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Cell biology

The cell is the basic unit of living organisms; while some organisms are made up of a single cell (e.g. protozoa and bacteria), others are made up of many cells, organised into tissues and organs, that perform specific functions.

Individual cells in humans and other eukaryotic organisms are organised into functional areas – organelles – that perform a specific function. Cells usually divide by mitosis to produce identical daughter cells to allow the development of tissue or the replacement of dying cells. However, for the purpose of reproduction, they divide by meiosis in which the daughter cells each possess half a full set of chromosomes. In developed tissues, mitosis occurs to replace those cells that have become damaged; if such cell division occurs in an unregulated fashion, cancer may result.

Organelles: structure and function

A cell contains specialised regions that take on specific functions. These organelles are discussed below; however, the biochemical interactions that occur are discussed in more detail in Chapter 3.

The cell membrane

The cell membrane is a **phospholipid bilayer** that surrounds the cell, defining its boundaries. The membrane contains many specialised molecules embedded within it, for the transport of molecules across it, as well as regulating the properties and behaviour of the membrane itself (Fig. 1.1).

Structure

The formation of the bilayer relies on the properties of the constituent phospholipids. They are made up of two parts:

1 A **polar head** region, which is soluble in water (**hydrophilic**). This region often contains a charged group which mediates its hydrophilic nature.
2 A **non-polar tail** region, which is insoluble in water (**hydrophobic**). This hydrophobic property results from the long uncharged fatty acyl chains.

These properties promote phospholipids to form a bilayer, in which the hydrophilic head regions are in contact with water while the hydrophobic tail regions accumulate in the middle of the bilayer. The fluidity of the membrane is regulated by the presence of **cholesterol**.

The cell membrane is a dynamic structure, with the various **protein** components free to rotate and diffuse laterally around the lipids, allowing their aggregation, which is often required for signalling. These proteins can be grouped into two types:

1 **Integral proteins** are embedded in the membrane.
2 **Peripheral proteins** are associated with the surface of the membrane as a result of non-covalent interactions.

Biomedical Science Lecture Notes, First Edition. Ian Lyons.

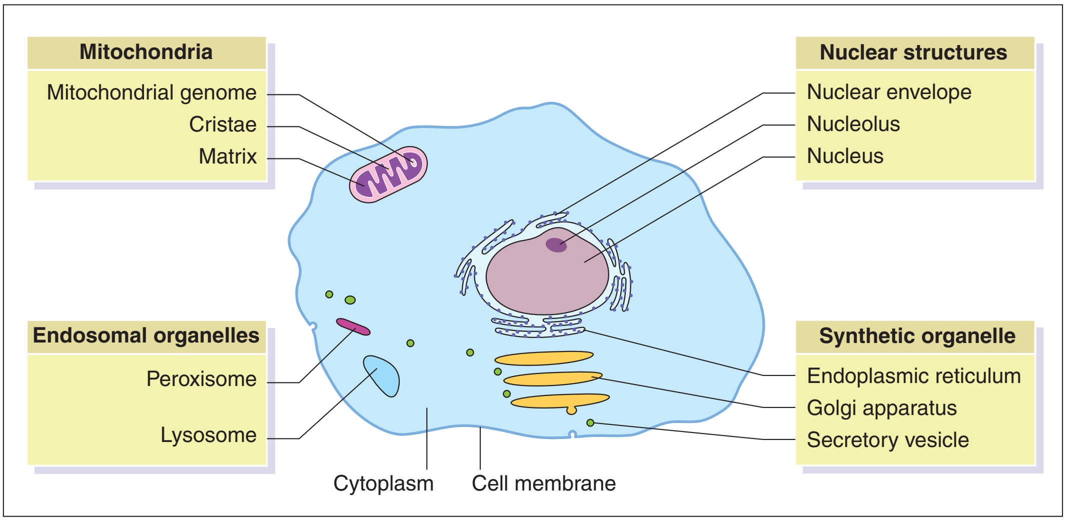


Figure 1.1 **The organelles in a eukaryotic cell**. There are various organelles within the cell that are specialised to perform specific functions, as can be reflected by their features. Many of the components shown – such as the mitochondria – are found in several copies within the cell, although they have been represented only a single time, for ease.

The precise proteins associated with the membrane differ depending on the cell specialisation, other external signals that have been received and the specific state of the individual cell.

Both proteins and lipids in the membrane may be **glycosylated**, whereby carbohydrates are added to the molecule. These carbohydrates may be involved in the interaction between the cell and the environment or other cells.

The cytoplasm

The organelles are contained in the cytoplasm, which is made up of a wide variety of ions and solutes, as well as a complex cytoskeletal structure. This structure maintains the cell shape and regulates various transport and trafficking pathways in the cell. The cytoplasm is also the site of most cellular metabolic reactions.

The nucleus

The nucleus contains almost all the genetic information necessary to produce any cell in the human body; this genetic information is densely packed as **chromatin**. The nucleus is separated from the rest of the cell by a **nuclear envelope**, which is made up of two lipid bilayers – the outer membrane being continuous with the **endoplasmic reticulum**. Transport of molecules across the nuclear membrane is tightly regulated by many **nuclear pores** in the nuclear envelope.

Despite its important function, the nucleus usually occupies a relatively small volume, often around 5% of the total cell volume.

The **nucleolus**, which is the site of synthesis of **ribosomal RNA** and the assembly of **ribosomes**, is one of the few structures visible in the nucleus (by light microscopy).

