

**ADVANCED CELL BIOLOGY//
MOLECULAR BIOLOGY OF CELLS**
01-146:470 + 16-148:514

•Monday 11.30.09 (8:40-10:00)
•SEC-118

• Class web page:
<http://lifesci.rutgers.edu/~denhardt/course/cellmolbiol.htm>

•Dr. Guy Werlen
•Dept. of Cell Biology & Neuroscience
•Nelson Bio. Labs., B333
•werlen@biology.rutgers.edu
•Office hours, Friday 3:30pm-5:00 pm

Lodish • Berk • Kaiser • Krieger • Scott • Bretscher • Ploegh • Matsudaira

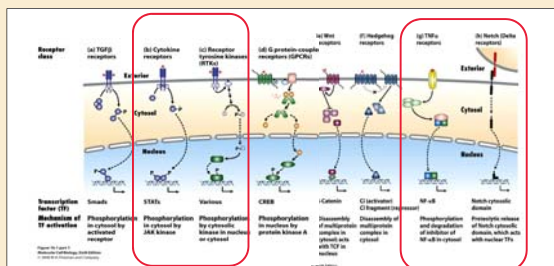
Molecular Cell Biology
6th Edition

Chapter 16:
Cell signaling II: Signal pathways that control gene activity

- 16.2 Cytokine Receptors and the Jak/STAT pathway.
- 16.3 Receptor tyrosine kinases.
- 16.4 Activation of Ras and the MAP kinase Pathways.
- 16.5 Phosphoinositides as Signal Transducers.
- 16.7 Pathways that involve Signal-Induced Protein Cleavage.

-Some slides (signaling in T cells) may also refer to chapter 24 .5,

Pathways that control gene expression



Distinct intracellular mechanisms transduce signals downstream of each of the eight major classes of cell-surface receptors. Direct activation of cytosolic transcription factors following TGF and cytokine receptor activation (a, b). Alternatively, receptor stimulation leads to the activation of cytosolic protein kinases that, by translocating into the nucleus activate nuclear transcription factors (c, d). In other pathways, active transcription factors are released from multiprotein complexes (e,f) or by proteolysis (g, h). Some receptor classes can trigger more than one intracellular pathway.

Cytokine receptors

Class I cytokine receptor (hematopoietin-receptor family)	<p>Receptors for erythropoietin, growth hormone, and IL-11 Receptors for IL-3, IL-5, and GM-CSF share a common chain, CD131 or β_2 (common beta chain) Receptors for IL-2, IL-4, IL-7, IL-9 and IL-15 share a common chain CD132 or γ_2 (common gamma chain). IL-2 receptor also has a third chain, a high-affinity subunit IL-2Rα (CD25)</p>
Class II cytokine receptor	Interferon- α , - β , and - γ receptor, IL-10 receptor
TNF-receptor family	Tumor necrosis factor (TNF) receptors I and II (CD40, Fas (Ap1), CD95, CD30, CD37, nerve growth factor receptor
Chemokine-receptor family	CCR1-10, CXCR1-5, XCR1, CX3CR1

Cytokine receptors belong to superfamilies of receptor proteins, each with a distinctive structure. Each superfamily member is a variant with distinct ligand specificity, performing a particular function on the cell that expresses it.

Many cytokine receptors are members of the hematopoietin-receptor superfamily, named after the first of its members to be defined. This large superfamily also known as the class I cytokine receptors, is divided into 3 subsets on the basis of differences in sequence and structure. These class I receptors are heteromers in which the α chain often defines ligand specificity, while the β & γ chains confer intracellular signaling function.

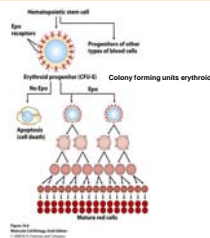
A smaller number comprises the class II cytokine receptor superfamily of which many bind interferons or interferon-like cytokines.

Members of the tumor necrosis factor receptor (TNFR) family form another superfamily of cytokine receptors. The ligand for this class of receptors act as trimers and may be associated with the cell membrane rather than being secreted.

The chemokine superfamily belongs to the very large family of G-protein coupled receptors (GPCR).

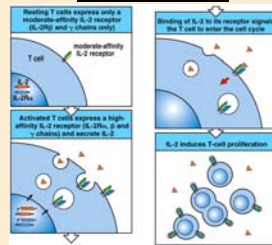
Cytokines control ontogeny, growth and activation of many hematopoietic cells

Red blood cells



Erythropoietin controls maturation of erythroid progenitor cells. Erythroid progenitor cells (CFU-E) are derived from hematopoietic stem cells. In the absence of Erythropoietin (Epo), CFU-E cells undergo apoptosis. Binding of Epo to its specific receptor (EpoR) on the surface of CFU-E induces gene transcription of anti-apoptotic proteins as well as proteins involved in cell division. Upon terminal differentiation, the EpoR is lost and mature red blood cells are no longer capable to respond to Epo.

White blood cells (f.ex. T lymphocytes)



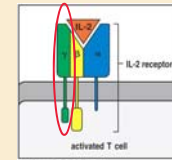
Interleukin-2 (class I cytokine) controls proliferation of naive T cells during an immune response and ensures clonal expansion of a particular T cell type. (cf. chapter 25.4)

Deficiency or mutations of cytokine receptors have dramatic consequences

Targeted deletion of EpoR, the receptor for erythropoietin leads to anemia and death of e13 old embryos



Deletion or mutation of the common γ chain of class I cytokine receptors induces a severe combined Immunodeficiency Syndrome. T cells fail to develop because many cytokines that share the γ -chain (IL-2, IL-4, IL-7, IL-9 & IL-15) can not activate their target cells.



Activation of JAK kinases is the proximal event that transduces cytokine signals intracellularly

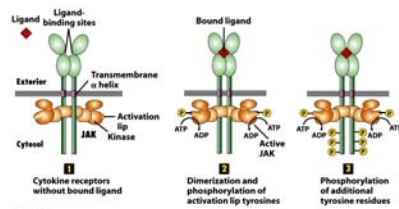


Figure 16-18 Molecular Cell Biology, Sixth Edition © 2008 W.H. Freeman and Company

The cytosolic domain of cytokine receptors tightly and irreversibly associates with separate JAK kinases. 1) In the absence of ligand, the receptors form dimers but the JAK kinases are poorly active because a structural "lip" protects the catalytic site. Ligand binding causes a conformational change that brings the active domains of the JAKs together. Ensues cross-phosphorylation on tyrosine residues located on the JAK's activation lip (2). This causes the lip to move out of the kinase catalytic site, thus increasing the ability of ATP to bind and the activated kinase can now phosphorylates several tyrosine residues in the receptor's cytosolic domain (3). The resulting phosphorylated tyrosines function as docking sites for inactive STAT transcription factors and other proteins that contain SH2 or PTB domains.

A tyrosine phosphorylated cytokine R can recruit many different SH2 containing proteins that will transduce the signal to distinct signaling pathways

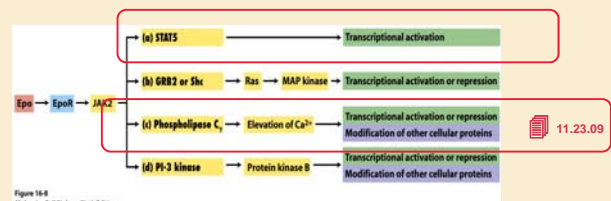
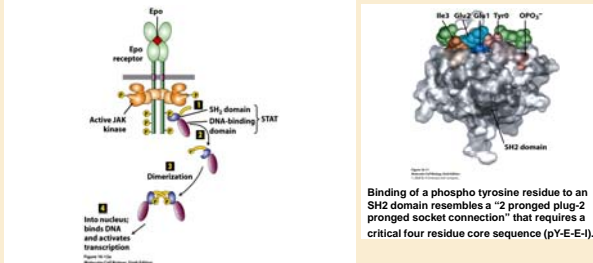


Figure 16-8 Molecular Cell Biology, Sixth Edition © 2008 W.H. Freeman and Company

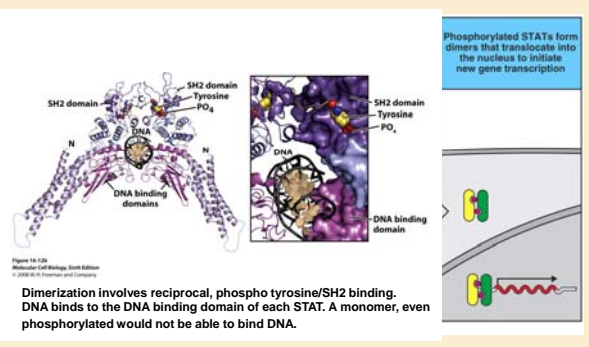
Four major pathways can transduce signals downstream of activated and phosphorylated EpoR-JAK complexes. Each pathway ultimately regulates the transcription of sets of different genes.

Phosphorylation of tyrosine residues of the cytokine receptor attracts STAT (signal transducers and transcription activators) proteins containing SH2 domains.

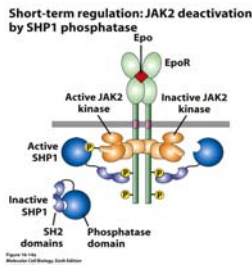


An inactive monomeric STAT transcription factor binds to phosphorylated tyrosine residues of the cytokine receptor following its activation (1). This brings STAT close to the active JAK kinases, that will phosphorylate a tyrosine residue in the C-terminal domain of STAT. Phosphorylated STAT dissociates from the receptor and spontaneously dimerizes (2 & 3). The STAT homodimer is stabilized by 2 SH2-phosphotyrosin interactions that prevent its re-binding to the cytokine receptor. Step4; the STAT dimer translocates into the nucleus where it binds to consensus promoter sequences and activates gene transcription.

Activated STATs dimerize, translocate to the nucleus and bind DNA on consensus promoter sites

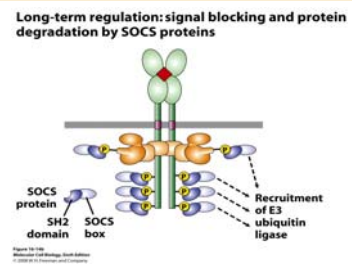


Short term deactivation of cytokine signaling via SHP1 phosphatase mobilization



In its inactive state the phosphatase domain of the phosphotyrosine phosphatase (PTP), SHP1 is masked by a regulatory domain containing a SH2 site. Upon activation, the SH2 domain of SHP1 binds to a phosphorylated tyrosine of the cytokine receptor, thus bring the phosphatase domain in close proximity to a critical tyrosine residue of JAK. Dephosphorylation of this tyrosine residue inactivates the kinase.

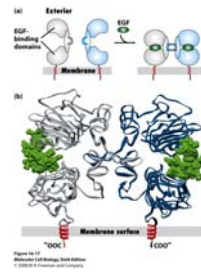
Long term cytokine deactivation by protein degradation via SOCS and the proteasome



SOCS (suppressor of cytokine signaling) binds to phosphorylated tyrosine residues of the cytokine receptor, thus blocking the association of STATs or other SH2 containing signal transducers with the cytokine receptor. SOCS recruits the E3 ubiquitin ligase, which targets the receptor for degradation by the proteasome.

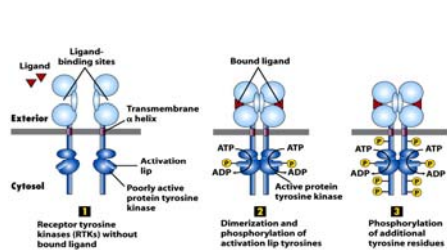
Receptor tyrosine kinases (RTK)

Ligand binding to a monomeric RTK induces a conformational change and dimerization



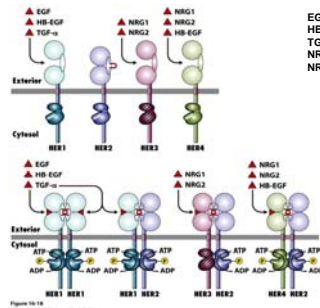
Dimerization of 2 identical ligand-bound RTK monomers occurs primarily through interactions of the activated loop segments

Intrinsic kinase activity regulates RTK signaling



Classical RTKs have intrinsic tyrosine kinase activity. Dimerization activates the tyrosine kinases, which cross-phosphorylate each other on tyrosine residues of the activation lip. This causes the lip to move out of the catalytic site of the respective kinase and allows ATP binding. The activated kinase phosphorylates additional Ys on the cytosolic domain of the RTK.

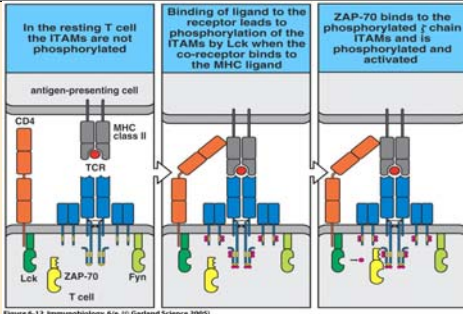
The HER receptors bind epidermal growth factor (EGF) or related growth factors



EGF= epidermal growth factor
 HB-EGF= heparin binding-EGF
 TGF= tumor-derived growth factor
 NRG1= neuregulin1
 NRG2= neuregulin2

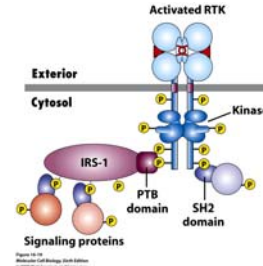
HER1 homodimerizes, while HER3 lacks a functional kinase domain and can signal only when dimerized to HER2. HER2 does not directly bind a ligand; it needs to heterodimerize with an activated HER1, HER3 or HER4. HER2 overexpression in breast epithelial cells leads to breast cancer (in 25% of breast cancer patients).

The T cell receptor (TCR) a atypical RTK



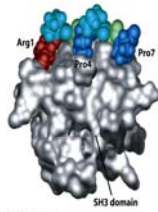
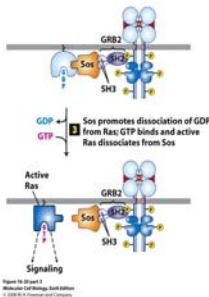
The TCR is a heterodimer of a α - and a β -chain that lack intrinsic tyrosine kinase activity. Several CD3 proteins are constitutively attached to the TCR. The CD3 isoforms control TCR expression, as well as signal transduction downstream of the TCR. A ligand for the TCR must at the same time bind to the CD4 (or CD8) coreceptor. This activates the tyrosine kinase Lck that is associated with CD4 (or CD8). Activated Lck will phosphorylate specific tyrosine residues in the ITAMs (immunoreceptor tyrosine-based activation motifs) of the CD3 isoforms. Phosphorylation of ITAMs will attract the SH2 domain containing tyrosine kinase, ZAP-70. ZAP-70 bound to CD30 can be phosphorylated and activated by Lck. For ref. see chapter 24.5 (figure 24-31).

Scaffold proteins ("adaptors") that contain SH2 or PTB domains associate to phosphorylated tyrosine residues of RTKs



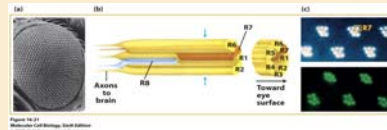
Cytosolic proteins that carry SH2- or PTB domains (in purple and respectively maroon) can bind to specific phosphorylated tyrosine residues of RTK. These proteins are the phosphorylated on tyrosines by associated or intrinsic receptor tyrosine kinases. Proteins that contain multiple tyrosine residues serve as scaffolds to nucleate many distinct SH2 or PTB containing signalin molecules.

Protein-protein interaction via Src homology 3 (SH3) domains



SH3 domains bind to proline-rich peptide sequences (X-P-(p/n)-P-X). SH3 domains of Grb2 binds to proline-rich domain of Sos.

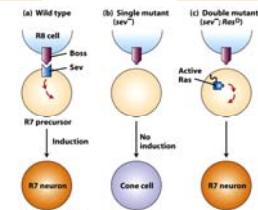
Genetic studies in *Drosophila* have identified key signaling molecules and mechanisms of the RTK/MAPK pathways



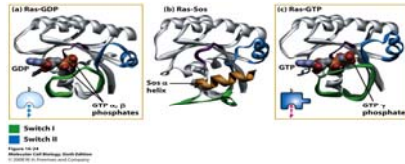
a) Several ommatidia constitute the eye of *Drosophila*. B) An ommatidia is a tubular structure formed by 8 photoreceptors. C) Ommatidia with the *sevenless* mutation lack the R7 photoreceptor cell.

a) The R8 cell in each ommatidia expresses a surface Protein, Boss, that binds to the RTK. Sev expressed on the surface of the neighboring R7 precursor cell.
b) This interaction activates a gene program that triggers the differentiation of the R7 precursor cell into a fully functional R7 photoreceptor cell. In the *sevenless* mutation the Boss-Sev interaction does not take place, consequently the R7 precursor cell stays immature.
c) Differentiation of R7 can be rescued by a constitutively active Ras protein.

Larval development

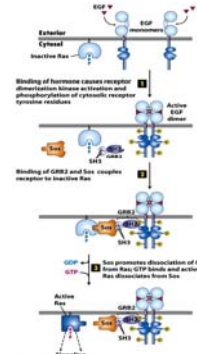


The binding of Sos to inactive Ras induces GDP/GTP exchange



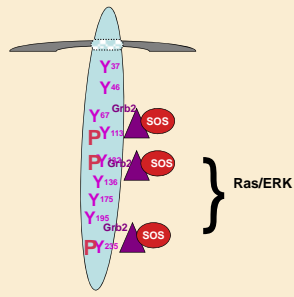
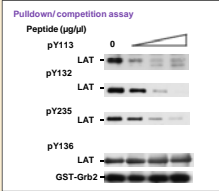
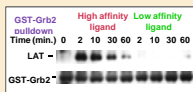
Sos pries Ras open by displacing the Switch I region, which allows GDP to diffuse out of the active pocket of Ras. GTP replaces GDP and the ensuing conformational change displaces Sos and promotes Ras interaction with downstream effectors.

Adaptor molecules and small G proteins link RTKs to the MAPK pathways



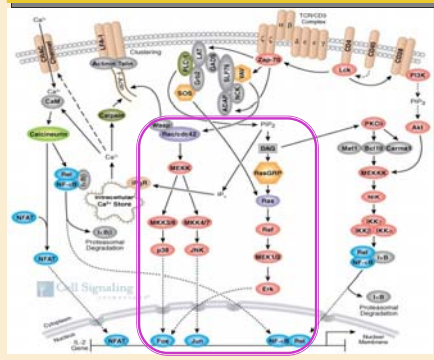
Activation of the small G-protein Ras follows ligand binding to RTKs. Similar to other RTK, activation of EGFR induces phosphorylation of intrinsic tyrosine residues that dock the SH-2 domain of the adaptor protein, GRB2. The SH3 domains of GRB2 associate to Sos, bringing this Ras activator closer to its substrate; the GDP bound to inactive Ras. Sos promotes the dissociation of GDP from Ras and following binding of GTP to Ras activates the G-protein.

Grb2 binding to LAT only occurs in response to high affinity ligand stimulation of OT-1 thymocytes



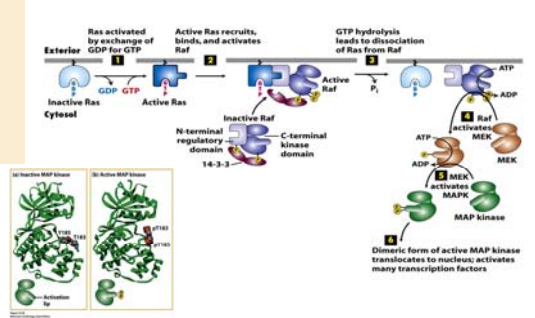
The T cell specific scaffold LAT (linker of activation of T cells) is phosphorylated in response to TCR engagement. The sequential phosphorylation of LAT's 9 distinct tyrosine residues nucleates various signaling molecules containing SH2 domains. Our laboratory has found that Grb2 binds to LAT only in response to high affinity ligands for the TCR, but not upon stimulation with a low affinity ligand. Furthermore, only Y113, Y132 & Y235 bind Grb2 but none of the other 6 tyrosine residues of LAT. For ref. see chapter 24.5 (figure 24-31) and Werlen et al, 2003, Science 299, 1859-1863.

The MAPK signaling pathways



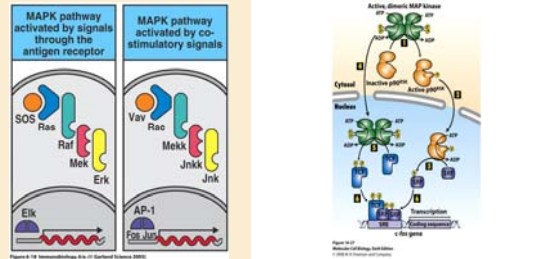
ERK, JNK and p38 are the most ubiquitous members of the MAPK family, they are regulated by a canonical module comprising a MAPKKK, a MAPKK and the MAPK. Each of the upstream components are specific to the ERK, JNK or p38 pathway. Each MAPK pathway is linked to a RTK, such as the TCR via a small G protein of the Ras or Rac families that is nucleated to scaffold proteins. (Cell Signaling Technology).

Mechanisms of MAPK activation



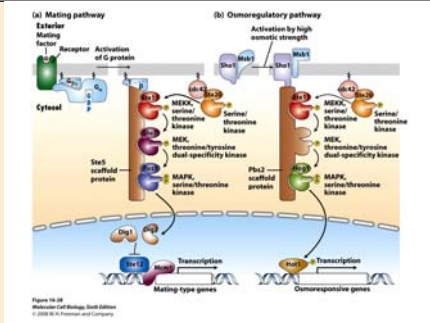
1) Ras is activated by exchange of GTP for GDP. 2) The new conformation of Ras allows its binding to, and activation of the Serine/threonine kinase, Raf. 3) The inactivation of Ras by GTP hydrolysis, allows further activation of Raf. 4) Raf phosphorylates a residue in the activation lip of the dual kinase, MEK. 5) Activated MEK, phosphorylates a tyrosine residue in the activation lip of ERK, which induces a conformational change of the lip and the exposure of a threonine residue that is also phosphorylated by MEK, thereby fully activating ERK. 6) ERK dimerizes and translocates to the nucleus.

MAPKs phosphorylate transcription factors and promote gene expression



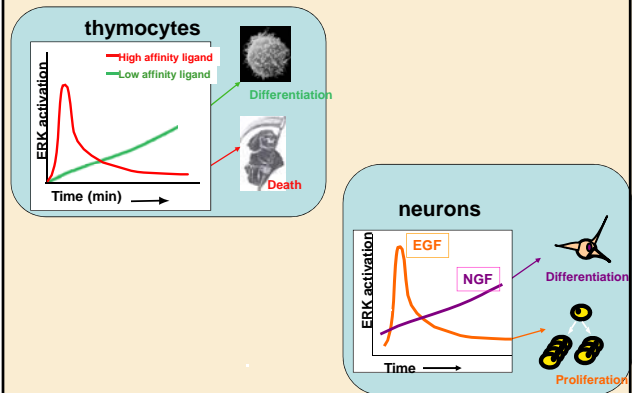
Each of the MAPK family member can activate specific as well as overlapping transcription factors. ERK activates directly the transcription factor, TCF (ternary complex factor), while SRF is activated via the kinase p90RSK. JNK phosphorylates Jun and thus regulates the activity of the transcription factor, AP-1.

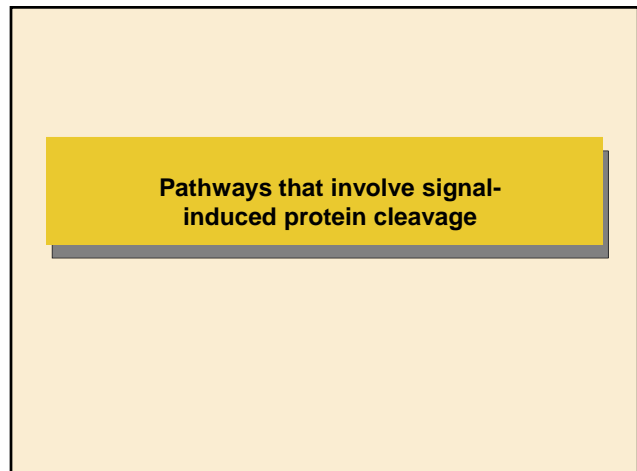
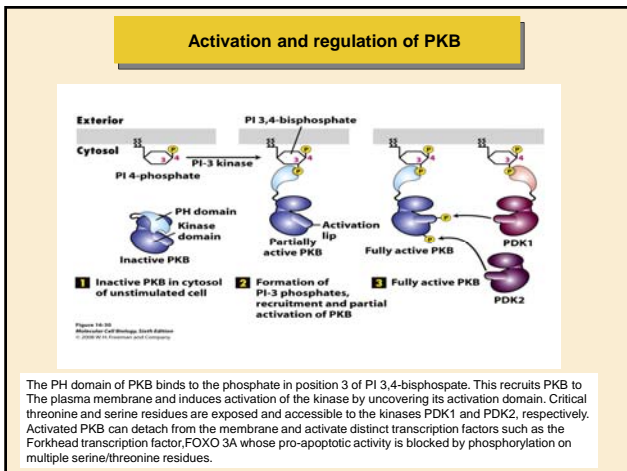
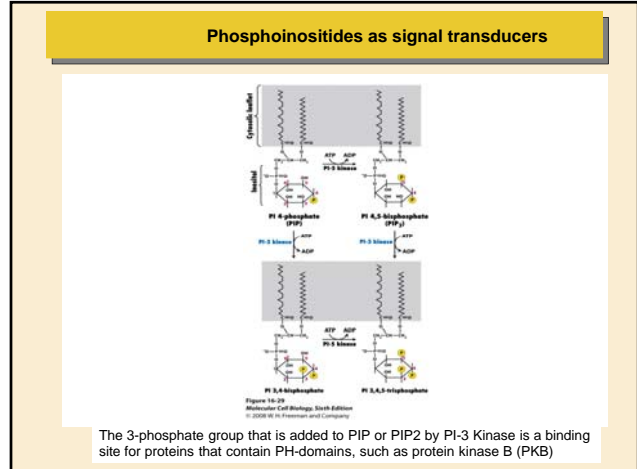
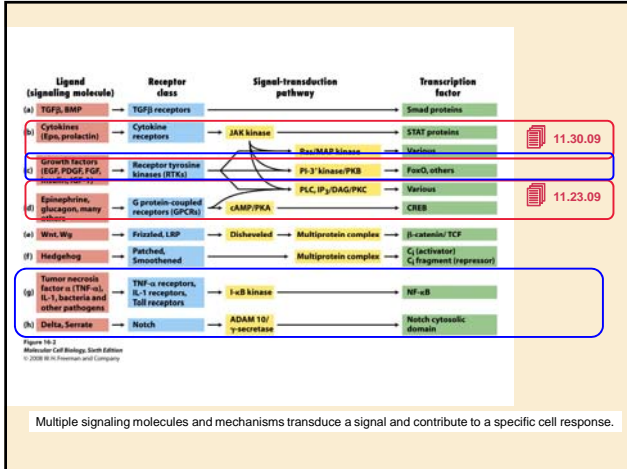
MAPK transduce mating and osmoregulatory signals in yeast



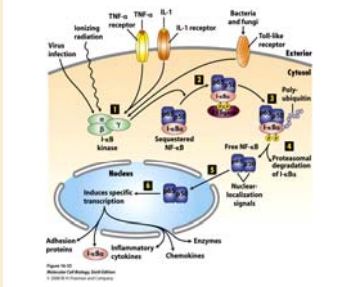
While the organization of MAPK cascades is similar from yeast to man, they are not connected to RTKs in yeast. A GPCR activates the Fus3 pathway and regulates the transcription of genes that are required for mating. Fus3 is a MAPK homologue. Hog1, a distinct MAPK is part of the osmoregulatory pathway and controls the transcription of genes required for osmotic homeostasis.

signaling kinetics specify cell responses

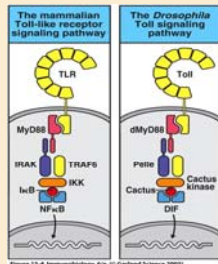




NF-κB signaling pathway

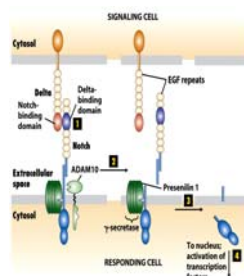


Activation of NF-κB requires E3 ligase targeted ubiquitination and subsequent proteasome degradation of I-κBα, an inhibitor of NF-κB.

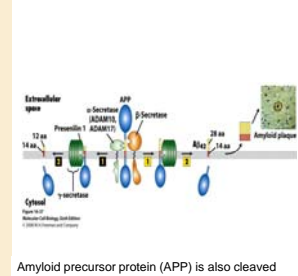


Homologies between the mammalian Toll-like receptor signaling pathway and the *Drosophila* Toll signaling pathway

Protein cleavage is the main signaling regulatory mechanism in the Notch and APP pathways

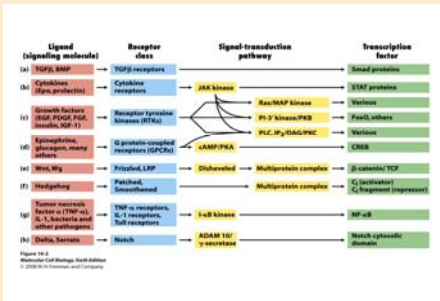


Notch is cleaved twice upon binding of delta to Notch

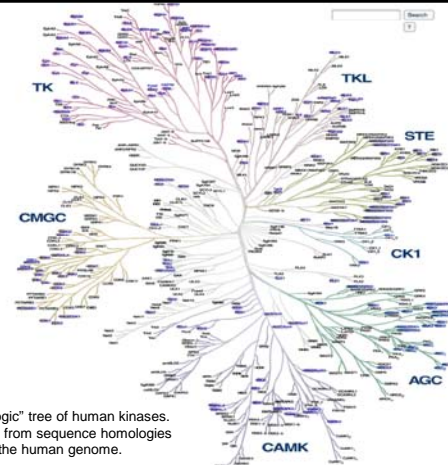


Amyloid precursor protein (APP) is also cleaved twice. Cleavage of the extracellular domain by β-Secretase instead of α-Secretase generates the Aβ42 peptides that form the large amyloid plaques found in Alzheimer patients.

Signal transduction: “the more you learn, the less it becomes clear” or the complexity of transducing accurately a signal that will induce a specific cell response



Multiple signaling molecules and mechanisms transduce a signal and contribute to a specific cell response.



“Genealogic” tree of human kinases. Deduced from sequence homologies found in the human genome.