Cell Biology

Unit 5 · Cell Signaling and Cell Communication

Cells talk to each other with molecules, and every reply a cell makes starts at a receptor. This unit follows a signal from the cell that sends it, through the receptor and the relay inside the target cell, to the response, and then looks at the junctions and the matrix that hold cells together. After it you can classify any signal, trace a GPCR or kinase pathway step by step, explain how drugs switch receptors on or off, and describe what lies between cells in animals, plants and bacteria.

Taught at 7 of 10 | Usually Sem 3 | 8 chapters

About this unit

This unit is about how cells send and receive messages, and about the structures that link cells to each other and to their surroundings. Chapter 1 sorts signals by how far they travel. Chapter 2 sorts receptors by where they sit. Chapters 3 to 5 follow the two big families of surface receptors inside the cell: G-protein coupled receptors with their second messengers, and the kinase pathways that start at enzyme-coupled receptors. Chapter 6 shows how drugs use the same receptors as agonists or antagonists. Chapters 7 and 8 turn to the physical contacts: cell junctions, adhesion molecules, the extracellular matrix and the cell wall.

Chapters

1. Cell communication: autocrine, paracrine and endocrine signals
2. Cell-surface and intracellular receptors
3. G-protein coupled receptors
4. Second messengers and signal amplification
5. Kinase pathways: PI3K/Akt, JAK/STAT and AMPK ADVANCED
6. Agonists and antagonists ADVANCED
7. Cell junctions and cell adhesion
8. Extracellular matrix and cell wall

How to use this book

- Read the lead paragraph and the figure of each chapter first; together they carry the one idea.
- Cover the solution of each worked example and work it out yourself on paper before reading on.
- Answer every "Check yourself" question in your head or on paper before you open it.

5.1 Cell communication: autocrine, paracrine and endocrine signals

A cell in your body is never alone. It receives chemical messages from other cells telling it to grow, divide, secrete, move or die. Biologists sort these messages by one simple question: how far does the signal travel from the cell that makes it to the cell that answers? A signal that acts back on its own maker is autocrine; one that acts on close neighbours is paracrine; one that travels through the blood to distant organs is endocrine. Add contact-dependent signalling, where the signal never leaves the membrane, and synaptic signalling by nerve cells, and you have the full map that every later chapter builds on.

The parts of any signalling event

Every signal has the same four parts. The **signalling cell** makes and releases a **signal molecule** (also called a ligand, from the Latin for "to bind"). The **target cell** carries a **receptor**, a protein that binds that ligand specifically. Binding changes the receptor's shape, and the target cell turns that change into a **response**: an enzyme switched on, a gene turned on, a channel opened.

The same ligand can give different responses in different cells. Acetylcholine slows the heart muscle cell but makes skeletal muscle contract, because the two cells carry different receptors for it. So the response depends on the receptor and the machinery behind it, not only on the signal.

Five modes, sorted by distance

1. **Endocrine.** Endocrine cells release **hormones** into the blood, which carries them throughout the body. Insulin from the pancreas, adrenaline from the adrenal medulla and thyroxine from the thyroid are examples. Because blood dilutes them, hormones act at very low concentrations, typically below 10^{-8} M, so their receptors must bind tightly. Endocrine signals are slow to arrive (seconds to minutes) but reach every tissue.
2. **Paracrine.** The signal diffuses through the fluid between cells and acts on cells nearby, usually within a short distance. It must not spread far, so the target cells take it up quickly, enzymes destroy it, or the extracellular matrix traps it. Growth factors during wound healing, histamine from mast cells in an allergy, and nitric oxide (NO) from blood-vessel lining are paracrine. NO lasts only about 5–10 seconds outside cells, which keeps it local.
3. **Autocrine.** The cell responds to the signal it released itself. A T cell activated by an antigen secretes the growth factor interleukin-2 (IL-2) and also carries IL-2 receptors, so it drives its own division. Autocrine loops also appear in groups of identical cells that must decide

together, and in cancer cells that make their own growth factors and stop needing outside permission to divide.

4. **Synaptic.** A nerve cell sends an electrical impulse along its axon and releases a **neurotransmitter** at a synapse, a gap of tens of nanometres to one target cell. This is a very local, very fast form of paracrine signalling. The neurotransmitter reaches a high concentration at the synapse (acetylcholine reaches about 5×10^{-4} M) and so its receptors can bind it with low affinity and let go quickly.

5. **Contact-dependent (juxtacrine).** The signal stays attached to the signalling cell's surface and acts only on a cell that touches it. The membrane protein Delta on one cell binding the receptor Notch on its neighbour is the classic case: it tells the neighbour not to become the same cell type, which spaces out nerve cells in a developing embryo.

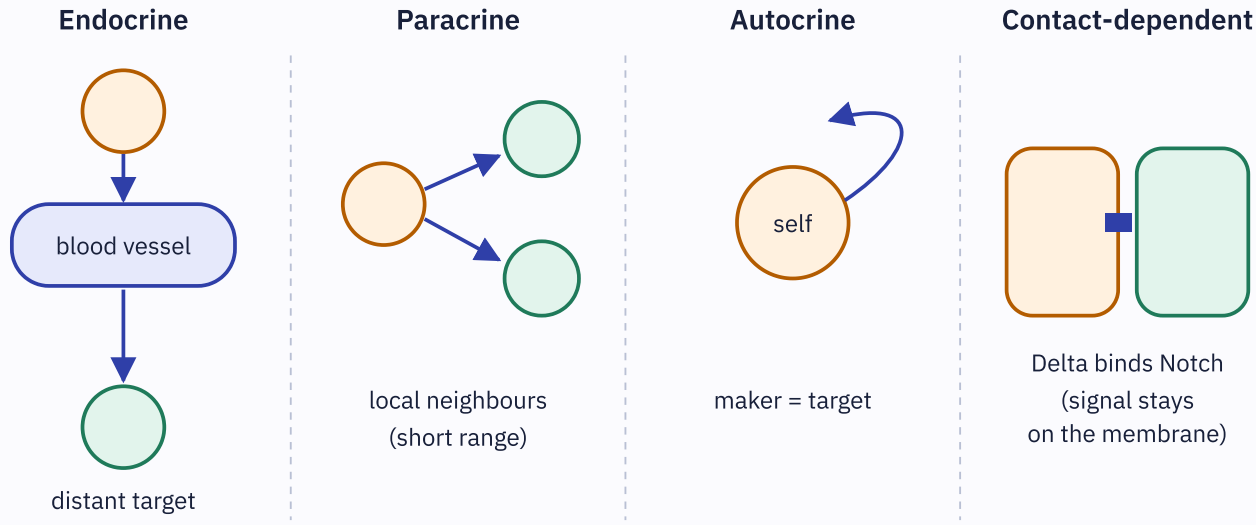


Figure 5.1.1 Four ways a signal reaches its target. Orange cells send, green cells receive. Notice that only the endocrine signal enters the blood, and only the contact-dependent signal never leaves the sender's membrane.

Why distance decides the design

Distance controls three things at once. First, **concentration**: a hormone diluted in about five litres of blood needs a high-affinity receptor, while a neurotransmitter released into a tiny synaptic gap can use a low-affinity one. Second, **speed**: synaptic signals act in milliseconds, endocrine ones in seconds to hours. Third, **reach**: one endocrine gland can coordinate the whole body, while a paracrine signal shapes one patch of tissue. The same molecule can work in more than one mode. Adrenaline is a hormone when the adrenal gland secretes it and a neurotransmitter when certain neurons release it.

A signal must also be switched off. Hormones are removed by the liver and kidneys; neurotransmitters are broken down (acetylcholinesterase destroys acetylcholine) or pumped

back into the nerve ending; paracrine factors are degraded or trapped locally. A signal that cannot be removed keeps the target switched on, which is often how a disease or a toxin works.

WORKED EXAMPLE

Question (6 marks). Classify each signal as endocrine, paracrine, autocrine, synaptic or contact-dependent, and give one reason for each. (a) Insulin from pancreatic β cells lowers blood glucose by acting on muscle and fat. (b) Platelets at a cut release platelet-derived growth factor, which makes nearby fibroblasts divide. (c) An activated T cell releases IL-2 and divides in response. (d) A motor neuron releases acetylcholine onto one muscle fibre. (e) In a fly embryo, a cell with Delta on its surface stops the cell it touches from becoming a nerve cell. (f) Endothelial cells release NO, which relaxes the smooth muscle around the same vessel.

1. (a) **Endocrine**: insulin enters the blood and acts on distant tissues.
2. (b) **Paracrine**: the growth factor diffuses only to nearby fibroblasts at the wound.
3. (c) **Autocrine**: the same cell makes the IL-2 and carries the IL-2 receptor.
4. (d) **Synaptic**: the neurotransmitter is released at a synapse onto one specific target.
5. (e) **Contact-dependent**: Delta stays bound to the sender's membrane, so only a touching cell responds.
6. (f) **Paracrine**: NO is a gas that diffuses a short distance and lasts only seconds, so it acts on the neighbouring muscle layer.

Award one mark per correct label with a reason. The reason, not the label, is what earns the mark in most university schemes.

EXAM TRAPS

- "Paracrine" and "autocrine" are not decided by the molecule but by who responds. IL-2 acting on the same T cell is autocrine; acting on a neighbouring T cell it is paracrine.
- Synaptic signalling is chemical, not purely electrical. The electrical impulse travels along the axon; the synapse itself uses a neurotransmitter.
- Do not write that hormones are always proteins. Steroids (cortisol, estrogen) and amines (adrenaline, thyroxine) are hormones too.
- The examiner's favourite question: "With neat diagrams, explain the different modes of cell signalling." Draw all modes, label sender, signal, receptor and target, and add one example each.

CHECK YOURSELF

– REMEMBER **Name the four parts of any signalling event.** 2 marks

Signalling cell, signal molecule (ligand), receptor on the target cell, and the response of the target cell.

– REMEMBER **Give one example each of an endocrine and a paracrine signal.** 2 marks

Endocrine: insulin, adrenaline or thyroxine. Paracrine: nitric oxide, histamine, or a growth factor at a wound.

– UNDERSTAND **Why do hormone receptors need higher affinity than neurotransmitter receptors?** 3 marks

Hormones are diluted in the blood to very low concentrations (typically below 10^{-8} M), so only a tight-binding receptor is occupied. At a synapse the neurotransmitter is concentrated (about 5×10^{-4} M), so a low-affinity receptor fills quickly and also releases the ligand fast, allowing rapid on-off signalling.

– UNDERSTAND **How is a paracrine signal kept local?** 3 marks

It is taken up quickly by target cells, destroyed by extracellular enzymes, trapped by the extracellular matrix, or is chemically short-lived (NO lasts about 5–10 seconds).

– APPLY **Acetylcholine slows the heart but contracts skeletal muscle. What does this tell you about signalling?** 3 marks

The response is set by the target cell, not by the signal. Heart muscle and skeletal muscle carry different acetylcholine receptors (a muscarinic GPCR in the heart, a nicotinic ion channel in skeletal muscle) linked to different machinery.

– APPLY **A tumour cell secretes a growth factor and carries its receptor. Which mode is this, and why is it dangerous?** 3 marks

Autocrine. The cell stimulates its own division and no longer depends on signals from other cells, so normal tissue control is lost.

– ANALYZE **Adrenaline acts both as a hormone and as a neurotransmitter. How can one molecule fit two modes?** 4 marks

The mode is defined by the route and range, not the chemistry. Released into the blood by the adrenal medulla it reaches all tissues (endocrine). Released at a nerve ending onto one target it acts locally and quickly (synaptic). The receptors are the same family; the delivery differs.

