E1 MEMBRANE LIPIDS

Key Notes

Membranes

Membranes form boundaries around the cell and around distinct subcellular compartments. They act as selectively permeable barriers and are involved in signaling processes. All membranes contain varying amounts of lipid and protein and some contain small amounts of carbohydrate.

Membrane lipids

In membranes the three major classes of lipids are the glycerophospholipids, the sphingolipids and the sterols. The glycerophospholipids have a glycerol backbone that is attached to two fatty acid hydrocarbon chains and a phosphorylated headgroup. These include phosphatidate, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylserine. The sphingolipids are based on sphingosine to which a single fatty acid chain is attached and either a phosphorylated headgroup (sphingomyelin) or one or more sugar residues (cerebrosides and gangliosides, the glycosphingolipids). The major sterol in animal plasma membranes is cholesterol, while the structurally related stigmasterol and β-sitosterol are found in plants.

Fatty acid chains

The fatty acid chains of glycerophospholipids and sphingolipids consist of long chains of carbon atoms which are usually unbranched and have an even number of carbon atoms (e.g. palmitate C16, stearate C18). The chains are either fully saturated with hydrogen atoms or have one or more unsaturated double bonds that are in the *cis* configuration (e.g. oleate C18:1 with one double bond).

Lipid bilayer

Membrane lipids are amphipathic since they contain both hydrophilic and hydrophobic regions. In the glycerophospholipids and the sphingolipids the fatty acid hydrocarbon chains are hydrophobic whereas the polar headgroups are hydrophilic. In cholesterol the entire molecule except for the hydroxyl group on carbon-3 is hydrophobic. In aqueous solution the amphipathic lipids arrange themselves into either micelles or more extensive bimolecular sheets (bilayers) in order to prevent the hydrophobic regions from coming into contact with the surrounding water molecules. The structure of the bilayer is maintained by multiple noncovalent interactions between neighboring fatty acid chains and between the polar headgroups of the lipids. In biological membranes there is an asymmetrical distribution of lipids between the inner and outer leaflets of the bilayer.

Membrane fluidity

Lipids are relatively free to move within the plane of the bilayer by either rotational or lateral motion, but do not readily flip from one side of the bilayer to the other (transverse motion). Increasing the length of the fatty acid chains or decreasing the number of unsaturated double bonds in the fatty acid chains leads to a decrease in the fluidity of the membrane. In animal membranes, increasing the amount of cholesterol also decreases the fluidity of the membrane.

Fluid mosaic model of membrane structure

The fluid mosaic model describes the structure of biological membranes, in which the membranes are considered as two-dimensional solutions of orientated lipids and globular proteins.

Lipid domains

Within biological membranes lipids and proteins cluster together in discrete domains. Lipid rafts are domains of the plasma membrane that are enriched in cholesterol, sphingomyelin and glycosphingolipids, as well as lipid modified proteins.

Related topics

Prokaryote cell structure (A1)
Eukaryote cell structure (A2)
Protein structure (B3)
Membrane proteins and carbohydrate (E2)
Transport of small molecules (E3)
Signal transduction (E5)
Nerve function (E6)
Structures and roles of fatty acids (K1)
Fatty acid breakdown (K2)
Triacylglycerols (K4)
Cholesterol (K5)
Electron transport and oxidative phosphorylation (L2)

Membranes

Membranes form boundaries both around the cell (the plasma membrane) and around distinct subcellular compartments (e.g. nucleus, mitochondria, lysosomes, etc.) (see Topics A1 and A2). They act as **selectively permeable barriers** allowing the inside environment of the cell or organelle to differ from that outside (see Topic E3). Membranes are involved in **signaling processes**; they contain specific receptors for external stimuli and are involved in both chemical and electrical signal generation (see Topics E5 and E6). All membranes contain two basic components: **lipids** and **proteins**. Some membranes also contain **carbohydrate**. The composition of lipid, protein and carbohydrate varies from one membrane to another. For example, the inner mitochondrial membrane has a larger amount of protein than lipid due to the presence of numerous protein complexes involved in oxidative phosphorylation and electron transfer (Topic L2), whereas the myelin sheath membrane of nerve cells, which serves to insulate the cell electrically, has a larger proportion of lipid (Topic E6).

Membrane lipids

Lipids were originally classified as biological substances that were insoluble in water but soluble in organic solvents such as chloroform and methanol. In addition to being structural components of membranes, lipids have several other biological roles. They serve as fuel molecules (Topic K2), as concentrated energy stores (e.g. triacylglycerol) (Topic K4) and as signaling molecules (Topic E5). Within membranes there are three major types of lipid: the **glycerophospholipids**, the **sphingolipids** and the **sterols**.

Glycerophospholipids

The glycerophospholipids are made up of three components: a **phosphorylated headgroup**, a three-carbon **glycerol backbone** and two **hydrocarbon fatty acid chains** (*Fig. 1*). The phosphorylated headgroup is attached to carbon-3 of the glycerol backbone, while the two fatty acid chains are attached to the other two carbon atoms. The simplest glycerophospholipid is phosphatidate (diacylglycerol 3-phosphate) which has only a phosphoric acid group esterified to carbon-3 of the glycerol. Although phosphatidate itself is present in small amounts in

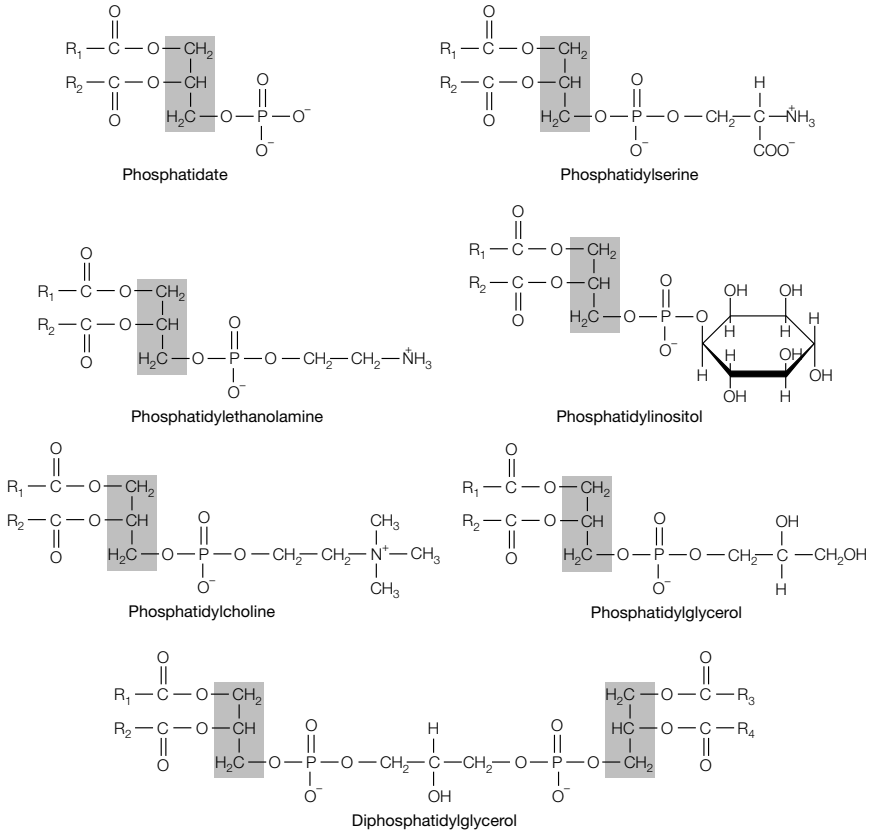

Fig. 1. Structures of membrane glycerophospholipids. R_1 and R_2 represent hydrocarbon chains of fatty acids.

membranes, the major glycerophospholipids are derived from it. In these other lipids the phosphate is further esterified to the hydroxyl group of one of several alcohols (choline, ethanolamine, glycerol, inositol or serine). The major glycerophospholipids found in membranes include phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylserine (*Fig. 1*). Diphosphatidylglycerol (or cardiolipin) is found predominantly in the inner mitochondrial membrane.

Sphingolipids

Sphingomyelins, the commonest sphingolipids, have a **sphingosine backbone** (*Fig. 2a*) in place of the glycerol in glycerophospholipids. Like the glycerophospholipids, they also have a phosphorylated headgroup (either choline or ethanolamine) and two hydrocarbon chains (*Fig. 2a*). One of the hydrocarbon chains comes from the sphingosine molecule, the other is a fatty acid as found in the glycerophospholipids except that it is bonded via an amide bond in sphingolipids. The sphingomyelins are present in the plasma membrane of most cells

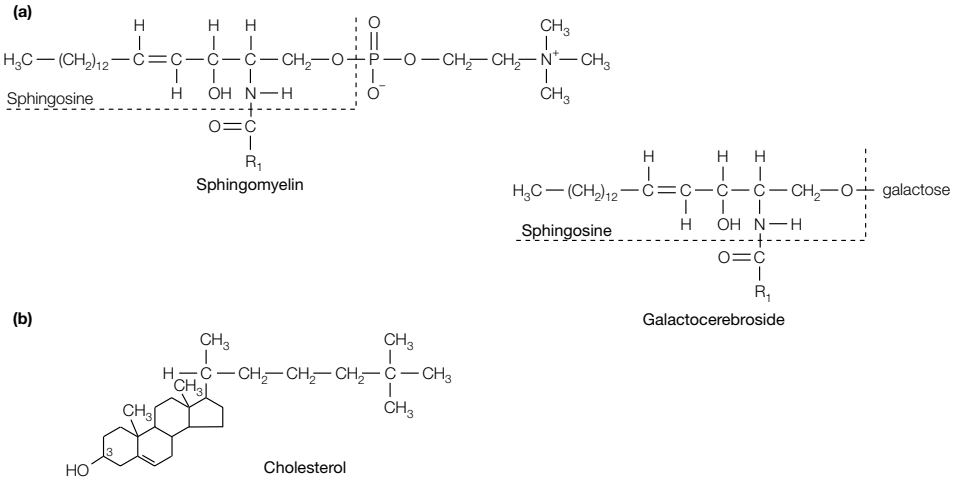

Fig. 2. Structures of (a) the sphingolipids sphingomyelin and galactocerebroside; (b) cholesterol. R_1 represents the hydrocarbon chain of fatty acids.

and are particularly abundant in the myelin sheath that surrounds nerve cells. The **glycosphingolipids**, such as the **cerebrosides** and **gangliosides**, are also derived from sphingosine, but in place of the phosphorylated headgroup they have one or more sugar residues. The galactocerebrosides have a single galactose residue (*Fig. 2a*) and are found predominantly in the neuronal cell membranes of the brain. The gangliosides have several sugar residues including at least one sialic acid (*N*-acetylneuraminic acid) residue and are a major constituent of most mammalian plasma membranes, being particularly abundant in brain cells. The lipid composition of the chloroplast thylakoid membranes in plant cells (see Topic A2) is highly distinctive: about 40% of the total lipids are **galactolipids** and 4% **sulfolipids**, with only 10% being phospholipids.

Sterols

The sterol **cholesterol** (*Fig. 2b*) is a major constituent of animal plasma membranes but is absent from prokaryotes. The fused ring system of cholesterol means that it is more rigid than other membrane lipids. As well as being an important component of membranes, cholesterol is the metabolic precursor of the steroid hormones (see Topic K5). Plants contain little cholesterol but have instead a number of other sterols, mainly **stigmasterol** and **β-sitosterol** which differ from cholesterol only in their aliphatic side-chains.

Fatty acid chains

The two fatty acid chains of glycerophospholipids and the single fatty acid chain and the hydrocarbon chain of the sphingosine in sphingolipids consist of long chains of carbon atoms. Usually these chains have an even number of carbon atoms (e.g. palmitate, C16; stearate, C18) and are unbranched. The chains can either be fully saturated with hydrogen atoms or unsaturated and have one or more double bonds that are usually in the *cis* configuration (e.g. oleate C18:1

which has 18 carbon atoms and one double bond; arachidonic acid C20:4 which has 20 carbon atoms and four double bonds) (for more details see Topic K1). The two fatty acid chains on a glycerophospholipid are usually not identical [e.g. 1-stearoyl-2-oleoyl-3-phosphatidylcholine (*Fig. 3*)].

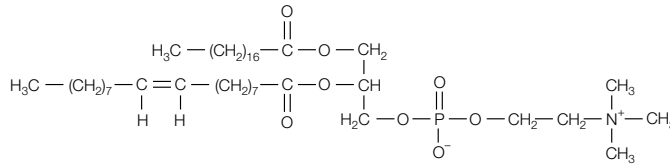

Fig. 3. Structure of 1-stearoyl-2-oleoyl-3-phosphatidylcholine showing the saturated stearoyl (C18:0) and the mono-unsaturated oleoyl (C18:1) hydrocarbon chains. The hydrogen atoms surrounding the C=C bond are in the cis configuration.

Lipid bilayer

Amphipathic (or amphiphilic) molecules contain both **hydrophilic** (water-loving) and **hydrophobic** (water-hating) regions. Membrane lipids are amphipathic molecules as they are made up of hydrophobic fatty acid chains and a hydrophilic polar headgroup. In the glycerophospholipids, the two hydrocarbon chains are hydrophobic whereas the glycerol backbone and the phosphorylated headgroup are hydrophilic. In the sphingolipids, the fatty acid chain and the hydrocarbon chain of the sphingosine are hydrophobic whereas the phosphorylated or sugar headgroup is hydrophilic. In the case of cholesterol, the entire molecule apart from the hydroxyl group on carbon-3 is hydrophobic in nature.

