Cell signalling

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Well, it worked for the metabolism section of the course, so why not cell signalling? Here are some notes on ligand-gated ion channels, G-protein coupled receptors and enzyme-linked receptors. The recommended text for this part of the course is Alberts et al., but I have to say that I prefer the treatment given in Lehninger. I'm not going to discuss steroid receptors here as they are located within the cell rather than on the plasma membrane, and are mainly concerned with the regulation of gene expression. They are discussed in the cancer notes in the section on regulation of gene expression in eukaryotes.

1 Ligand-gated ion channels

These do exactly what it says on the tin. They bind a ligand and allow an ion to pass across the membrane. This can be thought of as a form of facilitated diffusion, as transport happens down a concentration gradient. The advantage of ligand-gated ion channels over the receptors we will discuss in later sections is speed. They have a low affinity for their ligands. There are five examples we know, and they seem to come up time and again in past paper MCQs.

Name	**Ligand**	**Ion**	**Notes**
Nicotinic cholinergic receptor	Acetyl choline	Na^+, K^+, Ca^{2+}, etc.	Stimulated by nicotine in small amounts, desensitised by nicotine in large amounts (nicotine toxicity). Permeable to all small cations.
NMDA	Glutamate	Ca^{2+}	
AMPA	Glutamate	Na^+	RNA editing can change permeability to Ca^{2+} instead of Na^+
Kainate	Glutamate	Na^+	
GABA-A	γ-amino butyric acid	Cl^-	These are sometimes called 'negative' receptors since increasing permeability to Cl^- will hyper-polarise or repolarise a cell.

2 G-protein linked receptors

This is a large class of receptors (over 1000 in the human genome). They consist of four main parts, the receptor itself coupled to three intracellular subunits called α, β and γ, though the β and γ subunits always remain together so can be thought of as a single part. The receptor portion consists of a ligand binding site, seven trans-membrane spanning sections and an α subunit binding domain. The α subunit in turn consists of a GDP/GTP binding domain, which is occupied by GDP in the inactive form, a β/γ subunit binding domain, a receptor protein binding domain and a further domain whose purpose varies with the action of the subunit. The α subunit is bound to the membrane by a covalently attached palmitoyl group (via either a Cys or a Ser residue). Finally, the β/γ subunit has an α subunit binding domain and sometimes further domains whose purpose varies with the activity of the subunit[1].

Binding of the signalling molecule on the outside of the membrane causes a conformational change in the receptor which allows binding of an inactive $\alpha/\beta/\gamma$ complex. This causes a conformational change in the α subunit, which causes the catalysis of the conversion of the bound GDP to GTP. At this point, the three parts (receptor, α subunit and β/γ subunit) dissociate and become active. The key advantage of GPLRs is that they work at low agonist concentrations, as a large number of inactive $\alpha/\beta/\gamma$ complexes can bind to active receptors in turn before the ligand dissociates.

Examples of these kinds of receptors are α and β adrenergic receptors, serotonin receptors, muscarinic acetyl choline receptors and GABA-B receptors, whose ligands should be obvious from the names. We will discuss two main classes of G-protein linked receptors: those whose second messenger is cyclic AMP (cAMP) and those whose second messenger is IP_3 and DAG.

2.1 cAMP as a second messenger

The best characterised of the first class are the β-adrenergic receptors[2], which we will discuss in some detail. The α subunit of β-adrenergic receptors (also called G_s receptors) is called α_s because it *stimulates* adenylyl cyclase to catalyse the conversion of ATP to cyclic AMP (cAMP)[3]. Cyclic AMP allosterically activates cAMP-dependent protein kinase (also known as protein kinase A, or simply PKA). Thus the increase in [cAMP] driven by the activation of adenylyl cyclase activates PKA.

PKA has a number of effectors. In the glycogenolysis pathway PKA catalyses the phosphorylation of phosphorylase *b* kinase, which then becomes active and phosphorylates glycogen phosphorylase *b*, which becomes active[4]. In cardiomyocytes, PKA regulates Ca^{2+} channels by phosphorylation, though I couldn't find out the exact pathway. Finally, PKA regulates gene expression via cREB. The DNA sequence TGACGTCA (which is an auto-

[1]The β/γ subunit may also bind to the receptor subunit, though having now looked at four different textbooks I'm still not clear on this issue!

