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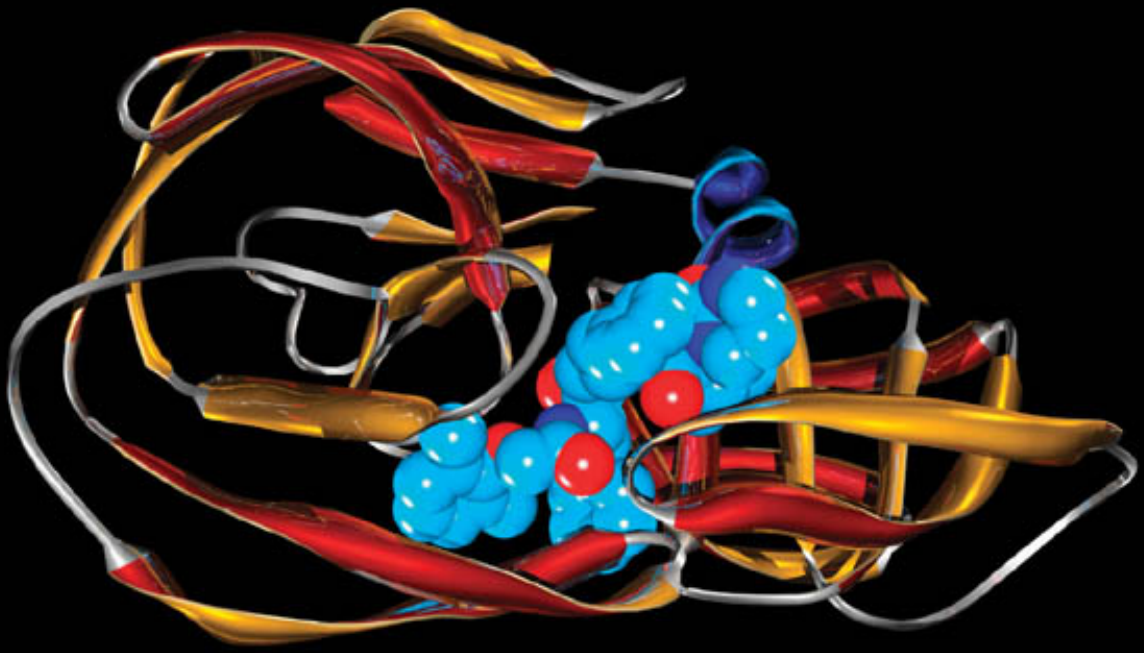
GRAHAM L. PATRICK



fifth edition

an introduction to MEDICINAL CHEMISTRY

OXFORD

2 Protein structure and function

The vast majority of drugs used in medicine are targeted to proteins, such as receptors, enzymes, and transport proteins. Therefore, it is important to understand protein structure in order to understand drug action on proteins. Proteins have four levels of structure: primary, secondary, tertiary, and quaternary.

2.1 The primary structure of proteins

The primary structure is the order in which the individual amino acids making up the protein are linked together through peptide bonds (Fig. 2.1). The 20 common amino acids found in humans are listed in Table 2.1, with the three- and one-letter codes often used to represent them. The structures of the amino acids are shown in Appendix 1. The primary structure of **Met-enkephalin** (one of the body's own painkillers) is shown in Fig. 2.2.

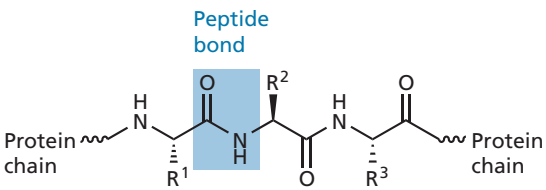


FIGURE 2.1 Primary structure of proteins (R^1, R^2, and R^3 = amino acid side chains).

The peptide bond in proteins is planar in nature as a result of the resonance structure shown in Fig. 2.3. This gives the peptide bond a significant double bond character, which prevents rotation. As a result, bond rotation in the protein backbone is only possible for the bonds on

TABLE 2.1 The 20 common amino acids found in humans

Synthesized in the human body			Essential to the diet		
Amino acid	Codes Three-letter	One-letter	Amino acid	Codes Three-letter	One-letter
Alanine	Ala	A	Histidine	His	H
Arginine	Arg	R	Isoleucine	Ile	I
Asparagine	Asn	N	Leucine	Leu	L
Aspartic acid	Asp	D	Lysine	Lys	K
Cysteine	Cys	C	Methionine	Met	M
Glutamic acid	Glu	E	Phenylalanine	Phe	F
Glutamine	Gln	Q	Threonine	Thr	T
Glycine	Gly	G	Tryptophan	Trp	W
Proline	Pro	P	Valine	Val	V
Serine	Ser	S			
Tyrosine	Tyr	Y			

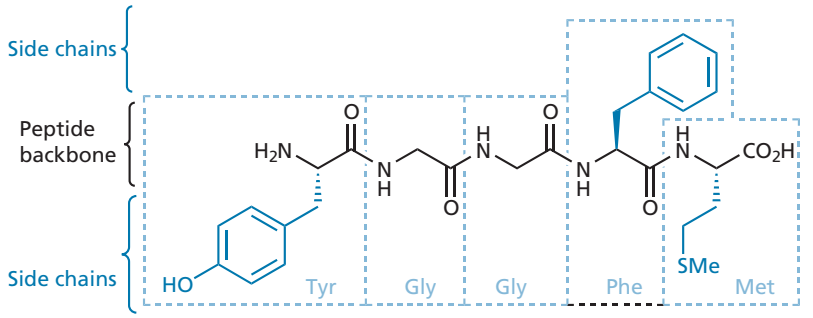


FIGURE 2.2 Met-enkephalin. The short hand notation for this peptide is H-Tyr-Gly-Gly-Phe-Met-OH or YGGFM.

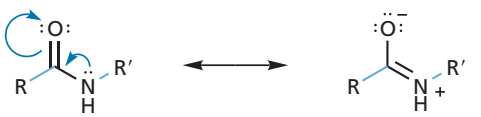


FIGURE 2.3 The planar peptide bond (bond rotation allowed for coloured bonds only).

trans conformation (favoured)

cis conformation (unfavoured)

FIGURE 2.4 *Trans* and *cis* conformations of the peptide bond.

either side of each peptide bond. This has an important consequence for protein tertiary structure.

There are two possible conformations for the peptide bond (Fig. 2.4). The *trans* conformation is the one that is normally present in proteins as the *cis* conformation leads to a steric clash between the residues. However, the *cis* conformation is possible for peptide bonds next to a proline residue.

2.2 The secondary structure of proteins

The secondary structure of proteins consists of regions of ordered structure adopted by the protein chain. In structural proteins, such as wool and silk, secondary structures are extensive and determine the overall shape and properties of such proteins. However, there are also regions of secondary structure in most other proteins. There are three main secondary structures: the α-helix, β-pleated sheet, and β-turn.

2.2.1 The α-helix

The α-helix results from coiling of the protein chain such that the peptide bonds making up the backbone are able to form hydrogen bonds between each other. These hydrogen bonds are directed along the axis of the helix, as shown in Fig. 2.5. The side chains of the component amino acids stick out at right angles from the helix, thus minimizing steric interactions and further stabilizing the structure. Other, less common, types of helices can occur in proteins, such as the 3(10)-helix, which is more stretched than the ideal α-helix, and the π-helix, which is more compact and extremely rare.

Test Your Understanding and Practise Your Molecular Modelling with Exercise 2.1.

2.2.2 The β-pleated sheet

The β-pleated sheet is a layering of protein chains one on top of another, as shown in Fig. 2.6. Here, too, the structure is held together by hydrogen bonds between the peptide chains. The side chains are situated at right angles to the sheets—once again to reduce steric interactions. The chains in β-sheets can run in opposite directions (antiparallel) or in the same direction (parallel) (Fig. 2.7).

2.2.3 The β-turn

A β-turn allows the polypeptide chain to turn abruptly and go in the opposite direction. This is important in allowing the protein to adopt a more globular compact shape. A hydrogen bonding interaction between the first and third peptide bond of the turn is important in stabilizing the turn (Fig. 2.8). Less abrupt changes in the direction of the polypeptide chain can also take place through longer loops, which are less regular in their structure, but often rigid and well defined.

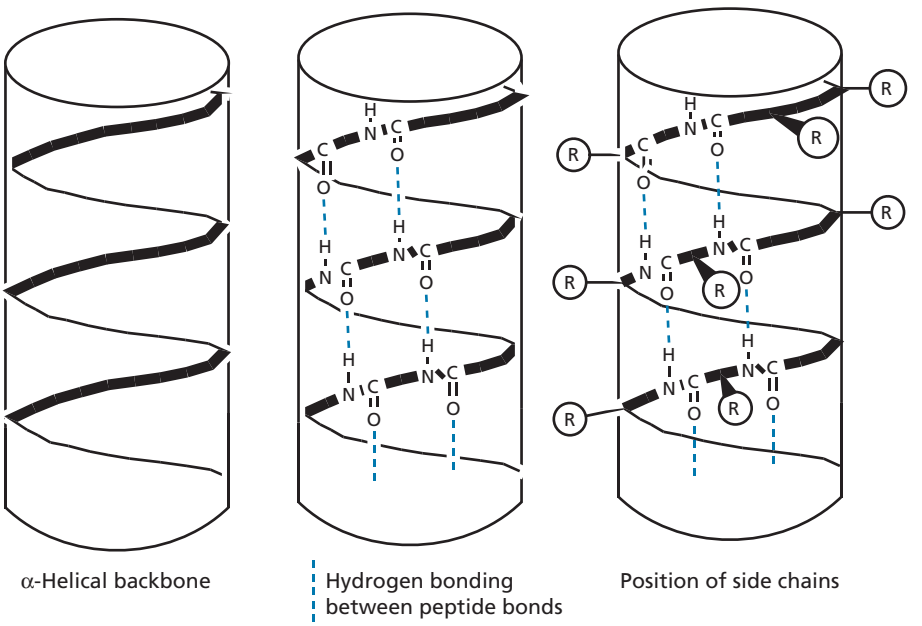


FIGURE 2.5 The α-helix for proteins showing intramolecular hydrogen bonds and the position of side chains.

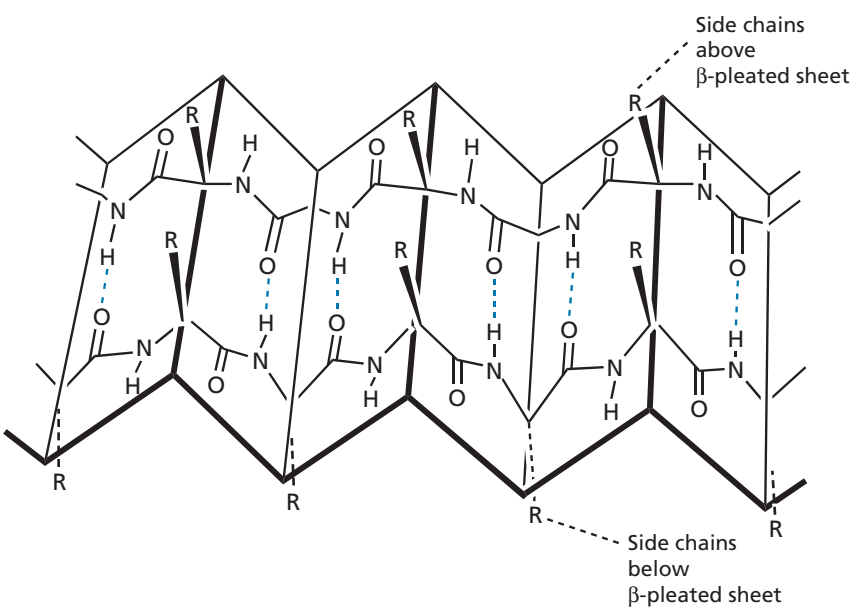


FIGURE 2.6 The β-pleated sheet (antiparallel arrangement).

2.3 The tertiary structure of proteins

The tertiary structure is the overall three-dimensional shape of a protein. Structural proteins are quite ordered in shape, whereas globular proteins, such as enzymes and receptors (Chapters 3 and 4), fold up to form more complex structures. The tertiary structure of enzymes and receptors is crucial to their function and also to their interaction with drugs; therefore, it is important to appreciate the forces that control tertiary structure.

Globular proteins often contain regions of ordered secondary structure, the extent of which varies from protein to protein. For example, **cyclin-dependent kinase 2** (a protein that catalyses phosphorylation reactions) has several regions of α-helices and β-pleated sheets

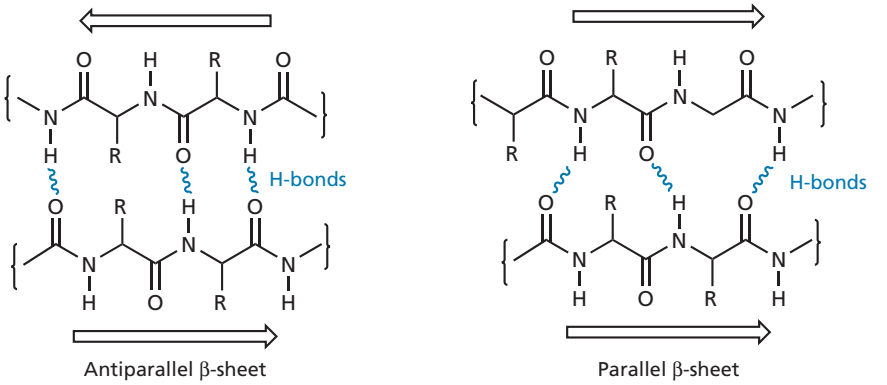


FIGURE 2.7 Hydrogen bonding in antiparallel and parallel β-sheets (the arrows are pointing to the *C*-terminal end of the chain).

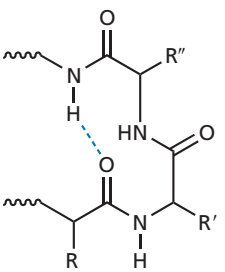


FIGURE 2.8 The β-turn showing hydrogen bonding between the first and third peptide bond.

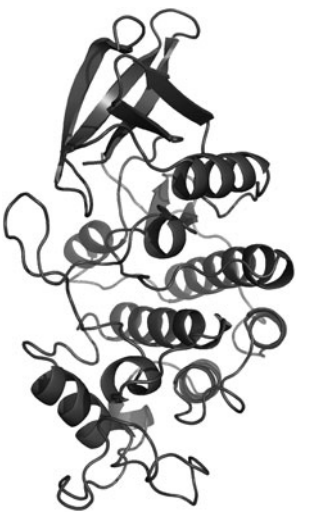


FIGURE 2.9 The pdb file (1hcl) for human cyclin-dependent kinase 2 (CDK2), where cylinders represent α-helices and arrows represent β-sheets. A pdb file contains the three-dimensional structural information for a protein and can be downloaded from the Brookhaven protein data bank. Each protein structure file is given a code, for example 1hcl.

Test Your understanding and practise your molecular modelling with Exercise 2.2.

(Fig. 2.9), whereas the digestive enzyme **chymotrypsin** has very little secondary structure. Nevertheless, the protein chains in both cyclin-dependent kinase 2 and chymotrypsin fold up to form a complex, but distinctive, globular shape. How does this come about?

At first sight, the three-dimensional structure of cyclin-dependent kinase 2 looks like a ball of string after a cat has been at it. In fact, the structure shown is a very precise shape which is taken up by every molecule of this protein, and which is determined by the protein's primary structure.* Indeed, it is possible to synthesize naturally occurring proteins in the laboratory which automatically adopt the same three-dimensional structure and function as the naturally occurring protein. The HIV-1 protease enzyme is an example (section 20.7.4.1).

This poses a problem. Why should a chain of amino acids take up such a precise three-dimensional shape? At first sight, it does not make sense. If we place a length of string on the table it does not fold itself up into a precise complex shape. So why should a chain of amino acids do such a thing?

The answer lies in the fact that a protein is not just a bland length of string. It contains a range of different chemical functional groups along its length—not only peptide links, but also the side chains of each amino acid. These can interact with each other such that there is either an attractive interaction or a repulsive interaction. Thus, the protein will twist and turn to minimize the unfavourable interactions and maximize the favourable ones until the most stable shape or conformation is found—the tertiary structure (Fig. 2.10).

* Some proteins contain species known as **cofactors** (e.g. metal ions or small organic molecules), which also have an effect on tertiary structure.

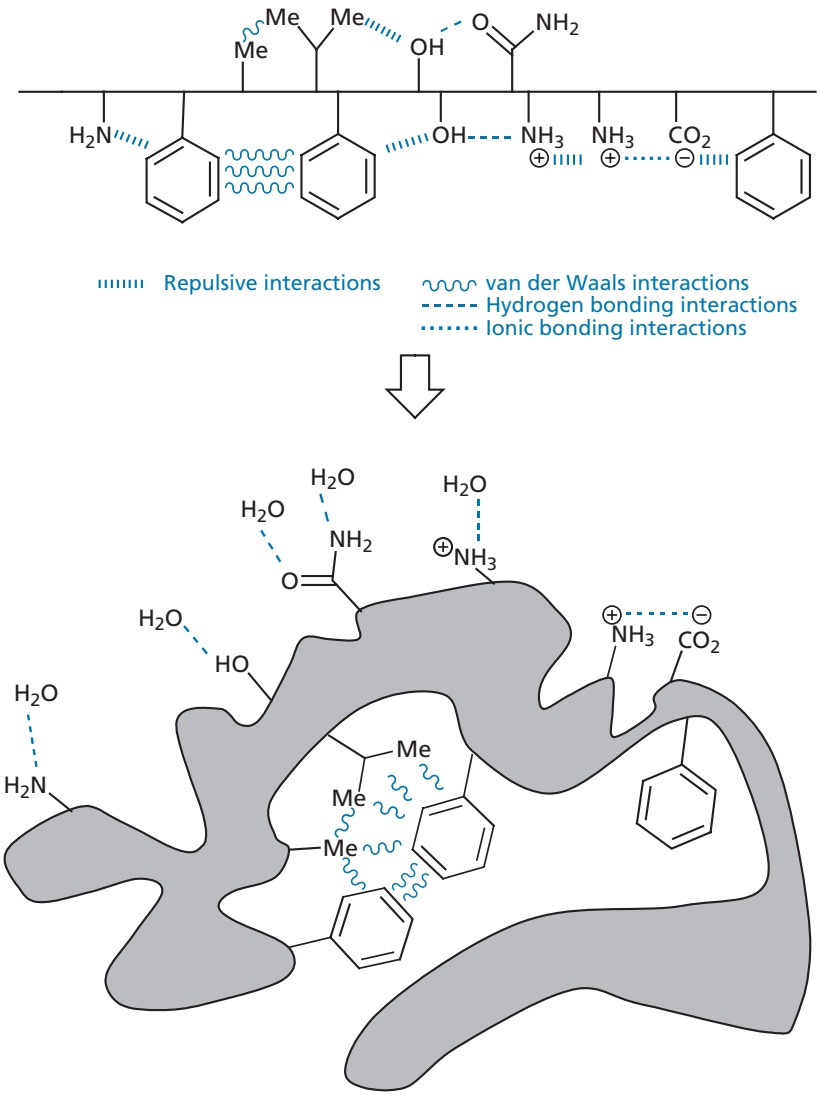


FIGURE 2.10 Tertiary structure formation as a result of intramolecular interactions.

With the exception of disulphide bonds, the bonding interactions involved in tertiary structure are the same as the **intermolecular bonds** described in section 1.3. The latter occur between different molecules, whereas the bonds controlling protein tertiary structure occur within the same molecule, and so they are called **intramolecular bonds**. Nevertheless, the principles described in section 1.3 are the same.

2.3.1 Covalent bonds—disulphide links

Cysteine has a residue containing a thiol group capable of forming a covalent bond in the protein tertiary structure. When two such residues are close together, a covalent disulphide bond can be formed as a result of oxidation. A covalent bridge is thus formed between two different parts of the protein chain (Fig. 2.11). It should be noted that the two cysteine residues involved in this bond formation may be far apart from each other in the primary structure of the protein, but are brought close together as a result of protein folding.

2.3.2 Ionic or electrostatic bonds

An ionic bond or salt bridge can be formed between the carboxylate ion of an acidic residue, such as aspartic acid or glutamic acid, and the aminium ion of a basic residue, such as lysine, arginine, or histidine (Fig. 2.12). This is the strongest of the intramolecular bonds.

2.3.3 Hydrogen bonds

Hydrogen bonds can be viewed as a weak form of ionic interaction as they involve interactions between atoms

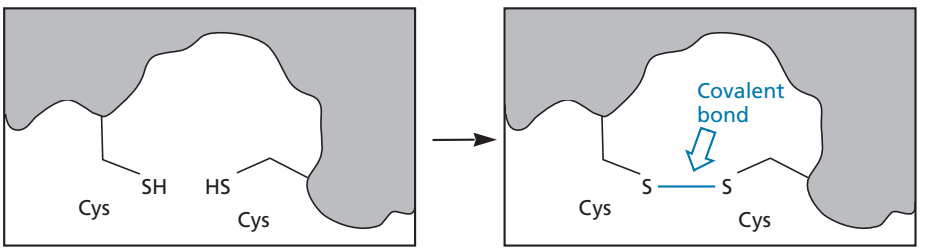


FIGURE 2.11 The formation of a disulphide covalent bond between two cysteine side chains.

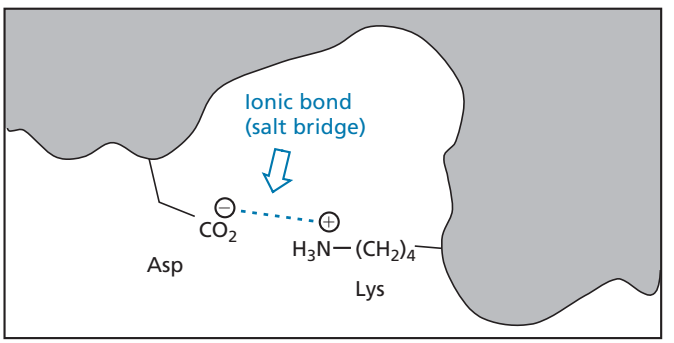


FIGURE 2.12 Ionic bonding between an aspartate side chain and a lysine side chain.

having partial charges. They can be formed between a large number of amino acid side chains, such as serine, threonine, aspartic acid, glutamic acid, glutamine, lysine, arginine, histidine, tryptophan, tyrosine, and asparagine. Two examples are shown in Fig. 2.13.

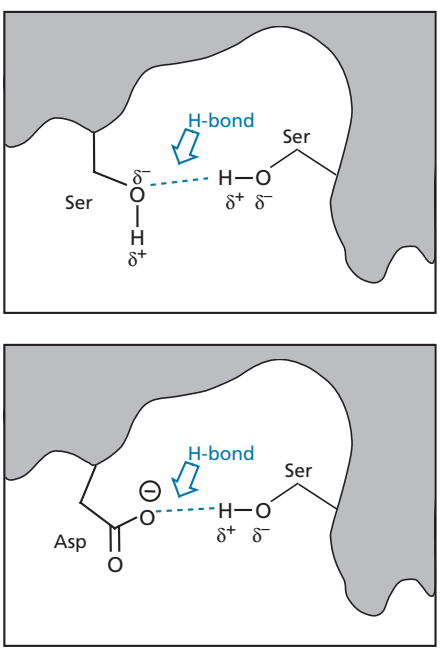


FIGURE 2.13 Hydrogen bonding between amino acid side chains.

2.3.4 Van der Waals and hydrophobic interactions

Van der Waals interactions are weaker interactions than hydrogen bonds and can take place between two hydrophobic regions of the protein. For example, they can take place between two alkyl groups (Fig. 2.14). The amino acids alanine, valine, leucine, isoleucine, phenylalanine, and proline all have hydrophobic side chains capable of interacting with each other by van der Waals interactions. The side chains of other amino acids, such as methionine, tryptophan, threonine, and tyrosine, contain polar functional groups, but the side chains also have a substantial hydrophobic character and so van der Waals interactions

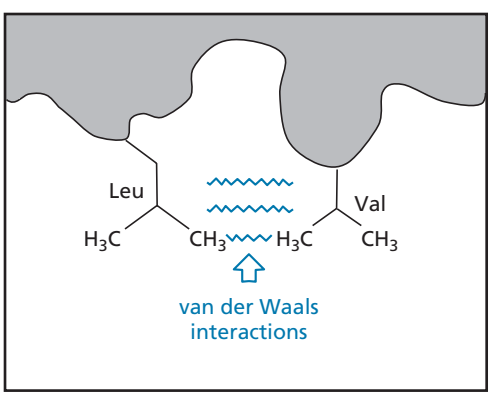


FIGURE 2.14 Van der Waals interactions between amino acid side chains.

are also possible for these amino acids. Hydrophobic interactions (section 1.3.6) are also important in the coming together of hydrophobic residues.

2.3.5 Relative importance of bonding interactions

We might expect the relative importance of the bonding interactions in protein tertiary structure to follow the same order as their strengths: covalent, ionic, hydrogen bonding, and, finally, van der Waals. In fact, the opposite is generally true. Usually, the most important bonding interactions are those due to van der Waals interactions and hydrogen bonding, while the least important interactions are those due to covalent and ionic bonding.

There are two reasons for this. Firstly, in most proteins there are more possible opportunities for van der Waals and hydrogen bonding interactions than for covalent or ionic bonding. We only need to consider the relative number of amino acids present in a typical globular protein to see why. The only amino acid that can form a covalent disulphide bond is cysteine, whereas there are many more amino acids that can interact with each other through hydrogen bonding and van der Waals interactions.

Having said that, there *are* examples of proteins with a large number of disulphide bridges, where the relative importance of the covalent link to tertiary structure is more significant. Disulphide links are also more significant in small polypeptides such as the peptide hormones **vasopressin** and **oxytocin** (Fig. 2.15). Nevertheless, in most proteins, disulphide links play a minor role in controlling tertiary structure.

As far as ionic bonding is concerned, there is only a limited number of amino acids with residues capable of forming ionic bonds, and so these, too, are outnumbered by the number of residues capable of forming hydrogen bonds or van der Waals interactions.

There is a second reason why van der Waals interactions are normally the most important form of bonding in tertiary structure. Proteins do not exist in a vacuum; they are surrounded by water. Water is a highly polar compound that interacts readily with polar, hydrophilic amino acid residues capable of forming hydrogen bonds

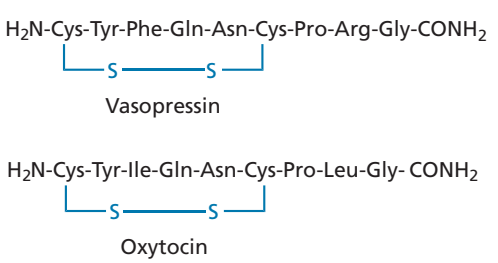


FIGURE 2.15 Vasopressin and oxytocin.

(Fig. 2.16). The remaining non-polar, hydrophobic amino acid residues cannot interact favourably with water, so the most stable tertiary structure will ensure that most of the hydrophilic groups are on the surface so that they interact with water and that most of the hydrophobic groups are in the centre so that they avoid water and interact with each other. As the hydrophilic amino acids form hydrogen bonds with water, the number of ionic and hydrogen bonds contributing to the tertiary structure is reduced leaving hydrophobic and van der Waals interactions to largely determine the three-dimensional shape of the protein.

For the reasons stated above, the centre of the protein must be hydrophobic and non-polar. This has important consequences. For example, it helps to explain why enzymes catalyse reactions that should be impossible in the aqueous environment of the human body. Enzymes contain a hollow, or canyon, on their surface called an **active site**. As the active site protrudes into the centre of the protein, it tends to be hydrophobic in nature and can provide a non-aqueous environment for the reaction taking place (Chapter 3).

Many other types of protein contain similar hollows or clefts that act as **binding sites** for natural ligands. They, too, are more hydrophobic than the surface and so van der Waals and hydrophobic interactions play an important role in the binding of the ligand. An understanding of these interactions is crucial to the design of effective drugs that will target these binding sites.

2.3.6 Role of the planar peptide bond

Planar peptide bonds indirectly play an important role in tertiary structure. Bond rotation in peptide bonds is hindered, with the *trans* conformation generally favoured, so the number of possible conformations that a protein can adopt is significantly restricted, making it more likely that a specific conformation is adopted. Polymers without peptide bonds do not fold into a specific conformation, because the entropy change required to form a highly ordered structure is extremely unfavourable. Peptide bonds can also form hydrogen bonds with amino acid side chains and play a further role in determining tertiary structure.

2.4 The quaternary structure of proteins

Only proteins that are made up of a number of protein subunits have quaternary structure. For example, **haemoglobin** is made up of four protein molecules—two identical alpha subunits and two identical beta subunits (not to be

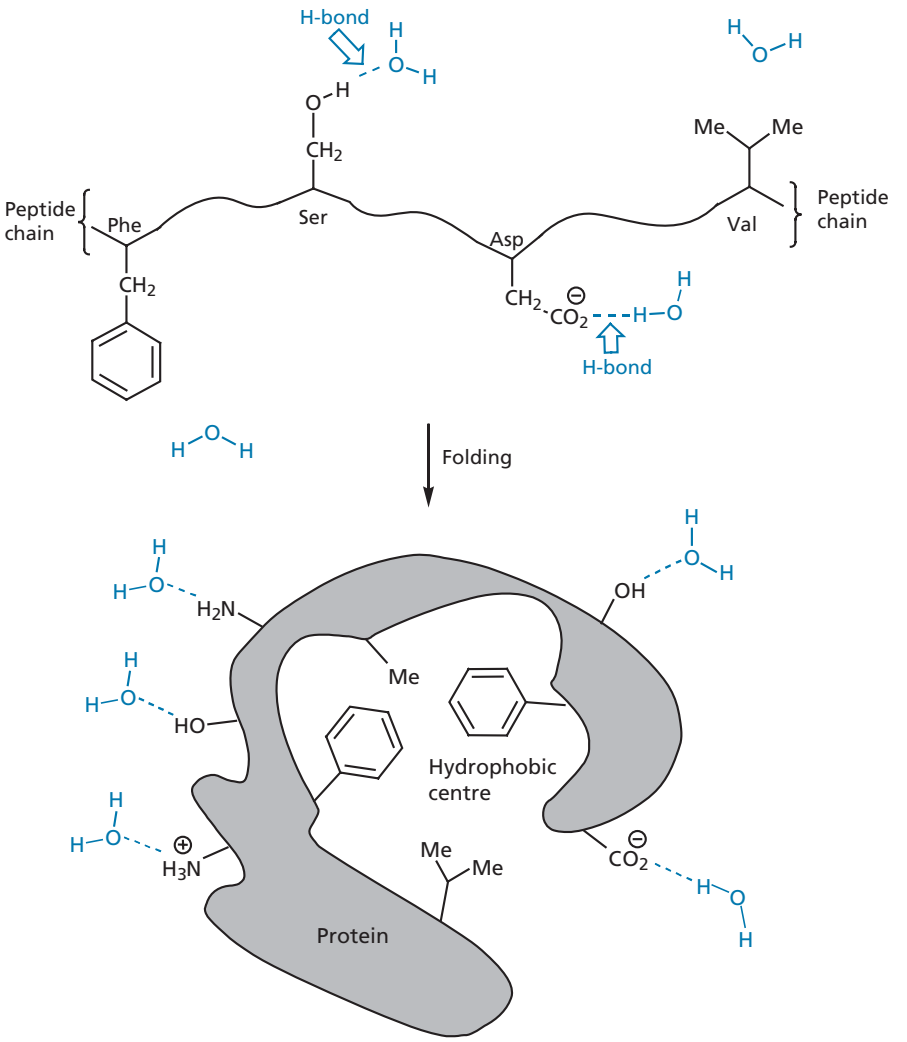


FIGURE 2.16 Bonding interactions with water.

confused with the alpha and beta terminology used in secondary structure). The quaternary structure of haemoglobin is the way in which these four protein units associate with each other.

As this must inevitably involve interactions between the exterior surfaces of proteins, ionic bonding can be more important to quaternary structure than it is to tertiary structure. Nevertheless, hydrophobic and van der Waals interactions have a role to play. It is not possible for a protein to fold up such that all its hydrophobic groups are placed towards the centre. Some of these groups may be stranded on the surface. If they form a small hydrophobic area on the protein surface, there is a distinct advantage for two protein molecules to form a dimer such that the two hydrophobic areas face each other rather than be exposed to an aqueous environment (Fig. 2.17). It is also

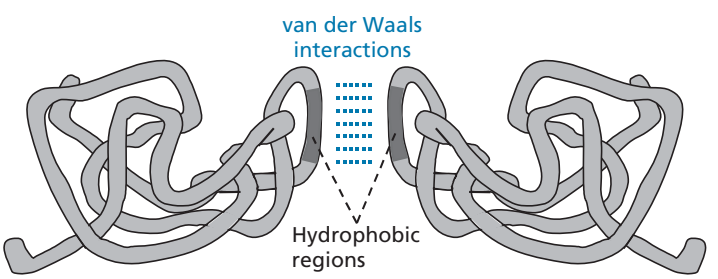


FIGURE 2.17 Quaternary structure involving two protein subunits.

possible for protein molecules to interlock in a quaternary structure (section 24.9.2).

2.5 Translation and post-translational modifications

The process by which a protein is synthesized in the cell is called **translation** (section 6.2.2). Many proteins are modified following translation (Fig. 2.18), and these modifications can have wide-ranging effects. For example, the *N*-terminals of many proteins are acetylated, making these proteins more resistant to degradation. Acetylation of proteins also has a role to play in the control of transcription, cell proliferation, and differentiation (section 21.7.3).

The fibres of **collagen** are stabilized by the hydroxylation of proline residues. Insufficient hydroxylation results in scurvy (caused by a deficiency of vitamin C). The glutamate residues of **prothrombin**, a clotting protein, are carboxylated to form γ-carboxyglutamate structures. In cases of vitamin K deficiency, carboxylation does not occur and excessive bleeding results. The serine, threonine, and tyrosine residues of many proteins are phosphorylated and this plays an important role in signalling pathways within the cell (sections 5.2–5.4).

