1. SYSTEMATIC CLASSIFICATION OF MICROORGANISMS

The systematic classification of microorganisms is a fundamental aspect of microbiology that helps organize and understand the diversity of microbial life. This classification is based on a hierarchy of taxonomic ranks, starting from the broadest category, the domain, down to the most specific, the species. The three domains are **Bacteria**, **Archaea**, and **Eukarya**, which are further divided into kingdoms, phyla, classes, orders, families, genera, and species[1].

Bacteria and **Archaea** are prokaryotic domains, characterized by cells without a nucleus or membrane-bound organelles. **Eukarya** includes all eukaryotic organisms, which have cells with a nucleus and organelles. Within these domains, organisms are further classified based on characteristics such as cell structure, metabolism, genetic makeup, and ecological roles[2].

Bacteria are incredibly diverse, ranging from the beneficial gut flora to pathogenic bacteria that cause diseases. They are classified into various phyla, such as **Proteobacteria** (which includes *E. coli*), **Firmicutes**, and **Actinobacteria**, based on their genetic and metabolic characteristics.

Archaea were once thought to be extreme bacteria but are now recognized as a distinct domain. They are known for living in extreme environments, like hot springs and salt lakes, but can also be found in more common habitats. They are classified into groups like **Euryarchaeota** and **Crenarchaeota**.

Eukarya includes all organisms with eukaryotic cells. Microorganisms in this domain include:

- **Protists**: A diverse group including algae, amoebas, and plasmodium.
- **Fungi**: Including yeasts, molds, and mushrooms, which play crucial roles in decomposition and nutrient cycling.
- **Algae**: Photosynthetic organisms that are important producers in aquatic ecosystems.

The classification system is dynamic and continually evolving as new methods, such as DNA sequencing, provide more detailed insights into the relationships between different organisms. This has led to revisions in the classification of many species and the discovery of new ones[3].

Taxonomy, the science of classification, not only organizes microorganisms into a structured system but also provides important information about their evolutionary history and relationships. It is a critical tool for microbiologists, allowing them to communicate about and study these organisms more effectively.

The systematic classification of microorganisms is a structured way of organizing and categorizing the vast diversity of microbial life based on shared characteristics and

evolutionary relationships. This system is known as taxonomy and involves several hierarchical levels, from the broadest to the most specific. Here's an overview of the classification:

1. **Domain**: The highest taxonomic rank, which includes **Bacteria**, **Archaea**, and **Eukarya**.
2. **Kingdom**: The next level, with five major kingdoms recognized: **Prokaryota** (e.g., bacteria and archaea), **Protoctista** (e.g., protozoa and algae), **Fungi**, **Plantae**, and **Animalia**.
3. **Phylum**: A division within a kingdom that groups organisms based on a set of broader traits.
4. **Class**: A category within a phylum that further narrows down similarities among organisms.
5. **Order**: Groups within a class that share even more specific characteristics.
6. **Family**: A rank within an order that brings together closely related genera.
7. **Genus**: A group within a family that contains species with highly similar features.
8. **Species**: The most specific level, representing a group of organisms that can interbreed and produce fertile offspring.

Each microorganism is given a unique scientific name using **binomial nomenclature**, which includes its genus and species names. This system is dynamic and can change with advancements in technology and our understanding of microbial life[1].

For example, the classification of *Escherichia coli* would be:

- **Domain**: Bacteria
- **Kingdom**: Eubacteria
- **Phylum**: Proteobacteria
- **Class**: Gammaproteobacteria
- **Order**: Enterobacteriales
- **Family**: Enterobacteriaceae
- **Genus**: Escherichia
- **Species**: coli

This systematic approach helps scientists communicate more effectively about microorganisms and understand their roles in various environments and processes.

PHYLOGENY

MOLECULAR PHYLOGENY

Phylogeny is a reference to the development of an organism evolutionarily. Molecular techniques allow for the evolutionary assessment of organisms using genomes or **ribosomal RNA** (**rRNA**) nucleotide sequences, generally believed to provide the most accurate information about the relatedness of microbes.

Nucleic acid hybridization or **DNA-DNA hybridization** is a commonly used tool for molecular phylogeny, comparing the similarities between genomes. The genomes of two organisms are heated up or "melted" to separate the complementary strand and then allowed to cool down. Strands that have complementary base sequences will re-anneal, while strands without complementation will remain upaired. Typically one source of DNA is labeled, usually with radioactivity, to allow for identification of each DNA source.

