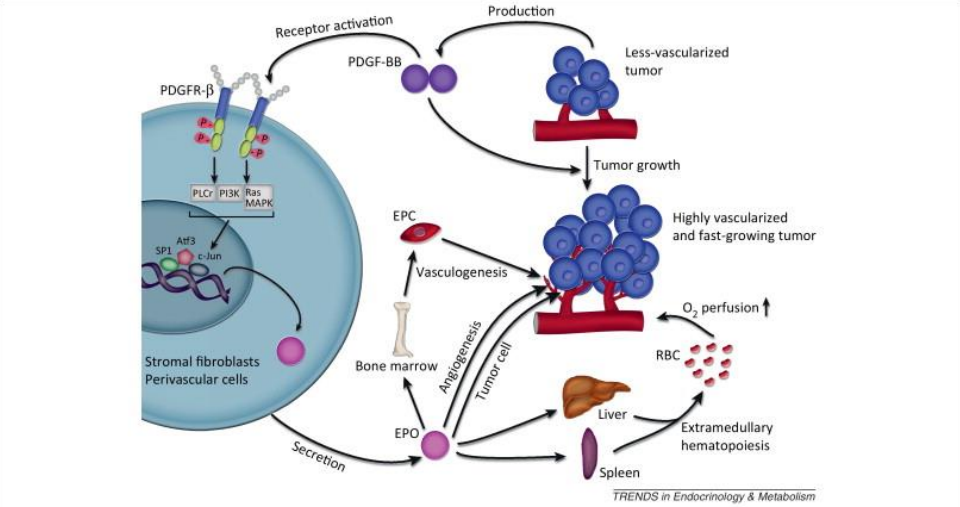
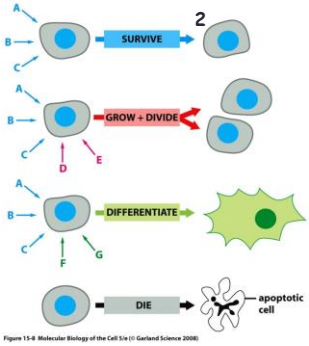
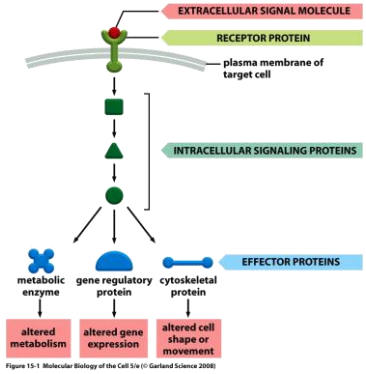
CELL COMMUNICATION : G-COUPLE RECEPTOR PROTEIN



- **Extracellular signal molecules**
  - Unicellular organisms
    - Response to extracellular signal molecules→ altered cell behavior
  - Multicellular organism
    - Response to signal molecules:
      - Altered metabolism
      - Altered tissue growth and differentiation
      - Protein synthesis and secretion
      - Altered intracellular and extracellular composition

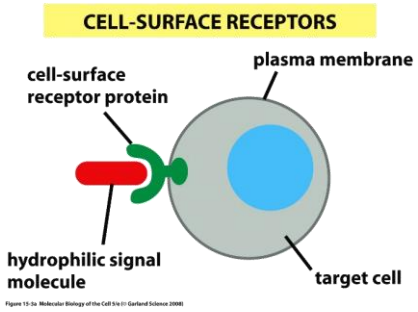


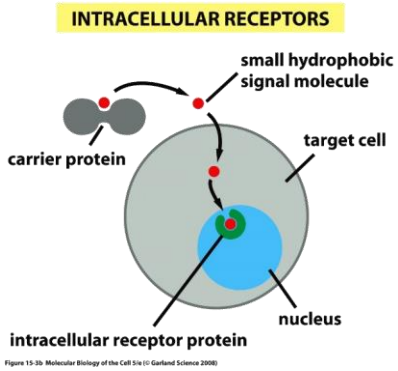
- Signal molecules : ligand bind to specific receptor - on/in target cell
- cell-surface receptors act as ***signal transducers*** by converting an extracellular ligand-binding event into intracellular signals that alter the behavior of the target cell



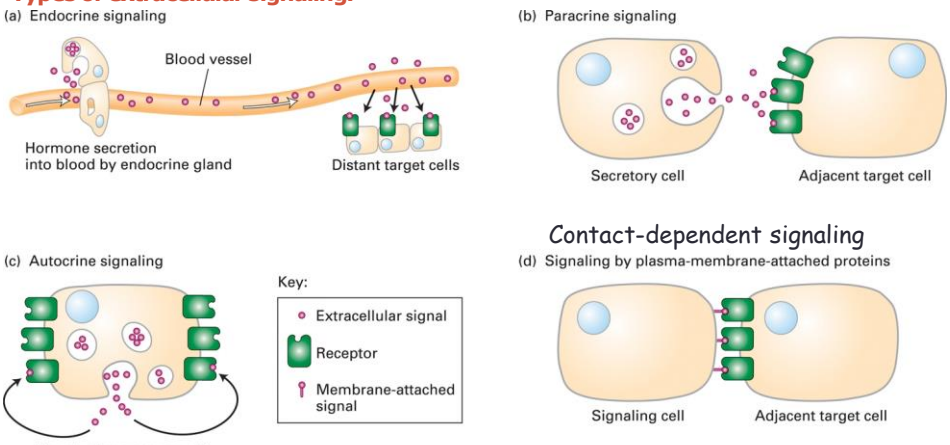
- Signal molecules ≤10^{-8}M → activate cell signalling & alter cell behavior

- Receptor :
  - Intracellular → hidrophobic/small signal molecules
  - Extracellular → hidrophilic signal molecules

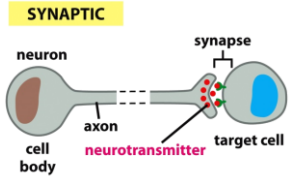




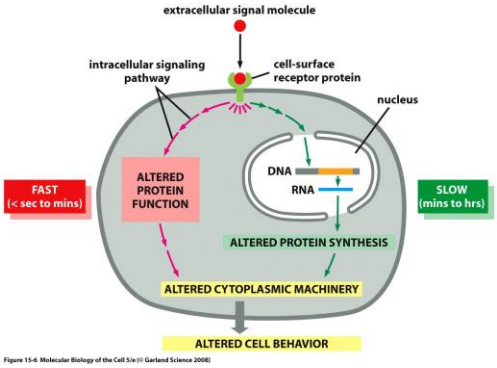
Types of extracellular signaling.



a) Endocrine signaling depends on endocrine cells, which secrete hormones into the bloodstream for distribution throughout the body.
b) Paracrine signaling depends on signals that are released into the extracellular space and act locally on neighboring cells.
c) Autocrine signaling: cells may also produce signals that they themselves respond to
d) Contact-dependent signaling requires cells to be in direct membrane–membrane contact.
e) Synaptic signaling is performed by neurons that transmit signals electrically along their axons and release neurotransmitters at synapses, which are often located far away from the neuronal cell body.

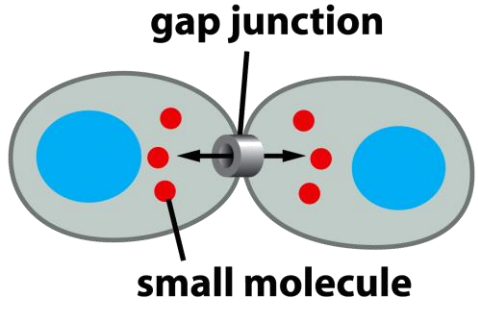


- Speed of a response to an extracellular signal depends on:
  - The mechanism of signal delivery
  - the nature of the target cell's response.



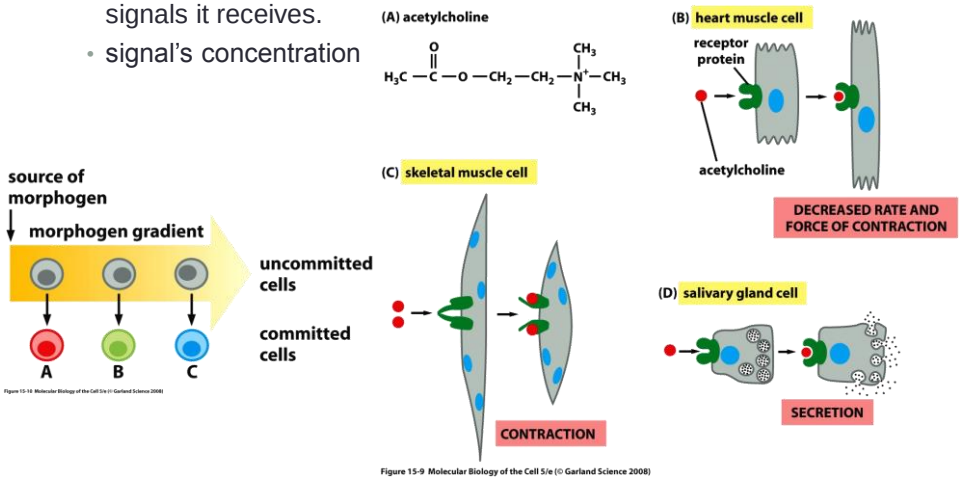
- Response :
  - rapid response:
    changes in proteins already present in the cell:
    - an allosteric change in a neurotransmitter-gated ion channel
    - protein phosphorylation
  - Slow response
    - changes in gene expression and the synthesis of new proteins

- Gap junction
  - Sharing signalling information
  - exchange of inorganic ions and other small watersoluble molecules : Ca^{2+}, cAMP
  - homogenize conditions in the communicating cells
    - nerve-muscle



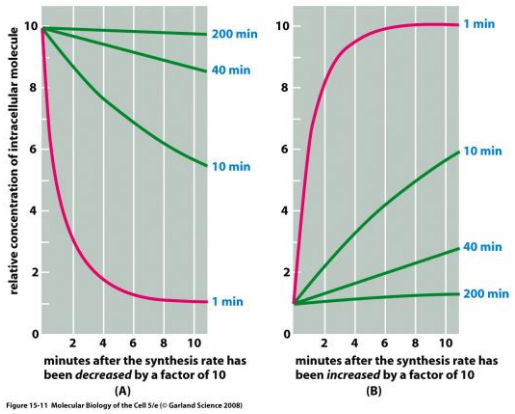
Different Types of Cells Usually Respond Differently to the Same Extracellular Signal Molecule

- A cell's response to extracellular signals depends on:
  - the receptor proteins
  - the intracellular machinery by which it integrates and interprets the signals it receives.
  - signal's concentration



A Cell Can Alter the Concentration of an Intracellular Molecule Quickly Only If the Lifetime of the Molecule Is Short

