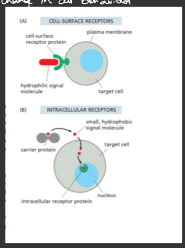
Cell Signalling (Day 14)

- In bacteria, communication b/w cell: quorum sensing | In multicellular organisms: extracellular signalling molecules

Receptors → bind signal molecule and activates intracellular pathway(s)
(may/may not be at cell surface)

signal far away → relay chains of molecules → process signal & distribute it inside cell → alter effector proteins & implement appropriate change in cell behaviour
(transcription factors, ion channels, parts of cytoskeleton)

only to immediate neighbour



Extracellular signals can act in 4 ways:

① Contact dependent	② Paracrine	③ Synaptic	④ Endocrine
• remain bound to surface of signalling cell • influence only cells in contact • important in developmental processes	• signalling molecules are local mediators — influence only cells in local environment of different types in same (multicellular)	• long range using neurotransmitters released by neurons on receiving cell/organ	• long distance using endocrine cells • secreted hormones into bloodstream

Receptor-binding — signals can be peptides, nucleotides, retinoids — regardless, bind to receptor to initiate response in target cell

(usually transmembrane receptor on target cell surface)

binding site shaped to recognise molecule with high specificity ⇒ act at very low concs of 10^{-8} M with high affinity

Cells respond to combination of signals

→ exposed to diverse cocktail of signals but responds selectively by expressing only specific receptors & internal signals that respond to signals required to regulate cell

→ can respond differently to different combinations — maybe differentiating in response to one & contracting with another

→ many cells require signal combination to keep living, otherwise apoptosis when deprived

Same signal – different effects in diff cells

heart muscle cells ← Acetylcholine → salivary gland cells; skeletal muscle cells

same receptor — different response

Extracellular signal has little information content — depends on differences in intracellular signalling proteins or effector proteins/genes activated

cells developmental history & genes it expresses

Fate of developing cells – Morphogen gradient

Depending on conc of signal — qualitatively different effects on the same cell type

↓

such a signal (morphogen) – diffuses out from localised cellular source (signalling centre) – generates conc. gradient

↓

cells with the highest conc. have most receptors activated (one dev pathway), those slightly further away encounter a lower conc & may follow another pathway

What happens when the signal ceases?

A signal exerts a transient effect by altering the conc of short-lived intracellular molecules (unstable) that undergo continual turnover

↓

once signal is gone — replacement of the molecules by new ones wipes out its effects – hence the rate at which cell responds to signal depends on rate of destruction, or turnover of the intracellular molecules that the signal affects

Intracellular Receptors

Nitric oxide and steroid hormones – intracellular receptors involved

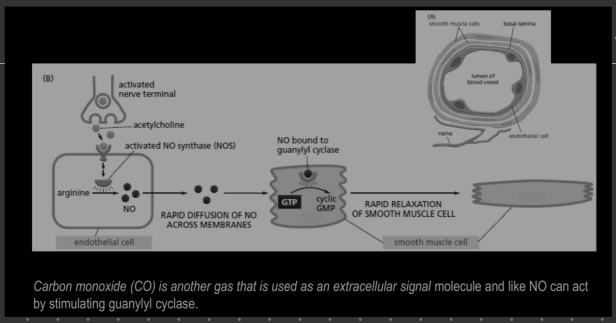
Acetylcholine in nerve cells in the blood vessel activate NO synthase in endothelial cells lining blood vessel

↓

endothelial cells form NO from arginine

↓

activates guanylyl cyclase to form cyclic GMP → causes muscle cells to relax



Carbon monoxide (CO) is another gas that is used as an extracellular signal molecule and like NO can act by stimulating guanylyl cyclase.

NOS → eNOS → endothelial cells

→ nNOS → nerve & muscle cells (produced due to influx of Ca^{2+})

→ iNOS (inducible) → activated macrophages in response to infection

CO → also acts like NO and activates guanyl cyclase.