The endoplasmic reticulum

The endoplasmic reticulum (ER) is a network of tubes continuous with the outer membrane of the nuclear envelopes. It produces lipids and protein for secretion or use in cellular organelles. There are two types of ER:

1. **Rough ER** is the site of protein production. Its 'rough' appearance under an electron microscope comes from the presence of protein-synthesising **ribosomes** on its surface. The resulting proteins are secreted into the ER lumen, from where they are transported for further modification
2. The **smooth ER** lacks ribosomes and is responsible for the production of lipids, which

contribute to the cellular membranes, and *also* the production of steroids. Smooth ER is associated with detoxifying reactions, particularly in the liver, and is also the site from which components are transported to other organelles by **vesicular transport**.

The proportions of rough and smooth ER may reflect the specialisation of the cell, e.g. in the adrenal cortex, which is responsible for the production of steroid hormones, the smooth ER accounts for most of the cell volume.

The Golgi apparatus

The Golgi apparatus modifies many cellular proteins, through the addition of carbohydrates.

It resembles a series of sac-like structures – **cisternae** – which receive proteins from the rough ER by **vesicular transport**. As proteins are transported between the different cisternae, they receive carbohydrate modifications; transport may be forwards or backwards between compartments, before the proteins are packaged into vesicles for secretion or transport to specific organelles. This process, **trafficking**, relies on the presence of specific signals in the proteins themselves, which target them to their final destination.

Mitochondria

Mitochondria synthesise ATP and other phosphate compounds that power cellular reactions through the catabolism of a variety of metabolic compounds.

Structure

Mitochondria are double-membrane organelles. The outer membrane is smooth whereas the inner membrane possesses a series of projections – **cristae –** that impinge on the interior of the mitochondria, known as the **matrix**.

Mitochondria contain a primitive genome that encodes some of the proteins not found in the nuclear genome that are necessary for their function. This, and their replication independently of the nucleus of the cell, may reflect their **endosymbiotic origin**. However, many of the proteins that mitochondria require are solely encoded in the main genome in the nucleus of the cell.

Unlike the main human genome, the mitochondrial genome is always inherited entirely from the mother; mitochondria in sperm do not contribute to the zygote.

DEFINITION Endosymbiotic theory of the origin of mitochondria

The mitochondrial genome has been sequenced and has many features consistent with a bacterial genome. It has been suggested that, at some point in the past, a bacterium became engulfed by a primitive cell and remained within it, resulting in a symbiosis between the two organisms. It is thought that this symbiotic bacterium evolved into the mitochondria of today.

Function

Mitochondria meet the energy needs of the cell. In the matrix the **TCA** (tricarboxylic acid) cycle occurs, which supplies the **electron transport chain** with the reduced co-factors necessary to generate **ATP**, the main energy currency of the cell.

The number of mitochondria present in the cell reflects its energy need; muscle cells and neurons have high energy demands and frequently have many mitochondria, whereas cells with lower energy requirements may possess very few.

CLINICAL Myoclonic epilepsy with ragged red fibres

Myoclonic epilepsy with ragged red fibres (MERRF) is a rare condition characterised by myoclonus, epilepsy and ataxia, usually manifesting in the teens or student age. Further symptoms include muscle weakness, deafness, dementia and seizure. The cause has been traced to a decrease in the function of the electron transport chain resulting from mutation in a mitochondrially-encoded protein. As such, the condition is maternally inherited, although the mother may express a weaker phenotype than her child.

Ribosomes

Ribosomes are minute organelles, around 20 nm in diameter, that synthesise amino acids. They are found free floating in the cytosol, or on the rough ER, where the proteins produced are destined for secretion or transport to another organelle.

Lysosomes and the endosomal compartment

The endosomal compartment consists of many membrane-bound compartments in the cytoplasm that package and transport molecules to other regions of the cell or to the external environment. They can also isolate reactions that may be damaging to the cell as a whole – often involving degradative enzymes.

Lysosomes

Lysosomes are membrane-enclosed compartments, involved in the degradation of some molecules and old organelles, so that the components can be broken down and reused to synthesise new macromolecules. They contain a variety of **acid hydrolases**, potent degradative enzymes that act in an acid environment. Consequently, the interior of the lysosome is acidic, typically pH 5.

In phagocytic cells, lysosomes have developed a more specific role. The enzymes can degrade bacteria and other pathogens that have been ingested to control infection.

Cellular processes

The cell survives and performs its role through the function of the various macromolecules within it. The synthesis and chemical properties of these macromolecules are discussed in Chapter 3, although the following major processes are discussed below:

- Transport across a membrane
- Enzymes – catalysis of the chemical reactions
- Trafficking of proteins to their site of action.

DEFINITION Osmosis

Osmosis can be defined as 'the flow of water across a semi-permeable membrane'. The cell membrane is permeable to water, though not to many of the solutes found within it. The presence of different solutes in different concentrations on either side of the membrane contributes to a concentration gradient. This gradient must be equilibrated by movement of water across the membrane. As such, the volume of a compartment is altered due to the flow of water down the concentration gradient.

Membrane transport

Membranes can regulate the movement of molecules across them. Although the phospholipid bilayer is insoluble to most polar molecules, non-polar molecules and small polar molecules can traverse the membrane by diffusion. This diffusion is crucial for the regulation of the cellular volume by **osmosis**.