BEYOND THE SYLLABUS

Bacteria also signal: in quorum sensing, each cell releases small autoinducer molecules, and when enough cells are present the concentration crosses a threshold and the whole population switches on genes together, for example for light production or biofilm formation. Cells also send exosomes, small membrane vesicles carrying proteins and RNA, which some researchers treat as a sixth mode of communication. Synthetic biologists now build artificial receptors (such as synthetic Notch, or synNotch) to programme engineered cells. None of this is needed for the exam; search "quorum sensing" and "extracellular vesicles signalling" to read further.

5.2 Cell-surface and intracellular receptors

A receptor is the protein that recognises a signal and starts the response. Where the receptor sits depends on the signal: a large or water-loving molecule such as insulin or adrenaline cannot cross the lipid bilayer, so its receptor must sit in the plasma membrane with a binding site facing outside. A small fat-soluble molecule such as cortisol or estrogen slips through the membrane, so its receptor waits inside the cell, in the cytosol or the nucleus. This split into cell-surface and intracellular receptors is the first classification every examiner asks for.

You need: the lipid bilayer and membrane proteins from Unit 2, *Plasma membrane structure: fluid mosaic model* and *Membrane proteins and lipids*.

Cell-surface receptors: three classes

All cell-surface receptors are transmembrane proteins. The ligand binds outside, and the receptor passes the message across the membrane by changing shape. They fall into three classes by what the inside part does.

1. **Ion-channel-coupled receptors** (ligand-gated channels). Binding opens a channel, ions flow, and the membrane voltage changes within milliseconds. The nicotinic acetylcholine receptor at the nerve–muscle junction lets Na^+ in and makes the muscle contract. Unit 2, *Ion channels: ligand-gated and voltage-gated*, covers how the channels work.
2. **G-protein coupled receptors** (GPCRs). The receptor activates a G protein inside the cell, which then switches an enzyme or channel on or off. This is the largest family; Chapter 5.3 is devoted to it.

3. **Enzyme-coupled receptors**. The inside part is an enzyme, or binds one directly. The main group is the **receptor tyrosine kinases** (RTKs), receptors for insulin, epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). Cytokine receptors, which borrow a separate kinase called JAK, belong here too (Chapter 5.5).

How a receptor tyrosine kinase works

An RTK has one membrane-spanning helix. The steps are:

1. The ligand binds and brings two receptor chains together (dimerisation). The insulin receptor is already a linked dimer, and ligand binding changes its shape instead.
2. Each kinase domain phosphorylates tyrosine residues on its partner: **autophosphorylation** (more exactly, cross-phosphorylation).
3. The phosphotyrosines become docking sites for signalling proteins that carry an **SH2 domain**, a module that binds a phosphorylated tyrosine.
4. One docked adaptor, Grb2, brings Sos, which activates the small G protein **Ras**. Ras starts the MAP kinase chain Raf → MEK → ERK, and ERK enters the nucleus to switch on genes for cell division. Unit 4, *Cancer and oncogenes such as Ras and Raf*, shows what happens when Ras is stuck on.

Intracellular receptors

Steroid hormones (cortisol, aldosterone, estrogen, progesterone, testosterone), thyroid hormone, retinoic acid and vitamin D are small and hydrophobic. They cross the membrane and bind receptors of the **nuclear receptor superfamily**. These receptors are transcription factors: each has a ligand-binding domain, a DNA-binding domain and a region that activates transcription.

Some wait in the cytosol. The glucocorticoid receptor, which binds cortisol, sits in the cytosol held inactive by chaperone proteins such as Hsp90. Cortisol binding releases the chaperones, the receptor moves into the nucleus, binds a short DNA sequence called a hormone response element, and turns genes on. Others, such as the thyroid hormone receptor, are already in the nucleus bound to DNA. Because they act by changing gene expression, their responses take tens of minutes to hours, unlike the seconds of most surface receptors.

Nitric oxide is a special case. It crosses membranes as a gas and binds an intracellular enzyme, soluble guanylyl cyclase, which makes the second messenger cGMP (Chapter 5.4). So "intracellular receptor" does not always mean a nuclear receptor.

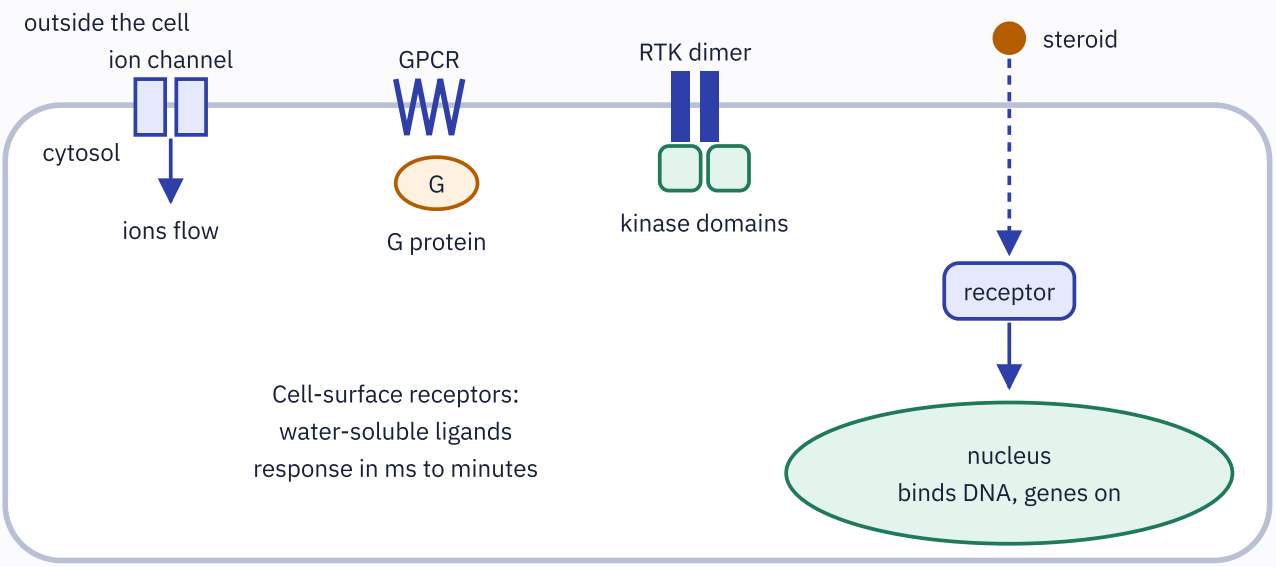


Figure 5.2.1 The three classes of cell-surface receptor sit in the membrane; a steroid crosses the membrane and binds an intracellular receptor, which carries it into the nucleus. Notice that only the intracellular route acts directly on DNA.

How tightly a receptor binds: occupancy

Binding is reversible: L + R ⇌ LR, where L is the ligand, R the free receptor and LR the bound complex. The **dissociation constant** K_d = [L][R]/[LR] has units of concentration. A small K_d means tight binding. The fraction of receptors occupied, θ (theta), follows from it:

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θ = [L] / ([L] + Kd)
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Here [L] is the free ligand concentration. When [L] = K_d, θ = 0.5, so K_d is the ligand concentration that fills half the receptors.

WORKED EXAMPLE

Question (5 marks). A hormone receptor has K_d = 2 nM. Find the fraction of receptors occupied when the hormone is at (a) 0.2 nM, (b) 2 nM, (c) 18 nM. What concentration gives 90% occupancy?

1. (a) $\theta = 0.2 / (0.2 + 2) = 0.2 / 2.2 = 0.091$, about 9%.
2. (b) $\theta = 2 / (2 + 2) = 0.50$, exactly half.
3. (c) $\theta = 18 / (18 + 2) = 18 / 20 = 0.90$, 90%.
4. For $\theta = 0.9$: $0.9([L] + 2) = [L]$, so $0.1[L] = 1.8$ and $[L] = 18$ nM. Going from 10% to 90% occupancy needs about an 81-fold rise in ligand.

The units cancel in θ, so any concentration unit works as long as [L] and K_d use the same one.

EXAM TRAPS

- Do not write that steroid receptors sit in the membrane. Classical steroid receptors are intracellular; they bind DNA.
- Small K_d means high affinity, not low. Students often reverse this.
- The insulin receptor is an RTK, not a GPCR, even though it is a "hormone receptor".
- Examiners often ask "classify receptors with examples". Give both levels: surface (three classes) and intracellular (cytosolic and nuclear), with one ligand each.

CHECK YOURSELF

– REMEMBER **Name the three classes of cell-surface receptor.** 3 marks

Ion-channel-coupled receptors, G-protein coupled receptors, enzyme-coupled receptors (mainly receptor tyrosine kinases).

– REMEMBER **What is an SH2 domain?** 1 mark

A protein module that binds a phosphorylated tyrosine, letting signalling proteins dock on an activated RTK.

– UNDERSTAND **Why can cortisol have an intracellular receptor but adrenaline cannot?** 3 marks

Cortisol is a small hydrophobic steroid that diffuses across the lipid bilayer. Adrenaline is charged and water-soluble, so it cannot cross; it must bind a receptor on the surface (a GPCR).

– UNDERSTAND **Describe how the glucocorticoid receptor reaches DNA.** 4 marks

Held in the cytosol by Hsp90 and other chaperones; cortisol binds; chaperones leave; receptor enters the nucleus; binds the hormone response element; activates transcription of target genes.

– APPLY **A receptor has K_d = 5 nM. What fraction is bound at 15 nM ligand?** 2 marks

θ = 15 / (15 + 5) = 0.75, so 75% of receptors are occupied.

– APPLY **An RTK has its tyrosines mutated to phenylalanine. What happens to signalling?** 3 marks

Phenylalanine cannot be phosphorylated, so no phosphotyrosine docking sites form, SH2 proteins such as Grb2 cannot bind, and Ras and the downstream pathway are not activated, even though the ligand still binds.

– ANALYZE **Why are responses through nuclear receptors slower than through ion channels?** 4 marks

An ion channel changes membrane voltage the moment it opens (milliseconds). A nuclear receptor must move to the nucleus, start transcription, and the mRNA must be processed and translated before new protein changes the cell, which takes minutes to hours.

BEYOND THE SYLLABUS

Many cancer drugs block RTKs. Imatinib inhibits the BCR-ABL kinase in chronic myeloid leukaemia, and gefitinib and erlotinib inhibit the EGF receptor in some lung cancers. Antibodies such as trastuzumab bind the HER2 receptor on the cell surface. Steroids also have fast "non-genomic" effects through membrane receptors, an active research area. This is not for the exam; search "tyrosine kinase inhibitors" and "nuclear receptor superfamily" to read on.

5.3 G-protein coupled receptors

G-protein coupled receptors (GPCRs) are the largest family of cell-surface receptors in animals. They detect adrenaline, histamine, serotonin, many neurotransmitters, light in the eye, smells and some tastes. About a third of approved drugs act on them. Every GPCR works the same way: the ligand changes the receptor's shape, the receptor switches on a G

protein inside the cell, and the G protein switches on an enzyme or ion channel. Learning one GPCR well teaches you all of them.

> You need: the three classes of surface receptor from Chapter 5.2.

Structure of the receptor

A GPCR is a single polypeptide chain that crosses the plasma membrane seven times as α-helices, so it is also called a **seven-transmembrane** (7TM) or serpentine receptor. The N-terminus is outside the cell and the C-terminus inside. The ligand binds in a pocket formed by the helices, or on the outer loops and N-terminus for large ligands. The inner loops and C-terminal tail face the cytosol and contact the G protein.

