

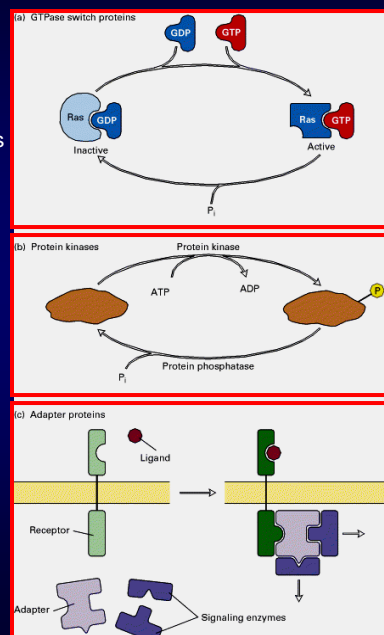
# Pharmacology-1 PHL 351

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## 8<sup>th</sup> Lecture

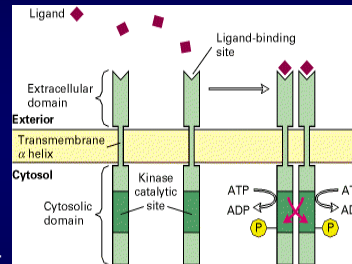
### Common intracellular signaling proteins

- (a) GTP-binding proteins with GTPase activity function as **molecular switches**.
- When bound to GTP they are active; when bound to GDP, they are inactive.
  - They fall into two categories, trimeric G proteins and Ras-like proteins.
- b) Protein kinases: modulate the activity or the binding properties of substrate proteins by phosphorylating serine, threonine, or tyrosine residues.
- The phosphorylated form of some proteins is active, whereas the dephosphorylated form of other proteins is active.
  - The combined action of kinases and phosphatases can cycle proteins between active and inactive states.
- c) Adapter proteins contain various protein-binding motifs that promote the formation of multiprotein signaling complexes.



## Kinase-linked Receptors, General structure & activation of receptor tyrosine kinases

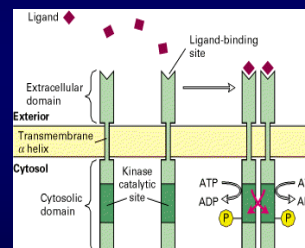
- ❖ Tyrosine-kinase (called *receptor tyrosine kinase*, more common) and guanylate cyclase-linked (much less common) receptors
- ❖ Actions: take minutes
- ❖ Examples: Growth factors, hormones (e.g. insulin) and cytokines
- ❖ Receptors for various hormones (e.g., insulin) and growth factors possess tyrosine kinase activity in their intracellular domain.
  - The intracellular domain incorporates both ATP- and substrate binding sites
- ❖ Cytokine receptors do not usually have intrinsic kinase activity, but associate, when activated by ligand binding, with kinases known as Jaks, which is the first step in the kinase cascade



## Kinase-linked Receptors,

### General structure and activation of receptor tyrosine kinases

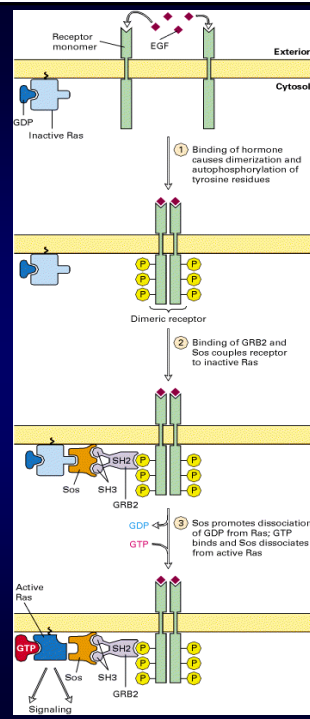
- ❖ The ligands for some RTKs, such as the receptor for EGF, are monomeric; ligand binding induces a conformational change in receptor monomers that promotes their dimerization.
- ❖ The ligands for other RTKs are dimeric; their binding brings two receptor monomers together directly.
- ❖ In either case, upon ligand binding, a tyrosine kinase activity is "switched on" at the intracellular portion.
- ❖ the kinase activity of each subunit of the dimeric receptor initially phosphorylates tyrosine residues near the catalytic site in the other subunit.
- ❖ Subsequently, tyrosine residues in other parts of the cytosolic domain are autophosphorylated.
- ❖ Protein phosphorylation leads to altered cell function via the assembly of other signal proteins



## Kinase-linked Receptors,

Activation of Ras following binding of a hormone (e.g., EGF) to an RTK.

