الجمهوريـــه الجزائريـــه الديمقراطيـــه الشعبيـــه
République Algérienne Démocratique et Populaire
وزارة التعليـــم العالـــي و البحـــث العلمـــي
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique

Université de Relizane
Faculté des Sciences et Technologies
N°74/U.R/F.S.T/2024
Relizane le 29/05/2024

جامعـــة غليـــزان
كليـــة العلـــوم والتكنولوجيـــا
الرقم:..74../ج.غ/ك.ع.ت/2024
غليزان في: .2024/05/29..

EXTRAIT DE PROCÈS-VERBAL DE LA RÉUNION DU CONSEIL SCIENTIFIQUE DE LA FACULTÉ SESSION ORDINAIRE DU 29 MAI 2024

Le Conseil Scientifique de la Faculté des Sciences et Technologies, réuni en session ordinaire le 29 mai 2024 sous la présidence du Professeur ELANDALOUSSI El Hadj, a pris acte des rapports favorables des experts désignés pour l'évaluation du polycopié de cours présenté par Dr **DJELLOULI Mustapha**, intitulé « **Cell Biology** ». Le CSF approuve le contenu scientifique du polycopié et autorise sa diffusion par voie d'affichage sur le site web de l'université ou son dépôt au niveau de la bibliothèque de la Faculté des Sciences et Technologies.

Le Président du Conseil Scientifique

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People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research

University of RELIZANE
Faculty of Science and Technology
Department of Biological Sciences

جامعة غليزان
RELIZANE UNIVERSITY

COURSE HANDOUT

Destined for first year students
Natural and Life Sciences

Titled

Cell Biology

Prepared by :

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Academic year : 2023/2024

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List of abbreviations

AFM	Atomic force microscopy
ATP	Adenosine triphosphate
cDNA	Complementary deoxyribonucleic acid
DIC	Differential interference contrast
DNA	Deoxyribonucleic acid
ER	Endoplasmic reticulum
FLIC	Fluorescence differential interference contrast
H_2O_2	Hydrogen peroxide
mRNA	Messenger ribonucleic acid
NSOM	Near-field scanning optical microscopy
O_{2^-}	Superoxide
OH	Hydroxyl radical
PEP	Phosphoenolpyruvate
RER	Rough endoplasmic reticulum
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rRNA	Ribosomal RNA
RuBisCo	Ribulose bisphosphate carboxylase/oxygenase
SEM	Scanning electron microscope
SER	Smooth endoplasmic reticulum
SPM	Scanned-probe microscopy
STM	Scanning tunneling microscopy

Chapter 1 : Generalities

1. The Science of Taxonomy

Taxonomy is the classification, description, identification, and naming of living organisms. Classification is the practice of organizing organisms into different groups based on their shared characteristics. The most famous early taxonomist was a Swedish botanist, zoologist, and physician named Carolus Linnaeus (1701–1778). In 1735, Linnaeus published Systema Naturae, an 11- page booklet in which he proposed the Linnaean taxonomy, a system of categorizing and naming organisms using a standard format so scientists could discuss organisms using consistent terminology. He continued to revise and add to the book, which grew into multiple volumes.

In his taxonomy, Linnaeus divided the natural world into three kingdoms: animal, plant, and mineral **(Figure 1)**. Within the animal and plant kingdoms, he grouped organisms using a hierarchy of increasingly specific levels and sublevels based on their similarities. The names of the levels in Linnaeus's original taxonomy were kingdom, class, order, family, genus (plural: genera), and species. Species was, and continues to be, the most specific and basic taxonomic unit.

1.1. Evolving trees of life (Phylogenies)

With advances in technology, other scientists gradually made refinements to the Linnaean system and eventually created new systems for classifying organisms. In the 1800s, there was a growing interest in developing taxonomies that took into account the evolutionary relationships, or phylogenies, of all different species of organisms on earth. One way to depict these relationships is via a diagram called a phylogenetic tree (or tree of life). In these diagrams, groups of organisms are arranged by how closely related they are thought to be. In early phylogenetic trees, the relatedness of organisms was inferred by their visible similarities, such as the presence or absence of hair or the number of limbs. Now, the analysis is more complicated. Today, phylogenic analyses include genetic, biochemical, and embryological comparisons, as will be discussed later in this chapter.

Linnaeus's tree of life contained just two main branches for all living things: the animal and plant kingdoms. In 1866, Ernst Haeckel, a German biologist, philosopher, and physician, proposed another kingdom, Protista, for unicellular organisms **(Figure 1)**. He later proposed a fourth kingdom, Monera, for unicellular organisms whose cells lack nuclei, like bacteria.

Nearly 100 years later, in 1969, American ecologist Robert Whittaker (1920–1980) proposed adding another kingdom—Fungi—in his tree of life. Whittaker's tree also contained a level of categorization above the kingdom level—the empire or superkingdom level—to distinguish between organisms that have membrane-bound nuclei in their cells (eukaryotes) and those that do not (prokaryotes). Empire Prokaryota contained just the Kingdom Monera. The Empire Eukaryota contained the other four kingdoms : Fungi, Protista, Plantae, and Animalia. Whittaker's five-kingdom tree was considered the standard phylogeny for many years.

Figure 1 shows how the tree of life has changed over time. Note that viruses are not found in any of these trees. That is because they are not made up of cells and thus it is difficult to determine where they would fit into a tree of life.

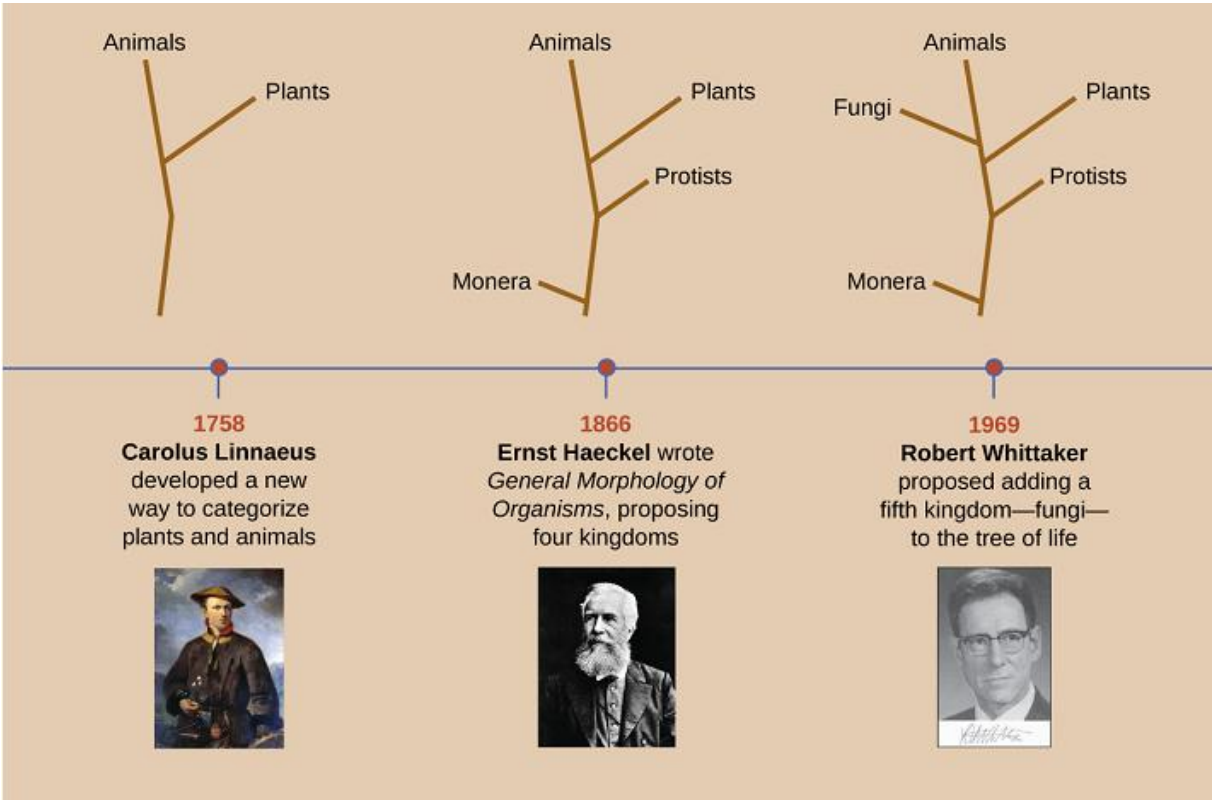


Figure 1 : This timeline shows how the shape of the tree of life has changed over the centuries. Even today, the taxonomy of living organisms is continually being reevaluated and refined with advances in technology.

1.2. The role of genetics in modern taxonomy

Haeckel's and Whittaker's trees presented hypotheses about the phylogeny of different organisms based on readily observable characteristics. But the advent of molecular genetics in the late 20th century revealed other ways to organize phylogenetic trees. Genetic methods

allow for a standardized way to compare all living organisms without relying on observable characteristics that can often be subjective. Modern taxonomy relies heavily on comparing the nucleic acids (deoxyribonucleic acid [DNA] or ribonucleic acid [RNA]) or proteins from different organisms. The more similar the nucleic acids and proteins are between two organisms, the more closely related they are considered to be.

In the 1970s, American microbiologist Carl Woese discovered what appeared to be a "living record" of the evolution of organisms. He and his collaborator George Fox created a genetics-based tree of life based on similarities and differences they observed in the small subunit ribosomal RNA (rRNA) of different organisms. In the process, they discovered that a certain type of bacteria, called archaebacteria (now known simply as archaea), were significantly different from other bacteria and eukaryotes in terms of the sequence of small subunit rRNA. To accommodate this difference, they created a tree with three Domains above the level of Kingdom: Archaea, Bacteria, and Eukarya **(Figure 2)**. Genetic analysis of the small subunit rRNA suggests archaea, bacteria, and eukaryotes all evolved from a common ancestral cell type. The tree is skewed to show a closer evolutionary relationship between Archaea and Eukarya than they have to Bacteria.

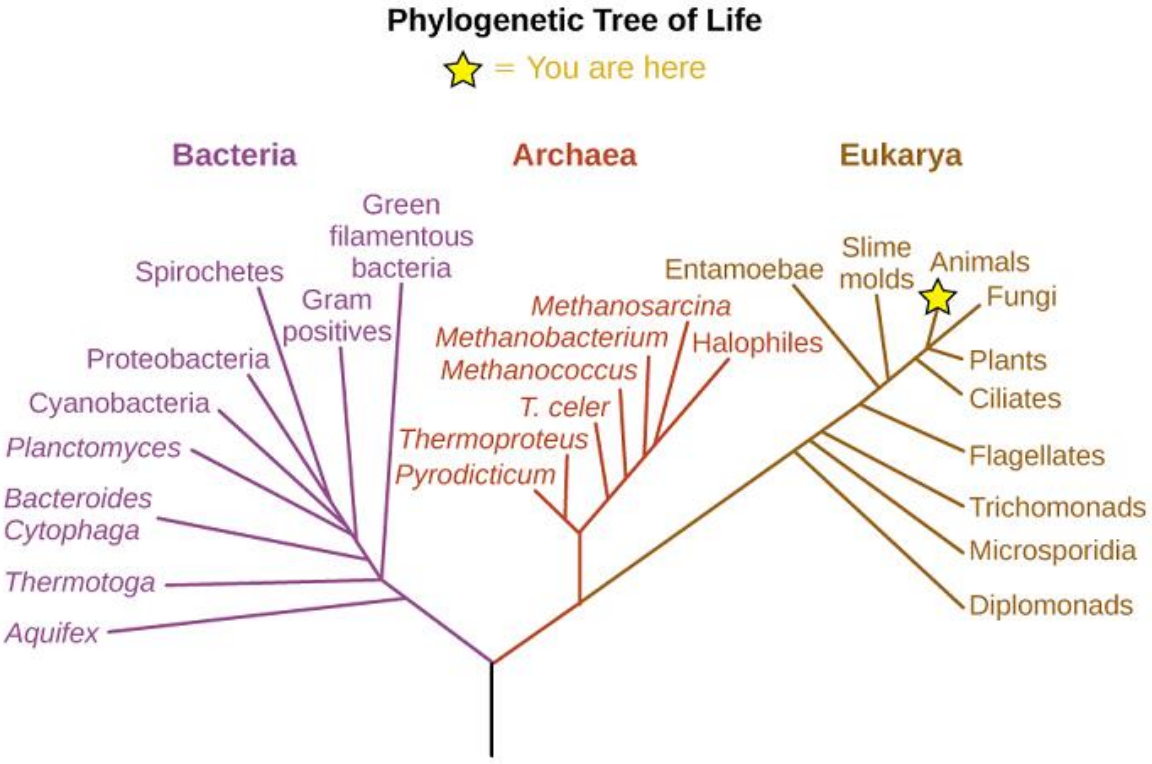


Figure 2 : Scientists continue to use analysis of RNA, DNA, and proteins to determine how organisms are related. One interesting, and complicating, discovery is that of horizontal gene transfer—when a gene of one species is absorbed into another organism's genome. Horizontal gene transfer is especially

common in microorganisms and can make it difficult to determine howorganisms are evolutionarily related. Consequently, some scientists now think in terms of “webs of life” rather than “trees of life.”

2. Cell theory

In biology, cell theory is a scientific theory which describes the properties of cells. These cells are the basic unit of structure in all organisms and also the basic unit of reproduction. With continual improvements made to microscopes over time, magnification technology advanced enough to discover cells in the 17th century. This discovery is largely attributed to Robert Hooke, and began the scientific study of cells, also known as cell biology. Over a century later, many debates about cells began amongst scientists. Most of these debates involved the nature of cellular regeneration, and the idea of cells as a fundamental unit of life.

Figure 3 :

Mathias Jakob Schleiden (1804–1881)

Figure 4 :

Theodor Schwann (1810–1882)

Matthias Jakob Schleiden (5 April 1804 – 23 June 1881) was a German botanist and cofounder of cell theory, along with Theodor Schwann (7 December 1810 – 11 January 1882) a German physiologist and Rudolf Ludwig Carl Virchow (13 October 1821 – 5 September 1902) was a German physician, anthropologist, pathologist, prehistorian, biologist, writer, editor, and politician, known for his advancement of public health. Credit for developing cell theory is usually given to these scientists- Schleiden and Schwann. While Rudolf Virchow contributed to the theory, he is not as credited for his attributions toward it. In 1838, Schleiden suggested that every structural part of a plant was made up of cells or the result of cells. He also suggested that cells were made by a crystallization process either within other cells or from the outside. However, this was not an original idea of Schlieden. He claimed this theory

as his own, though Barthelemy Dumortier had stated it years before him. This crystallization process is no longer accepted with modern cell theory. In 1839, Theodor Schwann states that along with plants, animals are composed of cells or the product of cells in their structures. This was a major advancement in the field of biology since little was known about animal structure up to this point compared to plants. From these conclusions about plants and animals, two of the three tenets of cell theory were postulated.

1. All living organisms are composed of one or more cells.

2. The cell is the most basic unit of life. Schleiden's theory of free cell formation through crystallization was refuted in the 1850s by Robert Remak, Rudolf Virchow, and Albert Kolliker. Robert Remak (26 July 1815 – 29 August 1865) was a Jewish Polish-German embryologist, physiologist, and neurologist, born in Posen, Prussia, who discovered that the origin of cells was by the division of pre-existing cells. In 1855, Rudolf Virchow added the third tenet to cell theory.

3. All cells arise only from pre-existing cells. However, the idea that all cells come from pre-existing cells had in fact already been proposed by Robert Remak; it has been suggested that Virchow plagiarized Remak and did not give him credit. Remak published observations in 1852 on cell division, claiming Schleiden and Schawnn were incorrect about generation schemes. He instead said that binary fission, which was first introduced by Dumortier, was how reproduction of new animal cells was made. Once this tenet was added, the classical cell theory was complete. Barthélemy Charles Joseph Dumortier (3 April 1797 in Tournai – 9 June 1878) was a Belgian who conducted a parallel career of botanist and Member of Parliament.

The modern version of the cell theory includes the ideas that :

1- Energy flow occurs within cells.

2- Heredity information (DNA) is passed on from cell to cell.

3- All cells have the same basic chemical composition.

3. Evolution and the origin of species

The theory of evolution is the unifying theory of biology, meaning it is the framework within which biologists ask questions about the living world. Its power is that it provides direction

for predictions about living things that are borne out in experiment after experiment. The Ukrainian-born American geneticist Theodosius Dobzhansky famously wrote that “nothing makes sense in biology except in the light of evolution. He meant that the tenet that all life has evolved and diversified from a common ancestor is the foundation from which we approach all questions in biology.

3.1. Understanding evolution

Evolution by natural selection describes a mechanism for how species change over time. That species change had been suggested and debated well before Darwin began to explore this idea. The view that species were static and unchanging was grounded in the writings of Plato, yet there were also ancient Greeks who expressed evolutionary ideas. In the eighteenth century, ideas about the evolution of animals were reintroduced by the naturalist Georges-Louis Leclerc Comte de Buffon who observed that various geographic regions have different plant and animal populations, even when the environments are similar. It was also accepted that there were extinct species.

During this time, James Hutton, a Scottish naturalist, proposed that geological change occurred gradually by the accumulation of small changes from processes operating like they are today over long periods of time. This contrasted with the predominant view that the geology of the planet was a consequence of catastrophic events occurring during a relatively brief past. Hutton’s view was popularized in the nineteenth century by the geologist Charles Lyell who became a friend to Darwin. Lyell’s ideas were influential on Darwin’s thinking: Lyell’s notion of the greater age of Earth gave more time for gradual change in species, and the process of change provided an analogy for gradual change in species. In the early nineteenth century, Jean-Baptiste Lamarck published a book that detailed a mechanism for evolutionary change. This mechanism is now referred to as an inheritance of acquired characteristics by which modifications in an individual are caused by its environment, or the use or disuse of a structure during its lifetime, could be inherited by its offspring and thus bring about change in a species. While this mechanism for evolutionary change was discredited, Lamarck’s ideas were an important influence on evolutionary thought.

3.2. Charles Darwin and Natural Selection

In the mid-nineteenth century, the actual mechanism for evolution was independently conceived of and described by two naturalists : Charles Darwin and Alfred Russel Wallace. Importantly, each naturalist spent time exploring the natural world on expeditions to the

tropics. From 1831 to 1836, Darwin traveled around the world on H.M.S. Beagle, including stops in South America, Australia, and the southern tip of Africa.

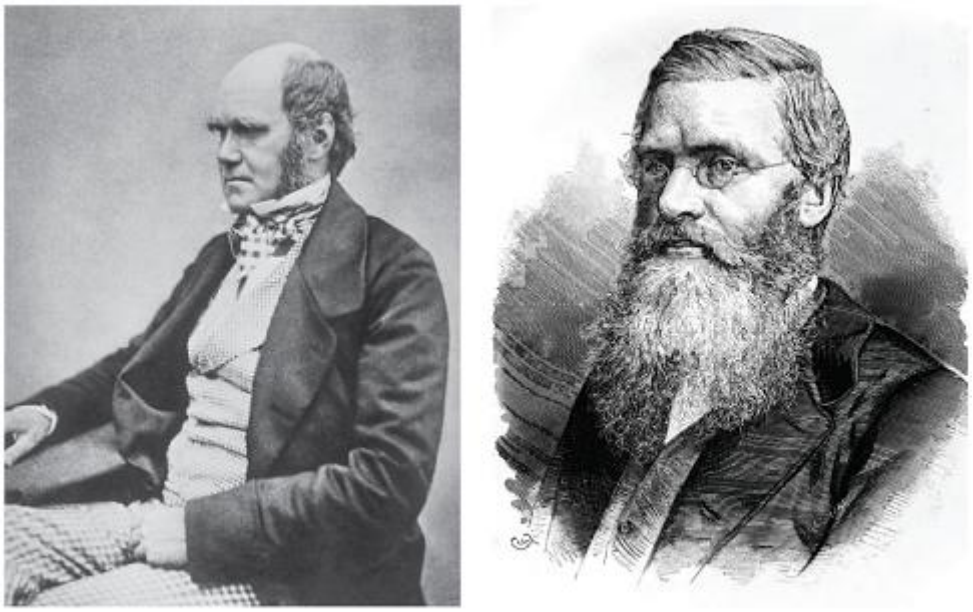


Figure 5 : Both (a) Charles Darwin and (b) Alfred Wallace wrote scientific papers on natural selection that were presented together before the Linnean Society in 1858.

Wallace traveled to Brazil to collect insects in the Amazon rainforest from 1848 to 1852 and to the Malay Archipelago from 1854 to 1862. Darwin's journey, like Wallace's later journeys to the Malay Archipelago, included stops at several island chains, the last being the Galápagos Islands west of Ecuador. On these islands, Darwin observed species of organisms on different islands that were clearly similar, yet had distinct differences. For example, the ground finches inhabiting the Galápagos Islands comprised several species with a unique beak shape **(Figure 6)**. The species on the islands had a graded series of beak sizes and shapes with very small differences between the most similar. He observed that these finches closely resembled another finch species on the mainland of South America. Darwin imagined that the island species might be species modified from one of the original mainland species. Upon further study, he realized that the varied beaks of each finch helped the birds acquire a specific type of food. For example, seed-eating finches had stronger, thicker beaks for breaking seeds, and insect-eating finches had spear-like beaks for stabbing their prey.

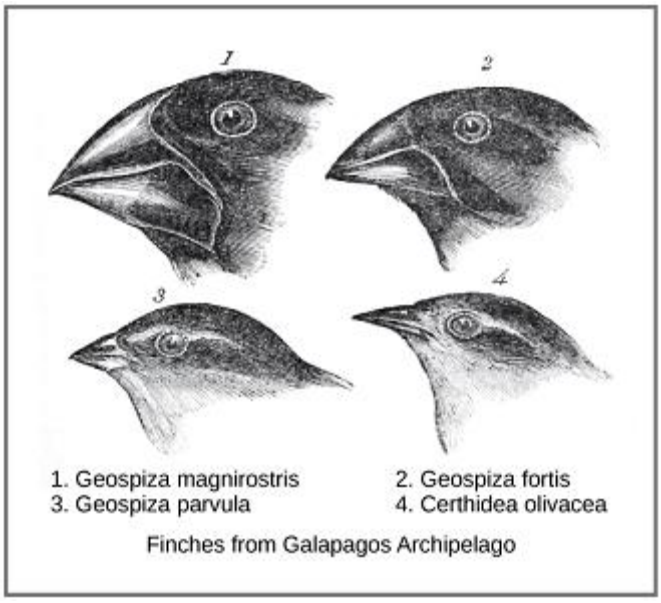


Figure 6 : Darwin observed that beak shape varies among finch species. He postulated that the beak of an ancestral species had adapted over time to equip the finches to acquire different food sources.

Wallace and Darwin both observed similar patterns in other organisms and they independently developed the same explanation for how and why such changes could take place. Darwin called this mechanism natural selection. Natural selection, also known as "survival of the fittest," is the more prolific reproduction of individuals with favorable traits that survive environmental change because of those traits; this leads to evolutionary change.

3.3. Evolution explains the origin of life

It is a common misunderstanding that evolution includes an explanation of life's origins. Conversely, some of the theory's critics believe that it cannot explain the origin of life. The theory does not try to explain the origin of life. The theory of evolution explains how populations change over time and how life diversifies the origin of species. It does not shed elight on the beginnings of life including the origins of the first cells, which is how life is defined. The mechanisms of the origin of life on Earth are a particularly difficult problem because it occurred a very long time ago, and presumably it just occurred once. Importantly, biologists believe that the presence of life on Earth precludes the possibility that the events that led to life on Earth can be repeated because the intermdiate stages would immediately become food for existing living things.

However, once a mechanism of inheritance was in place in the form of a molecule like DNA either within a cell or pre-cell, these entities would be subject to the principle of natural selection. More effective reproducers would increase in frequency at the expense of

inefficient reproducers. So while evolution does not explain the origin of life, it may have something to say about some of the processes operating once pre-living entities acquired certain properties.

Cell Structure

Your body has many kinds of cells, each specialized for a specific purpose. Just as a home is made from a variety of building materials, the human body is constructed from many cell types. For example, epithelial cells protect the surface of the body and cover the organs and body cavities within. Bone cells help to support and protect the body. Cells of the immune system fight invading bacteria. Additionally, blood and blood cells carry nutrients and oxygen throughout the body while removing carbon dioxide. Each of these cell types plays a vital role during the growth, development, and day-to-day maintenance of the body. In spite of their enormous variety, however, cells from all organisms—even ones as diverse as bacteria, onion, and human—share certain fundamental characteristics.

Chapter 2 : Methods of studying cells

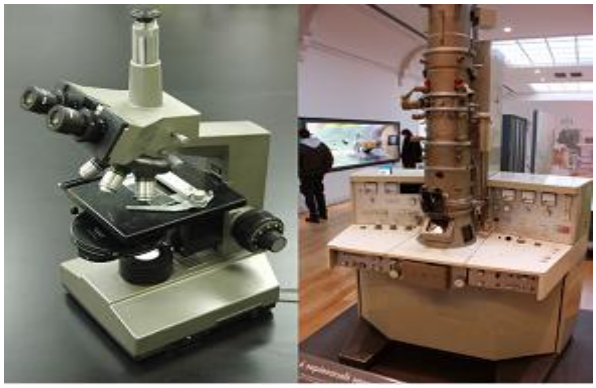
1. Microscopy

Cells vary in size. With few exceptions, individual cells cannot be seen with the naked eye, so scientists use microscopes (micro- = “small”; -scope = “to look at”) to study them. A microscope is an instrument that magnifies an object. Most photographs of cells are taken with a microscope; these images can also be called micrographs. The optics of a microscope’s lenses change the orientation of the image that the user sees. A specimen that is right-side up and facing right on the microscope slide will appear upside-down and facing left when viewed through a microscope, and vice versa. Similarly, if the slide is moved left while looking through the microscope, it will appear to move right, and if moved down, it will seem to move up. This occurs because microscopes use two sets of lenses to magnify the image. Because of the manner by which light travels through the lenses, this system of two lenses produces an inverted image (binocular, or dissecting microscopes, work in a similar manner, but they include an additional magnification system that makes the final image appear to be upright).

1.2. Light microscopes

To give you a sense of cell size, a typical human red blood cell is about eight millionths of a meter or eight micrometers (abbreviated as eight μm) in diameter; the head of a pin of is about

two thousandths of a meter (two mm) in diameter. That means about 250 red blood cells could fit on the head of a pin **(Figure 7)**.



a **b**

Figure 7 : Light and Electron Microscopes : (a) Most light microscopes used in a college biology lab can magnify cells up to approximately 400 times and have a resolution of about 200 nanometers. **(b)** Electron microscopes provide a much higher magnification, 100,000x, and a have a resolution of 50 picometers.

Most student microscopes are classified as light microscopes. Visible light passes and is bent through the lens system to enable the user to see the specimen. Light microscopes are advantageous for viewing living organisms, but since individual cells are generally transparent, their components are not distinguishable unless they are colored with special stains. Staining, however, usually kills the cells.

Light microscopes, commonly used in undergraduate college laboratories, magnify up to approximately 400 times. Two parameters that are important in microscopy are magnification and resolving power. Magnification is the process of enlarging an object in appearance. Resolving power is the ability of a microscope to distinguish two adjacent structures as separate : the higher the resolution, the better the clarity and detail of the image. When oil immersion lenses are used for the study of small objects, magnification is usually increased to 1,000 times. In order to gain a better understanding of cellular structure and function, scientists typically use electron microscopes.

1.3. Electron microscopes

In contrast to light microscopes, electron microscopes use a beam of electrons instead of a beam of light. Not only does this allow for higher magnification and, thus, more detail, it also provides higher resolving power. The method used to prepare the specimen for viewing with

an electron microscope kills the specimen. Electrons have short wavelengths (shorter than photons) that move best in a vacuum, so living cells cannot be viewed with an electron microscope **(Figure 7)**.

In a scanning electron microscope, a beam of electrons moves back and forth across a cell's surface, creating details of cell surface characteristics. In a transmission electron microscope, the electron beam penetrates the cell and provides details of a cell's internal structures. As you might imagine, electron microscopes are significantly more bulky and expensive than light microscopes.

