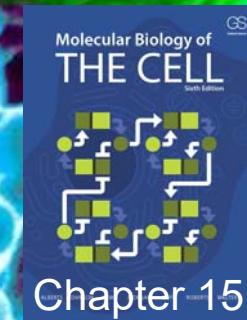


# Lecture 10

## Cell-cell Communication Part I

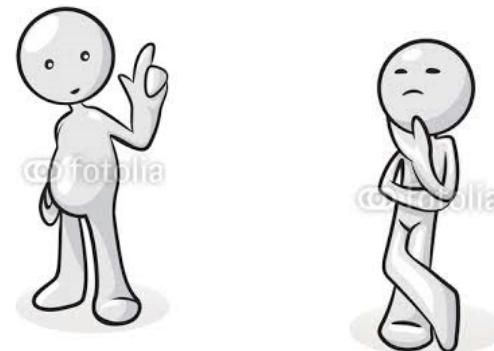
### Outline

- I. Overview of cell signaling -principles and players-
- II. Signal perception & transduction by intracellular receptors
- III. Signal perception & transduction by cell surface receptors
- IV. General methods to study cell signaling
- V. Positive and negative feedback in signaling  
& signaling kinetics



# What is cell communication?

Humans:



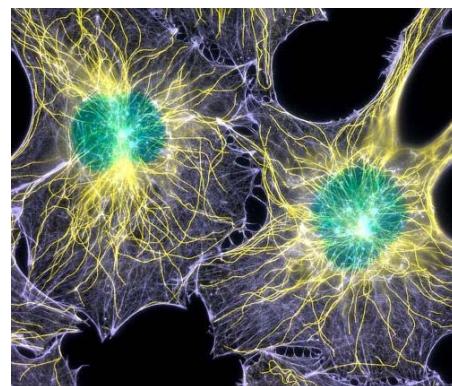
language  
body language

Insects:



pheromones  
touch  
noise

Cells:



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

# The seadevil-anglerfish: communication with light

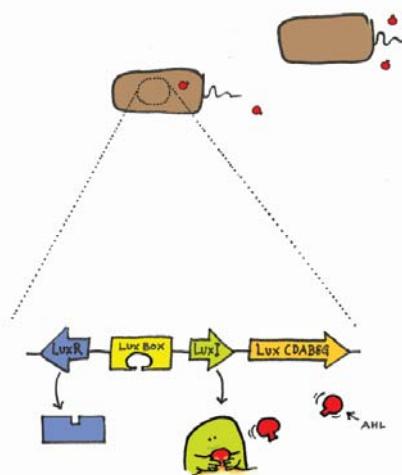


<https://video.nationalgeographic.com/video/weirdest-angler-fish>

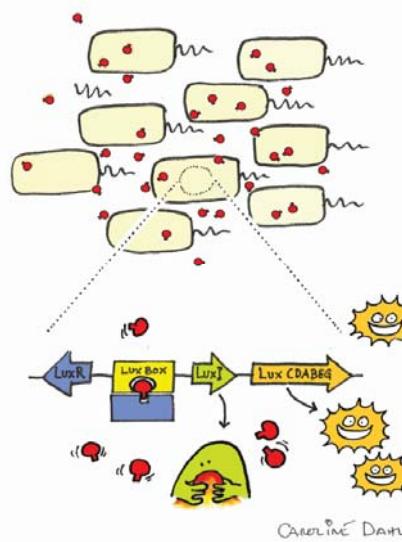
# Cell-cell communication: “Quorum sensing” in bacteria (determination of the density of the population)

Chemical signals secretion in correlation to the density of the cell population

A certain **density of the population** produces a **defined amount of a secreted effector** that triggers activation/deactivation of genes

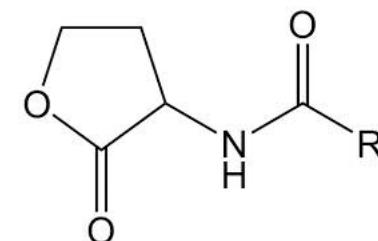


Low cell density

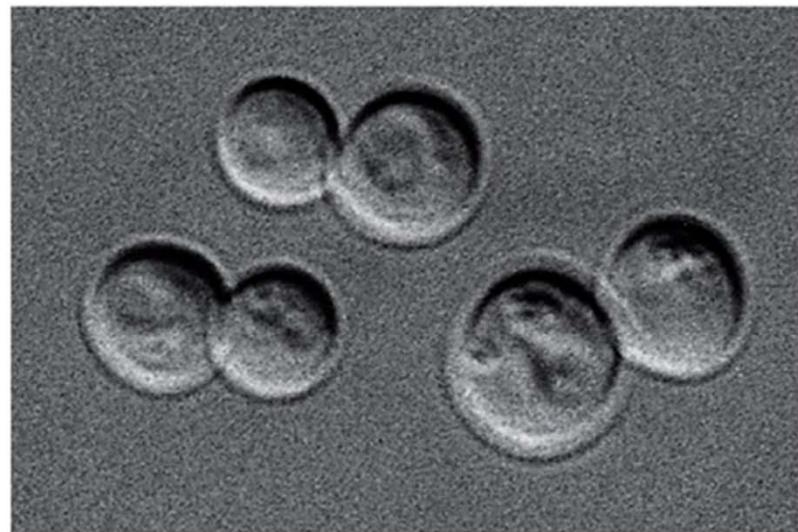


high cell density

The signaling molecule:  
AHL: acyl homoserine lactone

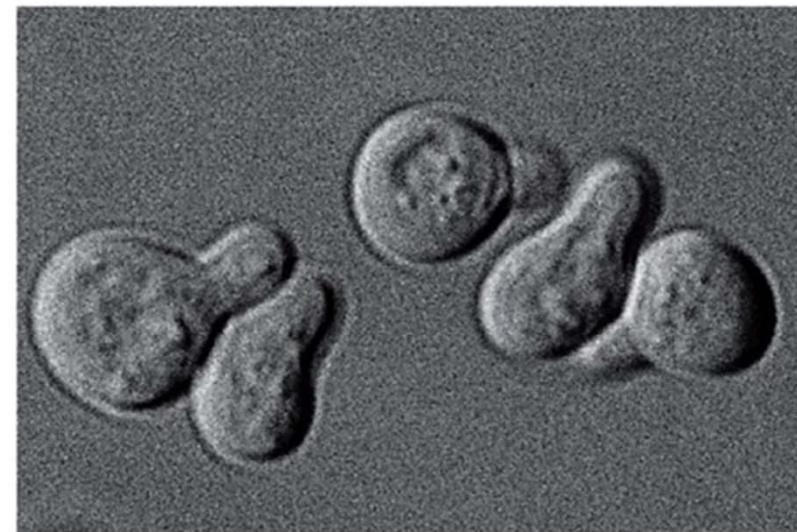


Cell-cell communication: Budding yeast mating corresponds to a secreted mating factor, the peptide  $\alpha$ -factor



(A)

2 haploid yeast cells



(B)

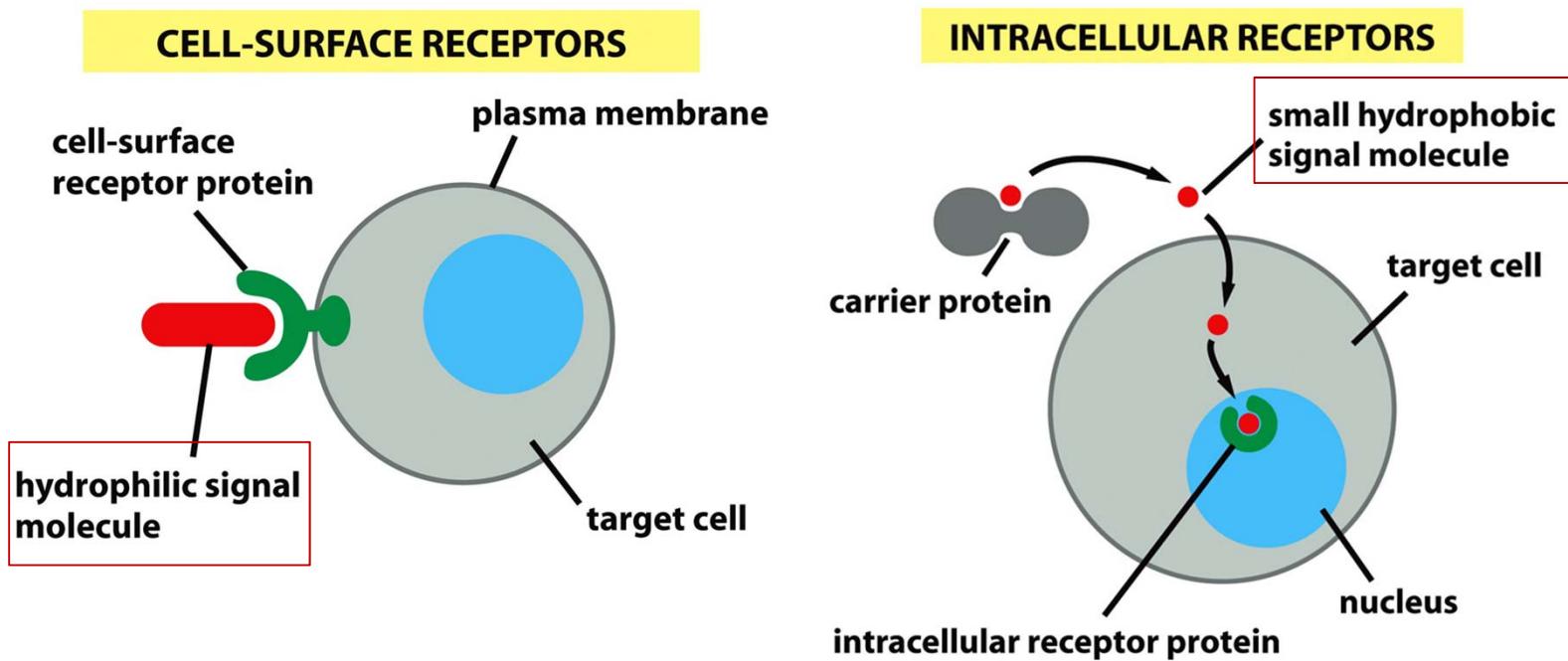
haploid cells fusion to  
become diploid cells

10  $\mu\text{m}$

## I. Overview of cell communication

- Chemical signaling involves ligands and receptors
- Two different types of receptors:
  - cell surface receptors
  - intracellular receptors

# Two different receptor classes work differently: cell-surface receptors vs. intracellular receptors

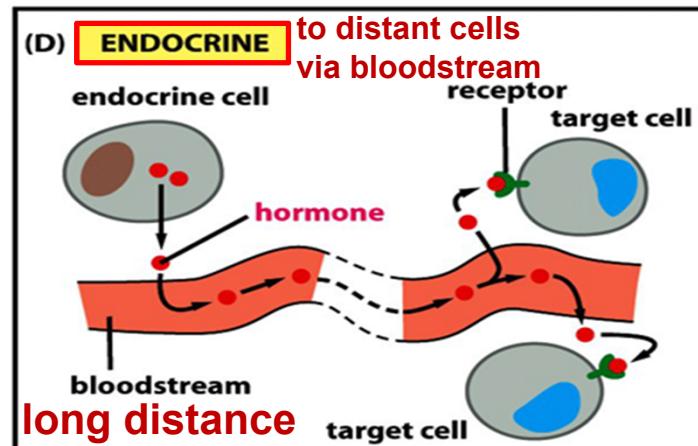
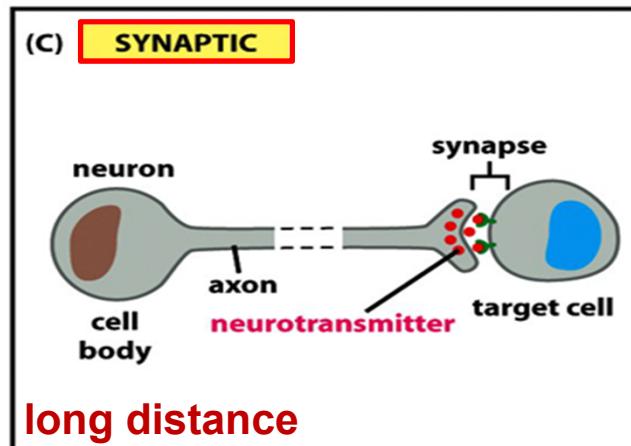
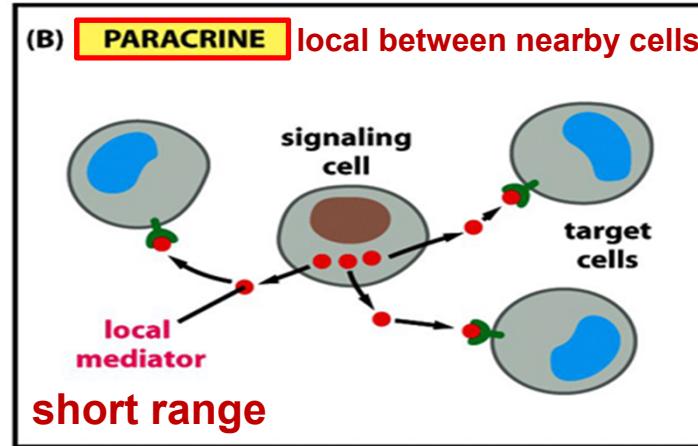
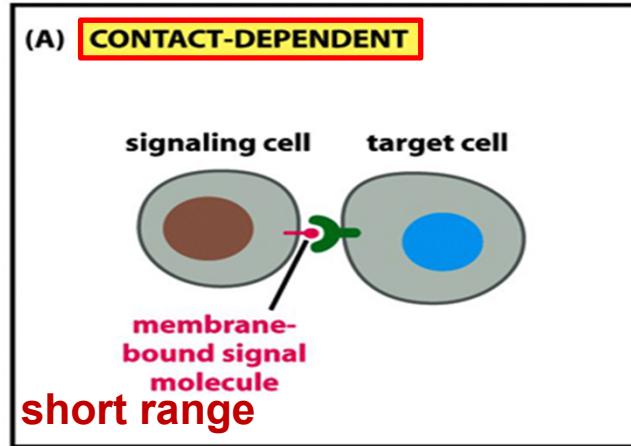


Regardless of the nature of the signal, perception by the *target cell* requires a receptor, which binds the signal molecule and then initiates a response in the target cell.

## Types of cell communication

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

## Four types of cell communication: for different distances, with different speeds



The same types of signaling molecules can be used in paracrine, synaptic, and endocrine signaling; but with crucial differences in speed and selectivity with which the signals are delivered to their targets !

