# 2.1 3 Measuring objects seen with a light microscope

**By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:**

- the preparation and examination of microscope slides for use in light microscopy
- the use and manipulation of the magnification formula

**KEY DEFINITIONS**

**eyepiece graticule:** a measuring device. It is placed in the eyepiece of a microscope and acts as a ruler when you view an object under the microscope.

**stage graticule:** a precise measuring device. It is a small scale that is placed on a microscope stage and used to calibrate the value of eyepiece divisions at different magnifications.

## Using graticules

- A microscope eyepiece can be fitted with a graticule.
- This graticule is transparent with a small ruler etched on it.
- As the specimen is viewed, the **eyepiece graticule** scale is superimposed on it and the dimensions of the specimen can be measured (just as you can measure a large object by placing a ruler against it) in eyepiece units (epu) (Figure 1).

The scale of the eyepiece graticule is arbitrary – it represents different lengths at different magnifications. The image of the specimen looks bigger at higher magnifications, but the actual specimen has not increased in size. The eyepiece scale has to be calibrated (its value worked out) for each different objective lens. See Table 1 for values of eyepiece divisions at different magnifications.

A **stage graticule** is used only to calibrate the eyepiece graticule.

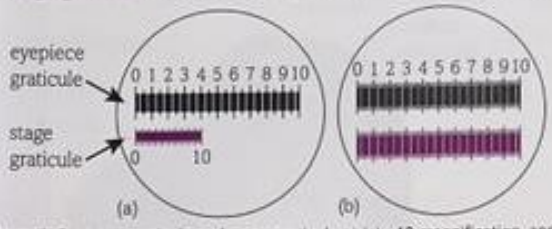



**Figure 1** Eyepiece graticule and stage graticule at (a) ×40 magnification, and (b) ×100 magnification.

## Using a stage graticule to calibrate the eyepiece graticule

A microscopic ruler on a special slide, called a stage graticule, is placed on the microscope stage.

This ruler is 1 mm long and divided into 100 divisions. Each division is 0.01 mm or 10 $\mu$m (**micrometres**).

1. Insert an eyepiece graticule into the ×10 eyepiece of your microscope. This ruler has a total of 100 divisions.
2. Place a stage graticule on the microscope stage and bring it into focus, using the low-power (×4) objective. Total magnification is now ×40.
3. Align the eyepiece graticule and the stage graticule as shown in Figure 1(a). Check the value of one eyepiece division at this magnification on your microscope.
4. In the example shown here, the stage graticule (which is 1 mm or 1000 $\mu$m) corresponds to 40 eyepiece divisions.
5. Therefore each eyepiece division = $\frac{1000}{40}$ $\mu$m = 25 $\mu$m.
6. Now use the ×10 objective lens on your microscope (total magnification is ×100) and focus on the stage graticule.
7. Align them both as shown in Figure 1(b).
8. In the example shown here, 100 eyepiece divisions now correspond with 1 mm or 1000 $\mu$m.
9. Therefore one eyepiece division = $\frac{1000}{100}$ $\mu$m = 10 $\mu$m.

| Magnification of eyepiece lens | Magnification of objective lens | Total magnification | Value of one eyepiece division (epu) ($\mu$m) |
|---|---|---|---|
| ×10 | ×4 | ×40 | 25 |
| ×10 | ×10 | ×100 | 10 |
| ×10 | ×40 | ×400 | 2.5 |
| ×10 | ×100 (oil-immersion lens) | ×1000 | 1.0 |

**Table 1** Values of eyepiece divisions at different magnifications for most modern microscopes used in schools.

**INVESTIGATION**

**Observing and measuring starch grains (amyloplasts) in potato tuber cells**

Wear eye protection.

