A1 Prokaryote cell structure

Key Notes

Prokaryotes

Prokaryotes are the most abundant organisms on earth and fall into two distinct groups, the bacteria (or eubacteria) and the archaea (or archaebacteria). A prokaryotic cell does not contain a membrane-bound nucleus.

Cell structure

Each prokaryotic cell is surrounded by a plasma membrane. The cell has no subcellular organelles, only infoldings of the plasma membrane called mesosomes. The deoxyribonucleic acid (DNA) is condensed within the cytosol to form the nucleoid.

Bacterial cell walls

The peptidoglycan (protein and oligosaccharide) cell wall protects the prokaryotic cell from mechanical and osmotic pressure. Some antibiotics, such as penicillin, target enzymes involved in the synthesis of the cell wall. Gram-positive bacteria have a thick cell wall surrounding the plasma membrane, whereas Gram-negative bacteria have a thinner cell wall and an outer membrane, between which is the periplasmic space.

Bacterial flagella

Some prokaryotes have tail-like flagella. By rotation of their flagella bacteria can move through their surrounding media in response to chemicals (chemotaxis). Bacterial flagella are made of the protein flagellin that forms a long filament which is attached to the flagellar motor by the flagellar hook.

Related topics

Eukaryote cell structure (A2)
Cytoskeleton and molecular motors (A3)
Amino acids (B1)
Membrane lipids (E1)
Membrane proteins and carbohydrate (E2)
Genes and chromosomes (F2)
Electron transport and oxidative phosphorylation (L2)

Prokaryotes

Prokaryotes are the most numerous and widespread organisms on earth, and are so classified because they have no defined membrane-bound nucleus. Prokaryotes comprise two separate but related groups: the **bacteria** (or eubacteria) and the **archaea** (or archaebacteria). These two distinct groups of prokaryotes diverged early in the history of life on Earth. The living world therefore has three major divisions or domains: bacteria, archaea and eukaryotes (see Topic A2). The bacteria are the commonly encountered prokaryotes in soil, water and living in or on larger organisms, and include *Escherichia coli* and the *Bacillus* species, as well as the cyanobacteria (photosynthetic blue-green algae). The archaea mainly inhabit unusual environments such as salt brines, hot acid springs, bogs and the ocean depths, and include the sulfur bacteria and the methanogens, although some are found in less hostile environments.

Cell structure

Prokaryotes generally range in size from 0.1 to 10 μm, and have one of three basic shapes: spherical (cocci), rod-like (bacilli) or helically coiled (spirilla). Like all cells, a prokaryotic cell is bounded by a **plasma membrane** that completely encloses the cytosol and separates the cell from the external environment. The plasma membrane, which is about 8 nm thick, consists of a lipid bilayer containing proteins (see Topics E1 and E2). Although prokaryotes lack the membranous subcellular organelles characteristic of eukaryotes (see Topic A2), their plasma membrane may be infolded to form **mesosomes** (*Fig. 1*). The mesosomes may be the sites of deoxyribonucleic acid (DNA) replication and other specialized enzymatic reactions. In photosynthetic bacteria, the mesosomes contain the proteins and pigments that trap light and generate adenosine triphosphate (ATP). The aqueous cytosol contains the macromolecules [enzymes, messenger ribonucleic acid (mRNA), transfer RNA (tRNA) and ribosomes], organic compounds and ions needed for cellular metabolism. Also within the cytosol is the prokaryotic 'chromosome' consisting of a single circular molecule of DNA which is condensed to form a body known as the **nucleoid** (*Fig. 1*) (see Topic F2).

Bacterial cell walls

To protect the cell from mechanical injury and osmotic pressure, most prokaryotes are surrounded by a rigid 3–25 nm thick **cell wall** (*Fig. 1*). The cell wall is composed of **peptidoglycan**, a complex of **oligosaccharides** and **proteins**. The oligosaccharide component consists of linear chains of alternating *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (NAM) linked β(1–4) (see Topic J1). Attached via an amide bond to the lactic acid group on NAM is a **D-amino acid**-containing tetrapeptide. Adjacent parallel peptidoglycan chains are covalently cross-linked through the tetrapeptide side-chains by other short peptides. The extensive cross-linking in the peptidoglycan cell wall gives it its strength and rigidity. The presence of D-amino acids in the peptidoglycan renders the cell wall resistant to the action of **proteases** which act on the more commonly

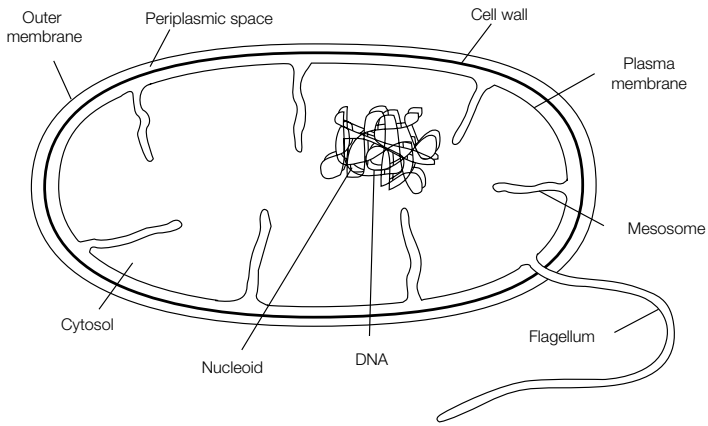

Fig. 1. Prokaryote cell structure.

occurring L-amino acids (see Topic B1), but provides a unique target for the action of certain **antibiotics** such as **penicillin**. Penicillin acts by inhibiting the enzyme that forms the covalent cross-links in the peptidoglycan, thereby weakening the cell wall. The β(1–4) glycosidic linkage between NAM and GlcNAc is susceptible to hydrolysis by the enzyme **lysozyme** which is present in tears, mucus and other body secretions.

Bacteria can be classified as either **Gram-positive** or **Gram-negative** depending on whether or not they take up the **Gram stain**. Gram-positive bacteria (e.g. *Bacillus polymyxa*) have a thick (25 nm) cell wall surrounding their plasma membrane, whereas Gram-negative bacteria (e.g. *Escherichia coli*) have a thinner (3 nm) cell wall and a second **outer membrane** (*Fig. 2*). In contrast with the plasma membrane, this outer membrane is very permeable to the passage of relatively large molecules (molecular weight > 1000 Da) due to **porin proteins** which form pores in the lipid bilayer. Between the outer membrane and the cell wall is the **periplasm**, a space occupied by proteins secreted from the cell.

Bacterial flagella

Many bacterial cells have one or more tail-like appendages known as **flagella**. By **rotating** their flagella, bacteria can move through the extracellular medium towards attractants and away from repellents, so called **chemotaxis**. Bacterial flagella are different from eukaryotic cilia and flagella in two ways: (1) each bacterial flagellum is made of the protein **flagellin** (53 kDa subunit) as opposed to tubulin (see Topic A3); and (2) it **rotates** rather than bends. An *E. coli* bacterium has about six flagella that emerge from random positions on the surface of the cell. Flagella are thin helical filaments, 15 nm in diameter and 10 μm long. Electron microscopy has revealed that the **flagellar filament** contains 11 subunits in two helical turns which, when viewed end-on, has the appearance of an 11-bladed propeller with a hollow central core. Flagella grow by the addition of new flagellin subunits to the end away from the cell, with the new subunits diffusing through the central core. Between the flagellar filament and the cell membrane is the **flagellar hook** composed of subunits of the 42 kDa hook protein that forms a short, curved structure. Situated in the plasma membrane is the basal body or **flagellar motor**, an intricate assembly of proteins. The flexible hook is attached to a series of protein rings which are embedded in the inner and outer membranes. The rotation of the flagella is driven by a flow of protons through an outer ring of proteins, called the stator. A similar proton-driven motor is found in the F_1F_0-ATPase that synthesizes ATP (see Topic L2).

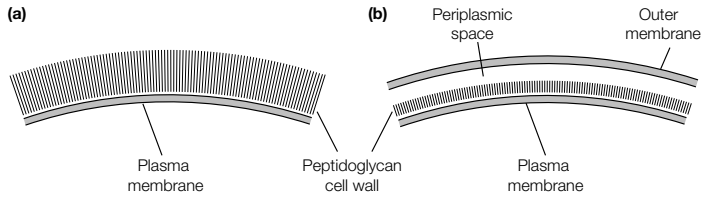

Fig. 2. Cell wall structure of (a) Gram-positive and (b) Gram-negative bacteria.

A2 EUKARYOTE CELL STRUCTURE

Key Notes

Eukaryotes

Eukaryotic cells have a membrane-bound nucleus and a number of other membrane-bound subcellular (internal) organelles, each of which has a specific function.

Plasma membrane

The plasma membrane surrounds the cell, separating it from the external environment. The plasma membrane is a selectively permeable barrier due to the presence of specific transport proteins and has receptor proteins that bind specific ligands. It is also involved in the processes of exocytosis and endocytosis.

Nucleus

The nucleus stores the cell's genetic information as DNA in chromosomes. It is bounded by a double membrane but pores in this membrane allow molecules to move in and out of the nucleus. The nucleolus within the nucleus is the site of ribosomal ribonucleic acid (rRNA) synthesis.

