Microbiology & Immunology

Microbiology and immunology is of high clinical significance. Many topics from these intertwined disciplines are covered on the MCAT. This content review is based upon the AAMC MCAT content guideline. The following topics will be discussed in the microbiology section. Immunology is discussed afterwards.

Microbiology

□ Introduction, Cell Theory, and Prokaryotic domains: Archaea and Bacteria

□ Prokaryotic domains: Archaea and Bacteria □ Bacterial morphology

□ Bacterial gene expression □ Bacterial cell walls and lipopolysaccharide

□ Caspsules, flagellar propulsion, and chemotaxis □ Pili, sporulation

□ Growth and Physiology of Prokaryotic Cells (aerobes and anaerobes)

□ Reproduction by fission □ Bacterial adaptation to environment through transformation, conjugation, and transduction (including virus structure and function) □ Transposons □ Animal Viruses including coronaviruses, retroviruses and HIV □ Prions and viroids

Introduction

With the development of the microscope in the 17th century, the stage was set for the discovery of the invisible world. The discovery of cells is widely attributed to Robert Hooke, who described "cells" in his book *Micrographia*. Despite this, Hooke could not explain the function of such cells and could not see any of their internal structures. Shortly afterwards, Anton van Leeuwenhoek observed motile organisms under microscopes with improved optics. He named the protozoa and bacteria that he observed "animalcules". Further, van Leeuwenhoek was the first to observe sperm and egg cells and to describe fertilization, a critical blow to the theory of spontaneous generation. These observations led to the development of the cell theory, a postulate that has three major tenants:

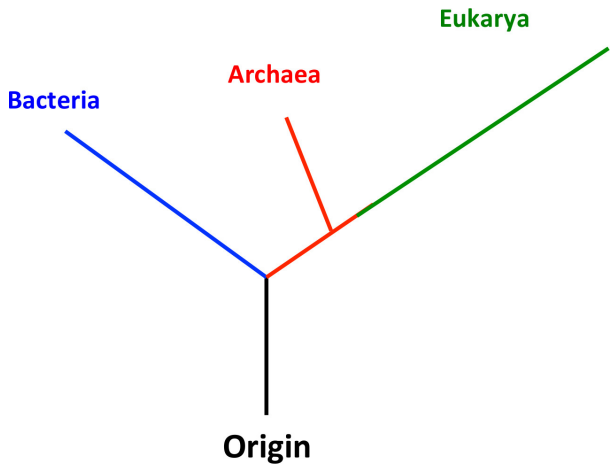
1) All living organisms are composed of cells.

2) The cell is the most fundamental unit of life.

3) All cells arise from pre-existing cells.

Modern corollaries to the original cell theory include the notion that the total activity of an organism is a function of the collective activity of individual cells and that most cells contain hereditary information (DNA) that is passed from cell to cell through division.

Traditionally, cells have been classified in two general categories: prokaryotes and eukaryotes. However, Carl Woese and colleagues evaluated the similarities and differences in ribosomal 16S RNA between organisms to define the tree of life as a taxonomy consisting of three domains: Archaea, Bacteria, and Eukarya. Initially thought to be similar to bacteria, the Archaea are as different from bacteria as plants are from animals.



Both Archaea and Bacteria are known as prokaryotes and their primary distinction from eukaryotes is the absence of a nuclear membrane. Although both Archaea and Bacteria lack nuclear membranes as well as membrane-bound organelles such as lysosomes and mitochondria, one major distinction between the two lies in the molecular composition of their lipid membranes. Importantly, Archaea possess branched lipids attached to glycerol through ether linkages, in contrast to the traditional phosphoester linkages seen in the Bacteria and Eukarya. This property contributes to the ability of many species of Archaea ("extremophiles") to withstand extreme environments (i.e. temperature and salt). Indeed, Archaea were initially identified as living in

extreme conditions such as hot springs and lakes with high salinity, but additional studies have shown that they are widely distributed in nature, including being part of the human gut microbiota. A further distinction between Archaea and Bacteria concerns the nature of the genetic material. Like Bacteria, Archaea have circular chromosomes. However, unlike Bacteria, the Archaea have histone-like proteins and utilize multiple DNA and RNA polymerases like the Eukarya. Despite this, the genome of the Archaea is not enclosed in a nuclear envelope.

Although widely accepted and applied, the three-domain system fails to address non-cellular life such as viruses and prions. In 2012, Luketa proposed a five-domain system that included acellular organisms with and without nucleic acid: Prionbiota and Vironbiota, respectively.

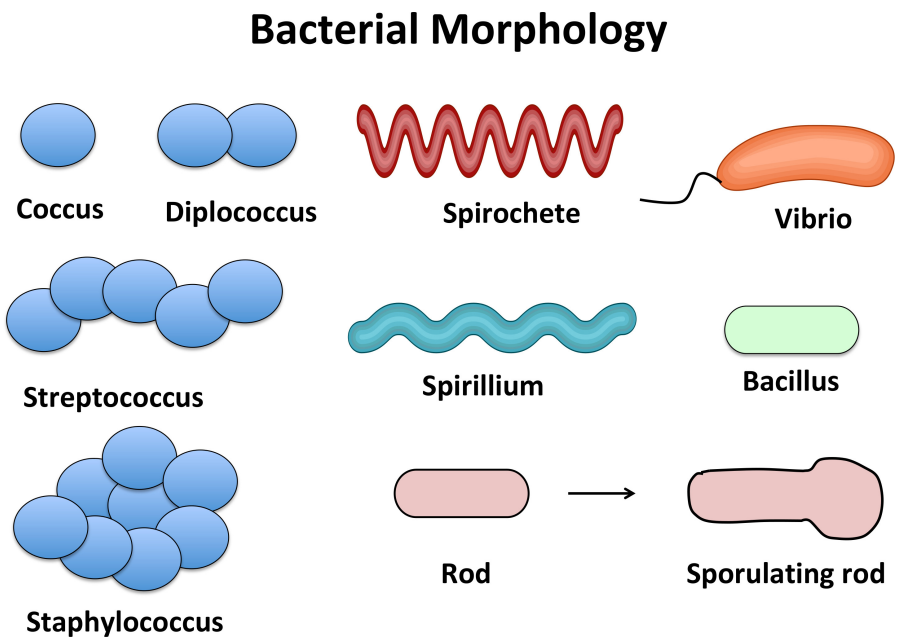
In contrast to Archaea and Bacteria, cells of the Eukarya domain are much larger and contain multiple membrane-bound organelles that have specialized functions. This necessarily demands for a larger genome. Below is a table comparing some significant features of the three major domains of life.

COMPARISON OF 3 MAJOR DOMAINS OF LIFE

PROPERTIES	ARCHAEA	BACTERIA	EUKARYA
Ester linked membrane linkages	NO	YES	NO
Nuclear envelope	NO	NO	YES
Membrane bound organelles	NO	NO	YES
Peptidoglycan in cell wall	NO	YES	NO
DNA associated with histones	YES	NO	YES
Live in extreme environments	YES	NO	NO
Response to antibiotics	NO	YES	NO
Circular genomes	YES	YES	NO

Bacteria

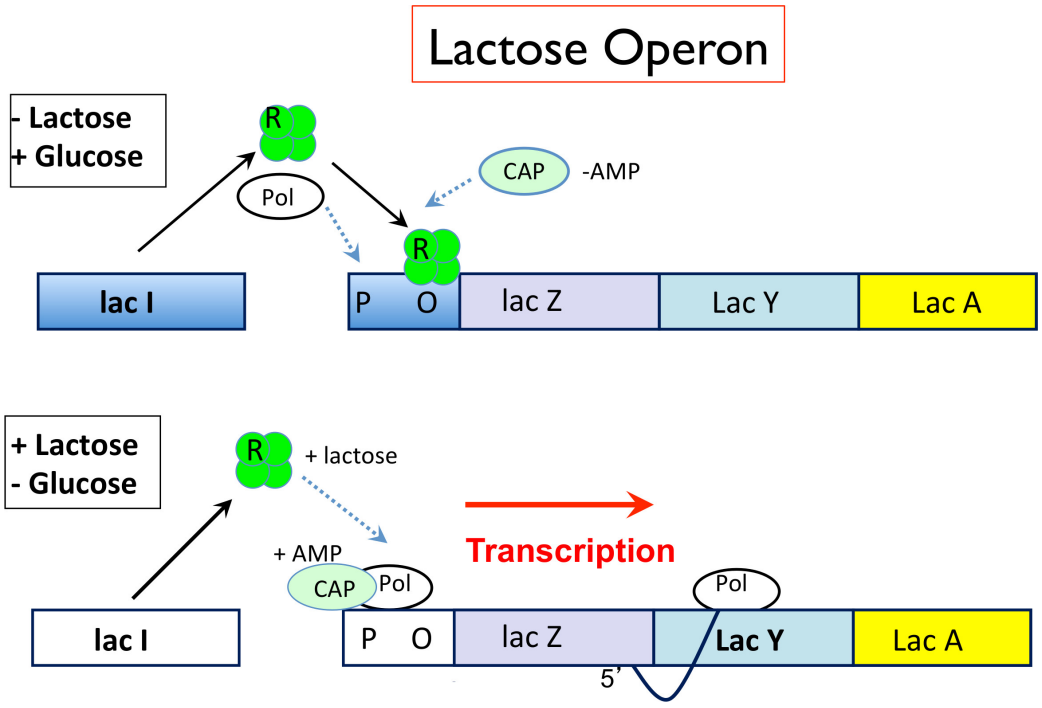
Bacteria comprise a large, diverse domain of life. They range in size from 0.1 μm (*Chlamydia*) to the largest known bacteria that is over 100 μm in length (*Thiomargarita namibiensis*). Various methodologies are used to distinguish among bacteria. This includes morphology, antigenicity, and genetic determinants (i.e. DNA sequences). Several types of morphological features of various bacteria are shown below. Notably, some bacteria contain flagella and some can form spores.



Bacterial gene expression

Bacterial genomes are less complex than eukaryotic ones on several levels. Importantly, bacteria have fewer genes and fewer non-coding DNA sequences. In contrast, eukaryotes possess introns and numerous sequences that fail to encode for any functional products. Further, most bacterial genes are organized into operons, a feature uncommon in eukaryotes. Operons are genetic units under the control of single promoters that regulate the expression of a number of genes participating in a common function. Operons coordinate numerous functions including the synthesis of amino acids as well as the metabolism of sugars such as arabinose and lactose. For example, the lactose (lac) operon discovered by Jacob and Monod encodes genes that regulate the metabolism of the disaccharide lactose in concert with the availability of glucose. The regulation of lactose metabolism in E. coli is shown below.

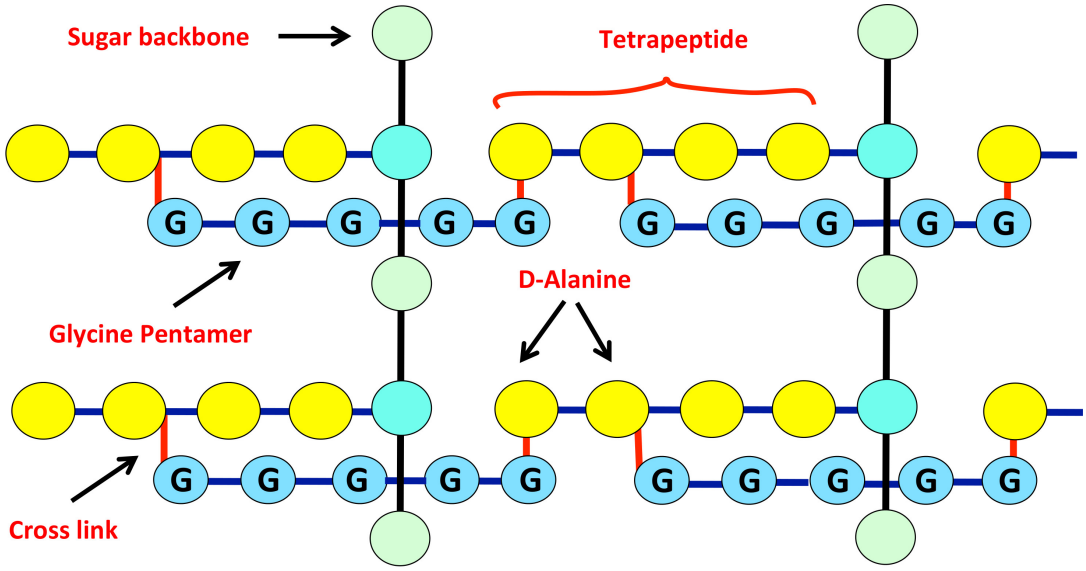
First recall that lactose is catabolized into galactose and glucose. The distal lac I gene encodes a tetrameric repressor (R) that binds to its operator sequence O, physically precluding RNA polymerase (Pol) from binding to the operon promoter, P. In the presence of lactose as the sole carbon source for growth (+ lactose, - glucose), lactose binds to the repressor. This changes the conformation of the repressor through allosterism, an event that prevents its binding to the operator. This allows for Pol transcription of the operon genes *lacZ*, *lacY*, and *lacA*. These genes are involved with transport of lactose and its subsequent catabolism. RNA polymerase function is dependent on the activity of the CAP protein that requires cAMP, a second messenger molecule that is elevated under low glucose conditions in E. coli.



Bacterial Cell Walls

Peptidoglycan (or murein) surrounds the cell membranes of most bacteria and forms a rigid, protective layer from the environment. Peptidoglycan is composed of a sugar backbone consisting of alternating residues of β-(1,4) linked N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) as depicted through the green and blue circles in the image shown below. Although not shown, there are no alpha linkages between the NAG and NAM sugars. The sugar backbone in peptidoglycan is cross-linked to

amino acid peptide bridges, including pentaglycine (G). This strong mesh-like structure provides bacteria with their shape.



PEPTIDOGLYCAN

The peptidoglycan structure is constantly being synthesized and degraded during cell growth. Indeed, all bacteria possessing peptidoglycan express autolysin enzymes that degrade peptidoglycan in preparation for cell division. Regeneration of the peptidoglycan is performed by transpeptidases and carboxypeptidases. Such enzymes are also known as penicillin-binding proteins as they inhibit the penicillin class of antibiotics (i.e. β lactams).

Bacteria can be largely distinguished from each other based upon the structure of their outer cell walls. Most bacteria are classified as either Gram-positive or Gram-negative as determined through the Gram staining technique. Importantly, the technique is based upon the nature of peptidoglycan in bacterial cell membranes. However, some bacteria (i.e. mycoplasma) are not identifiable by this technique as they do not contain peptidoglycan and are therefore considered Gram-indeterminate. The procedure is as follows:

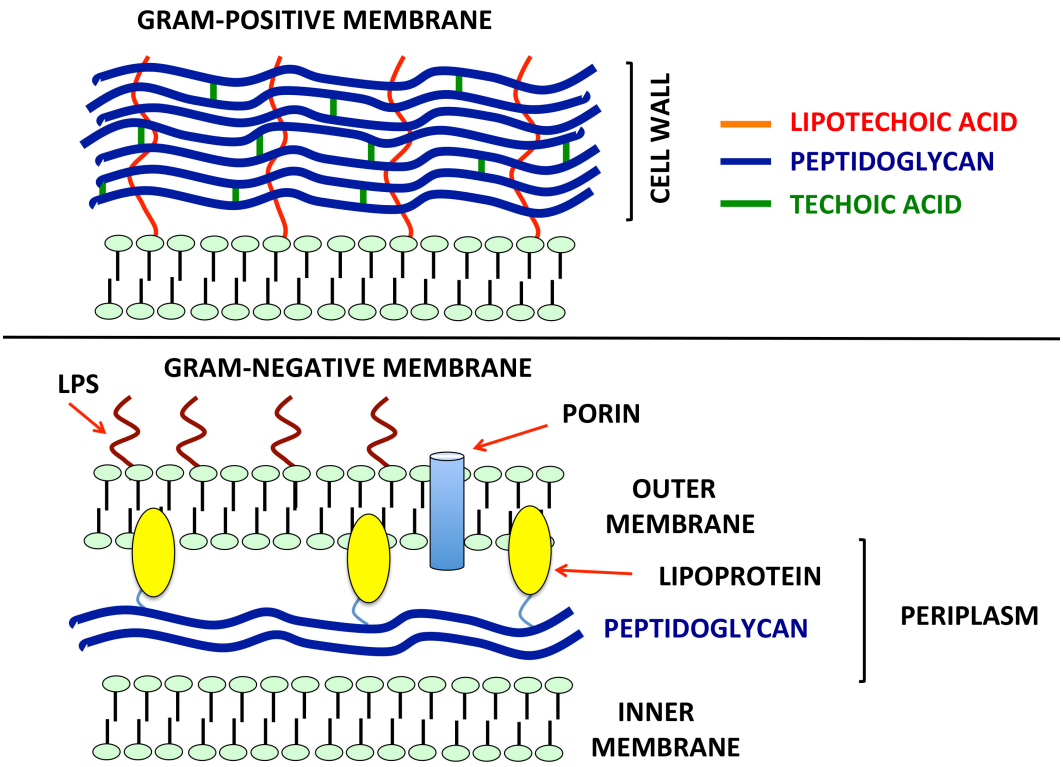
1) Heat fix bacterial sample onto a glass slide and stain with crystal violet (CV), a dye that is positively charged and diffuses through the cell wall and stains purple.