# Endocrine versus synaptic signalling

## Speed:

- **Endocrine:** needs diffusion and blood flow → **slow process**
- **Synaptic:** signals travel with 100 meters/sec → **fast process**

## Concentration of the signaling molecule & affinity of receptors:

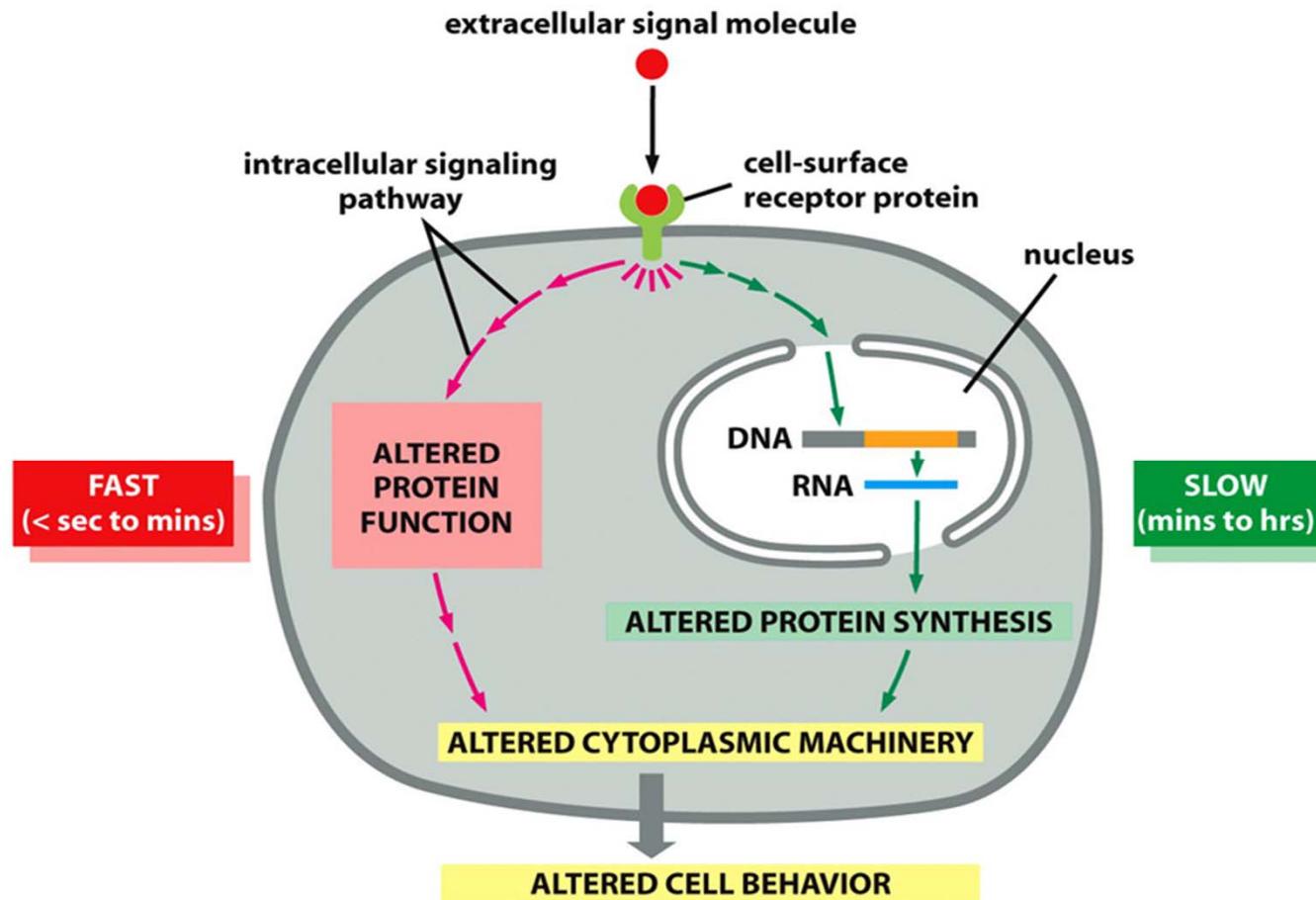
- **Endocrine:** signals are more diffused and must therefore work at low concentrations of the signaling molecule (nanomolar range). To catch one of the few signaling molecules, **receptors** need **high affinity** for ligands
- **Synaptic:** signaling molecules are at much higher concentration (micromolar) and more precise. Receptors bind with **low affinity** (low affinity = **fast release of ligands** → **allows for fast switching**)

**Another important factor is the response timing:  
effects in signaling can be slow and fast**

**Slow** effects: *de novo* protein synthesis in transcriptional response

**Fast** effects: change in protein behavior  
(activation/inactivation/modification)

# Slow and fast responses

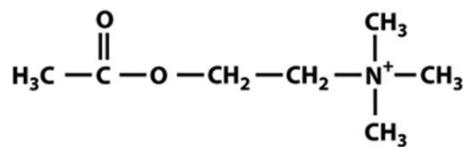


Alteration of protein function is immediate: **seconds or less after** signal reception

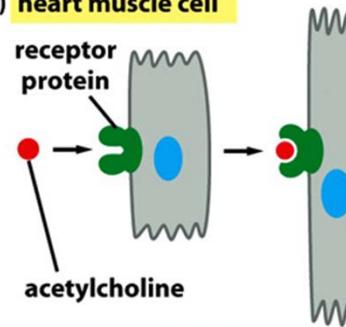
changes in gene expression and protein synthesis starts **1 hour or more after** signal reception

The same signals can trigger different effects,  
depending on the cell type

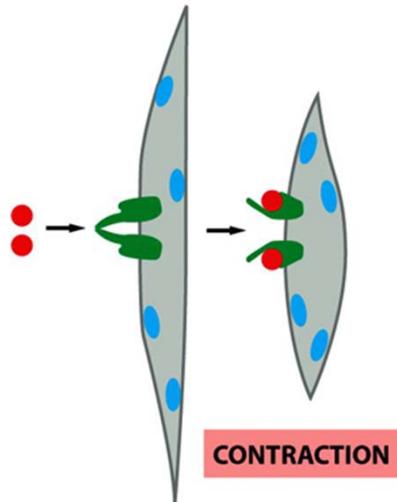
(A) acetylcholine



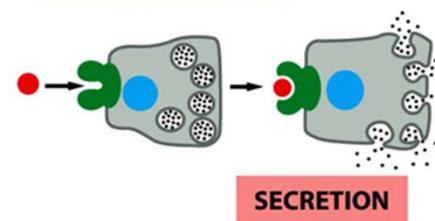
(B) heart muscle cell



(C) skeletal muscle cell



(D) salivary gland cell



DECREASED RATE AND  
FORCE OF CONTRACTION

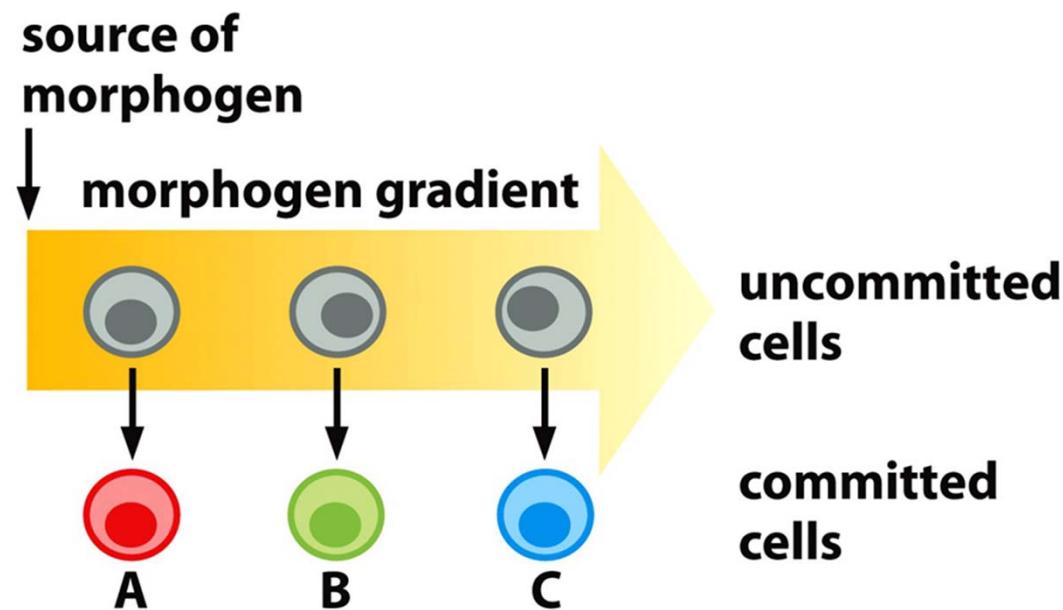
**Acetylcholine receptors** in heart muscle cells and salivary gland cells are **identical**  
However, they result in **different effector proteins activation**.  
**Acetylcholine receptors** in heart muscle and skeletal muscle are **different**.

## The same signals can trigger different effects

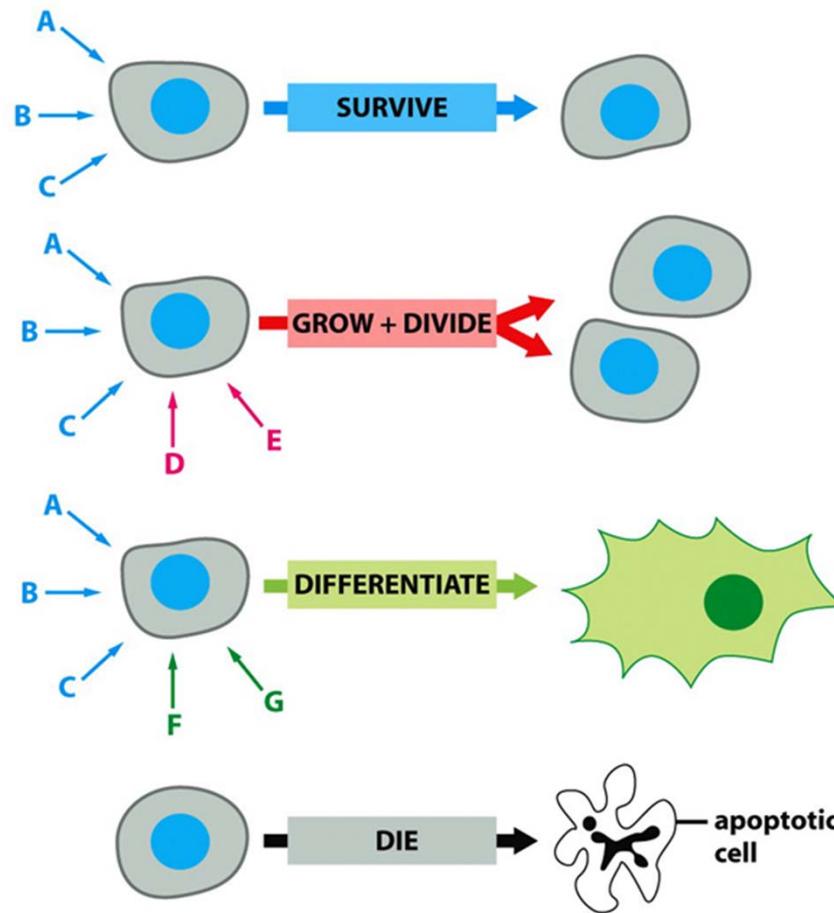
- Same signals can act on different receptors
- Same signals can act on same receptors  
but trigger different effectors, causing different reactions
- The same cell type can also react differently to different concentration of signals.  
(in this case, the signal is called “morphogen”).

One of the key challenges in cell biology is to understand how a cell integrates all of this signaling information in order to make decisions: to divide, to move, to differentiate, and so on...

# Morphogen in development



Animal cells are programmed to respond to specific combinations of signals: no signals → death

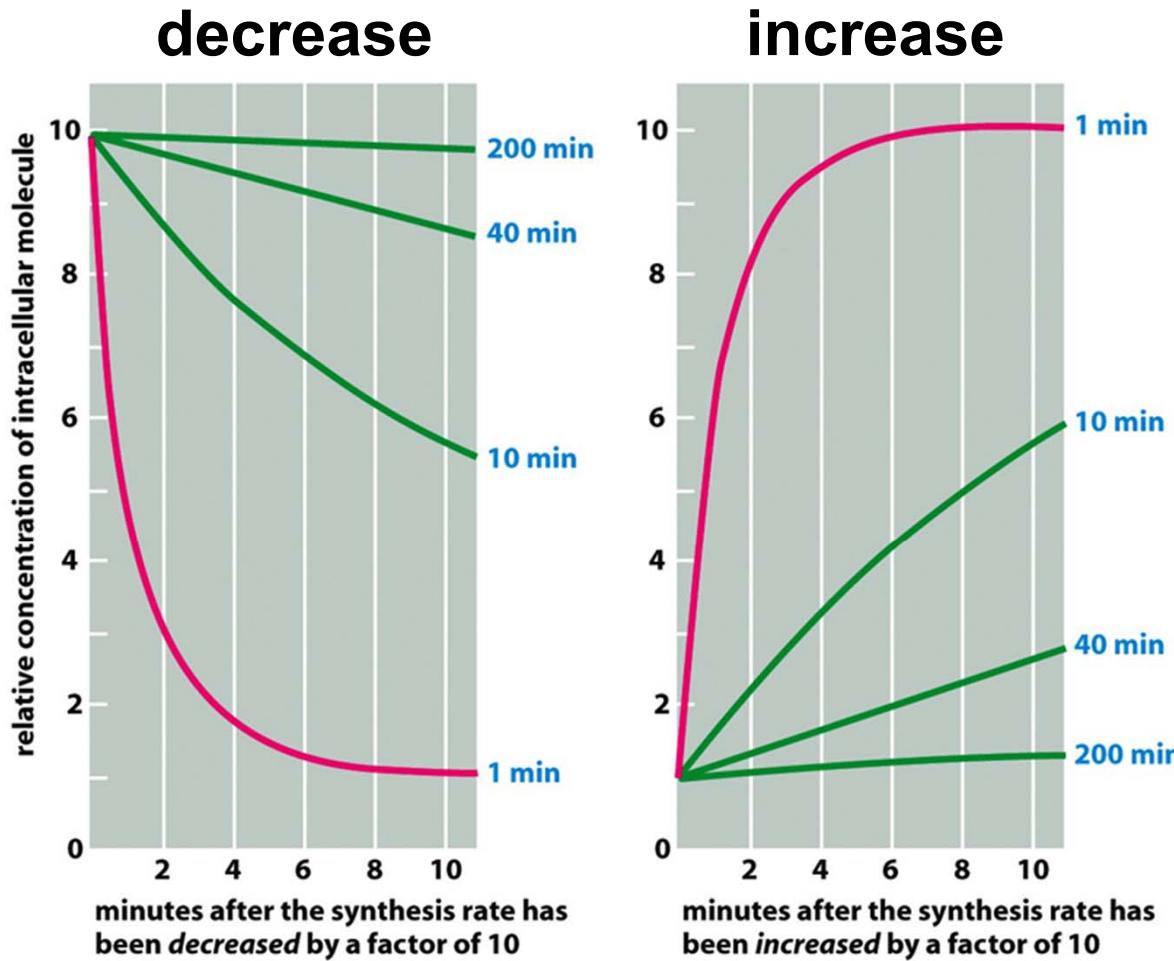


Cells can **integrate multiple signals from various receptors** to dictate **individual cell behavior**.

The amount and the activity of signaling molecules are important

- Many proteins in signaling have **short half lives:**  
**ensures/allows for quicker responses**  
**(...similar to the low affinity binding of the receptors)**
- Many signaling proteins have conversion between inactive and active states:  
**ensures/allows for quicker responses than *de novo* protein synthesis**

## Proteins that have **higher turn over rate** react to stimuli in a **faster** manner



high turn over: fast/high synthesis rate and quick degradation  
low turn over: slow/low synthesis rate and slow degradation

## II. Signal perception & transduction by intracellular receptors

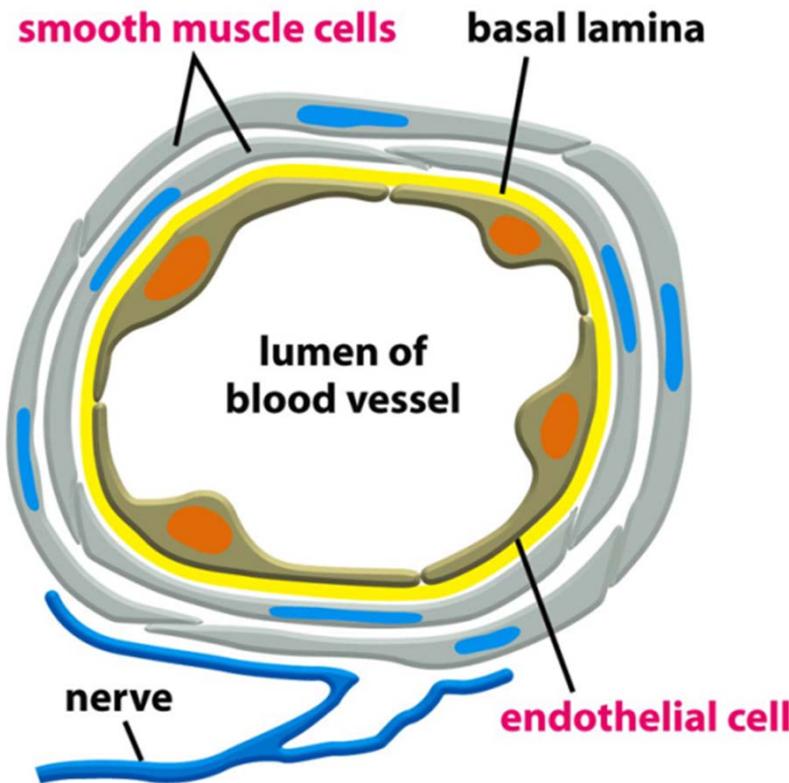
### Signaling molecules:

small hydrophobic molecules that can cross the plasma membrane.

