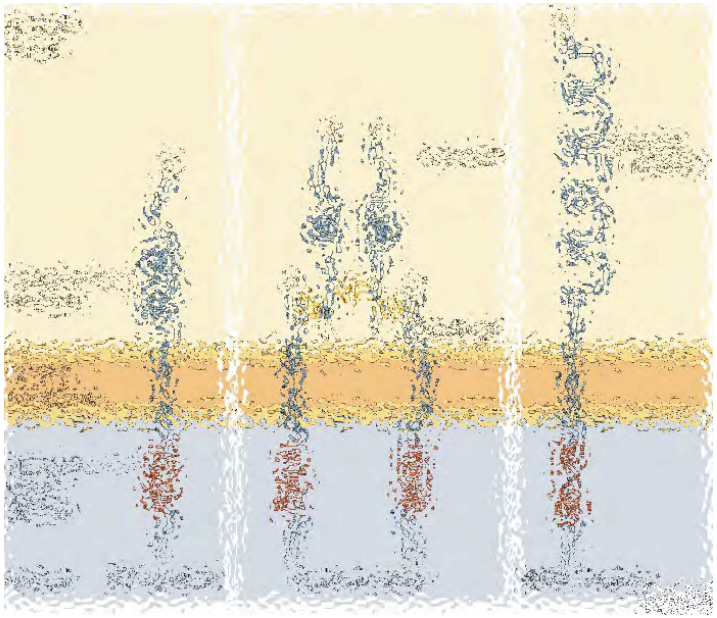
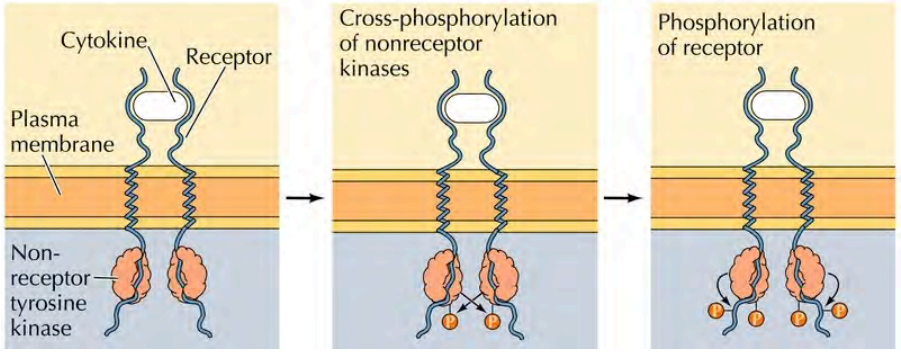
Enzyme-linked receptors fall into 3 categories:

- **Tyrosine Kinase Receptors**
  - Not only a receptor
  - Also an enzyme: Tyrosine kinase



- **Cytokine superfamily receptors**
  - No catalytic domain
  - Interact with non receptor protein-tyrosine kinases
    - Src family
    - JAK family



- **TGF-β receptors**

What is Cytokine?

- Secreted polypeptide or low molecular weight protein involved in *cell-to-cell signaling*.

- Acts in paracrine or autocrine fashion *through specific cellular receptors*.

- Can be produced by cells of any tissue and act on many *cells involved in immune and inflammatory response*.

Receptors Classification

RECEPTOR FAMILY	LIGANDS	
Class I cytokine receptors (hematopoietin)	IL-2	IL-21
	IL-3	IL-23
	IL-4	IL-27
	IL-5	GM-CSF
	IL-6	G-CSF
	IL-7	OSM
	IL-9	LIF
	IL-11	CNTF
	IL-12	Growth hormone
	IL-13	Prolactin
	IL-15	

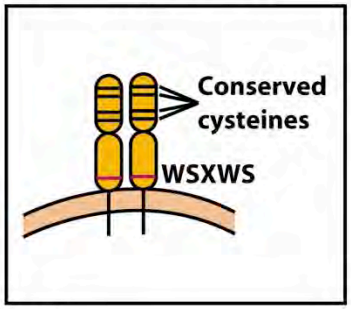


Figure 12-6b
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RECEPTOR FAMILY	LIGANDS
TNF receptors	TNF-α
	TNF-β
	CD27L
	CD30L
	CD40L
	Nerve growth factor (NGF)
	FAS

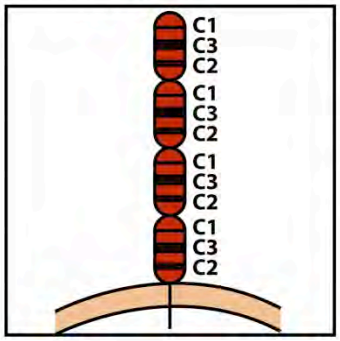

Figure 12-6d
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RECEPTOR FAMILY	LIGANDS
Class II cytokine receptors (interferon)	IFN-α
	IFN-β
	IFN-γ
	IL-10
	IL-19
	IL-20
	IL-22
	IL-24
	IL-26
	IL-28
	IL-29

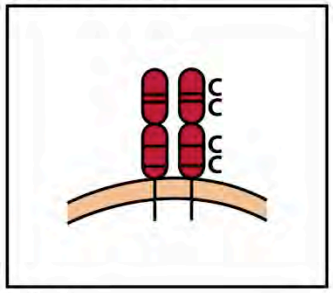

Figure 12-6c
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RECEPTOR FAMILY	LIGANDS
Immunoglobulin superfamily receptors	IL-1
	M-CSF
	C-Kit
	IL-18

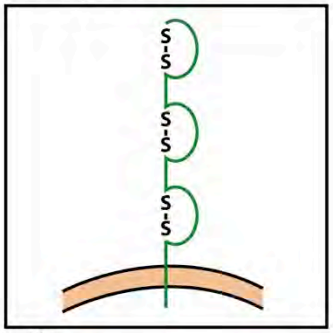

Figure 12-6a
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RECEPTOR FAMILY	LIGANDS
Chemokine receptors	IL-8
	RANTES
	MIP-1
	PF4
	MCAF
	NAP-2

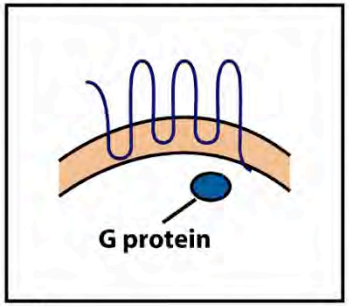

Figure 12-6e
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Cytokine receptors belong to families of receptor proteins, each with a distinctive structure.

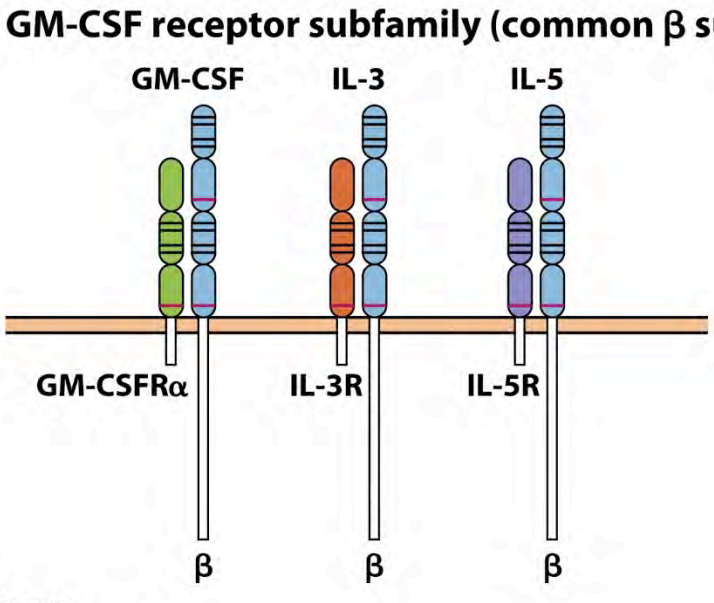

Figure 12-7a
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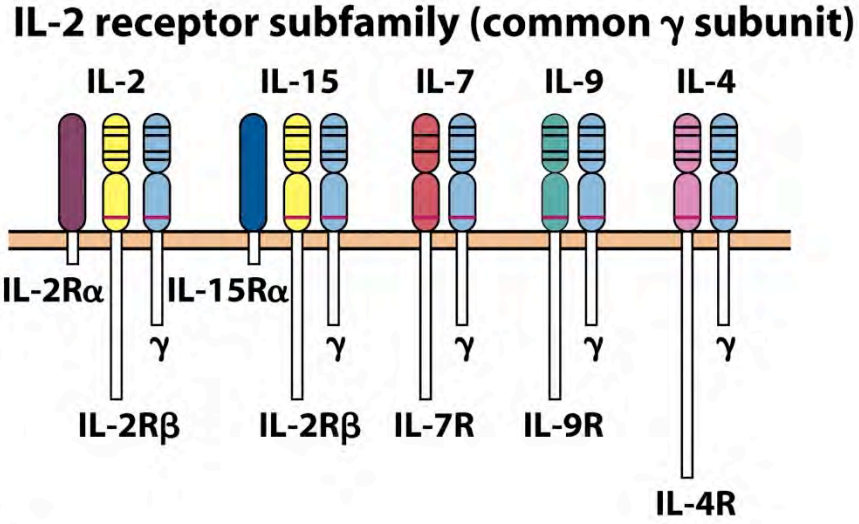

Figure 12-7c
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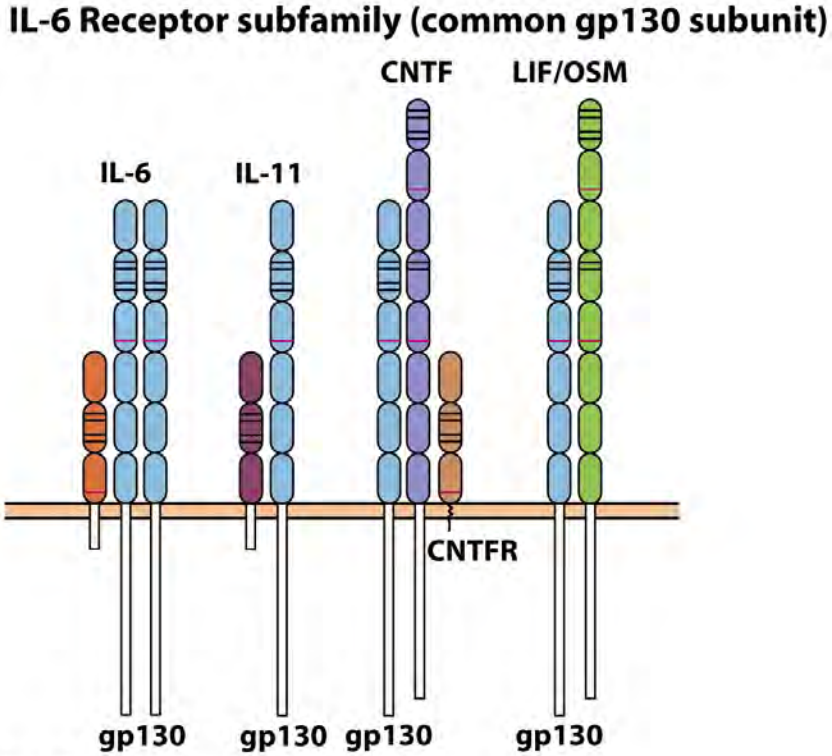

