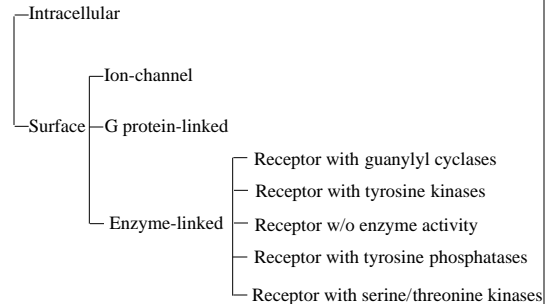


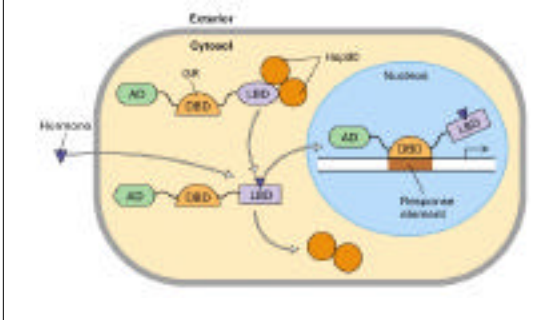
Ligands

- 1) Small lipophilic molecules that bind to intracellular receptors: steroids, thyroxine, and retinoic acids
- 2) lipophilic molecules that bind to cell surface receptors: prostaglandins
- 3) Hydrophilic molecules that bind to cell surface receptors:
 - a) peptides: growth hormones, cytokines
 - b) small charged molecules: epinephrine, histamine
- 4) Cell surface ligands that bind to cell surface receptors: TNF family, Boss, MHC

RECEPTORS



Gene Activation by the Glucocorticoid Receptor



G protein-linked cell surface receptors

Over 100 family members: Serotonin, Acetylcholine, Rhodopsin, Olfactory, Yeast mating factor.

Type I membrane, Pass plasma membrane seven times

Extracellular portion binds to ligands

Intracellular portion binds to trimeric G proteins

The signal-transducing G proteins

Function as signaling switches: Active G proteins bind GTPs

Inactive G proteins bind GDPs

1) Trimeric G proteins: G α , G β , G γ

Downstream effector molecules:

A) Adenylyl cyclase: use ATP to generate cAMP

cAMP dependent kinases: Glycogen breakdown, CREB

B) Phospholipase C- β : cut PIP2 into diacylglycerol and IP3

a) Activates PKC

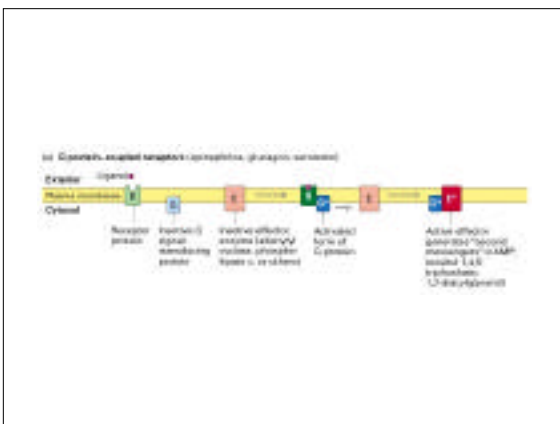
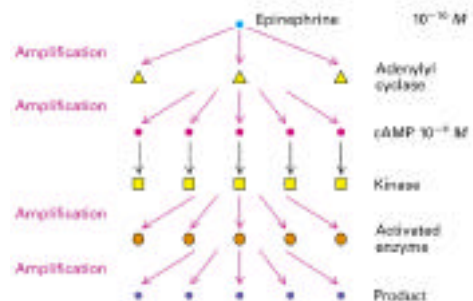
b) Releases calcium