NO relaxes smooth muscle cells

↳ nitroglycerine used to treat angina → converted to NO → reduces blood vessels, reduces O_2 requirement of heart

NO also helps kill invading microorganisms & in plants, helps in defensive responses to injury on infection

NO reversibly binds to iron in active site of enzyme guanylyl cyclase → cGMP → released into cytosol within seconds because turnover of cGMP high) rapid degradation to GMP (phosphodiesterase)

balanced production of cGMP

NO can also signal cells independent of cGMP, e.g. alter activity of an intracellular protein by covalently nitrosylating thiol (-SH) groups on cysteines in protein

Nuclear Receptors

Intracellular signals → hydrophobic small molecules → diffuse directly across plasma membrane of target cells and bind to intracellular receptors (transcription regulators)

(steroid sex hormones, thyroid hormones, retinoids & Vit. D → bind to respective intracellular receptor proteins and alter the ability of these proteins to control transcription of specific genes → intracellular receptors and effectors

part of nuclear receptor superfamily

↳ many identified by DNA sequencing only & ligand not known (orphan NR)

Related structure → domain swapping experiments suggest interchangeable modules – all intracellular models have similar str.

What happens when a receptor is activated?

Binding of a ligand to an intracellular ligand causes the ligand to clamp shut around it → inhibitory proteins dissociate → co-activator proteins bind to the transcription activating domain → thereby increasing gene transcription

Transcription Activation by Nuclear Receptors

- Nuclear receptors bind to DNA seq. adjacent to the gene the ligand regulates (maybe in cytosol or nucleus – bound to inhibitory protein complexes
- ligand binding – alters conformation of protein → inhibitory complex dissociates → receptor binds to coactivator proteins that stimulate gene expression

Sometimes, inhibits transcription.

Primary and Secondary response to steroid hormones.
↳ turns off primary response
or turns on 2° response

Cells can adjust their sensitivity to a signal

In response to many types of stimuli, cells are able to detect the same percentage of change in signal over a very wide range of stimulus strengths → responds to changes in the input signal rather than absolute amount of the signal.

Adaptation depends on negative feedback that operates with a short delay – strong response modifies the signalling machinery such that the machinery responds to the same level of signal with a lower intensity

How does desensitization happen?

↳ inactivation of receptors themselves — temporary sequestration by endocytosis of receptors into endosomes → sometimes, destroyed in lysosome (receptor down-regulation)

↳ inactivation by phosphorylation or methylation with a short delay following activation

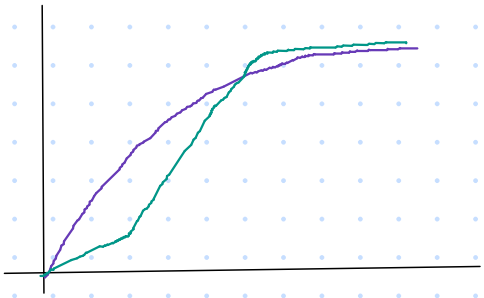
↳ downstream regulation → ↓ intracellular signalling molecule
↳ inhibitory proteins that prevent signal transduction

Responding abruptly to a gradually increasing conc of extracellular signal

Response to signal

① smoothly graded according to conc of signal

② independent of conc. (all or none)



Difficult to distinguish between two: measuring the effect of a signal on population of cells makes effect appear smoothly graded, even though individual response is all or none, due to variation among cells in the signal conc. at which switch occurs

↓

smoothly graded responses might steeply depend on signal strength giving appearance of switchlike behaviour

How is the gradation from smooth to all-or-none response achieved?

A variety of molecular mechanisms, mainly more than one signalling molecule required to be bound to its downstream target protein to induce a response

↳ more the no. of molecules required, sharpening of response to become almost all-or-none.

Responses are also sharpened when an intracellular signalling molecule activates one enzyme and at the same time, inhibits another molecule that catalyzes the opposite reaction

Classes of cell surface receptor proteins

Ion-channel coupled receptors = ∘ also called transmitter gated ion channel or ionotropic receptors

- ∘ involved in rapid synaptic signalling b/w nerve cells & their target nerve & muscle cells
- ∘ mediated by a small no of neuro transmitters that transiently open or close an ion channel formed by the protein to which they bind, briefly changing the ion-permeability and excitability of the target cell membrane

G protein coupled receptors – ① act by indirectly regulating the activity of a separate plasma membrane bound target protein (enzyme or ion channel)
② Trimeric GTP binding protein (G protein) mediates interaction between the activated receptor & the target protein
③ activation of target protein can change the conc. of one or more intracellular mediators or change ion permeability of plasma membrane.