Many of the proteins present on the surface of cells are linked to carbohydrates through asparagine residues. Such carbohydrates are added as post-translational modifications and are important to cell–cell recognition, disease processes, and drug treatments (section 10.7). The proteins concerned are called **glycoproteins** or **proteoglycans**, and are members of a larger group of molecules called **glycoconjugates.**

Several proteins are cleaved into smaller proteins or peptides following translation. For example, the **enkephalins** are small peptides which are derived from

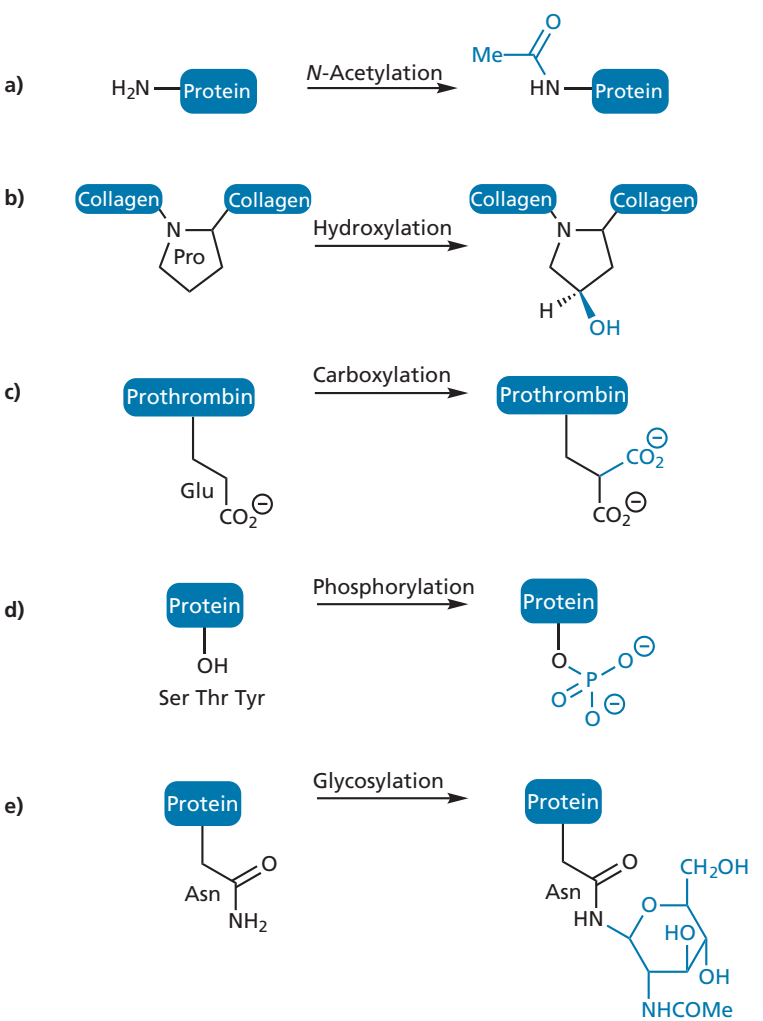


FIGURE 2.18 Examples of post-translational modifications carried out on proteins.

proteins in this manner (section 24.8). Active enzymes are sometimes formed by cleaving a larger protein precursor. Often, this serves to protect the cell from the indiscriminate action of an enzyme. For example, digestive enzymes are stored in the pancreas as inactive protein precursors and are only produced once the protein precursor is released into the intestine. In blood clotting, the soluble protein **fibrinogen** is cleaved to insoluble **fibrin** when the latter is required. Some polypeptide hormones are also produced from the cleavage of protein precursors. Finally, the cleavage of a viral polyprotein into constituent proteins is an important step in the life cycle of the HIV virus and has proved a useful target for several drugs currently used to combat AIDS (section 20.7.4).

2.6 Proteomics

A lot of publicity has been rightly accorded to the Human Genome Project, which has now been completed. The science behind this work is called **genomics** and it involves the identification of the genetic code in humans and other species. The success of this work has been hailed as a breakthrough that will lead to a new era in medicinal research. However, it is important to appreciate that this is only the start of a longer process. As we shall see in Chapter 6, DNA is the blueprint for the synthesis of proteins and so the task is now to identify all the proteins present in each cell of the body and, more importantly, how they interact with each other—an area of science known as **proteomics**. Proteomics is far more challenging than genomics because of the complexity of interactions that can take place between proteins (see Chapter 5). Moreover, the pattern and function of proteins present in a cell depend on the type of cell it is and this pattern can alter in the diseased state. Nevertheless, the race is now on to analyse the structure and function of proteins, many of which are completely new to science, and to see whether they can act as novel drug targets for the future. This is no easy task and it is made all the more difficult by the fact that it is not possible to simply derive the structure of proteins based on the known gene sequences. This is because different proteins can be derived from a single gene and proteins are often modified following their synthesis (section 2.5). There are roughly 40,000 genes, whereas a typical cell contains hundreds of thousands of different proteins. Moreover, knowing the structure of a protein does not necessarily suggest its function or interactions.

Identifying the proteins present in a cell usually involves analysing the contents of the cell and separating out the proteins using a technique known as two-dimensional gel electrophoresis. Mass spectrometry can then be used to study the molecular weight of each protein. Assuming a pure sample of protein is obtained, its primary structure can be identified by traditional sequencing techniques. The analysis of secondary and tertiary structures is trickier. If the protein can be crystallized, then it is possible to determine its structure by X-ray crystallography. Not all proteins can be crystallized, though, and even if they are, it is possible that the conformation in the crystal form is different from that in solution. In recent years nuclear magnetic resonance (NMR) spectroscopy has been successful in identifying the tertiary structure of some proteins.

There then comes the problem of identifying what role the protein has in the cell and whether it would serve as a useful drug target. If it does show promise as a target, the final problem is to discover or design a drug that will interact with it.

KEY POINTS

- The order in which amino acids are linked together in a protein is called the primary structure.
- The secondary structure of a protein refers to regions of ordered structure within the protein, such as α-helices, β-pleated sheets, or β-turns.
- The overall three-dimensional shape of a protein is called its tertiary structure.
- Proteins containing two or more subunits have a quaternary structure which defines how the subunits are arranged with respect to each other.
- Secondary, tertiary, and quaternary structures are formed to maximize favourable intramolecular and intermolecular bonds, and to minimize unfavourable interactions.
- Amino acids with polar residues are favoured on the outer surface of a protein because this allows hydrogen bonding interactions with water. Amino acids with non-polar residues are favoured within the protein because this maximizes van der Waals and hydrophobic interactions.
- Many proteins undergo post-translational modifications.
- Proteomics is the study of the structure and function of novel proteins discovered through genomics.

2.7 Protein function

We are now ready to discuss the various types of protein which act as drug targets.

2.7.1 Structural proteins

Structural proteins do not normally act as drug targets. However, the structural protein **tubulin** is an exception. Tubulin molecules polymerize to form small tubes called **microtubules** in the cell's cytoplasm (Fig 2.19). These

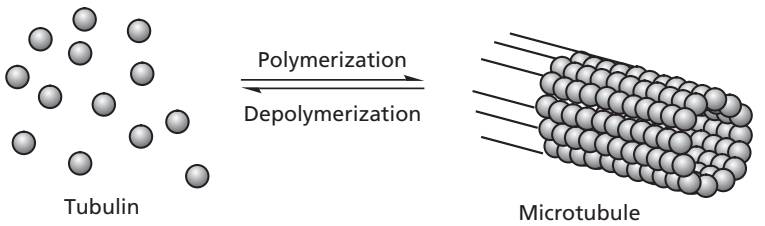


FIGURE 2.19 Polymerization of tubulin.

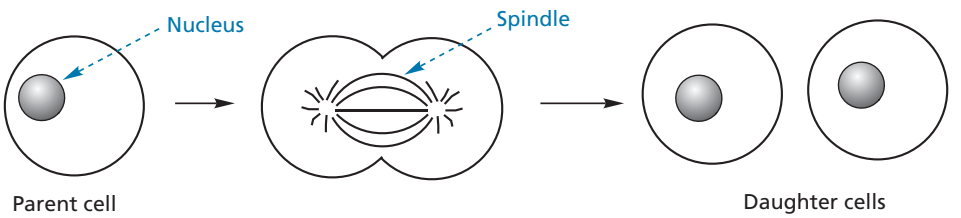


FIGURE 2.20 Cell division.

microtubules have various roles within the cell, including the maintenance of shape, exocytosis, and release of neurotransmitters. They are also involved in the mobility of cells. For example, inflammatory cells called **neutrophils** are mobile cells which normally protect the body against infection. However, they can also enter joints, leading to inflammation and arthritis.

Tubulin is also crucial to cell division. When a cell is about to divide, its microtubules depolymerize to give tubulin. The tubulin is then re-polymerized to form a structure called a **spindle** which then serves to push apart the two new cells and to act as a framework on which the chromosomes of the original cell are transferred to the nuclei of the daughter cells (Fig. 2.20). Drugs that target tubulin and inhibit this process are useful anticancer agents (section 10.2.2).

The structural proteins of viruses are important to the survival of the virus outside their host cell. Some of these proteins are proving to be interesting drug targets for the design of new antiviral agents and are discussed in more detail in sections 20.7.5 and 20.9.

2.7.2 Transport proteins

Transport proteins are present in the cell membrane and act as the cell's 'smugglers'—smuggling the important chemical building blocks of amino acids, sugars, and nucleic acid bases across the cell membrane such that the cell can synthesize its proteins, carbohydrates, and nucleic acids. They are also important in transporting important neurotransmitters (section 4.2) back into the neuron that released them so that the neurotransmitters only have a limited period of activity. But why is this smuggling operation necessary? Why can't these molecules pass through the membrane by themselves? Quite simply, the molecules concerned are polar structures and cannot pass through the hydrophobic cell membrane.

The transport proteins can float freely within the cell membrane because they have hydrophobic residues on their outer surface which interact favourably with the hydrophobic centre of the cell membrane. The portion of the transport protein that is exposed on the outer surface of the cell membrane contains a binding site that can bind a polar molecule, such as an amino acid, stow it away in a hydrophilic pocket, and ferry it across the membrane to release it on the other side (Fig. 2.21).

Transport proteins are not all identical; there are specific transport proteins for the different molecules that need to be smuggled across the membrane. The binding sites for these transport proteins vary in structure such that they can recognize and bind their specific guest. There are several important drugs which target transport proteins (section 10.1).

2.7.3 Enzymes and receptors

The most important drug targets in medicinal chemistry are enzymes and receptors. Chapters 3 and 4 are devoted to the structure and function of these proteins respectively.

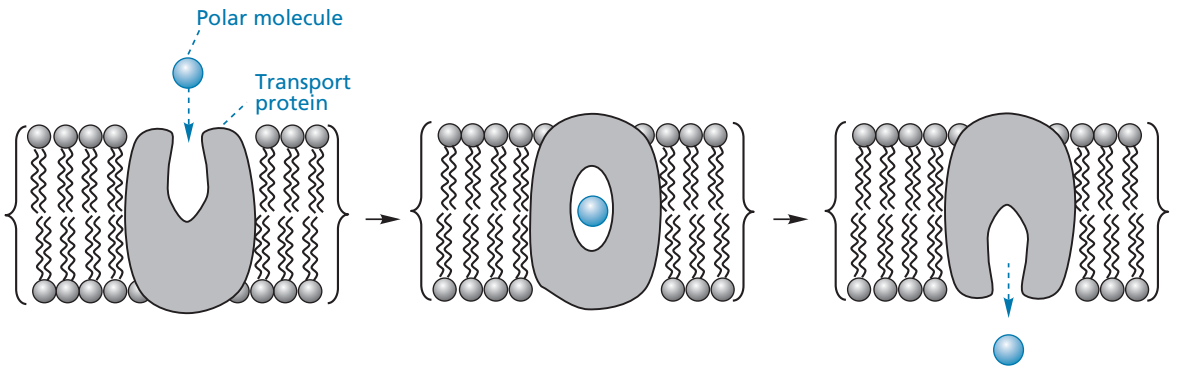


FIGURE 2.21 Transport proteins.

2.7.4 Miscellaneous proteins and protein–protein interactions

There are many situations in cell biology where proteins are required to interact with each other in order to produce a particular cellular effect. We have already seen an example of this in the polymerization of tubulin proteins in order to form microtubules (section 2.7.1). The structures of many important drug targets, such as ion channels, enzymes, and receptors consist of two or more protein subunits associated with each other. The signal transduction processes described in Chapter 5 show many instances where a variety of proteins, such as receptors, signal proteins, and enzymes, associate with each other in order to transmit a chemical signal into the cell. The actions of insulin are mediated through a protein–protein interaction (section 4.8.3). The control of gene expression involves the prior assembly of a variety of different proteins (section 4.9 and Box 8.2). An important part of the immune response involves proteins called antibodies interacting with foreign proteins (section 10.7.2). Cell–cell recognition involves protein–protein interactions—a process which is not only important in terms of the body's own proteins, but in the mechanism by which viruses invade human cells (sections 20.7.1, 20.8.1, and 20.9). Important processes that have an influence on tumour growth, such as angiogenesis and apoptosis (section 21.1), involve the association of proteins. Proteins called chaperones help to stabilize partially folded proteins during translation through protein–protein interactions. They are also important in the process by which old proteins are removed to the cell's recycling centre. Chaperones are particularly important when the cell experiences adverse environmental conditions which might damage proteins. It has been found that the synthesis of chaperones increases in tumour cells, which may reflect some of the stresses experienced in such cells, for example lack of oxygen, pH variation, and nutrient deprivation. Inhibiting chaperones could well lead to more damaged proteins and cell death. There are current studies looking into methods of inhibiting a chaperone protein called HSP90 (HSP stands for heat shock protein). Inhibition might prevent the synthesis of important receptors and enzymes involved in the process of cell growth and division and provide a new method of treating tumour cells (section 21.6.2.7). The inhibition of an enzyme acting as a chaperone protein is also being considered as a potential therapy for the treatment of Alzheimer's disease (section 22.15.2).

Protein–protein interactions are not limited to human biochemistry. Interfering with these interactions in other species could lead to novel antibacterial, antifungal, and antiviral agents. For example, HIV protease is an important enzyme in the life-cycle of the HIV virus and is an important target for antiviral agents (section 20.7.4). The enzyme consists of two identical proteins which bind together to produce the active site. Finding a drug that will prevent this association would be a novel method of inhibiting this enzyme.

To conclude, there is a lot of research currently underway looking at methods of inhibiting or promoting protein–protein interactions (section 10.5).

KEY POINTS

- Transport proteins, enzymes, and receptors are common drug targets.
- Transport proteins transport essential polar molecules across the hydrophobic cell membrane.
- Tubulin is a structural protein which is crucial to cell division and cell mobility.
- Many cell processes depend on the interactions of proteins with each other.

QUESTIONS

1. Draw the full structure of L-alanyl-L-phenylalanyl-glycine.
2. What is unique about glycine compared with other naturally-occurring amino acids?
3. Identify the intermolecular/intramolecular interactions that are possible for the side chains of the following amino acids; serine, phenylalanine, glycine, lysine, aspartic acid, and aspartate.
4. The chains of several cell membrane-bound proteins wind back and forth through the cell membrane, such that some parts of the protein structure are extracellular, some parts are intracellular, and some parts lie within the cell membrane. How might the primary structure of a protein help in distinguishing the portions of the protein embedded within the cell membrane from those that are not?
5. What problems might you foresee if you tried to synthesize L-alanyl-L-valine directly from its two component amino acids?
6. The tertiary structure of many enzymes is significantly altered by the phosphorylation of serine, threonine, or tyrosine residues. Identify the functional groups that are involved in these phosphorylations and suggest why phosphorylation affects tertiary structure.
7. What is the one-letter code for the polypeptide Glu–Leu–Pro–Asp–Val–Val–Ala–Phe–Lys–Ser–Gly–Gly–Thr?

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3 Enzymes: structure and function

In this chapter we discuss the structure and function of enzymes. Drug action at enzymes is discussed in Chapter 7 and in other chapters throughout the text.

3.1 Enzymes as catalysts

Enzymes are proteins which act as the body's catalysts—agents that speed up a chemical reaction without being consumed themselves. Without them, the cell's chemical reactions would either be too slow or not take place at all. An example of an enzyme-catalysed reaction is the reduction of **pyruvic acid** to **lactic acid**, which takes place when muscles are over-exercised, and is catalysed by an enzyme called **lactate dehydrogenase** (Fig. 3.1).

Note that the reaction is shown as an equilibrium. It is, therefore, more correct to describe an enzyme as an agent that speeds up the approach to equilibrium, because the enzyme speeds up the reverse reaction just as efficiently as the forward reaction. The final equilibrium concentrations of the starting materials and products are unaffected by the presence of an enzyme.

How do enzymes affect the rate of a reaction without affecting the equilibrium? The answer lies in the existence of a high-energy **transition state** that must be formed before the starting material (the **substrate**) can be converted to the product. The difference in energy between the transition state and the substrate is the **activation energy**, and it is the size of this activation energy that determines the rate of a reaction, rather than the difference in energy between the substrate and the product (Fig. 3.2). An enzyme acts to lower the activation energy by helping to stabilize the transition state. The energy of the substrate and products are unaffected, and therefore the equilibrium ratio of substrate to product is unaffected. We can relate energy to the rate and equilibrium constants with the following equations:

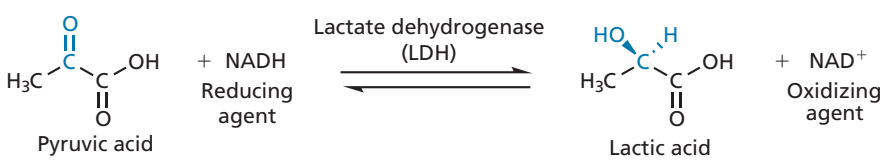


FIGURE 3.1 Reaction catalysed by lactate dehydrogenase.

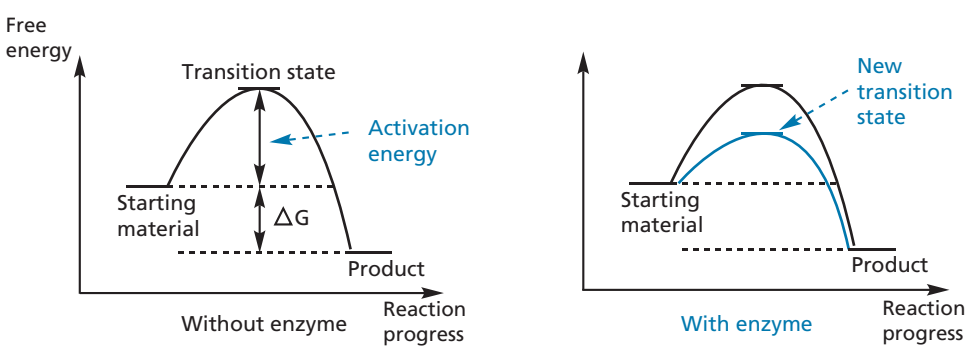


FIGURE 3.2 Graphs demonstrating the stabilization of a reaction's transition state by an enzyme.

$$\text{Energy difference} = \Delta G = -RT \ln K$$

where K is the equilibrium constant (= [products]/[reactants]), R is the gas constant (= 8.314 J mol^{-1} K^{-1}), and T is the temperature.

$$\text{Rate constant} = k = Ae^{-E/RT}$$

where E is the activation energy and A is the frequency factor.

Note that the rate constant k does not depend on the equilibrium constant K.

We have stated that enzymes catalyse reactions, but we have still to explain how.

3.2 How do enzymes catalyse reactions?

The factors involved in enzyme catalysis are summarized below and will be discussed in more detail in sections 3.2–3.5.

- Enzymes provide a reaction surface and a suitable environment.
- Enzymes bring reactants together and position them correctly so that they easily attain their transition-state configurations.
- Enzymes weaken bonds in the reactants.
- Enzymes may participate in the reaction mechanism.
- Enzymes form stronger interactions with the transition state than with the substrate or the product.

An enzyme catalyses a reaction by providing a surface to which a substrate can bind, resulting in the weakening of high-energy bonds. The binding also holds the substrate in the correct orientation to increase the chances of reaction. The reaction takes place, aided by the enzyme, to give a product which is then released (Fig. 3.3). Note again that it is a reversible process. Enzymes can catalyse both forward and backward reactions. The final equilibrium mixture will, however, be the same, regardless of whether we supply the enzyme with substrate or product. Substrates bind to, and react at, a specific area of the enzyme called the **active site**—usually quite a small part of the overall protein structure.

3.3 The active site of an enzyme

The active site of an enzyme (Fig. 3.4) has to be on or near the surface of the enzyme if a substrate is to reach it. However, the site could be a groove, hollow, or gully allowing the substrate to sink into the enzyme. Normally, the active site is more hydrophobic in character than the surface of the enzyme, providing a suitable environment for many reactions that would be difficult or impossible to carry out in an aqueous environment.

Because of the overall folding of the enzyme, the amino acid residues that are close together in the active site may be far apart in the primary structure. Several amino acids in the active site play an important role in enzyme function, which can be demonstrated by comparing the primary structures of the same enzyme from different organisms. Here, the primary structure differs from species to species as a result of **mutations** happening over millions of years. The variability is proportional to how far apart the organisms are on the evolutionary ladder. However, there are certain amino acids that remain constant, no matter the source of the enzyme. These are amino acids that are crucial to the enzyme's function and are often present in the active site. If one of these amino acids is altered through mutation, the enzyme could become useless and the cell bearing this mutation would have a poor chance of survival. Thus, the mutation would not be preserved. The only exception to this would be if the mutation introduced an amino acid which could either perform the same task as the original amino acid or improved substrate binding. This consistency of amino acids in the active site can often help scientists determine which amino acids are present in an active site, if this is not known already.

Amino acids present in the active site can have one of two roles:

- binding—the amino acid residue is involved in binding the substrate or a cofactor to the active site;

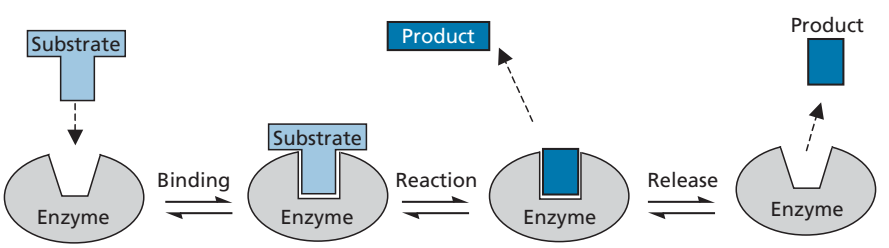


FIGURE 3.3 The process of enzyme catalysis.

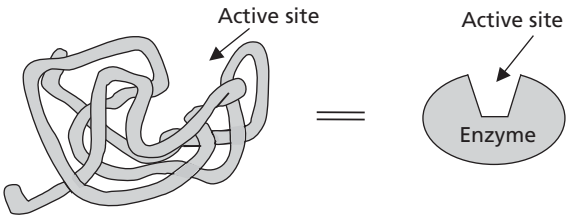


FIGURE 3.4 The active site of an enzyme.

- catalytic—the amino acid is involved in the mechanism of the reaction.

We shall study these in turn.

3.4 Substrate binding at an active site

The interactions which bind substrates to the active sites of enzymes include ionic bonds, hydrogen bonds, dipole–dipole, and ion–dipole interactions, as well as van der Waals and hydrophobic interactions (section 1.3). These binding interactions are the same bonding interactions responsible for the tertiary structure of proteins, but their relative importance differs. Ionic bonding plays a relatively minor role in protein tertiary structure compared with hydrogen bonding or van der Waals interactions, but it can play a crucial role in the binding of a substrate to an active site.

As intermolecular bonding forces are involved in substrate binding, it is possible to look at the structure of a substrate and postulate the probable interactions that it will have with its active site. As an example, consider **pyruvic acid**—the substrate for **lactate dehydrogenase** (Fig. 3.5).

If we look at the structure of pyruvic acid, we can propose three possible interactions by which it might bind to its active site—an ionic interaction involving the ionized carboxylate group, a hydrogen bond involving the ketonic oxygen, and a van der Waals interaction involving the methyl group. If these postulates are correct, it means that within the active site there must be **binding regions** containing suitable amino acids that can take part in these intermolecular interactions. Lysine, serine, and phenylalanine residues respectively would fit the bill. A knowledge of how a substrate binds to its active site is invaluable in designing drugs that will target specific enzymes (Chapter 7).

3.5 The catalytic role of enzymes

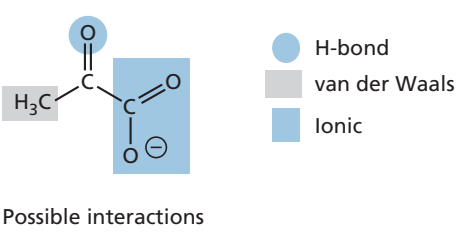
We now move on to consider the mechanism of enzymes and how they catalyse reactions. In general, enzymes catalyse reactions by providing binding interactions, acid/base catalysis, nucleophilic groups, and cofactors.

3.5.1 Binding interactions

In the past, it was thought that a substrate fitted its active site in a similar way to a key fitting a lock (**Fischer's lock and key hypothesis**). Both the enzyme and the substrate were seen as rigid structures, with the substrate (the key) fitting perfectly into the active site (the lock) (Fig. 3.6). However, this scenario does not explain how some enzymes can catalyse a reaction on a range of different substrates. It implies, instead, that an enzyme has an optimum substrate that fits it perfectly, whereas all other substrates fit less perfectly. This, in turn, would imply that the catalysed reaction is only efficient for the optimum substrate. As this is not the case for many enzymes, the lock and key analogy must be invalid.

It is now proposed that the substrate is not quite the ideal shape for the active site, and that it forces the active site to change shape when it enters—a kind of moulding process. This theory is known as **Koshland's theory of induced fit** as the substrate induces the active site to take up the ideal shape to accommodate it (Fig. 3.6).

For example, a substrate such as **pyruvic acid** might interact with specific binding regions in the active site of lactate dehydrogenase via one hydrogen bond, one ionic bond, and one van der Waals interaction (Fig. 3.7).



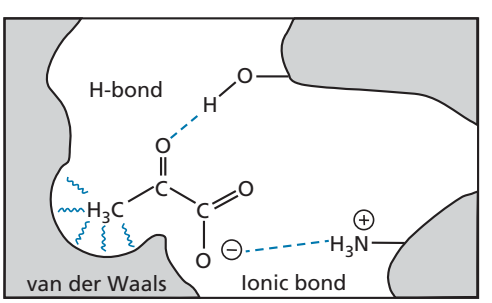


FIGURE 3.5 Binding interactions between pyruvic acid and lactate dehydrogenase.

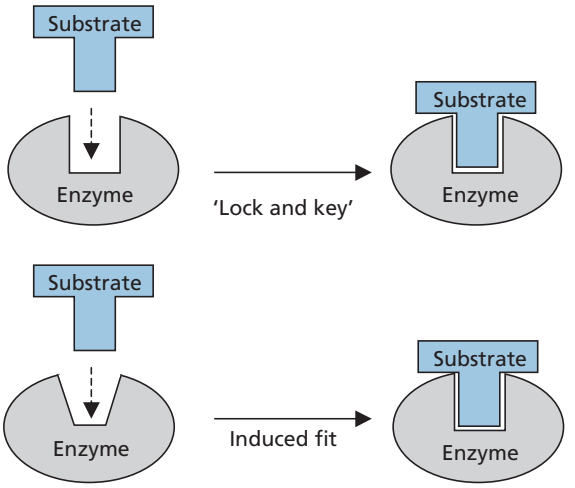


FIGURE 3.6 The 'lock and key' and 'induced fit' hypotheses for substrate–enzyme binding.

However, if the fit is not perfect, the three bonding interactions are not ideal either. For example, the binding groups may be slightly too far away from the corresponding binding regions in the active site. In order to maximize the strength of these bonds, the enzyme changes shape such that the amino acid residues involved in the binding move closer to the substrate.

This theory of induced fit helps to explain why some enzymes can catalyse reactions involving a wide range of substrates. Each substrate induces the active site into a shape that is ideal for it and, as long as the moulding process does not distort the active site so much that the reaction mechanism proves impossible, the reaction can proceed. The range of substrates that can be accepted depends on the substrates being the correct size to fit the active site and having the correct binding groups in the correct relative positions.

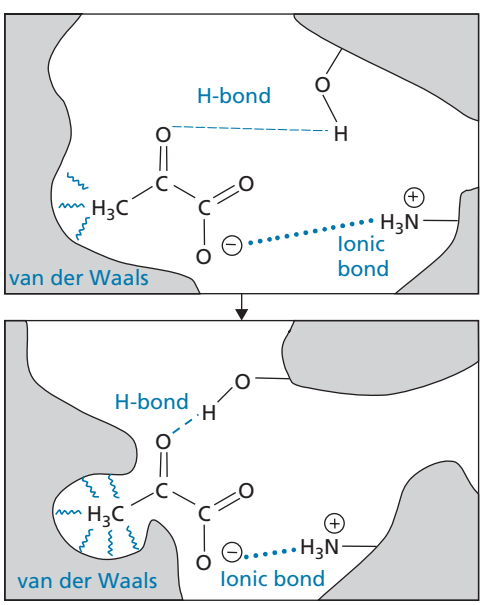


FIGURE 3.7 Example of an induced fit.

But note this. The substrate is not a passive spectator to the moulding process going on around it. As the enzyme changes shape to maximize bonding interactions, the same thing can happen to the substrate. It too may alter shape. Bond rotation may occur to fix the substrate in a particular conformation—and not necessarily the most stable one. Bonds may even be stretched and weakened. Consequently, this moulding process designed to maximize binding interactions may force the substrate into the ideal conformation for the reaction to follow and may also weaken the very bonds that have to be broken.

Once bound to an active site, the substrate is now held ready for the subsequent reaction. Binding has fixed the 'victim' (substrate) so that it cannot evade attack, and this same binding has weakened its defences (bonds) so that reaction is easier (a lower activation energy).

There is another point relating to substrate binding. The binding interactions with the active site must be sufficiently strong to hold the substrate for the subsequent reaction, but they cannot be too strong. If they were, the product might also be bound strongly and fail to depart the active site. This would block the active site of the enzyme and prevent it from catalysing another reaction. Therefore, a balance must be struck.

Finally, it is important to realize that the enzyme also binds the transition state involved in the enzyme-catalysed reaction. Indeed, the binding interactions involved are stronger than those binding the substrate, which means that the transition state is stabilized relatively more than the substrate. This results in a lower activation energy compared with the non-catalysed reaction.

3.5.2 Acid/base catalysis

Acid/base catalysis is often provided by the amino acid **histidine**, which contains an imidazole ring as part of its side chain. The imidazole ring acts as a weak base, which means that it exists in equilibrium between its protonated and free base forms (Fig. 3.8), allowing it to accept or donate protons during a reaction mechanism. This is important, as there are often very few water molecules present in an active site to carry out this role. Histidine is not the only amino acid residue that can provide acid/base catalysis. For example, a **glutamic acid** residue acts as a proton source in the reaction mechanism of the enzyme HMG-CoA reductase (Case study 1), while **aspartic acid** and **aspartate** residues act as proton donors and proton acceptors, respectively, in other enzyme-catalysed reactions (sections 7.4 and 20.7.4.1).

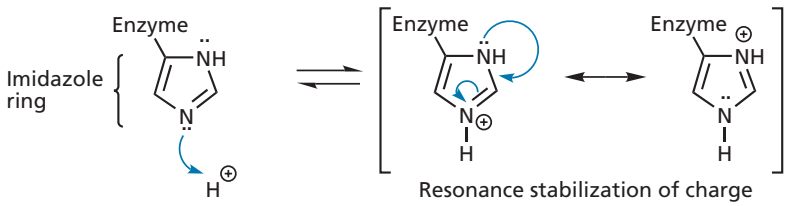


FIGURE 3.8 Histidine acting as a weak base.

Tyrosine acts as a proton source in the mechanism by which the enzyme 17β-hydroxysteroid type 1 catalyses the conversion of **estrone** to **estradiol**.

For additional material see Web article 1: steroids as novel anticancer agents.

3.5.3 Nucleophilic groups

The amino acids **serine** and **cysteine** are present in the active sites of some enzymes. These amino acids have nucleophilic residues (OH and SH respectively) which are able to participate in the reaction mechanism. They do this by reacting with the substrate to form intermediates that would not be formed in the uncatalysed reaction. These intermediates offer an alternative reaction pathway that may avoid a high-energy transition state and hence increase the rate of the reaction.

Normally, an alcoholic OH group, such as the one on serine, is not a good nucleophile. However, there is usually a histidine residue close by to catalyse the reaction. For example, the mechanism by which chymotrypsin hydrolyses peptide bonds (Fig. 3.9) involves a **catalytic triad** of amino acids—serine, histidine, and aspartic acid. Serine and histidine participate in the mechanism as a nucleophile and acid/base catalyst respectively. The aspartate group interacts with the histidine ring and serves to activate and orient it correctly for the mechanism.

The presence of a nucleophilic serine residue means that water is not required in the initial stages of the mechanism. This is important, firstly, because water is a

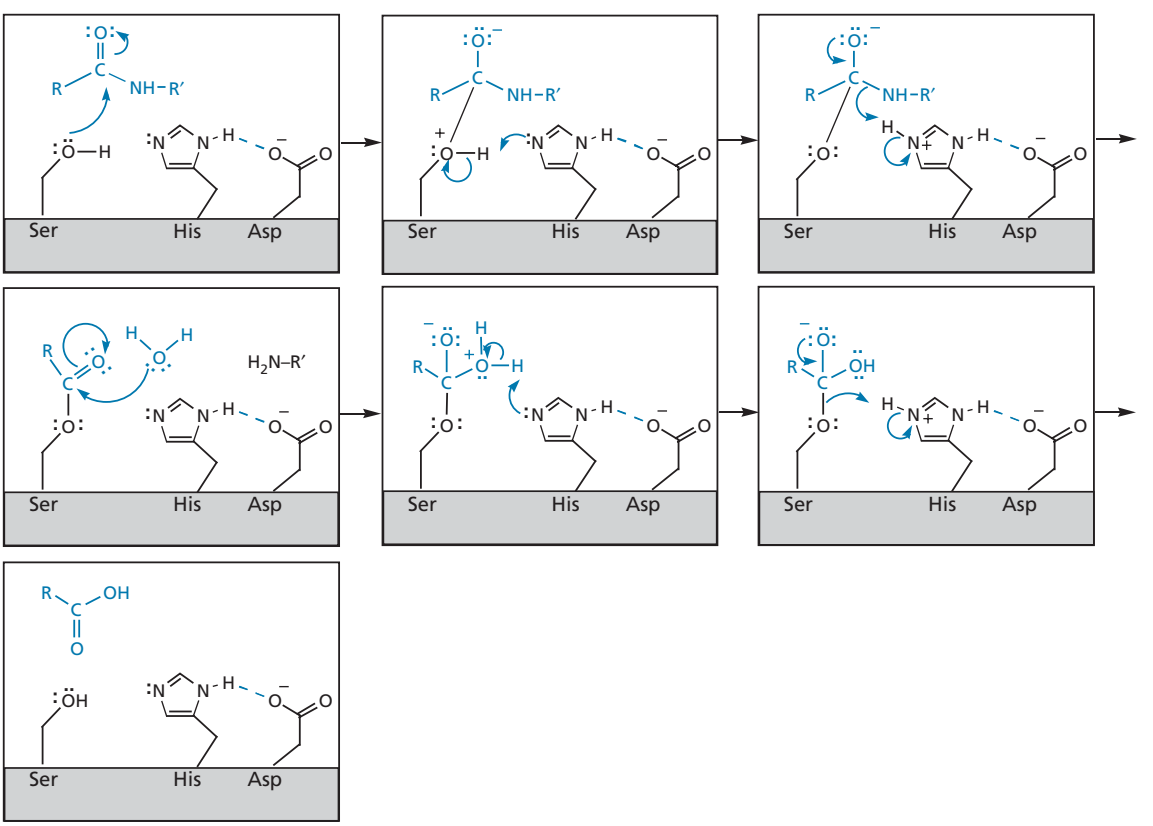


FIGURE 3.9 Hydrolysis of peptide bonds catalysed by the enzyme chymotrypsin.

poor nucleophile and may also find it difficult to penetrate the occupied active site. Secondly, a water molecule would have to drift into the active site and search out the carboxyl group before it could attack it. This would be something similar to a game of blind man's bluff. The enzyme, however, can provide a serine OH group positioned in exactly the right spot to react with the substrate. Therefore, the nucleophile has no need to search for its substrate: the substrate has been delivered to it.