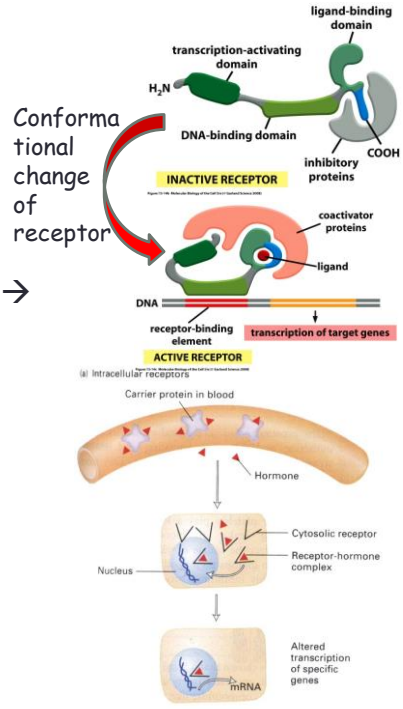
- During development ➔ transient extracellular signals →lasting effects
  - trigger a change in the cell's development through cell memory mechanisms.
- adult tissues
  - the response fades when a signal ceases.
  - effect is transient → signal effects by altering the concentrations of short-lived (unstable) intracellular molecules → undergoing continual turnover.
  - turnover rate can determine the promptness of the response when an extracellular signal arrives

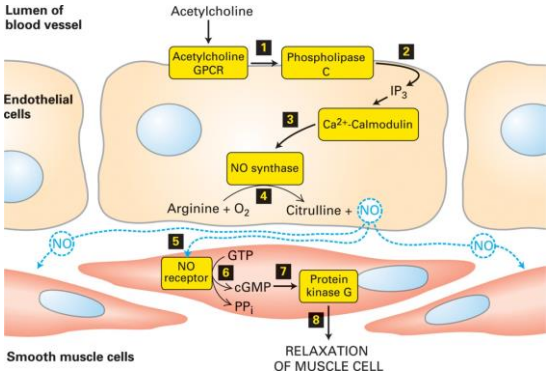


- Example : X and Y molecules are maintained by 1000 molecules
  - Y : synthesized and degraded every 100 mol/sec and lifetime each mole : 10 sec
  - X turnover rate: every 10 mol/sec. average lifetime:100 sec.
  - Activating Signal: 10 x fold increase synthesis of both x and Y ➔ Y 900; X 90

Intracellular Receptor

- Signal molecules:
  - small molecules (CO, NO),
  - hydrophobic molecules: estrogen, progesterone, testosterone, retinoic acid, vitamin D
- receptor: in cytoplasm or nucleus
- Intracellular receptor without ligand → inactive

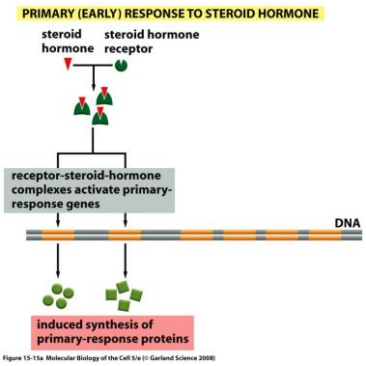


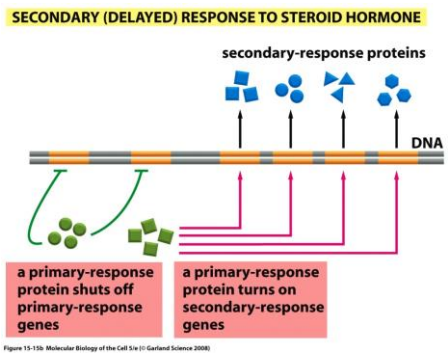


- The transcriptional response → multiple steps. :
  - direct stimulation of a small number of specific genes -~ ± 30 minutes → ***primary response***;

protein products – primary response

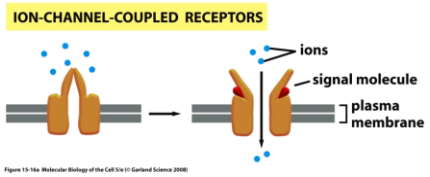
  - a delayed, ***secondary response***;
- some of the proteins from primary response → inhibit transcription of primary response genes→ **negative feedback**



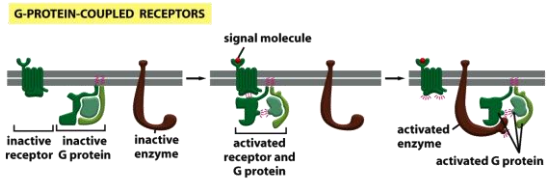


3 types of Cell-Surface Receptor Proteins

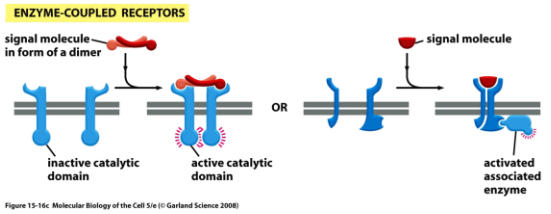
- **Ion-channel-coupled receptors**,
  - *transmitter-gated ion channels* or *ionotropic receptors*,



- **G-protein-coupled receptors**
  - act by indirectly regulating the activity of a separate plasma-membrane-bound target protein, which is generally either an enzyme or an ion channel.



- **Enzyme-coupled receptors**
  - either function directly as enzymes or associate directly with enzymes that they activate



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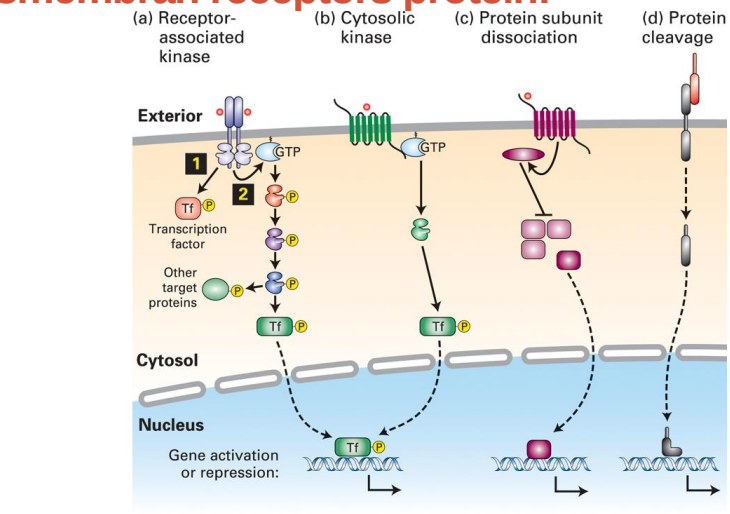
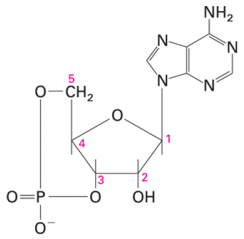
Transmembran receptors protein:



	(a) Receptor-associated kinase	(b) Cytosolic kinase	(c) Protein subunit dissociation	(d) Protein cleavage
Representative receptors and *pathways*	RTKs TGF-β receptors Cytokine receptors *JAK-STAT* *Ras/MAP Kinase*	GPCRs *cAMP/PKA/CREB*	*Wnt* *Hedgehog* *NF-kB*	*Notch/Delta*

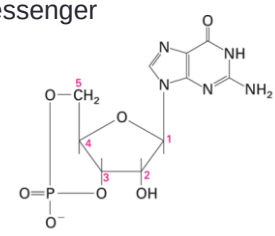
Relay signals from cell surface

- Relay signals from cell surface via :
  1. Small molecules : second messenger



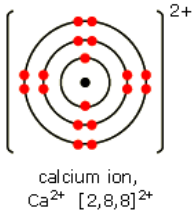
3′,5′-Cyclic AMP (cAMP)

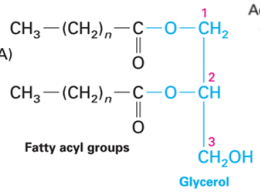
Activates protein kinase A (PKA)



3′,5′-Cyclic GMP (cGMP)

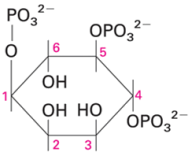
Activates protein kinase G (PKG) and opens cation channels in rod cells





1,2-Diacylglycerol (DAG)

Activates protein kinase C (PKC)



Inositol 1,4,5-trisphosphate (IP_3)

Opens Ca^{2+} channels in the endoplasmic reticulum

Relay signals from cell surface

- Relay signals from cell surface via :
  2. Network of intracellular signaling proteins
     - ***relay*** the signal
     - ***scaffold*** to bring two or more signaling proteins together
     - transform, or ***transduce***, the signal
     - ***amplify*** the signal it receives, :➔ chain reaction : signalling cascase
       - producing large amounts of a small intracellular mediator or
       - activating many copies of a downstream signaling protein.
     - ***Integrate*** *two or more signaling pathway*
     - ***spread*** the signal ➔ branches signalling stream
     - ***anchor*** one or more signaling proteins
     - ***modulate*** the activity of other signaling proteins ➔regulate the strength of signaling along a pathway.

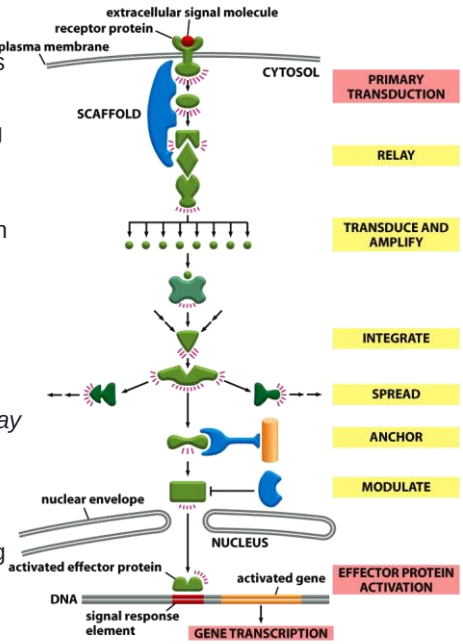


Figure 15-17 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Two important Molecular switches

- **Phosphorylation :**
  - **protein kinase & protein phosphatase**
- **GDP/GTP binding**
  - **GTPase-activating proteins (GAPs) & guanine nucleotide exchange factors (GEFs)**

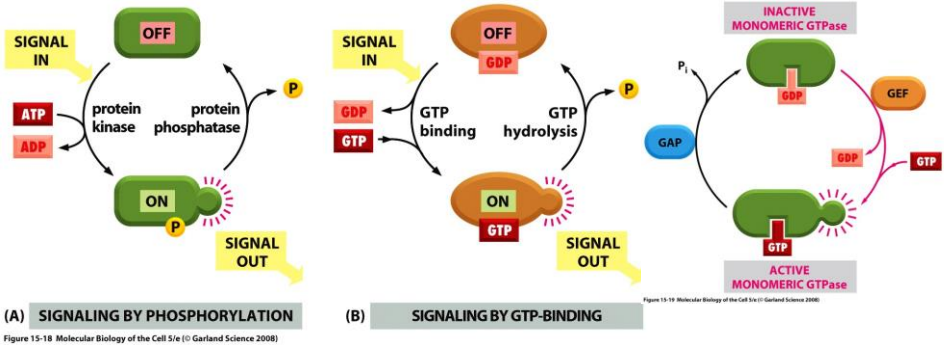
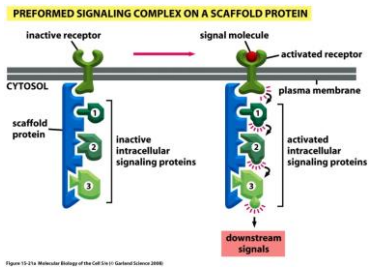
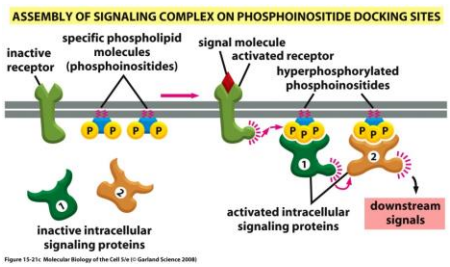