### **Examples:**

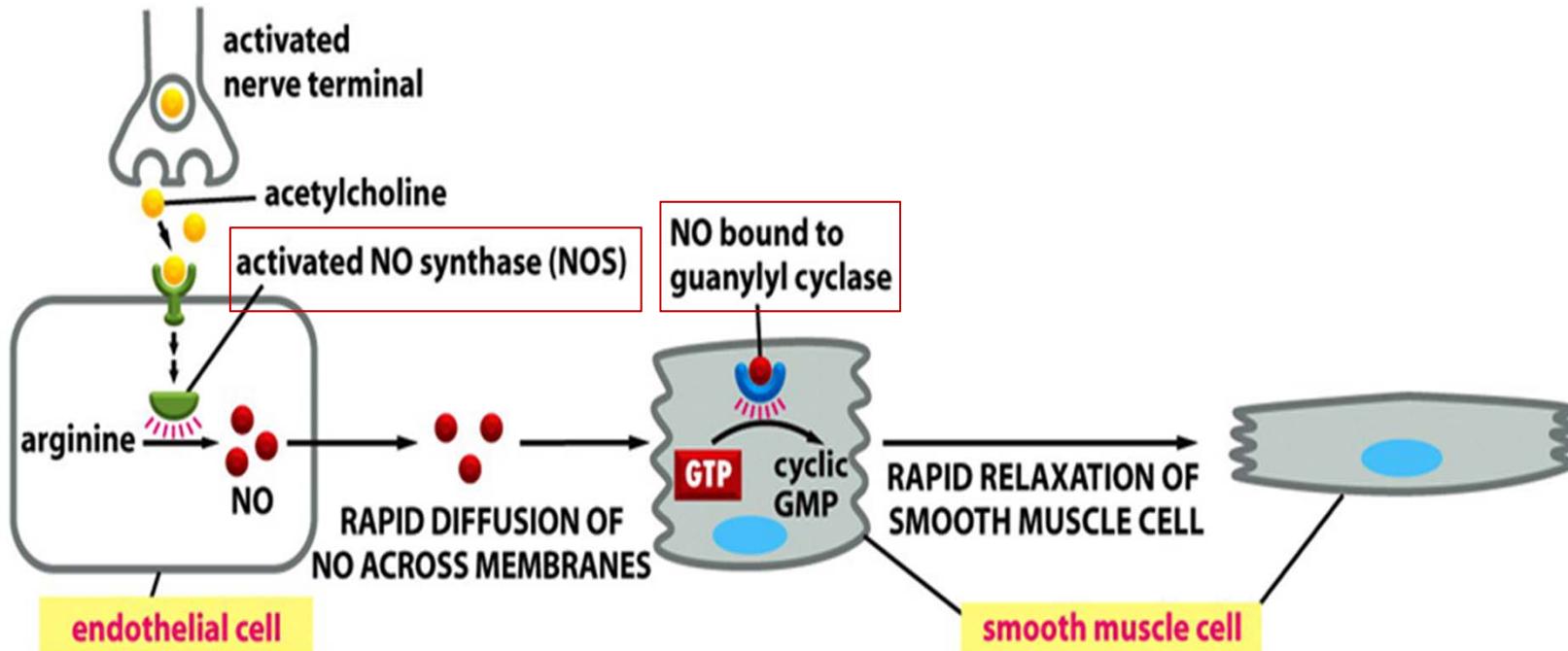
1. **Nitric oxide (NO) gas, carbon monoxide (CO) gas**
2. **Steroid hormones, thyroid hormones, retinoids, vitamin D, etc.**

# 1. Signalling via the gas nitric oxide (NO) in smooth muscle relaxation in blood vessels



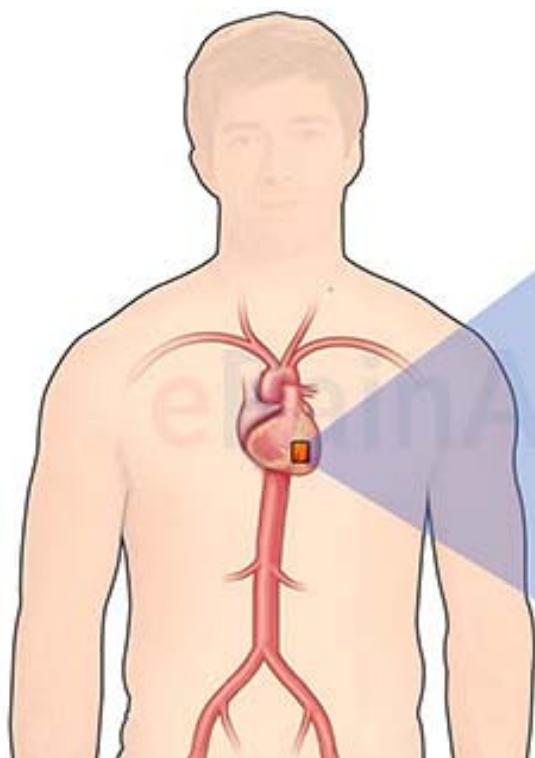
NO has a half life of **5-10 sec.**  
It is rapidly converted by water  
and oxygen into nitrates and nitrites

# 1. Signaling via the gas nitric oxide (NO) in smooth muscle relaxation in blood vessels



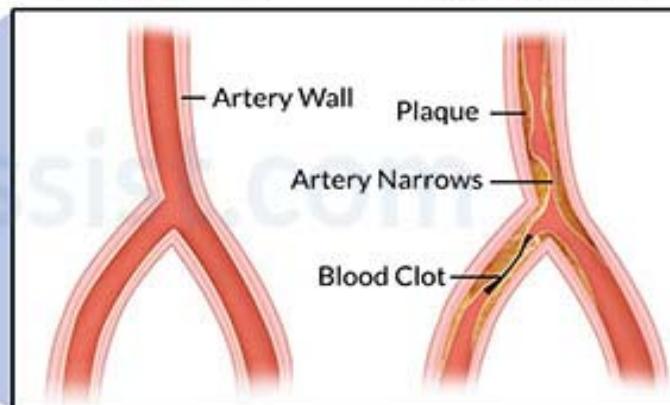
- Acetylcholine activates **NO synthase** in endothelial cell to produce NO.
- NO diffuses into smooth muscle cell and activates **guanylyl cyclase** to produce **cyclic GMP (cGMP)**, which triggers **muscle relaxation** and thus the **dilation of the blood vessel**

# Mechanism of nitroglycerin in treating angina pectoris

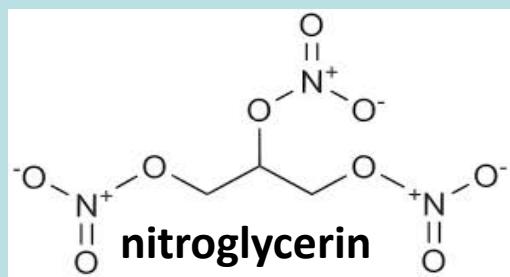


## Angina

Normal Artery      Blocked Artery

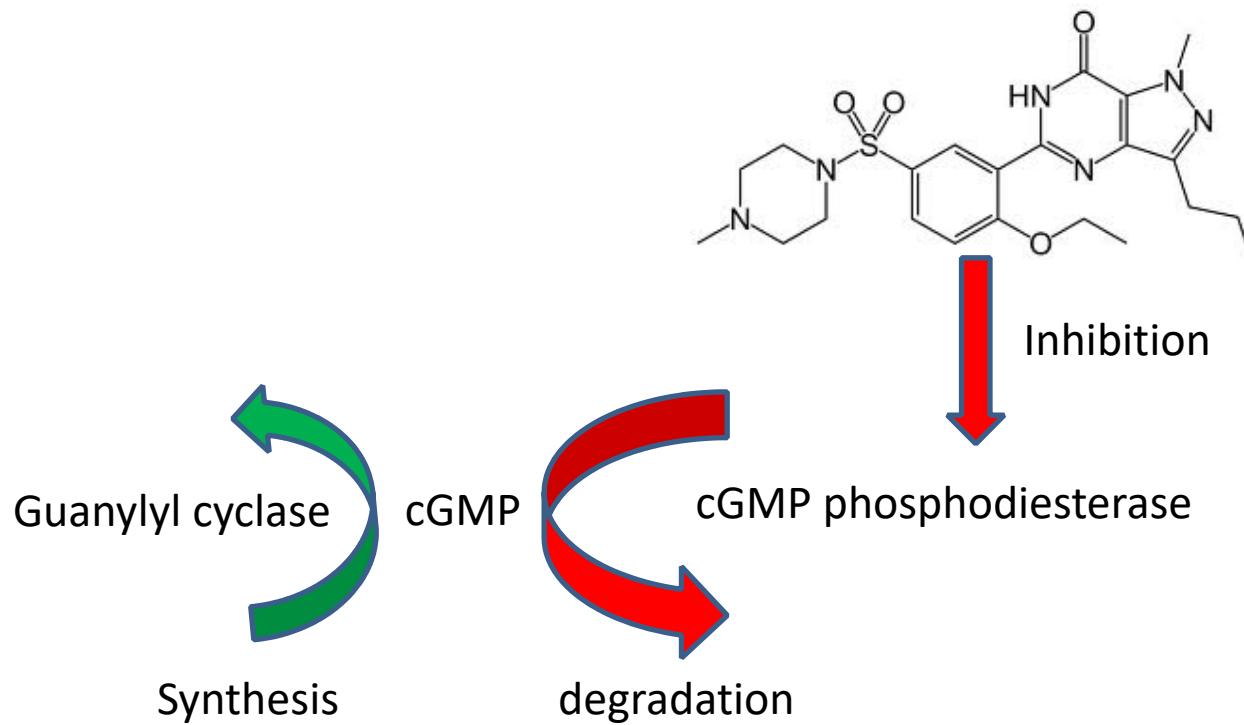


ePainAssist.com



→ NO gas → cGMP increase → Dilation of blood vessel reduces workload of heart

# Mechanism of Sildenafil (commercial name Viagra)



**Accumulation of cGMP due to inhibited degradation**  
causes prolonged blood vessel dilation

## 2. Signaling using nuclear receptors

Nuclear receptors: are Ligand-modulated gene regulatory proteins

Ligands of nuclear receptors:

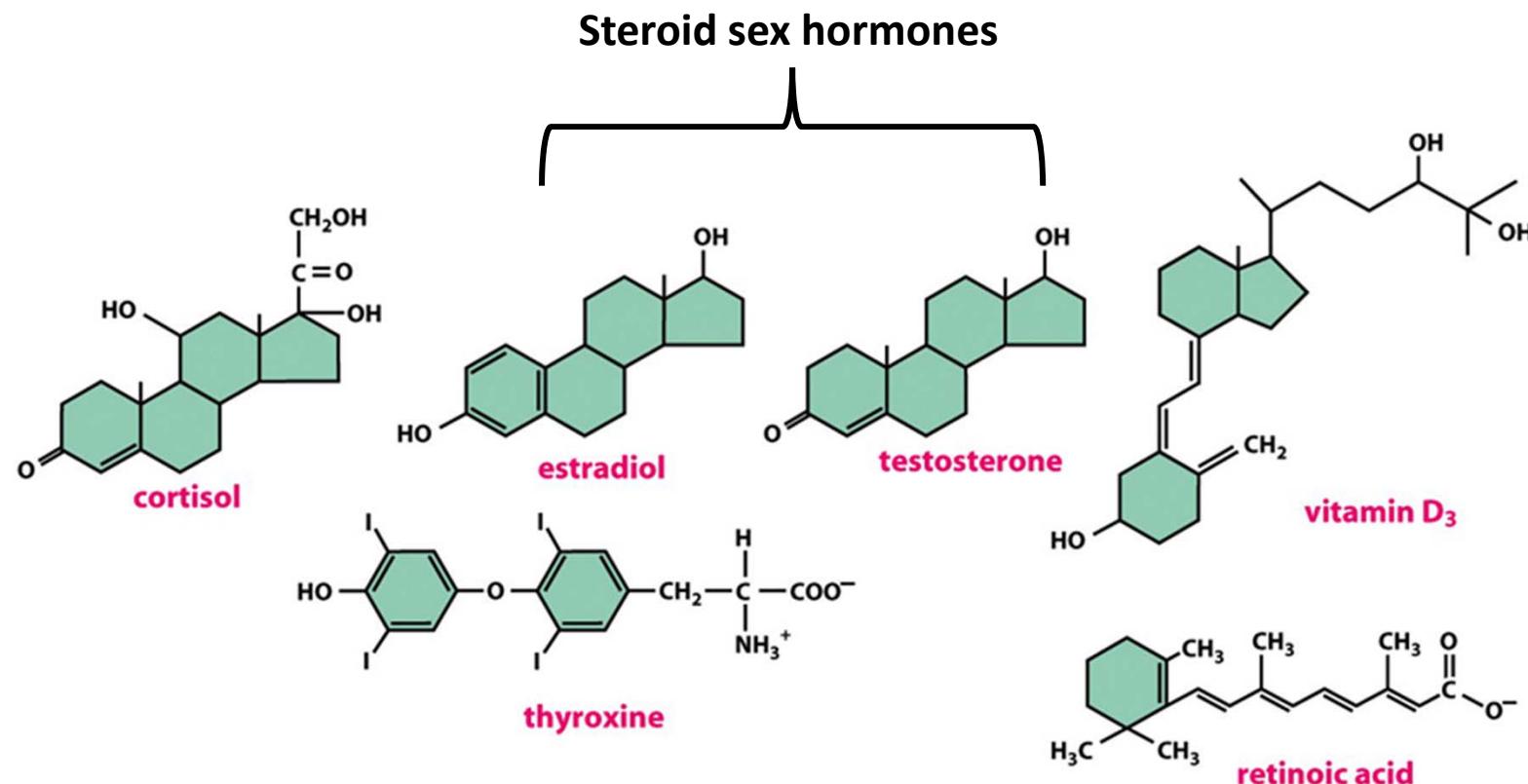
- **Steroid hormones** (made from cholesterol) :

- 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)

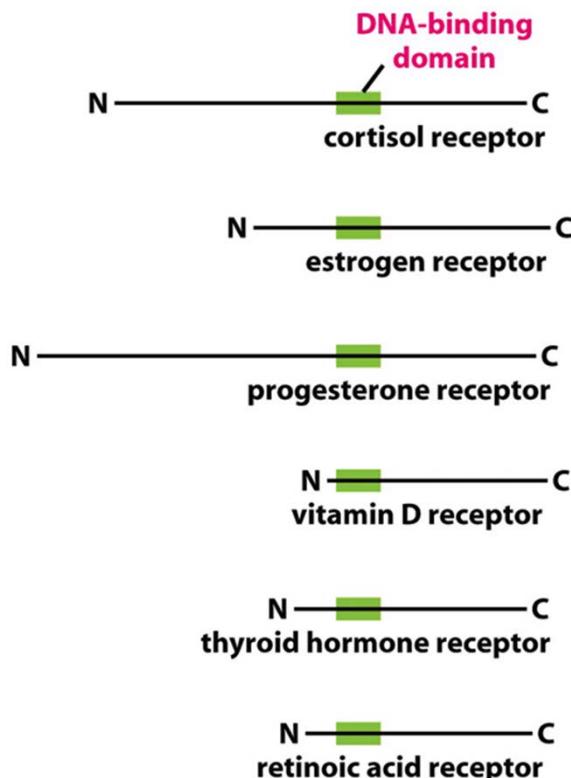
# Some nongaseous signal molecules that bind to intracellular receptors