1. Using a sharp knife, gently scrape a little material from the surface of a peeled raw potato and place it on to a microscope slide. You need a very thin layer on the slide. In that material will be potato tuber cells.
2. Place two drops of iodine/potassium iodide (KI) solution onto them and carefully add a coverslip.
3. Examine this slide under the microscope. Use low power first and then use high-power magnification. The amyloplasts stained with iodine solution will appear violet in colour.
4. Measure the length and width of three amyloplasts.

## WORKED EXAMPLE

If you are asked to measure a structure as it would appear with a light microscope with a graticule, then you will also be told the value of the divisions on the graticule. It is just like a ruler and allows you to measure the specimen.

Figure 2 shows a plant root in transverse section, seen under a light microscope. The smallest divisions on the graticule are 0.01 mm (10 $\mu$m) apart.

1. Use the graticule to measure the actual width of this root along the line AB.

   **Answer**
   The length of the line AB is 59 small divisions of the graticule × 10 $\mu$m = 590 $\mu$m = 0.59 mm.

2. Calculate the magnification of this image.

   **Answer**
   Measure the length of the image on the page with a ruler. It is approx. 59 mm = 59 000 $\mu$m. So the magnification = $\frac{59\,000}{590}$ = 100.

3. The eyepiece used in this microscope had a magnification factor of ×10. What was the magnification of the objective lens?

   **Answer**
   If the total magnification is ×100 and the eyepiece magnification is ×10, then the objective lens magnification is $\frac{100}{10}$ = 10.

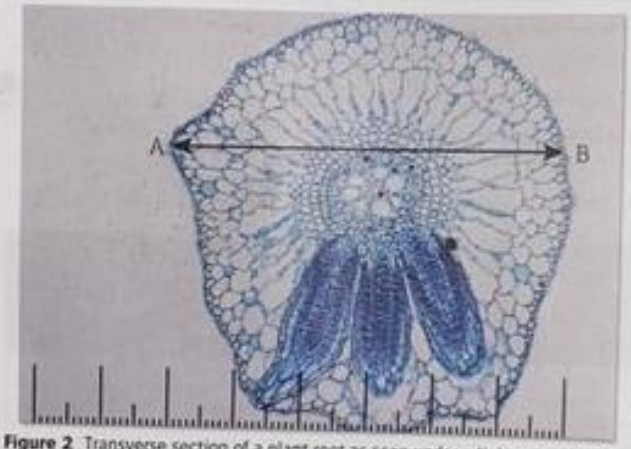
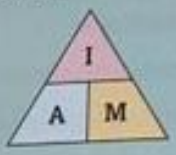



**Figure 2** Transverse section of a plant root as seen under a light microscope and with a graticule in place.

## LEARNING TIP

You have already seen the equation that **A**ctual size = **I**mage size/**M**agnification.
Therefore **M**agnification = **I**mage size/**A**ctual size
The IMA triangle shown in Figure 3 may help you to use and substitute that equation.




$$\text{Actual size} = \frac{\text{Image size}}{\text{Magnification}}$$

$$\text{Magnification} = \frac{\text{Image size}}{\text{Actual size}}$$

**Figure 3**

## Questions

1. If a nucleus measures 100 mm on a diagram, with a magnification of ×10 000, what is the actual size of the nucleus?
2. Draw up a table to show each of the following measurements in metres (m), millimetres (mm) and micrometres ($\mu$m): 5 $\mu$m, 0.3 m, 23 mm, 75 $\mu$m.
3. Express the following measurements in micrometres: (a) 5 cm, (b) 25 mm, and (c) 100 nm.
4. Express the following measurements in **nanometres**: (a) 0.5 mm, (b) 0.4 $\mu$m, and (c) 0.1 cm.
5. You want to examine and measure the sizes of some living, single-celled eukaryotic organisms in pond water.
   (a) Describe how you would make the slide, and how you would illuminate the specimen under the microscope.
   (b) Explain why it may be difficult to focus this specimen under the highest magnification of your microscope (see topic 2.1.2 as well to help you answer this question).

