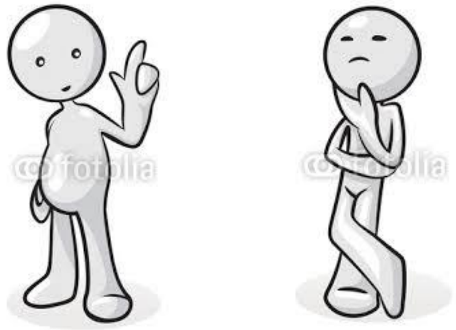
Lecture 10 Cell communication Part I

Outline

I. Overview of cell signaling
II. Intracellular signaling
III. General principles of cell surface signaling
IV. Several methods to study cell signaling
V. Positive and negative feedback in signaling and signaling kinetics

What is cell communication

Human:

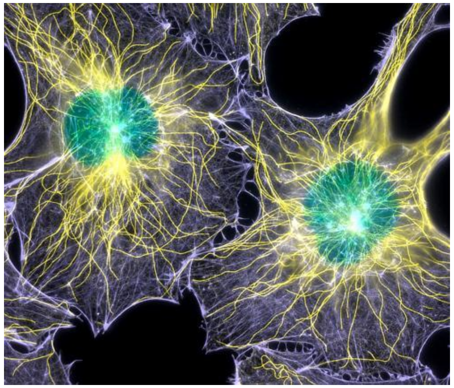


Language
Body language

Insect:

Pheromones
Touch
noise

Cell:



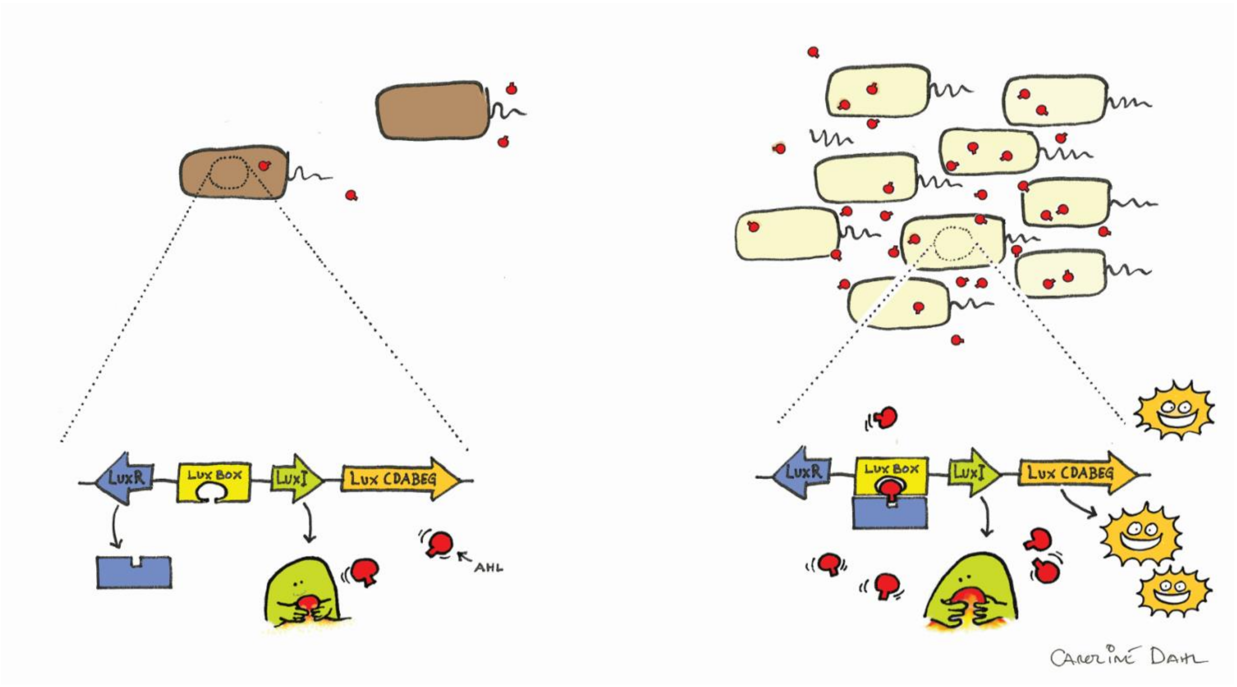
Physical: light, mechanical force, heat
Chemical: proteins, peptides, amino acid derivatives, nucleotides, steroids, retinoids, fatty acid derivatives, Gases(NO, CO), etc.

The seadevil- anglerfish

https://video.nationalgeographic.com/video/weirdest-angler-fish

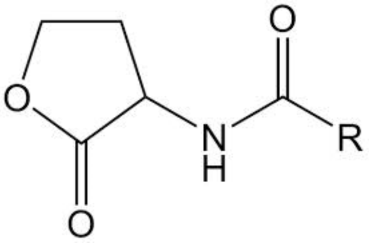
Quarum sensing in bacteria

Chemical signals secretion correlation to cell population

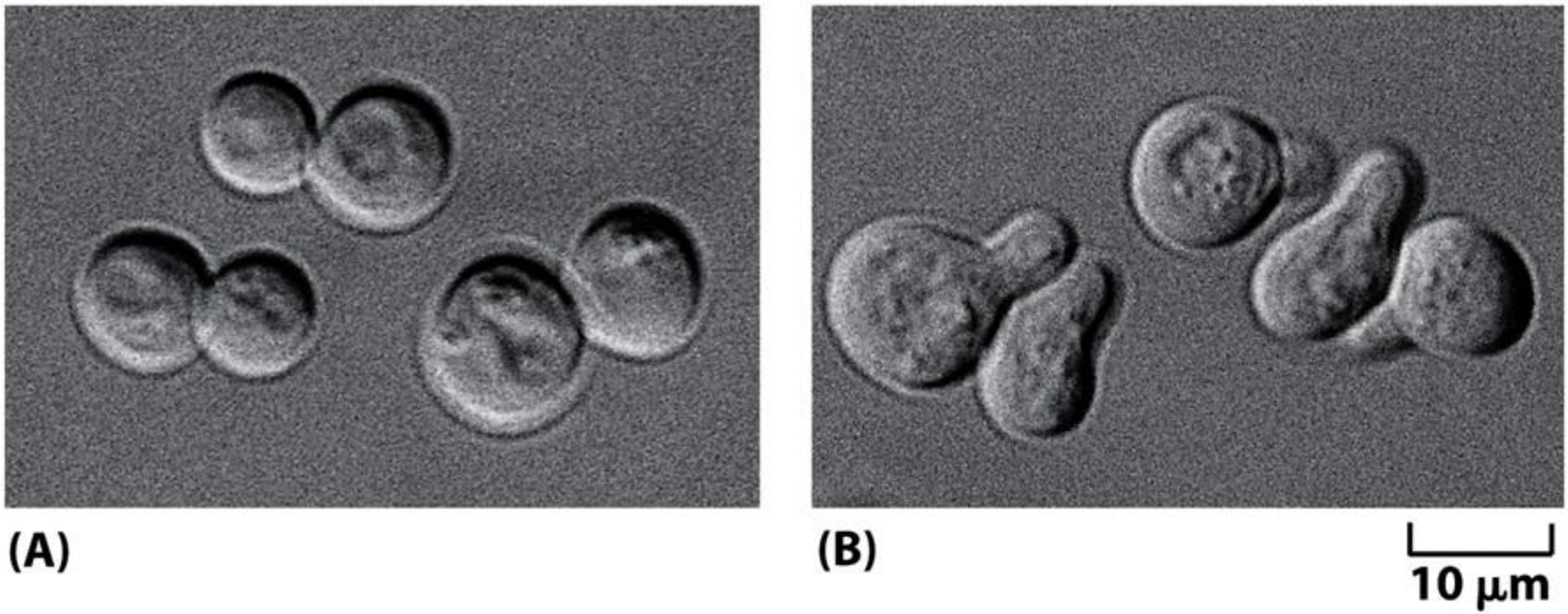


Low cell density high cell density

The signaling molecule --- AHL: acyl homoserine lactone



Budding yeast mating corresponds to *mating factor* (a peptide)



2 haploid yeast cells

haploid cells fusion to become diploid cells

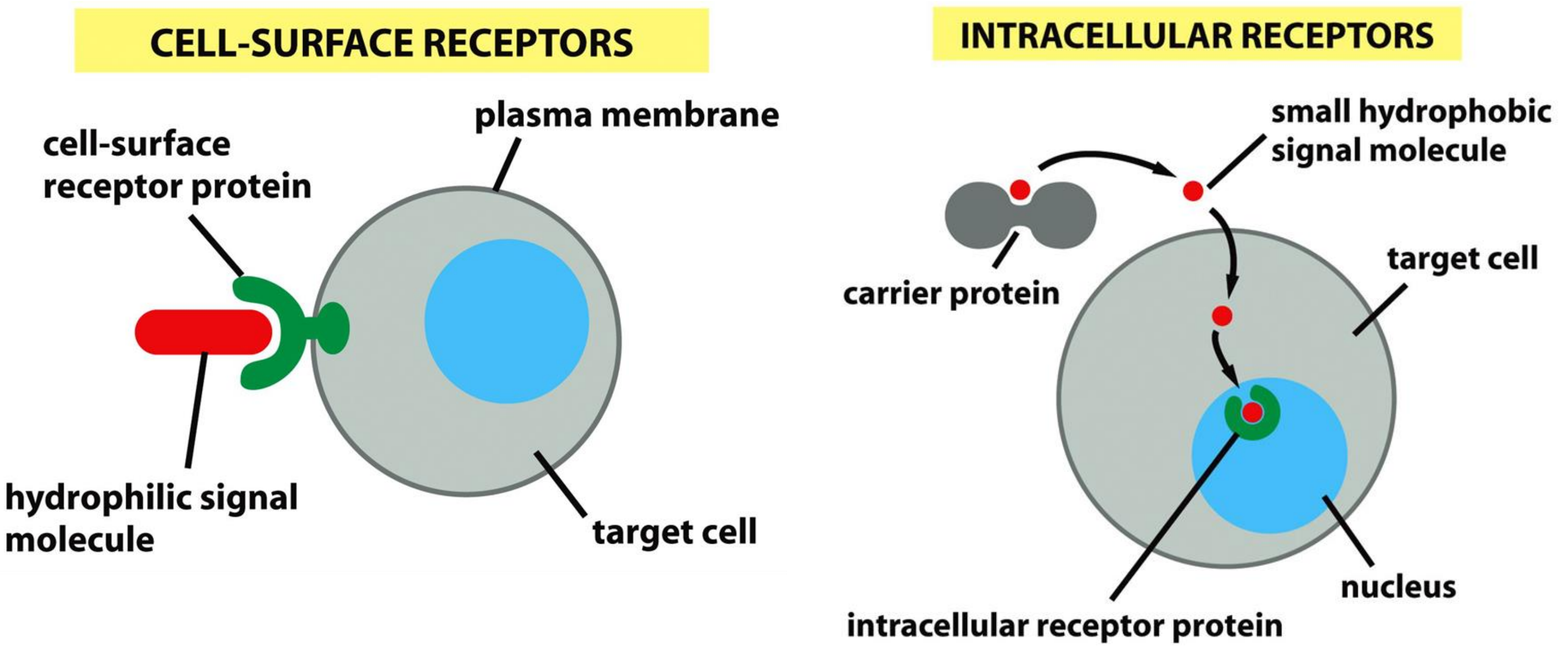
I. Overview of cell communication

Chemical signaling involves ligands and receptors

Two different types of receptors:

- ♥cell surface receptors
- ♥intracellular receptors

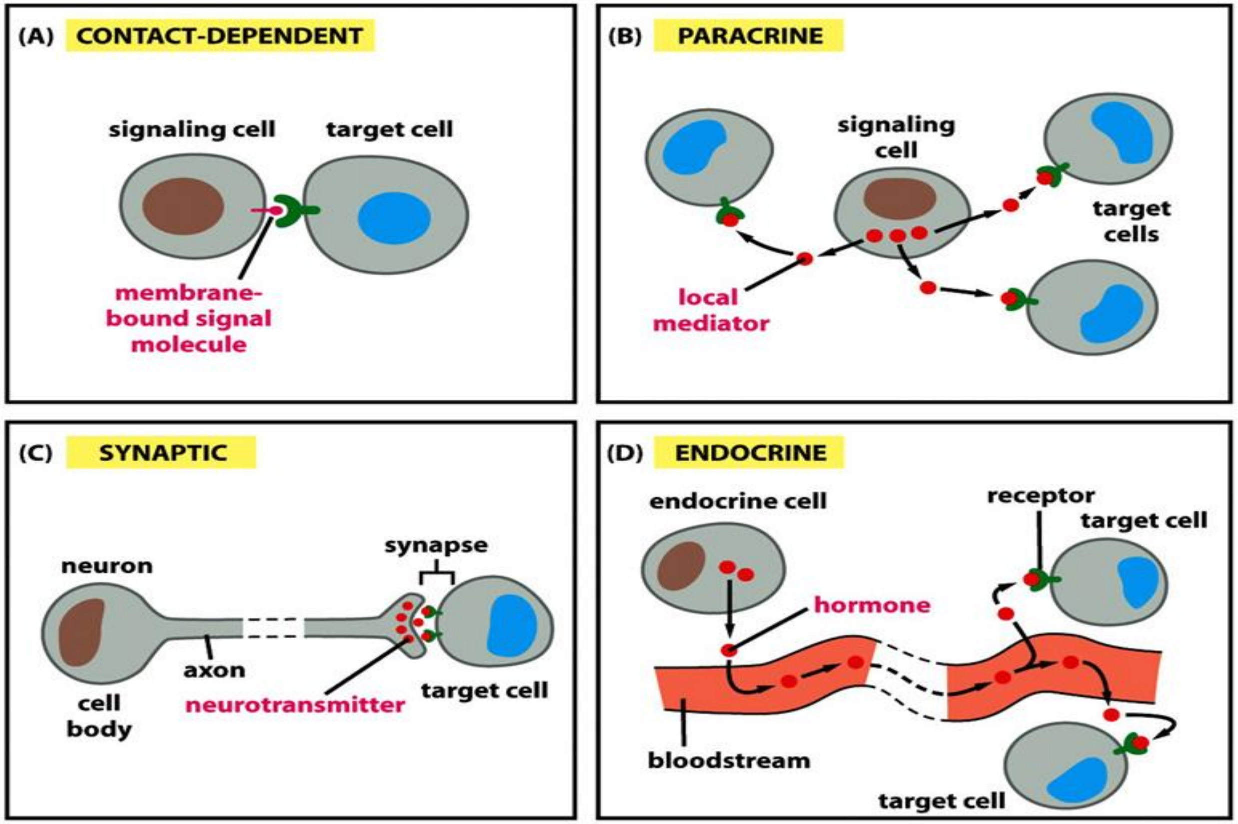
Two different receptors work differently



