Module 1

Introduction

The aim of this website is to describe cell signalling within its biological context. There has been an explosion in the characterization of signalling components and pathways. The next major challenge is to understand how cells exploit this large signalling toolkit to assemble the specific signalling pathways they require to communicate with each other. The primary focus is the biology of cell signalling. The emerging information on cell signalling pathways is integrated and presented within the context of specific cell types and processes. The beauty of cell signalling is the way different pathways are combined and adapted to control a diverse array of cellular processes in widely different spatial and temporal domains.

The first half of the website characterizes the components and properties of the major cell signalling pathways, with special emphasis on how they are switched on and off. Attention is also focused on the spatial and temporal aspects that determine how information is encoded and distributed to precise cellular locations. The second half of the website deals with the way these different signalling pathways are employed to control the life history of cells from their birth during the process of cell proliferation, their differentiation into specific cell types to carry out different cell functions, and finally their death through processes such as apoptosis. Cell signalling orchestrates all these cellular processes. Many of the same signalling systems that control development come into play again to regulate a wide range of specific processes in adult cells, such as contraction, secretion, metabolism, proliferation, information processing in neurons and sensory perception. These examples illustrate how cell signalling pathways are adapted and co-ordinated to regulate many different cellular processes. This intimate relationship between cell signalling and biology is providing valuable insights into the underlying genetic and phenotypic defects responsible for many of the major human diseases.

Overview of cell signalling mechanisms

Cells in organisms such as us constantly communicate with each other. This cellular discourse occurs through both electrical and chemical signals (Module 1: Figure cell communication). Communication through electrical signals is very fast and depends upon the presence of gap junctions to allow information to pass directly from one cell to its neighbour. Communication through chemical signals is by far the major form of information transfer between cells. One cell releases a chemical stimulus (e.g. a neurotransmitter, hormone or growth factor), which then alters the activity of target cells. The latter have receptors capable of detecting the incoming signal and transferring the information to the appropriate internal cell signalling pathway to bring about a change in cellular activity.

Communication through electrical signals

Communication through electrical signals is found mainly in excitable systems, particularly in the heart and brain. It is usually fast and requires the cells to be coupled together through low-resistance pathways such as the gap junctions (Module 1: Figure cell communication). In addition to passing electrical charge, the pores in these gap junctions are large enough for low-molecular-mass molecules such as metabolites and second messengers to diffuse from one cell to another.

Communication through chemical signals

Cells are enclosed within a lipophilic plasma membrane, which represents a formidable barrier that has to be crossed by all incoming signals. Hydrophobic hormones, such as the steroid hormones, can simply diffuse across this cell-surface barrier to gain access to protein receptors located in either the cytoplasm or the nucleus. More elaborate mechanisms are required for the water-soluble stimuli (e.g. hormones, neurotransmitters and growth factors) that cannot cross the plasma membrane (Module 1: Figure cell communication). The basic concept of a cell signalling pathway, therefore, concerns the mechanisms responsible for receiving this external information and relaying it through internal cell signalling pathways to activate the sensor and effector mechanisms that bring about a change in cellular responses (Module 1: Figure cell signalling mechanism). Cell signalling is a dynamic process in that there are ON mechanisms during which information flows down the signalling pathway in response to external stimuli (the green arrows in Module 1: Figure cell signalling mechanism), opposed by the OFF mechanisms that are responsible for switching off the signalling system once external stimuli are withdrawn (the red arrows in Module 1: Figure cell signalling mechanism). Some of these basic principles of cell signalling are explored in this introductory module, which will also briefly outline the contents of the other modules.

ON mechanisms

Most signalling pathways begin with the arrival of external cell stimuli usually in the form of a chemical signal, which is received by receptors at the cell periphery that function

Green text indicates links to content within this module; blue text indicates links to content in other modules

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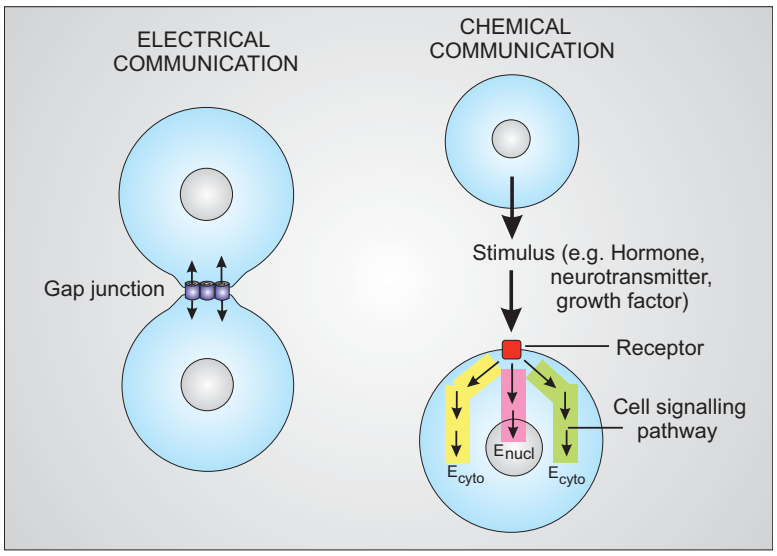
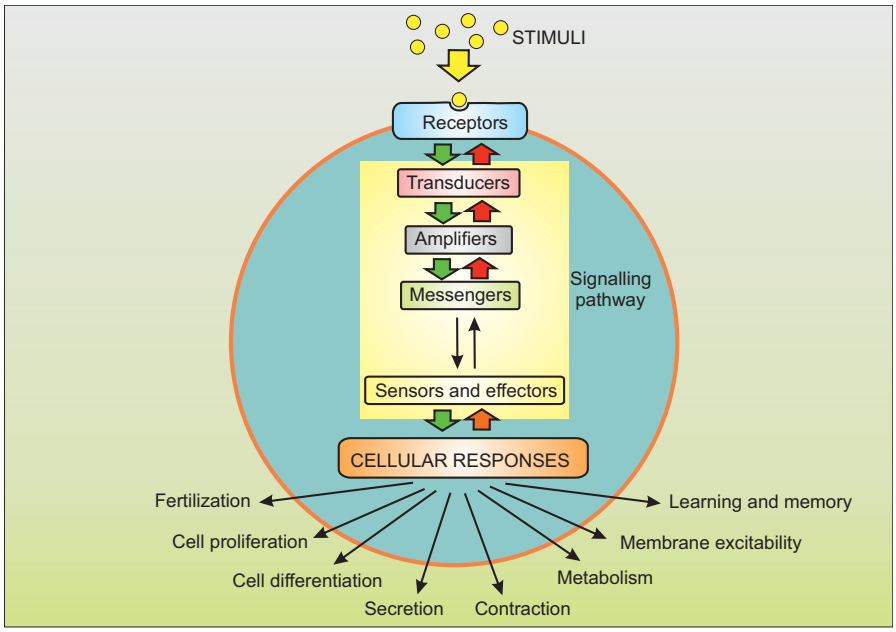
Module 1: | Figure cell communication



Cell communication through electrical and chemical signalling mechanisms.
Cells that are connected through the low-resistance gap junctions (shown on the left) can communicate rapidly with each other by passing electrical current or through the diffusion of low-molecular-mass second messengers such as cyclic AMP and inositol 1,4,5-trisphosphate ($InsP_3$). In the case of chemical communication (shown on the right), one cell releases a stimulus, which diffuses to a target cell that has receptors to detect the stimulus and to relay information along various cell signalling pathways to activate effectors either in the nucleus (E_{nucl}) or cytoplasm (E_{cyto}).

Module 1: | Figure cell signalling mechanism



The basic principle of a cell signalling pathway.
Stimuli (e.g. hormones, neurotransmitters or growth factors) acting on cell-surface receptors relay information through intracellular signalling pathways that can have a number of components. They usually begin with the activation of transducers that use amplifiers to generate internal messengers that either act locally or can diffuse throughout the cell. These messengers then engage sensors that are coupled to the effectors that are responsible for activating cellular responses. The green and red arrows indicate that cell signalling is a dynamic process consisting of ON mechanisms (green arrows) during which information flows down the pathway, opposed by the OFF mechanisms (red arrows) that switch off the different steps of the signalling pathway.

as molecular antennae embedded in the plasma membrane (**Module 1: Figure cell signalling mechanism**). These receptors then function to transfer information to a variety of **transducers and amplifiers** to produce **intracellular messengers**. These messengers stimulate the **sensors and effectors** responsible for activating cellular responses. These ON mechanisms responsible for transmitting information into the cell are counteracted by the **OFF mechanisms** that switch off this flow of information once stimuli are withdrawn. A related OFF mechanism is **receptor desensitization**, whereby receptors lose their sensitivity to external stimuli.

These cell signalling pathways utilize a variety of **information transfer mechanisms** such as diffusion, direct protein–protein interactions or covalent modifications, such as protein phosphorylation, acetylation and nitrosylation.

The effectiveness of information transfer is greatly enhanced through the **spatial and temporal aspects of cell signalling pathways**.

Each cell type has a unique repertoire of cell signalling components that will be referred to here as the cellular **signalsome**. During the final stages of development, cells express a particular phenotype, and this process of differentiation includes the expression of a distinctive set of signalling components (a cell-type-specific signalsome) required to control their particular functions. These signalsomes have a high degree of plasticity and are constantly being remodelled to cope with changing demands. Abnormal remodelling of cellular signalsomes creates signalling defects that have great significance for the onset of many diseases.

Signalling pathways do not operate in isolation, and a key element of cellular control mechanisms is the extensive cross-talk between signalling pathways.

These highly integrated signalling mechanisms act through different effectors (e.g. muscle proteins, secretory vesicles, transcription factors, ion channels and metabolic pathways) to control the activity of cellular processes such as development, proliferation, neural signalling, stress responses and apoptosis.

Cell stimuli

Cells are sensitive to an enormous variety of stimuli. Many of the stimuli are hormones that come in two main types. Classical hormones that are released from one group of cells and enter the circulation to act on another group of cells in a separate tissue. Then there are local hormones that usually are not dispersed through the circulation but they act within a local community of cells in an autocrine or paracrine manner. There are a small group of cell surface stimuli that do not leave the cell of origin but remain in the cell surface to activate receptors on neighbouring cells through a juxtacrine mechanism. Most stimuli are chemical in nature, such as hormones, neurotransmitters and growth factors. However, cells can also detect other modalities such as a wide range of sensory stimuli. In the following list, which is somewhat arbitrary, these stimuli have been placed in five main groups: neurotransmitters, hormones and local hormones, growth factors, cell-surface stimuli and sensory stimuli.

Neurotransmitters

Acetylcholine
ATP
Dopamine
γ-Aminobutyric acid (GABA)
Gastrin-releasing peptide (GRP)
Glutamate
5-Hydroxytryptamine (5-HT) (serotonin)
Kisspeptin
Neurotensin
Noradrenaline
Melanocortin
Melatonin
Orexin
Tachykinins
  Neurokinin A
  Neuropeptide K
  Substance P
Vasoactive intestinal polypeptide (VIP)

Hormones and Local Hormones

Activin
Adrenaline
Adenosine
Angiotensin II
Adiponectin
Adrenocorticotropic hormone (ACTH)
Aldosterone
Atrial-natriuretic factor (ANF)
Bombesin
Bradykinin
Brain-derived neurotrophic factor (BDNF)
Brain-type natriuretic peptide (BNP)
Calcitonin
Calcitonin gene-related peptide
Cardiotrophin (CT-1)
Chemokines
Cholecystokinin (CCK)
Ciliary neurotrophic factor (CNTF)
Colony-stimulating factor 1 (CSF-1)
Corticotropin-releasing factor (CRF)
Cortistatin (CST)
C-type natriuretic peptide (CNP)
1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]
Eicosanoids
Endocannabinoids
  Anandamide
  2-Arachidonoylglycerol (2-AG)
β-Endorphin
Endothelin
Erythropoietin (EPO)
Follicle-stimulating hormone (FSH)
Ftl ligand (FL)
Galanin
Gastrin
Ghrelin
Glucagon-like peptide 1 (GLP-1)
Glucagon
Gonadotropin-releasing hormone (GnRH)
Granulocyte colony-stimulating factor (G-CSF)
Granulocyte-macrophage colony-stimulating factor (GM-CSF)
Growth hormone (GH)
Growth hormone-releasing hormone (GHRH)
Guanylin
Hedgehog (Hh)
Histamine
Incretins
Inhibin
Insulin

Interferons (IFNs)
- Interferon-α (IFN-α)
- Interferon-β (IFN-β)
- Interferon-γ (IFN-γ)

Interleukins
- Interleukin-1 (IL-1)
- Interleukin-2 (IL-2)
- Interleukin-3 (IL-3)
- Interleukin-5 (IL-5)
- Interleukin-6 (IL-6)
- Interleukin-10 (IL-10)
- Interleukin-21 (IL-21)

Leptin
Leukaemia inhibitory factor (LIF)
β-Lipotropin
γ-Lipotropin
Luteinizing hormone (LH)
Lysophosphatidic acid (LPA)
α-Melanocyte-stimulating hormone (α-MSH)
β-Melanocyte-stimulating hormone (β-MSH)
γ-Melanocyte-stimulating hormone (γ-MSH)
Monocyte chemoattractant protein-1 (MCP-1)
Myostatin
Nerve growth factor (NGF)
Neuropeptide Y (NPY)
Neurotrophin-3 (NT-3)
Neurotrophin-4/5 (NT-4/5)
Nitric oxide (NO)
Oestradiol
Omega-3 fatty acid
Oncostatin (OSM)
Osteocalcin
Oxytocin
Oxyntomodulin (OXM)
Pathogen-associated molecular patterns (PAMPs)
Pancreatic polypeptide (PP)
Parathyroid hormone (PTH)
Parathyroid hormone (PTH)-related peptide (PTHrP)
Peptide YY (PYY)
Pituitary adenylyl cyclase-activating peptide (PACAP)
Platelet-activating factor (PAF)
Prolactin (PRL)
Protectin D1
Renin
Resistin
Resolvin E1
Resolvin E2
Resolvin D1
Secretin
Somatostatin (Sst)
Sphingosine-1-phosphate (S1P)
Stem cell factor (SCF)
Thrombin
Thrombopoietin (TPO)
Thyroid-stimulating hormone (TSH)
Thyrotropin-releasing hormone (TRH)
Thyroxine (T_4)
Transforming growth factor β (TGF-β)
Tumour necrosis factor α (TNFα)
Vasopressin
Wnt

Growth factors

Angiopoietin growth factors
- Ang1
- Ang2
- Ang3
- Ang4

Epidermal growth factors (EGFs)
- Amphiregulin (AR)
- Betacellulin (BTC)
- Epiregulin (EPR)
- Heparin-binding EGF-like growth factor (HB-EGF)
- Neuregulin
- Transforming growth factor-α (TGFα)

Insulin-like growth factors (IGFs)
- IGF-I
- IGF-II

Fibroblast growth factors (FGFs)
Hepatocyte growth factor (HGF)
Platelet-derived growth factors (PDGFs)
- PDGF-A
- PDGF-B
- PDGF-C
- PDGF-D

Progranulin (PGRN)
Vascular endothelial growth factors (VEGFs)
- VEGF-A
- VEGF-B
- VEGF-C
- VEGF-D

Placental growth factor (PLGF)

Cell surface stimuli

CD40L
CD86
Delta
Ephrin A
Ephrin B
FasL
ICOS
Jagged
MHC II-antigen complex
RANKL

Sensory stimuli

Light
Oxygen
Odorants
Sound
Tastants
Touch
Temperature

Stimuli can be released from cells in many different ways (Module 1: Figure formation and action of cell stimuli). A number of stimuli, particularly growth factors and cytokines, are carried in vesicles to the cell surface where they appear as a membrane-anchored cell surface stimulus

that can function as such or is a precursor that is cleaved by proteases such as the ADAM proteases to release the soluble stimulus through a process known as ectodomain shedding. Many stimuli, such as the eicosanoids, nitric oxide (NO), ATP and sphingosine 1-phosphate (S1P), are formed within the cytoplasm and leave the cell by diffusing across the plasma membrane. In some cases, this exit from the cell is facilitated by special transporters such as the ATP-binding cassette (ABC) transporters (Module 3: Figure ABC transporters). Many stimuli are synthesized as large precursors, such as pro-opiomelanocortin (POMP), which then undergo extensive, tissue-specific processing as they are cleaved into a number of individual hormones or neuropeptides.

Many stimuli such as hormones as neurotransmitters are packaged in to vesicles where they are stored before being released by exocytosis. These stimuli then have different modes of action (juxtacrine, autocrine, paracrine and endocrine), which have been defined on the basis of how far they travel to reach their cellular receptor targets.

Once these cell stimuli reach their targets they use a diverse number of cell signalling pathways to control cellular activity (Module 2: Figure cell signalling pathways). One way of describing this diversity is to consider the nature of the stimuli that feed into different cell signalling pathways. Some signalling mechanisms are used by many different signalling pathways, whereas other pathways respond to a specific set of stimuli. An example of the former is the cyclic AMP signalling pathway, which was the first signalling pathway to be clearly defined (Module 1: Figure stimuli for cyclic AMP signalling). The major stimuli for this signalling pathway fall into two main classes: neurotransmitters and hormones. They all act by engaging G protein-coupled receptors (GPCRs), which use heterotrimeric GTP-binding proteins (G proteins) to activate the amplifier adenylyl cyclase that converts ATP into the second messenger cyclic AMP (for further details see Module 2: Figure cyclic AMP signalling). Some of the stimuli that activate this signalling pathway belong to a group of lipid-derived stimuli known as the eicosanoids that include the prostaglandins, thromboxanes and leukotrienes (Module 1: Figure eicosanoids). The endocannabinoids are another group of lipid stimuli such as anandamide and 2-arachidonylglycerol (2-AG).

The inositol 1,4,5-trisphosphate ($InsP_3$)/diacylglycerol (DAG) signalling pathway is also used by a very large number of stimuli that are mainly neurotransmitters and hormones (Module 1: Figure stimuli for $InsP_3$/DAG signalling). These external stimuli bind to GPCRs, which are coupled to G proteins to activate the amplifier phospholipase C (PLC). PLC hydrolyses an inositol lipid to generate the two second messengers, $InsP_3$ and DAG (for further details see Module 2: Figure $InsP_3$ and DAG formation). This signalling pathway is also used by other groups of stimuli such as the growth and survival factors (Module 1: Figure stimuli for enzyme-linked receptors) and some of the Wnt stimuli that control development (Module 1: Figure stimuli for developmental signalling).

The cytokines are a diverse group of stimuli that function mainly in the control of haematopoiesis and immune responses, particularly during inflammation. There are over 40 members of the family that seem to function through two main receptor types (Module 1: Figure cytokines).

There are a number of peptides such as atrial natriuretic peptide (ANP), brain-type natriuretic peptide (BNP), C-type natriuretic peptide (CNP) and guanylin that act through the particulate guanylyl cyclases (pGCs).

There are a number of stimuli capable of opening ion channels (Module 1: Figure stimuli for ion channels). In this case, the ion channel carries out all the signalling functions.

Most of the stimuli described above arrive at the cell surface through a process of diffusion. In some cases, however, stimuli can be presented to receptors by special molecules. A classic example is the role of MHCII on the antigen-presenting cell that binds fragments of antigen that are then presented to the T cell receptor (Module 9: Figure TCR signalling). Another example is the role of CD14 in presenting lipopolysaccharide (LPS) to the Toll-like receptor (TLR) (Module 2: Figure Toll receptor signalling).

Juxtacrine

Direct cell-to-cell signalling achieved by a membrane-anchored stimulus in one cell acting on receptors located in a neighbouring cell (Module 1: Figure formation and action of cell stimuli). The following are examples where information is transmitted between cells through such a direct mechanism:

- Notch signalling pathway (Module 2: Figure Notch signalling).
- Eph receptor signalling (Module 1: Figure Eph receptor signalling).
- Classical cadherin signalling (Module 6: Figure classical cadherin signalling).
- Activation of the Fas receptor (FasR) by the Fas ligand (FasL) as occurs during activation of the extrinsic pathway of apoptosis (Module 11: Figure apoptosis).
- The inflammatory mediator TNFα can function in its transmembrane pro-form.

Autocrine

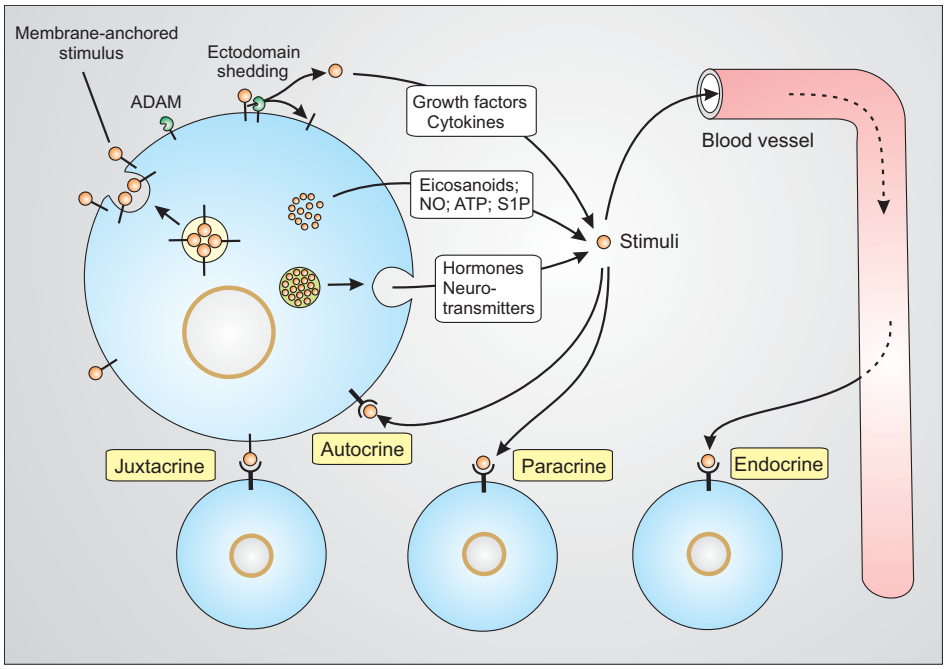
This is a local signalling mechanism whereby the cell that releases a stimulus has receptors capable of responding to that stimulus (Module 1: Figure formation and action of cell stimuli). An example of such an autocrine response is found in blood platelets that release eicosanoids that feed back to influence the progress of the platelet activation sequence (Module 11: Figure platelet activation).

Adenosine is another example of an autocrine factor that may function as an endogenous sleep-regulatory molecule.

Paracrine

Paracrine refers to a local signalling process whereby one cell releases a stimulus that diffuses away to act locally on cells in the immediate neighbourhood (Module 1: Figure formation and action of cell stimuli). The following are but a few of the many examples of paracrine signalling processes:

Module 1: | Figure formation and action of cell stimuli



Formation and mode of action of cell stimuli.
Cell stimuli are released from cells through different mechanisms: membrane-anchored stimuli are released by ectoderm shedding; stimuli formed in the cytoplasm pass out across the plasma membrane; stimuli packaged into vesicles are released by exocytosis. Such stimuli have four main modes of action. Membrane-anchored stimuli on the surface can activate receptors on neighbouring cells directly (juxtacrine). Stimuli that are released from the cell can feed back to activate receptors on the same cell (autocrine); they can diffuse to neighbouring cells (paracrine); or they enter the blood stream to act on cells further a field (endocrine).

- Neurotransmitters transfer information from the synaptic ending to the postsynaptic membrane during neuronal information transfer (see Step 3 in Module 10: Figure kinetics of neurotransmission).
- Acetylcholine (ACh) released from motor neurons triggers excitation-contraction coupling in skeletal muscle (Module 7: Figure skeletal muscle E-C coupling).
- Release of chemokines controls neutrophil chemotaxis during inflammatory responses (Module 11: Figure inflammation) and the migration of haematopoietic stem cells (HSCs) in the bone barrow (Module 8: Figure bone marrow).
- The H_2O_2 released from wounds during wound healing may function as a paracrine signal to attract leucocytes.
- Release of nitric oxide (NO) to control smooth muscle cells through the nitric oxide (NO)/cyclic GMP signalling pathway (Module 2: Figure NO and cyclic GMP signalling).
- Release of sphingosine 1-phosphate (S1P), which is released from endothelial cells as part of the sphingomyelin signalling pathway (Module 2: Figure sphingomyelin signalling) controls the activity of pericytes (Module 9: Figure angiogenesis signalling).
- Release of the growth factor platelet-derived growth factor B (PDGF-B) from tip cells during angiogenesis acts locally to control the proliferation of pericytes (Module 9: Figure angiogenesis signalling).
- ATP and acetylcholine (ACh) released from globus cells act locally to excite sensory nerve endings during O_2 sensing by the carotid body (Module 10: Figure carotid body chemoreception).

Orexin

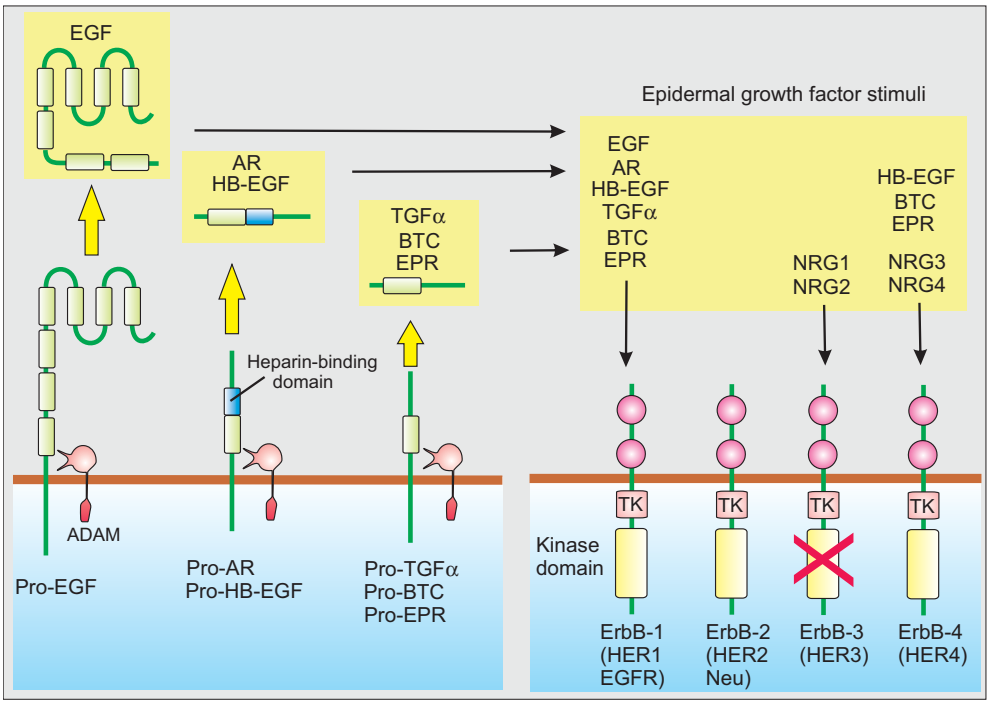
There are two orexins (orexin-A and orexin-B), which are peptide neurotransmitter that are synthesized and released from approximately 7,000 orexin neurons located in the lateral hypothalamus (Module 10: Figure brain anatomy). They are synthesized as a prepro-orexin precursor peptide that is then processed to form orexin-A (a 33-amino acid peptide) and orexin-B (a 28-amino acid peptide). Orexin-A acts through the OX_1R that is coupled to $G_{q/11}$ to activate phospholipase C (PLC) to give $InsP_3$ and DAG (Module 2: Figure $InsP_3$/DAG recycling), whereas the orexin-B acts through OX_2R that is also coupled to $G_{q/11}$, but can also act through $G_{i/o}$ to inhibit the cyclic AMP signalling pathway.

Orexin has a central role in regulating the sleep and wake regulatory mechanisms (Module 10: Figure sleep/wake cycle regulation).

Endocrine

During endocrine signalling, the stimulus is usually a hormone that is released from one cell to enter the blood stream to be carried around the body to act on cells expressing the appropriate receptors. There are numerous examples of how cellular activity is regulated through such endocrine control mechanisms:

Module 1: | Figure EGF stimuli and receptors



Epidermal growth factor (EGF) family of stimuli and their receptors.
Epidermal growth factor (EGF) stimuli precursors are located in the plasma membrane and are then cleaved by proteases, such as the ADAM proteases. The soluble growth factors then act on two of the main EGF receptor types. AR, amphiregulin; BTC, betacellulin; EPR, epiregulin; HB-EGF, heparin-binding EGF-like growth factor; TGFα, transforming growth factor-α.

- During the operation of the metabolic energy network, endocrine cells such as the insulin secreting β-cells and glucagon-secreting α-cells release insulin and glucagon respectively to alter the metabolic activity of liver and muscle cells (Module 7: Figure metabolic energy network).
- During the control of food intake and body weight, various endocrine cells in the gut release gut hormones that alter the activity of the neural circuits that control the feeding and satiety centres in the brain (Module 7: Figure control of food intake).

Ectodomain shedding

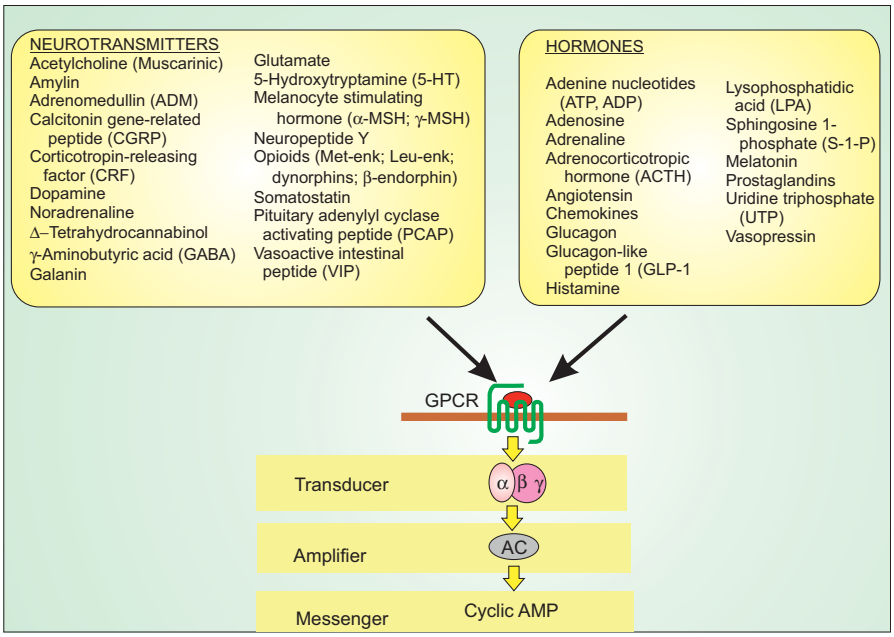
Many cell stimuli such as growth factors and cytokines are released from cells through a process of ectodomain shedding (Module 1: Figure formation and action of cell stimuli). The precursor of the growth factor is inserted into the surface membrane and is then cleaved by proteases to release the extracellular region as shown for the epidermal growth factor (EGF) stimuli (Module 1: Figure EGF stimuli and receptors). The different members of the EGF family are transported to the plasma membrane where they are anchored through a transmembrane region. ADAM proteases such as ADAM-12 hydrolyse the protein chain near the membrane to shed the extracellular region that is then free to diffuse away to act on the EGF receptors on neighbouring cells.

Ectodomain shedding can also be used to inactivate cell surface receptors by cleaving off the extracellular domains as occurs for the tumour necrosis factor α (TNFα) receptor. Mutations in the cleavage site of the TNF receptor that prevents its cleavage are the cause of TNF-receptor-associated periodic febrile syndrome (TRAPs).

Eicosanoids

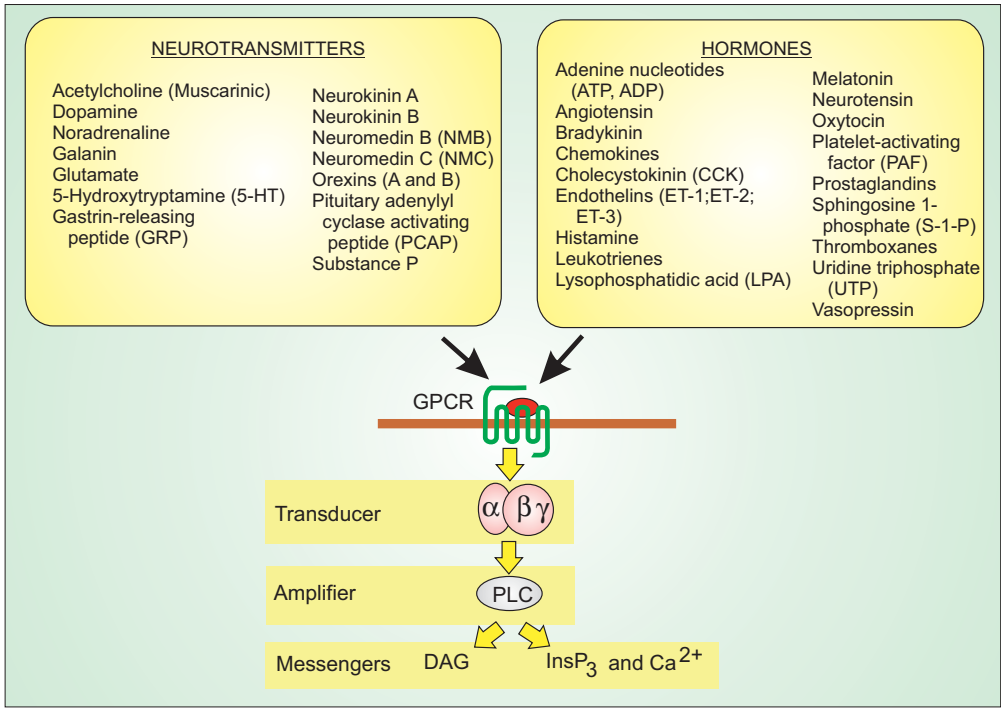
The eicosanoids are a group of stimuli that are derived from the metabolism of arachidonic acid (AA). AA is a fatty acid that is located on the *sn* – 2 position of many phospholipids. The enzyme phospholipase A_2 (PLA_2), which is activated by Ca^{2+} and by the mitogen-activated protein kinase (MAPK) signalling pathway, releases AA, which is then metabolized via two pathways to produce the prostanoids (prostaglandins and thromboxanes) and the leukotrienes (Module 1: Figure eicosanoids). The first step in the formation of the prostanoids is the enzyme cyclooxygenase (COX) that converts AA into the unstable cyclic endoperoxides prostaglandins PGG_2 and PGH_2. The latter is a precursor that is converted by various isomerases into the prostaglandins PGI_2, PGD_2, PGE_2 and $PGF_{2\alpha}$, and thromboxane A_2 (TXA_2). The formation of the leukotrienes begins with the enzyme 5-lipoxygenase (5-LO) that converts AA into the hydroxyperoxide 5-hydroperoxyeicosatetraenoic acid (5-HPETE). The activity of 5-LO depends upon a 5-lipoxygenase-activating protein (FLAP) that may function by presenting AA to 5-LO. The 5-HPETE is converted into leukotriene A_4 (LTA_4), which is the precursor for two enzymes. An LTC_4 hydrolase converts LTA_4 into LTB_4, whereas an LTC_4 synthase converts LTA_4 into LTC_4. This synthetic step

Module 1: | Figure stimuli for cyclic AMP signalling



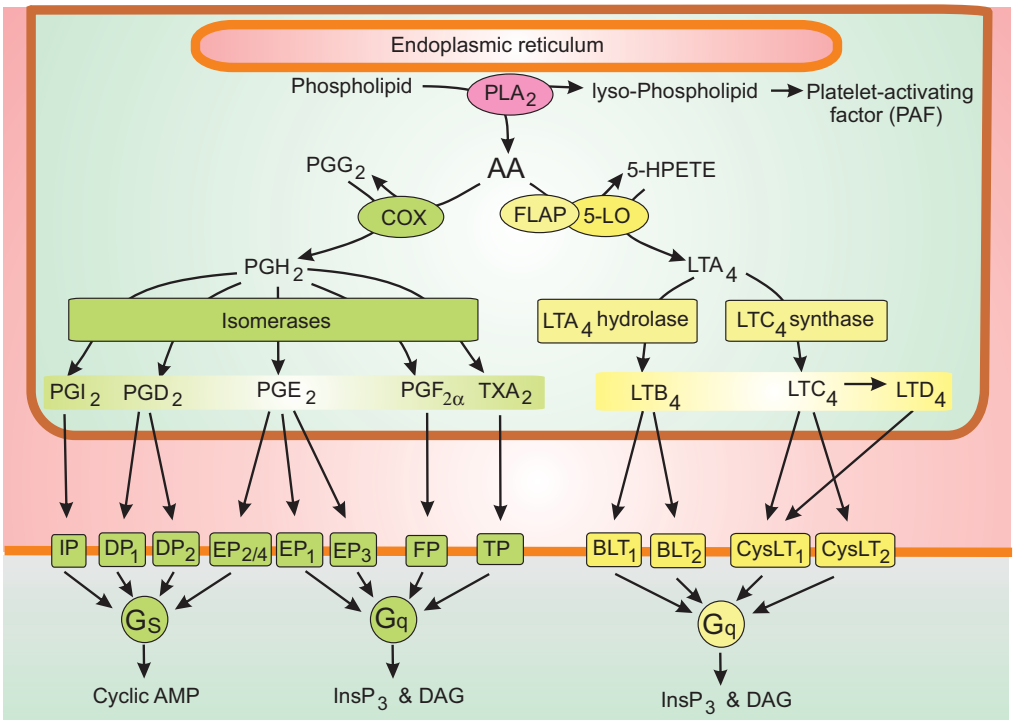
Cell stimuli that act through the cyclic AMP signalling pathway.
A large number of neurotransmitters and hormones act through G protein-coupled receptors (GPCRs) to engage the cyclic AMP signalling pathway. The GPCR acts through a heterotrimeric G protein, which has α and βγ subunits, to stimulate the amplifier adenylyl cyclase (AC) to generate the second messenger cyclic AMP.

Module 1: | Figure stimuli for $InsP_3$/DAG signalling



Cell stimuli that act through the inositol 1,4,5-trisphosphate ($InsP_3$)/diacylglycerol (DAG) signalling pathway.
A large number of neurotransmitters and hormones act through G protein-coupled receptors (GPCRs) to engage the $InsP_3$/DAG signalling pathway. The GPCRs that sense these different stimuli all feed into a family of heterotrimeric G proteins, which have α and βγ subunits that stimulate the amplifier phospholipase C (PLC) to generate the two second messengers, $InsP_3$ and DAG.

Module 1: | Figure eicosanoids



Synthesis and mode of action of the eicosanoids.
The eicosanoids are lipid-derived stimuli that are formed from arachidonic acid (AA), which is converted into the prostanoids (green pathway) and the leukotrienes (yellow pathway). The AA is produced by phospholipase A_2 (PLA_2), which attaches itself to the endoplasmic reticulum where it hydrolyses phospholipids to lysophospholipids with the release of AA. The AA is then metabolized via two separate pathways to produce the prostanoids and leukotrienes. The types of receptors and signalling pathways used by the eicosanoids are shown at the bottom.

depends upon the conjugation of LTA_4 with glutathione to form LTC_4, which is thus referred to as a cysteinyl-containing leukotriene, as are its derivatives LTD_4 and LTE_4.

These eicosanoids are lipophilic and can thus diffuse out from their cell of origin to act on neighbouring cells. Also shown in Module 1: Figure eicosanoids are the family of G protein-coupled receptors (GPCRs) that detect these eicosanoids. These receptors are connected to either the cyclic AMP signalling pathway (Module 1: Figure stimuli for cyclic AMP signalling) or the inositol 1,4,5-trisphosphate ($InsP_3$)/diacylglycerol (DAG) signalling pathway (Module 1: Figure stimuli for $InsP_3$/DAG signalling).

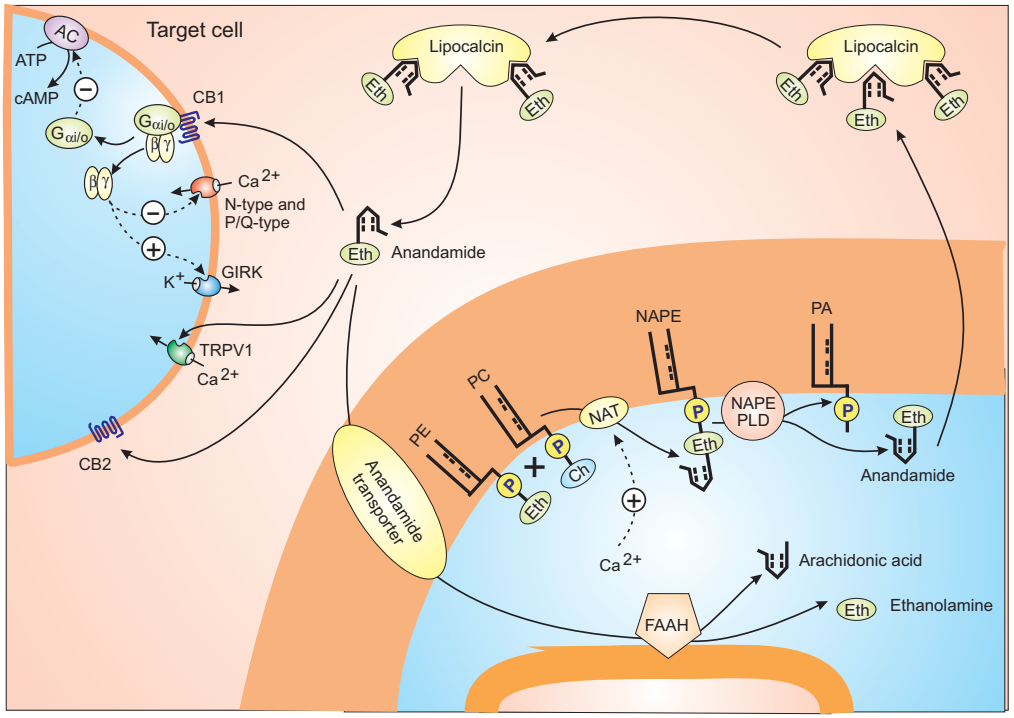
Inflammatory cells such as macrophages, mast cells and blood platelets produce large amounts of these eicosanoids during an inflammatory response. In macrophages, there is an increase in the expression of COX that results in an increase in the release of mediators such as platelet-activating factor (PAF) and PGE_2 (Module 11: Figure macrophage signalling). In blood platelets, PGI_2 acting on its receptor activates cyclic AMP formation, which then inhibits platelet activation (Module 11: Figure platelet activation)

Phospholipase A_2 (PLA_2)

Phospholipase A_2 (PLA_2) functions to cleave the *sn* – 2 ester bond of phospholipids to release the fatty acid to leave behind a lysophospholipid (Module 1: Figure eicosanoids). Both of these products have a role in forming stimuli for cell signalling. If the lysophospholipid is derived from phosphatidylcholine with an alkyl linkage in the *sn* – 1 position, it functions as a precursor for an acetyltransferase that converts it into platelet-activating factor (PAF). The free fatty acid that is released from the *sn* – 2 position is often arachidonic acid (AA), which is a precursor for the synthesis of the eicosanoids, such as the prostaglandins, thromboxanes and leukotrienes.

