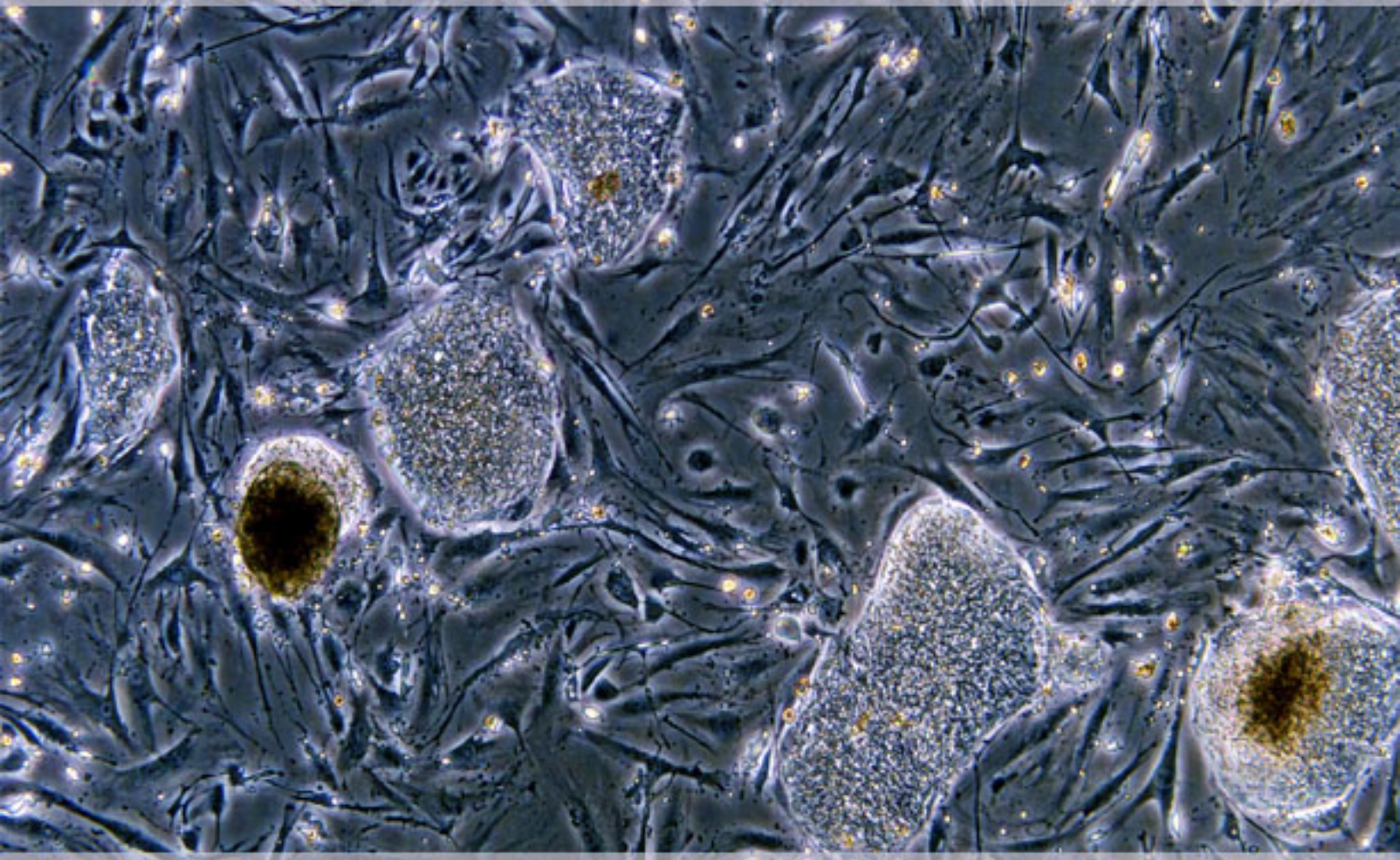
Essence of Cell in Biology



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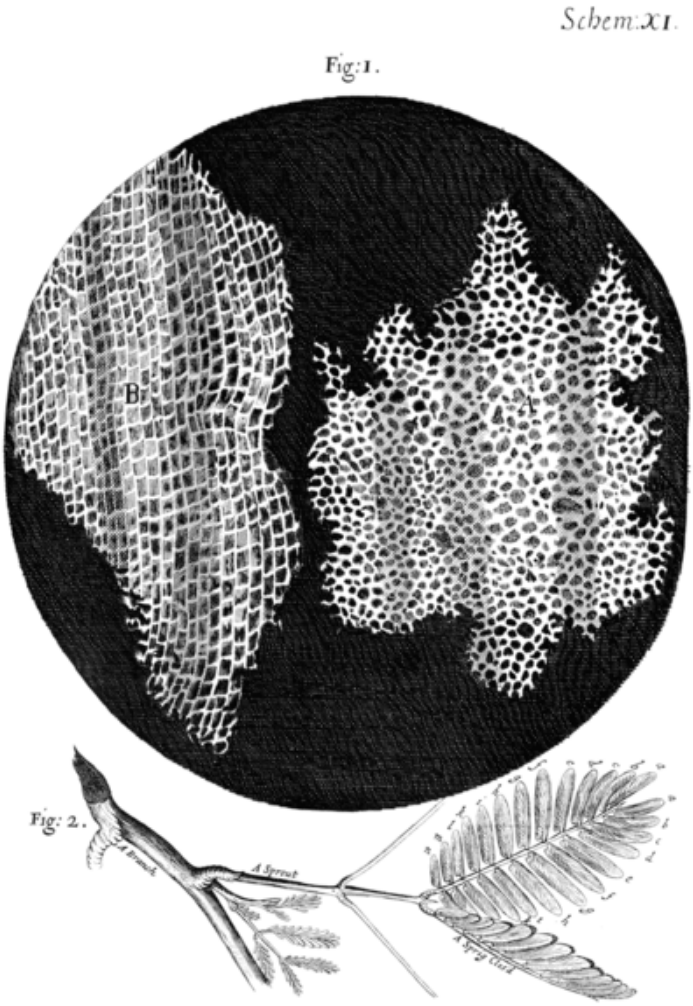
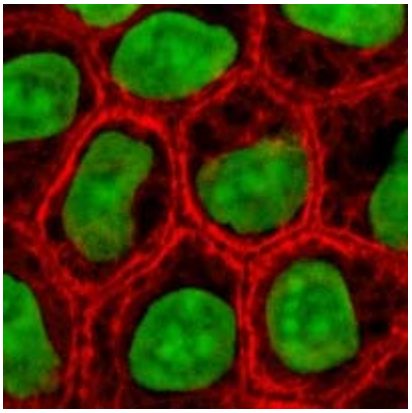
Cell (Biology)



Drawing of the structure of cork as it appeared under the microscope to Robert Hooke from Micrographia which is the origin of the word "**cell**" being used to describe the smallest unit of a living organism



Cells in culture, stained for keratin (red) and DNA (green)

The **cell** is the functional basic unit of life. It was discovered by Robert Hooke and is the functional unit of all known living organisms. It is the smallest unit of life that is classified as a living thing, and is often called the building block of life. Some organisms, such as most bacteria, are unicellular (consist of a single cell). Other organisms, such as humans, are multicellular. Humans have about 100 trillion or 10^{14} cells; a typical cell size is 10 μm and a typical cell mass is 1 nanogram. The largest cells are about 135 μm in the anterior horn in the spinal cord while granule cells in the cerebellum, the smallest, can be some 4 μm and the longest cell can reach from the toe to the lower brain stem (Pseudounipolar cells). The largest known cells are unfertilised ostrich egg cells which weigh 3.3 pounds.

In 1835, before the final cell theory was developed, Jan Evangelista Purkyně observed small "granules" while looking at the plant tissue through a microscope. The cell theory, first developed in 1839 by Matthias Jakob Schleiden and Theodor Schwann, states that all organisms are composed of one or more cells, that all cells come from preexisting cells, that vital functions of an organism occur within cells, and that all cells contain the hereditary information necessary for regulating cell functions and for transmitting information to the next generation of cells.

The word *cell* comes from the Latin *cellula*, meaning, a small room. The descriptive term for the smallest living biological structure was coined by Robert Hooke in a book he published in 1665 when he compared the cork cells he saw through his microscope to the small rooms monks lived in.

Anatomy

There are two types of cells: eukaryotic and prokaryotic. Prokaryotic cells are usually independent, while eukaryotic cells are often found in multicellular organisms.

Prokaryotic cells

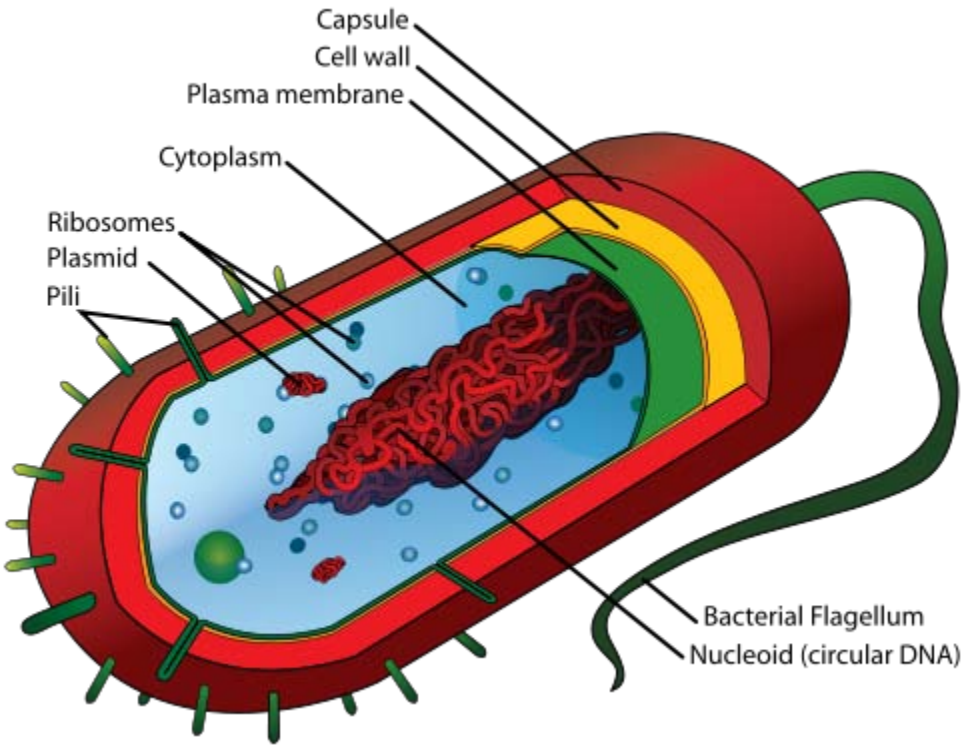


Diagram of a typical prokaryotic cell

The prokaryote cell is simpler, and therefore smaller, than a eukaryote cell, lacking a nucleus and most of the other organelles of eukaryotes. There are two kinds of prokaryotes: bacteria and archaea; these share a similar structure.

Nuclear material of prokaryotic cell consist of a single chromosome which is in direct contact with cytoplasm. Here the undefined nuclear region in the cytoplasm is called nucleoid.

A prokaryotic cell has three architectural regions:

- On the outside, flagella and pili project from the cell's surface. These are structures (not present in all prokaryotes) made of proteins that facilitate movement and communication between cells;
- Enclosing the cell is the cell envelope – generally consisting of a cell wall covering a plasma membrane though some bacteria also have a further covering layer called a capsule. The envelope gives rigidity to the cell and separates the interior of the cell from its environment, serving as a protective filter. Though most prokaryotes have a cell wall, there are exceptions such as *Mycoplasma* (bacteria) and *Thermoplasma* (archaea). The cell wall consists of *peptidoglycan* in bacteria, and acts as an additional barrier against exterior forces. It also prevents

the cell from expanding and finally bursting (cytolysis) from osmotic pressure against a hypotonic environment. Some eukaryote cells (plant cells and fungi cells) also have a cell wall;

- Inside the cell is the cytoplasmic region that contains the cell genome (DNA) and ribosomes and various sorts of inclusions. A prokaryotic chromosome is usually a circular molecule (an exception is that of the bacterium *Borrelia burgdorferi*, which causes Lyme disease). Though not forming a *nucleus*, the DNA is condensed in a *nucleoid*. Prokaryotes can carry extrachromosomal DNA elements called *plasmids*, which are usually circular. Plasmids enable additional functions, such as antibiotic resistance.

Eukaryotic cells

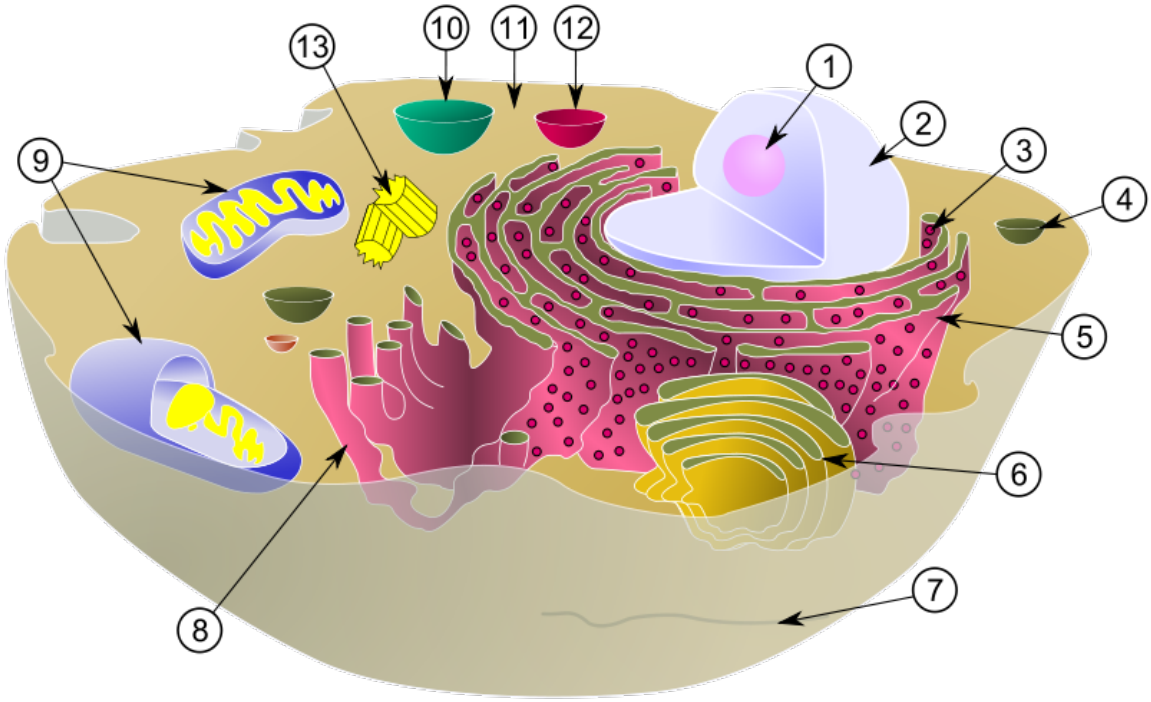


Diagram of a typical animal (eukaryotic) cell, showing subcellular components.
Organelles:
(1) nucleolus
(2) nucleus
(3) ribosome
(4) vesicle
(5) rough endoplasmic reticulum (ER)
(6) Golgi apparatus
(7) Cytoskeleton
(8) smooth endoplasmic reticulum
(9) mitochondria
(10) vacuole
(11) cytoplasm

(12) lysosome
(13) centrioles within centrosome

Eukaryotic cells are about 15 times wider than a typical prokaryote and can be as much as 1000 times greater in volume. The major difference between prokaryotes and eukaryotes is that eukaryotic cells contain membrane-bound compartments in which specific metabolic activities take place. Most important among these is a cell nucleus, a membrane-delineated compartment that houses the eukaryotic cell's DNA. This nucleus gives the eukaryote its name, which means "true nucleus." Other differences include:

- The plasma membrane resembles that of prokaryotes in function, with minor differences in the setup. Cell walls may or may not be present.
- The eukaryotic DNA is organized in one or more linear molecules, called chromosomes, which are associated with histone proteins. All chromosomal DNA is stored in the *cell nucleus*, separated from the cytoplasm by a membrane. Some eukaryotic organelles such as mitochondria also contain some DNA.
- Many eukaryotic cells are ciliated with *primary cilia*. Primary cilia play important roles in chemosensation, mechanosensation, and thermosensation. Cilia may thus be "viewed as sensory cellular antennae that coordinate a large number of cellular signaling pathways, sometimes coupling the signaling to ciliary motility or alternatively to cell division and differentiation."
- Eukaryotes can move using *motile cilia* or *flagella*. The flagella are more complex than those of prokaryotes.

Table 1: Comparison of features of prokaryotic and eukaryotic cells

	Prokaryotes	Eukaryotes
Typical organisms	bacteria, archaea	protists, fungi, plants, animals
Typical size	~ 1–10 μm	~ 10–100 μm (sperm cells, apart from the tail, are smaller)
Type of nucleus	nucleoid region; no real nucleus	real nucleus with double membrane
DNA	circular (usually)	linear molecules (chromosomes) with histone proteins
RNA-/protein-synthesis	coupled in cytoplasm	RNA-synthesis inside the nucleus protein synthesis in cytoplasm
Ribosomes	50S+30S	60S+40S
Cytoplasmatic structure	very few structures	highly structured by endomembranes and a cytoskeleton
Cell movement	flagella made of flagellin	flagella and cilia containing microtubules; lamellipodia and filopodia containing actin
Mitochondria	none	one to several thousand (though some lack mitochondria)
Chloroplasts	none	in algae and plants
Organization	usually single cells	single cells, colonies, higher multicellular

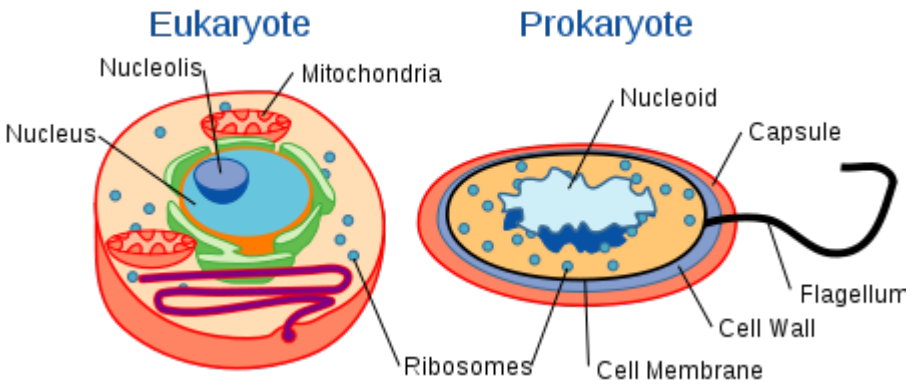
		organisms with specialized cells
Cell division	Binary fission (simple division)	Mitosis (fission or budding) Meiosis

Table 2: Comparison of structures between animal and plant cells

	Typical animal cell	Typical plant cell

Organelles	• Nucleus
	  ○ Nucleolus (within nucleus)
	• Rough endoplasmic reticulum (ER)
	• Smooth ER
	• Ribosomes
	• Cytoskeleton
	• Golgi apparatus
	• Cytoplasm
	• Mitochondria
	• Vesicles
	• Lysosomes
	• Centrosome
	  ○ Centrioles
		• Nucleus
		  ○ Nucleolus (within nucleus)
		• Rough ER
		• Smooth ER
		• Ribosomes
		• Cytoskeleton
		• Golgi apparatus (dictiosomes)
		• Cytoplasm
		• Mitochondria
		• Plastids and its derivatives
		• Vacuole(s)
		• Cell wall

Subcellular components



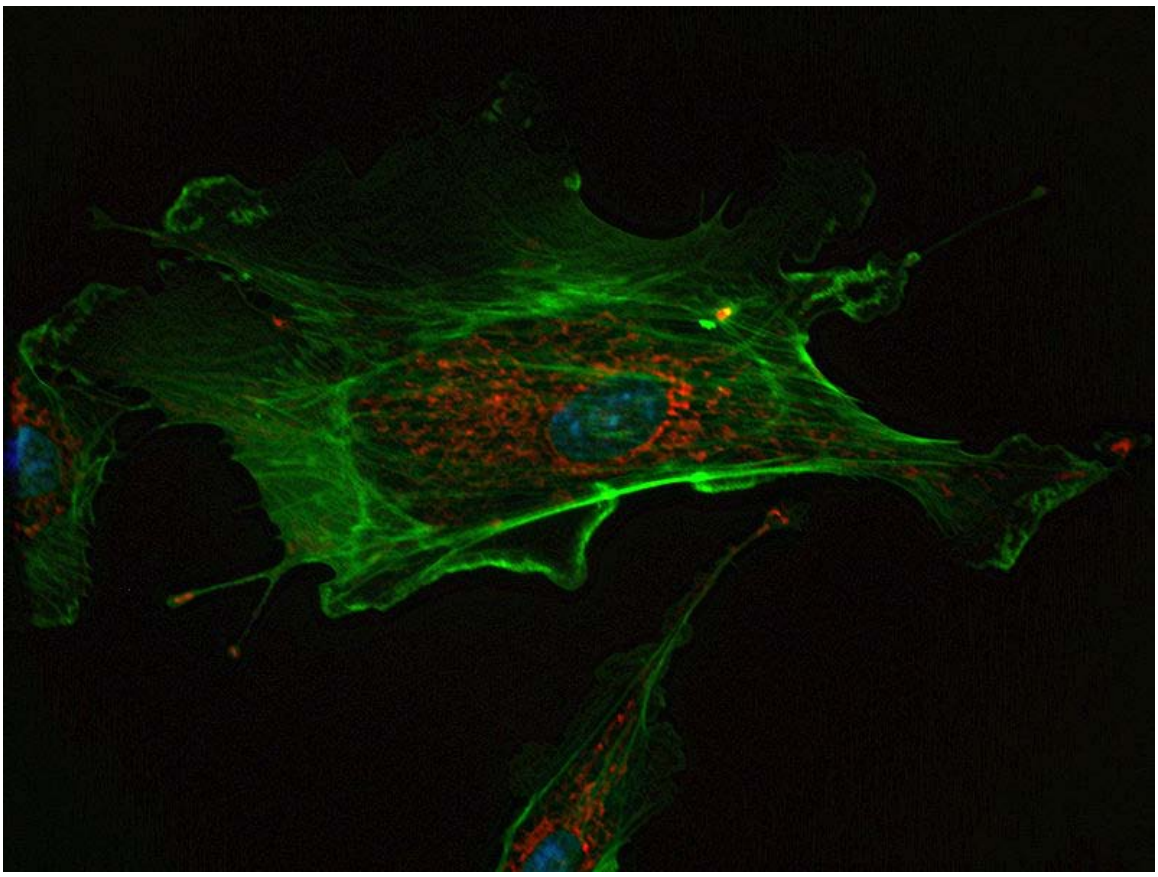
The cells of eukaryotes (left) and prokaryotes (right)

All cells, whether prokaryotic or eukaryotic, have a membrane that envelops the cell, separates its interior from its environment, regulates what moves in and out (selectively permeable), and maintains the electric potential of the cell. Inside the membrane, a salty cytoplasm takes up most of the cell volume. All cells possess DNA, the hereditary material of genes, and RNA, containing the information necessary to build various proteins such as enzymes, the cell's primary machinery. There are also other kinds of biomolecules in cells.

Membrane

The cytoplasm of a cell is surrounded by a cell membrane or *plasma membrane*. The plasma membrane in plants and prokaryotes is usually covered by a cell wall. This membrane serves to separate and protect a cell from its surrounding environment and is made mostly from a double layer of lipids (hydrophobic fat-like molecules) and hydrophilic phosphorus molecules. Hence, the layer is called a phospholipid bilayer. It may also be called a fluid mosaic membrane. Embedded within this membrane is a variety of protein molecules that act as channels and pumps that move different molecules into and out of the cell. The membrane is said to be 'semi-permeable', in that it can either let a substance (molecule or ion) pass through freely, pass through to a limited extent or not pass through at all. Cell surface membranes also contain receptor proteins that allow cells to detect external signaling molecules such as hormones.

Cytoskeleton



Bovine Pulmonary Artery Endothelial cell: nuclei stained blue, mitochondria stained red, and F-actin, an important component in microfilaments, stained green. Cell imaged on a fluorescent microscope.

The cytoskeleton acts to organize and maintain the cell's shape; anchors organelles in place; helps during endocytosis, the uptake of external materials by a cell, and cytokinesis, the separation of daughter cells after cell division; and moves parts of the

cell in processes of growth and mobility. The eukaryotic cytoskeleton is composed of microfilaments, intermediate filaments and microtubules. There is a great number of proteins associated with them, each controlling a cell's structure by directing, bundling, and aligning filaments. The prokaryotic cytoskeleton is less well-studied but is involved in the maintenance of cell shape, polarity and cytokinesis.

Genetic material

Two different kinds of genetic material exist: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Most organisms use DNA for their long-term information storage, but some viruses (e.g., retroviruses) have RNA as their genetic material. The biological information contained in an organism is encoded in its DNA or RNA sequence. RNA is also used for information transport (e.g., mRNA) and enzymatic functions (e.g., ribosomal RNA) in organisms that use DNA for the genetic code itself. Transfer RNA (tRNA) molecules are used to add amino acids during protein translation.

Prokaryotic genetic material is organized in a simple circular DNA molecule (the bacterial chromosome) in the nucleoid region of the cytoplasm. Eukaryotic genetic material is divided into different, linear molecules called chromosomes inside a discrete nucleus, usually with additional genetic material in some organelles like mitochondria and chloroplasts.

A human cell has genetic material contained in the cell nucleus (the nuclear genome) and in the mitochondria (the mitochondrial genome). In humans the nuclear genome is divided into 23 pairs of linear DNA molecules called chromosomes. The mitochondrial genome is a circular DNA molecule distinct from the nuclear DNA. Although the mitochondrial DNA is very small compared to nuclear chromosomes, it codes for 13 proteins involved in mitochondrial energy production and specific tRNAs.

Foreign genetic material (most commonly DNA) can also be artificially introduced into the cell by a process called transfection. This can be transient, if the DNA is not inserted into the cell's genome, or stable, if it is. Certain viruses also insert their genetic material into the genome.

Organelles

The human body contains many different organs, such as the heart, lung, and kidney, with each organ performing a different function. Cells also have a set of "little organs," called organelles, that are adapted and/or specialized for carrying out one or more vital functions. Both eukaryotic and prokaryotic cells have organelles but organelles in eukaryotes are generally more complex and may be membrane bound.

There are several types of organelles in a cell. Some (such as the nucleus and golgi apparatus) are typically solitary, while others (such as mitochondria, peroxisomes and lysosomes) can be numerous (hundreds to thousands). The cytosol is the gelatinous fluid that fills the cell and surrounds the organelles.

Cell nucleus – eukaryotes only - a cell's information center

The cell nucleus is the most conspicuous organelle found in a eukaryotic cell. It houses the cell's chromosomes, and is the place where almost all DNA replication and RNA synthesis (transcription) occur. The nucleus is spherical and separated from the cytoplasm by a double membrane called the nuclear envelope. The nuclear envelope isolates and protects a cell's DNA from various molecules that could accidentally damage its structure or interfere with its processing. During processing, DNA is transcribed, or copied into a special RNA, called messenger RNA (mRNA). This mRNA is then transported out of the nucleus, where it is translated into a specific protein molecule. The nucleolus is a specialized region within the nucleus where ribosome subunits are assembled. In prokaryotes, DNA processing takes place in the cytoplasm.