The G protein is a switch

The G protein that GPCRs use is **heterotrimeric**: three different subunits, α, β and γ. The α subunit binds a guanine nucleotide. With GDP bound, it is off. With GTP bound, it is on. β and γ stay tightly joined as one βγ unit. The α and γ subunits carry lipid tails that keep the G protein on the inner face of the membrane.

1. **Rest.** The G protein sits as αβγ with GDP on α. The receptor is empty.
2. **Activation.** The ligand binds; the receptor changes shape and binds the G protein. It acts as a **guanine nucleotide exchange factor** (GEF): it makes α release GDP. GTP, more plentiful in the cytosol, binds in its place.
3. **Splitting.** GTP-bound α changes shape and separates from βγ. Both α-GTP and βγ can now act on target proteins called **effectors**. One active receptor can switch on many G proteins one after another, the first step of amplification.
4. **Switching off.** The α subunit is itself a slow GTPase. It hydrolyses GTP to GDP within seconds to minutes, lets go of the effector, and rejoins βγ. **RGS proteins** (regulators of G-protein signalling) speed up this hydrolysis.

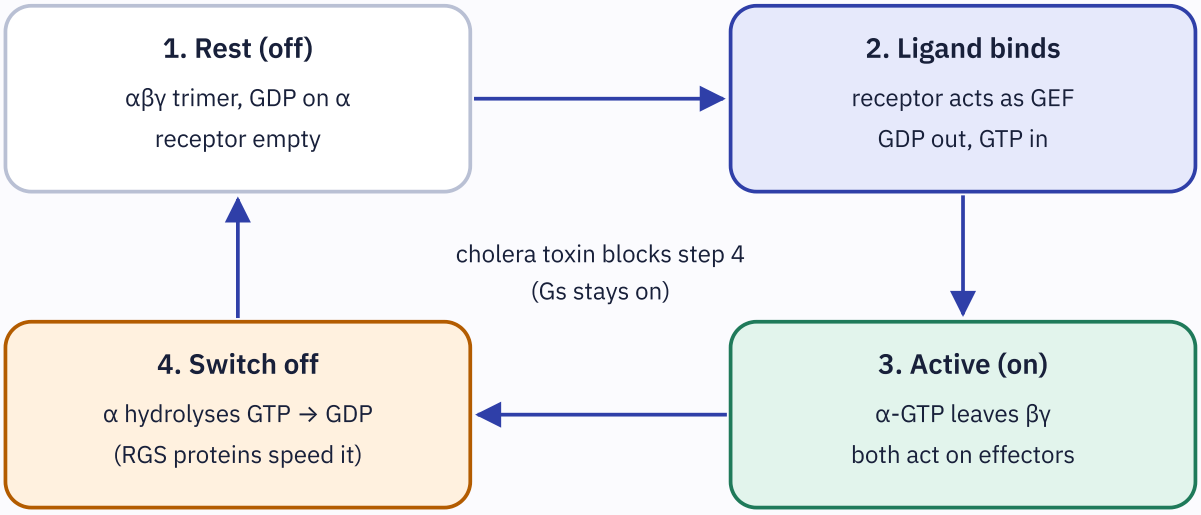


Figure 5.3.1 The G-protein cycle. The receptor only acts at step 2; the timer that ends the signal is the α subunit's own GTPase at step 4. Notice that anything that stops step 4 leaves the pathway on.

Main G proteins and their effectors

A TYPE	EFFECTOR	RESULT	EXAMPLE SIGNAL
Gs (stimulatory)	Adenylyl cyclase on	cAMP rises	Adrenaline at β-adrenergic receptors
Gi (inhibitory)	Adenylyl cyclase off; βγ opens K^+ channels	cAMP falls; heart slows	Acetylcholine at heart muscarinic receptors
Gq	Phospholipase C-β on	IP_3 and DAG; Ca^{2+} rises	Adrenaline at α_1 receptors; histamine H_1
Gt (transducin)	cGMP phosphodiesterase on	cGMP falls; channels close	Light at rhodopsin in rod cells

The second messengers in this table (cAMP, IP_3, DAG, Ca^{2+}) are the subject of Chapter 5.4. Notice that adrenaline uses different receptors in different tissues, which is how one hormone raises cAMP in the heart but Ca^{2+} in blood-vessel muscle.

Desensitisation: turning the receptor down

If adrenaline stays high, the cell stops responding as strongly. A **G-protein-coupled receptor kinase** (GRK) phosphorylates the active receptor's tail. **β-arrestin** then binds the phosphorylated tail, blocks the G protein from binding, and helps pull the receptor into the cell

by endocytosis. The receptor is later recycled or destroyed. This explains why some asthma inhalers that stimulate β_2 receptors lose effect with heavy use.

Toxins that jam the switch

Cholera toxin chemically modifies (ADP-ribosylates) the α subunit of Gs so it cannot hydrolyse GTP. Gs stays on, cAMP stays high in intestinal cells, chloride and water pour into the gut, and the patient loses litres of fluid as diarrhoea. **Pertussis toxin** (whooping cough) modifies Gi so the receptor can no longer activate it; Gi stays in the off state with GDP and is unable to inhibit adenylyl cyclase. Both toxins raise cAMP, but by opposite routes.

WORKED EXAMPLE

Question (6 marks). Liver cells are given adrenaline, and cAMP is measured. Predict the cAMP level, compared with untreated cells given adrenaline, in each case, and explain. (a) Cells pre-treated with cholera toxin. (b) Cells given GTPγS, a GTP analogue that α cannot hydrolyse. (c) Cells with a mutant receptor that binds adrenaline but cannot bind Gs. (d) Cells over-expressing an RGS protein that acts on Gs.

1. (a) **Higher and longer.** Gsα cannot hydrolyse GTP, so once activated it stays on and keeps adenylyl cyclase working.
2. (b) **Higher and longer**, for the same reason: α loads the analogue and cannot turn it to GDP. This is how researchers prove a response uses a G protein.
3. (c) **No rise.** Binding still happens, but the receptor cannot act as a GEF on Gs, so no GTP is loaded.
4. (d) **Lower and shorter.** Faster GTP hydrolysis shortens the time each Gsα spends on, so less cAMP is made.

The pattern to learn: anything that blocks GTP hydrolysis keeps the pathway on; anything that blocks GDP–GTP exchange keeps it off.

EXAM TRAPS

- The receptor does not bind GTP. The α subunit does; the receptor only makes α release GDP.
- Heterotrimeric G proteins (αβγ) are not the same as small G proteins such as Ras, which are single chains activated by Sos after an RTK (Chapter 5.2).
- βγ is not just a passive partner. It opens K^+ channels in the heart and recruits GRKs.
- Write "seven transmembrane α-helices", not "seven subunits". A GPCR is one chain.
- The favourite long question: "Explain the mechanism of GPCR signalling with a neat diagram, and how it is switched off."

CHECK YOURSELF

– REMEMBER **How many times does a GPCR cross the membrane?** 1 mark

Seven times, as α-helices; N-terminus outside, C-terminus inside.

– REMEMBER **Name the effector of Gs and of Gq.** 2 marks

Gs: adenylyl cyclase. Gq: phospholipase C-β.

– UNDERSTAND **Why is the α subunit called a timer?** 3 marks

It switches itself off by hydrolysing its bound GTP to GDP. The rate of this hydrolysis sets how long each activated α stays on, whatever the receptor does.

– UNDERSTAND **Explain desensitisation of a GPCR.** 4 marks

With prolonged ligand, GRK phosphorylates the active receptor; β-arrestin binds the phosphorylated receptor, blocks G-protein coupling, and promotes endocytosis, so fewer receptors respond.

– APPLY **Why does cholera cause watery diarrhoea?** 4 marks

Cholera toxin ADP-ribosylates Gsα in intestinal cells, blocking its GTPase. Gs stays active, adenylyl cyclase keeps making cAMP, and cAMP-driven chloride secretion draws water into the gut.

– APPLY **Pertussis toxin is added to heart cells, then acetylcholine. Does the heart slow?** 3 marks

No. The toxin stops the receptor activating Gi, so Gi is unable to inhibit adenylyl cyclase and its βγ does not open K^+ channels; the slowing effect is lost.

– ANALYZE **Adrenaline relaxes airway muscle but constricts some blood vessels. How can one ligand do both?** 4 marks

The tissues carry different adrenergic receptors. β_2 receptors couple to Gs and raise cAMP, which relaxes airway smooth muscle; α_1 receptors couple to Gq and raise Ca^{2+}, which contracts vascular smooth muscle. The receptor and G protein, not the ligand, set the response.

BEYOND THE SYLLABUS

The 2012 Nobel Prize in Chemistry went to Robert Lefkowitz and Brian Kobilka for work on GPCRs, including the crystal structure of a β_2 receptor bound to its G protein. Research now studies "biased agonists", drugs that steer a receptor towards G-protein or towards β-arrestin signalling, to keep a drug's benefit and lose its side effects. Cryo-electron microscopy has made new GPCR structures routine. This is not for the exam; search "biased agonism" and "GPCR cryo-EM" to read further.

5.4 Second messengers and signal amplification

The hormone that reaches a cell is the first messenger. It stays outside. Inside, the receptor and G protein make or release small molecules that spread the message through the cytosol: these are **second messengers**. The main ones are cyclic AMP (cAMP), cyclic GMP (cGMP), inositol 1,4,5-trisphosphate (IP_3), diacylglycerol (DAG) and calcium ions (Ca^{2+}). Because each enzyme in the chain makes many molecules of the next, a few hormone molecules can change millions of molecules inside the cell. That multiplication is **signal amplification**.

You need: the G-protein cycle and the table of Gs, Gi and Gq from Chapter 5.3.

The cAMP pathway

cAMP was the first second messenger discovered; Earl Sutherland received the 1971 Nobel Prize in Physiology or Medicine for the work, done on how adrenaline makes liver cells release glucose.

1. Active Gsα switches on **adenylyl cyclase**, a membrane enzyme that turns ATP into cAMP (and pyrophosphate).

2. cAMP activates **protein kinase A** (PKA). Inactive PKA is a tetramer of two regulatory (R) and two catalytic (C) subunits, R_2C_2. Each R binds two cAMP, so four cAMP release two free, active C subunits.
3. C subunits phosphorylate target proteins on serine and threonine. In the liver they start the glycogen breakdown cascade (worked example below). In the nucleus they phosphorylate the transcription factor **CREB** (cAMP response element binding protein), which turns on genes.
4. The signal ends when **cAMP phosphodiesterase** breaks cAMP to AMP, and protein phosphatases remove the phosphates PKA added. Methylxanthines such as theophylline, used in asthma, inhibit phosphodiesterase and so prolong cAMP.

The IP_3–DAG–Ca^{2+} pathway

1. Active Gqα switches on **phospholipase C-β** (PLCβ).
2. PLCβ cuts a minor membrane lipid, phosphatidylinositol 4,5-bisphosphate (PIP_2), into two messengers: water-soluble **IP_3**, which diffuses into the cytosol, and lipid **DAG**, which stays in the membrane.
3. IP_3 binds the **IP_3 receptor**, a Ca^{2+} channel in the endoplasmic reticulum (ER) membrane. Ca^{2+} rushes out of the ER into the cytosol.
4. DAG, together with Ca^{2+} and the membrane lipid phosphatidylserine, activates **protein kinase C** (PKC), which phosphorylates its own set of targets.
5. Ca^{2+} pumps return Ca^{2+} to the ER and out of the cell; DAG and IP_3 are converted back to lipids. The signal ends.

