

Signal Transduction

What is signal transduction?

Binding of ligands to a macromolecule (receptor)

"The secret life is molecular recognition; the ability of one molecule to "recognize" another through weak bonding interactions." Linus Pauling

Pleasure or Pain – it is the receptor ligand recognition

So why do cells need to communicate?

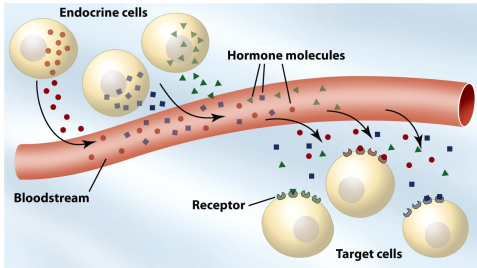
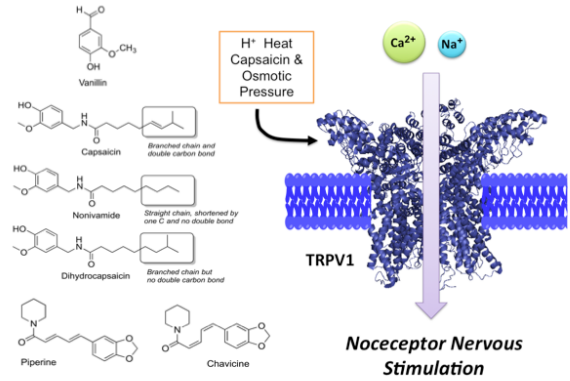


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-Coordination of movement

bacterial movement towards a chemical gradient green algae - colonies swimming through the water

- Coordination of metabolism - insulin glucagon effects on metabolism

-Coordination of growth - wound healing, skin, blood and gut cells

Hormones are chemical signals.

1) Every different hormone binds to a specific receptor and in binding a significant alteration in receptor conformation results in a biochemical response inside the cell

2) This can be thought of as an allosteric modification with two distinct conformations; bound and free.

Agonists and Antagonists

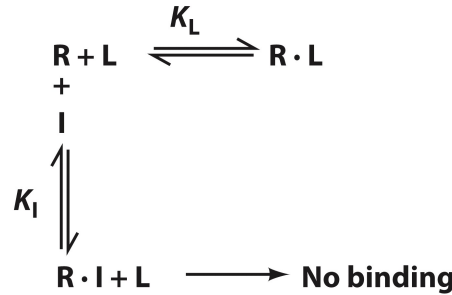
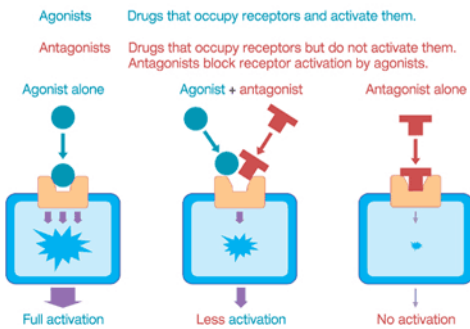
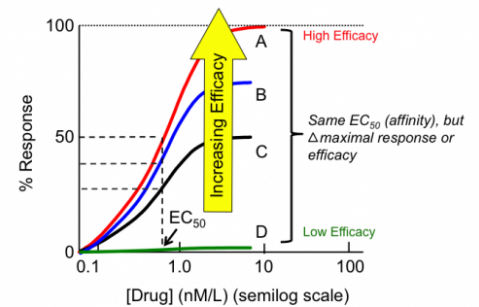
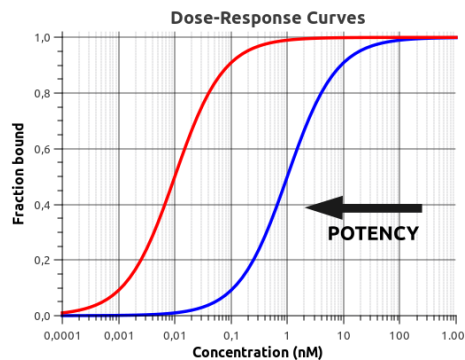


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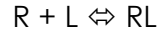
Log Dose Response

- Log dose response (Fractional Bound)
- Measures potency/efficacy of hormone, agonist or antagonist
- If measuring response, potency (efficacy) is shown differently

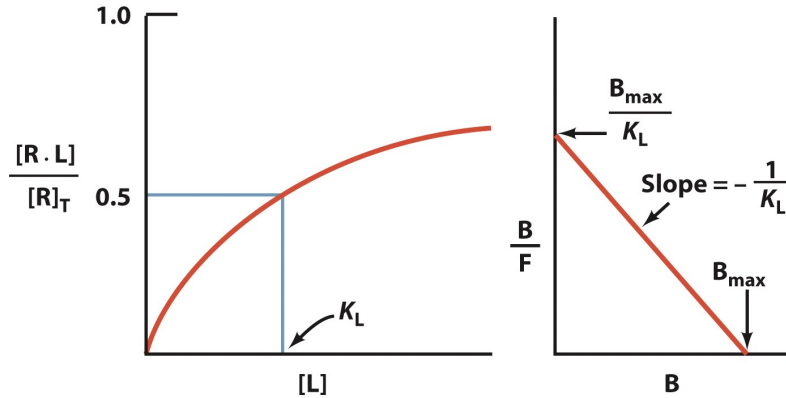


Scatchard Plot

Derived like kinetics

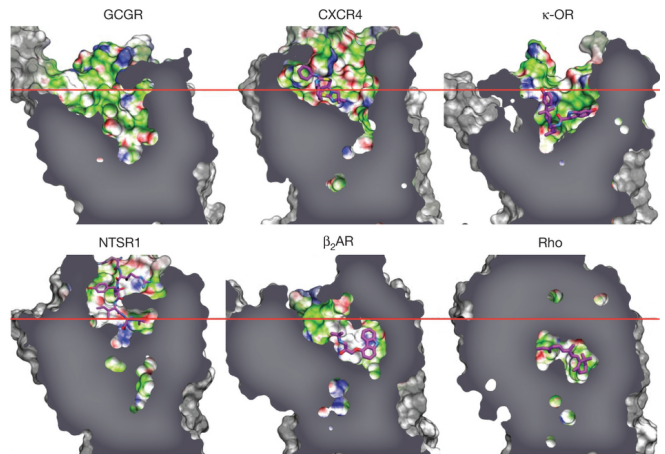


Used to measure receptor binding affinity K_D (K_L – 50% occupancy) in presence or absence of inhibitor/antagonist ($B = \text{Receptor bound to ligand}$)



3) The binding of the hormone leads to a transduction of the hormone signal into a biochemical response.

4) Hormone receptors are proteins and are typically classified as a cell surface receptor or an intracellular receptor. Each have different roles and very different means of regulating biochemical / cellular function.



Intracellular Hormone Receptors

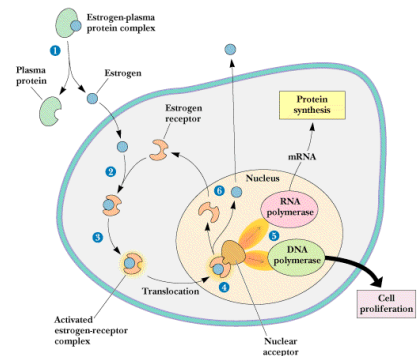
The steroid/thyroid hormone receptor superfamily (e.g. glucocorticoid, vitamin D, retinoic acid and thyroid hormone receptors)

- Protein receptors that reside in the cytoplasm and bind the lipophilic steroid/thyroid hormones.

Adrenocorticoids

- *Glucocorticoids* (cortisol)– metabolism, opposite of insulin similar to glucagon
- Mineralcorticoids (aldosterone) kidney function
- Sex Steroids – estrogens and testosterone

- These hormones are capable of freely penetrating the hydrophobic plasma membrane.
- Upon binding ligand the hormone-receptor complex translocates to the nucleus and bind to specific DNA sequences termed hormone response elements.
- The binding of the complex to and these DNA elements results in altered transcription rates of the associated gene.



Cell surface receptors

The cell surface receptors are a general classification of the proteins which specifically bind water soluble hormones.

- These receptors are very complex and varied. A key component of this class of receptors is that they possess at least one transmembrane spanning domain.
- From there all bets are off. The mechanism of the cell surface receptors varies depending on the type of hormone bound and the second messenger system involved.

There are three phases to a water soluble hormone action

- 1) hormone or first messenger
- 2) receptor binding and initiation of the second messenger system
- 3) amplification and cascade of the second messenger system

First messengers

Structure of 1st messengers vary greatly - we already know most of them

- 1) amino acid derivatives
 - tyrosine -> thyroid hormones T3/T4, epinephrine, dopamine
 - glutamate -> histamine
 - tryptophan -> serotonin
- 2) Peptides - usually made in pre/pro format, large families - insulin, glucagon, oxytocin, growth factors
- 3) Fatty acids and eicosanoids - can act as local mediators - TXA, LTE, phosphatidic acid, lysophosphatidic acid
- 4) Steroids - cholesterol derivatives
 - progesterone, estrogen, testosterone
 - site of action usually in nucleus w/ DNA binding protein
- 5) NO Nitric Oxide – small short lived gas molecule – responsible for bld pressure, synaptic signaling and more

Second messengers - Third phase of the signal transduction concept

A second messenger is a molecule produced in response to a 1st messenger binding to a receptor. -

Hallmark of a true signal is a specific and short lived response to an agonist

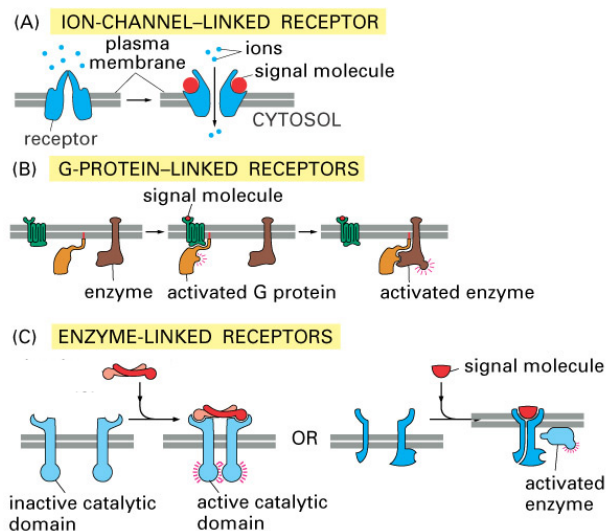
Leads to an amplification of original signal

second messenger may directly effect target protein / DNA or mostly leads to a chain of second messengers with a wide variety of effects

- specific control and response – cAMP
- Many different signaling proteins are involved (thousands). The design of the proteins organization comprise the signaling pathways

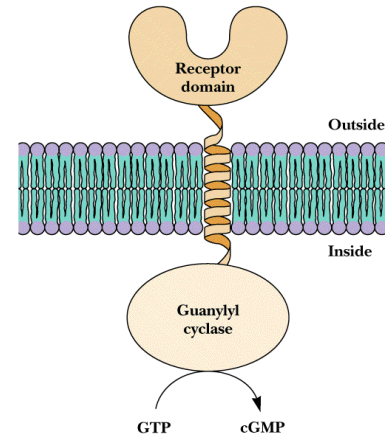
Receptors Ligand binding to receptors start intracellular signaling.

- Ligand-gated channels: Open or close in response to ligand to allow ions into cell initiating signaling. Nerve signaling
- GPCR: AKA 7 TM receptors, conformational change activates intracellular G proteins (not enz)
- Enzyme linked receptors – most have an enzyme activity or directly activate an enzyme



Receptors with integral enzyme activity

- Most receptors of this class are - single pass receptors
- guanylate cyclase (GTP → cGMP) responsible for vasodilation
 - Activated by NO (nitric oxide)
 - protein tyrosine kinase (tyrosine + ATP → tyrosine-P + ADP)
- interlukins and immunological receptors



Each receptor has three basic domains:

1 - Extracellular domain

- this is the ligand binding domain /often glycosylated
- often heavy in cystein rich domains and immunoglobulin like domains

2 - Transmembrane domain

- usually a single alpha helix (rich in hydrophobic amino acids)

3 - Intracellular domain -

- the intracellular portion becomes activated by tertiary structural changes
- many times autophosphorylates intracellular domain (tyrosine-P)
- once activated, many different proteins bind to intracellular domain

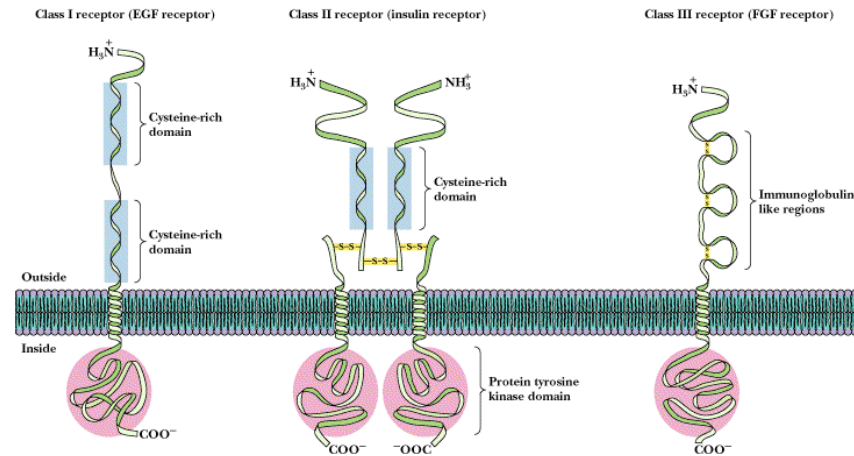
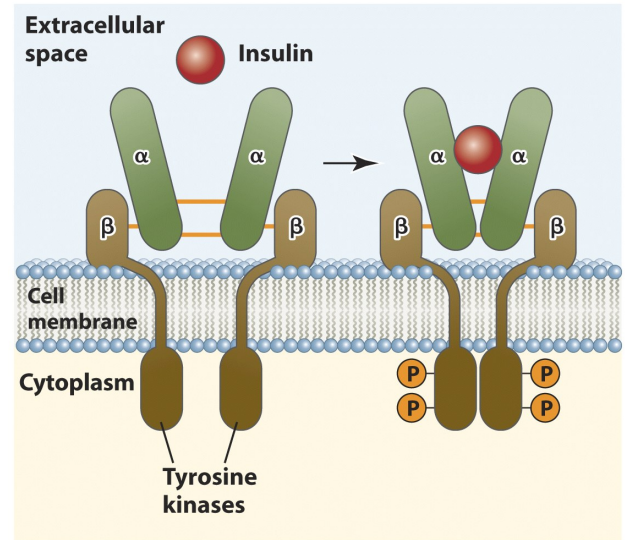
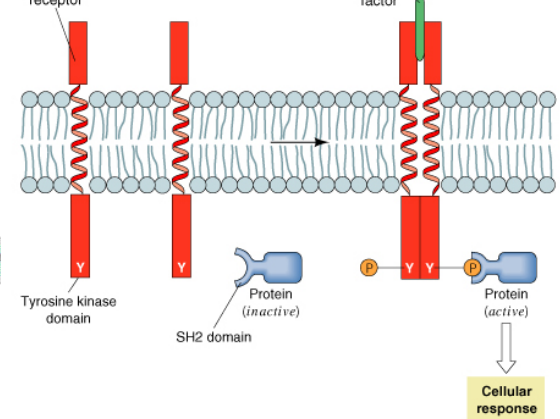


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Receptor Activation – Many of these types of receptors initiate cellular growth (proliferation) or differentiation (the act of converting from one precursor cell type to a mature form)

2% of genes (PTK) modify 33% of cellular protein!

