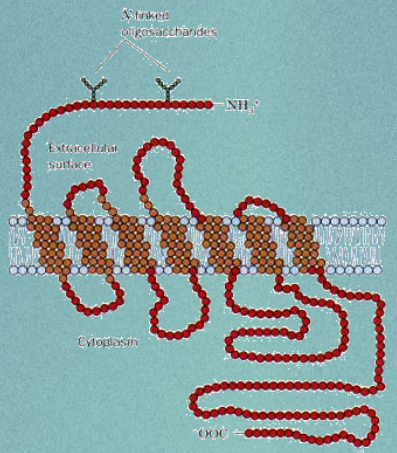
THE UNIVERSITY OF MELBOURNE
Faculty of Veterinary and Agricultural Sciences



Lecture 12
Receptors and signalling pathways 1: Signal transduction and second messengers

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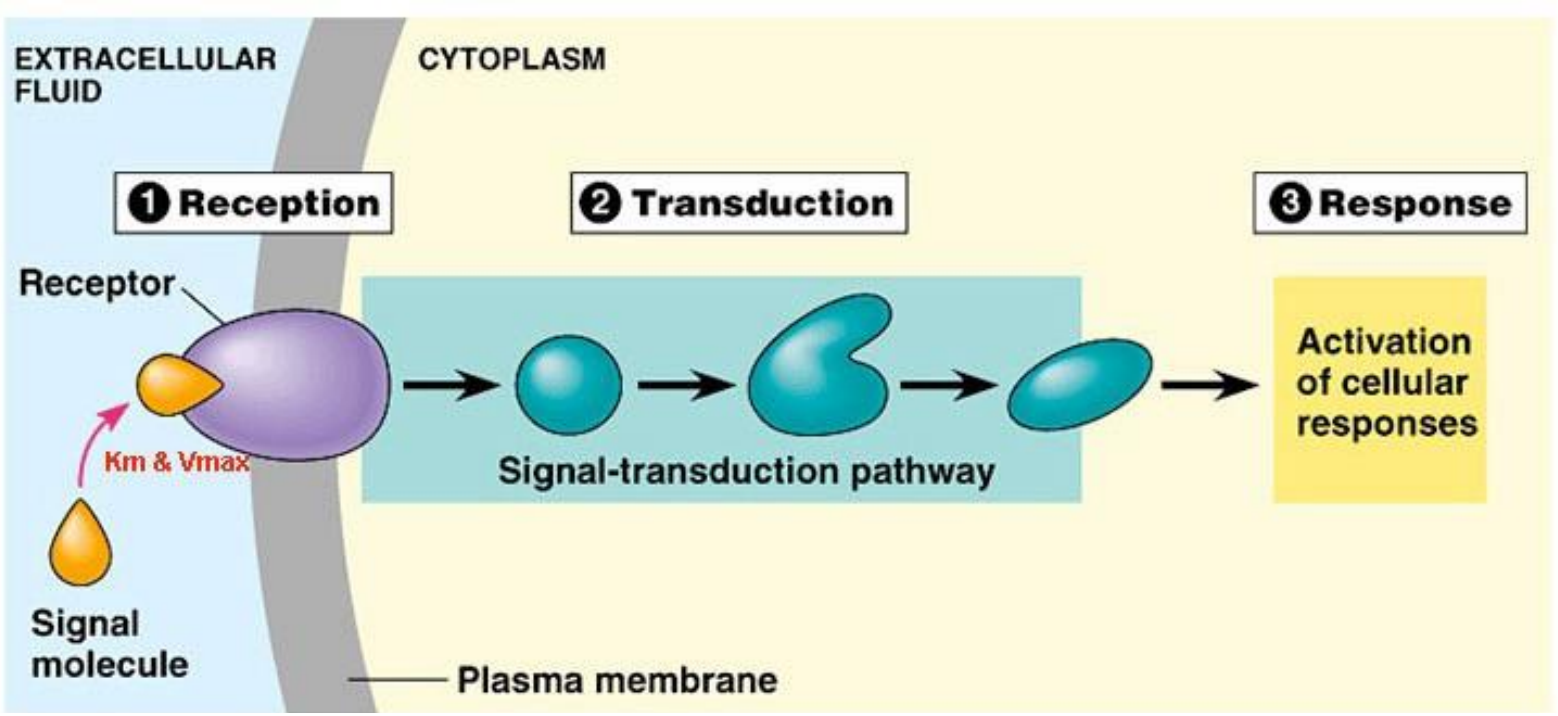
VETS30015 / VETS90121

Intended learning objectives

1. Describe the main signal transduction pathways involved in cell signalling
    1. Ligand-gated ion channels
    2. G-protein coupled receptors,
    3. Receptor enzymes (i.e., tyrosine kinase),
    4. Nuclear receptors (class I and class II)
    - Be able to give an example of each.

2. Explain through the use of examples how a G protein signal transduction pathway is regulated
3. Describe how bacterial toxins such as cholera toxin are able to interfere with heterotrimeric G protein signalling.

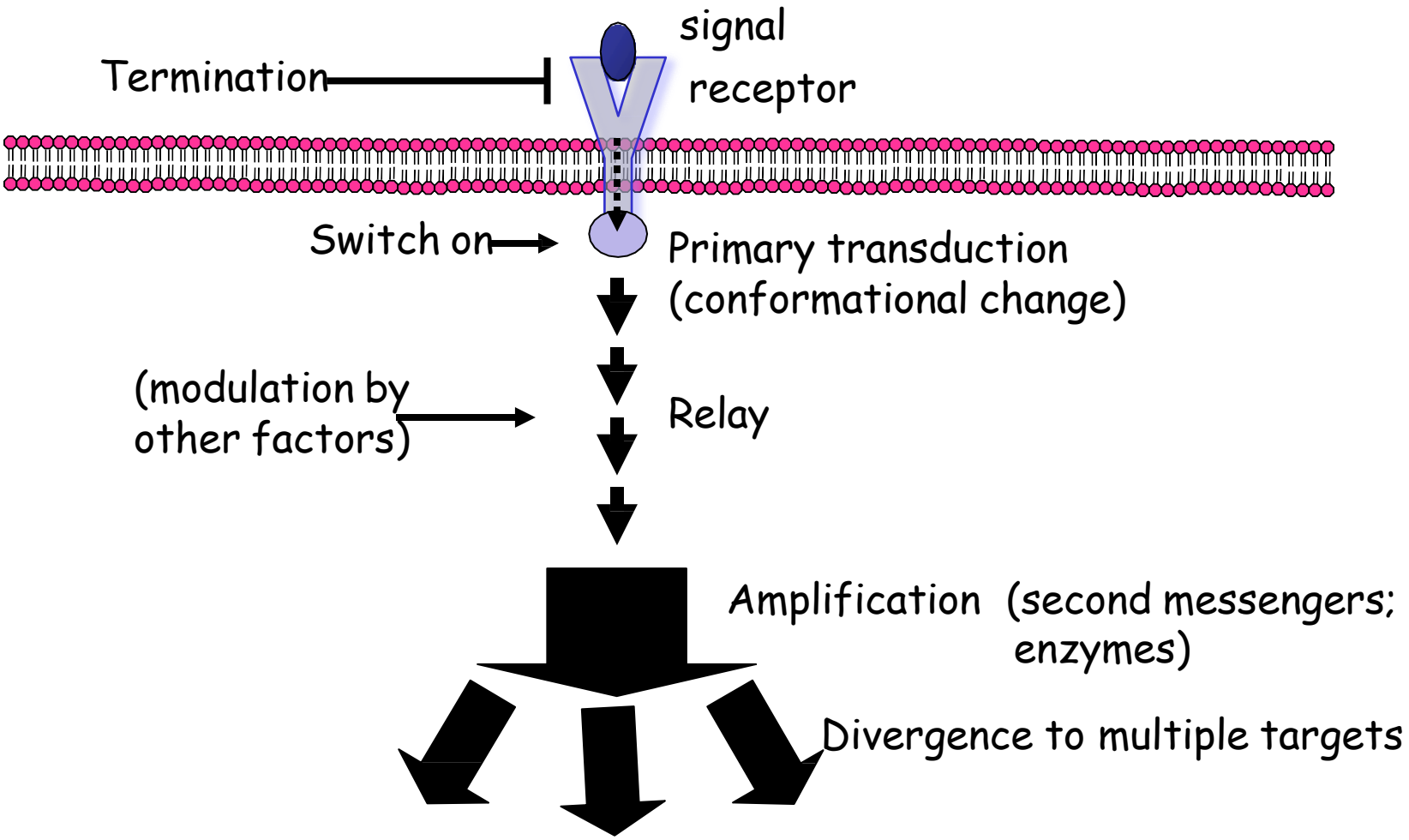
Signal transduction - conversion of signals into cellular responses