Nucleic acid sequencing, typically using the rRNAs from small ribosomal subunits, allows for direct comparison of sequences. The ribosomal sequence is seen as ideal because the genes encoding it do not change very much over time, nor does it appear to be strongly influenced by horizontal gene transfer. This makes it an excellent "**molecular chronometer**," or way to track genetic changes over a long period of time, even between closely related organisms.

Taxonomy refers to the organization of organisms, based on their relatedness. Typically, it involves some type of classification scheme, the identification of isolates, and the naming or nomenclature of included organisms. Many different classification schemes exist, although many have not been appropriate for comparison of microorganisms.

Classification Systems

A **phenetic classification** system relies upon the phenotypes or physical appearances of organisms.

Phylogenetic classification uses evolutionary relationships of organisms.

A **genotypic classification** compares genes or genomes between organisms.

The most popular approach is to use a **polyphasic** approach, which combines aspects of all three previous systems.

Microbial Species Currently there is no widely accepted "**species definition**" for microbes. The definition most commonly used is one that relies upon both genetic and phenotypic information (a polyphasic approach), with a threshold of 70% DNA-DNA hybridization and

97% 16S DNA sequence identity in order for two organisms to be deemed as belonging to the same species.

BACTERIA

The study of bacterial cell walls, internal components, and surface structures is a fundamental aspect of microbiology that provides insights into the physiology, classification, and pathogenicity of bacteria. Here's an overview of these components:

Cell Walls: Bacterial cell walls are complex structures that provide shape and protect the cell from osmotic lysis. They are primarily composed of peptidoglycan, a unique substance found only in bacteria. Peptidoglycan is a polysaccharide made of two glucose derivatives, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), which form long chains. These chains are cross-linked by peptide bridges, giving the cell wall its strength and rigidity. It is important to note that not all bacteria have a **cell wall**. Having said that though, it is also important to note that **most** bacteria (about 90%) have a cell wall and they typically have one of two types: a **gram positive** cell wall or a **gram negative** cell wall. The two different cell wall types can be identified in the lab by a differential stain known as the **Gram stain**.

Bacteria can be classified into two groups based on their cell wall structure:

- **Gram-positive bacteria**: Have a thick peptidoglycan layer and stain purple in the Gram stain procedure.
- **Gram-negative bacteria**: Have a thin peptidoglycan layer and an outer membrane, staining pink in the Gram stain procedure.

Internal Components: The internal components of bacteria include:

- **Cytoplasm**: The gel-like substance inside the cell membrane containing the cell's genetic material and ribosomes.
- **Nucleoid**: The region where the bacterial chromosome (DNA) is located.
- **Plasmids**: Small, circular DNA molecules that can replicate independently of the bacterial chromosome.
- **Ribosomes**: The sites of protein synthesis.
- **Inclusions**: Storage granules for nutrients or building blocks.

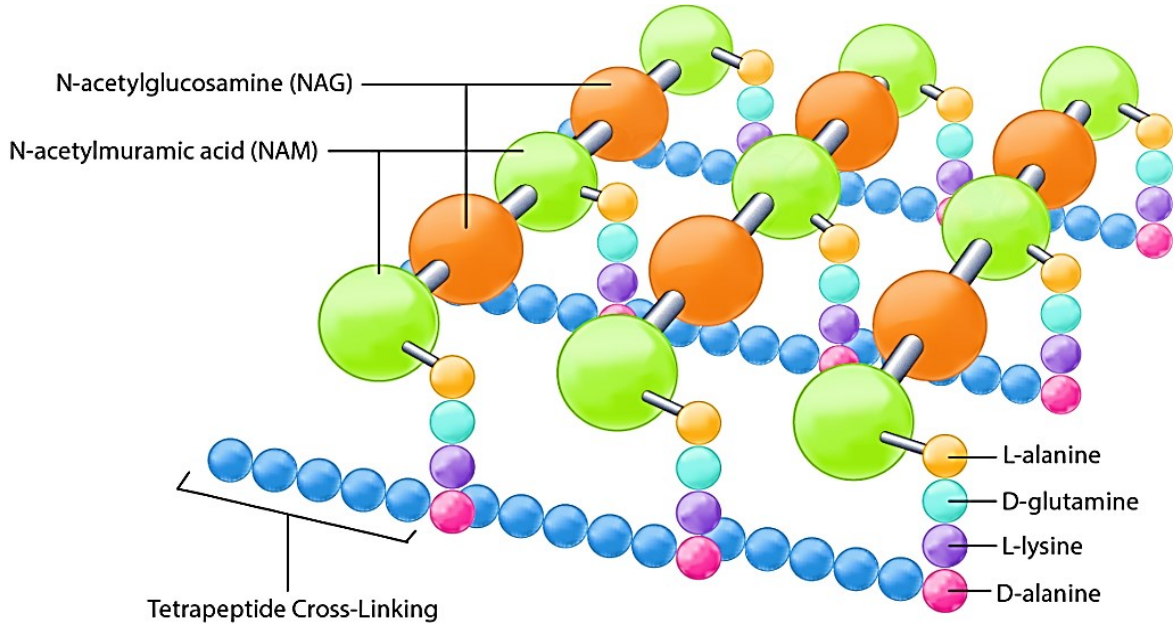
Surface Structures: Bacteria may have various surface structures that serve different functions:

- **Capsule**: A polysaccharide layer that protects the bacteria from desiccation, phagocytosis, and helps in attachment to surfaces[2].
- **Slime Layer**: Similar to the capsule but less organized, it also aids in protection and adherence.
- **S-Layer**: A regular array of protein or glycoprotein that can contribute to cell shape and protect against environmental stress.
- **Fimbriae and Pili**: Hair-like structures that help bacteria attach to surfaces and can play a role in the exchange of genetic material.

Understanding these structures is crucial for developing antibiotics and other treatments, as many target the unique components of bacterial cells.

Structure of Peptidoglycan

Let us start with peptidoglycan, since it is an ingredient that both bacterial cell walls have in common. Peptidoglycan is a polysaccharide made of two glucose derivatives, **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)**, alternated in long chains. The chains are cross-linked to one another by a **tetrapeptide** that extends off the NAM sugar unit, allowing a lattice-like structure to form. The four amino acids that compose the tetrapeptide are: **L-alanine, D-glutamine, L-lysine or *meso*-diaminopimelic acid (DPA),** and **D-alanine**. Typically only the L-isomeric form of amino acids are utilized by cells but the use of the mirror image D-amino acids provides protection from proteases that might compromise the integrity of the cell wall by attacking the peptidoglycan. The tetrapeptides can be **directly cross-linked** to one another, with the D-alanine on one tetrapeptide binding to the L-lysine/ DPA on another tetrapeptide. In many gram positive bacteria there is a cross-bridge of five amino acids such as glycine (**peptide interbridge**) that serves to connect one tetrapeptide to another. In either case the cross-linking serves to increase the strength of the overall structure, with more strength derived from **complete cross-linking**, where every tetrapeptide is bound in some way to a tetrapeptide on another NAG-NAM chain. While much is still unknown about peptidoglycan, research in the past ten years suggests that peptidoglycan is synthesized as a cylinder with a coiled substructure, where each coil is cross-linked to the coil next to it, creating an even stronger structure overall.



Peptidoglycan Structure.

ARCHAEA

The **Archaea** are a group of organisms that were originally thought to be bacteria (which explains the initial name of "archaeabacteria"), due to their physical similarities. More reliable genetic analysis revealed that the Archaea are distinct from both Bacteria and Eukaryotes, earning them their own domain in the Three Domain Classification originally proposed by Woese in 1977, alongside the *Eukarya* and the *Bacteria*.

