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, and 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 receptor. 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, help 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 and 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. G_s, G_i/G_o, G_q/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.

First, the receptor binds its neurotransmitter or hormone (frame 1 in Fig. 5.1). As a result, the receptor changes shape and exposes a new binding site on its inner surface (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 (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 (frame 4).

Binding of GTP results in another conformational change in the G-protein (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 (frame 6). Both the α-subunit and the βγ-dimer then depart the receptor.

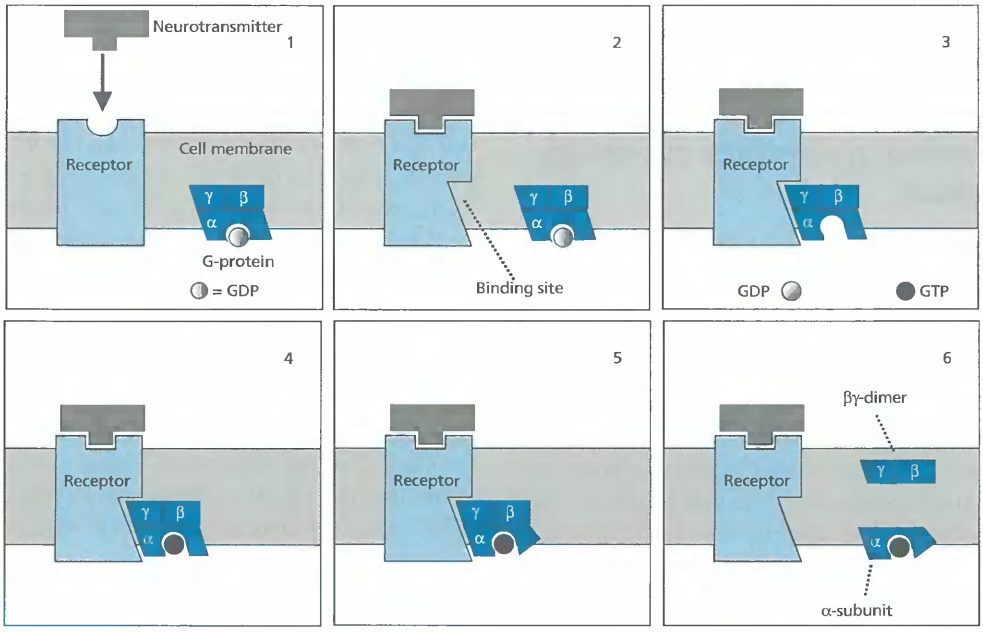


FIGURE 5.1 Activation of G-protein-coupled receptors and their interaction with G-proteins.

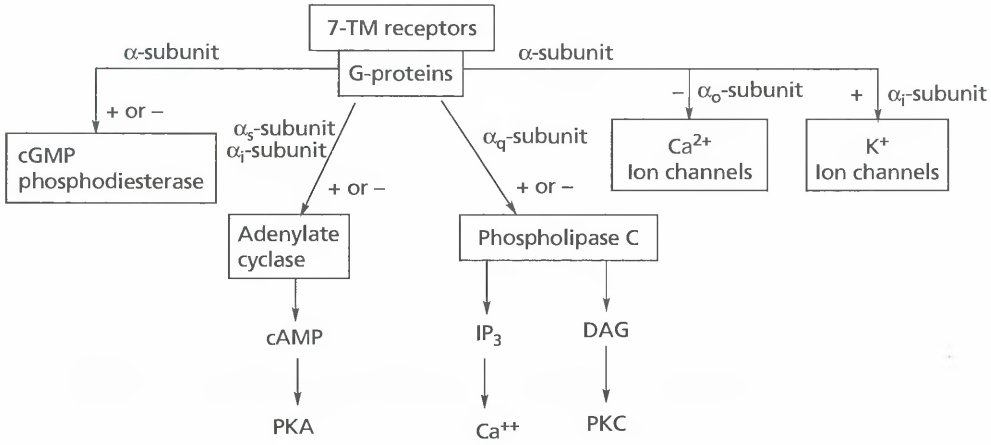


FIGURE 5.2 Signalling pathways arising from the splitting of different G-proteins.

