

Cells



All living things are made of cells, and cells are the smallest units that can be alive. **Life on Earth is classified into five kingdoms**, and they each have their own characteristic kind of cell. However the biggest division is between the cells of the **Prokaryote** kingdom (the bacteria) and those of the other four kingdoms (**Animals, Plants, Fungi** and **Protoctista**), which are all **eukaryotic** cells. **Prokaryotic cells are smaller and simpler than eukaryotic cells, and do not have a nucleus.**

Prokaryote = "before carrier bag" i.e. without a nucleus

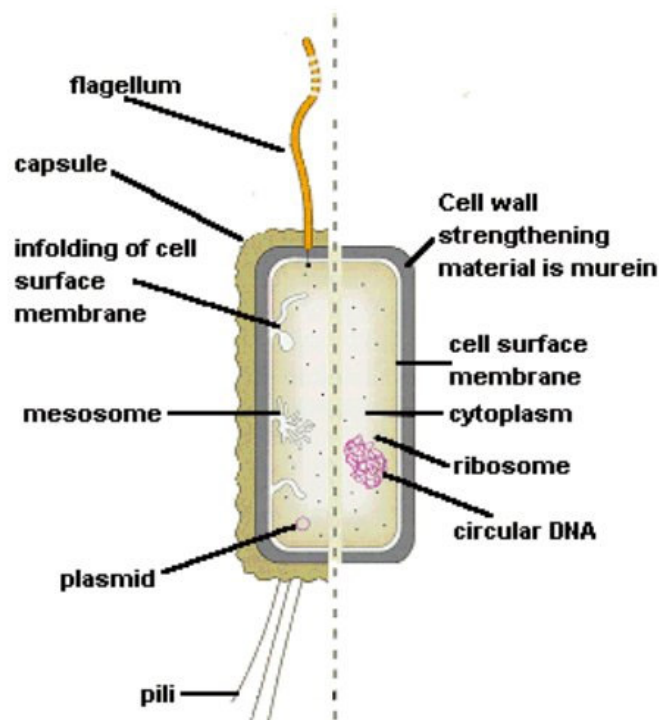
Eukaryote = "good carrier bag" i.e. with a nucleus

We'll examine these two kinds of cell in detail, based on structures seen in **electron micrographs** (= photos taken with an electron microscope). These show the individual **organelles** inside a cell.

Summary of the Differences Between Prokaryotic and Eukaryotic Cells

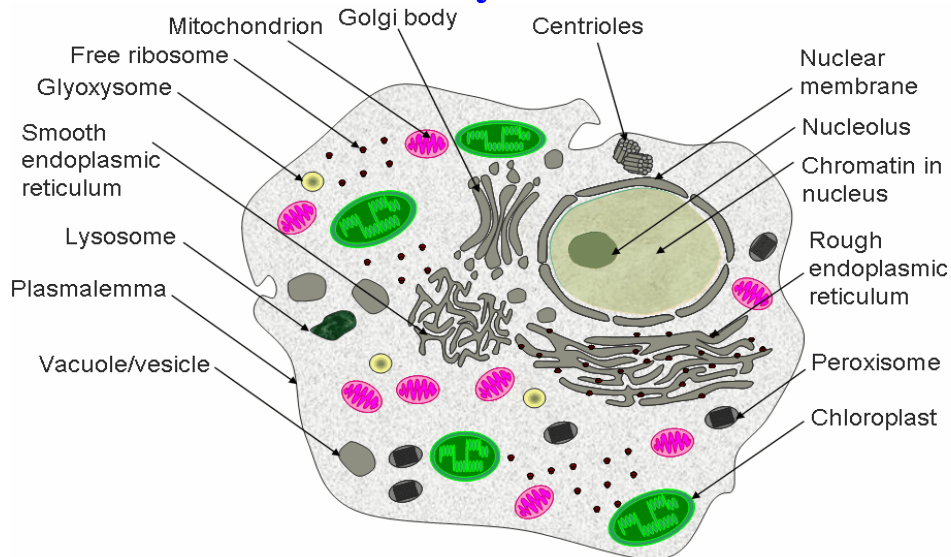
Prokaryotic Cells	Eukaryotic cells
Small cells (< 5 μm)	Larger cells (> 10 μm)
Always unicellular	Often multicellular
No nucleus or any membrane-bound organelles, such as mitochondria	Always have nucleus and other membrane-bound organelles
DNA is circular, without proteins	DNA is linear and associated with proteins to form chromatin
Ribosomes are small (70S)	Ribosomes are large (80S)
No cytoskeleton	Always has a cytoskeleton
Motility by rigid rotating flagellum (made of flagellin)	Motility by flexible waving cilia or flagellae (made of tubulin)
Cell division is by binary fission	Cell division is by mitosis or meiosis
Reproduction is always asexual	Reproduction is asexual or sexual
Huge variety of metabolic pathways	Common metabolic pathways

