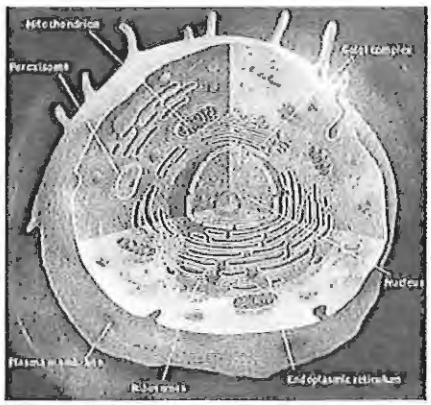
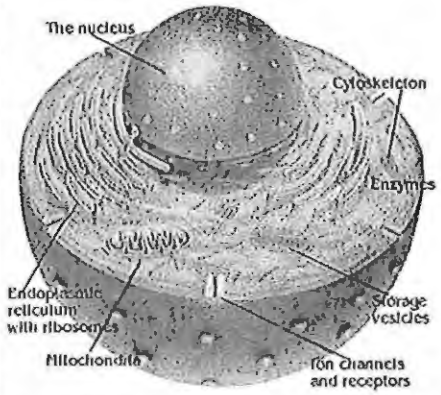
Overview of Signal Transduction



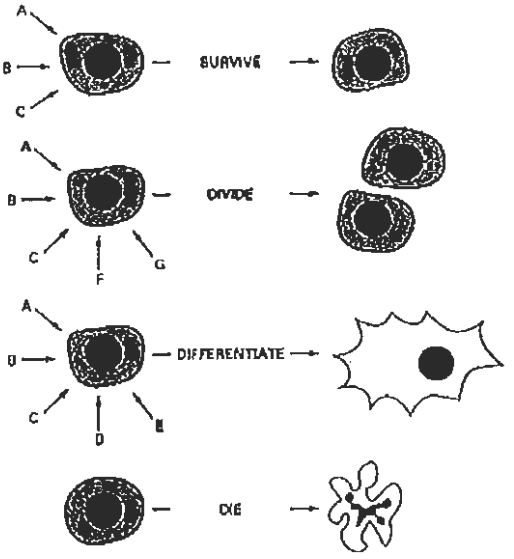


ดร. สุวรา วัฒนพิทยกุล
ภาควิชาเภสัชวิทยา คณะแพทยศาสตร์
มหาวิทยาลัยศรีนครินทรวิโรฒ

OUTLINE

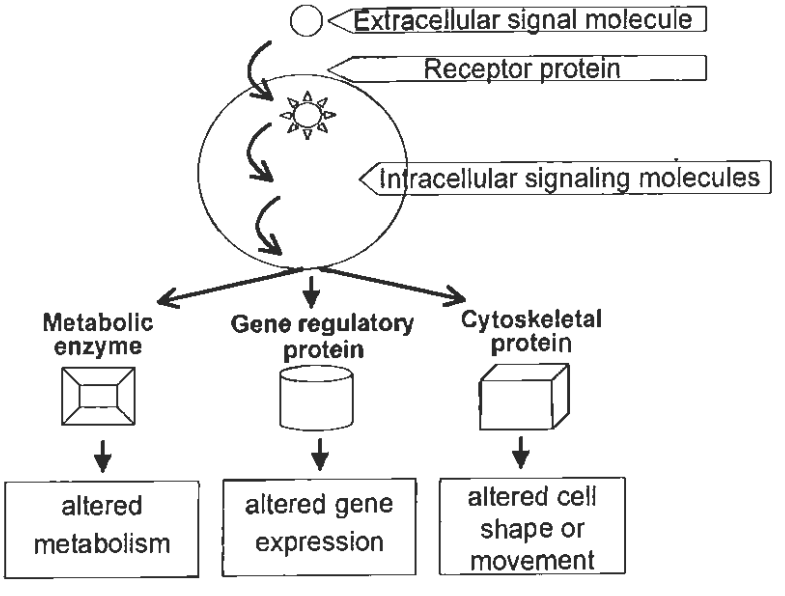
- General principles of cell communication
- Receptor-effector systems
  - Receptors and their effector systems
    - Membrane receptors
    - Nuclear receptors
- Specific Signal Transduction System
  - Tyrosine kinase
  - G protein
  - Apoptosis

Cell Signaling



- Multiple extracellular signals
- Each cell type displays a set of receptor proteins that enables it to respond to a corresponding set of signal molecules.
- These signal molecules work in combinations to regulate the behavior of the cell.
- If deprived of appropriate signals, most cells undergo a form of cell suicide known as programmed cell death, or apoptosis.

Mechanisms of Signal Transduction



Signal Transduction Receptors

I. RECEPTORS

- Membrane Receptors
  - G protein-coupled receptors
  - Ligand-gated ion channel receptors
  - Receptor tyrosine kinase
  - Cytokine receptors
- Intracellular receptors
  - Steroid hormone receptors
  - Thyroid hormone receptors
  - Vitamin D receptors
  - Retinoid receptors

Receptor-Effector Systems

II. EFFECTOR SYSTEMS (Intracellular Signal Transduction Pathways)

- second messengers
  - cAMP
  - cGMP
  - phospholipids and Ca^{2+}
- third messengers
  - protein kinases (PKA, PKC)
  - protein tyrosine kinases
  - serine/theronines kinases
- forth messengers
  - transcription factors

Tyrosine Kinase Signaling

- Receptor tyrosine kinases (RTKs)
  - Structural features
  - Classification
  - Activation
  - MAPK signaling pathway
- Non receptor protein tyrosine kinases (PTKs)
  - Classification
  - JAK/STAT pathway

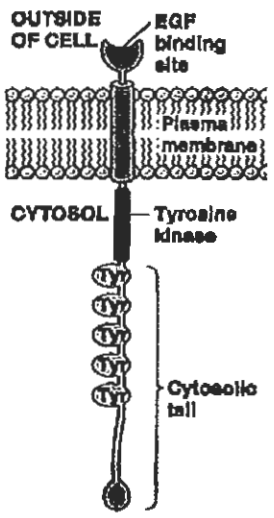
Important Abbreviations

CSF-1 colony-stimulation factor-1
EGF epidermal growth factor
ELAM-1 endothelial leukocyte adhesion molecule 1
EPO erythropoietin
ERK extracellular signal-regulated kinase
G-CSF granulocyte colony-stimulation factor
GAP GTPase-activating proteins
GEF GTP exchange factor
GM-CSF granulocyte-macrophage colony-stimulation factor
Grb2 growth factor receptor binding protein-2
ICAM-1 intercellular cell adhesion molecule 1
IFN interferon
IRF-1 interferon regulatory factor 1
MAP mitogen-activated protein
MAPK mitogen-activated protein kinase
MAPKK or MEK mitogen-activated protein kinase kinase
MEK MAP kinase/ERK kinase
MKK MAPKK (humans)
PDGF platelet-derived growth factor
PH pleckstrin homology
PLC-γ phospholypase C-γ
ras rat sarcoma viruses
Rho Ras homology
SAPK/JNK stress-activated protein kinase/Janus kinase or c-Jun N-terminal kinase
SH2, SH3 Src homology domain 2, 3, respectively
Shc Src homology and collagen
SOS son of sevenless
v-Src avian retroviral; Src Rous sarcoma virus
TH Tec homology
Tyk2 tyrosine kinase 2
VCAM-1 vascular cell adhesion molecule 1

Receptor Tyrosine Kinases

- These receptors traverse the membrane only once
- Receptor has intrinsic enzyme activity (kinase domain)
- Respond exclusively to peptide stimuli
  - cytokines
  - mitogen growth factors: e.g., platelet derived growth factor (PDGF), epidermal growth factor (EGF)

Structural Features of RTKs



(a) Structure of the epidermal growth factor (EGF) receptor

Four major domains:

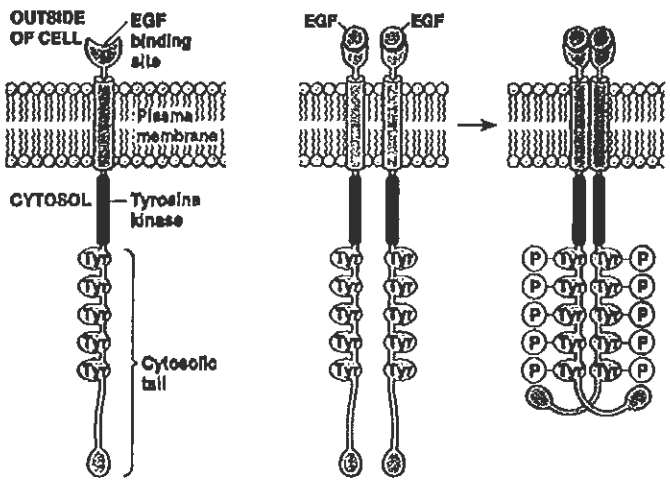
- Extracellular binding domain
- transmembrane domain
- Intracellular tyrosine kinase domain
- Intracellular regulatory domain

