PART I: PRELIMINARIES

1

ELEMENTS

Origins: great antiquity - RNA and DNA - 'selfish' genes. **Allosteric effectors**: conformational change - co-operative allostery - protein kinases and phosphatases. **Membranes**: lipids - fluid-mosaic structure - proteins - mobility. **Receptor molecules**: 7TM conformation - molecular structure - G-protein binding - desensitisisation. **Membrane signalling systems**: G-proteins - structure - collision-coupling biochemistry - various effectors - various second messengers. **Channels and gates**: TRPs, LGICs and VGICs - Na^+-channel, structure and functioning. **Concluding remarks**: ubiquity of molecular elements.

Three and a half thousand million years ago the first precursors of the prokaryocytes originated in already ancient oceans. Even before that time primitive self-replicating molecules had appeared in the primordial broth. It is likely that these earliest replicators were RNA rather than DNA. This is because some forms of RNA (the ribozymes) are known to have enzymic activity and replication proceeds more rapidly when enzyme-assisted. Although DNA replication is more efficient, it always depends on protein enzymes. These could hardly have been present in the primitive oceans. In consequence the more complex process of DNA replication must have evolved later. But whether with RNA or DNA, and *a fortiori* with the latter, effective replication depends on a multiplicity of molecules. It follows that these molecules must be kept in one another's vicinity. It may be that to begin with they were adsorbed on some common surface, perhaps a clay. But the most effective means of keeping a society of interacting molecules together is to enclose them in a tiny bag or vesicle.

The simplest contemporary prokaryocytes are the mycoplasmas. The smallest are only 0.3 μm in diameter and consist of no more than about 750 different types of protein. But even these tiny cells are more advanced than the protocells of three and a half billion years ago. For, in common with all contemporary cells, they use DNA rather than RNA as their hereditary material. But whether it is the simplest of living cells or their hypothetical ancestors that are being considered, one thing stands out: they stand out. They stand out from their surrounding environment. Their boundary membranes separate an 'internal' from an 'external' environment. Philosophically-inclined biologists trace the origins of the individual to this primordial period.

All organisms live in an environment. All organisms respond in one way or another to that environment. This is what distinguishes them from inanimate objects. It is clear that the boundary membrane between organism and environment must play a crucial role. It is here that specializations develop which are able to detect advantageous and disadvantageous changes. In other words, it is here that the simplest sensory systems originate. Informed of change in the external environment, the organism

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can react to its own advantage. Advantageous, that is, for the prospects of that organism leaving viable representatives in the next generation. Ultimately this can be traced down to the replicating molecules. Those which replicate most efficiently, that is leave more replicants in the next generation, mop up the available resources and survive.

Let us begin at the beginning; let us look at the elements from which sensory systems are constructed.

1.1 ALLOSTERIC EFFECTORS

Textbooks of biochemistry and/or molecular biology show that enzymic proteins have a complex three-dimensional structure. The covalently-bonded primary structure consisting of one or more amino acid chains is twisted into intricate conformations, the so-called secondary & tertiary structures. These structures are stabilized by numerous 'weak' forces: hydrogen bonds, Van der Waals and hydrophobic forces and so on. It should be stressed that these forces are individually weak. Whereas single covalent bonds have energies of about 100 kcal/mole (double and triple bonds have correspondingly higher energies), hydrogen bonds have energies of only 1 to 5 kcal/mole whilst hydrophobic and the very short range van der Waals forces are weaker still at only some 1 kcal/mol. But although they are very weak compared with covalent bonds, they are often very numerous. Large numbers of these weak forces hold the complex weave of a protein molecule in place.

This does, however, mean that the 'higher' structure of enzymic proteins is very fragile and easily disrupted. It also means that the structure can often shift from one stable conformation to another. It is this feature which underlies the phenomenon of **allostery**. In essence this means that when a molecule (or **ligand**) binds to one site on a protein's surface, it causes a conformation change which unmasks an active site somewhere else on its surface. In a sense this can be seen as the most primitive of all sensory systems. The protein molecule alters its behaviour in response to some factor in its environment (Figure 1.1).

Allosteric transitions play such crucial roles that they have been said to underly all cell biology. We shall meet them time after time in the following pages. Frequently such allosteric transitions occur in proteins consisting of more than one subunit. In these cases when a ligand binds to the allosteric site on one subunit, it causes a change which facilitates binding of ligands to allosteric sites on all the other subunits. This is known as **cooperative allostery** and can create a much stronger alteration in the behaviour of the allosteric protein.

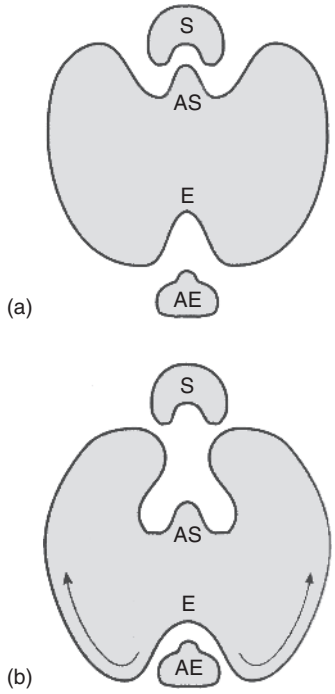

Figure 1.1 Conceptual diagram to show the effect of an allosteric effector on the activity of an enzyme. AE = allosteric effector; AS = active site; E = enzyme; S = substrate. When AE binds to the enzyme a change is induced in the latter's three-dimensional conformation (symbolized by arrows) so that AS is no longer accessible to the substrate molecule (S).

One of the most significant means of causing allosteric transitions is phosphorylation. We shall meet this mechanism again and again as we study the molecular bases of sensory systems, so it is worthwhile looking at it a little more closely here. The phosphorylation reaction is catalysed by a **protein kinase**. Protein kinases form a large family of several hundred members all of which share a 250 amino acid catalytic domain. The basic reaction is to transfer a phosphate group from ATP to a hydroxyl group on the side chain of an amino acid in the substrate

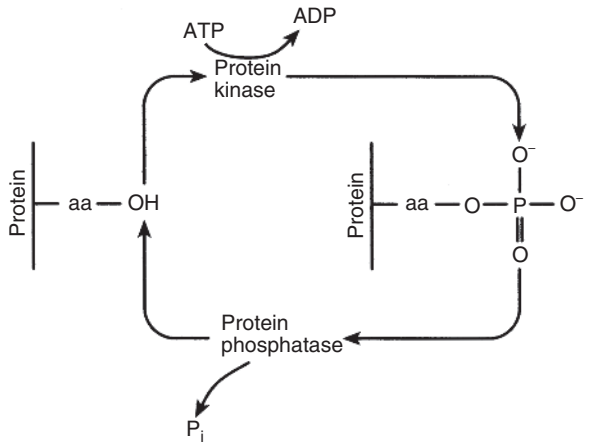

Figure 1.2 A protein chain is shown with an amino acid (serine, threonine or tyrosine) side chain: aa. Protein kinase uses a phosphate group from ATP to phosphorylate the side chain. Protein phosphatases later dephosphorylate the side chain.

protein. Only three amino acids – **serine, threonine and tyrosine** – present hydroxyl groups in their side chains, so only these amino acids are affected. The reaction is shown diagrammatically in Figure 1.2.

Whilst protein kinases in phosphorylating their substrate protein cause allosteric transitions, they are themselves subject to allosteric control. We need not in this book follow the biochemistry any further. The cascade of control mechanisms reaches deep into the biochemistry of the cell. But it is worth noting that other enzymes, the **protein phosphatases**, are present in the cytosol to undo the work which the kinases have done (Figure 1.2). These enzymes remove the phosphate from the substrate protein thus allowing it to relax back into its original conformation.

1.2 MEMBRANES

A second 'element' upon which all sensory systems are built is the biomembrane. Although the earliest membranes to form in primeval times may have been built of amino acids, all contemporary biological membranes (or biomembranes) consist of lipid bilayers with protein insertions. In addition, most membranes also contain carbohydrate. The lipids form a matrix or scaffolding in which the proteins are embedded, whilst the carbohydrates (where they exist) are attached either to the lipids (glycolipids) or to the proteins (glycoproteins) (Figure 1.3).

