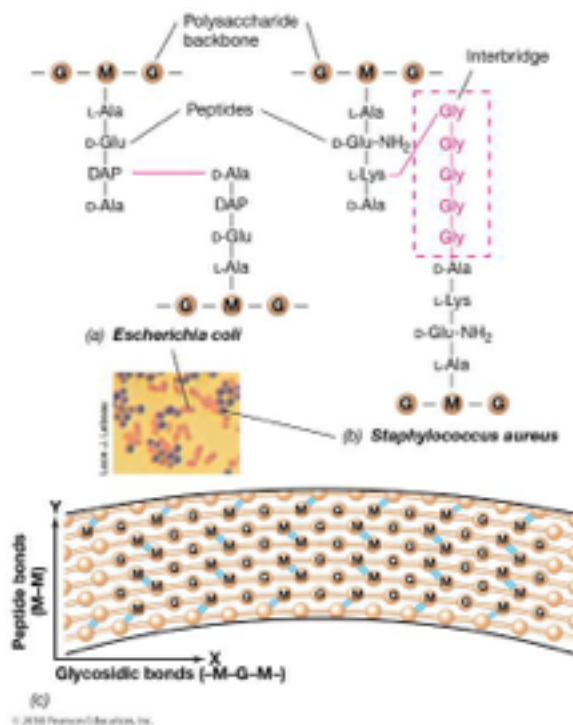
Typically composed of:

- Alternating repeats of two modified modified glucose residues (N-acetylglucosamine and N-acetylmuramic acid) in β -1,4 linkages;
- Amino acids L-alanine, D-alanine, D-glutamic acid, and either L-lysine or diaminopimelic acid (DAP);
- These constituents are connected in an ordered way to form the glycan



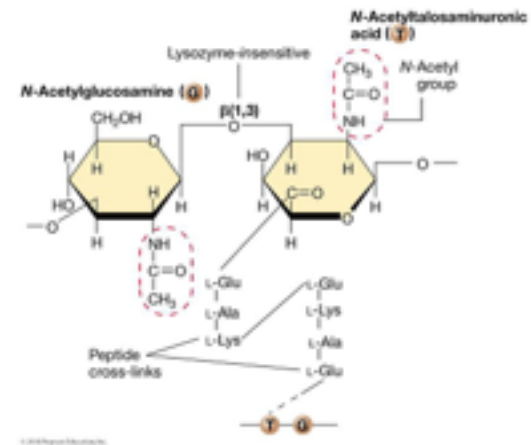
tetrapeptide and long chains of this basic unit form peptidoglycan.

- Cross-linked differently in Gram-negative bacteria and Gram-positive bacteria (often “interbridges”).
- Can be destroyed by lysozyme (enzymes that cleave glycosidic bond between sugars), which is found in human secretions (major defence against bacterial infection);
- 100+ distinct peptidoglycans have been described.
- Strands of peptidoglycan are biosynthesized adjacent to one another to form a sheet surrounding the cell, and individual strands are connected by peptide cross-links.
- In Gram-negative bacteria, the cross-link forms from the amino group of DAP of one glycan strand to the carboxyl group of the terminal D-alanine on the adjacent glycan strand.
- In Gram-positive bacteria, the cross-link often contains a short peptide “interbridge,” the kinds and numbers of amino acids in the interbridge varying between species (e.g. in *Staphylococcus aureus*, interbridge consists of five glycines).
- Structure of Gram-positive cell wall:
 - Up to 90 percent of cell wall composed of peptidoglycan;
 - Common to have teichoic acids (acidic substances) covalently bound to peptidoglycan. These acidic substances bind divalent metal ions (e.g., Ca^{+2} and Mg^{+2}) prior to their transport into cell.
 - Some teichoic acids are covalently bonded to membrane lipids rather than to peptidoglycan, and these are called lipoteichoic acids.
- Structure of Gram-negative cell wall:
 - Only a small amount of total cell wall consists of peptidoglycan, as most of wall is composed of the outer membrane.
 - Outer membrane not only made of phospholipid and



protein as is a lipid bilayer: instead, also contains polysaccharide, and lipid and polysaccharide are linked to form a complex. Thus, often call the outer membrane the lipopolysaccharide (LPS) layer.

Archaeal cell wall: certain cell walls contain a molecule that is remarkably similar to peptidoglycan, a polysaccharide called pseudomurein. The backbone of pseudomurein is formed from alternating repeats of N-acetylglucosamine (also present in peptidoglycan) and N-acetyltalosaminuronic acid; the latter replaces the N-acetylmuramic acid of peptidoglycan. Glycosidic bonds between the sugar derivatives are β -1,3 instead of β -1,4.



Cell surface structures

- Many *Bacteria* and *Archaea* secrete glycocalyxes on their cell surface that consist of polysaccharide layers (may be thick or thin, rigid or flexible). However, not part of cell wall because do not confer significant structural strength.
- If the layer is organized in a tight matrix that excludes small particles and is tightly attached, it is called a capsule. Capsules are readily visible by light microscopy if cells are treated with India ink.
- If the layer is more easily deformed and loosely attached (e.g. *Leuconostoc*), it is called a slime layer. Slime layers assist in attachment to surfaces, and thus play a role in development and maintenance of biofilms. Slime layers also act as virulence factors (molecules that contribute to the pathogenicity of a bacterial pathogen and protect against phagocytosis) and prevent dehydration/desiccation.
- Fimbriae and pili are thin filamentous protein structures, extend from the surface of a cell.
- Fimbriae enable organisms to stick to surfaces or form pellicles (thin sheets of cells on a liquid surface).
- Pili are typically longer, and fewer (1 or a few) found per cell than fimbriae. Two very important functions of pili include facilitating genetic exchange between cells in a process called conjugation (conjugative or sex pili) and allowing adherence to host tissues and supporting twitching motility (type IV pili).
- Hami: an unusual group of Archaea, the SM1 group, forms a unique attachment structure called a hamus (plural, hami) that resembles a tiny grappling hook. They assist in surface attachment, forming biofilms.

Cell inclusions

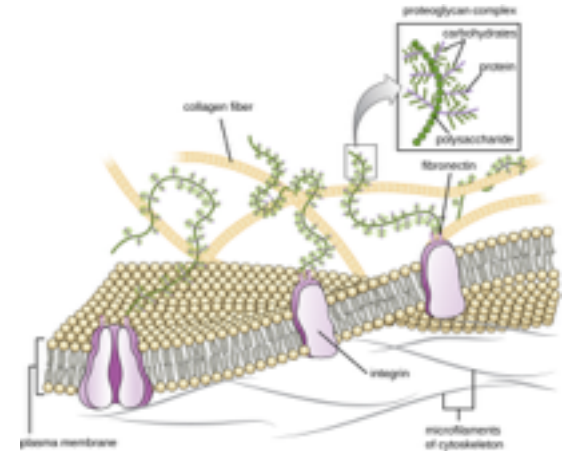
- Inclusions function as energy reserves, carbon reservoirs, and/or have special functions;
- Enclosed by a thin membrane that partitions off the inclusion in the cytoplasm;
- Reduces osmotic stress;
- Carbon storage polymers:
- Poly- β -hydroxybutyric acid (PHB): lipid polymer, stored as lipid droplets. PHB is produced by species of *Bacillus* and *Pseudomonas*. Industrially, PHB has also been used as a source of

biodegradable polymers for bioplastics.

- Glycogen: glucose polymer.
- Other types of stored energy or material:
 - Polyphosphate granules: inorganic phosphate storage;
 - Sulfur globules: elemental sulfur found in periplasm;
 - Carbonate minerals: *biomineralization* of barium, strontium, and magnesium;
 - Magnetosomes: biomineralized particles of the magnetic iron oxides; allow cell to undergo *magnetotaxis*: migration along magnetic field lines.

Gas vesicles

- Some *Bacteria* and *Archaea* are planktonic, meaning that they inhabit the water column of lakes and the oceans;
- Most planktonic organisms move up and down with changes in currents, but some can float because they contain gas vesicles, structures that confer buoyancy and allow the cells to position themselves in regions of the water column that best suit their metabolisms;
- Conical-shaped, gas-filled structures made of protein;
- Impermeable to water and solutes;
- Molecular structure:
 - Gas vesicles are composed of two proteins, GvpA and GvpC;
 - Function by decreasing cell density, increasing buoyancy;
 - Interestingly, GvpC has been tested as an adjuvant in vaccines with some promising success as conjugate for Chlamydia or Salmonella antigens. GvpC are non-toxic, exceptionally stable and bioengineerable (in archaea = no LPS contamination).

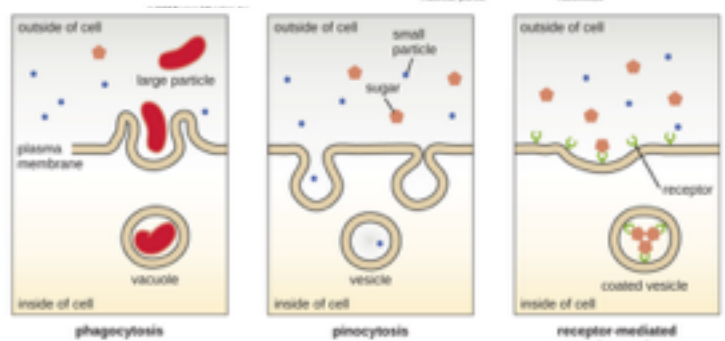
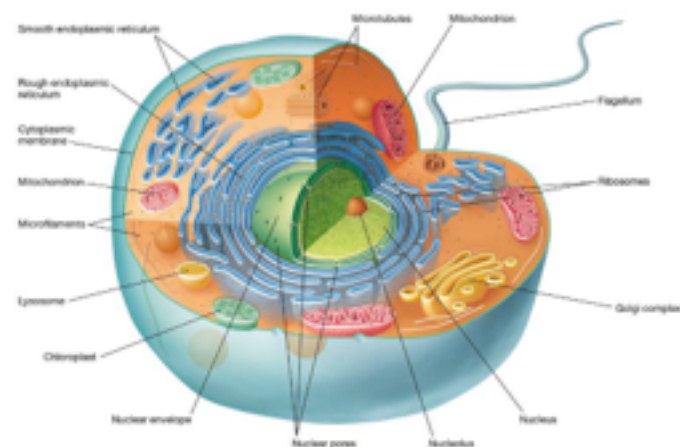


(B) Eukaryotic cells

- The processes of simple diffusion, facilitated diffusion, and active transport are used in both eukaryotic and prokaryotic cells. However, eukaryotic cells also have the unique ability to perform various types of endocytosis, the uptake of matter through plasma membrane invagination and vacuole/vesicle formation.

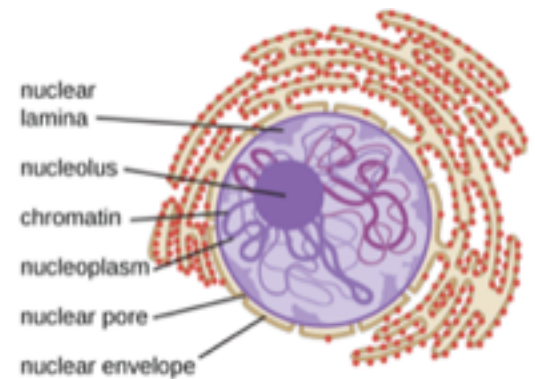
(1) Extracellular matrix:

- Occurs if no cell wall; stabilizes cell structure.
- Protect from stress
- Involved in cell communication
- Adhesion (fibronectin, integrins)
- Collagen provides strength
- Proteoglycans provide stickiness.



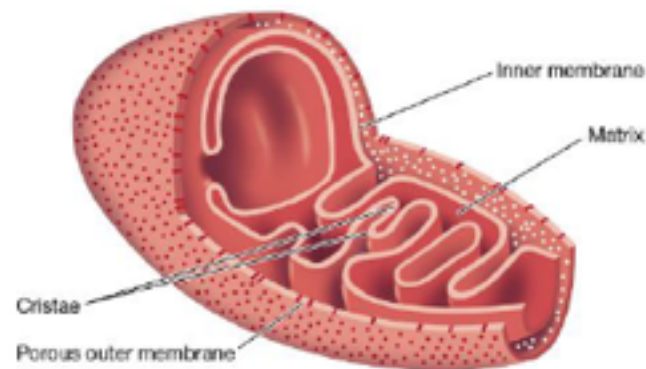
(2) Nucleus and cell division:

- Nucleus: contains the chromosomes
 - DNA is wound around histones = chromatin
 - Enclosed by two membranes that interact with nucleoplasm (inner membrane) and cytoplasm (outer membrane)
 - Within the nucleus is the nucleolus (site of ribosomal RNA synthesis)
 - Site of replication, transcription and transcript maturation.
- Cell division:
 - Mitosis is normal form of nuclear division in eukaryotic cells. It results in two diploid (two copies of each chromosome) daughter cells
 - Meiosis is a specialized form of nuclear division. It results in four haploid (one copy of each chromosome) gametes.



(3) Mitochondria, Hydrogenosomes, and Chloroplasts, specialists of energy metabolism

- Mitochondria: In aerobic eukaryotic cells, respiration occurs in the mitochondrion. The number of mitochondria per cell depends somewhat on the cell type and size (few to 1000+ per cell).
 - Respiration and oxidative phosphorylation for aerobic eukaryotes.
 - Surrounded by two membranes.
 - Cristae: folded internal membranes. Contain enzymes needed for respiration and ATP production.
 - Matrix: innermost area of mitochondrion. Contains citric acid enzymes (TCA cycle).
- Chloroplasts: chlorophyll-containing organelle found in phototrophic eukaryotes.
 - Relatively large; number of chloroplasts vary.
 - Like mitochondria, chloroplasts have a permeable outer membrane and a much less-permeable inner membrane (double membrane).
 - The innermost membrane surrounds the stroma, analogous to the matrix of the mitochondrion.
 - The stroma contains the enzyme ribulose biphosphate carboxylase (RubisCO), the key enzyme of the Calvin cycle, the series of biosynthetic reactions by which phototrophs convert CO₂ to organic compounds.
 - Chlorophyll and all other components needed for ATP synthesis in chloroplasts are located in a series of flattened membrane discs called thylakoids.
 - Like the cytoplasmic membrane, the thylakoid membrane is highly impermeable and its major function is to form a proton motive force that results in ATP synthesis.

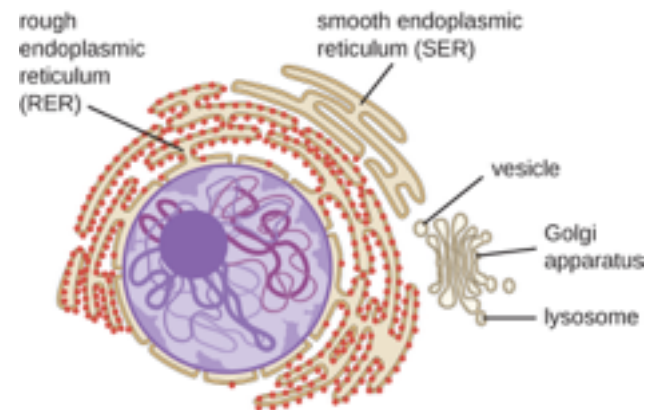


- Hydrogenosomes

- Some eukaryotic microorganisms are killed by oxygen and, like many *Bacteria* and *Archaea*, live an anaerobic lifestyle. Such cells lack mitochondria and some of them contain structures called hydrogenosomes
- Found in anaerobic, strict fermenters (e.g., *Trichomonas* and some protists);
- Similar size to mitochondria; but lack TCA cycle enzymes and cristae;
- Major function is oxidation of pyruvate to H_2 , CO_2 , and acetate.
- Some methane-producing *Archaea* live in some anaerobic eukaryotes and consume H_2 and CO_2 , producing CH_4 ; acetate is secreted.

- The endomembrane complex

- ER, golgi, lysosome
- Some protein translation
- Post translational modification
- Protein transport
- Lysosomes: contain digestive enzymes used for hydrolysis of food. Degrade and recycle damaged cell component, and separate lytic activity from cytoplasm.

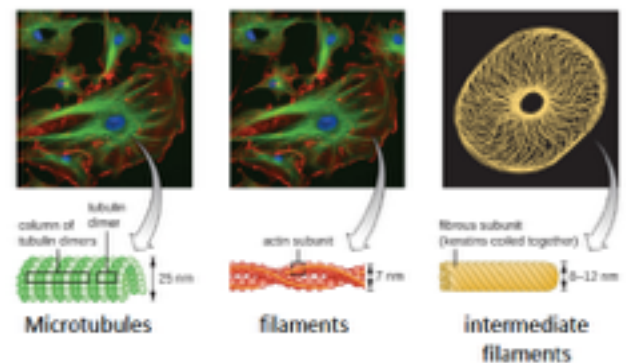


- Cytoskeleton

Provides (1) structural support, (2) transport network, (3) an anchor for organelles, and (4) cell motility.

- Peroxisome

- Produce hydrogen peroxide to break down molecules such as uric acid, amino acids, and fatty acids.
- Possess the enzyme catalase, which can degrade hydrogen peroxide.
- Plays role in lipid biosynthesis.
- Glyoxysomes are modified peroxisomes of yeasts and plant cells that perform several metabolic functions, including the production of sugar molecules.
- Glycosomes are modified peroxisomes made by certain trypanosomes, the pathogenic protozoans that cause Chagas disease and African sleeping sickness.



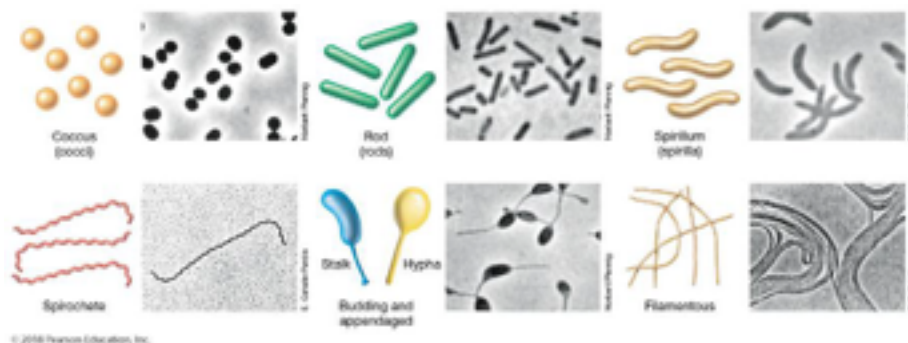
- Flagella and cilia

- Organelles of motility that allow cells to move by swimming
- Cilia are short flagella: structurally distinct from prokaryotic flagella and do not rotate; instead whip (flagella) or beat in synchrony (cilia).
- Both have a bundle of nine pairs of microtubules surrounding a central pair of microtubules.
- Dynein is attached to the microtubules and uses ATP to drive motility.

Describe all the cell morphologies and cellular arrangements similarities and differences between bacteria, archaea and eukaryotes.

Cell morphology refers to cell shape.

- Typically does not predict physiology, ecology, phylogeny, or other properties of a prokaryotic or archaeal cell.
- Morphology of a given microbe may be the result of selective forces that have shaped its evolution. Some examples include optimization for nutrient uptake (small cells and high surface-to-volume ratio), swimming motility in viscous environments or near surfaces (helical or spiral-shaped cells) and gliding motility (filamentous bacteria).
- A cell that is spherical or ovoid is called a coccus.
- A cylindrically shaped cell is called a rod or a bacillus.
- Some cells form curved or loose spiral shapes, called spirilla.
- Cells of some *Bacteria* and *Archaea* remain together in groups or clusters after cell division, and arrangements are often characteristic.



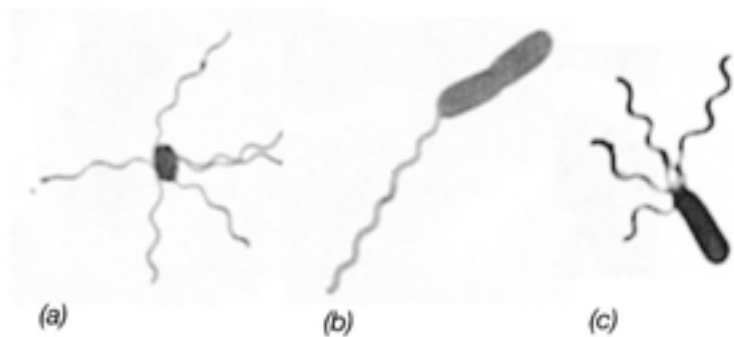
Common Prokaryotic Cell Arrangements		
Name	Description	Illustration
Coccus (pl. cocci)	Single coccus	
Diplococcus (pl. diplococci)	Pair of two cocci	
Tetrad (pl. tetrads)	Grouping of four cells arranged in a square	
Streptococcus (pl. streptococci)	Chain of cocci	
Staphylococcus (pl. staphylococci)	Cluster of cocci	
Bacillus (pl. bacilli)	Single rod	
Streptobacillus (pl. streptobacilli)	Chain of rods	

Describe the different modes of locomotion used by archaea, bacteria and single cell eukaryotes.

Several modes of locomotion:

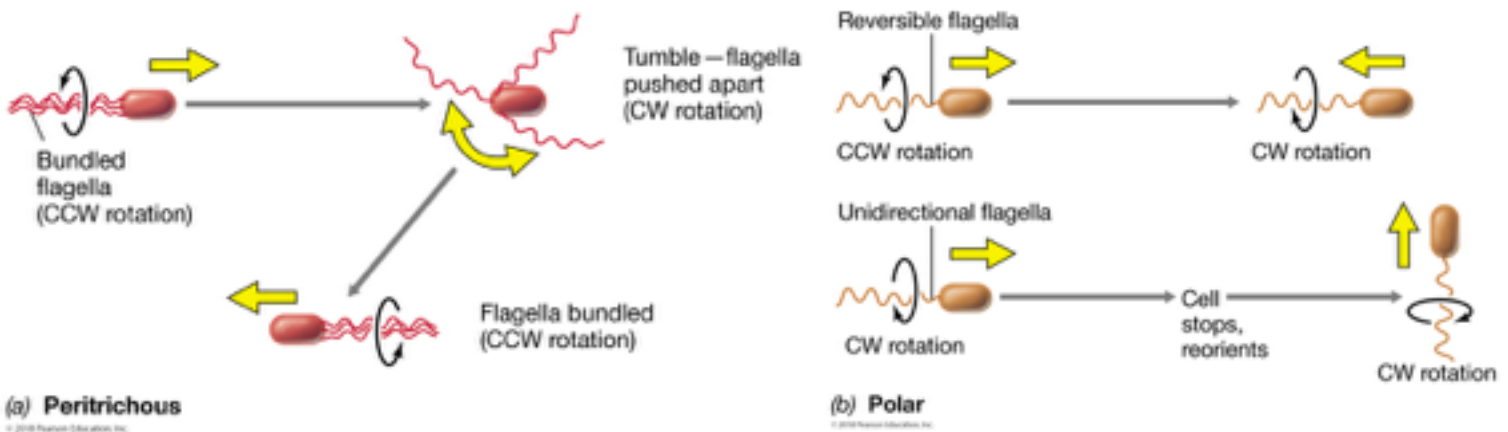
(1) Flagella, Archaeella, and Swimming Motility:

- Flagellar and flagellation: occurs in *Bacteria*. Long, thin appendages (15–20 nm wide) free at one end and anchored into the cell at the other end. Can be anchored to a cell in different arrangements: peritrichous (a), polar (b), lophotrichous (c), amphitrichous. A group of flagella (called a tuft) may arise at one end of the cell, a type of polar flagellation called lophotrichous. When a tuft of flagella emerges from both poles of the cell, flagellation is called amphitrichous.



