

# Chapter 12

## Cell 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:

1. An immediate change in the metabolism of the cell (e.g., increased **glycogenolysis** when a liver cell detects **adrenaline**);
2. An immediate change in the electrical charge across the plasma membrane (e.g., the source of **action potentials**);
3. A change in the gene expression — **transcription** — within the nucleus. (These responses take more time.)

It is the third category that is the topic of this chapter. So Chapter 10 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.

### 12.1. Steroid Receptors

Steroids are small **hydrophobic molecules** that can freely diffuse across the plasma membrane, through the cytosol, and into the nucleus.

#### (a) Receptors

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.

#### (b) Ligands

Some steroids that regulate gene expression are **glucocorticoids** (e.g., cortisol), **mineralocorticoids** (e.g., aldosterone), **sex hormones** such as **estradiol**, **progesterone**, **testosterone** and **ecdysone**.

#### (c) Mechanism

The steroid binds its receptor. The complex releases the HDACs and recruits **histone acetylases** (HATs) relieving chromosome repression and binds to a specific DNA sequence — the **Steroid Response Element (SRE)** — in the **promoters** of genes it will turn on.

## 12.2. 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.

### (a) Receptors

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**).

### (b) Mechanisms

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)**.

## 12.3. G-Protein-Coupled Receptors (GPCRs)

### (a) Receptors

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.

### (b) Ligands

Some of the many ligands that alter gene expression by binding GPCRs 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**).

### (c) Mechanisms

The ligand binds to a site on the extracellular portion of the receptor. 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** (shown here), and **inositol 1,4,5-trisphosphate (IP<sub>3</sub>)**. The second messenger, in turn, initiates a series of intracellular events (shown here as short arrows) such as phosphorylation and activation of enzymes and release of **Ca<sup>2+</sup>** into the cytosol from stores within the endoplasmic reticulum. In the case of cAMP, these enzymatic changes activate the **transcription factor CREB (cAMP response element binding protein)** 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.

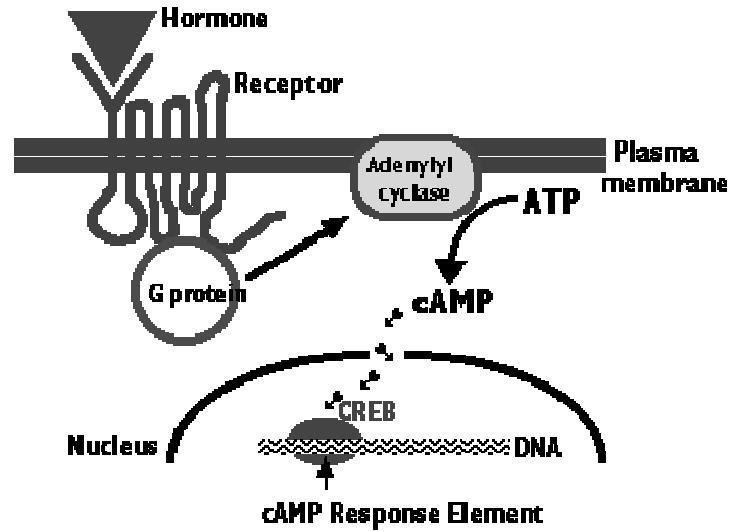


Fig.12.1. Mechanism of action of G-Proteins

## 12.4. Frizzled Receptors and Wnt Signalling

### (a) Receptors

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. Their effector site extends into the cytosol.