Key Points

- Light microscopes allow for magnification of an object approximately up to 400-1000 times depending on whether the high power or oil immersion objective is used.
- Light microscopes use visible light which passes and bends through the lens system.
- Electron microscopes use a beam of electrons, opposed to visible light, for magnification.
- Electron microscopes allow for higher magnification in comparison to a light microscope thus, allowing for visualization of cell internal structures.

Key Terms

- **Resolution :** The degree of fineness with which an image can be recorded or produced, often expressed as the number of pixels per unit of length (typically an inch).
- **Electron :** The subatomic particle having a negative charge and orbiting the nucleus; the flow of electrons in a conductor constitutes electricity.

1.4. General staining methods

Staining is a technique used in microscopy to enhance contrast in a microscopic image. Stains and dyes are frequently used to highlight structures in microbes for viewing, often with the aid of different microscopes. Stains may be used to define and examine different types of microbes, various stages of cellular life (e.g., the mitotic cycle), and even organelles within individual cells (e.g., mitochondria or chloroplasts).

In-vivo staining is the process of dyeing living tissue — in vivo means "in life" (as contrasted to in-vitro staining). When a certain cell or structure takes on contrasting color(s), its form (morphology) or position within a cell or tissue can be readily seen and studied. The usual

purpose is to reveal cytological details that might otherwise not be apparent; however, staining can also reveal where certain chemicals or specific chemical reactions are taking place within cells. In-vitro staining involves coloring cells or structures that have been removed from their biological context. Certain stains are often combined to reveal more details and features than a single stain could reveal alone, and a counterstain is a stain that increases visibility of cells or structures when the principal stain is not sufficient. Scientists and physicians can combine staining with specific protocols for fixation and sample preparation and can use these standard techniques as consistent, repeatable diagnostic tools.

There are an incredible number of stains that can be used in a variety of different methods. What follows here are some common aspects of the process of preparing for in-vitro staining.

- **Fixation :** This can itself consist of several steps. Fixation aims to preserve the shape of the cells (in this case, microbes) as much as possible. Sometimes heat fixation is used to kill, adhere, and alter the cells so they will accept stains. Most chemical fixatives generate chemical bonds between proteins and other substances within the sample, increasing their rigidity. Common fixatives include formaldehyde, ethanol, methanol, and picric acid.
- **Permeabilization :** This involves treatment of the cells with (usually) a mild surfactant. This treatment dissolves cell membranes, allowing larger dye molecules to enter the cell's interior.
- **Mounting :** This step usually involves attaching the samples to a glass microscope slide for observation and analysis. In some cases, cells may be grown directly on a slide. For samples of loose cells the sample can be directly applied to a slide.

At its simplest, the actual staining process may involve immersing the sample (before or after fixation and mounting) in dye solution, followed by rinsing and observation. Many dyes, however, require the use of a mordant — a chemical compound that reacts with the stain to form an insoluble colored precipitate. When the excess dye solution is washed away, the mordanted stain remains. There is an incredible array of stains that can be used at this step, from those that stain specific microbial types (see the figure below) to those that highlight sub-compartments or organelles of a cell, such as the nucleus or endoplasmic reticulum. Alternatively, negative staining can be employed. This is a simple staining method for bacteria, performed by smearing the cells onto the slide and then applying nigrosin (a black synthetic dye) or Indian ink (an aqueous suspension of carbon particles). After drying, the

microorganisms may be viewed in bright field microscopy as lighter inclusions contrast well against the dark environment surrounding them **(Figure 8)**.

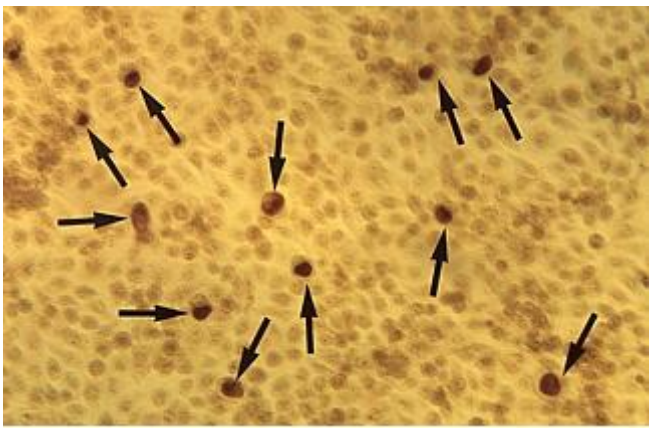


Figure 8 : Chlamydia Stain : Cells of the bacterial pathogen chlamydia (indicated by arrows) are highlighted by a stain called ‘’Geimsa’’.

Live, *in-vivo* staining microscopy shares many of these steps, with the exception of fixation, which invariably kills the microbe to be examined.

Key Points

- In-vivo staining, which visualizes cells that are alive, and in-vitro staining, which visualizes fixed cells, both have important uses.
- There is a vast array of stains that can be used on microbes that can highlight almost any characteristic of a cell, even organelles within a cell.
- Staining protocols can be complex, but they share some basic steps: preparation, fixation, staining, and mounting.

Key Terms

- ❖ **Surfactant :** a surface active agent, or wetting agent, capable of reducing the surface tension of a liquid; typically organic compounds having a hydrophilic “head” and a hydrophobic “tail”
- ❖ **Organelle :** a specialized structure found inside cells that carries out a specific life process (e.g., ribosomes, vacuoles)

2. Other types of microscopy

2.1. Dark-field microscopy

Dark-field microscopy is ideally used to illuminate unstained samples causing them to appear brightly lit against a dark background. This type of microscope contains a special condenser that scatters light and causes it to reflect off the specimen at an angle. Rather than illuminating the sample with a filled cone of light, the condenser is designed to form a hollow cone of light. The light at the apex of the cone is focused at the plane of the specimen; as this light moves past the specimen plane it spreads again into a hollow cone. The objective lens sits in the dark hollow of this cone; although the light travels around and past the objective lens, no rays enter it **(Figure 9)**.

The entire field appears dark when there is no sample on the microscope stage; thus the name dark-field microscopy. When a sample is on the stage, the light at the apex of the cone strikes it. The rays scattered by the sample and captured in the objective lens thus make the image.

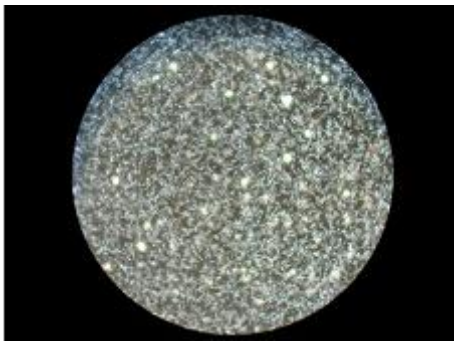


Figure 9 : Visualization of live bacteria : Spirochetes bacteria observed under dark field microscopy

Samples observed under dark-field microscopy should be carefully prepared since dust and other particles also scatter the light and are easily detected. Glass slides need to be thoroughly cleaned of extraneous dust and dirt. It may be necessary to filter sample media (agar, water, saline) to exclude confusing contaminants. Sample materials need to be spread thinly; too much material on the slide creates many overlapping layers and edges, making it difficult to interpret structures.

Dark-field microscopy has many applications in microbiology. It allows the visualization of live bacteria, and distinguishes some structure (rods, curved rods, spirals, or cocci) and movement.

Key Points

- In dark-field microscopy, the light reaches the specimen from an angle with the help of an opaque disk.
- The specimen appears lit up agains a dark background.
- Dark-field microscopy is most useful for extremely small living organisms that are invisible in the light microscope.

Key Terms

❖ **Condenser :** A lens (or combination of lenses) designed to gather light and focus it onto a specimen or part of a mechanism.

2.2. Phase-contrast microscopy

Phase-contrast microscopy visualizes differences in the refractive indexes of different parts of a specimen relative to unaltered light.

Key Points

- A phase-contrast microscope splits a beam of light into 2 types of light, direct and refracted (reflected) and brings them together to form an image of the specimen.
- Where the lights are “in-phase” the image is brighter, where the lights are “out of phase” the image is darker, and by amplifying these differences in the light, it enhances contrast.
- Phase-contrast microscopy allows for the detailed observation of living organisms, especially the internal structures.

Key Terms

❖ **Refractive index :** the ratio of the speed of light in air or vacuum to that in another medium.

Phase-contrast microscopy is a method of manipulating light paths through the use of strategically placed rings in order to illuminate transparent objects. Dutch physicist Fritz Zernike developed the technique in the 1930s; for his efforts he was awarded the Nobel Prize in 1953.

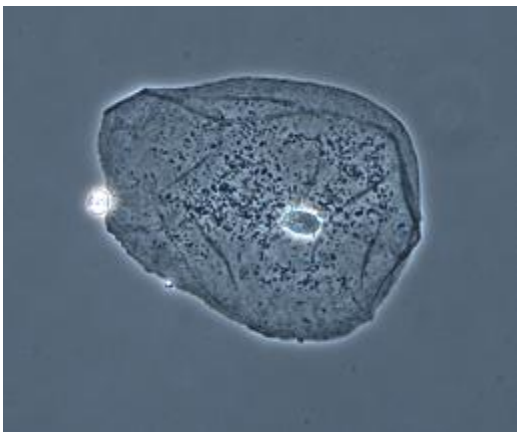


Figure 10 : Phase-contrast microscopy : Phase-contrast image of a cheek epithelial cell.

In phase-contrast microscopy, parallel beams of light are passed through objects of different densities. The microscope contains special condensers that throw light "out of phase" causing it to pass through the object at different speeds. Internal details and organelles of live, unstained organisms (e.g. mitochondria, lysosomes, and the Golgi body) can be seen clearly with this microscope **(Figure 10)**.

A phase ring in condenser allows a cylinder of light to pass through it while still in phase. Unaltered light hits the phase ring in the lens and is excluded. Light that is slightly altered by passing through a different refractive index is allowed to pass through. Light passing through cellular structures, such as chromosomes or mitochondria is retarded because they have a higher refractive index than the surrounding medium. Elements of lower refractive index advance the wave. Much of the background light is removed and light that constructively or destructively interfered is let through with enhanced contrast.

Phase-contrast microscopy allows the visualization of living cells in their natural state with high contrast and high resolution. This tool works best with a thin specimen and is not ideal for a thick specimen. Phase-contrast images have a characteristic grey background with light and dark features found across the sample. One disadvantage of phase-contrast microscopy is halo formation called halo-light ring.

2.3. Stereo light source

Interference microscopy uses a prism to split light into two slightly diverging beams that then pass through the specimen. It is thus based on measuring the differences in refractive index upon recombining the two beams. Interference occurs when a light beam is retarded or advanced relative to the other **(Figure 11)**.

There are three types of interference microscopy: classical, differential contrast, and fluorescence contrast. Since its introduction in the late 1960s differential interference contrast microscopy (DIC) has been popular in biomedical research because it produces high-resolution images of fine structures by enhancing the contrasted interfaces. The image produced is of a thin optical section and appears three-dimensional, with a shadow around it. This creates a contrast across the specimen that is bright on one side and darker on the other.

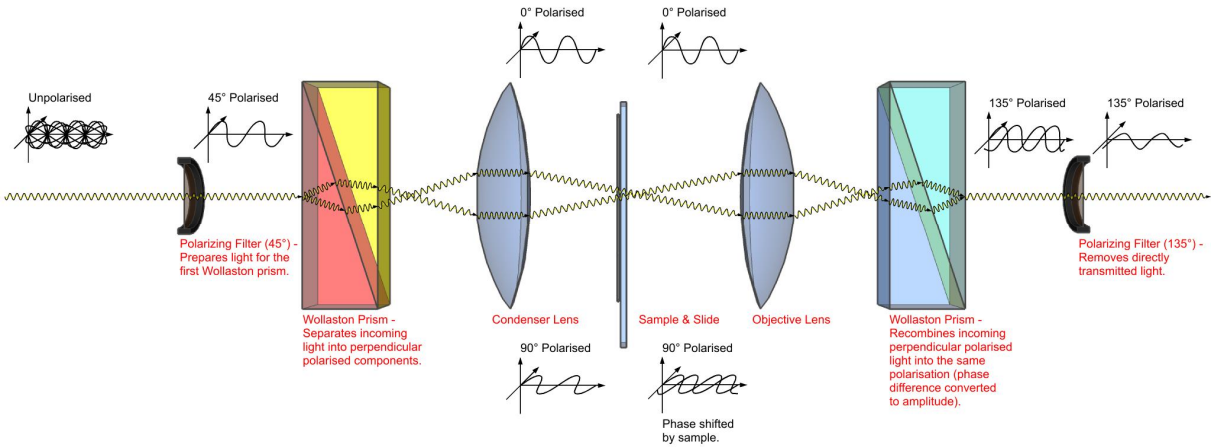


Figure 11 : Path of light in differential interference contrast microscopy (DIC) : Two parallel light beams pass through the specimen and combine to produce an image.

2.4. The interference microscope

The microscope is a bright field light microscope with the addition of the following elements: a polarizer between the light source and the condenser, a DIC beam-splitting prism, a DIC beam-combining prism, and an analyzer. Manipulating the prism changes the beam separation, which alters the contrast of the image. When the two beams pass through the same material across the specimen they produce no interference. When the two beams pass through different material across the specimen such as on the edges, they produce alteration when combined.

Fluorescence differential interference contrast (FLIC) microscopy was developed by combining fluorescence microscopy with DIC to minimize the effects of photobleaching on fluorochromes bound to the stained specimen. The same microscope is equipped to simulataneously image a specimen using DIC and fluorescence illumination.

Key Points

- Interference microscopy is superior to phase-contrast microscopy in its ability to eliminate halos and extra light.
- In differential interference contrast microscopy (DIC), the optical path difference is determined by the product of the refractive index difference (between the specimen and its surrounding medium) and the thickness traversed by a light beam between two points on the optical path.
- Images produced by DIC have a distinctive shadow-cast appearance

Key Terms

- ❖ **Photobleaching :** The destruction of a photochemical fluorescence by high-intensity light
- ❖ **Fluorochrome :** Any of various fluorescent dyes used to stain biological material before microscopic examination.

2.5. Fluorescence microscopy

The fluorescent microscope uses a high-pressure mercury, halogen, or xenon vapor lamp that emits a shorter wavelength than that emitted by traditional brightfield microscopy. These light sources produce ultraviolet light. When ultraviolet light hits an object, it excites the electrons of the object, and they give off light in various shades of color **(Figure 12)**. Since ultraviolet light is used a larger amount of information can be gathered; thus, the resolution of the object increases.

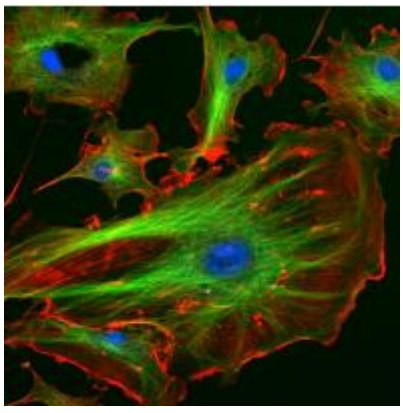


Figure 12 : Fluorescent cells.: Fixed endothelial cells stained with fluorescent dyes. Nuclei are stained blue with DAPI, microtubules are stained green by an antibody bound to FITC and actin filaments are labelled red with phalloidin bound to TRITC.

2.6. Fluorescent-antibody technique and dyes

This laboratory technique employs fluorescent dyes chemically linked to antibodies to help identify unknown microorganisms. This method uses the specificity of an antibody to its antigen to deliver a fluorescent dye to a target molecule. A filter is used to block the heat generated from the lamp and to match the fluorescent dye labeling the specimen. An additional barrier filter between the objective and the detector can filter out the remaining excitation light from fluorescent light.

Fluorescent dyes—molecules that absorb light of one wavelength and then re-emit it at a longer visible wavelength—can be used alone or in combination to gain specificity of the stained structure being visualized. The light emitted from the fluorophore is magnified through traditional objectives and ocular lenses. Staining organisms with these special dyes reduces the non-specific autofluorescence that some organisms can emit. Cells or organisms stained with fluorochromes appear colored against a dark background when fixed on a glass slide. Fluorescence microscopy does not allow examination of live microorganisms as it requires them to be fixed and permeabilized for the antibody to penetrate inside the cells.

Key Points

- In fluorescence microscopy, specimens are first stained with fluorochromes and then viewed through a compound microscope by using an ultraviolet (or near-ultraviolet) light source.
- Microorganisms appear as bright objects against a dark background.
- Fluorescence microscopy is used primarily in a procedure called fluorescent- antibody (FA) technique, or immunofluorescence.

Key Terms

- ❖ Autofluorescence : Self-induced fluorescence
- ❖ Halogen : any element of group 7, i.e. fluorine, chlorine, bromine, iodine and astatine, which form a salt by direct union with a metal.

2.7. Confocal microscopy

Confocal microscopy is a non-invasive fluorescent imaging technique that uses lasers of various colors to scan across a specimen with the aid of scanning mirrors. The point of illumination is brought to focus in the specimen by the objective lens. The scanning process

uses a device that is under computer control. The sequences of points of light from the specimen are detected by a photomultiplier tube through a pinhole. The output is built into an image and transferred onto a digital computer screen for further analysis. The technique employs optical sectioning to take serial slices of the image. The slices are then stacked (Z-stack) to reconstruct the three-dimensional image of the biological sample. Optical sectioning is useful in determining cellular localization of targets. The biological sample to be studied is stained with antibodies chemically bound to fluorescent dyes similar to the method employed in fluorescence microscopy. Unlike in conventional fluorescence microscopy where the fluorescence is emitted along the entire illuminated cone creating a hazy image, in confocal microscopy the pinhole is added to allow passing of light that comes from a specific focal point on the sample and not the other. The light detected creates an image that is in focus with the original sample. Confocal microscopy has multiple applications in microbiology such as the study of biofilms and antibiotic-resistant strains of bacteria. Development of modern confocal microscopes has been accelerated by new advances in computer and storage technology, laser systems, detectors, interference filters, and fluorophores for highly specific targets **(Figure 13)**.

Key Points

- Confocal microscopy requires immunoflurescence staining of biological samples.
- Confocal microscopy serves to control depth of field, eliminate background, and collect optical sections.
- The use of confocal microscopy has expanded to study both fixed and live cells with the ability to quantify targets.

Key Terms

❖ **Photomultiplier tube :** A vacuum tube that detects ultraviolet, visible, and near infrared light and multiplies it 100 million times.

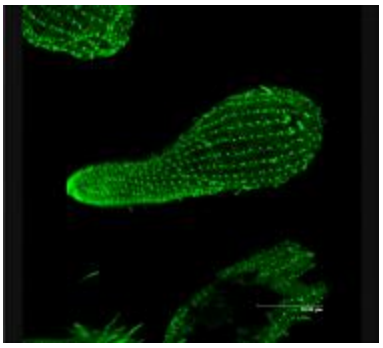


Figure 13 : Confocal microscopy : Tetrahymena cell, visualized using GFP-labeled anti-beta tubulin antibodies under confocal microscopy

2.8. Electron microscopy

Electron microscopy uses a beam of electrons as an energy source. An electron beam has an exceptionally short wavelength and can hit most objects in its path, increasing the resolution of the final image captured. The electron beam is designed to travel in a vacuum to limit interference by air molecules. Magnets are used to focus the electrons on the object viewed **(Figure 14)**.

Figure 14 : Electron microscope : A modern electron microscope

There are two types of electron microscopes. The more traditional form is the transmission electron microscope (TEM). To use this instrument, ultra-thin slices of microorganisms or viruses are placed on a wire grid and then stained with gold or palladium before viewing, to create contrast. The densely coated parts of the specimen deflect the electron beam and both dark and light areas show up on the image. TEM can project images in a much higher resolution—up to the atomic level of thinner objects.

The second and most contemporary form is the scanning electron microscope (SEM). It allows the visualization of microorganisms in three dimensions as the electrons are reflected when passed over the specimen. The same gold or palladium staining is employed.

Electron microscopy has multiple applications. It is ideal to :

- explore the in vivo molecular mechanisms of disease;
- visualize the three dimensional architecture of tissues and cells;
- determine the conformation of flexible protein structures and complexes;
- observe individual viruses and macromolecular complexes in their natural biological context.

Sample preparation can be critical to generate a successful image because the choice of reagents and methods used to process a sample largely depends on the nature of the sample and the analysis required.

Key Points

- A beam of electrons, instead of light, is used with an electron microscope.
- Electron microscopes have a greater magnification because the wavelengths of electrons are much smaller than those of visible light (0.005nm as opposed to 500nm respectively– one hundred thousand times smaller).
- There are two types of electron microscopes, scanning and transmission.
- The best compound light microscopes can magnify 2000x, electron microscopes can magnify up to 100,000x.

Key Terms

❖ **Electron beam :** a stream of electrons observed in vacuum tubes.

2.9. Scanned-probe microscopy

3-D Images

Scanned-probe microscopy (SPM) produces highly magnified and three-dimensional-shaped images of specimens in real time. SPM employs a delicate probe to scan the surface of the

specimen, eliminating the limitations that are found in electron and light microscopy. SPM covers several related technologies for imaging and measuring surfaces on a fine scale, down to the level of molecules and groups of atoms **(Figure 15)**.

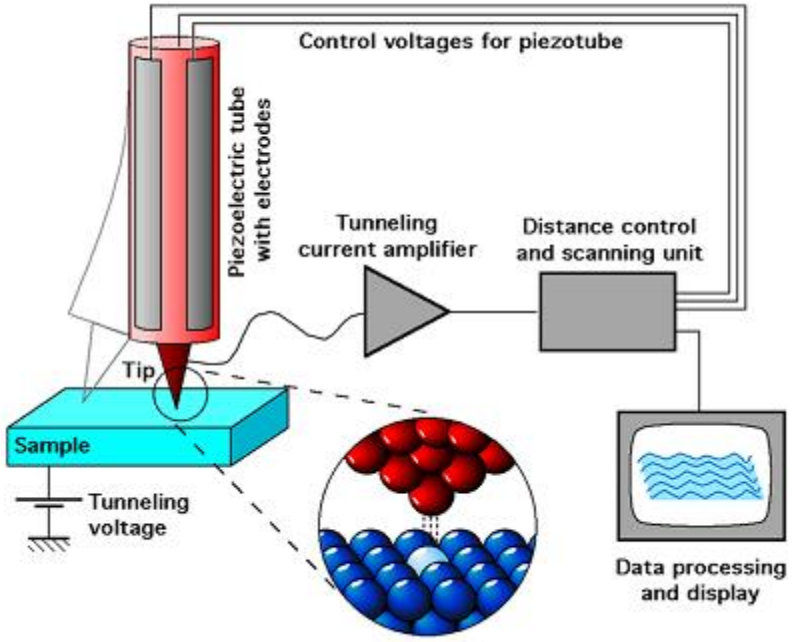


Figure 15 : Scanning tunneling microscopy : Schematic diagram of a scanning tunneling microscope

A scan may cover a distance of over 100 micrometers in the x and y directions and 4 micrometers in the z direction. SPM technologies share the concept of scanning a sharp probe tip with a small radius of curvature across the object surface. The tip is mounted on a flexible cantilever, allowing the tip to follow the surface profile. When the tip moves in proximity to the investigated object, forces of interaction between the tip and the surface influence the movement of the cantilever. Selective sensors detect these movements. Various interactions can be studied depending on the mechanics of the probe.

There are three common scanning probe techniques : atomic force microscopy (AFM) measures the interaction force between the tip and surface. The tip may be dragged across the surface, or may vibrate as it moves. The interaction force will depend on the nature of the sample, the probe tip and the distance between them. Scanning tunneling microscopy (STM) measures a weak electrical current flowing between tip and sample as they are held apart. Near-field scanning optical microscopy (NSOM) scans a very small light source very close to the sample. Detection of this light energy forms the image.

Key Points

- Scanned-probe microscopy has enabled researchers to create images of surfaces at the nanometer scale with a probe.
- The probe has an extremely sharp tip that interacts with the surface of the specimen.
- There are several variations of scanned-probe microscopy of which atomic force microscopy, scanning tunneling microscopy, and near-field scanning optical microscopy are most commonly used.

Key Terms

❖ **Micrometer :** An SI/MKS unit of measure, the length of one one-millionth of a meter. Symbols: µm, um, rm

Chapter 3 : Overview of prokaryotic and eukaryotic cells

1. Characteristics of prokaryotic cells

Key Points

- Prokaryotes lack an organized nucleus and other membrane-bound organelles.
- Prokaryotic DNA is found in a central part of the cell called the nucleoid.
- The cell wall of a prokaryote acts as an extra layer of protection, helps maintain cell shape, and prevents dehydration.
- Prokaryotic cell size ranges from 0.1 to 5.0 µm in diameter.
- The small size of prokaryotes allows quick entry and diffusion of ions and molecules to other parts of the cell while also allowing fast removal of waste products out of the cell.

Key Terms

❖ **Eukaryotic :** Having complex cells in which the genetic material is organized into membrane-bound nuclei.
❖ **Prokaryotic :** Of cells, lacking a nucleus.
❖ **Nucleoid :** the irregularly-shaped region within a prokaryote cell where the genetic material is localized.

Components of Prokaryotic Cells

All cells share four common components :

1. a plasma membrane : an outer covering that separates the cell's interior from its surrounding environment.

2. cytoplasm : a jelly-like cytosol within the cell in which other cellular components are found

3. DNA : the genetic material of the cell

4. ribosomes : where protein synthesis occurs

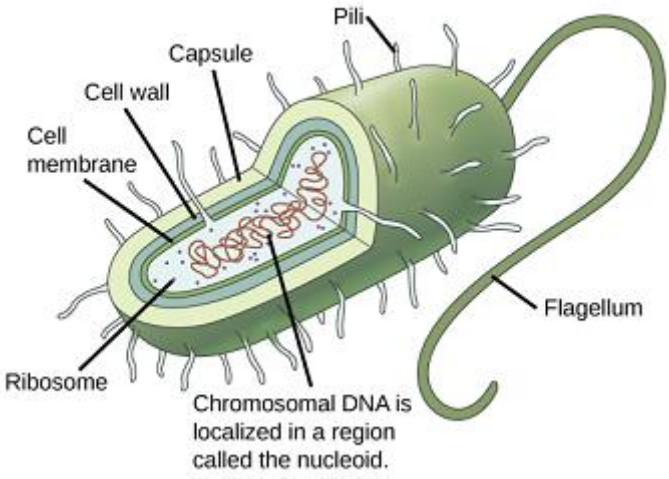


Figure 16 : General Structure of a prokaryotic cell : This figure shows the generalized structure of a prokaryotic cell.All prokaryotes have chromosomal DNA localized in a nucleoid, ribosomes, a cell membrane, and a cell wall.The other structures shown are present in some, but not all, bacteria.

However, prokaryotes differ from eukaryotic cells in several ways.

A prokaryote is a simple, single-celled (unicellular) organism that lacks an organized nucleus or any other membrane-bound organelle. We will shortly come to see that this is significantly different in eukaryotes. Prokaryotic DNA is found in a central part of the cell : the nucleoid.

Most prokaryotes have a peptidoglycan cell wall and many have a polysaccharide capsule. The cell wall acts as an extra layer of protection, helps the cell maintain its shape, and prevents dehydration. The capsule enables the cell to attach to surfaces in its environment. Some prokaryotes have flagella, pili, or fimbriae. Flagella are used for locomotion. Pili are used to exchange genetic material during a type of reproduction called conjugation. Fimbriae are used by bacteria to attach to a host cell **(Figure 16)**.

1.1. Cell Size

At 0.1 to 5.0 µm even 1 cm such as the case of *Thiomargarita magnifica* in diameter, prokaryotic cells are significantly smaller than eukaryotic cells, which have diameters ranging from 10 to 100 µm. The small size of prokaryotes allows ions and organic molecules that enter them to quickly diffuse to other parts of the cell. Similarly, any wastes produced within a prokaryotic cell can quickly diffuse out. This is not the case in eukaryotic cells, which have developed different structural adaptations to enhance intracellular transport **(Figure 17)**.