Prokaryotic Cells



- **Cytoplasm.** Contains all the enzymes needed for all metabolic reactions, since **there are no organelles**
- **Ribosomes.** The **smaller (70 S) type**.
- **Nuclear Body.** The region of the cytoplasm that contains DNA. It is **not surrounded by a nuclear membrane**.
- **DNA.** Always **circular** and **not associated with any proteins to form chromatin**.
- **Plasmid.** **Small loops of DNA**, used to exchange DNA between bacterial cells. **Used in genetic engineering**, they often contain **genes giving resistance to antibiotics**.
- **Cell membrane.** Made of phospholipids and proteins, **like eukaryotic membranes**.
- **Mesosome.** A tightly folded region of the cell membrane containing all the membrane-bound proteins required for respiration and photosynthesis. Can also be associated with the nucleoid.
- **Cell Wall.** Made of **murein** (not cellulose), which is a **glycoprotein** (i.e. a protein/carbohydrate complex, also called **peptidoglycan**). There are two kinds of cell wall, which can be distinguished by **Gram's stain**:
 - **Gram +ve** bacteria have a thick cell wall, stain purple, may have spores and **are sensitive to penicillin and lysosome** (an antibacterial enzyme found in tears and saliva).
 - **Gram -ve** bacteria have a thin cell wall with an outer lipid layer, have no spores and stain pink – these are thought to be more highly evolved.
- **Capsule.** A **thick polysaccharide layer outside the cell wall**. Used for sticking cells together, as a food reserve, as protection against desiccation and chemicals, and as protection against phagocytosis. Found only in some Gram +ve bacteria, **if a capsule is present, then flagellae are not**.
- **Flagellum.** A rigid rotating helical-shaped tail **used for propulsion**. The motor is embedded in the cell membrane and is driven by a H^+ gradient across the membrane. They always rotate clockwise - the only known example of a rotating motor in nature – rather like a propeller on a ship, it has to pass through the ‘hull’ of the cell *via* a waterproof seal.

Eukaryotic Cells



- **Cell Membrane (or Plasma Membrane).** This is a thin, flexible layer round the outside of all cells **made of phospholipids and proteins**. It **separates the contents of the cell from the outside environment, and controls the entry and exit of materials**. The membrane is examined in detail later.
- **Cytoplasm (or Cytosol).** This is the solution within the cell membrane. It contains enzymes for glycolysis (the first stage of respiration) and other metabolic reactions together with sugars, salts, amino acids, nucleotides and everything else needed for the cell to function. This is where the first stage of respiration (= **glycolysis**) takes place.
- **Nucleus.** This is the largest organelle. Surrounded by a **nuclear envelope**, which is a double membrane with **nuclear pores** - large holes containing proteins that control the exit of substances such as mRNA and ribosomes from the nucleus. The interior is called the **nucleoplasm**, which is full of **chromatin** - a DNA/protein complex containing the genes. During cell division the chromatin becomes condensed into discrete observable **chromosomes**. The **nucleolus** is a dark region of the nucleus, involved in making ribosomes and 'processing' m-RNA (i.e. removing introns)
- **80S Ribosomes.** These are the **smallest and most numerous of the cell organelles**, and are **the sites of protein synthesis**. They are composed of protein and RNA and are manufactured in the nucleolus of the nucleus. Ribosomes can be free in the cytoplasm, or (more commonly) are attached to the rough endoplasmic reticulum. They are often found in groups called **polysomes**. **NB All eukaryotic ribosomes are of the larger, 80S, type.**
- **Endoplasmic Reticulum (ER).** This collection of membrane channels forms an important **transport 'highway' within the cell**, allowing molecules to move from one place to another. It is attached to, and formed from, the outer membrane of the nucleus, and plays an important part in protein synthesis. **It comes in two distinct forms:**
 - **Rough Endoplasmic Reticulum (RER).** This is **studded with numerous 80S ribosomes** which give it its rough appearance. The ribosomes synthesise proteins which are processed in the SER (e.g. by modifying the polypeptide chain, or adding carbohydrates), before being exported from the cell via the **Golgi Body**.
 - **Smooth Endoplasmic Reticulum (SER).** Similar to the RER, but **without the ribosomes**. Series of membrane channels involved in the synthesis and transport of materials, mainly lipids and glycoproteins, needed by the cell.