In aqueous solution, amphipathic molecules will orientate themselves in such a way as to prevent the hydrophobic region coming into contact with the water molecules. In the case of those fatty acid salts which contain only one fatty acid chain (such as sodium palmitate, a constituent of soap), the molecules form a spherical micellar structure (diameter usually < 20 nm) in which the hydrophobic fatty acid chains are hidden inside the **micelle** and the hydrophilic headgroups interact with the surrounding water molecules (*Fig. 4a*). Because the two fatty acid chains of phospholipids are too bulky to fit into the interior of a micelle, the favored structure for most phospholipids in aqueous solution is a two-dimensional bimolecular sheet or **lipid bilayer** (*Fig. 4b*). Such lipid bilayers, in which the phospholipid molecules are orientated with their hydrophobic chains in the interior of the structure and their hydrophilic headgroups on the surfaces, can be relatively large structures of up to about 1 mm^2 in area. The two layers of lipids in the bilayer are referred to as the **inner and outer leaflets**. In biological membranes the individual lipid species are asymmetrically

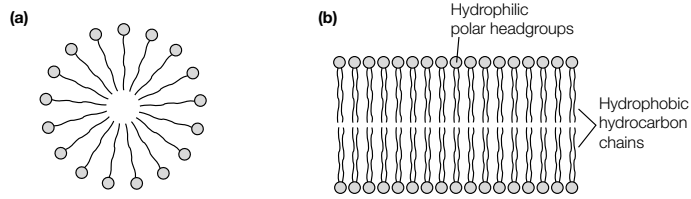

Fig. 4. Structure of (a) a micelle and (b) a lipid bilayer.

distributed between the two leaflets. For example, in the plasma membrane of erythrocytes, sphingomyelin and phosphatidylcholine are preferentially located in the outer leaflet, whereas phosphatidylethanolamine and phosphatidylserine are mainly in the inner leaflet.

Lipid bilayers will spontaneously self-assemble in aqueous solution. The major driving force behind this is the **hydrophobic effect** – the hydrophobic fatty acid chains avoid coming into contact with the water molecules. Once formed, the bilayer structure is maintained by multiple noncovalent interactions including hydrophobic interactions and van der Waals forces between the hydrocarbon chains, charge interactions and hydrogen bonding between the polar headgroups, and hydrogen bonding between the headgroups and the surrounding water molecules (see Topic B3 for a fuller description of these noncovalent interactions).

Membrane fluidity

Because there are no covalent bonds between the lipids in the bilayer, the membrane is not a static structure but has **fluidity**. The lipids are generally free to move within the plane of the inner or outer leaflet of the bilayer by either **rotational** or **lateral movement** (*Fig. 5*). However, they cannot readily flip from one leaflet of the bilayer to the other, so-called **transverse movement**, due to the unfavorable energetics involved in moving a hydrophilic headgroup through the hydrophobic interior of the bilayer. The fluidity of the bilayer can be altered in a number of ways. Upon heating above a characteristic **transition temperature**, the lipid bilayer will change from a gel-like consistency to a more fluid-like consistency. This transition temperature depends on the length of the fatty acid chains and on their degree of unsaturation. If the length of the fatty acid chains is increased, the fluidity of the bilayer will decrease due to the larger propensity for noncovalent interactions between the hydrocarbon chains. In contrast, if the degree of unsaturation in the fatty acid chains is increased, the fluidity of the bilayer will increase. This is because the double bonds which are in the *cis* configuration kink the hydrocarbon chain and disrupt the highly ordered packing of the fatty acid chains, thus reducing the number of interactions between neighboring lipids. An important regulator of membrane fluidity in mammalian systems is **cholesterol**. At physiological temperature (37°C), increasing the amount of cholesterol in the bilayer will lead to a decrease in the fluidity of the membrane since the rigid steroid ring system interferes with the lateral movement of the fatty acid chains.

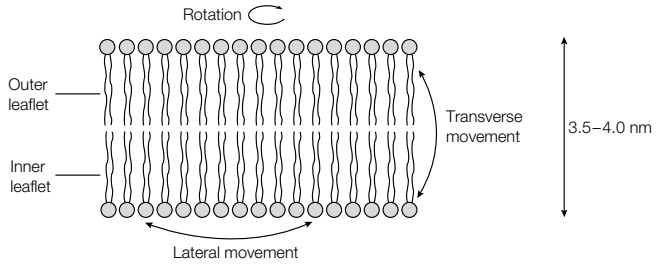

Fig. 5. Movement of lipids in membranes.

Fluid mosaic model of membrane structure

In 1972 S. Jonathan Singer and Garth Nicholson proposed the **fluid mosaic model** for the overall structure of biological membranes, in which the membranes can be viewed as two-dimensional solutions of orientated lipids and globular proteins (*Fig. 6*). The integral membrane proteins can be considered as 'icebergs' floating in a two-dimensional lipid 'sea'. They proposed that the bilayer organization of the lipids would act both as a solvent for the amphipathic integral membrane proteins and as a **permeability barrier**. They also proposed that some lipids may interact with certain membrane proteins, that these interactions would be essential for the normal functioning of the protein, and that membrane proteins would be free to diffuse laterally in the plane of the bilayer unless restricted in some way, but would not be able to flip from one side of the bilayer to the other. This model is now supported by a wide variety of experimental observations (see Topic E2).

Lipid domains

Biological membranes are not just homogeneous mixtures of lipids and proteins. Within them are discrete **domains** in which certain lipids and proteins cluster together to form structural and functional units. For example, the clustering together of **cholesterol, sphingomyelin and glycosphingolipids** in the outer leaflet of the bilayer, with other lipids in the inner leaflet gives rise to **lipid rafts**. Various lipid modified proteins (see Topic E2) can associate with lipid rafts and these structures have been implicated in various cellular processes, including **cell signaling** (see Topic E5) and the transport of proteins and lipids from the Golgi to the plasma membrane.

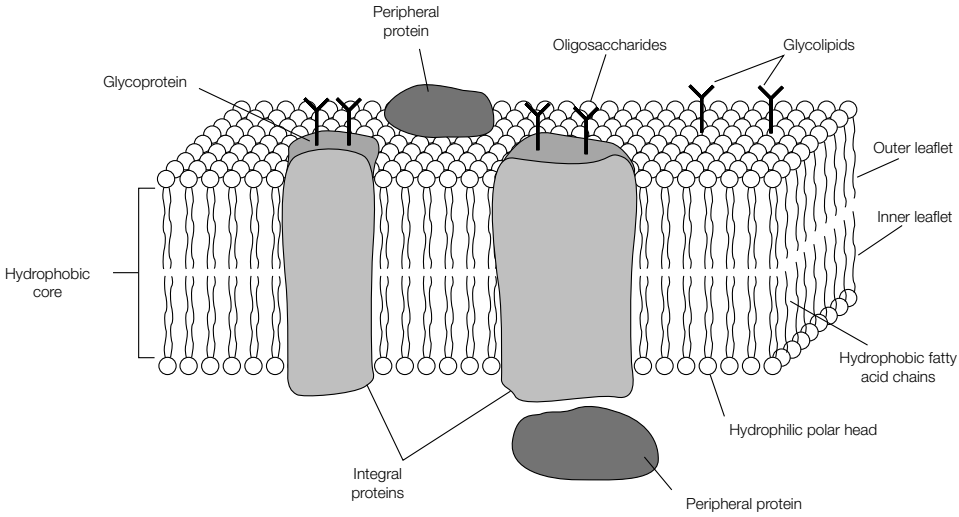

Fig. 6. The fluid mosaic model of membrane structure.

E2 Membrane proteins and carbohydrate

Key Notes

Integral membrane proteins

Integral (intrinsic) membrane proteins are tightly associated with the hydrophobic core of the lipid bilayer and can be removed from it only with organic solvents or detergents that disrupt the membrane structure. Most integral proteins have one (e.g. glycophorin) or more (e.g. bacteriorhodopsin) regions of the polypeptide chain that traverse the lipid bilayer. These regions consist mainly of amino acids with hydrophobic side-chains that fold into an α-helix and interact noncovalently with the surrounding lipids. Some integral proteins do not traverse the membrane but are attached covalently to a lipid that interacts with the hydrophobic interior of the bilayer. Integral proteins are asymmetrically distributed across the membrane and are usually free to move in the plane of the bilayer by lateral and rotational movement, but are not able to flip from one side of the membrane to the other (transverse movement).

Membrane protein purification and reconstitution

The first step in the purification of integral proteins is the disruption of the membrane structure by solubilizing it with a detergent (e.g. Triton X-100 or octyl glucoside). Once solubilized, the hydrophobic region of the protein is coated with a layer of amphipathic detergent molecules, allowing the protein to remain in aqueous solution and be purified as for a soluble globular protein. Once purified, integral proteins can be reincorporated into artificial lipid vesicles (liposomes) in order to study their function.

Peripheral membrane proteins

Peripheral membrane proteins are only loosely bound to the membrane and can readily be removed by washing the membranes with a solution of either high ionic strength or high pH. These treatments disrupt the noncovalent ionic and hydrogen bonds holding the peripheral proteins on the surface of the membrane. No part of a peripheral protein interacts with the hydrophobic interior of the bilayer. The cytoskeleton that covers the cytosolic surface of the plasma membrane is made up of a number of peripheral proteins and is important in maintaining and altering the shape of the cell.

Membrane carbohydrate

Sugar residues are found only on the extracellular side of the plasma membrane attached either to lipids to form glycolipids or to proteins to form glycoproteins. The carbohydrate forms a protective coat on the outer surface of the cell and is involved in intercellular recognition.

Related topics

Cytoskeleton and molecular motors (A3)
Bioimaging (A4)
Protein structure (B3)
Myoglobin and hemoglobin (B4)
Protein purification (B6)
Protein sequencing and peptide synthesis (B8)
Membrane lipids (E1)
Signal transduction (E5)
Protein targeting (E4)
Protein glycosylation (H5)
Cholesterol (K5)

Integral membrane proteins

Membrane proteins are classified as either **peripheral** (extrinsic) or **integral** (intrinsic) depending on how tightly they are associated with the membrane. Integral membrane proteins are tightly bound to the membrane through interactions with the hydrophobic core of the bilayer (see Topic E1; *Fig. 5*) and can be extracted from them only by using agents that disrupt the membrane structure, such as **organic solvents** (e.g. chloroform) or **detergents** (see below). Most integral proteins have one or more regions of the polypeptide chain that span the lipid bilayer and interact noncovalently with the hydrophobic fatty acid chains. However, some are anchored in the membrane by a covalently attached fatty acid or hydrocarbon chain (see below). Like lipids, integral proteins are **amphipathic**, having both hydrophobic and hydrophilic regions, and are **asymmetrically distributed** across the bilayer (see Topic E1).

Glycophorin

Because **erythrocytes** (red blood cells) do not contain any subcellular organelles (they are essentially a membranous sac for carrying hemoglobin; see Topic B4) their plasma membrane is a convenient model system for studies of membrane structure as it can readily be isolated from other membranes and intracellular components. One of the major glycoproteins in the plasma membrane of erythrocytes is **glycophorin A**; a 131 amino acid protein that was the first integral protein to be sequenced (see Topic B8). This revealed that the polypeptide chain of glycophorin consists of three domains:

1. An N-terminal region on the extracellular side of the membrane that contains all the N- and O-linked **glycosylation sites**;
2. A hydrophobic central region that is buried in the hydrophobic core of the bilayer; and
3. A C-terminal region rich in polar and charged residues that is exposed on the cytosolic side of the membrane (*Fig. 1a*).

As with the majority of transmembrane proteins, the hydrophobic membrane-spanning region consists mainly of amino acid residues with hydrophobic side-chains that are folded in an **α-helical conformation** (see Topic B3). As each amino acid residue adds 0.15 nm to the length of an α-helix, a helix of 25 residues would have a length of 3.75 nm, just enough to span the hydrophobic core of the bilayer. The hydrophobic side-chains of the residues in the helix protrude outwards from the helix axis to interact via hydrophobic bonds with the fatty acid chains. Either side of this hydrophobic α-helix are clusters of amino acids with charged side-chains which interact noncovalently with opposite charges on the polar headgroups of the membrane lipids.

Multiple membrane-spanning proteins

Some integral proteins have **multiple membrane-spanning α-helices**. **Bacteriorhodopsin**, a protein found in a photosynthetic bacterium, captures energy from light and uses it to pump protons across the bacterial membrane. Like numerous other integral proteins, such as the **G protein-coupled receptors** (see Topic E5), the polypeptide chain of bacteriorhodopsin loops backwards and forwards across the lipid bilayer seven times (*Fig. 1b*). Each of the **seven transmembrane α-helices** is linked to the next by a short hydrophilic region of the polypeptide chain that is exposed either on the extracellular or cytosolic side of the membrane. Other multiple membrane spanning proteins have from two to up to 14 transmembrane helices. For example, the **anion exchange band 3 protein** of

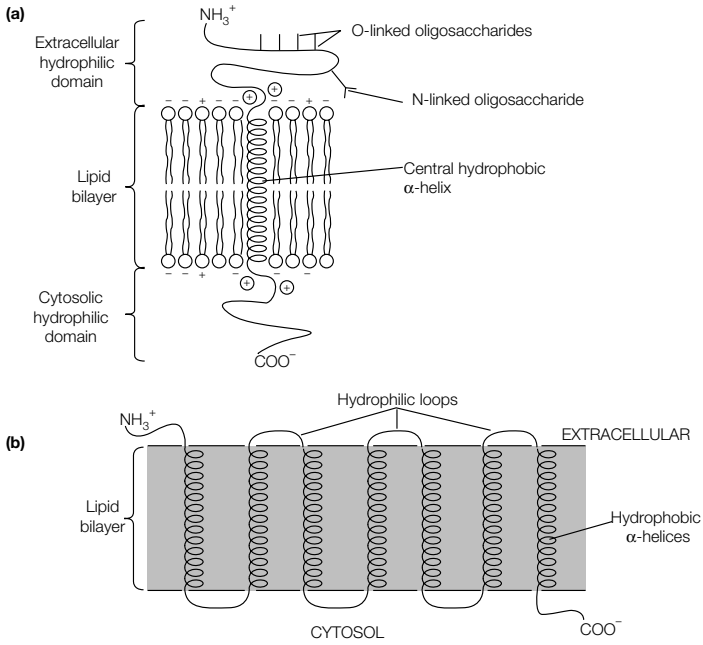

Fig. 1. Integral membrane proteins. (a) Single membrane-spanning region (e.g. glycophorin); (b) multiple membrane-spanning regions (e.g. bacteriorhodopsin).

the erythrocyte plasma membrane that transports Cl^- and HCO_3^- across the membrane loops backwards and forwards across the lipid bilayer up to 14 times.