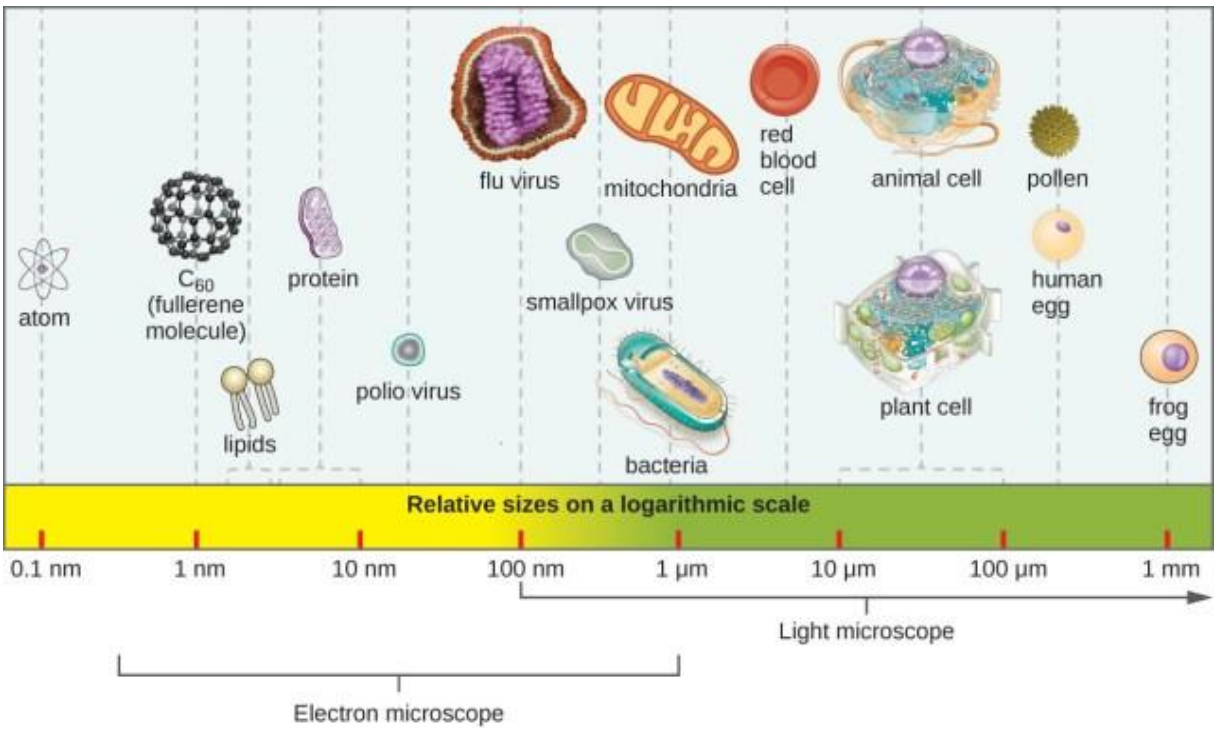


Figure 17 : Microbial size : This figure shows relative sizes of microbes on a logarithmic scale (recall that each unit of increase in a logarithmic scale represents a 10-fold increase in the quantity being measured).

Small size, in general, is necessary for all cells, whether prokaryotic or eukaryotic. Let's examine why that is so. First, we'll consider the area and volume of a typical cell. Not all cells are spherical in shape, but most tend to approximate a sphere. You may remember from your high school geometry course that the formula for the surface area of a sphere is $4\pi r$, while the formula for its volume is $4/3\pi r$. Thus, as the radius of a cell increases, its surface area increases as the square of its radius, but its volume increases as the cube of its radius (much more rapidly). Therefore, as a cell increases in size, its surface area-to-volume ratio decreases. This same principle would apply if the cell had the shape of a cube. If the cell grows too large, the plasma membrane will not have sufficient surface area to support the rate of diffusion

required for the increased volume. In other words, as a cell grows, it becomes less efficient. One way to become more efficient is to divide; another way is to develop organelles that perform specific tasks. These adaptations led to the development of more sophisticated cells called eukaryotic cells **(Figure 18)**.

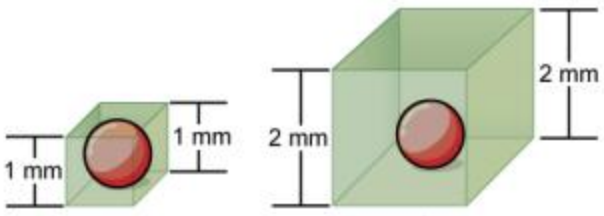


Figure 18 : Cell surface size : Notice that as a cell increases in size, its surface area-to-volume ratio decreases. When there is insufficient surface area to support a cell's increasing volume, a cell will either divide or die. The cell on the left has a volume of 1 mm^3 and a surface area of 6 mm^2, with a surface area-to-volume ratio of 6 to 1, whereas the cell on the right has a volume of 8 mm^3 and a surface area of 24 mm^2, with a surface area-to-volume ratio of 3 to 1.

1.2. Cell walls of prokaryotes

The cell wall of bacteria

Bacterial cells lack a membrane bound nucleus. Their genetic material is naked within the cytoplasm. Ribosomes are their only type of organelle. The term "nucleoid" refers to the region of the cytoplasm where chromosomal DNA is located, usually a singular, circular chromosome. Bacteria are usually single-celled, except when they exist in colonies. These ancestral cells reproduce by means of binary fission, duplicating their genetic material and then essentially splitting to form two daughter cells identical to the parent. A wall located outside the cell membrane provides the cell support, and protection against mechanical stress or damage from osmotic rupture and lysis.

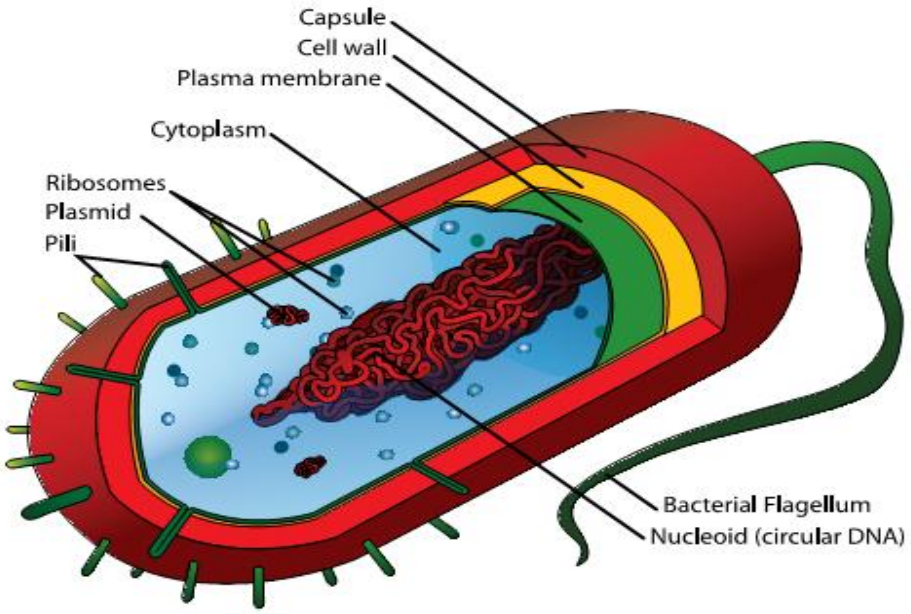


Figure 19 : Bacterial cell wall : The anatomy of bacterial cell structure.

The major component of the bacterial cell wall is peptidoglycan or murein. Peptidoglycan is a huge polymer of disaccharides (glycan) cross-linked by short chains of identical amino acids (peptides) monomers. The backbone of the peptidoglycan molecule is composed of two derivatives of glucose : N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) with a pentapeptide coming off NAM and varying slightly among bacteria. The NAG and NAM strands are synthesized in the cytosol of the bacteria. They are connected by inter-peptide bridges. They are transported across the cytoplasmic membrane by a carrier molecule called bactoprenol. From the peptidoglycan inwards all bacterial cells are very similar. Going further out, the bacterial world divides into two major classes : Gram positive ($Gram^+$) and Gram negative ($Gram^-$). The cell wall provides important ligands for adherence and receptor sites for viruses or antibiotics **(Figure 19)**.

Key Points

- A cell wall is a layer located outside the cell membrane found in plants, fungi, bacteria, algae, and archaea.
- A peptidoglycan cell wall composed of disaccharides and amino acids gives bacteria structural support.
- The bacterial cell wall is often a target for antibiotic treatment.

Key Terms

- **Binary fission :** The process whereby a cell divides asexually to produce two daughter cells.

1.3. Specialized external structures of prokaryotes

Endospores

An endospore is a dormant, tough, and non-reproductive structure produced by certain bacteria from the Firmicute phylum. Endospore formation is usually triggered by lack of nutrients, and usually occurs in Gram-positive bacteria. In endospore formation, the bacterium divides within its cell wall. One side then engulfs the other. Endospores enable bacteria to lie dormant for extended periods, even centuries. When the environment becomes more favorable, the endospore can reactivate itself to the vegetative state. Examples of bacteria that can form endospores include Bacillus and Clostridium. The endospore consists of the bacterium's DNA and part of its cytoplasm, surrounded by a very tough outer coating. Endospores can survive without nutrients. They are resistant to ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants. They are commonly found in soil and water, where they may survive for long periods of time. Bacteria produce a single endospore internally **(Figure 20)**.

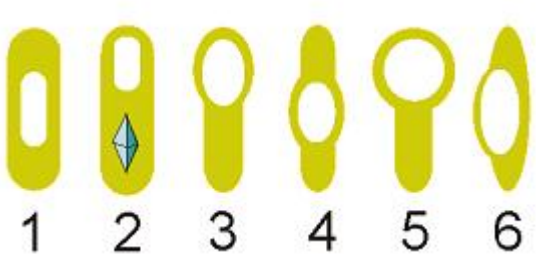


Figure 20 : Endospore morphology : Variations in endospore morphology: (1, 4) central endospore; (2, 3, 5) terminal endospore; (6) lateral endospore.

Viewing endospores under the light microscope can be difficult due to the impermeability of the endospore wall to dyes and stains. While the rest of a bacterial cell may stain, the endospore is left colorless. To combat this, a special stain technique called a Moeller stain is used. That allows the endospore to show up as red, while the rest of the cell stains blue. Another staining technique for endospores is the Schaeffer-Fulton stain, which stains endospores green and bacterial bodies red. There are variations in endospore morphology. Examples of bacteria having terminal endospores include *Clostridium tetani*, the pathogen

that causes the disease tetanus. Bacteria having a centrally placed endospore include *Bacillus* cereus, and those having a subterminal endospore include *Bacillus subtilis*. Sometimes the endospore can be so large that the cell can be distended around the endospore. This is typical of *Clostridium tetani* **(Figure 21)**.

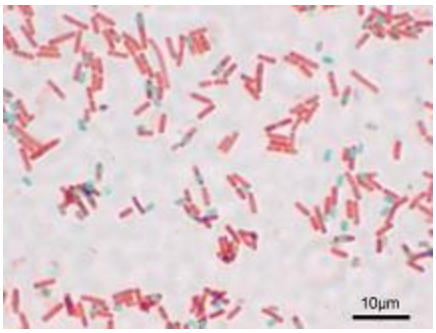


Figure 21 : *Bacillus subtilis* stained with the Schaeffer-Fulton stain.: A stained preparation of *Bacillus subtilis* showing endospores as green and the vegetative cell as red.

When a bacterium detects environmental conditions are becoming unfavorable it may start the process of endosporulation, which takes about eight hours. The DNA is replicated and a membrane wall known as a spore septum begins to form between it and the rest of the cell. The plasma membrane of the cell surrounds this wall and pinches off to leave a double membrane around the DNA, and the developing structure is now known as a forespore. Calcium dipicolinate is incorporated into the forespore during this time. Next the peptidoglycan cortex forms between the two layers and the bacterium adds a spore coat to the outside of the forespore. Sporulation is now complete, and the mature endospore will be released when the surrounding vegetative cell is degraded.

While resistant to extreme heat and radiation, endospores can be destroyed by burning or by autoclaving. Endospores are able to survive boiling at 100°C for hours, although the longer the number of hours the fewer that will survive. An indirect way to destroy them is to place them in an environment that reactivates them to their vegetative state. They will germinate within a day or two with the right environmental conditions, and then the vegetative cells can be straightforwardly destroyed. This indirect method is called Tyndallization. It was the usual method for a while in the late 19 century before the advent of inexpensive autoclaves. Prolonged exposure to ionising radiation, such as x-rays and gamma rays, will also kill most endospores.

Reactivation of the endospore occurs when conditions are more favourable and involves activation, germination, and outgrowth. Even if an endospore is located in plentiful nutrients,

it may fail to germinate unless activation has taken place. This may be triggered by heating the endospore. Germination involves the dormant endospore starting metabolic activity and thus breaking hibernation. It is commonly characterised by rupture or absorption of the spore coat, swelling of the endospore, an increase in metabolic activity, and loss of resistance to environmental stress.

As a simplified model for cellular differentiation, the molecular details of endospore formation have been extensively studied, specifically in the model organism *Bacillus subtilis*. These studies have contributed much to our understanding of the regulation of gene expression, transcription factors, and the sigma factor subunits of RNA polymerase.

Endospores of the bacterium *Bacillus anthracis* were used in the 2001 anthrax attacks. The powder found in contaminated postal letters was composed of extracellular anthrax endospores. Inhalation, ingestion or skin contamination of these endospores led to a number of deaths.

Geobacillus stearothermophilus endospores are used as biological indicators when an autoclave is used in sterilization procedures.

Bacillus subtilis spores are useful for the expression of recombinant proteins and in particular for the surface display of peptides and proteins as a tool for fundamental and applied research in the fields of microbiology, biotechnology and vaccination.

Key Points

- Examples of bacteria that can form endospores include *Bacillus* and *Clostridium*.
- Endospores can survive without nutrients. They are resistant to ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants.
- While resistant to extreme heat and radiation, endospores can be destroyed by burning or by autoclaving.

Key Terms

❖ **Endospore :** A dormant, tough, and non-reproductive structure produced by certain bacteria from the Firmicute phylum.

1.4. Specialized internal structures of prokaryotes

Ribosomes

Ribosomes are tiny spherical organelles that make proteins by joining amino acids together. Many ribosomes are found free in the cytosol, while others are attached to the rough endoplasmic reticulum. The purpose of the ribosome is to translate messenger RNA (mRNA) to proteins with the aid of tRNA. In eukaryotes, ribosomes can commonly be found in the cytosol of a cell, the endoplasmic reticulum or mRNA, as well as the matrix of the mitochondria. Proteins synthesized in each of these locations serve a different role in the cell. In prokaryotes, ribosomes can be found in the cytosol as well. This protein-synthesizing organelle is the only organelle found in both prokaryotes and eukaryotes, asserting the fact that the ribosome is a trait that evolved early on, most likely present in the common ancestor of eukaryotes and prokaryotes. Ribosomes are not membrane bound.

Ribosomes are composed of two subunits, one large and one small, that only bind together during protein synthesis. The purpose of the ribosome is to take the actual message and the charged aminoacyl-tRNA complex to generate the protein. To do so, they have three binding sites. One is for the mRNA; the other two are for the tRNA. The binding sites for tRNA are the A site, which holds the aminoacyl-tRNA complex, and the P site, which binds to the tRNA attached to the growing polypeptide chain **(Figure 22)**.

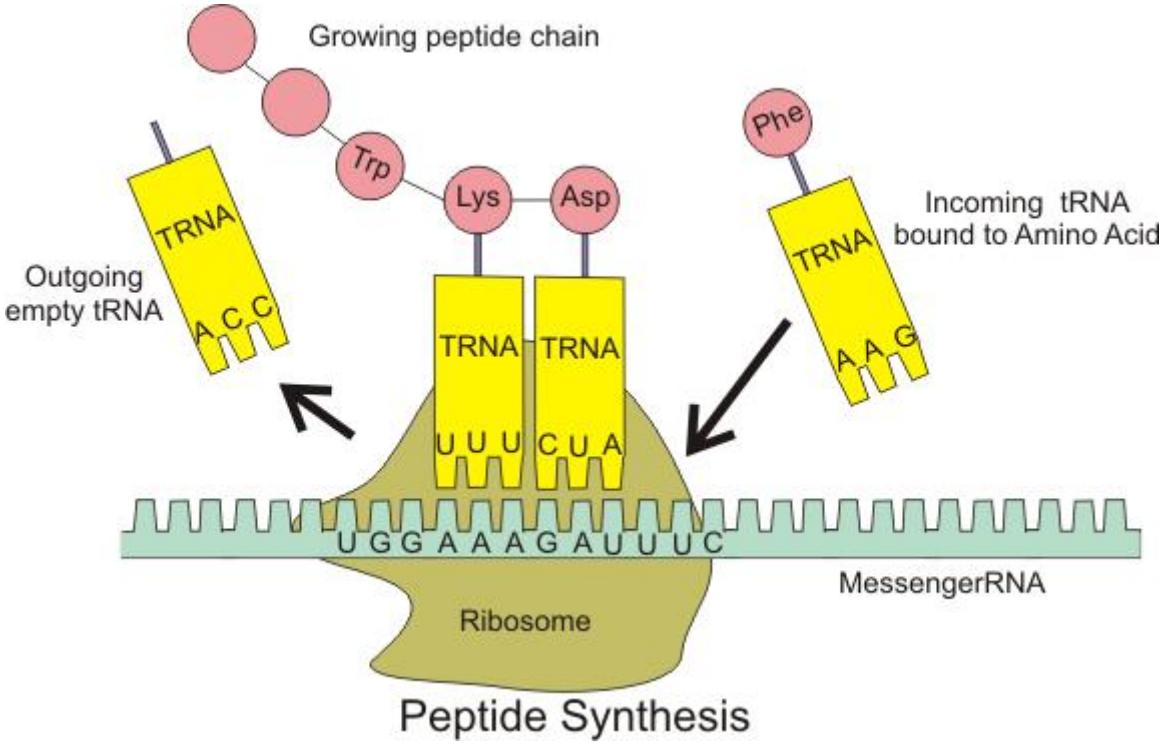


Figure 22 : Peptide synthesis by a ribosome.: The ribosome assembles amino acids into a protein. The specific amino acids are controlled by the mRNA sequence.

In most bacteria, the most numerous intracellular structure is the ribosome which is the site of protein synthesis in all living organisms. All prokaryotes have 70S (where S=Svedberg units) ribosomes while eukaryotes contain larger 80S ribosomes in their cytosol. The 70S ribosome is made up of a 50S and 30S subunits. The 50S subunit contains the 23S and 5S rRNA while the 30S subunit contains the 16S rRNA. These rRNA molecules differ in size in eukaryotes and are complexed with a large number of ribosomal proteins, the number and type of which can vary slightly between organisms. The ribosome is the most commonly observed intracellular multiprotein complex in bacteria.

Ribosome assembly consists of transcription, translation, the folding of rRNA and ribosomal proteins, the binding of ribosomal proteins, and the binding and release of the assembly components to make the ribosome. In vivo assembly of the 30S subunit has two intermediates (p130S and p230S) and the 50S subunit has three intermediates (p150S, p250S, and p350S). However, the reconstitution intermediates are not the same as in vitro. The intermediates of the 30S subunit yield 21S and 30S particles while the intermediates of the 50S subunit yield 32S, 43S, and 50S particles. The intermediates in the in vivo assembly are precursor rRNA which is different from in vitro which uses matured rRNA. To complete the mechanism of ribosome assembly, these precursor rRNA gets transformed in the polysomes.

Key Points

- All prokaryotes have 70S (where S=Svedberg units) ribosomes while eukaryotes contain larger 80S ribosomes in their cytosol.
- The 70S ribosome is made up of a 50S and 30S subunits.
- Ribosomes play a key role in the catalysis of two important and crucial biological processes. peptidyl transfer and peptidyl hydrolysis.
- Ribosomes are tiny spherical organelles that make proteins by joining amino acids together. Many ribosomes are found free in the cytosol, while others are attached to the rough endoplasmic reticulum.

Key Terms

- **Ribosome :** Small organelles found in all cells; involved in the production of proteins by translating messenger RNA.
- **Translation :** A process occurring in the ribosome, in which a strand of messenger RNA (mRNA) guides assembly of a sequence of amino acids to make a protein.

❖ **Svedberg :** The Svedberg unit (S) offers a measure of particle size based on its rate of travel in a tube subjected to high g-force.

Cell inclusions and storage granules

Key Points

- Sulfur granules are especially common in bacteria that use hydrogen sulfide as an electron source.
- When genes from one organism are expressed in another, the resulting protein sometimes forms inclusion bodies.
- Many bacteria store excess carbon in the form of polyhydroxyalkanoates or glycogen.

Key Terms

❖ Inclusion bodies: Inclusion bodies are nuclear or cytoplasmic aggregates of stainable substances, usually proteins.

Bacteria, despite their simplicity, contain a well-developed cell structure responsible for many unique biological properties not found among archaea or eukaryotes. Because of the simplicity of bacteria relative to larger organisms, and the ease with which they can be manipulated experimentally, the cell structure of bacteria has been well studied, revealing many biochemical principles that have been subsequently applied to other organisms. Most bacteria do not live in environments that contain large amounts of nutrients at all times. To accommodate these transient levels of nutrients, bacteria contain several different methods of nutrient storage that are employed in times of plenty, for use in times of want. For example, many bacteria store excess carbon in the form of polyhydroxyalkanoates or glycogen. Some microbes store soluble nutrients, such as nitrate in vacuoles. Sulfur is most often stored as elemental (S0) granules which can be deposited either intra- or extracellularly. Sulfur granules are especially common in bacteria that use hydrogen sulfide as an electron source. Most of the above mentioned examples can be viewed using a microscope, and are surrounded by a thin non-unit membrane to separate them from the cytoplasm.

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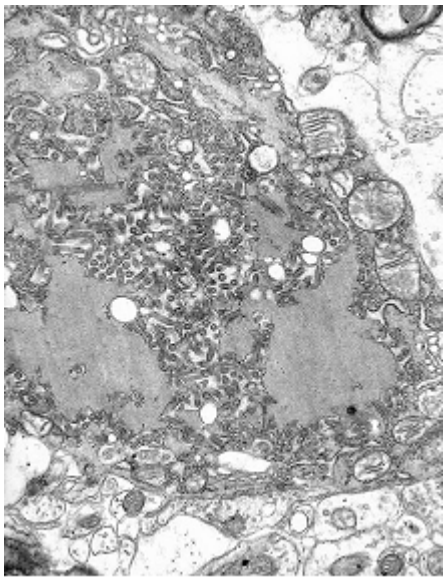


Figure 23 : Electron micrograph of the rabies virus.: This electron micrograph shows the rabies virus, as well as Negri bodies, or cellular inclusions.

When genes from one organism are expressed in another the resulting protein sometimes forms inclusion bodies. This is often true when large evolutionary distances are crossed; for example, a cDNA isolated from Eukarya and expressed as a recombinant gene in a prokaryote, risks the formation of the inactive aggregates of protein known as inclusion bodies. While the cDNA may properly code for a translatable mRNA, the protein that results will emerge in a foreign microenvironment. This often has fatal effects, especially if the intent of cloning is to produce a biologically active protein. For example, eukaryotic systems for carbohydrate modification and membrane transport are not found in prokaryotes.

The internal microenvironment of a prokaryotic cell (pH, osmolarity) may differ from that of the original source of the gene. Mechanisms for folding a protein may also be absent, and

hydrophobic residues that normally would remain buried may be exposed and available for interaction with similar exposed sites on other ectopic proteins. Processing systems for the cleavage and removal of internal peptides would also be absent in bacteria. The initial attempts to clone insulin in a bacterium suffered all of these deficits. In addition, the fine controls that may keep the concentration of a protein low will also be missing in a prokaryotic cell, and overexpression can result in filling a cell with ectopic protein that, even if it were properly folded, would precipitate by saturating its environment.

Carboxysomes

Carboxysomes are intracellular structures found in many autotrophic bacteria, including Cyanobacteria, Knallgasbacteria, Nitrosoand Nitrobacteria. They are proteinaceous structures resembling phage heads in their morphology; they contain the enzymes of carbon dioxide fixation in these organisms. It is thought that the high local concentration of the enzymes, along with the fast conversion of bicarbonate to carbon dioxide by carbonic anhydrase, allows faster and more efficient carbon dioxide fixation than is possible inside the cytoplasm. Similar structures are known to harbor the B -containing coenzyme glycerol dehydratase, the key enzyme of glycerol fermentation to 1,3-propanediol, in some Enterobacteriaceae, such as Salmonella.

Carboxysomes are bacterial microcompartments that contain enzymes involved in carbon fixation. Carboxysomes are made of polyhedral protein shells about 80 to 140 nanometres in diameter. These compartments are thought to concentrate carbon dioxide to overcome the inefficiency of RuBisCo (ribulose bisphosphate carboxylase/oxygenase) – the predominant enzyme in carbon fixation and the rate limiting enzyme in the Calvin cycle. These organelles are found in all cyanobacteria and many chemotrophic bacteria that fix carbon dioxide.

Carboxysomes are an example of a wider group of protein micro-compartments that have dissimilar functions but similar structures, based on homology of the two shell protein families. Using electron microscopy the first carboxysomes were seen in 1956, in the cyanobacterium Phormidium uncinatum. In the early 1960s, similar polyhedral objects were observed in other cyanobacteria. These structures were named polyhedral bodies in 1961; over the next few years they were also discovered in some chemotrophic bacteria that fixed carbon dioxide. Among these are *Halothiobacillus*, *Acidithiobacillus*, *Nitrobacter* and *Nitrococcus* **(Figure 24)**.

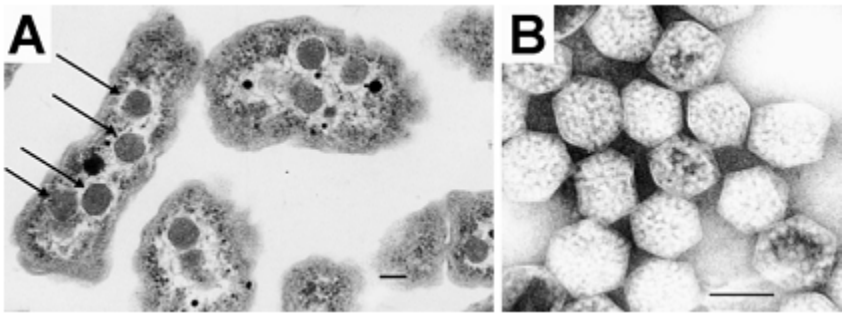


Figure 24 : Electron micrograph of a carboxysome : (A) A thin-section electron micrograph of H. neapolitanus cells with carboxysomes inside. In one of the cells shown, arrows highlight the visible carboxysomes. (B) A negatively stained image of intact carboxysomes isolated from *H. neapolitanus*.

Key Points

- Carboxysomes are proteinaceous structures resembling phage heads in their morphology and contain the enzymes of carbon dioxide fixation in these organisms.
- Carboxysomes are made of polyhedral protein shells about 80 to 140 nanometres in diameter.
- These organelles are found in all cyanobacteria and many chemotrophic bacteria that fix carbon dioxide.

Key Terms

❖ **Carboxysome :** A bacterial organelle that contains enzymes involved in carbon fixation.

Magnetosomes

Magnetosomes are intracellular organelles found in magnetotactic bacteria that allow them to sense and align themselves along a magnetic field (magnetotaxis). They contain 15 to 20 magnetite crystals that together act like a compass needle to orient magnetotactic bacteria in geomagnetic fields, thereby simplifying their search for their preferred microaerophilic environments. Each magnetite crystal within a magnetosome is surrounded by a lipid bilayer. Specific soluble and transmembrane proteins are sorted to the membrane. Recent research has shown that magnetosomes are invaginations of the inner membrane and not freestanding vesicles. Magnetite-bearing magnetosomes have also been found in eukaryotic magnetotactic algae, with each cell containing several thousand crystals **(Figure 25)**.