Hormone receptors exhibit:

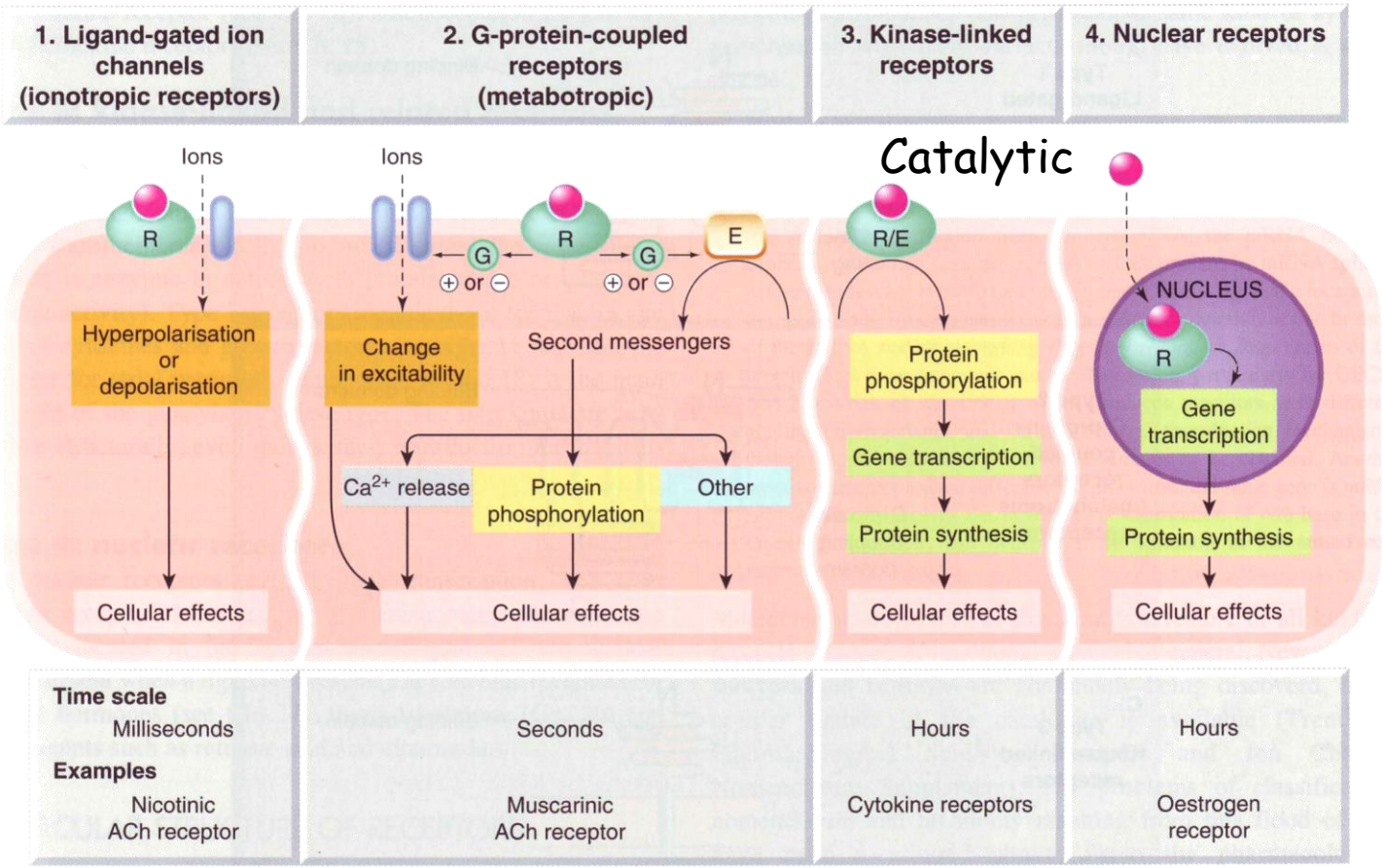
- Specificity, Affinity, Saturability, Reversibility (deactivation)
- Cell surface or intracellular
- Activate cellular responses (ion channel permeability, enzymes or changes in gene expression)

Cellular Signalling Cascade



(Then appropriate controls and feedback pathways terminate the response)

Main types of signal transduction pathways



No transduction Mechanism – ion current

Second messenger cAMP, cGMP, DAG, IP_3 and Ca^{++}

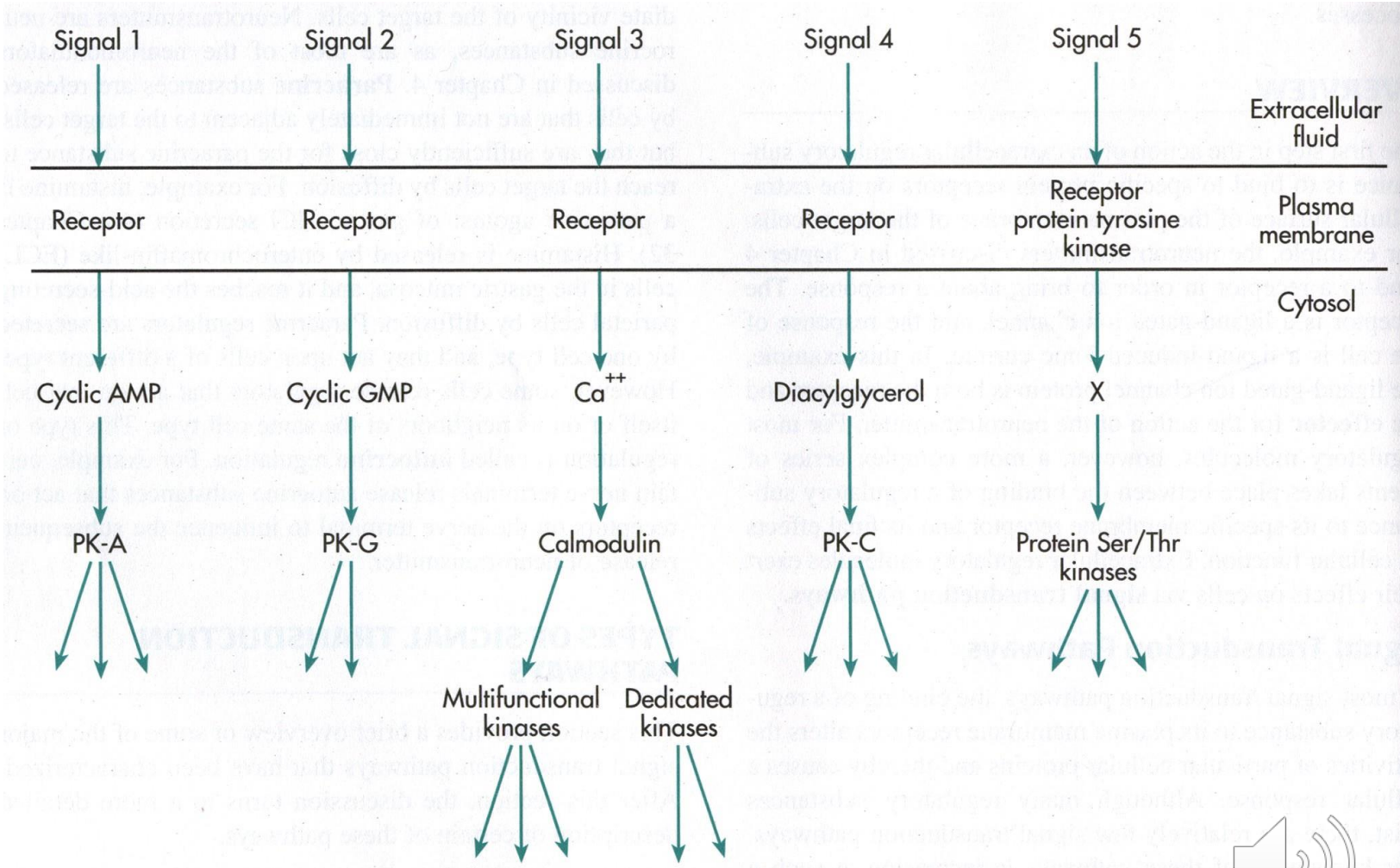
Receptor is an enzyme or part of enzyme complex

Receptor is cytosolic or nuclear

[R = receptor, G = G protein, E = enzyme]

