

Topic 5

Signal Transduction

Nelson & Cox (6th Ed)

Ch. 12 p433-459, 464-470, 471-473

Nelson & Cox (7th Ed)

Ch. 12 p437-456, 461-466, 471-475

What is Signal Transduction?

- In all multicellular organisms, there is a need for cells to communicate with each other, through extracellular signalling molecules, in order to coordinate a diverse array of tasks, including: cell specific functional outputs, growth and metabolism.
- Signal transduction, refers to the process of receiving and acting on these signals from outside the plasma membrane (extracellular).
- Universal property of ALL living cells.

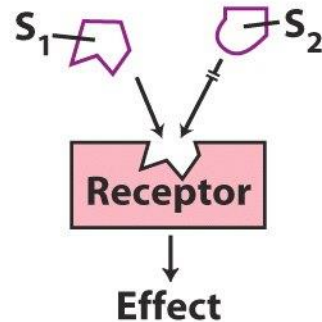
Signals Represent Information

- Signals are detected by specific receptors found within the: plasma membrane, cytoplasm, nuclear envelope or within the nucleus.
- Different signals represent an information code to which a cell will generate specific chemical responses.
- While there are many signals, and many responses...
...there are just a few evolutionarily conserved mechanisms for detecting and transducing these signals into an intracellular change.

Signal-Transducing Systems are Specific and Sensitive

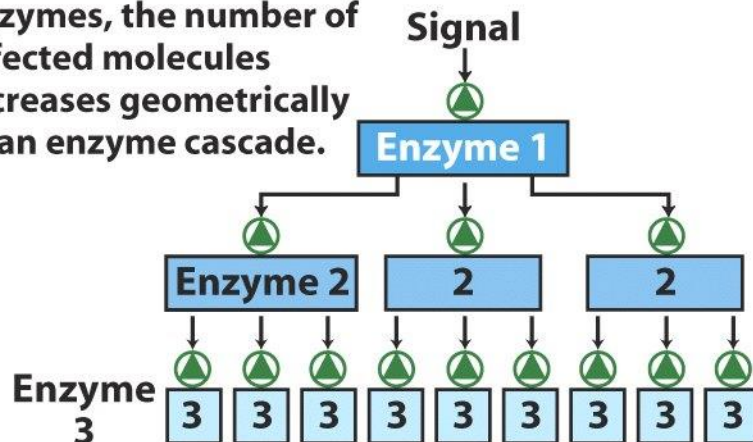
(a) Specificity

Signal molecule fits binding site on its complementary receptor; other signals do not fit.



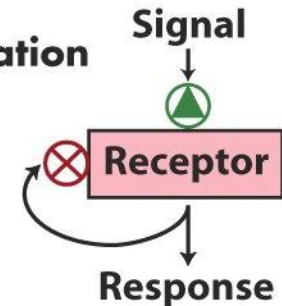
(b) Amplification

When enzymes activate enzymes, the number of affected molecules increases geometrically in an enzyme cascade.



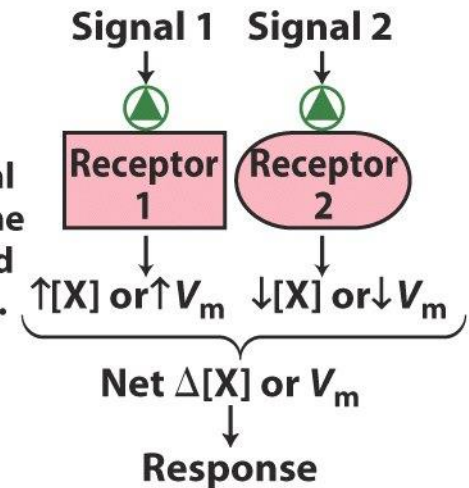
(c) Desensitization/Adaptation

Receptor activation triggers a feedback circuit that shuts off the receptor or removes it from the cell surface.



(d) Integration

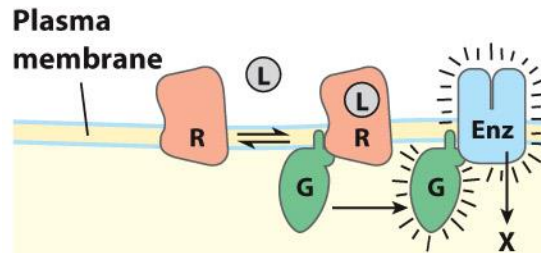
When two signals have opposite effects on a metabolic characteristic such as the concentration of a second messenger X , or the membrane potential V_m , the regulatory outcome results from the integrated input from both receptors.



Basic Signalling Mechanisms

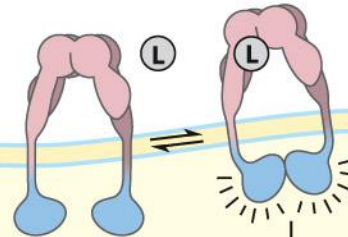
1. G protein-coupled receptor

External ligand (L) binding to receptor (R) activates an intracellular GTP-binding protein (G), which regulates an enzyme (Enz) that generates an intracellular second messenger (X).



2a. Receptor enzyme (tyrosine kinase)

Ligand binding activates tyrosine kinase activity by autophosphorylation.

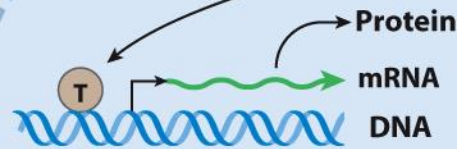


2b. Kinase activates transcription factor (T), altering gene expression.

Kinase cascade

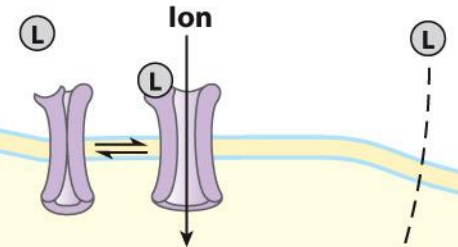
T

Nuclear envelope



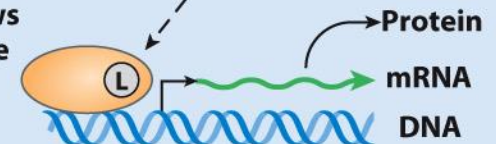
3. Gated ion channel

Channel opens or closes in response to concentration of signal ligand or membrane potential.

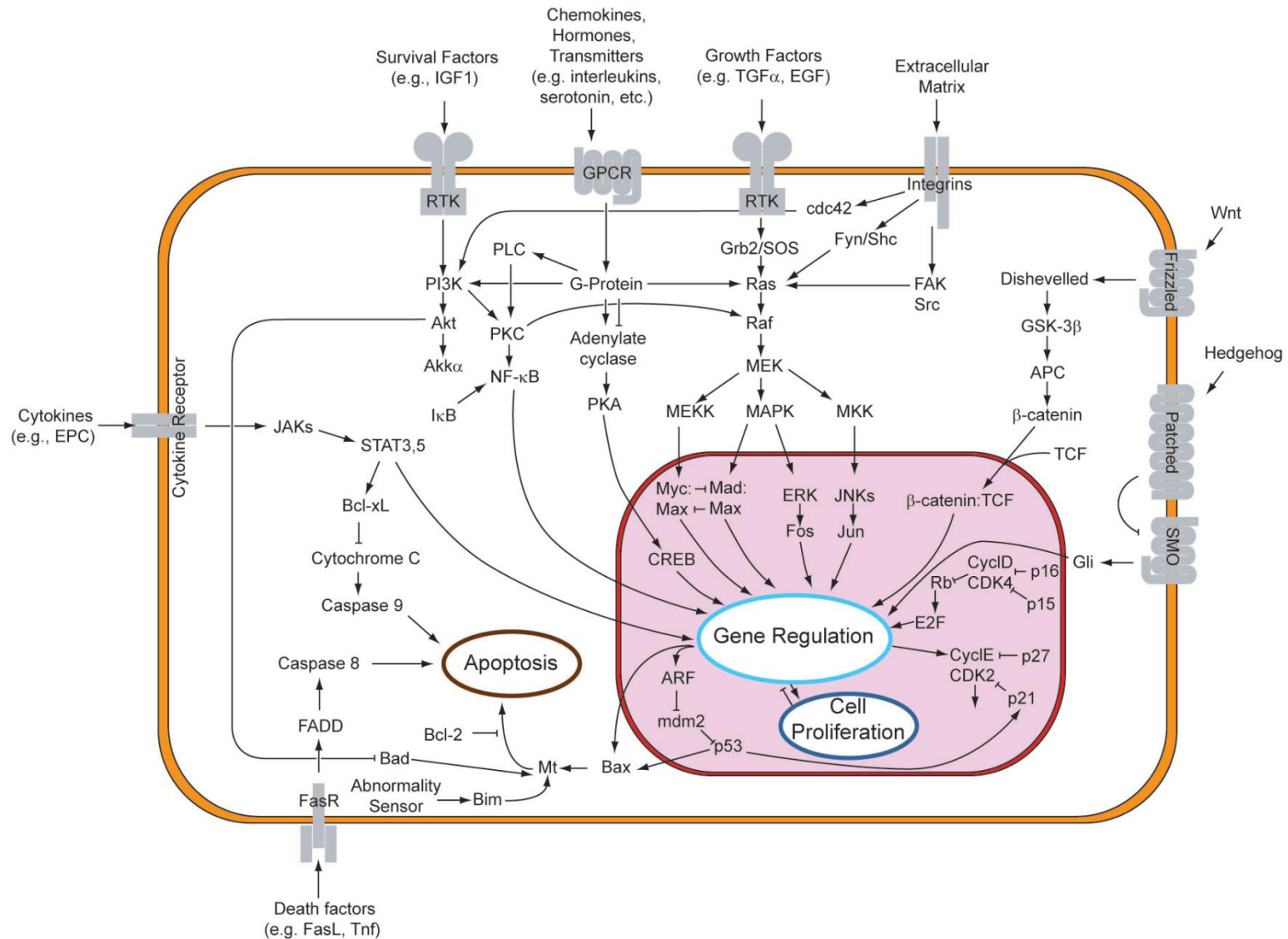


4. Nuclear receptor

Hormone binding allows the receptor to regulate the expression of specific genes.



Signalling Pathways - Complexity

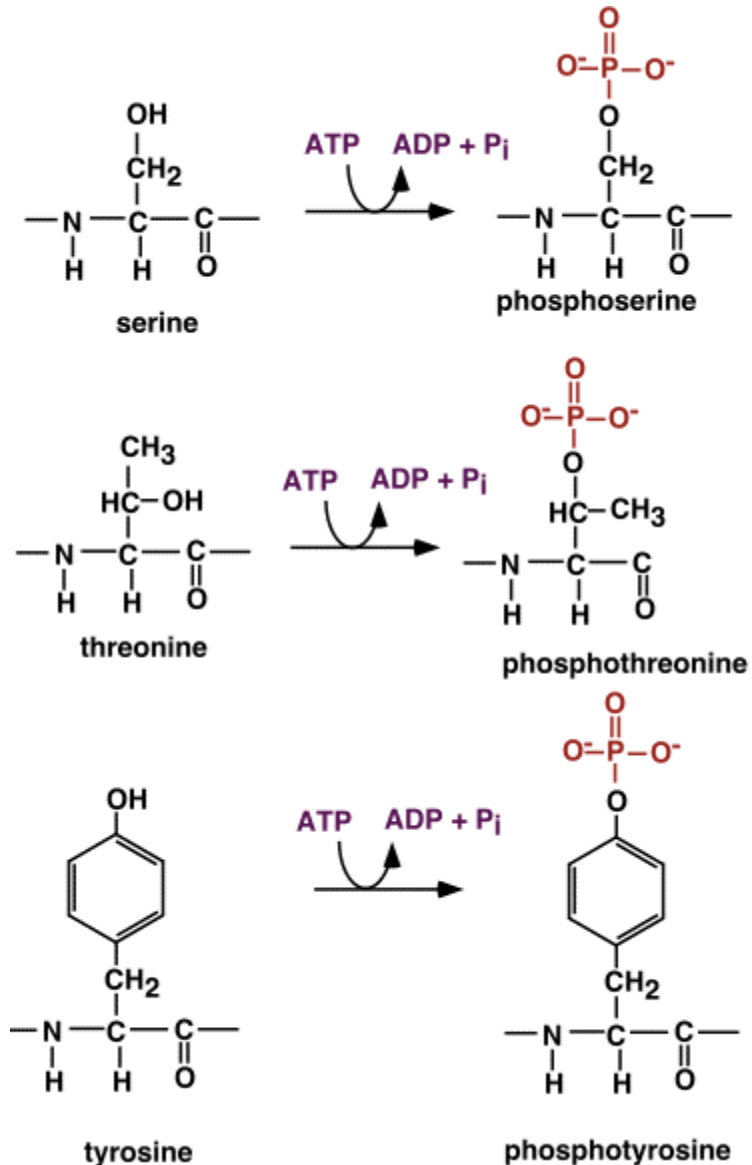


LEGENDS

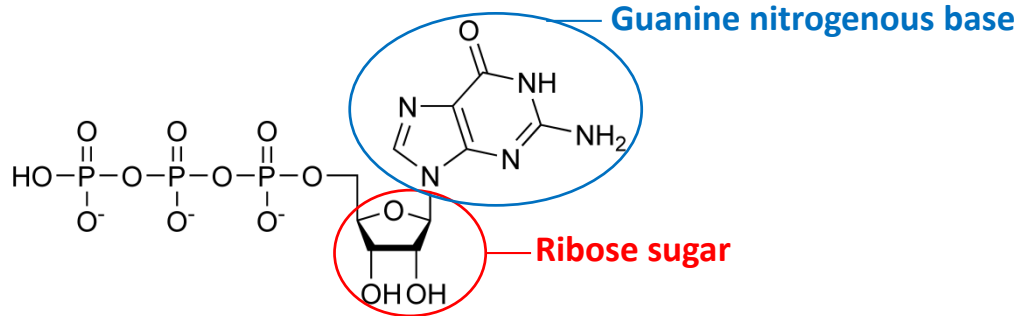


Protein Phosphorylation

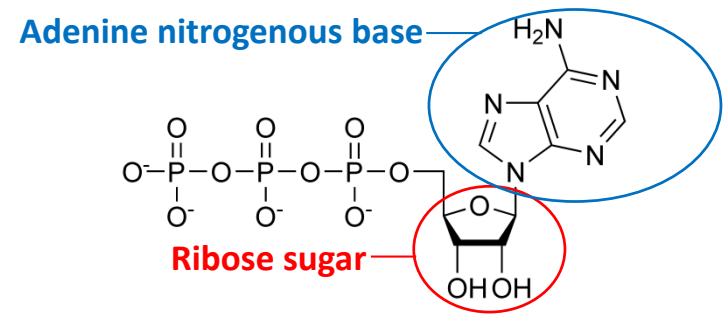
- Reversible phosphorylation of proteins is an important regulatory mechanism
- Enzymes called **kinases** (phosphorylate) and **phosphatases** (dephosphorylate) add/remove a phosphate group from specific amino acid residues in a protein
- Most commonly added to the amino acids: serine, threonine, tyrosine and histidine
- Phosphorylation of a protein can result in a conformational change in its structure
 - Due to: addition of the charged phosphate group
 - Activation/inactivation of the protein.