1.2.1 Lipids

It can be seen (Figure 1.3) that lipids form a bimolecular sheet. They fall into three major groups: phospholipids, glycolipids and steroids (especially cholesterol). There is no need to discuss their detailed structure in this book (standard biochemistry texts all provide good accounts). It is worth noting, however, that they are all *amphipathic* molecules, that is they are partially soluble in water and partially in organic solvents. A typical membrane lipid will have one end bearing an electrostatic charge, so that it can enter the water structure, and the other end covalently bonded with no electrostatic charges, so that it is at home in organic solvents. As both the extracellular and intracellular media are overwhelmingly aqueous, it follows that membrane-forming lipids line up with their hydrophilic heads projecting into the aqueous environment and their hydrophobic 'tails' facing inwards toward each other away from the watery exterior or interior. Some typical membrane lipids are shown in Figure 1.4.

From what has been said, and from Figures 1.3 and 1.4, it is clear that biological membranes are very delicate structures. Their constituent lipids are held in position by hydrophobic forces and by occasional electrostatic attractions between their 'head' groups. The extremely tenuous nature of the phospholipid bilayer means that at room temperature the individual molecules are in continuous motion. Indeed, the hydrophobic fatty-acid tails of the molecules have been likened to a basketful of snakes, squirming about in perpetual motion. The interior of the membrane is thus, to all intents and purposes, an organic fluid. We shall see that the extreme fluidity of the lipid matrix of biomembranes is of considerable significance in sensory systems when we come to consider G-protein signal transduction.

Not all the lipid constituents of biomembranes are, however, as labile as the phospholipids. Cholesterol, in particular, is a very different type of molecule. As shown in Figure 1.4, it consists of three different regions: a hydrophilic 'head' consisting of the hydroxyl

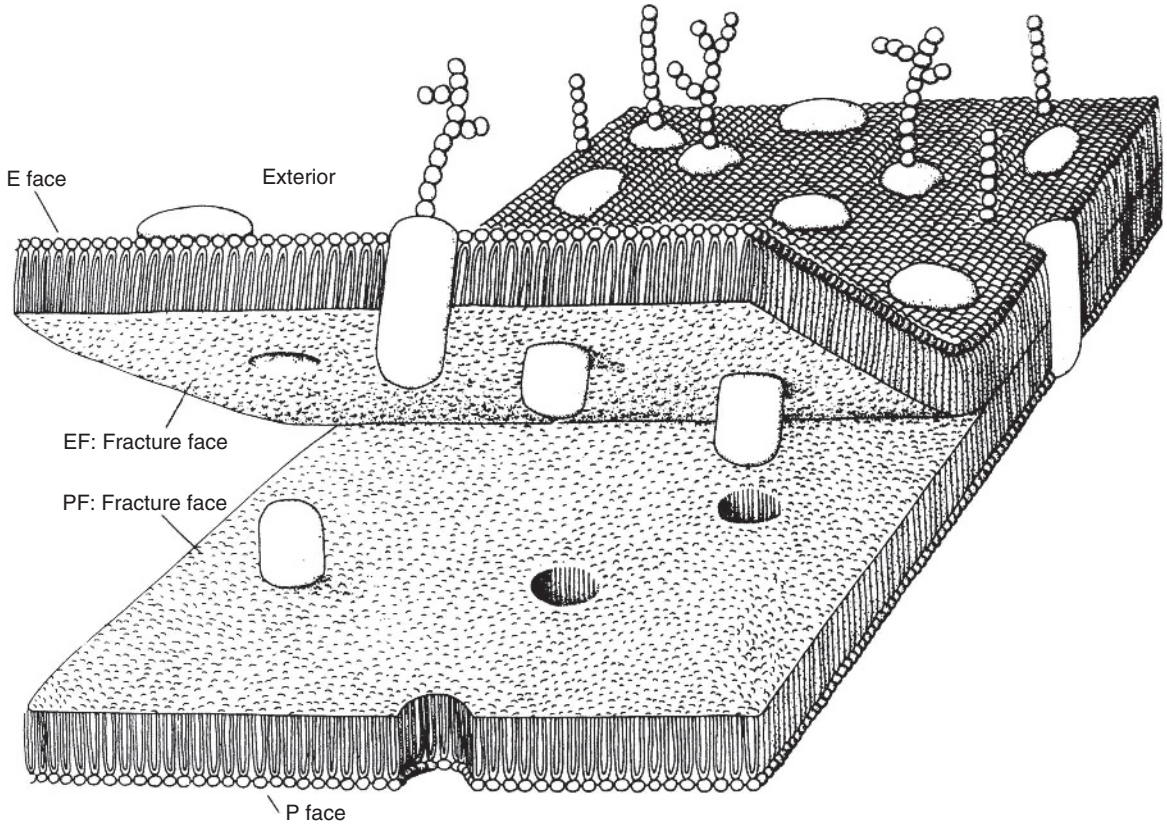

Figure 1.3 In this diagram the membrane has been quickly frozen to the temperature of liquid nitrogen and then broken open. The fracture plane runs along the centre of the lipid bilayer. The figure shows how proteins are embedded in the membrane and also the position of carbohydrate chain ('strings of sausages') projecting from the external face. Reproduced by permission of Executive of the Estate of Bunji Tagawa from Birgit Satir, 'The final steps of secretion', Scientific American, October 1975, p. 33.

group, a flat plate-like steroid ring and a flexible hydrophobic tail. The quantity of cholesterol present in biomembrane varies considerably. When it is present it stiffens the membrane and reduces its fluidity.

The fluidity of a membrane is, in fact, determined not only by the quantity of cholesterol that it contains but also by the length and saturation of the fatty acids that form its core. In artificial membranes formed of a single lipid species there is a sharp 'transition temperature', characteristic of the particular phospholipid, when the membrane changes from a fluid into a gel state. This temperature varies from place to place in a natural biomembrane depending on the amount of cholesterol and the saturation of the phospholipid 'tails'. A natural membrane can thus be envisaged as a mosaic of different fluidities.

1.2.2 Proteins

Embedded in this patchwork quilt of a membrane are the proteins. Although glycolipids (such as the cell adhesion molecules) are of great importance in intercellular recognition, the most important functional characteristics of biomembranes are conferred not by their lipids but by their proteins. The quantity of protein present varies from about 20% of the mass (myelin) to about 75% of the mass (mitochondrial inner membrane). Most membranes contain about 50% protein by mass.

The majority of proteins (as shown in Figure 1.4) are embedded in the membrane. They 'float' like icebergs in the variable phospholipid 'sea', or, to put it another way, they form a mosaic in the fluid