Types of cell communication

- Cell-cell contact
- Synaptic communication
- Paracrine/autocrine (local environment)
- Endocrine (long distance through blood stream)

Four types of cell communication



A and B are short-range

C and D are long-range

Endocrine versus synaptic

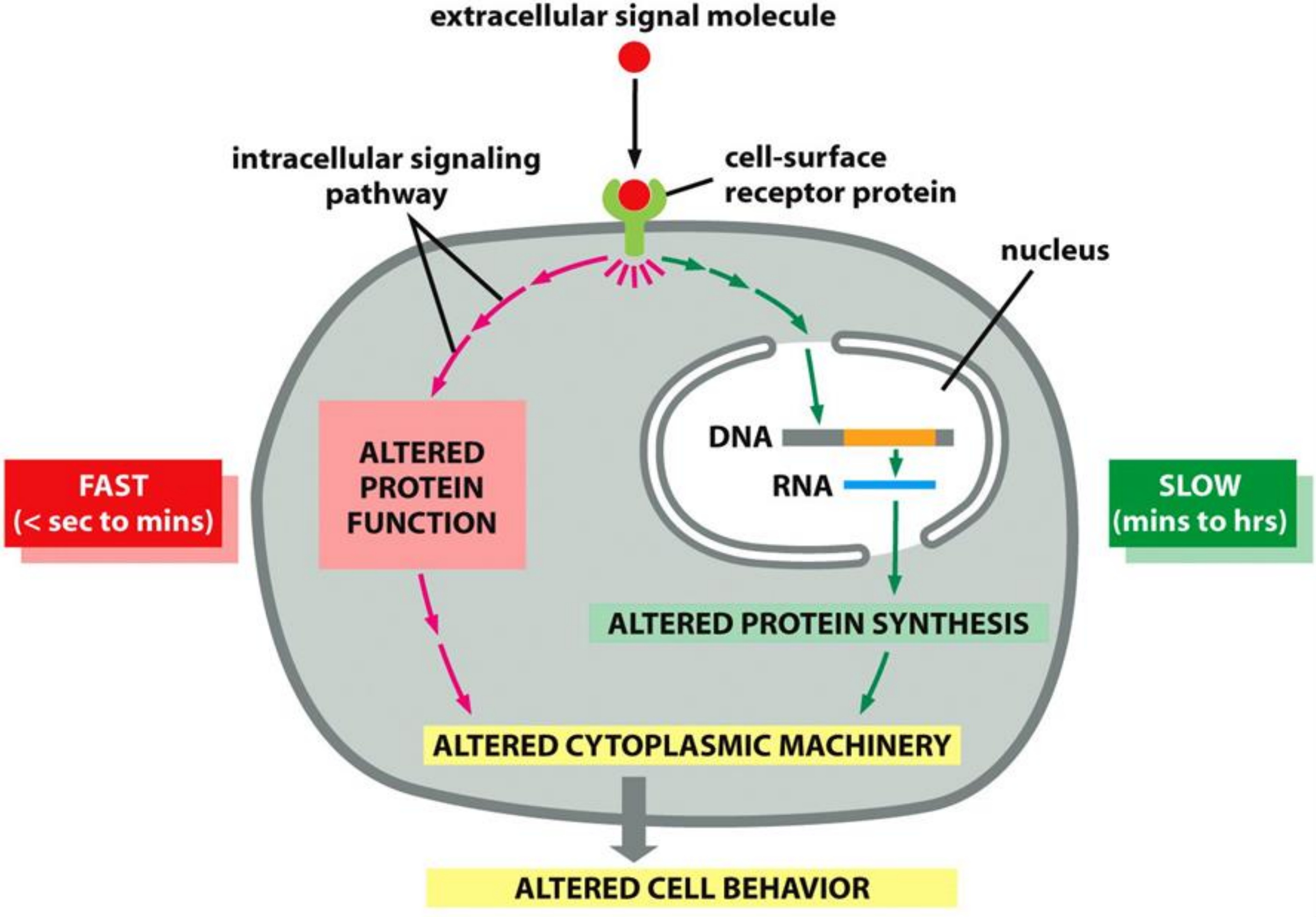
- Endocrine: need diffusion and blood flow, slow
- Synaptic: fast, 100meters/sec
- Endocrine: signals low concentration: 10^-8M and more diffused.
- Synaptic: signals higher concentration: 10^-4M and more precise.

Effects in signaling can be slow and fast

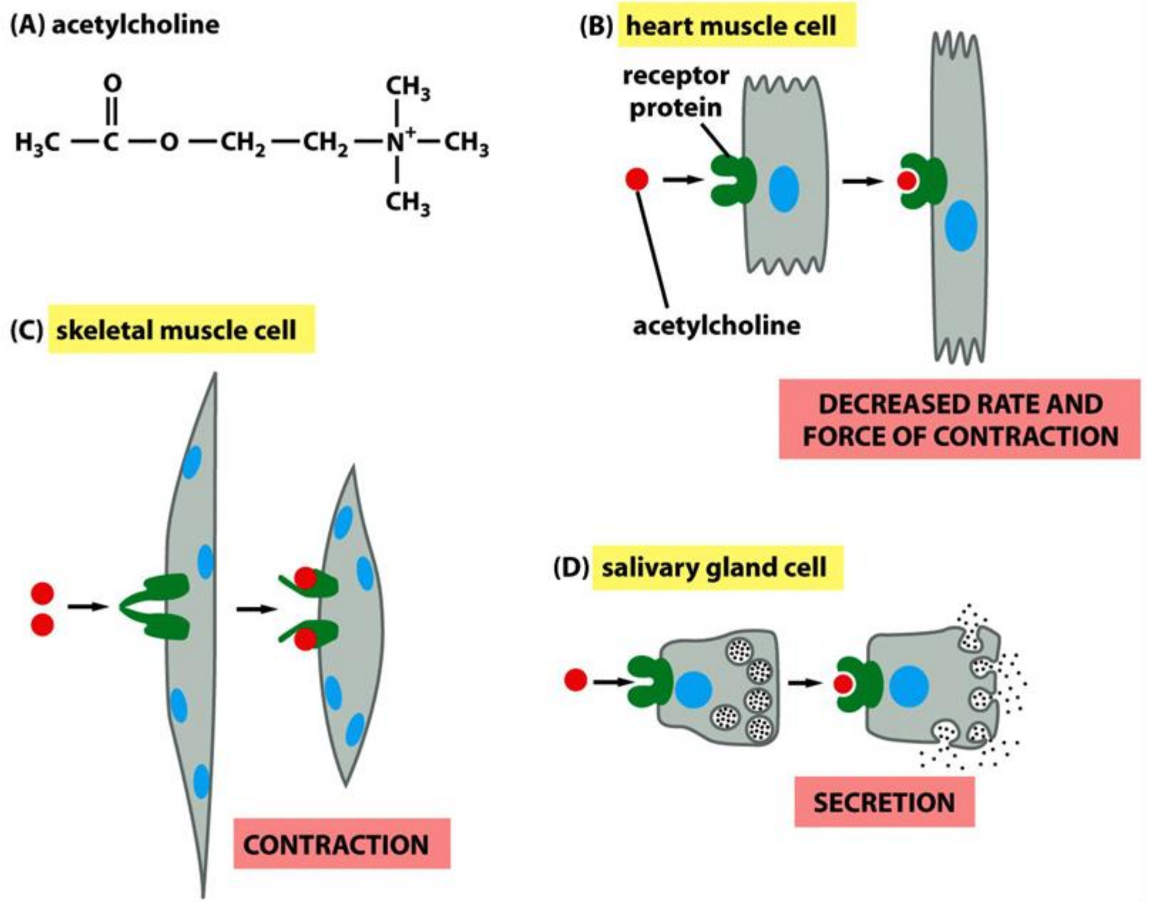
Slow: de novo protein synthesis in transcriptional response

Fast: change in protein behavior

Slow and fast responses



The same signals trigger different effects

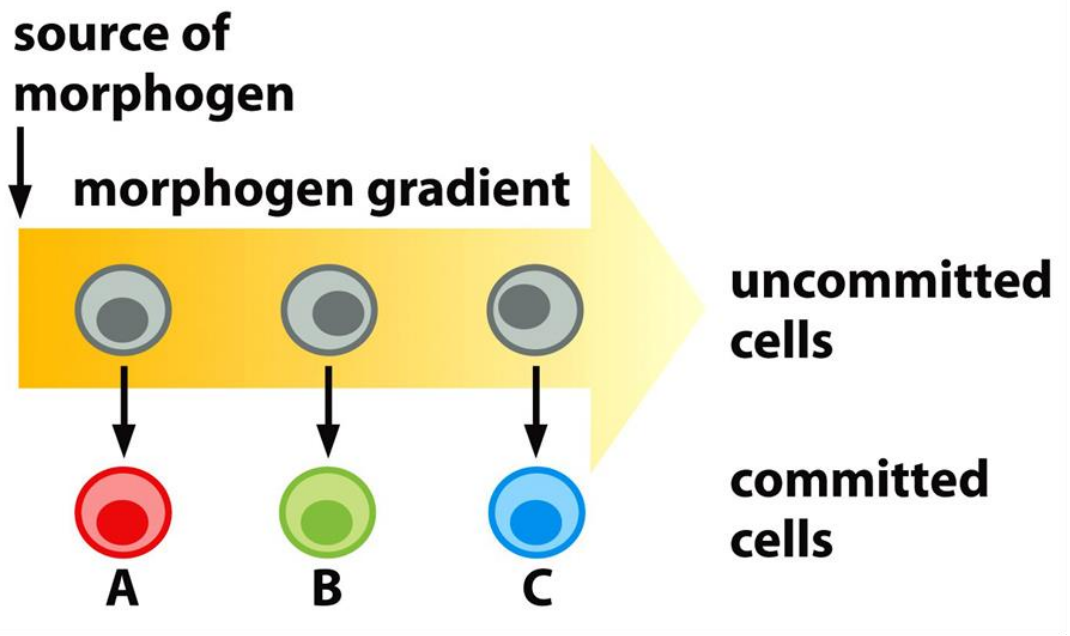


Acetylcholine receptors in heart muscle cells and salivary gland cells are identical
However, they result in different effector proteins activation.
Acetylcholine receptors in heart muscle and skeletal muscle are different.

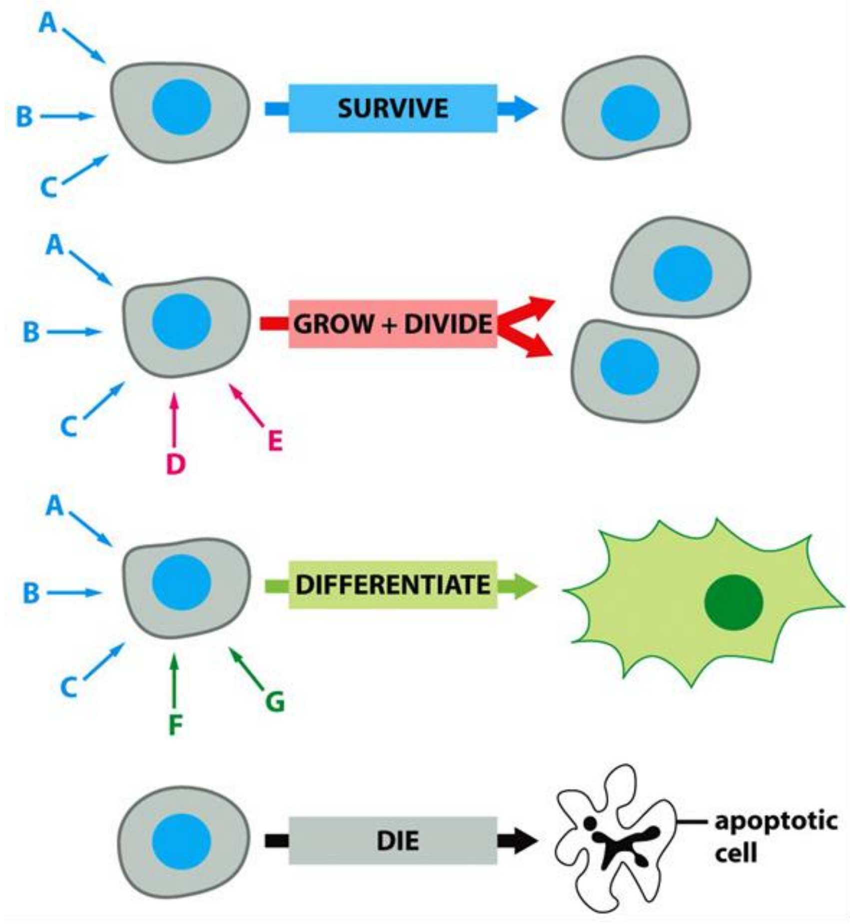
The same signals trigger different effects

- ♥Same signals act on different receptors
- ♥Same signals act on same Receptors but trigger different effectors
- ♥The same cell type reacts differently to different concentration of signals---the signal in this case is called---morphogen.