2) Addition of iodide to react with CV to trap it inside the cell.

3) Wash with ethanol

4) Counterstain with safranin. Gram-positive cells stain purple, and Gram-negative cells stain red.

As shown in the image below, Gram-negative bacteria have two membranes (outer and inner). In between lies the periplasmic space that consists of various lipoproteins, hydrolytic enzymes, and a thin peptidoglycan layer that poorly interacts with the crystal violet stain (hence Gram-negative). Porin molecules provide for passive transport of hydrophilic molecules across the outer membrane.

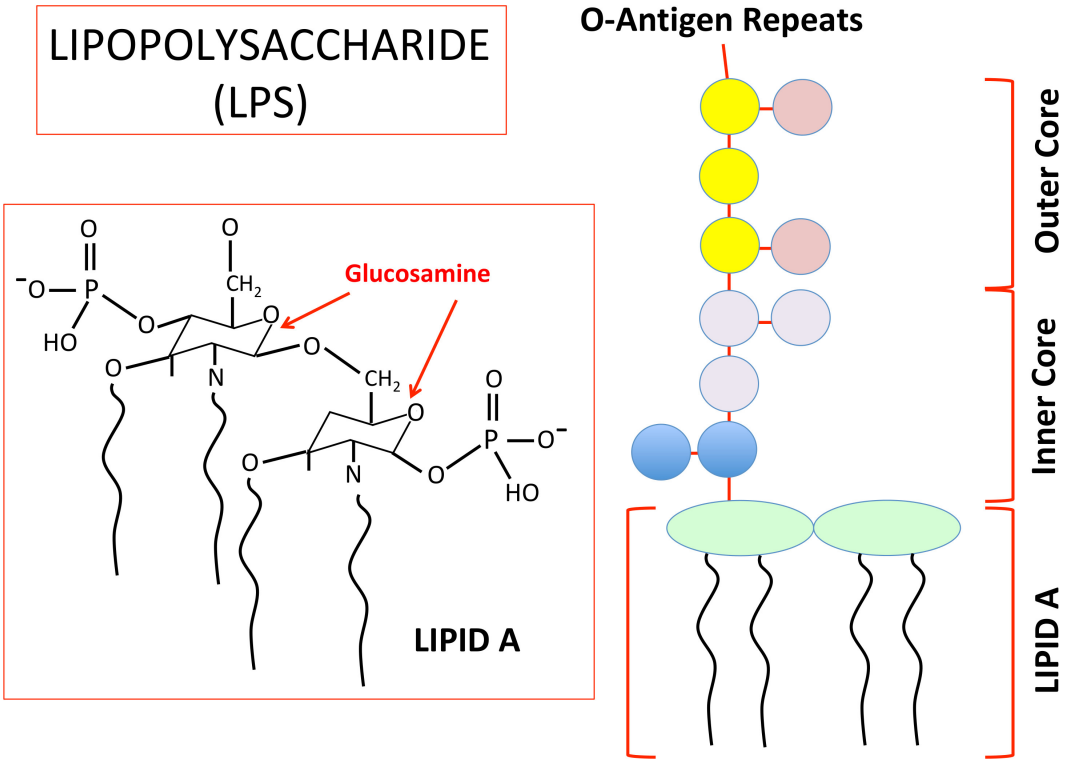


In contrast, Gram-positive bacteria have one cell membrane and a thick cell wall consisting of peptidoglycan as well as lipoic and lipotechoic acids that strongly associate with crystal violet. These polyol phosphate polymers are linked to NAM or amino acids in the peptide bridges of peptidoglycan or through membrane lipids. Lysozyme, an enzyme produced by bacteria as well as humans (i.e. mucus and tears), specifically degrades the peptidoglycan layer by hydrolyzing the glycosidic linkages between NAG and NAM. Additionally, polypeptides such as the M protein found in *Staphylococcus* associate with the peptidoglycan layer. This protein provides a defense mechanism against the host immune response by interfering with phagocytosis.

Lipopolysaccharide (LPS)

Lipopolysaccharide (LPS or endotoxin) is a major constituent of the outer membranes of Gram-negative bacteria and is often essential as mutations that prevent its formation can lead to cell death. As shown below, LPS is composed of a glucosamine disaccharide covalently linked to fatty acid chains embedded in the cell membrane (Lipid A). The O antigen is a repetitive glycan structure (colored circles) with hundreds of variations in different bacteria.

LPS gives the membrane structural integrity and acts as a barrier. Initially discovered as an "endotoxin", the Lipid A component of LPS is released upon bacterial lysis and when circulating, is causal for fever and even fatal septic shock. LPS elicits a strong immune response though its interaction with receptors on B cells, macrophages, and dendritic cells where it promotes the secretion of pro-inflammatory cytokines.



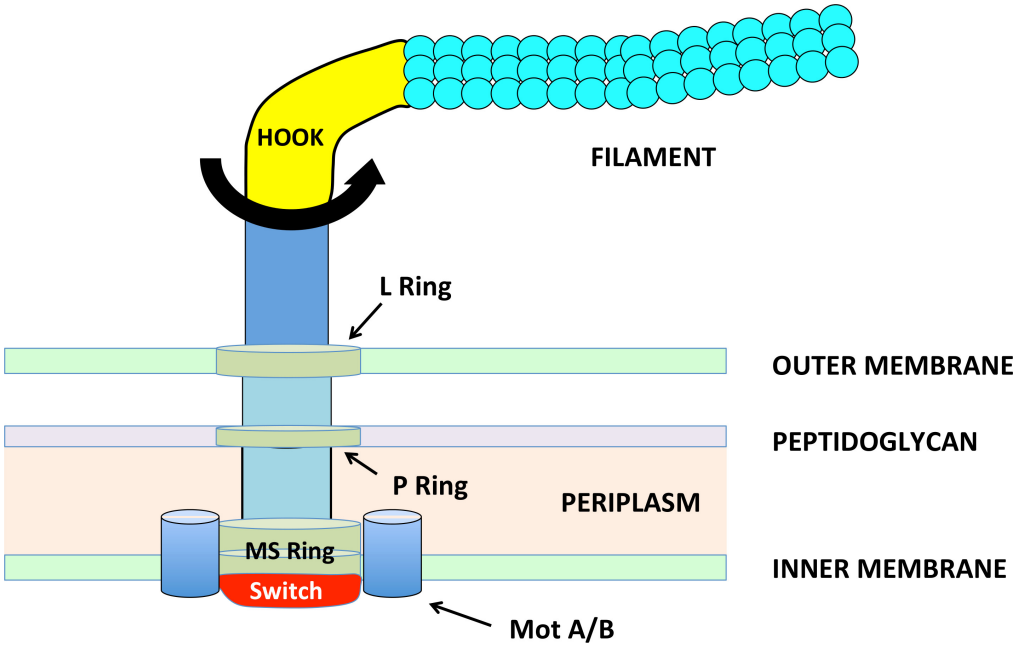
Capsules

Some Gram-negative and Gram-positive bacteria possess capsules, structures primarily composed of polysaccharides. Capsules lie outside of the cell envelope (cell membrane + cell wall) and can present as smooth and gelatinous. The slimy nature of the capsule (or glycocalyx) aids bacteria in adherence and is antigenic and anti-phagocytotic. Therefore, the capsule is an important virulence factor.

Flagella

Many bacteria contain one flagellum (monotrichous) or multiple flagella (lophotrichous). These long propeller-like appendages are used in bacterial locomotion. Indeed, in Latin, the word flagellum means "whip". Screw-like rotation (shown as CCW in the image) of the flagellum allows a bacterium to swim.

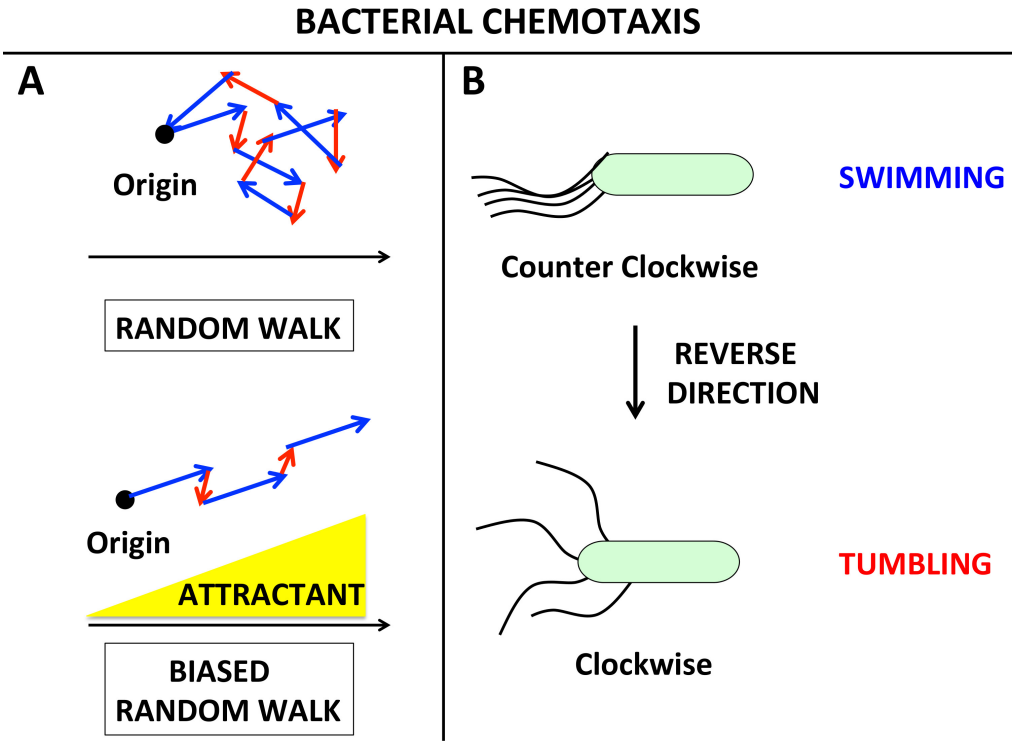
Bacterial flagella are composed of scores of proteins that comprise a rotary motor. A helical filament composed of monomers of the flagellin protein is attached to the hook that, in turn, is anchored in the membrane by the basal body (Gram-negative shown here). Unlike eukaryotic flagella that are driven by ATP, bacterial flagella derive their energy from the proton motive force of the cell membrane. This occurs through the action of the Mot A and B proteins.



These integral membrane proteins function as an engine as they couple the proton motive force of the membrane with the rotation of the hook and filament. The switch complex regulates the direction of rotation of the flagella, a key feature in bacterial chemotaxis.

Bacterial chemotaxis

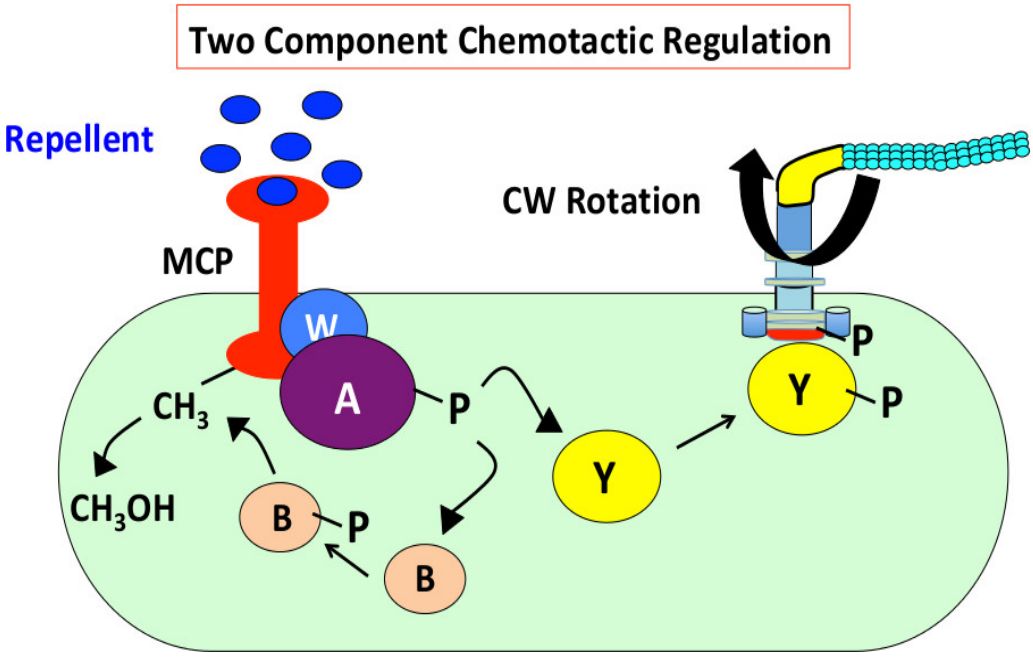
The AAMC MCAT Content Outline specifically mentions bacterial chemotaxis. Under normal conditions, bacteria swim in a random pattern or random walk where no net gain in movement is recorded over time. While swimming in a straight line, the flagella coordinately bundle to form a whip that propels the organism forward. Such smooth swimming (~0.8 seconds) is punctuated by tumbling events (0.1 seconds) where the flagella are unbundled and rotate in a clockwise manner. However, in response to gradients of attractants (i.e. food sources such as amino acid or sugars) or repellents (toxins), bacteria can sense their surroundings and either swim towards an attractant or away from a repellent. This biased random walk occurs through regulating the direction of rotation of the flagella such that they are bundled for longer periods of time in the presence of attractants.



Two component regulatory systems

The chemotactic behavior of bacteria is governed by a two-component regulatory system. These networks are comprised of a sensor kinase and a response regulator protein that controls a given cellular response. More than two-dozen such systems exist in bacteria and provide the organism with the ability to sense, respond, and adapt to changing environments. The image below illustrates this principle during bacterial chemotaxis in *E. coli* towards repellent molecules (i.e. butyrate). In this case, membrane receptors (MCP) sense repellents and initiate an intracellular phosphorelay system. Repellent ligand binding to receptor induces a conformational change in the receptor that activates the CheA sensor kinase protein in a manner dependent on the adaptor protein cheW. CheA autophosphorylates itself and then transfers this group to CheY, the response regulator in the two-component system. Phosphorylated CheY binds to the flagellar switch, creating a clockwise rotational bias that causes the cell to tumble and avoid the repellent. This represents the excitation state of the signaling pathway.

Adaptation to chemotactic stimuli is essential; otherwise excitatory signals would persist and perpetually bias swimming behavior. Once adapted after tumbling, the bacterium can reassess its new environment to make a new chemotactic response. In addition to phosphorylating CheY, CheA also phosphorylates CheB, a methylesterase enzyme that demethylates glutamate residues on the MCP receptors. CheB is antagonistic to CheR, a methyltransferase. Changing the degree of methylation of the MCP receptors



allows for the bacterium to adapt to the repellent signal. Repellent bound-MCP receptors in the demethylated state attenuate the phosphorylation of CheA, maintaining CheA-P levels observed in the baseline, unstimulated state. Thus, the two-component regulatory system consisting of CheA and CheY is regulated by the methylation status of the MCP receptors.