- **Golgi Body (or Apparatus).** Another **series of flattened membrane vesicles**, formed from the endoplasmic reticulum. Its job is to **transport proteins from the RER to the cell membrane for export**. Parts of the SER containing proteins fuse with one side of the Golgi body membranes, while at the other side small vesicles bud off and move towards the cell membrane, where they fuse, releasing their contents by **exocytosis**.
- **Vacuoles.** These are membrane-bound sacs containing a dilute solution. Most cells have small vacuoles that are formed as required, but plant cells usually have **one** very large permanent vacuole that fills most of the cell, so that the cytoplasm (and everything else) forms a thin layer round the outside. Plant cell vacuoles are filled with **cell sap**, and are very important in keeping the cell **turgid**. Some unicellular protists have **feeding vacuoles** for digesting food, or **contractile vacuoles** for expelling water (**osmoregulation**).
- **Lysosomes.** These are **small membrane-bound vesicles containing a cocktail of digestive enzymes**. They are used to break down unwanted chemicals, toxins, organelles or even whole cells, so that the materials may be recycled. They can also fuse with a feeding vacuole to digest its contents. Responsible for cell death – ‘**autolysis**’.
- **Cytoskeleton.** This is **a network of protein fibres** extending throughout all eukaryotic cells, used for support, transport and motility. The cytoskeleton is attached to the cell membrane and gives the cell its shape, as well as holding all the organelles in position. There are two types of protein fibres (**microfilaments** and **microtubules**); each has a corresponding protein that can carry a ‘cargo’ such as an organelle, chromosome or other cytoskeleton fibres along the fibre. They are responsible for chromosome movement in **mitosis** and the subsequent division of the cell, cytoplasmic streaming or **cyclosis** (in plants only), cilia and flagella movements, and muscle contraction.
- **Centriole.** This is a set of short microtubules (‘9+2’) **involved in cell division**. Before each division the centriole replicates itself and the two centrioles move to opposite ends of the cell, forming the **spindle** that organises and separates the chromosomes.
- **Cilia and Flagellae (or Undulipodia)** These are long flexible ‘tails’ present in some cells and **used for movement**. They are **surrounded by the cell membrane**, and are full of microtubules and motor proteins, so they are capable of complex swimming movements. There are two kinds:
 - **flagellae** (no relation of the bacterial flagellum) are longer than the cell, and there are usually only one or two of them, whilst
 - **cilia**, though identical in structure, are much smaller and there are usually very many of them.
- **Microvilli.** These are small finger-like extensions of the cell membrane found in some animal cells (e.g. the epithelial cells of the gut & kidney), where they **increase the surface area for absorption**. They are **just visible under the light microscope as the brush border**.
- **Cell Wall.** This is a thick layer outside the cell membrane used to give a cell strength and rigidity. Cell walls consist of a network of fibres, which give strength but are **freely permeable to solutes** (unlike membranes). (**A wickerwork basket is a good analogy.**) Plant cell walls are made mainly of **cellulose**, but also contain pectin, lignin and other polysaccharides too. It is built up in layers with the **middle lamella** separating the cell wall of adjacent cells. There are often channels through plant cell walls called **plasmodesmata**, which link the cytoplasm of adjacent cells.
 - Fungal cell walls are made of **chitin**.
 - **Animal cells do not have a cell wall.**

‘Semi-autonomous Organelles’:

There are two organelles which contain their own DNA (coding for about 50% of the organelle) and reproduce independently of the nucleus. They are said to be ‘**semi-autonomous organelles**’. Mitochondrial DNA mutates at a known, constant, rate and is **only** inherited from the mother, so it can be used to track purely female genetic lines. In the same way, the Y chromosome is (obviously) only passed on from father to son and so can be used to track the purely male genetic line.

