Signal Transduction

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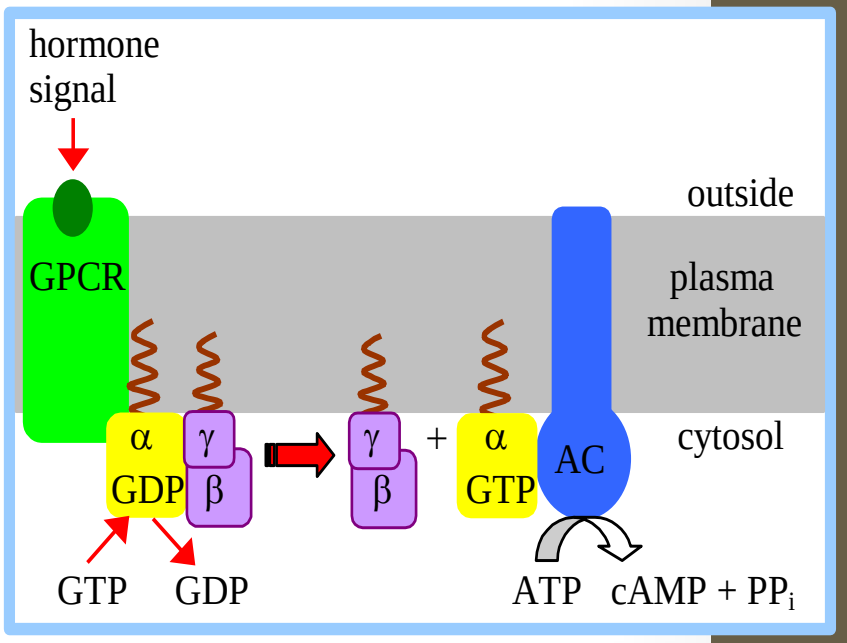
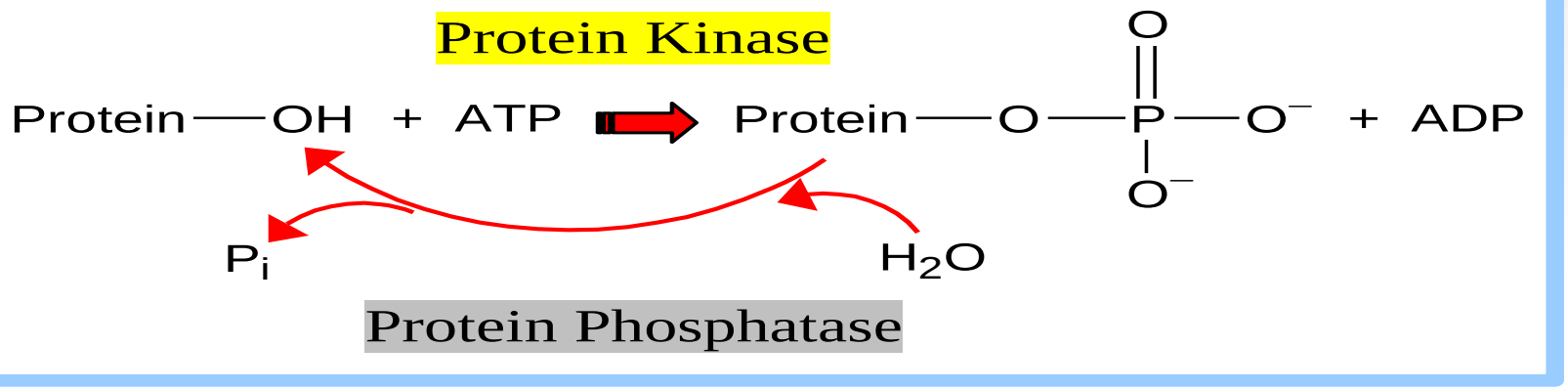
Integrative Physiology and Pharmacology

G Protein Signal Cascade

4. **Adenylate Cyclase**, activated by the stimulatory G_{α}-GTP, catalyzes synthesis of **cAMP**.

5. **Protein Kinase A** (cAMP Dependent Protein Kinase) catalyzes transfer of phosphate from ATP to serine or threonine residues of various cellular proteins, altering their activity.





Protein kinases and phosphatases are themselves regulated by complex signal cascades. For example:

- Some protein kinases are activated by **Ca^{++}-calmodulin**.
- **Protein Kinase A** is activated by **cyclic-AMP** (cAMP).

Protein Kinase A (cAMP-Dependent Protein Kinase) transfers P_i from ATP to OH of a Ser or Thr in a particular 5-amino acid sequence.

Protein Kinase A in the resting state is a complex of:

- 2 catalytic subunits (**C**)
- 2 regulatory subunits (**R**).

R_2C_2 : When each (**R**) binds 2 cAMP, a conformational change causes (**R**) to release (**C**).

The catalytic subunits can then catalyze phosphorylation of Ser or Thr on target proteins.

PKIs, Protein Kinase Inhibitors, modulate activity of the catalytic subunits (**C**).

Turn off of the signal:

1. **G_α** hydrolyzes GTP to GDP + P_i. (**GTPase**).

   The presence of **GDP** on G_α causes it to rebind to the inhibitory **$\beta\gamma$** complex.

   Adenylate Cyclase is no longer activated.

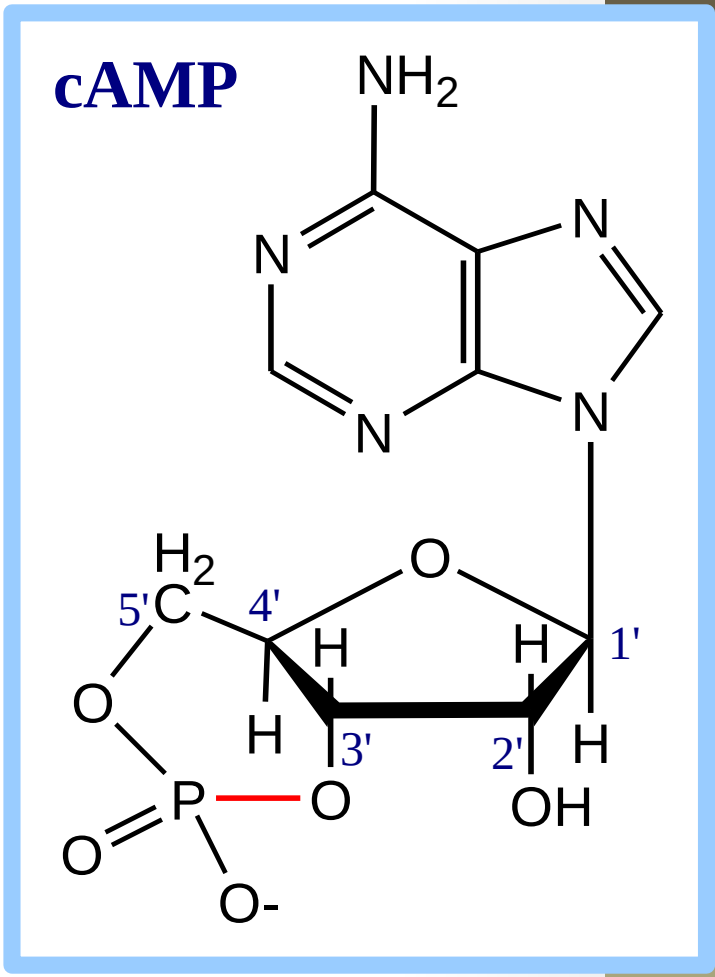
2. **Phosphodiesterases** catalyze hydrolysis of **cAMP → AMP**.

Phosphodiesterase enzymes catalyze:

cAMP + H_2O → AMP

The phosphodiesterase that cleaves cAMP is activated by phosphorylation catalyzed by Protein Kinase A.