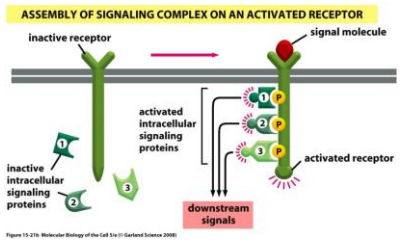
Figure 15-18 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Intracellular Signaling Complexes Enhance the Speed, Efficiency, and Specificity of the Response

- Scaffold protein
- Assembly of signaling complex on an activated receptor
- Assembly of signaling complex on phosphoinositide docking sites

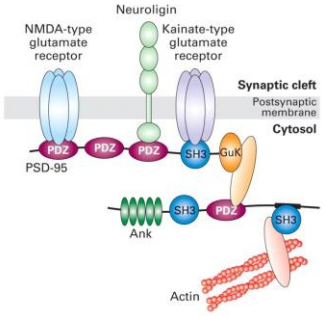


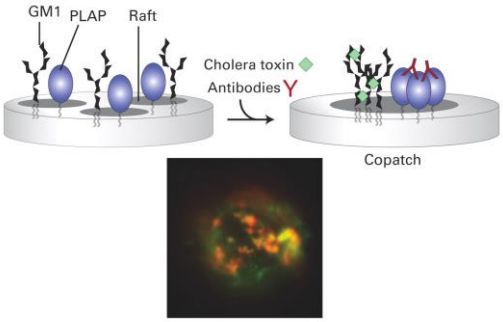


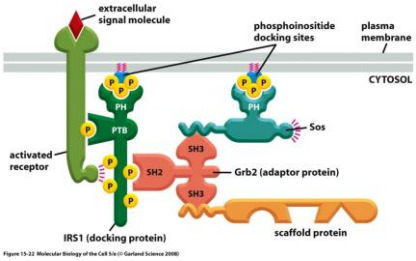


Receptor and signalling protein locallization

1. Clustering protein using adaptor protein domain
   - *Src homology 2 (SH2) or 3 (SH3) domains*
   - *phosphotyrosine-binding (PTB) domains*
   - *Pleckstrin homology (PH)* domains
2. Clustering protein using lipid raft

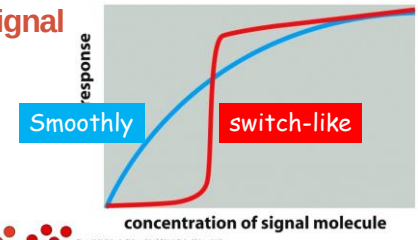




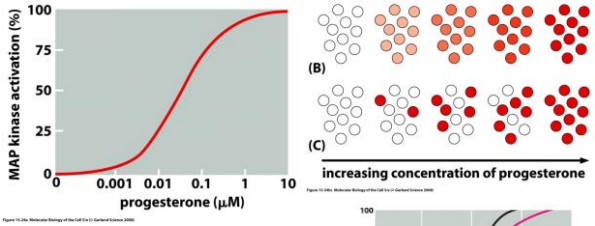


Multiple Mechanisms to Respond to a Gradually Increasing Concentration of an Extracellular Signal

- Smoothly graded vs switch-like signaling responses



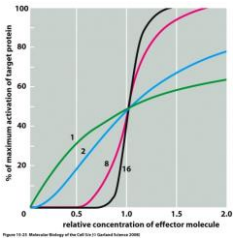
  - discontinuous or all-or-none



increased gradually in each individual cells

all-or-none way

  - Cooperative response



Feedback loops of intracellular signaling network

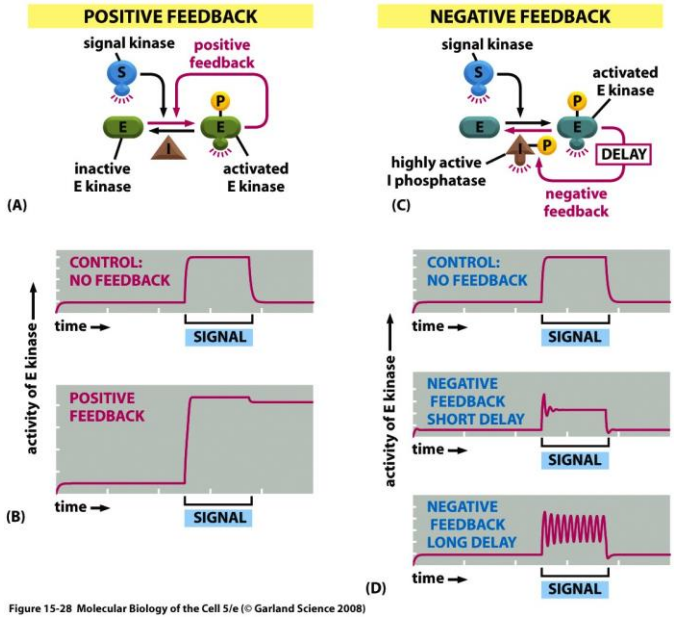


Figure 15-28 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Desensitization of a signal

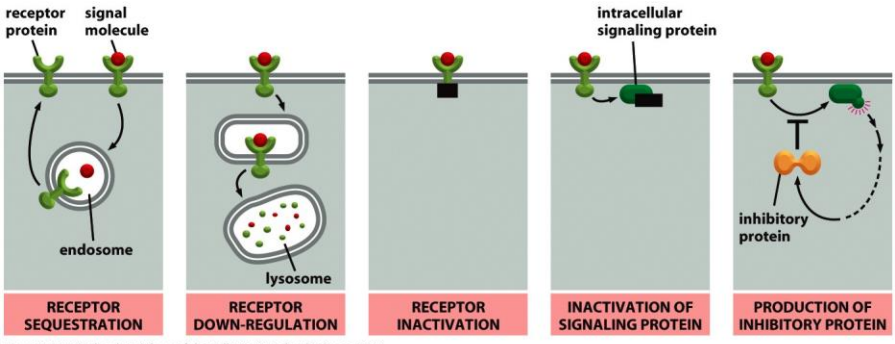
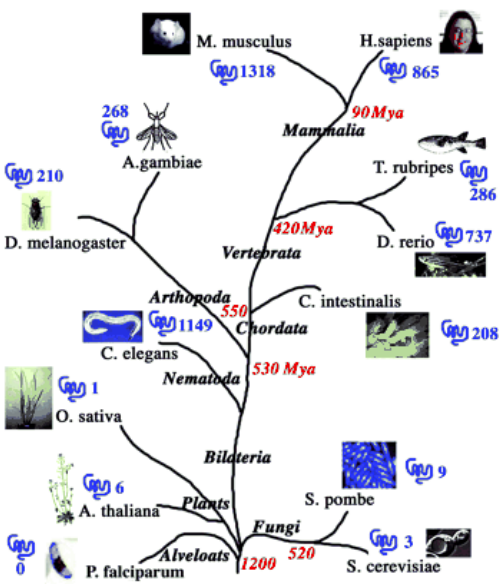
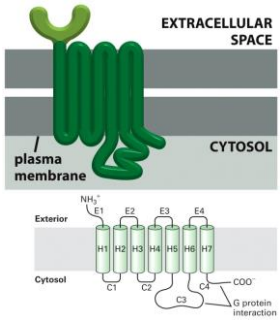


Figure 15-29 Molecular Biology of the Cell 5/e (© Garland Science 2008)

GPCR RECEPTOR

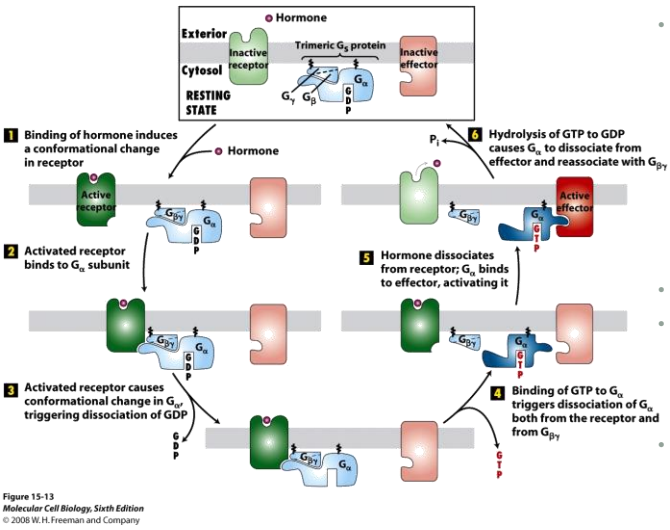




Nobel prize 1994: Alfred G. Gilman and Martin Rodbell for discovery of G-proteins and the role of these proteins in signal transduction in cells

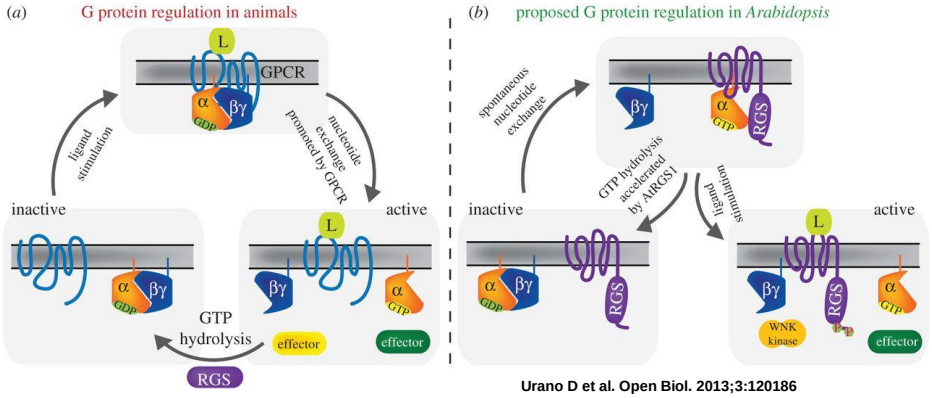
Perez, 2005
Phylogenetic GPCR tree of the different species. The numbers (in red) at the nodes indicate the time in million of years [(millions of years ago (Mya)] since the split at that node occurred [based upon the figure in Fredriksson and Schioth (2005)]. Blue GPCRs and numbers represent the number of GPCRs in the different main classes predicted in the various genomes [data taken from Table 2 in Fredriksson and Schioth (2005)].

