

Week 12

Cell Signalling

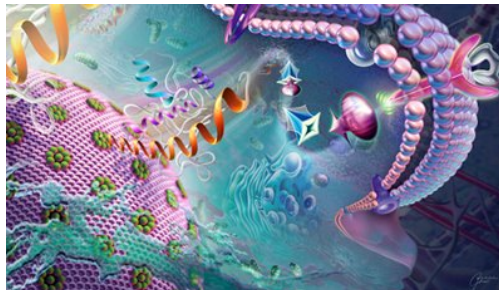


Suggested Reading: Chapter 15 Karp 9th ed.

15.1 Basic elements of cell signaling

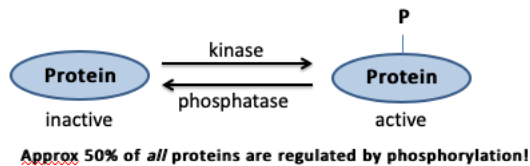
15.2 Survey of messengers and receptors

15.3 G-protein-coupled receptors



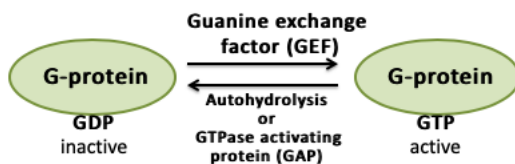
1

A reminder of kinase/phosphatase and G protein 'switches'



Protein kinases transfer a phosphate from ATP to a protein. This usually activates the protein.

Protein phosphatases remove the phosphate, reversing the 'switch'.



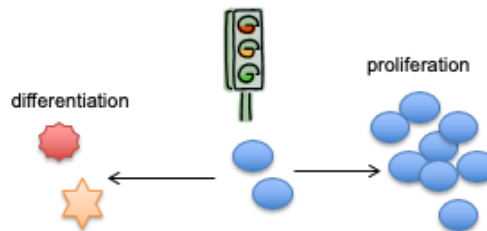
G-proteins are inactive when bound to GDP. The GEF enzyme swaps the GDP for a GTP, activating the G-protein.

There are two ways G-proteins switch 'off'. They have a slow intrinsic GTPase activity and will self-hydrolyze the GTP to GDP. If the proper GAP is present, the GAP will greatly speed up the process. So, G-proteins are more like 'timers' than 'switches'. We've already seen two of these (hint hint).

2

The importance of signalling

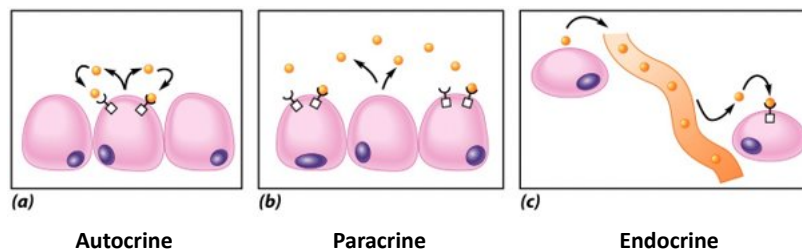
- Living organisms constantly receive and interpret signals from their environment. Signal transduction is the process of converting external signals into a cellular response through transmembrane receptors
- Cells of multi-cellular organisms receive signals from other cells, including signals for cell division and differentiation. The majority of cells in our bodies must constantly receive signals that keep them alive and functioning.
- Signal transduction allows for the alteration of gene expression and protein activity in response to environmental signals.
- It allows for communication between the different cells of an organism
- It allows for cells to adapt to their environment



3

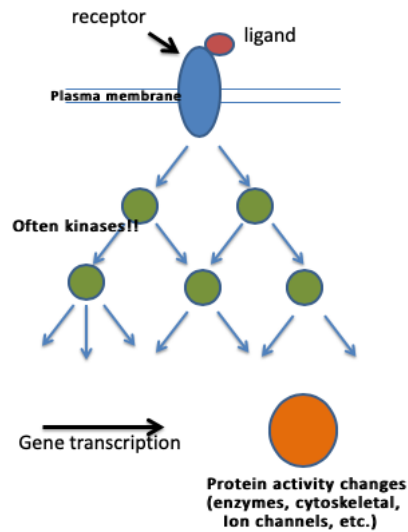
Types of extracellular signaling

- In **autocrine** signaling the cell has receptors on its surface that respond to an extracellular messenger it releases.
- In **paracrine** signaling, the extracellular messenger travel short distances to nearby cells through the extracellular space.
- In **endocrine** signaling, extracellular messengers (i.e. hormones) can travel long distances through the bloodstream and target distal cells.



4

The general idea of cell signalling



- 1. A ligand binds to a receptor on the PM. This causes a conformational change in the receptor on the cytosolic side (a process called signal **transduction**).
- 2. This triggers a cascade of effects in the cytosol. These cascades **amplify** the signal inside the cell. Often kinase activity is involved.
- 3. The final 'layer' of the proteins in the cascade trigger effects in the cell. These can include alteration of transcription of genes, or changes in the activity/function of proteins.

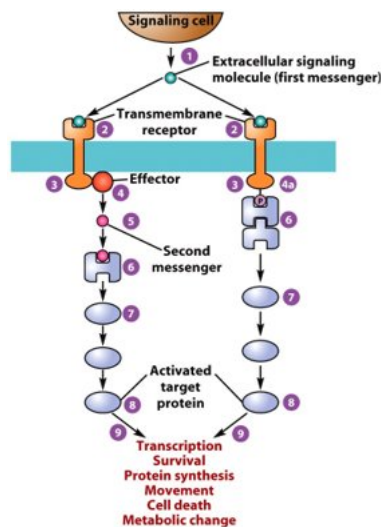
5

Two main ways signaling (in general) works

The activation of the receptor activates an effector protein.

The effector makes a soluble **second messenger** which diffuses into the cell.

The second messenger triggers the signal cascade leading to cell effects.



The activation of the receptor forms a 'recruiting station' that in turn recruits other proteins.

These proteins trigger the signal cascade.

Example: glucagon signaling

Example: MAP kinase cascade

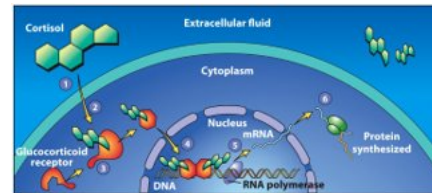
6

Types of ligands

Ligand = any molecule that binds to a receptor that triggers signaling

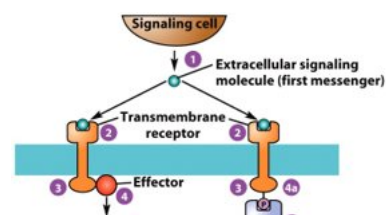
Hydrophobic ligands

- Often made from cholesterol
- can cross the cell membrane
- Thus, the receptors are *inside* the cell
- We've already seen an example of this (PEPCK activation in the transcription factor lecture)



Lipophobic ligands

- Many types (proteins, peptides, small molecules)
- Can't cross the cell membrane
- Bind to integral membrane receptors



7

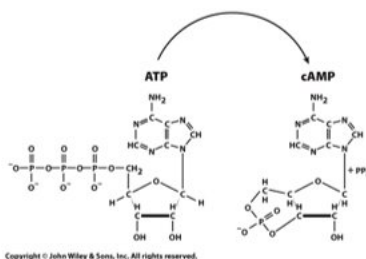
Second messengers made by different effectors

There are thousands of different receptors

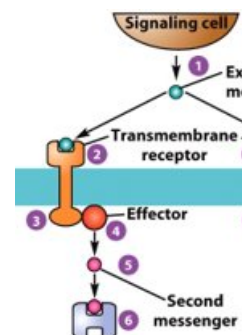
There are only a handful of primary second messengers, of which we'll discuss two

1. Cyclic AMP (cAMP)

- Made by the effector **adenylyl cyclase**
- Adenylyl cyclase is an integral membrane protein



cAMP can readily diffuse into the cytosol and trigger downstream effects

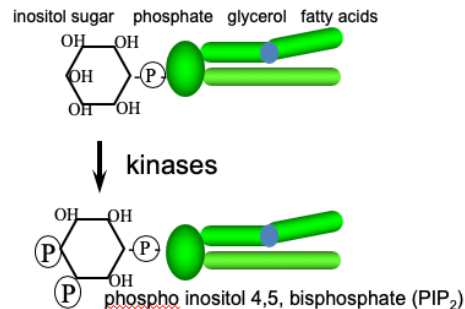


8

Second messengers made by different effectors

2. Inositol phosphates

- Derived from phosphatidylinositol
- The inositol portion can be phosphorylated by kinases
- Seven different possible P patterns

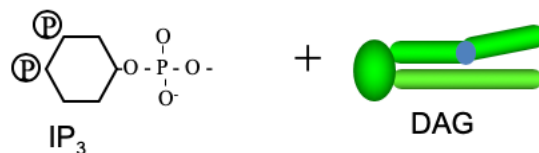


This is the substrate for the effector (next slide!)