Water is eventually required to hydrolyse the acyl group attached to the serine residue. However, this is a much easier step than the hydrolysis of a peptide link, as esters are more reactive than amides. Furthermore, the hydrolysis of the peptide link means that one half of the peptide can drift away from the active site and leave room for a water molecule to enter. A similar enzymatic mechanism is involved in the action of the enzyme **acetylcholinesterase** (section 22.12.3), **pancreatic lipase** (Box 7.2), and a viral protease enzyme carried by the hepatitis C virus (section 20.10).

The amino acid **lysine** has a primary amine group on its side chain which should make it a better nucleophilic group than serine or cysteine. However, the group is generally protonated at physiological pH, which precludes it acting as a nucleophile. Having said that, some enzymes have a lysine residue located in a hydrophobic pocket, which means that it is not protonated and can, indeed, act as a nucleophilic group.

3.5.4 Cofactors

Many enzymes require additional non-protein substances called cofactors for the reaction to take place. Deficiency of cofactors can arise from a poor diet resulting in the loss of enzyme activity and subsequent disease (e.g. scurvy). Cofactors are either metal ions (e.g. zinc) or small organic molecules called **coenzymes** (e.g. NAD^+, pyridoxal phosphate). Most coenzymes are bound by ionic bonds and other non-covalent bonding interactions, but some are bound covalently and are called **prosthetic groups**. Coenzymes are derived from water-soluble vitamins and act as the body's chemical reagents. For example, **lactate dehydrogenase** requires the coenzyme **nicotinamide adenine dinucleotide (NAD^+)** (Fig. 3.10) in order to catalyse the dehydrogenation of **lactic acid** to **pyruvic acid**. NAD^+ is bound to the active site along with lactic acid, and acts as the oxidizing agent. During the reaction it is converted to its reduced form (NADH) (Fig. 3.11). Conversely, NADH can bind to the enzyme and act as a reducing agent when the enzyme catalyses the reverse reaction.

$NADP^+$ and NADPH are phosphorylated analogues of NAD^+ and NADH, respectively, and carry out redox reactions by the same mechanism. NADPH is used almost exclusively for reductive biosynthesis, whereas NADH is used primarily for the generation of ATP.

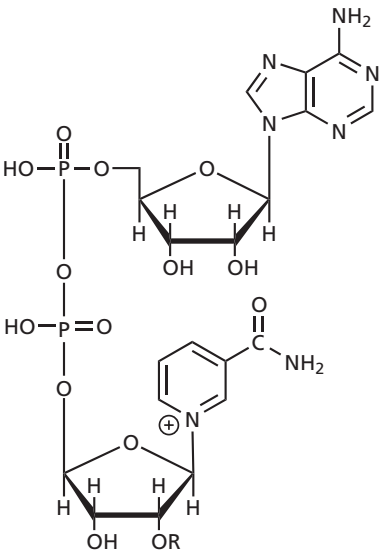


FIGURE 3.10 Nicotinamide adenine dinucleotide (R = H) and nicotinamide adenine dinucleotide phosphate (R = phosphate).

A knowledge of how the coenzyme binds to the active site allows the possibility of designing enzyme inhibitors that will fit the same region (see Case study 5 and section 21.6.2; see also Web article 1).

3.5.5 Naming and classification of enzymes

The name of an enzyme reflects the type of reaction it catalyses, and has the suffix '-ase' to indicate that it is an enzyme. For example, an **oxidase** enzyme catalyses an oxidation reaction. It is important to appreciate that enzymes can catalyse the forward and back reactions of an equilibrium reaction. This means that an oxidase enzyme can catalyse reductions, as well as oxidations. The reaction catalysed depends on the nature of the substrate, i.e. whether it is in the reduced or oxidized form.

Enzymes are classified according to the general class of reaction they catalyse and are coded with an EC number (Table 3.1).

3.5.6 Genetic polymorphism and enzymes

There are often subtle differences in the structure and properties of an enzyme between different individuals. This is owing to the fact that the DNA that codes for proteins (Chapter 6) is not identical from person to person. On average, there is a difference of one base pair in every thousand between individuals. This is known as **genetic polymorphism**. As the nucleic acid bases act as

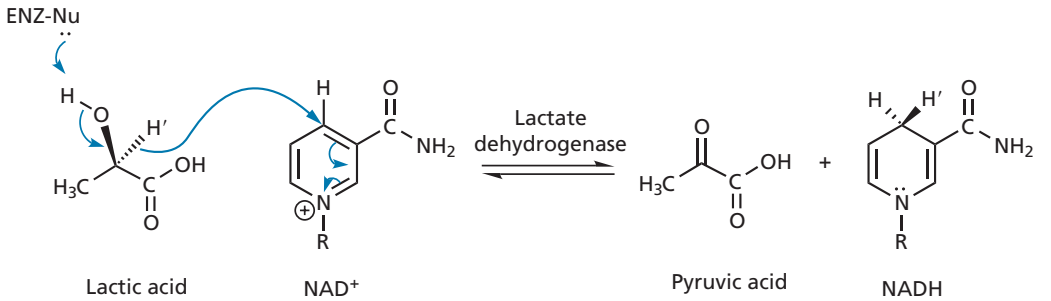


FIGURE 3.11 NAD^+ acting as a coenzyme.

TABLE 3.1 Classification of enzymes

EC number	Enzyme class	Type of reaction
E.C.1.x.x.x	Oxidoreductases	Oxidations and reductions
E.C.2.x.x.x	Transferases	Group transfer reactions
E.C.3.x.x.x	Hydrolases	Hydrolysis reactions
E.C.4.x.x.x	Lyases	Addition or removal of groups to form double bonds
E.C.5.x.x.x	Isomerases	Isomerizations and intramolecular group transfers
E.C.6.x.x.x	Ligases	Joining two substrates at the expense of ATP hydrolysis

Note: EC stands for Enzyme Commission, a body set up by the International Union of Biochemistry (as it then was) in 1955.

the code for amino acids in proteins, a difference at this level results in a different amino acid being introduced into the protein. Often, this has no observable effect on protein function, but not always. Some polymorphisms can adversely affect the proper functioning of an enzyme and lead to genetic disease. Others can have an influence on drug therapy. For example, individuals differ in their ability to metabolize drugs as a result of this phenomenon (section 11.5.6). Polymorphism can alter the sensitivity of an enzyme towards a drug, making the latter less effective. This is a particular problem in anticancer, antibacterial, and antiviral therapies where drug resistance can develop through the survival of cells containing less sensitive enzymes (Chapters 19–21).

3.6 Regulation of enzymes

Virtually all enzymes are controlled by agents which can either enhance or inhibit catalytic activity. Such control reflects the local conditions within the cell. For example, the enzyme **phosphorylase** *a* catalyses the breakdown of **glycogen** (a polymer of glucose monomers) to **glucose-1-phosphate** subunits (Fig. 3.12). It is stimulated by **adenosine 5′-monophosphate (AMP)** and inhibited by glucose-1-phosphate. Thus, rising levels of the product (glucose-1-phosphate) act as a self-regulating 'brake' on the enzyme.

But how does this control take place?

The answer is that many enzymes have a binding site which is separate from the active site called the **allosteric** binding site (Fig. 3.13). This is where the agents controlling the activity of the enzyme bind. When this occurs, an induced fit takes place which alters not only the allosteric binding site, but also the active site. Agents that inhibit the enzyme produce an induced fit that makes the active site unrecognizable to the substrate.

We might wonder why an agent inhibiting the enzyme has to bind to a separate, allosteric binding site and not to the active site itself. After all, if the agent could bind to the active site, it would directly block the natural substrate from entering. There are two explanations for this.

Firstly, many of the enzymes that are under allosteric control are at the start of a biosynthetic pathway (Fig. 3.14). A biosynthetic pathway involves a series of enzymes, all working efficiently to produce a final product. Eventually, the cell will have enough of the required material and will need to stop production. The most common control mechanism is known as **feedback control**, where the final product controls its own synthesis by inhibiting the first enzyme in the biochemical pathway. When there are low levels of final product in the cell, the first enzyme in the pathway is not inhibited and works normally. As the levels of final product increase, more and more of the enzyme is blocked and the rate of synthesis drops off in a graded fashion. Crucially, the final product has undergone many transformations from the original starting material and so it is no longer recognized by the active site of the first enzyme. A separate allosteric binding site is therefore needed which recognizes the final product. The biosynthesis of noradrenaline in section 23.4 is an example of a biosynthetic pathway under feedback control.

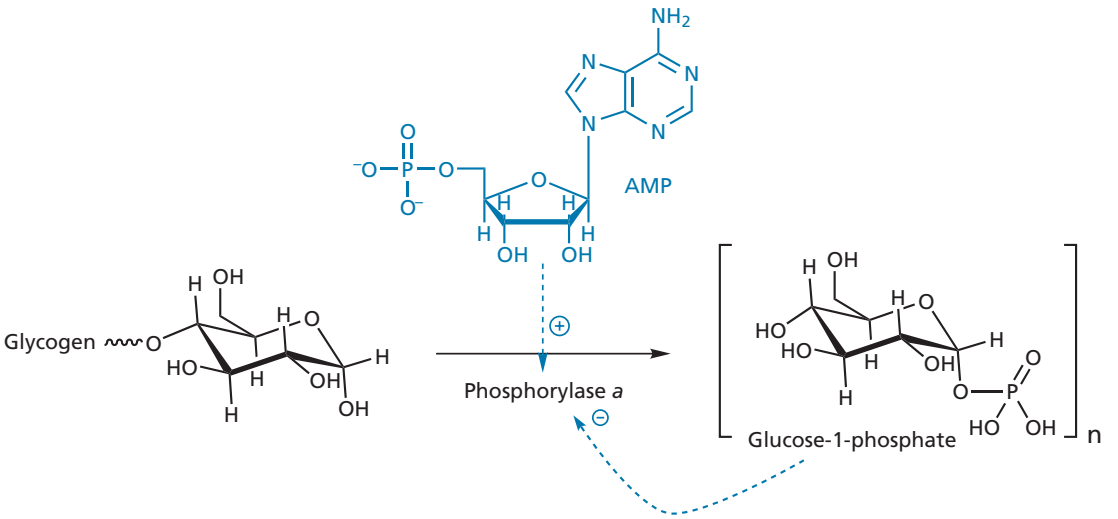


FIGURE 3.12 Internal control of the catalytic activity of phosphorylase *a* by glucose-1-phosphate and AMP.

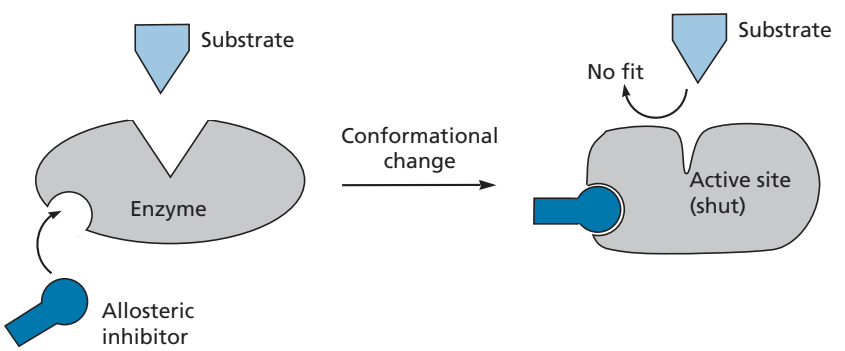


FIGURE 3.13 Allosteric inhibition of an enzyme.

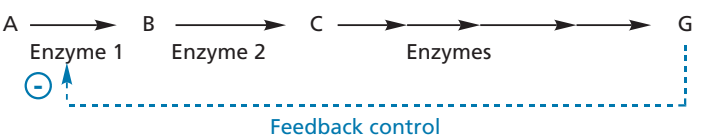


FIGURE 3.14 Feedback control of enzyme 1 by final product G.

Secondly, binding of the final product to the active site would not be a very efficient method of feedback control, as the product would have to compete with the enzyme's substrate. If levels of the latter increased, then the inhibitor would be displaced and feedback control would fail.

Many enzymes can also be regulated externally (Box 3.1). We shall look at this in more detail in Chapter 5, but, in essence, cells receive chemical messages from their environment which trigger a cascade of signals within the cell. In turn, these ultimately activate a set of enzymes known as **protein kinases**. The protein kinases play an important part in controlling enzyme activity within the cell by phosphorylating amino acids such as **serine, threonine**, or **tyrosine** in target enzymes—a covalent modification. For example, the hormone **adrenaline** is an external messenger which triggers a signalling sequence resulting in the activation of a protein kinase enzyme. Once activated, the protein kinase phosphorylates an inactive enzyme called **phosphorylase *b*** (Fig. 3.15). This enzyme now becomes active and is called **phosphorylase *a***. It catalyses the breakdown of glycogen and remains active until it is dephosphorylated back to phosphorylase ***b***.

In this case, phosphorylation of the target enzyme leads to activation. Other enzymes may be deactivated by phosphorylation. For example, **glycogen**

BOX 3.1 The external control of enzymes by nitric oxide

The external control of enzymes is usually initiated by external chemical messengers which do not enter the cell. However, there is an exception to this. It has been discovered that cells can generate the gas **nitric oxide** by the reaction sequence shown in Fig. 1, catalysed by the enzyme **nitric oxide synthase**.

Because nitric oxide is a gas, it can diffuse easily through cell membranes into target cells. There, it activates enzymes called **cyclases** to generate **cyclic GMP** from **GTP** (Fig. 2). Cyclic GMP then acts as a secondary messenger to influence other reactions within the cell. By this process, nitric oxide has an influence on a diverse range of physiological processes, including blood pressure, **neurotransmission**, and immunological defence mechanisms.

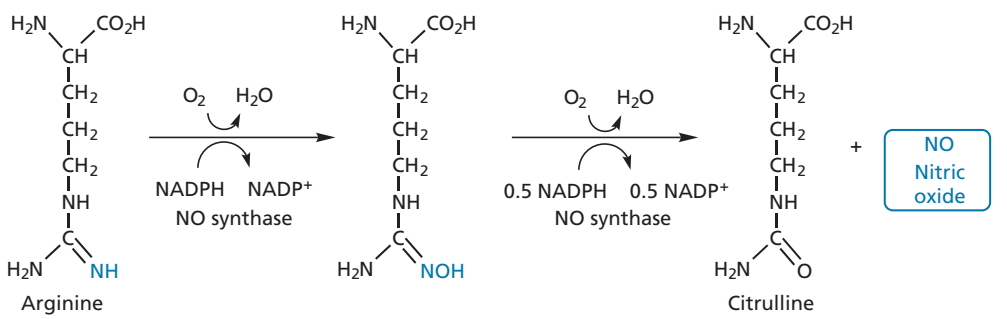


FIGURE 1 Synthesis of nitric oxide.

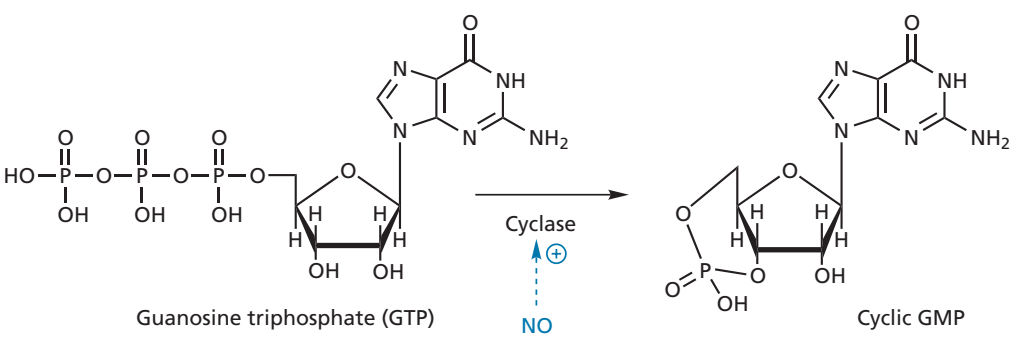


FIGURE 2 Activation of cyclase enzymes by nitric oxide (NO).

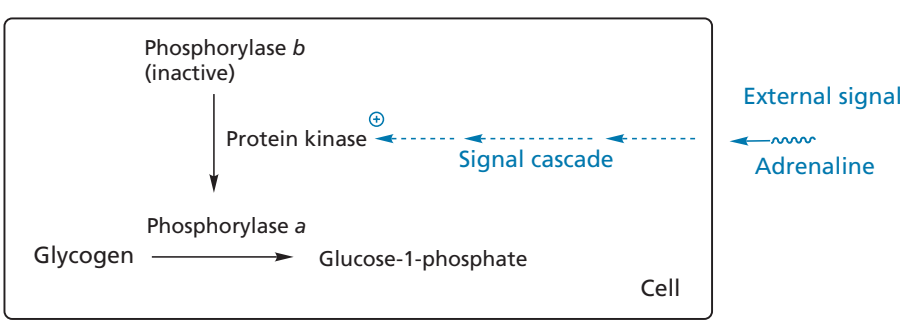


FIGURE 3.15 External control of phosphorylase *a*.

synthase—the enzyme that catalyses the *synthesis* of glycogen from glucose-1-phosphate—is inactivated by phosphorylation and activated by dephosphorylation. The latter is effected by the hormone **insulin**, which triggers a different signalling cascade from that of adrenaline.

Protein–protein interactions can also play a role in the regulation of enzyme activity. For example, signal proteins in the cell membrane are responsible for regulating the activity of membrane-bound enzymes (section 5.2).

3.7 Isozymes

Enzymes having a quaternary structure are made up of a number of polypeptide subunits. The combination of these subunits can differ in different tissues. Such variations are called **isozymes**. For example, there are five different isozymes of mammalian **lactate dehydrogenase** (LDH)—a tetrameric enzyme made up of four polypeptide subunits. There are two different types of subunits involved, which are labelled 'H' and 'M'. The former predominates in the LDH present in heart muscle, while the latter predominates in the LDH present in skeletal muscle. As there are two different types of subunit, five different isozymes are possible: HHHH, HHHM, HHMM, HMMM, and MMMM. Isozymes differ in their properties. For example, the M_4 isozyme in skeletal muscle catalyses the conversion of pyruvic acid to lactic acid and is twice as active as the H_4 isozyme in heart muscle. The H_4 isozyme catalyses the reverse reaction and is inhibited by excess pyruvic acid, whereas the M_4 isozyme is not.

KEY POINTS

- Enzymes are proteins that act as the body's catalysts by binding substrates and participating in the reaction mechanism.
- The active site of an enzyme is usually a hollow or cleft in the protein. There are important amino acids present in the active site that either bind substrates or participate in the reaction mechanism.
- Binding of substrate to an active site involves intermolecular bonds.
- Substrate binding involves an induced fit where the shape of the active site alters to maximize binding interactions. The binding process also orientates the substrate correctly and may weaken crucial bonds in the substrate to facilitate the reaction mechanism.
- The amino acid histidine is often present in active sites and acts as an acid/base catalyst. Glutamic acid, aspartic acid, and tyrosine also act as acid/base catalysts in some enzymes.
- The amino acids serine and cysteine act as nucleophiles in the reaction mechanisms of some enzymes. In some enzymes, lysine can act as a nucleophile.
- Cofactors are metal ions or small organic molecules (coenzymes) which are required by many enzymes. Coenzymes can be viewed as the body's chemical reagents.
- Prosthetic groups are coenzymes which are bound covalently to an enzyme.
- Enzymes are regulated by internal and/or external control.
- External control involves regulation initiated by a chemical messenger from outside the cell and which ultimately involves the phosphorylation of enzymes.
- Allosteric inhibitors bind to a different binding site from the active site and alter the shape of the enzyme such that the active site is no longer recognizable. Allosteric inhibitors are often involved in the feedback control of biosynthetic pathways.
- Isozymes are variations of the same enzyme. They catalyse the same reaction but differ in their primary structure, substrate specificity, and tissue distribution.
- The amino acid sequence in enzymes may differ between individuals as a result of genetic polymorphism. This may or may not result in a difference in enzyme activity.

3.8 Enzyme kinetics

3.8.1 The Michaelis-Menton equation

The Michaelis-Menten equation holds for an enzyme (E) which combines with its substrate (S) to form an enzyme–substrate complex (ES). The enzyme–substrate complex can then either dissociate back to E and S, or go on to form a product (P). It is assumed that formation of the product is irreversible.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

where k_1, k_2 *and* k_3 are rate constants.

For enzymes such as these, plotting the rate of enzyme reaction versus substrate concentration [S] gives a curve as shown in Fig. 3.16. At low substrate concentrations the rate of reaction increases almost proportionally to the substrate concentration, whereas at high substrate concentration the rate becomes almost constant and approaches a maximum rate ($rate_{max}$), which is independent of substrate concentration. This reflects a situation where there is more substrate present than active sites available; therefore, increasing the amount of substrate will have little effect.

The Michaelis-Menten equation relates the rate of reaction to the substrate concentration for the curve in Fig. 3.16.

$$\text{rate} = \text{rate}_{max} \frac{[S]}{[S] + K_M}$$

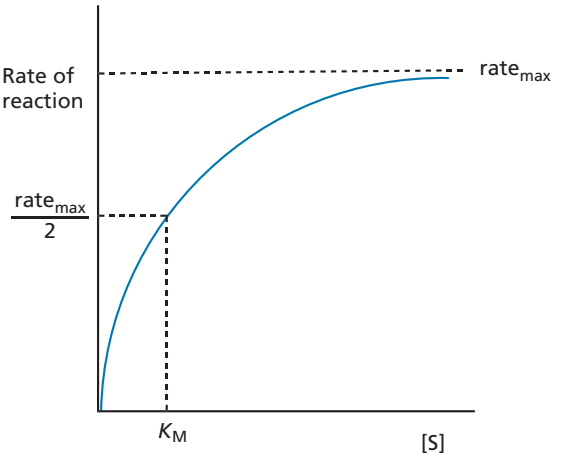


FIGURE 3.16 Reaction rate versus substrate concentration.

The derivation of this equation is not covered here, but can be found in most biochemistry textbooks. The constant K_M is known as the **Michaelis constant** and is equal to the substrate concentration at which the reaction rate is half of its maximum value. This can be demonstrated as follows. If Km = [S], then the Michaelis-Menten equation becomes:

$$\text{rate} = \text{rate}_{max}\frac{[S]}{[S]+[S]} = \text{rate}_{max}\frac{[S]}{2[S]} = \text{rate}_{max} \times \frac{1}{2}$$

The K_M of an enzyme is significant because it measures the concentration of substrate at which half the active sites in the enzyme are filled. This, in turn, provides a measure of the substrate concentration required for significant catalysis to occur.

K_M is also related to the rate constants of the enzyme-catalysed reaction:

$$K_M = \frac{k_2 + k_3}{k_1}$$

Consider now the situation where there is rapid equilibration between S and ES, and a slower conversion to product P. This means that the substrate binds to the active site and departs several times before it is finally converted to product.

$$E + S \underset{k_2 \text{fast}}{\overset{k_1 \text{fast}}{\rightleftharpoons}} ES \xrightarrow[\text{slow}]{k_3} E + P$$

Under these conditions, the dissociation rate (k_2) of ES is much greater than the rate of formation of product (k_3). k_3 now becomes insignificant relative to k_2 and the equation simplifies to:

$$K_M = \frac{k_2 + k_3}{k_1} = \frac{k_2}{k_1}$$

In this situation, K_M effectively equals the dissociation constant of ES and can be taken as a measure of how strongly the substrate binds to the enzyme.

$$[ES] \rightleftharpoons [E] + [S] \qquad \text{dissociation constant} = \frac{[E][S]}{[ES]}$$

A high value of K_M indicates weak binding because the equilibrium is pushed to the right; a low K_M indicates strong binding because the equilibrium is to the left. K_M is also dependent on the particular substrate involved and on environmental conditions, such as pH, temperature, and ionic strength.

The maximum rate is related to the total concentration of enzyme ($[E]_{total} = [E] + [ES]$) as follows:

$$\text{rate}_{max} = k_3[E]_{total}$$

A knowledge of the maximum rate and the enzyme concentration allows the determination of k_3. For example, the enzyme **carbonic anhydrase** catalyses the formation of hydrogen carbonate and does so at a maximum rate of 0.6 moles of hydrogen carbonate molecules formed per second for a solution containing 10^{-6} moles of the enzyme. Altering the above equation, k_3 can be determined as follows:

$$k_3 = \frac{\text{rate}_{max}}{[E]_{total}} = \frac{0.6}{10^{-6}}\frac{\text{M s}^{-1}}{\text{M}} = 600\,000\,\text{s}^{-1}$$

Therefore, each enzyme is catalysing the formation of 600,000 hydrogen carbonate molecules per second. The turnover number is the time taken for each catalysed reaction to take place, i.e. 1/600 000 = 1.7 µs.

3.8.2 Lineweaver-Burk plots

A problem related to Michaelis-Menton kinetics is the fact that there may not be sufficient data points to

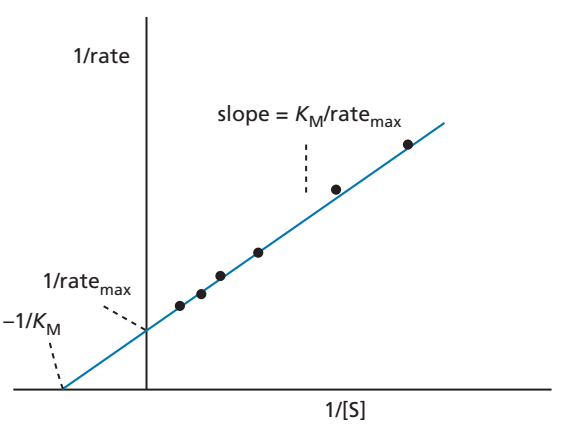


FIGURE 3.17 Lineweaver-Burk plot.

determine whether the curve of the Michaelis-Menton plot has reached a maximum value or not. This means that values for the maximum rate and K_M are likely to be inaccurate. More accurate values for these properties can be obtained by plotting the reciprocals of the rate and the substrate concentration to give a **Lineweaver-Burk plot** (Fig. 3.17):

$$\frac{1}{\text{rate}} = \frac{K_M}{\text{rate}_{max}} \cdot \frac{1}{[S]} + \frac{1}{\text{rate}_{max}} \qquad (y = m.x + c)$$

The maximum rate can then be obtained from the intersect of the line with the y-axis, while K_M can be obtained from the slope of the line or the intersect with the x-axis.

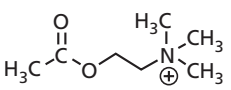
KEY POINTS

- The Michaelis-Menten equation relates the rate of an enzyme-catalysed reaction to substrate concentration.
- The Michaelis constant is equal to the substrate concentration at which the rate of the enzyme catalysed reaction is half of its maximum value.
- A Lineweaver-Burk plot provides more accurate values for the maximum rate and K_M.

QUESTIONS

1. Enzymes can be used in organic synthesis. For example, the reduction of an aldehyde is carried out using aldehyde dehydrogenase. Unfortunately, this reaction requires the use of the cofactor NADH, which is expensive and is used up in the reaction. If ethanol is added to the reaction, only catalytic amounts of cofactor are required. Why?

2. Acetylcholine is the substrate for the enzyme acetylcholinesterase. Suggest what sort of binding interactions could be involved in holding acetylcholine to the active site.



Acetylcholine

3. The ester bond of acetylcholine is hydrolysed by acetylcholinesterase. Suggest a mechanism by which the enzyme catalyses this reaction.

4. Suggest how binding interactions might make acetylcholine more susceptible to hydrolysis.

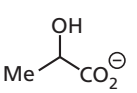
5. 17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is an enzyme that catalyses the conversion of estrone to estradiol in the presence of the cofactor NADH. The initial rate data for the enzyme-catalysed reaction in the absence of an inhibitor is as follows:

   Substrate concentration (10^{-2} mol dm^{-3}) 5 10 25 50 100

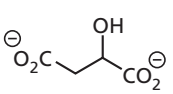
   Initial rate (10^{-1} mol dm^{-3} s^{-1}) 28.6 51.5 111 141 145

   Create a Michaelis Menton plot and a Lineweaver-Burk plot. Use both plots to calculate the values of K_M and the maximum rate of reaction. Identify which plot is likely to give the more accurate results and explain why this is the case.

6. Lactate dehydrogenase has a 1000-fold selectivity for lactate as a substrate over malate. However, if a mutation occurs that alters an active site glutamine residue to an arginine residue, the enzyme shows a 10,000-fold selectivity for malate over lactate. Explain this astonishing transformation.



Lactate



Malate

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4 Receptors: structure and function

In this chapter we discuss the structure and function of receptors. Drug action at receptors is discussed in Chapter 8 and in other chapters throughout the text.

4.1 Role of the receptor

Receptors are proteins which are, by far, the most important drug targets in medicine. They are implicated in ailments such as pain, depression, Parkinson's disease, psychosis, heart failure, asthma, and many other problems. What are these receptors and what do they do?

In a complex organism there has to be a communication system between cells. After all, it would be pointless if individual heart cells were to contract at different times. The heart would then be a wobbly jelly and totally useless in its function as a pump. Communication is essential to ensure that all heart muscle cells contract at the same time. The same is true for all the organs and tissues of the body if they are to operate in a coordinated and controlled fashion.

Control and communication come primarily from the brain and spinal column (the **central nervous system**), which receives and sends messages via a vast network of nerves (Fig. 4.1). The detailed mechanism by which nerves transmit messages along their length need not concern us here (see Appendix 4). It is sufficient for our purposes to think of the message as being an electrical pulse which travels down the nerve cell (**neuron**) towards the target, whether that be a muscle cell or another neuron. If that was all there was to it, it would be difficult to imagine how drugs could affect this communication system. However, there is one important feature that is crucial to our understanding of drug action. Neurons do not connect directly to their target cells. They stop just short of the cell surface. The distance is minute, about 100 Å, but it is a space that the electrical 'pulse' is unable to jump.

Therefore, there has to be a method of carrying the message across the gap between the nerve ending and the target cell. The problem is solved by the release of a chemical messenger called a **neurotransmitter** from the nerve cell (Fig. 4.2). Once released, this chemical messenger diffuses across the gap to the target cell, where it binds and interacts with a specific protein (receptor) embedded in the cell membrane. This process of binding leads to a series or cascade of secondary effects, which results either in a flow of ions across the cell membrane or in the switching on (or off) of enzymes inside the target cell. A biological response then results, such as the contraction of a muscle cell or the activation of fatty acid metabolism in a fat cell.

The first person to propose the existence of receptors was Langley in 1905. Up until that point, it was thought that drugs acted to prevent the release of the neurotransmitter from the neuron, but Langley was able to show that certain target cells responded to the drug nicotine, even when the neurons supplying those cells were dead.

So far, we have talked about cellular communication involving neurons and neurotransmitters, but cells also receive chemical messages from circulating **hormones**. Once again, receptors are responsible for binding these messengers and triggering a series of secondary effects.

We shall consider these secondary effects and how they result in a biological action in Chapter 5, but, for the moment, the important thing to note is that the communication system depends crucially on a chemical messenger. As a chemical process is involved, it should be possible for other chemicals (drugs) to interfere or interact with the process.

4.2 Neurotransmitters and hormones

There are a large variety of messengers that interact with receptors and they vary significantly in structure and complexity. Some neurotransmitters are simple molecules, such as monoamines (e.g. **acetylcholine**, **noradrenaline**, **dopamine**, and **serotonin)** or amino acids (e.g.

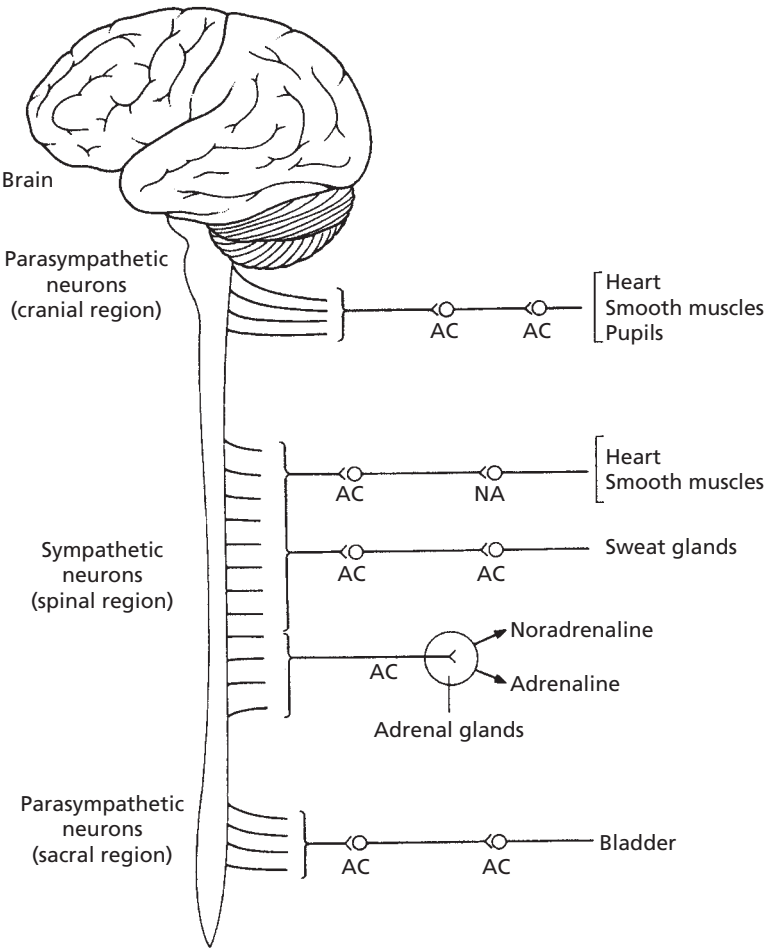


FIGURE 4.1 The central nervous system (AC = acetylcholine; NA = noradrenaline). Taken from Mann, J. (1992) *Murder, Magic, and Medicine*. Oxford University Press, with permission.

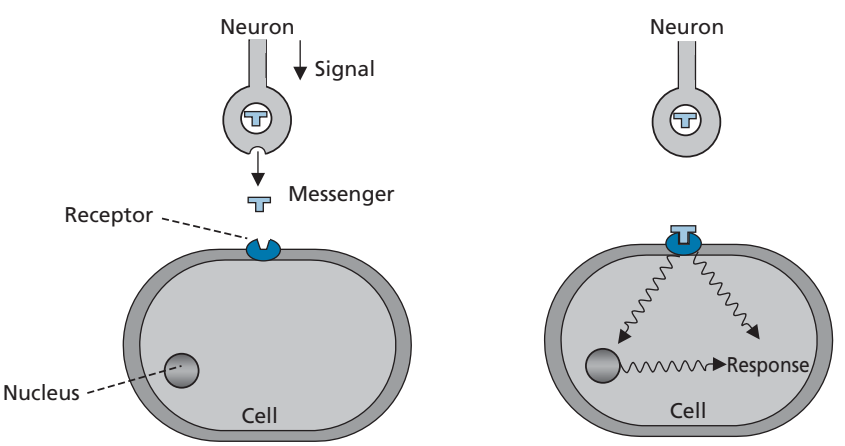


FIGURE 4.2 Neurotransmitters act as chemical messengers that bind to receptors and trigger reactions within a cell.