**...all of them are small and hydrophobic!**

# Common features of nuclear receptors

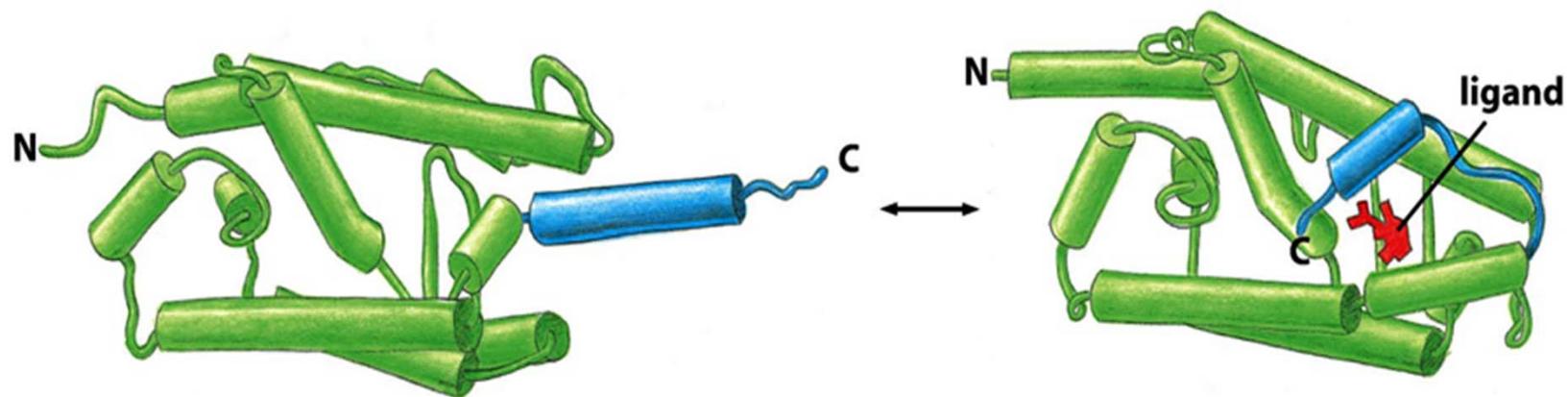
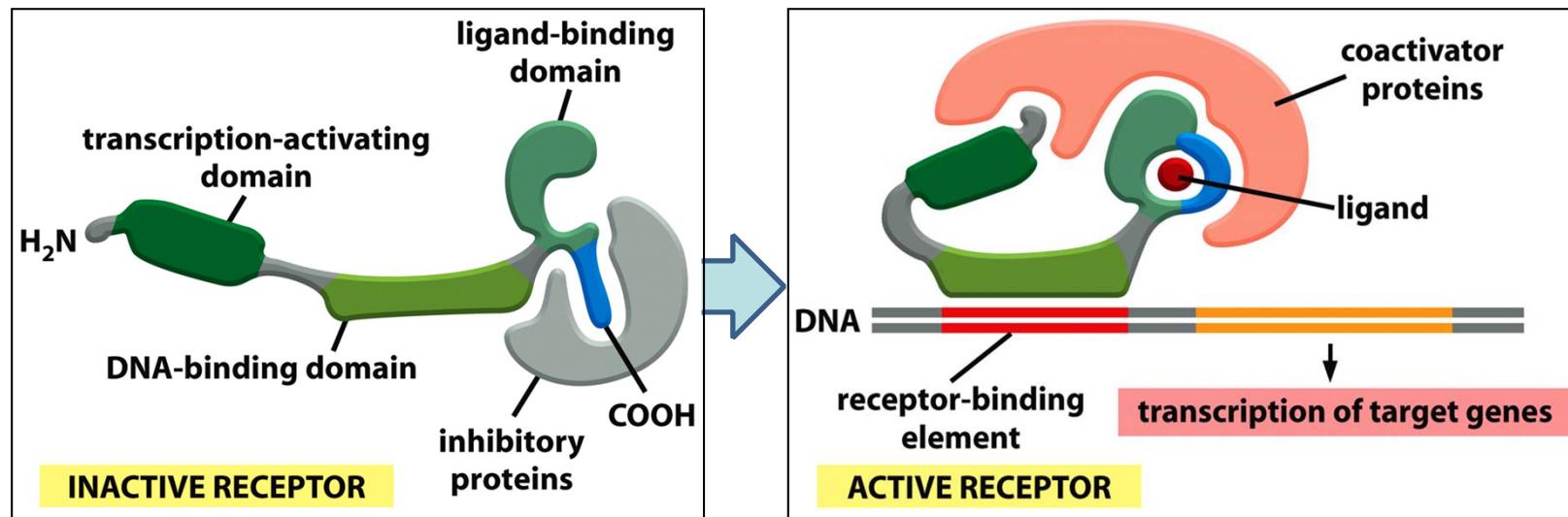
- Work either as homodimer or heterodimer
- Serve both as **ligand** receptor and as gene **transcription factor**



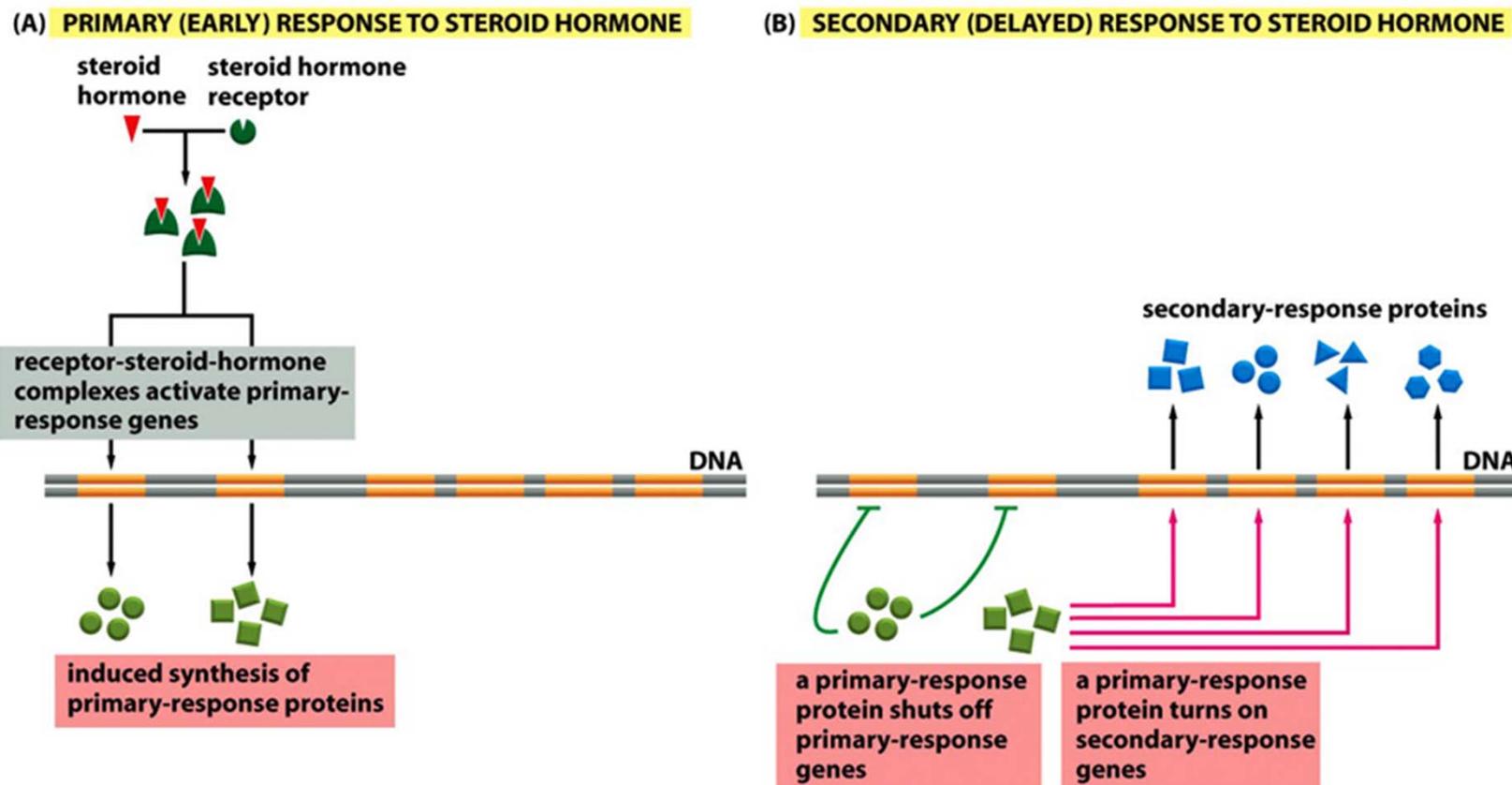
All of these receptors possess  
3 functional domains:

- **DNA binding domain**
- **Gene transactivation domain**
- **Ligand binding domain**

# A model for how nuclear receptors work



# Hormone receptors trigger both, primary and secondary responses

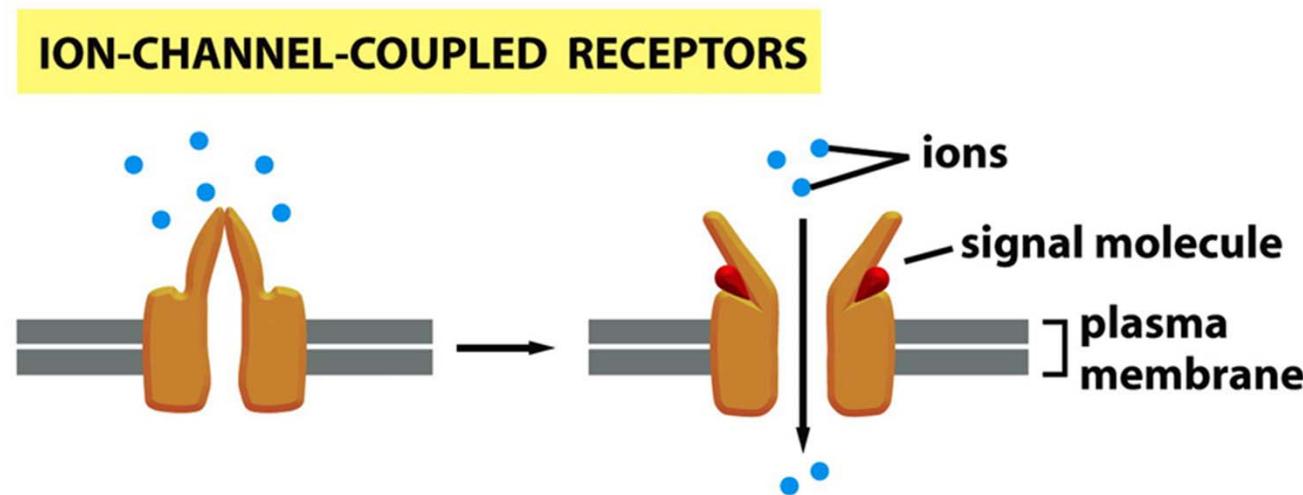


### III. Signal perception & transduction by cell surface receptors

- Ion-channel coupled receptor
- G-protein coupled receptor
- Enzyme-coupled receptor
- Other types

# The major **three** classes of cell surface receptors

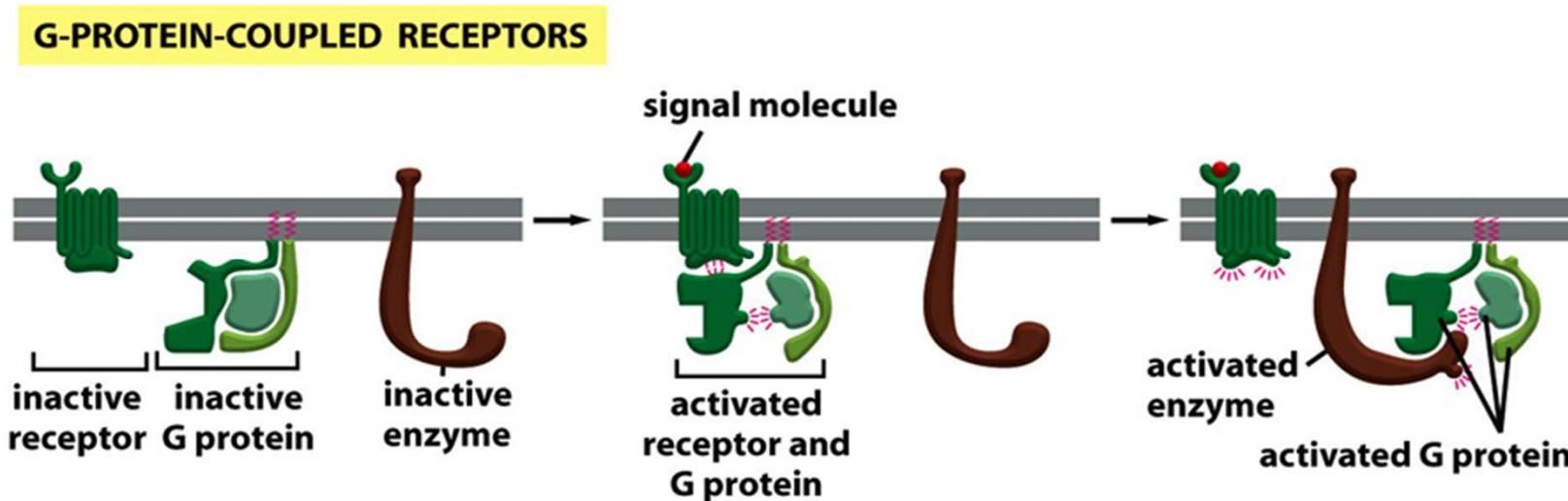
## 1.) Ion-channel-coupled receptors



- The signaling molecule (transmitter) **binds** to the receptor and **triggers** the opening/closure of the channel.
- This is why such receptors are also called: transmitter-gated ion channels!

# The major **three** classes of cell surface receptors

## 2.) G (GTP-binding)-protein-coupled receptors (GPCRs)

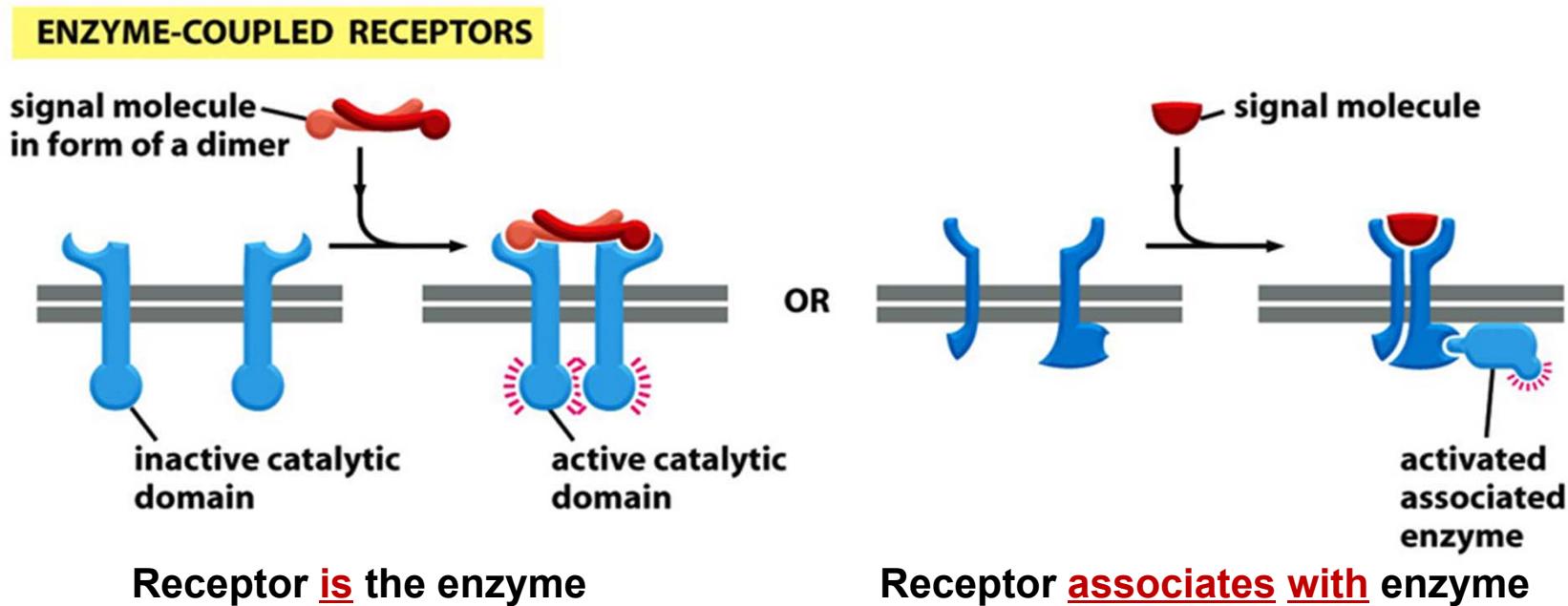


The **activation** of a G-protein-coupled receptor triggers **indirectly** the activation of another membrane protein.