In peritrichous flagellation, flagella are inserted around the cell surface.

- Archaeella: occurs in *Archaea*. Half the diameter of bacterial flagella (10–13 nm). Move by rotation, and composed of several different filament proteins with little homology to bacterial flagellin. Speeds vary from 0.1–10x *Escherichia coli*; structurally similar to type IV pili.
- Flagella structure:
 - Helical in shape;
 - Consists of several components: filament composed of flagellin.
 - The flagellum motor is a reversible rotating machine composed of several proteins and is anchored in the cytoplasmic membrane and cell wall.
 - Increase or decrease rotational speed relative to strength of proton motive force.
- Movement of flagellated bacteria:



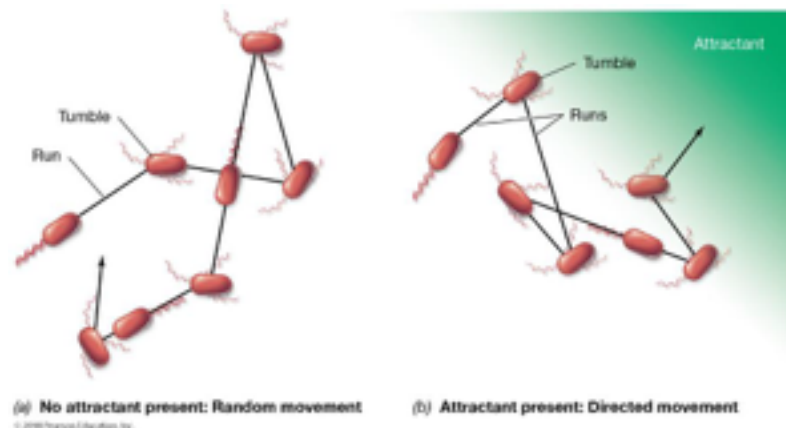
- Structure of Archaeella:
 - Simpler than flagella;
 - Uses ATP rather than the proton motive force as energy source.

(2) Gliding motility:

- Some bacteria are motile but lack flagella. Most of these non-swimming yet still motile cells move by gliding.
- Bacteria only; no *Archaea*.
- Unlike flagellar motility, in which cells stop and then start off in a different direction, gliding motility is a slower and smoother form of movement and typically occurs along the long axis of the cell.
- Cells glide out and move away from the center of the colony
- Requires surface contact.
- Advantageous for finding food sources.
- Mechanism of gliding motility:

Different mechanisms:

- Excretion of polysaccharide slime from pores onto the outer surface of the cell (cyanobacteria). The slime contacts both the cell surface and the solid surface against which the cell moves;
- Type IV pili/twitching motility (retracts and extend pili and twitches at the same time);
- Gliding-specific proteins (adhesion complexes or other specialized proteins).



(3) Chemotaxis:

- Taxis: directed movement in response to chemical or physical gradients.
- Chemotaxis: directed movement of an organism toward (positive chemotaxis) or away from (negative chemotaxis) a chemical gradient.
- Phototaxis: response to light.
- Aerotaxis: response to oxygen.
- Osmotaxis: response to ionic strength.
- Hydrotaxis: response to water.
- Monitor/sample environment with chemoreceptors that sense attractants and repellents;
- Chemotaxis in peritrichously flagellated bacteria (e.g., *E. coli*):
- How do bacteria sense the chemical gradient?
- Bacteria respond to temporal, rather than spatial, differences in chemical concentration (while moving, cells “monitor” their environment by sampling chemicals periodically and comparing the concentration of a particular chemical with that sensed a few moments before).
- It uses complex receptor-cell signalling and gene regulation to achieve direction.

Explain why archaea were deemed sufficiently different from bacterial and eukaryotic cells to warrant their own kingdom of life.

- Carl Woese, a microbiologist studying the genetic sequencing of organisms, developed a new sequencing method that involved splitting the RNA into fragments that could be sorted and compared to other fragments from other organisms. The more similar the patterns between species were, the more closely related the organisms.
- He sequenced a variety of different species and happened upon a group of methanogens that had vastly different patterns than any known prokaryotes or eukaryotes. These methanogens were much more similar to each other than they were to other organisms sequenced, leading Woese to propose the new domain of Archaea.
- His experiments showed that the Archaea were more similar to eukaryotes than prokaryotes, even though they were more similar to prokaryotes in structure. This led to the conclusion that Archaea and Eukarya shared a more recent common ancestor than Eukarya and Bacteria in general. Although Archaea are prokaryotic, they are more closely related to Eukarya and thus cannot be placed within either the Bacteria or Eukarya domains.

Summary of Cell Structures

Cell Structure	Prokaryotes			Cell wall composition	Motility structures	Membrane-bound organelles	Endomembrane system	Ribosomes
	Bacteria	Archaea	Eukaryotes					
Size	~0.5-1 μM	~0.5-1 μM	~5-20 μM	• Peptidoglycan, or • None	Rigid spiral flagella composed of flagellin	No	No	70S
Surface area-to-volume ratio	High	High	Low	• Pseudopeptidoglycan, or • Glycopeptide, or • Polysaccharide, or • Protein (S-layer), or • None	Rigid spiral flagella composed of archaeal flagellins	No	No	70S
Nucleus	No	No	Yes	• Cellulose (plants, some algae) • Chitin (molluscs, insects, crustaceans, and fungi) • Silica (some algae) • Most others lack cell walls	Flexible flagella and cilia composed of microtubules	Yes	Yes (ER, Golgi, lysosomes)	• 80S in cytoplasm and rough ER • 70S in mitochondria, chloroplasts
Genome characteristics	• Single chromosome • Circular • Haploid • Lacks histones	• Single chromosome • Circular • Haploid • Contains histones	• Multiple chromosomes • Linear • Haploid or diploid • Contains histones					
Cell division	Binary fission	Binary fission	Mitosis, meiosis					
Membrane lipid composition	• Ester-linked • Straight-chain fatty acids • Bilayer	• Ether-linked • Branched isoprenoids • Bilayer or monolayer	• Ester-linked • Straight-chain fatty acids • Sterols • Bilayer					

Compare and contrast autotrophs and heterotrophs.

- Autotroph: an organism capable of biosynthesizing all cell material from CO₂ as the sole carbon source (e.g. photosynthesis by plants, some bacteria)
- Heterotroph: an organism that uses organic compounds as a carbon source (animals, protists, fungi).

Compare and contrast aerobic and anaerobic respiration.

Define fermentation and explain why it does not require oxygen.

- Fermentation is a form of anaerobic catabolism in which organic compounds both donate electrons and accept electrons, and redox balance is achieved without need for external electron acceptors.
- Does not require oxygen because uses an organic molecule (commonly pyruvate) as a final electron acceptor. Thus, does not involve an electron transport system and thus does not require a final oxygen acceptor such as oxygen in aerobic respiration.
- If respiration does not occur, NADH must be reoxidized to NAD⁺ for reuse as an electron carrier for glycolysis, the cell's only mechanism for producing any ATP, to continue.

Compare and contrast fermentation and anaerobic respiration.

	Aerobic Respiration	Anaerobic Respiration
Definition	Aerobic respiration uses oxygen.	Anaerobic respiration is respiration without oxygen; the process uses a respiratory electron transport chain but does not use oxygen as the electron acceptors.
Cells that use it	Aerobic respiration occurs in most cells.	Anaerobic respiration occurs mostly in prokaryotes
Amount of energy released	High (36-38 ATP molecules)	Lower (Between 36-2 ATP molecules)
Stages	Glycolysis, Krebs cycle, Electron Transport Chain	Glycolysis, Krebs cycle, Electron Transport Chain
Products	Carbon dioxide, water, ATP	Carbon dioxide, reduced species, ATP
Site of reactions	Cytoplasm and mitochondria	Cytoplasm and mitochondria
Reactants	glucose, oxygen	glucose, electron acceptor (not oxygen)
combustion	complete	incomplete
Production of Ethanol or Lactic Acid	Does not produce ethanol or lactic acid	Produce ethanol or lactic acid

Type of Metabolism	Example	Final Electron Acceptor	Pathways Involved in ATP Synthesis (Type of Phosphorylation)	Maximum Yield of ATP Molecules
Aerobic respiration	<i>Pseudomonas denitrificans</i>	O_2	EMP glycolysis (SLP)	2
			Krebs cycle (SLP)	2
			Electron transport and chemiosmosis (OP)	34
			Total	38
Anaerobic respiration	<i>Paracoccus denitrificans</i>	NO_3^- , SO_4^{2-} , Fe^{3+} , CO_2 , - other inorganics	EMP glycolysis (SLP)	2
			Krebs cycle (SLP)	2
			Electron transport and chemiosmosis (OP)	1-32
			Total	5-36
Fermentation	<i>Candida utilis</i>	Organics (usually pyruvate)	Fermentation	0
			Total	2

Module 3: Microbial Growth and Regulation

Part 1: Microbial Growth

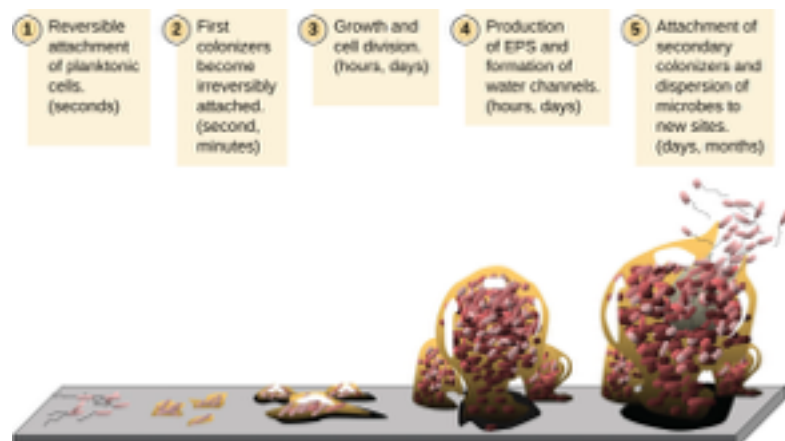
Differentiate between sessile growth, planktonic growth and biofilms.

- Whether dividing by binary fission or some form of budding, microbial cells can grow either in suspension or attached to surfaces. The suspended lifestyle, called *planktonic growth*, is the way many bacteria live in nature. Many other microorganisms show *sessile growth*, meaning that they grow attached to a surface.
- These attached cells can develop into a *biofilm*, which is a community of microorganisms (mostly bacteria) that is fixed on a surface and maintained there via the secretion of an adhesive and protective matrix of polysaccharides. Biofilms trap nutrients for microbial growth and help prevent detachment of cells on dynamic structures, such as in flowing systems.

Describe the formation, biochemistry and characteristics of biofilms.

- Biofilm formation has four basic stages:

- (1) Attachment: random collision of cells with a surface accounts for the initial cell attachment. Cell attachment is facilitated by structures such as flagella and pili or by proteins on the cell surface.
- (2) Colonization
- (3) Development: attachment of



a cell to a surface is a signal for the expression of biofilm-specific genes. The latter include genes encoding proteins that produce intercellular signaling molecules and extracellular polysaccharides that initiate matrix formation.

(4) Dispersal: biofilms are not static entities and cells can be released from the biofilm through an active process of dispersal.

- Some form multilayered sheets with different organisms in each individual layer. These biofilms are called *microbial mats*; mats composed of various phototrophic and chemotrophic bacteria are common in hot springs and in intertidal regions.
- They are ubiquitous, concern all matter of life (animal, plants, fungi, aquatic or soil environments, etc...) or inert material (technologies: medical devices, blockage of petrol pipeline by sludge, biofilm on boats reduce glide, etc.).
- What are biofilms composed of?
 - The composition can be homogenous or heterogenous (i.e. multiple bacterial species).
 - They are complex and dynamic systems with a growth and death balance.
 - Even within a homogenous biofilm, certain cells express different genes and perform different functions within the community. For example, some cells can display greater motility whilst others sporulate. This is akin to multicellularity in some aspects.
 - The composition can depend on the nature of material on which biofilms are formed (biotic or abiotic).
 - The molecular constitution of biofilms is largely constituted of:

(A) extracellular polymeric substances (EPS), which are polysaccharides in gram-positive and mostly cellulose in gram-negative;

(B) proteins (type IV pili);

(C) eDNA (extracellular DNA): under the control of quorum sensing, certain bacteria will release their DNA extracellularly. Other bacteria can die and de facto release their DNA as they die. DNA is negatively charged and if it accumulates around bacteria, may protect them from certain antimicrobials which are generally cationic molecules (such as antibiotics). DNA is sticky and may help the biofilm structure and coherence, as well as make it difficult for immune cells or competitors to penetrate the structure. This could favour the exchange of genetic material within the biofilm communities, and could serve as a source of nutrients in oligotrophic conditions.

★ The composition of a biofilm can vary within a single bacterial species. For example, staphylococci can have a polysaccharide, protein/eDNA, fibrin or amyloid biofilm.

- They are a common growth form for bacteria because the intensely interwoven nature of the structure prevents harmful chemicals (e.g. antibiotics) from penetrating, prevent bacterial grazing by protists, and prevent washing away of cells.
- This dynamic system, its cell differentiation and complex architecture (matrix can be filamentous or not) make the bacterial colonies 10-1000 times more resistant to antimicrobials.

Biofilm formation in *P. aeruginosa*

- Signals guide bacteria in transitioning from planktonic growth to life in a semisolid matrix. The actual switch from planktonic to biofilm growth in many bacteria is triggered by cellular accumulation of the regulatory molecule cyclic diguanosine monophosphate (c-di-GMP).

- C-di-GMP is widely distributed only in Bacteria. Its synthesis and degradation depend on environmental and cellular cues, and its synthesis triggers physiological events (e.g. c-di-GMP binds proteins that reduce activity of flagellar motor, regulates attachment proteins on cell surface, and mediates biosynthesis of extracellular matrix polysaccharides).
- Besides the intracellular activities triggered by c-di-GMP, intercellular communication by quorum sensing necessary for development and maintenance of *P. aeruginosa* biofilms.
- As acyl homoserine lactones (AHLs) accumulate, they signal to adjacent *P. aeruginosa* cells that population is growing. This also triggers expression of genes necessary for biofilm formation, including those that increase extracellular polysaccharide and c-di-GMP synthesis.
- Elevated c-di-GMP initiates extracellular polysaccharide production and leads to decreased flagellar function.

Biofilm formation in *V. cholerae*

- Also uses both inter- and intracellular signaling to control biofilm formation
- While signaling by c-di-GMP activates expression of genes for biofilm formation, quorum sensing acts in an opposite manner compared to *P. aeruginosa*. Accumulation of quorum signaling molecules *represses* biofilm formation genes, *activates* flagellar and virulence genes.
- Thus, biofilm formation is triggered by low cell densities, repressed by high cell densities. It is more likely to occur when *V. cholerae* is found in its natural environment where resources are more scarce, thus leading to smaller populations, compared to intestinal cells where nutrients are more plentiful. This allows cell to attach to marine surfaces (e.g., plankton, crustaceans, sediments) for better access to nutrients and protection.

Name different categories of microbes with requirements for growth with or without oxygen: obligate aerobe, obligate anaerobe, facultative anaerobe, aerotolerant anaerobe, microaerophile.

Group	Relationship to O ₂	Type of metabolism	Example ^a	Habitat ^b
Aerobes				
Obligate	Required	Aerobic respiration	<i>Micrococcus luteus</i> (B)	Skin, dust
Facultative	Not required, but growth better with O ₂	Aerobic respiration, anaerobic respiration, fermentation	<i>Escherichia coli</i> (B)	Mammalian large intestine
Microaerophilic	Required but at levels lower than atmospheric	Aerobic respiration	<i>Spirillum volutans</i> (B)	Lake water
Anaerobes				
Aerotolerant	Not required, and growth no better when O ₂ present	Fermentation	<i>Streptococcus pyogenes</i> (B)	Upper respiratory tract
Obligate	Harmful or lethal	Fermentation or anaerobic respiration	<i>Methanobacterium formicicum</i> (A)	Sewage sludge, anoxic lake sediments

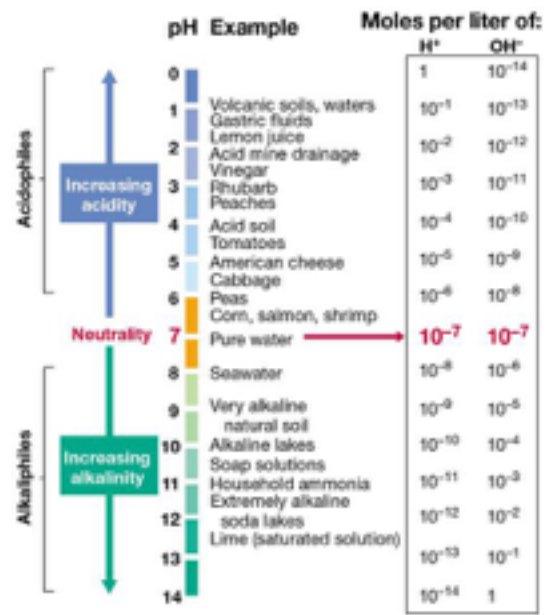
^aLetters in parentheses indicate phylogenetic status (B, Bacteria; A, Archaea). Representatives of either domain of prokaryotic cells are known in each category. Most eukaryotes are obligate aerobes, but facultative aerobes (for example, yeast) and obligate anaerobes (for example, certain protozoa and fungi) are known.

^bListed are typical habitats of the example organisms; many others could be listed.

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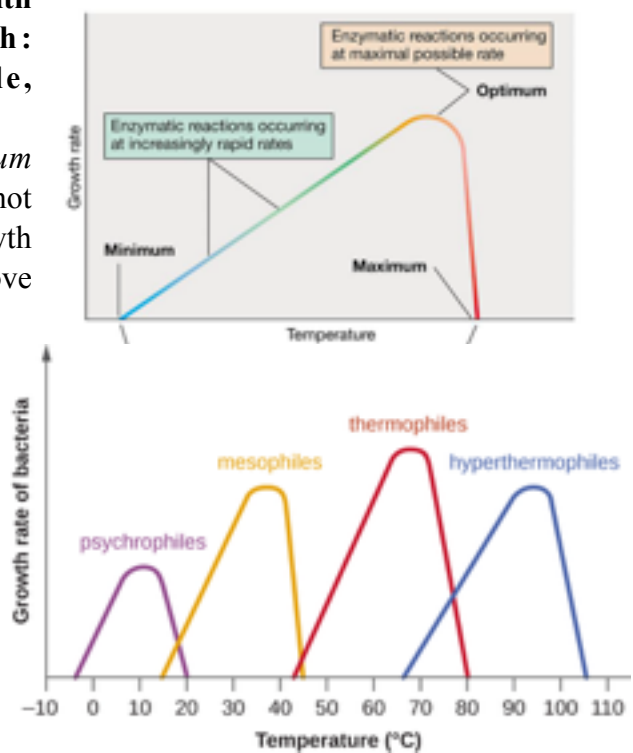
Describe the different categories of microbes with pH requirements for growth: acidophiles, neutrophiles, and alkaliphiles.

- Most natural environments have a pH between 3 and 9, and organisms with pH growth optima in this range are most common.
- The intracellular pH must stay relatively close to neutral (pH 5–9) even if the external pH is highly acidic or basic.
- Microbial culture media typically contain buffers to maintain constant pH.
- *Neutrophiles*: organisms that grow optimally at pH 5.5–7.9.
- *Acidophiles*: organisms that grow best at low pH (< 5.5). Stability of the cytoplasmic membrane is a critical factor. When the pH is raised to neutrality, the cytoplasmic membranes of strongly acidophilic bacteria are destroyed and the cells lyse. This indicates that these organisms are not just acid-tolerant but that high concentrations of protons are actually required for cytoplasmic membrane stability. This would make them obligate acidophiles because their membranes are destroyed at neutral pH.
- *Alkaliphiles*: organisms that grow best at high pH (≥ 8). Found in soda lakes and high-carbonate soils. Certain alkaliphiles are used commercially (e.g. they excrete hydrolytic enzymes such as proteases and lipases that maintain their activities at alkaline pH. These enzymes are added to laundry detergents to remove protein and fat stains). Some use sodium (Na^+) motive force rather than proton motive force to drive transport reactions and rotate its flagellum.



Describe different categories of microbes with temperature requirements for growth: psychrophile, psychrotrophs, mesophile, thermophile, hyperthermophile.

- *Cardinal temperatures* refer to the *minimum* temperature below which bacterial growth is not possible, an *optimum* temperature at which growth is most rapid, and a *maximum* temperature above which growth is not possible. The range for any given organism is typically < 40 °C.
- Microorganisms can be classified into groups by their growth temperature optima:
(A) *Psychrophiles*: low temperature optima; an optimal growth temperature of 15 °C or



lower, a maximum growth temperature below 20 °C, and a minimum growth temperature of 0 °C or lower. Found in constantly cold environments.