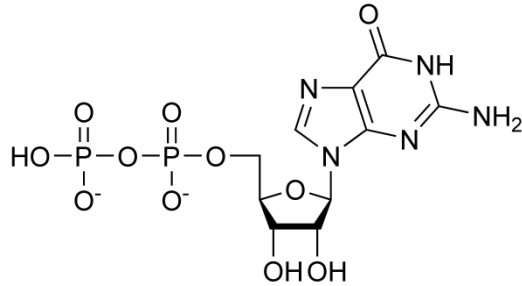
Guanosine 5'-triphosphate (GTP)



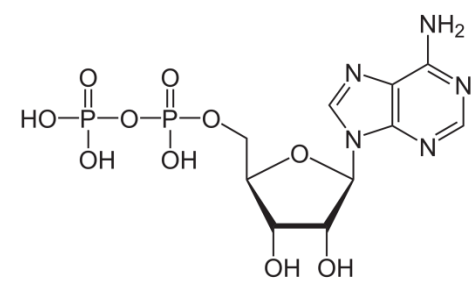
Adenosine 5'-triphosphate (ATP)



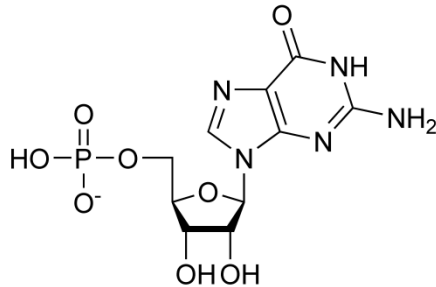
Guanosine 5'-diphosphate (GDP)



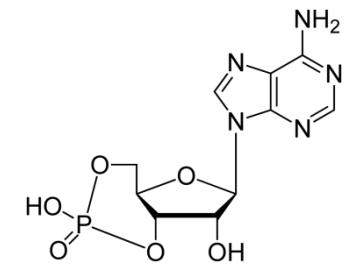
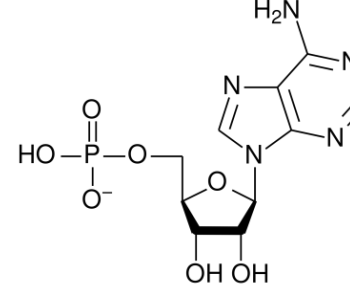
Adenosine 5'-diphosphate (ADP)



Guanosine 5'-monophosphate (GMP)



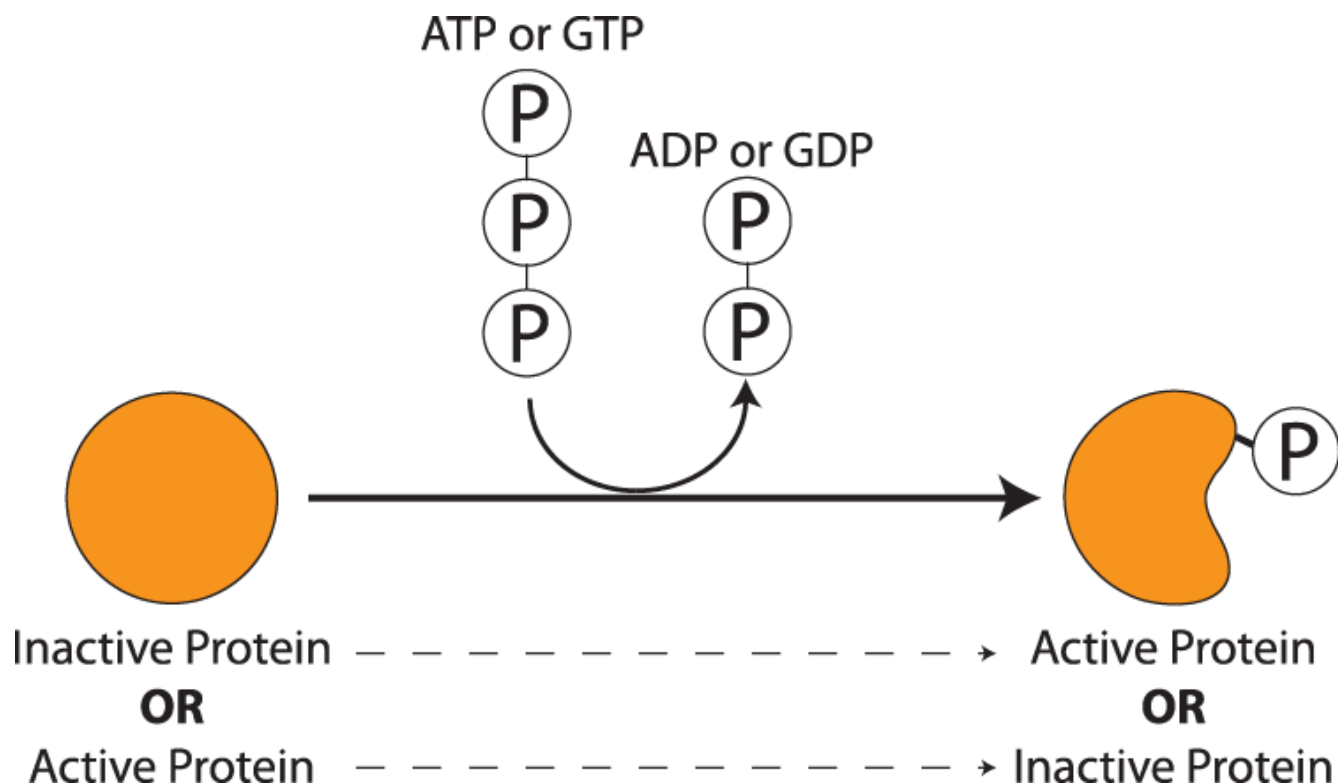
Adenosine 5'-monophosphate (AMP)



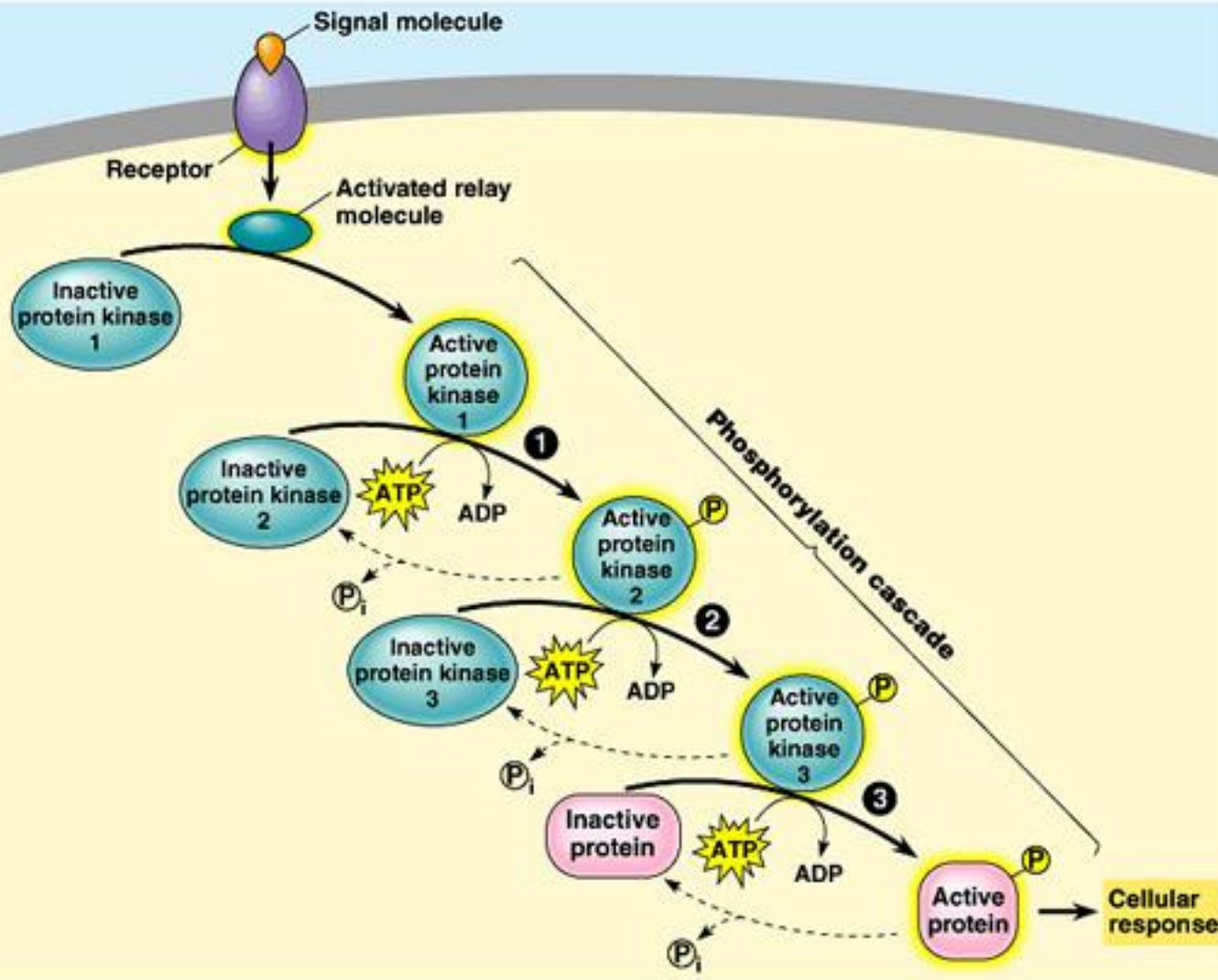
Cyclic adenosine 5'-monophosphate (cAMP)

Protein Kinases

- A specialised enzyme that chemically adds a phosphate group to specific amino acid residues in a protein.



Phosphorylation Cascade



Each Kinase activates a following kinase

Each active kinase can:

- Stimulate multiple pathways (signal divergence)
- Amplify the signal

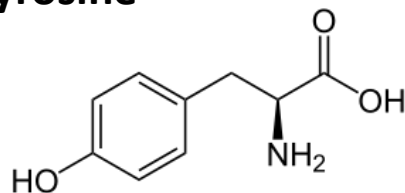
1. G-Protein Coupled Receptors

- Indirectly activate enzymes through GTP-binding proteins (called G Proteins)
- More than 1000 types
- *E.g.* β -adrenergic Receptor (adrenaline)
 - Found in the membranes of muscle, liver, neural and adipose cells

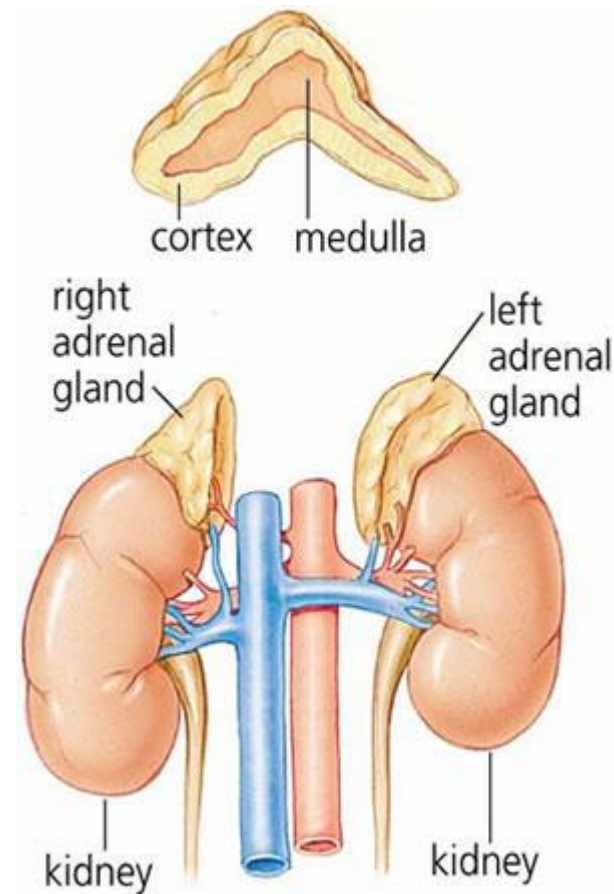
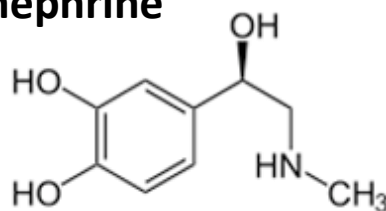
Epinephrine (Adrenalin)

