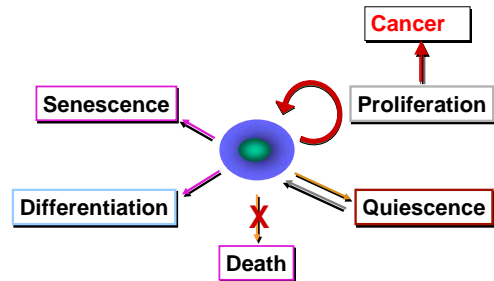


细胞信号转导 (Cell Signaling)

陈晔光
清华大学生命科学学院
ygchen@tsinghua.edu.cn

Fates of a cell

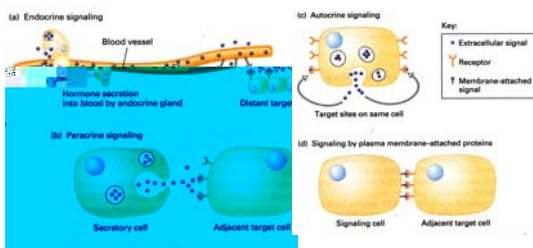


- General introduction
 - Common features of signal transduction
 - Cell surface signal transducers, receptors
- Ion channels
- Secondary messengers
 - cAMP
 - cGMP
 - Lipids
 - Calcium
- G proteins
 - Trimeric G proteins
 - Monomeric G proteins, Tyr kinase/MAP kinase
- Protein modules

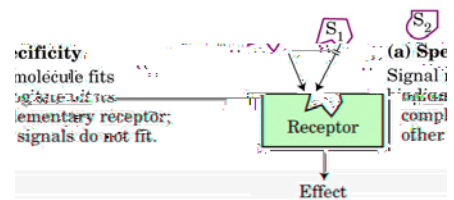
What is "Signal Transduction"?

This expression first made its mark in the biological literature around 1974. Physical scientists and electronic engineers had earlier used the term to describe the conversion of energy or information from one form into another. Signal transduction at the cellular level refers to the movement of signals from outside the cell to inside; cascade of information from the plasma membrane to the nucleus in response to an extracellular stimulus in living organisms.

General schemes of intercellular signaling



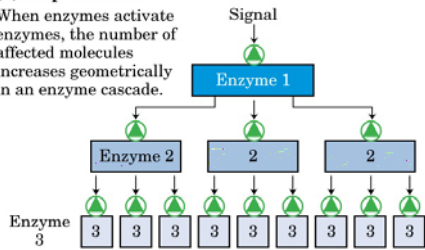
Four basic features of signal-transducing systems



Four basic features of signal-transducing systems

(b) Amplification

When enzymes activate enzymes, the number of affected molecules increases geometrically in an enzyme cascade.

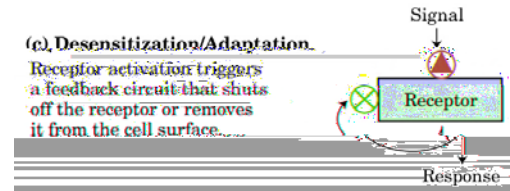


7

Four basic features of signal-transducing systems

(c) Desensitization/Adaptation

Receptor activation triggers a feedback circuit that shuts off the receptor or removes it from the cell surface.



8

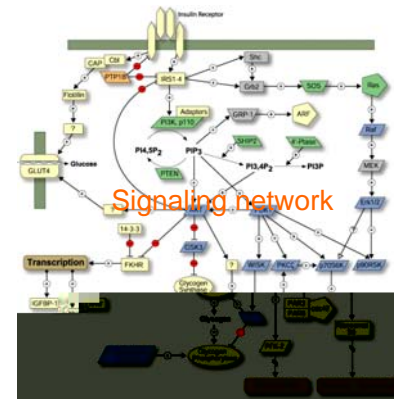
Four basic features of signal-transducing systems

(d) Integration

When two signals have opposite effects on a metabolic characteristic, such as the concentration of a second messenger X, or the membrane potential, the regulatory outcome results from the integrated effects of both signals.