- In order to adapt to life in the cold, psychrophiles produce enzymes that function optimally in the cold and that may be denatured or otherwise inactivated at even very moderate temperatures. Several cold-active enzymes show a greater content of α -helices and smaller content of β -sheets in their secondary structures. This allows for greater flexibility for catalyzing their reactions at cold temperatures. Cold-active enzymes also have more polar and fewer hydrophobic amino acids and fewer weak bonds (e.g. hydrogen and ionic bonds) compared to a corresponding enzyme in mesophiles.
- Another characteristic feature of psychrophiles is that their cytoplasmic membranes remain functional at low temperatures because of their higher unsaturated and shorter-chain fatty acid content, which helps membrane remain in a semifluid state at low temperatures (allowing transport and bioenergetic functions). Some psychotrophic bacteria even contain *polyunsaturated fatty acids*, which remain flexible at very low temperatures unlike monounsaturated or fully saturated fatty acids, which tend to stiffen.
- Other molecular adaptations include *cold shock proteins*, which are a type of molecular chaperone and have several functions that include maintaining cold-sensitive proteins in active conformation, binding specific mRNAs and facilitating their translation under cold conditions. There are also *cryoprotectants* (e.g. antifreeze proteins, certain solutes such as glycerol or certain sugars), which are produced in large amounts at cold temperatures and help prevent formation of ice crystals which could puncture cytoplasmic membrane. Highly psychotrophic bacteria often produce abundant levels of *exopolysaccharide* cell surface slime, and these slime layers confer cytoprotection.

(B) *Thermophiles*: organisms with growth temperature optima between 45°C and 80°C.

(C) *Hyperthermophiles*: organisms with optima greater than 80°C. They inhabit hot environments, including boiling hot springs and seafloor hydrothermal vents, that can experience temperatures in excess of 100°C.

Above 65°C, only prokaryotic life forms thrive, but an extensive diversity is present.

In order for thermophiles and hyperthermophiles to survive high temperatures, their enzymes and other proteins are stable and actually function optimally at high temperatures. This characteristic is due to:

- Critical amino acid substitutions in a few locations that affect protein structure and function to provide heat-tolerant folds in proteins.
- Increased number of ionic bonds between basic and acidic amino acids and highly hydrophobic interiors that help to resist unfolding in the aqueous cytoplasm.
- Production of solutes (e.g. di-inositol phosphate, diglycerol phosphate) which help to stabilize proteins against thermal denaturation.

Besides enzymes and other macromolecules in cell, the cytoplasmic membranes of thermophiles and hyperthermophiles must be heat-stable.

- In Bacteria, the cytoplasmic membrane has a higher content of long-chain and saturated fatty acids and fewer unsaturated fatty acids. Saturated fatty acids form a stronger hydrophobic

environment than unsaturated fatty acids, and longer-chain fatty acids have a higher melting point than shorter-chain fatty acids. These properties all increase membrane stability.

- Archaea do not contain fatty acids but instead have C₄₀ hydrocarbons made of repeating isoprene units bonded to glycerol phosphate. Also, structure of cytoplasmic membrane of many hyperthermophiles forms a lipid monolayer rather than a bilayer. This monolayer structure covalently links both halves of the membrane, preventing it from melting at high growth temperatures of hyperthermophiles.

Enzymes from thermophiles and hyperthermophiles are commercially useful. These heat-stable enzymes catalyze biochemical reactions at high temperatures and are more stable than enzymes from mesophiles. For example, Taq polymerase is used for polymerase chain reaction (PCR), a technique for amplifying DNA and a major tool of modern biology.

Name different categories of microbes with specific growth requirements other than oxygen, pH, and temperature, such as altered salinity (halophiles) and light.

- *Osmolarity*: water availability is an important factor affecting growth of microorganisms. It is expressed in water activity (a_w), the ratio of vapour pressure of air in equilibrium with a substance or solution to vapour pressure of pure water. Values of a_w vary between 0 (no free water, driest) and 1 (pure water, wettest).
- *Halophiles*: organisms that grow best at $a_w = 0.98$ (seawater); have a specific requirement for NaCl.
- *Halotolerant*: organisms that can tolerate some additional dissolved solutes but generally grow best in the absence of the added solute.
- *Extreme halophiles*: organisms that require very high levels (15% to 30%) of NaCl; often unable to grow at lower concentrations.
- *Osmophiles*: organisms that live in environments high in sugar as solute.
- *Xerophiles*: organisms able to grow in very dry environments. Lowest $a_w = 0.61$; physiochemical constraints on obtaining water at lower a_w . Deserts can have an $a_w = 0.1$ during the day, but they absorb water at night and it does rain sometimes, which is sufficient to support bacterial growth.
- *Compatible solutes*: an organism switching from high a_w to low a_w can maintain its osmolarity by pumping in or synthesizing compatible solutes in its cytoplasm. It is called compatible because its presence does not inhibit the organism's biochemistry.

Explain what is quorum sensing.

- A regulatory system that monitors the population level and controls gene expression based on cell density. It is a regulatory mechanism that assesses population density.
- Therefore, in low cell population density, single bacteria produce autoinducers which are released into their external environment. By contrast, in high cell-population density, there will be an accumulation of autoinducers released into the external environment that signals the bacteria to express certain genes.

- Therefore, quorum sensing allows a population to communicate and act upon a certain trigger such as the release of light in *V. fischeri*. Quorum sensing is also a model used to observe the first steps in multicellular communication.

Explain the roles and advantages in cell-to-cell communication and coordination of cellular activities through quorum sensing.

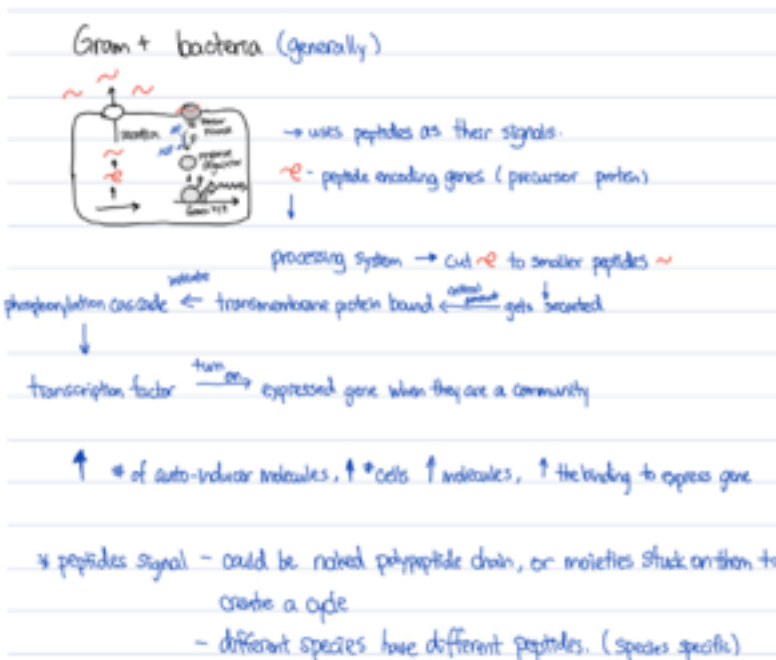
- Many bacteria use this approach to ensure that sufficient cell numbers are present before initiating activities that require a certain cell density to work effectively (e.g. release of toxins in unison to cause disease).
- In some species, regulatory pathways are controlled by the density of cells of their own kind, making quorum sensing an effective means to assess population density, and thus, determine if a particular pathway is to be initiated or not.

Explain what is an autoinducer.

An autoinducer is a signalling molecule such as a hormone or a pheromone, that when trapped at abundant levels in regions of high cell-population density, acts as an indicator for cells to express a certain phenotype. This is seen in *V. fischeri* when the autoinducer (AHL) or signal for light is found at abundant levels within the squid's specialized light organ.

Describe molecular mechanisms of quorum sensing in Gram – and Gram + bacteria (use one example for each).

- In gram-negative bacteria, we can look at the mechanism for quorum sensing using the Lux system in *V. fischeri*:



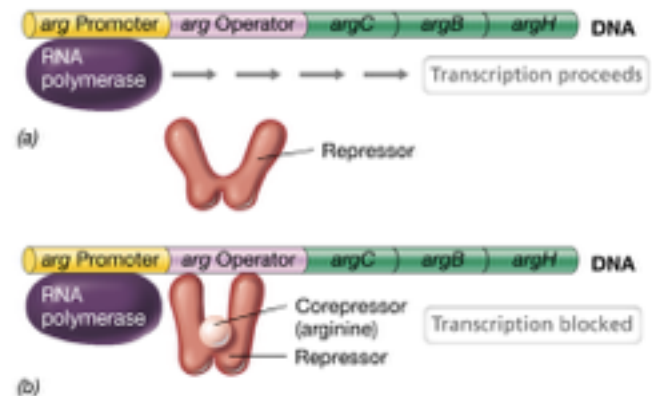
Part 2: Mechanisms of Regulation

Compare and contrast differences and similarities in the transcriptional control mechanisms between bacteria, archaea and eukarya.

(1) Negative and positive gene regulation in prokaryotes:

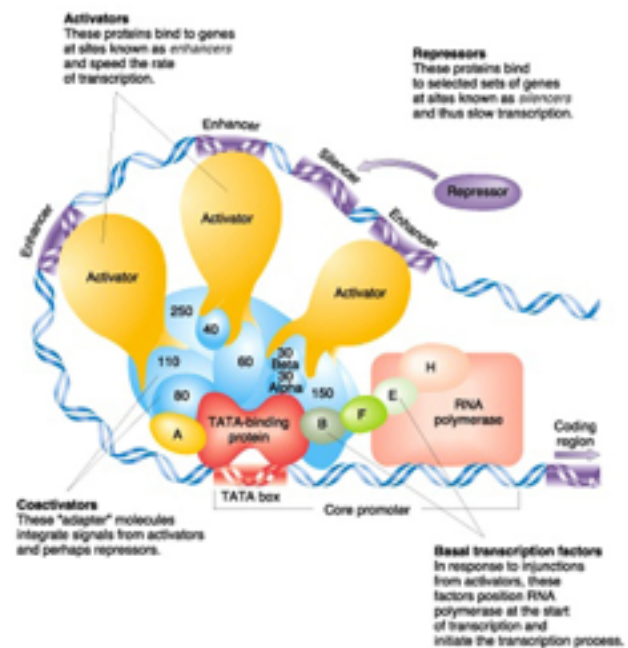
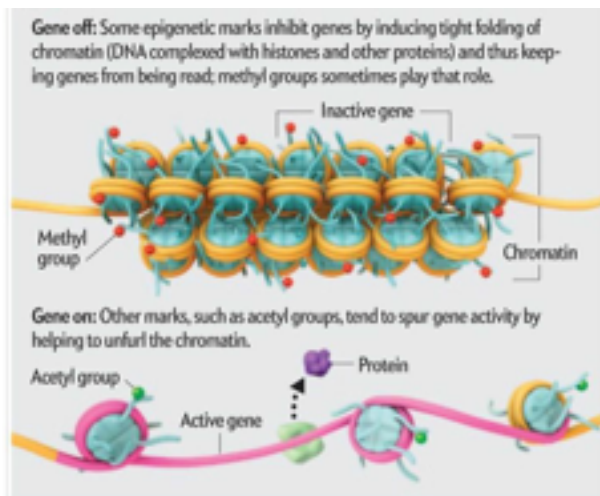
- Negative gene regulation *prevents* transcription, resulting in less mRNA available for translation and thus a lesser amount of its protein product in cell.

- Repression: In (a), the *operon* (cluster of consecutive genes whose expression is under control of a single regulatory site/operator) is transcribed because *repressor* is unable to bind to the operator. In (b), after a *corepressor* (small molecule) binds to repressor, the repressor binds to the operator and blocks transcription; mRNA and the proteins it encodes are not made. For *argCBH* operon, the amino acid arginine is the corepressor that binds to the arginine repressor.



- Induction: a repressor protein bound to operator blocks the binding of RNA polymerase. An inducer molecule binds to repressor and inactivates it so it can no longer bind to operator. RNA polymerase then transcribes DNA and makes mRNA for this operon.
- In positive gene regulation, regulatory protein is *activator* that activates binding of RNA polymerase to DNA. In absence of *inducer*, neither activator protein nor RNA polymerase can bind to the DNA. Inducer molecule binds to activator protein, which in turn binds to activator-binding site. This recruits RNA polymerase to bind to promoter and begin transcription.

(2) Eukaryotic gene regulation:



Overview of transcriptional regulation



Compare and contrast differences in the genetic and genomic structures between bacterial, archaeal and eukaryotic cells.

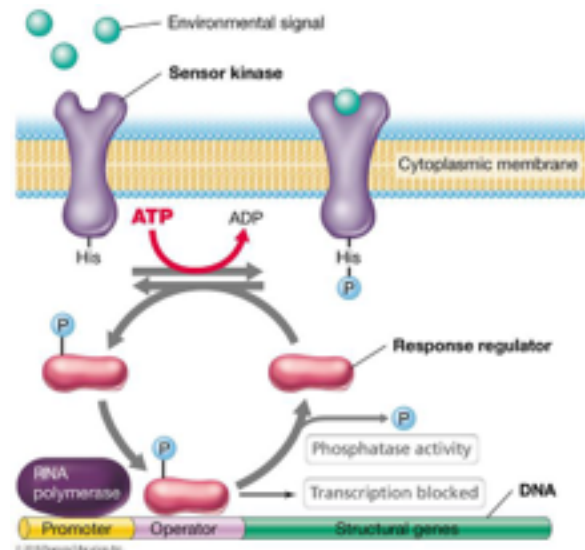
- Operons occur in prokaryotic and archeal cells, but not in eukaryotes where each gene made on individual mRNAs, each gene having its own promoter.
- Enhancers are eukaryotic proteins (transcription factors) that bind DNA sequence elements located as far away as several thousand base pairs from a gene. They they can help activate transcription of the relevant gene.
- Eukaryotic genes have introns (non-coding sequences), mostly code for a single protein, whereas prokaryotic genes lacks introns and they form this operon which codes for number of proteins.

	Bacteria	Archaea	Eukarya
Nuclear membrane	No (nucleoid)	No (nucleoid)	Yes
Genome structure	Single circular chromosome (1n)	Single circular chromosome (1n)	Single or multiple linear chromosome(s) (1n or 2n)
Plasmids	Common	Common	Rare
Gene structure	Operons, no introns	Introns present in some genes	Intronic sequences present in most cells
RNA polymerase	Single polymerase	Single polymerase, eukaryal-like RNA Pol II	Three main polymerases (RNA pol I, II and III)
Histones	Histone-like proteins	Yes, nucleosomes are tetramers	Yes, nucleosomes are octamers
Regulation system	Activator and repressor proteins	Activator and repressor proteins	Chromatin modifiers and transcription factors

Describe what is a two-component system.

- A two-component system is a signal transduction system that contains two parts: a specific *sensor kinase protein* usually located in cytoplasmic membrane, and a *response regulator protein*, present in cytoplasm.

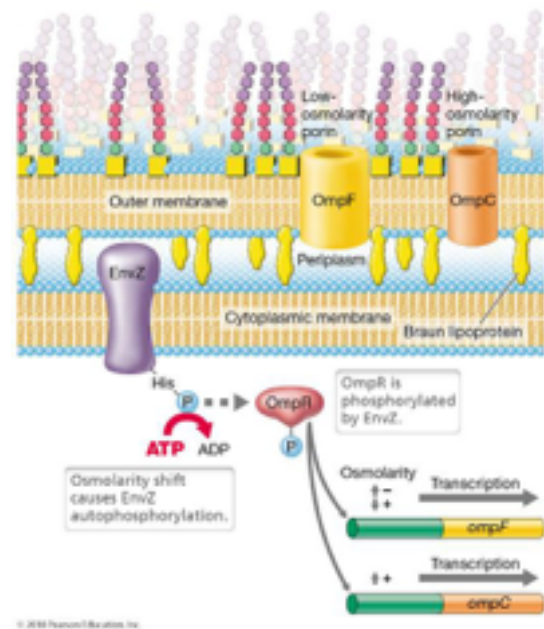
- (1) A kinase is an enzyme that phosphorylates compounds, typically using phosphate from ATP. Sensor kinases detect a signal from environment and autophosphorylate. Phosphate then transferred from sensor to another protein in cell.
 - (2) The response regulator is a DNA-binding protein that regulates transcription in positive or negative fashion.
- This system also has a feedback loop in order to complete the regulatory circuit and terminate the response. It uses a phosphatase, an enzyme that removes the phosphate from the response regulator at a constant rate.



In this example, regulation is negative; phosphorylated response regulator functions as a repressor that binds DNA, thus blocking transcription. Once dephosphorylated, response regulator is released and transcription is permitted.

Explain how a two-component system functions (mechanism) using an example.

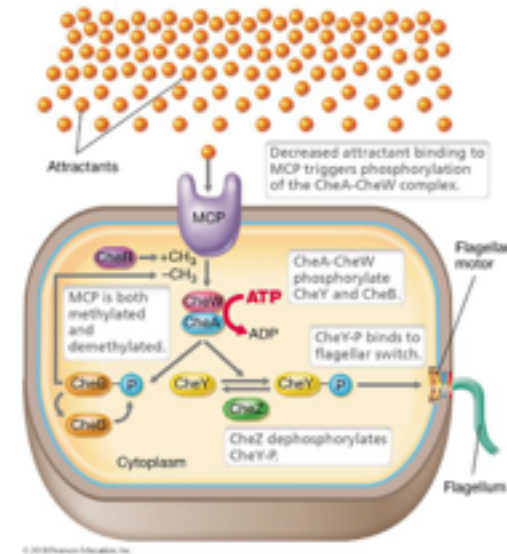
- In *E. coli*, osmolarity of environment controls the relative levels of proteins OmpC and OmpF in outer membrane. These proteins are *porins*, proteins that allow metabolites to cross outer membrane of gram-negative bacteria. If osmotic pressure is *low*, synthesis of OmpF (porin with larger pore) increases; if osmotic pressure is *high*, OmpC (porin with smaller pore) made in larger amounts.
- EnvZ, a sensor kinase in cytoplasmic membrane, detects changes in osmotic pressure. EnvZ autophosphorylates and transfers its phosphate group to OmpR, the response regulator of this system.
- In low osmotic pressure, phosphorylated OmpR *activates* transcription of *OmpF* gene. In high osmotic pressure, OmpR *represses* transcription of *OmpF* gene and *activates* transcription of *ompC* instead.



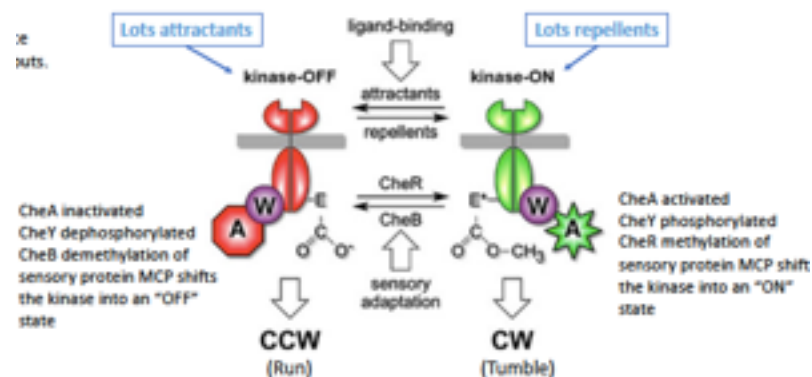
Describe in details and discuss at length all the mechanisms of stress responses in bacteria we have seen throughout the course.

- (1) Regulation of chemotaxis: modified two-component system used in chemotaxis to:

- Sense temporal changes in attractants or repellents:
 - Sensory proteins in cytoplasmic membrane sense attractants and repellents, and interact with cytoplasmic sensor kinases.
 - Methyl-accepting chemotaxis proteins (MCPs) bind attractant or repellent and initiate flagellar rotation, and interact with CheA (sensor kinase) and CheW (an adaptor protein).
- Controlling flagellar rotation: controlled by CheY protein. CheY results in counterclockwise rotation and runs. CheY-P results in clockwise rotation and tumbling.
- Adaptation: Stop responding and reset (feedback loop). Allows the system to reset itself to continue to sense the presence of a signal.
 - Relies on response regulator CheB;
 - Involves modification of MCPs: methylation stops response to attractants and increases response to repellants. The opposite is true; unmethylated MCPs are highly sensitive to attractants and insensitive to repellents.

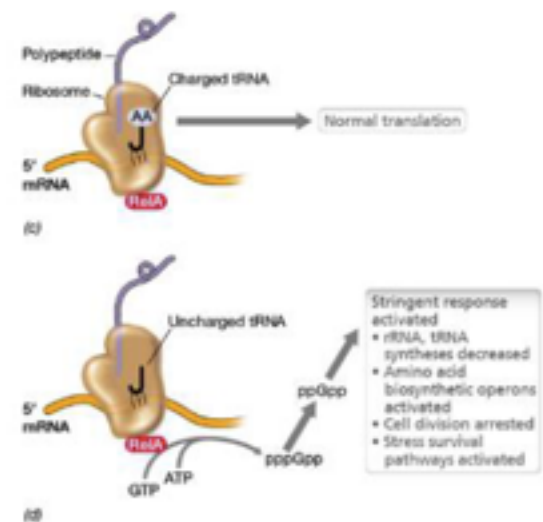


- During sensory adaptation, changes in receptor methylation level shift signaling complexes toward the opposing state to restore a balance between CCW and CW signal outputs.
 - If the concentration of attractant is high, CheA is not activated and the kinase is off.
 - Gradually, CheR will methylate MCPs, switching the kinase system into an ON state even though the attractants concentration stays high.
 - Eventually CheA will autophosphorylate and activate CheY. CheY-P will cause the cell to tumble.



(2) Stringent response:

- Used to survive nutrient deprivation, environmental stress, and antibiotics;
- Shuts down macromolecule synthesis and activates stress survival pathways.
- Environment/habitat determines response. Example: voiding *E. coli* in feces reduces nutrients, initiates ppGpp synthesis, stringent response occurs.



(3) Phosphate (Pho) region:

- P essential for DNA, RNA, membrane synthesis, energy

generation, and cell signaling;

- Inorganic phosphate (PO_4^{3-} or P_i) often limiting in environment;
- Two-component regulatory system regulating extracellular enzymes, P_i transporters, and storage enzymes;
- Represses many genes and controls some aspects of pathogenesis.

(4) Heat shock response

- Heat shock proteins: counteract damage of denatured proteins and help cell recover from temperature stress;
- Very ancient proteins
- Induced by heat, exposure to ethanol or ultraviolet (UV) radiation;
- Three major classes: Hsp70 (DnaK in *E. coli*), Hsp60 (GroEL in *E. coli*), and Hsp10 (GroES in *E. coli*);
- Largely controlled by alternative sigma factor RpoH.