- Is a hormone/neurotransmitter released from specific neurons and the adrenal medulla
- Is a catecholamine synthesised from the amino acid tyrosine
- Is a major transmitter of the autonomic nervous system (controls unconscious actions)
 - Sympathetic nervous system
 - Produces your 'flight or fight' response
 - Binds to adrenergic receptors (G-protein coupled receptors)

Tyrosine



Epinephrine



Carlyn Iverson

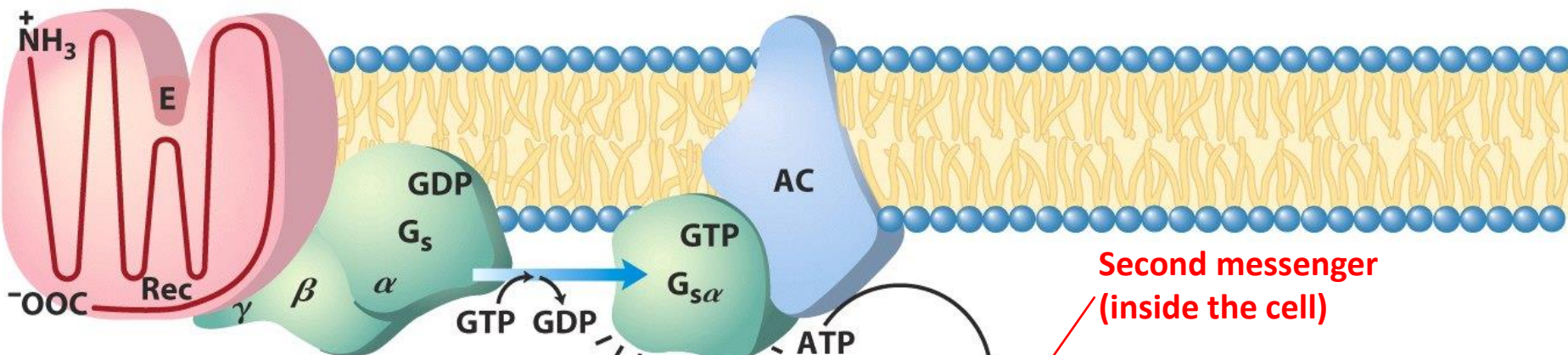
G-Protein Coupled Receptors

- Three components to this signal transduction mechanism:
 - Plasma membrane receptor composed of 7 transmembrane protein helices
 - A GTP-binding protein (G-protein)
 - An enzyme in the cytoplasm that generates a “second messenger”
- Ligand-binding on the outside of the cell activates the receptors
 - Stimulates G-protein to swap GDP for GTP
 - GTP-bound G-protein then dissociates from receptor, binds to nearby enzyme, altering it's activity, and now produces a second messenger molecule

First messenger
(Initial signal - outside the cell)

①

Epinephrine binds to its specific receptor.



②

The occupied receptor causes replacement of the GDP bound to G_s by GTP, activating G_s .

③

G_s (α subunit) moves to adenylyl cyclase and activates it.

④

Adenylyl cyclase catalyzes the formation of cAMP.

cyclic nucleotide phosphodiesterase

Second messenger
(inside the cell)

cAMP
5'-AMP

⑤

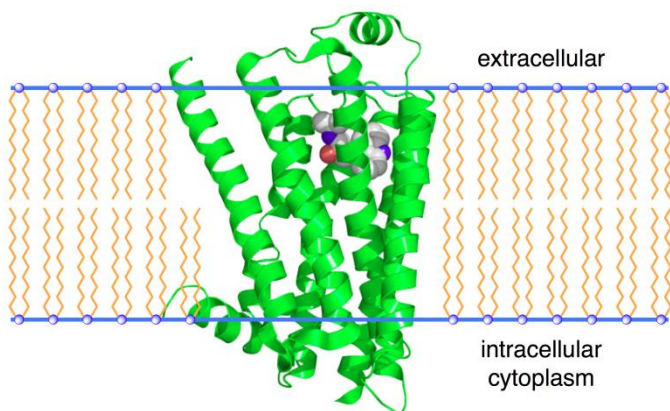
cAMP activates PKA.

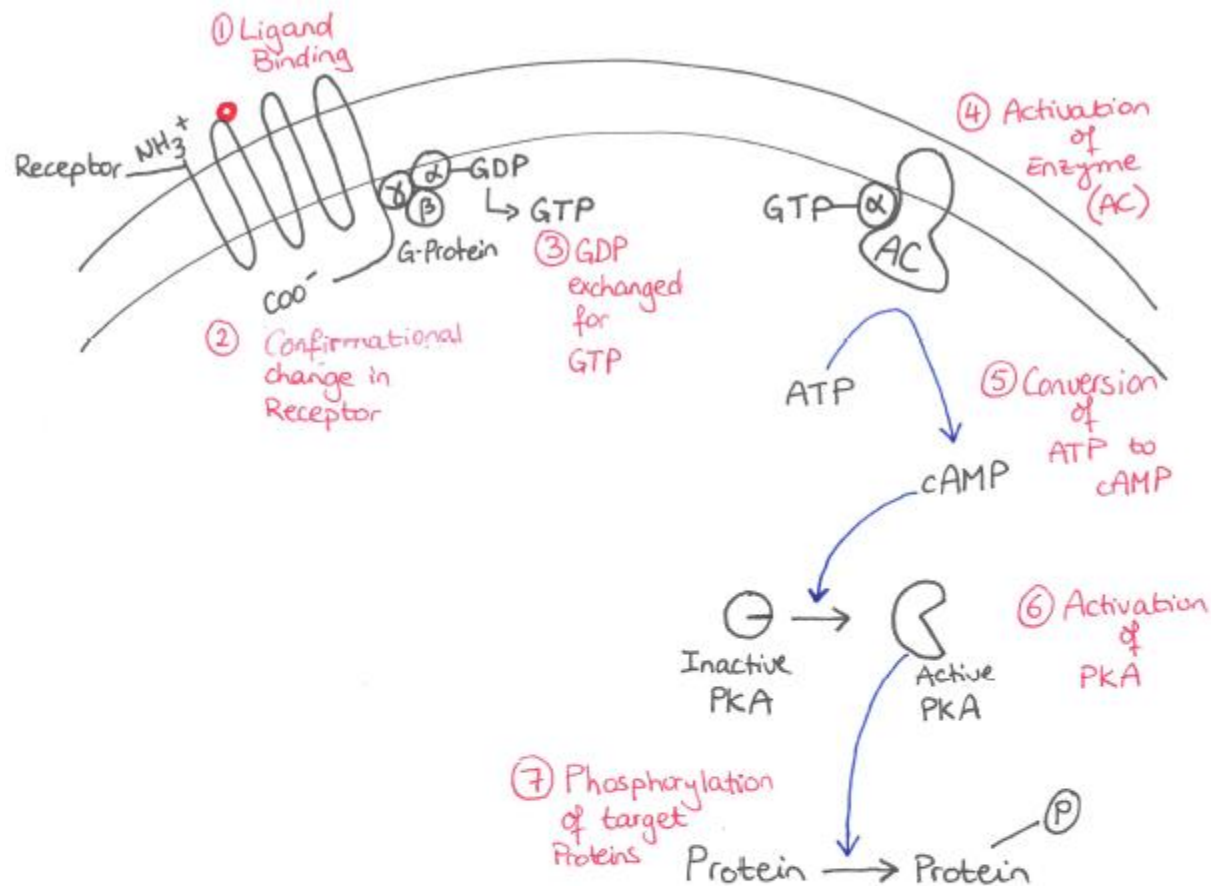
⑥

Phosphorylation of cellular proteins by PKA causes the cellular response to epinephrine.

⑦

cAMP is degraded, reversing the activation of PKA.





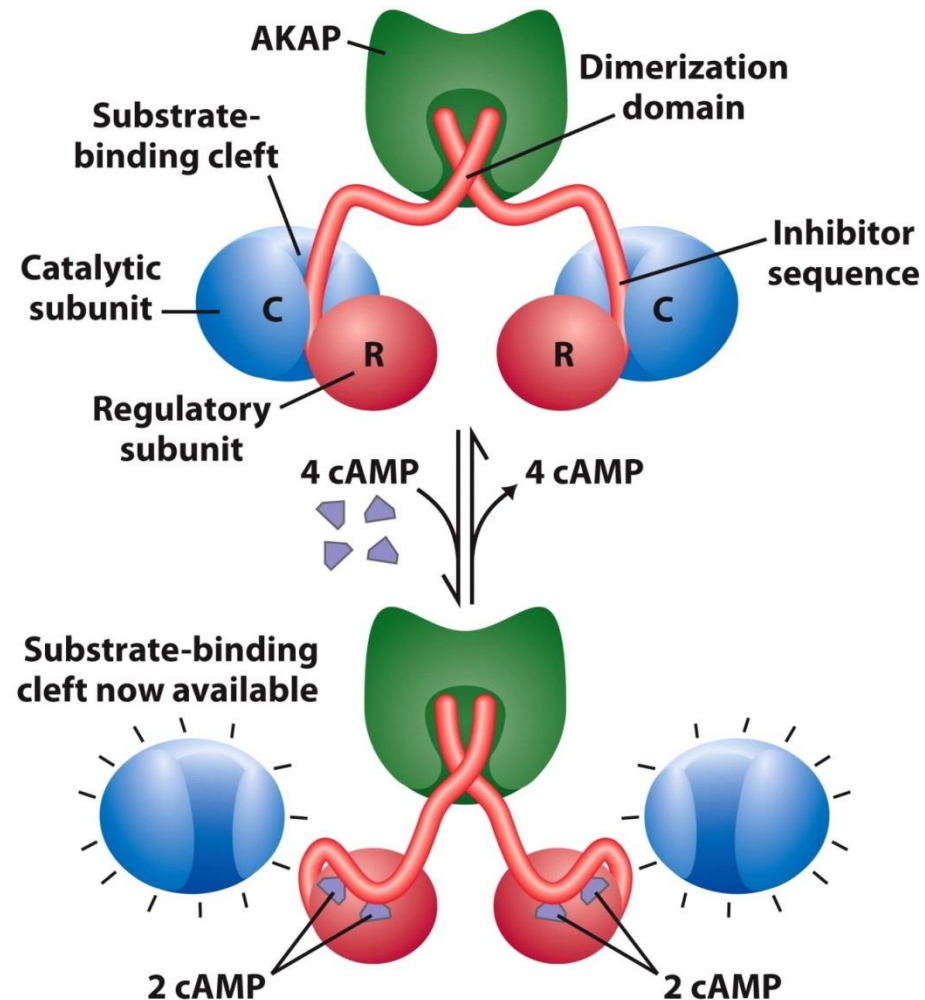
⑧ NET RESPONSE

Phosphorylation of target proteins induces cellular response

Amongst other things,
can induce
phosphorylation cascade

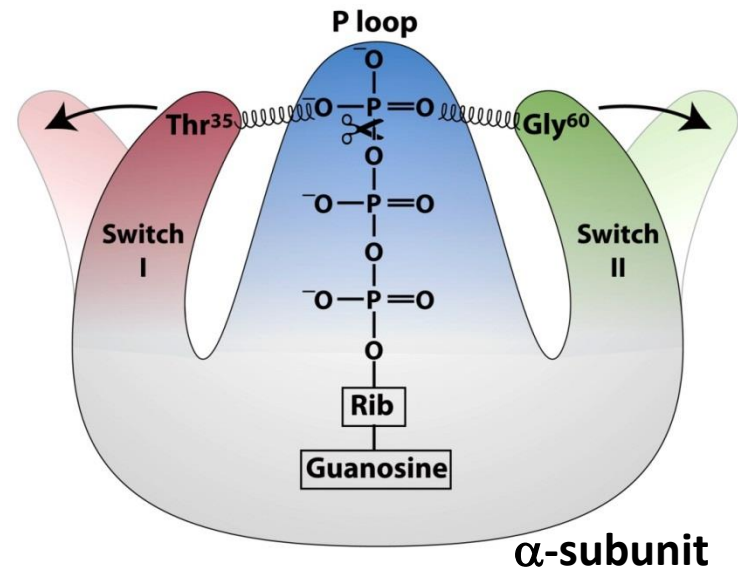
Protein Kinase A

- Elevated levels of cytoplasmic cAMP are required for activation of PKA
- cAMP binds to the regulatory subunits on PKA, removing them from the **active site** and allowing activation of PKA



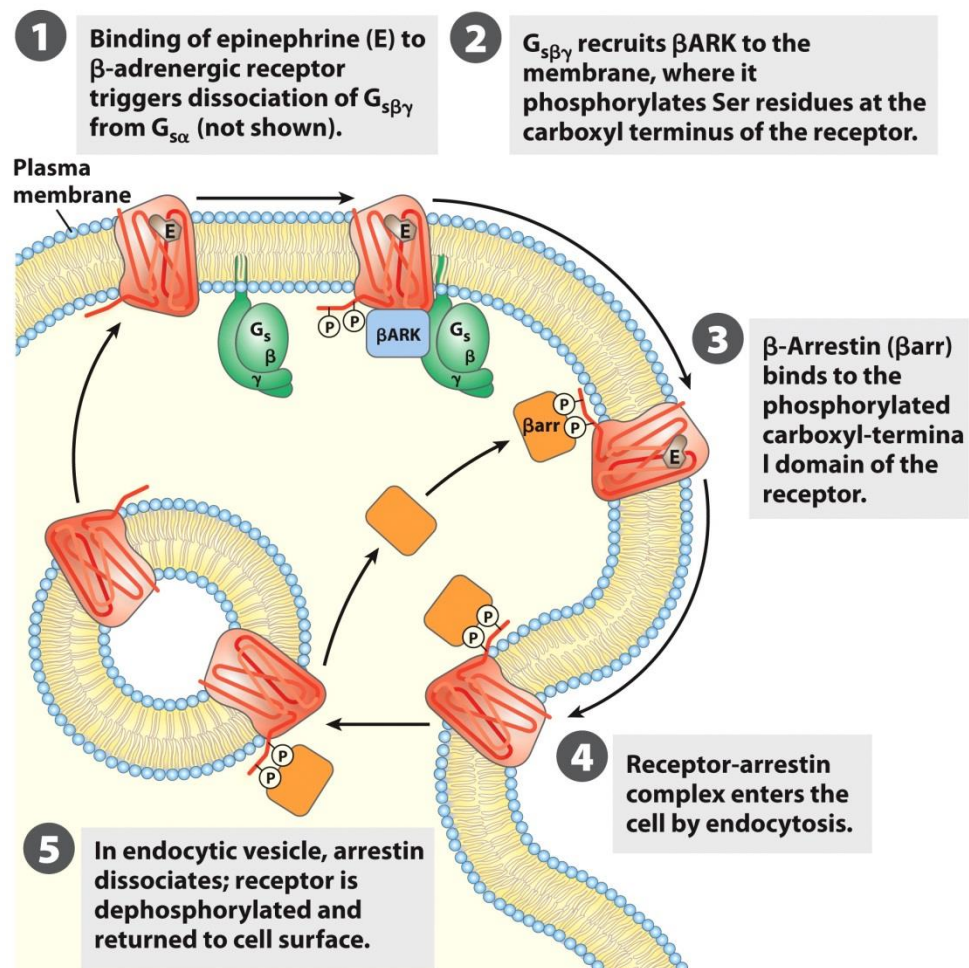
Inactivation of Signal Transduction (Epinephrine)

