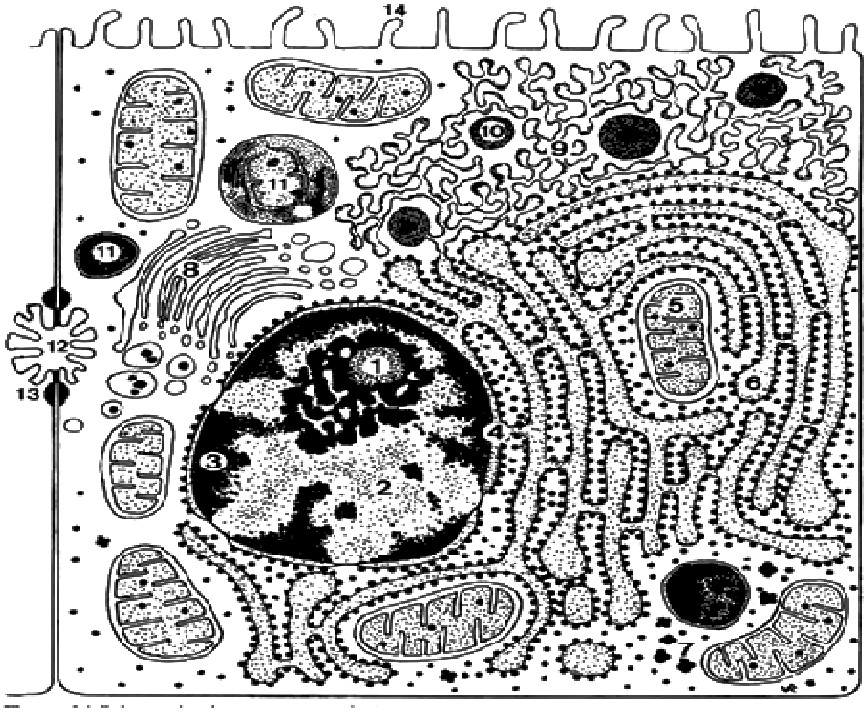
UNIVERSITY OF BAMENDA

DEPARTMENT OF BIOLOGICAL SCIENCES

BIOS 2101

CELL BIOLOGY



Dr. Martin A. Salah

Chapter 1

Introduction to Cell Biology

Both **Living** and **Nonliving Things** are composed of **molecules** made from chemical elements such as **carbon, hydrogen, oxygen**, and **nitrogen**. The organization of these molecules into **Cells** is one feature that distinguishes Living Things from all other matter. The **cell** is the s**mallest unit** of **matter** that can carry on all the processes of **life**.

1.1. The Cell Theory

Every living thing-from the tiniest **bacterium** to the largest **whale**-are made of one or more cells. Before the seventeenth century, no one knew that Cells existed. Most Cells are too small to be seen with the unaided eye. They were not discovered until after the invention of the microscope in the early seventeenth century.

1.1.1. Invention and Development of Microscopy

One of the First **Microscopes** was made by the Dutch drapery store owner **Anton Von Leewenhoek**. With his hand-held microscope, Leewenhoek became the first person to observe and describe **microscopic organisms** and living cells.

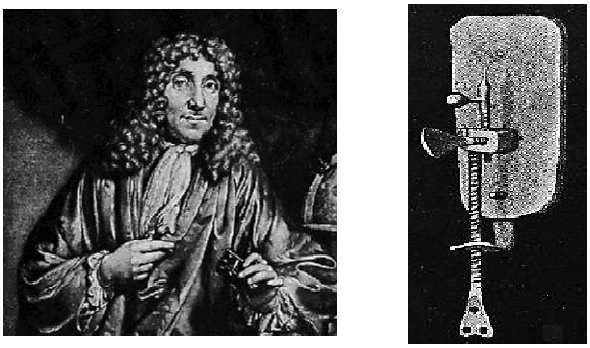


Fig.1.2. Anton van Leeuwenhoek in1676 (left) with his handcrafted microscope (right)

In 1665, the English Scientist **Robert Hooke** used a microscope to examine a thin slice of cork and described it as consisting of "a great many little boxes". It was after his observation that Hook called what he saw "**Cells**". They looked like "little boxes" and reminded him of the small rooms in which monks lived, so he called the "**Cells**".