Figure 12-7b
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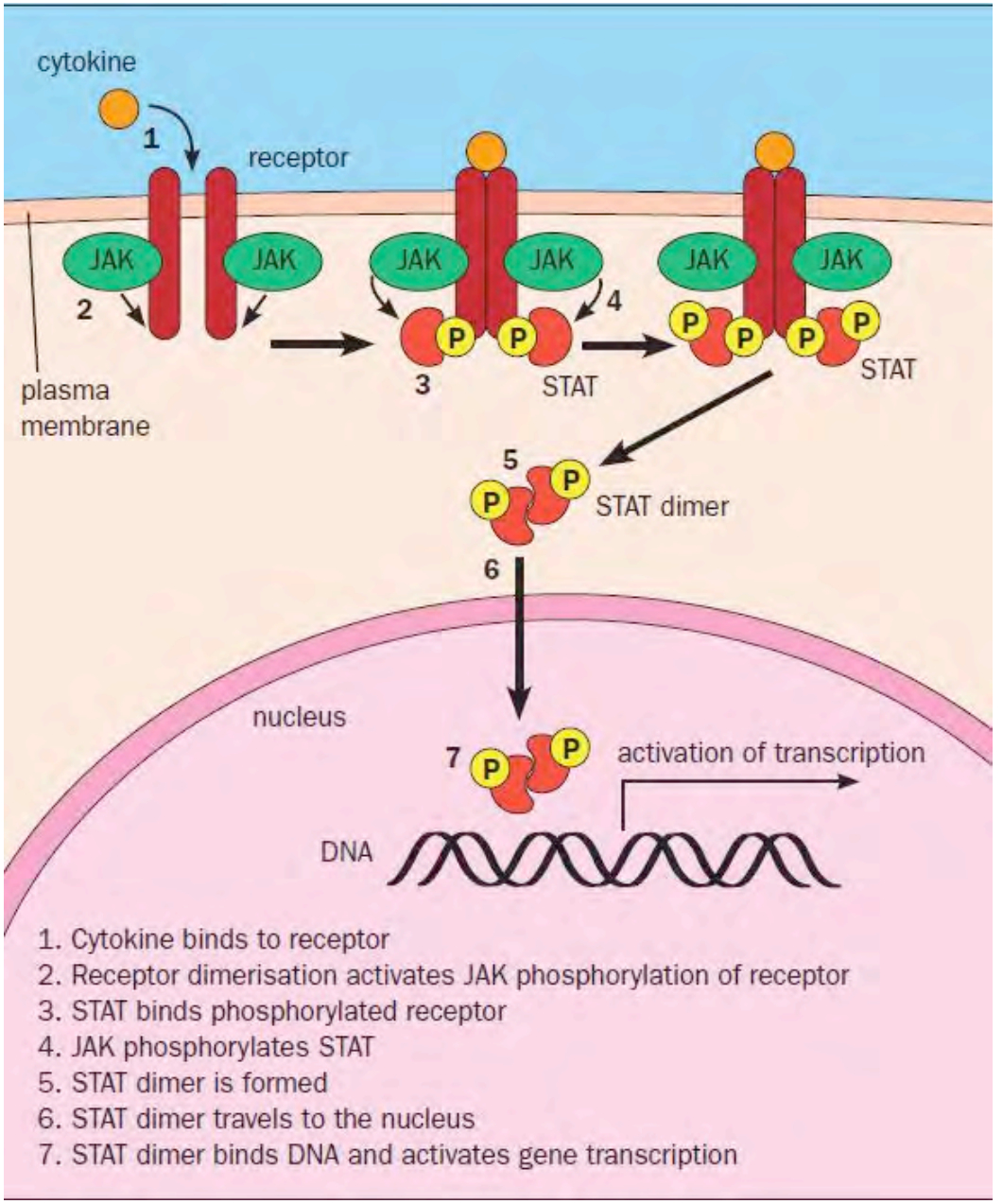
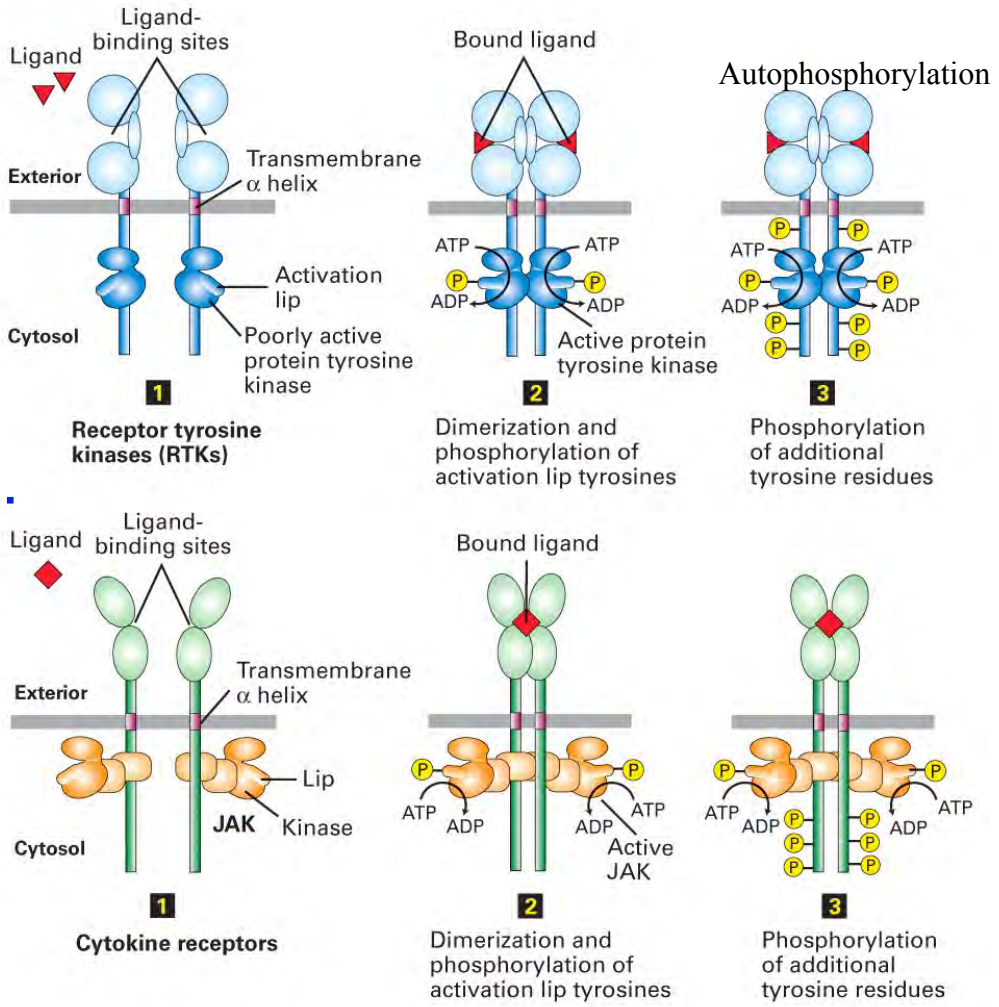
Cytokine receptors subfamilies have shared signaling subunits

Cytokine Receptor Signaling

- Similar to Receptor Tyrosine Kinase signaling
- Receptor clustering
- **Cytokine receptors do NOT have any enzymatic activity, but bind cytosolic kinases**
- Phosporylation and activation of JAK kinases
- Binding of STAT to p-Receptor via SH2 domain
- Phosphorylation of STAT by JAK kinase
- Translocation of p-STAT into nucleus
- Activation of transcription
- Feedback regulation: SHP1 and SOCS



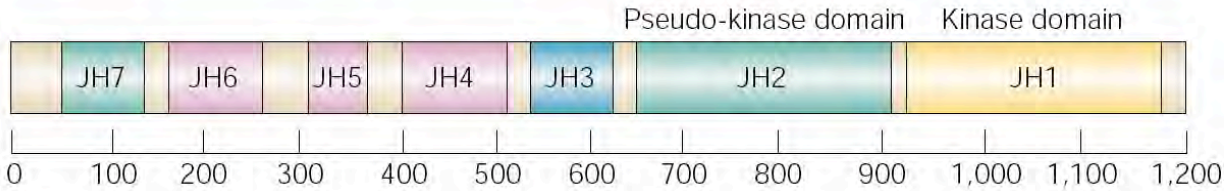
Cytokine Receptors and Receptor Tyrosine Kinases Share Many Signaling Features



- Ligand binding to both cytokine receptors and receptor tyrosine kinases triggers formation of functional oligomers
- In some cases, the ligand induces association of two monomeric receptor subunits diffusing in the plan of the plasma membrane; in other cases, the receptor is a dimer in the absence of ligand and ligand binding alters the conformation of the extracellular domains of the two subunits
- In either cases, formation of the functional dimeric receptor causes the cytosolic kinases to phosphorylate the second kinase

The JAK-family of tyrosine kinases

- Family members
  - JAK1 (135 kDa)
  - JAK2 (130 kDa)
  - JAK3 (120 kDa)
  - Tyk2 (140 kDa)



- Common feature
  - C-terminal kinase + pseudokinase
  - ≠ RTK by lacking transmembrane domains and SH2, SH3, PTB, PH
  - several regions homologous between JAK-members
  - Associated with cytokine receptors (type in and II)
- Function
  - Associated with cytokine receptors in non-stimulated cells in an inactive form

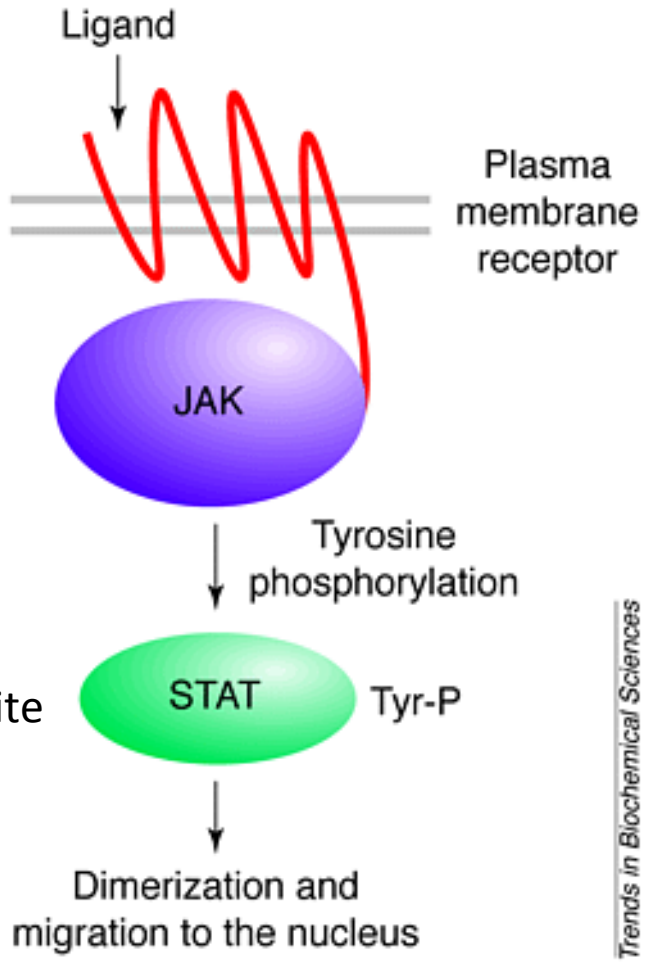
STAT proteins

STATs: Signal Transducers and Activators of Transcription

1. Transducers for signals from many cytokines
   Broad spectrum of biological effects

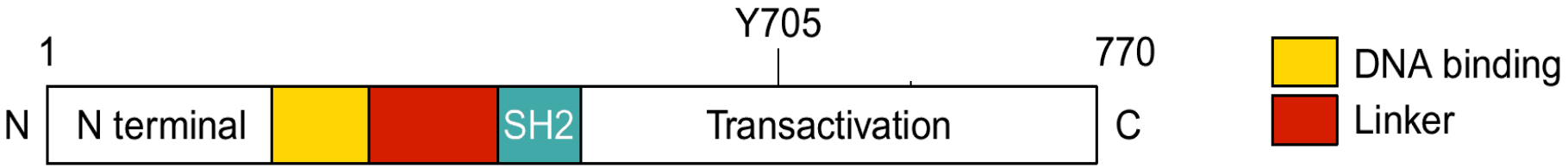
2. Transcriptional activators
   activation at the cell membrane, response in the nucleus

Rapid signal response
   The activation/deactivation cycle of STAT molecules is quite short, about 15 min for an individual molecule.