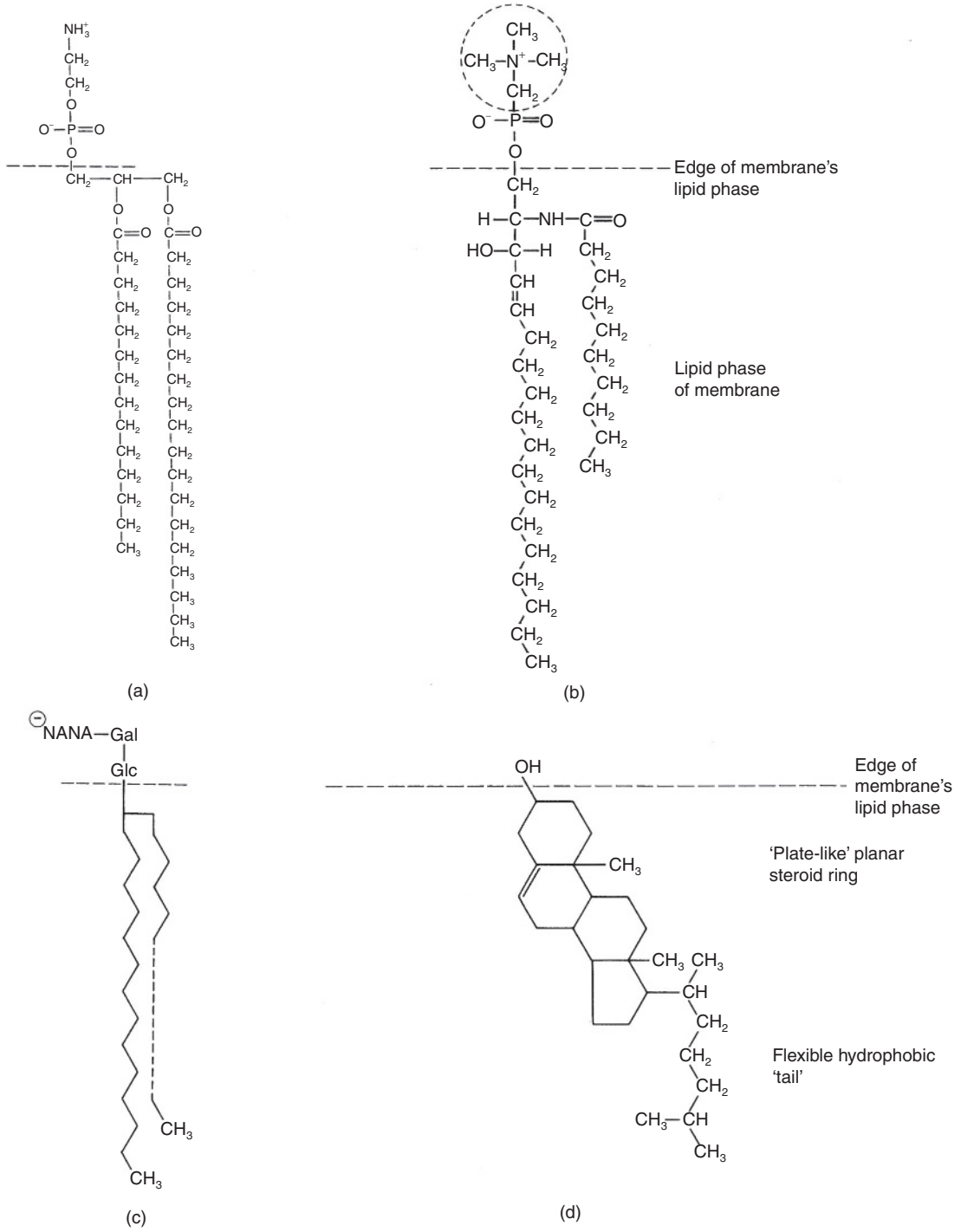

Figure 1.4 Some common membrane lipids. (a) Phosphatidyl choline (lecithin); (b) Sphingomyelin; (c) a ganglioside (the dashed line represents a lengthy CH_2 chain); (d) Cholesterol. Gal = galactose; Glc = glucose; NANA = *N*-acetylneuraminic acid.

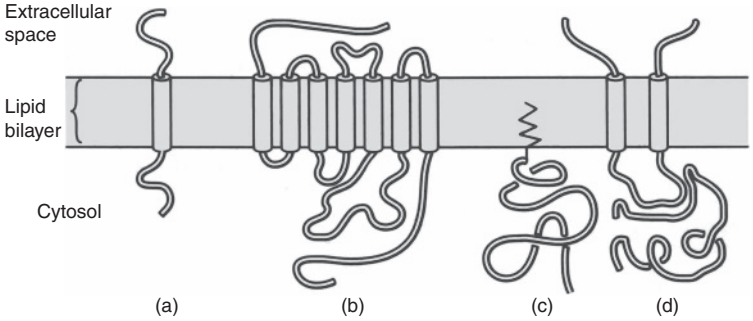

Figure 1.5 Some of the ways in which proteins are associated with membranes. The intramembranous cylinders represent alpha helices. (a) A single alpha helix passes through the membrane; (b) a number of alpha helices pass and repass the membrane; (c) the protein is held to the cytoplasmic leaflet of the bilayer by a fatty acid chain or prenyl group (significant examples of this are provided by G-proteins); (d) a membrane embedded protein is non-covalently attached to another protein in the cytosol.

phospholipid matrix. This concept is, for this reason, called the 'fluid-mosaic' model. In most cases the proteins project all the way through the bilayer and extend into both the intracellular and extracellular spaces. In other cases the proteins are attached to the membrane by a fatty acid chain, phospholipid or prenyl group. In these latter cases the protein itself is located in the cytosol. Some of these various means of attachment are shown in Figure 1.5. We shall see that membrane-bound proteins form the basic elements of all sensory receptors.

Transmembrane proteins are constructed in such a way that they have hydrophobic domains embedded in the membrane and hydrophilic domains projecting into the aqueous intracellular and/or extracellular compartments (Figure 1.5). In comparison with globular proteins of the aqueous cytosol the intramembranous domains of membrane proteins are, in a sense, inside out: their hydrophobic amino acid residues point outwards, their hydrophilic residues are tucked inside toward their cores. This ensures that the proteins stick in the membrane. Very commonly, as shown in Figure 1.5, the intramembranous domains consist of alpha-helical segments. Again, the vast majority of the amino acid residues making up these intramembranous alpha-helices are hydrophobic.

Studies which involve the incorporation of enzymatic proteins into artificial lipid bilayers show that the activity of such proteins is conditioned by their lipid environment. Features of the bilayer such as length of fatty acid chains, degree of saturation and the nature of the lipid 'heads' all influence the biological activity of the enzyme. Just as water-soluble enzymes are affected by features of the aqueous environment (pH, salt concentration, etc.), so lipid-embedded enzymes are affected by the precise nature of their lipids which surround them.

1.2.3 Mobility of Proteins

We have already likened membrane proteins to icebergs floating in a lipid sea. It is not surprising, then, to find that many have considerable lateral mobility. In the next section we shall see that this mobility has been pressed into service, with great effect, in the development of signalling systems based on proteins shuttling in the plane of the membrane. Protein diffusion coefficients range from about 10^{-9} cm^2/s for visual pigments in rod-cell outer-segments to about 10^{-11} cm^2/s for proteins in other membranes. In the first case a protein would travel about 0.1 μm (0.1 micron) per second, in the latter case 0.001 μm/s. There are a number of reasons for these great differences in mobility. First, it may be due to the differential lipid constitution of the membrane which, as indicated above, affects its fluidity. Or it may be that the protein is part of a large multiplex of other proteins and thus rendered too bulky to move easily. Alternatively, it may be impeded by structures external to the membrane, cell junctions, desmosomes, tight junctions and so on. Last, but far from least, it may be that the membrane protein is anchored to an element of the submembranous cytoskeleton.

1.3 MEMBRANE SIGNALLING SYSTEMS

The boundary membranes of cells, located as they are between the external environment and the internal environment of the cell's cytosol, have developed

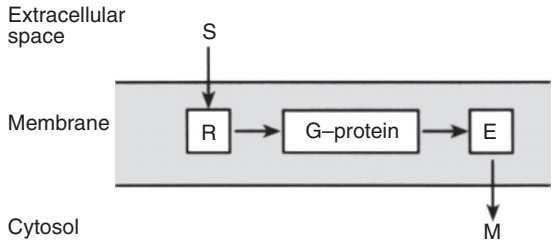

Figure 1.6 Schematic diagram to show G-protein signalling system. S = stimulus; R = membrane receptor; E = effector (enzyme, ion channel, etc.); M = second messenger.

many biochemical mechanisms which 'transduce' external events into 'messages' released into the cytosol. Most of these 'mechanisms' take the form of protein molecules embedded in the membrane. Some are ion channels which open directly in response to mechanical stimuli, others depend on the mobility of other proteins in the biomembrane. In essence the latter depend on the presence of a receptor molecule which can recognize an environmental stimulant and a means of transforming this recognition into a message which can diffuse into the receptor cell's cytosol.