Pili

Pili, or fimbriae, are hair-like structures on the surface of bacteria. They have smaller diameters, shorter lengths, and are not filamentous like flagella. Further, pili are not associated with bacterial movement as flagella are, but rather can cause a twitching motion in some species of bacteria. Like flagella, pili are antigenic. Composed of the protein pilin, pili promote the adherence of bacteria to various surfaces, including the urogenital tract in humans. Fimbriae are required to initiate the formation of biofilms on surfaces.

Sporulation

In response to nutrient deprivation, bacteria initiate a variety of responses in order to achieve homeostasis. This includes the activation of chemotactic motility to seek nutrients, and the secretion of antibiotics and hydrolase enzymes to neutralize competing microbes and to degrade any surrounding macromolecules that might be potential sources of energy. Further, some bacteria activate a genetic competence system that promotes the uptake of foreign DNA, a process that allows the bacteria to potentially change its genetic composition in its mission to adapt and survive.

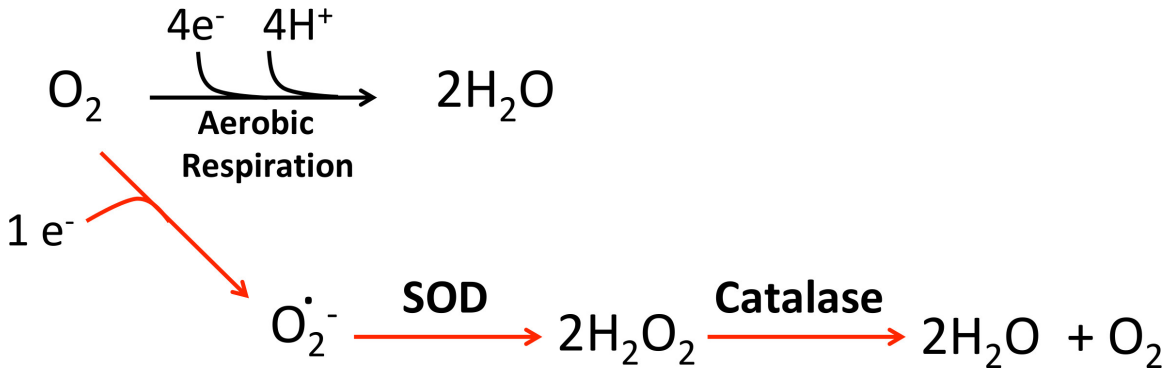
As a last ditch effort to adapt to its environment, some Gram-positive bacteria, but not Gram-negative ones, can exit the normal growth cycle and sporulate. Sporulation has been most widely studied in species of Bacillus, which includes *Bacillus subtilis* and *Bacillus anthracis*, the causative agent for anthrax. During sporulation an asymmetric division septum is created. Through the activation of a series of gene expression programs, developmental and morphogenic pathways are initiated, allowing for collaboration between the mother cell and forespore that culminates in the formation of a mature spore.

Growth and Physiology of Prokaryotic Cells

In order for bacterial growth to occur, an adequate supply of macromolecules and energy must be present. Bacteria derive the energy required to generate macromolecules for growth and division through multiple methodologies. In fact, bacteria are often classified according to their metabolic requirements.

In animal cells the reduction of oxygen into water during electron transport provides a major source of energy that can be used for the synthesis of macromolecules. However, in some bacteria such as Clostridium botulinum, the agent responsible for botulism as well as the source of cosmetic Botox, oxygen is a poison. Thus, Clostridium botulinum is classified as an obligate anaerobe. These bacteria derive their energy from pathways such as glycolysis and electron transport in the cell membrane, In contrast to animal cells that use oxygen as the terminal acceptor, obligate anaerobes use alternative electron acceptors such as sulfate and nitrate.

As oxygen is very reactive and can generate free radical species that damage cells, the ability of obligate anaerobes to survive in oxygen depends on their capacity to limit the superoxide radicals created through oxygen reactivity. Therefore, the inability of obligate anaerobes to tolerate oxygen is linked to low expression of enzymes such as superoxide dismutase (SOD) and catalase (See below). In this light, those obligate anaerobes that express higher levels of enzymes capable of handling genotoxic oxygen radicals can grow in atmospheres composed of higher levels of oxygen than those obligate anaerobes that fail to express these enzymes.



In contrast to obligate anaerobes, obligate aerobes require oxygen for growth and completely oxidize carbon into CO_2 through aerobic respiration. However, most bacteria are classified as facultative anaerobes as they can grow in the presence or absence of oxygen. Chemotrophic bacteria tend to live in extreme environments (i.e. deep in the ocean, lava beds) and can oxidize metals for energy. They also synthesize organic compounds from CO_2.

Parasitic and symbiotic bacteria

In addition to their energetic requirements, bacteria can also be classified as to how they interact with other organisms. Bacteria that infect a host and use its resources to live and multiply are termed parasitic. Some parasitic bacteria cause diseases such as cholera and the plague. Many parasitic bacteria cause food-borne illnesses as well as sexually transmitted diseases. This includes *Salmonella typhimurium*, a Gram-negative intracellular parasite that is transmitted to humans and animals through undercooked food as well as syphilis and gonorrhea.

Symbiotic bacteria form a mutually beneficial relationship with their host. One classic example of this is bacteria from the genus Rhizobia, a Gram-negative soil bacterium. Mutual signals form the plant and bacteria provide the capacity for the bacteria to infect the host plant. Bacterial growth generates nodules that serve as the sites for fixation of atmospheric nitrogen into ammonia through expression of the nitrogenase enzyme:

$$N_2 + 16ATP + 8e^- + 8H^+ = 2NH_3 + H_2 + 16ADP + 16P_i$$

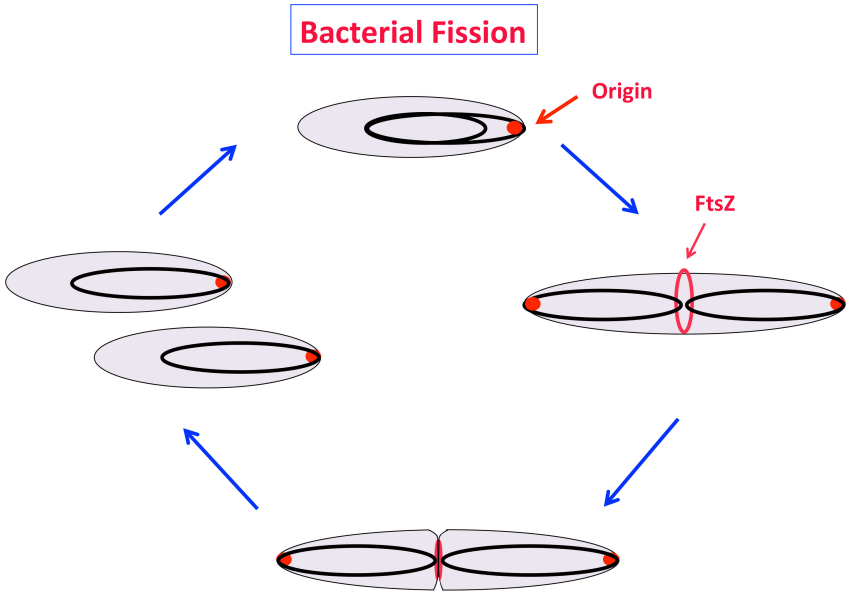
The Rhizobia-produced ammonia is used as a substrate for the production of amino acids such as glutamine and asparagine. In return, the plants provide the bacteria with carbohydrates and oxygen.

Reproduction by fission

In contrast to eukaryotic cells that reproduce by the process of mitosis, bacteria reproduce by the asexual process of binary fission. The duration of bacterial fission depends on the species and the growth media, but E coli can complete the process in as little as 20 minutes. During fission, the circular, tightly wound bacterial chromosome is duplicated by DNA polymerases. DNA replication begins at the single origin, an AT rich DNA sequence that is associated with the plasma membrane.

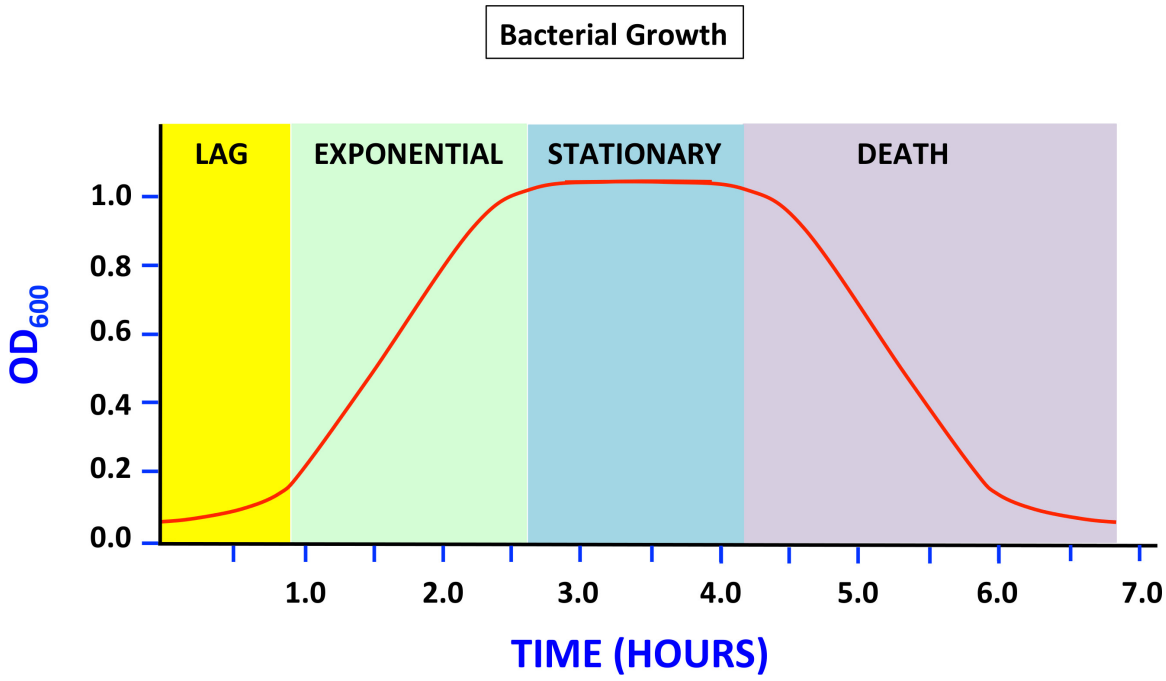
After duplication, the DNA migrates towards opposite pole of the growing bacterial cell. Next, the FtsZ protein initiates the formation of a septum that will split the cell into two. FtsZ resembles the eukaryotic tubulin protein and forms a ring (Z ring) that is considered as part of a prokaryotic cytoskeleton. FtsZ generates a spatial scaffold for the septum and may also provide the force (energy) required to divide the cell through its ability to hydrolyze GTP. Because FtsZ appears as a master regulator of septum formation, it is an attractive point for regulating the cellular response to DNA damage. Indeed,

genotoxic stress to bacteria induces a wide pattern of gene expression including that of SulA, an FtsZ inhibitory protein. The SulA-FtsZ complex inhibits the formation of the Z ring and abrogates cell division.



Growth of Bacteria

The growth of bacteria in culture is often divided into four phases: lag, exponential, stationary, and death. During the lag phase, the bacteria begin to adapt to their environment and synthesize macromolecules in preparation for



exponential growth. During the exponential, or logarithmic growth phase, the bacteria have adapted to their conditions. The growth rate (number of doublings per unit time) is defined as the slope of the line during this phase. During the growth rate there are no limitations on the availability of nutrients. However, during the stationary phase nutrient availability becomes rate limiting. Further, cells produce growth-inhibitory substances such as organic acids (i.e. lactic acid). As a consequence, new cell formation is inhibited. Lastly, the death phase is marked by nutrient depletion or other perturbations to the culture (i.e. oxygen deprivation for obligate aerobes).

Bacterial growth can be modeled mathematically through the following equation:

$$N_t = N_O \times 2^{T/D}$$

In this equation, N_t = Total number of cells after time = T; N_O = initial number of cells, D = doubling time.. Note that OD_{600} is the optical density and reflects the turbidity of the culture. The OD_{600} is a measure of the cell number.

Bacterial adaptation to the environment

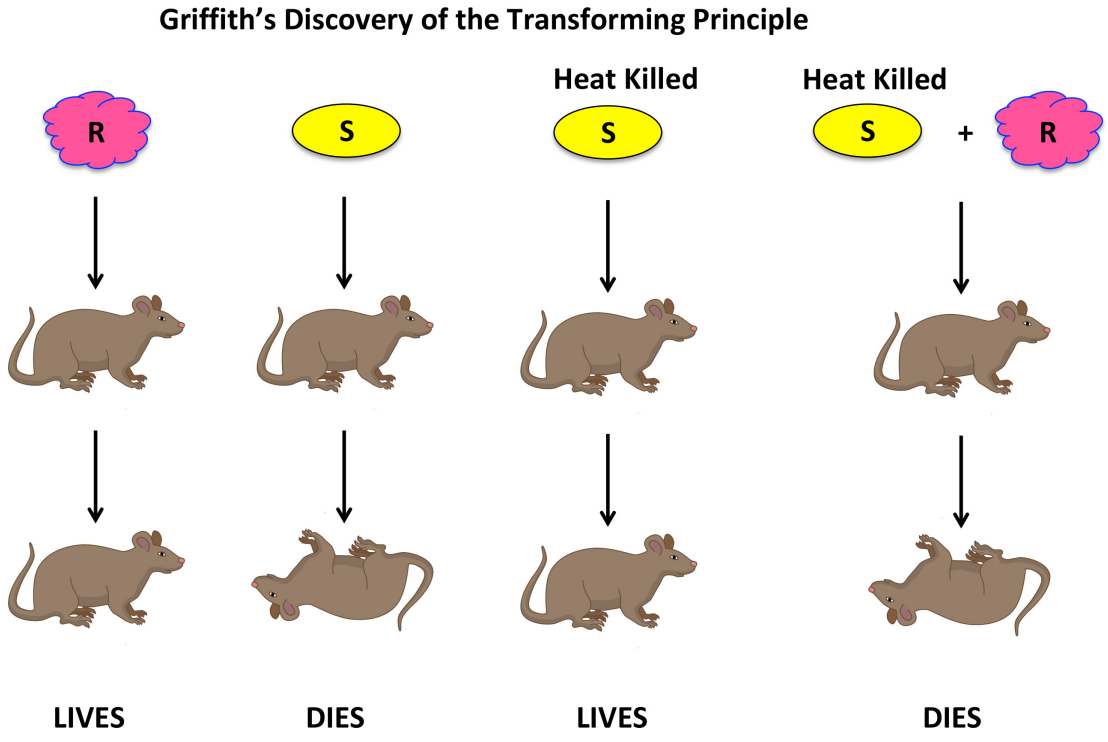
With the exception of mutations that fail to be corrected by a robust DNA repair apparatus, the daughter chromosomes derived from bacterial fission are identical to the mother chromosome; there is little to no genetic diversity. As such, this lack of genetic variability could limit the bacterium in its capacity to adapt to the inevitable changing environment, a feature that would hinder natural selection. However, bacteria display a high degree of genetic adaptability as seen through the processes of transformation, transduction, and conjugation as well as through movement of transposable elements. During these horizontal transfers of genetic information, bacteria acquire new genetic material.

Transformation

The ability of bacteria to take up DNA from their environment is known as transformation. The source of DNA is often derived from other bacteria (i.e. deceased) and can come in the form of linear DNA or circular, episomal DNA known as plasmids.