C) Directly regulates Ion channels

2) Monomeric G proteins: Ras superfamily

Signal Amplification via Second Messengers

cAMP, cGMP, Ca²⁺, and Phospholipids



Receptor Tyrosine kinases

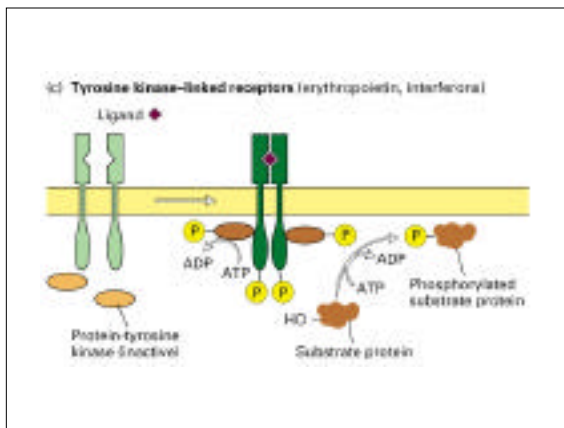
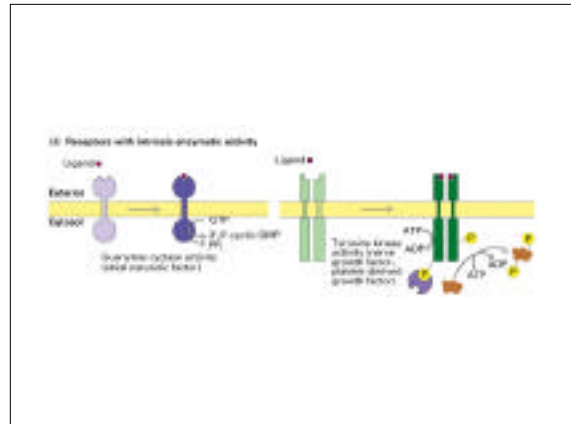
A single hydrophobic transmembrane domain
 An extracellular domain for ligand binding
 A cytoplasmic tail contains a tyrosine kinase domain and tyrosin residues
 Ligand binding will cause dimerization of the receptors, which will induce trans-phosphorylation on tyrosine residues.
 Signals transduced through binding of SH2-containing proteins to phosphotyrosines.

- A) Adaptor proteins: Grb2, Shc, NCK, and Crk.
- B) Enzymes: Src, GAP, Syp, PI3K, PLC γ

Receptors without intrinsic enzyme activity

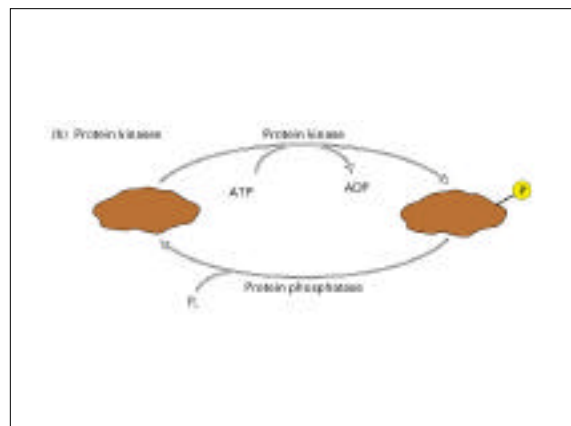
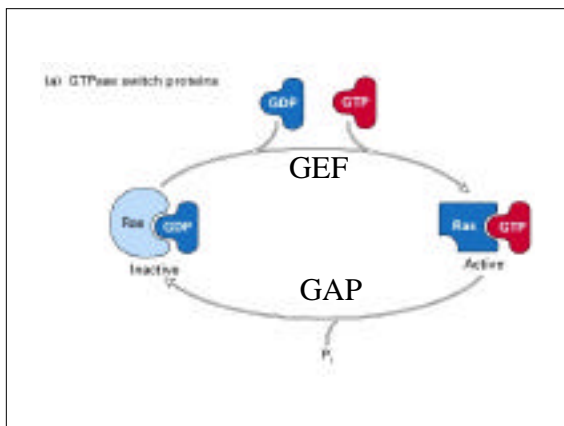
- A) Cytokine receptors
- B) Antigen receptors