Rang et al, 2003

Signal transduction pathways & second messengers

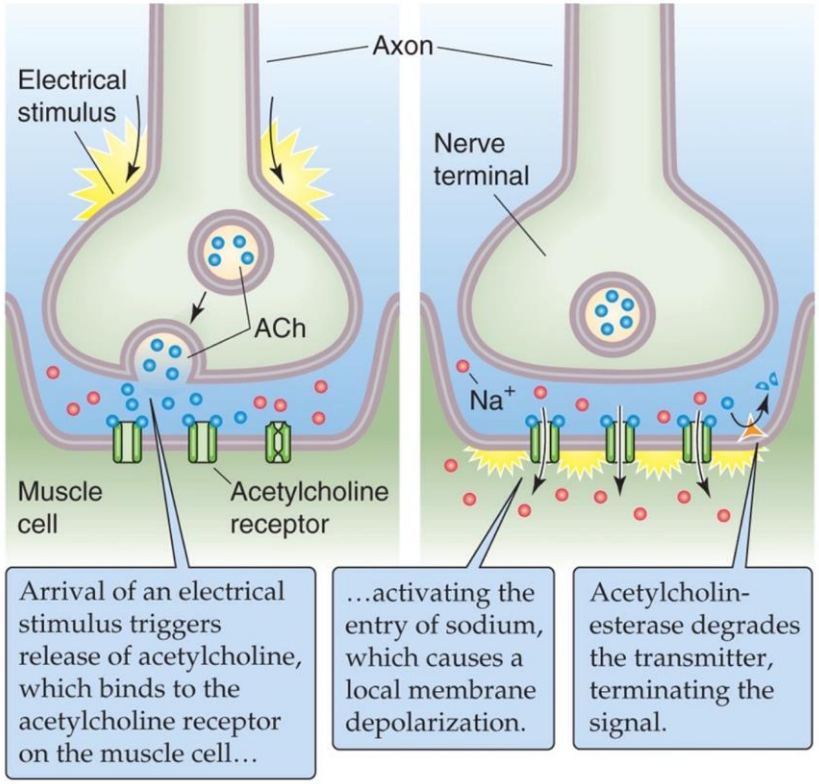


- Berne, Levy, Koeppen & Stanton 2004

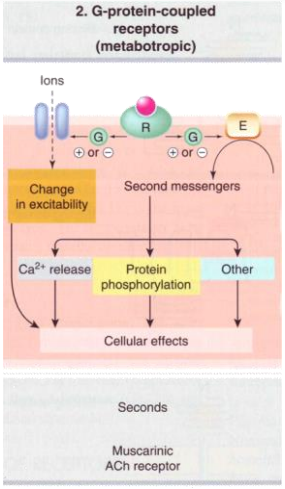
Ligand gated ion channels

- Many ion channels open or close in response to binding a ligand.
  - Some ion channels are gated by extracellular ligands; some by intracellular ligands.
- External ligands

  E.g. Acetylcholine (ACh).
  - Ionotropic receptor
    - Nicotinic Ach receptor
    - Opens Na+ ion channel
    - Depolarization



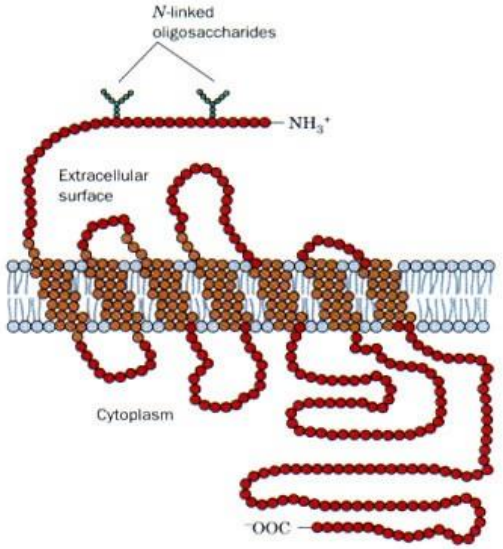
G-protein coupled receptors



G proteins (**guanine nucleotide-binding proteins**)

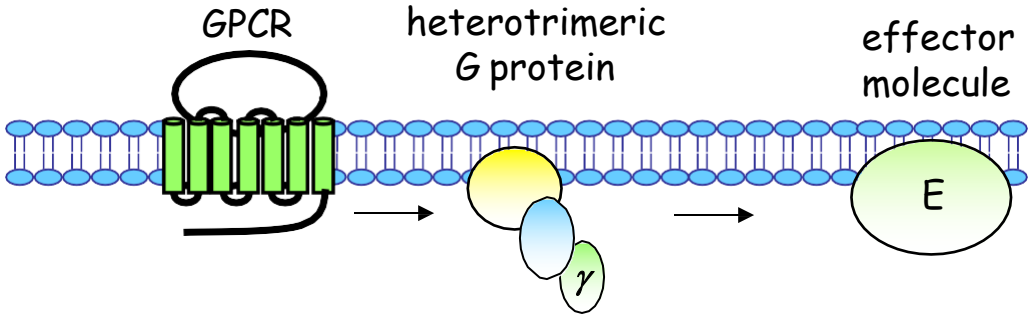
- Largest family of cell surface receptors
  - (>1000 genes encode for GPCRs)
- 7 transmembrane domains
- Coupled to intracellular effector systems via G- proteins
- Includes receptors for many hormones and transmitters
  - Examples:

    muscarinic AChR; adrenergic receptor

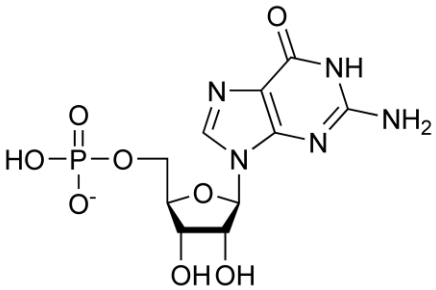


G proteins & GPCRs

Basic components of a GPCR-mediated signalling system

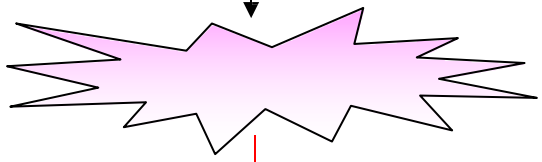


e.g $AT_{1\,(angiotensin)}$ adrenergic muscarinic	At least 23 α, 12 γ and 6 β subunits	Phospholipase A_2 Phospholipase C Adenylate cyclase calcium channels



G proteins (guanine nucleotide-binding proteins)

