Corso di
PRINCIPI E TECNICHE DI RIGENERAZIONE CELLULARE

Modulo di Biologia Molecolare

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Struttura del corso

Parte propedeutica

Basi della segnalazione inter-intra cellulare.

Parte "core":

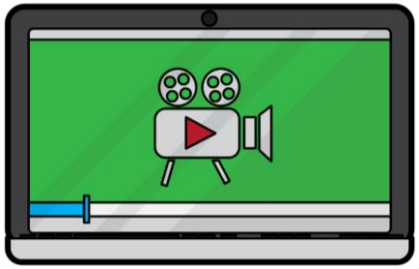
Il processo di "Rigenerazione tissutale" come modello di integrazione e crosstalk delle diverse vie di segnalazione in organismi modello.
"Golden standards": rigenerazione degli arti, rigenerazione epatica, rigenerazione intestinale, rigenerazione cardiaca, rigenerazione polmonare
Applicazione traslazionale, tramite vettori virali, lipidici e bionanovettori.
Modelli cellulari avanzati per lo studio in vitro

Parte "applicativa" (Prof. Sorrentino):

Morfologia/istologia di organi eletti a modello e i principi alla base dell'ingegneria tissutale e della medicina rigenerativa: analisi critica della letteratura scientifica piu' recente sull'argomento.

Come?

✓Lezioni Frontali



✓Video

Article

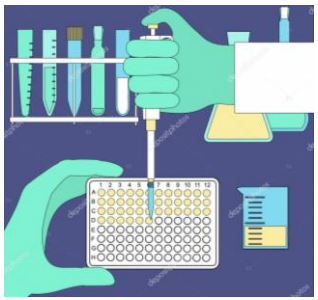
The oldest gnathostome teeth

https://doi.org/10.1038/s41586-022-05166-2
Received: 24 April 2020
Accepted: 29 July 2022
Published online: 28 September 2022
Check for updates

Plamen S. Andreev[1,2,7], Ivan J. Sansom[3,7], Qiang Li[1,2,7], Wenjin Zhao[2,4,5], Jianhua Wang[1], Chun-Chieh Wang[6], Lijian Peng[1], Liantao Jia[2], Tuo Qiao[2,4] & Min Zhu[2,4,5]

Mandibular teeth and dentitions are features of jawed vertebrates that were first acquired by the Palaeozoic ancestors[1–3] of living chondrichthyans and osteichthyans. The fossil record currently points to the latter part of the Siluri

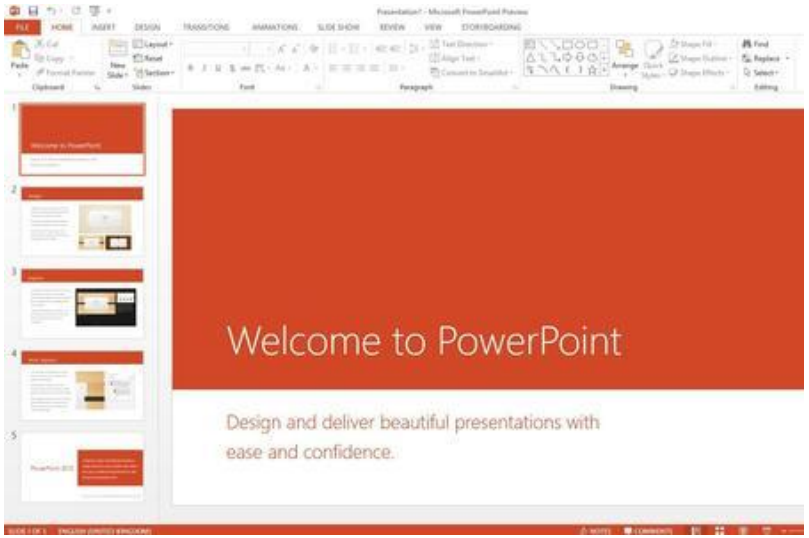
✓Commenti di Articoli



✓Schede tecniche

Moodle+MsTeams

- ✓ Presentazioni PPT (PDF) di ogni lezione



- ✓ Video-lezione registrata in audio e video

Testi consigliati

- ✓ B. Alberts et al – Molecular Biology of the Cell– Garland Publishing

REVIEW

nature biotechnology

CRISPR-Cas systems for editing, regulating and targeting genomes

Jeffry D Sander[1,2] & J Keith Joung[1,2]

NEWS & VIEWS

CANCER

T cells home in on brain tumours

Immunotherapies activate T cells to destroy tumours, but the approach has failed in some brain cancers. A strategy to improve migration of T cells across the blood–brain barrier could overcome this limitation.