Signalling through GPCR



- GPCR ~ GEF
- GPCR-ligand ➔ Activate Trimeric G proteins (3 subunit: α,β,γ)
- In animals: transmit extracellular signals, such as hormones, neurotransmitters, chemokines, lipid mediators, light, tastes and odorants
  in Plants: i.e. ABA-induced stomatal
- movements
- G-α-subunit of G-trimeric protein →bind to a specific regulator of G protein signaling (RGS).
- RGS ~ GAPs

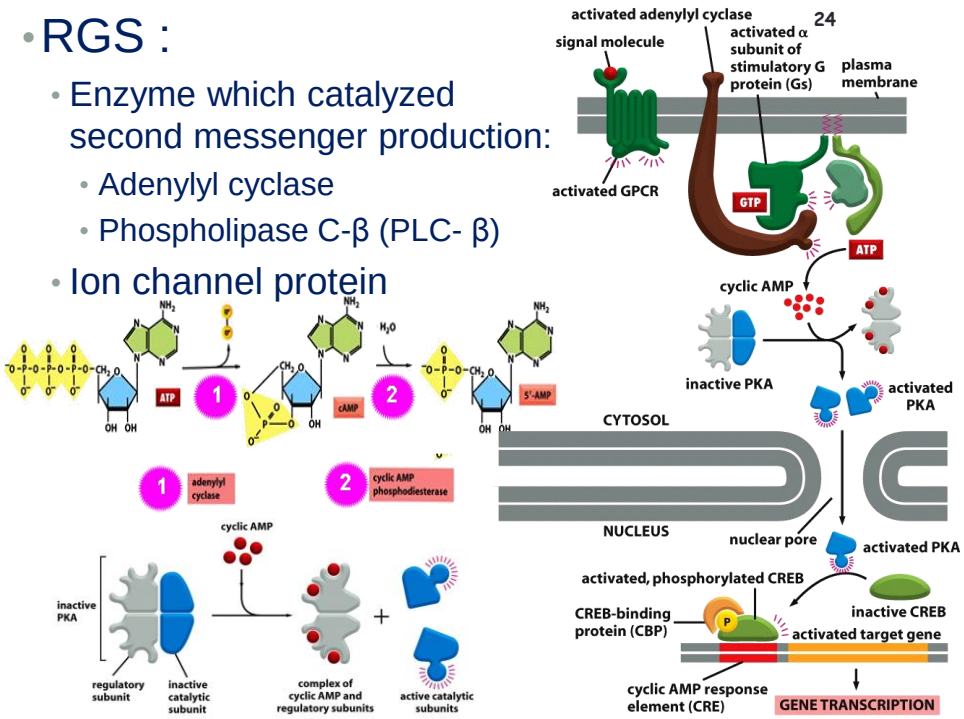
The 'G' cycle of animals versus Arabidopsis.



Urano D et al. Open Biol. 2013;3:120186

The 'G' cycle of animals versus *Arabidopsis*. (*a*) G protein regulation in mammalian cells. In the absence of ligand, G protein forms an inactive heterotrimer with Gβγ dimer (bottom left). Ligand-bound GPCR promotes GDP dissociation and GTP binding on G protein (top). GTP-bound Gα dissociates from Gβγ dimer, and both activated Gα and freely released Gβγ modulate activity of the effectors (bottom right). Gα hydrolyses GTP to GDP, and re-binds to Gβγ to return to its inactive state. (*b*) G protein regulation modelled in *Arabidopsis*. *Arabidopsis* G protein (AtGPA1) can spontaneously dissociate GDP and activate itself (bottom left). AtGPA1 does not hydrolyse its GDP rapidly; however, AtRGS1, a 7TM-RGS protein, promotes the GTP hydrolysis of AtGPA1 (top). d-glucose or other stimuli functions on AtRGS1 directly or indirectly, and decouples AtGPA1 from AtRGS1 (bottom right). Once released from AtRGS1, AtGPA1 does not hydrolyse its GTP efficiently, maintaining its active state and modulating the effector activities.

- RGS :
  - Enzyme which catalyzed second messenger production:
    - Adenylyl cyclase
    - Phospholipase C-β (PLC- β)
  - Ion channel protein



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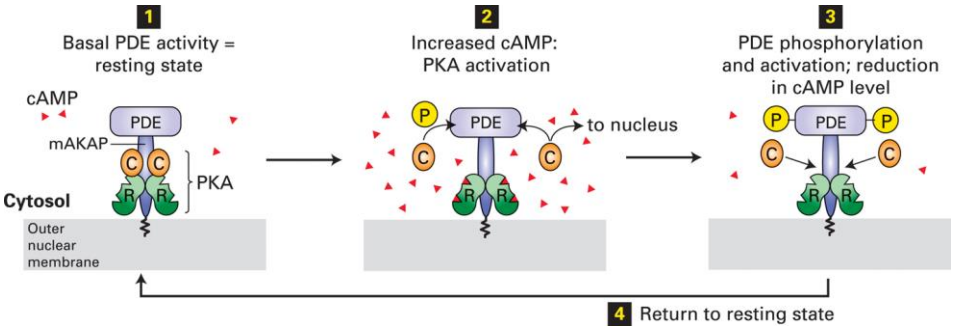
cAMP regulator

- level cAMP level ↑→ good however distract cell function → regulator protein : PDE ➔ localized together with PKA to the nuclear membrane by : A kinase-associated protein : AKAP



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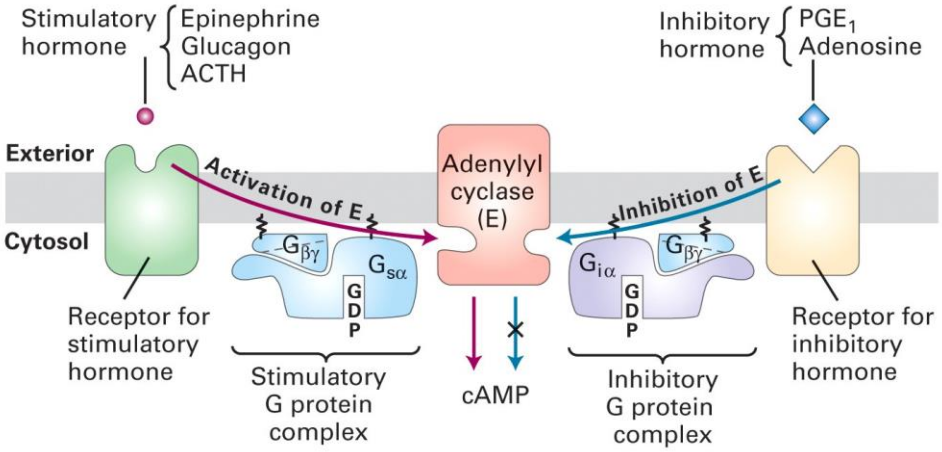
Adenylyl cyclase activation and inhibition



Glycogen breakdown in muscle and liver cells is stimulated by epinefrin through GPCR

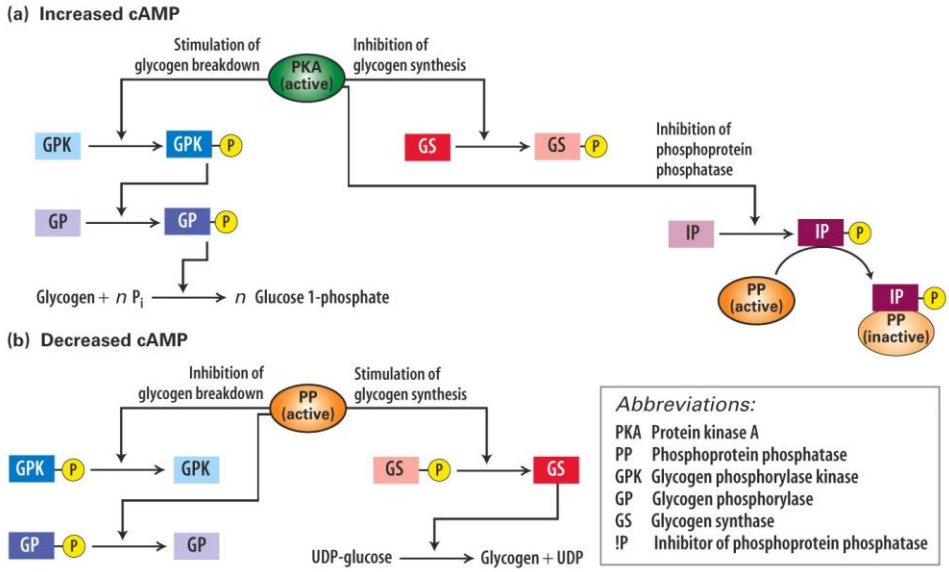


Table 15–1 Some Hormone-induced Cell Responses Mediated by Cyclic AMP

TARGET TISSUE	HORMONE	MAJOR RESPONSE
Thyroid gland	thyroid-stimulating hormone (TSH)	thyroid hormone synthesis and secretion
Adrenal cortex	adrenocorticotrophic hormone (ACTH)	cortisol secretion
Ovary	luteinizing hormone (LH)	progesterone secretion
Muscle	adrenaline	glycogen breakdown
Bone	parathormone	bone resorption
Heart	adrenaline	increase in heart rate and force of contraction
Liver	glucagon	glycogen breakdown
Kidney	vasopressin	water resorption
Fat	adrenaline, ACTH, glucagon, TSH	triglyceride breakdown

Table 15–2 Some Cell Responses in Which GPCRs Activate PLCβ

TARGET TISSUE	SIGNAL MOLECULE	MAJOR RESPONSE
Liver	vasopressin	glycogen breakdown
Pancreas	acetylcholine	amylase secretion
Smooth muscle	acetylcholine	muscle contraction
Blood platelets	thrombin	platelet aggregation