(5) The general stress response: the RpoS regulon

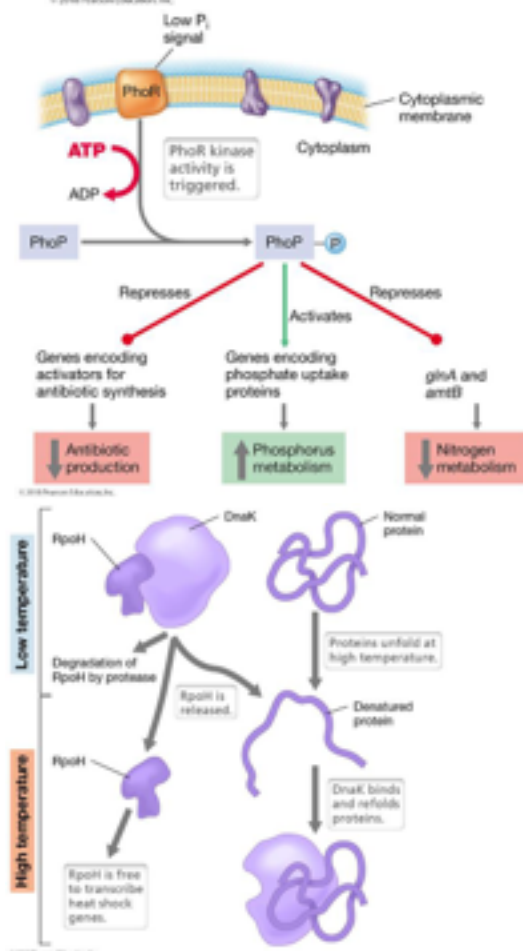
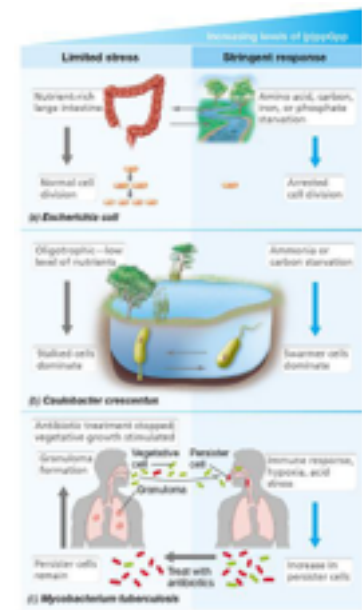
- Allows cells to withstand harsh conditions (e.g., extreme pH, oxidative stress);
- Controlled by alternative sigma factor RpoS (stationary phase sigma factor)
- RpoS regulon includes 400+ genes: nutrient limitation, resistance to DNA damage, biofilm formation, responses to osmotic, oxidative, and acid stress.

Explain the concepts of persistence and dormancy in antibiotic resistant bacteria.

- *Persistence*: population of antibiotic-sensitive bacteria produces rare cells that are *transiently* tolerant to multiple antibiotics.
- *Persisters* are genetically identical but dormant (viable but do not grow). Dormancy prevents antibiotic from killing cell. When treatment is stopped, cells emerge from dormancy and grow. Believed to be cause of recurring *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa* infections.

Describe the mechanism of persistence using the Toxin-Antitoxin module system.

- Toxin-Antitoxin (TA) modules:
 - Genetic loci that encode a toxin whose production inhibits cell growth and an antitoxin.
 - Found in almost all Bacteria and many Archaea;
 - Play a role in normal physiology and pathogenicity;



- Toxic activity thought to promote cellular adaption by slowing cell growth to ensure survival during stress.
- *hipAB* genes encode TA module that triggers persistence in *E. coli*.

HipA: toxin that inhibits translation

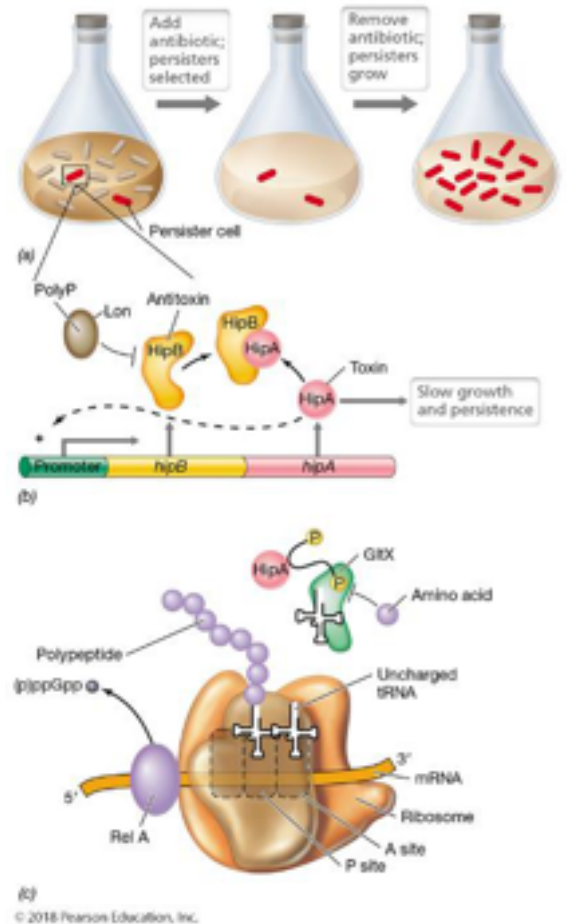
HipB: antitoxin

- Normally HipA and HipB form a stable complex.
- If Lon is activated, HipB is degraded, translation is inhibited, and growth arrested.
- HipA targets glutamyl-tRNA synthetase (GltX), leading to ribosome stalling and activation of RelA.
- Stalling inhibits translation and production of the alarmone (p)ppGpp (guanosine tetraphosphate and guanosine pentaphosphate) by RelA and induction of stringent response pathway.

Cells that have triggered stringent response become dormant.

Part 4: Viruses and their replication

Describe the characteristics used to identify viruses as obligate intracellular parasites.



Characteristics of Viruses

Infectious, acellular pathogens

Obligate intracellular parasites with host and cell-type specificity

DNA or RNA genome (never both)

Genome is surrounded by a protein capsid and, in some cases, a phospholipid membrane studded with viral glycoproteins
(naked viruses) (enveloped viruses)

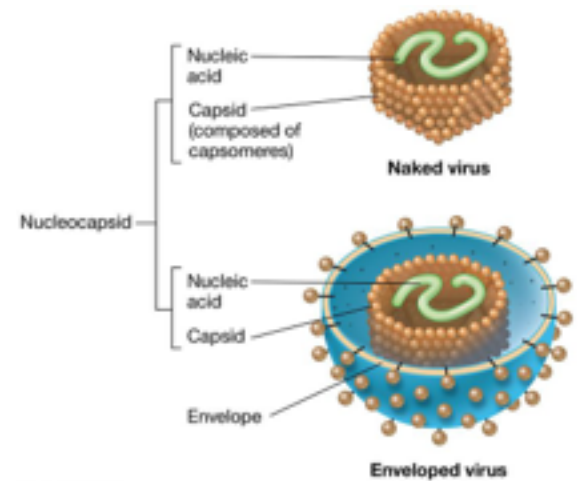
Lack genes for many products needed for successful reproduction, requiring exploitation of host-cell genomes to reproduce

- * Not considered living entities, viruses rely on host cell for energy, metabolic intermediates, and protein synthesis, and so they are obligate intracellular parasites.

Describe the structures and composition of viruses.

- Most viruses are smaller than prokaryotic cells; range from 0.02 to 0.3 μm .
- Viral components and activities:

- Capsid: the protein shell that surrounds the genome of a virus particle.
- Naked viruses (e.g., most bacterial viruses) have no other layers.
- Enveloped viruses (e.g., many animal viruses) have an outer layer consisting of a phospholipid bilayer (from host cell membrane) and viral proteins.
- Nucleocapsid: nucleic acid + protein in enveloped viruses.

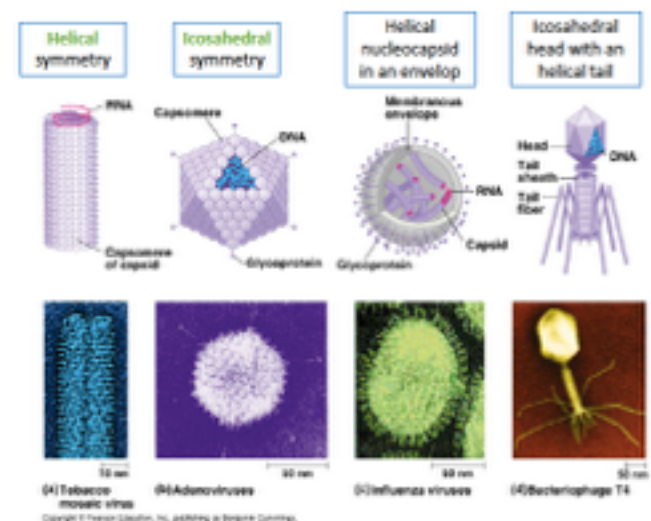


- Structure of the virion:

- (1) Helical
 - (2) Icosahedral
 - (3) Complex
- (a) Naked
 - (b) Enveloped

- Virion structure: naked viruses

- Helical symmetry: length of virus determined by length of nucleic acid. Width of virus determined by size and packaging of capsomeres (subunit of capsid).



- Icosahedral symmetry: most efficient arrangement of subunits in a closed shell. Requires fewest capsomeres.

- Virion structure: enveloped viruses

- Have lipoprotein membrane surrounding nucleocapsid;
- RNA or DNA genomes;
- Envelope proteins attach to and infect animal host cell;
- Relatively few enveloped plant or bacterial viruses because of cell walls surrounding cell membrane;
- Entire virion enters animal cell during infection.
- Enveloped viruses exit more easily.

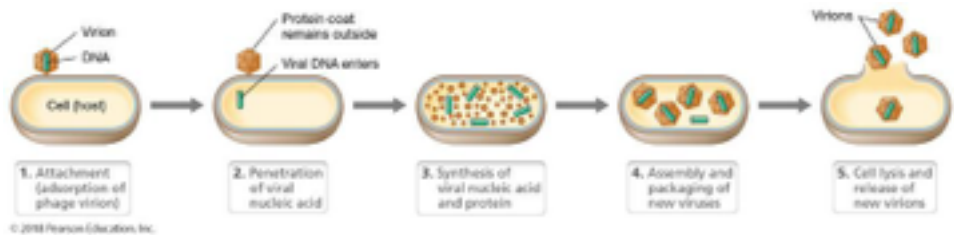
- Enzymes inside virions:

- Lysozyme: makes hole in cell wall to allow nucleic acid entry. Also lyses bacterial cell to release new virions.
- Neuraminidases: destroy glycoproteins and glycolipids. Allows liberation of viruses from cell.

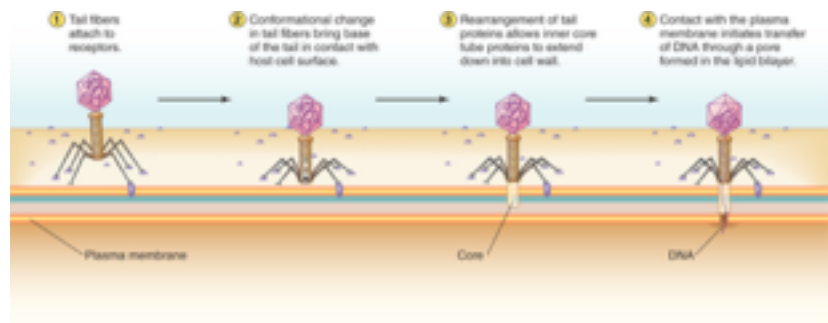
- Nucleic acid polymerases: RNA replicases are RNA-dependent RNA polymerases, and RNA reverse transcriptase are RNA-dependent DNA polymerase in retroviruses.

Differentiate among bacteriophages, plant viruses, and animal viruses.

- Bacteriophage: a type of virus that infects bacteria.
- Major difference between prokaryotic and eukaryotic viruses is nucleic acid entry in prokaryotes and virion entry in eukaryotes.

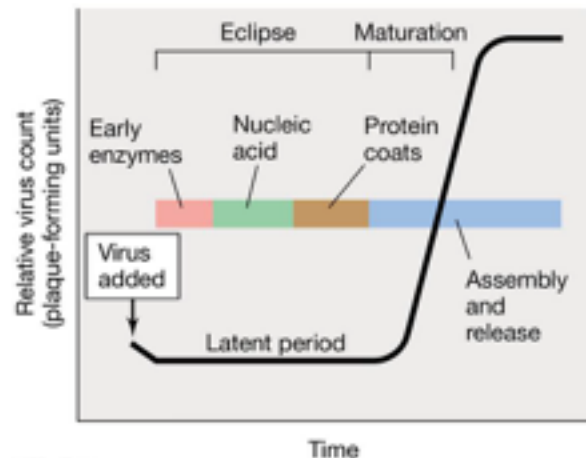


Phases of viral replication in a permissive (supportive) host
attachment (adsorption) of the virion
penetration (entry, injection) of the virion nucleic acid
synthesis of virus nucleic acid and protein by host cell metabolism as redirected by virus
assembly of capsids and packaging of viral genomes into new virions
release of mature virions from host cell



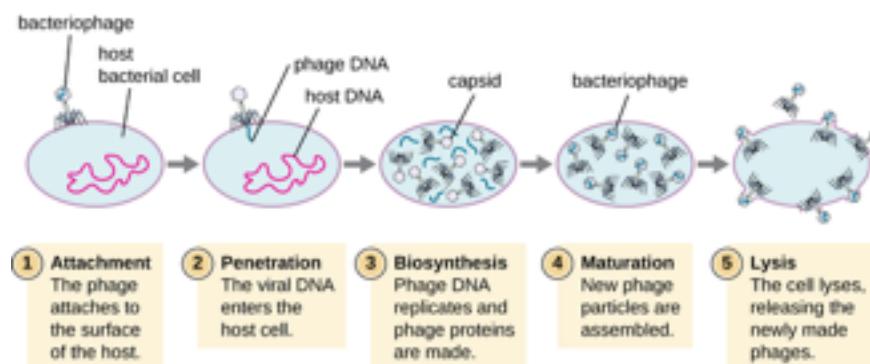
Describe the general characteristics of viral life cycles and its different phases.

- Virus replication is typically characterized by a one-step growth curve: increase occurs when cells burst.
- Eclipse: genome replicated and proteins translated
- Maturation: packaging of nucleic acids in capsids
- Latent period: eclipse + maturation;
- Release: cell lysis, budding, or excretion
- Burst size: number of virions released.

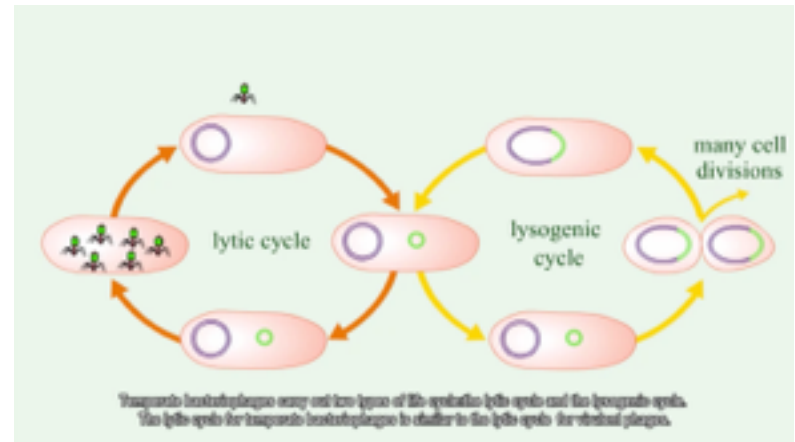
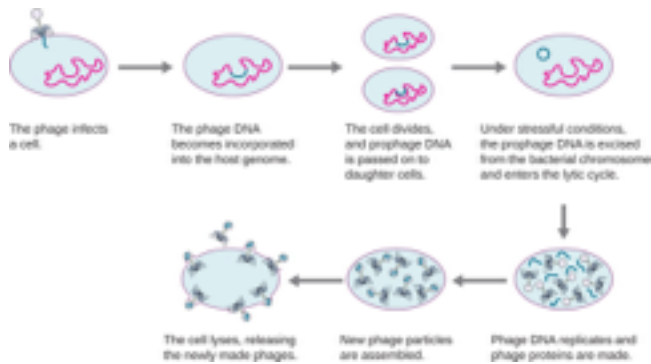


Describe the lytic and lysogenic life cycles of bacteriophages.

- The lytic cycle of a bacteriophage

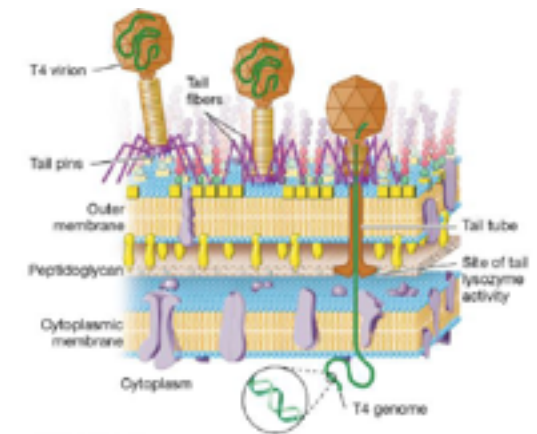


- The lysogenic cycle of a bacteriophage



Describe how phages, using T4 as an example, deliver DNA into its host.

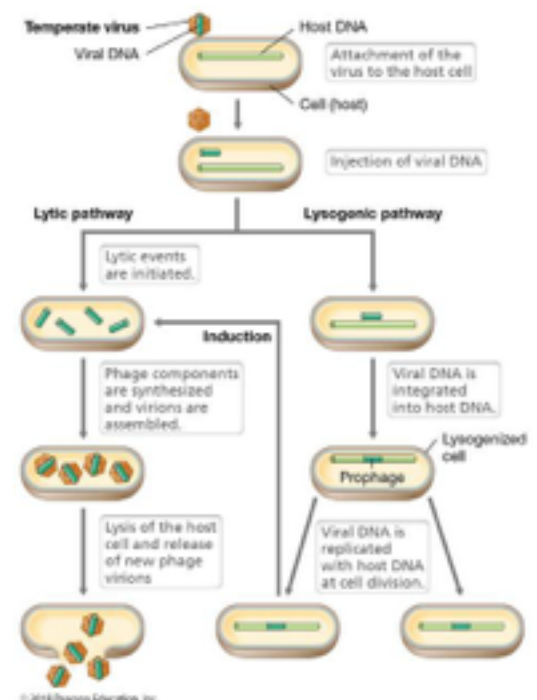
- Virions attach to cells via tail fibers that interact with polysaccharides on E. coli LPS layer.
- Tail fibers retract, and tail pins contact cell wall.
- T4 lysozyme forms small pore in peptidoglycan.
- Tail sheath contracts, and viral DNA passes into cytoplasm.
- Capsid stays outside.



Describe the replication process of animal viruses (what is particular to animal infections).

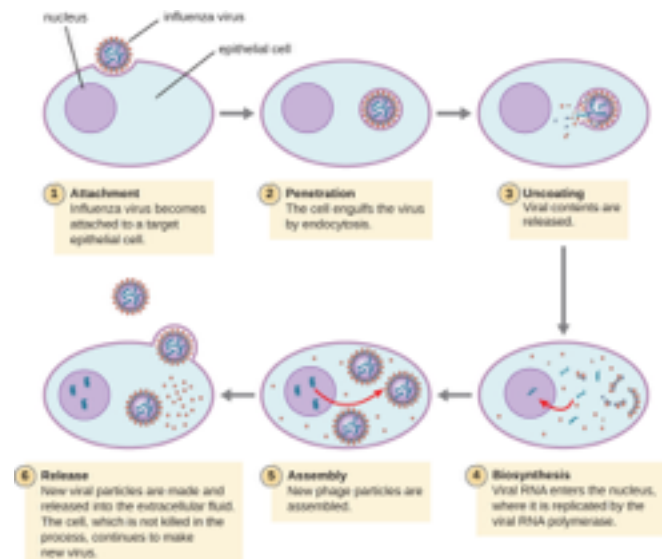
Describe the mechanism of entry utilized by naked and enveloped viruses.

- Major tenets (capsid and DNA/RNA genome, infection and takeover of host, assembly and release) are universal, regardless of the nature of the host.
- Most human viral diseases are caused by RNA viruses.
- Bind specific host cell receptors, typically used for cell-cell contact or immune function.
- Different tissues and organs express different cell surface proteins: often viruses only infect certain tissues.



- Two key differences:
 - Entire virion enters the animal cell.
 - Eukaryotic cells contain a nucleus, the site of replication for many animal viruses.