Morphogen in development



Cell is programmed to respond to specific combinations of signals



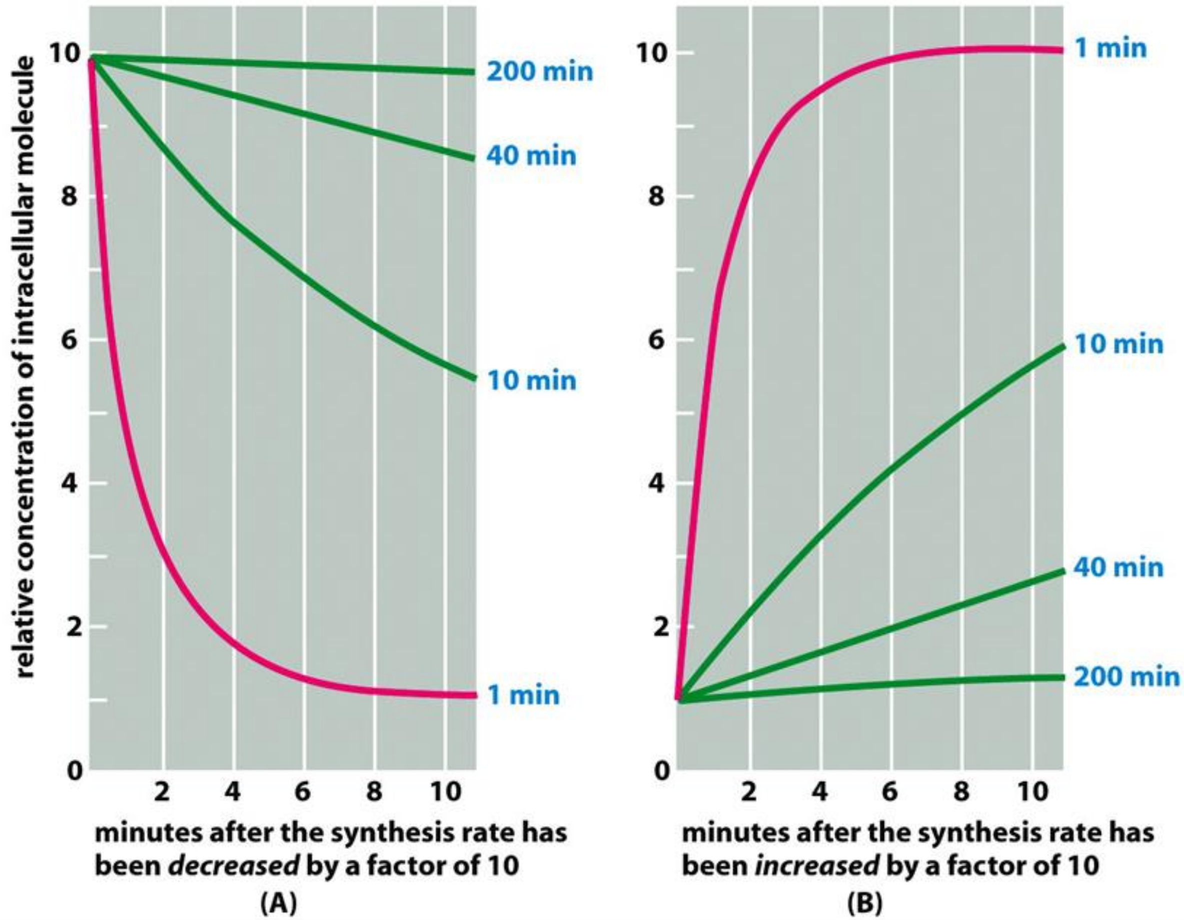
A cell can integrate multiple receptor signaling to dictate individual cell behavior.

The amount and activity of signaling molecules are important

•Many proteins in signaling have short half lives---**ensure quicker response**

•Many signaling proteins have conversion between inactive and active states---**quicker response than de novo protein synthesis**

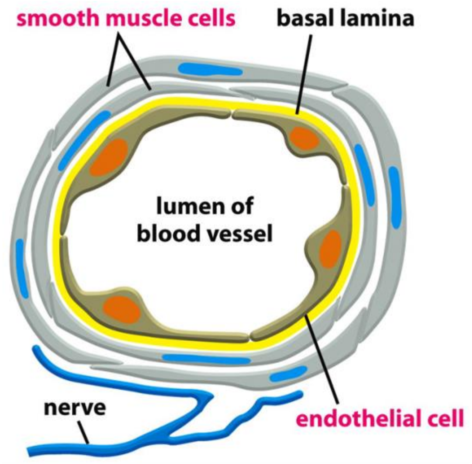
Proteins that have faster turn over Rate react to stimuli in a faster manner



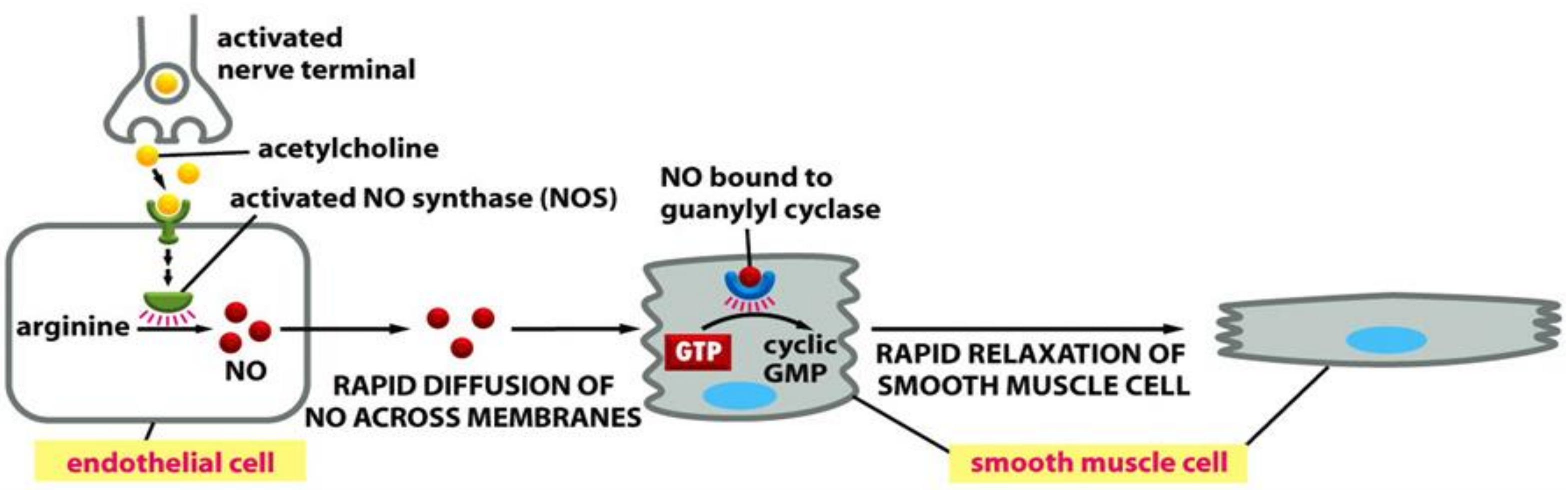
II. Intracellular receptors

- Signaling molecules are hydrophobic and can cross plasma membrane.
- Examples:
  1. NO gas, CO gas
  2. steroid hormones, thyroid hormones, retinoids, vitamin D, etc.

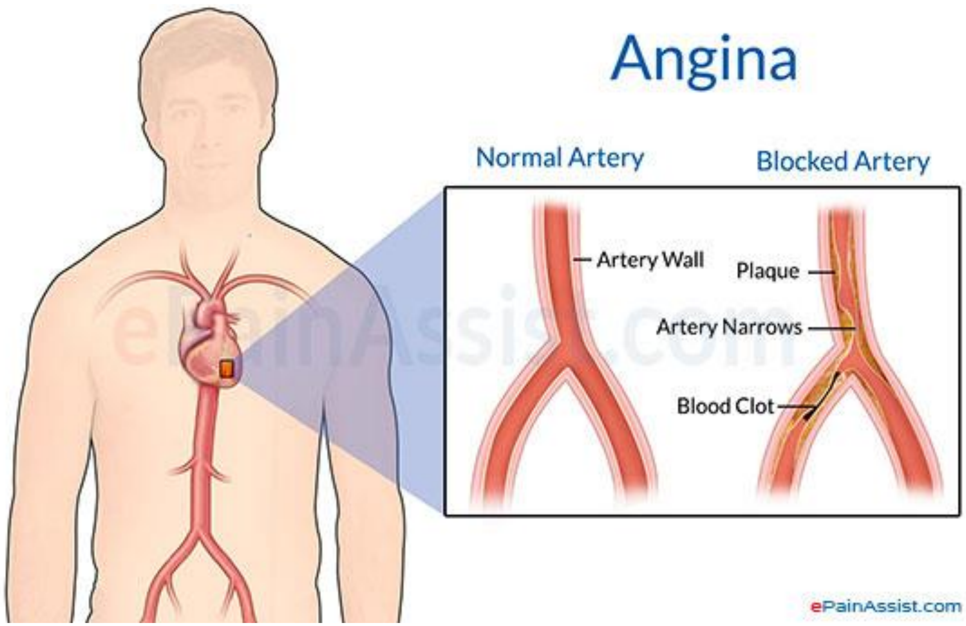
1. Signaling of NO in smooth muscle relaxation in blood vessel

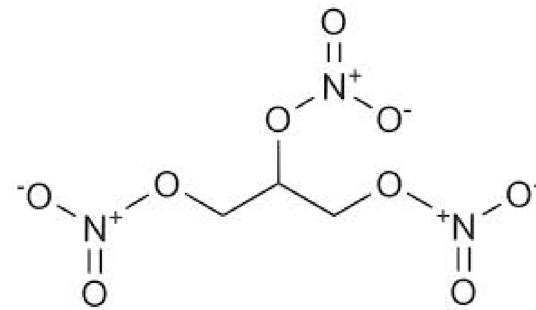


NO has a half life of 5-10 sec.
It is rapidly converted by water and oxygen into nitrates and nitrites



