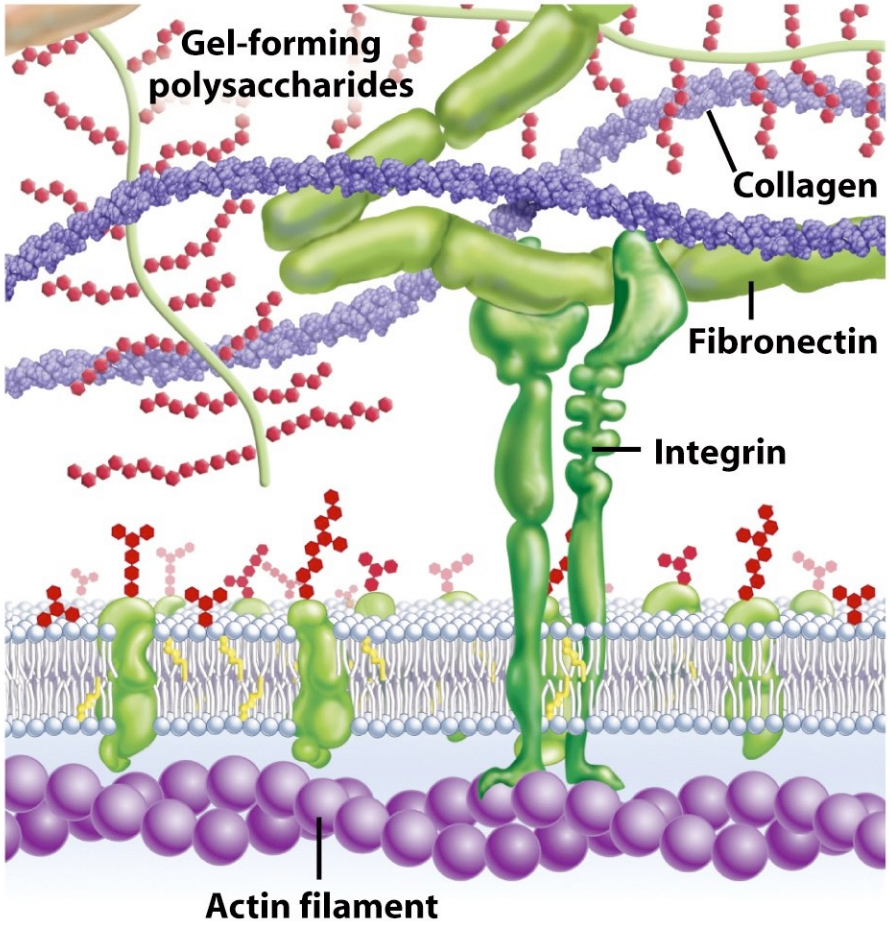
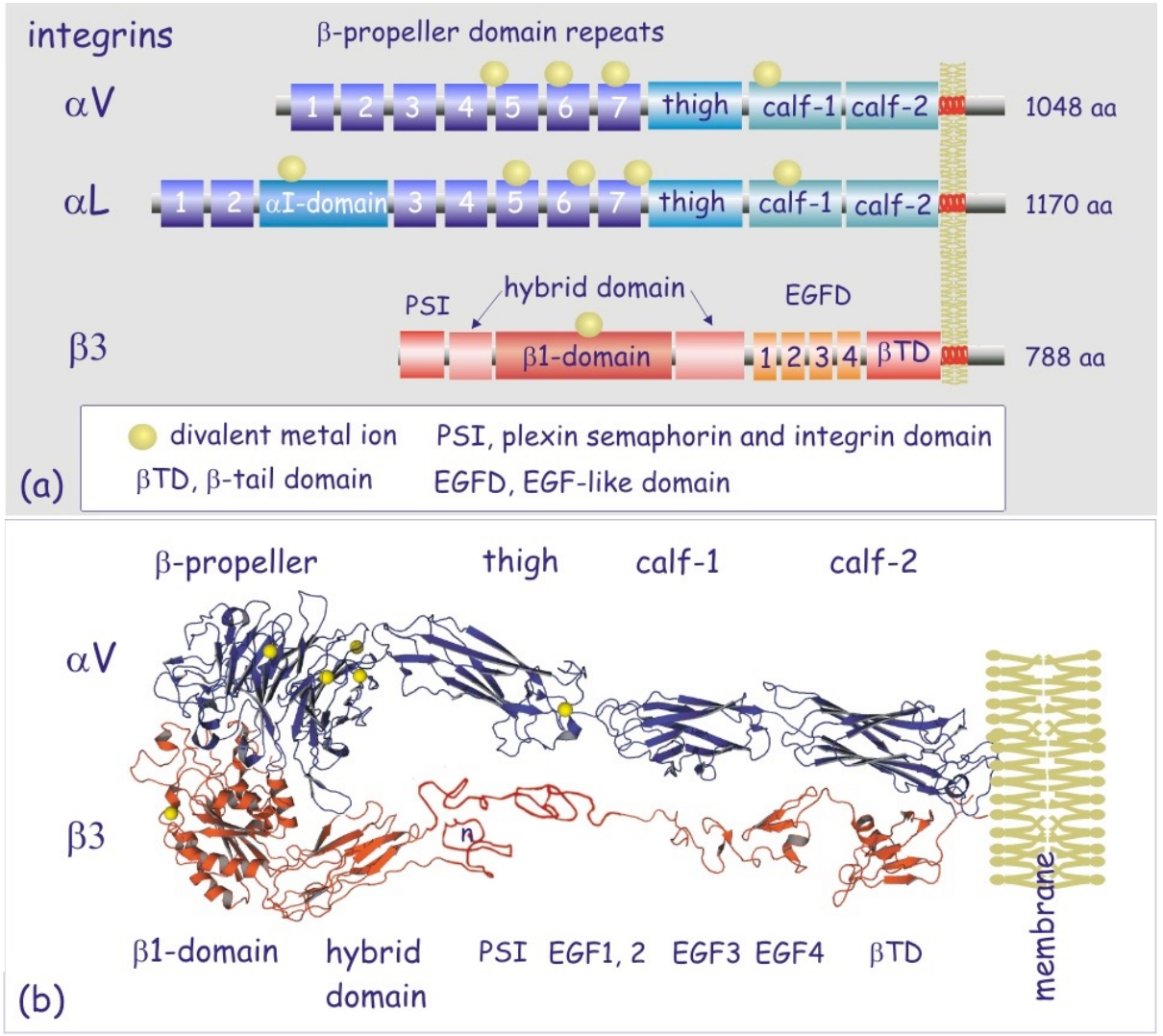
Integrins



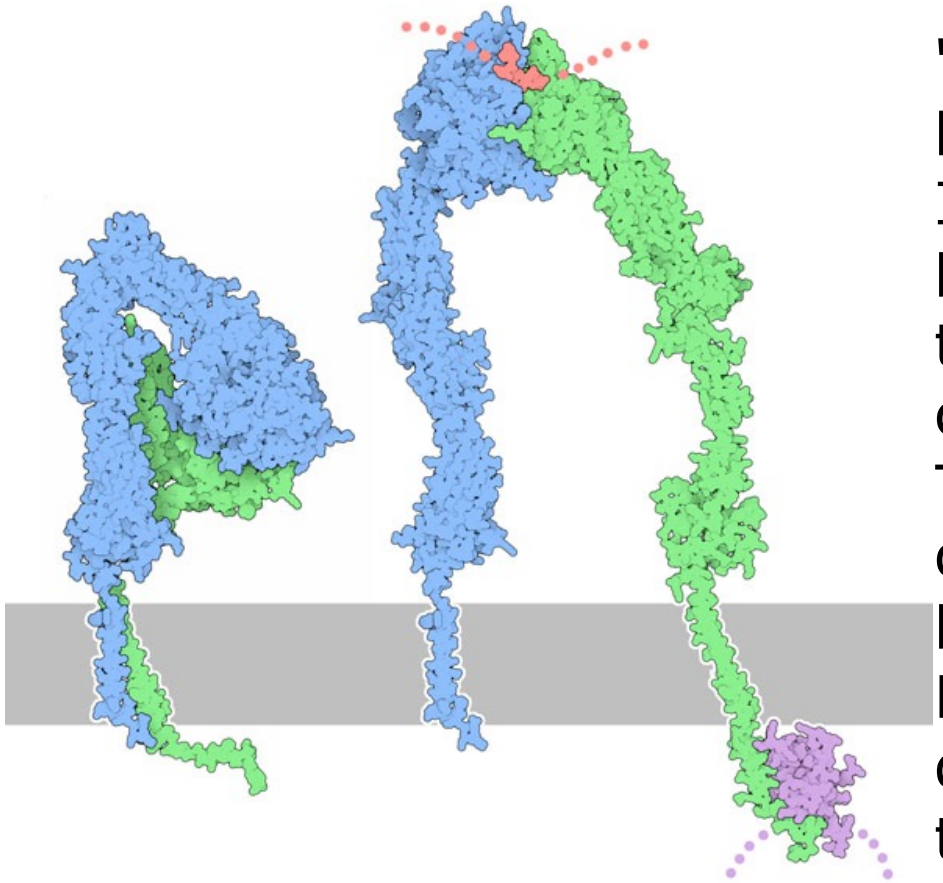
Integrins are transmembrane receptors that mediate the attachment between a cell and other cells or the extracellular matrix (ECM) components such as fibronectin, vitronectin, collagen, and laminin. In addition to transmitting mechanical forces across otherwise vulnerable membranes, they are involved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

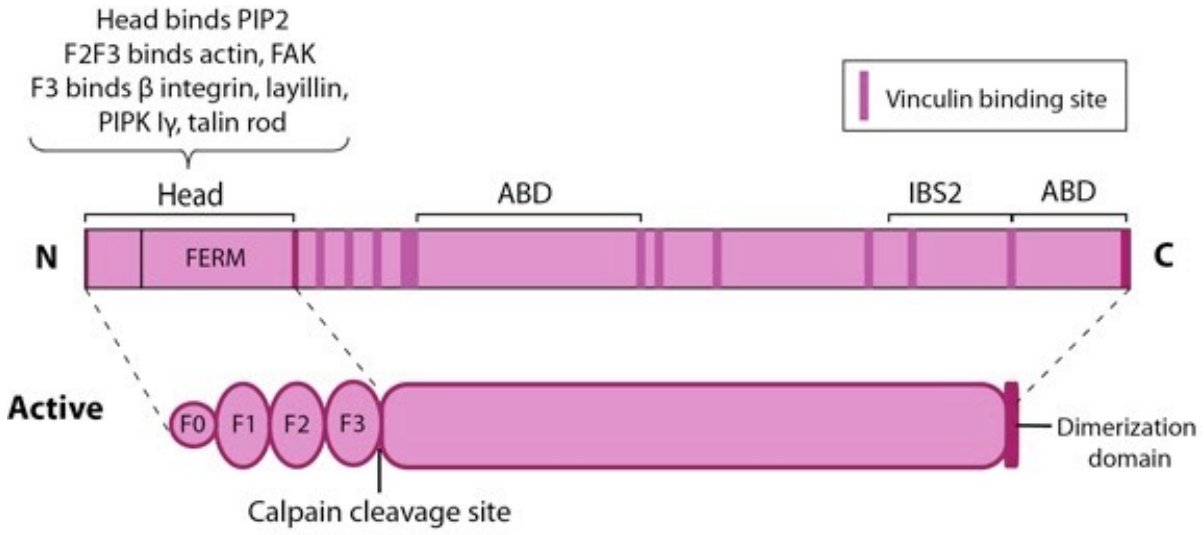
In cells, the priming is accomplished by **talin**, which binds to the β tail of the integrin dimer and changes its conformation.

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

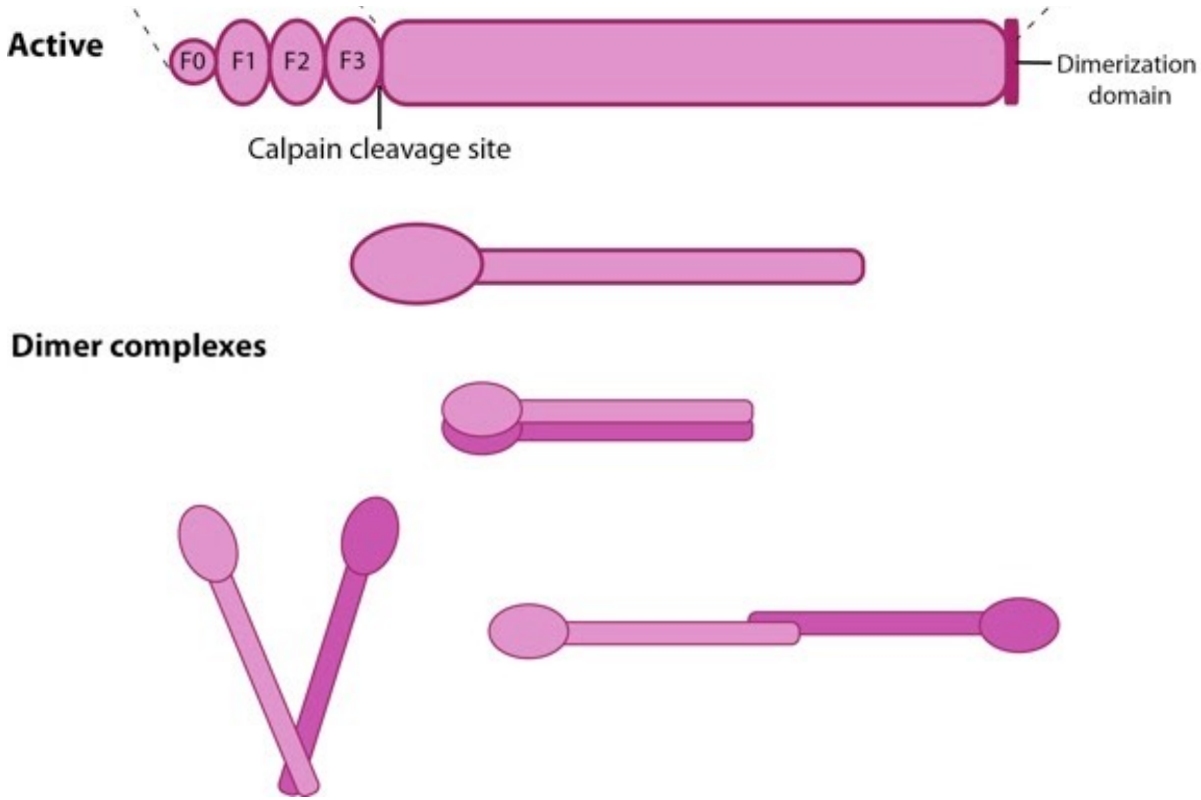
Talin is a 270kDa cytoskeletal protein concentrated at regions of cell–substratum contact and, in lymphocytes, at cell–cell contacts. It is a structural platform that is required for the initial linkage between the contractile cytoskeleton and sites of integrin/fibronectin adhesion



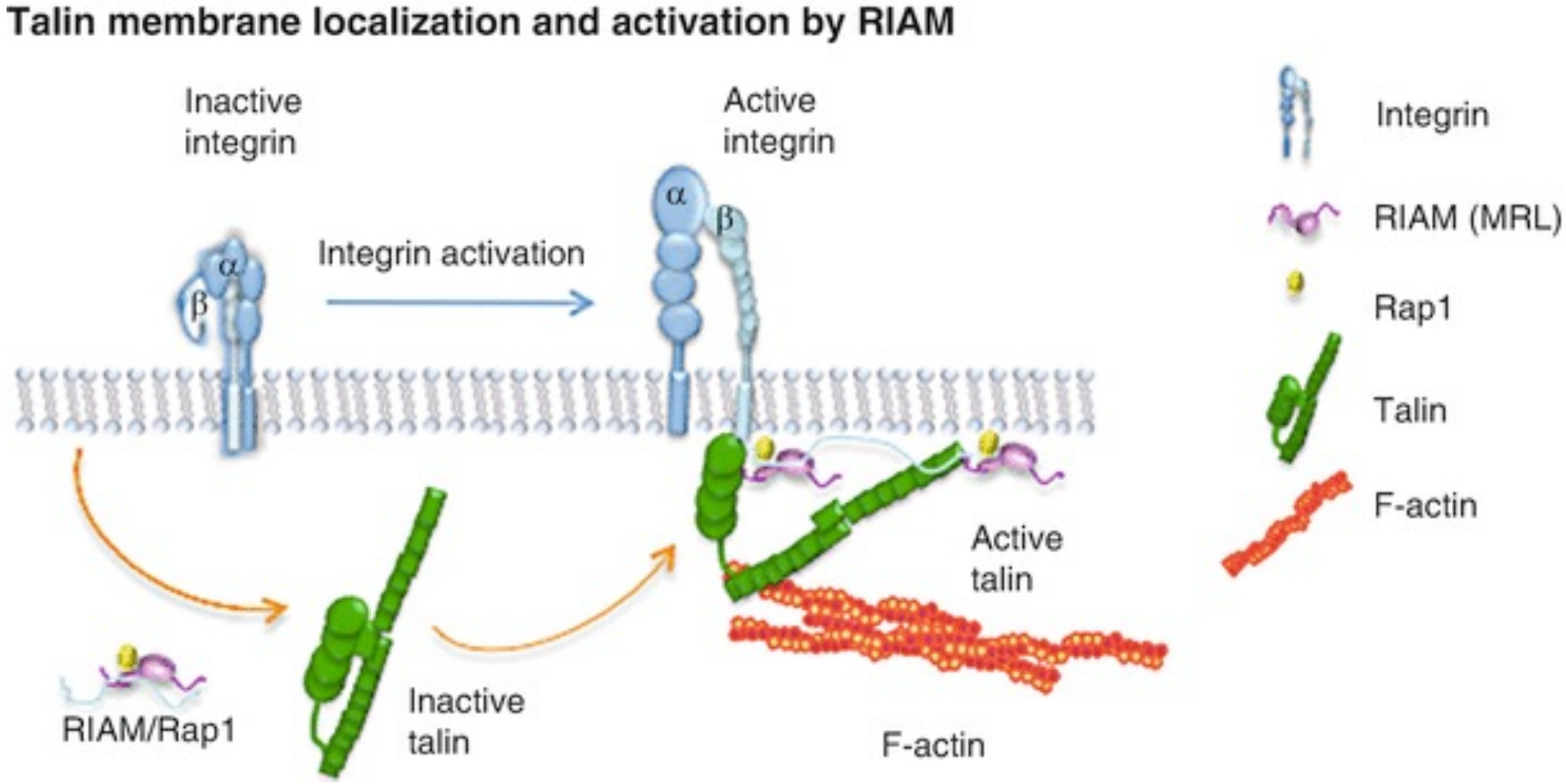
Integrin tail binding occurs via the F3 phosphotyrosine binding (PTB) domain via a unique interaction with the integrin membrane proximal region, which is sufficient for integrin activation. The basic patches on all subdomains can dock onto the plasma membrane and further enhance integrin activation.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