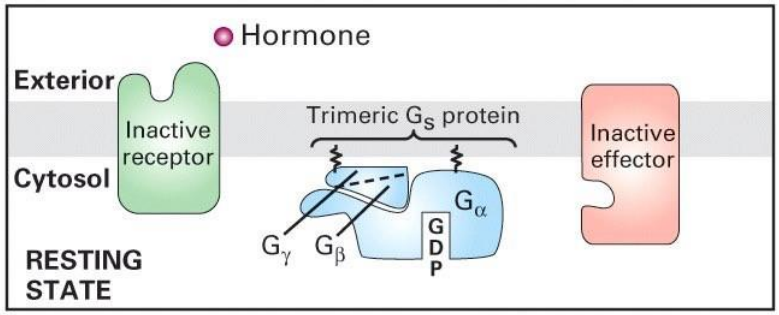
second messenger generation

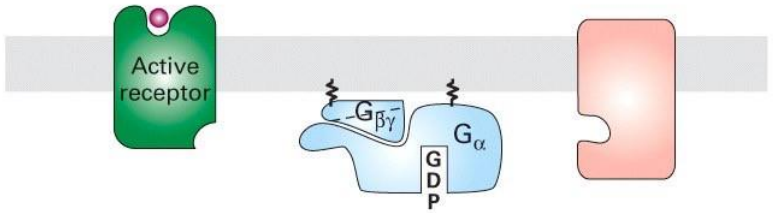


phosphorylation of cytosolic and nuclear proteins

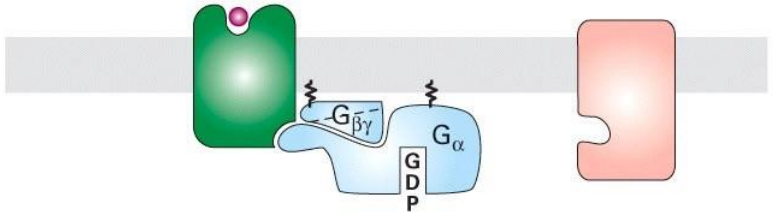
cellular response to stimulus

GPCRs: Function of the G protein

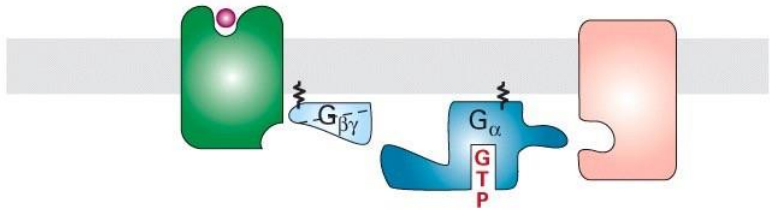




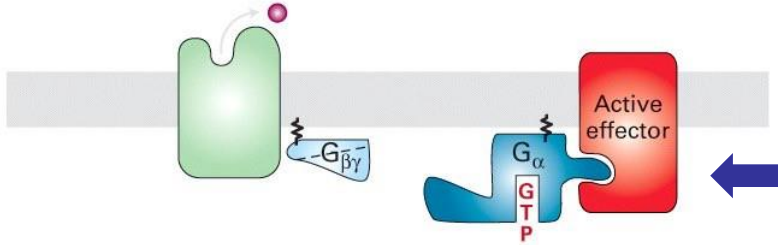
1 Binding of hormone induces a conformational change in receptor



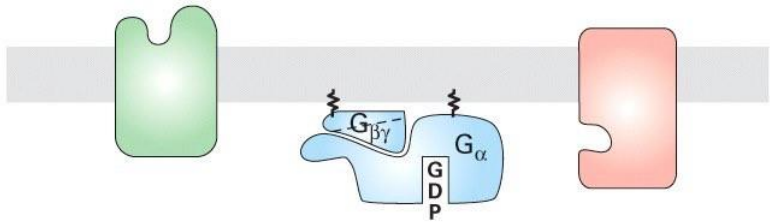
2 Activated receptor binds to G_α subunit



3 Binding induces conformational change in G_α; bound GDP dissociates and is replaced by GTP; G_α dissociates from $G_{\beta\gamma}$



4 Hormone dissociates from receptor; G_α binds to effector, activating it



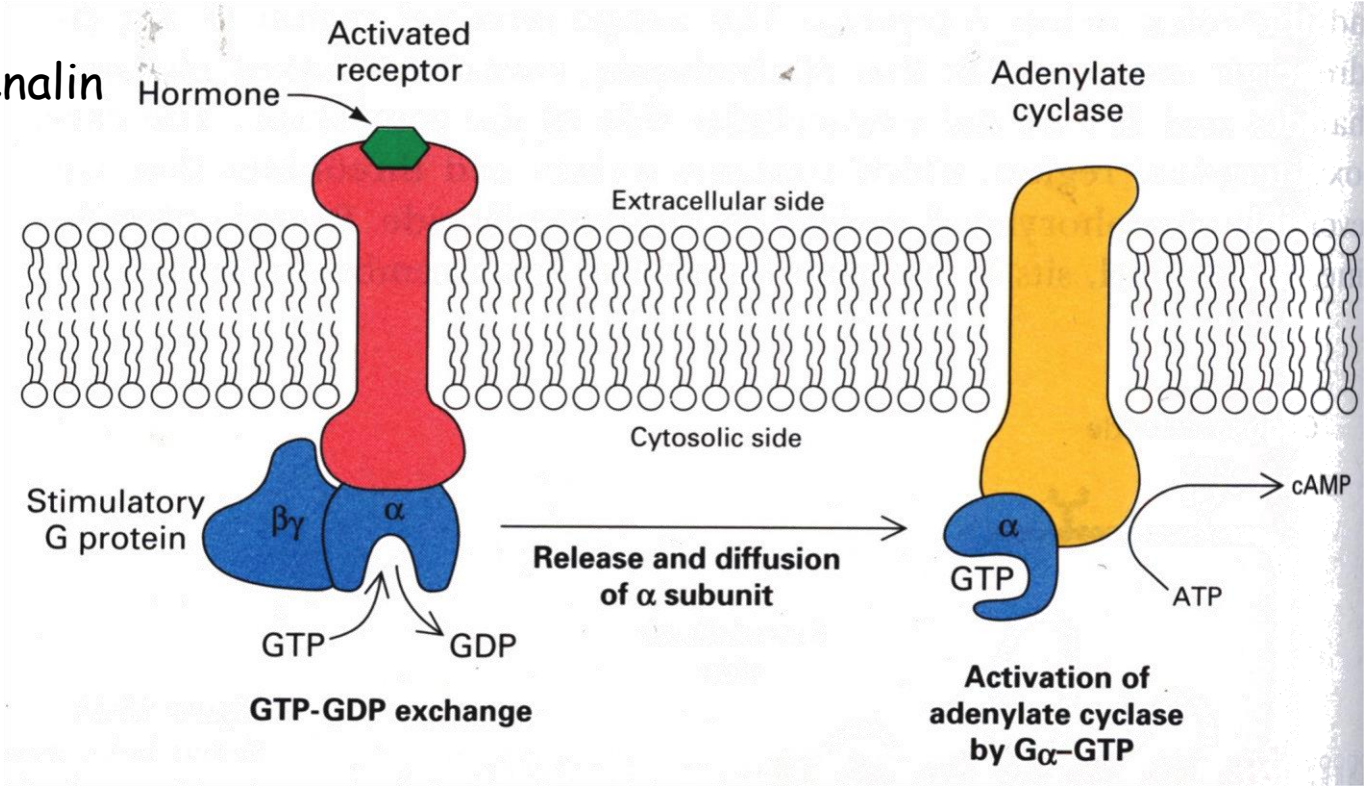
5 Hydrolysis of GTP to GDP causes G_α to dissociate from effector and reassociate with $G_{\beta\gamma}$

Response depends on the effector protein

G$\beta\gamma$ subunits can also activate downstream effectors

G protein activation

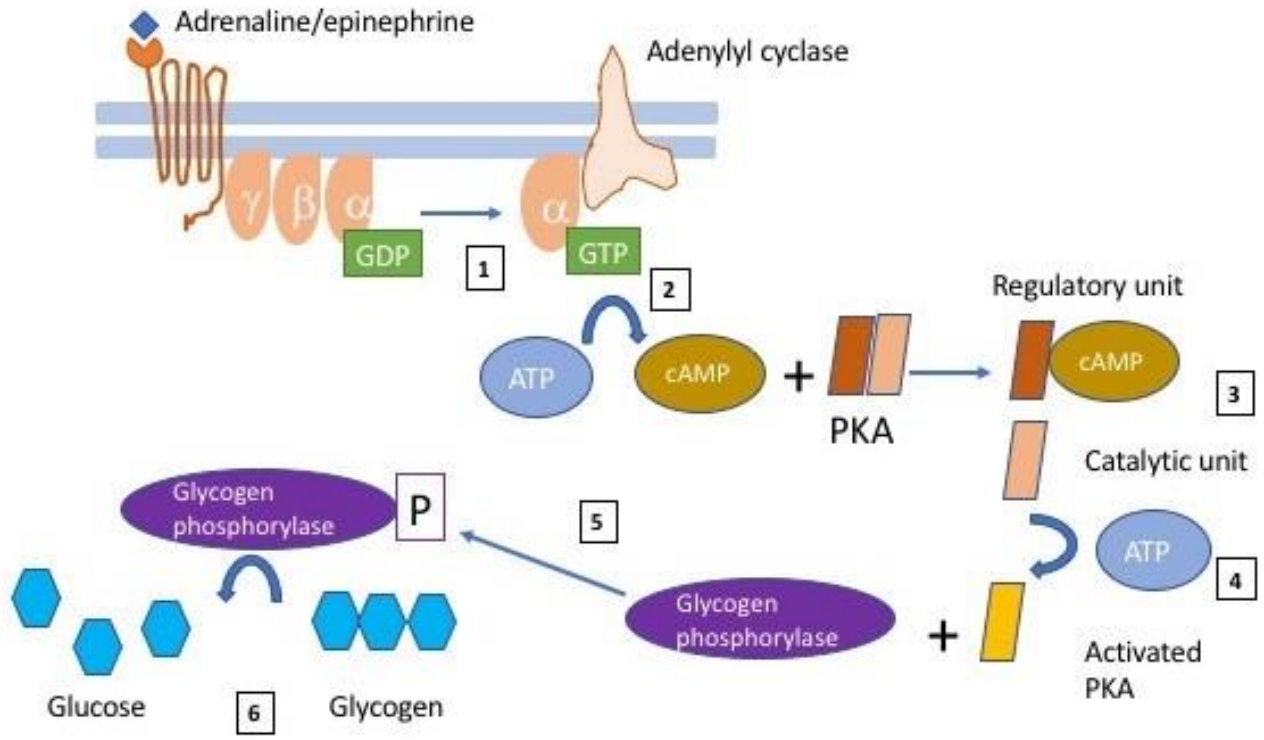
Adrenalin



Stryer 1995

Adrenaline/adrenergic receptor

1. G protein α subunit with GTP activates adenylyl cyclase
2. Adenylyl cyclase hydrolyses ATP to cAMP
3. cAMP binds to PKA regulatory subunit and releases the PKA catalytic unit
4. Catalytic unit is phosphorylated
5. Activated PKA phosphorylates glycogen phosphorylase enzyme
6. Glycogen broken down to glucose