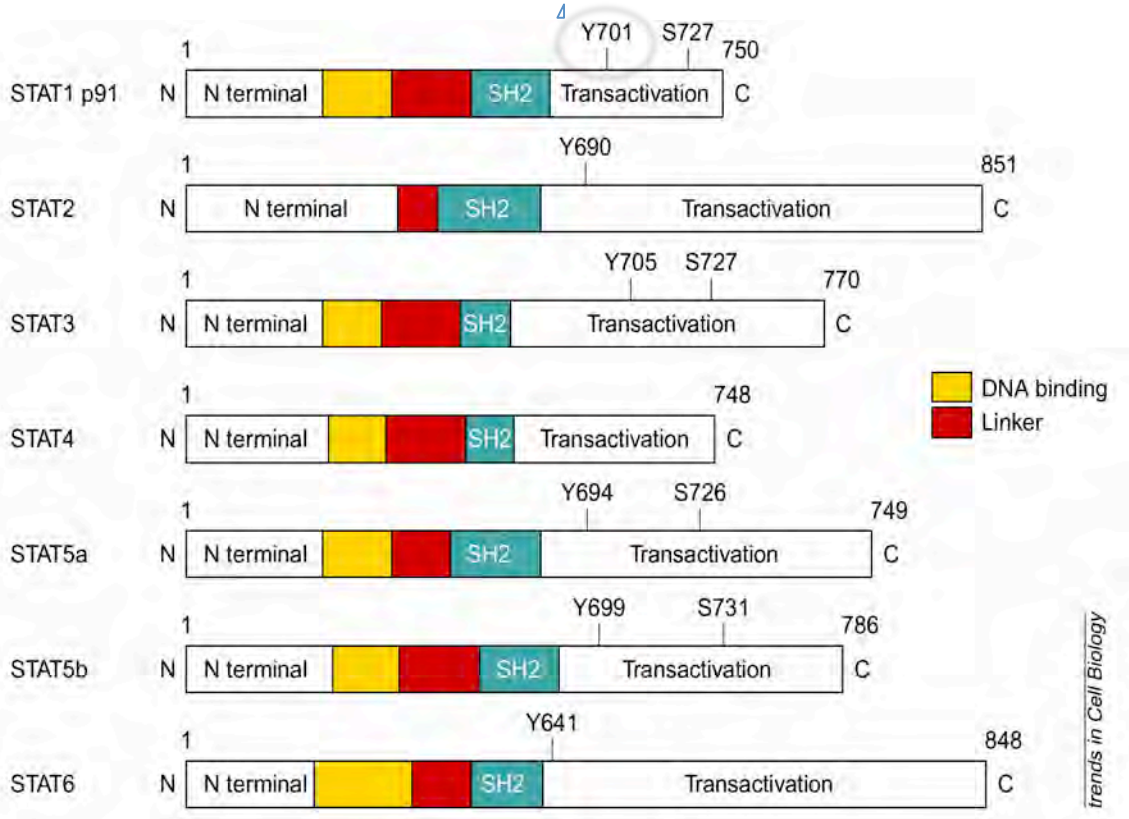


STAT proteins

- All STAT proteins contain an N-terminal SH2 domain that binds to phosphotyrosine in the receptor's cytosolic domain, a central DNA binding domain and a C-terminal domain with a critical tyrosine residue



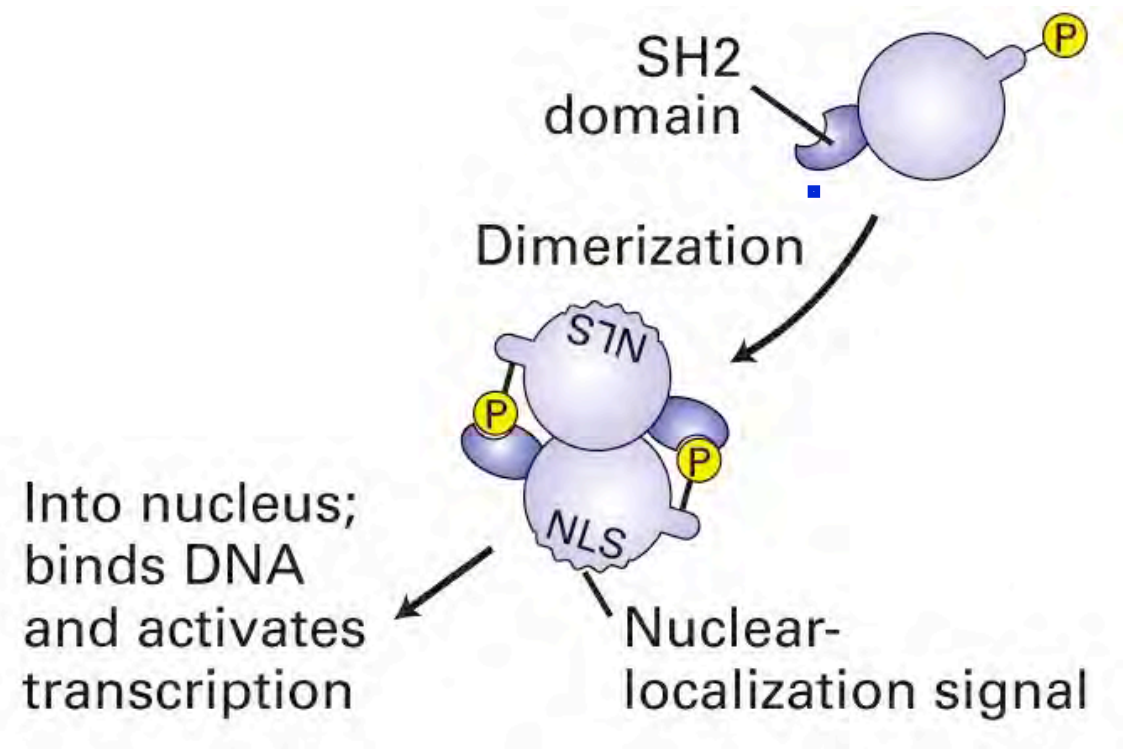
STAT-family members



- STAT1 - involved in IFNα/β- and IFNγ-response
- STAT2 - involved in IFNα/β-response. Mainly acting as partner for STAT1/p48
- STAT3 - involved in response to several cytokines including IL6. It activates several genes involved in acute phase response
  - **Important in growth regulation, embryonic development & organogenesis**
  - **Activation of STAT3 correlated with cell growth, link to cancer, binds c-Jun**
- STAT4 - involved in IL12-response
- STAT5a & 5b - involved in response to several cytokines including prolactin, IL-2, and regulates expression of milk proteins in breast tissue in response to prolactin
- STAT6 - involved in IL4-response
- non-mammalian family members (e.g. Drosophila)

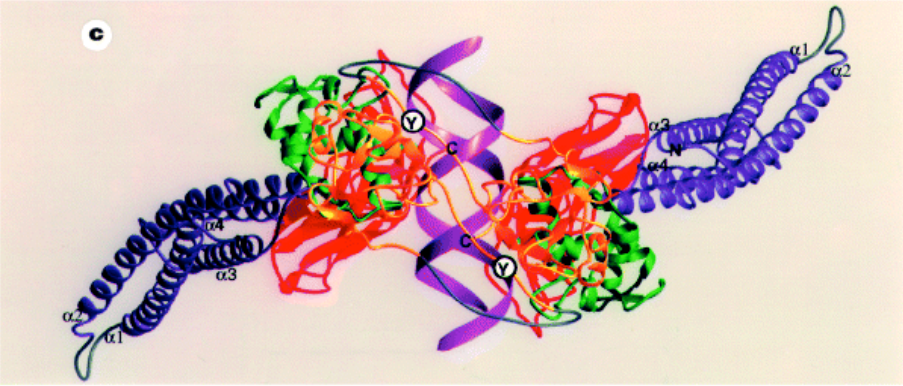
STAT proteins

- Once the STAT is bound to the receptor, the C-terminal tyrosine is phosphorylated by an associated JAK kinase
- The phosphorylated STAT dissociates from the receptor, and two activated STATs form a dimer and then enters the nucleus



STATs - structure and function

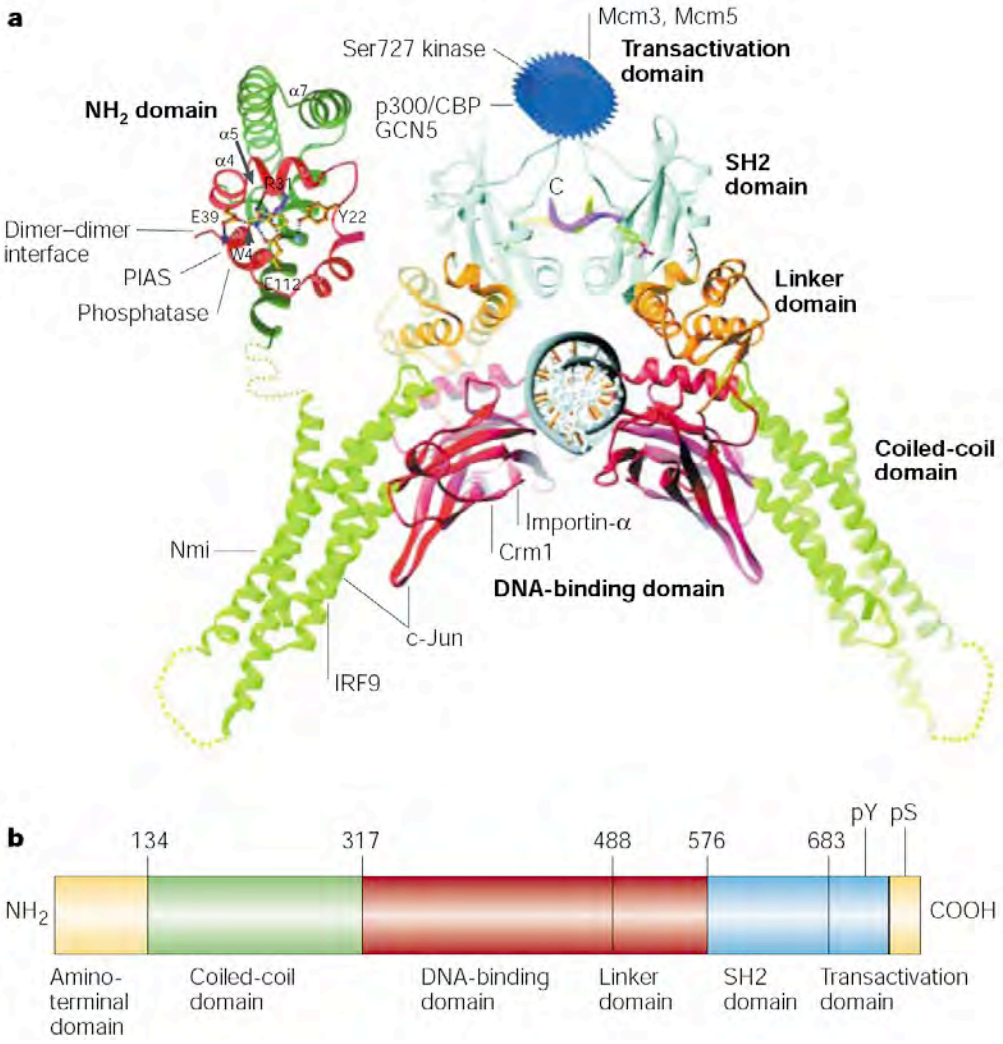
- **SH2-domain**
  - Three important functions in STATs:
    - important for recruitment of STAT to receptor
    - important for interaction with the JAK kinase
    - important for dimerization of STATs to an active DNA-binding form
- **Tyr-701**
  - conserved key Tyr residue located just C-terminal to SH2
  - essential for dimerization to an active DNA-binding form
  - function: Tyr^P binding for SH2 in partner



- **DNA-binding domain**

DBD located in the middle of the protein
Unique motif - All DBDs bind similar motifs in DNA symmetric inverted half sites.