Diagram of a cell nucleus

Mitochondria and Chloroplasts – eukaryotes only - the power generators

Mitochondria are self-replicating organelles that occur in various numbers, shapes, and sizes in the cytoplasm of all eukaryotic cells. Mitochondria play a critical role in generating energy in the eukaryotic cell. Mitochondria generate the cell's energy by oxidative phosphorylation, using oxygen to release energy stored in cellular nutrients (typically pertaining to glucose) to generate ATP. Mitochondria multiply by splitting in two. Respiration occurs in the cell mitochondria. Organelles that are modified chloroplasts are broadly called plastids, and are involved in energy storage through photosynthesis, which uses solar energy to generate carbohydrates and oxygen from carbon dioxide and water. Mitochondria and chloroplasts each contain their own genome, which is separate and distinct from the nuclear genome of a cell. Both organelles contain this DNA in circular plasmids, much like prokaryotic cells, strongly supporting the evolutionary theory of endosymbiosis; since these organelles contain

their own genomes and have other similarities to prokaryotes, they are thought to have developed through a symbiotic relationship after being engulfed by a primitive cell.

Endoplasmic reticulum – eukaryotes only

The endoplasmic reticulum (ER) is the transport network for molecules targeted for certain modifications and specific destinations, as compared to molecules that will float freely in the cytoplasm. The ER has two forms: the rough ER, which has ribosomes on its surface and secretes proteins into the cytoplasm, and the smooth ER, which lacks them. Smooth ER plays a role in calcium sequestration and release.

Golgi apparatus – eukaryotes only

The primary function of the Golgi apparatus is to process and package the macromolecules such as proteins and lipids that are synthesized by the cell. It is particularly important in the processing of proteins for secretion. The Golgi apparatus forms a part of the endomembrane system of eukaryotic cells. Vesicles that enter the Golgi apparatus are processed in a cis to trans direction, meaning they coalesce on the cis side of the apparatus and after processing pinch off on the opposite (trans) side to form a new vesicle in the animal cell.

Diagram of an endomembrane system

Ribosomes

The ribosome is a large complex of RNA and protein molecules. They each consist of two subunits, and act as an assembly line where RNA from the nucleus is used to synthesise proteins from amino acids. Ribosomes can be found either floating freely or bound to a membrane (the rough endoplasmatic reticulum in eukaryotes, or the cell membrane in prokaryotes).

Lysosomes and Peroxisomes – eukaryotes only

Lysosomes contain digestive enzymes (acid hydrolases). They digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. Peroxisomes have enzymes that rid the cell of toxic peroxides. The cell could not house these

destructive enzymes if they were not contained in a membrane-bound system. These organelles are often called a "suicide bag" because of their ability to detonate and destroy the cell.

Centrosome – the cytoskeleton organiser

The centrosome produces the microtubules of a cell – a key component of the cytoskeleton. It directs the transport through the ER and the Golgi apparatus. Centrosomes are composed of two centrioles, which separate during cell division and help in the formation of the mitotic spindle. A single centrosome is present in the animal cells. They are also found in some fungi and algae cells.

Vacuoles

Vacuoles store food and waste. Some vacuoles store extra water. They are often described as liquid filled space and are surrounded by a membrane. Some cells, most notably *Amoeba*, have contractile vacuoles, which can pump water out of the cell if there is too much water. The vacuoles of eukaryotic cells are usually larger in those of plants than animals.

Structures outside the cell wall

Capsule

A gelatinous capsule is present in some bacteria outside the cell wall. The capsule may be polysaccharide as in pneumococci, meningococci or polypeptide as *Bacillus anthracis* or hyaluronic acid as in streptococci. Capsules are not marked by ordinary stain and can be detected by special stain. The capsule is antigenic. The capsule has antiphagocytic function so it determines the virulence of many bacteria. It also plays a role in attachment of the organism to mucous membranes.

Flagella

Flagella are the organelles of cellular mobility. They arise from cytoplasm and extrude through the cell wall. They are long and thick thread-like appendages, protein in nature. Are most commonly found in bacteria cells but are found in animal cells as well.

Fimbriae (pili)

They are short and thin hair like filaments, formed of protein called pilin (antigenic). Fimbriae are responsible for attachment of bacteria to specific receptors of human cell (adherence). There are special types of pili called (sex pili) involved in conjunction.

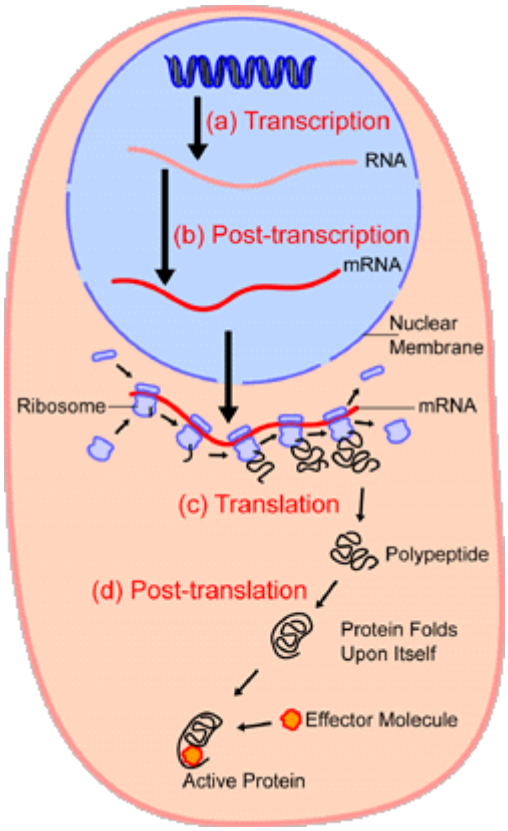
Functions

Growth and metabolism

Between successive cell divisions, cells grow through the functioning of cellular metabolism. Cell metabolism is the process by which individual cells process nutrient molecules. Metabolism has two distinct divisions: catabolism, in which the cell breaks down complex molecules to produce energy and reducing power, and anabolism, in which the cell uses energy and reducing power to construct complex molecules and perform other biological functions. Complex sugars consumed by the organism can be broken down into a less chemically complex sugar molecule called glucose. Once inside the cell, glucose is broken down to make adenosine triphosphate (ATP), a form of energy, through two different pathways.

The first pathway, glycolysis, requires no oxygen and is referred to as anaerobic metabolism. Each reaction is designed to produce some hydrogen ions that can then be used to make energy packets (ATP). In prokaryotes, glycolysis is the only method used for converting energy.

The second pathway, called the Krebs cycle, or citric acid cycle, occurs inside the mitochondria and can generate enough ATP to run all the cell functions.



An overview of protein synthesis.
Within the nucleus of the cell (*light blue*), genes (DNA, *dark blue*) are transcribed into RNA. This RNA is then subject to post-transcriptional modification and control, resulting in a mature mRNA (*red*) that is then transported out of the nucleus and into the cytoplasm (*peach*), where it undergoes translation into a protein. mRNA is translated by ribosomes (*purple*) that match the three-base codons of the mRNA to the three-base anti-codons of the appropriate tRNA. Newly synthesized proteins (*black*) are often further modified, such as by binding to an effector molecule (*orange*), to become fully active.

Creation

Cell division involves a single cell (called a *mother cell*) dividing into two daughter cells. This leads to growth in multicellular organisms (the growth of tissue) and to procreation (vegetative reproduction) in unicellular organisms.

Prokaryotic cells divide by binary fission. Eukaryotic cells usually undergo a process of nuclear division, called mitosis, followed by division of the cell, called cytokinesis. A diploid cell may also undergo meiosis to produce haploid cells, usually four. Haploid cells serve as gametes in multicellular organisms, fusing to form new diploid cells.

DNA replication, or the process of duplicating a cell's genome, is required every time a cell divides. Replication, like all cellular activities, requires specialized proteins for carrying out the job.

Protein synthesis

Cells are capable of synthesizing new proteins, which are essential for the modulation and maintenance of cellular activities. This process involves the formation of new protein molecules from amino acid building blocks based on information encoded in DNA/RNA. Protein synthesis generally consists of two major steps: transcription and translation.

Transcription is the process where genetic information in DNA is used to produce a complementary RNA strand. This RNA strand is then processed to give messenger RNA (mRNA), which is free to migrate through the cell. mRNA molecules bind to protein-RNA complexes called ribosomes located in the cytosol, where they are translated into polypeptide sequences. The ribosome mediates the formation of a polypeptide sequence based on the mRNA sequence. The mRNA sequence directly relates to the polypeptide sequence by binding to transfer RNA (tRNA) adapter molecules in binding pockets within the ribosome. The new polypeptide then folds into a functional three-dimensional protein molecule.

Movement or motility

Cells can move during many processes: such as wound healing, the immune response and cancer metastasis. For wound healing to occur, white blood cells and cells that ingest bacteria move to the wound site to kill the microorganisms that cause infection.

At the same time fibroblasts (connective tissue cells) move there to remodel damaged structures. In the case of tumor development, cells from a primary tumor move away and spread to other parts of the body. Cell motility involves many receptors, crosslinking, bundling, binding, adhesion, motor and other proteins. The process is divided into three steps – protrusion of the leading edge of the cell, adhesion of the leading edge and de-adhesion at the cell body and rear, and cytoskeletal contraction to pull the cell forward. Each step is driven by physical forces generated by unique segments of the cytoskeleton.

Evolution

The origin of cells has to do with the origin of life, which began the history of life on Earth.

Origin of the first cell

There are several theories about the origin of small molecules that could lead to life in an early Earth. One is that they came from meteorites. Another is that they were created at deep-sea vents. A third is that they were synthesized by lightning in a reducing atmosphere; although it is not clear if Earth had such an atmosphere. There are essentially no experimental data defining what the first self-replicating forms were. RNA is generally assumed to be the earliest self-replicating molecule, as it is capable of both storing genetic information and catalyzing chemical reactions. But some other entity with the potential to self-replicate could have preceded RNA, like clay or peptide nucleic acid.

Cells emerged at least 4.0–4.3 billion years ago. The current belief is that these cells were heterotrophs. An important characteristic of cells is the cell membrane, composed of a bilayer of lipids. The early cell membranes were probably more simple and permeable than modern ones, with only a single fatty acid chain per lipid. Lipids are known to spontaneously form bilayered vesicles in water, and could have preceded RNA. But the first cell membranes could also have been produced by catalytic RNA, or even have required structural proteins before they could form.

Origin of eukaryotic cells

The eukaryotic cell seems to have evolved from a symbiotic community of prokaryotic cells. DNA-bearing organelles like the mitochondria and the chloroplasts are almost certainly what remains of ancient symbiotic oxygen-breathing proteobacteria and cyanobacteria, respectively, where the rest of the cell seems to be derived from an ancestral archaean prokaryote cell – a theory termed the endosymbiotic theory.

There is still considerable debate about whether organelles like the hydrogenosome predated the origin of mitochondria, or viceversa.

Sex, as the stereotyped choreography of meiosis and syngamy that persists in nearly all extant eukaryotes, may have played a role in the transition from prokaryotes to eukaryotes. An 'origin of sex as vaccination' theory suggests that the eukaryote genome

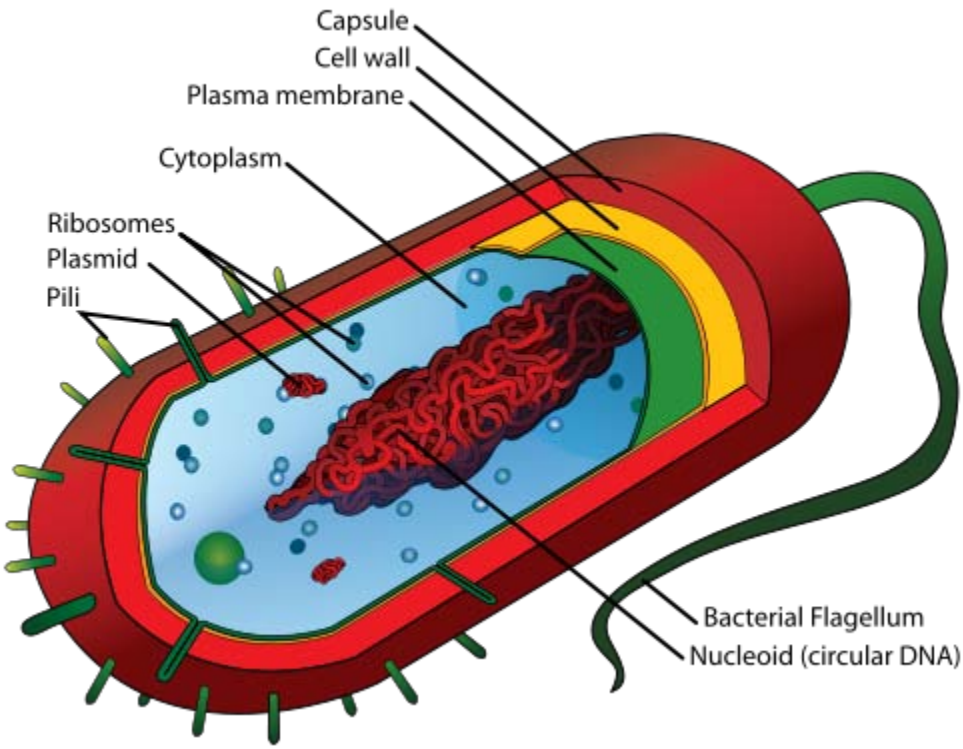
accreted from prokaryan parasite genomes in numerous rounds of lateral gene transfer. Sex-as-syngamy (fusion sex) arose when infected hosts began swapping nuclearized genomes containing co-evolved, vertically transmitted symbionts that conveyed protection against horizontal infection by more virulent symbionts.

History

- 1632–1723: Antonie van Leeuwenhoek teaches himself to grind lenses, builds a microscope and draws protozoa, such as *Vorticella* from rain water, and bacteria from his own mouth.
- 1665: Robert Hooke discovers cells in cork, then in living plant tissue using an early microscope.
- 1839: Theodor Schwann and Matthias Jakob Schleiden elucidate the principle that plants and animals are made of cells, concluding that cells are a common unit of structure and development, and thus founding the cell theory.
- The belief that life forms can occur spontaneously (*generatio spontanea*) is contradicted by Louis Pasteur (1822–1895) (although Francesco Redi had performed an experiment in 1668 that suggested the same conclusion).
- 1855: Rudolf Virchow states that cells always emerge from cell divisions (*omnis cellula ex cellula*).
- 1931: Ernst Ruska builds first transmission electron microscope (TEM) at the University of Berlin. By 1935, he has built an EM with twice the resolution of a light microscope, revealing previously unresolvable organelles.
- 1953: Watson and Crick made their first announcement on the double-helix structure for DNA on February 28.
- 1981: Lynn Margulis published *Symbiosis in Cell Evolution* detailing the endosymbiotic theory.

Chapter- 2

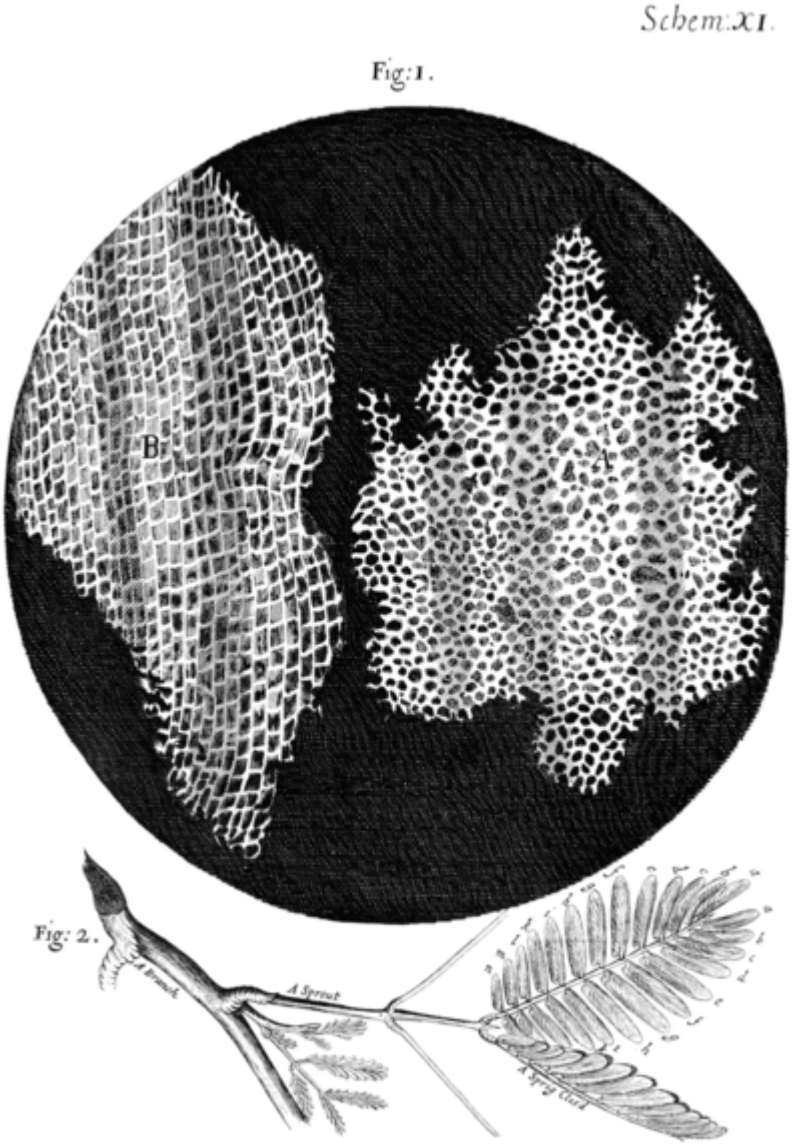
Cell Theory



A prokaryote

Cell theory refers to the idea that cells are the basic unit of structure in every living thing. Development of this theory during the mid 17th century was made possible by advances in microscopy. This theory is one of the foundations of biology. The theory says that new cells are formed from other existing cells, and that the cell is a fundamental unit of structure, function and organization in all living organisms.

History



Drawing of the structure of cork by Robert Hooke that appeared in *Micrographia*

The cell was discovered by Robert Hooke in 1665. He examined (under a coarse, compound microscope) very thin slices of cork and saw a multitude of tiny pores that he remarked looked like the walled compartments a monk would live in. Because of this association, Hooke called them cells, the name they still bear. However, Hooke did not know their real structure or function. Hooke's description of these cells (which were actually non-living cell walls) was published in *Micrographia*.. His cell observations gave no indication of the nucleus and other organelles found in most living cells.

The first man to witness a live cell under a microscope was Antony van Leeuwenhoek (although the first man to make a compound microscope was Zacharias Janssen), who in 1674 described the algae *Spirogyra* and named the moving organisms animalcules, meaning "little animals".. Leeuwenhoek probably also saw bacteria. Cell theory was in contrast to the vitalism theories proposed before the discovery of cells.

The idea that cells were separable into individual units was proposed by Ludolph Christian Treviranus and Johann Jacob Paul Moldenhawer. All of this finally led to Henri Dutrochet formulating one of the fundamental tenets of modern cell theory by declaring that "The cell is the fundamental element of organization"

The observations of Hooke, Leeuwenhoek, Schleiden, Schwann, Virchow, and others led to the development of the cell theory. The cell theory is a widely accepted explanation of the relationship between cells and living things. The cell theory states:

- All living things or organisms are made of cells and their products.
- New cells are created by old cells dividing into two.
- Cells are the basic building units of life.

The cell theory holds true for all living things, no matter how big or small, or how simple or complex. Since according to research, cells are common to all living things, they can provide information about all life. And because all cells come from other cells, scientists can study cells to learn about growth, reproduction, and all other functions that living things perform. By learning about cells and how they function, you can learn about all types of living things.

Credit for developing cell theory is usually given to three scientists: Theodor Schwann, Matthias Jakob Schleiden, and Rudolf Virchow. In 1839, Schwann and Schleiden suggested that cells were the basic unit of life. Their theory accepted the first two tenets of modern cell theory. However the cell theory of Schleiden differed from modern cell theory in that it proposed a method of spontaneous crystallization that he called "Free Cell Formation". In 1858, Rudolf Virchow concluded that all cells come from pre-existing cells, thus completing the classical cell theory.

Classical interpretation

1. All living organisms are made up of one or more cells.
2. Cells are the basic unit of life.
3. All cells arise from pre-existing cells.
4. The cell is the unit of structure, physiology, and organization in living things.
5. The cell retains a dual existence as a distinct entity and a building block in the construction of organisms.

Modern interpretation

The generally accepted parts of modern cell theory include:

1. The cell is the fundamental unit of structure and function in living organisms.
2. All cells arise from pre-existing cells by division.
3. Energy flow (metabolism and biochemistry) occurs within cells.
4. Cells contain hereditary information (DNA) which is passed from cell to cell during cell division.
5. All cells are basically the same in chemical composition in organisms of similar species.
6. All known living things are made up of one or more cells.
7. Some organisms are made up of only one cell and are known as unicellular organisms.
8. Others are multicellular, composed of a number of cells.
9. The activity of an organism depends on the total activity of independent cells.

Types of cells

Cells can be subdivided into the following subcategories:

1. *Prokaryotes*: Prokaryotes lack a nucleus (though they do have circular DNA) and other membrane-bound organelles (though they do contain ribosomes). Bacteria and Archaea are two domains of prokaryotes.
2. *Eukaryotes*: Eukaryotes, on the other hand, have distinct nuclei bound by a nuclear membrane and membrane-bound organelles (mitochondria, chloroplasts, lysosomes, rough and smooth endoplasmic reticulum, vacuoles). In addition, they possess organized chromosomes which store genetic material.

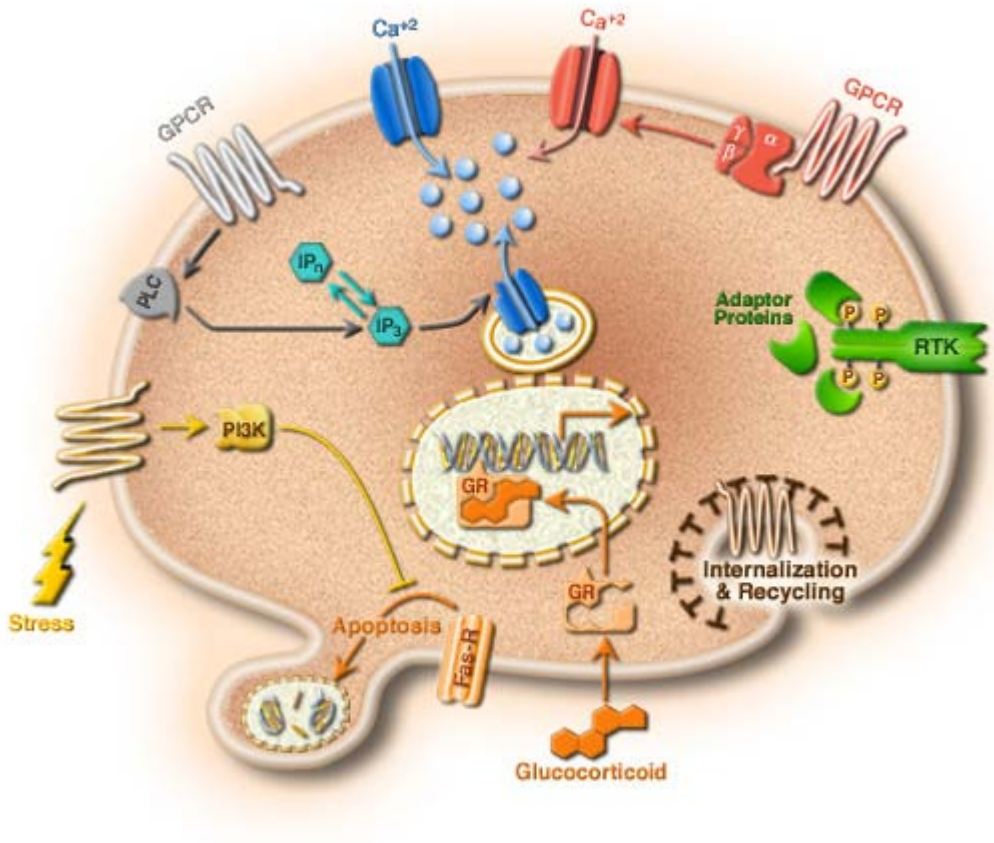
Chapter- 3

Cell Biology and Cell Division

Cell biology

Cell biology (formerly **cytology**, from the Greek *kytos*, "container") is a scientific discipline that studies cells – their physiological properties, their structure, the organelles they contain, interactions with their environment, their life cycle, division and death. This is done both on a microscopic and molecular level. Cell biology research encompasses both the great diversity of single-celled organisms like bacteria and protozoa, as well as the many specialized cells in multicellular organisms such as humans.

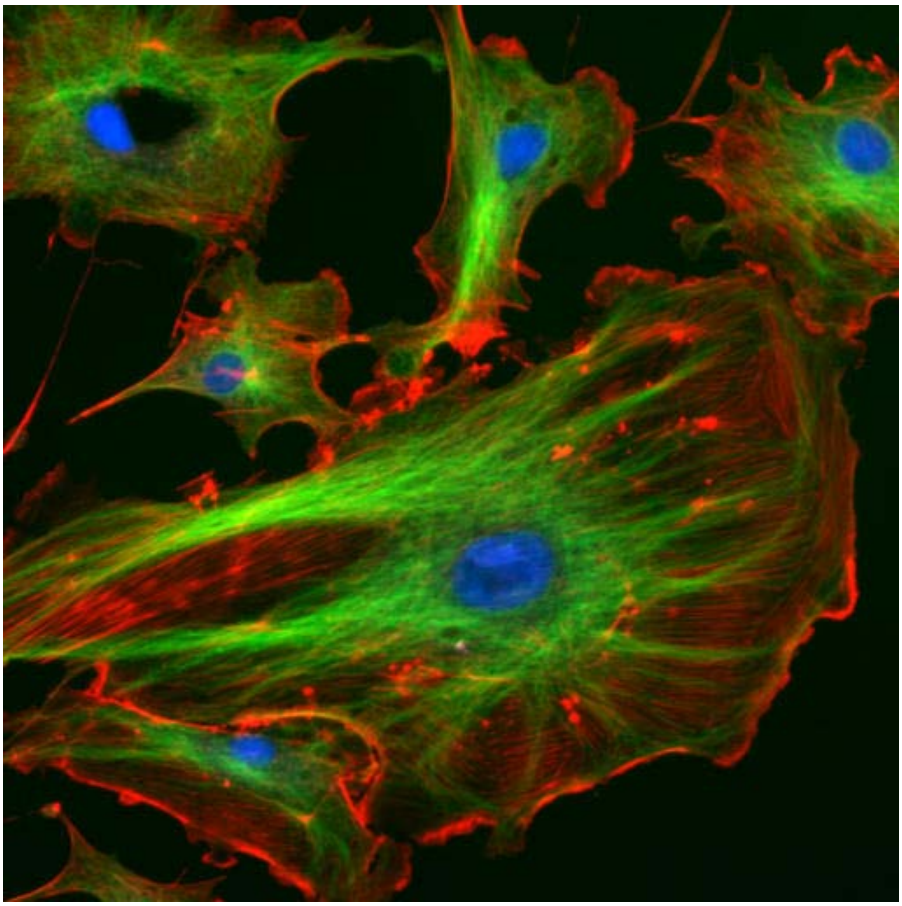
Knowing the components of cells and how cells work is fundamental to all biological sciences. Appreciating the similarities and differences between cell types is particularly important to the fields of cell and molecular biology as well as to biomedical fields such as cancer research and developmental biology. These fundamental similarities and differences provide a unifying theme, sometimes allowing the principles learned from studying one cell type to be extrapolated and generalized to other cell types. Hence, research in cell biology is closely related to genetics, biochemistry, molecular biology, immunology, and developmental biology.