Similarities to Bacteria

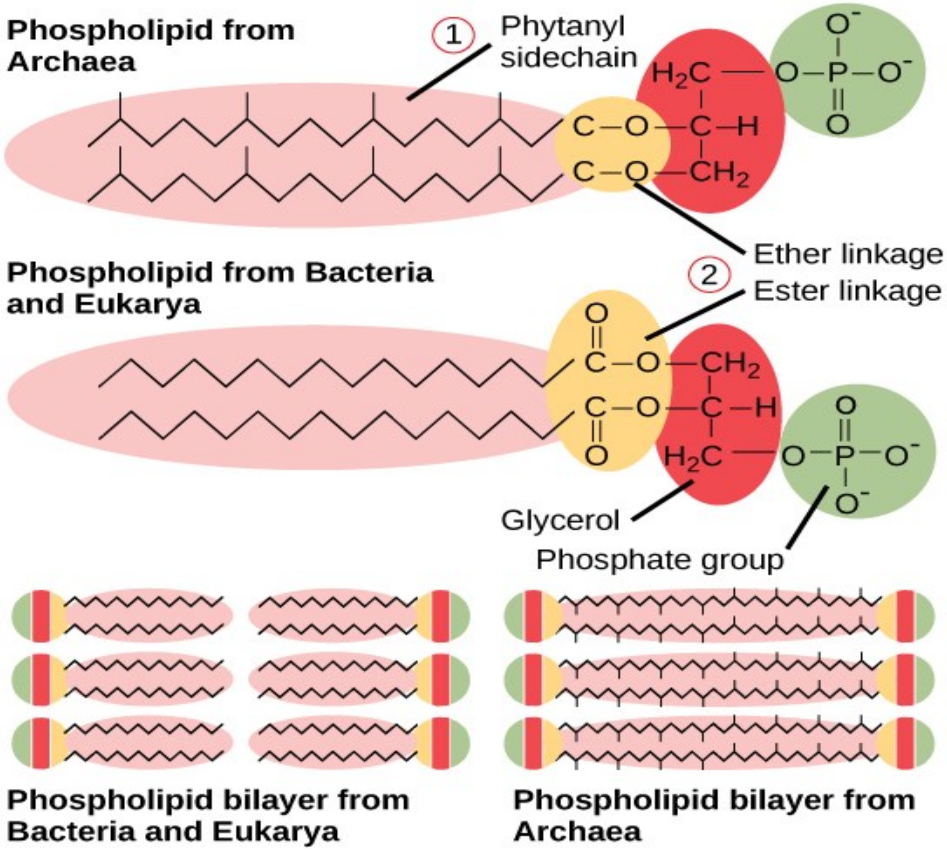
So, why were the archaea originally thought to be bacteria? Perhaps most importantly, they lack a nucleus or other membrane-bound organelles, putting them into the prokaryotic category (if you are using the traditional classification scheme). Most of them are unicellular, they have 70S sized ribosomes, they are typically a few micrometers in size, and they reproduce asexually only. They are known to have many of the same structures that bacteria can have, such as plasmids, inclusions, flagella, and pili. Capsules and slime layers have been found but appear to be rare in archaea. While archaea were originally isolated from extreme environments, such as places high in acid, salt, or heat, earning them the name "extremophiles," they have more recently been isolated from all the places rich with bacteria: surface water, the ocean, human skin, soil, etc.

Key Differences

Plasma Membrane

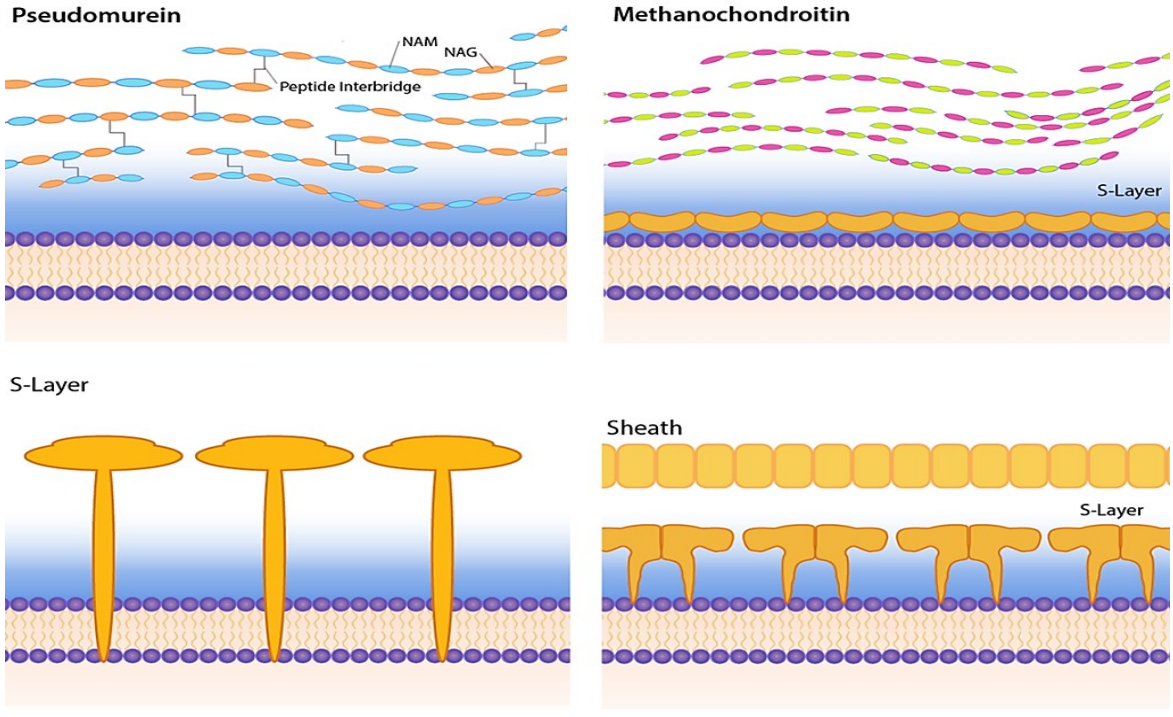
There are several characteristics of the plasma membrane that are unique to *Archaea,* setting them apart from other domains. One such characteristic is chirality of the glycerol linkage between the phopholipid head and the side chain.