9

Second messengers made by different effectors

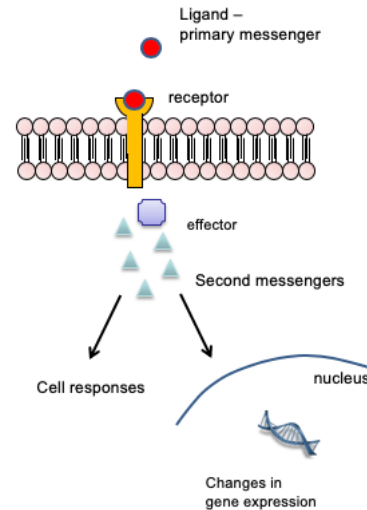
- The main lipid second messengers are produced by the effector phosphatidylinositol-specific **phospholipase C**.
 - abbreviated **PI-PLC**
- produces two signal molecules:
 - **Diacylglycerol (DAG)**, which stays within membrane
 - and **Inositol triphosphate (IP₃)**, highly soluble, enters cytoplasm



10

Lipophobic (water soluble) signals

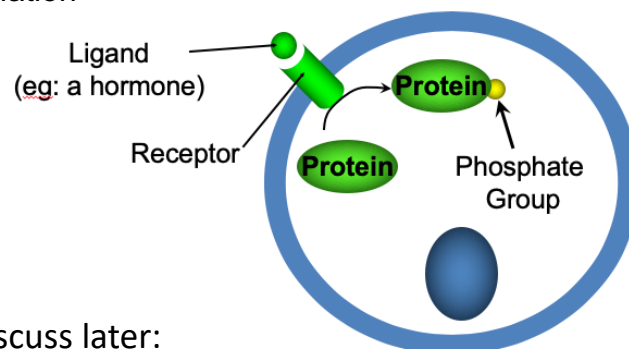
- Extracellular signals are *transduced* and *amplified*
- *Transduction*:
 - Process by which a signal crosses the membrane or is transferred to other molecules
- *Amplification*:
 - Process by which the effect of a signal is multiplied/increased



11

Signal transduction can occur two ways

- Occurs one of two ways:
 - via direct phosphorylation

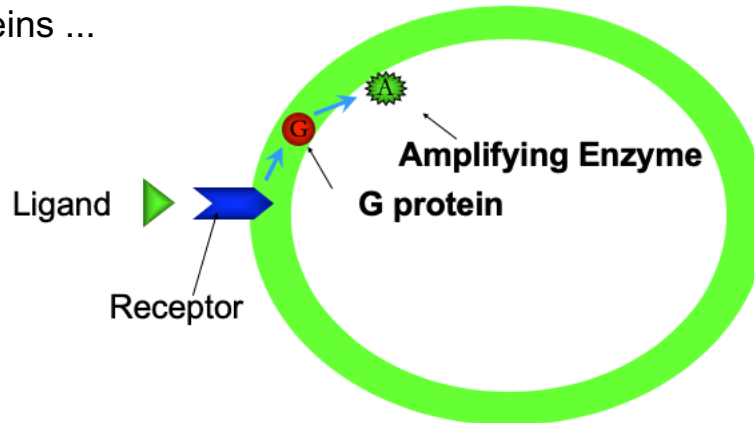


- An example we will discuss later:
RTK Receptors

12

Signal transduction can occur two ways

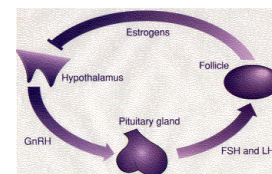
- A far more common form of transduction is via “G” proteins ...



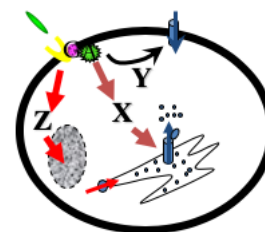
13

Why do cells amplify a signal?

- Amplification of the signal permits
 - the initial signaling molecule (hormone) to be in limited concentrations and still be effective
 - one hormone to activate numerous enzymes
 - e.g. each protein kinase (a second messenger) can activate several MORE kinase molecules.
 - coordination of several different pathways simultaneously, as all are induced by a single signal



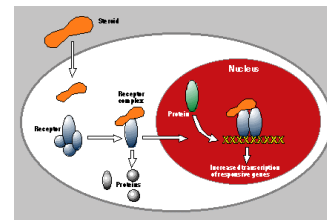
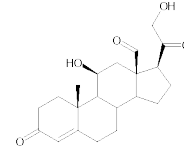
Concentration of a hormone is very dilute when it reaches the target tissue



14

The steroid hormones (endocrine signals)

- Steroid hormones - small and fat soluble
 - made from cholesterol
 - travel through bloodstream bound to carrier proteins
 - enter cells by diffusion
 - include glucocorticoids (including cortisol), estradiol, testosterone, progesterone.
- Easily enter cell
 - high-affinity receptors present in cytoplasm or nucleus
 - hormone receptor complex binds to DNA at promoter regions upstream of certain genes
 - transcriptional enhancement * → gene expression increases
 - relatively slow, longer term response

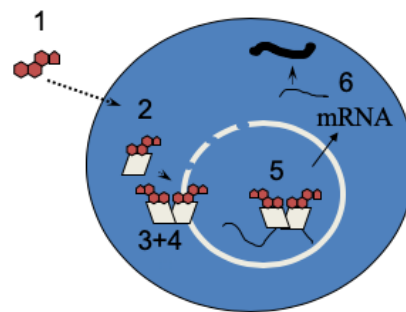


* the receptor-steroid complex releases HDACs and recruits histone acetylases, relieving chromosome repression

15

Review: action of cortisol on PEPCK gene expression

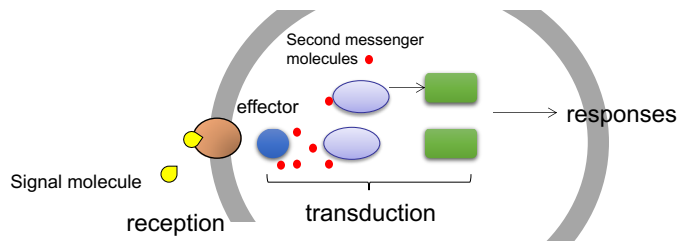
- cortisol is released from adrenal gland in response to stress. Cells respond by gene expression, causing change in sugar metabolism, providing more energy to the cell.
- action:
 1. diffuses into cell
 2. binds specific receptor (protein)
 3. receptor dimerizes
 4. dimer enters nucleus
 5. dimer binds to GRE response element on PEPCK promoter (and other genes)
 6. increases transcription rate, enzyme amount and enzyme activity → gluconeogenesis



16

Lipophobic hormones (signaling molecules) cannot enter the cell

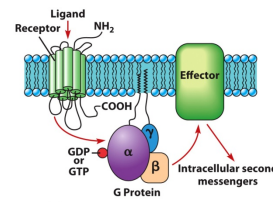
- water-soluble hormones, proteins, neurotransmitters, cell-cell contacts
- process of **Signal transduction** occurs; the external signal is different from internal cellular signals
- **Signaling pathways**; a series of sequential steps
 1. recognition of stimulus by cell-surface receptor (specific, sensitive)
 2. transfer across the membrane
 3. **effector** (e.g. adenylyl cyclase) molecules or a series of molecules on interior of membrane or cytosol create the next message (cAMP, or calcium ions); usually a series of intervening steps before ultimate response (activations/inactivations)
 4. cessation of the response when signal disappears. (eg. cAMP phosphodiesterase)



17

G-Protein Coupled Receptors

- “G-protein” = GTP-binding protein
- Many hormones work through GPCRs
- As many as 2000 different GPCRs
 - many putative GPCR genes identified
 - no known ligands for many
- *Caenorhabditis* (nematode):
 - 1/20 of all the proteins are GPCRs
- Humans:
 - one quarter of all prescription drugs work through GPCRs

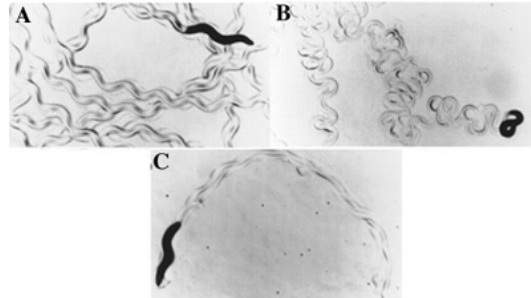


18

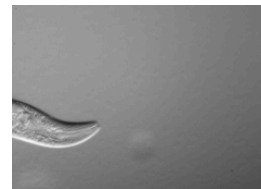
GPCRs are easily studied using mutant nematodes

Mutations in *C. elegans* G proteins can cause easily detected changes in the worm's movements:

- A** – normal worm
 - Sinusoidal movement
- B** – mutant G-protein causes hyperactivity
 - Sidewinding, twisting
- C** – another mutant G-protein causes lethargy
 - Slow, limited tracks



Tracks left in the bacterial food of wild type animals (A), hyperactive *goa-1* animals (B) and lethargic *egl-30* animals (C).