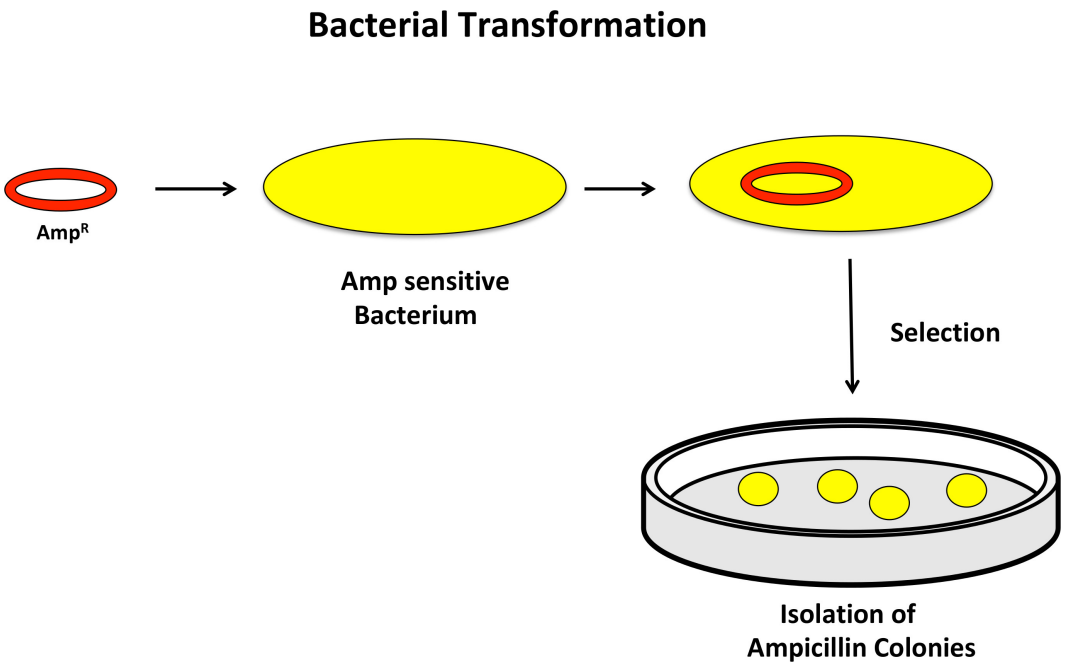
While trying to develop a vaccine against pneumonia, Frederick Griffith discovered transformation in 1928 with a seminal experiment using Streptococcus pneumonia. At the time, scientists knew from Mendel's experiments that traits were inherited from one generation to another. However, conventional thought at the time held that proteins would serve as the genetic material as they possessed the diversity through being composed of 20 different amino acids, as opposed to the nucleic acids that are composed of four bases.

Through the use of two different strains of S. pneumonia, abbreviated as R and S, Griffith discovered the process of transformation (outlined below). The S strain, or smooth strain, represents a variant of S. pneumonia that expresses polysaccharides on its capsule surface. Such a structure, as earlier described, provides protection against the immune system of the host. Therefore, the S strain is virulent and can infect and kill the host, a mouse in the case of the Griffith experiment. In contrast, the R strain, or rough strain, does not have a capsule and can be killed by the host immune system; the animal lives. When Griffith killed the S strain with heat prior to injecting into the mouse host, the animal lived. However, when the cellular components of the heat-killed S strain bacteria were incubated with the non-virulent R strain and injected into the host, Griffith observed that the animals died. That is, the R strain was "transformed" by components of the S strain. Over fifteen years later in 1944, Oswald Avery and colleagues determined that the transformation principle was



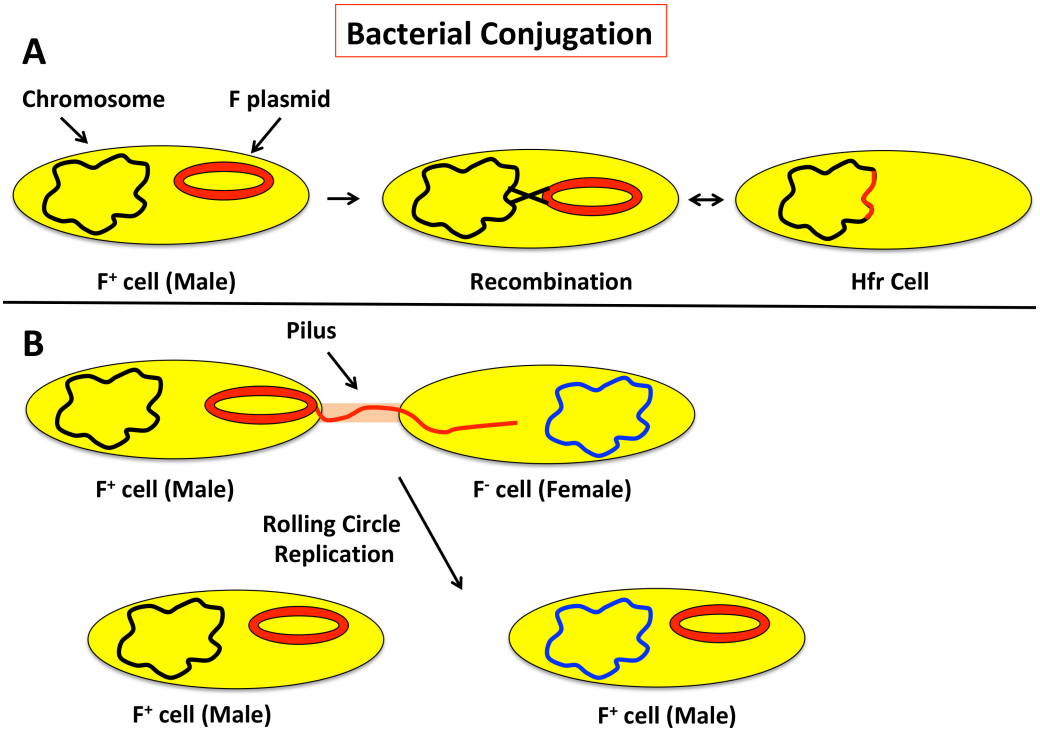
deoxyribonucleic acid.

Today bacteria are routinely transformed in laboratories with plasmids encoding genes that promote antibiotic resistance. Plasmids are extrachromosomal hereditary determinants that replicate autonomously. They are usually closed circular double stranded DNA molecules that contain genes that encode virulence factors as well as antibiotic resistance genes. Upon transformation and selection on media containing ampicillin, those bacteria that have taken up the plasmid via transformation can be selected for. This is shown below in the image.



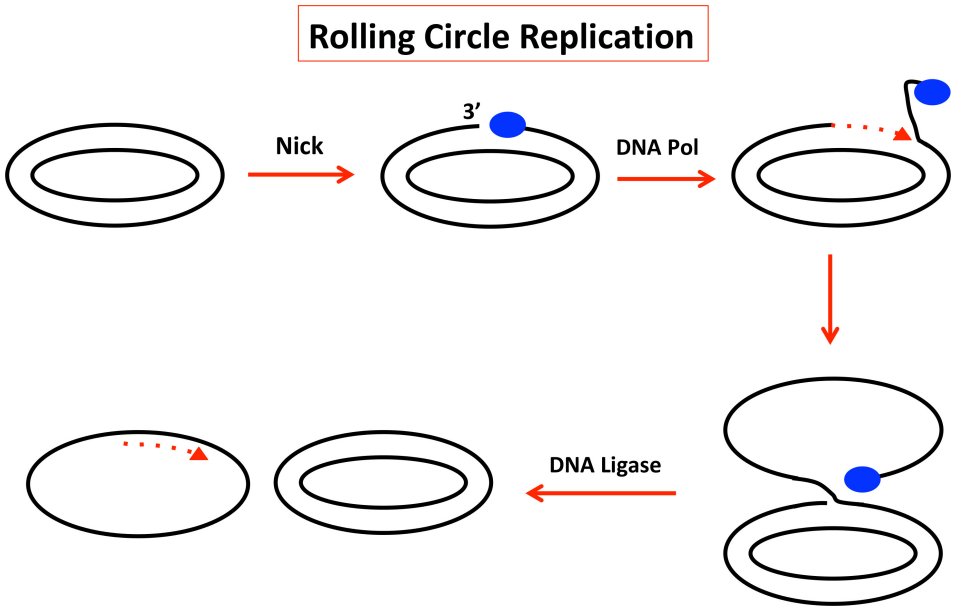
Bacterial Conjugation

During the process of bacterial conjugation, a bacterium directly transfers DNA to another bacterium through a pilus. In some ways, the process resembles sex. During conjugation, the F plasmid is transferred from a male bacterium (F^+) to a female (F^-) recipient. The F plasmid is large (~100 kb) and contains genes, including those that direct the formation of the pilus structure. In addition, the F plasmid contains DNA sequences that are homologous to the chromosome. This allows for the exchange of genetic information between



the F plasmid and the chromosome through the process of homologous recombination. This generates a cell capable of high frequency of recombination, or an Hfr cell (Panel A).

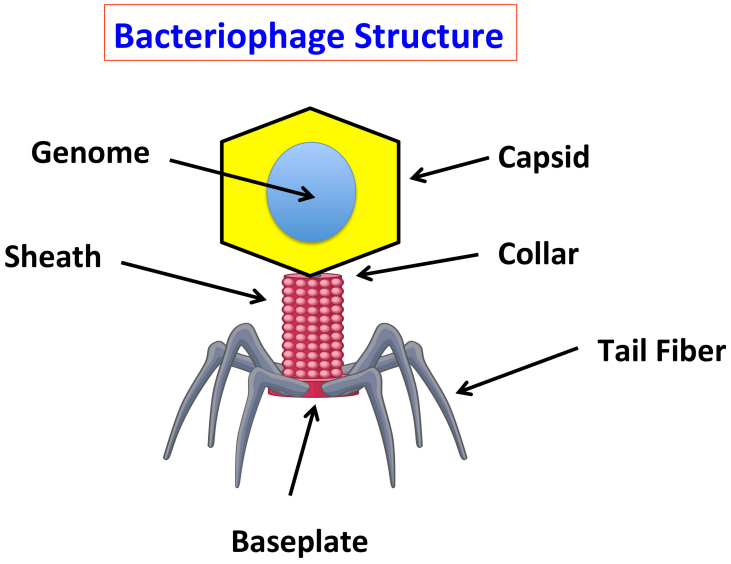
The transfer of the F plasmid from a male cell to a female recipient occurs through the rolling circle mechanism of DNA replication. This is shown in the image. The F plasmid encodes for a protein that nicks the DNA. A DNA polymerase is recruited to the 5' end of the nicked plasmid and replicates the



DNA while displacing the complementary strand through collaboration with a helicase enzyme. This displaced lagging strand will ultimately be replicated through Okazaki fragments to generate a replica F plasmid.

Transduction

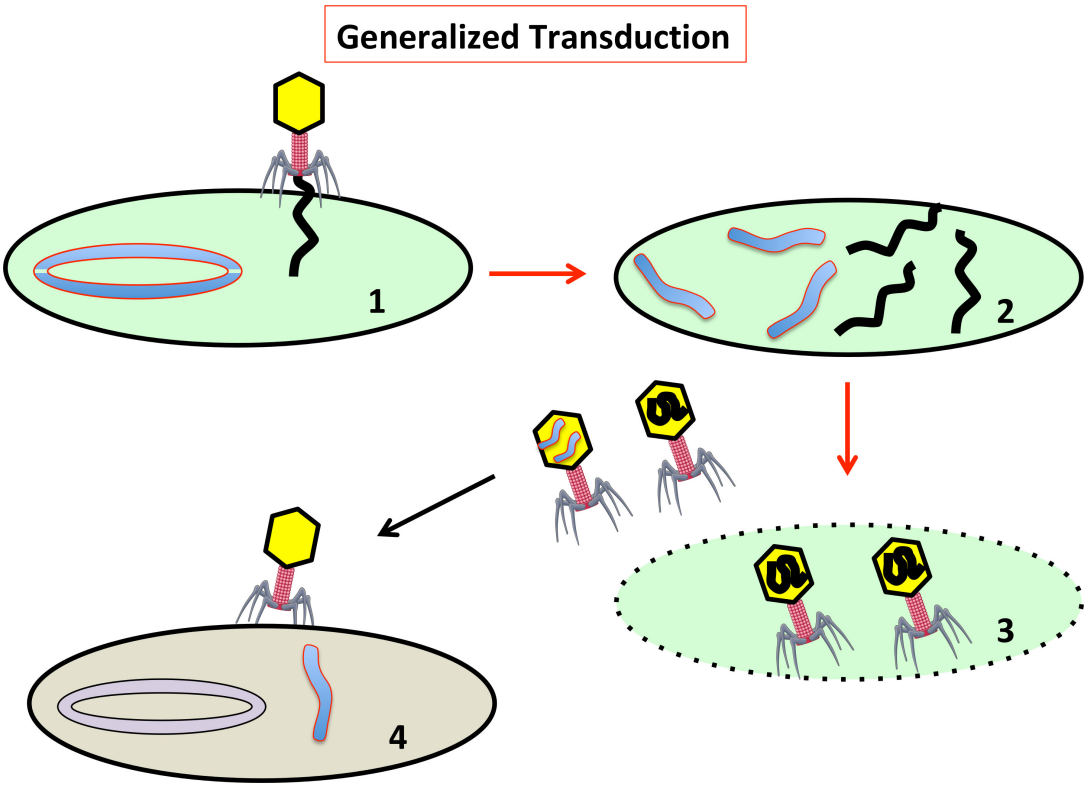
Bacteria can acquire new genetic information from prokaryotic viruses known as bacteriophage through the process of transduction. The general organization of a typical bacteriophage is shown below. Bacteriophage possess no organelles and harbor their nucleic acid genomes (DNA or RNA) in capsid structures and, after attaching to surface receptors through their tail fibers, inject the genome into the host through a syringe-like mechanism.



Once inside the host, the viral genome can enter either a lytic or lysogenic life cycle. In the lytic stage, the viral genome duplicates itself and forms new virions through the use of host machinery (i.e. ribosomes). This often occurs through killing the host and releasing new viral particles. In the lysogenic life cycle, the viral genome integrates into the host chromosome. Here, it does not express the genes to make new virions, but rather is passively replicated along with the host chromosome. In response to various forms of stress (i.e. UV radiation and lack of nutrients), the viral genome can be activated for the lytic cycle.

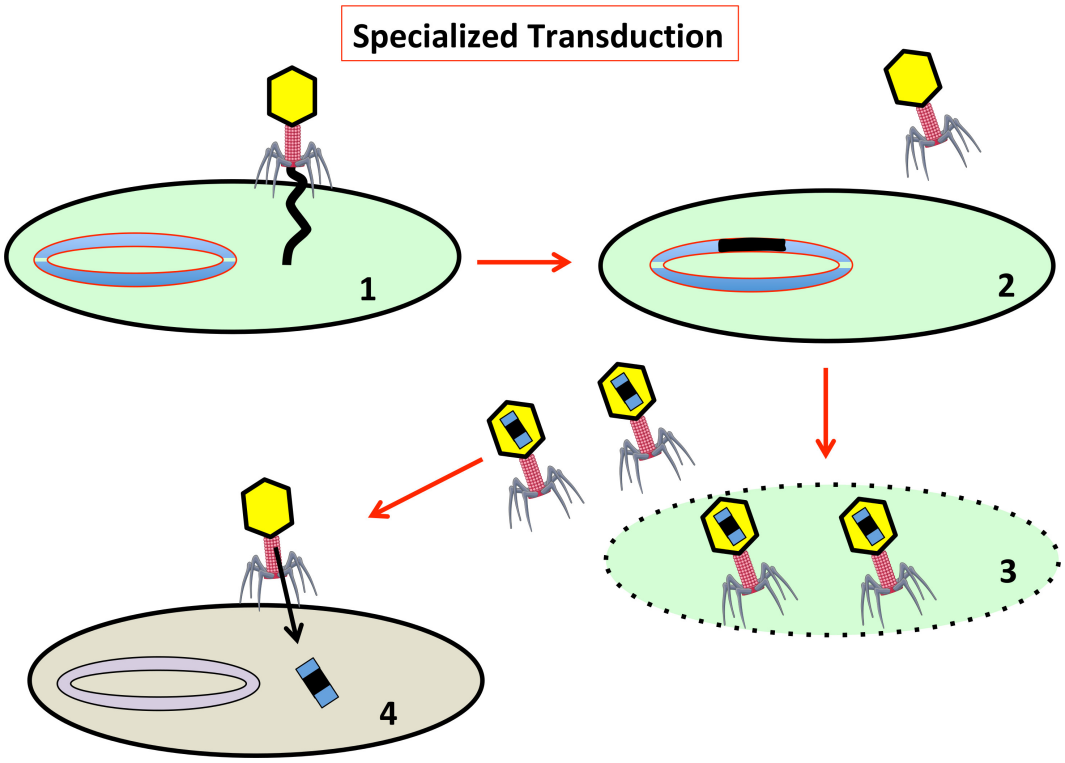
There are two types of transduction (generalized and specialized); each one depends on the nature the viral life cycle.

