BME 42-620 Engineering Molecular Cell Biology

Lecture 21:

Cell Signaling (II)

Chapter 15

Final Exam Papers

1) R. Delanoue & I. Davis, Dynein anchors its mRNA cargo after apical transport in the Drosophila blastoderm embryo, *Cell*, 122:97-106, 2005.

2) D. Levy & R. Heald, Nuclear size is regulated by importin α and Ntf2 in Xenopus, *Cell*, 143:288, 2010.

3) S. Ally, A. G. Larson, et al, Opposite-polarity motors activate one another to trigger cargo transport in live cells, *Journal of Cell Biology*, 187:1071-1082, 2009.

4) Y. Shimamoto, Y. T. Maeda, et al, Insights into the micromechanical properties of the metaphase spindle, *Cell*, 145:1062-1074, 2011.

5) C. A. Wilson, M. A. Tsuchida, et al, Myosin II contributes to cell-scale actin network treadmilling through network disassembly, *Nature*, 465:373-377, 2010.

6) A. Levskaya, O. D. Weiner, W. A. Lim, C. A. Voigt, Spatiotemporal control of cell signaling using a light-switchable protein interaction, *Nature*, 461:997-1001, 2009.

Final Exam Time & Location

- Available final exam dates
  - Dec. 9, 11 (morning)
  - Dec. 14, 15, 16
  - Dec. 10 may be possible

- Location
  - Mellon Institute 411 (in the former Lane Center)
  - Other locations possible

Final Exam Presentation Format (I)

- Each presentation should include three sections
  - Background
  - Data presentation
  - Critical review

- Time allocation

  - Background section: approximately 15 minutes
  - Data presentation: ~45-60 minutes
  - Critical review section: approximately 10 minutes

Final Exam Presentation Format (II)

- Organization

  - For each group, approximately one student → one section

  - Background section should be brief;
    Give details but be selective

  - Data presentation should include a slide summarizing main messages
    All figures in the main text must be covered

  - Critical review can accompany data presentation

  - Review section may include
    Whether the data and methods are sound
    Whether the logic development is sound
    Limitations, white space
    Writing style

Final Exam Presentation Format (III)

- Each presentation will be graded based on

  - Accuracy, clarity, logic, & completeness of presentation of all sections

  - Quality of slides (as the final report); Give proper citations

- For each group, the presentation PPT file will serve as the final group report.

- Each student should turn in a two-page report following the standard instructions of reading assignments.

Outline

- Overview of cell signaling
- Classification of signaling related proteins
- Receptors
- Signaling protein transducers
- Second messengers

- Overview of cell signaling
- Classification of signaling related proteins
- Receptors
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- Second messengers

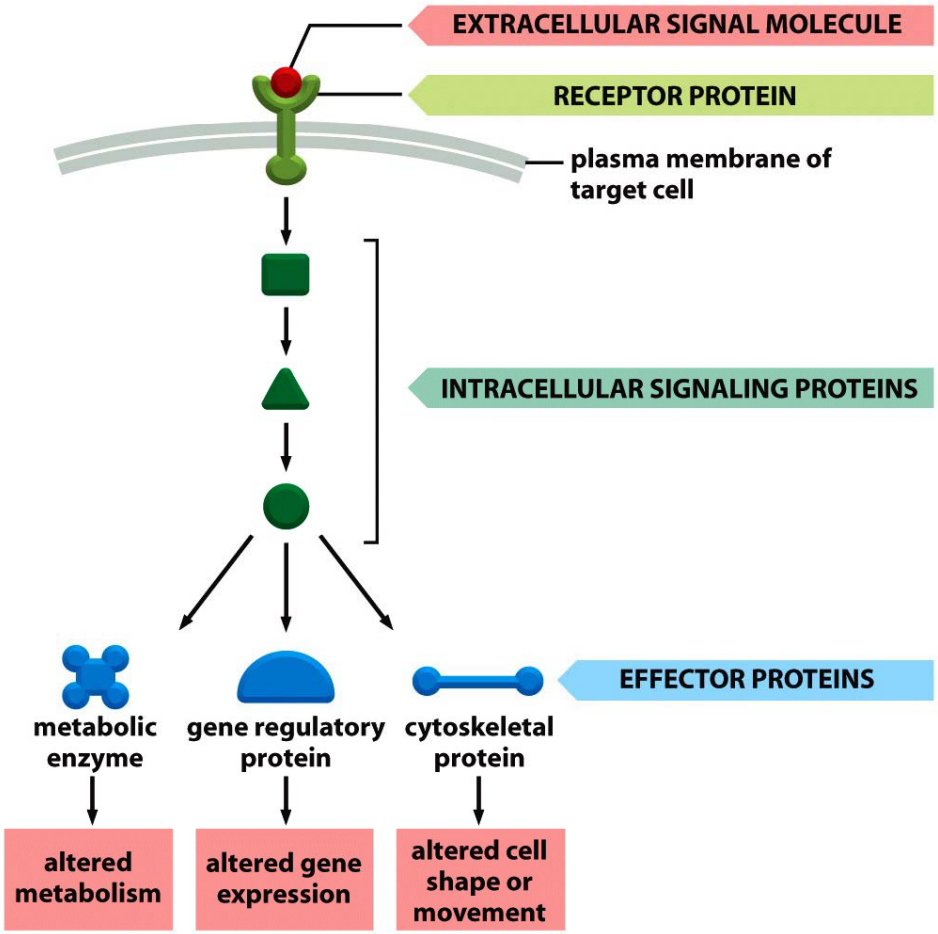
Overview of Cell Signaling

- Sources of extracellular signal
  - Non-cellular environment
  - Cellular environment (cell-cell communication)
  - Hundreds of types of signals

- Cells signaling
  - Stimulus sensing; communication
  - Information processing; decision making

- ↓Receptors
  ↓Signal transducers
  ↓Effector proteins

- Signaling pathways regulate nearly all cellular functions.



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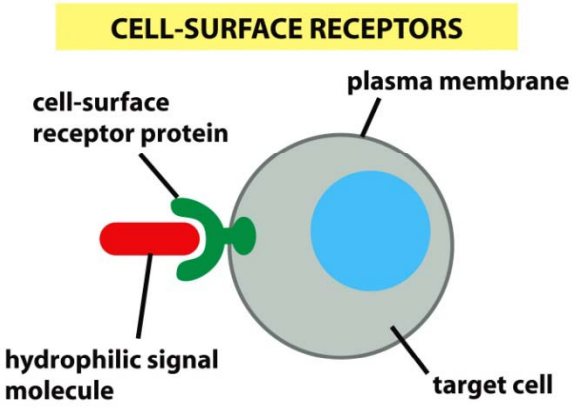
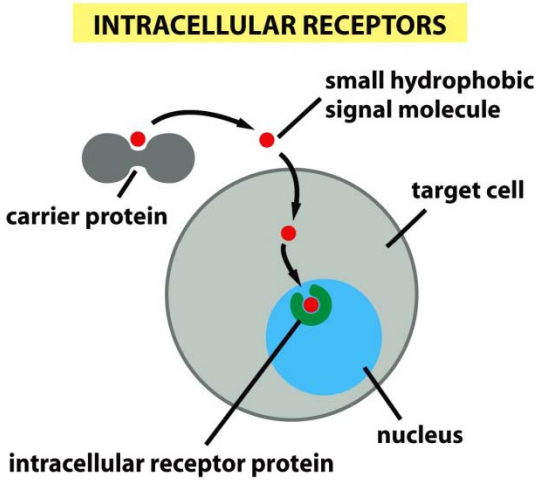
Membrane & Intracellular Receptors

- Receptors bind signaling molecules (ligands)
- Receptors are highly sensitive and specific.
  - Typical signal molecule concentration $<10^{-8}$ M
  - More than 1500 human genes encode receptors
- Most receptors are at the cell surface.
- Some receptors are intracellular (e.g. light, gas receptors).





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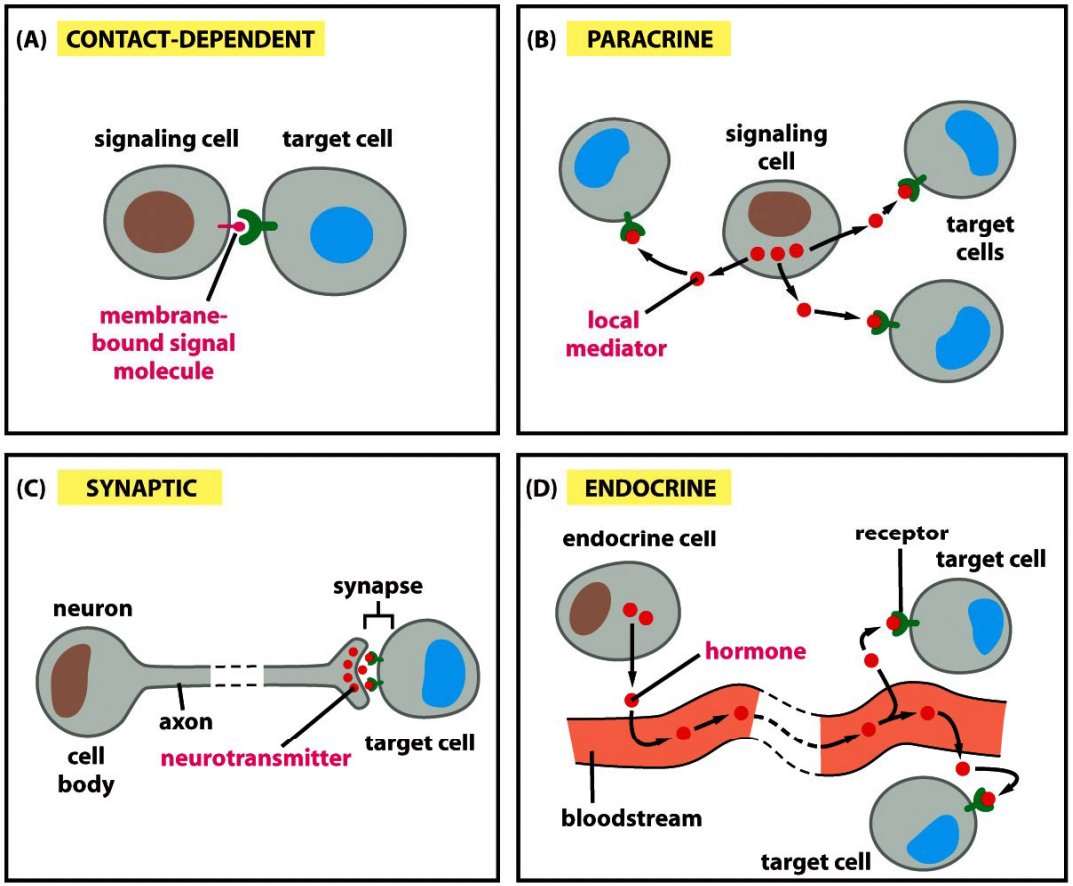
General Principles of Signaling (I)

- Four forms of intercellular signaling
- Paracrine signaling acts locally over different types of cells.
- Autocrine signaling acts locally over the same types of cells including themselves.
- Endocrine signaling acts over long distance.