Science AAAS

Cell Signaling in Space and Time: Where Proteins Come Together and When They're Apart
John D. Scott and Tony Pawson
Science **326**, 1220 (2009);
DOI: 10.1126/science.1175668

- ✓ Per le lezioni che tratteranno argomenti particolarmente innovativi e non sufficientemente descritti nei libri di testo, sara' fornito agli studenti opportuno materiale didattico, caricato sul Moodle del Corso.

Esame

✓ Colloquio orale


articles

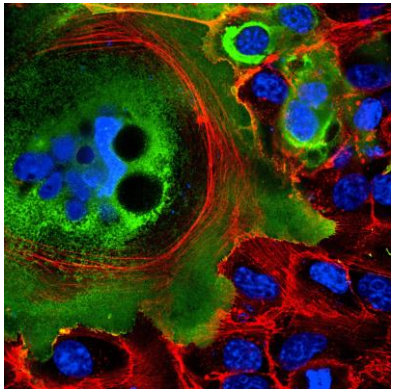
Structure of importin-β bound to the IBB domain of importin-α

news feature

Reservoirs dog AIDS therapy

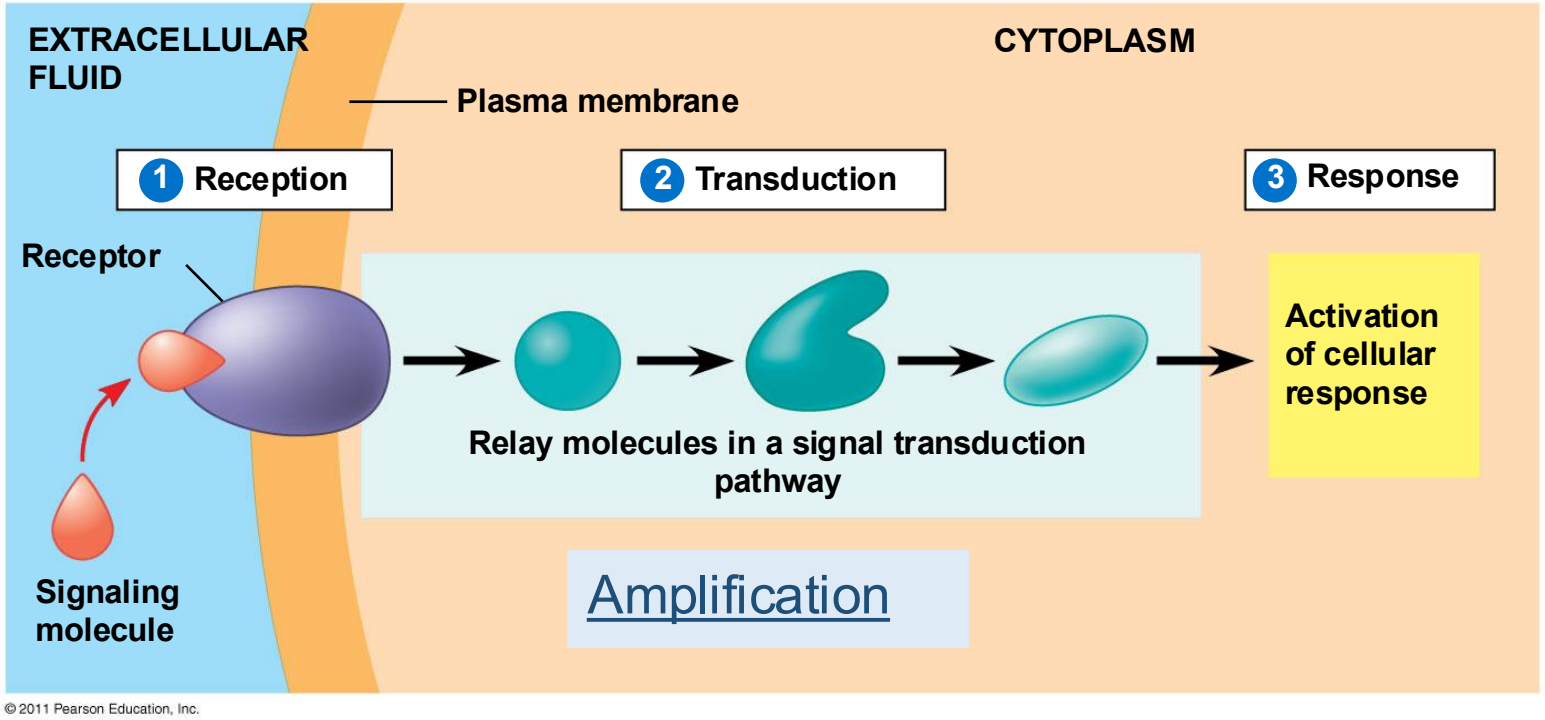
Pools of latent HIV, lurking in the cells of infected people, remain u
even by powerful drug combinations. Paul Smaglik reports on how
forcing researchers to rethink their strategies for fighting the virus.