Thus **cAMP stimulates its own degradation**, leading to rapid turnoff of a cAMP signal.



3. **Receptor desensitization** varies with the hormone.

- In some cases the **activated receptor** is **phosphorylated** via a G-protein Receptor Kinase.
- The phosphorylated receptor then may bind to a protein **β-arrestin.**
- **β-Arrestin** promotes **removal of the receptor** from the membrane by clathrin-mediated endocytosis.
- **β-Arrestin** may also bind a cytosolic **Phosphodiesterase**, bringing this enzyme close to where cAMP is being produced, contributing to signal turnoff.

4. **Protein Phosphatase** catalyzes removal by hydrolysis of phosphates that were attached to proteins via Protein Kinase A.

- **Different** isoforms of G_α have different signal roles. E.g.:
  - The **stimulatory $G_{s\alpha}$**, when it binds GTP, **activates** Adenylate cyclase.
  - An **inhibitory $G_{i\alpha}$**, when it binds GTP, **inhibits** Adenylate cyclase.

    Different effectors & their receptors induce **$G_{i\alpha}$** to exchange GDP for GTP than those that activate **$G_{s\alpha}$**.
- The complex of **$G_{\beta,\gamma}$** that is released when G_α binds GTP is itself an effector that binds to and **activates or inhibits** several other proteins.

  E.g., **$G_{\beta,\gamma}$ inhibits** one of several isoforms of **Adenylate Cyclase**, contributing to rapid signal turnoff in cells that express that enzyme.

Small GTP-binding proteins include (roles indicated):

- **initiation & elongation factors** (protein synthesis).
- **Ras** (growth factor signal cascades).
- **Rab** (vesicle targeting and fusion).
- **ARF** (forming vesicle coatomer coats).
- **Ran** (transport of proteins into & out of the nucleus).
- **Rho** (regulation of actin cytoskeleton)

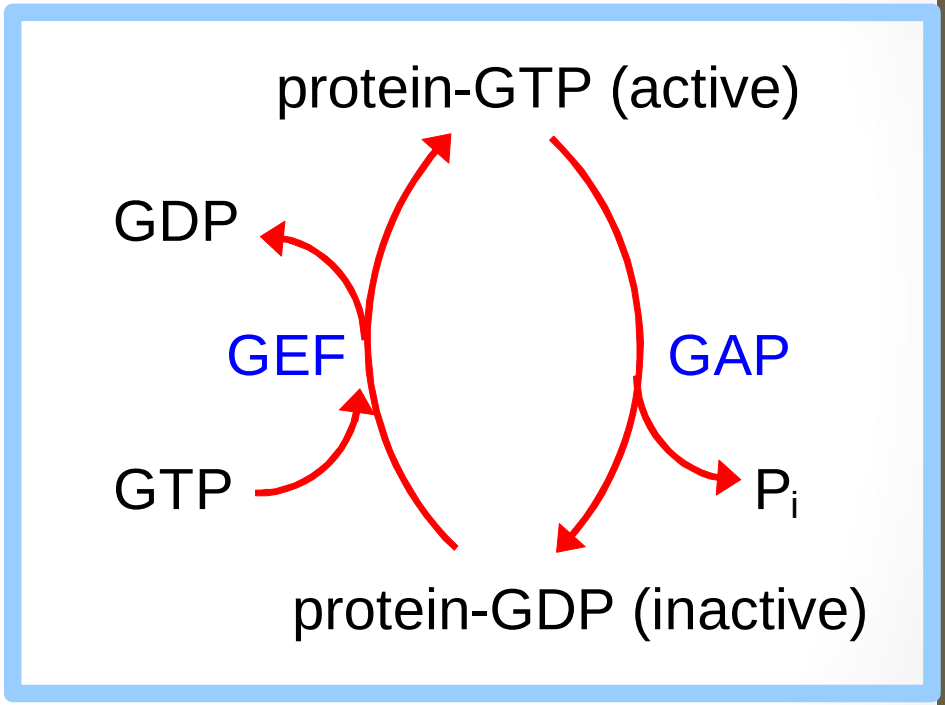
All GTP-binding proteins differ in conformation depending on whether GDP or GTP is present at their nucleotide binding site.

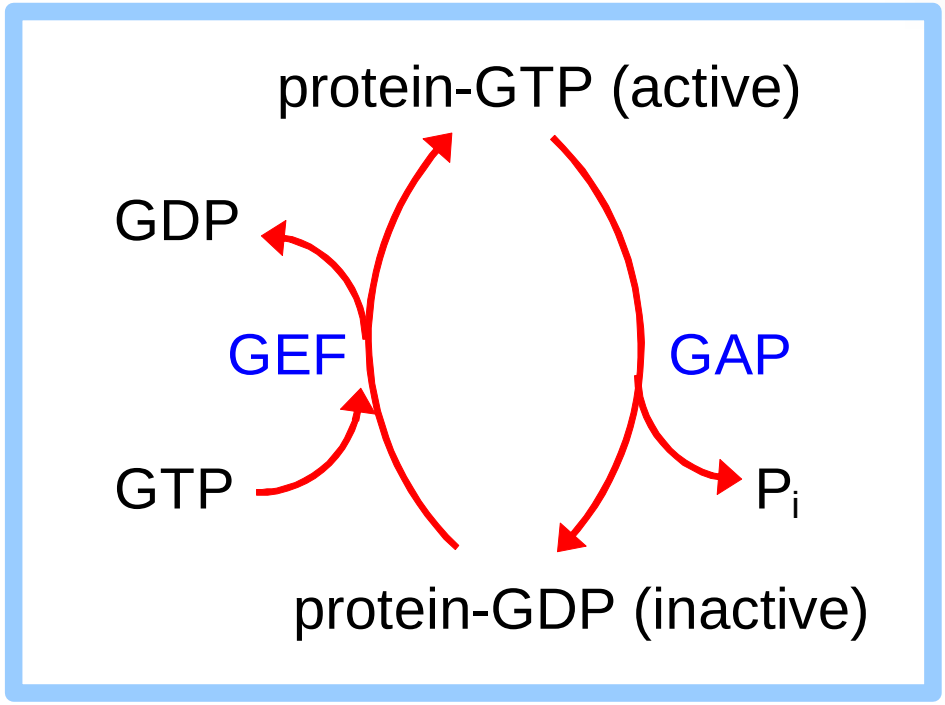
Generally, **GTP** binding induces the **active** state.

Most GTP-binding proteins depend on **helper proteins**:

GAPs,

GTPase **A**ctivating **P**roteins, promote GTP hydrolysis.

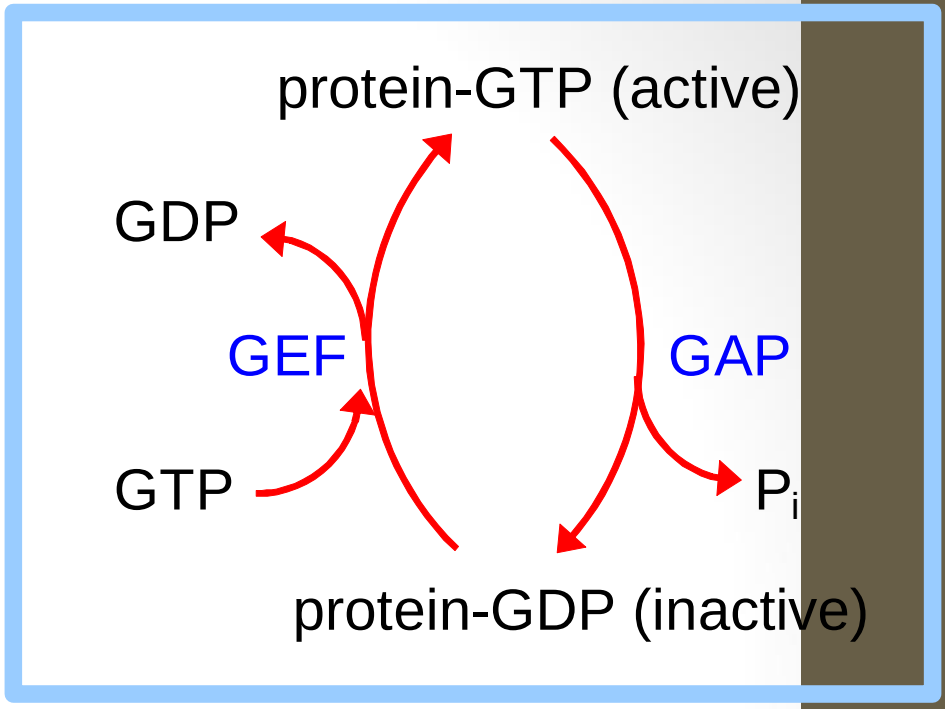




- **G_α** of a heterotrimeric G protein has innate capability for GTP hydrolysis.