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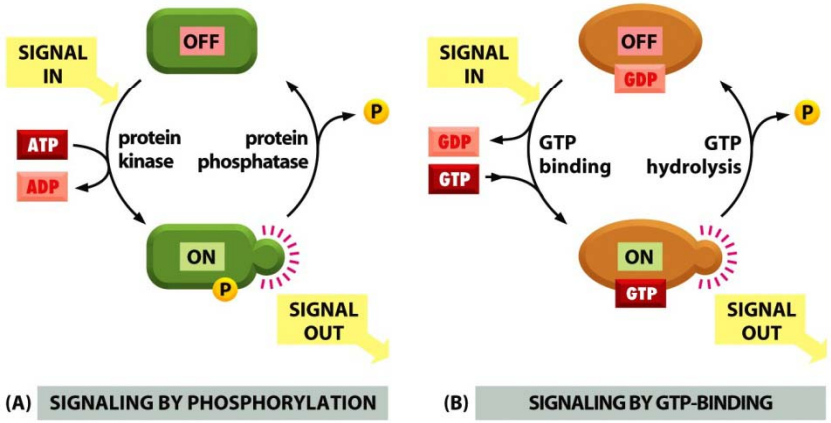
General Principles of Signaling (II)

- Many signaling proteins act as molecular switches
- Two ways to activate/deactivate signaling proteins
- Human genomes encodes ~520 kinases and ~150 phosphatases
- Two main types of kinases
  - tyrosine kinase
  - serine/threonine kinase
- Two types of GTP-binding proteins
  - Trimeric G proteins
  - Monomeric GTPases



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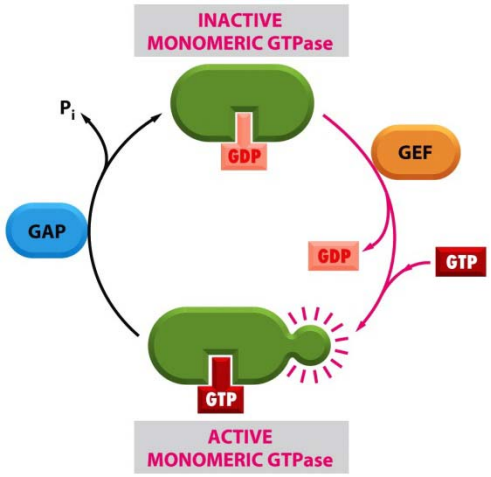
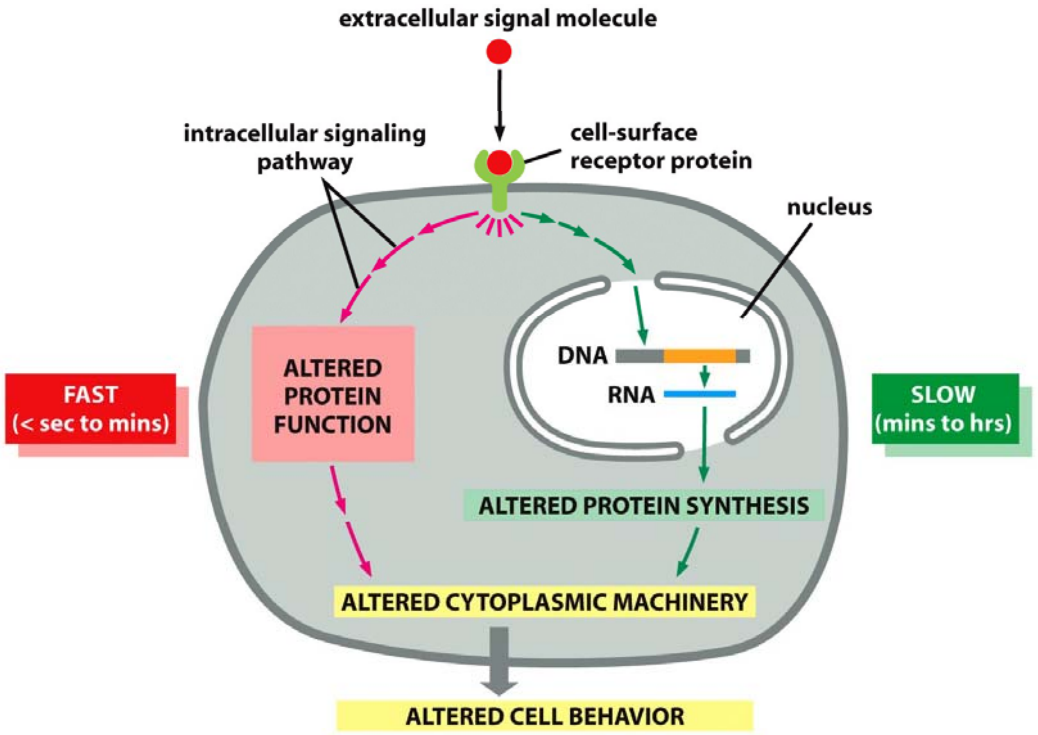


General Principles of Signaling (III)

- Different pathways have different rates of response.
- Pathways involve gene expression regulation are usually slow.



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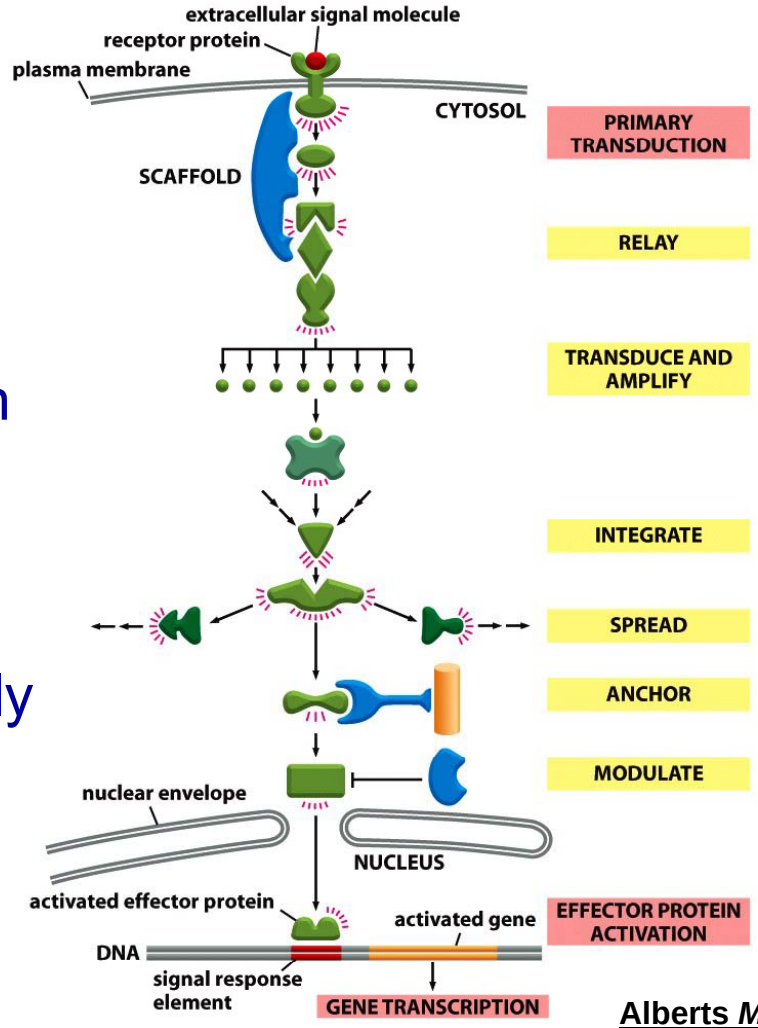
General Principles of Signaling (IV)

- Cascade of signaling events
  - ↓Receptors
  - ↓Signal transducers
  - ↓Effector proteins
- Relay, integration, and distribution of signals require transducers.
- Signaling pathways regulate nearly all cellular functions.



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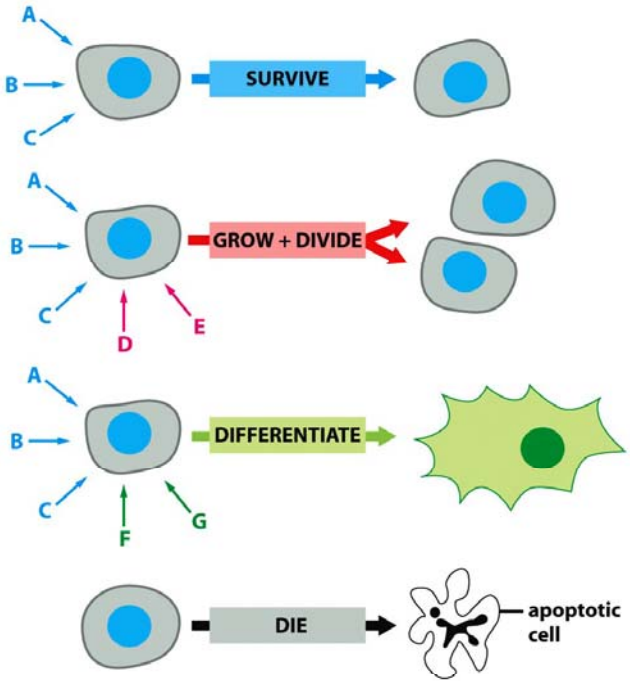
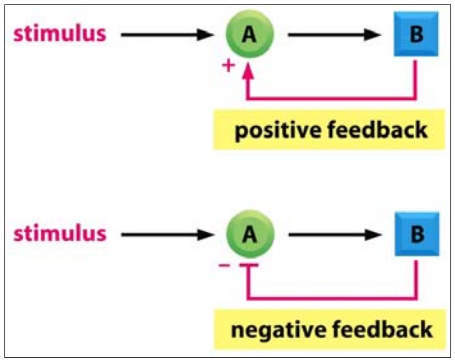
Specific Reponses of Cells to Signaling

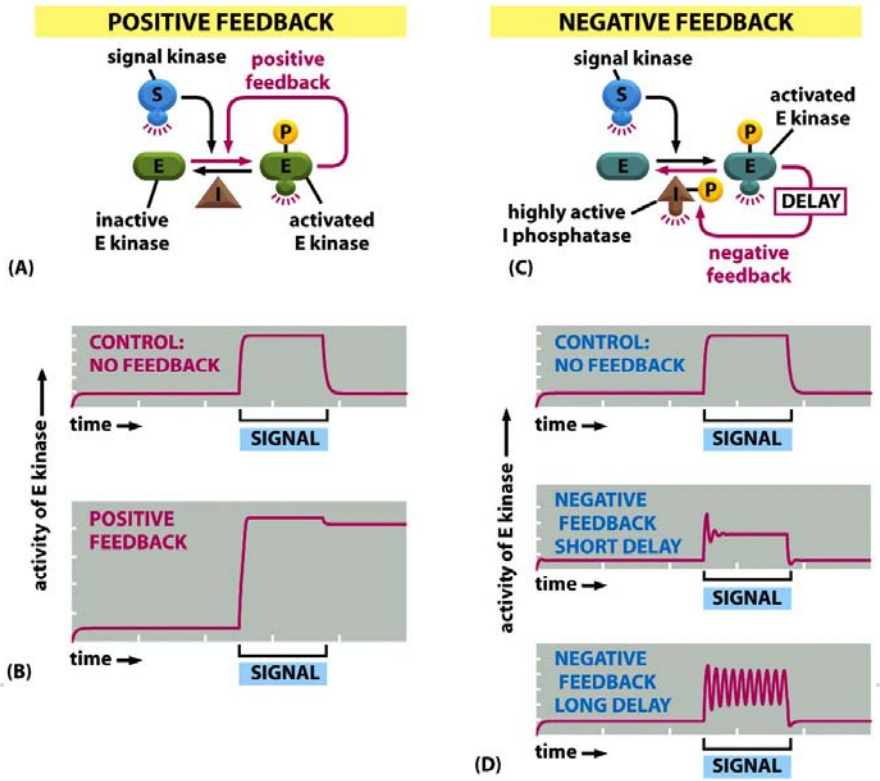
- A cell in a multicellular organism may be exposed to hundreds of signals.
- Different types of cells respond differently to the same type of signals.
- A major challenge is to understand how the cells process such information and make decisions.



