Signal Transduction II

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Signal Molecule

G-protein coupled receptor

Receptor tyrosine kinase

G protein

Adenylyl cyclase

cAMP

PKA

Gene regulatory proteins

CaM Kinase

PKC

MAPK

PKB

Many target proteins

PLC

IP3

DAG

Calmodulin

Ca2+

Grb2

Ras-GEF

Ras

MAPKKK

MAPKK

PI-3 kinase

PI(3,4,5)P3

PDK1
Key contents for signal transduction:

I. Signaling molecules:

Diversity, Receptors, GTPase switch proteins, protein kinases, adaptor proteins, protein phosphatases

II. Signaling Pathways:

A. Cell-surface receptor pathways (receptors that are ion channels, Signaling through G-protein-coupled receptors, Signaling through Enzyme-linked receptors, Signaling that involved in proteolysis)

B. Intracellular receptor pathways (Nitric oxide pathway, Nuclear receptor pathway)

III. The regulation of cell signaling:

A. The interaction between different signaling pathways (Convergence, Divergence, Crosstalk)

B. The adaptation of targeting cells: Receptor sequestration, down-regulation, inactivation, Production of inhibitory protein, Inactivation of signaling protein
3. Signaling through Enzyme-linked receptors

3.1 receptor tyrosine kinase pathway

3.2 Cytokine receptors pathway

3.3 TGFβ receptors pathway
# 3. Signaling through Enzyme-linked receptors

<table>
<thead>
<tr>
<th>Receptor Class/Pathway*</th>
<th>Distinguishing Characteristics</th>
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<tbody>
<tr>
<td><strong>RECEPTORS WITH INTRINSIC OR ASSOCIATED ENZYMATIC ACTIVITY</strong></td>
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</table>
| TGFβ receptors (14, 15) | **Ligands:** Transforming growth factor β superfamily (TGFβ, BMPs), activin, inhibins (mammals); Dpp (*Drosophila*)  
**Receptors:** Intrinsic protein serine/threonine kinase activity in cytosolic domain (type I and II)  
**Signal transduction:** Direct activation of cytosolic Smad transcription factors |
| Cytokine receptors (14, 15) | **Ligands:** Interferons, erythropoietin, growth hormone, some interleukins (IL-2, IL-4), other cytokines  
**Receptors:** Single transmembrane α helix; conserved multi-β strand fold in extracellular domain; JAK kinase associated with intracellular domain  
**Signal transduction:** (1) Direct activation of cytosolic STAT transcription factors; (2) PI-3 kinase pathway; (3) IP$_3$/DAG pathway; (4) Ras-MAP kinase pathway |
| Receptor tyrosine kinases (14) | **Ligands:** Insulin, epidermal growth factor (EGF), fibroblast growth factor (FGF), neurotrophins, other growth factors  
**Receptor:** Single transmembrane α helix; intrinsic protein tyrosine kinase activity in cytosolic domain  
**Signal transduction:** (1) Ras–MAP kinase pathway; (2) IP$_3$/DAG pathway; (3) PI-3 kinase pathway |
3.1 Receptor tyrosine kinase pathway

**Ligands:** Insulin, epidermal growth factor (GF), Fibroblast GF, neurotrophins and other GFs.

**Receptors:** Single transmembrane α helix; intrinsic protein tyrosine kinase activity in cytosolic domain; dimerization and phosphorylation induced by binding to ligand.

**Signal transduction:**

A. MAP kinase pathway

B. IP3/DAG pathway.

C. PI-3 kinase pathway
Nobel Prize for discovery of growth factors

Rita Levi-Montalcini (c. 1986) b. 1909

1950: Study of transplantable mouse sarcoma

- Grafted the tumor on developing chicken embryo
- Embryo sensory ganglia innervated the transplant
- Sensory and sympathetic nerve fibers invaded the tumor and branched along the tumor cells but did not establish synaptic junctions
- Under these conditions, the embryo showed aberrant growth and distribution of nerve fibers
- This suggested the tumor released a humoral factor that acts on sensory and sympathetic ganglia

Purified nerve growth factor (NGF)

Stanley Cohen (c. 1986) b. 1922

1956:

- Promotes outgrowth @ 1-10 ng
- Increases ganglia by enhancing differentiation and preventing cell death
- Promotes differentiation of tumor cells such as neuroblastoma cells in vitro
- Removes NGF degeneration