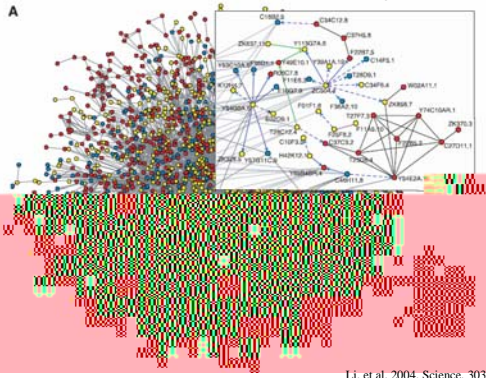
Response



Signaling network

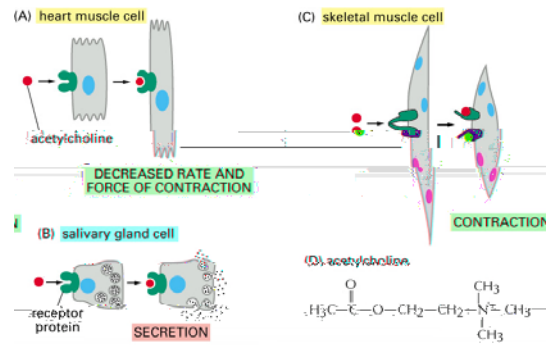
10

Protein interactome network in C. elegans

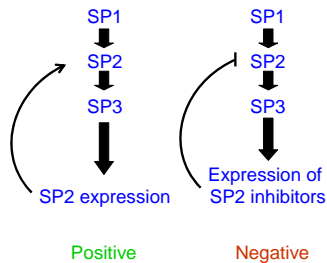


Li, et al., 2004, Science, 303:540

Context-specificity of cell signaling: Different cells respond differently to the same extracellular signal

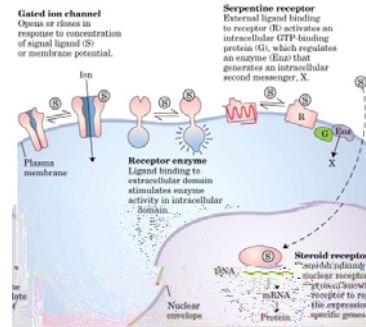


Positive and negative feedback mechanisms



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Four general types of signal transducers



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Ligand-activated cell-surface receptors

- ¼ Ion-channel receptors: acetylcholine
- ¼ G-protein-coupled receptors: epinephrine, glucagon, serotonin
- ¼ Tyrosine kinase-linked receptors: interferons
- ¼ Tyrosine kinase receptors: EGF, PDGF, insulin
- ¼ Tyrosine phosphatase receptors: CD45
- ¼ Serine/threonine kinase receptors: TGF E BMP
- ¼ Guanylate cyclase receptor: atrial natriuretic factor

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General introduction

- Common features of signal transduction
- Cell surface signal transducers, receptors

Ion channels

Secondary messengers

- cAMP
- cGMP
- Lipids
- Calcium

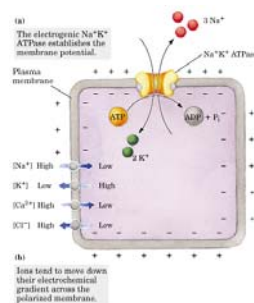
G proteins

- Trimeric G proteins
- Monomeric G proteins, Tyr kinase/MAP kinase

Protein modules

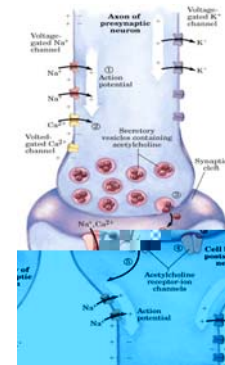
16

Transmembrane electrical potential



17

Role of voltage-gated and ligand-gated ion channels in neural transmission

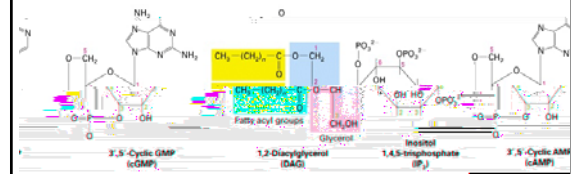


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General introduction
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 cAMP
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 Lipids
 Calcium
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 Trimeric G proteins
 Monomeric G proteins, Tyr kinase/MAP kinase
 Protein modules

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Second messengers



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Secondary messengers:

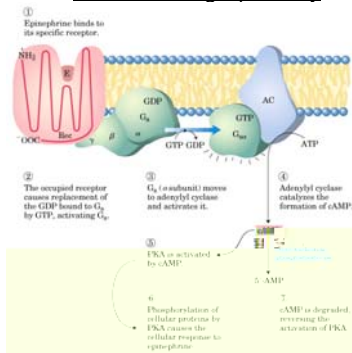
cAMP
 cGMP
 Lipids
 Calcium

21

Produced by Chromaffin cells in adrenal medulla
 Functions:
 Increase heart rate, blood pressure,
 sweating, rate of respiration
 Stimulate conversion of glycogen to glucose