Feedback Loops in Signaling Networks

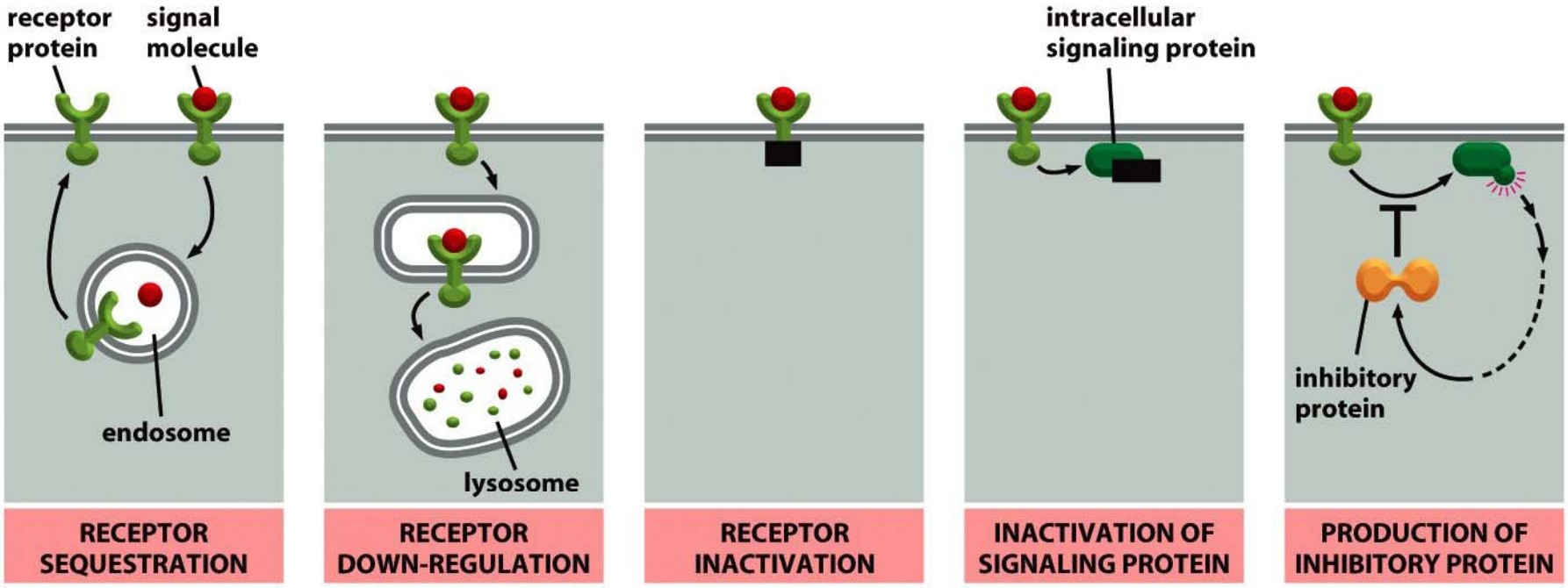
- Two types of feedback loops
  - Positive feedback
  - Negative feedback
- Positive feedback loop
  - Bistability
- Negative feedback loop
  - Robustness to noise





Adaptation of Sensitivity to Signaling

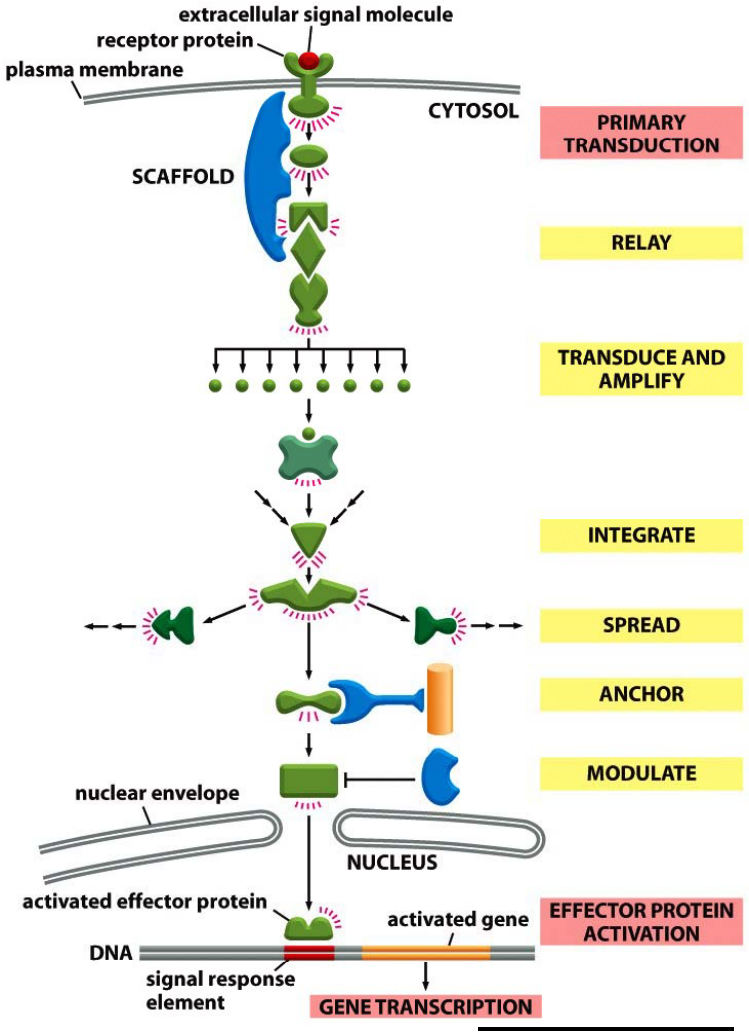
- Cells can adapt to external stimuli through sensitivity adjustment.



- Overview of cell signaling
- **Classification of signaling related proteins**
- Receptors
- Signaling protein transducers
- Second messengers

Overview of Cell Signaling

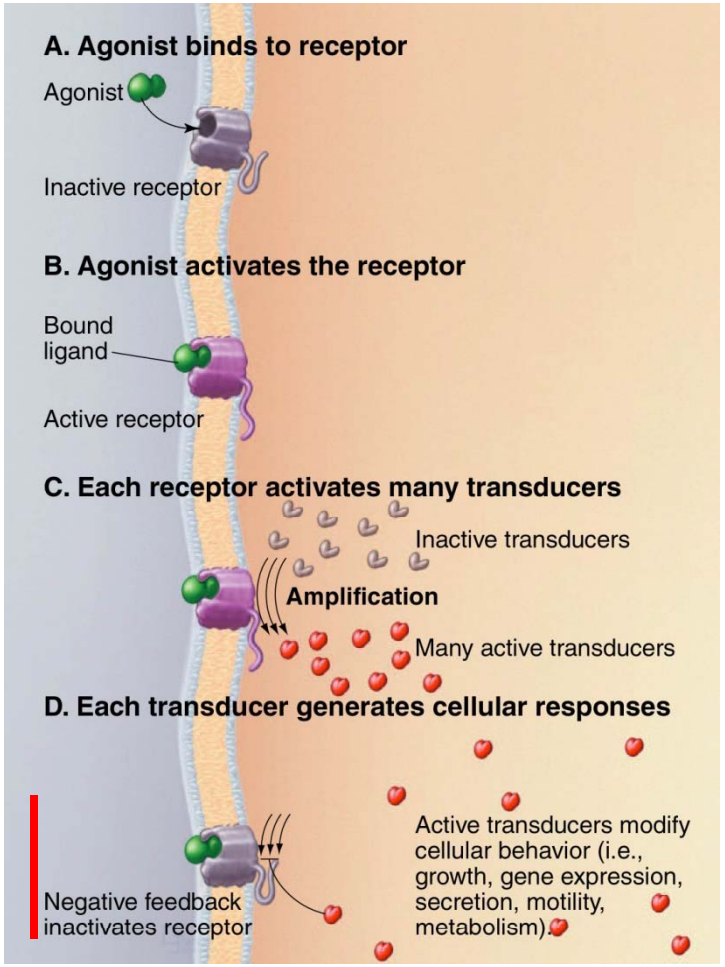
- Cascade of signaling events
  - ↓Receptors
  - ↓Signal transducers
  - ↓Effector proteins
- Relay, integration, and distribution of signals require transducers.
- Signaling pathways regulate nearly all cellular functions.



Transducers in Signaling

- Signaling proteins
  - Kinases
  - Phosphatases
  - GTPases
  - Adapters

- Second messengers
  - cAMP, cGMP
  - Lipids
  - Calcium
  - NO (nitrogen monoxide)



- Overview of cell signaling
- Classification of signaling related proteins
- Receptors
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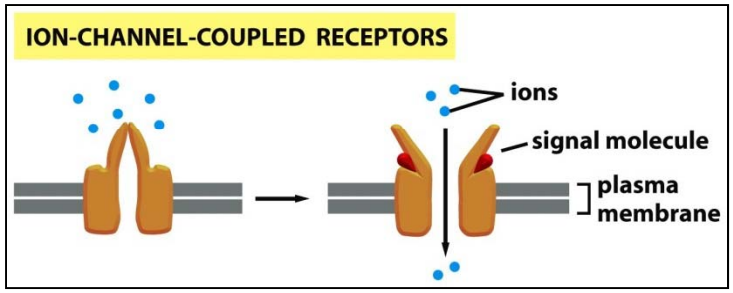
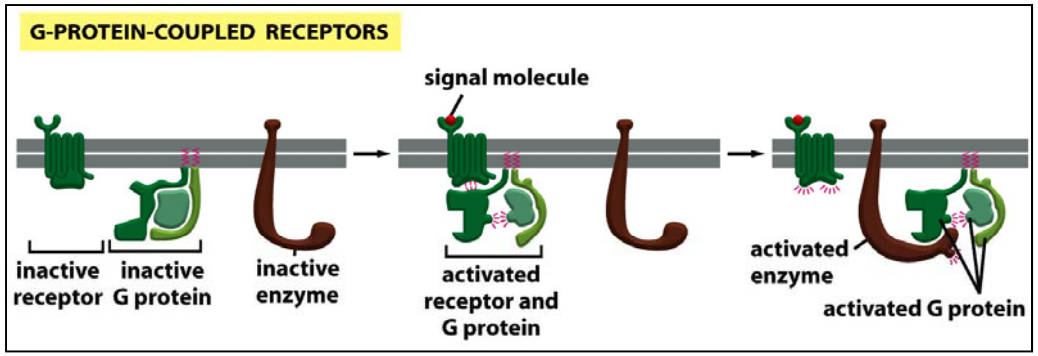
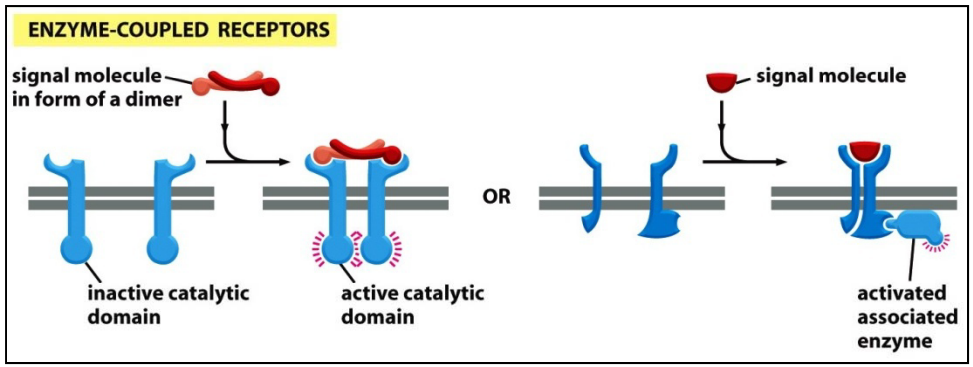
Membrane Receptors