2.1

# 4 The ultrastructure of eukaryotic cells: membrane-bound organelles

**By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:**

- **the ultrastructure of eukaryotic cells and the functions of the different cellular components**
- **the importance of the cytoskeleton**
- **photomicrographs of cellular components in a range of eukaryotic cells**

All animal, plant, fungal and protoctist cells are eukaryotic. This means that they have (see Figure 1):

- a nucleus surrounded by a nuclear envelope and containing DNA organised and wound into linear chromosomes
- an area inside the nucleus called the nucleolus, containing RNA, where chromosomes unwind; the nucleolus is also involved in making ribosomes
- jelly-like cytoplasm in which the organelles are suspended
- a cytoskeleton – a network of protein filaments (actin or microtubules) within the cytoplasm that move organelles from place to place within the cell; allow some cells (amoebae and lymphocytes) to move; and allow contraction of muscle cells
- a plasma membrane (also called cell surface membrane or cytoplasmic membrane)
- membrane-bound organelles, other than the nucleus, such as mitochondria, the Golgi apparatus and endoplasmic reticulum
- small vesicles
- ribosomes, which are organelles without membranes, where proteins are assembled.

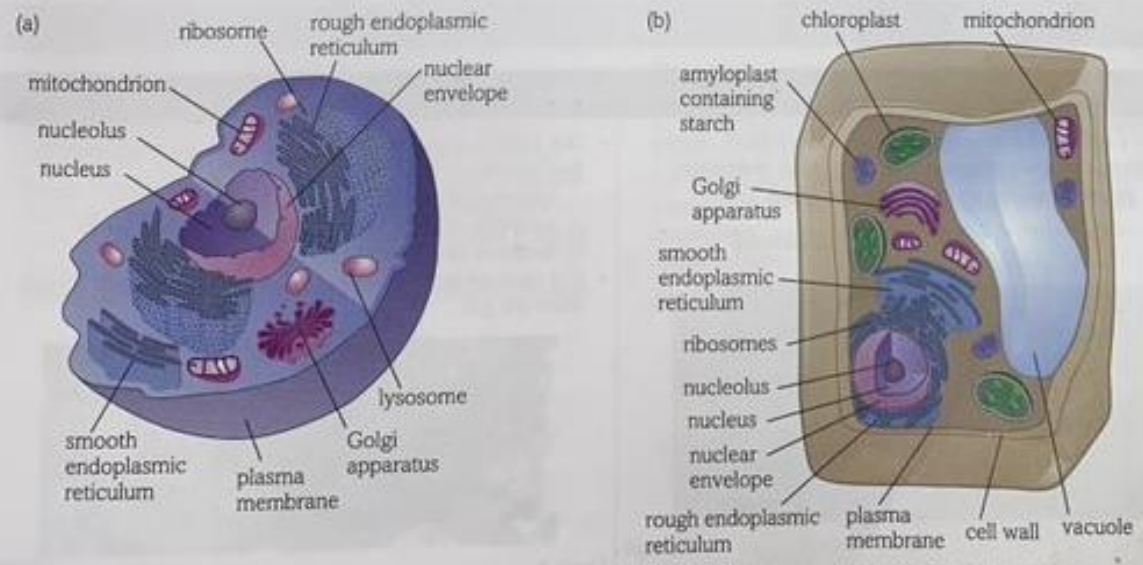
## Organelles

Cells are the fundamental units or building blocks of all living organisms. You will see in topics 2.6.4 and 2.6.5 that cells become specialised to do particular jobs. Within every cell there are various organelles, each having specific functions. This provides a division of labour, which means that every cell can carry out its many functions efficiently.

## Membrane-bound organelles

Most of the organelles within eukaryotic cells are membrane bound, which means they are covered by a membrane (similar in structure to the plasma membrane or cell surface membrane); see topic 2.5.1. This keeps each organelle separate from the rest of the cell, so that it is a discrete compartment. Membrane-bound organelles are a feature of eukaryotic cells; prokaryotic cells do not have them.