The receptor–ligand complex is able to activate several G-proteins in this way before the ligand departs and 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, and this results in the synthesis of several hundred cAMP 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 $\beta\gamma$-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). PKA is inactive in the resting state and consists of four protein subunits, two of which are regulatory and two of which are catalytic. Its activation occurs as shown in Fig. 5.7.

The regulatory subunits have two binding sites for cAMP, and when this binds, the catalytic subunits are split from the protein complex and become active. Once the protein kinase catalytic subunits become active, they catalyse the phosphorylation and activation of further enzymes with functions specific to the particular cell or organ in question. For example, a protein kinase activates lipase enzymes in fat cells that 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 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 **glycogen** breakdown and glycogen synthesis in a liver cell are regulated as shown in Fig. 5.8.

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 synthesis of cAMP and the activation of PKA by the mechanism

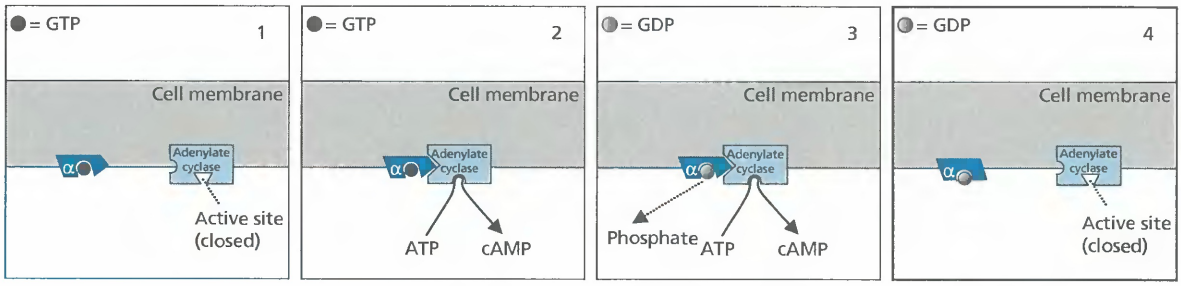


FIGURE 5.3 Interaction of α_s-subunit with adenylate cyclase, and activation of the enzyme.

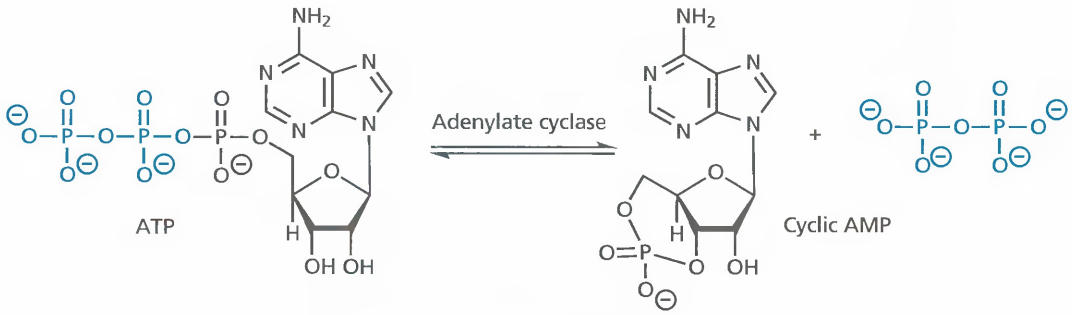


FIGURE 5.4 Synthesis of cyclic AMP.

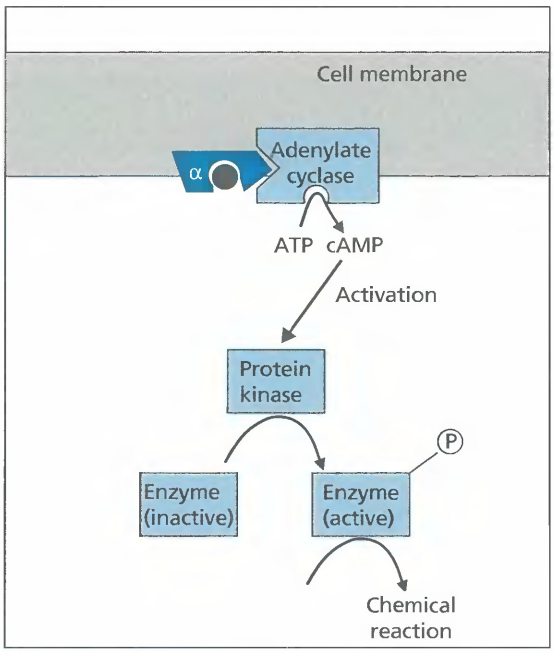


FIGURE 5.5 Activation of protein kinase A by cyclic AMP, phosphate. Ⓟ = phosphate