Peptide growth factors

Stanley Cohen

b. 1922

Rita Levi-Montalcini

b. 1909
Epidermal Growth Factor
Stanley Cohen

- Used mouse submaxillary gland as source of NGF
- Noted another activity
- Factor caused precocious incisor eruption, early eyelid opening and lung maturation - epithelial cells
- Purification with these assays
The structure of Receptor tyrosine kinase

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

(b) Activation of the EGF receptor
Diversity of receptor structure
Different receptors interact with downstream molecules with unique SH2 domains
Activation of PLC after activation of EGF receptor

PLC is activated by EGF.

EGF activates PI3 kinase.

EGF activates MAP kinase.
MAP kinase pathway

Activation of Ras after activation of EGF receptor

- Grb2 is an adaptor protein
- Sos is a guanine nucleotide exchange factor
- Sos displaces GDP
Activation of the MAP kinase pathway

1. Ras activated by exchange of GDP for GTP.
2. Active Ras recruits, binds, and activates Raf.
3. GTP hydrolysis leads to dissociation of Ras from Raf.
4. Raf activates MEK.
5. MEK activates MAPK.
6. Dimeric form of active MAP kinase translocates to nucleus; activates many transcription factors.

Raf → Mek → MAP kinase
Induction of gene transcription by activated MAP kinase
IP3/DAG pathway is activated by EGF
Phosphatidylinositol 3 kinase (PI3K) pathway

- Identified by its association with activated PDGFR
- Consists of 85kDa and 110kDa subunits
- Phosphorylated inositol at the 3 position of the inositol ring
- Activation of phosphatidylinositol kinase 1 (PDK1)
Different receptors interact with downstream molecules with unique SH2 domains.
**Generation of PI3 phosphates**

- **Inositol**
  - PI 4-phosphate (PIP)
    - PI-3 kinase
      - ATP, ADP
    - PIP-5 kinase
      - ATP, ADP
- **PI 4,5-bisphosphate (PIP₂)**
  - ATP, ADP

**Generation of IP3/DAG**

- **Cytosolic leaflet**
  - Phosphatidylinositol (PI)
    - ATP, ADP, PI-4 kinase
    - ATP, ADP, PIP-5 kinase
  - Inositol 1,4,5-trisphosphate (IP₃)
  - Phospholipase C

- **1,2-Diacylglycerol (DAG)**
  - ATP, ADP
Phosphatidylinositol pathway
Recruitment and activation of PKB by phosphatidyl inositols

- In unstimulated cell PKB is in cytosol
- Formation of PI 3, 4-bisphosphate
- The PH domain of PKB docks to the 3-phosphate → partial activation
- PDK1 also binds PI 3, 4-bisphosphate
- PDK1 phosphorylation results in activation of PKB

PDK is phosphoinositol dependent kinase

PKB, Akt I, influences cell survival
The major signaling pathways activated by GPCRs and RTKs

- G-protein-linked receptor
- G protein
  - adenyl cyclase
  - cyclic AMP
  - PKA
  - gene regulatory proteins
- G protein
  - phospholipase C
  - IP₃
  - Ca²⁺
  - calmodulin
  - CaM-kinase
  - gene regulatory proteins
  - many target proteins
- Grb2
  - Ras-GEF
  - Ras
  - MAP-kinase-kinase-kinase
  - MAP-kinase
  - PDK1
  - PKB
  - many target proteins
- PI 3-kinase
  - PI(3,4,5)P₃
  - many target proteins
The interactome map within cells
3. Signaling through Enzyme-linked receptors

3.1 receptor tyrosine kinase pathway

3.2 Cytokine receptors pathway

3.3 TGFβ receptors pathway
3.2 Cytokine receptors pathway

**Ligands:** Interferons, erythropoitin (EPO), some interleukins (IL-2, IL-4) and others.

**Receptors:** Single transmembrane a helix; JAK kinase associated with intracellular domain.

**Signal transduction:**

A. Direct activation of cytosolic **STAT** (Signal transducer and activator of transcription) transcription factors.

B. PI3 kinase pathway

C. MAP kinase pathway

D. IP3/DAG pathway.

**Basic function:** Proliferation, differentiation and maturation of blood cells and immune cells.
Erythropoietin and red blood cells