There is a large family of PLA_2 enzymes that function to provide these two signalling precursors. Humans have about 15 enzymes that differ with regard to their cellular distribution and how they are activated. Some of the enzymes are secreted ($sPLA_2$). The cytosolic forms fall into two main groups: the Ca^{2+}-sensitive ($cPLA_2$) and Ca^{2+}-insensitive ($iPLA_2$) groups. In the case of $cPLA_2$, enzyme activity is activated by Ca^{2+} that acts through Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which phosphorylates Ser-515 causing the enzyme to translocate to internal membranes such as the endoplasmic reticulum. Full activation of the enzyme is also dependent upon the mitogen-activated protein kinase (MAPK) signalling pathway. Mast cells provide an example of how PLA_2 functions to generate cell signalling stimuli (Module 11: Figure mast cell signalling). PLA_2 may also function in osmosensing by responding to cell swelling to produce lipid messengers that activate channels such as TRPV4.

Module 1: | Figure anandamide



Anandamide formation and signalling function

Anandamide formation begins with an *N*-acetyltransferase (NAT) removing an arachidonyl moiety from phosphatidylcholine (PC) and attaches it to phosphatidylethanolamine (PE) to form *N*-arachidonyl-phosphatidylethanolamine (NAPE), which is the anandamide precursor. A NAPE phospholipase D (NAPE PLD) hydrolyses this precursor to leave phosphatidic acid (PA) in the membrane and releasing anandamide. The anandamide diffuses across the membrane and attaches to lipocalcin, which carries it to its target cells where it acts by binding to the cannabinoid receptor 1 or 2 (CB1 or CB2). Anandamide is inactivated by entering cells through the anandamide transport and is hydrolysed to arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH).

Endocannabinoids

The active ingredient in the *Cannabis* plant is Δ^9-tetrahydrocannabinol (THC), which has marked psychoactive properties. The brain contains the cannabinoid receptor CB1, which not only responds to THC but also responds to the endogenous cannabinoids (endocannabinoids). The two main endocannabinoids appear to be anandamide and 2-arachidonoylglycerol (2-AG), but other related molecules such as noladin ether, *N*-arachidonoyldopamine and virodhamine have been identified. A feature of all of these endocannabinoids is that they contain an arachidonyl moiety.

The signalling function of the endocannabinoids are carried out by two cannabinoid receptors (CB1 and CB2), which are typical **G-protein-coupled receptor (GPCR)** (**Module 1: Table G protein-coupled receptors**). Most of the CB1 receptors are located in the brain, but can occur elsewhere. On the other hand, the CB2 receptors are found mainly on immune cells. In the brain the **endocannabinoid retrograde signalling mechanism** is particularly important (**Module 10: Figure endocannabinoid retrograde signalling**).

Dysregulation of the endocannabinoid signalling system occurs during **obesity** and may contribute to metabolic abnormalities such as **insulin resistance** and the onset of Type 2 diabetes.

Anandamide

Anandamide [*N*-arachidonylethanolamine (AEA)] is one of the major endocannabinoids that functions in the control of a number of important processes, including white fat cell insulin resistance (Module 12: Figure insulin resistance), and contributes to the process of Ca^{2+} and synaptic plasticity during learning and memory in neurons (Module 10: Figure Ca^{2+}-induced synaptic plasticity). Like many other endocannabinoids, anandamide contains an arachidonyl moiety that is derived from phosphatidylcholine (PC) in the plasma membrane (Module 1: Figure anandamide). Anandamide formation begins with an N-acetyltransferase (NAT) removing this arachidonyl moiety from phosphatidylcholine (PC) and transferring it to the ethanolamine head group of phosphatidylethanolamine (PE) to form N-arachidonyl-phosphatidylethanolamine (NAPE). This anandamide precursor is then cleaved by a specific NAPE phospholipase D (PLD) to leave phosphatidic acid (PA) in the membrane and releasing anandamide. Since it is lipophilic, the anandamide can easily cross the plasma membrane where it appears to associate with lipocalcin that acts as a vehicle to carry anandamide to neighbouring cells where it can carry out its paracrine functions.

The formation of anandamide is sensitive to Ca^{2+}, which acts by stimulating NAT.

At its target cells, anandamide can act through a number of receptors, both classical G-protein-linked receptors (GPCRs) and receptor-operated channels such as TRPV1 and TRPV4. There are two cannabinoid receptors CB1 and CB2. The CB1 receptor, which is coupled to the heterotrimeric G-proteins G_i or G_o, acts by dissociating the G-protein complex to form $G_{\alpha i}$ or $G_{\alpha o}$ and the $\beta\gamma$ dimer that have different functions. The $G_{\alpha i}$ or $G_{\alpha o}$ act to inhibit adenylyl cyclase (AC) to reduce the activity of the cyclic AMP signalling pathway, whereas $\beta\gamma$ either inhibits Ca^{2+} channels, such as the N-, P/Q- and L-type channels, or it activates the GIRK K^+ channels. Anandamide can also act directly on TRPV1 and TRPV4 channels that introduce Ca^{2+} into the cell. These various actions of anandamide in neurons seem to play a role in triggering long-term depression (LTD). It is of interest that low levels of dietary omega-3 fatty acids, which provide the arachidonic acid precursor for endocannabinoid formation, causes a decrease in LTD and might be responsible for various neuropsychiatric diseases such as depression.

The action of anandamide is terminated by a two-step process. First, it is brought back into cells by an anandamide transporter, which accelerates its transport across the plasma membrane (Module 1: Figure anandamide). The next step is for the anandamide to be hydrolysed by fatty acid amide hydrolase (FAAH) located on inner membranes.

2-Arachidonoylglycerol (2-AG)

2-Arachidonylglycerol (2-AG) is one of the endocannabinoids that appears to be formed through two mechanisms. First, it is produced during phosphoinositide metabolism when PtdIns4,5-P_2 is hydrolysed by phospholipase C (PLC) to give $InsP_3$ and DAG (Module 2: Figure $InsP_3$/DAG recycling). These two second messengers are then recycled back to the precursor lipid PtdIns4,5P_2 through a series of steps. The DAG can be converted into phosphatidic acid (PA) by DAG kinase or it can be hydrolysed by a Ca^{2+}-sensitive DAG lipase to form 2-AG.

The action of 2-AG is terminated by a monoacylglycerol lipase (MAGL) (Module 2: Figure $InsP_3$/DAG recycling).

Growth factors

There are a large number of growth factors that act by stimulating the cell cycle signalling mechanisms responsible for inducing cell proliferation (Module 9: Figure cell cycle signalling mechanisms). As indicated below, many of these growth factors are grouped together into families:

Angiopoietin growth factors

- Ang1
- Ang2
- Ang3
- Ang4

Epidermal growth factors (EGFs)

- Amphiregulin (AR)
- Betacellulin (BTC)
- Epiregulin (EPR)
- Heparin-binding EGF-like growth factor (HB-EGF)
- Neuregulin
- Transforming growth factor-α (TGFα)

Insulin-like growth factors (IGFs)

- IGF-I
- IGF-II

Fibroblast growth factors (FGFs)
Hepatocyte growth factor (HGF)
Platelet-derived growth factors (PDGFs)

- PDGF-A
- PDGF-B
- PDGF-C
- PDGF-D

Vascular endothelial growth factors (VEGFs)

- VEGF-A
- VEGF-B
- VEGF-C
- VEGF-D
- Placental growth factor (PLGF)

Angiopoietin growth factors

There are four angiopoietins (Ang1–4) that function in controlling a variety of processes:

- The primary function of the angiopoietins is to control angiogenesis. The maturation and stability of endothelial cells is controlled by pericytes that release angiopoietin 1 (Ang1), which acts through TIE2 receptors (see step 7 in Module 9: Figure angiogenesis signalling). Angiopoietin 2 (Ang2) released by proliferating endothelial cells facilitates the loosening of cell contacts by inhibiting the activation of the TIE2 receptors by Ang1 (see step 9 in Module 9: Figure angiogenesis signalling).
- Cell adhesion of haematopoietic stem cells (HSCs) to the stem cell niche is provided by an interaction between Ang1 and its TIE2 receptor (Module 8: Figure HSC regulation).

Epidermal growth factors (EGFs)

There are a number of EGF stimuli that have a shared function of activating EGF receptors (Module 1: Figure EGF stimuli and receptors). The founder member of this family is EGF, but there are a number of related proteins such as amphiregulin (AR), betacellulin (BTC), epiregulin (EPR), heparin-binding EGF-like growth factor (HB-EGF), neuregulins (NRGs) and transforming growth factor-α (TGFα). These EGFs are also characterized by having an extracellular EGF motif that consists of six spatially conserved cysteine residues that form three intramolecular disulphide bonds. In addition, amphiregulin (AR) and heparin-binding EGF-like growth factor (HB-EGF) have an N-terminal heparin-binding domain. All these EGF stimuli are made as precursors that are anchored to the plasma membrane through a transmembrane domain. A process of ectodomain shedding is then responsible for releasing the extracellular region through the action of proteases such as the ADAM proteases.

These EGF stimuli act through the epidermal growth factor receptors (EGFRs) by promoting both homo- and heterodimerization of the ErbB receptor subunits, which are typical protein tyrosine kinase-linked receptors (PTKRs).

Neuregulins (NRGs)

The neuregulins (NRGs) are coded for by four genes that give rise to the neuregulin family that act through the EGF receptor family (Module 1: Figure EGF stimuli and receptors). The neuregulin family are derived from four genes (*NRG1*, *NRG2*, *NRG3* and *NRG4*). Most information is available for NRG1, which exist in numerous splice forms (Types I to VI).

Type I NRG1: this isoform is also known as heregulin, Neu differentiation factor (NDF) or acetylcholine receptor inducing activity (ARIA). The last name relates to a role for NRG1 in activating ErbB receptors to drive the expression of nicotinic acetylcholine receptors during synapse formation at neuromuscular junctions in skeletal muscle.

Type II NRG1: this isoform is also known as glial growth factor-2 (GGF2). In the brain, neuregulin-1 may act through ErbB to control the expression of NMDA receptors and PSD95 (Module 12: Figure schizophrenia).

There are numerous reports linking the *NRG1* gene to schizophrenia.

Insulin-like growth factors (IGFs)

The insulin-like growth factors (IGFs), which are produced in a variety of cells such as the liver, brain and in osteoblasts. In the case of the liver, the formation and release of IGFs is stimulated by growth hormone (GH). There are two IGFs: IGF-I and IGF-II. The IGFs are particularly important for controlling early development. IGF-II is mainly responsible for placental growth of multiple foetal organs whereas IGF-I regulates growth of the brain in both the foetus and adult. A remarkably feature of this IGF control system is the way it can adjust growth to the supply of nutrients. IGF-I has both neuroprotective and myelinogenetic actions. IGF-II is one of the most abundant growth factors and is stored in bone where it regulates bone formation by the osteoblasts (Module 7: Figure osteoblast function). IGF-I also has an important role in controlling protein synthesis during both physiological cardiac hypertrophy and during the pathological hypertrophy responsible for heart disease (Module 12: Figure physiological and pathological hypertrophy). IGF-I also function in muscle repair and regeneration by stimulating protein synthesis in satellite cells (Module 8: Figure satellite cell activation).

The action of IGFs is tightly regulated by the IGF-binding proteins (IGFBPs), which are found in both the serum and in the bone matrix.

IGF-binding proteins (IGFBPs)

IGF-binding proteins act by preventing IGF from binding to its IGF receptors. For the most part, therefore, they are thought of as negative regulators of the IGFs. However, there are indications that they may also help in cell activation by functioning as a local store of IGFs that can be released under appropriate conditions as occurs for IGFBP-4 (see below). In many cases, the IGFBPs are differentially regulated during development suggesting that they may have specific functions in controlling different events during the developmental sequence.

There are six IGFBPs (IGFBP1–6):

- IGFBP-1. One of its functions is to control bone formation.
- IGFBP-2. It is secreted by osteoblasts to regulate the function of IGF in controlling bone development. IGFBP-2 functions in bone remodelling where it may play a unique role in facilitating the interaction between IGF-II and its receptor. When IGFBP-2 binds to IGF-II it undergoes a conformational change that enhances its affinity for the glycosaminoglycans in the bone extracellular matrix (ECM) and thus positions IGF-II to act on its receptor. IGFBP-2 also modulates the action of IGF-I in regulating brain development. IGF-I has both neuroprotective and myelinogenetic actions so it is of interest that the levels of IGFBP-2 are increased in various pathological conditions such as multiple scelerosis.
- IGFBP-4 inhibits IGF action in a variety of cell types including bone cells. In the latter case, the IGFBP-4–IGF complex functions as a reservoir of IGF that is released following proteolysis of IGFBP-4 by pregnancy-associated plasma protein (PAPP-A).
- IGFBP-5. This isoform is the most highly conserved of the IGFBP family and is not normally found in the circulation, but is located mainly in the bone matrix where it can influence IGF activity. There are indications that the binding of IGFBP-5 to the extracellular matrix (ECM) reduces its IGF affinity thus releasing this growth factor. IGFBP-5 is also susceptible to hydrolysis by various proteases such as ADAM-9, which would also help to release IGF.

Fibroblast growth factors (FGFs)

There is a large family of fibroblast growth factors (FGFs). These FGFs are characterized by their ability to bind heparin and heparan sulphate proteoglycans (HSPG) that function as cofactors for the effective activation of FGF receptors. The diffusion of FGFs is limited through their affinity for HSPG and are thus likely to act in a paracrine manner on cells close to their site of release. In humans there are 22 *FGF* genes *FGF 1–23* with FGF 15 missing because the gene in mouse was found to be an orthologue of human *FGF19*. The numbering system was introduced to clear up difficulties that arose from individual FGFs having multiple names often reflecting different cellular origins. For example FGF1 has been called acidic FGF (aFGF), endothelial cell growth factor (ECGF), retina-derived growth factor (RDGF), eye-derived growth factor-II (EDGF-II) and brain-derived growth factor-II (BDGF-II). Similarly, FGF2 has been called basic FGF (bFGF), eye-derived growth factor-I (EDGF-I) and brain-derived growth factor-I (BDGF-I).

FGF23 is produced by osteoblasts and released to act on the kidney where it reduces the reabsorption of phosphate. The expression of FGF23 is controlled by the vitamin

D hormone 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] (Module 7: Figure vitamin D receptor activation).

FGFs act through FGFR receptors (FGFR1–4), which are members of the protein tyrosine kinase-linked receptors (PTKRs) (Module 1: Figure tyrosine kinase-linked receptors). The FGFs are particularly important in the control of development. Not only do they promote cell growth but they also regulate cell survival, migration and differentiation. During limb development, for example, the mesenchyme releases FGF10 to induce the formation of the ectoderm ridge, which then releases FGF8 to feed information back to the mesoderm. A large number of these FGFs are expressed in the eye where they control lens development and function. They also function in wound healing and tissue repair and are important regulators of haematopoietic stem cell (HSC) self renewal (Module 8: Figure HSC regulation).

Heparan sulphate proteoglycans (HSPG)

The heparan sulphate (HS) proteoglycans (HSPGs) play an important role in cell signalling by binding growth factors such as fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF), which thus serves to restrict their action to areas close to their sites of release. An example of this is seen during angiogenesis where PDGF released from the tip cells functions locally to control the proliferation of the pericytes (Module 9: Figure angiogenesis signalling). HSPG is a sulphated linear polymer containg repeating disaccharide subunits of D-glucosamine and hexuronic acid. These polymers are found both in the extracellular matrix and on the surface of cells.

The heparan sulphate proteoglycans syndecan-3 and syndecan-4 are expressed in satellite cells.

Hepatocyte growth factor (HGF)

Hepatocyte growth factor (HGF), which is also known as scatter factor, belongs to the plasminogen family and is a mesenchymal-derived heparin-binding growth factor that acts through the hepatocyte growth factor receptor (HGFR), which is also known as the MET receptor. The closely related ligand macrophage-stimulating protein (MSP) acts on the RON receptor, which resembles the MET receptor. HGF is released as a 97 kDa precursor that is then cleaved by proteases such as urokinase-type plasminogen activator (uPA) to give the active disulphide-linked heterodimer. Each monomer has a hairpin loop followed by four kringle domains, which are double-looped structures stabilized by disulphide bridges.HGF is released mainly from mesenchymal cells and acts in a paracrine manner on neighbouring epithelial cells to induce invasive growth. Such invasive growth is a normal part of processes that occur during embryonic development and tissue repair. For example, HGF controls the proliferation of satellite cells during repair of skeletal muscle (Step 2 in Module 8: Figure satellite cell function). HGF also plays an important role in the control of male and female gonodal function. In carrying out its normal actions it can stimulate proliferation, disrupt intercellular junctions, induce migration and protect cells against apoptosis. This multitasking capacity of HGF to orchestrate the cellular processes required for tissue remodelling is potentially dangerous because it can have pathological consequences when control over this HGF/MET pathway is taken over by tumour cells to drive metastasis.

Platelet-derived growth factors (PDGFs)

There are four platelet-derived growth factor genes that code for the four isoforms (PDGF-A, PDGF-B, PDGF-C and PDGF-D). The biologically active form of PDGF depends on these four different proteins combining to form either homodimers (AA, BB, CC, DD) or heterodimers (AB) that are connected together by disulphide bonds. These dimeric forms then function to activate the platelet-derived growth factor receptor (PDGFR) by bringing together the two subunits (Module 1: Figure PDGFR activation).

The PDGFs function in the development of connective tissue by stimulating the proliferation of smooth muscle cells and the pericytes of blood vessels. As such, it plays an important role in angiogenesis where PDGF-B is released by the tip cell to stimulate the proliferation and migration of pericytes (Module 9: Figure angiogenesis signalling). PDGF also stimulates the proliferation of mesangial cells (Module 7: Figure mesangial cell).

Progranulin (PGRN)

Progranulin (PGRN) is a secreted glycoprotein that has been implicated in a number of cellular processes such as inflammation, cell proliferation, neurite growth and cell survival. The protein consists of repeating granulin (GRN) peptides each of which has six cysteines and these can interact to form six intramolecular disulphide bonds. The correct folding of the protein is very dependent on an ER chaperone network consisting of protein disulphide isomerases (PDIs) such as ERp5 and ERp57, which interact with Ca^{2+}-binding proteins such as calreticulin. The individual granulin peptides of PGRN are released by proteases such as elastase, which can be regulated by secretory leukocyte protease inhibitor (SLPI). PGRN can also be cleared by binding to sortilin 1 (SORT1), which is a trafficking protein that transports PGRN to the lysosomes.

Just how PGRN acts is unclear. No obvious PRGN signalling receptors have been identified, but there are indications that it can bind to tumour necrosis factor (TNF) receptors. Some of the actions of PGRN appear to depend on activation of the MAPK signalling and PtdIns 3-kinase signalling pathways. A decrease in PGRN levels has also been associated with an upregulation of the Fz2 receptor that is coupled to the non-canonical Wnt/Ca^{2+} signalling system.

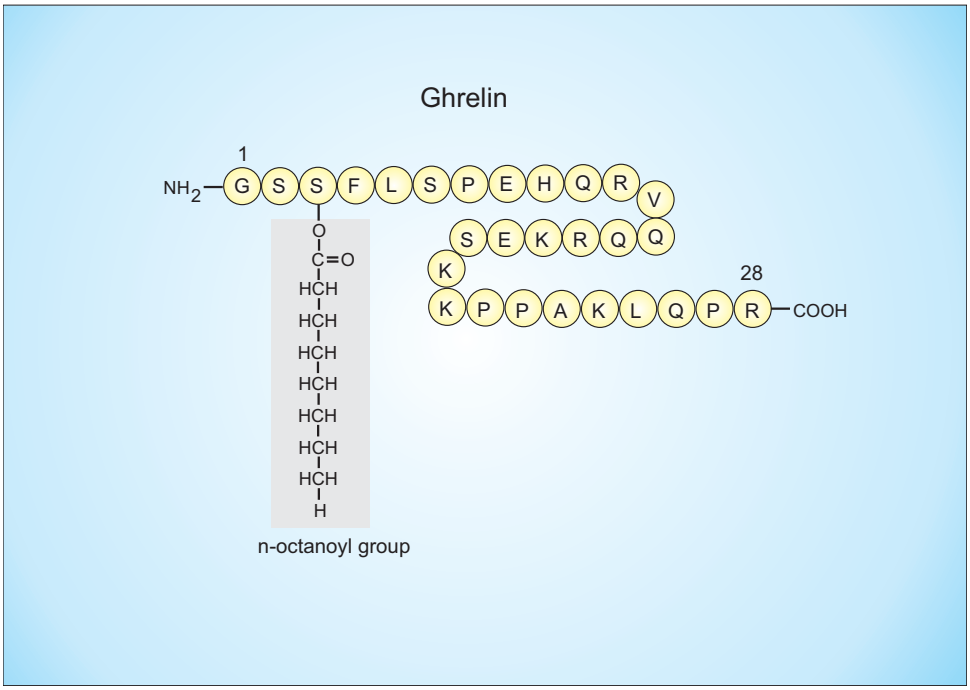
PGRN has both neurotrophic and anti-inflammatory responses. Such an action is particularly important in the brain where it acts to inhibit microglial inflammatory responses.

Frontotemporal dementia (FTD) has been linked to mutations in the *GRN* gene that encodes PGRN.

Vascular endothelial growth factor (VEGF)

The vascular endothelial growth factor (VEGF) family, which has five members, functions in angiogenesis (Module 9: Figure angiogenesis).

Module 1: | Figure ghrelin



Structure of human ghrelin.
Human ghrelin is a 28-amino-acid peptide that has n-octanoic acid attached to Ser-3. Redrawn from Figure 3 in Kojima et al. (2005).

Neurotrophins

The neurotrophins are a family of proteins that function in the nervous system to control processes such as neurogenesis, synaptic plasticity during learning, neuronal survival and differentiation. There are four main members: brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). These neurotrophins form homodimers that then act through two types of receptor, the Trk receptors or the p75 neurotrophin receptor ($p75^{NTR}$).

BDNF functions in learning and memory by activating the protein synthesis necessary for the growth of spines during Ca^{2+}-induced synaptic plasticity (Module 10: Figure Ca^{2+}-induced synaptic plasticity).

Adipokine hormones

Adipokines such as leptin and adiponectin are hormones released from the white fat cells of adipose tissue as part of the regulatory network for the control of food intake and body weight (Module 7: Figure control of food intake). Other cells within adipose tissue release hormones such as resistin.

Leptin

Leptin, which is the product of the *ob* gene, is produced and released by white fat cells (Module 7: Figure lipolysis and lipogenesis). After its release it circulates in the plasma where its level reflects energy homoeostasis by increasing with overfeeding and declining with starvation. Leptin is a satiety signal that acts to decrease feeding through the mechanisms that function to control food intake and body weight (Module 7: Figure control of food intake). This role of reducing food intake is reduced in obesity apparently due to the development of 'leptin resistance'.

Leptin acts on two main types of leptin-sensitive neurons. First, it inhibits the orexigenic NPY/AgRP neurons located in the arcuate nucleus (ARC) (See Step 5 in Module 7: Figure control of food intake). Secondly, it activates the POMC/CART neurons that stimulate the satiety centre. In addition, it may also activate anorexigenic neurons located in the nucleus of the solitary tract (NTS).

Leptin is a Type I cytokine (Module 1: Figure cytokines), which acts through the Ob receptor (Ob-R). This Ob-R exists as six isoforms (Ob-Ra–f) that result from alternate splicing. The inhibition of food intake by leptin is carried out by the Ob-Rb isoform, which has a long cytoplasmic domain that recruits components of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling pathway. The activated STATs then enter the nucleus where they activate transcription of genes such as the suppressor of cytokine signalling proteins (SOCS) (Module 2: Figure JAK/STAT function). In the hypothalamic neuronal targets of leptin, there is a marked up-regulation of SOCS-3.

Leptin may reduce insulin secretion by stimulating the activity of phosphodiesterase PDE3B, thereby reducing the level of cyclic AMP (Module 7: Figure β-cell signalling).

Adiponectin

Adiponectin, like leptin, is released from white fat cells (Module 7: Figure control of food intake). Unlike leptin, which circulates in the plasma at ng/ml levels, adiponectin is present in μg/ml levels. It also differs from leptin in that

its levels decrease in obesity but increases during weight loss. The precise function of adiponectin is unclear, but it appears to have a role in protecting cells against glucose intolerance and insulin resistance (Module 12: Figure insulin resistance).

There are two adiponectin receptors (AdipoR1 and AdipoR2). The AdipoR1 is found in skeletal muscle whereas the type 2 receptor is expressed in liver. These AdipoRs appear to act by promoting the entry of external Ca^{2+}, which then acts through CaMKKβ to phosphorylate AMPK as part of the AMP signalling pathway (Module 2: Figure AMPK control of metabolism).

Resistin

Resistin, which appears to be released from macrophages and stromal cells located in adipose tissue, has been implicated in insulin resistance and the development of diabetes during obesity.

Gut hormones

In addition to its function in digestion and nutrient absorption, the gastrointestinal tract is also an endocrine organ in that it contains a large number of cells that synthesize and secrete gut hormones. The stomach has various endocrine cells that contribute to the neural and endocrine functions of the stomach:

- Enterochromaffin-like cells (ECLs) release histamine.
- D cells secrete somatostatin.
- G cells secrete gastrin.

The intestine also has endocrine cells:

- Enterochromaffin cells release 5-hydroxytryptamine (5-HT).

The gastrointestinal tract and pancreas also releases a range of hormones that contribute to control of food intake and body weight (Module 7: Figure control of food intake):

- The X/A-like cells of the stomach release the hormone ghrelin.
- The duodenum release cholecystokinin (CCK).
- L-cells located in the intestine and colon release glucagon-like peptide 1 (GLP-1) and oxyntomodulin (OXM).
- The peptide YY (PYY) is released fron the enteroendocrine L cells.
- The pancreatic islets of Langerhans release both insulin and pancreatic polypeptide (PP).

Ghrelin

Ghrelin is a 28-amino-acid peptide that is released mainly from the X/A-like cells in the stomach (Module 7: Figure stomach structure), but is also produced from other regions of the gastrointestinal tract. An octanyl group is attached at Ser-3 (Module 1: Figure ghrelin) and this acylation is essential for ghrelin to interact with its growth-hormone-secretagogue receptor (GHS-R). Ghrelin has also been located in ghrelin-containing neurons in the brain. It mediates its action through the ghrelin receptor (GHS-R), which is a typical G protein-coupled receptor (GPCR), that is coupled through G_q to activate the inositol 1,4,5-trisphosphate ($InsP_3$) signalling cassette.

Ghrelin is a multifunction hormone capable of stimulating a variety of processes:

- Ghrelin that is released in anticipation of eating functions in the control of food intake and body weight by activating the NPY/AgRP neurons that control the feeding centre (Module 7: Figure control of food intake).
- Ghrelin acts on pituitary somatotrophs to release growth hormone (GH).
- Ghrelin may act on the stomach to control acid secretion and gastric movement.

High levels of ghrelin have been found in both anorexia nervosa and in Prader-Willi syndrome (PWS).

Glucagon-like peptide 1 (GLP-1)

The precursor pre-proglucagon is synthesized and released by intestinal L cells following feeding (Module 7: Figure L cell). This precursor is then processed by prohormone convertase 1 and 2 to different hormones depending on the tissue. In the pancreas, it is converted into glucagon, whereas in the CNS and intestine it is processed to form glucagon-like peptide 1 (GLP-1) and oxyntomodulin (OXM). GLP-1 acts on the GLP-1 receptor (GLP-1R) to activate the cyclic AMP signalling pathway. The ability of GLP-1 to reduce food intake might be mediated through GLP-1Rs located in the hypothalamus.

One of the main targets of GLP-1 is the insulin-secreting β-cell where it facilitates the glucose-induced release of insulin (Module 7: Figure β-cell signalling).

Circulating GLP-1 is inactivated by dipeptidyl peptidase 4 (DPP4).

Cholecystokinin (CCK)

Cholycystokinin (CCK) is a major gut hormone that is released into the circulation by enteroendocrine cells located primarily in the duodenum and also in the intestine (Module 7: Figure control of food intake). CCK is a highly versatile stimulus, functioning either as a neurotransmitter or as a hormone:

- CCK regulates fluid secretion by the exocrine pancreas (Module 7: Figure control of pancreatic secretion).
- CCK functions as a local hormone to activate afferent terminals of the vagus nerve to transmit satiety signals to the nucleus of the solitary tract (NTS) (Module 7: Figure control of food intake). CCK has a major role in regulating the control of food intake and body weight.
- CCK activates smooth muscle contraction to control stomach emptying.
- CCK released within the CNS is thought to modulate dopaminergic activity.
- CCK is expressed in various hippocampal interneurons such as the basket cells (Module 10: Figure hippocampal interneurons).

Oxyntomodulin (OXM)

Oxyntomodulin (OXM) is produced from the common precursor pre-proglucagon that is synthesized and released by intestinal L cells following feeding

(Module 7: Figure L cell). OXM functions in the control of food intake and body weight by reducing food intake. Some of the actions of OXM resemble that of glucagon-like peptide 1 (GLP-1). It may act through the GLP-1 receptors (GLP-1R) that are expressed in the hypothalamus to reduce food intake. In addition, OXM may act locally like cholecystokinin (CCK) to control stomach emptying.

Pancreatic polypeptide (PP)

Pancreatic polypeptide (PP), which is a member of the PP-fold family of peptides that include peptide YY (PYY) and neuropeptide Y (NPY), is produced and released from cells located on the periphery of the islets of Langerhans (Module 7: Figure control of food intake). One of the primary actions of PP is to contribute to the control of food intake and body weight. PP levels in the plasma are highest in the evening, but decline to low levels in the early morning. The levels increase following feeding and play a role in reducing food intake.

Peptides belonging to the PP-fold act through the PP-fold receptors Y_1–Y_6, which are G protein-coupled receptors (Module 1: Table G protein-coupled receptors). PP appears to act through the Y_4 and Y_5 receptors.

Neuropeptide Y (NPY)

Neuropeptide Y (NPY) is a member of the pancreatic polypeptide-fold (PP-fold) family that includes peptide YY (PYY) and pancreatic polypeptide (PP). NPY functions in the control of food intake and body weight (Module 7: Figure control of food intake). It is a potent orexigenic neuropeptide and its levels and release from the NPY/AgRP neurons increases after feeding. NPY is also expressed in various hippocampal interneurons such as the bis-stratified cell (Module 10: Figure hippocampal interneurons).

Peptide YY (PYY)

Peptide YY (PYY), which is a member of the pancreatic polypeptide-fold (PP-fold) family that includes neuropeptide Y (NPY) and pancreatic polypeptide (PP), is released from enteroendocrine L cells located in the intestine and colon (Module 7: Figure small intestine). The $PYY_{1–36}$, which is the peptide secreted by the L cells, is rapidly converted into $PYY_{3–36}$ by removal of the N-terminal Tyr-Pro residues by dipeptidyl peptidase 4 (DPP4). Peptides belonging to the PP-fold act through G protein-coupled receptors Y_1–Y_6 (Module 1: Table G protein–coupled receptors). PYY appears to act through the Y_2 and Y_5 receptors.

Pro-opiomelanocortin (POMC)

Pro-opiomelanocortin (POMC) is a large polypeptide hormone precursor that is cleaved to give a number of biologically active peptides that can function either as hormones or as neurotransmitters. The following are some of the major peptides that are derived from the selective processing of POMC:

Adrenocorticotropic hormone (ACTH)
β-Endorphin
β-Lipotropin
γ-Lipotropin
α-Melanocyte-stimulating hormone (α-MSH)
β-Melanocyte-stimulating hormone (β-MSH)
γ-Melanocyte-stimulating hormone (γ-MSH)

The hormones and transmitters derived from POMC have a number of functions:

- During melanogenesis, keratinocytes synthesize pro-opiomelanocortin (POMC) that is processed to form α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH), which then stimulate the melanocytes (Module 7: Figure melanogenesis).
- In the anterior pituitary, the pro-opiomelanocortin (POMC) gene is transcribed and is then cleaved to produce the adrenocorticotropic hormone (ACTH) that is released from the corticotrophs (Module 10: Figure corticotroph regulation).
- Neurons within the ARC, which are anorexigenic, express POMC that is the precursor for α-melanocyte stimulating hormone (α-MSH) that functions in the control of food intake and body weight. The α-MSH then acts on the melanocortin 4 receptor (MC4R) on the second-order neurons to activate the satiety centre thus decreasing food intake and weight loss (see inset in Module 7: Figure control of food intake).

Mutations in the POMC gene in the region that encodes α-MSH, which is released from the POMC/CART neurons in the hypothalamus during the control of food intake and body weight (Step 7 in Module 7: Figure control of food intake), is responsible for early-onset obesity.

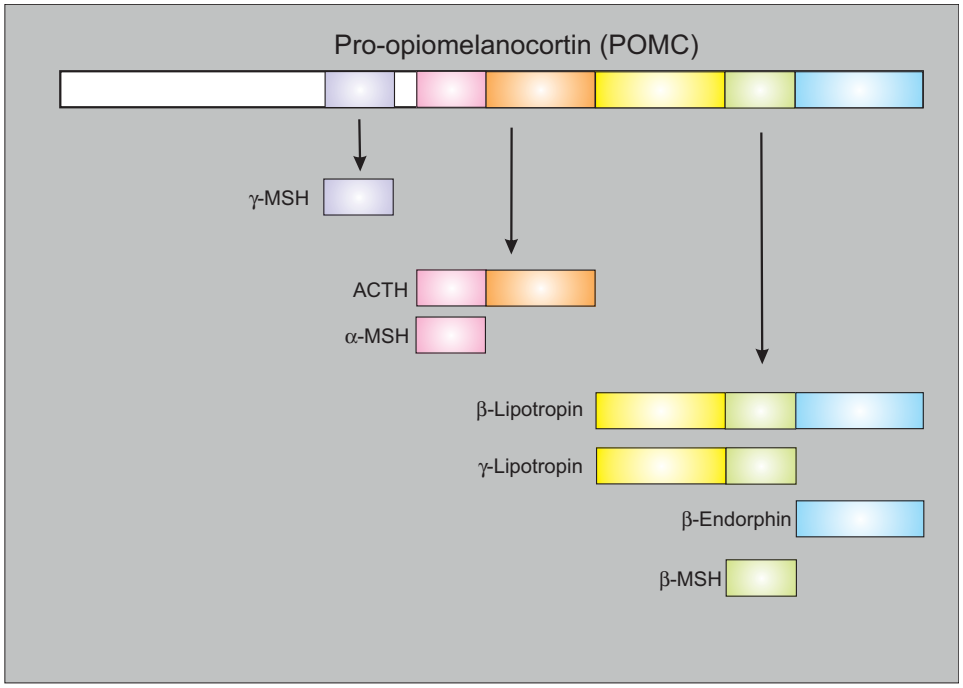
Cytokines

The cytokines are a heterogeneous group of soluble (8–60 kDa) glycoproteins that function as stimuli that use various cell signalling pathways to regulate many different cellular processes. The haematopoietic cytokines orchestrate the development of haematopoietic cells (Module 8: Figure haematopoietic cytokines). Cytokines are also important mediators of immune and inflammatory responses where they have both paracrine and autocrine functions. However, some cytokines also function as hormones to control other systems such as the brain, where they have been implicated in processes such as sleep, and in endocrine glands and reproductive organs. It has proved difficult to classify cytokines because of their wide structural and functional diversity. This difficulty is compounded by the fact that some cytokines can have different functions depending on their location. In addition to driving proliferation, they can also promote survival. They commit cells to a particular pathway of differentiation; they induce maturation and can continue to exert control over the activity of mature cells. There is also considerable diversity with regard to the structure and function of the cytokine receptors.

Most of the cytokines fall into the following groups:

- Chemokines
- Interleukins

Module 1: | Figure pro-opiomelanocortin



Pro-opiomelanocortin (POMC).
Pro-opiomelanocortin (POMC) is a large polypeptide hormone precursor that is cleaved into a number of biologically active peptides that can act as hormones or neurotransmitters.

Interleukin-1 (IL-1)
Interleukin-2 (IL-2)
Interleukin-3 (IL-3)
Interleukin-5 (IL-5)
Interleukin-6 (IL-6)
Interleukin-10 (IL-10)
Interleukin-21 (IL-21)

- Thymic stromal lymphopoietin (TSLP)
- Interferons (IFNs)

Interferon-α (IFN-α)
Interferon-β (IFN-β)
Interferon-γ (IFN-γ)

- Colony-stimulating factors (CSFs), haematopoietins and neuropoietins

Cardiotrophin (CT-1)
Ciliary neurotrophic factor (CNTF)
Colony-stimulating factor 1 (CSF-1). Also known as macrophage colony-stimulating factor (M-CSF)
Erythropoietin (EPO)
Ftl ligand (FL)
Granulocyte colony-stimulating factor (G-CSF)
Granulocyte-macrophage colony-stimulating factor (GM-CSF)
Leukaemia inhibitory factor (LIF)
Oncostatin (OSM)
Stem cell factor (SCF)
Thrombopoietin (TPO)

- Growth hormone (GH)
- Prolactin (PRL)
- Tumour necrosis factor α (TNFα)

Interleukins

The interleukins are a heterogeneous group of cytokines (approximately 35) that have many different functions. Most of the interleukins act through cytokine receptors to activate the JAK/STAT signalling pathway (Module 1: Figure cytokines). However, there are exceptions in that interleukin 1 (IL-1) acts through the Toll receptor signalling pathway (Module 2: Figure Toll receptor signalling).

Interleukin-1 (IL-1)

Interleukin-1 (IL-1) is one of the inflammatory cytokines that are released in response to infection or cell injury by cells of the innate immune system, such as the macrophages (Module 11: Figure inflammation). IL-1 that is stored in keratinocytes is released rapidly after wounding of the skin and signals quickly to surrounding cells that the external barrier is damaged.

IL-1 acts through the IL-1 receptor (IL-1R), which is part of a Toll-like receptor (TLR) superfamily. The IL-1R belongs to the group of non-enzyme-containing receptors that function by recruiting various signal transducing components (Module 1: Figure cytokines). The external domain contains three immunoglobulin-like domains, whereas the cytoplasmic domain has the Toll/IL-1R (TIR) domain that has three box motifs that are highly conserved in all of the receptors. It is these box motifs that are responsible for recruiting the components of the

Toll receptor signalling pathway (Module 2: Figure Toll receptor signalling).

Interleukin-2 (IL-2)

Interleukin-2 (IL-2) has a somewhat specific function in controlling the activity of T cells. When T cells are activated they release IL-2 that feeds back in an autocrine manner to provide a link between the earlier events initiated by the T cell receptor (TCR) and the cell cycle components necessary to initiate DNA synthesis (Module 9: Figure T cell signalling map).

The IL-2 signalling pathway resembles that of many other cytokines in that it is carried out by the JAK/STAT signalling pathway (Module 1: Figure cytokines). The interleukin-2 receptor (IL-2R) is a heterotrimer composed of IL-2α, IL-2β and γ_c-subunits. The γ_c is a common subunit that is used by other receptors such as the heterodimeric IL-4 receptor (Module 1: Figure type I cytokine receptors). Upon binding IL-2, this heterotrimeric IL-2R recruits the transducers Jak1 and Jak3 to activate the transcription factors STAT3, STAT5a and STAT5b (Module 2: Figure JAK/STAT heterogeneity). These STATs are transcription factors that function in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

Interleukin-3 (IL-3)

Interleukin-3 (IL-3) is released from activated T cells and is an example of a haematopoietic cytokine that functions to control haematopoiesis (Module 8: haematopoietic cytokines). It not only operates as a growth factor to control the proliferation of the stem cells and early progenitor cells, but it also guides the subsequent differentiation and maturation of the myeloid progenitor cells that form the eosinophils, neutrophils, megakaryocytes and blood platelets.

The interleukin-3 receptor (IL-3R) is a heterotetramer composed of two IL-3α and two β_c subunits (Module 1: Figure type I cytokine receptors). This β_c is a promiscuous transducing subunit that is also used by IL-5 and GM-CSF. Upon binding IL-3, this β_c subunit recruits the transducer Jak2 to activate STAT5a and STAT5b (Module 2: Figure JAK/STAT heterogeneity). These STATs are transcription factors that function in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

Interleukin-5 (IL-5)

Interleukin-5 (IL-5) is an example of an haematopoietic cytokine that functions to control haematopoiesis (Module 8: Figure haematopoietic cytokines). Unlike many of the other haematopoietic cytokines, it has little effect on the proliferation of the stem cells and early progenitors, but it comes into effect later on to control the differentiation and maturation of the eosinophils.

The interleukin-5 receptor (IL-5R) is a heterotetramer composed of two IL-5α and two β_c subunits (Module 1: Figure type I cytokine receptors). This β_c is a promiscuous transducing subunit that is also used by IL-3 and GM-CSF. Upon binding IL-5, this β_c subunit recruits the transducer Jak2 to activate STAT5a and STAT5b (Module 2: Figure JAK/STAT heterogeneity). These STATs are transcription factors that function in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

Interleukin-6 (IL-6)

Interleukin-6 (IL-6) was originally identified as a B cell differentiation factor, but is now known to act on many other cell types. It has also been implicated in chronic inflammation and cancer. One of its primary actions is to function as a haematopoietic cytokine to control many aspects of haematopoiesis (Module 8: Figure haematopoietic cytokines). It not only operates as a growth factor to control the proliferation of the stem cells and early progenitor cells, but it also guides the subsequent differentiation and maturation of the myeloid progenitor cells that form the megakaryocytes and blood platelets.