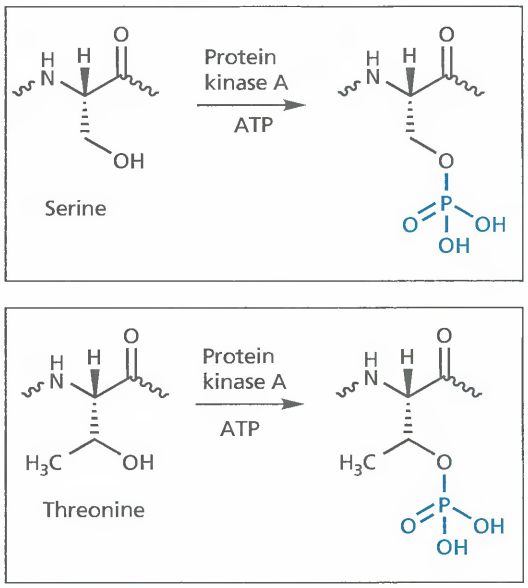


FIGURE 5.6 Phosphorylation of serine and threonine residues in protein substrates.

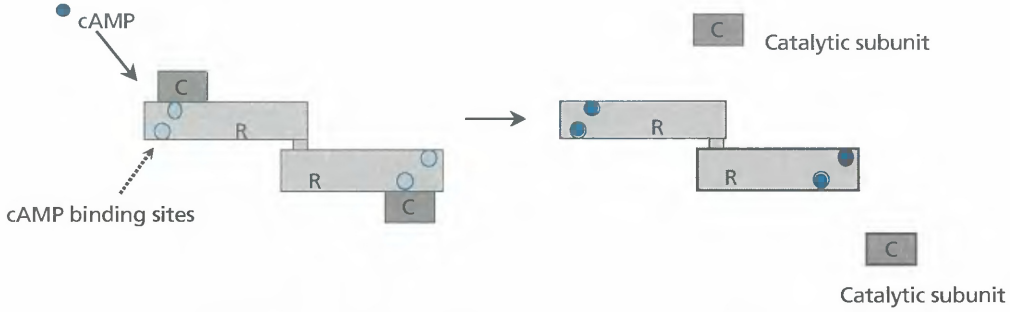


FIGURE 5.7 Activation of protein kinase A by cyclic AMP.

already discussed. The catalytic subunit of PKA now phosphorylates three enzymes within the cell, as follows:

- An enzyme called **phosphorylase kinase** is phosphorylated and is activated as a result. 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 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