No intrinsic enzymes activity,
 Signals transduced through associated kinases
 Nonreceptor tyrosine kinases: Src family kinases, Jak family kinases
 a) Phosphorylate receptor tails to create binding sites for SH2 containing proteins
 b) Directly phosphorylate downstream molecules

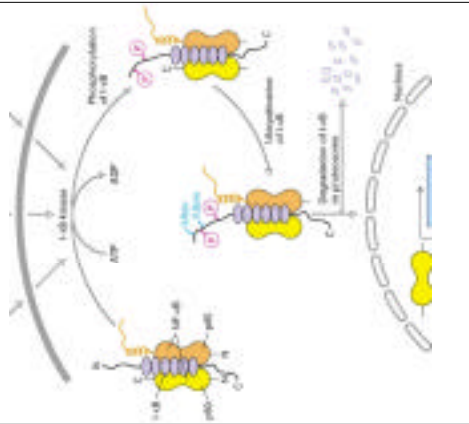


Signaling Triggers

- Dimerization or Oligomerization
- GTP/GDP Switch
- Phosphorylation or Dephosphorylation
- Translocation
- Cleavage or Degradation



Degradation of I- κ B and translocation of NF- κ B are Key Steps in NF- κ B Activation

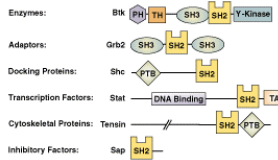


Important Concepts in Signal Transduction

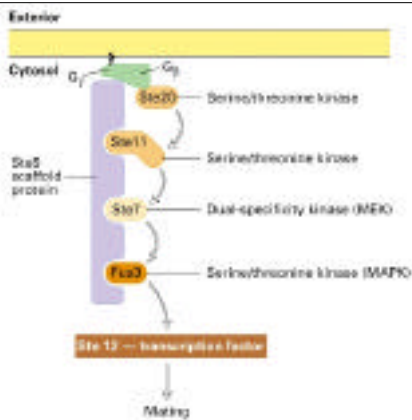
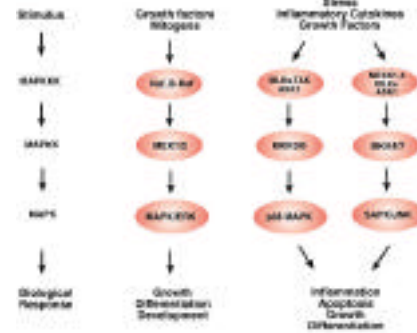
Structure (Domain, Motif)
 Cascade
 Complex
 Specificity
 Network

SH2 Domain
 PTB Domain
 SH3 Domain
 PH Domain
 14-3-3 Domain
 FYVE Domain
 Death Domain
 DED Domain
 CARD Domain
 TIR Domain
 LRR Domain
 Pyrin Domain
 Zn finger Domain
 Ring Finger Domain
 TRAF domain
 PDZ Domain
 SAM Domain
 WD40 Domain

Protein Domains



MAPK signaling cascades



Methods for Studying Signal Transduction

Interaction

Two Hybrid Interaction (One Hybrid, Two Hybrid, Three Hybrid)
 Co-precipitation (Immunoprecipitation, Biochemical Purification, Western, Mass Spec.)
 Expression Cloning (protein-DNA or protein-protein including antibody based screening)

Expression

Differential and Subtractive Hybridizations
 Differential Display
 Representational Difference Analysis
 Gene-Chips
 Protein-Chips

Homology

Low Stringent Hybridization
 PCR
 Database (Genomic, cDNA, EST)
 Computer Cloning (Sequence Homology, Structural Homology, Domain, Motif)