Classification of RTKs

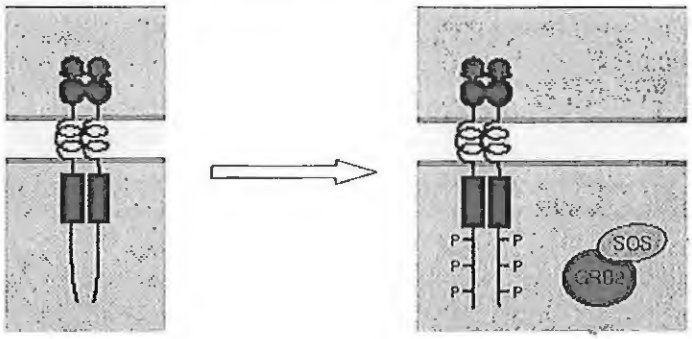
Class	Examples	Structural Features of Class
I	EGF receptor, NEU/HER2, HER3	cysteine-rich sequences
II	insulin receptor, IGF-1 receptor	cysteine-rich sequences; characterized by disulfide-linked heterotetramers
III	PDGF receptors, c-Kit	contain 5 immunoglobulin-like domains; contain the kinase insert
IV	FGF receptors	contain 3 immunoglobulin-like domains as well as the kinase insert; acidic domain
V	vascular endothelial cell growth factor (VEGF) receptor	contain 7 immunoglobulin-like domains as well as the kinase insert domain
VI	hepatocyte growth factor (HGF) and scatter factor (SC) receptors	heterodimeric like the class II receptors except that one of the two protein subunits is completely extracellular. The HGF receptor is a proto-oncogene that was originally identified as the Met oncogene
VII	neurotrophin receptor family (trkA, trkB, trkC) and NGF receptor	contain no or few cysteine-rich domains; NGFR has leucine rich domain

Receptor Tyrosine Kinases

Structure of RTK dimerization autophosphorylation



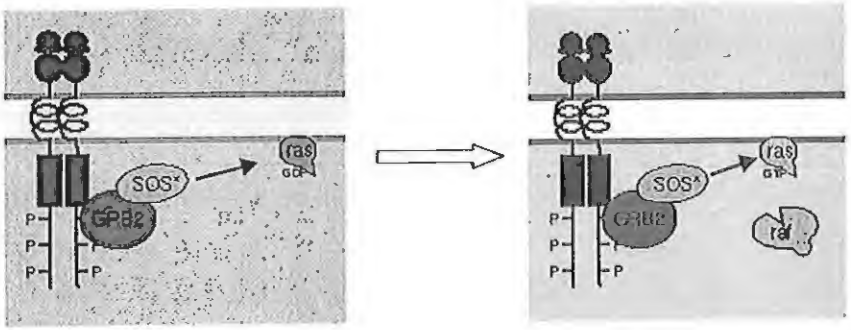
Receptor Tyrosine Kinase Signaling



- Ligand binding
- Receptor dimerization
  - increase activity of kinase domain
  - create "docking site"

- Autophosphorylation
- Recruitment of SH2-containing protein (adapter protein, e.g. GRB2)

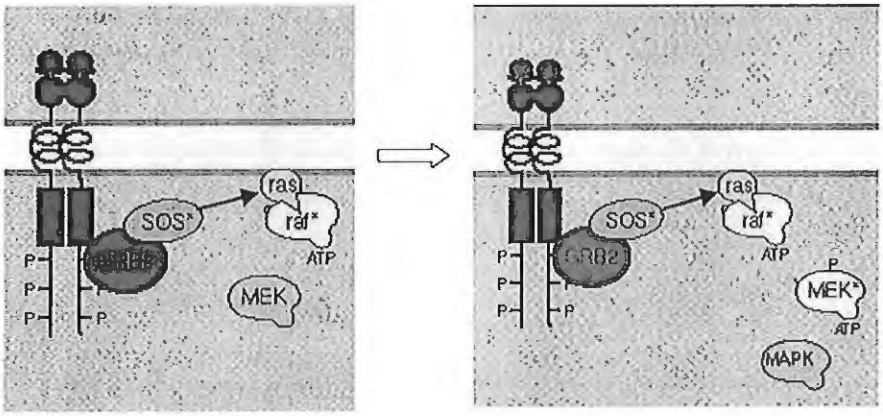
Receptor Tyrosine Kinase Signaling



- Binding of the adaptor proteins to the phosphorylated tyrosine residues (*docking sites*)

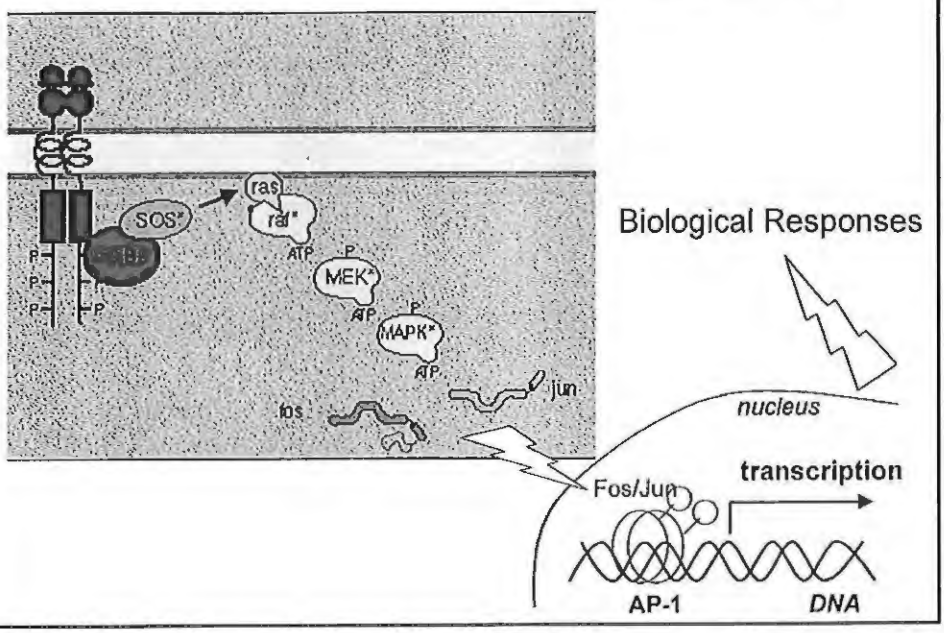
- Ras is activated, followed by the activation of Raf

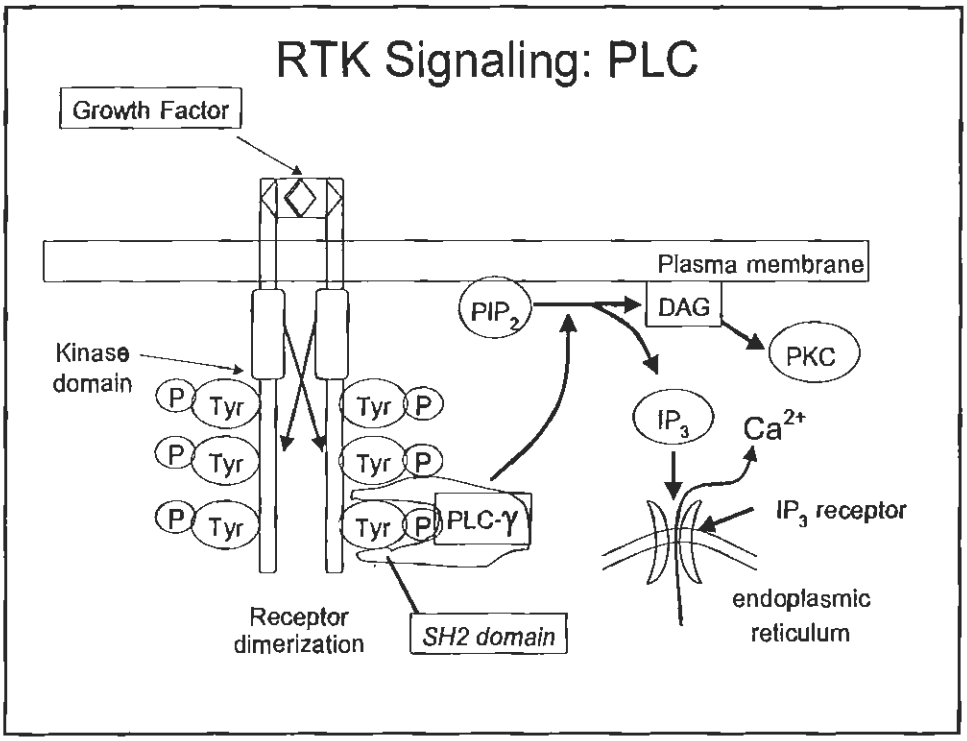
Receptor Tyrosine Kinase Signaling



- Activation of the proteins in the cascade of MAPK signaling pathway

Receptor Tyrosine Kinase Signaling





Signaling Through Protein Tyrosine Kinases
(phosphorylation & dephosphorylation)