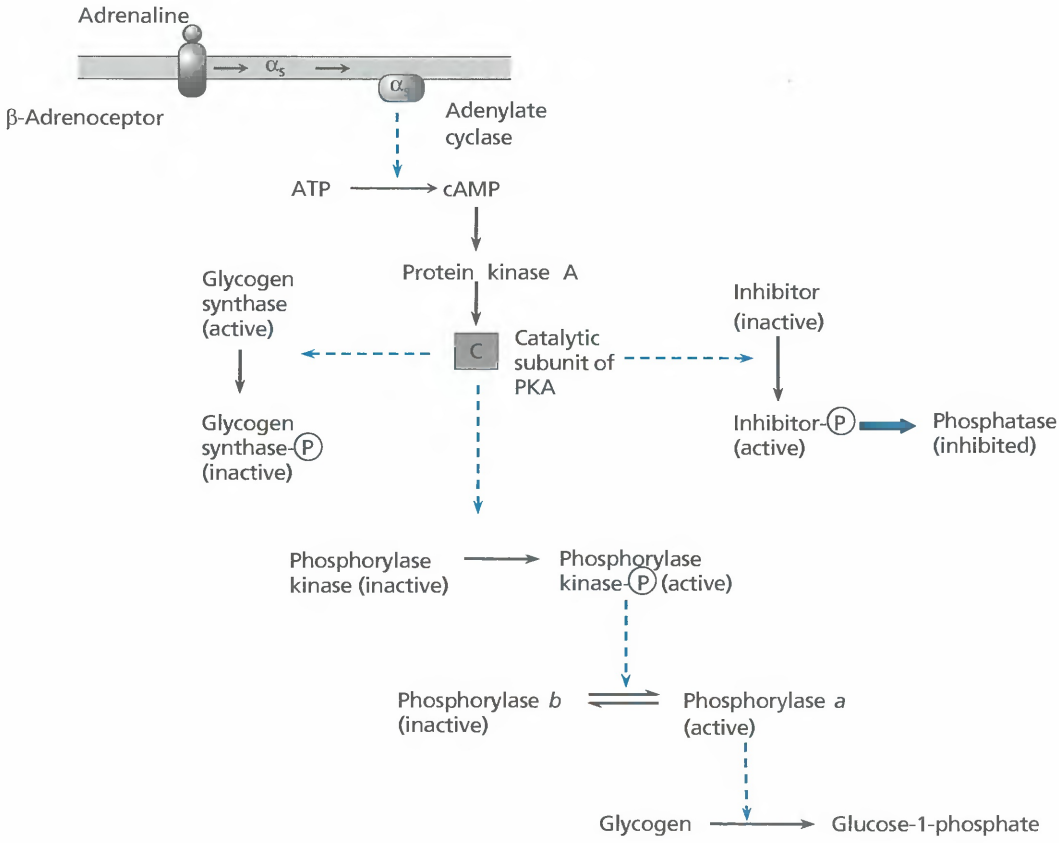


FIGURE 5.8 Regulation of glycogen synthesis and metabolism in a liver cell.

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, and this 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.

Since 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 its β-adrenoceptor to activate adenylate cyclase since the β-adrenoceptor binds the G_s-protein. On the other hand, noradrenaline interacts with its α_2-adrenoceptor to inhibit adenylate cyclase because this receptor binds the G_i-protein. This example illustrates the point that it is the receptor, rather than the neurotransmitter or hormone, that determines which G-protein is activated.

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 the 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.

- First, 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.
- Second, 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.
- Third, 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 in the liver, **adrenaline** activates G_s-linked β_2-adrenoceptors leading to lipolysis in fat cells, 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 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 in 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, since 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 to the α-subunit. Therefore, regulation by the dimers becomes more important when a greater number of receptors are activated.

By now it should be becoming 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 a whole myriad of 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 any positively charged residues 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. 5.9).