Endoplasmic reticulum

This interconnected network of membrane vesicles is divided into two distinct parts. The rough endoplasmic reticulum (RER), which is studded with ribosomes, is the site of membrane and secretory protein biosynthesis and their post-translational modification. The smooth endoplasmic reticulum (SER) is involved in phospholipid biosynthesis and in the detoxification of toxic compounds.

Golgi apparatus

The Golgi apparatus, a system of flattened membrane-bound sacs, is the sorting and packaging center of the cell. It receives membrane vesicles from the RER, further modifies the proteins within them, and then packages the modified proteins in other vesicles which eventually fuse with the plasma membrane or other subcellular organelles.

Mitochondria

Mitochondria have an inner and an outer membrane separated by the intermembrane space. The outer membrane is more permeable than the inner membrane due to the presence of porin proteins. The inner membrane, which is folded to form cristae, is the site of oxidative phosphorylation, which produces ATP. The central matrix is the site of fatty acid degradation and the citric acid cycle.

Chloroplasts

Chloroplasts in plant cells are surrounded by a double membrane and have an internal membrane system of thylakoid vesicles that are stacked up to form grana. The thylakoid vesicles contain chlorophyll and are the site of photosynthesis. Carbon dioxide (CO_2) fixation takes place in the stroma, the soluble matter around the thylakoid vesicles.

Lysosomes

Lysosomes in animal cells are bounded by a single membrane. They have an acidic internal pH (pH 4–5), maintained by proteins in the membrane that pump in H^+ ions. Within the lysosomes are acid hydrolases; enzymes involved in the degradation of macromolecules, including those internalized by endocytosis.

Peroxisomes

Peroxisomes contain enzymes involved in the breakdown of amino acids and fatty acids, a byproduct of which is hydrogen peroxide. This toxic compound is rapidly degraded by the enzyme catalase, also found within the peroxisomes.

Cytosol

The cytosol is the soluble part of the cytoplasm where a large number of metabolic reactions take place. Within the cytosol is the cytoskeleton, a network of fibers (microtubules, intermediate filaments and microfilaments) that maintain the shape of the cell.

Plant cell wall

The cell wall surrounding a plant cell is made up of the polysaccharide cellulose. In wood, the phenolic polymer called lignin gives the cell wall additional strength and rigidity.

Plant cell vacuole

The membrane-bound vacuole is used to store nutrients and waste products, has an acidic pH and, due to the influx of water, creates turgor pressure inside the cell as it pushes out against the cell wall.

Related topics

Cytoskeleton and molecular motors (A3)
Bioimaging (A4)
Transport of small molecules (E3)
Transport of macromolecules (E4)
Signal transduction (E5)
Genes and chromosomes (F2)
Protein targeting (H4)
Electron transport and oxidative phosphorylation (L2)
Photosynthesis (L3)

Eukaryotes

A eukaryotic cell is surrounded by a **plasma membrane**, has a membrane-bound nucleus and contains a number of other distinct **subcellular organelles** (*Fig. 1*). These organelles are membrane-bounded structures, each having a unique role and each containing a specific complement of proteins and other molecules. Animal and plant cells have the same basic structure, although some organelles and structures are found in one and not the other (e.g. chloroplasts, vacuoles and cell wall in plant cells, lysosomes in animal cells).

Plasma membrane

The plasma membrane envelops the cell, separating it from the external environment and maintaining the correct ionic composition and osmotic pressure of the cytosol. The plasma membrane, like all membranes, is impermeable to most substances but the presence of specific proteins in the membrane allows certain molecules to pass through, therefore making it **selectively permeable** (see Topic E3). The plasma membrane is also involved in communicating with other cells, in particular through the binding of ligands (small molecules such as hormones, neurotransmitters, etc.) to **receptor proteins** on its surface (see Topic E5). The plasma membrane is also involved in the **exocytosis** (secretion) and **endocytosis** (internalization) of proteins and other macromolecules (see Topic E4).

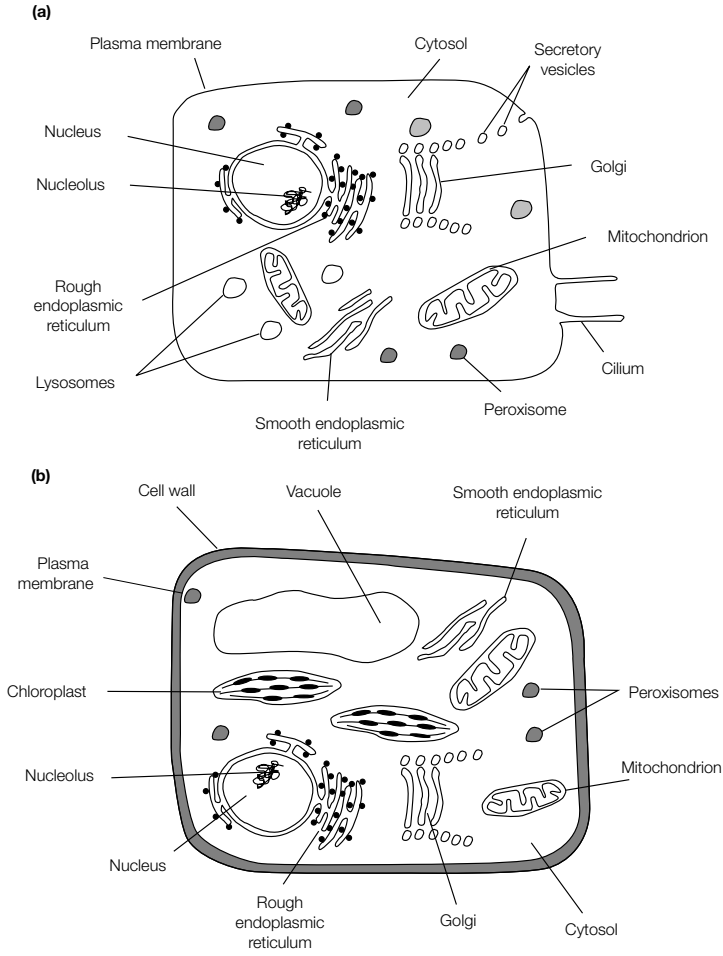

Fig. 1. Eukaryote cell structure. (a) Structure of a typical animal cell, (b) structure of a typical plant cell.

Nucleus

The nucleus is bounded by two membranes, the **inner and outer nuclear membranes**. These two membranes fuse together at the **nuclear pores** through which molecules [messenger ribonucleic acid (mRNA), proteins, ribosomes, etc.] can move between the nucleus and the cytosol. Other proteins, for example those involved in regulating gene expression, can pass through the pores from the cytosol to the nucleus. The outer nuclear membrane is often continuous with the rough endoplasmic reticulum (RER). Within the nucleus the **DNA** is tightly coiled around **histone proteins** and organized into complexes called **chromosomes** (see Topic F2). Visible under the light microscope (see Topic A4) is the **nucleolus**, a subregion of the nucleus which is the site of ribosomal ribonucleic acid (rRNA) synthesis.

Endoplasmic reticulum

The endoplasmic reticulum (ER) is an interconnected network of membrane vesicles. The **rough endoplasmic reticulum (RER)** is studded on the cytosolic face with **ribosomes**, the sites of **membrane and secretory protein biosynthesis** (see Topic H3). Within the lumen of the RER are enzymes involved in the **post-translational modification** (glycosylation, proteolysis, etc.) of membrane and secretory proteins (see Topic H5). The **smooth endoplasmic reticulum (SER)**, which is not studded with ribosomes, is the site of **phospholipid biosynthesis**, and is where a number of **detoxification reactions** take place.

Golgi apparatus

The Golgi apparatus, a system of flattened membrane-bound sacs, is the **sorting and processing center** of the cell. Membrane vesicles from the RER, containing membrane and secretory proteins, fuse with the Golgi apparatus and release their contents into it. On transit through the Golgi apparatus, further **post-translational modifications** to these proteins take place and they are then sorted and packaged into different vesicles (see Topic H5). These vesicles bud off from the Golgi apparatus and are transported through the cytosol, eventually fusing either with the plasma membrane to release their contents into the extracellular space (a process known as **exocytosis**; see Topic E4) or with other internal organelles (e.g. lysosomes).

Mitochondria

A mitochondrion has an **inner and an outer membrane** between which is the **intermembrane space** (*Fig. 2a*). The outer membrane contains **porin proteins** which make it permeable to molecules of up to 10 kDa. The inner membrane, which is considerably less permeable, has large infoldings called **cristae** which protrude into the **central matrix**. The inner membrane is the site of oxidative phosphorylation and electron transport involved in ATP production (see Topic L2). The central matrix is the site of numerous metabolic reactions including the citric acid cycle (see Topic L1) and fatty acid breakdown (see Topic K2). Also within the matrix is found the mitochondrial DNA which encodes some of the mitochondrial proteins.

Chloroplasts

Chloroplasts, present exclusively in plant cells, also have **inner and outer membranes**. In addition, there is an extensive internal membrane system made up of **thylakoid vesicles** (interconnected vesicles flattened to form discs) stacked upon each other to form **grana** (*Fig. 2b*). Within the thylakoid vesicles is the green pigment **chlorophyll** (see Topic M4), along with the enzymes that trap light energy and convert it into chemical energy in the form of ATP (see Topic L3). The **stroma**, the space surrounding the thylakoid vesicles, is the site of carbon dioxide (CO_2) fixation – the conversion of CO_2 into organic compounds. Chloroplasts, like mitochondria, contain DNA which encodes some of the chloroplast proteins.