Diffusion

Diffusion is the movement of molecules down a concentration gradient (osmosis is diffusion of water). Diffusion continues until the concentration gradient has been abolished and a dynamic equilibrium established, where the flow across the membrane in each direction is the same. There are a number of factors that govern the rate of diffusion:

- The rate of diffusion is proportional to the square of the **distance**.
- The **size –** small particles diffuse faster.
- The rate of diffusion is proportional to the **concentration gradient**.
- A **thicker membrane** will slow the rate of diffusion.

Non-polar molecules (e.g. steroid hormones) can dissolve within the bilayer and are capable of diffusing across the membrane, as are small particles such as H_2O and O_2. Polar molecules (and large molecules in general) cannot pass unaided through the lipid bilayer.

Mechanisms of membrane transport

Large or charged molecules cannot diffuse across the lipid bilayer and must be helped by specific transport proteins; some molecules must be transported against the concentration gradient, through **active transport**. Membrane transport is achieved through the presence of channels and carrier proteins in the bilayer.

Channels are pores that let specific water-soluble molecules across the membrane while in solution. Channels may be gated to restrict the flow of their target molecule to specific periods. Gating may be in response to a ligand (e.g. acetylcholine), voltage or another stimulus.

Carrier proteins bind their target molecule and transport it across the bilayer by a conformational shift. Three different types of transport can be distinguished:

1 **Facilitated diffusion** allows the movement of molecules that cannot diffuse directly through the lipid bilayer to move down their concentration gradient. The binding of the ligand to its carrier protein is sufficient to induce a conformation change so that it can move the molecule(s) across the membrane without using the cell's energy. Alternatively, the transporter may 'flip' between two states, allowing the movement of molecules across the lipid bilayer if they are bound at the moment of conformational change. Facilitated diffusion is used by many ion exchangers (e.g. Cl^-, HCO_3^- exchanger) and by transporters of larger molecules (e.g. the glucose transporters)
2 **Primary active transport** is the transport of molecules against their concentration gradient, directly using ATP to power the process. This generates a large concentration gradient that can power other cellular processes. It can also allow the compartmentalisation of harmful molecules and their removal from the cell. The **Na^+/K^+ ATPase** works through primary active transport.
3 **Secondary active transport** is the movement of a molecule against its concentration gradient that is powered by a different concentration gradient generated by primary active transport, e.g. the Na^+/Ca^{2+} exchanger transports Ca^{2+} against its concentration gradient due to the flow of Na^+ down the gradient, which was previously generated through the action of the Na^+/K^+ ATPase.

Enzymes and catalysis

Enzymes are **proteins** that catalyse a chemical reaction. All enzymes contain an **active site** in which catalysis of the reaction takes place. This active site is defined by the amino acid residues in the molecule and the folding patterns that emerge. Two distinct types of region can be identified in the active site:

1 The **binding sites** hold the substrate on the active site.
2 The **catalytic site** is the region of the active site where the reaction takes place.

Two distinct theories of how the active site contributes to catalysis have been put forward:

1 The 'lock-and-key' hypothesis
2 The induced fit hypothesis

The 'lock-and-key' hypothesis

This hypothesis suggests that there is a high complementarity between the enzyme and its substrate – similar to a lock and key. The specificity is determined by the amino acid residues in the active site that are not involved in binding or catalysis of the substrate. These interactions may be the result of complementary shape, the presence of chemically complementary charges, or a combination of both.

The induced fit hypothesis

The induced fit hypothesis suggests that interactions between an enzyme and its substrate result in a conformational change, 'steric strain' that promotes the catalysis of the reaction. In multi-subunit proteins, the binding of a ligand to one subunit may induce a conformational change in the other subunits to promote binding further, e.g. in haemoglobin.

Multienzymes

Enzymes are often found in large multimeric complexes. These **multienzymes** consist of several copies of each subunit and co-factors that are required. **Pyruvate dehydrogenase** is an example of a multienzyme.

Multienzymes may also express different subunits in a tissue-specific manner. These **isoenzymes** may have different kinetic properties or slightly different kinetic functions. **Lactate dehydrogenase** is a tetramer made up of two different types of subunit: 'H' form subunits are found predominantly in the heart, whereas 'M' form subunits are found in the muscles. The different subunits have different properties – 'H' subunits catalyse the conversion of lactate to pyruvate, whereas the 'M' forms are required for anaerobic glycolysis and catalyse the conversion of pyruvate to lactate. As a result of these two subunit types, five different proteins may be produced: H_4, H_3M, H_2M_2, HM_3, M_4.

Co-factors

Co-factors are non-protein molecules that aid the function of an enzyme:

- **Metal ions** are frequently used as electron donors/acceptors because they can exist in a variety of oxidation states.
- **Organic molecules** are referred to as **coenzymes** and bind a substrate required for the reaction, or may be reduced or oxidised during the enzymatic reaction. NAD^+ and $NADP^+$ are examples of co-factors that become reduced, and can be used in other reactions.

Protein trafficking

Proteins must be targeted to the correct sites in the cell to perform a function. This information is contained in the amino acid sequence of the protein. The following are two broad methods by which this information is encoded:

1. **The signal peptide** results from a chain of around 15–60 amino acids typically located at one end of the polypeptide. This signal sequence encodes the destination of the protein and is recognised by various proteins to trigger the transport of the protein. After targeting of the protein, the signal peptide is be cleaved from the polypeptides by enzymes known as **signal peptidases**.
2. **Signal patches** are a less well-understood method of targeting. The fully folded protein encodes a region in its structure that is recognised and targets the protein to a specific location. Signal patches are difficult to identify, because they rely on the folded nature of the protein, and are not identifiable solely from the amino acid sequence.