Lipid-anchored proteins

A large number of integral proteins in eukaryotes do not traverse the membrane but are anchored in one or other leaflet of the bilayer through covalent attachment to a hydrocarbon chain. Several proteins, including the **prion protein** (the causative agent of mad cow disease), are stably anchored at the cell surface through covalent linkage of their C-terminal amino acid to the headgroup of a phosphatidylinositol lipid via an ethanolamine–phosphate–trimannose–glucosamine bridge, so-called **glycosyl phosphatidylinositol (GPI)-anchored** proteins (*Fig. 2a*). This complex structure is built up by sequential addition of the individual sugar residues and ethanolamine phosphate to phosphatidylinositol. A C-terminal hydrophobic signal peptide is removed from the protein in the lumen of the RER and the preformed GPI anchor added to the newly exposed C-terminal amino acid (see Topic H4 for details).

Other proteins are transiently attached to the cytosolic face of the membrane either by amide linkage of a myristate (C14:0) molecule to an N-terminal Gly residue (**myristoylated** proteins; *Fig. 2b*), or by thioether linkage of a 15-carbon **farnesyl** or a 20-carbon **geranylgeranyl** polyunsaturated hydrocarbon to a C-terminal Cys residue (**prenylated** proteins; *Fig. 2c*). Farnesyl and geranylgeranyl are synthesized from isopentenyl pyrophosphate, the precursor of cholesterol

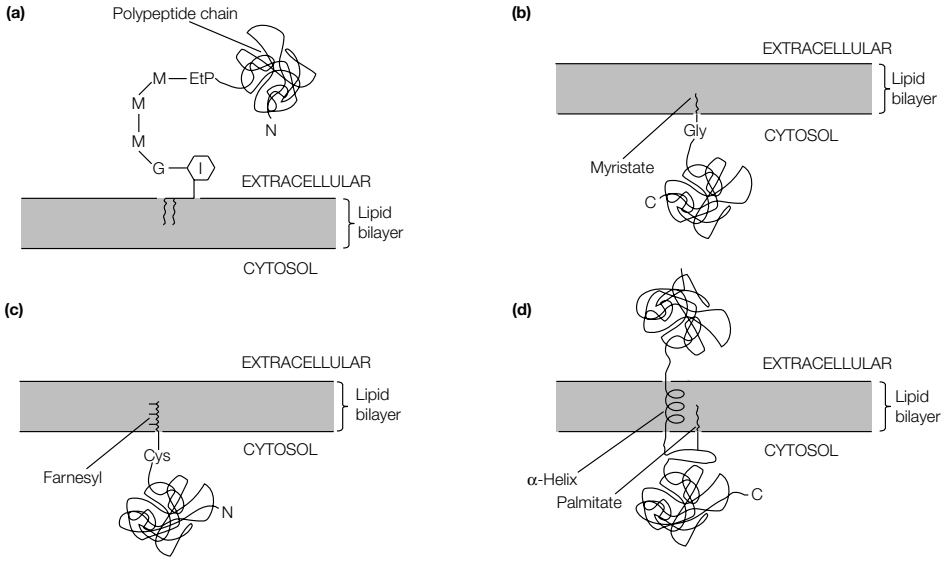

Fig. 2. Lipid-modified proteins. (a) A glycosyl phosphatidylinositol-anchored protein (G, glucosamine; M, mannose; EtP, ethanolamine phosphate); (b) a myristoylated protein; (c) a prenylated protein; (d) a palmitoylated protein.

(see Topic K5). Some proteins are also modified on Cys residues with covalently attached palmitate (C16:0) (**palmitoylated** proteins). These include some with membrane-spanning polypeptides (*Fig. 2d*), some prenylated proteins and some myristoylated proteins. Many of the proteins involved in cell signaling, such as the G proteins and the Ras family of proteins are lipid modified (see Topic E5).

Integral membrane protein movement and distribution

Many proteins are free to **move laterally** in the plane of the bilayer. One experiment used to show this involved fusing cultured mouse cells with human cells under appropriate conditions to form a hybrid cell known as a **heterokaryon** (*Fig. 3a*). The mouse cells were labeled with mouse protein-specific antibodies to which the green-fluorescing dye **fluorescein** had been covalently attached, whilst the human cells were labeled with the red-fluorescing dye **rhodamine** (see Topic A4). Upon cell fusion, the mouse and human proteins as seen under the **fluorescence microscope** (see Topic A4) were segregated on the two halves of the heterokaryon (*Fig. 3a*). After 40 min at 37°C, however, the mouse and human proteins had completely intermingled. Lowering the temperature to below 15°C inhibited this process, indicating that the proteins are free to diffuse laterally in the membrane and that this movement is slowed as the temperature is lowered. It should be noted, though, that some integral membrane proteins are not free to move laterally in the membrane because they interact with the cytoskeleton inside the cell (see below).

The distribution of proteins in membranes can be revealed by electron microscopy using the **freeze–fracture technique** (*Fig. 3b*). In this technique, a membrane specimen is rapidly frozen to the temperature of liquid nitrogen

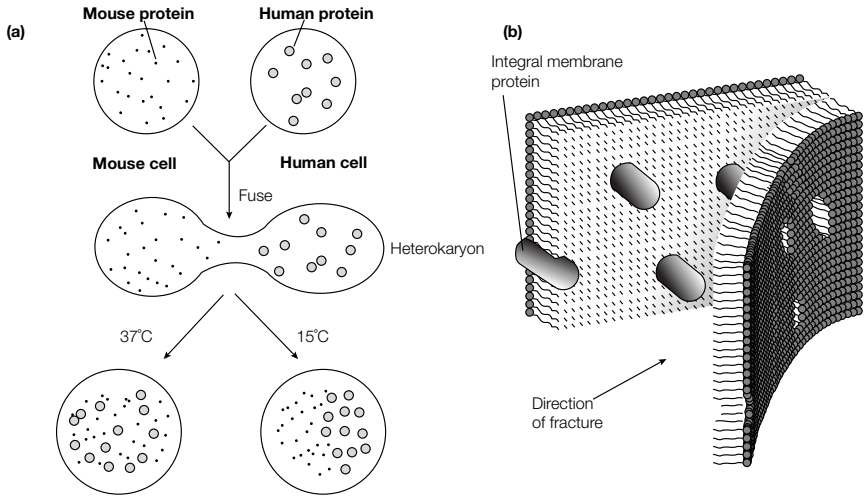

Fig. 3. (a) Movement and (b) distribution (as shown by freeze–fracture electron microscopy) of integral membrane proteins.

(–196°C) and then fractured by a sharp blow. The bilayer often splits into monolayers, revealing the interior. The exposed surface is then coated with a film of carbon and shadowed with platinum in order for the surface to be viewed in the electron microscope (see Topic A4). The fractured surface of the membrane is revealed to have numerous randomly distributed protuberances that correspond to integral membrane proteins.

Membrane protein purification and reconstitution

The first step in the **purification** of an integral membrane protein is to disrupt its interactions with other integral proteins and the lipids in the membrane. This is commonly achieved by adding a **detergent** which solubilizes the membrane. In order to solubilize the membrane but not denature the protein, gentle detergents such as Triton X-100 or octyl glucoside are used (*Fig. 4*), rather than the harsh detergent SDS. As the detergent molecules are themselves amphipathic they readily intercalate into the lipid bilayer and disrupt the hydrophobic interactions.

Once solubilized, the hydrophobic region of the integral protein is coated with a layer of detergent molecules which enables the protein to remain in solution (*Fig. 5a*). The solubilized protein can then be purified as for a water-soluble

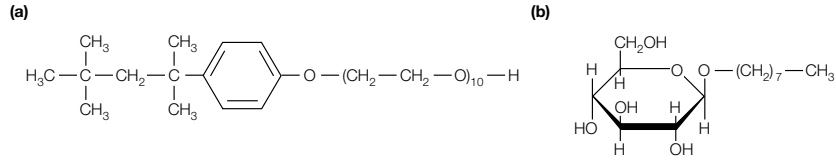

Fig. 4. Structures of (a) Triton X-100 and (b) octyl glucoside.

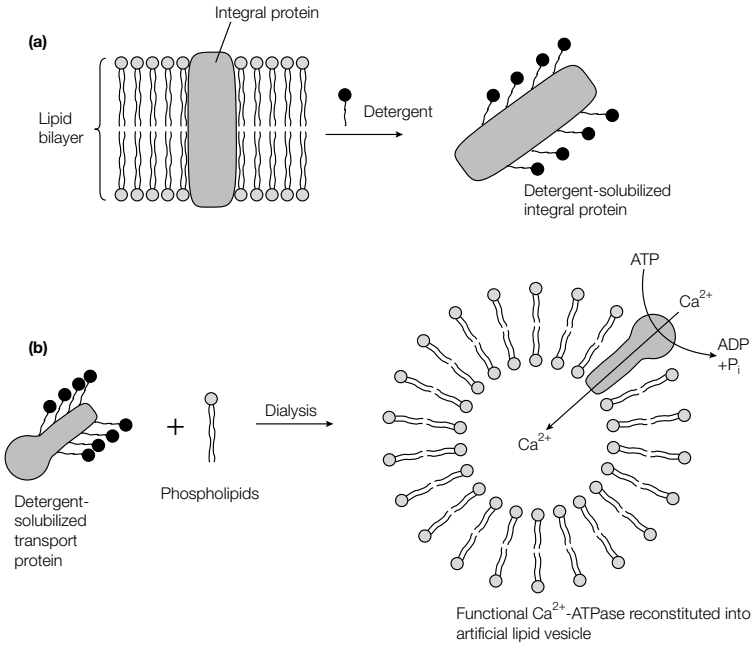

Fig. 5. (a) Detergent solubilization and (b) reconstitution into artificial lipid vesicles of an integral membrane protein.

globular protein (see Topic B6) as long as detergent is kept in the buffers to prevent aggregation and loss of the protein. Once purified, an integral protein can be reincorporated into artificial lipid vesicles (**liposomes**) in order to study its function (*Fig. 5b*). If phospholipids are added to the protein in detergent solution and the detergent dialyzed away, phospholipid vesicles containing the protein will spontaneously form. The function of the protein can then be studied. For example, if the **Ca^{2+}-ATPase** is reincorporated into lipid vesicles, its function (i.e. transport of Ca^{2+} upon ATP hydrolysis) can be studied by monitoring Ca^{2+} on the inside of the vesicle upon addition of Ca^{2+} and ATP to the outside (*Fig. 5b*).

Peripheral membrane proteins

Peripheral membrane proteins are less tightly bound to the lipid bilayer than integral membrane proteins and can be readily removed by washing the membranes with a solution of **high ionic strength** (e.g. 1 M NaCl) or **high pH**. These procedures leave the lipid bilayer intact but disrupt the ionic and hydrogen bond interactions that hold the peripheral proteins on the surface of the membrane. No part of a peripheral protein interacts with the hydrophobic core of the bilayer. Peripheral membrane proteins can be found either on the outer or the inner surface of the bilayer and can be associated with the membrane through noncovalent interactions with either the lipid headgroups and/or other proteins in the membrane (see Topic E1; *Fig. 5*). Once removed from the membrane, peripheral proteins behave as water-soluble globular proteins and can be purified as such (see Topic B6).

Cytoskeleton

The cytosolic surface of the erythrocyte plasma membrane is covered by a network of peripheral membrane proteins that make up the **cytoskeleton** (*Fig. 6*) (see Topic A3). The major component of this cytoskeleton is **spectrin** which folds into a triple-stranded **α-helical coiled coil** to form long chains. The spectrin chains are attached to the plasma membrane through interactions with two other peripheral proteins, **ankyrin** and **protein band 4.1**. Ankyrin forms a cross-link between spectrin and the cytosolic domain of the integral anion exchanger band 3 protein, while band 4.1 promotes the binding of **actin filaments** (see Topic A3) to the spectrin chains linking them to the cytosolic domain of glycophorin. The cytoskeleton gives the erythrocyte plasma membrane great strength and flexibility, and is important in maintaining and altering the shape of the cell. In other mammalian cells the cytoskeleton has similar functions but consists of numerous other proteins and criss-crosses throughout the cytoplasm (see Topic A3).

Membrane carbohydrate

The extracellular surface of the plasma membrane is often covered with a protective coat of **carbohydrate**. The sugar residues of this carbohydrate coat can be attached either to certain lipids such as the **glycosphingolipids** (see Topic E1), or to the polypeptide chains of peripheral or integral membrane proteins. These **glycolipids** and **glycoproteins** are abundant in the plasma membrane of eukaryotic cells but are virtually absent from most intracellular membranes, particularly the inner mitochondrial membrane and the chloroplast lamellae. In glycoproteins the sugar residues can be attached to the polypeptide chain either through the hydroxyl group in the side-chain of Ser or Thr residues as **O-linked oligosaccharides**, or through the amide group in the side-chain of Asn as **N-linked oligosaccharides** (see Topic H5). The carbohydrate on the extracellular face of the membrane not only serves a **protective role** but is also involved in **intercellular recognition** and in maintaining the asymmetry of the membrane.