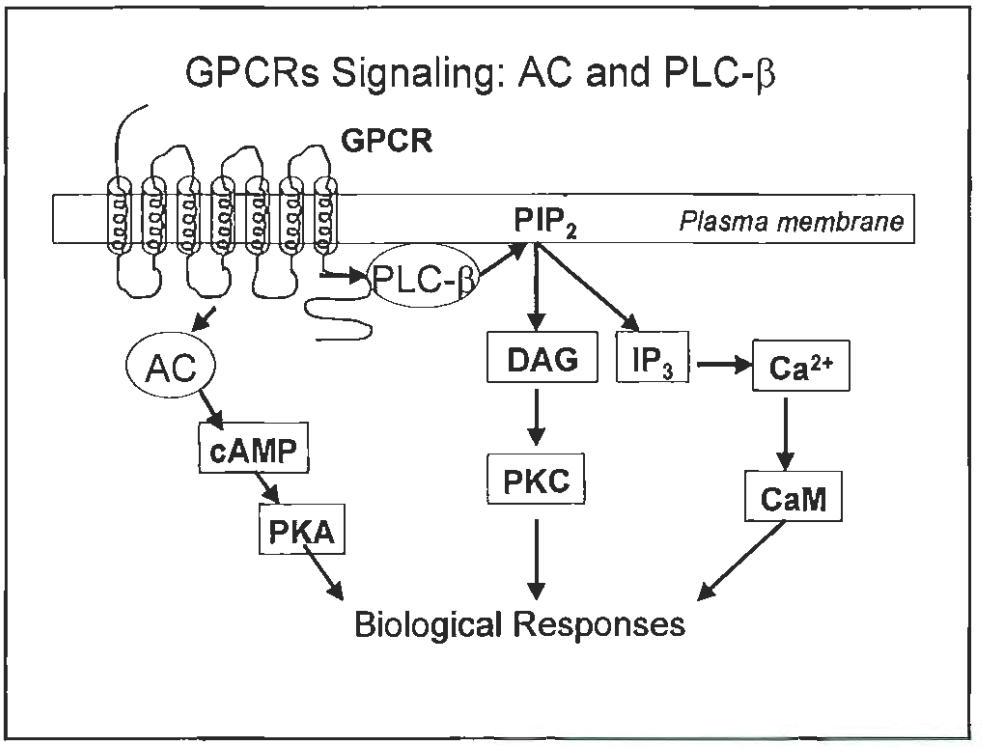
- Tyrosine kinase adds a phosphate group (Pi) specifically to tyrosine residue
- Phosphatase removes Pi
- Phosphorylation state alters shape (conformation) of protein and changes its function

MAPK

- MAPK: a familly serine/theronine kinases
- MAPK subfamilies
  - ERKs
    - Raf-MEK-ERK pathway
    - Cell proliferation, survival and differentiation
  - SAPK/JNK
    - Stress: e.g., UV
    - CD40: a receptor related to the TNF and IL-1 receptors which binds CD40 ligand and elicits a variety of effects in B cells
  - P38
    - Inflammation
    - Cell death

Ras, Raf and MAP Kinase Pathway

- Actvated in response to growth factors and other stimuli resulted in proliferation, differentiation, cell survival, inflammation, and cell death
- MAP Kinases (mitogen-activated protein kinases) is a family of protein-serine/threonine kinases
- The first effector protein of this pathway is Ras, a GTP-binding protein
- Activation of Ras leads to activation of Raf protein serine/threonine kinase, which phosphorylates and activates MAP Kinase and down stream signaling molecules



GPCR-linked Effector Systems

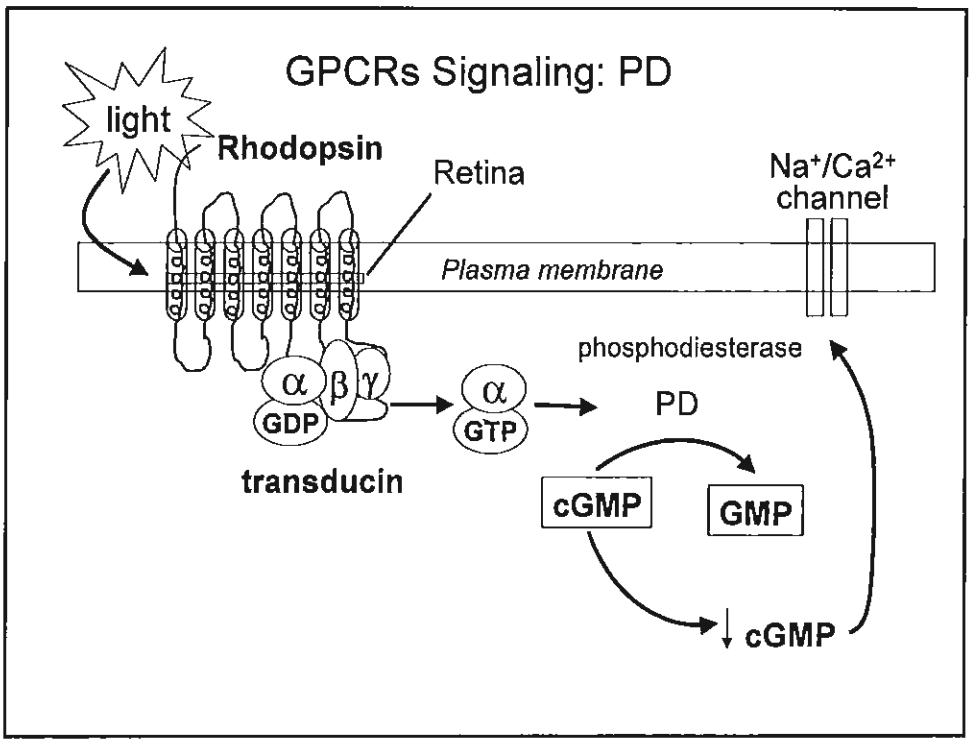
3. GPCRs that are coupled to transducin that activate a phosphodiesterase

   3.1 leading to a decrease in the level of cGMP

   - results in the closing of a Na^+/Ca^{2+} channel --> hyperpolarization of the cell
   - e.g., role of vitamin A in vision

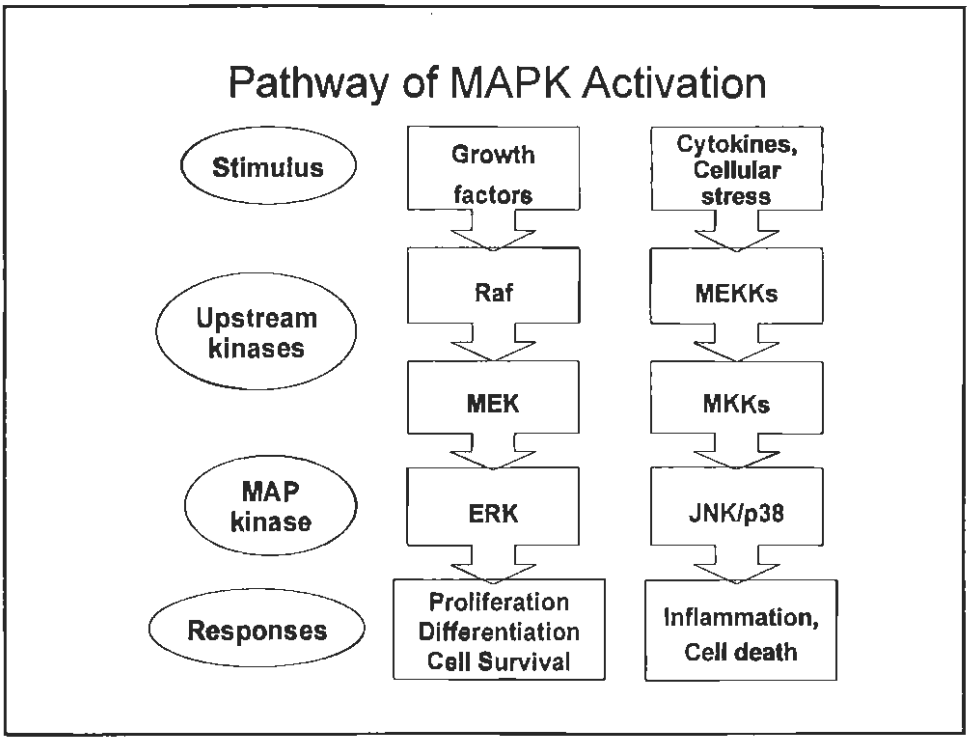
   3.2 leading to increase in cGMP formation