Targeting of protein to the endoplasmic reticulum

All protein synthesis is initiated on free-floating ribosomes, yet the protein synthesis must be targeted to the correct location as a result of a signal peptide encoded at the start of the amino acid chain. This ER-targeting sequence produces a series of changes:

- The signal peptide rapidly folds around the ribosome such that it pauses in translation.
- The signal peptide allows the binding of the ribosome to specific pores in the ER.
- Cleavage of the signal peptide permits the continuation of translation, with the protein product being secreted into the ER lumen.

Targeting of proteins to other compartments

Many different signals have been identified that target proteins to different compartments. Often, these rely on signals in the protein sequence; however, the protein may promote an additional modification that targets the protein. This can be seen in lysosomal targeting, where the addition of the sugar mannose 6-phosphate is responsible for the targeting of the protein to the lysosome. Deficiencies in protein targeting may contribute to serious diseases.

Cellular transport and secretion

CLINICAL I-cell disease

I-cell disease is a very rare inherited disease that results from the presence of non-functioning lysosomes. These become engorged because they are unable to break down their contents. The cells become non-viable, which is manifested grossly in skeletal deformities, restricted joint movements and hepatosplenomegaly. The condition is currently untreatable and therapy focuses on limiting symptoms. Nevertheless, death results early in infancy.

I-cell disease results from a deficiency in the enzyme *N*-acetylglucosamine-1-phosphotransferase, which is involved in the carbohydrate modifications targeting a protein to the lysosomes. Instead the enzymes are secreted from the cell and unable to perform their essential function in the lysosome.

Vesicular transport

Transport of molecules to their target in the cell often requires the passage of a molecule through a lipid bilayer. Vesicular transport allows the transport of a molecule from the interior of one membrane-bound organelle to the interior of another in a lipid capsule.

The future contents of a vesicle are often aggregated together on the lipid bilayer and the vesicle is formed around them. The newly formed vesicle is then transported to the target, where the lipid bilayer of the vesicle fuses with the membrane, releasing its contents.

Vesicular transport is important in the **maintenance of cellular membranes**; fusion of a vesicle to a membrane contributes to the lipid bilayer. Fusion of a vesicle with the cell membrane also results in release of the vesicular contents – as is seen in the **constitutive** and **regulatory** secretory pathways.

Exocytosis and endocytosis

Vesicular transport to and from the cell membrane is required for the release or accumulation of molecules by the cell, as well as the redistribution of the lipid bilayer:

- **Exocytosis** is the process by which vesicles fuse with the cell membrane releasing their contents into the external environment. Exocytosis is also important for the contribution of a vesicle-derived bilayer and membrane-associated protein to the cell membrane.
- **Endocytosis** is the reverse of exocytosis, whereby vesicles are generated on the surface of the cell. This allows the recovery of membrane or cell surface proteins from the cell surface or, through the aggregation of receptor-bound molecules, the transport of specific molecules into the cell. Two distinct forms of receptor-mediated endocytosis exist:
  - **pinocytosis** results from the ingestion of small vesicles, typically 100 nm in diameter; pinocytosis can occur in all cell types
  - **phagocytosis** is the ingestion of large particles into specialised vesicles, known as **phagosomes**; this process is restricted to a few specialised cell types and allows the uptake of particles that may be over 1 μm in size.

> **DEFINITION Clathrin-mediated transport**
>
> Clathrin is a protein associated with the formation of endocytic vesicles. It binds to the membrane proteins via a series of specific adapter proteins. The clathrin molecules interact with each other to form a lattice structure that progressively deforms the membrane into a vesicle. Formation of the vesicle allows it to bud from the membrane, at which point the clathrin coat dissociates and the vesicle travels to the endosomal compartment.

Transcytosis

Epithelial cells are found at barriers between the body and the environment. There is often a necessity to transport molecules from one side of the cell to the other. This process is mediated by specific receptors that trigger receptor-mediated endocytosis of the target molecules and their transport to the endosomal compartment. Molecules are then transported to the other side of the cell by exocytosis.

Secretion

Two major secretory pathways have evolved to regulate the secretion of proteins from the cell:

1. The **constitutive secretory pathway** is present in all cells. This is the default secretory pathway that targets proteins to the cell membrane, if they do not possess a signal that targets them elsewhere.
2. The **regulated secretory pathway** is seen only in cells specialised for secretion – such as in neurons or hormone-secreting cells. Such cells tend to produce large amounts of their secretory product and store them in **secretory granules**.

Cell division and the cell cycle

Everyone develops from a single zygote as a result of cell division, differentiation and cell death. During the proliferation required for this development, cells divide to produce two daughter cells that contain the full complement of chromosomes – **diploid** cells. This form of cell division is named **mitosis**.

During reproduction, a sperm and an ovum, which express only one copy of each chromosome, must fuse to produce a **zygote** that bears a full complement of 46 chromosomes. The production of the **haploid** cells that contain only one copy of each chromosome is essential for reproduction, and occurs by **meiosis**.

Some cells are constantly replenished throughout an individual's life as a result of cell division, e.g. epithelial cells are constantly sloughed off as a result of wear and tear and must be replenished by the division of stem cells and their differentiation.