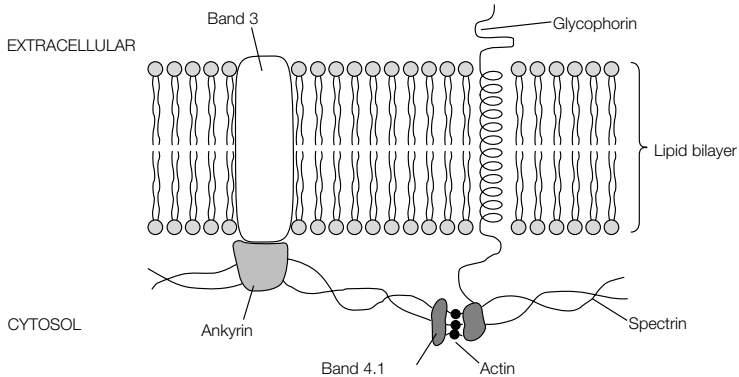

Fig. 6. The erythrocyte cytoskeleton.

E3 TRANSPORT OF SMALL MOLECULES

Key Notes

Membrane permeability

The plasma membrane is a selectively permeable barrier. Some molecules (water, gases, urea) can pass directly through the bilayer unaided, whereas other molecules (sugars, amino acids, ions, etc.) require the presence of integral membrane transport proteins.

Passive transport

The movement of molecules across a membrane by passive transport does not require an input of metabolic energy. The molecule moves from a high concentration to a lower concentration. Passive transport by simple diffusion does not require the presence of integral membrane proteins. The rate of movement of a molecule (e.g. water, gases, urea) by simple diffusion is directly proportional to its concentration gradient across the membrane. Passive transport by facilitated diffusion requires the presence of specific integral membrane proteins to facilitate the movement of the molecule (e.g. glucose, other sugars, amino acids) across the membrane. The transport protein (e.g. the erythrocyte glucose transporter) is specific for a particular molecule, is saturable, displays binding kinetics, and is influenced by the temperature, pH and inhibitors.

Active transport

Active transport of a molecule across a membrane against its concentration gradient requires an input of metabolic energy. In the case of ATP-driven active transport, the energy required for the transport of the molecule (Na^+, K^+, Ca^{2+} or H^+) across the membrane is derived from the coupled hydrolysis of ATP (e.g Na^+/K^+-ATPase). In ion-driven active transport, the movement of the molecule to be transported across the membrane is coupled to the movement of an ion (e.g. Na^+, H^+) down its concentration gradient. If both the molecule to be transported and the ion move in the same direction across the membrane, the process is called symport (e.g. Na^+/glucose transporter); if the molecule and the ion move in opposite directions it is called antiport (e.g. erythrocyte band 3 anion transporter).

Glucose transport into intestinal epithelial cells

The transport of glucose across the polarized epithelial lining cells of the intestine involves its symport across the apical membrane by the Na^+/glucose transporter, with the energy for the movement of the glucose coming from the movement of Na^+ down its concentration gradient. The concentration of Na^+ ions inside the cell is maintained at a low level by the action of a Na^+/K^+-ATPase on the basolateral membrane. The glucose then exits from the cell across the basolateral membrane by facilitated diffusion through the glucose transporter. The movement of Na^+ and glucose across the cell sets up a difference in osmotic pressure causing water to follow by simple diffusion, which forms the basis of glucose rehydration therapy.

Related topics

- Protein structure (B3)
- Introduction to enzymes (C1)
- Enzyme kinetics (C3)
- Membrane lipids (E1)
- Membrane proteins and carbohydrate (E2)
- Nerve function (E6)
- Monosaccharides and disaccharides (J1)
- Glycolysis (J3)

Membrane permeability

A pure phospholipid bilayer, with its hydrophobic interior (see Topic E1), is **permeable** to water, gases (O_2, CO_2, N_2) and small uncharged polar molecules (e.g. urea, ethanol), but is **impermeable** to large uncharged polar molecules (e.g. glucose), ions (Na^+, K^+, Cl^-, Ca^{2+}) and charged polar molecules (e.g. amino acids, ATP, glucose 6-phosphate). The first group of molecules can cross a biological membrane unaided and without an input of energy, whereas the latter group require the presence of **integral membrane transport proteins** and, in some cases, an **input of energy** to travel through the otherwise impermeable membrane barrier. Thus the plasma membrane and the membranes of internal organelles are **selectively permeable barriers**, maintaining a distinct internal environment.

Passive transport

The passive transport of molecules across a membrane does not require an input of metabolic energy. The rate of transport (diffusion) is proportional to the concentration gradient of the molecule across the membrane. There are two types of passive transport: **simple diffusion** and **facilitated diffusion**.

Simple diffusion

Only relatively small uncharged or hydrophobic molecules (H_2O, O_2, CO_2, other gases, urea and ethanol) cross the lipid bilayer by simple diffusion. No membrane proteins are involved, so there is no specificity. The molecule in aqueous solution on one side of the membrane dissolves into the lipid bilayer, crosses it, and then dissolves into the aqueous solution on the opposite side. The rate of diffusion is directly proportional to the concentration gradient of the molecule across the membrane and the process is not saturable (*Fig. 1a*).

Facilitated diffusion

Unlike simple diffusion, the facilitated (or carrier-mediated) diffusion of a molecule across a biological membrane is dependent on **specific integral membrane**

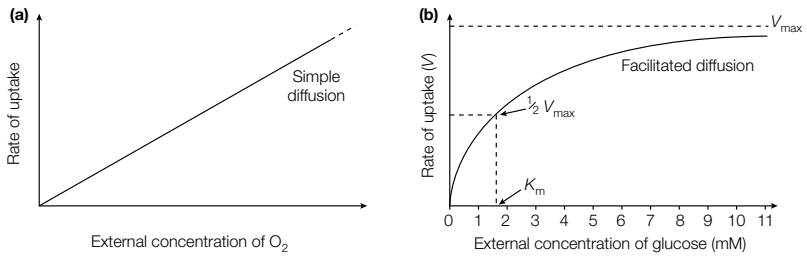

Fig. 1. Kinetics of (a) simple and (b) facilitated diffusion.

proteins, often called **uniporters** (see Topic E2). The molecule binds to the protein on one side of the membrane, the protein then undergoes a **conformational change**, transports the molecule across the membrane and then releases it on the other side. Molecules transported across membranes in this way include hydrophilic molecules such as glucose, other sugars and amino acids. The transport proteins are **specific** for one particular molecule or a group of structurally similar molecules. The transport proteins are capable of being saturated, display Michaelis–Menten-type binding kinetics (K_m and V_{max}) (*Fig. 1b*), and are influenced by temperature, pH and inhibitor molecules in a similar manner to enzymes (see Topics C1 and C3).

An example of facilitated diffusion is the uptake of glucose into erythrocytes by the glucose transporter. The **erythrocyte glucose transporter** is an integral membrane protein of mass 45 kDa that is asymmetrically orientated in the plasma membrane. This uniporter protein structure traverses the membrane with 12 α-helices (see Topics B3 and E2) which form a central pore through which the glucose molecule is passed upon **conformational changes** in the protein (*Fig. 2*). All the steps in the transport of glucose into the cell are **freely reversible**, the direction of movement of glucose being dictated by the relative concentrations of glucose on either side of the membrane. In order to maintain the concentration gradient across the membrane, the glucose is rapidly phosphorylated inside the cell by hexokinase to glucose 6-phosphate (see Topic J3) which is no longer a substrate for the glucose transporter. The erythrocyte glucose transporter is highly specific for D-glucose (K_m 1.5 mM), the nonbiological L-isomer being transported at a barely measurable rate. D-Mannose and D-galactose, which differ from D-glucose in the configuration at one carbon atom (see Topic J1), are transported at intermediate rates. Thus the transporter has a higher affinity (lower K_m) for glucose than for other sugars.

Many cells, such as erythrocytes and those in the kidney, contain water-channel proteins, **aquaporins**, that accelerate the osmotic flow of water. Each aquaporin protein is a tetramer of identical 28 kDa subunits, with each subunit containing six transmembrane α-helices. The water molecules move through a central pore in each subunit. Aquaporins allow cells to move large quantities of water rapidly across their plasma membrane.

Active transport

The active transport of molecules requires an **input of metabolic energy**. This can be derived either from direct coupling to the **hydrolysis of ATP** or by coupling to the **movement of an ion** down its concentration gradient.

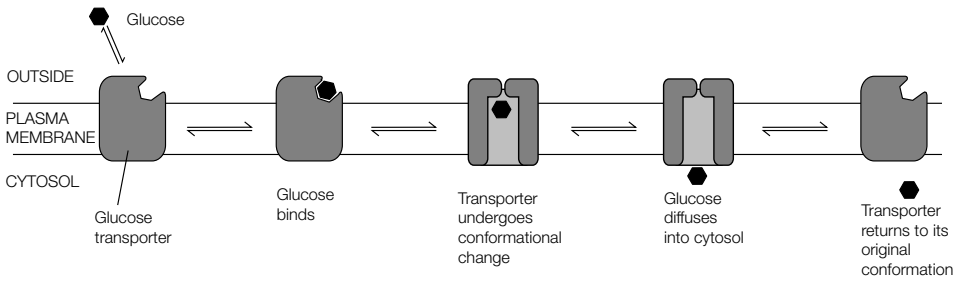

Fig. 2. Facilitated diffusion of glucose into erythrocytes.

ATP-driven active transport

In this case, the energy required for the transport of the molecule across the membrane is derived from the **coupled hydrolysis of ATP**, for example the movement of Na^+ and K^+ ions by the **Na^+/K^+-ATPase**. All cells maintain a high internal concentration of K^+ and a low internal concentration of Na^+. The resulting **Na^+/K^+ gradient** across the plasma membrane is important for the active transport of certain molecules, and the maintenance of the **membrane electrical potential** (see Topic E6). The movement across the membrane of Na^+, K^+, Ca^{2+} and H^+, as well as a number of other molecules, is directly coupled to the hydrolysis of ATP.

Structure and action of the Na^+/K^+-ATPase

The Na^+/K^+-ATPase is an integral membrane protein consisting of 110 kDa α and 55 kDa β subunits. The functional unit is either a heterotetramer ($\alpha_2\beta_2$) or, more likely, a heterodimer (αβ; *Fig. 3*). Upon hydrolysis of one molecule of ATP to ADP and P_i (the P_i transiently binds to an aspartyl residue in the protein), the protein undergoes a conformational change and three Na^+ ions are pumped out of the cell across the plasma membrane and two K^+ ions are pumped in the opposite direction into the cell. Both ions are being moved up their concentration gradients across the membrane; hence the requirement for an input of energy. No transport occurs unless ATP is hydrolyzed, and no ATP is hydrolyzed if there is no Na^+ and K^+ to transport (i.e. it is a **coupled system**).

Ion-driven active transport

In this case, the movement of the molecule to be transported across the membrane is coupled to the **movement of an ion**, usually either Na^+ or H^+. The energy for the movement of the molecule across the membrane against its concentration gradient comes from the movement of the ion down its concentration gradient. If both the molecule and the ion move in the same direction, it is termed **symport**, and the protein involved in the process is called a symporter (e.g. Na^+/glucose transporter; *Fig. 4a*); if the molecule and the ion move in the opposite direction, it is termed **antiport**, and the protein involved in the process is called an antiporter (e.g. erythrocyte band 3 anion transporter; *Fig. 4b*).

Glucose transport into intestinal epithelial cells

The cells lining the lumen of the intestine are **polarized**, that is they have two distinct sides or domains which have different lipid and protein compositions. The **apical** or **brush border** membrane facing the lumen is highly folded into **microvilli** to increase the surface area available for the absorption of nutrients. The rest of the plasma membrane, the **basolateral** surface, is in contact with neighboring cells and the blood capillaries (*Fig. 5*). Movement between adjacent

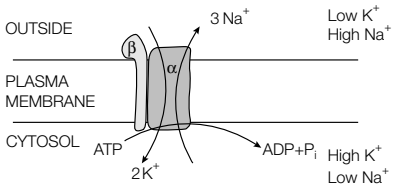

Fig. 3. The Na^+/K^+-ATPase, shown as an αβ heterodimer.

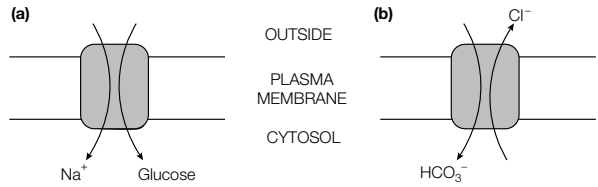

Fig. 4. Ion-driven cotransport mechanisms. (a) Symport process involving a symporter (e.g. Na^+/glucose transporter); (b) antiport process involving an antiporter (e.g. erythrocyte band 3 anion transporter).

epithelial cells is prevented by the formation of **tight junctions** around the cells near the apical domain. Thus any nutrient molecules in the lumen of the intestine have to pass through the cytosol of the epithelial cell in order to enter the blood.

Glucose (or other sugars and amino acids) are transported across the apical membrane from a relatively low concentration in the lumen of the intestine to a relatively high concentration in the cytosol of the epithelial cell by a **glucose/Na^+ symporter protein** (*Fig. 5*). This is a form of **ion-driven active transport**; the energy for the movement of glucose against its concentration gradient comes from the movement of Na^+ down its concentration gradient. The blood flowing through the capillaries on the basolateral side of the epithelial cell maintains a concentration gradient of glucose across this membrane allowing the glucose to move out of the cell by **facilitated diffusion** through a **glucose transporter (a uniporter)** which is similar to the erythrocyte glucose transporter (see above). The relatively low concentration of Na^+ inside the epithelial cell is maintained by a **Na^+/K^+-ATPase** (see above) on the basolateral membrane, an example of **ATP-driven active transport** (*Fig. 5*).