- Most extracellular signal molecules bind to specific membrane receptors.
- Three largest classes of receptors, defining three transduction mechanisms.
- Two common strategies used to transfer signals
  - conformation changes
  - clustering

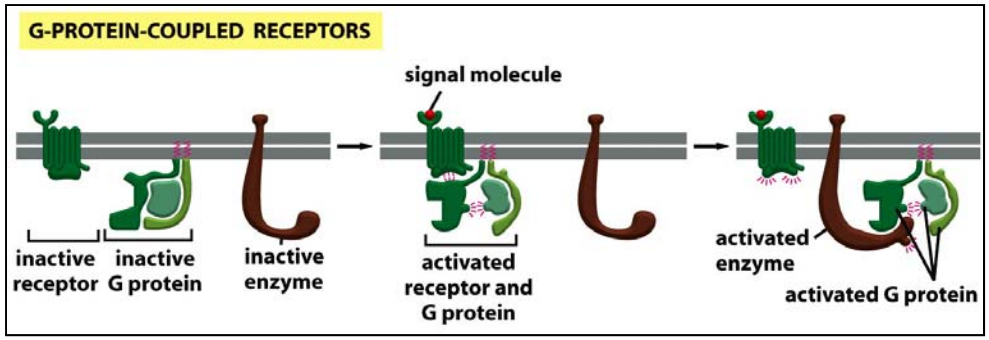




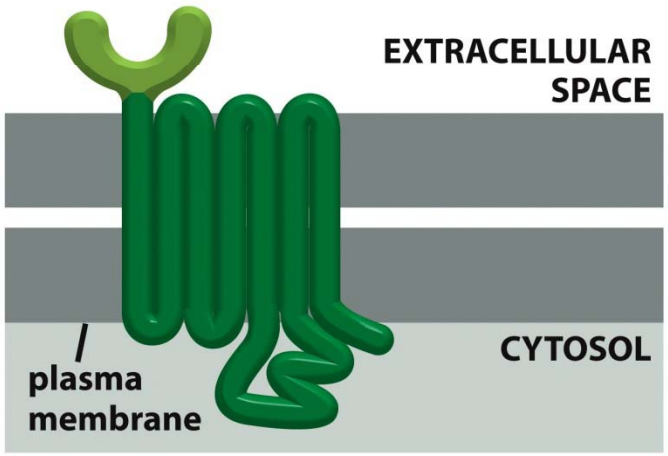


G-Protein Coupled Receptors (I)

- Signal molecules of GPCR include
  - photons
  - molecules of taste and smell
  - hormones, neurotransmitters, …
  - proteins, small peptides, etc…



- Function
  - Nearly all human senses: sight, smell, taste
  - Behavior and mood regulation
  - Regulation of immune system and inflammation
  - Nervous system regulation



- Half of known drugs work through GPCR directly or indirectly

Different Trimeric G-Protein Families

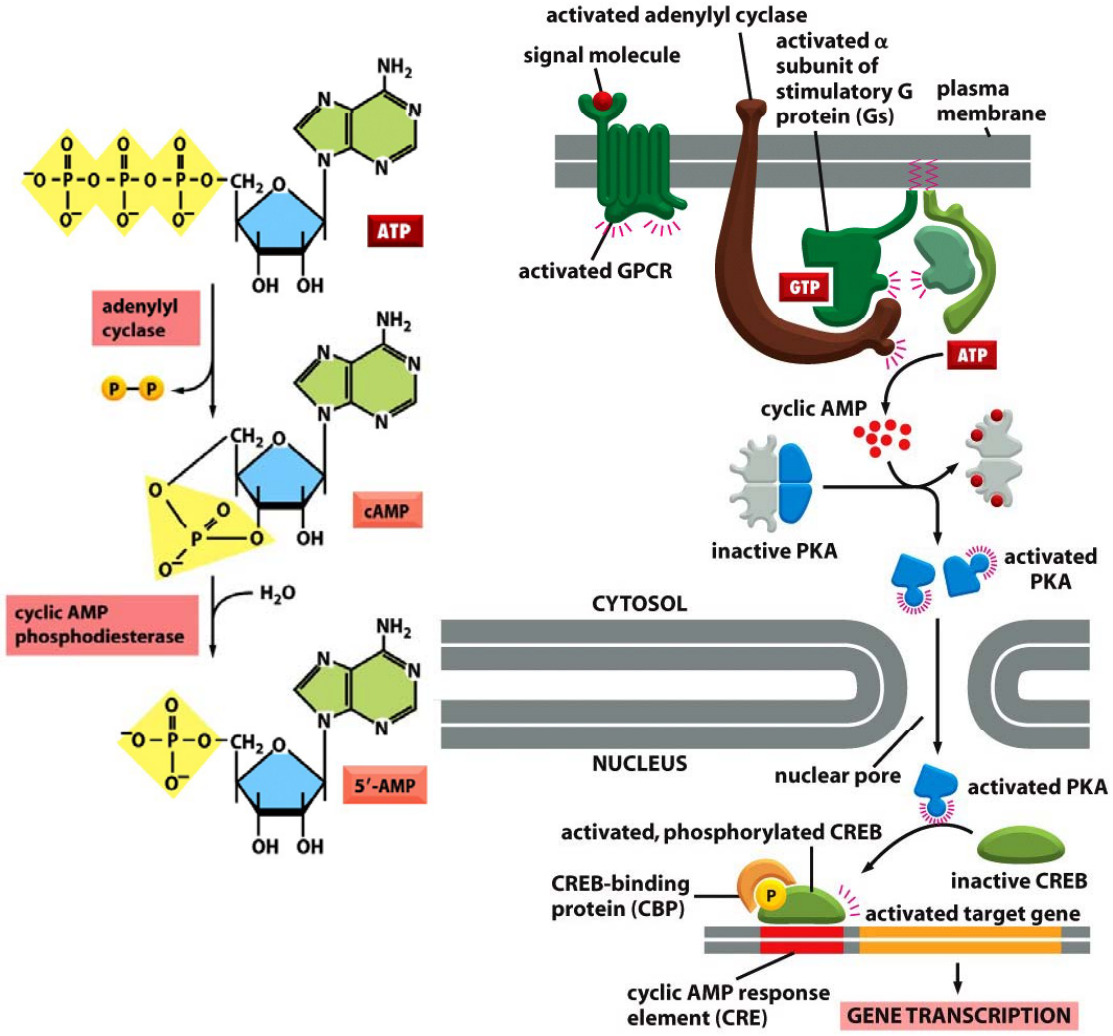
Table 15–3 Four Major Families of Trimeric G Proteins*

FAMILY	SOME FAMILY MEMBERS	SUBUNITS THAT MEDIATE ACTION	SOME FUNCTIONS
I	G_s	α	activates adenylyl cyclase; activates Ca^{2+} channels
	G_{olf}	α	activates adenylyl cyclase in olfactory sensory neurons
II	G_i	α	inhibits adenylyl cyclase
		βγ	activates K^+ channels
	G_o	βγ	activates K^+ channels; inactivates Ca^{2+} channels
		α and βγ	activates phospholipase C-β
	G_t (transducin)	α	activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
III	G_q	α	activates phospholipase C-β
IV	$G_{12/13}$	α	activates Rho family monomeric GTPases (via Rho-GEF) to regulate the actin cytoskeleton

*Families are determined by amino acid sequence relatedness of the α subunits. Only selected examples are included. About 20 α subunits and at least 6 β subunits and 11 γ subunits have been described in humans.

Example: Regulation of cAMP by G Proteins

- Cyclic AMP is synthesized from ATP by adenylyl cyclase.
- Cyclic AMP is degraded by cAMP phosphodiesterases through hydrolysis.



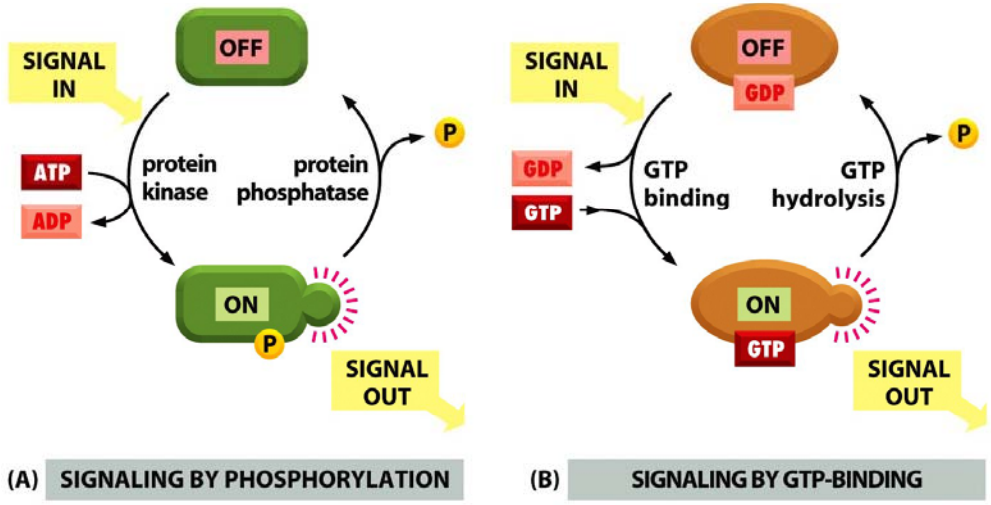
Enzyme Coupled Receptors

- Enzyme coupled receptors:
  - receptor serine/threonine kinases
  - receptor tyrosine kinase
  - cytokine receptors
  - guanylyl cyclase receptors

- Latent gene regulatory pathway receptors
  - Notch receptors
  - Hedgehog receptors
  - TNF receptors
  - Toll-like receptors

Protein Kinase & Phosphatase (I)