- **Mitochondria (sing. Mitochondrion)**. These are sausage-shaped organelles (2-5µm long), where **aerobic respiration** takes place in eukaryotic cells. Mitochondria are **surrounded by a double membrane**: the outer membrane is quite permeable, but the inner membrane is highly folded into **cristae**, which give it a large surface area. It is studded with **ATPase**, the enzyme which is the main site of ATP synthesis. This is where the last stage of respiration – the **ETC** takes place. The space enclosed by the inner membrane is called the **mitochondrial matrix** and contains small circular strands of DNA and 70S ribosomes. This is the site of the **TCA or Krebs’s cycle** stage of respiration.
- **Chloroplasts. Bigger and fatter than mitochondria (so settle first when cells are homogenised and centrifuged)**, chloroplasts are **the site of photosynthesis**, so are only found in photosynthetic cells (plants and algae). Like mitochondria a **double membrane encloses them**, but chloroplasts also contain membranes arranged in disks called **thylakoids**. Thylakoids contain chlorophyll and other photosynthetic pigments and **carry out the light reactions of photosynthesis**. The thylakoids are then stacked into piles called **grana**. The space between the inner membrane and the thylakoid is called the **stroma** – the site of the light-independent (or ‘carbon-fixing’) stage of photosynthesis. **Chloroplasts also contain starch grains, 70S ribosomes and circular DNA.**

Endosymbiosis (= probable evolution of mitochondria and chloroplasts)

*Prokaryotic cells are far older and more diverse than eukaryotic cells. Prokaryotic cells have probably been around for 3.5 billion years, while eukaryotic cells arose only about 1 billion years ago. It is thought that eukaryotic cell organelles like mitochondria and chloroplasts are derived from prokaryotic cells that became incorporated inside larger prokaryotic cells. This idea is called **endosymbiosis**, and is supported by these observations:*

- *Organelles contain circular DNA, like bacteria cells.*
- *Contain 70S ribosomes, like bacteria cells.*
- *Organelles have double membranes, as though a single-membrane cell had been engulfed and surrounded by a larger cell.*
- *Organelles reproduce by binary fission, like bacteria.*
- *Organelles are very like some bacteria that are alive today.*

Cell Fractionation

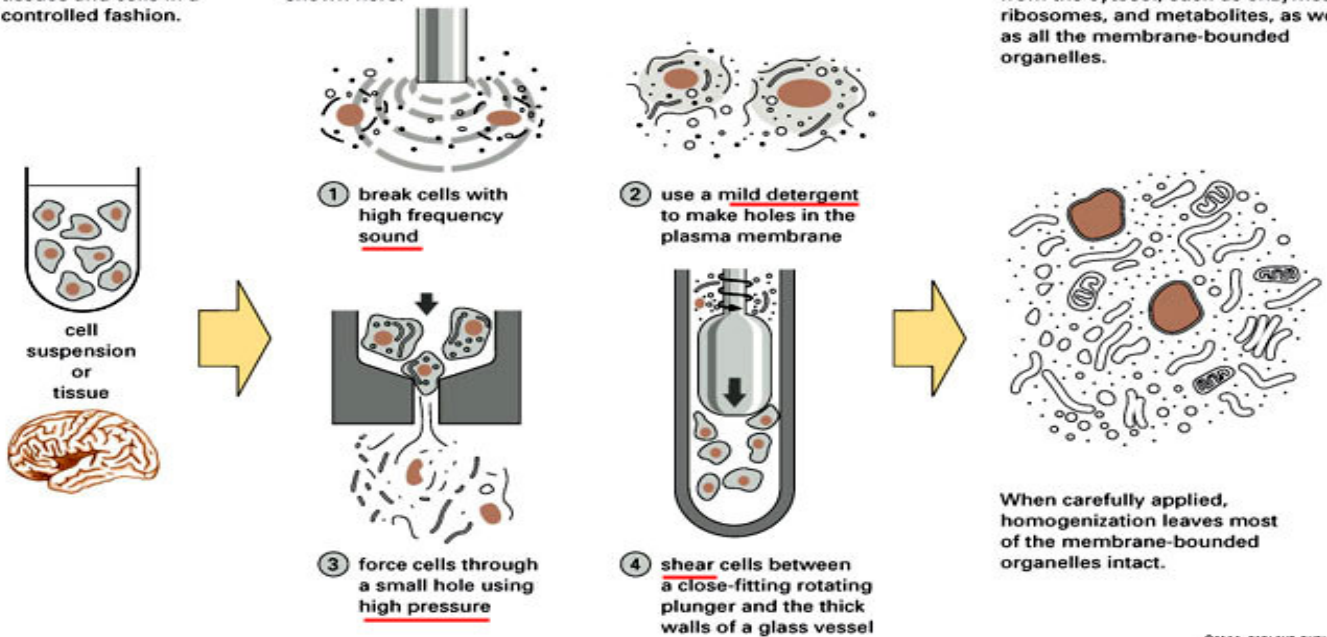
This means separating different parts and organelles of a cell, so that they can be studied in detail. All the processes of cell metabolism (such as respiration or photosynthesis) have been studied in this way. The most common method of fractionating cells is to use **differential centrifugation**. This begins with breaking open the cells (**homogenisation**):