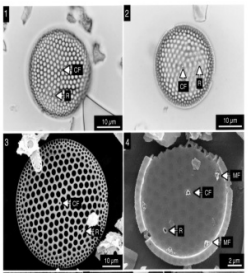


Fig.1.2. Robert Hooke's image in the background of his microscope (left) and his original honey-comb resembling slides at different magnification powers (right)

1.1.2. The Development of the Ideas of the Cell Theory

In 1838, German Botanist **Matthias Schleiden** studied a variety of **plants** and concluded that "***all plants are composed of cells***". The next year, (1939) the German Zoologist **Theodor Schwann** reported that "***animals are also made of cells***" and proposed *a cellular basis for all life*. In 1855, German Physician **Rudolf Virchow** induced that "*The Animal arises only from an Animal and the Plant Only from a Plant*" or " ***That Cells only arise from pre-existing Cells***". His statement contradicted the idea that life could arise from Nonliving Matter. "*The Theory of Spontaneous Generation*" The process by which life begins when ethers enter nonliving things.

Matthias Schleiden (1804-81),

Theodor Schwann, (1810-1882)

Rudolf Virchow (1821-1902)

Fig.1.3. Authors of the Cell Theory

The combine Work of **Schleiden, Schwann**, and **Virchow** make up what is now known as the modern **Cell Theory**. The Cell Theory consist of three Principles:

A. All living Organism are composed of one or more cells.

B. Cells are the basic units of anatomy and physiology in an organism.

C. All Cells come only from reproduction of pre- existing cells.

1.2. The Evolution of the Cell

Prokaryotic cells appeared about 3.5 billion years ago. These cells had no nucleus, simple, circular DNA, no internal organelles. They were **heterotrophic** and used **fermentation** to extract energy from the molecules formed as the result of the heat and light in the early

atmosphere. Fermentation produced **carbon dioxide** and so the concentration of this gas rose in the atmosphere, which further blocked sunlight and continued the cooling trend on Earth. **Photosynthesis** began about 3.3 billion years ago with **autotrophic prokaryotes**. While the earliest photoautotrophic prokaryotes used hydrogen sulphide gas and produced **sulphur**, later cells used water and produced oxygen gas, the concentration of which rose in the atmosphere.

1.2.3. Endosymbiosis and Origin of Eukaryotes

The endosymbiosis theory postulates that

1. The **mitochondria** of eukaryotes evolved from aerobic bacteria (probably related to the **rickettsias**) living within their host cell.
2. The **chloroplasts** of eukaryotes evolved from endosymbiotic **cyanobacteria**.
3. Eukaryotic **cilia** and **flagella** may have arisen from endosymbiotic **spirochetes**.
4. The basal bodies from which eukaryotic cilia and flagella develop would have been able to create the mitotic spindle and thus made **mitosis** possible.

The evidence for mitochondria and chloroplasts are as follows:

1. Both **mitochondria** and **chloroplasts** can arise only from pre-existing mitochondria and chloroplasts. They cannot be formed in a cell that lacks them because **nuclear genes** encode only some of the proteins of which they are made.
2. Both mitochondria and chloroplasts have their own **genome** and it resembles that of bacteria not that of the nuclear genome. Both genomes consist of a **single circular molecule of DNA**. There are no **histones** associated with the DNA.
3. Both mitochondria and chloroplasts have their own **protein-synthesizing machinery**, and it more closely resembles that of **bacteria** than that found in the cytoplasm of eukaryotes.
    a. The first amino acid of their transcripts is always **fMet** as it is in bacteria (not **methionine** [Met] that is the first amino acid in eukaryotic proteins).
    b. A number of antibiotics (e.g., **streptomycin**) that act by blocking protein synthesis in bacteria also block protein synthesis within mitochondria and chloroplasts. They do not interfere with protein synthesis in the cytoplasm of the eukaryotes.
    c. Conversely, inhibitors (e.g., **diphtheria toxin**) of protein synthesis by eukaryotic ribosomes do not — sensibly enough — have any effect on bacterial protein synthesis nor on protein synthesis within mitochondria and chloroplasts.
    d. The antibiotic **rifampicin**, which inhibits the RNA polymerase of bacteria, also inhibits the RNA polymerase within mitochondria. It has no such effect on the RNA polymerase within the eukaryotic nucleus.

1.3. Diversity of Cellular Structures

Not all cells are alike. Even cells within the same organism show Enormous Diversity in **Size, Shape**, and **Internal Organization**. Your Body contains at least 200 Different Cell Types.