<http://www.ucl.ac.uk/lmcb/pages/nurrish.html>

19

G-Protein Coupled Receptors

- A family of integral proteins, all have seven trans-membrane α -helix segments
- All work in the same way
 - through the heterotrimeric G-proteins
 - α , β , γ subunits
 - turn on effector molecule which makes the second messenger
 - E.g. epinephrine and glucagon turn on adenylyl cyclase to make cAMP (2nd messenger).
 - others (e.g. acetylcholine) use phosphoinositol, DAG second messengers
 - others (e.g. photoreceptors) use cyclic GMP

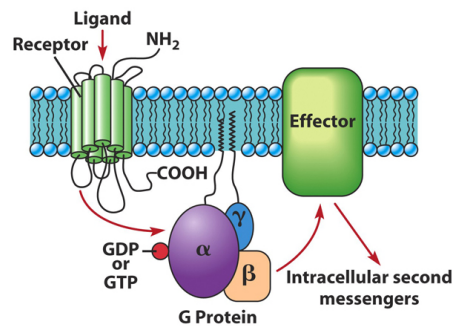


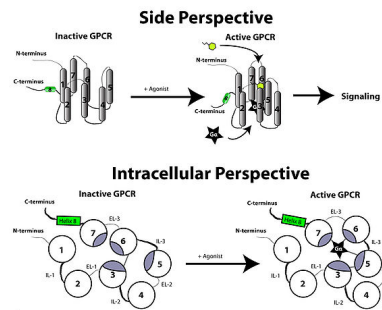
Figure 15-34 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Note: $G\alpha$ and $G\gamma$ are lipid-anchored proteins

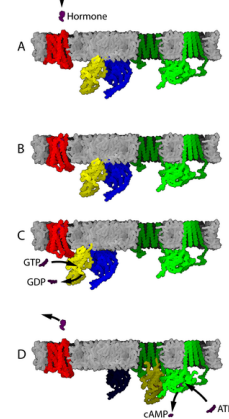
20

Some visual models of how GPCRs work

- Binding of the ligand causes a conformational change in GPCR – allowing G protein to interact
- The downstream effector proteins are nearby



Images acquired from http://en.wikipedia.org/wiki/G_protein-coupled_receptor



21

GPCR action in 8 steps

1. When receptor combines with ligand, receptor changes shape and binds the α subunit of the G protein
2. *Activation of the G protein:* α subunit then exchanges a GDP for a GTP, entering activated state
 - the receptor/ligand can activate several G proteins, as long as ligand is bound
3. *Relay:* the α subunit dissociates from β, γ and associates with effector, producing second message
 - β, γ stay together
 - second message is made for duration of binding

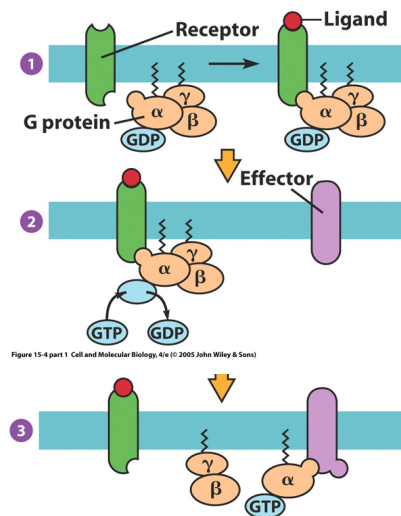


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22

GPCR action in 8 steps

4. Activated effector produces second messenger (eg. adenylyl cyclase makes cyclic AMP = cAMP)

5. α subunit hydrolyzes GTP into GDP, thereby deactivating itself

6. α subunit binds other two subunits - now inactive

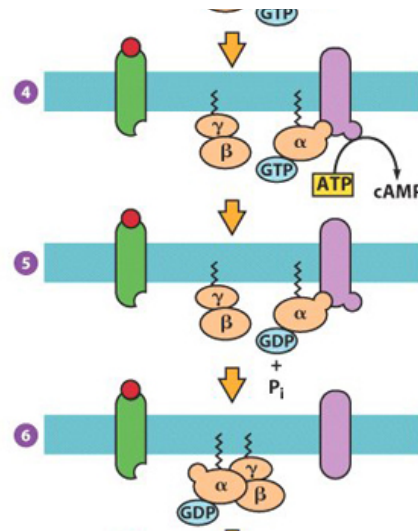


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23

GPCR action in 8 steps

To prevent overstimulation, activated receptors need to be blocked from interacting with G-proteins

7. G-protein-coupled receptor kinase (GRK) phosphorylates receptor

8. Arrestin protein binds to phosphorylated receptor to prevent G-proteins from binding – “desensitization”

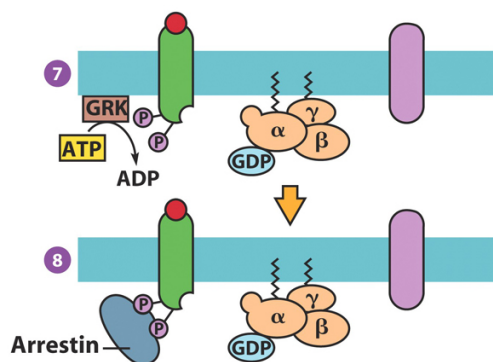


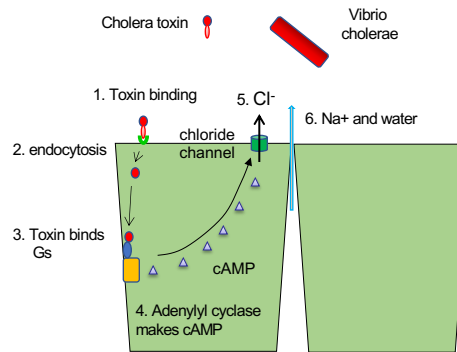
Figure 15-4 part 7 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

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24

G-proteins have different effects

- Different types of G-proteins, each with different $G\alpha$ subunits.
 - $G_s\alpha$ stimulates adenylyl cyclase
 - $G_q\alpha$ activates phospholipase C
 - $G_i\alpha$ inactivates adenylyl cyclase
- The $G_{\beta\gamma}$ complex can also activate other effectors.



Cholera toxin specifically binds $G_s\alpha$

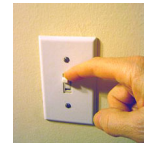
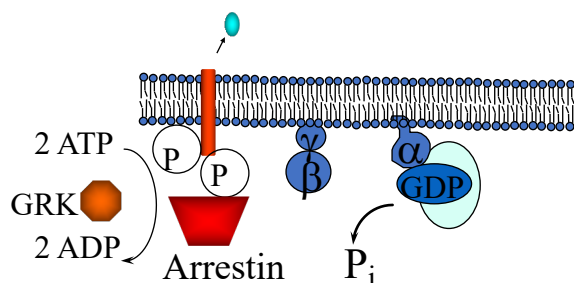
Locks it into the active state (cannot hydrolyze GTP)

Adenylyl cyclase remains active all the time

cAMP induces chloride channel to remain open, and water follows by osmosis

25

Stopping signalling



- G-protein hydrolyzes GTP, inactivates itself
- receptor is phosphorylated, becomes inactive
- phosphorylated receptor binds arrestin,
- arrestin binds to clathrin – endocytosis of receptor occurs

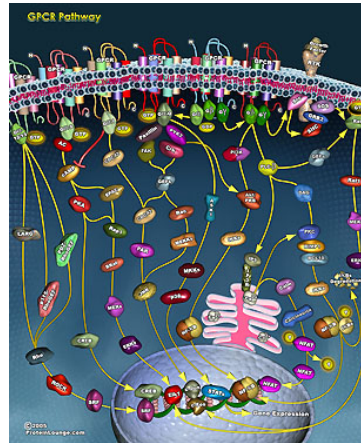
26

What you should know after this section

- Five types of cell signalling
- Difference between lipophilic and lipophobic signal molecules
- Define signaling transduction
- Why and how are signals amplified?
- Describe a G-Protein Couple Receptor's structure and function
- The role of GPCRs in inducing second messengers
- How does GPCR-mediated signalling stop?