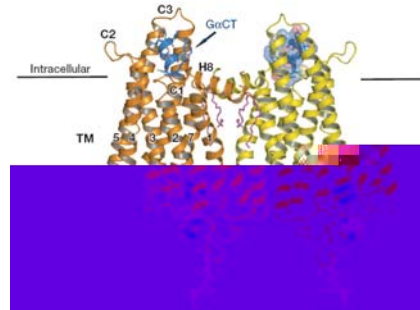
22

Transduction of the epinephrine signal: the Eadrenergic pathway



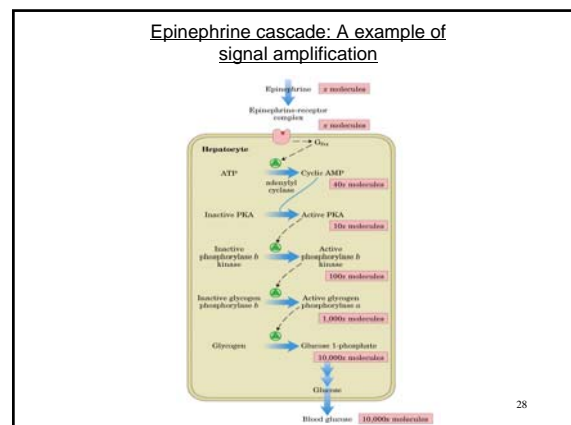
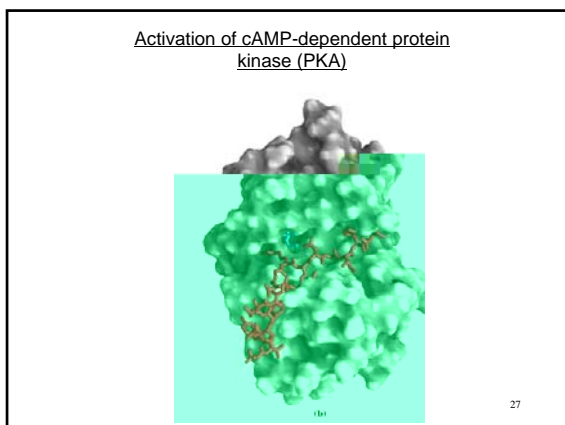
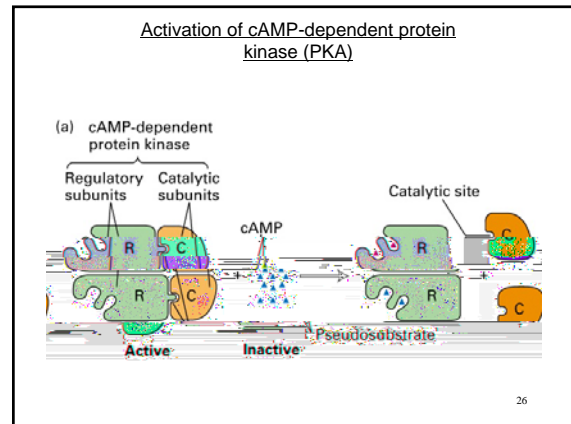
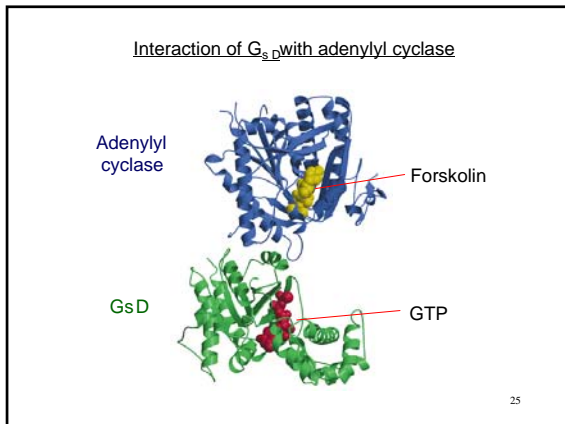
23

Structure of Opsin associated with the C-terminal Gs D



Scheerer et al. Nature, 2008

24

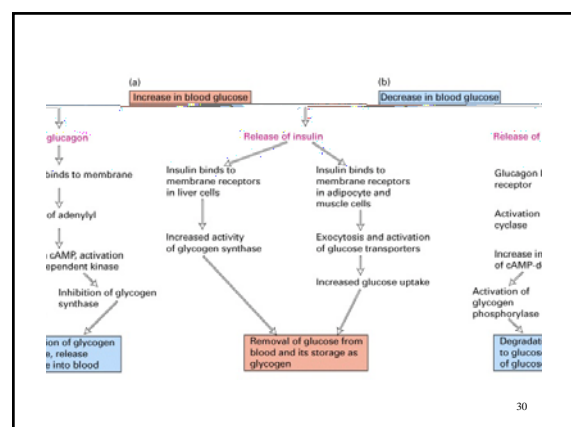


cAMP as a secondary messenger

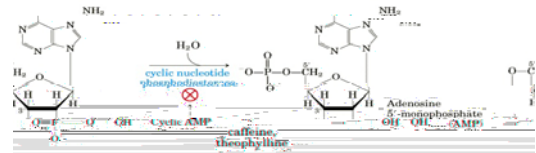
Tissue	Hormone binding to cAMP	Effect
Adipose	Epinephrine, ACTH	Increase in hydrolysis of triglycerides; decrease in lipogenesis
Liver	Epinephrine, norepinephrine, thyrotropin...	Increase in conversion of glycogen to glucose; synthesis of glycogen; increase in gluconeogenesis; synthesis of... glucose
Ovarian follicle	FSH, LH	Increase in synthesis of estrogen, progesterone...
Adrenal cortex	ACTH	Increase in synthesis of aldosterone, cortisol
Cardiac muscle cells	Epinephrine	Increase in contraction rate
Thyroid	TSH	Secretion of thyroxine
Bone cells	Parathyroid hormone	Increase in resorption of calcium from bone
Skeletal muscle	Epinephrine	Conversion of glycogen to glucose
Intestine	Epinephrine	Fluid secretion
Kidney	Vasopressin	Resorption of water
Blood platelets	Prostaglandin I	Inhibition of aggregation and secretion

SOURCE: E. W. Sutherland, 1972, Science 177:401.