Enzyme-coupled receptors – ① function directly as enzymes or associate with enzymes they can activate
② single-pass transmemb. proteins with ligan-binding site outside cell and catalytic site inside
③ heterogeneous in structure
④ mostly are, or associate with protein kinases

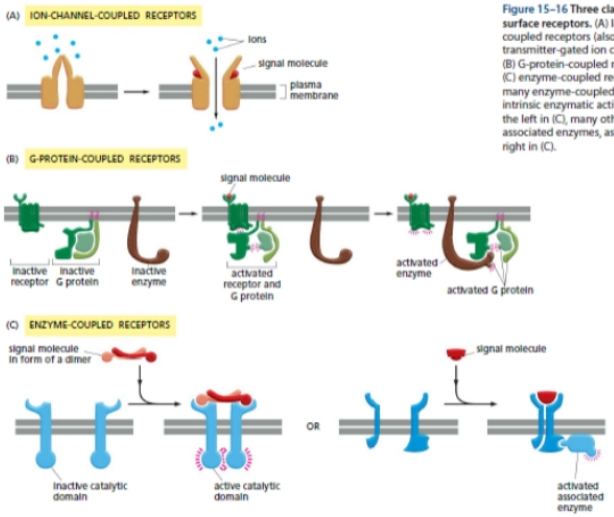


Figure 15–16 Three classes of cell-surface receptors. (A) Ion-channel-coupled receptors (also called transmitter-gated ion channels), (B) G-protein-coupled receptors, and (C) enzyme-coupled receptors. Although many enzyme-coupled receptors have intrinsic enzymatic activity, as shown on the left in (C), many others rely on associated enzymes, as shown on the right in (C).

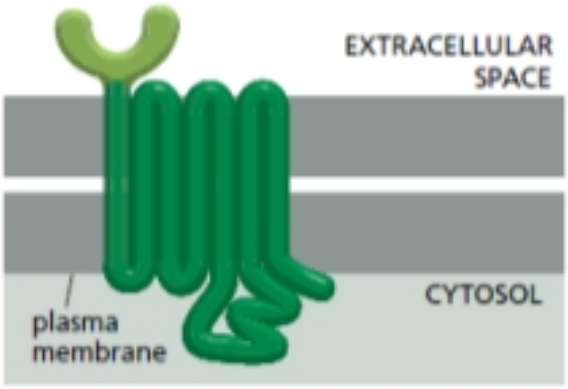


Figure 15–30 A G-protein-coupled receptor (GPCR). GPCRs that bind protein

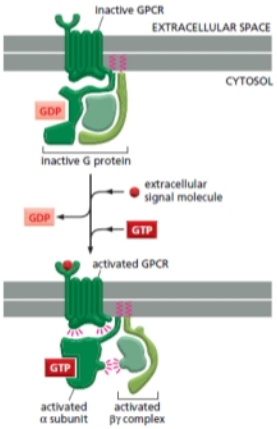
Signalling through G protein coupled receptors

- found in all eukaryotes
- mediate most responses to extracellular signals
- senses of smell, taste (except sour) and sight depend on them
- more than 700 GPCRs in humans
- signal molecules that act are varied in structure
- same signal molecule can activate different GPCR family members
- all GPCRs have similar structure
- single polypeptide chain that threads back and forth across lipid bilayer 7 times

- all use G proteins to relay signals into cell interior
- eg; rhodopsin & olfactory receptors

signaling (RGS).