- Epo interaction with precursor stem cells

- Cloning of erythropoietin major success of biotechnology

M. Socolovsky et al., *Blood* 98: 3261
Janus Kinase: A tyrosine kinase tightly bound to cytosolic domain of Epo receptor

Janus, Roman two faced God of Gate
Janus Kinase/STAT signaling pathway

STAT (Signal transducer and activator of transcription)

The JAK - STAT signaling pathway

- Activated by erythropoietin
- STAT transcription factor
- The SH2 domain of STAT binds to phosphorylated tyrosine
- Phosphorylated STATs dimerize and move to nucleus
- Binding to interferon stimulated response element (ISRE) or interferon γ activated sequence (GAS).

Erythropoietin (a cytokine) may activate several pathways.

EPO → EpoR → JAK2 → ?

1. **STAT5** → Transcriptional activation
2. **GRB2 or Shc** → Ras → MAP kinase → Transcriptional activation or repression
3. **Phospholipase Cγ** → Elevation of Ca^{2+} → Transcriptional activation or repression; modification of other cellular proteins
4. **PI-3 kinase** → Protein kinase B → Transcriptional activation or repression; modification of other cellular proteins
Expression of erythrocytes in mutant mice

Targeted disruption of Epo receptor or JAK2

These mice are wild type for JAK2 $^{+/+}$

These mice are wild type for EpoR $^{+/+}$

Note: JAK2 is downstream of EpoR

Wu et al., 1995 Cell 85:59

Neubauer et al., 1998 Cell 93:307
General strategy for gene targeting in mice

Step 1 Gene targeting in ES cells

1. ES cell culture
   Embryonic stem (ES) cells are cultivated from mouse pre-implantation embryos (blastocysts).

2. Construction of targeting vector
   The vector contains pieces of DNA that are homologous to the target gene, as well as inserted DNA which changes the target gene and allows for positive-negative selection.

3. ES cell transfection
   The cellular machinery for homologous recombination allows the targeting vector to enable the target vector to find and recombine with the target gene.

4. Proliferation of targeted ES cell
   Selection for presence of neo and absence of HSV-1k enriches targeted ES cells.
General strategy for gene targeting in mice

Step 2 From gene targeted ES cells to gene targeted mice

5. Injection of ES cells into blastocysts
The targeted ES cells are injected into blastocysts... where they mix and form a mosaic with the cells of the inner cell mass from which the embryo develops. The injected blastocysts are implanted into a surrogate mother where they develop into mosaic embryos.

6. Birth and breeding of mosaic mice
The mosaic mice mate with normal mice to produce both gene targeted and normal offspring.

Gene targeted mice – called "knockout mice" when the targeted gene is inactivated.
GFP-knock-in mouse → Tissue-specific Cre-transgenic mouse
The Nobel Prize in Physiology or Medicine 2007

"for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

Mario R. Capecchi

Sir Martin J. Evans

Oliver Smithies

Harvard Ph.D. '67,
Jim Watson
"... the one thing that I think is extremely important, is that anyone can do it, if given a chance, if given the opportunity."

Telephone interview with Mario R. Capecchi immediately following the announcement of the 2007 Nobel Prize in Physiology or Medicine, 8 October 2007. The interviewer is Adam Smith, Editor-in-Chief of Nobelprize.org.

Kirk R. Thomas and Mario R. Capecchi
Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells
The legends of Nobel winners @ Harvard

Welcome to Bio-Lab!
Harvard Bio-Lab
The Nobel Prize in Physiology or Medicine 2013 was awarded jointly to James E. Rothman, Randy W. Schekman and Thomas C. Südhof "for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells".

James E. Rothman
Randy W. Schekman
Thomas C. Südhof

Harvard Ph.D. ’76,
One half jointly to Bruce A. Beutler and Jules A. Hoffmann "for their discoveries concerning the activation of innate immunity" and the other half to Ralph M. Steinman "for his discovery of the dendritic cell and its role in adaptive immunity".
The Nobel Prize in Chemistry 2009

"for studies of the structure and function of the ribosome"

Venkatraman Ramakrishnan

Photo: MRC Laboratory of Molecular Biology

Venkatraman Ramakrishnan

1/3 of the prize

United Kingdom

MRC Laboratory of Molecular Biology

Thomas A. Steitz

Harvard Ph.D. 66,

1/3 of the prize

USA

Yale University

Ada E. Yonath

Credits: Micheline Pelletier/Corbis

Ada E. Yonath

1/3 of the prize

Israel

Weizmann Institute of Science
The Nobel Prize in Chemistry 2008

"for the discovery and development of the green fluorescent protein, GFP"

<table>
<thead>
<tr>
<th>Name</th>
<th>Country</th>
<th>Prize Share</th>
<th>Institute/University</th>
<th>Birth Year</th>
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<tbody>
<tr>
<td>Osamu Shimomura</td>
<td>USA</td>
<td>1/3 of the prize</td>
<td>Marine Biological Laboratory (MBL) Woods Hole, MA, USA; Boston University Medical School Massachusetts, MA, USA</td>
<td>1928 (in Kyoto, Japan)</td>
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<tr>
<td>Martin Chalfie</td>
<td>USA</td>
<td>1/3 of the prize</td>
<td>Columbia University New York, NY, USA</td>
<td>1947</td>
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<tr>
<td>Roger Y. Tsien</td>
<td>USA</td>
<td>1/3 of the prize</td>
<td>University of California San Diego, CA, USA; Howard Hughes Medical Institute</td>
<td>1952</td>
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</tbody>
</table>
The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA

Andrew Z. Fire
1/2 of the prize
School of Medicine
Stanford University
USA
b. 1959
Ph. D, MIT

Craig C. Mello
1/2 of the prize
Medical School
University of Massachusetts
Worcester, MA, USA
b. 1960
Ph. D, ’90 Harvard
Ph. D, ’90 Harvard

Dan Stinchcomb
V617F mutations of JAK2 in chronic myeloproliferative disorders

![Diagram of JAK2 signaling pathway]

**Diagram Description:**

- **JAK2** (Janus Kinase 2) is a protein involved in signaling pathways that regulate cell growth and differentiation.
- **V617F** mutation occurs in JAK2 Exon 12 and is associated with chronic myeloproliferative disorders.
- The diagram illustrates the activation of signaling pathways involving JAK2, Stat (Signal Transducer and Activator of Transcription), PI3K (Phosphatidylinositol 3-kinase), Akt (Serine/threonine protein kinase), mTOR (Mammalian target of rapamycin), and other proteins.
- The mutation affects the function of these pathways, leading to increased cell proliferation and survival.

**Key Biological Processes:**

1. **JAK2-Stat Signaling:** JAK2 phosphorylates Stat proteins, leading to their activation and nuclear translocation.
2. **PI3K-Akt-mTOR Pathway:** PI3K activates Akt, which in turn phosphorylates mTOR, controlling cell growth and metabolism.
3. **ERK Pathway:** ERK (Extracellular signal-regulated kinase) is activated in the presence of JAK2V617F mutation, promoting proliferation.

**Clinical Relevance:**

- Mutations in JAK2 are a hallmark of chronic myeloproliferative disorders, including polycythemia vera, essential thrombocythemia, and chronic myeloid leukemia.
- Understanding the molecular mechanisms underlying these mutations is crucial for the development of targeted therapies.
The role of JAK2 in gastric carcinogenesis

Downregulation of miR-375 in 90% of gastric cancer

JAK2 is a miR-375 target gene in gastric cancer cells

Overexpression of miR-375 inhibits the proliferation of gastric cancer cells

The potential role of JAK2 in gastric cancer

Ding et al., *Cell Research* 2010
美国哈佛大学校园（2003年1月）
3. Signaling through Enzyme-linked receptors

3.1 receptor tyrosine kinase pathway

3.2 Cytokine receptors pathway

3.3 TGFβ receptors pathway
3.3 TGFβ receptors pathway

**Ligands:** Transforming growth factor β superfamily (TGFβ), bone morphogenetic protein, activin, inhibins, Nodal (mammals) and Dpp (*Drosophila*), etc.

**Receptors:** Intrinsic serine/threonine kinase protein activity in cytosolic domain (type I and II), Type III is β glycan without kinase activity.

**Signal transduction:** Direct activation of cytosolic Smad (mammalian homologues of C. elegan Sma and Drosophila Mad) transcription factors.