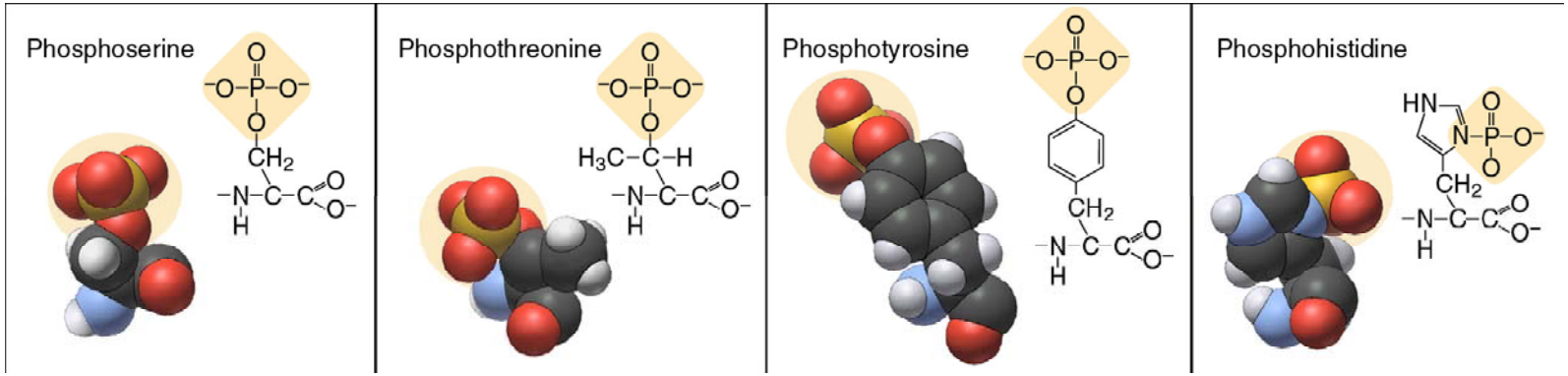
Protein ⇌ (**Kinase** / **Phosphatase**) **Phosphorylated protein**



Presence/absence of a single phosphate group turns on/off a signaling protein

Protein Kinase & Phosphatase (II)

- Normally part of a signaling cascade
- Often serve as signal amplifiers
- Human genomes encodes ~520 kinases and ~150 phosphatases
- Two main types of kinases
  - serine/threonine kinase (>99%)
  - tyrosine kinase

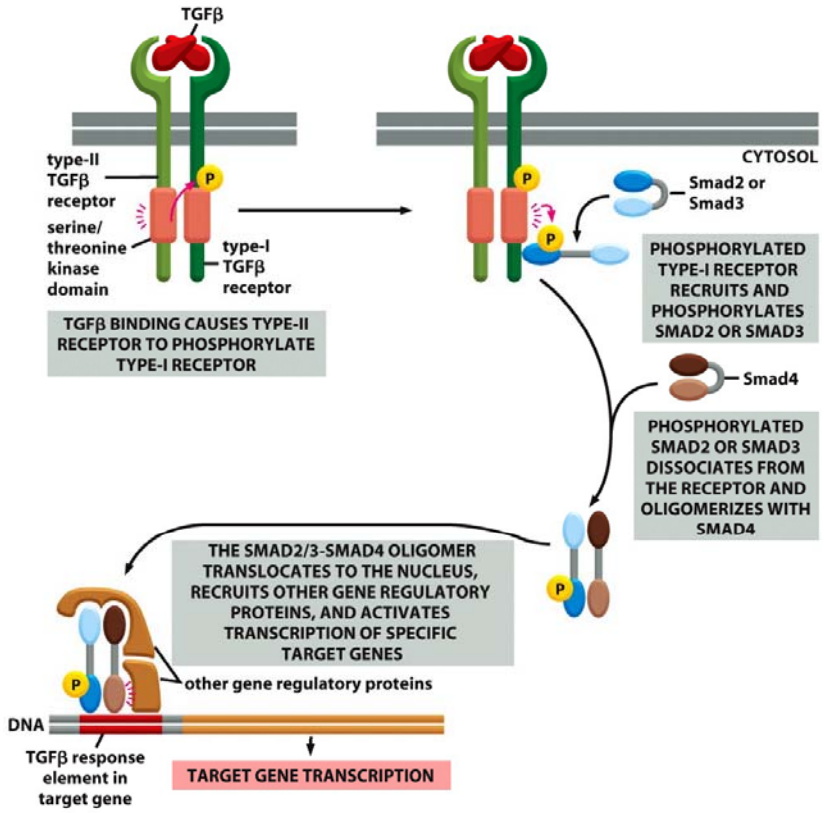


Common	Common	Rare	Very rare

Abundance in eukaryotes

Receptor Serine/Threonine Kinases

- Binds to about 40 human proteins, e.g. TGF-β and bone morphogenetic protein.
- TGF-β acts through receptor serine/threonine kinase and Smads.
- TGF-β
  - Embryonic development signaling.
  - Inhibits proliferation of most adult cells.
  - Stimulate extracellular matrix production
  - Regulate cell death in development.
  - Regulate tissue repair and immune response in adults.

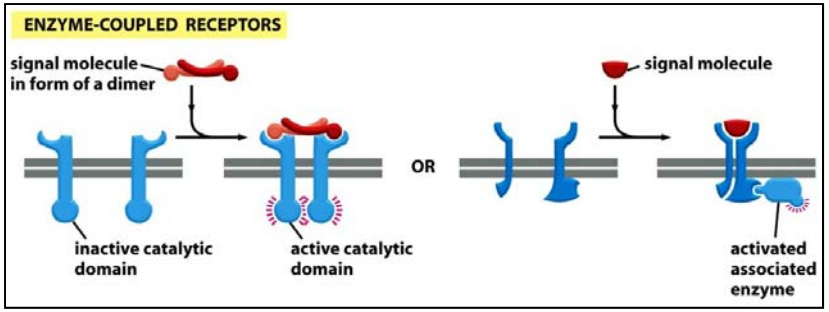


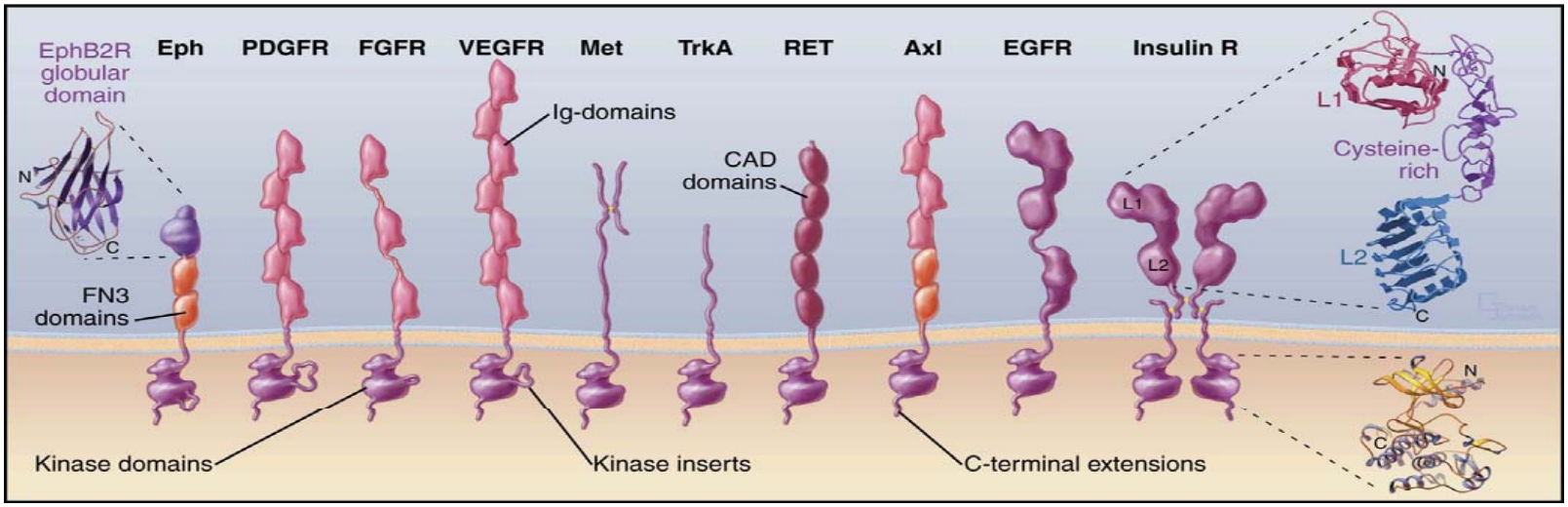
Smad: Sma in C. elegans & Mad in Drosophila

Receptor Tyrosine Kinase (I)

- Phosphorylate tyrosines on themselves and a small set of intracellular signaling proteins.

- Receptor tyrosine kinase
  - extracellular ligand-binding domain
  - cytoplasmic tyrosine kinase domain
  - single transmembrane helix





Receptor Tyrosine Kinase (II)

Table 15–4 Some Signal Proteins That Act Via RTKs

SIGNAL PROTEIN	RECEPTORS	SOME REPRESENTATIVE RESPONSES
Epidermal growth factor (EGF)	EGF receptors	stimulates cell survival, growth, proliferation, or differentiation of various cell types; acts as inductive signal in development
Insulin	insulin receptor	stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factors (IGF1 and IGF2)	IGF receptor-1	stimulate cell growth and survival in many cell types
Nerve growth factor (NGF)	Trk A	stimulates survival and growth of some neurons
Platelet-derived growth factors (PDGF AA, BB, AB)	PDGF receptors (α and β)	stimulate survival, growth, proliferation, and migration of various cell types
Macrophage-colony-stimulating factor (MCSF)	MCSF receptor	stimulates monocyte/macrophage proliferation and differentiation
Fibroblast growth factors (FGF1 to FGF24)	FGF receptors (FGFR1–FGFR4, plus multiple isoforms of each)	stimulate proliferation of various cell types; inhibit differentiation of some precursor cells; act as inductive signals in development
Vascular endothelial growth factor (VEGF)	VEGF receptors	stimulates angiogenesis
Ephrins (A and B types)	Eph receptors (A and B types)	stimulate angiogenesis; guide cell and axon migration

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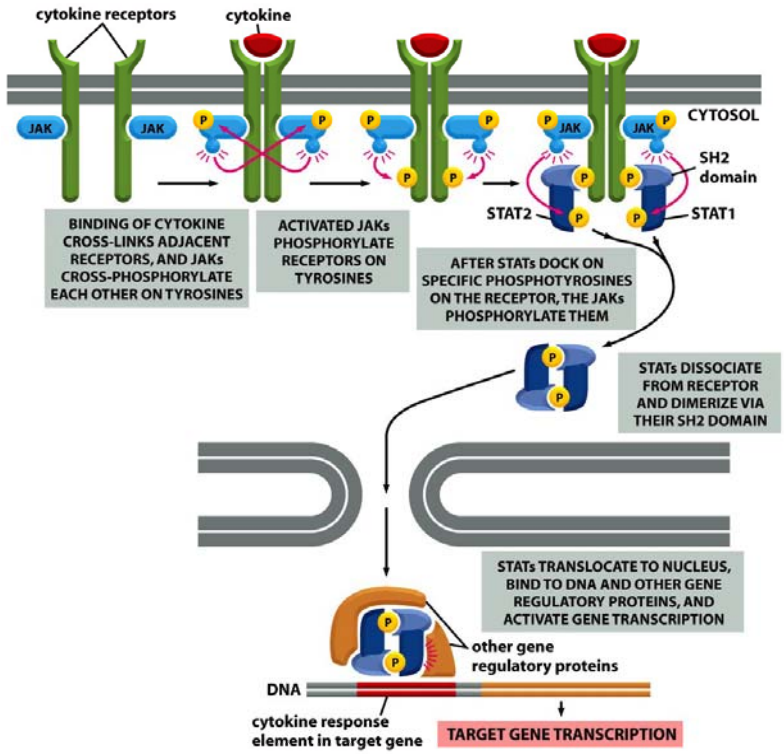
Cytokine Receptors