A “G-protein” mediates between the receptor and the membrane protein **that’s why** it is called “**G-protein-coupled**”.

# The major **three** classes of cell surface receptors

## 3.) Enzyme-coupled receptors



These receptors either are enzymes that are **activated by the signaling molecule** or they associate with enzymes which they activate.

# The concept of second messenger

- The **first** messenger: **extracellular signals** (signaling molecule)
- **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 they **relay the signals**

Examples: **cAMP**

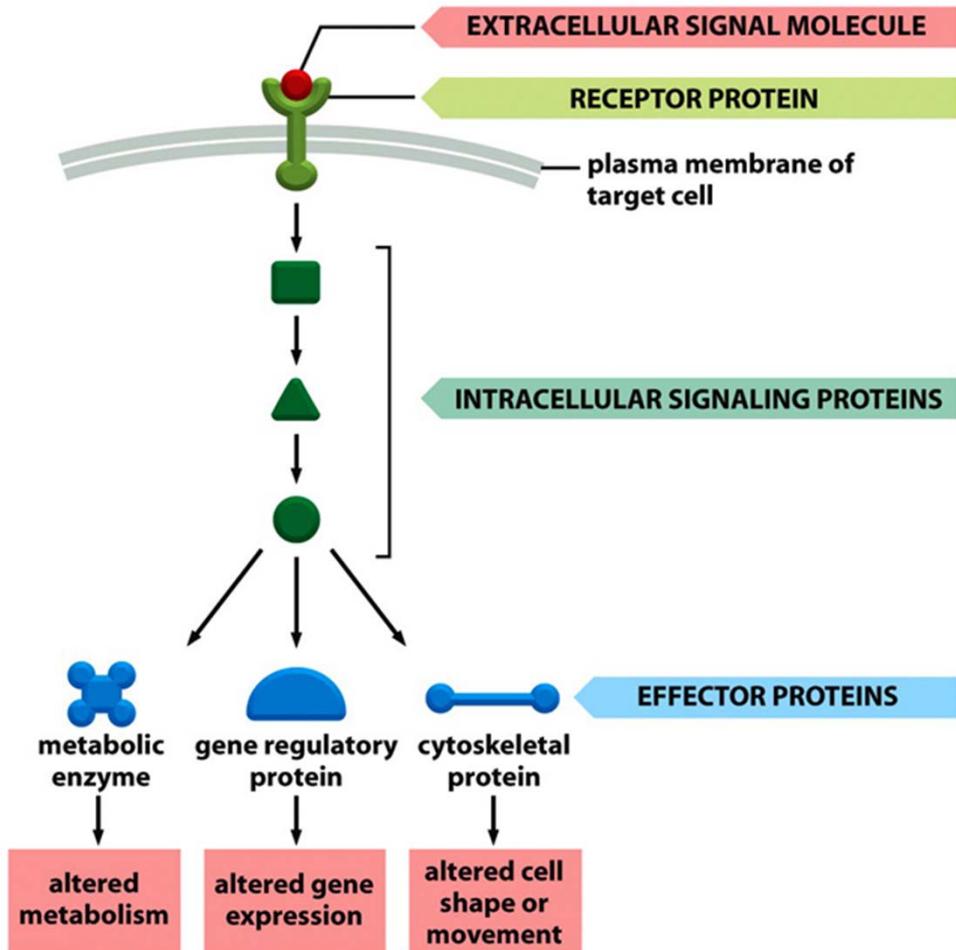
**cGMP**

**Ca<sup>2+</sup>**

**Diacylglycerol (DAG)**

**Inositol triphosphate (IP<sub>3</sub>)**

# Relay of signals from cell surface receptors



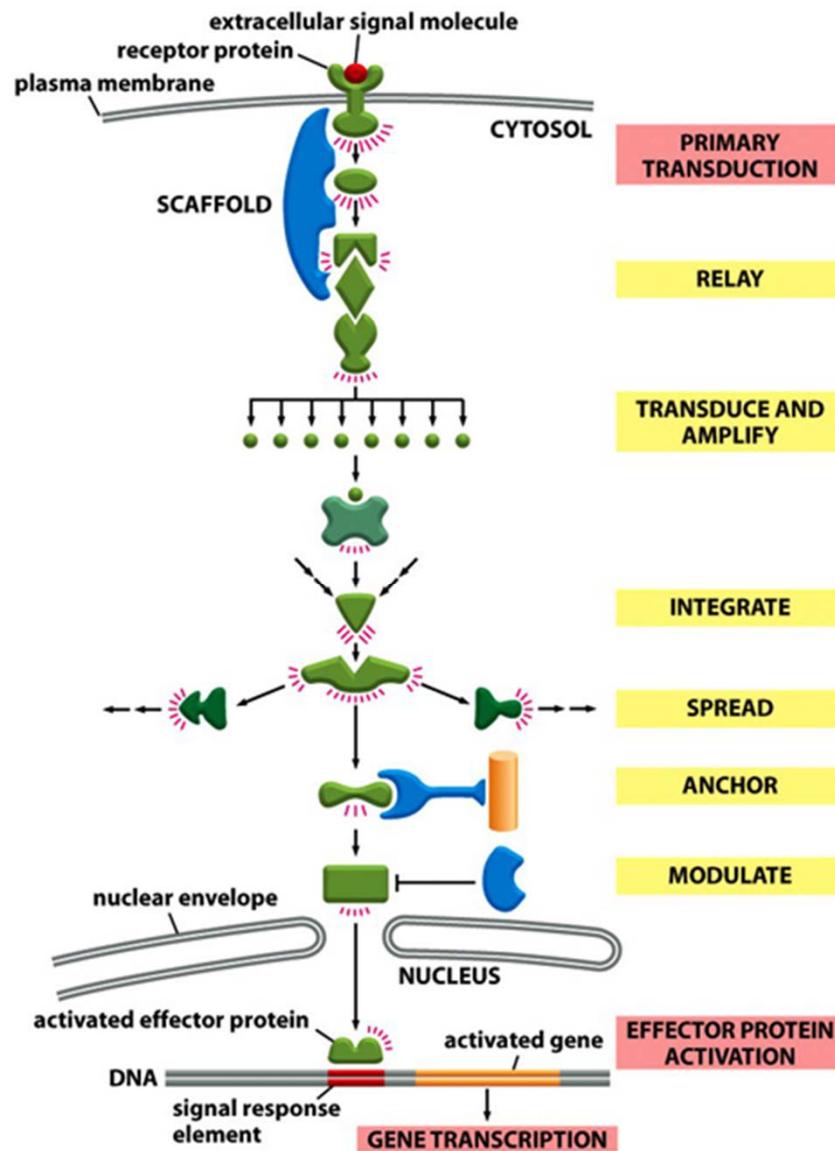
Intracellular signaling pathway:

- activated by an extracellular signal (binding to a receptor protein)

The receptor activates one or more intracellular signaling pathways.  
(involving a series of signaling proteins)

Finally, one or more of the intracellular signaling proteins alters the activity of effector proteins and thereby the behavior of the cell.

# Signaling proteins can have various functions



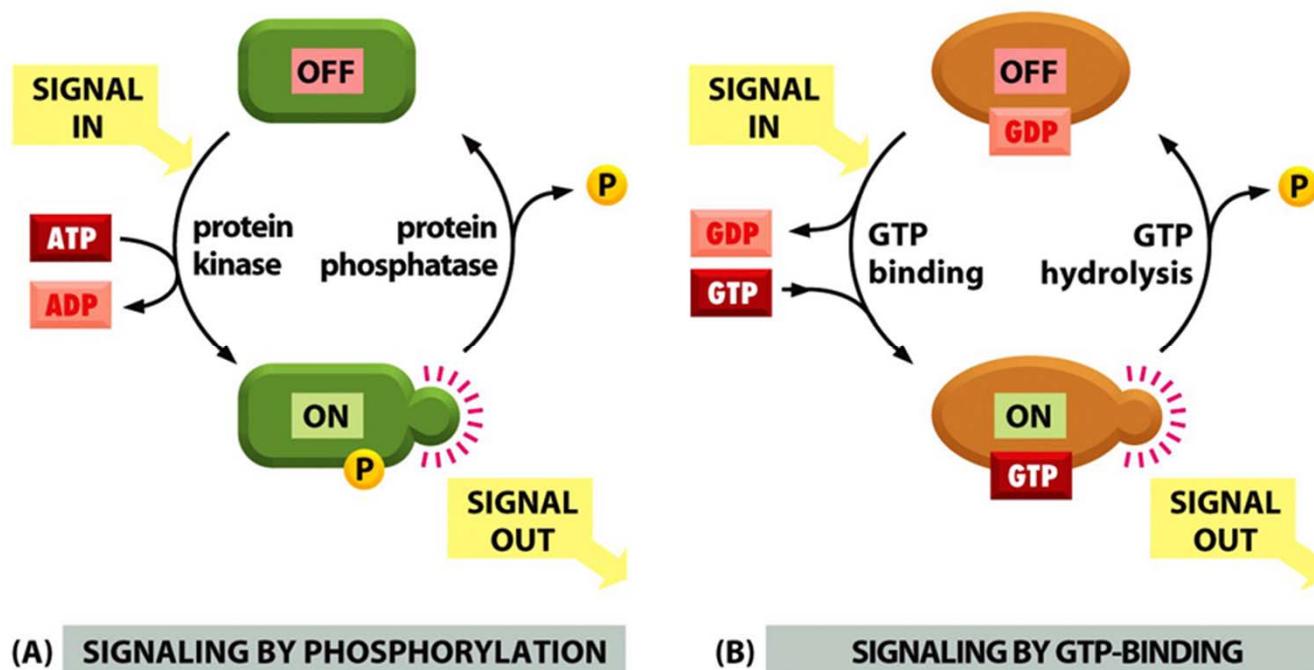
- Scaffolds form signaling-complexes
- Relay signals to the next component
- Change signals into a different form
- Amplification of the signal
- Integration of signals from multiple pathways
  - Spread signals from one pathway to another (**crosstalk**)
- Anchor signals to structures
- Modulate the activity of 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.
- **Amplify** 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 Ca<sup>2+</sup> binding
- Ubiquitination, etc. → Remember lecture 9?



# Protein phosphorylation

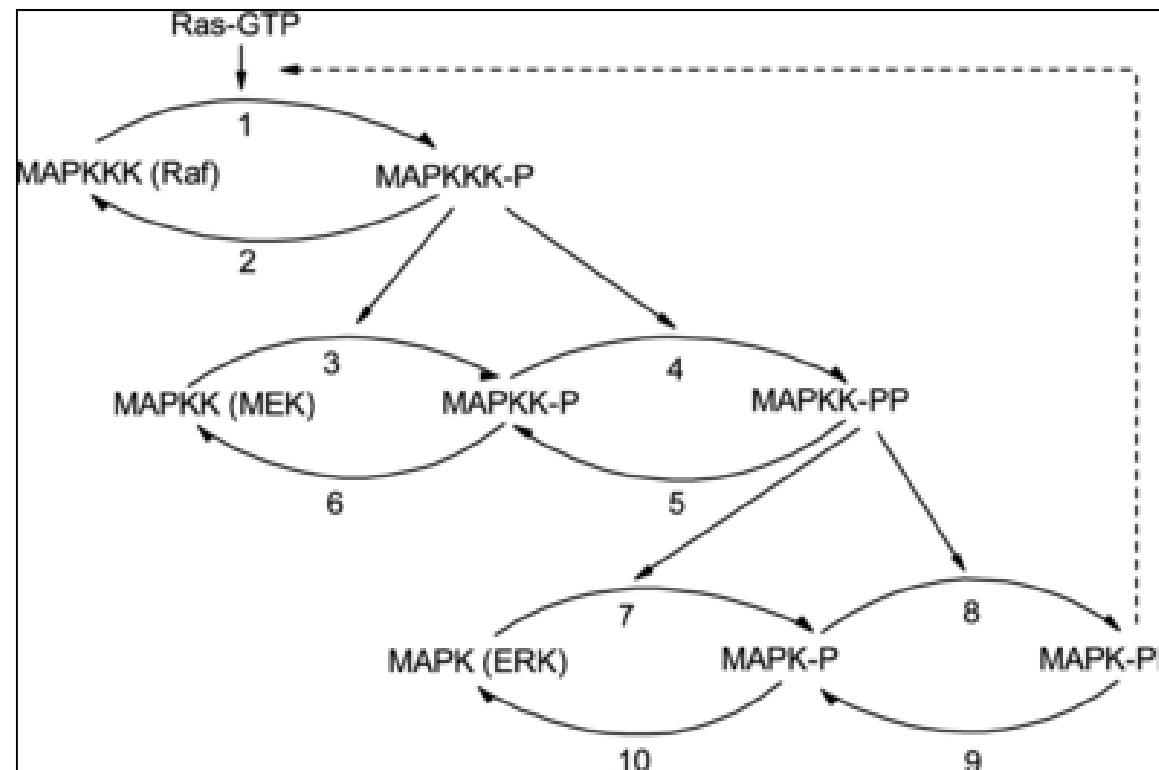
- It is one major way of post-translational modification to regulate protein activity
- >30% of all human genome proteins can be phosphorylated
- >520 human kinases (kinome) and >150 protein phosphatases
- Two categories:  
Serine/Threonine kinase; Tyrosine kinase
- Protein kinases are major therapeutic targets in human diseases:  
e.g. : Acute leukemia:  
Gleevec targets BCR-ABL kinase



# Phosphorylation cascade

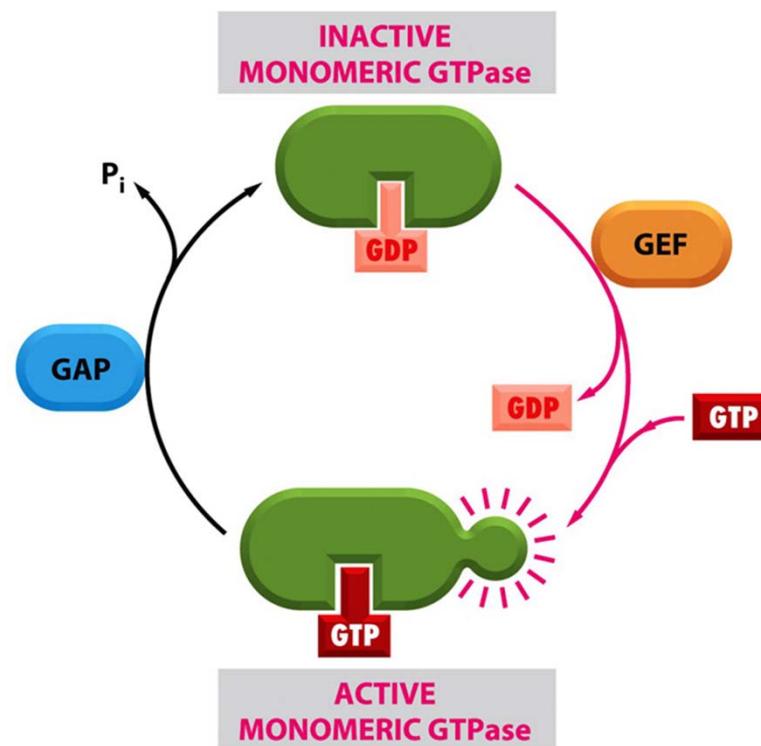
Signaling protein itself is a kinase which can phosphorylate and activate downstream effectors