The cell cycle

The life of the cell can be described in the cell cycle, which is split into four stages: **G1**, **M**, **G2** and **S**. Cells may also leave the cell cycle and become

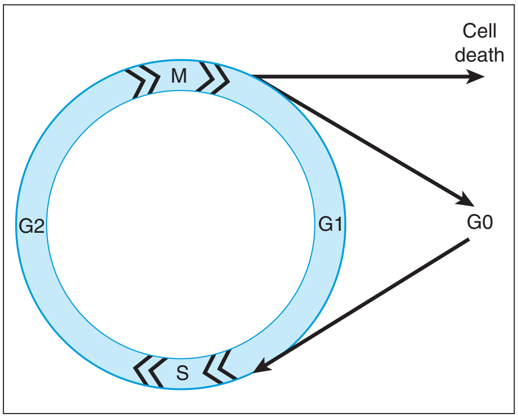


Figure 1.2 The cell cycle. The cell cycle consists of four phases that occur in sequence. Mitosis occurs in the M phase and is followed by the G1 phase. The cell spends most of its time in this phase, although the length of the phase may vary depending on the cell. The S phase is the point at which the synthesis of DNA occurs, so that each chromosome is made up of two identical chromatids and is followed by a short G2 phase before the cell division. This cycle can continue, or the cell may exit the cycle into G0 when it is fully differentiated and not considered part of the cell cycle. However, rarely, cells may re-enter the cycle from G0. Cells may also leave the cell cycle through cell death.

non-dividing differentiated cells, **G0**, or through cell death (Fig. 1.2):

- G1 and G2 reflect the parts of the cell cycle where the cells are not dividing and there is no synthesis of DNA. This is the period in which cells spend the most time.
- The S stage of the cycle is the period during which DNA is synthesised.
- The M stage of the cycle is the point at which cell division occurs.

Cell division

There are structures and features of meiosis and mitosis that are common to both, and reflect key processes in cell division:

- The **aggregation of chromosomes** must occur after DNA replication. The DNA becomes tightly packaged so that each chromosome becomes easily distinguishable.
- **Migration of the centrioles** to opposite ends of the cell is essential to form the **spindle**. These structures are made up of microtubules and exhibit a nine plus two arrangement: nine pairs of microtubules arranged in a circle around an additional two microtubules.

DEFINITION The spindle

The spindle is a microtubule structure associated with cell division. The microtubules are organised by two centrioles, which are positioned at either end of the cell and form a network of microtubules between them. This spindle becomes associated with the chromosomes and regulates their movement to the opposite poles of the cell, ensuring that the correct chromosome complement occurs with both mitotic and meiotic cell division.

Mitosis

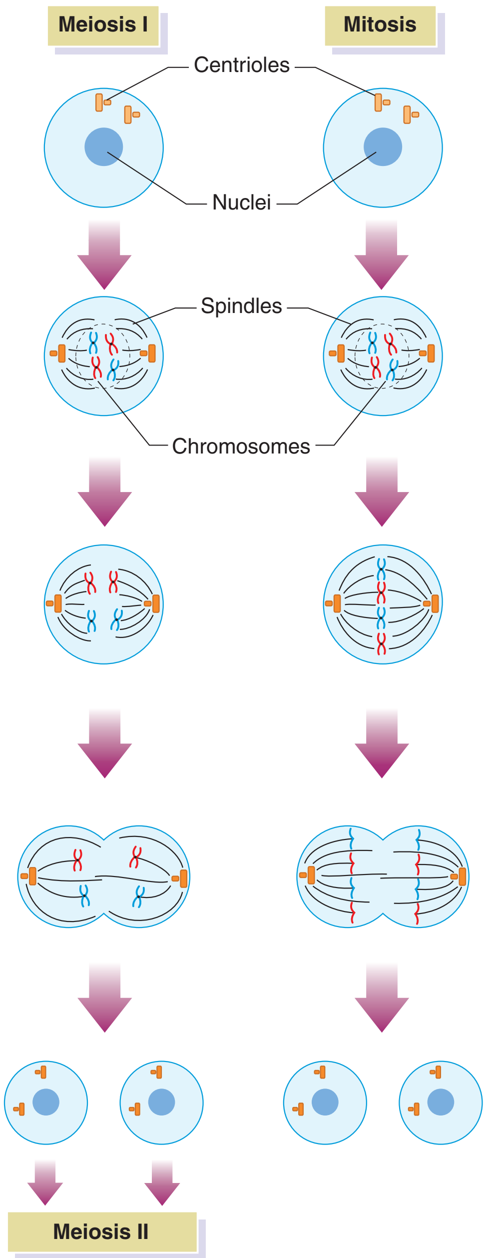
Mitosis occurs as a series of stages. As mitosis is a dynamic process the determination of when one stage becomes the next is difficult; however, there are certain key features that can be identified at each stage of mitosis (Fig. 1.3):

- **Interphase** – the period during which the cell is not dividing. During this period the chromosomes exist in their normal disaggregated state.
- **Prophase** – the first stage of replication. The centrioles migrate to the poles and the spindle begins to form. The nuclear envelope begins to break down and the DNA condenses such that the individual chromosomes are visible.
- **Metaphase –** the chromosomes align along the centre of the cell, where they attach to the spindle.
- **Anaphase –** the spindle pulls the chromosome, such that one **chromatid** from each chromosome migrates to each pole. The cytoplasm begins to contract as the cells start to divide.
- **Telophase –** the cells become two separate daughter cells, as cleavage of the cytoplasm is completed.