Activation Protein kinase A by cAMP

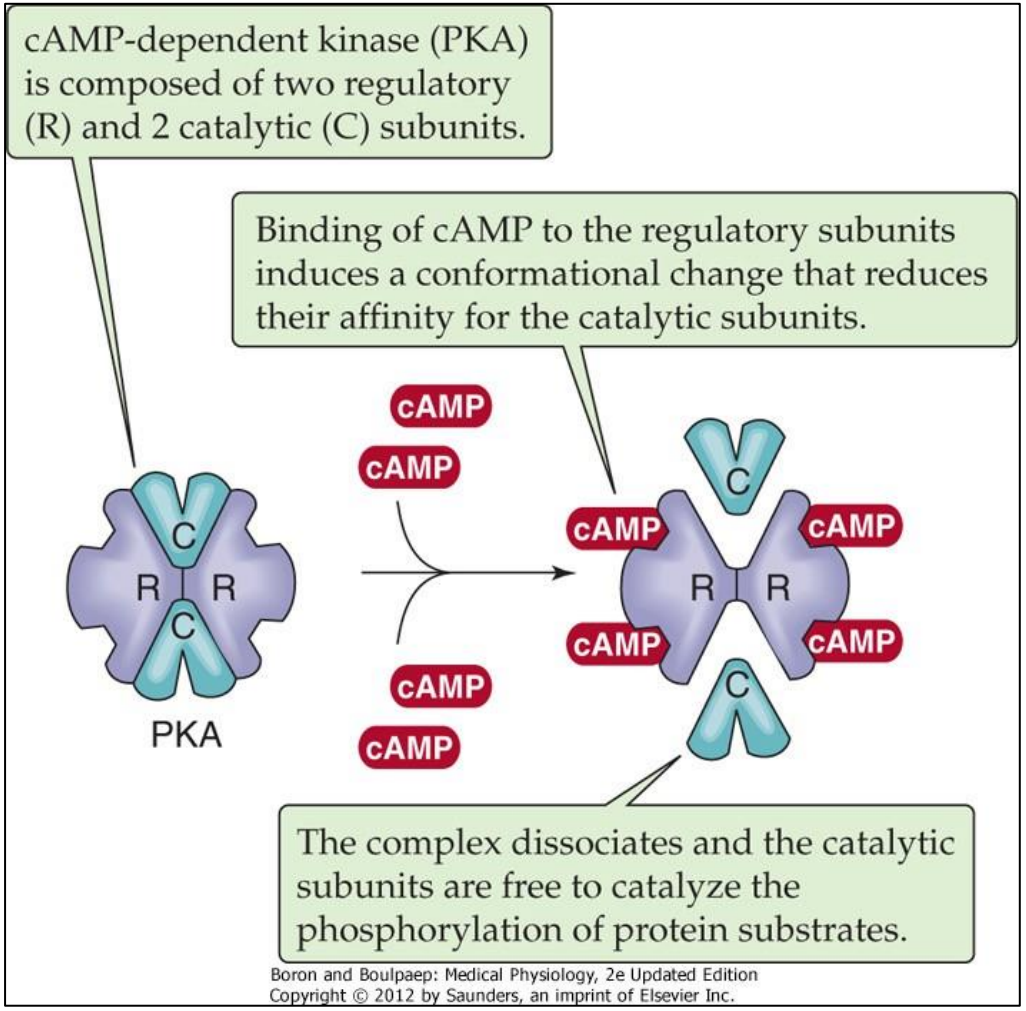
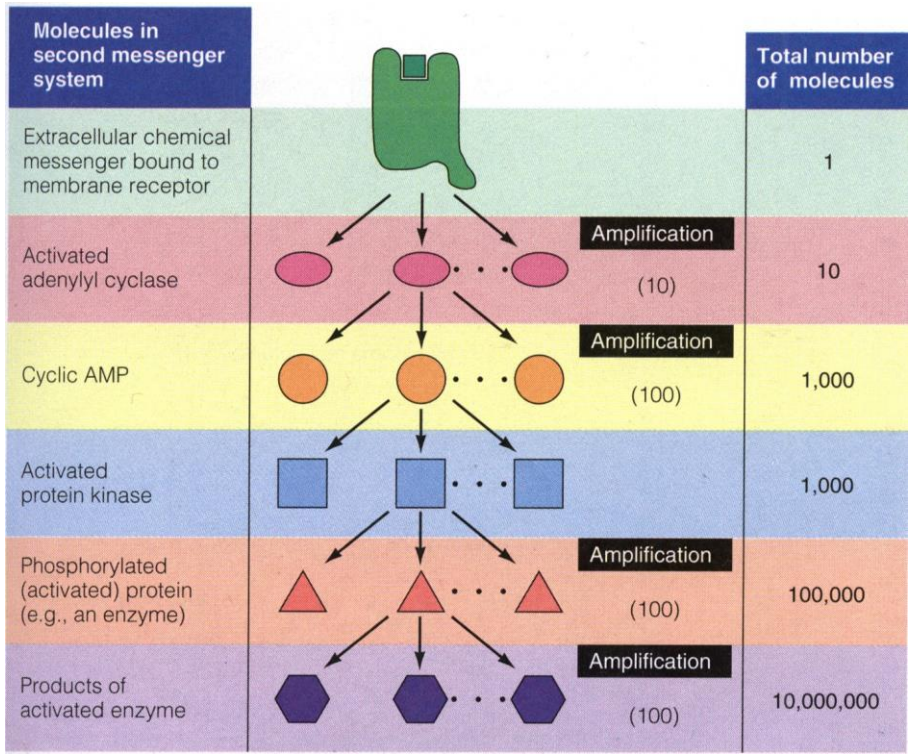
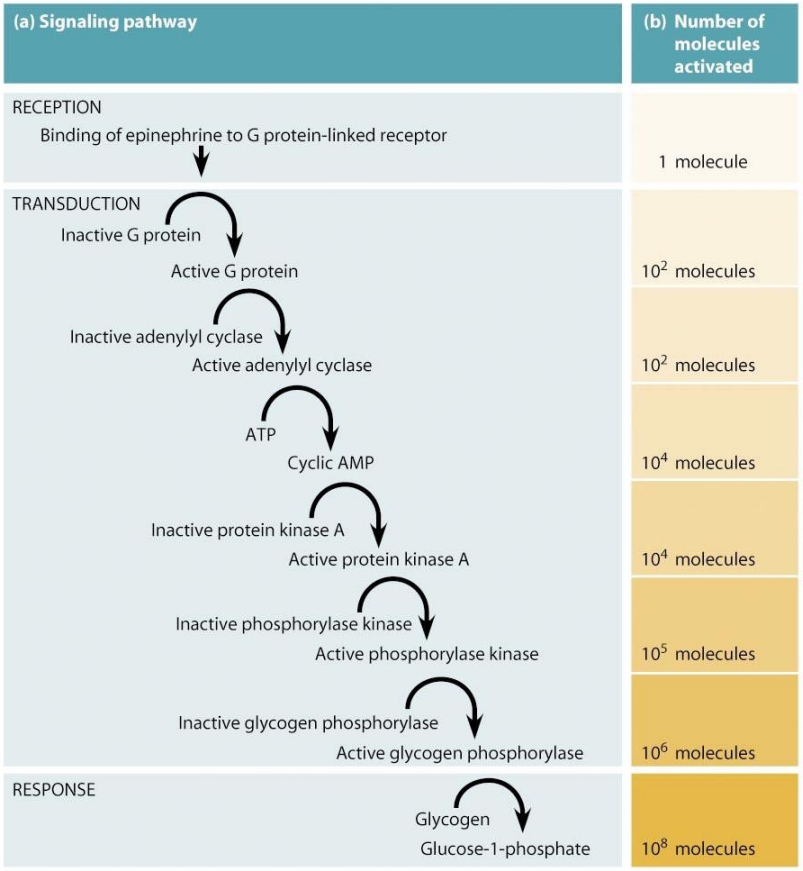


Figure 3-6 Activation of protein kinase A by cAMP.