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

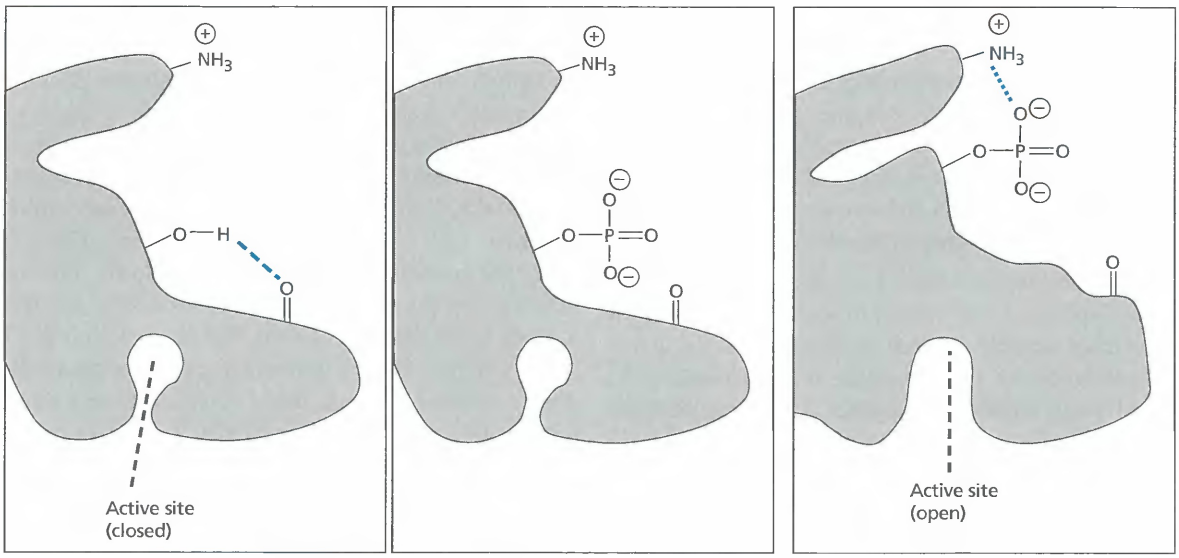


FIGURE 5.9 Conformational changes in a protein, induced by phosphorylation.

ligand binding. Since 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 that 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 **Gq-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

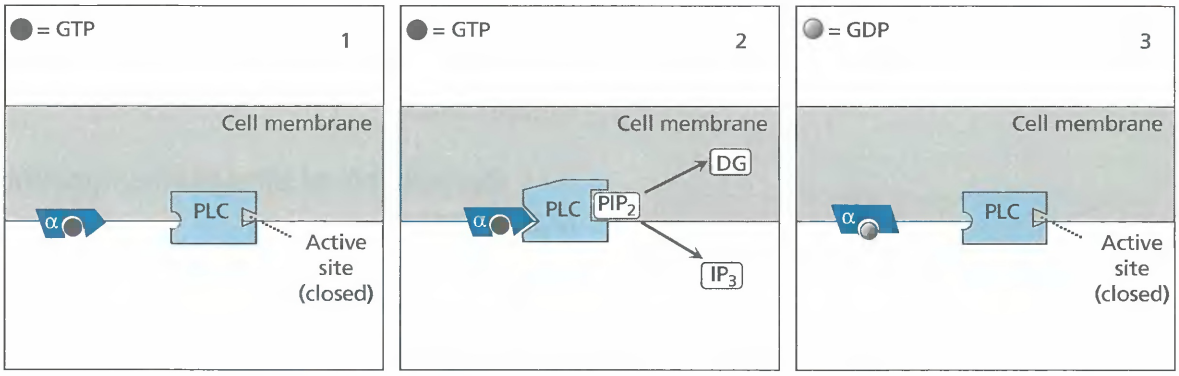


FIGURE 5.10 Activation of phospholipase C by an α_q-subunit.

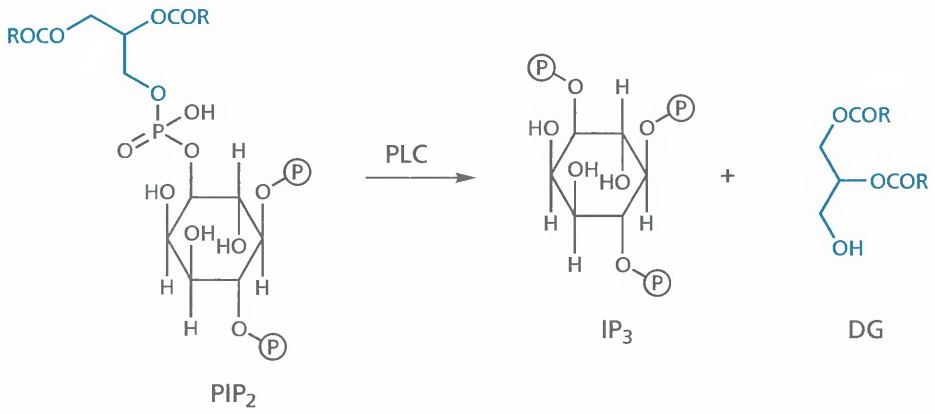


FIGURE 5.11 Hydrolysis of PIP_2 to inositol triphosphate (IP_3) and diacylglycerol (DG). Ⓟ = phosphate

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** (PIP2) (an integral part of the cell membrane structure) to generate the two secondary messengers **diacylglycerol** (DG) and **inositol triphosphate** (IP_3) (Figs. 5.10–5.11).

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. 5.12). 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.

5.3.3 Action of the secondary messenger—inositol triphosphate

Inositol triphosphate is a hydrophilic molecule and moves into the cytoplasm (Fig. 5.13). 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.