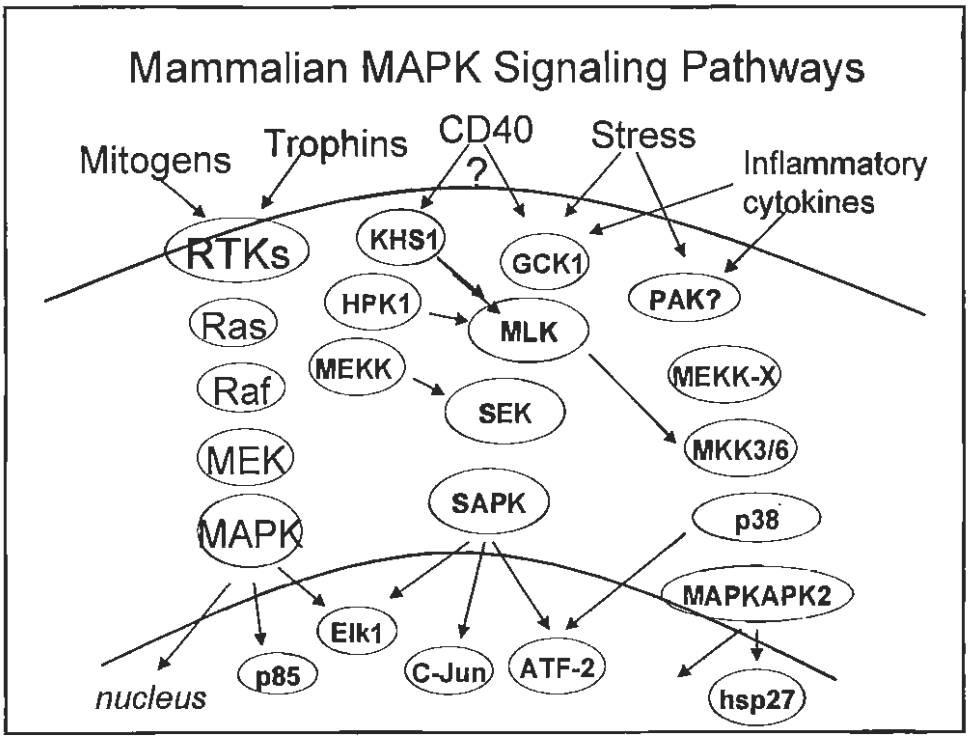
   - e.g., angiotensin type II (AT_2) receptor



GPCR-linked Effector Systems

4. GPCRs signaling to MAPK/ERK
   - Proliferative pathway


Pathway of MAPK Activation
Stimulus
Growth factors
Cytokines, Cellular stress
Upstream kinases
Raf
MEKKs
MEK
MKKs
MAP kinase
ERK
JNK/p38
Responses
Proliferation Differentiation Cell Survival
Inflammation, Cell death

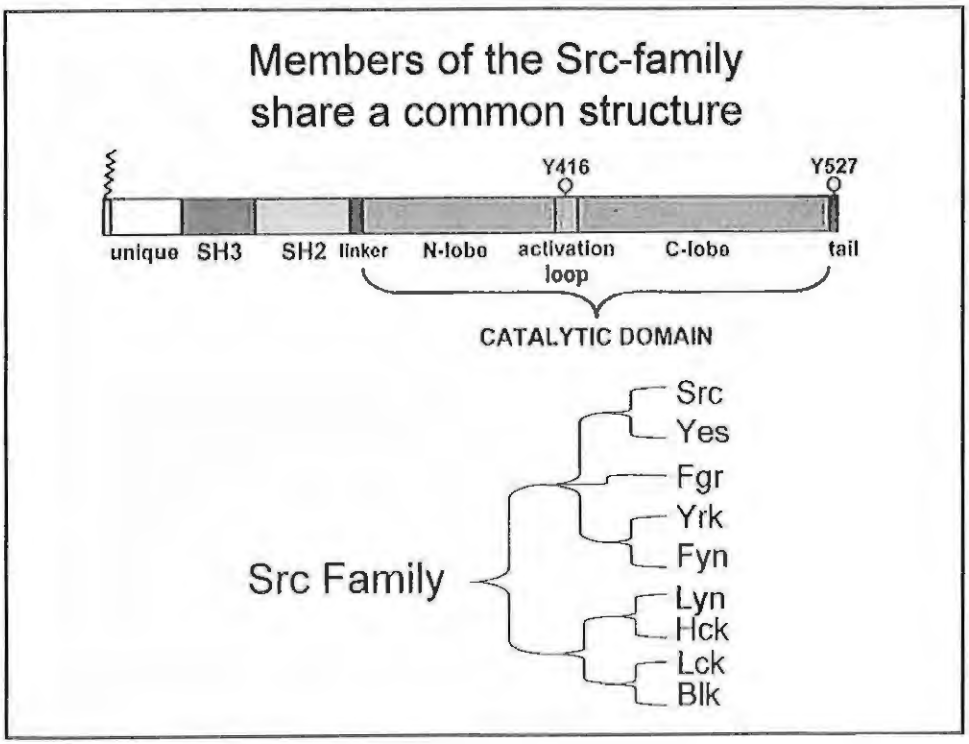

Mammalian MAPK Signaling Pathways
Mitogens
Trophins
CD40
?
Stress
Inflammatory cytokines
RTKs
KHS1
GCK1
PAK?
HPK1
Ras
MLK
MEKK
MEKK-X
Raf
SEK
MKK3/6
MEK
SAPK
p38
MAPK
MAPKAPK2
Elk1
p85
C-Jun
ATF-2
hsp27
nucleus

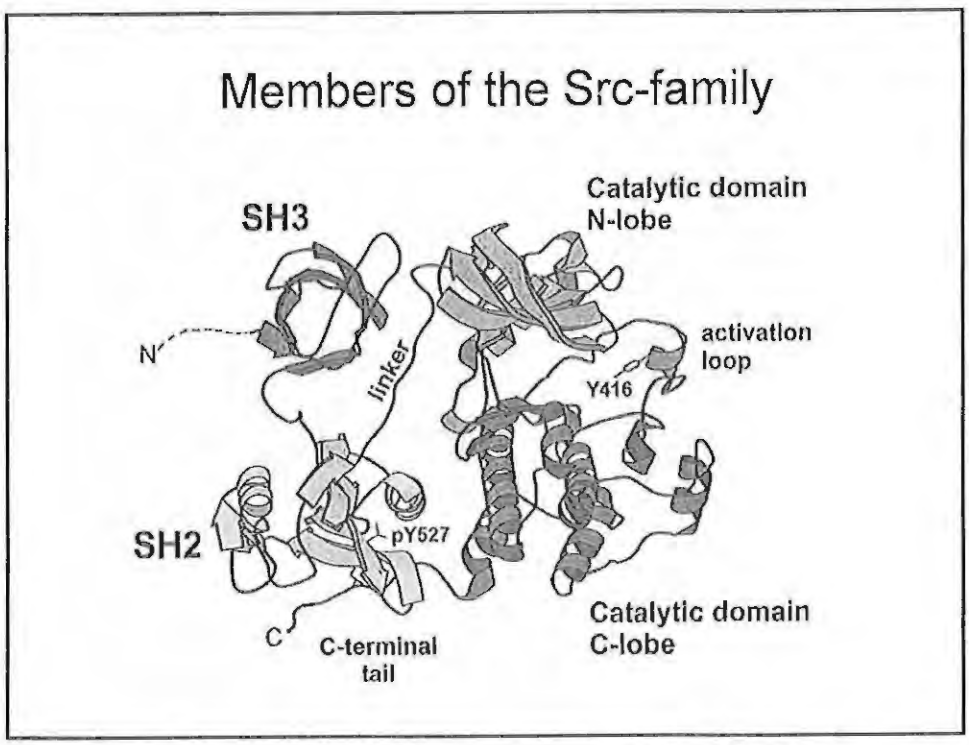
Non-receptor protein tyrosine kinases (PTKs)

- Most PTKs couple to the receptors that lack intrinsic enzymatic activity e.g.,
  - cytokine recptors
  - CD4 and CD8 cell surface glycoprotein of T cells
  - T cell antigen receptor (TCR)
- PTKs are classified into families
  - ***Src****
  - ***Jak****
  - Fps/Fes
  - Tec/Btk
  - Syk/ZAP70

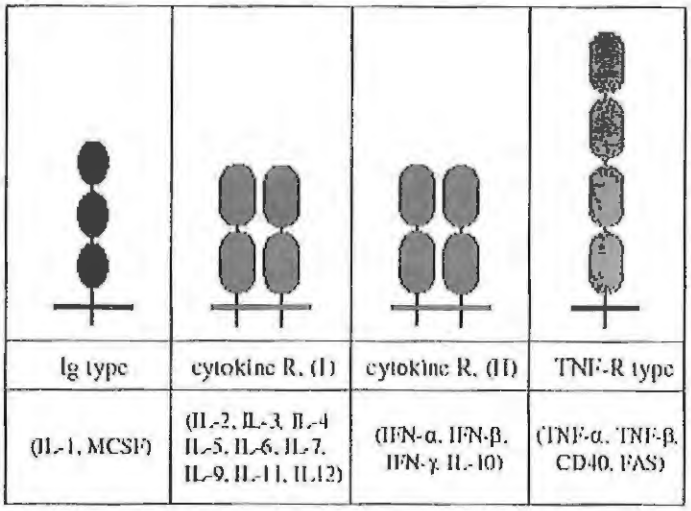
PTKs: Src

- SH2 domain
  - ~ 100 amino acids ---> binding pocket
  - binds to phosphorylated tyrosine residues of the receptor
- SH3 domain
  - ~ 60 amino acids
  - protein-protein interaction
  - 10-residue consensus sequence: XPXXPPPFXP (X = any amino acid; P = proline; F = phenylalanine)


Members of the Src-family
share a common structure
Y416
Y527
unique
SH3
SH2
linker
N-lobe
activation loop
C-lobe
tail
CATALYTIC DOMAIN
Src Family
Src
Yes
Fgr
Yrk
Fyn
Lyn
Hck
Lck
Blk