As this type of receptor is of such importance and ubiquity, let us examine it in some detail. The system consists of three parts: the receptor molecule itself, a means of signalling its activation to a membrane-bound 'effector' and the effector itself. The system is shown schematically in Figure 1.6.

1.3.1 Receptor Molecules

The most important type of receptor molecule is the so-called '7TM' 'serpentine' receptor. It is called a 7TM receptor as it makes seven passes through the membrane or, in other words, has seven transmembrane domains (Figure 1.7). Its sinuous course through the membrane is also reminiscent of a serpent. 7TM receptors are found not only in receptor cells but also in the subsynaptic membranes of metabotropic synapses. Many, but not all, 7TM receptors are evolutionarily related. They form an extremely large superfamily of proteins, indeed it has been found that some 2% of the mamamalian genome codes for these proteins.

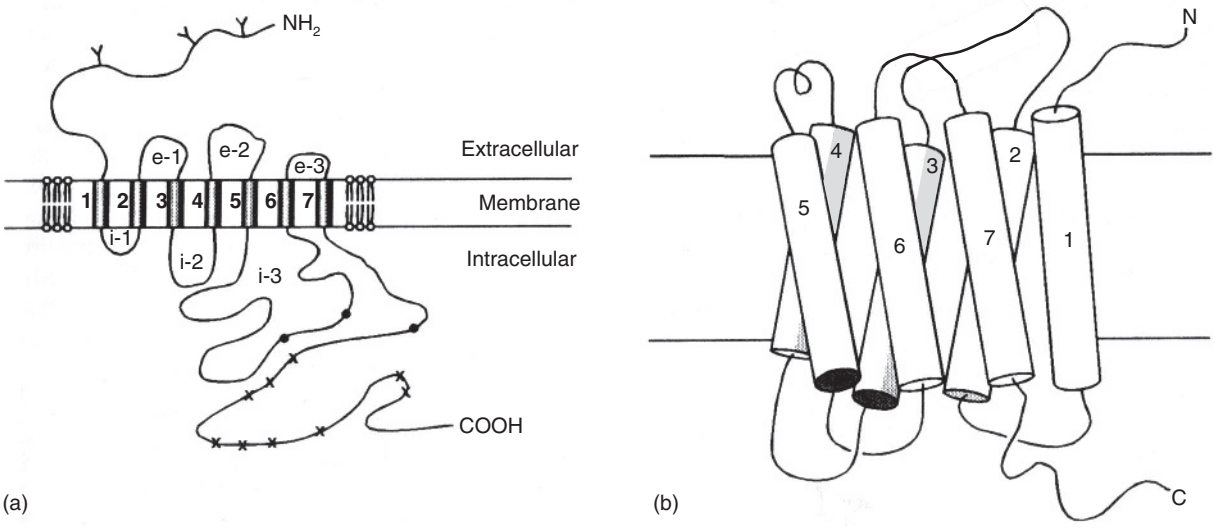

Figure 1.7 Architecture of a 7TM receptor. (a) Schematic view: the seven transmembrane (TM) helices are shown as columns in the membrane numbered 1–7. The N-terminal sequence is extracellular and normally has carbohydrate sequences attached (glycosylated). This is represented by Y's. The extracellular loops are labelled e-1, e-2, e-3 and these may also sometimes by glycosylated. The intracellular loops i-1, i-2, i-3 provide recognition surfaces for specific G-proteins. The dark spots signify phosphorylation sites for protein kinase and the crosses represent sites which specific desensitising protein kinases affect. (b) Three-dimensional conformation of the receptor in the membrane.

The plan view of Figure 1.7a shows how the seven transmembrane segments traverse the membrane. It also shows that there are also large extracellular and intracellular domains. Figure 1.7b shows that, when looked at in three dimensions, the seven transmembrane segments form the pillars of a hollow column, orientated rather like a 'barrel of staves'.

The 7TM receptors not only share a common architectural theme but also a common membrane-bound means of signal amplification. This mechanism (as we shall see in the next section) capitalizes on the lateral mobility of proteins, in this case G-proteins, in biomembranes and on the fact that the lipid bilayer holds such proteins in close proximity to each other so that they cannot diffuse away into the cytosol. Because 7TM receptors are almost always coupled to a G-protein system they are often known as G-protein coupled receptors (*GPCRs*). Careful structure–function analysis of the 7TM receptors shows that the first, second and third cytoplasmic loops (i-1, i-2 and i-3) and the carboxy-terminal tail (Figure 1.6a) are crucial to G-protein binding, with the third loop particularly involved in the recognition of particular G-proteins. Ultimately, when a receptor has been overexposed to its agonist, it becomes markedly less responsive. This is known as **desensitization**. We shall come across it many times in the following pages. Desensitization is brought about by specific protein kinases (Section 1.1) phosphorylating the hydroxyl groups of serine, threonine and tyrosine residues in the carboxyterminal tail of the receptor. These residues are symbolized by crosses in Figure 1.7a. They are additional to serine, threonine and tyrosine residues, which are affected by by nonspecific protein kinase (symbolized by spots). These phosphorylations, as we saw in Section 1.1, alter the three-dimensional conformation of the receptor. Sensitivity is restored by dephosphorylation by one of the many phosphatase enzymes that populate the cytosol.

1.3.2 G-proteins

When a 7TM receptor molecule located in the membrane of a sensory cell is activated by some change in the external environment it undergoes a **conformational change**. Recent successes in analysing the type-example of a GPCR, the β_2-adrenergic receptor, at a 3.4/3.7 Å resolution has allowed molecular biologists the first detailed look into the precise chemical mechanisms which produce this change. The conformational change triggers an allosteric change in the associated **G-protein (Guanine-binding protein)** causing it to release its bound GDP which is quickly replaced by GTP. Once this has happened the G-protein complex splits into its constituent units (see below), which are released to travel in the membrane to activate an effector molecule also located in the membrane. This often (not always) leads to the release of a second messenger into the cytosol. The process is shown schematically in Figure 1.9.

The G-proteins involved in signal transduction are members of another large superfamily of proteins. They have been described as precisely engineered time-switches which can turn on and off the activity of other molecules. All the G-proteins are switched 'on' by binding to GTP and switched 'off' by the hydrolysis of GTP to GDP. This hydrolysis is catalysed by the GTPase activity of the G-protein itself. The process is comparatively slow, ranging from a few seconds to a few tens of seconds.

The G-proteins of biological membranes all have a heterotrimeric structure. They consist of a large alpha subunit (circa 45 kDa) and smaller beta and gamma subunits (Figure 1.8). The alpha subunit possesses the GTPase activity and in the inactive (or 'off') state it holds a GDP molecule in its active site. The smaller beta and gamma subunits are bound closely together; indeed it is impossible to separate them in physiological conditions. In the inactive state the beta–gamma complex is firmly attached

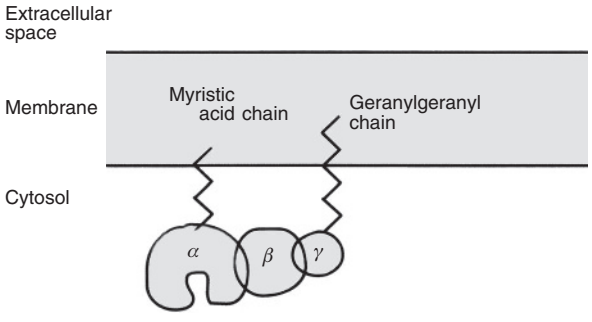

Figure 1.8 Conformation of a heterotrimeric membrane-bound G-protein. The α-subunit is shown with a cavity representing a site for GDP or GTP.