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Inactivation of cAMP



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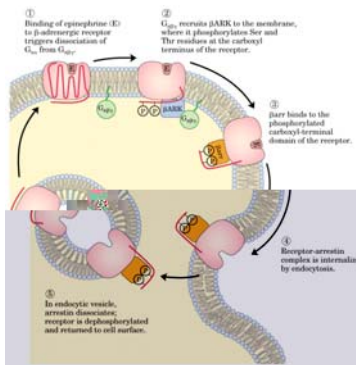
Medicine: Adrenergic receptor

Cardiac muscle cells possess β_1 receptor, whose activation increases heart rate. Propranolol (1-(S)-propranolol), an β -selective antagonist, can slow heart contraction and is used to treat cardiac arrhythmia and angina.

Smooth muscle cells have β_2 receptors, whose activation promotes relaxation. Terbutaline (1-(3,5-dihydroxyphenyl)-2-tert-butylaminoethanol), an agonist selective for β_2 , is used in the treatment of asthma.

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Desensitization of the Adrenergic receptor



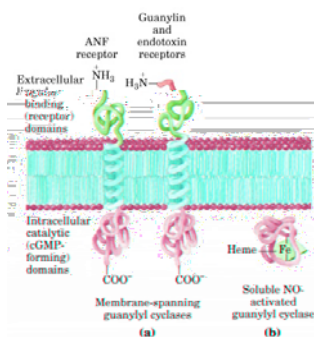
33

Secondary messengers:

- cAMP
- cGMP
- Lipids
- Calcium

34

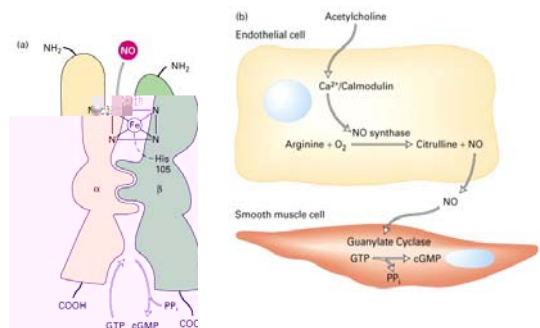
Guanylyl cyclases



Endotoxin, produced by *E. coli* and other gram-negative bacteria in intestine, stimulates cGMP production, increases Cl⁻ secretion and consequently decreases reabsorption of water by the intestinal epithelium, producing diarrhea.

35

NO (Nitric Oxide) signaling



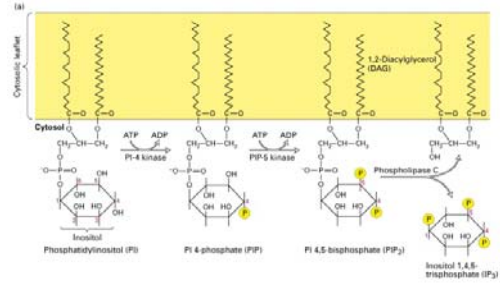
36

Secondary messengers:

- cAMP
- cGMP
- Lipids
- Calcium

37

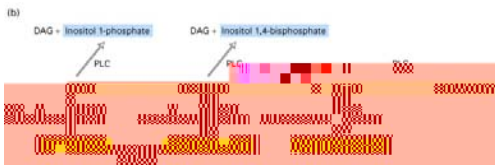
Lipids as messengers



172G 6¼G

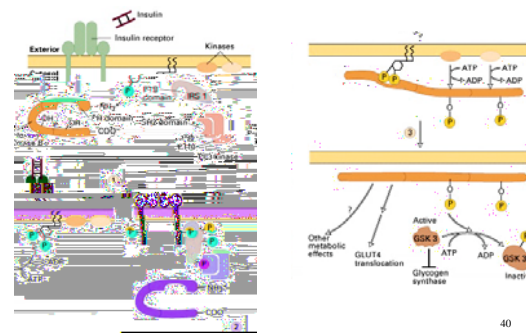
38

Lipid phosphorylation



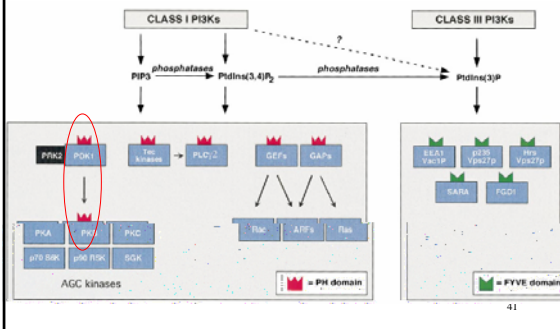
39

Insulin regulates glucose metabolism via PI3K



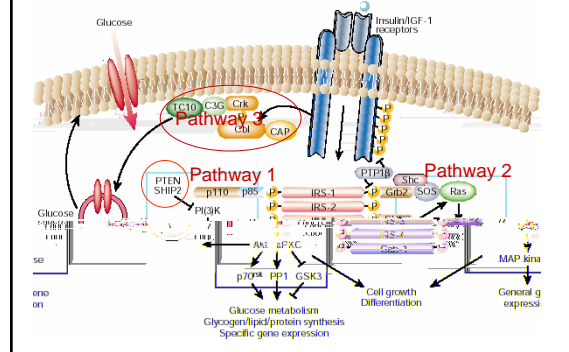
40

PH domain-containing proteins

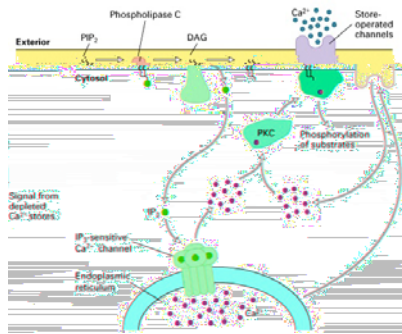


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Glucose metabolism controlled by Insulin signaling

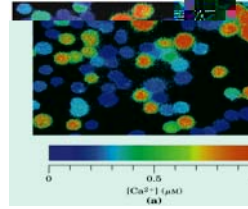


Lipids and calcium as secondary messengers



43

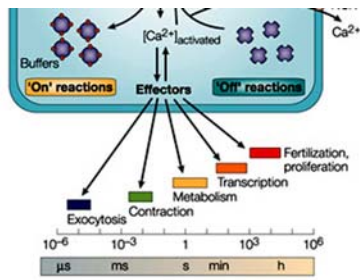
Triggering of oscillations in intracellular $[Ca^{2+}]$ by extracellular signals



Norepinephrine treatment of hepatocytes

44

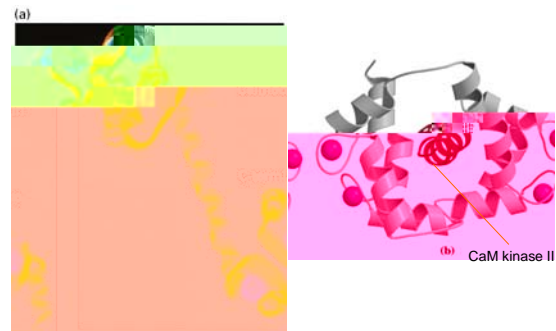
Calcium activated proteins



Nature Reviews | Molecular Cell Biology

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Calmodulin



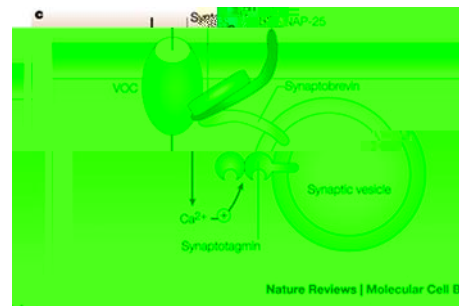
46

Calcium activated proteins

- ¼ Calmodulin: CaM kinase; phosphorylase b kinase (glycogen breakdown)
- ¼ Protein kinase C
- ¼ Calcineurin: protein phosphatase 1B
- ¼ Troponin - muscle contraction
- ¼ Synaptotagmin (neurotransmitter release)
- ¼ Guanylyl cyclase

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Calcium activated proteins



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TABLE 20-4 Cellular Responses to Hormones Induced by Ras in Involves GTP-binding to Raf-1 Kinase

Target	Effect	Effect
Parathyroid hormone	Acetylcholine	Secretion of P-glycoprotein, weak in anion and ATPase
Parathyroid hormone-related protein	Acetylcholine	Secretion of P-glycoprotein
Parathyroid hormone-related protein	Acetylcholine	Secretion of insulin
Parathyroid hormone-related protein	Acetylcholine	Secretion of insulin
Parathyroid hormone-related protein	Acetylcholine	Secretion of insulin
Liver	Angiogenesis	Conversion of glucose to glucose
Blood platelets	Aggregation, shape change, secretion of hormones	Aggregation, shape change, secretion of hormones
Macrophages	Angiogenesis	Histamine secretion
Fibroblasts	Peptide growth factors, such as fibroblast and PDGF	DNA synthesis, cell division
Spermatogenesis	Angiogenesis	Rise of fertilization membrane

Adapted from: EF GTPase, 2002, Nov 20; doi:10.1006/jcbp.2002.1044-4502; 312-315.