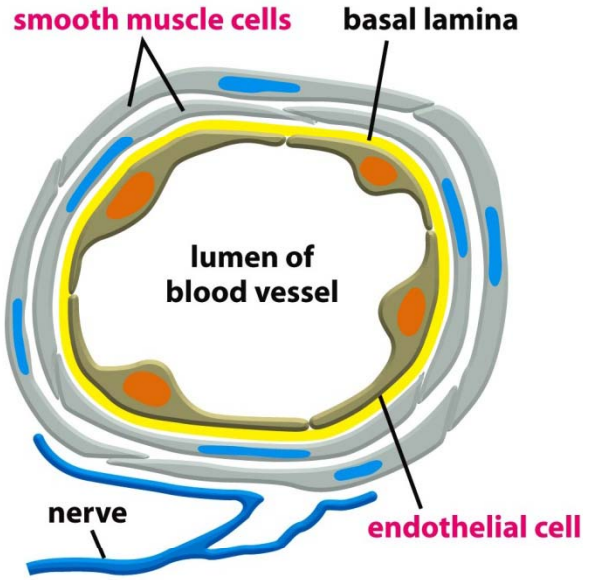
- Cytokines are polypeptide hormones or growth factors that act as a local mediator in cell-cell communication.
- Immune cells secrete cytokines when pathogens are encountered.
- Cytokines recruit immune cells in response to pathogens.
- Cytokine receptors activate the JAK-STAT signaling pathway.
- JAK-STAT pathway provides a fast track to the nucleus.

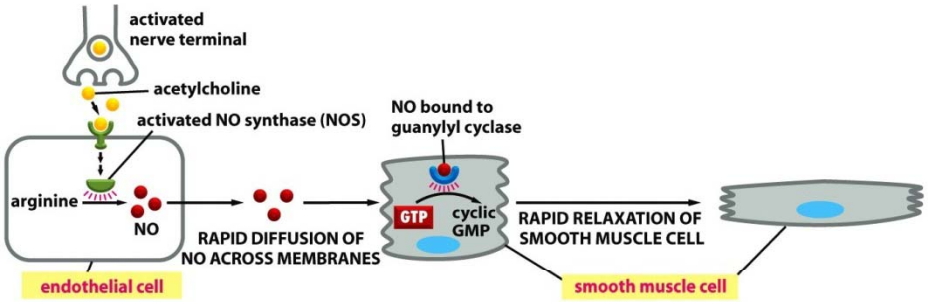


JAK: Janus kinases
STAT: signal transducer and activators of transcription

Intracellular Receptor: Guanylyl Cyclase Receptors

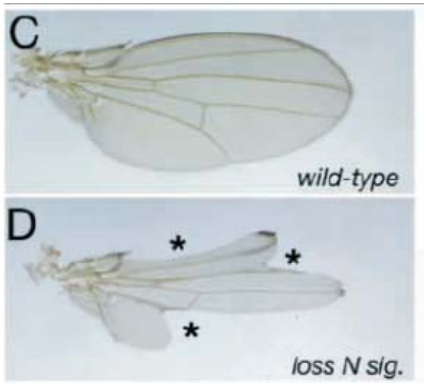
- Soluble guanylyl cyclase is a mammalian NO/CO sensor.
- NO signaling is critical to many physiological processes involving cardiovascular and neuronal systems.
- Related drugs work by blocking the breakdown of cGMP.



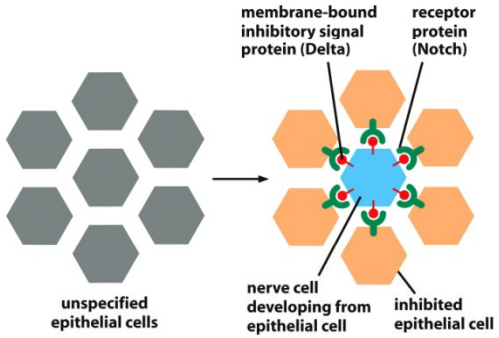


Notch Receptors (I)

- Latent gene regulatory proteins are activated by protein degradation.
- Protein ligand: Delta (fly), LAG-2 (worm); Receptors: Notch, Lin-12 (worm)
- Most widely used in
  - cell fate regulation (development)
  - pattern formation (development)
  - tissue renewal (post-development)
- Main function: lateral inhibition
  - Amplify and consolidate molecular differences between adjacent cells during embryonic development

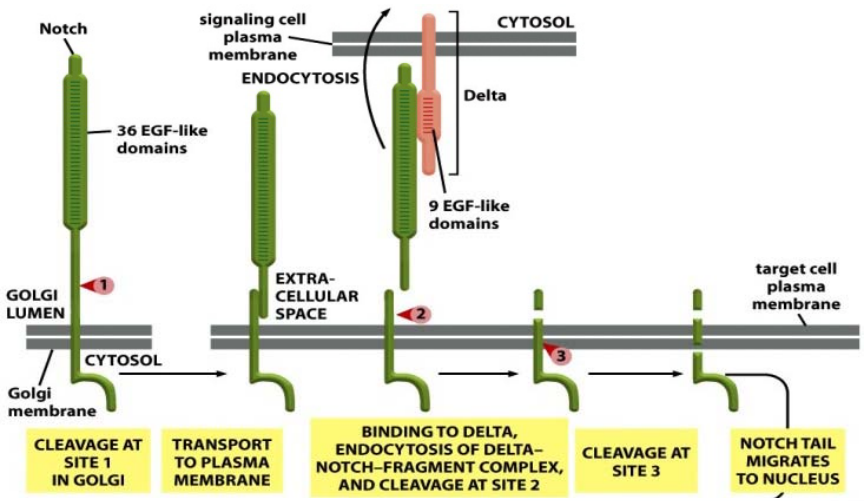


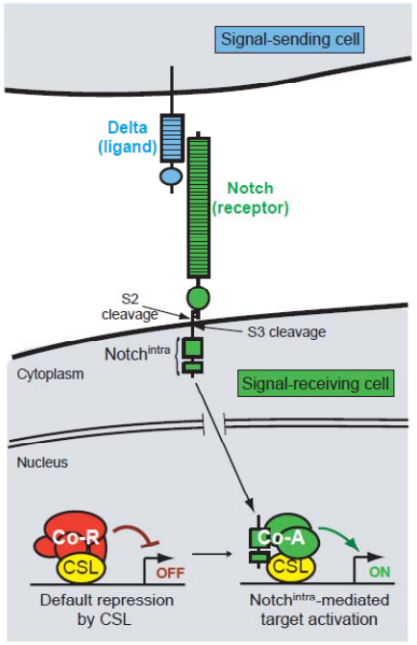
Lai, *Development*, 131:965, 2004



Notch Receptors (II)

- Binding of Delta triggers cleavage of Notch.
- Released Notch tail migrates into the nucleus to convert Rbpsuh protein from a transcriptional repressor into a transcriptional activator.
- Activation of Notch is irreversible.
- The simplest known pathway from cell surface to nucleus.

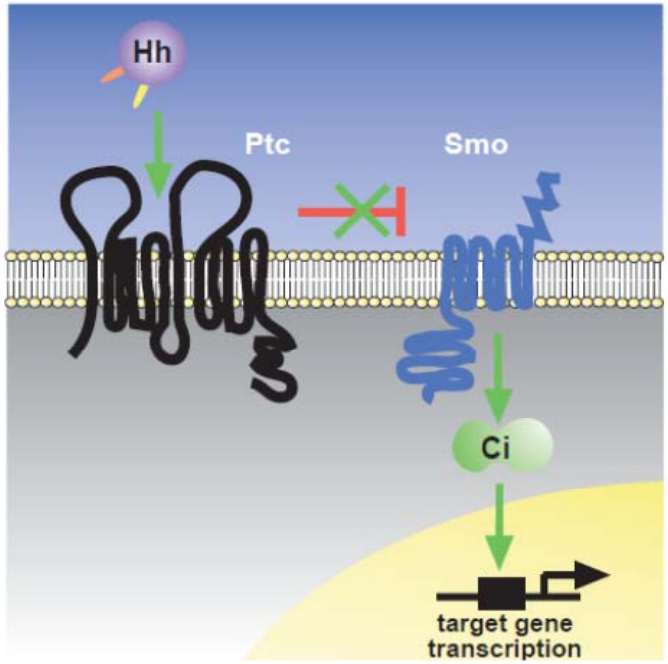




Lai, *Development*, 131:965, 2004

Hedgehog Receptors

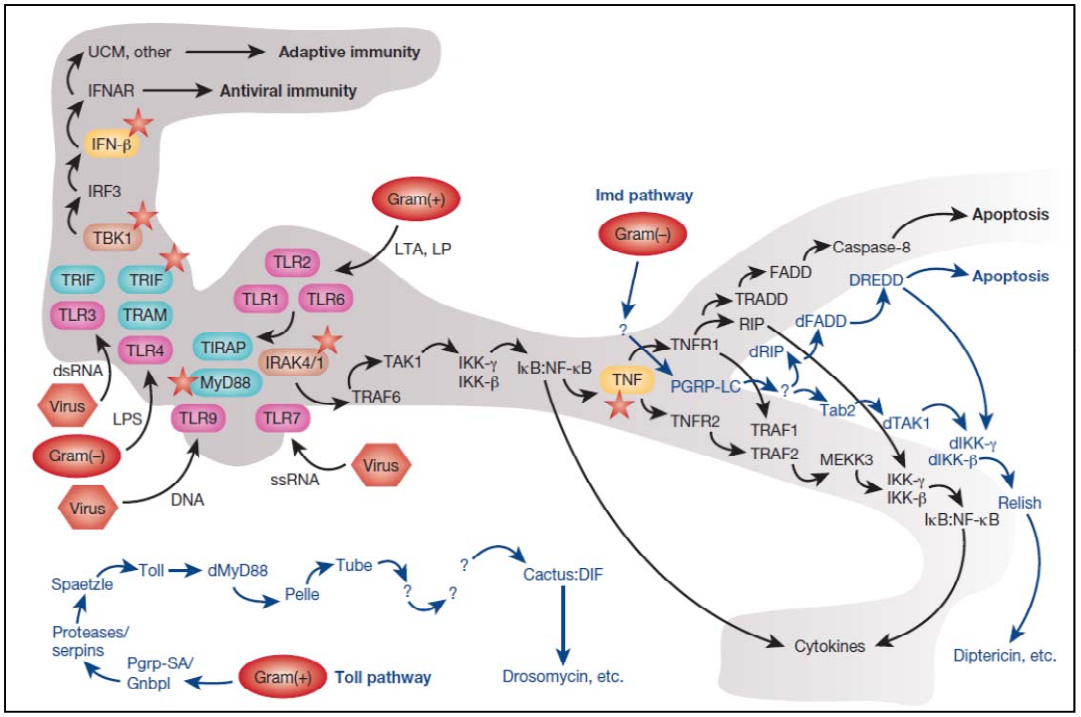
- Protein ligand: Hh; Receptors: Ptc & Smo
- Hh binds and inactivate Ptc, which activates Smo and gene transcription.
- Main functions
  - Regulates cellular differentiation in embryonic development
  - Maintaining stem cells in postembryonic tissues (tissue renewal)
- Mutation of Hh causes developmental defects.
- Mutation of Ptc and Smo causes skin cancer.