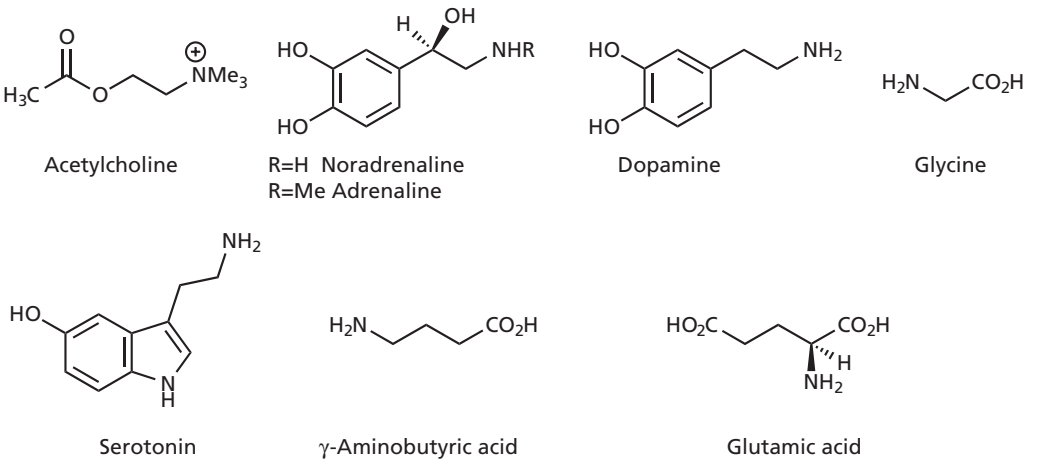


FIGURE 4.3 Examples of neurotransmitters and the hormone adrenaline.

γ-aminobutyric acid [GABA], **glutamic acid,** and **glycine**) (Fig. 4.3). Even the calcium ion can act as a chemical messenger. Other chemical messengers are more complex in structure and include lipids, such as **prostaglandins**; purines, such as **adenosine** or **ATP** (Chapter 6); **neuropeptides**, such as **endorphins** and **enkephalins** (section 24.8); peptide hormones, such as **angiotensin** or **bradykinin**; and even enzymes, such as **thrombin**.

In general, a neuron releases mainly one type of neurotransmitter, and the receptor which awaits it on the target cell will be specific for that messenger. However, that does not mean that the target cell has only one type of receptor protein. Each target cell has a large number of neurons communicating with it and they do not all use the same neurotransmitter (Fig. 4.4). Therefore, the target cell will have other types of receptors specific for those other neurotransmitters. It may also have receptors waiting to receive messages from chemical messengers that have longer distances to travel. These are the hormones released into the circulatory system by various glands in the body. The best known example of a hormone is **adrenaline**. When danger or exercise is anticipated, the adrenal medulla gland releases adrenaline into the bloodstream where it is carried round the body, preparing it for vigorous exercise.

Hormones and neurotransmitters can be distinguished by the route they travel and by the way they are released, but their action when they reach the target cell is the same. They both interact with a receptor and a message is received. The cell responds to that message and adjusts its internal chemistry accordingly, and a biological response results.

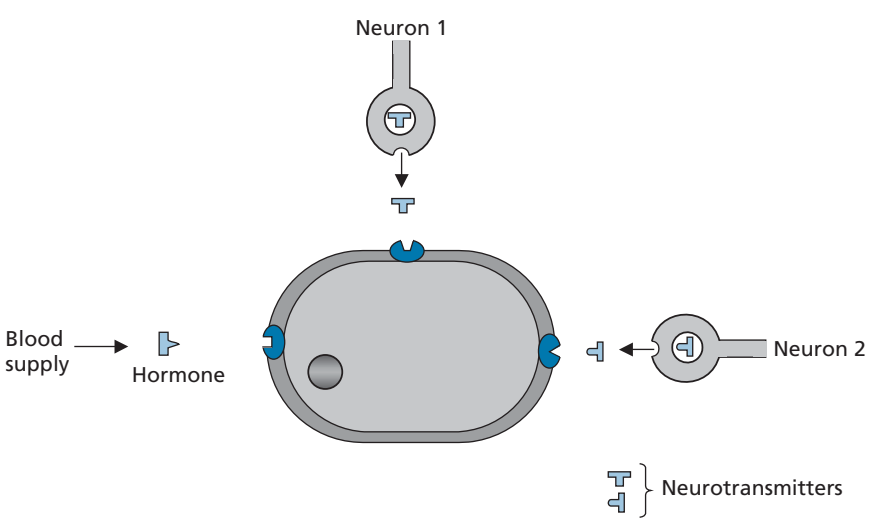


FIGURE 4.4 Target cell containing various receptors specific to different types of messenger.

4.3 Receptor types and subtypes

Receptors are identified by the specific neurotransmitter or hormone which activates them. Thus, the receptor activated by **dopamine** is called the **dopaminergic receptor**, the receptor activated by **acetylcholine** is called the **cholinergic receptor**, and the receptor activated by **adrenaline** or **noradrenaline** is called the **adrenergic receptor** or **adrenoceptor**.

However, not all receptors activated by the same chemical messenger are exactly the same throughout the body. For example, the adrenergic receptors in the lungs are slightly different from the adrenergic receptors in the heart. These differences arise from slight variations in amino acid composition; if the variations are in the binding site, it allows medicinal chemists to design drugs which can distinguish between them. For example, adrenergic drugs can be designed to be 'lung' or 'heart' selective. In general, there are various *types* of a particular receptor and various *subtypes* of these, which are normally identified by numbers or letters. Having said that, some of the early receptors that were discovered were named after natural products which bound to them, for example the muscarinic and nicotinic types of cholinergic receptor (section 22.4).

Some examples of receptor types and subtypes are given in Fig. 4.16. The identification of many of these subtypes is relatively recent and the current emphasis in medicinal chemistry is to design drugs that are as selective as possible for receptor types and subtypes so that the drugs are tissue selective and have fewer side effects.

4.4 Receptor activation

A receptor is a protein molecule usually embedded within the cell membrane with part of its structure exposed on the outside of the cell. The protein surface is a complicated shape containing hollows, ravines, and ridges. Somewhere within this complicated geography there is an area that has the correct shape to accept the incoming messenger. This area is known as the **binding site** and is analogous to the active site of an enzyme (section 3.3). When the chemical messenger fits into this site it 'switches on' the receptor molecule and a message is received (Fig. 4.5). However, there is an important difference between enzymes and receptors in that the chemical messenger does not undergo a chemical reaction. It fits into the binding site of the receptor protein, passes on its message, and then leaves unchanged. If no reaction takes place, what *has* happened? How does the chemical messenger tell the receptor its message and how is this message conveyed to the cell? The first thing to note is that when the messenger fits the binding site of the protein receptor it causes the binding site to change shape. This is known as an **induced fit**. This, in turn, has wider ramifications as there is a knock-on effect which causes the overall protein to change shape. But how does an induced fit happen and what is the significance of the receptor changing shape?

4.5 How does the binding site change shape?

As we have seen, the binding site of a receptor changes shape when a chemical messenger fits into it. This is not a moulding process in which the binding site wraps itself around the messenger. Instead, the induced fit is brought about by the intermolecular binding interactions that can take place between the messenger and the binding site. This is exactly the same process that occurs when a substrate binds to the active site of an enzyme (section 3.5.1), but, in this situation, no catalysed reaction follows binding.

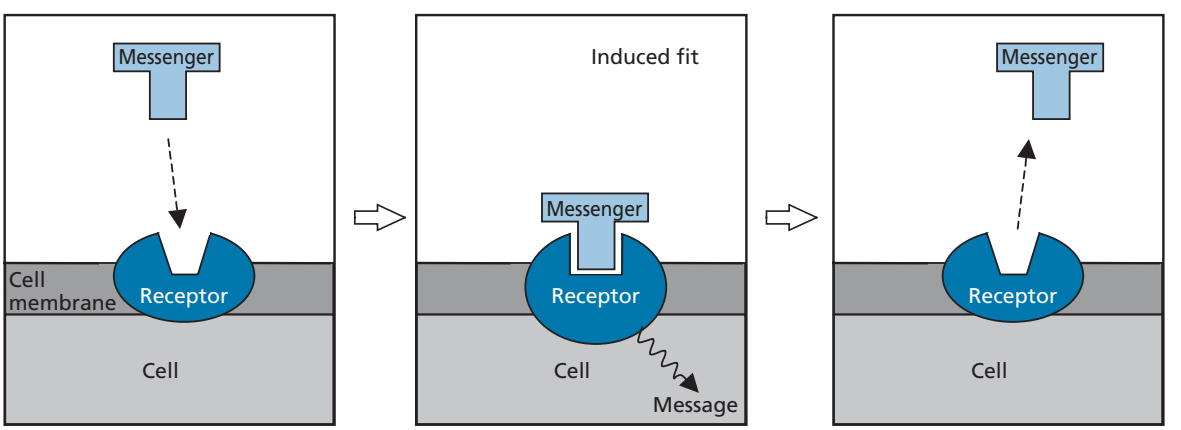


FIGURE 4.5 Binding of a chemical messenger to a protein receptor.

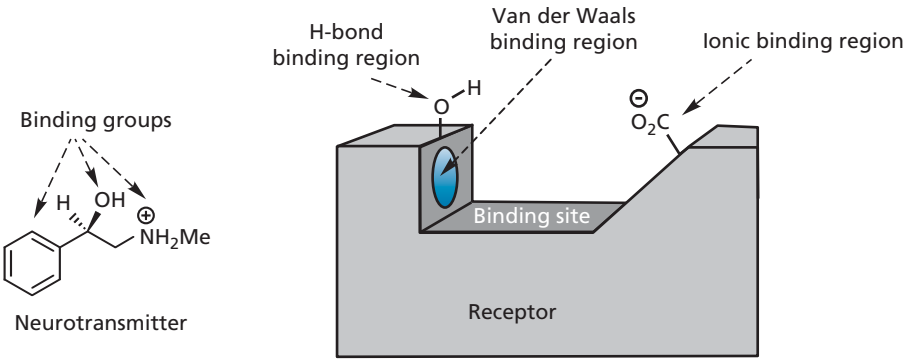


FIGURE 4.6 A hypothetical receptor and neurotransmitter.

To illustrate how binding interactions result in an induced fit, let us consider a hypothetical neurotransmitter and a hypothetical binding site as shown in Fig. 4.6. The neurotransmitter has an aromatic ring that can take part in van der Waals interactions, an alcohol OH group that can take part in hydrogen bonding interactions, and a charged nitrogen centre that can take part in ionic or electrostatic interactions. These functional groups are the messenger's **binding groups**.

The hypothetical binding site contains three **binding regions** which contain functional groups that are complimentary to the binding groups of the messenger. The messenger fits into the binding site such that intermolecular interactions take place between the messenger's binding groups and the receptor's binding regions (Fig. 4.7). However, the fit is not perfect. In the diagram, there are good van der Waals and hydrogen bond interactions, but the ionic interaction is not as strong as it could be. The ionic binding region is close enough to have a weak interaction with the messenger, but not close enough for the optimum interaction. The receptor protein therefore alters shape to bring the carboxylate group closer to the positively charged nitrogen and to obtain a stronger interaction. As a result, the shape of the binding site is altered and an induced fit has taken place.

The illustration shown here is a simplification of the induced fit process and, in reality, both the messenger and the binding site take up different conformations or shapes to maximize the bonding forces between them. As with enzyme–substrate binding, there is a fine balance involved in receptor–messenger binding. The bonding forces must be large enough to change the shape of the binding site, but not so strong that the messenger is unable to leave. Most neurotransmitters bind quickly to their receptors then 'shake themselves loose' once their message has been received.

We have now seen how a chemical messenger can cause an induced fit in the binding site of a receptor protein. However, this induced fit has a knock-on effect which alters the overall shape of the protein. It is this overall shape change that is crucial to the activation of a receptor and in its ability to trigger an amazing 'domino effect' which affects the cell's internal chemistry. This domino effect involves several different proteins and enzymes, and ultimately produces an observed biological effect. The process by which this takes place is called **signal transduction** and is covered in more detail in Chapter 5.

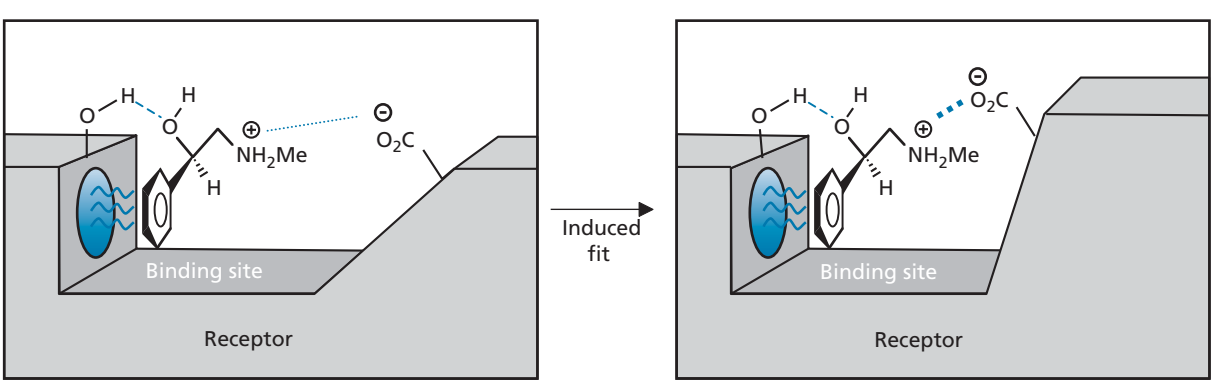


FIGURE 4.7 Binding of a hypothetical neurotransmitter to a binding site resulting in an induced fit.

Signal amplification is an important feature of this process as it means that a relatively small number of neurotransmitter molecules can have a dramatic effect on the cell's internal chemistry. In this chapter, we shall focus on the structure of different receptors and the process by which they are activated and trigger the signal transduction process.

There are three different types (or families) of membrane-bound receptors:

- ion channel receptors;
- G-protein-coupled receptors;
- kinase-linked receptors.

We shall consider each of these in turn in sections 4.6–4.8.

KEY POINTS

- Most receptors are membrane-bound proteins that contain an external binding site for hormones or neurotransmitters. Binding results in an induced fit that changes the receptor conformation. This triggers a series of events that ultimately results in a change in cellular chemistry.
- Neurotransmitters and hormones do not undergo a reaction when they bind to receptors. They depart the binding site unchanged once they have passed on their message.
- The interactions that bind a chemical messenger to the binding site must be strong enough to allow the chemical message to be received, but weak enough to allow the messenger to depart.
- Binding groups are the functional groups present on a messenger molecule which are used for binding it to the receptor binding site.
- Binding regions are regions of the receptor binding site which contain functional groups capable of forming intermolecular bonds to the binding groups of a messenger molecule.

4.6 Ion channel receptors

4.6.1 General principles

Some neurotransmitters operate by controlling ion channels. What are these ion channels and why are they necessary? Let us look again at the structure of the cell membrane.

As described in section 1.2.1, the membrane is made up of a bilayer of phospholipid molecules so the middle of the cell membrane is 'fatty' and hydrophobic. Such a barrier makes it difficult for polar molecules or ions to move in or out of the cell. Yet, it is important that these species should cross. For example, the movement of sodium and potassium ions across the membrane is crucial to the function of nerves (Appendix 4). It seems an intractable problem, but, once again, the ubiquitous proteins provide the answer by forming ion channels.

Ion channels are complexes made up of five protein subunits which traverse the cell membrane (Fig. 4.8). The centre of the complex is hollow and lined with polar amino acids to give a hydrophilic tunnel, or pore.

Ions can cross the fatty barrier of the cell membrane by moving through these hydrophilic channels or tunnels. But there has to be some control. In other words, there has to be a 'lock gate' that can be opened or closed as required. It makes sense that this lock gate should be controlled by a receptor protein sensitive to an external chemical messenger, and this is exactly what happens. In fact, the receptor protein is an integral part of the ion channel complex and is one or more of the constituent protein subunits. In the resting state, the ion channel is closed (i.e. the lock gate is shut). However, when a chemical messenger binds to the external binding site of the receptor protein, it causes an induced fit which causes the protein to change shape. This, in turn, causes the overall protein complex to change shape, opening up

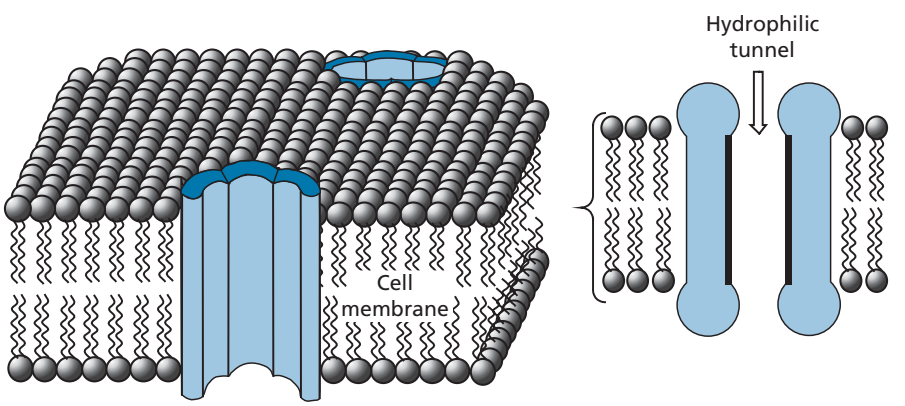


FIGURE 4.8 The structure of an ion channel. The bold lines show the hydrophilic sides of the channel.

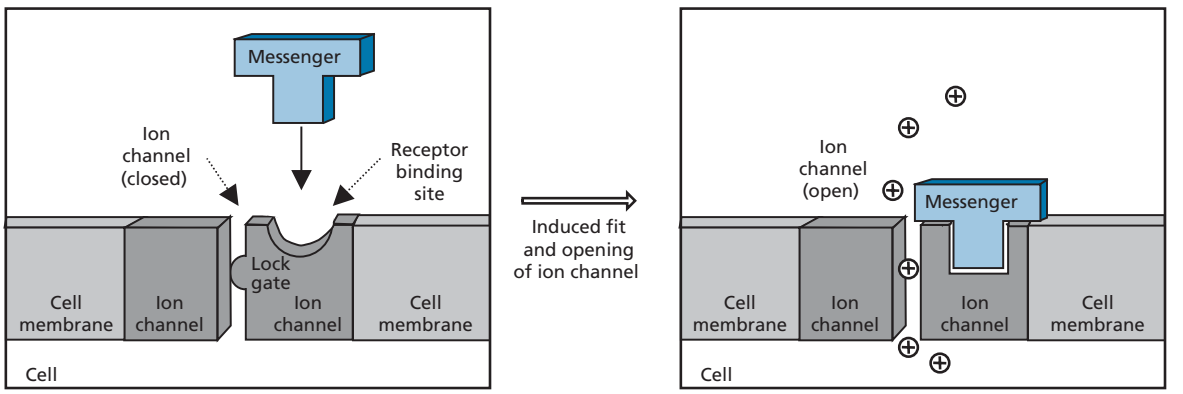


FIGURE 4.9 Lock-gate mechanism for opening ion channels.

the lock gate and allowing ions to pass through the ion channel (Fig. 4.9). We shall look at this in more detail in section 4.6.3.

The operation of an ion channel explains why the relatively small number of neurotransmitter molecules released by a neuron is able to have such a significant biological effect on the target cell. By opening a few ion channels, several thousand ions are mobilized for each neurotransmitter molecule involved. Moreover, the binding of a neurotransmitter to an ion channel results in a rapid response, measured in a matter of milliseconds. This is why the synaptic transmission of signals between neurons usually involves ion channels.

Ion channels are specific for certain ions. For example there are different cationic ion channels for sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) ions. There are also anionic ion channels for the chloride ion (Cl^-). The ion selectivity of different ion channels is dependent on the amino acids lining the ion channel. It is interesting to note that the mutation of just one amino acid in this area is sufficient to change a cationic-selective ion channel to one that is selective for anions.

4.6.2 Structure

The five protein subunits that make up an ion channel are actually **glycoproteins** (sections 2.5 and 10.7.1), but we will refer to them here as proteins. The protein subunits in an ion channel are not identical. For example, the ion channel controlled by the nicotinic cholinergic receptor is made up of five subunits of four different types [α (×2) β, γ, δ]; the ion channel controlled by the glycine receptor is made up of five subunits of two different types [α (×3), β (×2)] (Fig. 4.10).

The receptor protein in the ion channel controlled by glycine is the α-subunit. Three such subunits are present, all of which are capable of interacting with glycine. However, the situation is slightly more complex in the nicotinic ion channel controlled by the neurotransmitter acetylcholine. Most of the binding site is on the α-subunit, but there is some involvement from neighbouring subunits. In this case, the ion channel complex as a whole might be viewed as the receptor.

Let us now concentrate on the individual protein subunits. Although there are various types of these, they all fold up in a similar manner such that the protein chain traverses the cell membrane four times. This means that

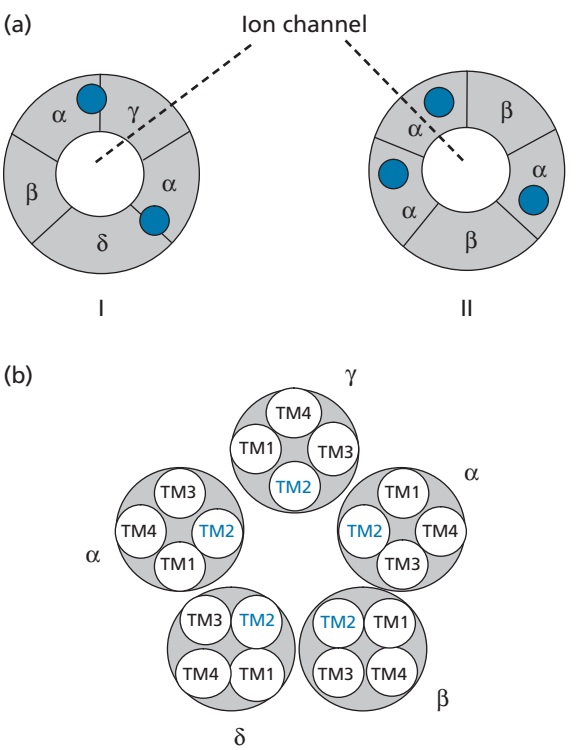


FIGURE 4.10 (**a**) Pentameric structure of ion channels (transverse view). I, ion channel controlled by a nicotinic cholinergic receptor; II, ion channel controlled by a glycine receptor. The coloured circles indicate ligand binding sites. (**b**) Transverse view of I, including transmembrane regions.

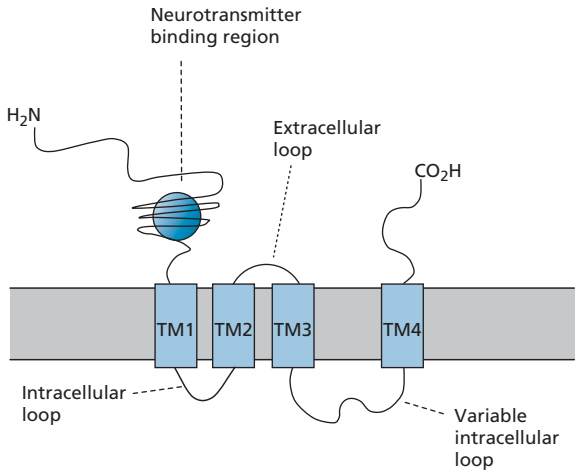


FIGURE 4.11 Structure of the four transmembrane (4-TM) receptor subunit.

each subunit has four transmembrane (TM) regions which are hydrophobic in nature. These are labelled TM1–TM4. There is also a lengthy *N*-terminal extracellular chain which (in the case of the α-subunit) contains the ligand-binding site (Fig. 4.11).

The subunits are arranged such that the second transmembrane region of each subunit faces the central pore of the ion channel (Fig. 4.10). We shall see the significance of this when we look at the next section.

4.6.3 Gating

When the receptor binds a ligand, it changes shape which has a knock-on effect on the protein complex, causing the ion channel to open—a process called **gating** (Fig. 4.12).

The binding of a neurotransmitter to its binding site causes a conformational change in the receptor, which eventually opens up the central pore and allows ions to flow. This conformational change is quite complex, involving several knock-on effects from the initial binding process. This must be so, as the binding site is quite far from the lock gate. Studies have shown that the lock gate is made up of five kinked α-helices where one helix (the 2-TM region) is contributed by each of the five protein subunits. In the closed state the kinks point towards each other. The conformational change induced by ligand binding causes each of these helices to rotate such that the kink points the other way, thus opening up the pore (Fig. 4.13).

4.6.4 Ligand-gated and voltage-gated ion channels

The ion channels that we have discussed so far are called **ligand-gated ion channels** as they are controlled by chemical messengers (**ligands**). There are other types of ion channel which are not controlled by ligands, but are instead sensitive to the potential difference that exists across a cell membrane—the **membrane potential**. These ion channels are present in the axons of excitable cells (i.e. neurons) and are called **voltage-gated ion channels.** They are crucial to the transmission of a signal along individual neurons and are important drug targets for local anaesthetics. A description of these ion channels is given in Appendix 4.

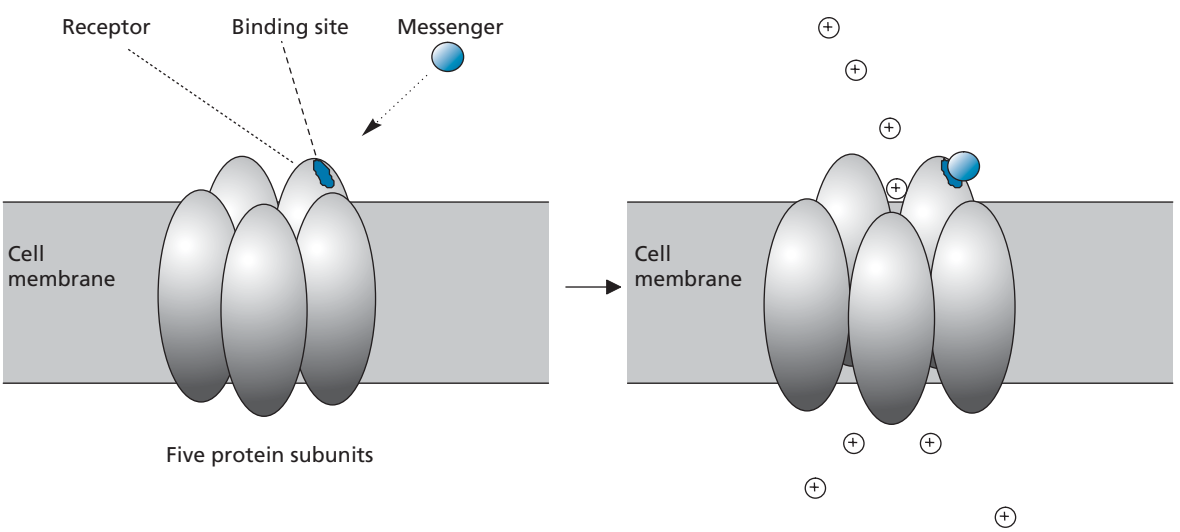


FIGURE 4.12 Opening of an ion channel (gating).

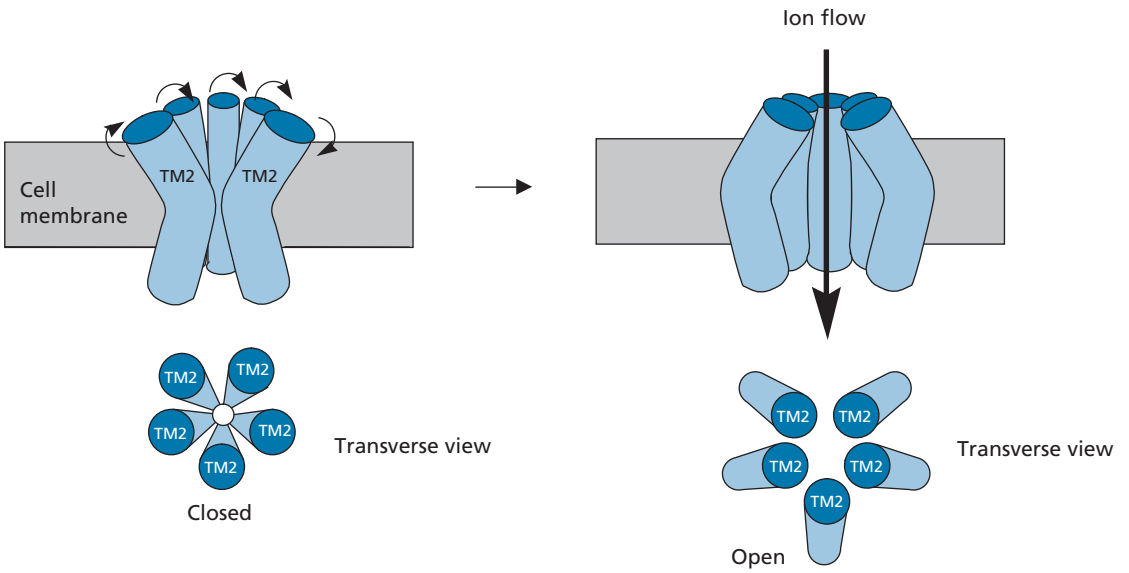


FIGURE 4.13 Opening of the 'lock gate' in an ion channel.

KEY POINTS

- Receptors controlling ion channels are an integral part of the ion channel. Binding of a messenger induces a change in shape, which results in the rapid opening of the ion channel.
- Receptors controlling ion channels are called ligand-gated ion channel receptors. They consist of five protein subunits with the receptor binding site being present on one or more of the subunits.
- Binding of a neurotransmitter to an ion channel receptor causes a conformational change in the protein subunits such that the second transmembrane domain of each subunit rotates to open the channel.

4.7 G-protein-coupled receptors

4.7.1 General principles

The **G-protein-coupled receptors** are some of the most important drug targets in medicinal chemistry. Indeed, some 30% of all drugs on the market act by binding to these receptors. In general, they are activated by hormones and slow-acting neurotransmitters. They include the **muscarinic receptor** (section 22.11), **adrenergic receptors** (section 23.2), and **opioid receptors** (section 24.4). The response from activated G-protein-coupled receptors is measured in seconds. This is slower than the response of ion channels, but faster than the response of kinase-linked receptors (section 4.8), which takes a matter of minutes. There are a large number of different G-protein-coupled receptors interacting with important neurotransmitters, such as acetylcholine, dopamine, histamine, serotonin, glutamate, and noradrenaline. Other G-protein-coupled receptors are activated by peptide and protein hormones, such as the enkephalins and endorphins.

G-protein-coupled receptors are membrane-bound proteins that are responsible for activating proteins called **G-proteins** (Fig. 4.14). These latter proteins act as **signal proteins** because they are capable of activating or deactivating membrane-bound enzymes (sections 5.1–5.2). Consequently, activation of the receptor by a chemical messenger influences the reactions that take place within the cell.

The receptor protein is embedded within the membrane, with the binding site for the chemical messenger exposed on the outer surface. On the inner surface, there is another binding site which is normally closed (Fig. 4.14, frame 1). When the chemical messenger binds to its binding site, the receptor protein changes shape, opening up the binding site on the inner surface. This new binding site is recognized by the G-protein, which then binds (Fig. 4.14, frame 2). The G-protein is attached to the inner surface of the cell membrane and is made up of three protein subunits, but once it binds to the receptor the complex is destabilized and fragments to a monomer and a dimer (Fig. 4.14, frame 3). These then interact with membrane-bound enzymes to continue the signal transduction process (sections 5.1–5.3).

There are several different G-proteins, which are recognized by different types of receptor. Some of the activated subunits from these G-proteins have an inhibitory effect on a membrane-bound enzyme, while others have a stimulatory effect. Nevertheless, the mechanism by which the G-protein is activated by fragmentation is the same.

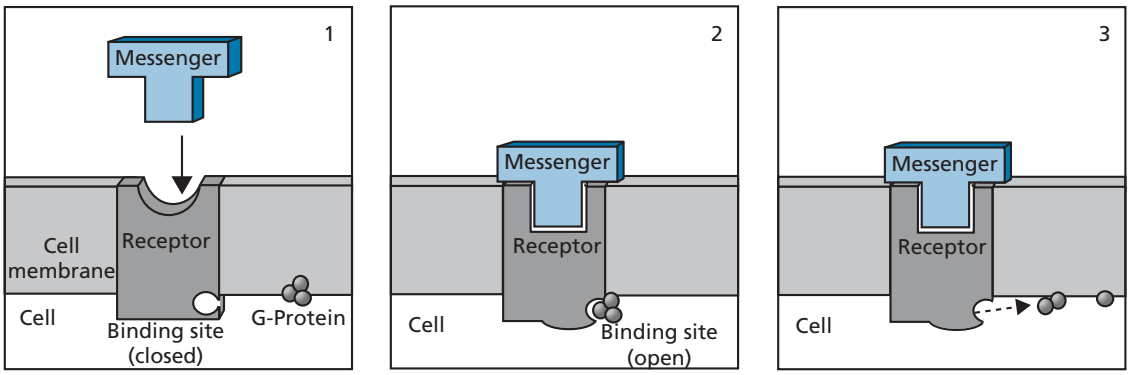


FIGURE 4.14 Activation of a G-protein-coupled receptor and G-protein.

There is a substantial amplification of the signal in this process, as one activated receptor activates several G-proteins.

4.7.2 Structure

The G-protein-coupled receptors fold up within the cell membrane such that the protein chain winds back and forth through the cell membrane seven times (Fig. 4.15). Each of the seven transmembrane sections is hydrophobic and helical in shape, and it is usual to assign these helices with roman numerals (I, II, etc.) starting from the *N*-terminus of the protein. Owing to the number of transmembrane regions, the G-proteins are also called **7-TM receptors**. The binding site for the G-protein is situated on the intracellular side of the protein and involves part of the *C*-terminal chain, as well as part of the variable intracellular loop (so called because the length of this loop varies between different types of receptor). As one might expect, the binding site for the neurotransmitter or hormone messenger is on the extracellular portion of the protein. The exact position of the binding site varies from receptor to receptor. For example, the binding site for the adrenergic receptor is in a deep binding pocket between the transmembrane helices, whereas the binding site for the glutamate receptor involves the *N*-terminal chain and is situated above the surface of the cell membrane.

