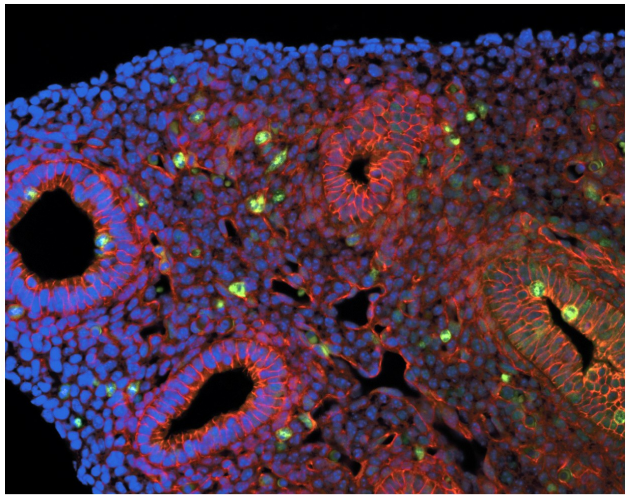
Molecular and Cellular Biology

08.2. Cell Signalling II:

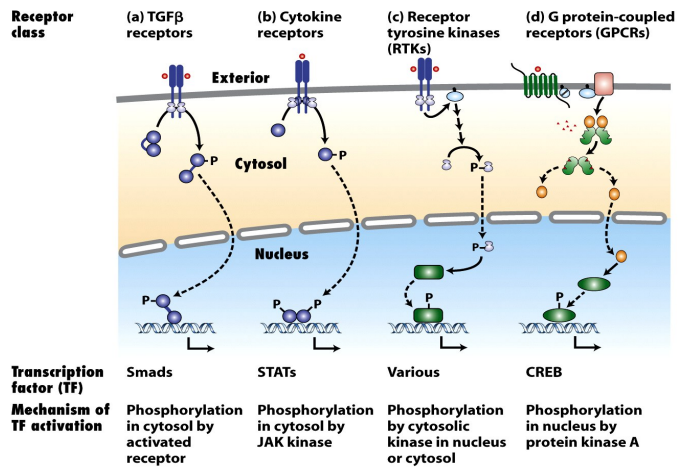
Signal Transduction Pathways that Control Gene Activity

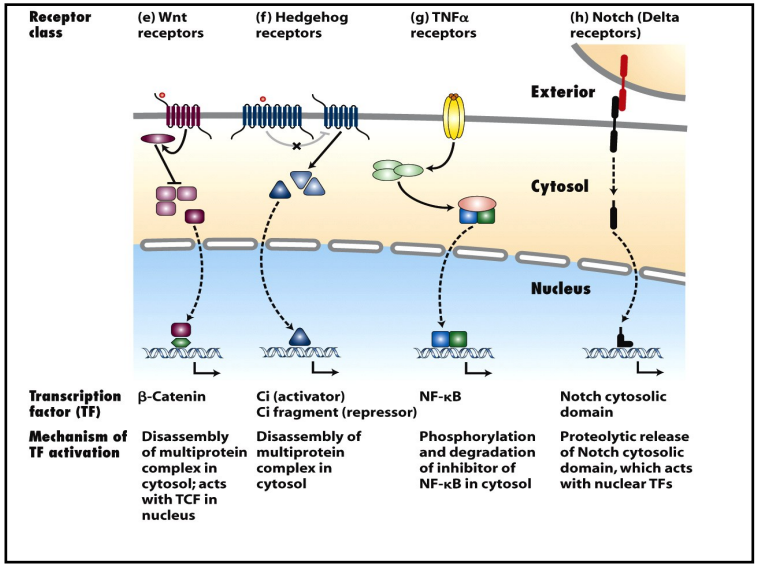
- Receptor Kinases etc-

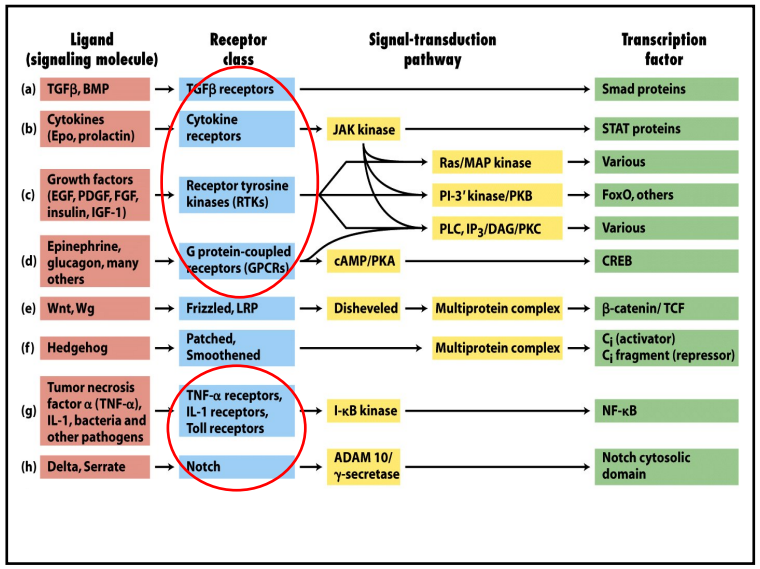
Prof. Dr. Klaus Heese

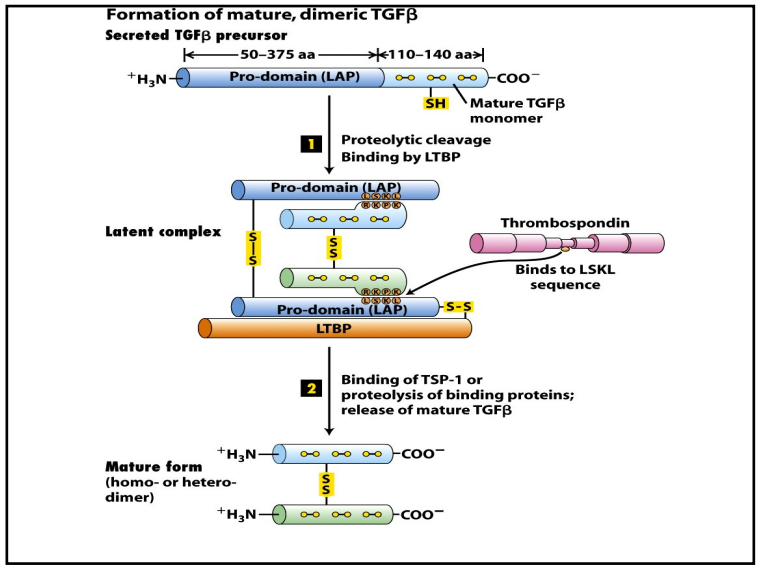


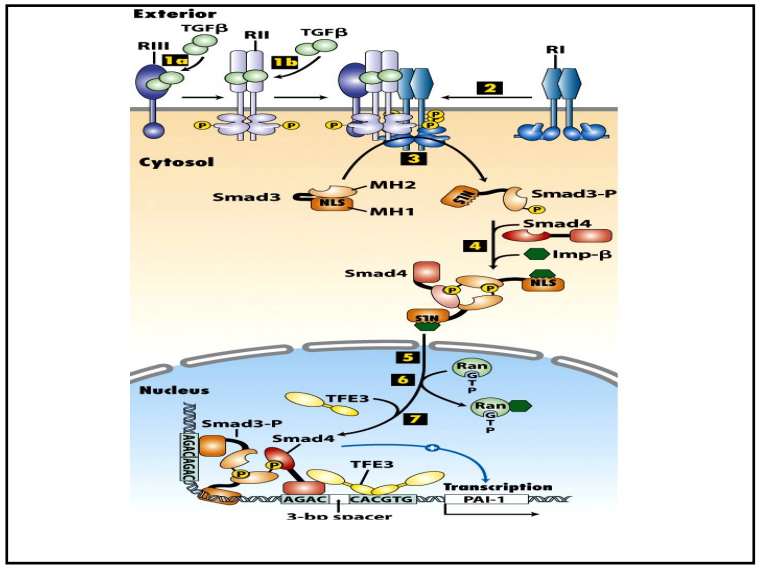
Overview of various Ligand-Receptor Signalling Pathways