1.3.1. Cell Size

A few types of cells are large enough to be seen by the unaided eye. The **Female Egg** is the largest cell in the body, and can be seen without the aid of a microscope. Most cells are visible only with a microscope. Most cells are small for **two reasons**:

1. Cells are limited in size by the **ratio** between their **outer surface area** and their **volume**. A small cell has more surface area than a large cell for a given volume of **cytoplasm.** This is important because the nutrients, **oxygen**, and other materials a cell requires must enter through it surface. As a cell grows larger at some point its surface area becomes too Small to allow these materials to enter the cell quickly enough to meet the cell's need.
2. The cell's nucleus can only control a certain amount of living, active cytoplasm.

1.3.2. Cell Shape

Cells come in a variety of Shapes. Notice the **neurons** on the wall, the basic cell of our Nervous System. This diversity of form reflects a diversity of function. Most Cells have a Specific **Shape.** The shape of a cell depends on it's **function.** Cells of the Nervous System that carry information from your toes to your brain are long and threadlike. Blood Cells are shaped like round disk that can squeeze through tiny blood vessels.

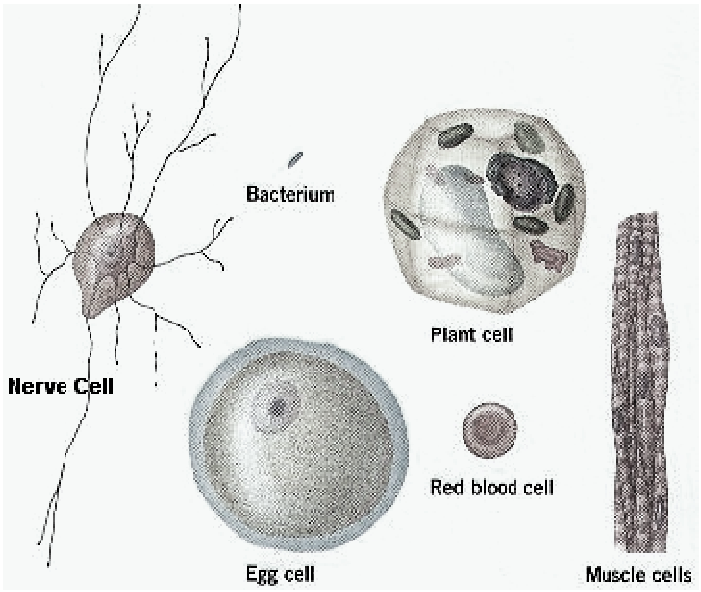


Fig.1.5. Diversity of Cellular Structures

Chapter 2

Methods of Cell Isolation and Study

There are four techniques applied in the cell study:

1. Biochemical Tests
2. Microscopy
3. Chromatography
4. Cell Fractionation

2.1. Methods of Cell Study

2.1.1. Biochemical Tests

These five tests identify the main biologically important chemical compounds. For each test take a small amount of the substance to test, and shake it in water in a test tube. If the sample is a piece of food, then grind it with some water in a pestle and mortar to break up the cells and release the cell contents. Many of these compounds are insoluble, but the tests work just as well on a fine suspension.

(a) Starch (Iodine Test)

To approximately 2 cm^3 of test solution add two drops of iodine/potassium iodide solution. A **blue-black colour** indicates the presence of starch as a starch-poly-iodide complex is formed. Starch is only slightly soluble in water, but the test works well in a suspension or as a solid.

(b) Reducing Sugars (Benedict's Test)

All monosaccharides and most disaccharides (except sucrose) will reduce **copper (II) sulphate**, producing a precipitate of **copper (I) oxide** on heating, so they are called **reducing sugars**. **Benedict's reagent** is an aqueous solution of copper (II) sulphate, **sodium carbonate** and **sodium citrate**. To approximately 2 cm^3 of test solution add an equal quantity of Benedict's reagent. Shake, and heat for a few minutes at 95°C in a water bath. A precipitate indicates reducing sugar. The colour and density of the precipitate gives an indication of the amount of reducing sugar present, so this test is semi-quantitative. The original pale blue colour means no reducing sugar, a green precipitate means relatively little sugar; a brown or red precipitate means progressively more sugar is present.

(c) Non-reducing Sugars (Benedict's Test)

Sucrose is called a **non-reducing sugar** because it does not reduce copper sulphate, so there is no direct test for sucrose. However, if it is first hydrolysed (broken down) to its constituent monosaccharides (glucose and fructose), it will then give a positive **Benedict's test**. So

sucrose is the only sugar that will give a **negative Benedict's test** before hydrolysis and a positive test afterwards. First test a sample for reducing sugars, to see if there are any present before hydrolysis. Then, using a separate sample, boil the test solution with dilute hydrochloric acid for a few minutes to hydrolyse the glycosidic bond. Neutralise the solution by gently adding small amounts of solid sodium hydrogen carbonate until it stops fizzing, then test as before for reducing sugars.

(d) Lipids (Emulsion Test)

Lipids do not dissolve in water, but do dissolve in ethanol. This characteristic is used in the emulsion test. Do not start by dissolving the sample in water, but instead shake some of the test sample with about 4 cm^3 of ethanol. Decant the liquid into a test tube of water, leaving any undissolved substances behind. If there are lipids dissolved in the ethanol, they will precipitate in the water, forming a cloudy white emulsion. The test can be improved by adding the dye **Sudan III,** which stains **lipids red**.

(e) Protein (Biuret Test)

To about 2 cm^3 of test solution add an equal volume of **biuret solution,** down the side of the test tube. A blue ring forms at the surface of the solution, which disappears on shaking, and the solution turns lilac-purple, indicating protein. The colour is due to a complex between nitrogen atoms in the peptide chain and Cu^{2+} ions, so this is really a test for peptide bonds.

2.1.2. Microscopy

Of all the techniques used in biology microscopy is probably the most important. The vast majority of living organisms are too small to be seen in any detail with the human eye, and cells and their organelles can only be seen with the aid of a microscope. Cells were first seen in **1665 by Robert Hooke** (who named them after monks' cells in a monastery), and were studied in more detail by **Leeuwehoek** using a primitive microscope.

(a) Units of Measurement

The standard **SI units** of measurement used in microscopy are:

metre	m	= 1 m
millimetre	mm	= 10^{-3} m
micrometre	μm	= 10^{-6} m
nanometre	nm	= 10^{-9} m
picometre	pm	= 10^{-12} m
angstrom	Å	= 10^{-10} m (obsolete)

(b) Magnification and Resolving Power

By using more lenses microscopes can magnify by a larger amount, but this doesn't always mean that more detail can be seen. The amount of detail depends on the **Resolving power** of a microscope, which is the smallest separation at which two separate objects can be distinguished (or resolved). It is calculated by the formula:

$$\text{resolving power} = \frac{0.6\lambda}{n.a.}$$

where λ is the wavelength of light, and *n.a.* is the **numerical aperture** of the lens (which ranges from about 0.5 to 1.4). So the resolving power of a microscope is ultimately limited by the wavelength of light (400-600nm for visible light). To improve the resolving power a shorter wavelength of light is needed, and sometimes microscopes have blue filters for this purpose (because blue has the shortest wavelength of visible light).

(c) Different Kinds of Microscope

(i) Light Microscope

This is the oldest, simplest and most widely-used form of microscopy. Specimens are illuminated with light, which is focussed using glass lenses and viewed using the eye or photographic film. Specimens can be living or dead, but often need to be stained with a coloured dye to make them visible. Many different stains are available that stain specific parts of the cell such as DNA, lipids, cytoskeleton, etc. All light microscopes today are **compound microscopes**, which means they use several lenses to obtain high magnification. Light microscopy has a resolution of about 200 nm, which is good enough to see cells, but not the details of cell organelles. There has been a recent resurgence in the use of light microscopy, partly due to technical improvements, which have dramatically improved the resolution far beyond the theoretical limit. For example **fluorescence microscopy** has a resolution of about 10 nm, while **interference microscopy** has a resolution of about 1 nm.

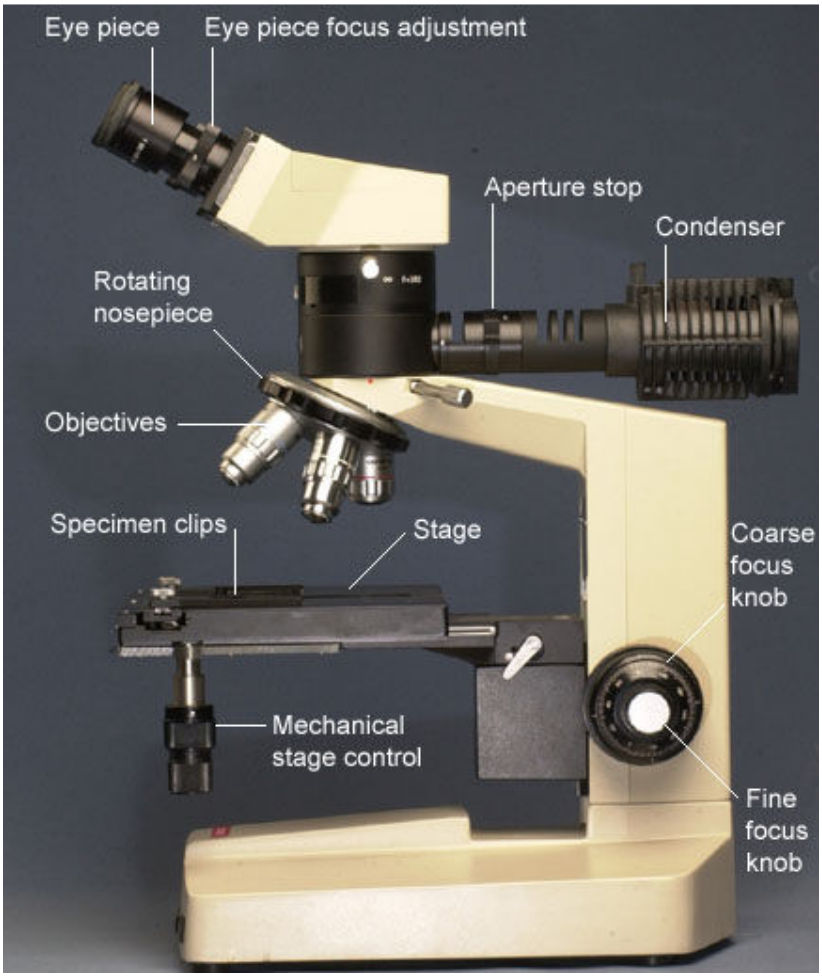


Fig.2.1.A Modern Light Microscope

(ii) Electron Microscope

This uses a beam of electrons, rather than electromagnetic radiation, to "illuminate" the specimen. This may seem strange, but electrons behave like waves and can easily be produced (using a hot wire), focussed (using electromagnets) and detected (using a phosphor screen or photographic film). A beam of electrons has an effective wavelength of less than 1 nm, so can be used to resolve small **sub-cellular ultrastructure**. The development of the electron microscope in the 1930s revolutionised biology, allowing organelles such as **mitochondria, ER** and **membranes** to be seen in detail for the first time.

The main problem with the electron microscope is that specimens must be fixed in plastic and viewed in a vacuum, and must therefore be dead. Other problems are that the specimens can be damaged by the electron beam and they must be stained with an electron-dense chemical (usually heavy metals like osmium, lead or gold). Initially there was a problem of **artefacts** (i.e. observed structures that were due to the preparation process and were not real), but improvements in technique have eliminated most of these.

There are two kinds of electron microscope:

1. The **Transmission Electron Microscope** (TEM) works much like a light microscope, transmitting a beam of electrons through a thin specimen and then focussing the electrons to form an image on a screen or on film. This is the most common form of electron microscope and has the best resolution.
2. The **Scanning Electron Microscope** (SEM) scans a fine beam of electron onto a specimen and collects the electrons scattered by the surface. This has poorer resolution, but gives excellent 3-dimentional images of surfaces.
3. **X-ray Microscope.** This is an obvious improvement to the light microscope, since x-rays have wavelengths a thousand time shorter than visible light, and so could even be used to resolve atoms. Unfortunately there are no good x-ray lenses, so an image cannot be focussed, and useable x-ray microscopes do not yet exist. However, x-rays can be used without focussing to give a **Diffraction Pattern**, which can be used to work out the structures of molecules, such as those of **proteins** and **DNA**.
4. **Scanning Tunnelling Microscope (or Atomic Force Microscope).** This uses a very fine needle to scan the surface of a specimen. It has a resolution of about 10 pm, and has been used to observe individual atoms for the first time.

Transmission Electron Microscope

Scanning Electron Microscope

Fig. 2.2. Electron Microscopes

(iii) Comparison of Light and Electron Microscopes

	light microscope	**electron microscope**
illumination and source	light from lamp	electrons from hot wire
focusing	glass lenses	electromagnets
detection	eye or film	phosphor screen or film
magnification	1 500 x	500 000 x
resolution	200 nm	1 nm
specimen	living or dead	dead
staining	coloured dyes	heavy metals
cost	cheap to expensive	very expensive