4.7.3 The rhodopsin-like family of G-protein-coupled receptors

The G-protein-coupled receptors include the receptors for some of the best-known chemical messengers in medicinal chemistry (e.g. glutamic acid, GABA, noradrenaline, dopamine, acetylcholine, serotonin, prostaglandins, adenosine, endogenous opioids, angiotensin, bradykinin, and thrombin). Considering the structural variety of the chemical messengers involved, it is remarkable that the overall structures of the G-protein-coupled receptors

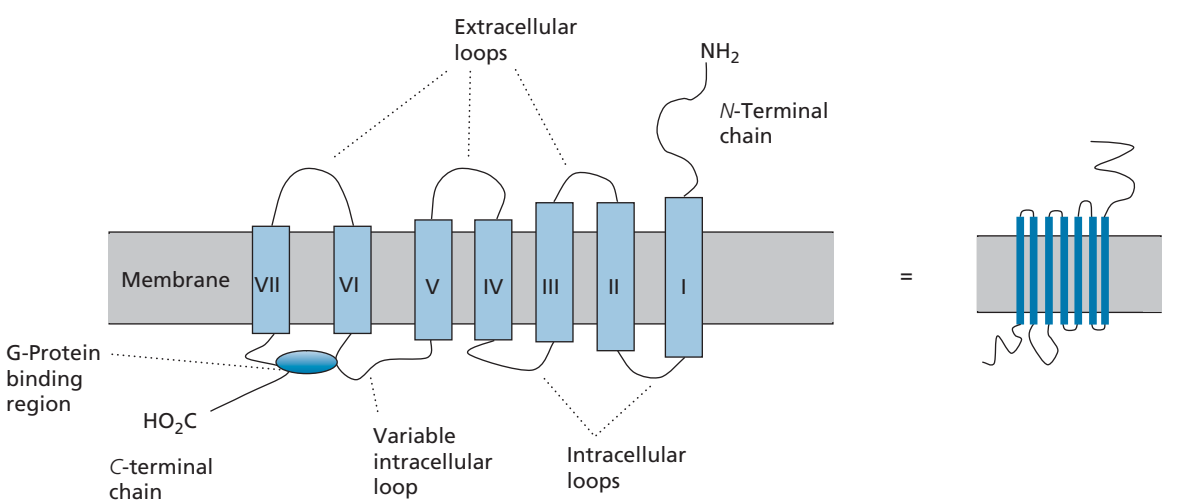


FIGURE 4.15 Structure of G-protein-coupled receptors.

are so similar. Nevertheless, despite their similar overall structure, the amino acid sequences of the receptors vary quite significantly. This implies that these receptors have evolved over millions of years from an ancient common ancestral protein. Comparing the amino acid sequences of the receptors allows us to construct an evolutionary tree and to group the receptors of this superfamily into various sub-families, which are defined as class A (rhodopsin-like receptors), class B (secretin-like receptors), and class C (metabotropic glutamate-like and pheromone receptors). The most important of these, as far as medicinal chemistry is concerned, is the rhodopsin-like family—so called because the first receptor of this family to be studied in detail was the rhodopsin receptor itself, a receptor involved in the visual process. A study of the evolutionary tree of rhodopsin-like receptors throws up some interesting observations (Fig. 4.16).

First of all, the evolutionary tree illustrates the similarity between different kinds of receptors based on their relative positions on the tree. Thus, the muscarinic, α-adrenergic, β-adrenergic, histamine, and dopamine receptors have evolved from a common branch of the evolutionary tree and have greater similarity to each other than to any receptors arising from an earlier evolutionary branch (e.g. the **angiotensin receptor**). Such receptor similarity may prove a problem in medicinal chemistry. Although the receptors are distinguished by different neurotransmitters or hormones in the body, a drug may not manage to make that distinction. Therefore, it is important to ensure that any new drug aimed at one kind of receptor (e.g. the dopamine receptor) does not interact with a similar kind of receptor (e.g. the muscarinic receptor).

Receptors have further evolved to give receptor *types* and *subtypes* which recognize the same chemical messenger, but are structurally different. For example, there are two types of adrenergic receptor (α and β), each of which has various subtypes (α_1, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, β_3). There are two types of cholinergic receptor—nicotinic (an ion channel receptor) and muscarinic (a 7-TM receptor). Five subtypes of the muscarinic cholinergic receptor have been identified.

The existence of receptor subtypes allows the possibility of designing drugs that are selective for one receptor subtype over another. This is important, because one receptor subtype may be prevalent in one part of the body (e.g. the gut), while a different receptor subtype is prevalent in another part (e.g. the heart). Therefore, a drug that is designed to interact selectively with the receptor subtype in the gut is less likely to have side effects on the heart. Even if the different receptor subtypes are present in the same part of the body, it is still important to make drugs as selective as possible because different receptor subtypes frequently activate different signalling systems, leading to different biological results.

A closer study of the evolutionary tree reveals some curious facts about the origins of receptor subtypes. As one might expect, various receptor subtypes have diverged from a common evolutionary branch (e.g. the dopamine subtypes D2, D3, D4). This is known as **divergent evolution** and there should be close structural similarity between these subtypes. However, receptor subtypes are also found in separate branches of the tree. For example, the dopamine receptor subtypes ($D1_A$, $D1_B$, and D5) have developed from a different evolutionary branch. In other words, the ability of a receptor to bind dopamine has developed in different evolutionary branches—an example of **convergent evolution**.

Consequently, there may sometimes be greater similarities between receptors which bind different ligands but which have evolved from the same branch of the tree

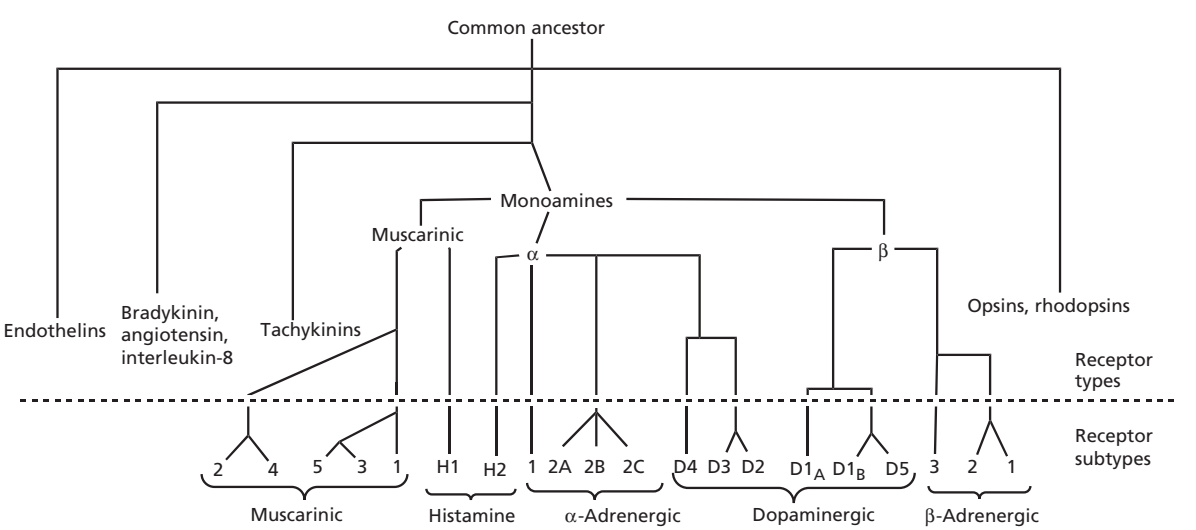


FIGURE 4.16 Evolutionary tree of G-protein-coupled receptors.

than there are between the various subtypes of receptors which bind the same ligand. For example, the histamine H_1 receptor resembles a muscarinic receptor more closely than it does the histamine H_2 receptor. Again, this has important consequences in drug design because there is an increased possibility that a drug aimed at a muscarinic receptor may also interact with a histamine H_1 receptor and lead to unwanted side effects.

As these receptors are membrane bound, it is not easy to crystallize them for X-ray crystallographic studies. However, the X-ray crystal structures of the β_2 and β_1 adrenoceptors have now been determined.

4.7.4 Dimerization of G-coupled receptors

There is strong evidence that some G-coupled receptors can exist as dimeric structures containing identical or different types of receptor—homodimers or heterodimers respectively. The presence of these receptor dimers appears to vary between different tissues and this has important consequences for drug design. An agent that is selective for one type of receptor would not normally affect other types. However, if receptor heterodimers are present, a 'communication' is possible between the component receptors such that an agent interacting with one half of the dimer may affect the activity of the other half. This is discussed further in section 24.9 with respect to opioid receptors.

KEY POINTS

- G-protein-coupled receptors activate signal proteins called G-proteins. Binding of a messenger results in the opening of a binding site for the signal protein. The latter binds and fragments, with one of the subunits departing to activate a membrane-bound enzyme.
- The G-protein-coupled receptors are membrane-bound proteins with seven transmembrane sections. The *C*-terminal chain lies within the cell and the *N*-terminal chain is extracellular.
- The location of the binding site differs between different G-protein-coupled receptors.
- The rhodopsin-like family of G-protein-coupled receptors includes many receptors that are targets for currently important drugs.
- Receptor types and subtypes recognize the same chemical messenger, but have structural differences, making it possible to design drugs that are selective for one type (or subtype) of receptor over another.
- Receptor subtypes can arise from divergent or convergent evolution.
- It is possible for some G-protein coupled receptors to exist as dimeric structures.

4.8 Kinase-linked receptors

4.8.1 General principles

Kinase-linked receptors are a superfamily of receptors which activate enzymes directly and do not require a G-protein (Fig. 4.17). **Tyrosine kinase receptors** are important examples of kinase-linked receptors and are proving to be highly important targets for novel anticancer drugs (section 21.6.2). In these structures, the protein concerned plays the dual role of receptor and enzyme. The receptor protein is embedded within the cell membrane, with part of its structure exposed on the outer surface of the cell and part exposed on the inner surface. The outer surface contains the binding site for the chemical messenger and the inner surface has an active site that is closed in the resting state. When a chemical messenger binds to the receptor it causes the protein to change shape. This results in the active site being opened up, allowing the protein to act as an enzyme within the cell. The reaction that is catalysed is a phosphorylation reaction where tyrosine residues on

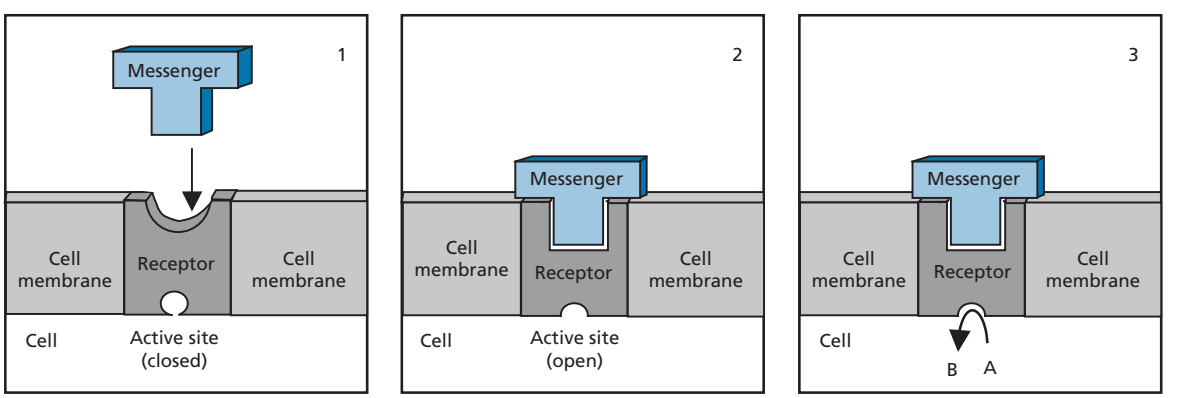


FIGURE 4.17 Enzyme activation.

a protein substrate are phosphorylated. An enzyme that catalyses phosphorylation reactions is known as a kinase enzyme and so the protein is referred to as a tyrosine kinase receptor. ATP is required as a cofactor to provide the necessary phosphate group. The active site remains open for as long as the messenger molecule is bound to the receptor, and so several phosphorylation reactions can occur, resulting in an amplification of the signal. A curiosity of this enzyme-catalysed reaction is that the substrate for the reaction is the receptor itself. This is explained more fully in section 4.8.3.

The kinase-linked receptors are activated by a large number of polypeptide hormones, growth factors, and cytokines. Loss of function of these receptors can lead to developmental defects or hormone resistance. Overexpression can result in malignant growth disorders.

4.8.2 Structure of tyrosine kinase receptors

The basic structure of a tyrosine kinase receptor consists of a single extracellular region (the *N*-terminal chain) that includes the binding site for the chemical messenger, a single hydrophobic region that traverses the membrane as an α-helix of seven turns (just sufficient to traverse the membrane), and a *C*-terminal chain on the inside of the cell membrane (Fig. 4.18). The *C*-terminal region contains the catalytic binding site. Examples of tyrosine kinase receptors include the receptor for **insulin,** and receptors for various **cytokines** and **growth factors**.

4.8.3 Activation mechanism for tyrosine kinase receptors

A specific example of a tyrosine kinase receptor is the receptor for a hormone called **epidermal growth factor** (EGF). EGF is a **bivalent ligand** which can bind to two receptors at the same time. This results in **receptor dimerization**, as well as activation of enzymatic activity. The dimerization process is important because the active site on each half of the receptor dimer catalyses the phosphorylation of accessible tyrosine residues on the other half (Fig. 4.19). If dimerization did not occur, no phosphorylation would take place. Note that these phosphorylations occur on the intracellular portion of the receptor protein chain. The relevance of these phosphorylation reactions will be explained in section 5.4.1. The important point to grasp at this stage is that an external chemical messenger has managed to convey its message to the interior of the cell without itself being altered or having to enter the cell.

Dimerization and auto-phosphorylation are common themes for receptors in this family. However, some of the receptors in this family already exist as dimers or tetramers, and only require binding of the ligand. For example, the **insulin** receptor is a heterotetrameric complex (Fig. 4.20).

4.8.4 Tyrosine kinase-linked receptors

Some kinase receptors bind ligands and dimerize in a similar fashion to the ones described above, but do not have inherent catalytic activity in their *C*-terminal chain. However, once they have dimerized, they can bind and activate a tyrosine kinase enzyme from the cytoplasm. The **growth hormone** (GH) receptor is an example of this type of receptor and is classified as a tyrosine kinase-linked receptor (Fig. 4.21).

KEY POINTS

- Kinase-linked receptors are receptors which are directly linked to kinase enzymes. Messenger binding results in the opening of the kinase-active site, allowing a catalytic reaction to take place.
- Tyrosine kinase receptors have an extracellular binding site for a chemical messenger and an intracellular enzymatic

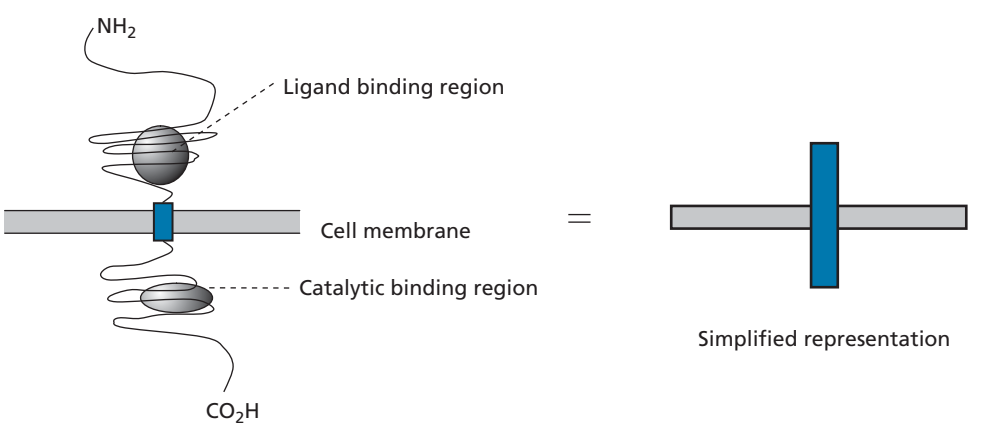


FIGURE 4.18 Structure of tyrosine kinase receptors.

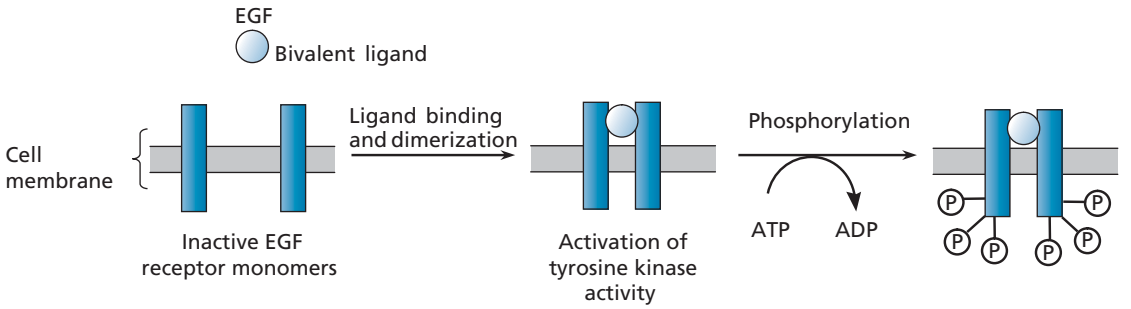


FIGURE 4.19 Activation mechanism for the epidermal growth factor (EGF) receptor.

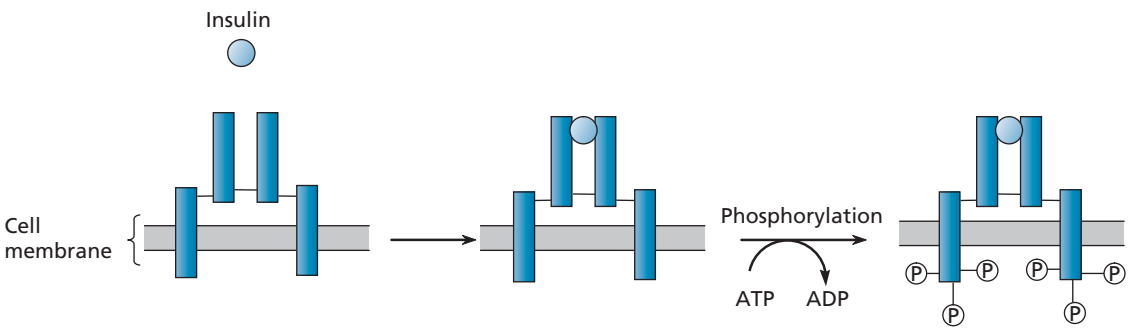


FIGURE 4.20 Ligand binding and activation of the insulin receptor.

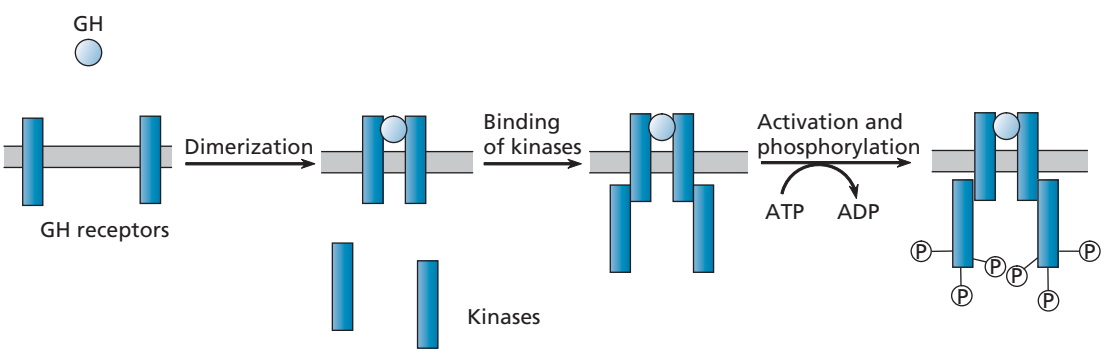


FIGURE 4.21 Activation of the growth hormone (GH) receptor.

active site which catalyses the phosphorylation of tyrosine residues in protein substrates.

- Ligand binding to the epidermal growth factor (EGF) receptor results in dimerization and opening of the active sites. The active site on one half of the dimer catalyses the phosphorylation of tyrosine residues present on the *C*-terminal chain of the other half.
- The insulin receptor is a preformed heterotetrameric structure which acts as a tyrosine kinase receptor.
- The growth hormone receptor dimerizes on binding its ligand, then binds and activates tyrosine kinase enzymes from the cytoplasm.

4.9 Intracellular receptors

Not all receptors are located in the cell membrane. Some receptors are within the cell and are defined as intracellular receptors. There are about 50 members of this group and they are particularly important in directly regulating gene transcription. As a result, they are often called **nuclear hormone receptors** or **nuclear transcription factors.** The chemical messengers for these receptors include steroid hormones, thyroid hormones, and retinoids. In all these cases, the messenger has to pass through the cell membrane in order to reach its receptor so it has to be hydrophobic in nature. The response time

resulting from the activation of the intracellular receptors is measured in hours or days, and is much slower than the response times of the membrane-bound receptors.

The intracellular receptors all have similar general structures. They consist of a single protein containing a ligand binding site at the *C*-terminus and a binding region for DNA near the centre (Fig. 4.22). The DNA binding region contains nine cysteine residues, eight of which are involved in binding two zinc ions. The zinc ions play a crucial role in stabilizing and determining the conformation of the DNA binding region. As a result, the stretches of protein concerned are called the **zinc finger domains.** The DNA binding region for each receptor can identify particular nucleotide sequences in DNA. For example, the zinc finger domains of the **estrogen receptor** recognize the sequence 5′-AGGTCA-3′, where A, G, C, and T are adenine, guanine, cytosine, and thymine.

The mechanism by which intracellular receptors work is also very similar (Fig. 4.23). Once the chemical messenger (ligand) has crossed the cell membrane, it seeks out its receptor and binds to it at the ligand binding site. An induced fit takes place which causes the receptor to change shape. This, in turn, leads to a dimerization of the ligand–receptor complex. The dimer then binds to a protein called a **co-activator** and, finally, the whole complex binds to a particular region of the cell's DNA. As there are two receptors in the complex and two DNA binding regions, the complex recognizes two identical sequences of nucleotides in the DNA separated by a short distance. For example, the estrogen ligand–receptor dimer binds to a nucleotide sequence of 5′-AGGTCANNNTGACCT-3′ where N can be any nucleic acid base. Depending on the complex involved, binding of the complex to DNA either triggers or inhibits the start of transcription, and affects the eventual synthesis of a protein.

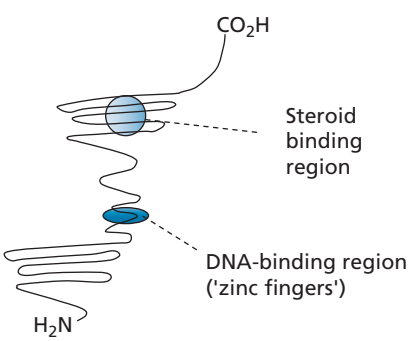


FIGURE 4.22 Structure of intracellular receptors.

4.10 Regulation of receptor activity

The role of allosteric binding sites in regulating the activity of enzymes was covered in section 3.6. Allosteric binding sites also play a role in regulating or modulating the activity of various receptors. These include ligand-gated ion channels, such as the nicotinic and the γ-aminobutyric acid receptors, and several G-protein-coupled receptors, such as the muscarinic, adenosine, and dopamine receptors. Structures that interact with these sites are called **allosteric modulators** and can either enhance or decrease the effect of the chemical messenger on the receptor (sections 8.2.7 and 8.3.2).

4.11 Genetic polymorphism and receptors

Genetic polymorphism was discussed in section 3.5.6 with respect to enzymes. Polymorphism is also responsible for receptors having subtle differences in structure and activity between individuals. In some cases, this can lead to diseases such as cancer (section 21.1.3).

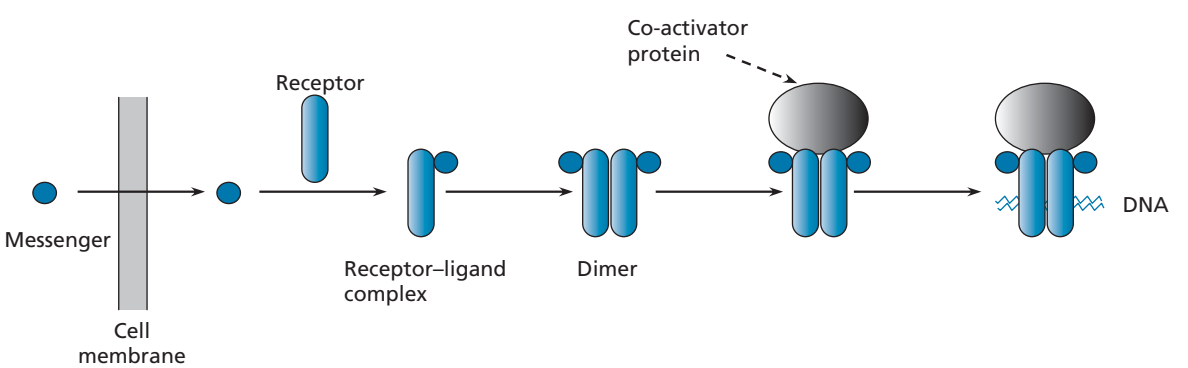


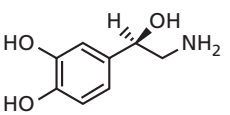
FIGURE 4.23 From messenger to control of gene transcription.

KEY NOTES

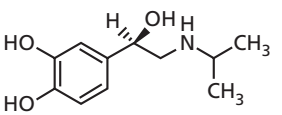
- Intracellular receptors are located within the cell and are important in controlling transcription.
- The chemical messengers for intracellular receptors must be sufficiently hydrophobic to pass through the cell membrane.
- The binding of a ligand with an intracellular receptor results in dimerization and the formation of a transcription factor complex which binds to a specific nucleotide sequence on DNA.

QUESTIONS

1. Explain the distinction between a binding site and a binding region.
2. Consider the structures of the neurotransmitters shown in Fig. 4.3 and suggest what type of binding interactions could be involved in binding them to a receptor binding site. Identify possible amino acids in the binding site which could take part in each of these binding interactions.
3. There are two main types of adrenergic receptor: the α and β–adrenoceptors. Noradrenaline shows slight selectivity for the α-receptor, whereas isoprenaline shows selectivity for the β-adrenoceptor. Adrenaline shows no selectivity and binds equally well to both the α- and β-adrenoceptors. Suggest an explanation for these differences in selectivity.



Noradrenaline



Isoprenaline

4. Suggest why the transmembrane regions of many membrane-bound proteins are α-helices.

FURTHER READING

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5 Receptors and signal transduction

In Chapter 4, we discussed the structure and function of receptors. In this chapter, we consider what happens once a receptor has been activated. The interaction of a receptor with its chemical messenger is only the first step in a complex chain of events involving several secondary messengers, proteins, and enzymes that ultimately leads to a change in cell chemistry. These events are referred to as **signal transduction**. Unfortunately, a full and detailed account of these processes would fill a textbook in itself so the following account is focused mainly on the signal transduction processes that result from activation of G-protein-coupled receptors and kinase receptors. The signal transduction pathways following activation of G-protein-coupled receptors are of particular interest as 30% of all drugs on the market interact with these kinds of receptors. The transduction pathways for kinase receptors are also of great interest as they offer exciting new targets for novel drugs, particularly in the area of anticancer therapy (section 21.6.2). An understanding of the pathways and the various components involved helps to identify suitable drug targets.

5.1 Signal transduction pathways for G-protein-coupled receptors

G-protein-coupled receptors activate a signalling protein called a G-protein, which then initiates a signalling cascade involving a variety of enzymes. The sequence of events leading from the combination of receptor and ligand (the chemical messenger) to the final activation of a target enzyme is quite lengthy, so we shall look at each stage of the process in turn.

5.1.1 Interaction of the receptor–ligand complex with G-proteins

The first stage in the process is the binding of the chemical messenger or ligand to the receptor, followed by the binding of a G-protein to the receptor–ligand complex (Fig. 5.1). G-proteins are membrane-bound proteins situated at the inner surface of the cell membrane and are made up of three protein subunits (α, β, and γ). The α-subunit has a binding pocket which can bind guanyl nucleotides (hence the name G-protein) and which binds **guanosine diphosphate (GDP)** when the G-protein is in the resting state. There are several types of G-protein (e.g. Gs, Gi/Go, Gq/G_{11}) and several subtypes of these. Specific G-proteins are recognized by specific receptors. For example, G_s is recognized by the β-adrenoceptor, but not the α-adrenoceptor. However, in all cases, the G-protein acts as a molecular 'relay runner' carrying the message received by the receptor to the next target in the signalling pathway.

We shall now look at what happens in detail.

Firstly, the receptor binds its neurotransmitter or hormone (Fig. 5.1, frame 1). As a result, the receptor changes shape and exposes a new binding site on its inner surface (Fig. 5.1, frame 2). The newly exposed binding site now recognizes and binds a specific G-protein. Note that the cell membrane structure is a fluid structure and so it is possible for different proteins to 'float' through it. The binding process between the receptor and the G-protein causes the latter to change shape, which, in turn, changes the shape of the guanyl nucleotide binding site. This weakens the intermolecular bonding forces holding GDP and so GDP is released (Fig. 5.1, frame 3).

However, the binding pocket does not stay empty for long because it is now the right shape to bind **GTP** (**guanosine triphosphate**). Therefore, GTP replaces GDP (Fig. 5.1, frame 4).

Binding of GTP results in another conformational change in the G-protein (Fig. 5.1, frame 5), which weakens the links between the protein subunits such that the α-subunit (with its GTP attached) splits off from the β and γ-subunits (Fig. 5.1, frame 6). Both the α-subunit and the βγ-dimer then depart the receptor.

The receptor–ligand complex is able to activate several G-proteins in this way before the ligand departs and

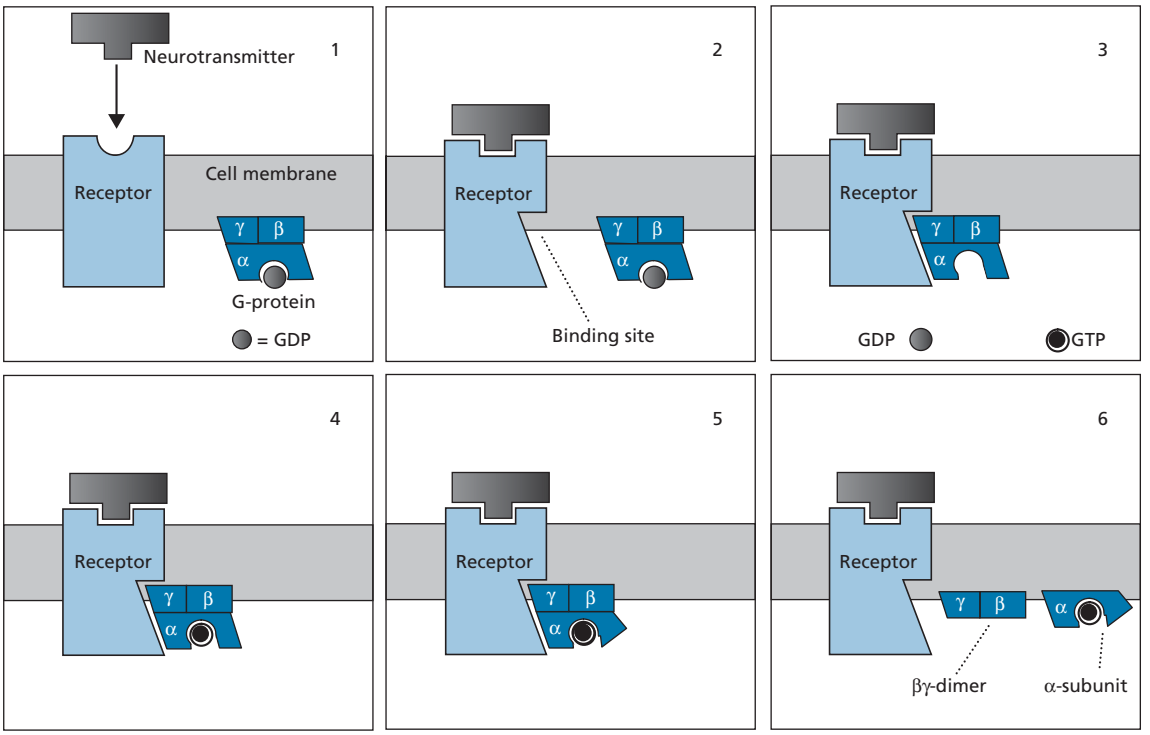


FIGURE 5.1 Activation of G-protein-coupled receptors and their interaction with G-proteins.

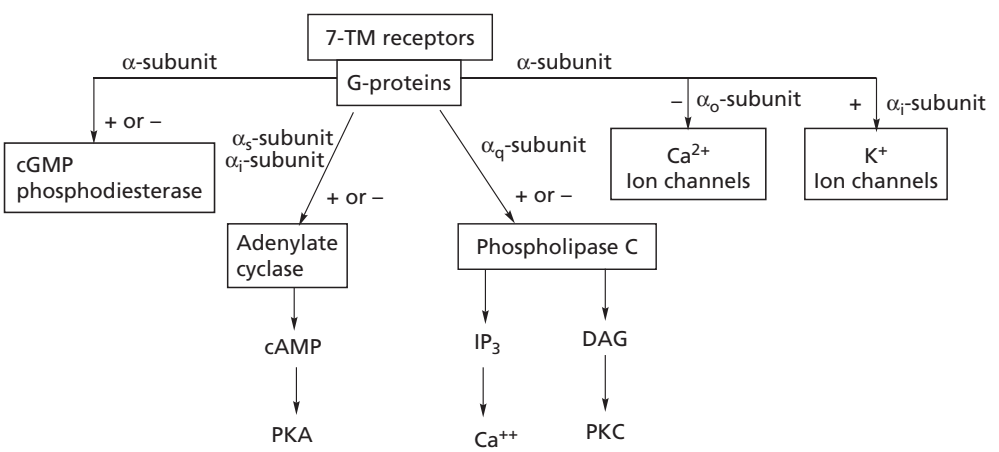


FIGURE 5.2 Signalling pathways arising from the splitting of different G-proteins.

switches off the receptor. This leads to an amplification of the signal.

Both the α-subunit and the βγ-dimer are now ready to enter the second stage of the signalling mechanism. We shall first consider what happens to the α-subunit.

5.1.2 Signal transduction pathways involving the α-subunit

The first stage of signal transduction (i.e. the splitting of a G-protein) is common to all of the 7-TM receptors. However, subsequent stages depend on what type of G-protein is involved and which specific α-subunit is formed (Fig. 5.2). Different α-subunits—there are at least 20 of them—have different targets and different effects:

- α_s stimulates adenylate cyclase;
- α_i inhibits adenylate cyclase and may also activate potassium ion channels;
- α_o activates receptors that inhibit neuronal calcium ion channels;
- α_q activates phospholipase C.

We do not have the space to study all these pathways in detail. Instead, we shall concentrate on two—the activation of **adenylate cyclase** and the activation of **phospholipase C**.