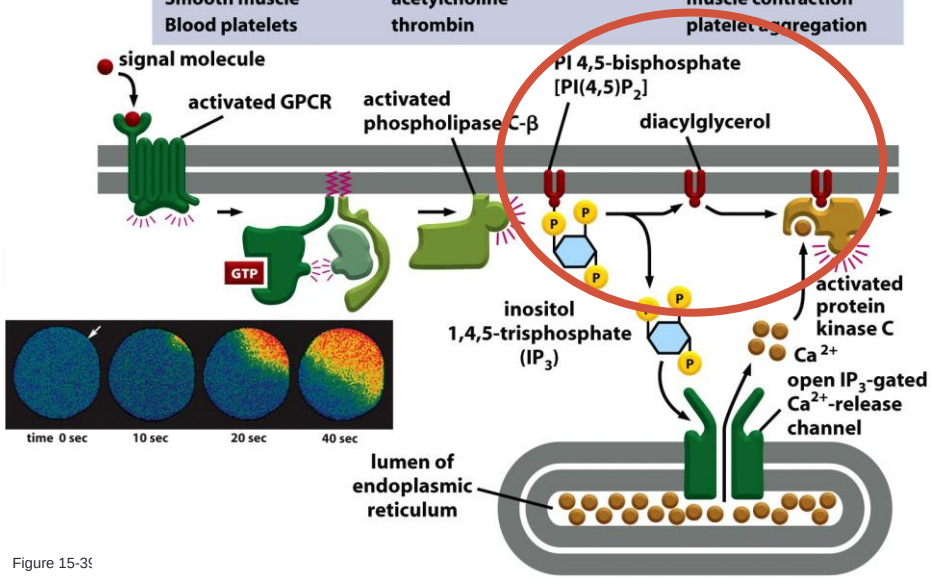
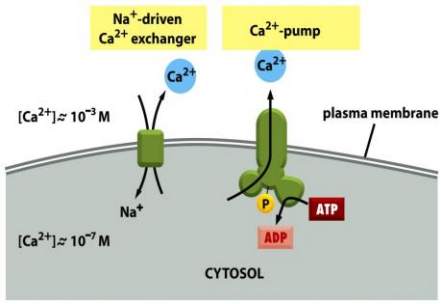
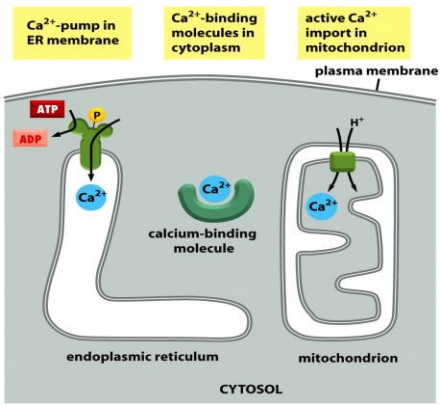


Figure 15-3

Calsium – ubiquitous intracellular mediator

- Ca^{2+}-ion
  - Cytosol : ~10^{-7} M; extracellular: 10^{-3} M
  - sudden rise in Cytosolic Ca^{2+}-ion conc in egg cells
    - initiates embryonic development;
    - Contraction in muscle,
    - Secretion in secretory cells
- Mechanisms to keep lower conc of Ca^{2+}-ion in cytosol
  - Ca^{2+}-pump
  - Antiporter Ca^{2+}/Na+
  - Symporter Ca^{2+}/H+
  - Calsium binding molecules i.e. calmodulin
  -





Ca2+/Calmodulin-Dependent Protein Kinases (CaM-Kinases) Mediate Many of the Responses to Ca2+ Signals in Animal Cells

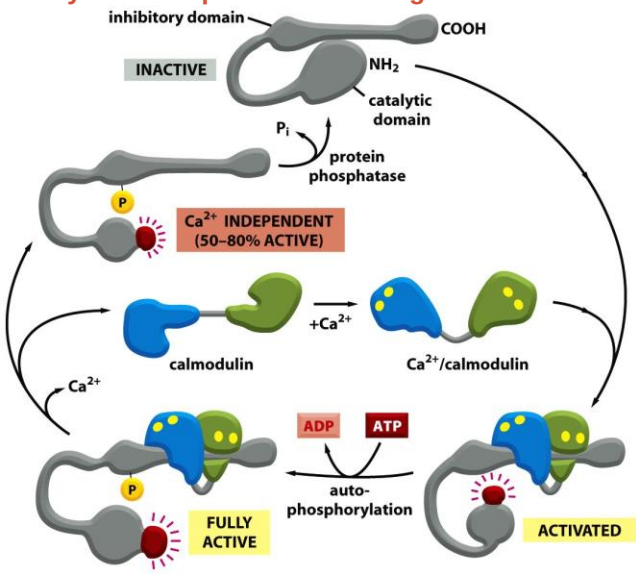


Figure 15-44 *Molecular Biology of the Cell* (© Garland Science 2008)

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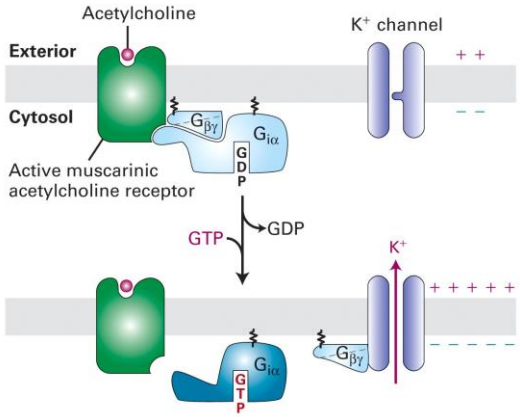
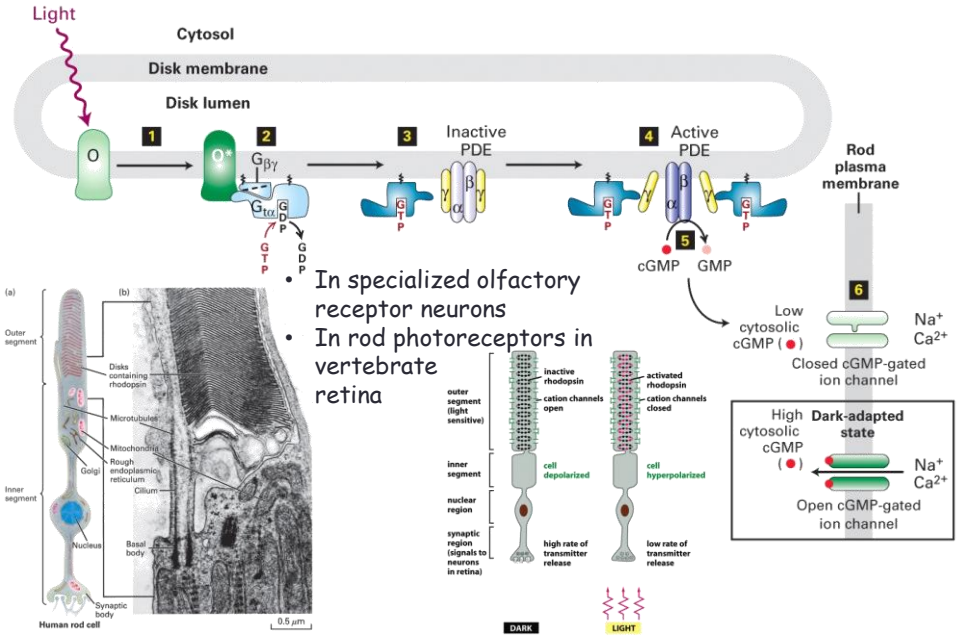
Some GPCR regulate ion channels

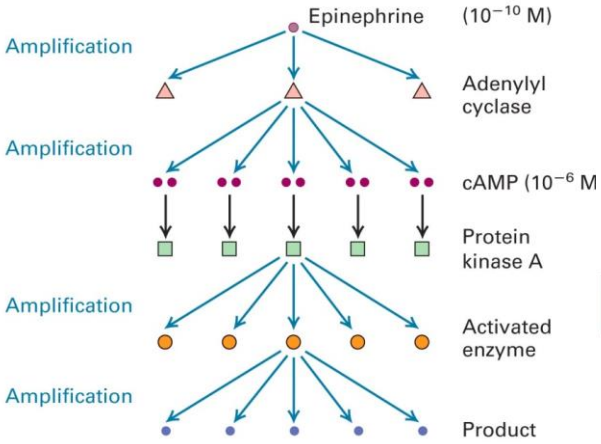
- Glutamate, serotonin and nicotinic acetylcholine receptors ➔ ion channel coupled receptors on skeletal muscle and nerve cells
- GPCR activation ➔ activate/inactivate ion channel → potential membrane

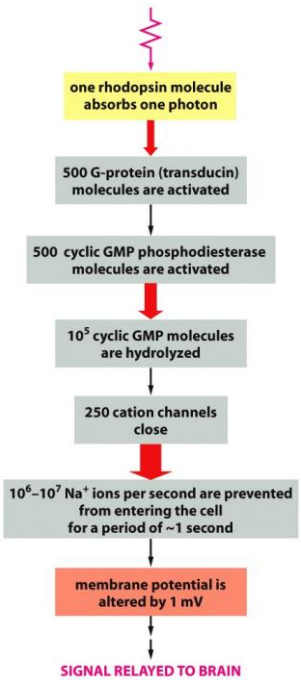


GPCRs regulate cyclic-Nucleotide-Gated Ion Channels



- **Intracellular Mediators and Enzymatic Cascades Amplify Extracellular Signals**





GPCR Desensitization Depends on Receptor Phosphorylation

- three general modes of desensitization
  - *receptor inactivation*
  - *receptor sequestration*,
  - *receptor down-regulation*

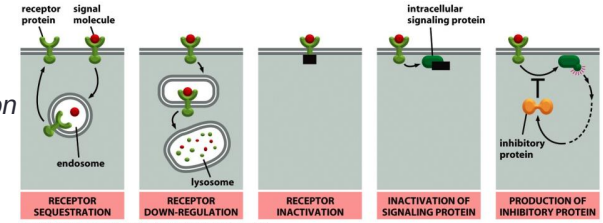
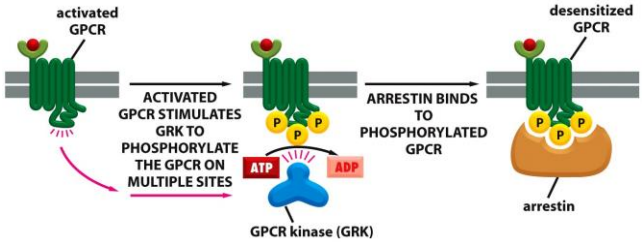


Figure 15-29 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- In each case, the desensitization of the GPCRs depends on their phosphorylation by PKA, PKC, or a member of the family of **GPCR kinases** (**GRKs**)



3 types of Cell-Surface Receptor Proteins

- **Ion-channel-coupled receptors**,
  - *transmitter-gated ion channels* or *ionotropic receptors,*

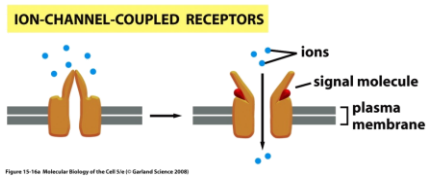


Figure 15-16a Molecular Biology of the Cell 5/e (© Garland Science 2008)

- **G-protein-coupled receptors**
  - act by indirectly regulating the activity of a separate plasma-membrane-bound target protein, which is generally either an enzyme or an ion channel.