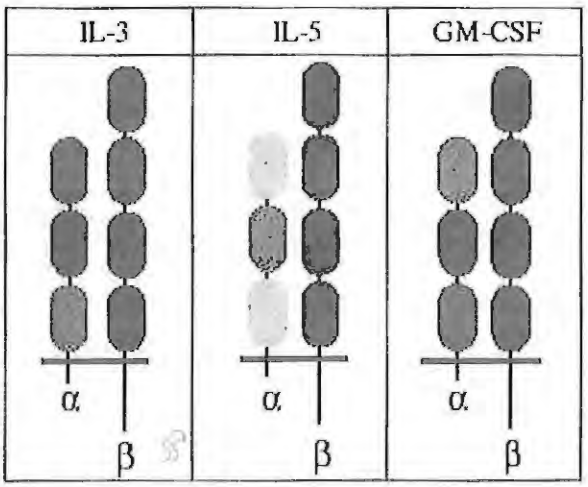

Members of the Src-family
SH3
Catalytic domain
N-lobe
N
linker
activation
loop
Y416
SH2
pY527
C
C-terminal
tail
Catalytic domain
C-lobe

Cytokine Signaling

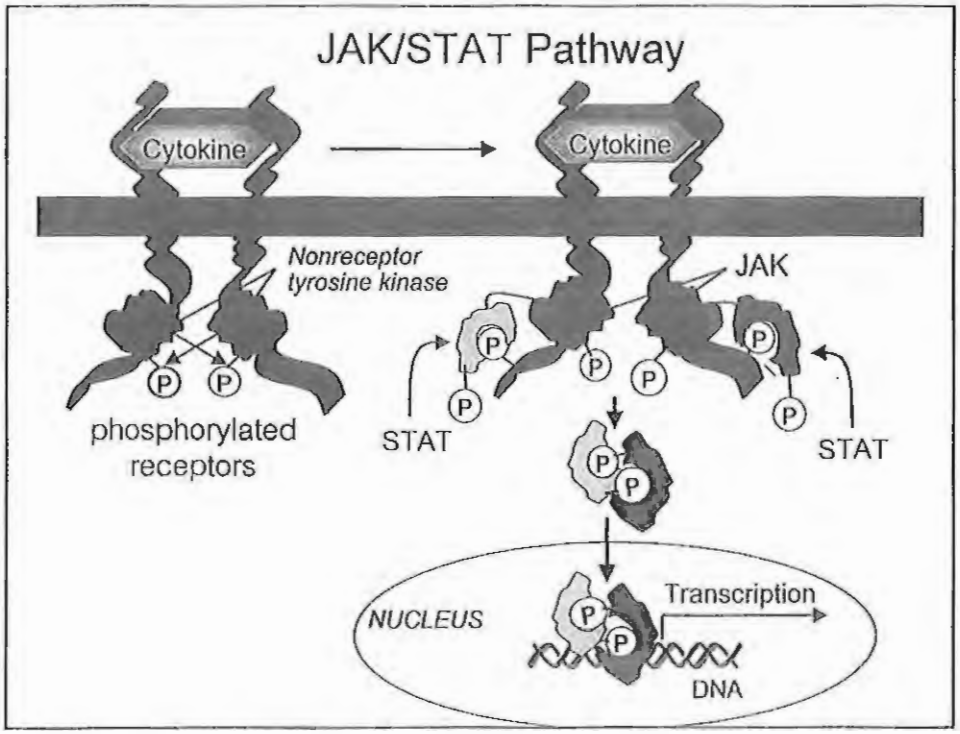


the domain structure of non chemokine, cytokine receptors

Cytokine Signaling



- different α chains but signal transduction is mediated by a common β chain.



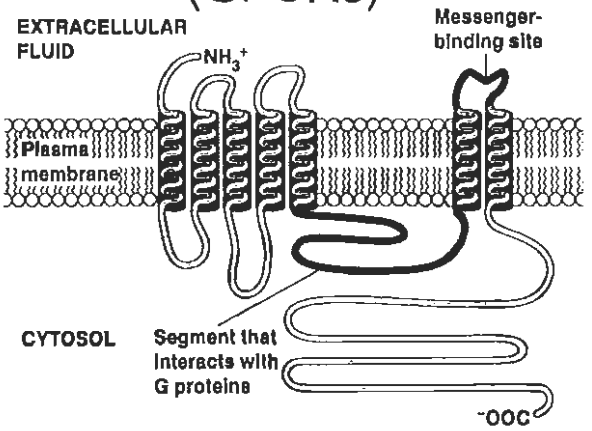
JAK/STAT UTILIZATION

RECEPTOR FAMILY	RECEPTOR	JAKs	STATs
gp140	IL-3, IL-5, GM-CSF	Jak2	Stat5
gp130	IL-6, IL-11, OSM, CNTF G-CSF, LIF, CNTF, CT-1 IL-12 Leptin	Jak1, -2, Tyk2 Jak2, Tyk2 Jak2	Stat1,-3,-5 Stat4 Stat3,-5
IL-2	IL-2, IL-7, IL-9, IL-15 IL-4 IL-13	Jak1, -3 Jak1, -3 Jak1, -2, Tyk2	Stat5, -3, -1 Stat6 Stat6
Growth Hormone	GH TPO PRO, EPO	Jak2 Jak2 Jak2	Stat5, -3, -1 Stat3,-5 Stat5
Interferon	IFNα, IFNβ IFNγ IL-10	Jak1, Tyk2 Jak1,-2 Jak1, Tyk2	Stat1, -2, -3, -5 Stat1 Stat3,-1
RTKs	EGF/ErbB, TGFα, PDGF CSF-1 Insulin bFGF HGF		Stat1, -3, -5 Stat5,-3 Stat1,-3 Stat3
GPCR	Angiotensin Serotonin a-Thrombin CXCR4	Jak2, Tyk2 Jak2 Jak2,-3	Stat1,-2,-3 Stat3 Stat3

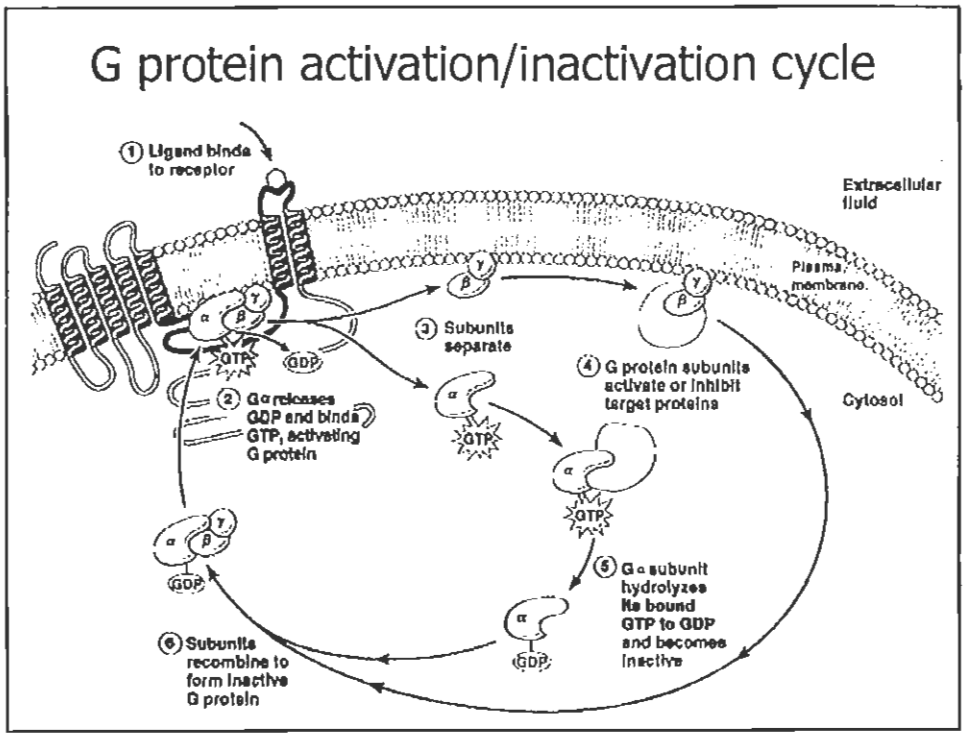
G protein-coupled receptors (GPCRs)

- Diverse physiological functions
  - small biogenic amines: 5-HT, dopamine, acethylcholine (Ach)
  - glycoprotein hormones: TSH, FSH, luteinizing hormine/choriogonadotropin (LH/CG)
  - sensory systems: vision, smell and taste
  - miscellaneous ligands: neurotransmitters, nucleotides, prostanoids, Ca^{2+}, and lipid
  - certain chemokine receptors: CCR-5 receptor

G protein-coupled receptors (GPCRs)



- Seven transmembrane alpha helices
- The primary messenger binds to the extracellular portion of the receptor
- This binding causes an intracellular portion of the receptor to activate an adjacent G protein.