After mitosis the cells return to the G1 phase of the cell cycle.

Meiosis

Meiosis generates **haploid** cells, which contain only one of each pair of chromosomes. This process occurs through two special cell divisions that produce four haploid (1*n*) cells from a single parent diploid cell (2*n*).



Interphase

During interphase, the cells are at rest. For ease only two pairs of chromosome are represented within the cells.

Prophase

During prophase, the processes that occur during meiosis and mitosis are very similar. The spindle starts to form, following the migration of the centrioles, and the nuclear envelope starts to degrade. The chromosomes start to aggregate.

Metaphase

The crucial difference between the two forms of cell division is that within mitosis the chromosomes align on the equator of the cell. During meiosis the chromosomes match up in their homologous pairs, such that one of each chromosome pair is situated either side of the equator.

Anaphase

The spindle pulls the chromosomes to the poles. In mitosis, one chromatid from each chromosome is pulled to either pole, whereas, in meiosis, one chromosome from each chromosome pair is pulled, halving the number of chromosomes in the daughter cells. Contraction of the cytoplasm occurs during anaphase.

Telophase

Separation of the cells is completed and the nuclear structures start to reform. In meiosis, however, a second round of cell division occurs immediately; meiosis II is similar to mitosis and completes the production of four haploid cells from a single diploid cell.

Figure 1.3 Mitosis and Meiosis. The two different forms of cell division have similar steps. Whilst mitosis produces two identical daughter cells, meiosis produces two cells with half the number of chromosomes of its parent cell.

Meiosis I

The first division in meiosis results in the separation of homologous chromosomes after the replication of the DNA to produce chromosomes that have two chromatids, as for mitosis. The resultant division is referred to as 'reduction division' because it reduces the number of chromosomes in the cell from 46 to 23:

- **Prophase I** – the chromosomes condense and then pair up with their homologous chromosomes such that one chromosome of each homologous pair lies on either side of the midline of the cell. At this stage recombination between the homologous chromosomes may occur as a result of '**crossing over**'.
- **Anaphase I** – as a result of the random division of maternal and paternal chromosomes one either side of the midline, the separation of the chromosomes leads to each daughter cell possessing one chromosome from each pair, although it is random whether each chromosome is maternally or paternally derived.

Meiosis II

The second division in meiosis closely resembles mitosis. Each of the chromosomes aligns on the midline of the cell, and the chromatids separate to the poles. The result of the two divisions of meiosis is the formation of four daughter cells, each with half the chromosome complement of their parent cells.

> **DEFINITION Chromosome structure**
>
> For most of the cell cycle, chromosomes are diffusely distributed in the nucleus; however, during cell division they become tightly condensed. Although the molecular mechanisms underlying this packaging are covered in detail in Chapter 2, the gross structure is important to the process of cell division. The tightly packaged chromosome consists of two identical **chromatids**. These are joined together at a region known as the **centromere**. During the anaphase of mitosis and meiosis the chromosomes or chromatids are pulled to the poles by the spindle:
>
> - Separation of chromatids results in the production of two identical daughter cells.
> - Separation of chromosome pairs results in the movement of both chromatids to the same pole. This occurs in meiosis I to generate a haploid cells.

Cell differentiation

Cells may leave the cell cycle and become differentiated, expressing specific patterns of genes such that the cells can perform a particular function. The genes expressed result in specific protein complements, e.g. in nerve cells, they will be very different from those in a muscle cell.

The zygote is **totipotent** and has the potential to become any cell in the body or placenta. The potency of a cell decreases as it becomes **pluripotent**, capable of generating a wide variety of cell types, although not all (e.g. a haematopoietic stem cell can generate all types of blood cell, but is unable to generate other tissues, such as epithelium or nervous tissue). Finally, a cell becomes **unipotent** when it is irreversibly committed to a single fate.

Regulation of differentiation

Differentiation is regulated by various developmental signals received by the cell from its surrounding environment (e.g. signalling molecules such as transforming growth factor β (TGF-β)) which interact with the existing pattern of gene expression in the cell.

Cell growth

Cells in the body, and the tissues that contain them, may grow and shrink in response to stimuli, while remaining at the same stage of differentiation. Both atrophy (the reduction in size of a tissue) and hypertrophy (the growth in size) are physiological occurrences, although they may also occur pathologically.

Atrophy

The progressive reduction in the size of a tissue or organ – atrophy – is an important process during development. Shortly after birth, the ductus arteriosus must decrease to prevent blood bypassing the lungs; the tissue then atrophies, forming the ligamentum arteriosum.

Many pathologies are associated with a decrease in size of an organ or tissue, which may represent a generalised effect or be specific to a tissue.

Generalised atrophy can occur as a result of chronic starvation and can also be seen in the advanced stages of malignant disease (e.g. cachexia).

CLINICAL Cachexia

Cachexia is associated with a variety of chronic diseases, and is characterised by loss of weight, weakness and a generalised deterioration of the condition of the body. The mechanism underlying cachexia is thought to be associated with the high levels of cytokines and hormones.

Tissue-specific atrophy reflects changes affecting a specific tissue or organ system in the body, and is due to changes directly affecting that organ, e.g. skeletal muscles atrophy if they are not used; the size of the individual myocytes (and therefore the force that the muscle can generate) reduces rapidly.