For example: Ras-Raf-MEK-ERK pathway



# GTP-binding proteins (G-proteins)

- Large trimeric GTP-binding proteins
- Small monomeric GTPase



## Characteristics of signal transduction

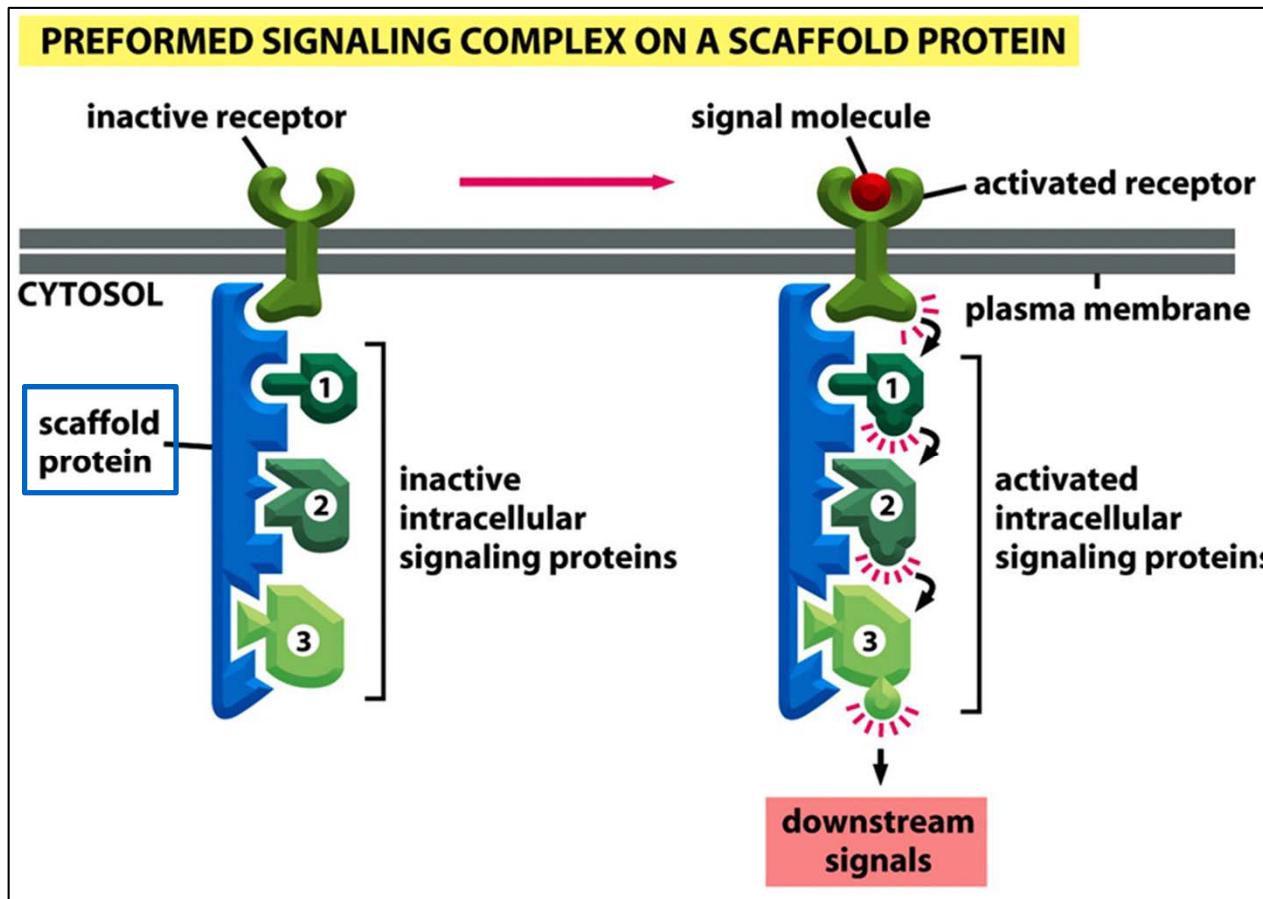
- Specificity
- Efficiency
- Reversibility
- Saturation
- High binding affinity

High speed and specificity in signaling is achieved  
by the formation of “**signaling complexes**”

Signaling complexes can be established in different ways:

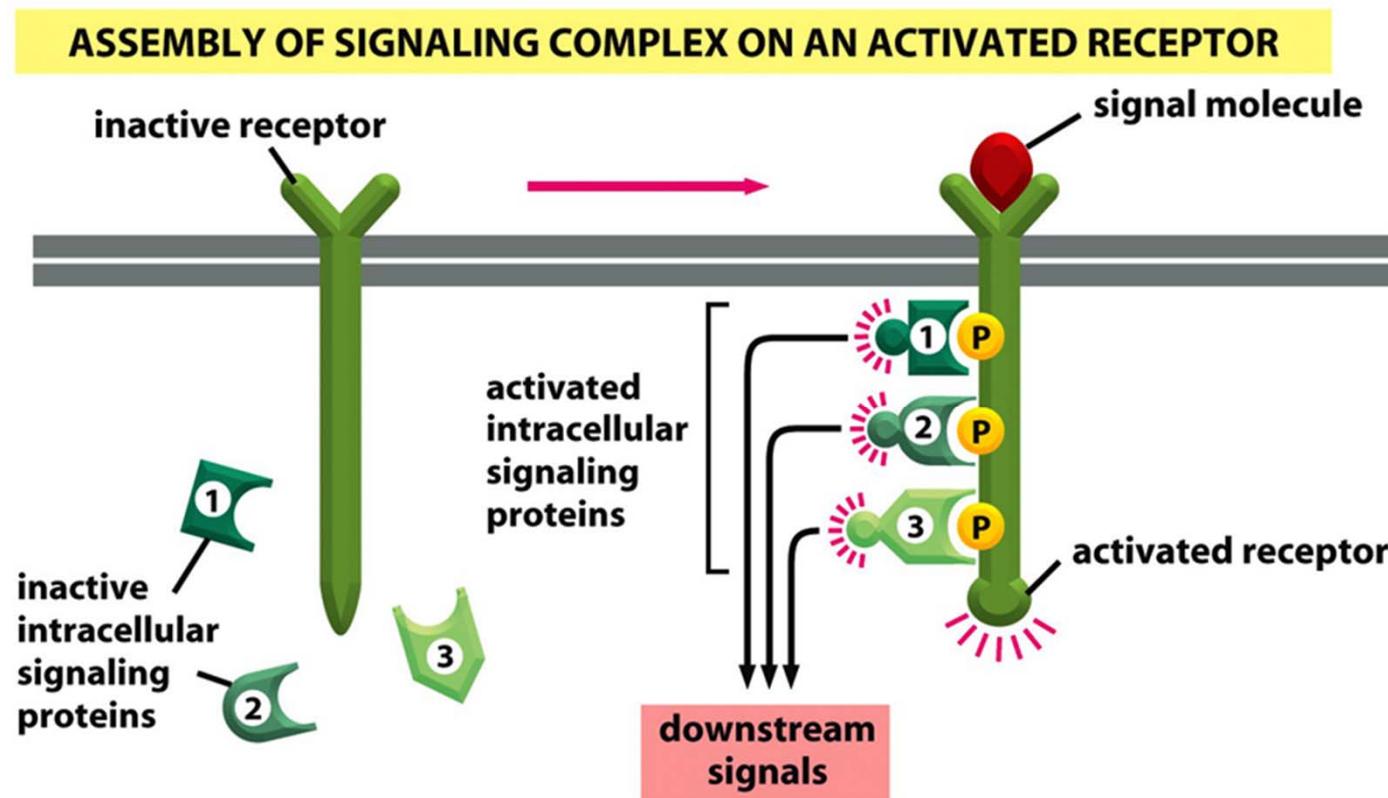
- 1) assembly of the complex via scaffolding proteins  
**on an inactive receptor**
- 2) assembly of the complex **on an activated receptor**
- 3) assembly of the complex **at the membrane**  
on phosphoinositide docking sites

## 1) Formation of a “signaling complex” on an inactive receptor



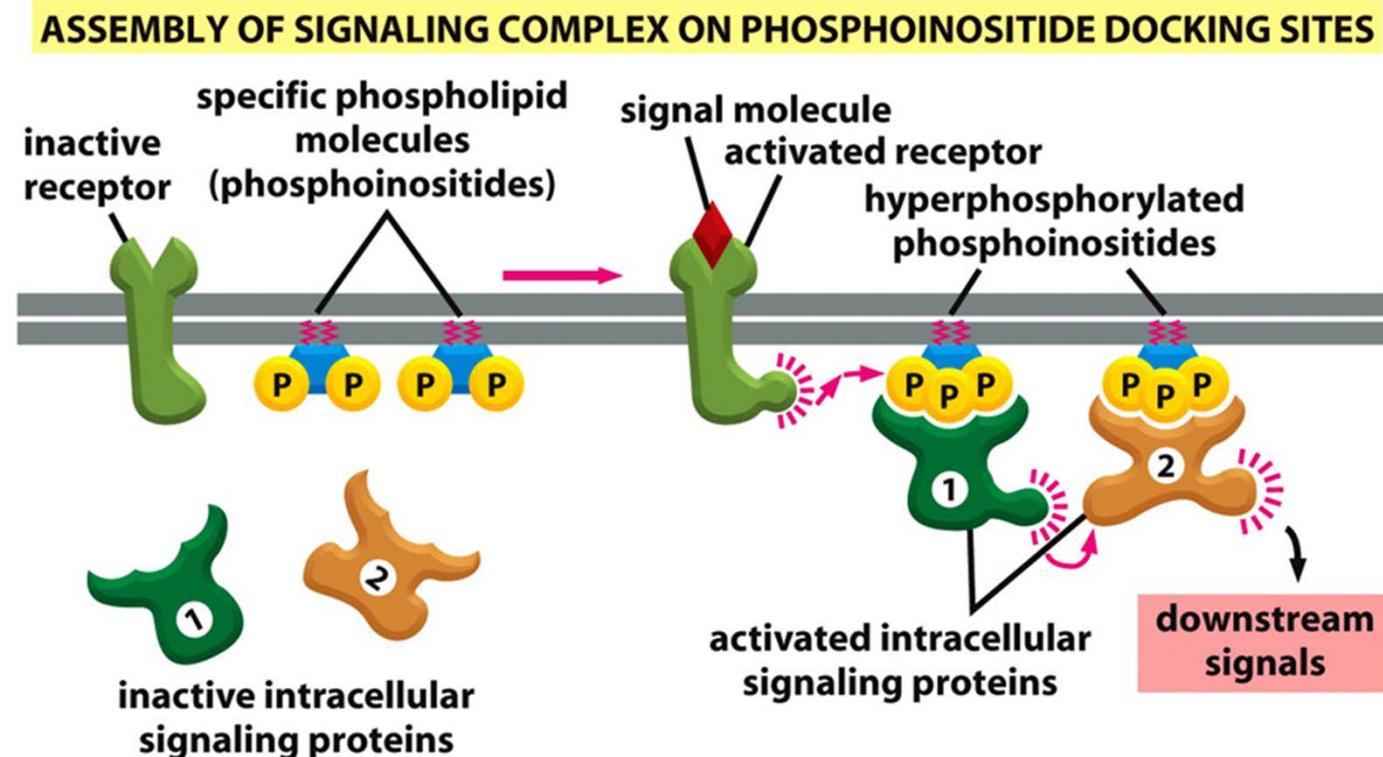
- Receptor and some intracellular signaling proteins, which he activates in sequence, are pre-assembled into a signaling complex on the inactive receptor by a **scaffold protein**

## 2) Formation of a “signaling complex” on an activated receptor



- A signaling complex **assembles transiently on a receptor** only **after** the binding of an extracellular signal molecule has activated the receptor.
- The activated receptor phosphorylates itself at multiple sites, which then act as docking sites for intracellular signaling proteins.

### 3) Formation of a signaling complex at the membrane on phosphoinositide docking sites

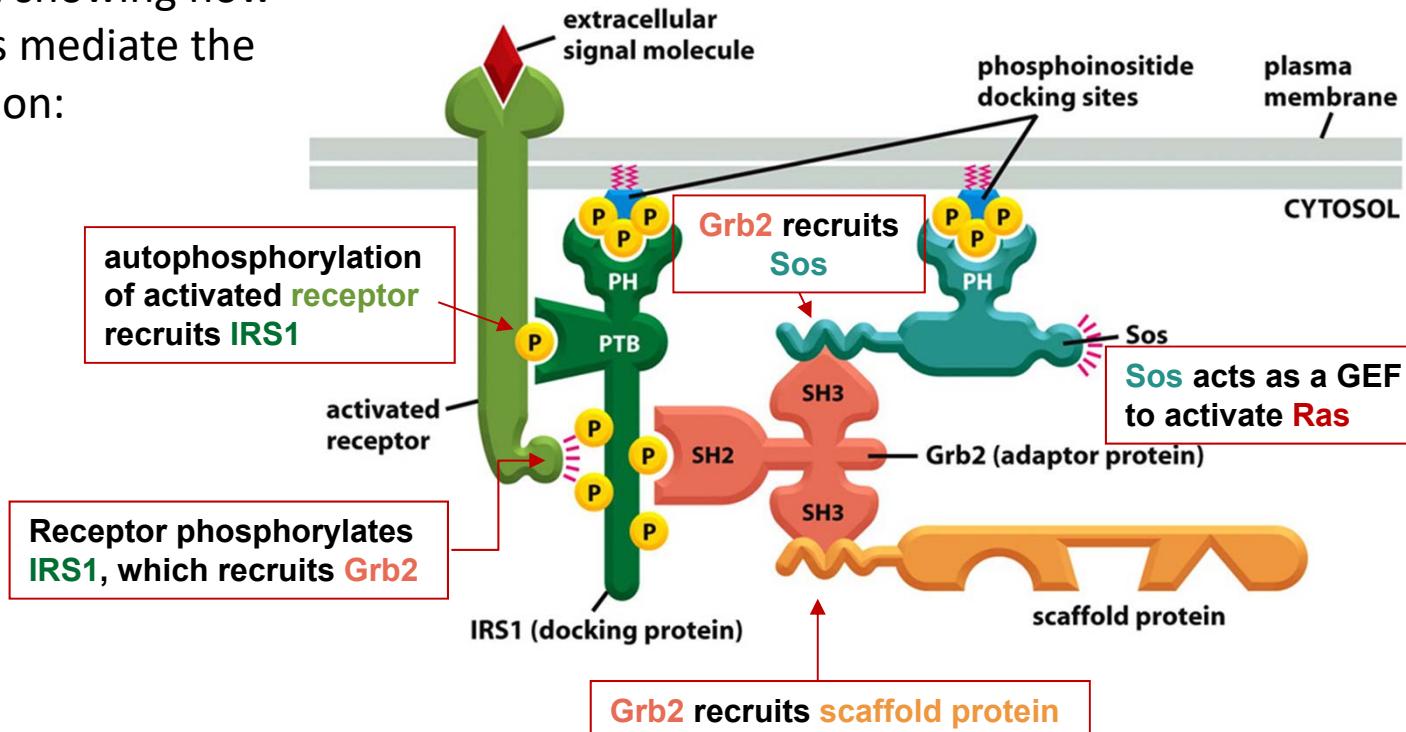


- Activation of a receptor leads to the phosphorylation of specific phospholipids (phosphoinositides) in the adjacent plasma membrane.
- These phosphoinositides then serve as docking sites for specific intracellular signaling proteins, which can now interact with each other.

