Cellular Signalling

Cells must be ready to respond to essential signals in their environment. These are often chemicals in the extracellular fluid (ECF) from distant locations in a multicellular organism (**endocrine** signalling by **hormones**); nearby cells (**paracrine** stimulation by **cytokines**); or even secreted by themselves (**autocrine** stimulation).

They may also respond to molecules on the surface of adjacent cells (e.g. producing contact inhibition). Signalling molecules may trigger an immediate change in the metabolism of the cell (e.g., increased **glycogenolysis** when a liver cell detects **adrenaline**); and an immediate change in the electrical charge across the plasma membrane (e.g., the source of **action potentials**); as well as a change in the gene expression (transcription) within the nucleus.

This chapter examines some of the major pathways by which the arrival of a chemical signal at the cells turns on a new pattern of gene expression. Two categories of signalling molecules (**steroids** and **nitric oxide**) diffuse into the cell where they bind **internal** receptors. The others, e.g., proteins, bind to receptors displayed at the surface of the cell. These are transmembrane proteins whose extracellular portion has the binding site for the signalling molecule (the **ligand**) and intracellular portion activates proteins in the cytosol that in different ways eventually regulate gene transcription in the nucleus.

Steroid Receptors

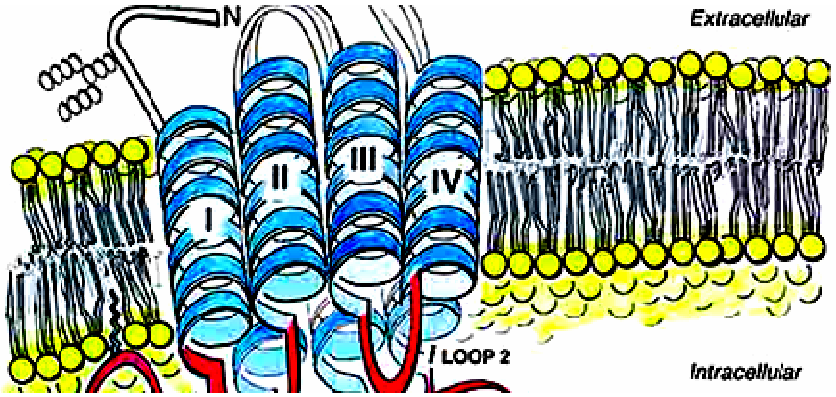
Steroids are small hydrophobic molecules that can freely diffuse across the plasma membrane, through the cytosol, and into the nucleus. Steroid receptors are dimers of **zinc-finger** proteins that reside within the nucleus (except for the **glucocorticoid receptor** which resides in the cytosol until it binds its **ligand**). Until their ligand finds them, some steroid receptors within the nucleus associate with **histone deacetylases** (HDACs), keeping gene expression repressed in those regions of the chromosome. Ligands are some steroids that regulate gene expression**.** Steroids include **glucocorticoids,** (e.g., cortisol) **mineralocorticoids,** (e.g., aldosterone) **sex hormones** such as estradiol, progesterone, testosterone and **ecdysone .** The steroid binds its receptor and the complex releases the HDACs and recruits **histone acetylases** (HATs) relieving chromosome repression; It then binds to a specific DNA sequence, the **Steroid Response Element** (**SRE**) — in the **promoters** of genes it will turn on.

Nitric Oxide (NO) Receptors

NO diffuses freely across cell membranes. There are so many other molecules with which it can interact, that it is quickly consumed close to where it is synthesized. Thus NO acts in a **paracrine** or even **autocrine** fashion — affecting only cells near its point of synthesis. The signalling functions of NO begin with its binding to protein receptors in the cell. The binding sites can be either a metal ion in the protein or one of its S atoms (e.g., on cysteine). In either case, binding triggers an **allosteric change** in the protein which, in turn, triggers the formation of a "**second messenger**" within the cell. The most common protein target for NO seems to be **guanylyl cyclase**, the enzyme that generates the second messenger **cyclic GMP** (**cGMP**).