[2]Both β_1 and β_2 adrenergic receptors work through the same mechanism, so it is safe to merely refer to 'β-adrenergic receptors' without causing confusion.

[3]The version of adenylyl cyclase which is activated in this step is an integral membrane protein.

[4]The active form of glycogen phosphorylase *b* is sometimes called glycogen phosphorylase *a*.

complementary sequence) is called a cAMP response element (cRE). PKA phosphorylates the transcription factor cRE binding protein (cREB), which causes it to bind cREs and activate transcription of these genes.

In addition to the stimulatory G_s protein associated with the β-adrenergic receptor, there is also the G_i protein, which *inhibits* the action of adenylyl cyclase. G_s is permanently activated by the cholera toxin, while G_i is permanently inactivated by the pertussis toxin.

2.2 IP_3/DAG as a second messenger

We now come on to a third GPLR called G_q. In this case the active alpha subunit binds to the integral membrane protein phospholipase C. Phospholipase C in turn catalyses the cleavage of the membrane bound molecule phosphatidylinositol 4,5 bisphosphate (PIP_2) into diacylglycerol (DAG), which remains in the membrane, and inositol 1,4,5 trisphosphate (IP_3), which dissociates into the cytoplasm. IP_3 then binds to a ligand-gated ion channel on the endoplasmic reticulum, releasing sequestered Ca^{2+}. Back at the surface of the plasma membrane, DAG and Ca^{2+} together activate protein kinase C. PKC is a serine and threonine protein kinase which has lots of different isozymes in different tissues with tissue-specific roles.

You may have noticed that the ligand-gated ion channels on the ER sounded a lot like the ryanodine receptors we met in physiology in the muscle lectures. The two receptors are indeed closely related, the key difference being that the ligand of ryanodine receptors is Ca^{2+} itself (calcium-induced calcium release).

There are many other effectors of Ca^{2+} in addition to protein kinase C, probably the most important of which is calmodulin, which binds to other proteins, for example calmodulin-regulated protein kinases (CaM kinases). The one we need to know about is CaM kinase IV, which is a transcription factor. Another class of Ca^{2+} effectors is the synaptotagmins, which are Ca^{2+} sensors for vesicle modulated sectretion such as acetyl choline at the NMJ or insulin from pancreatic β cells.

IP_3 is inactivated by a phosphatase, which dephosphorylates the phosphate at the 5 position, making inositol 1,4 bisphosphate (IP_2).

In addition to the members of the phospholipase C family which are activated by G-protein linked receptors, there are also members which contain SH2 domains and are thus activated by receptor tyrosine kinases. Thus there are two completely different methods for initiating the IP_3/DAG pathway. Receptor tyrosine kinases are discussed below in the section on enzyme-linked receptors.

2.3 Whatever happened to those β/γ subunits?

While it is true that most of the current work to date has focussed on the actions of the α subunit of heterotrimeric G-proteins, it turns out that the β/γ subunit is an important regulator in some other G-proteins. One example of this is the presynaptic G-protein linked receptor that opiates bind to, which regulates the activity of certain ion channels. Another is the muscarinic acetyl choline receptor in the heart, where the β/γ subunit activates K^+ channels, thus hyperpolarising the cell. This will clearly have the effect of

reducing the rate of depolarisation of the pacemaker cells in the SA node, thus slowing the heart rate.

3 cGMP pathways

Adenosine is not the only purine to act as a second messenger in its cyclic mononucleoside form. Cyclic GMP is also used as a second messenger in three different scenarios.

1. Guanylyl cyclase acts as a membrane-bound receptor for atrial natriuritic peptide, among other things. It has a single membrane spanning domain with an extracellular ligand-binding site at the N-terminal end and an intracellular catalytic domain which converts GTP to cGMP. Another example is the guanylin receptor, which regulates Cl^- secretion in the intestine. It also binds a bacterial endotoxin produced by E. coli and some other gram-negative bacteria, causing severe diarrhoea.

2. Soluble guanylyl cyclase is a heterotetrameric protein composed of two regulatory subunits and two catalytic subunits. Oxidation of the regulatory subunits by nitric oxide (NO) causes activation of the enzyme in a vasodilatory pathway.