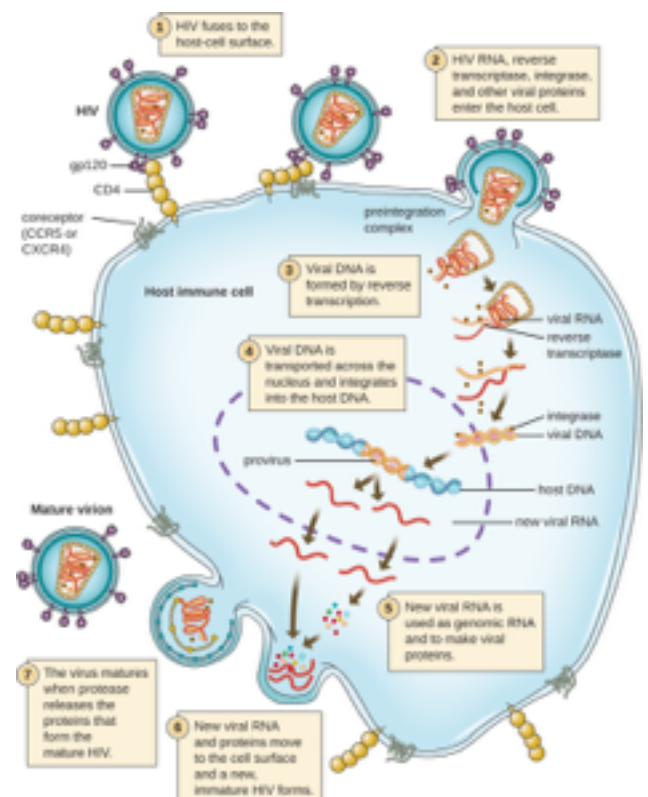
- Lytic cycle in animal cells:



- Lysogenic cycle in animal cells

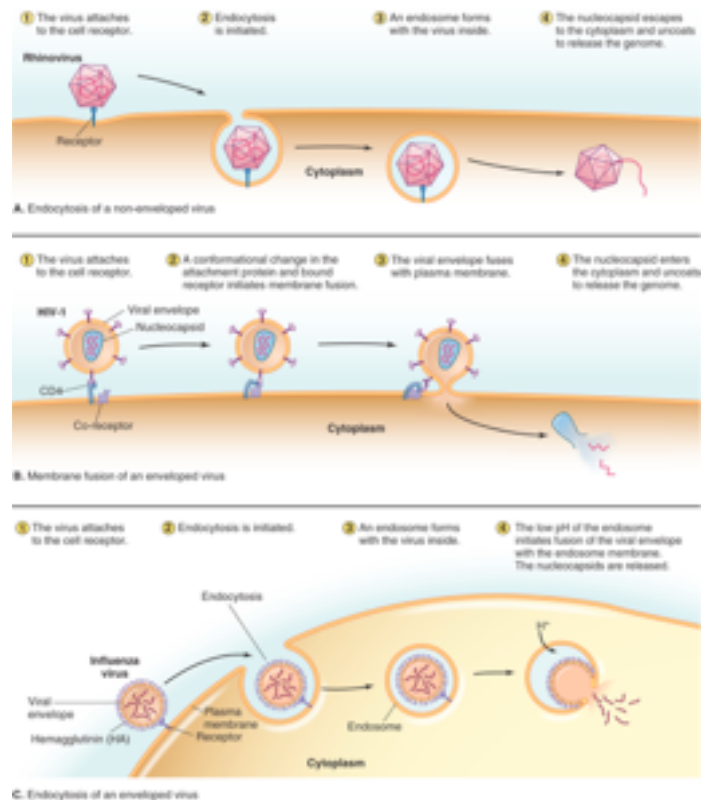
HIV infection:

- The adhesion of the virus gp120 envelop protein to the cell surface marker CD4 (only present on T helper cells) and its coreceptor is mandatory for infection.
- The viral envelop fuses with the lipid bilayer of the host cell, the viral capsid is delivered in the cytoplasm.
- The capsid is digested by proteases and the ssRNA genome delivered into to the nucleus with the reverse transcriptase enzyme necessary to convert ssRNA in cDNA which can then be converted to dsDNA by the host cell.
- An integrase, also delivered with the virus, helps the transduction of the virus into the host genome.
- New viral mRNA and proteins are produced by the host cells, virions assemble within the cytoplasm and envelop proteins are integrated into the host plasma membrane.
- Encapsulated new viral particles exocytose, the vesicle with which they exocytose forms the new envelop, the virions are mature and can infect new cells.



Explain what is the difference between a virulent and temperate virus.

- Virulent: Viruses always lyse and kill host after infection.
- Temperate: Viruses replicate their genomes in tandem with host genome and without killing host, establishing long-term, stable relationship.
 - Can be lytic/virulent;
 - Can enter **lysogeny**: most viral genes are not transcribed, viral genome is replicated with host chromosome and passed to daughter cells;
 - **Lysogeny**: host cell that harbours temperate virus. Can result in lysogenic conversion with new properties (e.g., virulence in pathogens).



Describe unique characteristics of retroviruses and latent viruses.

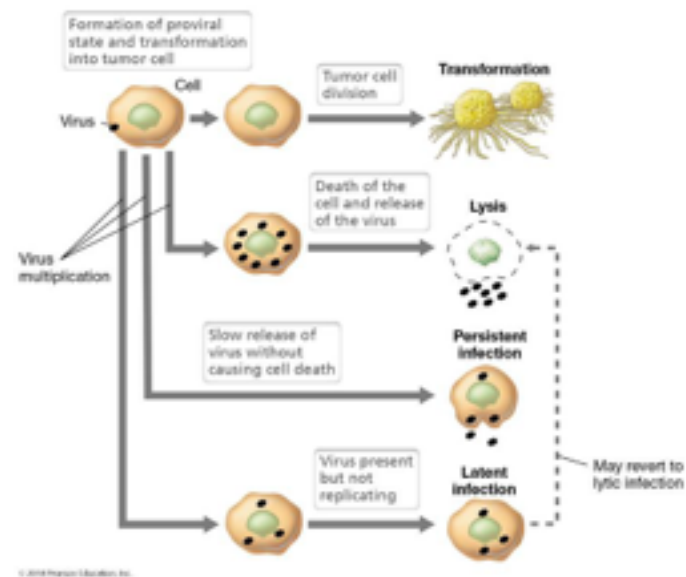
- Retroviruses are structurally complex animal viruses that contain an RNA genome. However, unlike other RNA viruses, the genome is replicated inside the host cell by way of a DNA intermediate.
- The term *retrovirus* refers to the fact that these viruses transfer information from RNA to DNA (in contrast to genetic information flow in cells, which occurs from DNA to RNA).
- Retroviruses use the enzyme reverse transcriptase to carry out this unusual process: this retroviral enzyme can produce DNA from an RNA template.
- Enveloped viruses and carry several enzymes within the virion. These include reverse transcriptase, integrase, and a retroviral-specific protease. The genome of the retrovirus is unique and consists of two identical single-stranded RNAs.

Discuss human viruses and their virus-host cell interactions.

Discriminate between persistent infections, latent or chronic infections.

- Persistent infections: when the virus is never completely cleared, can occur when the virus:
 - Does not kill the host;
 - Immunosuppresses the host;
 - Regulated viral or host gene expression.

- Latent infection: when lysogenic viruses become dormant within a host (e.g. chickenpox, after an acute infection, the viral genome integrated into the host can stay dormant for years to “wake up” 50 years later and cause another form of disease; shingles).
- Chronic infection: similar to latent infection but with multiple episodes of infections or constant low levels of infection. As for latent infections, the virus is never quite cleared. e.g. HIV is considered a chronic infection. Infected patients generally have low level infection with immune interference and antigenic blood presence, but no symptoms. The virus can mutate rapidly, thwarting the immune system, which helps with persistence.



Name the possible outcome of viral infections in animal cells.

- Virulent infection: lysis of host cell, most common.
- Latent infection: Viral DNA exists in host genome and virions are not produced; host cell is unharmed unless/until virulence is triggered.
- Persistent infections: Release of virions from host cell by budding does not result in cell lysis. Infected cell remains alive and continues to produce virus
- Transformation: conversion of normal cell into tumour cell.

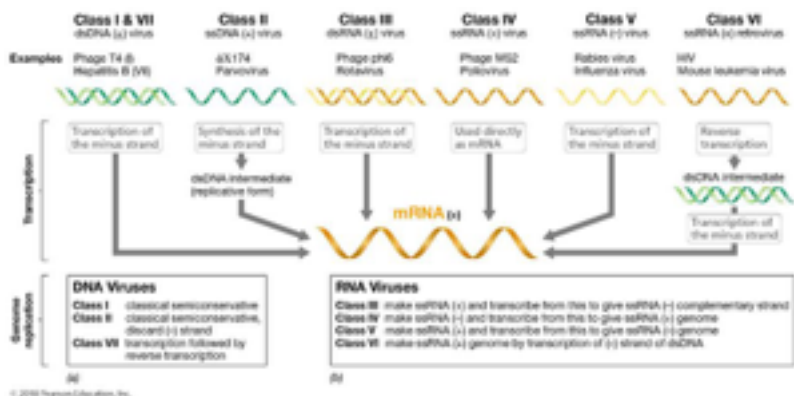
Explain the process of transduction.

- Transfer of genetic material from one bacteria (or *Archaea* and *Eukarya*) to another.
- *Generalized transduction*: occurs when a random piece of bacterial chromosomal DNA is transferred by the phage during the lytic cycle. (random process)
- *Specialized transduction*: occurs at the end of the lysogenic cycle, when the prophage is excised and the bacteriophage enters the lytic cycle (process not so random, since viral DNA integration is not random).

Describe viral genomes' constitution and classification.

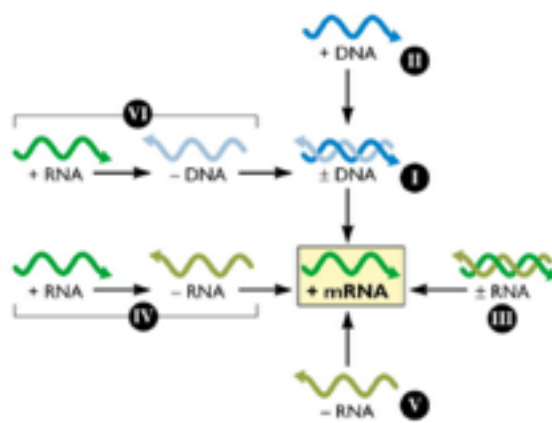
- Size of viral genome:
 - Varies 1000-fold from smallest to largest.
 - smallest circovirus: 1.75-kilobase single strand
 - largest Pandoravirus: 2.5-megabase pairs. Infects some marine amoebae, larger than some bacteria.
 - RNA genomes typically smaller than DNA viruses.
- Viral genomes:

- The **Baltimore classification**, developed by David Baltimore, is a virus classification system that groups viruses into families



The Baltimore Scheme

Baltimore's original scheme [Expression of animal virus genomes, Bacteriol Rev. 1971 Sep;35(3):235-41] omitted the gapped DNA genome which had not yet been discovered.

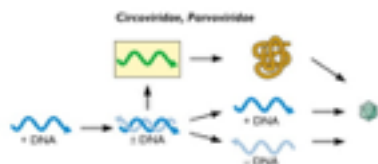


according to their genomes (7 groups in roman numerals). Classified based on:
 - Either DNA or RNA genomes

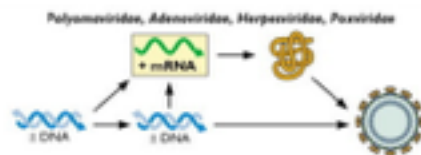
- Single-stranded or double- stranded; single-stranded may be plus sense (same as mRNA) or minus sense (complementary to mRNA);
- Either linear or circular;
- One or multiple pieces;
- Usually smaller than those of cells.

(1) DNA genomes:

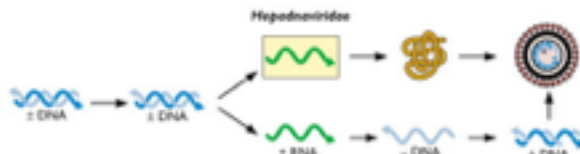
Single-stranded DNA genomes



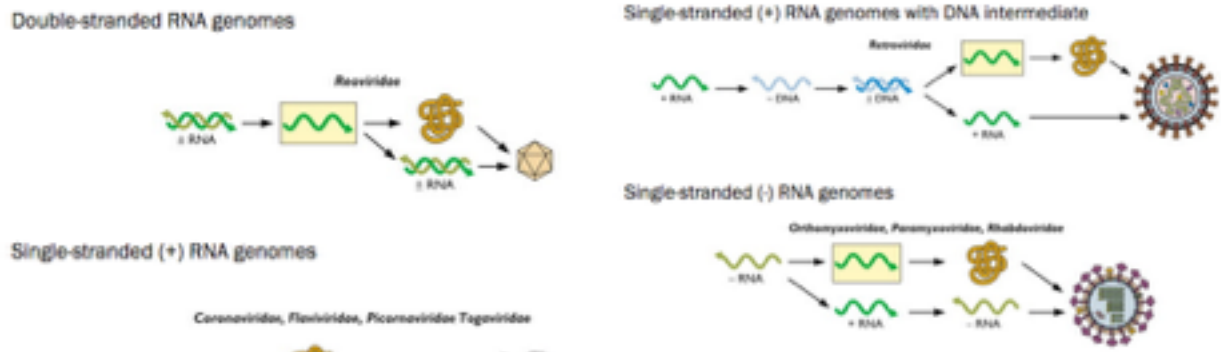
Double-stranded DNA genomes



Double-stranded gapped DNA genomes



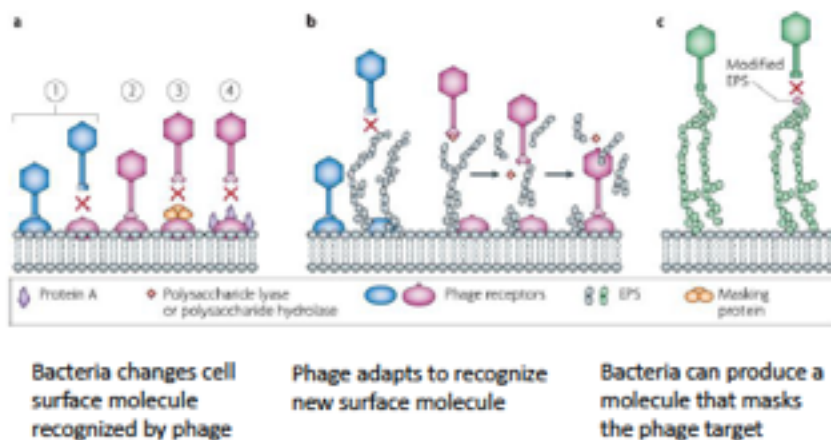
(2) RNA genomes:



Describe the viral defence mechanism of bacteria and how bacteriophages can circumvent them.

Prokaryotes possess mechanisms to diminish viral infections:

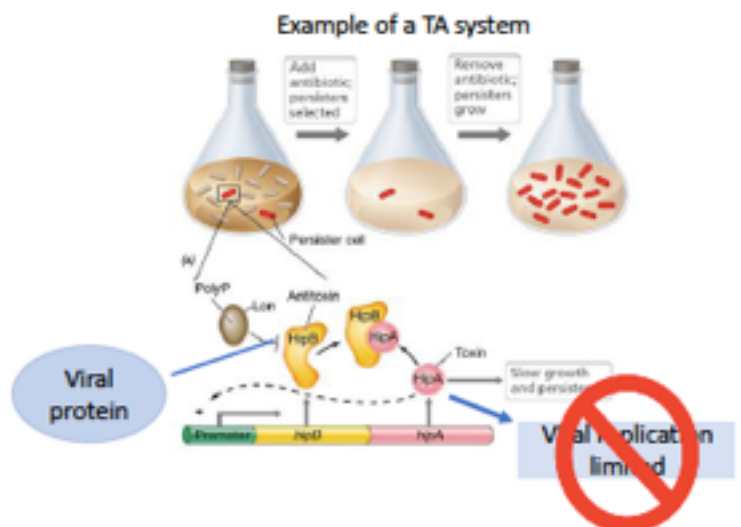
(1) Modification of cell-host recognition



(2) Toxin-antitoxin system

Antiviral defence using the TA systems: if the infecting virus inhibits the host cell's transcription and/or translation, which is often the case, the unstable antitoxin disappears and the toxin exerts its toxic effect to limit viral replication (the toxin acts as an antiviral).

Prokaryotic viruses can bypass this defence system by expressing a product similar to the antitoxin and thereby inhibit host translation



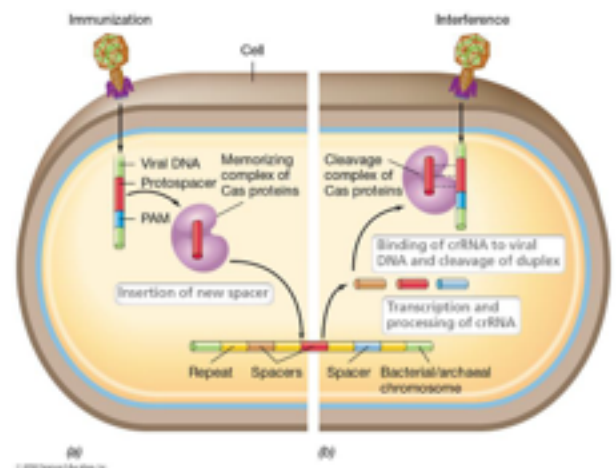
shutoff.

(3) Antiviral CRISPR-Cas

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats that protect from bacteriophage infection.

- Regions contain short repeats of constant DNA sequences alternating with short variable spacers corresponding to “memory” of viral or other foreign DNA.
- Some viruses have evolved to avoid CRISPR:
 - Mutation of PAM regions
 - Production of Cas inhibitors
 - Phage-encoded CRISPR in a phage that infects *Vibrio cholerae*.

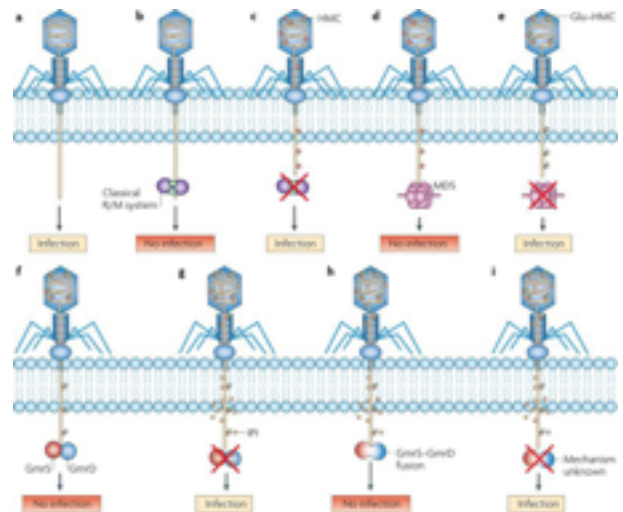
★ When immunized cell encounters same virus, Cas proteins destroy incoming DNA.



(4) Restriction modification systems

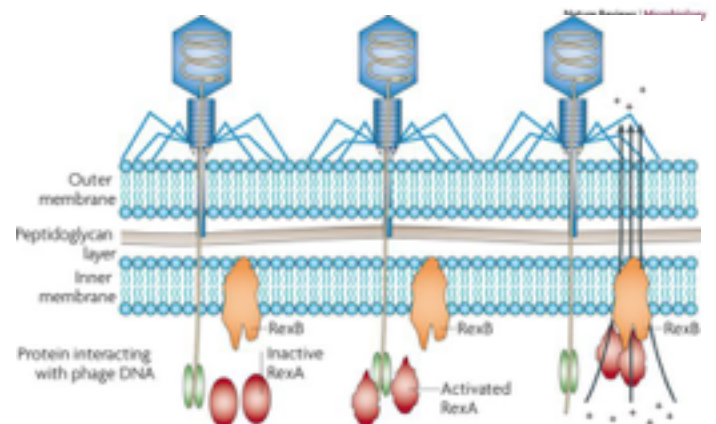
Going from a to g:

- Normal infection
- Bacteria digest viral DNA/RNA using restriction enzymes
- Virus counteract with undigestible modified nucleic acid
- Bacteria finds a way to digest modified nucleic acids
- Virus finds another way to protect its nucleic acids
- Bacteria finds another way to get around it
- Etc...



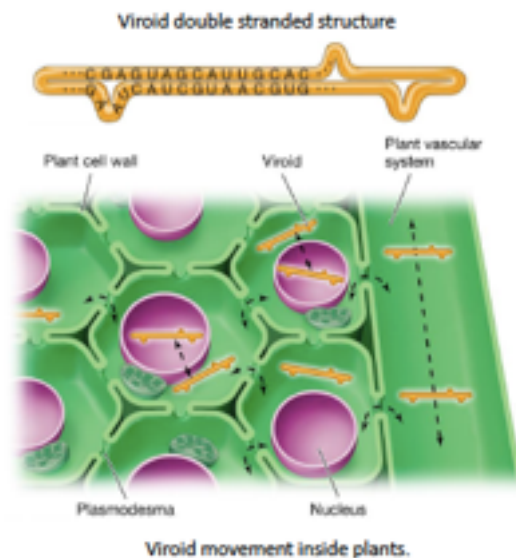
(5) *E. coli* Rex system

- Two-component system (RexA sensor kinase, RexB is regulator).
- RexA recognizes phage DNA protein complex and activates RexB
- RexB is an ion channel, it opens upon activation.
- Cations escape the cell, disrupting the membrane potential and killing the cell.



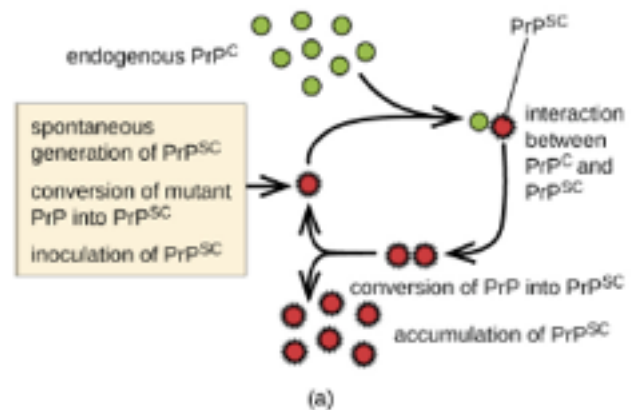
Describe viroids and their unique characteristics.

- Viroids: infectious RNA molecules that lack a protein component:
 - Small, circular, ssRNA molecules;
 - Smallest known pathogens (246–399 bp);
 - Cause a number of important plant diseases;
 - Do not infect animals or microorganisms.
- Structure and function:
 - Forms hairpin-shaped double stranded molecule with closed ends, providing stability outside host cell;
 - Enters plant through wound (e.g., insect, mechanical damage);
 - Move between cells through plasmodesmata (thin cytoplasmic strands);
 - Completely dependent on plant RNA polymerases for replication;
 - Catalytic self-cleaving activity releases individual viroids after replication.



Describe prions and their unique characteristics.

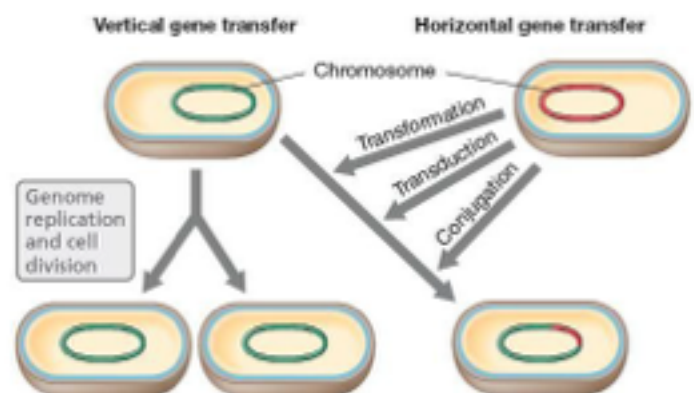
- Prions are misfolded proteins that aggregate, accumulate and eventually cause cell death.
- Normal proteins (PrP^C) can spontaneously misfold or mutated and become infectious (PrP^{Sc}).
- The misfolded or mutated protein complexes cause other normal proteins to misfold and aggregate, leading to cell death and tissue damage.
- Transmission of the infectious prions is mainly through eating contaminated tissue or feed, blood transfusion or via genetic transmission of mutant allele.