**Basic function:** Negative regulation of cell proliferation, loss of TGFβ signaling contributes abnormal cell proliferation and malignancy.
Smads family

- **R-Smads (receptor regulated Smads):** Smad2 and Smad3, etc.

- **Co-Smads (common Samds):** Smad4

- **I-Smads (Inhibitory Smads):** Smad6 and Smad7.
TGFβ binding involves serine phosphorylation

- TGFβ binds type III / type II receptor
- Type II (kinase) dimerizes with type I and phosphorylates it
- Phosphorylation of type I results in activation of kinase activity
- Phosphorylation of Smad leads to dimerization and translocation to the nucleus

TGFβ signaling pathway

- Smads complex induces expression of plasminogen activator inhibitor (PAI)

TFE3: Transcription factor E3

Ran: small G protein

TGFβ signaling pathway (2009)

Gene responses: tumor suppression
- Cytostasis: CDK inhibition, Myc inhibition
- Differentiation: ID1 regulation
- Apoptosis: cell death signaling

Gene responses: other effects
- Phenotypic plasticity: EMT inducers
- Environment: ECM, cytokines, proteases
- Signaling: receptors, transducers, TFs

Stress responses
- Migration
- Cell shape
- Cell-cell contacts
TGFβ pathway in physiology and pathology

Implications for a wider spectrum of disorders, including cancer, fibrosis, healing and inflammatory and cardiovascular diseases.

<table>
<thead>
<tr>
<th>Gene</th>
<th>OMIM number</th>
<th>Syndrome</th>
<th>OMIM number</th>
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<tbody>
<tr>
<td>ENG</td>
<td>131195</td>
<td>HHT1</td>
<td>187300</td>
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<tr>
<td>ACVRL1</td>
<td>601284</td>
<td>HHT2</td>
<td>600376</td>
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<td>SMAD4</td>
<td>600993</td>
<td>JPS</td>
<td>174900</td>
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<td>JPS and HHT syndrome</td>
<td>175050</td>
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<td>BMPR1A</td>
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<td>Camurati-Engelmann disorder</td>
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<td>TGFBRI2</td>
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<td>MFS2</td>
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<td>FBN1</td>
<td>134797</td>
<td>MFS1</td>
<td>154700</td>
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</table>

FBN1 is included although it is not a signaling component. It is an extracellular matrix protein that may influence TGFβ signaling by acting as a pool or activating matrix for the ligand.

HHT: hereditary hemorrhagic telangiectasia
JPS: juvenile polyposis syndrome
MFS: Marfan syndrome
ENG: Type III receptor
ACVRL1: Type I receptor

The Role of TGFβ in Cancer. In normal and premalignant cells, TGFβ enforces homeostasis and suppresses tumor progression directly through cell-autonomous tumor-suppressive effects (cytostasis, differentiation, apoptosis) or indirectly through effects on the stroma (suppression of inflammation and stroma-derived mitogens). However, when cancer cells lose TGFβ tumor-suppressive responses, they can use TGFβ to their advantage to initiate immune evasion, growth factor production, differentiation into an invasive phenotype, and metastatic dissemination or to establish and expand metastatic colonies.
Overview of Enzyme-linked receptors
4. Intracellular receptor -- Nitric oxide pathway

**Ligands:** Nitric oxide.

**Receptors:** NO receptor

**Signal transduction:** Generation of cGMP

**Basic function:** Vasodilation and neurotransmitter
Regulation of contractility of arterial smooth muscle by NO and cGMP
NO pathway in physiology and pathology

- **Cardiovascular diseases**

- **Neurological diseases**: Brain ischemia and chronic degenerative diseases of the nervous system

- **Erectile dysfunction** (impotence): Sildenafil, popularly known by the trade name **Viagra**, stimulates erections primarily by enhancing signaling through the nitric oxide pathway in the penis.
The Nobel Prize in Physiology or Medicine 1998

for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system

Robert F. Furchgott  Louis J. Ignarro  Ferid Murad
5. Intracellular receptor -- Nuclear receptor pathway

**Ligands:** steroid hormones, thyroxine, retinoid acids and fatty acid etc.

**Receptors:** Highly conserved DNA-binding domain, somewhat conserved hormone-binding domain, and a variable domain; located within cytosol or nucleus.