Mechanism of nitroglycerin in treating angina pectoris





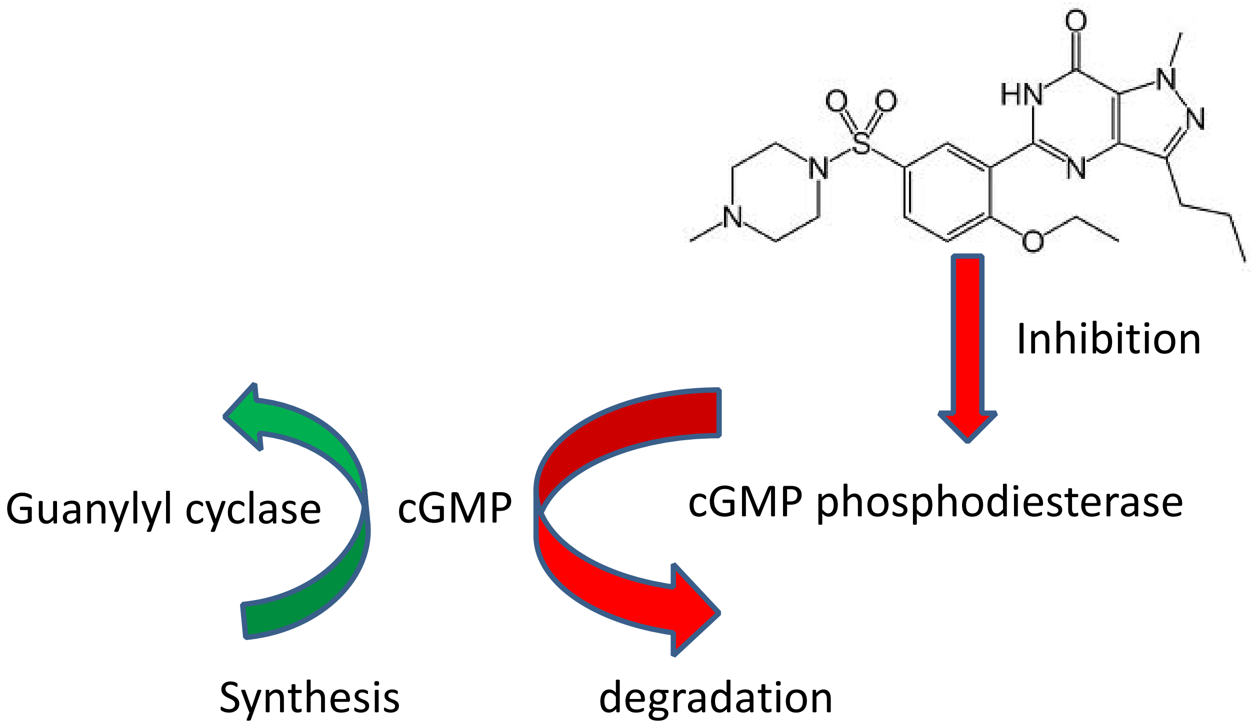
NO gas

Increase in cGMP

Dilation of blood vessel

Reduce workload of heart

Mechanism of Sildenafil---commercial name Viagra

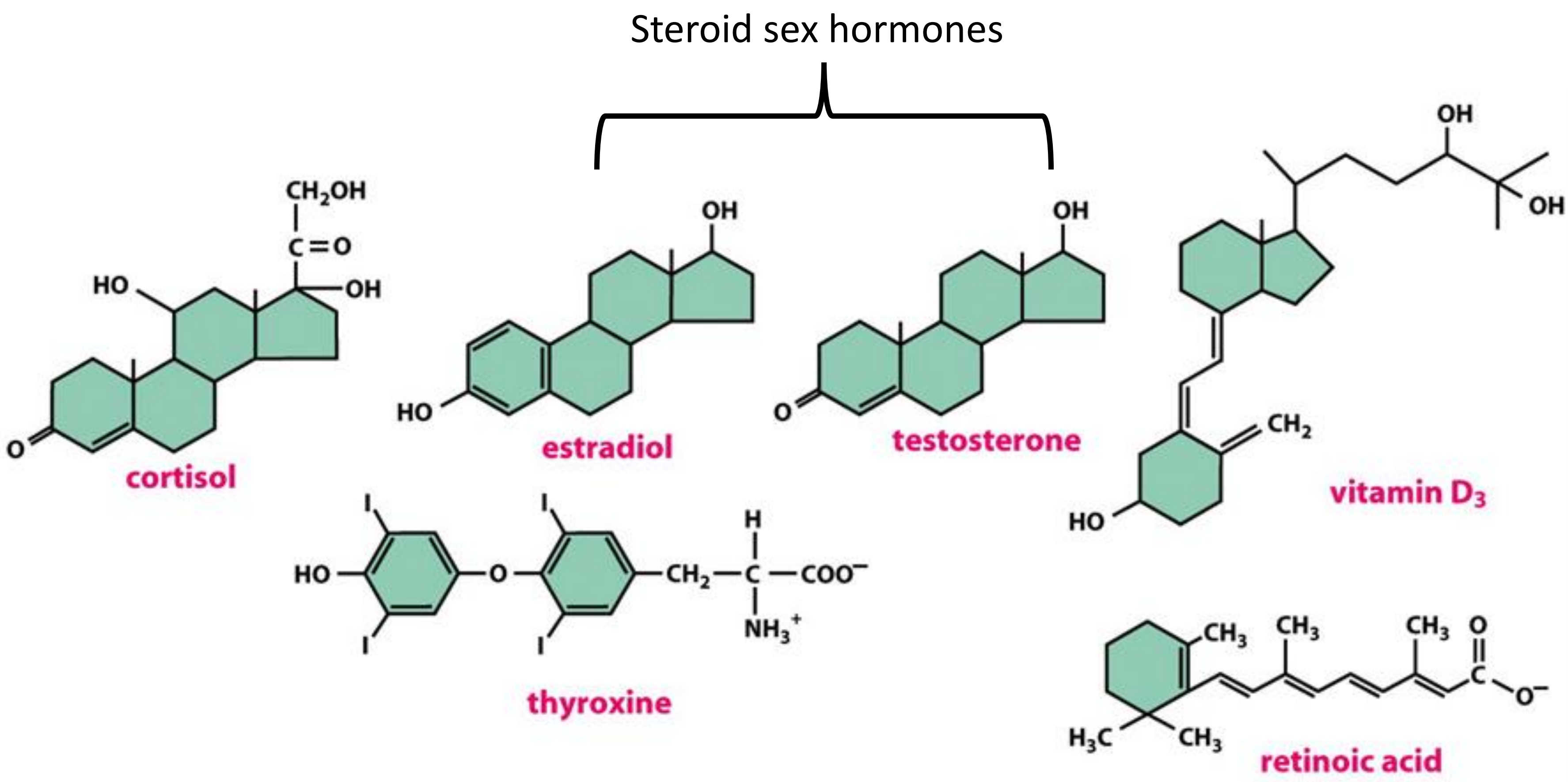


Accumulation of cGMP causes prolonged blood vessel dilation

2. Signaling via nuclear receptor

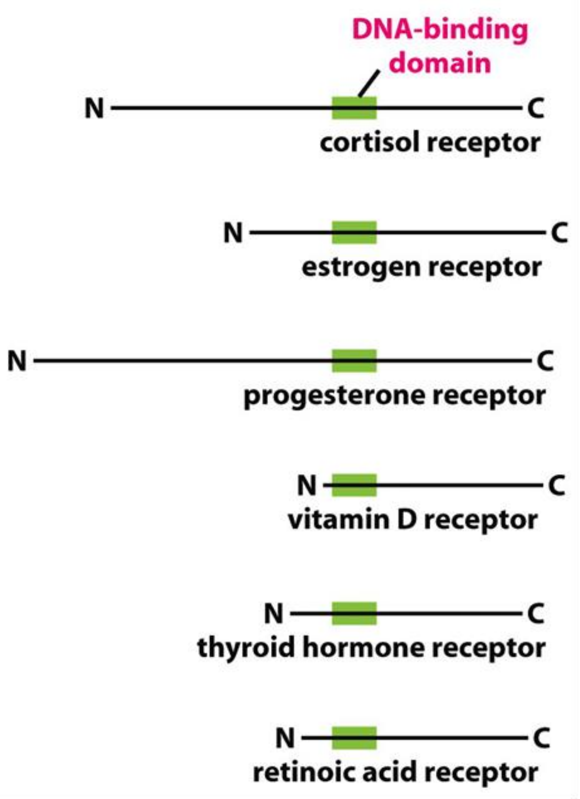
- ♦ Steroid hormones (made from cholesterol) :
  - ♥ cortisol (secreted from cortex to adrenal gland)
  - ♥ Sex hormones (estradiol, testosterone, progesterone
  - ♥ Vitamin D (synthesized in the skin under sunlight)
  - ♥ molting hormone ecdysone (insects)
- ♦ Thyroid hormone: (made from tyrosine)
- ♦ Retinoids (made from vitamin A)

Some nongaseous signal molecules that bind to intracellular receptors



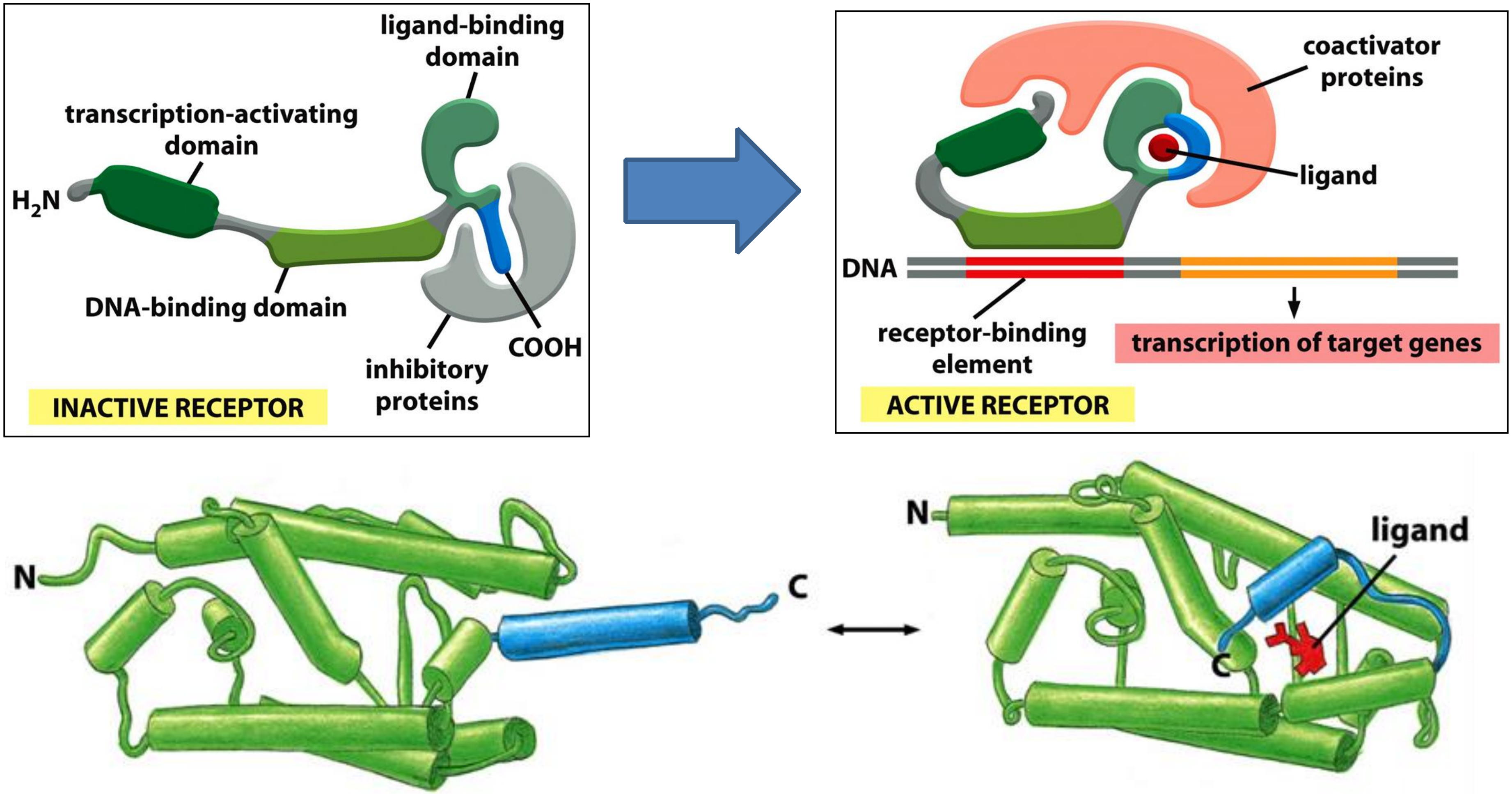
Common features of nuclear receptors

- Work either as homodimer of heterodimer
- Serve both as ligand receptor and gene transcription factor

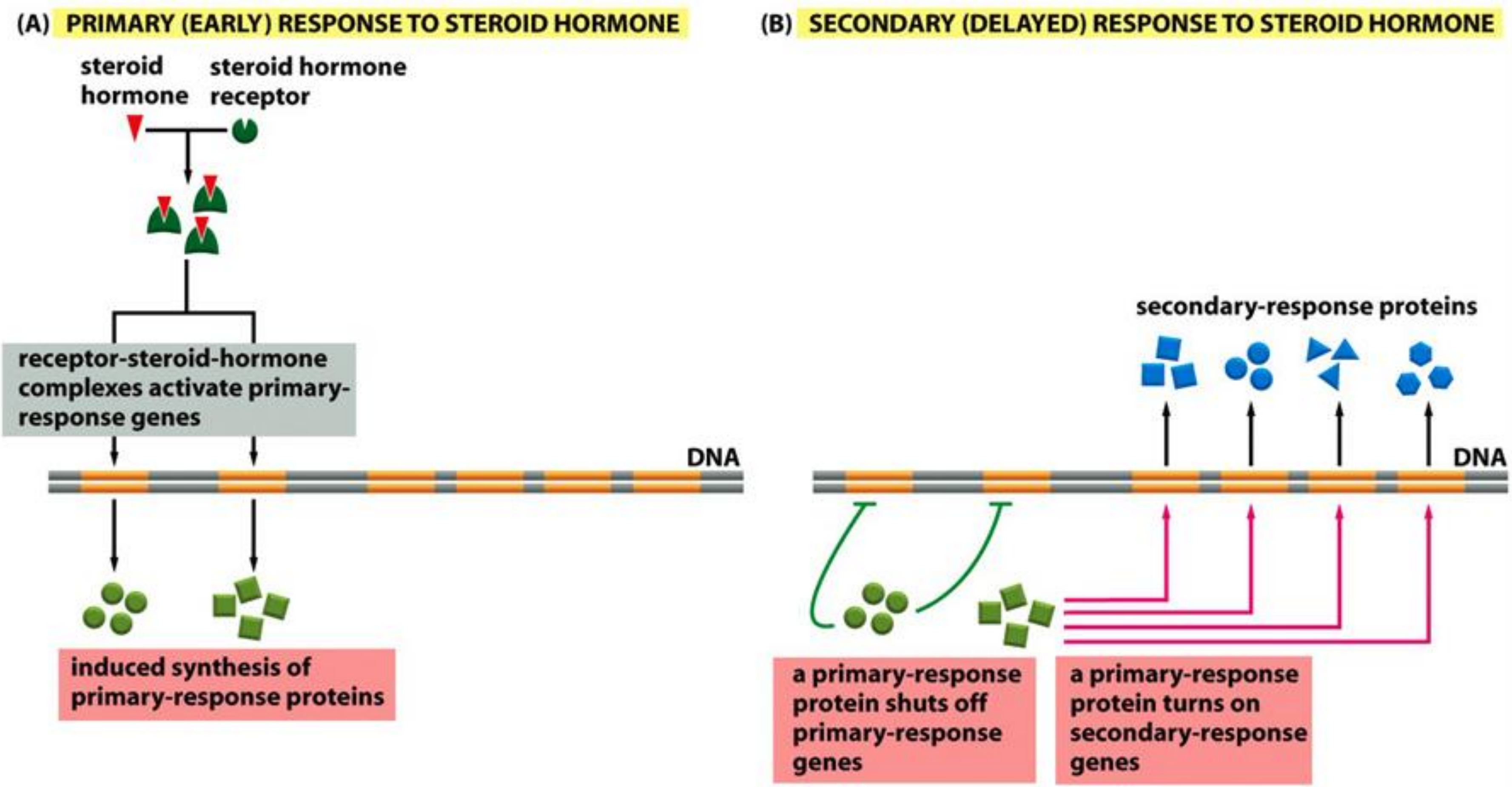


All these receptors have DNA binding domain and gene transactivation domain

A model for how nuclear factor works



Hormone receptors trigger both primary and secondary responses



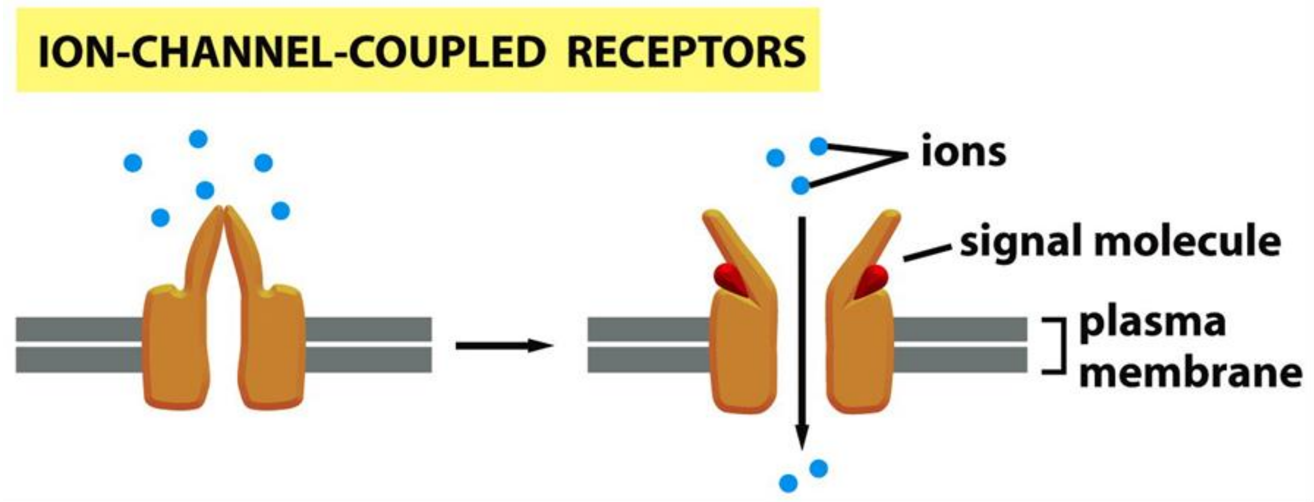
III. Cell surface receptor signaling

- ♥ Ion-channel coupled receptor
- ♥ G-protein coupled receptor
- ♥ Enzyme-coupled receptor