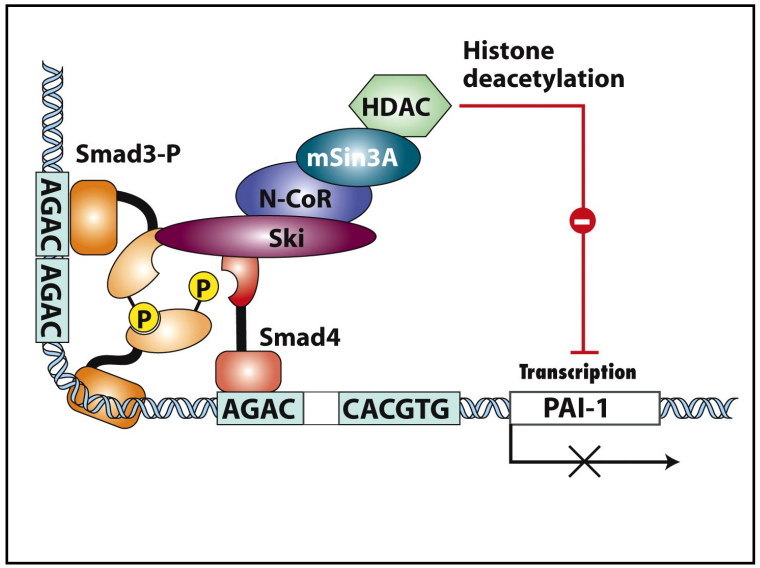


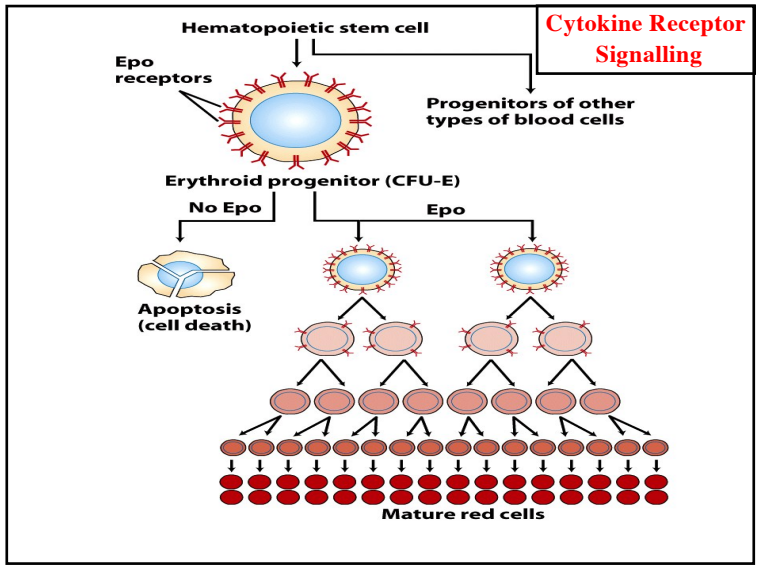


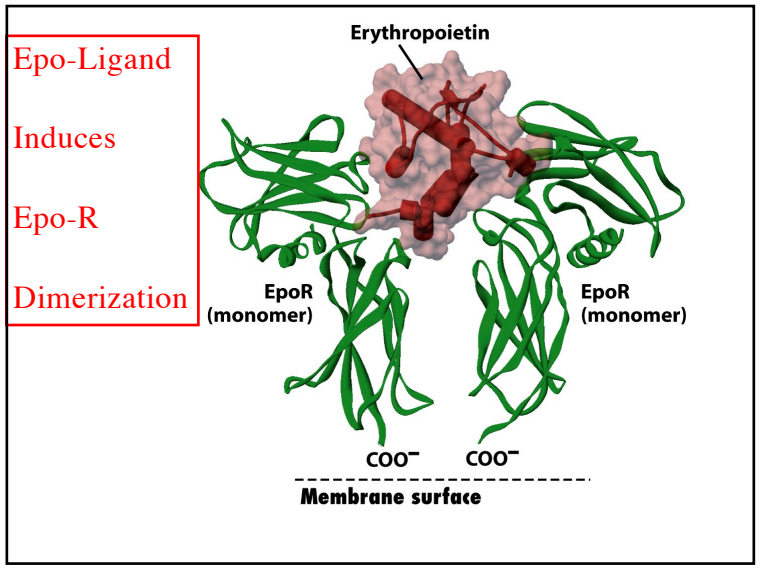

Ligand (signaling molecule)
Receptor class
Signal-transduction pathway
Transcription factor
(a) TGFβ, BMP
TGFβ receptors
Smad proteins
(b) Cytokines (Epo, prolactin)
Cytokine receptors
JAK kinase
STAT proteins
(c) Growth factors (EGF, PDGF, FGF, insulin, IGF-1)
Receptor tyrosine kinases (RTKs)
Ras/MAP kinase
Various
PI-3′ kinase/PKB
FoxO, others
PLC, IP3/DAG/PKC
Various
(d) Epinephrine, glucagon, many others
G protein-coupled receptors (GPCRs)
cAMP/PKA
CREB
(e) Wnt, Wg
Frizzled, LRP
Disheveled
Multiprotein complex
β-catenin/ TCF
(f) Hedgehog
Patched, Smoothened
Multiprotein complex
Ci (activator) Ci fragment (repressor)
(g) Tumor necrosis factor α (TNF-α), IL-1, bacteria and other pathogens
TNF-α receptors, IL-1 receptors, Toll receptors
I-κB kinase
NF-κB
(h) Delta, Serrate
Notch
ADAM 10/ γ-secretase
Notch cytosolic domain