G protein

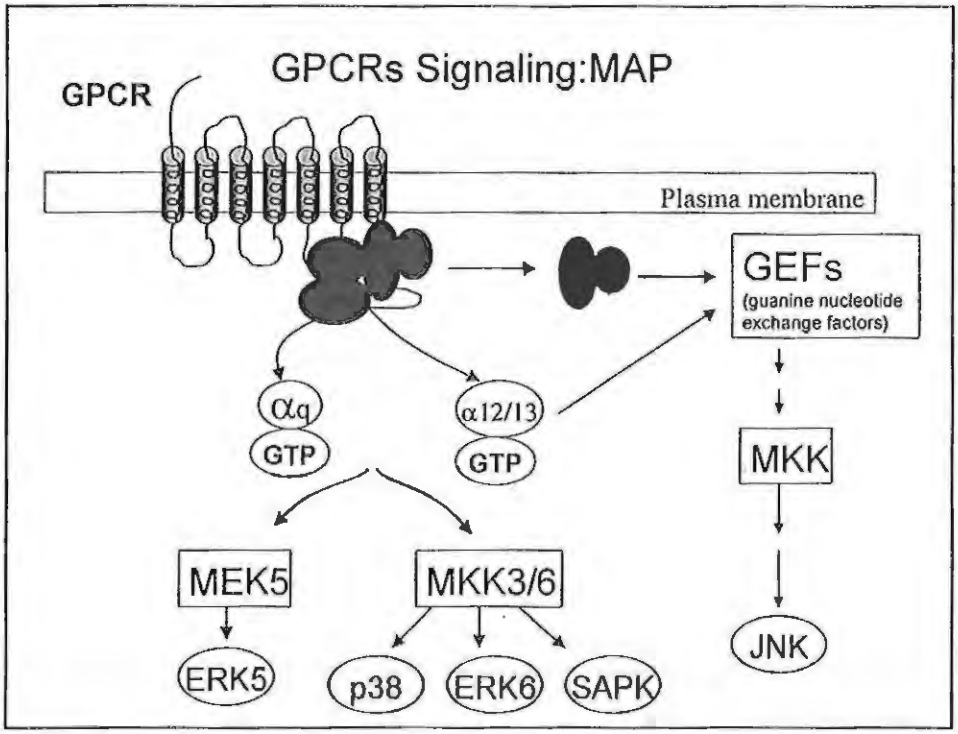
- G protein subunits
  - alpha (Gα)
  - beta (Gβ)
  - gamma (Gγ)
- Inactive State: Gα-GDP
- Active State: Gα-GTP

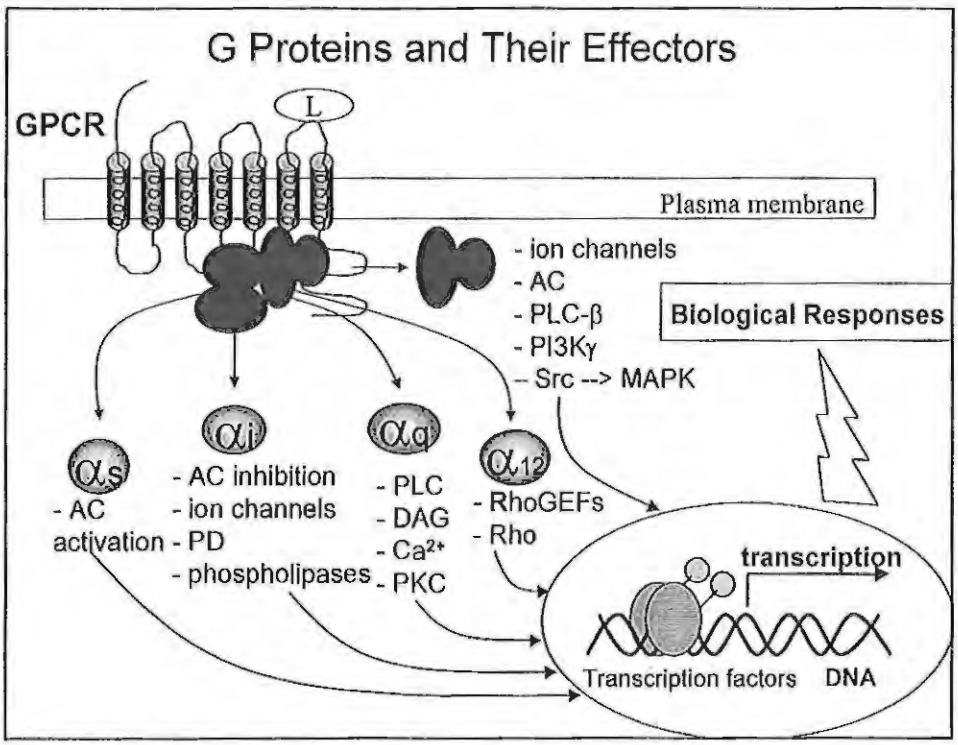
GPCR-linked Effector Systems

1. GPCRs that modulate adenylate cyclase (AC) activity
   1) Gs: increase the production of cAMP
      - leading to an activation of protein kinase A (PKA)
      - e.g., beta-adrenergic receptors, glucagon, odorant molecule receptors
   2) Gi: decrease the production of cAMP
      - repress adenylate cyclase activity
      - e.g., alpha-adrenergic receptors

GPCR-linked Effector Systems

2. GPCRs that activate phospholipase C-gamma (PLC-β)
   - leading to the hydrolysis of polyphosphoinositides (PIP_2) generating the second messengers, diacylglycerol (DAG) and inositoltrisphosphate (IP_3)
   - e.g., angiotensin type I (AT_1) receptor, bradykinin, vasopressin receptors

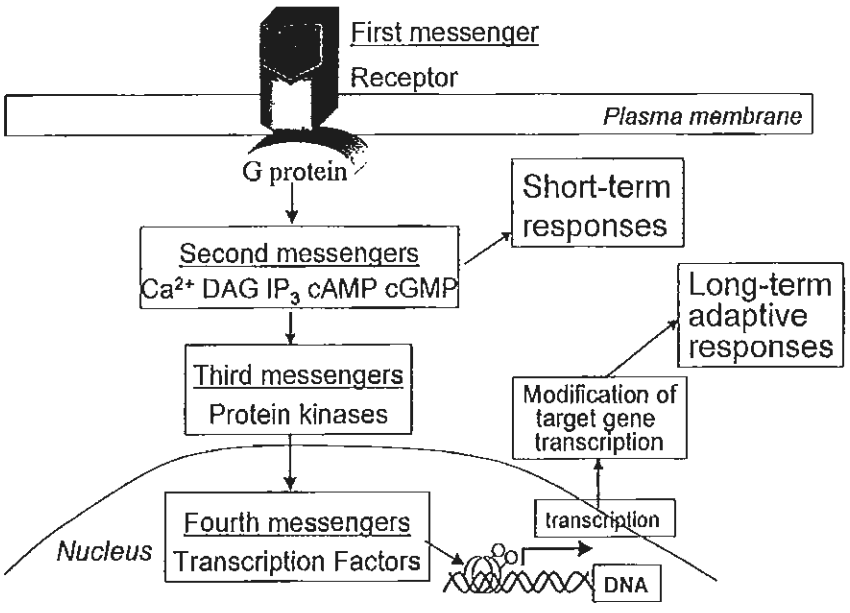

GPCRs Signaling:MAP
GPCR
Plasma membrane
GEFs
(guanine nucleotide exchange factors)
αq
GTP
α12/13
GTP
MKK
MEK5
MKK3/6
JNK
ERK5
p38
ERK6
SAPK


G Proteins and Their Effectors
GPCR
L
Plasma membrane
- ion channels
- AC
- PLC-β
- PI3Kγ
- Src --> MAPK
Biological Responses
αs
- AC activation
αi
- AC inhibition
- ion channels
- PD
- phospholipases
αq
- PLC
- DAG
- Ca2+
- PKC
α12
- RhoGEFs
- Rho
transcription
Transcription factors
DNA

G Proteins and Their Effectors

G Protein	Effectors	Results
I. αs	• Adenylate cyclase (AC), activation	• Increase cAMP
II. αi	• AC, inhibition	• Decrease cAMP
	• Phospholipases • Phosphodiesterase	• Increase or decrease enzyme activity
	• Ion channels	• Open or close
III. αq	• Phospholipase C-gamma (PLC-γ)	• Hydrolysis of PIP_2 → DAG and IP_3
	• PLC-β	• Activation of PKC
IV. $\alpha 12/13$	• Rho, RhoGEFs (guanine nucleotide exchange factors)	• Catalyze the exchange of GDP for GTP
V. $\beta\gamma$	• Ion channels	• Open or closed
	• PI3Kγ, PLC-β, AC, JNK	• Activation or Inhibition