27

Week 13 Cell Signalling II



- Suggested reading – Karp 9th edition, Chapter 15
- Regulation of blood glucose levels
- GPCRs in sensory perception

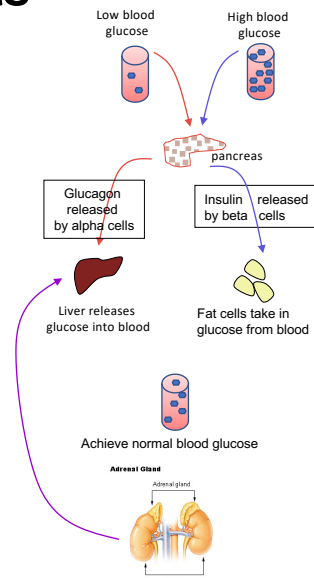
28

Regulating glucose metabolism glucagon and epinephrine effects

- Utilization of glucose
 - primary energy source
 - stored as insoluble polymer, glycogen
 - glycogen conversion to glucose is promoted by hormones
 - glucagon (released from pancreas), boosts blood glucose
 - epinephrine (adrenal gland), boosts blood glucose during stress

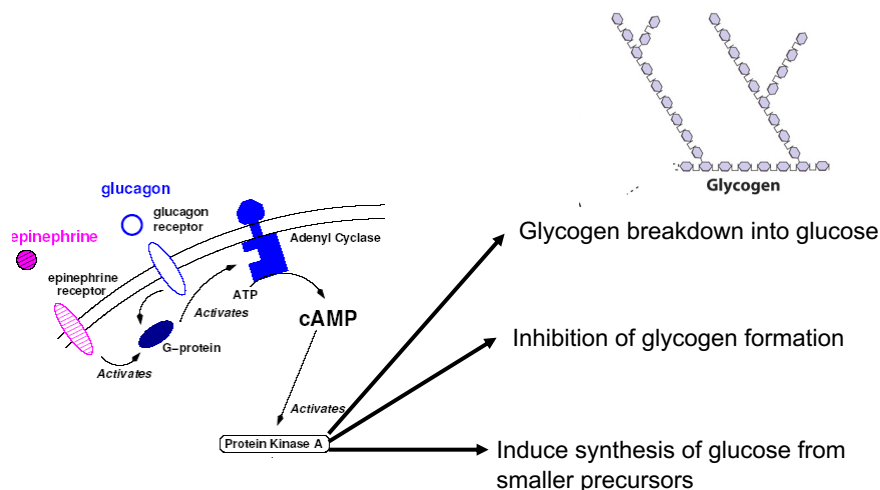
To produce glucose:

- Promote breakdown of glycogen to glucose-1-phosphate (first step in catabolism)
- Glucose is either catabolized or sent to bloodstream for delivery to other places.
- Inhibit glycogen synthase: this enzyme makes glycogen, so it has to be turned off in order for the cells to release or burn glucose.



29

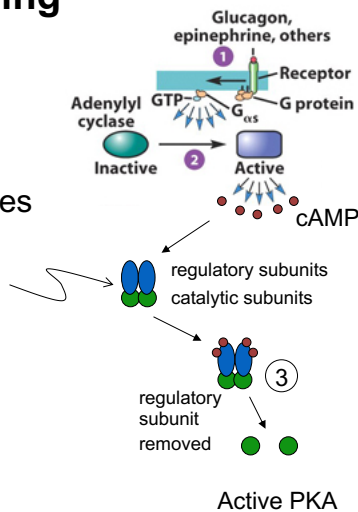
Glucagon and epinephrine (adrenaline) bind to different GPCRs, but the GPCRs then both activate adenylyl cyclase



30

Glucagon/epinephrine signalling

1. Hormone binds to receptor which binds to G-protein
2. Activation of effector: **Adenylyl cyclase**, formation of cAMP, diffuses into cytoplasm
3. cAMP binds to **Protein kinase A (PKA)** & activates it – how?

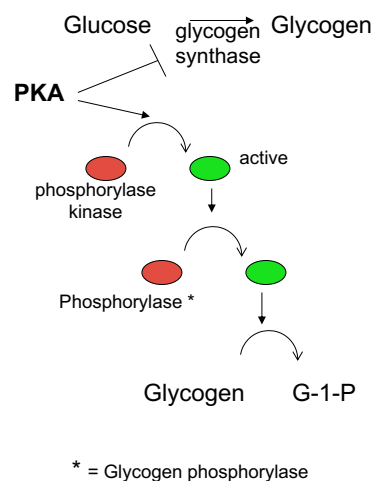


PKA is a tetramer with 2 regulatory subunits and 2 catalytic subunits; cAMP removes the inhibitory regulatory subunits

31

Glucagon/epinephrine signalling

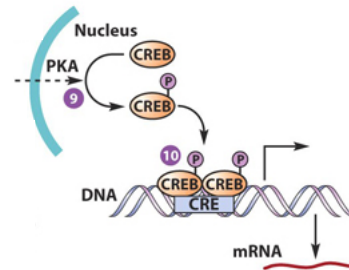
4. PKA phosphorylates target1: **Glycogen synthase**, inactivating it. Glycogen no longer produced
5. At same time, PKA phosphorylates the enzyme **Phosphorylase kinase**, activating it
6. the phosphorylase kinase then phosphorylates target enzyme: **Phosphorylase**, activating it,
7. phosphorylase catalyzes glycogen break-down, glucose-1-phosphate is released



32

In the nucleus... glucagon/epinephrine signalling

8. also at same time, in the nucleus, PKA phosphorylates transcription factor: **cyclic AMP response element binding protein (CREB)**
9. phosphorylated CREB dimerizes and binds to **cAMP response element (CRE)**, turning on PEPCK gene, gluconeogenesis increases



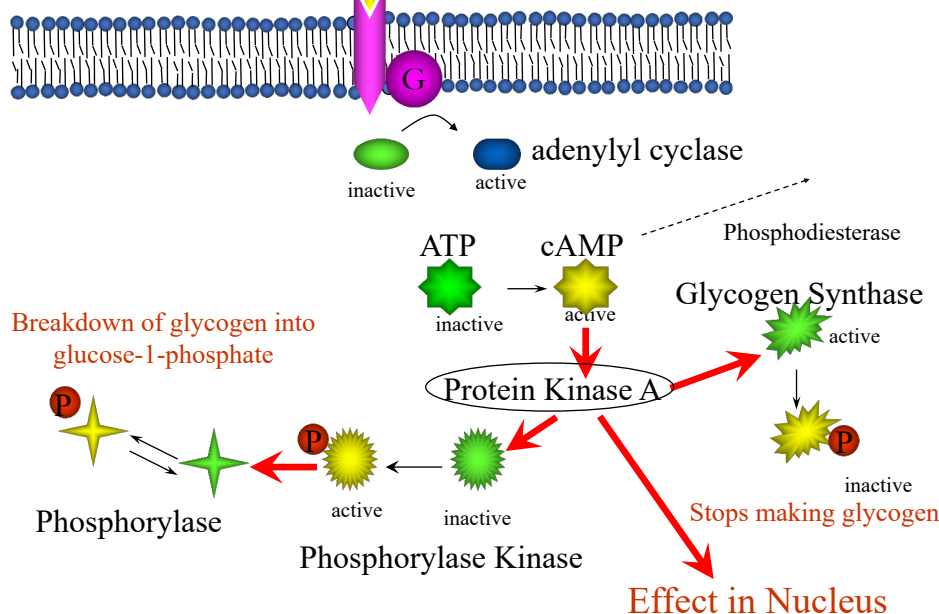
Which is a faster way to yield glucose?