  Other types

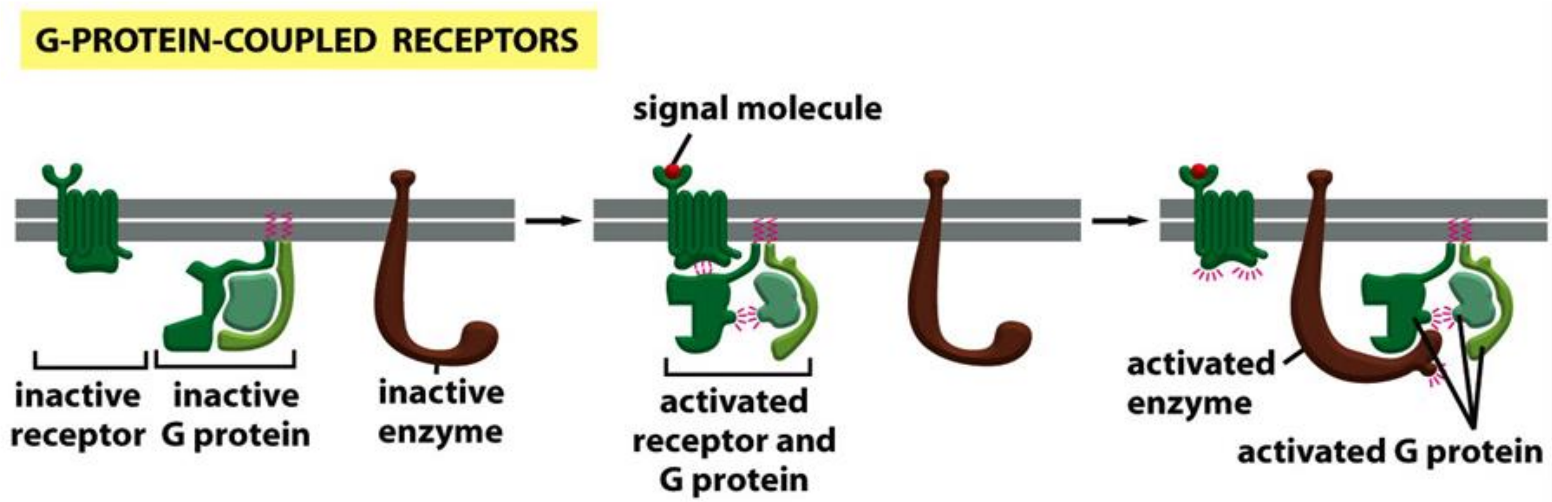
The major three classes of cell surface receptors

1.



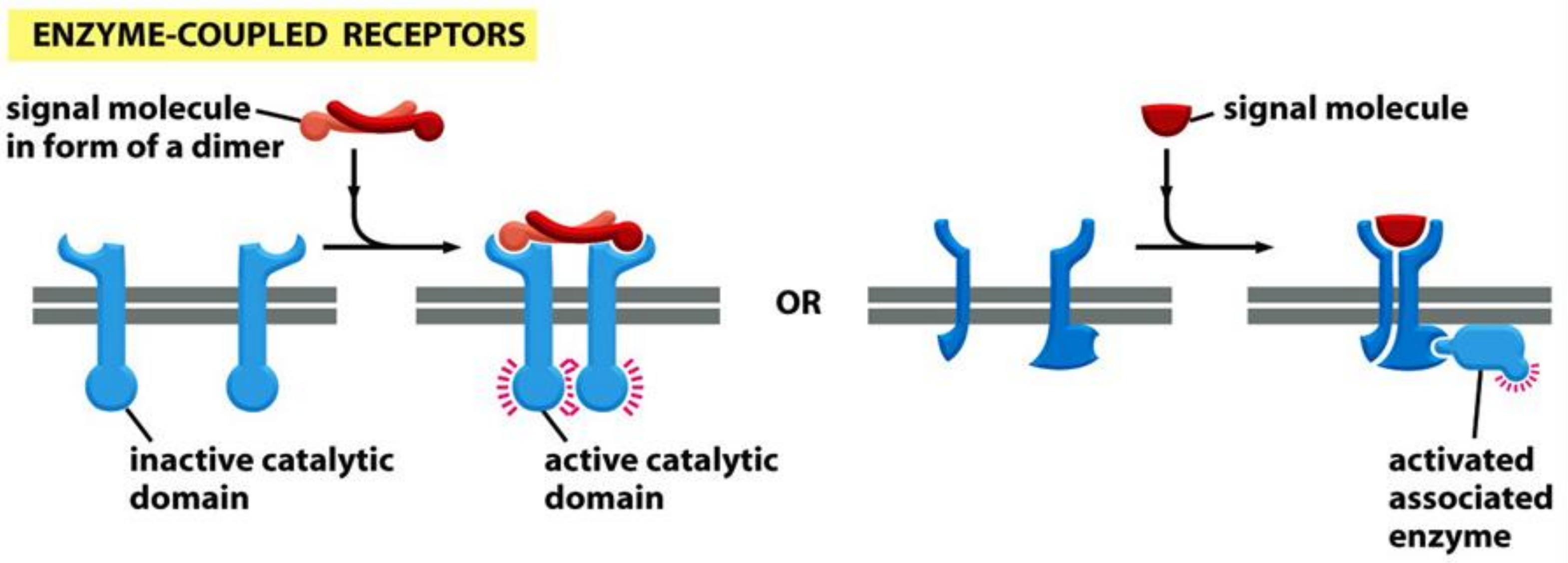
The major three classes of cell surface receptors

2.



The major three classes of cell surface receptors

3.



The concept of second messenger

- ♦The first messenger---extracellular signals
- ♦Second messenger--- small molecules generated in large numbers after receptor activation. They are either hydrophilic or lipid diffusing.
- ♦Second messenger work on effector proteins and relay signals.
  - ♥cAMP
  - ♥cGMP
  - ♥Ca^{2+}
  - ♥diacylglycerol (DAG)
  - ♥Inositol triphosphate (IP3)

Relay of signals from cell surface receptors

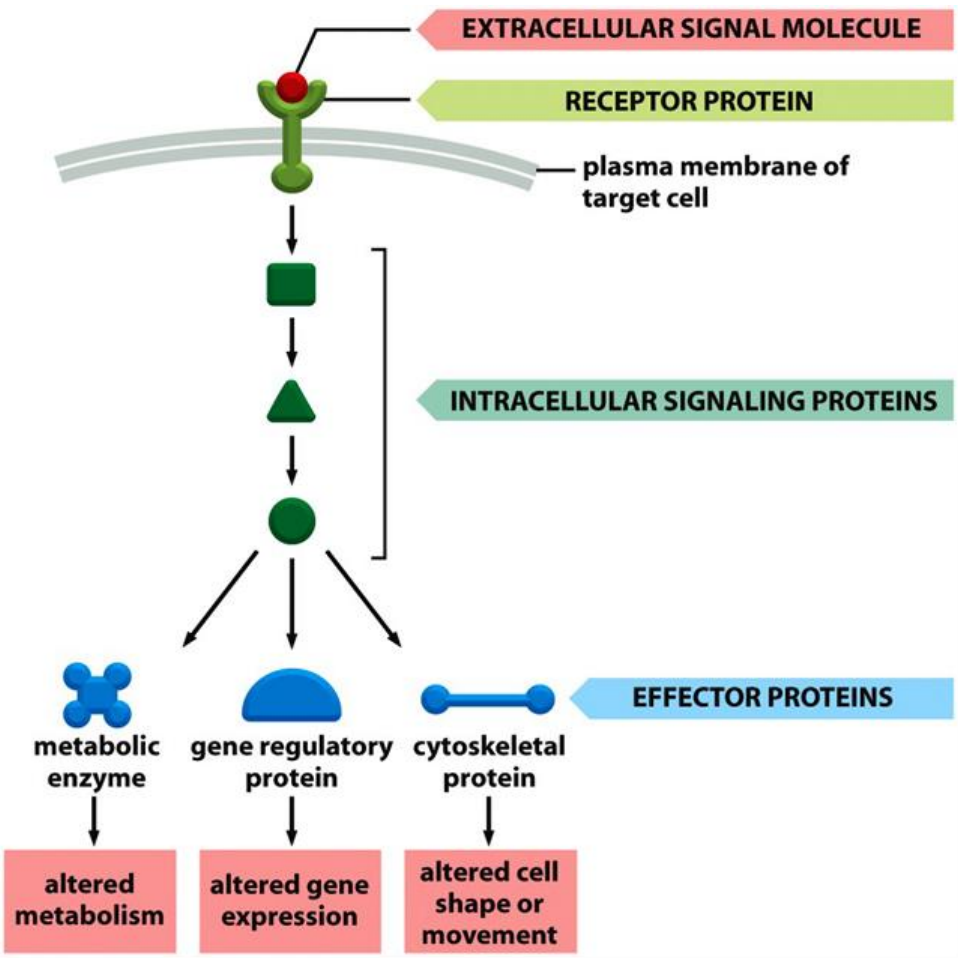
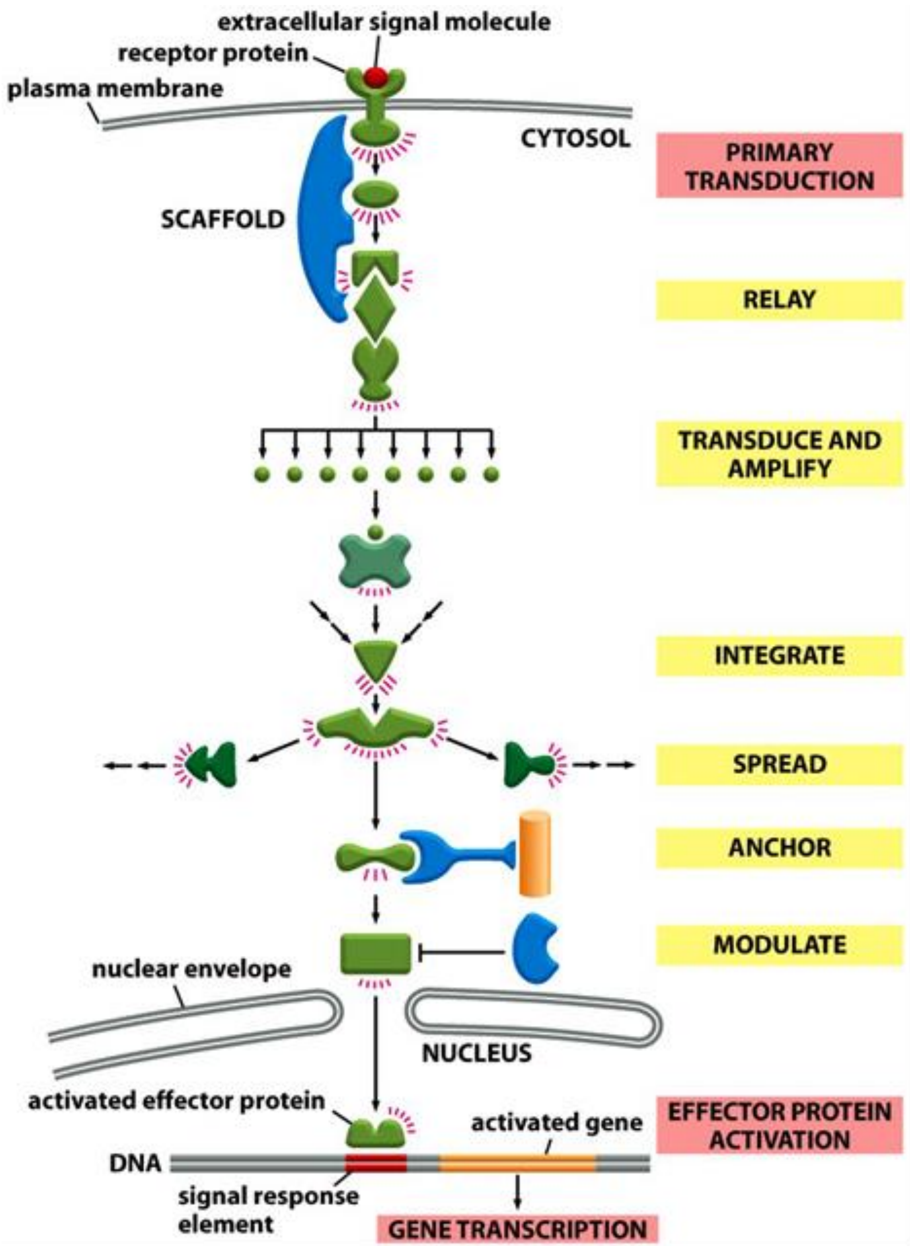


Diagram to show various functions for the signaling proteins

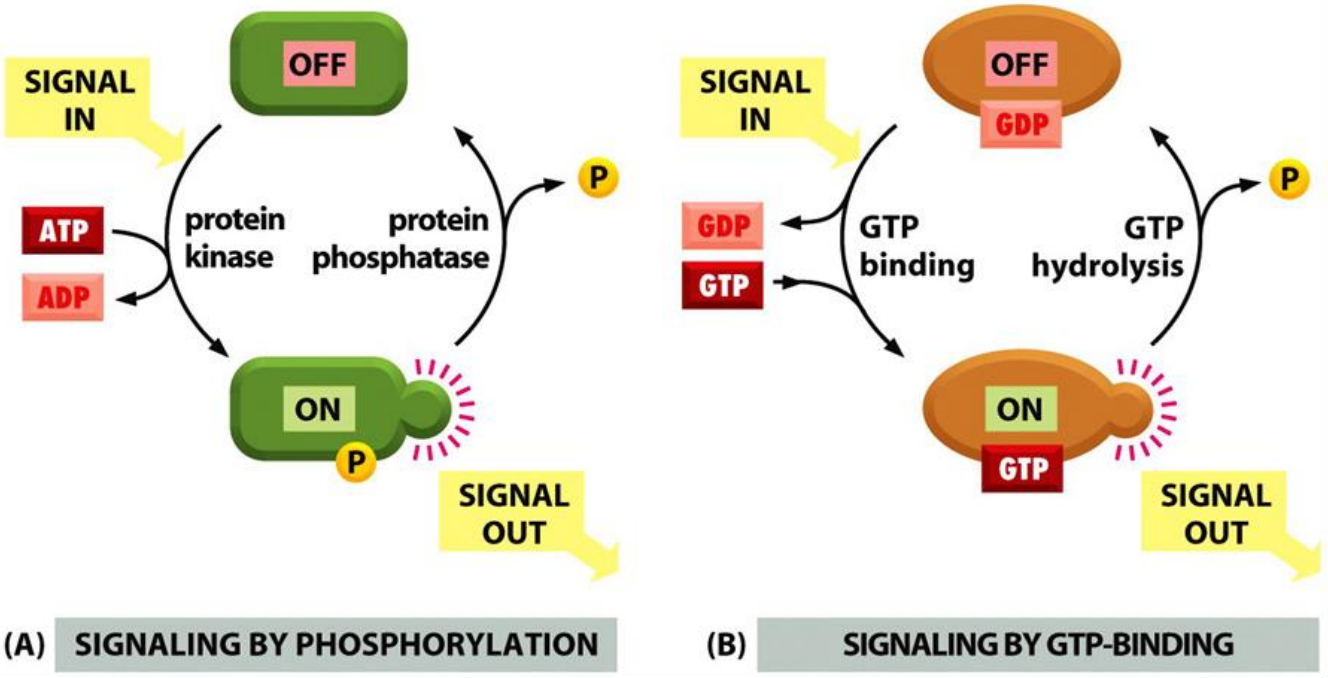


Functions for intracellular signaling proteins

- **Relay** signals to the next component
- Act as a **scaffold** to bring two signaling proteins more quickly and efficiently
- **transform** the signal into a different form.
- **Amply** the signal it receives---signaling cascade
- **Integrate** signals from two or more pathways
- Spread signals from one pathway to another---**crosstalk**
- **Anchor** signaling proteins to a specific structure
- **Modulate** the activity of signaling proteins

important types of switches to regulate protein activity

- **Protein phosphorylation**
- **GTP-binding**
- cAMP or Ca2+ binding
- Ubiquitination, etc.