Function

In vitro Systems
 Cell Culture Systems
 Transgenic or knockout Animals

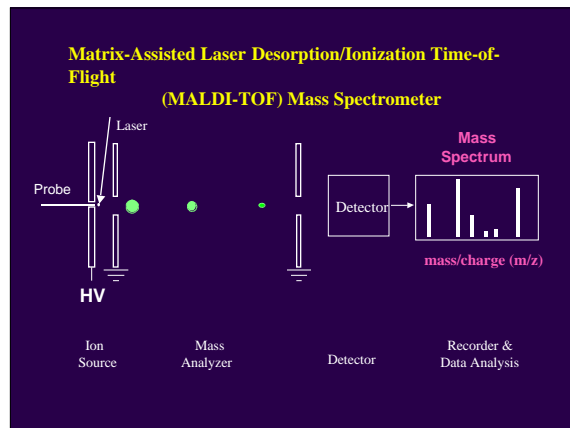
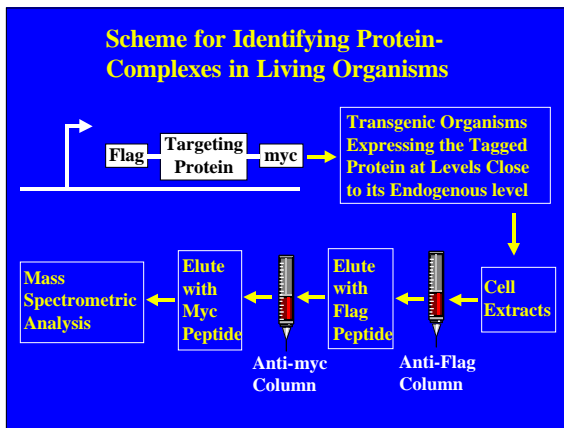
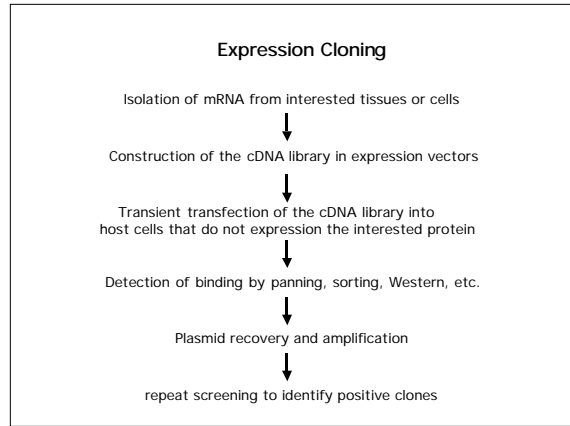
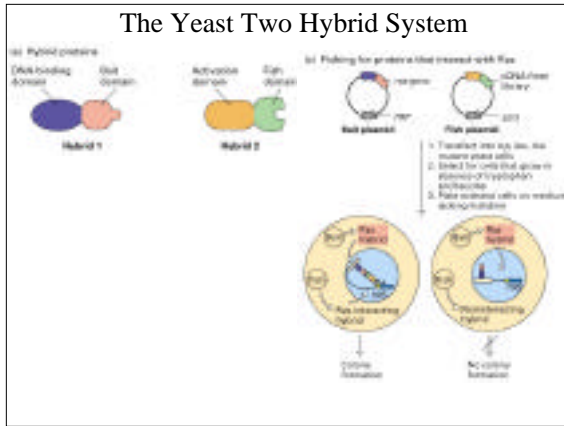
Sense or Antisense approach
 RNAi approach
 Dominant Active or Dominant Negative

Readouts

Binding
 Phosphorylation
 Translocation
 Gene Expression
 Other Modifications

Cell Growth
 Cell Transformation
 Cell Differentiation
 Cell Apoptosis

Development
 Survival
 Environmental Response
 Behavior



PepFrag Search Results

Mass of a protein: 156.7 kDa

Mass of a parent peptide after complete trypsin digestion : 2405 +/- 2.0

Database: *GENPEPT*, Kingdom: Fungi

MGNGRHA 2 mass = 156462.5 Da
putative pol polyprotein (NCBI gi: 538067)- Magnaporthe grisea
 TELCR QTGVQQLLSYHPETDGGTER ANQEV mass = 2505.5 Da

SCE9747 30 mass = 156649.6 Da
Yer105p (NCBI gi: 603343) - Saccharomyces cerevisias
 KLIQK VLEGDAGTEETISQLEVDQSR GVLHT mass = 2405.5 Da