Magnetotactic bacteria usually mineralize either iron oxide magnetosomes, which contain crystals of magnetite (Fe_3O_4), or iron sulfide magnetosomes, which contain crystals of greigite (Fe_3O_4). Several other iron sulfide minerals have also been identified in iron sulfide

magnetosomes — including mackinawite (tetragonal Fe_3S_4) and a cubic Fe_3S_4 — which are thought to be precursors of Fe_3S_4.

Figure 25 : Magnetospirilli with black magnetosome chains faintly visible : There is a broad range of shapes and groups of magnetic bacteria. However, cultivation of these organisms in the laboratory is often difficult. Only a few strains of magnetotactic bacteria have been isolated in pure culture, a tiny minority of the vast diversity of naturally occurring populations from largely unexplored natural habitats such as the marine environment.

Key Points

- Magnetosomes contain 15 to 20 magnetite crystals that together act like a compass needle to orient magnetotactic bacteria in geomagnetic fields, thereby simplifying their search for their preferred microaerophilic environments.
- The particle morphology of magnetosome crystals varies, but is consistent within cells of a single magnetotactic bacterial species or strain.
- Each magnetite crystal within a magnetosome is surrounded by a lipid bilayer. Specific soluble and transmembrane proteins are sorted to the membrane.

Key Terms

- **Magnetotaxis :** The supposed ability to sense a magnetic field and coordinate movement in response, later discovered to be natural magnetism: such creatures orient themselves magnetically even after death.
- **Magnetosome :** A membranous prokaryotic organelle, containing mineral crystals, present in magnetotactic bacteria.

Gas vesicles

Gas vesicles are spindle-shaped structures found in some planktonic bacteria that provides buoyancy to these cells by decreasing their overall cell density. Positive buoyancy is needed to keep the cells in the upper reaches of the water column, so that they can continue to perform photosynthesis. They are made up of a shell of protein that has a highly hydrophobic inner surface, making it impermeable to water (and stopping water vapor from condensing

inside), but permeable to most gases. Because the gas vesicle is a hollow cylinder, it is liable to collapse when the surrounding pressure becomes too great **(Figure 26)**.

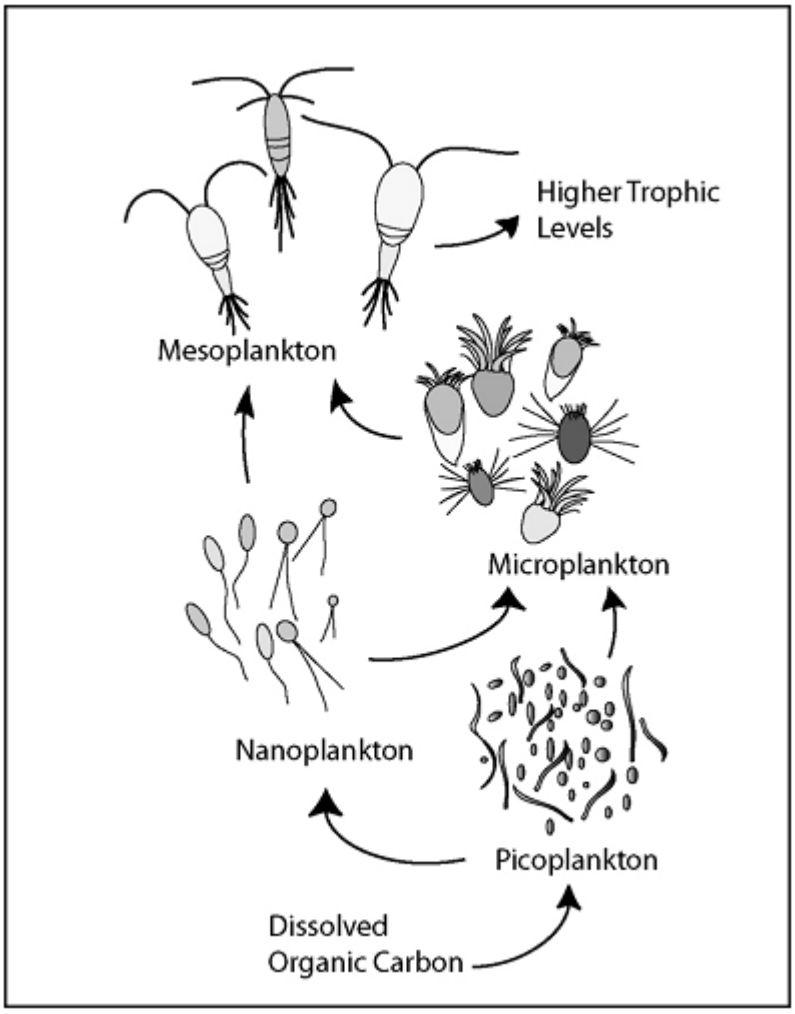


Figure 26 : Illustration of a microbial loop : Gas vesicles provide bouyancy for some planktonic bacteria by decreasing their overall cell density.

Natural selection has fine-tuned the structure of the gas vesicle to maximize its resistance to buckling by including an external strengthening protein, GvpC, rather like the green thread in a braided hosepipe. There is a simple relationship between the diameter of the gas vesicle and pressure at which it will collapse – the wider the gas vesicle the weaker it becomes. However, wider gas vesicles are more efficient. They provide more buoyancy per unit of protein than narrow gas vesicles. Different species produce gas vesicles of different diameters, allowing them to colonize different depths of the water column (fast growing, highly competitive species with wide gas vesicles in the top most layers; slow growing, dark-adapted, species with strong narrow gas vesicles in the deeper layers). The diameter of the gas vesicle will also help determine which species survive in different bodies of water. Deep lakes that experience

winter mixing will expose the cells to the hydrostatic pressure generated by the full water column. This will select for species with narrower, stronger gas vesicles.

Key Points

- They are made up of a shell of protein that has a highly hydrophobic inner surface, making it impermeable to water (and stopping water vapour from condensing inside), but permeable to most gases.
- Natural selection has fine tuned the structure of the gas vesicle to maximize its resistance to buckling, including an external strengthening protein, GvpC, rather like the green thread in a braided hosepipe.
- The diameter of the gas vesicle will also help determine which species survive in different bodies of water.

Key Terms

- **Gas vesicle :** Gas vesicles are spindle-shaped structures found in some planktonic bacteria that provide buoyancy to these cells by decreasing their overall cell density.
- **Gas gangrene :** a bacterial infection that produces gas in tissues in necrotizing or rotting tissues.

2. Internal structures of eukaryotic cells

Unlike prokaryotic cells, eukaryotic cells have: 1) a membrane-bound nucleus; 2) numerous membrane-bound organelles such as the endoplasmic reticulum, Golgi apparatus, chloroplasts, mitochondria, and others; and 3) several, rod-shaped chromosomes. Because a eukaryotic cell's nucleus is surrounded by a membrane, it is often said to have a "true nucleus." The word "organelle" means "little organ," and, as already mentioned, organelles have specialized cellular functions, just as the organs of your body have specialized functions.

At this point, it should be clear to you that eukaryotic cells have a more complex structure than prokaryotic cells. Organelles allow different functions to be compartmentalized in different areas of the cell. Before turning to organelles, let's first examine two important components of the cell: the plasma membrane and the cytoplasm **(Figure 27)**.

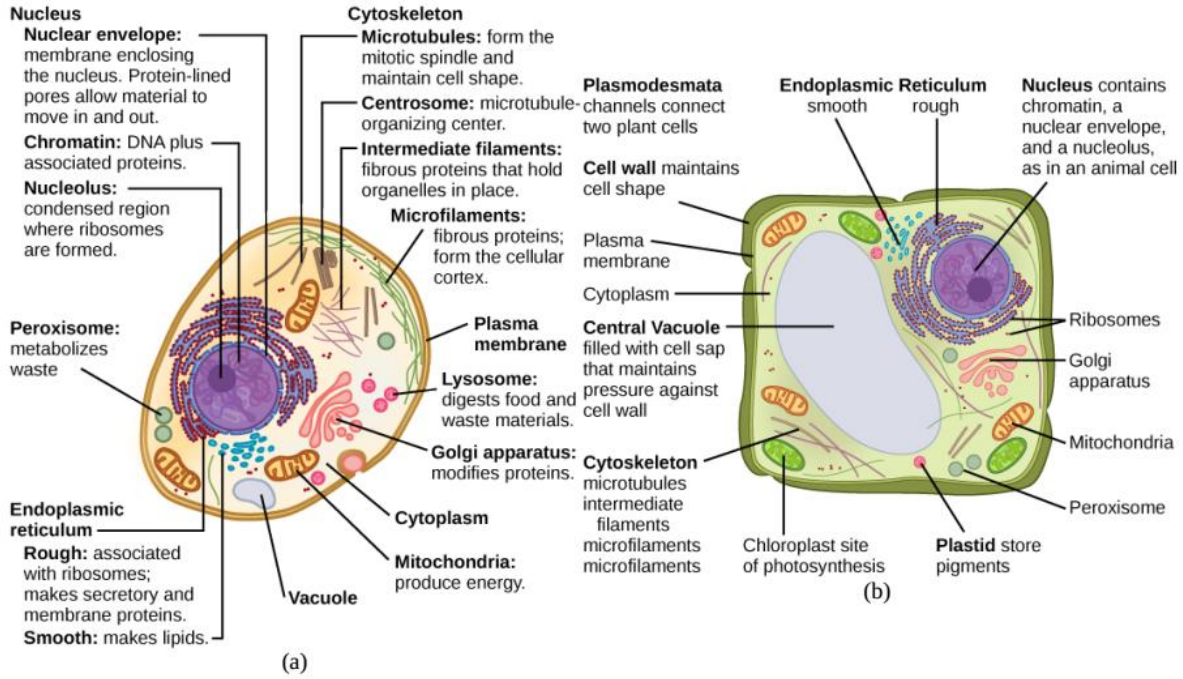


Figure 27 : These figures show the major organelles and other cell components of (a) a typical animal cell and (b) a typical eukaryotic plant cell. The plant cell has a cell wall, chloroplasts, plastids, and a central vacuole—structures not found in animal cells. Plant cells do not have lysosomes or centrosomes.

2.1. The Plasma Membrane

Like prokaryotes, eukaryotic cells have a plasma membrane **(Figure 28)**, a phospholipid bilayer with embedded proteins that separates the internal contents of the cell from its surrounding environment. A phospholipid is a lipid molecule with two fatty acid chains and a phosphate-containing group. The plasma membrane controls the passage of organic molecules, ions, water, and oxygen into and out of the cell. Wastes (such as carbon dioxide and ammonia) also leave the cell by passing through the plasma membrane.

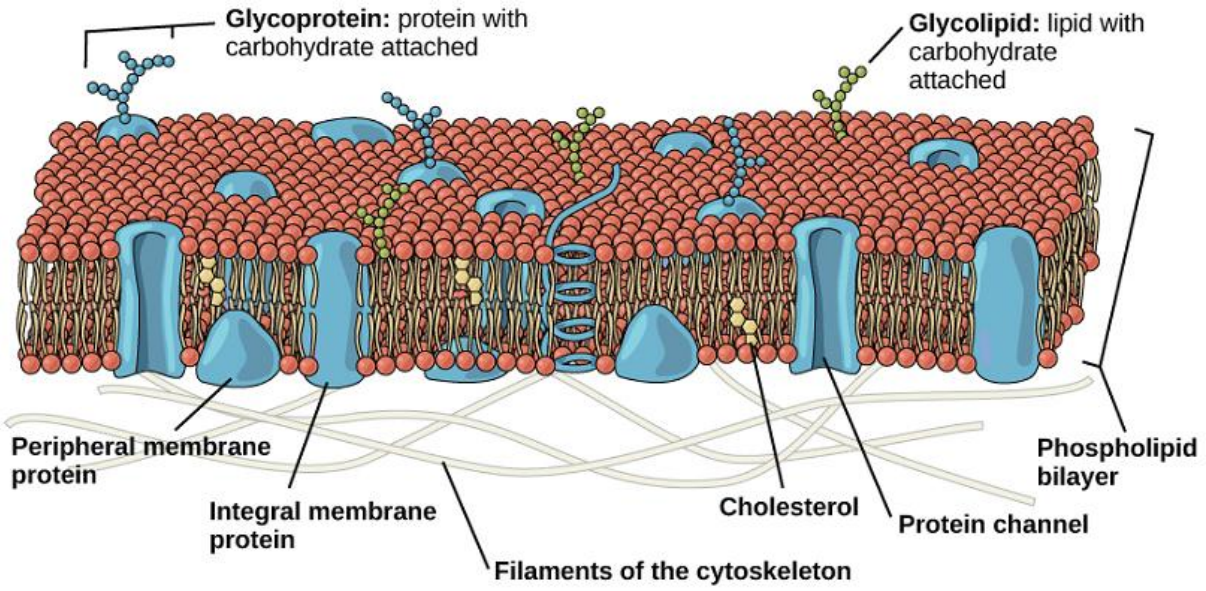


Figure 28 : The eukaryotic plasma membrane is a phospholipid bilayer with proteins and cholesterol embedded in it

2.2. The Cytoplasm

The cytoplasm is the entire region of a cell between the plasma membrane and the nuclear envelope (a structure to be discussed shortly). It is made up of organelles suspended in the gel-like cytosol, the cytoskeleton, and various chemicals. Even though the cytoplasm consists of 70 to 80 percent water, it has a semi-solid consistency, which comes from the proteins within it. However, proteins are not the only organic molecules found in the cytoplasm. Glucose and other simple sugars, polysaccharides, amino acids, nucleic acids, fatty acids, and derivatives of glycerol are found there, too. Ions of sodium, potassium, calcium, and many other elements are also dissolved in the cytoplasm. Many metabolic reactions, including protein synthesis, take place in the cytoplasm.

2.3. The Nucleus

Typically, the nucleus is the most prominent organelle in a cell **(Figure 29)**. The nucleus (plural = nuclei) houses the cell's DNA and directs the synthesis of ribosomes and proteins.

One of the main differences between prokaryotic and eukaryotic cells is the nucleus. As previously discussed, prokaryotic cells lack an organized nucleus while eukaryotic cells contain membrane-bound nuclei (and organelles) that house the cell's DNA and direct the synthesis of ribosomes and proteins.

The nucleus stores chromatin (DNA plus proteins) in a gel-like substance called the nucleoplasm. To understand chromatin, it is helpful to first consider chromosomes. Chromatin describes the material that makes up chromosomes, which are structures within the nucleus that are made up of DNA, the hereditary material. You may remember that in prokaryotes, DNA is organized into a single circular chromosome. In eukaryotes, chromosomes are linear structures. Every eukaryotic species has a specific number of chromosomes in the nuclei of its body's cells. For example, in humans, the chromosome number is 46, while in fruit flies, it is eight. Chromosomes are only visible and distinguishable from one another when the cell is getting ready to divide. In order to organize the large amount of DNA within the nucleus, proteins called histones are attached to chromosomes; the DNA is wrapped around these histones to form a structure resembling beads on a string. These protein-chromosome complexes are called chromatin.

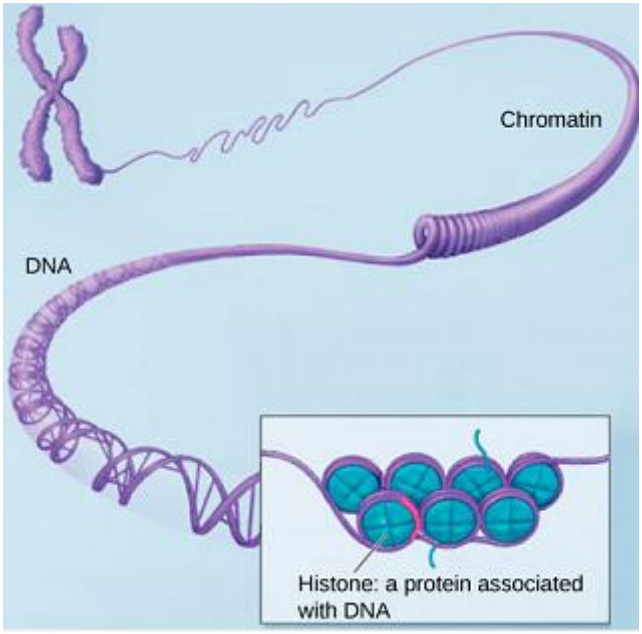


Figure 29 : DNA is highly organized : This image shows various levels of the organization of chromatin (DNA and protein). Along the chromatin threads, unwound protein-chromosome complexes, we find DNA wrapped around a set of histone proteins.

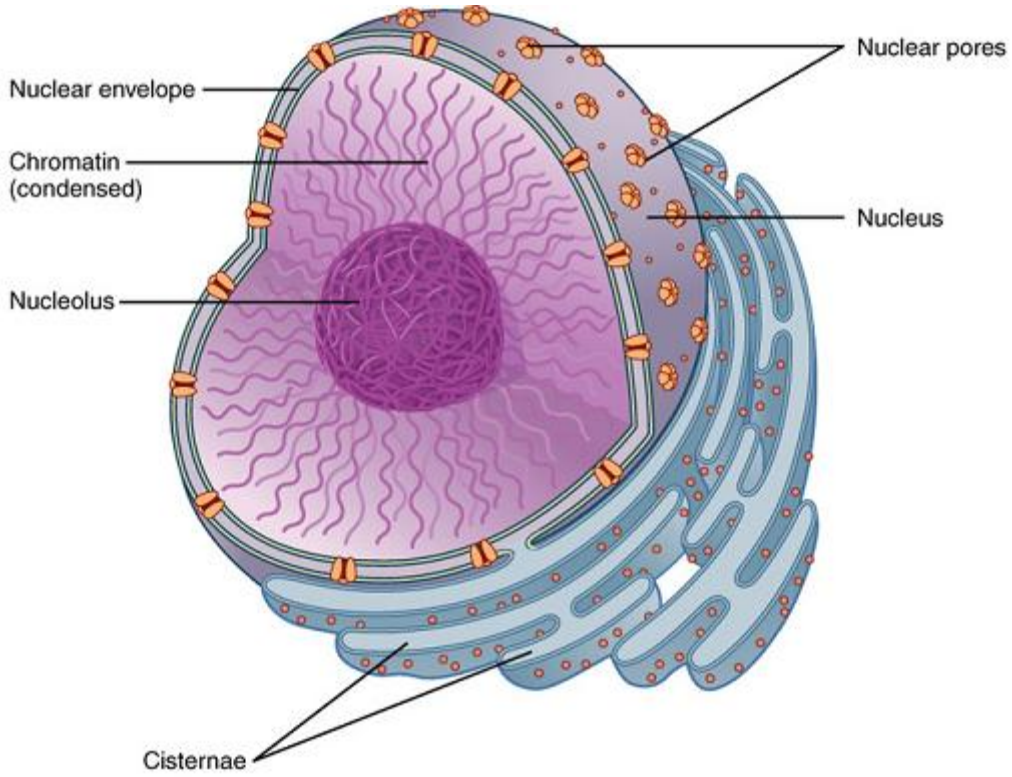


Figure 30 : The nucleus stores the hereditary material of the cell : The nucleus is the control center of the cell. The nucleus of living cells contains the genetic material that determines the entire structure and function of that cell.

The nucleoplasm is also where we find the nucleolus. The nucleolus is a condensed region of chromatin where ribosome synthesis occurs. Ribosomes, large complexes of protein and ribonucleic acid (RNA), are the cellular organelles responsible for protein synthesis. They receive their "orders" for protein synthesis from the nucleus where the DNA is transcribed into messenger RNA (mRNA). This mRNA travels to the ribosomes, which translate the code provided by the sequence of the nitrogenous bases in the mRNA into a specific order of amino acids in a protein **(Figure 30)**.

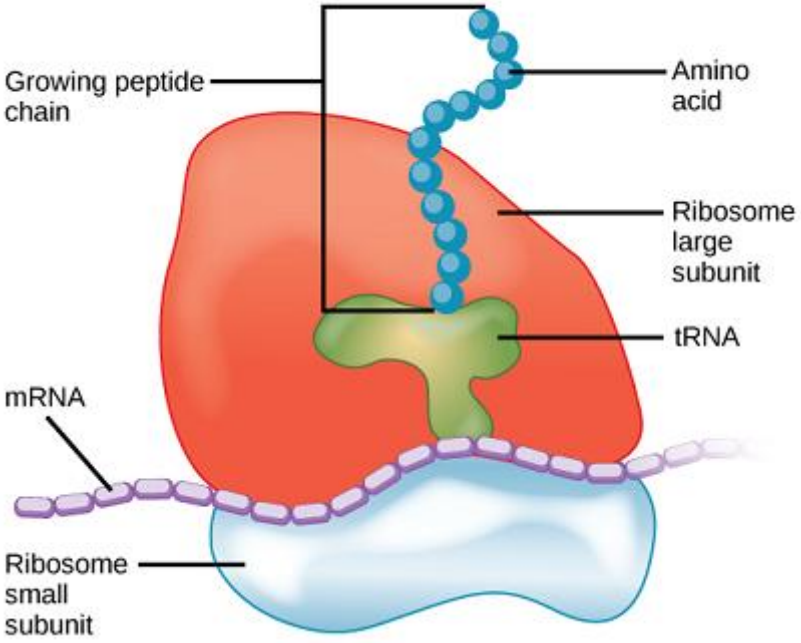


Figure 31 : Ribosomes are responsible for protein synthesis : Ribosomes are made up of a large subunit (top) and a small subunit (bottom). During protein synthesis, ribosomes assemble amino acids into proteins.

Lastly, the boundary of the nucleus is called the nuclear envelope. It consists of two phospholipid bilayers : an outer membrane and an inner membrane. The nuclear membrane is continuous with the endoplasmic reticulum, while nuclear pores allow substances to enter and exit the nucleus **(Figure 31)**.

2.4. Mitochondria

One of the major features distinguishing prokaryotes from eukaryotes is the presence of mitochondria. Mitochondria are doublemembraned organelles that contain their own ribosomes and DNA. Each membrane is a phospholipid bilayer embedded with proteins. Eukaryotic cells may contain anywhere from one to several thousand mitochondria, depending on the cell's level of energy consumption. Each mitochondrion measures 1 to 10 micrometers (or greater) in length and exists in the cell as an organelle that can be ovoid to worm-shaped to intricately branched.

2.4.1. Mitochondria Structure

Most mitochondria are surrounded by two membranes, which would result when one membrane-bound organism was engulfed into a vacuole by another membrane-bound organism. The mitochondrial inner membrane is extensive and involves substantial infoldings called cristae that resemble the textured, outer surface of alpha-proteobacteria. The matrix and inner membrane are rich with the enzymes necessary for aerobic respiration.

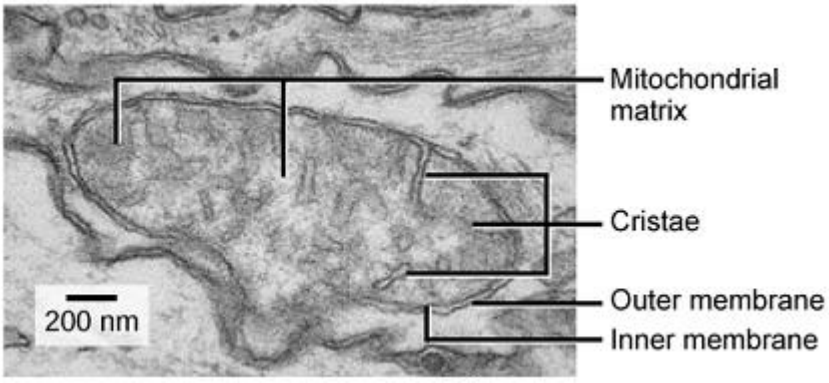


Figure 32 : Mitochondrial structure : This electron micrograph shows a mitochondrion as viewed with a transmission electron microscope.

Mitochondria have their own (usually) circular DNA chromosome that is stabilized by attachments to the inner membrane and carries genes similar to genes expressed by alpha-proteobacteria. Mitochondria also have special ribosomes and transfer RNAs that resemble these components in prokaryotes. These features all support the hypothesis that mitochondria were once free-living prokaryotes **(Figure 32)**.

2.4.2. Mitochondria Function

Mitochondria are often called the “powerhouses” or “energy factories” of a cell because they are responsible for making adenosine triphosphate (ATP), the cell’s main energy-carrying molecule. ATP represents the short-term stored energy of the cell. Cellular respiration is the process of making ATP using the chemical energy found in glucose and other nutrients. In mitochondria, this process uses oxygen and produces carbon dioxide as a waste product. In fact, the carbon dioxide that you exhale with every breath comes from the cellular reactions that produce carbon dioxide as a by-product.

It is important to point out that muscle cells have a very high concentration of mitochondria that produce ATP. Your muscle cells need a lot of energy to keep your body moving. When your cells don’t get enough oxygen, they do not make a lot of ATP. Instead, the small amount

of ATP they make in the absence of oxygen is accompanied by the production of lactic acid. In addition to the aerobic generation of ATP, mitochondria have several other metabolic functions. One of these functions is to generate clusters of iron and sulfur that are important cofactors of many enzymes. Such functions are often associated with the reduced mitochondrion derived organelles of anaerobic eukaryotes.

2.4.3. Origins of Mitochondria

There are two hypotheses about the origin of mitochondria: *endosymbiotic* and *autogenous*, but the most accredited theory at present is endosymbiosis. The endosymbiotic hypothesis suggests mitochondria were originally prokaryotic cells, capable of implementing oxidative mechanisms. These prokaryotic cells may have been engulfed by a eukaryote and became endosymbionts living inside the eukaryote.

Key Points

- Mitochondria contain their own ribosomes and DNA; combined with their double membrane, these features suggest that they might have once been free-living prokaryotes that were engulfed by a larger cell.
- Mitochondria have an important role in cellular respiration through the production of ATP, using chemical energy found in glucose and other nutrients.
- Mitochondria are also responsible for generating clusters of iron and sulfur, which are important cofactors of many enzymes.

Key Terms

- **Alpha-proteobacteria :** A taxonomic class within the phylum Proteobacteria — the phototropic proteobacteria.
- **Adenosine triphosphate :** a multifunctional nucleoside triphosphate used in cells as a coenzyme, often called the "molecular unit of energy currency" in intracellular energy transfer
- **Cofactor :** an inorganic molecule that is necessary for an enzyme to function.

2.5. Comparing plant and animal cells

Key Points

- Centrosomes and lysosomes are found in animal cells, but do not exist within plant cells.

- The lysosomes are the animal cell's "garbage disposal", while in plant cells the same function takes place in vacuoles.
- Plant cells have a cell wall, chloroplasts and other specialized plastids, and a large central vacuole, which are not found within animal cells.
- The cell wall is a rigid covering that protects the cell, provides structural support, and gives shape to the cell.
- The chloroplasts, found in plant cells, contain a green pigment called chlorophyll, which captures the light energy that drives the reactions of plant photosynthesis.
- The central vacuole plays a key role in regulating a plant cell's concentration of water in changing environmental conditions.

Key Terms

- **Protest :** Any of the eukaryotic unicellular organisms including protozoans, slime molds and some algae; historically grouped into the kingdom Protoctista.
- **Autotroph :** Any organism that can synthesize its food from inorganic substances, using heat or light as a source of energy
- **Heterotrophy :** an organism that requires an external supply of energy in the form of food, as it cannot synthesize its own.

2.6. Animal cells versus plant cells

Each eukaryotic cell has a plasma membrane, cytoplasm, a nucleus, ribosomes, mitochondria, peroxisomes, and in some, vacuoles; however, there are some striking differences between animal and plant cells. While both animal and plant cells have microtubule organizing centers (MTOCs), animal cells also have centrioles associated with the MTOC: a complex called the centrosome. Animal cells each have a centrosome and lysosomes, whereas plant cells do not. Plant cells have a cell wall, chloroplasts and other specialized plastids, and a large central vacuole, whereas animal cells do not.