BREAKING CELLS AND TISSUES

The first step in the purification of most proteins is to disrupt tissues and cells in a controlled fashion.

Using gentle mechanical procedures, called homogenization, the plasma membranes of cells can be ruptured so that the cell contents are released. Four commonly used procedures are shown here.

The resulting thick soup (called a homogenate or an extract) contains large and small molecules from the cytosol, such as enzymes, ribosomes, and metabolites, as well as all the membrane-bounded organelles.

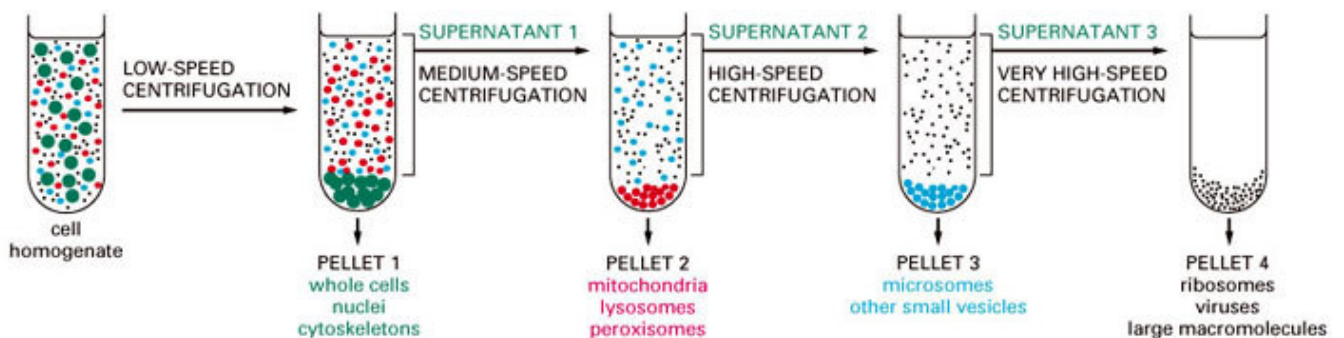


The resulting 'soup' is then centrifuged at progressively higher speeds and for longer periods of time. **Note the order in which the organelles separate:**
N.B. Chloroplasts separate just ahead of mitochondria (being larger and heavier):