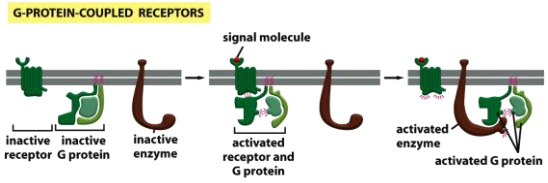


Figure 15-16b Molecular Biology of the Cell 5/e (© Garland Science 2008)

- **Enzyme-coupled receptors**
  - either function directly as enzymes or associate directly with enzymes that they activate

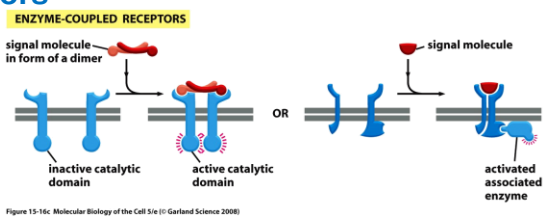


Figure 15-16c Molecular Biology of the Cell 5/e (© Garland Science 2008)

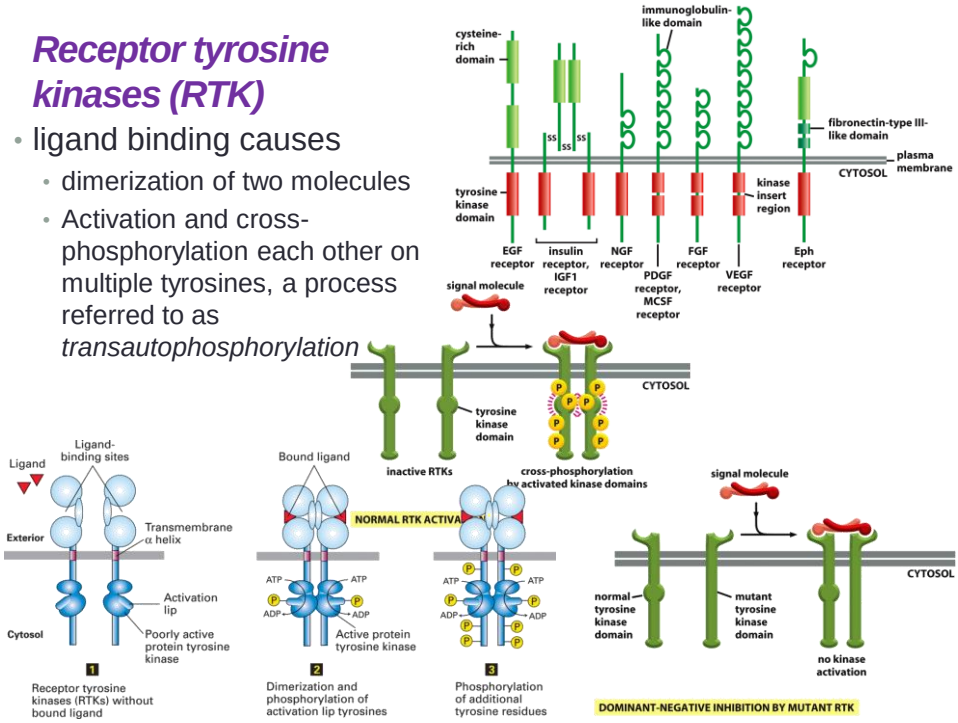
ENZYME-COUPLED RECEPTORS

Six principal classes of enzyme-coupled receptors

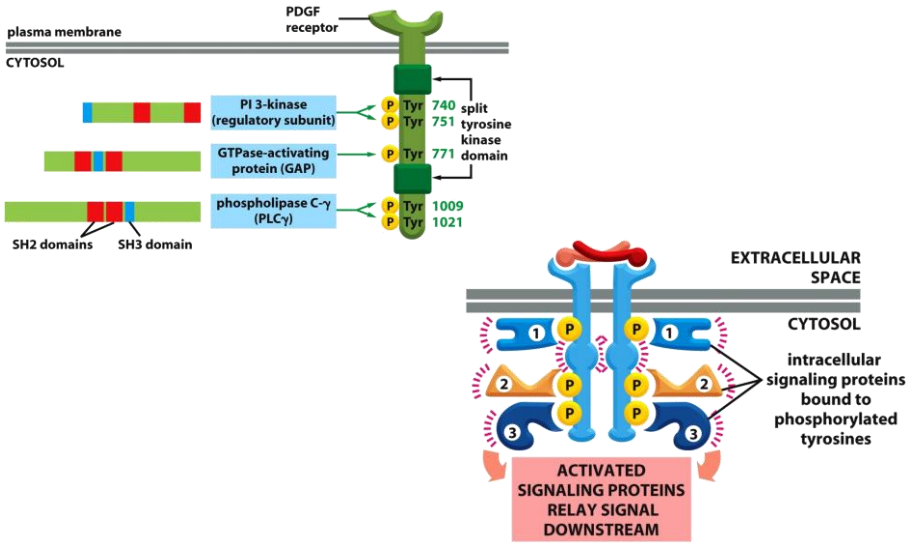
1. ***Receptor tyrosine kinases*** **directly phosphorylate specific tyrosines on themselves and on a small set of intracellular signaling proteins.**
2. ***Tyrosine-kinase-associated receptors*** **have no intrinsic enzyme activity but directly recruit cytoplasmic tyrosine kinases to relay the signal.**
3. ***Receptor serine/threonine kinases*** **directly phosphorylate specific serines or threonines on themselves and on latent gene regulatory proteins with which they are associated.**
4. ***Histidine-kinase-associated receptors*** **activate a two-component signaling pathway in which the kinase phosphorylates itself on histidine and then immediately transfers the phosphoryl group to a second intracellular signaling protein.**
5. ***Receptor guanylyl cyclases*** **directly catalyze the production of cyclic GMP in the cytosol, which acts as a small intracellular mediator in much the same way as cyclic AMP.**
6. ***Receptorlike tyrosine phosphatases*** **remove phosphate groups from tyrosines of specific intracellular signaling proteins.**

Receptor tyrosine kinases (RTK)

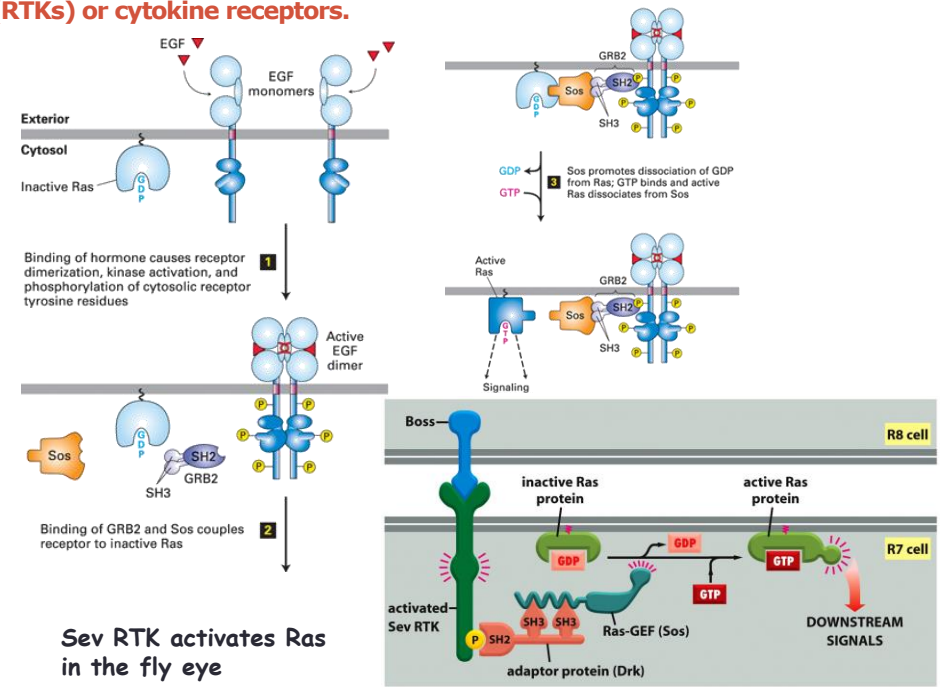
- ligand binding causes
  - dimerization of two molecules
  - Activation and cross-phosphorylation each other on multiple tyrosines, a process referred to as *transautophosphorylation*



Proteins with SH2 Domains Bind to Phosphorylated Tyrosines



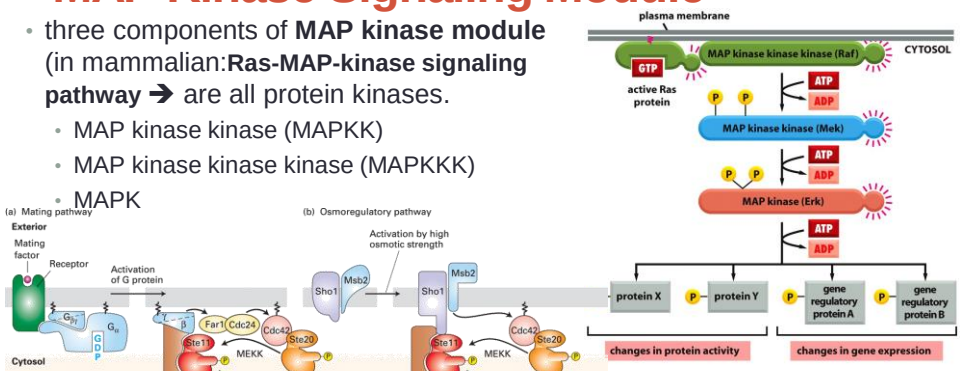
Activation of Ras following ligand binding to receptor tyrosine kinases (RTKs) or cytokine receptors.



Sev RTK activates Ras in the fly eye

MAP Kinase Signaling Module

- three components of **MAP kinase module** (in mammalian:**Ras-MAP-kinase signaling pathway** ➔ are all protein kinases.
  - MAP kinase kinase (MAPKK)
  - MAP kinase kinase kinase (MAPKKK)
  - MAPK



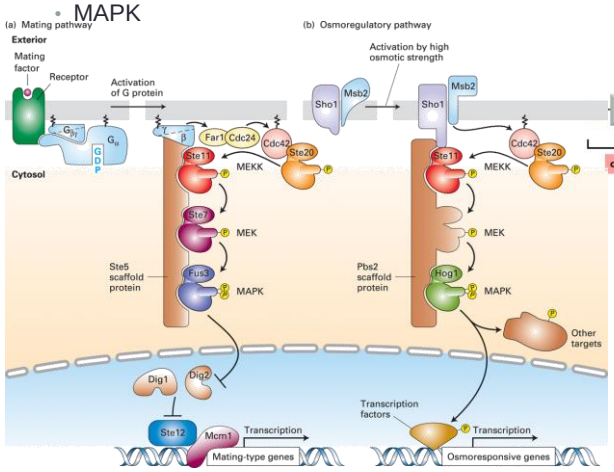
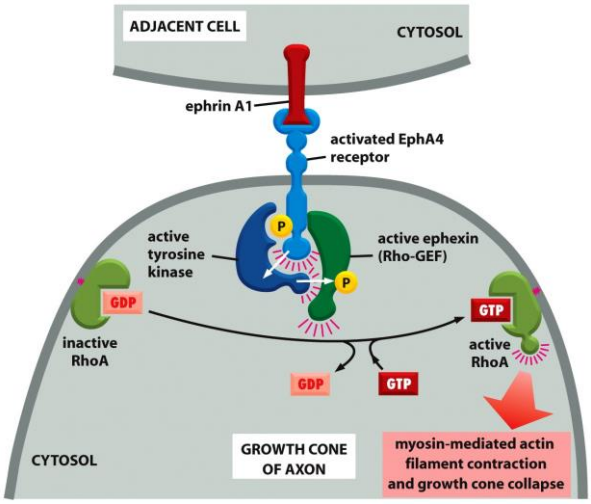