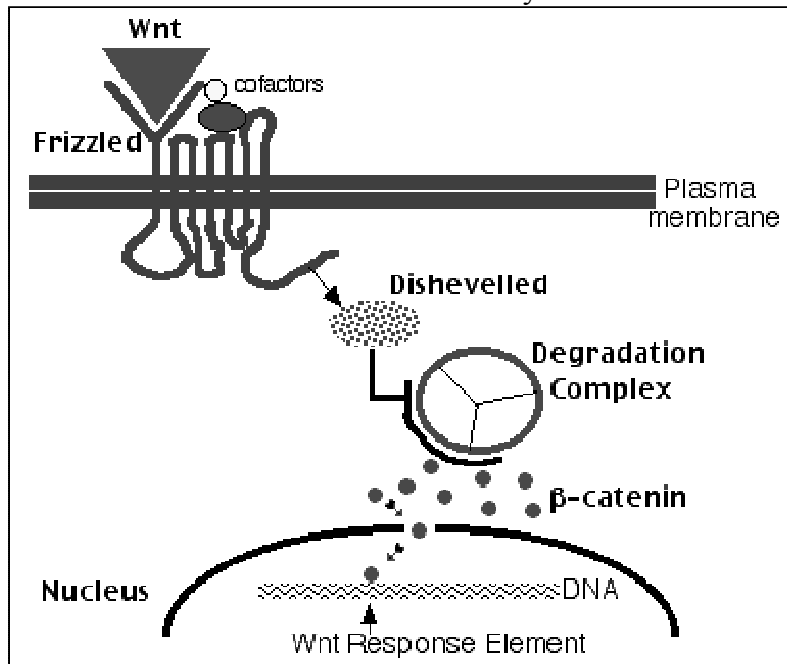


Fig.12.2. Frizzled Receptors and Wnt Signalling

### (b) Ligands

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 *Int-1* in mice.

### (c) The Roles of $\beta$ -catenin

$\beta$ -catenin molecules connect **actin filaments** to the **cadherins** that make up **adherens junctions** that bind cells together. Any excess  $\beta$ -catenin is quickly destroyed by a multiprotein **degradation complex**. (One component is the protein encoded by the **APC proto-oncogene**.) The degradation complex phosphorylates  $\beta$ -catenin so it can have ubiquitin molecules attached to prepare it for destruction in **proteasomes**. But undegraded  $\beta$ -catenin takes on a second function: it becomes a potent **transcription factor**.

### (d) Mechanism

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  $\beta$ -catenin **degradation complex** so  $\beta$ -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.

## 12.5. 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.

### 15.5.1 Receptor Tyrosine Kinases (RTKs)

#### (a) Receptors

The receptors are transmembrane proteins that span the plasma membrane just once.

#### (b) Ligands

Some ligands that trigger RTKs: 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 **achondroplasia** — the most common type of dwarfism.) and Macrophage Colony-Stimulating Factor (M-CSF)

#### (c) Mechanisms

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.

**Example:** 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**.

### (d) 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 = cancer. For the RTKs, this is done by quickly engulfing and destroying the ligand-receptor complex by **receptor-mediated endocytosis**.

### (e) 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 **PDGF** is **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 (Erbbitux®) 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 **SRC** encodes a mutated version of a normal tyrosine kinase associated with the inner face of the plasma membrane.

## 12.5.2. JAK-STAT Pathways

### (a) Receptors

These consist of 2 identical single-pass transmembrane proteins (i.e., homodimers) embedded in the plasma membrane. Each of their cytoplasmic ends binds a molecule of a **Janus kinase** ("JAK").

### (b) Ligands

Many ligands trigger JAK-STAT pathways: **Interferons**, Most of the **interleukins**, **Growth hormone (GH)**, **Prolactin (PRL)**, Erythropoietin (**EPO**), Thrombopoietin, and Granulocyte-Macrophage Colony-Stimulating Factor (**GM-CSF**)

### (c) Mechanisms

Binding of the ligand activates the JAK molecules which phosphorylate certain **tyrosine** (Tyr) residues on each other as well as on one or another of several **STAT** ("Signal Transducer and Activator of Transcription) 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.

## 12.6. Transforming Growth Factor-beta (TGF- $\beta$ ) Receptors

### (a) 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.

### (b) Ligands

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.

### (c) Mechanisms

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**.

### (d) Tumour-Suppressor Genes

The TGF- $\beta$  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- $\beta$  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**.

## 12.7. Tumour Necrosis Factor-alpha (TNF- $\alpha$ ) Receptors and the NF- $\kappa$ B Pathway

TNF- $\alpha$  is made by macrophages and other cells of the immune system.

### (a) Receptors

Trimers of 3 identical cell-surface transmembrane proteins.

### (b) Ligands

- TNF- $\alpha$  (hence the name)
- Lymphotoxin (LT; also known as TNF- $\beta$ )

### (c) Mechanisms

NF- $\kappa$ B resides in the cytosol bound to an **inhibitor** called I $\kappa$ B. Binding of ligand to the receptor triggers phosphorylation of I $\kappa$ B. I $\kappa$ B then becomes **ubiquinated** and destroyed by **proteasomes**. This liberates NF- $\kappa$ B 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- $\kappa$ B 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**. The immunosuppressive and anti-inflammatory effects of **glucocorticoids** are caused by their enhancing the production of I $\kappa$ B. NF- $\kappa$ B also turns on genes needed for cell proliferation, **cell adhesion**, and **angiogenesis**.

## 12.8. 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 costimulator molecules also present in the plasma membrane) causes a rise in intracellular Ca<sup>2+</sup> which activates **calcineurin**, a phosphatase which removes phosphate from **NF-AT** ("Nuclear Factor of Activated 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.