Signal amplification through G proteins





Important regulatory control points

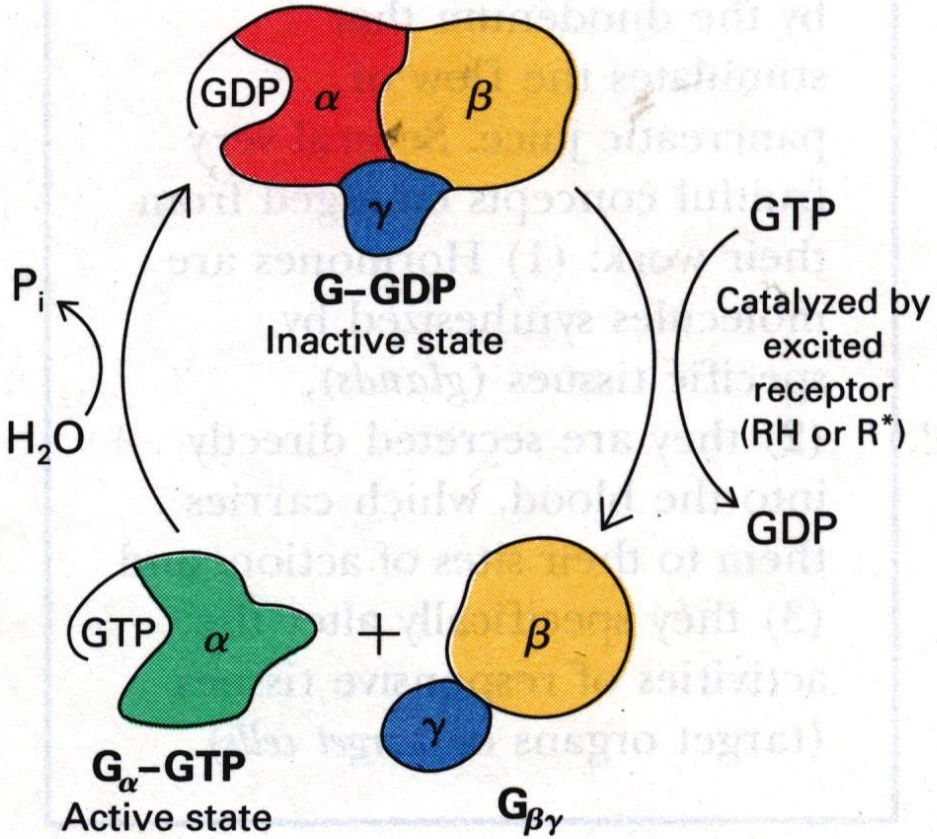
1. Ligand- Receptor interaction

- Activation via the G protein system occurs only while hormone-receptor conjugates persist
- Depends on hormone concentration & lifespan in blood
- Many receptor hormone conjugates are internalized and 'new' receptors expressed
  - Ligand-induced receptor internalisation through clathrin-coated pits

- Proteins called β-arrestins bind to GPCR and downregulate a response to prolonged hormone exposure (desensitization)
  - Inactivate GPCR
  - Promote removal by endocytosis

Important regulatory control points
G proteins molecular switch

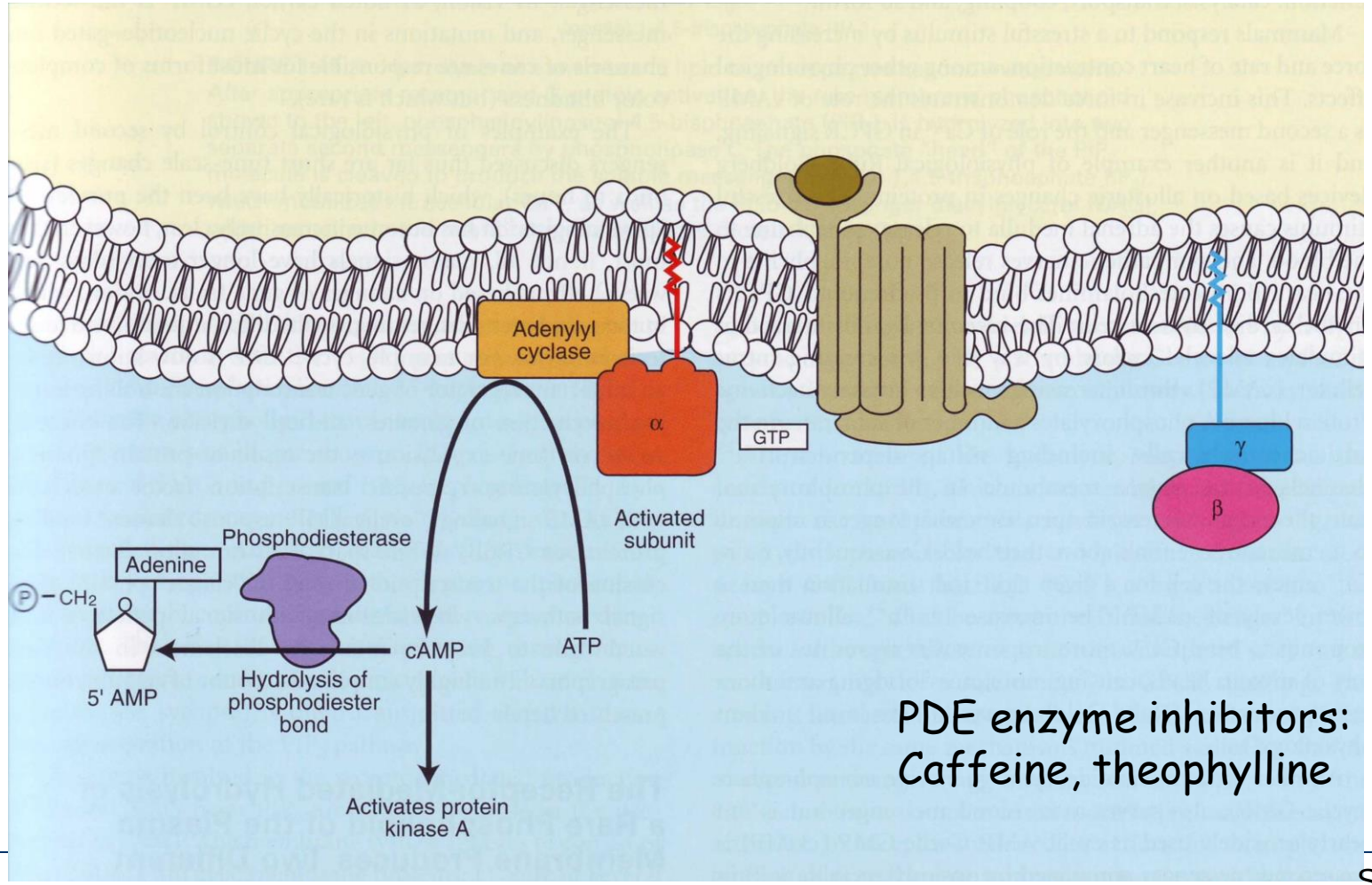
2. "Ground state" G$\alpha\beta\gamma$ protein reforms resulting in the deactivation of adenylate cyclase.