Understanding cells in terms of their molecular components.

Processes

Movement of proteins



Endothelial cells under the microscope. Nuclei are stained blue with DAPI, microtubles are marked green by an antibody and actin filaments are labelled red with phalloidin.

Each type of protein is usually sent to a particular part of the cell. An important part of cell biology is the investigation of molecular mechanisms by which proteins are moved to different places inside cells or secreted from cells.

Most proteins are synthesized by ribosomes in the cytoplasm. This process is known as protein biosynthesis. Biosynthesis (also called biogenesis) is an enzyme-catalyzed process in cells of living organisms by which substrates are converted to more complex products (also simply known as protein translation). Some proteins, such as those to be incorporated in membranes (known as membrane proteins), are transported into the "rough" endoplasmic reticulum (ER) during synthesis. This process can be followed by transportation and processing in the Golgi apparatus. From the Golgi, membrane proteins can move to the plasma membrane, to other sub-cellular compartments, or they can be

secreted from the cell. The ER and Golgi can be thought of as the "membrane protein synthesis compartment" and the "membrane protein processing compartment", respectively. There is a semi-constant flux of proteins through these compartments. ER and Golgi-resident proteins associate with other proteins but remain in their respective compartments. Other proteins "flow" through the ER and Golgi to the plasma membrane. Motor proteins transport membrane protein-containing vesicles along cytoskeletal tracks to distant parts of cells such as axon terminals.

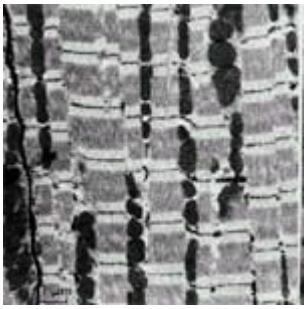
Some proteins that are made in the cytoplasm contain structural features that target them for transport into mitochondria or the nucleus. Some mitochondrial proteins are made inside mitochondria and are coded for by mitochondrial DNA. In plants, chloroplasts also make some cell proteins.

Extracellular and cell surface proteins destined to be degraded can move back into intracellular compartments upon being incorporated into endocytosed vesicles some of which fuse with lysosomes where the proteins are broken down to their individual amino acids. The degradation of some membrane proteins begins while still at the cell surface when they are cleaved by secretases. Proteins that function in the cytoplasm are often degraded by proteasomes.

Other cellular processes

- Active transport and Passive transport - Movement of molecules into and out of cells.
- Autophagy - The process whereby cells "eat" their own internal components or microbial invaders.
- Adhesion - Holding together cells and tissues.
- Reproduction - Made possible by the combination of sperm made in the testiculi(contained in some male cells nucleus') and the egg made in the ovary(contained in the nucleus of a female cell). When the sperm breaks through the hard outer shell of the egg a new cell embryo is formed, which, in humans, grows to full size in 9 months.
- Cell movement: Chemotaxis, Contraction, cilia and flagella.
- Cell signaling - Regulation of cell behavior by signals from outside.
- DNA repair and Cell death
- Metabolism: Glycolysis, respiration, Photosynthesis
- Transcription and mRNA splicing - gene expression.

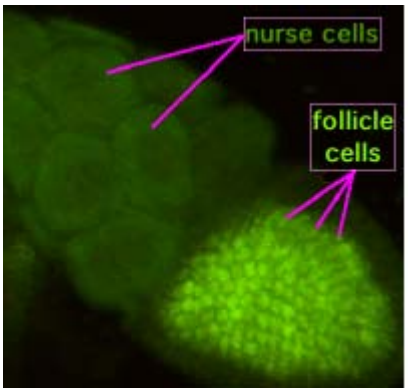
Internal cellular structures



Electron micrograph.

- Chloroplast - key organelle for photosynthesis (only found in plant cells)
- Cilia - motile microtubule-containing structures of eukaryotes
- Cytoplasm - contents of the main fluid-filled space inside cells
- Cytoskeleton - protein filaments inside cells
- Endoplasmic reticulum - major site of membrane protein synthesis
- Flagella - motile structures of bacteria, archaea and eukaryotes
- Golgi apparatus - site of protein glycosylation in the endomembrane system
- Lipid bilayer - fundamental organizational structure of cell membranes
- Membrane lipid and protein barrier
- Mitochondrion - major energy-producing organelle by releasing it in the form of ATP
- Nucleus - holds most of the DNA of eukaryotic cells and controls all cellular activities
- Organelle - term used for major subcellular structures
- Ribosome - RNA and protein complex required for protein synthesis in cells
- Vesicle - small membrane-bounded spheres inside cells

Techniques used to study cells



Cells may be observed under the microscope. This includes the Optical Microscope, Transmission Electron Microscope, Scanning Electron Microscope, Fluorescence Microscope, and by Confocal Microscopy.

Several different techniques exist to study cells.

- Cell culture is the basic technique of growing cells in a laboratory independent of an organism.
- Immunostaining, also known as immunohistochemistry, is a specialized histological method used to localize proteins in cells or tissue slices. Unlike regular histology, which uses stains to identify cells, cellular components or protein classes, immunostaining requires the reaction of an antibody directed against the protein of interest within the tissue or cell. Through the use of proper controls and published protocols (need to add reference links here), specificity of the antibody-antigen reaction can be achieved. Once this complex is formed, it is identified via either a "tag" attached directly to the antibody, or added in an additional technical step. Commonly used "tags" include fluorophores or enzymes. In the case of the former, detection of the location of the "immuno-stained" protein occurs via fluorescence microscopy. With an enzymatic tag, such as horse radish peroxidase, a chemical reaction is carried out that results in a dark color in the location of the protein of interest. This darkened pattern is then detected using light microscopy.
- Computational genomics is used to find patterns in genomic information
- DNA microarrays identify changes in transcript levels between different experimental conditions.
- Gene knockdown mutates a selected gene.
- In situ hybridization shows which cells are expressing a particular RNA transcript.
- PCR can be used to determine how many copies of a gene are present in a cell.
- Transfection introduces a new gene into a cell, usually an expression construct

Purification of cells and their parts Purification may be performed using the following methods:

- Cell fractionation
    - Release of cellular organelles by disruption of cells.
    - Separation of different organelles by centrifugation.
- Flow cytometry
- Immunoprecipitation
- Proteins extracted from cell membranes by detergents and salts or other kinds of chemicals.

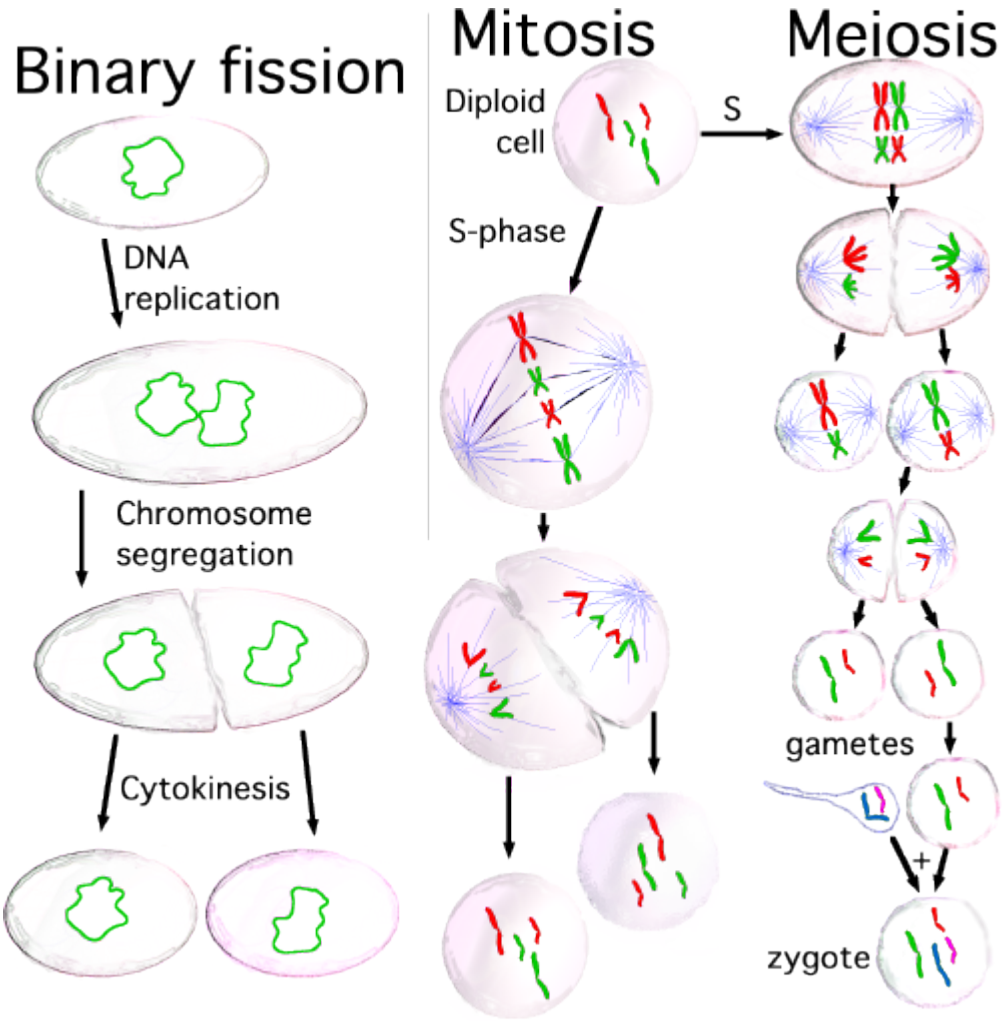
Cell division

Cell division is the process by which a *parent cell* divides into two or more *daughter cells*. Cell division is usually a small segment of a larger cell cycle. This type of cell division in eukaryotes is known as mitosis, and leaves the daughter cell capable of dividing again. The corresponding sort of cell division in prokaryotes is known as binary fission. In another type of cell division present only in eukaryotes, called meiosis, a cell is permanently transformed into a gamete and cannot divide again until fertilization. Right before the parent cell splits, it undergoes DNA replication.

For simple unicellular organisms such as the amoeba, one cell division is equivalent to reproduction-- an entire new organism is created. On a larger scale, mitotic cell division can create progeny from multicellular organisms, such as plants that grow from cuttings. Cell division also enables a sexually reproducing organisms to develop from the one-celled zygote, which itself was produced by cell division from gametes. And after growth, cell division allows for continual construction and repair of the organism. A human being's body experiences about 10,000 trillion cell divisions in a lifetime.

The primary concern of cell division is the maintenance of the original cell's genome. Before division can occur, the genomic information which is stored in chromosomes must be replicated, and the duplicated genome separated cleanly between cells. A great deal of cellular infrastructure is involved in keeping genomic information consistent between "generations".

Variants



Three types of cell division

Cells are classified into two categories: simple, non-nucleated prokaryotic cells, and complex, nucleated eukaryotic cells. By dint of their structural differences, **eukaryotic** and **prokaryotic** cells do not divide in the same way.

Furthermore, the pattern of cell division that transforms eukaryotic stem cells into gametes (sperm in males or ova in females) is different from that of eukaryotic somatic (non-germ) cells.

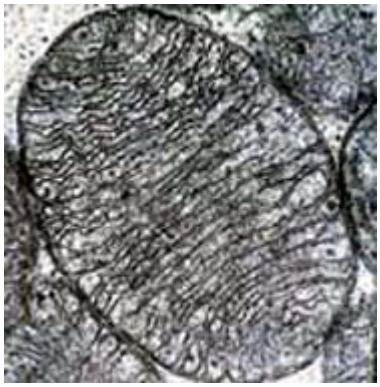
Degradation

Multicellular organisms replace worn-out cells through cell division. In some animals, however, cell division eventually halts. In humans this occurs on average, after 52 divisions, known as the Hayflick limit. The cell is then referred to as senescent. Cells stop

dividing because the telomeres, protective bits of DNA on the end of a chromosome required for replication, shorten with each copy, eventually being consumed, as described in telomere shortening. Cancer cells, on the other hand, are not thought to degrade in this way, if at all. An enzyme called telomerase, present in large quantities in cancerous cells, rebuilds the telomeres, allowing division to continue indefinitely.

Chapter- 4

Endosymbiotic Theory



Electron micrograph of a mitochondrion showing its mitochondrial matrix and membranes

The **endosymbiotic theory** concerns the origins of mitochondria, plastids (e.g. chloroplasts), and possibly other organelles of eukaryotic cells. According to this theory, certain organelles originated as free-living bacteria that were taken inside another cell as endosymbionts. Mitochondria developed from proteobacteria (in particular, Rickettsiales or close relatives) and chloroplasts from cyanobacteria.

History

The endosymbiotic (from the greek: endo- meaning inside and -symbiosis meaning cohabiting) theory was first articulated by the Russian botanist Konstantin Mereschkowski in 1905. Mereschkowski was familiar with work by botanist Andreas Schimper, who had observed in 1883 that the division of chloroplasts in green plants closely resembled that of free-living cyanobacteria, and who had himself tentatively proposed (in a footnote) that green plants had arisen from a symbiotic union of two organisms. Ivan Wallin extended the idea of an endosymbiotic origin to mitochondria in the 1920s. These theories were initially dismissed or ignored. More detailed electron

microscopic comparisons between cyanobacteria and chloroplasts (for example studies by Hans Ris), combined with the discovery that plastids and mitochondria contain their own DNA (which by that stage was recognized to be the hereditary material of organisms) led to a resurrection of the idea in the 1960s.

The endosymbiotic theory was advanced and substantiated with microbiological evidence by Lynn Margulis in a 1967 paper, *The Origin of Mitosing Eukaryotic Cells*. In her 1981 work *Symbiosis in Cell Evolution* she argued that eukaryotic cells originated as communities of interacting entities, including endosymbiotic spirochaetes that developed into eukaryotic flagella and cilia. This last idea has not received much acceptance, because flagella lack DNA and do not show ultrastructural similarities to bacteria or archaea. According to Margulis and Dorion Sagan, "Life did not take over the globe by combat, but by networking" (i.e., by cooperation). The possibility that peroxisomes may have an endosymbiotic origin has also been considered, although they lack DNA. Christian de Duve proposed that they may have been the first endosymbionts, allowing cells to withstand growing amounts of free molecular oxygen in the Earth's atmosphere. However, it now appears that they may be formed *de novo*, contradicting the idea that they have a symbiotic origin.

It is believed that over millennia these endosymbionts transferred some of their own DNA to the host cell's nucleus during the evolutionary transition from a symbiotic community to an instituted eukaryotic cell (called "serial endosymbiosis"). This hypothesis is thought to be possible because it is known today from scientific observation that transfer of DNA occurs between bacteria species, even if they are not closely related. Bacteria can take up DNA from their surroundings and have a limited ability to incorporate it into their own genome.

Evidence

Evidence that mitochondria and plastids arose from bacteria is as follows:

- New mitochondria and plastids are formed only through a process similar to binary fission. In some algae, such as *Euglena*, the plastids can be destroyed by certain chemicals or prolonged absence of light without otherwise affecting the cell. In such a case, the plastids will not regenerate.
- They are surrounded by two or more membranes, and the innermost of these shows differences in composition from the other membranes of the cell. The composition is like that of a bacterial cell membrane.
- Both mitochondria and plastids contain DNA that is different from that of the cell nucleus and that is similar to that of bacteria (in being circular in shape and in its size).
- DNA sequence analysis and phylogenetic estimates suggest that nuclear DNA contains genes that probably came from plastids.
- These organelles' ribosomes are like those found in bacteria (70S).
- Proteins of organelle origin, like those of bacteria, use N-formylmethionine as the initiating amino acid.

- Much of the internal structure and biochemistry of plastids, for instance the presence of thylakoids and particular chlorophylls, is very similar to that of cyanobacteria. Phylogenetic estimates constructed with bacteria, plastids, and eukaryotic genomes also suggest that plastids are most closely related to cyanobacteria.
- Mitochondria have several enzymes and transport systems similar to those of bacteria.
- Some proteins encoded in the nucleus are transported to the organelle, and both mitochondria and plastids have small genomes compared to bacteria. This is consistent with an increased dependence on the eukaryotic host after forming an endosymbiosis. Most genes on the organellar genomes have been lost or moved to the nucleus. Most genes needed for mitochondrial and plastid function are located in the nucleus. Many originate from the bacterial endosymbiont.
- Plastids are present in very different groups of protists, some of which are closely related to forms lacking plastids. This suggests that if chloroplasts originated *de novo*, they did so multiple times, in which case their close similarity to each other is difficult to explain.
- Many of these protists contain "primary" plastids that have not yet been acquired from other plastid-containing eukaryotes.
- Among eukaryotes that acquired their plastids directly from bacteria (known as Primoplantae), the glaucophyte algae have chloroplasts that strongly resemble cyanobacteria. In particular, they have a peptidoglycan cell wall between the two membranes.
- Mitochondria and plastids are similar in size to bacteria.

Secondary endosymbiosis

Primary endosymbiosis involves the engulfment of a bacterium by another free living organism. Secondary endosymbiosis occurs when the product of primary endosymbiosis is itself engulfed and retained by another free living eukaryote. Secondary endosymbiosis has occurred several times and has given rise to extremely diverse groups of algae and other eukaryotes. Some organisms can take opportunistic advantage of a similar process, where they engulf an alga and use the products of its photosynthesis, but once the prey item dies (or is lost) the host returns to a free living state. Obligate secondary endosymbionts become dependent on their organelles and are unable to survive in their absence. RedToL, the Red Algal Tree of Life Initiative funded by the National Science Foundation highlights the role red algae or Rhodophyta played in the evolution of our planet through secondary endosymbiosis.

One possible secondary endosymbiosis in process has been observed by Okamoto & Inouye (2005). The heterotrophic protist *Hatena* behaves like a predator until it ingests a green alga, which loses its flagella and cytoskeleton, while *Hatena*, now a host, switches to photosynthetic nutrition, gains the ability to move towards light and loses its feeding apparatus.

The process of secondary endosymbiosis left its evolutionary signature within the unique topography of plastid membranes. Secondary plastids are surrounded by three (in euglenophytes and some dinoflagellates) or four membranes (in haptophytes, heterokonts, cryptophytes, and chlorarachniophytes). The two additional membranes are thought to correspond to the plasma membrane of the engulfed alga and the phagosomal membrane of the host cell. The endosymbiotic acquisition of a eukaryote cell is represented in the cryptophytes; where the remnant nucleus of the red algal symbiont (the nucleomorph) is present between the two inner and two outer plastid membranes.

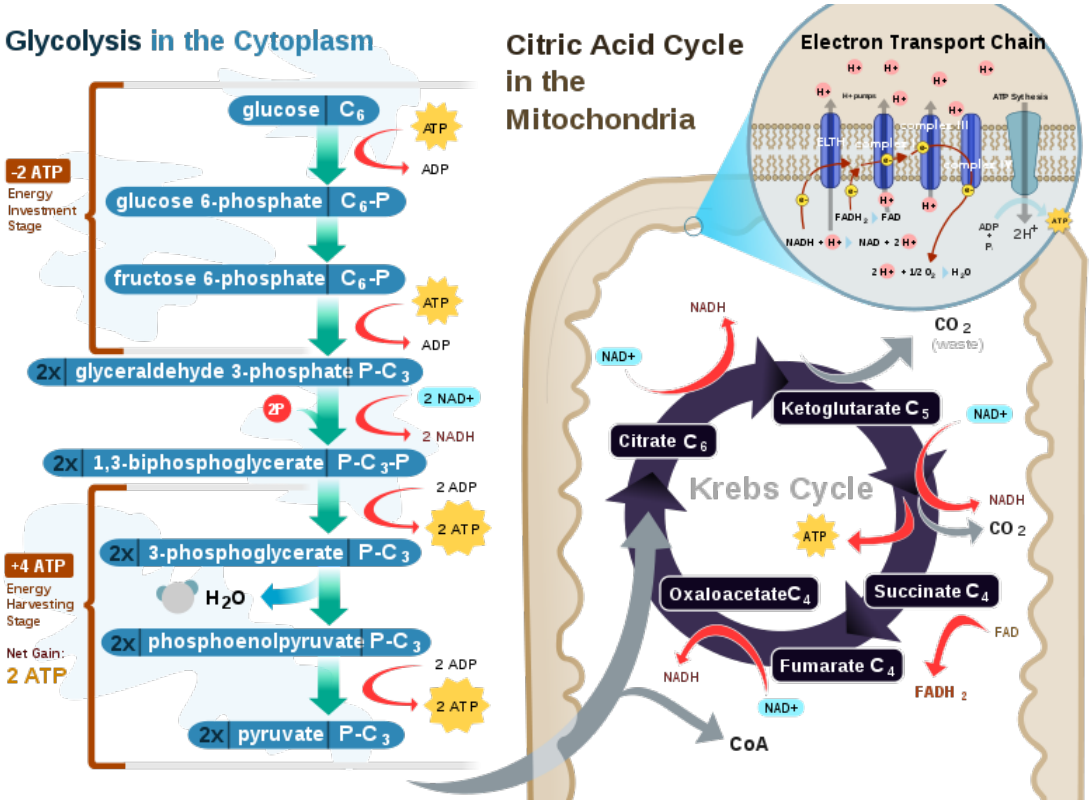
Despite the diversity of organisms containing plastids, the morphology, biochemistry, genomic organisation, and molecular phylogeny of plastid RNAs and proteins suggest a single origin of all extant plastids – although this theory is still debated.

Problems

- Neither mitochondria nor plastids can survive in oxygen or outside the cell, having lost many essential genes required for survival. The standard counterargument points to the large timespan that the mitochondria/plastids have co-existed with their hosts. In this view, genes and systems that were no longer necessary were simply deleted, or in many cases, transferred into the host genome instead. (In fact these transfers constitute an important way for the host cell to regulate plastid or mitochondrial activity.) For example, most plastids are not able to produce respiratory proteins necessary for respiration. Like any living cell, plastids would die if energy is not provided to them by respiration.
- A large cell, especially one equipped for phagocytosis, has vast energetic requirements, which cannot be achieved without the internalisation of energy production (due to the decrease in the surface area to volume ratio as size increases). This implies that, for the cell to gain mitochondria, it could not have been a eukaryote, and must have been a bacterium. This in turn implies that the emergence of the eukaryotes and the formation of mitochondria were achieved simultaneously. This may be explained by possibly a very close symbiotic relationship between two types of bacteria which eventually led to gene exchange and engulfing of the mitochondria precursors through partial fusion or engulfing by the host bacteria.
- Genetic analysis of small eukaryotes that lack mitochondria shows that they all still retain genes for mitochondrial proteins. This implies that all these eukaryotes once had mitochondria. This objection can be answered if, as suggested above, the origin of the eukaryotes coincided with the formation of mitochondria. Alternatively, we may postulate extinction of all other descendants of a mitochondrion-free ancestral eukaryote, perhaps due to competition from the symbiotic clade, or oxygen poisoning as levels continued to rise.

Chapter- 5

Cellular Respiration



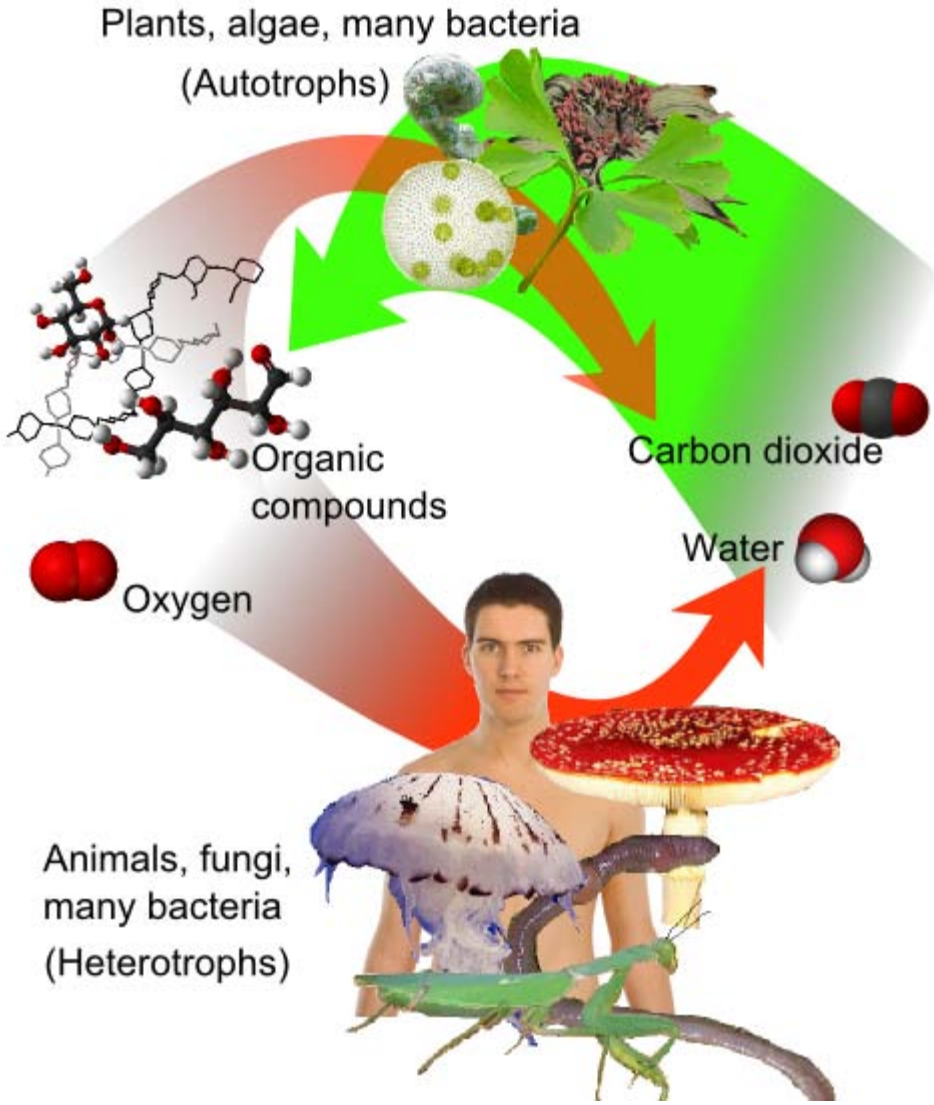
Cellular respiration in a typical eukaryotic cell.

Cellular respiration takes place in the mitochondria. *Cellular respiration'* is the set of the metabolic reactions and processes that take place in the cells of organisms to convert biochemical energy from nutrients into adenosine triphosphate (ATP), and then release waste products. The reactions involved in respiration are catabolic reactions that involve the redox reaction(oxidation of one molecule and the reduction of another). Respiration is one of the key ways a cell gains useful energy to fuel cellular reformations.

Nutrients commonly used by animal and plant cells in respiration include sugar, amino acids and fatty acids, and a common oxidizing agent (electron acceptor) is molecular oxygen (O_2). Bacteria and archaea can also be lithotrophs and these organisms may respire using a broad range of inorganic molecules as electron donors and acceptors, such as sulfur, metal ions, methane or hydrogen. Organisms that use oxygen as a final electron acceptor in respiration are described as aerobic, while those that do not are referred to as anaerobic.

The energy released in respiration is used to synthesize ATP to store this energy. The energy stored in ATP can then be used to drive processes requiring energy, including biosynthesis, locomotion or transportation of molecules across cell membranes.

Aerobic respiration



Aerobic respiration (red arrows) is the main means by which both plants and animals utilize energy in the form of organic compounds that was previously created through photosynthesis (green arrow).