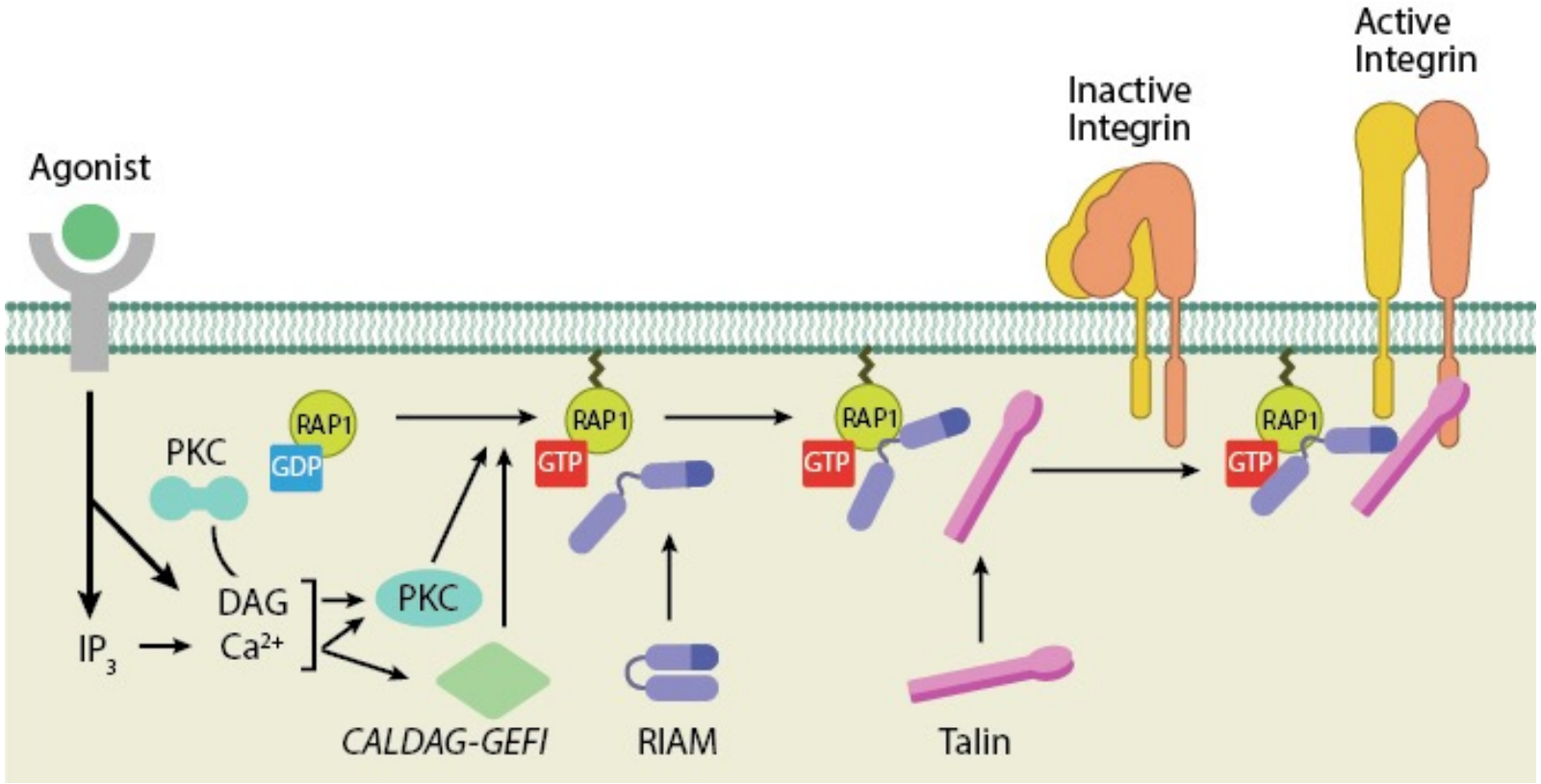


In resting cells, most integrins are kept inactive, possibly owing to conformational constraints in the cytoplasmic tails. A small proportion of the integrin dimers display the thermodynamically unfavourable, active conformation and can bind their ligand.

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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(e-mail: e.caron@ic.ac.uk)

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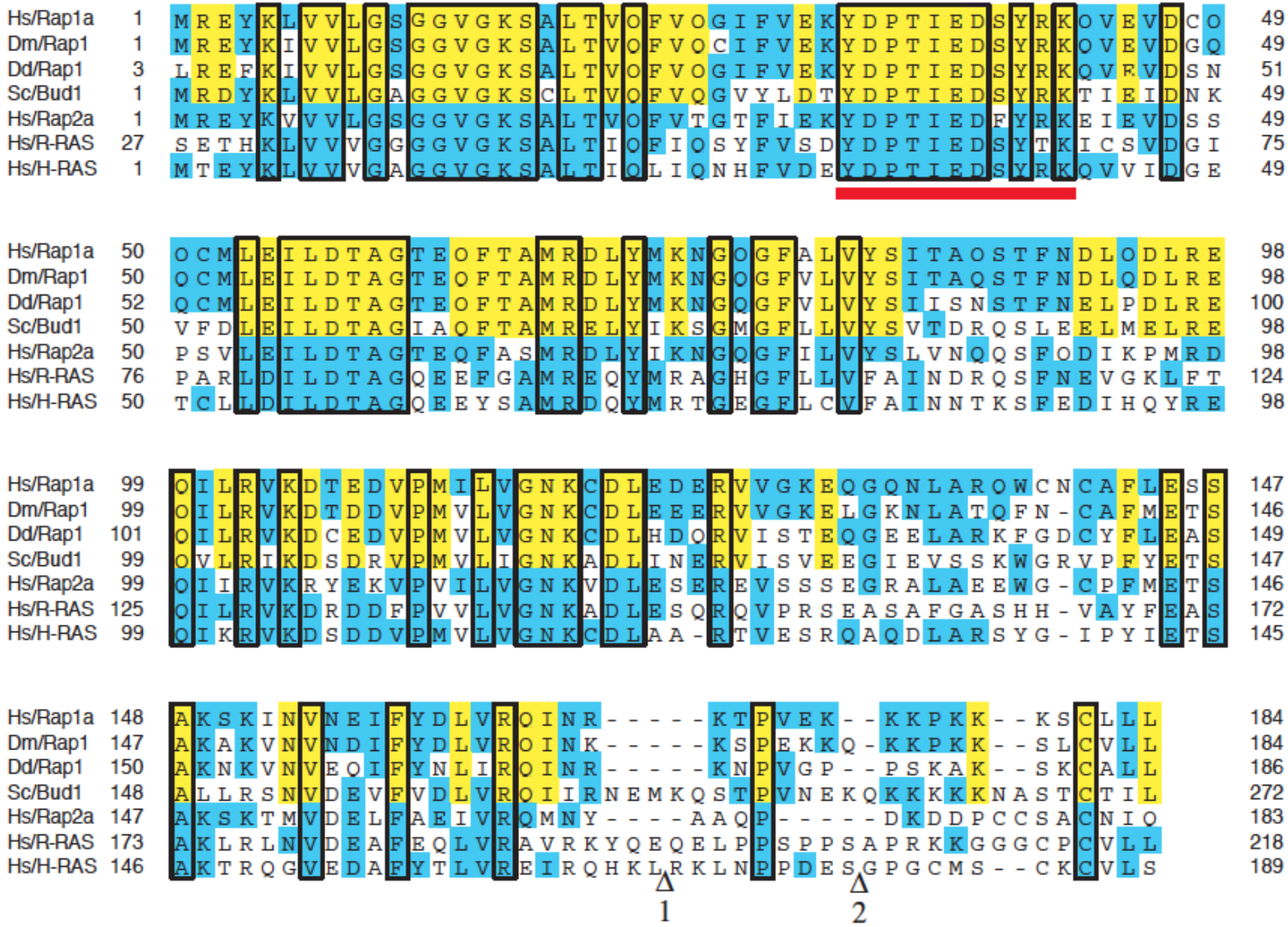
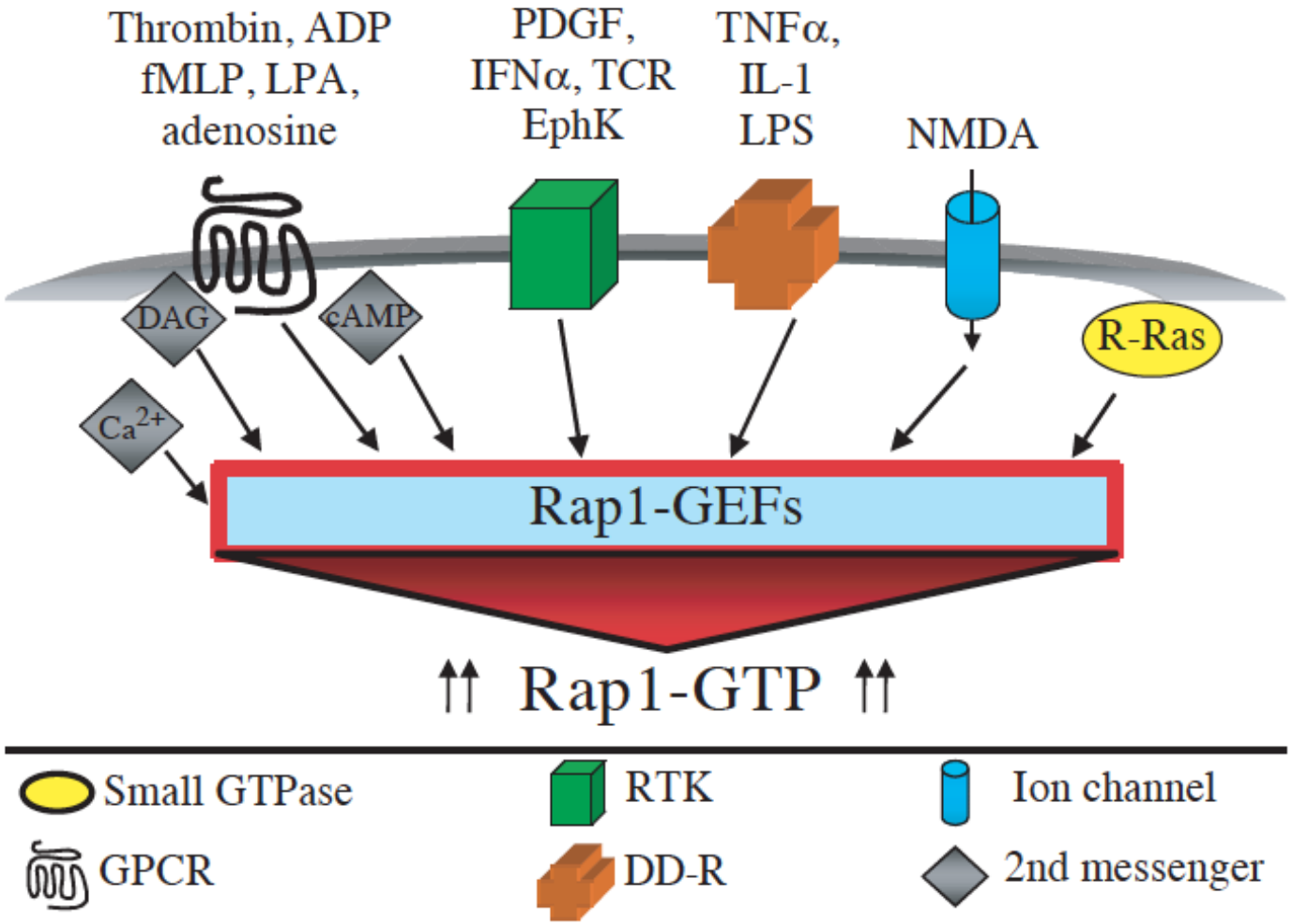
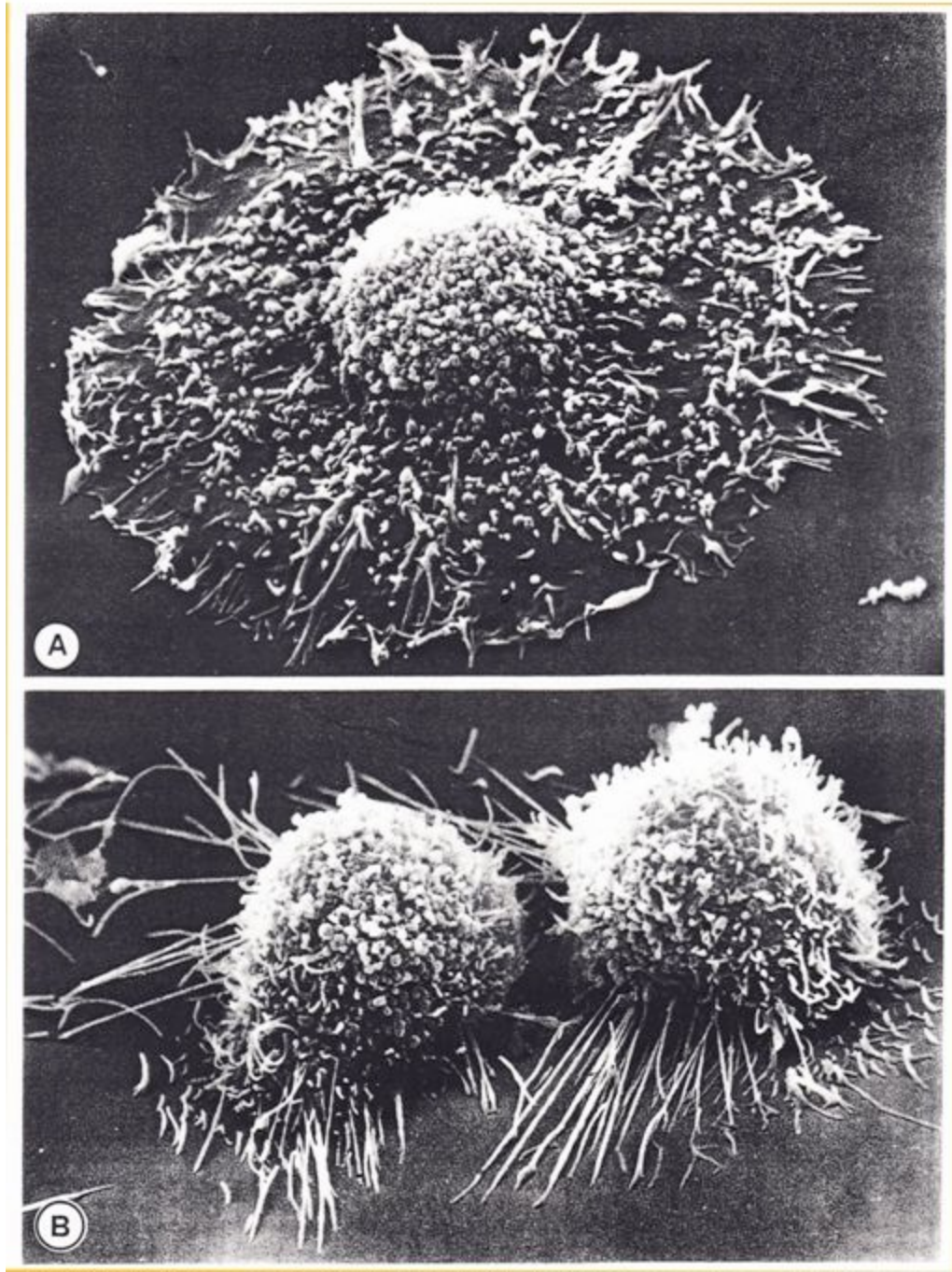
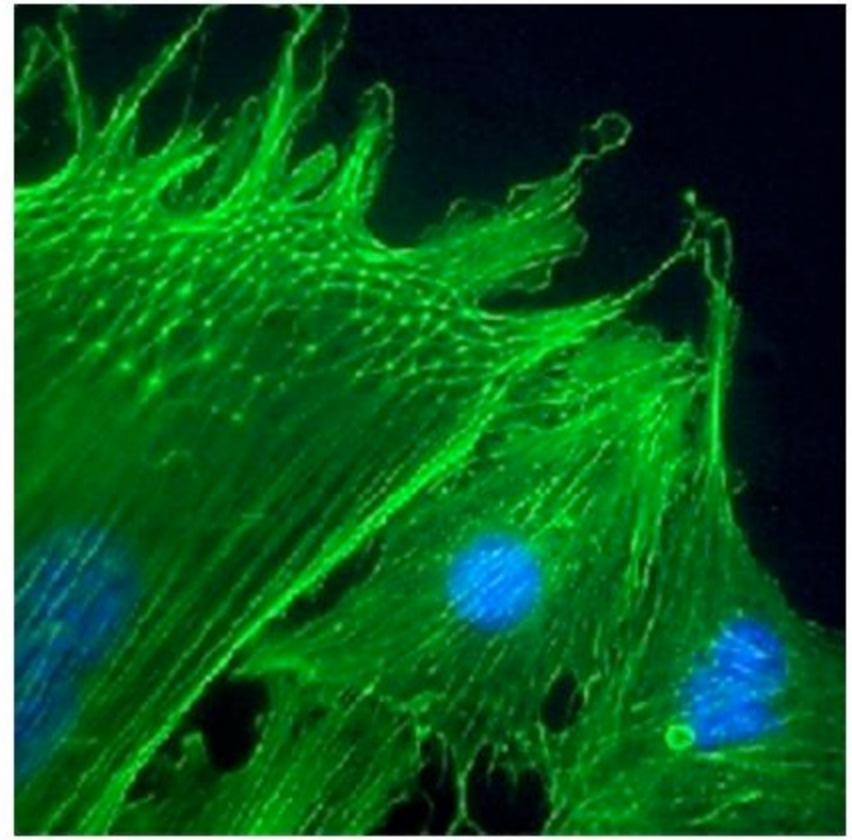
doi:10.1242/jcs.00238