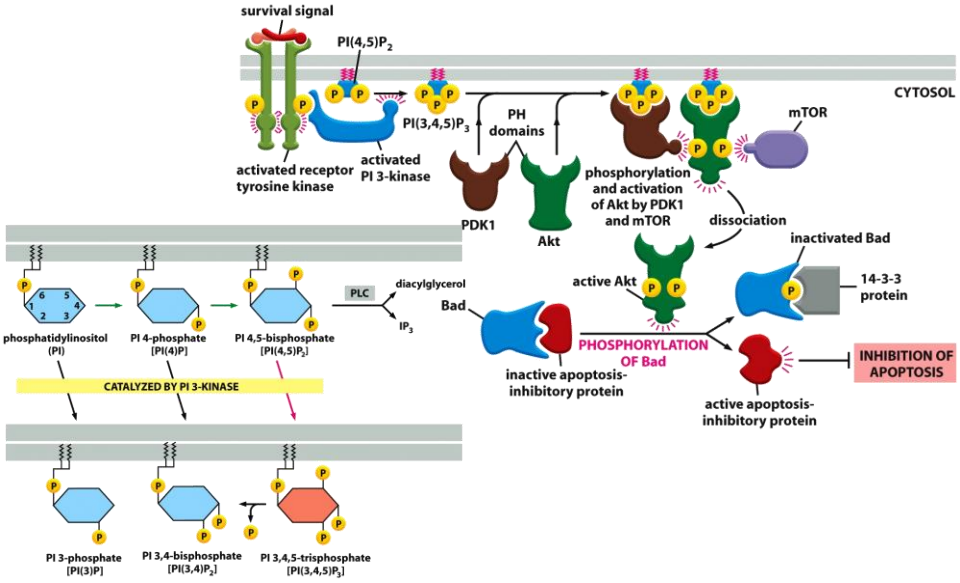
Figure 15-60 *Molecular Biology of the Cell* (© Garland Science 2008)

Scaffold Proteins Help Prevent Cross-Talk Between Parallel MAP Kinase Modules

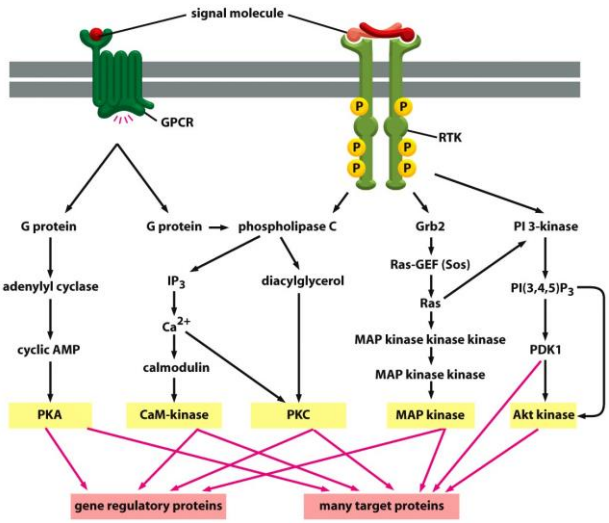
- **Rho Family GTPases Functionally Couple Cell-Surface Receptors to the Cytoskeleton**



PI 3-Kinase Produces Lipid Docking Sites in the Plasma Membrane

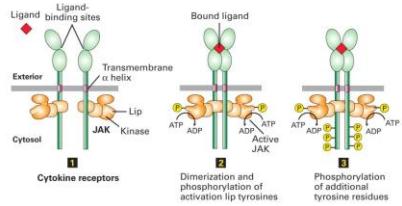


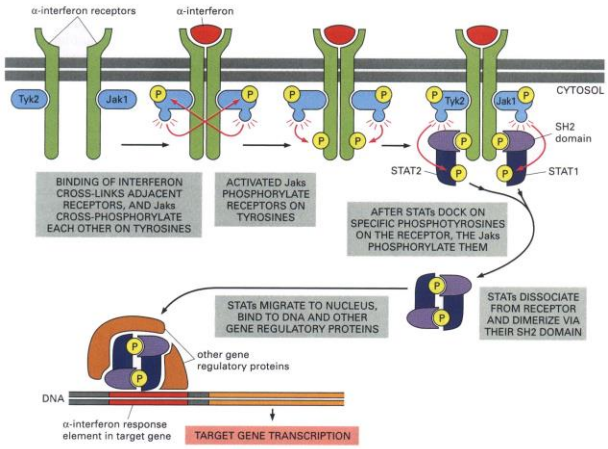
parallel intracellular signaling pathways activated by GPCRs & RTKs

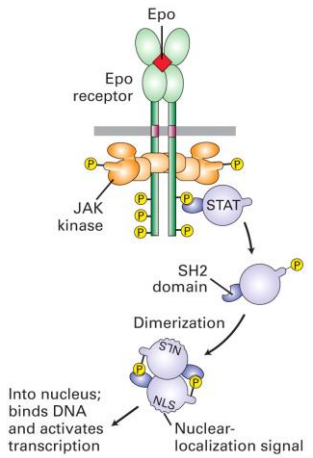


Other signaling pathway: JAK-STAT

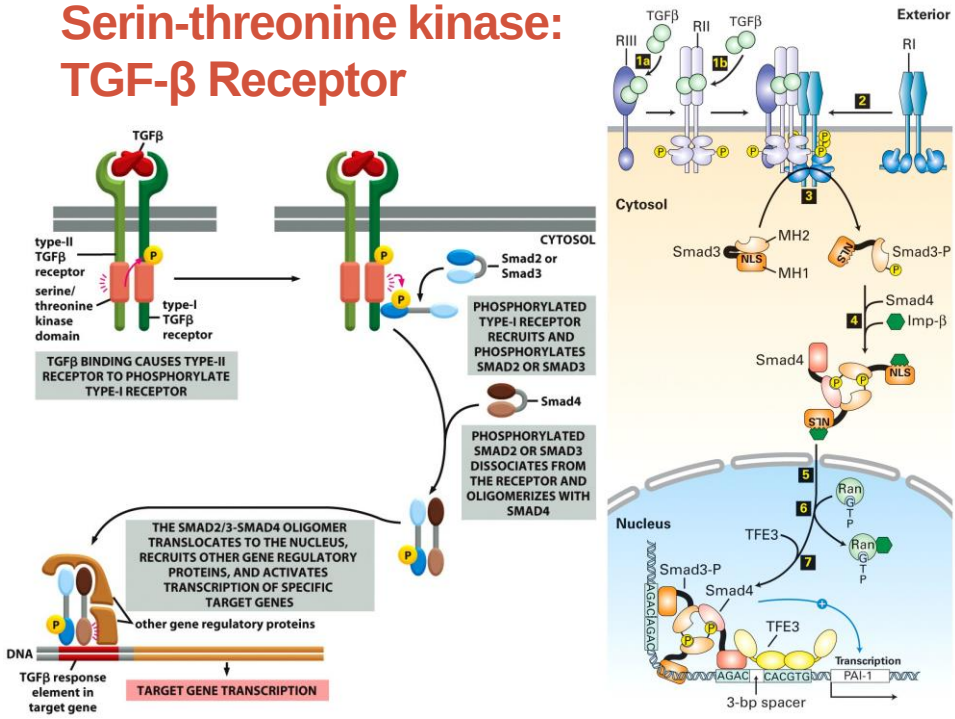
- cytoplasmic tyrosin kinase protein: Janus kinase
- interferon, prolactin receptors





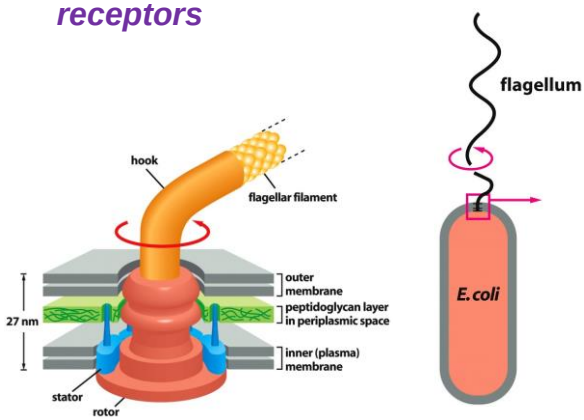


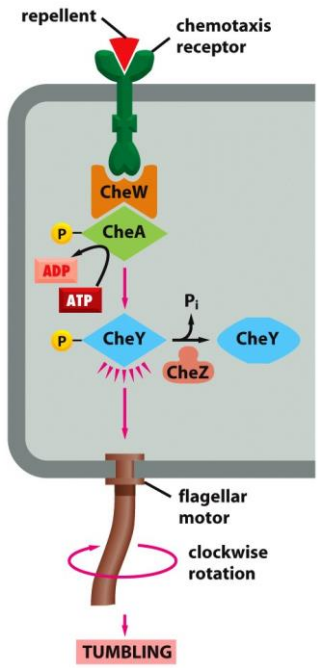
Serin-threonine kinase: TGF-β Receptor



Histidine-kinase-associated receptors

- i..e Bcterial chemotaxis is mediated by ***Histidine-kinase-associated receptors***



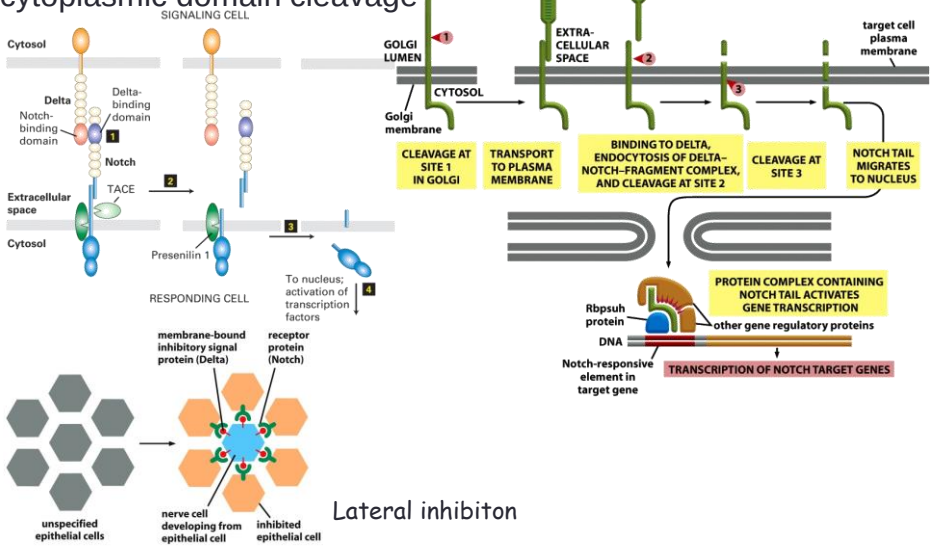


Other signaling pathways: SIGNALING PATHWAYS DEPENDENT ON REGULATED PROTEOLYSIS OF LATENT GENE REGULATORY PROTEINS

- signaling pathway that:
  - Mediated by Receptor protein Notch
  - Activate Wnt
  - Activate Hedgehog
  - Activate NFkB