Aerobic respiration requires oxygen in order to generate energy (ATP). Although carbohydrates, fats, and proteins can all be processed and consumed as reactant, it is the preferred method of pyruvate breakdown in glycolysis and requires that pyruvate enter the mitochondrion in order to be fully oxidized by the Krebs cycle. The product of this process is energy in the form of ATP (Adenosine triphosphate), by substrate-level phosphorylation, NADH and $FADH_2$

Simplified reaction: $C_6H_{12}O_6\ (aq) + 6\ O_2\ (g) \rightarrow 6\ CO_2\ (g) + 6\ H_2O\ (l)$
$\Delta G = -2880$ kJ per mole of $C_6H_{12}O_6$

The negative ΔG indicates that the reaction can happen spontaneously

The reducing potential of NADH and $FADH_2$ is converted to more ATP through an electron transport chain with oxygen as the "terminal electron acceptor". Most of the ATP produced by aerobic cellular respiration is made by oxidative phosphorylation. This works by the energy released in the consumption of pyruvate being used to create a chemiosmotic potential by pumping protons across a membrane. This potential is then used to drive ATP synthase and produce ATP from ADP and a phosphate group. Biology textbooks often state that 38 ATP molecules can be made per oxidised glucose molecule during cellular respiration (2 from glycolysis, 2 from the Krebs cycle, and about 34 from the electron transport system). However, this maximum yield is never quite reached due to losses (leaky membranes) as well as the cost of moving pyruvate and ADP into the mitochondrial matrix and current estimates range around 29 to 30 ATP per glucose.

Aerobic metabolism is 19 times more efficient than anaerobic metabolism (which yields 2 mol ATP per 1 mol glucose). They share the initial pathway of glycolysis but aerobic metabolism continues with the Krebs cycle and oxidative phosphorylation. The post glycolytic reactions take place in the mitochondria in eukaryotic cells, and in the cytoplasm in prokaryotic cells.

Glycolysis

Glycolysis is a metabolic pathway that is found in the cytosol of cells in all living organisms and is anaerobic (that is, oxygen is not required). The process converts one molecule of glucose into two molecules of pyruvate(pyruvic acid), it makes energy in the form of two net molecules of ATP. Four molecules of ATP per glucose are actually produced; however, two are consumed for the preparatory phase. The initial phosphorylation of glucose is required to destabilize the molecule for cleavage into two pyruvate. During the pay-off phase of glycolysis, four phosphate groups are transferred to ADP by substrate-level phosphorylation to make four ATP, and two NADH are produced when the pyruvate are oxidized. The overall reaction can be expressed this way:

$$\text{Glucose} + 2\ NAD^+ + 2\ P_i + 2\ ADP \rightarrow 2\ \text{pyruvate} + 2\ NADH + 2\ ATP + 2\ H^+ + 2\ H_2O$$

Oxidative decarboxylation of pyruvate

The pyruvate is oxidized to acetyl-CoA and CO_2 by the Pyruvate dehydrogenase complex, a cluster of enzymes—multiple copies of each of three enzymes—located in the mitochondria of eukaryotic cells and in the cytosol of prokaryotes. In the process one molecule of NADH is formed per pyruvate oxidized, and 3 molecules of ATP are formed for each molecule of pyruvate(pyruvic acid.) This step is also known as the *link reaction*, as it links glycolysis and the Krebs cycle.

Citric acid cycle

This is also called the *Krebs cycle* or the *tricarboxylic acid cycle*. When oxygen is present, acetyl-CoA is produced from the pyruvate molecules created from glycolysis. Once acetyl-CoA is formed, two processes can occur, aerobic or anaerobic respiration. When oxygen is present, the mitochondria will undergo aerobic respiration which leads to the Krebs cycle. However, if oxygen is not present, fermentation of the pyruvate molecule will occur. In the presence of oxygen, when acetyl-CoA is produced, the molecule then enters the citric acid cycle (Krebs cycle) inside the mitochondrial matrix, and gets oxidized to CO_2 while at the same time reducing NAD to NADH. NADH can be used by the electron transport chain to create further ATP as part of oxidative phosphorylation. To fully oxidize the equivalent of one glucose molecule, two acetyl-CoA must be metabolized by the Krebs cycle. Two waste products, H_2O and CO_2, are created during this cycle.

The citric acid cycle is an 8-step process involving 18 different enzymes. Throughout the entire cycle, acetyl-CoA changes into citrate, cis-aconitate, isocitrate, α-ketoglutarate, succinyl-CoA, succinate, fumarate, malate, and finally, oxaloacetate. The net energy gain from one cycle is 3 NADH, 1 $FADH_2$, and 1 GTP; the GTP may subsequently be used to produce ATP. Thus, the total energy yield from one whole glucose molecule (2 pyruvate molecules) is 6 NADH, 2 $FADH_2$, and 2 ATP.

Oxidative phosphorylation

In eukaryotes, oxidative phosphorylation occurs in the mitochondrial cristae. It comprises the electron transport chain that establishes a proton gradient (chemiosmotic potential) across the inner membrane by oxidizing the NADH produced from the Krebs cycle. ATP is synthesised by the ATP synthase enzyme when the chemiosmotic gradient is used to drive the phosphorylation of ADP. The electrons are finally transferred to exogenous oxygen and, with the addition of two protons, water is formed.

the table below are for one glucose molecule being fully oxidized into carbon dioxide. It is assumed that all the reduced coenzymes are oxidized by the electron transport chain and used for oxidative phosphorylation.

Step	coenzyme yield	ATP yield	Source of ATP
Glycolysis preparatory phase		-2	Phosphorylation of glucose and fructose 6-phosphate uses two ATP from the cytoplasm.
Glycolysis pay-off phase		4	Substrate-level phosphorylation
	2 NADH	6	Oxidative phosphorylation
Oxidative decarboxylation of pyruvate	2 NADH	6	Oxidative phosphorylation
		2	Substrate-level phosphorylation
Krebs cycle	6 NADH	18	Oxidative phosphorylation
	2 $FADH_2$	4	Oxidative phosphorylation
Total yield		**38 ATP**	From the complete oxidation of one glucose molecule to carbon dioxide and oxidation of all the reduced coenzymes.

Although there is a theoretical yield of 38 ATP molecules per glucose during cellular respiration, such conditions are generally not realized due to losses such as the cost of moving pyruvate (from glycolysis), phosphate, and ADP (substrates for ATP synthesis) into the mitochondria. All are actively transported using carriers that utilise the stored energy in the proton electrochemical gradient.

- Pyruvate is taken up by a specific, low km transporter to bring it into the mitochondrial matrix for oxidation by the pyruvate dehydrogenase complex.
- The **phosphate translocase** is a symporter and the driving force for moving phosphate ions into the mitochondria is the proton motive force.
- The **adenine nucleotide carrier** is an antiporter and exchanges ADP and ATP across the inner membrane. The driving force is due to the ATP (-4) having a more negative charge than the ADP (-3) and thus it dissipates some of the electrical component of the proton electrochemical gradient.

The outcome of these transport processes using the proton electrochemical gradient is that more than 3 H^+ are needed to make 1 ATP. Obviously this reduces the theoretical efficiency of the whole process and the likely maximum is closer to 28-30 ATP molecules. In practice the efficiency may be even lower due to the inner membrane of the mitochondria being slightly leaky to protons. Other factors may also dissipate the proton gradient creating an apparently leaky mitochondria. An uncoupling protein known as thermogenin is expressed in some cell types and is a channel that can transport protons. When this protein is active in the inner membrane it short circuits the coupling between the electron transport chain and ATP synthesis. The potential energy from the proton gradient is not used to make ATP but generates heat. This is particularly important in brown fat thermogenesis of newborn and hibernating mammals.

Fermentation

Without oxygen, pyruvate (pyruvic acid) is not metabolized by cellular respiration but undergoes a process of fermentation. The pyruvate is not transported into the mitochondrion, but remains in the cytoplasm, where it is converted to waste products that may be removed from the cell. This serves the purpose of oxidizing the electron carriers so that they can perform glycolysis again and removing the excess pyruvate. This waste product varies depending on the organism. In skeletal muscles, the waste product is lactic acid. This type of fermentation is called lactic acid fermentation. In yeast, the waste products are ethanol and carbon dioxide. This type of fermentation is known as alcoholic or ethanol fermentation. The ATP generated in this process is made by substrate-level phosphorylation, which does not require oxygen.

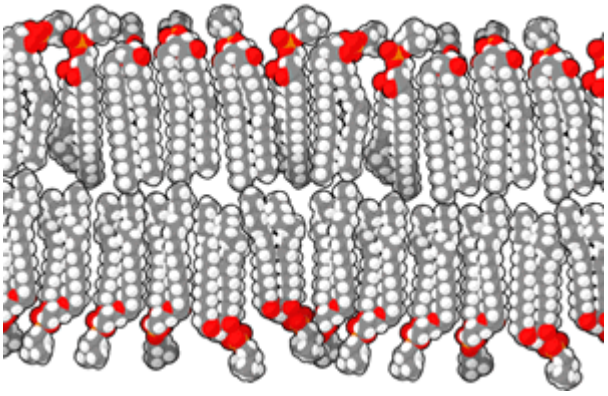
Fermentation is less efficient at using the energy from glucose since 2 ATP are produced per glucose, compared to the 38 ATP per glucose produced by aerobic respiration. This is because the waste products of fermentation still contain plenty of energy. Ethanol, for example, can be used in gasoline (petrol) solutions. Glycolytic ATP, however, is created more quickly. For prokaryotes to continue a rapid growth rate when they are shifted from an aerobic environment to an anaerobic environment, they must increase the rate of the glycolytic reactions. For multicellular organisms, during short bursts of strenuous activity, muscle cells use fermentation to supplement the ATP production from the slower aerobic respiration, so fermentation may be used by a cell even before the oxygen levels are depleted, as is the case in sports that do not require athletes to pace themselves, such as sprinting.

Anaerobic Respiration

Anaerobic respiration is used by some microorganisms in which neither oxygen (aerobic respiration) nor pyruvate or pyruvate derivative (fermentation) is the final electron acceptor. Rather, an inorganic acceptor (for example, Sulfur) is used.

Chapter- 6

Lipid Bilayer



This fluid lipid bilayer cross section is made up entirely of phosphatidylcholine.

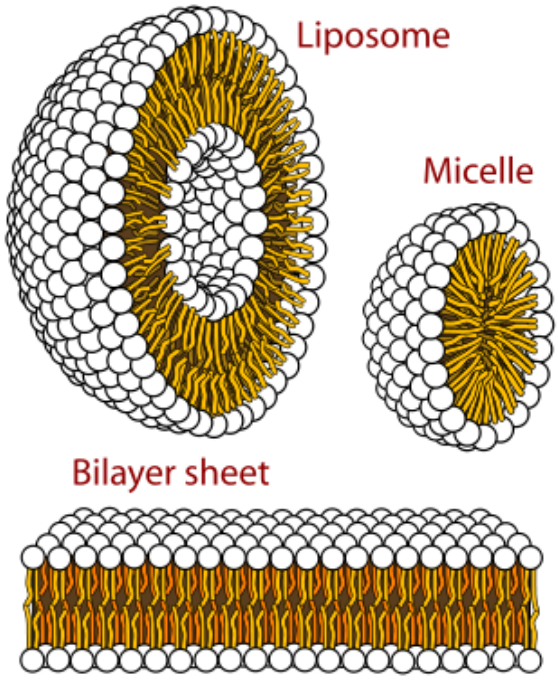
The **lipid bilayer** is a thin membrane made of two layers of lipid molecules. These membranes are flat sheets that form a continuous barrier around cells. The cell membrane of almost all living organisms and many viruses are made of a lipid bilayer, as are the membranes surrounding the cell nucleus and other sub-cellular structures. The lipid bilayer is the barrier that keeps ions, proteins and other molecules where they are needed and prevents them from diffusing into areas where they should not be. Lipid bilayers are ideally suited to this role because, even though they are only a few nanometers thick, they are impermeable to most water-soluble (hydrophilic) molecules. Bilayers are particularly impermeable to ions, which allows cells to regulate salt concentrations and pH by pumping ions across their membranes using proteins called ion pumps.

Natural bilayers are usually made mostly of phospholipids, which have a hydrophilic head and two hydrophobic tails. When phospholipids are exposed to water, they arrange themselves into a two-layered sheet (a bilayer) with all of their tails pointing toward the center of the sheet. The center of this bilayer contains almost no water and also excludes molecules like sugars or salts that dissolve in water but not in oil. This assembly process is similar to the coalescing of oil droplets in water and is driven by the same force, called the hydrophobic effect. Because lipid bilayers are quite fragile and are so thin that they are invisible in a traditional microscope, bilayers are very challenging to study.

Experiments on bilayers often require advanced techniques like electron microscopy and atomic force microscopy.

Phospholipids with certain head groups can alter the surface chemistry of a bilayer and can, for example, mark a cell for destruction by the immune system. Lipid tails can also affect membrane properties, for instance by determining the phase of the bilayer. The bilayer can adopt a solid gel phase state at lower temperatures but undergo phase transition to a fluid state at higher temperatures. The packing of lipids within the bilayer also affects its mechanical properties, including its resistance to stretching and bending. Many of these properties have been studied with the use of artificial "model" bilayers produced in a lab. Vesicles made by model bilayers have also been used clinically to deliver drugs.

Biological membranes typically include several types of lipids other than phospholipids. A particularly important example in animal cells is cholesterol, which helps strengthen the bilayer and decrease its permeability. Cholesterol also helps regulate the activity of certain integral membrane proteins. Integral membrane proteins function when incorporated into a lipid bilayer. Because bilayers define the boundaries of the cell and its compartments, these membrane proteins are involved in many intra- and inter-cellular signaling processes. Certain kinds of membrane proteins are involved in the process of fusing two bilayers together. This fusion allows the joining of two distinct structures as in the fertilization of an egg by sperm or the entry of a virus into a cell.

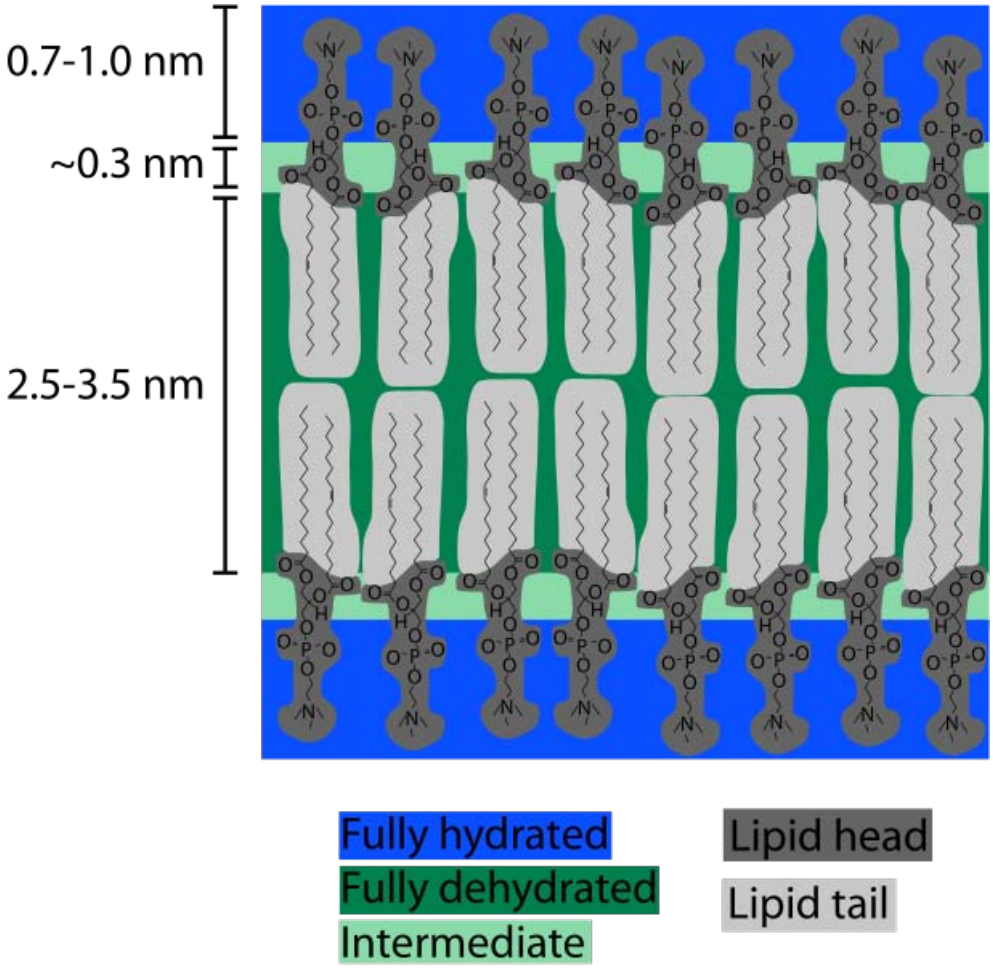


The three main structures phospholipids form in solution; the liposome (a closed bilayer), the micelle and the bilayer.

Structure and organization

A lipid bilayer is a sheet of lipids two molecules thick, arranged so that the hydrophilic phosphate heads point “out” to the water on either side of the bilayer and the hydrophobic tails point “in” to the core of the bilayer. This arrangement results in two “leaflets” which are each a single molecular layer. Lipids self-assemble into this structure because of the hydrophobic effect, which creates an energetically unfavorable interaction between the hydrophobic lipid tails and the surrounding water. Thus, a lipid bilayer is typically held together by entirely non-covalent forces that do not involve formation of chemical bonds between individual molecules.

There are some similarities between this structure and a common soap bubble, although there are also important differences. As illustrated, both structures involve two single-molecule layers of an amphiphilic substance. In the case of a soap bubble, the two soap monolayers coat an intervening water layer. The hydrophilic heads are oriented “in” toward this water core, while the hydrophobic tails point “out” to the air. In the case of a lipid bilayer, this structure is reversed with heads out and tails in. Another important difference between lipid bilayers and soap bubbles is their relative size. Soap bubbles are typically hundreds of nanometers thick, on the same order as the wavelength of light, which is why interference effects cause rainbow colors on a bubble surface. A single lipid bilayer, on the other hand, is around five nanometers thick, much smaller than the wavelength of light and is therefore invisible to the eye, even with a standard light microscope.



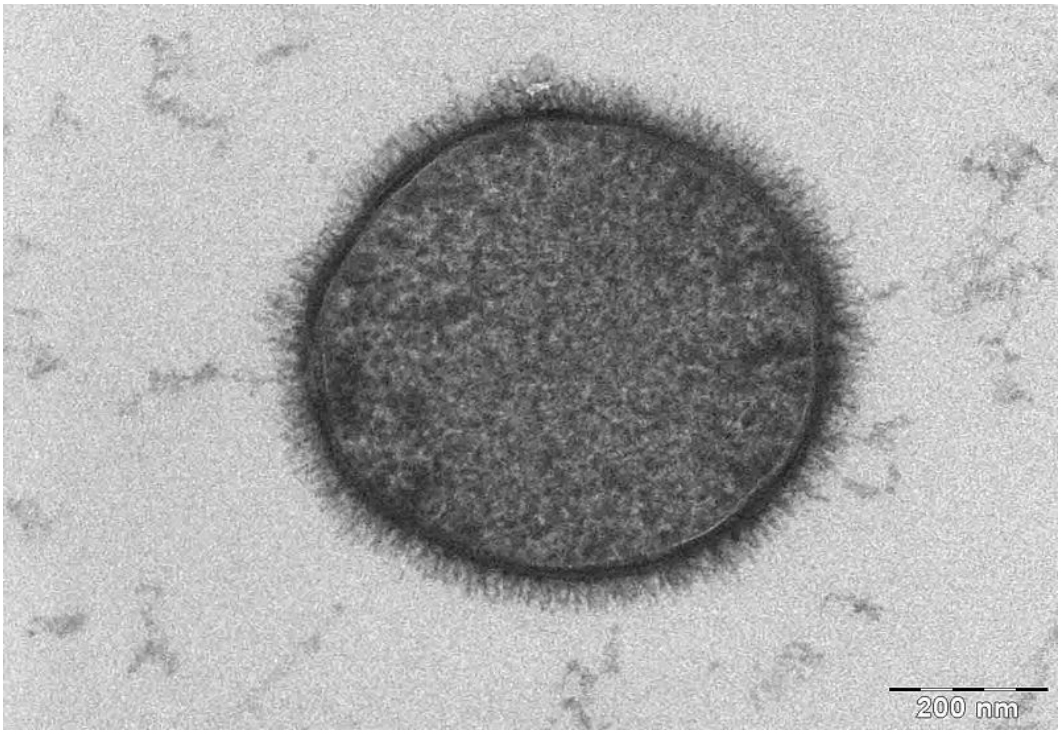
Schematic cross sectional profile of a typical lipid bilayer. There are three distinct regions: the fully hydrated headgroups, the fully dehydrated alkane core and a short intermediate region with partial hydration

Cross section analysis

The lipid bilayer is very thin compared to its lateral dimensions. If a typical mammalian cell (diameter ~10 micrometre) were magnified to the size of a watermelon (~1 ft/30 cm), the lipid bilayer making up the plasma membrane would be about as thick as a piece of office paper. Despite being only a few nanometers thick, the bilayer consists of several distinct chemical regions across its cross-section. These regions and their interactions with the surrounding water have been characterized over the past several decades with x-ray reflectometry, neutron scattering and nuclear magnetic resonance techniques.

The first region on either side of the bilayer is the hydrophilic headgroup. This portion of the membrane is completely hydrated and is typically around 8-9Å thick. In phospholipid bilayers the phosphate group is located within this hydrated region, approximately 5Å outside the hydrophobic core. In some cases, the hydrated region can extend much further, for instance in lipids with a large protein or long sugar chain grafted to the head.

One common example of such a modification in nature is the lipopolysaccharide coat on a bacterial outer membrane, which helps retain a water layer around the bacterium to prevent dehydration.



TEM image of a bacterium. The furry appearance on the outside is due to a coat of long chain sugars attached to the cell membrane. This coating helps trap water to prevent the bacterium from becoming dehydrated.

Next to the hydrated region is an intermediate region which is only partially hydrated. This boundary layer is approximately 3 Å thick. Within this short distance, the water concentration drops from 2M on the headgroup side to nearly zero on the tail (core) side. The hydrophobic core of the bilayer is typically 3-4 nm thick, but this value varies with chain length and chemistry. Core thickness also varies significantly with temperature, particularly near a phase transition.

Asymmetry

In many naturally occurring bilayers, the compositions of the inner and outer membrane leaflets are different. In human red blood cells, the inner (cytoplasmic) leaflet is largely composed of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol and its phosphorylated derivatives. By contrast, the outer (extracellular) leaflet is based on phosphatidylcholine, sphingomyelin and a variety of glycolipids, In some cases, this asymmetry is based on where the lipids are made in the cell and reflects their initial orientation. The biological functions of lipid asymmetry are imperfectly understood, although it is clear that it is used in several different situations. For example, when a cell undergoes apoptosis, the phosphatidylserine — normally localised to the cytoplasmic

leaflet — is transferred to the outer surface: there it is recognised by a macrophage which then actively scavenges the dying cell.

Lipid asymmetry arises, at least in part, from the fact that most phospholipids are synthesised and initially inserted into the inner monolayer: those that constitute the outer monolayer are then transported to the inner monolayer by a class of enzymes called flippases,. Other lipids, such as sphingomyelin, appear to be synthesised at the external leaflet. Flippases are members of a larger family of lipid transport molecules which also includes flopases, which transfer lipids in the opposite direction, and scramblases, which randomize lipid distribution across lipid bilayers (as in apoptotic cells). In any case, once lipid asymmetry is established it does not normally dissipate quickly because spontaneous flip-flop of lipids between leaflets is extremely slow.

It is possible to mimic this asymmetry in the laboratory in model bilayer systems. Certain types of very small artificial vesicle will automatically make themselves slightly asymmetric, although the mechanism by which this asymmetry is generated is very different from that in cells. By utilizing two different monolayers in Langmuir-Blodgett deposition or a combination of Langmuir-Blodgett and vesicle rupture deposition it is also possible to synthesize an asymmetric planar bilayer. This asymmetry may be lost over time as lipids in supported bilayers can be prone to flip-flop.

Phases and phase transitions

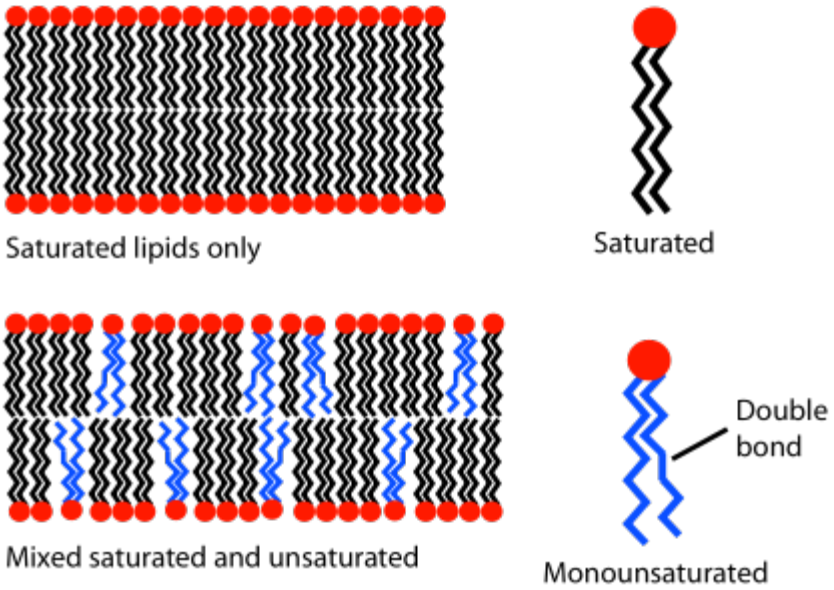


Diagram showing the effect of unsaturated lipids on a bilayer. The lipids with an unsaturated tail (blue) disrupt the packing of those with only saturated tails (black). The resulting bilayer has more free space and is consequently more permeable to water and other small molecules.

At a given temperature a lipid bilayer can exist in either a liquid or a gel (solid) phase. All lipids have a characteristic temperature at which they transition (melt) from the gel to

liquid phase. In both phases the lipid molecules are prevented from flip-flopping across the bilayer, but in liquid phase bilayers a given lipid will exchange locations with its neighbor millions of times a second. This random walk exchange allows lipid to diffuse and thus wander across the surface of the membrane. Unlike liquid phase bilayers, the lipids in a gel phase bilayer are locked in place.

The phase behavior of lipid bilayers is largely determined by the strength of the attractive Van der Waals interactions between adjacent lipid molecules. Longer tailed lipids have more area over which to interact, increasing the strength of this interaction and consequently decreasing the lipid mobility. Thus, at a given temperature, a short-tailed lipid will be more fluid than an otherwise identical long-tailed lipid. Transition temperature can also be affected by the degree of unsaturation of the lipid tails. An unsaturated double bond can produce a kink in the alkane chain, disrupting the lipid packing. This disruption creates extra free space within the bilayer which allows additional flexibility in the adjacent chains. An example of this effect can be noted in everyday life as butter, which has a large percentage saturated fats, is solid at room temperature while vegetable oil, which is mostly unsaturated, is liquid.

Most natural membranes are a complex mixture of different lipid molecules. If some of the components are liquid at a given temperature while others are in the gel phase, the two phases can coexist in spatially separated regions, rather like an iceberg floating in the ocean. This phase separation plays a critical role in biochemical phenomena because membrane components such as proteins can partition into one or the other phase and thus be locally concentrated or activated. One particularly important component of many mixed phase systems is cholesterol, which modulates bilayer permeability, mechanical strength and biochemical interactions.