  It has the essential arginine residue normally provided by a GAP for small GTP-binding proteins.

GEFs, **G**uanine **N**ucleotide **E**xchange Factors, promote GDP/GTP exchange.



- An activated **receptor** (GPCR) normally serves as **GEF** for a heterotrimeric G-protein.
- Alternatively, **AGS** (Activator of G-protein Signaling) proteins may activate some heterotrimeric G-proteins, independent of a receptor.

Some AGS proteins have GEF activity.

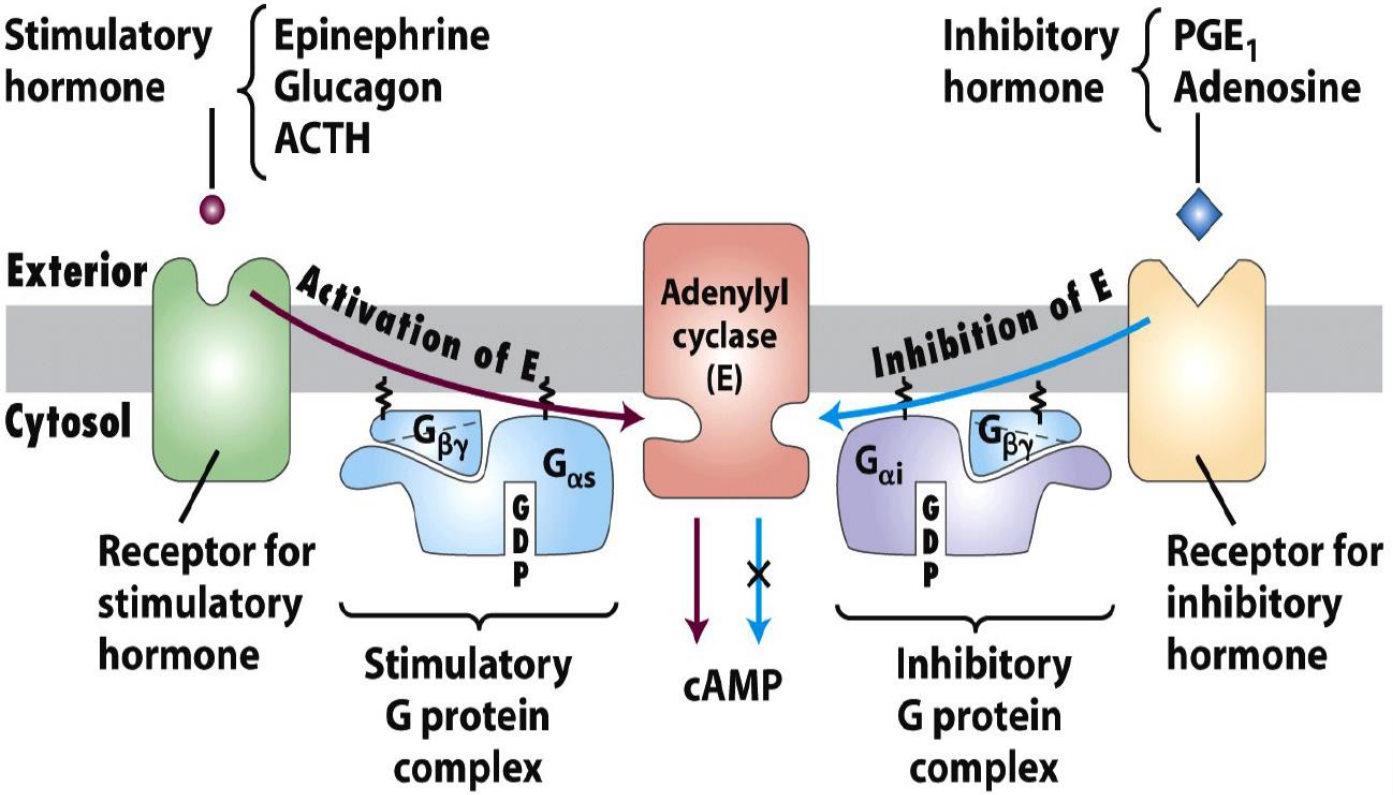

Stimulatory hormone
Epinephrine
Glucagon
ACTH
Inhibitory hormone
PGE1
Adenosine
Exterior
Cytosol
Activation of E
Inhibition of E
Adenylyl cyclase (E)
Gβγ
Gαs
GDP
Gαi
Receptor for stimulatory hormone
Receptor for inhibitory hormone
Stimulatory G protein complex
cAMP
Inhibitory G protein complex

Figure 15-21

3. Three major classes of surface receptors for signaling, cont.:

C. Enzyme-linked receptors:

1. Tyrosine kinase-linked receptors (TKRs).

***A.* Overview of TKRs:**

1. **Cell surface receptors that are directly linked to intracellular enzymes (kinases).**

2. **Includes receptors for most growth factors (NGF, EGF. PDGF), insulin, and Src.**

3. **Common structure: N terminal extracellular ligand-binding domain, single TM domain, cytosolic C-terminal domain with tyrosine kinase activity.**

4. **Can be single polypeptide or dimer.**

Examples of tyrosine kinase-linked receptors (TKRs):