✓ Commento e/o interpretazione articoli scientifici



✓ Interpretazione di dati sperimentali

The basic mechanism of *Intra*-cellular signal transduction



Cells have many receptor proteins on their plasma membrane surface, and molecules that bind specifically to these receptors are called signaling molecules. When bound with signaling molecules, receptors go through structural changes and are activated, which then changes the shape, movement and functions of the cell by activating intracellular signal transduction proteins, and regulates gene expression through the relocation of intracellular signaling molecules to the nucleus.

nature REVIEWS MOLECULAR CELL BIOLOGY

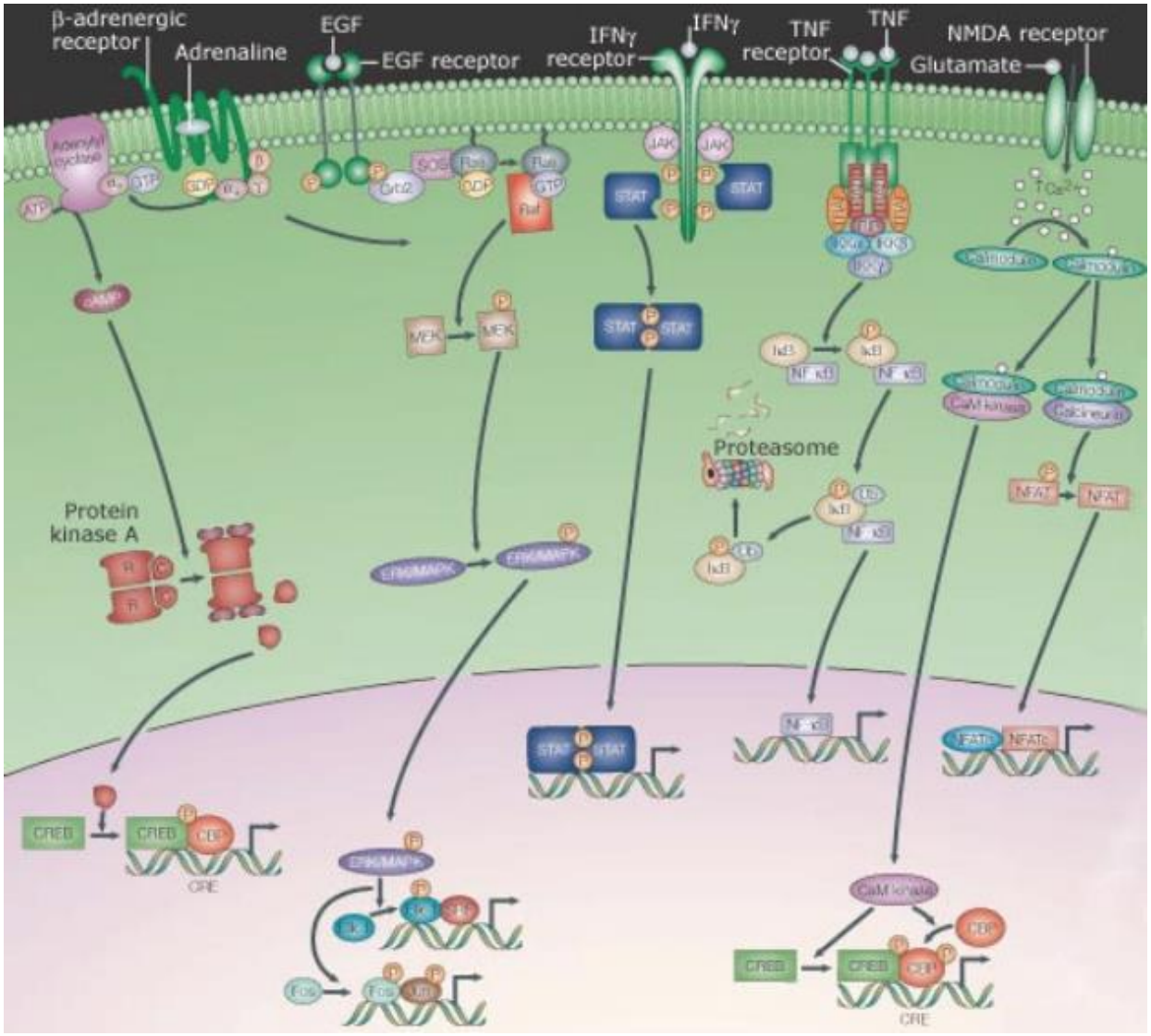
Signalling from the membrane to the nucleus