Surface chemistry

While lipid tails primarily modulate bilayer phase behavior, it is the headgroup that determines the bilayer surface chemistry. Most natural bilayers are composed primarily of phospholipids, although sphingolipids such as sphingomyelin and sterols such as cholesterol are also important components. Of the phospholipids, the most common headgroup is phosphatidylcholine (PC), accounting for about half the phospholipids in most mammalian cells. PC is a zwitterionic headgroup, as it has a negative charge on the phosphate group and a positive charge on the amine but, because these local charges balance, no net charge.

Other headgroups are also present to varying degrees and can include phosphatidylserine (PS) phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). These alternate headgroups often confer specific biological functionality that is highly context-dependent. For instance, PS presence on the extracellular membrane face of erythrocytes is a marker of cell apoptosis, whereas PS in growth plate vesicles is necessary for the nucleation of hydroxyapatite crystals and subsequent bone mineralization. Unlike PC, some of the other headgroups carry a net charge, which can alter the electrostatic interactions of small molecules with the bilayer.

Biological roles

Containment and separation

The primary role of the lipid bilayer in biology is to separate aqueous compartments from their surroundings. Without some form of barrier delineating "self" from "non-self" it is difficult to even define the concept of an organism or of life. This barrier takes the form of a lipid bilayer in all known life forms except for a few species of archaea which utilize a specially adapted lipid monolayer. It has even been proposed that the very first form of life may have been a simple lipid vesicle with virtually its sole biosynthetic capability being the production of more phospholipids. The partitioning ability of the lipid bilayer is based on the fact that hydrophilic molecules cannot easily cross the hydrophobic bilayer core, as discussed in Transport across the bilayer below.

Prokaryotes have only one lipid bilayer- the cell membrane (also known as the plasma membrane). Many prokaryotes also have a cell wall, but the cell wall is composed of proteins or long chain carbohydrates, not lipids. In contrast, eukaryotes have a range of organelles including the nucleus, mitochondria, lysosomes and endoplasmic reticulum. All of these sub-cellular compartments are surrounded by one or more lipid bilayers and, together, typically comprise the majority of the bilayer area present in the cell. In liver hepatocytes for example, the plasma membrane accounts for only two percent of the total bilayer area of the cell, whereas the endoplasmic reticulum contains more than fifty percent and the mitochondria a further thirty percent.

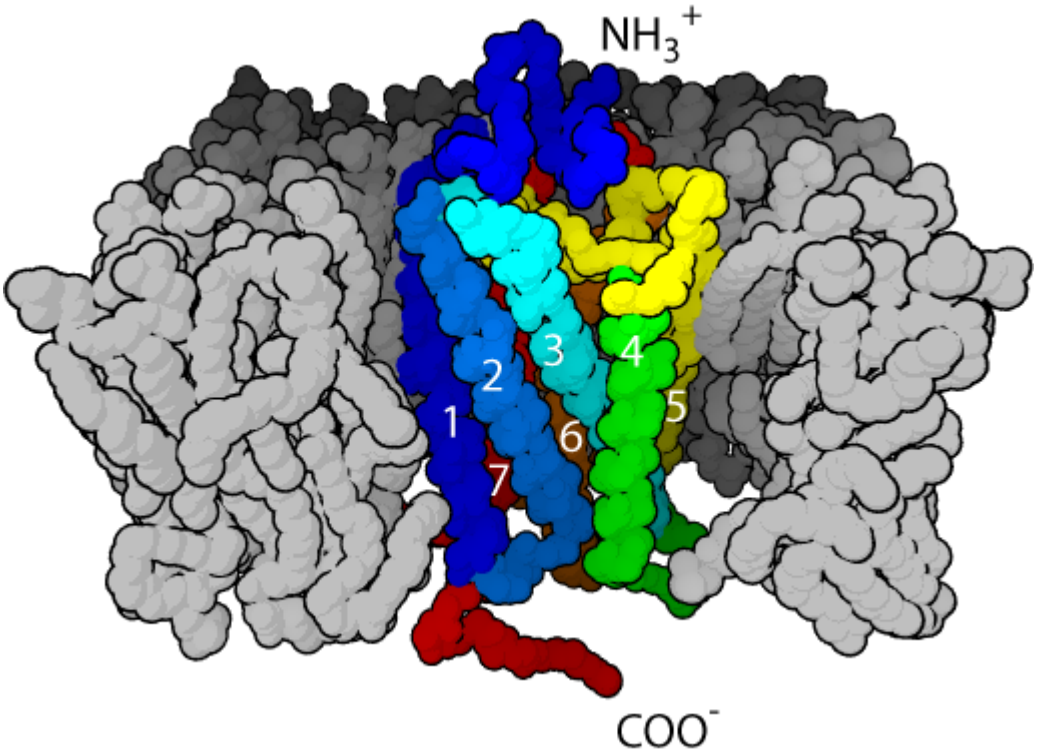


Illustration of a GPCR signaling protein. In response to a molecule such as a hormone binding to the exterior domain (blue) the GPCR changes shape and catalyzes a chemical reaction on the interior domain (red). The gray feature is the surrounding bilayer.

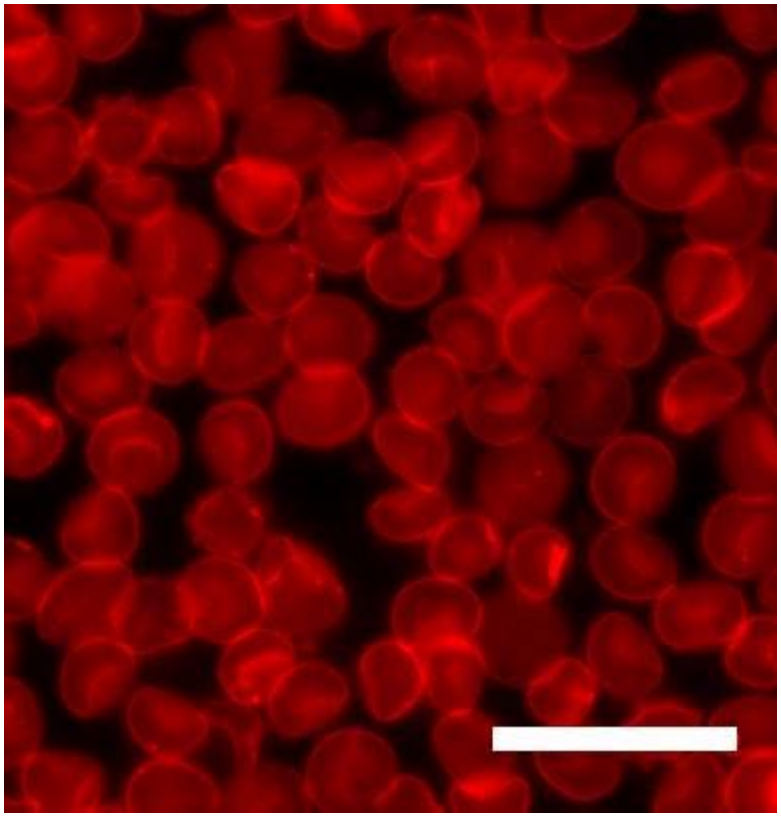
Signaling

Probably the most familiar form of cellular signaling is synaptic transmission, whereby a nerve impulse that has reached the end of one neuron is conveyed to an adjacent neuron via the release of neurotransmitters. This transmission is made possible by the action of synaptic vesicles loaded with the neurotransmitters to be released. These vesicles fuse with the cell membrane at the pre-synaptic terminal and release its contents to the exterior of the cell. The contents then diffuse across the synapse to the post-synaptic terminal.

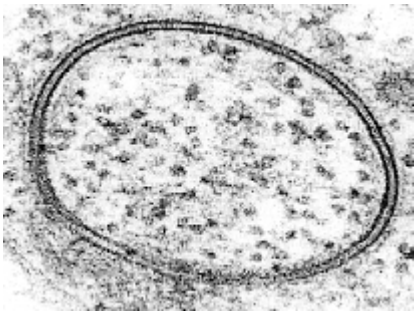
Lipid bilayers are also involved in signal transduction through their role as the home of integral membrane proteins. This is an extremely broad and important class of biomolecule. It is estimated that up to a third of the human proteome may be membrane proteins. Some of these proteins are linked to the exterior of the cell membrane. An example of this is the CD59 protein, which identifies cells as “self” and thus inhibits their destruction by the immune system. The HIV virus evades the immune system in part by grafting these proteins from the host membrane onto its own surface. Alternatively, some membrane proteins penetrate all the way through the bilayer and serve to relay individual signal events from the outside to the inside of the cell. The most common class of this type of protein is the G protein-coupled receptor (GPCR). GPCRs are responsible for much of the cell’s ability to sense its surroundings and, because of this important role, approximately 40% of all modern drugs are targeted at GPCRs.

In addition to protein- and solution-mediated processes, it is also possible for lipid bilayers to participate directly in signaling. A classic example of this is phosphatidylserine-triggered phagocytosis. Normally, phosphatidylserine is asymmetrically distributed in the cell membrane and is present only on the interior side. During programmed cell death a protein called a scramblase equilibrates this distribution, displaying phosphatidylserine on the extracellular bilayer face. The presence of phosphatidylserine then triggers phagocytosis to remove the dead or dying cell.

Characterization methods



Human red blood cells viewed through a fluorescence microscope. The cell membrane has been stained with a fluorescent dye. Scale bar is 20μm.

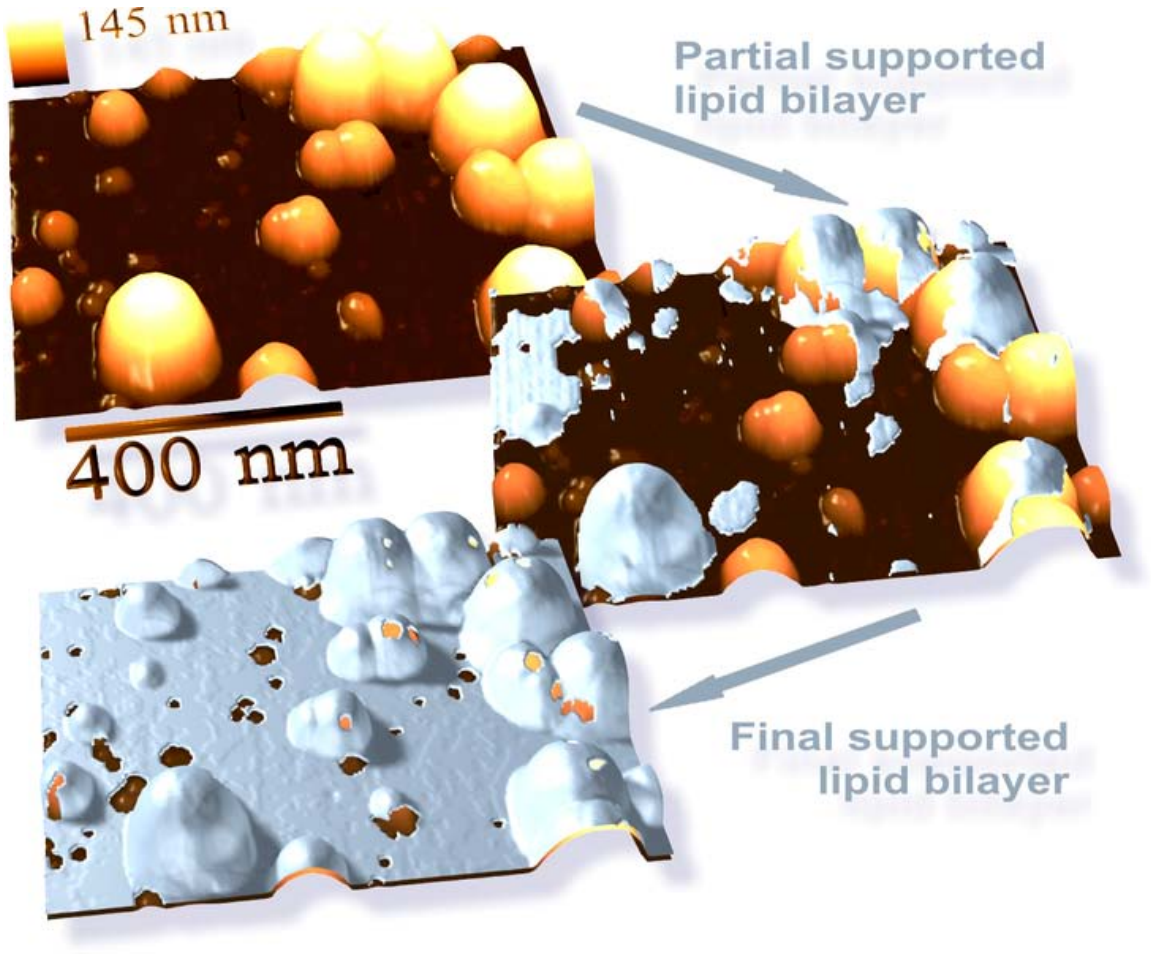


Transmission Electron Microscope (TEM) image of a lipid vesicle. The two dark bands around the edge are the two leaflets of the bilayer. Historically, similar images confirmed that the cell membrane is a bilayer

The lipid bilayer is a very difficult structure to study because it is so thin and fragile. In spite of these limitations dozens of techniques have been developed over the last seventy years to allow investigations of its structure and function.

Electrical measurements are a straightforward way to characterize an important function of a bilayer: its ability to segregate and prevent the flow of ions in solution. By applying a voltage across the bilayer and measuring the resulting current, the resistance of the bilayer is determined. This resistance is typically quite high since the hydrophobic core is impermeable to charged species. The presence of even a few nanometer-scale holes results in a dramatic increase in current. The sensitivity of this system is such that even the activity of single ion channels can be resolved.

Electrical measurements do not provide an actual picture like imaging with a microscope can. Lipid bilayers cannot be seen in a traditional microscope because they are too thin. In order to see bilayers, researchers often use fluorescence microscopy. A sample is excited with one wavelength of light and observed in a different wavelength, so that only fluorescent molecules with a matching excitation and emission profile will be seen. Natural lipid bilayers are not fluorescent, so a dye is used that attaches to the desired molecules in the bilayer. Resolution is usually limited to a few hundred nanometers, much smaller than a typical cell but much larger than the thickness of a lipid bilayer.



3d-Adapted AFM images showing formation of transmembrane pores (holes) in supported lipid bilayer

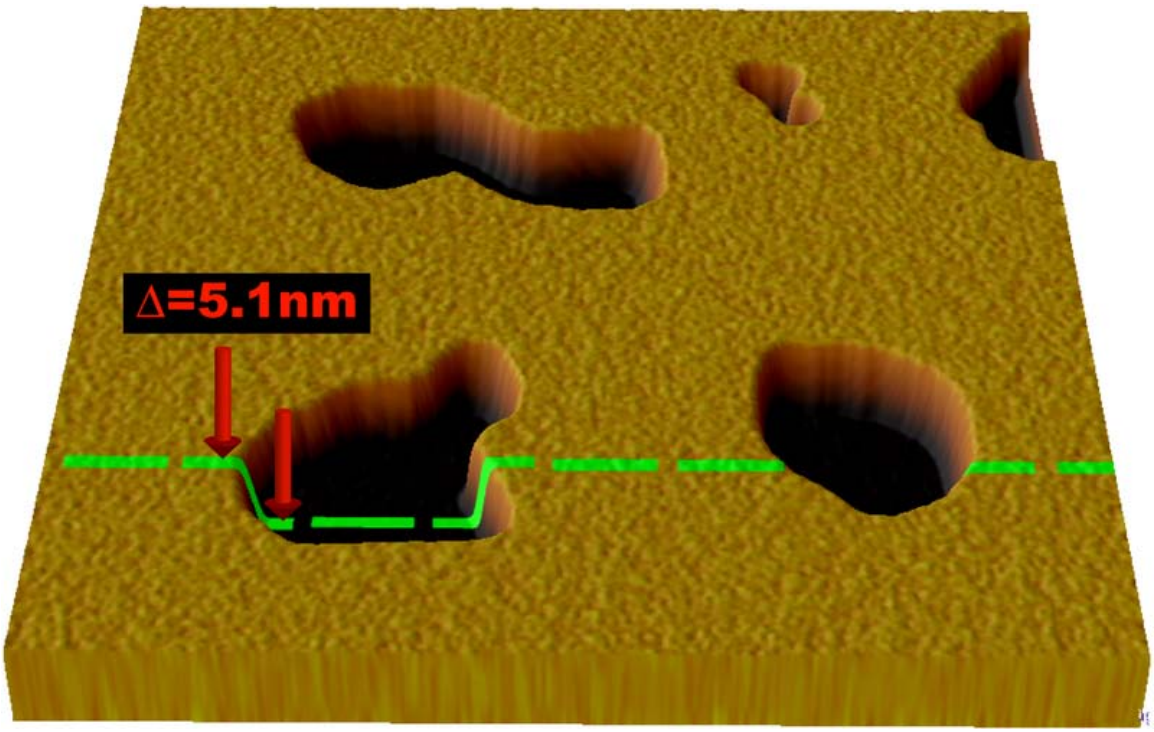


Illustration of a typical AFM scan of a supported lipid bilayer. The pits are defects in the bilayer, exposing the smooth surface of the substrate underneath.

Electron microscopy offers a higher resolution image. In an electron microscope, a beam of focused electrons interacts with the sample rather than a beam of light as in traditional microscopy. In conjunction with rapid freezing techniques, electron microscopy has also been used to study the mechanisms of inter- and intracellular transport, for instance in demonstrating that exocytotic vesicles are the means of chemical release at synapses.

^{31}P-NMR(nuclear magnetic resonance) spectroscopy is widely used for studies of phospholipid bilayers and biological membranes in native conditions. The analysis of ^{31}P-NMR spectra of lipids could provide a wide range of information about lipid bilayer packing, phase transitions (gel phase, physiological liquid crystal phase, ripple phases, non bilayer phases), lipid head group orientation/dynamics, and elastic properties of pure lipid bilayer and as a result of binding of proteins and other biomolecules.

In addition, a specific H-N...(O)-P experiment (transition by scalar coupling 3JH-P~5Hz) could provide a direct information about formation of hydrogen bonds between amid protons of protein to phosphate of lipid headgroups, which is useful in studies of protein/membrane interactions.

A new method to study lipid bilayers is Atomic force microscopy (AFM). Rather than using a beam of light or particles, a very small sharpened tip scans the surface by making physical contact with the bilayer and moving across it, like a record player needle. AFM is a promising technique because it has the potential to image with nanometer resolution at room temperature and even under water or physiological buffer, conditions necessary

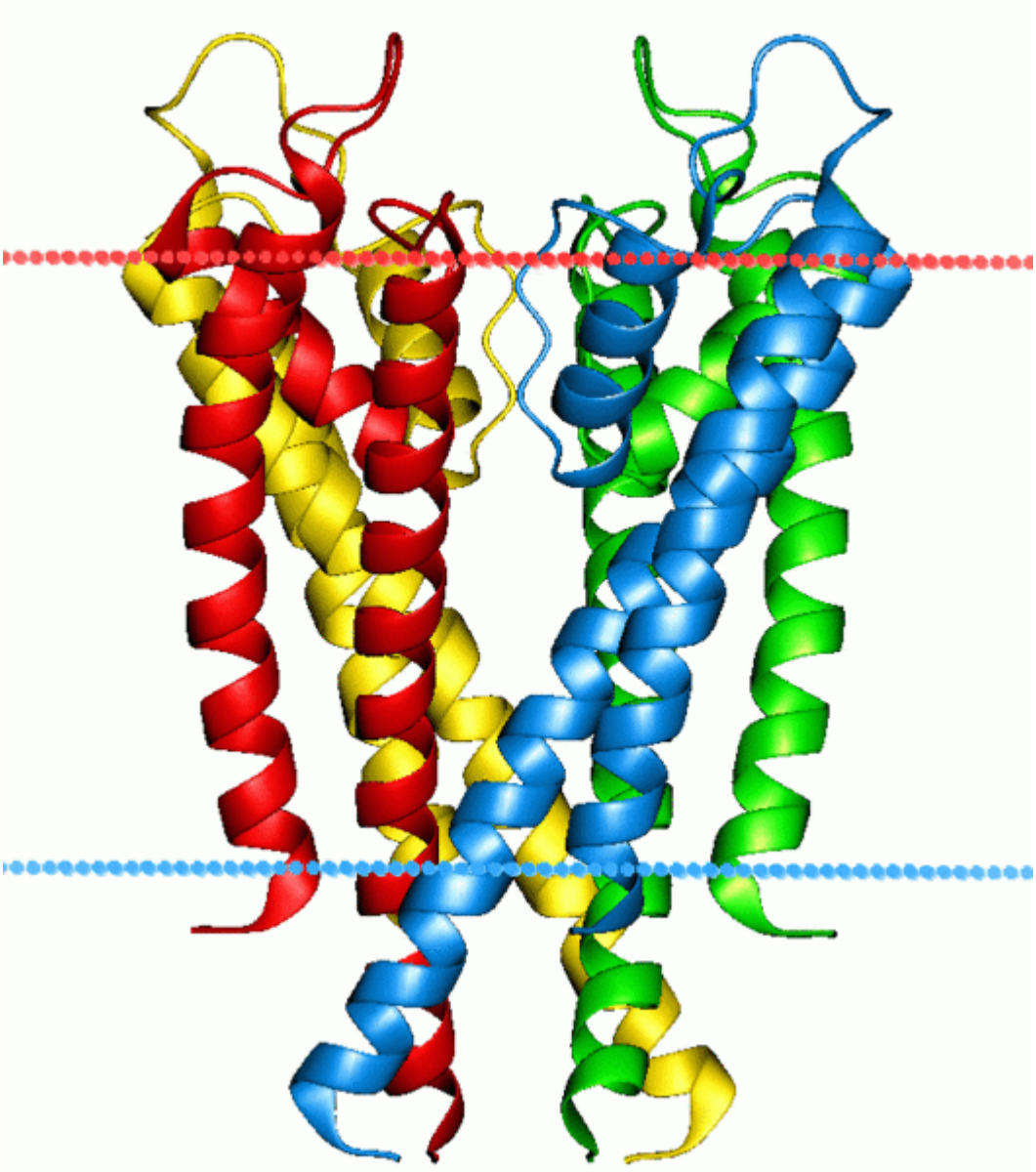
for natural bilayer behavior. Utilizing this capability, AFM has been used to examine dynamic bilayer behavior including the formation of transmembrane pores (holes) and phase transitions in supported bilayers. Another advantage is that AFM does not require fluorescent or isotopic labeling of the lipids, since the probe tip interacts mechanically with the bilayer surface. Because of this, the same scan can image both lipids and associated proteins, sometimes even with single-molecule resolution. AFM can also probe the mechanical nature of lipid bilayers.

Lipid bilayers exhibit high levels of birefringence where the refractive index in the plane of the bilayer differs from that perpendicular by as much as 0.1 refractive index units. This has been used to characterise the degree of order and disruption in bilayers using dual polarisation interferometry to understand mechanisms of protein interaction.

Transport across the bilayer

Passive diffusion

Most polar molecules have low solubility in the hydrocarbon core of a lipid bilayer and consequently have low permeability coefficients across the bilayer. This effect is particularly pronounced for charged species, which have even lower permeability coefficients than neutral polar molecules. Anions typically have a higher rate of diffusion through bilayers than cations. Compared to ions, water molecules actually have a relatively large permeability through the bilayer, as evidenced by osmotic swelling. When a cell or vesicle with a high interior salt concentration is placed in a solution with a low salt concentration it will swell and eventually burst. Such a result would not be observed unless water was able to pass through the bilayer with relative ease. The anomalously large permeability of water through bilayers is still not completely understood and continues to be the subject of active debate. Uncharged apolar molecules diffuse through lipid bilayers many orders of magnitude faster than ions or water. This applies both to fats and organic solvents like chloroform and ether. Regardless of their polar character larger molecules diffuse more slowly across lipid bilayers than small molecules.



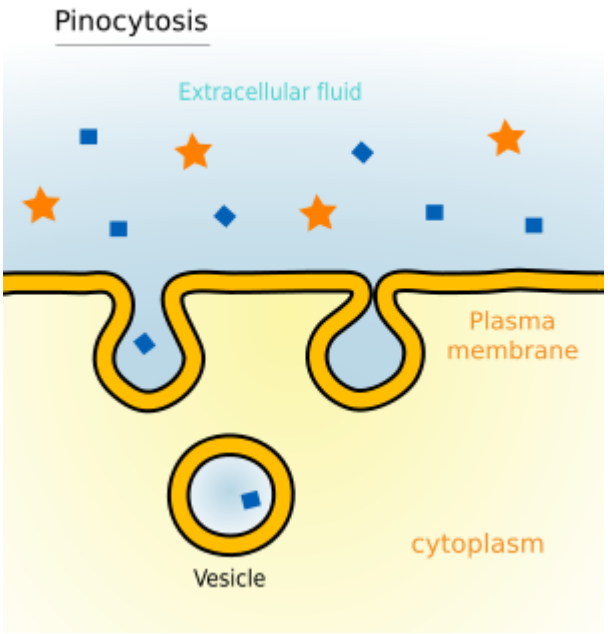
Structure of a potassium ion channel. The alpha helices penetrate the bilayer (boundaries indicated by red and blue lines), opening a hole through which potassium ions can flow

Ion pumps and channels

Two special classes of protein deal with the ionic gradients found across cellular and sub-cellular membranes in nature- ion channels and ion pumps. Both pumps and channels are integral membrane proteins that pass through the bilayer, but their roles are quite different. Ion pumps are the proteins that build and maintain the chemical gradients by utilizing an external energy source to move ions against the concentration gradient to an area of higher chemical potential. The energy source can be ATP, as is the case for the Na^+-K^+ ATPase. Alternatively, the energy source can be another chemical gradient

already in place, as in the Ca^{2+}/Na^{+} antiporter. It is through the action of ion pumps that cells are able to regulate pH via the pumping of protons.

In contrast to ion pumps, ion channels do not build chemical gradients but rather dissipate them in order to perform work or send a signal. Probably the most familiar and best studied example is the voltage-gated Na^{+} channel, which allows conduction of an action potential along neurons. All ion pumps have some sort of trigger or "gating" mechanism. In the previous example it was electrical bias, but other channels can be activated by binding a molecular agonist or through a conformational change in another nearby protein.