(A)



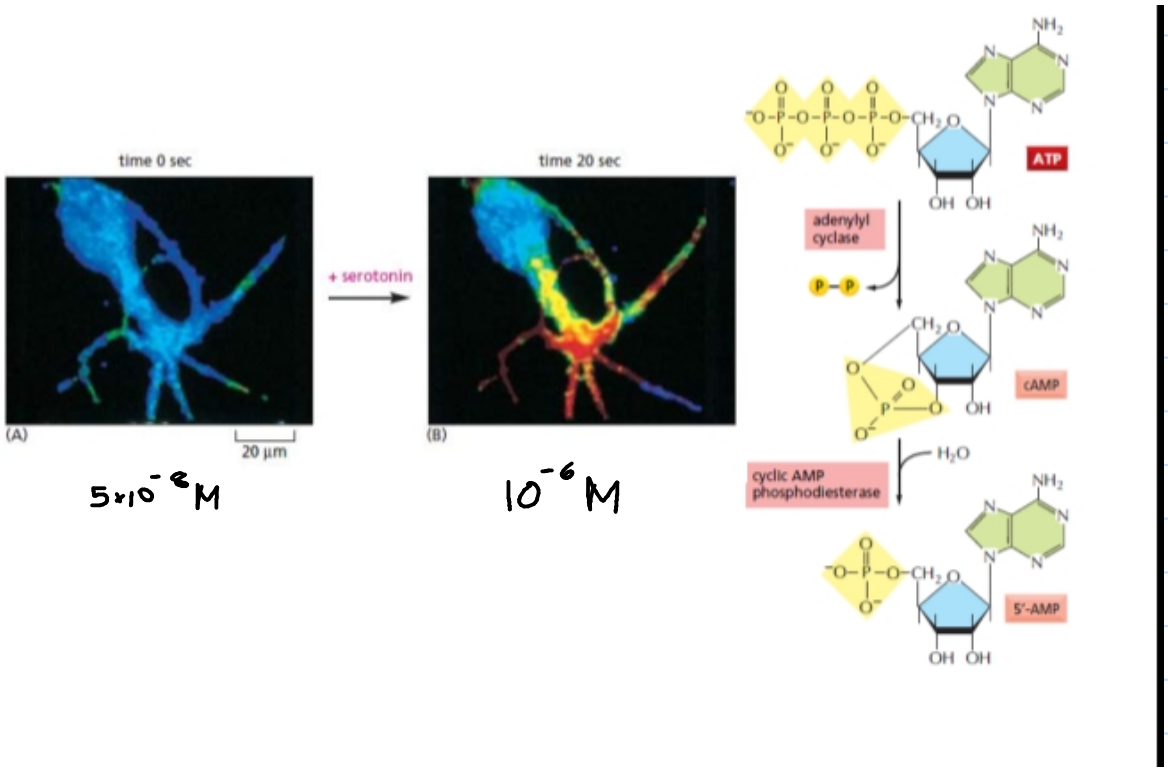
Role of G proteins in signal relay

- extracellular signalling molecule binds to GPCR → conformational change allows it to activate G protein
- G protein is present on cytoplasmic face of membrane, where it functionally couples GPCR to enzymes or ion channels in the membrane
- sometimes, physically associated with receptor before activation, sometimes after
- 3 protein subunits — (α, β, γ)
- in the unstimulated state, α subunit has GDP bound & GPCR inactive
  - ↳ activated GPCR → acts like a Guanine Nuc. EF & removes bound GDP from α-subunit → allows GTP to bind in its place → activated G protein
- α subunit → GTPase → hydrolyses bound GTP to become inactive → time for which G protein remains active depends on this
  - usually short because GTPase activity is greatly enhanced by binding of the subunit to sp. regulators of G protein (RGS)

Regulation of cAMP by G proteins

- cAMP acts as a small intracellular mediator in all prokaryotic and animal cells
- normal conc. in the cytosol is 10^{-3} M, but on extracellular signal → ↑ 20x
- rapid synthesis & rapid breakdown need to balanced for rapid response
- cAMP is synthesised from ATP by a plasma-memb. bound enzyme adenylyl cyclase, and it is rapidly & continuously destroyed by cyclic AMP phosphodiesterase that can hydrolyse cyclic AMP to 5'AMP
- many extracellular signal molecules work by increasing cAMP conc. & they do so by increasing the activity of adenylyl cyclase against a steady background of phosphodiesterase activity.