STAT-DBD structure



- Symmetry-axis through DNA, each monomer contacts a separate half site
- The dimer forms a C-shaped ”clamp” around DNA.
- The dimer is kept together by reciprocal SH2- Tyr^P interactions between the SH2 domain in one monomer and the phosphorylated Tyr in the other.
- The SH2 domain in each monomer is closely linked to the core DBD and is itself close to DNA, and is assumed also to contribute to DNA-binding.
- N-terminal coiled-coil region not close to DNA, probably involved in prot-prot interaction with flexible position

Specificity in response

- each cytokine activates a subgroup STAT
- some cytokines activate only one specific STAT

What does mediate specificity?

1. the SH2 - receptor interaction specific for certain combinations swaps-experiments of SH2 between STATs change specificity affinity of the SH2-receptor interaction is affected by the sequence context of the Tyr

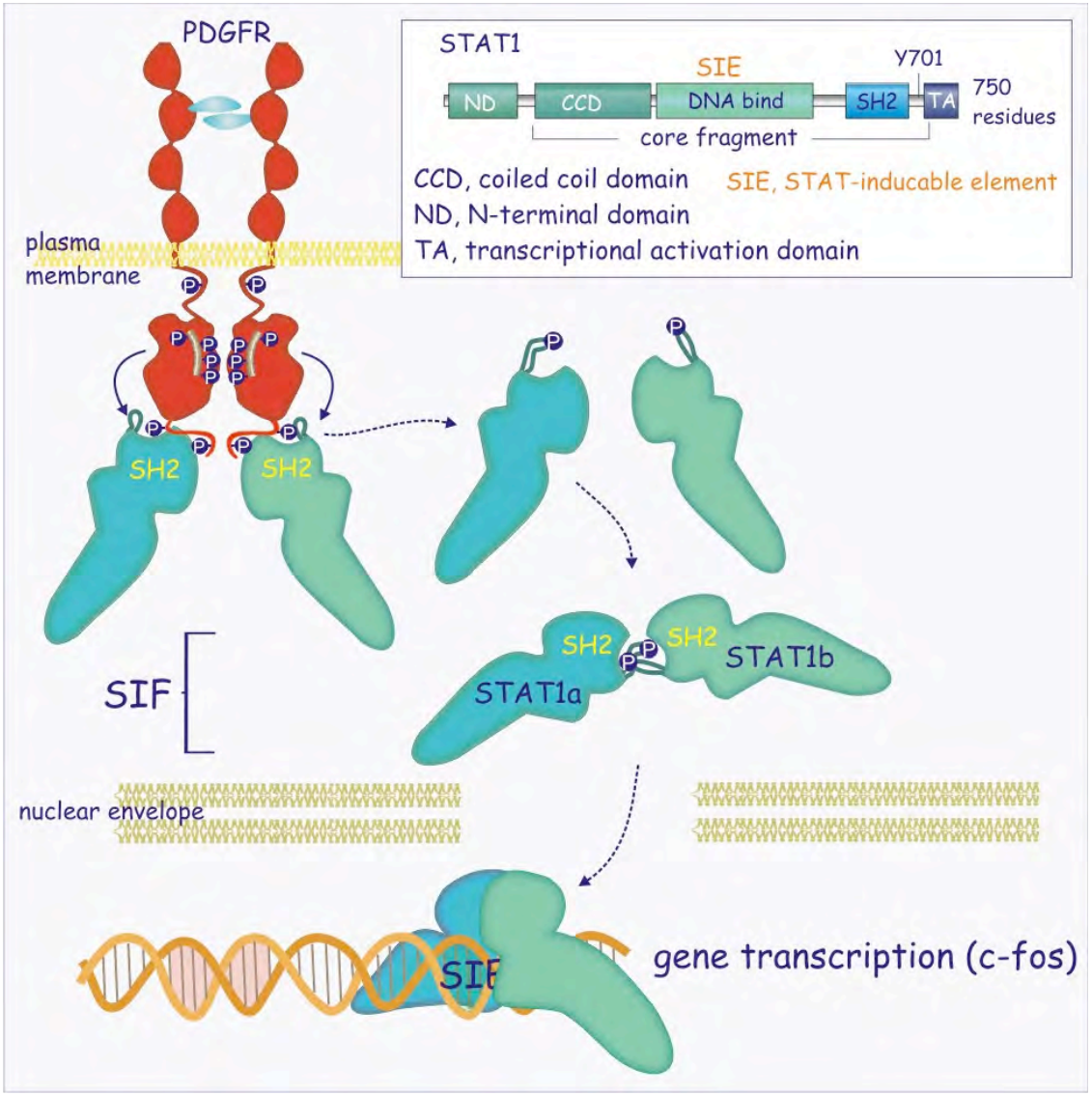
2. different STAT-dimers bind different response elements in the genome and turn on different genes

STAT1 knock-out mice illustrate biological specificity: $STAT1^{-/-}$
phenotype: total lack of IFN-response → highly sensitive to virus-infection

Several signalling pathways linked

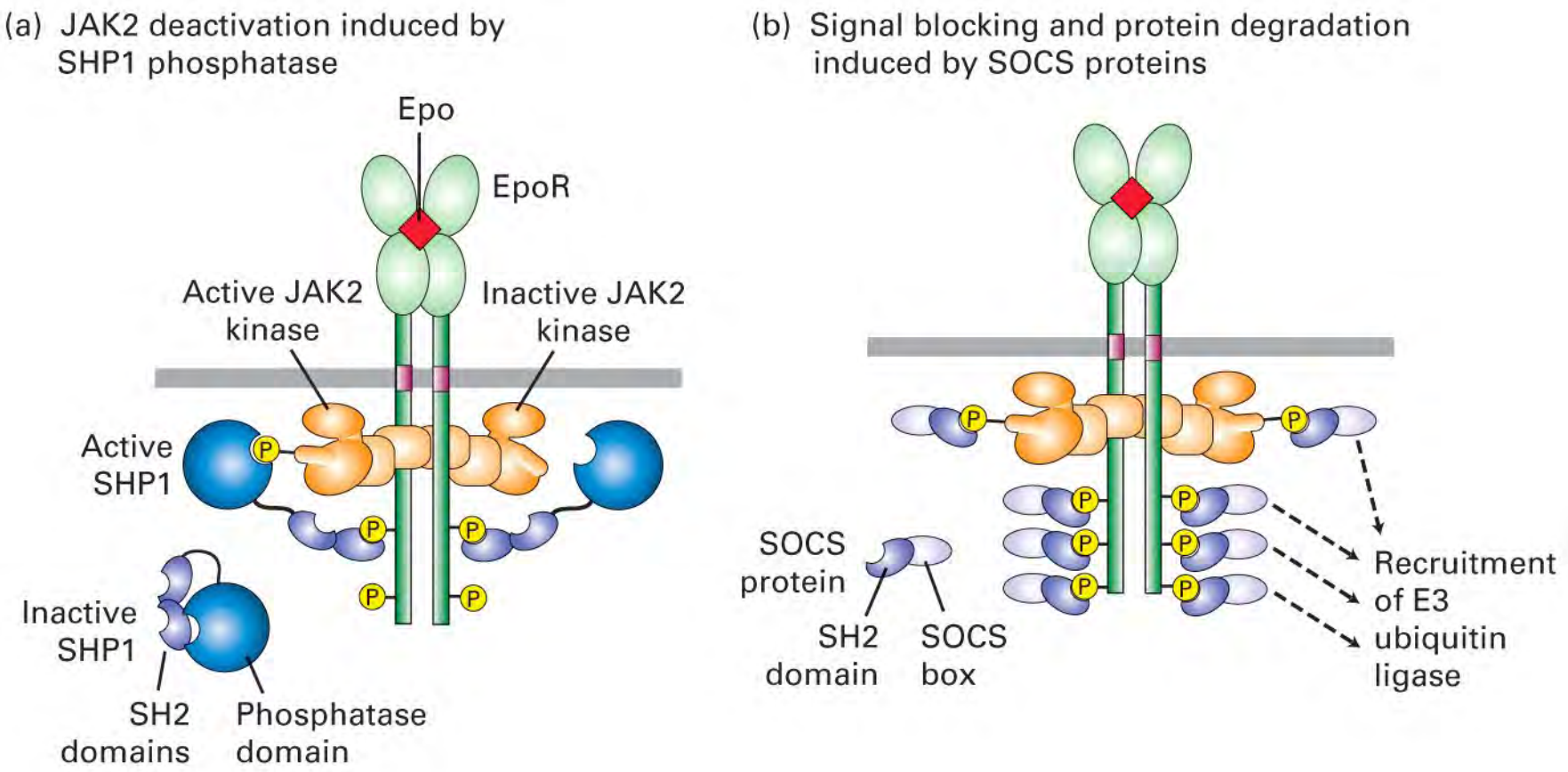
- STATs may also be Tyr-phosphorylated and hence activated by other receptor families
  - receptor tyrosine kinases (RTKs) may phosphorylate STATs
    - EGF stimulation → activation of STAT1, STAT3
  - non-receptor tyrosine kinases such as Src and Abl may also phosphorylate STATs
  - G-protein coupled 7TMS receptors such as angiotensine receptor (?)
- STAT may also be modified by Ser-phosphorylation
  - DNA-binding reduced (STAT3)
- JAKs may activate other signalling pathways than STATs
  - Tyr^P will recruit several protein-substrates and lead to phosphorylation and activation of other signalling pathways
    - e.g. JAK activation → activation of MAP-kinases
    - e.g. substrates: IRS-1, SHC, Grb2, HCP, Syp, Vav

Direct phosphorylation of STAT transcription factors.



Through their SH2 domains, STAT1a and STAT1b bind to the tyrosine-phosphorylated receptor and become phosphorylated. They then form a dimer, (called a Sis-inducible factor, SIF) which translocates to the nucleus, where it binds to a Sis-inducible element (SIE) within the fos promoter.

Negative Regulation of the JAK-STAT pathway

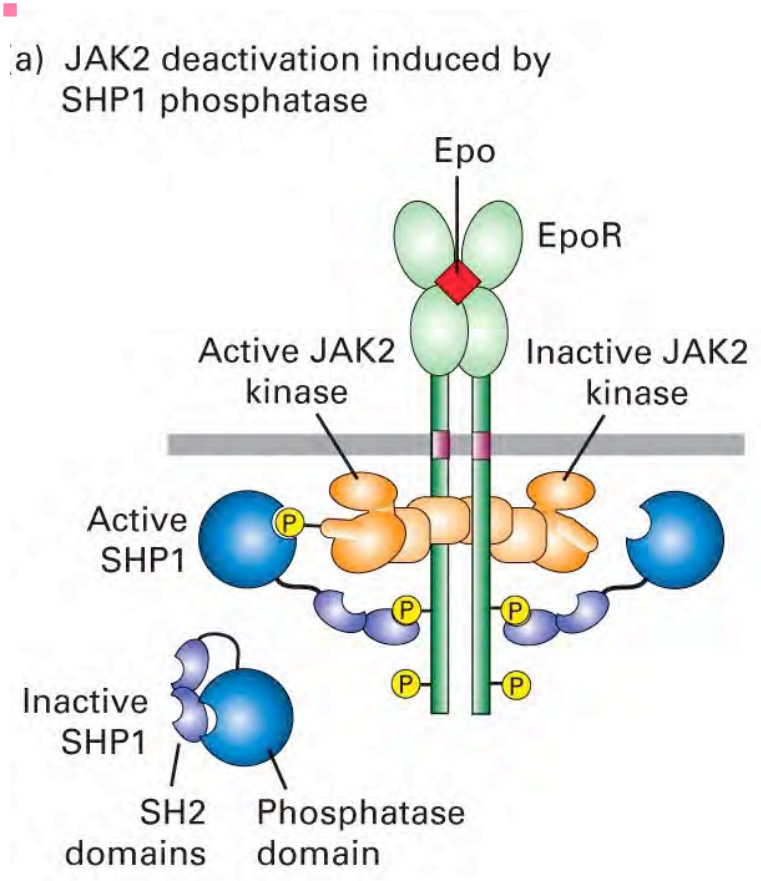


- Signal-induced transcription of target genes can not last for too long and needs de-sensitized
- Signaling from cytokine receptor is usually dampened by two classes of proteins: short term regulation by SHP1 phosphatase and long term regulation by SOCS proteins