Schematic illustration of pinocytosis, a type of endocytosis

Endocytosis and exocytosis

Some molecules or particles are too large or too hydrophilic to effectively pass through a lipid bilayer. Other molecules could pass through the bilayer but must be transported rapidly in such large numbers that channel-type transport is impractical. In both cases these types of cargo can be moved across the cell membrane through fusion or budding of vesicles. When a vesicle is produced inside the cell and fuses with the plasma membrane to release its contents into the extracellular space this process is known as exocytosis. In the reverse process a region of the cell membrane will dimple inwards and eventually pinch off, enclosing a portion of the extracellular fluid to transport it into the cell. Endocytosis and exocytosis rely on very different molecular machinery to function, but the two processes are intimately linked and could not work without each other. The primary mechanism this interdependence is the sheer volume of lipid material involved. In a typical cell, an area of bilayer equivalent to the entire plasma membrane will travel through the endocytosis/exocytosis cycle in about half an hour. If these two processes

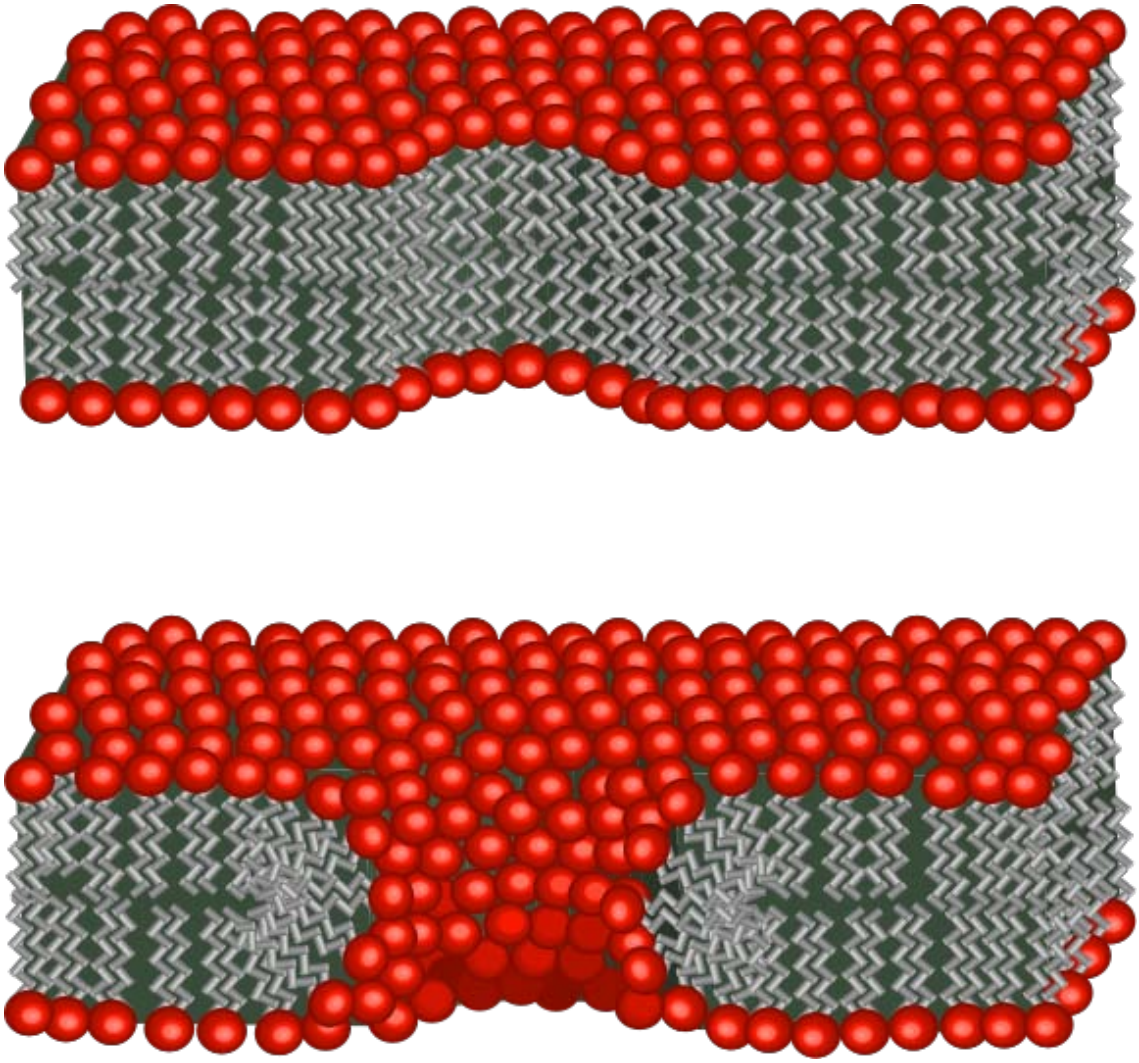
were not balancing each other the cell would either balloon outward to an unmanageable size or completely deplete its plasma membrane within a matter of minutes.

Electroporation

Electroporation is the rapid increase in bilayer permeability induced by the application of a large artificial electric field across the membrane. Experimentally, electroporation is used to introduce hydrophilic molecules into cells. It is a particularly useful technique for large highly charged molecules such as DNA which would never passively diffuse across the hydrophobic bilayer core. Because of this, electroporation is one of the key methods of transfection as well as bacterial transformation. It has even been proposed that electroporation resulting from lightning strikes could be a mechanism of natural horizontal gene transfer.

This increase in permeability primarily affects transport of ions and other hydrated species, indicating that the mechanism is the creation of nm-scale water-filled holes in the membrane. Although electroporation and dielectric breakdown both result from application of an electric field, the mechanisms involved are fundamentally different. In dielectric breakdown the barrier material is ionized, creating a conductive pathway. The material alteration is thus chemical in nature. In contrast, during electroporation the lipid molecules are not chemically altered but simply shift position, opening up a pore which acts as the conductive pathway through the bilayer as it is filled with water.

Mechanics



Schematic showing two possible conformations of the lipids at the edge of a pore. In the top image the lipids have not rearranged, so the pore wall is hydrophobic. In the bottom image some of the lipid heads have bent over, so the pore wall is hydrophilic.

Lipid bilayers are large enough structures to have some of the mechanical properties of liquids or solids. The area compression modulus K_a, bending modulus K_b, and edge energy Λ, can be used to describe them. Solid lipid bilayers also have a shear modulus, but like any liquid, the shear modulus is zero for fluid bilayers. These mechanical properties affect how the membrane functions. K_a and K_b affect the ability of proteins and small molecules to insert into the bilayer, and bilayer mechanical properties have been shown to alter the function of mechanically activated ion channels. Bilayer mechanical properties also govern what types of stress a cell can withstand without tearing. Although lipid bilayers can easily bend, most cannot stretch more than a few percent before rupturing.

As discussed in the Structure and organization section, the hydrophobic repulsion between lipid tails and water is the primary force holding lipid bilayers together. Thus, the elastic modulus of the bilayer is primarily determined by how much extra area is exposed to water when the lipid molecules are stretched apart. It is not surprising given this understanding of the forces involved that studies have shown that K_a varies strongly with solution conditions but only weakly with tail length and unsaturation. Because the forces involved are so small, it is difficult to experimentally determine K_a. Most techniques require sophisticated microscopy and very sensitive measurement equipment.

In contrast to K_a, which is a measure of how much energy is needed to stretch the bilayer, K_b is a measure of how much energy is needed to bend or flex the bilayer. Formally, bending modulus is defined as the energy required to deform a membrane from its intrinsic curvature to some other curvature. Intrinsic curvature is defined by the ratio of the diameter of the head group to that of the tail group. For two-tailed PC lipids, this ratio is nearly one so the intrinsic curvature is nearly zero. If a particular lipid has too large a deviation from zero intrinsic curvature it will not form a bilayer and will instead form other phases such as micelles or inverted micelles. Typically, K_b is not measured experimentally but rather is calculated from measurements of K_a and bilayer thickness, since the three parameters are related.

Λ is a measure of how much energy it takes to expose a bilayer edge to water by tearing the bilayer or creating a hole in it. The origin of this energy is the fact that creating such an interface exposes some of the lipid tails to water, but the exact orientation of these border lipids is unknown. There is some evidence that both hydrophobic (tails straight) and hydrophilic (heads curved around) pores can coexist.

Fusion

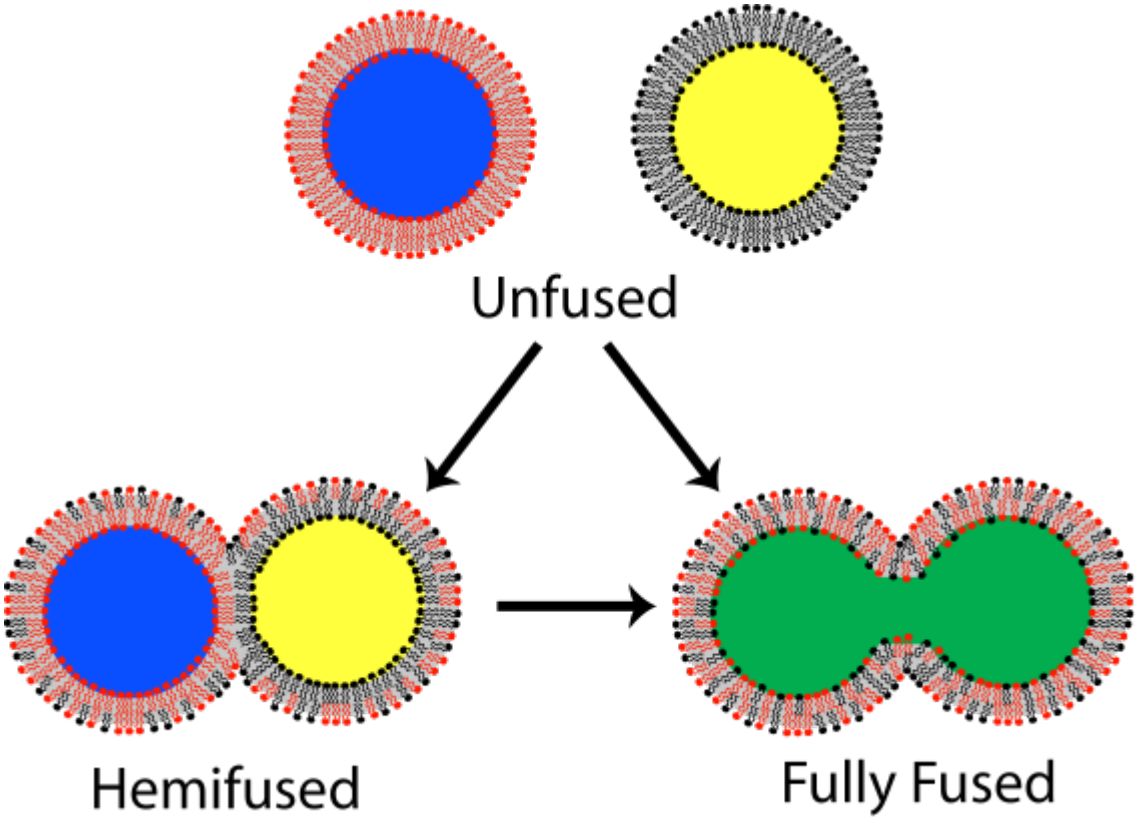
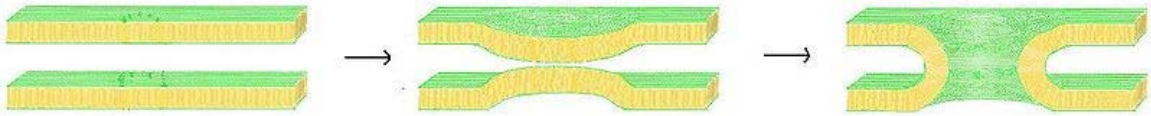


Illustration of lipid vesicles fusing showing two possible outcomes: hemifusion and full fusion. In hemifusion only the outer bilayer leaflets mix. In full fusion both leaflets as well as the internal contents mix.

Fusion is the process by which two lipid bilayers merge, resulting in one connected structure. If this fusion proceeds completely through both leaflets of both bilayers, a water-filled bridge is formed and the solutions contained by the bilayers can mix. Alternatively, if only one leaflet from each bilayer is involved in the fusion process, the bilayers are said to be hemifused. Fusion is involved in many cellular processes, particularly in eukaryotes since the eukaryotic cell is extensively sub-divided by lipid bilayer membranes. Exocytosis, fertilization of an egg by sperm and transport of waste products to the lysozome are a few of the many eukaryotic processes that rely on some form of fusion. Even the entry of pathogens can be governed by fusion, as many bilayer-coated viruses have dedicated fusion proteins to gain entry into the host cell.

There are four fundamental steps in the fusion process. First, the involved membranes must aggregate, approaching each other to within several nanometers. Second, the two bilayers must come into very close contact (within a few angstroms). To achieve this close contact, the two surfaces must become at least partially dehydrated, as the bound surface water normally present causes bilayers to strongly repel. The presence of ions, particularly divalent cations like magnesium and calcium, strongly affects this step. One

of the critical roles of calcium in the body is regulating membrane fusion. Third, a destabilization must form at one point between the two bilayers, locally distorting their structures. The exact nature of this distortion is not known. One theory is that a highly curved "stalk" must form between the two bilayers. Proponents of this theory believe that it explains why phosphatidylethanolamine, a highly curved lipid, promotes fusion. Finally, in the last step of fusion, this point defect grows and the components of the two bilayers mix and diffuse away from the site of contact.



Schematic illustration of the process of fusion through stalk formation.

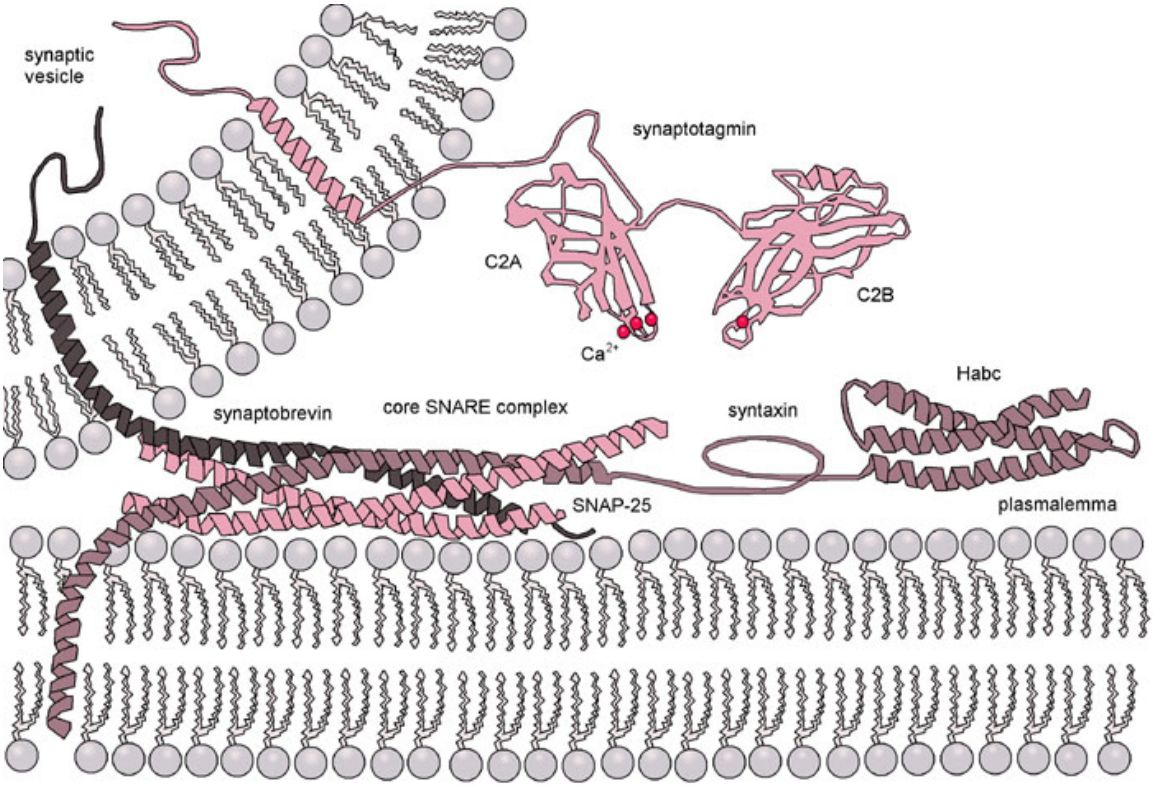


Diagram of the action of SNARE proteins docking a vesicle for exocytosis. Complementary versions of the protein on the vesicle and the target membrane bind and wrap around each other, drawing the two bilayers close together in the process.

The situation is further complicated when considering fusion *in vivo* since biological fusion is almost always regulated by the action of membrane-associated proteins. The first of these proteins to be studied were the viral fusion proteins, which allow an

enveloped virus to insert its genetic material into the host cell (enveloped viruses are those surrounded by a lipid bilayer; some others have only a protein coat).Eukaryotic cells also use fusion proteins, the best studied of which are the SNAREs. SNARE proteins are used to direct all vesicular intracellular trafficking. Despite years of study, much is still unknown about the function of this protein class. In fact, there is still an active debate regarding whether SNAREs are linked to early docking or participate later in the fusion process by facilitating hemifusion.

In studies of molecular and cellular biology it is often desirable to artificially induce fusion. The addition of polyethylene glycol (PEG) causes fusion without significant aggregation or biochemical disruption. This procedure is now used extensively, for example by fusing B-cells with melanoma cells. The resulting “hybridoma” from this combination expresses a desired antibody as determined by the B-cell involved, but is immortalized due to the melanoma component. Fusion can also be artificially induced through electroporation in a process known as electrofusion. It is believed that this phenomenon results from the energetically active edges formed during electroporation, which can act as the local defect point to nucleate stalk growth between two bilayers.

Model systems

Lipid bilayers can be created artificially in the lab to allow researchers to perform experiments that cannot be done with natural bilayers. These synthetic systems are called model lipid bilayers. There are many different types of model bilayers, each having experimental advantages and disadvantages. They can be made with either synthetic or natural lipids. Among the most common model systems are:

- Black lipid membranes (BLM)
- Supported lipid bilayers (SLB)
- Tethered Bilayer Lipid Membranes (t-BLM)
- Vesicles

Commercial applications

To date, the most successful commercial application of lipid bilayers has been the use of liposomes for drug delivery, especially for cancer treatment. (Note- the term “liposome” is essentially synonymous with “vesicle” except that vesicle is a general term for the structure whereas liposome only refers to artificial, not natural vesicles) The basic idea of liposomal drug delivery is that the drug is encapsulated in solution inside the liposome then injected into the patient. These drug-loaded liposomes travel through the system until they bind at the target site and rupture, releasing the drug. In theory, liposomes should make an ideal drug delivery system since they can isolate nearly any hydrophilic drug, can be grafted with molecules to target specific tissues and can be relatively non-toxic since the body possesses biochemical pathways for degrading lipids.

The first generation of drug delivery liposomes had a simple lipid composition and suffered from several limitations. Circulation in the bloodstream was extremely limited due to both renal clearing and phagocytosis. Refinement of the lipid composition to tune fluidity, surface charge density and surface hydration resulted in vesicles that adsorb fewer proteins from serum and thus are less readily recognized by the immune system. The most significant advance in this area was the grafting of polyethylene glycol (PEG) onto the liposome surface to produce "stealth" vesicles which circulate over long times without immune or renal clearing.

The first stealth liposomes were passively targeted at tumor tissues. Because tumors induce rapid and uncontrolled angiogenesis they are especially "leaky" and allow liposomes to exit the bloodstream at a much higher rate than normal tissue would. More recently work has been undertaken to graft antibodies or other molecular markers onto the liposome surface in the hope of actively binding them to a specific cell or tissue type. Some examples of this approach are already in clinical trials.

Another potential application of lipid bilayers is the field of biosensors. Since the lipid bilayer is the barrier between the interior and exterior of the cell it is also the site of extensive signal transduction. Researchers over the years have tried to harness this potential to develop a bilayer-based device for clinical diagnosis or bioterrorism detection. Progress has been slow in this area and, although a few companies have developed automated lipid-based detection systems, they are still targeted at the research community. These include Biacore Life Sciences, which offers a disposable chip for utilizing lipid bilayers in studies of binding kinetics and Nanion Inc which has developed an automated patch clamping system. Other, more exotic applications are also being pursued such as the use of lipid bilayer membrane pores for DNA sequencing by Oxford Nanolabs. To date, this technology has not proven commercially viable.

A supported lipid bilayer (SLB) as described above has achieved commercial success as a screening technique to measure the permeability of drugs. This **p**arallel **a**rtificial **m**embrane **p**ermeability **a**ssay PAMPA technique measures the permeability across specifically formulated lipid cocktail(s) found to be highly correlated with Caco-2 cultures, the gastrointestinal tract, blood-brain barrier and skin.

History

By the early twentieth century scientists had come to believe that cells are surrounded by a thin oil-like barrier, but the structural nature of this membrane was not known. Two experiments in 1925 laid the groundwork to fill in this gap. By measuring the capacitance of erythrocyte solutions, Hugo Fricke determined that the cell membrane was 3.3 nm thick. Although the results of this experiment were accurate, Fricke misinterpreted the data to mean that the cell membrane is a single molecular layer. Prof. Dr. Evert Gorter (1881–1954) and F. Grendel of Leiden University approached the problem from a different perspective, spreading the erythrocyte lipids as a monolayer on a Langmuir-Blodgett trough. When they compared the area of the monolayer to the surface area of the cells, they found a ratio of two to one. Later analyses showed several errors and incorrect

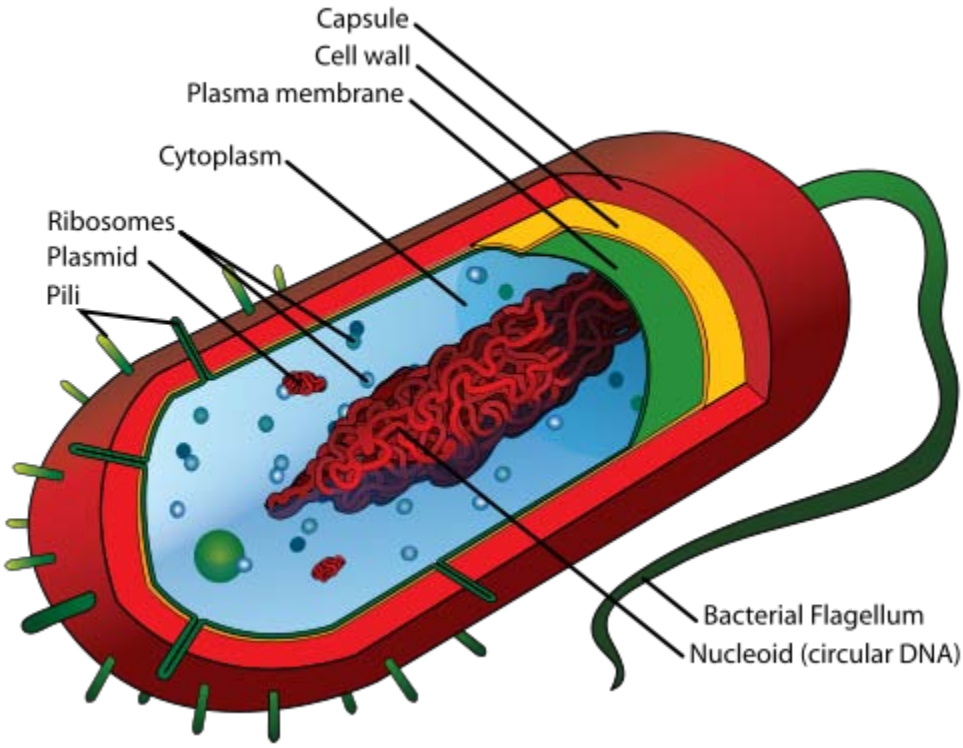
assumptions with this experiment but, serendipitously, these errors canceled out and from this flawed data Gorter and Grendel drew the correct conclusion- that the cell membrane is a lipid bilayer.

This theory was confirmed through the use of electron microscopy in the late 1950s. Although he did not publish the first electron microscopy study of lipid bilayers J. David Robertson was the first to assert that the two dark electron-dense bands were the headgroups and associated proteins of two apposed lipid monolayers. In this body of work, Robertson put forward the concept of the "unit membrane." This was the first time the bilayer structure had been universally assigned to all cell membranes as well as organelle membranes.

Around the same time the development of model membranes confirmed that the lipid bilayer is a stable structure that can exist independently of proteins. By "painting" a solution of lipid in organic solvent across an aperture, Mueller and Rudin were able to create an artificial bilayer and determine that this exhibited lateral fluidity, high electrical resistance and self-healing in response to puncture, all of which are properties of a natural cell membrane. A few years later, Alec Bangham showed that bilayers, in the form of lipid vesicles, could also be formed simply by exposing a dried lipid sample to water. This was an important advance since it demonstrated that lipid bilayers form spontaneously via self assembly and do not require a patterned support structure.

Chapter- 7

Prokaryote



Cell structure of a bacterium, one of the two domains of prokaryotic life.

The **prokaryotes** are a group of organisms that lack a cell nucleus (= karyon), or any other membrane-bound organelles. They differ from the eukaryotes, which have a cell nucleus. Most are unicellular, but a few prokaryotes such as myxobacteria have multicellular stages in their life cycles. The word *prokaryote* comes from the Greek *πρό- (pro-)* "before" + *καρυόν (karyon)* "nut or kernel".

The prokaryotes are divided into two domains: the bacteria and the archaea. Archaea were recognized as a domain of life in 1990. These organisms were originally thought to

live only in inhospitable conditions such as extremes of temperature, pH, and radiation but have since been found in all types of habitats.

Relationship to eukaryotes

A distinction between prokaryotes and eukaryotes (meaning true kernel, also spelled "eucaryotes") is that eukaryotes do have "true" nuclei containing their DNA, whereas the genetic material in prokaryotes is not membrane-bound. Eukaryotic organisms may be unicellular, as in amoebae, or multicellular, as in plants and animals. The difference between the structure of prokaryotes and eukaryotes is so great that it is sometimes considered to be the most important distinction among groups of organisms. However, a criticism of this classification is that the word "prokaryote" is based on what these organisms are not (they are not eukaryotic), rather than what they are (either archaea or bacteria). In 1977, Carl Woese proposed dividing prokaryotes into the Bacteria and Archaea (originally Eubacteria and Archaebacteria) because of the major differences in the structure and genetics between the two groups of organisms. This arrangement of Eukaryota (also called "Eukarya"), Bacteria, and Archaea is called the three-domain system replacing the traditional two-empire system.

The cell structure of prokaryotes differs greatly from that of eukaryotes. The defining characteristic is the absence of a nucleus. Also the size of Ribosomes in prokaryotes are smaller than in eukaryotes, which is now where respiration takes place.The genomes of prokaryotes are held within an irregular DNA/protein complex in the cytosol called the nucleoid, which lacks a nuclear envelope. Prokaryotes generally lack membrane-bound cell compartments: such as mitochondria and chloroplasts. Instead processes such as oxidative phosphorylation and photosynthesis take place across the prokaryotic plasma membrane. However, prokaryotes do possess some internal structures, such as cytoskeletons, and the bacterial order Planctomycetes have a membrane around their nucleoid and contain other membrane-bound cellular structures. Both eukaryotes and prokaryotes contain large RNA/protein structures called ribosomes, which produce protein. Prokaryotes are usually much smaller than eukaryotic cells.

Prokaryotes also differ from eukaryotes in that they contain only a single loop of stable chromosomal DNA stored in an area named the nucleoid, while eukaryote DNA is found on tightly bound and organized chromosomes. Although some eukaryotes have satellite DNA structures called plasmids, these are generally regarded as a prokaryote feature, and many important genes in prokaryotes are stored on plasmids.

Prokaryotes have a larger surface-area-to-volume ratio giving them a higher metabolic rate, a higher growth rate and consequently a shorter generation time compared to Eukaryotes.

Sociality

While prokaryotes are still commonly imagined to be strictly unicellular, most are capable of forming stable aggregate communities. When such communities are encased in a stabilizing polymer matrix ("slime"), they may be called "biofilms". Cells in biofilms often show distinct patterns of gene expression (phenotypic differentiation) in time and space. Also, like multicellular eukaryotes, these changes in expression appear to often result from cell-to-cell signaling, a phenomenon known as quorum sensing.