Hs/Rap1a 1 MREYKLVVLGSGGVGKSALTVQFVQGIFVEKYDPTIEDSYRKQVEVDCQ 49
Dm/Rap1 1 MREYKIVVLGSGGVGKSALTVQFVQCIFVEKYDPTIEDSYRKQVEVDGQ 49
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
Hs/Rap1a 50 QCMLEILDTAGTEQFTAMRDLYMKNGQGFALVYSITAQSTFNDLQDLRE 98
Dm/Rap1 50 QCMLEILDTAGTEQFTAMRDLYMKNGQGFVLVYSITAQSTFNDLQDLRE 98
Dd/Rap1 52 QCMLEILDTAGTEQFTAMRDLYMKNGQQFVLVYSIISNSTFNELPDLRE 100
Sc/Bud1 50 VFDLEILDTAGIAQFTAMRELYIKSGMGFLLVYSVTDRQSLEELMELRE 98
Hs/Rap2a 50 PSVLEILDTAGTEQFASMRDLYIKNGQGFILVYSLVNQQSFQDIKPMRD 98
Hs/R-RAS 76 PARLDILDTAGQEEFGAMREQYMRAGHGFLLVFAINDRQSFNEVGKLFT 124
Hs/H-RAS 50 TCLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHQYRE 98
Hs/Rap1a 99 QILRVKDTEDVPMILVGNKCDLEDERVVGKEQGQNLARQWCNCAFLESS 147
Dm/Rap1 99 QILRVKDTDDVPMVLVGNKCDLEEERVVGKELGKNLATQFN-CAFMETS 146
Dd/Rap1 101 QILRVKDCEDVPMVLVGNKCDLHDQRVISTEQGEELARKFGDCYFLEAS 149
Sc/Bud1 99 QVLRIKDSDRVPMVLIGNKADLINERVISVEEGIEVSSKWGRVPFYETS 147
Hs/Rap2a 99 QIIRVKRYEKVPVILVGNKVDLESEREVSSSEGRALAEEWG-CPFMETS 146
Hs/R-RAS 125 QILRVKDRDDFPVVLVGNKADLESQRQVPRSEASAFGASHH-VAYFEAS 172
Hs/H-RAS 99 QIKRVKDSDDVPMVLVGNKCDLAA-RTVESRQAQDLARSYG-IPYIETS 145
Hs/Rap1a 148 AKSKINVNEIFYDLVRQINR-----KTPVEK--KKPKK--KSCLLL 184
Dm/Rap1 147 AKAKVNVNDIFYDLVRQINK-----KSPEKKQ-KKPKK--SLCVLL 184
Dd/Rap1 150 AKNKVNVEQIFYNLIRQINR-----KNPVGP--PSKAK--SKCALL 186
Sc/Bud1 148 ALLRSNVDEVFVDLVRQIIRNEMKQSTPVNEKQKKKKKNASTCTIL 272
Hs/Rap2a 147 AKSKTMVDELFAEIVRQMNY----AAQP-----DKDDPCCSACNIQ 183
Hs/R-RAS 173 AKLRLNVDEAFEQLVRAVRKYQEQELPPSPPSAPRKKGGGCPCVLL 218
Hs/H-RAS 146 AKTRQGVEDAFYTLVREIRQHKLRKLNPPDESGPGCMS--CKCVLS 189
Δ 1
Δ 2

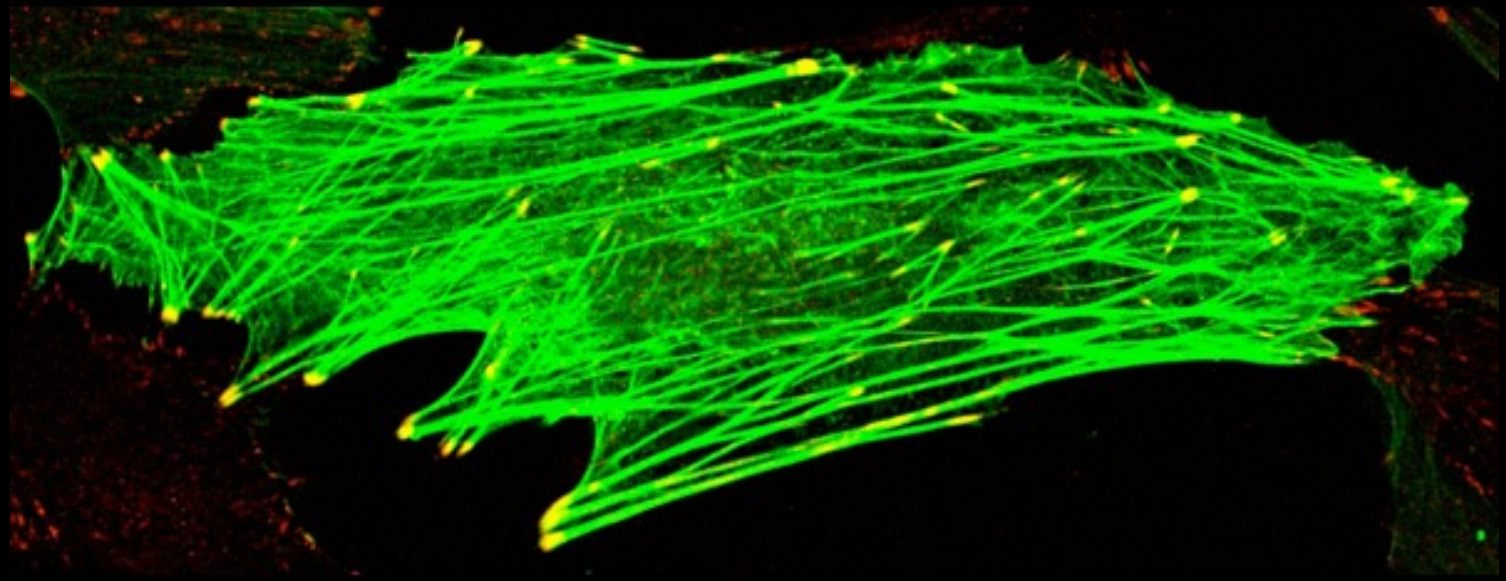


Many receptors and second messengers are coupled to the activation of Rap1 guanine nucleotide exchange factors (Rap1GEFs), and an increase in the cellular levels of active, GTP-bound Rap1.


A
B



FAK KINASE



- PTK2 protein tyrosine kinase 2 (PTK2)/Focal Adhesion Kinase (FAK) is a focal adhesion-associated protein kinase involved in cellular adhesion and spreading processes.
- With the exception of certain types of blood cells, most cells express FAK.
- FAK activity elicits intracellular signal transduction pathways that promote the turn-over of cell contacts with the extracellular matrix, promoting cell migration.
- FAK is required during development: its KO is lethal

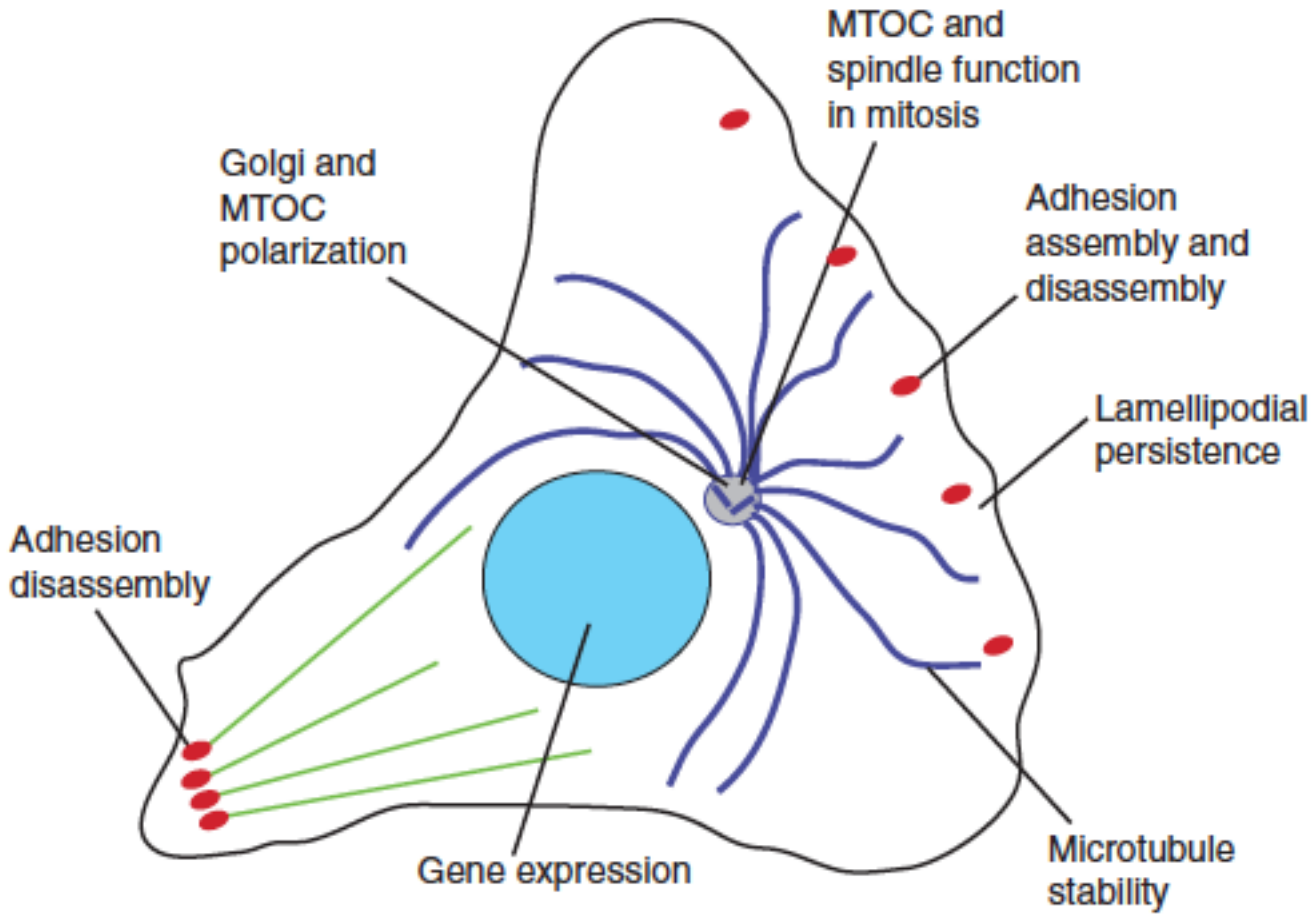


Fig. 2. Major cellular functions of FAK. A migrating cell with the leading edge (right) and trailing edge (left) is shown. Cell-ECM adhesions (red), stress fibers (green), microtubules (dark blue), the MTOC (grey) and nucleus (blue) are illustrated. Black lines denote cellular targets of FAK signaling.

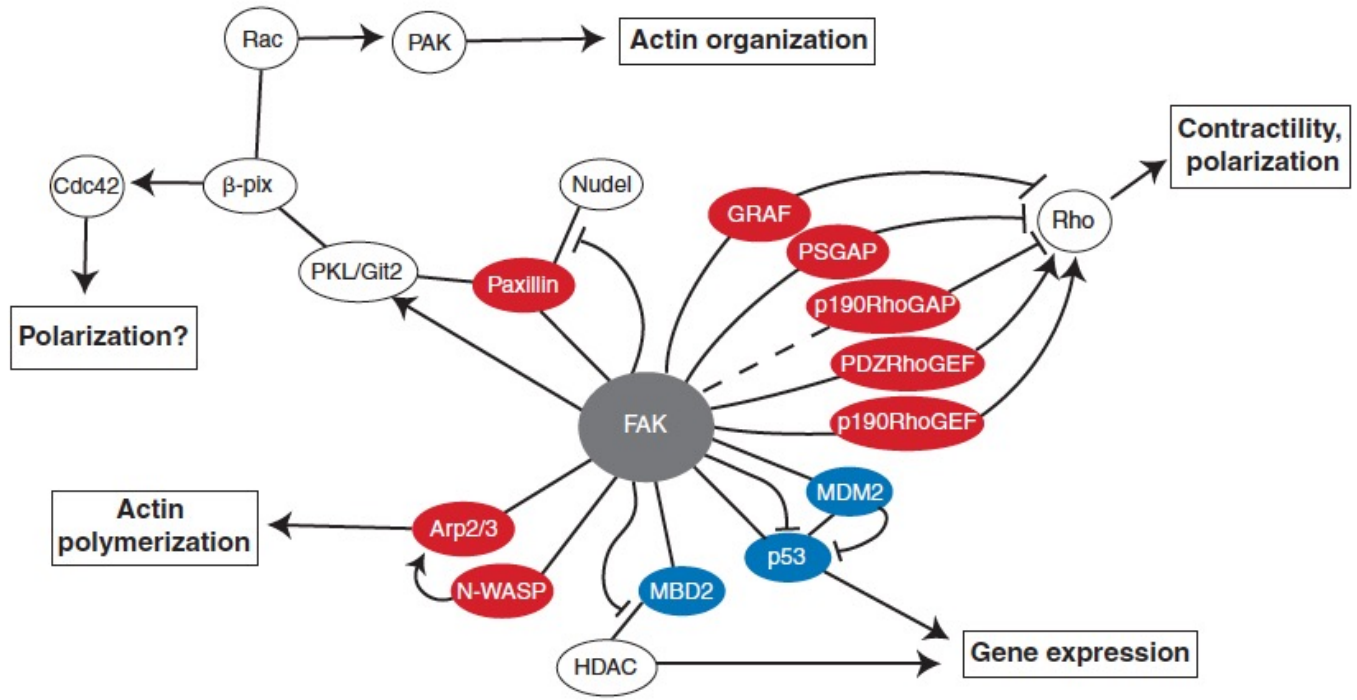


Fig. 3. Networks of FAK signaling that control the actin cytoskeleton and gene expression. Solid lines link proteins that physically interact and dashed lines denote indirect protein-protein interactions. Red proteins are FAK binding partners that are implicated in the regulation of the actin cytoskeleton and blue proteins are FAK binding partners that are implicated in regulating gene expression. Positive (arrows) and negative (bars) regulation of downstream proteins and/or events is indicated.

Cellular functions of FAK kinases: insight into molecular mechanisms and novel functions

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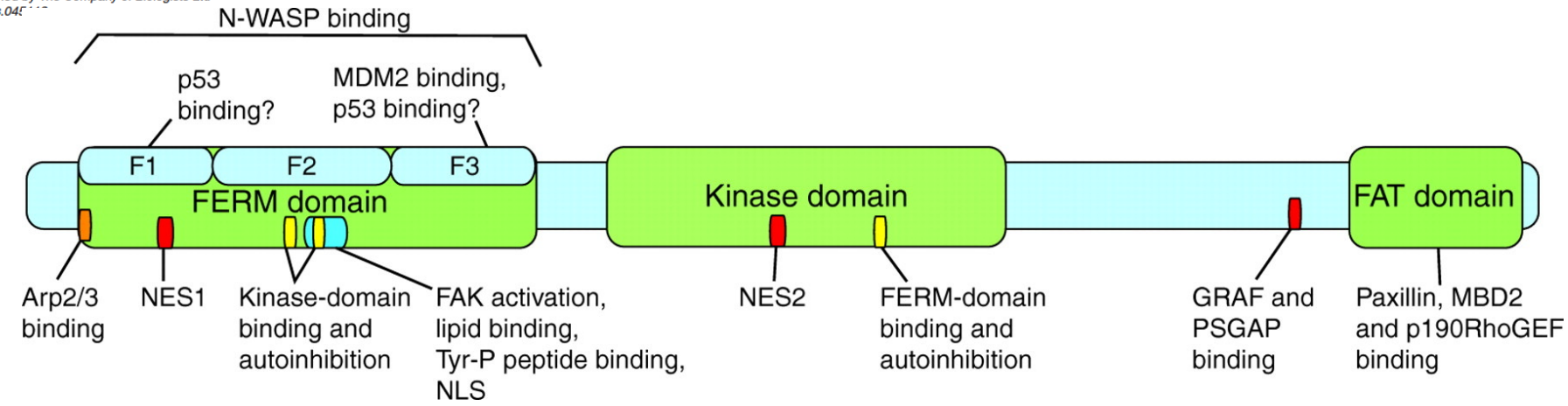

Sequence and structural analysis reveals 4 distinct domains:
① an N-terminal FERM domain;
② a centrally located catalytic tyrosine kinase domain;
③ a C-terminal focal-adhesion targeting (FAT) domain (a four-helix bundle);
④ and an unstructured proline-rich region between the catalytic and FAT domains

The FERM domain docks with the catalytic domain to autoinhibit kinase activity, but also interacts with other molecules to control FAK signaling. The FAT domain and proline-rich region are also docking sites for binding partners that function in localization and downstream signaling.