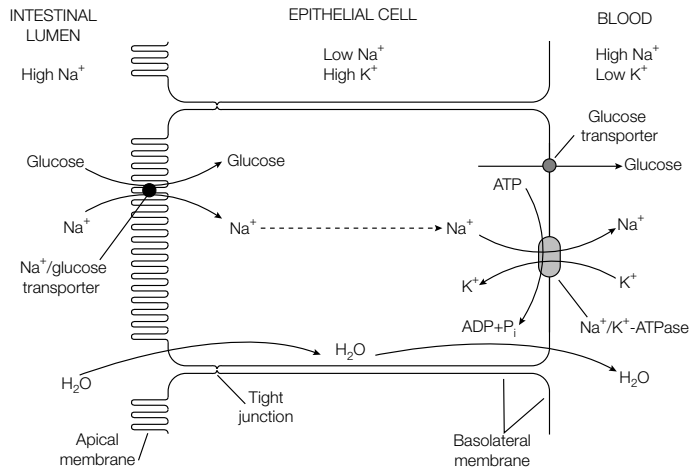

Fig. 5. Transport of glucose and water across intestinal epithelial cells.

Glucose rehydration therapy

The movement of Na^+ and glucose from the lumen of the intestine across the epithelial cell to the blood sets up a difference in **osmotic pressure** across the cell. As a result, water flows through the cell, across the apical and basolateral membranes by **simple diffusion**. Hence the uptake of water requires both Na^+ and glucose (or amino acids) to be present in the lumen of the intestine. The presence of water alone in the lumen of the intestine is much less effective. This is the basis of **glucose rehydration therapy** as a remedy for dehydration; a solution of glucose and salt (NaCl) is administered to the patient. This is a simple, inexpensive but extremely important treatment which has saved the lives of many infants in developing countries who would have otherwise died of the effects of dehydration, usually associated with diarrhea.

E4 TRANSPORT OF MACROMOLECULES

Key Notes

Exocytosis

Exocytosis is the secretion of proteins out of the cell across the plasma membrane into the extracellular space. Proteins destined to be secreted are synthesized on ribosomes bound to the RER membrane and are then transported in membrane-bound vesicles to the Golgi apparatus where they are sorted and packaged up into secretory vesicles. All cells continuously secrete proteins via the constitutive pathway, although many cells (e.g. of the pancreas, nerve cells) also secrete proteins via the regulated secretory pathway in response to certain stimuli.

Endocytosis

Endocytosis is the uptake of macromolecules from the extracellular space into the cell across the plasma membrane via the formation of an intracellular vesicle pinching off from the plasma membrane.

Phagocytosis

Phagocytosis is the uptake of large particles (bacteria and cell debris). The particle binds to receptors on the surface of the phagocytic cell and the plasma membrane then engulfs the particle and ingests it via the formation of a large endocytic vesicle, a phagosome. Most protozoa utilize phagocytosis as a form of feeding, whereas in multicellular organisms only a few specialized cells (e.g. macrophages and neutrophils) can undergo phagocytosis.

Pinocytosis

Pinocytosis is the nonspecific uptake of extracellular fluid via small endocytic vesicles that pinch off from the plasma membrane. This is a constitutive process occurring in all eukaryotic cells.

Receptor-mediated endocytosis

Receptor-mediated endocytosis is the selective uptake of extracellular macromolecules (such as cholesterol) through their binding to specific cell surface receptors. The receptor–macromolecule complex then accumulates in clathrin-coated pits and is endocytosed via a clathrin-coated vesicle.

Clathrin-coated pits and vesicles

Both endocytosis of material at the plasma membrane and exocytosis from the Golgi apparatus involve the formation of clathrin-coated pits and vesicles. On the cytosolic side of the membrane these structures have an electron-dense coat consisting mainly of the protein clathrin, the polypeptides of which form a three-legged structure known as a triskelion. The clathrin triskelions assemble into a basket-like convex framework that causes the membrane to invaginate at that point and eventually to pinch off and form a vesicle (endosome). In endocytosis these clathrin-coated vesicles migrate into the cell where the clathrin coats are lost before delivering their contents to the lysosomes.

Related topics

Eukaryote cell structure (A2)
Bioimaging (A4)
Membrane proteins and carbohydrate (E2)
Transport of small molecules (E3)
Signal transduction (E5)
Translation in eukaryotes (H3)
Protein targeting (H4)
Protein glycosylation (H5)
Cholesterol (K5)
Lipoproteins (K6)

Exocytosis

A cell often needs to secrete larger molecules than can be accommodated by the transport systems dealt with in Topic E3. **Exocytosis** refers to the movement of proteins out of the cell across the plasma membrane into the extracellular space. Proteins destined to be **secreted** from the cell are translated on ribosomes attached to the RER (see Topic H3). **Membrane-bound vesicles** containing these proteins then bud off from the RER, migrate through the cytosol and fuse with the membrane of the Golgi apparatus (*Fig. 1*). On transport through the endoplasmic reticulum and Golgi apparatus (see Topic A2), various post-translational modifications to the proteins take place, such as glycosylation (see Topic H5).

The **Golgi apparatus** is the major sorting center of the cell where proteins and lipids are packaged into discrete vesicles and then targeted to the appropriate part of the cell (see Topic H4). For example, vesicles containing lysosomal proteins will be targeted to the lysosomes (see Topic A2). If the vesicles contain no specific targeting signal they will migrate through the cytosol to the plasma membrane, the so-called default or **constitutive secretory pathway** (*Fig. 1*). Transport vesicles destined for the plasma membrane in the constitutive secretory pathway leave the Golgi apparatus in a steady stream. The membrane proteins and lipids in these vesicles provide new material for the plasma membrane, while the soluble proteins inside the vesicles are secreted to the extracellular space. All cells have this constitutive secretory pathway.

In certain cells, however, an additional secretory pathway exists, the **regulated secretory pathway**. This pathway is found mainly in cells that are specialized for secreting products rapidly on demand in response to a particular stimulus (*Fig. 1*). For example, the hormone insulin and digestive enzymes are secreted by the pancreas, while neurotransmitters are secreted by nerve cells. In these cells such substances are initially stored in secretory vesicles which form

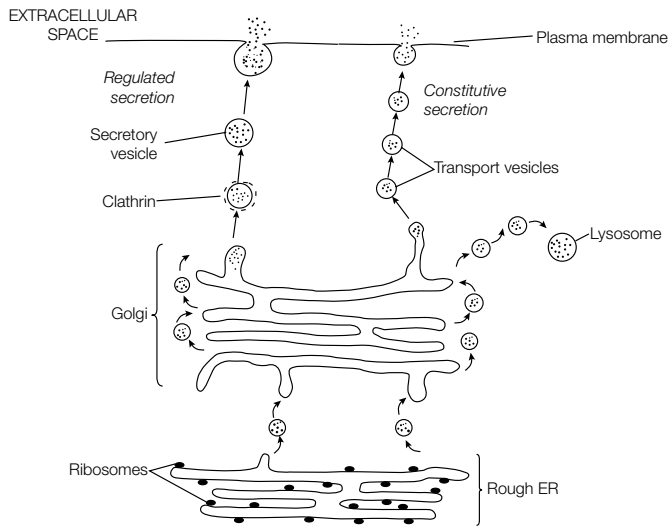

Fig. 1. Exocytosis of proteins by the constitutive and regulated secretory pathways.

by **clathrin-coated** budding (see below) from the Golgi apparatus. The clathrin coat then dissociates and the vesicles remain in the cytosol until signaled to release their contents on fusion with the plasma membrane.

Endocytosis

Endocytosis is the uptake of extracellular macromolecules across the plasma membrane into the cell. The material to be ingested is progressively enclosed by a small portion of the plasma membrane, which first invaginates and then pinches off to form an intracellular vesicle containing the ingested macromolecule. Endocytosis can be divided into three distinct types depending on the size of the ingested macromolecule and whether specific cell surface receptors are involved. These three processes are: **phagocytosis**, **pinocytosis** and **receptor-mediated endocytosis**.

Phagocytosis

Phagocytosis is the ingestion of large particles such as bacteria and cell debris via large endocytic vesicles called **phagosomes**. In order to be ingested the particle must first bind to the surface of the phagocyte, usually through specialized cell surface receptors. Once bound to the receptors, the phagocyte is stimulated to begin engulfing the particle with its plasma membrane, thereby enclosing it within a phagosome (*Fig. 2a*). The phagosome then fuses with a lysosome (see Topic A2) and the ingested particle is broken down. Utilizable material will be transported into the cytosol, while indigestible substances will remain in the lysosomes, forming residual bodies. In protozoa, phagocytosis is a form of feeding, where the ingested material is broken down in the lysosomes and utilized as food. In multicellular organisms only a few specialized cells are capable of phagocytosis. **Macrophages** and **neutrophils** (white blood cells) use phagocytosis to protect the organism against infection by ingesting invading microorganisms. Macrophages are also involved in scavenging dead and damaged cells and cell debris.

Pinocytosis

Unlike phagocytosis, which is a regulated form of endocytosis carried out by a small number of cell types, **pinocytosis** is a constitutive process that occurs continuously in all eukaryotic cells. Small areas of the plasma membrane are ingested in the form of small **pinocytic vesicles** that are later returned to the cell

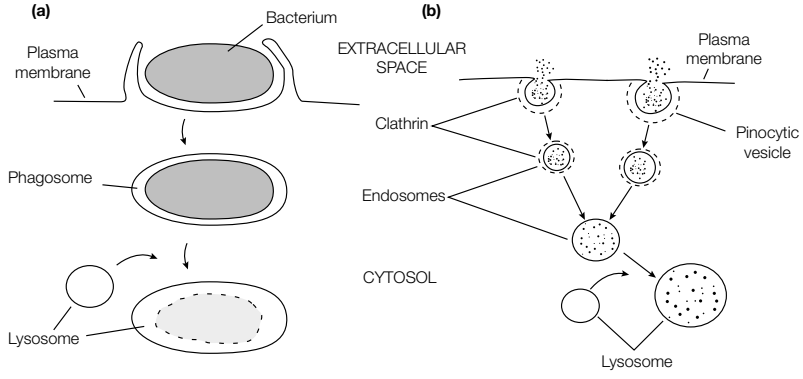

Fig. 2 (a) Phagocytosis and (b) pinocytosis.

surface. As the pinocytic vesicle forms, a small amount of extracellular fluid is enclosed in the vesicle and also taken up into the cell (*Fig. 2b*). This nonspecific uptake of extracellular fluid is often referred to as **cell-drinking** or **fluid-phase endocytosis**. The initial endocytosis begins by the formation of a **clathrin-coated pit** (see below) at specialized regions of the plasma membrane (*Fig. 2b*). The resulting **clathrin-coated endocytic vesicle** (**endosome**) is then endocytosed, the clathrin coat dissociates and the endosome fuses with a lysosome (see below).

Receptor-mediated endocytosis

Receptor-mediated endocytosis is the selective uptake of macromolecules from the extracellular fluid via **clathrin-coated pits and vesicles**. This process, which takes place in most animal cells, involves the macromolecule binding specifically to a **cell surface receptor** (which is an integral membrane protein; see Topics E2 and E5). Once bound to the receptor, the receptor–macromolecule complex accumulates in a clathrin-coated pit and is then endocytosed in a clathrin-coated vesicle (*Fig. 3*, and see below). Receptor-mediated endocytosis provides a way of selectively concentrating particular macromolecules that are at low concentrations in the extracellular fluid, thereby increasing the efficiency of their uptake without having to take in large quantities of extracellular fluid. One of the best studied and understood receptor-mediated endocytic processes is the uptake of **cholesterol** by mammalian cells (see Topics K5 and K6). Many **viruses** and other **toxins** gain entry to animal cells by receptor-mediated endocytosis. Although the cells do not purposely have cell surface receptors that recognize the viral particle, the virus has evolved to express a protein on its surface that mimics the correct ligand recognized by the receptor, thus allowing the virus to bind and be internalized.

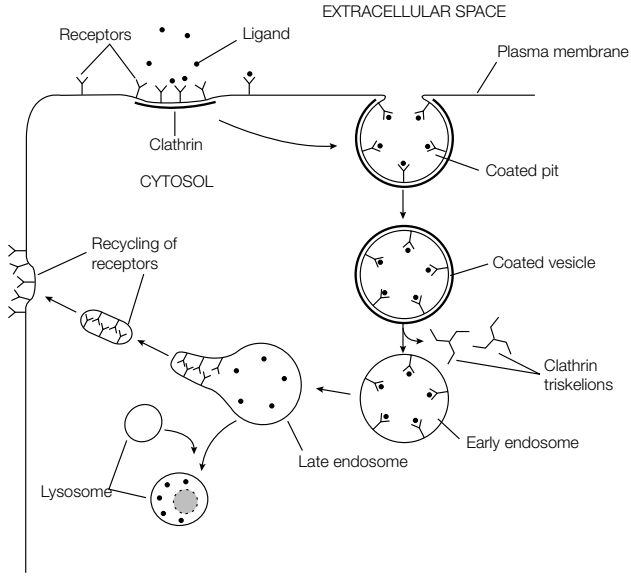

Fig. 3. Receptor-mediated endocytosis involves clathrin-coated pits and vesicles.

Clathrin-coated pits and vesicles

Clathrin-coated pits and vesicles are involved in both the exocytosis of proteins from the Golgi apparatus and the endocytosis of material at the plasma membrane. Electron micrographs (see Topic A4) of clathrin-coated pits reveal that these pits are invaginations of the plasma membrane that are coated on their cytosolic surface with a densely packed material made up predominantly of the protein **clathrin**. This protein, which has been highly conserved throughout evolution, consists of three large and three small polypeptide chains that together form a three-legged structure called a **triskelion**. These clathrin triskelions assemble into a basket-like convex framework of hexagons and pentagons to form the coated pits. The assembly of the clathrin coat is thought to drive the membrane to invaginate at that point. As further clathrin triskelions are added to the structure a complete cage forms, pinching off a region of the membrane and forming a clathrin-coated vesicle. In endocytosis these vesicles then migrate into the cell, shed their clathrin coat and become **early endosomes** (*Fig. 3*). The early endosomes then migrate towards the Golgi apparatus and the nucleus, becoming **late endosomes** before fusing with the lysosomes.