Atrophy restricted to a specific tissue or site can be the result of a variety of causes, including:

- Ischaemia – lack of blood flow and related nutrients to nourish the tissue
- Disuse
- Neuropathy – lack of nervous impulse to stimulate the tissue
- Idiopathic.

Increased growth

The increased growth of a tissue can be broken down into hyperplasia (increase in cell number) and hypertrophy (increase in cell size). Both occur physiologically in response to external stimuli and are usually reversible when the external stimulus is withdrawn.

Hypertrophy

Hypertrophy is commonly seen where there is relatively little cell turnover (e.g. muscle). It can also occur in response to a pathological change in another part of the body, e.g. in aortic stenosis the left ventricle is required to work harder to overcome the additional pressure cause by the constriction; as a result left ventricular hypertrophy occurs.

Hypertrophy may become a pathological process: in those who exercise, generalised cardiac hypertrophy is often seen. However, the coronary circulation may not develop sufficiently rapidly to respond to the additional tissue mass. Athletic heart syndrome may occur in young individuals, in whom there is a sudden failure of the coronary circulation to adequately supply the myocardium. Here what was initially a physiologically advantageous hypertrophy has developed into a pathological condition.

Hyperplasia

Increased growth through an increase in cell numbers is commonly seen in tissues that are constantly being renewed. This allows an increase in production of the required terminally differentiated cell, e.g. increased production of red blood cells in those exposed to chronic hypoxic conditions. Hyperplasia is often seen in endocrine tissues, when increased production of a hormone associated with that tissue is needed.

Cancer

There may be changes in a cell that contribute to its excessive multiplication. This change, **neoplasia**, is different to the changes seen in hyperplasia, because there is no useful function attributed to the cell growth – the cells involved will often have an abnormal histological appearance. The growth is rarely reversible. Cancerous growth is a serious cause of death – it is currently the second biggest killer in Britain – and can occur in almost every tissue in the body. Tumours may be defined as benign or malignant.

Benign tumours

Benign tumours are well defined from the surrounding tissue and often encapsulated. They are frequently harmless because they do not spread to other sites and in rare cases (e.g. skin warts) may regress. Benign tumours may cause illness as a result of local and systemic effects:

- **Local effects** may result from the occupation of space, generating pressure on a location (e.g. intracranial tumours pressing on the brain). Similarly, tumours may obstruct crucial vessels or ducts, restricting blood flow depending on their location.
- **Systemic effects** may occur in hormone-secreting tumours, e.g. a **phaeochromocytoma** is an adrenaline-secreting tumour that leads to severe hypertension.

Malignant tumours

Malignant tumours are distinguished from benign tumours because they directly invade the host tissue. They can spread rapidly, and may produce **metastases** in distant sites. The cells in malignant tumours are usually less well differentiated than benign tumours and are often made up of histologically abnormal cells, showing features of stem cells.

Spread of malignant tumours

Local invasion into the tissue surrounding the tumour may be aided by the de-differentiation of the cells:

- They express altered adhesion molecules; this has two effects to:
  - increase dissociation and release from the primary tumour
  - promote adherence to the seeding site in new tissue.
- They secrete enzymes to degrade the extracellular matrix.
- The reduced dependence of the tumour for growth factors (which are often tissue-specific) aid its survival and proliferation.

There are a variety of routes through which a malignant tumour may spread into local tissue:

- Direct invasion into tissue
- Growth into a body cavity
- Growth along a fascial plane
- Growth into blood or lymphatic vessels – this process can contribute to the formation of metastases.

Metastasis

Metastasis is the generation of secondary tumours at distant sites. This form of spread is found only in malignant tumours and is often associated with a very poor prognosis. There are three main routes through which metastases can occur:

1. Cells may enter the bloodstream and lodge in a remote tissue site.
2. Lymphatic involvement can lead to cells entering the lymph nodes and from there entering the bloodstream
3. Tumour cells may seed in body cavities; they may grow in suspension in fluid within the cavity and from there seed to a distant organ.

The distribution of metastases will often reflect the location of the primary tumour and the route of spread. Locations of metastatic growth also rely on specific interactions between the tumour and the tissue, which contributes to the specific locations favoured by particular tumours, e.g. melanomas typically form metastases in the liver or brain.

The liver is a common site of metastases for most tumours (except brain tumours); this is thought to be due to its rich blood supply to carry cells, and also due to the expression of many growth factors that aid the survival and promote the proliferation of metastatic tumour cells.

The local effects of metastases are similar to the local effect for other tumours, although, as they are disseminated and invasive, destruction of the host tissue as a result of the outgrowth of the metastases also occurs.

The genetic basis of malignancy

Tumours occur as a result of the accumulated genetic changes within a cell that lead to its de-differentiation and uncontrolled proliferation. These changes require the loss of **tumour-suppressor genes** and the activation of **oncogenes**. Tumours are generally the result of malignant changes in a single cell, reflecting the rarity of the events.

As a long period of time is required for such events to occur, most tumours become more common with age – as the pro-malignant changes develop. There are a variety of factors and stimuli that have been identified to increase the likelihood of cancer:

- **Chemical stimuli** can increased the risk of cancer by damaging DNA, e.g. smoking is linked with the occurrence of lung cancer.
- A variety of **physical stimuli** has been identified that promotes malignant changes:
  - **ionising radiation** (e.g. X-rays) causes single-stranded breaks in DNA; if such damage is incorrectly repaired by cellular mechanisms, it may contribute to cancer
  - **ultraviolet (UV) light** can promote base changes in the DNA; UV commonly leads to cytosine conversion to the RNA base uracil; DNA repair mechanisms, however, recognise the uracil as incorrect and will replace it with a thymine base. The exposure to UV light has been linked with an increased risk of developing skin cancer.