Conserved **interaction domains** are important for protein binding  
(example: activation of the insulin receptor)

- Src homology 2 (SH2) domain
- Phosphotyrosine-binding domains (PTB)
- Src homology 3 (SH3): **bind proline rich domains**
- Pleckstrin homology (PH): **bind phosphoinositides**

Diagram showing how domains mediate the interaction:



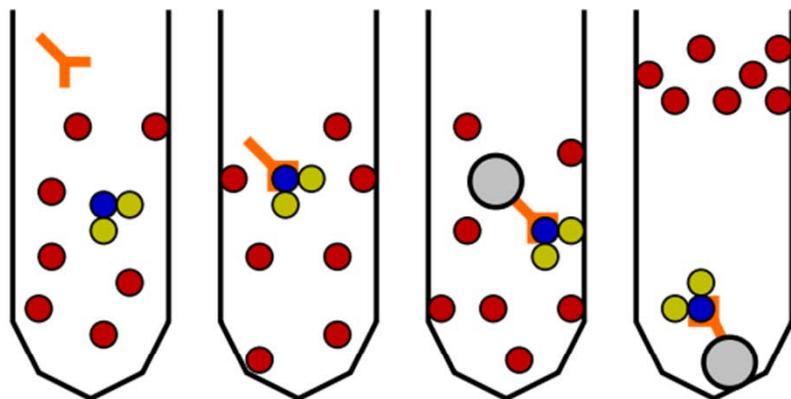
## IV. General methods to study cellular signaling (General methods for cell biological applications)

- **Protein co-immunoprecipitation (co-IP)**  
aka “pull-down” assays
  - general test for protein-protein interaction
- **SDS-PAGE** (Polyacrylamide gel electrophoresis) followed by Western blotting (**WB**) and immunodetection using specific antibodies  
(e.g. phospho-specific antibodies)
- ***In vitro* protein activity studies**
  - enzymatic assays
- **short hairpin (shRNA) or short interfering (siRNA) induced “knock-down”**  
aka RNA interference (RNAi)
- **inhibitors**
- **Rescue analysis**
- **Live cell imaging analysis and spectromicroscopical analysis**

## 1. Co-immunoprecipitation (co-IP)

**Application:**

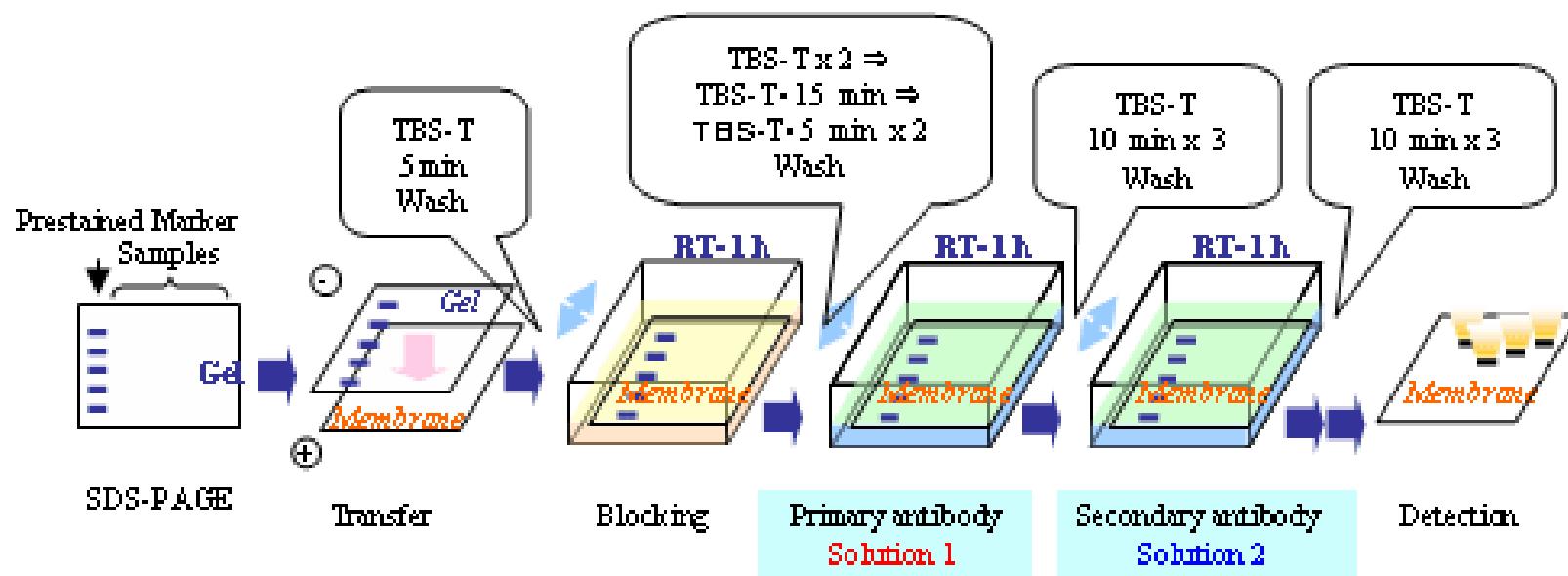
- receptor-ligand interaction
- kinase-substrate interaction
- find other protein interaction partners
- protein-protein interactions in general



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

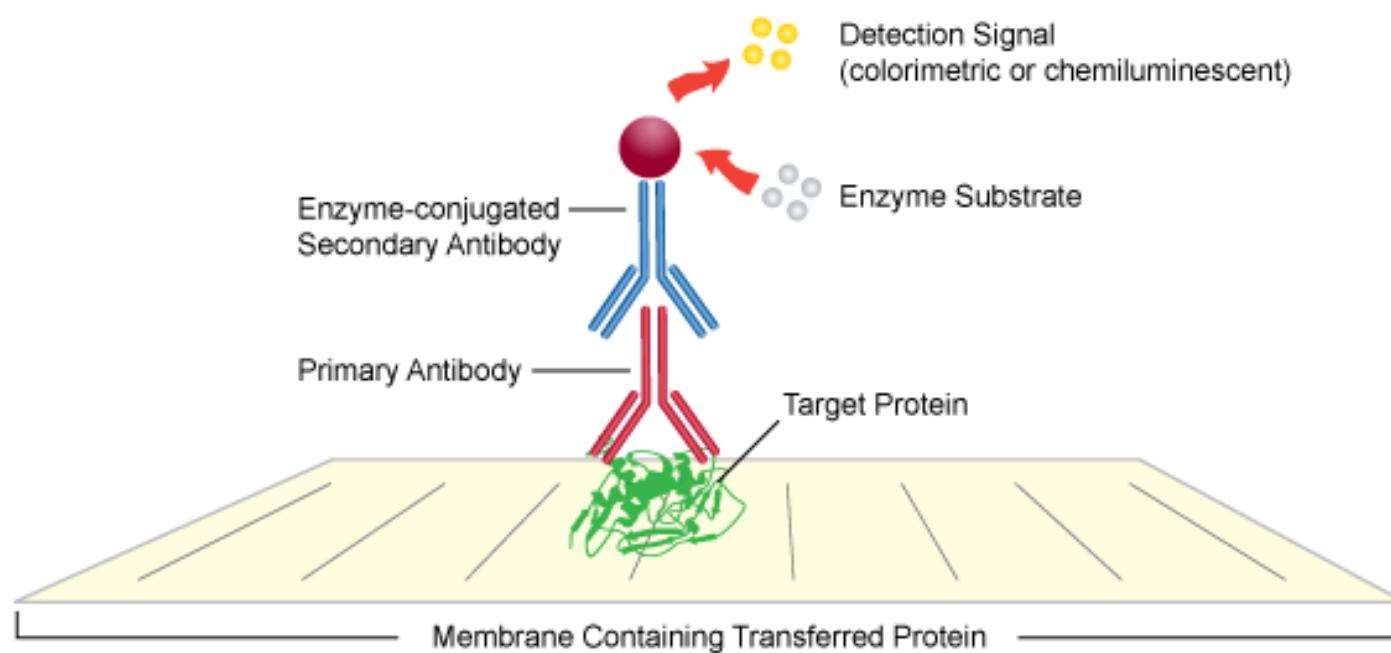
## 2. Western blotting (WB)

(SDS-PAGE followed by WB and immunodetection)



## 2. Western blotting (WB) (SDS-PAGE followed by WB and immunodetection)

### Detection in Western Blots



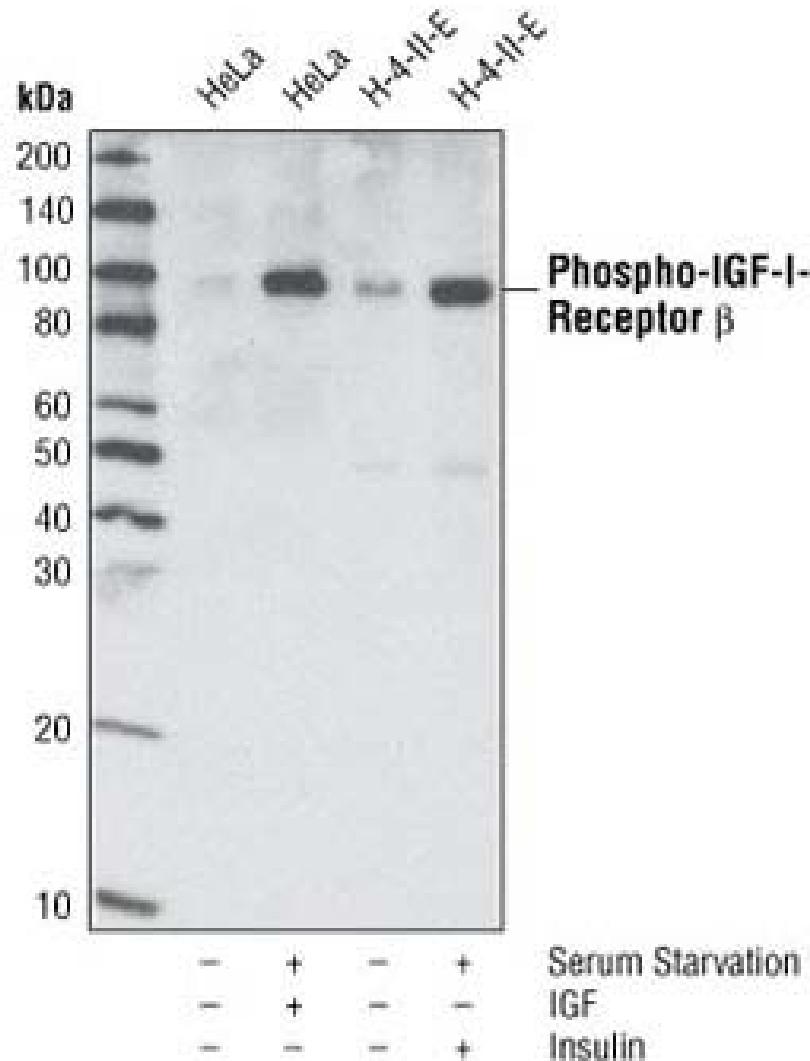
**Diagram 2:** Illustration of detection in Western Blots.

## 2. Western blotting (WB) (SDS-PAGE followed by WB and immunodetection)

### Example:

test for IGF receptor activation by IGF and insulin, the principal factor controlling cell growth.

(using anti phospho antibodies for detection)



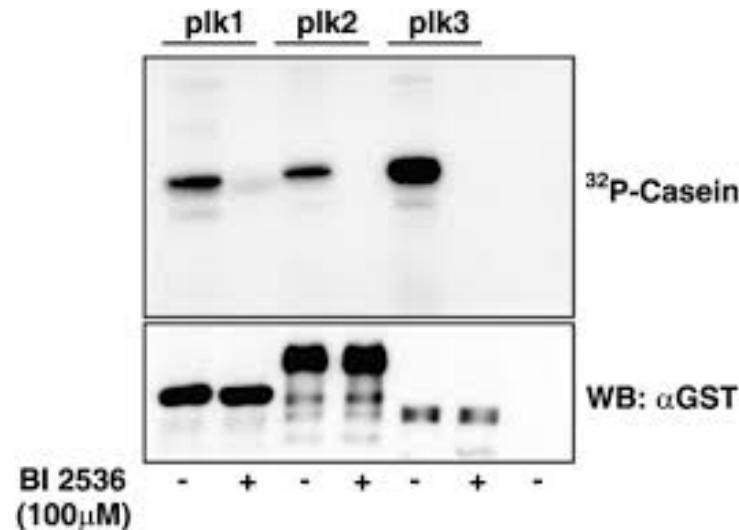
### 3. *In vitro* protein activity assay (enzymatic activity assay)

#### Procedure:

- (1). Purify protein *in vitro* (either isolation or production of recombinant proteins)
- (2). Set up *in vitro* protein assay with substrates and necessary components such as ATP, etc
- (3). Analyze protein activity by comparing signal strength.

#### Concept:

analysis of the kinase activity of **three polo-like kinases (PLKs)** using  $^{32}\text{P}$  in a substrate (**casein**) **phosphorylation reaction** in the absence (-) or presence (+) of the kinase inhibitor BI 2536



#### Detection:

Upper panel:  $^{32}\text{P}$ -phosphorylated casein

Lower panel: control using anti-GST antibodies to proof that PLKs (GST-PLKs) were present in the respective (-/+) samples (loading control)

## 4. shRNA/siRNA induced “knock-down”of inhibitors

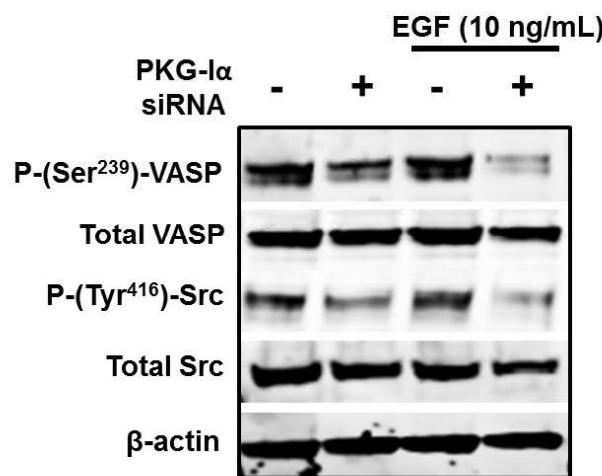
- **sh (short hairpin) RNA/si (small interfering) RNA**  
mRNA “**knockdown**” via **RNAi**
- **shRNA & siRNA** trigger degradation of  
specific target mRNA
- Many enzymes have relatively specific inhibitors.

## 4. shRNA/siRNA induced “knock-down” of inhibitors

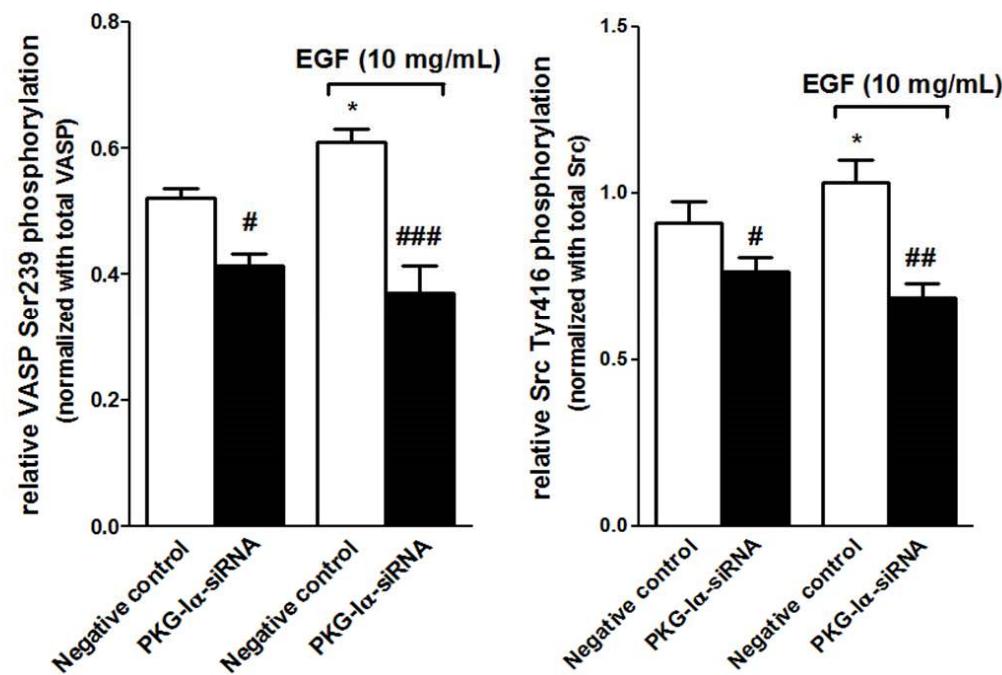
Example:

cGMP-dependent protein kinase G (PKG- $\lambda$ ) phosphorylates VASP and Src upon EGF perception by the respective receptor

A.