- the cytoplasmic conversion of glycogen to glucose,
- or the nuclear transcription of gluconeogenesis genes

33

Glucagon, Epinephrine

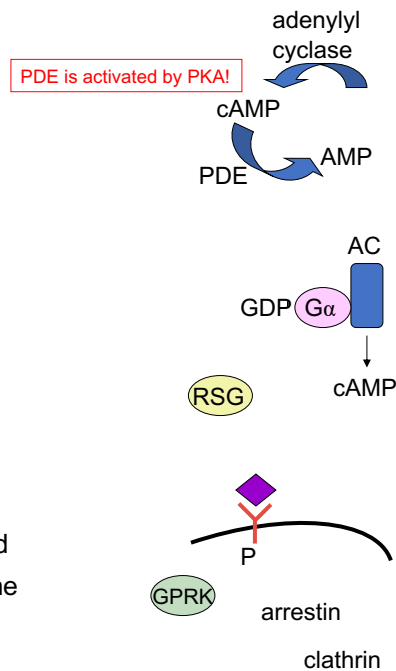
Note: these hormones bind to different receptors



34

Termination of the signal

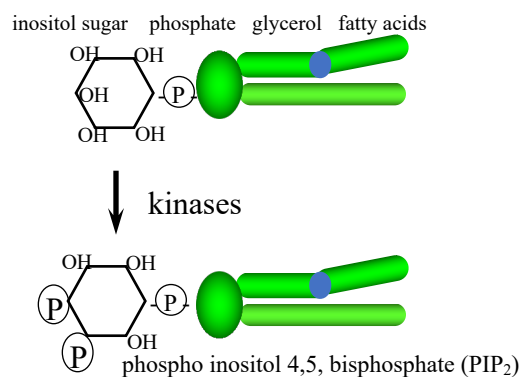
1. cAMP is broken down by **phosphodiesterase**
2. **Phosphatases** reverse the phosphorylation of the three proteins:
 1. phosphorylase kinase
 2. glycogen synthase and
 3. phosphorylase
3. **Adenylyl cyclase** is deactivated when the α subunit of the G-protein hydrolyzes the GTP back to GDP (α subunit re-associates with γ and β) – this hydrolysis is a timed event - aided by another protein: **RSG** (regulator of G protein signaling)*
4. inactivation of the receptor, a 2-step process of **desensitization**. This means that the cell stops responding, *even when ligand is still present around the cell*
 - a. Phosphorylation by **G-protein receptor kinase (GPRK)**, inactivates the receptor,
 - b. The phosphorylated receptor binds another protein called **arrestin**, which acts as **adaptor** for **clathrin**, allowing receptors to be internalized, thus further desensitizing the cell



35

Lipid Second Messengers

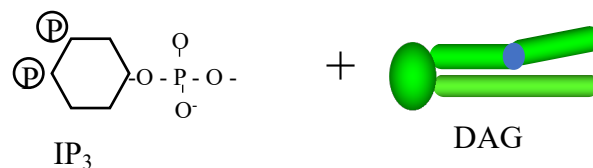
- GPCR-induced intracellular messengers
- Made by the phosphorylation and hydrolysis of the membrane glycerophosphate **phosphatidylinositol**



36

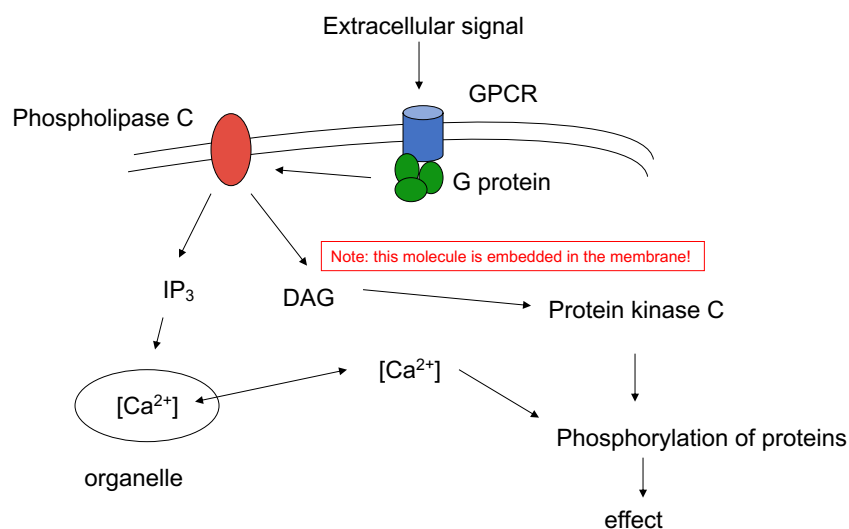
Lipid Second Messengers

- The main lipid second messengers are produced by phosphatidylinositol-specific **phospholipase C**.
 - abbreviated **PI-PLC**
 - produces two signal molecules:
 - Diacylglycerol (DAG)**, which stays within membrane
 - and **Inositol triphosphate (IP₃)**, highly soluble, enters cytoplasm



37

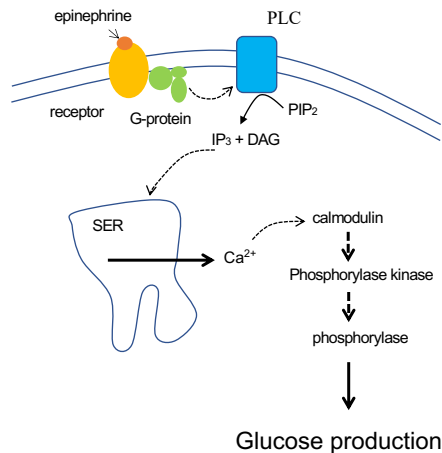
Overview of phospholipase C action



38

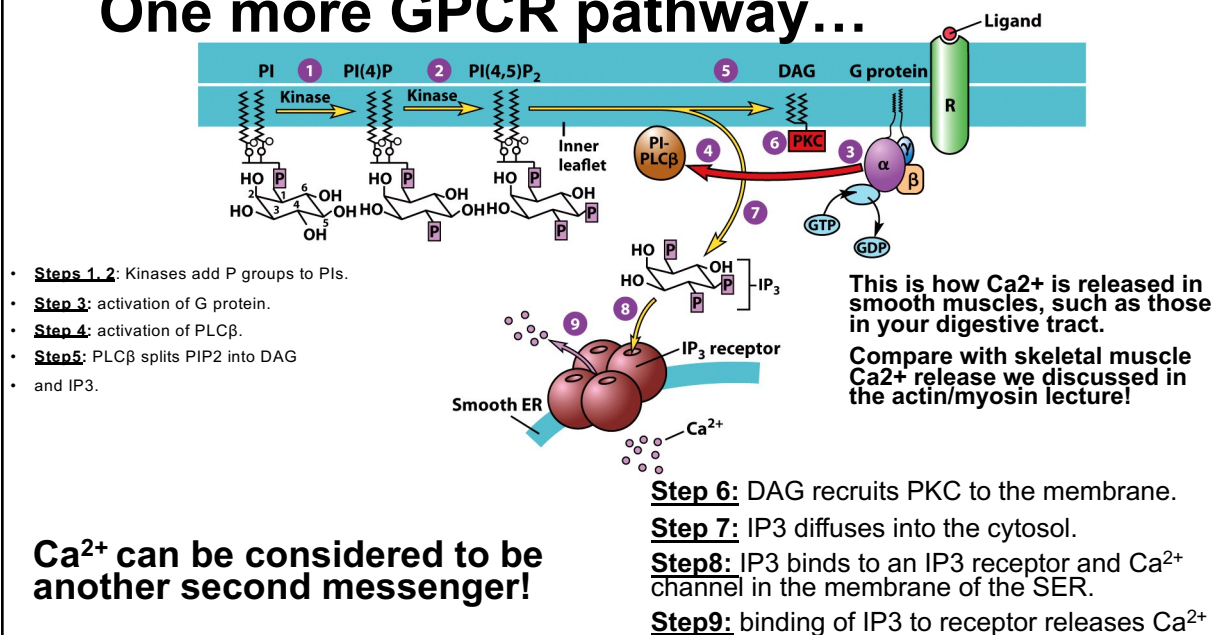
Epinephrine can induce lipid second messengers

- Epinephrine also triggers DAG and IP₃ second messengers, through different G-proteins
- IP₃ causes intracellular stores of calcium to be released – the Ca²⁺ binds and activates proteins that are in the gluconeogenesis pathway
- DAG activates PKC and also opens Ca²⁺ channels on cell's membrane
- → activate gluconeogenesis enzymes



39

One more GPCR pathway...