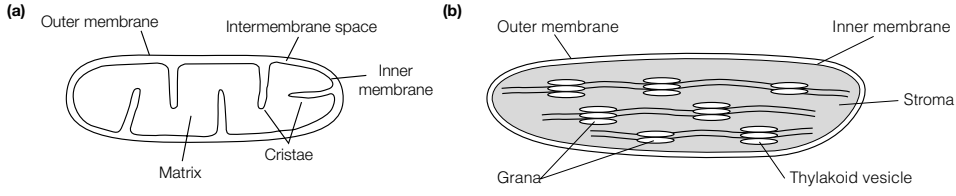

Fig. 2. Structure of (a) a mitochondrion and (b) a chloroplast.

Lysosomes

Lysosomes, which are found only in animal cells, have a single boundary membrane. The internal pH of these organelles is **mildly acidic** (pH 4–5), and is maintained by integral membrane proteins which pump H^+ ions into them (see Topic E3). The lysosomes contain a range of hydrolases that are optimally active at this acidic pH (and hence are termed **acid hydrolases**) but which are inactive at the neutral pH of the cytosol and extracellular fluid. These enzymes are involved in the degradation of host and foreign macromolecules into their monomeric subunits; **proteases** degrade proteins, **lipases** degrade lipids, **phosphatases** remove phosphate groups from nucleotides and phospholipids, and **nucleases** degrade DNA and RNA. Lysosomes are involved in the degradation of extracellular macromolecules that have been brought into the cell by **endocytosis** (see Topic E4) as well as in the degradation and recycling of normal cellular components.

Peroxisomes

These organelles have a single boundary membrane and contain enzymes that degrade fatty acids and amino acids. A byproduct of these reactions is **hydrogen peroxide**, which is toxic to the cell. The presence of large amounts of the enzyme **catalase** in the peroxisomes rapidly converts the toxic hydrogen peroxide into harmless H_2O and O_2:

$$2H_2O_2 \xrightarrow{\textit{Catalase}} 2H_2O + O_2$$

Cytosol

The cytosol is that part of the **cytoplasm** not included within any of the subcellular organelles, and is a major site of cellular metabolism, containing a large number of different enzymes and other proteins. For example, glycolysis (see Topic J3), gluconeogenesis (see Topic J4), the pentose phosphate pathway (see Topic J5) and fatty acid synthesis (see Topic K3) all take place in the cytosol. The cytosol is not a homogeneous 'soup' but has within it the **cytoskeleton**, a network of fibers criss-crossing through the cell that helps to maintain the shape of the cell. The cytoskeletal fibers include **microtubules** (30 nm in diameter), **intermediate filaments** (10 nm in diameter) and **microfilaments** (8 nm in diameter) (see Topic A3). Also found within the cytosol of many cells are **inclusion bodies** (granules of material that are not membrane-bounded) such as glycogen granules in liver and muscle cells, and droplets of triacylglycerol in the fat cells of adipose tissue.

Plant cell wall

Surrounding the plasma membrane of a plant cell is the cell wall, which imparts strength and rigidity to the cell. This is built primarily of **cellulose**, a rod-like **polysaccharide** of repeating glucose units linked β(1–4) (see Topic J1). These cellulose molecules are aggregated together by hydrogen bonding into bundles of fibers, and the fibers in turn are cross-linked together by other polysaccharides. In wood another compound, **lignin**, imparts added strength and rigidity to the cell wall. Lignin is a complex water-insoluble phenolic polymer.

Plant cell vacuole

Plant cells usually contain one or more **membrane-bounded vacuoles**. These are used to store nutrients (e.g. sucrose), water, ions and waste products (especially excess nitrogen-containing compounds). Like lysosomes in animal cells, vacuoles have an **acidic pH** maintained by H^+ pumps in the membrane and contain a variety of **degradative enzymes**. Entry of water into the vacuole causes it to expand, creating hydrostatic pressure (**turgor**) inside the cell which is balanced by the mechanical resistance of the cell wall.

A3 Cytoskeleton and molecular motors

Key Notes

Cytoskeleton

Eukaryotic cells have an internal scaffold, the cytoskeleton, that controls the shape and movement of the cell and the organelles within it. The cytoskeleton consists of microfilaments, intermediate filaments and microtubules.

Microfilaments

Microfilaments are 5–9 nm diameter helical polymers of the protein actin that have a mechanically supportive function in the cell.

Intermediate filaments

Intermediate filaments are 7–11 nm diameter rope-like fibers made from a family of intermediate filament proteins that provide mechanical strength and resistance to shear stress.

Microtubules

Microtubule filaments are hollow cylinders of 25 nm diameter made of the protein tubulin. The wall of the microtubule is made up of a helical array of alternating α- and β-tubulin subunits. The mitotic spindle involved in separating the chromosomes during cell division is made of microtubules. Colchicine and vinblastine inhibit microtubule formation, whereas taxol stabilizes microtubules. Through interfering with mitosis, some of these compounds are used as anticancer drugs.

Molecular motors

Molecular motors or motor proteins bind to cytoskeletal filaments and use energy derived from the hydrolysis of ATP to move along them. The head region or motor domain which hydrolyses ATP binds to the filament, while the tail region binds the cargo. The major types of motor proteins are the myosins, the kinesins and the dyneins.

Muscle structure

Each cell within vertebrate striated muscle contains within its sarcoplasm many parallel myofibrils which in turn are made up of repeating sarcomere units. Within the sarcomere are the alternating dark A band and light I band, in the middle of which are the H zone and Z line, respectively. A myofibril contains two types of filaments: the thick filaments consisting of myosin, and the thin filaments consisting of actin, tropomyosin and troponin. When muscle contracts, the thick and thin filaments slide over one another, shortening the length of the sarcomere.

Myosin

The protein myosin consists of two heavy polypeptide chains and two pairs of light chains arranged as a double-headed globular region attached to a two-stranded α-helical coiled-coil. Myosin molecules spontaneously assemble into filaments, hydrolyze ATP and bind actin.

Actin

Actin, the major constituent of the thin filaments, can exist as monomeric globular G-actin or as polymerized fibrous F-actin. The actin filaments are connected to the thick filaments by cross-bridges formed by the S1 heads of myosin.

The generation of force in muscle

The cyclic formation and dissociation of complexes between the actin filaments and the S1 heads of myosin leads to contraction of the muscle. On binding to actin, myosin releases its bound Pi and ADP. This causes a conformational change to occur in the protein which moves the actin filament along the thick filament. ATP then binds to myosin, displacing the actin. Hydrolysis of the ATP returns the S1 head to its original conformation.

Troponin and tropomyosin

Tropomyosin lies along the thin filament and prevents the association of myosin with actin in the resting state. Ca^{2+} ions released into the sarcoplasm from the sarcoplasmic reticulum in response to a nerve stimulation bind to the TnC subunit of troponin and cause a conformational change in the protein. This movement is transmitted by an allosteric mechanism through the TnI and TnT subunits of troponin to tropomyosin, causing the latter to move out of the way and allowing the actin and myosin to associate.

Cilia

Eukaryotic cilia are hair-like protrusions on the surface of the cell that consist mainly of microtubules. The microtubule fibers in a cilium are bundled together in a characteristic 9 + 2 arrangement within the axoneme. The outer nine microtubule doublets look like a figure eight with a smaller circle, subfiber A, and a larger circle, subfiber B.

Dynein

Dynein is a very large protein that forms cross-bridges with the B subfibers and possesses ATPase activity. Two dynein arms protrude from subfiber A which, upon hydrolysis of ATP, move along adjacent B subfibers. Due to extensible nexin links between the doublets, this sliding motion is converted into a local bending of the cilium.

Related topics

Eukaryote cell structure (A2)
Bioimaging (A4)
Protein structure (B3)
Regulation of enzyme activity (C5)
Glycolysis (J3)
The urea cycle (M3)

Cytoskeleton

In the cytosol of eukaryotic cells is an **internal scaffold**, the cytoskeleton. The cytoskeleton is important in maintaining and altering the shape of the cell, in enabling cells such as sperm and white blood cells to move from one place to another, in transporting intracellular vesicles, and in pulling the chromosomes apart at mitosis and then dividing the cell in two. Three types of **filaments** make up the cytoskeleton: microfilaments, intermediate filaments and microtubules, each with distinct mechanical properties and dynamics.

Microfilaments

The **microfilaments** (also known as **actin filaments**), diameter 5–9 nm, have a mechanically supportive function, determining the shape of the cell's surface and they are involved in whole cell movement. Microfilaments are two-stranded helical polymers of the protein **actin** which appear as flexible structures organized into a variety of linear bundles and more extensive networks. Through their interaction with myosin, the microfilaments form contractile assemblies that are involved in various intracellular movements such as cytoplasmic streaming and the formation of membrane invaginations.

Intermediate filaments

The **intermediate filaments** (7–11 nm in diameter) provide mechanical strength and resistance to shear stress. They are made of **intermediate filament proteins**, which constitute a large and heterogeneous family, that form rope-like fibers. The skin in higher animals contains an extensive network of intermediate filaments made up of the protein **keratin** that has a two-stranded α-helical coiled-coil structure, while the nuclear lamina, a meshwork just beneath the inner nuclear membrane, is formed from another type of intermediate filament.

Microtubules

The third type of cytoskeletal filaments, the **microtubules**, determines the position of membrane-bound organelles and directs their intracellular transport. For example, the **mitotic spindle** involved in separating the replicated chromosomes during mitosis is an assembly of microtubules. Microtubules are hollow cylindrical structures with an outer diameter of 25 nm that are built from the protein **tubulin** (*Fig. 1*). The rigid wall of a microtubule is made up of a helical array of alternating α- and β-tubulin subunits, each 50 kDa in size. A cross-section through a microtubule reveals that there are 13 tubulin subunits per turn of the filament. Microtubules in cells are formed by the addition of α- and β-tubulin molecules to pre-existing filaments or nucleation centers. One end of the microtubule is usually attached to a **microtubule-organizing center** called a **centrosome**. The drugs **colchicine** and **vinblastine** inhibit the polymerization of microtubules, thus blocking cell processes such as cell division that depend on functioning microtubules. Another compound, **taxol**, stabilizes tubulin in microtubules and promotes polymerization. Some of these compounds, such as vinblastine and taxol, are being used as **anticancer drugs** since they block the proliferation of rapidly dividing cells by interfering with the mitotic spindle.

Molecular motors

Numerous accessory proteins associate with the cytoskeleton, including the molecular motors or **motor proteins**. These proteins bind to a cytoskeletal filament and use the energy derived from repeated cycles of ATP hydrolysis to move along it; thus they convert chemical energy into motion. There are many

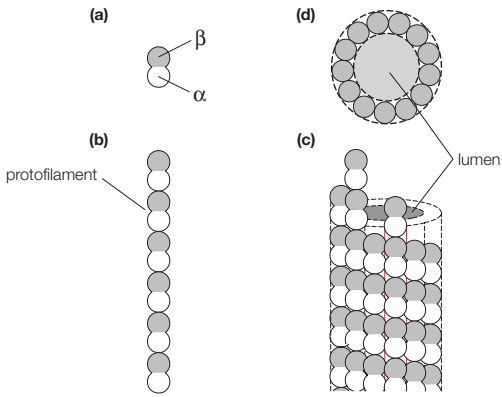

Fig. 1. The structure of a microtubule. (a) Tubulin consists of a*- and* b*-subunits. (b) A tubulin protofilament consisting of many adjacent subunits. (c) The microtubule is formed from 13 protofilaments aligned in parallel. (d) Cross-section of the hollow microtubule.*

different types of motor proteins in eukaryotic cells that differ in the type of filament to which they bind, the direction in which they move along the filament and the cargo they carry. The motor proteins associate with the filaments through a **head region** or **motor domain** that binds and hydrolyzes ATP, while the **tail region** binds the cargo that is transported. There are three types of motor proteins: the **myosins** that bind to actin filaments, and the **kinesins** and **dyneins** that bind to microtubules.

Muscle structure

The best understood force-generating process in biological systems is the contraction of **vertebrate striated muscle**, so named because it appears striated (striped) under phase-contrast microscopy (see Topic A4). This muscle is composed of numerous multinucleate cells that are bounded by an electrically excitable plasma membrane. Each cell contains within its **sarcoplasm** (cytosol) many parallel **myofibrils**, each approximately 1 μm in diameter. The sarcoplasm is also rich in ATP, creatine phosphate (see Topic M3) and glycolytic enzymes (see Topic J3). The functional unit of the myofibril is the **sarcomere** which repeats every 2.3 μm along the fibril axis (*Fig. 2a*). A dark **A band** and a light **I band** alternate regularly along the length of the myofibril. The central region of the A band, the **H zone**, is less dense than the rest of the band. Within the middle of the I band is a very dense narrow **Z line**. A cross-section of a

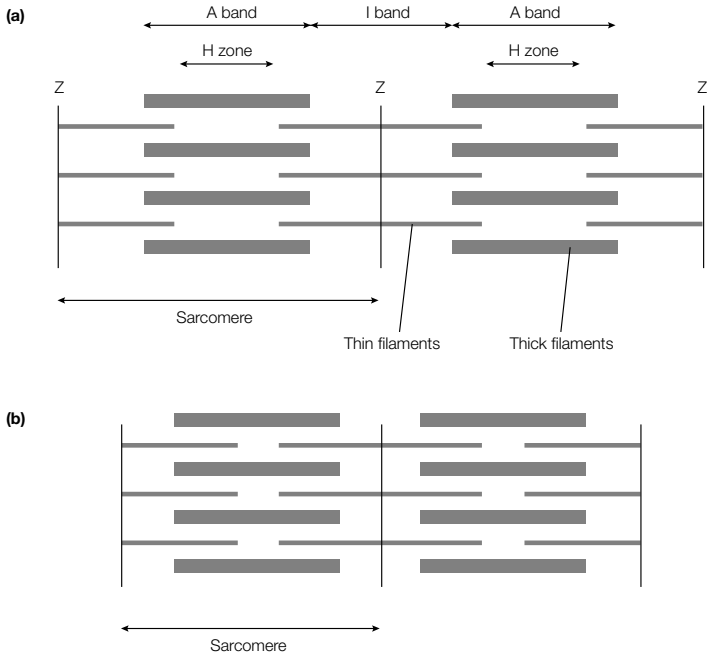

Fig. 2. Schematic diagram showing the appearance of vertebrate striated muscle as it appears under phase-contrast microscopy. (a) Relaxed, (b) contracted.

myofibril reveals that there are two types of interacting filaments. The **thick filaments** of diameter approximately 15 nm are found only in the A band (*Fig. 2a*) and consist primarily of the protein **myosin**, while the **thin filaments** of approximately 9 nm diameter contain **actin**, **tropomyosin** and the **troponin complex**.

When muscle contracts it can shorten by as much as a third of its original length. Information obtained from X-ray crystallographic (see Topic B3), and light- and electron-microscopic studies (see Topic A4) led to the proposal of the **sliding filament model** to explain muscle contraction. The thick and thin filaments were seen not to change in length during muscle contraction, but the length of the **sarcomere** was observed to decrease as the thick and thin filaments slide past each other (*Fig. 2*). Thus, as muscle contracts, the sizes of the H zone and the I band are seen to decrease. The force of the contraction is generated by a process that actively moves one type of filament past neighboring filaments of the other type.

Myosin

Myosin is a large protein (520 kDa) consisting of six polypeptide chains: two heavy chains (220 kDa each), and two pairs of light chains (20 kDa each). This large protein has three biological activities:

1. Myosin molecules spontaneously assemble into **filaments** in solutions of physiological ionic strength and pH;
2. Myosin is an **ATPase**, hydrolyzing ATP to ADP and P_i;
3. Myosin binds the **polymerized form of actin**.

Myosin consists of a double-headed globular region joined to a long rod. The rod is a two-stranded α-helical coiled-coil formed by the two heavy chains, while the globular heads are also part of each heavy chain with the light chains attached (*Fig. 3a*). Limited **proteolysis** of myosin with **trypsin** results in its dissection into two fragments: **light meromyosin** (**LMM**) and **heavy meromyosin** (**HMM**) (*Fig. 3b*). Functional studies of these two fragments reveal that LMM can still form filaments but lacks ATPase activity, whereas HMM does not form filaments but possesses ATPase activity and can bind to actin. HMM can be further split into two identical **globular subfragments** (**S1**) and one **rod-shaped subfragment** (**S2**) by another protease, **papain** (*Fig. 3b*). The S1 subfragment, whose structure has been determined by X-ray crystallography, contains an ATPase site, an actin-binding site and two light chain-binding sites. The proteolytic cleavage of myosin occurs at **flexible hinge regions** within the protein that separate the globular S1 domains from the rod-like S2 and LMM domains (*Fig. 3c*). These hinges have a crucial role to play in the contraction of muscle.

Actin

Actin, the major constituent of the thin filaments, exists in two forms. In solutions of low ionic strength it exists as a 42 kDa monomer, termed **G-actin** because of its globular shape. As the ionic strength of the solution rises to that at the physiological level, G-actin polymerizes into a fibrous form, **F-actin**, that resembles the thin filaments found in muscle. Although actin, like myosin, is an **ATPase**, the hydrolysis of ATP is not involved in the contraction–relaxation cycle of muscle but rather in the assembly and disassembly of the actin filament.

On the thick filaments, **cross-bridges** emerge from the filament axis in a regular helical array towards either end, whereas there is a bare region in the middle that is devoid of cross-bridges (*Fig. 4*). In muscle depleted of ATP, the myosin cross-bridges interact with the surrounding actin filaments. The absolute

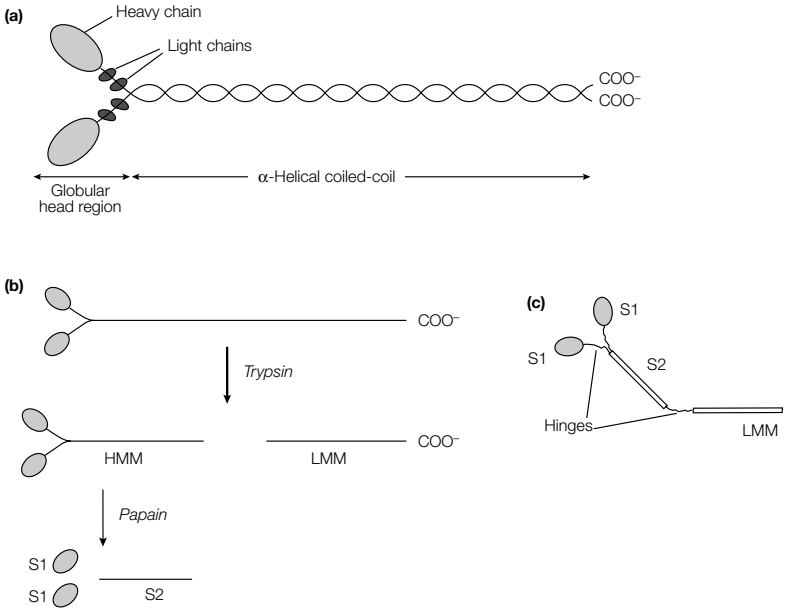

Fig. 3. Structure of myosin (a) showing the association of the two heavy and two pairs of light chains, (b) showing the proteolytic fragmentation of myosin, and (c) showing the hinge regions between domains.

direction of the actin and myosin molecules reverses halfway between the Z lines. Thus, as the two thin filaments that bind the cross-bridges at either end of a thick filament move towards each other, sliding over the thick filament, the distance between the Z lines shortens and the muscle contracts (*Fig. 4*).