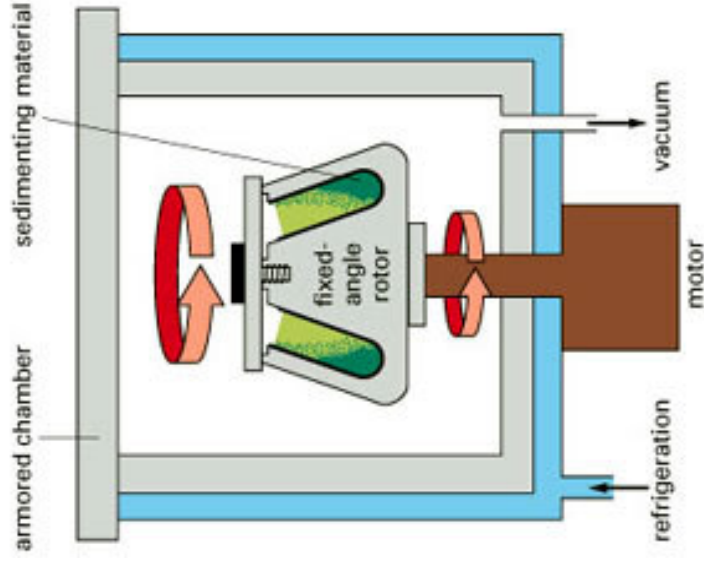
DIFFERENTIAL CENTRIFUGATION

Repeated centrifugation at progressively higher speeds will fractionate cell homogenates into their components.

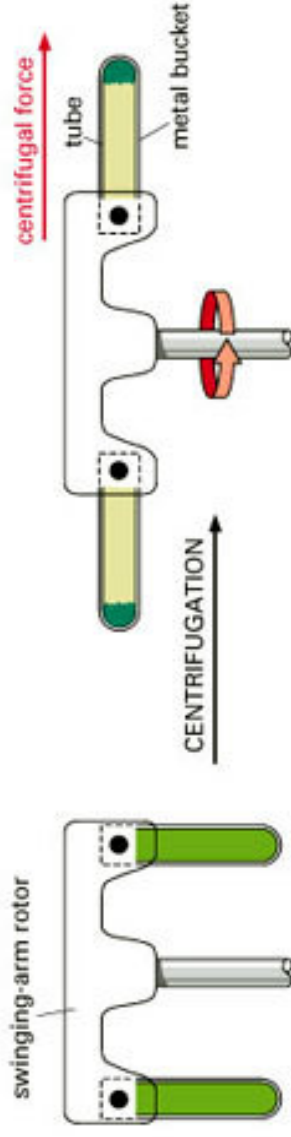
Centrifugation separates cell components on the basis of size and density. The larger and denser components experience the greatest centrifugal force and move most rapidly. They sediment to form a pellet at the bottom of the tube, while smaller, less dense components remain in suspension above, called the supernatant.



THE CENTRIFUGE

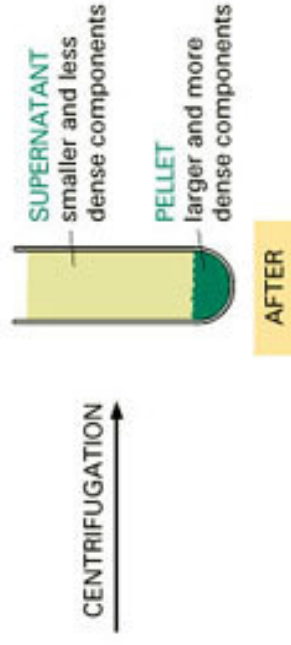
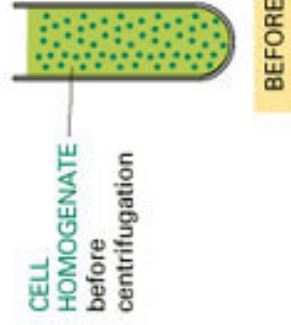


Centrifugation is the most widely used procedure to separate the homogenate into different parts, or fractions. The homogenate is placed in test tubes and rotated at high speed in a centrifuge (sometimes called an ultracentrifuge). Present-day ultracentrifuges rotate at speeds up to 100,000 revolutions per minute and produce enormous forces, as high as 600,000 times gravity.



Many cell fractionations are done in a second type of rotor, a swinging-arm rotor.

The metal buckets that hold the tubes are free to swing outward as the rotor turns.



At such speeds, centrifuge chambers must be refrigerated and evacuated so that friction does not heat up the homogenate. The centrifuge is surrounded by thick armor plating, since an unbalanced rotor can shatter with an explosive release of energy. A fixed-angle rotor can hold larger volumes than a swinging-arm rotor, but the pellet forms less evenly.