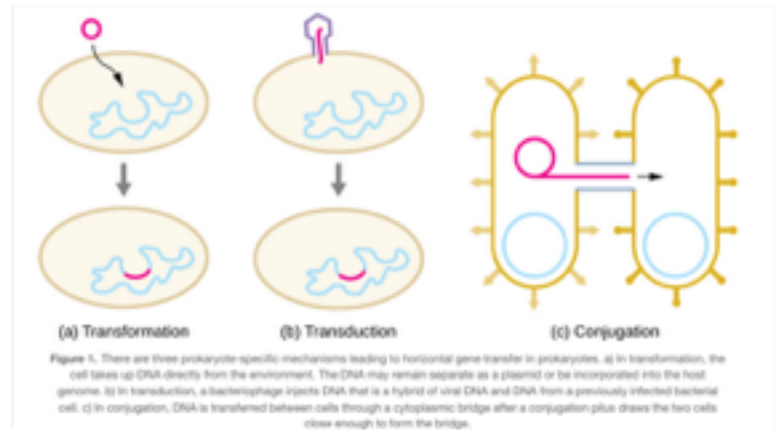
Module 4: Genetics and Genomics

Explain how asexual gene transfer can result in prokaryotic genetic diversity.

- *Horizontal gene transfer* between closely or sometimes more distantly related species (only small pieces of DNA at a time).
- Also mutations, viral infections introducing



- or pilfering genetic material and mobile elements such as transposons.
- Transformation: naked DNA is taken up from the environment.
 - Transduction: genes are transferred between cells in a virus.
 - Conjugation: use of a hollow tube called a conjugation pilus to transfer genes between cells.



Discuss the benefits and trade-off of sexual vs asexual reproduction.

Sexual	Asexual
<ul style="list-style-type: none"> • Slower to produce offsprings • Generates diversity <ul style="list-style-type: none"> • Material from 2 parents • Crossover • Independent assortment of chromosomes during meiosis • Chromosomal segregation not always correct 	<ul style="list-style-type: none"> • Generates more offsprings more quickly • Generates less diversity <ul style="list-style-type: none"> • Identical genome passed down to daughter cells

Both are vertical transmission of the genetic material

Explain in detail each of these processes (mechanism): transformation, transduction, conjugation and transposition.

(1) Transformation

- Many bacteria are naturally “competent” and can bind DNA in the environment, transport across the plasma membrane and make it single stranded.
- Double stranded DNA would normally be digested by endo or exonucleases in the cytoplasm, but these nucleases are mostly ineffective against ssDNA (single-stranded DNA).
- This ssDNA can recombine with the genome. The DNA that was added to the wildtype genome is called “recombinant DNA”.
- Whatever was coded on this recombinant DNA that is now incorporated into the bacterial genome becomes an integral part of the bacterial genome and can change the phenotype (new toxin, enzyme, structural protein...)
- However, there is relatively little environmental DNA, it is rarely a whole coding piece and recombination requires that the “recombinant DNA” be at least in part similar to the genomic

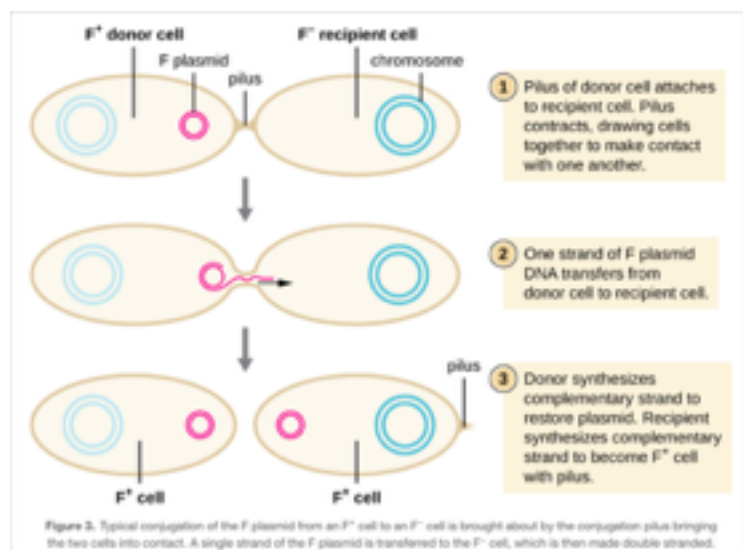
DNA for a length of nucleotides for recombination to occur. Also, although less efficient, nucleases still digest the DNA fragments.

- So transformation is not very efficient as introducing diversity. Regardless, it is still an important mechanisms for the acquisition of tolerance to Abx or virulence.

(2) Conjugation

- DNA is transferred from one cell to another by a needle-like structure, the conjugation pilus, linking the 2 cells together.
- The conjugation pilus is called the F pilus.
- In *E. coli*, the capacity to form a conjugation or F pilus is encoded on a plasmid. F⁺ cells have the plasmid and can make a pilus- they are DONOR cells. F⁻ cells do not have the plasmid – they are RECIPIENT cells.

- Conjugation of the F plasmid

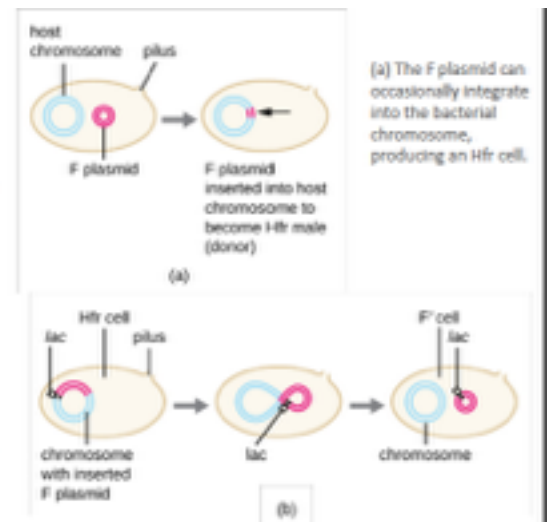


Conjugation of F' and Hfr cells

When conjugation does not transfer a plasmid but **chromosomal DNA** to another cell

- Hfr: high frequency recombination

(b) Imprecise excision of the F plasmid from the chromosome of an Hfr cell may lead to the production of an F' plasmid that carries chromosomal DNA adjacent to the integration site. This F' plasmid can be transferred to an F⁻ cell by conjugation.

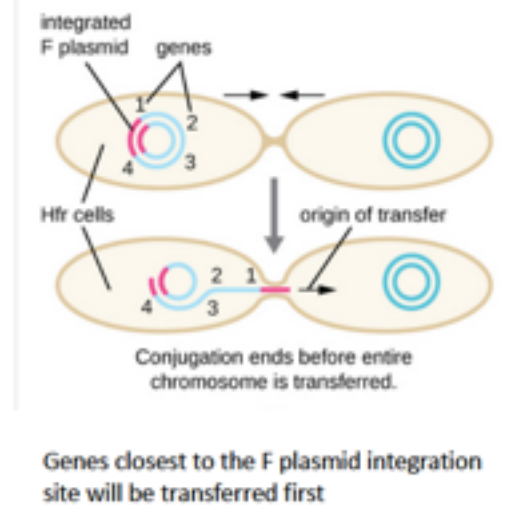


- Consequences and applications of conjugation:
- Plasmids are integral part of the bacterial genome;

- They code for many functional gene groups or operons, such as resistance to an Abx or virulence factors such as toxins.
- Plasmids containing Abx resistance genes are called R plasmids
- Single R plasmids commonly contain multiple genes conferring resistance to multiple antibiotics.
- Plasmids can be manipulated by genetic engineering for research, industrial, environmental or clinical purposes. They can be transferred into bacteria, archaea, single cell eukaryotes, insects, animals and plants in order to genetically manipulate these organisms to our advantages.

Conjugation of F⁻ and Hfr cells

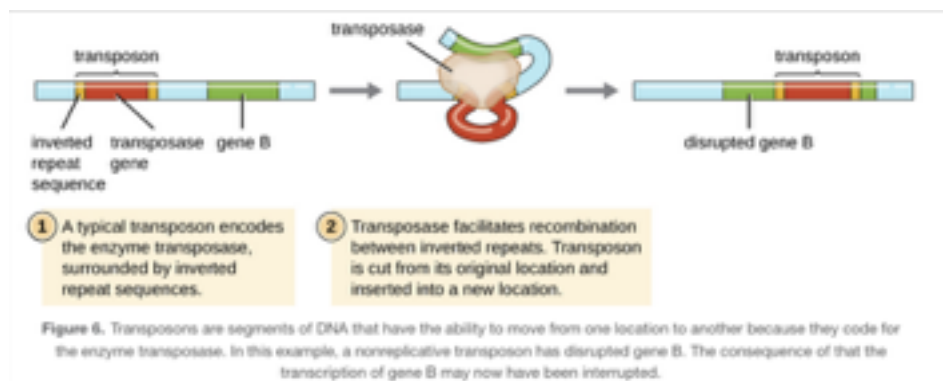
- It is possible that a bacteria may attempt to transfer the whole chromosome to an F⁻ cell
- However, since contact between cells is transient and short lived, this rarely happens.
- But it may be sufficient to transfer one or several operons and therefore whole new biochemical pathway to the F⁻ cells.



(3) Transduction

- **Generalized transduction:** any piece of DNA can be transferred from one infected cell to another if it is erroneously incorporated into the capsid when the virions are assembling.
- **Specialized transduction:** a specific piece of DNA is lifted from the infected cells from either side of where the lysogenic phage had integrated into the host cells. These sequences are then encapsulated and transported to other cells and will integrate in the newly infected cells at the viral integration site.
- Transformed cells may acquire new phenotypic abilities, such as virulence, if enough DNA material coding for virulence factors was exchanged.
- Transduction is a major contributor to genetic hypermobility and both phage and bacterial evolution.
- These principles are used when developing gene therapies or using viral transduction in research in order to generate cells that overexpress a protein or for RNA inhibition, when you want to diminish the expression of a protein.

(4)



Term	Definition
Conjugation	Transfer of DNA through direct contact using a conjugation pilus
Transduction	Mechanism of horizontal gene transfer in bacteria in which genes are transferred through viral infection
Transformation	Mechanism of horizontal gene transfer in which naked environmental DNA is taken up by a bacterial cell
Transposition	Process whereby DNA independently excises from one location in a DNA molecule and integrates elsewhere

Transposition

- Transposons are genetically mobile elements;
- They exist in both prokaryotic and eukaryotic cells, however, in our human genomes, they are mostly degenerated and not functional. They form repeat elements and structural elements of our genome.
- In bacteria, transposons contribute to genetic diversity through shuffling sequences around the genome; possibly disrupting functional genetic units or creating novel sequences that may eventually prove beneficial.

Explain how microorganisms have developed such a huge diversity of organisms using genetics and genomics as a premise for your explanation.

Explain what is vertical transmission and horizontal (or lateral) transmission of genetic material.

- Vertical transmission is the transfer of genetic traits from one generation to the next (from mother to daughter).
- Horizontal (or lateral) transmission refers to gene transfer from one cell to another between closely or sometimes more distantly related species.

Describe the notions of pan genome and core genome.

- Core genome: shared by all strains of the same species.
- Pan genome: core genome plus genes that are not shared by all the strain in a same species
E.g. One *E. coli* strain may have 5,000 coding sequences but the pan genome for *Escherichia* contains about 25,000 coding sequences.

Explain what are chromosomal islands and describe why we think they are of foreign origin.

- Chromosomal islands (or genomic islands) are clusters of genes for specialized functions that are not essential for survival.
- This means certain strains of the species will possess such an island whilst other will not not, creating more genetic diversity within a single species.
- Chromosomal islands are considered to be of foreign origin for 3 main reasons:

- (1) The extra genes are often “flanked” by inverted repeats (indicating they might have been inserted there by transposition).
- (2) The base composition and codon bias of the island may differ substantially to that of the rest of the genome.
- (3) Chromosomal islands are found only in certain strains of the species but not, again suggesting their presence was not via evolution of vertically transmitted DNA, but by an insertion event potentially through lateral transfer (HGT).
 - Comparison of the genomes of pathogenic bacteria with those of their harmless or less virulent relatives often reveals chromosomal islands that encode virulence factors: special proteins, toxins, enzymes, or other molecules or structures that facilitate disease symptoms. Some virulence genes are carried on lysogenic bacteriophages or plasmids; however, many others are clustered in chromosomal regions called *pathogenicity islands*.

Explain the difference between genomics and metagenomics.

DNA	Genome the total complement of genetic information of a cell or a virus
	Metagenome the total genetic complement of all the cells present in a particular environment
	Epigenome the total number of possible epigenetic changes
	Methylome the total number of methylated sites on the DNA (whether epigenetic or not)
	Mobilome the total number of mobile genetic elements in a cell

- Metagenomics: the genomic analysis of pooled DNA or RNA from an environmental sample containing organisms that have not been isolated; same as environmental genomics.
- Genomics: the discipline that maps, sequences, analyzes, and compares genomes.

Explain how genomics and metagenomics can help us identify chromosomal islands or biosynthetic gene networks (BGCs) and even new microorganisms, and what are the advantages in looking for this information.

- Identification of new biochemical pathway (E.g. production of antibiotics or other useful chemical);
- Identification of new chromosomal islands: pathogenicity islands, also biodegradation, nitrogen fixation, magnetosome, ...
- Identification of new genes and new organisms: better understanding of diversity, function, morphology, etc... and phylogenic analysis

Module 5: Microbe-Human Interactions and the Immune system

Immune System Assignment

Compare and contrast the innate and adaptive immune response.

<i>Hallmark</i>	<i>Innate</i>	<i>Adaptive</i>
Level of specificity	non-specific	antigen-specific
Involvement of memory	No involvement since it is non-specific	Memory cells - Remember the aggressor and acts specifically against certain antigens.
Time for first response to an antigen	Immediately or within hours	After 4-7 days if the innate system unable to destroy pathogens.
Time for secondary/ repeated response to an antigen	Same as last time	Rapid, if the antigen was being recognized
Level of self- discrimination	Heavily based on self vs. non-self discrimination, has to be PERFECT	Discriminate between pathogen vs. non-pathogen structures.
Duration of the response	First line of defense	Takes up to a week to kick in
Key cells involved	Epidermis(skin cell as the physical defense) Inflammatory cells - site of infection Defense cells - help to defend body: macrophages (tissue) Neutrophil granulocytes(blood and tissue) Natural Killer Cells Dendritic cells Basophils Eosinophils	Cell-mediated immune response: Scavenger cell - taking up and digesting the marked pathogens T-cells(T lymphocytes) - special defense in the tissue B-cells(B lymphocytes) - antibodies reproduction Other antigen presenting cells

Name physical barriers (at least 3) that help keep the bacteria out.

Skin, mucous membranes, microbiota in the gut.

Describe the functions of key cells in the immune system (Macrophage, NK cell, B cell, T cell, Mast cell, Dendritic cell, Neutrophil, Eosinophils).

<i>Cell type</i>	<i>Role During an Infection (identify the effector mechanism employed by the cells to stop infection)</i>	<i>Arm of the Response (innate or adaptive)</i>
Macrophage	Engulfs and digests foreign substances, microbes, and anything else that does not have the type of proteins specific to healthy body cells on its surface, in process of phagocytosis. Also plays a role in adaptive immunity by presenting antigen of a pathogen to the corresponding helper T cell.	Innate immunity

NK cell	Type of lymphocyte that provides rapid responses to virally infected cells. Unlike other lymphocytes such as T cells, an NK cell can recognize stressed cells without antibodies or the presence of specific proteins on the cell surface of infected cells. NK cells can create pores in the cell membrane of a target cell and cause apoptosis or osmotic cell lysis.	Innate immunity
B cell	Functions in the humoral immunity component of the adaptive immune system by producing antigen-specific immunoglobulins (i.e. antibodies). These antibodies neutralize pathogens by recognizing the specific antigen on them and binding them, resulting in the destruction of the pathogen by other parts of the immune system (e.g. macrophages) or by neutralizing its target directly. Also, present antigens to trigger specific helper T cells.	Adaptive immunity
T cell	Broadly speaking, can be divided into two different types: killer T-cells and helper T-cells. Killer T-cells destroy virus-infected cells and tumor cells by recognizing a specific antigen. Helper T-cells assist in many WBCs processes, including maturation of B cells and activation of killer T-cells and macrophages.	Adaptive immunity
Mast cell	Play a key role in the inflammatory process by releasing compounds that induce inflammation. Expresses immunoglobulin E (IgE) which defends against several parasitic pathogens. Also recruits neutrophils and macrophages by release of histamine.	Innate immunity
Dendritic cell	Antigen-presenting cells: process antigen material and present it on the cell surface to the T cells of the immune system. They act as messengers between the innate and adaptive immune systems.	Innate immunity
Neutrophil	Most abundant type of phagocyte, usually the first cells to arrive at the site of infection. Also, release granules with antimicrobial properties (process of degranulation).	Innate immunity

Eosinophils	Upon activation, secrete a range of highly toxic proteins and free radicals that are highly effective in killing parasites.	Innate immunity
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Describe the functions of key cytokines in the immune system (IFN- α /b, IFN- γ , TNF- α , IL-12, IL-6 and IL-10).

- IFN- α is produced by macrophages and B cells. Involved in innate immune response against viral infection.
- IFN- β is produced by fibroblasts. They have antiviral activity, involved in immune response.
- IFN- γ is secreted by T helper cells, cytotoxic T cells, macrophages, mucosal epithelial cells, and NK cells. It is important for innate and adaptive immunity against viral, bacterial and protozoal infections. Able to directly inhibit viral replication.
- TNF- α Produced primarily by macrophages can be produced by CD4+, lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neuron cell types. TNF- α regulates immune cells primarily, and can induce fever, apoptotic cell death, cachexia, and inflammation. It can also inhibit the formation of tumors and viral replication.
- IL-12 is produced by dendritic cells, macrophages, neutrophils, and human B-lymphoblastoid cells. It stimulates the growth and function of T cells and stimulates the production of IFN- γ , and TNF- α .
- IL-6 is produced by osteoblasts, and in some smooth muscle cells in blood vessels. It can also be secreted by macrophages in response to microbial molecules which induced intracellular signaling to stimulate inflammatory cytokine production.
- IL-10 is primarily produced by monocytes and to a lesser extent, lymphocytes. It affects immunoregulation and inflammation by reducing the quantity of some cytokines and antigens.

Explain what are antigens and what is antigen presentation and what is antigenic variation.

- An antigen is a molecule capable of interacting with specific components of the immune system and that often functions as an immunogen to elicit an adaptive immune response.
- Antigen presentation occurs in antigen-presenting cells such as dendritic cells and B cells. The antigen-presenting cell digests protein from either the inside or the outside of a pathogen and then displays the antigenic peptide fragment on its cell surface. Once the antigenic peptide fragment is located on the antigen-presenting cell's surface, it can act as a recognition factor for T cells. Now, T cells are able to recognize that cells containing this antigenic peptide are pathogens and will, therefore, signal the immune response. Antigen presentation is critical during an immune response since it enables for T cells to continuously monitor and recognize any signs of infection or abnormal cell growth.

- Antigenic variation refers to the mechanism by which an infectious agent such as a protozoan, bacterium or virus alters its surface proteins in order to avoid a host immune response.

Explain how pathogens can avert immune cell function (antigenic variation, cytokine inhibition, cell signaling inhibition, cell death inhibition).

- Antigenic variation: when organism is exposed to particular antigen (i.e. a protein on the surface of a bacterium) an immune response is stimulated and antibodies are generated to target that specific antigen. The immune system will then "remember" that particular antigen, and defenses aimed at that antigen become part of the immune system's acquired immune response. If the same pathogen tries to re-infect the same host the antibodies will act rapidly to target the pathogen for destruction. However, if the pathogen can alter its surface antigens, it can evade the host's acquired immune system. This will allow the pathogen to re-infect host while immune system generates new antibodies to target the newly identified antigen.

Part 1: Microbial symbiosis with humans

Distinguish between microbiota and microbiome.

- A microbiome is a functional collection of different microbes in a particular environmental system (e.g., the human microbiome).
- Scientists use the term microbiota to describe all the microbes in a microhabitat (e.g., skin microbiota).

Discuss, in a general way, what we know of the diversity and dynamics of microbial populations in humans so far.

- There are approximately 10^{13} microbes in the human microbiome living in complex communities.
- These are very early studies, and they reveal that there are complex interactions between host and its microbiota.
- No one species is the most abundant across all individuals (diversity between individuals is high).
- Particular microbial groups typically dominate certain niches.
- Similarities between individuals are more evident at higher taxonomic levels (phyla).
- Specialized microbial ecological niches have evolved with the immune system.
- This close collaboration has inevitably influenced the development of the immune system.
- In addition, tissues such as mucous membranes are most exposed to antigens of all kinds, the good as the bad, imposing a certain paradox on the immune system: how to differentiate the two.
- Lung, skin and gut mucosa (Peyer's patch), appendices.

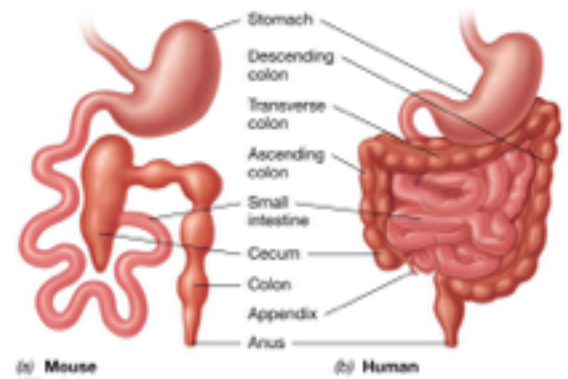
- Also form follicular structures where T and/or B cells are concentrated.
- Skin $\sim 2\text{m}^2$
- Lungs $\sim 140\text{m}^2$
- GI tract $\sim 400\text{m}^2$

Discuss, in a general way, what is missing in our knowledge on the human microbiota and what are the research challenges faced by the scientific community (includes the advantages and disadvantages of using mice models).

- Human Microbiome Study Groups have formed most of our understanding of the functions of the human microbiome.
- The Human Microbiome Project (HMP) surveyed hundreds of medical students over several years to determine a baseline for healthy human microbiomes. Lacked diversity and metadata (dietary habits, amount of proteins, lipids, sugars ingested, lifestyle, genetic background, etc...)
- Later projects revealed more diversity in non-US born subjects and lacked data on diet or other lifestyle attributes.

Mouse Models:

- While there are significant differences between mice and humans, mice have been used to good effect to study human gut microbiome interactions.
- Mice have a larger cecum than humans.
- Most fermentation is completed in the mouse cecum, rather than the human large intestine.