The interleukin-6 receptor (IL-6R) is the prototype of an IL-6 subfamily of receptors that are characterized by sharing the transducing subunit glycoprotein 130 (gp130) (Module 1: Figure type I cytokine receptors). Other members of this subfamily include receptors for IL-11, cardiotrophin (CT-1), ciliary neurotrophic factor (CNTF), leukaemia inhibitory factor (LIF) and oncostatin (OSM). All of these receptors have two α-subunits that are specific for each cytokine and two transducing subunits, which are either gp130 homodimers as in the case of IL-6R and IL-11R or are heterodimers with a gp130 subunit being paired with some other subunit such as the LIFR for the receptors that respond to LIF or CNTF. Upon binding the appropriate cytokine, these IL-6 subfamily of receptors recruit the transducer Jak1 to activate STAT3 (Module 2: Figure JAK/STAT heterogeneity) which is a transcription factor that functions in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

Mutations in gp130 are responsible for the onset of inflammatory hepatocellular adenomas (IHCAs).

Activation of the IL-6R by IL-6 may contribute to schizophrenia by altering the phenotype of the inhibitory interneurons that drive brain rhythms (Module 12: Figure schizophrenia).

Interleukin-10 (IL-10)

Interleukin-10 (IL-10) is a type II cytokine that belongs to a subfamily containing IL-19, 1L-20, IL-22, IL-24, IL-26, IL-28 and IL-29. Although members of this family are related to each other by having a common genomic organization and similar receptors, they all have very different functions. IL-10 is a potent anti-inflammatory and anti-immune factor that is released by T helper cells, various regulatory T cells, dendritic cells, macrophages, mast cells and eosinophils. One of the main functions of IL-10 is to reduce the expression of the MHCII complex and it also reduces the maturation of dendritic cells. By reducing the presentation of antigens, IL-10 reduces the release of IL-2, IL-4, IL-5 and interferon-γ (IFN- γ). It can also reduce the formation of inflammatory mediators such as IL-1, IL-6 and TNF. Many of these actions to dampen down inflammatory and immune responses seem to depend on the ability of IL-10 to inhibit the activity of the nuclear factor κB (NF-κB) signalling pathway that is induced by various inflammatory mediators.

The IL-10 receptor (IL-10R) consists of two IL-10R1 subunits and two IL-10R2 subunits. Upon binding IL-10, these receptor subunits recruit the transducers Jak1 and tyrosine kinase 2 (Tyk2) that function to activate STAT3 (Module 2: Figure JAK/STAT heterogeneity) which is a transcription factor that functions in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

Interleukin-17 (IL-17)

Interleukin-17 (IL-17) released from $CD4^+$ T helper 17 (Th17) cells coordinates the recruitment of neutrophils.

Interleukin-21 (IL-21)

Interleukin-21 (IL-21) is a potent regulator of various immune cells such as germinal centre B-cells, natural killer (NK) cells and cytotoxic T-cells. The IL-21 receptor (IL-21R), which resembles that of other type I cytokines such as IL-2R (Module 1: Figure type I cytokine receptors), responds to IL-21 by activating the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function). It uses Jak1 and Jak3 to induce the STAT3 homodimer to activate its target genes. The main function of IL-21 is to stimulate proliferation as occurs during B-cell differentiation in the lymph node (Module 8: Figure B cell maturation signalling).

Thymic stromal lymphopoietin (TSLP)

Thymic stromal lymphopoietin (TSLP) is a cytokine that has been implicated in Atopic dermatitis (AD) where it plays a major role in activating the itch sensation. TSLP is generated in the keratinocytes in the skin and in the bronchial epithelial cells in asthma. The keratinocytes have proteinase-activated receptor 2 (PAR_2) that are coupled through $G_{q/11}$ to activate phospholipase C3β (PLC3β). The PLCβ3 then generates inositol 1,4,5-trisphosphate ($InsP_3$) that releases Ca^{2+} from the endoplasmic reticulum (ER) that then results in activation of Orai1, which is a store-operated channel (SOC) that promotes the entry of external Ca^{2+} (Module 3: Figure SOC signalling components). The increase in cytosolic Ca^{2+} then activates NFAT that enters the nucleus where it acts to increase the expression of TSLP. The latter then diffuses out to interact with receptors on the sensory neurons in the skin to induce the itch sensation by activating TRPA1 channels (Module 10: Figure Itch signal transduction mechanism).

Interferons (IFNs)

The interferons are cytokines that modulate immune responses and have a special role in dealing with viral infections. There are three main IFNs. The type I IFNs, interferon-α (IFN-α) and interferon-β (IFN-β), which have a relatively high antiviral potency, are released from endothelial cells and macrophages in responses to infection by viruses and bacteria. Interferon-γ (IFN-γ) is a type II IFN.

Interferon-α (IFN-α)

Type I interferon-α (IFN-α) and interferon-β (IFN-β) are produced by endothelial cells and macrophages in responses to viral infections (Module 2: Figure viral recognition). Once released they act in both an autocrine and paracrine manner to stimulate their IFN receptors to activate a battery of genes that help combat the infection. One of the gene products is double-stranded RNA-dependent protein kinase (PKR), which phosphorylates and inhibits the initiation factor eIF2 and the resulting decrease in protein synthesis helps to reduce viral replication (Module 9: Figure regulation of eIF-2α cycling). IFN-α and IFN-β also activate the 2–5A synthetase, which produces the oligoadenylate that switches on a latent ribonuclease that degrades double-stranded RNA (ssRNA).

Interferon-γ (IFN-γ)

Interferon-γ (IFN-γ) consists of two peptide chains (143 amino acids) that have two N-linked glycosylations. It is produced by helper T cells and NK cells that have been activated by interleukin-2 (IL-2) and interleukin-12 (IL-12). It acts through the type II interferon-γ receptor (IFNγR), which is coupled to the JAK/STAT signalling pathway , to influence many different responses such as an increased expression of the class I MHC complex and an increase in the activity of macrophages, neutrophils and NK cells.

An important function of IFN-γ is to regulate the expression of the Vitamin D receptor (VDR). Expression of the VDR is reduced in a large proportion of the population in the Mediterranean island of Sardinia that suffer from Multiple sclerosis (MS). These MS patients have reduced expression of the *Ifng* gene that encodes IFN-γ.

Cardiotrophin (CT-1)

Cardiotrophin (CT-1) was originally discovered in the heart, but is now known to function in many other cell types. In the heart, it can activate proliferation and survival. It can also have pathological consequences by contributing to myocyte hypertrophy and collagen synthesis that are part of the cardiac remodelling events that result in heart disease. The cardiotrophin receptor (CT-1R) is a member of the IL-6 subfamily of receptors that are characterized by sharing the transducing subunit glycoprotein 130 (gp130) (Module 1: Figure type I cytokine receptors). The CT-1R has two CT-1Rα subunits and it uses LIFRβ and gp130 as transducing subunits. Upon binding CT-1, the CT-1R recruits Jak1 to activate STAT3 (Module 2: Figure JAK/STAT heterogeneity), which is a transcription factor that functions in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

Ciliary neurotrophic factor (CNTF)

Ciliary neurotrophic factor (CNTF) is expressed primarily in the nervous system where it functions as a neurotrophic factor. It is also a potent survival factor for both neurons and astrocytes. CNTF has been tested as a therapeutic agent to control certain neurodegenerative disorders such as motor neuron disease, but patients were found to suffer severe weight loss suggesting that CNTF may play a role in the control of food intake and body weight. CNTF has been implicated in neurogenesis where it may mediate the ability of dopamine to stimulate proliferation in both the subventricular zone and the dentate gyrus. The CNTF receptor (CNTFR) is a member of the IL-6 subfamily of receptors that are characterized by sharing the transducing

subunit glycoprotein 130 (gp130) (Module 1: Figure type I cytokine receptors). The CNTFR has two CNTFRα subunits and it uses LIFRβ and gp130 as transducing subunits. Upon binding CNTF, the CNTFR recruits Jak1 to activate STAT3 (Module 2: Figure JAK/STAT heterogeneity), which is a transcription factor that functions in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

Erythropoietin (EPO)

Erythropoietin (EPO) is an example of a haematopoietic cytokine that functions to control haematopoiesis (Module 8: Figure haematopoietic cytokines). EPO is synthesized predominantly by the proximal kidney tubule cells and is released into the circulation to control the differentiation and maturation of erythrocytes.

The EPO receptor (EPOR) is composed of two identical subunits (Module 1: Figure type I cytokine receptors). The typical cytokine receptor modules that make up the extracellular domains co-operate with each other to bind a single EPO molecule to induce the conformational changes in the intracellular box motifs that recruit and activate Jak2. The latter then activates STAT5a and STAT5b (Module 2: Figure JAK/STAT heterogeneity), which are transcription factors that function in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

Ftl ligand (FL)

FL is one of the haematopoietic cytokines that functions in the control of haematopoiesis (Module 8: Figure haematopoietic cytokines). It is an example of a membrane-anchored stimulus that functions through a juxtacrine mechanism to control stem cells and progenitors cells. In addition, it controls the subsequent differentiation and maturation of dendritic cells.

The Ftl ligand (FL) acts on the FL receptor (FLR), which is a typical protein tyrosine-linked receptors (PTKRs) (Module 1: Figure tyrosine kinase-linked receptors).

Granulocyte colony-stimulating factor (G-CSF)

Granulocyte colony-stimulating factor (G-CSF), which is also known as colony-stimulating factor-3 (CSF-3), is one of the haematopoietic cytokines that functions to control haematopoiesis (Module 8: Figure haematopoietic cytokines). One of its primary functions is to control the differentiation and maturation of the neutrophils.

The G-CSF receptor (G-CSFR) is composed of two identical subunits (Module 1: Figure type I cytokine receptors). The extracellular region has a number of domains: a terminal immunoglobulin-like domain, a cytokine receptor module and three fibronectin type-III-like domains. Some of these domains are used to bind G-CSF to induce the conformational change in the intracellular box motifs that recruit and activate Jak2. The latter then activates STAT5a and STAT5b (Module 2: Figure JAK/STAT heterogeneity), which are transcription factors that function in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

Granulocyte-macrophage colony-stimulating factor (GM-CSF)

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is one of the colony-stimulating factors (CSFs) and is also known as CSF-2. GM-CSF was first identified as one of the haematopoietic cytokines that functions to control haematopoiesis (Module 8: Figure haematopoietic cytokines). It not only operates as a growth factor to control the proliferation of the early progenitor cells but it also guides the subsequent differentiation and maturation of the neutrophilic granulocytes and macrophages.

The GM-CSF receptor (GM-CSFR) is a heterotetramer composed of two GM-CSFα and two β_c subunits (Module 1: Figure type I cytokine receptors). This β_c is a promiscuous transducing subunit that is also used by IL-3 and IL-5. Upon binding GM-CSF, this β_c subunit recruits the transducer Jak2 to activate STAT5a and STAT5b (Module 2: Figure JAK/STAT heterogeneity). These STATs are transcription factors that function in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

Growth hormone (GH)

Growth hormone (GH) consists of a single protein chain (approximately 190 amino acids), which is synthesized and released by somatotrophs (Module 10: Figure somatotroph regulation). It acts to promote growth, but it can also modulate various metabolic processes (protein, lipid and carbohydrate). The action of GH can be either direct (e.g. in fat cells where it induces triacylglycerol breakdown and reduces their ability to take up lipids) or indirect, as occurs through its ability to stimulate liver cells to produce and release insulin-like growth factor I (IGF-I)

Leukaemia inhibitory factor (LIF)

Leukaemia inhibitory factor (LIF) is a multifunctional cytokine that was found first through its ability to suppress the proliferation of a myeloid leukaemic cell line. However, it was found subsequently to have many other functions: it maintains embryonic stem cells in an undifferentiated state, it facilitates the implantation of blastocysts, it functions in the autonomic nervous system, adipocytes, liver, osteoclasts and it assists in the release of adrenocorticotropic hormone (ACTH) by the corticotrophs.

The LIF receptor (LIFR) is a member of the IL-6 subfamily of receptors that are characterized by sharing the transducing subunit glycoprotein 130 (gp130) (Module 1: Figure type I cytokine receptors). The LIFR has two LIFRα subunits and the transducing subunits LIFRβ and gp130. Upon binding LIF, the LIFR recruits Jak1 to activate STAT3 (Module 2: Figure JAK/STAT heterogeneity), which is a transcription factor that functions in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

During osteoclastogenesis, LIF acts like PTH to increase the expression of RANKL by the supporting cells.

Oncostatin (OSM)

Oncostatin (OSM) is a cytostatic cytokine that inhibits cell proliferation and may thus act as a tumour suppressor. It

was discovered through its ability to inhibit the growth of melanoma cells in culture. It is a 28 kDa glycoprotein that is released from T cells and monocytes. There are two OSM receptors that are members of the IL-6 subfamily of receptors that are characterized by sharing the transducing subunit glycoprotein 130 (gp130) (Module 1: Figure type I cytokine receptors). The OSMR has two OSMRα subunits that then combine with two transducing β subunits, one of these is gp130 and the other is either LIFR (Type I OSMR) or OSMRβ (Type II OSMR). Upon binding OSM, these OSMRs recruit the Jak transducers to activate STAT3 (Module 2: Figure JAK/STAT heterogeneity), which is a transcription factor that functions in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

Prolactin (PRL)

Prolactin (PRL) is a small protein (19 amino acids), which has certain structural similarities to growth hormone (GH). It is released into the circulation from the lactotrophs (Module 10: Figure lactotroph regulation). PRL functions to stimulate lactation and can also influence maternal behaviour. However, it is made in other locations and has many other functions. The prolactin receptor, which is located primarily in mammary gland, liver and ovary, is a member of the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT heterogeneity).

Stem cell factor (SCF)

Stem cell factor (SCF), which is also known as steel factor, is produced by many cell types (bone marrow stromal cells, endothelial cells, placental cells). SCF functions during the early stages of haematopoiesis where it contributes to maintain haematopoietic cell self renewal (Module 8: Figure HSC regulation) and continues to control the formation of erythroid cells and mast cells (Module 8: Figure haematopoietic cytokines). SCF is also released from keratinocytes during the control of melanogenesis by the melanocytes (Module 7: Figure melanogenesis).

SCF is the ligand for the c-KIT receptor (also known as CD117), which is one of the protein tyrosine-linked receptors (PTKRs) (Module 1: Figure tyrosine kinase-linked receptors).

Mutations in SCF or its receptor c-KIT cause piebaldism. Mutations in c-KIT are also found in many gastrointestinal stromal tumours.

Thrombopoietin (TPO)

Thrombopoietin (TPO) is produced predominantly by hepatocytes but can also be released from kidney proximal tubule cells, fibroblasts and endothelial cells. TPO is an example an haematopoietic cytokine that functions to control haematopoiesis (Module 8: Figure haematopoietic cytokines). It operates very early to regulate the proliferation of the stem and progenitor cells. Later on it guides the subsequent differentiation and maturation of the megakaryocytes and blood platelets.

The TPO receptor (TPOR) is composed of two identical subunits (Module 1: Figure type I cytokine receptors). Each extracellular domain, which has typical cytokine receptor modules, functions together to bind a single EPO molecule to induce a conformational change in the intracellular box motifs that recruit and activate Jak2. The latter then activates STAT5a and STAT5b (Module 2: Figure JAK/STAT heterogeneity), which are transcription factors that function in the JAK/STAT signalling pathway (Module 2: Figure JAK/STAT function).

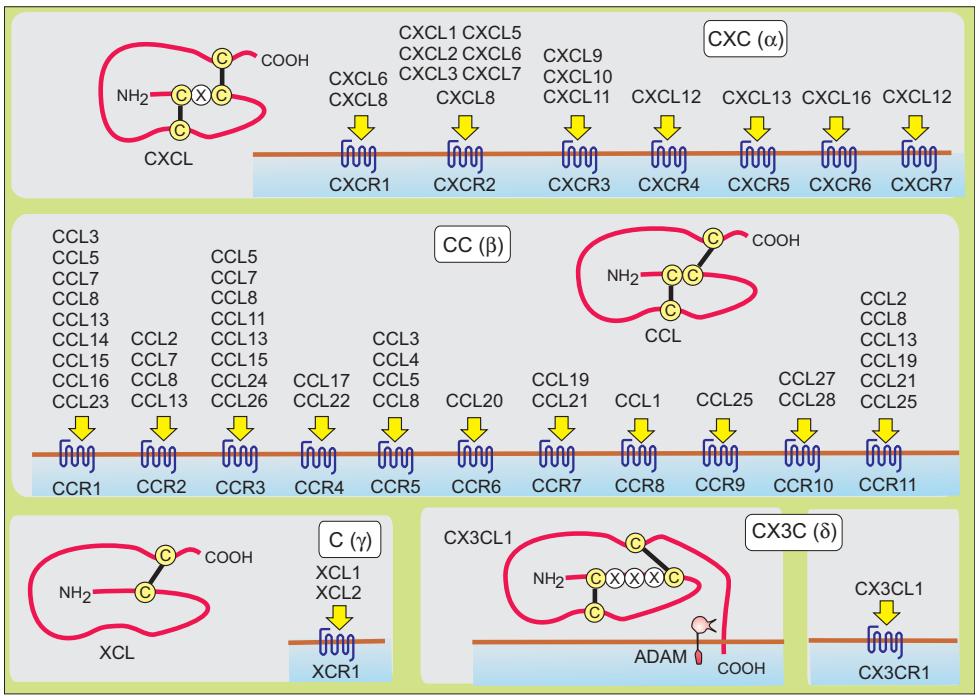
Chemokines

The chemokines are low-molecular-mass proteins that have a variety of functions. There are approximately 50 chemokines that have been divided into four families: CXC (α), CC (β), C (γ) and CX3C (δ) (Module 1: Figure chemokines). These families are subdivided on the basis of the position of conserved cysteine residues located in the N-terminal region (see the models for each family). For example, the CXC family has two cysteine residues (C) separated by a single non-cysteine amino acid residue (X). This nomenclature is used to refer to both the ligands (L) and their receptors (R). Consequently, CXCL1 refers to the first ligand of the CXC family and CXCR1 is the first receptor of the same family. The same terminology applies to the other families. The CX3C (δ) family is unusual in that it has a single member CX3CL1 that is tethered to membranes through its C-terminal region. It can act on its CX3CR1 either in this tethered form or as a freely diffusible molecule after it has been released from the membrane by proteases such as A Disintigrin or the ADAM protease family.

Chemokines first came to prominence as chemoattractants during inflammation, but subsequently were found to have signalling functions in many other cellular processes:

- Chemokines establish gradients to attract various inflammatory cells such as neutrophils (Module 11: Figure inflammation).
- Chemokines play a role in haematopoiesis where they regulate the migration of cells in lymphoid organs, bone marrow and thymus. For example, haematopoietic stem cell (HSC) migration is controlled by CXCL12 [stromal-derived factor-1 (SDF-1)] that acts through the CXCR4 chemokine receptor (Step 1 in Module 8: Figure bone marrow). SDF-1 plays a role in renal cell carcinoma (RCC).
- The chemokine CXCL12 controls preosteoclast chemotaxis (Module 8: Figure preosteoclast chemotaxis).
- T cell chemotaxis is driven by a chemokine gradient (Module 9: Figure T cell chemotaxis).
- Microglia use chemokines to develop neural inflammatory responses (Module 7: Figure microglia interactions). A chemokine signalling network contributes to the neuronal-microglial interactions (Module 7: Figure neuronal chemokine function) that may contribute to neuropathic pain.
- A relationship between chemokines and cancer has revealed that chemokines such as CXCL12 and its receptor CXCR4 may play a critical role in the onset of metastasis. In addition, the chemokine CCL19 may

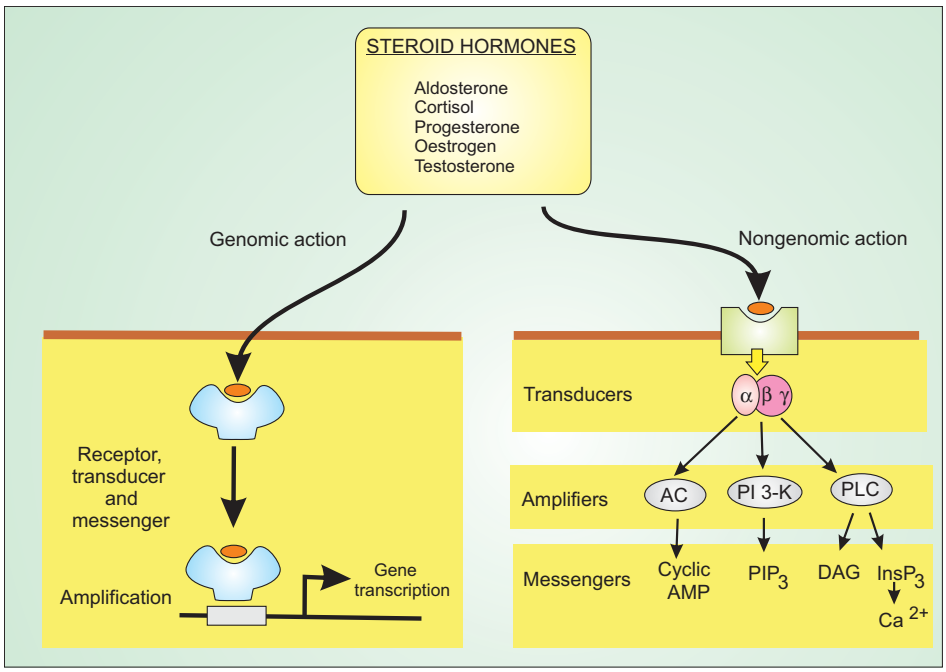
Module 1: | Figure chemokines



Summary of the four chemokine families and their G protein-coupled receptors.

The four chemokine families CXC (α), CC (β), C (γ) and CX3C (δ) are subdivided on the basis of conserved cysteine residues located in the N-terminal region (see the models for each family). For example, the ligands for the CXC family (CXCL) have two cysteine residues separated by a single amino acid (X). There are seven receptors CXCR1–7 for the CXCLs. A similar terminology applies to the other families. The CX3C (δ) family is unusual in that it has a single member CX3CL1 that is tethered to membranes through its C-terminal region. It can act on its CX3CR1 either in this tethered form or after it has been released from the membrane by proteases. This Figure is based on information contained in Box 1 from Rostène et al. (2007).

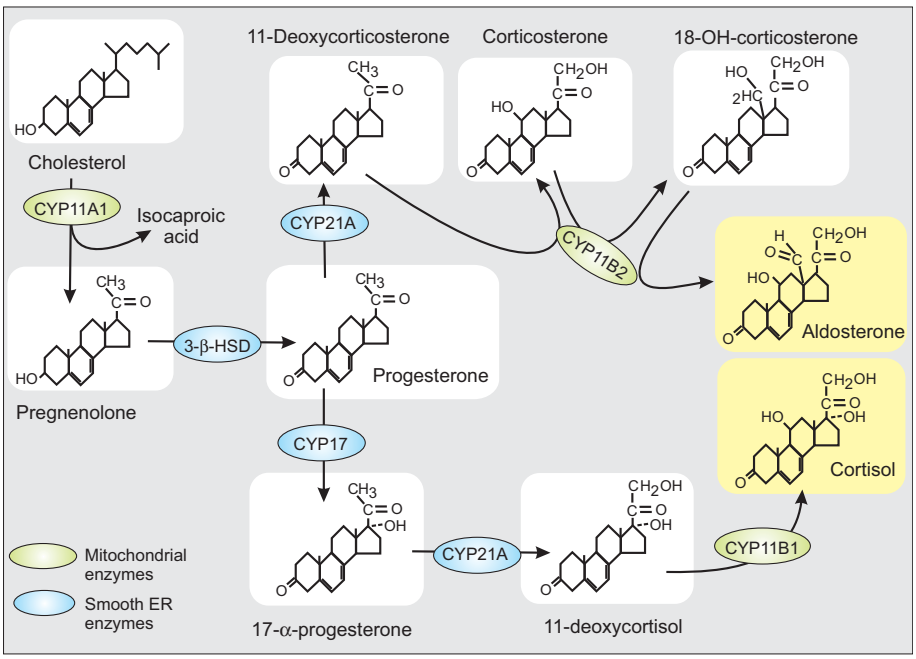
Module 1: | Figure steroid stimuli



Steroid stimuli operate through genomic or non-genomic mechanisms.

The genomic action depends upon steroids passing through the plasma membrane to engage specific receptors that are transcription factors that activate gene transcription. In the non-genomic action, the steroid hormone engages cell-surface receptors to activate conventional signalling pathways.

Module 1: | Figure aldosterone and cortisol biosynthesis



Biosynthesis of mineralocorticoids and corticosteroids.
The mineralocorticoid aldosterone and the corticosteroid cortisol are synthesized from cholesterol through a sequence of reactions driven by a battery of enzymes. The association of the enzymes with either the mitochondrion (green) or the smooth endoplasmic reticulum (ER) (blue) is shown in more detail for aldosterone synthesis in Module 7: Figure glomerulosa cell signalling. See the text for further details.

function to attract leukaemic T-cells into the brain in patients with T cell acute lymphoblastic leukaemia.

- Retinal pigmented epithelial cells release the chemokines eotaxin-1 (CCL11), eotaxin-2 (CCL24) and eotaxin-3 (CCL26) to activate the CCR3 receptors on the choroidal endothelial cells to stimulate angiogenesis. Abnormal activation of this signalling pathway may be responsible for the choroidal neovascularization responsible for age-related macular degeneration (AMD).
- The chemokine monocyte chemoattractant protein-1 (MCP-1) is released by white fat cells to attract macrophages to adipose tissue where they release TNFα that sets up an inflammatory response that contributes to insulin resistance (Module 12: Figure insulin resistance).
- In the germinal centre the chemokines CXCL12 and CXCL13 direct the migration of B-cells between the dark and light zones during B-cell differentiation in the lymph node (Module 8: Figure germinal centre).

Steroids

Steroid hormones are hydrophobic stimuli responsible for regulating a number of physiological processes such as reproduction (oestrogen and testosterone), glucose metabolism and stress responses (cortisol) and salt balance (aldosterone). Steroids have two main modes of action, genomic and non-genomic (Module 1: Figure steroid stimuli). The genomic action depends on the fact that the steroids are hydrophobic and thus can pass through the plasma membrane to bind to intracellular receptors, which are transcription factors capable of activating gene transcription. The non-genomic action depends on steroids binding to cell-surface receptors that are then coupled to conventional intracellular signalling pathways.

Aldosterone and cortisol biosynthesis illustrates how steroids are synthesized from cholesterol through a series of reactions carried out by enzymes associated with either the mitochondrion or the smooth endoplasmic reticulum (Module 1: Figure aldosterone and cortisol biosynthesis).

Aldosterone and cortisol biosynthesis

Aldosterone and the corticosteroids (cortisol and corticosterone) are synthesized in the zona glomerulosa and the zona fasciculata/reticularis cells of the adrenal gland respectively (Module 7: Figure adrenal gland). Like other steroids, they are synthesized in a series of steps carried out by a number of different enzymes (Module 1: Figure aldosterone and cortisol biosynthesis):

- CYP11A1 is a side chain cleavage enzyme that initiates the process by cleaving off the side chain of cholesterol to produce pregnenolone.
- 3β-HSD is a short-chain hydroxysteroid dehydrogenase that converts pregnenolone into progesterone. The two synthetic pathways now diverge.
- CYP21A is a steroid 21-hydroxylase that converts progesterone into 11-deoxycorticosterone.
- CYP11B2 (11β-hydroxylase), which is highly expressed in the zona glomerulosa cells, is an aldosterone synthase that carries out the last three steps of converting 11-deoxycorticosterone into corticosterone, 18-OH-corticosterone and finally aldosterone.

- CYP17 is a steroid 17α-hydroxylase and 17,20-lyase that converts progesterone into 17α-progesterone that begins the synthetic sequence that results in the formation of cortisol.
- CYP21A converts 17α-progesterone into 11-deoxycortisol.
- CYP11B1, which is strongly expressed in the zona fasciculata/reticularis carries out the final step of cortisol synthesis by converting 11-deoxycortisol into cortisol.

Aldosterone

Aldosterone is mainly synthesized and released by the zona glomerulosa cells in the cortical region of the adrenal gland (Module 7: Figure adrenal gland). However, it can also be produced by other cells located in the nervous system, heart, kidney and blood vessels. This extra-adrenal production of aldosterone may be particularly important for tissue repair. The enzymes responsible for aldosterone and cortisol biosynthesis are located on the mitochondrion and smooth endoplasmic reticulum (Module 1: Figure aldosterone and cortisol biosynthesis). One of the primary genomic actions of aldosterone is to regulate Na^+ reabsorption by the distal convoluted tubule (DCT) (Module 7: Figure kidney tubule function) and the colon (Module 7: Figure colon function).

Cortisol

Cortisol is one of the glucocorticoids produced by the cortisol biosynthetic mechanisms located in zona fasciculata/reticularis cells of the adrenal gland (Module 1: Figure aldosterone and cortisol biosynthesis). It has multiple functions in the cardiovascular, neural and immunological systems. It has both immunosuppressive and anti-inflammatory responses.

The glucocorticoids have anti-inflammatory effects mediated in part by an increase in the transcription and synthesis of inhibitor of nuclear factor κB (NF-κB) (IκB) that then functions to inhibit NF-κB by promoting its retention in the cytosol.

Glucocorticoids also increase the production of other anti-inflammatory molecules such as interleukin-10 (IL-10), TNF-α, COX2 and phospholipase A_2 (PLA_2).

Through their ability to modulate the immune system, glucocorticoids such as prednisone, dexamethasone and hydrocortisone are used to treat many inflammatory conditions such as asthma, allergies, dermatitis, rheumatoid arthritis, leukaemias and lymphomas.

Corticosterone

Corticosterone is produced by the cortisol biosynthetic mechanisms located in zona fasciculata/reticularis cells of the adrenal gland (Module 1: Figure aldosterone and cortisol biosynthesis). It functions to increase glycogen formation by enhancing the conversion of amino acids into carbohydrates.

Oestrogens

Oestrogens are the female sex hormones that act primarily in sexual development and reproduction. However, they have many other functions, particularly in the cardiovascular system. There are a number of oestrogens such as oestrone (E1), oestradiol (E2) and oestriol (E3). Most attention has focused on E2 that acts through oestrogen receptor-α (ERα) and oestrogen receptor-β (ERβ) encoded by the Esr1 and Esr2 genes respectively, which are typical of the nuclear receptors (Module 4: Table nuclear receptor toolkit).

In addition to these genomic actions mediated by nuclear receptors, E2 also has a non-genomic action in that it can stimulate the G protein coupled receptor 20 (GPR20). Such an action seems to be particularly important in regulating both the expression and the channel gating of plasma membrane epithelial Na^+ channels (ENaC).

Receptors

In order to respond to the myriad cell stimuli outlined in the previous section, cells have evolved an equally impressive battery of cell-surface receptors. These receptors have two main functions: they have to detect incoming stimuli and then transmit this information to the internal transducers that initiate the signalling pathway (Module 1: Figure cell signalling mechanism). Receptors vary enormously in the way they carry out this transfer of information across the plasma membrane. The diverse receptor types can be separated into two main groups depending on the number of membrane-spanning regions they have to embed them into the membrane:

- Single membrane-spanning receptors

Protein tyrosine kinase-linked receptors (PTKRs)
Serine/threonine kinase-linked receptors (S/TKRs)
Particulate guanylyl cyclases (pGCs)
Non-enzyme-containing receptors

- Multi-membrane-spanning receptors

G protein-coupled receptors (GPCRs)
Ion channel receptors
Sigma receptors

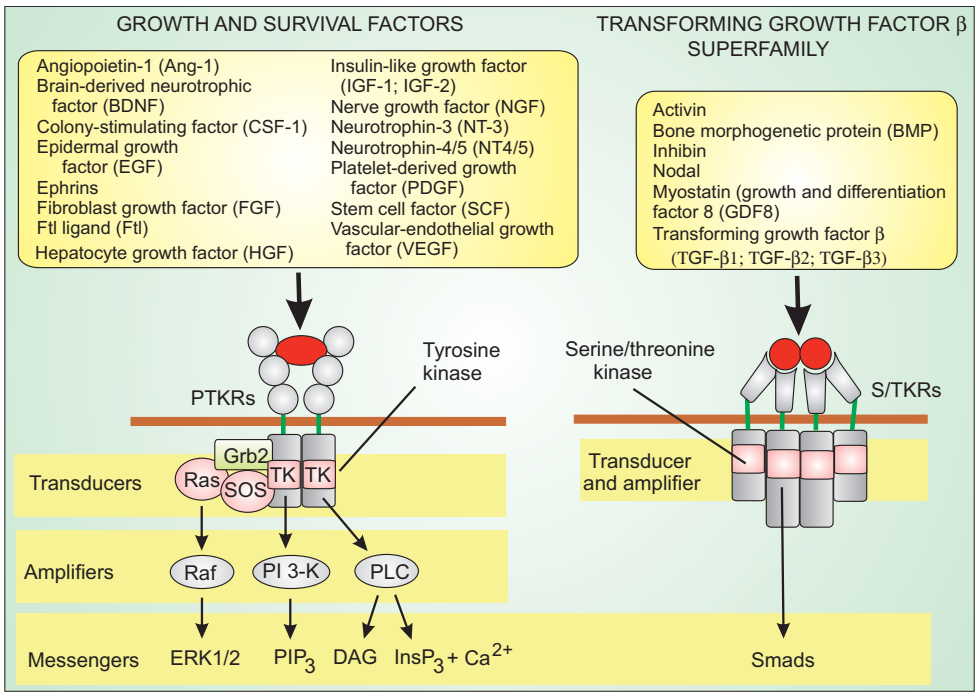
There are a large number of single membrane-spanning proteins, which fall into two groups:

- Type I transmembrane proteins, which are orientated with an extracellular N-terminus and intracellular C-terminus. Most of the single-spanning receptors belong to this group.
- Type II transmembrane proteins, which are orientated with an extracellular C-terminus and intracellular N-terminus.

Protein tyrosine kinase-linked receptors (PTKRs)

The protein tyrosine kinase-linked receptors (PTKRs) are the classical example of single-membrane-spanning receptors that contain an enzymatic activity (Module 1: Figure stimuli for enzyme-linked receptors). There are about 20 subfamilies of these PTKRs, which have the same basic structural features (Module 1: Figure tyrosine kinase-linked receptors). The extracellular domain is responsible for binding the growth or survival factors, whereas the cytosolic region contains the tyrosine kinase domain that is the transducer responsible for initiating the process of signal transduction. One of the characteristics

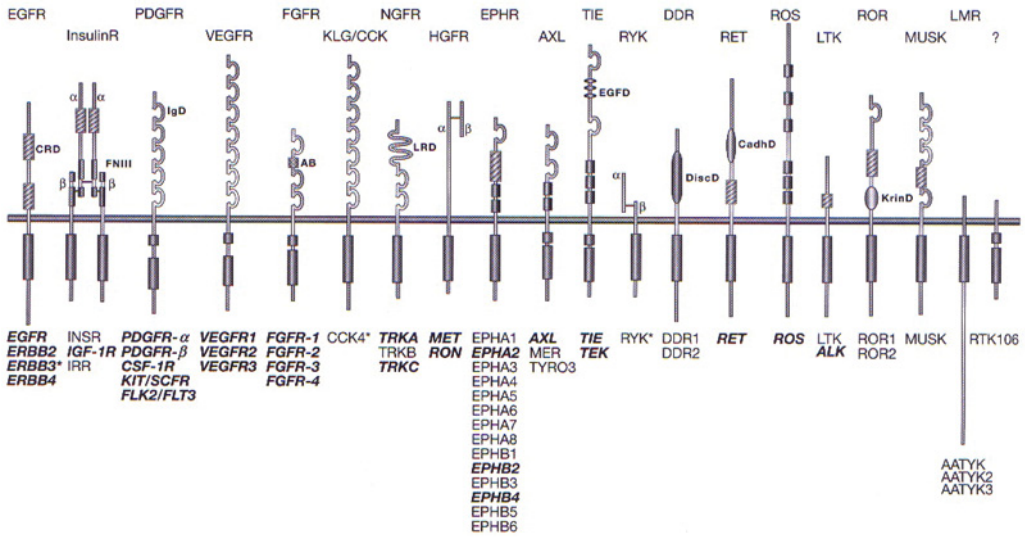
Module 1: | Figure stimuli for enzyme-linked receptors



Growth and survival factors engage enzyme-linked receptors.

These single-membrane-spanning receptors have an extracellular ligand-binding domain and an intracellular domain that contains either a tyrosine kinase (TK) or a serine/threonine kinase. The protein tyrosine kinase-linked receptors (PTKRs), which usually function as dimers, assemble a complex of scaffolding proteins (green) and transducers (red) that relay information out to amplifiers that function in different signalling pathways. The serine/threonine kinase-linked receptors (S/TKRs) function as tetramers and have a much simpler signalling pathway in that the serine/threonine kinase functions as both the transducer and amplifier to generate the Smad messengers.

Module 1: | Figure tyrosine kinase-linked receptors



Tyrosine kinase-linked receptors.

There are 20 subfamilies of tyrosine kinase-linked receptors that resemble each other by having a single transmembrane domain that embeds the receptors in the plasma membrane. The receptors normally function as dimers. The insulin receptor pre-exists as a dimer, whereas the others dimerize when they bind their external stimuli. The cytoplasmic portion has the tyrosine kinase that functions in signal transduction. During activation by external stimuli, the receptors dimerize and then phosphorylate each other to provide phosphorylated binding sites that draw in various signalling components (e.g. see Module 1: Figure PDGFR activation). Reproduced from *Handbook of Cell Signaling*, Volume 1 (edited by R.A. Bradshaw and E.A. Dennis), Heldin, C.-H., Protein tyrosine kinase receptor signalling overview, pp. 391–396. Copyright (2003), with permission from Elsevier; see Heldin 2003.

of these receptors is that they can transmit information down a number of cell signalling pathways by assembling a number of transducers and amplifiers. There are a number of variations on this basic structural organization as illustrated by the following examples of some of the main PTKRs:

- Anaplastic lymphoma kinase (ALK) receptor
- Epidermal growth factor receptor (EGFR)
- Colony-stimulating factor-1 receptor (CSFR-1)
- Fibroblast growth factor receptor (FGFR)
- Hepatocyte growth factor receptor (HGFR)
- Insulin receptor
- Insulin-like growth factor receptor (IGFR)
- Platelet-derived growth factor receptor (PDGFR)
- TIE receptors
- Trk receptors
- Vascular endothelial growth factor receptor (VEGFR)

Anaplastic lymphoma kinase (ALK) receptor

The anaplastic lymphoma kinase (ALK) receptor belongs to the protein tyrosine kinase-linked receptors (PTKRs) family (Module 1: Figure tyrosine kinase-linked receptors). ALK is expressed mainly in the central and peripheral nervous system. Little is known about the stimulus responsible for activating this receptor. However, it has come to prominence as a major predisposition gene for childhood neuroblastomas.

Epidermal growth factor receptor (EGFR)

The epidermal growth factor receptor (EGFR), which is one of the protein tyrosine kinase-linked receptors (PTKR) (Module 1: Figure tyrosine kinase-linked receptors), has four members (Module 1: Figure EGF stimuli and receptors). There is a problem with regard to terminology in that they have been given different names and some of these are shown on the Figure. In much of the literature, the ErbB terminology is now preferred and will be used in this case. All of these receptors have a similar domain structure: they are single membrane-spanning proteins with an N-terminal extracellular ligand-binding domain and a C-terminal region that has a kinase domain and numerous tyrosine docking sites that participate in the process of signal transduction. The external ligand-binding domain has two cysteine-rich regions. Despite these similarities, these receptors have different properties. For example there are marked differences in the way they respond to the large family of epidermal growth factors (EGFs) (Module 1: Figure EGF stimuli and receptors). ErbB1 is the most catholic in its tastes and responds to most of the ligands. There is no known ligand for ErbB2, which thus is an example of an orphan receptor. However, there is much interest in c-ErbB2 because it is one of the common oncogenes found in breast cancer. ErbB3, which interacts with the neuregulins NRG1 and NRG2, is unusual in that its kinase domain is non-functional. This does not mean that ErbB3 is prevented from signalling because it does so by forming functional dimers by interacting with one of the other ErbB receptors as described later. ErbB4 binds to a number of EGFs.

The way in which these receptors function resembles that for many of the other PTKRs as exemplified for the platelet-derived growth factor receptor (PDGFR) (Module 1: Figure PDGFR activation). The various EGFs induce two chains to form both homo- and heterodimers. This dimerization then induces the receptor phosphorylation events that initiate the onset of cell signalling. The Cbl down-regulation of cell signalling components mechanism (Module 1: Figure receptor down-regulation) is responsible for the down-regulation of these ErbB receptors.

The EGF receptor family play an important role in mammary gland development where ErbB1 controls outgrowth of the ducts, whereas ErbB2 and ErbB3 regulate alveolar morphogenesis and lactation.

Members of the ErbB family of receptors are often amplified or activated through mutations in various cancers.