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Focal Adhesions Require Catalytic Activity of Src Family Kinases To Mediate Integrin-Matrix Adhesion

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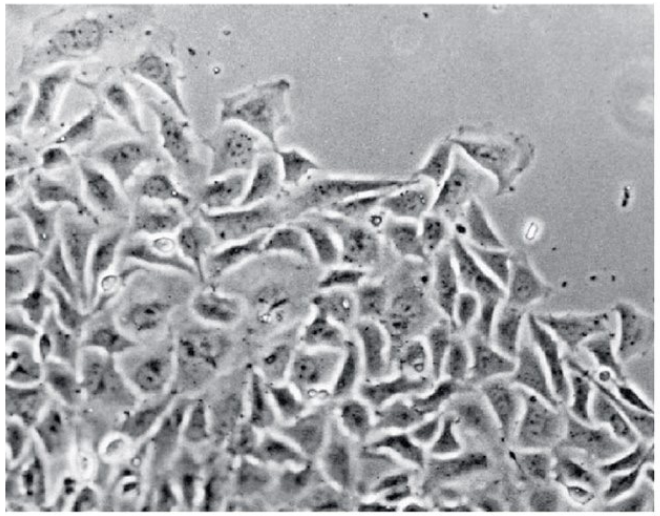
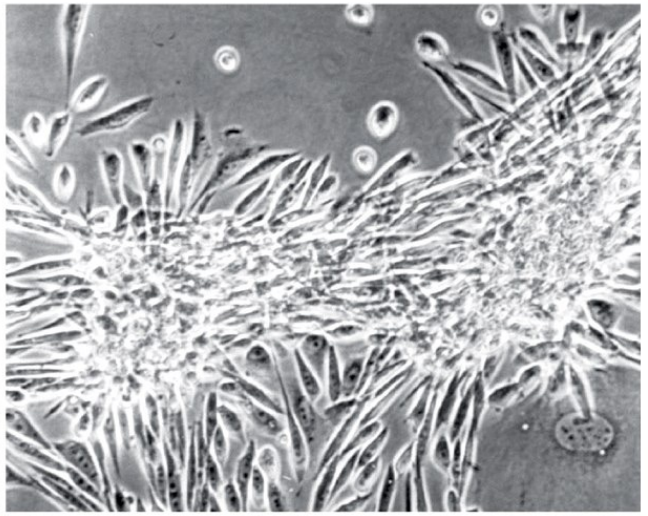
The Ben May Institute for Cancer Research and Center for Molecular Oncology,[1] Committee on Cell Physiology,[2] and Committee on Cancer Biology,[3] The University of Chicago, Chicago, Illinois 60637

Received 9 July 2001/Returned for modification 17 August 2001/Accepted 20 November 2001

No contact inhibition of cell division

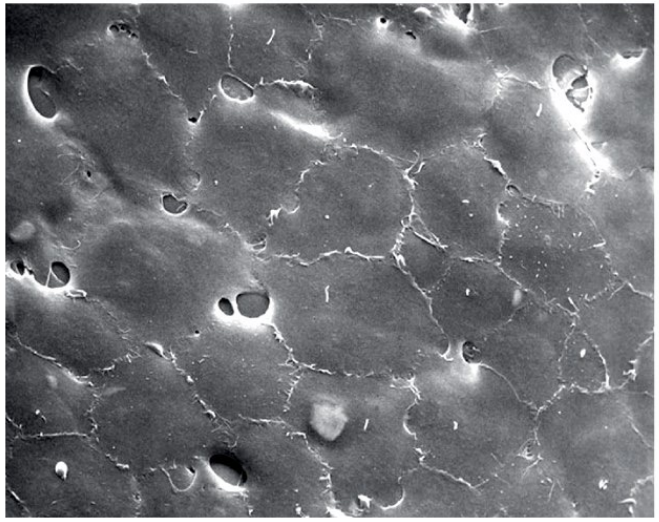
Normal

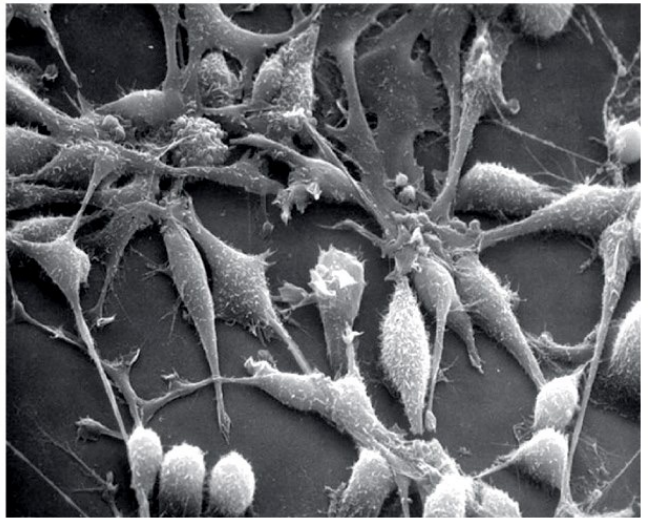




Cancer

Normal

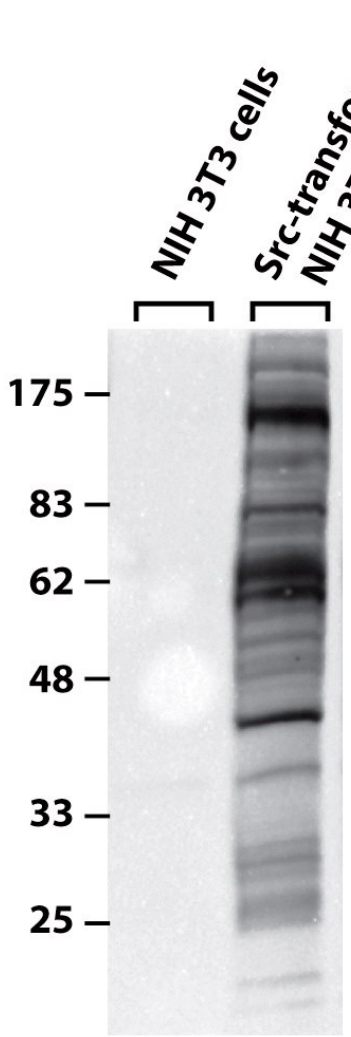




Cancer

Normally, Src kinase intrinsic activity is low

What makes Src so active in transformed cells?



Western Blot with antibody that recognizes Tyr phosphorylated proteins

The structures of c-src and v-src provided an important clue!

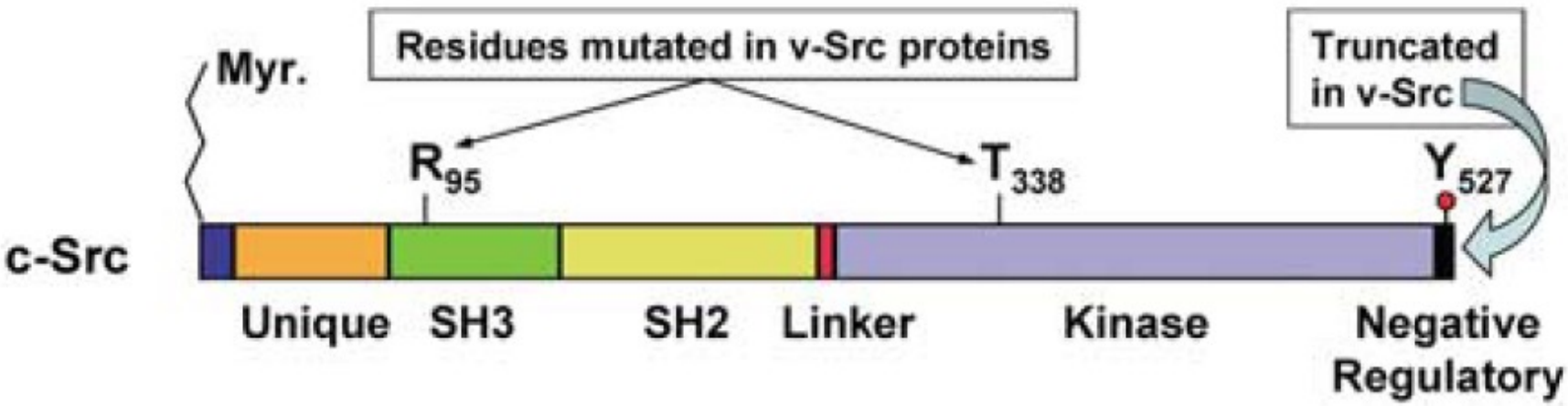
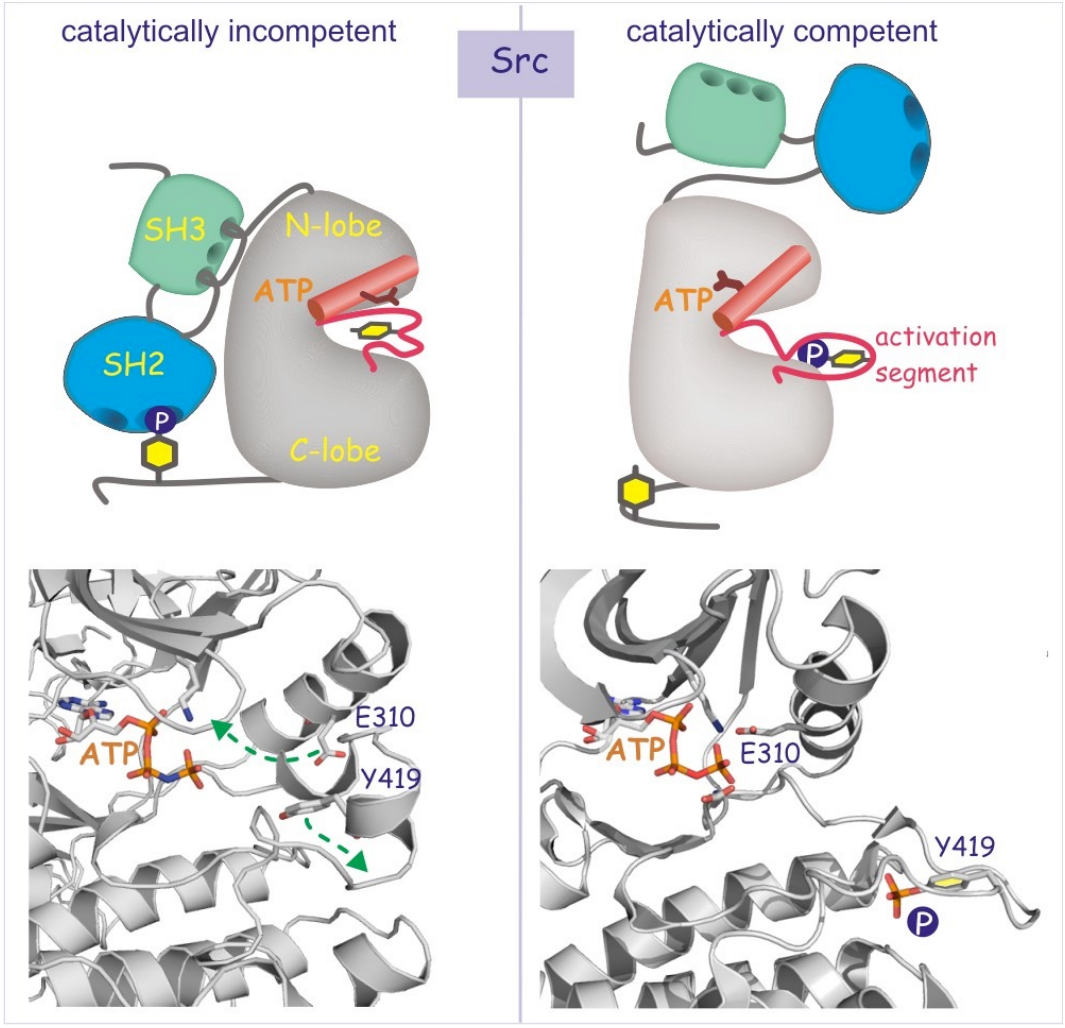


Figure 1 Domain structure of Src family kinases

Regulation of Src kinase activity

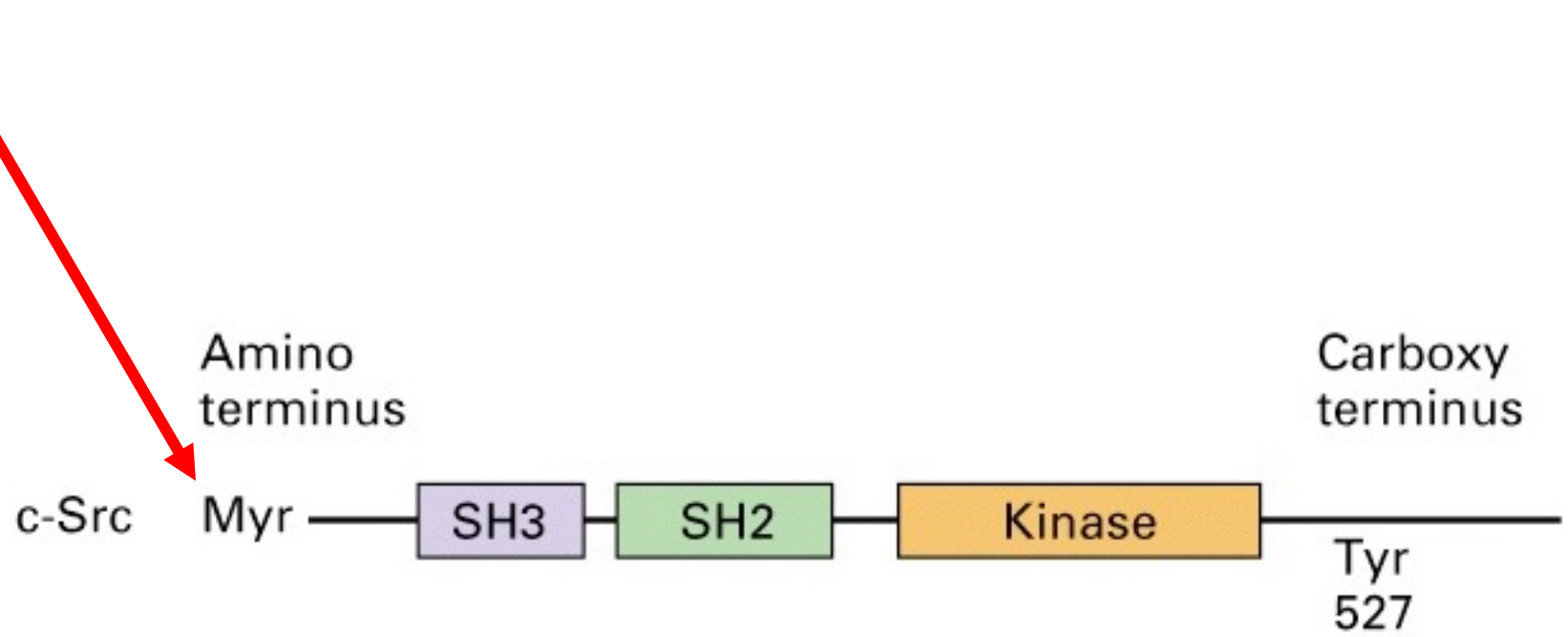


Phosphorylation of the C-terminal tyrosine of Src causes binding of its own SH2 domain. This event places the SH3 domain adjacent to the N-terminal lobe of the kinase domain which affects the coordination of ATP (orange). Detachment of the SH2 domain, through dephosphorhylation of the carboxy-terminal tyrosine (or through binding of the SH2 domain to tyrosine phosphates of other proteins) removes this restraint.