5.2 Signal transduction involving G-proteins and adenylate cyclase

5.2.1 Activation of adenylate cyclase by the α_s-subunit

The α_s-subunit binds to a membrane-bound enzyme called adenylate cyclase (or adenylyl cyclase) and 'switches' it on (Fig. 5.3). This enzyme now catalyses the synthesis of a molecule called cyclic AMP (cAMP) (Fig. 5.4). cAMP is an example of a **secondary messenger** which moves into the cell's cytoplasm and carries the signal from the cell membrane into the cell itself. The enzyme will continue to be active as long as the α_s-subunit is bound, resulting in the synthesis of several hundred cyclic AMP molecules—representing another substantial amplification of the signal. However, the α_s-subunit has intrinsic GTP-ase activity (i.e. it can catalyse the hydrolysis of its bound GTP to GDP) and so it deactivates itself after a certain time period and returns to the resting state. The α_s-subunit then departs the enzyme and recombines with the βγ-dimer to reform the G_s-protein while the enzyme returns to its inactive conformation.

5.2.2 Activation of protein kinase A

cAMP now proceeds to activate an enzyme called protein kinase A (PKA) (Fig. 5.5). PKA belongs to a group of enzymes called the **serine–threonine kinases** which catalyse the phosphorylation of serine and threonine residues in protein substrates (Fig. 5.6).

Protein kinase A catalyses the phosphorylation and activation of further enzymes with functions specific to the particular cell or organ in question, for example lipase enzymes in fat cells are activated to catalyse the breakdown of fat. The active site of a protein kinase has to be capable of binding the region of the protein substrate which is to be phosphorylated, as well as the cofactor ATP which provides the necessary phosphate group.

There may be several more enzymes involved in the signalling pathway between the activation of PKA and the activation (or deactivation) of the target enzyme. For example, the enzymes involved in the breakdown and synthesis of glycogen in a liver cell are regulated as shown in Fig. 5.7.

Adrenaline is the initial hormone involved in the regulation process and is released when the body requires immediate energy in the form of **glucose**. The hormone initiates a signal at the β-**adrenoceptor** leading to the

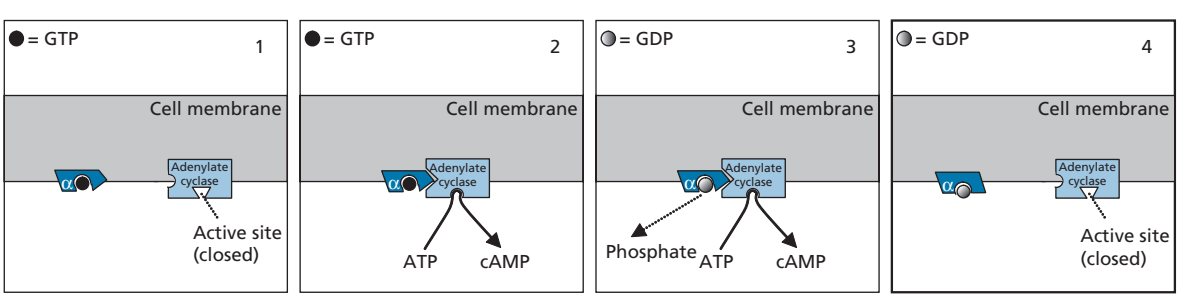


FIGURE 5.3 Interaction of α_s-subunit with adenylate cyclase and activation of the enzyme.

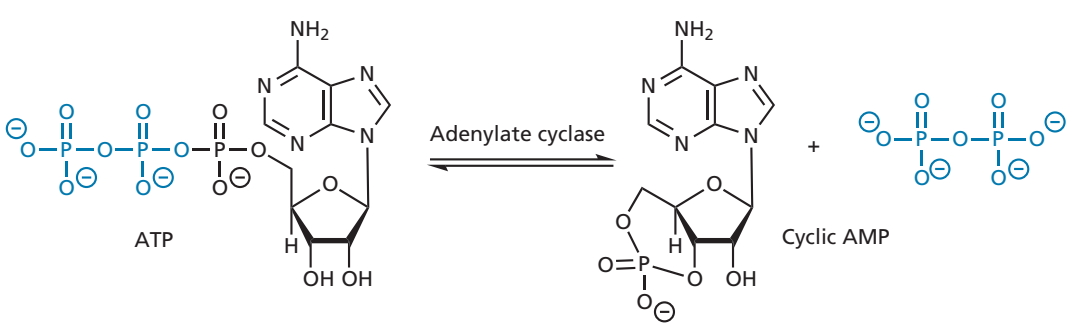


FIGURE 5.4 Synthesis of cyclic AMP.

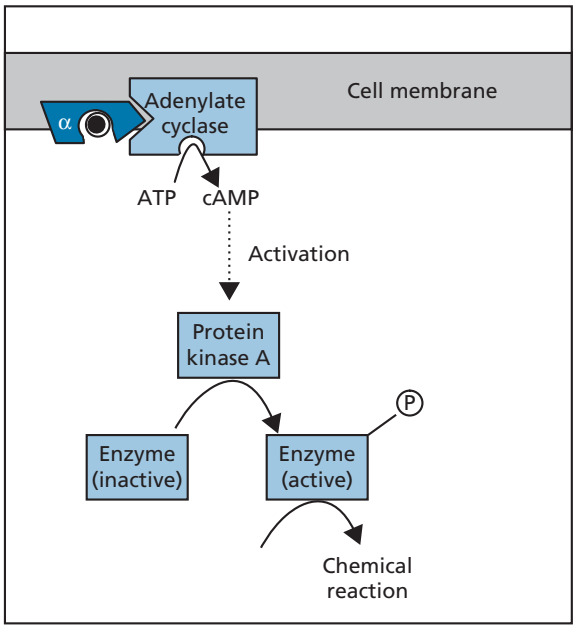


FIGURE 5.5 Activation of protein kinase A by cyclic AMP (Ⓟ = phosphate).

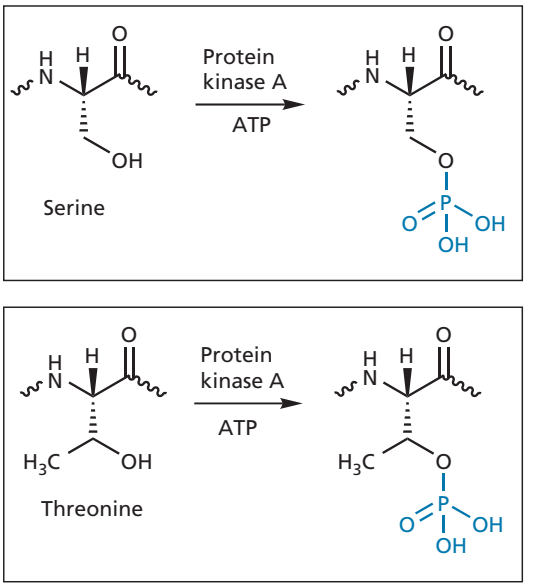


FIGURE 5.6 Phosphorylation of serine and threonine residues in protein substrates.

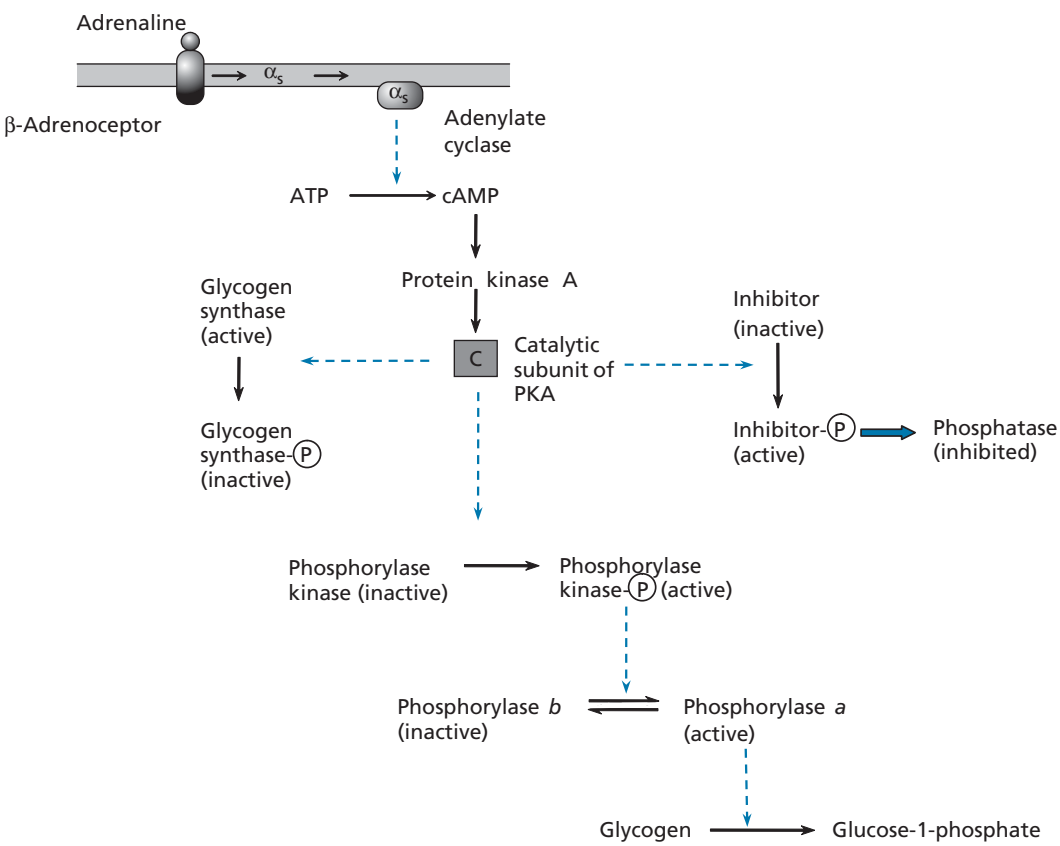


FIGURE 5.7 Regulation of glycogen synthesis and metabolism in a liver cell.

synthesis of cAMP and the activation of PKA by the mechanism already discussed. The catalytic subunit of PKA now phosphorylates three enzymes within the cell, as follows:

- an enzyme called **phosphorylase kinase** is phosphorylated and activated. This enzyme then catalyses the phosphorylation of an inactive enzyme called **phosphorylase *b*** which is converted to its active form, **phosphorylase *a***. Phosphorylase *a* now catalyses the breakdown of glycogen by splitting off glucose-1-phosphate units;
- **glycogen synthase** is phosphorylated to an inactive form, thus preventing the synthesis of glycogen;
- a molecule called **phosphorylase inhibitor** is phosphorylated. Once phosphorylated, it acts as an inhibitor for the **phosphatase** enzyme responsible for the conversion of phosphorylase *a* back to phosphorylase *b*. The lifetime of phosphorylase *a* is thereby prolonged.

The overall result of these different phosphorylations is a coordinated inhibition of glycogen synthesis and enhancement of glycogen metabolism to generate glucose in liver cells. Note that the effect of adrenaline on other types of cell may be quite different. For example, adrenaline activates β-adrenoceptors in fat cells leading to the activation of protein kinases, as before. This time, however, phosphorylation activates **lipase** enzymes which then catalyse the breakdown of fat to act as another source of glucose.

5.2.3 The G_i-protein

We have seen how the enzyme adenylate cyclase is activated by the α_s-subunit of the G_s-protein. Adenylate cyclase can also be inhibited by a different G-protein—the G_i-protein. The G_i-protein interacts with different receptors from those that interact with the G_s-protein, but the mechanism leading to inhibition is the same as that leading to activation. The only difference is that the α_i-subunit released binds to adenylate cyclase and inhibits the enzyme rather than activates it.

Receptors that bind G_i-proteins include the **muscarinic M_2 receptor** of cardiac muscle, **α_2-adrenoceptors** in smooth muscle, and **opioid receptors** in the central nervous system.

The existence of G_i- and G_s-proteins means that the generation of the secondary messenger cAMP is under the dual control of a brake and an accelerator, which explains the process by which two different neurotransmitters can have opposing effects at a target cell. A neurotransmitter which stimulates the production of cAMP forms a receptor–ligand complex which activates a G_s-protein, whereas a neurotransmitter which inhibits the production of cAMP forms a receptor–ligand complex which activates a G_i-protein. For example, **noradrenaline** interacts with the **β-adrenoceptor** to activate a G_s-protein, whereas **acetylcholine** interacts with the muscarinic receptor to activate a G_i-protein.

As there are various different types of receptor for a particular neurotransmitter, it is actually possible for that neurotransmitter to activate cAMP in one type of cell but inhibit it in another. For example, noradrenaline interacts with the β-adrenoceptor to activate adenylate cyclase because the β-adrenoceptor binds the G_s-protein. However, noradrenaline interacts with the α_2-adrenoceptor to inhibit adenylate cyclase because this receptor binds the G_i-protein. This example illustrates that it is the receptor that determines which G-protein is activated and not the neurotransmitter or hormone,

It is also worth pointing out that enzymes such as adenylate cyclase and the kinases are never fully active or inactive. At any one time, a certain proportion of these enzymes are active and the role of the G_s- and G_i-proteins is to either increase or decrease that proportion. In other words, the control is graded rather than all or nothing.

5.2.4 General points about the signalling cascade involving cyclic AMP

The signalling cascade involving the G_s-protein, cAMP and PKA appears very complex and you might wonder whether a simpler signalling process would be more efficient. There are several points worth noting about the process as it stands.

- Firstly, the action of the G-protein and the generation of a secondary messenger explains how a message delivered to the outside of the cell surface can be transmitted to enzymes within the cell—enzymes that have no direct association with the cell membrane or the receptor. Such a signalling process avoids the difficulties involved in a messenger molecule (which is commonly hydrophilic) having to cross a hydrophobic cell membrane.
- Secondly, the process involves a molecular 'relay runner' (the G-protein) and several different enzymes in the signalling cascade. At each of these stages, the action of one protein or enzyme results in the activation of a much larger number of enzymes. Therefore, the effect of one neurotransmitter interacting with one receptor molecule results in a final effect several factors larger than one might expect. For example, each molecule of **adrenaline** is thought to generate 100 molecules of cAMP and each cAMP molecule starts off an amplification effect of its own within the cell.
- Thirdly, there is an advantage in having the receptor, the G-protein, and adenylate cyclase as separate entities.

The G-protein can bind to several different types of receptor–ligand complexes. This means that different neurotransmitters and hormones interacting with different receptors can switch on the same G-protein leading to activation of adenylate cyclase. Therefore, there is an economy of organization involved in the cellular signalling chemistry, as the adenylate cyclase signalling pathway can be used in many different cells and yet respond to different signals. Moreover, different cellular effects will result depending on the type of cell involved (i.e. cells in different tissues will have different receptor types and subtypes, and the signalling system will switch on different target enzymes). For example, **glucagon** activates G_s-linked receptors in the liver leading to **gluconeogenesis**, **adrenaline** activates G_s-linked β_2-adrenoceptors in fat cells leading to **lipolysis**, and **vasopressin** interacts with G_s-linked vasopressin (V_2) receptors in the kidney to affect sodium/water resorption. Adrenaline acts on $G_{i/o}$-linked α_2-adrenoceptors leading to contraction of smooth muscle and **acetylcholine** acts on $G_{i/o}$-linked M_2 receptors leading to relaxation of heart muscle. All these effects are mediated by the cAMP signalling pathway.

- Finally, the dual control of 'brake/accelerator' provided by the G_s- and G_i-proteins allows fine control of adenylate cyclase activity.

5.2.5 The role of the βγ-dimer

If you've managed to follow the complexity of the G-protein signalling pathway so far, well done. Unfortunately, there's more! You may remember that when the G-protein binds to a receptor–ligand complex, it breaks up to form an α-subunit and a βγ-dimer. Until recently, the βγ-dimer was viewed merely as an **anchor** for the α-subunit to ensure that it remained bound to the inner surface of the cell membrane. However, it has now been found that the βγ-dimers from both the G_i- and the G_s-proteins can themselves activate or inhibit adenylate cyclase. There are actually six different types (or isozymes) of adenylate cyclase, and activation or inhibition depends on the isozyme involved. Moreover, adenylate cyclase is not the only enzyme that can be controlled by the βγ-dimer. The βγ-dimer is more promiscuous than the α-subunits and can affect several different targets, leading to a variety of different effects. This sounds like a recipe for anarchy. However, there is some advantage in the dimer having a signalling role, as it adds an extra subtlety to the signalling process. For example, it is found that higher concentrations of the dimer are required to result in any effect compared with the α-subunit. Therefore, regulation by the dimers becomes more important when a greater number of receptors are activated.

By now it should be clear that the activation of a cellular process is more complicated than the interaction of one type of neurotransmitter interacting with one type of receptor. In reality, the cell is receiving myriad signals from different chemical messengers via various receptors and receptor–ligand interactions. The final signal depends on the number and type of G-proteins activated at any one time, as well as the various signal transduction pathways that these proteins initiate.

5.2.6 Phosphorylation

As we have seen above, phosphorylation is a key reaction in the activation or deactivation of enzymes. Phosphorylation requires ATP as a source for the phosphate group and occurs on the phenolic group of tyrosine residues when catalysed by **tyrosine kinases,** and on the alcohol groups of serine and threonine residues when catalysed by **serine–threonine kinases**. These functional groups are all capable of participating in hydrogen bonding, but if a bulky phosphate group is added to the OH group, hydrogen bonding is disrupted. Furthermore, the phosphate group is usually ionized at physiological pH and so phosphorylation introduces two negatively charged oxygens. These charged groups can now form strong ionic bonds with a suitably positioned positively charged group in the protein causing the enzyme to change its tertiary structure. This change in shape results in the exposure or closure of the active site (Fig. 5.8).

Phosphorylation by kinase enzymes also accounts for the **desensitization** of G-protein-linked receptors. Phosphorylation of serine and threonine residues occurs on the intracellular *C*-terminal chain after prolonged ligand binding. As the *C*-terminal chain is involved in G-protein binding, phosphorylation changes the conformation of the protein in that region and prevents the G-protein from binding. Thus, the receptor–ligand complex is no longer able to activate the G-protein.

KEY POINTS

- G-proteins consist of three protein subunits, with the α-subunit bound to GDP. There are several types of G-protein.
- Receptor–ligand binding opens a binding site for the G-protein. On binding, GDP is exchanged for GTP, and the G-protein fragments into an α-subunit (bearing GTP) and a βγ-dimer.
- G-proteins are bound and split for as long as the chemical messenger is bound to the receptor, resulting in a signal amplification.
- An α_s-subunit binds to adenylate cyclase and activates it such that it catalyses the formation of cAMP from ATP. The reaction proceeds for as long as the α_s-subunit is bound, representing another signal amplification. An α_i-subunit inhibits adenylate cyclase.
- The α-subunits eventually hydrolyse bound GTP to GDP and depart adenylate cyclase. They combine with their respective βγ-dimers to reform the original G-proteins.

- cAMP acts as a secondary messenger within the cell and activates PKA. PKA catalyses the phosphorylation of serine and threonine residues in other enzymes leading to a biological effect determined by the type of cell involved.
- The signalling cascade initiated by receptor–ligand binding results in substantial signal amplification and does not require the original chemical messenger to enter the cell.
- The overall activity of adenylate cyclase is determined by the relevant proportions of G_s and G_i-proteins that are split, which, in turn, depends on the types of receptors that are being activated.
- The βγ-dimer of G-proteins has a moderating role on the activity of adenylate cyclase and other enzymes when it is present in relatively high concentration.
- Tyrosine kinases are enzymes which phosphorylate the phenol group of tyrosine residues in enzyme substrates. Serine–threonine kinases phosphorylate the alcohol groups of serine and threonine in enzyme substrates. In both cases, phosphorylation results in conformational changes that affect the activity of the substrate enzyme.
- Kinases are involved in the desensitization of receptors.

5.3 Signal transduction involving G-proteins and phospholipase C

5.3.1 G-protein effect on phospholipase C

Certain receptors bind G_s- or G_i-proteins and initiate a signalling pathway involving adenylate cyclase (section 5.2). Other 7-TM receptors bind a different G-protein called a **G_q-protein**, which initiates a different signalling pathway. This pathway involves the activation or deactivation of a membrane-bound enzyme called phospholipase C. The first part of the signalling mechanism is the interaction of the G-protein with a receptor–ligand complex as described previously in Fig. 5.1. This time, however, the G-protein is a G_q-protein rather than a G_s or G_i-protein, and so an α_q-subunit is released. Depending on the nature of the released α_q-subunit, phospholipase C is activated or deactivated. If activated, phospholipase C catalyses the hydrolysis of **phosphatidylinositol diphosphate** (PIP_2) (an integral part of the cell membrane structure) to generate the two secondary messengers **diacylglycerol** (DG) and **inositol triphosphate** (IP_3) (Figs 5.9 and 5.10).

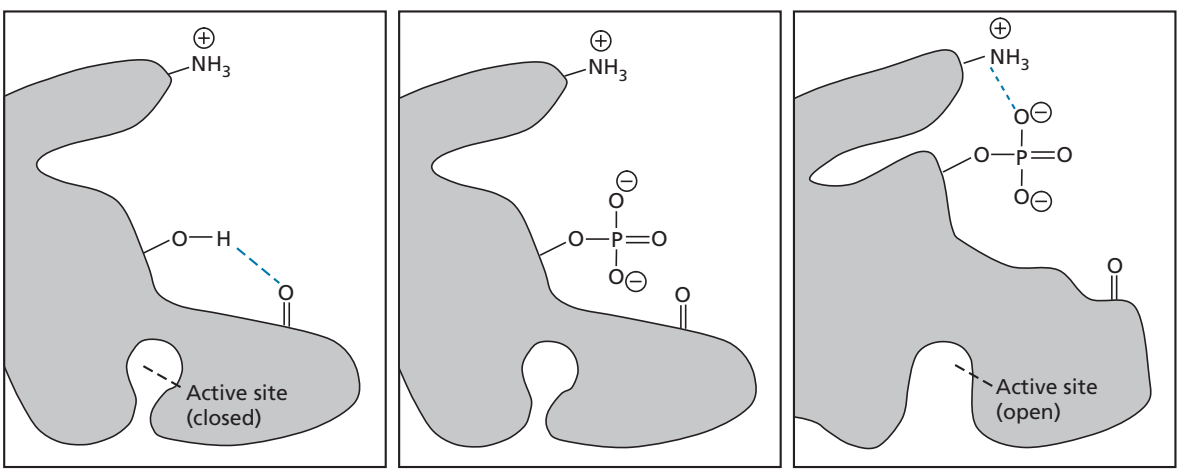


FIGURE 5.8 Conformational changes in a protein, induced by phosphorylation.

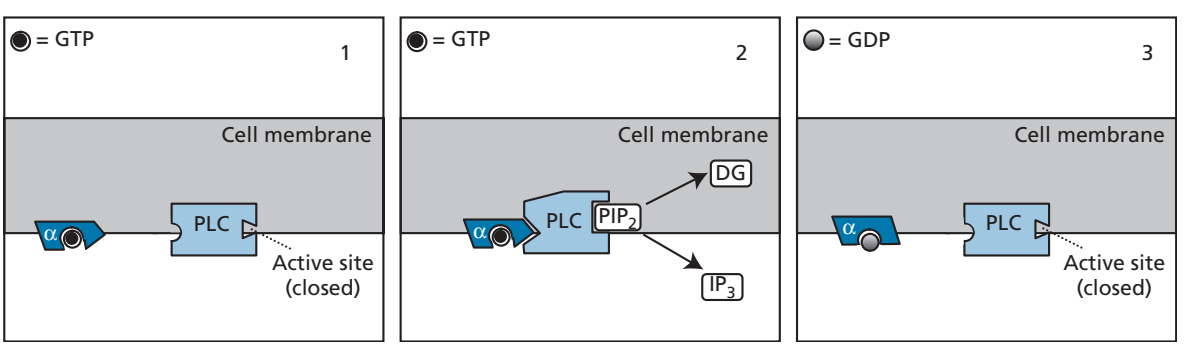


FIGURE 5.9 Activation of phospholipase C by an α_q-subunit.

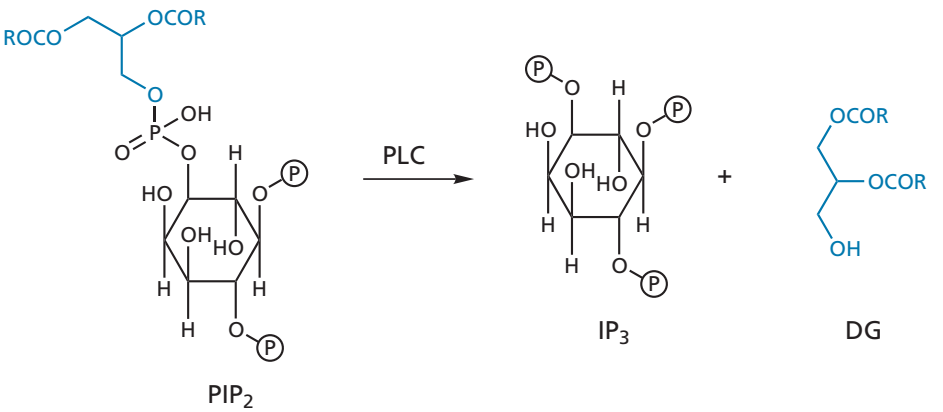


FIGURE 5.10 Hydrolysis of PIP_2 to inositol triphosphate (IP_3) and diacylglycerol (DG) (Ⓟ = phosphate).

5.3.2 Action of the secondary messenger: diacylglycerol

Diacylglycerol is a hydrophobic molecule and remains in the cell membrane once it is formed (Fig. 5.11). There, it activates an enzyme called **protein kinase C** (PKC) which moves from the cytoplasm to the cell membrane and then catalyses the phosphorylation of serine and threonine residues of enzymes within the cell. Once phosphorylated, these enzymes are activated and catalyse specific reactions within the cell. These induce effects such as tumour propagation, inflammatory responses, contraction or relaxation of smooth muscle, the increase or decrease of neurotransmitter release, the increase or decrease of neuronal excitability, and receptor desensitizations.

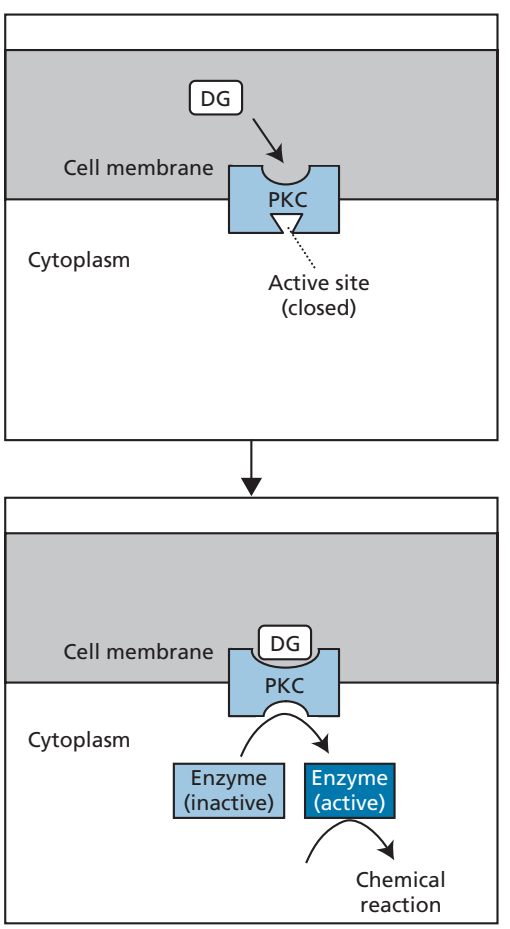


FIGURE 5.11 Activation of protein kinase C (PKC) by diacylglycerol (DG).

5.3.3 Action of the secondary messenger: inositol triphosphate

Inositol triphosphate is a hydrophilic molecule and moves into the cytoplasm (Fig. 5.12). This messenger works by mobilizing calcium ions from calcium stores in the endoplasmic reticulum. It does so by binding to a receptor and opening up a calcium ion channel. Once the ion channel is open, calcium ions flood the cell and activate calcium-dependent protein kinases which, in turn, phosphorylate and activate cell-specific enzymes. The released calcium ions also bind to a calcium binding protein called **calmodulin**, which then activates calmodulin-dependent protein kinases that phosphorylate and activate other cellular enzymes. Calcium has effects on contractile proteins and ion channels, but it is not possible to cover these effects in detail in this text. Suffice it to say that the release of calcium is crucial to a large variety of cellular functions including smooth muscle and cardiac muscle contraction, secretion from exocrine glands, transmitter release from nerves, and hormone release.

5.3.4 Re-synthesis of phosphatidylinositol diphosphate

Once IP_3 and DG have completed their tasks, they are recombined to form phosphatidylinositol diphosphate (PIP_2). Oddly enough, they cannot be linked directly and both molecules have to undergo several metabolic

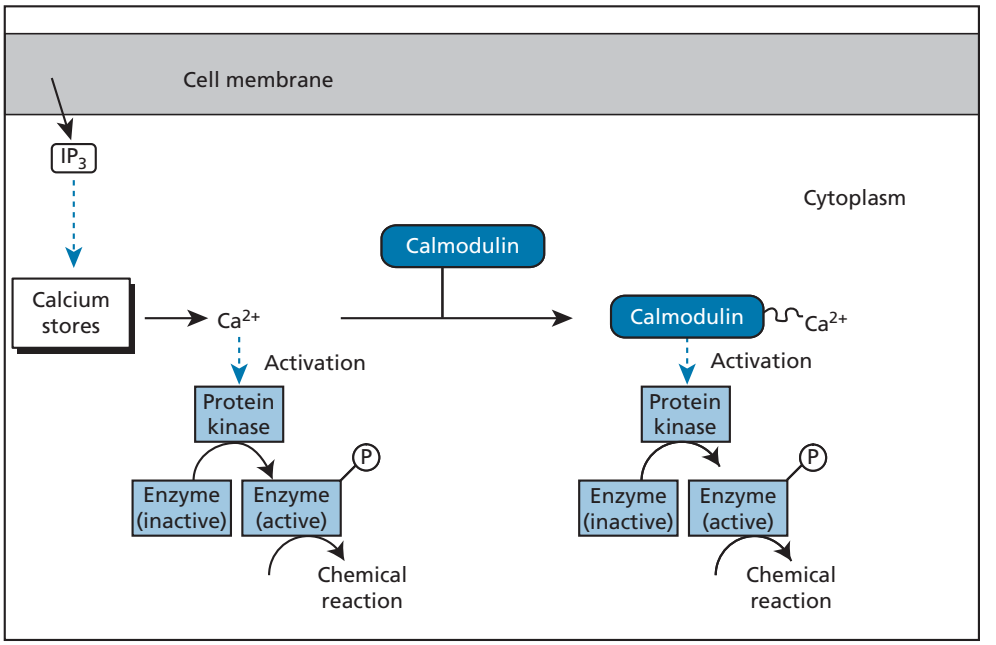


FIGURE 5.12 Signal transduction initiated by inositol triphosphate (IP_3). (Ⓟ = phosphate)

steps before re-synthesis can occur. For example, IP_3 is dephosphorylated in three steps to inositol which is then used as one of the building blocks for the re-synthesis of PIP_2 (Fig. 5.13). It is thought that **lithium salts** control the symptoms of manic depressive illness by interfering with this complex synthesis. They do so by inhibiting the monophosphatase enzyme responsible for the final dephosphorylation leading to inositol.

KEY POINTS

- G_q-proteins are split in a similar manner to G_s and G_i-proteins. The α_q-subunit affects the activity of phospholipase C which catalyses the hydrolysis of PIP_2 to form the secondary messengers IP_3 and DG.
- DG remains in the cell membrane and activates PKC, which is a serine–threonine kinase.
- IP_3 is a polar molecule which moves into the cytoplasm and mobilizes calcium ions. The latter activate protein kinases both directly and via the calcium binding protein calmodulin.
- IP_3 and DG are combined in a series of steps to reform PIP_2. Lithium salts are believed to interfere with this process.

5.4 Signal transduction involving kinase-linked receptors

5.4.1 Activation of signalling proteins and enzymes

We saw in section 4.8 that the binding of a chemical messenger to a kinase-linked receptor activates kinase activity such that a phosphorylation reaction takes place on the receptor itself. In the case of a tyrosine kinase, this involves the phosphorylation of tyrosine residues. We now continue that story.

Once phosphorylation has taken place, the phosphotyrosine groups and the regions around them act as binding sites for various signalling proteins or enzymes. Each phosphorylated tyrosine region can bind a specific signalling protein or enzyme. Some of these signalling proteins or enzymes become phosphorylated themselves once they are bound and act as further binding sites for yet more signalling proteins (Fig. 5.14).

Not all of the phosphotyrosine binding regions can be occupied by signalling proteins at one time so the type

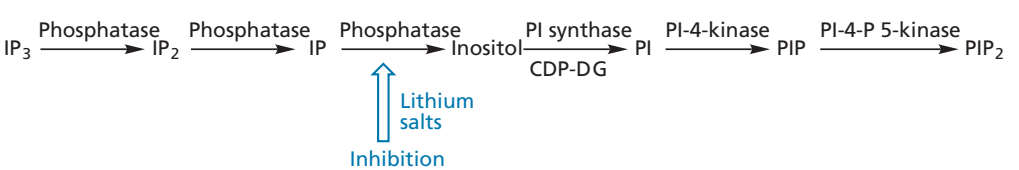


FIGURE 5.13 Re-synthesis of PIP_2 from IP_3 (CDP-DG = cytidine diphosphate-diacylglycerol).