2.7. The Centrosome

The centrosome is a microtubule-organizing center found near the nuclei of animal cells. It contains a pair of centrioles, two structures that lie perpendicular to each other. Each centriole is a cylinder of nine triplets of microtubules. The centrosome (the organelle where all microtubules originate) replicates itself before a cell divides, and the centrioles appear to have

some role in pulling the duplicated chromosomes to opposite ends of the dividing cell. However, the exact function of the centrioles in cell division isn't clear, because cells that have had the centrosome removed can still divide; and plant cells, which lack centrosomes, are capable of cell division **(Figure 33)**.

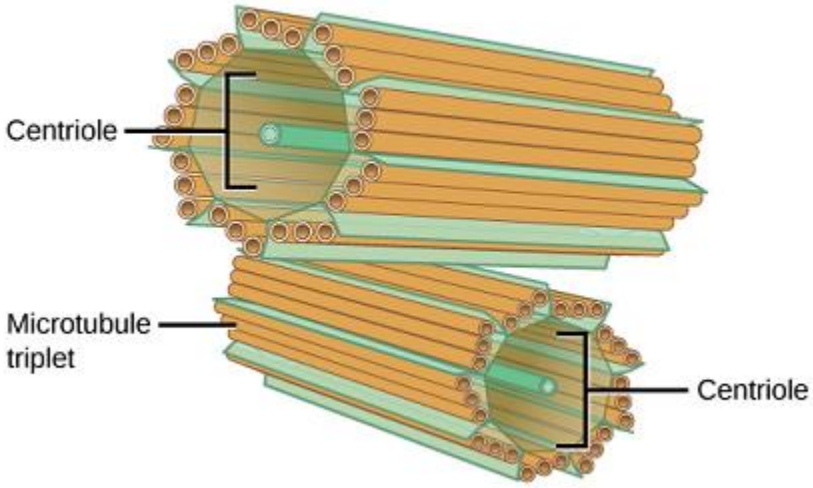


Figure 33 : The centrosome structure : The centrosome consists of two centrioles that lie at right angles to each other. Each centriole is a cylinder made up of nine triplets of microtubules. Nontubulin proteins (indicated by the green lines) hold the microtubule triplets together

2.8. Lysosomes

Animal cells have another set of organelles not found in plant cells: lysosomes. The lysosomes are the cell's "garbage disposal." In plant cells, the digestive processes take place in vacuoles. Enzymes within the lysosomes aid the breakdown of proteins, polysaccharides, lipids, nucleic acids, and even worn-out organelles. These enzymes are active at a much lower pH than that of the cytoplasm. Therefore, the pH within lysosomes is more acidic than the pH of the cytoplasm. Many reactions that take place in the cytoplasm could not occur at a low pH, so the advantage of compartmentalizing the eukaryotic cell into organelles is apparent.

2.9. The cell wall

The cell wall is a rigid covering that protects the cell, provides structural support, and gives shape to the cell. Fungal and protistan cells also have cell walls. While the chief component of prokaryotic cell walls is peptidoglycan, the major organic molecule in the plant cell wall is cellulose, a polysaccharide comprised of glucose units. When you bite into a raw vegetable, like celery, it crunches. That's because you are tearing the rigid cell walls of the celery cells with your teeth.

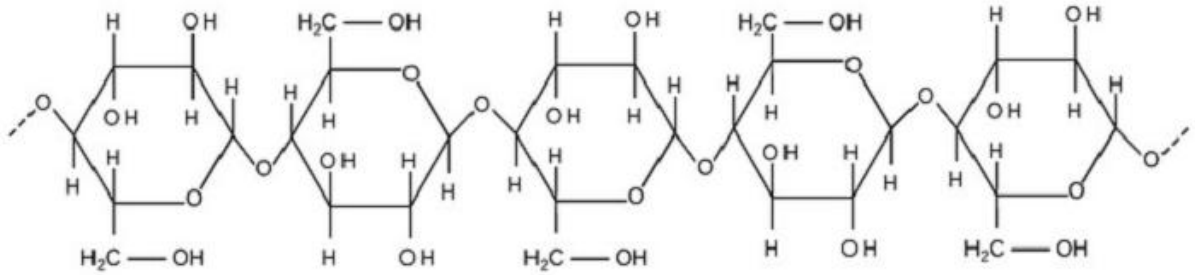


Figure 34 : Cellulose : Cellulose is a long chain of β-glucose molecules connected by a 1-4 linkage. The dashed lines at each end of the figure indicate a series of many more glucose units. The size of the page makes it impossible to portray an entire cellulose molecule.

2.10. Chloroplasts

Like mitochondria, chloroplasts have their own DNA and ribosomes, but chloroplasts have an entirely different function. Chloroplasts are plant cell organelles that carry out photosynthesis. Photosynthesis is the series of reactions that use carbon dioxide, water, and light energy to make glucose and oxygen. This is a major difference between plants and animals; plants (autotrophs) are able to make their own food, like sugars, while animals (heterotrophs) must ingest their food **(Figure 35)**.

Like mitochondria, chloroplasts have outer and inner membranes, but within the space enclosed by a chloroplast's inner membrane is a set of interconnected and stacked fluid-filled membrane sacs called thylakoids. Each stack of thylakoids is called a granum (plural = grana). The fluid enclosed by the inner membrane that surrounds the grana is called the stroma.

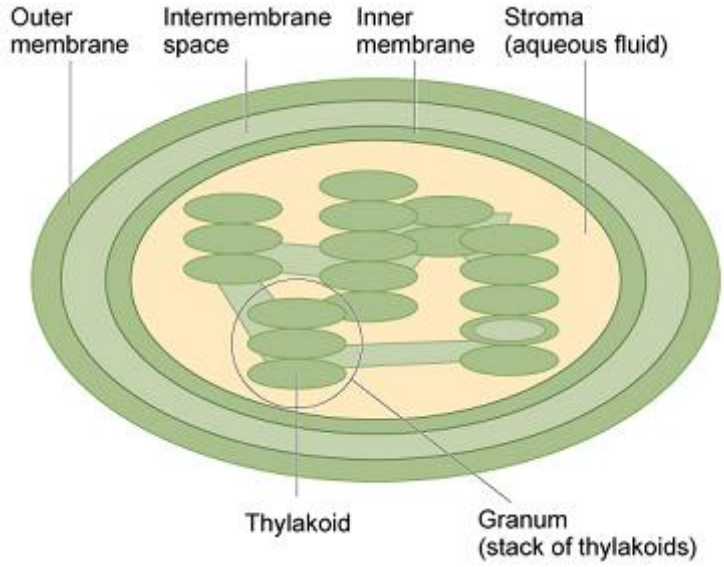


Figure 35 : The chloroplast structure : The chloroplast has an outer membrane, an inner membrane, and membrane structures called thylakoids that are stacked into grana.

The chloroplasts contain a green pigment called chlorophyll, which captures the light energy that drives the reactions of photosynthesis. Like plant cells, photosynthetic protists also have chloroplasts. Some bacteria perform photosynthesis, but their chlorophyll is not relegated to an organelle.

2.11. The central vacuole

The central vacuole plays a key role in regulating the cell's concentration of water in changing environmental conditions. When you forget to water a plant for a few days, it wilts. That's because as the water concentration in the soil becomes lower than the water concentration in the plant, water moves out of the central vacuoles and cytoplasm. As the central vacuole shrinks, it leaves the cell wall unsupported. This loss of support to the cell walls of plant cells results in the wilted appearance of the plant. The central vacuole also supports the expansion of the cell. When the central vacuole holds more water, the cell gets larger without having to invest a lot of energy in synthesizing new cytoplasm.

2.12. The endoplasmic reticulum

The endomembrane system (endo = "within") is a group of membranes and organelles **(Figure 36)** in eukaryotic cells that works together to modify, package, and transport lipids and proteins. It includes the nuclear envelope, lysosomes, and vesicles, which we've already mentioned, and the endoplasmic reticulum and Golgi apparatus, which we will cover shortly. Although not technically within the cell, the plasma membrane is included in the endomembrane system because, as you will see, it interacts with the other endomembranous organelles. The endomembrane system does not include the membranes of either mitochondria or chloroplasts.

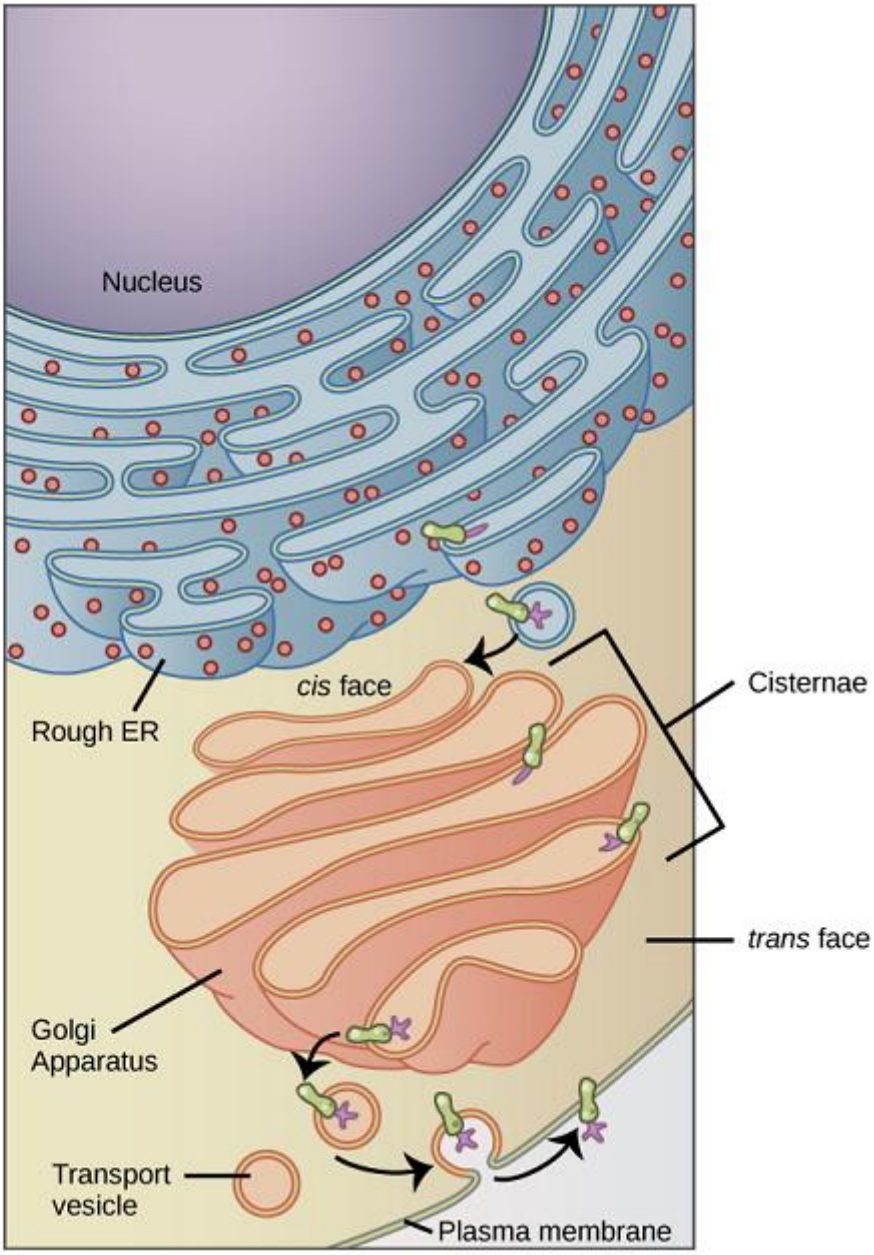


Figure 36 : "Membrane and secretory proteins are synthesized in the rough endoplasmic reticulum (RER). The RER also sometimes modifies proteins. In this illustration, a (green) integral membrane protein in the ER is modified by attachment of a (purple) carbohydrate. Vesicles with the integral protein bud from the ER and fuse with the cis face of the Golgi apparatus. As the protein passes along the Golgi's cisternae, it is further modified by the addition of more carbohydrates. After its synthesis is complete, it exits as integral membrane protein of the vesicle that bud from the Golgi's trans face and when the vesicle fuses with the cell membrane the protein becomes integral portion of that cell membrane.

Key Points

- If the endoplasmic reticulum (ER) has ribosomes attached to it, it is called rough ER; if it does not, then it is called smooth ER.

- The proteins made by the rough endoplasmic reticulum are for use outside of the cell.
- Functions of the smooth endoplasmic reticulum include synthesis of carbohydrates, lipids, and steroid hormones; detoxification of medications and poisons; and storage of calcium ions.

Key Terms

❖ **Lumen :** The cavity or channel within a tube or tubular organ.

❖ **Reticulum :** A network

The endoplasmic reticulum (ER) is a series of interconnected membranous sacs and tubules that collectively modifies proteins and synthesizes lipids. However, these two functions are performed in separate areas of the ER: the rough ER and the smooth ER. The hollow portion of the ER tubules is called the lumen or cisternal space. The membrane of the ER, which is a phospholipid bilayer embedded with proteins, is continuous with the nuclear envelope.

2.12.1. Rough ER

The rough endoplasmic reticulum (RER) is so named because the ribosomes attached to its cytoplasmic surface give it a studded appearance when viewed through an electron microscope. Ribosomes transfer their newly synthesized proteins into the lumen of the RER where they undergo structural modifications, such as folding or the acquisition of side chains. These modified proteins will be incorporated into cellular membranes—the membrane of the ER or those of other organelles —or secreted from the cell (such as protein hormones, enzymes). The RER also makes phospholipids for cellular membranes. If the phospholipids or modified proteins are not destined to stay in the RER, they will reach their destinations via transport vesicles that bud from the RER's membrane. Since the RER is engaged in modifying proteins (such as enzymes, for example) that will be secreted from the cell, the RER is abundant in cells that secrete proteins. This is the case with cells of the liver, for example **(Figure 37)**.

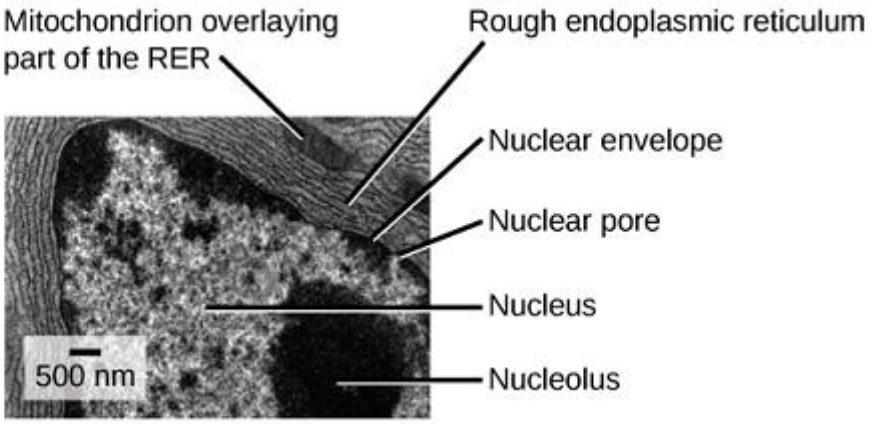


Figure 37 : Rough endoplasmic reticulum : This transmission electron micrograph shows the rough endoplasmic reticulum and other organelles in a pancreatic cell.

2.12.2. Smooth ER

The smooth endoplasmic reticulum (SER) is continuous with the RER but has few or no ribosomes on its cytoplasmic surface. Functions of the SER include synthesis of carbohydrates, lipids, and steroid hormones; detoxification of medications and poisons; and storage of calcium ions. In muscle cells, a specialized SER called the sarcoplasmic reticulum is responsible for storage of the calcium ions that are needed to trigger the coordinated contractions of the muscle cells.

2.13. The Golgi apparatus

We have already mentioned that vesicles can bud from the ER and transport their contents elsewhere, but where do the vesicles go?. Before reaching their final destination, the lipids or proteins within the transport vesicles still need to be sorted, packaged, and tagged so that they wind up in the right place. Sorting, tagging, packaging, and distribution of lipids and proteins takes place in the Golgi apparatus (also called the Golgi body), a series of flattened membranes.

The receiving side of the Golgi apparatus is called the cis face. The opposite side is called the trans face. The transport vesicles that formed from the ER travel to the cis face, fuse with it, and empty their contents into the lumen of the Golgi apparatus. As the proteins and lipids travel through the Golgi, they undergo further modifications that allow them to be sorted. The most frequent modification is the addition of short chains of sugar molecules. These newly-modified proteins and lipids are then tagged with phosphate groups or other small molecules so that they can be routed to their proper destinations **(Figure 38)**.

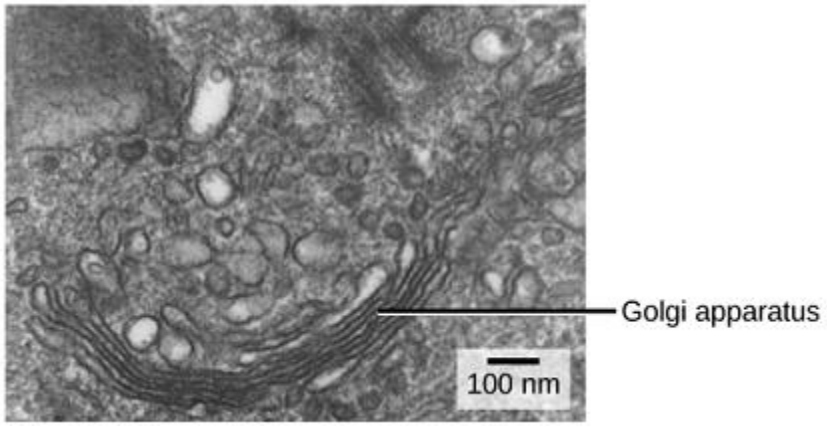


Figure 38 : The Golgi apparatus sorts and packages cellular products : The Golgi apparatus in this white blood cell is visible as a stack of semicircular, flattened rings in the lower portion of the image. Several vesicles can be seen near the Golgi apparatus.

Finally, the modified and tagged proteins are packaged into secretory vesicles that bud from the trans face of the Golgi. While some of these vesicles deposit their contents into other parts of the cell where they will be used, other secretory vesicles fuse with the plasma membrane and release their contents outside the cell.

In another example of form following function, cells that engage in a great deal of secretory activity (such as cells of the salivary glands that secrete digestive enzymes or cells of the immune system that secrete antibodies) have an abundance of Golgi. In plant cells, the Golgi apparatus has the additional role of synthesizing polysaccharides, some of which are incorporated into the cell wall and some of which are used in other parts of the cell.

Key Points

- The Golgi apparatus is a series of flattened sacs that sort and package cellular materials.
- The Golgi apparatus has a cis face on the ER side and a trans face opposite of the ER.
- The trans face secretes the materials into vesicles, which then fuse with the cell membrane for release from the cell.

Key Terms

❖ **Vesicle :** A membrane-bound compartment found in a cell.

2.14. Peroxisomes

A type of organelle found in both animal cells and plant cells, a peroxisome is a membrane-bound cellular organelle that contains mostly enzymes. Peroxisomes perform important functions, including lipid metabolism and chemical detoxification. They also carry out oxidation reactions that break down fatty acids and amino acids **(Figure 39)**.

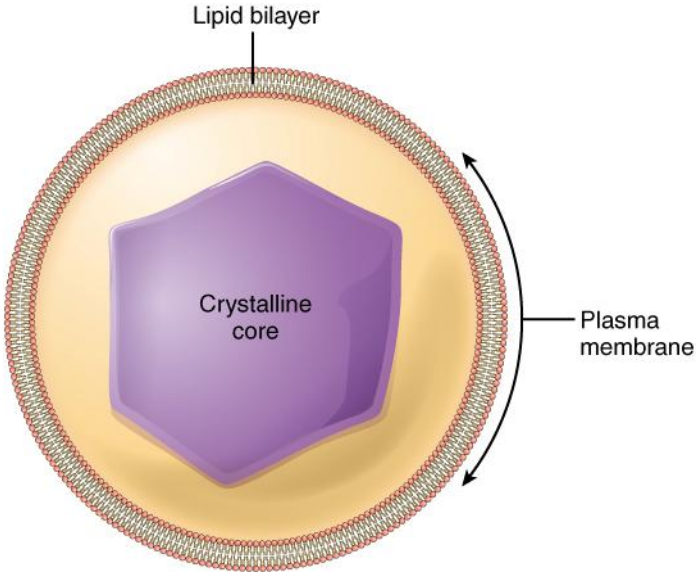


Figure 39 : Peroxisomes : Peroxisomes are membrane-bound organelles that contain an abundance of enzymes for detoxifying harmful substances and lipid metabolism.

In contrast to the digestive enzymes found in lysosomes, the enzymes within peroxisomes serve to transfer hydrogen atoms from various molecules to oxygen, producing hydrogen peroxide (H_2O_2). In this way, peroxisomes neutralize poisons, such as alcohol, that enter the body. In order to appreciate the importance of peroxisomes, it is necessary to understand the concept of reactive oxygen species.

Reactive oxygen species (ROS), such as peroxides and free radicals, are the highly-reactive products of many normal cellular processes, including the mitochondrial reactions that produce ATP and oxygen metabolism. Examples of ROS include the hydroxyl radical OH, H_2O_2, and superoxide (O_{2^-}). Some ROS are important for certain cellular functions, such as cell signaling processes and immune responses against foreign substances. Many ROS, however, are harmful to the body. Free radicals are reactive because they contain free unpaired electrons; they can easily oxidize other molecules throughout the cell, causing cellular damage and even cell death. Free radicals are thought to play a role in many destructive processes in the body, from cancer to coronary artery disease.

Peroxisomes oversee reactions that neutralize free radicals. They produce large amounts of the toxic H_2O_2 in the process, but contain enzymes that convert H_2O_2 into water and oxygen. These by-products are then safely released into the cytoplasm. Like miniature sewage treatment plants, peroxisomes neutralize harmful toxins so that they do not cause damage in the cells. The liver is the organ primarily responsible for detoxifying the blood before it travels throughout the body; liver cells contain an exceptionally high number of peroxisomes.

Key Points

- Lipid metabolism and chemical detoxification are important functions of peroxisomes.
- Peroxisomes are responsible for oxidation reactions that break down fatty acids and amino acids.
- Peroxisomes oversee reactions that neutralize free radicals, which cause cellular damage and cell death.
- Peroxisomes chemically neutralize poisons through a process that produces large amounts of toxic HO, which is then converted into water and oxygen.
- The liver is the organ primarily responsible for detoxifying the blood before it travels throughout the body; as a result, liver cells contain large amounts of peroxisomes.

Key Terms

- **Enzyme :** a globular protein that catalyses a biological chemical reaction.
- **Free radical :** Any molecule, ion or atom that has one or more unpaired electrons; they are generally highly reactive and often only occur as transient species.

2.15. Lysosomes

A lysosome has three main functions: the breakdown/digestion of macromolecules (carbohydrates, lipids, proteins, and nucleic acids), cell membrane repairs, and responses against foreign substances such as bacteria, viruses and other antigens. When food is eaten or absorbed by the cell, the lysosome releases its enzymes to break down complex molecules including sugars and proteins into usable energy needed by the cell to survive. If no food is provided, the lysosome's enzymes digest other organelles within the cell in order to obtain the necessary nutrients.

In addition to their role as the digestive component and organelle-recycling facility of animal cells, lysosomes are considered to be parts of the endomembrane system. Lysosomes also use

their hydrolytic enzymes to destroy pathogens (disease-causing organisms) that might enter the cell. A good example of this occurs in a group of white blood cells called macrophages, which are part of your body's immune system. In a process known as phagocytosis or endocytosis, a section of the plasma membrane of the macrophage invaginates (folds in) and engulfs a pathogen. The invaginated section, with the pathogen inside, then pinches itself off from the plasma membrane and becomes a vesicle. The vesicle fuses with a lysosome. The lysosome's hydrolytic enzymes then destroy the pathogen **(Figure 40)**.

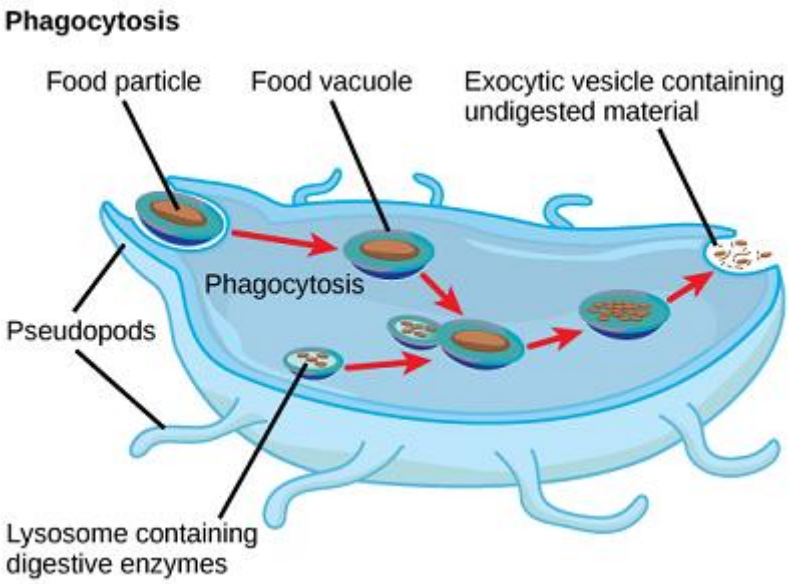


Figure 40 : Lysosomes digest foreign substances that might harm the cell : A macrophage has engulfed (phagocytized) a potentially pathogenic bacterium and then fuses with a lysosomes within the cell to destroy the pathogen. Other organelles are present in the cell but for simplicity are not shown.

A lysosome is composed of lipids, which make up the membrane, and proteins, which make up the enzymes within the membrane. Usually, lysosomes are between 0.1 to 1.2µm, but the size varies based on the cell type. The general structure of a lysosome consists of a collection of enzymes surrounded by a single-layer membrane. The membrane is a crucial aspect of its structure because without it the enzymes within the lysosome that are used to breakdown foreign substances would leak out and digest the entire cell, causing it to die.

Lysosomes are found in nearly every animal-like eukaryotic cell. They are so common in animal cells because, when animal cells take in or absorb food, they need the enzymes found in lysosomes in order to digest and use the food for energy. On the other hand, lysosomes are not commonly-found in plant cells. Lysosomes are not needed in plant cells because they have

cell walls that are tough enough to keep the large/foreign substances that lysosomes would usually **digest out of the cell.**

Key Points

- Lysosomes breakdown/digest macromolecules (carbohydrates, lipids, proteins, and nucleic acids), repair cell membranes, and respond against foreign substances such as bacteria, viruses and other antigens.
- Lysosomes contain enzymes that break down the macromolecules and foreign invaders.
- Lysosomes are composed of lipids and proteins, with a single membrane covering the internal enzymes to prevent the lysosome from digesting the cell itself.

Key Terms

- **Enzyme :** a globular protein that catalyses a biological chemical reaction.
- **Lysosome :** An organelle found in all types of animal cells which contains a large range of digestive enzymes capable of splitting most biological macromolecules.

2.16. The cytoskeleton

If you were to remove all the organelles from a cell, would the plasma membrane and the cytoplasm be the only components left? No. Within the cytoplasm, there would still be ions and organic molecules, plus a network of protein fibers that help maintain the shape of the cell, secure some organelles in specific positions, allow cytoplasm and vesicles to move within the cell, and enable cells within multicellular organisms to move. Collectively, this network of protein fibers is known as the cytoskeleton. There are three types of fibers within the cytoskeleton: microfilaments, intermediate filaments, and microtubules **(Figure 41)**. Here, we will examine each.