Important regulatory control points

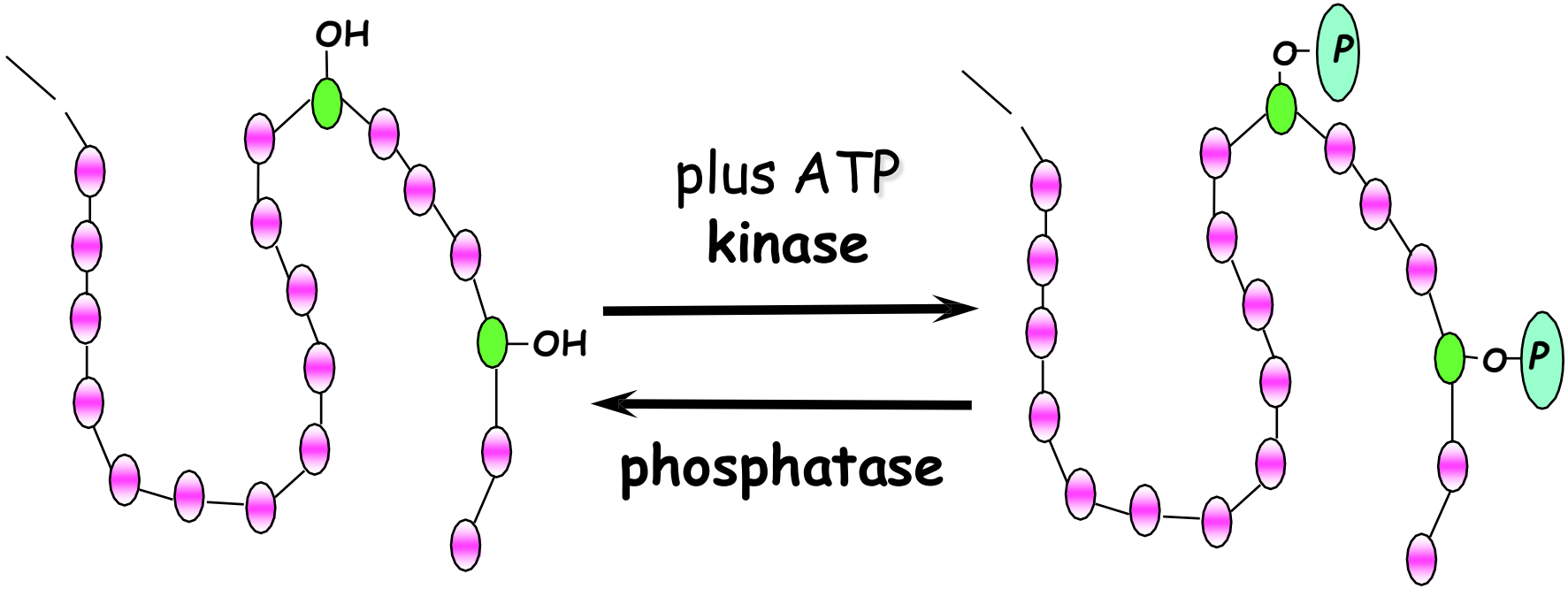
3. **Degradation of cAMP is catalysed by specific a phosphodiesterase**
   - cAMP → AMP
4. **Intracellular protein phosphatases**
   - Remove the phosphate groups from the key enzymes affected by their addition in the first place.
     - Eg, deactivation of protein kinase A etc
   - The balance between kinases (adding phosphates) and phosphatases (removing) activity plays a major role in the control of signalling events.

Phosphodiesterase enzyme breaks down cAMP (regulatory control)



What do kinases do??