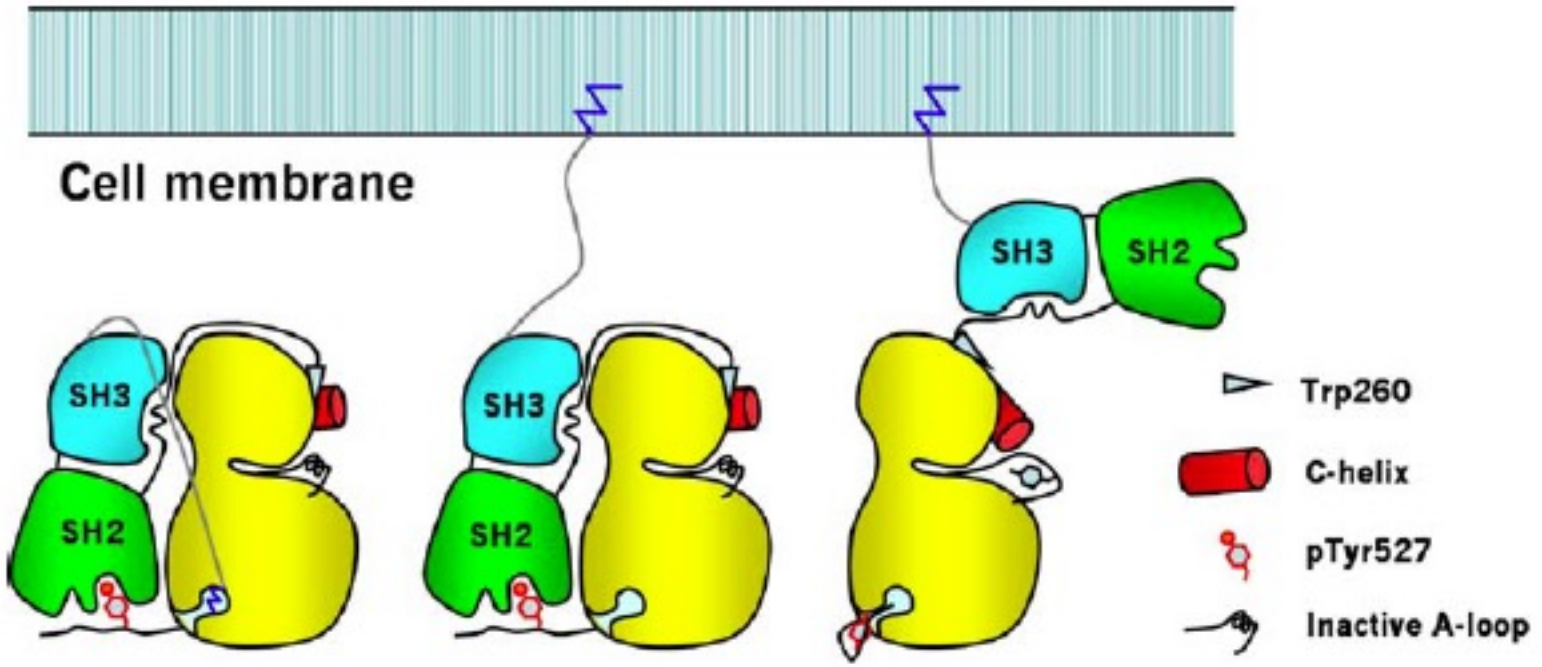
Subsequent phosphorylation of tyrosine-419 in the activation segment liberates the entry path for substrate; the protein kinase is now catalytically competent.

Where is Src within cells?

This is a covalently attached lipid: what might that mean?

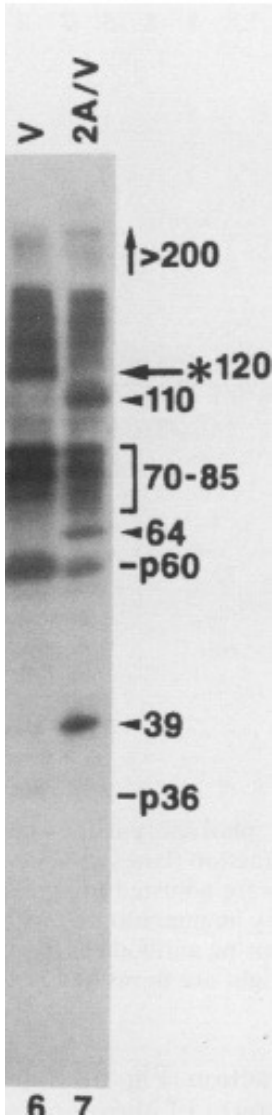


A more detailed model of Src activation



Cowen-Jacob et al. Structure 13, 861-871 (2005)

Identifying The Targets of Src: 1989…



Reynolds et al. MCB (1989)

Western blotting with anti-phosphotyrosine antibodies

V = v-Src transfected cells

2A/V = non-myristylated v-Src transfected cells

p120 catenin: modulates cell-cell adhesion

Identifying the targets of Src

Few Examples

- STAT3: modulates cell-cell adhesion
- p120 catenin: modulates cell-cell adhesion
- Cortactin A: regulates actin polymerization
- Focal Adhesion Kinase: involved in cell-matrix interactions

Mike Schaller, ex-UNC

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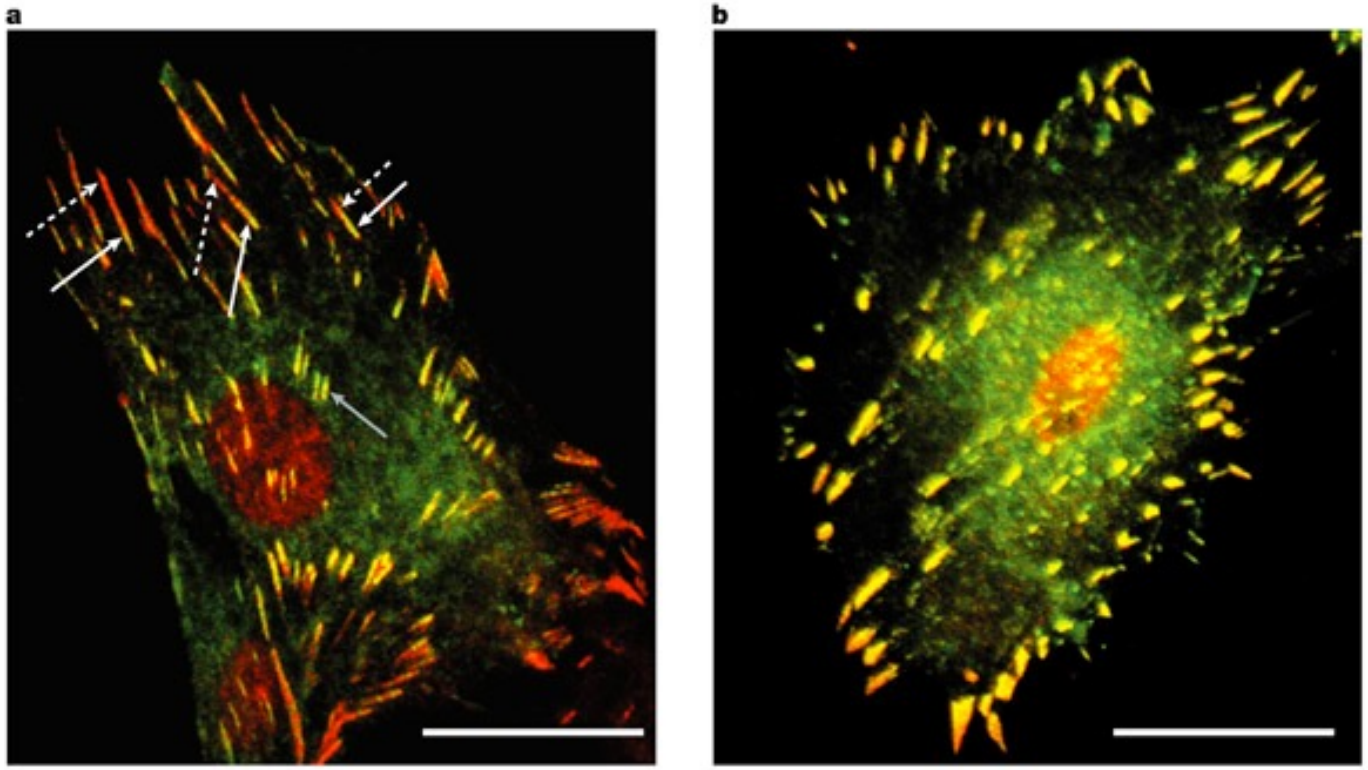
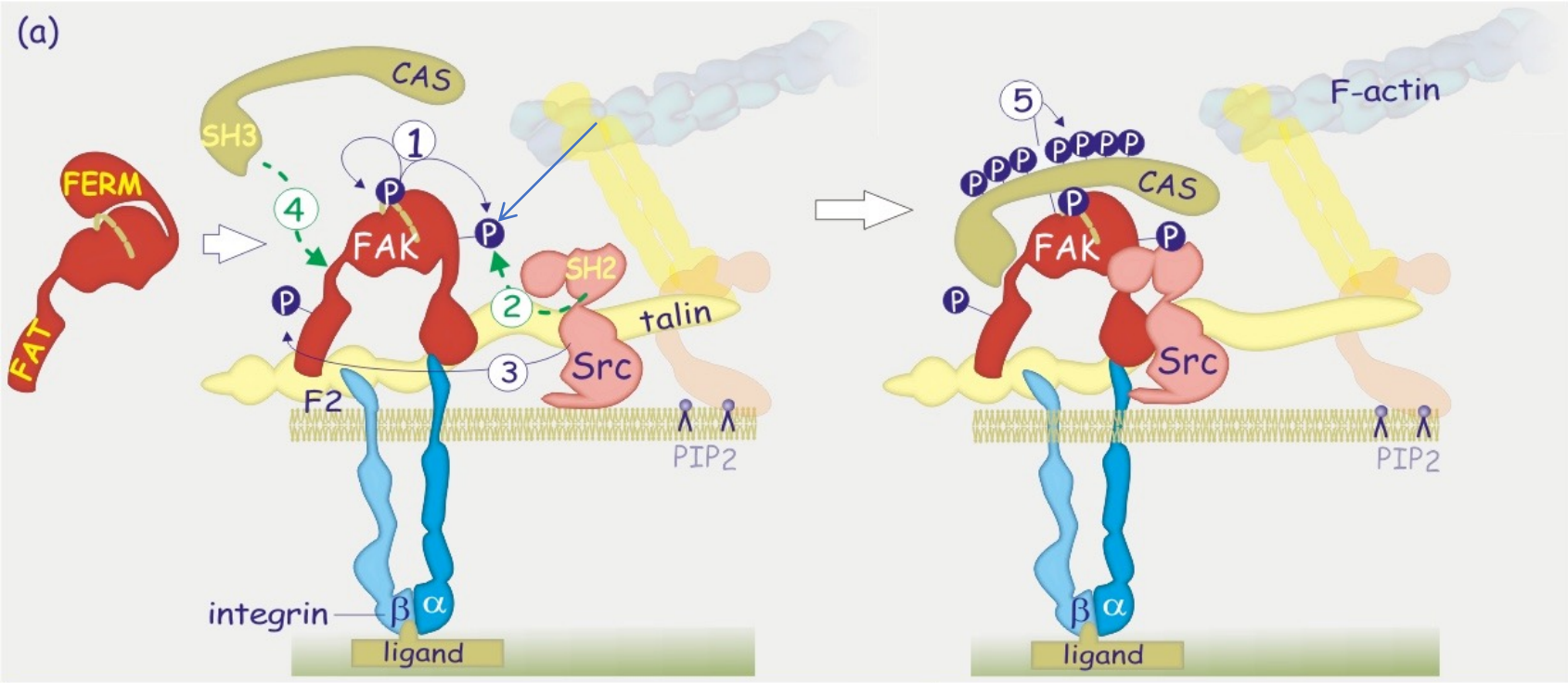
(A) In the case of inactive src, the focal adhesions are enlarged (at the termini of stabilized actin cables).
FAK is already at the membrane proximal region (broken arrow) and FAK–v-Src-KD is localized at the membrane-distal region.



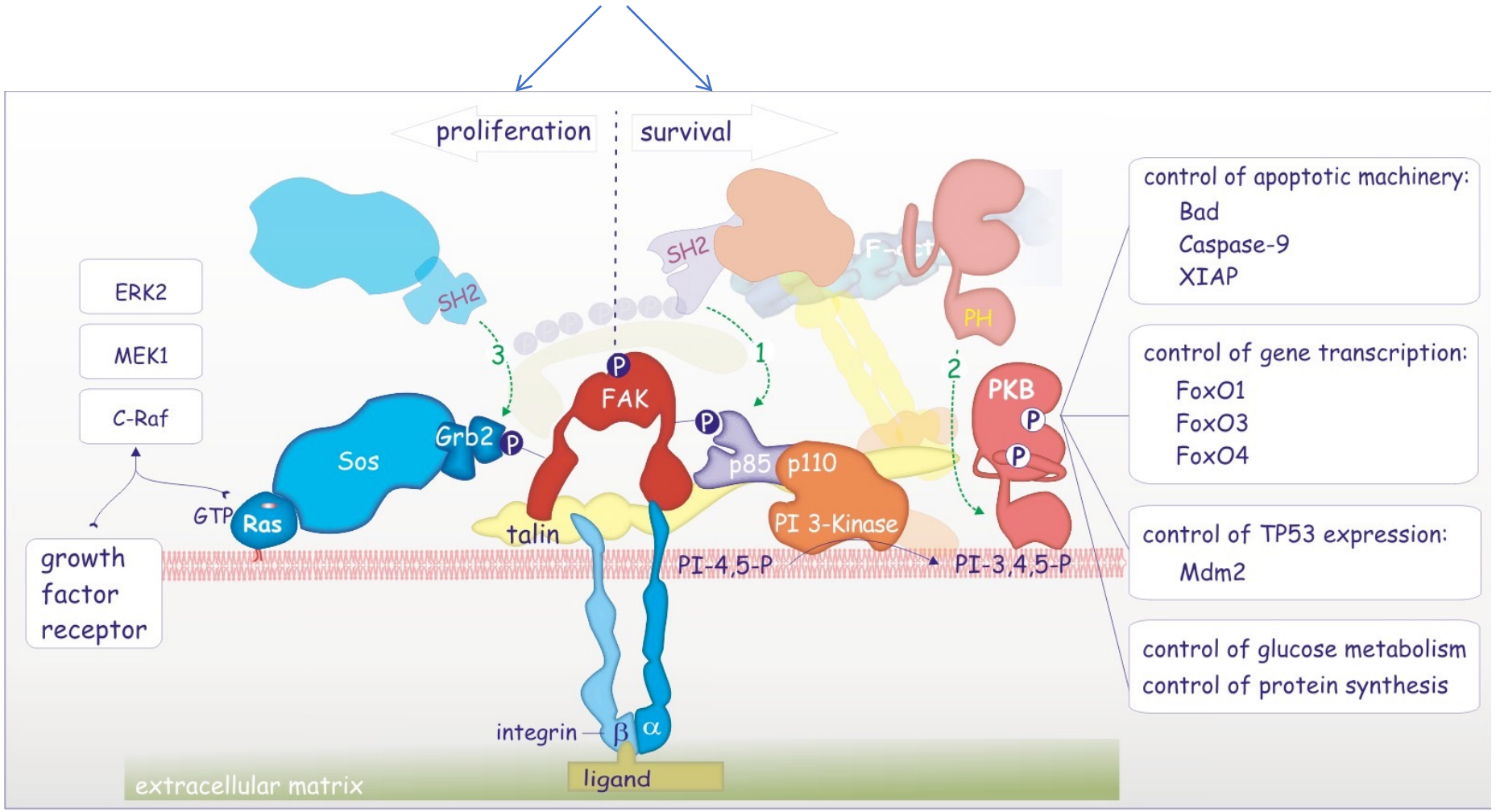
(B) FAK (red) and v-Src (green) co-localize in smaller adhesion structures at the cell periphery when v-Src is active, and focal adhesions (and the associated actin filaments) are dynamically regulated. These adhesion characteristics indicate that v-Src-KD impairs adhesion turnover and that focal-adhesion size is linked to Src-dependent dynamic regulation

Integrin signaling complex

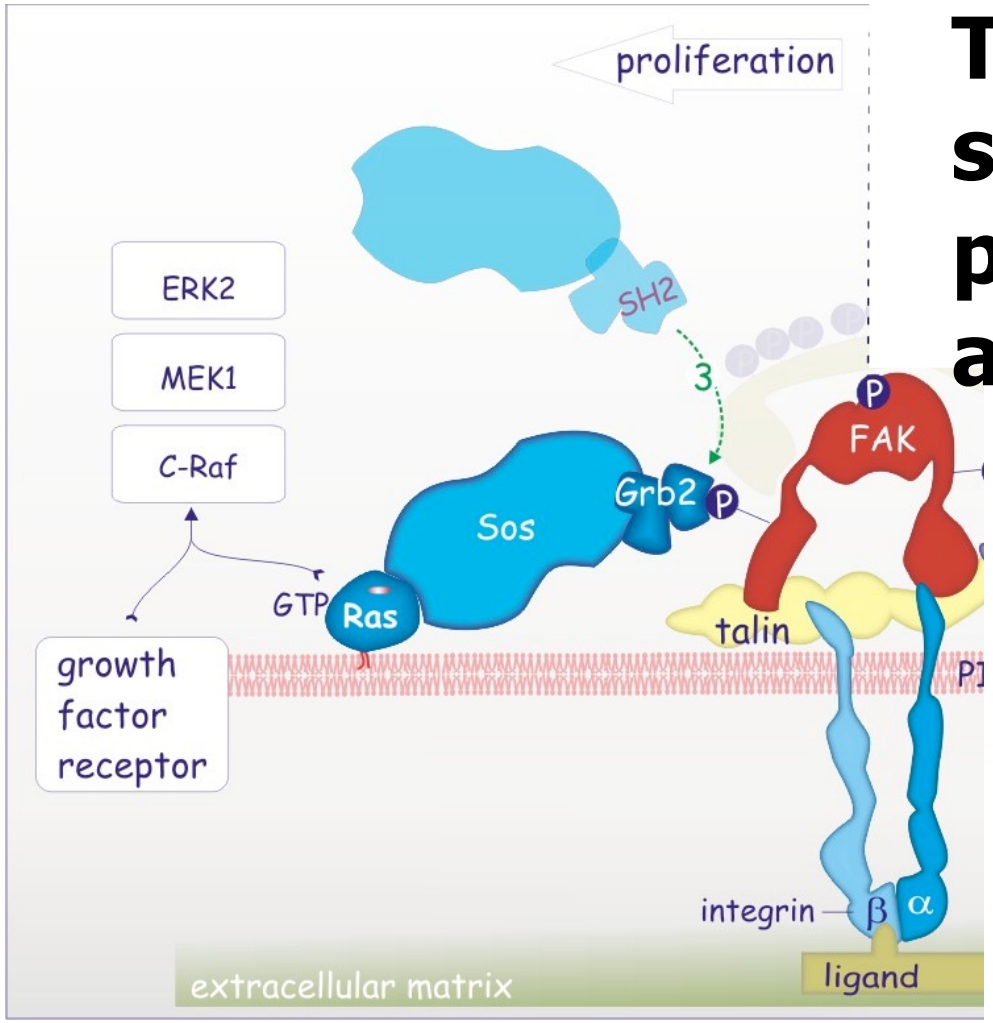


The focal adhesion kinase FAK associates with talin. Autophosphorylation of FAK then generates a docking site for the SH2 domain of Src which phosphorylates FAK at Y925. Src and FAK next phosphorylate the FAK-associated docking protein CAS at multiple sites.
An integrin-signalling complex is formed that acts in a manner similar to growth factor-receptor signaling complexes, i.e. attachment of adaptors and effectors and tyrosine phosphorylation substrates.