Signaling from Cytokine Receptors Is Modulated by Negative Signals

SHP1 Phosphatase

- Mutant mice lacking SHP1 phosphatase die because of producing excess amount of erythrocytes and other blood cells. SHP1 negatively regulates signaling from several types of cytokine receptors in several types of progenitor cells
- Binding of an SH2 domain SHP1 to a particular phospho-tyrosine in the activated receptor unmasks its phosphatase catalytic site and brings it near the phosphrylated tyrosine in the lip region of JAK2
- Removal of the phosphate from this tyrosine inactivates the JAK kinase

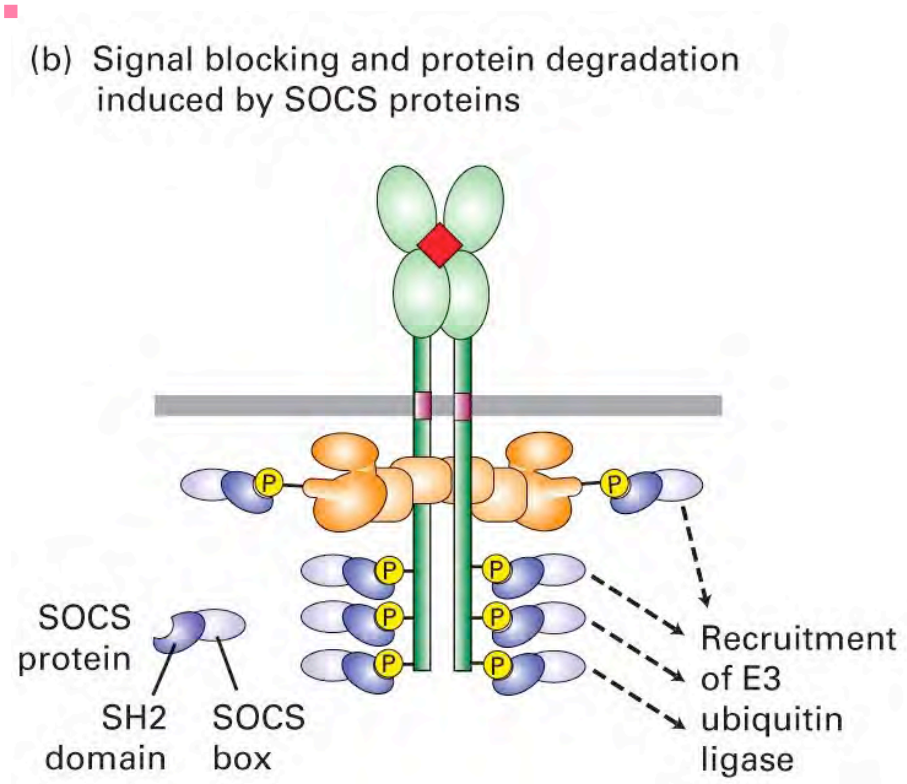


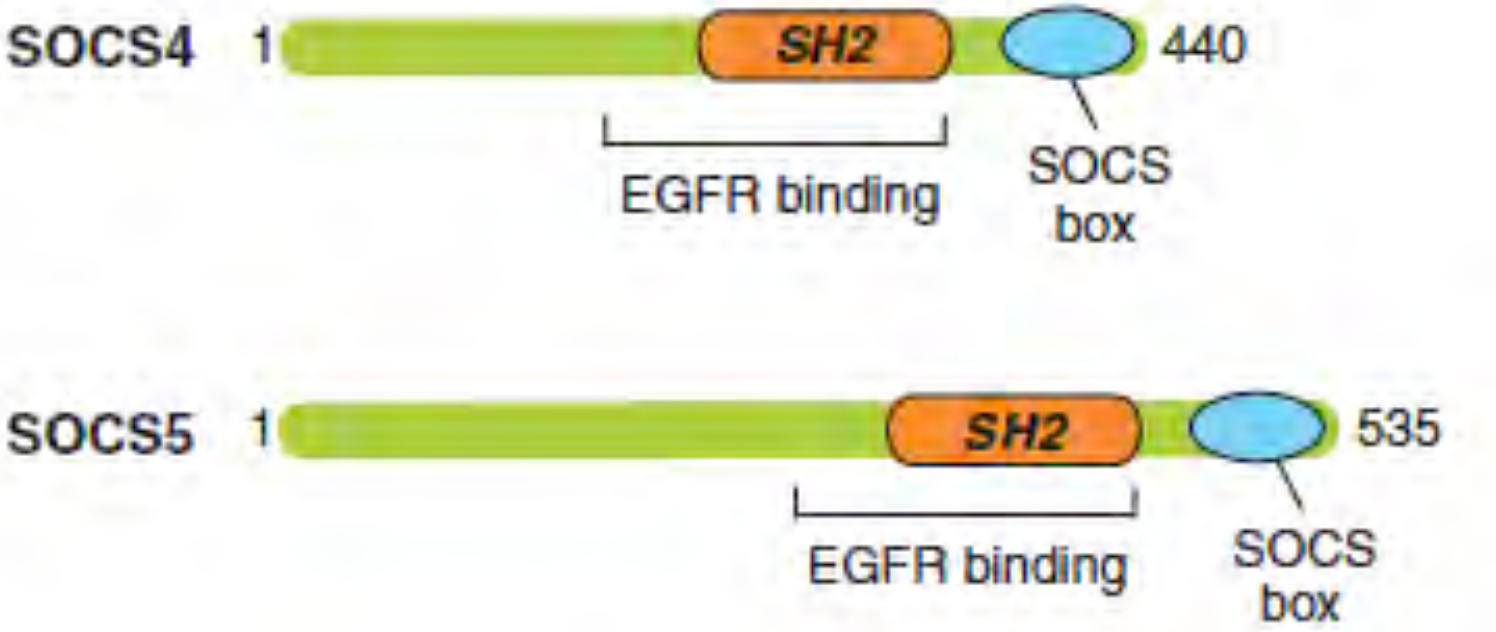
Signaling from Cytokine Receptors Is Modulated by Negative Signals

STAT proteins induce a class of small proteins termed SOCS proteins. These negative regulators are also known as CIS proteins

CIS proteins act in two ways to negatively regulate cytokine receptor stimulated signaling:

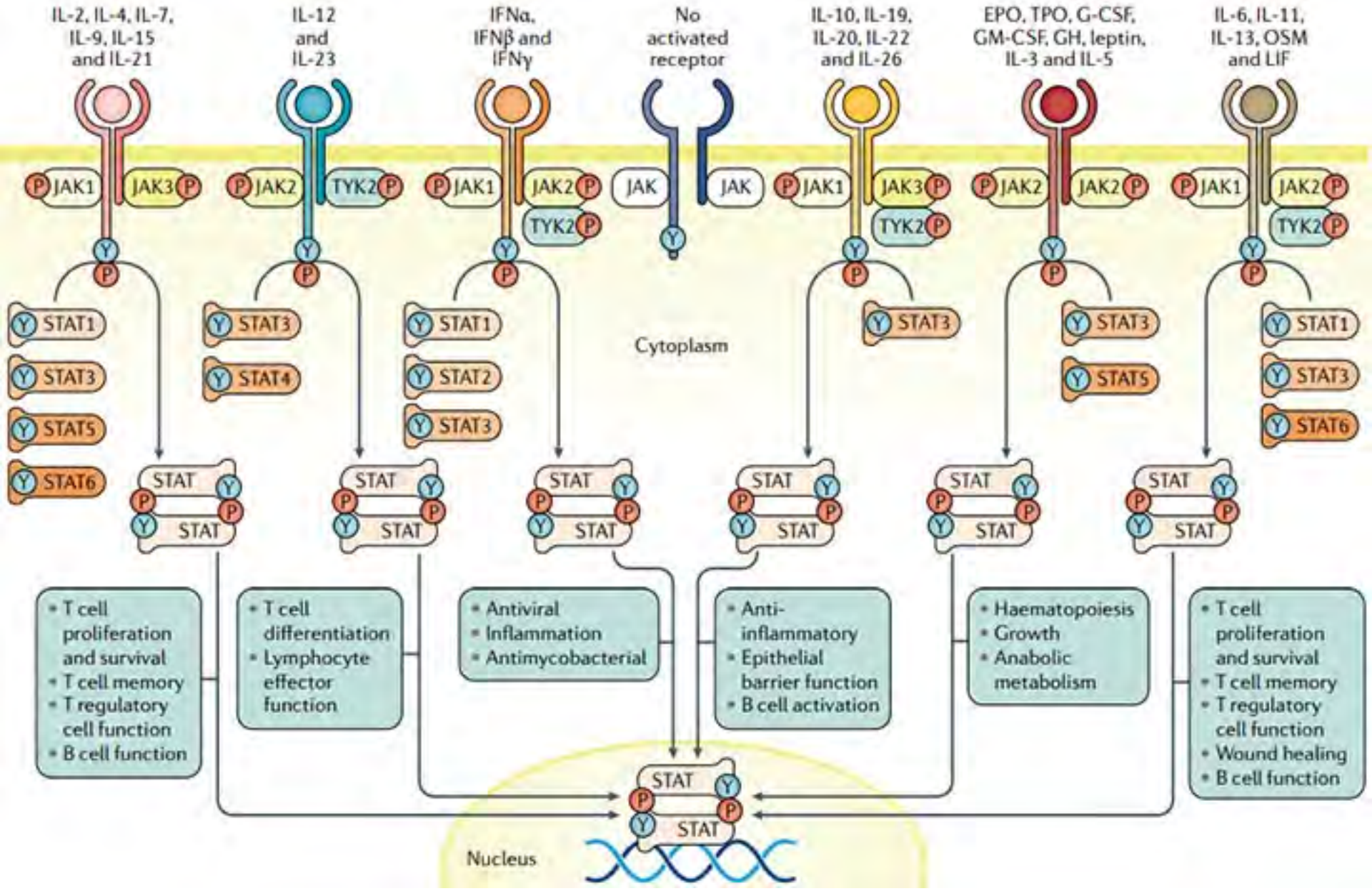
- The SH2 domain in several SOCS proteins bind to phosphotyrosines on an activated receptor, preventing binding of other SH2-containing signaling proteins and thus inhibiting receptor signaling
- SOCS-1 can bind to critical phosphotyrosine in the activation lip of activated JAK2 kinase thereby inhibiting its catalytic activity
- All SOCS proteins contain a SOCS box that recruits components of E3 ubiquitin ligases. As a result of SOCS-1 binding, JAK2 becomes polyubiquitinated and then degraded in proteasomes and thus terminate the signaling permanently