In generalized transduction, the virus is in the lytic stage, the life cycle where it is actively making viral particles and lysing the host. As shown in the image, the bacteriophage attaches to the cell and injects it DNA nucleic acid (1). The viral genome is replicated as the host genome becomes hydrolyzed (2). The cell is lysed and new virions are released (3). In some cases, the new virion packages the DNA from the bacterial host. Upon infection of a new bacterium, the new virus transfers DNA from one bacterium to another (4). If the conditions favor lysogeny, the newly introduced DNA molecule can integrate into the host genome.



Specialized Transduction

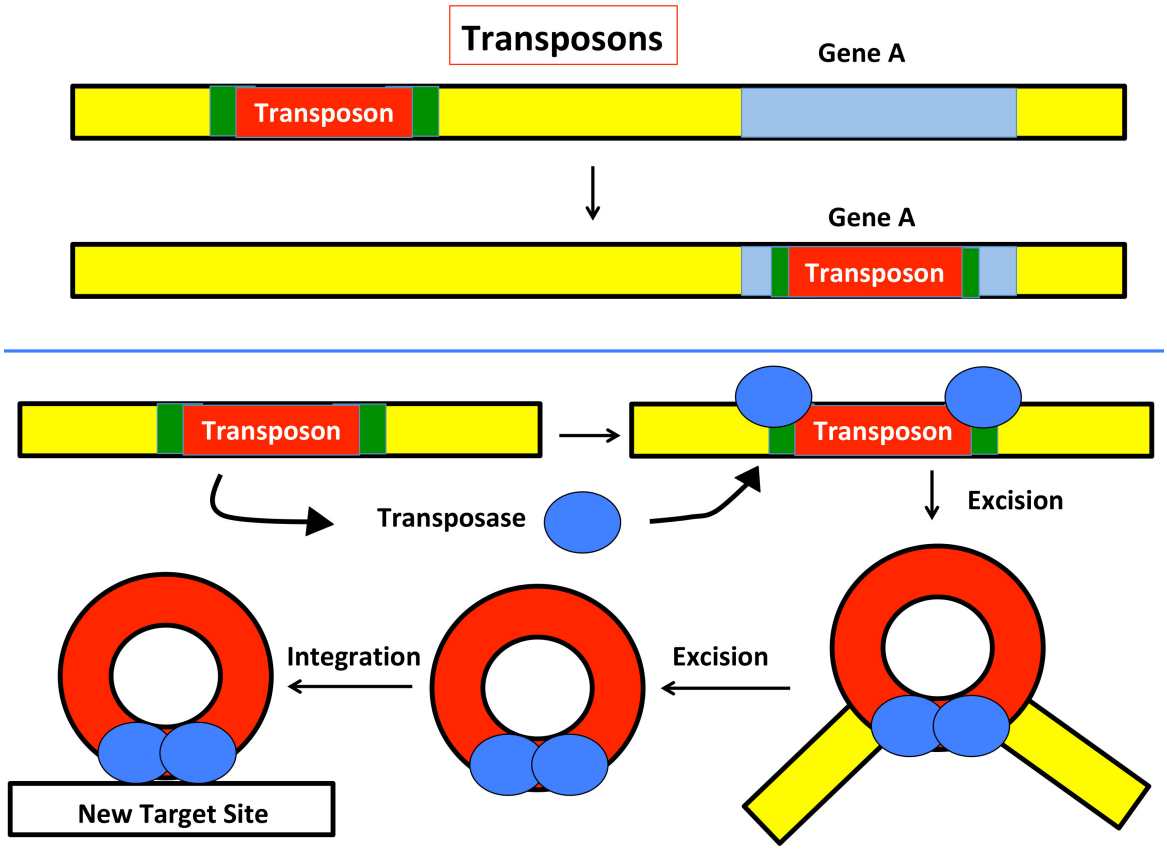
In specialized transduction, the virus uses the lysogenic life cycle. After injection of its nucleic acid genome (1), the DNA gets incorporated via homologous recombination into the host chromosome as a prophage (2). The silenced DNA is replicated along with the chromosome and distributed to new bacteria via fission (lysogeny). In response to changes in environmental conditions, the lysogenic phage can be activated into the lytic cycle. However, upon removal of the phage genome from the host, surrounding chromosomal DNA from the host is inadvertently excised (3). This results in the generation of new phage particles that contain host DNA. The newly formed virions infect new bacterial hosts and inject host DNA sequences (4). The initial bacteria's DNA sequences have been horizontally transferred to a new bacterium through specialized transduction.



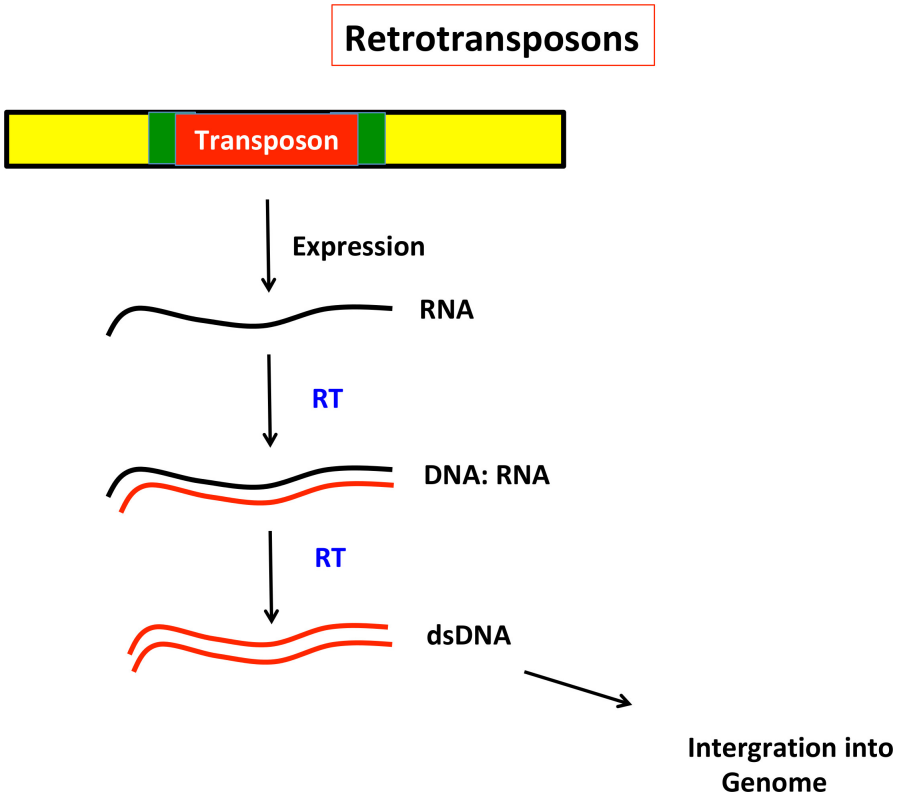
Transposons

Transposons, or "jumping genes", are segments of DNA that are horizontally transferred from one organism to another or within the same organism. Through integration from one chromosomal spot to another, transposons can increase the size of the genome as well as create mutations by inserting into functional coding sequences. Although Barbara McClintock discovered transposons in corn, they are now known to exist in animal and bacterial cells.

As shown below, Type II transposons move around from one place to another in a genome through a "cut and paste" mechanism that uses a transposase enzyme encoded by sequences within the transposon (Top Panel in image below). When inserted into coding sequences at other chromosomal loci, the transposition event can be mutagenic. The transposase enzyme binds to inverted repeat sequences flanking the transposon. This creates a DNA-protein complex that is excised. In some cases, flanking host sequences are also excised and when inserted at a new target site, mutagenesis occurs.

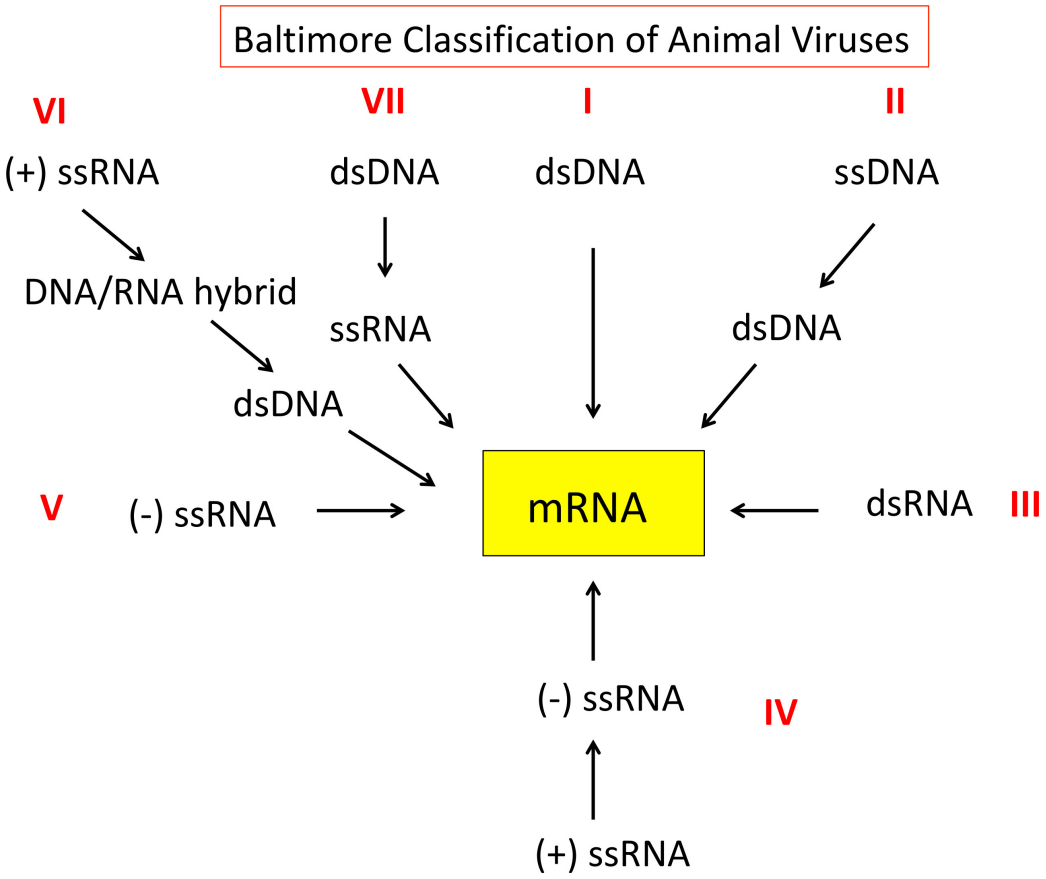


Type I transposons are also known as retrotransposons and are found in human cells. Unlike Type II transposons, Type I transposons are synthesized through an RNA intermediate. In this scenario, the transposon encodes for the reverse transcriptase enzyme (RT). This is an important enzyme, particularly as it used in various molecular biology techniques. After RNA polymerase copies the RNA into a single stranded DNA molecule, RT generates a DNA:RNA hybrid that is degraded by an intrinsic RNAse activity of the enzyme. As RT is also a DNA-dependent DNA polymerase, a double stranded (ds) DNA molecule that can integrate into another host is generated, an event that propagates the transposon.



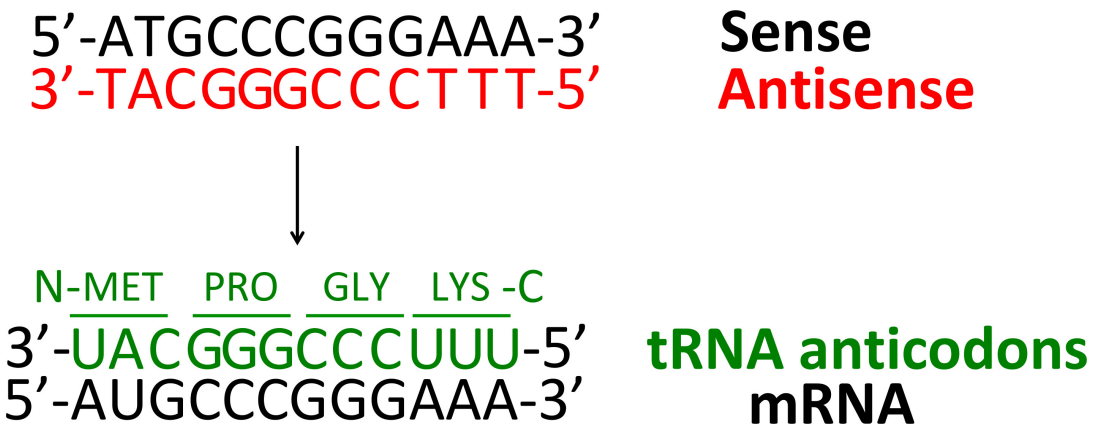
Animal Viruses

Animal viruses can be classified in various ways including their morphology, tropism, and nucleic acid genome. The Baltimore classification distinguishes viruses based upon their genetic material (single or double stranded DNA or RNA) as well as their mode of replication. The key thing is to focus on how the virus generates its mRNA molecules for replication and the production of new viral particles.



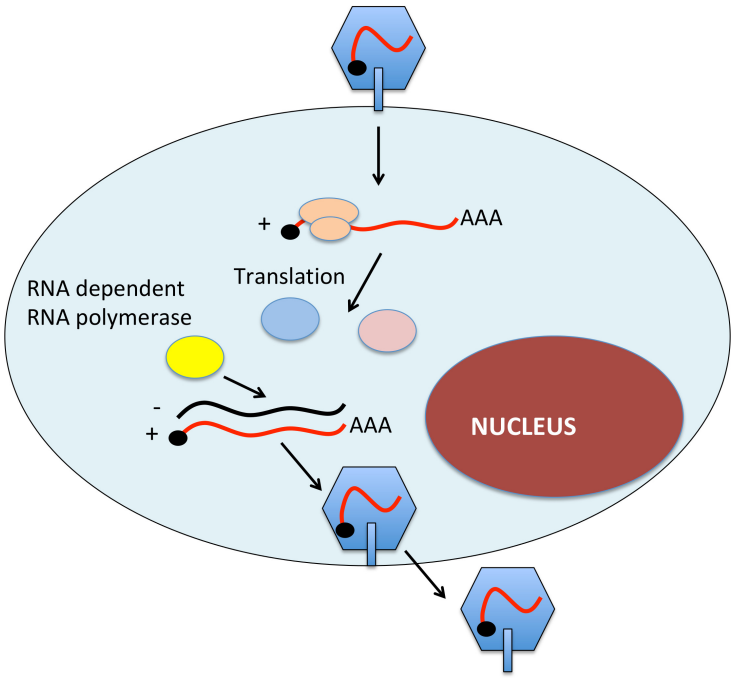
RNA viruses are classified by the nature of their nucleic acid genomes and exist in three major forms: single stranded viruses (coronaviruses, polio, and influenza), double stranded RNA viruses (rotavirus), and retroviruses such as HIV. Single stranded RNA viruses exist in either the + or – form. The genomes of + strand viruses are equivalent to that of the "sense" strand of an mRNA. This is the sequence that will dictate the primary protein structure.

Upon translation via host ribosomes, + strand viruses express an RNA-dependent RNA polymerase that can be used to propagate its genome. On the other hand, - strand viruses, such as measles can be thought of as antisense messages. Thus, in order for – strand viruses to express their genes, they must first be converted into a + strand. As the host DNA-dependent RNA polymerase is disqualified from performing this reaction, - strand viruses must package (and encode) their own polymerase.