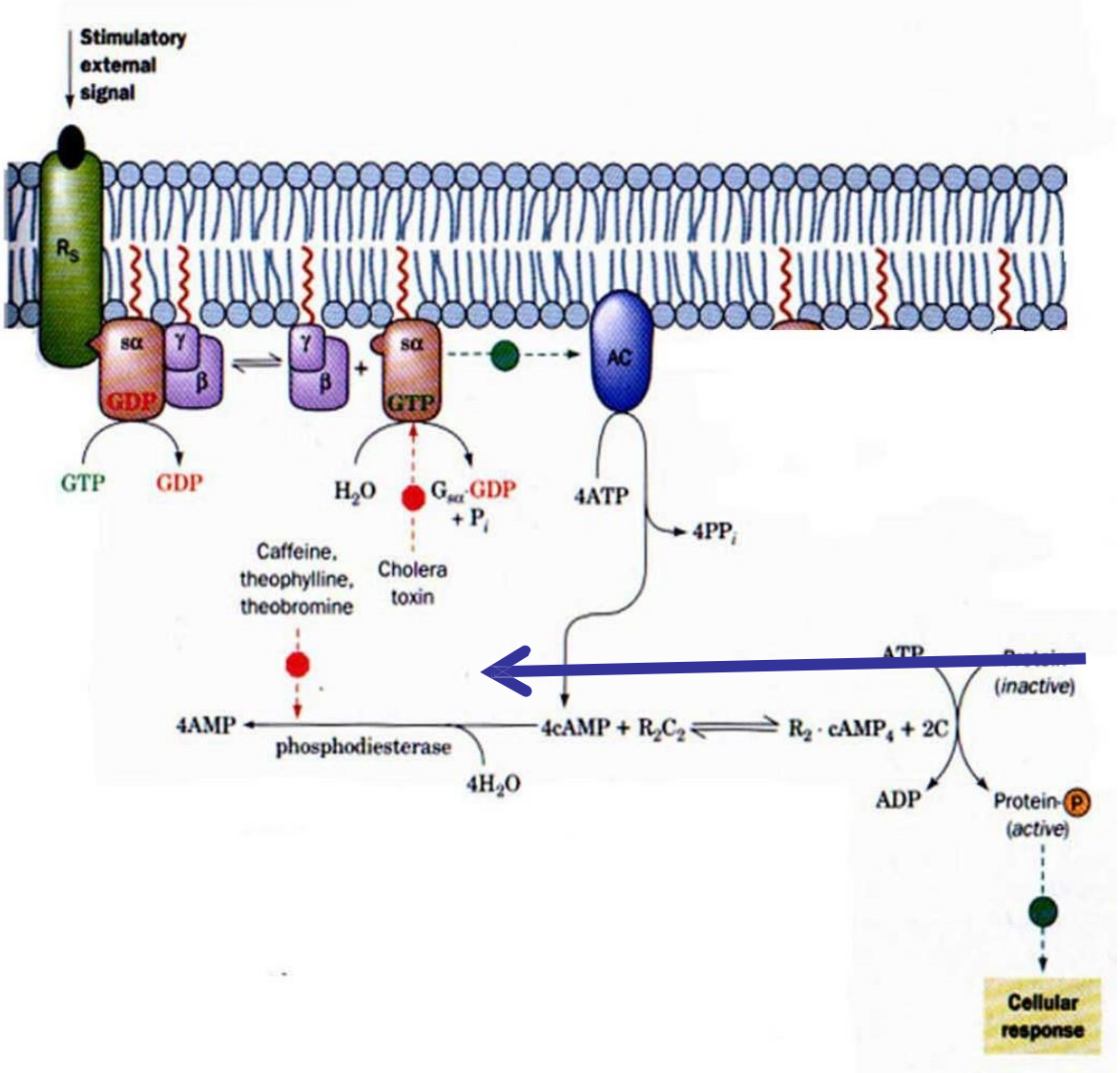
Reversible phosphorylation



tyrosine, threonine or serine residue

conformational change
change in function
change in location

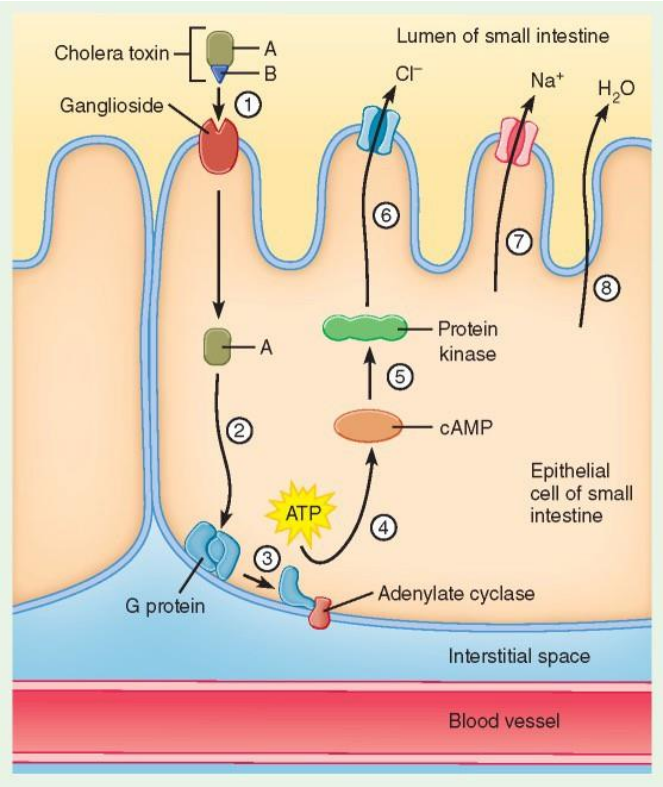
Cholera toxin and G proteins



Cholera toxin inhibits G protein hydrolysis -Switched on longer

Effect of Cholera toxin on salt and water balance

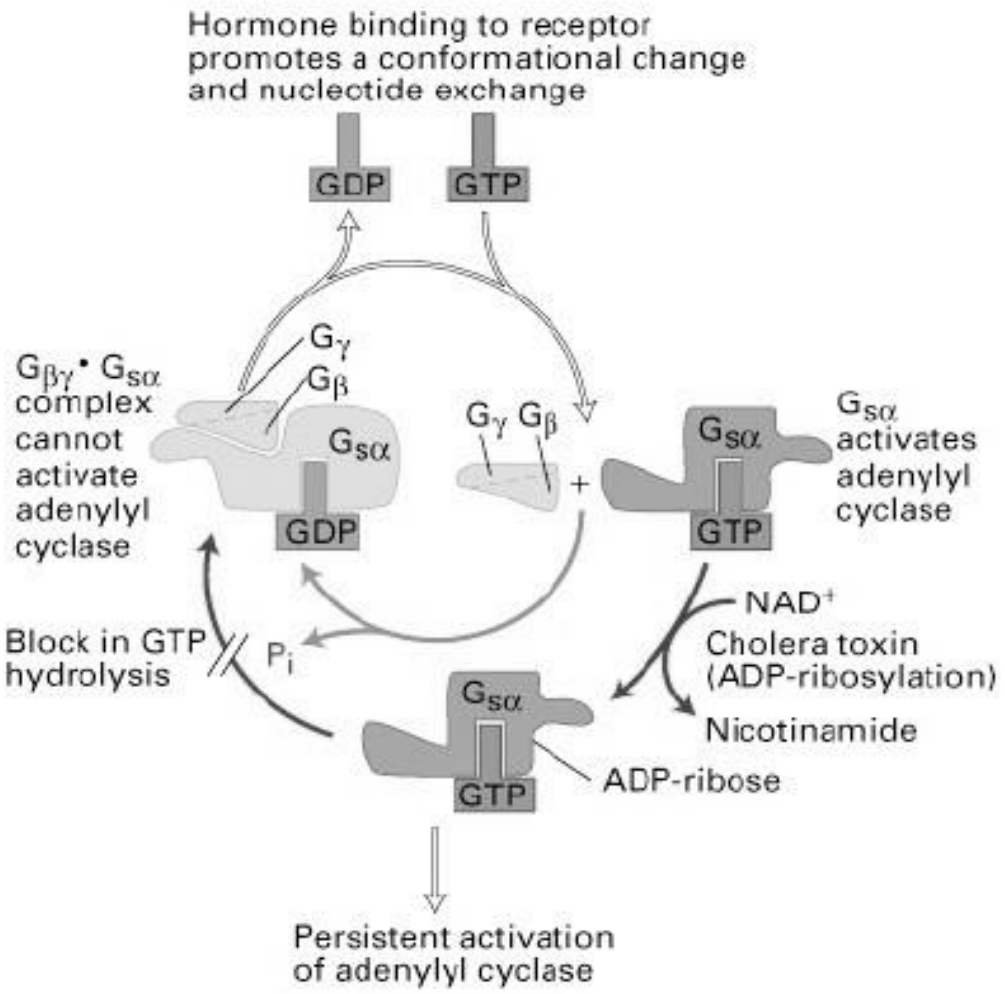
1. Cholera toxin enters intestinal crypt cells across the apical membrane
2. The toxin α subunit detaches and catalyses ADP ribosylation (addition of a ribosome unit) of the Gαs protein coupled to adenylyl cyclase
   - Inhibits GTPase activity and GTP cannot be hydrolysed back to GDP
3. GTP remains permanently bound to Gαs subunit
   - Adenylyl cyclase remains activated
4. cAMP levels 100x higher than normal
5. Cytosolic PKA is over-activated
6. PKA phosphorylates cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel proteins ATP-mediated secretion of Cl^- ions
7. Leads to secretion of other ions and
8. Water loss of up to 2L/h



Consequence: severe dehydration and diarrhoea

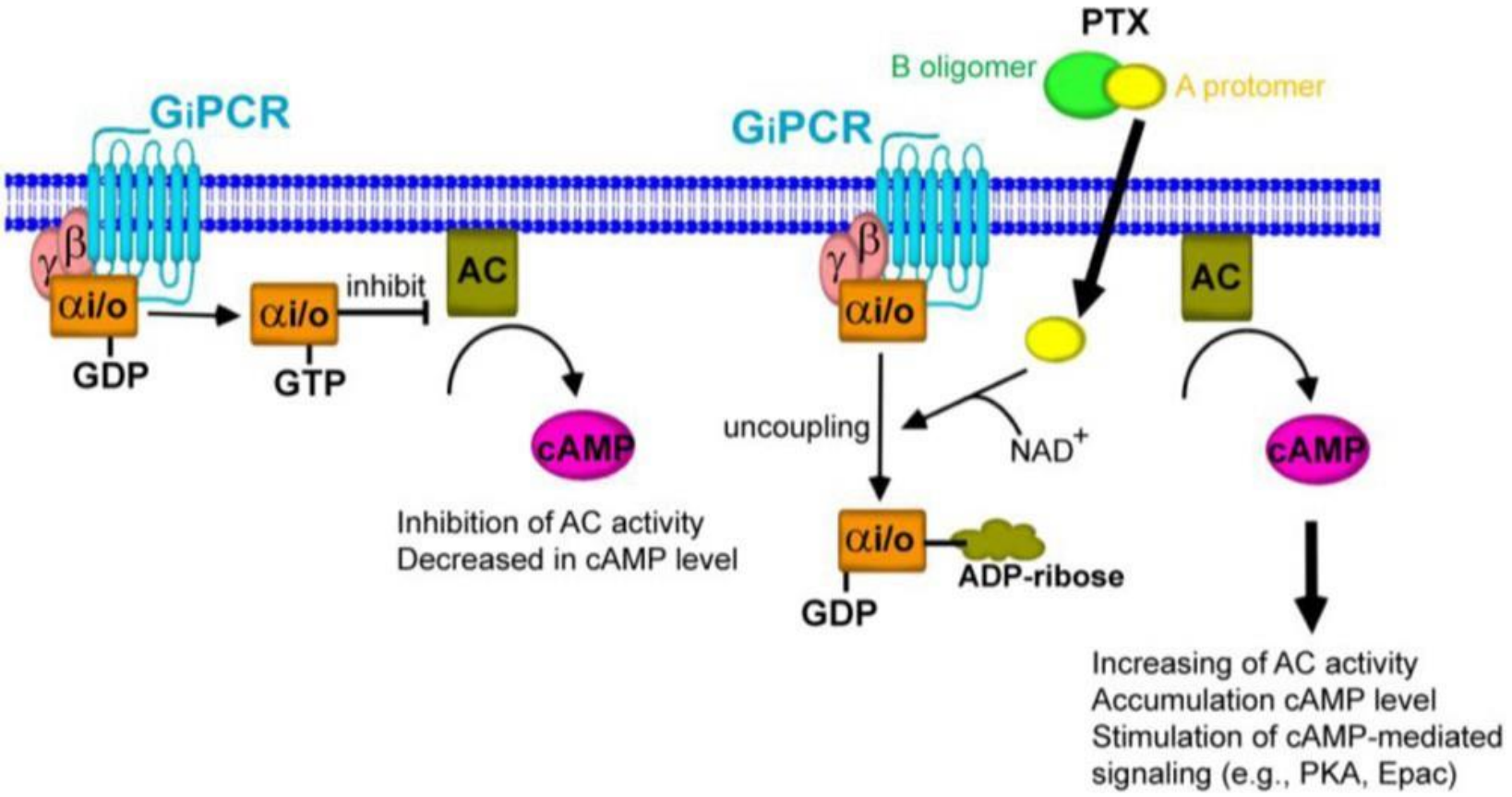
Cholera toxin

- Cholera toxin ADP-ribosylates the Ga subunit of the G protein that activates adenylyl cyclase
- Ga can no longer hydrolyze GTP



http://yakko.bme.virginia.edu/biom204/slides/cholera_bnw.pdf

Pertussis toxin – increases cAMP by blocking Gi



Toxins **2011**, *3*(7), 884-899

Summary

Key events in signal transduction:

1. **Recognition** (Receptor specificity)

2/3. Transduction / transmission

- Ligand binding causes a conformational change in the receptor
- Triggers catalytic activities to the receptor or causes the receptor to interact with cytoplasmic or membrane enzymes.
- Second messengers
- Activate catalytic cascade & effectors

4. **Modulation of an effector.**
   - protein kinases, phosphatases - altering enzyme activity.
5. **Appropriate response of cell to initial stimulus.**
   - Summation and integration of multiple signalling pathways.
6. **Termination of response.**
   - Inbuilt controls and feedback pathways.