3. In rods and cones on the retina, light causes the activity of cGMP phosphodiesterase to increase (via the α subunit of Rhodopsin, a heterotrimeric G-protein linked receptor which is stimulated by light instead of a ligand), thus decreasing [cGMP]. This causes cation channels which had been kept open by cGMP to close, resulting in a decrease in cytosolic $[Ca^{2+}]$ (since Ca^{2+} continues to exit the cell through the Na^+/Ca^{2+} exchanger). The decrease in $[Ca^{2+}]$ results in activation of guanylyl cyclase, thus completing the loop. I'm not sure how this signals to the brain as I'd expect it to hyperpolarise the membrane, but I guess an action potential must be triggered somehow.

4 Enzyme-linked receptors

Although this section is called "enzyme-linked receptors", we will actually only discuss protein tyrosine kinase receptors. The first guanylyl cyclase receptor mentioned in the previous section is an example of another kind of enzyme-linked receptor.

Receptor tyrosine kinases have single membrane spanning domains with extracellular ligand binding domains and intracellular tyrosine kinase domains. In their active ligand-bound form they form dimers which phosphorylate each other at certain (not all) tyrosine residues, a process called autophosphorylation. The phosphorylated tyrosine residues are recognised by *src* homology 2 (SH2) domains[5] on other proteins. This is a very specific process, so not any SH2 domain can bind any phosphorylated tyrosine residue. The surrounding structure must also be compatible.

We will only discuss the phosphatidylinositol 3,4,5 trisphosphate pathway, though this is but one example of many pathways activated by protein tyrosine kinase receptors. The

[5]The gene for *src* was one of the first identified oncogenes.

phosphorylated tyrosine residues on PDGF or EGF receptors are recognised by the SH2 domain on phosphatidylinositol 3-kinase (PI3K). PI3K is a dimeric protein with a regulatory subunit containing an SH2 domain and a catalytic subunit that catalyses the addition of a phosphate to PIP_2 at the 3 position, making phosphatidylinositol 3,4,5 trisphosphate (PIP_3). PIP_3 remains bound to the membrane and attracts proteins containing PIP_3 binding domains. As far as we know, migration of PIP_3 binding proteins to the membrane is by simple diffusion rather than being mediated by actin or other motor proteins.

An immunologically important one is Bruton's tyrosine kinase (Btk), which is involved in the proliferation of leukocytes. People with mutated Btk genes can have severe immunodeficiencies. A fungal metabolite called wortmannin is an anti-inflammatory agent which works by inhibiting PI3K in neutrophils. This is a key target for drug design.

Last but certainly not least, there are two PIP_3 effectors (also called PIP_3 receptors) in the insulin pathway (though here we discuss only one). The insulin pathway differs from classic protein tyrosine kinase receptors in that it is always dimerised, but only activated when its agonist (insulin) is bound. Also, instead of recruiting PI3K directly, the insulin receptor first of all recruits insulin receptor substrates containing SH2 domains. IRS-1 in particular is phosphorylated on several tyrosine residues by the intracellular catalytic part of the insulin receptor and these phosphorylated tyrosine residues in turn recruit other proteins with SH2 domains, one of which is PI3K. PI3K then catalyses the conversion of PIP_2 to PIP_3, which activates phospholipid dependent kinase 1 (PDK-1). PDK-1 phosphorylates and thus activates protein kinase B (PKB), which phosphorylates glycogen synthase kinase 3 (GSK3). GSK3 is responsible for phosphorylating glycogen synthase, which as we saw in the metabolism lectures will inactivate it, but GSK3 itself is inactivated by phosphorylation by PKB, thus the net result is *increased* glycogen synthesis.

PKB also phosphorylates a whole host of other important proteins which we meet in the cell cycle section of the course (see the Cancer notes for details).

- S6kinase, a translational activator. This activates it, stimulating translation.
- PKC. This activates it, stimulating the things discussed in Section 2.2.
- p21. This inhibits it, prompting cells to enter the S phase of the cell cycle.
- bad, a pro-apoptotic protein. This inhibits it, thus inhibiting apoptosis.

This whole pathway is inhibited by the protein PTEN[6], which removes a phosphate from PIP_3, converting it back to PIP_2.

There is also a large class of receptors which do not contain tyrosine kinase activity within them but instead recruit a tyrosine kinase from the cytoplasm when they dimerise following binding of the agonist. With this one exception, their method of action is as described above. Examples include interleukin and interferon receptors.

[6]PTEN is short for phosphatase and tensin homolog deleted on chromosome ten. You can see why they wanted to shorten the name!