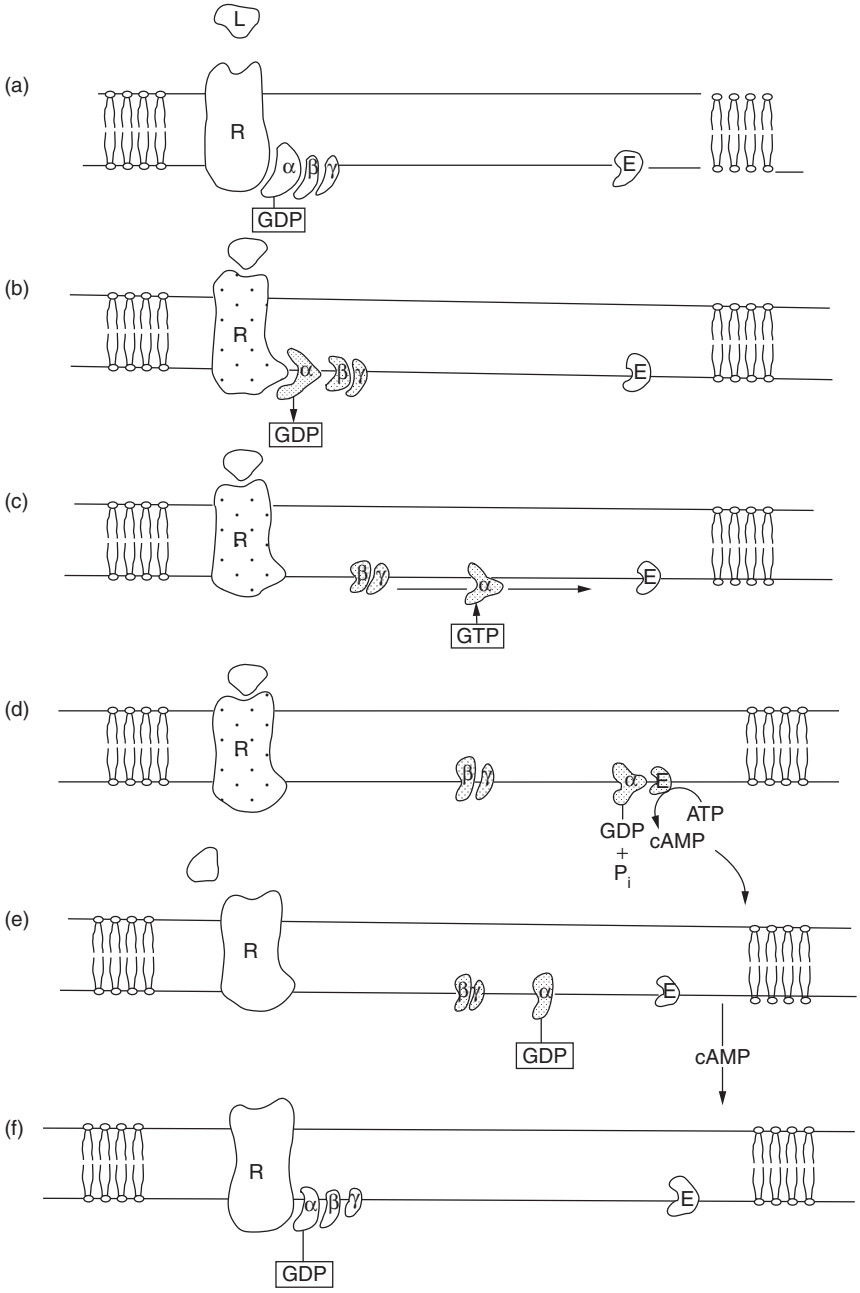

Figure 1.9 G-protein signalling system in a biological membrane. (a) Resting phase. (b) Ligand attaches and activates receptor. This causes a conformational change which releases the α-subunit of the G-protein. It also causes a conformation change in this subunit so that GDP is released from its binding site and the $\beta\gamma$-dyad set free. (c) The α-subunit is activated by accepting GTP and travels to the effector. (d) The α-subunit docks with and activates the effector which, in turn, catalyses the production of a second messenger, for instance cAMP, which diffuses into the cytosol. The α-subunit also catalyses the dephosphorylation of GTP to GDP and Pi. (e) Dephosphorylation of GTP deactivates the α-subunit which consequently detaches from the effector. (f) The receptor is deactivated by loss of its ligand and by other biochemical reactions (see text) and the receptor-G-protein complex is reformed. Stippling = activation; E = effector; L = ligand; R = GPCR.

to the alpha subunit. The gamma subunit is linked to the cytoplasmic leaflet of the biomembrane by a 20 carbon geranylgeranyl tail (related to cholesterol) and the alpha subunit is also linked into the membrane by a 14 carbon fatty-acid (myristic acid). These linkages ensure that the G-protein complex is both held in the plane of the membrane and is also able to move easily in that plane.

Alpha subunits are very variable. Those found in the olfactory system are, for example, distinctively different from those in the visual system. The beta and gamma subunits are less heterogeneous; even so, a number of different types are known. This molecular heterogeneity makes it possible to 'design' flexible and well-adapted signalling systems. We shall discuss this further when we meet with specific instances in later chapters.

When a GPCR is activated, it undergoes a conformational change which is transmitted to the alpha subunit of its attached G-protein complex. This, in turn, undergoes an allosteric transition releasing its GDP, separating from the receptor and from its beta and gamma subunits. As there is normally a plentiful supply of GTP in the cytosol, it diffuses in to occupy the vacant site on the alpha subunit. The beta–gamma complex goes its separate way. The alpha subunit with its attached GTP is now able to interact with an 'effector' in the membrane, an enzyme such as adenylyl cyclase, perhaps, or phospholipase C-β (PLC-β) or an ion channel such as TRP or mAChR. The enzyme may be switched on or off, the ion channel up or down regulated. Again we shall look at specific examples in later chapters. But this interaction only lasts so long as the alpha subunit retains its GTP. But, as we noted above, the alpha subunit is in fact a GTPase. So, very soon, the GTP attached to the alpha subunit is hydrolysed to GDP. When this happens, the alpha subunit changes its conformation once again and can no longer activate the effector. Soon the alpha-GDP meets up with the beta–gamma dyad, forms once again the timeric complex and reattaches to the deactivated receptor, thus completing the cycle (Figure 1.9).

Until recently it was believed that the beta–gamma complex played no real part in membrane signalling. That assumption is now being questioned. There is evidence that the complex may have an independent role. It is possible that it is able to inhibit the activity of free alpha subunits, or it may have an independent effect on membrane effectors.

1.3.3 Effectors and Second Messengers

There are various classes of effector molecule: cyclase enzymes, phospholipases, phosphodiesterases, membrane channels. We shall meet instances of all these effectors in this book. Similarly, there are various types of second messenger: cAMP, cGMP, inositol triphosphate (IP_3 or $InsP_3$), diacylglycerol (DAG) and the ubiquitous Ca^{2+} ion. In this section we examine only two classes of effector, the adenylyl cyclases (ACs) and phospholipase C-β (=PIP_2-phospholipase) both of which engender important 'second messengers'. We shall consider the role of phosphodiesterases and membrane channels and the other second messengers as appropriate in later parts of this book.

1.3.3.1 Adenylyl Cyclases

These enzymes catalyse the formation of cAMP: a ubiquitous and, perhaps, the most important 'second messenger' in animal cells. In turn, the most important role of cAMP is to activate the cAMP-dependent protein kinase (PKA). Once activated this multimeric enzyme phosphorylates (with the help of ATP) one or other of the many biologically active proteins present in the cell - enzymes, receptor and channel proteins, nuclear histones, transcription factors. The phosphorylation is normally of a serine, threonine or tyrosine residue, and the effect is either to inhibit (note the desensitization of G-coupled receptors already mentioned above) or activate the protein. Dephosphorylation back to the original status is by one of the many phosphatase enzymes with which the cytosol abounds.

Molecular biological techniques have shown there to be at least six different adenylyl cyclases in mammalian cells. All have a molecular weight of about 120–130 kDa and examination of hydrophobic sequences indicates that there are twelve transmembrane segments. The six cyclases differ in their sensitivity to the beta–gamma complex of G-proteins and to the calcium binding protein, calmodulin. Type 1 AC is, for instance, stimulated by Ca^{2+}calmodulin

and inhibited by the beta–gamma dimer whilst Type 2 AC does not respond to the first and is stimulated by the second.