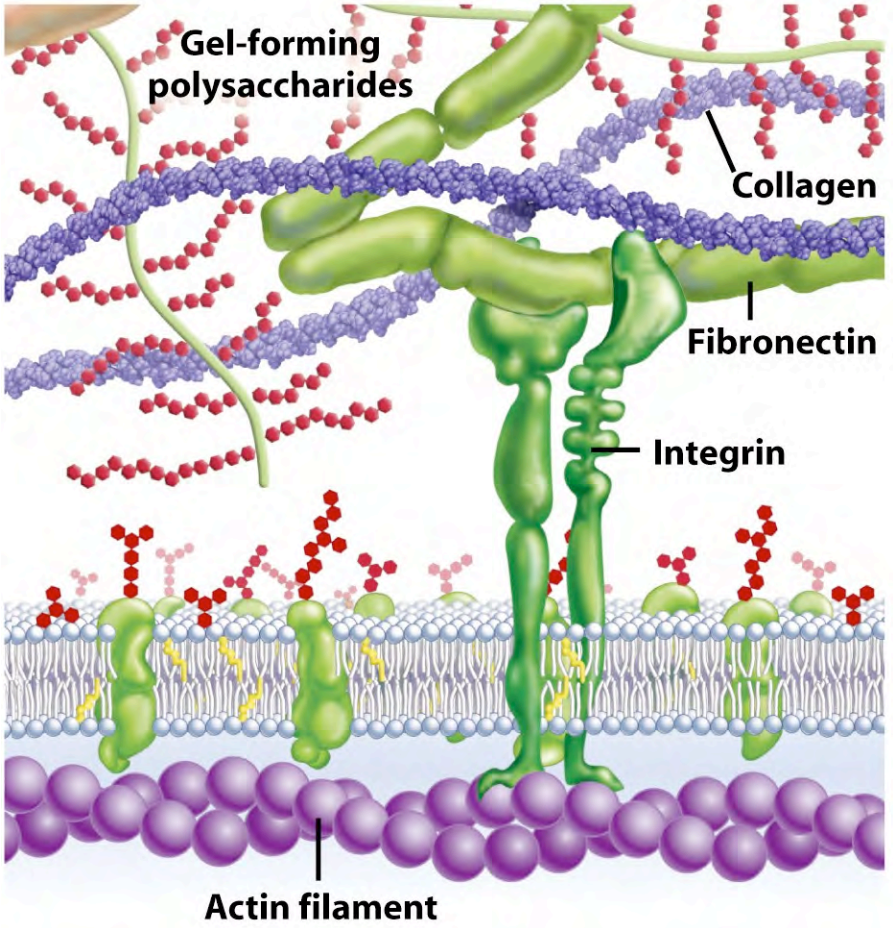


SOCS4 and SOCS5 bind to the EGFR through their respective SH2 domains, which share 87% sequence homology and a poorly defined Nterminal region.

The SOCS box recruits an E3 ligase and thereby leads to EGFR ubiquitylation.


IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21
IL-12 and IL-23
IFNα, IFNβ and IFNγ
No activated receptor
IL-10, IL-19, IL-20, IL-22 and IL-26
EPO, TPO, G-CSF, GM-CSF, GH, leptin, IL-3 and IL-5
IL-6, IL-11, IL-13, OSM and LIF
JAK1
JAK3
JAK2
TYK2
JAK
STAT1
STAT3
STAT5
STAT6
STAT4
STAT2
Cytoplasm
STAT
• T cell proliferation and survival
• T cell memory
• T regulatory cell function
• B cell function
• T cell differentiation
• Lymphocyte effector function
• Antiviral
• Inflammation
• Antimycobacterial
• Anti-inflammatory
• Epithelial barrier function
• B cell activation
• Haematopoiesis
• Growth
• Anabolic metabolism
• T cell proliferation and survival
• T cell memory
• T regulatory cell function
• Wound healing
• B cell function
Nucleus

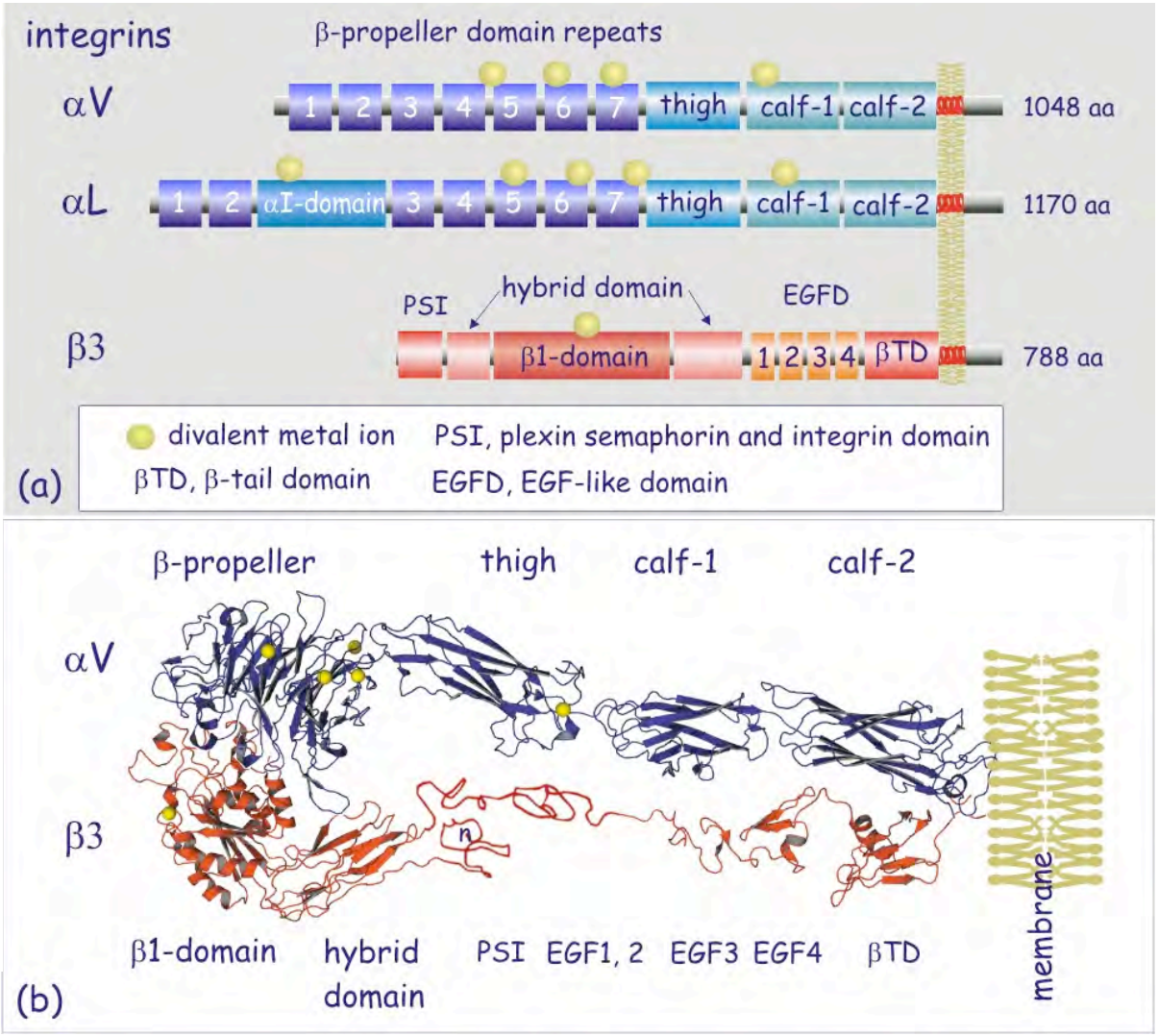
Integrins



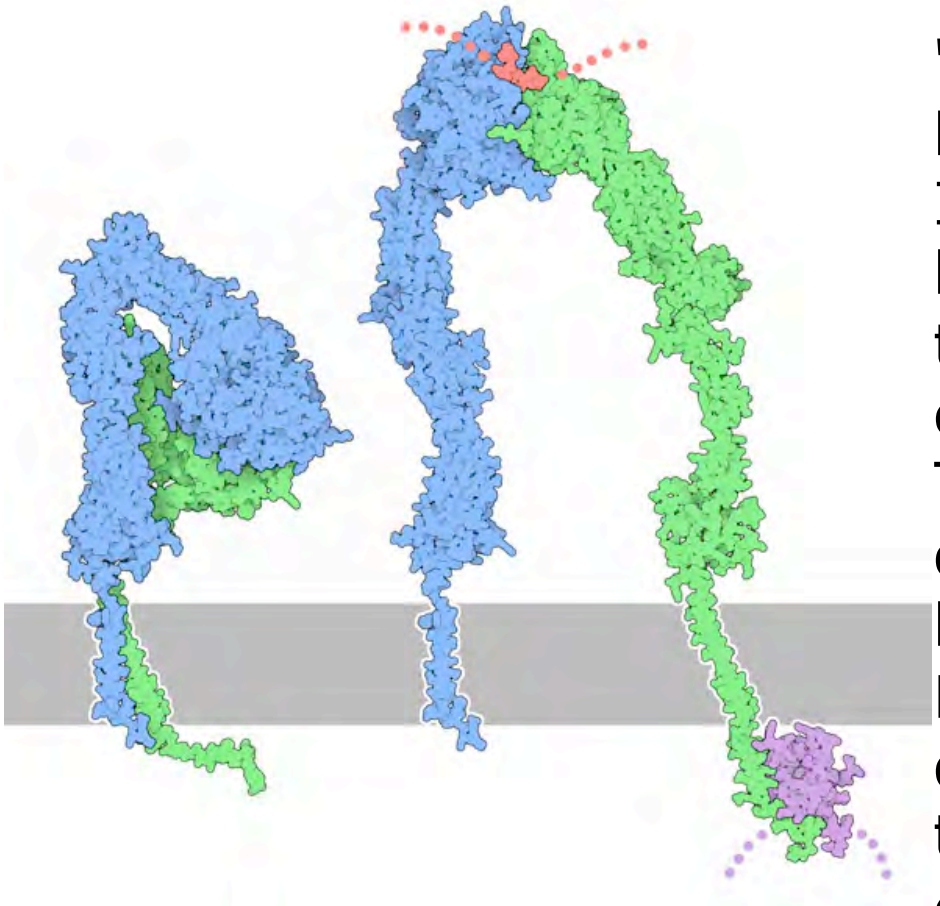
Integrins are transmembrane receptors that mediate the attachment between a cell and other cells or the extracellular matrix (ECM) components such as fibronectin, vitronectin, collagen, and laminin. In addition to transmitting mechanical forces across otherwise vulnerable membranes, they are involved in cell signaling and the regulation of cell cycle, shape and motility.

Domain architecture of integrins

Integrins are heterodimers containing two distinct chains, called the α (alpha) and β (beta) subunits.
In mammals, 18α and 8β subunits have been characterized.
The α and β subunits each penetrate the plasma membrane and possess small cytoplasmic domains.



Integrins activation



Integrin dimers are in a "bent" conformation which prevents them from interacting with their ligands. Therefore, integrin dimers must be 'unbent' in order to allow their binding to the ECM.

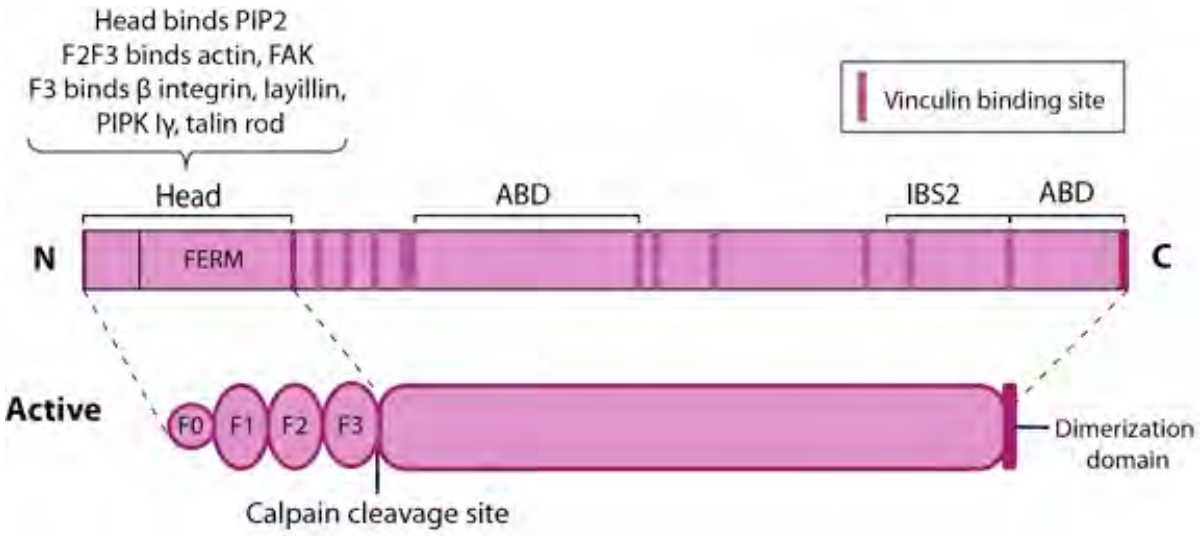
In cells, the priming is accomplished by **talin**, which binds to the β tail of the integrin dimer and changes its conformation.