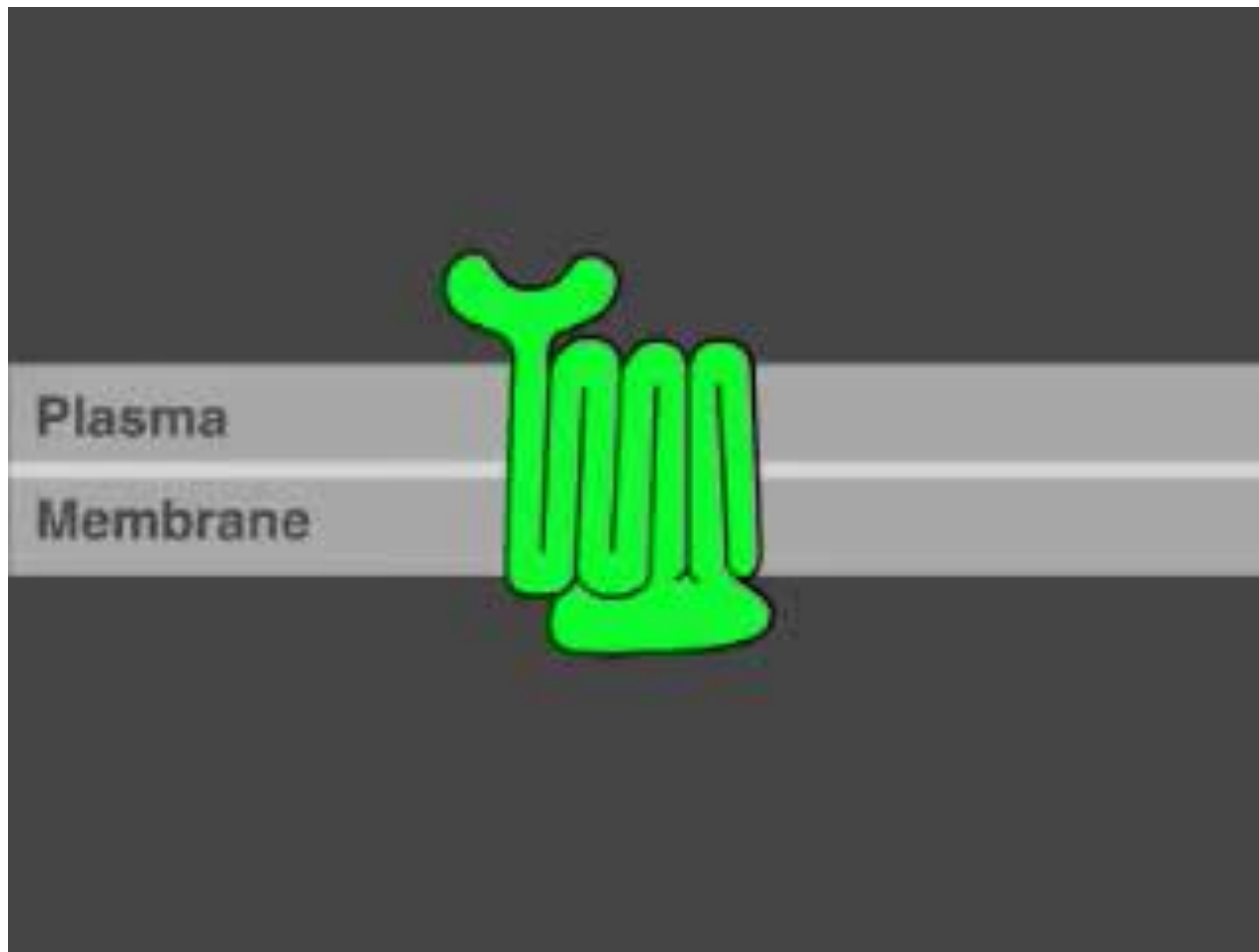
- When the concentration of epinephrine drops below the disassociation constant (K_d) for that receptor, the hormone dissociates
- Self-inactivation of α -subunit occurs through hydrolysis of GTP to GDP by intrinsic GTPase activity (enhanced by regulators of G protein signalling (**RGSs**)).
- Inactivation of cAMP through hydrolysis to 5'-AMP
- Reduced cAMP levels will naturally cause many kinases to revert to their inactive form
- Phosphatases reverse the phosphorylation of signalling proteins



Desensitisation of Signal Transduction (Epinephrine)

- Desensitisation is critical to the bodies ability to respond to new signals:
 - When you cut yourself, at first you feel tremendous pain, but quickly this pain is replaced by a general throbbing
- Adrenergic receptors:
 - β -adrenergic receptor kinase (β APK) phosphorylates the cytoplasmic side of the transmembrane receptor
 - Phosphorylation leads to the binding of the protein β -arrestin (β arr)
 - β arr binding:
 - Prevents further interactions with G-proteins
 - Leads to removal of receptor (eventually returned to membrane)







**Some G proteins
activate
adenylate cyclase**

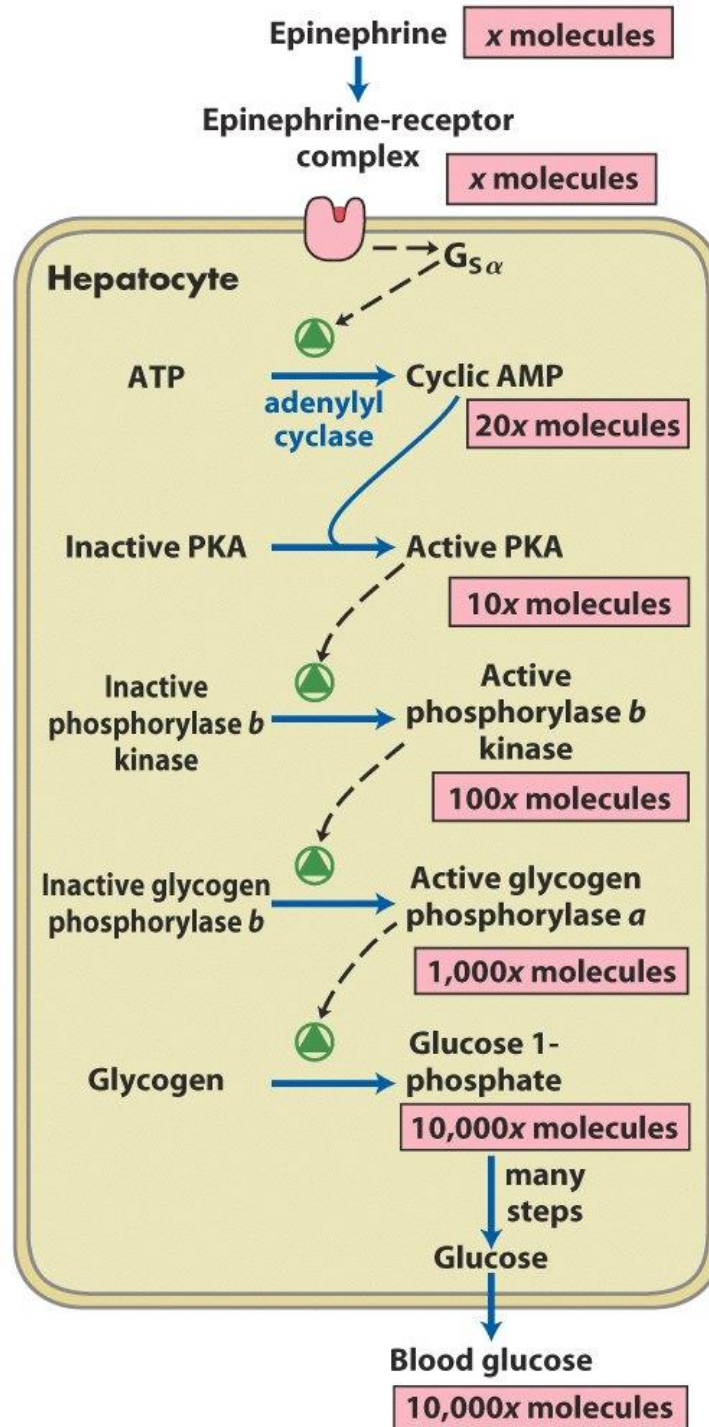


TABLE 12–2**Some Enzymes and Other Proteins Regulated by cAMP-Dependent Phosphorylation (by PKA)**

Enzyme/protein	Sequence phosphorylated*	Pathway/process regulated
Glycogen synthase	RA S CTSSS	Glycogen synthesis
Phosphorylase <i>b</i> kinase α subunit β subunit	VEFRRL S I RTKR S GSV }	Glycogen breakdown
Pyruvate kinase (rat liver)	GVLRRAS V AZL	Glycolysis
Pyruvate dehydrogenase complex (type L)	GYLRRAS V	Pyruvate to acetyl-CoA
Hormone-sensitive lipase	PMRR S V	Triacylglycerol mobilization and fatty acid oxidation
Phosphofructokinase-2/fructose 2,6-bisphosphatase	LQRRRG S SIPQ	Glycolysis/gluconeogenesis
Tyrosine hydroxylase	FIGRRQ S L	Synthesis of L-dopa, dopamine, norepinephrine, and epinephrine
Histone H1	AKRKAS G PPVS	DNA condensation
Histone H2B	KKAKAS R KESYSVYVYK	DNA condensation
Cardiac phospholamban (cardiac pump regulator)	AIRRA S T	Intracellular [Ca ²⁺]
Protein phosphatase-1 inhibitor-1	IRRRR P T P	Protein dephosphorylation
PKA consensus sequence [†]	xR[RK]x[S T]B	Many

*The phosphorylated S or T residue is shown in red. All residues are given as their one-letter abbreviations (see Table 3–1).

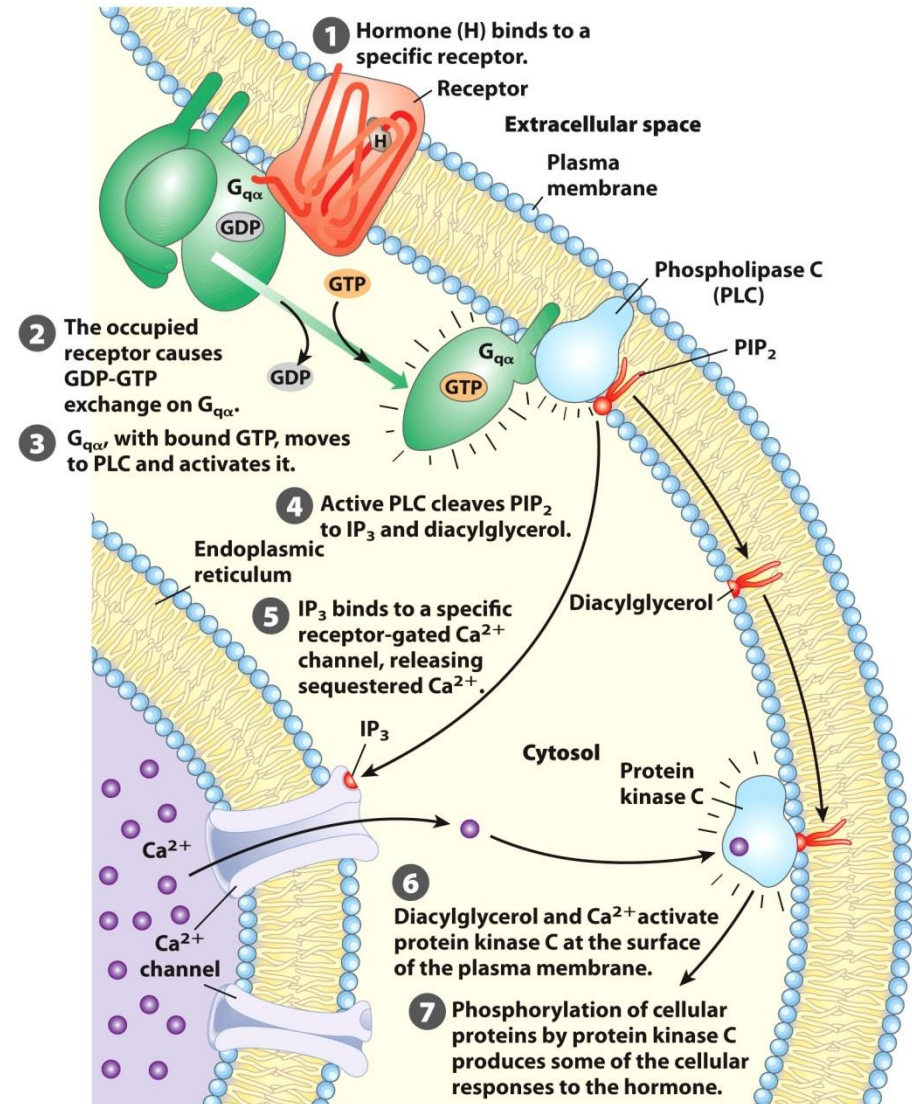
[†]x is any amino acid; B is any hydrophobic amino acid. See Box 3–3 for conventions used in displaying consensus sequences.