Electron microscopy has enabled scientists to ascertain the *structure* of these organelles by making and examining several sections through an organelle in order to build up a 3D picture of it. Biochemistry research has enabled scientists to find the *functions* of each organelle.




**ure 1** Structure of (a) generalised animal cell and (b) generalised plant cell, showing structures visible with a transmission electron microscope.

## Nucleus, nuclear envelope and nucleolus

| Structure | Function |
|---|---|
| • The nucleus is surrounded by a double membrane, called the nuclear envelope. There are pores in the nuclear envelope.<br>• The nucleolus does not have a membrane around it. It contains RNA.<br>• Chromatin is the genetic material, consisting of DNA wound around histone proteins. When the cell is not dividing, chromatin is spread out or extended. When the cell is about to divide, chromatin condenses and coils tightly into chromosomes (see topic 2.6.2). These make up nearly all the organism's genome (see Figure 2). | • The nuclear envelope separates the contents of the nucleus from the rest of the cell.<br>• In some regions the outer and inner nuclear membranes fuse together. At these points some dissolved substances and ribosomes can pass through.<br>• The pores enable larger substances, such as messenger RNA (mRNA) to leave the nucleus (see topic 2.3.3). Substances, such as some steroid hormones, may enter the nucleus, from the cytoplasm, via these pores.<br>• The nucleolus is where ribosomes are made.<br>• Chromosomes contain the organism's genes.<br>In summary, the nucleus:<br>• is the control centre of the cell<br>• stores the organism's genome<br>• transmits genetic information<br>• provides the instructions for protein synthesis. |

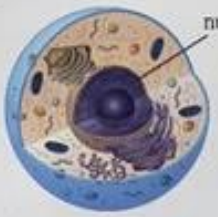



**Figure 2**

## Rough endoplasmic reticulum (RER)

| Structure | Function |
|---|---|
| • This is a system of membranes, containing fluid-filled cavities (cisternae) that are continuous with the nuclear membrane.<br>• It is coated with ribosomes. | • RER is the intracellular transport system: the cisternae form channels for transporting substances from one area of a cell to another.<br>• It provides a large surface area for ribosomes, which assemble amino acids into proteins (see topic 2.3.3). These proteins then actively pass through the membrane into the cisternae and are transported to the Golgi apparatus for modification and packag |

## Smooth endoplasmic reticulum (SER)

| Structure | Function |
|---|---|
| • This is a system of membranes, containing fluid-filled cavities (cisternae) that are continuous with the nuclear membrane.<br>• There are no ribosomes on its surface (see Figure 3). | • SER contains enzymes that catalyse reactions involved with lipid metabolism, such as:<br>○ synthesis of cholesterol<br>○ synthesis of lipids/phospholipids needed by the cell<br>○ synthesis of steroid hormones.<br>• It is involved with absorption, synthesis and transport of lip (from the gut). |

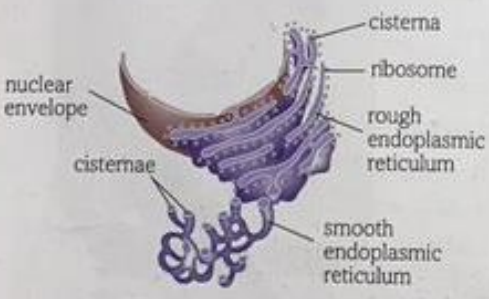



**Figure 3**

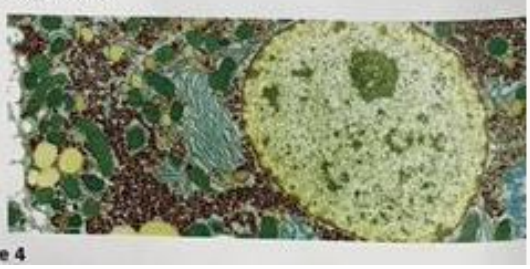


**Figure 4**

## Golgi apparatus

| Structure | Function |
|---|---|
| This consists of a stack of membrane-bound flattened sacs. Secretory vesicles bring materials to and from the Golgi apparatus (see Figure 5). | • Proteins are modified for example by:<br>○ adding sugar molecules to make glycoproteins<br>○ adding lipid molecules to make lipoproteins<br>○ being folded into their 3D shape.<br>• The proteins are packaged into vesicles that are pinched off and then:<br>○ stored in the cell or<br>○ moved to the plasma membrane, either to be incorporated into the plasma membrane, or exported outside the cell. |