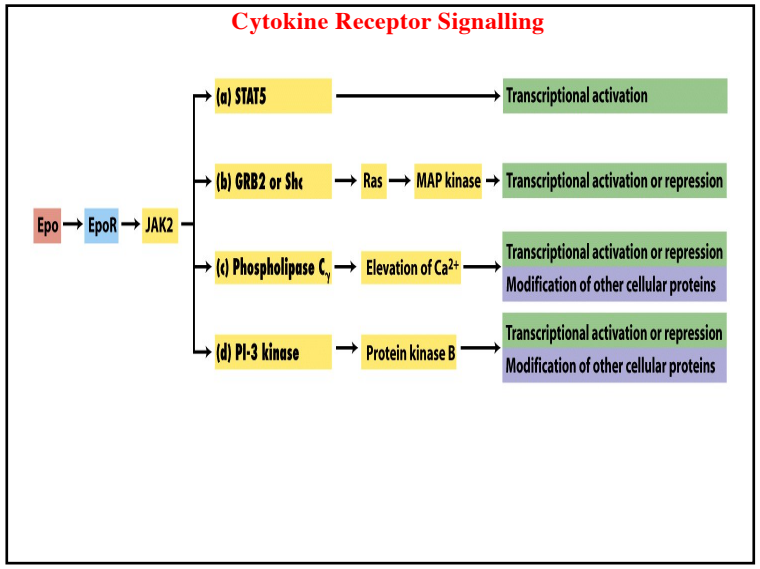

Formation of mature, dimeric TGFβ
Secreted TGFβ precursor
50–375 aa
110–140 aa
+H3N
Pro-domain (LAP)
COO−
SH
Mature TGFβ monomer
1
Proteolytic cleavage
Binding by LTBP
Pro-domain (LAP)
Latent complex
Thrombospondin
Binds to LSKL sequence
Pro-domain (LAP)
S-S
LTBP
2
Binding of TSP-1 or proteolysis of binding proteins; release of mature TGFβ
Mature form (homo- or hetero-dimer)
+H3N
COO−
+H3N
COO−

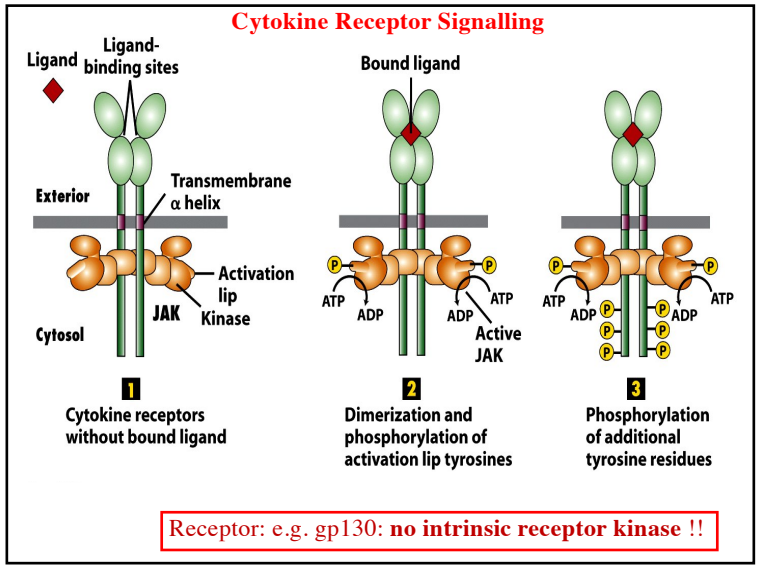

Exterior
TGFβ
RIII
RII
TGFβ
RI
1c
1b
2
Cytosol
3
Smad3
MH2
MH1
NLS
Smad3-P
Smad4
4
Imp-β
Smad4
NLS
5
6
Ran GTP
Nucleus
TFE3
7
Ran GTP
Smad3-P
Smad4
TFE3
Transcription
AGAC
CACGTG
PAI-1
3-bp spacer

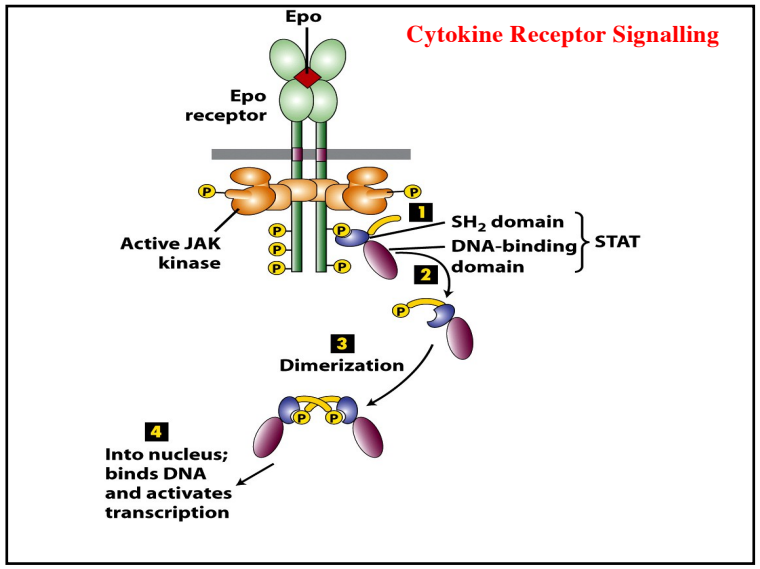

Histone deacetylation
HDAC
mSin3A
N-CoR
Ski
Smad3-P
AGAC
AGAC
P
P
Smad4
Transcription
AGAC
CACGTG
PAI-1

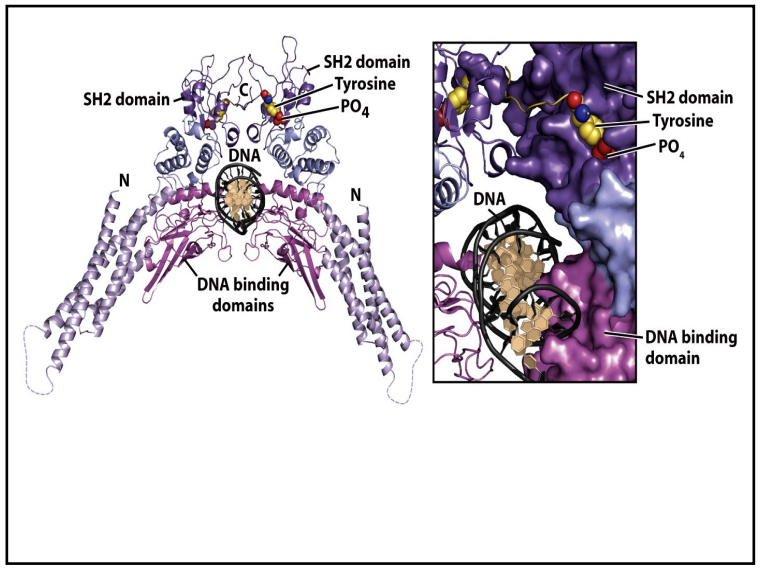

Cytokine Receptor Signalling
Hematopoietic stem cell
Epo receptors
Progenitors of other types of blood cells
Erythroid progenitor (CFU-E)
No Epo
Epo
Apoptosis (cell death)
Mature red cells

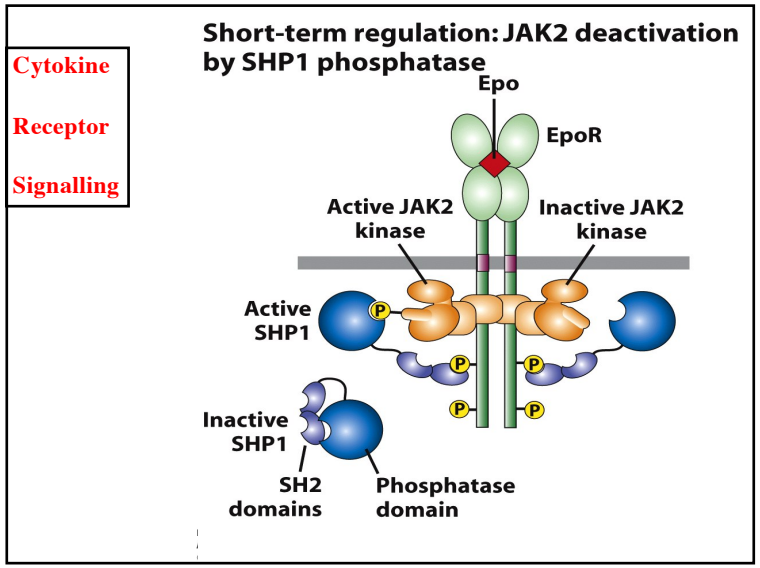

Epo-Ligand
Induces
Epo-R
Dimerization
Erythropoietin
EpoR (monomer)
EpoR (monomer)
COO⁻
COO⁻
Membrane surface

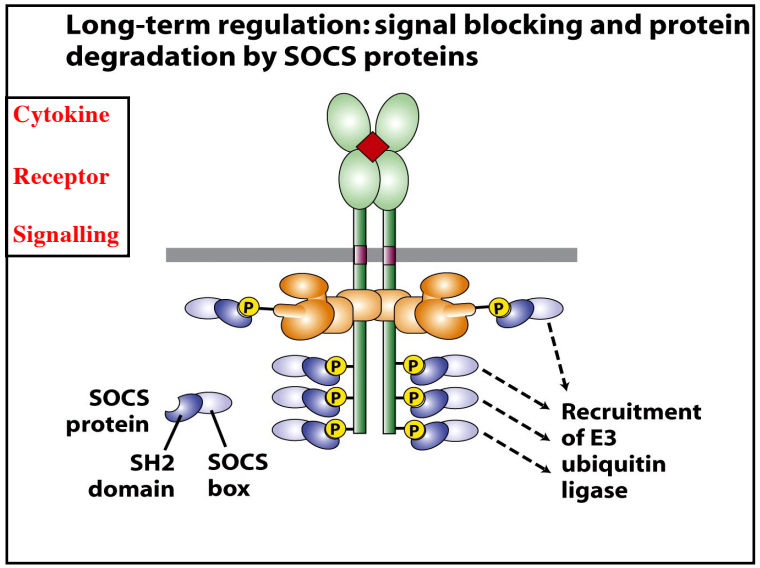

Cytokine Receptor Signalling
Epo
EpoR
JAK2
(a) STAT5
Transcriptional activation
(b) GRB2 or Shc
Ras
MAP kinase
Transcriptional activation or repression
(c) Phospholipase Cγ
Elevation of Ca2+
Transcriptional activation or repression
Modification of other cellular proteins
(d) PI-3 kinase
Protein kinase B
Transcriptional activation or repression
Modification of other cellular proteins

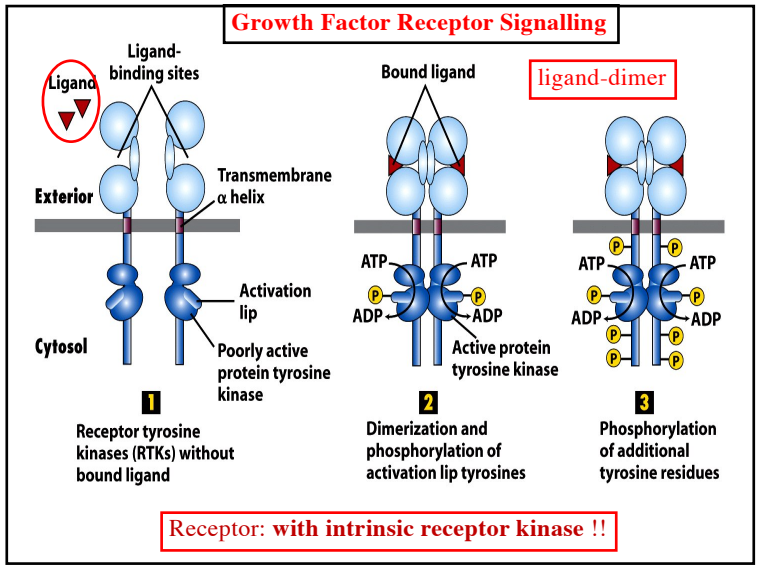

Cytokine Receptor Signalling
Ligand
Ligand-binding sites
Exterior
Transmembrane α helix
Activation lip
JAK
Kinase
Cytosol
Bound ligand
ATP
ADP
Active JAK
1
Cytokine receptors without bound ligand
2
Dimerization and phosphorylation of activation lip tyrosines
3
Phosphorylation of additional tyrosine residues
Receptor: e.g. gp130: no intrinsic receptor kinase !!

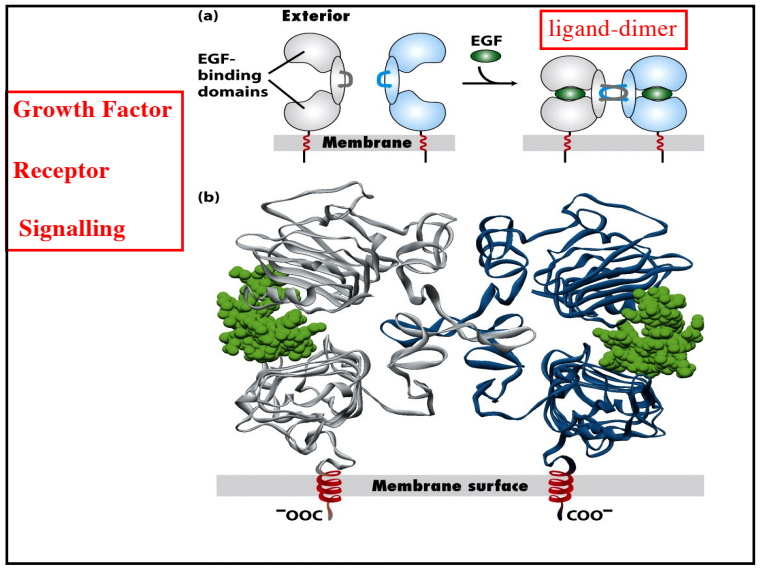

Cytokine Receptor Signalling
Epo
Epo receptor
Active JAK kinase
1
SH2 domain
DNA-binding domain
STAT
2
3
Dimerization
4
Into nucleus; binds DNA and activates transcription

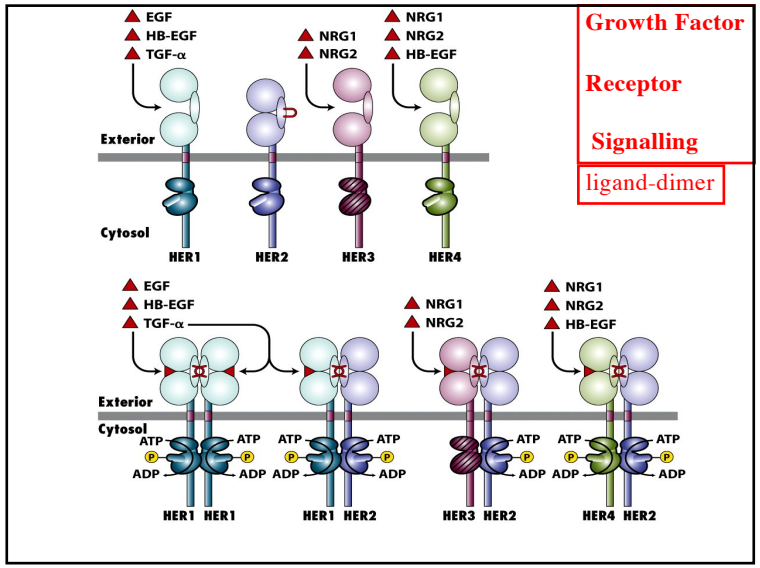

SH2 domain
Tyrosine
PO4
SH2 domain
C
DNA
N
N
DNA binding domains
SH2 domain
Tyrosine
PO4
DNA
DNA binding domain

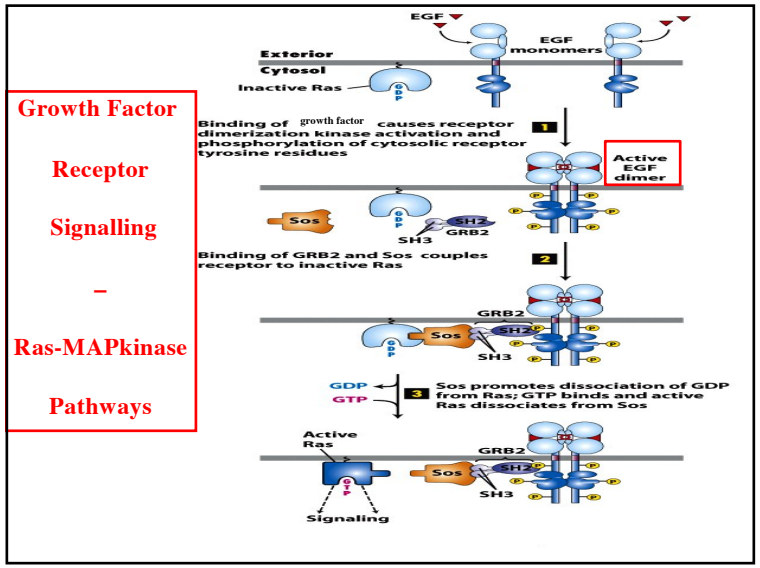

Cytokine Receptor Signalling
Short-term regulation: JAK2 deactivation by SHP1 phosphatase
Epo
EpoR
Active JAK2 kinase
Inactive JAK2 kinase
Active SHP1
Inactive SHP1
SH2 domains
Phosphatase domain

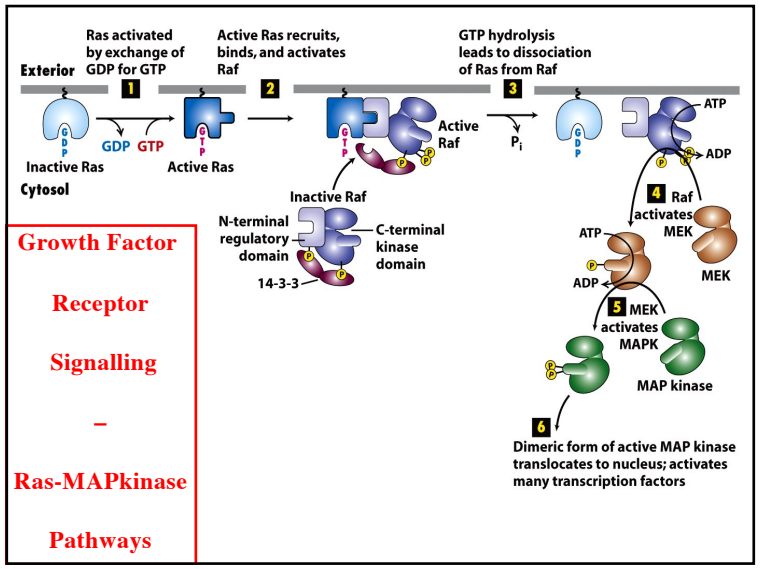

Cytokine Receptor Signalling
Long-term regulation: signal blocking and protein degradation by SOCS proteins
SOCS protein
SH2 domain
SOCS box
Recruitment of E3 ubiquitin ligase

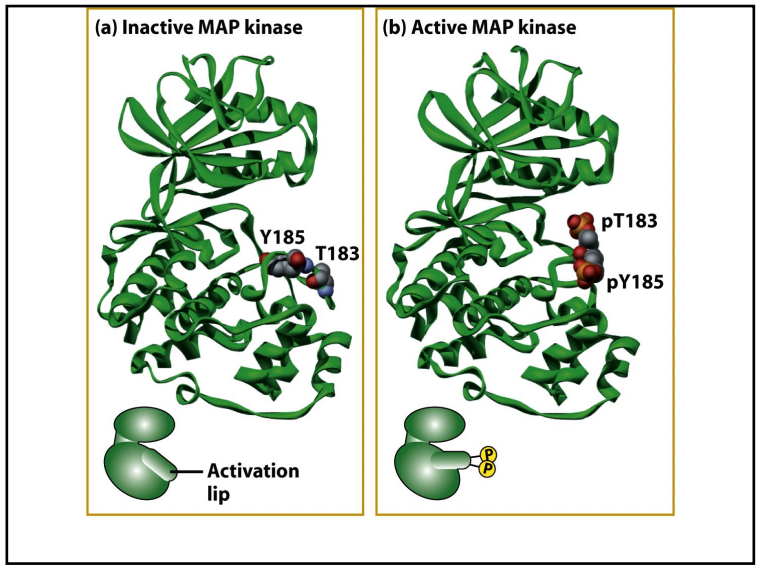

Growth Factor Receptor Signalling
Ligand
Ligand-binding sites
Exterior
Transmembrane α helix
Activation lip
Cytosol
Poorly active protein tyrosine kinase
1
Receptor tyrosine kinases (RTKs) without bound ligand
Bound ligand
ligand-dimer
ATP
ADP
Active protein tyrosine kinase
2
Dimerization and phosphorylation of activation lip tyrosines
3
Phosphorylation of additional tyrosine residues
Receptor: with intrinsic receptor kinase !!

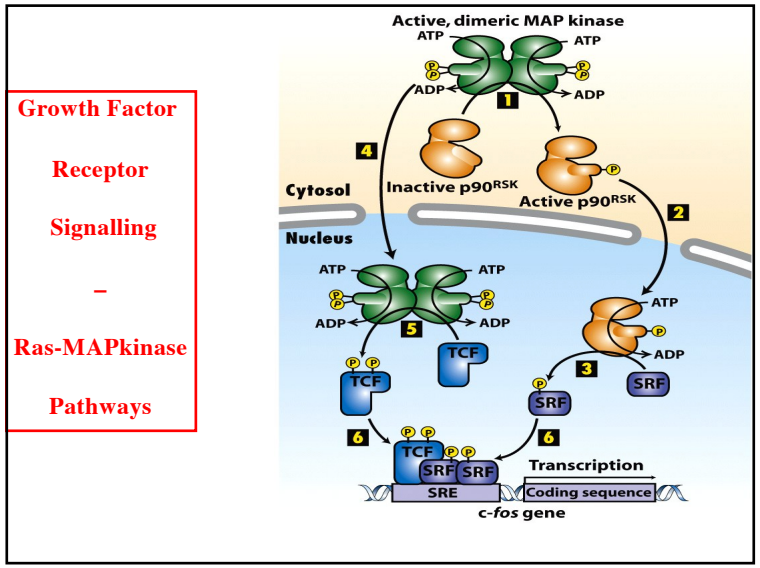

Growth Factor Receptor Signalling
(a)
Exterior
EGF-binding domains
EGF
ligand-dimer
Membrane
(b)
Membrane surface
⁻OOC
COO⁻

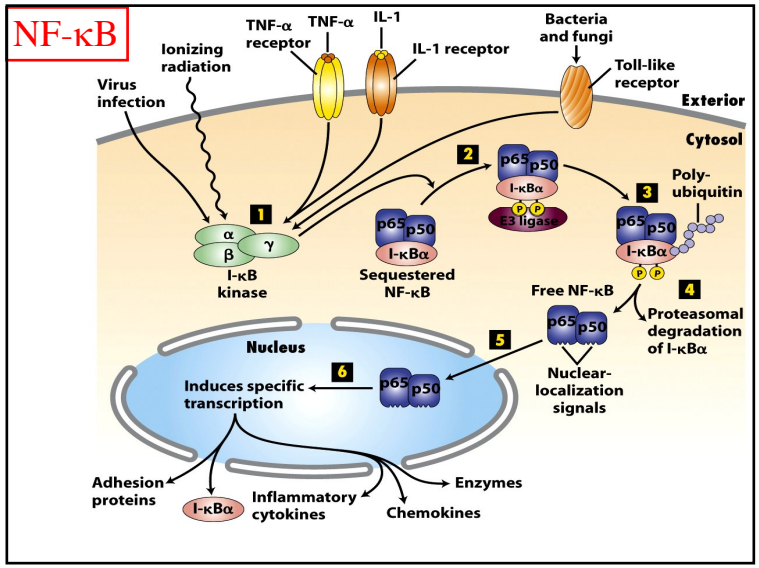

Growth Factor Receptor Signalling
ligand-dimer
EGF
HB-EGF
TGF-α
NRG1
NRG2
HB-EGF
Exterior
Cytosol
HER1
HER2
HER3
HER4
ATP
ADP
HER1 HER1
HER1 HER2
HER3 HER2
HER4 HER2


Growth Factor Receptor Signalling – Ras-MAPkinase Pathways
EGF
EGF monomers
Exterior
Cytosol
Inactive Ras
GDP
Binding of growth factor causes receptor dimerization kinase activation and phosphorylation of cytosolic receptor tyrosine residues
1
Active EGF dimer
Sos
SH3
GRB2
Binding of GRB2 and Sos couples receptor to inactive Ras
2
SH2
GTP
GDP
3
Sos promotes dissociation of GDP from Ras; GTP binds and active Ras dissociates from Sos
Active Ras
Signaling

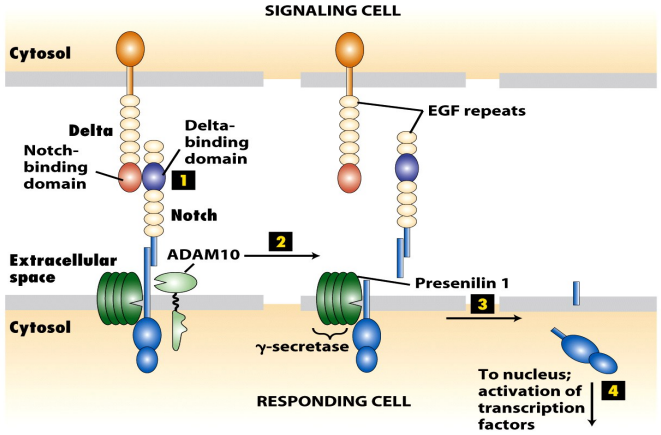

Growth Factor
Receptor
Signalling
–
Ras-MAPkinase
Pathways
Exterior
Cytosol
Inactive Ras
GDP
GTP
Active Ras
Ras activated by exchange of GDP for GTP
Active Ras recruits, binds, and activates Raf
GTP hydrolysis leads to dissociation of Ras from Raf
Inactive Raf
Active Raf
N-terminal regulatory domain
C-terminal kinase domain
14-3-3
ATP
ADP
Pi
Raf activates MEK
MEK
MEK activates MAPK
MAP kinase
Dimeric form of active MAP kinase translocates to nucleus; activates many transcription factors


(a) Inactive MAP kinase
(b) Active MAP kinase
Y185
T183
pT183
pY185
Activation lip


Growth Factor
Receptor
Signalling
–
Ras-MAPkinase
Pathways
Active, dimeric MAP kinase
ATP
ADP
Cytosol
Nucleus
Inactive p90RSK
Active p90RSK
TCF
SRF
SRE
Coding sequence
Transcription
c-fos gene

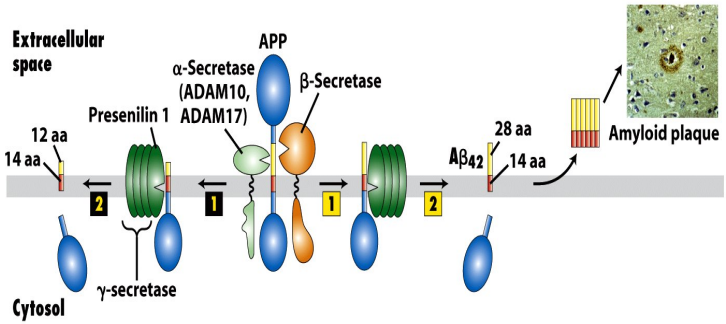

NF-κB
Ionizing radiation
Virus infection
TNF-α receptor
TNF-α
IL-1
IL-1 receptor
Bacteria and fungi
Toll-like receptor
Exterior
Cytosol
I-κB kinase
Sequestered NF-κB
I-κBα
p65
p50
E3 ligase
Poly-ubiquitin
Free NF-κB
Proteasomal degradation of I-κBα
Nuclear-localization signals
Nucleus
Induces specific transcription
Adhesion proteins
Inflammatory cytokines
Enzymes
Chemokines

Cell Signalling by Proteolysis / Cleavage / Shedding:
Regulated Intramembrane Proteolysis / RIP-tide mechanism

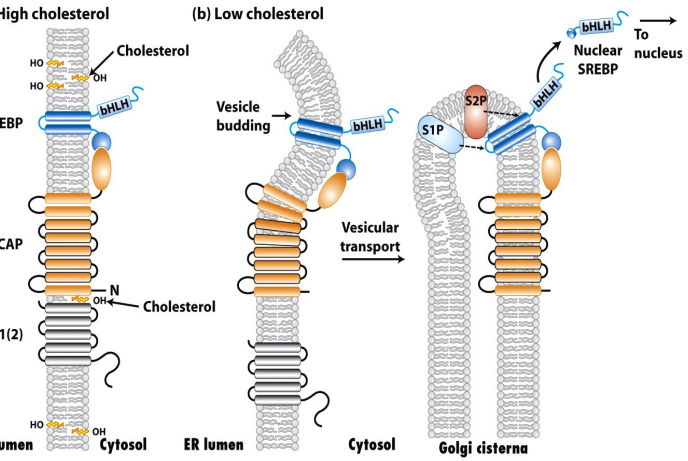


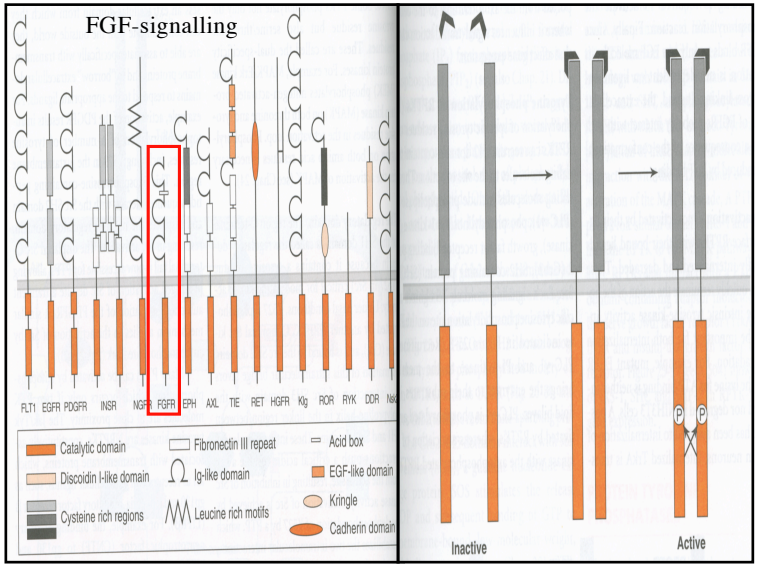
The Amyloid Precursor Protein (APP), Cleavage,

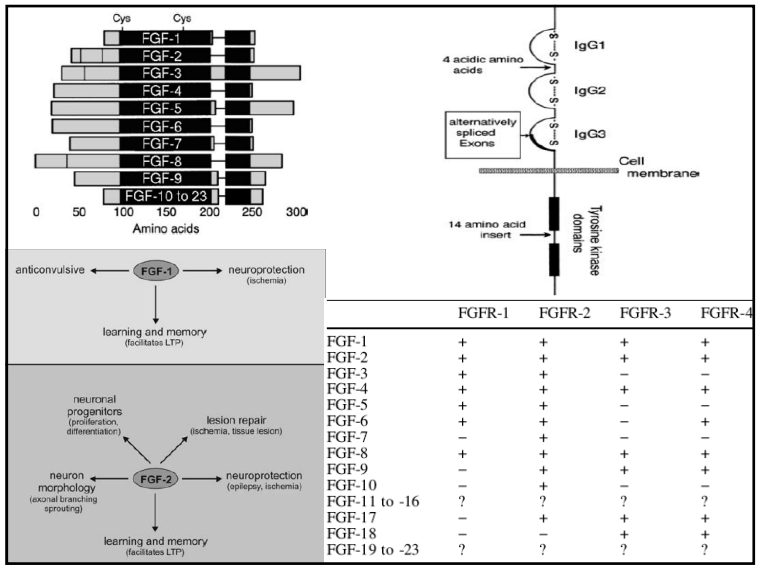
and Alzheimer's disease



Cholesterol, Proteolysis, and Gene Transcription







	FGFR-1	FGFR-2	FGFR-3	FGFR-4
FGF-1	+	+	+	+
FGF-2	+	+	+	+
FGF-3	+	+	–	–
FGF-4	+	+	+	+
FGF-5	+	+	–	–
FGF-6	+	+	–	+
FGF-7	–	+	–	–
FGF-8	+	+	+	+
FGF-9	–	+	+	+
FGF-10	–	+	–	–
FGF-11 to -16	?	?	?	?
FGF-17	–	+	+	+
FGF-18	–	–	+	+
FGF-19 to -23	?	?	?	?

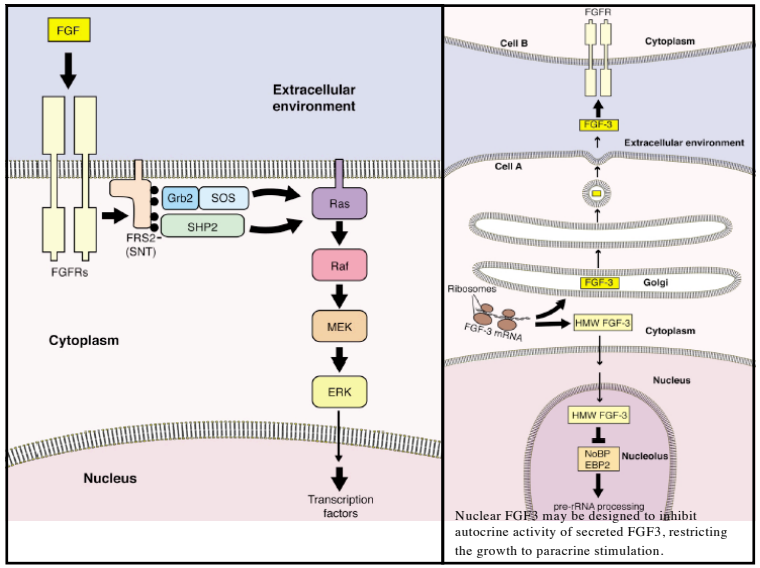
FGF2 = bFGF

The FGF-family comprises heparin-binding proteins that promote mitogenesis of mesoderm- and neuroectoderm-derived cells

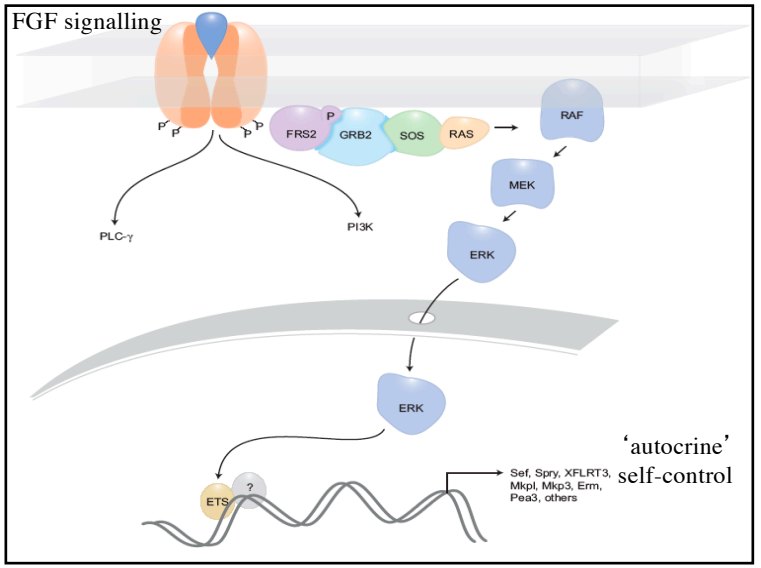
21.5, 22 and 24 kDa = HMW FGF2, nuclear
18kDa FGF2, cytoplasm, cell surface, extracellular

Proliferation, Angiogenesis

18kDa FGF2 (but not HMW FGF2) up-regulates IL-6 and IL-6R in Schwann Cells ----> implication for neuro-regeneration (e.g.: after peripheral nerve lesion / crushed sciatic nerves.
With regard to the functional role of FGF-2 after peripheral nerve injury, evidence from in vivo studies suggests that the molecule could mediate neurotrophic effects on axotomized motor and sensory neurons. This notion is supported by the finding that nerve lesion results in an up-regulation of FGF-2 expression in motoneurons, dorsal root ganglia, and sympathetic ganglia and that exogenously applied FGF-2 can prevent the lesion-induced neuron death. At the lesion site itself FGF-2 might be involved in the myelination.

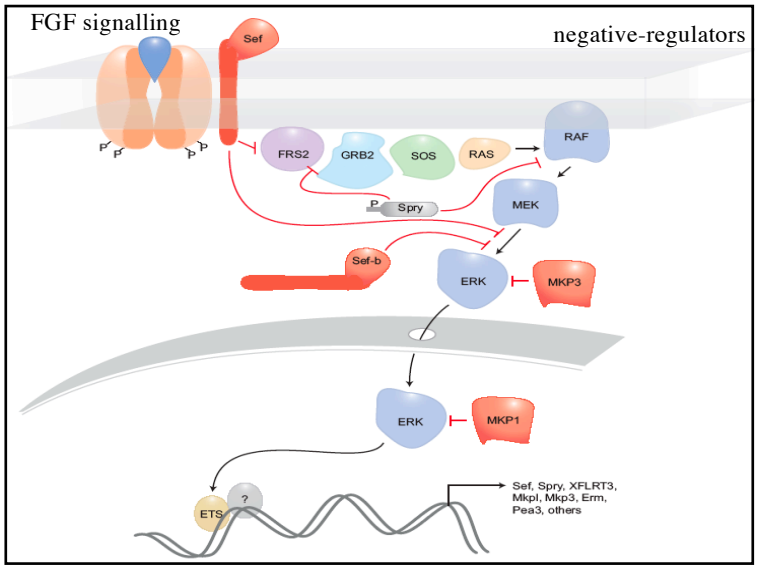


Nuclear FGF3 may be designed to inhibit autocrine activity of secreted FGF3, restricting the growth to paracrine stimulation.



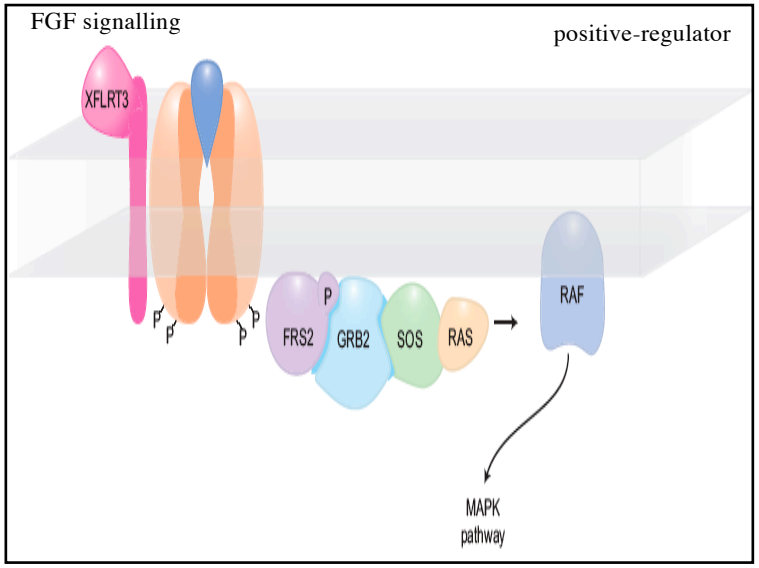
FGF signalling

negative-regulators

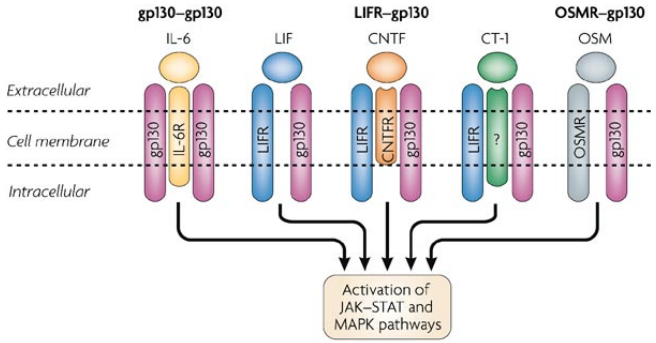


FGF signalling

positive-regulator



IL-6 Receptor Family
Neuro-Immune Functions

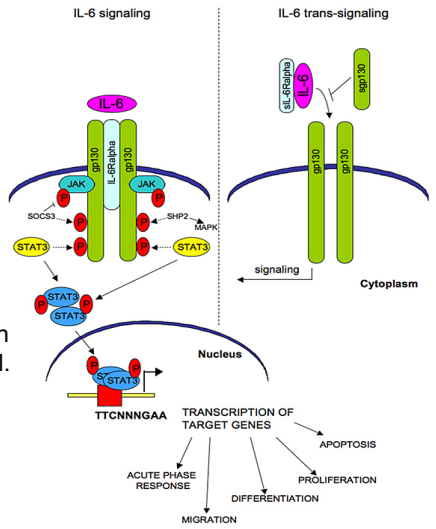


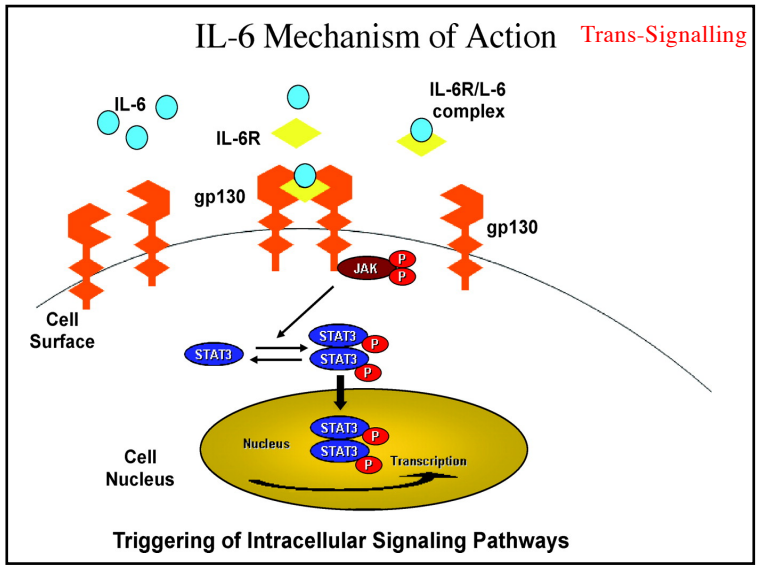
Classic vs Trans-signaling

Classic-: when target cell express IL-6R (IL-6R$^{+/+}$).

Trans-: when target cell does not express IL-6R (IL-6R$^{-/-}$).

This way, IL-6R$^{-/-}$ cells can also receive IL-6 signal.





IL-6 signalling: from cell surface to the nucleus

Upon binding of IL-6/IL-6R complex, gp130 forms homo-dimer and is subsequently phosphorylated by Janus kinase (JAK) family protein.

Phosphorylated gp130 then binds to STAT3, then STAT3 becomes also phosphorylated by JAK → phosphorylated STAT3 (p-STAT3).

p-STAT3 then forms a homo-dimer (p-STAT3/p-STAT3)

Dimerized STAT3 translocates into the nucleus and then binds to DNA on STAT3 responsive elements in the promoter region → activation of transcription machinery.