However, mice have a short life cycle and well-defined genetic lines; they can be raised in a germ-free environment and we can manipulate their microbiota.

- antibiotic therapy
- strict dietary control
- fecal transplants
- germ-free environment.

Describe the different microbial microhabitat in the gastro-intestinal tract, the characteristic of the microbiota present and what mechanisms the GI tract has in order to clear or contain the microorganisms present.

- Humans are monogastric and omnivorous.
- Microbes in gut affect early development, health, and predisposition to disease.
- Colonization of gut begins at birth.
- Consists of stomach, small intestine, and large intestine
- Responsible for digestion of food, absorption of nutrients, and production of nutrients by the indigenous microbial flora.

- Contains 10^{13} to 10^{14} microbial cells.

- The stomach and small intestine:

- Microbial populations in different areas of the GI tract are influenced by diet and the physical conditions in the area.

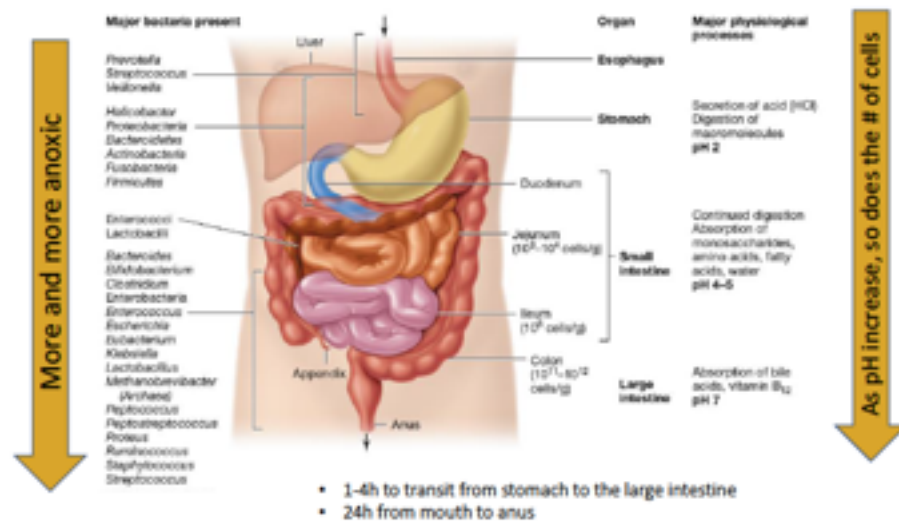
- The acidity of the stomach and the duodenum of the small intestine (\sim pH 2) prevent many organisms from colonizing the GI tract; however, there is a rich microbiome in the healthy stomach.

- Firmicutes, Bacteroidetes, and Actinobacteria are common in the gastric fluid, while Firmicutes and Proteobacteria are common in the mucus layer of the stomach.
- *Helicobacter pylori* was discovered in the 1980s and has since been found in \sim 50% of the world's population! When present, it is found in the gastric mucosa.
- Intestinal microorganisms carry out a variety of essential metabolic reactions that produce various compounds.

- The large intestine:

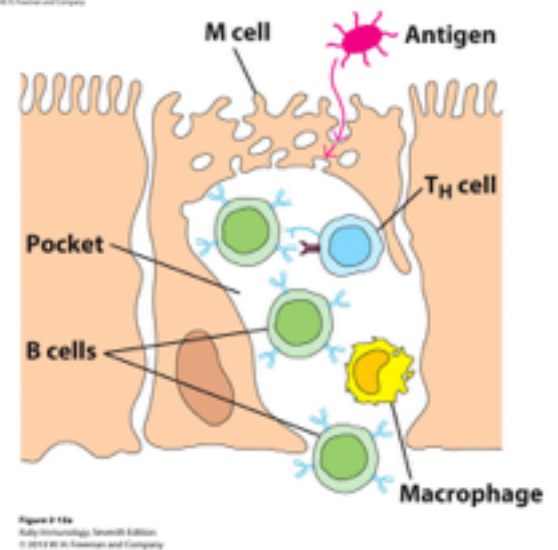
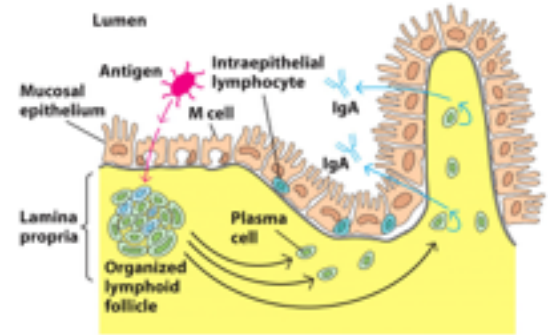
- The colon is essentially an in vivo fermentation vessel (anoxic so no respiration using O_2 as final acceptor), with the microbiota using nutrients derived from the digestion of food.
- Most organisms are restricted to the lumen of the large intestine, while others are in the mucosal layers.

- The vast majority (\sim 98 percent) of all human gut phylotypes fall into one of three major bacterial phyla: (1) Firmicutes, (2) Bacteroidetes, and (3) Proteobacteria.
- Individuals may have mostly Firmicutes, mostly Bacteroidetes, or a mix of the two. This may regulate metabolism and the host's propensity for obesity.
- In contrast to the limited phylum-level diversity, the species diversity in the mammalian gut is enormous (3,500 to 35,000 species but no more than \sim 200 in one individual).
- Archaea, yeasts, fungi and protists are either absent or form a very little part of the gut community in humans.



Describe what are the roles and effects of the commensal flora in the GI tract and how it may change through time or under various selective pressures (bottle fed infant vs breast-fed infant, meat diet vs complex fiber diet, etc...)

- Many microbial metabolites or transformation products that can be generated in the gut have significant influence on host physiology: (1) vitamin production, (2) modification of steroids, (3) amino acid biosynthesis.
- Educating the immune system:
 - Several bacterial species are anti-inflammatory and actively participate in suppressing the activation of innate immune cells, stimulate the secretion of mucus by the endothelial (goblet) cells and the secretion of IgA in the lumen.
 - Axenic animals (without intestinal flora) or gnotobiotic animals (introduction of specific microbial species to an axenic animal) have an atrophy of all peripheral lymphoid organs, low levels of serum Ig, and decreased immune responses of all types relative to a normal animal, indicating that commensals also participate in the education of adaptive immunity cells.
 - The only entryway into tissues for the commensals are the M cells. But if they can be transferred from the lumen to the lamina propria by the M cells, the local secretion of inhibitory cytokines such as TGF β and the presence of Treg inhibit the inflammatory response and even favour the differentiation of resident TH in Treg.



Describe how the microbiota of any ecological niche on the human body can both protect against pathogenesis and at times participate in diseases. What needs to happen for an infectious or opportunistic agent to cause disease (especially in the gut).

Immune response to a gut infection

By constantly monitoring intestinal antigens, the immune cells of the gastric mucosa are always on the alert and eliminate threats locally, **without our knowledge**.

For there to be an immune response the following conditions must be present:

1- There must be stimulation of the innate cells.

- Physical barriers must be broken
- The balance between pathogenic and commensal must be broken (ecological niche favorable to pathogens)
- Or a deregulation of the innate system favorable to pathogens

2- The severity of the pathology is a consequence of the host / pathogen relationship

- Virulence of micro Φ influence immune response
- Aggressiveness, opportunistic micro Φ , toxin, quantity of pathogens ingested?
- Quality and number of commensals
- Presence of immune deficiency (over or under activation)
- Break in tolerance (food allergy?)

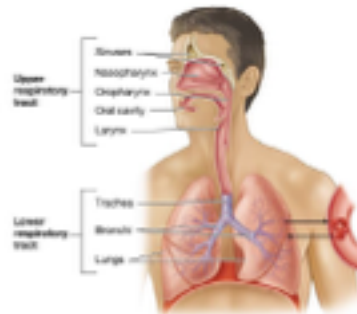
Describe the different microbial microhabitat in the oral cavity and airways, the characteristic of the microbiota present and what mechanisms the oral cavity and airways have in order to clear or contain the microorganisms present.

The oral cavity is a **complex, heterogeneous microbial habitat**.

- Saliva contains **antimicrobial enzymes**.
- But high concentrations of nutrients near surfaces in the mouth promote localized microbial growth.

Microenvironments of the Respiratory Tract

- Microbes **thrive in the upper respiratory tract**.
 - Bacteria continually enter the upper respiratory tract from the air during breathing.
 - Most are **trapped in the mucus** of the nasal and oral passages and expelled with nasal secretions or swallowed and then killed in the stomach.
- The lower respiratory tract has **no normal microbiota** in healthy adults.
 - **Ciliated mucosal cells** move particles up and out of the lungs.



Particularity of the lungs

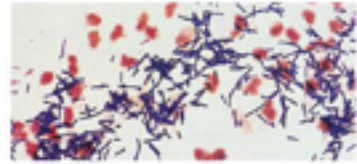
- The anatomy of the lungs, allowing gas exchange, is very delicate. **A strong immune response causes tissue damage that could kill**. The immune response must therefore be particularly well controlled and almost surgeon-like in its precision.
- The epithelial cells are able to defend themselves largely by themselves!
 - Secretion of **mucus**
 - Secretion of **surfactants** that normally decrease surface tension but can also be microbicides
 - Can detect pathogens and secrete **microbicidal polypeptides**, or **cytokines** such as IL-25 and IL-33, following their activation
- A large population of resident alveolar **macrophage** constantly **clear debris and intruders** by phagocytosis.
- **DCs**, present in the respiratory epithelium, also seem to set the tone for the immune response with their ability to **present Ag to cells of adaptive immunity**.

Describe the different microbial microhabitat in the urogenital tract, the characteristic of the microbiota present and what mechanisms it has in order to clear or contain the microorganisms present.

- Kidney and bladder normally sterile.
- **Altered conditions** can cause potential pathogens in the urethra (such as *Escherichia coli* and *Proteus mirabilis*) to multiply and cause disease.
- *E. coli* and *P. mirabilis* frequently cause urinary tract infections in women, for example, when the pH changes.



- The vagina of the adult female is **weakly acidic** and contains significant amounts of **glycogen**.
- *Lactobacillus acidophilus*, a resident organism in the vagina, ferments the glycogen, producing lactic acid.
- Lactic acid maintains a local acidic environment (pH[~]5.0).
- *L. acidophilus* only colonizes the vagina at puberty and disappears during menopause. Before puberty and after menopause, pH is neutral (no lactic acid being produced), so the microbiota changes as women become fertile or lose their fertility.
- Yeasts like *Candida* are commensals that can cause opportunistic infections, for example, when *L. acidophilus* is displaced and can't protect the mucosa anymore (after Abx trx, douching, or sexual intercourse (semen is alkaline)).



(P)
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L. acidophilus



- Male genitalia microbiota **understudied**.
- **Circumcision** is the largest influence on the composition of the penis microbiome, fewer anaerobic bacteria within six months after the men in a study were circumcised. <https://doi.org/10.1371/journal.pone.0098422>
- **Age and sexual activity** are other factors affecting microbiota composition.
- Species found resemble that of the gut (Bacteroidetes and Firmicutes)
- Unknown yet if commensal microbiota present is preventative of diseases like chlamydia or contributes to its colonization by providing nutrients for *C. trachomatis*.
- Female and male partners seem to **share genital microbiota**. Anaerobes associated with bacterial vaginosis also found on uncircumcised penis.



WIKIMEDIA, [ALT SEX/TSA/DIGAST](#)

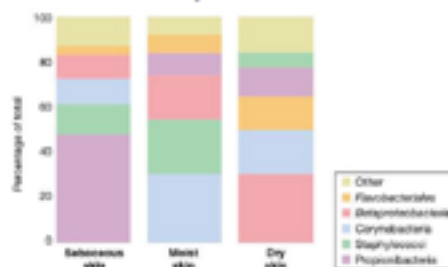
<http://www.the-scientist.com/news-opinion/peeing-the-penis-microbiome-37438>

Describe the different microbial microhabitat in the skin, the characteristic of the microbiota present.

- There are approximately 1 million resident bacteria per square centimeter of skin for a total of about 10^{10} skin microorganisms covering the average adult.
- The skin surface varies greatly in chemical composition and moisture content

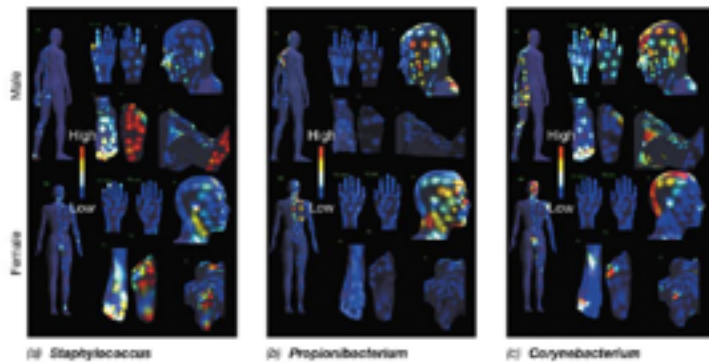
- three microenvironments

- dry skin
- moist skin
- sebaceous skin

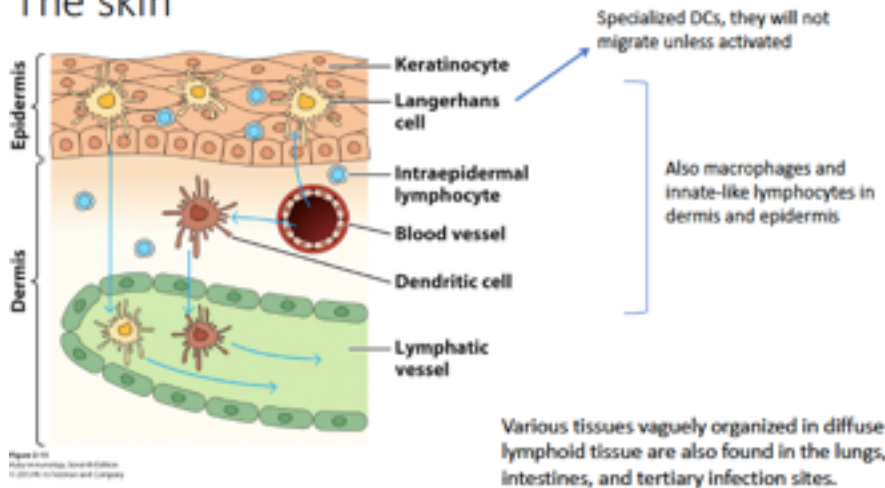


Composition is influenced by

- environmental factors (e.g., weather, pets)
- host factors (e.g., age (puberty), personal hygiene)
- Each microenvironment shows a unique microbiota.



The skin



Describe disorders of the microbiota in the GI tract (IBD) and what using mouse models have shown about the capacity of the microbiota to impact our health.

Inflammatory Bowel Disease (IBD)

- Failure to develop a tolerance to commensal bacteria in the gut early in life? Leaky gut?
- Chronic inflammation of the gut and disruption of homeostasis (dysbiosis).
- Antibiotic use increases the risk of developing IBD
- Links with Western diet (rich in animal proteins which can produce harmful metabolites also associated with colon cancer), high fiber diet seems to be beneficial
- Once developed, IBD may be transmissible between family members.
- Individuals with IBD have lower gut microbiome diversity.
- Example of IBDs: Crohn's disease and ulcerative colitis, where an adaptive T cell response against commensal gut bacteria is detected when it should not (break in tolerance).

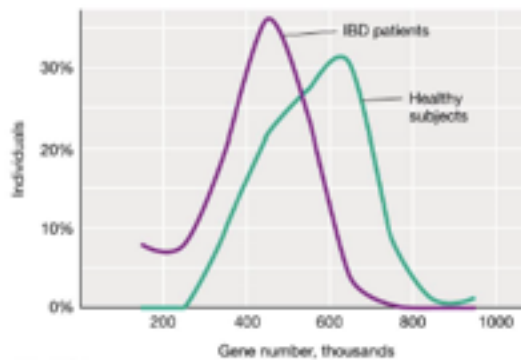


Figure 24.18 Reduced functional capacity of the gut microbiome of patients with inflammatory bowel disease. Metagenomic analysis of human gut microbiota in healthy subjects and patients with inflammatory bowel disease (IBD) revealed a tendency toward fewer nonredundant bacterial genes in patients with IBD.

The Role of the Gut Microbiota in Obesity:

Mouse Models

- Normal mice have 40 percent more fat than germ-free mice with the same diet.
- When germ-free mice were given normal mouse microbiota, they started gaining weight.
- Mice that are genetically obese have different microbiota than normal mice. Obese mice have more *Firmicutes*.

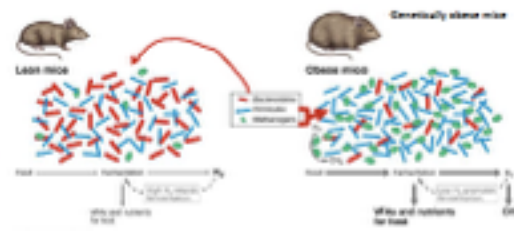


Figure 24.19 Differences in gut microbial communities between lean and obese mice. Obese mice have more methanogens, a 50% reduction in Bacteroidetes, and a proportional phylum-wide increase in Firmicutes. Nutrient production from fermentation is higher in obese mice due to removal of H_2 by methanogens.

VFA: volatile fatty acids

The Gut Microbiota and Human Obesity

- Like the mouse model, obese humans have more *Firmicutes* than non-obese humans.
- The nature and transferability of gut microbiota is dependent on diet as well genetics.

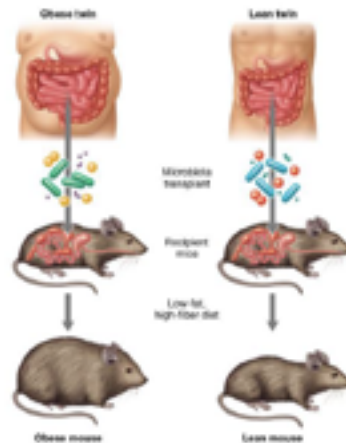


Figure 24.20 Transfer of an obese condition by fecal transplant. Transplanting fecal material from the gut contents of a paired identical human twin study group (one twin was obese and the other lean) to germ-free mice showed that the obese twin microbiota made the mouse obese. Conversely, transfer of gut contents from the lean twin did not contribute to an obese phenotype. Adapted from Kitaura, YK, et al. *Science* 302. DOI:10.1126/science.1161224.

Distinguish between probiotics and prebiotics.

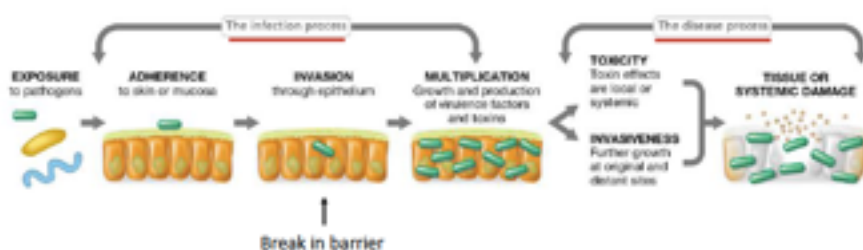
- **Probiotics** are live organisms that confer a health benefit to the host.
 - Species of *Bifidobacterium* and *Lactobacillus* bacteria are commonly used probiotics found in yogurt and probiotic drinks.
 - They may work by taking up space or nutrients, limiting the ability of pathogens to colonize the gut.
- **Prebiotics** are typically carbohydrates that are indigestible by human hosts, but provide nutrition for fermentative gut bacteria, presumably to promote the growth of “good” bacteria.

Part 2: Weapons of mass infection

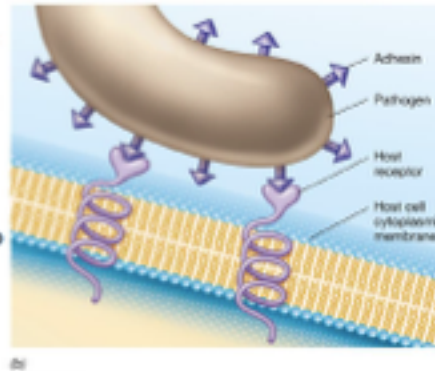
Describe what conditions need to be met for an infection to occur, ie the infection and disease process (in humans or animals).

25.1 Microbial Adherence

Adherence is the enhanced ability of microbes to attach to host tissues. It is necessary, but not sufficient, to start disease.



- There are many different receptors coating both the pathogen and tissues where the bacteria or virus binds.
- **Adhesins** are glycoproteins or lipoproteins found on the pathogen's surface that enable it to bind to host cells.

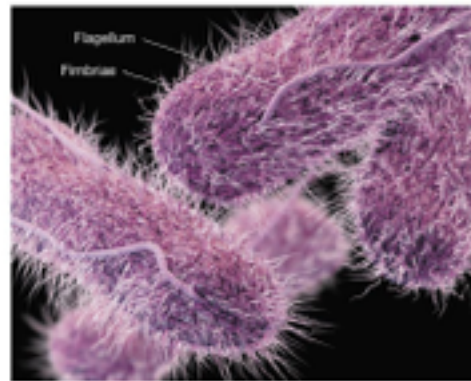


- Adherence Structures: **Capsules**
- The bacterial capsule forms a thick coating outside the plasma membrane and cell wall and serves two important functions in bacterial pathogenicity.
 - The capsule is both sticky and contains specific receptors to facilitate attachment on host tissues.
 - Capsules, such as those found in *Streptococcus pneumoniae*, protect the bacteria from ingestion by white blood cells.

- Adherence Structures:

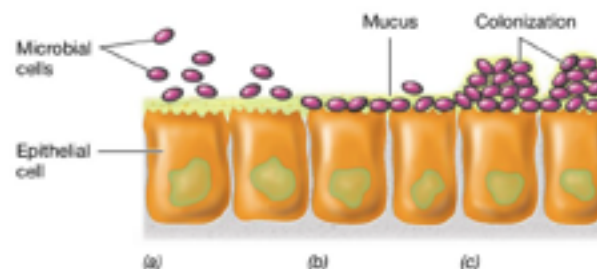
Fimbriae, Pili, and Flagella

- Fimbriae, Flagella, and pili are bacterial cell surface protein structures that function in attachment.



- **Colonization** is the growth of microorganisms after they've gained access to host tissues.
 - The process begins at birth.

- Typically starts with mucous membranes, or tightly packed epithelial cells coated in mucus, a thick liquid secretion of glycoproteins



Distinguish between the terms infection, bacteremia and septicemia.