Coronaviruses

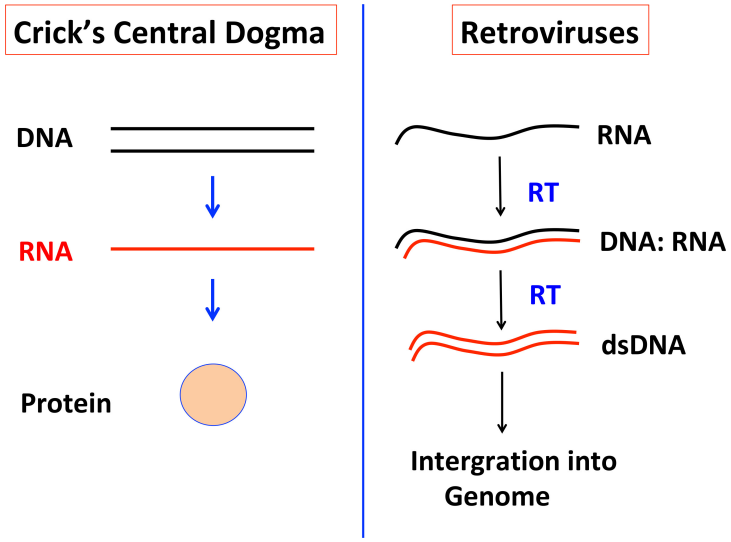
Coronaviruses (SARS, MERS, and COVID-19) are singles stranded RNA viruses with + polarity. That is, their genome behaves like an mRNA molecule,



complete with CAP and poly A tail. The novel COVID-19 virus attaches to cells via the angiotensin II receptor, a protein that is abundantly expressed in respiratory tissue.

Retroviruses

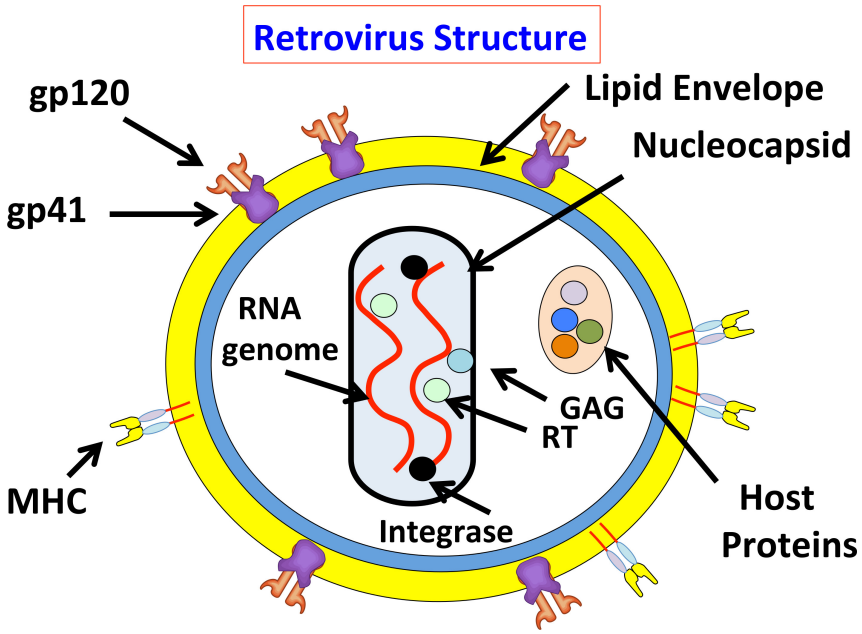
Retroviruses, including the AIDS (acquired immune deficiency syndrome) virus are animal viruses that resemble type I retrotransposons, especially with respect to their use of reverse transcriptase. The discovery of retroviruses and their unique life cycle took the world by surprise, especially as they appeared to violate the tenants of Crick's central dogma, or the flow of genetic material from DNA to RNA to protein. Crick's theory was based upon Beadle and Tatum's "one gene-one enzyme" hypothesis. In contrast, retroviruses are RNA viruses that insert copies of their DNA genome into their host cells, forming pro viruses through the action of the reverse transcriptase.



Structure and function of the HIV retrovirus

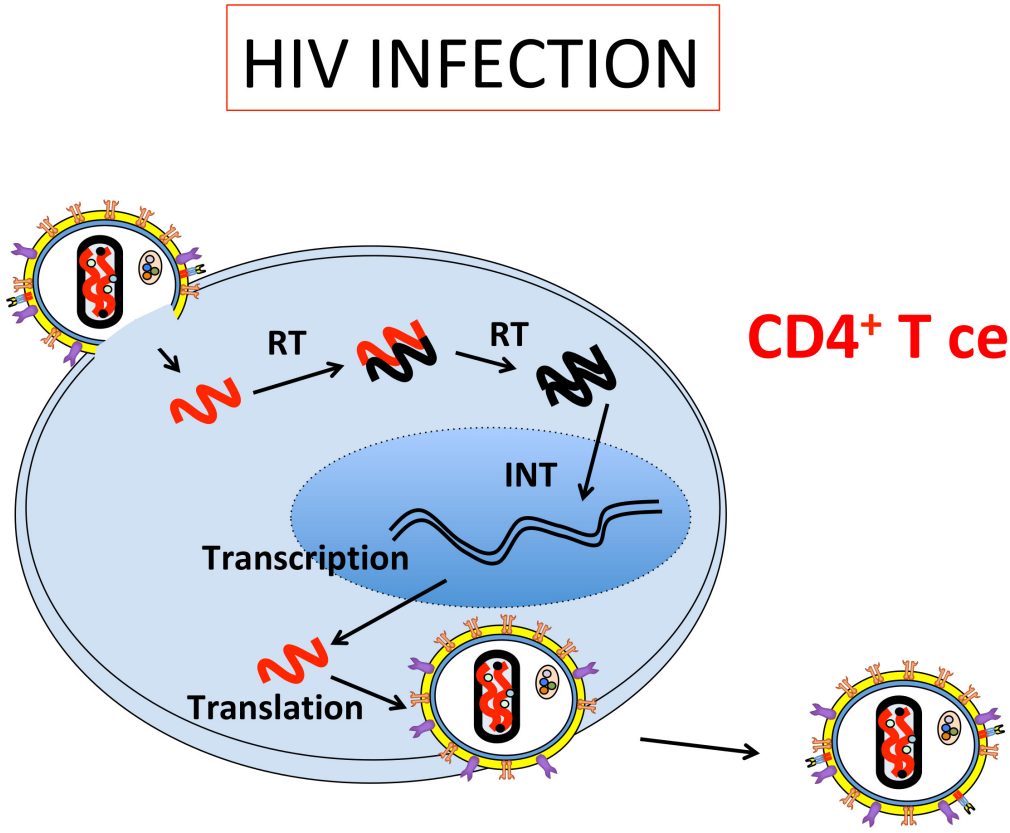
The structure of retroviruses is more complex than bacteriophages. A simplified view of the HIV retrovirus is shown below. The lipid envelope allows for the retrovirus to enter and exit cells; it is composed of host cell membrane proteins that are derived from viral fusion with the host membrane upon exit of a mature virion from the cell. Embedded in the envelope are multiple proteins including the glycoprotein gp120. Importantly, gp120 is essential for HIV entry into host cells through its interaction with host proteins including the CD4 T cell receptor. Thus, HIV infects CD4-positive ($CD4^+$) T

cells, or those T cells that express CD4 on their surface. gp120 associates with gp41 to form the Env protein, the only HIV-derived protein on the viral surface. The complex promotes the fusion of the HIV viral membrane with the host cell membrane, providing the first step towards infecting the host cell. As Env is the only HIV-encoded protein on the surface, it is an attractive target for developing anti-viral therapeutics. Additional envelope proteins include major histocompatibility complex (MHC), an important T cell component expressed by the host. The role of MHC in T cell function will be described in the immunology section below.



The HIV virion contains two copies of its RNA genome enclosed within a nucleocapsid that also contains GAG proteins on the inner surface. Packaged within this structure are host cell proteins such as proteases, reverse transcriptase (RT), and integrase enzymes.

Upon recognition of the CD4 receptor, as well as other T cell surface proteins, the HIV virion fuses with the cell membrane and releases its + stranded RNA genome along with other capsid factors such as reverse transcriptase (RT) and integrase (INT). HIV also infects macrophages and gains entry through the CCR5 receptor. As discussed above, RT performs its major functions: 1) Generation of an RNA:DNA hybrid through an RNA-dependent DNA polymerase activity; 2) RNAse H-dependent nuclease activity of RT generates a single stranded DNA (ssDNA) molecule; 3) Formation of a double stranded DNA viral genome that is integrated into the host genome as a prophage with the assistance of the integrase (INT) protein. Transcription of the nine HIV genes in its genome generates an RNA molecule that is processed and translated into proteins that build new HIV virions. The newly assembled virions fuse with the cell membrane where it acquires its envelope.



Prions (Proteinaceous infectious particles)

Prions are infectious proteins that are causal for a number of neurodegenerative diseases in humans and animals, including "mad cow" disease. During the 1960s, the hypothesis that some forms of encephalopathy

are caused by infections protein agents devoid of nucleic acids was put forward. First proposed by Alper and Griffith, their seminal paper entitled "Does the agent of Scrapie replicate without nucleic acid" initiated the prion hypothesis. In the early 1980s Prusiner purified the actual infectious protein called PrP^{C}. Although PrP^{C} is found normally throughout the body, it can adopt an infectious conformation called PrP^{SC}, where the SC stands for "scrapie", a neurodegenerative disease in sheep. PrP^{SC} forms extracellular aggregates (amyloid plaques) in neuronal tissue. Prion replication occurs through remodeling of the conformation of PrP^{C} into Prp^{SC}, an altered structure that serves as a template to convert more PrP^{C} molecules into the disease-causing conformation.

Prior research is ongoing and several outstanding questions remain. Because the infectious nature of prion infectivity also depends on genetic susceptibility of the host as well as possible co-factors, including nucleic acids, some have questioned if the prion protein in of and by itself is sufficient to cause neurodegenerative diseases. Further, additional proteins including alpha synuclein have been found to have prion-like properties.

Viroids

Viroids are composed of single stranded, circular RNA molecules up to 401 nucleotides. They have the distinction of being the smallest known infectious agent and replicate through a rolling circle mechanism. Found only in plants, viroids possess no protein structures characteristic of viruses.

IMMUNITY

The following topics are discussed in this section:

□ Introduction; hematopoiesis and lymphoid organs □ Innate immune system cells-Macrophages and Phagocytes □ Adaptive immune system □ Antibody structure and function □ Classes of antibodies □ Diversity and somatic recombination □ Collaboration between B and T cells (clonal expansion, major histocompatibility complex □ Allergic response □ Autoimmunity

□ Immunological techniques (Polyclonal and Monoclonal antibodies; ELISA technique; flow cytometry)

Immune System

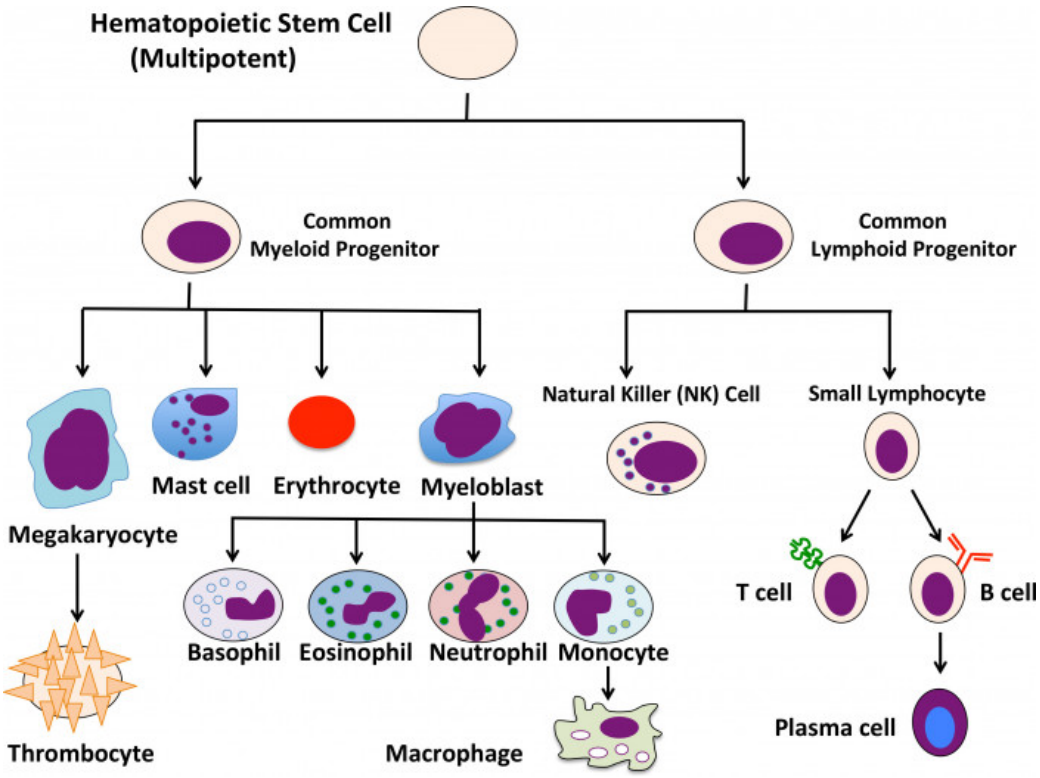
Immunity confers the ability of an organism to defend itself against foreign particles or organisms that can render harm. Human immunity is comprised of both innate (cells of myeloid lineage) and adaptive systems (T and B lymphocytes). The principle function of the human immune system is to distinguish self from non-self. The factors that make up the innate (non-specific) immune system are constitutive, able to rapidly respond to foreign threats, and do not change over the lifespan of the organism. In contrast, the adaptive immune response is specific, records a "memory" of invading pathogens, and utilizes B and T cells of lymphoid origin (lymphocytes). In bacteria, host restriction modification and the CRISPR-Cas system represent primitive forms of innate and adaptive immunity, respectively.

Cells of the immune system are localized throughout the body. Further, as many of these cells migrate in response to chemical cues indicative of infection, lymphoid organs such as the bone marrow, spleen, thymus, and lymph nodes coordinate immune responses. In the fetus, the liver is responsible for hematopoiesis and acts as a primary lymphoid organ. In adults, the liver produces various factors involved in innate immunity, but the site of hematopoiesis switches over to the bone marrow after birth. The bone marrow is a primary immune organ and produces myeloid and lymphoid cells (B and T cells). Myeloid cells such as white blood cells function in innate immunity. This is described in more detail below. Bone marrow-derived T cells migrate to the thymus, a primary lymphoid organ that discriminates between T cells that recognize "self from non-self". Those T cells that recognize host proteins (self) are eliminated in the thymus via the apoptotic process of negative selection. This is further discussed below. After selection, mature T cells migrate to

MAJOR LYMPHOID ORGANS

TYPE	SITE	FUNCTION
1°	Liver	Fetal production of lymphoid cells
1°	Bone marrow	Hematopoietic production of myeloid and lymphoid cells
1°	Thymus	Receives bone marrow T cells; site where self is selected from non-self
2°	Lymph nodes Spleen	Sites of antigen activation of lymphocytes

secondary lymphoid tissue. The importance of the thymus as a critical lymphoid organ is seen in DiGeorge syndrome, a condition where genetic loss of thymus function produces a severe immunodeficiency. In addition to T cells, the bone marrow generates the antibody producing B cells. Once produced, naïve B cells migrate to secondary lymphoid tissues where they collaborate with T cells to coordinate the adaptive immune response. In addition to the lymph nodes and spleen, tonsils, Peyer's patches, adenoids, and even the skin comprise the secondary lymphoid tissues where adaptive immune responses occur.