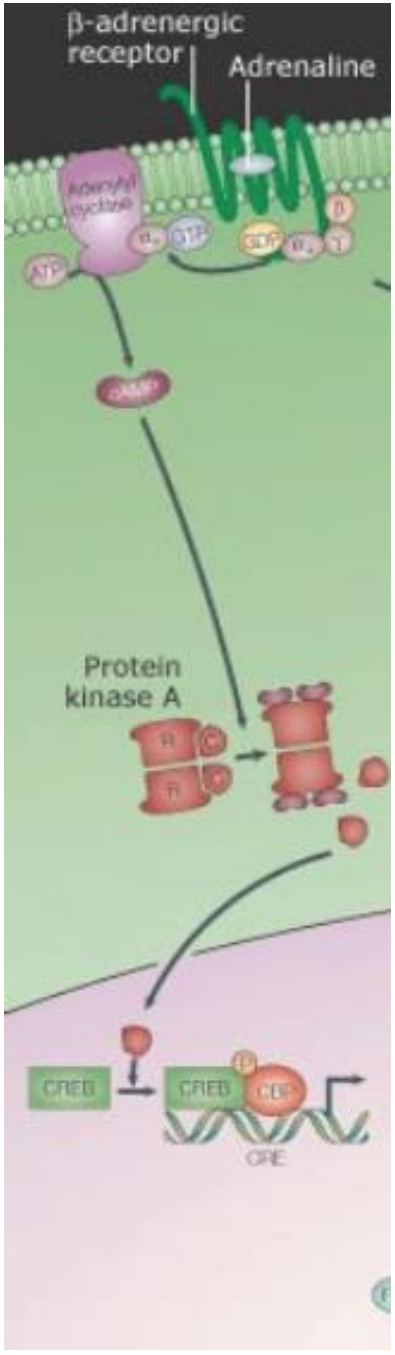
Signalling from cell-surface receptors through relay systems

Receptors that after engaging their extracellular ligand undergo a conformational change, which induces them to oligomerize.

This results in the production of second messengers (ions, lipids) or post-translational modification (phosphorylation, proteolysis) of cytoplasmic proteins.

A series of protein-protein interactions then relay the signal to the nucleus, whereupon the activity of transcription factors is altered.