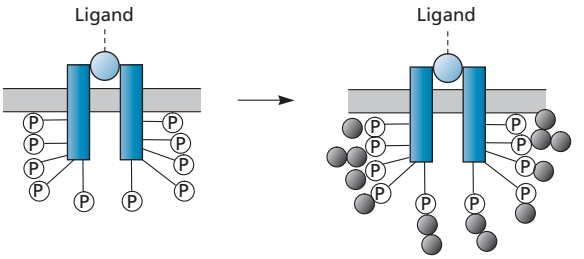


FIGURE 5.14 Binding of signalling proteins (indicated by dark circles) to activated kinase-linked receptors. (Ⓟ = phosphate)

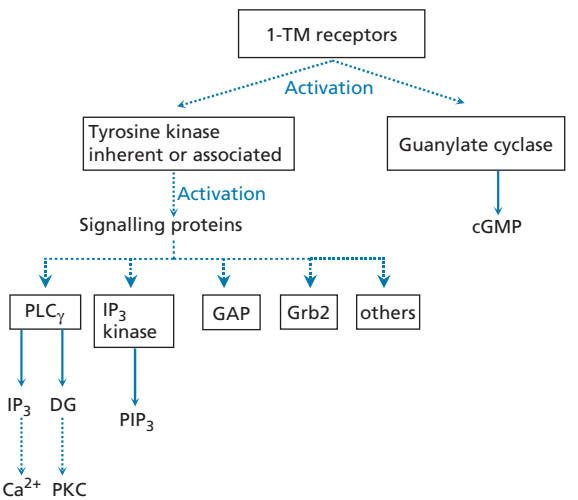


FIGURE 5.15 Signalling pathways from 1-TM receptors.

of signalling that results depends on which signalling proteins *do* manage to bind to the kinase receptors available. There is no room in an introductory text to consider what each and every signalling protein does, but most are the starting point for phosphorylation (kinase) cascades along the same principles as the cascades initiated by G-proteins (Fig. 5.15). Some growth factors activate a specific subtype of **phospholipase C** (PLCγ), which catalyses phospholipid breakdown leading to the generation of IP_3 and subsequent calcium release by the same mechanism as described in section 5.3.3. Other signalling proteins are chemical 'adaptors', which serve to transfer a signal from the receptor to a wide variety of other proteins, including many involved in cell division and differentiation. For example, the principal action of **growth factors** is to stimulate transcription of particular genes through a kinase signalling cascade (Fig. 5.16). A signalling protein called **Grb2** binds to a specific phosphorylated site of the receptor–ligand complex and becomes phosphorylated itself. A membrane protein called **Ras** (with a bound molecule of **GDP**) interacts with the receptor–ligand-signal protein complex and functions in a similar way to a G-protein (i.e. GDP is lost and GTP is gained). Ras is now activated and activates a serine–threonine kinase called **Raf**, initiating a serine–threonine kinase cascade which finishes with the activation of **mitogen-activated protein (MAP) kinase**. This phosphorylates and activates proteins called **transcription factors** which enter the nucleus and initiate gene expression resulting in various responses, including cell division. Many cancers can arise from malfunctions of this signalling cascade if the kinases involved become permanently activated, despite the absence of the initial receptor signal. Alternatively, some cancer cells over-express kinases and, as a result, the cell becomes super-sensitive to signals that stimulate growth and division. Consequently, inhibiting the kinase receptors or targeting the signalling pathway is proving to be an important method of designing new drugs for the treatment of cancer (section 21.6).

5.4.2 Small G-proteins

The Ras signal protein described in section 5.4.1 is an example of a class of signal proteins called the **small G-proteins**, so called because they are about two-thirds the size of the G-proteins described in sections 5.1–5.3. There are several subfamilies of small G-proteins (Ras, Rho, Arf, Rab, and Ran) and they can be viewed as being similar to the α-subunit of the larger G-proteins. Like the α-subunits, they are able to bind either GDP in the resting state or GTP in the activated state. Unlike their larger cousins, the small G-proteins are not activated by direct interaction with a receptor, but are activated downstream of receptor activation through intermediary proteins, which are classed as **guanine nucleotide exchange factors** (GEF). For example, activation of Ras (as shown in Fig. 5.16) requires the prior involvement of the protein Grb2 following receptor activation. Like the α-subunits, small G-proteins can autocatalyse the hydrolysis of bound GTP to give bound GDP, resulting in a return to the resting state. However, this process can be accelerated by helper proteins known as **GTPase activating proteins** (GAPs). This means that the level of activity of small G-proteins is under simultaneous brake and accelerator control involving GAP and GEF respectively.

The small G-proteins are responsible for stimulating cell growth and differentiation through different signal transduction pathways. Many cancers are associated with defects in small G-proteins, such as the Ras protein. *Ras* is the gene coding for the Ras protein and is one of the genes most commonly mutated in human tumours. There are three Ras proteins in mammalian cells: H-, K-, and N-Ras. Mutations which result in the inability of these proteins to autocatalyse the hydrolysis of bound GTP can occur. As a result, they remain permanently activated, leading, in turn, to permanent cell growth and division (see also section 21.6.1).

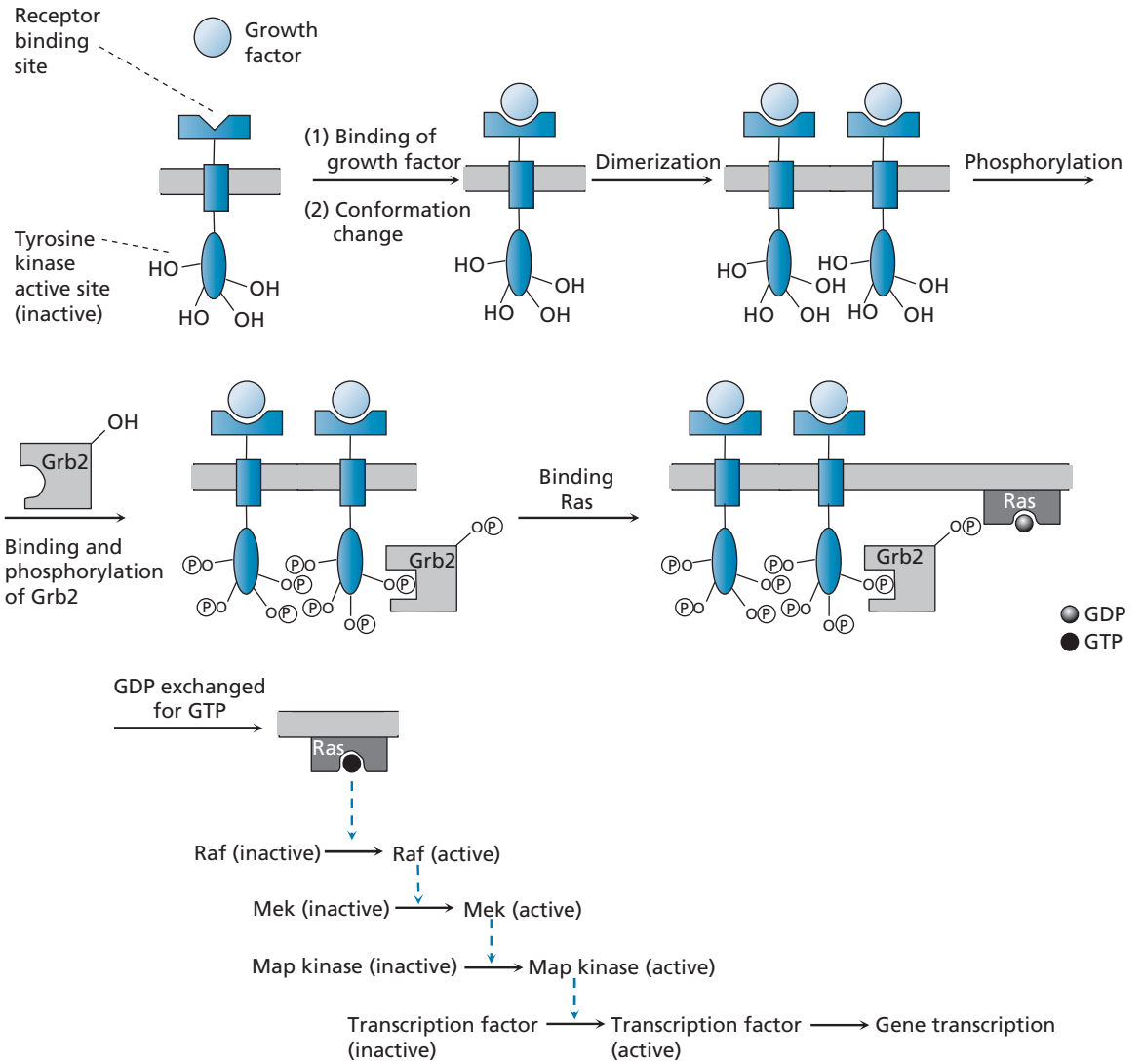


FIGURE 5.16 From growth factor to gene transcription. (Ⓟ = phosphate)

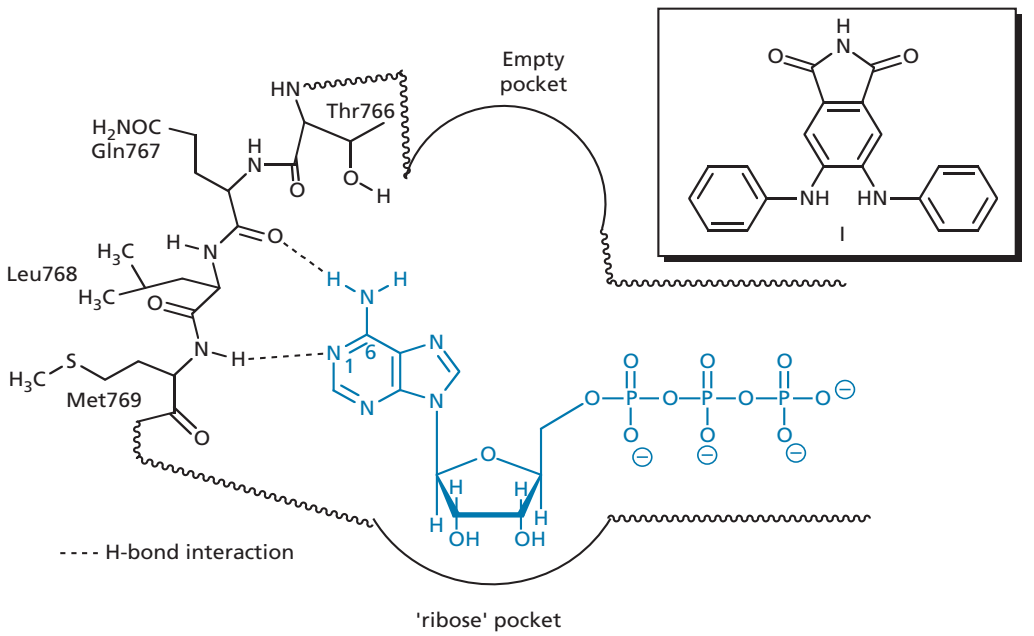
5.4.3 Activation of guanylate cyclase by kinase receptors

Some kinase receptors have the ability to catalyse the formation of **cyclic GMP** from **GTP**. Therefore, they are both receptor and enzyme (guanylate cyclase). The membrane-bound receptor/enzyme spans the cell membrane and has a single transmembrane segment. It has an extracellular receptor binding site and an intracellular guanylate cyclase active site. Its ligands are **α-atrial natriuretic peptide** and **brain natriuretic peptide**. Cyclic GMP appears to open sodium ion channels in the kidney, promoting the excretion of sodium.

KEY POINTS

- The phosphorylated tyrosine residues on activated kinase receptors act as binding sites for various signalling proteins and enzymes which are activated in turn.
- Small G-proteins are similar in nature to G-proteins, binding GDP in the resting state, and GTP in the activated state. They are single proteins activated by guanine nucleotide exchange factors.
- Some kinase receptors have an intracellular active site capable of catalysing the formation of cyclic GMP from GTP.

QUESTIONS



1. A model binding site for ATP was created for endothelial growth factor (EGF) receptor kinase, which demonstrates how ATP is bound (see above). Structure I is known to inhibit the binding of ATP. Suggest how structure I might bind.
2. Small G-proteins like Ras have an autocatalytic property. What does this mean and what consequences would there be (if any) should that property be lost?
3. Farnesyl transferase is an enzyme which catalyses the attachment of a long hydrophobic chain to the Ras protein. What do you think the purpose of this chain is and what would be the effect if the enzyme was inhibited?
4. Consider the signal transduction pathways shown in Fig. 5.16 and identify where signal amplification takes place.
5. The enzyme cAMP phosphodiesterase hydrolyses cAMP to AMP. What effect would an inhibitor of this enzyme have on glucose-1-phosphate production (Fig. 5.7)?
6. An enzyme was produced by genetic engineering where several of the serine residues were replaced by glutamate residues. The mutated enzyme was permanently active, whereas the natural enzyme was only active in the presence of a serine–threonine protein kinase. Give an explanation.
7. Suggest why tyrosine kinases phosphorylate tyrosine residues in protein substrates, but not serine or threonine residues.
8. Antibodies have been generated to recognize the extracellular regions of growth factor receptors. Binding of the antibody to the receptor should block the growth factor from reaching its binding site and block its signal. However, it has been observed that antibodies can sometimes trigger the same signal as the growth factor. Why should this occur? Consult section 10.7.2 to see the structure of an antibody.

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Titles for general further reading are listed on p.763.

6 Nucleic acids: structure and function

In this chapter we discuss the structure and function of nucleic acids. Drug action at nucleic acids is discussed in Chapter 9 and in other chapters throughout the text. Although most drugs act on protein structures, there are several examples of important drugs which act directly on nucleic acids. There are two types of nucleic acid—**DNA** (deoxyribonucleic acid) and **RNA** (ribonucleic acid). We first consider the structure of DNA.

6.1 Structure of DNA

Like proteins, DNA has a primary, secondary, and tertiary structure.

6.1.1 The primary structure of DNA

The primary structure of DNA is the way in which the DNA building blocks are linked together. Whereas proteins have over 20 building blocks to choose from, DNA has only four—the **nucleosides deoxyadenosine**, **deoxyguanosine**, **deoxycytidine**, and **deoxythymidine** (Fig. 6.1). Each nucleoside is constructed from two components—a **deoxyribose** sugar and a base. The sugar is the same in all four nucleosides and only the base is different. The four possible bases are two bicyclic purines (**adenine** and **guanine**) and two smaller pyrimidine structures (**cytosine** and **thymine**) (Fig. 6.2).

The nucleoside building blocks are joined together through phosphate groups which link the 5′-hydroxyl group of one nucleoside unit to the 3′-hydroxyl group of the next (Fig. 6.3). With only four types of building block available, the primary structure of DNA is far less varied than the primary structure of proteins. As a result, it was long thought that DNA had only a minor role to play in cell biochemistry, as it was hard to see how such an apparently simple molecule could have anything to do with the mysteries of the genetic code. The solution to this mystery lies in the secondary structure of DNA.

6.1.2 The secondary structure of DNA

Watson and Crick solved the secondary structure of DNA by building a model that fitted all the known experimental results. The structure consists of two DNA chains arranged together in a double helix of constant diameter (Fig. 6.4). The double helix has a major groove and a minor groove, which are of some importance to the action of several anticancer agents acting as intercalators (section 9.1).

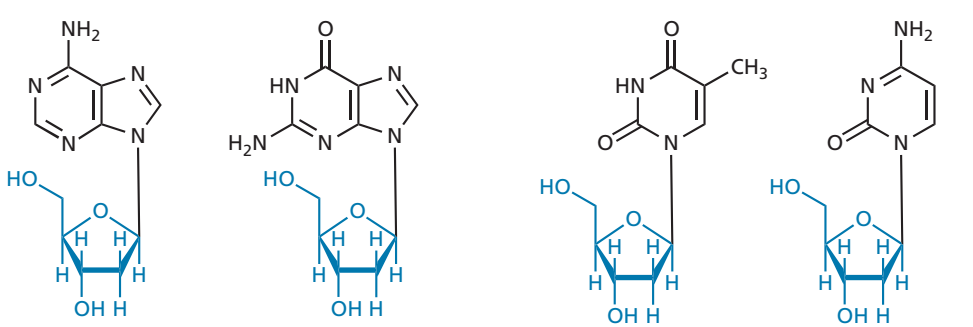


FIGURE 6.1 Nucleosides—the building blocks of DNA.

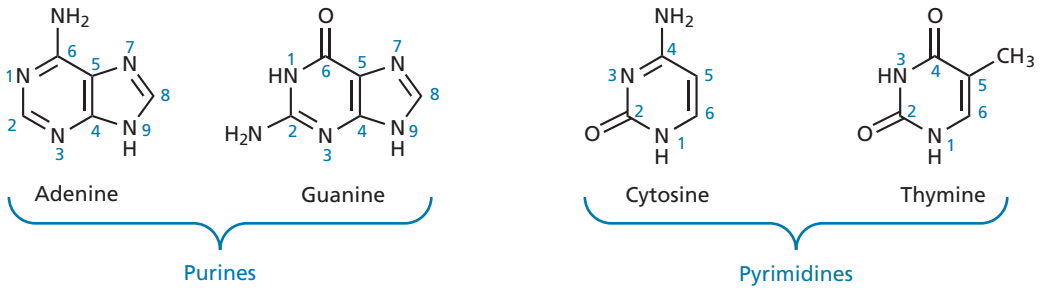


FIGURE 6.2 The nucleic acid bases for DNA.

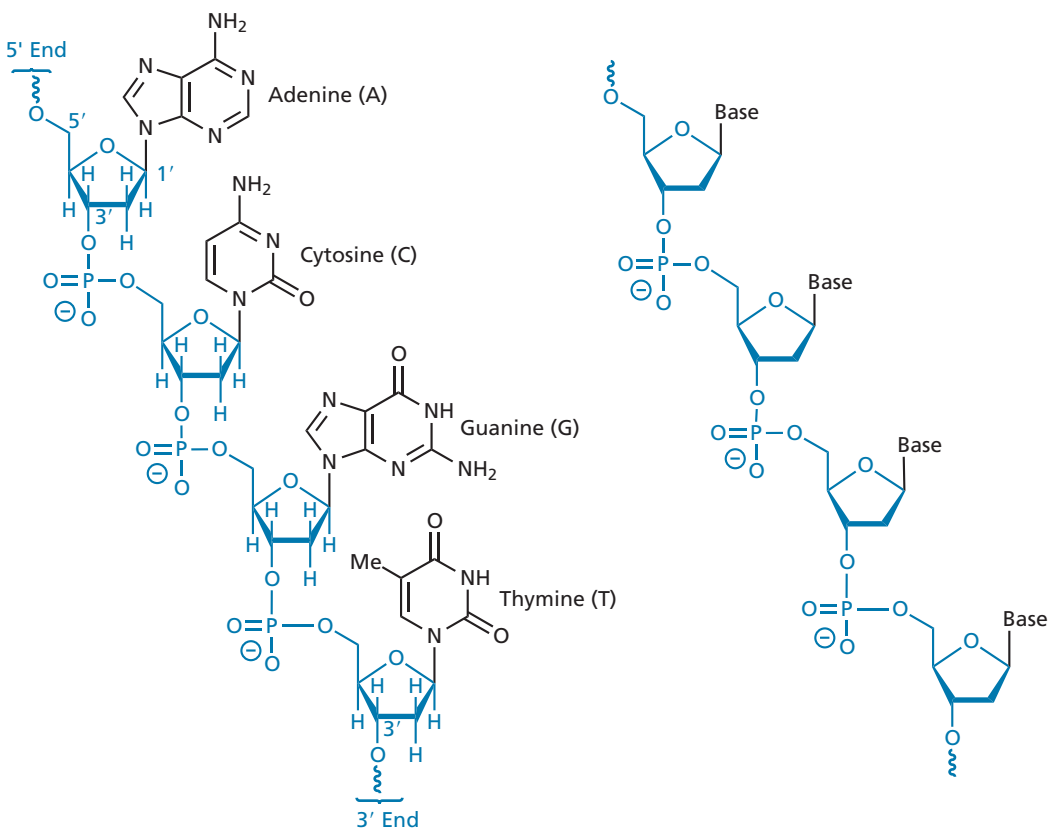


FIGURE 6.3 Linkage of nucleosides through phosphate groups.

The structure relies crucially on the pairing up of nucleic acid bases between the two chains. Adenine pairs with thymine via two hydrogen bonds, whereas guanine pairs with cytosine via three hydrogen bonds. Thus, a bicyclic purine base is always linked with a smaller monocyclic pyrimidine base to allow the constant diameter of the double helix. The double helix is further stabilized by the fact that the base pairs are stacked one on top of each other, allowing hydrophobic interactions between the faces of the heterocyclic rings. The polar sugar–phosphate backbone is placed to the outside of the structure and can form favourable polar interactions with water.

The fact that adenine always binds to thymine and cytosine always binds to guanine means that the chains are complementary to each other. It is now possible to see how **replication** (the copying of genetic information) is feasible. If the double helix unravels, a new chain can be constructed on each of the original chains (Fig. 6.5). In other words, each of the original chains acts as a template for the construction of a new and identical double helix. The mechanism by which this

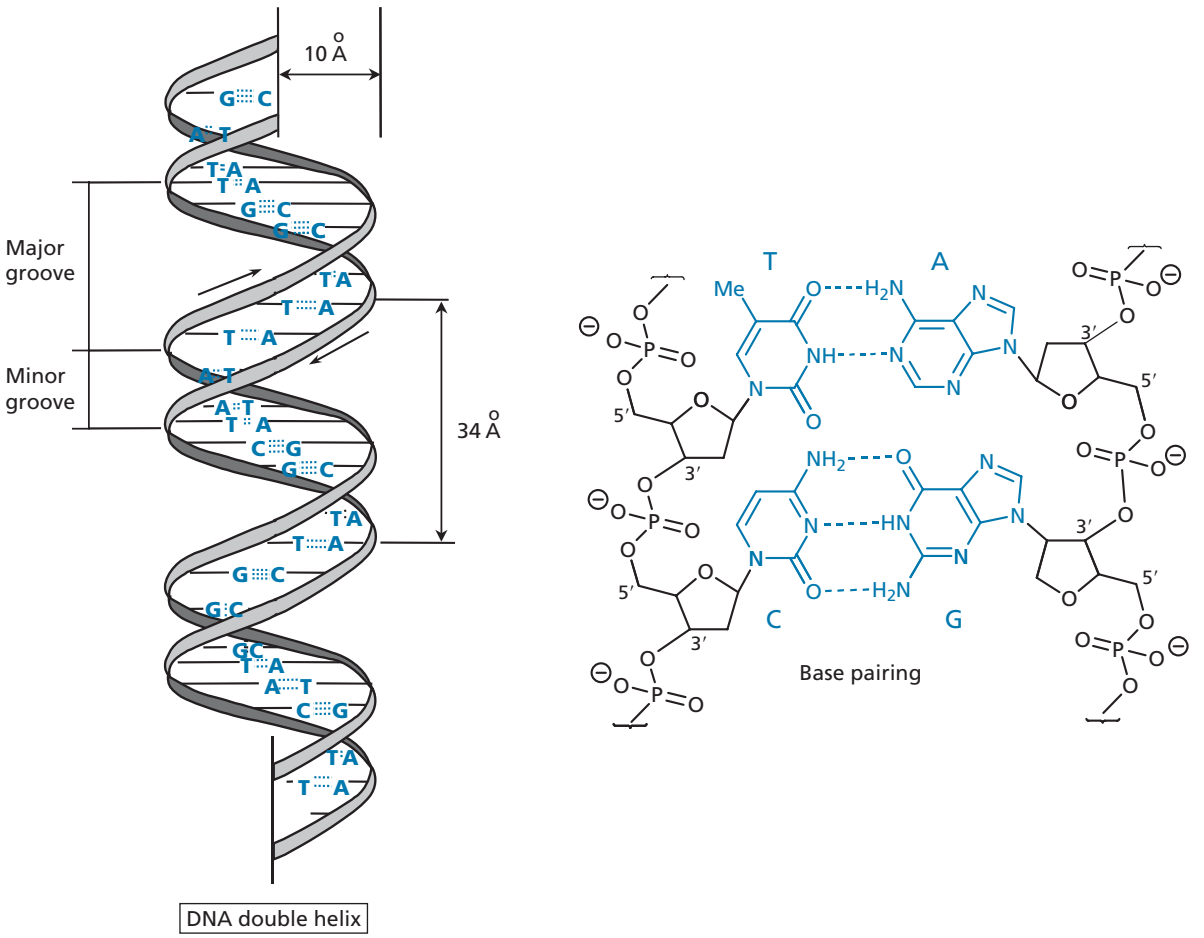


FIGURE 6.4 The secondary structure of DNA.

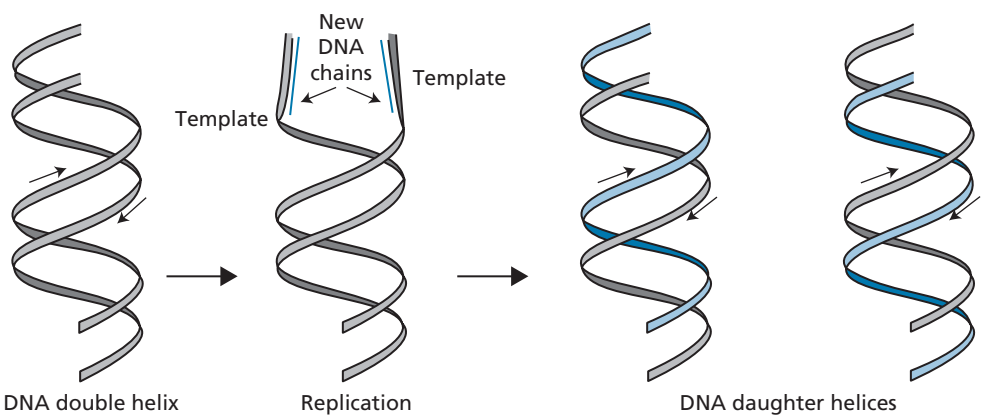


FIGURE 6.5 Replication of DNA chains.

takes place is shown in Figs 6.6 and 6.7. The template chain has exposed bases which can base-pair by hydrogen bonding with individual **nucleotides** in the form of triphosphates. Once a nucleotide has base-paired, an enzyme-catalysed reaction takes place where the new nucleotide is spliced on to the growing complementary chain with the loss of a diphosphate group—the latter acting as a good leaving group. Note that the process involves each new nucleotide reacting with the 3′ end of the growing chain.

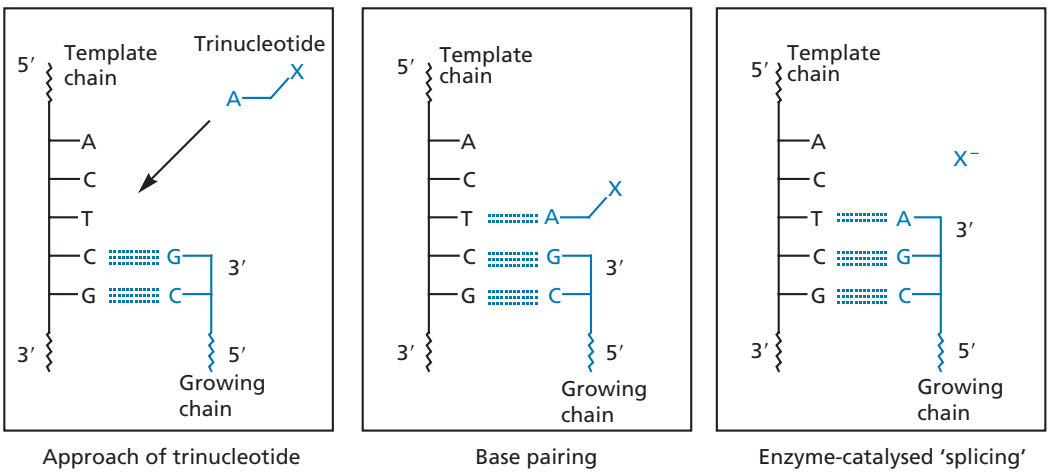


FIGURE 6.6 Base pairing of a trinucleotide and extension of the growing DNA chain.

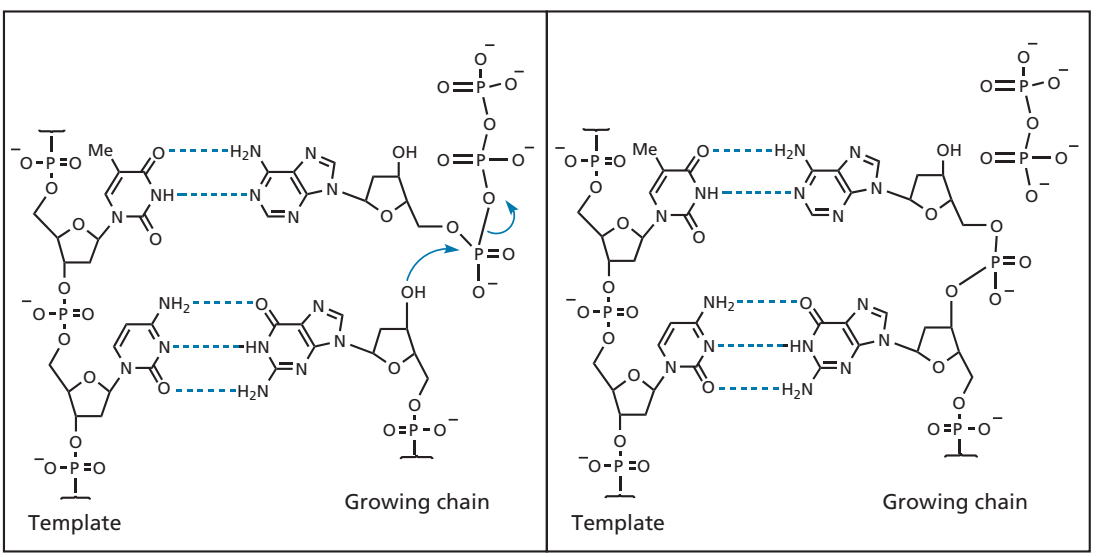


FIGURE 6.7 Mechanism by which a nucleotide is linked to the growing DNA chain.

We can now see how genetic information is passed on from generation to generation, but it is less obvious how DNA codes for proteins. How can only four nucleotides code for over 20 amino acids? The answer lies in the **triplet code**. In other words, an amino acid is coded not by one nucleotide, but by a set of three. There are 64 (4^3) ways in which four nucleotides can be arranged in sets of three—more than enough for the task required. Appendix 2 shows the standard genetic code for the various triplets. We shall look at how this code is interpreted to produce a protein in section 6.2.

6.1.3 The tertiary structure of DNA

The tertiary structure of DNA is often neglected or ignored, but it is important to the action of the quinolone group of antibacterial agents (section 9.2) and to several anticancer agents (sections 9.1 and 9.2). DNA is an extremely long molecule: so long, in fact, that it would not fit into the nucleus of the cell if it existed as a linear molecule. It has to be coiled into a more compact three-dimensional shape which *can* fit into the nucleus—a process known as **supercoiling**. This process requires the action of a family of enzymes called **topoisomerases**, which can catalyse the seemingly impossible act of passing one stretch of DNA helix across another stretch. They do this by temporarily cleaving one, or both, strands of the DNA helix to create a temporary gap, then resealing the strand(s) once the crossover has taken place. Supercoiling allows the efficient storage of DNA, but the DNA has to be uncoiled again if replication and transcription (section 6.2.2) are to take place. If uncoiling did not take place, the

unwinding process (catalysed by **helicase** enzymes) that takes place during replication and transcription would lead to increased tension due to increased supercoiling of the remaining DNA double helix. You can demonstrate the principle of this by pulling apart the strands of rope or sisal. The same topoisomerase enzymes are responsible for catalysing the uncoiling process, so inhibition of these enzymes would effectively block transcription and replication.

Topoisomerase II is a mammalian enzyme that is crucial to the effective replication of DNA. The enzyme binds to parts of DNA where two regions of the double helix are in near proximity (Fig. 6.8). The enzyme binds to one of these DNA double helices and tyrosine residues are used to nick both strands of the DNA (Fig. 6.9). This results in a temporary covalent bond between the enzyme and the resulting 5′ end of each strand, thus stabilizing the DNA. The strands are now pulled in opposite directions to form a gap through which the intact DNA region can be passed. The enzyme then reseals the strands and departs.

Topoisomerase I is similar to topoisomerase II in that it relieves the torsional stress of supercoiled DNA during replication, transcription, and the repair of DNA. The difference is that it cleaves only one strand of DNA, whereas topoisomerase II cleaves both strands. The enzyme catalyses a reversible transesterification reaction similar to that shown in Fig. 6.9, but where the tyrosine residue of the enzyme is linked to the 3′ phosphate end of the DNA strand rather than the 5′ end. This

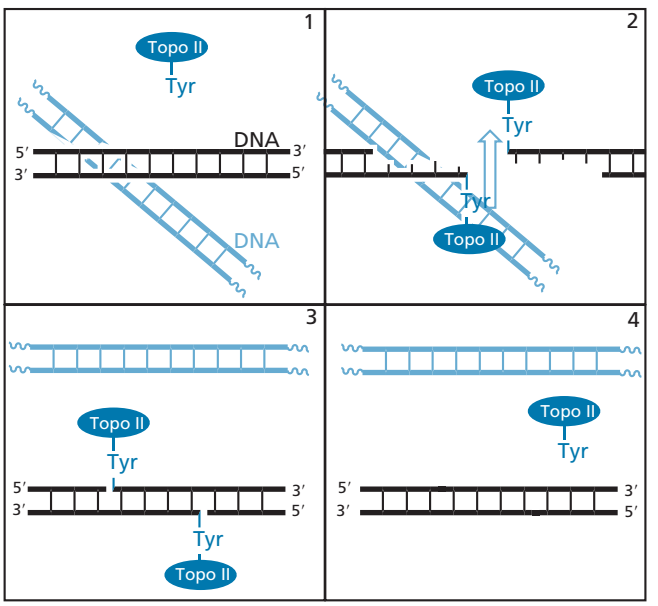


FIGURE 6.8 Method by which topoisomerase II catalyses the crossover of DNA strands. Note that the same enzyme bonds covalently to each DNA strand.

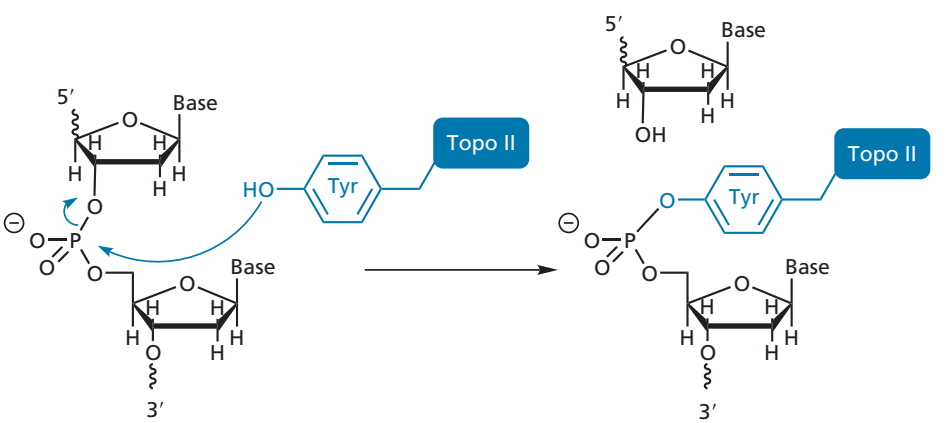


FIGURE 6.9 Mechanism by which topoisomerase II splits a DNA chain.

creates a 'cleavable complex' with a single-strand break. Relaxation of torsional strain takes place either by allowing the intact strand to pass through the nick or by free rotation of the DNA about the uncleaved strand. Once the torsional strain has been relieved, the enzyme rejoins the cleaved strand of DNA and departs.

Topoisomerase IV is a bacterial enzyme that carries out the same process as the mammalian enzyme topoisomerase II and is an important target for the fluoroquinolone antibacterial agents (section 9.2).

6.1.4 Chromatins

So far, we have focused on the structure of DNA. However, DNA is not an isolated macromolecule within the nucleus of the cell. It is associated with a variety of proteins, such as histones, in a structure called a chromatin (Fig. 21.5). The histones and associated DNA form a structure called a **nucleosome**, which occurs regularly along the length of the chromatin and plays a crucial role in the regulation of DNA transcription (section 21.7.3).