Integrin signaling

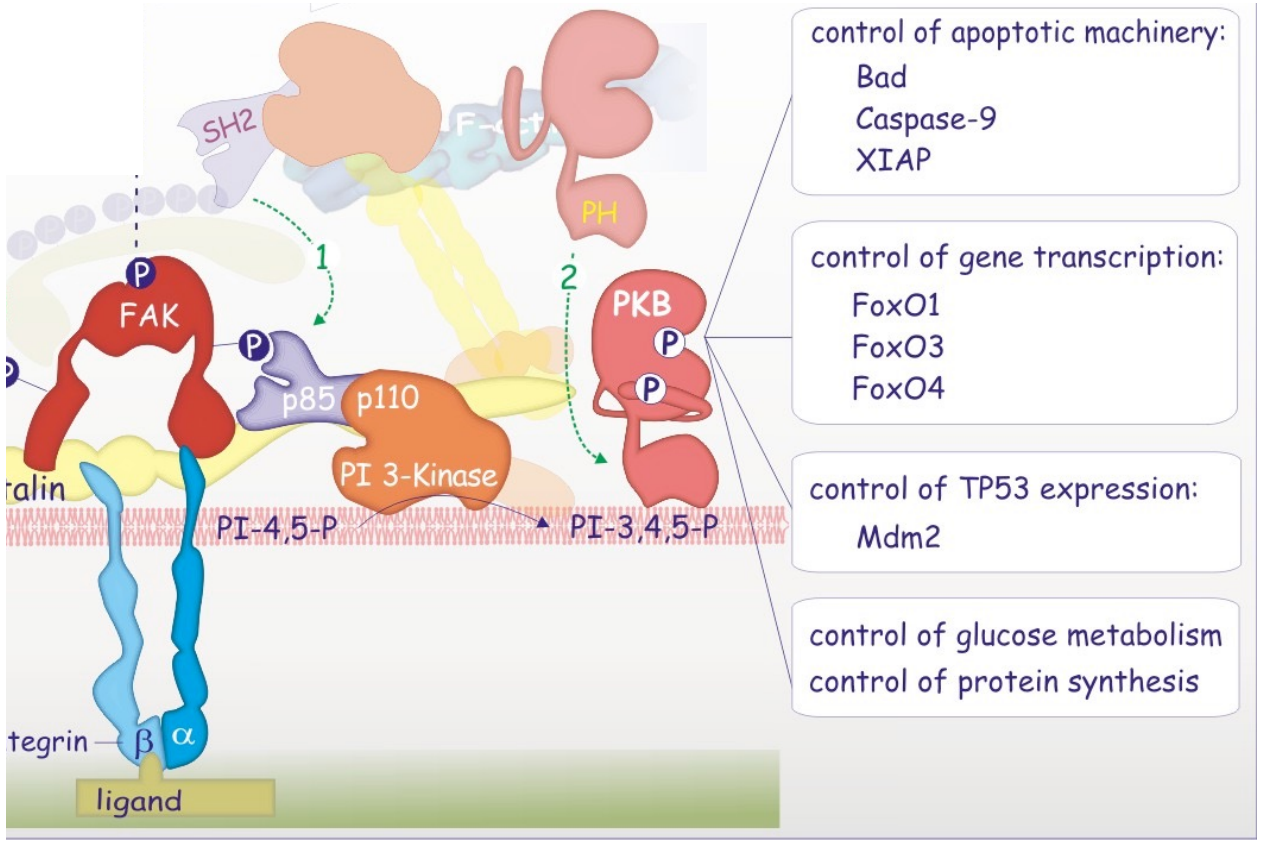


The focal adhesion site promotes cell proliferation through activation of Ras



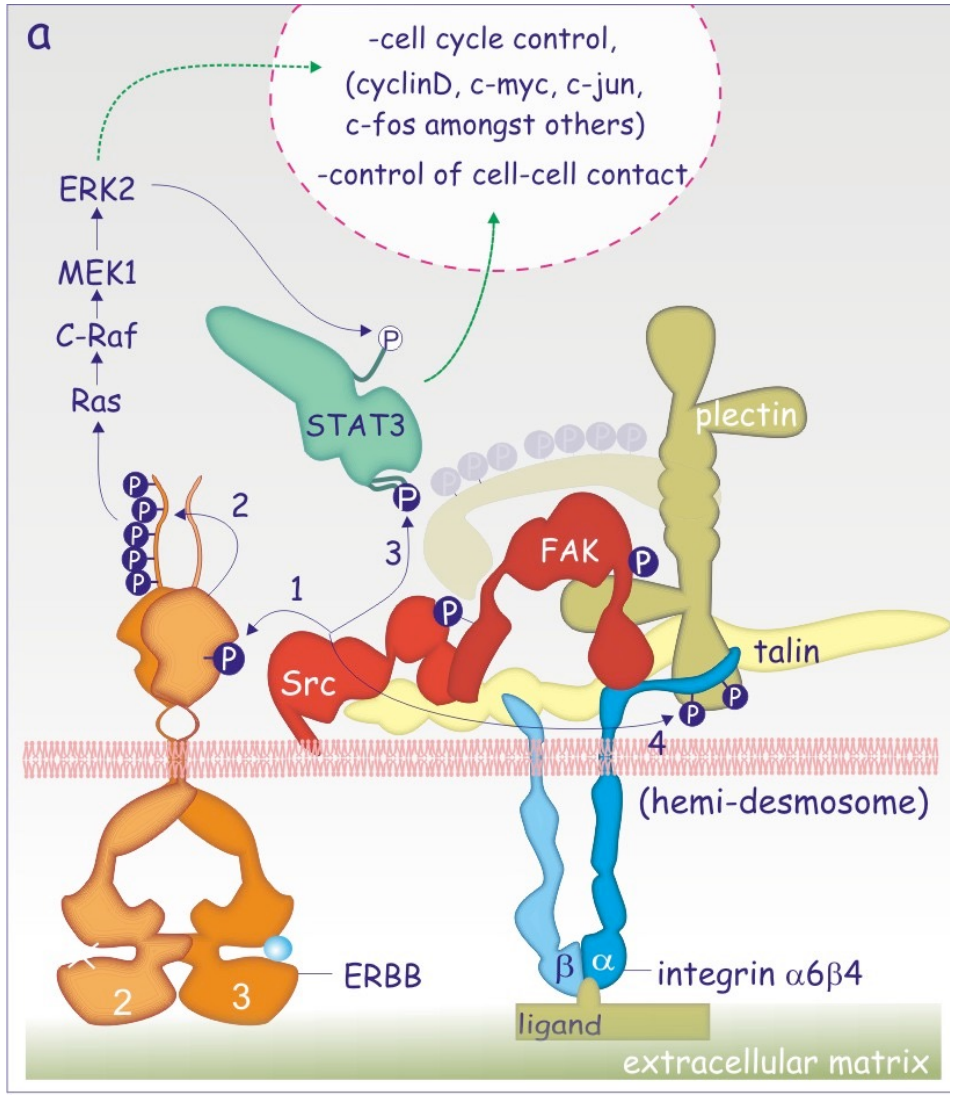
Phosphorylated focal adhesion kinase (FAK) is a binding site for Grb2. This interaction recruits the Ras guanine exchange factor Sos, leading to activation of Ras.
Ras-GTP initiates the activation of the Raf-ERK pathway, necessary for initiation of the cell cycle.

The focal adhesion site promotes cell survival through activation of PKB



Phosphorylated focal adhesion kinase (FAK) binds the SH2 domain of the regulatory subunit (p85) of PI 3-kinase. Subsequent production of PIP3 provides a binding site for PKB (and PDK1). After its activation PKB phosphorylates a large number of proteins that directly or indirectly deal with cell death.

Adhesion-mediated cell cycle control

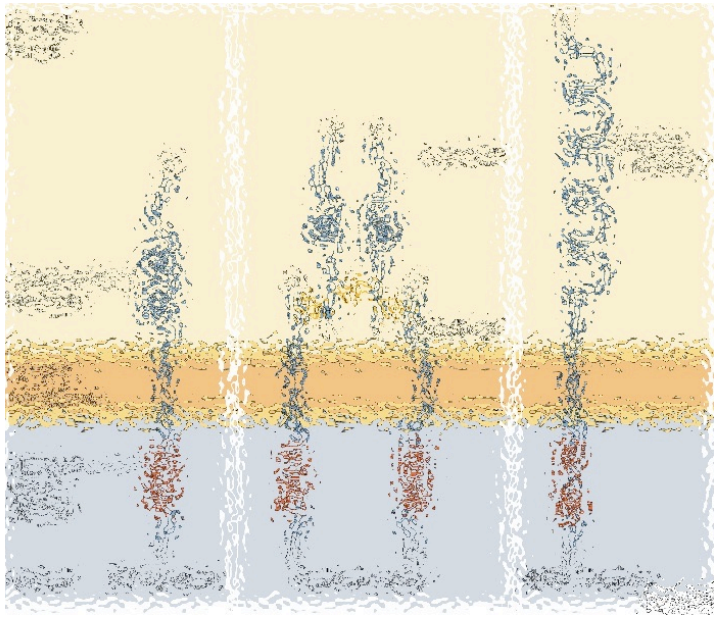


In epithelial cells, integrin α6β4, forms a special adhesion complex named *hemi-desmosome*. These complexes are linked to intermediate filaments via **plectin**.
ERBB2/3 receptors are recruited into these complexes leading to phosphorylation of ERBB2 by Src bound to FAK.
Src also phosphorylates STAT3 and this signal is enforced by a second phosphorylation on serine through ERK2. Both phosphorylations enhance its transcriptional activity.

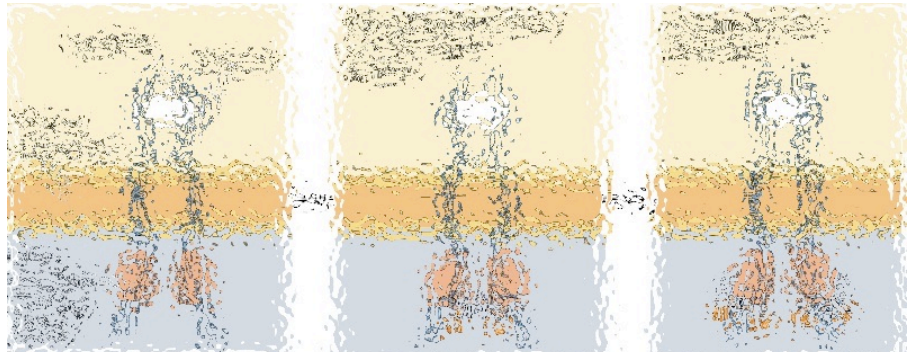
In the case of breast tumor cells, this pathway promotes cellular invasion.

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 <u>non</u> receptor protein-tyrosine kinases
    - Src family
    - JAK family



- **TGF-β receptors**

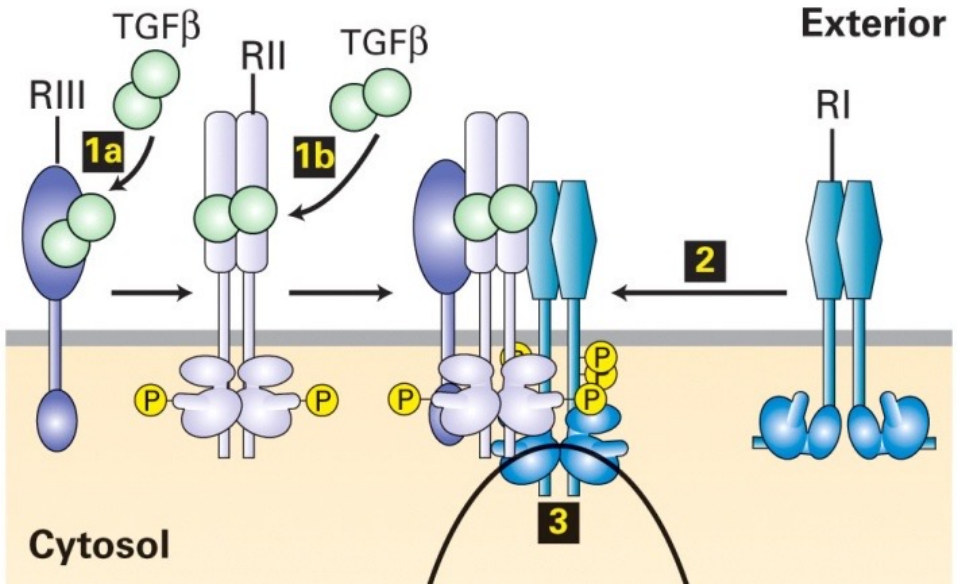
TGFβ

- TGFβ (Transforming Growth Factor β) superfamily proteins play important roles in regulating development of vertebrates and invertebrates
  - Bone Morphogenic Protein (BMP) is one of the TGFβ superfamily important in regulating formation of mesoderm and the earliest blood forming cells
  - GDF11
  - TGFβ-1 is another member of the TGFβ superfamily proteins which can induce a transformed phenotype of certain cells in culture
- There are three human TGFβ isoforms known to have potent anti-proliferative effects on many types of mammalian cells. Mutation of TGFβ will result in releasing cells from growth inhibition (frequently occurs in human tumors)
- TGFβ also promotes expression of cell-adhesion molecules and extracellular matrix molecules

TGFβ Signaling Receptors Have Serine/Threonine Kinase Activity

- Three different polypeptides with apparent molecular weights of 55, 85 and 280 kDa were purified, referred to as types RI, RII and RIII TGFβ receptors
- Type RIII TGFβ receptor is a **cell-surface proteoglycan**, also called **β-glycan** which bind and concentrate TGFβ near the cell surface
- Type RI and type RII receptors are dimeric transmembrane proteins with serine/threonine kinases as part of their cytosolic domains

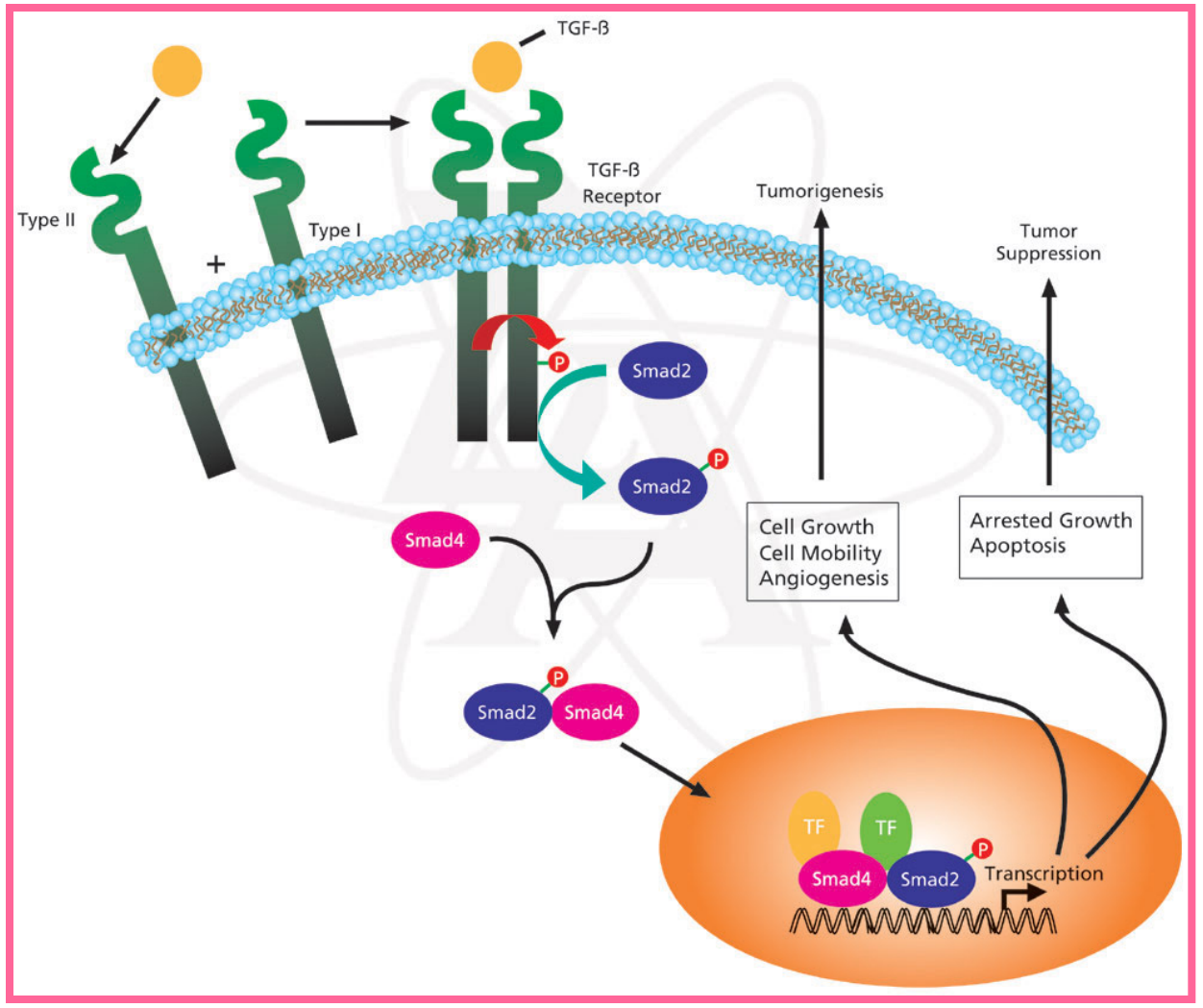
- RII is a constitutively active kinase that phosphrylates itself in the absence of TGFb
- Binding of TGFβ induces the formation of two copies each of RI and RII. A RII then phophorylates serine/threonine of RI adjacent to the cytoplasm and thus activate the RI kinase activity