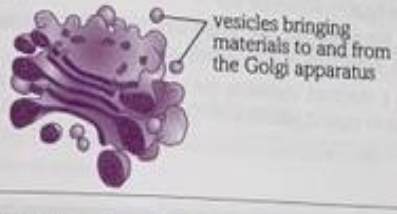



**Figure 5**

## Mitochondria (singular: mitochondrion)

| Structure | Function |
|---|---|
| • These may be spherical, rod-shaped or branched, and are 2–5 $\mu$m long.<br>• They are surrounded by two membranes with a fluid-filled space between them. The inner membrane is highly folded into cristae.<br>• The inner part of the mitochondrion is a fluid-filled matrix. (see Figure 6). | • Mitochondria are the site of ATP (energy currency) production during aerobic respiration.<br>• They are self-replicating, so more can be made if the cell's energy needs increase.<br>• They are abundant in cells where much metabolic activity takes place, for example in liver cells and at synapses between neurones where neurotransmitter is synthesised and released. |

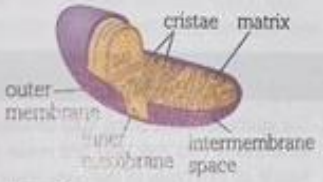



Figure 6

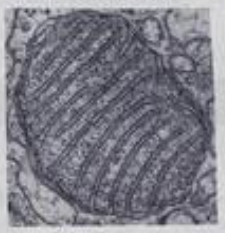


**Figure 7**

## Chloroplasts

| Structure | Function |
|---|---|
| • These are large organelles, 4–10 $\mu$m long.<br>• They are found only in plant cells and in some protoctists.<br>• They are surrounded by a double membrane or envelope. The inner membrane is continuous with stacks of flattened membrane sacs called thylakoids (resembling piles of plates), which contain chlorophyll. Each stack or pile of thylakoids is called a granum (plural: grana). The fluid-filled matrix is called the stroma.<br>• Chloroplasts contain loops of DNA and starch grains (see Figure 8). | • Chloroplasts are the site of photosynthesis.<br>• The first stage of photosynthesis, when light energy is trapped by chlorophyll and used to make ATP, occurs in the grana. Water is also split to supply hydrogen ions.<br>• The second stage, when hydrogen reduces carbon dioxide, using energy from ATP, to make carbohydrates, occurs in the stroma. Chloroplasts are abundant in leaf cells, particularly the palisade mesophyll layer. |

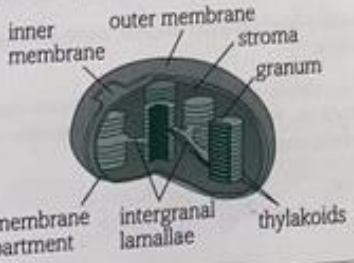



**Figure 8**

**Figure 9**

## Vacuole

| Structure | Function |
|---|---|
| The vacuole is surrounded by a membrane called the tonoplast, and contains fluid. | • Only plant cells have a large permanent vacuole.<br>• It is filled with water and solutes and maintains cell stability because when full it pushes against the cell wall, making the cell turgid.<br>• If all the plant cells are turgid then this helps to support the plant, especially in non-woody plants.<br>There is a practical involving the pigments in beetroot cell vacuoles in topic 2.5.5. |