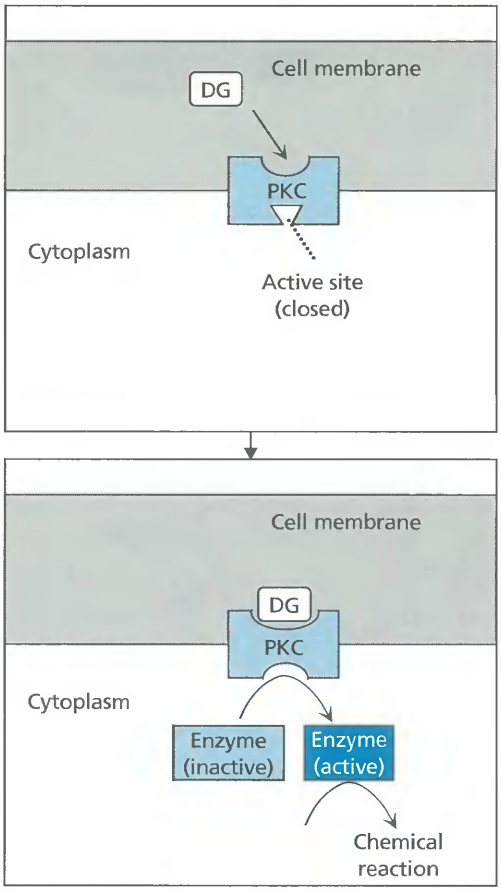


FIGURE 5.12 Activation of protein kinase C (PKC) by diacylglycerol (DG).

5.3.4 Resynthesis 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 steps before resynthesis 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 resynthesis of PIP_2 (Fig. 5.14). 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 that 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.

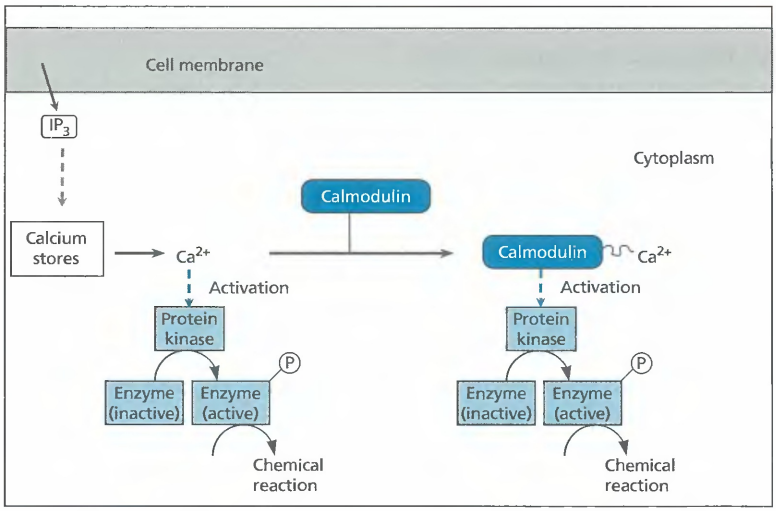


FIGURE 5.13 Signal transduction initiated by inositol triphosphate (IP_3).

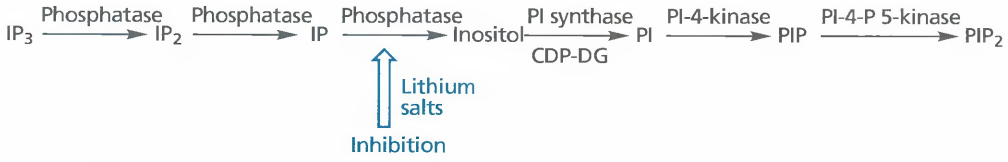


FIGURE 5.14 Resynthesis of PIP_2 from inositol triphosphate (IP_3) (CDP-DG = cytidine diphosphate-diacylglycerol).

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. 5.15).