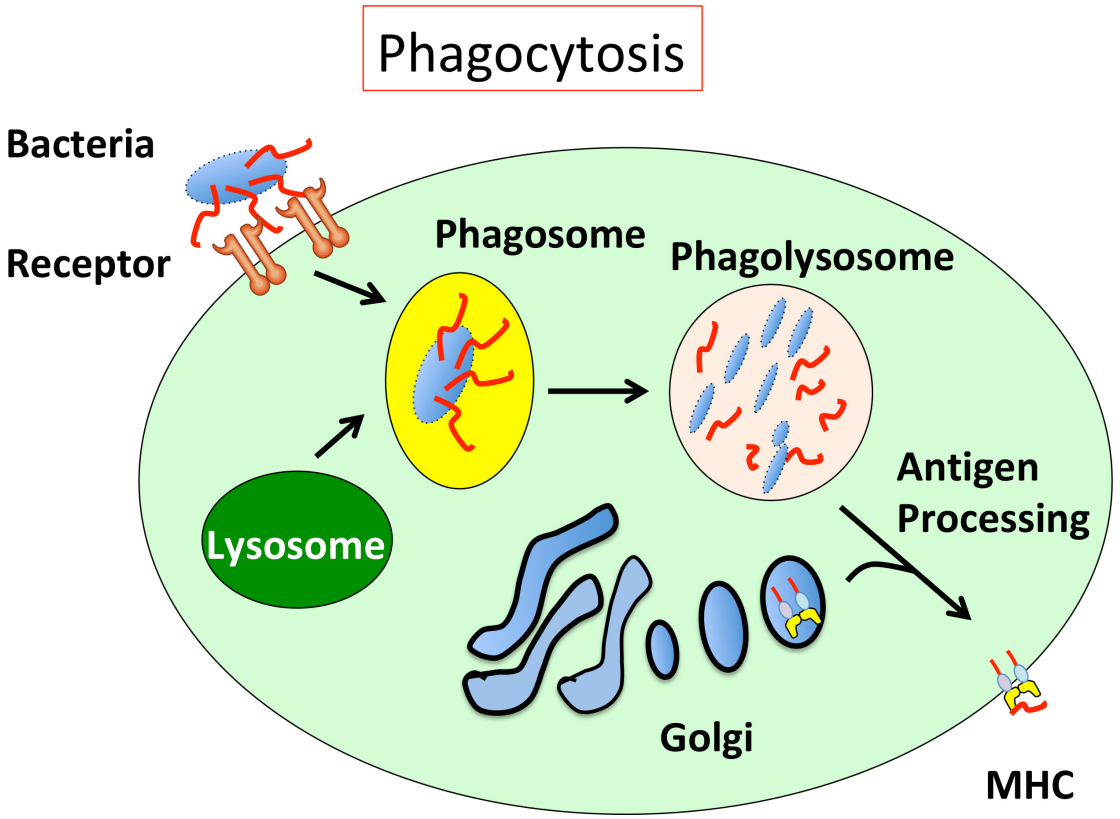
Innate Immunity

Hematopoietic stem cells (HSCs) are multipotent precursors derived from the red marrow of the bone which itself is derived from the embryonic mesoderm. In humans, cells of the innate immune system (i.e. white blood cells) are derived from a common myeloid progenitor. Many types of white blood cells function as professional phagocytes, cells specialized in the ability to engulf foreign particles such as bacteria, viruses, and other dead cells. Phagocytes such

as monocytes, neutrophils, macrophages, and dendritic cells are attracted to sites of infection through chemical signals. Here, they perform chemotaxis through amoeboid movement, a process distinct from bacterial chemotaxis that occurs through flagellar rotation.

In its simplest terms, innate immunity consists of skin, mucous membranes, stomach acid as well as the lysozyme that is secreted in tears. However, many pathogens can escape such initial barriers to elicit an immune response. In response to pathogens, an inflammatory response is initiated. This allows for an influx of fluids that include a large number of antibodies, some of which can recognize various epitopes on the pathogen. Once coated with antibodies, the organism is said to be "opsinized". Phagocytes recognize the constant region of antibodies bound to the pathogen, providing a link between innate and adaptive immunity. (Antibody structure is described in detail below.)

When phagocytes encounter foreign particles such as bacteria or viruses, they use cell surface receptors to recognize patterns typically presented on the foreign surface. Such patterns include sugars such as mannose, lectin, and LPS. This interaction promotes the engulfment of the bacteria and the formation of a phagosome and the subsequent activation of the pro-inflammatory response through the secretion of cytokines, peptide molecules that provide the language of cell-to-cell communication in the immune system. The phagosome



combines with the lysosome and is acidified. Here, various hydrolytic enzymes functioning at low pH degrade the bacterium. Peptide antigens in the phagolysosome combine with the major histocompatibility complex (MHC) protein that is secreted from the Golgi apparatus. MHC binds to peptide antigens derived from bacterial lysis and presents them on the surface of antigen presenting cells (APCs) such as dendrites and macrophages. APCs circulate in the lymphatic system and present MHC-bound antigens to lymphoid T and B cells (lymphocytes) in lymph nodes, providing a link between innate and the adaptive immunity that will generate a lasting immune response against the foreign entity.

A survey of key players in innate immunity is described below.

Granulocytes (Polymorphonuclear cells)

Neutrophils, basophils, eosinophils, and mast cells comprise the granulocytes. These white blood cells possess granules that store various antimicrobial factors (i.e. enzymes such as protease and peroxidases) for release during an immune response. These granulocytes are also known as polymorphonuclear cells due to their distinctive globular nuclei.

Neutrophils are the most abundant of the white blood cells and their elevated levels in clinical tests often indicate the presence of infection. They use an amoeboid movement and are usually the first recruited cells to sites of infection. After ingestion of the foreign body and formation of the phagosome, neutrophils undergo a respiratory burst, a period of rapid production and release of reactive oxygen species that are used in host defense.

Basophils are the least abundant of the white blood cells and function in the reaction to allergic conditions such as asthma and hay fever. **Eosinophils** reside in tissues and release various factors including the vasodilator histamine and anticoagulant heparin.

Mast cells can be found throughout the body and are often concentrated in those tissues that interface with the environment (i.e. mucosal tissue) as well as around blood vessels and nerves. Mast cells are intimately associated with allergies. Upon IgE binding to an allergen (i.e. pollen or proteins form tree nuts), mast cell receptors interact with the immunoglobulin and initiate signaling pathways that release mediators such as histamine and tryptase. This initiates an inflammatory response. In some cases, swelling (i.e. angioedema) or urticaria (hives) can be severe enough to initiate an anaphylactic response.

Monocytes

Monocytes are the largest of the white blood cells and circulate for a few days before entering tissues where they differentiate into either macrophages or dendritic cells. Monocytes perform phagocytosis, antigen presentation and cytokine signaling.

Macrophages

Macrophages are derived from monocytes. They perform phagocytosis and release chemokines that recruit additional immune cells to sites of infection. Macrophages are also antigen presenting cells and often reside in various tissues where they are often dubbed "resident histiocytes". Macrophages that reside in a particular type of tissue are often given distinct names. For example, alveolar macrophages (dust cells) are located in the lungs and Kupffer cells are located in the liver. Sinus histiocytes are located in the lymph nodes. Therefore, a particular cell type might not be labeled as a macrophage, but it is still a macrophage.

Dendritic cells

Dendritic cells are formed from myeloid precursors and are concentrated in regions of the body that are exposed to the environment (skin, mucosal lining). Their chief function is to process and present antigens to T cells in secondary lymphoid tissue. Dendritic cells therefore interface the innate and adaptive immune systems.

Dendritic cells assess the environment through pattern recognition receptors such as toll like receptors (TLRs). In response to various ligands (flagellin, viral surfaces, nucleic acids), TLRs dimerize and recruit various adapter molecules to initiate a signal transduction pathway that ultimately induces a pro-inflammatory response through the activation of the NF-κβ transcription factor. TLRs are also activated by viral nucleic acids (i.e. double stranded RNA). In this scenario, interferons are expressed. Interferons are proteins that are released from infected cells and block viral protein synthesis and activate and regulate macrophages and T cells.

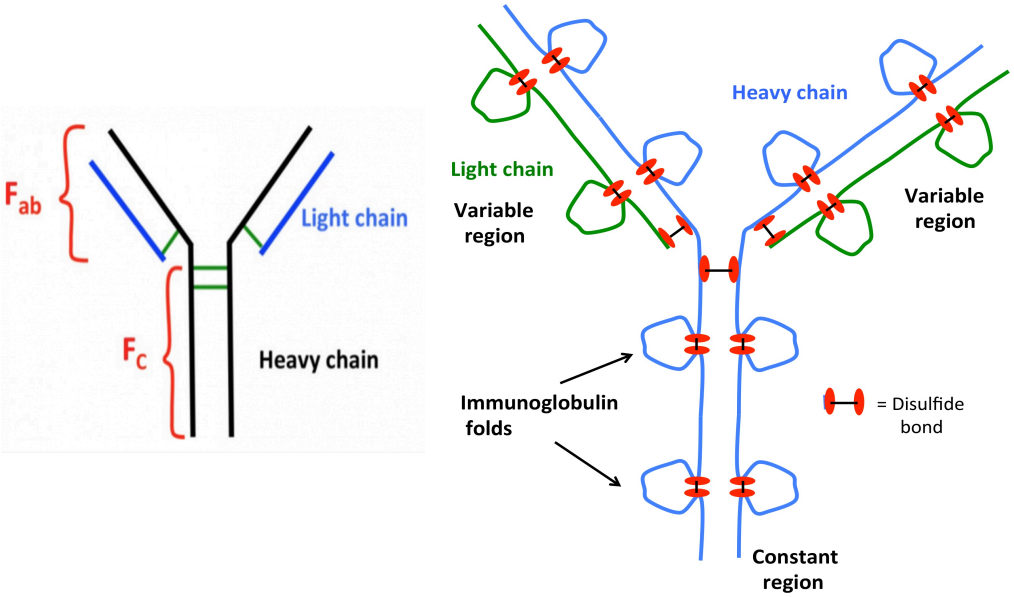
Adaptive immunity

Adaptive immunity is synonymous with acquired immunity and refers to the ability to recognize specific antigens and to record a memory of them for future response. This occurs through somatic gene rearrangements that generate a diverse repertoire of antibodies and T cell receptors.

Antibody Structure & Function

Antibodies, or immunoglobulins (IgG), are well known for having a "Y" shaped three dimensional structure (see below). Antibodies are heterodimers of two heavy and two light chains linked together by disulfide bonds. The heavy chain migrates near 50 kD and the light chain migrates near 35 kD in SDS polyacrylamide gel electrophoresis experiments (SDS PAGE).

Antibodies have two variable regions and one constant region. The variable regions consist of both heavy and light chain sequences and bind to the antigen. The variable region recognizes antigen by binding to an epitope. Epitopes can be sugars, lipids, nucleic acids, or proteins and peptides. In the context of proteins an epitope can be as little as five contiguous amino acids. Variable regions are localized to a Fab fragment derived from proteolysis.



The constant region contains heavy chain sequences and is localized to the F_c portion of the antibody. The constant region determines the class of antibody (i.e. IgM, IgG, and IgE). Each class has a different function as shown below. Some antibody constant regions interact with components of the complement system, a group of proteins that are involved with innate immunity.

Classes of Antibodies

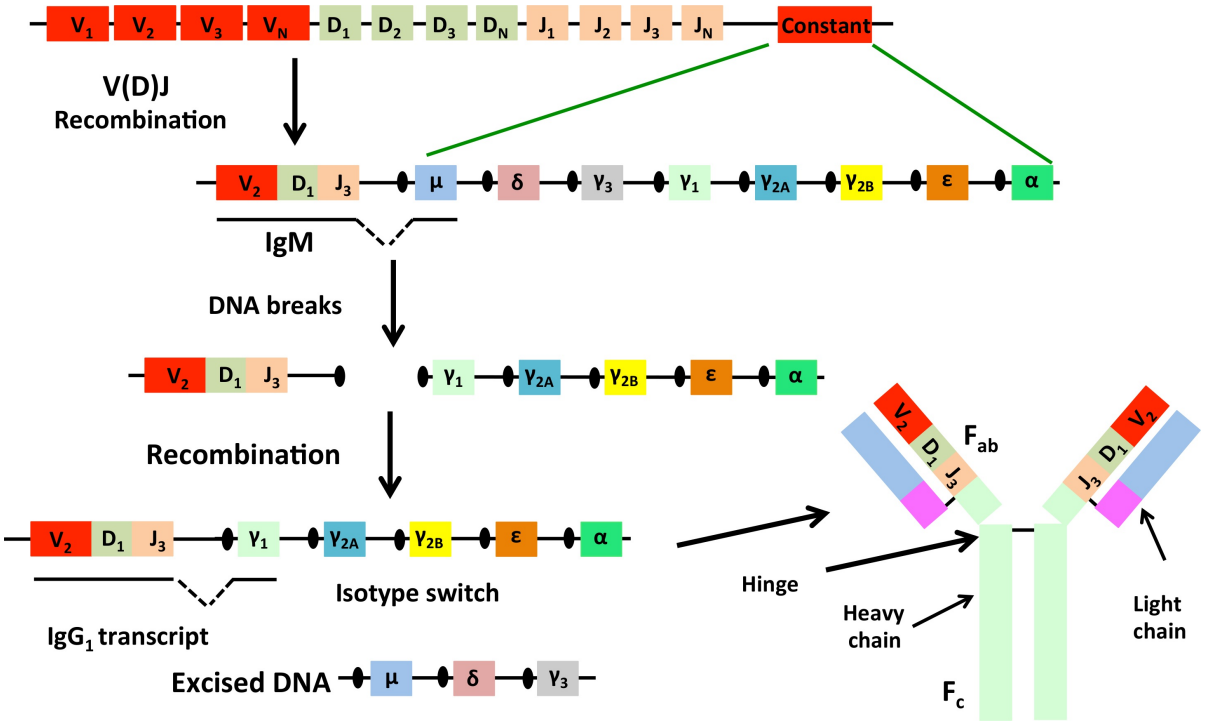
Class	Cellular Role
IgM	Functions in primary immune response; binds B cell receptor
IgG	Major serum immunoglobulin; crosses placenta
IgA	Enriched in mucous membranes
IgE	Binds to mast cells and basophils; mediates allergic response
IgD	Co-expressed with IgM on B cells

Antibody diversity and somatic recombination

Scientists had long known that the number of antibody proteins far exceeded the number of genes required to encode them. As the number of antibodies is in the millions, how could the genome encode such a large number of proteins? Numerous theories were put forward, but Tonegawa solved the problem when he discovered that somatic gene rearrangements occurred in B cells to generate numerous combinations of antibodies. An analogous process occurs in T cells to generate the T cell receptor.

Two distinct DNA repair pathways are required to generate antibodies in B cells. Specifically, both V(D)J and class (or isotype) switch recombination are required to generate the immunoglobulin heavy chain. In contrast, the light

chain (as well as the T cell receptor) is made only via V(D)J recombination. The basics are shown below.



Chromosome 14 in humans contains multiple variable (V), diversity (D), and joining (J) segments of DNA that encode for various portions of antibodies. During immune cell development, RAG enzymes generate DNA double stranded breaks (DSBs). By deleting various portions of DNA through the creation of DSBs, numerous combinations of V, D, and J segments can be re-formed through DNA repair that can generate many different structures that recognize antigen. Therefore, V(D)J recombination creates immunological diversity.

Antibodies "start out" as IgM and then can have their constant regions replaced with various effector classes, including IgA, IgG, IgD, and IgE. This mostly occurs through isotype switching. The image shows the "switch" from IgM to IgG. In response to various cytokines, the AID enzyme creates DNA breaks in the chromosome. After excision of sequences and DNA repair, the IgG constant region coding sequence replaces the previous IgM constant portion. IgM antibodies that switch to IgG have identical variable regions, but different constant regions. As the constant regions are formed exclusively from the heavy chain, the primary structures of IgM and IgA are different.

Collaboration between B and T cells

During the development of B and T cells, the immunological property of tolerance is achieved through elimination of cells that possess antibody and T cell receptors that react with "self". This occurs in the bone marrow (B cells) and thymus (T cells).

In collaboration with T cells, B cells normally produce and secrete antibodies. A schematic of this is shown below. Prior to encountering an antigen, a naïve B cell expresses its antibody on the cell surface. Step 1: Upon encountering an antigen (i.e. viral protein), endocytosis brings the receptor-antigen complex into the cells where it is digested into fragments (Step 2). Peptide fragments are displayed on the major histocompatibility complex (MHC, Red) and recognized by T cells expressing a matching T cell receptor (Green) (Step 3). In response to cytokine secretion (Step 4), the B cells differentiate and multiply as plasma cells where they secrete the antibody for maximal immune response (Steps 5, 6). This is known as clonal expansion.