**Signal transduction:** activation of receptors’ transcription factor activity by ligand binding.

**Basic function:** Biological homeostasis and sex regulation, etc.
Intracellular receptors (Steroid hormone receptors)
6. Signaling that involved in proteolysis

4.1 NF-κB (nuclear factor κB) pathway

4.2 Notch/Delta signaling pathway

4.3 Hedgehog (Hh) pathway

4.4 Wnt pathway
III. The network of cell signaling

The major signaling pathways relevant to cell growth in human cells.
III. The regulation of cell signaling

The interaction between different signaling pathways

Convergence

Divergence

Crosstalk

The adaptation of targeting cells: Desensitization

Receptor sequestration

Receptor down-regulation

Receptor inactivation

Inactivation of signaling protein

Production of inhibitory protein
The major signaling pathways activated by GPCRs and RTKs

- **Signal Molecule**
- **G-protein coupled receptor**
- **Receptor tyrosine kinase**

**G protein**
- **Adenylyl cyclase**
  - cAMP
    - PKA

**G protein**
- **PLC**
  - IP3
    - Ca^2+ (Calmodulin)
  - DAG
    - CaM Kinase
    - PKC

**Grb2**
- **Ras-GEF**
  - Ras
    - MAPKKK
    - MAPKK
    - MAPK
    - PDK1
    - PI(3,4,5)P3
    - PI-3 kinase

**Many target proteins**
- Gene regulatory proteins

**PKB**
The major signaling pathways activated by GPCRs and RTKs

- G-protein coupled receptor
- Receptor tyrosine kinase

**Convergence**
- G protein
- PLC
- DAG
- PKC
- CaM Kinase
- Calmodulin
- cAMP
- Adenylyl cyclase
- PKA

**Divergence**
- Grb2
- Ras-GEF
- Ras
- MAPKKK
- MAPKK
- MAPK
- PI(3,4,5)P3
- PI-3 kinase
- Grb2
- Ras-GEF
- Ras
- MAPKKK
- MAPKK
- MAPK
- PI(3,4,5)P3
- PI-3 kinase
- PKB

**Crosstalk**
- IP3
- Ca2+
- Calmodulin
- CaM Kinase
- Adenylyl cyclase
- PKA

**Gene regulatory proteins**
- Many target proteins
Erythropoietin (a cytokine) may activate several pathways

EPO → EpoR → JAK2

(a) STAT5 → Transcriptional activation
(b) GRB2 or Shc → Ras → MAP kinase → Transcriptional activation or repression
(c) Phospholipase Cγ → Elevation of Ca²⁺ → Transcriptional activation or repression; modification of other cellular proteins
(d) PI-3 kinase → Protein kinase B → Transcriptional activation or repression; modification of other cellular proteins
Five ways in which target cells can become desensitized to a signal molecule.
The inactivation mechanisms shown here for both the receptor and the intracellular signaling protein often involve phosphorylation of the protein that is inactivated, although other types of modification are also known to occur. In bacterial chemotaxis, desensitization depends on methylation of the receptor protein.
GPCR Desensitization Depends on Receptor Phosphorylation

**Receptor inactivation:** They can become altered so that they can no longer interact with G proteins.

**Receptor sequestration:** They can be temporarily moved to the interior of the cell (internalized) so that they no longer have access to their ligand.

**Receptor down-regulation:** They can be destroyed in lysosomes after internalization.
The roles of G-protein-linked receptor kinases (GRKs) and arrestins in receptor desensitization.
Down regulation of the **EGFR**

- cDNA expression vector for EGFR with GFP-tag
- Transfect cells
- Observe GFP signal after stimulation of cells with EGF
Sequestration and down-regulation of EGFR

- At 0sec both EGF and EGFR are at cell surface
- At 30sec both EGF and EGFR have been internalized
- At 30sec both have been targeted to lysosomes; proteolysis initiated
Sequestration and down-regulation of EGFR

**Imaging analysis**

**Western analysis**

**NIH 3T3**

EGF receptors (% remaining)

Time (h)

0.5 1.0 2.0

EGF receptors (% remaining)

EGF

EGFR

Min

0 15

Hours

1 2 4

kDa

203

115

wt 1

GFP

wt 2
Thank you!