Talin binding alters the angle of tilt of the β3 chain transmembrane helix which primes integrins.

Moreover, talin proteins are able to dimerize and thus are thought to trigger the clustering of integrin dimers which leads to the formation of a focal adhesion.

Talin

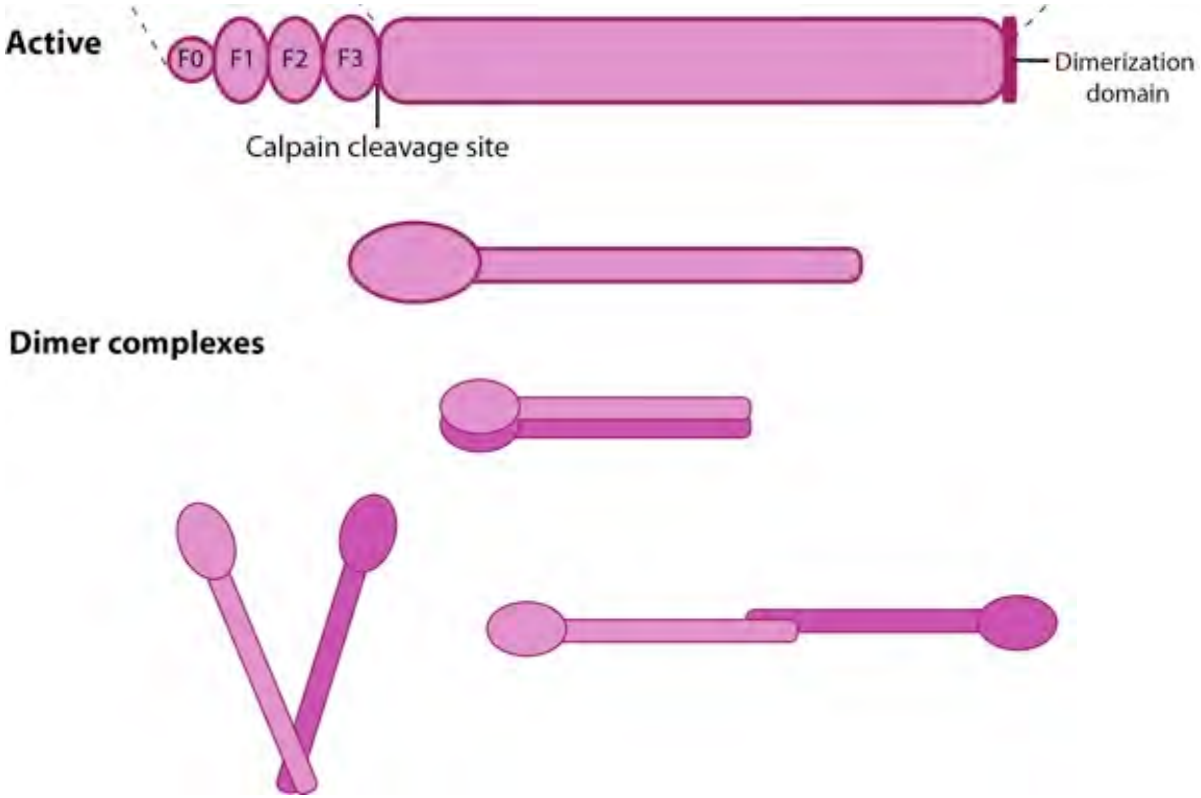
Talin is a 270kDa cytoskeletal protein concentrated at regions of cell–substratum contact and, in lymphocytes, at cell–cell contacts. It is a structural platform that is required for the initial linkage between the contractile cytoskeleton and sites of integrin/fibronectin adhesion



Integrin tail binding occurs via the F3 phosphotyrosine binding (PTB) domain via a unique interaction with the integrin membrane proximal region, which is sufficient for integrin activation. The basic patches on all subdomains can dock onto the plasma membrane and further enhance integrin activation. Specific interactions through basic residues on F3 are also essential for integrin clustering.

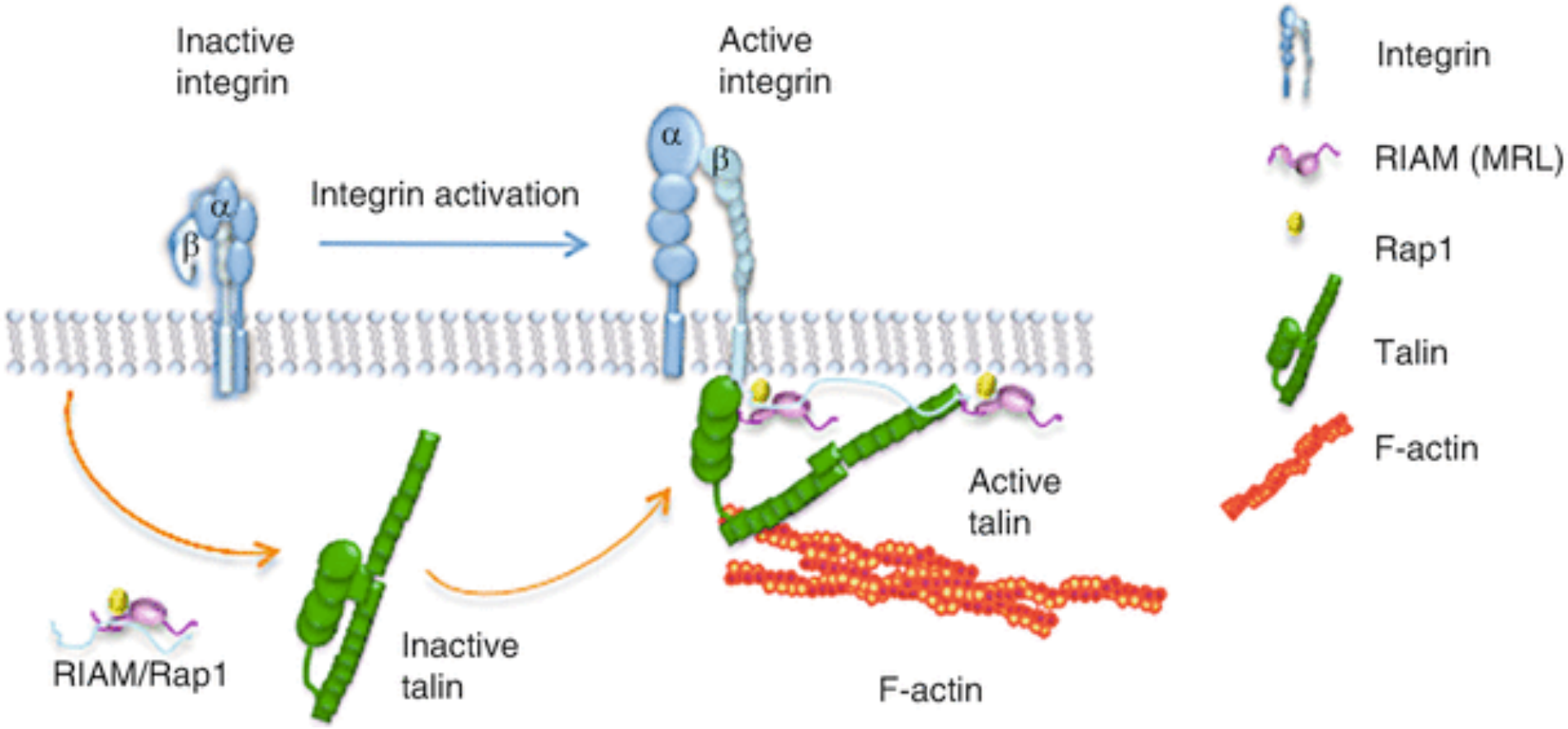
The rod contains an additional integrin-binding site (IBS2), two actin-binding sites (ABD) and several vinculin-binding sites that are shown to be exposed by stretch in response to force. Talin also contains numerous potential phosphorylation sites which are suggested to directly or indirectly regulate the association of talin with other factors

Talin activation and membrane recruitment



Talin is in an autoinhibited form in the cytosol due to the intermolecular association between the F3 subdomain and a helical bundle in the rod region. This not only blocks integrin binding site on F3 but also F2 and F3 binding to membrane. Activation likely involves binding to membrane phospholipids such as phosphatidylinositol 4,5-bis-phosphate (PIP2), vinculin and F-actin or calpain cleavage. This enhances talin's affinity for the β-integrin subunit by revealing binding sites.

Talin membrane localization and activation by RIAM

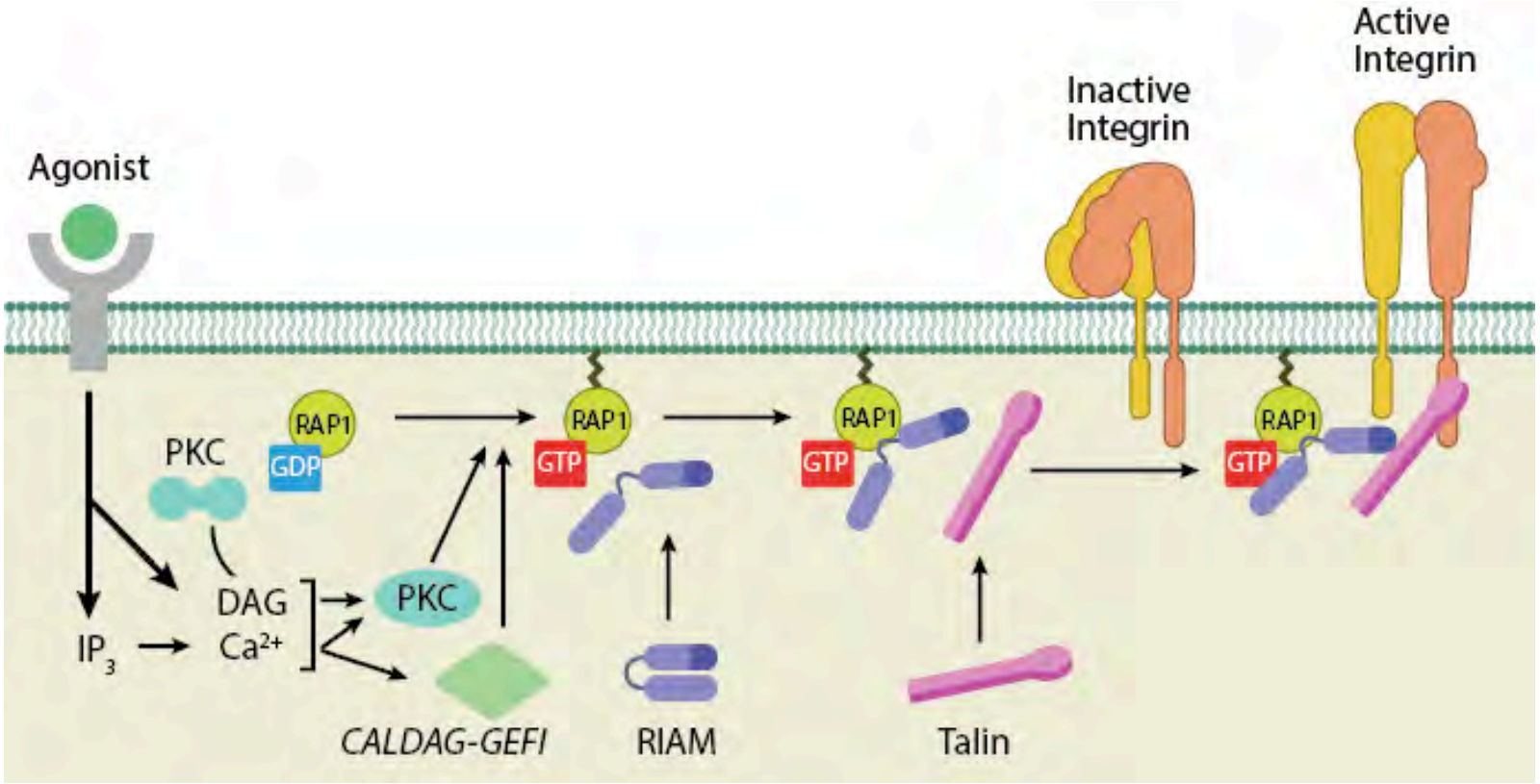


In resting cells, most integrins are kept inactive, possibly owing to conformational constraints in the cytoplasmic tails. A small proportion of the integrin dimers display the thermodynamically unfavourable, active conformation and can bind their ligand.

Upon agonist stimulation, Rap1 is transiently converted to the active GTP-bound form and directly or indirectly brings talin to the integrin cytoplasmic tail, maintaining them in their active conformation.

Rap1 activity is therefore required for ligand binding and outside-in signalling to take place, by the anchoring of the ligand-bound integrin to the actin cytoskeleton.

Talin activation and membrane recruitment



Ligand occupancy in certain cell-surface receptors (agonists) causes phospholipid hydrolysis releasing diacylglycerol (DAG) and inositol triphosphate (IP3). IP3 increases cytosolic levels of calcium ions; DAG and Ca2+ can promote GTP-loading and membrane translocation of Rap1 either by activating Ca2+ and DAG-regulated GEF (CALDAG-GEF or Rap-GEF) or protein kinase C (PKC). Activated Rap1 in turn, recruits Rap1-GTP-interacting adaptor molecule (RIAM) along with its binding partner, talin to the plasma membrane.

Rap-1

(Ras-proximate-1 or Ras-related protein 1)

It is a small GTPase which belongs to Ras-related protein family; there are two isoforms of the Rap1 protein, each encoded by a separate gene, RAP1A and RAP1B.

Rap1 plays a unique, Ras-independent role in eukaryotic cells. Activated by virtually all receptor types and second messengers, Rap1 controls adhesion-related functions such as phagocytosis,cell-cell contacts and functional activation of integrins through inside-out signalling.

Cellular functions of the Rap1 GTP-binding protein: a pattern emerges

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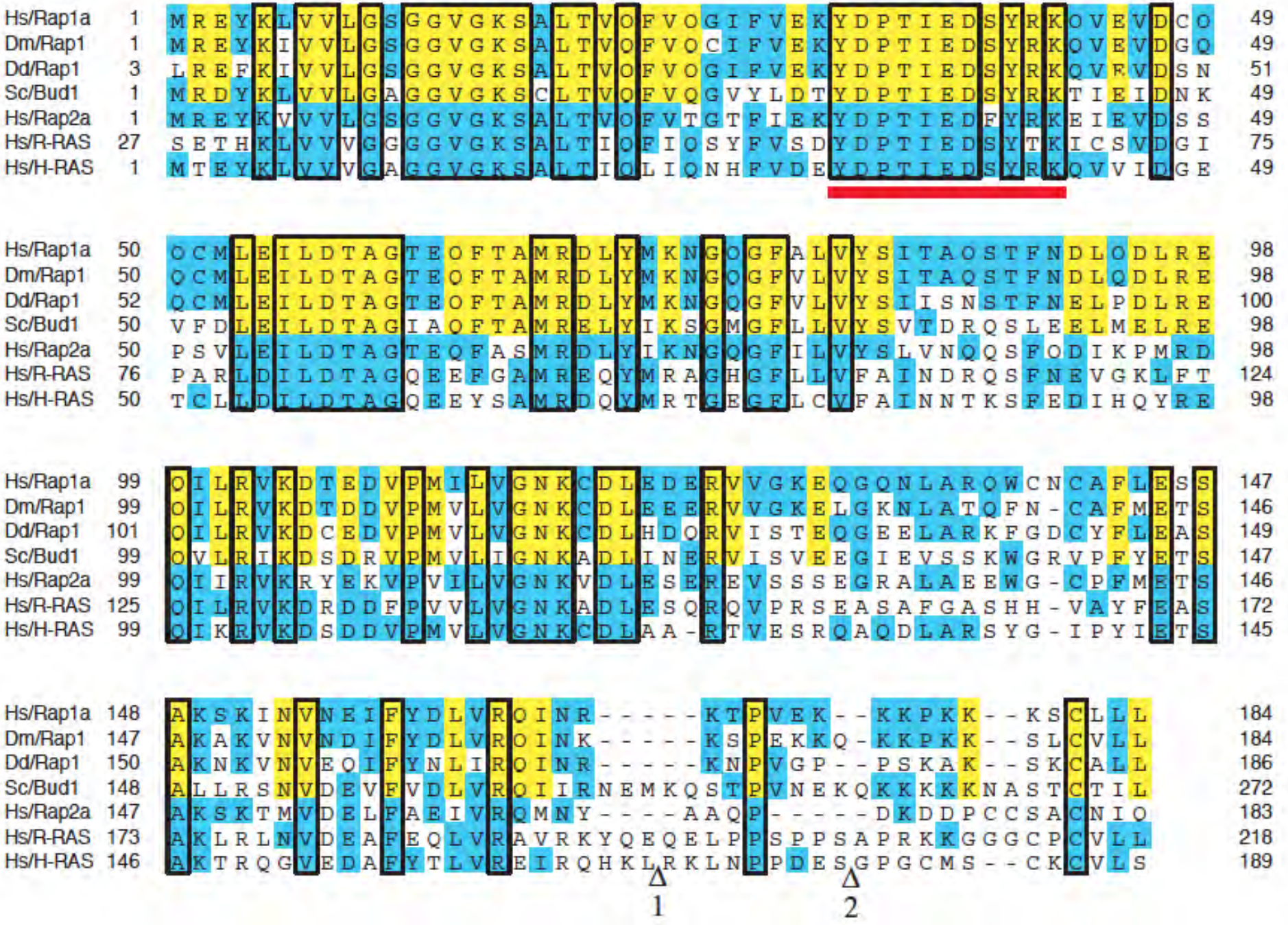
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Dd/Rap1 3 LREFKIVVLGSGGVGKSALTVQFVQGIFVEKYDPTIEDSYRKQVEVDSN 51
Sc/Bud1 1 MRDYKLVVLGAGGVGKSCLTVQFVQGVYLDTYDPTIEDSYRKTIEIDNK 49
Hs/Rap2a 1 MREYKVVVLGSGGVGKSALTVQFVTGTFIEKYDPTIEDFYRKEIEVDSS 49
Hs/R-RAS 27 SETHKLVVVGGGGVGKSALTIQFIQSYFVSDYDPTIEDSYTKICSVDGI 75
Hs/H-RAS 1 MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGE 49
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Sc/Bud1 50 VFDLEILDTAGIAQFTAMRELYIKSGMGFLLVYSVTDRQSLEELMELRE 98
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Hs/R-RAS 76 PARLDILDTAGQEEFGAMREQYMRAGHGFLLVFAINDRQSFNEVGKLFT 124
Hs/H-RAS 50 TCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHQYRE 98
Hs/Rap1a 99 QILRVKDTEDVPMILVGNKCDLEDERVVGKEQGQNLARQWCNCAFLESS 147
Dm/Rap1 99 QILRVKDTDDVPMVLVGNKCDLEEERVVGKELGKNLATQFN-CAFMETS 146
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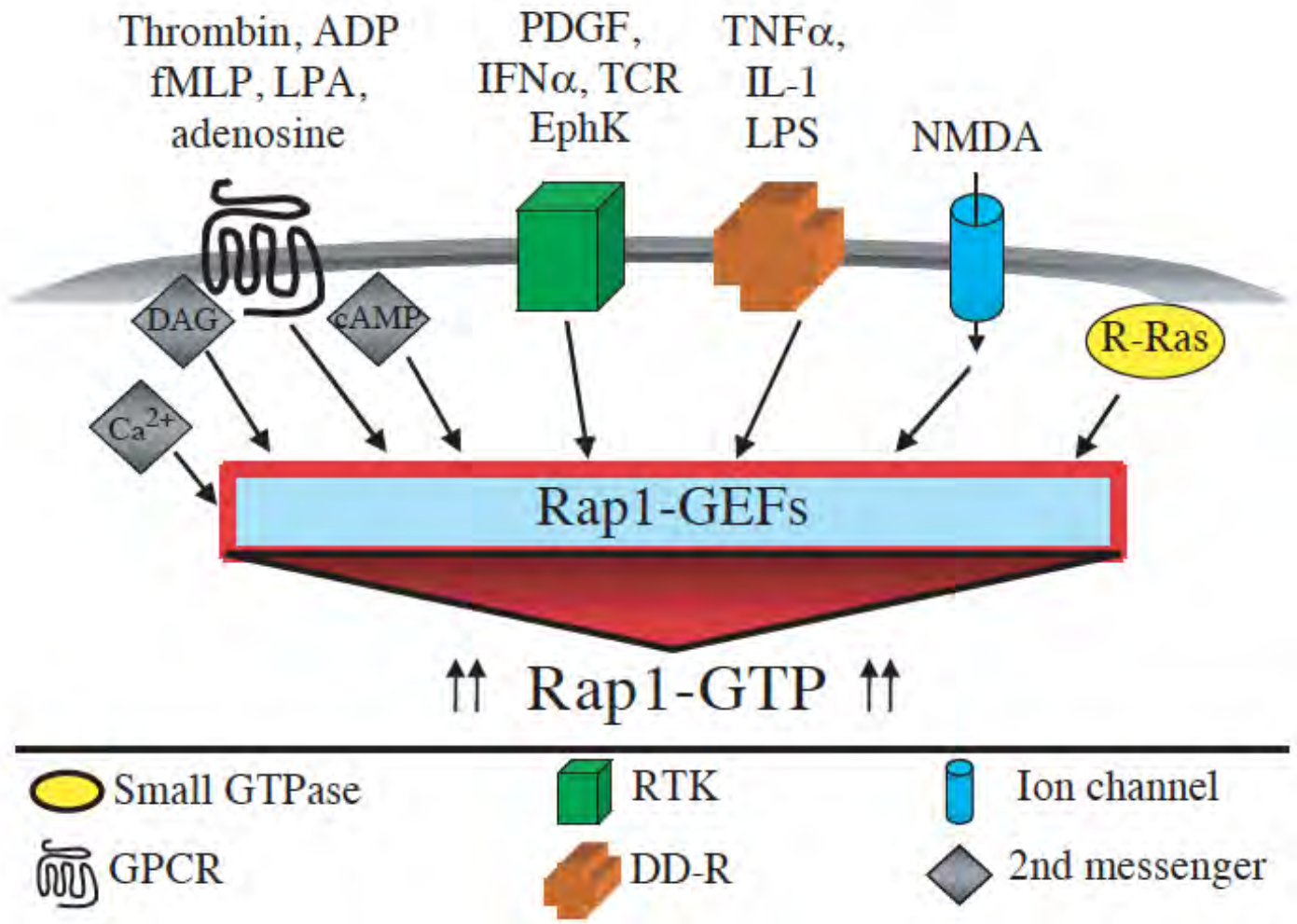
Δ 1
Δ 2



Many receptors and second messengers are coupled to the activation of Rap1 guanine nucleotide exchange factors (Rap1GEFs), and an increase in the cellular levels of active, GTP-bound Rap1.