1.3.3.2 Phospholipase C-β (=PLC-β or PIP_2-phospholipase)

The activation of this second important effector results in the production of two second messengers: inositol triphosphate (IP_3) and diacylglycerol (DAG). Both these second messengers are derived by the cleavage by PLC-β of the phospholipid, phosphatidyl-inositol 4,5 biphosphate (PIP_2), which is predominantly located in the inner leaflet of the plasma membrane (Figure 1.10).

Figure 1.10 shows a membrane receptor picking up some external signal that leads via a G-protein mechanism to the activation of membrane-embedded PLC-β. This then reacts with PIP_2 to produce IP_3 and DAG. IP_3 is a water soluble molecule and hence it readily diffuses away into the cytosol. Here it may interact with receptors in the membranes of the endoplasmic reticulum (ER) leading to a release of Ca^{2+}. These ions have many and varied effects on cellular biochemistry. Ultimately IP_3 is inactivated by inositol triphosphatase. DAG, on the other hand, is hydrophobic and hence remains behind in the membrane.

We have not finished with the system yet. For the DAG left behind also has a job to do. It interacts with other membrane-bound proteins. There are two important cases: protein C kinase (PKC) and the transient receptor protein (TRP). These reactions are Ca^{2+}-dependent. Consequently, when the Ca^{2+} concentration of the cytosol rises (an effect, as we have just seen, of IP_3) DAG activates PKC and/or TRP. In the case of PKC, the activation requires the presence of phosphatidyl serine as well. This phospholipid is also located in the membrane's inner leaflet. The aroused PKC can now activate proteins that elicit specific biochemical responses. In neurons a number of effects have been demonstrated, including synthesis and secretion of neurotransmitters, alterations to the sensitivity of receptors and the functioning of the cytoskeleton. We shall outline the affect of DAG on TRP in the next section.

It can be seen from the above account that G-protein systems provide an extremely flexible means of transforming an external signal into a second messenger which can diffuse into the cytosol. The second messenger may take a number of forms (depending on the effector enzyme) but by far the most common is cyclic AMP (cAMP). Alternatively, as noted above, the alpha subunit may affect the operation of a membrane channel and this in its turn may alter the electrical polarity of the membrane.

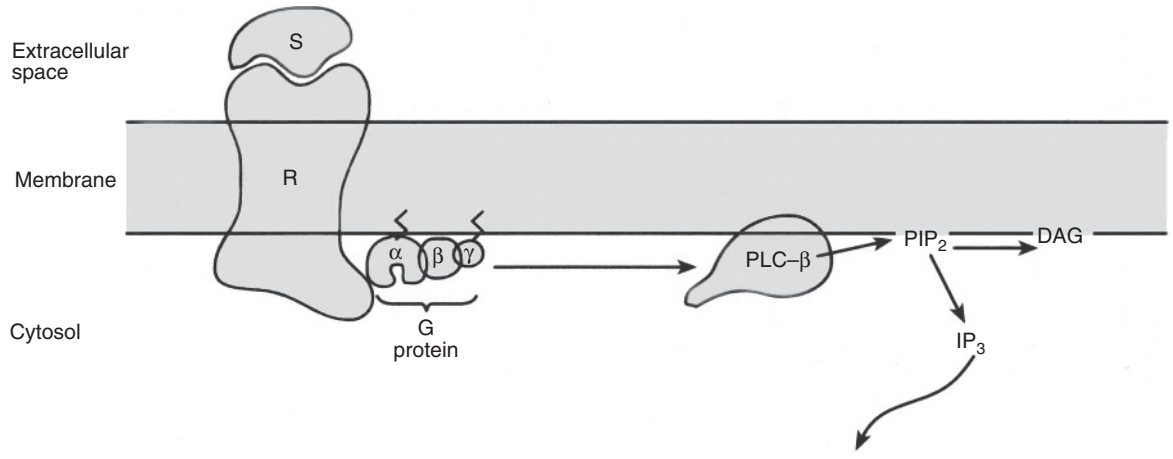

Figure 1.10 Second messenger formation via PLC-β. The activated receptor leads via the G-protein coupling system to activation of PLC-β. PLC-β catalyses the splitting of the membrane lipid PIP_2 into IP_3 and DAG. IP_3 diffuses into the cytosol; DAG remains in the membrane where it may exert further effects.

1.4 CHANNELS AND GATES

We saw above that biomembranes consist of proteins embedded in a lipid bilayer. The bilayer effectively prevents hydrophilic substances crossing the membrane. Embedded proteins act both as pumps and gated channels. We shall see in Chapter 2 that the pumps create a concentration gradient of small inorganic ions and hence a voltage gradient across the membrane. Other embedded proteins form hydrophilic channels through which inorganic ions and other water soluble materials can travel. Some of these channels, the so-called 'leak' channels, allow ions, such as potassium ions, to flow along their concentration gradients into or out of the cell; others play a more active role and act as gates controlling these flows. When these gates open, ions flow down their electrochemical gradients and the voltage across the membrane consequently drops. This change in voltage is known as **receptor potential** and in most cases is the first sign that an environmental change has been detected.

We shall meet many examples of these channels and gates in the following pages. There are several types: those directly affected by the environmental change, those activated by ligands (ligand-gated ion channels or *LGICs*) and those controlled by the voltage across the membrane (*VGICs*). The very large superfamily of TRP channels contains members which are responsive to all three types of stimuli.

1.4.1 TRP Channels

TRP channels were first discovered in *Drosophila,* where photoreceptors carrying certain gene mutations exhibited a **transient receptor potential** to continuous light, hence the acronym *TRP*. The channels responsible for this effect, the TRP channels, were isolated and their sequences analysed and used to search for similar channels in other organisms. It turns out that TRP channels are found throughout the living world, from Archaea to humans. Evolution has pressed them into many uses. In yeast TRP channels sense hypertonicity; in Nematode worms they sense noxious chemicals and touch; in mice they detect pheromones; in humans they are involved in sensing temperature, taste and are believed to play a crucial part in detecting sound These are just a few of the rôles TRP channels play. We have already seen that they were first found playing a part in photoreception; they also have a fundamental rôle in mechanosensitivity. TRP channels are, moreover, not restricted to reacting to happenings in the external environment. They are deeply involved in responding to changes in the internal environment too. They are involved in detecting pH, osmolarity, vasodilation, growth cone guidance and numerous other aspects of the body's physiology.

Their very widespread distribution in the living world and the many uses to which they have been put indicates that they evolved very early in life's history. Indeed, they are believed to represent the most ancient of the cell's sensors. Metazoan TRPs have evolved into two major groups, Group 1 and Group 2 (Figure 1.11). Group 1 consists of five families (TRPC, TRPN, TRPM, TRPV and TRPA) while Group 2 consists of two families (TRPP and TRPML). Because their origin is so ancient and their common ancestor so far in the past, these families show rather little similarity in their amino acid sequence. That they are all members of the same superfamily of molecules is, however, shown by their sharing common tertiary and quaternary structures.

We have already noted that the tertiary structure involves six transmembrane domains (S1–S6) (Figure 1.12a). The so-called quaternary structure consists of four of these 6TM subunits, which may be similar or dissimilar, grouped around a central pore (Figure 1.12b). The architecture of this transmembrane pore is similar to that of the bacterial KcsA channel first worked out by MacKinnon and colleagues in *Streptomyces lividans*, and the total tetrameric structure is similar to that of the 6TM K^+-channels, which play such important roles in the biophysics of nerve cell membranes. Each of the subunits is orientated so that transmembrane segments S5 and S6 line the pore, forming a structure very much like an inverted wigwam, and between them provide a filter which selects cations, especially Ca^{2+} (Figure 1.12c).