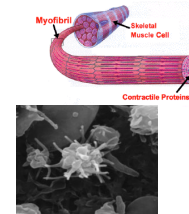


40

Intracellular signalling molecules activate different responses in different cells

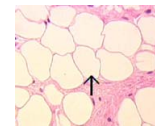
a. IP_3

- i. Muscle cells contraction
- ii. blood platelet aggregation of platelets

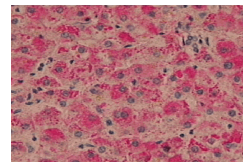


b. Protein Kinase C

- cell growth, differentiation, metabolism.
- i. mast cells histamine release
- ii. adipose tissue fat synthesis
- iii. liver cells glycogen hydrolysis



Glycogen stained pink

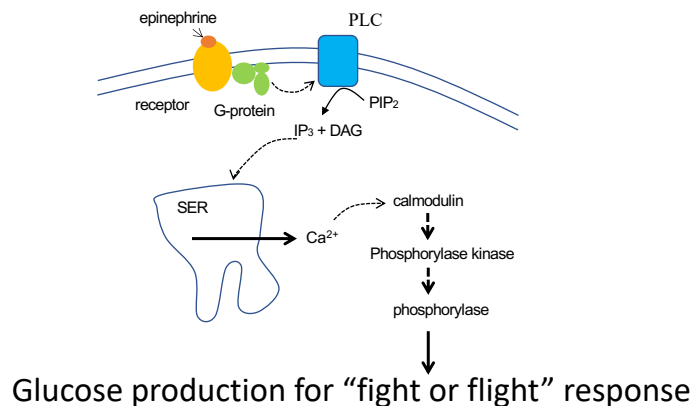


41

c. cAMP in different cells

i. Liver:

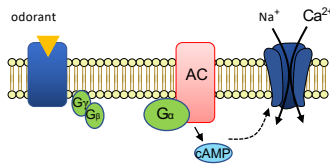
epinephrine -glycogen hydrolysis, glucose synthesis,
glucagon -reduction in glycogen production



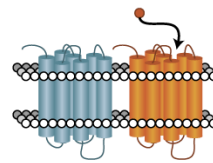
42

GPCRs in odorant reception

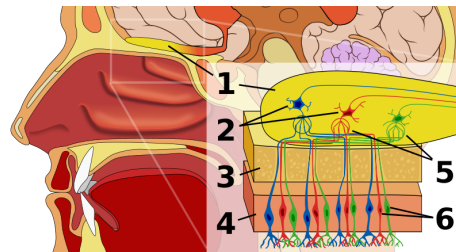
- Our sense of smell is based on GPCRs on olfactory sensory neurons.
- Olfactory receptors have affinity for more than one odorant molecule;
- 1 odorant molecule may bind to >1 receptor, but with different affinity



1. Odorant molecule binds to GPCR
2. G-protein stimulates adenylyl cyclase (AC)
3. cAMP opens calcium-sodium channels
4. Membrane depolarization triggers electrical signal in sensory neuron



Microsoft Clip Art



Odorants bind to different receptors, stimulating odorant sensory neurons; olfactory neurons then relay the mix of signals to brain

43

GPCRs for odorant detection

Humans and dogs

- Humans have ~400 odorant receptors; dogs have ~1100
- Dogs can sniff out some cancers
- Some cancer cells produce unusual amounts of certain metabolites
- 6 dogs trained to detect bladder cancer from urine samples
- 22/54 (41%) accuracy rate; one "failure" turned out to be from a person with a previously undiagnosed kidney tumor

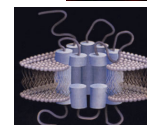
Electronic noses

- layer of olfactory receptor proteins from animal noses on a microelectrode

Human sperm cells have odorant receptors

– for homing in on the egg?

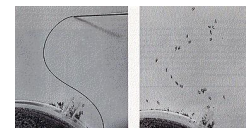
<http://www.youtube.com/watch?v=IZA9R0uSGWc>



44

Insects and pheromone detection

- GPCRs used to detect minute amounts of sex-pheromone - males seeking females
- Understanding pheromone-receptor interactions means we can control pest insects with pheromone mimics
- Ants following chemical trails to food sources use GPCRs to detect the paths
- Conflicting evidence on whether humans have pheromones, but we do produce odorants that can affect behaviour.



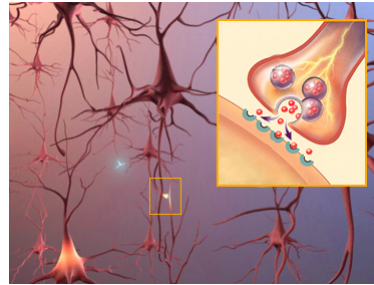
45

What you should know after this section

- Which hormones induce cells to take in or release glucose
- Outline the signaling pathway following glucagon reception that results in glucose production
- Outline how the cell terminates all components of glucagon-induced signaling
- Outline how epinephrine activates phospholipase C, the second messengers involved and the downstream events
- Outline how acetylcholine signaling induces muscle contraction
- How does vasopressin induce aquaporins to be mobilized?
- How do GPCRs affect odorant reception?

46

Week 13 Cell Signaling III

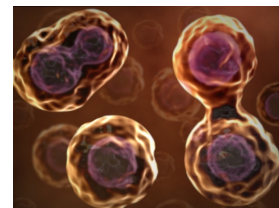


- **Suggested reading – Karp 9th edition, Chapter 15**
- Protein tyrosine phosphorylation
- Signaling by the insulin receptor
- Calcium as a 2nd messenger
- Convergence, divergence, crosstalk

47

Receptor Tyrosine Kinases (RTKs)

- **Receptor is an enzyme**
 - a protein tyrosine kinase that phosphorylates target proteins at particular tyrosines
 - > 90 types of RTK receptors in humans
 - all have similar structure – single trans-membrane segment
 - present in all multicellular organisms
 - involved in regulation of:
 - Growth (growth factor receptors)
 - Cell division (defects lead to cancer)
 - Cell survival and death
 - Cell attachments, migrations

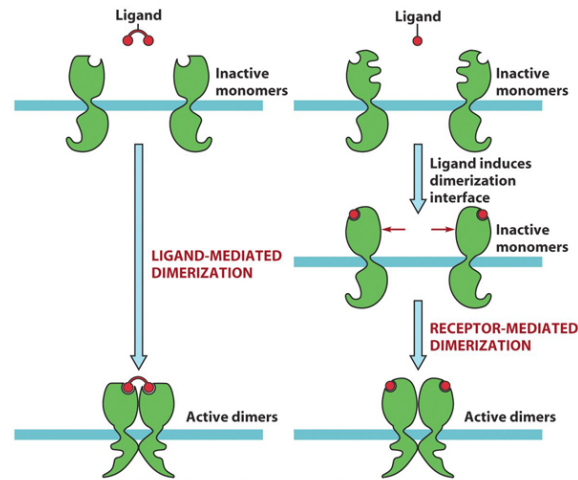


<http://topnews.in/health/files/cell-growth.jpg>

48

RTKs function as dimers

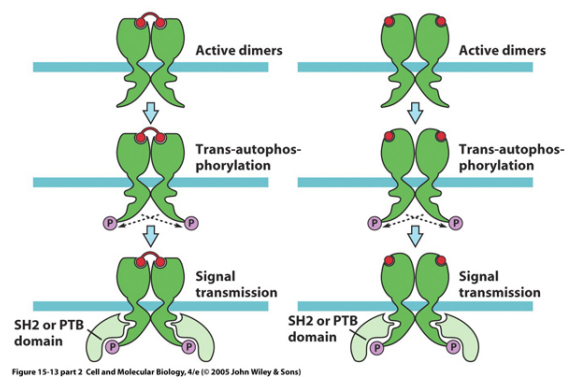
- Binding of ligand induces dimerization either by:
- One ligand binding to both monomers or
- One ligand binds to each monomer, which causes them to dimerize



49

RTKs phosphorylate each other

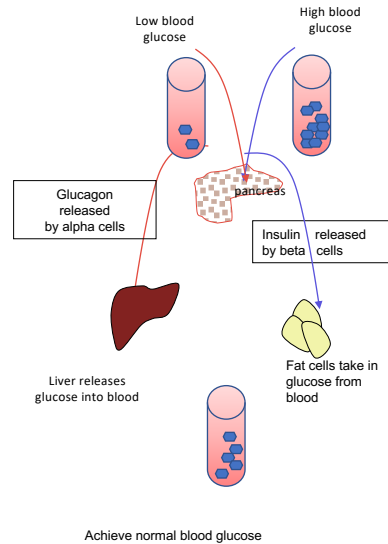
- The two monomers, once joined, trans-autophosphorylate each other
- Once phosphorylated, they are activated and can bind to other signaling molecules



50

Insulin receptor action through the RTK

- Insulin is produced when blood glucose increases after eating.
- It causes glucose uptake by liver, muscle, fat cells
- Acts to decrease blood sugar, cells produce glycogen or fat, gluconeogenesis is inhibited.



51

Insulin receptor action through the RTK

1. Receptor binds ligand on exterior of the cell.

- the insulin receptor is a heterodimer - two extracellular alpha chains with ligand binding sites and two beta chains with kinase activity, all are linked by disulfide bridges.