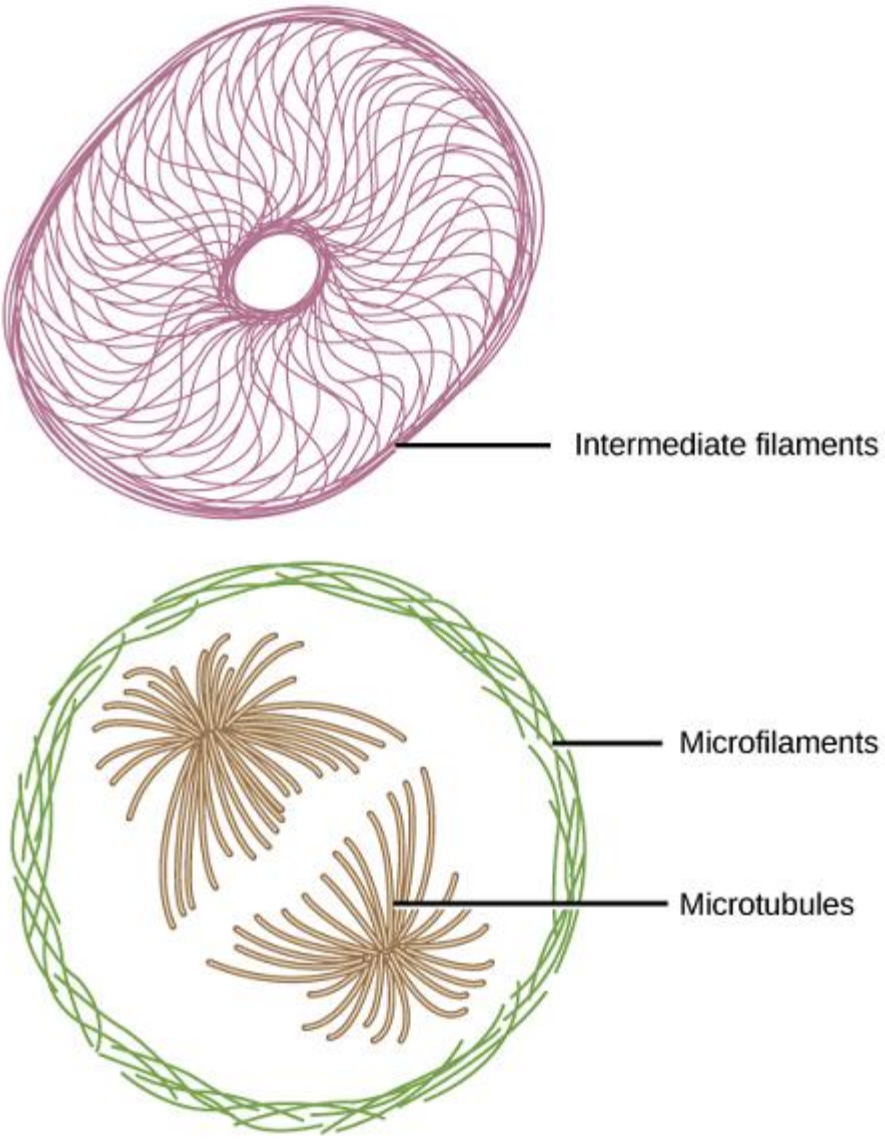


Figure 41 : Microfilaments thicken the cortex around the inner edge of a cell; like rubber bands, they resist tension. Microtubules are found in the interior of the cell where they maintain cell shape by resisting compressive forces. Intermediate filaments are found throughout the cell and hold organelles in place.

2.16.1. Microfilaments

Of the three types of protein fibers in the cytoskeleton, microfilaments are the narrowest. They function in cellular movement, have a diameter of about 7 nm, and are made of two intertwined strands of a globular protein called actin **(Figure 42)**. For this reason, microfilaments are also known as actin filaments.

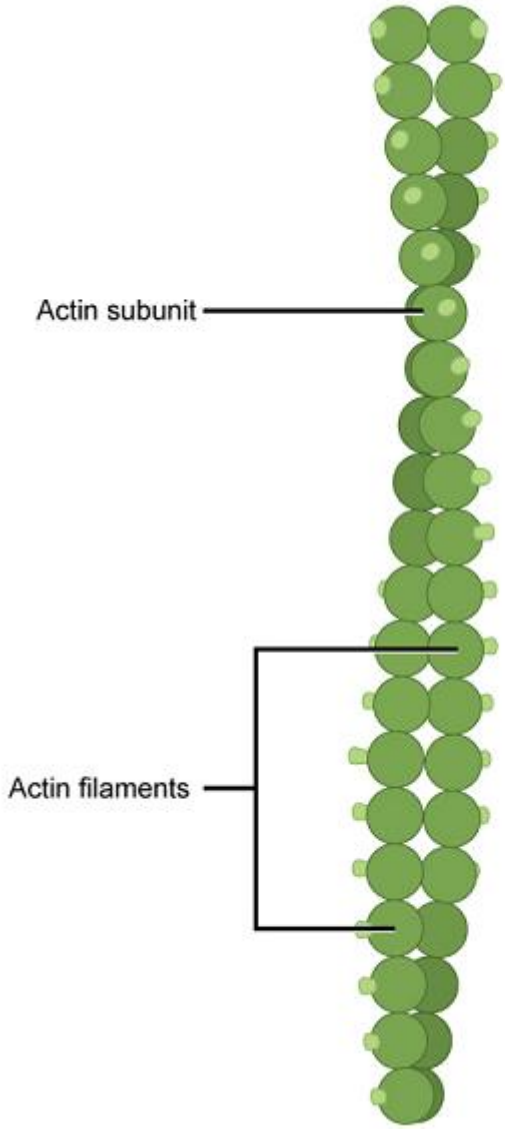


Figure 42 : Microfilaments are made of two intertwined strands of actin.

Actin is powered by ATP to assemble its filamentous form, which serves as a track for the movement of a motor protein called myosin. This enables actin to engage in cellular events requiring motion, such as cell division in animal cells and cytoplasmic streaming, which is the circular movement of the cell cytoplasm in plant cells. Actin and myosin are plentiful in muscle cells. When your actin and myosin filaments slide past each other, your muscles contract.

Microfilaments also provide some rigidity and shape to the cell. They can depolymerize (disassemble) and reform quickly, thus enabling a cell to change its shape and move. White blood cells (your body's infection-fighting cells) make good use of this ability. They can move to the site of an infection and phagocytize the pathogen.

2.16.2. Microtubules

Microtubules are part of the cell's cytoskeleton, helping the cell resist compression, move vesicles, and separate chromosomes at mitosis.

Key Points

- Microtubules help the cell resist compression, provide a track along which vesicles can move throughout the cell, and are the components of cilia and flagella.
- Cilia and flagella are hair-like structures that assist with locomotion in some cells, as well as line various structures to trap particles. The structures of cilia and flagella are a "9+2 array," meaning that a ring of nine microtubules is surrounded by two more microtubules. Microtubules attach to replicated chromosomes during cell division and pull them apart to opposite ends of the pole, allowing the cell to divide with a complete set of chromosomes in each daughter cell.

Key Terms

- **Microtubule :** Small tubes made of protein and found in cells; part of the cytoskeleton
- **Flagellum :** a flagellum is a lash-like appendage that protrudes from the cell body of certain prokaryotic and eukaryotic cells
- **Cytoskeleton :** A cellular structure like a skeleton, contained within the cytoplasm.

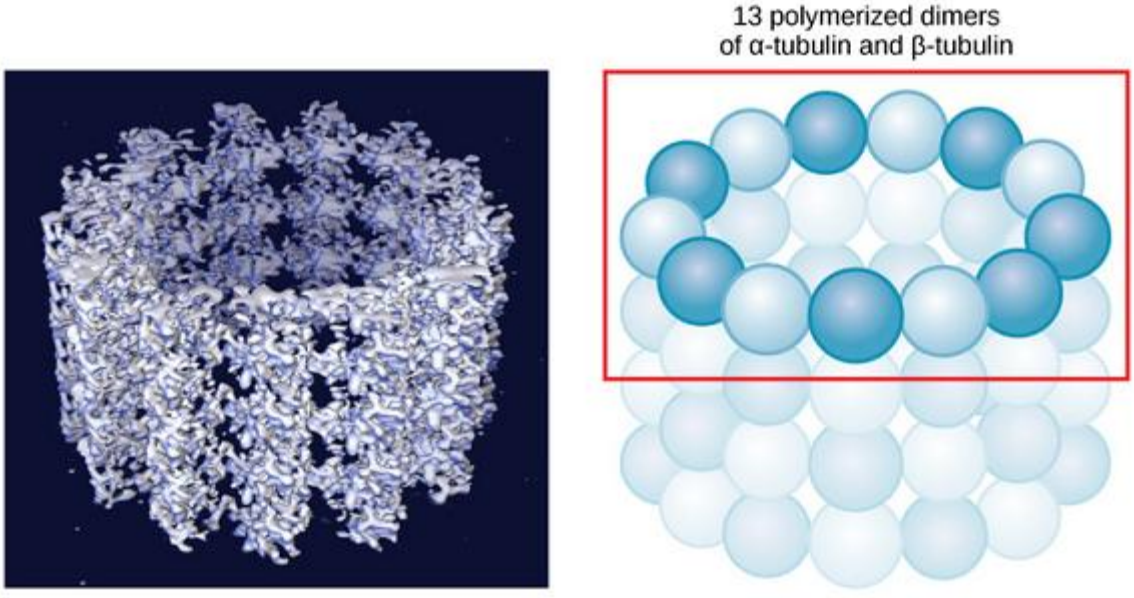


Figure 43 : Microtubule structure : Microtubules are hollow, with walls consisting of 13 polymerized dimers of α-tubulin and β-tubulin (right image). The left image shows the molecular structure of the tube.

As their name implies, microtubules are small hollow tubes. Microtubules, along with microfilaments and intermediate filaments, come under the class of organelles known as the cytoskeleton. The cytoskeleton is the framework of the cell which forms the structural supporting component. Microtubules are the largest element of the cytoskeleton. The walls of the microtubule are made of polymerized dimers of α-tubulin and β-tubulin, two globular proteins. With a diameter of about 25 nm, microtubules are the widest components of the cytoskeleton. They help the cell resist compression, provide a track along which vesicles move through the cell, and pull replicated chromosomes to opposite ends of a dividing cell. Like microfilaments, microtubules can dissolve and reform quickly.

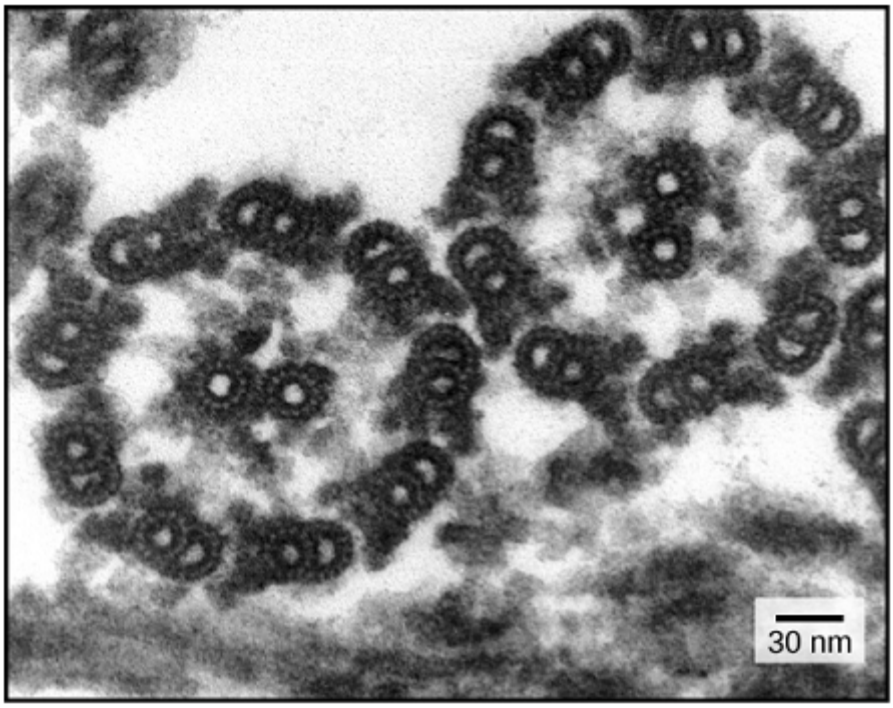


Figure 44 : Microtubules are the structural component of flagella : This transmission electron micrograph of two flagella shows the 9 + 2 array of microtubules: nine microtubule doublets surround a single microtubule doublet.

Microtubules are also the structural elements of flagella, cilia, and centrioles (the latter are the two perpendicular bodies of the centrosome). In animal cells, the centrosome is the microtubule-organizing center. In eukaryotic cells, flagella and cilia are quite different structurally from their counterparts in prokaryotes.

2.16.3. Intermediate Filaments

Intermediate filaments (IFs) are cytoskeletal components found in animal cells. They are composed of a family of related proteins sharing common structural and sequence features.

Intermediate filaments have an average diameter of 10 nanometers, which is between that of 7 nm actin (microfilaments), and that of 25 nm microtubules, although they were initially designated 'intermediate' because their average diameter is between those of narrower microfilaments (actin) and wider myosin filaments found in muscle cells. Intermediate filaments contribute to cellular structural elements and are often crucial in holding together tissues like skin.

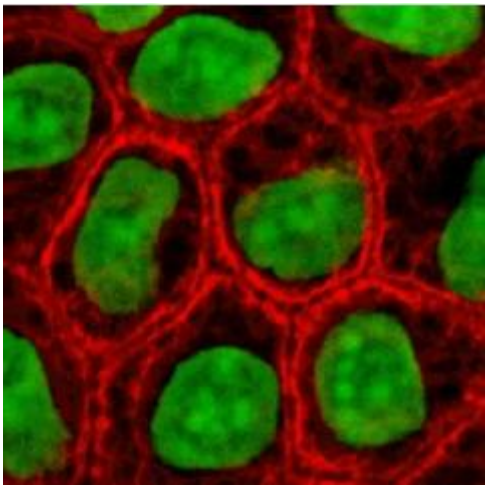


Figure 45 : Stained keratin intermediate filaments : Keratin cytoskeletal intermediate filaments are concentrated around the edge of the cells and merge into the surface membrane. This network of intermediate filaments from cell to cell holds together tissues like skin.

Flagella and Cilia

Flagella (singular = flagellum) are long, hair-like structures that extend from the plasma membrane and are used to move an entire cell (for example, sperm, Euglena). When present, the cell has just one flagellum or a few flagella. When cilia (singular = cilium) are present, however, many of them extend along the entire surface of the plasma membrane. They are short, hair-like structures that are used to move entire cells (such as paramecia) or substances along the outer surface of the cell (for example, the cilia of cells lining the Fallopian tubes that move the ovum toward the uterus, or cilia lining the cells of the respiratory tract that trap particulate matter and move it toward your nostrils).

Despite their differences in length and number, flagella and cilia share a common structural arrangement of microtubules called a "9 + 2 array." This is an appropriate name because a single flagellum or cilium is made of a ring of nine microtubule doublets surrounding a single microtubule doublet in the center.

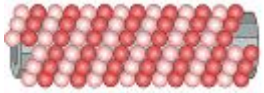
2.16.4. The plasma membrane and cellular signaling

Among the most sophisticated functions of the plasma membrane is its ability to transmit signals via complex proteins. These proteins can be receptors, which work as receivers of extracellular inputs and as activators of intracellular processes, or markers, which allow cells to recognize each other.

Membrane receptors provide extracellular attachment sites for effectors like hormones and growth factors, which then trigger intracellular responses. Some viruses, such as Human Immunodeficiency Virus (HIV), can hijack these receptors to gain entry into the cells, causing infections.

Membrane markers allow cells to recognize one another, which is vital for cellular signaling processes that influence tissue and organ formation during early development. This marking function also plays a later role in the "self"-versus-"non-self" distinction of the immune response. Marker proteins on human red blood cells, for example, determine blood type (A, B, AB, or O).

Table 1 : Cytoskeleton structure

	Microtubules	**Intermediate filaments**	**Microfilaments**
Fiber diameter	About 25 nm	8 to 11 nm	Around 7 nm
Protein composition	Tubulin, with two subunits, alpha and beta tubulin	One of different types of proteins such as lamin, vimentin, and keratin	Actin
Shape	Hollow cylinders made of two protein chains twisted around each other	Protein fiber coils twisted Into each other	Two actin chains twisted around one another
Main functions	Organelle and vesicle movement; form mitotic spindles during cell reproduction; cell motility (in cilia and flagella)	Organize cell shape; Positions organelles in cytoplasm structural support of the nuclear envelope and sarcomeres; involved in cellto-cell and cell-to- matrix junctions	Keep cellular shape; Allows movement of certain cells by forming cytoplasmatic extensions or contraction of actin fibers; involved in some cell-to-cell or cell-to- matrix junctions
Representation			

2.17. Extracellular matrix of animal cells

The extracellular matrix of animal cells holds cells together to form a tissue and allow tissues to communicate with each other **(Figure 46)**.

Key Points

- The extracellular matrix of animal cells is made up of proteins and carbohydrates.
- Cell communication within tissue and tissue formation are main functions of the extracellular matrix of animal cells.
- Tissue communication is kick-started when a molecule within the matrix binds a receptor; the end results are conformational changes that induce chemical signals that ultimately change activities within the cell.

Key Terms

- **Collagen :** Any of more than 28 types of glycoprotein that forms elongated fibers, usually found in the extracellular matrix of connective tissue.
- **Proteoglycan :** Any of many glycoproteins that have heteropolysaccharide side chains
- **Extracellular matrix :** All the connective tissues and fibres that are not part of a cell, but rather provide support.

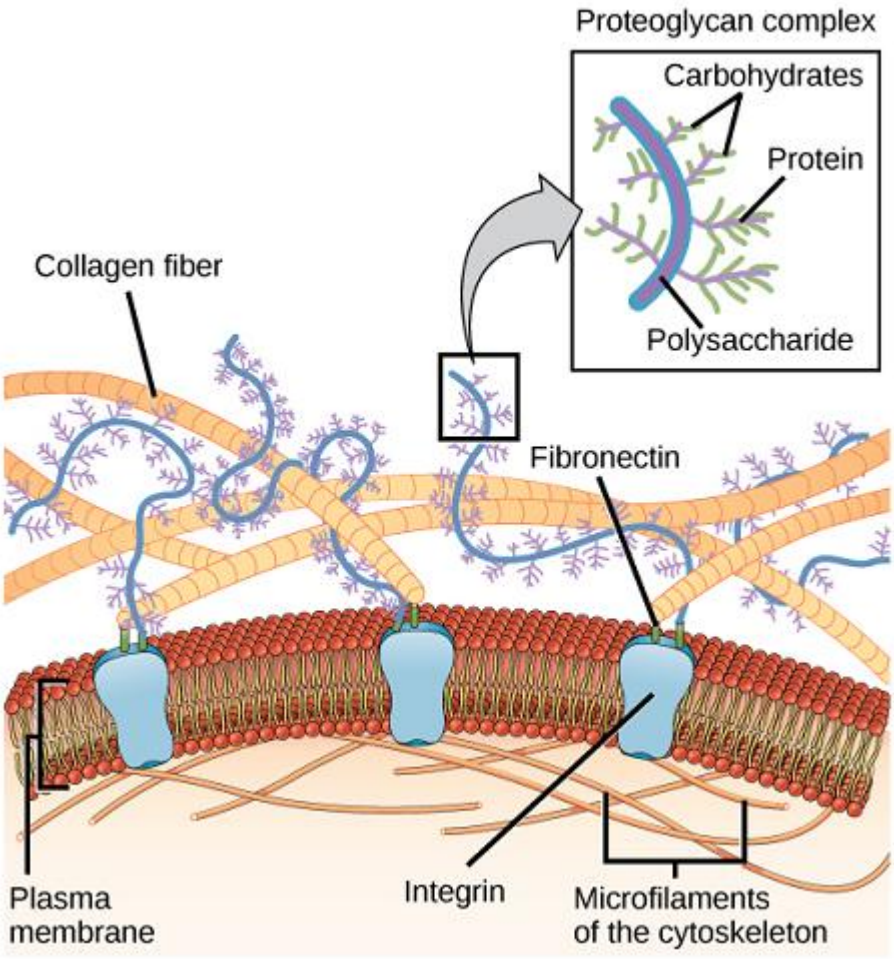


Figure 46 : The Extracellular matrix : The extracellular matrix consists of a network of proteins and carbohydrates.

Most animal cells release materials into the extracellular space. The primary components of these materials are proteins. Collagen is the most abundant of the proteins. Its fibers are interwoven with carbohydrate-containing protein molecules called proteoglycans. Collectively, these materials are called the extracellular matrix. Not only does the extracellular matrix hold the cells together to form a tissue, but it also allows the cells within the tissue to communicate with each other.

How does this cell communication occur? Cells have protein receptors on the extracellular surfaces of their plasma membranes. When a molecule within the matrix binds to the receptor, it changes the molecular structure of the receptor. The receptor, in turn, changes the conformation of the microfilaments positioned just inside the plasma membrane. These conformational changes induce chemical signals inside the cell that reach the nucleus and turn “on” or “off” the transcription of specific sections of DNA. This affects the production of associated proteins, thus changing the activities within the cell.

An example of the role of the extracellular matrix in cell communication can be seen in blood clotting. When the cells lining a blood vessel are damaged, they display a protein receptor called tissue factor. When a tissue factor binds with another factor in the extracellular matrix, it causes platelets to adhere to the wall of the damaged blood vessel and stimulates the adjacent smooth muscle cells in the blood vessel to contract (thus constricting the blood vessel). Subsequently, a series of steps are initiated which then prompt the platelets to produce clotting factors.

3. The Cytoplasmic membrane of prokaryotic and eukaryotic cells

3.1. Components of plasma membranes

Key Points

- The principal components of the plasma membrane are lipids (phospholipids and cholesterol), proteins, and carbohydrates.
- The plasma membrane protects intracellular components from the extracellular environment.
- The plasma membrane mediates cellular processes by regulating the materials that enter and exit the cell.
- The plasma membrane carries markers that allow cells to recognize one another and can transmit signals to other cells via receptors.

Key Terms

- **Plasma membrane :** The semipermeable barrier that surrounds the cytoplasm of a cell.
- **Receptor :** A protein on a cell wall that binds with specific molecules so that they can be absorbed into the cell.

3.1.1. Structure of plasma membranes

The plasma membrane (also known as the cell membrane or cytoplasmic membrane) is a biological membrane that separates the interior of a cell from its outside environment.

The primary function of the plasma membrane is to protect the cell from its surroundings. Composed of a phospholipid bilayer with embedded proteins, the plasma membrane is selectively permeable to ions and organic molecules and regulates the movement of substances in and out of cells. Plasma membranes must be very flexible in order to allow

certain cells, such as red blood cells and white blood cells, to change shape as they pass through narrow capillaries.

The plasma membrane also plays a role in anchoring the cytoskeleton to provide shape to the cell, and in attaching to the extracellular matrix and other cells to help group cells together to form tissues. The membrane also maintains the cell potential.

In short, if the cell is represented by a castle, the plasma membrane is the wall that provides structure for the buildings inside the wall, regulates which people leave and enter the castle, and conveys messages to and from neighboring castles. Just as a hole in the wall can be a disaster for the castle, a rupture in the plasma membrane causes the cell to lyse and die **(Figure 47)**.

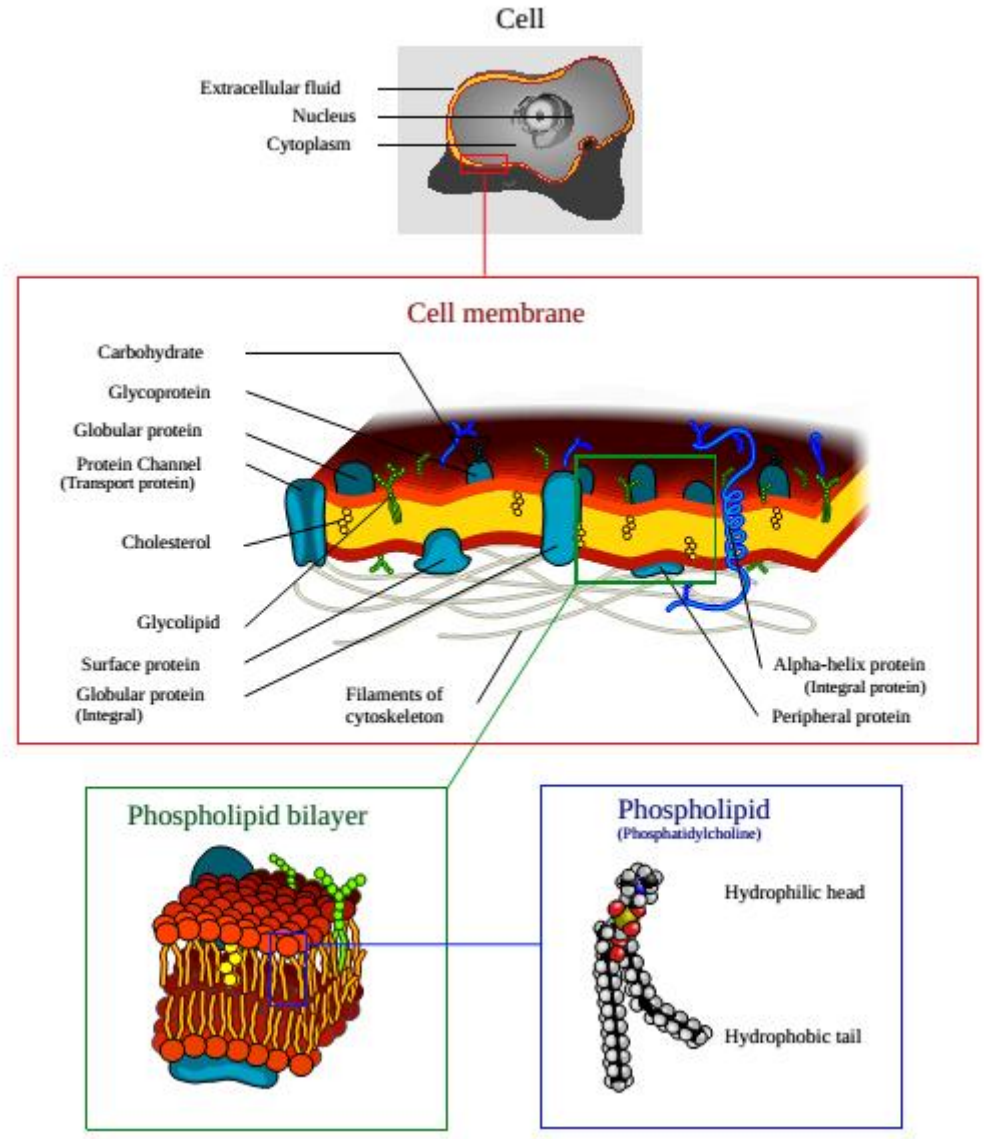


Figure 47 : The plasma membrane : The plasma membrane is composed of phospholipids and proteins that provide a barrier between the external environment and the cell, regulate the transportation of molecules across the membrane, and communicate with other cells via protein receptors.

3.1.2. The plasma membrane and cellular transport

The movement of a substance across the selectively permeable plasma membrane can be either "passive"—i.e., occurring without the input of cellular energy —or "active"—i.e., its transport requires the cell to expend energy.

The cell employs a number of transport mechanisms that involve biological membranes :

1. Passive osmosis and diffusion: transports gases (such as O_2 and CO_2 and other small molecules and ions

2. Transmembrane protein channels and transporters : transports small organic molecules such as sugars or amino acids

3. Endocytosis : transports large molecules (or even whole cells) by engulfing them

4. Exocytosis : removes or secretes substances such as hormones or enzymes

3.1.3. Transport across the cell membrane

3.1.3.1. Facilitated transport

Facilitated transport is a type of passive transport. Unlike simple diffusion where materials pass through a membrane without the help of proteins, in facilitated transport, also called facilitated diffusion, materials diffuse across the plasma membrane with the help of membrane proteins. A concentration gradient exists that would allow these materials to diffuse into the cell without expending cellular energy. However, these materials are ions or polar molecules that are repelled by the hydrophobic parts of the cell membrane. Facilitated transport proteins shield these materials from the repulsive force of the membrane, allowing them to diffuse into the cell.

The material being transported is first attached to protein or glycoprotein receptors on the exterior surface of the plasma membrane. This allows the material that is needed by the cell to be removed from the extracellular fluid. The substances are then passed to specific integral proteins that facilitate their passage. Some of these integral proteins are collections of beta-

pleated sheets that form a channel through the phospholipid bilayer. Others are carrier proteins which bind with the substance and aid its diffusion through the membrane.