Binding of a ligand can have different effects on different cells, based on:

- Whether the receptor for that ligand is present?
- The subtype of receptor present (e.g. α- or β-adrenergic receptors: epinephrine)
- The type of G-protein present at the receptor (e.g. excitatory or inhibitory)
- The set of target proteins present (this is specific to each cell)
- Compartmentalisation of the receptor and its target ligands (effectors)

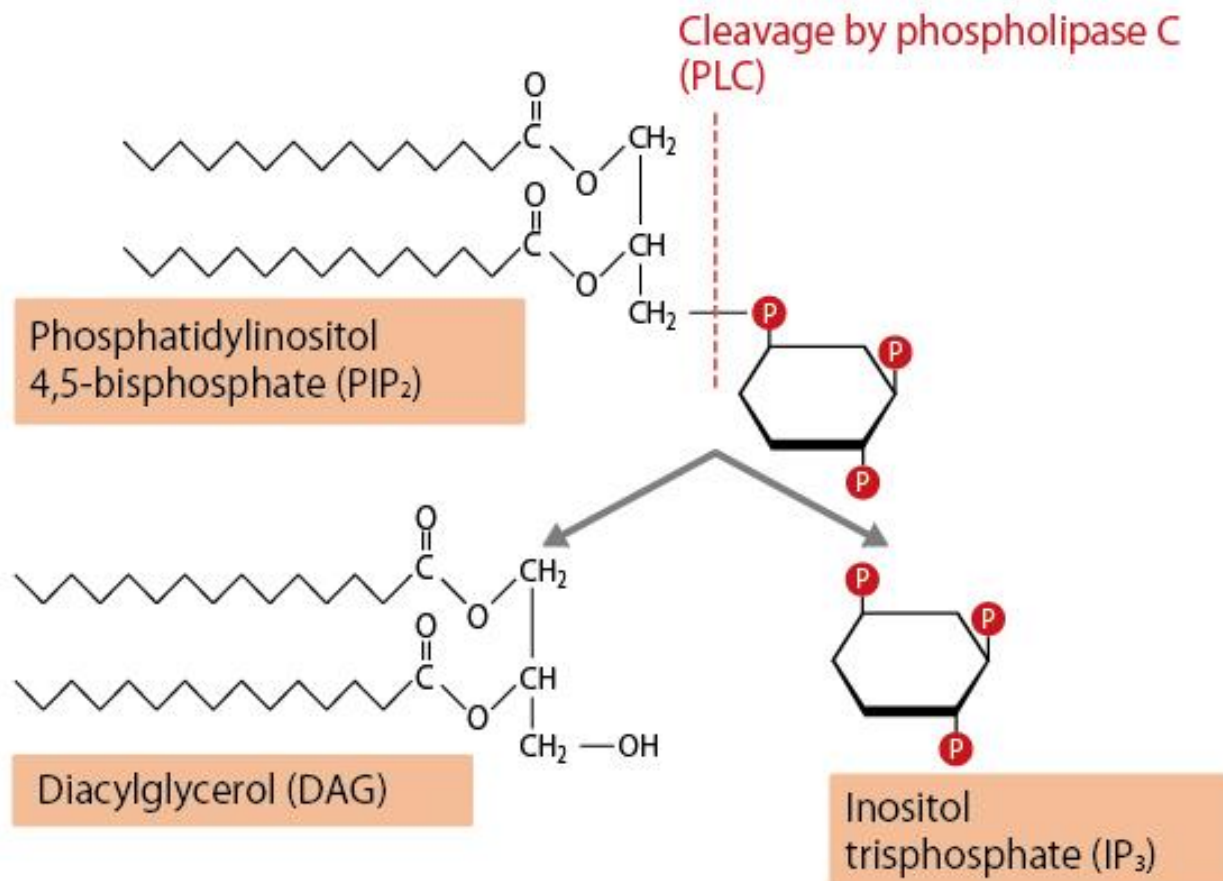
Glycerophospholipid Signalling – PIP₂

- Second class of G-protein coupled receptors:
 - Activation of phospholipase C (PLC) by G-protein cleaves specific glycerophospholipids
 - Commonly: phosphatidylinositol 4,5-bisphosphate (PIP₂)
 - PIP₂ broken into inositol triphosphate (IP₃) and diacylglycerol (DAG).
 - IP₃ (water soluble) diffuses to the endoplasmic reticulum causing the opening of Ca²⁺ channels (increases cytosolic Ca²⁺ levels)
 - Induces numerous outputs including activation of protein kinase C (PKC)
 - DAG also activates PKC
 - PKC phosphorylates proteins



Cleavage of PLP_2 by Phospholipase C

Slide 31 & 32,
Topic 4



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2. Receptor Tyrosine Kinase

- Integral membrane proteins with three domains
 - Extracellular ligand-binding domain
 - Trans-membrane domain
 - Intracellular enzyme = a tyrosine-specific protein kinase
 - When a ligand binds, the kinase becomes active leading to phosphorylation of tyrosine residues within target proteins, changing their activity state
- *E.g.* Insulin Receptor

Insulin

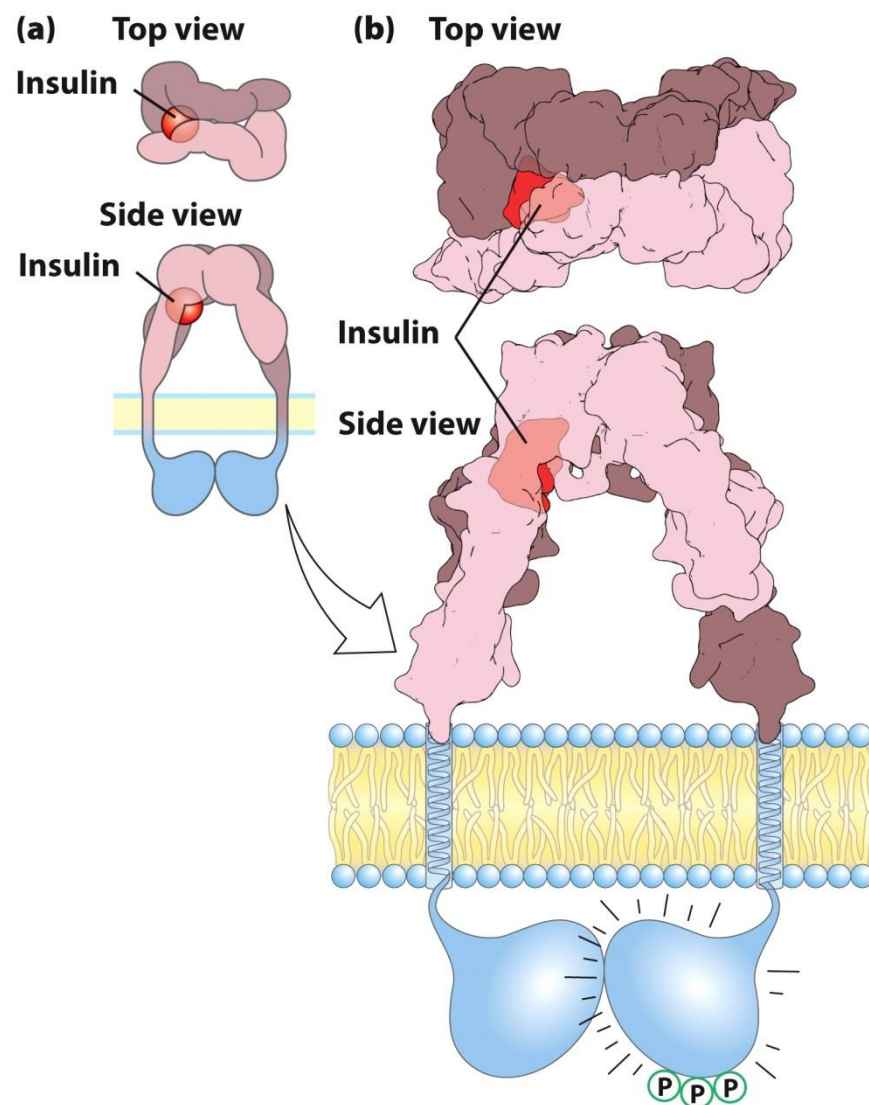
- Hormone synthesised/released from the beta cells of the pancreas in response to high blood glucose levels
 - Insulin causes:
 - Modulation of genes involved in cell growth and division
 - Stimulation of glycogen synthesis
 - Uptake of glucose by liver, muscle and adipose cells
 - Inhibition of glucagon synthesis/release
 - Uptake of amino acids by cells for protein synthesis
 - Up-taken glucose is used for:
 - 1st as energy
 - 2nd Energy storage as glycogen
 - 3rd energy storage as fats

Insulin Receptor

- Four subunits:
 - 2 x α subunits, protrude outside of the membrane, bind hormone
 - 2 x β subunits, span the plasma membrane and protrude into the cytoplasm. Contain tyrosine kinase activity.

Insulin Signal Transduction

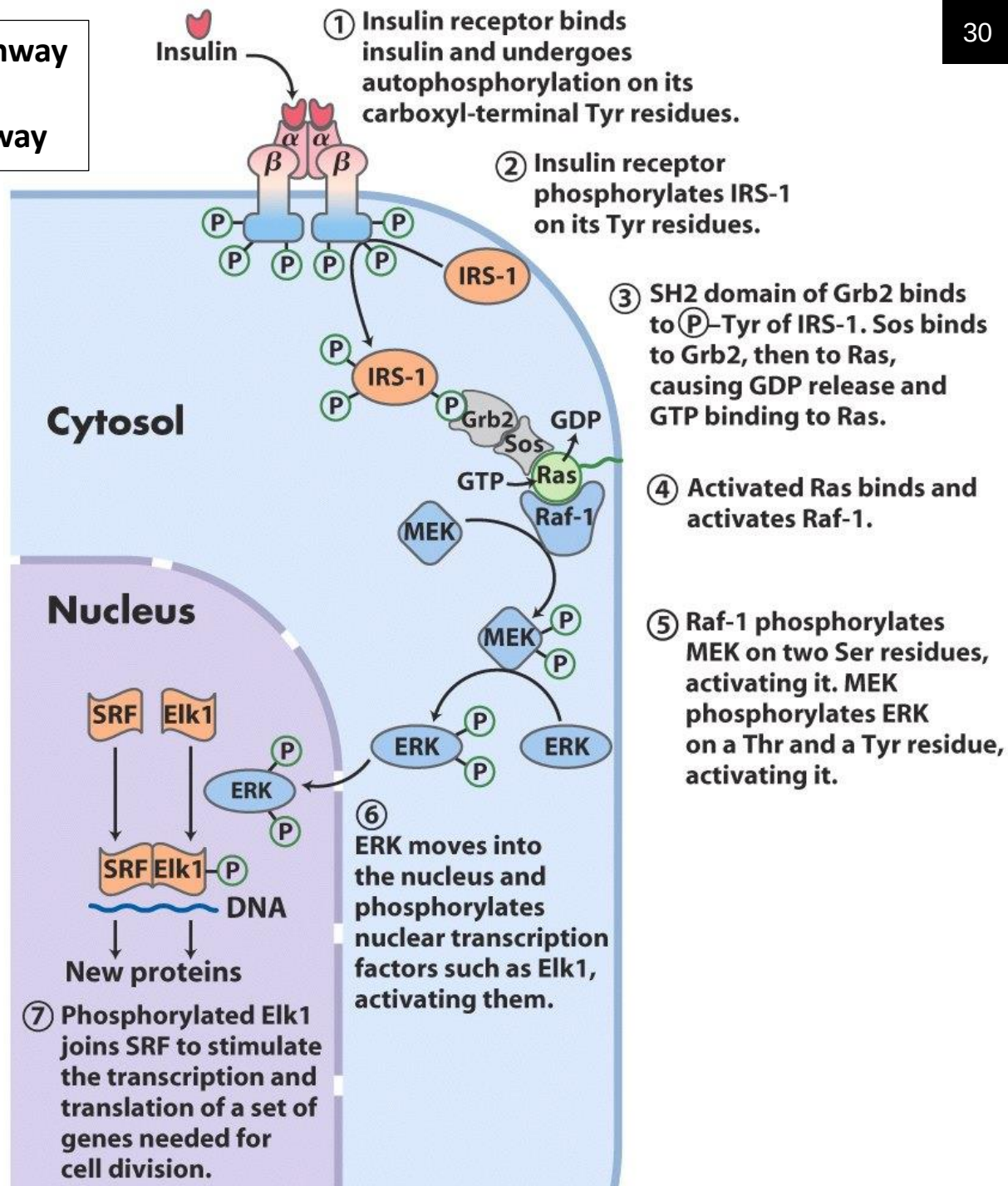
- Hormone binds to receptor (2 x α and 2 x β subunits)
- Each β unit phosphorylates tyrosine residues in the other β unit, transferring phosphate from ATP onto the hydroxy groups of tyrosine.
- This “auto-phosphorylation” opens up the active site of the kinase so that the enzyme can phosphorylate Tyr residues of other target proteins.