- In archaea it is in the **L-isomeric form**, while bacteria and eukaryotes have the **D-isomeric form**.
- A second difference is the presence of an **ether-linkage** between the glycerol and the side chain, as opposed to the **ester-linked lipids** found in bacteria and eukaryotes. The ether-linkage provides more chemical stability to the membrane.
- A third and fourth difference are associated with the side chains themselves, unbranched fatty acids in bacteria and eukaryotes, while **isoprenoid chains** are found in archaea. These isoprenoid chains can have **branching side chains**.
- Lastly, the plasma membrane of Archaea can be found as **monolayers**, where the isoprene chains of one phospholipid connect with the isoprene chains of a phospholipid on the opposite side of the membrane.
- Bacteria and eukaryotes only have **lipid bilayers**, where the two sides of the membrane remain separated.



Cell Wall

Like bacteria, the archaeal cell wall is a semi-rigid structure designed to provide protection to the cell from the environment and from the internal cellular pressure. While the cell walls of bacteria typically contain peptidoglycan, that particular chemical is lacking in archaea. Instead, archaea display a wide variety of cell wall types, adapted for the environment of the organism. Some archaea lack a cell wall altogether. While it is not universal, a large number of Archaea have a proteinaceous **S-layer** that is considered to be part of the cell wall itself (unlike in Bacteria, where an S-layer is a structure in addition to the cell wall). For some Archaea the S-layer is the only cell wall component, while in others it is joined by additional. The archaeal S-layer can be made of either protein or glycoprotein, often anchored into the plasma membrane of the cell. The proteins form a two-dimensional crystalline array with a smooth outer surface. A few S-layers are composed of two different S-layer proteins. While archaea lack peptidoglycan, a few contain a substance with a similar chemical structure, known as **pseudomurein**. Instead of NAM, it contains **N-acetylalosaminuronic acid (NAT)** linked to NAG, with peptide interbridges to increase strength. **Methanochondroitin** is a cell wall polymer found in some archaeal cells, similar in composition to the connective tissue component chondroitin, found in vertebrates. Some archaea have a **protein sheath** composed of a lattice structure similar to an S-layer. These cells are often found in filamentous chains, however, and the protein sheath encloses the entire chain, as opposed to individual cells.



Cell Wall Structures

Ribosomes

While archaea have ribosomes that are 70S in size, the same as bacteria, it was the rRNA nucleotide differences that provided scientists with the conclusive evidence to argue that archaea deserved a domain separate from the bacteria. In addition, archaeal ribosomes have a different shape than bacterial ribosomes, with proteins that are unique to archaea. This provides them with resistance to antibiotics that inhibit ribosomal function in bacteria.

Structures

Many of the structures found in bacteria have been discovered in archaea as well, although sometimes it is obvious that each structure was evolved independently, based on differences in substance and construction.

Cannulae, a structure unique to archaea, have been discovered in some marine archaeal strains. These hollow tube-like structures appear to connect cells after division, eventually leading to a dense network composed of numerous cells and tubes. This could serve as a means of anchoring a community of cells to a surface.

Hamus (pl. hami)

Another structure unique to archaea is the **hamus**, a long helical tube with three hooks at the far end. Hami appear to allow cells to attach both to one another and to surfaces, encouraging the formation of a community.

Pilus (pl. pili)

Pili have been observed in archaea, composed of proteins most likely modified from the bacterial pilin. The resulting tube-like structures have been shown to be used for attachment to surfaces.