3.1.3.2. Channels

The integral proteins involved in facilitated transport are collectively referred to as transport proteins; they function as either channels for the material or carriers. In both cases, they are transmembrane proteins. Channels are specific for the substance that is being transported. Channel proteins have hydrophilic domains exposed to the intracellular and extracellular fluids; they additionally have a hydrophilic channel through their core that provides a hydrated opening through the membrane layers. Passage through the channel allows polar compounds to avoid the nonpolar central layer of the plasma membrane that would otherwise slow or prevent their entry into the cell. Aquaporins are channel proteins that allow water to pass through the membrane at a very high rate **(Figure 48)**.

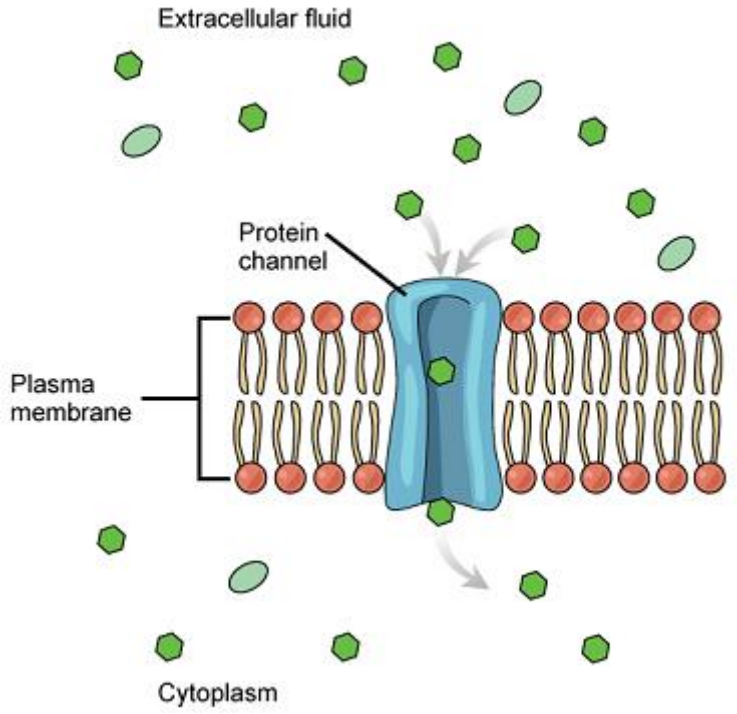


Figure 48 : Channel proteins in facilitated transport : Facilitated transport moves substances down their concentration gradients. They may cross the plasma membrane with the aid of channel proteins.

Channel proteins are either open at all times or they are “gated,” which controls the opening of the channel. The attachment of a particular ion to the channel protein may control the opening or other mechanisms or substances may be involved. In some tissues, sodium and chloride ions pass freely through open channels, whereas in other tissues, a gate must be

opened to allow passage. An example of this occurs in the kidney, where both forms of channels are found in different parts of the renal tubules. Cells involved in the transmission of electrical impulses, such as nerve and muscle cells, have gated channels for sodium, potassium, and calcium in their membranes. Opening and closing of these channels changes the relative concentrations on opposing sides of the membrane of these ions, resulting in the facilitation of electrical transmission along membranes (in the case of nerve cells) or in muscle contraction (in the case of muscle cells).

3.1.3.3. Carrier proteins

Another type of protein embedded in the plasma membrane is a carrier protein. This protein binds a substance and, in doing so, triggers a change of its own shape, moving the bound molecule from the outside of the cell to its interior; depending on the gradient, the material may move in the opposite direction. Carrier proteins are typically specific for a single substance. This adds to the overall selectivity of the plasma membrane. The exact mechanism for the change of shape is poorly understood. Proteins can change shape when their hydrogen bonds are affected, but this may not fully explain this mechanism. Each carrier protein is specific to one substance, and there are a finite number of these proteins in any membrane. This can cause problems in transporting enough of the material for the cell to function properly **(Figure 49)**.

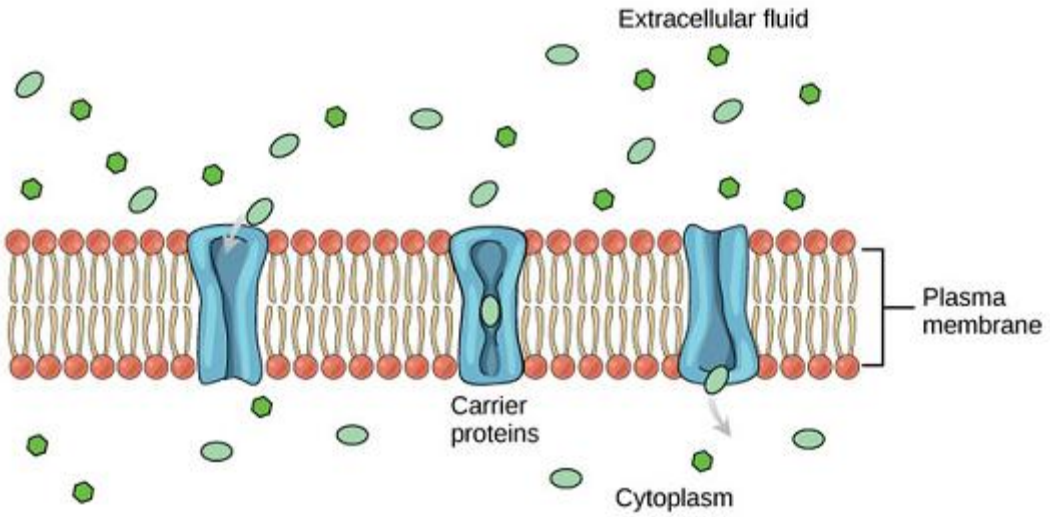


Figure 49 : Carrier proteins : Some substances are able to move down their concentration gradient across the plasma membrane with the aid of carrier proteins. Carrier proteins change shape as they move molecules across the membrane.

An example of this process occurs in the kidney. Glucose, water, salts, ions, and amino acids needed by the body are filtered in one part of the kidney. This filtrate, which includes glucose, is then reabsorbed in another part of the kidney. Because there are only a finite number of carrier proteins for glucose, if more glucose is present than the proteins can handle, the excess is not transported; it is excreted from the body in the urine. In a diabetic individual, this is described as "spilling glucose into the urine." A different group of carrier proteins called glucose transport proteins, or GLUTs, are involved in transporting glucose and other hexose sugars through plasma membranes within the body.

Channel and carrier proteins transport material at different rates. Channel proteins transport much more quickly than do carrier proteins. Channel proteins facilitate diffusion at a rate of tens of millions of molecules per second, whereas carrier proteins work at a rate of a thousand to a million molecules per second.

Key Points

- A concentration gradient exists that would allow ions and polar molecules to diffuse into the cell, but these materials are repelled by the hydrophobic parts of the cell membrane.
- Facilitated diffusion uses integral membrane proteins to move polar or charged substances across the hydrophobic regions of the membrane.
- Channel proteins can aid in the facilitated diffusion of substances by forming a hydrophilic passage through the plasma membrane through which polar and charged substances can pass.
- Channel proteins can be open at all times, constantly allowing a particular substance into or out of the cell, depending on the concentration gradient; or they can be gated and can only be opened by a particular biological signal.
- Carrier proteins aid in facilitated diffusion by binding a particular substance, then altering their shape to bring that substance into or out of the cell.

Key Terms

- **Facilitated diffusion :** The spontaneous passage of molecules or ions across a biological membrane passing through specific transmembrane integral proteins.
- **Membrane protein :** Proteins that are attached to, or associated with the membrane of a cell or an organelle.

3.1.3.4. Primary active transport

The sodium-potassium pump maintains the electrochemical gradient of living cells by moving sodium in and potassium out of the cell. The primary active transport that functions with the active transport of sodium and potassium allows secondary active transport to occur. The secondary transport method is still considered active because it depends on the use of energy as does primary transport **(Figure 50)**.

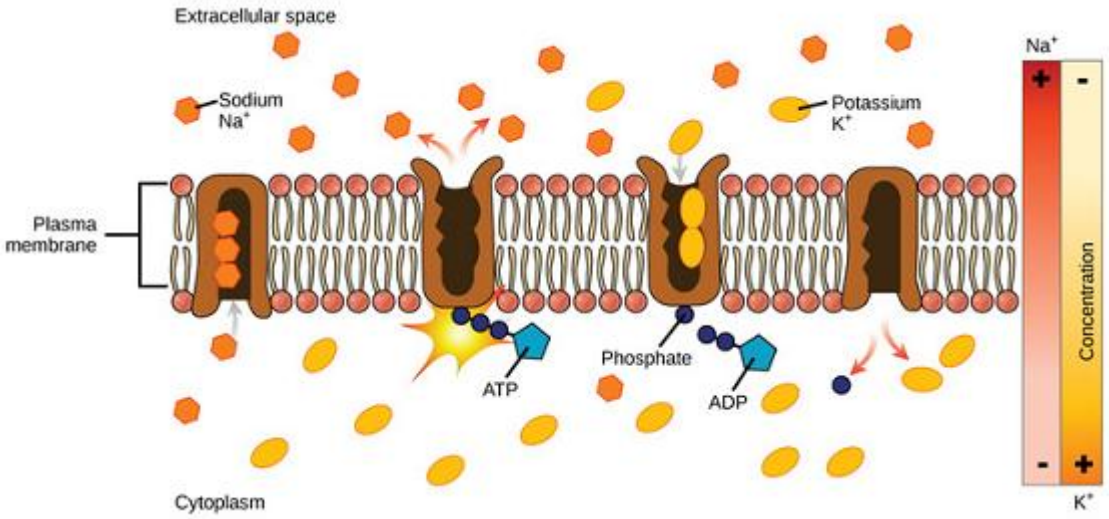


Figure 50 : Active transport of sodium and potassium : Primary active transport moves ions across a membrane, creating an electrochemical gradient (electrogenic transport).

One of the most important pumps in animals cells is the sodium-potassium pump (Na^+/K^+ ATPase), which maintains the electrochemical gradient (and the correct concentrations of Na and K) in living cells. The sodium-potassium pump moves two K into the cell while moving three Na out of the cell. The Na^+/K^+ ATPase exists in two forms, depending on its orientation to the interior or exterior of the cell and its affinity for either sodium or potassium ions. The process consists of the following six steps :

- With the enzyme oriented towards the interior of the cell, the carrier has a high affinity for sodium ions. Three sodium ions bind to the protein.
- ATP is hydrolyzed by the protein carrier, and a low-energy phosphate group attaches to it.
- As a result, the carrier changes shape and re-orients itself towards the exterior of the membrane. The protein's affinity for sodium decreases, and the three sodium ions leave the carrier.

- The shape change increases the carrier's affinity for potassium ions, and two such ions attach to the protein. Subsequently, the low-energy phosphate group detaches from the carrier.
- With the phosphate group removed and potassium ions attached, the carrier protein repositions itself towards the interior of the cell.
- The carrier protein, in its new configuration, has a decreased affinity for potassium, and the two ions are released into the cytoplasm. The protein now has a higher affinity for sodium ions, and the process starts again.

Several things have happened as a result of this process. At this point, there are more sodium ions outside of the cell than inside and more potassium ions inside than out. For every three ions of sodium that move out, two ions of potassium move in. This results in the interior being slightly more negative relative to the exterior. This difference in charge is important in creating the conditions necessary for the secondary process. The sodium-potassium pump is, therefore, an electrogenic pump (a pump that creates a charge imbalance), creating an electrical imbalance across the membrane and contributing to the membrane potential.

Key Points

- The sodium-potassium pump moves K^+ into the cell while moving Na^+ at a ratio of three Na^+ for every two K^+ ions.
- When the sodium-potassium- ATPase enzyme points into the cell, it has a high affinity for sodium ions and binds three of them,
- hydrolyzing ATP and changing shape.
- As the enzyme changes shape, it reorients itself towards the outside of the cell, and the three sodium ions are released.
- The enzyme's new shape allows two potassium to bind and the phosphate group to detach, and the carrier protein repositions itself towards the interior of the cell.
- The enzyme changes shape again, releasing the potassium ions into the cell.

Key Terms

❖ **Electrogenic pump :** An ion pump that generates a net charge flow as a result of its activity.

❖ **Na^+/K^+ ATPase :** An enzyme located in the plasma membrane of all animal cells that pumps sodium out of cells while pumping potassium into cells.

ABC transporters

ATP-binding cassette transporters (ABC-transporters) are members of a protein superfamily that is one of the largest and most ancient families with representatives in all extant phyla from prokaryotes to humans. ABC transporters are transmembrane proteins that utilize the energy of adenosine triphosphate (ATP) hydrolysis to carry out certain biological processes including translocation of various substrates across membranes and non-transport-related processes such as translation of RNA and DNA repair. They transport a wide variety of substrates across extra- and intracellular membranes, including metabolic products, lipids and sterols, and drugs. Proteins are classified as ABC transporters based on the sequence and organization of their ATP-binding cassette (ABC) domain(s).

ABC transporters are involved in tumor resistance, cystic fibrosis and a range of other inherited human diseases along with both bacterial (prokaryotic) and eukaryotic (including human) development of resistance to multiple drugs. Bacterial ABC transporters are essential in cell viability, virulence, and pathogenicity.

ABC transporters are divided into three main functional categories. In prokaryotes, importers mediate the uptake of nutrients into the cell. The substrates that can be transported include ions, amino acids, peptides, sugars, and other molecules that are mostly hydrophilic. The membrane-spanning region of the ABC transporter protects hydrophilic substrates from the lipids of the membrane bilayer thus providing a pathway across the cell membrane. In gram-negative bacteria, exporters transport lipids and some polysaccharides from the cytoplasm to the periplasm. Eukaryotes do not possess any importers. Exporters or effluxers, which are both present in prokaryotes and eukaryotes, function as pumps that extrude toxins and drugs out of the cell. The third subgroup of ABC proteins do not function as transporters, but rather are involved in translation and DNA repair processes.

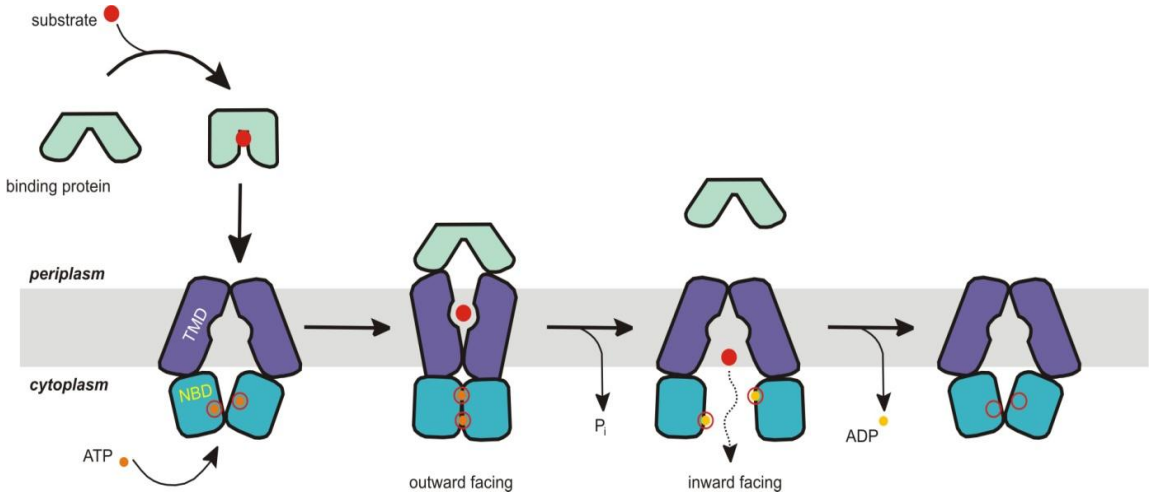


Figure 51 : Mechanism of ABC transport : Proposed mechanism of transport for ABC importers. This alternating-access model was based on the crystal structures of ModBC-A

In bacterial efflux systems, certain substances that need to be extruded from the cell include surface components of the bacterial cell (e.g. capsular polysaccharides, lipopolysaccharides, and teichoic acid), proteins involved in bacterial pathogenesis (e.g. hemolysis, heme-binding protein, and alkaline protease), heme, hydrolytic enzymes, S-layer proteins, competence factors, toxins, antibiotics, bacteriocins, peptide antibiotics, drugs and siderophores. They also play important roles in biosynthetic pathways, including extracellular polysaccharide biosynthesis and cytochrome biogenesis.

Key Points

- ABC transporters use the energy of ATP hydrolysis to transport substrates across cell membranes.
- Bacterial ABC transporters are essential in cell viability, virulence, and pathogenicity.
- The substrates that can be transported include ions, amino acids, peptides, sugars, and other molecules that are mostly hydrophilic.

Key Terms

- **Membrane :** A flexible enclosing or separating tissue forming a plane or film and separating two environments (usually in a plant or animal).
- **Hydrolysis :** A chemical process of decomposition involving the splitting of a bond and the addition of the hydrogen cation and the hydroxide anion of water.

- **ATP-binding cassette (ABC) domain :** The ATP-binding cassette (ABC) family is a group of proteins which bind and hydrolyse ATP in order to transport substances across cellular membranes.

Siderophores

Iron is essential for almost all living organisms as it is involved in a wide variety of important metabolic processes. However, iron is not always readily available; therefore, microorganisms use various iron uptake systems to secure sufficient supplies from their surroundings. There is considerable variation in the range of iron transporters and iron sources utilized by different microbial species. Pathogens, in particular, require efficient iron acquisition mechanisms to enable them to compete successfully for iron in the highly iron-restricted environment of the host's tissues and body fluids.

Siderophores are small, high-affinity iron chelating compounds secreted by microorganisms such as bacteria, fungi, and grasses. Siderophores are amongst the strongest soluble Fe binding agents known. Iron is essential for almost all life, because of its vital role in processes like respiration and DNA synthesis. However, despite being one of the most abundant elements in the Earth's crust, the bioavailability of iron in many environments such as the soil or sea is limited by the very low solubility of the Fe ion. This ion state is the predominant one of iron in aqueous, non-acidic, oxygenated environments, and accumulates in common mineral phases such as iron oxides and hydroxides (the minerals that are responsible for red and yellow soil colours). Hence, it cannot be readily utilized by organisms. Microbes release siderophores to scavenge iron from these mineral phases by formation of soluble Fe complexes that can be taken up by active transport mechanisms. Many siderophores are nonribosomal peptides, although several are biosynthesised independently.

Siderophores are amongst the strongest binders to Fe known, with enterobactin being one of the strongest of these. Because of this property, they have attracted interest from medical science in metal chelation therapy, with the siderophore desferrioxamine B gaining widespread use in treatments for iron poisoning and thalassemia.

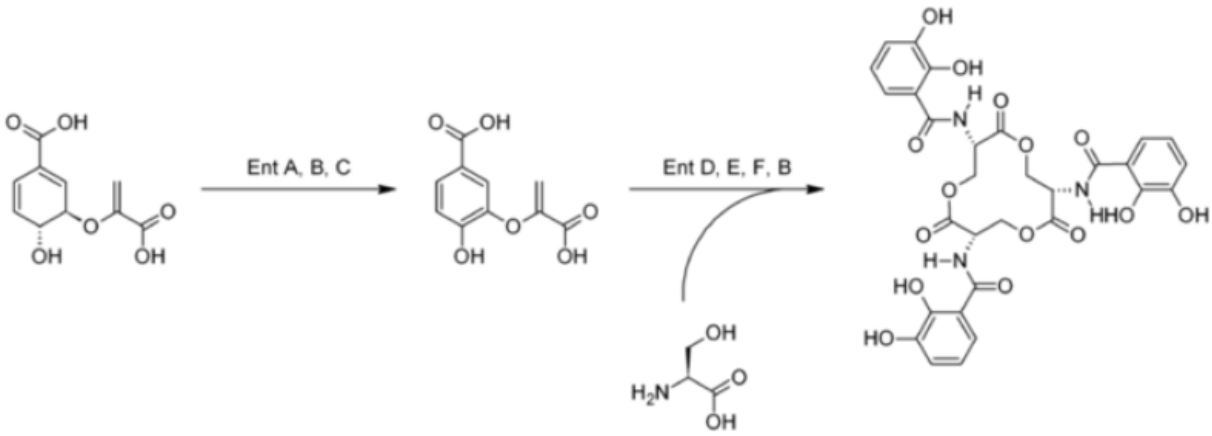


Figure 52 : Synthesis of enterobactin : Enterobactin (also known as Enterochelin) is a high affinity siderophore that acquires iron for microbial systems. It is primarily found in Gram-negative bacteria, such as *Escherichia coli* and *Salmonella typhimurium*.

Iron is tightly bound to proteins such as hemoglobin, transferrin, lactoferrin, and ferritin. There are great evolutionary pressures put on pathogenic bacteria to obtain this metal. For example, the anthrax pathogen Bacillus anthracisreleases two siderophores, bacillibactin and petrobactin, to scavenge ferric iron from iron proteins. While bacillibactin has been shown to bind to the immune system protein siderocalin, petrobactin is assumed to evade the immune system and has been shown to be important for virulence in mice **(Figure 52)**.

Besides siderophores, some pathogenic bacteria produce hemophores (heme binding scavenging proteins) or have receptors that bind directly to iron/heme proteins. In eukaryotes, other strategies to enhance iron solubility and uptake are the acidification of the surrounding (e.g. used by plant roots) or the extracellular reduction of Fe into the more soluble Fe ions.

Siderophores usually form a stable, hexadentate, octahedral complex with Fe preferentially compared to other naturally occurring abundant metal ions, although if there are less than six donor atoms water can also coordinate. The most effective siderophores are those that have three bidentate ligands per molecule, forming a hexadentate complex and causing a smaller entropic change than that caused by chelating a single ferric ion with separate ligands.

Siderophores are usually classified by the ligands used to chelate the ferric iron. The majors groups of siderophores include the catecholates (phenolates), hydroxamates and carboxylates (e.g. derivatives of citric acid). Citric acid can also act as a siderophore. The wide variety of siderophores may be due to evolutionary pressures placed on microbes to produce structurally different siderophores, which cannot be transported by other microbes' specific active transport systems, or in the case of pathogens deactivated by the host organism.

Key Points

- Siderophores are important for some pathogenic bacteria for their acquisition of iron. Many siderophores are nonribosomal peptides, although several are biosynthesised independently.
- The wide variety of siderophores may be due to evolutionary pressures placed on microbes to produce structurally different siderophores which cannot be transported by other microbes' specific active transport systems, or in the case of pathogens deactivated by the host organism.
- Microbes release siderophores to scavenge iron from these mineral phases by formation of soluble Fe^{3+} complexes that can be taken up by active transport mechanisms.

Key Terms

❖ **Siderophores :** Sidereophores are small, high-affinity iron chelating compounds secreted by microorganisms such as bacteria and fungi, and also grasses. Siderophores are amongst the strongest soluble Fe^{3+} binding agents known.

Group translocation

Key Points

- PEP is known as a multi-component system that always involves enzymes of the plasma membrane and those in the cytoplasm. An example of this transport is found in *E. coli* cells.
- The Tat pathway is a protein export, or secretion pathway, that serves to actively translocate folded proteins across a lipid membrane bilayer.
- Systems for secreting proteins across the bacterial outer membrane may be quite complex and play key roles in pathogenesis.

Key Terms

❖ **Phosphotransferase system :** A distinct method used by bacteria for sugar uptake where the source of energy is from phosphoenolpyruvate (PEP).

❖ **Tat pathway :** A protein export or secretion pathway found in plants, bacteria, and archaea.

With some exceptions, bacteria lack membrane-bound organelles as found in eukaryotes, but they may assemble proteins onto various types of inclusions such as gas vesicles and storage granules. Bacteria may have a single plasma membrane (Gram-positive bacteria) or an inner membrane plus an outer membrane separated by the periplasm (Gram-negative bacteria). Proteins may be incorporated into the plasma membrane. They can also be trapped in either the periplasm or secreted into the environment, according to whether or not there is an outer membrane. The basic mechanism at the plasma membrane is similar to the eukaryotic one. In addition, bacteria may target proteins into or across the outer membrane. Systems for secreting proteins across the bacterial outer membrane may be quite complex. The systems play key roles in pathogenesis. These systems may be described as type I secretion, type II secretion, etc. In most Gram-positive bacteria, certain proteins are targeted for export across the plasma membrane and subsequent covalent attachment to the bacterial cell wall.

PEP group translocation, also known as the phosphotransferase system or PTS, is a distinct method used by bacteria for sugar uptake where the source of energy is from phosphoenolpyruvate (PEP). It is known as a multi-component system that always involves enzymes of the plasma membrane and those in the cytoplasm. An example of this transport is found in E. coli cells. The system was discovered by Saul Roseman in 1964.

The twin-arginine translocation pathway (Tat pathway) is a protein export or secretion pathway found in plants, bacteria, and archaea. In contrast to the Sec pathway which transports proteins in an unfolded manner, the Tat pathway serves to actively translocate folded proteins across a lipid membrane bilayer. In bacteria, the Tat translocase is found in the cytoplasmic membrane and serves to export proteins to the cell envelope or to the extracellular space. In Gram-negative bacteria the Tat translocase is composed of three essential membrane proteins: TatA, TatB, and TatC. In the most widely studied Tat pathway, that of the Gram negative bacterium Escherichia coli, these three proteins are expressed from an operon with a fourth Tat protein, TatD, which is not required for Tat function. A fifth Tat protein TatE that is homologous to the TatA protein is present at a much lower level in the cell than TatA. It is not believed to play any significant role in Tat function.

The Tat pathways of Gram-positive bacteria differ in that they do not have a TatB component. In these bacteria the Tat system is made up from a single TatA and TatC component, with the TatA protein being bifunctional and fulfilling the roles of both *E. coli* TatA and TatB. Not all bacteria carry the tatABC genes in their genome. However, of those that do, there seems to be no discrimination between pathogens and nonpathogens. Despite that fact, some pathogenic

bacteria such as *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Yersinia pseudotuberculosis*, and *E. coli* O157:H7 rely on a functioning Tat pathway for full virulence in infection models. In addition, a number of exported virulence factors have been shown to rely on the Tat pathway. One such category of virulence factors are the phospholipase C enzymes, which have been shown to be Tat-exported in *Pseudomonas aeruginosa* and thought to be Tat-exported in *Mycobacterium tuberculosis* **(Figure 53)**.

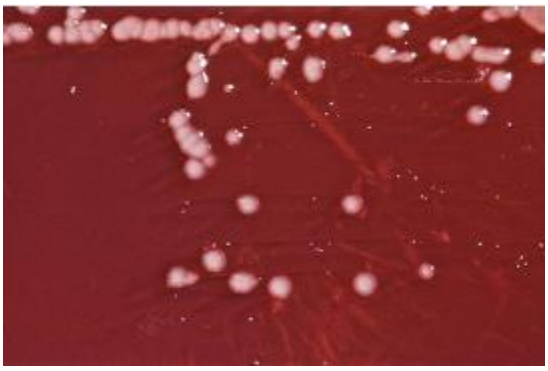


Figure 53 : *Pseudomonas aeruginosa* : *P. aeruginosa* is capable of growth in diesel and jet fuel, where it is known as a hydrocarbonusing microorganism (or "HUM bug"), causing microbial corrosion. It creates dark, gellish mats sometimes improperly called "algae" because of their appearance.

3.1.3.5. Protein export and secretion

3.1.3.5.1. Endocytosis

Endocytosis is a type of active transport that moves particles, such as large molecules, parts of cells, and even whole cells, into a cell. There are different variations of endocytosis, but all share a common characteristic: the plasma membrane of the cell invaginates, forming a pocket around the target particle. The pocket pinches off, resulting in the particle being contained in a newly-created intracellular vesicle formed from the plasma membrane **(Figure 54)**.

3.1.3.5.2. Phagocytosis

Phagocytosis (the condition of "cell eating") is the process by which large particles, such as cells or relatively large particles, are taken in by a cell. For example, when microorganisms invade the human body, a type of white blood cell called a neutrophil will remove the invaders through this process, surrounding and engulfing the microorganism, which is then destroyed by the neutrophil **(Figure 54)**.