Mitogen-activated protein kinase pathway

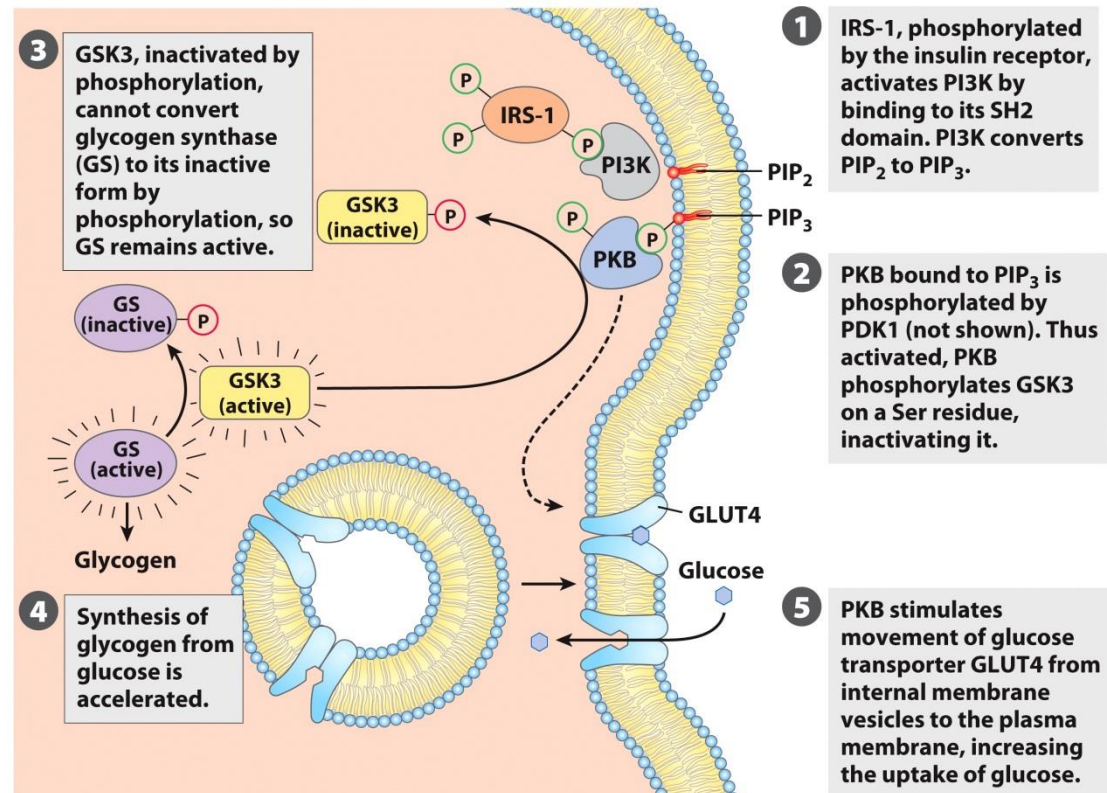
MAPK/ERK or Ras-Raf-MEK-ERK pathway

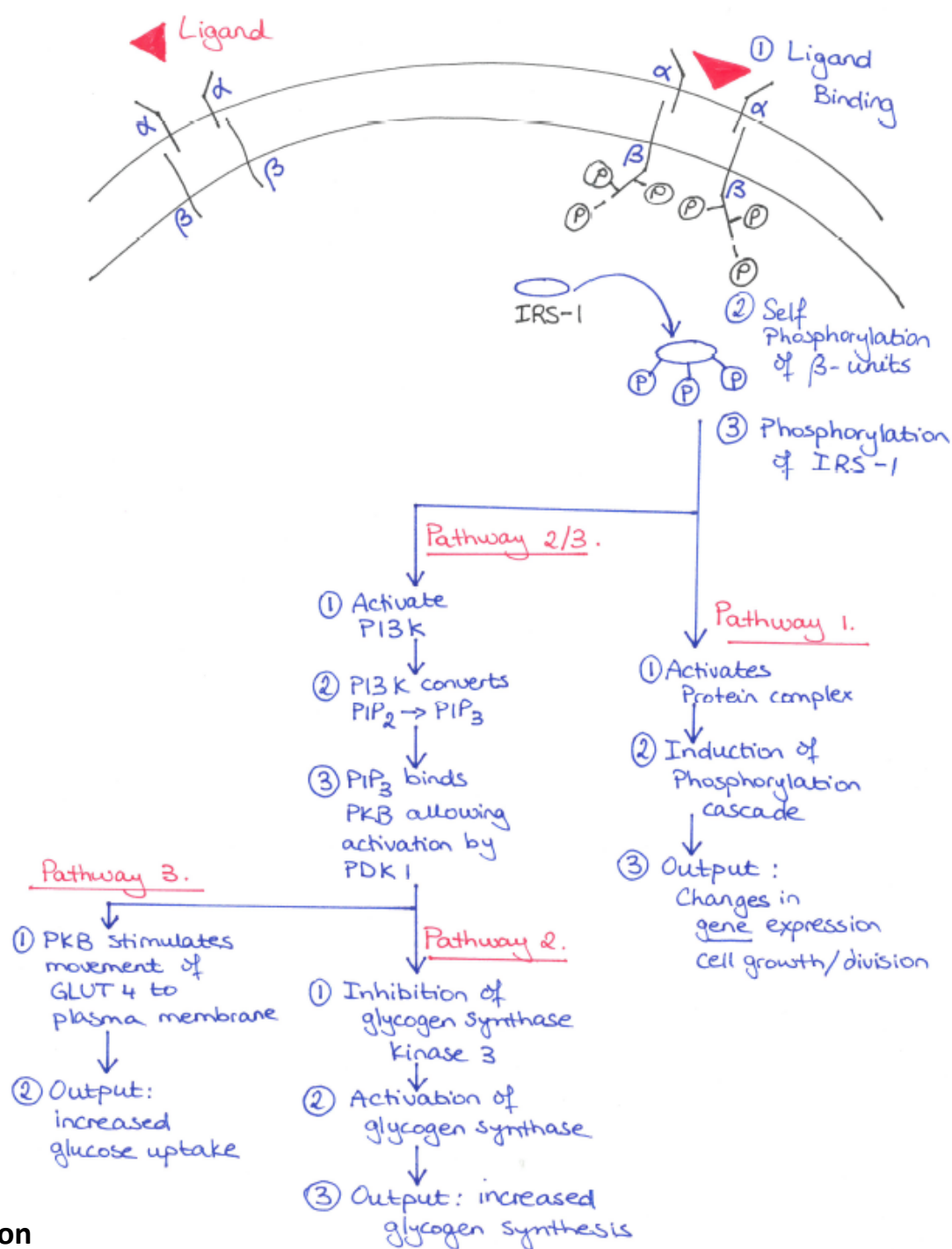
- Insulin binding causing activation of kinase activity through auto-phosphorylation
- Activated kinase phosphorylates Insulin receptor substrate-1 (**IRS-1**)
- IRS-1** activates a number of pathways
- Pathway 1:**
 - IRS-1** activates a specific protein complex
 - Induces phosphorylation cascade (activation of numerous intermediate protein kinases)
 - Output: alterations in the expression of genes associated with cell growth and division



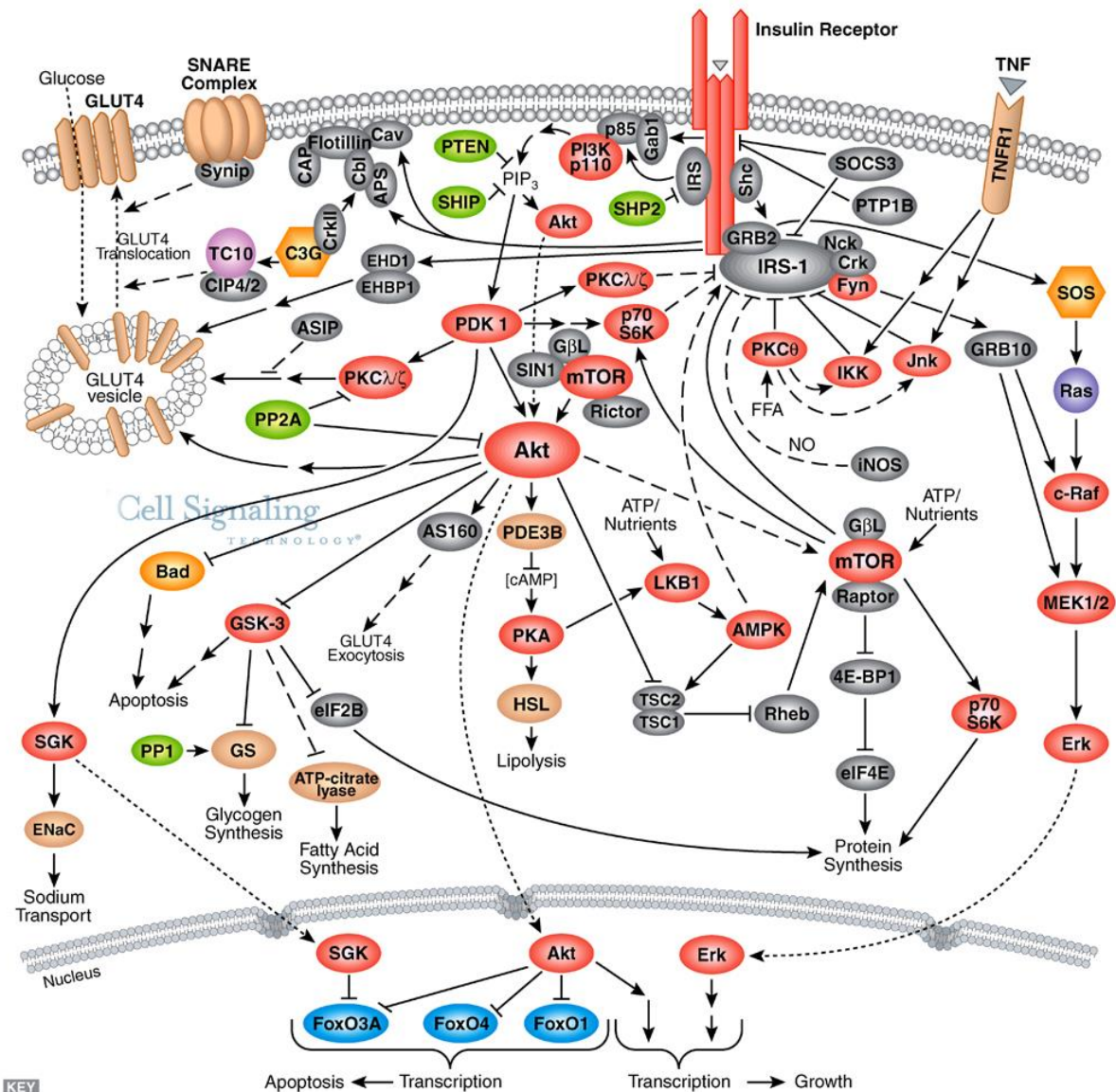
- **IRS-1** activates phosphoinositide 3-kinase (**PI3K**)
- **PI3K** converts the membrane lipid **PIP₂** to **PIP₃**
- **PIP₃** binds protein kinase B (**PKB**) allowing its activation (phosphorylated by PDK1)
- **Pathway 2:**
 - Inhibits the activity of **glycogen synthase kinase 3**
 - Activation of **glycogen synthase**
 - Output: increase glycogen synthesis (e.g. storage of glucose)
- **Pathway 3:**
 - **PKB** stimulates the movement of glucose transporters (**GLUT4**) to the plasma membrane
 - Output: increased glucose uptake.

PI3K/Akt (PKB) pathway





True Complexity of Pathways



KEY

 Kinase Phosphatase

 Transcription Factor

→ Direct Stimulatory Modification

—| Direct Inhibitory Modification

→ → Multistep Stimulatory Modification

→ —| Multistep Inhibitory Modification

— — ➔ Tentative Stimulatory Modification

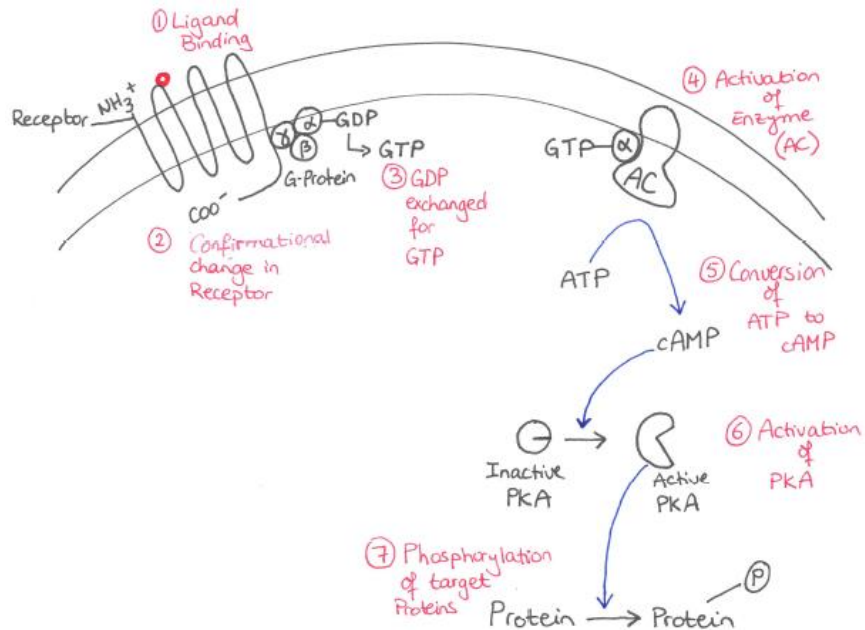
 Transcriptional Stimulation Transcriptional Inhibition