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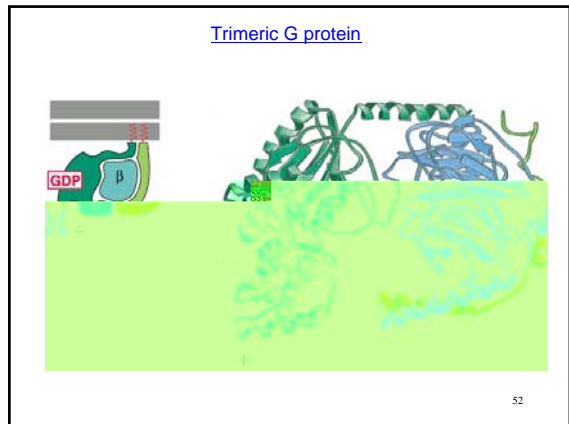
General introduction
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 Monomeric G proteins, Tyr kinase/MAP kinase
 Protein modules

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GTP-binding proteins: A superfamily

- ¾ Trimeric G proteins: G_s, G_i, G_q, G_t
- ¾ Small GTP-binding proteins
 - 9 Ras: cell growth
 - 9 Rac, Rho, Cdc42: cell migration
 - 9 Rab: membrane trafficking
 - 9 ARF: membrane trafficking
 - 9 Ran: Nuclear transport
- ¾ Other GTP-binding proteins: Dynamin, EF-Tu

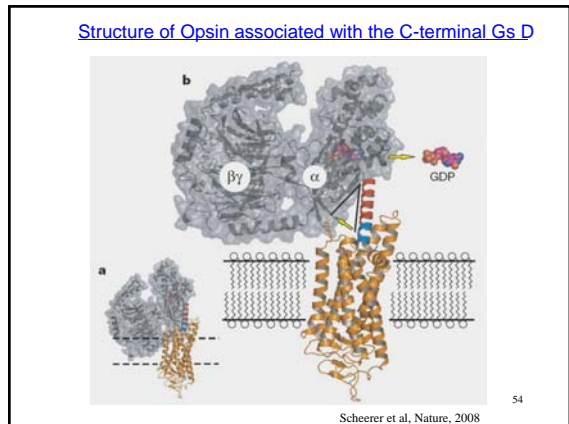
51



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Self-inactivation of G_s

53



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Toxins produced by bacteria that cause cholera (affecting Gs) and whooping cough (pertussis) (affecting Gi)

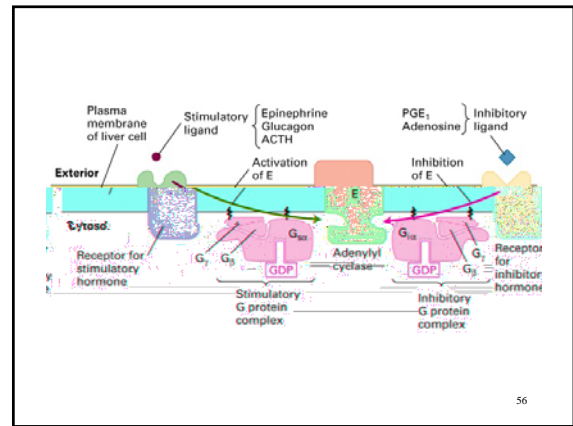
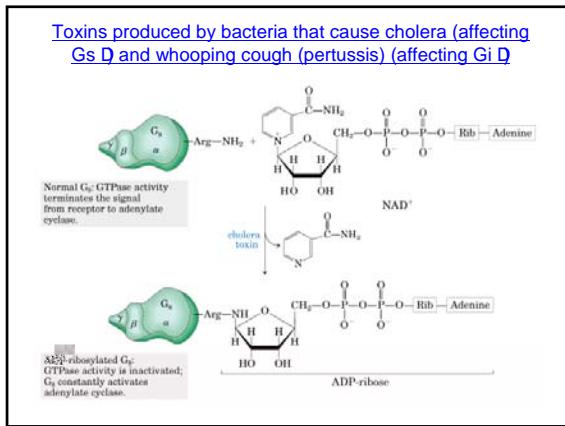


TABLE 20-5 Properties of Mammalian G Proteins Linked to GPCRs

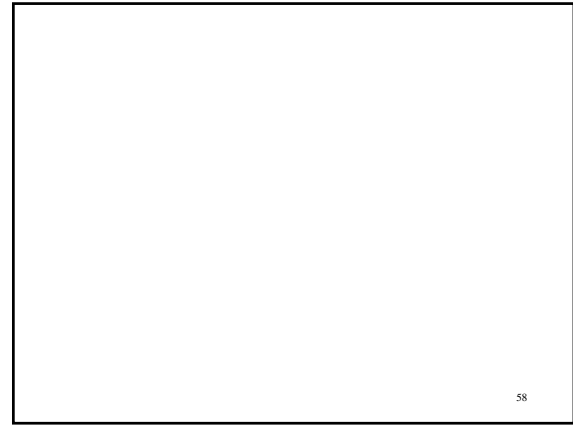
G _α Subclass*	Effect	Associated Effector Protein	2nd Messenger
G _s	↑	adenylyl cyclase	cAMP
	↑	Ca ²⁺ channel	Ca ²⁺
	↓	Na ⁺ channel	Change in membrane potential
G _i	↓	adenylyl cyclase	cAMP
	↑	K ⁺ channel	Change in membrane potential
	↓	Ca ²⁺ channel	Ca ²⁺
G _q	↑	Phospholipase C	IP ₃ , DAG
	↑	Phospholipase C	IP ₃ , DAG
G ₁₂	↓	Ca ²⁺ channel	Ca ²⁺
	↑	cGMP phosphodiesterase	cGMP
G ₁₃	↑	Phospholipase C	IP ₃ , DAG
	↓	Adenylyl cyclase	cAMP