It can be seen from Figure 1.12a that both N- and C-terminal ends of the polypeptide chain are located in the cytoplasm. These cytoplasmic domains are highly diverse and confer different properties on the different families. There is evidence (as yet not fully conclusive) that stretching forces in the membrane pull open the pore and that the selectivity filter

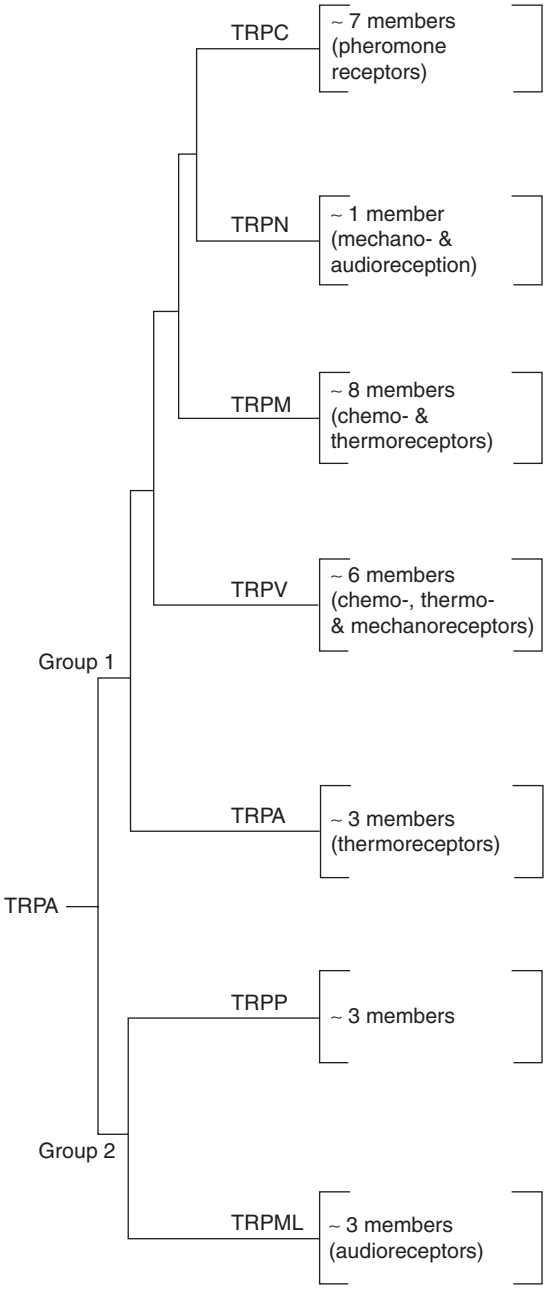

Figure 1.11 Schematic to show the evolutionary relationships of TRP channels. The number of members in each family as presently known is given and the involvement of the different TRP channels in sensory systems is indicated. Nomenclature: TRPA (ankyrin); TRPC (canonical); TRPM (melastatin), TRPML (mucolipin); TRPN (no mechanoreceptor potential C: NOMPC); TRPP (polycystin); TRPV (vanilloid)). Further explanation in text. Adapted from Venkatachalam and Montell (2007).

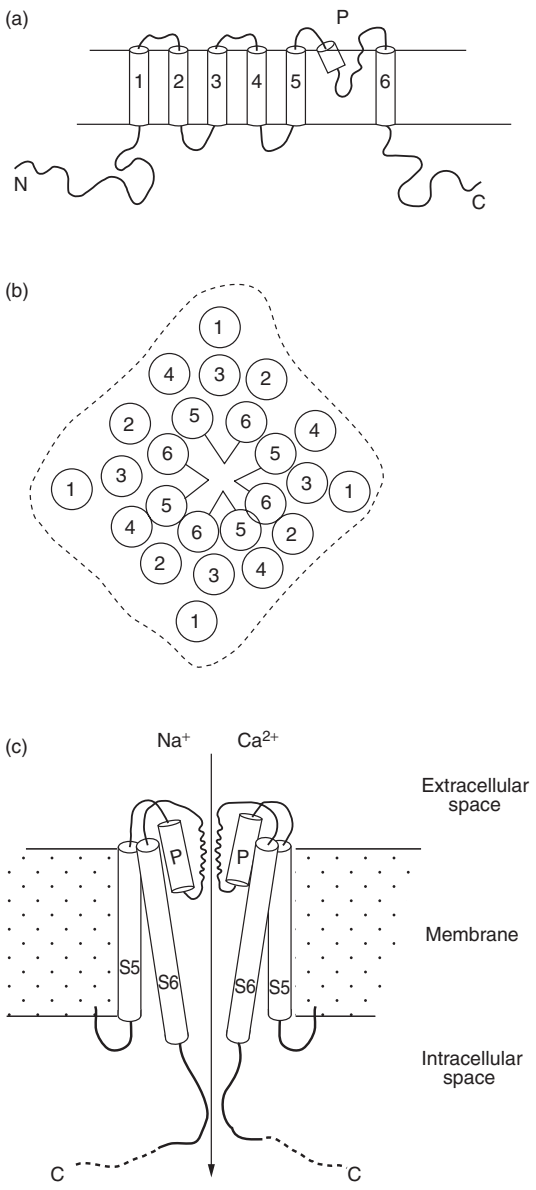

Figure 1.12 Structure of TRP channel. (a) Six helices (represented by cylinders) span the membrane. Between helix five and six there is a short helix followed by a series of amino acids (represented by zig-zag) which constitute the selectivity filter (P). (b) Plan view to show the disposition of the four subunits of the complete channel in the membrane. The pore (P) complex (represented by a 'hairpin') in each subunit faces inward to form the channel. (c) Detail of the channel. For simplicity only two of the subunits are shown and of these only the S5 and S6 transmembrane segments. The channel is a little over 5 Å in diameter and the selectivity filter consists of a group of amino acid residues represented by the 'saw-tooth' section between P and S6.

then ensures that Ca^{2+} and, to a lesser extent, Na^{+} stream inward down their steep concentration gradients. This, as we shall see in Chapter 2, would depolarize the membrane (detectable as a receptor potential). Changes in ambient temperature also open TRP channels, although the biochemical mechanism is as yet not known. Thus TRP channels are directly involved in sensing **mechanical** and **thermal** stimuli.

Some TRP channels depend on 7TM receptors detecting other stimulus modalities – chemical or electromagnetic. Once a 7TM receptor is activated by an appropriate stimulus, a collision-coupling mechanism operates via PLC-β and DAG (as indicated in the previous section) to open TRP channels (Figure 1.10).

TRP channels are rapidly shut again by Ca^{2+}, which only exists in a free state for a few microseconds within the cytoplasm before being bound. Because TRP channels are responsive to many different types of stimuli, they are thought to play an integrative role in a cell's response to its environment. We shall meet TRP channels frequently in the following pages.

1.4.2 Ligand-Gated Ion Channels (LGICs)

There are many varieties of LGIC. The most intensively investigated is the nicotinic acetyl choline receptor (nAChR). This consists of a massive (268 kDa) pentameric protein embedded in the membrane. The five subunits consist of two (461 amino acid) α-subunits, one (493 amino-acid) β-subunit, one (506 amino acid) γ-subunit and one (522) δ-subunit. Each subunit makes four transmembrane passes (Figure 1.13a) and the five subunits are compactly assembled to surround a central ion pore (Figure 1.13b). When the ligand, in this case acetylcholine (ACh) attaches to binding sites on the two alpha subunits, the channel opens and univalent cations flow long their electrochemical gradients. Charged amino acids on TM2 (Figure 1.13) line the pore and select the ions that can pass through the channel.