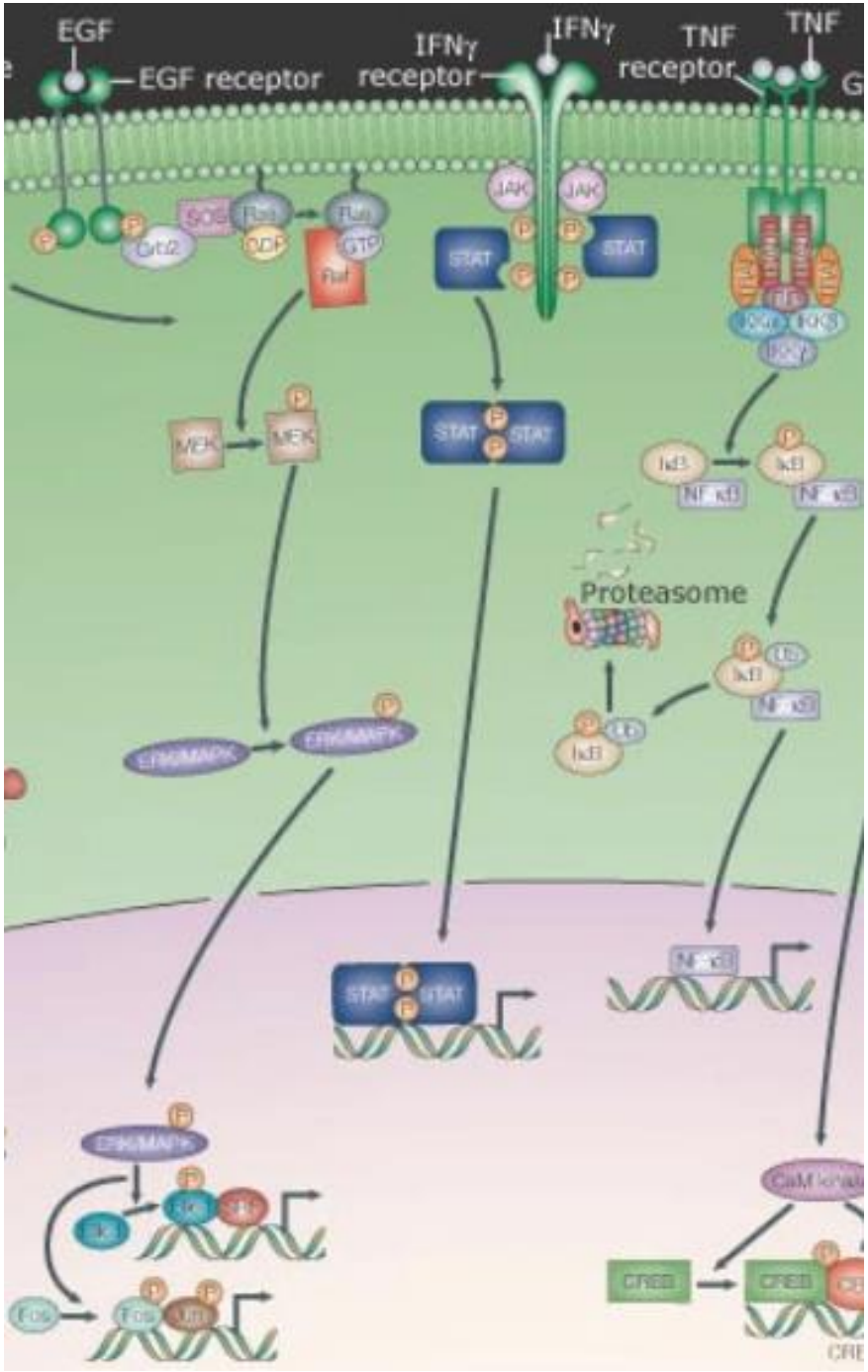




Signalling from the membrane to the nucleus
Signalling from cell-surface receptors through relay systems

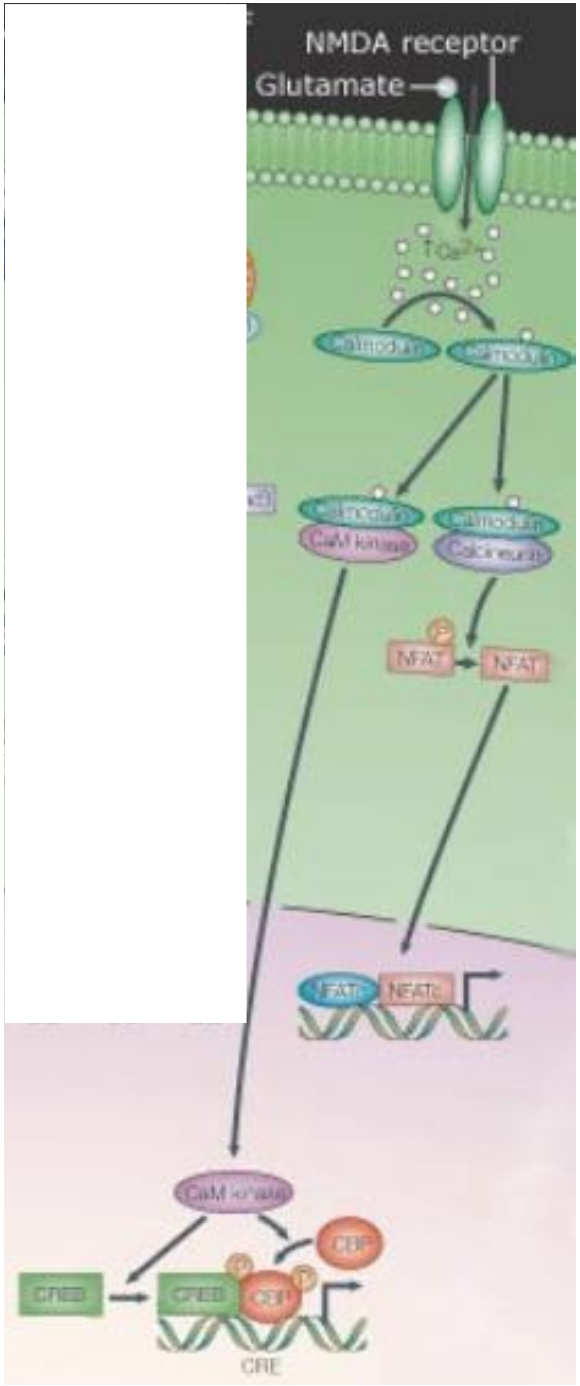
1- Seven-transmembrane receptors

Ligand binding to the extracellular domain of 7TM receptors induces a conformational change, which causes a heterotrimeric G-protein that is associated with the receptor to dissociate into an α monomer and a $\beta\gamma$ dimer. The monomer and the dimer can both induce downstream signalling events.