E5 SIGNAL TRANSDUCTION

Key Notes

Cell signaling

Cells communicate with one another in multicellular organisms using extracellular signaling molecules or hormones. The hormone is secreted by the signaling cell and then binds to a receptor on the target cell, initiating a response in that cell. In endocrine signaling the hormone acts at a distant site in the body from where it was produced, in paracrine signaling the hormone acts on nearby cells, and in autocrine signaling the hormone acts on the same cell from which it was secreted.

Signaling molecules with intracellular receptors

Some lipophilic hormones (e.g. the steroid hormones, thyroxine, retinoic acid and vitamin D) diffuse across the plasma membrane and interact with intracellular receptors of the nuclear receptor family in the cytosol or nucleus. The gases nitric oxide (NO) and carbon monoxide (CO) are signaling molecules that cross the plasma membrane and stimulate the intracellular enzyme guanylyl cyclase to produce cGMP.

Signaling molecules with cell surface receptors

Other lipophilic hormones (e.g. the prostaglandins) and hydrophilic hormones (e.g. the peptide hormones insulin and glucagon and the biogenic amines epinephrine and histamine) bind to receptor proteins in the plasma membrane.

Cell surface receptors

Cell surface receptors are integral membrane proteins located in the plasma membrane that bind the hormone (ligand) with high affinity and specificity. On binding the ligand, the receptor may undergo a conformational change or dimerize and transmit the information into the cell (signal tranduction).

Enzyme-linked receptors

Enzyme-linked receptors (e.g. the insulin receptor) have an intrinsic enzyme activity. Ligand binding causes autophosphorylation of tyrosine residues in the cytoplasmic domain of these receptor tyrosine kinases. These modified tyrosines are then recognized by other proteins in the cytosol. Other receptors have serine/threonine kinase activity, while some lack intrinsic kinase activity and associate with cytoplasmic tyrosine kinases (e.g. Src family kinases). Many enzyme-linked receptors interact through small binding domains (e.g. SH2, SH3, PTB, PH, PDZ) with scaffold proteins inside the cell which organize groups of proteins into signaling complexes.

Ion channel-linked receptors

Ion channel-linked receptors change conformation to allow ions to flow across the membrane thereby altering the membrane potential.

G protein-coupled receptors

G protein-coupled receptors (GPCRs) contain seven transmembrane α-helices and activate trimeric G [guanosine triphosphate (GTP)-binding] proteins that in turn lead to the production of an intracellular second messenger. Trimeric G proteins contain three subunits: α, β and γ. The Gα subunit is a GTPase switch protein. Different GPCRs interact with different trimeric G proteins leading to different intracellular signaling events.

GTPase switch proteins

There are two main classes of GTPase switch proteins: the Gα subunit of the trimeric G proteins and the monomeric G proteins (e.g. Ras, Rho, Rab). They are attached to the cytosolic face of the plasma membrane by a covalently attached lipid and cycle through an inactive GDP bound form and an active GTP bound form.

Second messengers

Intracellular signaling molecules (second messengers) are produced in response to the activation of various cell surface receptors. The second messengers cAMP and 3′,5′-cyclic guanosine monophosphate (cGMP) are produced by adenylate cyclase and guanylate cyclase, respectively. Activation of phospholipase C leads to the production of the second messengers inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG) which in turn cause the release of Ca^{2+} from intracellular stores and activate protein kinase C, respectively.

Calcium ions

Many extracellular signals induce an increase in the level of Ca^{2+} ions in the cytosol. When triggered Ca^{2+} channels, such as voltage-dependent Ca^{2+} channels in the plasma membrane, IP_3-gated Ca^{2+} channels and ryanodine receptors in the ER and sarcoplasmic reticulum, allow Ca^{2+} ions to flow into the cytosol. The rise in cytosolic Ca^{2+} results in altered cellular events through Ca^{2+}-binding proteins, such as troponin C in muscle and calmodulin.

Regulated proteolysis

Some cell surface receptors undergo regulated intramembrane proteolysis upon binding ligand, with the release of the cytoplasmic domain which translocates to the nucleus.

Related topics

Cytoskeleton and molecular motors (A3)
Amino acids (B1)
Introduction to enzymes (C1)
Membrane proteins and carbohydrate (E2)
Nerve function (E6)
Regulation of transcription by RNA Pol II (G6)
Control of glycogen metabolism (J7)
Structures and roles of fatty acids (K1)
Triacylglycerols (K4)
Cholesterol (K5)

Cell signaling

In multicellular organisms there is a need for the cells to **communicate** with one another in order to **coordinate** their growth and metabolism. The principal way by which cells communicate with each other is by means of **extracellular signaling molecules** or **hormones**. These molecules are synthesized and secreted by **signaling cells** and produce a specific response in **target cells** that have **specific receptors** for the signaling molecule. Different cells can respond differently to the same signaling molecule depending on the type of receptor and the intracellular reactions initiated (see below). Cell signaling can be classified into three distinct types based on the distance over which the signaling

molecule acts. In **endocrine signaling**, the signaling molecule (e.g. insulin) acts on target cells distant from its site of synthesis in cells of an endocrine organ (e.g. the pancreas; *Fig. 1a*). The endocrine cells secrete the signaling molecule into the bloodstream (if an animal) or the sap (if a plant) which carries it to the target cells elsewhere in the organism. In **paracrine signaling**, the signaling molecule affects only target cells close to the cell from which it was secreted (*Fig. 1b*). The communication from one nerve cell to another by chemical neurotransmitters is an example of paracrine signaling (see Topic E6). The third type of cell signaling is **autocrine signaling**, where a cell responds to a molecule that it has produced itself (*Fig. 1c*).

Signaling molecules with intracellular receptors

Small lipophilic (lipid-soluble) hormones diffuse across the plasma membrane and then interact with **intracellular receptors** in the cytosol or nucleus. The receptors are all structurally related, being part of the **nuclear receptor superfamily**. The resulting hormone–receptor complex often binds to regions of the DNA and affects the transcription of certain genes (see Topic G6). Small lipophilic hormones with intracellular receptors include the **steroid hormones** which are synthesized from cholesterol (see Topic K5) (e.g. the female sex hormones estrogen and progesterone), **thyroxine** which is produced by thyroid cells and is the principal iodinated compound in animals, **retinoic acid** which is derived from vitamin A, and **vitamin D** which is synthesized in the skin in response to sunlight (see Topic K5).

An important and remarkable example of a small signaling molecule that passes readily across the target cell plasma membrane is the gas **nitric oxide** (NO). This is used in both animals and plants. NO is synthesized by the deamination of arginine (see Topic B1), catalyzed by the enzyme **NO synthase**. NO rapidly diffuses out of the cell where it is produced and into neighboring cells. It only acts locally as it has a short half-life, about 5–10 s. In many target cells, NO binds to the active site of **guanylyl cyclase**, stimulating the enzyme to produce the small intracellular mediator cGMP (see below). **Nitroglycerine**, which is used to treat patients with **angina** (pain resulting from inadequate blood flow to the heart muscle), is converted to NO, which relaxes the blood vessels thereby reducing the workload on the heart. **Carbon monoxide** (CO) is another gas that is used as a signaling molecule, again by stimulating guanylyl cyclase.

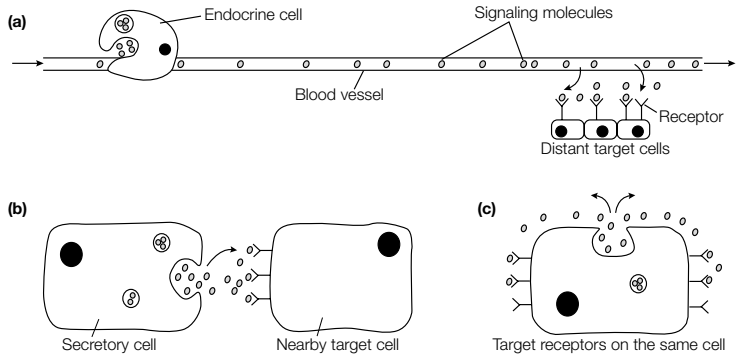

Fig. 1. Cellular signaling. (a) Endocrine, (b) paracrine, (c) autocrine.

Signaling molecules with cell surface receptors

Hydrophilic (water-soluble) molecules (which cannot diffuse across the hydrophobic interior of the lipid bilayer) bind to receptors in the plasma membrane. These include the **peptide hormones,** such as **insulin** and **glucagon**, and the small charged molecules, often **biogenic amines**, such as **epinephrine** (**adrenalin**) and **histamine,** that are derived from amino acids and function as hormones and neurotransmitters (see Topic E6). Some lipophilic (lipid-soluble) hormones also bind to receptors located in the plasma membrane. These include the **prostaglandins**, a family of structurally similar compounds that are found in both vertebrates and invertebrates. Prostaglandins are synthesized from **arachidonic acid** (a 20-carbon fatty acid with four unsaturated double bonds) (see Topic K1) and act as paracrine signaling molecules. **Aspirin** and other **anti-inflammatory agents** inhibit the synthesis of prostaglandins.

Cell surface receptors

Hydrophilic and some lipophilic hormones bind to cell surface receptors. These are **integral membrane proteins** situated in the plasma membrane (see Topic E2) that bind the signaling molecule (**ligand**) with high affinity. The ligand binds to a specific site on the receptor in much the same way as a substrate binds to an enzyme (see Topic C1). Binding of the ligand to the receptor may cause a **conformational change** in the receptor or promote **dimerization** of two receptors that initiates a sequence of reactions in the target cell (often referred to as **signal transduction**) leading to a change in cellular function. The distribution of receptors varies on different cells, and there is often more than one type of receptor for a particular ligand, allowing different target cells to respond differently to the same signaling molecule. Cell surface receptors can be classified into three major classes depending on how they transfer the information from the ligand to the interior of the cell: enzyme-linked receptors, ion channel-linked receptors and G protein-coupled receptors.

Enzyme-linked receptors

Numerous receptors have intrinsic or tightly associated **enzyme activity**. On binding of the ligand to their extracellular face, such receptors undergo a conformational change and activate an **enzyme activity**. In the case of the **insulin receptor** which is a complex of two α- and two β-subunits held together by disulfide bonds, the polypeptide hormone insulin (the ligand) binds to the extracellular face of the α-subunits (*Fig. 2*). The receptor then undergoes a conformational change leading to the **autophosphorylation** (self-phosphorylation) of the

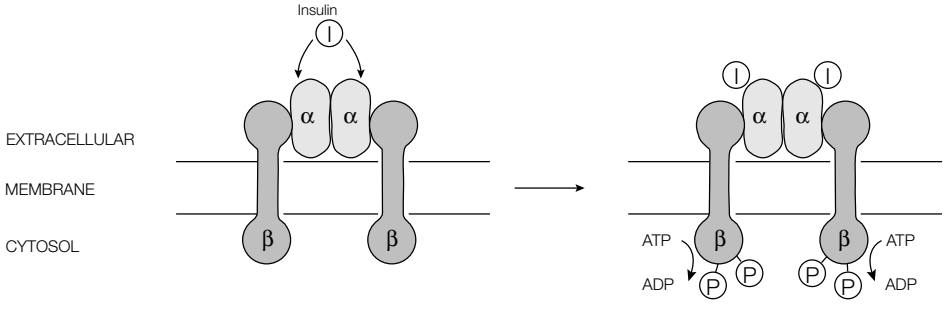

Fig. 2. Signal transduction through an enzyme-linked receptor such as the insulin receptor.

cytosolic domain of the β-subunit. Specifically the hydroxyl groups in the side-chains of certain **tyrosine residues** are phosphorylated, with **ATP** being the phosphate donor. The phosphorylated receptor is then recognized by other proteins in the cytosol that in turn modulate various intracellular events, allowing the cell to respond to the hormone appropriately (see Topic J7). In addition, the β-subunit can directly phosphorylate other target proteins within the cell.

The insulin receptor is an example of a **receptor tyrosine kinase**, while the **transforming growth factor-β** (TGF-β) family of receptors have **serine/threonine kinase activity** in their cytosolic domain. Other receptors with tightly associated enzyme activity include various **cytokine receptors** that bind interferons, growth hormone, some interleukins and other cytokines. For many enzyme-linked receptors, ligand binding induces the **oligomerization** (formation of dimers or higher oligomers) and it is this rearrangement of the cytosolic domains that enables the neighboring kinase domains of the receptor chains to cross-phosphorylate each other in the process of **autophosphorylation**.

Some enzyme-linked receptors, as well as other receptors, interact with **scaffold proteins** inside the cell which organize groups of proteins into **signaling complexes** *(Fig. 3)*. The proteins within these signaling complexes assemble through the interactions of a variety of highly conserved, small binding domains such as the **Src homology 2 (SH2) domains** and **phosphotyrosine-binding (PTB) domains** that bind to phosphorylated tyrosine residues, **Src homology 3 (SH3) domains** that bind to short proline-rich amino acid sequences, and **pleckstrin homology (PH) domains** that bind to the headgroups of inositol phospholipids that have been additionally phosphorylated by **phosphatidylinositol 3-kinase (PI 3-kinase)**. Some scaffold proteins contain multiple **PDZ domains**, each of which binds to a specific motif on a receptor or signaling protein. The binding of these scaffold proteins to the activated receptor may help to relay the signal onward or may decrease the signaling process, providing negative feedback.