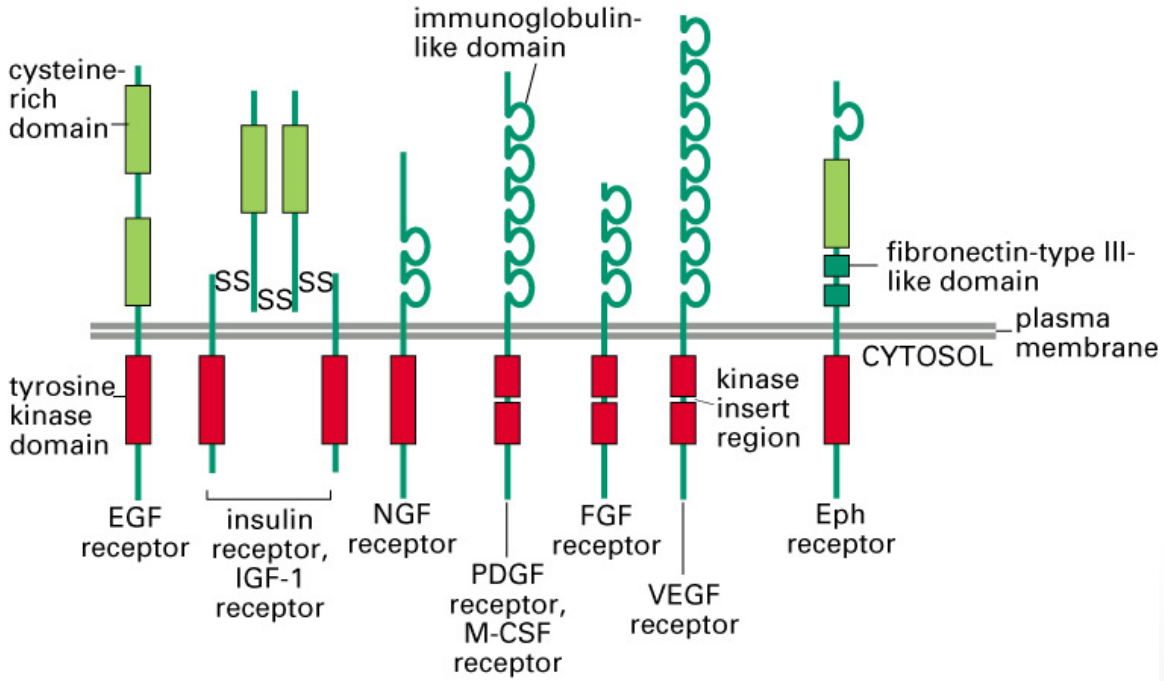


Figure 15–49. Molecular Biology of the Cell, 4th Edition.

3. Three major classes of surface receptors for signaling, cont.:

C. Enzyme-linked receptors, cont.:

1. Tyrosine kinase-linked receptors (TKRs)

***B.* Mechanism of activation of TKRs:**

i. **ligand binding induces receptor dimerization (receptor crosslinking).**

ii. **dimerization leads to autophosphorylation of the receptor (cross-phosphorylation).**

iii. **phosphorylation increases kinase activity & also creates specific new binding sites.**

iv. **proteins that bind to these new binding sites transmit intracellular signals.**

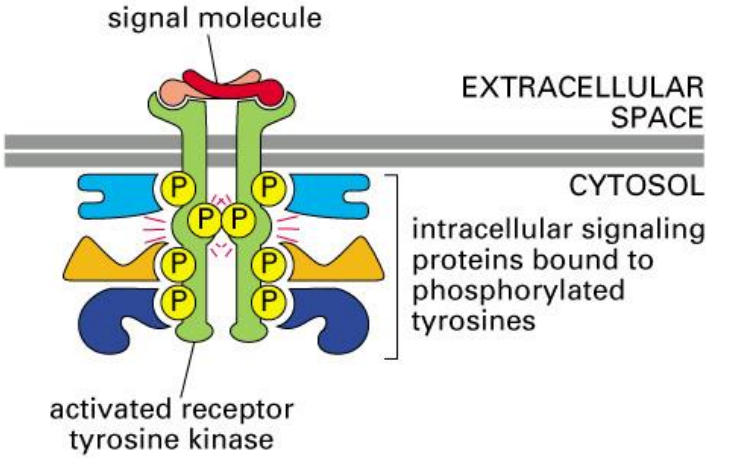


Figure 15–52. Molecular Biology of the Cell, 4th Edition.

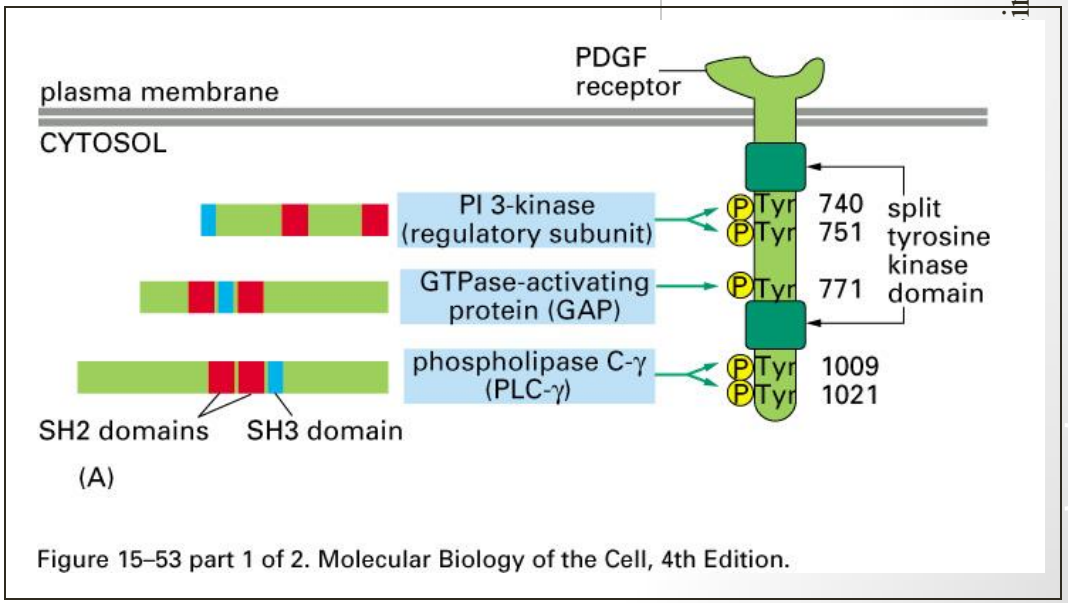


Figure 15–53 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

How receptor tyrosine kinases work together with monomeric GTPases:

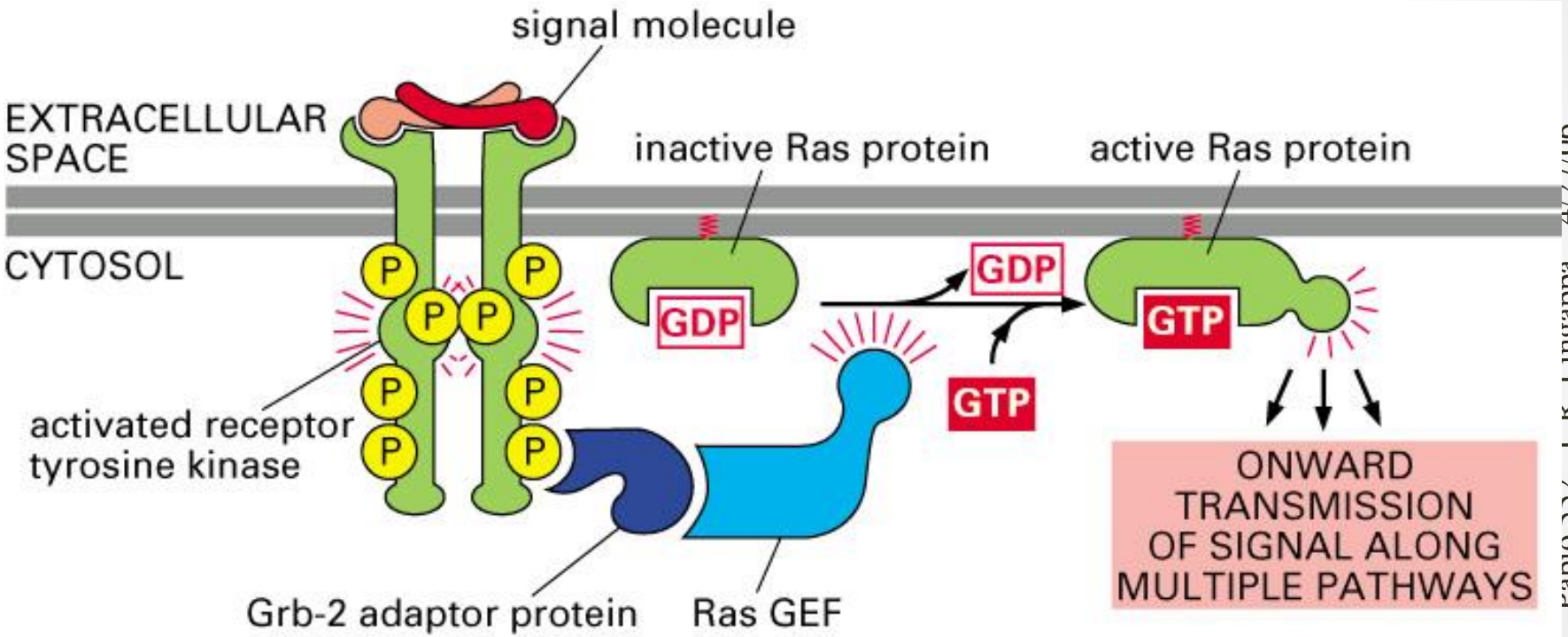
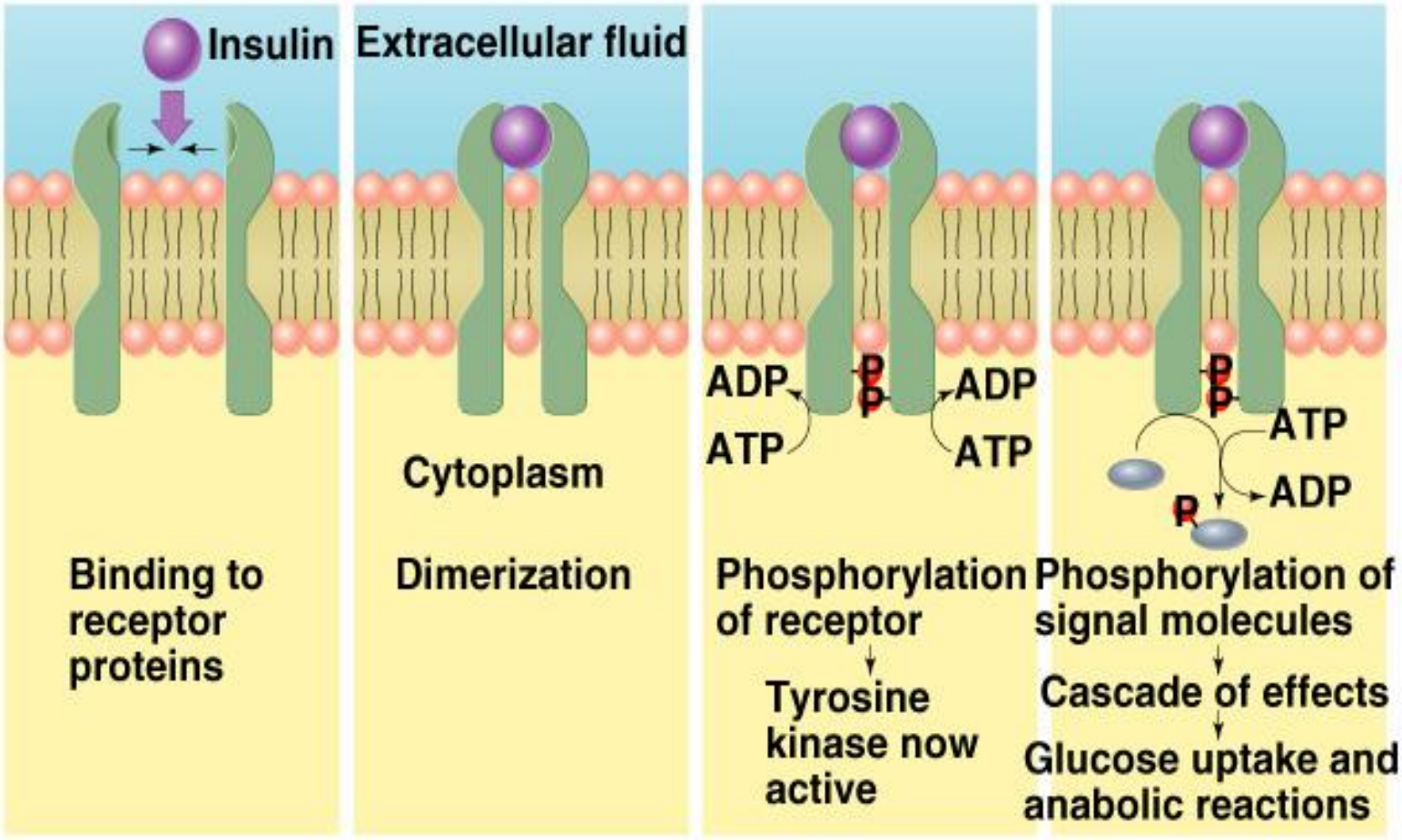


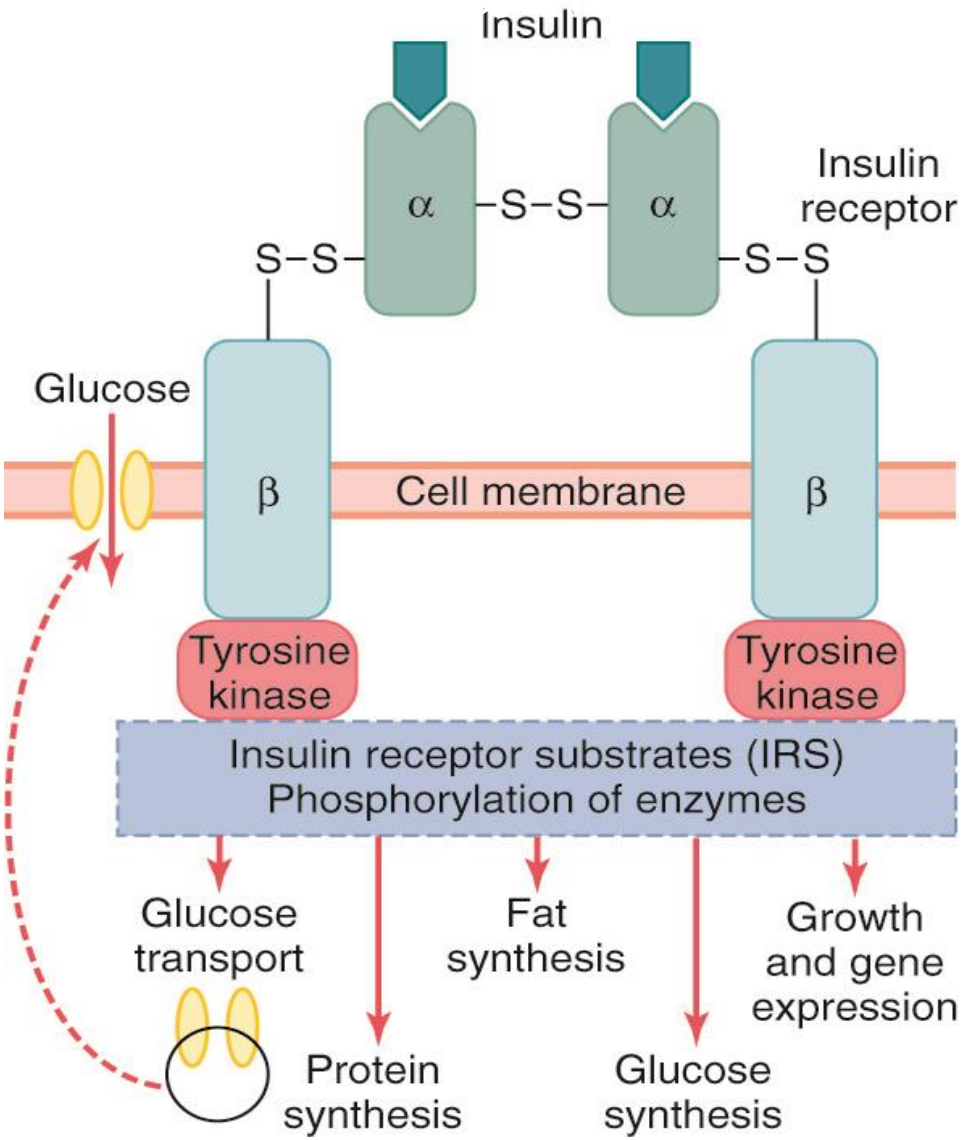
Figure 15–55. Molecular Biology of the Cell, 4th Edition.