Calcium and calmodulin

Resting cytosolic Ca^{2+} is about 10^{-7} M (0.1 μM), while outside the cell and inside the ER it is about 10^{-3} M (1 mM). This 10,000-fold gradient, kept by ATP-driven pumps (Unit 2, *Active transport*), means that opening a few channels raises cytosolic Ca^{2+} fast. Ca^{2+} acts mainly through **calmodulin**, a small protein with four Ca^{2+}-binding sites. Ca^{2+}-calmodulin changes shape and activates enzymes such as the Ca^{2+}/calmodulin-dependent kinases (CaM-kinases). In muscle, Ca^{2+} triggers contraction (Unit 6, *Muscle cells*).

cGMP

cGMP is made by guanylyl cyclase. Nitric oxide activates the soluble form inside smooth muscle, cGMP activates protein kinase G, and the muscle relaxes, widening the blood vessel. Sildenafil works by inhibiting phosphodiesterase 5 (PDE5), which breaks cGMP. In rod cells of the eye the logic runs the other way: light lowers cGMP through transducin (Chapter 5.3).

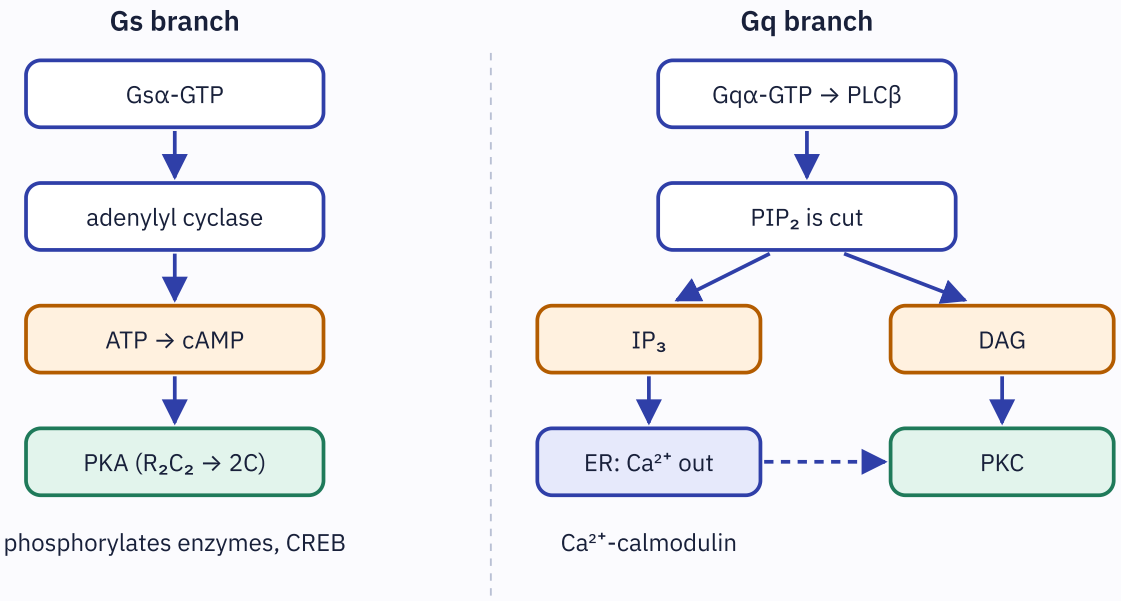


Figure 5.4.1 The two main GPCR second-messenger branches. Orange boxes are the second messengers. Notice that the Gq branch makes two messengers from one lipid, and that PKC needs both of them (DAG directly, Ca^{2+} via the dashed arrow).

Why a cascade amplifies

Each enzymatic step is a multiplier. One active receptor activates many G proteins in turn; each adenylyl cyclase makes many cAMP; each kinase phosphorylates many substrate molecules. Multiplying the gains of several steps gives a huge total gain. A cascade also gives control points: the cell can damp any step (phosphodiesterase, phosphatases, RGS proteins), and different branches can be combined.

WORKED EXAMPLE

Question (6 marks). In a liver cell, adrenaline acts through a β-adrenergic receptor, Gs, adenylyl cyclase, cAMP, PKA, phosphorylase kinase and glycogen phosphorylase, which releases glucose 1-phosphate from glycogen. Use these illustrative gains: each receptor activates 10 Gs; each Gs–cyclase pair makes 100 cAMP; PKA needs 4 cAMP to release 2 C subunits; each C activates 10 phosphorylase kinase; each phosphorylase kinase activates 10 phosphorylase; each phosphorylase releases 1,000 glucose 1-phosphate. How many glucose 1-phosphate molecules come from one adrenaline-bound receptor?

1. Gs: 1 × 10 = 10 active Gs.
2. cAMP: 10 × 100 = 1,000 cAMP.
3. PKA: 1,000 cAMP ÷ 4 per holoenzyme = 250 holoenzymes, giving 250 × 2 = 500 C subunits.
4. Phosphorylase kinase: 500 × 10 = 5,000.
5. Glycogen phosphorylase: 5,000 × 10 = 50,000.
6. Glucose 1-phosphate: 50,000 × 1,000 = 5×10^7 molecules.

Answer: about 50 million glucose 1-phosphate molecules from one receptor, an overall gain of 5×10^7. Notice that the PKA step divides by 2 (four cAMP give two C), the only step that is not a gain. The real gains differ from cell to cell; the method is what the examiner checks.

EXAM TRAPS

- IP_3 releases Ca^{2+} from the ER, not from mitochondria and not from outside the cell.
- DAG stays in the membrane; IP_3 diffuses. Students often swap them.
- PKA's regulatory subunits bind cAMP; the catalytic subunits do the phosphorylating. cAMP does not bind the catalytic subunits.
- Phosphodiesterase ends the cAMP signal; it is not part of the activation path.
- Favourite question: "What are second messengers? Explain the cAMP and IP_3/DAG pathways." Name all five messengers and draw both branches.

CHECK YOURSELF

– REMEMBER **List five second messengers.** 2 marks

cAMP, cGMP, IP_3, DAG, Ca^{2+}.

– REMEMBER **Which enzyme makes cAMP and which destroys it?** 2 marks

Adenylyl cyclase makes cAMP from ATP; cAMP phosphodiesterase breaks it to AMP.

– UNDERSTAND **How does cAMP activate PKA?** 3 marks

Inactive PKA is R_2C_2. Two cAMP bind each R subunit; the R subunits change shape and release the two C subunits, which are then active kinases.

– UNDERSTAND **Why can a small rise in Ca^{2+} influx make a large signal?** 3 marks

Resting cytosolic Ca^{2+} is very low (about 10^{-7} M) against about 10^{-3} M outside and in the ER, so opening channels raises cytosolic Ca^{2+} many-fold quickly, and calmodulin then switches on many enzymes.

– APPLY **A drug blocks phosphodiesterase in heart cells given adrenaline. Predict the effect on cAMP and PKA.** 3 marks

cAMP is not broken down, so it stays high for longer; more PKA stays active and its effects (for example stronger heart contraction) last longer.

– APPLY **With gains of 20, 50 and 10 at three steps, what is the total gain?** 2 marks

20 × 50 × 10 = 10,000. Gains multiply, they do not add.

– ANALYZE **Why does a cell use a multi-step cascade instead of letting the receptor act directly on glycogen phosphorylase?** 4 marks

Each step multiplies the signal, giving large amplification from very few hormone molecules. Each step is also a control point that can be damped or combined with other signals, and different branches can spread one signal to several responses (metabolism, gene expression).

BEYOND THE SYLLABUS

Second messengers do not flood the cell evenly. Anchoring proteins called AKAPs (A-kinase anchoring proteins) hold PKA near particular targets, so cAMP signals form local "nanodomains". Researchers watch cAMP and Ca^{2+} in living cells with fluorescent sensors such as the GCaMP calcium indicators. This is not for the exam; search "cAMP compartmentalisation" and "genetically encoded calcium indicators" to read further.

5.5 Kinase pathways: PI3K/Akt, JAK/STAT and AMPK

Many signals act not through second messengers alone but through chains of protein kinases, enzymes that add phosphate groups to other proteins. Three chains appear again and again in physiology and disease: PI3K/Akt, which tells a cell to survive, grow and take up glucose; JAK/STAT, which carries cytokine signals straight to the nucleus; and AMPK, which senses low energy and switches the cell to saving mode. Elsewhere this chapter is extra reading.

> You need: receptor tyrosine kinases and SH2 domains from Chapter 5.2.

PI3K/Akt: the survival and growth pathway

1. Insulin or a growth factor activates its RTK. The phosphorylated receptor (or, for insulin, the docking protein IRS-1) binds **phosphoinositide 3-kinase** (PI3K) through SH2 domains.
2. PI3K adds a phosphate at position 3 of the inositol ring of the membrane lipid PIP_2, making **PIP_3**.
3. PIP_3 is a docking site for proteins with a **PH domain** (pleckstrin homology domain). Two of them arrive: the kinase PDK1 and the kinase **Akt** (also called protein kinase B).
4. PDK1 phosphorylates Akt on threonine 308, and the complex mTORC2 phosphorylates it on serine 473. Akt is now fully active and leaves the membrane.
5. Akt phosphorylates many targets: it inactivates the pro-death protein BAD (so the cell survives), keeps FOXO transcription factors out of the nucleus, activates mTORC1 (more protein synthesis and growth), and in muscle and fat cells moves GLUT4 glucose transporters to the surface.
6. The phosphatase **PTEN** removes the 3-phosphate from PIP_3 and ends the signal. PTEN is a tumour suppressor; losing it leaves Akt on, which is common in cancer (Unit 4, *Cancer and oncogenes*).

JAK/STAT: a short road to the nucleus

Receptors for cytokines such as interferons, erythropoietin and many interleukins have no kinase domain of their own. Instead, a **Janus kinase** (JAK) is bound to each receptor chain inside the cell. Ligand binding brings the chains together, and the two JAKs phosphorylate each other and then the receptor tails. **STAT** proteins (signal transducers and activators of transcription) dock on the phosphotyrosines through their SH2 domains and are phosphorylated by JAK. Phosphorylated STATs let go, pair up through their SH2 domains, enter the nucleus and

switch on genes. The pathway is turned off by phosphatases and by **SOCS** proteins (suppressors of cytokine signalling), which STAT itself induces: a negative feedback loop. Erythropoietin, for example, signals through JAK2 and STAT5 to make red blood cells.

AMPK: the energy sensor

AMP-activated protein kinase (AMPK) has three subunits: α (the kinase), β and γ. The γ subunit binds AMP, ADP or ATP. When ATP falls and AMP rises, AMP binding helps the upstream kinase LKB1 phosphorylate α on threonine 172 and protects that phosphate from removal. Active AMPK switches off ATP-using processes (fatty acid synthesis by inhibiting acetyl-CoA carboxylase; protein synthesis by inhibiting mTORC1) and switches on ATP-making ones (glucose uptake, fatty acid oxidation). Exercise activates AMPK in muscle, and the diabetes drug metformin activates it indirectly.