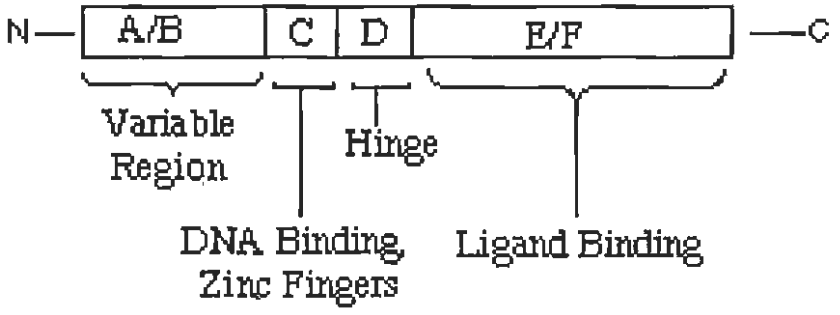
Transcriptional Regulation by GPCRs



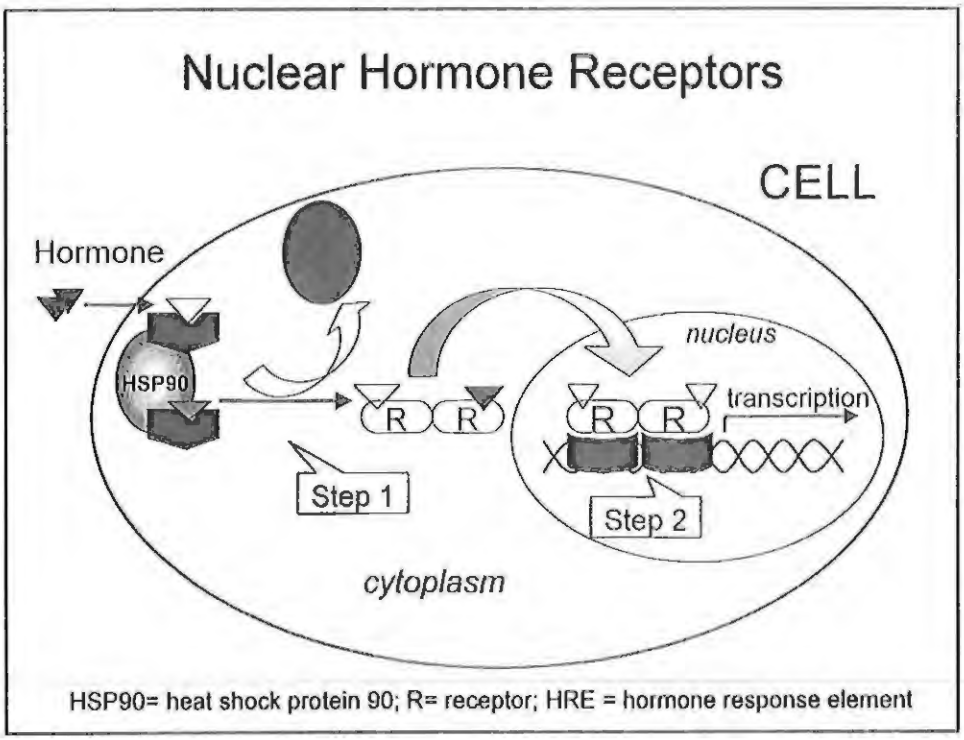
Intracellular Receptors

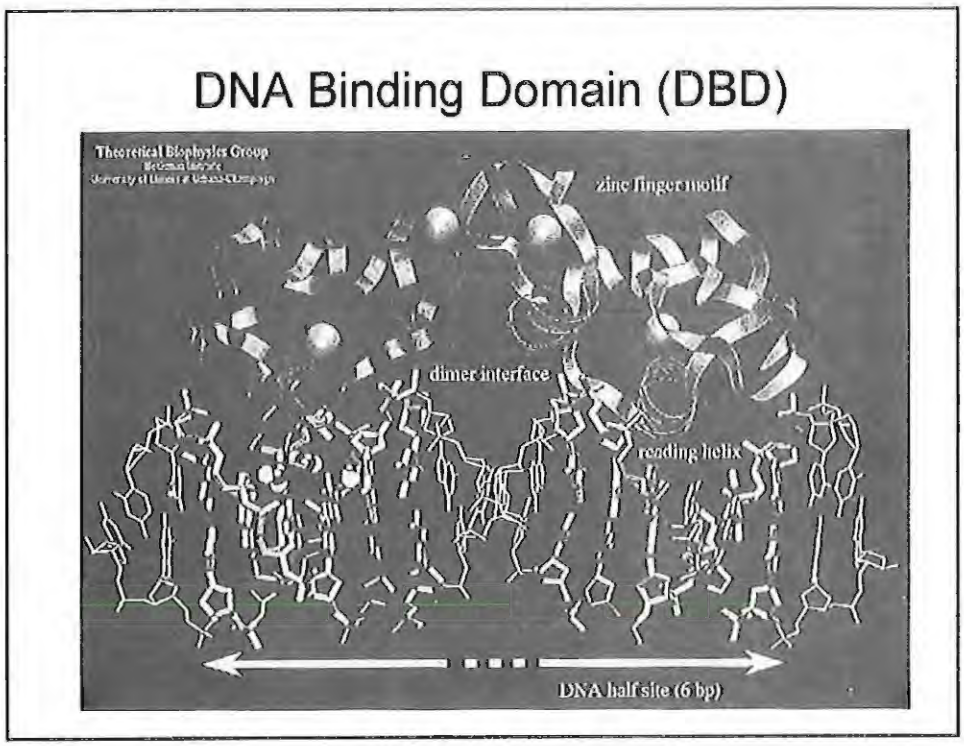
- Nuclear hormone receptors
  - control development and differentiation of skin, bone and behavioral centers in the brain
  - continually regulate reproductive tissues
  - are ligand-activated transcription factors that regulate gene expression by interacting with specific DNA sequences upstream of their target genes
  - have a two-step mechanism of action
    1) binding of the hormone to its receptor
    2) receptor binding to DNA and regulation of transcription

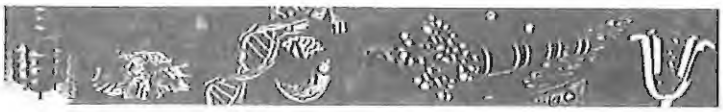
Nuclear Hormone Receptors



Structure of Steroid Hormone Receptors


Nuclear Hormone Receptors
CELL
Hormone
HSP90
R
R
nucleus
R
R
transcription
Step 1
Step 2
cytoplasm
HSP90= heat shock protein 90; R= receptor; HRE = hormone response element


DNA Binding Domain (DBD)
Theoretical Biophysics Group
zinc finger motif
dimer interface
reading helix
DNA half site (6 bp)



»Apoptosis

- Programmed cell death
- Characeterized by blebbling, vacuole formation, chromatin condensation, and DNA fragmentation
- Signaling pathways in apoptosis
  - External signals : death receptors - death effectors
  - intracellular signals: cytochrome C
- To die or not to die?

Necrosis versus Apoptosis

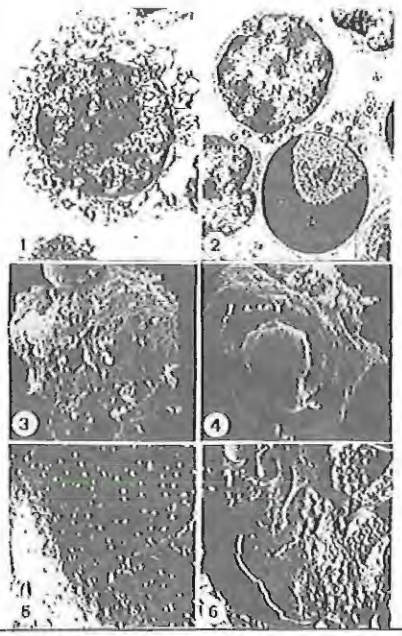


FIGURE 1: A necrotic cell: the disruption of plasma membrane and organelles is observable.

FIGURE 2
An apoptotic (A) and a normal (N) cell. The characteristic chromatin rearrangement appears in A.

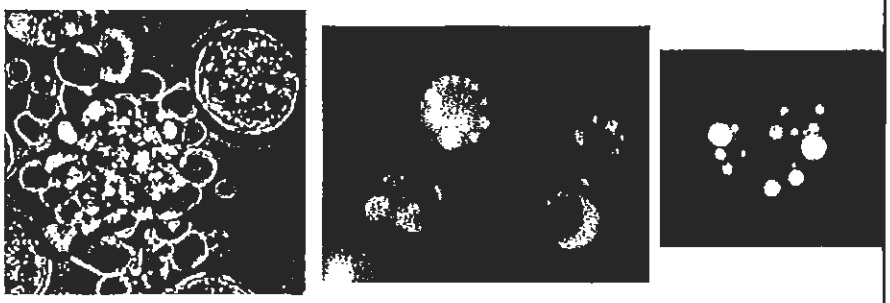
FIGURE 3
A necrotic cell. Numerous lesions appear on the cell surface.

FIGURE 4
An apoptotic cell. Surface blebbing is evident.

FIGURE 5
A normal cell, nuclear envelope. The regular distribution of nuclear pores is visible.

FIGURE 6
An apoptotic cell. The nuclear envelope shows a characteristic clustering (asterisc) of nuclear pores.