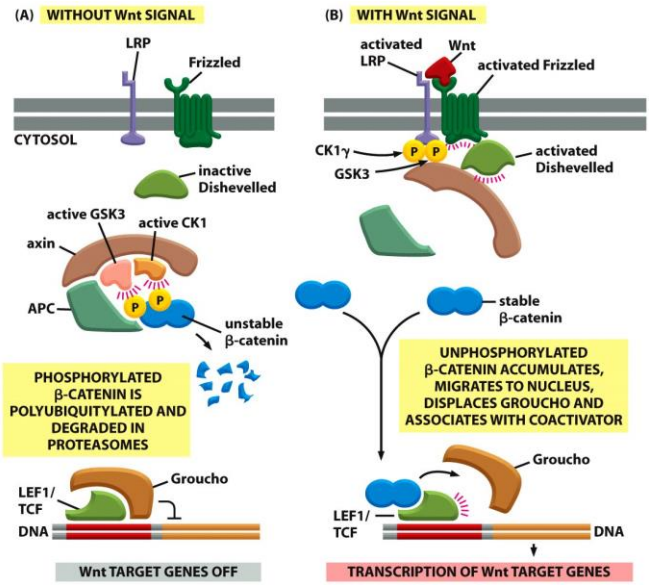
Notch receptor

- Receptor activation ➔ cytoplasmic domain cleavage



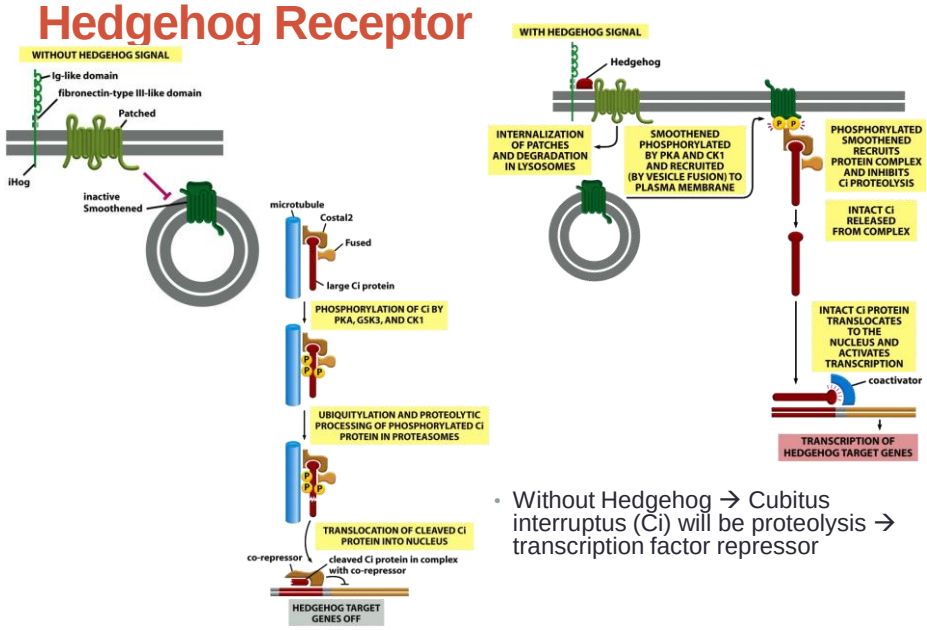
Lateral inhibiton

Wnt receptor



- Bind to frizzled receptor and inhibit b-catenin degradation
- Wnts activate at least three types of intracellular signaling pathways:
- (1) The *Wnt*/b-*catenin pathway* (also known as the *canonical Wnt pathway*) is centered on the latent gene regulatory protein b-*catenin*.
- (2) The *planar polarity*
- *pathway* coordinates the polarization of cells in the plane of a developing epithelium and depends on Rho family GTPases.
- (3) The Wnt/Ca2+ pathway stimulates an increase of intracellular Ca2+, with consequences of the sort we described earlier for other pathways.

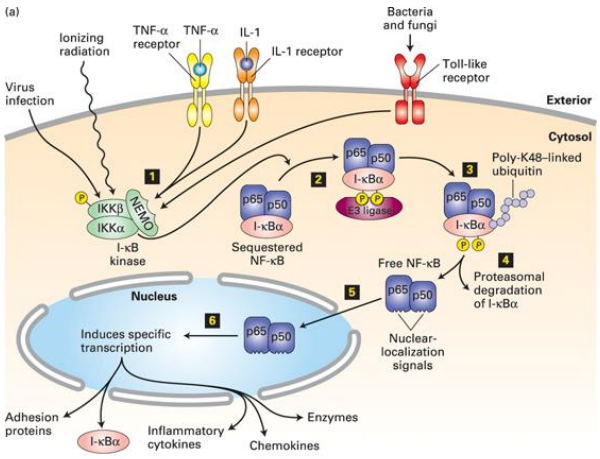
Hedgehog Receptor



- Without Hedgehog → Cubitus interruptus (Ci) will be proteolysis → transcription factor repressor

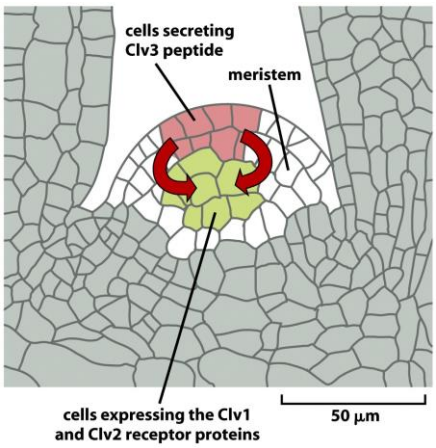
NFkB pathway

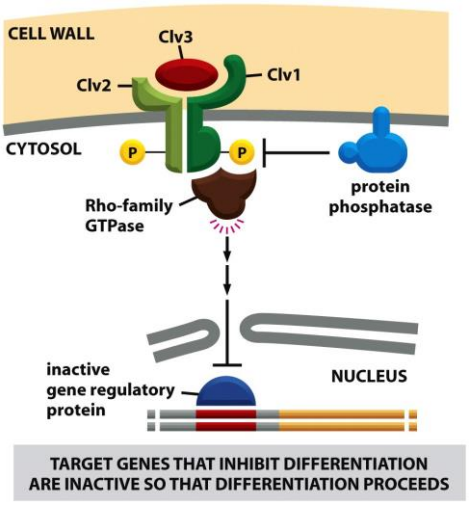
- Jalur yang bergantung NF-κB
- Respon inflamasi, dan dalam proses perkembangan
- Ligan TNF-α & IL-1 berikatan dengan reseptor → aktivasi NF-κB (IκB terdegradasi)
- NF-κB inaktif terikat IκB



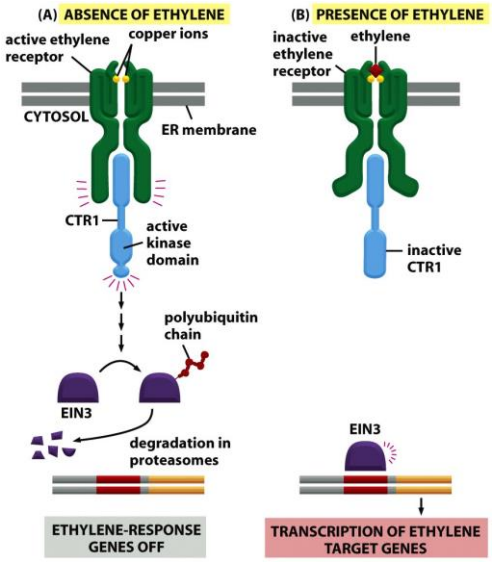
Signaling pada tumbuhan

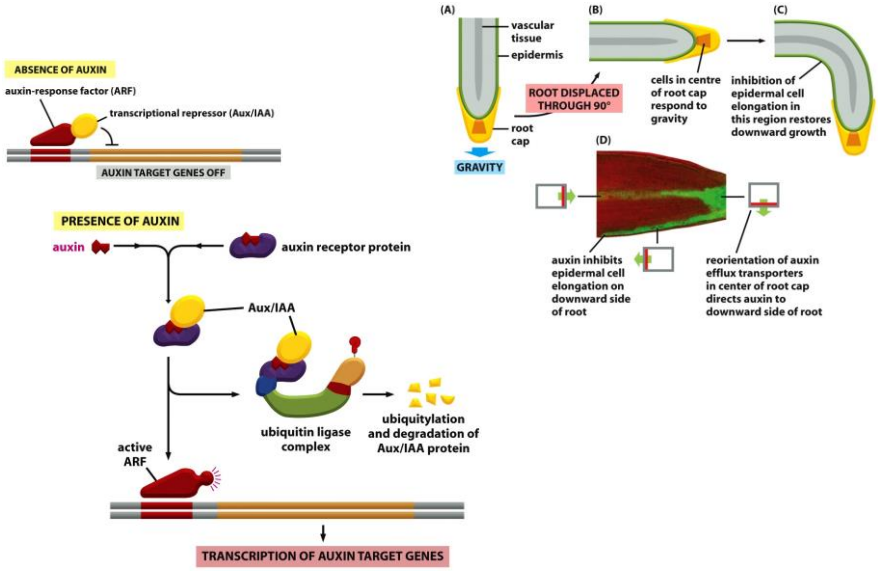
- Reseptor serin threonin kinase
  - Memiliki tandem leucine-rich repeats (LRR) pada domain ekstrasel dari rese





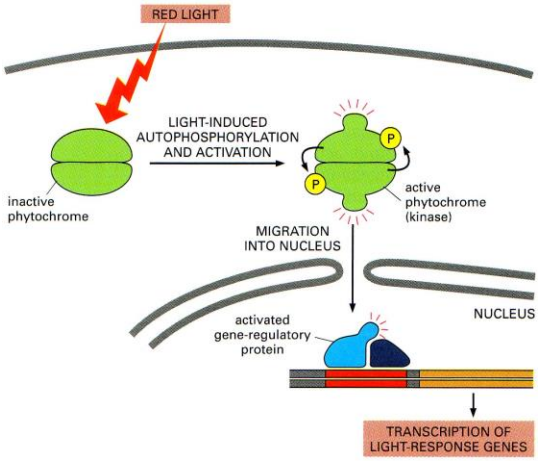
- **Reseptor histidin kinase**
  - Reseptor untuk etilen





Fotoprotein

- Fitokrom
  - Dimer, berada dalam sitoplasma
  - Serin threonin kinase
  - Responsif terhadap cahaya merah dan far-red
  - Diaktivasi oleh cahaya merah → fosforilasi sendiri → aktivasi protein lain



- Fotoprotein yang responsif terhadap cahaya biru
  - Fototrofin
    - Terikat membran
    - Berperan dalam fototropisme
  - Kriptokrom
    - Flavoprotein
    - Menyerupai enzim sensitif cahaya biru, foliasis (→ perbaikan kerusakan DNA yang terkena UV pada organisma kecuali mamalia)
    - Kriptokrom tidak berperan dalam perbaian kerusakan DNA