Protein phosphorylation

♥It is one major way of post-translational modification to regulate protein activity

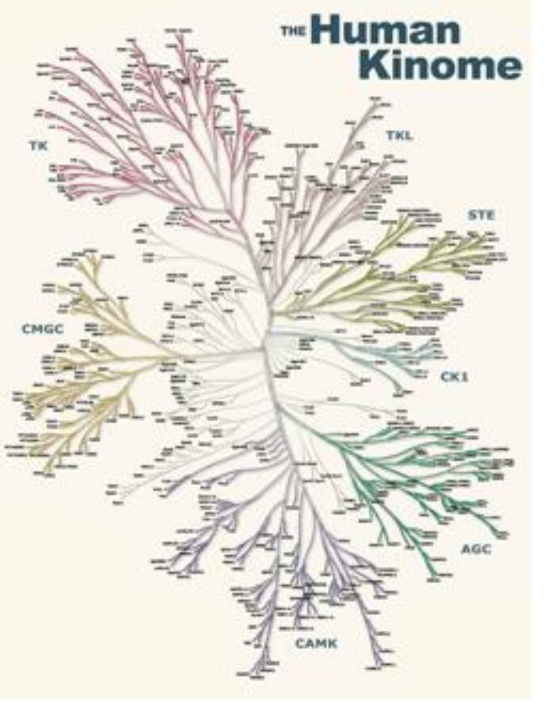
♥>30% of all human genome proteins can be phosphorylated

♥>520 human kinases (kinome) and >150 protein phosphatases

♥Two categories: Serine/Threonine kinase; Tyrosine kinase

♥Protein kinases are major therapeutic targets in human diseases

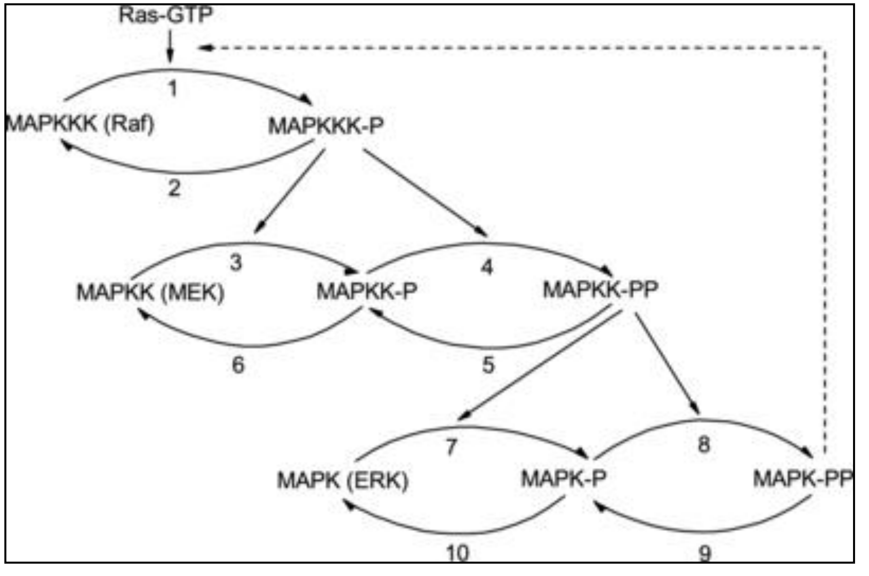
e.g. : Acute leukemia --- Gleevec targets BCR-ABL kinase



Phosphorylation cascade

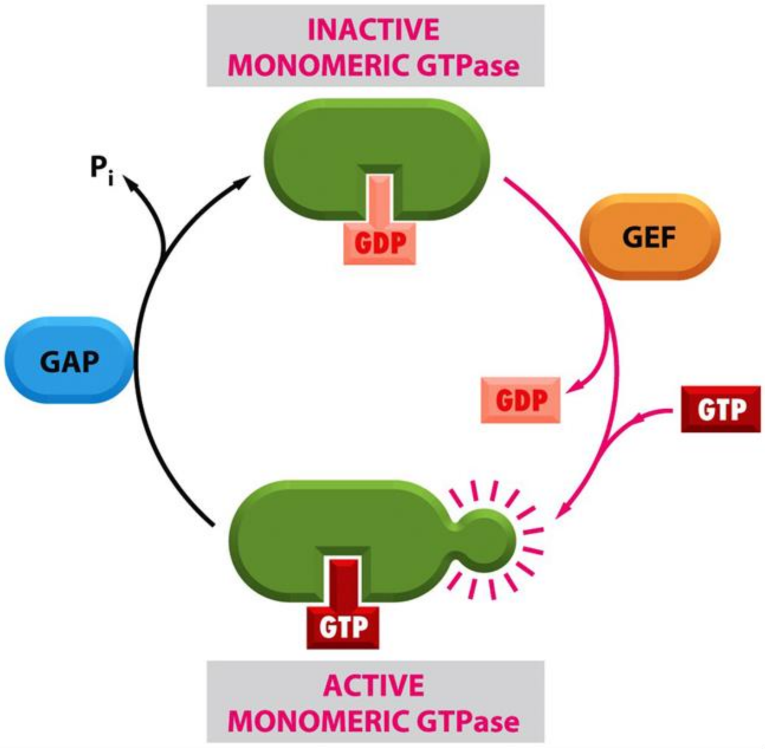
Signaling protein itself is a kinase which can phosphorylate and activate downstream effectors

For example: Ras-Raf-MAP kinase pathway



GTP-binding proteins (G-proteins)

- Large trimeric GTP-binding proteins
- Small monomeric GTPase

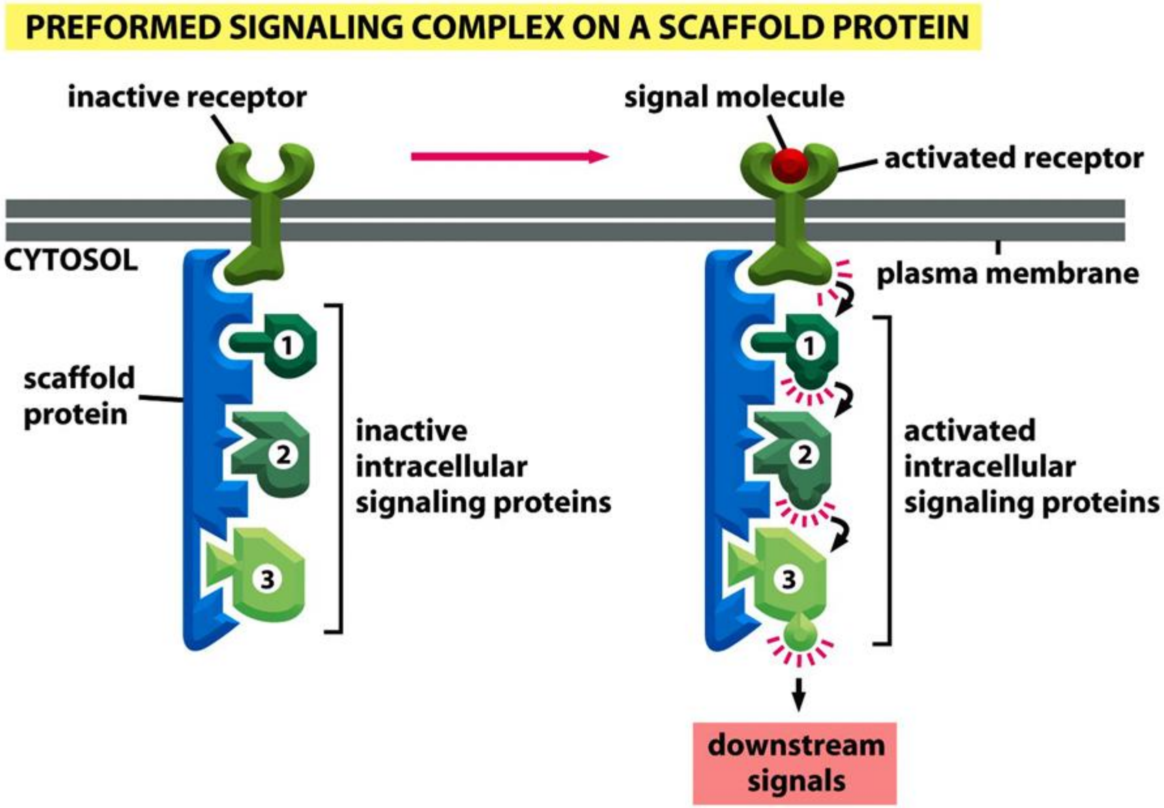


Characteristics of signal transduction

- Specifity
- Efficiency
- Reversibility
- Saturation
- High binding affinity

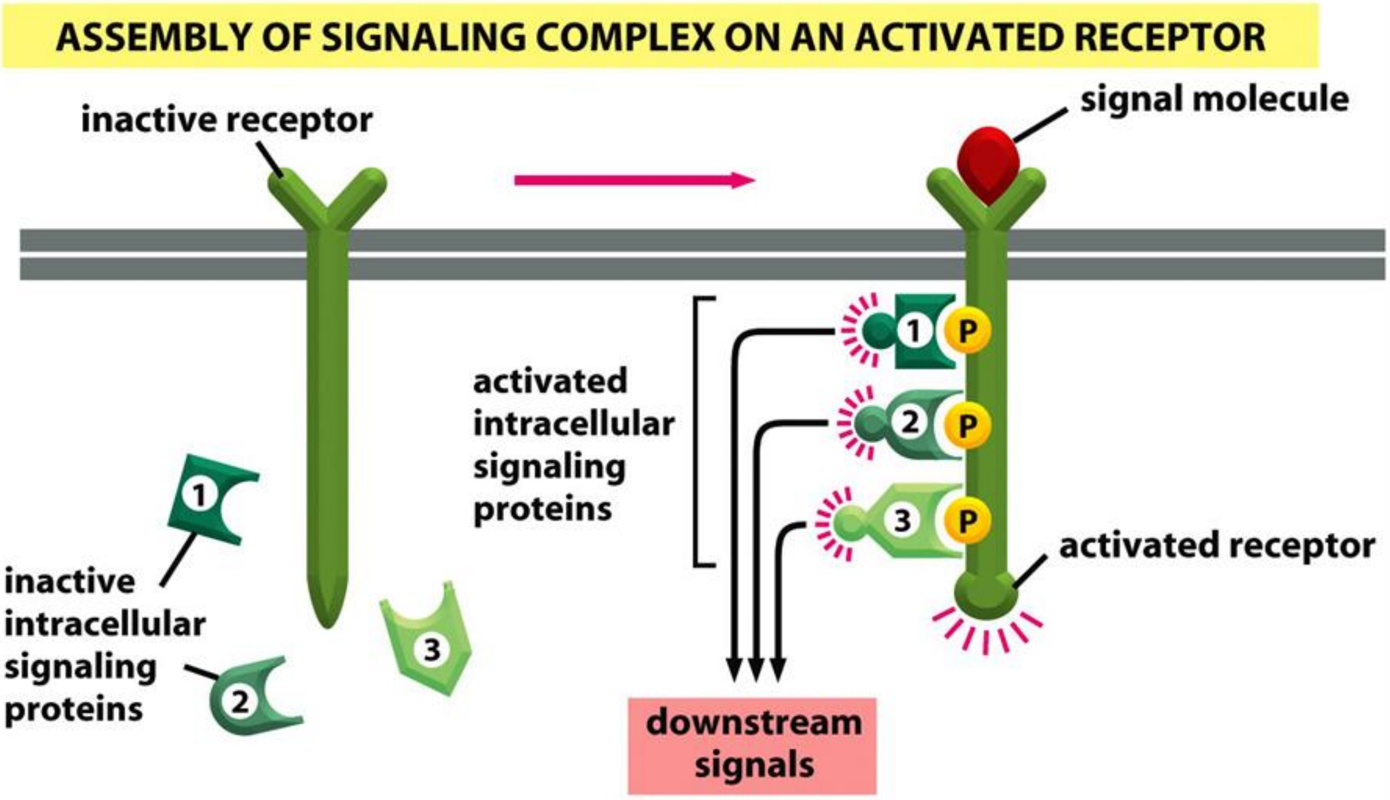
How to achieve high speed and specificity in signaling