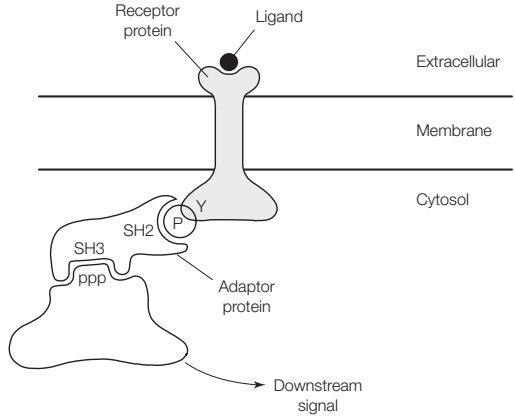

Fig. 3. A hypothetical signaling complex. Binding of ligand to the extracellular face of the cell surface receptor results in phosphorylation of a tyrosine residue in its cytosolic domain. The phosphorylated tyrosine residue is recognized by an SH2 domain in the adaptor protein. Elsewhere in the adaptor protein is an SH3 domain that binds to a proline-rich sequence (PPP) in another signaling protein, such that the signal is relayed into the cell.

Some cell surface receptors require tyrosine phosphorylation for their activity and yet lack a tyrosine kinase domain. These receptors act through **cytoplasmic tyrosine kinases** (or nonreceptor tyrosine kinases) which associate with the receptor and phosphorylate various target proteins. The largest family of cytoplasmic tyrosine kinases is the **Src family** that includes Src, Yes, Fyn and Lck, which all contain SH2 and SH3 domains and are located on the cytoplasmic surface of the plasma membrane.

Ion channel-linked receptors

Ion channel-linked receptors (transmitter-gated ion channels or ionotrophic receptors) are involved in the rapid synaptic signaling between electrically excitable cells. Here, binding of the ligand causes a conformational change in the protein such that a specific **ion channel** is opened (*Fig. 4*). This allows a certain ion to flow through that subsequently alters the **electric potential** across the membrane. For example, at the nerve–muscle junction the **neurotransmitter acetylcholine** binds to specific receptors that allow Na^+ ions to flow into, and K^+ ions out of, the target cell (see Topic E6 for more detail).

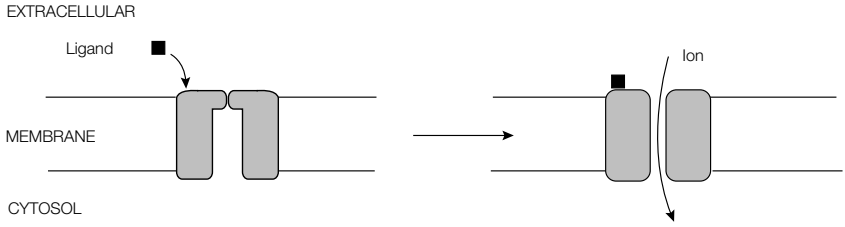

Fig. 4. Signal transduction through an ion channel-linked receptor.

G protein-coupled receptors

G protein-coupled receptors (**GPCRs**) form a very large group of cell surface receptors that are coupled to signal-transducing trimeric G proteins. All GPCRs contain **seven membrane-spanning** α-helical regions with their N-terminus on the extracellular face of the plasma membrane and their C-terminus on the cytoplasmic face (*Fig. 5*). The GPCR family includes receptors for numerous hormones and neurotransmitters, light-activated receptors (rhodopsins) in the eye, and thousands of odorant receptors in the mammalian nose. On binding its ligand, a GPCR activates the signal-transducing **trimeric G proteins [guanyl nucleotide (GTP)-binding proteins]** which in turn activate or inhibit an effector protein. The trimeric G proteins consist of three subunits: α, β and γ. The Gα subunit is a **GTPase switch protein** that alternates between an active (on) state

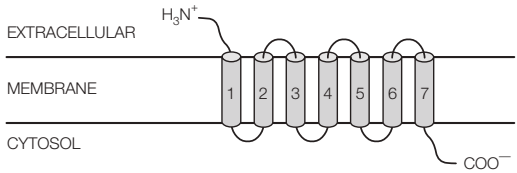

Fig. 5. G protein-coupled receptors (GPCRs) contain seven transmembrane α-helical regions (cylinders 1-7), with their N-terminus on the extracellular side of the membrane and their C-terminus in the cytosol.

and an inactive (off) state (see below). In the human genome there are multiple copies of each of the α, β and γ subunits, providing diversity in the signaling through GPCRs.

The Gα and Gγ subunits are linked to the cytosolic surface of the plasma membrane by covalently attached lipids (see Topic E2). In the resting state, when no ligand is bound to the GPCR, the Gα subunit is bound to GDP and complexed with Gβγ (*Fig. 6*). Binding of the ligand to the GPCR changes its conformation, causing it to bind to the Gα subunit in such a way that the GDP is displaced and GTP becomes bound. The Gα subunit then dissociates from Gβγ, but both remain anchored to the membrane. The Gα subunit with GTP bound then interacts with and activates an associated **effector protein**, such as adenylyl cyclase, or in some cases regulates the opening of an ion channel causing a change in the membrane potential. However, this activation is short-lived, as the GTP is rapidly, within seconds, hydrolyzed to GDP by the intrinsic GTPase activity in the Gα subunit (see below). The Gα subunit, now with GDP bound, dissociates from the effector protein, deactivating it, and reassociates with Gβγ, ready for another round of activation and nucleotide exchange (*Fig. 6*).

The hormone **epinephrine** binds to several different GPCRs. On binding to **β-adrenergic receptors** on the surface of liver and adipose cells, epinephrine promotes glycogenolysis and lipolysis, respectively (see Topics J7 and K4). On

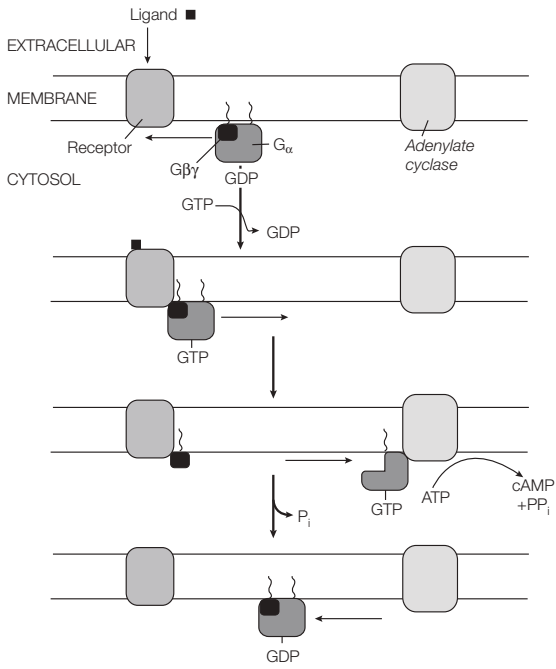

Fig. 6. Signal transduction through a G protein-coupled receptor (see text for details).

smooth muscle cells lining the blood vessels in the intestine, skin and kidneys, epinephrine binds to the **α_2-adrenergic receptor** causing the arteries to constrict. The β-adrenergic receptors are coupled to a **stimulatory G protein** (G_s) that activates the membrane-bound adenylyl cyclase, whereas the α_1-adrenergic receptor is coupled to an **inhibitory G protein** (G_i) that inhibits adenylyl cyclase, while the G_q protein coupled to the α_2-adrenergic receptor activates a different effector protein. Thus through binding to different receptors and activating different G proteins one ligand can trigger a variety of different actions in different target cells.

GTPase switch proteins

The **GTPase family** of proteins are a group of **intracellular switch proteins** (or on–off molecular switches) of which there are two main classes: the Gα subunit of the **trimeric G proteins** (see above) and the **monomeric G proteins** such as the **Ras, Rho and Rab families**. Ras, Rho and Rab proteins that couple to the activated receptor through adaptor scaffold proteins (see above) act as transducers and bifurcation signaling proteins, changing the nature of the signal and sending it along multiple downstream pathways. In the case of Ras, a downstream serine/threonine phosphorylation cascade is activated, that includes the **mitogen-activated protein kinase (MAP-kinase)**. Like the G_α subunits, the Ras proteins are attached to the cytosolic face of the plasma membrane by a covalently attached lipid group (see Topic E2). When the GTPase has guanosine diphosphate (GDP) bound, it is in the 'off' state. Activation, via a cell surface receptor or a **guanine nucleotide-exchange factor** (GEF), leads to the GDP being exchanged for GTP, converting the GTPase to the 'on' state (*Fig. 7)*. The activated GTPase with its bound GTP then dissociates from the receptor and binds to and activates an effector enzyme (e.g. adenylyl cyclase) which in turn catalyzes the formation of a second messenger (e.g. cAMP). The GTPase then hydrolyzes the bound GTP, causing it to revert back to the 'off' state (*Fig. 7*). **Cholera toxin** acts by inhibiting its intrinsic GTPase activity (*Fig. 7*), with the result that once activated to the GTP-bound state the GTPase cannot be turned off again.

Second messengers

The binding of ligands to many receptors leads to a short-lived increase in the concentration of certain **intracellular signaling molecules** called **second**

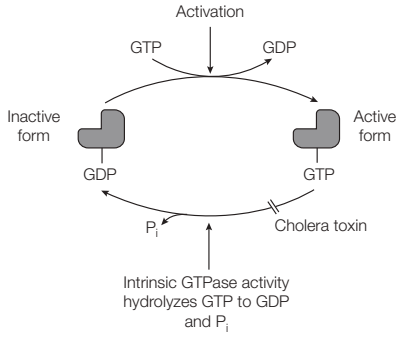

Fig. 7. Cycling of the GTPase switch proteins between the active and inactive forms.

messengers. (The hormone/ligand can be considered as the first messenger.) The major second messengers are 3',5'-cyclic AMP (**cAMP**), 3',5'-cyclic GMP (**cGMP**), **inositol 1,4,5-trisphosphate** (IP_3), **1,2-diacylglycerol** (DAG) and **Ca^{2+}**. The elevation in the level of one or other of these second messengers then leads to a rapid alteration in cellular function.

cAMP and cGMP are derived from ATP and GTP by the actions of **adenylyl cyclase** and **guanylyl cyclase**, respectively. For example, the action of epinephrine and glucagon on glycogen metabolism is mediated through the second messenger cAMP which in turn activates the **cAMP-dependent protein kinase**, **protein kinase A** (see *Fig. 3* in Topic J7). cAMP and cGMP are short-lived as they are rapidly broken down by **phosphodiesterases**.

IP_3 and DAG are derived from the membrane lipid **phosphatidylinositol 4,5-bisphosphate** (which is a phosphorylated derivative of phosphatidylinositol; see Topic E1) by the action of **phospholipase C** which is also located in the plasma membrane and, like adenylyl cyclase, is activated via G proteins by GPCRs (*Fig. 8*). One of the main actions of the polar IP_3 is to diffuse through the cytosol and interact with Ca^{2+} channels in the membrane of the ER (*Fig. 8*), causing the release of stored Ca^{2+} ions which in turn mediate various cellular responses. The DAG produced by the hydrolysis of phosphatidylinositol 4,5-bisphosphate, along with Ca^{2+} ions released from the ER, activates **protein kinase C**, a membrane-bound serine/threonine protein kinase, that phosphorylates various target proteins, again leading to alterations in a variety of cellular processes (*Fig. 8*).

Calcium ions

Many extracellular signals induce an increase in the level of **Ca^{2+} ions** in the cytosol. For example, in muscle cells, Ca^{2+} triggers contraction (see Topic A3). The concentration of Ca^{2+} in the cytosol is usually kept very low (approx. 0.1 μM), whereas its concentration in the extracellular fluid and in the lumen of the ER is high (approx. 1 mM). Thus there is a **gradient of Ca^{2+} ions** across the

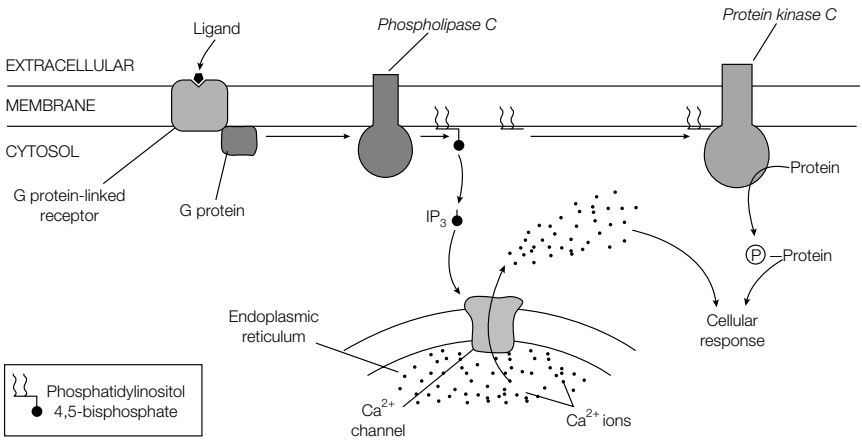

Fig. 8. Generation of the intracellular second messengers inositol 1,4,5-trisphosphate (IP_3), 1,2-diacylglycerol (DAG) and Ca^{2+}.

plasma membrane and ER membrane, such that when **Ca^{2+} channels** in these membranes are triggered to open, Ca^{2+} ions rapidly flow into the cytosol, raising the Ca^{2+} concentration by 10–20-fold and triggering Ca^{2+}-responsive proteins inside the cell. There are three main types of Ca^{2+} channel: **voltage-dependent Ca^{2+} channels** in the plasma membrane that open in response to membrane depolarization, for example in nerve cells (see Topic E6); **IP_3-gated Ca^{2+}-channels** in the ER (see above); and **ryanodine receptors** (so called because they are inhibited by the plant alkaloid ryanodine) that release Ca^{2+} from the sarcoplasmic reticulum in muscle cells (see Topic A3) or the ER of other cells. Ca^{2+} pumps, such as the Ca^{2+}-ATPase, in the ER and plasma membrane help to maintain the low concentration of Ca^{2+} ions in the cytosol of resting cells. **Ca^{2+}-binding proteins** serve as transducers of the cytosolic Ca^{2+} signal. These Ca^{2+}-binding proteins include **troponin C** in skeletal muscle (see Topic A3) and **calmodulin**, a ubiquitous protein found in all eukaryotic cells. Calmodulin functions as a **multipurpose intracellular Ca^{2+} receptor**, mediating many Ca^{2+}-regulated processes, and undergoes a conformational change upon binding Ca^{2+}. The activated calmodulin can then bind to a number of different target proteins, including a family of **Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases)** which then phosphorylate serines or threonines on other proteins.