• Invasion and Systemic Infection

• Invasiveness

- ability of a pathogen to grow in host tissue at densities that inhibit host function

• Bacteremia: the presence of bacteria in the bloodstream

• Septicemia: bloodborne systemic infection

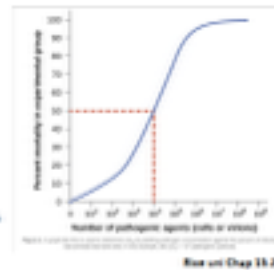
- may lead to massive inflammation, septic shock, and death

• Infection: any situation in which a microorganism (not a member of the local flora) is established and growing in a host

Explain what virulence and attenuation mean.
Explain what LD₅₀ and ID₅₀ are.

25.3 Pathogenicity, Virulence, and Attenuation

- Pathogens use various strategies to establish virulence.
 - **Virulence** is the relative ability of a pathogen to cause disease.
- Measuring virulence
 - Virulence can be estimated from experimental studies of the **LD₅₀** (lethal dose₅₀).
 - the amount of an agent that kills 50 percent of the animals in a test group
 - Highly virulent pathogens show little difference in the number of cells required to kill 100 percent of the population as compared to 50 percent of the population.
- **Attenuation**
 - the decrease or loss of virulence
- **Attenuated** strains of various pathogens are valuable to clinical medicine because they are often used for the production of viral vaccines.



Describe the cell structures or biochemical armoury that microorganisms can deploy to infect us (virulence factors):

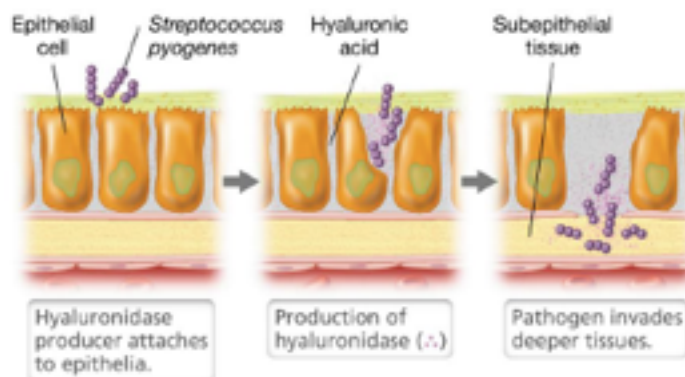
- For adhering to cells and tissues (adhesins, capsule, fimbriae, pili and flagella)
- For invading cells and tissues (enzymes, cytolytic exotoxins)
- For helping their growth and toxicity (virulence plasmid, exotoxins, endotoxins, anti-phagocytic proteins, immune inhibitors).

25.5 Enzymes as Virulence Factors

Invasiveness requires a pathogen break down host tissues. This is often done with *enzymes* that attack host cells.

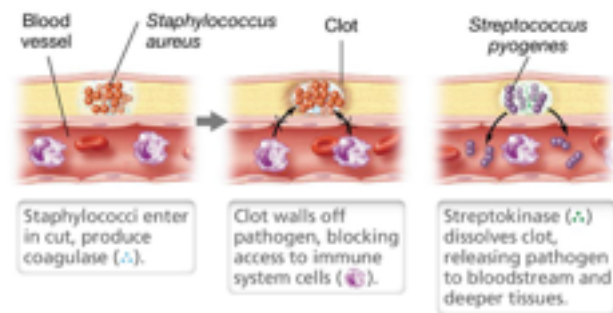
Tissue-Destroying Enzymes

- **Hyaluronidase** breaks down host tissues.



(a) Hyaluronidase

- **Coagulase** and **streptokinase** manipulate clotting. Coagulase forms clots, while streptokinase breaks them down.



(b) Coagulase and streptokinase

25.6 AB-Type Exotoxins

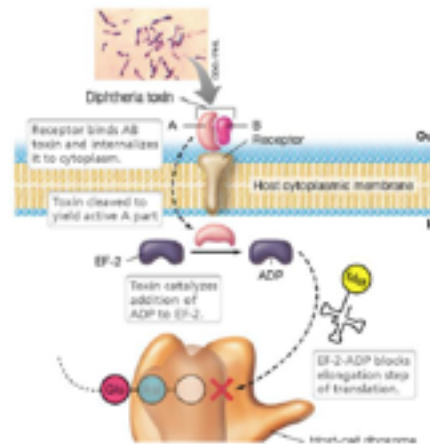
- Toxicity is the ability of an organism to cause disease by means of a toxin that inhibits host cell function or kills host cells.
- **Exotoxins**
 - proteins released from the pathogen cell as it grows
 - three categories
 - **cytolytic toxins**
 - **AB toxins**
 - **Superantigen toxins**

Describe the virulence mechanisms of the following toxins and their effect at the cellular, tissue or systemic levels when appropriate:

- Diphtheria toxin

25.6 AB-Type Exotoxins

- **Diphtheria Exotoxin: Blockage of Protein Synthesis**
 - The toxin destroys healthy tissues in the respiratory system.
- **AB toxin that is made up of an Active (A) domain and a binding (B) domain**
 - The A domain adds an ADP-ribosyl group to EF-TU, which prevents its function in translation.



<https://www.omicsonline.org/norway/diphtheria-peer-reviewed-pdf-ppt-articles/>

- **Botulinum and Tetanus toxins**

- **Neurological Exotoxins: Botulinum and Tetanus Toxins**
- *Clostridium tetani* and *Clostridium botulinum* produce potent AB exotoxins that affect nervous tissue.
 - Botulinum toxin consists of several related AB toxins that are the most potent biological toxins known.
 - Tetanus toxin is also an AB protein neurotoxin.

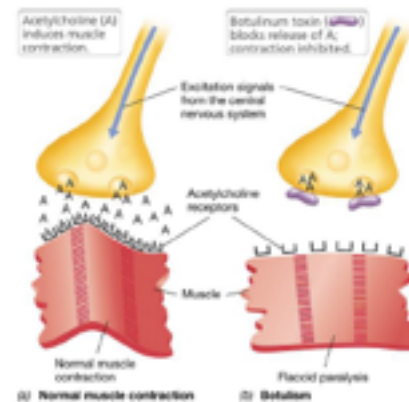
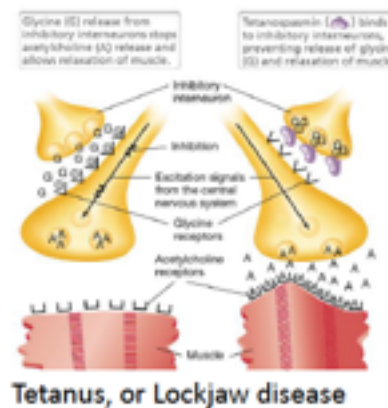


Figure 25.13 The activity of botulinum toxin.

25.6 AB-Type Exotoxins

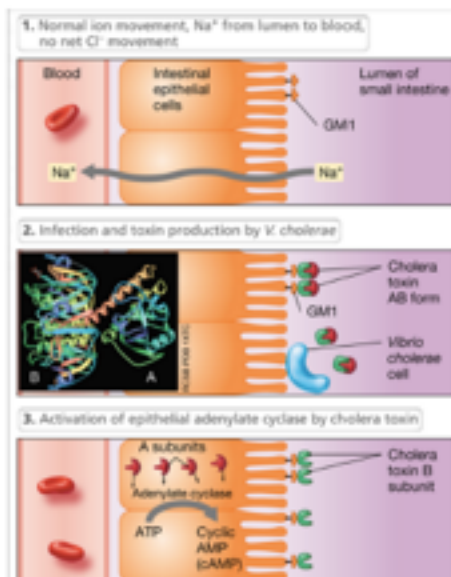
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 - Botulinum toxin consists of several related AB toxins that are the most potent biological toxins known.
 - Tetanus toxin is also an AB protein neurotoxin.



Tetanus, or Lockjaw disease

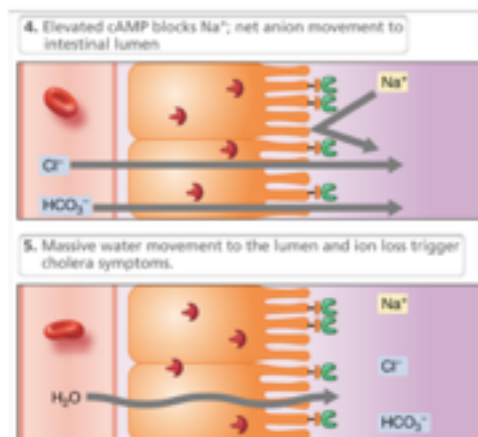
- **Cholera toxin**

25.6 AB-Type Exotoxins



Other enterotoxins

- exotoxins whose activity affects the small intestine
- generally cause massive secretion of fluid into the intestinal lumen, resulting in vomiting and diarrhea
- example: **cholera toxin**



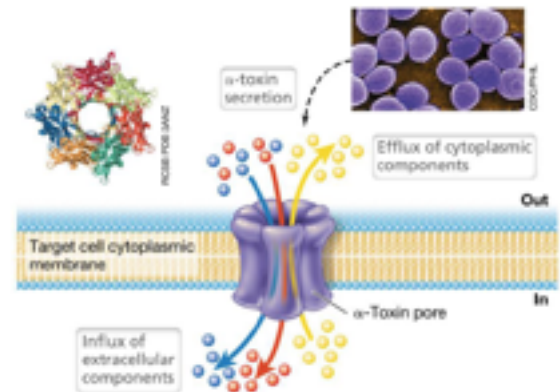
- Cytolytic toxins (hemolysins and Staph alpha-toxin)

• Cytolytic Exotoxins

- work by degrading cytoplasmic membrane integrity, causing cell lysis and death
- Toxins that lyse red blood cells are called *hemolysins*.

• Cytolytic Exotoxins

- *Staphylococcal α-toxin* kills nucleated cells and lyses erythrocytes.



- Endotoxins

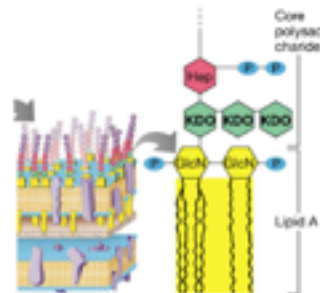
25.8 Endotoxins

• Endotoxin Structure and Biology

- the **lipopolysaccharide** portion of the cell envelope of certain gram-negative Bacteria, which is a toxin when solubilized
- generally less toxic than exotoxins

• *Limulus* amoebocyte lysate (LAL)

- Presence of endotoxin can be detected by the *Limulus* amoebocyte lysate (LAL) assay.
- Overharvesting of horseshoe crabs is a concern, as their blood is used in this assay.



• 4min <https://www.youtube.com/watch?v=5tavelk1lkg>

• Endotoxins are very different from Exotoxins

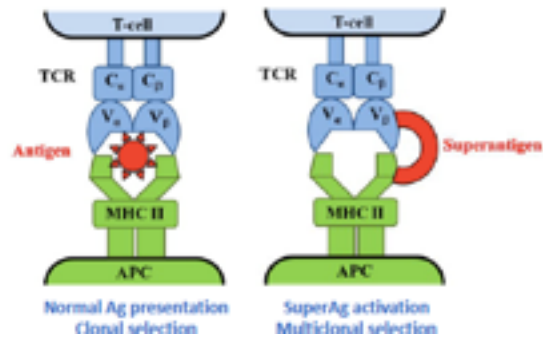
Property	Exotoxins	Endotoxins
Chemistry	Proteins, secreted by certain gram-positive or gram-negative Bacteria; generally heat-labile	Lipopolysaccharide–lipoprotein complexes, released on cell lysis as part of the outer membrane of gram-negative Bacteria; extremely heat-stable
Mode of action; symptoms	Specific; usually binds to specific cell receptors or structures; either cytotoxin, enterotoxin, or neurotoxin with defined, specific action on cells or tissues	General; fever, diarrhea, vomiting
Toxicity	Often highly toxic in picogram to microgram quantities, sometimes fatal	Moderately toxic in tens to hundreds of microgram amounts, rarely fatal
Immune response	Highly immunogenic; stimulate the production of neutralizing antibody (antitoxin)	Relatively poor immunogen; immune response not sufficient to neutralize toxin
Toxoid potential ^a	Heat or chemical treatment may destroy toxicity, but treated toxin (toxoid) remains immunogenic	None
Fever potential	Nonpyrogenic; does not produce fever in the host	Pyrogenic; often induces fever in the host
Genetic origin	Often encoded on extrachromosomal elements or lysogenic bacteriophages	Encoded by chromosomal genes

^aA toxoid is a modified toxin that is no longer toxic, but can still elicit an immune response against the toxin. [AP Section 28.9.](#)

Describe what are superantigens and how they can be so devastating during a *S. aureus* infection.

• Superantigens

- cause an overstimulation of the immune system
- can lead to shock and death
- generally due to a localized infection, but with systemic effects



Describe what is herd immunity.

Herd immunity: the resistance of a population to a pathogen as a result of the immunity of a large portion of the population.

Describe what is active immunity (vs passive immunity).

Name what are the characteristics of a perfect vaccine.

Characteristics of the perfect vaccine

- Safe
- Effective and protective
 - Induces a humoral response (neutralizing antibody production)
 - Induces a cellular response (CTLs, Th2, or Th1 depending on the pathogen)
- Long-term protection
- Convenient delivery method
- Cheap

Describe the 4 main classes of vaccines and what their advantages and disadvantages are:

28.9 Classes of vaccines



1. Live attenuated vaccine

The microbe got a serious ass-whopping, but is still strong enough to be effective.
ex. MMR vaccine (measles-mumps-rubella)



2. Inactivated ("killed") vaccine

Microbes dead. History finished. That's it.
ex. polio vaccine



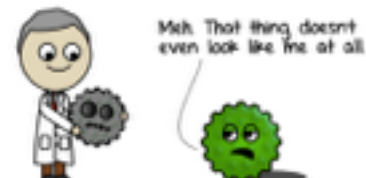
3. Subunit vaccine

The vaccine is made from various pieces of the microbe.
ex. vaccines against diphtheria and tetanus



4. Conjugate vaccine

It's a 'fake' microbe. Sort of like a vaccine dressed for Halloween.
ex. vaccine against meningitis



Advantages:

- Maintains their ability to replicate, stimulating both humoral and cellular responses.
- Do not often need boosters

Disadvantages:

- Potentially could return to a pathogenic state (mutation)
- May have more harmful side effects
- Often necessary to transport/store these vaccines at 4 ° C

Inactivated vaccines

Chemically or by heat

Advantages:

- Can not return to a pathogenic form
- Often more stable / easy transportation

Disadvantages:

- Often requires a booster
- Does not replicate in the host, so no cellular immunity (humoral only, but without mucosal IgA)
- Exposures to possible chemicals / adjuvants often necessary
- Potentially dangerous if the pathogen is not fully killed / inactivated
- The chemical or heat treatment of the microorganism could denature the antigen, ie the structure of the epitope.

Subunit vaccines

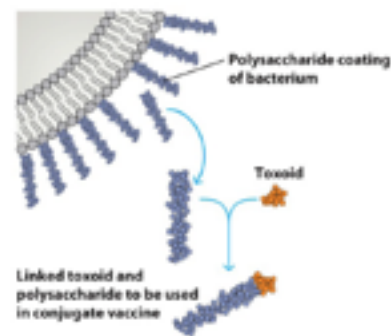
- Uses purified macromolecules, derived from pathogens
- Exotoxins / inactivated toxoids
- Inactivated polysaccharides
- Inactivated surface glycoproteins
- Recombinant protein antigen

Advantages/ Disadvantages:

- Similar to inactivated or killed virus vaccines
- No T helper cells activation
- Thymo-independent activation type 2 = mainly IgM, less isotypic switching to IgG (which are more specific and better antibodies) and little memory

Conjugated vaccines

- Normally, a vaccine can not be effective if it is only one part of the microorganism
- Some molecules simply are not sufficiently immunogenic to cause a good immune response
- But when coupled with another Ag, the response is stimulated more effectively. Example:
 - The polysaccharide which alone would stimulate only B cells, are coupled with an Ag that also stimulates Th2, which will stimulate more B by combined recognition.
 - The under 2 years are not mature enough to generate Thymo-dependent Ab. It is therefore necessary to conjugate a thymo-independent Ag to a thymo-dependent Ag in order to recruit Th2 help to stimulate Ab production.



Ex: Meningitis and pneumococcal vaccine

Describe what are recombinant and DNA vaccines.

Recombinant vaccines

- Uses an inactivated pathogen or other recombinant vectors. (Mice were protected from rabies following ingestion of spinach leaves infected with alfalfa mosaic virus transfected with a gene encoding a rabies virus peptide.)
- Genetically modify to transport the genes of another pathogen and express them

Advantages:

- All the benefits of an attenuated vaccine
- Less risk - do not use the actual pathogen, but completely another organism

Disadvantages:

- Some of the attenuated vaccine problems are still present (especially stability problems)

DNA vaccines

- Plasmids carrying the pathogenic genes injected into the muscle tissue
- Host cells internalizes the DNA and produce the immunogenic proteins in vivo.
- Allows the presentation of Ag on class I MHCs, stimulating the production of cytotoxic T cells (CTLs)

Advantages:

- Induces humoral and cellular responses (CTLs)
- Extended exposure to Ag = better memory
- VERY stable (no refrigeration required)
- Plasmids can be manipulated at will to generate / modify the Ag

Disadvantages:

- Currently unknown! Technique too recent.

Describe what are adjuvants.

Vaccine adjuvants

Animal model adjuvants are constituents of bacteria, particularly of their cell wall:

- Complete Freund's adjuvant = oil and water emulsion containing killed mycobacteria.
- Polysaccharide, bacterial DNA (unmethylated CpG), ...
- Used in animal models, but not in humans

Human adjuvants are chemicals that can help generate an answer:

- Alum-good for stimulating Th2, but not Th1
- MF59-emulsion of oil and water, slow delivery of Ag
- AS04-alum plus a TLR4 agonist (encourages the Th1 response)
- Currently researching the use of *cytokine adjuvants*, to direct the response of the vaccine to an inflammatory and cellular (Th1) or humoral (Th2) response

Explain what adjuvants are for in vaccine formulations.

Vaccine adjuvants

- Used when **weak antigens**
- Proinflammatory adjuvants can **stimulate the recruitment of immune cells**, increasing the effectiveness of the vaccine
- Some adjuvants **slow down the release of Ag** in the medium and prolong their interaction with immune cells, increasing the effectiveness of the vaccine

Example:

DPT (diphtheria, pertussis, tetanus)

Pertussis toxoid is not immunogenic by itself, so add an adjuvant if injected alone.

On the other hand, when it is administered together with diphtheria and tetanus toxoids, it serves as an adjuvant and a response is stimulated against the 3 toxoids effectively.

Name or identify targets of antiviral drugs (be as precise as possible)

28.11 Antimicrobial Drugs That Target Nonbacterial Pathogens

- **Antiviral drugs**
 - Most antiviral drugs also target host structures, resulting in toxicity.
 - Most successful and commonly used antivirals are the **nucleoside analogs**. (e.g., AZT)
 - block reverse transcriptase and production of viral DNA
 - also called **nucleoside reverse transcriptase inhibitors (NRTIs)**
 - **Non nucleoside reverse transcriptase inhibitors (NNRTIs)** bind directly to RT and inhibit reverse transcription.
 - **Protease inhibitors** inhibit the processing of large viral proteins into individual components.
 - **Fusion inhibitors** prevent viruses from successfully fusing with the host cell.
 - **Neuraminidase inhibitors** (e.g., Tamiflu) successfully limit influenza infection.
 - **Interferons** are small proteins that prevent viral multiplication by stimulating antiviral proteins in uninfected cells.

Name or identify targets of antifungal drugs (be as precise as possible).

28.11 Antimicrobial Drugs That Target Nonbacterial Pathogens

- Drugs that target **eukaryotic pathogens**
 - **Fungi** pose special problems for chemotherapy because they are eukaryotic.
 - Much of the cellular machinery is the same as that of animals and humans.
 - As a result, many antifungals are topical.
- A few drugs target unique metabolic processes unique to fungi, such as cell wall synthesis.
 - **Ergosterol inhibitors** target the unique fungal plasma membrane component ergosterol.
 - **Echinocandins** inhibit 1,3 β -D glucan synthase and are used to treat *Candida* infections.
- Other drugs target **chitin biosynthesis**, target **folate biosynthesis**, or disrupt **microtubule aggregation**.
- Like antibiotic resistance, **antifungal resistance** is on the rise.

Category	Target	Examples	Use
Allylamines	Ergosterol synthesis	Terbinafine	Oral, topical
Antimetic antibiotic	Mitosis inhibitor	Griseofulvin	Oral
Azoles	Ergosterol synthesis	Clotrimazole Fluconazole Itraconazole	Topical Oral Topical
Chitin synthesis inhibitor	Chitin synthesis	Nikkomycin Z	Experimental
Echinocandins	Cell wall synthesis	Caspofungin	Intravenous
Nucleic acid analogs	DNA synthesis	5-Fluorocytosine	Oral
Polynes	Ergosterol synthesis	Amphotericin B Nystatin	Oral, intravenous Oral, topical
Polyoxins	Chitin synthesis	Polyoxin A and B	Agricultural

Targets are important

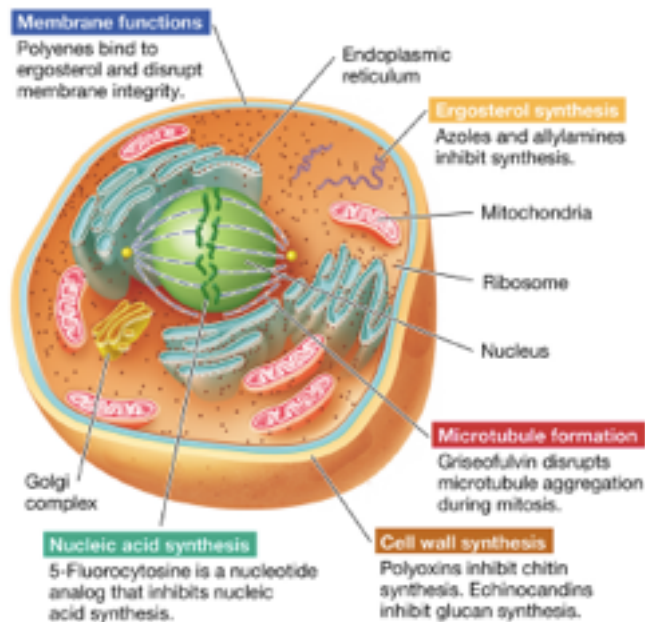


Figure 28.32 Targets of some antifungal agents.