2- Enzyme-linked cell-surface receptors

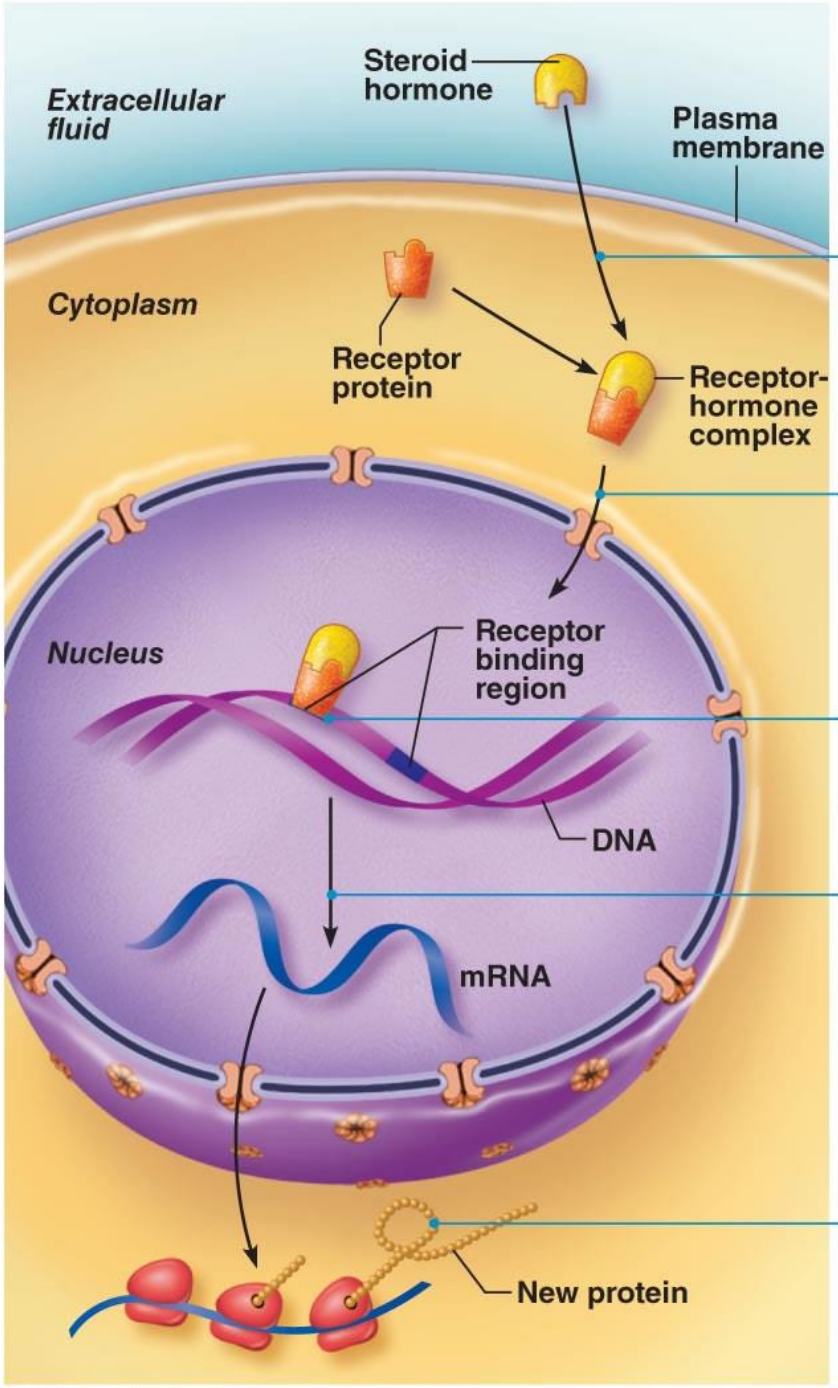
These receptors either have intrinsic enzymatic activity, or they can recruit proteins having catalytic function. On binding their ligands, the receptors undergo a conformational change that activates or represses their intrinsic activity, or the activity of the proteins that they recruit.



3- Ligand-gated ion channels

These receptors function as channels that open or close in response to ligand binding.
When they are open - or in the active state - they allow the passage of selected ions through the pore. This is a transient process, as the channels become desensitized.

4- Intracellular receptors



① The steroid hormone diffuses through the plasma membrane and binds an intracellular receptor.

② The receptor-hormone complex enters the nucleus.

③ The receptor-hormone complex binds a specific DNA region.