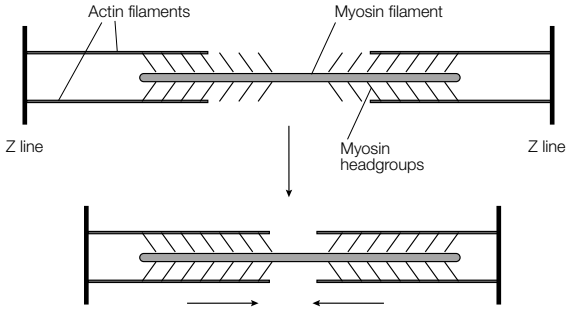

Fig. 4. Schematic diagram showing the interaction of the myosin thick filaments and the actin thin filaments during skeletal muscle contraction.

The generation of force in muscle

The cyclic formation and dissociation of cross-bridges between actin and the S1 heads of myosin leads to contraction of the muscle because of **conformational changes** that take place in the myosin S1 head. In resting muscle, the S1 heads are unable to interact with the actin in the thin filaments because of steric interference by the regulatory protein **tropomyosin** (*Fig. 5a*). The myosin has bound to it ADP and P_i. When the muscle is stimulated, the tropomyosin moves out of the way, allowing the S1 heads projecting out from the thick filament to attach to the actin in the thin filament (*Fig. 5b*). On binding of myosin–ADP–P_i to actin, first the P_i and then the ADP are released. As the ADP is released, the S1 head undergoes a **conformational change in the hinge region** between the S1 and S2 domains that alters its orientation relative to the actin molecule in the thin filament (*Fig. 5c*). This constitutes the **power stroke** of muscle contraction and

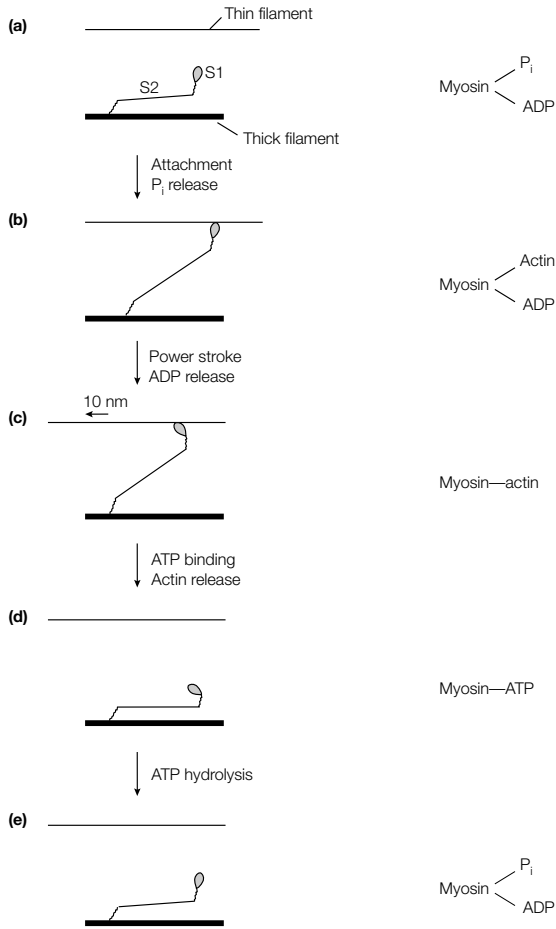

Fig. 5. Mechanism for the generation of force in muscle as an S1 head of a myosin thick filament interacts with an actin thin filament.

results in the thin filament moving a distance of approximately 10 nm relative to the thick filament towards the center of the sarcomere. ATP then binds to the S1 head which leads to the rapid release of the actin [i.e. dissociation of the thin and thick filaments (*Fig. 5d*)]. The ATP is then hydrolyzed to ADP and P_i by the free S1 head, which is returned to its original conformation ready for another round of attachment (*Fig. 5e*), conformational change and release.

Troponin and tropomyosin

Troponin and tropomyosin mediate the regulation of muscle contraction in response to **Ca^{2+}**. These two proteins are present in the thin filament, alongside the actin, and constitute about a third of its mass. **Tropomyosin** is an elongated protein of 70 kDa that forms a two-stranded α-helical rod which lies nearly parallel to the long axis of the thin filament. **Troponin** is a complex of three polypeptide chains: **TnC** (18 kDa) which binds Ca^{2+}, **TnI** (24 kDa) which binds to actin and **TnT** (37 kDa) which binds to tropomyosin. On muscle stimulation by a nerve impulse, Ca^{2+} ions are released from the **sarcoplasmic reticulum** (a specialized form of the ER found in muscle cells; see Topic A2) into the cytosol, raising the cytosolic Ca^{2+} concentration from the resting concentration of less than 1 μM to about 10 μM. The Ca^{2+} binds to sites on TnC, causing a **conformational change** in this polypeptide which is transmitted through the other components of the troponin complex to the tropomyosin. The tropomyosin then moves out of the way, allowing the S1 head of myosin to interact with the actin and initiate a cycle of contraction. Thus, Ca^{2+} controls muscle contraction by an **allosteric mechanism** (see Topic C5) involving troponin, tropomyosin, actin and myosin.

Cilia

The hair-like protrusions or cilia on the surfaces of certain eukaryotic cells, such as those lining the respiratory passages, consist mainly of **microtubules**. Cilia are involved in moving a stream of liquid over the surface of the cell. Free cells such as protozoa and sperm from various species can be propelled by either cilia or a flagellum. In eukaryotic cells, flagella differ from cilia only in being much longer. Electron microscopic studies have shown that virtually all eukaryotic cilia and flagella have the same basic design; a bundle of fibers called an **axoneme** surrounded by a membrane that is continuous with the plasma membrane (*Fig. 6*). The microtubule fibers in an axoneme are in a characteristic **9 + 2 array**, with a peripheral group of nine pairs of microtubules surrounding two singlet microtubules (*Fig. 6*). Each of the nine outer doublets appears like a figure eight, the smaller circle is termed **subfiber A**, the larger circle, **subfiber B**. Subfiber A is joined to a central sheath by radial spokes, while neighboring microtubule doublets are held together by **nexin** links. Two **dynein** arms emerge from each subfiber A, with all the arms in a cilium pointing in the same direction (*Fig. 6*).

Dynein

Dynein is a very large protein (1000–2000 kDa) consisting of one, two or three heads depending on the source. Like the heads of myosin, the heads of dynein form **cross-bridges**, in this case with the B subfibers, and possess **ATPase activity**. The binding of ATP to dynein causes it to dissociate from the B subfiber. On hydrolysis of the ATP to ADP and P_i, the dynein binds again with the B subfiber with the subsequent release of the P_i and ADP (a cycle very similar to that which occurs with the binding of the S1 heads of myosin to ATP). This ATPase cycle leads to the movement of the cilium as the outer doublets of the axoneme slide past each other. The force between adjacent doublets is generated by the dynein

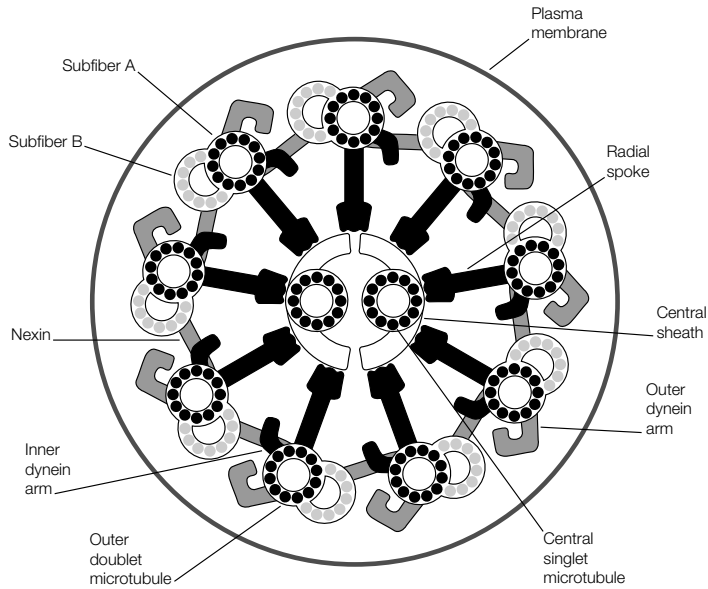

Fig. 6. Cross-sectional diagram of a cilium.

cross-bridges. Thus, the dynein arms on subfiber A of one doublet walk along subfiber B of the adjacent doublet. Unlike in muscle, where the myosin and actin filaments slide past each other, in a cilium the radial spokes resist the sliding motion, which instead is converted into a local bending. The highly extensible protein, **nexin**, keeps adjacent doublets together during this process.