6.1.5 Genetic polymorphism and personalized medicine

The process of replication is not 100% perfect and, occasionally, a mutation can occur. If the mutation does not prove fatal, it will be carried on from generation to generation. This leads to different individuals having subtly different gene sequences. On average, there is a difference of one base pair in every thousand base pairs between individuals. This is known as **genetic polymorphism**. As the nucleic acid bases act as the code for amino acids in proteins, a difference at this level results in a different amino acid being introduced into a protein, which may or may not have an effect on that protein's activity or function (sections 3.5.6 and 4.11). Genetic polymorphism has important consequences with respect to the susceptibility of individuals to disease and also to the kinds of drug therapies that are best suited for individuals. A detailed knowledge of a patient's genome opens up the possibility of predicting and preventing disease, as well as choosing the ideal drug therapy for that patient should a disease occur. This is known as **personalized medicine** (see also sections 15.1.4.4 and 21.1.11).

KEY POINTS

- The primary structure of DNA consists of a sugar phosphate backbone with nucleic acid bases attached to each sugar moiety. The sugar is deoxyribose and the bases are adenine, thymine, cytosine, and guanine.
- The secondary structure of DNA is a double helix where the nucleic acid bases are stacked in the centre and paired up such that adenine pairs with thymine, and cytosine pairs with guanine. Hydrogen bonding is responsible for the base-pairing and there are van der Waals interactions between the stacks of bases. Polar interactions occur between the sugar phosphate backbone and surrounding water.
- The DNA double helix is coiled up into a tertiary structure. The coiling and uncoiling of the double helix requires topoisomerase enzymes.
- The copying of DNA from one generation to the next is known as replication. Each strand of a parent DNA molecule acts as the template for a new daughter DNA molecule.
- The genetic code consists of nucleic acid bases, which are read in sets of three during the synthesis of a protein. Each triplet of bases codes for a specific amino acid.
- Knowing a patient's genome opens up the possibility of predicting disease and identifying the best therapies for that individual. This is known as personalized medicine.

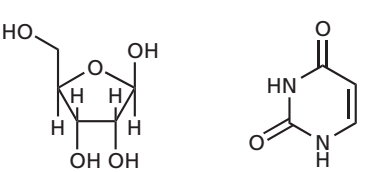
6.2 Ribonucleic acid and protein synthesis

6.2.1 Structure of RNA

The primary structure of RNA is the same as that of DNA, with two exceptions: **ribose** (Fig. 6.10) is the sugar component rather than **deoxyribose**, and **uracil** (Fig. 6.10) replaces thymine as one of the bases.

Base-pairing between nucleic acid bases can occur in RNA, with adenine pairing to uracil, and cytosine pairing to guanine. However, the pairing is between bases within the same chain and it does not occur for the whole length of the molecule (e.g. Fig. 6.11). Therefore, RNA is not a double helix, but it does have regions of helical secondary structure.

Because the secondary structure is not uniform along the length of the RNA chain, more variety is allowed in RNA tertiary structure. There are three main types of RNA molecules with different cellular functions: **messenger RNA** (mRNA), **transfer RNA** (tRNA), and **ribosomal RNA** (rRNA). These three molecules are crucial to the process by which protein synthesis takes place. Although DNA contains the genetic code for proteins, it cannot produce these proteins directly. Instead, RNA



Ribose Uracil

FIGURE 6.10 Ribose and uracil.

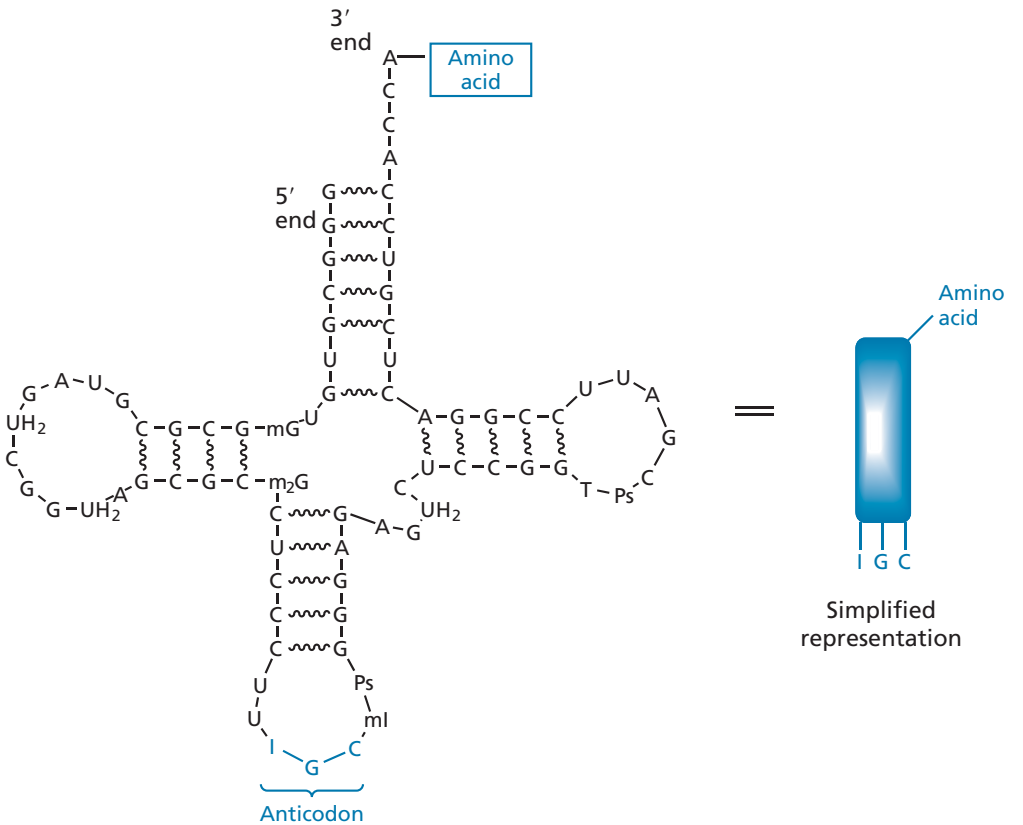


FIGURE 6.11 Yeast alanine transfer RNA. The wiggly lines indicate base pairing (mI = methylinosine, UH_2 = dihydrouridine, T = ribothymidine, Ps = pseudouridine, mG = methylguanosine, m_2G = dimethylguanosine).

takes on that role, acting as the crucial 'middle man' between DNA and proteins. This has been termed the **central dogma** of molecular biology.

The bases adenine, cytosine, guanine, and uracil are found in mRNA and are predominant in rRNA and tRNA. However, tRNA also contains a number of less common nucleic acids—see, for example, Fig. 6.11.

6.2.2 Transcription and translation

A molecule of mRNA represents a copy of the genetic information required to synthesize a single protein. Its role is to carry the required code out of the nucleus to a cellular organelle called the **endoplasmic reticulum**. This is where protein production takes place on bodies called **ribosomes**. The segment of DNA which is copied is called a gene and the process involved is called **transcription**. The DNA double helix unravels and the stretch that is exposed acts as a template on which the mRNA can be built (Fig. 6.12). Once complete, the mRNA departs the nucleus to seek out a ribosome, while the DNA re-forms its double helix.

Ribosomal RNA is the most abundant of the three types of RNA and is the major component of ribosomes. These can be looked upon as the production sites for protein synthesis—a process known as **translation.** The ribosome binds to one end of the mRNA molecule, then travels along it to the other end, allowing the triplet code to be read, and catalysing the construction of the protein molecule one amino acid at a time (Fig. 6.13). There are two segments to the mammalian ribosome, known as the 60S and 40S subunits. These combine to form an 80S ribosome. In bacterial cells, the ribosomes are smaller and consist of 50S and 30S subunits combining to form a 70S ribosome. The terms 50S, etc. refer to the sedimentation properties of the various structures. These are related qualitatively to size and mass, but not quantitatively—that is why a 60S and a 40S subunit can combine to form an 80S ribosome.

rRNA is the major component of each subunit, making up two thirds of the ribosome's mass. The 40S subunit contains one large rRNA molecule along with several proteins, whereas the 60S subunit contains three different sized rRNAs; again, with accompanying proteins. The secondary structure of rRNA includes extensive stretches of base pairing (**duplex regions**), resulting in a well-defined tertiary structure. It was thought at one time that rRNA only played a structural role and that the proteins were acting as enzymes to catalyse translation. The rRNA molecules certainly do have a crucial structural role, but

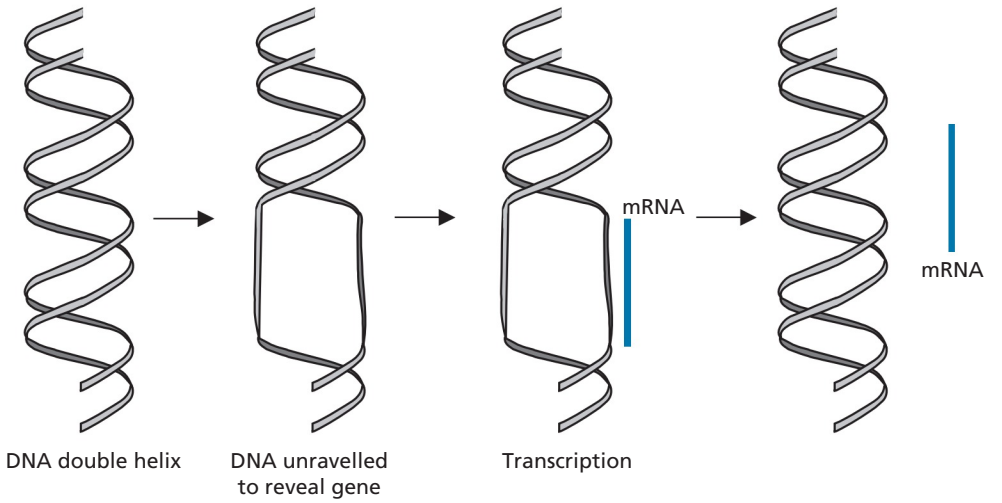


FIGURE 6.12 Formation of mRNA.

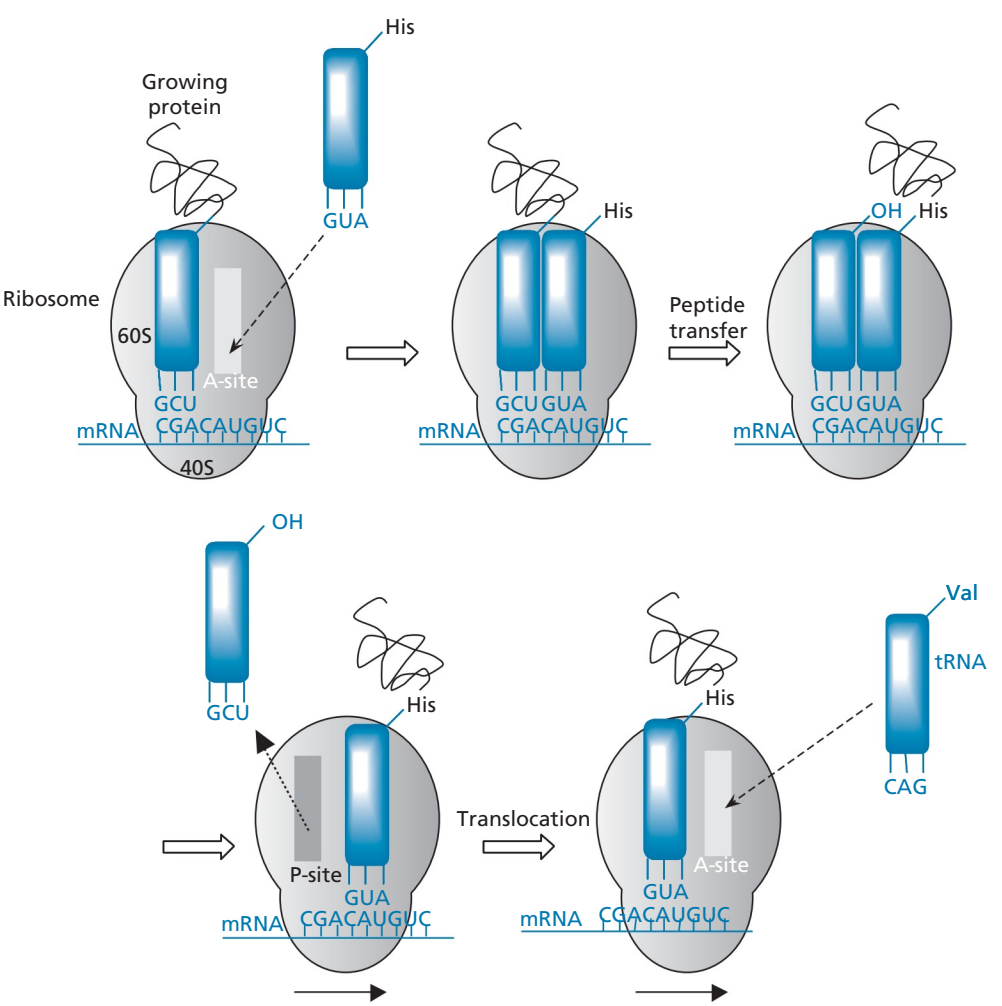


FIGURE 6.13 Protein synthesis—translation.

it is now known that they, rather than the ribosomal proteins, have the major catalytic role. Indeed, the key sites in the ribosome where translation takes place are made up almost entirely of rRNA. The proteins are elongated structures which meander through the ribosome structure and are thought to have a fine-tuning effect on the translation process.

Transfer RNA is the crucial adaptor unit which links the triplet code on mRNA to a specific amino acid. This means there has to be a different tRNA for each amino acid. All the tRNAs are clover-leaf in shape, with two different binding regions at opposite ends of the molecule (see Fig. 6.11). One binding region is for the amino acid, where a specific amino acid is covalently linked to a terminal adenosyl residue. The other is a set of three nucleic acid bases **(anticodon)** which will base-pair with a complementary triplet on the mRNA molecule. A tRNA having a particular anticodon will always have the same amino acid attached to it.

Let us now look at how translation takes place in more detail. As rRNA travels along mRNA, it reveals the triplet codes on mRNA one by one. For example, in Fig. 6.13 the triplet code CAU is revealed along with an associated binding site called the A site. The A stands for aminoacyl and refers to the attached amino acid on the incoming tRNA. Any tRNA molecule can enter this site but it is accepted only if it has the necessary anticodon capable of base-pairing with the exposed triplet on mRNA. In this case, tRNA having the anticodon GUA is accepted and brings with it the amino acid histidine. The peptide chain that has been created so far is attached to a tRNA molecule which is bound to the P binding site (standing for peptidyl). A grafting process then takes place, catalysed by rRNA, where the peptide chain is transferred to histidine (Fig. 6.14). The tRNA occupying the P binding site now departs and the ribosome shifts along mRNA to reveal the next triplet (a process called **translocation**), and so the process continues until the whole strand is read. The new protein is then released from the ribosome, which is now available to start the process again. The overall process of transcription and translation is summarized in Fig. 6.15.

6.2.3 Small nuclear RNA

After transcription, mRNA molecules are frequently modified before translation takes place. This involves a splicing operation where the middle section of mRNA (the **intron**) is excised and the ends of the mRNA molecule (the **exons**) are spliced together (Fig. 6.16).

Splicing requires the aid of an RNA-protein complex called a **spliceosome**. The RNA molecules involved in this complex are called **small nuclear RNAs** (snRNAs). As the name indicates, these are small RNA molecules with fewer than 300 nucleotides that occur in the nucleus of the cell. The role of the snRNAs in the spliceosome is to base-pair with particular segments of mRNA such that the mRNA can be manipulated and aligned properly for the splicing process. Splice sites are recognized by their nucleotide sequences, but, on occasion, a mutation in DNA may introduce a new splice site somewhere else on mRNA. This results in faulty splicing, an altered mRNA, and a defective protein. About 15% of genetic diseases are thought to be due to mutations that result in defective splicing.

6.3 Genetic illnesses

A number of genetic illnesses are due to genetic abnormalities that result in the non-expression of particular proteins or the expression of defective proteins. For example, **albinism** is a condition where the skin, hair, and eyes lack pigment; it is associated with a deficiency of an enzyme called **tyrosinase**. This is a copper-containing enzyme that catalyses the first two stages in the synthesis

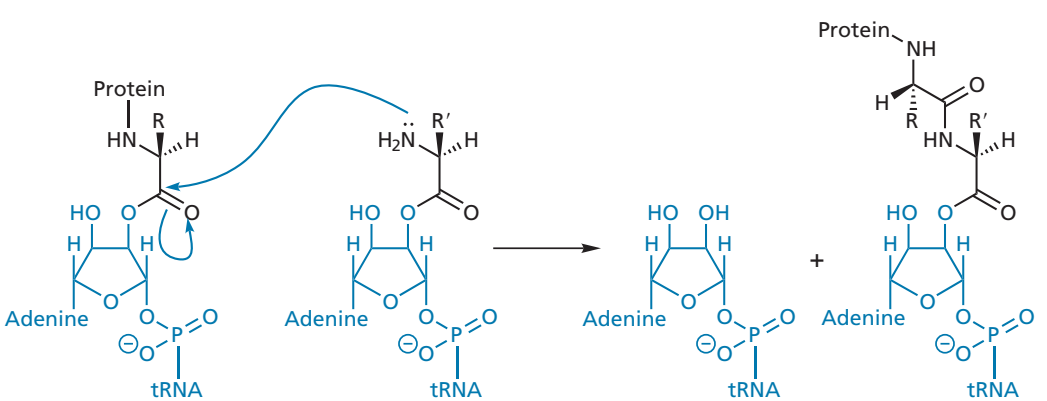


FIGURE 6.14 Mechanism by which a growing protein is transferred to the next amino acid.

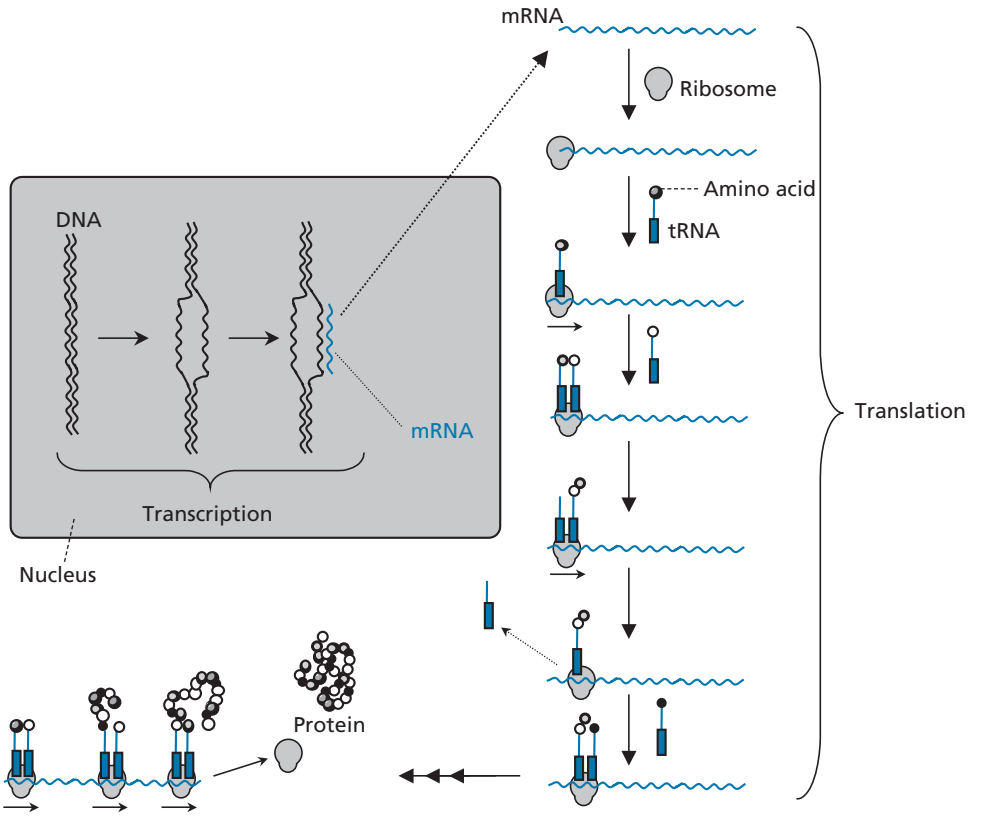


FIGURE 6.15 Transcription and translation.

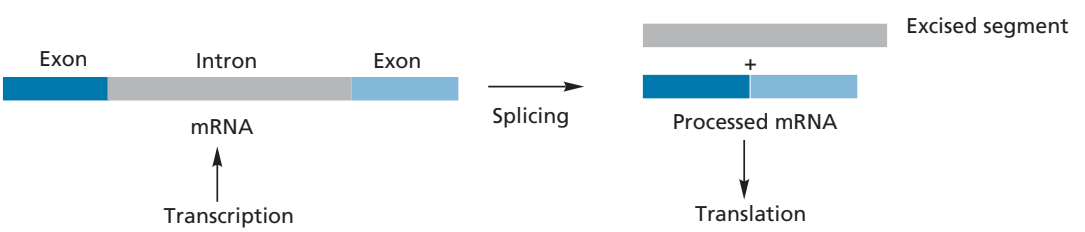


FIGURE 6.16 Splicing messenger RNA (mRNA).

of the pigment **melanin**. Over 90 mutations of the tyrosinase gene have been identified which lead to the expression of inactive enzyme. Mutations in the triplet code result in one or more amino acids being altered in the resulting protein, and if these amino acids are important to the activity of the enzyme, activity is lost. Mutations which alter amino acids in the active site are the ones most likely to result in loss of activity.

Phenylketonuria is a genetic disease caused by the absence or deficiency of an enzyme called **phenylalanine hydroxylase**. This enzyme normally converts phenylalanine to tyrosine. In its absence the blood levels of phenylalanine rise substantially, along with alternative metabolic products, such as phenylpyruvate. If left untreated, this disease results in severe mental retardation.

Haemophilias are inherited genetic diseases in which one of the blood coagulation factors is deficient. This results in uncontrolled bleeding after an injury. In the past, people with this disease were likely to die in their youth. Nowadays, with the proper treatment, affected individuals should have a normal life expectancy. Treatment in severe cases involves regular intravenous infusion with the relevant coagulation factor. In less severe cases, transfusions can be used when an injury has taken place. The coagulation factors used to be typically derived from blood plasma, but this meant that people with haemophilia were susceptible to infection from infected blood samples. For example, during the period 1979–1985 more than 1200 people in the UK were infected with HIV as a result of receiving infected blood

products. For the same reason, they were also prone to viral infections caused by hepatitis B and C. During the 1990s, recombinant DNA technology (section 6.4) successfully produced blood coagulation factors and these are now the agents of choice as they eliminate the risk of infection. Unfortunately, some patients produce an immune response to the infused factor, which can preclude their use. At present, clinical trials are under way to test whether gene therapy can be used as a treatment. This involves the introduction of a gene which will code for the normal coagulation factor so that it can be produced naturally in the body (section 6.4).

Muscular dystrophy is another genetic disease that affects 1 in every 3500 males and is characterized by the absence of a protein called **dystrophin**. This has an important structural role in cells and its absence results in muscle deterioration. Gene therapy is also being considered for this disease.

Many cancers are associated with genetic defects which result in molecular signalling defects in the cell. This is covered more fully in Chapter 21.

6.4 Molecular biology and genetic engineering

Over the last few years, rapid advances in molecular biology and genetic engineering have had important repercussions for medicinal chemistry. It is now possible to clone specific genes and to include these genes into the DNA of fast-growing cells such that the proteins encoded by these genes are expressed in the modified cell. As the cells are fast-growing this leads to a significant quantity of the desired protein, which permits its isolation, purification, and structural determination. Before these techniques became available, it was extremely difficult to isolate and purify many proteins from their parent cells owing to the small quantities present. Even if one was successful, the low yields inherent in the process made an analysis of the protein's structure and mechanism of action very difficult. Advances in molecular biology and recombinant DNA techniques have changed all that.

Recombinant DNA technology allows scientists to manipulate DNA sequences to produce modified DNA or completely novel DNA. The technology makes use of natural enzymes called **restriction enzymes** and **ligases** (Fig. 6.17). The restriction enzymes recognize a particular sequence of bases in each DNA molecule and split a specific sugar phosphate bond in each strand of the double helix. With some restriction enzymes, the break is not a clean one; there is an overlap between the two chains resulting in a tail of unpaired bases on each side of the break. The bases on each tail are complementary and can still recognize each other, so they are described as 'sticky' ends. The same process is carried out on a different molecule of DNA and the molecules from both processes are mixed together. As these different molecules have the same sticky ends, they recognize each other such that base pairing takes place in a process called **annealing**. Treatment with the ligase enzyme then repairs the sugar phosphate backbone and a new DNA molecule is formed.

If the DNA molecule of interest does not have the required sequence recognized by the restriction enzyme, a synthetic DNA **linker** that *does* contain the sequence can be added to either end of the molecule using a ligase enzyme. This is then treated with the restriction enzyme as before (Fig. 6.18).

There are many applications for this technology, one of which is the ability to amplify and express the gene for a particular human protein in bacterial cells. In order to do this it is necessary to introduce the gene to the bacterial cell. This is done by using a suitable **vector** which will carry the gene into the cell. There are two suitable

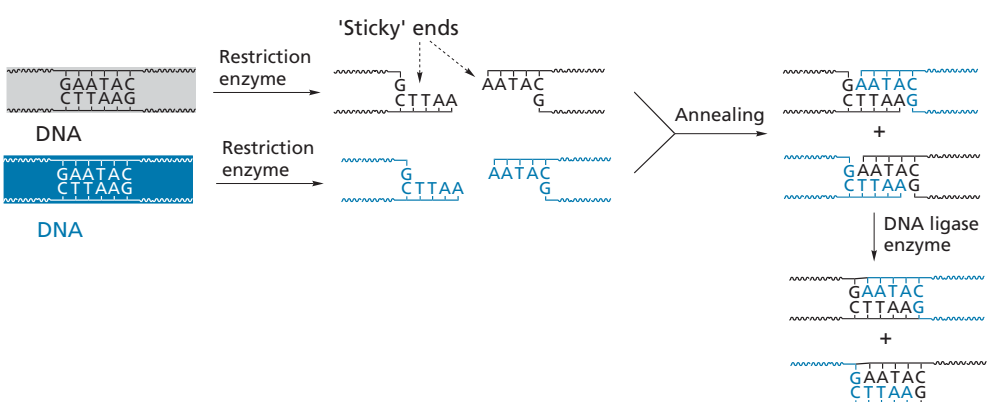


FIGURE 6.17 Recombinant DNA technology.

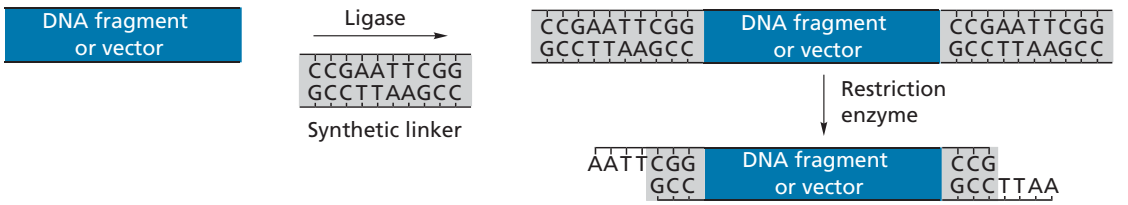


FIGURE 6.18 Attaching sequences recognized by restriction enzymes.

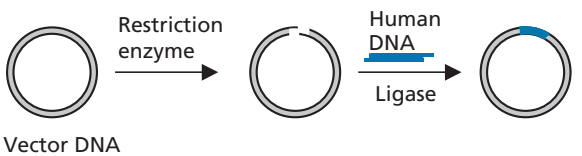


FIGURE 6.19 Inserting a human gene into a plasmid by recombinant DNA technology.

vectors—**plasmids** and **bacteriophages**. Plasmids are segments of circular DNA that are transferred naturally between bacterial cells and allow the sharing of genetic information. Because the DNA is circular, the DNA representing a human gene can be inserted into the vector's DNA by the same methods described above (Fig. 6.19). Bacteriophages (phages for short) are viruses that infect bacterial cells. There are a variety of these, but the same recombinant DNA techniques can be used to insert human DNA into viral DNA.

Whichever vector is used, the modified DNA is introduced into the bacterial cell where it is cloned and amplified (Fig. 6.20). For example, once a phage containing modified nucleic acid infects a bacterial cell, the phage takes over the cell's biochemical machinery to produce multiple copies of itself and its nucleic acid.

Human genes can be introduced to bacterial cells such that the gene is incorporated into bacterial DNA and expressed as if it were the bacteria's own. This allows the production of human proteins in much greater quantity than would be possible by any other means. Such proteins could then be used for medicinal purposes, as described in the following sections. Modified genes can also be introduced and expressed to produce modified proteins to see what effect a mutation would have on the structure and function of a protein.

The following are some of the applications of genetic engineering to the medical field.

Harvesting important proteins The genes for important hormones or growth factors, such as **insulin** and **human growth factor**, have been included in fast-growing unicellular organisms. This allows the harvesting of these proteins in sufficient quantities that they can be marketed and administered to patients who are deficient in these important hormones. Genetic engineering has also been crucial in the production of monoclonal antibodies (section 14.8.3).

Genomics and the identification of new protein drug targets Nowadays, it is relatively easy to isolate and identify a range of signalling proteins, enzymes, and receptors by **cloning** techniques. This has led to the identification of a growing number of isozymes and receptor subtypes which offer potential drug targets for the future. The **Human Genome Project** involved the mapping of human DNA (completed in 2000) and has led to the discovery of previously unsuspected new proteins. These, too, may offer potential drug targets. The study of the structure and function of new proteins discovered from genomics is called **proteomics** (section 2.6).

Study of the molecular mechanism of target proteins Genetic engineering allows the controlled

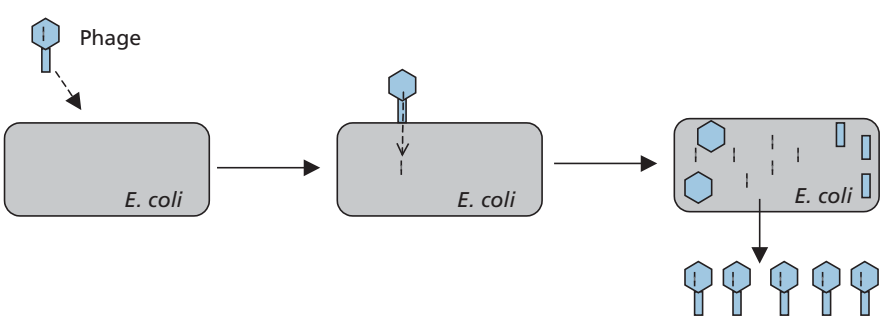


FIGURE 6.20 Infecting *Escherichia coli* with a phage.

mutation of proteins such that specific amino acids are altered. This allows researchers to identify which amino acids are important to enzyme activity or to receptor binding. In turn, this leads to a greater understanding of how enzymes and receptors operate at the molecular level.

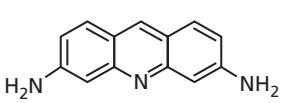
Somatic gene therapy involves the use of a carrier virus to smuggle a healthy gene into cells in the body where the corresponding gene is defective. Once the virus has infected the cell, the healthy gene is inserted into the host DNA where it undergoes transcription and translation. This approach has great therapeutic potential for cancers, AIDS, and genetic abnormalities, such as cystic fibrosis. However, the approach is still confined to research laboratories and there is still a long way to go before it is used clinically. There are several problems still to be tackled, such as how to target the viruses specifically to the defective cells, how to insert the gene into DNA in a controlled manner, how to regulate gene expression once it is in DNA, and how to avoid immune responses to the carrier virus. Progress in this field was set back significantly in 1999 as a result of the death of a teenage volunteer during a clinical trial in the USA. This was attributed to an over-reactive immune response to the carrier virus used in the trial. Consequently, there are now studies looking into the use of artificial viruses which would be less likely to cause an immune response. Non-viral delivery systems are also being studied involving caged molecules called **cyclodextrins**. In addition, lipids, polyaminoesters, glycine polymers, and carbon buckyballs are being investigated as carriers.

KEY POINTS

- The primary structure of RNA is similar to that of DNA, but it contains ribose instead of deoxyribose. Uracil is used as a base in place of thymine, and other bases may be present in smaller quantities.
- Base pairing and sections of helical secondary structure are possible within the structure of RNA.
- There are three main types of RNA—messenger RNA, transfer RNA, and ribosomal RNA.
- Transcription is the process by which a segment of DNA is copied as mRNA. mRNA carries the genetic information required for the synthesis of a protein from the nucleus to the endoplasmic reticulum.
- rRNA is the main constituent of ribosomes where protein synthesis takes place. A ribosome moves along mRNA revealing each triplet of the genetic code in turn.
- tRNA interprets the coded message in mRNA. It contains an anticodon of three nucleic acid bases which binds to a complementary triplet on mRNA. Each tRNA carries a specific amino acid, the nature of which is determined by the anticodon.
- The process of protein synthesis is called translation. The growing protein chain is transferred from one tRNA to the amino acid on the next tRNA and is only released once the complete protein molecule has been synthesized.
- Genetic engineering has been used in the production of important hormones for medicinal purposes, the identification of novel drug targets, the study of protein structure and function, and gene therapy.

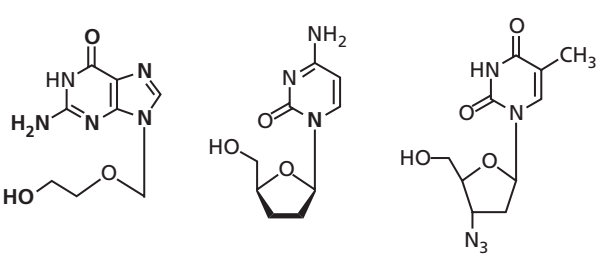
QUESTIONS

1. Proflavine is a topical antibacterial agent which intercalates bacterial DNA and was used to treat wounded soldiers in the Far East during World War II. What role (if any) is played by the tricyclic ring and the primary amino groups? The drug cannot be used systemically. Suggest why this is the case.



Proflavine

2. The following compounds are antiviral drugs that mimic natural nucleosides. What nucleosides do they mimic?



3. Adenine is an important component of several important biochemicals. It has been proposed that adenine was synthesized early on in the evolution of life when the Earth's atmosphere consisted of gases, such as hydrogen cyanide and methane. It has also been possible to synthesize adenine from hydrogen cyanide. Consider the structure of adenine and identify how cyanide molecules might act as the building blocks for this molecule.

4. The genetic code involves three nucleic acid bases coding for a single amino acid (the triplet code). Therefore, a mutation to a particular triplet should result in a different amino acid. However, this is not always the case. For any triplet represented by XYZ, which mutation is least likely to result in a change in amino acid—X, Y, or Z?

5. The amino acids serine, glutamate, and phenylalanine were found to be important binding groups in a receptor binding site (see Appendix 1 for structures). The triplet codes for these amino acids in the

mRNA for this receptor were AGU, GAA, and UUU respectively. Explain what effect the following mutations might have, if any:

AGU to ACU; AGU to GGU; AGU to AGC
GAAto GAU; GAA to AAA; GAA to GUA
UUU to UUC; UUU to UAU; UUU to AUU

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