-----> Translocation

→ Separation of Subunits or Cleavage Products

→

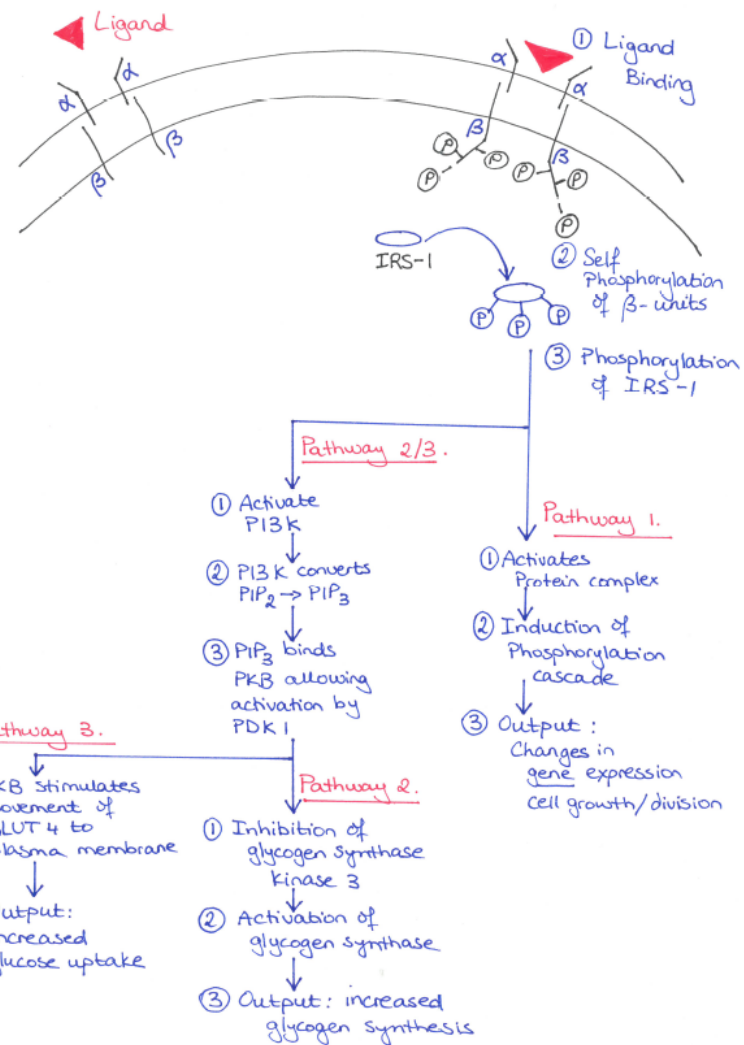
GPCR vs RPK



⑧ NET RESPONSE

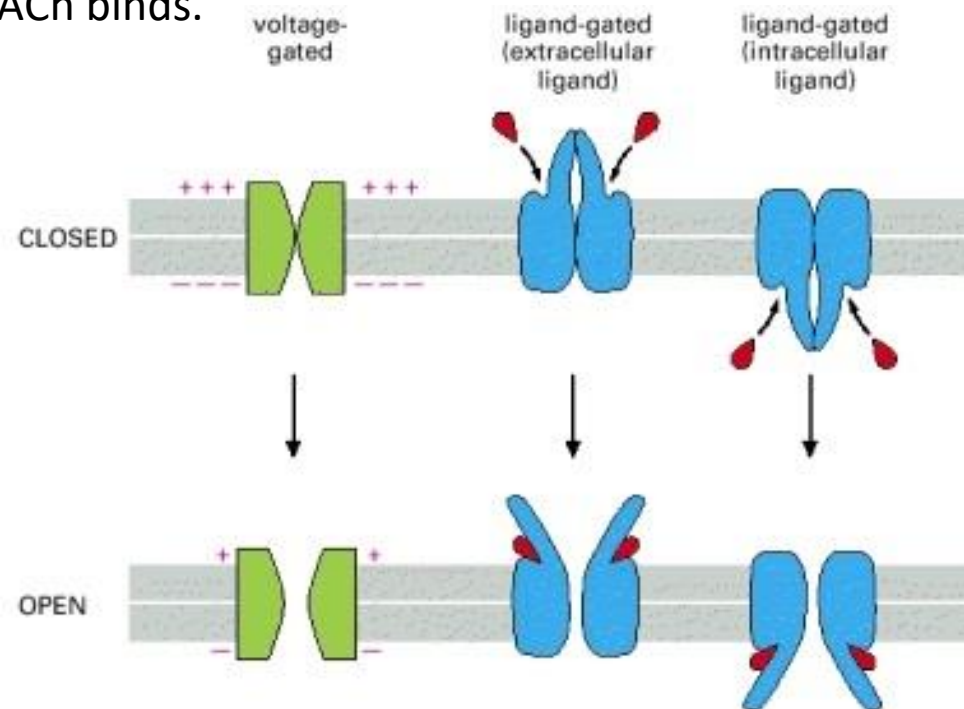
Phosphorylation of target proteins induces cellular response

Amongst other things, can induce phosphorylation cascade

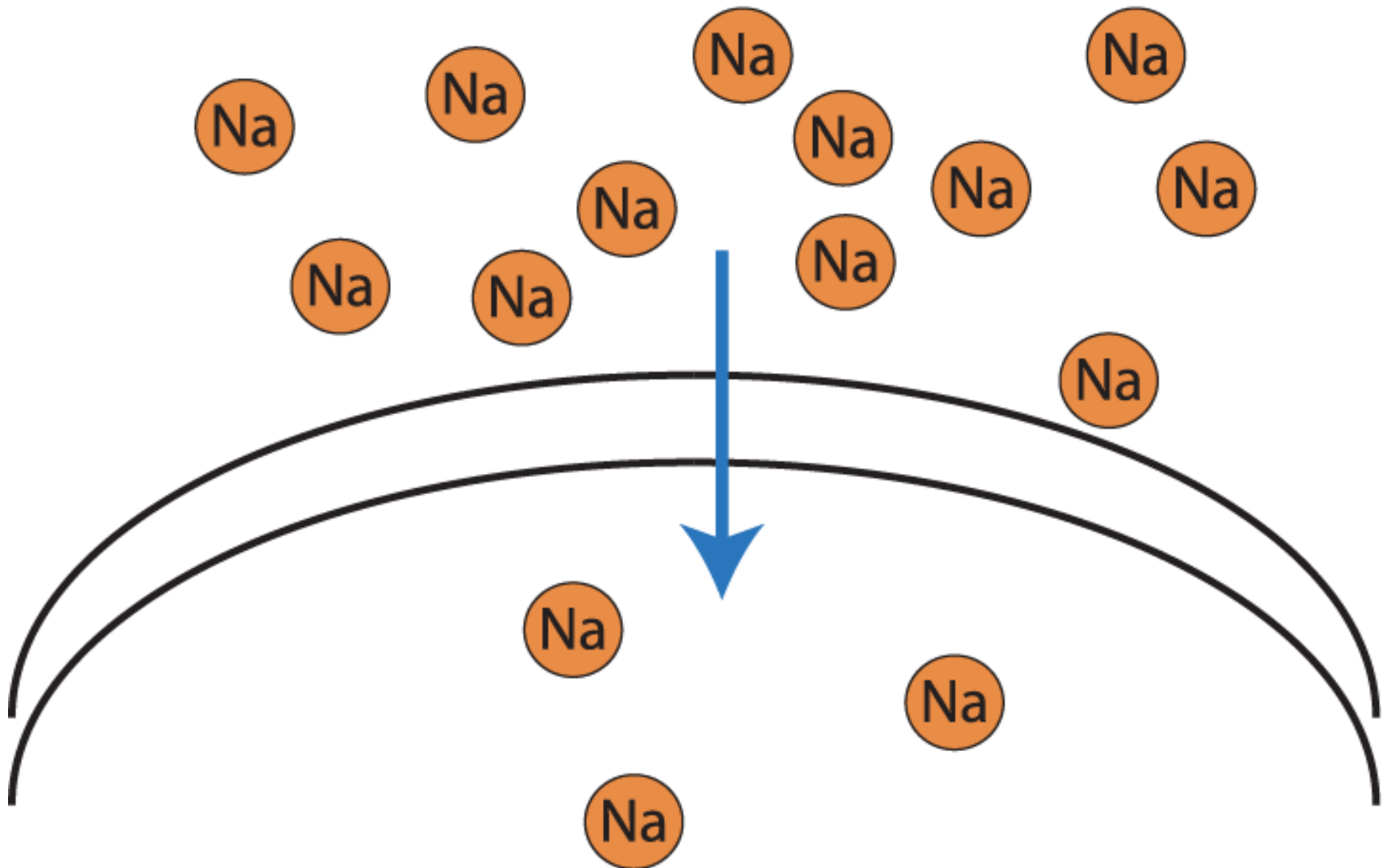


3. Gated Ion Channels

- Gated ion channels are usually triggered to open by either: **voltage** or the binding of a **ligand**
- Examples:
 - Voltage-gated channels:** sodium (Na^+), potassium (K^+), chloride (Cl^-) and calcium (Ca^{2+})
 - Ligand-gated channels:** acetylcholine receptor (AChR). The ion channel component of the AChR opens when ACh binds.
- ACh = Neurotransmitter
 - Released by neurons
 - Diffuses across synaptic cleft to:
 - Post-synaptic neuron
 - muscle cells