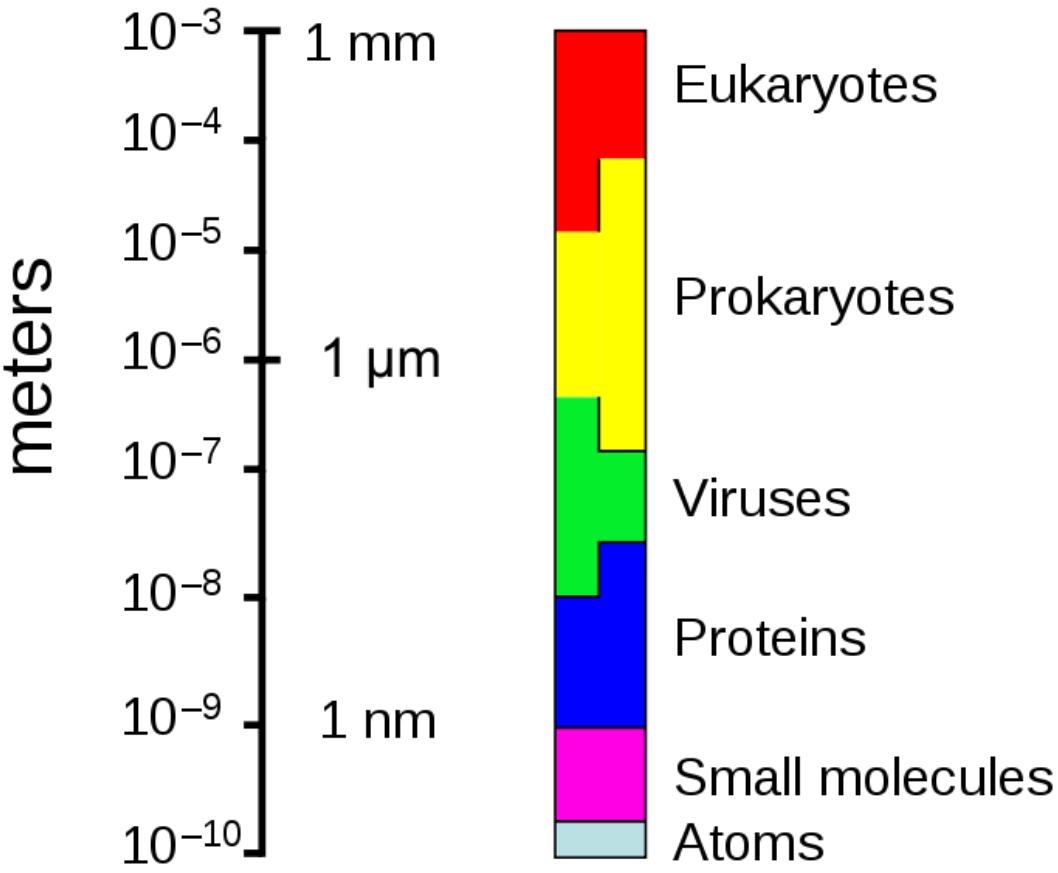
Biofilms may be highly heterogeneous and structurally complex and may attach to solid surfaces, or exist at liquid-air interfaces, or potentially even liquid-liquid interfaces. Bacterial biofilms are often made up of microcolonies (approximately dome-shaped masses of bacteria and matrix) separated by "voids" through which the medium (e.g. water) may flow relatively uninhibited. The microcolonies may join together above the substratum to form a continuous layer, closing the network of channels separating microcolonies. This structural complexity—combined with observations that oxygen limitation (a ubiquitous challenge for anything growing in size beyond the scale of diffusion) is at least partially eased by movement of medium throughout the biofilm—has led some to speculate that this may constitute a circulatory system and many researchers have started calling prokaryotic communities multicellular (for example). Differential cell expression, collective behavior, signaling, programmed cell death, and (in some cases) discrete biological dispersal events all seem to point in this direction. However, these colonies are seldom if ever founded by a single founder (in the way that animals and plants are founded by single cells), which presents a number of theoretical issues. Most explanations of co-operation and the evolution of multicellularity have focused on high relatedness between members of a group (or colony, or whole organism). If a copy of a gene is present in all members of a group, behaviors that promote cooperation between members may permit those members to have (on average) greater fitness than a similar group of selfish individuals.

Should these instances of prokaryotic sociality prove to be the rule rather than the exception it would have serious implications for the way we view prokaryotes in general and the way we deal with them in medicine. Bacterial biofilms may be 100x more resistant to antibiotics than free-living unicells and may be nearly impossible to remove from surfaces once they have colonized them. Other aspects of bacterial cooperation—such as bacterial conjugation and quorum-sensing mediated pathogenicity—present additional challenges to researchers and medical professionals seeking to treat the associated diseases.

Reproduction

Bacteria and archaea reproduce through asexual reproduction, usually by binary fission or budding. Genetic exchange and recombination still occur, but this is a form of horizontal gene transfer and is not a replicative process, simply involving DNA being transferred between two cells, as in bacterial conjugation.

Structure



The sizes of prokaryotes relative to other organisms and biomolecules

Recent research indicates that all prokaryotes actually do have cytoskeletons, albeit more primitive than those of eukaryotes. Besides homologues of actin and tubulin (MreB and FtsZ) the helically arranged building block of the flagellum, flagellin, is one of the most significant cytoskeletal proteins of bacteria as it provides structural backgrounds of chemotaxis, the basic cell physiological response of bacteria. At least some prokaryotes also contain intracellular structures which can be seen as primitive organelles. Membranous organelles (a.k.a. intracellular membranes) are known in some groups of prokaryotes, such as vacuoles or membrane systems devoted to special metabolic properties, e.g. photosynthesis or chemolithotrophy. Additionally, some species also contain protein-enclosed microcompartments, which have distinct physiological roles (e.g. carboxysomes or gas vacuoles).

Most prokaryotes are between 1 µm and 10 µm, but they can vary in size from 0.2 µm to 750 µm (Thiomargarita namibiensis).

Prokaryotic cell Structure

Flagellum
Cell membrane
Cell wall (except genus Mycoplasma)
Cytoplasm
Ribosome
Nucleoid
Glycocalyx
Inclusions

Morphology of prokaryotic cells

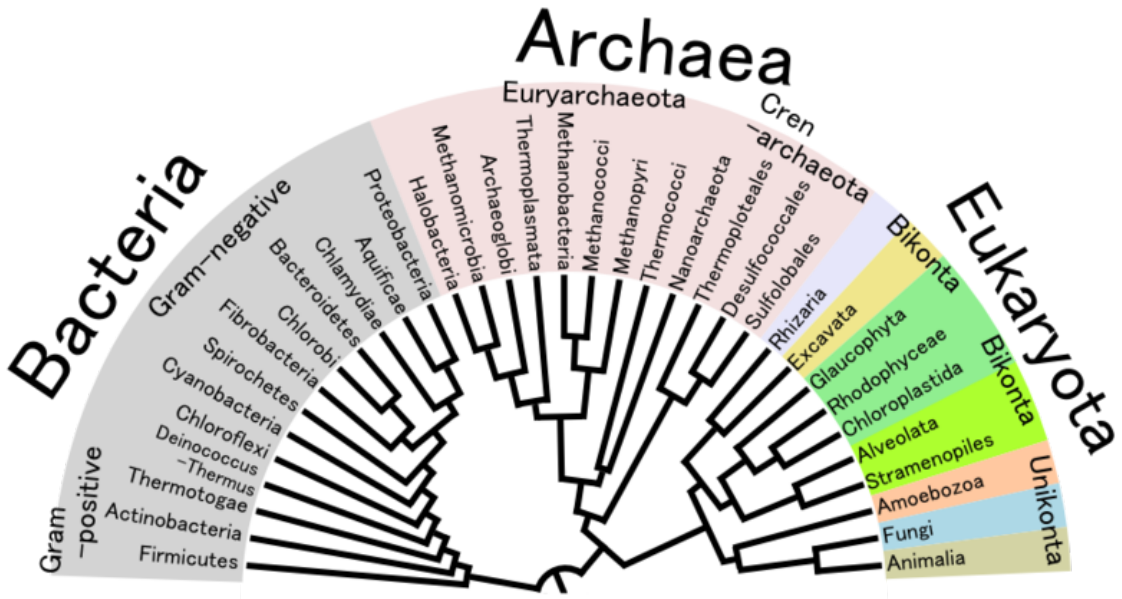
Prokaryotic cells have various shapes; the four basic shapes are:

- Cocci - spherical
- Bacilli - rod shaped
- Spirochaete - spiral shaped
- Vibrio - comma shaped

Environment

Prokaryotes live in nearly all environments on earth where there is liquid water. Some archaea and bacteria thrive in harsh conditions, such as high temperatures (thermophiles) or high salinity (halophiles). Organisms such as these are referred to as extremophiles. Many archaea grow as plankton in the oceans. Symbiotic prokaryotes live in or on the bodies of other organisms, including humans.

Evolution of prokaryotes



Phylogenetic tree showing the diversity of prokaryotes, compared to eukaryotes.

The current model of the evolution of the first living organisms is that these were some form of prokaryotes, which may have evolved out of protobionts. The eukaryotes are generally thought to have evolved later in the history of life. However, some authors have questioned this conclusion, arguing that the current set of prokaryotic species may have evolved from more complex eukaryotic ancestors through a process of simplification. Others have argued that the three domains of life arose simultaneously, from a set of varied cells that formed a single a gene pool. This controversy was summarized in 2005:

There is no consensus among biologists concerning the position of the eukaryotes in the overall scheme of cell evolution. Current opinions on the origin and position of eukaryotes span a broad spectrum including the views that eukaryotes arose first in evolution and that prokaryotes descend from them, that eukaryotes arose contemporaneously with eubacteria and archeabacteria and hence represent a primary line of descent of equal age and rank as the prokaryotes, that eukaryotes arose through a symbiotic event entailing an endosymbiotic origin of the nucleus, that eukaryotes arose without endosymbiosis, and that eukaryotes arose through a symbiotic event entailing a simultaneous endosymbiotic origin of the flagellum and the nucleus, in addition to many other models, which have been reviewed and summarized elsewhere.

The oldest known fossilized prokaryotes were laid down approximately 3.5 billion years ago, only about 1 billion years after the formation of the Earth's crust. Even today, prokaryotes are perhaps the most successful and abundant life forms. Eukaryotes only appear in the fossil record later, and may have formed from endosymbiosis of multiple prokaryote ancestors. The oldest known fossil eukaryotes are about 1.7 billion years old.

However, some genetic evidence suggests eukaryotes appeared as early as 3 billion years ago.

While Earth is the only place in the universe where life is known to exist, some have suggested that there is evidence on Mars of fossil or living prokaryotes; but this possibility remains the subject of considerable debate and skepticism.

Prokaryotes have diversified greatly throughout their long existence. The metabolism of prokaryotes is far more varied than that of eukaryotes, leading to many highly distinct prokaryotic types. For example, in addition to using photosynthesis or organic compounds for energy, as eukaryotes do, prokaryotes may obtain energy from inorganic compounds such as hydrogen sulfide. This enables prokaryotes to thrive in harsh environments as cold as the snow surface of Antarctica, and as hot as undersea hydrothermal vents and land-based hot springs.

Chapter- 8

Transfection

Transfection is the process of deliberately introducing nucleic acids into cells. The term is used notably for non-viral methods in eukaryotic cells. It may also refer to other methods and cell types, although other terms are preferred: "transformation" is more often used to describe non-viral DNA transfer in bacteria, non-animal eukaryotic cells and plant cells - a distinctive sense of transformation refers to spontaneous genetic modifications (mutations to cancerous cells (Carcinogenesis), or under stress (UV irradiation)). "Transduction" is often used to describe virus-mediated DNA transfer. The word *transfection* is a blend of *trans*- and *infection*.

Genetic material (such as supercoiled plasmid DNA or siRNA constructs), or even proteins such as antibodies, may be transfected.

Transfection of animal cells typically involves opening transient pores or "holes" in the cell membrane, to allow the uptake of material. Transfection can be carried out using calcium phosphate, by electroporation, or by mixing a cationic lipid with the material to produce liposomes, which fuse with the cell membrane and deposit their cargo inside.

Transfection can result in unexpected morphologies and abnormalities in target cells.

Terminology

The meaning of the term has evolved. The original meaning of transfection was "infection by transformation", *i.e.* introduction of DNA (or RNA) from a prokaryote-infecting virus or bacteriophage into cells, resulting in an infection. Because the term transformation had another sense in animal cell biology (a genetic change allowing long-term propagation in culture, or acquisition of properties typical of cancer cells), the term transfection acquired, for animal cells, its present meaning of a change in cell properties caused by introduction of DNA.

Methods

There are various methods of introducing foreign DNA into a eukaryotic cell: some rely on physical treatment (electroporation, nanoparticles, magnetofection), other on chemical materials or biological particles (viruses) that are used as carriers.

Chemical-based transfection

Chemical-based transfection can be divided into several kinds: cyclodextrin, polymers, liposomes, or nanoparticles

- One of the cheapest methods uses **calcium phosphate**, originally discovered by F. L. Graham and A. J. van der Eb in 1973. HEPES-buffered saline solution (HeBS) containing phosphate ions is combined with a calcium chloride solution containing the DNA to be transfected. When the two are combined, a fine precipitate of the positively charged calcium and the negatively charged phosphate will form, binding the DNA to be transfected on its surface. The suspension of the precipitate is then added to the cells to be transfected (usually a cell culture grown in a monolayer). By a process not entirely understood, the cells take up some of the precipitate, and with it, the DNA.

- Other methods use **highly branched organic compounds**, so-called dendrimers, to bind the DNA and get it into the cell.

- A very efficient method is the inclusion of the DNA to be transfected in **liposomes**, i.e. small, membrane-bounded bodies that are in some ways similar to the structure of a cell and can actually fuse with the cell membrane, releasing the DNA into the cell. For eukaryotic cells, transfection is better achieved using **cationic liposomes** (or mixtures), because the cells are more sensitive. Popular agents were DOTMA and DOPE , and now - more effectively - Lipofectamine and UptiFectin .

- Another method is the use of **cationic polymers** such as DEAE-dextran or polyethylenimine. The negatively charged DNA binds to the polycation and the complex is taken up by the cell via endocytosis. Popular agents of this type are the Fugene or LT-1 , and JetPEI .

- Other proprietary chemical transfection reagents: PromoFectin, GenePORTER, Hilymax . Effectene or Altogen's cell line specific reagents.

Non chemical methods

- Electroporation is a popular method, although requiring an instrument and affecting the viability of many cell types, that also creates micro-sized holes transiently in the plasma membrane of cells under an electric discharge.

- Similarly, transfection applying sonic forces to cells, referred as Sono-poration.

- Optical transfection is a method where a tiny (~1 μm diameter) hole is transiently generated in the plasma membrane of a cell using a highly focused laser. This technique was first described in 1984 by Tsukakoshi et al., who used a frequency tripled Nd:YAG to generate stable and transient transfection of normal rat kidney cells. In this technique, one cell at a time is treated, making it particularly useful for single cell analysis.

- Gene electrotransfer is a technique that enables transfer of genetic material into prokaryotic or eukaryotic cells. It is based on a physical method named electroporation, where transient increase in the permeability of cell membrane is achieved when submitted to short and intense electric pulses.

- Impalefection is a method of introducing DNA bound to a surface of a nanofiber that is inserted into a cell.

Particle-based methods

- A direct approach to transfection is the **gene gun**, where the DNA is coupled to a nanoparticle of an inert solid (commonly gold) which is then "shot" directly into the target cell's nucleus.

- Magnetofection, or Magnet assisted transfection is a transfection method, which uses magnetic force to deliver DNA into target cells. Nucleic acids are first associated with magnetic nanoparticles. Then, application of magnetic force drives the nucleic acid particle complexes towards and into the target cells, where the cargo is released.

- Impalefection is carried out by impaling cells by elongated nanostructures such as carbon nanofibers or silicon nanowires which have been functionalized with plasmid DNA.

Viral methods

DNA can also be introduced into cells using viruses as a carrier. In such cases, the technique is called viral transduction, and the cells are said to be transduced. This can be done using insect cells.

Other (and hybrid) methods

Other methods of transfection include nucleofection, heat shock.

Stable and transient transfection

For most applications of transfection, it is sufficient if the transfected genetic material is only transiently expressed. Since the DNA introduced in the transfection process is usually not integrated into the nuclear genome, the foreign DNA will be diluted through mitosis or degraded.

If it is desired that the transfected gene actually remains in the genome of the cell and its daughter cells, a stable transfection must occur. To accomplish this, a marker gene is co-transfected, which gives the cell some selectable advantage, such as resistance towards a certain toxin. Some (very few) of the transfected cells will, by chance, have integrated the foreign genetic material into their genome. If the toxin is then added to the cell culture, only those few cells with the marker gene integrated into their genomes will be able to proliferate, while other cells will die. After applying this selective stress (selection pressure) for some time, only the cells with a stable transfection remain and can be cultivated further.

A common agent for selecting stable transfection is Geneticin, also known as G418, which is a toxin that can be neutralized by the product of the neomycin resistant gene.

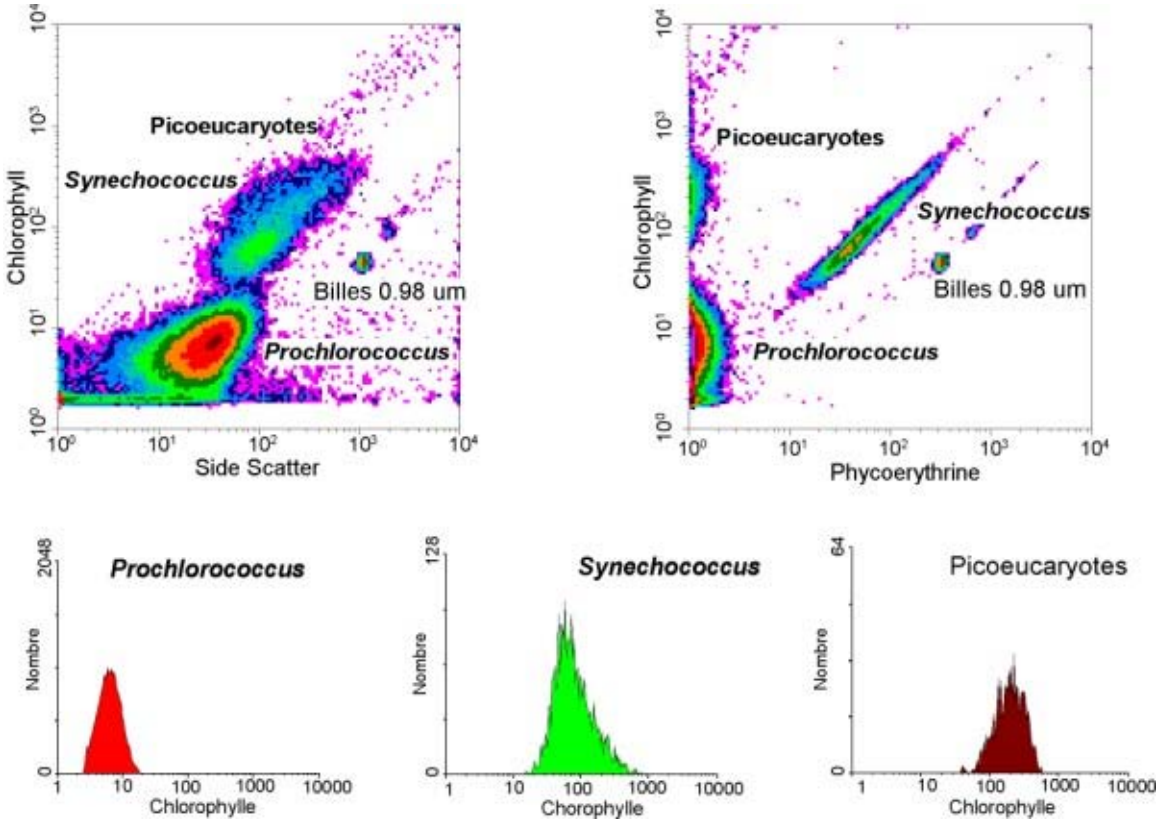
RNA transfection

RNA can also be transfected into cells to transiently express its coded protein, or to study RNA decay kinetics. The later application is referred as siRNA transfection or **RNA silencing**, and has become a major application in research (to replace the "knock-down" experiments, to study the expression of proteins, i.e. of Endothelin-1) with potential applications in gene-therapy.

A limitation of the silencing approach rely on the toxicity of the transfection for cells, and its suspected effect on the expression of other genes/proteins.

Chapter- 9

Flow Cytometry



Analysis of a marine sample of photosynthetic picoplankton by flow cytometry showing three different populations (*Prochlorococcus*, *Synechococcus*, and picoeukaryotes)

Flow cytometry (abbreviated: **FCM**) is a technique for counting and examining microscopic particles, such as cells and chromosomes, by suspending them in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of up to thousands of particles per second. Flow cytometry is routinely used in the diagnosis of health disorders, especially blood cancers, but has many other applications in both

research and clinical practice. A common variation is to physically sort particles based on their properties, so as to purify populations of interest.

History

The first impedance-based flow cytometry device, using the Coulter principle, was disclosed in U.S. Patent 2,656,508, issued in 1953, to Wallace H. Coulter. The first fluorescence-based flow cytometry device (ICP 11) was developed in 1968 by Wolfgang Göhde from the University of Münster and first commercialized in 1968/69 by German developer and manufacturer Partec through Phywe AG in Göttingen. At that time, absorption methods were still widely favored by other scientists over fluorescence methods . Soon after, flow cytometry instruments were developed, including the Cytofluorograph (1971) from Bio/Physics Systems Inc. (later: Ortho Diagnostics), the PAS 8000 (1973) from Partec, the first FACS instrument from Becton Dickinson (1974), the ICP 22 (1975) from Partec/Phywe and the Epics from Coulter (1977/78).

Name of the technology

The original name of the flow cytometry technology was "pulse cytophotometry" (German: *Impulszytophotometrie*). Only 20 years later in 1988, at the Conference of the American Engineering Foundation in Pensacola, Florida, the name was changed to "flow cytometry", a term that quickly became popular.

Principle of flow cytometry

A beam of light (usually laser light) of a **single wavelength** is directed onto a hydrodynamically-focused stream of fluid. A number of detectors are aimed at the point where the stream passes through the light beam: one in line with the light beam (Forward Scatter or FSC) and several perpendicular to it (Side Scatter (SSC) and one or more fluorescent detectors). Each suspended particle from 0.2 to 150 micrometers passing through the beam scatters the ray, and fluorescent chemicals found in the particle or attached to the particle may be excited into emitting light at a longer wavelength than the light source. This combination of scattered and fluorescent light is picked up by the detectors, and, by analysing fluctuations in brightness at each detector (one for each fluorescent emission peak), it is then possible to derive various types of information about the physical and chemical structure of each individual particle. FSC correlates with the cell volume and SSC depends on the inner complexity of the particle (i.e., shape of the nucleus, the amount and type of cytoplasmic granules or the membrane roughness). Some flow cytometers on the market have eliminated the need for fluorescence and use only light scatter for measurement. Other flow cytometers form images of each cell's fluorescence, scattered light, and transmitted light.

Front of desktop flow cytometer - the Becton-Dickinson FACSCalibur.

Flow cytometers

Modern flow cytometers are able to analyze several thousand particles every second, in "real time," and can actively separate and isolate particles having specified properties. A flow cytometer is similar to a microscope, except that, instead of producing an image of the cell, flow cytometry offers "high-throughput" (for a large number of cells) automated quantification of set parameters. To analyze solid tissues, a single-cell suspension must first be prepared.

A flow cytometer has 5 main components:

- a flow cell - liquid stream (sheath fluid), which carries and aligns the cells so that they pass single file through the light beam for sensing
- a measuring system - commonly used are measurement of impedance (or conductivity) and optical systems - lamps (mercury, xenon); high-power water-cooled lasers (argon, krypton, dye laser); low-power air-cooled lasers (argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet) resulting in light signals

- a detector and Analogue-to-Digital Conversion (ADC) system - which generates FSC and SSC as well as fluorescence signals from light into electrical signals that can be processed by a computer
- an amplification system - linear or logarithmic
- a computer for analysis of the signals.

The process of collecting data from samples using the flow cytometer is termed 'Acquisition'. Acquisition is mediated by a computer physically connected to the flow cytometer, and the software which handles the digital interface with the cytometer. The software is capable of adjusting parameters (i.e. voltage, compensation, etc.) for the sample being tested, and also assists in displaying initial sample information while acquiring sample data to insure that parameters are set correctly. Early flow cytometers were, in general, experimental devices, but technological advances have enabled widespread applications for use in a variety of both clinical and research purposes. Due to these developments, a considerable market for instrumentation, analysis software, as well as the reagents used in acquisition such as fluorescently-labeled antibodies has developed.

Modern instruments usually have multiple lasers and fluorescence detectors (the current record for a commercial instrument is **4 lasers and 18 fluorescence detectors**). Increasing the number of lasers and detectors allows for multiple antibody labeling, and can more precisely identify a target population by their phenotypic markers. Certain instruments can even take digital images of individual cells, allowing for the analysis of fluorescent signal location within or on the surface of cell.

Data analysis

Gating

The data generated by flow-cytometers can be plotted in a single dimension, to produce a histogram, or in two-dimensional dot plots or even in three dimensions. The regions on these plots can be sequentially separated, based on fluorescence intensity, by creating a series of subset extractions, termed "gates." Specific gating protocols exist for diagnostic and clinical purposes especially in relation to hematology.

The plots are often made on logarithmic scales. Because different fluorescent dyes' emission spectra overlap, signals at the detectors have to be compensated electronically as well as computationally. Data accumulated using the flow cytometer can be analyzed using software, e.g., WinMDI(deprecated), Flowjo, FCS Express, VenturiOne or CellQuest Pro. Once the data is collected, there is no need to stay connected to the flow cytometer. For this reason, analysis is most often done on a separate computer. This is especially necessary in core facilities where usage of these machines is in high demand.

Computational analysis

Recent progress on automated population identification using computational methods has offered an alternative to traditional gating strategies. Automated identification systems could potentially help findings of rare and hidden populations. Representative automated methods include FLOCK in Immunology Database and Analysis Portal (ImmPort) , FLAME in GenePattern and flowClust ,, in Bioconductor. Collaborative efforts have resulted in an open project called FlowCAP (Flow Cytometry: Critical Assessment of Population Identification Methods,) to provide an objective way to compare and evaluate the flow cytometry data clustering methods, and also to establish guidance about appropriate use and application of these methods.

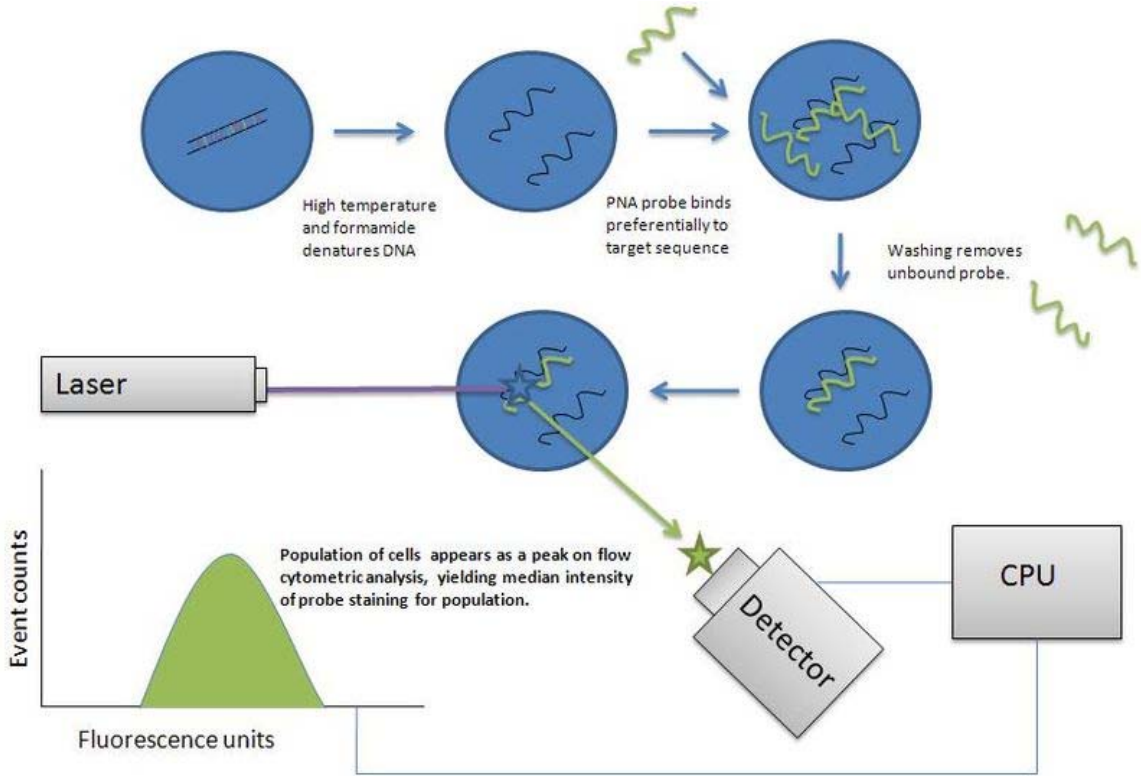
Fluorescence-activated cell sorting

Fluorescence-activated cell sorting is a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. It is a useful scientific instrument, as it provides fast, objective and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest. The acronym FACS is trademarked and owned by Becton, Dickinson and Company. While many immunologists use this term frequently for all types of sorting and non-sorting applications, it is not a generic term for flow cytometry. The first cell sorter was invented by Mack Fulwyler in 1965, using the Coulter principle, a relatively difficult technique and one no longer used in modern instruments. The technique was expanded by Len Herzenberg who was responsible for coining the term FACS. Herzenberg won the Kyoto Prize in 2006 for his work in flow cytometry.