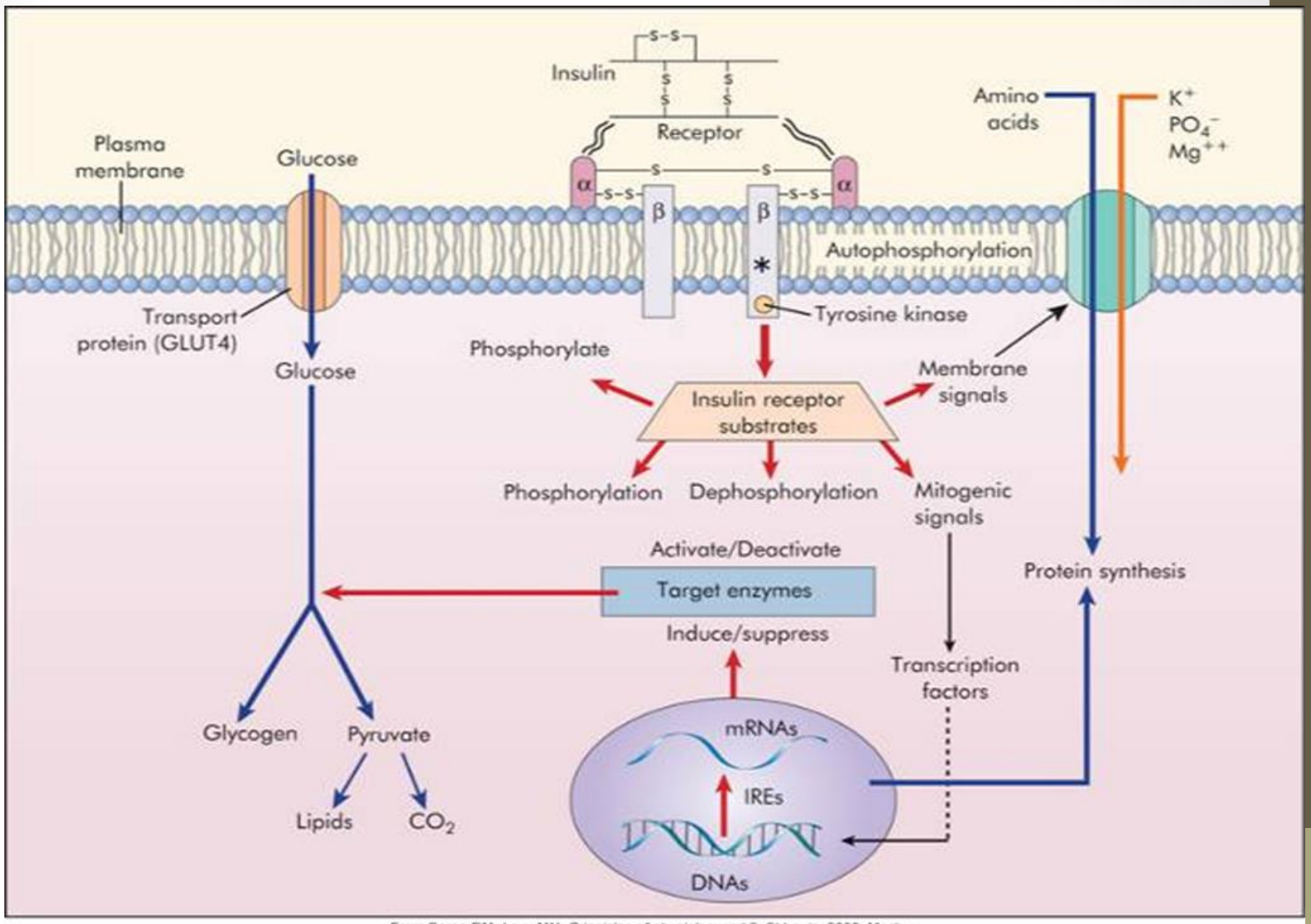
Tyrosine Kinase

- Insulin receptor consists of 2 units that dimerize when they bind with insulin.
  - Insulin binds to ligand–binding site on plasma membrane, activating enzymatic site in the cytoplasm.
- Autophosphorylation occurs, increasing tyrosine kinase activity.
- Activates signaling molecules.
  - Stimulate glycogen, fat and protein synthesis.
  - Stimulate insertion of GLUT-4 carrier proteins.

Tyrosine Kinase

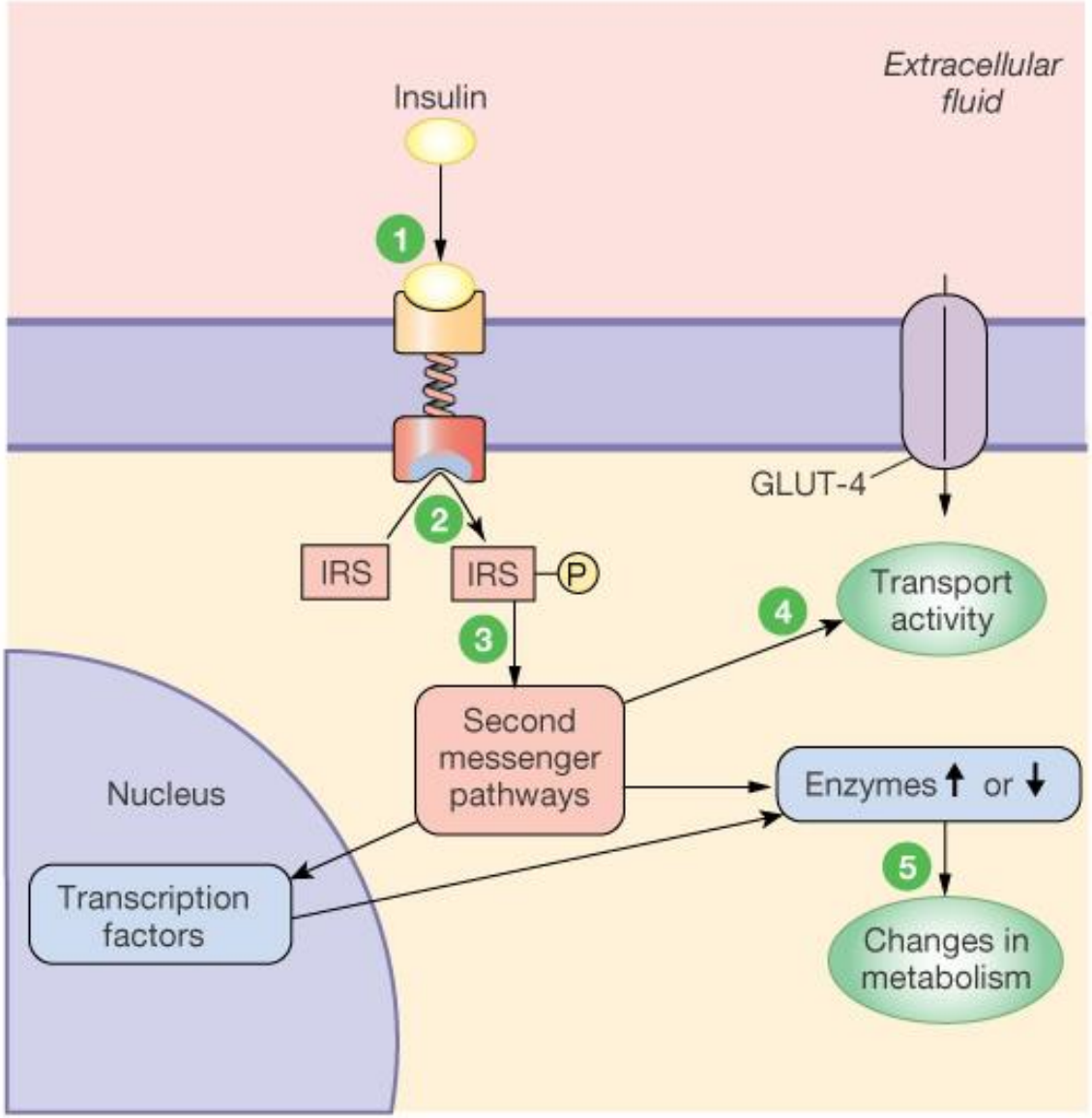



Insulin
α
S–S
α
Insulin receptor
S–S
S–S
Glucose
β
Cell membrane
β
Tyrosine kinase
Tyrosine kinase
Insulin receptor substrates (IRS)
Phosphorylation of enzymes
Glucose transport
Protein synthesis
Fat synthesis
Glucose synthesis
Growth and gene expression