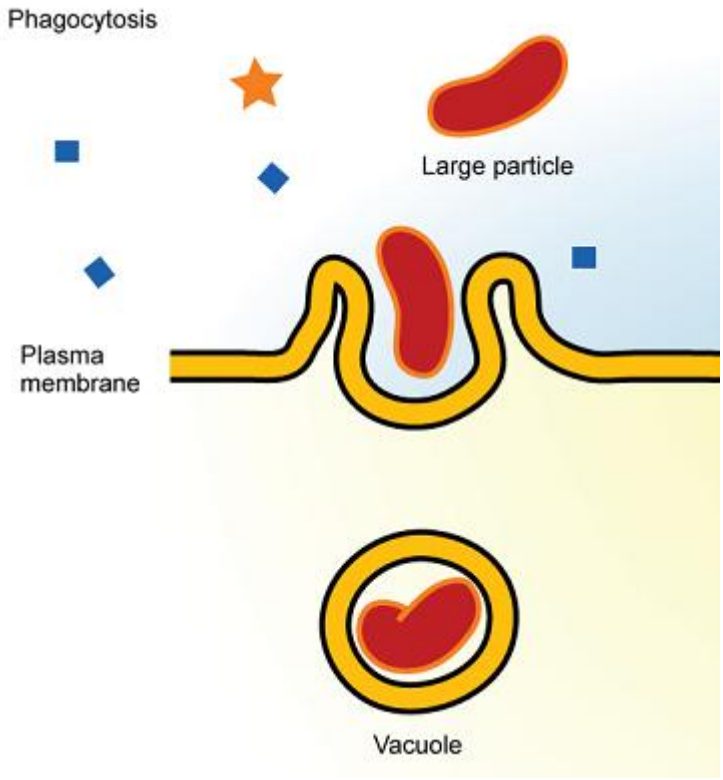


Figure 54 : Phagocytosis : In phagocytosis, the cell membrane surrounds the particle and engulfs it

In preparation for phagocytosis, a portion of the inward-facing surface of the plasma membrane becomes coated with a protein called clathrin, which stabilizes this section of the membrane. The coated portion of the membrane then extends from the body of the cell and surrounds the particle, eventually enclosing it. Once the vesicle containing the particle is enclosed within the cell, the clathrin disengages from the membrane and the vesicle merges with a lysosome for the breakdown of the material in the newlyformed compartment (endosome). When accessible nutrients from the degradation of the vesicular contents have been extracted, the newly-formed endosome merges with the plasma membrane and releases its contents into the extracellular fluid. The endosomal membrane again becomes part of the plasma membrane.

3.1.3.5.3. Pinocytosis

A variation of endocytosis is called pinocytosis. This literally means “cell drinking” and was named at a time when the assumption was that the cell was purposefully taking in extracellular fluid. In reality, this is a process that takes in molecules, including water, which the cell needs from the extracellular fluid. Pinocytosis results in a much smaller vesicle than does phagocytosis, and the vesicle does not need to merge with a lysosome **(Figure 55)**.

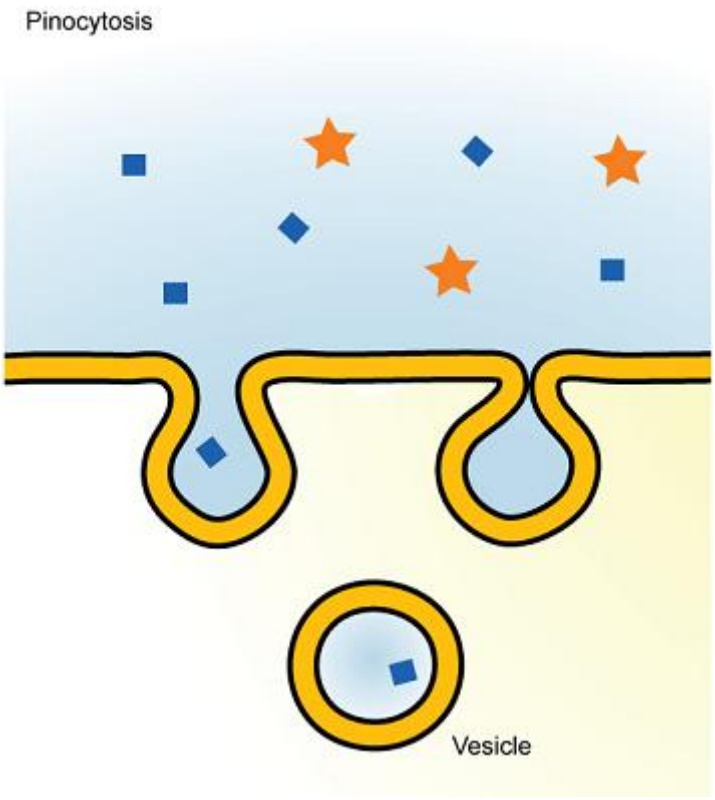


Figure 55 : Pinocytosis : In pinocytosis, the cell membrane invaginates, surrounds a small volume of fluid, and pinches off

Potocytosis, a variant of pinocytosis, is a process that uses a coating protein, called caveolin, on the cytoplasmic side of the plasma membrane, which performs a similar function to clathrin. The cavities in the plasma membrane that form the vacuoles have membrane receptors and lipid rafts in addition to caveolin. The vacuoles or vesicles formed in caveolae (singular caveola) are smaller than those in pinocytosis. Potocytosis is used to bring small molecules into the cell and to transport these molecules through the cell for their release on the other side of the cell, a process called transcytosis.

3.1.3.5.4. Receptor-mediated endocytosis

A targeted variation of endocytosis, known as receptor-mediated endocytosis, employs receptor proteins in the plasma membrane that have a specific binding affinity for certain substances. In receptor-mediated endocytosis, as in phagocytosis, clathrin is attached to the cytoplasmic side of the plasma membrane. If uptake of a compound is dependent on receptor-mediated endocytosis and the process is ineffective, the material will not be removed from the tissue fluids or blood. Instead, it will stay in those fluids and increase in concentration. Some human diseases are caused by the failure of receptor-mediated endocytosis. For example, the form of cholesterol termed low-density lipoprotein or LDL (also referred to as “bad”

cholesterol) is removed from the blood by receptor-mediated endocytosis. In the human genetic disease familial hypercholesterolemia, the LDL receptors are defective or missing entirely. People with this condition have life-threatening levels of cholesterol in their blood, because their cells cannot clear LDL particles from their blood.

Although receptor-mediated endocytosis is designed to bring specific substances that are normally found in the extracellular fluid into the cell, other substances may gain entry into the cell at the same site. Flu viruses, diphtheria, and cholera toxin all have sites that cross-react with normal receptor-binding sites and gain entry into cells **(Figure 56)**.

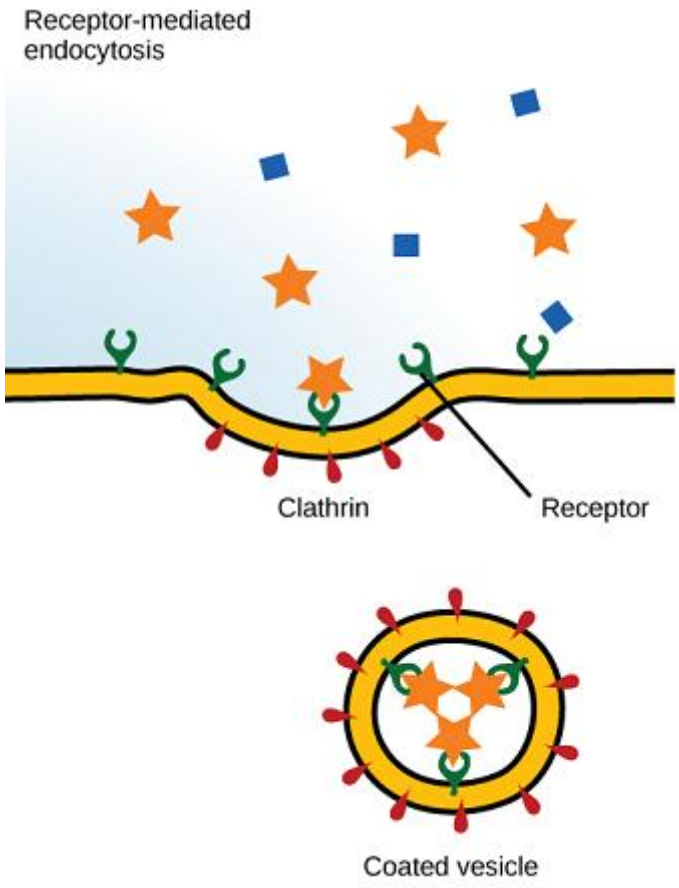


Figure 56: Receptor-Mediated Endocytosis : In receptor-mediated endocytosis, uptake of substances by the cell is targeted to a single type of substance that binds to the receptor on the external surface of the cell membrane.

Key Points

- Endocytosis consists of phagocytosis, pinocytosis, and receptor -mediated endocytosis.
- Endocytosis takes particles into the cell that are too large to passively cross the cell membrane.

- Phagocytosis is the taking in of large food particles, while pinocytosis takes in liquid particles.
- Receptor-mediated endocytosis uses special receptor proteins to help carry large particles across the cell membrane.

Key Terms

- **Endosome :** An endocytic vacuole through which molecules internalized during endocytosis pass en route to lysosomes
- **Neutrophil :** A cell, especially a white blood cell that consumes foreign invaders in the blood.

Chapter 4 : The cell cycle

The cell cycle is an ordered series of events involving cell growth and cell division that produces two new daughter cells. Cells on the path to cell division proceed through a series of precisely timed and carefully regulated stages of growth, DNA replication, and division that produce two genetically identical cells. The cell cycle has two major phases: interphase and the mitotic phase **(Figure 57)**. During interphase, the cell grows and DNA is replicated. During the mitotic phase, the replicated DNA and cytoplasmic contents are separated and the cell divides.

Interphase

During interphase, the cell undergoes normal processes while also preparing for cell division. For a cell to move from interphase to the mitotic phase, many internal and external conditions must be met. The three stages of interphase are called G , S, and G .

G Phase

The first stage of interphase is called the G phase, or first gap, because little change is visible. However, during the G stage, the cell is quite active at the biochemical level. The cell is accumulating the building blocks of chromosomal DNA and the associated proteins, as well as accumulating enough energy reserves to complete the task of replicating each chromosome in the nucleus.

S Phase

Throughout interphase, nuclear DNA remains in a semi-condensed chromatin configuration. In the S phase (synthesis phase), DNA replication results in the formation of two identical copies of each chromosome—sister chromatids—that are firmly attached at the centromere region. At this stage, each chromosome is made of two sister chromatids and is a duplicated

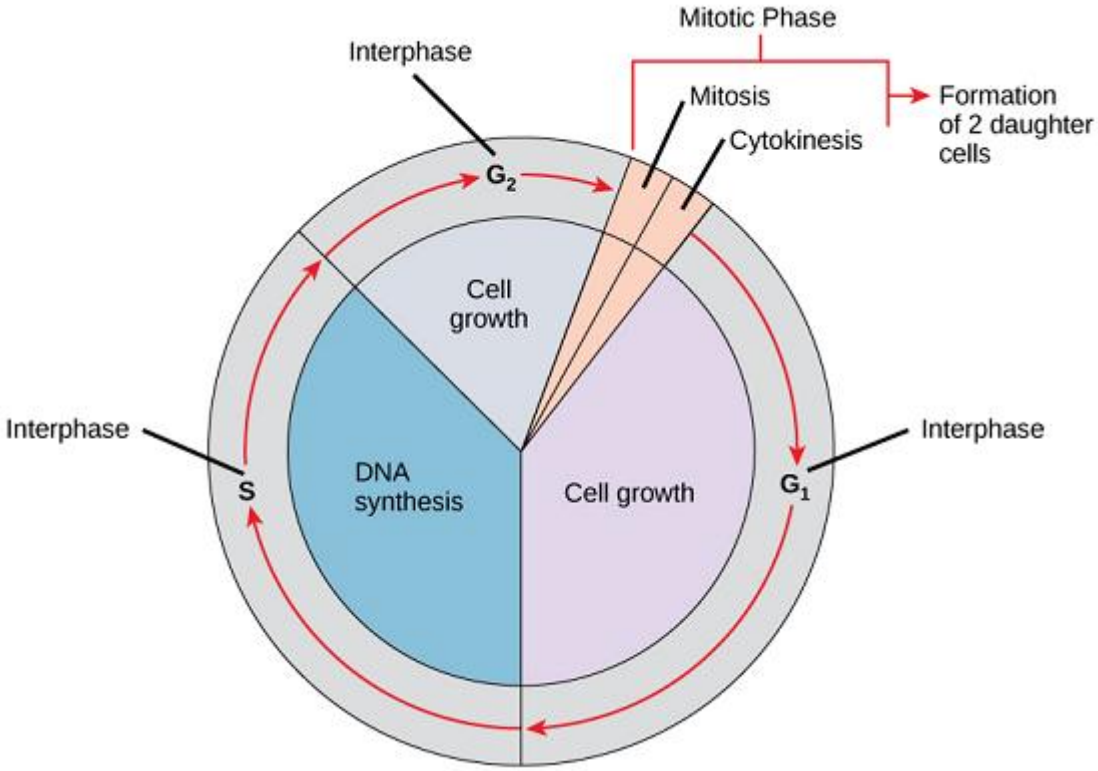


Figure 57 : The different phases of Mitosis

chromosome. The centrosome is duplicated during the S phase. The two centrosomes will give rise to the mitotic spindle, the apparatus that orchestrates the movement of chromosomes during mitosis. The centrosome consists of a pair of rod-like centrioles at right angles to each other. Centrioles help organize cell division. Centrioles are not present in the centrosomes of many eukaryotic species, such as plants and most fungi **(Figure 57)**.

G Phase

In the G phase, or second gap, the cell replenishes its energy stores and synthesizes the proteins necessary for chromosome manipulation. Some cell organelles are duplicated, and

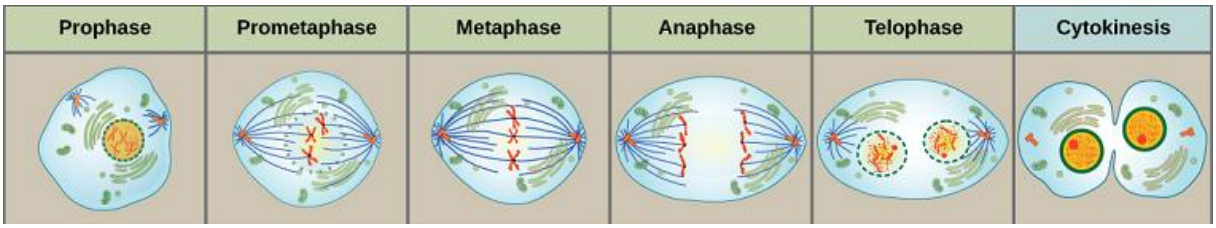
the cytoskeleton is dismantled to provide resources for the mitotic spindle. There may be additional cell growth during G. The final preparations for the mitotic phase must be completed before the cell is able to enter the first stage of mitosis.

The Mitotic Phase

To make two daughter cells, the contents of the nucleus and the cytoplasm must be divided. The mitotic phase is a multistep process during which the duplicated chromosomes are aligned, separated, and moved to opposite poles of the cell, and then the cell is divided into two new identical daughter cells. The first portion of the mitotic phase, mitosis, is composed of five stages, which accomplish nuclear division. The second portion of the mitotic phase, called cytokinesis, is the physical separation of the cytoplasmic components into two daughter cells.

Mitosis

Mitosis is divided into a series of phases—prophase, prometaphase, metaphase, anaphase, and telophase—that result in the division of the cell nucleus **(Figure 58)**.



Chromosomes condense and become visible. Spindle fibers emerge from the centrosomes. Nuclear envelope breaks down. Centrosomes move toward opposite poles.	Chromosomes continue to dense. Kinetochores appear at the centromeres. Mitotic spindle microtubules attach to kinetochores.	Chromosomes are lined up at the metaphase plate. Each sister chromatid is attached to a spindle fiber originating from opposite poles.	Centromeres split in two. Sister chromatids are pulled toward opposite poles. Certain spindle fibers begin to elongate the cell.	Chromosomes arrive at opposite poles and begin to decondense. Nuclear envelope material surrounds each set of chromosomes. The mitotic spindle breaks down Spindle fibers continue to push	Animal cells : a cleavage furrow separates the daughter cells. Plant cells : a cell plate, the precursor to a new cell wall, separates the daughter cells.

				pole apart	

Chapter 5 : Introduction to 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.

1. 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.

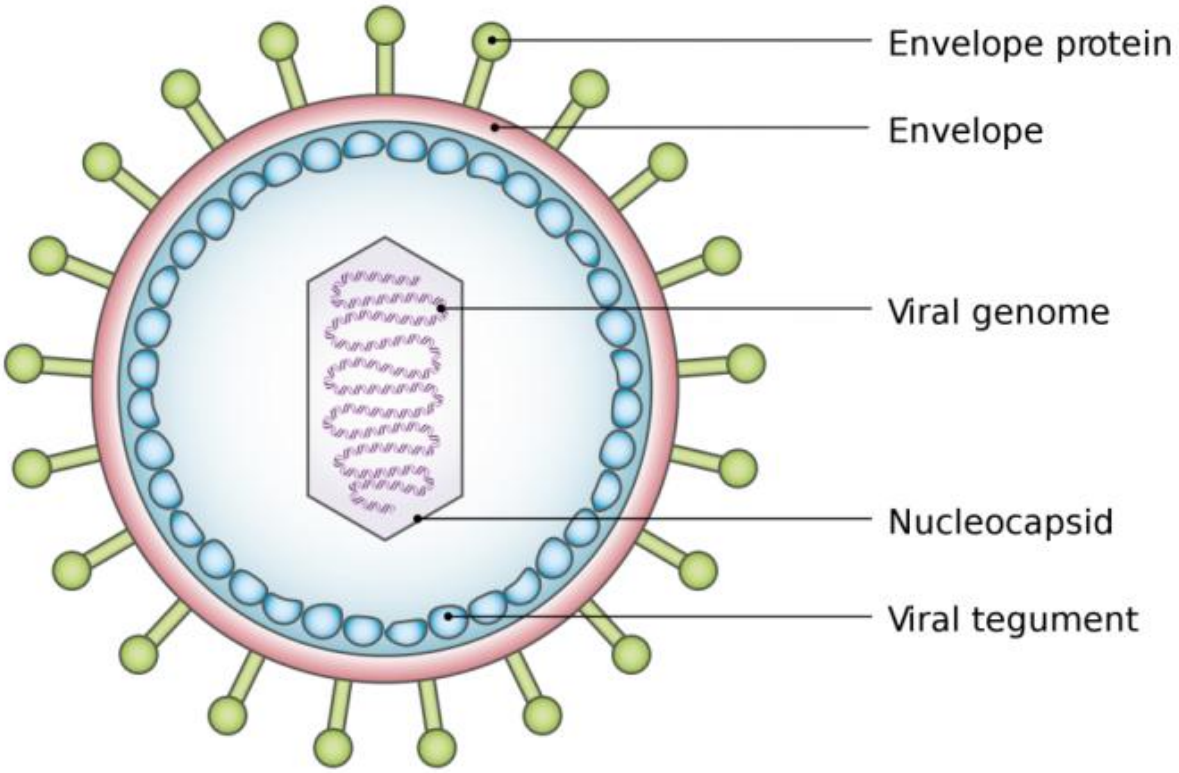


Figure 57 : General structure of a virus

2. 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.

3. 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.

4. 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.

4.1. 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.

4.2. 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.

4.3. 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.

4.4. 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.

4.5. 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.

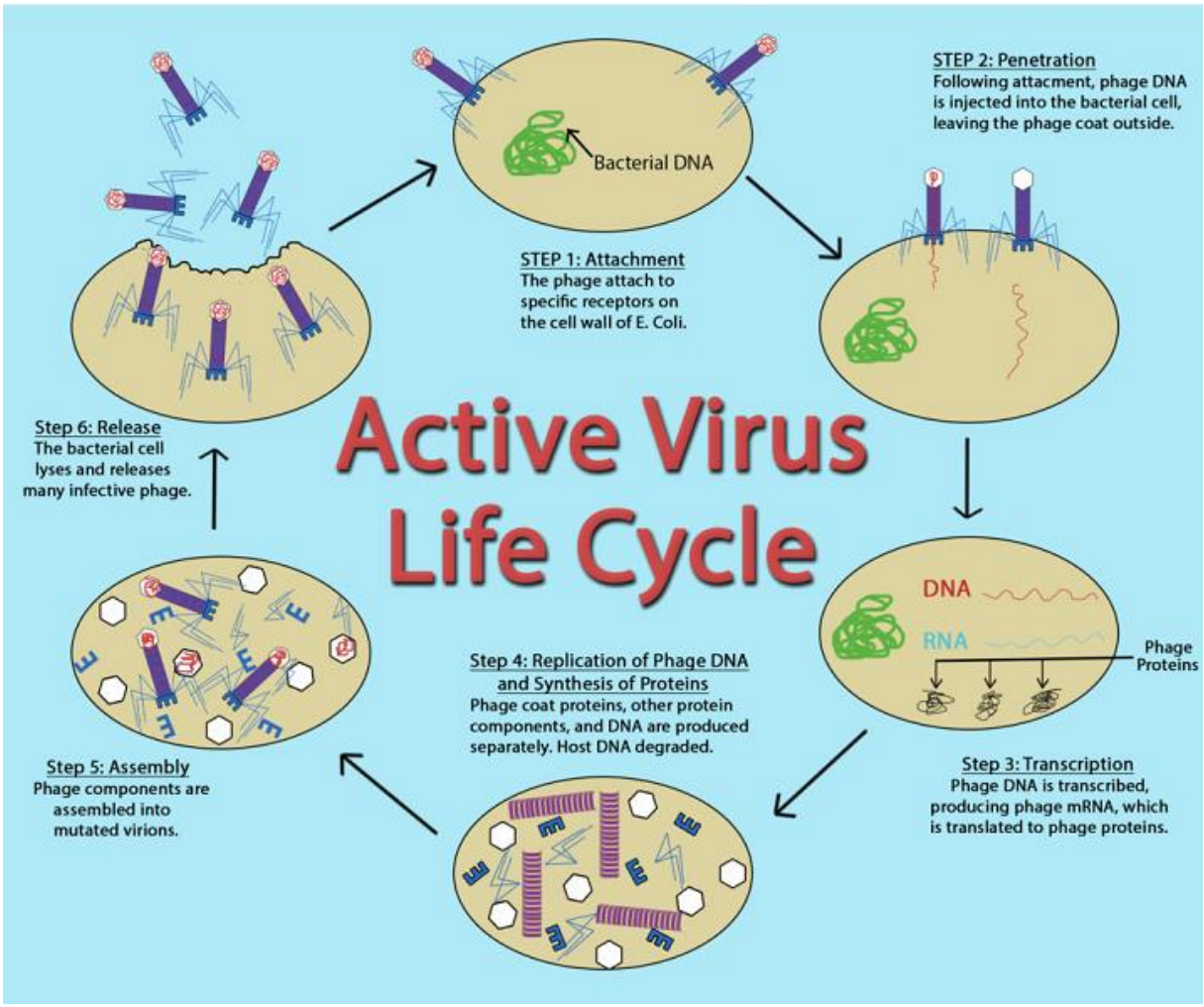


Figure 58 : Active virus life cycle by John Kellogg

5. 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.

A phage employing lysogeny still undergoes the first two steps of a typical replication cycle, attachment and penetration. Once the viral DNA has been inserted into the cell it integrates with the host DNA, forming a prophage. The infected bacterium is referred to as a lysogenor

lysogenic bacterium. In this state, the virus enjoys a stable relationship with its host, where it does not interfere with host cell metabolism or reproduction. The host cell enjoys immunity from reinfection from the same virus.

Exposure of the host cell to stressful conditions (i.e. UV irradiation) causes induction, where the viral DNA excises from the host cell DNA. This event triggers the remaining steps of the lytic cycle, synthesis, maturation, and release, leading to lysis of the host cell and release of newly formed virions.

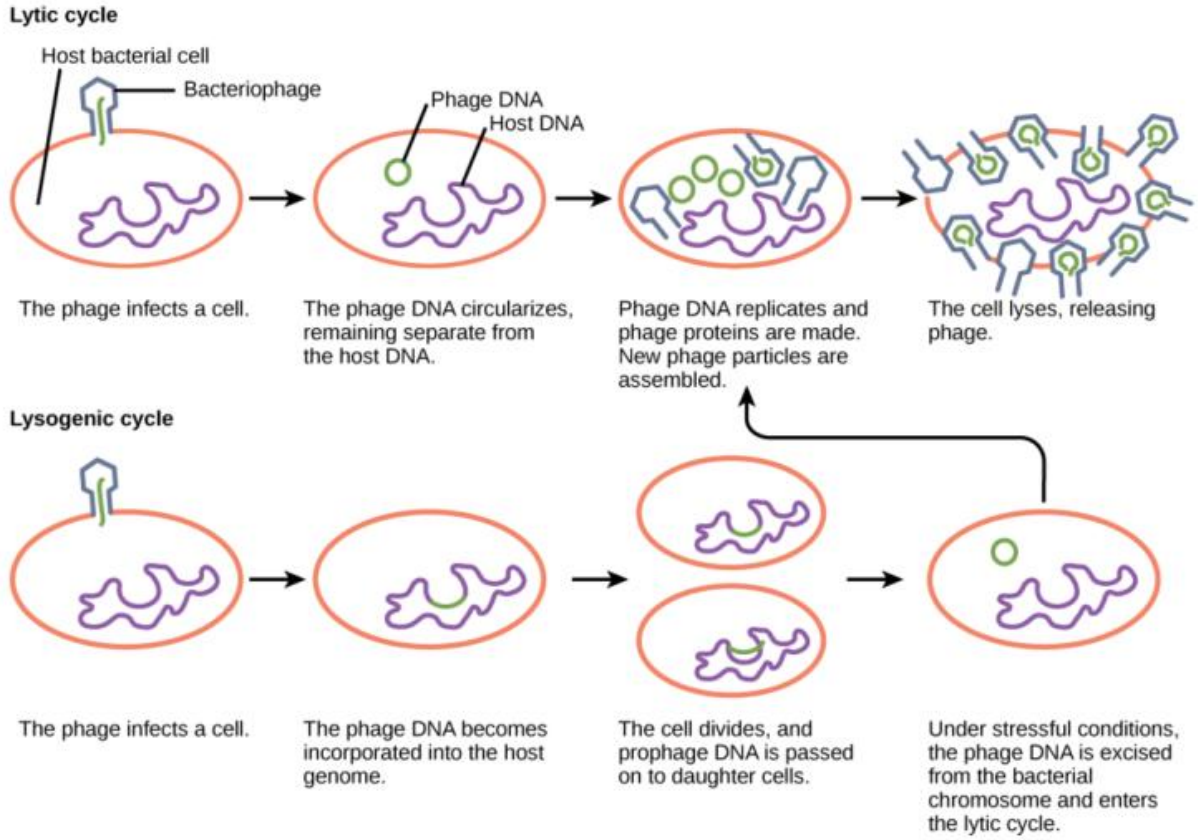


Figure 59 : Lytic cycle versus lysogenic cycle of replication

So, what dictates the replication type that will be used by a temperate phage? If there are plenty of host cells around, it is likely that a temperate phage will engage in the lytic cycle of replication, leading to a large increase in viral production. If host cells are scarce, a temperate phage is more likely to enter lysogeny, allowing for viral survival until host cell numbers increase. The same is true if the number of phage in an environment greatly outnumber the host cells, since lysogeny would allow for host cells numbers to rebound, ensuring long term viral survival.

Lysogens can experience a benefit from lysogeny as well, since it can result in lysogenic conversion, a situation where the development of a prophage leads to a change in the host's phenotype. One of the best examples of this is for the bacterium *Corynebacterium diphtheriae*, the causative agent of diphtheria. The diphtheria toxin that causes the disease is encoded within the phage genome, so only *C. diphtheriaelysogens* cause diphtheria.

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