TGFβ Receptor Signaling:
a logic resembling the STAT-family

STAT-related logic

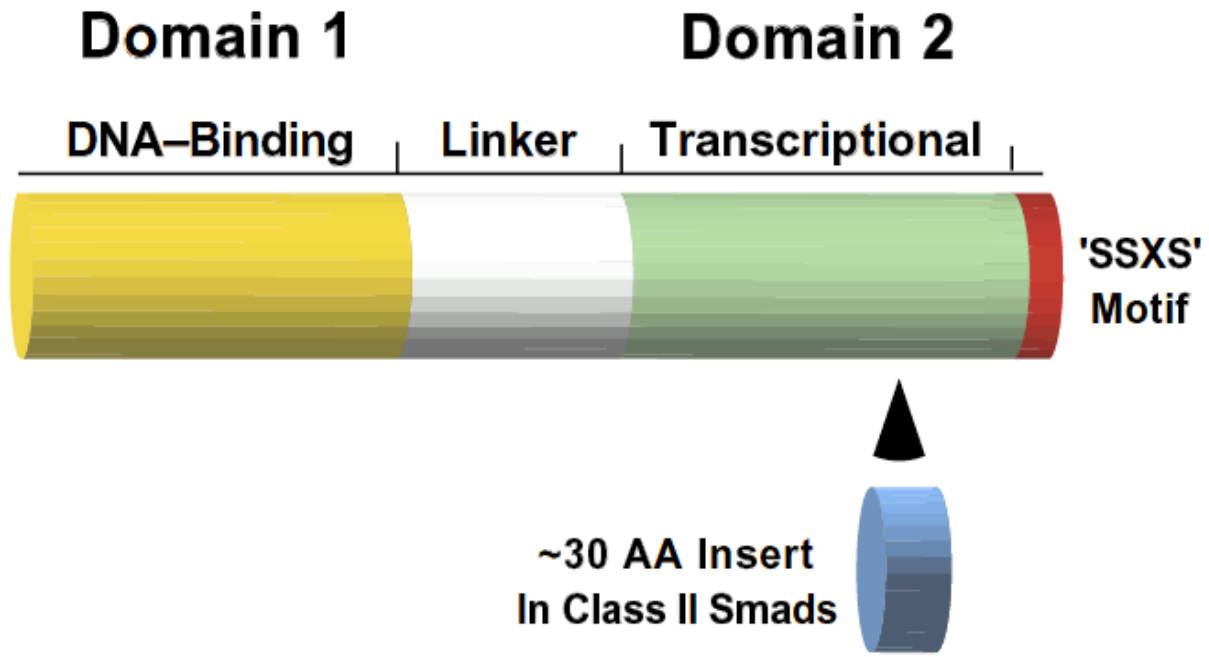
- TGFß-receptors are activated by binding of ligand (TGFb).
- Activated receptor kinases phosphorylate specific Smad-factors
- Phosphorylated Smad-factors associate with a common Smad-factor (Smad4)
- The generated heteromeric complexes migrate to the nucleus as transcription factors



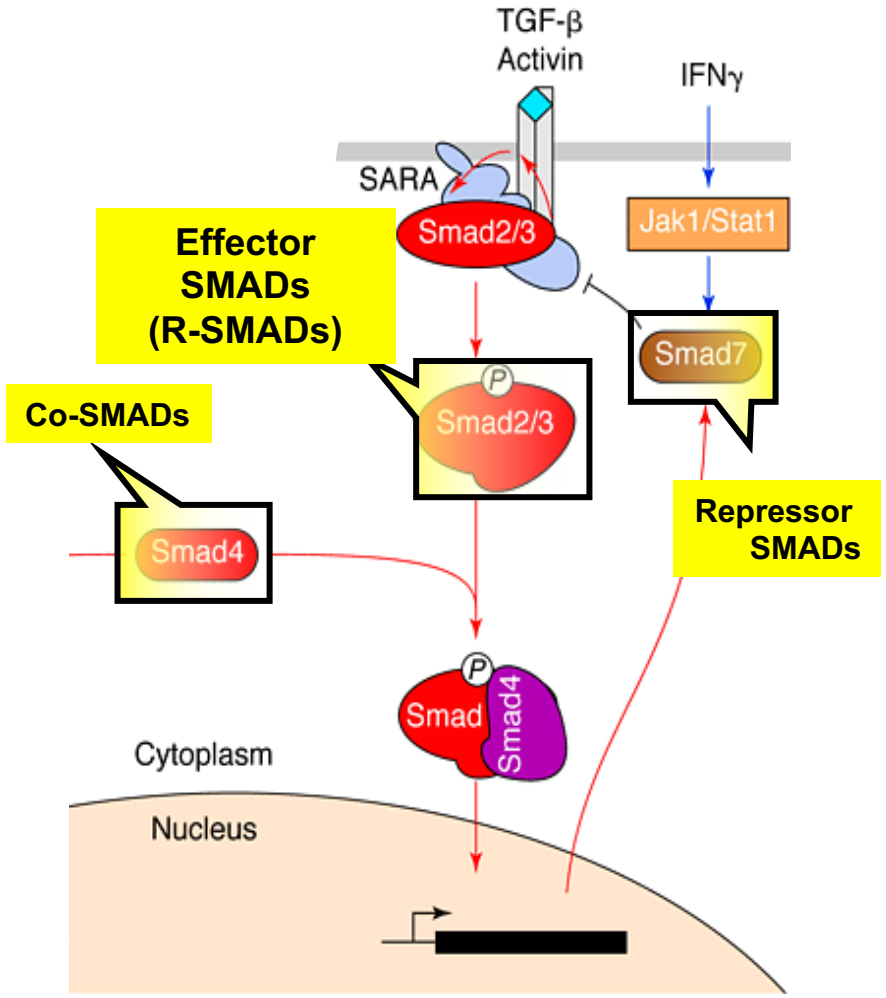
Classification

- Smad-factors: design and classification

  - Nine different Smad-factors identified in vertebrates
  - common conserved domains: N-terminal MH1-domain (DBD) + C-terminal MH2-domain



Three groups of SMADs

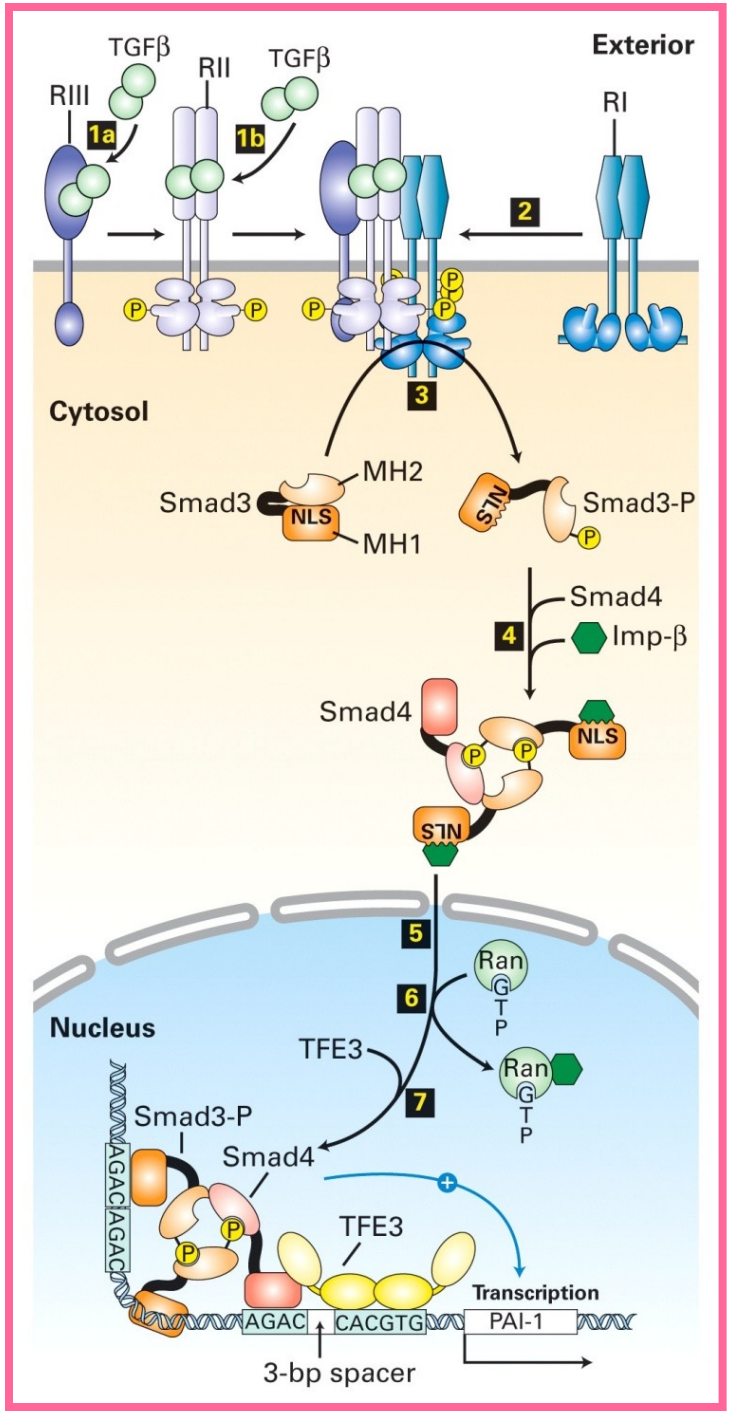


1: **Effector SMADs** (also called the Receptor-SMADs) are ser-phosphorylated by the activated receptor. Smad1, 5, 8, 9 are phosphorylated in response to bone morphogenetic morphogenetic protein (BMP) and growth and differentiation factor (GDF); Smad2 and 3 are phosphorylated in response to the activin/nodal branch of the TGF-β pathway.

2: **Regulatory or co-SMADs** (common SMADs). They are Smad4 and Smad4β.

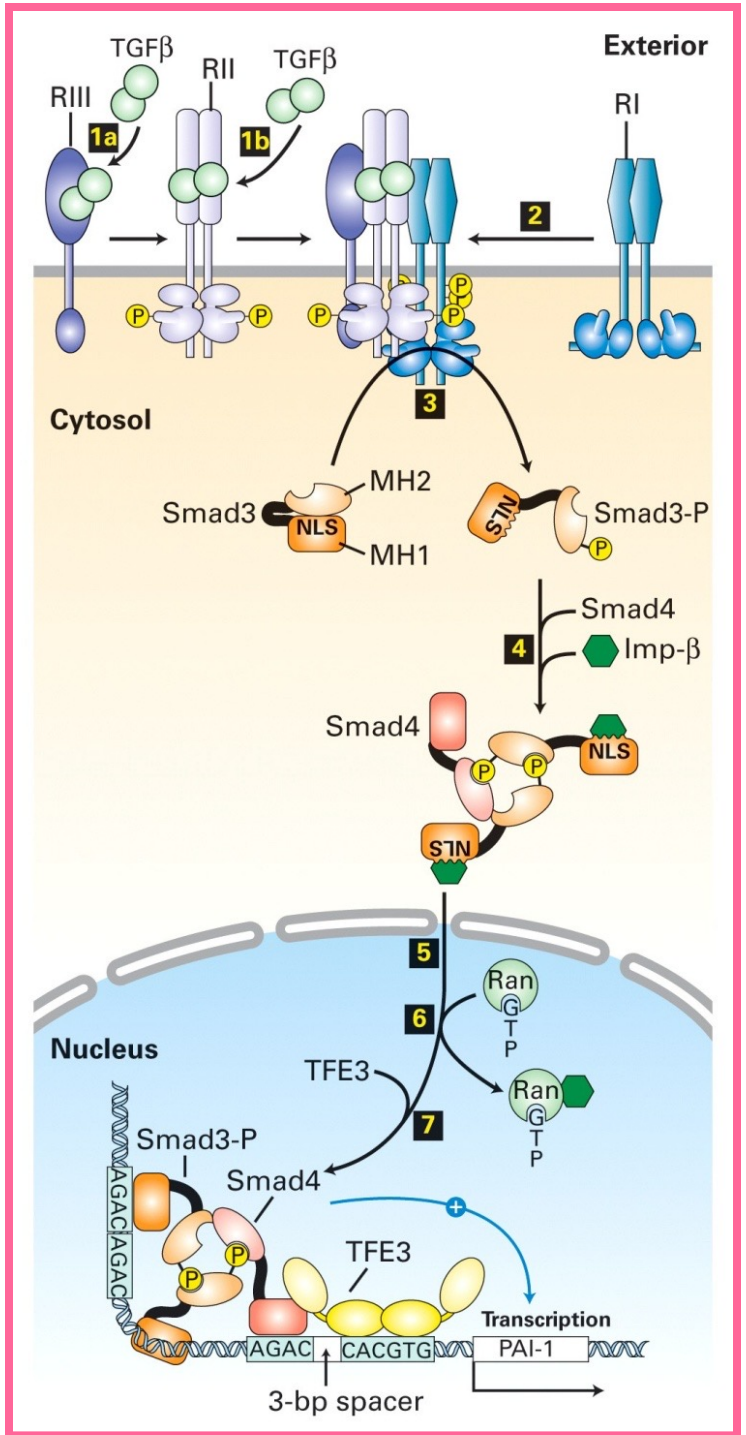
The regulatory Smad4 binds to all effector SMADs in the formation of transcriptional complexes, but it does not appear to be required for nuclear translocation of the effector molecules.

3: Two **inhibitory SMADs**, Smad6 and Smad7, provide negative regulation of the pathway by blocking Smad4 binding.



Activated Type I TGFβ Receptors Phosphorylate Smad Transcription Factors

- R-Smads contain two domains, MH1 and MH2, separated by a flexible linker region. The N-terminus of the MH1 contains a specific DNA binding segment and a NLS sequence
- When R-Smads are in inactive state, the NLS is masked and the MH1 and MH2 domains associate in a way that they can not bind to DNA or to a co-Smad
- Phosphorylation of three serine residues near the C-terminus of a R-Smad (Smad2 or Smad3) by activated type I TGFβ receptors separates the domains, allowing binding of importin β to the NLS



A complex containing two molecules of Smad3 (or Smad2) and one molecule of a co-Smad (Smad4) forms in the cytosol

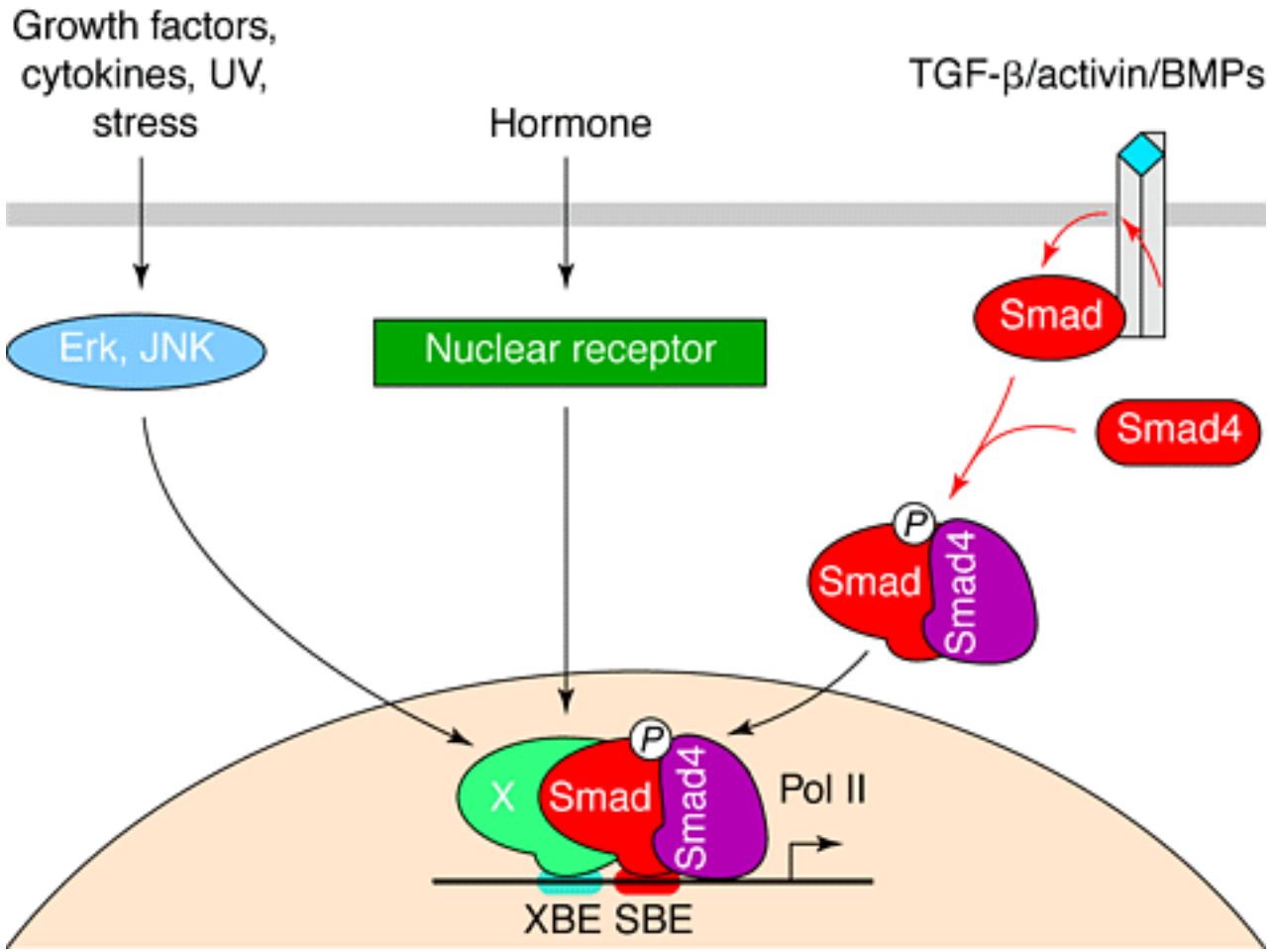
The complex is stabilized by binding two phosphorylated serines in both the Smad3 and the Smad4 MH2 domains