Not all of the phosphotyrosine binding regions can be occupied by signalling proteins at the one time, and so the type 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. 5.16). 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 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. 5.17). 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

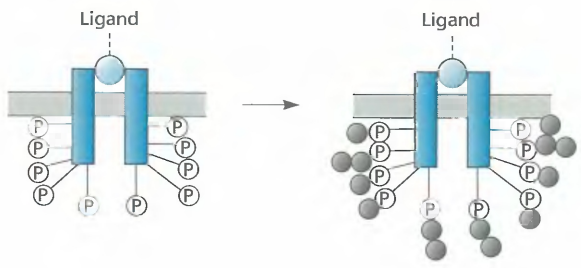


FIGURE 5.15 Binding of signalling proteins (indicated by dark circles) to activated kinase-linked receptors.

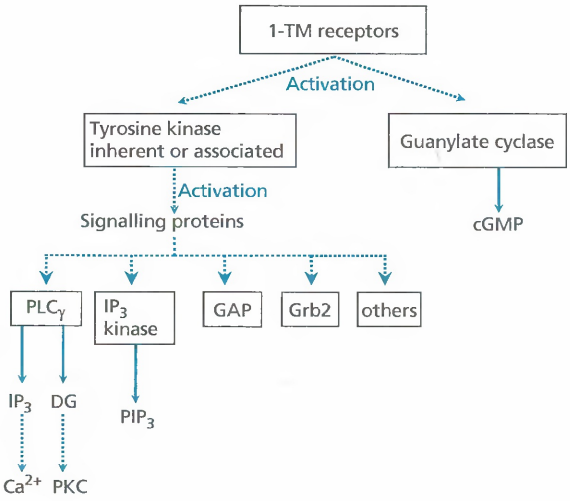


FIGURE 5.16 Signalling pathways from one transmembrane region (1-TM) receptors.