- **Trauma** has been implicated in cancer, because the increased cell growth may promote malignant changes. This is extremely rarely seen in the development of skin cancers at burn sites.

There are a number of tumours that are associated with childhood and for which the frequency decreases with age. These tumours are likely to affect young people for one of two main reasons:

- The tumour is **derived from a stem cell**, the number of which decrease as the individual ages.
- The individual has **inherited a mutation** associated with the development of the tumour (e.g. the Wilms' tumour gene is associated with the development of renal cancer in children).

Oncogenes

Oncogenes are genes that are often activated in cancerous cells. These genes have a physiological role during development; however, their inappropriate expression can lead to uncontrolled proliferation. Oncogenes may be activated as a result of a variety of changes:

- Mutations in the genes may **alter the nature or activity of the protein product**, which can contribute to malignant changes within the cell, e.g. the molecule CD117 is a receptor for cytokine stem cell factor and signalling through it stimulates cell survival and proliferation. Mutation of this protein so that it signals without binding its ligand is associated with the development of cancer, in particular leukaemia and some forms of testicular germ cell cancer.
- **Over-expression of a gene** can lead to cancerous changes. This may be seen as the result of a chromosomal translocation, leading the oncogene to become translocated to a site on a different chromosome that is highly expressed. The 'Philadelphia translocation' is associated with many leukaemias and results from a translocation between chromosome 9 and chromosome 22.

Tumour-suppressor genes

The activation of oncogenes in not sufficient to result in malignancy. In most cases tumorigenesis also requires loss of **tumour-suppressor genes**. These genes are often associated with DNA repair or regulation of the cell cycle, preventing damaged cells proliferating.

A commonly lost tumour-suppressor gene is *p53*, which is involved in regulating the entry of cells into the cell cycle. It acts to detect DNA damage, preventing damaged cells from entering the cell cycle.

Several changes are required in an individual cell for malignancy to result. Many oncogenes must be activated and several tumour-suppressor genes must be lost. This is known as the 'multi-step theory of malignancy'.

Infectious causes of cancer

There are specific viral infections that have been established as causing cancer – in particular, the **human papillomaviruses (HPVs)**. The production of viral proteins is aided by the proliferation of cells; HPVs inactivate a number of tumour-suppressor genes (e.g. *p53*) so increasing the entry of cells into the cell cycle to aid replication of HPV, although at the same time this can lead to tumour formation. Most HPV strains are associated with warts, although others are associated with more serious conditions such as cervical cancers – in particular HPV-16 and HPV-18.

Infectious diseases may cause an increased risk of cancer through repeated damage to a tissue – repeated replication of the tissue can increase the likelihood of errors occurring during DNA replication, and thus enhance the risk of malignant changes. This is illustrated by **hepatitis B** infection; chronic carriers are at an increased risk of primary hepatocellular carcinoma due to the constant damage and regeneration of the liver as a result of the immune response against the infection.

Treatment of cancer

Treatment of tumours focuses on destruction or removal of the neoplastic cells from the body. Three methods may be used:

1. **Surgical excision** of the tumour
2. **Radiotherapy** to destroy localised tumours
3. **Chemotherapy** to destroy tumour cells and treat metastases, because they cannot be removed adequately by surgical/radiotherapeutic means.

Mechanisms of chemotherapy

Chemotherapy targets features of the cells that are different from normal differentiated cells, primarily through targeting dividing cells. This contributes to the high toxicity, because many stem cells in the body are also affected. Despite the severe side effects, chemotherapy is the only method that can effectively target metastatic cancer. The drugs used in chemotherapy act in a variety of manners to promote the destruction of cancerous cells:

- **Alkylating agents** (e.g. cisplatin) bind to and cross-link DNA to prevent further replication of the tumour cells.
- **Antimetabolites** (e.g. methotrexate) replace key components in the synthesis of DNA or RNA. Methotrexate blocks the synthesis of purine bases. However, this action also contributes to the side effects of the drug, such as myelosuppression and nephrotoxicity.
- **Antitumour antibiotics** act primarily through binding to DNA to prevent its uncoiling, which leads to breakage of the helix and inhibits replication of the cells. Such drugs (e.g. doxorubicin) may also inhibit **topoisomerases**.
- **Spindle tubule inhibitors** (e.g. vincristine) destroy microtubules, preventing spindle formation that is required for the chromosome segregation in cell division. Similar to other chemotherapy agents, they are associated with severe side effects of nephrotoxicity and bone marrow suppression.

Monoclonal antibodies in cancer therapy

Owing to their de-differentiated state, many malignant cancers express molecules that are not seen commonly in adult individuals (although they may be expressed during embryonic development and early life). These molecules, if expressed on the cell surface, may be used to target the cancer specifically (e.g. Herceptin targets HER2 which is expressed on some breast cancers).

RELATED CHAPTERS

Chapter 2 Molecular biology and genetics
Chapter 3 Biochemistry
Chapter 4 Physiology
Chapter 5 Pharmacology
Chapter 10 Endocrinology