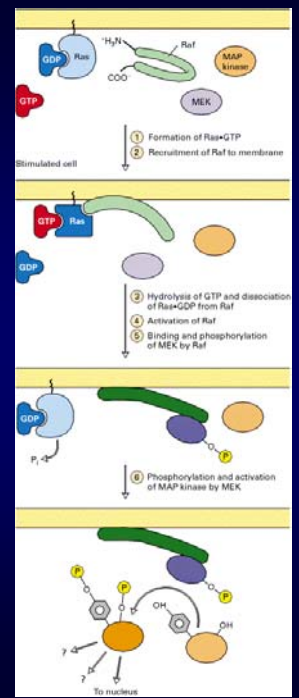
1. The adapter protein GRB2 binds to a specific phosphotyrosine on the activated RTK and to Sos, which in turn interacts with the inactive Ras-GDP.
  2. The guanine nucleotide – exchange factor (GEF) activity of Sos then promotes formation of active Ras-GTP.
- Note that Ras is tethered to the membrane by a farnesyl anchor



## Kinase-linked Receptors,

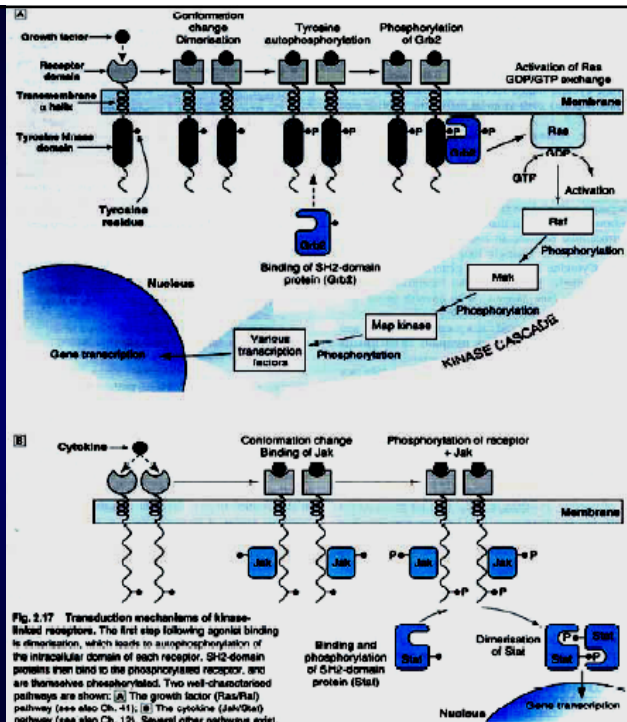
Kinase cascade that transmits signals downstream from activated Ras protein

1. Activated Ras binds to the N-terminal domain of Raf, a serine/threonine kinase.
2. Raf binds to and phosphorylates MEK, a dual-specificity protein kinase that phosphorylates both tyrosine and serine residues.
3. MEK phosphorylates and activates MAP kinase, another serine/threonine kinase.
4. MAP kinase phosphorylates many different proteins, including nuclear transcription factors, that mediate cellular responses.



## Kinase-linked Receptors, all

See RDRM p 43 →  
and p42, 44



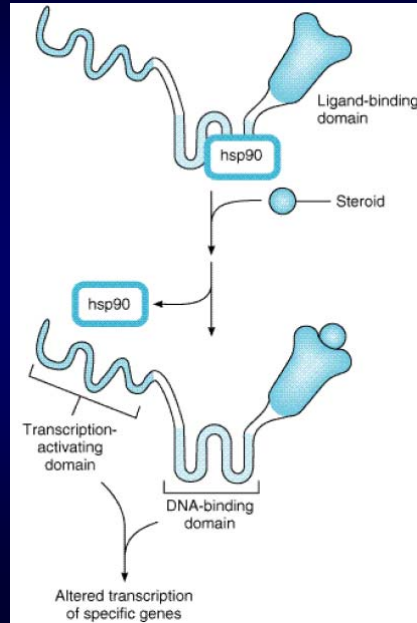
## Intracellular Receptors

- ❖ These receptors could be cytosolic or nuclear
- ❖ Several biologic signals are sufficiently lipid-soluble to cross the plasma membrane and act on intracellular receptors.
- ❖ One of these is a gas, nitric oxide (NO), that acts by stimulating an intracellular enzyme, guanylyl cyclase, which produces cyclic guanosine monophosphate (cGMP), which stimulates a cGMP-dependent protein kinase.
- ❖ Another class of ligands—including corticosteroids, mineralocorticoids, sex steroids, vitamin D, and thyroid hormone—stimulates the transcription of genes in the nucleus by
  - ❖ binding to nuclear receptors
  - ❖ This binding of hormone exposes a normally hidden domain of the receptor protein, thereby permitting the latter to bind to a particular nucleotide sequence on a gene and to regulate its transcription.
  - ❖ End result is an alteration in gene transcription and therefore protein synthesis
- ❖ Actions: slow-acting (hours), long lasting