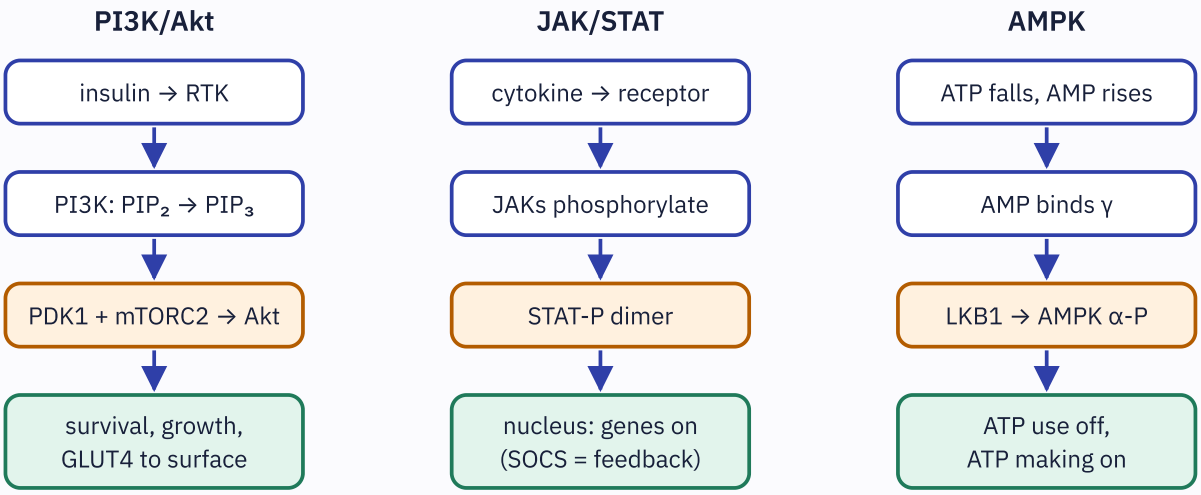


Figure 5.5.1 The three kinase pathways. The first two start at a receptor; AMPK starts from the cell's own energy state. Notice that Akt drives growth through mTORC1 while AMPK brakes it, so the two pathways pull mTORC1 in opposite directions.

WORKED EXAMPLE

Question (5 marks). The enzyme adenylate kinase keeps 2 ADP ⇌ ATP + AMP near equilibrium, with $K = [ATP][AMP]/[ADP]^2 \approx 1$. A resting muscle cell has ATP = 5.0 mM, ADP = 0.50 mM. (a) Find AMP. (b) During exercise ATP falls to 4.4 mM and ADP rises to 0.95 mM. Find AMP now and compare the percentage changes in ATP and AMP. Why is AMP, not ATP, the signal AMPK reads?

1. (a) $[AMP] = K[ADP]^2/[ATP] = 1 \times (0.50)^2 / 5.0 = 0.25 / 5.0 = 0.050$ mM.
2. (b) $[AMP] = (0.95)^2 / 4.4 = 0.9025 / 4.4 = 0.205$ mM. Check the total: 4.4 + 0.95 + 0.205 = 5.555 mM, the same as 5.0 + 0.50 + 0.05 = 5.55 mM, so adenine nucleotides are conserved.
3. ATP change: (5.0 – 4.4)/5.0 = 12% fall. AMP change: 0.205/0.050 = 4.1 times, a 310% rise.
4. Because AMP depends on the square of ADP, a small fall in ATP gives a large rise in AMP. AMP is therefore a far more sensitive signal of energy stress than ATP.

EXAM TRAPS

- PI3K phosphorylates a lipid ($PIP_2 \rightarrow PIP_3$), not a protein. Akt is the protein kinase.
- Cytokine receptors are not RTKs; the kinase (JAK) is a separate protein bound to them.
- AMPK is not activated by cAMP. The name refers to AMP; do not confuse it with PKA.
- PTEN is a phosphatase that opposes PI3K; losing PTEN activates, not blocks, Akt signalling.

CHECK YOURSELF

– REMEMBER **What does PI3K make, and which domain binds it?** 2 marks

PIP_3 from PIP_2; PIP_3 is bound by PH domains (on Akt and PDK1).

– REMEMBER **Expand JAK and STAT.** 2 marks

Janus kinase; signal transducer and activator of transcription.

– UNDERSTAND **How do STATs reach the nucleus?** 3 marks

They dock by SH2 domains on phosphorylated receptor tyrosines, are phosphorylated by JAK, then dimerise through SH2–phosphotyrosine contacts and move into the nucleus to bind DNA.

– APPLY **A tumour has lost PTEN. Predict PIP_3, Akt and cell survival.** 3 marks

PIP_3 stays high, Akt stays active, BAD is kept inactive, so the cells resist apoptosis and keep growing.

– APPLY **Why does exercise raise glucose uptake in muscle even without insulin?** 3 marks

Exercise uses ATP, AMP rises, AMPK is activated and promotes glucose uptake (GLUT4 to the surface) through a route independent of insulin and Akt. (Ca^{2+} signals from contraction help too.)

– ANALYZE **Compare how JAK/STAT and cAMP/PKA reach gene expression.** 4 marks

JAK/STAT is short: the transcription factor itself is phosphorylated at the receptor and goes straight to the nucleus. cAMP/PKA goes through G protein, cyclase, second messenger and kinase before PKA phosphorylates CREB; it amplifies more but has more steps.

BEYOND THE SYLLABUS

Each pathway is a drug target. JAK inhibitors such as ruxolitinib treat myelofibrosis and some autoimmune diseases, and a single JAK2 mutation (V617F) drives the blood cancer polycythaemia vera. PI3K inhibitors are used in some breast cancers. This is not for the exam; search "mTOR signalling network" to see how Akt and AMPK meet.

5.6 Agonists and antagonists

A receptor does not know whether the molecule in its pocket is the body's own hormone or a tablet. Any molecule that binds a receptor and switches it on is an **agonist**; any that binds and blocks it without switching it on is an **antagonist**. Most drugs work this way: salbutamol inhalers are agonists at airway β_2 receptors, and propranolol, a "beta blocker", is an antagonist at heart β receptors. Other colleges meet it in pharmacology.

You need: K_d and occupancy from Chapter 5.2.

Two separate properties: affinity and efficacy

Affinity is how tightly a molecule binds, measured by K_d. **Efficacy** is how well the bound molecule activates the receptor. An antagonist can have high affinity and zero efficacy. This split

gives four kinds of ligand:

1. **Full agonist**: gives the maximum response the system can make. Adrenaline at β receptors; morphine at μ-opioid receptors.
2. **Partial agonist**: even when every receptor is occupied, gives less than the maximum. Buprenorphine at μ-opioid receptors. Because it occupies receptors, it also blocks a full agonist from acting fully.
3. **Antagonist** (neutral): binds, gives no response, and blocks agonists. Naloxone reverses opioid overdose this way; atropine blocks muscarinic acetylcholine receptors.
4. **Inverse agonist**: some receptors are partly active even when empty (constitutive activity). An inverse agonist lowers that activity below the resting level.

Competitive and non-competitive antagonists

A **competitive** antagonist binds reversibly to the same site as the agonist. Enough agonist outcompetes it, so the maximum response is still reached, but more agonist is needed: the dose-response curve shifts to the right without lowering the top. A **non-competitive** antagonist either binds elsewhere on the receptor (an allosteric site) or binds the agonist site irreversibly. Adding more agonist cannot overcome it, so the maximum response falls.