*A single G_α may be associated with more than one of these effectors.

Table II. Properties of mammalian G protein β- and γ-subunits

Subunit	Mass (kDa × 10 ⁻³)	% Amino acid identity ^a	Tissue distribution	Effector/role
β				
β ₁	37.3	100	Ubiquitous	Required for G _α -receptor interaction
β ₂	37.3	90	Nearly ubiquitous	
β ₃	37.2	83	Nearly ubiquitous	
β ₄	37.2	89	Nearly ubiquitous	
				Modulate activation of certain adenylyl cyclases by G _{αs} or calmodulin
				Support of agonist-induced receptor phosphorylation and desensitization
γ ₁	8.4	100	Retina, other (?)	↑ Phospholipase C
γ ₂	7.9	38	Brain, adrenal, other (?)	
γ ₃	8.5	36	Brain, testis	↑ K ⁺ channels (?)
γ ₄	(?partial)	(34)	(Kidney, retina (?))	↑ Phospholipase A ₂ (?)
γ ₅	7.3	25	Liver, other (?)	
γ ₆	7.5	35	Brain, other (?)	

^a% Amino acid identity: comparison is with the first-listed member of each family.



Lipid modification of trimeric G proteins

P: palmitoylation; M: myristoylation; F: farnesylation; GG: geranylgeranylation

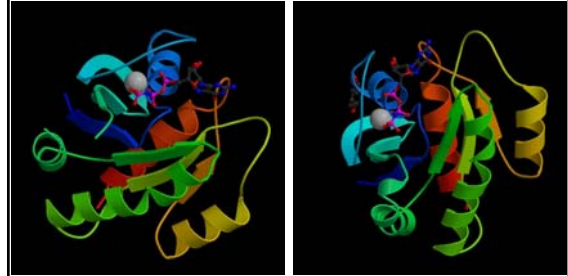
Small GTP-binding proteins

- ¼ Ras: cell growth
- ¼ Rac, Rho, Cdc42: cell migration
- ¼ Rab: membrane trafficking
- ¼ ARF: membrane trafficking
- ¼ Ran: Nuclear transport

61

Ras

R Oncogenes discovered from murine sarcoma viruses (Harvey virus - H-ras, Kirsten virus - K-ras) and N-ras;
 R Activated in 10-50% of human tumors (G12V, Q61L, both of which are resistant to GAP).



Conserved motifs in Ras-like proteins

N-terminus 4-31aa	Guanine nucleotide binding domain 160 aa					Extension 13-49 aa	C-terminus
	PM1 GxxxGKs	G1 F	PM2 T	PM3 DxxG	G2 nKxD	G3 ExSA	Caax xCC CxC

PM: phosphate/Mg²⁺ binding regions; G: guanine base binding regions;
 Caax: a=aliphatic, x= any residue

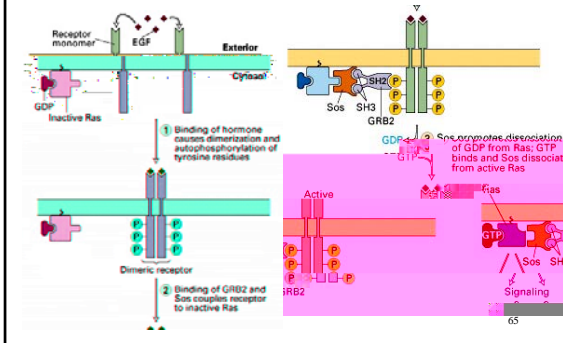
63

The GTP/GDP cycle of small GTP-binding proteins



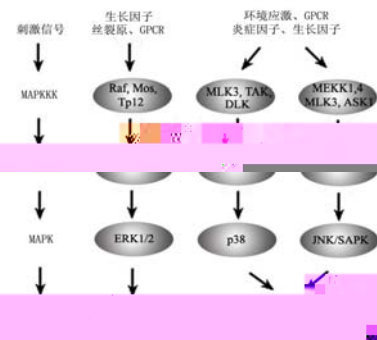
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Ras mediates signaling from Receptor Tyrosine Kinase (RTK)



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MAP kinase pathways



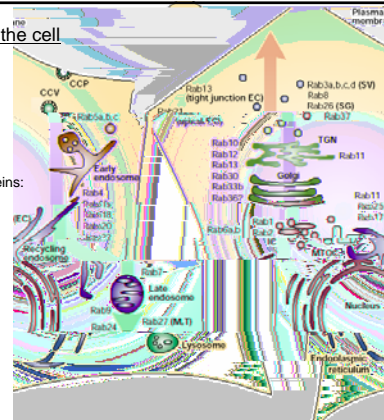
66

The MAP kinase pathways: p38 as an example

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Rab proteins in the cell

Functions of Rab proteins:
Tethering and guiding
vesicle fusion;
Vesicle formation.