Many other types of LGICs are known. These are activated by a variety of ligands (5HT, glycine, GABA, etc.) and all these major types are subdivided into numerous subtypes. So far as sensory systems are concerned, the most significant LGICs are those

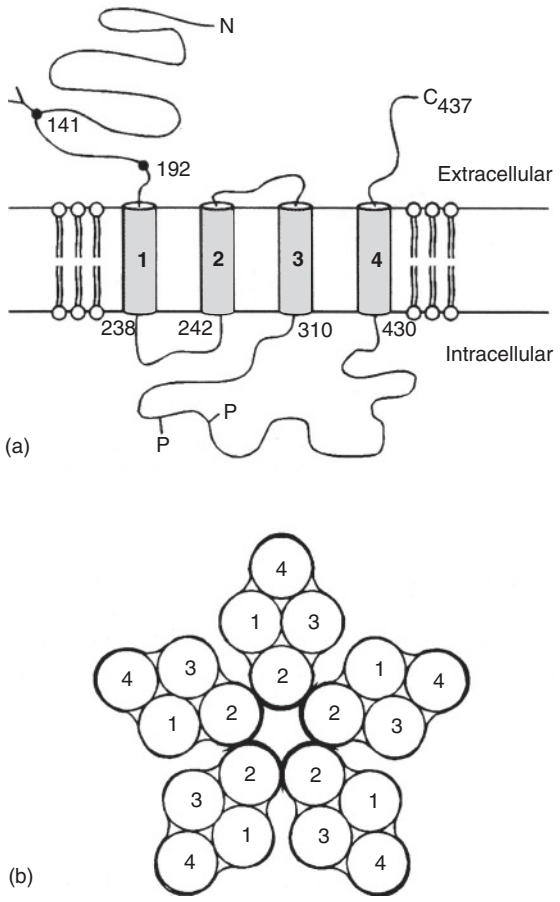

Figure 1.13 (a) Schematic view of the α-subunit of the nAChR. Four helices represented by cylinders span the membrane. Both C and N terminals are extracellular. (b) Plan view. The pentameric structure of the entire receptor is seen from above. It is believed that helix 2 of each subunit forms the lining of the pore.

found in olfactory and photoreceptor cells, which are activated by cyclic nucleotides (CNGs). They are similar to TRP channels and, like these channels, the subunit protein makes six passes through the membrane whilst the total channel consists of four of these subunits.

1.4.3 Voltage-Gated Ion Channels (VGICs)

There are also many types of VGIC. They are all activated by changes in membrane potential. They differ in the ion that they allow to pass. Thus there is

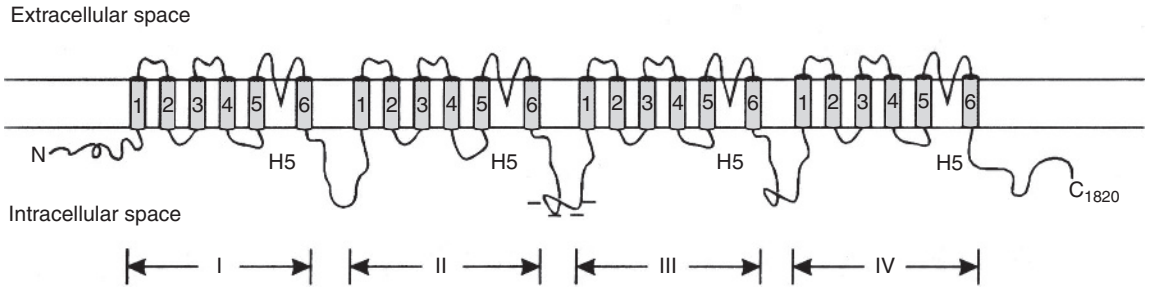

Figure 1.14 Schematic diagram of the disposition of the Na^+-channel protein in a membrane. The four domains are labelled I, II, III, IV. In life the four domains are clustered in the third dimension to form a huge protein with a central canal.

a large variety of voltage-sensitive K^+-channels and also various types of Cl^-, Ca^{2+} and Na^+ channel. In this section we shall confine ourselves to just one type of voltage-sensitive Na^+-channel. This is the channel that is responsible for the rising phase of the action potential and is thus the defining element of exciteable tissues such as nerve and striated muscle.

We shall see in the next chapter that the resting potential across most cell membranes is about 50 or 60 mV (inside negative to outside). This may not seem very much. It must be remembered, however, that biological membranes are very thin – no more than 6 or 7 nm. Hence the voltage drop is in fact very steep. A potential gradient of 60 mV in 6 nm works out as 10^5 V/cm. Voltage-sensitive proteins are very delicately poised in this intense electric field. Any change in the potential gradient will affect their conformation and thus the degree of opening or closure of any embedded ion channel.

The structure of the Na^+-channel has been the subject of intense research. It is nowadays well known. It is shown diagammatically in Figure 1.14. It consists of a single massive polypeptide (1820 amino acids) which, as the plan view in Figure 1.14 shows, consists of four successive domains. The domains are all homologous with each other and each has six membrane-spanning helices. The fourth helix (S4) in each domain contains a number of positively-charged amino acid residues (especially arginine and lysine) and it is consequently believed to form the 'voltage sensor' which is sensitive to any voltage change across the membrane. Between the fifth (S5) and sixth (S6) membrane-spanning helix in each domain the polypeptide chain is believed to form a 'hairpin' structure (H5) and to be inserted into the membrane. When the protein forms up into its 3D form of a hollow cylinder, the hairpins line the pore and confer ion selectivity. Finally, the intracellular segment of polypeptide between homologous domains III and IV is responsible for inactivating the channel.

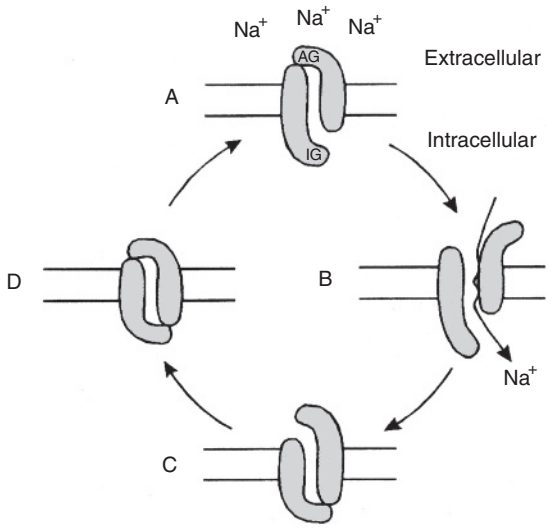

Figure 1.15 Conformation cycle of the sodium channel. (A) In the 'resting' membrane the sodium channel is closed. The activation gate (AG) is shut and the inactivation gate (IG) is open. (B) When the membrane is depolarized the voltage drop is sensed by a 'voltage sensor' and the activation gate opens. Sodium ions flow down their electrochemical gradient. They hop from one site in the channel to the next (as indicated) and hence proceed in single file. (C) After about 1 ms the inactivation gate closes. (D) As the membrane returns to its resting potential the activation gate closes and the inactivation gate reopens.

The physiology of the Na^+-channel has also been intensively studied. It can be shown that when the voltage across the membrane drops below some threshold value, the channel opens for about 1 ms and allows Na^+ ions carrying about 2 pA of current to pass. Once the 1 ms opening time has passed, the channel closes and will not open again while the membrane remains depolarized. This 'inactivation' is due, as we saw above, to the segment of polypeptide between domains III and IV blocking the channel. The channel protein thus exists in three major conformations: closed, open and inactivated. This cycle is shown in Figure 1.15.

The exact time of opening and closing of a channel is not strictly predictable: it is, in other words, stochastic. Furthermore, any patch of excitable membrane will have a large population of Na^+-channels and each individual channel will have a slightly different opening threshold. As an excitable membrane is depolarized more and more, Na^+-channels open. The influx of Na^+ions depolarizes and ultimately repolarizes the membrane, a phenomenon which electrophysiologists record as the action potential. The biophysics of the action potential will be discussed in the next chapter (Section 2.6).

1.5 CONCLUDING REMARKS

In this chapter we have laid some of the groundwork. Biochemical detail has been deliberately omitted. Interested students should consult one or other of the many excellent texts on biochemistry and/or molecular biology. We shall see that the elements discussed above appear again and again at the core of the specialized and often highly elaborate sensory systems which have evolved in the animal kingdom. In particular we shall find that the biochemistry of biomembranes, receptors, G-protein systems and channels is linked to mechanisms controlling the electrical polarity of the membranes of sensory cells and endings. It is to this matter that we turn in the next chapter.