From Berne RM, Levy MN: *Principles of physiology*, ed 3, St Louis, 2000, Mosby.

Insulin Action on Cells:



1. Insulin binds to tyrosine kinase receptor.
2. Receptor phosphorylates insulin-receptor substrates (IRS).
3. Second messenger pathways alter protein synthesis and existing proteins.
4. Membrane transport is modified.
5. Cell metabolism is changed.

Signaling Overview

3. Three major classes of surface receptors for signaling, cont.:

C. Enzyme-linked receptors, cont.:

2. TKs non-covalently associated with receptor (includes cytokine receptors, T & B cell receptors) = NRTKs

Cytokine receptors, as well as T and B cell receptors, stimulate tyrosine kinases that are non-covalently associated with receptor.

A. Overview

1. **N-term. extracell. ligand-binding domain, transmemb α helix,C-term. cytosolic domain**
2. **Cytosolic domain has no catalytic (kinase) activity**
3. **Acts in conjuction with a non-receptor tyrosine kinase that is activated as a result of ligand binding.**
4. **Activation is similar to that of RTKs: ligand binding causes cross phosphorylation of associated tyrosine kinases that phosphorylate the receptor, providing phosphotyrosine binding sites for recruitment of proteins with SH2 domains.**

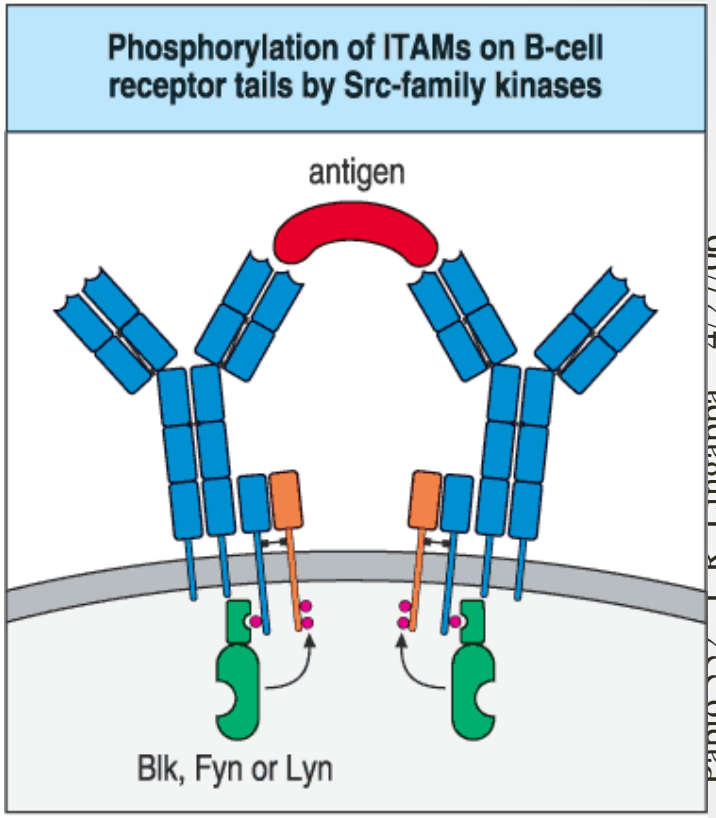


Fig 6.9 © 2001 Garland Science

From Janeway, Immunobiology, 5th edition

3. Three major classes of surface receptors for signaling, cont.:

C. Enzyme-linked receptors, cont.:

B. **Two kinds of kinases associate with NRTKs:**

1. Src family protein kinases - important for B and T cell signaling

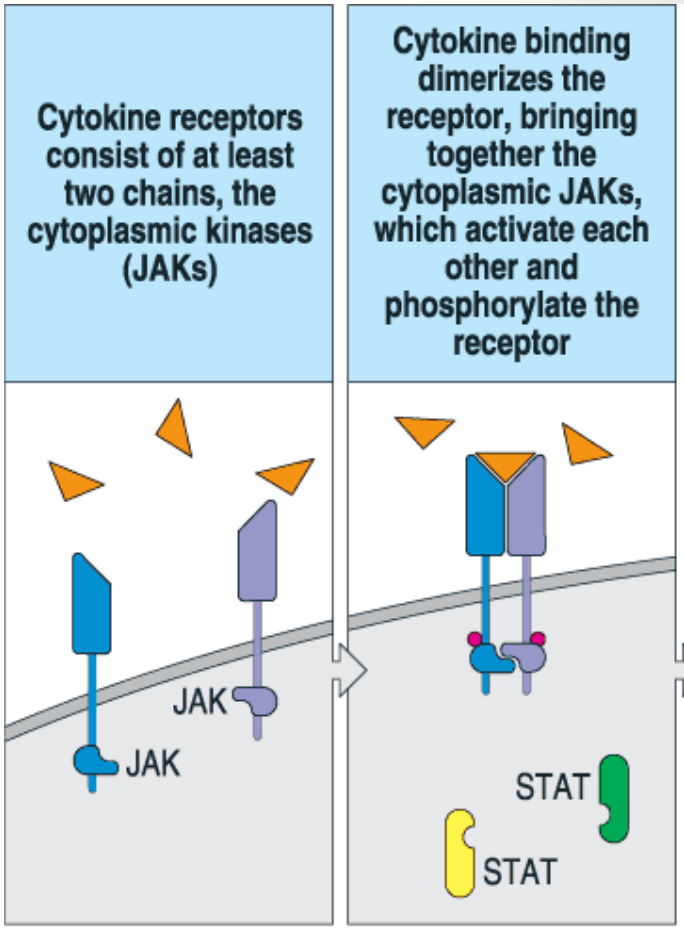
2. Janus kinases (JAK) - universally required for signaling from cytokine receptors.

C. Receptors can be linked to or associated with other enzymes, besides TKs, i.e.

Protein-tyrosine phosphatases (remove phosphates, thereby terminate signals initiated by protein-tyrosine kinases).