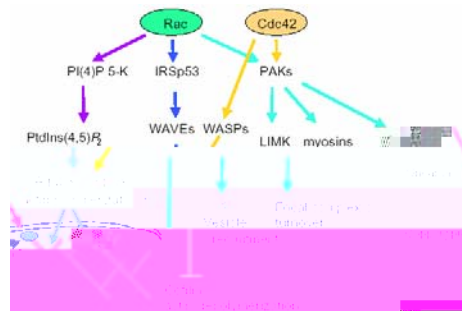


Steps of cell migration

69

Ridley, 2001

Rac-induced lamellipodium extension



70

Ridley, 2001

Rho-induced actin reorganization

71

Ridley, 2001

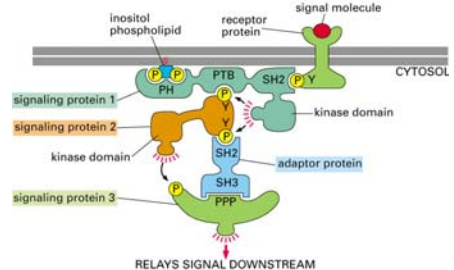
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Protein Modules: protein building blocks

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Interactions between intracellular signaling proteins are mediated by modular binding domains



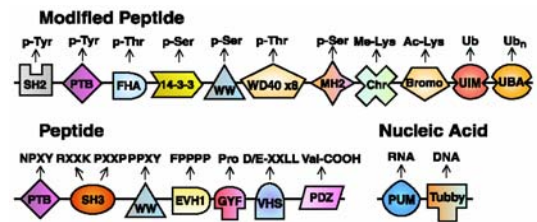
BINDING DOMAIN	MOTIF RECOGNIZED
PH = Pleckstrin homology domain	PIP ₂ = phosphorylated inositol phospholipid
PTB = phosphotyrosine-binding domain	P-Y = phosphotyrosine
SH2 = Src homology 2 domain	
SH3 = Src homology 3 domain	PPP = proline-rich motif

Protein modules

PTB domains: ~ 100-150 aa, bind to NPXY motifs: Shc, IRS-1
 PDZ domains: ~80-90 aa, recognize short peptide motifs (4-5 residues) at the C-terminus of membrane proteins, usually containing a hydrophobic residue at the very end; protein-protein interaction: Dishevelled, FAP
 SH2 (src homology): ~100 aa, binds to phosphotyrosine residues: Src, Grb2, Shc, STAT
 SH3: binds to proline-rich sequences (PXXP): Src, Nck
 WW domains: bind to Pro-rich sequences (XPPXY): Nedd4 (E3 ubiquitin ligase), Smurf, Dystrophin
 Death domains: Fas
 LIM domains: recognize turn-based motifs
 PH (Pleckstrin-homology) domains: associate with phosphoinositides (PI_{3,4}P₂; PI_{4,5}P₂; PI_{3,4,5}P₃), target proteins to the plasma membrane: Akt, SOS
 FYVE domains: associate with phosphoinositides (PI₃P), target proteins to endosomes: EEA1, SARA

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Building blocks - modular interaction domains

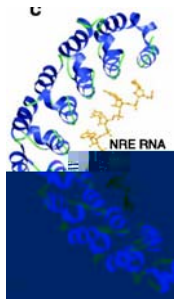


There are about 115 SH2 and 253 SH3 domains encoded by the human genome.

76
Pawson and Nash, 2003

Repeated motifs

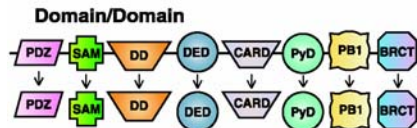
Some interaction domains are assembled from repeated motifs (up to 50 copies): HEAT, TPR, Arm, ankyrin, leucine-rich, Pumilio repeat.



77
Pawson and Nash, 2003

Protein interaction domains

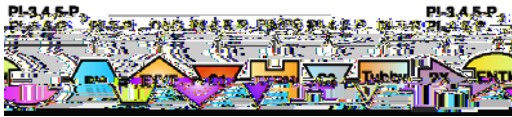
Domains bind to short peptide motifs, whereas a number of modular domains undergo homo- or heterotypic domain-domain interactions.



78
Pawson and Nash, 2003

Protein-lipid interaction domains

Phospholipid



79
Pawson and Nash, 2003

Specificity of SH2 binding to phosphotyrosine

80
Pawson, 1995

谢谢!

81