- GPCRs that act by increasing cAMP are coupled to a stimulatory G protein (G_s) → activates adenylyl cyclase → ↑ cAMP
- another G protein (inhibitory) inhibits adenylyl cyclase, but acts mainly by directly regulating ion channels



- e.g. serotonin acts through a GPCR to cause rapid rise in cAMP levels

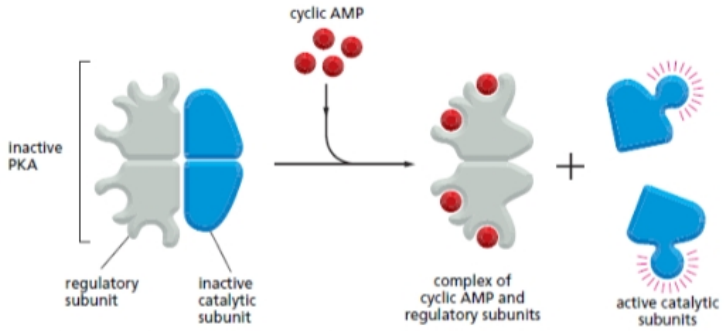
Toxins

Cholera toxin
1. catalyses transfer of ADP ribose sugar from NAD^+ to α-subunit of G_s
2. alters the α-subunit so that it can no longer hydrolyse bound GTP
3. adenylyl cyclase stimulated indefinitely
4. ↑ cAMP → large efflux of Cl^- & H_2O into gut → diarrhoea

Pertussis toxin
1. catalyses ADP ribosylation of α subunit of G_i → prevents protein from interacting with receptors
2. G protein retains bound GDP and is unable to react with with target proteins

Cyclic AMP-dependent protein Kinase (PKA) mediates most of the effects of cAMP

- in most animal cells, cAMP exerts its effects by activating cAMP- dependent PKA
- Kinase phosphorylates specific serines & threonines on selected targeted proteins include including intracellular signalling proteins & effector proteins
- in the inactive state → two catalytic subunits & two regulatory subunits
- binding of regulatory subunits alters their conformation, causing them to dissociate from the complex
- released catalytic subunits are activated to phosphorylate specific target proteins



The activation of cyclic- AMP-dependent protein kinase (PKA).
The binding of cyclic AMP to the regulatory subunits of the PKA tetramer induces a conformational change, causing these subunits to dissociate from the catalytic subunits, thereby activating the kinase activity of the catalytic subunits. The release of the catalytic subunits requires the binding of more than two cyclic AMP molecules to the regulatory subunits in the tetramer. This requirement greatly sharpens the response of the kinase to changes in cyclic AMP concentration. Mammalian cells have at least two types of PKAs: type I is mainly in the cytosol, whereas type II is bound via its regulatory subunits and special anchoring proteins to the plasma membrane, nuclear membrane, mitochondrial outer membrane, and microtubules. In both types, once the catalytic subunits are freed and active, they can migrate into the nucleus (where they can phosphorylate gene regulatory proteins), while the regulatory subunits remain in the cytoplasm.

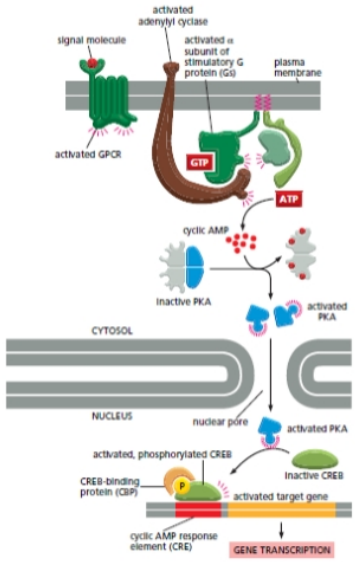


Figure 15–36 How a rise in intracellular cyclic AMP concentration can alter gene transcription. <AGAT> The binding of an extracellular signal molecule to its GPCR activates adenylyl cyclase via G_s and thereby increases cyclic AMP concentration in the cytosol. The rise in cyclic AMP concentration activates PKA, and the released catalytic subunits of PKA can then enter the nucleus, where they phosphorylate the gene regulatory protein CREB. Once phosphorylated, CREB recruits the coactivator CBP, which stimulates gene transcription. In some cases, at least, the inactive CREB protein is bound to the cyclic AMP response element (CRE) in DNA before it is phosphorylated (not shown).

This signaling pathway controls many processes in cells, ranging from hormone synthesis in endocrine cells to the production of proteins required for the induction of long-term memory in the brain. We will see later that CREB can also be activated by some other signaling pathways, independent of cAMP.