The cell suspension is entrained in the center of a narrow, rapidly flowing stream of liquid. The flow is arranged so that there is a large separation between cells relative to their diameter. A vibrating mechanism causes the stream of cells to break into individual droplets. The system is adjusted so that there is a low probability of more than one cell per droplet. Just before the stream breaks into droplets, the flow passes through a fluorescence measuring station where the fluorescent character of interest of each cell is measured. An electrical charging ring is placed just at the point where the stream breaks into droplets. A charge is placed on the ring based on the immediately-prior fluorescence intensity measurement, and the opposite charge is trapped on the droplet as it breaks from the stream. The charged droplets then fall through an electrostatic deflection system that diverts droplets into containers based upon their charge. In some systems, the charge is applied directly to the stream, and the droplet breaking off retains charge of the same sign as the stream. The stream is then returned to neutral after the droplet breaks off.

Fluorescent labels

A wide range of fluorophores can be used as labels in flow cytometry. These each have a characteristic peak excitation and emission wavelength. Also, the emission spectra of the labels often overlap. Consequently, the combination of labels which can be used depends on the wavelength of the lamp(s) or laser(s) used to excite the fluorochromes and on the detectors available



Use of flow cytometry to measure copy number variation of a specific DNA sequence (Flow-FISH)

Measurable parameters

This list is very long and constantly expanding.

- volume and morphological complexity of cells
- cell pigments such as chlorophyll or phycoerythrin
- total DNA content (cell cycle analysis, cell kinetics, proliferation, etc.)
- total RNA content
- DNA copy number variation (by Flow-FISH)
- chromosome analysis and sorting (library construction, chromosome paint)
- protein expression and localization
- Protein modifications, phospho-proteins

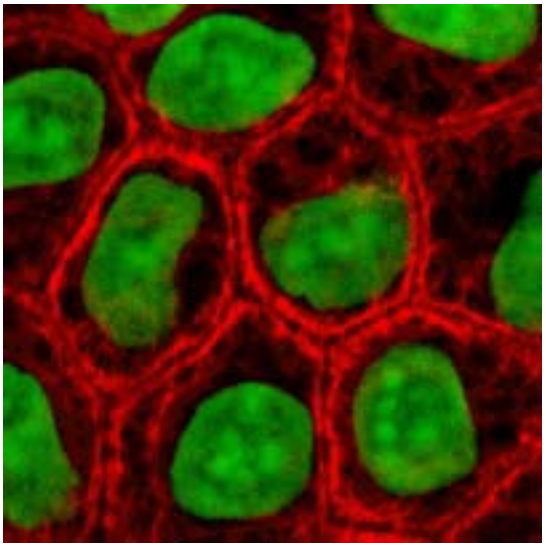
- transgenic products *in vivo*, particularly the Green fluorescent protein or related fluorescent * cell surface antigens (Cluster of differentiation (CD) markers)
- intracellular antigens (various cytokines, secondary mediators, etc.)
- nuclear antigens
- enzymatic activity
- pH, intracellular ionized calcium, magnesium, membrane potential
- membrane fluidity
- apoptosis (quantification, measurement of DNA degradation, mitochondrial membrane potential, permeability changes, caspase activity)
- cell viability
- monitoring electropermeabilization of cells
- oxidative burst
- characterising multidrug resistance (MDR) in cancer cells
- glutathione
- various combinations (DNA/surface antigens, etc.)
- cell adherence (for instance pathogen-host cell adherence)

Applications

The technology has applications in a number of fields, including molecular biology, pathology, immunology, plant biology and marine biology. It has broad application in medicine (especially in transplantation, hematology, tumor immunology and chemotherapy, genetics and sperm sorting for sex preselection). In marine biology, the auto-fluorescent properties of photosynthetic plankton can be exploited by flow cytometry in order to characterise abundance and community structure. In protein engineering, flow cytometry is used in conjunction with yeast display and bacterial display to identify cell surface-displayed protein variants with desired properties. It is also used to determine ploidy of grass carp fry.

Chapter- 10

Cell Culture



Epithelial cells in culture, stained for keratin (red) and DNA (green)

Cell culture is the complex process by which cells are grown under controlled conditions. In practice, the term "cell culture" has come to refer to the culturing of cells derived from multicellular eukaryotes, especially animal cells. However, there are also cultures of plants, fungi and microbes, including viruses, bacteria and protists. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture.

Animal cell culture became a common laboratory technique in the mid-1900s, but the concept of maintaining live cell lines separated from their original tissue source was discovered in the 19th century.

History

The 19th-century English physiologist Sydney Ringer developed salt solutions containing the chlorides of sodium, potassium, calcium and magnesium suitable for maintaining the beating of an isolated animal heart outside of the body. In 1885 Wilhelm Roux removed a

portion of the medullary plate of an embryonic chicken and maintained it in a warm saline solution for several days, establishing the principle of tissue culture. Ross Granville Harrison, working at Johns Hopkins Medical School and then at Yale University, published results of his experiments from 1907–1910, establishing the methodology of tissue culture.

Cell culture techniques were advanced significantly in the 1940s and 1950s to support research in virology. Growing viruses in cell cultures allowed preparation of purified viruses for the manufacture of vaccines. The injectable polio vaccine developed by Jonas Salk was one of the first products mass-produced using cell culture techniques. This vaccine was made possible by the cell culture research of John Franklin Enders, Thomas Huckle Weller, and Frederick Chapman Robbins, who were awarded a Nobel Prize for their discovery of a method of growing the virus in monkey kidney cell cultures.

Concepts in mammalian cell culture

Isolation of cells

Cells can be isolated from tissues for *ex vivo* culture in several ways. Cells can be easily purified from blood, however only the white cells are capable of growth in culture. Mononuclear cells can be released from soft tissues by *enzymatic digestion* with enzymes such as collagenase, trypsin, or pronase, which break down the extracellular matrix. Alternatively, pieces of tissue can be placed in growth media, and the cells that grow out are available for culture. This method is known as *explant culture*.

Cells that are cultured directly from a subject are known as ***primary cells***. With the exception of some derived from tumors, most primary cell cultures have limited lifespan. After a certain number of population doublings (called the Hayflick limit) cells undergo the process of senescence and stop dividing, while generally retaining viability.

An established or **immortalised** *cell line* has acquired the ability to proliferate indefinitely either through random mutation or deliberate modification, such as artificial expression of the telomerase gene. There are numerous well established cell lines representative of particular cell types.

Maintaining cells in culture

Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37°C, 5% CO_2 for mammalian cells) in a cell incubator. Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes being expressed.

Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the growth medium. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrients. The growth factors used to supplement media are often derived from animal blood, such as calf serum. One

complication of these blood-derived ingredients is the potential for contamination of the culture with viruses or prions, particularly in biotechnology medical applications. Current practice is to minimize or eliminate the use of these ingredients wherever possible, but this cannot always be accomplished. Alternative strategies involve sourcing the animal blood from countries with minimum BSE/TSE risk such as Australia and New Zealand, and using purified nutrient concentrates derived from serum in place of whole animal serum for cell culture.

Plating density (number of cells per volume of culture medium) plays a critical role for some cell types. For example, a lower plating density makes granulosa cells exhibit estrogen production, while a higher plating density makes them appear as progesterone producing theca lutein cells.

Cells can be grown in *suspension* or *adherent* cultures. Some cells naturally live in suspension, without being attached to a surface, such as cells that exist in the bloodstream. There are also cell lines that have been modified to be able to survive in suspension cultures so that they can be grown to a higher density than adherent conditions would allow. Adherent cells require a surface, such as tissue culture plastic or microcarrier, which may be coated with extracellular matrix components to increase adhesion properties and provide other signals needed for growth and differentiation. Most cells derived from solid tissues are adherent. Another type of adherent culture is *organotypic culture* which involves growing cells in a three-dimensional environment as opposed to two-dimensional culture dishes. This 3D culture system is biochemically and physiologically more similar to *in vivo* tissue, but is technically challenging to maintain because of many factors (e.g. diffusion).

Cell line cross-contamination

Cell line cross-contamination can be a problem for scientists working with cultured cells. Studies suggest that anywhere from 15–20% of the time, cells used in experiments have been misidentified or contaminated with another cell line. Problems with cell line cross contamination have even been detected in lines from the NCI-60 panel, which are used routinely for drug-screening studies. Major cell line repositories including the American Type Culture Collection (ATCC) and the German Collection of Microorganisms and Cell Cultures (DSMZ) have received cell line submissions from researchers that were misidentified by the researcher. Such contamination poses a problem for the quality of research produced using cell culture lines, and the major repositories are now authenticating all cell line submissions. ATCC uses short tandem repeat (STR) DNA fingerprinting to authenticate its cell lines.

To address this problem of cell line cross-contamination, researchers are encouraged to authenticate their cell lines at an early passage to establish the identity of the cell line. Authentication should be repeated before freezing cell line stocks, every two months during active culturing and before any publication of research data generated using the cell lines. There are many methods for identifying cell lines including isoenzyme

analysis, human lymphocyte antigen (HLA) typing, Chromosomal analysis, Karyotyping, Morphology and STR analysis.

One significant cell-line cross contaminant is the immortal HeLa cell line.

Manipulation of cultured cells

As cells generally continue to divide in culture, they generally grow to fill the available area or volume. This can generate several issues:

- Nutrient depletion in the growth media
- Accumulation of apoptotic/necrotic (dead) cells.
- Cell-to-cell contact can stimulate cell cycle arrest, causing cells to stop dividing known as contact inhibition or senescence.
- Cell-to-cell contact can stimulate cellular differentiation.

Among the common manipulations carried out on culture cells are media changes, passaging cells, and transfecting cells. These are generally performed using tissue culture methods that rely on sterile technique. Sterile technique aims to avoid contamination with bacteria, yeast, or other cell lines. Manipulations are typically carried out in a biosafety hood or laminar flow cabinet to exclude contaminating micro-organisms. Antibiotics (e.g. penicillin and streptomycin) and antifungals (e.g. Amphotericin B) can also be added to the growth media.

As cells undergo metabolic processes, acid is produced and the pH decreases. Often, a pH indicator is added to the medium in order to measure nutrient depletion.

Media changes

In the case of adherent cultures, the media can be removed directly by aspiration and replaced.

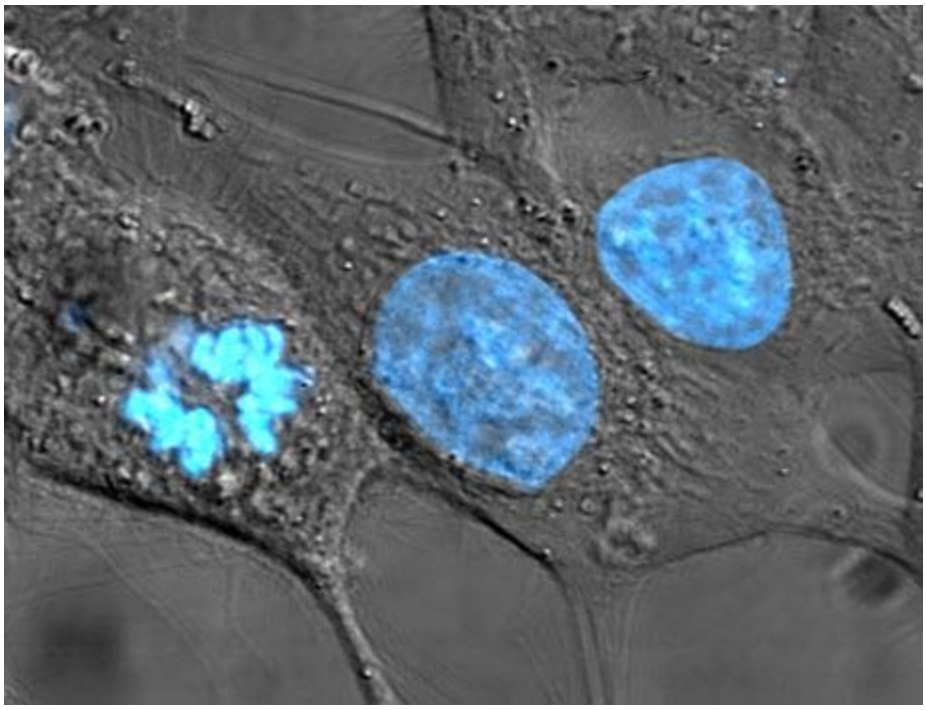
Passaging cells

Passaging (also known as subculture or splitting cells) involves transferring a small number of cells into a new vessel. Cells can be cultured for a longer time if they are split regularly, as it avoids the senescence associated with prolonged high cell density. Suspension cultures are easily passaged with a small amount of culture containing a few cells diluted in a larger volume of fresh media. For adherent cultures, cells first need to be detached; this is commonly done with a mixture of trypsin-EDTA, however other enzyme mixes are now available for this purpose. A small number of detached cells can then be used to seed a new culture.

Transfection and transduction

Another common method for manipulating cells involves the introduction of foreign DNA by transfection. This is often performed to cause cells to express a protein of interest. More recently, the transfection of RNAi constructs have been realized as a convenient mechanism for suppressing the expression of a particular gene/protein. DNA can also be inserted into cells using viruses, in methods referred to as transduction, infection or transformation. Viruses, as parasitic agents, are well suited to introducing DNA into cells, as this is a part of their normal course of reproduction.

Established human cell lines



One of the earliest human cell lines, descended from Henrietta Lacks, who died of the cancer that those cells originated from, the cultured HeLa cells shown here have been stained with Hoechst turning their nuclei blue.

Cell lines that originate with humans have been somewhat controversial in bioethics, as they may outlive their parent organism and later be used in the discovery of lucrative medical treatments. In the pioneering decision in this area, the Supreme Court of California held in *Moore v. Regents of the University of California* that human patients have no property rights in cell lines derived from organs removed with their consent.

Generation of hybridomas

It is possible to fuse normal cells with an immortalised cell line. This method is used to produce monoclonal antibodies. In brief, lymphocytes isolated from the spleen (or possibly blood) of an immunised animal are combined with an immortal myeloma cell

line (B cell lineage) to produce a hybridoma which has the antibody specificity of the primary lymphoctye and the immortality of the myeloma. Selective growth medium (HA or HAT) is used to select against unfused myeloma cells; primary lymphoctyes die quickly in culture and only the fused cells survive. These are screened for production of the required antibody, generally in pools to start with and then after single cloning.

Applications of cell culture

Mass culture of animal cell lines is fundamental to the manufacture of viral vaccines and other products of biotechnology

Biological products produced by recombinant DNA (rDNA) technology in animal cell cultures include enzymes, synthetic hormones, immunobiologicals (monoclonal antibodies, interleukins, lymphokines), and anticancer agents. Although many simpler proteins can be produced using rDNA in bacterial cultures, more complex proteins that are glycosylated (carbohydrate-modified) currently must be made in animal cells. An important example of such a complex protein is the hormone erythropoietin. The cost of growing mammalian cell cultures is high, so research is underway to produce such complex proteins in insect cells or in higher plants, use of single embryonic cell and somatic embryos as a source for direct gene transfer via practicle bombardment, transit gene expression and confocal microscopy observation is one of its applications. It also offers to confirm single cell origin of somatic embryos and the asymmetry of the first cell division, which starts the process.

Tissue culture and engineering

Cell culture is a fundamental component of tissue culture and tissue engineering, as it establishes the basics of growing and maintaining cells *ex vivo*. The major application of human cell culture is in stem cell industry where mesenchymal stem cells can be cultured and cryopreserved for future use.

Vaccines

Vaccines for polio, measles, mumps, rubella, and chickenpox are currently made in cell cultures. Due to the H5N1 pandemic threat, research into using cell culture for influenza vaccines is being funded by the United States government. Novel ideas in the field include recombinant DNA-based vaccines, such as one made using human adenovirus (a common cold virus) as a vector, such as adjuvants.

Culture of non-mammalian cells

Plant cell culture methods

Plant cell cultures are typically grown as cell suspension cultures in liquid medium or as callus cultures on solid medium. The culturing of undifferentiated plant cells and calli requires the proper balance of the plant growth hormones auxin and cytokinin.

Bacterial and yeast culture methods

For bacteria and yeast, small quantities of cells are usually grown on a solid support that contains nutrients embedded in it, usually a gel such as agar, while large-scale cultures are grown with the cells suspended in a nutrient broth.

Viral culture methods

The culture of viruses requires the culture of cells of mammalian, plant, fungal or bacterial origin as hosts for the growth and replication of the virus. Whole wild type viruses, recombinant viruses or viral products may be generated in cell types other than their natural hosts under the right conditions. Depending on the species of the virus, infection and viral replication may result in host cell lysis and formation of a viral plaque.

Common cell lines

Human cell lines

- National Cancer Institute's 60 cancer cell lines

- DU145 (Prostate cancer)
- Lncap (Prostate cancer)
- MCF-7 (breast cancer)
- MDA-MB-438 (breast cancer)
- PC3 (Prostate cancer)
- T47D (breast cancer)
- THP-1 (acute myeloid leukemia)
- U87 (glioblastoma)
- SHSY5Y Human neuroblastoma cells, cloned from a myeloma
- Saos-2 cells (bone cancer)

Primate cell lines

- Vero (African green monkey *Chlorocebus* kidney epithelial cell line initiated 1962)

Rat tumor cell lines

- GH3 (pituitary tumor)
- PC12 (pheochromocytoma)

Mouse cell lines

- MC3T3 (embryonic calvarial)

Plant cell lines

- Tobacco BY-2 cells (kept as cell suspension culture, they are model system of plant cell)

Other species cell lines

- zebrafish ZF4 and AB9 cells.
- *Madin-Darby Canine Kidney (MDCK)* epithelial cell line
- Xenopus A6 kidney epithelial cells.

List of cell lines

Cell line	Meaning	Organism	Origin tissue	Morphology	Link
293-T		Human	Kidney (embryonic)		Derivative of HEK 293ECACC
3T3 cells	"3-day transfer, inoculum 3 x 10^5 cells"	Mouse	Embryonic fibroblast		Also known as NIH 3T3 ECACC
721		Human	Melanoma		
9L		Rat	Glioblastoma		
A2780		Human	Ovary	Ovarian Cancer	ECACC
A2780ADR		Human	Ovary	Adriamycin-resistant derivative	ECACC
A2780cis		Human	Ovary	Cisplatin-resistant derivative	ECACC
A172		Human	glioblastoma	malignant glioma	ECACC
A20		Murine	B lymphoma	B lymphocyte	
A253		Human	Head and neck carcinoma	submandibular duct	

A431		Human	Skin epithelium	squamous carcinoma	ECACCCell Line Data Base
A-549		Human	Lungcarcinoma	Epithelium	DSMZECACC
ALC		Murine	bone marrow	Stroma	PubMed
B16		Murine	Melanoma		ECCAC
B35		Rat	Neuroblastoma		ATCC
BCP-1 cells		Human	PBMC	HIV+ Lymphoma	ATCC
BEAS-2B	Bronchial epithelium + Adenovirus 12-SV40 virus hybrid (Ad12SV40)	Human	Lung	Epithelial	ATCC
bEnd.3	*Brain endothelial*	Mouse	Brain / Cerebral cortex	Endothelium	ATCC
BHK-21	"Baby Hamster Kidney Fibroblast cells"	Hamster	Kidney	fibroblast	ECACCOlympus
BR 293		Human	Breast	Breast cancer	
BxPC3	Biopsy xenograph of pancreatic carcinoma line 3	Human	pancreatic adenocarcinoma	Epithelial	ATCC
C3H-10T1/2		Mouse	Embryonic mesenchymal cell line		ECACC
C6/36		Asian tiger mosquito	larval tissue		ECACC
Cal-27		Human	Tongue	squamous cell carcinoma	
CHO	*Chinese hamster ovary*	hamster	Ovary	Epithelium	ECACCICLC
COR-L23		Human	Lung		ECACC
COR-		Human	Lung		ECACC

L23/CPR					
COR-L23/5010		Human	Lung		ECACC
COR-L23/R23		Human	Lung	Epithelial	ECACC
COS-7	*Cercopithecus aethiops, origin-defective SV-40*	Ape - *Cercopithecus aethiops* (Chlorocebus)	Kidney	fibroblast	ECACCATCC
COV-434		Human	Ovary	Metastatic granulosa cell carcinoma	ECACC
CML T1	*Chronic Myelod Leukaemia T-lymphocyte 1*	Human	CML acute phase	T cell leukaemia	Blood
CMT	*canine mammary tumor*	Dog	Mammary gland	Epithelium	
CT26		Murine	Colorectal Carcinoma	Colon	
D17		canine	osteosarcoma		ECACC
DH82		canine	histiocytosis	monocyte/macrophage	ECACC J Vir Meth
DU145		Human	Androgen insensitive carcinoma	Prostate	PubMed
DuCaP	Dura mater Cancer of the Prostate	Human	Metastatic Prostate Cancer	Epithelial	EAC { Ehrlich Ascites Carcinoma } mice
EL4		Mouse		T cell leukaemia	ECACC
EM2		Human	CML blast crisis	Ph+ CML line	Cell Line Data Base
EM3		Human	CML blast crisis	Ph+ CML line	Cell Line Data Base
EMT6/AR1		Mouse	Breast	Epithelial-like	ECACC

EMT6/AR10.0		Mouse	Breast	Epithelial-like	ECACC
FM3		Human	Metastatic lymph node	melanoma	
H1299		Human	Lung	Lung cancer	
H69		Human	Lung		ECACC
HB54		hybridoma	hybridoma	secretes L243 mAb (against HLA-DR)	Human Immunology
HB55		hybridoma	hybridoma	secretes MA2.1 mAb (against HLA-A2 and HLA-B17)	Journal of Immunology
HCA2		Human	fibroblast		Journal of General Virology
HEK-293	*Human embryonic kidney*	Human	Kidney (embryonic)	Epithelium	ATCC
HeLa	*Henrietta Lacks*	Human	Cervical cancer	Epithelium	DSMZECACC
Hepa1c1c7	clone 7 of clone 1 hepatoma line 1	Mouse	Hepatoma	Epithelial	ECACC ATCC
HL-60	*Human leukemia*	Human	Myeloblast	bloodcells	ECACCDSMZ
HMEC	*Human mammary epithelial cell*	Human		Epithelium	ECACC
HT-29		Human	Colon epithelium	Adenocarcinoma	ECACC Cell Line Data Base
Jurkat		Human	T-Cell-Leukemia	white blood cells	ECACC DSMZ
JY cells		Human	Lymphoblastoid	EBV immortalised B cell	
K562 cells		Human	Lymphoblastoid	CML blast crisis	ECACC
Ku812		Human	Lymphoblast	erythroleukemi	ECACC

			oid	a	LGCstandards
KCL22		Human	Lymphoblastoid	CML	
KG1		Human	Lymphoblastoid	AML	
KYO1	Kyoto 1	Human	Lymphoblastoid	CML	DSMZ
LNCap	Lymph node Cancer of the Prostate	Human	prostatic adenocarcinoma	Epithelial	ECACCATCC
Ma-Mel 1, 2, 3....48		Human		a range of melanoma cell lines	
MC-38		Mouse		Adenocarcinoma	
MCF-7	*Michigan Cancer Foundation-7*	Human	Mammary gland	Invasive breast ductal carcinoma	ER+, PR+
MCF-10A	*Michigan Cancer Foundation*	Human	mammary gland	Epithelium	ATCC
MDA-MB-231	M.D. Anderson - Metastatic Breast	Human	Breast	Cancer	ECACC
MDA-MB-468	M.D. Anderson - Metastatic Breast	Human	Breast	Cancer	ECACC
MDA-MB-435	M.D. Anderson - Metastatic Breast	Human	Breast	melanoma or carcinoma (disputed)	Cambridge Pathology ECACC
MDCK II	*Madin Darby canine kidney*	Dog	Kidney	Epithelium	ECACC ATCC
MDCK II	*Madin Darby canine kidney*	Dog	Kidney	Epithelium	ATCC
MOR/0.2R		Human	Lung		ECACC
MONO-MAC 6		Human	WBC	myeloid metaplasic AML	Cell Line Data Base

MTD-1A		Mouse		Epithelium	
MyEnd	*Myocardial endothelial*	Mouse		Endothelium	
NCI-H69/CPR		Human	Lung		ECACC
NCI-H69/LX10		Human	Lung		ECACC
NCI-H69/LX20		Human	Lung		ECACC
NCI-H69/LX4		Human	Lung		ECACC
NIH-3T3	*NIH, 3-day transfer, inoculum 3 x 10^5 cells*	Mouse	embryo	fibroblast	ECACCATCC
NALM-1			peripheral blood	blast-crisis CML	Cancer Genetics and Cytogenetics
NW-145				Melanoma	ESTDAB
OPCN / OPCT cell lines	Onyvax Prostate Cancer....			Range of prostate tumour lines	Asterand
Peer		Human	T cell leukemia		DSMZ
PNT-1A / PNT 2				Prostate tumour lines	ECACC
RenCa	Renal Carcinoma	Mouse		renal carcinoma	
RIN-5F		Mouse	Pancreas		
RMA/RMAS		Mouse		T cell tumour	
Saos-2 cells		Human		Osteosarcoma	ECACC
Sf-9	*Spodoptera frugiperda*	insect - *Spodoptera frugiperda* (moth)	Ovary		DSMZECACC
SkBr3		Human		Breast carcinoma	
T2		Human		T cell leukemia/B cell line hybridoma	DSMZ
T-47D		Human	Mammary gland	ductal carcinoma	

T84		Human	colorectal Carcinoma / Lungmetastasis	Epithelium	ECACCATCC
THP1 cell line		Human	Monocyte	AML	ECACC
U373		Human	Glioblastoma-astrocytoma	Epithelium	
U87		Human	glioblastoma-astrocytoma	Epithelial-like	Abcam
U937		Human	Leukaemic monocytic lymphoma		ECACC
VCaP	Vertebra Prostate Cancer	Human	Metastatic prostate cancer	Epithelial	ECACC ATCC
Vero cells	'Vera Reno' ('Green kidney') / 'Vero' ('truth')	African Green Monkey	Kidney epithelium		ECACC
WM39		Human	skin	Primary melanoma	
WT-49		Human	Lymphoblastoid		
X63		Mouse	Melanoma		
YAC-1		Mouse	Lymphoma		Cell Line Data Base ECACC
YAR		Human	B-cell	EBV transofrmed	Human Immunology