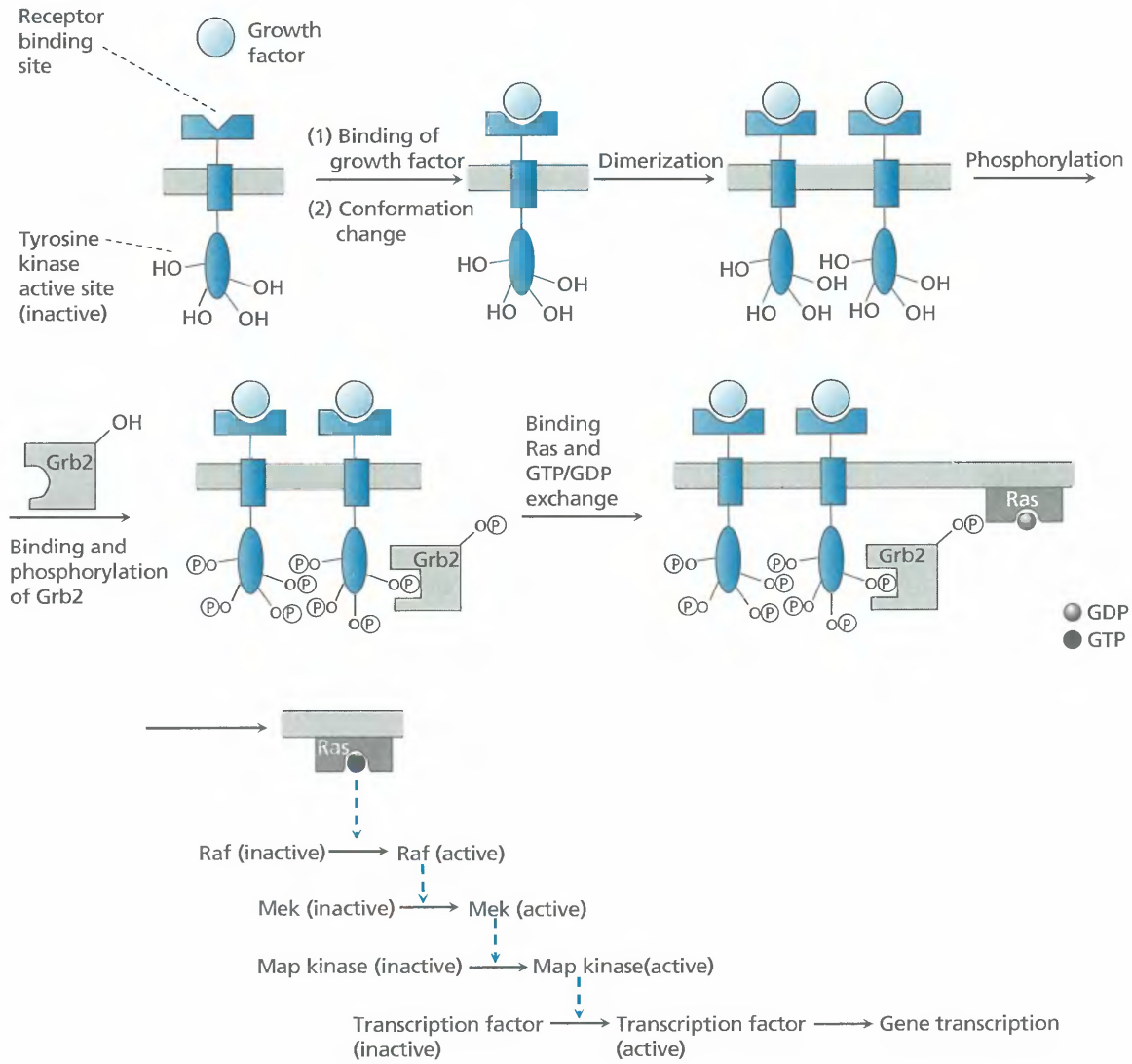


FIGURE 5.17 From growth factor to gene transcription.

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 in Fig. 5.17 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 the 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 can occur which result in the inability of these proteins to autocatalyse the hydrolysis of bound GTP. As a result they remain permanently activated, leading in turn to permanent cell growth and division—see also section 21.6.1.

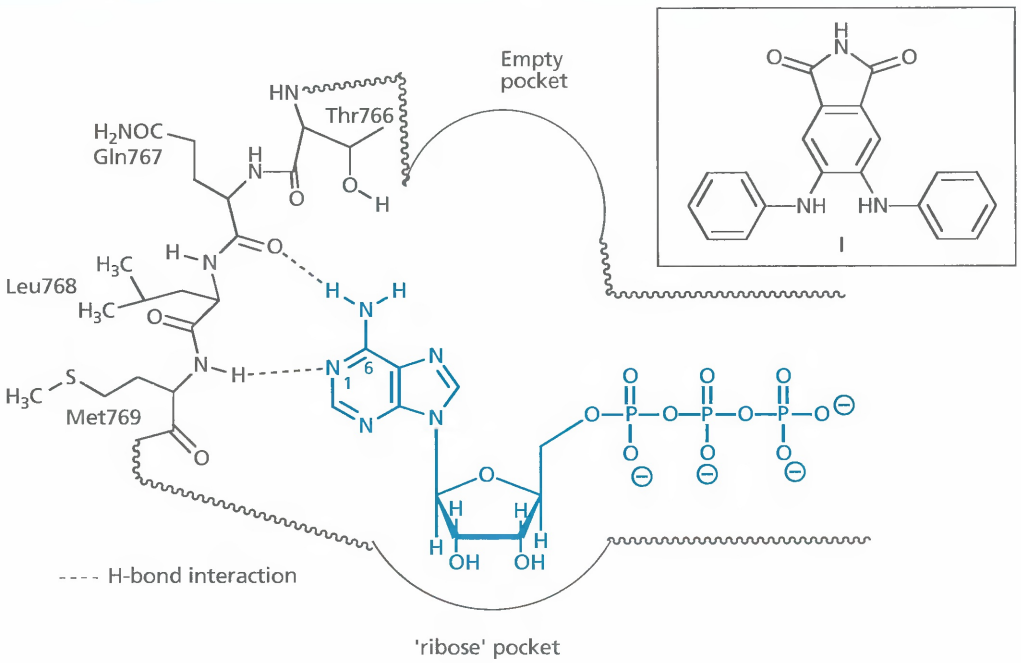
5.4.3 Activation of guanylate cyclase by kinase receptors

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 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 is the purpose of this chain and what would be the effect if the enzyme was inhibited?
4. Consider the signal transduction pathways shown in Fig. 5.17 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. 5.8)?

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.

FURTHER READING

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Titles for general further reading are listed on p. 725.