## Nuclear Receptors, an example

### ❖ Mechanism of glucocorticoid action.

- ❖ A heat-shock protein, hsp90, binds to the glucocorticoid receptor polypeptide in the absence of hormone and prevents folding into the active conformation of the receptor.
- ❖ Binding of a hormone ligand (steroid) causes dissociation of the hsp90 stabilizer and permits conversion of glucocorticoid receptor to the active configuration.
- ❖ The active glucocorticoid receptor binds to a particular nucleotide sequence on a gene → altered transcription of certain genes



## Non-receptor Mechanisms

### 1. Actions on Enzymes

- Enzymes = Biological catalysts
  - Speed chemical reactions
  - Are not changed themselves
- Drugs altering enzyme activity alter processes catalyzed by the enzymes
- Examples
  - Cholinesterase inhibitors
  - Monoamine oxidase inhibitors

### 2. Drugs Which Interact With Carrier/Transporter Proteins

- ❖ Carriers/transporters e.g., norepinephrine carrier → target for maprotiline  
serotonin transporter → target for fluoxetine

### 3. Changing Physical Properties

- Mannitol
  - Changes osmotic balance across membranes
  - Causes urine production (osmotic diuresis)

## Non-receptor Mechanisms

4. Changing Cell Membrane Permeability (Ion Channels)
  - Lidocaine
    - Blocks sodium channels
  - Verapamil, nifedipine
    - Block calcium channels
  - Adenosine
    - Opens potassium channels
5. Combining With Other Chemicals
  - Examples
    - Antacids
    - Chelation of heavy metals

## Factors Modifying Drug Action

1. Alteration in Concentration of Drug That Reaches the Receptor
  - Patients may differ in the rate of absorption of a drug, in distributing it through body compartments, or in clearing the drug from the blood.
  - By altering the concentration of drug that reaches relevant receptors, such pharmacokinetic differences may alter the clinical response.
  - Some differences can be predicted on the basis of age, weight, sex, disease state, liver and kidney function, and genetic differences
2. Variation in Concentration of an Endogenous Receptor Ligand
  - This mechanism contributes greatly to variability in responses to pharmacologic antagonists.
  - For example, propranolol, a  $\beta$ -adrenoceptor antagonist, will markedly slow the heart rate of a patient whose endogenous catecholamines are elevated (as in pheochromocytoma) but will not affect the resting heart rate of a well-trained marathon runner.
  - A partial agonist may exhibit even more dramatically different responses: Saralasin, a weak partial agonist at angiotensin II receptors, lowers blood pressure in patients with hypertension caused by increased angiotensin II production and raises blood pressure in patients who produce small amounts of angiotensin.

## Factors Modifying Drug Action

### 3. Alterations in Number or Function of Receptors

- Changes in drug responsiveness can be caused by increases or decreases in the number of receptor sites or by alterations in the efficiency of coupling of receptors to distal effector mechanisms. These changes may be caused by:
  - the agonist ligand itself, e.g., clonidine ( $\alpha_2$ -adrenoceptor agonist) down-regulates  $\alpha_2$ -adrenoceptors
  - other hormones; for example, thyroid hormones increase both the number of receptors in rat heart muscle and cardiac sensitivity to catecholamines.
  - genetic factors which can play an important role in altering the number or function of specific receptors.

### 4. Changes in Components of Response Distal to the Receptor

- Although a drug initiates its actions by binding to receptors, the response observed in a patient depends on the functional integrity of biochemical processes in the responding cell and physiologic regulation by interacting organ systems.
- Before initiating therapy with a drug, the prescriber should be aware of **patient characteristics that may limit the clinical response (see next 2 slides)**

## Factors Modifying Drug Action, patients characteristics that may limit the clinical response

### a. Age: pediatric or geriatric

- **Pediatric patients**
  - Higher proportion of water
  - Lower plasma protein levels
    - More available drug
  - Immature liver/kidneys
    - Liver often metabolizes more slowly
    - Kidneys may excrete more slowly
- **Geriatric patients**
  - Chronic disease states
  - Decreased plasma protein binding
  - Slower metabolism
  - Slower excretion
  - Dietary deficiencies
  - Use of multiple medications
  - Lack of compliance

## Factors Modifying Drug Action

- b. Weight
  - Big patients "spread" drug over larger volume
- c. Gender
  - Difference in sizes
  - Difference in fat/water distribution
  - Women usually, require a relatively smaller dose than man. Drugs should be avoided as far as possible during pregnancy and lactation to prevent harmful effect on the fetus and the baby.
- d. Genetic effects
  - Lack of specific enzymes
  - Lower metabolic rate
- e. Psychological factors
  - Placebo effect
- f. Pathology
  - Drug may aggravate underlying pathology
  - Hepatic disease may slow drug metabolism
  - Renal disease may slow drug elimination
  - Acid/base abnormalities may change drug absorption or elimination