Fibroblast growth factor receptor (FGFR)

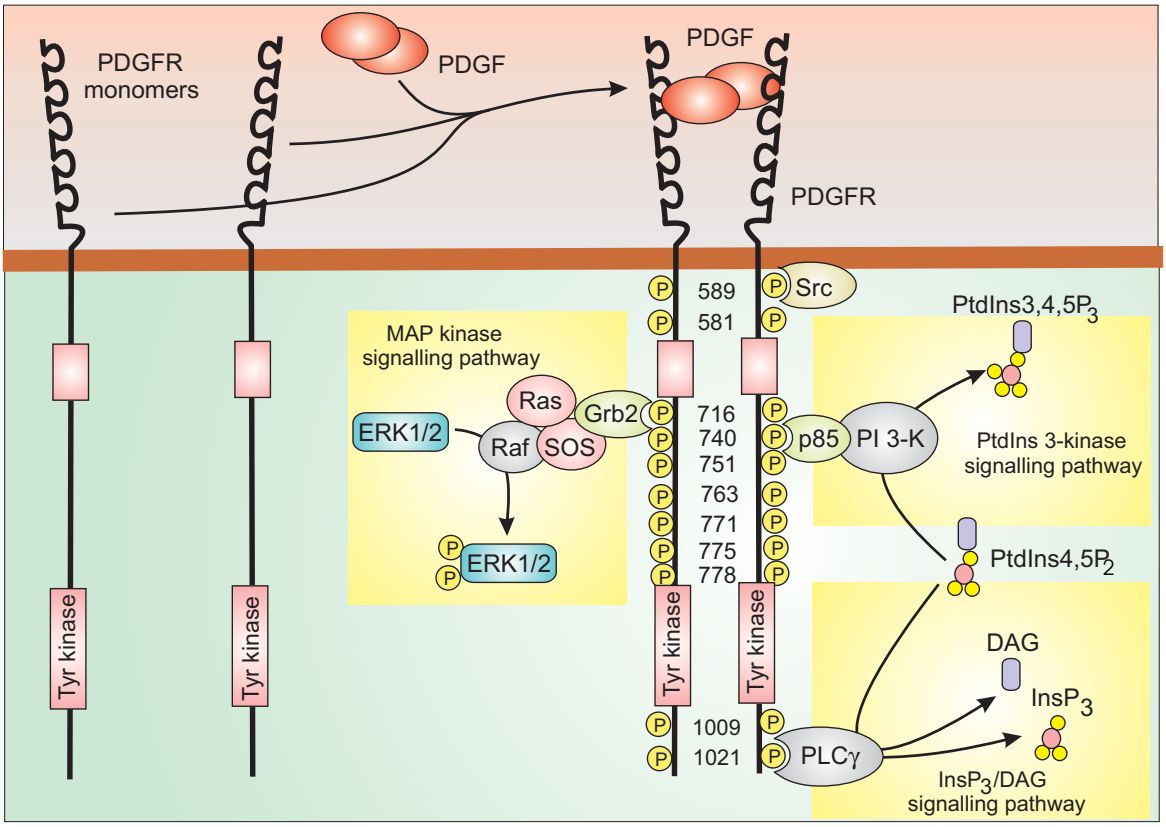
The fibroblast growth factor receptors (FGFRs) are typical protein tyrosine kinase-linked receptors (PTKR) (Module 1: Figure tyrosine kinase-linked receptors). There are four FGFRs (FGFR1–4). The cytosolic region of the molecule has a split tyrosine kinase domain. The extracellular domain has three immunoglobulin-like (Ig-like) domains. The affinity of the different FGFRs for the large FGF family is varied by splicing events in the third Ig-like domain. The first two Ig-like domains are separated by a stretch of acidic amino acids and a heparin-binding domain that interacts with heparan sulphate proteoglycans (HSPGs) that also bind to the family of fibroblast growth factors (FGFs). The HSPG facilitates the interaction between the FGFs and their FGFRs to induce the dimeric receptor complexes necessary to recruit and engage different signalling pathways.

Hepatocyte growth factor receptor (HGFR)

There are two closely related hepatocyte growth factor receptors (HGFRs), MET and RON. MET belongs to the family of protein tyrosine kinase-linked receptors (PTKR) (Module 1: Figure tyrosine kinase-linked receptors). MET is the receptor that responds to hepatocyte growth factor (HGF) whereas RON responds to macrophage-stimulating protein (MSP). Most attention has been focused on MET that is coded for by the p190 c-met proto-oncogene. MET is a disulphide-linked heterodimer that originates from a single protein. A small part is cleaved off to form the α-subunit that is attached to the transmembrane β chain. (Module 1: Figure tyrosine kinase-linked receptors). The extracellular region of this β chain has a Sema domain, a cysteine-rich domain called the Met-related sequence (MRS) and four immunoglobulin-like (Ig-like) structures (IPT domain). The intracellular part has the catalytic tyrosine kinase domain and regulatory domain, which has tyrosine residues at 1349 and 1356 that provide docking sites to recruit the components of signalling pathways.

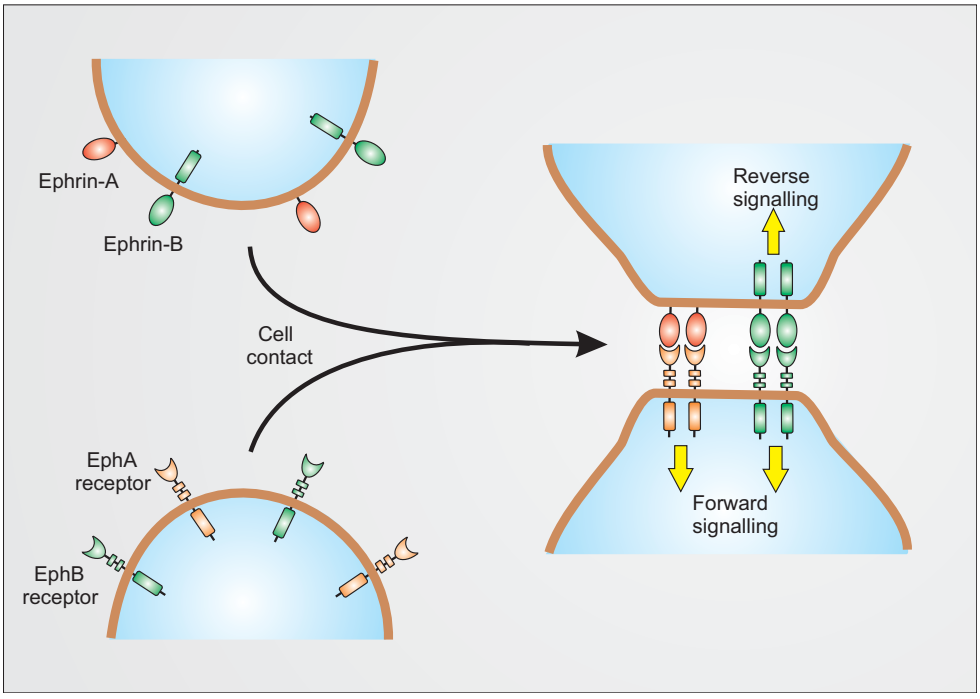
The juxtamembrane region of the β chain has two phosphorylation sites that function in receptor down-regulation. Phosphorylation of Ser-985 inhibits the activity of the receptor tyrosine kinase whereas Tyr-1003

Module 1: | Figure PDGFR activation



Activation of the platelet-derived growth factor receptor (PDGFR).
The PDGFR is a typical protein tyrosine kinase-linked receptor. The PDGF dimer binds together two PDGFR monomers, which then phosphorylate each other on multiple tyrosine residues that then provide docking sites for components of a number of prominent signalling pathways. An animated version of this Figure is available.

Module 1: | Figure Eph receptors



Eph receptor/ephrin bidirectional signalling.
The ephrins are cell-surface proteins that interact with Eph receptors on neighbouring cells to set up both forward and reverse signalling. These two signalling modes are described in more detail in Module 1: Figure Eph receptor signalling

phosphorylation is a binding site for the ubiquitin ligase Cbl that functions in the Cbl down-regulation of cell signalling components (Module 1: Figure receptor down-regulation).

Both the MET and RON receptors play an important role in the invasive growth of carcinomas.

Insulin-like growth factor receptor (IGFR)

There are two insulin-like growth factor receptors (IGFRs) that are capable of responding to insulin-like growth factors (IGFs). The IGF type I receptor (IGF-IR) resembles the insulin receptor both in its structure and mode of activation (Module 2: Figure insulin receptor). Like the insulin receptor, IGF-IR has a heterotetrameric $\alpha_2\beta_2$ structure. The two IGFs (IGF-I and IGF-II) bind with equal affinities to the two extracellular α-subunits to induce the conformational change responsible for receptor activation. The β-subunits, which are mostly intracellular, have the tyrosine kinase domain and motifs that interact with components of the different signalling pathways activated by these receptors.

The IGF type II receptor (IGF-IIR) is a single protein that is embedded in the membrane through a transmembrane region. The extracellular domain has fifteen cysteine-based repeats. The primary function of the IGF-IIR, which is strongly expressed during embryonic development, is to remove IGF-II and is thus a negative regulator of this IGF isoform. The *IGF-IIR* gene is maternally imprinted.

Platelet-derived growth factor receptor (PDGFR)

The platelet-derived growth factor receptor (PDGFR) is a classical protein tyrosine kinase-linked receptor (PTKR) (Module 1: Figure tyrosine kinase-linked receptors). There are two isoforms, α and β, which have different affinities for platelet-derived growth factor (PDGF). The extracellular region has five immunoglobulin-like (Ig-like) domains that function in ligand binding (domains I–III) and receptor dimerization (domain IV) (Module 1: Figure PDGFR activation). The intracellular region has a split tyrosine kinase domain and numerous tyrosine residues that are phosphorylated during receptor activation, which is initiated when a dimeric PDGF molecule brings together two PDGFRs. Once dimerization occurs, a transphosphorylation process begins whereby the kinase domain on one chain phosphorylates numerous tyrosine residues on the neighbouring chain. These phosphorylated tyrosine residues then provide docking sites for various signalling components to generate multiple output signals (Module 1: Figure PDGFR activation):

- Growth factor receptor-bound protein 2 (Grb2) recruits components of the extracellular-signal-regulated kinase (ERK) pathway (Module 2: Figure ERK signalling) that promotes proliferation.
- The p85 subunit of Class I PtdIns 3-kinase recruits the PtdIns 3-kinase signalling cassette (Module 2: Figure PtdIns 3-kinase signalling) that promotes survival and protein synthesis.
- Phospholipase Cγ (PLCγ) induces both the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette and the diacylglycerol (DAG)/protein kinase C (PKC) signalling cassette that contributes to cell proliferation (Module 2: Figure PLC structure and function).

Ephrin (Eph) receptor signalling

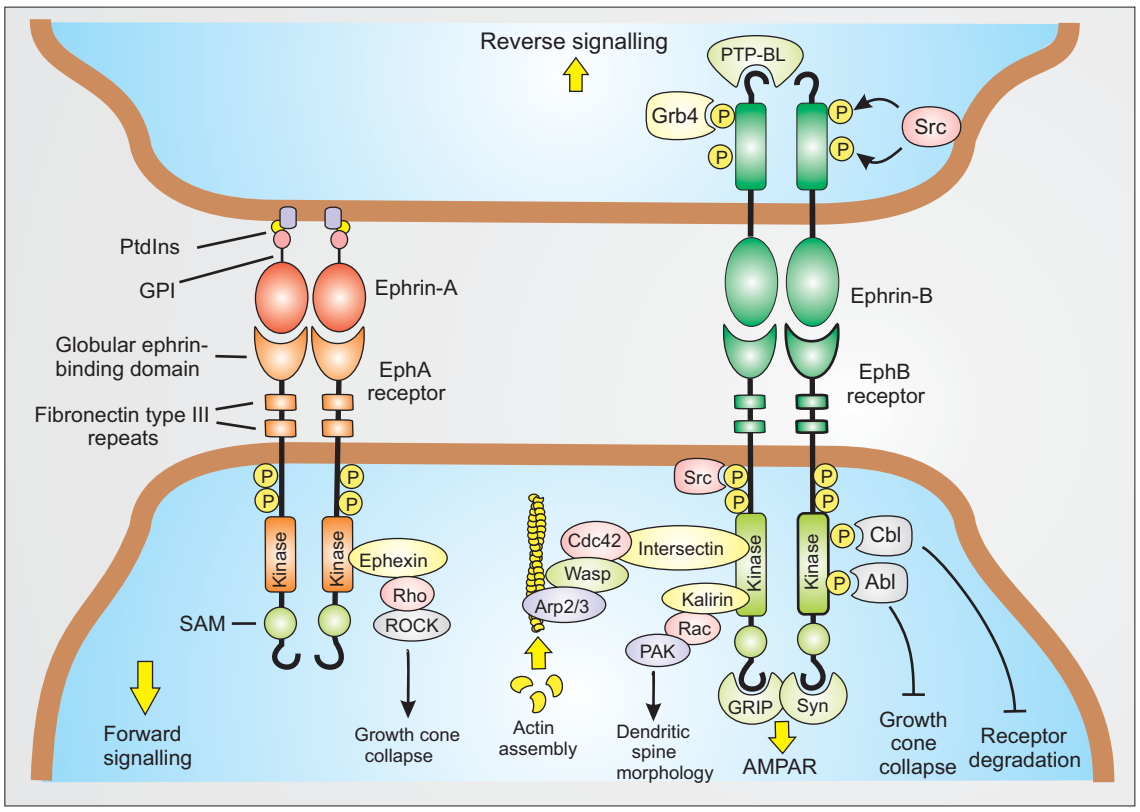
The ephrin (Eph) receptors constitute one of the largest family of protein tyrosine kinase-linked receptors (PTKRs) (Module 1: Figure tyrosine kinase-linked receptors). These receptors function to transfer information between cells that come into contact with each other and are particularly important for spatial patterning during a variety of developmental processes such as axonal guidance, cell morphogenesis and bone cell differentiation. The Eph receptor family is divided into ten A types (EphA1–EphA10) and six B types (EphB1–EphB6). The stimuli for these Eph receptors are the ephrins, which are expressed on the surface of cells (Module 1: Figure Eph receptors). These ephrins are divided into A and B types determined by their ability to bind to either the EphA or EphB receptors. There are six ephrin-A ligands (ephrin-A1–ephrin-A6), which are attached to the membrane through a glycosylphosphatidylinositol (GPI) anchor, and three transmembrane ephrin-B ligands (ephrin-B1–ephrin-B3). The ephrin-B ligands, which have a cytoplasmic domain, are unusual in that they have a dual function. Not only do they function as a ligand to activate the EphB receptors, but also they function as a receptor in that the cytoplasmic domain can convey information in the reverse direction. The Eph receptor/ephrin complex is a bidirectional signalling system and, through the forward and reverse signalling modes, information can be conveyed to both interacting cells.

Craniofrontonasal syndrome (CFNS) is caused by a loss-of-function mutation of the gene that encodes ephrin-B1.

The Eph receptors have an N-terminal ephrin-binding domain followed by a cysteine-rich region and then two fibronectin type III domains (Module 1: Figure Eph receptor signalling). These ectodomains are connected through a typical transmembrane domain to the cytoplasmic domains. There is a relatively long juxtamembrane segment that connects to the kinase domain. At the C-terminal region there is a SAM domain that may participate in receptor dimerization. Finally there is a PDZ domain-binding motif.

When cells approach each other, the ephrin-A ligands bind to the EphA receptors and the ephrin-B ligands bind to the EphB receptors and the resulting interactions induce dimerization as is typical of other PTKRs (Module 1: Figure stimuli for enzyme-linked receptors). In addition to dimers, the Eph receptor/ephrin complex can form higher-order aggregates, and this clustering may be facilitated by interactions between the fibronectin type III domains and the SAM domain. As the receptors are brought together, transphosphorylation by the kinase domains results in the phosphorylation of multiple sites, which then provides the binding motifs to recruit a range of signalling transducers. One of the main functions of Eph receptor signalling is to modulate the dynamics of cell movement by altering both actin remodelling and cell adhesion. A number of the downstream signalling pathways are thus directed towards

Module 1: | Figure Eph receptor signalling



Signal transduction by the Eph receptor/ephrin system.
Activation of the Eph receptors depends upon their dimerization following their binding to the ephrin ligands. The ephrin-A ligands are attached to PtdIns in the membrane through a glycosylphosphatidylinositol (GPI) anchor. The ephrin-B ligands are transmembrane proteins that have a cytoplasmic domain capable of reverse signalling. For forward signalling by the Eph receptors, transphosphorylation by the kinase domains of tyrosine residues provide binding sites for various transducing elements, of which many function to control actin remodelling.

the control of actin assembly. There are subtle differences in the action of EphA and EphB receptors that appear to be adapted to control different cellular processes.

One of the functions of EphA receptors in retinal ganglion neurons is to induce growth cone collapse by activating the Rho signalling mechanism (Module 2: Figure Rho signalling). The activated EphA receptor binds to the Rho guanine nucleotide exchange factor (GEF) ephexin, which is responsible for stimulating Rho (Module 1: Figure Eph receptor signalling). The Rho·GTP then activates the Rho kinase (ROCK) that stimulates the actin–myosin contractions responsible for collapsing the growth cone. Aggregating platelets communicate with each other through the bidirectional Eph signalling system (Step 11 in Module 11: Figure platelet activation).

Most information is available for the forward signalling pathways initiated by the EphB receptors. While the EphA receptors are mainly linked to Rho activation, the EphB receptors are coupled to the Rho GEFs kalirin and intersectin that activate Rac (Module 2: Figure Rac signalling) and Cdc42 (Module 2: Figure Cdc42 signalling) respectively. Cdc42 acts through Wiskott-Aldrich syndrome protein (WASP) and the actin-related protein complex (Arp2/3 complex) to regulate actin assembly (Module 1: Figure Eph receptor signalling). Kalirin, which is found in neuronal dendrites as part of the postsynaptic density (PSD) signalling elements, acts through Rac and the p21-activated kinase (PAK) to control spine morphogenesis (Step 2 in Module 10: Figure postsynaptic density).

TIE receptors

The two angiopoietin receptors TIE1 and TIE2 respond to the angiopoietin growth factors (Ang1–4) that control angiogenesis. The TIE receptors are typical protein tyrosine kinase-linked receptors (PTKRs) (Module 1: Figure tyrosine kinase-linked receptors) that function to generate a number of cell signalling pathways (Module 1: Figure stimuli for enzyme-linked receptors).

Trk receptors

The Trk receptors, which mediate the action of the different neurotrophins (BDNF, NGF, NT-3 and NT-4/5), are typical protein tyrosine kinase-linked receptors (PTKRs) (Module 1: Figure tyrosine kinase-linked receptors). There are three Trk receptors:

- TrkA responds to nerve growth factor (NGF)
- TrkB responds to brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5)
- TrkC responds to neurotrophin-3 (NT-3)

Like other PTKRs, the Trk receptors act through a number of cell signalling pathways (Module 1: Figure stimuli for enzyme-linked receptors).

Trk stands for tropomyosin-receptor kinase, which harks back to its original discovery of *trk* that is one of the oncogenic growth factor receptors. The latter is formed by the fusion of the first seven exons of tropomyosin to the transmembrane and cytoplasmic domains of what is now known to be the TrkA receptor. Trk has been identified in thyroid papillary carcinomas and in colon carcinoma.

The p75 neurotrophin receptor ($p75^{NTR}$), which can also bind neurotrophins, can influence the binding affinity and specificity of the Trk receptors. In addition, $p75^{NTR}$ is coupled to separate signalling pathways that function to promote neuronal cell death.

Serine/threonine-kinase linked receptors (S/TKRs)

The serine/threonine kinase-linked receptors (S/TKRs) are typical single membrane-spanning receptors (Module 1: Figure stimuli for enzyme-linked receptors). They have an extracellular domain that binds members of the transforming growth factor superfamily. The cytoplasmic domain has a serine/threonine protein kinase region that functions as both a transducer and amplifier in that it is activated by the receptor to phosphorylate the Smads, thus producing many copies of these messengers (for further details see Module 2: Figure TGF-βR activation)

Particulate guanylyl cyclases (pGCs)

The particulate guanylyl cyclases (pGCs) are a family of seven single membrane-spanning receptors (pGC-A–pGC-G). These receptors consist of an extracelllar domain, a short transmembrane segment and an intracellular domain that contains the catalytic guanylyl cyclase (GC) region that converts GTP into cyclic GMP (Module 2: Figure NO and cyclic GMP signalling). The extracellular domain binds a range of peptides such as atrial natriuretic factor (ANF), brain-type natriuretic peptide (BNP), C-type natriuretic peptide (CNP) and guanylin. These peptides act through different pGC receptor types. For example, ANP and BNP act through pGC-A, whereas guanylin and uroguanylin, which are released within the intestine, act on pGC-C to stimulate intestinal secretion (Module 7: Figure intestinal secretion). In the case of the adrenal zona glomerulosa cells, ANP increases the formation of cyclic GMP to control the release of aldosterone (Module 7: Figure glomerulosa cell signalling).For these receptors, the guanylyl cyclase region of the cytoplasmic domain functions both as a transducer and as an amplifier.

The remaining receptor isoforms pGC-B, -D, -E, -F and -G are orphan receptors, as there are no known stimuli. pGC-E and pGC-F are expressed in photoreceptors in the eye, where they function to produce cyclic GMP in phototransduction (Step 11 in Module 10: Figure phototransduction).

Certain strains of *Escherichia coli,* which secrete the STa toxin, increase intestinal secretion and cause diarrhoea by activating the cyclic GMP signalling pathway by stimulating the particulate guanylyl cyclase C (pGC-C) receptor that is normally activated by guanylin (Module 7: Figure intestinal secretion).

Non-enzyme-containing receptors

There are a number of membrane-spanning receptors that have cytoplasmic domains that lack enzyme activity. During receptor activation, the role of signal transduction is carried out by the cytoplasmic domains binding various transducers and amplifiers that relay information out to the cell signalling pathways (Module 1: Figure cytokines). Such signalling mechanisms used are the large number of Type I and Type II cytokines and the various apoptotic and inflammatory mediators:

Cytokines: there are a large number of cytokines that act through the cytokine receptors to recruit the Janus kinases (JAKs), which are tyrosine kinases that double up as transducers and amplifiers. They phosphorylate the signal transducers and activators of transcription (STATs), which are transcription factors that act as the messenger to carry information into the nucleus (for further information see Module 2: Figure JAK/STAT function).

Apoptotic and inflammatory mediators: there are a number of inflammatory and apoptotic mediators that use non-enzyme-containing receptors. One group act through the tumour necrosis factor (TNF) family of receptors as exemplified by tumour necrosis factor (TNF) that acts on a trimeric receptor (Module 1: Figure cytokines), which is a multifunctional receptor in that it recruits a number of transducers and amplifiers to relay information to different signalling pathways:

- Pro-caspase 8 is converted into caspase 8, which functions as an initiator caspase in the extrinsic pathway of apoptosis (Module 11: Figure apoptosis). The DR6 receptor in neurons activates caspase-3 and caspase-6 (Module 12: Figure amyloid cascade hypothesis)
- The sphingomyelinase (SMase) enzymes are recruited to the active receptor to initiate the sphingomyelin signalling pathway by producing the second messenger ceramide (Module 2: Figure sphingomyelin signalling)
- The nuclear factor κB (NF-κB) signalling pathway is initiated by binding a complex of scaffolding proteins and enzymes [e.g. inhibitory κB kinase (IKK)], which constitute a transducing complex that activates the transcription factor NF-κB (Module 2: Figure NF-κB activation)

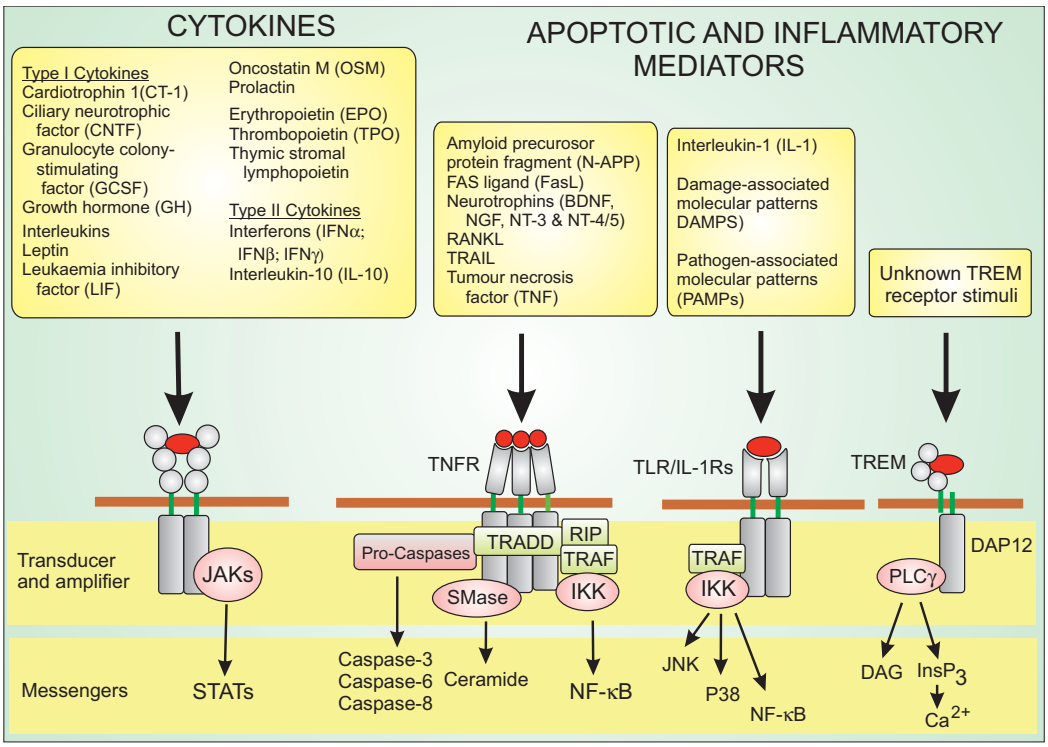
Another group of inflammatory mediators such as interleukin-1 (IL-1), the pathogen-associated molecular patterns (PAMPs) and the endogenous damage-associated molecular patterns (DAMPs) act through the TLR/IL-1 receptor superfamily. There is a large family of Toll-like receptors (TLRs) that closely resemble the IL-1 receptor (IL-1R). These receptors relay information through the nuclear factor κB (NF-κB) signalling pathway, the JNK signalling pathway and the p38 signalling pathway.

Integrin signalling is another example of a signalling system based on membrane-spanning receptors that lack enzyme activity.

Toll-like receptors (TLRs)

There are families of toll-like receptors (TLRs) that includes the interleukin-1 (IL-1) receptor (IL-1R). All of these receptors are integral membrane glycoproteins that

Module 1: | Figure cytokines



Summary of cytokine signalling pathways.
Cytokines and various apoptotic and inflammatory mediators act on non-enzyme-containing receptors that consist of variable numbers of subunits. Each monomer has a single transmembrane domain with an extracellular ligand-binding domain and an inner cytoplasmic domain that lacks enzyme activity but can associate with a number of transducing elements that generate different messengers. Further details of the organization of the type I cytokine receptors are shown in Module 1: Figure class I cytokine receptors. Details of the signal transduction mechanisms induced by TNFRs and TLR/IL-1Rs are shown in (Module 2: Figure NF-κB activation) and (Module 2: Figure Toll receptor signalling) respectively.

have an external ligand-binding domain and an internal cytoplasmic domain that lack enzyme activity. The IL-1R and TLRs are included within this TLR/IL-1R family by virtue of the fact that they display considerable homology with regard to their cytoplasmic domains. They belong to the non-enzyme-containing receptors that function by recruiting various signal transducing components (Module 1: Figure cytokines). This cytoplasmic domain has the Toll/IL-1R (TIR) domain that has three conserved boxes that are highly conserved in all of the receptors. The main difference between them lies in the external domain. In the case of the IL-1R, the external domain contains three immunoglobulin-like domains. By contrast, this region of the TLRs contains a number of leucine-rich repeat (LRR) motifs. It is this region of the molecule that is responsible for binding the pathogen-associated molecular patterns (PAMPs), which are the breakdown products of pathogens. The TLRs are also sensitive to damage-associated molecular patterns (DAMPS). When the TLRs detect these DAMPS and PAMPs, they alert cells to the existence of damaging pathogens (Module 11: Figure formation and action of PAMPs). Information from the TLRs on the plasma membrane is relayed through the Toll receptor signalling pathway (Module 2: Figure Toll receptor signalling). The TLRs located on the endosomal membranes function in virus recognition and antiviral responses (Module 2: Figure virus recognition). The TLRs on microglia in the brain respond to pathogens by releasing inflammatory mediators (Module 7: Figure microglia interactions).

Tumour necrosis factor (TNF) receptor (TNF-R)

The superfamily of tumour necrosis factor (TNF) receptors (TNF-Rs) has many members (e.g. TNF-R itself, Fas, DR3-6, p75 neurotrophin receptor ($p75^{NTR}$) and RANK. As a group, they are responsible for controlling many cellular processes such as apoptosis and various inflammatory responses. They are fairly versatile receptors in that they can relay information out through a number of signalling pathways (Module 1: Figure cytokines). The TNF-R has two subunits, TNF-R1 and TNF-R2, which are usually co-expressed in cells. Some members of this family are often referred to as death receptors because one of their actions is to stimulate caspase-8 to induce apoptosis (Module 11: Figure TNFα apoptotic signalling). However, these receptors can also contribute to inflammatory responses through their ability to activate the nuclear factor κB (NF-κB) signalling pathway (Module 2: Figure NF-κB activation).

The DR6 receptor responds to the N-terminal amyloid precursor protein (APP) fragment (N-APP) that functions in both axonal pruning and neuronal apoptosis (see step 11 in Module 12: Figure amyloid cascade hypothesis). Alterations in the activity of the DR6 activity has been implicated in Alzheimer's disease as part of the amyloid cascade hypothesis.

Fas

The Fas receptor, which is also known as CD95, is a member of the superfamily of tumour necrosis factor α (TNFα) receptors (TNFα-Rs). Fas is a member of the death receptor family capable of triggering apoptosis through the extrinsic pathway (Module 11: Figure TNFα apoptotic signalling).

Fas has cysteine-rich extracellular domains, while their cytosolic regions contain a death domain (DD). When Fas engages the Fas ligand (FasL, also known as CD95L), they form trimers and are then able to activate the extrinsic pathway of apoptosis (Module 11: Figure apoptosis).

Fas ligand (FasL)

The Fas ligand (FasL), which is also known as CD45L, is a homotrimer made up of three type II transmembrane proteins. When it binds to Fas located in another cell through a juxtacrine mechanism, it causes Fas to trimerize and this induces apoptosis in the target cell (Module 11: Figure TNFα apoptotic signalling).

Cytokine receptors

There are a number of different receptor types that respond to the large number of cytokines. By far the majority of the cytokines act through the Type I cytokine receptors (Module 1: Figure type I cytokine receptors), which are typical non-enzyme-containing receptors (Module 1: Figure cytokines). Their structural organization is characterized by extracellular ligand-binding domains and cytoplasmic domains that lack enzyme activity but have a variable number of Box motifs that associate with the Janus tyrosine kinases (Jaks) that relay information to the downstream Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling pathway (Module 2: Figure JAK/STAT function). When stimuli bind to the external binding sites, they induce a conformational change that bring together the box motifs and their associated Jaks, which are then close enough for them to phosphorylate each other to initiate the signalling pathway. This transducing unit can have a variable number of participating subunits (Module 1: Figure type I cytokine receptors). The simplest cases are the homodimers, as exemplified by the receptors for EPO, TPO and G-CSF. The receptor for IL-2 has a more complex heterotrimeric arrangement composed of α- , β- and γ_c-subunits. The common γ-subunit (γ_c) is also used by other receptors such as the heterodimeric IL-4 receptor. There are other cytokine receptors that have four subunits: there are α-subunits, which facilitate ligand binding and are thus specific for particular cytokines, and there are more promiscuous transducing subunits (e.g. β_c, gp130 and LIFR) that are shared by a number of receptors. The glycoprotein 130 (gp130) is a particularly good example of such promiscuity because it is shared by the IL-6 subfamily of receptors that respond to a number of cytokines (IL-6, IL-11, LIF, CNTF and CT-1).

Some of the other cytokines such as colony-stimulating factor 1 (CSF-1), Ftl ligand (FL) and stem cell factor (SCF) respond through protein tyrosine kinase-linked receptors (PTKRs) (Module 1: Figure stimuli for enzyme-linked receptors). Tumour necrosis factor (TNF) and related cytokines act on a trimeric receptor (Module 1: Figure cytokines), which is more versatile in that it recruits a number of transducers and amplifiers to relay information to different signalling pathways (Module 1: Figure cytokines).

p75 neurotrophin receptor ($p75^{NTR}$)

The p75 neuotrophin receptor ($p75^{NTR}$), which is capable of binding all of the neurotrophins, is a member of the tumour necrosis factor receptor (TNF-R) superfamily of non-enzyme-containing receptors (Module 1: Figure cytokines). The extracellular domain has four negatively charged cysteine-rich repeats that bind to the neurotrophins. The cytoplasmic domain has a death domain (DD) similar to that on other death receptors such as the Fas receptor and the p55 TNF receptor (Module 11: Figure TNFα apoptotic signalling). The presence of DD probably means that $p75^{NTR}$ stimulates apoptosis using mechanisms similar to those employed by Fas or TNFα.

Triggering receptor expressed in myeloid cells (TREM)

There are a family of triggering receptors expressed in myeloid cells (TREMs) and TREM-like (TREML) receptors that are encoded by genes located on human chromosome 6p21.1. These TREMs, which function to control the innate immune system, are expressed on various myeloid cells such as the brain microglia, macrophages, neutrophils, megakaryocytes, dendritic cells (DCs) and osteoclasts. This innate response, which is usually activated by toll-like receptors (TLRs), has to be fine-tuned in order to prevent excessive inflammation. The TREMs act as such regulators in that they can either amplify or dampen such innate immune responses. In general, triggering receptors expressed in myeloid cell 1 (TREM-1) acts to enhance inflammation whereas triggering receptors expressed in myeloid cell 2 (TREM-2) has a more anti-inflammatory role.

Triggering receptor expressed in myeloid cell 1 (TREM-1)

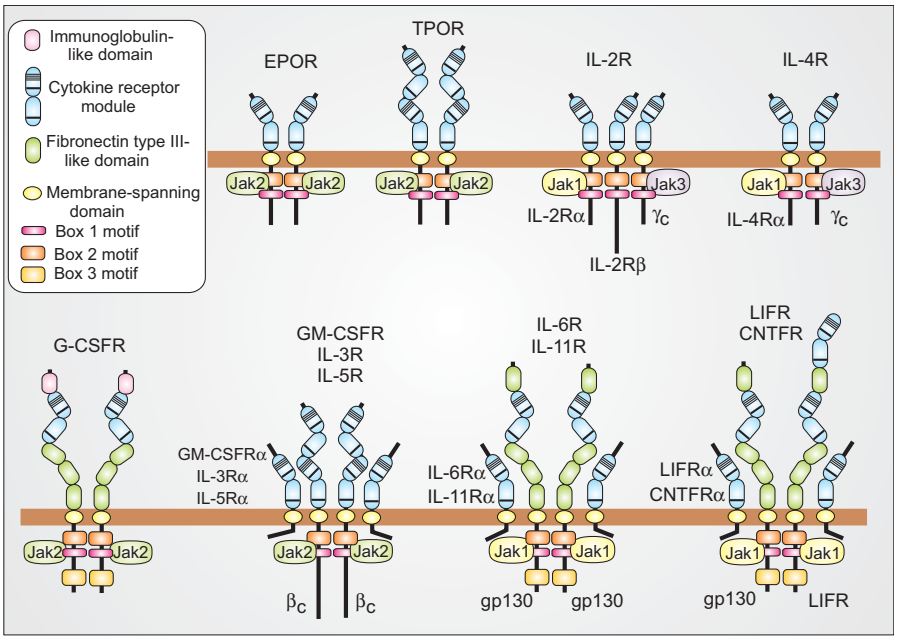
Triggering receptor expressed in myeloid cell 1 (TREM-1) has a complex role in microbial sepsis in that low levels acting through a neutrophil respiratory burst can improve survival whereas more intense stimulation can enhance the inflammatory response associated with sepsis. During the course of an infection, soluble TREM-1 is released. Activation of TREM-1 is also active in enhancing inflammation in Inflammatory Bowel Disease (IBD).

Triggering receptor expressed in myeloid cell 2 (TREM-2)

The triggering receptor expressed in myeloid cells 2 (TREM-2) functions primarily as a negative regulator of innate immunity.

An example of the anti-inflammatory action of TREM-2 is found in the brain where it suppresses the ability of the microglia and macrophages to release inflammatory mediators such as TNF and IL-6. In experimental autoimmune encephalomyelitis (EAE), which is a mouse model of multiple sclerosis (MS), there is a marked up-regulation of TREM-2 in both macrophages and the microglia. TREM2 is a transmembrane glycoprotein that has an extracellular immunoglobulin-like domain, a transmembrane do-

Module 1: | Figure type I cytokine receptors



Class I cytokine receptors

Structural organization of type I cytokine receptors that are characterized by variable extracellular ligand-binding domains and cytoplasmic domains that lack enzyme activity but have a variable number of Box motifs that associate with the Janus tyrosine kinases (Jaks) that relay information to the downstream Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling pathway. The box describes the nature of the receptor domains. Information for this figure was taken from the *Handbook of Cell Signaling*, Vol. 3 (edited by R.A. Bradshaw and E.A. Dennis), Pixley, F.J. and Stanley, E.R., Cytokines and cytokine receptors regulating cell survival, proliferation, and differentiation in hematopoiesis, pp. 615–623. Copyright (2003), with permission from Elsevier; see Pixley and Stanley (2003).

main and a short cytoplasmic region, which is associated with DNAX-activating protein 12 (DAP12), which is also known as TYRO protein tyrosine kinase binding protein (TYROBP). The latter has a typical immunoreceptor tyrosine-based activation motif (ITAM) that associates with various amplifiers such as phospholipase Cγ (PLCγ) (Module 1: Figure cytokines). The nature of the stimuli that act through TREM-2 are still somewhat uncertain. It appears to bind to surface ligands on pathogens and it may also interact with Hsp60 on the surface of astrocytes.

The TREM-2 expressed on developing osteoclasts functions in osteoclastogenesis by switching on the Ca^{2+} signal that activates the transcription factor nuclear factor of activated T cells (NFAT) (Module 8: Figure osteoclastogenesis). The related receptor signal-regulatory protein (SIRPβ1) has a similar role, and their actions will thus be considered together. When activated, TREM-2 and SIRPβ1 interact with DNAX-activating protein 12 (DAP12), which is an adaptor that has a typical immunoreceptor tyrosine-based activation motif (ITAM) (Module 8: Figure osteoclastogenesis). This ITAM region has tyrosine residues that are phosphorylated by the non-receptor tyrosine kinase Src. These specific phosphotyrosine residues recruit Syk that then activates phospholipase Cγ1 (PLCγ1) to switch on the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette. The resulting Ca^{2+} signal appears as a typical series of repetitive Ca^{2+} transients (Module 8: osteoclast Ca^{2+} oscillations). These Ca^{2+} oscillations are responsible for activating the transcription factor NFATc1 by the well-established mechanism that depends upon the Ca^{2+}-dependent activation of calcineurin (Module 4: Figure NFAT activation).

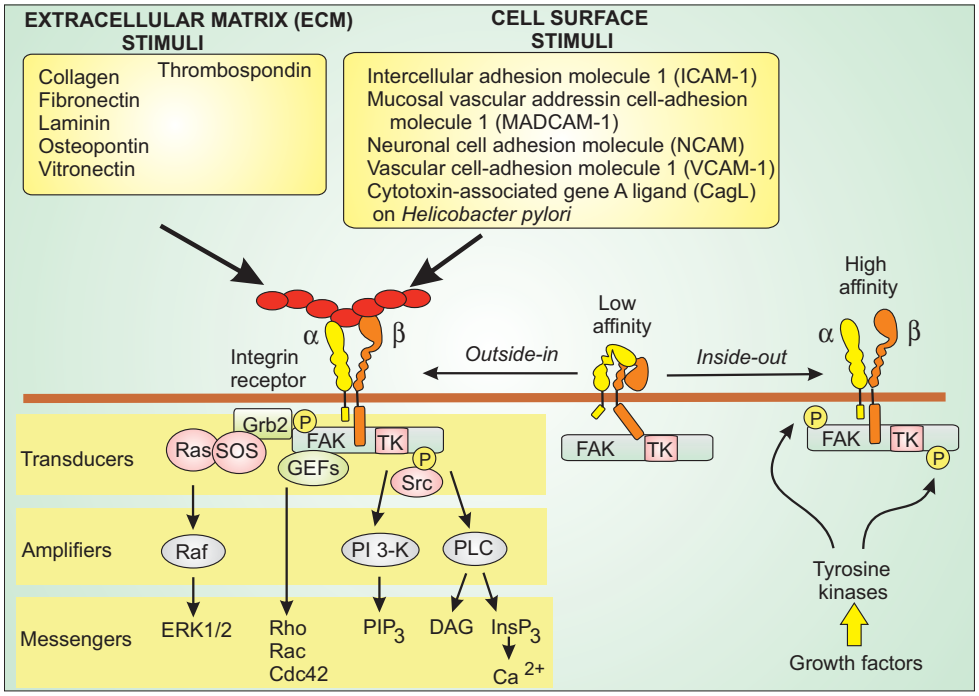
Mutations in the DAP12 gene (*TYROBP*) and in the TREM-2 gene have been linked to polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy syndrome (PLOSL), which is also known as Nasu-Hakola disease (NHD). Since PLOSL causes disorders of both bone and the central nervous system, it is evident that the TREM2/DAP12 signalling pathway may also have an important function in the development and maintenance of brain function.

A variant of TREM-2 may contribute to the inflammation in Alzheimer's disease (Module 12: Figure Inflammation and Alzheimer's disease).

Integrin signalling

The integrins are cell-surface receptors that function in cell adhesion either to the extracellular matrix (ECM) or to specific cell-surface ligands during cell–cell interactions (Module 1: Figure integrin receptor). Integrins have two main functions. Firstly, they provide a link between the internal cytoskeleton and the ECM as occurs at focal adhesion complexes and podosomes. Secondly, they have a very important signalling function that is unusual because they can signal in both directions. In the conventional *outside-in* mode, external stimuli activate the integrin receptor, which then engages various transducing elements to transmit signals into the cell. In the *inside-out* mode, intracellular signals coming from other receptors, often growth factor

Module 1: | Figure integrin receptor



Integrin receptor activation by extracellular matrix (ECM) and cell-surface stimuli.
The integrin receptor is a heterodimer composed of an α- and a β-subunit. In the absence of stimulation (low-affinity state), the extracellular domains are bent over, but extend out to a high-affinity state following *outside-in* or *inside-out* signalling. During the former, the large extracellular domain interacts with various components of either the extracellular matrix (ECM) or cell-surface ligands. Integrins have very short cytoplasmic domains that lack enzyme activity. During receptor activation, the cytoplasmic domain of the β-subunit interacts with transducers such as focal adhesion kinase (FAK), which has tyrosine kinase (TK) activity, enabling an autophosphorylation to create binding sites to activate a number of transducing elements. During *inside-out* signalling, other receptors activate tyrosine kinases that phosphorylate FAK to induce a high-affinity state that then enables the integrin receptor to bind to other adhesion molecules.