Flagellum (pl. flagella)

The archaeal **flagellum**, while used for motility, differs so markedly from the bacterial flagellum that it has been proposed to call it an "**archaellum**," to differentiate it from its bacterial counterpart.

What is similar between the bacterial flagellum and the archaeal flagellum?

Both are used for movement, where the cell is propelled by rotation of a rigid filament extending from the cell. After that the similarities end.

What are the differences?

- The rotation of an archaeal flagellum is powered by ATP, as opposed to the proton motive force used in bacteria.

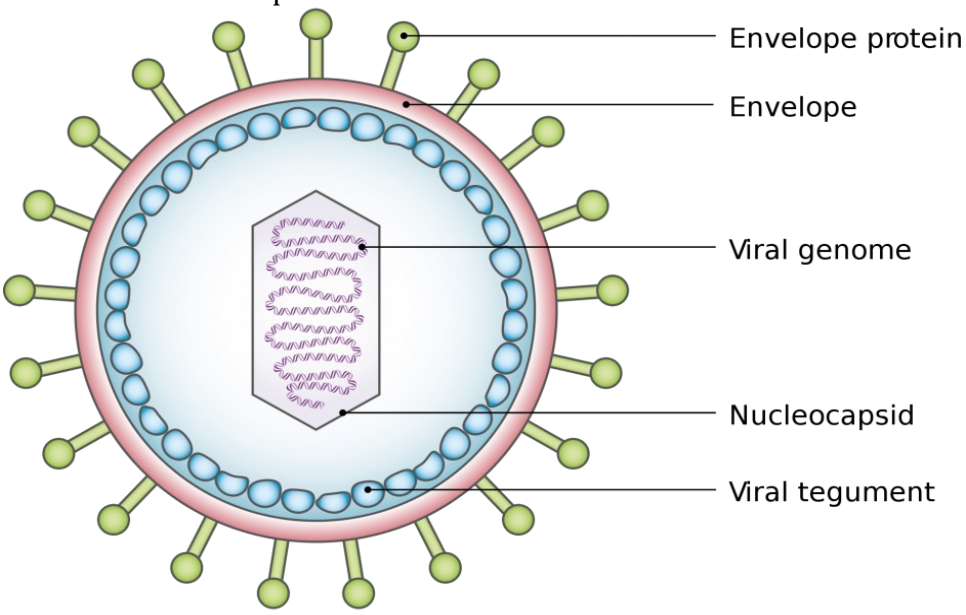
- The proteins making up the archaeal flagellum are similar to the proteins found in bacterial pili, rather than the bacterial flagellum.
- The archaeal flagellum filament is not hollow so growth occurs when flagellin proteins are inserted into the base of the filament, rather than being added to the end.
- The filament is made up of several different types of flagellin, while just one type is used for the bacterial flagellum filament.
- Clockwise rotation pushes an archaeal cell forward, while counterclockwise rotation pulls an archaeal cell backwards.
- An alternation of runs and tumbles is not observed.

Classification

Currently there are two recognized phyla of archaea: **Euryarchaeota** and **Proteoarchaeota**. Several additional phyla have been proposed (Nanoarchaeota, Korarchaeota, Aigarchaeota, Lokiarchaeota), but have yet to be officially recognized, largely due to the fact that the evidence comes from environmental sequences only.

VIRUSES

Viruses are typically described as **obligate intracellular parasites**, acellular infectious agents that require the presence of a host cell in order to multiply. Viruses that have been found to infect all types of cells – humans, animals, plants, bacteria, yeast, archaea, protozoa…some scientists even claim they have found a virus that infects other viruses! But that is not going to happen without some cellular help.



Virus Characteristics

Viruses can be extremely simple in design, consisting of nucleic acid surrounded by a protein coat known as a **capsid**. The capsid is composed of smaller protein components referred to as **capsomers**. The capsid+genome combination is called a **nucleocapsid**.

Viruses can also possess additional components, with the most common being an additional membranous layer that surrounds the nucleocapsid, called an **envelope**. The envelope is actually acquired from the nuclear or plasma membrane of the infected host cell, and then modified with viral proteins called **peplomers**. Some viruses contain viral enzymes that are necessary for infection of a host cell and coded for within the viral genome. A complete virus, with all the components needed for host cell infection, is referred to as a **virion**.