The importin β–bound heteromeric R-Smad3/Smad4 complex translocates into nucleus

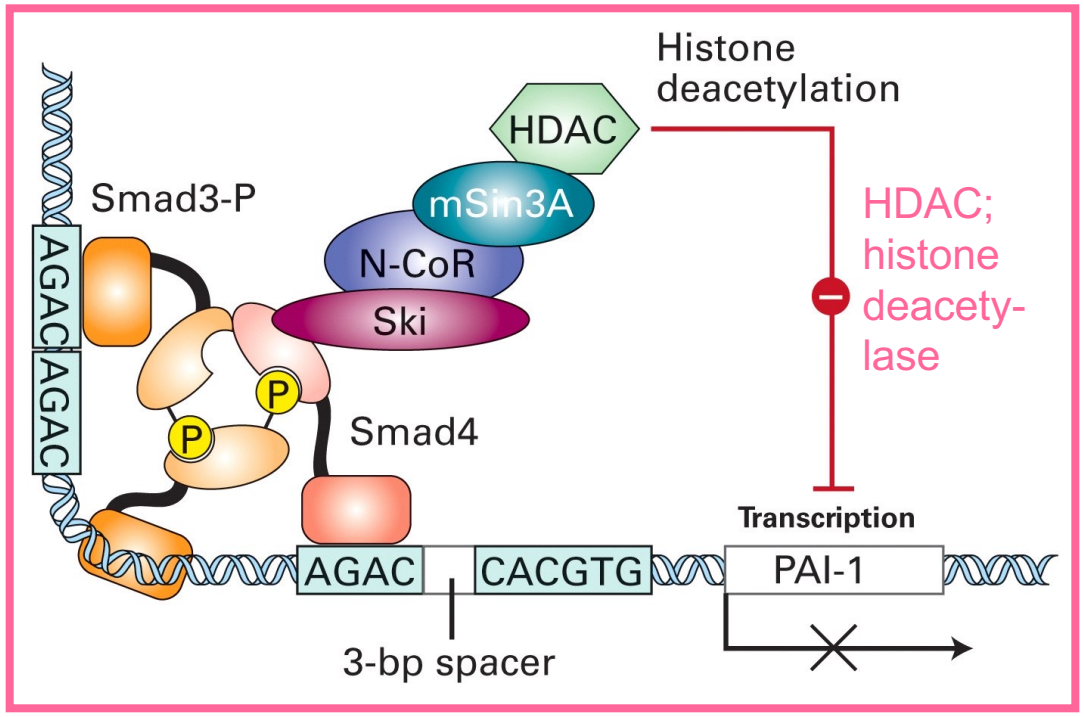
After importin β dissociates from the complex in the nucleus, the Smad2 (or 3)/Smad4 will cooperate with other transcription factors to turn on specific target genes

In the nucleus, R-Smads are continuously dephosphorylated, resulting in the dissociation of the R-Smad /co-Smad complex and export of these Smads from the nucleus. Therefore, the concentration of the active Smads in the nucleus closely reflects the levels of the activated TGFβ receptors on the cell surface

The Smad-factors activate their target genes in combination with other TFs



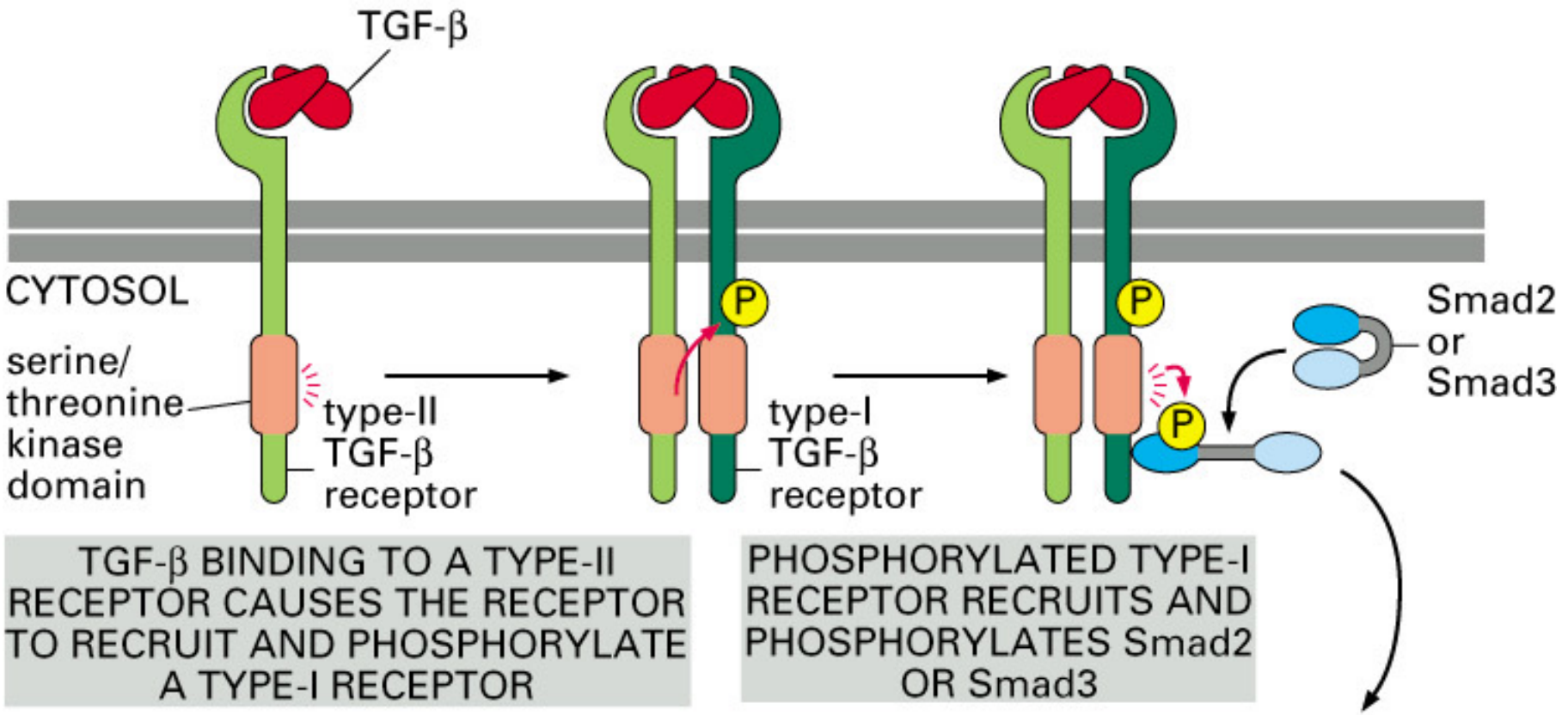
Oncoproteins and I-Smads Regulate Smad Signaling via Negative Feedback Loop



- Smad signaling is regulated by additional intracellular proteins including *SnoN and Ski (Ski stands for "Sloan-Kettering Cancer Institute")*
- These proteins are oncoproteins since they cause abnormal cell proliferation when over expressed in cultured fibroblasts

- SnoN and Ski can bind to Smad2/Smad4 or Smad3/Smad4 complex after TGFβ stimulation
- Binding of SnoN and Ski to Smad2/Smad4 or Smad3/Smad4 will block transcriptional activation of target genes, making cells resistant to growth inhibition induced by TGFβ
- PAI-1 gene: encodes plasminogen activator inhibitor-1

Summary


TGF-β
CYTOSOL
serine/
threonine
kinase
domain
type-II
TGF-β
receptor
P
type-I
TGF-β
receptor
P
P
Smad2
or
Smad3
TGF-β BINDING TO A TYPE-II RECEPTOR CAUSES THE RECEPTOR TO RECRUIT AND PHOSPHORYLATE A TYPE-I RECEPTOR
PHOSPHORYLATED TYPE-I RECEPTOR RECRUITS AND PHOSPHORYLATES Smad2 OR Smad3

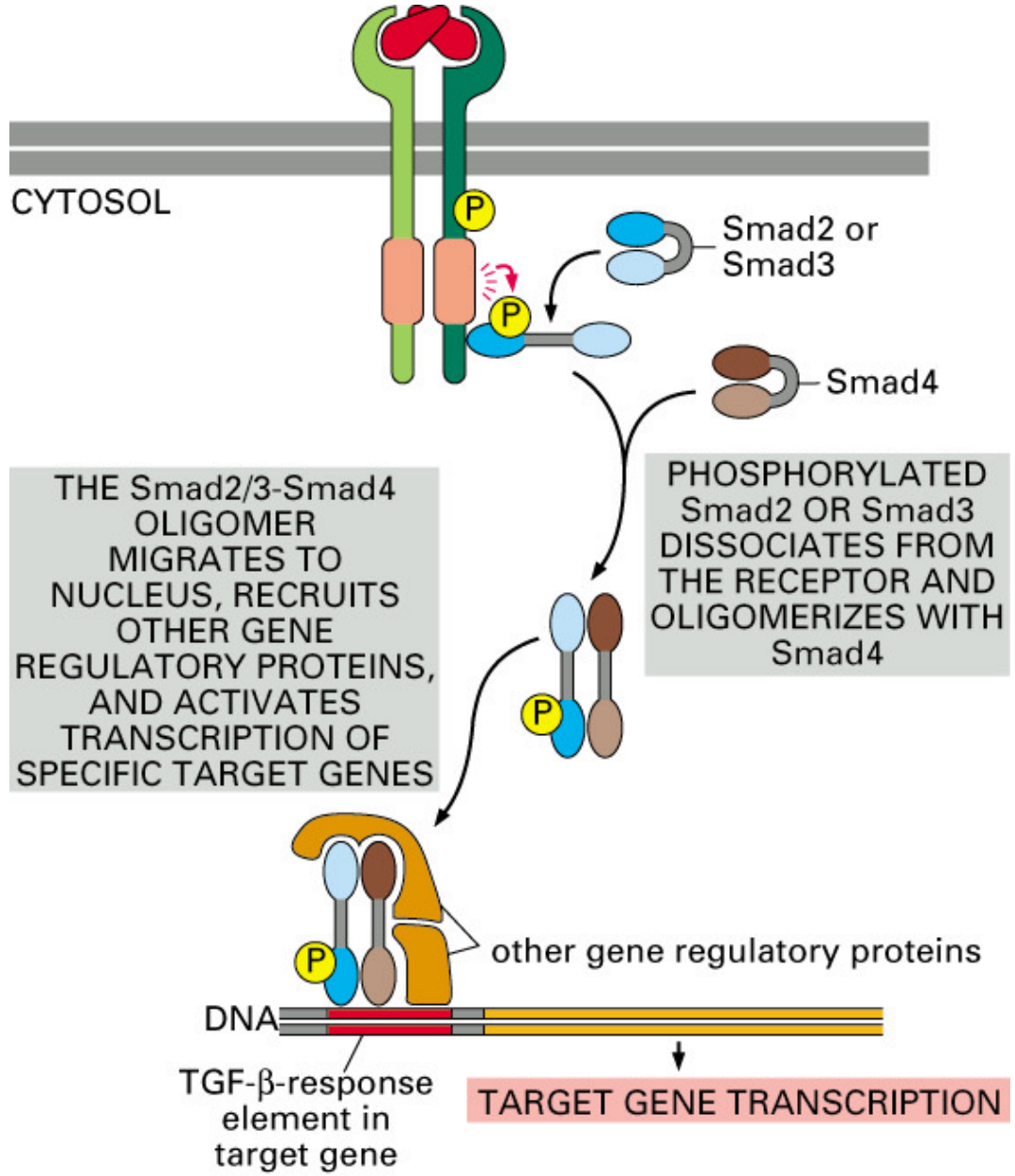
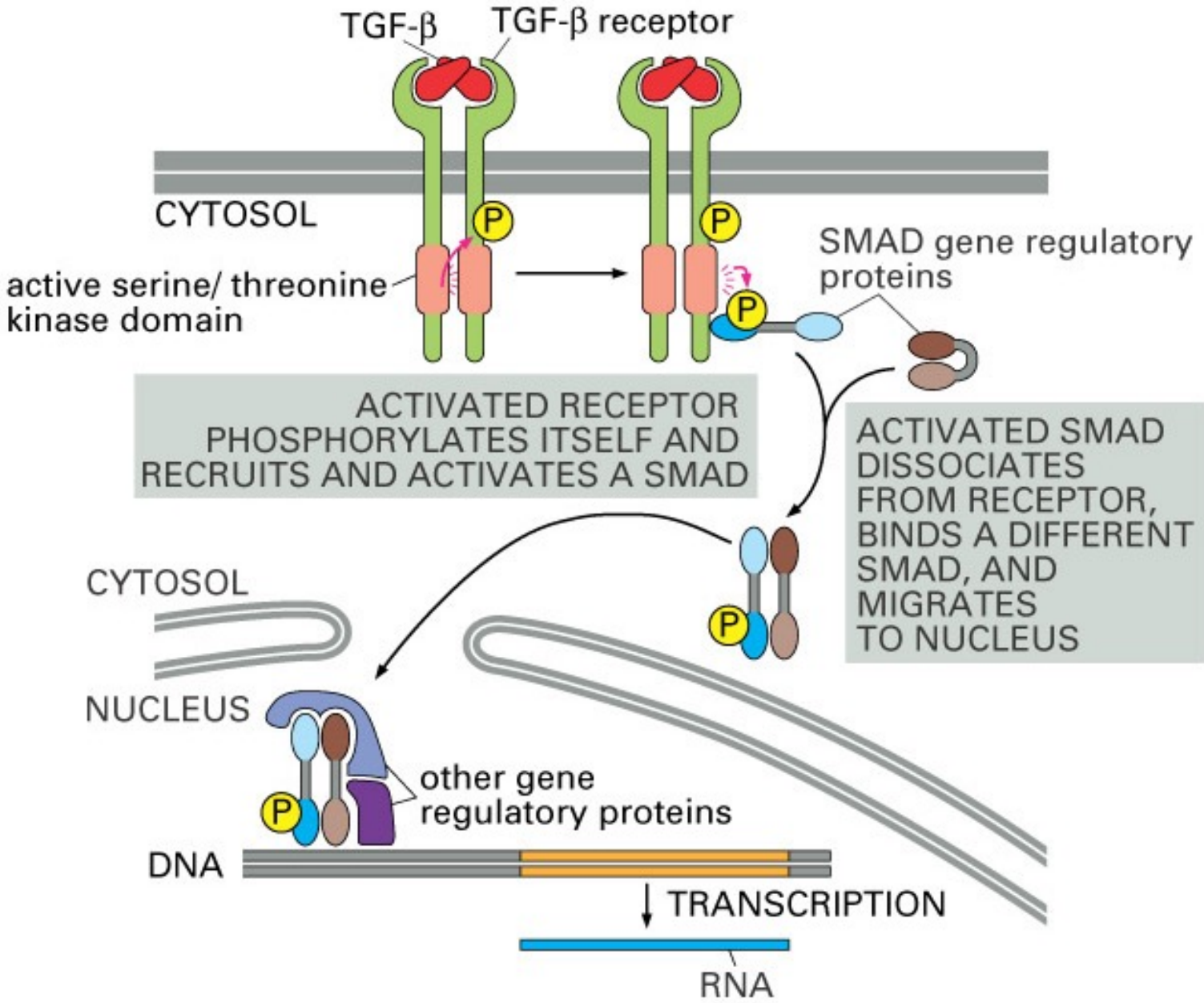


Figure 15–65 part 2 of 2. Molecular Biology of the Cell, 4th Edition.


TGF-β
TGF-β receptor
CYTOSOL
active serine/ threonine kinase domain
P
SMAD gene regulatory proteins
ACTIVATED RECEPTOR PHOSPHORYLATES ITSELF AND RECRUITS AND ACTIVATES A SMAD
ACTIVATED SMAD DISSOCIATES FROM RECEPTOR, BINDS A DIFFERENT SMAD, AND MIGRATES TO NUCLEUS
CYTOSOL
NUCLEUS
other gene regulatory proteins
DNA
TRANSCRIPTION
RNA