A defect or absence in any one of the proteins within the axoneme (e.g. dynein, nexin, etc.) results in cilia that are immotile, so called **immotile-cilia syndrome**. Patients suffering from this disease have chronic pulmonary disorders due to the cilia in the respiratory tract being unable to sweep out bacteria and other foreign particles. In addition, males with this genetic defect are infertile because their sperm are unable to move due to flagella inactivity.

A4 BIOIMAGING

Key Notes

Light microscopy

In light microscopy, a beam of light is focused through a microscope using glass lenses to produce an enlarged image of the specimen. In a compound light microscope the specimen is illuminated from below with the beam of light being focused on to it by the condenser lens. The incident light that passes through the specimen is then focused by the objective lens on to its focal plane, creating a magnified image.

Fixing and staining specimens

The specimen to be viewed by microscopy is first fixed with alcohol or formaldehyde, embedded in wax and then cut into thin sections with a microtome before being mounted on a glass slide and viewed under the microscope. Subcellular organelles cannot readily be distinguished under the light microscope without first staining the specimen with a chemical, such as hematoxylin or eosin. The location of an enzyme in a specimen can be revealed by cytochemical staining using a substrate which is converted into a colored product by the enzyme.

Phase-contrast microscopy

Phase-contrast microscopy and the more complex differential interference contrast microscopy can be used to visualize living cells. The microscope is adapted to alter the phase of the light waves to produce an image in which the degree of brightness of a region of the specimen depends on its refractive index.

Fluorescence microscopy

In fluorescence microscopy, fluorescent compounds (which absorb light at the exciting wavelength and then emit it at the emission wavelength) are attached to a secondary antibody which binds to the primary antibody that is itself specific for the subcellular structure under investigation. Upon illumination at the exciting wavelength, the fluorescent compound emits light, revealing where the primary antibody has bound.

Confocal scanning microscopy

This variation of fluorescence microscopy uses a laser to focus light of the exciting wavelength on to the specimen so that only a thin section of it is illuminated. The laser beam is moved through the sample, producing a series of images which are then reassembled by a computer to produce a three-dimensional picture of the specimen.

Green fluorescent protein

The naturally green fluorescent protein (GFP) from a jellyfish can be tagged on to other proteins and used to visualize the location and movement of proteins in living cells by fluorescent microscopy.

Fluorescence resonance energy transfer

Interactions between one protein and another can be monitored by fluorescence resonance energy transfer (FRET) by labeling the two proteins of interest with different fluorochromes. The emission spectrum of one fluorochrome overlaps with the excitation spectrum of the other such that, when the two proteins are in close proximity, light can be transferred from one fluorochrome to the other.

Transmission electron microscopy

In electron microscopy, a beam of electrons is focused using electromagnetic lenses. The specimen is mounted within a vacuum so that the electrons are not absorbed by atoms in the air. In transmission electron microscopy, the beam of electrons is passed through a thin section of the specimen that has been stained with heavy metals. The electron-dense metals scatter the incident electrons, thereby producing an image of the specimen.

Scanning electron microscopy

In scanning electron microscopy, the surface of a whole specimen is coated with a layer of heavy metal and then scanned with an electron beam. Excited molecules in the specimen release secondary electrons which are focused to produce a three-dimensional image of the specimen.

Related topics

Eukaryote cell structure (A2)
Membrane proteins and carbohydrate (E2)
Antibodies as tools (D4)
DNA cloning (I4)
Polymerase chain reaction (I6)

Light microscopy

In light microscopy, **glass lenses** are used to focus a beam of light on to the **specimen** under investigation. The light passing through the specimen is then focused by other lenses to produce a **magnified image**.

Standard (bright-field) light microscopy is the most common microscopy technique in use today and uses a **compound microscope**. The specimen is illuminated from underneath by a lamp in the base of the microscope (*Fig. 1*), with the light being focused on to the plane of the specimen by a **condenser lens.** Incident light coming through the specimen is picked up by the **objective lens** and focused on to its focal plane, creating a magnified image. This image is further magnified by the eyepiece, with the total magnification achieved being the sum of the magnifications of the individual lenses. In order to increase the resolution achieved by a compound microscope, the specimen is often overlaid

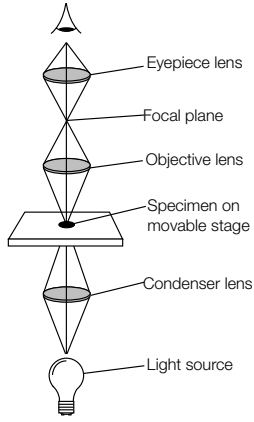

Fig. 1. Optical pathway of a compound microscope.

with **immersion oil** into which the objective lens is placed. The limit of resolution of the light microscope using visible light is approximately 0.2 μm.

Fixing and staining specimens

In standard light microscopy the specimen to be examined is usually first fixed with a solution containing alcohol or formaldehyde. These compounds denature proteins and, in the case of formaldehyde, introduce covalent cross-links between amino groups on adjacent molecules which stabilize protein–protein and protein–nucleic acid interactions. The **fixed specimen** may then be embedded in paraffin wax or a resin and cut into **thin sections** (0.5–10 μm thick) using a **microtome**. Each section is mounted on a glass slide and then positioned on the movable specimen stage of the microscope. The various subcellular constituents (nucleus, mitochondria, cytosol, etc.) absorb about the same degree of visible light, making it difficult to distinguish them under the light microscope without first staining the specimen. Many **chemical stains** bind to biological molecules; for example, **hematoxylin** binds to the basic amino acids arginine and lysine in proteins, and **eosin** binds to acidic molecules (such as DNA and the side-chains of the amino acids aspartate and glutamate). Another way of visualizing specific structures within cells is **cytochemical staining** in which an enzyme catalyzes the production of many molecules of a localized, colored reaction product from a colorless precursor. The colored product can then be seen in the light microscope wherever the enzyme is present. For example, peroxisomes can be visualized by using a cytochemical stain for catalase (see Topic A2).

Phase-contrast microscopy

When light passes through a living cell, the phase of the light wave is changed according to the **refractive index** of the cell: light passing through a relatively thick or dense part of the cell, such as the nucleus, is retarded; consequently its **phase** is shifted relative to light that has passed through an adjacent thinner region of the cytoplasm. Both **phase-contrast microscopy** and, in a more complex way, **differential interference contrast microscopy** (or Nomarski interference microscopy), exploit the interference effects produced when the two sets of light waves recombine, thereby creating an image of the cell's structure. As these types of microscopy do not require specimens to be fixed or stained they are useful for examining the structure and movement of larger organelles (nucleus, mitochondria, etc.) in living cells.

Fluorescence microscopy

In fluorescence microscopy, the light microscope is adapted to detect the light emitted by a **fluorescent compound** that is used to stain selectively components within the cell. A chemical is said to be fluorescent if it absorbs light at one wavelength (the **excitation wavelength**) and then emits light at a longer wavelength (the **emission wavelength**). Two commonly used compounds in fluorescent microscopy are **rhodamine** and **Texas red**, which emit red light, and **fluorescein**, which emits green light. First, an antibody against the antigen of interest (so-called **primary antibody**; see Topic D4) is added to the specimen. A fluorescent compound is chemically coupled to a **secondary antibody** that recognizes the primary antobody. Then the fluorescently-tagged secondary antibody is added to the tissue section or permeabilized cell, and the specimen is illuminated with light at the exciting wavelength (*Fig. 2*). The structures in the specimen to which the antibody has bound can then be visualized. Fluorescence microscopy can also be applied to living cells, which allows the movement of the cells and structures within them to be followed with time (see Topic E2 for an example of this).

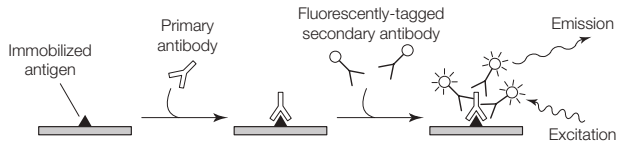

Fig. 2. Labeling of protein with a fluorescently-tagged antibody for fluorescent microscopy. The primary antibody recognizes the antigen of interest and binds to it in the specimen. Several molecules of the secondary antibody bind to the primary antibody providing amplification of the signal. The secondary antibody is covalently coupled to a fluorescent dye that emits light when illuminated at its excitation wavelength.

Confocal scanning microscopy

Confocal scanning microscopy is a refinement of normal fluorescence microscopy which produces clearer images of whole cells or larger specimens. In normal fluorescence microscopy, the fluorescent light emitted by the compound comes from molecules above and below the **plane of focus**, blurring the image and making it difficult to determine the actual three-dimensional molecular arrangement. With the confocal scanning microscope, only molecules in the plane of focus fluoresce due to the use of a focused **laser beam** at the exciting wavelength. The laser beam is moved to different parts of the specimen, allowing a series of images to be taken at different depths through the sample. The images are then combined by a computer to provide the complete three-dimensional image. **Deconvolution microscopy** achieves the same image-sharpening effect as confocal scanning microscopy but through a different process.