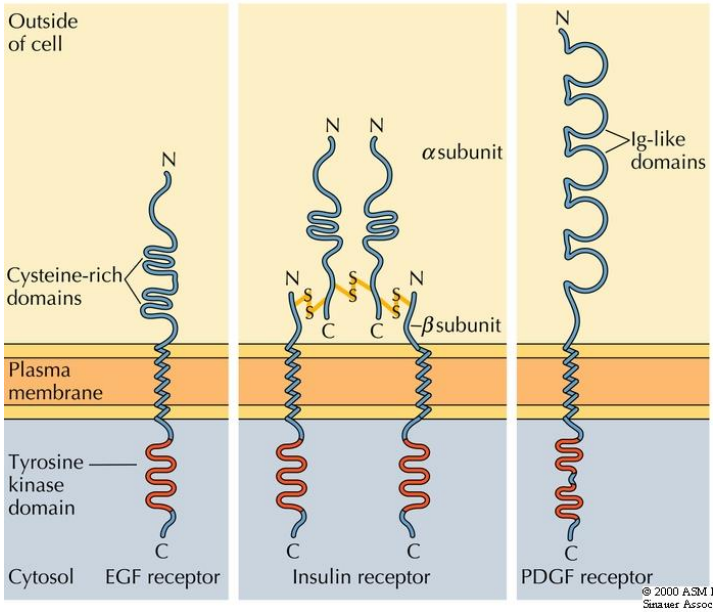
④ Binding initiates transcription of the gene to mRNA.

⑤ The mRNA directs protein synthesis.

Enzyme-linked receptors fall into 3 categories:

1- Tyrosine Kinase Receptors

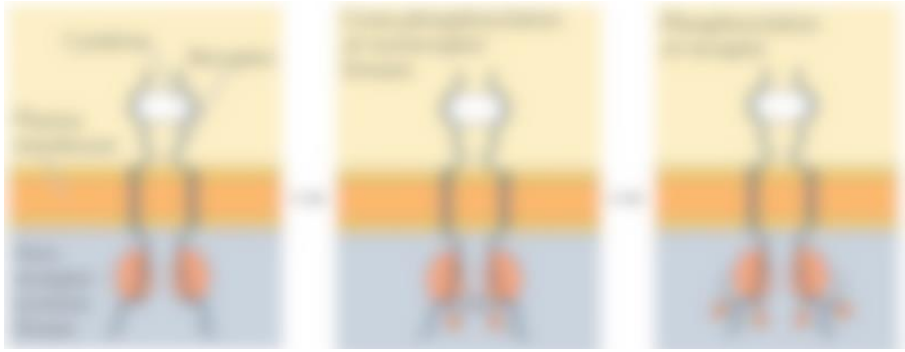
- Not only a receptor
- Also an enzyme: Tyrosine kinase