## Lysosomes

| Structure | Function |
|---|---|
| • These are small bags, formed from the Golgi apparatus. Each is surrounded by a single membrane.<br>• They contain powerful hydrolytic (digestive) enzymes.<br>• They are abundant in phagocytic cells such as neutrophils and macrophages (types of white blood cell) that can ingest and digest invading pathogens such as bacteria (see topic 4.1.5). | • Lysosomes keep the powerful hydrolytic enzymes separate from the rest of the cell.<br>• Lysosomes can engulf old cell organelles and foreign matter, digest them and return the digested components to the cell for reuse. |

## Cilia and undulipodia

| Structure | Function |
|---|---|
| • These are protrusions from the cell and are surrounded by the cell surface membrane.<br>• Each contains microtubules (see 'Cytoskeleton', in topic 2.1.5).<br>• They are formed from centrioles (see 'Centrioles', in topic 2.1.5). | • The epithelial cells lining your airways each have many hundreds of cilia that beat and move the band of mucus.<br>• Nearly all cell types in the body have one cilium that acts as an antenna. It contains receptors and allows the cell to detect signals about its immediate environment.<br>• The only type of human cell to have an undulipodium (a longer cilium) is a spermatozoon. The undulipodium enables the spermatozoon to move. |

**LEARNING TIP**

Sometimes the undulipodium of eukaryotic cells is called a flagellum (from the Latin word meaning 'whip'). However, some bacteria (prokaryotes) also have a flagellum or flagella (more than one flagellum), and as the internal structure of the prokaryotic flagellum is different from that of the eukaryotic 'flagellum', the eukaryotic structure should really be called an undulipodium.

**DID YOU KNOW?**

Scientists have recently discovered that nearly all of our cells have at least one cilium. Cells lining the kidney tubules each have one, and these cilia monitor the flow of urine. Brain cells also have one, and these are important for enabling learning. Individuals with genetic diseases that prevent the formation of these cilia have learning difficulties.

**LEARNING TIP**

Remember that there is a level of organisation within an organism. Organelles are small structures inside cells. Do not confuse them with organs, which are large structures made of many cells and tissues.

### Questions

1. Name one substance that passes out of pores in the nuclear envelope, to the cell cytoplasm.
2. Name one substance that passes into the nucleus via the pores in the nuclear envelope.
3. Describe the role of the nucleolus.
4. State three functions of the nucleus.
5. Name one type of human cell that does not contain a nucleus.
6. Suggest why hydrolytic enzymes within cells need to be inside a vesicle.
7. If you carried out a physical training programme, how and why would you expect the number of mitochondria in your muscle cells to change?

2.1

# 5 Other features of eukaryotic cells

**By the end of this topic, you should be able to demonstrate and apply your knowledge and understanding of:**

- **the ultrastructure of eukaryotic cells and the function of the different cellular components**
- **the importance of the cytoskeleton**

## Organelles without membranes

Ribosomes and the cytoskeleton, including centrioles, are not covered by membranes.

The tables below explain how the structures of organelles without a membrane help them to carry out their functions.

### Ribosomes

| Structure | Function |
|---|---|
| • Small spherical organelles, about 20 nm in diameter.<br>• Made of ribosomal RNA.<br>• Made in the nucleolus, as two separate subunits, which pass through the nuclear envelope into the cell cytoplasm and then combine.<br>• Some remain free in the cytoplasm and some attach to the endoplasmic reticulum. | • Ribosomes bound to the exterior of RER are mainly for **synthesising** proteins that will be exported outside the cell.<br>• Ribosomes that are free in the cytoplasm, either singly or in clusters, are primarily the site of assembly of proteins that will be used inside the cell.<br>Topic 2.3.3 outlines how proteins are made. |