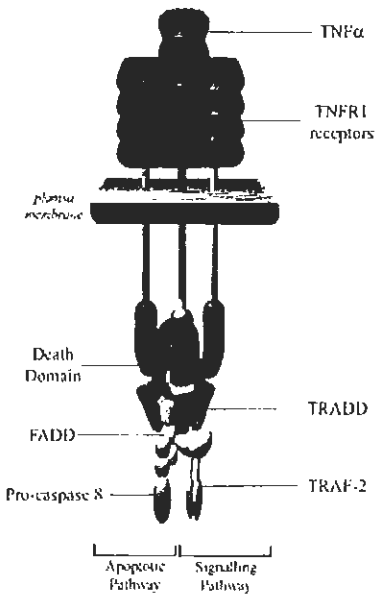
Apoptosis



SF21 cell undergoing apoptosis

human malignant B-lymphocytes. Apoptosis was detected by fluorescence microscopy after staining of DNA with acridine orange.

TNFR - Death Receptor



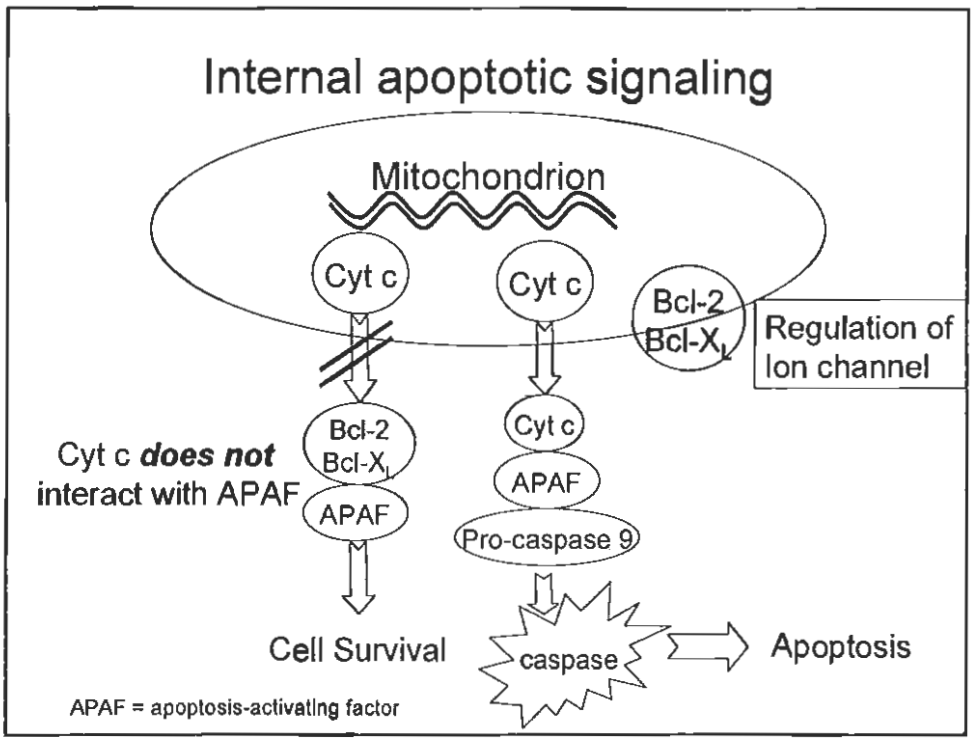
Prototype of death receptor are:

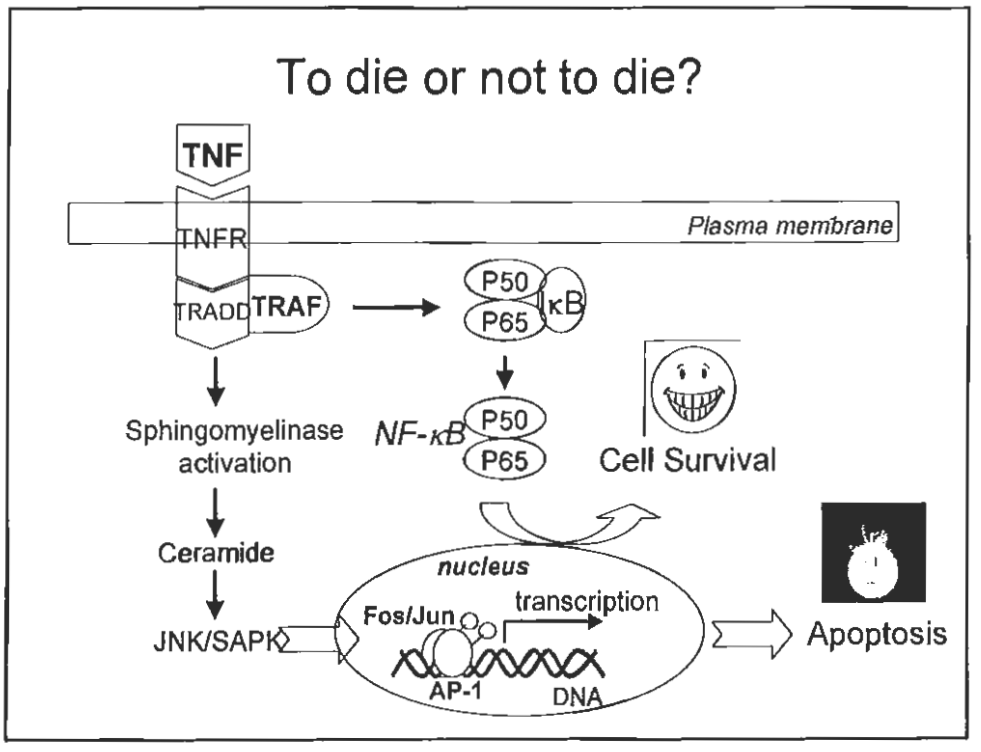
1. CD95 (Fas Apo1): FADD
2. TNFR-I: TRADD

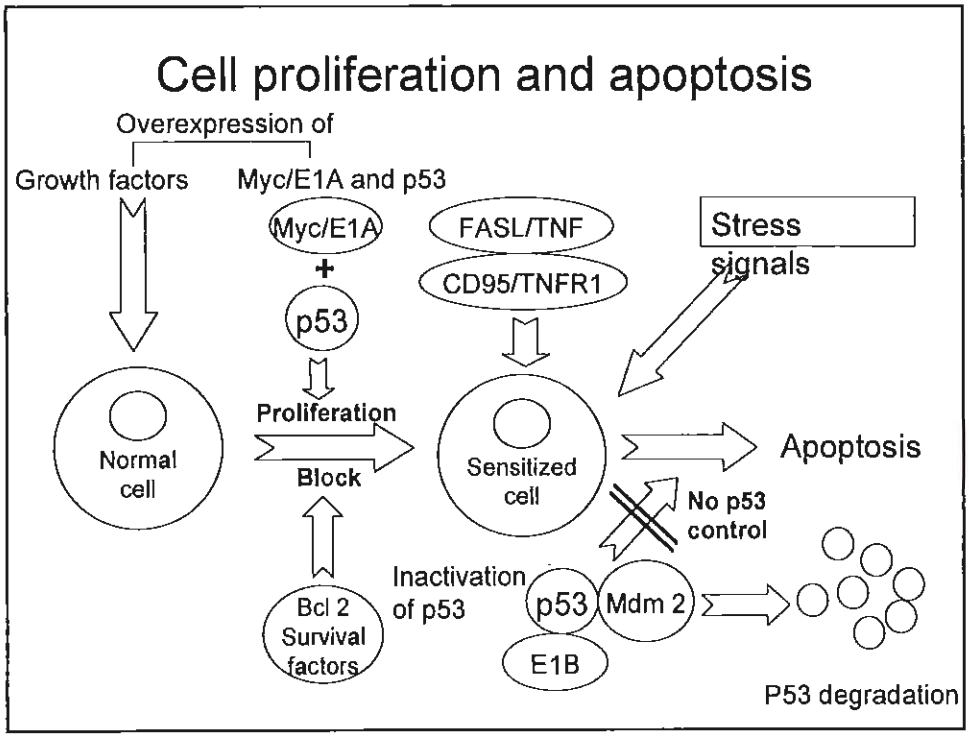
TNFR = tumor necrosis factor receptor

TRADD = TNFR-associated death domain

FADD = Fas death domain


Internal apoptotic signaling
Mitochondrion
Cyt c
Cyt c
Bcl-2
Bcl-X$_L$
Regulation of Ion channel
Cyt c does not interact with APAF
Bcl-2
Bcl-X$_L$
APAF
Cyt c
APAF
Pro-caspase 9
Cell Survival
caspase
Apoptosis
APAF = apoptosis-activating factor


To die or not to die?
TNF
TNFR
Plasma membrane
TRADD
TRAF
P50
P65
κB
Sphingomyelinase activation
NF-κB
P50
P65
Cell Survival
Ceramide
nucleus
transcription
Fos/Jun
JNK/SAPK
AP-1
DNA
Apoptosis


Cell proliferation and apoptosis
Overexpression of
Growth factors
Myc/E1A and p53
Myc/E1A
+
p53
FASL/TNF
CD95/TNFR1
Stress signals
Normal cell
Proliferation
Block
Sensitized cell
Apoptosis
No p53 control
Inactivation of p53
Bcl 2 Survival factors
p53
Mdm 2
E1B
P53 degradation