Lum & Beachy, *Science*, 304:1755, 2004

Toll-like Receptors (TLRs)

- Mammals have TLRs that recognize specific foreign molecules.
- Main function
  - To sense and respond to infection
- At the core of our inherited resistance to disease



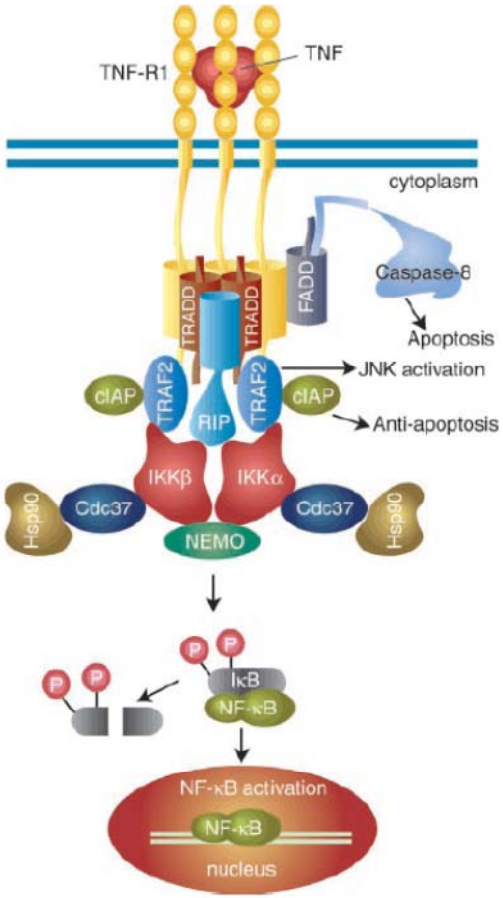
Beutler, *Nature*, 430:257, 2004

Tumor Necrosis Factor (TNF) Receptors

- Binding of TNF with its receptors triggers mutiple signaling pathways.

- Function
  - -Triggering apoptosis of tumor cells
  - -Mediate inflammatory response
  - -Regulate immune system function

- Inappropriate TNF signaling has been implicated in many human diseases.



Chen et al, Science, 296:1634, 2002.

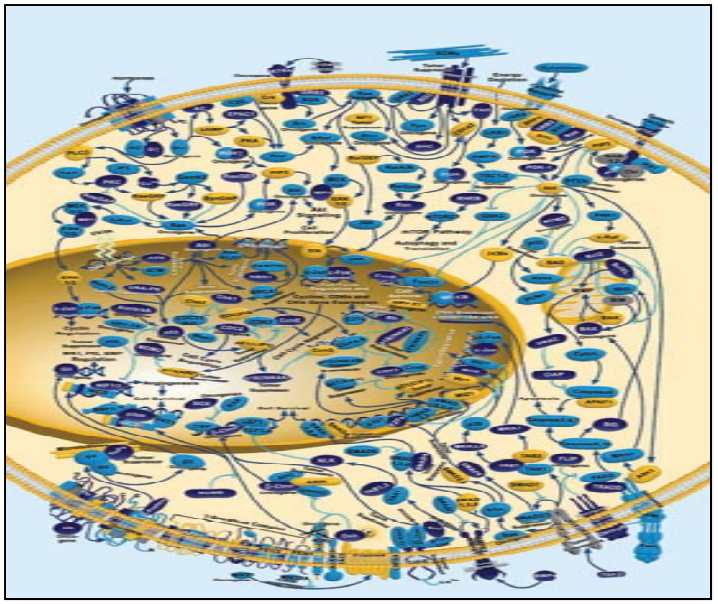
NF-kB Pathway

- Activation of Toll-like receptors or TNF receptors triggers a signaling cascade that releases NFkB.
- NFkB proteins regulate transcriptions of hundreds of genes participate in immune responses.
- Excessive or inappropriate inflammation response can cause tissue damage and severe pain.
- Chronic inflammation can lead to cancer.

Challenges in Analyzing Signaling Pathways

- Hundreds of signaling pathways.
- Pathways frequently branch and converge.
- Positive and negative feedback loops are common.
- Outcomes of signaling pathways can be spatial and temporal dependent.
- Analysis typically uses graph models.

Human cancer pathways



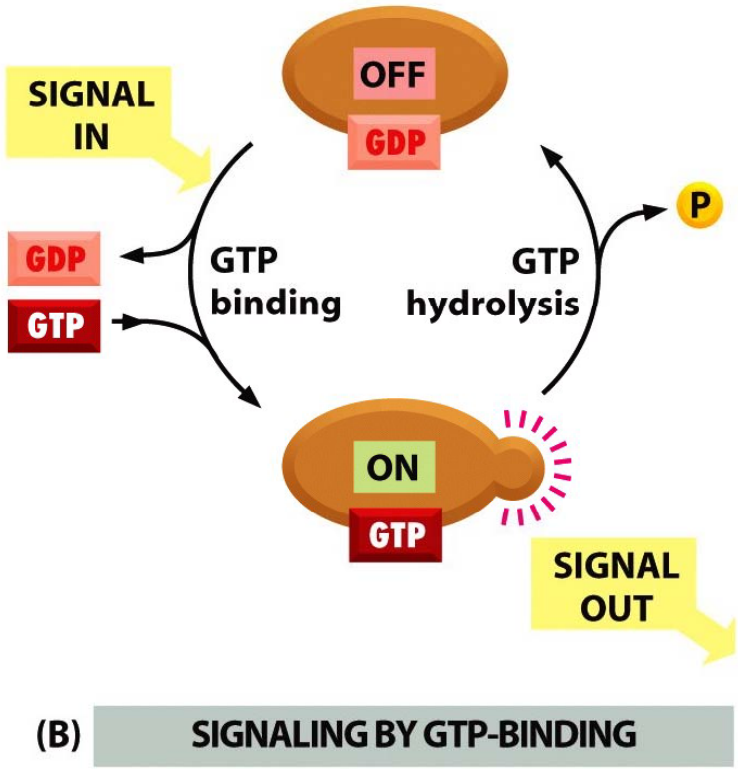
References

- J. Hancock, Cell Signaling, 3rd ed., Oxford University Press, 2010.
- F. Marks et al, Cell Signal Processing, Garland Science, 2008.

- Overview of cell signaling
- Classification of signaling related proteins
- Receptors
- Signaling protein transducers
- Second messengers

GTP-Binding Proteins

- Trimeric G-protein & Monomeric small GTPase
- Large family of related proteins
- Evolved from a common ancestor by gene duplication and divergence
- Use GTP binding and hydrolysis to switch between two states of activity

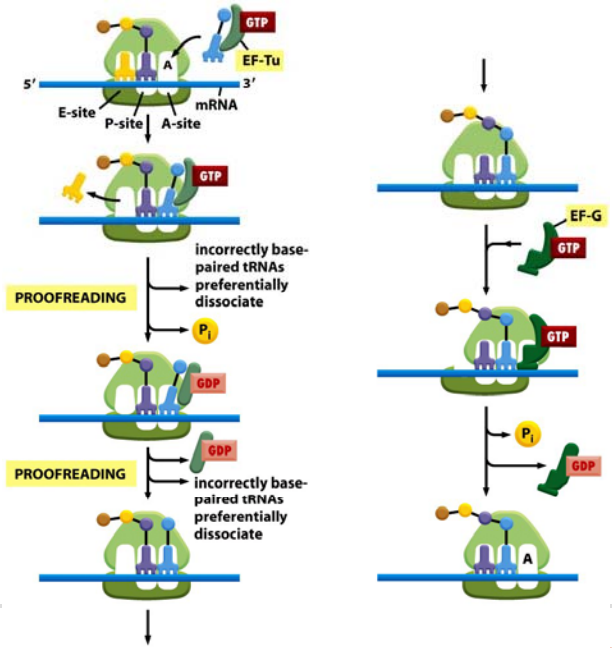


(B) SIGNALING BY GTP-BINDING

Monomeric GTPases

Participate in many cellular activities:

• Membrane traffic	Arf, Rab, Sar
• Nuclear transport	Ran
• Signal transduction	Ras
• Regulation of the cytoskeleton	Rho
• Protein synthesis	EF-Tu
• Protein translocation into ER	SRP



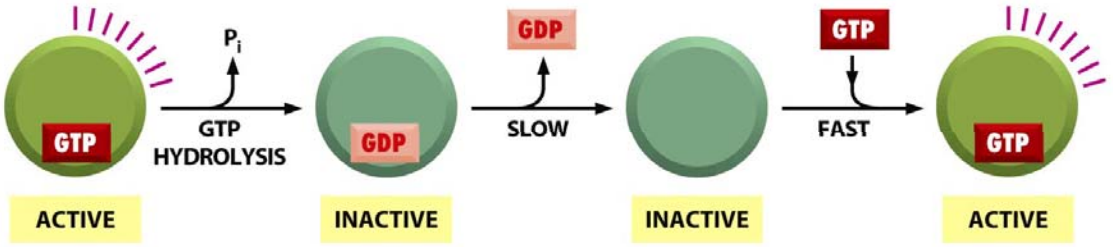
Actin Regulation

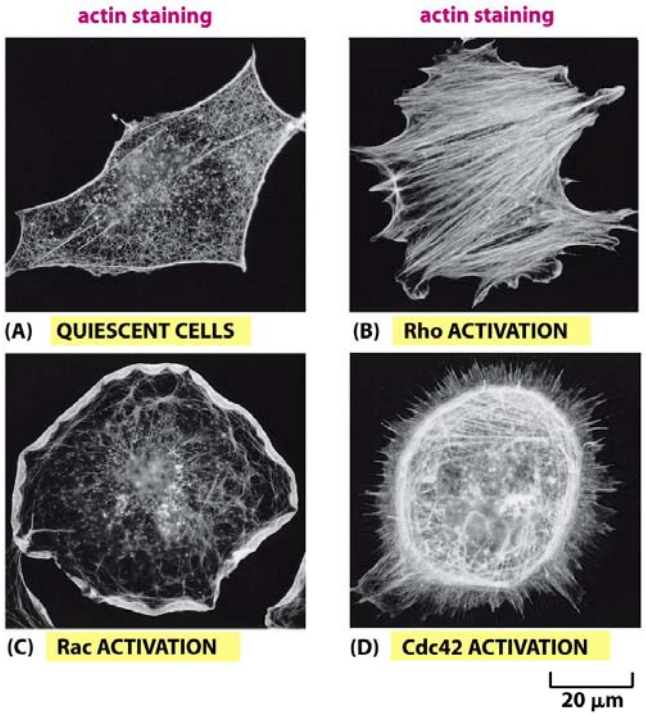
- GTPase: Molecule switch; Family of proteins that are activated by GTP binding and inactivated by GTP hydrolysis and phosphate dissociation.