2. Activation of the receptor

- insulin receptor is unusual in existing in dimer state even before binding the ligand
- tyrosine kinase activity, *trans-autophosphorylation*.
- it also phosphorylates another protein, **Insulin Receptor Substrate (IRS)**

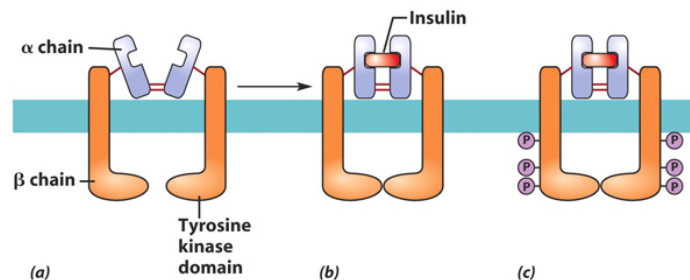


Figure 15-20 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

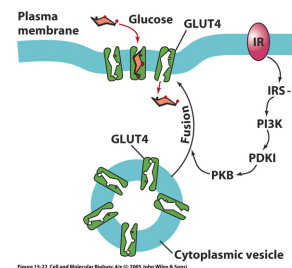
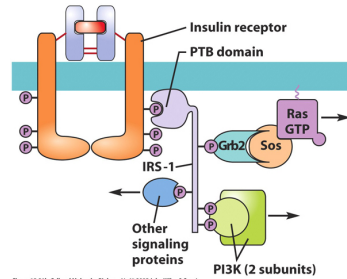
52

Insulin receptor action through the RTK

3. Other proteins recognize, bind and are activated by the phosphorylated tyrosines on receptor and IRS

4. Final actions of insulin:

- a. Cell proliferation via the effectors:
 - PLC (and its second messengers: IP_3 , DAG)
 - Protein kinase C
- b. Increased glucose import into cells via:
 - (Protein kinase B)* induces movement of glucose transporters to cell membrane
 - Stimulation of glycogen synthesis



* - you don't need to remember yet another PK

53

Diabetes – defects in insulin signaling

Diabetes – high levels of glucose in blood, loss of glucose in urine, excessive loss of water from frequent urination, high blood pressure

Two types:

- Type I – no insulin produced by pancreas
- Type II – insulin resistance in cells that should take in glucose

High joule diet → high insulin production → loss of sensitivity to insulin

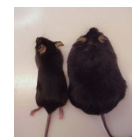
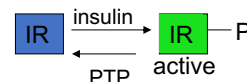
Several possible causes for loss of sensitivity

- faulty insulin receptor
- faulty intracellular signaling

How to increase sensitivity to insulin?

- remove the negative regulator (the off switch) for the insulin receptor
- protein tyrosine phosphatase (PTP) removes P from insulin receptor

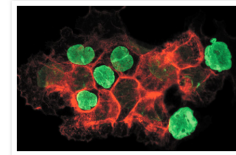
PTP gene knockout in mice produces insulin-sensitive animals that can eat high fat diets without developing diabetes (and becoming obese)



54

epidermal growth factor receptor (EGFR)

- A **growth factor** (also known as a cytokine) is a naturally occurring substance capable of stimulating cellular growth, proliferation and cellular differentiation
- **Epidermal growth factor** or **EGF** is a growth factor that plays an important role in the regulation of cell growth, proliferation, and differentiation by binding to its receptor EGFR
 - This signaling results in changes in gene expression
 - Transcription factors are activated - genes involved in cell proliferation are transcribed
 - Signal is turned off by receptor being ubiquitinated and shunted to lysosome
- Mutations that lead to EGFR overexpression (known as up-regulation) or overactivity have been associated with a number of cancers, including lung cancer.



Cells labeled with antibodies detecting EGFR on cell surface (red)



or this?



55

MAP-Kinase signaling pathway

- A **growth factor** (also known as a cytokine)
- is a naturally occurring substance capable of stimulating cellular growth, proliferation and cellular differentiation, and cell survival
- **Epidermal growth factor** or **EGF** is a growth factor that plays an important role in the regulation of cell growth, proliferation, and differentiation by binding to its receptor EGFR
 - This signaling results in changes in gene expression
 - Transcription factors are activated - genes involved in cell proliferation such as the cyclins are transcribed



or this?

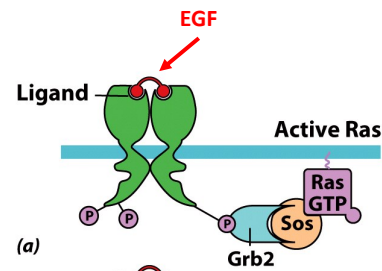


Mutations that lead to EGFR overexpression (known as up-regulation) or over-activity have been associated with a number of cancers, including lung cancer.

56

Growth factors such as EGF activate the MAP kinase pathway

MAP kinase = mitogen activated protein kinase.
Serine / threonine specific kinases.
Mitogens are chemical substances (usually proteins) that induce mitosis



Steps:

1. EGF binds and activates EGFR
2. EGFR autophosphorylates its tyrosines
3. The phosphorylated tyrosines serve as a docking station for Grb2/Sos, which is recruited to the membrane
4. SOS is a GEF that activates RAS

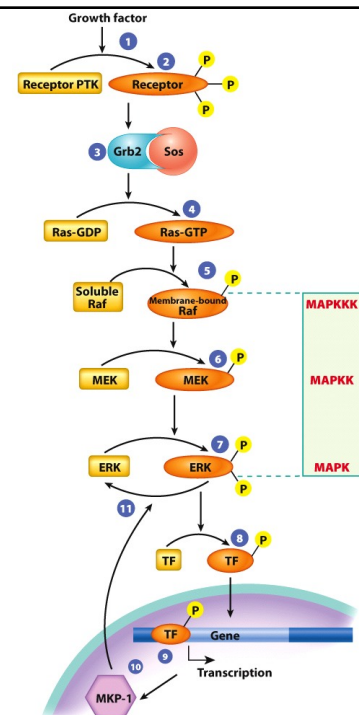
- **Ras** is a monomeric G protein embedded in the membrane by a lipid group. Ras is active when bound to GTP and inactive when bound to GDP.
- **Grb2** acts as an adaptor protein that links EGFR and Sos.
- **Sos** is a GEF that activates G-proteins.

57

Ras recruitment sets off a kinase signal cascade

5. Once Ras is recruited to the membrane, it recruits a serine/threonine kinase called Raf
6. Raf phosphorylates and activates MEK
7. MEK phosphorylates and activates ERK
8. ERK phosphorylates transcription factors, which enter the nucleus and start transcription of target genes that initiate the cell cycle (e.g. cyclin D)

30% of cancers have a mutation in this pathway. Thus these genes (and others) are referred to as *oncogenes*.

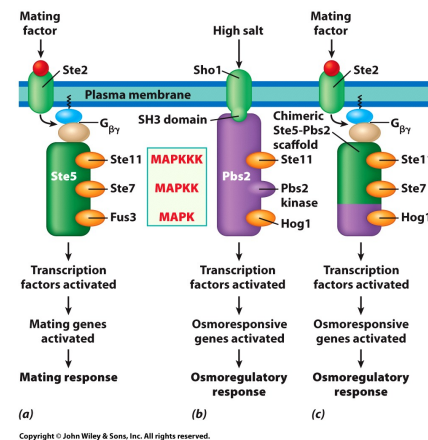


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58

The MAP-kinase pathway has been recruited for different purposes in other organisms

- In yeast, the pathway has different components, but is mostly the same idea. It can regulate yeast mating and osmoregulation.
- In plants, it regulates response to pathogens.
- In flies, it regulates the development of photoreceptors.



(don't memorize figure!)

59

Other second messengers: Calcium

- Ca^{2+} can be let into the cytosol by **voltage dependent channels** in nerves, some muscles and by **receptor-mediated calcium channels** (like we just saw with the smooth muscle)
- **fertilization**, calcium channels open up in oocytes following fertilization.
 - prevent further sperm entry anywhere around the large cell (rapid, long-distance)
 - begins activation of cyclins, starting cell division (coordinating effect)
- Calcium can also bind to **calmodulin** which modulates the activity of other pathways such as adenyl cyclase and other kinases

Remember that $[\text{Ca}^{2+}]$ is low in the cytosol through the action of Ca^{2+} pumps and exchangers.