### Centrioles

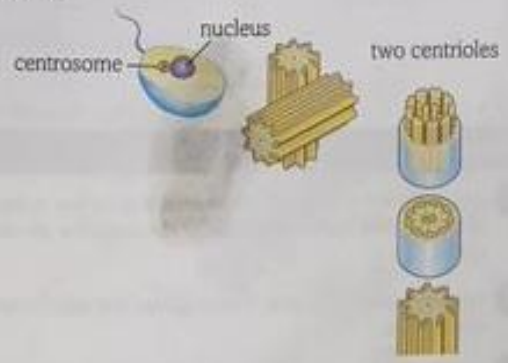
| Structure | Function |
|---|---|
| • The centrioles consist of two bundles of microtubules at right angles to each other. The microtubules are made of tubulin protein subunits, and are arranged to form a cylinder (see Figure 1).<br>  <br> | • Before a cell divides, the spindle, made of threads of tubulin, forms from the centrioles.<br>• Chromosomes attach to the middle part of the spindle (see topic 2.6.2) and motor proteins walk along the tubulin threads, pulling the chromosomes to opposite ends of the cell.<br>Centrioles are involved in the formation of cilia and undulipodia:<br>• Before the cilia form, the centrioles multiply and line up beneath the cell surface membrane.<br>• Microtubules then sprout outwards from each centriole, forming a cilium or undulipodium.<br>Centrioles are usually absent from cells of (higher) plants but may be present in some unicellular green algae, such as *Chlamydomonas*. |

**Figure 1** Centrioles.

## Cytoskeleton

| Structure | Function |
| --- | --- |
| A network of protein structures within the cytoplasm.<br>It consists of:<br>• rod-like microfilaments made of subunits of the protein actin; they are polymers of actin and each microfilament is about 7 nm in diameter<br>• intermediate filaments about 10 nm in diameter<br>• straight, cylindrical microtubules, made of protein subunits called tubulin; about 18–30 nm in diameter.<br>• The **cytoskeletal motor proteins**, myosins, kinesins and dyneins, are molecular motors. They are also enzymes and have a site that binds to and allows **hydrolysis** of ATP as their energy source. | The protein microfilaments within the cytoplasm give support and mechanical strength, keep the cell's shape stable and allow cell movement.<br>Microtubules also provide shape and support to cells, and help substances and organelles to move through the cytoplasm within a cell.<br>• They form the track along which motor proteins (dynein and kinesin) walk and drag organelles from one part of the cell to another.<br>• They form the spindle before a cell divides. These spindle threads enable chromosomes to be moved within the cell.<br>• Microtubules also make up the cilia, undulipodia and centrioles.<br>**Intermediate filaments** are made of a variety of proteins. They:<br>• anchor the nucleus within the cytoplasm<br>• extend between cells in some tissues, between special junctions, enabling cell–cell signalling and allowing cells to adhere to a basement membrane, therefore stabilising tissues. |

## Cellulose cell wall

| Structure | Function |
| --- | --- |
| The cell wall of plants is on the outside of the plasma membrane. It is made from bundles of cellulose fibres.<br>  <br>**Figure 2**<br>There is more information on the structure of cellulose in Chapter 2.2. | Absent from animal cells, the cell wall is strong and can prevent plant cells from bursting when turgid (swollen).<br>The cell walls of plant cells:<br>• provide strength and support<br>• maintain the cell's shape<br>• contribute to the strength and support of the whole plant<br>• are permeable and allow solutions (solute and solvent) to pass through.<br>Fungi have cell walls that contain chitin, not cellulose. |

**DID YOU KNOW?**

There are many different types of kinesin. In the human genome there are 45 genes for kinesin proteins. Each type has a differently shaped tail domain so that it can attach to a different type of 'cargo' to move it within the cell.

### Questions

1. Describe how the functions of ribosomes that are free in the cytoplasm differ from the functions of ribosomes that are attached to RER.
2. Name the monomeric units of microtubules that occur in the cytoskeleton.
3. Describe the functions of the cellulose cell wall found in plant cells.
4. On a dull day, the chloroplasts inside palisade leaf cells are moved up to near the surface, to absorb more light. By what mechanism do you think the chloroplasts are moved?