Serine/ threonine kinases, i.e. TGF-β

Guanylyl cyclases



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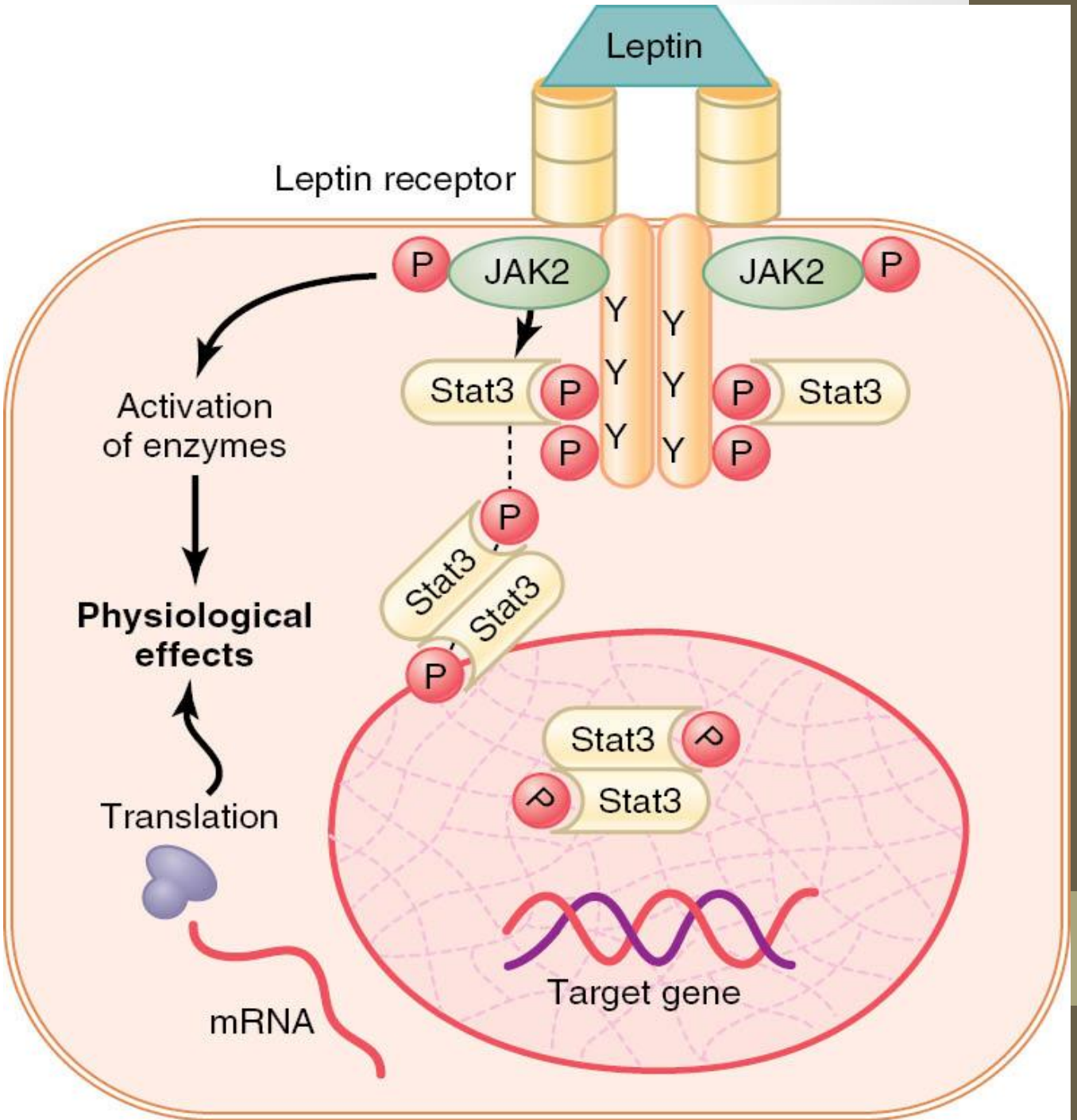
Pabio 552, J. R. Lingappa 4/27/06

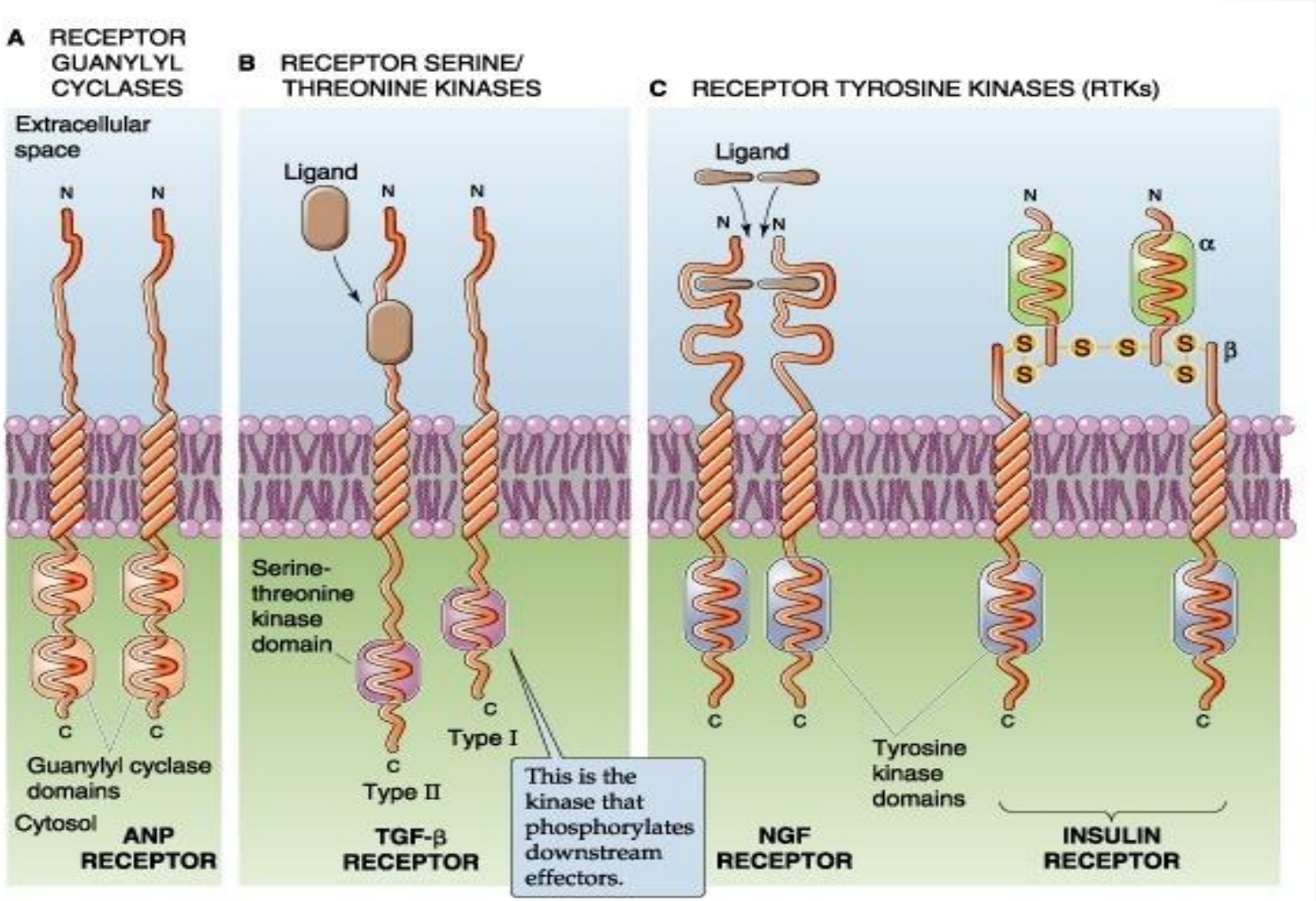
Enzyme-linked Receptor (the Leptin receptor)
JAK= Janus Kinase
STAT= Signal Transducer and Activator of Transcription



Enzyme-linked receptor (the leptin receptor)

- The receptor exists as a homodimer (two identical parts)
- Leptin binds to the extracellular part of the receptor
- This causes activation of the intracellular associated janus kinas 2
- This causes phosphorylation of signal transducer and activator of transcription (STAT) proteins
- This then activates the transcription of target genes and synthesis of proteins
- JAK 2 phosphorylation also activates several other enzyme systems that mediate some of the more rapid effects of leptin

Enzyme linked receptors



Tyrosine Kinase