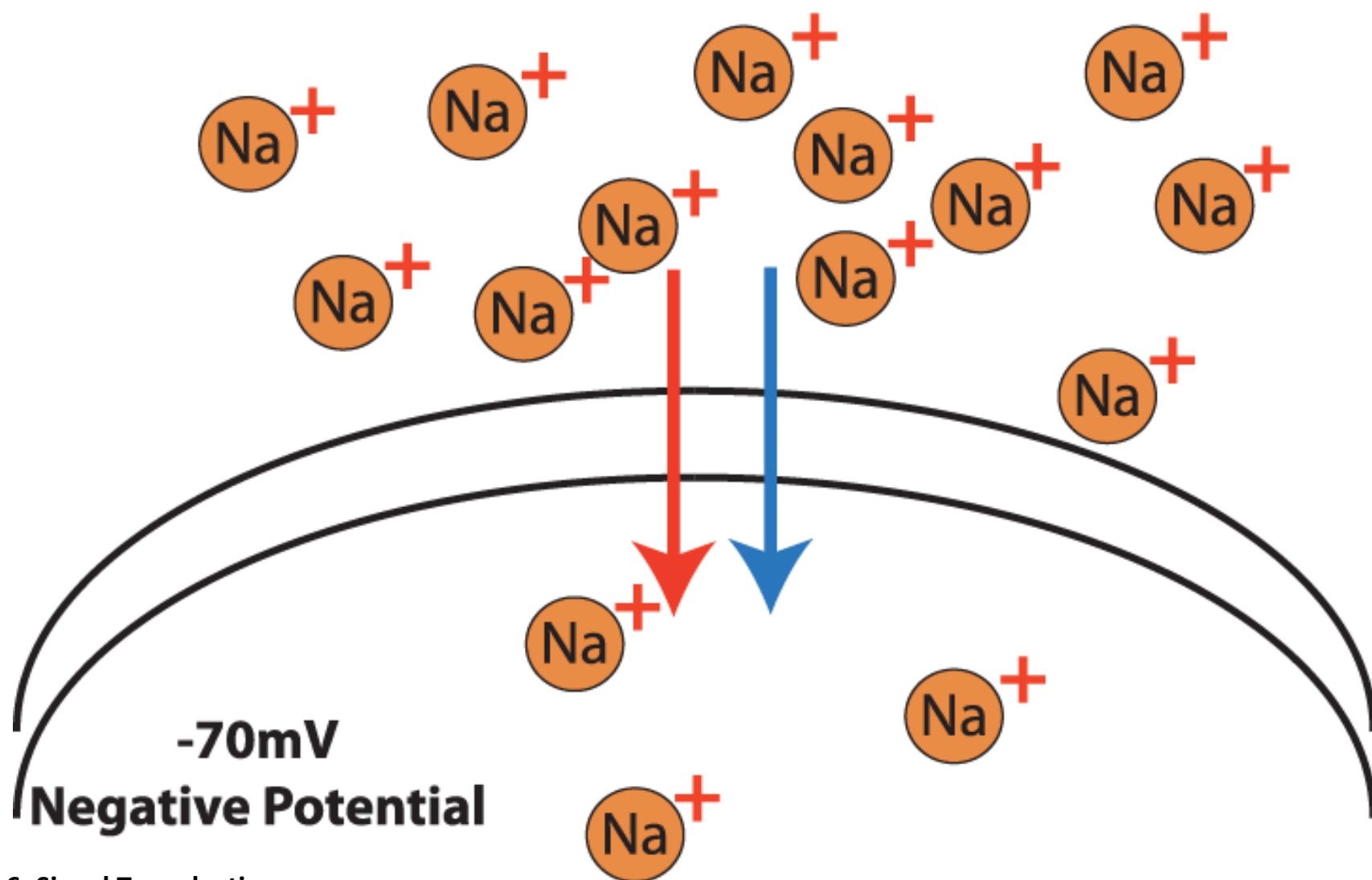


Concentration or Chemical Gradient



Electrical (Charge) Gradient

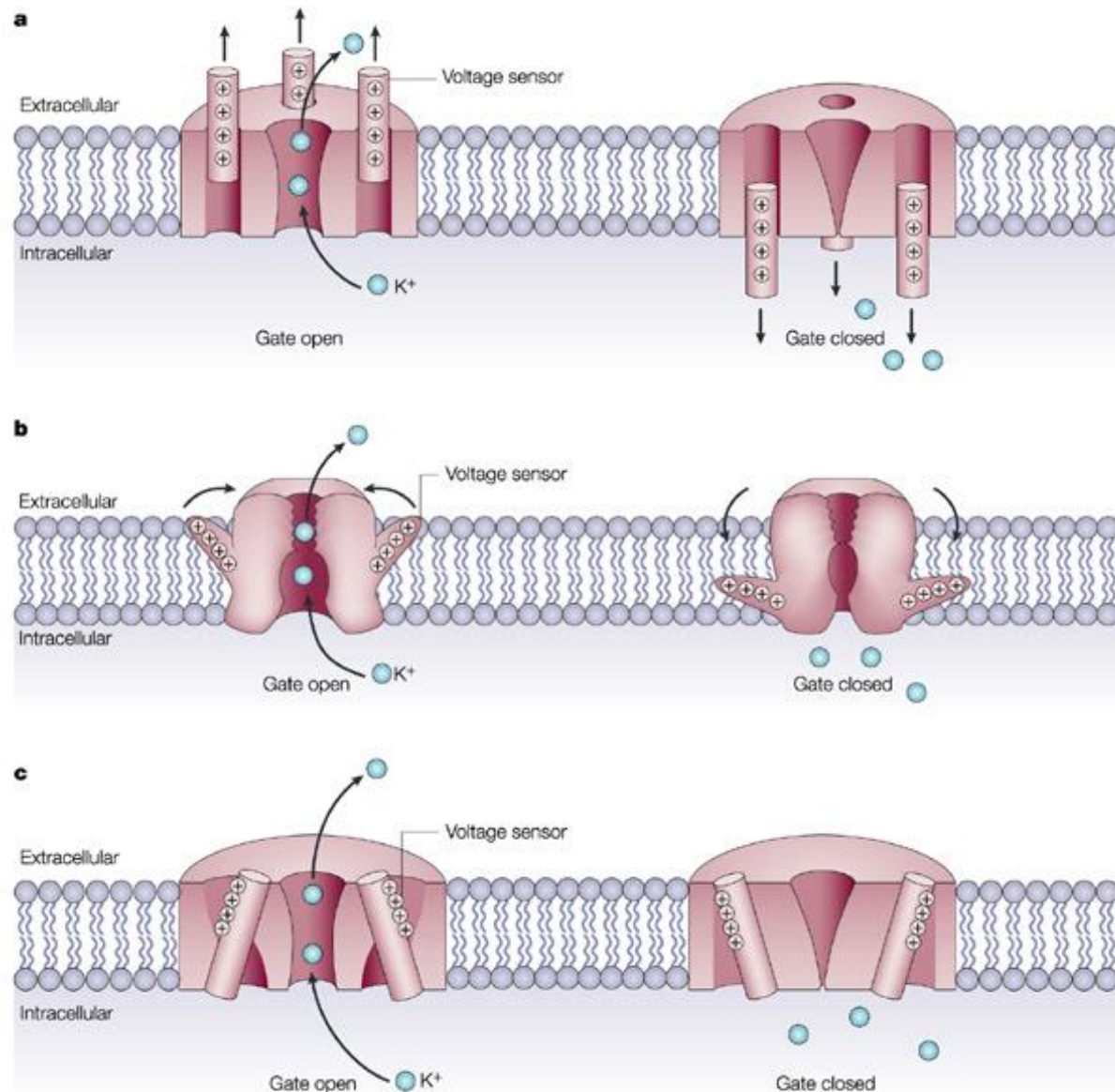
Therefore, ions follow there electrochemical gradient



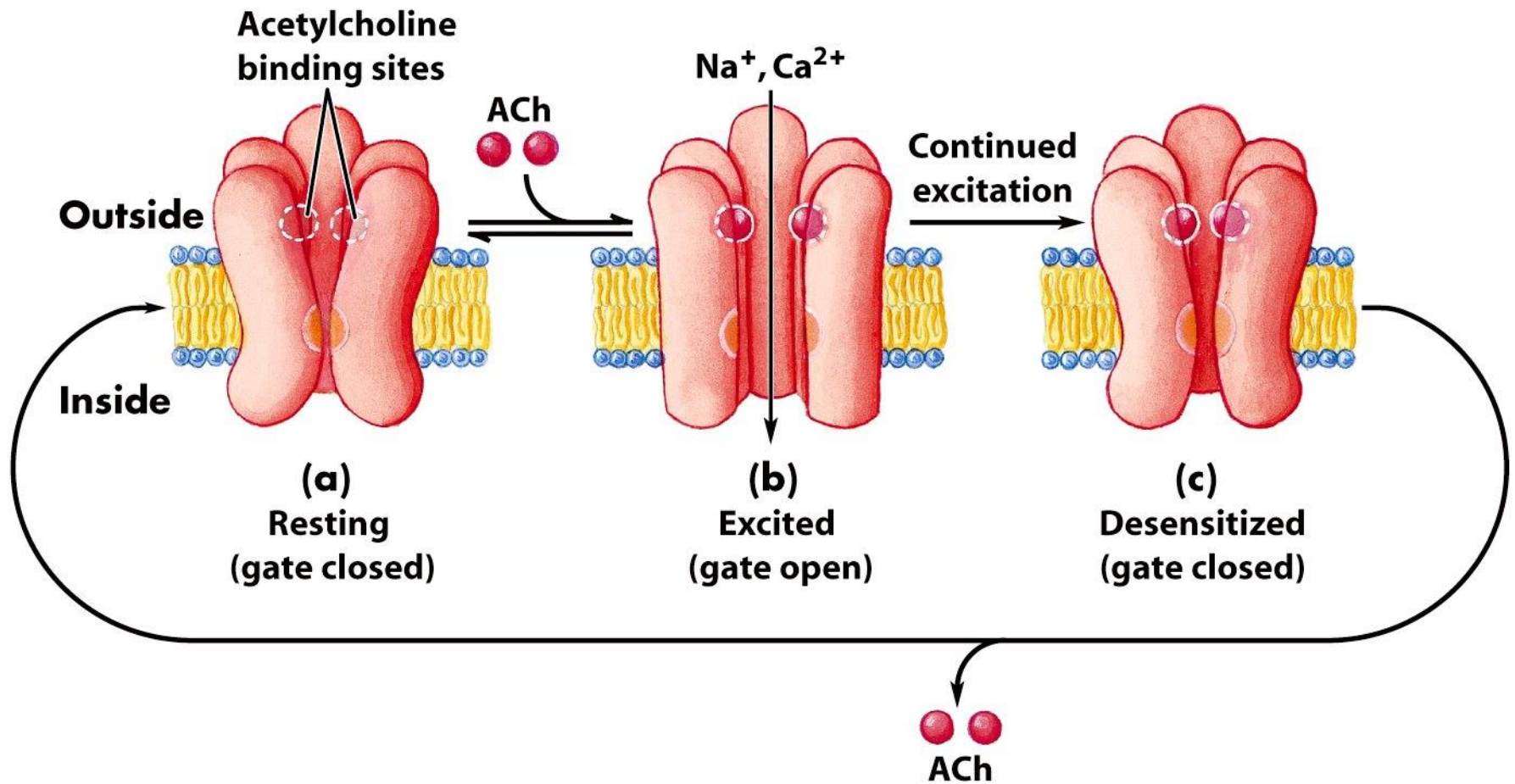
Gated Ion Channels

Possible models of channel opening

- A. The conventional model: voltage sensors move across the membrane like a 'peg in a hole'.
- B. Paddle model: paddles, composed of an α -helical hairpin, pivot against the membrane like levers.
- C. The transporter-like model: the voltage sensors do not translocate across the membrane, but simply pivot along their longitudinal axis.

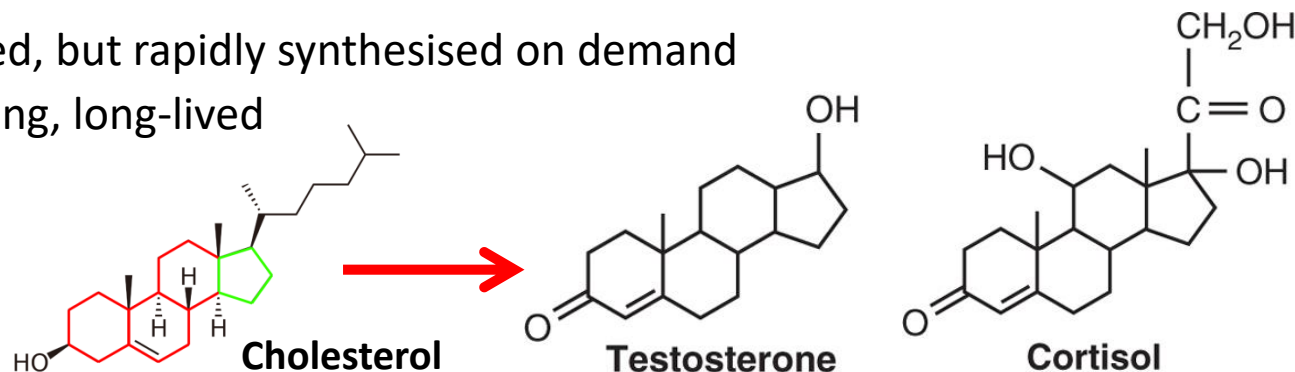


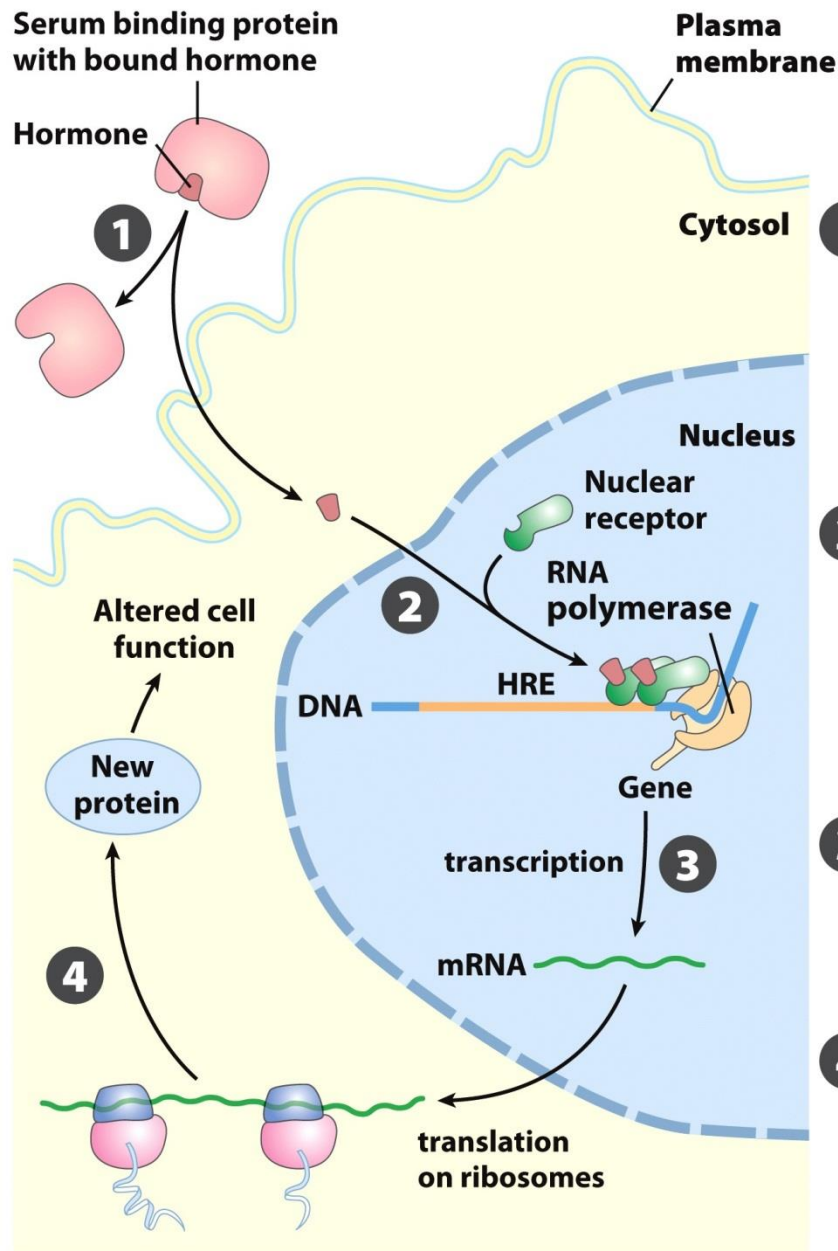
Ligand Gated Channels



4. Steroid and Nuclear Receptors

- **Water soluble ligands**
 - *E.g.* insulin, adrenaline
 - Can't diffuse into cells
 - Bind to plasma membrane bound receptors (e.g. **G-protein couples receptors**)
- **Lipid soluble ligands (steroids)**
 - *E.g.* testosterone, estrogen, cortisol etc.
 - Hydrophobic
 - Require carrier protein in blood (inactive until released)
 - Can freely diffuse across the cell membrane
 - Bind to protein receptors within the cytoplasm or nucleus
 - Commonly alter gene expression
 - Typically synthesised from cholesterol
 - Generally not stored, but rapidly synthesised on demand
 - Normally, slow acting, long-lived





1 Hormone, carried to the target tissue on serum binding proteins, diffuses across the plasma membrane and binds to its specific receptor protein in the nucleus.

2 Hormone binding changes the conformation of the receptor; it forms homo- or heterodimers with other hormone-receptor complexes and binds to specific regulatory regions called hormone response elements (HREs) in the DNA adjacent to specific genes.

3 Receptor attracts coactivator or corepressor protein(s) and, with them, regulates transcription of the adjacent gene(s), increasing or decreasing the rate of mRNA formation.

4 Altered levels of the hormone-regulated gene product produce the cellular response to the hormone.

You should be able to....

- Outline what is meant by the term “signal transduction”
- Describe the process of phosphorylation and dephosphorylation of proteins and its role in signal transduction
- Describe, using examples, different mechanisms for signal transduction (i.e. G-protein coupled receptor, receptor tyrosine kinase, gated ion channels and steroid receptors)
- Compare and contrast different signal transduction mechanisms

What's next?

- ✓ • Topic 1 – Foundations in Biochemistry
 - ✓ • Topic 2 – Amino Acids
 - ✓ • Topic 3 – Proteins
 - ✓ • Topic 4 – Lipids and Membranes
-
- ✓ • Topic 5 – Signal Transduction
 - Topic 6 – Enzymes
 - Topic 7 – DNA structure, replication and repair
 - Topic 8 – Transcription
 - Topic 9 – Translation

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