60

Types of Calcium Responses

- **voltage dependent channels** in nerves, some muscles
- receptor-mediated calcium channels
- **fertilization**, calcium channels open up in oocytes following fertilization.
 - prevent further sperm entry anywhere around the large cell (rapid, long-distance)
 - begins activation of cyclins, starting cell division (coordinating effect)



Polyspermy block:
only 1 sperm gets in

Calcium-sensitive fluorescent dye shows wave of calcium spreads through cytoplasm over 1 minute period

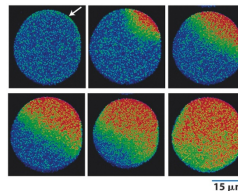


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61

Calcium-induced calcium release

1. Calcium ions enter cell through activated channel
2. Ca^{2+} binds to the ryanodine receptor on ER
3. Ca^{2+} released from the SER (or the vacuole in plants) diffuse down concentration gradient, diffuse throughout cell
4. calcium ions bind to calmodulin (calcium-binding protein)
5. Activated calmodulin binds to many more effectors → intracellular effects
6. one of the effects is to initiate the pumping of calcium back out of the cytoplasm to SER, the plant vacuole and the exterior of cell: termination of signal is therefore fast

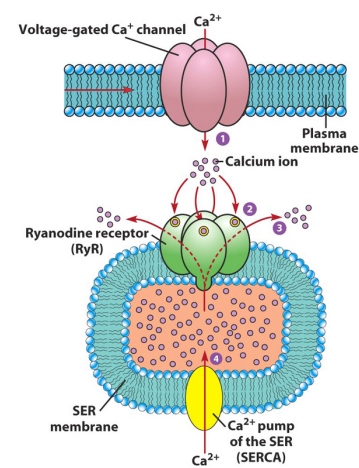
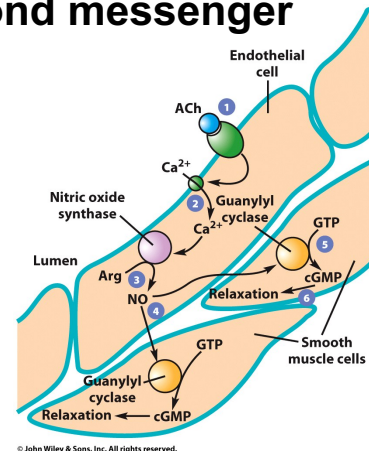


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62

Nitric oxide as a hormone, cGMP as a second messenger

- Nitric oxide (NO) – an inorganic gas – can act as an extracellular messenger and as a second messenger
- NO is synthesized by nitric oxide synthase within cells
- NO acts as autocrine and paracrine hormone
- NO activates guanylyl cyclase to make cGMP
- In blood vessels, acetylcholine stimulus can induce NO synthase
 - cGMP causes smooth muscle cells (around blood vessels) to relax
 - nitroglycerine applied under the tongue to patients with congestive heart problems, produces NO to relax arteries



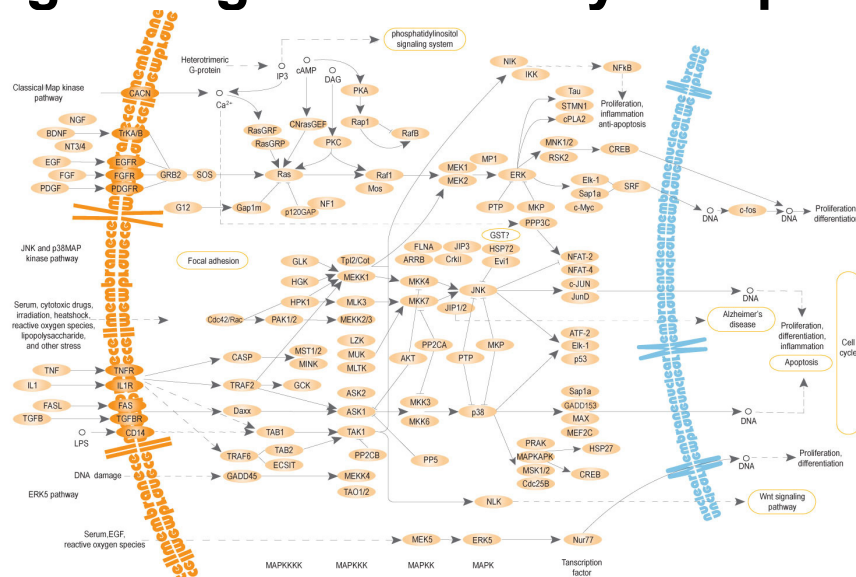
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drugs that inhibit **cGMP phosphodiesterase** can be used to relax blood vessels – e.g. viagra

63

Cell signalling can be crazy complex

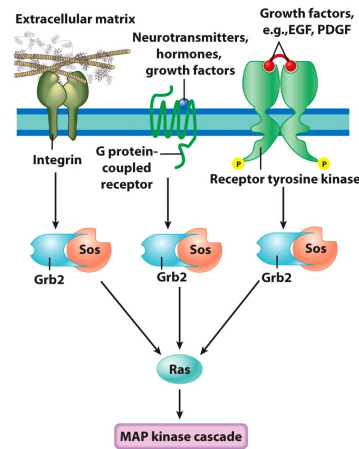
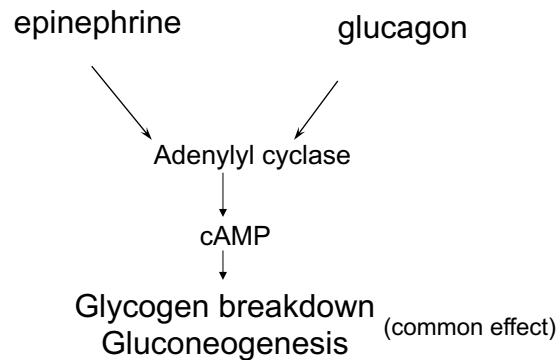


All these proteins regulate cell division and proliferation. Don't memorize, obviously...

64

Interconnections of signalling pathways

- 1. **Convergence.** two receptors cause the same secondary signal to be activated.

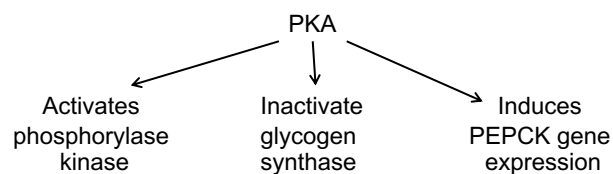


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65

Interconnections of signalling pathways

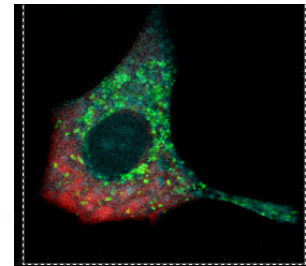
- 2. **Divergence.** one ligand has several effects or one signal propagates several responses
 - e.g. Protein kinase A (PKA, induced by epinephrine receptor) turns on **three** different proteins



66

Why is signalling so important?

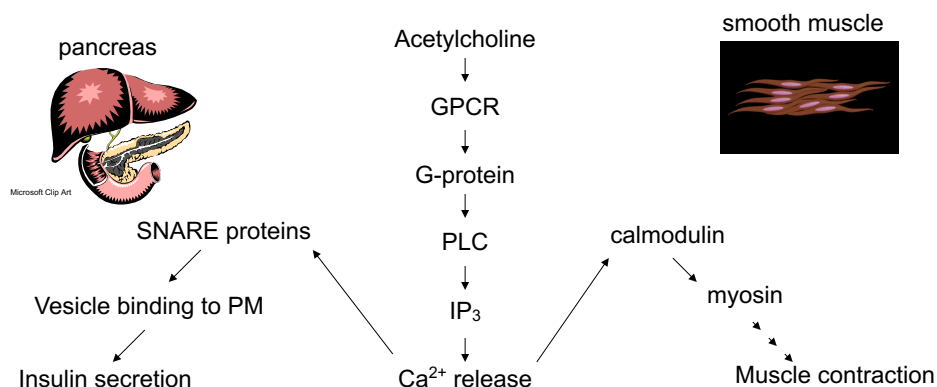
- cells can keep signals separate
 - second messengers diffuse rapidly, but some steps, including the kinases (such as PKA) are kept in certain locations in the cell.
 - PKA is actually held in place by special binding proteins (called **AKAPs**), focusing its effect in the cell.
 - some pathways are kept together in one location in the cell by special **scaffolding proteins**, limiting the location of the downstream cell response.
- How a cell responds to a signal:
 - Depends on: number of receptors; number of other signals; type of cell (because different cells have different downstream signaling molecules).



67

Same pathways, different effects

- ~2000 GPCRs, but only a handful of G-proteins – so how do cells exhibit so many different responses?
- The same intracellular signaling pathway causes different effects in different cell types because cells in different tissues have different downstream proteins to respond to the signal



68

What you should know after this section

- Describe an RTK and how it differs from a GPCR
- How does insulin promote glucose uptake?
- How is insulin insensitivity associated with diabetes, and how can you alter insulin sensitivity?
- How is calcium associated with preventing polyspermy?
- Describe calcium-induced calcium release – where is the calcium receptor?
- How does nitric oxide affect blood flow?
- What do signals get amplified?
- Define signal convergence, divergence, and cross-talk.