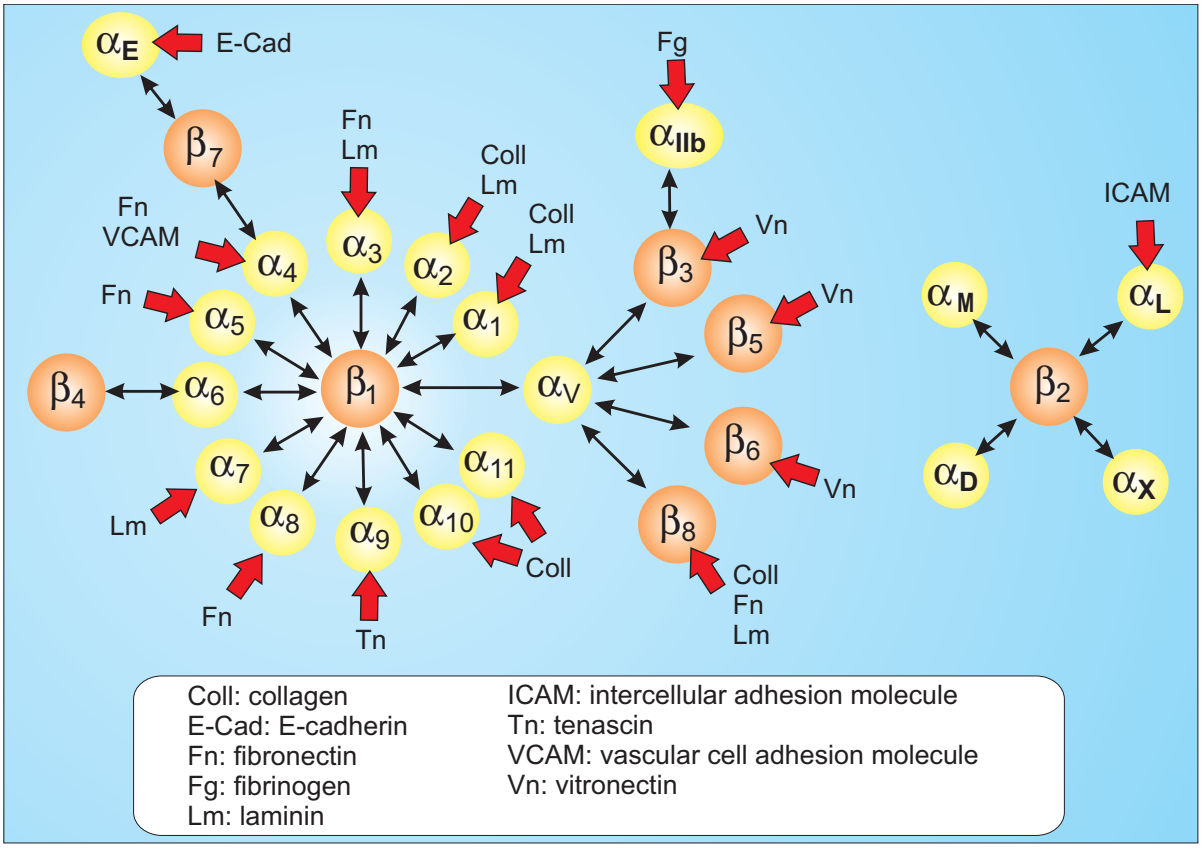
receptors, induce a conformational change in the integrin receptors that greatly enhance their affinity for their external ligands. An example of inside-out signalling is found in osteoclasts, where the colony-stimulating factor-1 receptor (CSF-1R) functions to sensitize the integrin receptors (Module 8: Figure osteoclastogenesis).

Integrins are composed of transmembrane α- and β-subunits that come together to form a functional heterodimer (Module 1: Figure integrin receptor). There are 18 α-subunits and eight β-subunits that can combine to form 24 different heterodimers that have their own binding specificities and characteristic expression patterns (Module 1: Figure integrin heterodimeric complexes). The following examples illustrate the expression of different combinations in specific cell types:

- α_2/β_1 and $\alpha IIb/\beta_3$ are expressed in blood platelets (Module 11: Figure platelet activation). The calcium and integrin-binding protein 1 (CIB1) appears to regulate $\alpha IIb/\beta_3$ by binding to its cytoplasmic tail.
- α_v/β_3 is expressed in osteoclasts and functions in bone resorption (Module 7: Figure osteoclast function)
- α_L/β_2, which is also known as lymphocyte function-associated antigen-1 (LFA1), is expressed on lymphocytes and contributes to the immunological synapse
- α_4/β_7, which is also known as very late antigen 4 (VLA4), is expressed on lymphocytes
- α_7/β_1 is expressed in cardiac cells, where it functions in the link between the sarcolemma and the sarcomeres (Module 12: Figure cardiac contractile elements)
- α_5/β_1 responds to the cytoxin-associated gene A (CagA) ligand (CagL) on the end of the nano-syringe that is used by *Helicobacter pyloris* to inject proteins into its host cell (Module 12: Figure *H pylori* nano-syringe). CagA is thought to be a bacterial oncoprotein responsible for inducing stomach cancer.

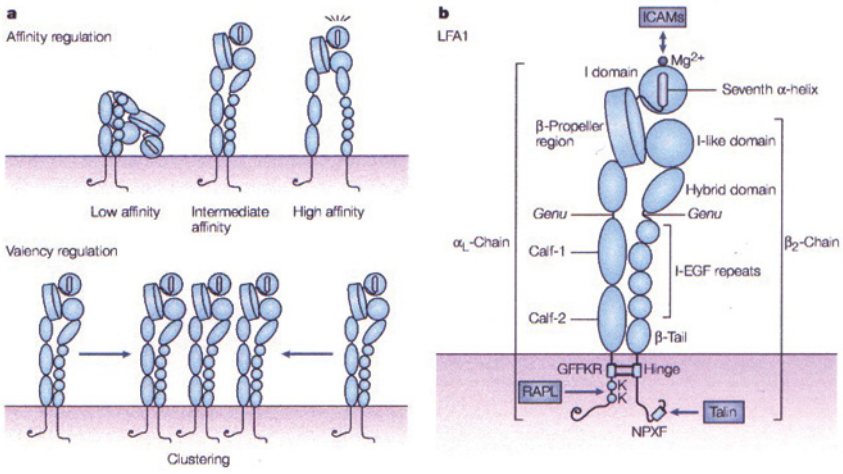
The integrins have large extracellular regions that interact with specific sequences on the extracellular matrix proteins (cell–matrix interactions) or specific cell-surface ligands (cell–cell interactions). The intracellular cytoplasmic domain is relatively short (40–70 amino acids) and lacks enzyme activity. There is considerable information on integrin structure and the dramatic conformational changes that occur during the formation of adhesion complexes (Module 1: Figure integrin receptor structure). The structure of the α_L subunit illustrates the large cytoplasmic head that consists of a series of domains beginning with an I domain followed by the β-propeller region and then three domains that end in the transmembrane domain. One of the linkers between these three domains is known as *genu* (knee), because it is the region where the molecule bends over when it assumes the low-affinity state. Finally, there is a short cytoplasmic domain, which has a GFFKR hinge

Module 1: | Figure integrin heterodimeric complexes



The integrin family of α- and β-subunits.
The double-headed arrows link together the 24 known α–β combinations. The red arrows illustrate the stimuli that activate these different heterodimeric complexes. This figure is based on Figure 3a from Brancaccio et al. 2006.

Module 1: | Figure integrin receptor structure



The structure and regulation of the α_L/β_2 integrin receptor of lymphocytes.
a. The function of integrin receptors can be modulated through a change in either affinity or valency. Changes in conformation bring about different affinity states, whereas valency regulation depends upon their lateral mobility and clustering. b. Structural domains of the α_L and β_2 chains. See text for further details. Reproduced by permission from Macmillan Publishers Ld: *Nature Reviews Immunology*. Kinashi, T. (2005) Intracellular signalling controlling integrin activation in lymphocytes. 5:546–559. Copyright (2005); http://www.nature.com/nri; Kinashi 2005.

motif and the KK motif that binds to RAPL. The β_2 begins with an I-like domain followed by a hybrid domain, which is linked through a *genu* to four integrin epidermal growth factor (I-EGF) repeats. The latter are connected to a β-tail, which connects to the transmembrane region and the cytoplasmic tail region. The latter has a hinge region and a NPXF motif that binds to talin.

External stimuli such as intercellular adhesion molecule (ICAM) in the case of the α_L/β_2 integrin in lymphocytes, bind to the I domain of the α_L subunit through a reaction that depends upon Mg^{2+} occupying the metal-ion-dependent adhesion site (MIDAS). The molecule then undergoes the large conformational changes that bring about the changes in affinity.

In order to transmit information into the cell, the relatively short cytoplasmic domains of the integrin receptors function as a signalling platform to assemble various transducing elements (Module 1: Figure integrin receptor). An important component of the transducing mechanism are kinases such as the integrin-linked kinase (ILK) and focal adhesion kinase (FAK), which can relay information down conventional signalling pathways such as the mitogen-activated protein kinase (MAPK) signalling pathway, the PtdIns 3-kinase signalling pathway, the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette and the signalling pathways activated by the monomeric G proteins (Rho, Rac and Cdc42) that are critical for remodelling the actin cytoskeleton.

More detailed information on the signalling and skeletal functions of integrins is presented in the following sections:

- Blood platelet aggregation (Steps 8–10 in Module 11: Figure platelet activation)
- Cardiac cell contractile and cytoskeletal elements (Module 12: Figure cardiac contractile elements)
- Osteoclast activation in bone resorption (Step 1 in Module 7: Figure osteoclast function)
- Focal adhesion complex formation (Module 6: Figure integrin signalling)

Glanzmann's thrombasthenia is a bleeding disorder that has been linked to mutations in the β_3 integrin subunit.

G protein-coupled receptors (GPCRs)

The G protein-coupled receptors (GPCRs) represent a very large superfamily of receptors that are capable of responding to an enormous number and variety of extracellular stimuli (light, odorants, neurotransmitters, hormones and proteases) (Module 1: Table G protein-coupled receptors). As their name implies, they are coupled to the heterotrimeric G proteins that function as the transducers to relay information to different signalling pathways such as the cyclic AMP signalling pathway (Module 1: Figure stimuli for cyclic AMP signalling) and the inositol 1,4,5-trisphosphate ($InsP_3$)/diacylglycerol (DAG) signalling pathway (Module 1: Figure stimuli for $InsP_3$/DAG signalling).

The GPCRs are characterized by having seven-membrane-spanning regions with the N-terminus facing the outside and the C-terminus lying in the cytoplasm. The external ligands, which usually bind to a pocket formed by the external regions of some of the transmembrane domains, induce a conformational change in the receptor that is then transmitted through the membrane to activate the GTP-binding proteins (G proteins). These G proteins fall into two main groups: the heterotrimeric G proteins and the monomeric G proteins. It is the heterotrimeric G proteins that are the main transducers responsible for transferring information from the GPCRs to a number of signalling pathways. This transduction process is discussed in further detail in Module 2 (see Module 2: Figure heterotrimeric G protein signalling). The receptor activates the G proteins by functioning as a guanine nucleotide exchange factor (GEF) to induce the exchange of GDP for GTP (Module 2: Figure G protein binary switching). When the G protein is bound to GTP it activates a variety of downstream effectors including adenylyl cyclase and phospholipase C.

Many oncogenic growth factors are known to act through GPCRs and many human cancers have mutations in these GPCRs.

Ca^{2+}-sensing receptor (CaR)

The Ca^{2+}-sensing receptor (CaR) belongs to the family of G protein-coupled receptors (GPCRs) (Module 1: Table G protein-coupled receptors). The primary function of the CaR is to regulate parathyroid hormone (PTH) synthesis and release, but it is also expressed in many other cell types, such as bone cells, neurons, intestine, kidney, skin, pancreas and heart. It is a typical GPCR with the usual seven transmembrane domains, a large extracellular N-terminal domain and a C-terminal domain of 216 amino acids, some of which have potential phosphorylation sites for protein kinase C (PKC) and protein kinase A (PKA). The CaR operates as a dimer, with the two subunits linked together by two disulfide bonds (Module 7: Figure PTH secretion). In the parathyroid gland, the CaR dimers are located on caveolae where they appear to be linked to both caveolin and the scaffolding protein filamin. The CaR can also form heterodimers with the mGluR1 and mGluR5 receptors in the brain. Unlike many other GPCRs, the CaR does not desensitize and responds continuously to the prevalling Ca^{2+} concentration.

In addition to responding to Ca^{2+}, CaR is also sensitive to a number of other agonists that fall into three main groups. The first group consists of related inorganic ions (Mg^{2+} and Gd^{3+}) or organic polycations (neomycin and spermine) that act in much the same way as Ca^{2+} to directly activate the receptor. The other two groups function indirectly as allosteric regulators that alter the affinity of the receptor, either positively (calcimimetics) or negatively (calcilytics).

In the parathyroid gland, CaR functions as a 'calciostat' in that it is very sensitive to small fluctuations in the plasma level of Ca^{2+}. It has the potential of relaying information to the parathyroid cell through different signalling pathways. The main mechanism appears to be through the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette. It may also act to inhibit the Ca^{2+}-inhibitable isoform of adenylyl cyclase (AC)

Module 1: | Table G protein-coupled receptors
G protein-coupled receptors (GPCRs) and their associated heterotrimeric G proteins and downstream signalling pathways.

G protein-coupled receptor	Heterotrimeric G protein	Signalling pathway
Acetylcholine (muscarinic) receptors		
M1	$G_{q/11}$	Stimulate phospholipase Cβ
M2	G_i	Inhibit adenylyl cyclase
M3	$G_{q/11}$	Stimulate phospholipase Cβ
M4	G_i	Inhibit adenylyl cyclase
M5	$G_{q/11}$	Stimulate phospholipase Cβ
Angiotensin receptors		
AT_1	$G_{q/11}$	Stimulate phospholipase Cβ
AT_2	?	Decrease in MAPK signalling
Adenosine receptors		
A_1	G_i	Inhibit adenylyl cyclase
A_{2A}	G_s	Stimulate adenylyl cyclase
A_{2B}	G_s	Stimulate adenylyl cyclase
A_3	G_i	Inhibit adenylyl cyclise
α_1-Adrenoreceptors		
α_{1A}	$G_{q/11}$	Stimulate phospholipase Cβ
α_{1B}	$G_{q/11}$	Stimulate phospholipase Cβ
α_{1D}	$G_{q/11}$	Stimulate phospholipase Cβ
α_2-Adrenoreceptors		
α_{2A}	G_i	Inhibit adenylyl cyclase
α_{2B}	G_i	Inhibit adenylyl cyclase
α_{2C}	G_i	Inhibit adenylyl cyclase
α_{2C}	G_i	Inhibit adenylyl cyclase
β-Adrenoreceptors		
β_1	G_s	Stimulate adenylyl cyclase
β_2	G_s	Stimulate adenylyl cyclase
β_3	G_s	Stimulate adenylyl cyclase
Bombesin receptors		
BB_1	$G_{q/11}$	Stimulate phospholipase Cβ
BB_2	$G_{q/11}$	Stimulate phospholipase Cβ
BB_3	$G_{q/11}$	Stimulate phospholipase Cβ
Bradykinin receptors		
B_1 (BK_1)	$G_{q/11}$	Stimulate phospholipase Cβ
B_2 (BK_2)	$G_{q/11}$	Stimulate phospholipase Cβ
Calcitonin receptor		
CTR	G_s	Stimulate adenylyl cyclase
Calcitonin gene-related peptide		
$CGRP_1$	G_s	Stimulate adenylyl cyclase
$CGRP_2$	G_s	Stimulate adenylyl cyclase
Amylin	G_s	Stimulate adenylyl cyclase
Adrenomedullin	G_s	Stimulate adenylyl cyclase
Cannabinoid receptors		
CB1	G_i	Inhibit adenylyl cyclase, close Ca^{2+} channels and open K^+ channels
CB2	G_i	Inhibit adenylyl cyclase, close Ca^{2+} channels and open K^+ channels
Ca^{2+}-sensing receptor		
CaR	$G_{q/11}$ and G_i	Stimulate phospholipase Cβ and inhibit adenylyl cyclase
Chemokine receptors (See Module 1: Figure chemokines)		
CCR1	G_i	Inhibit adenylyl cyclase
CCR2	$G_{q/11}$ and G_i	Stimulate phospholipase Cβ and inhibit adenylyl cyclase
CCR3	G_i	Inhibit adenylyl cyclase
CCR4		
CCR5	$G_{q/11}$ and G_i	Stimulate phospholipase Cβ and inhibit adenylyl cyclase
CCR6	$G_{q/11}$ and G_i	Stimulate phospholipase Cβ and inhibit adenylyl cyclase
CCR7		
CCR8	G_i	Inhibit adenylyl cyclase
CCR9	G_i	Inhibit adenylyl cyclase
CCR10	G_i	Inhibit adenylyl cyclase
CXCR1	G_i	Inhibit adenylyl cyclase
CXCR2	G_i	Inhibit adenylyl cyclase
CXCR3		
CXCR4	G_i	Inhibit adenylyl cyclase
CXCR5		
CXCR6		
CXCR7		
XCR1	G_i	Inhibit adenylyl cyclase
CX_3CR1	G_i	Inhibit adenylyl cyclase
Cholecystokinin and gastrin receptors		
CCK_A (responds to CCK)	$G_{q/11}$	Stimulate phospholipase Cβ
CCK_B (responds to gastrin)	$G_{q/11}$	Stimulate phospholipase Cβ
Corticotropin-releasing factor receptors		
CRF-R_1	G_s	Stimulate adenylyl cyclase
CRF-$R_{2\alpha}$	G_s	Stimulate adenylyl cyclase

Module 1 | Table continued

G protein-coupled receptor	Heterotrimeric G protein	Signalling pathway
CRF-$R_{2\beta}$	G_s	Stimulate adenylyl cyclase
CRF-$R_{2\gamma}$	G_s	Stimulate adenylyl cyclase
Dopamine receptors		
D_1	G_s	Stimulate adenylyl cyclase
D_2	G_i	Inhibit adenylyl cyclase
D_3	G_i	Inhibit adenylyl cyclase
D_4	G_i	Inhibit adenylyl cyclase
D_5	G_s	Stimulate adenylyl cyclase
Gastrin receptor (see **Cholecystokinin and gastrin**)		
Endothelin receptors		
ET_A	$G_{q/11}$	Stimulate phospholipase Cβ
ET_B	$G_{q/11}$	Stimulate phospholipase Cβ
Metabotropic glutamate receptors (mGluRs)		
Group I		
$mGluR_1$	$G_{q/11}$	Stimulate phospholipase Cβ
$mGluR_5$	$G_{q/11}$	Stimulate phospholipase Cβ
Group II		
$mGluR_2$	G_i	Inhibit adenylyl cyclase
$mGluR_3$	G_i	Inhibit adenylyl cyclise
Group III		
$mGluR_4$	G_i	Inhibit adenylyl cyclase
$mGluR_6$	G_i	Inhibit adenylyl cyclase
$mGluR_7$	G_i	Inhibit adenylyl cyclase
$mGluR_8$	G_i	Inhibit adenylyl cyclise
GABA receptor		$GABA_A$ and $GABA_B$ are receptor-operated channels (see Module 3: Table receptor-operated channel toolkit)
$GABA_B$	G_S	Stimulate adenylyl cyclase
Galanin receptors		
GalR1	G_i	Inhibit adenylyl cyclase
GalR2	$G_{q/11}$	Stimulate phospholipase Cβ
GalR3	G_i	K^+ channel modulation
Ghrelin receptor (growth-hormone-secretagogue receptor)		
GHS-R	$G_{q/11}$	Stimulate phospholipase Cβ
Gonadotropin-releasing hormone (GnRH) receptor		
GnRHR	$G_{q/11}$	Stimulate phospholipase Cβ
Growth hormone-releasing hormone (GHRH) receptor		
GHRH-R	G_s	Stimulate adenylyl cyclase
Kisspeptin receptor		
GPR54	$G_{q/11}$	Stimulate phospholipase Cβ (Module 10: Figure GnRH neuron)
Histamine receptors		
H_1	$G_{q/11}$	Stimulate phospholipase Cβ
H_2	G_s	Stimulate adenylyl cyclase
H_3	G_i	Inhibit adenylyl cyclase
H_4	G_i	Inhibit adenylyl cyclise
5-Hydroxytryptamine (5-HT) receptors		
$5\text{-}HT_{1A}$	G_i	Inhibit adenylyl cyclase
$5\text{-}HT_{1B}$	G_i	Inhibit adenylyl cyclase
$5\text{-}HT_{1C}$	G_i	Inhibit adenylyl cyclase
$5\text{-}HT_{2A}$	$G_{q/11}$	Stimulate phospholipase Cβ
$5\text{-}HT_{2B}$	$G_{q/11}$	Stimulate phospholipase Cβ
$5\text{-}HT_{2C}$	$G_{q/11}$	Stimulate phospholipase Cβ
$5\text{-}HT_3$	–	A receptor-operated Ca^{2+} channel (see Module 3: Table receptor-operated channel toolkit)
$5\text{-}HT_4$	G_s	Stimulate adenylyl cyclase
Leukotriene receptors		
BLT_1	$G_{q/11}$	Stimulate phospholipase Cβ
BLT_2	$G_{q/11}$	Stimulate phospholipase Cβ
BLT_3	$G_{q/11}$	Stimulate phospholipase Cβ
BLT_4	$G_{q/11}$	Stimulate phospholipase Cβ (see Module 1: Figure eicosanoids for details of leukotriene formation)
Neurotensin receptors		
NT1	$G_{q/11}$	Stimulate phospholipase Cβ
NT2	$G_{q/11}$	Stimulate phospholipase Cβ
Sphingosine 1-phosphate receptors		(see Module 2: Figure sphingomyelin signalling
EDG-1	G_i	Inhibit adenylyl cyclase
EDG-3	$G_{q/11}$	Stimulate phospholipase Cβ
EDG-5	$G_{q/11}$	Stimulate phospholipase Cβ

Module 1 | Table continued

G protein-coupled receptor	Heterotrimeric G protein	Signalling pathway
EDG-6	$G_{q/11}$	Stimulate phospholipase Cβ
EDG-8	G_i	Inhibit adenylyl cyclase
Lysophosphatidic acid receptors		
EDG-2	Gi	Inhibit adenylyl cyclase
EDG-4	$G_{q/11}$	Stimulate phospholipase Cβ
EDG-7	$G_{q/11}$	Stimulate phospholipase Cβ
Mas-related G protein-coupled receptor (Mrgpr)		
MrgprA3		
MrgprC11	$G_{q/11}$	Activate PLC in Itch sensitive neurons (Module 10: Figure Itch signal transduction mechanism)
Melanocortin receptors		
MC1R (activated by α-MSH)	G_s	Stimulate adenylyl cyclase (see Module 7: Figure melanogenesis)
MC2R (activated by ACTH)	G_s	Stimulate adenylyl cyclase
MC3R	G_s	Stimulate adenylyl cyclase
MC4R (activated by α-MSH)	G_s	Stimulate adenylyl cyclase (see Module 7: Figure control of food intake)
MC5R	G_s	Stimulate adenylyl cyclase
Melatonin receptors		
MT_1	$G_{q/11}$	Stimulate phospholipase Cβ
MT_2	G_i	Inhibit adenylyl cyclase
MT_3	$G_{q/11}$	Stimulate phospholipase Cβ
Olfactory receptors (ORs)		
Approximately 1000 OR genes have been identified	G_s	Stimulate adenylyl cyclase
PP-fold peptides		
Y_1	G_i	Inhibit adenylyl cyclase
Y_2	G_i	Inhibit adenylyl cyclase
Y_3	G_i	Inhibit adenylyl cyclase
Y_4	G_i	Inhibit adenylyl cyclase
Y_5	G_i	Inhibit adenylyl cyclase
Pituitary adenylyl cyclase-activating peptide (PACAP) (see **Vasoactive intestinal peptide**)		
Platelet-activating factor (PAF) receptor		
PAFR	$G_{q/11}$	Stimulate phospholipase Cβ
Prostanoid receptors		
EP_1	$G_{q/11}$	Stimulate phospholipase Cβ
EP_2	G_s	Stimulate adenylyl cyclase
EP_3	$G_{q/11}$	Stimulate phospholipase Cβ
EP_4	G_s	Stimulate adenylyl cyclase
DP	G_s	Stimulate adenylyl cyclase
FP	$G_{q/11}$	Stimulate phospholipase Cβ
IP	G_s	Stimulate adenylyl cyclase
TP	$G_{q/11}$	Stimulate phospholipase Cβ (see Module 1: Figure eicosanoids for details of prostanoid formation and action)
Proteinase-activated receptors		
PAR_1	$G_{q/11}$	Stimulate phospholipase Cβ
PAR_2	$G_{q/11}$	Stimulate phospholipase Cβ (see Module 7: Figure melanogenesis)
PAR_3	?	?
PAR_4	$G_{q/11}$	Stimulate phospholipase Cβ
P2 receptors		
$P2Y_1$	$G_{q/11}$	Stimulate phospholipase Cβ
$P2Y_2$	$G_{q/11}$	Stimulate phospholipase Cβ
$P2Y_4$	$G_{q/11}$	Stimulate phospholipase Cβ
$P2Y_6$	$G_{q/11}$	Stimulate phospholipase Cβ
$P2Y_{11}$	$G_{q/11}$	Stimulate phospholipase Cβ
$P2Y_{12}$	G_i	Inhibit adenylyl cyclase ($P2X_{1–7}$ family members are ion channels; see Module 3: Table receptor-operated channel toolkit)
Opioid receptors		
μ (β-endorphin)	G_i	Inhibit adenylyl cyclase
	$G_{i/o}$	Open K^+ channels
	G_o	Close Ca^{2+} channels
δ (β-endorphin)	G_i	Inhibit adenylyl cyclase
	$G_{i/o}$	Open K^+ channels
	G_o	Close Ca^{2+} channels
κ (Dynorphin)	G_i	Inhibit adenylyl cyclase
	$G_{i/o}$	Open K^+ channels
	G_o	Close Ca^{2+} channels
Orexin receptors		
OX_1R	$G_{q/11}$	Stimulate phospholipase Cβ
OX_2R	$G_{q/11}$ & $G_{i/o}$	Stimulate phospholipase Cβ and inhibits adenylyl cyclase

Module 1 | Table continued

G protein-coupled receptor	Heterotrimeric G protein	Signalling pathway
Somatostatin receptors		
sst_1	G_i	Inhibit adenylyl cyclase
sst_{2A} and sst_{2B}	G_i	Inhibit adenylyl cyclase
sst_3	G_i	Inhibit adenylyl cyclase
sst_4	G_i	Inhibit adenylyl cyclase
sst_5	G_i	Inhibit adenylyl cyclise
Tachykinin receptors		
NK_1 (substance P)	$G_{q/11}$	Stimulate phospholipase Cβ
NK_2 (neurokinin A)	$G_{q/11}$	Stimulate phospholipase Cβ
NK_3 (neurokinin B)	$G_{q/11}$	Stimulate phospholipase Cβ
Taste receptors		
T1R1 + T1R3 (umami)	$G_{q/11}$	Stimulate phospholipase Cβ
T1R2 + T1R3 (sweet)	$G_{q/11}$	Stimulate phospholipase Cβ
T2Rs (bitter)	$G_{q/11}$	Stimulate phospholipase Cβ
Thyroid-stimulating hormone (TSH) receptor		
TSH-R	G_s	Stimulate adenylyl cyclase
Thyrotropin-releasing hormone (TRH) receptor		
TRH-R	$G_{q/11}$	Stimulate phospholipase Cβ
Vasoactive intestinal peptide (VIP) and pituitary adenylyl cyclase-activating peptide (PACAP)		
$VPAC_1$	G_s	Stimulate adenylyl cyclase
$VPAC_2$	$G_{q/11}$	Stimulate phospholipase Cβ
PAC_1	$G_{q/11}$	Stimulate phospholipase Cβ
Vasopressin and oxytocin receptors		
V_{1a}	$G_{q/11}$	Stimulate phospholipase Cβ
V_{1b}	$G_{q/11}$	Stimulate phospholipase Cβ
V_2	G_s	Stimulate adenylyl cyclase
OT	$G_{q/11}$	Stimulate phospholipase Cβ

There are a large number of G protein-coupled receptors (GPCRs), which, as their name implies, are coupled to heterotrimeric G proteins that can act through a number of signalling pathways (Module 2: Figure heterotrimeric G protein signalling). Data for this table were taken from *The Sigma–RBI Handbook of Receptor Classification and Signal Transduction* edited by K.J. Watling (2001) Sigma–Aldrich Research Biochemicals Incorporated.

(Module 2: Table adenylyl cyclases) via a pertussis-insensitive G protein (G_i). When expressed in other cell types, CaR has also been found to activate the mitogen-activated protein kinase (MAPK) signalling pathway and phospholipase A_2 (PLA_2).

The CaR is widely distributed throughout the brain on both neurons and glial cells where it controls a wide range of neural processes: early in development, when the extracellular level of Ca^{2+} is known to be elevated, the CaR controls both axonal growth and dendritic branching; neural migration during development; neuronal excitability by regulating various Ca^{2+}-sensitive channels and this control of excitability might contribute to alterations in synaptic plasticity. A possible role in the regulation of learning and memory is made all the more plausible by the fact that the CaR is activated by β amyloids and would thus support the calcium hypothesis of Alzheimer's disease (Module 12: Figure amyloids and Ca^{2+} signalling).

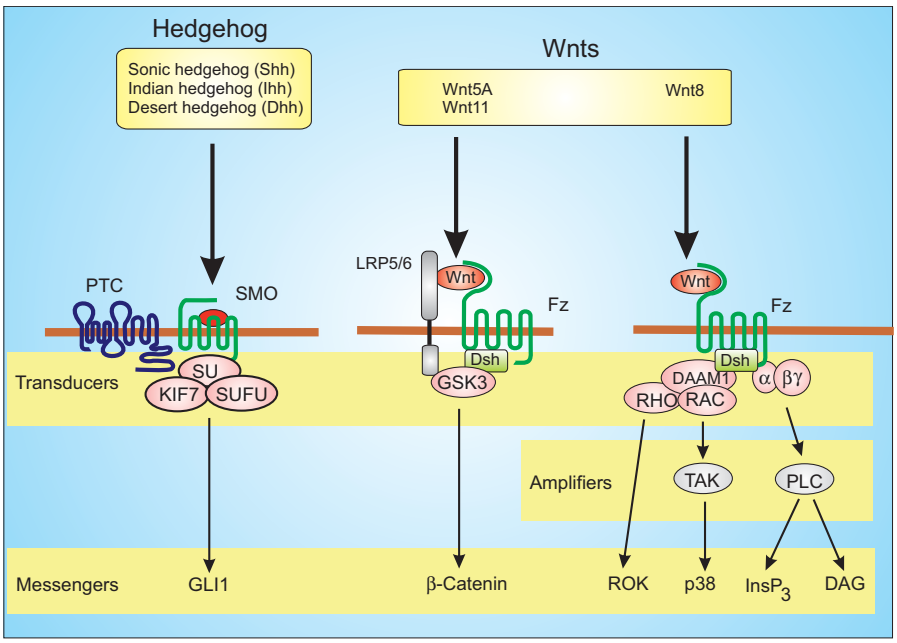
Various diseases characterized by an alteration in Ca^{2+} homoeostasis have been linked to inherited mutations in the CaR:

- Familial hypocalciuretic hypercalcaemia (FHH)
- Neonatal severe hyperparathyroidism (NSHPT)
- Autosomal dominant hypocalcaemia (ADH)
- Type V Barrter's disease

Transducers and amplifiers

Transducers and amplifiers are considered together because their activities are intimately connected and sometimes the two functions reside in the same molecule. The transducers and amplifiers are connected to the receptors, where they receive information coming in from the outside and transform it into the internal messengers (Module 1: Figure cell signalling mechanism). There are many different mechanisms of information transduction. One of the classical mechanisms is found for the G protein-coupled receptors (GPCRs) that use heterotrimeric G proteins to relay information to amplifiers such as adenylyl cyclase (Module 1: Figure stimuli for cyclic AMP signalling) or phospholipase C (PLC) (Module 1: Figure stimuli for $InsP_3$/DAG signalling). The monomeric G proteins also function as transducers for the tyrosine kinase-linked receptors (Module 1: Figure stimuli for enzyme-linked receptors). In this case, the tyrosine kinase associated with the receptor functions together with G proteins such as Son-of-sevenless (SoS) to carry out the transduction event. For receptors that have serine/threonine kinase activity, the enzyme functions as both a transducer and an amplifier. In the case of those receptors that lack enzymatic activity, the transducers and amplifiers are drawn on to the receptor as occurs for the cytokine receptors (Module 1: Figure cytokines) and for the Hedgehog and Frizzled receptors (Module 1: Figure stimuli for developmental signalling).

Module 1: | Figure stimuli for developmental signalling



Stimuli that regulate development act through a number of cell signalling pathways.
The Hedgehog proteins and Wnts are stimuli that control development. They both use a seven-membrane-spanning receptor that resembles the G protein-coupled receptor used by other signalling systems, but in this case the receptor has evolved to activate other signalling pathways. In the case of Hedgehog, the smoothened (SMO) receptor engages a transducing complex that activates the transcription factor GLI1. The signalling pathways for Wnts are carried out by Frizzled (Fz) receptors that are coupled to different signalling pathways. Some of the Wnts activate the transcription factor β-catenin, whereas other Wnts bind to Fz receptors that relay information to other signalling pathways.

Finally, the ion channel receptors are an example where a single protein combines the function of receptor, transducer and amplifier (Module 1: Figure stimuli for ion channels).

These different transducers and amplifiers produce many different intracellular messengers that carry information to the internal sensors and effectors. The cell signalling pathways responsible for producing these messengers are described in more detail in Module 2: Cell Signalling Pathways.

Ion channel receptors

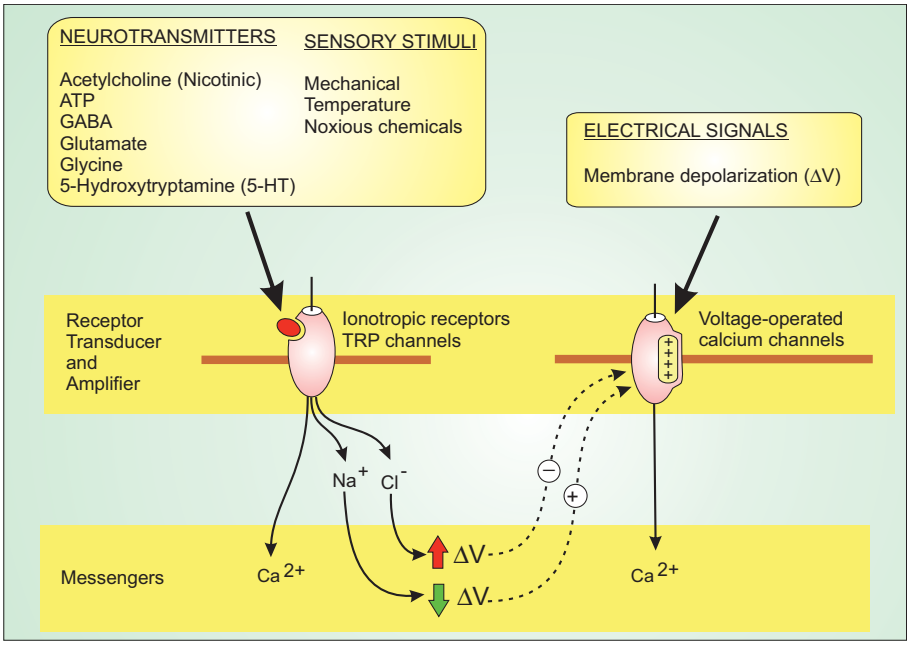
Ion channels play a crucial role in many aspects of cell signalling. One of their most obvious roles is to function as receptors for a number of external stimuli (Module 1: Figure stimuli for ion channels). These receptors are multifunctional in that they detect the incoming stimulus, they transduce the information into channel opening and, by virtue of conducting large amounts of charge, they markedly amplify the signal. Such amplification is the reason such channel receptors are such effective transducers of sensory information.

Module 3: Ion Channels describes the properties of these ion channel receptors and it also describes how some ion channels are important effectors for different intracellular messengers. Their sensitivity to cyclic nucleotides such as cyclic AMP and cyclic GMP is a critical component of sensory transduction.

Sigma receptors

There are two sigma (σ) receptors: sigma-1 receptors (Sig-1R) and sigma-2 receptors (Sig-2R). These receptors, which are widely distributed in the brain and in many peripheral organs, are located mainly in the mitochondrial-associated ER membranes (MAMs) that are specialized functional zones where regions of the endoplasmic reticulum (ER) come into close contact with the mitochondria (Module 5: Figure mitochondrial-associated ER membranes). Most information is available for Sig-1R, which has two transmembrane domains with the N- and C-termini located in the lumen of the ER. There are two steroid-binding domains that come together to form a pocket, which is the binding site for a number of agents such as neurosteroids, neuroleptics, dextrobenzomorphans, methamphetamine and cocaine. Within the MAM, the Sig-1R interacts with both the inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) on the ER membrane and the Ca^{2+}-sensitive chaperone protein BiP, which is located within the ER lumen. When the luminal level of Ca^{2+} declines, the Sig-1R dissociates from BiP and then acts as a chaperone to stabilize the activity of the $InsP_3R$ and thus regulates the transfer of Ca^{2+} from the ER to the mitochondrion. A similar response occurs following stimulation by Sig-1R agonists such as progesterone and this also results in a relocation of the receptor to the plasma membrane where it regulates the activity of a number of channels (Module 5: Figure mitochondrial-associated ER membranes):

Module 1: | Figure stimuli for ion channels



Function of ion channels as receptors for cell stimuli.

Ion channels are sensitive to a range of modalities and can thus be considered to be receptors. Unlike other receptors, they combine all of the components of the signalling pathway in a single protein. When they detect the incoming stimulus (receptor function), they undergo a conformational change (transducer function) to gate large quantities of ions (amplification function). With regard to messenger function, those receptors that gate Ca^{2+} contribute to Ca^{2+} signalling pathways. The gating of ions also changes membrane potential (ΔV) and this can have a messenger function by altering the activity of voltage-operated Ca^{2+} channels (VOCs).

- NMDA receptor (NMDAR)
- Different members of the large family of voltage-dependent K^+ (K_v) channels (Module 3: Table voltage-dependent K^+ channels). There are a number of examples where the Sig-1R supresses the K^+ current and would thus lead to excitation. In the case of small cell lung carcinoma cells, Sig-1Rs inhibited the $K_V1.3$ delayed rectifier K^+ channel resulting in cell cycle arrest. This observation is consistent with the observation that the $K_v1.3$ channel palys a critical role in maintaining the membrane potential that promotes the T cell receptor (TCR) Ca^{2+} signalling that drives cell proliferation (Module 9: Figure T cell Ca^{2+} signalling).

The fact that these receptors are sensitive to psychostimulants, such as methamphetamine and cocaine, has attracted considerable interest as to how they may function in drug addiction. Stimulation of sigma receptors increases dopamine (DA) transmission in the shell of the nucleus accumbens (NAc), which is a part of the brain responsible for the reinforcing effects of drugs such as cocaine that are abused by humans.

In addition to drug addiction, the Sig-1Rs have also been linked to a number of disease states such as Alzheimer's disease (AD), amnesia, amyotrophic lateral sclerosis (ALS), retinal degeneration, and cancer

Intracellular messengers

The role of intracellular messengers is to carry information generated at the cell surface to the internal sensors and effectors (Module 1: Figure cell signalling mechanism). These messengers can take many forms. The concept of an internal messenger first emerged in the cyclic AMP signalling pathway (Module 1: Figure stimuli for cyclic AMP signalling) where the external stimulus was considered to be the first messenger, whereas the cyclic AMP formed during information transduction was referred to as the second messenger. However, the term 'second messenger' can be confusing because there are examples where there are additional messengers within a signalling pathway. Therefore, to avoid confusion, the term 'intracellular messenger' will be used to refer to the agents that carry information within the cell.

Intracellular messengers can take many different forms:

1. Ca^{2+} is one of the major intracellular messengers that transmits information for the Ca^{2+} signalling pathways (Module 2: Figure Ca^{2+} modules).
2. Cyclic AMP that functions in the cyclic AMP signalling pathway (Module 2: Figure cyclic AMP signalling).
3. Ca^{2+}-mobilizing second messengers:
   - Inositol 1,4,5-trisphosphate ($InsP_3$) that functions as a messenger linking receptors to the Ca^{2+} signalling pathway (Module 2: Figure $InsP_3$ and DAG formation).
   - Cyclic ADP-ribose (cADPR) that releases Ca^{2+} from internal stores (Module 2: Figure cADPR/NAADP function)

- Nicotinic acid–adenine dinucleotide phosphate (NAADP) (Module 2: Figure cADPR/NAADP function).

4. Lipid messengers:
   - Diacylglycerol (DAG) is a messenger in the phosphoinositide signalling pathway that links receptor activation to protein kinase C and protein phosphorylation (Module 2: Figure $InsP_3$ and DAG formation).
   - PtdIns3,4,5P_3 is the lipid messenger that functions in the PtdIns 3-kinase signalling pathway (Module 2: Figure PtdIns 3-kinase signalling).
   - PtdIns4,5P_2 is the lipid messenger that functions as a membrane messenger to activate many cellular processes (Module 2: Figure PtdIns4,5P_2 signalling).
   - Phosphatidic acid (PA) is a messenger for the phospholipase D signalling pathway (Module 2: Figure PLD signalling).
   - Ceramide functions in the sphingomyelin signalling pathway (Module 2: Figure sphingomyelin signalling).

5. Protein kinase messengers. Certain protein kinases can carry information to different regions of the cell:
   - The mitogen-activated protein kinase (MAPK) signalling pathway generates active kinases such as extracellular-signal-regulated kinase 1/2 (ERK1/2) (Module 2: Figure ERK signalling) and c-Jun N-terminal kinase (JNK) (Module 2: Figure JNK signalling) that carry information into the cell and very often into the nucleus.

6. Transcription factors that are activated at the cell surface or within the cytoplasm function as messengers carrying information into the nucleus:
   - Nuclear factor κB (NF-κB) carries information from the cell surface into the cell (Module 2: Figure NF-κB activation).
   - The signal transducers and activators of transcription (STATs) transfer information from cell-surface receptors into the nucleus (Module 1: Figure cytokines).
   - Smads transfer information from the transforming growth factor β (TGF-β) receptor superfamily into the nucleus (Module 2: Figure Smad signalling).
   - β-Catenin functions as a messenger in the canonical Wnt pathway (Module 2: Figure Wnt canonical pathway).
   - GLI1 functions in the Hedgehog signalling pathway (Module 2: Figure Hedgehog signalling pathway).

The function of all of these intracellular messengers is to transmit information to the sensors and effectors that are responsible for the final function of the cell signalling pathways to activate a whole host of cellular processes.

Sensors and effectors

The intracellular messengers that are produced by the different signalling pathways function to regulate cellular processes (Module 1: Figure cell signalling mechanism). Just as the cell has receptors to detect external stimuli, the cell contains internal sensors to detect these intracellular messengers. Typical examples of such sensors are the Ca^{2+}-binding proteins that detect increases in Ca^{2+} and relay this information to different effectors to control processes such as contraction and secretion. Some sensors are also effectors. For example, there are enzymes that respond to messengers such as cyclic AMP and cyclic GMP that not only detect the messenger, but also carry out various effector functions. While some of these effectors might be relatively simple, consisting of a single downstream effector system, there are more complicated effectors made up of multiple components such as those driving processes such as exocytosis, phagocytosis, actin remodelling and gene transcription.

The activation of these sensors and effectors completes the flow of information down the cell signalling pathways (green arrows in Module 1: Figure cell signalling mechanism). The operation of such sensors and effectors is described in more detail in Module 4: Sensors and Effectors.

OFF mechanisms

Signalling pathways are composed of the ON mechanisms that generate a flow of information into the cell and the OFF mechanisms that switch off this internal flow of information, enabling cells to recover from stimulation (Module 1: Figure cell signalling mechanism). Module 5: OFF Mechanisms describes how the intracellular messengers and their downstream effectors are inactivated. The second messengers cyclic AMP and cyclic GMP are inactivated by phosphodiesterases. Inositol 1,4,5-trisphosphate ($InsP_3$) metabolism is carried out by both inositol trisphosphatase and inositol phosphatases. Diacylglycerol (DAG) metabolism also occurs through two enzyme systems, DAG kinase and DAG lipase.

In the case of Ca^{2+} signalling, recovery is carried out by the Ca^{2+} pumps and exchangers that remove Ca^{2+} from the cytoplasm. Many of these second messengers activate downstream effectors through protein phosphorylation, and these activation events are reversed by corresponding protein phosphatases.

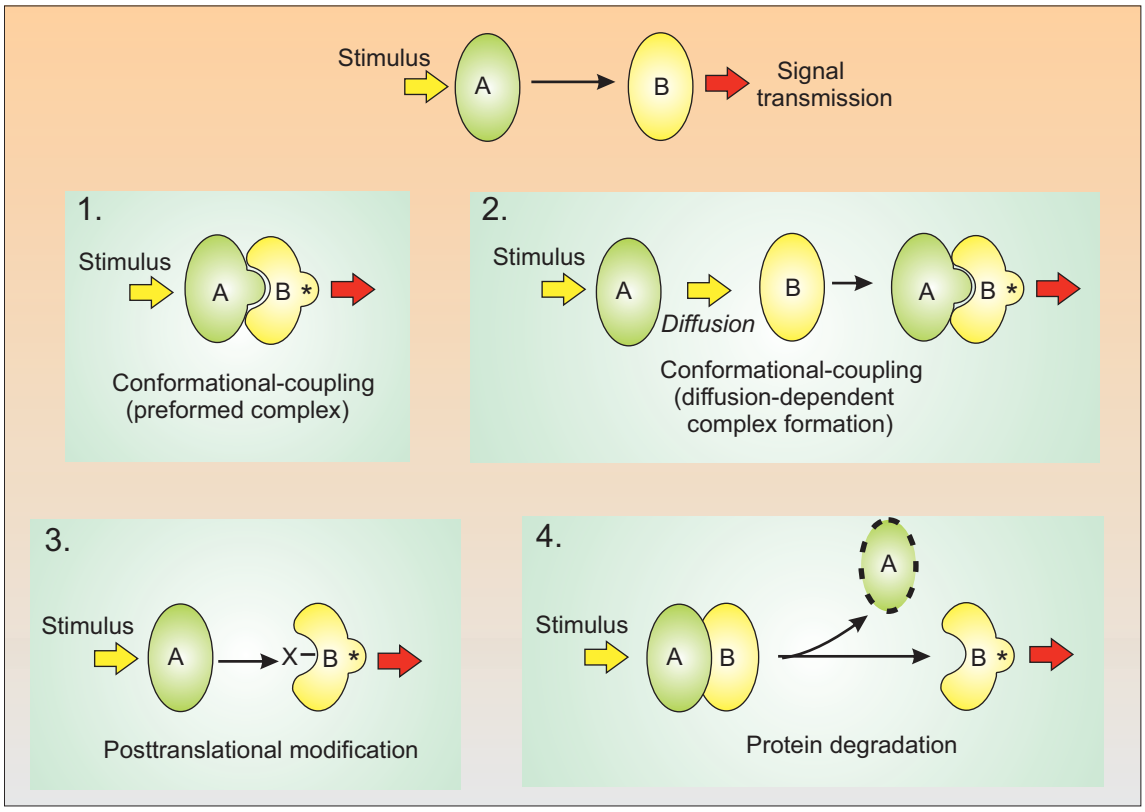
Information transfer mechanisms

The function of the cell signalling pathways is to transmit information from the cell periphery to the internal effectors, such as the contractile proteins, membrane vesicles, ion channels, metabolic pathways and cell cycle proteins that are responsible for activating cellular responses. There are a number of mechanisms whereby information is transmitted through these pathways (**Module 1: Figure signal transmission mechanisms**).

Conformational-coupling mechanisms

Information can be transferred from one signalling element to the next through a process of conformational coupling. If the components, which are usually proteins, are already associated with each other, then this transfer mechanism can be very fast (mechanism 1 in **Module 1: Figure signal transmission mechanisms**). A classical example of such a conformational-coupling mechanism occurs during

Module 1: | Figure signal transmission mechanisms



Different modes of signal transmission.
Cell signalling pathways depend upon different types of signal transmission mechanisms that transfer information from one component to the next. A stimulus arriving at component A is transferred to component B. There are four main signal transmission mechanisms, as described in the text.

excitation–contraction coupling in skeletal muscle, where the $Ca_V1.1$ L-type channel is pre-coupled to the ryanodine receptor (RYR1) (Module 3: Figure L-type channel/RYR1 complex). Another example is the association of voltage-operated Ca^{2+} channels with the proteins responsible for exocytosis of synaptic vesicles (Module 4: Figure Ca^{2+}-induced membrane fusion).

Conformational coupling is also used when information is being transferred by diffusion of signalling elements. Low-molecular-mass second messengers (e.g. Ca^{2+}, cyclic AMP, cyclic GMP and reactive oxygen species) or proteins such as the phosphorylated extracellular-signal-regulated kinase 1/2 (ERK1/2) or various activated transcription factors that translocate from the cytoplasm into the nucleus carry information as they diffuse through the cell. In order to transmit this information, these diffusing elements use a conformation-coupling mechanism to transmit information when they bind to downstream elements (mechanism 2 in Module 1: Figure signal transmission mechanisms).

Post-translational modifications

Signalling systems use a variety of post-translational protein modifications in order to transmit information along signalling pathways (mechanism 3 in Module 1: Figure signal transmission mechanisms). The basic mechanism is for a stimulus to activate component A, which then acts on component B to bring about a conformational change through some post-translational modification. These modifications, which function in signal transmission, are often very specific in that they are directed towards particular amino acids that can by altered in many different ways:

- Protein glycosylation
- Protein phosphorylation
- Protein oxidation
- Protein acetylation
- Protein methylation
- Sumoylation
- Ubiquitination

Protein glycosylation

A number of signalling proteins can be glycosylated through the attachment of a β-*N*-acetylglucosamine (GlcNAc) residue. An *O*-GlcNAc transferase (OGT) uses UDP-GlcNAc, which is the end product of hexosamine biosynthesis, to form the O-linked β-*N*-acetylglucosamine (O-GlcNAc) post-translational modification, which is then reversed by a *O*-GlcNAcase. The UDP-GlcNAc may function as a nutrient sensor because its levels fluctuate depending on the availability of metabolites such as glucose and free fatty acids (FFAs). The elevation of these metabolites during obesity may increase the activity of OGT resulting in the glycosylation and

alteration of insulin receptor signalling components resulting in insulin resistance (Module 12: Figure insulin resistance).

Protein phosphorylation

Protein kinases and phosphatases alter the activity of proteins by either adding or removing phosphate groups respectively. Cells express an enormous number of protein kinases responsible for phosphorylating signalling components as a mechanism of signal transmission. In some cases, there are a series of kinases that phosphorylate each other to set up a signalling cascade. A classical example is the mitogen-activated protein kinase (MAPK) signalling pathway (Module 2: Figure MAPK signalling). The kinases are divided into two main groups defined by the amino acids that they phosphorylate. There are tyrosine kinases and serine/threonine kinases. These kinases come in many different forms and can either function as part of cell-surface receptors or as non-receptor-linked kinases operating in different regions within the cell. These kinases can come into play right at the beginning of certain signalling pathways as occurs for the protein tyrosine kinase-linked receptors (PTKRs) and the serine/threonine kinase-linked receptors (S/TKRs) (Module 1: Figure stimuli for enzyme-linked receptors).

By far the majority of the signalling kinases are not linked to receptors, but operate within the cells as part of an internal signalling cascade. The Src family of non-receptor protein tyrosine kinases, such as Src, Lck, Lyn, Fyn and Syk, are particularly important in initiating signalling events in T cells (Module 9: Figure TCR signalling) and mast cells (Module 11: Figure FcεRI mast cell signalling). The Tec tyrosine kinase family also plays an important role in the early transmission of information in lymphocytes.

Most signalling pathways use non-receptor serine/threonine protein kinases at some point during the processes of signal transmission. The following are examples of some of the major kinases that function by modifying protein properties through phosphorylation of serine and/or threonine residues:

- Adaptor-associated kinase 1 (AAK1)
- AMP-activated protein kinase (AMPK)
- β-Adrenergic receptor kinase 1 (βARK1)
- Casein kinase I (CKI)
- Calcium/calmodulin-dependent serine protein kinase (CASK)
- CDK-activating kinase (CAK)
- Checkpoint kinases
- C-terminal Src kinase (CSK)
- Cyclin-dependent kinases (CDKs); see Cell cycle signalling
- Cyclin-dependent kinase 5 (CDK5)
- Cyclin-dependent kinase (CDK)-activating kinase (CAK)
- Cyclic GMP-dependent protein kinase (cGK)
- DNA-dependent protein kinase (DNA-PK)
- Glycogen synthase kinase-3 (GSK-3)
- Integrin-linked kinase (ILK)
- Large tumour suppressor (Lats)
- Leucine-zipper-interacting kinase (ZIPK)
- LKB1
- Mammalian sterile-20-like kinase (MST)
- Membrane-associated guanylate kinases (MAGUKs)
- Myosin light chain kinase (MLCK)
- Myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK)
- p21-activated kinase (PAK)
- PKR (protein kinase R)-like ER kinase (PERK)
- Protein kinase A (PKA)
- Protein kinase B (PKB)
- Protein kinase C (PKC)
- Rho kinase (ROK)
- Polo-like kinases (Plks)
- Ribosomal S6 kinase 1 (S6K1)
- WNK protein kinase

Cyclin-dependent kinase 5 (CDK5)

Although cyclin-dependent kinase 5 (CDK5) was originally identified as one of the CDKs that function in the control of cell cycle signalling, it is now known to have different functions mainly restricted to post-mitotic neurons. The p35 and p39 activators of CDK5 are expressed exclusively in neurons. The p35 is cleaved by calpain to form p25 that can induce a prolonged activation of CDK5. The phosphorylation of Tau by the CDK5/p25 complex is a major factor in causing neurodegeneration. CDK5 is also thought to phosphorylate a number of other substrates such as p21-activated kinases 1 (PAK1), Src, Synapsin 1, MUNC18, Amphyphysin 1, DARPP32 and the glucocorticoid receptor (GR). Some of these substrates function in vesicle transport and endocytosis. For example the phosphorylation of MUNC18 will alter synaptic vesicle transport while phosphorylated Amphyphysin 1 will promote endocytosis (Module 4: Figure scission of endocytic vesicles).

Phosphorylation of GR results in the activation of the *Hdac2* gene and the resulting increase in the expression of HDAC2 may contribute to Alzheimer's disease (AD).

The p25, which activates CDK5, may also be neurotoxic in its own right in that it can contribute to neuronal death and has been linked to various neurodegenerative diseases including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and has also been found to associate with the Lewy bodies, a hallmark of Parkinson's disease (PD).

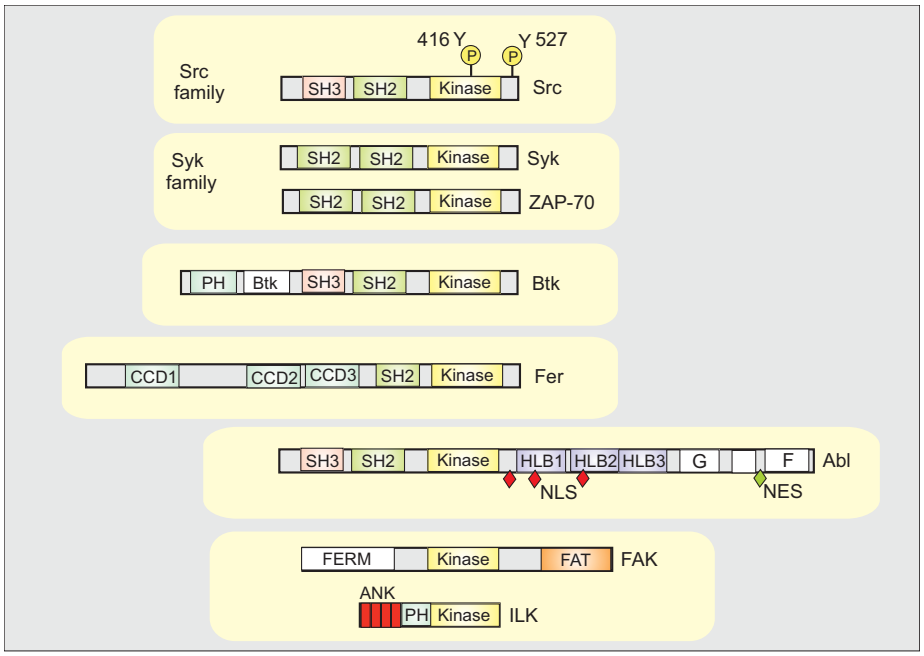
Non-receptor protein tyrosine kinases

There are a large number of non-receptor protein tyrosine kinases with a diverse range of signalling functions. In addition to having a tyrosine kinase domain, they contain protein interaction domains that enable them to interact with both upstream and downstream signalling elements (Module 1: Figure non-receptor tyrosine kinases). Some of the kinases, which have more general functions, are described here, whereas others are described in relation to signalling processes where they perform more specific functions:

- Src family

  Src
  Blk

Module 1: | Figure non-receptor tyrosine kinases



Domain structure of non-receptor protein tyrosine kinases.
These non-receptor protein tyrosine kinases are characterized by having a kinase domain together with a variable number of protein–protein interaction domains, which enable them to interact with upstream and downstream components of different signalling pathways. The presence of nuclear localization signals (NLS) and a nuclear export signal (NES) enables Abl to move between the cytoplasm and nucleus where it carries out its different signalling functions. Fer contains three coiled-coil domains (CCDs). See the text for further details. Phosphorylation of two residues on Src is critical for its activation (Module 1: Figure Src activation).

Fgr
Hck
Fyn
Lck
Lyn
Yes

- Spleen tyrosine kinase (Syk) family

  Syk
  ζ-Associated protein of 70 kDa (ZAP70)

- Tec tyrosine kinase family

  Tec
  Bruton's tyrosine kinase (Btk)
  Inducible T cell kinase (Itk)

- C-terminal Src kinase (CSK)
- Fer
- Abelson tyrosine kinase (Abl) family

  Abl
  Abl-related gene (*arg*) product (Arg)

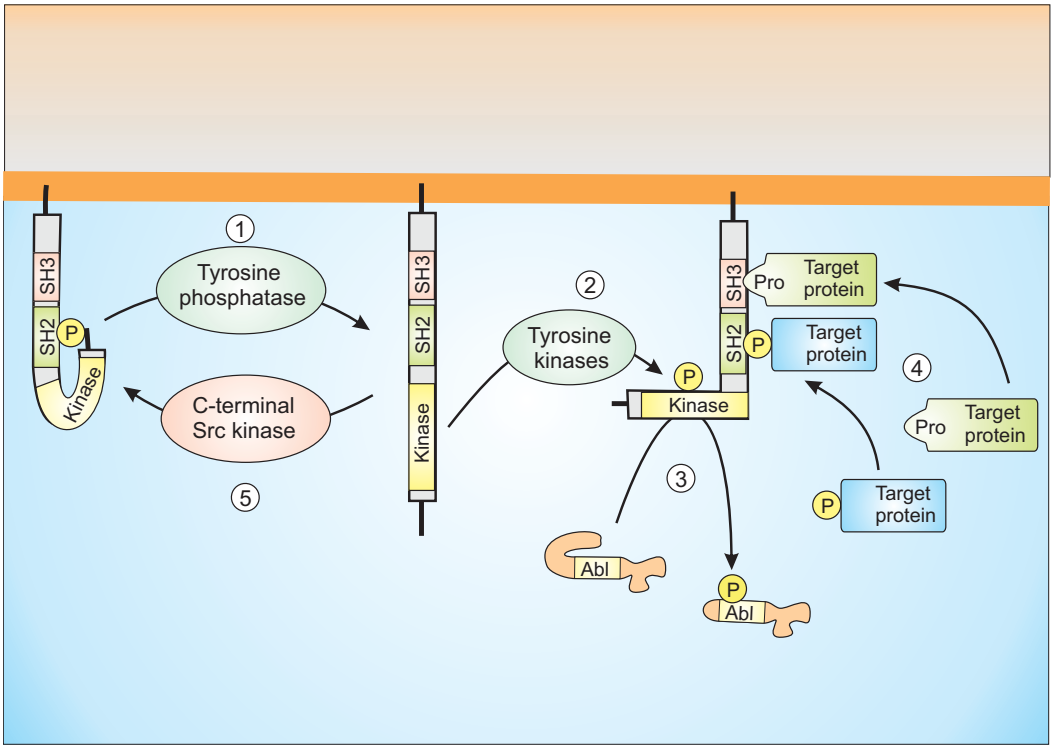
- Janus kinases (JAKs); see Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling pathway
- Focal adhesion kinase (FAK)
- Proline-rich tyrosine kinase 2 (Pyk2)

Src

Src is the prototype of the Src protein tyrosine kinase family (Src, Blk, Fyn, Fgr, Hck, Lck, Lyn, Yes). These tyrosine kinases function both as adaptors to assemble signalling complexes and as a tyrosine kinase to phosphorylate components of such signalling complexes. Their structure has domains responsible for this dual adaptor and enzymatic function (Module 1: Figure non-receptor tyrosine kinases). The kinases are attached to the membrane through the N-terminal region and this is then followed by an Src homology 3 (SH3) domain and then an Src homology 2 (SH2) domain. The kinase region is located in the C-terminal part of the molecule where there are two tyrosine residues (Tyr-416 and Tyr-527) that are critical for regulating the activity of Src. The SH2 domain not only enables Src to interact with other signalling molecules, but also participates in an intramolecular interaction that regulates Src activity. The regulation of Src, which resembles that found in other members of the family, depends upon the following processes (Module 1: Figure Src activation):

1. In the inactive conformation, the phosphorylated Tyr-527 at the C-terminal end forms an intramolecular interaction with the SH2 domain. During activation, tyrosine phosphatases remove this inhibitory phosphate and the molecule opens out.
2. Various tyrosine kinases phosphorylate Tyr-416 located within the kinase domain, resulting in an increase in enzyme activity.

Module 1: | Figure Src activation



Activation of the non-receptor tyrosine kinase Src.
Src is the prototype of the family of Src tyrosine kinases that have a similar activation mechanism. 1. A C-terminal phosphotyrosine group is removed to open up the molecule. 2. Tyrosine kinases phosphorylate a residue within the kinase domain resulting in an increase in enzyme activity. 3. The activated kinase domain can phosphorylate various target proteins such as Abl. 4. The SH2 and SH3 domains can bind various target proteins. 5. C-terminal Src kinase (CSK) phosphorylates the C-terminal tyrosine residue to reinstate the inactive conformation.

3. The activated tyrosine kinase domain can now phosphorylate various substrates such as Abl (Module 1: Figure Abl signalling).
4. The active conformation of the molecule also makes the SH2 and SH3 domains available to interact with a number of target proteins to assemble signalling complexes.
5. Src is inactivated by the C-terminal Src kinase (CSK) that phosphorylates the C-terminal Tyr-527 to reinstate the inactive conformation (Module 1: Figure Src activation).

Src performs a wide range of functions:

- It activates the non-receptor protein tyrosine kinase Abl (Module 1: Figure Abl signalling)
- It acts together with the proline-rich tyrosine kinase 2 (Pyk2) to promote formation of the osteoclast podosome (Module 7: Figure osteoclast podosome)
- It plays a role in relaying information from integrin receptors to PtdIns 3-kinase at the focal adhesion complex (Module 6: Figure integrin signalling)
- During osteoclastogenesis, colony-stimulating factor-1 (CSF-1) acts on the colony-stimulating factor-1 receptor (CSF-1R) and recruits Src, which forms a complex with c-Cbl and PtdIns 3-kinase (PtdIns 3-K) (Module 8: Figure osteoclast differentiation). Src also phosphorylates the typical immunoreceptor tyrosine-based activation motifs (ITAMs) on the Fc receptor γ (FcRγ) and DNAX-activating protein 12 (DAP12) adaptors, which co-ordinate activation of the Ca^{2+} signalling pathway in the developing osteoclast.
- It phosphorylates and activates the Tec tyrosine kinase family.

C-terminal Src kinase (CSK)

C-terminal Src kinase (CSK) inactivates Src by phosphorylating Tyr-527 at its C-terminal end (Module 1: Figure non-receptor tyrosine kinases). Once this residue is phosphorylated, the C-terminal end containing the kinase domain bends around so that this phosphorylated group interacts with the Src homology 2 (SH2) domain. Src is inactive in the folded configuration (Module 1: Figure Src activation). CSK plays a similar role in inactivating Lck, which is one of the early T cell receptor transducers (Module 9: Figure TCR signalling)

Fyn

In blood platelets, Fyn acts together with Lyn by binding to the collagen receptor glycoprotein VI (GPVI) where they phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) on the Fc receptor γ (FcRγ) chains to provide binding sites for phospholipase C γ2 (PLC γ2) (Step 2 in Module 11: Figure platelet activation). Fyn also binds to the adaptor protein SLAM-associated protein (SAM), which mediates the action of the

signalling lymphocyte activation molecule (SLAM) family of co-stimulatory molecules.

In mast cells, Fyn is recruited to the FcεRI receptor where it phosphorylates Gab2 and Bruton's tyrosine kinase (Btk), which contribute to the activation of PtdIns 3-kinase, which then phosphorylates PtdIns4,5P_2 to form the lipid messenger PtdIns3,4,5P_3 (Module 11: Figure FcεRI mast cell signalling).

Another important action of Fyn is to regulate the function of the classical cadherins. Stability of the cadherin/β-catenin/actin complex is controlled by phosphorylation of Tyr-142 on β-catenin by Fyn and this regulates its interaction with α-catenin and the actin cytoskeleton (Module 6: Figure classical cadherin signalling).

Lck

Lck is one of the main non-receptor protein tyrosine kinases expressed in T cells, where it plays an early role as one of the T cell receptor transducers (Module 9: Figure TCR signalling). As for Src (see Step 5 in Module 1: Figure Src activation), Lck is inactive when the molecule is folded by an intramolecular interaction between the Src homology 2 (SH2) domain and a phosphate group at the C-terminal end of the molecule. In the case of T cells, Lck is activated by the transmembrane tyrosine kinase CD45, which dephosphorylates an inhibitory phosphate group on Tyr-505. Conversely, the molecule is inactivated when Tyr-505 is phosphorylated by the C-terminal Src kinase (CSK).

Lyn

Lyn functions in the activation of B cells, mast cells and blood platelets. In mast cells, Lyn acts to phosphorylate the immunoreceptor tyrosine-based activation motifs (ITAMs) on the FcεRI receptor to provide binding sites to recruit Syk (Module 11: Figure FcεRI mast cell signalling). Lyn also phosphorylates and activates Syk as part of the cascade of signals that lead to mast cell activation. Another of its functions is to phosphorylate and activate the Tec tyrosine kinase family.

In the case of blood platelets, Lyn binds to the collagen receptor glycoprotein VI (GPVI) and phosphorylates ITAMs on the Fc receptor γ (FcRγ) chains to provide binding sites for phospholipase Cγ2 (PLCγ2) (Step 2 in Module 11: Figure platelet activation). Lyn also plays a role in B-cell antigen receptor (BCR) activation (Module 9: Figure B cell activation).

Syk

Syk (spleen tyrosine kinase) is a non-receptor protein tyrosine kinase (Module 1: Figure non-receptor tyrosine kinases) that plays a role in signal transduction in mast cells and in B cells. In mast cells, phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) on the FcεRI receptor by Lyn provides binding sites that recruit Syk, which is also phosphorylated and activated by Lyn (Module 11: Figure FcεRI mast cell signalling). The activated Syk then phosphorylates the scaffolding proteins linker for activation of T cells (LAT) and Src homology 2 domain-containing protein of 76 kDa (SLP-76) to provide binding sites for other signalling components. Syk also phosphorylates phospholipase Cγ1 (PLCγ1) and Bruton's tyrosine kinase (Btk), which interact with each other during the activation of PLCγ1. Syk performs a similar function in the activation of B cells (Module 2: Figure ROS effects on Ca^{2+} signalling) and during osteoclastogenesis (Module 8: Figure osteoclast differentiation).

ζ-Associated protein of 70 kDa (ZAP70)

The ζ-associated protein of 70 kDa (ZAP70) is a non-receptor protein tyrosine kinase that functions in T cell activation (Module 1: Figure non-receptor tyrosine kinases). It is one of the key components of the T cell receptor transducers that are drawn into the large signalling complex (Module 9: Figure TCR signalling). ZAP70 uses its Src homology 2 (SH2) domains to bind to that the immunoreceptor tyrosine-based activation motifs (ITAMs) that are phosphorylated by Lck. Once in place, ZAP70 then phosphorylates scaffolding components such as linker for activation of T cells (LAT) and SH2-domain-containing protein of 76 kDa (SLP-76), which are drawn into the growing central supramolecular activation cluster (c-SMAC) and function as docking sites to communicate to the different signalling cassettes.

Fer

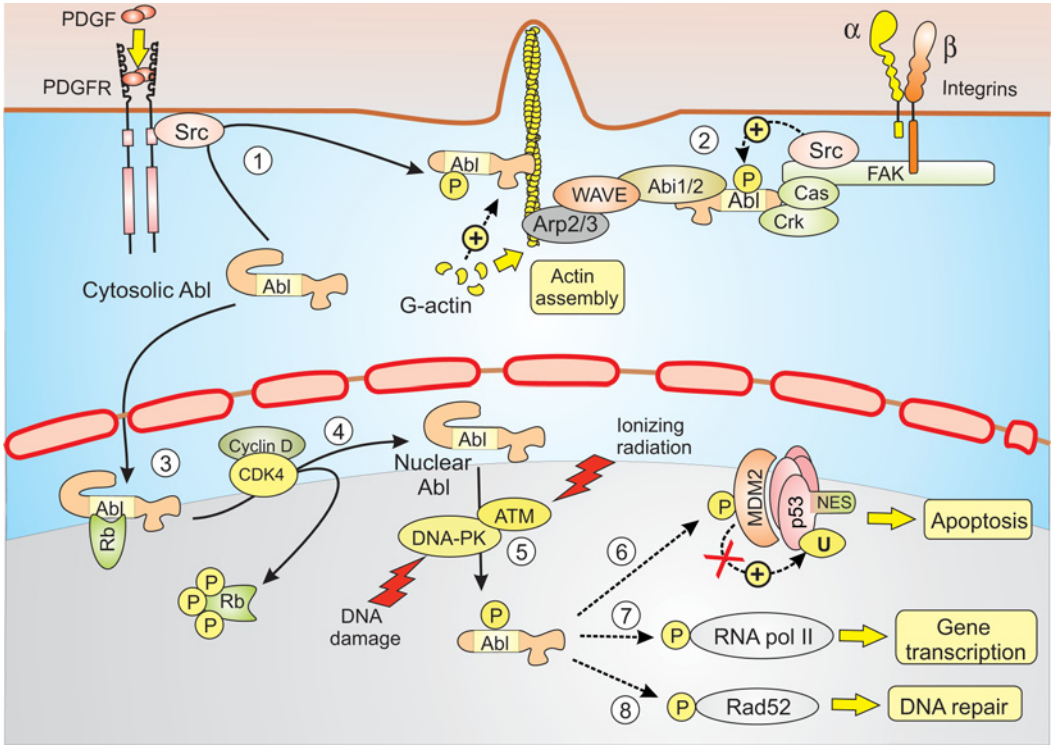
Fer is a non-receptor tyrosine kinase that plays a role in regulating the function of the classical cadherins (Module 6: Figure classical cadherin signalling). It has the typical conserved kinase domain together with three coiled-coil domains (CCD1–CCD3) and a Src homology 2 (SH2) domain (Module 1: Figure non-receptor tyrosine kinases).

Abl

The Abelson tyrosine kinase (Abl) takes its name from the fact that the c-*abl* gene was first identified as part of the Abelson murine leukaemia virus and was subsequently found to be a component of the human BCR-ABL oncogene. The Abl component of the BCR-ABL oncogene is a multitasking signalling molecule capable of operating in both the cytoplasm and nucleus to regulate diverse cellular processes, including actin remodelling, chemotaxis, gene expression, T cell receptor signalling, DNA repair and apoptosis.

The ability of Abl to regulate multiple cellular processes depends on its complex domain structure (Module 1: Figure non-receptor tyrosine kinases). The N-terminal region begins with an Src homology 3 (SH3) domain and an Src homology 2 (SH2) domain, which are followed by the kinase domain. Like other non-receptor protein tyrosine kinases, there is an internal autoinhibition whereby the SH3 domain bends round to interact with the kinase domain. The SH3 domain is used by Abl to interact with a plethora of proteins that have the PXXP motif such as 3BP-1 (a Rac GTPase-activating protein), Abelson-interactor (Abi), ATM (ataxia telangiectasia mutated), Cbl, DNA-dependent protein kinase (DNAPK), NR2D subunit of the*N*-methyl-d-aspartate (NMDA) receptor, scramblase-1 and Wiskott-Aldrich syndrome protein (WASP) verprolin homologous (WAVE). Many of these binding partners are either substrates of the Abl kinase or they are upstream kinases that phosphorylate and activate

Module 1: | Figure Abl signalling



Abl signalling functions in the cytoplasm and nucleus.
The non-receptor protein tyrosine kinase Abl is a multitasking signalling component that contributes to actin remodelling in the cytoplasm and also acts within the nucleus to regulate processes such as apoptosis, gene transcription and DNA repair. Details of the numbered events are described in the text.

Abl. Like the SH3 domain, the SH2 domain of Abl interacts with a number of signalling components, such as the Eph receptor (Module 1: Figure Eph receptor signalling), Cbl, Crk-associated substrate (Cas) and RNA polymerase II.

The middle of the molecule contains three domains containing high-mobility group (HMG)-like boxes (HLB1–HLB3) that can bind to A/T-rich DNA regions (Module 1: Figure non-receptor tyrosine kinases). The C-terminal region contains binding sites for globular (G-) and filamentous (F-) actin. Interposed between these two sites is a C-terminal domain-interacting domain (CTD-ID) that is a binding site for the CTD of mammalian RNA polymerase II. The region containing the HLB domains also have a number of proline-rich motifs that enable Abl to interact with SH3 domain-containing proteins such as Abelson-interactor (Abi), Crk, growth factor receptor-bound protein 2 (Grb2), Nck and proline/serine/threonine phosphatase-interacting protein 1 (PSTPIP1).

Abl can regulate a number of cellular processes, both in the cytoplasm and in the nucleus. It can move between these two compartments by virtue of having nuclear localization signals (NLSs) and a nuclear export signal (NES) (Module 1: Figure non-receptor tyrosine kinases). The signalling function of Abl is described in Module 1: Figure Abl signalling:

1. Abl located in the cytoplasm is activated by Src associated with protein tyrosine kinase-linked receptors such as the platelet-derived growth factor receptor (PDGFR). Src phosphorylates Abl, relieving the autoinhibition and thus enabling the molecule to open out to begin its signalling role in actin remodelling. Abl can bind to both G- and F-actin, but how this facilitates actin assembly is unclear.
2. Abl can also be activated by integrin receptors, where it can contribute to actin assembly by forming a complex with Abelson-interactor (Abi), Wiskott-Aldrich syndrome protein (WASP) verprolin homologous (WAVE) and the actin-related protein 2/3 complex (Arp2/3 complex). Such complex formation is found at focal adhesion complexes (Module 6: Figure integrin signalling).
3. In addition to its function in the cytoplasm, Abl also operates within the nucleus. Its nuclear function seems to depend upon its interaction with the pocket protein retinoblastoma susceptibility gene Rb (Module 1: Figure Abl signalling).
4. The inhibitory effect of Rb on nuclear Abl is relieved by the phosphorylation of Rb by the cyclin D/cyclin-dependent kinase 4 (CDK4) complex, which is a component of the cell cycle signalling pathway (Module 9: Figure cell cycle signalling mechanisms).
5. Nuclear Abl can be activated by a number of stressful stimuli, such as ionizing radiation acting through

Module 1: | Table protein acetylation toolkit
Protein acetylation toolkit.

Histone acetyltransferases (HATs)	
p300	
CBP	
PCAF	
TIP60	Tat interactive protein 60 (TIP60) functions in the nucleus where it has both transcriptional and DNA repair functions
Histone deacetylases (HDACs)	
Class I HDACs	Reside in the nucleus where they act to deacylate histones
HDAC1	
HDAC2	
HDAC3	The p65 subunit of NF-κB is acetylated by acetylated PCAF and deacetylated by HDAC3
HDAC8	
Class IIa HDACs	Regulate gene transcription by shuttling in and out of the nucleus
HDAC4	
HDAC5	
HDAC7	
HDAC9	
Class IIb HDACs	
HDAC6	Associates with microtubules and deacylates tubulin, Hsp90 and cortactin
HDAC10	
Class III HDACs	
SIRT1	
SIRT2	
SIRT3	Found mainly in the mitochondrion where it regulates the activity of a number of enzymes (Module 5: Figure mitochondrial Ca^{2+} signalling)
SIRT4	
SIRT5	
SIRT6	
SIRT7	
SIRT8	
Class IV HDACs	
HDAC11	

ATM (ataxia telangiectasia mutated) or by DNA damage acting through DNA-dependent protein kinase (DNAPK).

6. Abl inhibition of mouse double minute-2 (MDM2) prevents the degradation of p53 by the ubiquitin ligase mouse double minute-2 (MDM2) and this enhances the transcription of the genes that induce apoptosis (Module 4: Figure p53 function).
7. Abl can phosphorylate and activate RNA polymerase II, which contributes to gene expression (Module 1: Figure Abl signalling).
8. Abl can phosphorylate and activate Rad52, which contributes to DNA repair.

Abl inhibition of mouse double minute-2 (MDM2)

One function of Abl is to regulate the activity of the mouse double minute-2 (MDM2), which is a ubiquitin ligase that targets the transcription factor p53 for degradation (Module 4: Figure p53 function). A variety of cell stresses activate p53, which is at the heart of a signalling network that controls the cell cycle network, senescence and apoptosis (Module 9: Figure proliferation signalling network). MDM2 plays a role in this network by virtue of its ability to destabilize p53 by targeting it for degradation. This MDM2 inhibitory action is alleviated by Abl, which is activated by various stressful stimuli (Step 5 in Module 1: Figure Abl signalling). One of the actions of Abl is to increase the transcriptional activity of p53. The proline-rich regions of Abl bind to p53 and may act either by stabilizing the interaction of p53 with DNA or by masking the lysine target sites that are ubiquitinated by MDM2. Another proposed action is for Abl to phosphorylate MDM2 to inhibit the ubiquitination of p53 (Step 6 in Module 1: Figure Abl signalling).

Protein oxidation

The redox signalling pathway generates reactive oxygen species such as superoxide and hydrogen peroxide that carry out their second messenger functions by oxidizing specific thiol groups on specific cysteine residues in target proteins (Module 2: Figure reversible and irreversible ROS oxidations).

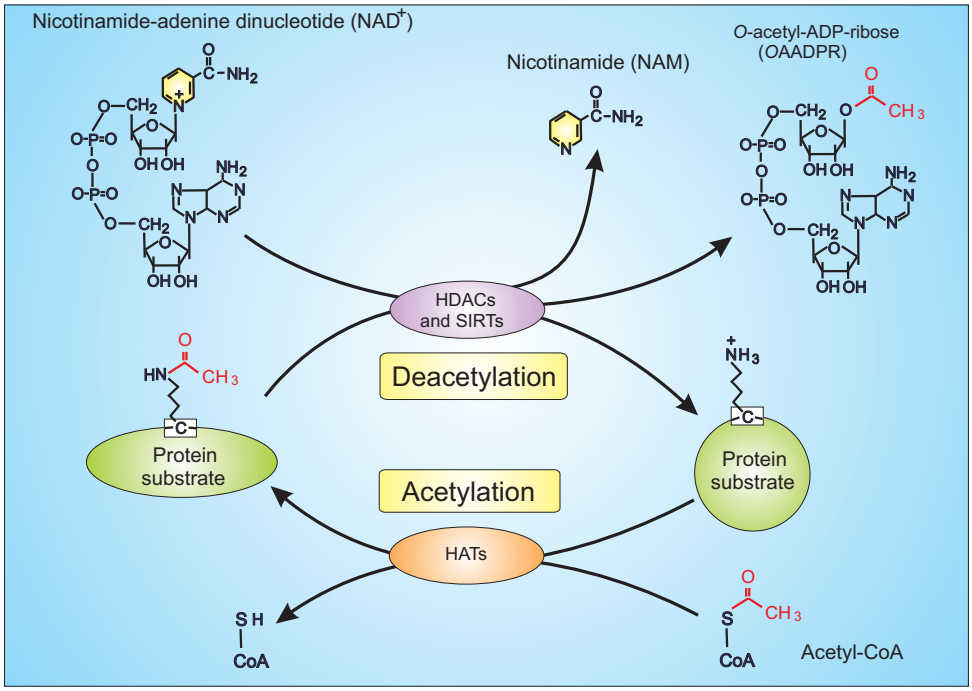
Protein acetylation

Protein acetylation plays a particularly important role in chromatin remodelling and is also responsible for regulating the activity of many other proteins particularly transcription factors. The reversible acetylation of lysine residues on target proteins is carried out by an extensive protein acetylation toolkit (Module 1: Table protein acetylation toolkit).

The histone acetyltransferases (HATs) function to acetylate lysine residues on substrate proteins (Module 1: Figure protein acetylation). This acetylation reaction is reversed by deacetylases such as the histone deacetylases (HDACs) and the sirtuins. This reversible process of protein acetylation has a number of functions:

- Acetylation of histones, often acting in combination with nuclear receptors, opens up chromatin to make it accessible to a whole variety of transcription factors and thus constitutes an ON mechanism. Such a role for acetylation is evident during the activation of the myocyte enhancer factor-2 (MEF2) (see Step 9 in Module 4: Figure MEF2 activation).

Module 1: | Figure protein acetylation



Protein acetylation.
Reversible acetylation of lysine residues on proteins is carried out by histone acetylases (HATs) that add an acetate group (shown in red), which is removed by histone deacetylases (HDACs) and the SIRTs. See text for further details,

- The activity of the transcription factor PGC-1α, which functions as part of the AMPK signalling pathway, is controlled by acetylation through SIRT1 and deacetylation by GCN5 (Module 2: Figure AMPK control of metabolism).
- The protein CLOCK, which is a component of the circadian clock, is an acetyltransferase that acetylates its partner BMAL1 to make the latter more susceptible to the feedback inhibition by CRY (see Step 4 in Module 6: Figure circadian clock molecular mechanisms).
- The movement of kinesin-1 along microtubules is enhanced by the acetylation of the α-tubulin subunits.
- There is an important role for p53 acetylation in regulating the transcriptional activity of p53 (Module 4: Figure p53 function).
- Acetylation of components such as histones, p53 and ATM play a role in the G1 checkpoint signalling to DNA double-strand breaks (Module 9: Figure G1 checkpoint signalling).
- Acetylation of ULK1 by TIP60 functions to control autophagy (Module 11: Figure autophagy).

Histone acetyltransferases (HATs)

The histone acetyltransferases (HATs) carry out the transfer of an acetyl group from acetyl-CoA to the ε-amino group on specific lysine residues of target proteins (Module 1: Figure protein acetylation). The enzyme ATP citrate lyase (ACL) relates energy balance to HAT activity through the control of the nuclear production of acetyl-CoA, which is the substrate for acetyltransferase activity.

Despite the name, the target proteins acetylated by HATs are not restricted to histones and this important post-translational modification alters the activity of many different proteins often acting in tandem with protein phosphorylation. Histone acetylation is of particular importance in that it remodels chromatin to make it easier for transcription factors to act at promoter sites to initiate gene transcription. The following are typical examples of such HATs:

- p300
- CREB binding protein (CBP)
- p300/CBP association factor (PCAF)
- General control of amino-acid synthesis (GCN5)
- Tat interactive protein 60 (TIP60)

Histone deacetylases (HDACs)

The histone deacetylases (HDAC1–11) and sirtuins (SIRT1–8) have been classified into five groups (Module 1: Table protein acetylation toolkit). These HDACs remove the acetyl group from the ε-amino group on specific acetylated lysine residues of target proteins (Module 1: Figure protein acetylation). The deacylation reaction is coupled to the hydrolysis of nicotinamide–adenine dinucleotide (NAD^+) to form nicotinamide (NAM) and *O*-acetyl-ADP-ribose (OAADPR) during which the acetyl group is transferred from the protein substrate to the OAADPR. Since NAD^+ is required for this reaction, this enzymatic reaction is closely linked to the energy status of the cell as defined by the NAD^+/NADH ratio and is thus a part of the NAD signalling system (Module 2: Figure NAD-dependent signalling pathways).

One of the primary functions of protein deacylation by HDACs is to regulate gene transcription. HDACs contribute to the epigenetic changes responsible for chromatin remodelling. In effect, they repress gene transcription by two mechanisms as exemplified by the actions of HDAC2 and HDAC4. By repressing gene transcription, these HDACs constitute a typical OFF mechanism.

HDAC activation functions in the control of many different transcriptional processes:

- Inhibition of the HDACs, which enhances histone acetylation and chromatin remodelling, is necessary for neuronal gene transcription (Module 10: Figure neuronal gene transcription) and has been shown to restore memory in a mouse neurodegeneration model.
- A cardiac histone deacetylase (HDAC) shuttle plays an important role in the onset of cardiac hypertrophy (Module 12: Figure hypertrophy signalling mechanisms).
- HDAC is part of the repressor complex that inhibits the transcription of Wnt genes (Module 2: Figure Wnt canonical pathway).
- HDAC3 associates with the nuclear receptor co-repressor 1 (N-CoR1) to control the circadian clock molecular mechanisms.

HDAC2

HDAC2 is an example of a class I HDAC that is a resident nuclear protein that acts to inhibit gene transcription by deacylating histones resulting in chromosome condensation, which prevents transcription factors gaining access to their promoter sites. Just how HDAC2 is regulated is unclear. As a resident nuclear protein, the activity of HDAC2 may be regulated by altering its expression level. In neurons, the expression of HDAC2 is activated by the glucocorticoid receptor (GR) that binds to the glucocorticoid responsive element (GRE) on the promotor region of the *Hdac2* gene. HDAC2 functions to regulate neuronal gene transcription (Module 10: Figure neuronal gene transcription).

Inhibitors such as sodium butyrate and suberoylanilide hydroxamic acid (SAHA) can reduce the activity of the class 1a HDACs. These HDAC inhibitors have been successfully used to improve some of the symptoms of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and amytrophic lateral sclerosis (ALS).

HDAC4

HDAC4 is an example of a classIIa HDAC that regulates gene transcription by shuttling in and out of the nucleus. It binds directly to various transcription factors to repress their activity. In the case of the Type IIa HDACs, activation of gene transcription depends on HDAC inactivation, which usually occurs through its translocation out of the nucleus. This export from the nucleus can be induced either by HDAC phosphorylation by protein kinases (Module 4: Figure MEF2 activation) or by HDAC oxidation through redox signalling pathways. The cardiac histone deacetylase (HDAC) shuttle is a good example of the important role that the HDACs play in regulating gene transcription (Module 12: Figure hypertrophy signalling mechanisms). HDAC4 also has an important role in regulating neuronal gene transcription (Module 10: Figure neuronal gene transcription).

HDAC phosphorylation

The export of HDAC from the nucleus can be induced by protein phosphorylation. For example, Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) phosphorylates Ser-259 and Ser-498 of HDAC5, which then translocates out of the nucleus in association with 14-3-3 protein. An example of such a signalling mechanism is evident during the activation of the myocyte enhancer factor-2 (MEF2) (Module 4: Figure MEF2 activation). Phosphorylation of HDAC4 and its removal from the nucleus is also a critical event for the cardiac histone deacetylase (HDAC) shuttle during the onset of cardiac hypertrophy (Module 12: Figure hypertrophy signalling mechanisms).

HDAC oxidation

The export of HDAC from the nucleus can be induced through protein oxidation by various messengers of the redox signalling pathway. The HDACs have hyperreactive cysteine residues that can be oxidized by reactive oxygen species (ROS) or by reactive nitrogen species (RNS). In the case of cortical neurons, nitrosylation reactions at Cys-262 and Cys-274 inactivate HDAC2 by causing it to leave the nucleus. A redox-dependent pathway may also function to export HDAC4 from the nucleus in cardiac cells. The two cysteine residues (Cys-667 and Cys-669) on HDAC4 are oxidized by reactive oxygen species (ROS) to form an intramolecular disulphide bond and the resulting conformational change results in the oxidized HDAC4 being exported from the nucleus.

Sirtuins

The sirtuins are an important family of histone deacetylases (HDACs). There are seven members of the sirtuin family that have both NAD^+-dependent deacetylase (Module 1: Table protein acetylation toolkit) and ADP-ribosylase activity. Sirtuins deacetylation function requires the coenzyme NAD^+ as a co-substrate to form nicotinamide (NAM) and *O*-acetyl-ADP-ribose (OAADPR); the acetyl group is transferred from the protein substrate to the OAADPR (Module 1: Figure protein acetylation). Since NAD^+ is required for this reaction, this enzymatic reaction is closely linked to the energy status of the cell as defined by the NAD^+/NADH ratio and is thus a part of the NAD signalling system (Module 2: Figure NAD-dependent signalling pathways).

A number of environmental factors, such as the availability of food and cell stress, can regulate sirtuin expression through the activity of a large number of transcription factors (**c-Myc**, **FOXO3**, **p53** and **E2F1**) and certain miRNAs (**miR-34** and **miR-199**).

The activity of SIRT1 is inhibited by Deleted in breast cancer 1 (DBC1) and this inhibition is removed following activation of the **AMPK signalling pathway**.

The sirtuins can deacylate a wide range of substrates (PGC-1α, p53, FOXO, NF-κB, myoD and histones), which reflects their multiple roles:

- One of its important functions is to regulate **p53 acetylation**.
- It has also been implicated in gene silencing, senescence, apoptosis and fat metabolism.
- In the case of **brown fat cells**, SIRT1 inhibits the activity of PGC-1α by reversing the p300-dependent acetylation reactions (**Module 8: Figure brown fat cell differentiation**).
- SIRT1 functions in the **PER regulatory loop** of the **circadian clock**.
- The mitochondrial sirtuins have multiple roles particularly in the mitochondria. Sirtuin 3 (SIRT3) seems to regulate the expression of peroxisome-proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α). Both SIRT1 and SIRT3 can be imported into the mitochondria where they may regulate mitochondrial metabolism. SIRT3 deacylates the acetyl-CoA synthetase 2 that converts acetate into acetyl-CoA, whereas SIRT4 ribosylates and inhibits the glutamate dehydrogenase that converts glutamate into α-ketoglutarate.
- The activity of PGC-1α is regulated by reversible acetylation: it is acetylated by general control of amino-acid synthesis (GCN5) and is deacetylated and activated by SIRT1 (Module 2: Figure AMPK control of metabolism). The energy sensor AMP-activated kinase (AMPK) translates changes in energy stress into altered SIRT1 activity by regulating the intracellular level of its co-substrate nicotinamide adenine dinucleotide (NAD^+) (see step 3 in Module 2: Figure AMPK control of metabolism).
- SIRT5 influences two proteins involved in cellular metabolism: cytochrome *c* and carbamoyl phosphate synthetase 1 (CPS1). The latter is the rate-limiting first step of the urea cycle in that helps to clear the ammonia generated by amino acid metabolism.

Protein methylation

Protein function can be modified by methylation of arginine or lysine residues by enzymes such as a family of protein arginine methyltransferases (PRMTs) and Smyd-2. Such methylation reactions are reversed by demethylases such as the histone lysine-specific demethylase (LSD1) that removes the methyl group from p53.

Methylation is used to regulate a number of different proteins and cellular processes:

- Modifying the activity of the transcriptional regulator peroxisome-proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α), which controls the differentiation of brown fat cells (Module 8: Figure brown fat cell differentiation).
- There is a role for p53 methylation in regulating gene transcription.
- Histone methylation of lysine and arginine residues in the N-terminal tail of histone H3 has a role in chromatin remodelling. When such methylation reactions occur within gene promoter regions, they can activate or repress gene transcription depending on their location. During replicative senescence, Suv39h1 methylates histones to enhance formation of heterochromatin that will silence DNA. An abbreviation system has been developed to describe both the location and the nature of the methylation reaction. The nitrogen groups of lysine and arginine can be modified by one or more methyl groups. The lysine can be mono- , di- or trimethylated (me1, me2 and me3) whereas the arginine can be mono- or di-methylated with the added complication that this may be either symmetrical (me2s) or asymmetrical (me2a). In the case of histone H3, the trimethylation of arginine at position 9 (H3K9me3) represses transcription whereas di- and trimethylation of Arg-4 (H3K4me2 and H3K4me3) promotes transcription.
  The H3K36me3-specific histone methyltransferase Wolf–Hirschhorn syndrome candidate 1 (WHSC1) plays an important role in development. In embryonic stem cells (ES), it interacts with cell-type-specific transcription factors such as Nanog, Sall1 and Sall4. It also interacts with the cardiac-specific transcription factor Nkx2. Defects in WHSC1 appear to play a role in Wolf-Hirschhorn syndrome (WHS).
- The transcriptional co-repressor switch independent (SIN3), which functions in chromatin remodelling, assembles a large core complex that can contain various methyl transferases such as the histone H3-specific enzyme ERG-associated protein with SET domain (ESET) and the histone 3 lysine 4 (H3K4) methyl transferase (ALL-1).

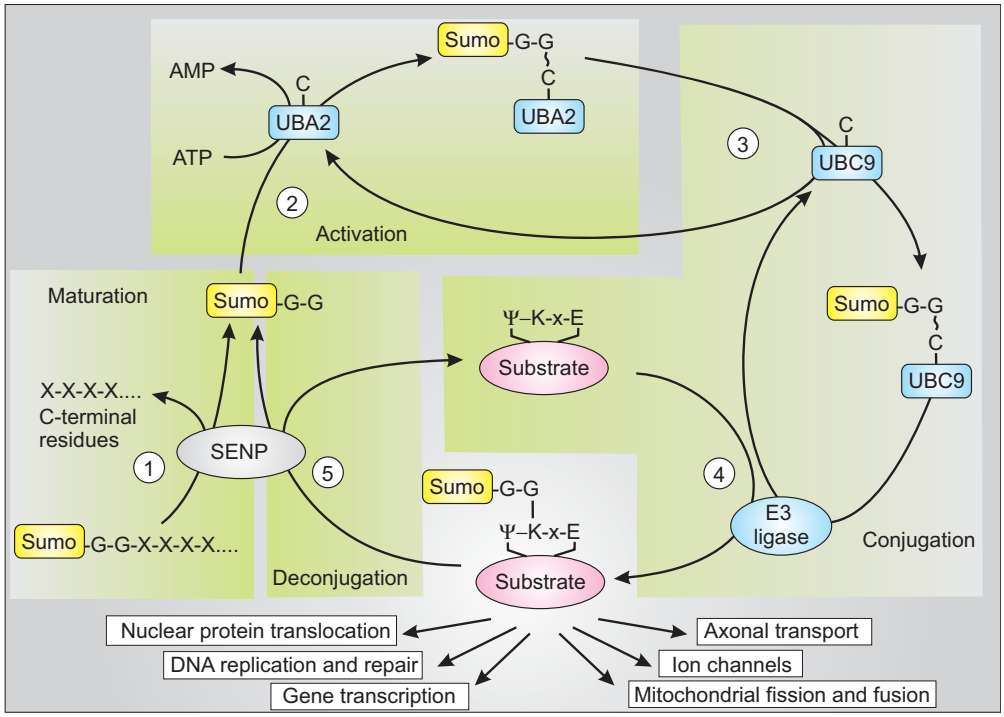
Sumoylation

Sumoylation is an example of a post-translation modification mechanism whereby the function of a protein is altered by the covalent attachment of a small ubiquitin-related modifier (SUMO) (Module 1: Figure sumoylation). The addition of SUMO induces a change in the activity, stability or location of its target proteins. The nature of these targets and the components responsible for the sumoylation reaction are summarized in Module 1: Table sumoylation toolkit. There are four human SUMO proteins: the first three are widely expressed, whereas SUMO-4 is restricted to certain cell types (kidney, spleen and nymph nodes). In most cases, a single SUMO molecule is added to the substrate proteins, but both SUMO-3 and SUMO-4 can form SUMO chains through the formation of SUMO–SUMO isopeptide bonds.

The sumoylation reaction occurs through the following series of discrete steps as shown in Module 1: Figure sumoylation:

1. The immature SUMO proteins have a variable length (2–11 amino acids) C-terminal region that is attached to an invariant Gly-Gly motif. This SUMO precursor undergoes a maturation process that entails the proteolytic cleavage of the C-terminal extension to expose these two glycine residues. This cleavage is carried out by the sentrin-specific proteases (SENPs)

Module 1: | Figure sumoylation



Sumoylation

Addition of the small ubiquitin-related modifier (SUMO) to protein substrates alters their signalling functions to control many cellular processes. As described in the text, the sumoylation reaction can be divided into a series of discrete steps: maturation, activation, conjugation and deconjugation.

(Module 1: Table sumoylation toolkit). The SUMO is now in a form to enter the sumoylation reaction, which is a reversible protein modification that depends on the formation of an isopeptide bond between the C-terminal glycine and the ε-amino group on a specific lysine residue in the substrate.

2. The first step in the sumoylation reaction is the activation of SUMO by the E1 activating enzyme AOS1-UBA2 which uses the energy of ATP to form a thioester bond between the C-terminal carboxy group of SUMO and a Cys-173 residue on UBA2 (Module 1: Figure sumoylation).
3. The conjugation process begins with the transfer of SUMO from UBA2 to the E2 conjugating enzyme UBC9.
4. The final conjugation step, which is carried out by various SUMO E3 ligases such as the PIAS family and RanBP2 (Module 1: Table sumoylation toolkit), results in the transfer of SUMO from UBC9 to its various substrates. These substrates have consensus SUMO acceptor sites such as ΨKxE or ΨKxExxpSP, where Ψ is an aliphatic branched amino acid and x is any amino acid (Module 1: Figure sumoylation).
5. The same SENPs that function in maturation are responsible for reversing sumoylation by removing the SUMO groups from the substrate proteins.

Sumoylation of substrate proteins contribute to the control of a number of cellular processes such as protein translocation across the nucleus, gene transcription, DNA replication and repair, mitochondrial fission and fusion, ion channels and the axonal transport of proteins. In carrying out these functions, sumoylation can alter substrate protein activity in different ways. The addition of a SUMO group can bring about a conformational change in the substrate or it can alter its interaction with other proteins. In addition, the SUMO group can provide additional binding sites to interact with other downstream effector proteins, which have a SUMO-interacting/binding motif (SIM/SBM). The enzymes that function in the conjugation process also have this SIM/SBM motif. The following examples illustrate how sumoylation can regulate a variety of cell signalling components and cellular responses:

- Sumoylation causes RanGAP1 to translocate from the cytoplasm to the nucleus by interacting with the nucleoporin RanBP2, which also functions as a SUMO E3 ligase.
- The stability of sarco/endo-plasmic reticulum Ca^{2+}-ATPase (SERCA) isoform SERCA2a in cardiac cells is enhanced following sumoylation by SUMO1. The proteasomal degradation of SERCA2a is reduced following the addition of SUMO1 to lysines 480 and 585. A decline in the level of SUMO1 seems to be responsible for the decrease in SERCA2a that occurs during congestive heart failure (CHF). *SUMO-1* gene transfer has proved to be an effective therapy in animal models of CHF.
- Sumoylation regulates synaptic function by controlling the endocytosis of kainate receptors. When the subunit

Module 1 | Table sumoylation toolkit
Sumoylation toolkit.

Sumoylation components	Comments
Small ubiquitin-related modifier (SUMO)	
SUMO-1	
SUMO-2	Can form SUMO chains through formation of SUMO–SUMO isopeptide bonds. Closely resembles SUMO-3 (97% identity)
SUMO-3	Closely resembles SUMO-2 and can also form SUMO chains
SUMO-4	
SUMO-specific isopeptidases	
Sentrin-specific proteases	Function in the maturation and deconjugation reactions (Module 1: Figure sumoylation)
SENP1	Translocates between the nucleus and cytoplasm
SENP2	Found in the nuclear pore complexes
SENP3	
SENP5	Found in the nucleolus and cytoplasm
SENP6	
SENP7	
Sumoylation-activating enzymes	
E1 activating enzyme AOS1-UBA2	Forms a thioester bond between SUMO and UBA2 (Step 2 in Module 1: Figure sumoylation)
E2 conjugating enzyme UBC9	Transfers SUMO from UBA2 to UBC9 (Step 3 in Module 1: Figure sumoylation)
E3 ligases	There are a number of ligases that transfer SUMO from UBC9 to downstream substrates (Step 4 in Module 1: Figure sumoylation)
PIAS family	Protein inhibitor of activated STAT (PIAS) family that contain the SP-RING motif
PIAS1	
PIAS3	
PIASxα	
PIASxβ	
PIASxγ	
RanBP2	A nuclear pore protein
MMS21	This SP-RING ligase (also known as NSE2) functions in DNA repair
SUMO substrates	Characterized by having ΨKxE or ΨKxExxpSP SUMO-acceptor sites
RanGAP1	
PML	
Sp100	
IκBα	
p53	
HSF1	Heat shock factor-1
SNIP1	Smad nucleus interaction protein-1
MEF2	

GluR6 is Sumoylated following receptor activation, it is internalized and degraded.

- The promyelocytic leukaemia (PML) protein is Sumoylated when it functions to assemble the PML nuclear bodies (PML NBs).
- Translocation of the transcriptional co-repressor C-terminal binding protein (CtBP) from the cytoplasm into the nucleus is controlled by sumoylation.
- The transcriptional activity of p53 may be repressed by a process of p53 sumoylation.
- The half-life of axin, which is a negative regulator of the canonical Wnt/β-catenin pathway, is prolonged following sumoylation.

Ubiquitination

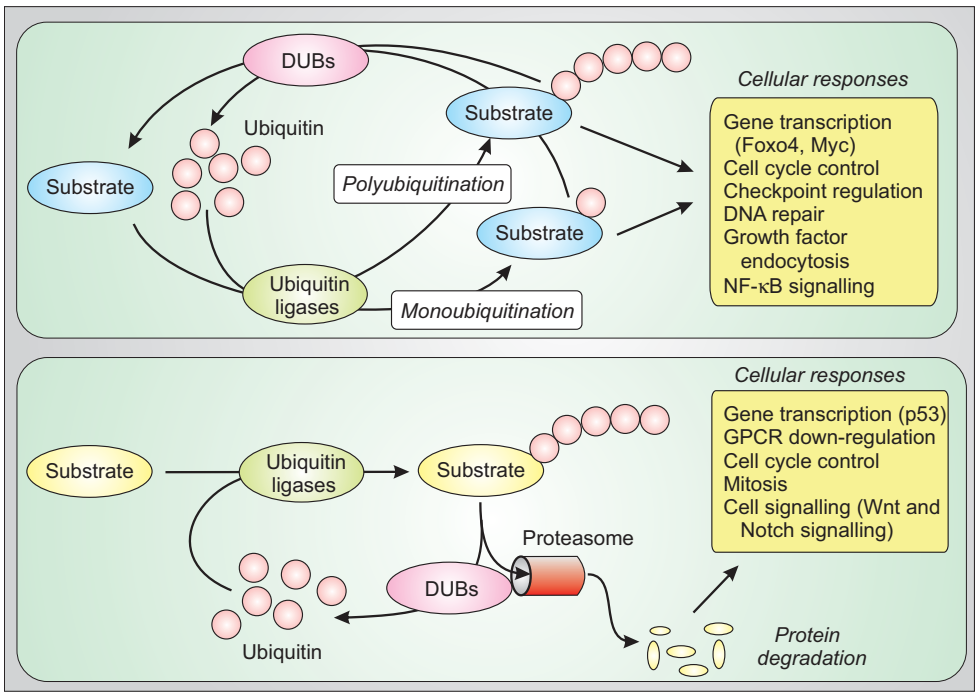
Ubiquitination is a protein modification mechanism that depends on the covalent addition of the highly conserved 76-residue polypeptide protein ubiquitin to specific target proteins (Module 1: Figure protein ubiquitination). This process of protein ubiquitination has two important functions. First, it is used in a ubiquitin signalling system whereby the reversible ubiquitination of certain cell protein substrates, which are components of cell signalling pathways, functions to control a number of cellular responses (see top panel in Module 1: Figure protein ubiquitination). A set of activating, conjugating and ligase enzymes are responsible for adding ubiquitin to the substrate protein. In some cases, only a single ubiquitin is added (monoubiquitination) whereas in others there are multiple additions (polyubiquitination) with ubiquitin molecules being added to each other to form a chain. This post-translational modification alters the conformation of the target proteins to enable them to participate in various signalling pathways to control cellular responses. The cell signalling response is terminated by removal of the ubiquitin groups by deubiquitinating enzymes (DUBs).

The ubiquitin–proteasome system carries out the other major function of protein ubiquitination. In this case, the addition of ubiquitin to protein substrates marks them out for degradation by the proteasome (see lower panel in Module 1: Figure protein ubiquitination). Before the protein enters the proteasome, DUBs strip off the attached ubiquitin that are then recycled for further rounds of protein degradation. Such protein degradation can markedly influence cellular responses when the substrates are components of cell signalling pathways.

Ubiquitin signalling system

The ubiquitin signalling system is highly versatile in that it controls the activity of a number of cellular responses (see top panel in Module 1: Figure protein ubiquitination). This signalling system is based on the reversible ubiquitination of protein substrates, which are components of cell signalling pathways. The following examples illustrate the multiple functions of this signalling system:

Module 1: | Figure protein ubiquitination



Protein ubiquitination in cell signalling and protein degradation.
Covalent modification of proteins by ubiquitin has two important functions. As shown in the top panel, reversible ubiquitination of protein substrates alters their conformation enabling them to carry out various signalling functions in the control of cellular responses. The bottom panel illustrates how the ubiquitination of certain proteins marks them out for degradation by the proteasome. Before they enter the proteasome, deubiquitinases (DUBs) remove the ubiquitin, which is recycled back to be used again by the ligases. The way in which these ligases function to ubiquitinate proteins is shown in Module 1: Figure ubiquitin–proteasome system.

- ubiquitin signalling and gene transcription
- ubiquitin signalling and cell cycle regulation
- ubiquitin signalling and cell signal transduction
- ubiquitin signalling and endocytosis

Ubiquitin signalling and cell signal transduction

The reversible ubiquitination of various signalling components contributes to the processing of information in the following cell signalling pathways:

- In the tumour necrosis factor α (TNFα) signalling pathway, ubiquitination contributes to the formation of a macromolecular cell signalling complex (Module 2: Figure NF-κB activation).
- In the Toll receptor signalling pathway, the reversible ubiquitination of TRAF6 functions to assemble a signal transducing complex (Module 2: Figure Toll receptor signalling).

Ubiquitin signalling and cell cycle regulation

There are many cell cycle stages when ubiquitination functions to regulate either progression or cell cycle arrest at specific checkpoints that are activated during DNA damage. The ubiquitin–proteasome system functions during the cyclin E control of G_1 progression and DNA synthesis and during chromosome separation at anaphase (Module 9: Figure chromosome separation). These are examples where ubiquitin-dependent protein degradation has a signalling function. By contrast, the following are examples where the reversible ubiquitin signalling system functions in cell cycle control and particularly in checkpoint signalling:

- The reversible ubiquitination of Cdc20 functions in spindle-assembly checkpoint signalling (Module 9: Figure spindle assembly checkpoint).
- A ubiquitin signalling system based on the Fanconi anaemia/BRCA pathway functions in both DNA repair and cell cycle control (Module 9: Figure Fanconi anemia pathway).

Ubiquitin signalling and endocytosis

The ubiquitin signalling system plays a role in the processes of endocytosis and is particularly important with regard to the process of down-regulating various receptors.

- The Cbl down-regulation of signalling components is evident during the endocytosis of protein tyrosine kinase-linked receptors (PTKRs). For example, the epidermal growth factor receptor (EGFR) and the Met receptor, are down-regulated through the action of the adaptor protein Cbl, which binds to the activated receptor and initiates its degradation through a ubiquitination process (Module 1: Figure receptor down-regulation).
- The receptor down-regulation of G protein-coupled receptors (GPCRs) depends upon the reversible

ubiquitination of arrestin (Module 1: Figure homologous desensitization).

Deubiquitinating enzymes (DUBs)

The deubiquitinating enzymes (DUBs) function to remove ubiquitin from ubiquitinated proteins that function in a large number of cellular responses (Module 1: Figure protein ubiquitination). There are a large number of DUBs (approximately 100) that fall into five subfamilies:

Ubiquitin-specific proteases (Usps)

There are 58 Usps:

- Usp7, which is also known as herpesvirus-associated ubiquitin-specific protease (HAUSP), is a p53-binding protein that functions in p53 deubiquitination and degradation (Module 4: Figure p53 function). The Usp7/HAUSP deubiquinates both p53 and the ubiquitin ligase mouse double minute-2 (MDM2).
- Usp7/HAUSP reverses the FOXO4 monoubiquitination that facilitates its entry into the nucleus (Module 4: Figure FOXO control mechanisms).
- Usp28 functions to protect the transcription factor Myc from Myc degradation (Module 4: Figure Myc as a gene activator).
- Ubiquitin-specific protease 44 (Usp44) deubiquitinates Cdc20 during spindle-assembly checkpoint signalling (Module 9: Figure spindle assembly checkpoint).
- Ubiquitin-specific protease Y (UBPY) and associated molecule with the Src homology 3 (SH3) domain of STAM (AMSH) remove ubiquitin from cargo proteins during early endosome protein sorting and intraluminal vesicle formation (Module 4: Figure intraluminal endosomal vesicle formation).

Ubiquitin carboxy-terminal hydrolases (UCHs)

There are 4 UCHs

Ovarian tumour-like proteases (OTUs)

There are 14 OTUs

Machado-Jacob-Disease proteases (MJDs)

There are 4 MJDs

JAMM-domain DUBs

There are 14 DUBs that are metalloenzymes that contain JAMM domains. An example is the BRCA1-BRCA2-containing complex (BRCC) that plays a role in stabilizing the activity of NLRP3, which is a key component of the inflammasome.

Four of these subfamilies (Usps, UCHs, OTUs, MJDs) are cysteine proteases that are characterized by a catalytic triad of cysteine, histidine and asparagine residues.

These DUBs have two main functions. First, there are the housekeeping DUBs that function in the ubiquitin–proteasome system to maintain the pool of ubiquitin by removing it from proteins just before they are degraded by the proteasome (Module 1: Figure ubiquitin–proteasome system). Secondly, the DUBs have multiple roles to play in the operation of the ubiquitin signalling system (Module 1: Figure protein ubiquitination).

Protein degradation

Protein degradation is used as a mechanism to control transmission of information through certain signalling pathways. This degradation can occur through either the ubiquitin–proteasome system or through a variety of proteases.

Ubiquitin–proteasome system

The ubiquitin–proteasome system represents a major mechanism for degrading most short-lived intracellular proteins and features significantly in a number of signalling pathways. It is based on the protein ubiquitin, which is a highly conserved 76-residue polypeptide. One of its main functions is to direct substrate proteins for destruction by the 26S proteasome. However, it has additional functions in that it can regulate various cell processes such as membrane trafficking.

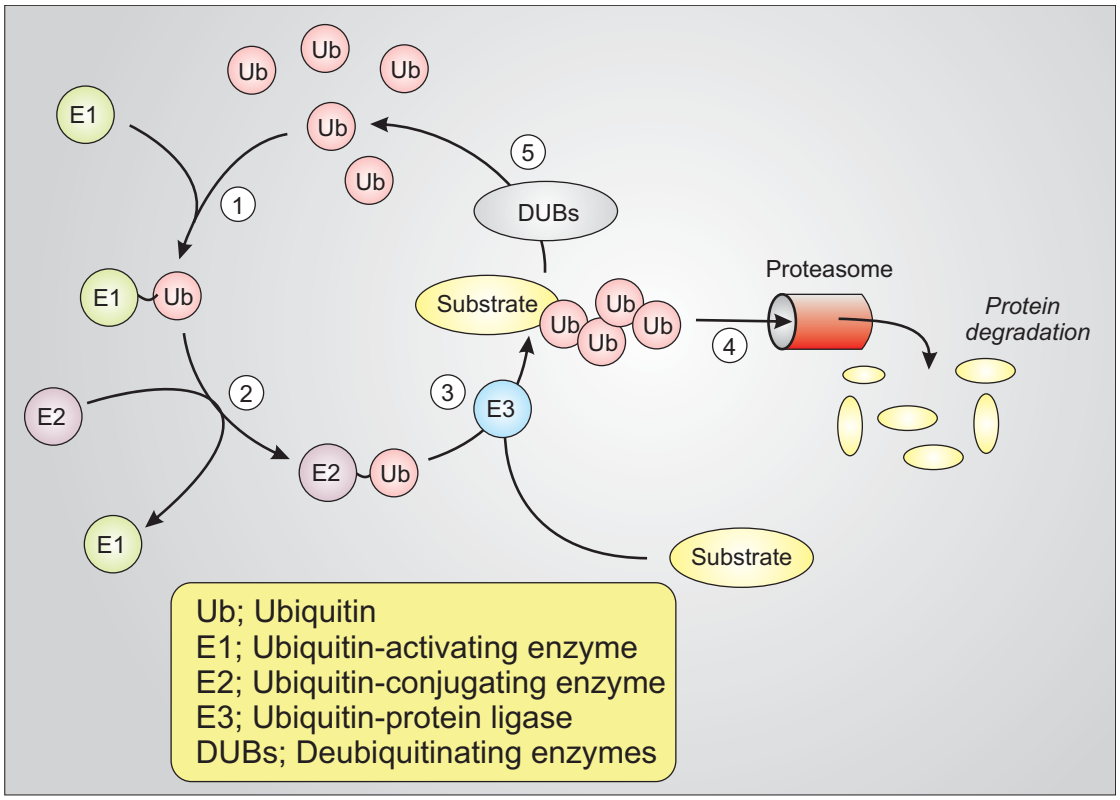
Its function in protein degradation depends on an orderly sequence of reactions as outlined in Steps 1–5 in Module 1: Figure ubiquitin–proteasome system:

1. The first step is the adenylation of ubiquitin (Ub) by the ubiquitin-activating enzyme E1 to form an Ub–AMP intermediate that then interacts with an internal thiol to form an E1–Ub thioester.
2. The Ub attached to E1 is then transferred to one of the ubiquitin-conjugating enzymes (E2) to form an E2–Ub thioester.
3. The ubiquitin is now in a form to be transferred to the protein substrate through a reaction that occurs in conjunction with a ubiquitin/protein ligase E3. The linkage is formed between the C-terminus of ubiquitin and lysine ε-amino groups of the substrate protein. Transfer of a single Ub to the substrate is usually not sufficient to mark the protein for degradation. Instead, there are multiple rounds of ubiquitination (i.e. repetition of Steps 1–3) with each new Ub being added to the preceding molecule to form a multi-ubiquitin chain.
4. The polyubiquitinated protein is recognized by the proteasome and is degraded.
5. The covalent attachment of ubiquitin to proteins is a reversible process. There are a family of deubiquitinating enzymes (DUBs) that can remove ubiquitin before the target protein enters the proteasome.

There are numerous examples of how the ubiquitin-proteasome system is used in signal transmission:

- Activation of the nuclear factor κB (NF-κB) signalling pathway (Module 2: Figure NF-κB activation).
- Levels of the transcription factor p53 are controlled through p53 ubiquitination and degradation carried out by the oncogene mouse double minute-2 (MDM2) (Module 4: Figure p53 function).
- The mouse double minute-2 (MDM2) ubiguitin ligase functions in the receptor down-regulation of G protein-coupled receptors (GPCRs) (Module 1: Figure homologous desensitization).
- The ubiquitin–proteasome system functions at a number of steps during the cell cycle. The cyclin-dependent kinase (CDK) inhibitor p27 is degraded by SCF^{Skp2}

Module 1: | Figure ubiquitin–proteasome system



Operation of the ubiquitin–proteasome system of protein degradation.
The ubiquitin–proteasome system functions in the controlled degradation of proteins. Ubiquitin molecules are attached to the protein substrate to form a chain of ubiquitin molecules which is recognized by the proteasome and degraded. The enzymatic steps responsible for the ubiquitination process are described in the text. This figure was based on Figure 1 in Hochstrasser 2003. Reproduced from *Handbook of Cell Signaling*, Volume 1 (edited by R.A. Bradshaw and E.A. Dennis), Hochstrasser, M. (2003) The ubiquitin–proteasome system, pp. 347–350. Copyright (2003), with permission from Elsevier.

during the cyclin E control of G_1 progression and DNA synthesis. This onset of DNA synthesis is also dependent on the degradation of the cyclin E/CDK2 complex by SCF^{Fbw7}. Chromosome separation at anaphase is controlled by the degradation of Emi2 by the ubiquitination complex SCF (Module 9: Figure chromosome separation).

- The process of chromosome separation at anaphase depends on the anaphase-promoting complex (APC) that ubiquitinates and thus degrades securin to release the enzyme separase that is responsible for hydrolysing cohesin molecules (Module 9: Figure chromosome separation).
- Operation of the spindle-assembly checkpoint signalling pathway depends on the ubiquitination and activation of the Cdc20 protein (Module 9: Figure spindle assembly checkpoint).
- In the Wnt signalling pathways, phosphorylated β-catenin is ubiquitinated by the $SCF^{\beta\text{-}TrCP}$ ubiquitin ligase and is then degraded under resting conditions (Steps 2 and 3 in Module 2: Figure Wnt canonical pathway).
- One of the mechanisms for the down-regulation of cell signalling depends upon a process of signalsome degradation as exemplified by the Cbl down-regulation of signalling components (Module 1: Figure receptor down-regulation).
- The ubiquitin ligase Nedd4-2 regulates the membrane expression of the epithelial Na^+ channels (ENaC).
- Inactivation of Notch signalling depends on the proteasomal degradation of phosphorylated NICD after its ubiquitinylation by the nuclear ubiquitin ligase SEL-10 (Step 9 in Module 2: Figure Notch signalling).
- A number of ubiquitin ligases [Itch , Deltex , Mind bomb (Mib) and Neutralized (Neur)] participate in the modulation of Notch signalling (Module 2: Figure Notch modulation).
- The ubiquitin ligase Cullin 3 plays a key role in the regulation of stress-sensing transcription factor nuclear factor erythroid 2 related factor 2 (NRF-2) (Module 4: Figure NRF-2 antioxidant function).
- HMG-CoA reductase degradation protein 1 (HRD1) is an E3 ligase that carries out the hydrolysis of the transcription factor ATF6 that is responsible for ER stress signalling (Module 2: Figure ER stress signalling).

Ubiquitin ligases

The E3 ubiquitin ligases play a critical role in recognizing protein substrates that are to be degraded by the

proteasome (Module 1: Figure ubiquitin–proteasome system). These ligases can function either as single entities or as part of a much larger multisubunit complex. Some of the E3 ligases require phosphorylation of their substrates as occurs for the SCF ubiquitin ligases. The latter is a large complex and its name reflects some of its components (Skp1, Cdc53 or Cullin, and F-box protein). The variable component in this complex is the F-box protein, which defines the substrate specificity of the different SCF complexes. In the cases where the function of a particular complex is known, the SCF abbreviation is given a superscript to indicate its function such as SCF^{Skp2}. There are a large number of ubiquitin ligases functioning in cell signalling pathways:

- Mouse double minute-2 (MDM2) is an ubiquitin ligase that targets the transcription factor p53 for degradation (Module 4: Figure p53 function). It also functions in the receptor down-regulation of G protein-coupled receptors (GPCRs) (Module 1: Figure homologous desensitization) and the protein acetylase TIP60.
- SCF^{Skp2} degrades phosphorylated p27 as part of cyclin E control of G_1 progression and DNA synthesis.
- The Smad ubiquitin-regulatory factor 1 (Smurf1) is a component of the Smad signalling toolkit (Module 2: Table Smad signalling toolkit). Smurf1 also promotes the ubiquitination and degradation of Rho during the process of neutrophil chemotaxis (Module 11: Figure neutrophil chemotactic signalling).
- The ubiquitin ligase Cbl functions in the Cbl down-regulation of signalling components (Module 1: Figure receptor down-regulation).
- Mind bomb (Mib), Neutralized (Neur), Itch (a Hect domain E3 ligase) and Deltex (a Ring finger E3 ligase) function in Notch signalling (Module 2: Figure Notch modulation).
- The Neuronal-expressed developmentally down-regulated gene 4-2 (Nedd4-2) ubiquitin ligase regulates the rate of membrane insertion of the epithelial Na^+ channel (ENaC).
- When Cyclin E controls G1 progression and DNA synthesis, the cyclin E/CDK2 complex is degraded by SCF^{Fbw7}.
- The ubiquitin E3 ligase Parkin can mono- and polyubiquitinate both lysine-48 and lysine-63 residues. One of its numerous substrates is parkin interacting substrate (PARIS). Another of its substrates is NEMO (Module 2: Figure NF-κB activation) that functions in the expression of optic atrophy 1 (OPA1), which functions to maintain healthy mitochondria (Module 5: Figure OPA1 and mitochondrial cristae remodelling). Mutations in the *Parkin* gene have been linked to Parkinson's disease (PD).
- Mutations in the *Ube3a* gene codes for an E6-AP ubiquitin ligase have been linked to the neurodevelopmental syndrome Angelman syndrome (AS).

Proteases

There are a number of proteases that have important signalling functions:

- ADAM proteases
- Calpains
- Caspases
- Dipeptidyl peptidase type IV (DPP-IV)
- Insulin-degrading enzyme (IDE)
- Matrix metalloproteinases (MMPs)
- Presenilins
- α-Secretase
- β-Secretase
- γ-Secretase complex
- Separase
- Urokinase-type plasminogen activator (uPA)

ADAM proteases

The ADAM (a disintegrin and metalloprotease) family are part of a large zinc protease superfamily. In humans, there are 23 ADAM genes (Module 1: Table ADAM proteases) that code for proteases that have diverse functions including cell migration, neural and muscle development, immune surveillance and fertilization. Much attention has been focused on their role in a variety of cell signalling functions. Some of the ADAMs have a specific role in the process of protein ectoderm shedding to release various cell stimuli such as growth factors and cytokines (Module 1: Figure formation and function of cell stimuli).

The ADAM proteins, which are transported to the cell surface in vesicles, have a single transmembrane region that anchors them in the plasma membrane. The large extracellular domain begins with a metalloprotease domain that is followed by the disintegrin domain that can bind to integrin receptors. There also is a cysteine-rich domain and an EGF-like domain. The cytoplasmic domain has phosphorylation sites and proline-rich regions capable of binding proteins containing SH3 domains. The ADAM proteases function in a variety of cell signalling processes:

- ADAM-17 and ADAM-10 initiate the proteolytic events that hydrolyse the Notch receptor during the Notch signalling pathway (see Step 3 in Module 2: Figure Notch signalling). ADAM-17 also functions to release TGF-α during the process of skin development.
- ADAM-2 plays an early role in the process of sperm-induced oocyte activation.
- ADAM-9 may function to hydrolyse IGFBP-5 to release insulin-like growth factor (IGF) in bone.
- ADAM-12 may function in the ectoderm shedding of epidermal growth factor receptors (EGFRs) (Module 1: Figure EGF stimuli and receptors).
- ADAM proteases function to release the membrane-anchored CX3CL1 chemokine (Module 1: Figure chemokines). Release of the soluble form of CX3CL1 functions in neuronal-microglial interactions (Module 7: neuronal chemokine function).

Calpains

The calpains are Ca^{2+}-activated non-lysosomal proteases (CAPNs) that have been implicated in the control of cytoskeletal remodelling, cell cycle progression and apoptosis. They are cysteine proteases that have a catalytic site similar to that found in the caspases, cathepsins and papain.

Module 1: | Table ADAM proteases
Human ADAM protease toolkit.

ADAM proteases	Comments
ADAM-1	Fertilinα: non-functional in humans
ADAM-2	Fertilinβ: catalytically inactive. Functions in fertilization
ADAM-3	Cyritestin: non-functional in humans
ADAM-6	
ADAM-7	
ADAM-8	Functions in monocytes
ADAM-9	Also known as Meltrin γ or MDC9. Functions in cell migration
ADAM-10	Also known as Kuzbanian or MADM. Functions in neural and cardiac development
ADAM-11	Also known as MDC
ADAM-12	Also known as Meltrin α. Two splice forms: ADAM-12L remains membrane-anchored whereas ADAM-12S is released
ADAM-15	Also known as Metargidin
ADAM-17	Also known as TACE [Tumour-necrosis factor (TNF) α-converting enzyme]. Multiple functions including ectodomain shedding (see Module 1: Figure formation and action of cell stimuli)
ADAM-18	Also known as tMDCIII
ADAM-19	Also known as Meltrin β. Processes the neuroregulin precursor
ADAM-20	
ADAM-21	
ADAM-22	Also known as MDC2
ADAM-23	Functions in neural development
ADAM-28	Also known as MDC-Ls. Functions in immune surveillance
ADAM-29	
ADAM-30	
ADAM-32	
ADAM-33	

There are approximately 15 mammalian calpains which are divided into typical calpains (nine members) and atypical calpains (six members). The sensitivity to Ca^{2+} of the typical CAPNs depends on EF-hand motifs located in the C-terminal region. The atypical CAPNs lack these EF-hands and it is not clear how they respond to Ca^{2+}. Most cells constitutively express two calpains called μ-calpain and m-calpain (calpain 1 and calpain 2). The former is a heterodimer formed between CAPN1 and a smaller CAPN4 subunit and is sensitive to Ca^{2+} concentrations of about 50 μM. The μ-calpain, which is also a heterodimer formed by CAPN2 and CAPN4, requires higher levels of Ca^{2+} (about 200 μM) for its activation. Ten Ca^{2+} ions are bound to the penta-EF-hand domains that are distributed throughout the molecule.

The intrinsically unstructured protein calpastatin is a potent inhibitor of the calpains. Calpastatin acts at very low doses and plays an important role in protecting cells when calpains are over-activated when Ca^{2+} concentrations are abnormally high, as occurs during glutamate-induced excitotoxic cell death of neurons or during ischaemia/repurfusion injury in cardiac cells.

Calpains are thought to function in cell signalling by cleaving various substrates, but these are still to be clearly defined. Despite the lack of information on the physiological role of calpains, they have been linked to several human diseases. Mutations in calpain 3 have been linked to limb girdle muscular dystrophy type 2A. Mutations in the atypical calpain 10 have been linked to Type 2 diabetes.

Caspases

Caspases are a family of cysteinyl aspartate-specific proteases, which function in the caspase cascade responsible for apoptosis (Module 11: Figure apoptosis). Caspases 3, 7 and 9 are potently inhibited by X-chromosome-linked inhibitor of apoptosis protein (XIAP).

Dipeptidyl peptidase type 4 (DPP-4)

A protease that functions in the metabolism of various hormones such as growth hormone-releasing hormone (GHRH) and glucagon-like peptide 1 (GLP-1).

Insulin-degrading enzyme (IDE)

The insulin-degrading enzyme (IDE) is part of the amyloid cascade hypothesis. It is released from the microglia to hydrolyse β amyloids to reduce their deleterious effects on neuronal survival (Module 12: Figure amyloid cascade hypothesis).

Matrix metalloproteinases (MMPs)

The matrix metalloproteinases (MMPs) are a large family of zinc-containing endopeptidases that cleave components of the extracellular matrix (ECM), growth factors (e.g. transforming growth factor β) and cell adhesion components such as the cadherins and integrins, and they can release the apoptotic ligand Fas (Module 1: Table MMPs and their inhibitors) . They play a particularly important role in remodelling the ECM during tissue development such as angiogenesis.

These different proteases have a multidomain organization that contains a number of similar domains (Module 1: Figure MMP structure). The N-terminal region begins with a secretion sequence (S) followed by a prodomain, which is cleaved during the activation of the enzyme by plasmin or by other members of the MMP family. There is a conserved catalytic domain that is connected through a hinge region (H) to a haemopexin-like domain, which consists of four twisted β-sheets to form a propeller structure similar to that found in the serum protein haemopexin. This haemopexin-like domain acts together with the hinge region to recognize substrates and to unravel their structure so that they become accessible to the catalytic domain. The matrilysins lack the hinge region and the haemopexin-like domain. The membrane-type MMPs have C-terminal specialization such as a transmembrane domain (TMD) or

Module 1 | Table MMPs and their inhibitors
The matrix metalloproteinase (MMP) family and their endogenous inhibitors.

MMP and MMP inhibitors	Comments
MATRIX METALLOPROTEINASES (MMPs)	
Collagenases	Cleave collagens
MMP1	Collagens (I, II, III, VII, VIII and X), L-selectin, proteoglycans, entactin, ovostatin, MMP-2 and MMP-9
MMP-8 (neutrophil collagenase)	Collagens (I, II, III, V, VII, VIII and X), aggrecan and fibronectin
MMP-13	Collagens (I, II, III, IV, IX, X and XIV), plasminogen, aggregan, fibrinogen and MMP-9
MMP-18	
Gelatinases	Cleave denatured collagens (gelatins), laminins and certain chemokines
MMP-2 (gelatinase A)	Can activate MMP-1 and MMP-9 by cleaving off the prodomain
MMP-9 (gelatinase B)	Released by osteoclasts to hydrolyse the bone matrix (Module 7: Figure osteoclast function)
Stromelysins	Function to cleave collagen, fibronectin, laminin, gelatine and casein, and can activate various MMPs by removing the prodomain
MMP-3 (stromelysin-1)	Collagens (III, IV, V and IX), perlecan, decorin, laminin, elastin, cytostatin, plasminogen, MMP-2, MMP-7, MMP-8 and MMP-13
MMP-10 (stromelysin-2)	Collagens (III-V), aggrecan, elastin, MMP-1 and MMP-6
Matrilysins	Differs from the other MMPs by the absence of the haemopexin domain
MMP-7 (matrilysin-1)	Cleaves ECM components E-cadherin and pro-α-defensin
MMP-26 (matrilysin-2)	Cleaves collagen IV, fibronectin and fibrinogen, and activates MMP-9
Membrane-type MMPs (MT-MMPs)	
MMPs with transmembrane domains	
MMP-14 (MT1-MMP)	
MMP-15 (MT2-MMP)	
MMP-16 (MT3-MMP)	
MMP-24 (MT5-MMP)	
MMPs with glycosylphosphatidylinositol anchors	
MMP-17 (MT4-MMP)	
MMP-25 (MT6-MMP)	
Miscellaneous MMPs	A heterogeneous group that are usually expressed in specific cells or during specific events
MMP-11	
MMP-12 (macrophage metalloelastase)	Collagen IV, elastin, fibronectin, vitronectin, laminin, entactin and fibrin
MMP-19	Collagen type I
MMP-20 (enamelysin)	
MMP-21	
MMP-22	
MMP-23	
MMP-28 (epilysin)	
MMP INHIBITORS	
Tissue inhibitors of metalloproteinases (TIMPS)	
TIMP-1	
TIMP-2	
TIMP-3	
TIMP-4	
Reversion-inducing cysteine-rich protein with Kazal motifs (RECK)	A membrane-anchored inhibitor of MMPs

Most of the information for this table was taken from Catania et al. (2006).

a glycosylphosphatidylinositol residue that anchors them to the membrane. The gelatinases contain fibronectin domains located within the catalytic domain.

The matrix metalloproteinases (MMPs) are secreted as a proMMP, and the prodomain has to be cleaved before the enzyme can function. This cleavage is carried out by enzymes such as plasmin, by certain of soluble MMPs or by one of the membrane-type MMPs (shown in green in Module 1: Figure MMP activation and function), which cleave off the prodomain. The activated MMPs degrade components of the extracellular matrix (ECM), as shown in the figure, but can also act on other substrates such as growth factors, cadherins and integrins. This hydrolytic activity is inhibited by soluble tissue inhibitors of metalloproteinases (TIMPs) or by the membrane-bound reversion-inducing cysteine-rich protein with Kazal motifs (RECK).

The MMPs have been implicated in a large number of disease states:

MMP-2 and MMP-9 play a critical role in the late stages of cancer cells, which secrete matrix proteinases that degrade the extracellular matrix and thus greatly enhance the metastatic potential by enabling tumour cells to invade other tissues.

Alterations in the expression of the MMPs and their inhibitors are a major contributor to diabetic nephropathy.

MMPs have been implicated in the development of rheumatoid arthritis and osteoarthritis.

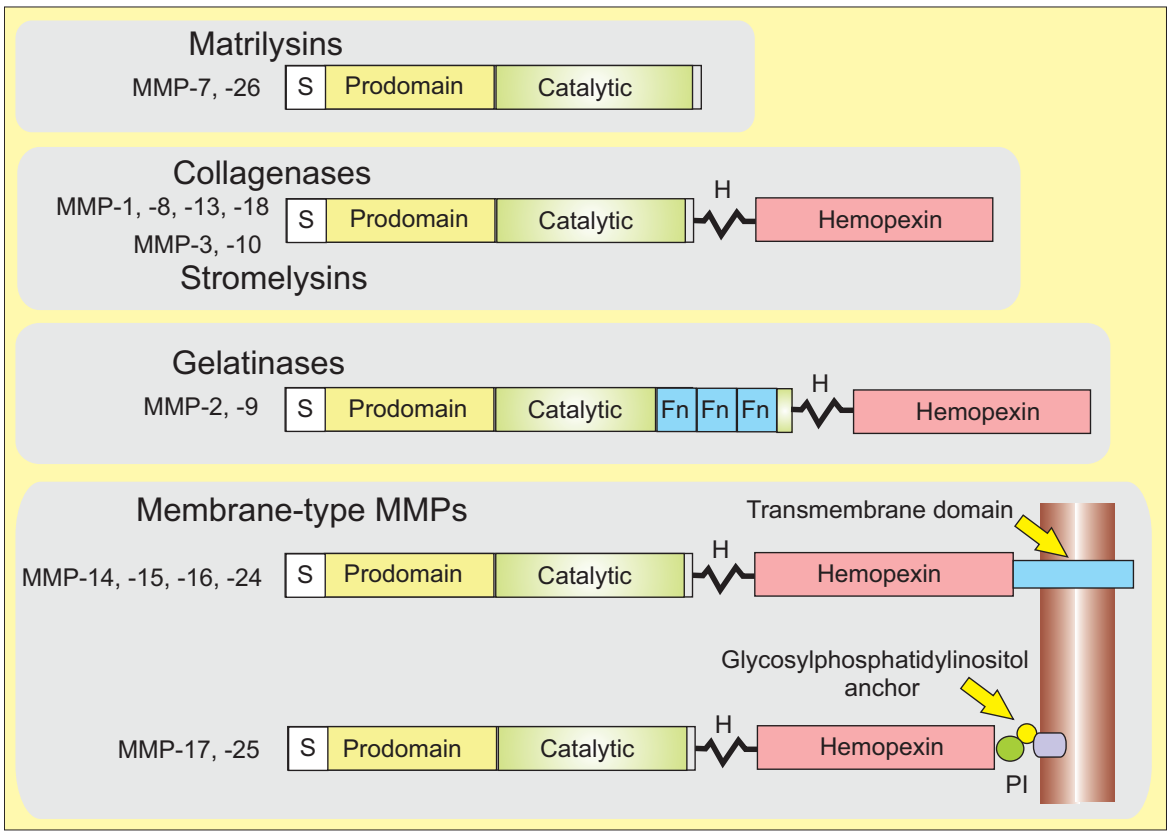
Tissue inhibitors of metalloproteinases (TIMPs)

The tissue inhibitors of metalloproteinases (TIMPs) are specific inhibitors of the matrix metalloproteinases (MMPs) (Module 1: Table MMPs and their inhibitors).

Reversion-inducing cysteine-rich protein with Kazal motifs (RECK)

The reversion-inducing cysteine-rich protein with Kazal motifs (RECK) is a membrane-anchored inhibitor of some of the matrix metalloproteinases (MMPs) such as MMP-2,

Module 1: | Figure MMP structure



Domain structure of the matrix metalloproteinases (MMPs).
The different groups of matrix metalloproteinases (MMPs), which are described in Module 1: Table MMPs and their inhibitors, have a similar structural organization as described in the text. S, secretion sequence; Fn, fibronectin-like domain; PI, PtdIns.

MMP-9 and MMP-14 (Module 1: Table MMPs and their inhibitors)

Separase
Separation of chromosome at anaphase is controlled by separase, which is a caspase-related protease that cleaves the cohesin molecules that hold chromosomes together on the mitotic spindle (Module 9: Figure chromosome separation).

Urokinase-type plasminogen activator (uPA)
One of the functions of urokinase-type plasminogen activator (uPA) is to hydrolyse the hepatocyte growth factor (HGF) precursor.

DNA methylation

Methylation of DNA is the basis of the epigenetic mechanism responsible for gene silencing. The methyl group is usually attached to the N5 position on cytosine (C) that is linked to a guanine (G) through a phosphodiester (p) that forms the CpG dinucleotide complex. Such CpGs often occur as clusters where they are referred to as CpG islands. CpG dinucleotides are often located in the 5' regulatory regions of many genes where they function in gene silencing once they have been methylated by DNA methyltransferases.

DNA methyltransferase

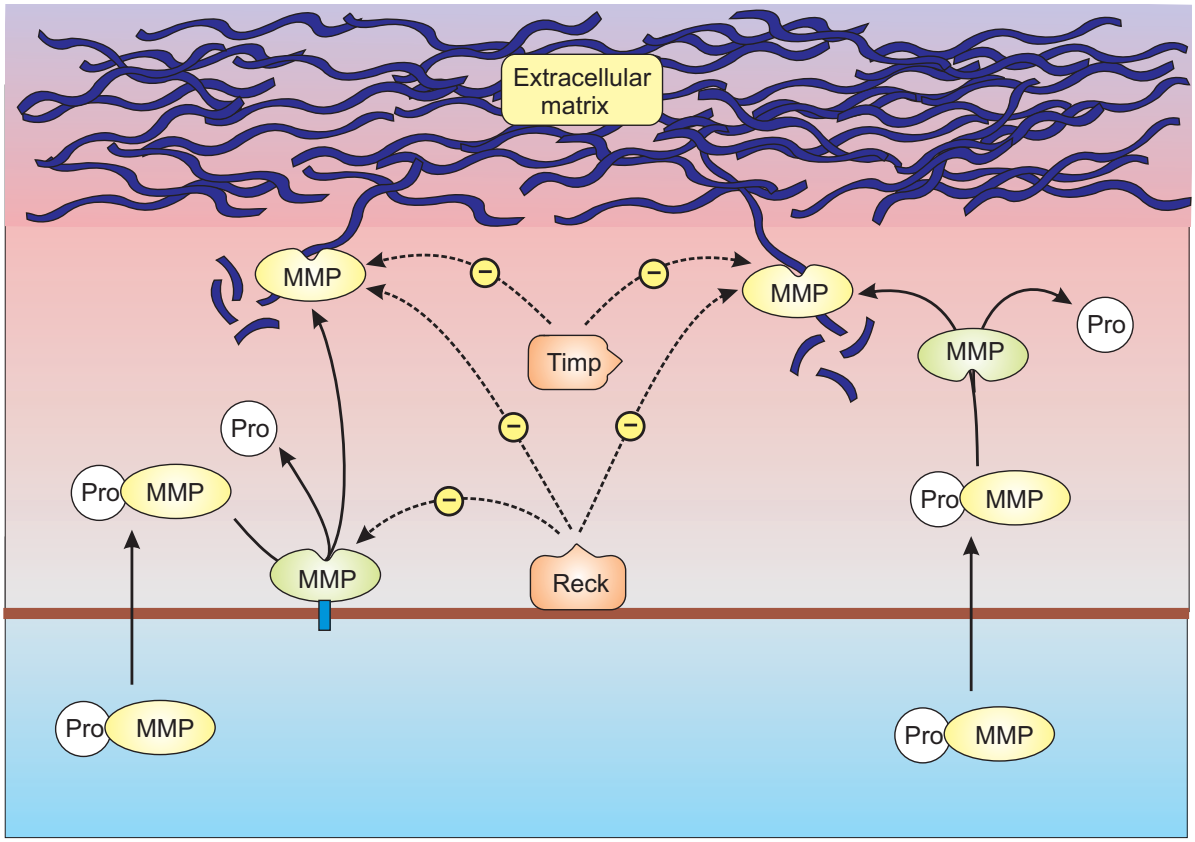
This family of enzymes catalyses the transfer of a methyl group from S-adenosyl methionine (SAM) to cytosine residues on DNA.

DNA methyltransferase 1 (DNMT1)
The primary function of DNA methyltransferase 1 (DNMT1) is to maintain the DNA methylation patterns established by the DNMT3 *de novo* methyltransferases. DNMT1 is the somatic isoform and there is an oocyte isoform (DNMT1o), which is found in the cytoplasm of the oocyte and then translocates to the cell nucleus during development.DNMT1 associates with the transcriptional repressor methyl-CpG-binding protein 2 (MeCP2) that functions in gene silencing (Module 4: Figure MeCP2 activation).

tRNA aspartic acid methyl transferase 1 (TRDMT1)
This RNA cytosine methyltransferase was originally thought to be a DNA methyltransferase and was known as DNA methyltransferase 2 (DNMT2). Subsequently, it was found to methylate the aspartic acid tRNA.

DNA methyltransferase 3 (DNMT3)
The DNA methyltransferase 3 (DNMT3) family consists of three members: DNMT3a, DNMT3b and DNMT3L. DNMT3a and DNMT3b are responsible for setting up the DNA methylation patterns during development and

Module 1: | Figure MMP activation and function



Activation and function of the matrix metalloproteinases (MMPs).
The matrix metalloproteinases (MMPs) are activated either by certain soluble MMPs or by one of the membrane-type MMPs (shown in green), which cleave off the prodomain (Pro). The activated MMPs degrade components of the extracellular matrix (ECM). This hydrolytic activity is inhibited by soluble tissue inhibitors of metalloproteinases (TIMPs) or by the membrane-bound reversion-inducing cysteine-rich protein with Kazal motifs (RECK).

are thus known as *de novo* DNA methyltransferases. DNMTL was included in the family on the basis of its homology with other DNA methyltransferases, but it lacks enzyme activity. However, it facilitates the *de novo* methyltransferases by enhancing their DNA binding and enzyme activities.

In embryonic stem cells, DNMT 3a and DNMT 3b are silenced by the miR-290-295 cluster (Module 8: Figure ES cell miRNAs).

Desensitization of cell signalling

There are a number of diverse mechanisms for desensitizing cell signalling mechanisms. These can operate at many levels along a signalling pathway, but most attention has focused on receptor desensitization and receptor down-regulation.

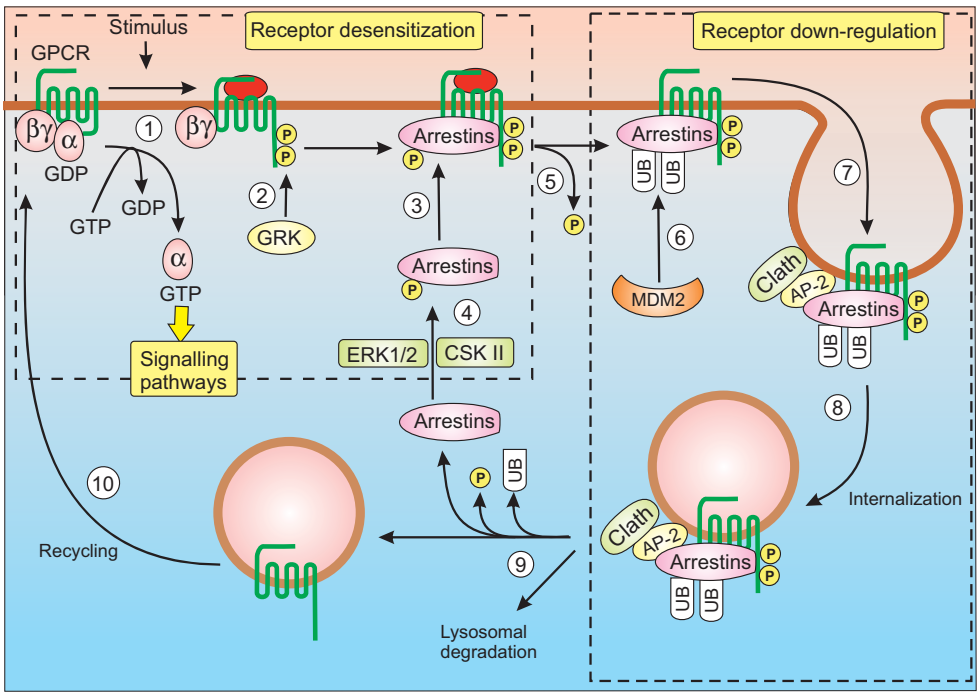
Receptor desensitization

Receptor desensitization has been studied in some detail in the case of G protein-coupled receptors (GPCRs), which often desensitize rapidly following agonist-induced activation. The initial rapid formation of second messengers such as cyclic AMP can be reduced within minutes. After removal of the agonist, the receptor can recover its former sensitivity equally quickly. For short periods of agonist stimulation, this rapid desensitization/resensitization sequence occurs without a change in the number of surface receptors. There are two types of desensitization: homologous desensitization and heterologous desensitization.

Homologous desensitization

In homologous desensitization, only those receptors that are being activated undergo desensitization, which is carried out by the combined actions of G protein receptor kinases (GRKs) and a family of proteins called arrestins. A good example of this occurs during the formation of cyclic GMP in phototransduction when rhodopsin is phosphorylated by rhodopsin kinase (GRK1) (Step 2 in Module 10: Figure phototransduction). In the case of β-adrenergic receptors, the phosphorylation of the activated receptor is carried out by β-adrenergic receptor kinase 1 (βARK1). The phosphorylated residues on the GPCRs provide binding sites for accessory proteins called arrestins that inhibit further receptor activity by preventing the receptor from binding the heterotrimeric G proteins (Module 2: Figure heterotrimeric G protein signalling). The following sequence of events illustrates how this GRK-arrestin regulatory system operates in homologous receptor desensitization (Module 1: Figure homologous desensitization):

Module 1: | Figure homologous desensitization



Homologous desensitization and down-regulation of G protein-coupled receptors (GPCRs).
Homologous desensitization has two components. Receptor desensitization occurs soon after agonist stimulation through a sequence of reactions that are readily reversible (Steps 1–4 in hatched box on the left). If agonist stimulation persists, receptor down-regulation occurs when the receptors are internalized (Steps 5–8 in hatched box on the right). The receptors that enter the clathrin-coated pits are either recycled or degraded. See text for further details.

1. When a G protein coupled receptor (GPCR) detects a stimulus it undergoes a conformation change that enables it to function as a GTP exchange factor (GEF) for heterotrimeric G proteins such that the GDP on the Gα subunit is exchanged for GTP. The active Gα–GTP complex is then able to stimulate a number of signalling pathways (see Module 1: Table G protein-coupled receptors).
2. The change in the conformation of the active receptor opens up sites that are phosphorylated by one of the G protein receptor kinases (GRKs).
3. Arrestin then binds to this phosphorylated receptor and results in receptor desensitization in that the bound arrestin physically prevents the receptor from activating further G proteins.
4. Before the arrestin can bind to the activated receptor it has to be phosphorylated and this is carried out by various kinases such as ERK1/2, which is a component of the ERK pathway, or casein kinase II (CSKII).

This process of receptor desensitization is rapid and it can be reversed equally rapidly. However, if the stimulus is particular strong or persistent, receptor down-regulation occurs through a process of endocytosis as the receptors enter clathrin-coated pits.

Receptor down-regulation

In contrast with receptor desensitization, which is often fast and there is no reduction in the number of cell-surface receptors, receptor down-regulation is usually a slower process that depends upon the removal of receptors from the cell surface by endocytosis. The activated receptors are concentrated into clathrin-coated pits, which are then pinched off from the surface by the cytoplasmic GTPase dynamin.

Receptor down-regulation has been described for many different receptor types. The sequence of events that occurs for G protein-coupled receptors (GPCRs) is shown in Module 1: Figure homologous desensitization:

5. The Steps 1–4 are described in the section on the reversible process of receptor desensitization. Here we deal with the process of receptor down-regulation that begins with a protein phosphatase removing the phosphate moiety from arrestin.
6. The dephosphorylated arrestin is then ubiquitinated by the E3 ubiquitin ligase mouse double minute-2 (MDM2) that sets the stage for the onset of endocytosis.
7. The receptor complex enters the clathrin-coated pit by arrestin binding to clathrin using adaptor protein-2 (AP2) as an adaptor protein.
8. The membrane and associated receptor complex are internalized through a process driven by the GTPase dynamin.
9. The endocytic vesicle can then be directed to separate pathways. In one pathway, it can enter a lysosomal degradation pathway resulting in receptor

proteolysis. Alternatively, the arrestin can be deubiquitinated and the arrestin is returned to the cytoloplasm. The receptor is also dephosphorylated.

10. The receptor is then recycled back to the plasma membrane and can once again participate in cell signalling.

Receptor down-regulation is not restricted to G protein-coupled receptors (GPCRs), but also applies to the protein tyrosine kinase-linked receptors (PTKRs). The Cbl down-regulation of signalling components is an example of how such PTKRs are down-regulated.

Heterologous desensitization

Heterologous desensitization occurs through a mechanism that depends on the phosphorylation of G protein-coupled receptors by various kinases that are activated by other signalling pathways. A classic example is the ability of protein kinase A (PKA) to inactivate the β2 adrenoceptor by phosphorylating a single residue in the third cytoplasmic loop. This phosphorylation does not require the receptor to be activated, as occurs for homologous desensitization. It thus provides a mechanism for cross-talk between different receptor systems. Activation of one receptor, which generates cyclic AMP and activates PKA, can thus phosphorylate and inactivate other receptor types.

G protein receptor kinases (GRKs)

The family of G protein receptor kinases (GRKs) function in the homologous desensitization of G protein-coupled receptors (Module 1: Figure homologous desensitization).

- GRK1 is also known as rhodopsin kinase and functions in the inactivation of rhodopsin (see Step 2 in Module 10: Figure phototransduction).
- GRK2 is expressed widely in cells and normally resides in the cytoplasm. During receptor activation, it interacts with the G protein βγ subunit that puts it in position to phosphorylate receptors in a number of cell types:
- GRK2 and β-arrestin 1 interact to control β-adrenergic responses in cardiac cells.
- The GRK2 in lymphocytes functions to control the CCR5 receptor that responds to CCL4 and CCL5 (Modules 1: Figure chemokines).
- Many brain areas express GRK2 that controls the sensitivity of the many G protein-coupled receptors that modulate neural activity.

GRK3, which has properties similar to GRK2, is expressed in airway smooth muscle cells where it modulates the activity of the acetylcholine-dependent contractions (Module 7: Figure bronchiole-arteriole contraction).

GRK4 is expressed mainly in the testis, but is also found in kidney tubules. Polymorphic variants of GRK4 result in constitutive activation and this causes a decrease in Na^+ secretion and hypertension.

GRK5, which is expressed widely, modulates the sensitivity of acetylcholine receptors that controls the contraction of airway smooth muscle cells (Module 7: Figure bronchiole-arteriole contraction).

GRK6 is expressed widely. It is particularly evident in immune cells. Ablation of GRK6 or β-arrestin 2 can reduce the chemotactic response of lymphocytes to CXCL12 that acts through CXCR4 receptors.

GRK4–6 lack a βγ-binding domain, but can associate with the membrane through either PtdIns4,5P_2-binding domains or through a palmitate lipid modification.

GRK7 is an iodopsin kinase.Levels of GRK2 and GRK6 are low in lymphocytes from patients with rheumatoid arthritis.Mice that are heterozygous for GRK2, were found to be more sensitive to autosomal encephalomyelitis, which is a model for multiple sclerosis.Up-regulation of both GRK2 and GRK3 have been described in patients suffering from depression. One suggestion is that the increased levels of these two GRKs may result in desensitization of dopamine receptors.There also is the suggestion that the abnormal expression of neuronal Ca^{2+} sensor-1 (NCS-1), which has been noted in the prefrontal cortex of patients with schizophrenia and bipolar dosorders, may desensitize D2 dopamine receptors by acting through GRK2.

Arrestins

Arrestins are multifunctional adaptor proteins that act together with the G protein receptor kinases (GRKs) to regulate the activity of G protein-coupled receptors (GPCRs). There are four arrestin isoforms. Arrestin 1 and arrestin 4 are found only in rods and cones where they regulate the activity of rhodopsin.The other two isoforms, β-arrestin 1 (arrestin 2) and β-arrestin 2 (arrestin 3), are expressed more widely and function to regulate the activity of GPCRs. These two isoforms are fairly similar but there also are examples were they act specifically on certain receptors. For example, β-arrestin 2 is specific for β2 adrenoceptor whereas β-arrestin 1 is specific for proteinase-activated receptor 1 (PAR1). With regard to receptor desensitization, β-arrestins have two main functions. First, they function in homologous desensitization by associating with active receptors to inhibit G protein activation (Steps 1–4 in Module 1: Figure homologous desensitization). Secondly, they function in receptor down-regulation by linking the receptor complex to AP2 and clathrin during the process of endocytosis (Steps 5–8 in Module 1: Figure homologous desensitization). The arrestins function as clathrin-associated sorting proteins (CLASPs) by binding to calthrin and AP2 to guide GPCRs into the coated pits ready for internalization (Module 4: Figure cargo sorting signals).

In addition to this role in receptor desensitization, arrestins can also function as scaffolding/targeting protein to assemble a variety of signalling components. This signalling role is particularly evident in the way the GPCRs can activate signalling pathways normally associated with protein tyrosine kinase-linked receptors. For example, the arrestins can assemble components of the different mitogen activated protein kinase (MAPK) signalling pathways such as the ERK pathway and the JNK3 pathway. The omega-3 fatty acid receptor GPR120 uses arrestin-2 to inhibit various inflammatory responses (Module 2: Figure omega-3 fatty acids).

β-Adrenergic receptor kinase 1 (βARK1)

β-Adrenergic receptor kinase 1 (βARK1), which is also known as GRK2, is one of the G protein receptor kinases (GRKs) that functions in receptor desensitization.

Cbl down-regulation of signalling components

One of the main functions of the adaptor protein Cbl is to function as an E3 ubiquitin ligase with a particularly important role in the down-regulation of many cell signalling components. The ubiquitin–proteasome system is one of the major protein degradation pathways in cells (Module 1: Figure ubiquitin–proteasome system). Cbl uses this system to down-regulate a variety of signalling proteins. The best example of this process is the Cbl-dependent down-regulation of protein tyrosine kinase-linked receptors (PTKRs) such as the epidermal growth factor receptor (EGFR), hepatocyte growth factor receptors (HGFRs), colony-stimulating factor-1 receptor (CSF-1R), neurotrophin and vascular endothelial growth factor (VEGF) (Module 1: Figure receptor down-regulation). However, this mechanism is not restricted to PTKRs, because Cbl can also down-regulate other receptors (e.g. FcεRI, α_5 integrin subunit and components of the T cell receptor). Cbl is also able to down-regulate many other downstream signalling components, such as various non-receptor protein tyrosine kinases (e.g. Src, Syk, Hck, Fgr, Lyn and c-Abl), Bim, Sprouty 2 (SPRY2) and signal transducer and activator of transcription 5 (STAT 5). There are some substrates that are ubiquitinated by Cbl, but are not degraded, such as the p85 regulatory subunit of PtdIns 3-kinase, Vav, Crk-like (CrkL) and phospholipase Cγ1 (PLCγ1). The description of Cbl structure and regulation reveals the existence of numerous domains (Module 6: Figure Cbl structure) that enable it to interact with proteins to carry out its role in terminating cell signalling. Its role in degradation occurs through a series of steps as illustrated by its action on PTKRs (Module 1: Figure receptor down-regulation):

1. The growth factor brings together two receptor subunits, and their tyrosine kinase regions phosphorylate each other to provide the phosphorylated binding sites that draw in various signalling components (for details see Module 1: Figure PDGFR activation).
2. One of these components is the adaptor protein called growth factor receptor-bound protein 2 (Grb2).
3. The cytosolic Cbl attaches to the activated receptor, the tyrosine kinase-binding (TKB) domain attaches to one of the phosphorylated residues (e.g. phosphorylated Tyr-1045 on the EGFR), whereas the proline-rich region (Pro) attaches to growth factor receptor-bound protein (Grb2) (Module 1: Figure receptor down-regulation).
4. Once Cbl is attached, it is activated by phosphorylation by a variety of tyrosine kinases and in particular by non-receptor protein tyrosine kinases, such as Src, Hck, Fyn and Syk. These phosphorylation events enable Cbl to activate its ubiquitin ligase activity that is located in the RING finger domain, which is inhibited by Sprouty 2 (Spry2). The phosphorylation of Spry2 causes its displacement from the RING domain to the TKB domain. Phosphorylation of two tyrosine residues (Tyr-368 and Tyr-371) in the L region plays a critical role in switching on the ubiquitination activity of Cbl.
5. The displacement of Spry2 frees up the RING domain, which then binds to the E2 conjugating enzyme.
6. The E2/RING complex then ubiquitinates a variety of residues dispersed throughout the receptor and also on Spry2.
7. Once the receptor is ubiquitinated, it is directed towards coated pits through a process that is facilitated by the adaptor Cbl-interacting protein of 85 kDa (CIN85). A proline-rich sequence located close to the C-terminus binds to CIN85, which functions to target receptor complexes to the clathrin-coated vesicles by binding to endophilin that associate with clathrin adaptor protein-2 (AP2).
8. The clathrin-coated pits are taken in to form vesicles which fuse to form multivesicular bodies where the receptors are sorted for either recycling back to the plasma membrane or sending to the lysosome for destruction. The Cbl-dependent ubiquitination determines this decision by targeting the receptors for lysosomal degradation.

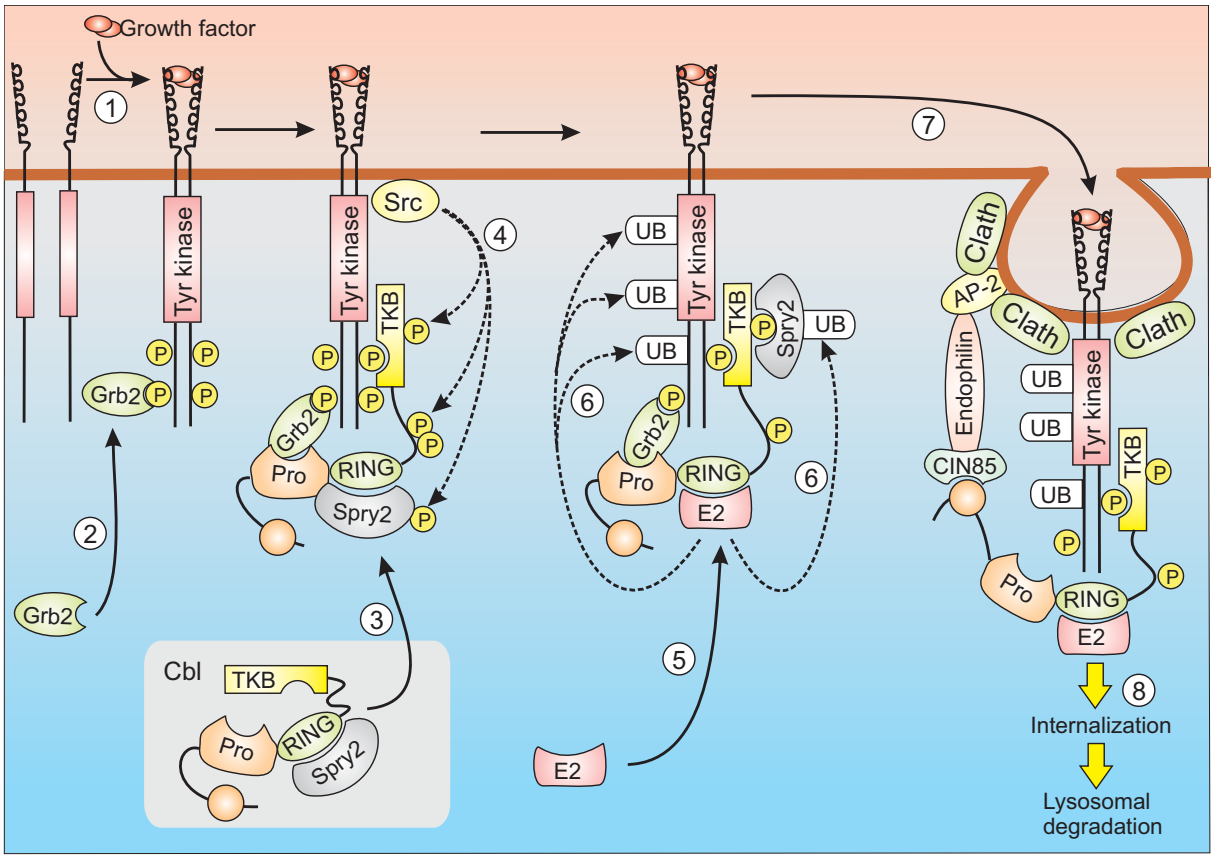
Spatial and temporal aspects of cell signalling

Cell signalling pathways are highly organized with regard to both space and time. With regard to the spatial aspects, the basic principle is that signalling components are linked together through modular protein–protein domains, which are often held in close apposition using a variety of structural devices such as protein scaffolds, lipid rafts and caveolae. These organized complexes of signalling components maximize the flow of information between signalling components.

This spatial organization of signalling molecules can lead to highly localized signalling events, and these are referred to as the elementary events of signalling. They have been particularly well characterized for the Ca^{2+} signalling pathway because they can be visualized in real time. Such localized elementary events can have a localized action or they can be recruited to create more global signals. Such globalization phenomena are not necessarily restricted to individual cells, but they can spread from cell to cell through gap junctions. Such intercellular communication can co-ordinate the activity of cell communities.

The temporal aspects of signalling concern the way information is organized in the time domain. Many biological processes are rhythmical. Of particular importance are the cellular oscillators that set up oscillating intracellular signals that can operate over an enormous range of frequencies to drive many different cellular processes. Membrane oscillators set up rapid membrane potential oscillations that can drive neural processing of information and pacemaker activity in contractile systems such as the heart and smooth muscle. Cytosolic oscillators (second to minute range) set up oscillations in intracellular Ca^{2+} to control many cellular processes. An important feature of such oscillations is the way information can be encoded and decoded depending on the frequency, amplitude or

Module 1: | Figure receptor down-regulation



Cbl-dependent down-regulation of protein tyrosine kinase-linked receptors (PTKRs).
The down-regulation of protein tyrosine kinase-linked receptors (PTKRs), such as the epidermal growth factor receptor (EGFR)and the Met receptor, is regulated by the adaptor protein Cbl, which binds to the activated receptor and initiates its degradation through a ubiquitination process that proceeds through Steps 1–8 as described in the text.

shape of the individual transients. At the other end of the temporal scale is the circadian clock, which is a transcriptional oscillator that is responsible for driving the 24 h diurnal rhythm.

The organization of signalling systems in both time and space is described in **Module 6: Spatial and Temporal Aspects of Signalling**.

Signalsome

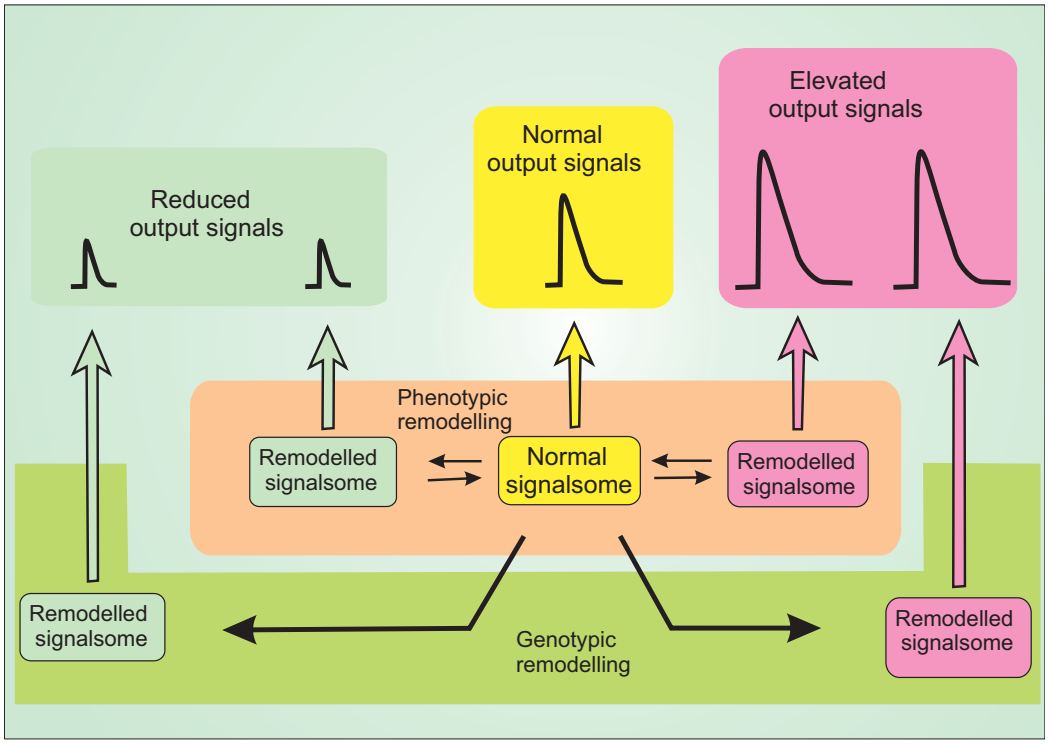
The genome contains a very large repertoire of signalling components, from which each cell type assembles a unique set of components that will be referred to as a signalsome. During the process of differentiation at the end of development, each specialized cell selects out those components that are particularly suited to provide the signalsome most appropriate to control its unique functions. Many cells express combinations of the different signalling pathways to provide the cell-specific signalsomes that are necessary to regulate their particular functions. For example, skeletal muscle (**Module 7: Figure skeletal muscle E-C coupling**) selects out one of the Ca^{2+} signalling modules (i.e. module 4 in **Module 2: Figure Ca^{2+} modules**) to control contraction, the cyclic AMP signalling pathway (**Module 2: Figure cyclic AMP signalling**) to control glycogen breakdown and the PtdIns 3-kinase signalling pathway (**Module 2: Figure PtdIns 3-kinase signalling**) to control glycogen synthesis.

An important feature of signalsomes is that they are constantly being remodelled. Phenotypic and genotypic remodelling of the signalsome can alter the nature of the output signal (Module 1: Figure remodelling the signalsome). Such remodelling of cell signalling systems can have both beneficial and pathological consequences. An important role for such remodelling is to maintain signalsome stability and also to adjust the properties of the signalsome to cope with changing demands on the cell. There is increasing evidence that signalling systems can regulate the transcription of their own signalling components. There are examples of such phenotypic remodelling being either beneficial or pathological.

The following modules deal with different aspects of these cell-specific signalsomes:

- **Module 7: Cellular Processes** describes how different signalsomes function to control specific **mammalian cell types** (**Module 7: Table cell inventory**). The cell-specific signalsomes function to process information into a form that activates different effectors to give changes in specific cellular responses such as cell growth, contraction, secretion and metabolism.

Module 1: | Figure remodelling the signalsome



Phenotypic and genotypic remodelling of the signalsome.
The normal signalsome shown in yellow generates a characteristic output signal. This normal signalsome can undergo phenotypic remodelling through processes such as protein phosphorylation or through alterations in the expression levels of individual signalling components to produce signalsomes that generate either reduced or enhanced signals. Similar changes can also occur through genotypic remodelling where mutations in signalling components alter the nature of the output signals of the signalsome.

- Module 8: Development describes the role of signalling pathways in controlling development during which different cell types emerge. An important aspect of cell differentiation is the selection and expression of those signalling components that are required to control adult cell functions.
- Module 9: Cell Cycle and Proliferation describes the control mechanisms that operate during the cell cycle to control cell division.
- Module 10: Neuronal Signalling describes the function of different neuronal cells, which all have very different signalsomes to carry out their particular functions within the neural circuits in the brain.
- Module 11: Cell Stress, Inflammatory Responses and Cell Death describes how each cell signalsome contains systems that can cope with a variety of cell stresses that result in responses such as inflammatory responses, autophagy, senescence and apoptosis.
- Module 12: Signalling Defects and Disease describes how alterations in the signalsome result in various disease states. A remarkable feature of cell signalling systems is their plasticity. Signalsomes are not fixed in stone, but are highly plastic, and the properties of their output signals can be adjusted through both phenotypic and genotypic remodelling of the signalsome (Module 1: Figure remodelling the signalsome). It will be argued that the operation of the signalsome is under constant review and contributes to signalsome stability. However, cells are able to adapt to changing demands by remodelling cellular signalsomes. Expression of the signalling components that make up the cell-specific signalsomes are under constant review. The remodelling process is usually carefully orchestrated, but there are many examples of signalsome remodelling defects that result in a whole range of different diseases.

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