- activated by growth factors
- The receptor itself has a tyrosine kinase activity
- other than insulin binding of hormone/growth factor ligand leads to dimerization
- leads to many changes in second messenger biological activities

Tyrosine Kinase Autophosphorylation

To understand the mechanism of autophosphorylation, let's look at the structure of the enzyme portion of a protein tyrosine kinase (PTK):

AMPNP binding to phosphorylated PTK domain

γ -phosphate group next to OH of substrate ready for transfer

- 3 PTK Tyr are phos in 18 aa activation loop (shown in orange pTyr in magenta)
- Non-phos loop fills kinase active site, once phosphorylated, the loop is removed, substrate and ATP align and bind

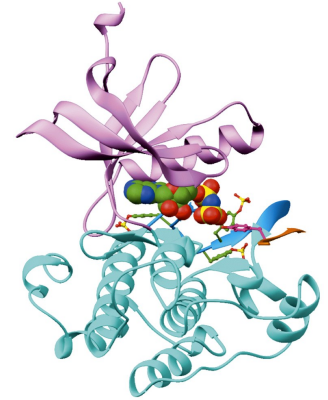


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PTK Phosphorylation Induces Structural Changes

Insulin Receptor (Kinase domain) aligned by C-term lobes.

- Green backbone blue loop = phos x3
- Yellow and red loop = non-phospho
- 21° rotation positions residues for substrate binding and reaction (see last slide)...

Many intracellular receptor binding sites for other proteins based on Rous Sarcoma Virus

- Src is a transforming causing protein with 3 distinct domains Src Homology
- SH1 - contains tyrosine kinase of Src specific for this protein
- SH2 - binds phosphotyrosine
 - pTyr (neg charge) binds acidic Arg, Glu and Ile deep in pocket why only pTyr not others bind SH2?
- SH3 - believed to bind to cytoskeleton or portion of plasma membrane that is high in proline amino acid residues

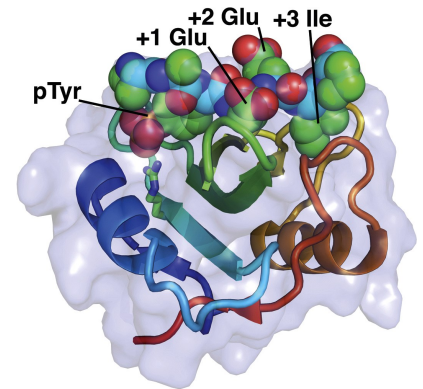


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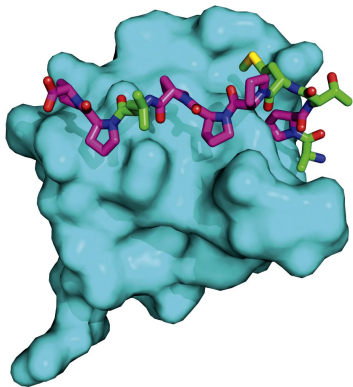


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Many intracellular receptor binding sites for other proteins based on Rous Sarcoma Virus

- SH3 - believed to bind to cytoskeleton or portion of plasma membrane that is high in proline amino acid residues.
- Consensus sequence -XPpXP- where X is an aliphatic aa and P is always proline; p is usually a Pro

Therefore once the receptor is activated and phosphorylated there will be a slug of proteins recruited to the cell membrane.

- This may result in the receptor phosphorylation of the protein (PLC β)
- Another action is that a number of critical components are brought together where they can form a functional complex and initiate a series of events (Ras MAPK)