The **EC_{50}** is the agonist concentration giving half the maximum response; a lower EC_{50} means higher **potency**. For a competitive antagonist B with dissociation constant K_B, the factor by which the EC_{50} shifts, the **dose ratio** r, is given by the Gaddum–Schild equation:

```
r = 1 + [B] / KB
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where [B] is the antagonist concentration. A plot of log(r − 1) against log[B], the Schild plot, is a straight line of slope 1 for a truly competitive antagonist.

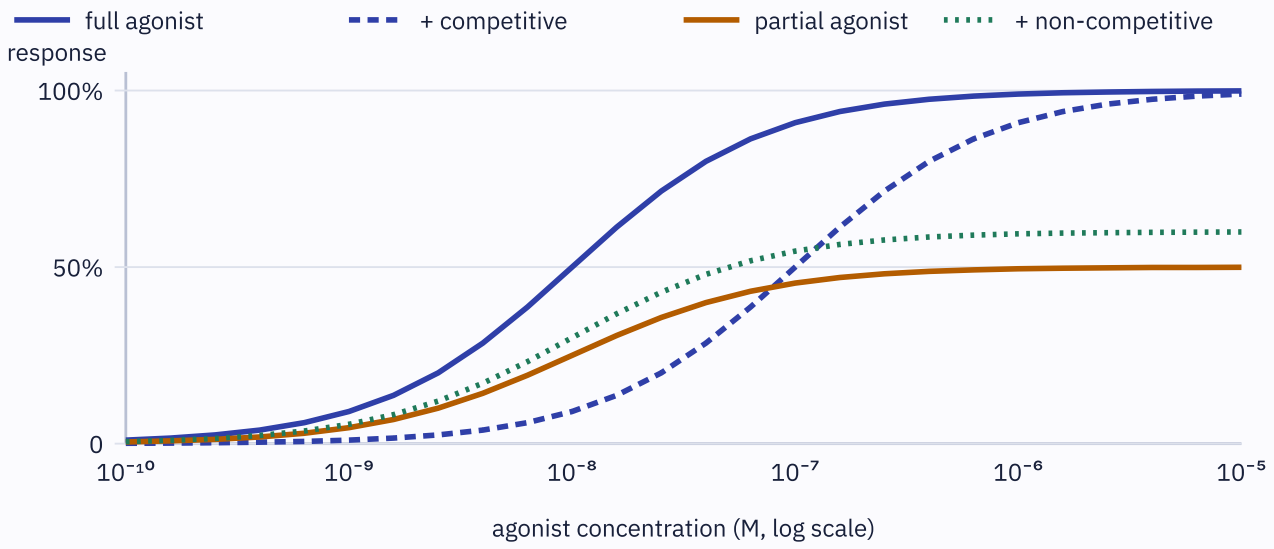


Figure 5.6.1 Dose-response curves computed for an agonist with EC_{50} = 10 nM. The competitive antagonist (dashed blue, dose ratio 10) shifts the curve right but still reaches 100%; the partial agonist tops out at 50%; the non-competitive antagonist lowers the maximum to 60%. Notice that only the competitive case can be overcome by more agonist.

WORKED EXAMPLE

Question (5 marks). An agonist has EC_{50} = 10 nM. A competitive antagonist with K_B = 2 nM is added at 18 nM. (a) Find the new EC_{50}. (b) What antagonist concentration would shift the EC_{50} to 1 µM? (c) Will the maximum response change?

1. (a) r = 1 + 18/2 = 1 + 9 = 10. New EC_{50} = 10 × 10 nM = 100 nM.
2. (b) Needed r = 1 µM / 10 nM = 1000 nM / 10 nM = 100. So $[B]/K_B$ = 99 and [B] = 99 × 2 nM = 198 nM.
3. (c) No. A competitive antagonist is surmountable; at high enough agonist concentration the full response returns.

EXAM TRAPS

- High affinity does not mean high efficacy. Antagonists often bind more tightly than the natural agonist.
- A partial agonist acts as an antagonist when a full agonist is present.
- Potency (EC_{50}) and efficacy (maximum) are different; a curve can shift without changing its top.
- A competitive antagonist shifts the curve right; a non-competitive one lowers the maximum.

CHECK YOURSELF

– REMEMBER **Define agonist and antagonist.** 2 marks

An agonist binds a receptor and activates it; an antagonist binds and does not activate it, blocking agonist action.

– REMEMBER **Give one clinical antagonist and its receptor.** 1 mark

Propranolol (β-adrenergic), atropine (muscarinic), or naloxone (μ-opioid).

– UNDERSTAND **What is an inverse agonist?** 2 marks

A ligand that lowers the activity of a receptor that is partly active on its own, below the resting level.

– APPLY **K_B = 5 nM and [B] = 45 nM. By what factor does the EC_{50} rise?** 2 marks

r = 1 + 45/5 = 10; the EC_{50} rises ten-fold.

– APPLY **Why can naloxone reverse a morphine overdose?** 3 marks

Naloxone is a competitive antagonist at μ-opioid receptors with high affinity; it displaces morphine from the receptors and gives no activation itself, so breathing recovers.

– ANALYZE **From a set of dose-response curves, how would you tell a competitive from a non-competitive antagonist?** 4 marks

Competitive: parallel right shift, same maximum, Schild slope 1. Non-competitive: maximum falls and is not restored by more agonist; the curve may also shift.

BEYOND THE SYLLABUS

Modern pharmacology adds allosteric modulators, which bind away from the agonist site and turn the receptor's response up or down, and biased agonists that favour one downstream pathway. This is not for the exam; search "allosteric modulator GPCR" and "Schild analysis" to read further.

5.7 Cell junctions and cell adhesion

Cells in a tissue are not loose bags. They are bolted to each other and to the matrix below them by specialised contact points called **cell junctions**, built from **cell adhesion molecules** (CAMs) in the membrane and anchored to the cytoskeleton inside. Junctions do four jobs: seal the gaps between cells (tight junctions), hold cells together against pulling forces (adherens junctions and desmosomes), fix cells to the matrix (hemidesmosomes and focal adhesions), and let small molecules pass directly from one cell to the next (gap junctions). The skin, the gut lining and the heart all depend on them.

You need: actin filaments and intermediate filaments from Unit 3, *Cytoskeleton: microtubules, actin and intermediate filaments*.

The adhesion molecule families

1. **Cadherins**: cell–cell adhesion that needs Ca^{2+} (the name comes from "calcium-dependent adhesion"). A cadherin usually binds the same cadherin on the next cell (homophilic binding). E-cadherin holds epithelial cells together.
2. **Integrins**: two chains, α and β, that bind the extracellular matrix (fibronectin, laminin, collagen) outside and the cytoskeleton inside. They also send signals in both directions.
3. **Selectins**: bind sugar groups on other cells, also Ca^{2+}-dependent. They let white blood cells roll along the wall of a blood vessel during inflammation.
4. **Immunoglobulin-superfamily CAMs**: Ca^{2+}-independent, such as N-CAM in nerve tissue and ICAM-1 on blood-vessel lining, which binds the integrins of white blood cells.

The junctions of an epithelial cell, from top to bottom

1. **Tight junction** (occluding junction): a belt near the top where rows of the proteins **claudin** and **occludin** seal neighbouring membranes together. It stops fluid leaking between cells,

so material must pass through the cells by transporters. It also acts as a fence that keeps apical and basolateral membrane proteins apart.

2. **Adherens junction**: just below, a belt of cadherins linked inside through **catenins** to a ring of actin filaments. It holds cells together and lets a sheet of cells fold, as in forming the neural tube.
3. **Desmosome**: a button-like spot weld. The desmosomal cadherins (desmogleins and desmocollins) link through plaque proteins such as plakoglobin and desmoplakin to **intermediate filaments** (keratin in skin). Desmosomes are common in tissues under mechanical stress, such as skin and heart muscle.
4. **Gap junction**: a cluster of channels joining the cytoplasm of two cells. Six **connexin** proteins form a **connexon** (hemichannel) in one membrane; two connexons dock to form a complete channel. Ions and small molecules such as sugars, amino acids, nucleotides, cAMP, IP_3 and Ca^{2+} pass, but proteins and nucleic acids do not. Textbooks usually quote a size cut-off near 1,000 daltons; measured limits vary with the connexin type and are often lower. Gap junctions let heart muscle cells contract together by passing ions.
5. **Hemidesmosome**: at the base, integrins (α6β4) bind laminin in the basal lamina and link inside to keratin filaments. **Focal adhesions** are the matching actin-linked integrin contacts of cells such as fibroblasts.

Plants have no gap junctions. Their cells connect through **plasmodesmata**, channels through the cell wall lined by the plasma membrane, which allow larger molecules than gap junctions, including some proteins and RNAs.

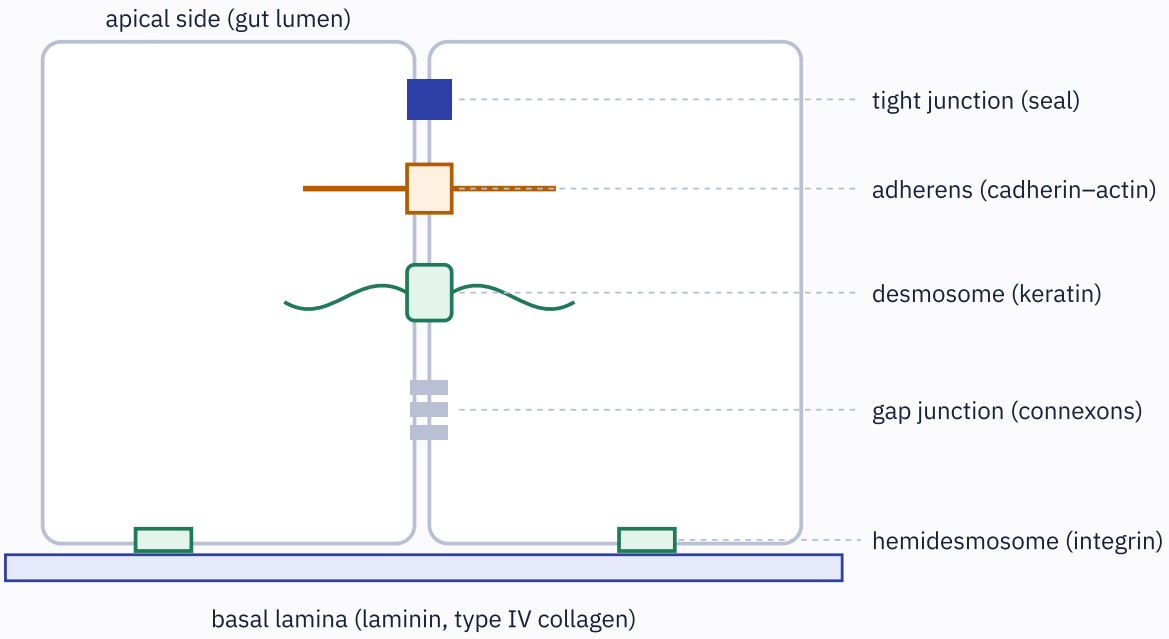


Figure 5.7.1 The junctions between two gut epithelial cells, in their usual order from the lumen (top) to the basal lamina (bottom). Orange lines are actin, green curves are intermediate filaments. Notice that the two anchoring junctions use different filaments: adherens junctions use actin, desmosomes and hemidesmosomes use intermediate filaments.

WORKED EXAMPLE

Question (6 marks). Interpret each experiment. (a) A sheet of epithelial cells falls apart when EDTA (which removes Ca^{2+}) is added, and re-forms when Ca^{2+} is restored. (b) A fluorescent dye of 400 daltons injected into one heart muscle cell appears in its neighbours within minutes, but a 10,000-dalton fluorescent protein stays in the injected cell. (c) An electron-dense tracer added to the gut lumen stops at a point near the top of the spaces between the cells. (d) A patient's own antibodies against desmoglein 3 cause skin blisters.

1. (a) Cell–cell adhesion depends on Ca^{2+}, so it is mediated by **cadherins** (adherens junctions and desmosomes). Removing Ca^{2+} makes cadherins lose their shape and binding.
2. (b) The cells are connected by **gap junctions**. Small molecules pass through connexon channels; a protein is far too large.
3. (c) The tracer is stopped by **tight junctions**, which seal the space between cells at the apical end.
4. (d) Desmoglein 3 is a desmosomal cadherin. Antibodies block it, **desmosomes** fail, and skin cells separate under friction. This disease is pemphigus vulgaris.

EXAM TRAPS

- Desmosomes link to intermediate filaments, not actin. Adherens junctions link to actin.
- Hemidesmosomes use integrins, not cadherins, because they bind the matrix, not another cell.
- Tight junctions seal; they do not allow molecules through like gap junctions. Do not swap the two.
- Six connexins make one connexon (half a channel); two connexons make one gap-junction channel.
- Favourite question: "Classify cell junctions with a neat diagram." Use three groups: occluding (tight), anchoring (adherens, desmosome, hemidesmosome, focal adhesion), communicating (gap junction, plasmodesmata).

CHECK YOURSELF

– REMEMBER **Name the proteins that form tight junctions.** 2 marks

Claudins and occludin.

– REMEMBER **Which adhesion molecules need Ca^{2+}?** 2 marks

Cadherins and selectins (integrins need divalent cations too, but the classic Ca^{2+}-dependent cell–cell molecules are the cadherins).

– UNDERSTAND **Give two functions of tight junctions.** 2 marks

They seal the space between cells so solutes cannot leak across the sheet, and they act as a fence keeping apical and basolateral membrane proteins separate.

– UNDERSTAND **How is a gap junction channel built?** 3 marks

Six connexins form a connexon in each cell's membrane; connexons of two neighbouring cells dock end to end, forming a continuous aqueous channel between the two cytoplasms.

– APPLY **Why do heart muscle cells need gap junctions?** 3 marks

Ions pass through gap junctions, so the electrical excitation spreads quickly from cell to cell and the heart muscle contracts together.

– ANALYZE **Loss of E-cadherin is common in tumours that spread. Explain why.** 4 marks

E-cadherin holds epithelial cells together. Without it, cells detach from their neighbours, can move away and invade, which is a step towards metastasis.

BEYOND THE SYLLABUS

Junctions are also signalling hubs: β-catenin, part of the adherens junction, doubles as a transcription co-activator in the Wnt pathway, and integrins sense matrix stiffness, a field called mechanobiology. Researchers engineer leaky or tight barriers in "organ-on-a-chip" models of the gut and blood–brain barrier. This is not for the exam; search "mechanotransduction integrins" and "epithelial–mesenchymal transition".

5.8 Extracellular matrix and cell wall

Outside the plasma membrane, most cells build a structure of their own. Animal cells secrete an **extracellular matrix** (ECM): a network of protein fibres in a hydrated sugar gel that gives tissues strength, holds cells in place and carries signals. Plant, fungal and bacterial cells build a rigid **cell wall** instead, which resists the pressure of water entering by osmosis. The materials differ (collagen in animals, cellulose in plants, peptidoglycan in bacteria, chitin in fungi), but the idea is the same: a fibre for strength set in a filler.

You need: integrins and hemidesmosomes from Chapter 5.7, and plant versus animal cells from Unit 1.

The animal extracellular matrix

1. **Collagen**, the most abundant protein in mammals, gives tensile strength (resistance to pulling). Three polypeptide chains wind into a **triple helix**. Every third residue is glycine, giving a repeating Gly-X-Y sequence in which X is often proline and Y often hydroxyproline. Glycine, the smallest amino acid, is the only one that fits in the crowded centre of the helix. Hydroxyproline is made after translation by prolyl hydroxylase, an enzyme that needs vitamin C; without vitamin C collagen is weak and the disease scurvy follows (bleeding gums, poor wound healing). Type I collagen forms fibrils in skin, bone and tendon; type IV forms a sheet-like network in the basal lamina.
2. **Elastin** gives stretch and recoil to skin, lungs and large arteries.
3. **Glycosaminoglycans** (GAGs) are long unbranched chains of repeating disaccharides, strongly negative because of sulfate and carboxyl groups. Hyaluronan, chondroitin sulfate, heparan sulfate and keratan sulfate are examples. Except hyaluronan, GAGs are attached to core proteins as **proteoglycans**, such as aggrecan in cartilage. Their negative charges attract cations and water, forming a gel that resists compression, which is why cartilage cushions joints.

4. **Adhesive glycoproteins** connect cells to the matrix. **Fibronectin** carries an Arg-Gly-Asp (**RGD**) sequence that integrins recognise. **Laminin** is the main adhesive protein of the basal lamina.
5. The **basal lamina** (basement membrane) is a thin mat of type IV collagen, laminin and proteoglycans under every epithelium. It supports the epithelium, filters (in the kidney glomerulus) and guides cells during repair.

The ECM is constantly remodelled. **Matrix metalloproteinases** (MMPs) cut matrix proteins during growth and wound healing; cancer cells use them to invade. The matrix also stores growth factors and signals to cells through integrins, linking this chapter back to the signalling chapters of this unit.

The plant cell wall

The **primary wall** is laid down while the cell grows. It is made of **cellulose microfibrils** (long chains of glucose joined by β-1,4 bonds, bundled into crystalline fibres) cross-linked by **hemicelluloses** such as xyloglucan and embedded in **pectin**, a gel of acidic polysaccharides. Cellulose is made at the plasma membrane by cellulose synthase complexes, which move along tracks set by cortical microtubules underneath. Between neighbouring cells lies the **middle lamella**, rich in pectin (calcium pectate), which glues them together. After growth stops, many cells add a thick **secondary wall** inside the primary wall, with more cellulose and often **lignin**, which makes wood hard and waterproof. Plasmodesmata cross all these layers.

Bacterial and fungal walls

The bacterial wall is **peptidoglycan** (murein): chains of alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), joined by β-1,4 bonds and cross-linked by short peptides. **Gram-positive** bacteria have a thick peptidoglycan layer that holds the crystal violet stain. **Gram-negative** bacteria have a thin layer between two membranes, the outer one carrying lipopolysaccharide (LPS). Penicillin blocks the transpeptidase enzymes that make the peptide cross-links, and lysozyme in tears cuts the NAM–NAG bond. Fungal walls are made mainly of **chitin** (a polymer of NAG) and glucans.

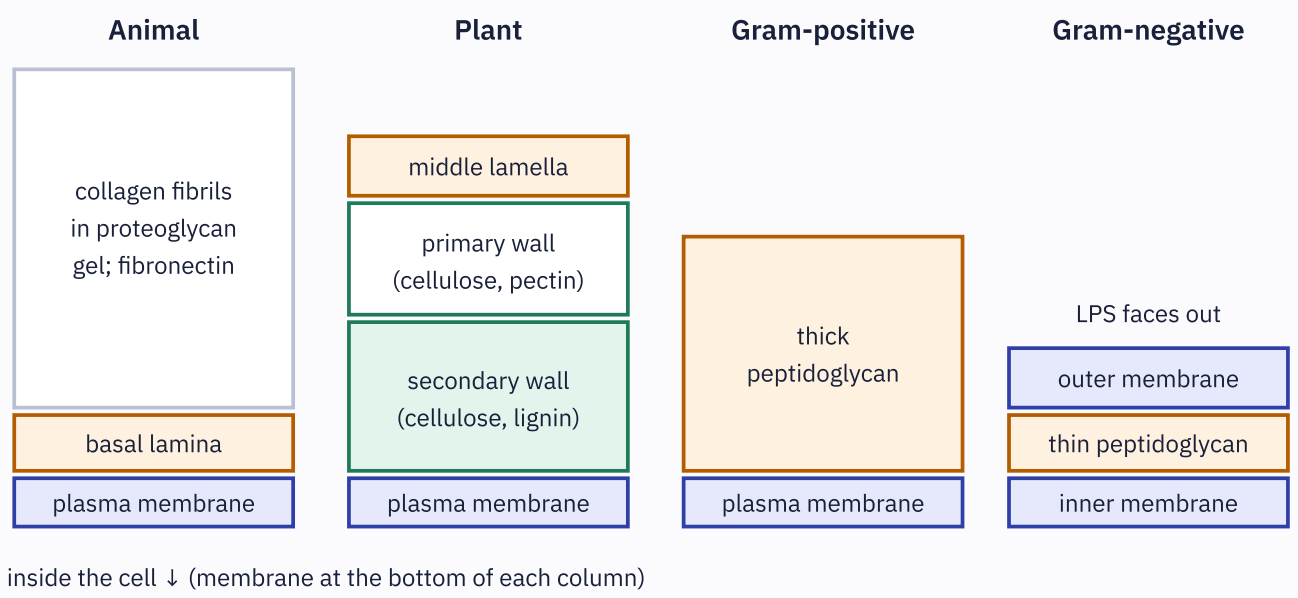


Figure 5.8.1 What lies outside the plasma membrane in four kinds of cell, membrane at the bottom. Notice that the plant secondary wall is laid down inside the primary wall, nearest the membrane, and that the Gram-negative wall has a second membrane outside its thin peptidoglycan.

WORKED EXAMPLE

Question (6 marks). (a) A synthetic collagen-like peptide is $(\text{Pro-Hyp-Gly})_{10}$. How many residues and how many glycines does one chain have, and how many glycines are in its triple helix? Why must glycine be every third residue? (b) Predict the result of treating each sample with the enzymes given: plant leaf tissue with pectinase alone; plant leaf tissue with cellulase plus pectinase in an osmotically balanced solution; a Gram-positive bacterium with lysozyme in water.

1. (a) Each repeat has 3 residues, so 10 × 3 = 30 residues per chain, of which 10 are glycine. The triple helix has 3 chains, so 3 × 10 = 30 glycines.
2. Glycine has only a hydrogen as its side chain. In the triple helix every third residue faces the crowded central axis, and only glycine is small enough to fit there.
3. (b) Pectinase dissolves the pectin of the middle lamella, so the cells separate from each other but keep their walls (this is how single cells are prepared from leaves).
4. Cellulase plus pectinase removes the whole wall, giving **protoplasts**: wall-less cells that survive only because the solution is osmotically balanced.
5. Lysozyme cuts the NAM–NAG bonds of the thick peptidoglycan; without its wall the bacterium takes in water and bursts (lyses) in water.

EXAM TRAPS

- The secondary wall is inside the primary wall, not outside it. It is added between the primary wall and the membrane.
- Collagen gives tensile strength; proteoglycans resist compression. Do not swap them.
- Cellulose is β-1,4 linked glucose; starch is α-1,4. The bond type, not the sugar, makes the difference.
- Gram-positive means thick peptidoglycan and no outer membrane; Gram-negative means thin peptidoglycan with an outer LPS membrane.
- Favourite question: "Describe the components of the extracellular matrix and their functions." List collagen, elastin, GAGs and proteoglycans, fibronectin and laminin, with one function each.

CHECK YOURSELF

– REMEMBER **Name the repeating tripeptide pattern of collagen.** 1 mark

Gly-X-Y, with X often proline and Y often hydroxyproline.

– REMEMBER **What are the layers of a plant cell wall, from outside in?** 2 marks

Middle lamella, primary wall, secondary wall, then the plasma membrane.

– UNDERSTAND **Why does vitamin C deficiency weaken connective tissue?** 3 marks

Prolyl hydroxylase needs vitamin C to make hydroxyproline. Without it, collagen chains form unstable triple helices and weak fibres, causing scurvy.

– UNDERSTAND **How do proteoglycans make cartilage resist compression?** 3 marks

Their GAG chains are densely negatively charged, so they attract cations and hold large amounts of water, forming a gel that pushes back when squeezed.

– APPLY **Why is penicillin harmless to human cells?** 2 marks

It blocks the transpeptidases that cross-link peptidoglycan. Human cells have no peptidoglycan and no such enzymes.

– APPLY **A cell line lacks the integrins that recognise RGD. Predict its behaviour on a fibronectin-coated dish.** 3 marks

The cells cannot bind fibronectin's RGD sequence, so they attach poorly, do not spread, and lose integrin-dependent survival signals.

– ANALYZE **Compare the animal ECM with the plant cell wall as "fibre in a filler" composites.** 4 marks

Animal: collagen fibres (tension) in a proteoglycan gel (compression), flexible and remodelled by MMPs, linked to cells by integrins. Plant: cellulose microfibrils (tension) in hemicellulose and pectin, rigid, resisting turgor pressure, stiffened by lignin in secondary walls. Both pair a strong fibre with a hydrated filler.

BEYOND THE SYLLABUS

Tissue engineers grow cells on scaffolds made from collagen, hyaluronan or decellularised organs, which keep the native ECM after the cells are washed away. Plant cell walls are the raw material of second-generation biofuels, where lignin is the main barrier to breaking cellulose into sugar. This is not for the exam; search "decellularised scaffold" and "lignocellulose pretreatment" to read further.

Unit summary

TERM	MEANING OR FORMULA	CHAPTER
Endocrine / paracrine / autocrine	Signal via blood to distant cells / to nearby cells / back to the cell that made it	5.1
Contact-dependent signalling	Membrane-bound signal acts only on a touching cell (Delta–Notch)	5.1
K_d	$K_d = [L][R]/[LR]$; ligand concentration that fills half the receptors	5.2
Fractional occupancy	$\theta = [L] / ([L] + K_d)$	5.2
Receptor tyrosine kinase	Ligand → dimerisation → cross-phosphorylation → SH2 docking → Ras → MAP kinases	5.2
Nuclear receptor	Intracellular receptor for steroids and thyroid hormone; a ligand-activated transcription factor	5.2
GPCR	Seven-transmembrane receptor acting as GEF for a heterotrimeric G protein (αβγ)	5.3
Gs / Gi / Gq	Adenylyl cyclase on / adenylyl cyclase off / phospholipase C-β on	5.3
Second messengers	cAMP, cGMP, IP_3, DAG, Ca^{2+}	5.4
PKA activation	$R_2C_2 + 4$ cAMP → $R_2(cAMP)_4 + 2$ C (active)	5.4
Cascade gain	Total gain = product of the gains of each step	5.4
PI3K/Akt	PIP_2 → PIP_3 (PI3K); PTEN reverses; Akt drives survival and growth	5.5
Adenylate kinase	2 ADP ⇌ ATP + AMP; $[AMP] \approx [ADP]^2/[ATP]$, so AMP signals energy stress to AMPK	5.5
Dose ratio (Gaddum–Schild)	$r = 1 + [B]/K_B$ for a competitive antagonist	5.6
Junction types	Occluding (tight); anchoring (adherens, desmosome, hemidesmosome); communicating (gap junction, plasmodesmata)	5.7
Collagen	Triple helix, Gly-X-Y repeat; hydroxyproline needs vitamin C	5.8

TERM	MEANING OR FORMULA	CHAPTER
Peptidoglycan	NAG–NAM chains (β-1,4) cross-linked by peptides; thick in Gram-positive	5.8

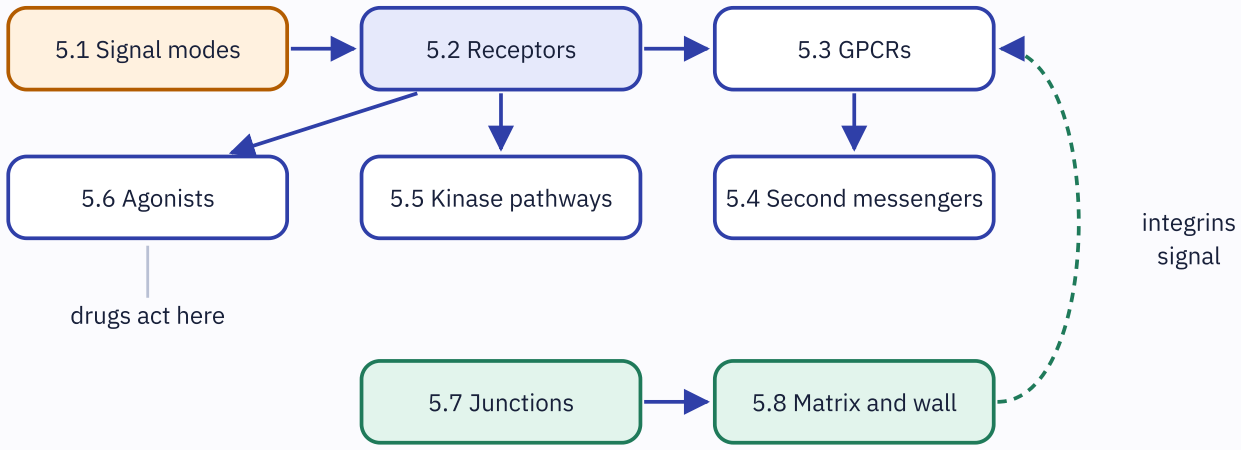


Figure 5.9.1 How the chapters connect. Signalling (orange to blue) runs from the mode of delivery to the receptor and then branches; the contact chapters (green) feed back into signalling because integrins carry messages from the matrix into the cell.

1. Signals are sorted by range: endocrine (blood), paracrine (neighbours), autocrine (self), synaptic (one target via a synapse) and contact-dependent (touch).
2. Water-soluble signals use cell-surface receptors (ion channel, GPCR, enzyme-coupled); small hydrophobic signals such as steroids use intracellular receptors that act on DNA.
3. K_d measures affinity; $\theta = [L]/([L] + K_d)$ gives the fraction of receptors bound.
4. A GPCR is a GEF for a heterotrimeric G protein; the α subunit's own GTPase ends the signal, and toxins that block it keep the pathway on.
5. Second messengers (cAMP, cGMP, IP_3, DAG, Ca^{2+}) carry the signal inside the cell, and each enzyme step multiplies it.
6. PI3K/Akt promotes survival and growth, JAK/STAT carries cytokine signals straight to genes, and AMPK reads AMP as a sign of low energy.
7. Agonists activate receptors (fully or partly); competitive antagonists shift the dose-response curve right, non-competitive ones lower the maximum.
8. Junctions seal (tight), anchor (adherens, desmosome, hemidesmosome) or connect (gap junction); cadherins need Ca^{2+} and integrins bind the matrix.
9. The ECM is collagen fibres in a proteoglycan gel; plant walls are cellulose in hemicellulose and pectin; bacterial walls are peptidoglycan.

Exam question bank

Remember and understand

- UNDERSTAND **Explain the different modes of cell signalling with neat diagrams and examples.** 8 marks

  Define signalling cell, ligand, receptor, response. Endocrine (insulin, adrenaline; blood; low concentration, high-affinity receptors). Paracrine (NO, growth factors; local; kept local by uptake and breakdown). Autocrine (IL-2 in T cells; cancer). Synaptic (acetylcholine; fast, high local concentration). Contact-dependent (Delta–Notch). Diagram of each.

- UNDERSTAND **Classify receptors with examples. Describe how an intracellular steroid receptor works.** 12 marks

  Surface: ion-channel (nicotinic AChR), GPCR (β-adrenergic), enzyme-coupled (insulin receptor, EGF receptor; cytokine receptors with JAK). Intracellular: nuclear receptor superfamily (cortisol, estrogen, thyroid hormone, vitamin D). Glucocorticoid receptor: cytosolic with Hsp90; ligand binding; release; nuclear entry; binding hormone response element; transcription. Slow response. Diagram.

- UNDERSTAND **Describe the structure of tight junctions, desmosomes and gap junctions and give the function of each.** 10 marks

  Tight: claudin and occludin strands; seal and fence. Desmosome: desmoglein and desmocollin, plaque (plakoglobin, desmoplakin), keratin filaments; mechanical strength; pemphigus. Gap: connexins → connexon → channel; small molecules and ions pass; electrical coupling in heart. Labelled diagram of an epithelial cell.

- REMEMBER **List the components of the animal extracellular matrix and describe the structure of collagen.** 8 marks

  Collagen, elastin, GAGs and proteoglycans, fibronectin, laminin, basal lamina. Collagen: three chains, triple helix, Gly-X-Y, proline and hydroxyproline, vitamin C and prolyl hydroxylase, fibril-forming type I versus network type IV.

Apply

- APPLY **Trace the action of adrenaline on a liver cell from receptor to glucose release, and calculate the amplification for given gains.** 12 marks

  β-adrenergic GPCR → Gs (GDP/GTP exchange) → adenylyl cyclase → cAMP → PKA (R_2C_2, 4 cAMP release 2 C) → phosphorylase kinase → glycogen phosphorylase → glucose 1-phosphate. Multiply gains, halving at the PKA step. Termination: GTPase, phosphodiesterase, phosphatases. Spans Chapters 5.3 and 5.4.

- APPLY **Explain the IP_3–DAG pathway and the role of calcium and calmodulin.** 10 marks

  Gq → PLCβ → PIP_2 split → IP_3 (to ER IP_3 receptor, Ca^{2+} release) and DAG (membrane, with Ca^{2+} and phosphatidylserine activates PKC). Ca^{2+} gradient (about 10^{-7} M inside versus 10^{-3} M outside). Calmodulin with four Ca^{2+} sites activates CaM-kinases. Termination by pumps.

- APPLY **Explain how cholera toxin and pertussis toxin act, and predict their effects on cAMP.** 8 marks

  G-protein cycle. Cholera: ADP-ribosylates Gsα, blocks GTP hydrolysis, Gs stays on, cAMP high, chloride and water secretion, diarrhoea. Pertussis: ADP-ribosylates Giα, blocks activation by receptor, Gi stays off, inhibition of cyclase lost, cAMP rises. Same result by opposite routes.

- APPLY **A receptor has K_d = 4 nM. A competitive antagonist with K_B = 1 nM is present at 9 nM. Find the occupancy by agonist alone at 4 nM and 36 nM, and the EC_{50} shift caused by the antagonist.** 8 marks

  θ = 4/8 = 0.5; θ = 36/40 = 0.9. Dose ratio r = 1 + 9/1 = 10, so the curve shifts ten-fold right with the same maximum. Spans Chapters 5.2 and 5.6.

Analyze and evaluate

- ANALYZE **Compare GPCR and receptor tyrosine kinase signalling in structure, activation, messengers, speed and switching off.** 12 marks

  GPCR: 7TM single chain, GEF for αβγ, second messengers, fast, ended by GTPase, desensitised by GRK and arrestin. RTK: single helix, dimerisation and cross-phosphorylation, SH2 docking, Ras–MAPK and PI3K/Akt, slower and often transcriptional, ended by phosphatases (PTEN for PIP_3) and endocytosis. Table and diagram.

– ANALYZE **Discuss how the PI3K/Akt and AMPK pathways together decide whether a cell grows.** 10 marks

PI3K/Akt from insulin and growth factors activates mTORC1 (growth, protein synthesis), GLUT4 translocation, survival via BAD and FOXO. AMPK from high AMP (adenylate kinase squares ADP) inhibits mTORC1 and acetyl-CoA carboxylase. The cell grows only when growth signals are present and energy is sufficient. PTEN loss and metformin as examples.

– ANALYZE **"The extracellular matrix is more than glue." Evaluate with examples.** 10 marks

Mechanical: collagen tension, proteoglycan compression. Signalling: integrins bind RGD of fibronectin and laminin, survival signals, matrix stiffness. Storage of growth factors. Remodelling by MMPs in wound healing and cancer invasion. Basal lamina filtering and guiding repair. Links Chapters 5.7 and 5.8 to the signalling chapters.

– ANALYZE **Compare the animal ECM, the plant cell wall and the bacterial cell wall, and explain why penicillin and lysozyme act only on bacteria.** 12 marks

Fibre and filler in each: collagen in proteoglycan; cellulose in hemicellulose and pectin with lignin; peptidoglycan NAG–NAM with peptide cross-links. Gram-positive versus Gram-negative. Penicillin blocks transpeptidase; lysozyme cuts NAM–NAG; neither target exists in animal or plant cells.

Glossary

Agonist — A ligand that binds a receptor and activates it.

Akt — Protein kinase B; activated by PIP_3, PDK1 and mTORC2; drives survival and growth.

AMPK — AMP-activated protein kinase; switches the cell to energy-saving mode when AMP rises.

Antagonist — A ligand that binds a receptor without activating it and blocks agonists.

Autocrine — Signalling in which a cell responds to a signal it released.

Basal lamina — Thin mat of type IV collagen, laminin and proteoglycans under an epithelium.

β-arrestin — Protein that binds a phosphorylated GPCR, stops G-protein coupling and promotes endocytosis.

Cadherin — Ca^{2+}-dependent cell–cell adhesion protein of adherens junctions and desmosomes.

Calmodulin	Small Ca^{2+}-binding protein (four sites) that activates enzymes when Ca^{2+} rises.
cAMP	Cyclic AMP; second messenger made by adenylyl cyclase, activates PKA.
Connexon	Ring of six connexins forming half of a gap-junction channel.
Desmosome	Spot junction linking the intermediate filaments of neighbouring cells.
EC_{50}	Agonist concentration giving half the maximum response; a measure of potency.
Endocrine	Signalling by hormones carried in the blood to distant cells.
Fibronectin	Matrix glycoprotein with an RGD sequence that integrins bind.
G protein (heterotrimeric)	αβγ switch, on with GTP and off with GDP, activated by GPCRs.
GEF	Guanine nucleotide exchange factor; makes a G protein release GDP so GTP can bind.
Glycosaminoglycan	Long negatively charged disaccharide chain that holds water in the matrix.
Integrin	αβ transmembrane receptor that binds the matrix and links to the cytoskeleton.
IP_3	Inositol 1,4,5-trisphosphate; releases Ca^{2+} from the ER.
JAK/STAT	Cytokine pathway in which Janus kinases phosphorylate STAT transcription factors.
K_d	Dissociation constant; ligand concentration at which half the receptors are bound.
Middle lamella	Pectin-rich layer cementing neighbouring plant cells.
Paracrine	Signalling to nearby cells through the extracellular fluid.
Peptidoglycan	Bacterial wall polymer of NAG and NAM cross-linked by peptides.
PI3K	Phosphoinositide 3-kinase; makes PIP_3 from PIP_2.
PKA	Protein kinase A; R_2C_2 tetramer activated by cAMP.
Proteoglycan	Core protein carrying GAG chains; resists compression.
Second messenger	Small intracellular molecule that relays a signal from a receptor (cAMP, cGMP, IP_3, DAG, Ca^{2+}).
Tight junction	Claudin–occludin seal between epithelial cells near their apical end.

Sources and further reading

- Alberts B. et al., *Molecular Biology of the Cell*, Garland Science / W. W. Norton: the chapters on cell signalling and on cell junctions and the extracellular matrix (from memory; check the edition you are prescribed).
- Lodish H. et al., *Molecular Cell Biology*, W. H. Freeman: the chapters on signal transduction and on integrating cells into tissues (from memory; check).
- Karp G., *Cell and Molecular Biology: Concepts and Experiments*, Wiley: the chapters on cell signalling and on interactions between cells and their environment (from memory; check).
- Cooper G. M., *The Cell: A Molecular Approach*, Sinauer / Oxford University Press: the chapter on cell signalling (from memory; check).
- Alberts B. et al., *Molecular Biology of the Cell*, 4th edition (2002), "General Principles of Cell Communication", free on NCBI Bookshelf: ncbi.nlm.nih.gov/books/NBK26813 (source of the hormone and acetylcholine concentrations and the nitric oxide half-life used in Chapter 5.1).
- Wikipedia, "Gap junction": en.wikipedia.org/wiki/Gap_junction (consulted for the connexon structure and the range of size limits quoted in Chapter 5.7).

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