- Rho GTPase:
  cdc42: its activation triggers actin polymerization and bundling at filopodia.

  Rho: its activation promotes actin bundling.

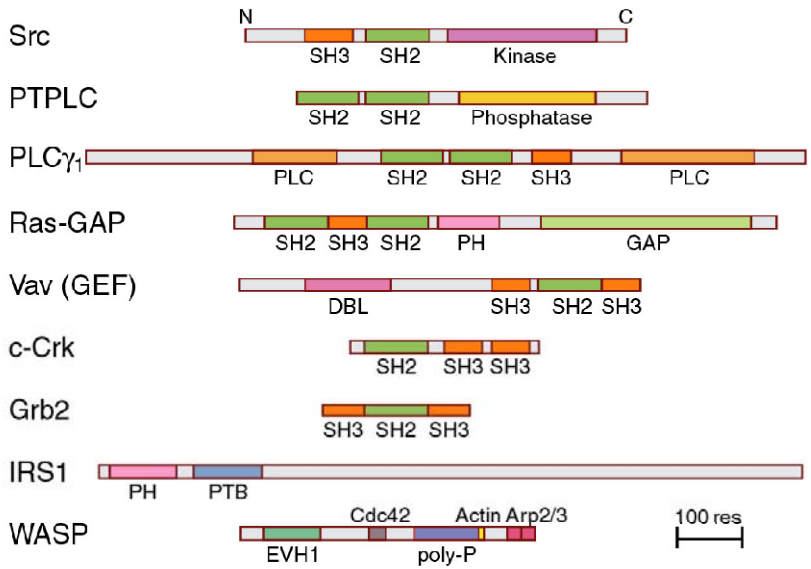
  Rac: its activation promotes polymerization at the cell periphery.





Adaptor Domains (I)

- Adaptor domains mediate interactions of proteins with each other and with membrane.
- These domains are compactly folded and incorporated into a variety of proteins.
- Adaptors facilitate the formation of protein complexes and make signal transduction more reliable.



SH1 : tyrosine kinase domain

SH2: Src homology 2, binds phosphotyrosine peptides

SH3: Src homology 3 binds polyproline type II helices

Adaptor Domains (II)

Table 25-4

ADAPTER DOMAINS

Domain Name	Size (Residues)	Consensus Ligands	Example of Proteins with Domain
EH (Eps15 homology)	95	S/T-N-P-F-Φ	Clathrin adapter proteins, synaptojanin I
EVH1 (Ena-VASP homology)	110	D/E-Φ-P-P-P-P	WASp, VASP, Ena
PH (Pleckstrin homology)	100	PIP_2, PIP_3	Kinases, scaffolds, GEFs, GAPs, PLCδ, dynamin
PDZ	100	-x-x-S/T-x-V-COOH -x-x-Φ-x-Φ-COOH	Scaffolds for channels and transduction enzymes
PTB (phosphotyrosine binding)	125	-Φ-x-N-P-x-pY-	IRS1, Shc scaffold proteins
SH2 (Src homology 2)	100	-pY-x-x-Φ-	Transduction enzymes and scaffold proteins
SH3 (Src homology 3)	60	(+) -R/K-x-x-P-x-x-P- (−) -x-P-x-x-P-x-R/K-	Tyrosine kinases, phosphatases, Grb2, PLCγ, spectrin, myosin I
WW	38–40	-P-P-x-Y-	Peptidyl prolyl isomerase, ubiquitin ligase
14-3-3	250	-R-S-X-pS-x-P-	14-3-3 isoforms

- Overview of cell signaling
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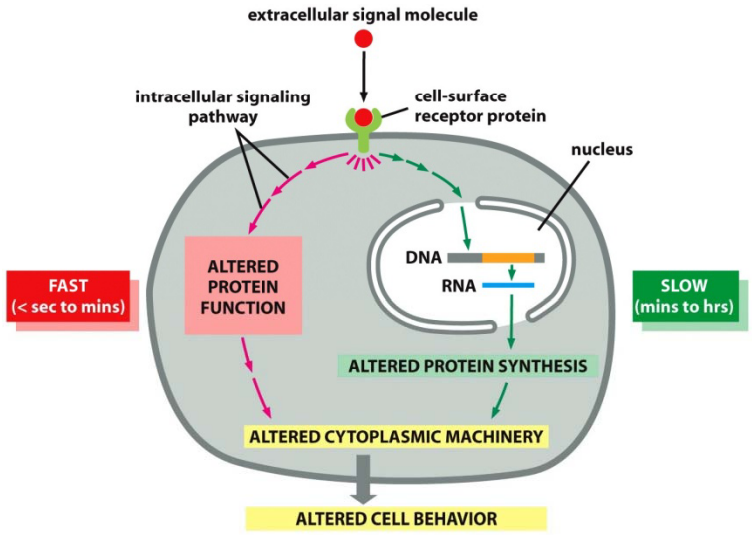
- Second messengers

Overview of Second Messengers (I)

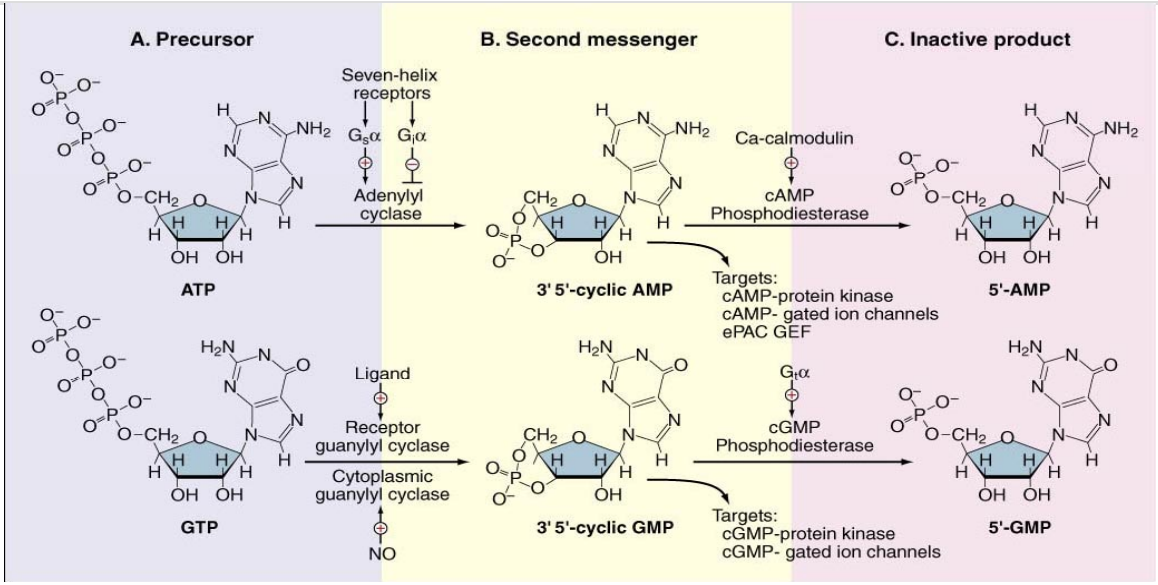
- Types of second messengers
  - Cyclic nucleotides: cAMP, cGMP
  - Calcium
  - Lipids
  - Nitric oxide
- Small molecules.
- Information encoded by local concentrations.
- Advantages
  - Range (e.g. broadcasting)
  - Response speed (up to ms)
- Second messengers are interrelated.



Overview of Second Messengers (II)

- Production (source)
- Localization
- Target
- Degradation (sink)

Cyclic Nucleotide (I)



- Producer:
  - cAMP → adenylyl cyclase
  - cGMP → guanylyl cyclase

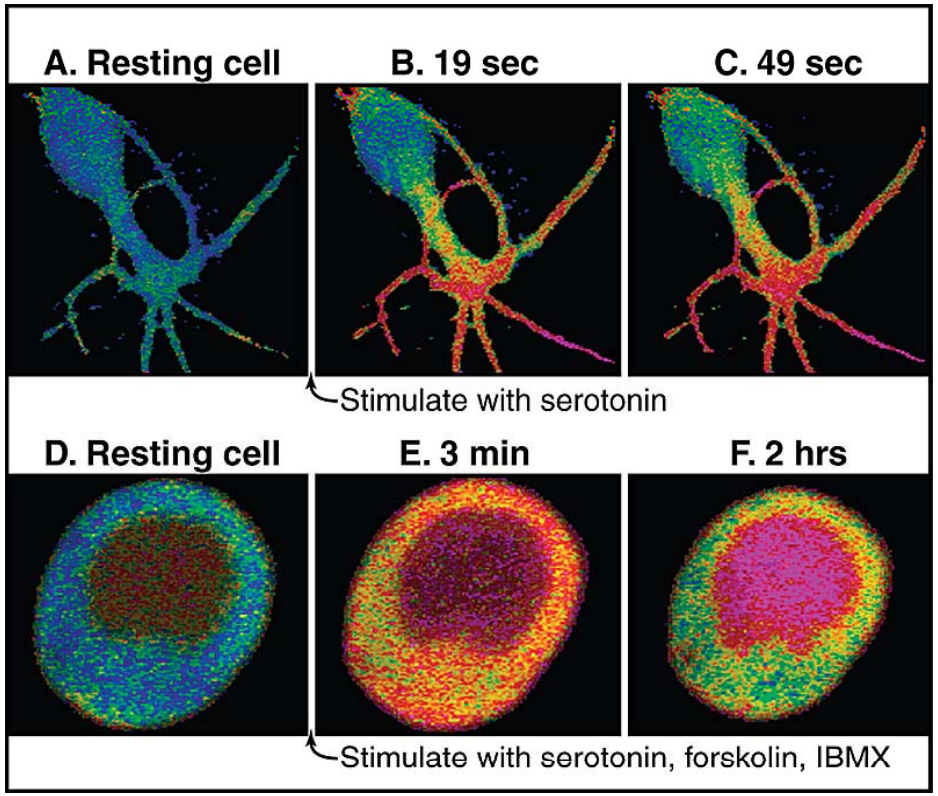
- Degrader:
  - cAMP phosphodiesterase
  - cGMP phospoodiesterase

cAMP (I)

- Diffuse rapidly through cytoplasm as in free solution
- May be modulated locally (through upstream G-proteins)
- Concentration in resting cell ~10^{-8}M
- Can amplify signal by 100-fold on time scale of ms.
- Targets:
  - kinase
  - cyclic nucleotide-gated ion channels
  - Exchange factors for small GTPases (Rap1, Rap2)

cAMP (II)

- cAMP regulates PKA
- PKA targets metabolic enzymes, transcription factors and ion channels
- Guanylyl cyclase (cGMP producer) is activated by NO and CO



Questions ?