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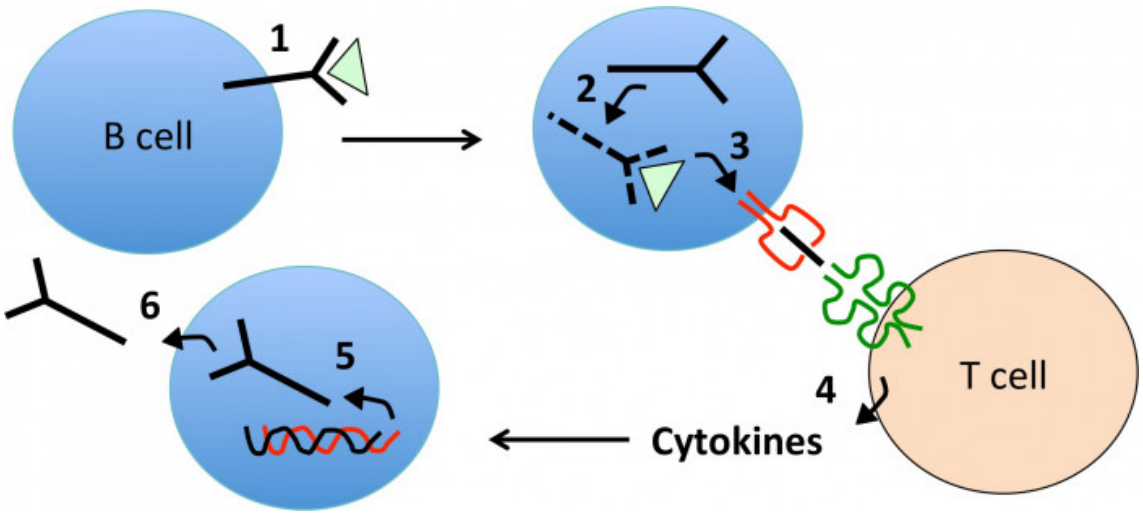
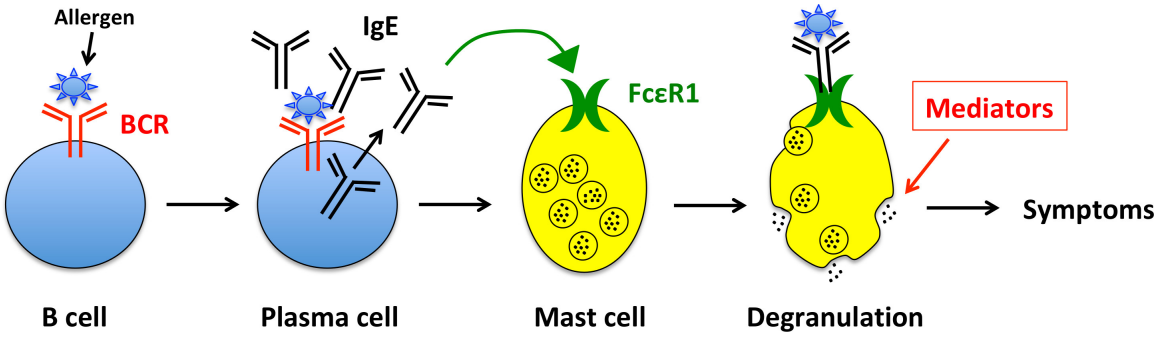


Allergic response

Mast cells interact with B cells during the allergic response. Allergens bind to B cell receptors (BCRs). This causes differentiation into plasma cells that secrete IgE. Plasma cells are terminally differentiated B cells that release one type of

antibody. Soluble IgE binds to the high affinity FcεR1 receptor primarily present on mast cells. This results in degranulation and release of chemical mediators such as histamine and tryptase. As a result a variety of allergic symptoms can be seen depending on the severity of the reaction. This includes angioedema, hives (urticaria), reduced blood pressure, dyspnea, anaphylaxis, and sneezing.



Autoimmunity

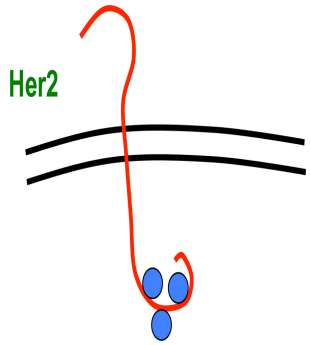
Normally, lymphoid B and T cells expressing auto-antigens on their surface are recognized and processed for negative selection via apoptosis. This prevents the immune system from recognizing and attacking the "self." In autoimmune diseases, this process of immunological tolerance is compromised as the body fails to adequately eliminate B and T cells recognizing self antigens. For example, autoantibodies against various cell factors such as DNA, insulin, and myelin are known to be causal for the autoimmune disorders lupus, type I diabetes, and multiple sclerosis, respectively. The mechanisms behind autoimmune diseases vary from condition to condition as multiple mechanisms are posited to be causal for disease: 1) The sequestration of antigens during development in places such as the eye lens, central nervous system, and sperm, shields these tissues and their epitopes from pre-existing auto-reactive antibodies. Upon injury or infection and consequent release of an antigen no longer sequestered, a self immune response is triggered; 2) "Molecular mimicry" suggests that when foreign peptides from exogenous antigens with sufficient similarities to a host peptide are recognized as self, a cross-activate B and T cell response is observed.

Immunological Techniques

Many features of the immune system have been exploited in the development of techniques to measure various biological parameters. This includes the presence of various proteins within extracts or on the surface of cells. Notably, the development of antibodies against specific proteins allows for the identification of their presence or absence.

Polyclonal and Monoclonal antibodies

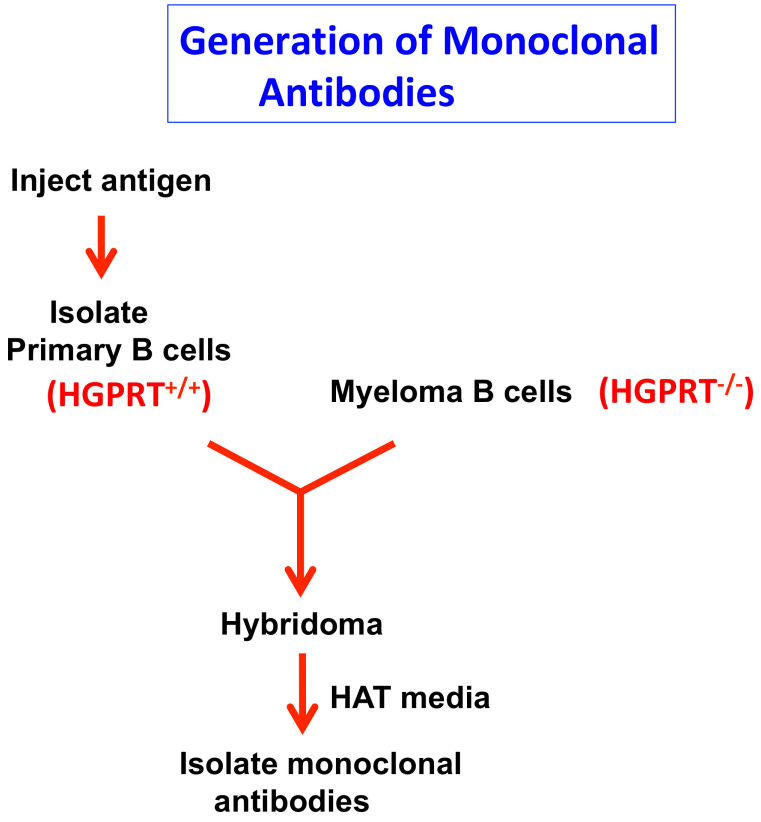
Polyclonal antibodies are comprised of multiple immunoglobulin molecules that recognize a single antigen at different sites (epitopes) of the molecule. Monoclonal antibodies (MAbs) are generated from clonal cells (i.e. cells of the same origin) and have a monovalent affinity towards an antigen through the recognition of a single epitope. This is in contrast to polyclonal antibodies that recognize multiple epitopes on the same antigen. The generation of a homogeneous population of MAbs represents a major advance in medical research and was awarded the Nobel Prize in medicine. Multiple monoclonal antibodies have been approved by the FDA for clinical use. This includes Herceptin, an antibody that targets the Her2 tyrosine kinase and is used for the treatment of breast cancer (**Fig. 1**).



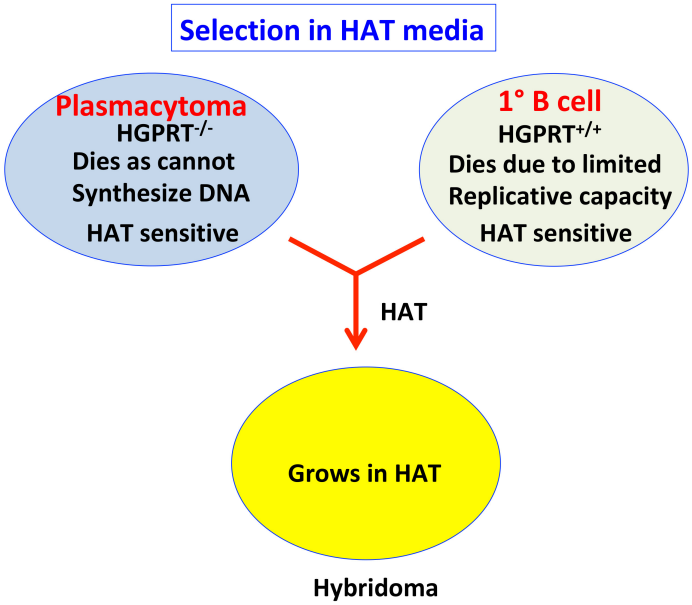
Structure of Her2 receptor.
Tyrosine phosphorylation occurs in cytoplasm and is shown in blue.

MAbs are generated from a cell line (hybridoma) derived from the fusion of two cell types. The first cell type, a primary B cell, cannot grow on its own but can produce antibodies. The second cell type, a B cell derived from a myeloma,

cannot produce antibodies, but can grow in its own. The strategy for generating MAbs is as follows. When transformed, myeloma B cells (or plasmacytomas) deficient in antibody production due to lack of the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT) are fused with freshly isolated,

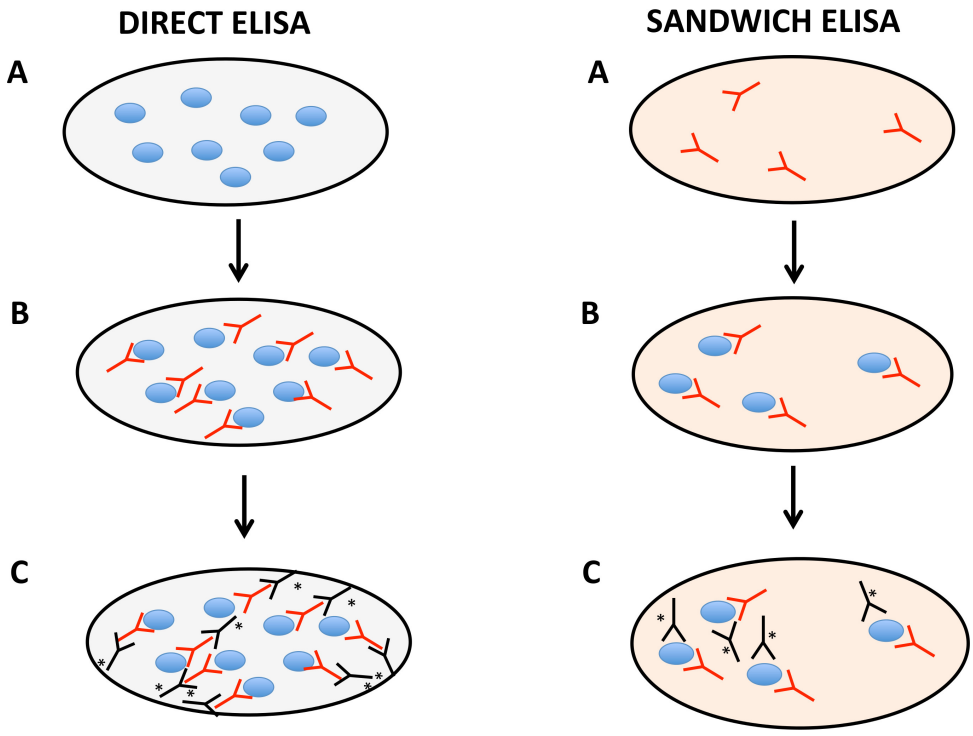


primary B cells derived from mice immunized with an antigen of interest, hybridoma cell lines are created (see Figure). Sequences containing clusters of charged and polar amino acids are often used as antigens for this procedure, as they are highly immunogenic. The primary B cells, which cannot replicate in vitro, produce antibodies and express HGPRT, an enzyme involved in the nucleotide salvage pathway, a cellular method to generate nucleotides de novo. Fused hybridoma cells are cultured in selective HAT media containing the nucleotide precursor hypoxanthine, the folate antagonist aminopterin, and thymidine. Aminopterin inhibits dihydrofolate reductase (DHFR), an enzyme responsible for generating uracil from thymidine. This prevents de novo synthesis of DNA, the second pathway for generating nucleotides. The generation of the hybridoma generates a fused cell type that can synthesize DNA and has unlimited replicative capacity. Moreover, this cell line secretes only one antibody type, a monoclonal antibody that recognizes one epitope on an antigen.



ELISA

Enzyme-linked immunosorbent (ELISA or ELISA assay) is an important biochemical diagnostic test used in labs. They are used to determine the presence and amount of various proteins and metabolites in biological tissues. The direct and sandwich ELISA will be discussed here.



In the direct ELISA, a solution containing the antigen to be measured is placed in a plastic coated well (solid state support) (**STEP A**). The antigen adheres to the surface through charged interactions with the well. Afterwards, the plate is coated with a protein (i.e. serum albumin) to saturate all binding sites. After washing to release all unbound material, a primary antibody is added that is specific for the antigen of interest (**STEP B**). After washing away the unbound antibody, a secondary antibody is added (**STEP C**). Importantly, the secondary antibody recognizes the constant region of the primary antibody (not the variable region) and is labeled with a flour (*) that can be detected and quantitated. In some cases, the primary antibody can be labeled with a flour. In some cases, the secondary antibody is linked to an enzyme that performs a reaction that generates a product that can be detected by a spectrophotometer. Hence, the name "enzyme-linked" in ELISA. We will use the name "flour" as a general term.

In the sandwich ELISA, a "capture" antibody specific for an antigen of interest is added to the well (**STEP A**). After binding, serum albumin is added to block all potential binding sites on the surface. Afterwards, the well is washed and a mixture (i.e. cell extract or serum sample) containing the antigen of interest is added to the well. This allows for the formation of an antibody-antigen complex (**STEP B**). After washing, a second antibody specific for the antigen is added to the well. This antibody is labeled with a fluor for detection. The binding of both antibodies to the antigen forms the basis of the "sandwich" (**STEP C**).

Flow cytometry

Flow cytometry is used to detect specific characteristics of cells in solution. It is often used to detect the presence of certain cell types in a biological sample. For example, imagine if a doctor was trying to diagnose a patient with mastocytosis, a condition where mast cells inappropriately accumulate in the bone marrow. As mast cells express CD117 on their surface, treatment of the sample from the bone marrow with an anti-CD117 antibody would label mast cells with the antibody. If the antibody is fluorescently labeled, then the flow cytometer can specifically detect and quantitate the CD117+ cells in a population. Flow cytometry is also used to measure cell size and purified cell populations. A cartoon of a flow cytometer is shown below.

In the procedure, a cell suspension is labeled with a fluorescent antibody and applied to the flow cytometer. The cells funnel down a chamber in single file and pass through a laser beam of light. As the light shines on the passing cells, those cells possessing the specific fluorescent label will emit light that can be used to quantitate the number of cells in the population. In addition, light is scattered in a forward and side fashion and this information is also used to generate information regarding cell size.