Regulated proteolysis

There are several unusual signaling pathways for relaying signals from cell surface receptors to the interior of the cell that involve **regulated proteolysis**. Amongst these pathways is that mediated by the receptor protein **Notch** and the pathway activated by secreted **Hedgehog** proteins that have been highly conserved across evolution and play crucial roles in animal development. In the case of the transmembrane Notch receptor, binding of its **ligand Delta** to the extracellular face leads to a proteolytic cleavage first in the region adjacent to the membrane and then a second cleavage within the hydrophobic transmembrane region (*Fig. 9*). The released cytoplasmic domain then migrates to the nucleus where it activates the transcription of various target genes (*Fig. 9*). Proteolytic

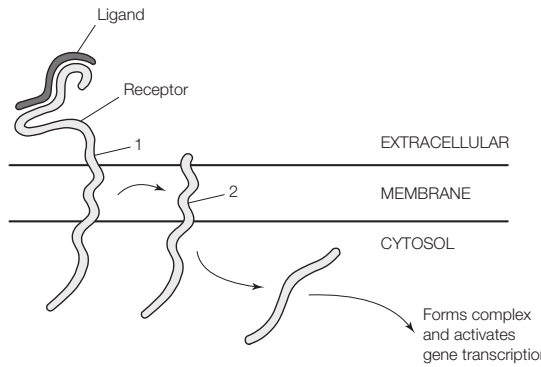

Fig. 9. Cell signaling through the Notch receptor. Binding of ligand results in proteolytic cleavage of the receptor (1) on the extracellular face of the membrane. The resulting membrane-bound stub is then cleaved within the transmembrane domain (2), releasing the cytosolic tail which forms a complex with other proteins and activates gene transcription in the nucleus.

cleavage within the hydrophobic transmembrane region is unusual but more and more proteins are being identified that are subject to this **regulated intramembrane proteolysis** (RIP). As peptide bonds in the receptor protein are cleaved in this process, the receptor cannot be reused.

E6 Nerve function

Key Notes

Nerve cells

Nerve cells, or neurons, consist of a cell body from which the dendrites and axon extend. The dendrites receive information from other cells; the axon passes this information on to another cell, the post-synaptic cell. The axon is covered in a myelin membranous sheath except at the nodes of Ranvier. The axon ends at the nerve terminal where chemical neurotransmitters are stored in synaptic vesicles for release into the synaptic cleft.

The action potential

An electric membrane potential exists across the plasma membrane due to the unequal distribution of Na^+ and K^+ ions which is generated by the Na^+/K^+-ATPase. Upon stimulation, neurons depolarize their membrane potential from the resting state (–60 mV) to +40 mV, generating an action potential. The action potential is caused by Na^+ ions flowing into the cell through voltage-sensitive Na^+ channels. The resting membrane potential is restored by K^+ ions flowing out of the cell through voltage-sensitive K^+ channels. The poison tetrodotoxin acts by blocking the Na^+ channel.

Neurotransmitters

Chemical neurotransmitters, such as acetylcholine, the biogenic amines and small peptides, are stored in the pre-synaptic nerve terminal in synaptic vesicles. When the action potential reaches the nerve terminal it causes the synaptic vesicles to fuse with the plasma membrane in a Ca^{2+}-dependent manner and to release their contents by exocytosis. The neurotransmitter then diffuses across the synaptic cleft, binds to specific receptors on the post-synaptic cell membrane and initiates a response in that cell.

Related topics

Enzyme inhibition (C4)
Membrane lipids (E1)
Membrane proteins and carbohydrate (E2)
Transport of small molecules (E3)
Transport of macromolecules (E4)
Signal transduction (E5)

Nerve cells

In eukaryotes, probably the most rapid and complex signaling is mediated by **nerve impulses**. Nerve cells (**neurons**) consist of a cell body with numerous projections of the plasma membrane, called **dendrites** (*Fig. 1*). These interact with other cells and receive information from them in the form of nerve impulses. The cell body then assimilates the information derived from a number of dendritric contacts and passes on the information as another nerve impulse down the large **axon** (*Fig. 1*). The axon ends at the **synapse** where it makes contact with the **post-synaptic** (target) **cell**. The axon is covered in places by a membranous **myelin** sheath, made up mainly of the lipid sphingomyelin (see Topic E1), which acts as an electrical insulator, enabling the nerve impulses to be transmitted over long distances, sometimes more than 1 m in larger animals. Every millimeter or so along the axon the myelin sheath is interrupted by

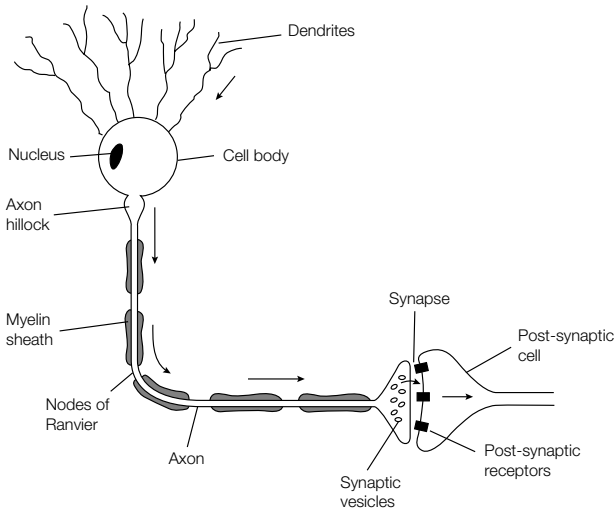

Fig. 1. Schematic diagram of a typical nerve cell.

unmyelinated regions called the **nodes of Ranvier** (*Fig. 1*). The end of the axon, the nerve terminal, is full of **synaptic vesicles** that store the chemical **neurotransmitters**, such as acetylcholine. When a nerve impulse reaches the nerve terminal the synaptic vesicles release their contents into the **synaptic cleft**, the space between the pre- and post-synaptic cells. The neurotransmitter then diffuses across the space and interacts with receptors on the surface of the post-synaptic cell, causing a signal to be transduced in that cell.

The action potential

An electric potential (the **membrane potential**) exists across the plasma membrane of all cells. Most cells are electrically inactive as this membrane potential does not vary with time. However, neurons and muscle cells are **electrically active** as their membrane potential *can* vary with time. In all cells the membrane potential is generated through the action of the **Na^+/K^+-ATPase** (see Topic E3), with a high concentration of K^+ inside the cell and a high concentration of Na^+ outside. Neurons vary their electric potential by controlled changes in the **permeability** of the plasma membrane to Na^+ and K^+ ions. Upon stimulation, the membrane potential of a neuron rises rapidly from the **resting potential** of –60 mV (millivolts) to approximately +40 mV (*Fig. 2a*); the membrane is said to **depolarize** and an **action potential** is generated. In order for this to occur, the membrane potential has to be depolarized beyond a critical **threshold level** (approximately –40 mV). With time, the membrane potential returns to the resting potential. The action potential is propagated along the axon starting from the axon hillock (*Fig. 1*).

The action potential arises from large, transient changes in the permeability of the plasma membrane of the neuron to Na^+ and K^+ ions. Two types of **voltage-sensitive ion channels** are present in the membrane: one is selectively permeable to Na^+ ions, the other to K^+ ions (*Fig. 3a*). These **integral membrane proteins** (see Topic E2) are sensitive to the membrane potential, undergoing

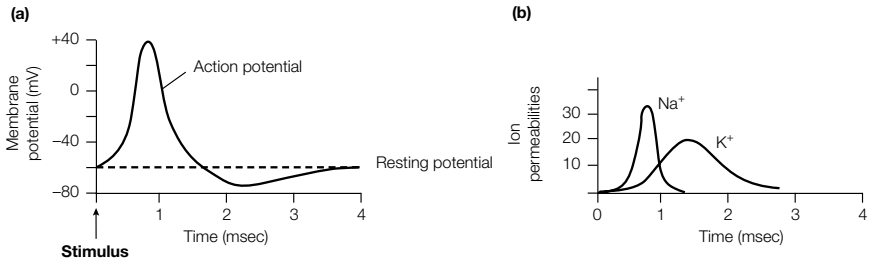

Fig. 2. The action potential. (a) Depolarization of the membrane potential; (b) changes in the permeability of the plasma membrane to Na^+ and K^+.

conformational changes as the potential alters (see Topic E3). First, the conductance of the membrane to Na^+ changes. Depolarization of the membrane beyond the threshold level causes a conformational change in the Na^+ channel, allowing Na^+ ions to flow down their concentration gradient from the outside of the cell into the interior (*Fig. 3b*). The entry of Na^+ further depolarizes the membrane, causing more Na^+ channels to open, resulting in a rapid influx of Na^+ and a change in the membrane potential from –60 mV to +40 mV in a millisecond. The Na^+ channels then spontaneously close, and the K^+ channels open, allowing K^+ ions to flow out of the cell and restore the negative resting potential within a few milliseconds (*Fig. 3c*). The wave of depolarization is propagated along the axon

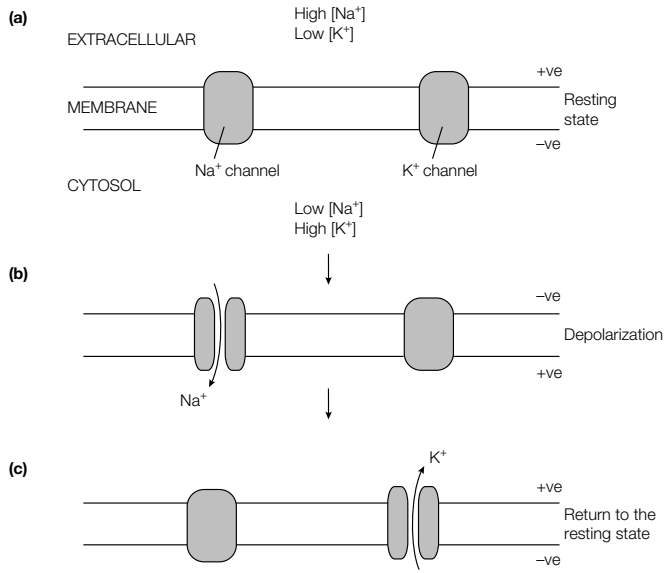

Fig. 3. Mechanism of depolarization of the nerve membrane by the opening and closing of selective Na^+ and K^+ ion channels.

by the opening of Na^+ channels on the nerve terminal side of the initial depolarized region (*Fig. 4*). The action potential can only move in that direction as the Na^+ channels have a **refractory period** when they are insensitive to further stimulation. Only approximately one in a million of the Na^+ and K^+ ions in a neuron flow across the plasma membrane during the action potential. Thus, this is a very efficient way of signaling over long distances.

The neurotoxin, **tetrodotoxin**, a highly potent poison from the puffer fish, blocks the conduction of nerve impulses along axons and so leads to respiratory paralysis by binding very tightly to the Na^+ channel and blocking its action.

Neurotransmitters When the action potential reaches the nerve terminal it causes the release of a **chemical neurotransmitter** from the synaptic vesicles. The mammalian nervous system employs numerous substances as neurotransmitters. These include the **amino acids** glutamate and glycine, **acetylcholine**, the **biogenic amines** such as **epinephrine** and **dopamine**, and a variety of **small peptides** such as the **enkephalins**. For example, acetylcholine is stored in **synaptic vesicles**, a specialized form of secretory vesicle, and is released into the **synaptic cleft** by **exocytosis** (see Topic E4) in a Ca^{2+}-dependent manner (*Fig. 5*). The acetylcholine molecules then diffuse across to the plasma membrane of the **post-synaptic cell** where they bind to specific **receptors**. The acetylcholine receptor is a 250 kDa complex of four polypeptide chains that forms a **gated channel** through the membrane (see Topic E5). On binding of two acetylcholine molecules, the channel opens, allowing Na^+ and K^+ ions to flow in and out of the cell, respectively. The resulting depolarization of the post-synaptic membrane initiates a new action potential in that cell. The acetylcholine in the synaptic cleft is rapidly broken down by the enzyme **acetylcholinesterase** which is the target of compounds such as **diisopropylphosphofluoridate** (see Topic C4 for its structure and mechanism of action), used as a component of some nerve gases.

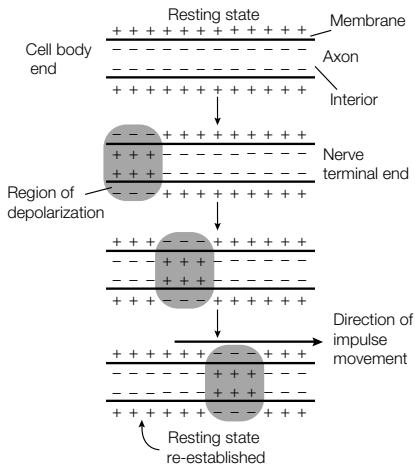

Fig. 4. Propagation of the action potential along an axon.

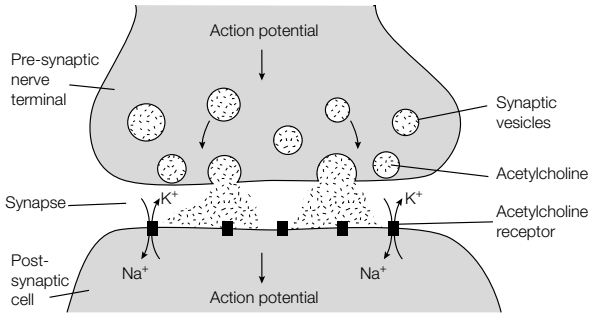

Fig. 5. Release of a neurotransmitter into the synaptic cleft.