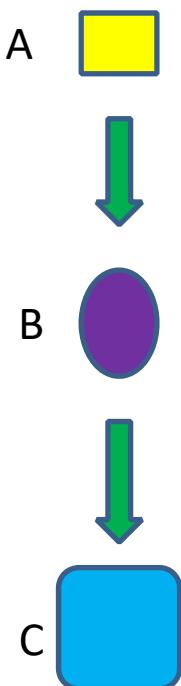
B.



## 5. Phenotype rescue assay

### Application:

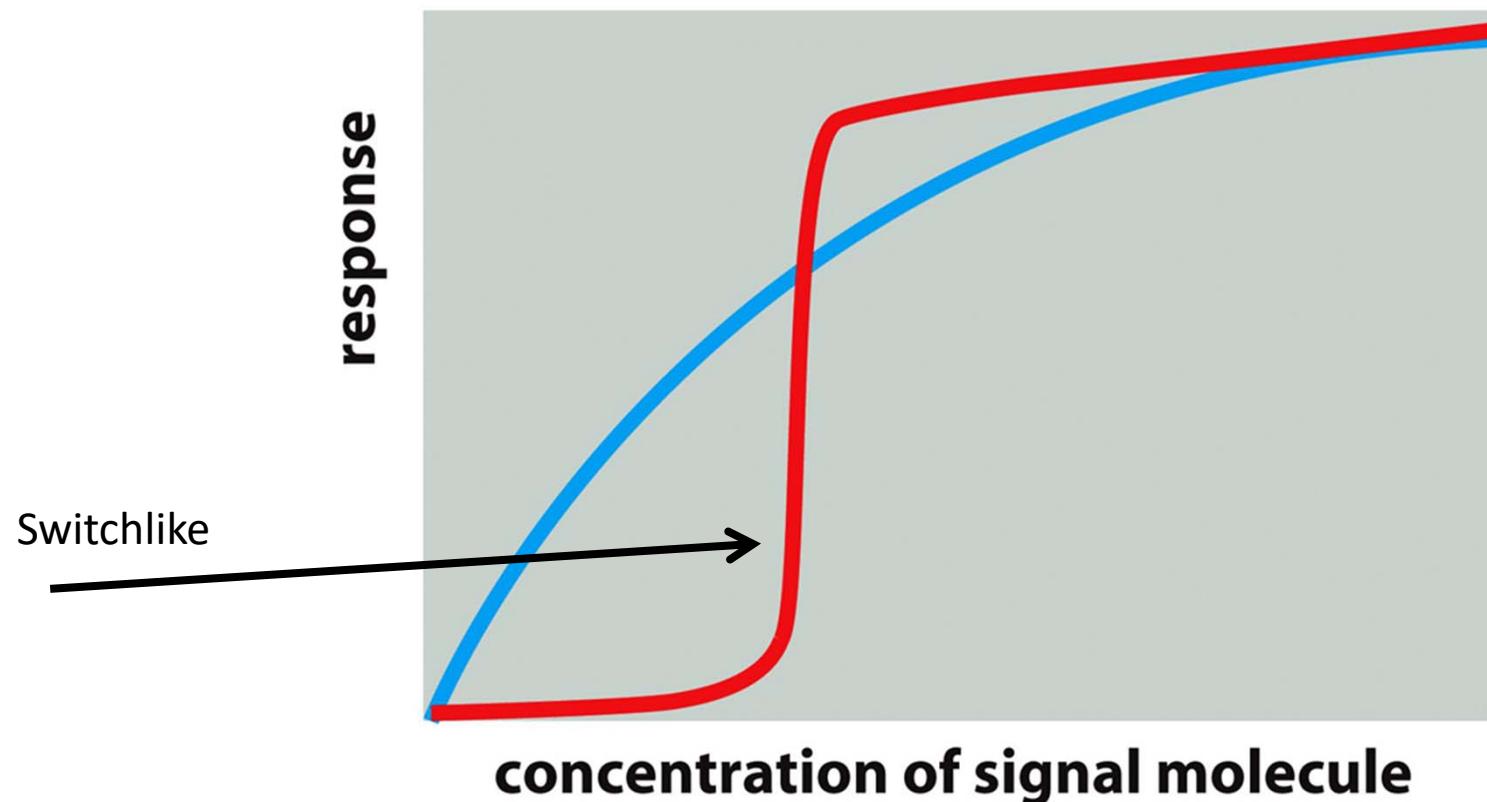
- To prove that one signaling protein locates **upstream or downstream** of another



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

## V. Positive and negative feedback in signaling & signaling kinetics

Signaling can cause both, a “all-or-none” response  
or a “smoothly-graded” response”



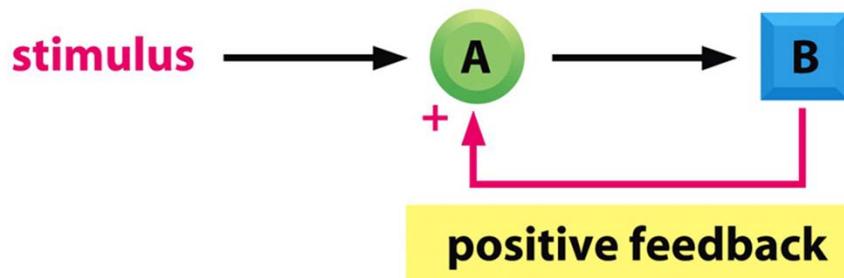
But how is this achieved?

## What causes switch-like “all-or-none” response?

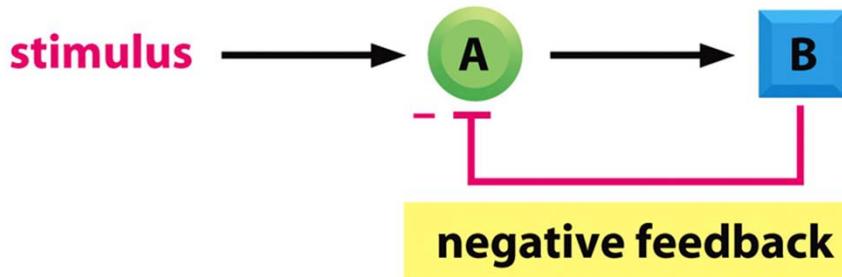
1. “All-or-none” response could be due to a **cooperative response to reach a certain threshold:**  
**Examples:** - all **4 cAMPs** must bind to PKA,  
- **multiple sites** must be **phosphorylated**
2. “All-or-none” response could be due to **concerted effects** of activation of one enzyme and simultaneous inhibition of another enzyme that catalyses the opposing reaction.
3. “All-or-none” response needs **positive feedback** response (more product more effect).

# Positive and negative feedback

## Principle:



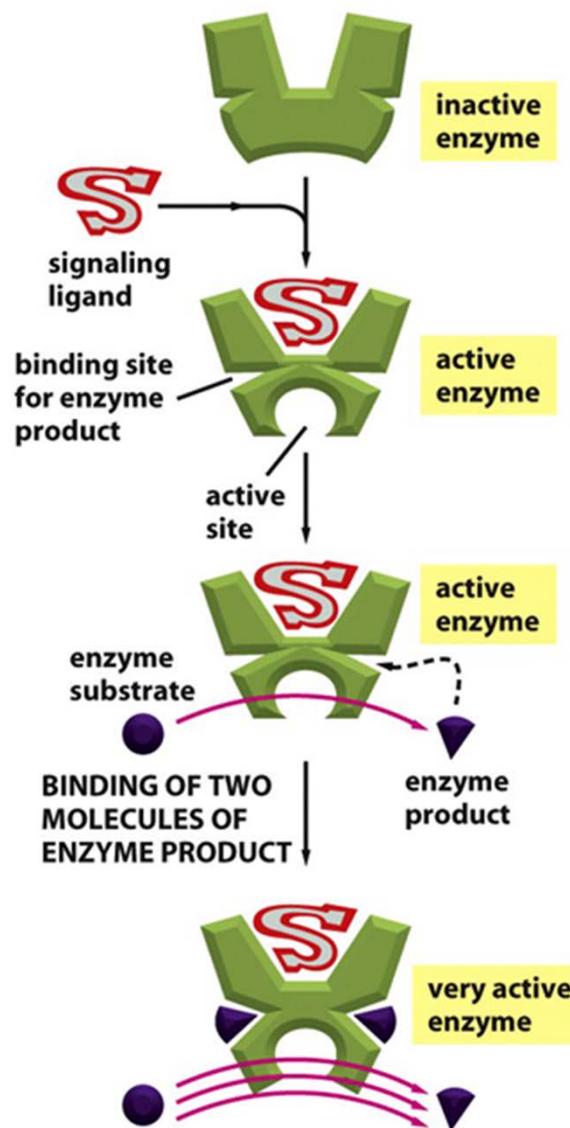
A stimulus activates protein A, which, in turn, activates protein B. Protein B then acts back to increase the activity of A.



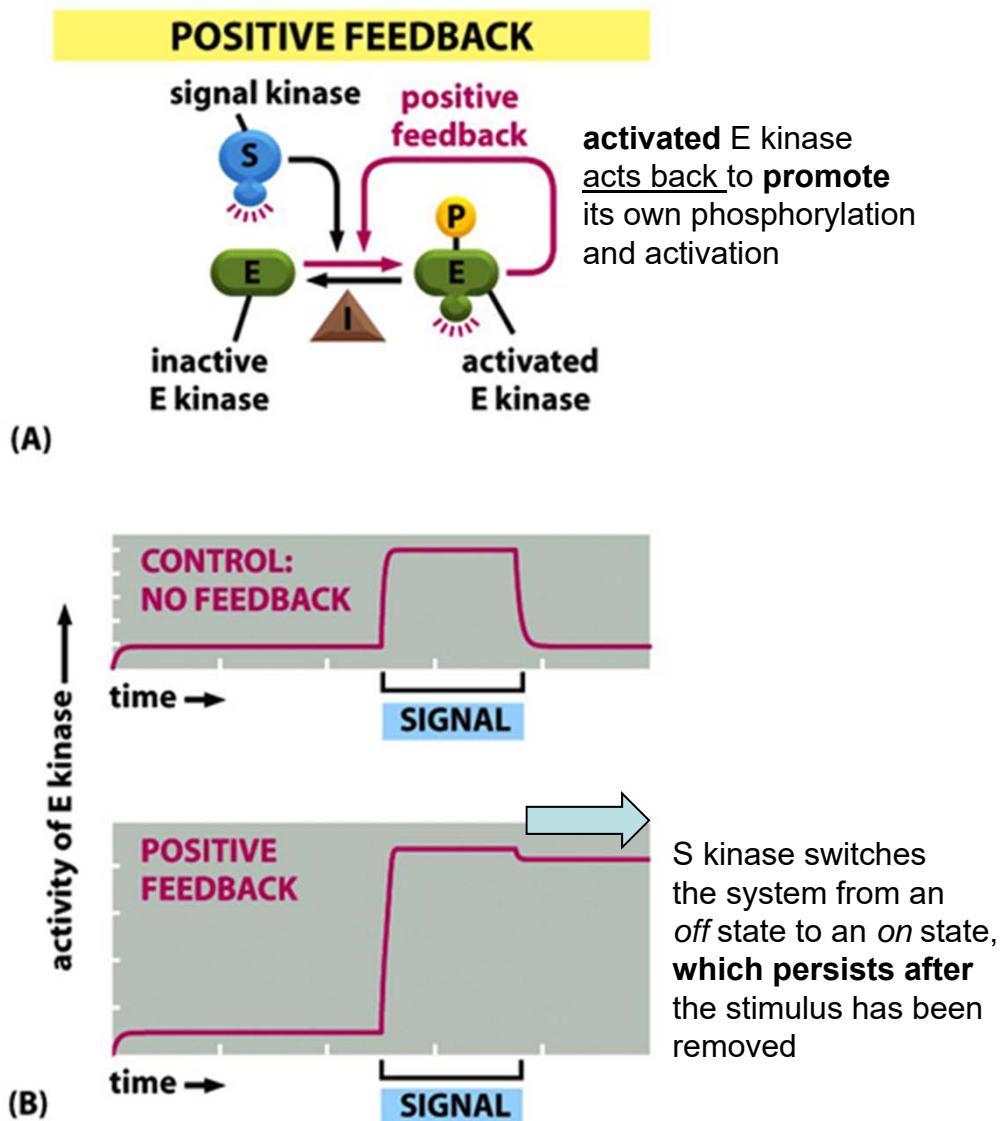
A stimulus activates protein A, which, in turn, activates protein B. Protein B then acts back to decrease the activity of A.

Such reactions can convert a short-term signal in a long-term answer!

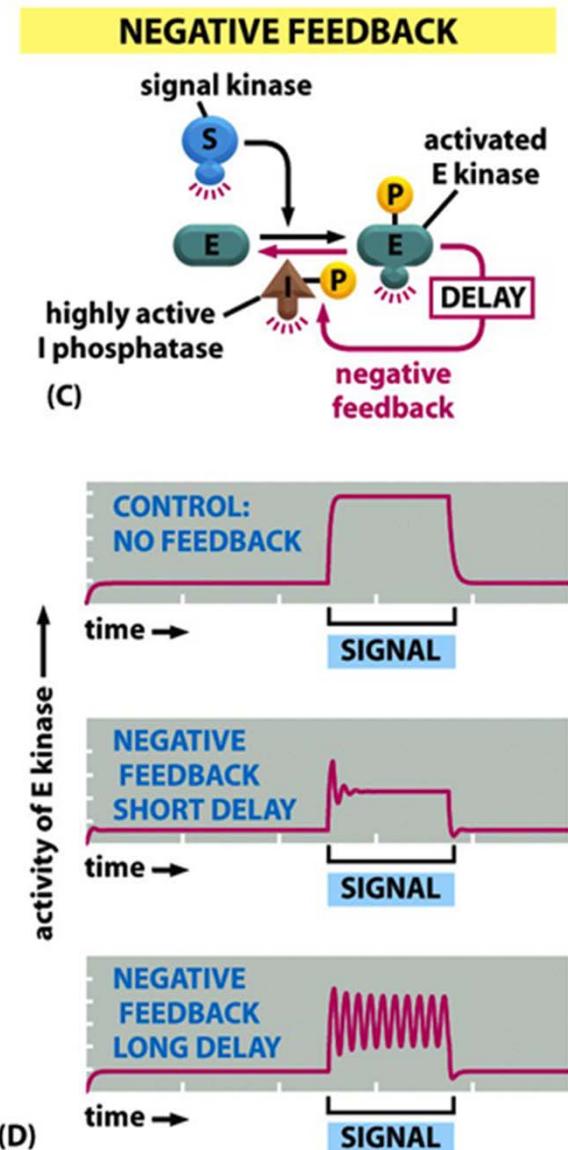
# Positive feedback gives switch-like response



# Different results from positive and negative feedback



# Different results from positive and negative feedback



basal activity of the I phosphatase dephosphorylates (deactivates) the activated E at a steady, low rate

With a **short delay**, the system shows a **strong, brief response** when the signal is abruptly changed, and the feedback then drives the response back down to a lower level.

With a **long delay**, the feedback produces sustained oscillations for **as long as the stimulus is present**.

## Negative feedback allows adaptation/desensitization for cells

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