(1)



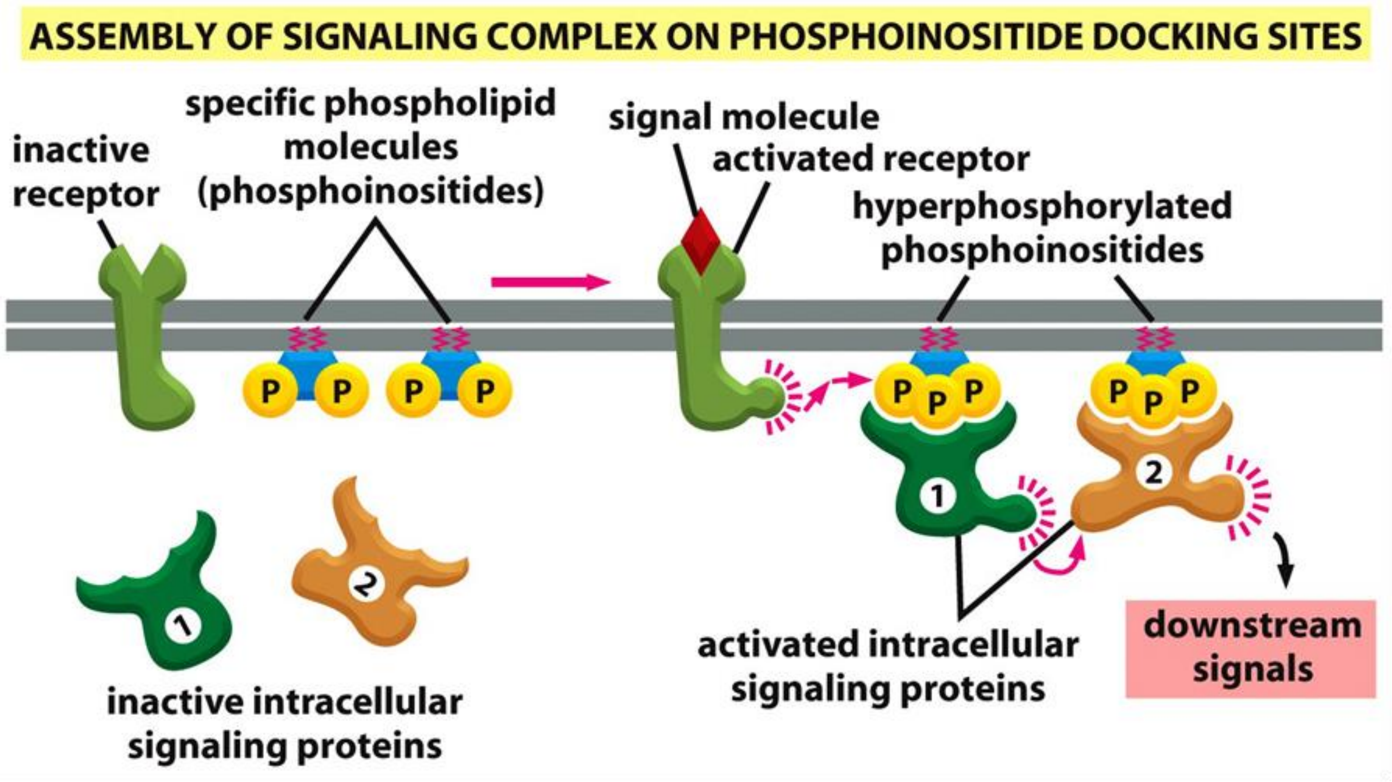
How to achieve high speed and specificity in signaling

(2)



How to achieve high speed and specificity in signaling

(3)



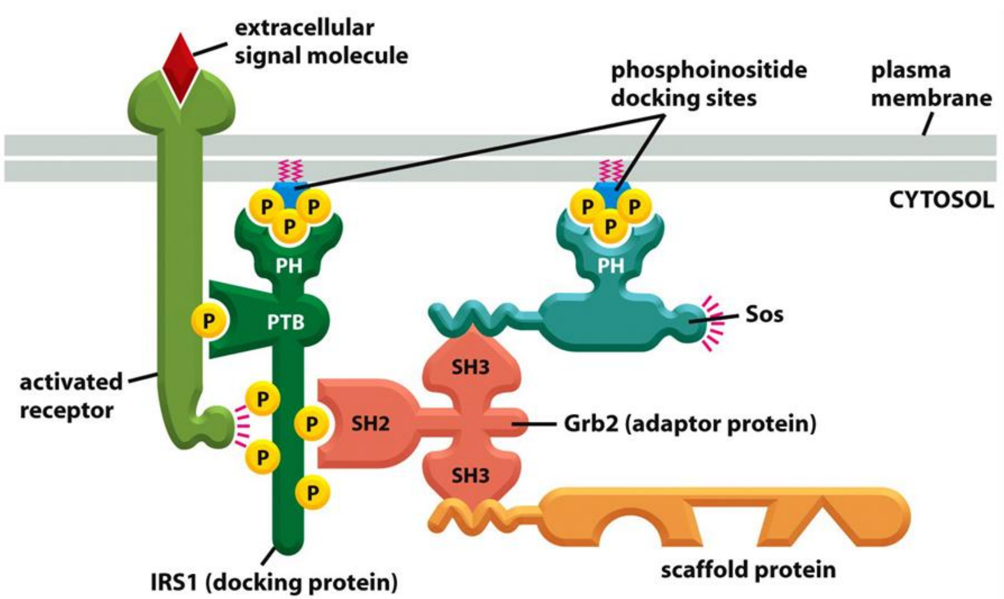
Conserved *interaction domains* are important in protein binding

- Scr homology 2 (SH2) domain
- Phosphotyrosine-binding domains (PTB)

Bind phosphotyrosine

- Scr homology 3 (SH3) : bind proline rich domain
- Pleckstrin homology (PH): bind phosphoinositides

Diagram to Show how Domains Mediate the Interaction:

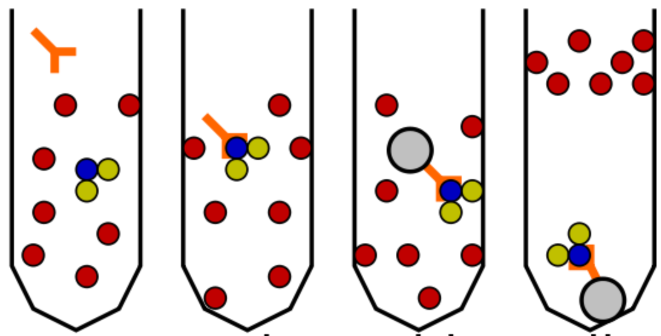


IV. Several methods to study signal transduction

- Protein co-immunoprecipitation
- Western blotting, phospho-specific antibody
- In vitro protein activity studies
- shRNA/siRNA, inhibitors
- Rescue analysis

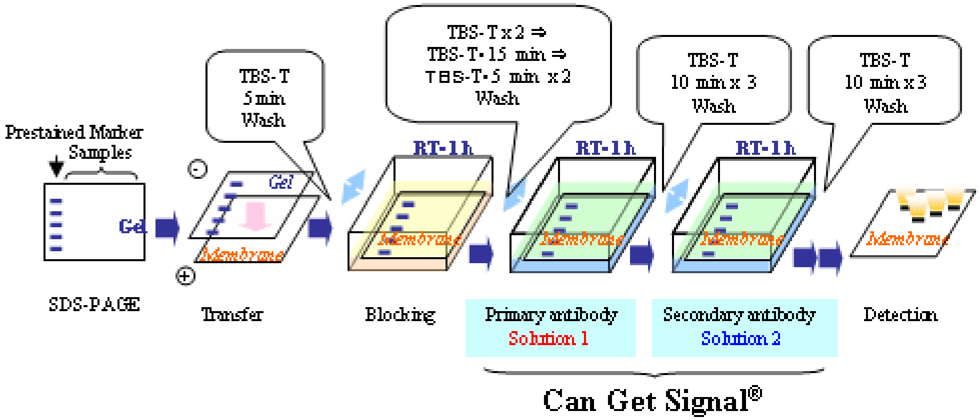
1. co-IP

applications: receptor-ligand interaction
kinase-substrate interaction
other protein interaction partners



1. Researcher adds antibody.
2. Antibody binds target.
3. Protein A beads bind antibody.
4. Centrifugation sediments beads.

2. Western blotting



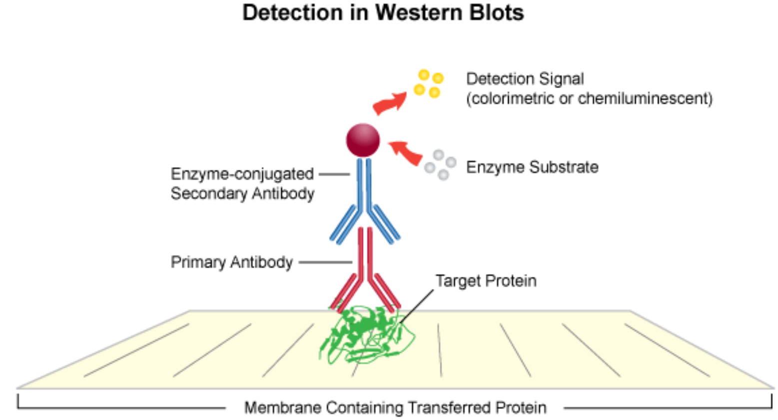
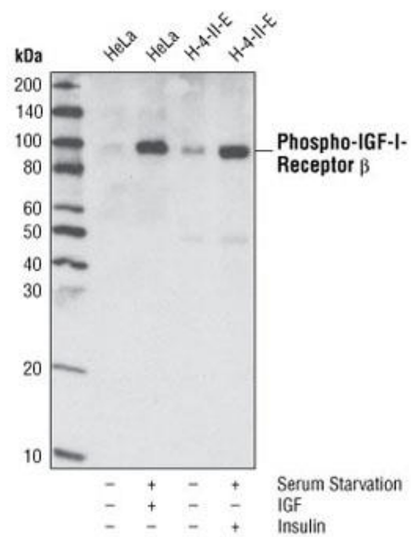


Diagram 2: *Illustration of detection in Western Blots.*

e. g. IGF receptor activation

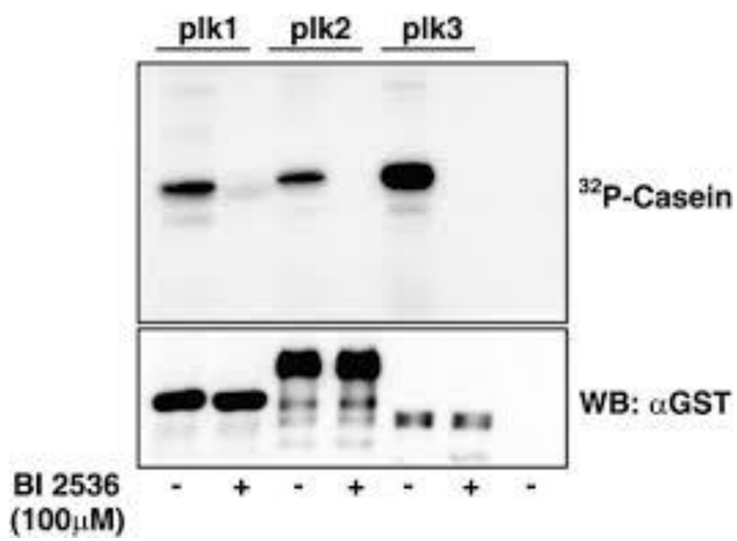


3. In vitro protein activity assay

(1). Purify protein in vitro

(2). Set up in vitro protein assay with substrates and necessary components such as ATP, etc

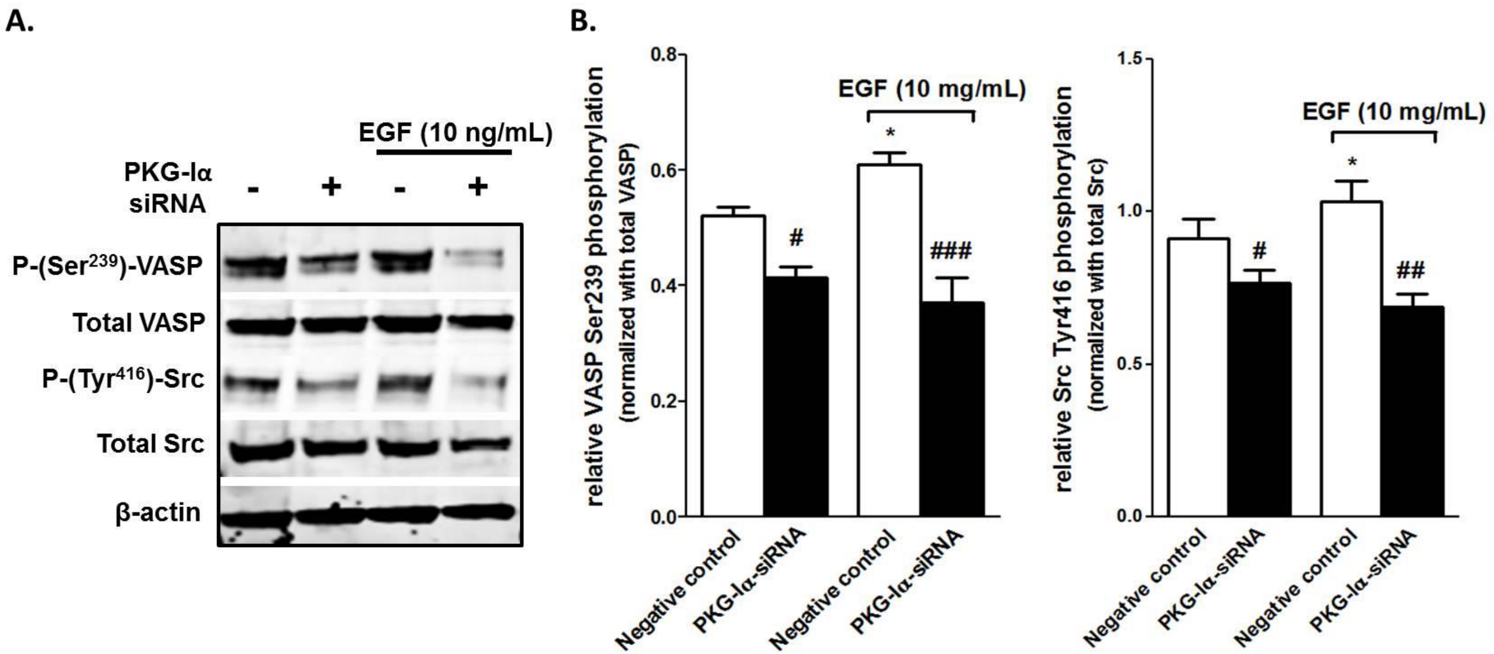
(3). Analyze protein activity by comparing signal strength.