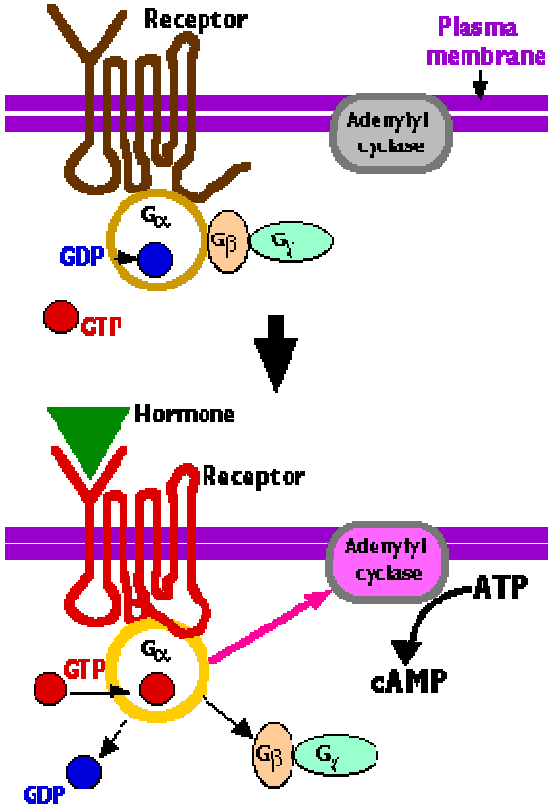
G-Protein-Coupled Receptors (GPCRs)

These are transmembrane proteins that wind 7 times back and forth through the plasma membrane. Their ligand-binding site is exposed outside the surface of the cell. Their effector site extends into the cytosol.



G-Protein-Coupled Receptors

Some of the many ligands that alter gene expression by binding GPCRs are protein and peptide **hormones** such as **thyroid-stimulating hormone** (TSH), **ACTH**, **Serotonin** and GABA (which affect gene expression in addition to their role as **neurotransmitters**). The ligand binds to a site on the extracellular portion of the receptor. The binding of the ligand to the receptor activates a **G protein** associated with the cytoplasmic C-terminal. This initiates the production of a **"second messenger"**. The most common of these are **cyclic AMP**, (**cAMP**) which is produced by **adenylyl cyclase** from **ATP** and **inositol 1,4,5-trisphosphate** (**IP_3**). The second messenger, in turn, initiates a series of intracellular events such as phosphorylation and activation of enzymes, release of Ca^{2+} into the cytosol from stores within the endoplasmic reticulum. In the case of cAMP, these enzymatic changes activate the **transcription factor CREB** (**c**AMP **r**esponse **e**lement **b**inding protein) and bound to its **response element** 5' TGACGTCA 3' in the promoters of genes that are able to respond to the ligand, activated CREB turns on gene **transcription**. The cell begins to produce the appropriate gene products in response to the signal it had received at its surface. In addition to their roles in affecting gene expression, GPCRs regulate many **immediate** effects within the cell that do not involve gene expression.



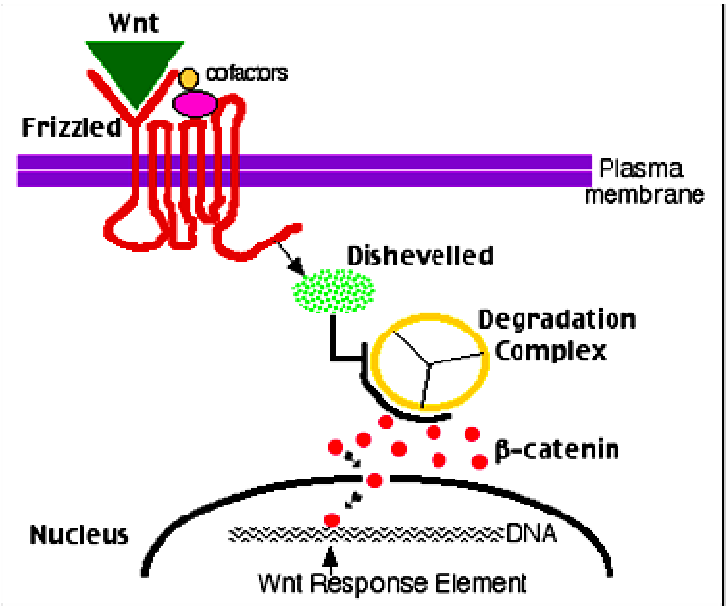
G-Protein-Coupled Receptors

Frizzled Receptors and Wnt Signalling

Frizzled receptors, like GPCRs, are transmembrane proteins that wind 7 times back and forth through the plasma membrane. Their ligand-binding site is exposed outside the surface of the cell and their effector site extends into the cytosol.Their ligands are **Wnt proteins.** These get their name from two of the first to be discovered, proteins encoded by *wingless* (*wg*) in Drosophila and its homolog and *Int-1* in mice.

The roles of β-catenin

β-catenin molecules connect **actin filaments** to the **cadherins** that make up **adherens junctions** that bind cells together. Any excess β-catenin is quickly destroyed by a multiprotein **degradation complex**. (One component is the protein encoded by the APC **proto-oncogene**.) The degradation complex phosphorylates β-catenin so it can have **ubiquitin** molecules attached to prepare it for destruction in **proteasomes**. But undegraded β-catenin takes on a second function: it becomes a potent **transcription factor.** The binding of a **Wnt** ligand to **Frizzled** (done with the aid of cofactors) activates Frizzled. This, in turn, activates a cytosolic protein called **Dishevelled**. Activated Dishevelled **inhibits** the β-catenin **degradation complex** so β-catenin escapes destruction by proteasomes and is free to enter the nucleus where it binds to the promoters and/or enhancers of its target genes. Wnt-controlled gene expression plays many roles in **embryonic development** as well as regulating activities in the adult body.



Frizzled Receptors and Wnt Signalling

Cytokine Receptors

Dozens of cytokine receptors have been discovered. Most of these fall into one or the other of two major families: Receptor **Tyrosine Kinases** (RTKs) and receptors that trigger a **JAK-STAT pathway**.

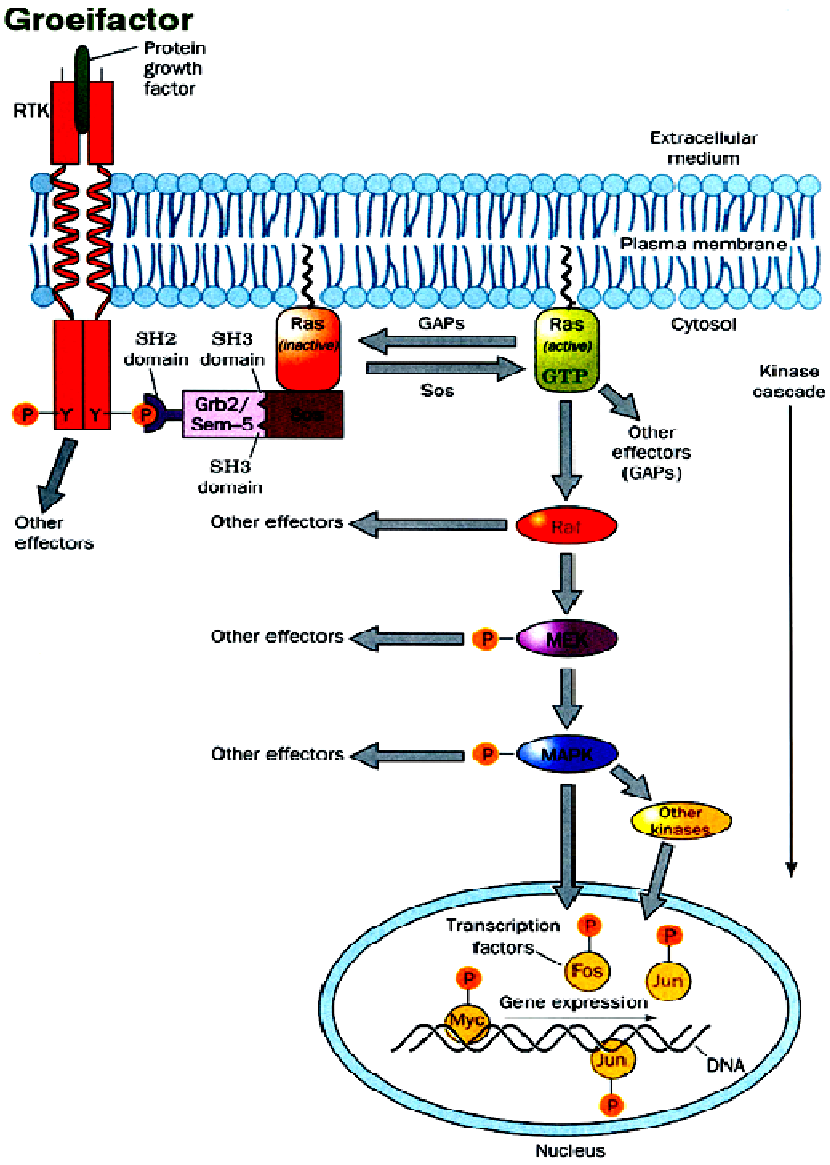
Receptor Tyrosine Kinases (RTKs)

The receptors are transmembrane proteins that span the plasma membrane just once. Some ligands that trigger RTKs are Insulin**,** Vascular Endothelial Growth Factor (**VEGF**), Platelet-Derived Growth Factor (**PDGF**), Epidermal Growth Factor (**EGF**), Fibroblast Growth Factor (FGF). (A mutation in its receptor causes **chondroplasia**— the most common type of dwarfism.) and Macrophage Colony-Stimulating Factor (M-CSF). Binding of the ligand to two adjacent receptors forms an active dimer. This activated dimer is a **tyrosine kinase**; an enzyme that attaches phosphate groups to certain **tyrosine** (Tyr) residues — first on itself, then on other proteins converting them into an active state. Many of these other proteins are also tyrosine kinases (the **human genome** encodes 90 different tyrosine kinases) and in this way a cascade of expanding phosphorylations occurs within the cytosol.

Some of these cytosolic tyrosine kinases act directly on gene transcription by entering the nucleus and transferring their phosphate to **transcription factors** thus activating them.e.g. The cytosol of B cells contains **Btk** ("Bruton's tyrosine kinase") which is essential to turning on appropriate gene expression when a **B cell encounters antigen**. Inherited mutations in the gene encoding Btk cause **X-linked agammaglobulinemia** in boys. These boys cannot manufacture antibodies and suffer recurrent bacterial infections unless given periodic injections of immune globulin (IG). Others act indirectly through the production of **second messengers**.

Turning RTKs Off

A cell must also be able to **stop** responding to a signal. For growth factor receptors, failure to do so could lead to uncontrolled mitosis which can result in cancer. For the RTKs, this is done by quickly engulfing and destroying the ligand-receptor complex by **receptor-mediated endocytosis.**



Tyrosine Kinase (RTKs) cell signalling

Proto-Oncogenes

One might expect that anything which leads to the inappropriate expression of receptors that trigger cell division could lead to cancer (uncontrolled cell division). And, in fact, the gene encoding the **receptor** for **PDGF** is ***c-sis***; it is a **proto-oncogene**, and mutated versions participate in making the cell cancerous. The genes encoding **receptors** for **EGF** are also proto-oncogenes and are expressed at abnormally high levels in several human cancers. Two monoclonal antibodies that target these receptors trastuzumab (Herceptin) that inactivates HER2 ("Human Epidermal growth factor Receptor 2") and cetuximab (Erbitux) that inactivates HER1 show promise against breast cancer. Two tyrosine kinase inhibitors **gefitinib** (Iressa) and **erlotinib** (Tarceva) block the action of the EGF receptors on the cells of certain lung cancers and have shown some promise against these cancers. Mutant versions of some of the "second-order" kinases are also associated with cancer:

The oncogene ***v-src*** encodes a mutated version of a normal tyrosine kinase associated with the inner face of the plasma membrane. The fusion protein BCR/ABL produced by the **Philadelphia chromosome** activates constitutively (all the time) the cytosolic tyrosine kinase ABL that normally would be activated only when the cell is stimulated by a growth factor (e.g., PDGF). The result: **chronic myelogenous leukemia** (CML). A promising treatment: **Imatinib mesylate** (Gleevec also known STI571). This molecule fits into the active site of the ABL protein preventing ATP from binding there. Without ATP as a phosphate donor, the ABL protein cannot phosphorylate its substrate(s).

RAS and its target ***RAF***. These kinases participate in a signalling path that links RTKs to gene activation. In mammals, this path promotes mitosis. Mutations in ***RAS*** and/or ***RAF*** are associated with many types of cancer so *RAS* and *RAF* are **proto-oncogenes**.

JAK-STAT Pathways

These are also single-pass **transmembrane proteins** embedded in the plasma membrane. Many ligands trigger JAK-STAT pathways such as **Interferons**. Most of the **interleukins**, (e.g., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-11, IL-12, and IL-13), Growth hormone (**GH**), Prolactin (**PRL**), Erythropoietin (**EPO**), Thrombopoietin and Granulocyte-Macrophage Colony-Stimulating Factor (**GM-CSF**)

Binding of the ligand causes two receptors to form a **dimer**. The dimer activates a **Janus kinase** (**"JAK"**) which phosphorylates certain **tyrosine** (Tyr) residues on one or another of several **STAT** ("**S**ignal **T**ransducer and **A**ctivator of **T**ranscription) proteins. These, in turn, form dimers which enter the nucleus and bind to specific DNA sequences in the promoters of genes that begin transcription. The JAK-STAT pathways are much shorter and simpler than the pathways triggered by RTKs and so the response of cells to these ligands tends to be much more rapid.

Transforming Growth Factor-beta (TGF-β) Receptors

Two types of single-pass transmembrane proteins that, when they bind their ligand, become **kinases** that attach phosphate groups to **serine** and/or **threonine** residues of their target proteins. Ligands for these receptors include transforming Growth Factor-beta (hence the name), activins, Bone Morphogenic Proteins (**BMPs**) and myostatin, an inhibitor of skeletal muscle growth.

The ligand binds to the extracellular portion of the receptors, which then phosphorylate one or more **SMAD** proteins in the cytosol. The SMAD proteins move into the nucleus where they form

dimers with another SMAD protein designated **SMAD4**. These dimers bind to a DNA sequence (CAGAC) in the **promoters** of target genes and — with the aid of other transcription factors — enhance, or repress, as the case may be, gene transcription.

Tumour-Suppressor Genes

The TGF-β signalling pathway suppresses the **cell cycle** in several ways. So it is not surprising that defects in the pathway are associated with cancer. Mutations in the genes encoding the TGF-β receptors as well as of the SMAD proteins are found in many cancers including pancreatic and **colon cancer**. Thus all these genes qualify as **tumour-suppressor genes.**

Tumour Necrosis Factor-alpha (TNF-α) Receptors and the NF-κB Pathway

TNF-α is made by **macrophages** and other cells of the immune system. They are trimers of 3 identical cell-surface transmembrane proteins with the ligands being TNF-α (hence the name) and Lymphotoxin (LT; also known as TNF-β). NF-κB resides in the cytosol bound to an **inhibitor** called IκB. Binding of ligand to the receptor triggers phosphorylation of IκB then becomes **ubiquinated** and destroyed by **proteasomes**. This liberates NF-κB so that it is now free to move into the nucleus where it acts as a transcription factor binding to the promoters and/or enhancers of more than 60 genes: NF-κB got its name from its discovery as a transcription factor bound to the enhancer of the **kappa light chain** antibody gene. However, it also turns on the genes encoding **IL-1** and other **cytokines** that promote **inflammation**. NF-κB also turns on genes needed for cell proliferation, **cell adhesion,** and **angiogenesis**.

The T-Cell Receptor for Antigen (TCR)

T cells use a transmembrane dimeric protein as a receptor for a particular combination of antigen fragment nestled in the cleft of a glycoprotein encoded by genes in the major histocompatibility complex. Activation of the TCR (when aided by **co-stimulator** molecules also present in the plasma membrane) causes a rise in intracellular Ca^{2+} which activates **calcineurin**, a p**hosphatase** which removes phosphate from **NF-AT** ("**N**uclear **F**actor of **A**ctivated **T** cells"). Dephosphorylated NF-AT enters the nucleus, and with the help of accessory transcription factors (e.g., AP-1), binds to the promoters of some 100 genes expressed in activated T cells.

The immunosuppressant drugs **FK506** and **cyclosporine** inhibit calcineurin thus reducing the threat of transplant rejection by T cells.

The signalling systems discussed in this chapter are only some of the major players, and even the description of these is greatly oversimplified because many of these signalling pathways interconnect with one another (creating "cross talk"). While cross talk might seem to create problems (for our comprehension as well as for the cell!), in fact it is probably essential to precisely modulate the genetic response to a variety of ligands reaching the cell at the same time and in varying intensities.