Green fluorescent protein

Visualization of proteins in living cells has been revolutionized by the discovery of a naturally fluorescent protein found in the **jellyfish** *Aquorea victoria*. In this 238 amino acid protein, called **green fluorescent protein** (GFP), certain amino acid side-chains have spontaneously cyclized to form a green-fluorescing chromophore. Using recombinant DNA techniques (see Topics I4 and I6), the DNA encoding GFP can be tagged on to the DNA sequences encoding other proteins, and then introduced into living cells in culture or into specific cells of a whole animal. Cells containing the introduced gene will then produce the **protein tagged with GFP** which will fluoresce green under the fluorescent microscope. The localization and movement of the GFP-tagged protein can then be studied in living cells in real time. Multiple variations of GFP have been engineered which emit light at different wavelengths, e.g. cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP), allowing several proteins to be visualized simultaneously in the same cell.

Fluorescence resonance energy transfer

Interactions between one protein and another can be monitored by **fluorescence resonance energy transfer** (FRET) (*Fig. 3*). The two proteins of interest are each labeled with a **different fluorochrome** (tagged with different variants of GFP, see above), chosen so that the emission spectrum of one fluorochrome overlaps with the excitation spectrum of the other. If the two proteins come into very **close proximity** (closer than 2 nm), the energy of the absorbed light can be transferred directly from one fluorochrome to the other. Thus, when the sample is illuminated at the excitation wavelength of the first fluorochrome, light is emitted at the emission wavelength of the second. If the two proteins fail to come into close proximity then no transfer of fluorescence occurs.

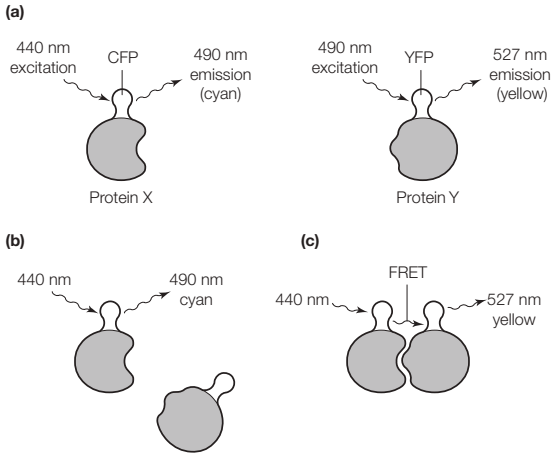

Fig. 3. Fluorescence resonance energy transfer (FRET). To determine whether two proteins interact inside the cell, the proteins are first tagged with two different variants of GFP. (a) In this example, protein X is coupled to cyan fluorescent protein (CFP), which is excited at 440 nm and emits blue light at 490 nm, while protein Y is coupled to yellow fluorescent protein (YFP), which is excited at 490 nm and emits yellow light at 527 nm. (b) If protein X and Y do not interact, illuminating the sample at 440 nm yields fluorescence at 490 nm from CFP only. (c) When protein X and Y interact, FRET now occurs. Illuminating the sample at 440 nm excites CFP, whose emission in turn excites YFP, resulting in the emission of yellow light at 527 nm.

Transmission electron microscopy

In contrast with light microscopy where optical lenses focus a beam of light, in electron microscopy **electromagnetic lenses** focus a beam of **electrons**. Because electrons are absorbed by atoms in the air, the specimen has to be mounted in a **vacuum** within an evacuated tube. The resolution of the electron microscope with biological materials is at best 0.10 nm. In **transmission electron microscopy**, a beam of electrons is directed through the specimen and electromagnetic lenses are used to focus the **transmitted electrons** to produce an image either on a viewing screen or on photographic film (*Fig. 4a*). As in standard light microscopy, thin sections of the specimen are viewed. However, for transmission electron microscopy the sections must be much thinner (50–100 nm thick). Since electrons pass uniformly through biological material, unstained specimens give very poor images. Therefore, the specimen must routinely be stained in order to scatter some of the incident electrons which are then not focused by the electromagnetic lenses and so do not form the image. **Heavy metals** such as gold and osmium are often used to stain biological materials. In particular **osmium tetroxide** preferentially stains certain cellular components, such as membranes, which appear black in the image. The transmission electron microscope has sufficiently high resolution that it can be used to obtain information about the shapes of purified proteins, viruses and subcellular organelles.

Antibodies can be tagged with electron-dense gold particles in a similar way to being tagged with a fluorescent compound in fluorescence microscopy, and then bound to specific target proteins in the thin sections of the specimen. When viewed in the electron microscope, small dark spots due to the gold particles are seen in the image wherever an antibody molecule has bound to its antigen (see Topic D4) and so the technique can be used to localize specific antigens.

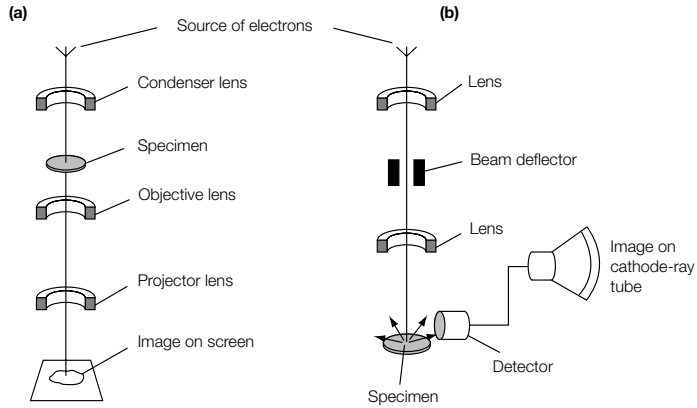

Fig. 4. Principal features of (a) a transmission electron microscope and (b) a scanning electron microscope.

Scanning electron microscopy

In **scanning electron microscopy**, an (unsectioned) specimen is fixed and then coated with a thin layer of a **heavy metal** such as **platinum**. An electron beam then **scans** over the specimen, exciting molecules within it that release secondary electrons. These secondary electrons are focused on to a scintillation detector and the resulting image displayed on a cathode-ray tube (*Fig. 4b*). The scanning electron microscope produces a **three-dimensional image** because the number of secondary electrons produced by any one point on the specimen depends on the angle of the electron beam in relation to the surface of the specimen. The resolution of the scanning electron microscope is 10 nm, some 100-fold less than that of the transmission electron microscope.

A5 CELLULAR FRACTIONATION

Key Notes

Isolating cells and their parts: overview

Animal and plant tissues contain a mixture of cell types, and most cells contain multiple subcellular organelles. In order to study cells and organelles in isolation, it is desirable to have a homogeneous population of cells.

Flow cytometry

Individual cells can be identified using a flow cytometer. Antibodies, coupled to fluorescent compounds, that bind to molecules on the surface of particular types of cells can be used to separate cells from each other in a fluorescence-activated cell sorter (FACS).

Cell culture

Cells can be grown in culture under appropriate conditions with defined growth medium. Primary cultures are prepared directly from tissues, whereas secondary cultures have been made to proliferate and will grow for weeks or months in culture.

Subcellular fractionation

Subcellular fractionation is the breaking open of a cell (e.g. by homogenization) and the separation of the various organelles from one another, usually by centrifugation.

Differential velocity centrifugation

Differential velocity centrifugation separates the subcellular organelles on the basis of their size. A centrifuge is used to generate powerful forces to separate the various organelles which pellet to the bottom of the centrifuge tube. At lower forces, nuclei, mitochondria, chloroplasts and lysosomes pellet, whereas higher forces are needed to pellet the endoplasmic reticulum, Golgi apparatus and plasma membrane.

Equilibrium density-gradient centrifugation

This procedure uses a gradient of a dense solution (e.g. sucrose solution) to separate out subcellular organelles on the basis of their density. An ultracentrifuge is used to sediment the organelles to an equilibrium position in the gradient where their density is equal to that of the sucrose.

Marker proteins

A convenient way of determining the purity of an organelle preparation is to measure the activity of a marker protein or enzyme in the various subcellular fractions. A marker protein is one that is found within only one particular compartment of the cell.

Related topics

Eukaryote cell structure (A2)
Bioimaging (A4)
Protein purification (B6)
Electrophoresis of proteins (B7)
Introduction to enzymes (C1)
Antibodies as tools (D4)

Isolating cells and their parts: overview

Most animal and plant tissues contain a mixture of cell types, and most cells contain multiple **subcellular organelles** (see Topic A2). Although microscopy techniques (see Topic A4) can be used to visualize organelles and large molecules

inside cells, many studies on cell structure and function require samples of a particular type of cell, subcellular organelle or components within them. Most biochemical procedures require obtaining large numbers of cells and then physically disrupting them to isolate their components. **Tissue samples** will often provide large quantities of material but will contain a heterogeneous mix of cells. Techniques have been developed whereby **homogeneous populations of cells** can be isolated, grown in culture to amplify them, and subsequently studied or fractionated into their component parts.