4. shRNA/siRNA, inhibitors

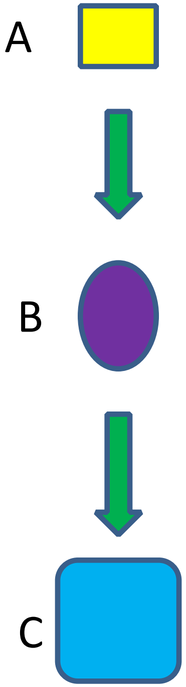
- shRNA- short hairpin RNA, siRNA-small interference RNA
- They work by triggering target mRNA degradation.
- Many enzymes have relatively specific inhibitors.

For example:



5. rescue assay

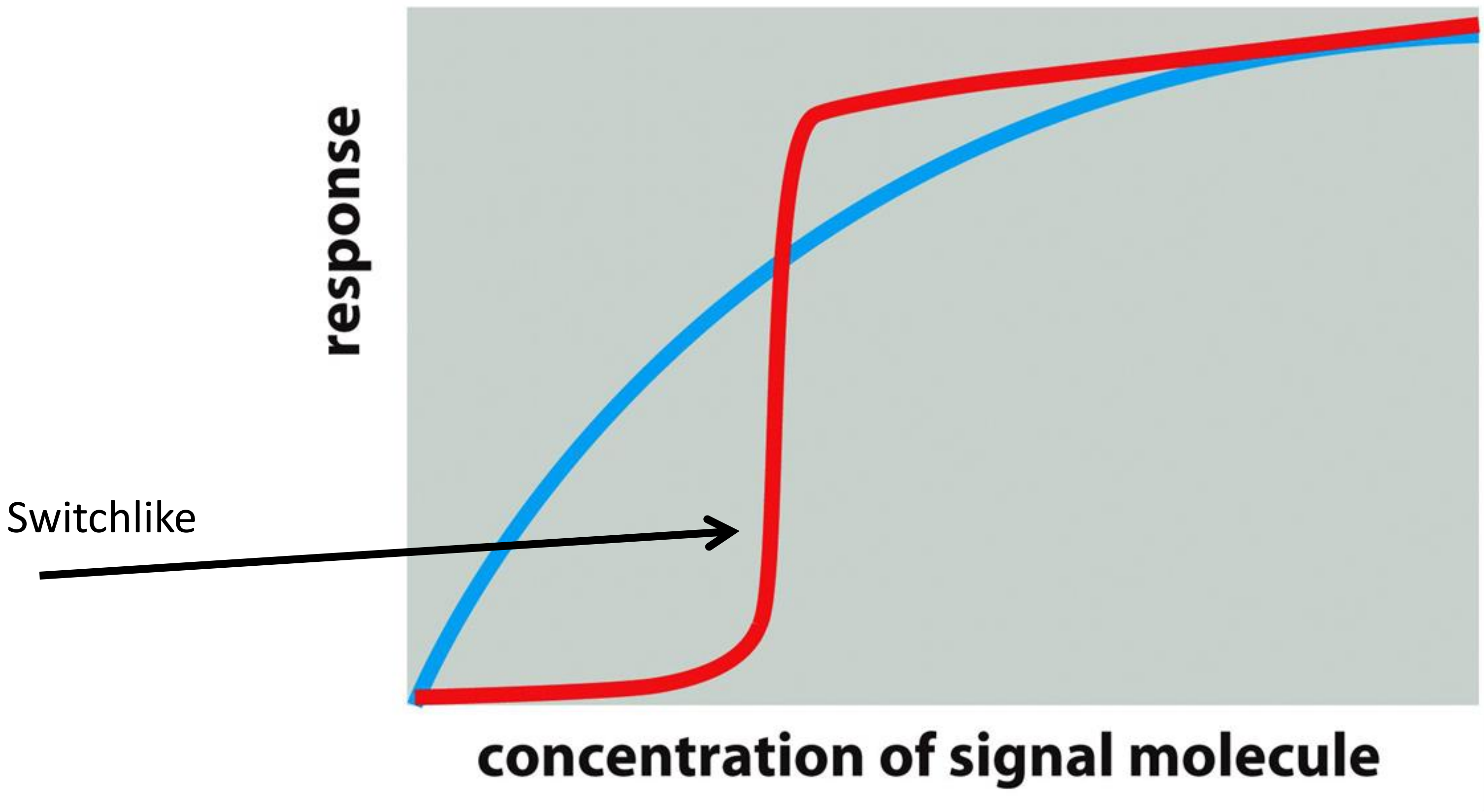
- How to prove one signaling protein locate upstream of downstream of another?



1. Deletion of A or B leads to a certain defect
2. Expression of activated C can rescue this defect.

V. signaling kinetics

Signaling can be both *All or none* and *smoothly graded* response



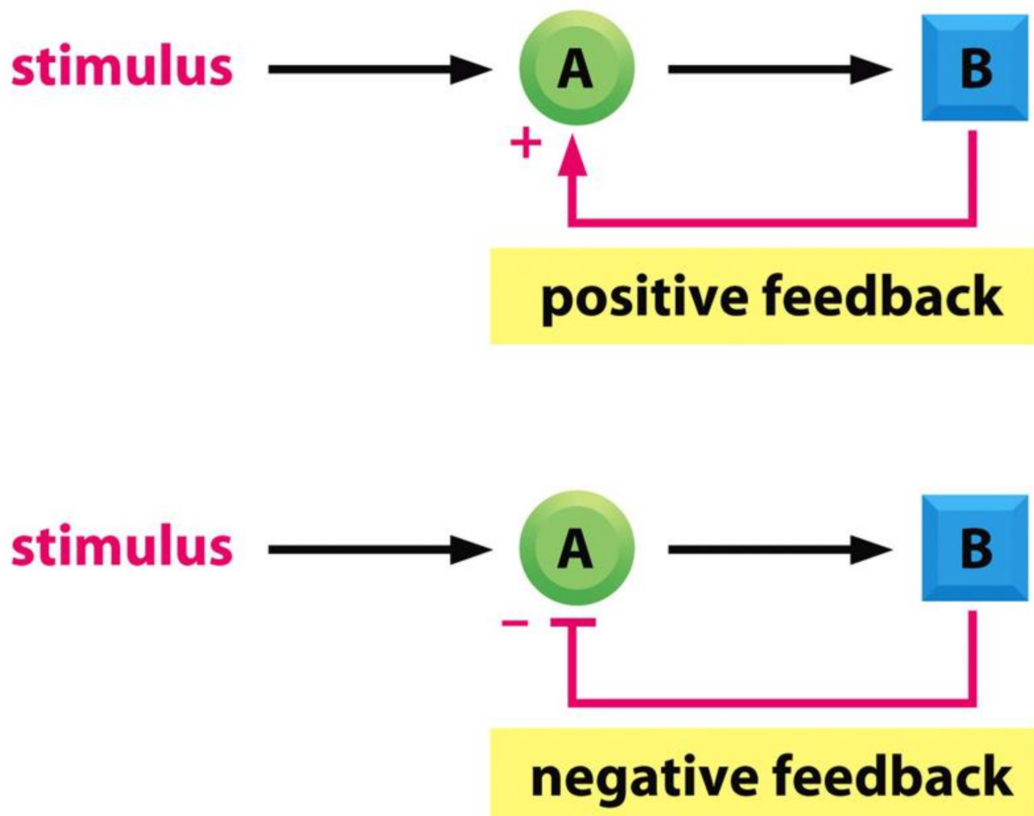
What causes switchlike responses?

♥ All of none could be due to cooperative response(e.g. 4 cAMPs bind to PKA)

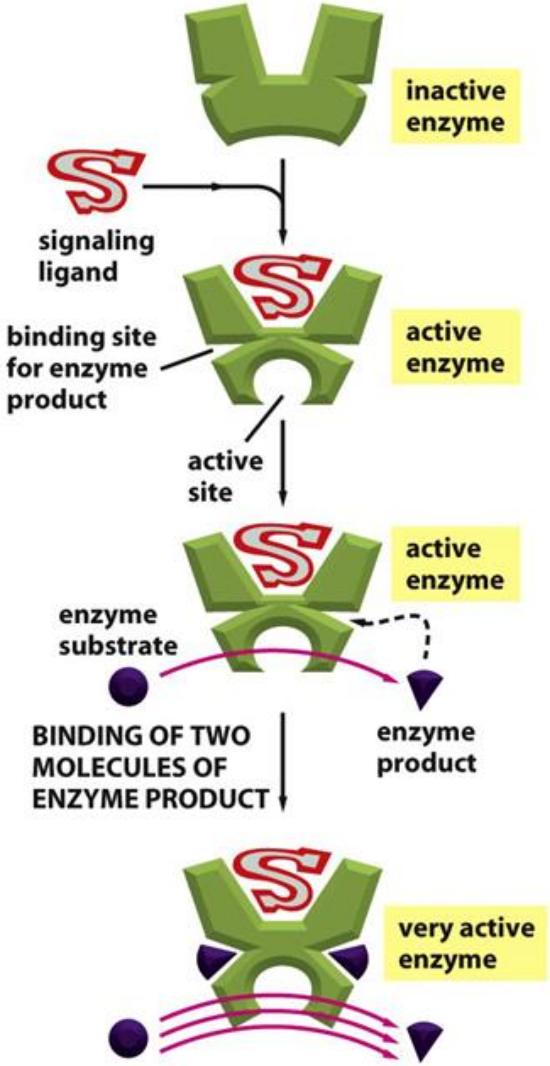
♥ Or it could be due to concerted effect of a simultaneous inhibition for the opposite reaction.

♥ It needs positive feedback response.

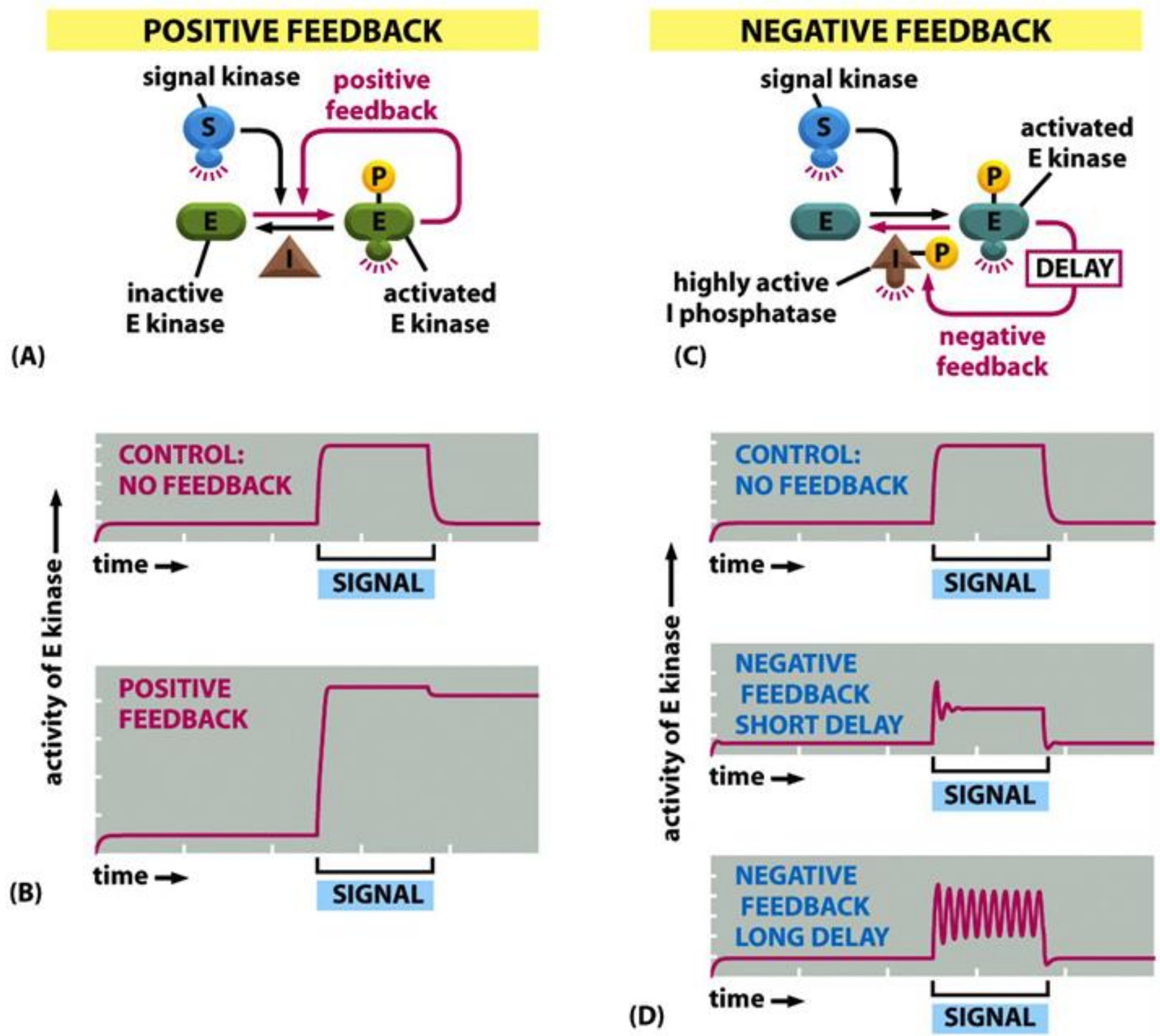
Positive and negative feedback



Positive feedback gives switchlike response



Different results from positive and negative feedback



Negative feedback allows adaptation/desensitization for cells

- Detects changes of concentration of signals.
- There are several ways to achieve these: