Signaling: Protein Tyrosine Kinases

- found in all multicellular eukaryotic organisms
  - control survival, growth and differentiation
- types:
  - receptor tyrosine kinases (RTKs) - EGFR / TRKs
  - non-receptor tyrosine kinases (NRTKs) - Src / Jak
- catalyze transfer of γ phosphate of ATP to tyrosine residues on protein subsrates
  - modulation of enzyme activity
  - creation of binding sites for downstream signaling molecules

Hubbard and Till (2000) Annu. Rev. Biochem. 69:373-98.

Transmembrane receptor types involved in signaling via phosphorylation

- receptors with intrinsic enzyme activity
  - receptor tyrosine kinases (RTK) (EGFR / TRKs)
  - receptor serine/threonine kinases (TGFßR)
- "binary" receptors" - lack intrinsic catalytic activity but associate with cytosolic non-receptor protein tyrosine kinases
  - JAK PTK family (cytokine Rs / IFN-γ)
  - Src (T-cell receptors and others)

Hunter (2000) Cell 100:113-127

RTK signaling

- Regulate cellular:
  - proliferation
  - differentiation
  - migration
  - metabolic changes
- Major discoveries:
  - first RTK ligand - nerve growth factor
  - first RTK - epidermal growth factor receptor

1986 Nobel Prize: Physiology or Medicine

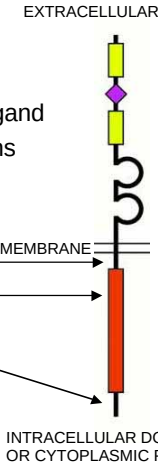
Rita Levi-Montalcini
Born: Turin, Italy, 1909

Stanley Cohen
Born: Brooklyn NY, 1922

Cowan (2001) Annu. Rev. Neurosci. 24:551-600

RTK: General Structure

- extracellular domain:
  - binds to (soluble) polypeptide ligand
  - diverse array of globular domains
- transmembrane helix
- cytoplasmic portion
  - juxtamembrane region
  - tyrosine kinase domain
  - carboxyterminal tail

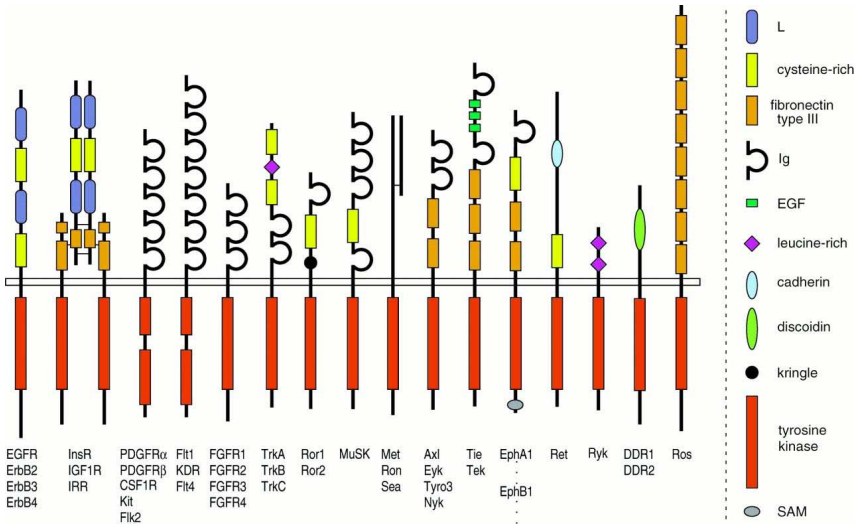


Example:

Neurotrophin Receptor (TrkA)

Ligand = nerve growth factor (NGF)

Receptor Protein Tyrosine Kinase Subfamilies



Activation of RTK

- **ligand-mediated oligomerization**
- **tyrosine phosphorylation** (signal transduction is TOTALLY dependent upon PTK activity)
  - autophosphorylation of tyrosine in activation loop of RTK & enhancement of intrinsic RTK catalytic activity (some)
  - autophosphorylation of tyrosines in RTK justamembrane, kinase insert and C-terminal region which creates binding sites to recruit downstream adaptor/signaling proteins *(Src-homolgy 2 (SH2) and phosphotyrosine-binding (PTB) domains recognize phosphotyrosines)*
  - recruitment of RTK-associated proteins with or without additional phosphorylation leading to signal propagation

RTK substrates/associated proteins

- **adapter proteins** *(lack a catalytic domain; serve as intermediate between RPTK and downstream signaling pathways)*
  - **Grb2**
  - **Shc** (linkTrkAvia Grb2 to MAPK pathway…)
  - **many others…** (SNT / SH2-B)
- **enzymes**
  - **phosphatidylinositol 3-kinase** (PI3K)
  - **phospholipases** (PLC-γ)
  - **protein tyrosine phosphatases** (SH-PTP-2)
  - **Src** (cytosolic tyrosine kinase)

How can interaction with RTK influence protein activity?

- **activation or inhibition by phosphorylation** *(ex. PLCγ activation)*
- **allosteric activation** *(ex. PI-3 kinase)*
- **localization of other proteins to RTK which may lead to their subsequent phosphorylation or may simply bring additional proteins in close proximity to their substrates** *(ex. Shc brings Grb2 which brings Sos to Ras to kick-off the MAPK pathway)*

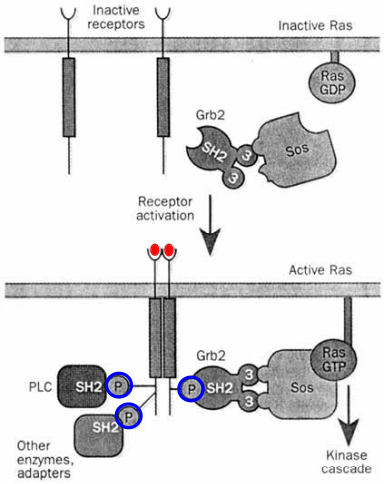
Schematic of RTK downstream signaling

MAPK pathway activation

- Grb2 (SH2 domain) binds to RTK
- Sos (guanine nucleotide exchange factor) binds to Grb2 (SH3 domain)
- Sos activates Ras (GDP-GTP exchange)
- Activation of Ras leads to activation of a mitogen-activated protein kinase cascade (constitutive Ras activation is found in a variety of human tumors)

Other

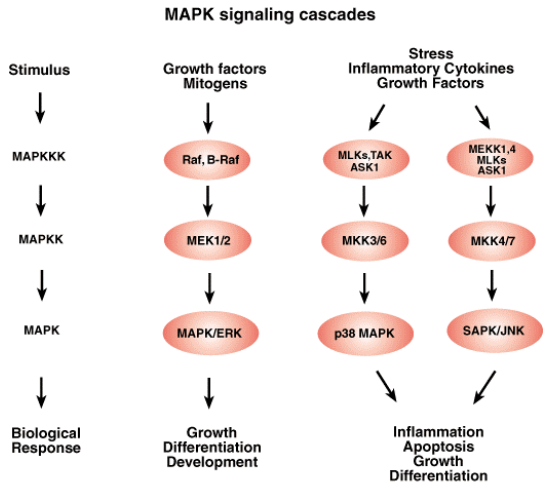
- PLCγ, other enzymes, adapters...



McCormick (1993) Nature 363:15-16.

Description of MAPK modules

- regulate cellular proliferation, differentiation and survival
- targets of the receptor tyrosine kinase family (& GPCRs)
- three MAPKs in mammals (ERK / p38 / JNK)
- Upstream signals lead to activation of **MAP3K** (serine/threonine kinase) which P's and activates a **MAP2K** (dual specificity kinase) which P's and activates a **MAPK** (seriine/threonine kinase)



Neurotrophins

(NGF/ BDNF/NT-3/NT-4)

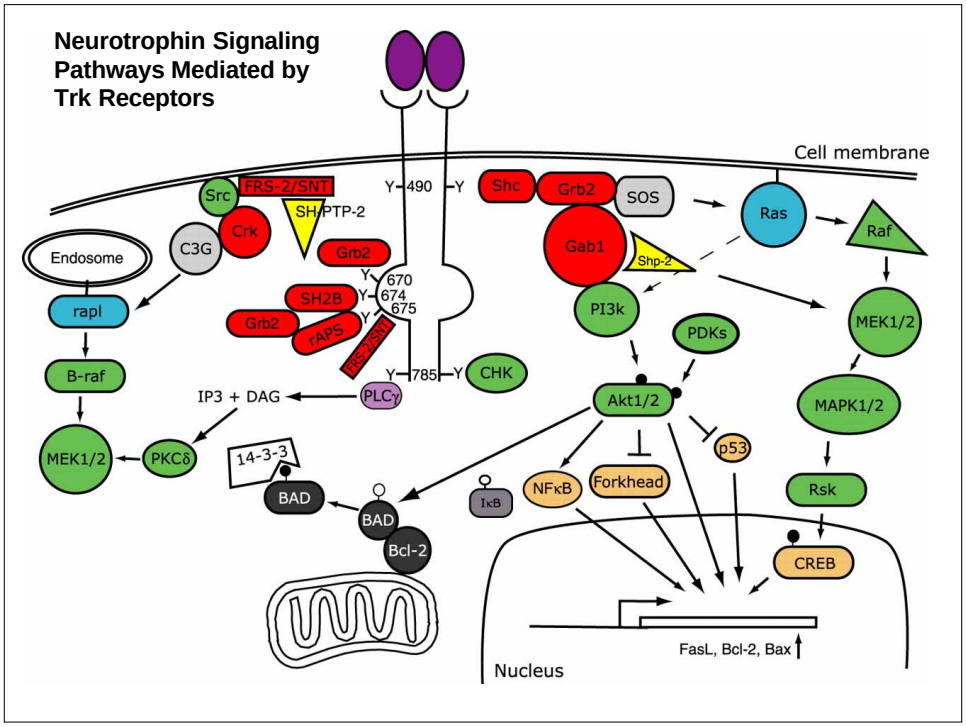
- Essential for development of the vertebrate nervous system
- Neurotrophins bind to 2-different receptors:
  - Trks (tropomyosin-related kinase receptor tyrosine kinases)
    - Types:
      - TrkA: NGF (NT-3)
      - TrkB: BDNF / NT-4
      - TrkC: NT-3
    - Function: transmit + signals to enhance survival / differentiation
  - p75 neurotrophin R or $p75^{NTR}$ (TNF family member)
    - Bind ALL neurotrophins
    - Function: can be + or -

TrkA-mediated signaling

- Cell culture systems:
  - PC12 cells
  - Primary neuronal (sympathetic) cultures
    - mass
    - compartmentalized
- Approaches:
  - Receptor mutants *(overexpressed)*
  - Pharmacological inhibitors
  - Ectopic overexpression
  - Mutants *(constitutively active / dominant-negative / selective effector)*
  - Ab microinjection
- Conclusions:
  - A component may be proven to be sufficient but yet not be necessary for a given neurotrophin-mediated response
  - Pathways are complex and often redundant

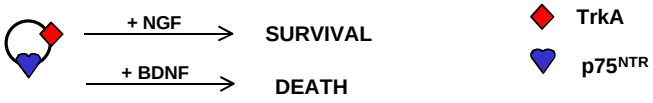
Trk-mediated signaling

- PI3K:
  - Ras -dependent & -independent (via Gab1)
  - Akt targets (serine/threonine kinase)
    - Bad / Forkhead / IκB
- Ras:
  - PI3K pathway activation
  - MEK/MAP targets
    - Ribosomal S6 kinase (RSK) / CREB / Bcl2
- Other routes to MAPK pathway activation
  - PLCγ: Generation of DAG and IP3 and activation of PKCδ
  - SNT adaptor: activation of Src (cytosolic tyrosine kinase)
- Other…



Roles for p75^{NTR}

- The signaling capacity and biological role of p75^{NTR} is a function of of cellular Trk activation status
  - In the absence of Trk activation, neurotrophin interaction with p75^{NTR} signals apoptosis



Elimination of neurons to prevent neuronal mistargeting

  - Trk signaling silences p75^{NTR}-mediated apoptotic pathways
  - p75^{NTR} is essential for apoptosis following growth factor withdrawal in some cells
  - p75^{NTR} refines the ligand specificity of Trk R's

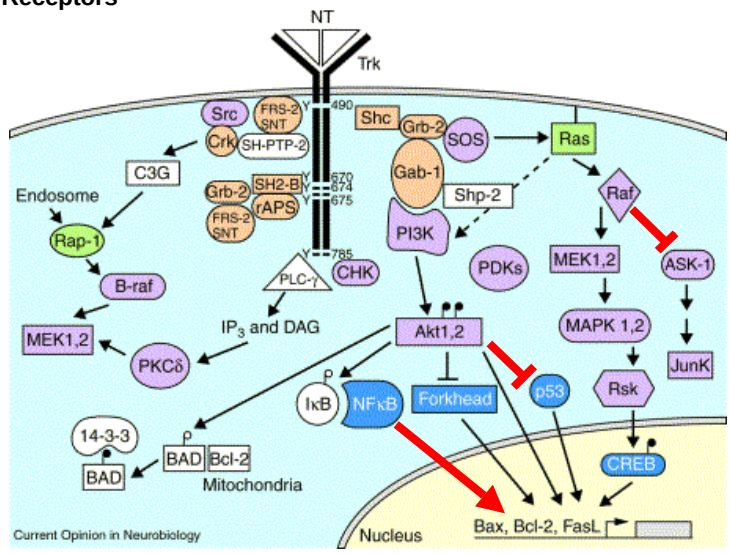
p75^{NTR}-mediated signaling

- **Pro-apoptotic**
  - JNK (Jun amino-terminal kinase)-p53-BAX
  - Activation of the cell stress pathway of apoptosis
    (Trk activation silences this pathway)

- **Pro-survival** (with Trk)
  - Activation of the NFκB pathway

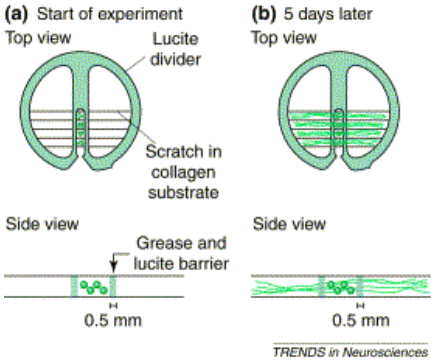
Neurotrophin Signaling Pathways Mediated by Trk Receptors

Trk + p75^{NTR} + Appropriate neurotrophin



Importance of Location in Neurotrophin Signal Transduction

- Use of compartmentalized cultures
  - Can apply neurotrophin separately to
    - Cell body
    - Distal axons
- Neurotrophins applied to distal axons
  - result in the rapid appearance of phosphorylated Trks in the cell body (formation of "signaling endosomes")
  - sufficient to support survival



Heerssen&Segal (2002) 25:160-165

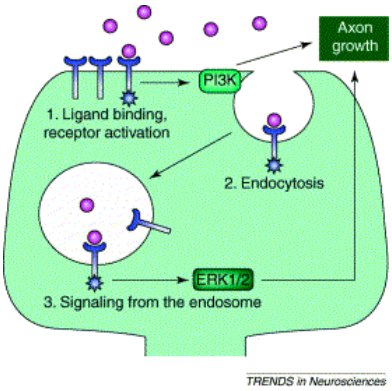
Local signaling at the axon terminal

axonal elongation:

- PI3K lipid signaling
  - pharmacologic inhibitor - inhibits axonal elongation
- Local Erk1/2 activation
  - pharmacologic inhibitor - inhibits axonal elongation

endosome formation:

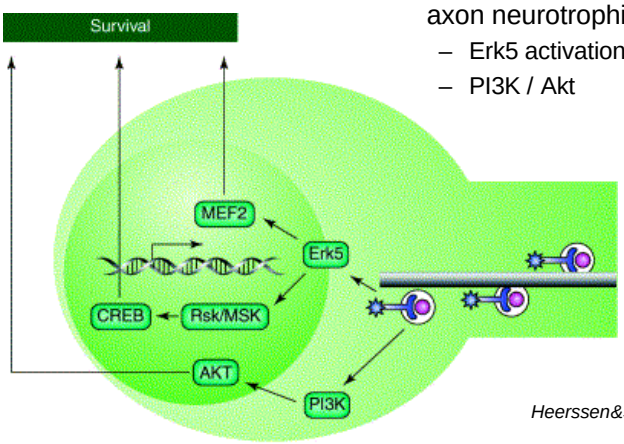
- PI3K lipid signaling
  - Phamacologic inhibitor - disrupts ligand-dependent internalization



Heerssen&Segal (2002) 25:160-165

Signaling at the cell body

- Cell body effects resulting from distal axon neurotrophin application:
  - Erk5 activation (not Erk1/2)
  - PI3K / Akt

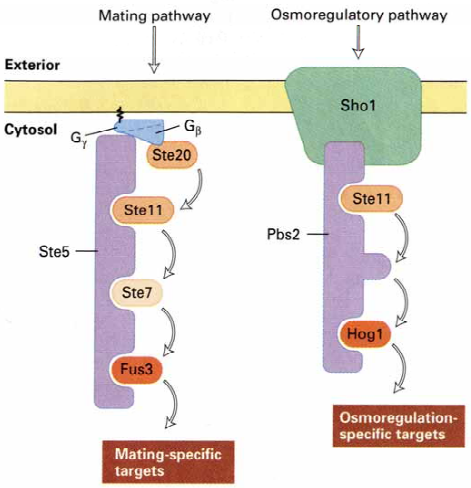


Heerssen&Segal (2002) 25:160-165

TRENDS in Neurosciences

Role for Scaffolding Proteins

- first example in yeast
- selective regulation of different MAPK pathways (strengthen weak interactions between kinases)
- enhance activation of pathway components (enhance the rate of phosphate transfer)
- insulate the MAP kinase pathway (prevent crosstalk with functionally unrelated modules)



MAP kinase signaling in mammals: Are protein scaffolds involved?

Examples in mammals

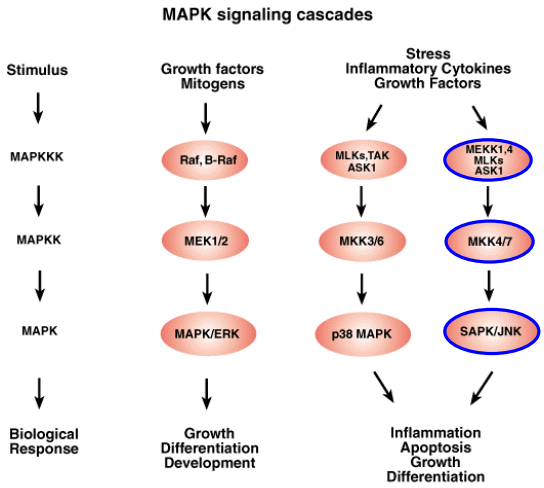
- C-Jun NH_2-terminal kinase (JNK) group
- Kinase suppressor of Ras (KSR)

Overlap in JNK pathway substrate specificity with other MAP kinases

Formation of signaling complexes

- physical interaction between components
- assembly on anchor or scaffold proteins

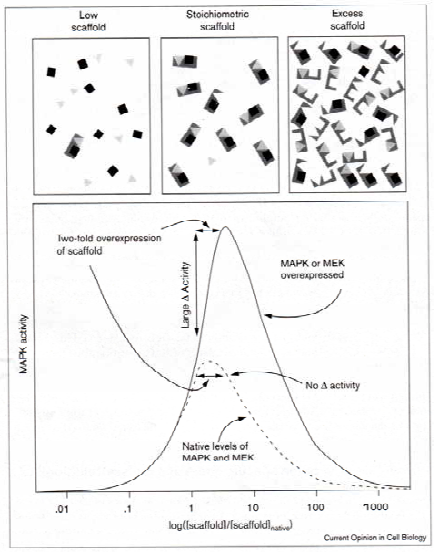
Identification of JNK interacting protein-1 (JIP-1) - "A cytoplasmic inhibitor of the JNK signal transduction pathway"



Dickens et al., (1997) Science 277:693-696

Scaffolds can look like inhibitors !

- Titrate the [scaffold] close to the concentration of binding components for optimal signaling
- Too much scaffold would dilute the pathway components (eg. problem with overexpression experiments)



Burack & Shaw (2000) Curr Op Cell Biol 12:211-216

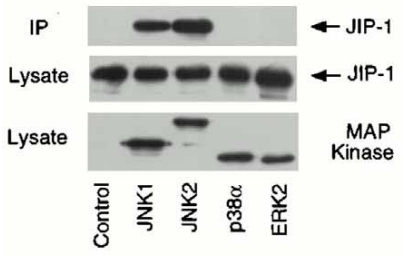
JIP-1 binds selectively to JNK MAPKs, MKK7 (MAPKK) and MLK MAPKKKs

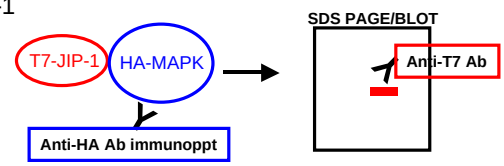
Expression of epitope-tagged MAPKKK, MAPKK or MAPK in cells to determine if epitope-tagged JIP-1 shows binding specificity

Example:

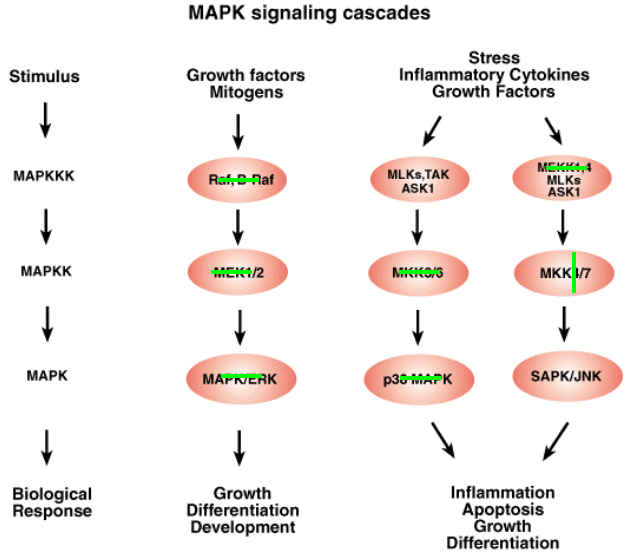
- T7-tagged JIP-1 expressed in cells with HA-tagged MAPK
- HA-tagged MAPKs immunoprecipitated with HA-Ab; SDS gel & immunoblot
- T7-tagged JIP-1 detected by western blotting using a T7-Ab
- Control: verification that JIP-1 and MAP kinases were expressed

Whitmarsh et al. (1998) Science 281:1671-1674





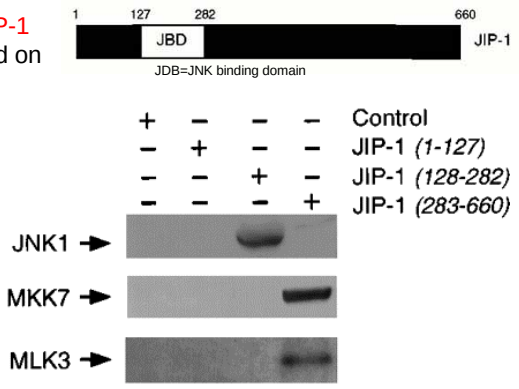
Summary of JIP-1 specificity



Components of the JNK signaling pathway interact directly with JIP-1

***in vitro* binding assay**

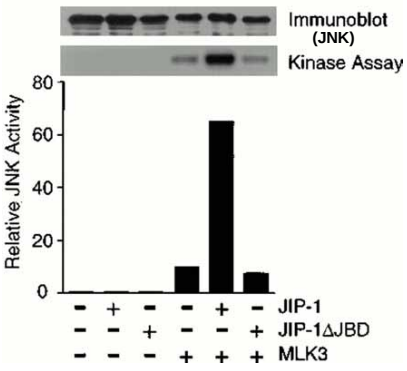
- GST (control) and GST-JIP-1 fusion proteins immobilized on glutathione agarose
- incubate with bacterially expressed:
  - JNK1
  - Flag-tagged-MKK7
  - Flag-tagged-MLK3
- SDS gel / immunoblot
  - anti-Flag-MKK7 Ab
  - anti-Flag-MLK3 Ab
  - JNK-Ab



Whitmarsh et al. (1998) Science 281:1671-1674

Coexpression of JIP-1 with upstream components of the JNK pathway

Effect of JIP-1 overexpression on JNK activation by MLK3



Whitmarsh et al. (1998) Science 281:1671-1674

Model: Interaction of JIP-1 with the JNK MAP kinase module