Flow cytometry

Different cells can be identified by measuring the light they scatter, or the fluorescence they emit, as they pass a laser beam in a **flow cytometer**. In a **fluorescence-activated cell sorter** or **FACS** (*Fig. 1*), an instrument based on flow cytometry, cells can be identified and separated from each other. The cells of

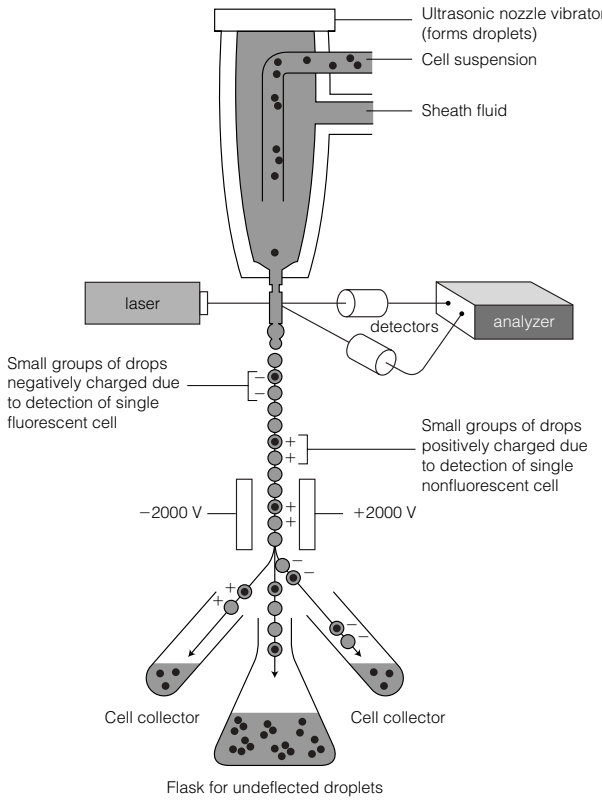

Fig. 1. A fluorescence-activated cell sorter. An antibody specific for a particular cell surface protein is linked to a fluorescent molecule and then added to a mixture of cells. When the individual cells pass through a laser beam they are monitored for fluorescence. Droplets containing single cells are given a positive or negative charge, depending on whether the cell has bound the fluorescently-tagged antibody or not. The droplets containing a single cell are then deflected by an electric field into collection tubes according to their charge.

interest are first labeled with an **antibody** which is specific for a particular cell surface molecule. The antibody is coupled to a fluorescent dye (see Topic A4), such that when the individual cells pass a laser beam in single file in a narrow stream, the fluorescence of each cell is measured. A vibrating nozzle then forms tiny droplets each containing a single cell which are given a positive or negative charge depending on whether the cell they contain is fluorescing. A strong electric field deflects the different charged droplets into separate containers so that each container eventually has a **homogeneous population of cells** with respect to the cell surface molecule tagged with fluorescent antibody. These homogeneous populations can then be used for biochemical analysis or grown in culture. The DNA and RNA content of a cell can also be measured by flow cytometry.

Cell culture

Isolated cells can be grown in a plastic **culture dish** under appropriate conditions with **defined growth medium**. Cultures prepared directly from the tissues of an organism are referred to as **primary cultures**, while cells that have been made to proliferate to form large numbers and which can be repeatedly subcultured for weeks or months are referred to as **secondary cultures**. Many cells in culture retain the differentiated properties appropriate to their origin. For example, fibroblasts continue to secrete collagen, and nerve cells extend axons. Cultured cells provide a large number of identical cells that can be used for a variety of cell biological and biochemical studies.

Subcellular fractionation

In order to study macromolecules and metabolic processes within cells, it is often helpful to isolate one type of **subcellular organelle** (see Topic A2) from the rest of the cell contents by subcellular fractionation. Initially, the plasma membrane (and cell wall if present) has to be ruptured. To do this, the tissue or cell sample is suspended in an isotonic sucrose solution (0.25–0.32 M) buffered at the appropriate pH, and the cells are then broken open by **homogenization** in a blender or homogenizer, by **sonication,** or by subjecting them to high pressures (**French press** or **nitrogen bomb**). The initial homogenization, and the following subcellular fractionation, are usually carried out at 4°C in order to minimize enzymic degradation of the cell's constituents. The sample of broken cells is often strained through muslin or other fine gauze to remove larger lumps of material before proceeding further.

Differential velocity centrifugation

In differential velocity centrifugation, the various subcellular organelles are separated from one another on the basis of their **size**. A **centrifuge** is used to generate powerful forces; up to 100 000 times the force of gravity (*g*). The homogenized sample is placed in an appropriate centrifuge tube which is then loaded in the **rotor** of the centrifuge and subjected to centrifugation (*Fig. 2a*). At first relatively low *g* forces are used for short periods of time but then increasingly higher *g* forces are used for longer time periods. For example, centrifugation at 600*g* for 3 min would pellet the **nuclei**, the largest organelles (*Fig. 2b*). The supernatant from this step is removed to a fresh tube and then centrifuged at 6000*g* for 8 min to pellet out **mitochondria**, **peroxisomes** and, if present, **lysosomes** or **chloroplasts**. Centrifugation of this next supernatant at 40 000*g* for 30 min will pellet out the **plasma membrane**, and fragments of the **endoplasmic reticulum** and **Golgi apparatus**. A final centrifugation at 100 000*g* for 90 min would result in a **ribosomal pellet** and a supernatant that is essentially free of particulate matter and is considered to be the true soluble **cytosolic fraction**.

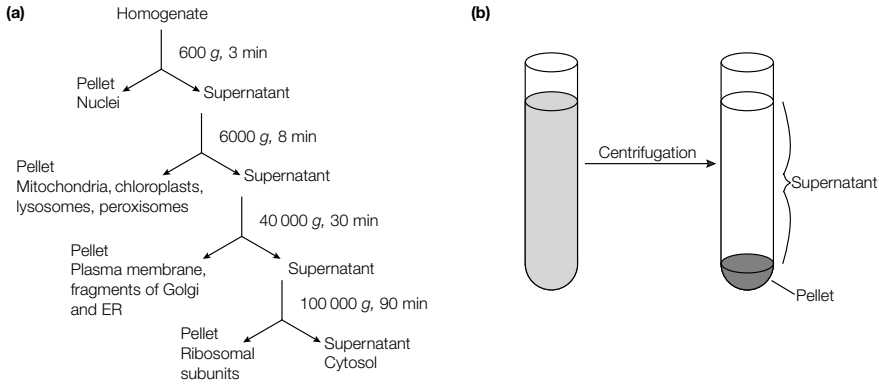

Fig. 2. Cell fractionation by differential velocity centrifugation. (a) Scheme for subcellular fractionation of a tissue sample, (b) appearance of a sample in the centrifuge tube before and after centrifugation.

However, the fractions isolated by differential velocity centrifugation are not usually entirely free of other subcellular organelles and so may need to be purified further. For separations at low *g* forces, a preparative centrifuge is used which has a rotor spinning in air at ambient pressure. However, an ultracentrifuge is required for separations at higher *g* forces. The chamber of the ultracentrifuge is kept in a high vacuum to reduce friction, and subsequent heating, which would otherwise occur between the spinning rotor and air.

Equilibrium density-gradient centrifugation

Equilibrium density-gradient centrifugation is often used to purify further organelles following their partial separation by differential velocity centrifugation. In this procedure the organelles are separated on the basis of their **density**, not their size. The impure organelle fraction is loaded at the top of a centrifuge tube that contains a gradient of a **dense solution** (e.g. a sucrose solution; *Fig. 3*). The sucrose solution is most concentrated (dense) at the bottom of the tube, and decreases in concentration (and density) towards the top of the tube. During centrifugation (e.g. 160 000*g* for 3 h) the various organelles move down the tube to an **equilibrium position** where their density is equal to that of the sucrose at that position. The **forces of sedimentation** tend to make the organelles move further down the tube but, if they do so, they enter a region of higher density

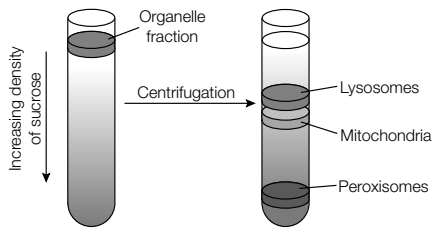

Fig. 3. Separation of organelles by equilibrium density-gradient centrifugation.

than the organelle density and so they float back to their previous position. Mitochondria, lysosomes and peroxisomes all differ in density and so can be effectively separated from one another by density-gradient centrifugation (*Fig. 3*). Similarly, the rough endoplasmic reticulum, Golgi apparatus and plasma membrane can be separated using a gradient of lower density. The more dense **cesium chloride** is used to make the density gradient for the separation of denser particles such as DNA, RNA and proteins by equilibrium centrifugation.

Marker proteins

When the cell sample has been fractionated, the purity of the different organelle preparations needs to be assessed. One way in which this can be done is by assessing **morphology** in the electron microscope (see Topic A4). A more readily available alternative though is to measure the activity of (to assay for) a particular **enzyme** (see Topic C1) which is characteristic of that organelle and is not found elsewhere in the cell. For example, **catalase** is a good marker enzyme for peroxisomes, **succinate dehydrogenase** for mitochondria, **cathepsin C** or **acid phosphatase** for lysosomes, and **alkaline phosphatase** for the plasma membrane. Thus, the presence of catalase in a fraction of lysosomes would indicate its contamination by peroxisomes. A good indication of the **purity**/degree of contamination of an organelle preparation can be ascertained by measuring the activity of such enzymes in the various isolated fractions. Alternatively, a marker protein can be detected following SDS PAGE (see Topic B7) and western blotting with a specific antibody (see Topic D4).