Virus Genome

While cells contain double-stranded DNA for their genome, viruses are not limited to this form. While there are **dsDNA** viruses, there are also viruses with single-stranded DNA (**ssDNA**), double-stranded RNA (**dsRNA**), and single-stranded RNA (**ssRNA**). In this last category, the ssRNA can either positive-sense (**+ssRNA**, meaning it can transcribe a message, like mRNA) or it can be negative-sense (**-ssRNA**, indicating that it is complementary to mRNA). Some viruses even start with one form of nucleic acid in the nucleocapsid and then convert it to a different form during replication.

Virus Structure

Viral nucleocapsids come in two basic shapes, although the overall appearance of a virus can be altered by the presence of an envelope, if present. **Helical viruses** have an elongated tube-like structure, with the capsomers arranged helically around the coiled genome. **Icosahedral viruses** have a spherical shape, with icosahedral symmetry consisting of 20 triangular faces. The simplest icosahedral capsid has 3 capsomers per triangular face, resulting in 60 capsomers for the entire virus. Some viruses do not neatly fit into either of the two previous categories because they are so unusual in design or components, so there is a third category known as **complex viruses**. Examples include the poxvirus with a brick-shaped exterior and a complicated internal structure, as well as bacteriophage with tail fibers attached to an icosahedral head.

Virus Replication Cycle

While the replication cycle of viruses can vary from virus to virus, there is a general pattern that can be described, consisting of five steps:

1. **Attachment** – the virion attaches to the correct host cell.
2. **Penetration or Viral Entry** – the virus or viral nucleic acid gains entrance into the cell.
3. **Synthesis** – the viral proteins and nucleic acid copies are manufactured by the cells' machinery.
4. **Assembly** – viruses are produced from the viral components.
5. **Release** – newly formed virions are released from the cell.

Attachment

Outside of their host cell, viruses are inert or metabolically inactive. Therefore, the encounter of a virion to an appropriate host cell is a random event. The attachment itself is highly specific, between molecules on the outside of the virus and receptors on the host cell surface. This accounts for the specificity of viruses to only infect particular cell types or particular hosts.

Penetration or Viral Entry

Many unenveloped (or **naked**) viruses inject their nucleic acid into the host cell, leaving an empty capsid on the outside. This process is termed penetration and is common with bacteriophage, the viruses that infect bacteria. With the eukaryotic viruses, it is more likely for the entire capsid to gain entrance into the cell, with the capsid being removed in the cytoplasm. An unenveloped eukaryotic virus often gains entry via **endocytosis**, where the host cell is compelled to engulf the capsid resulting in an endocytic vesicle. An enveloped eukaryotic virus gains entrance for its nucleocapsid when the viral envelope fuses with the host cell membrane, pushing the nucleocapsid past the cell membrane. If the entire nucleocapsid is brought into the cell then there is an uncoating process to strip away the capsid and release the viral genome.

Synthesis

The synthesis stage is largely dictated by the type of viral genome, since genomes that differ from the cell's dsDNA genome can involve intricate viral strategies for genome replication and protein synthesis. Viral specific enzymes, such as RNA-dependent RNA polymerases, might be necessary for the replication process to proceed. Protein production is tightly controlled, to insure that components are made at the right time in viral development.

Assembly

The complexity of viral assembly depends upon the virus being made. The simplest virus has a capsid composed of 3 different types of proteins, which self-assembles with little difficulty. The most complex virus is composed of over 60 different proteins, which must all come together in a specific order. These viruses often employ multiple assembly lines to create the different viral structures and then utilize scaffolding proteins to put all the viral components together in an organized fashion.

Release

The majority of viruses lyse their host cell at the end of replication, allowing all the newly formed virions to be released to the environment. Another possibility, common for enveloped viruses, is **budding**, where one virus is released from the cell at a time. The cell membrane is modified by the insertion of viral proteins, with the nucleocapsid pushing out through this modified portion of the membrane, allowing it to acquire an envelope.

Bacteriophage

Viruses that infect bacteria are known as bacteriophage or **phage**. A **virulent phage** is one that always lyses the host cell at the end of replication, after following the five steps of replication described above. This is called the **lytic cycle** of replication. There are also **temperate phage**, viruses that have two options regarding their replication. Option 1 is to mimic a virulent phage, following the five steps of replication and lysing the host cell at the end, referred to as the lytic cycle. But temperate phage differ from virulent phage in that they have another choice: Option 2, where they remain within the host cell without destroying it. This process is known as **lysogeny** or the **lysogenic cycle** of replication.