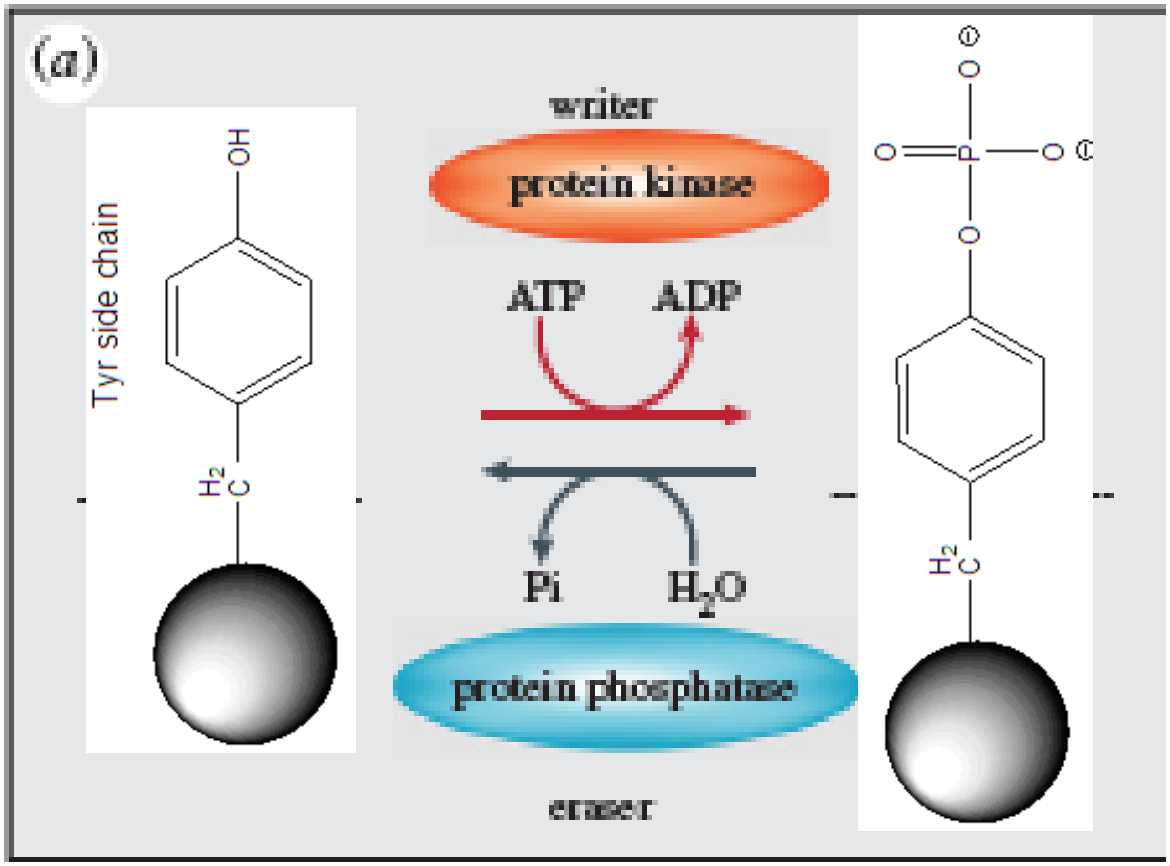
2- TGF-β receptors

3- Cytokine superfamily receptors

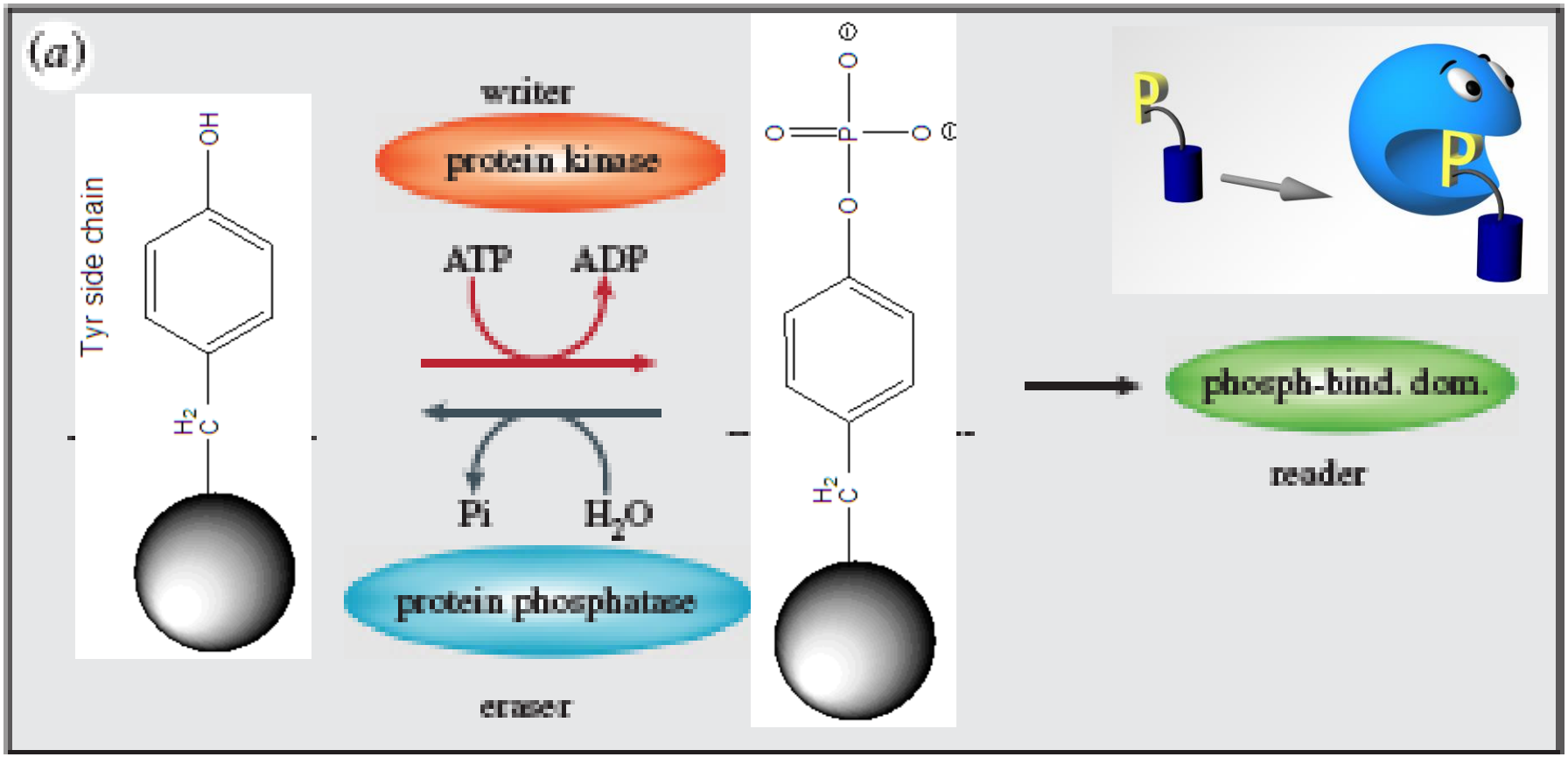
- No catalytic domain
- Interact with non receptor protein-tyrosine kinases
  - Src family
  - JAK family



Key concepts



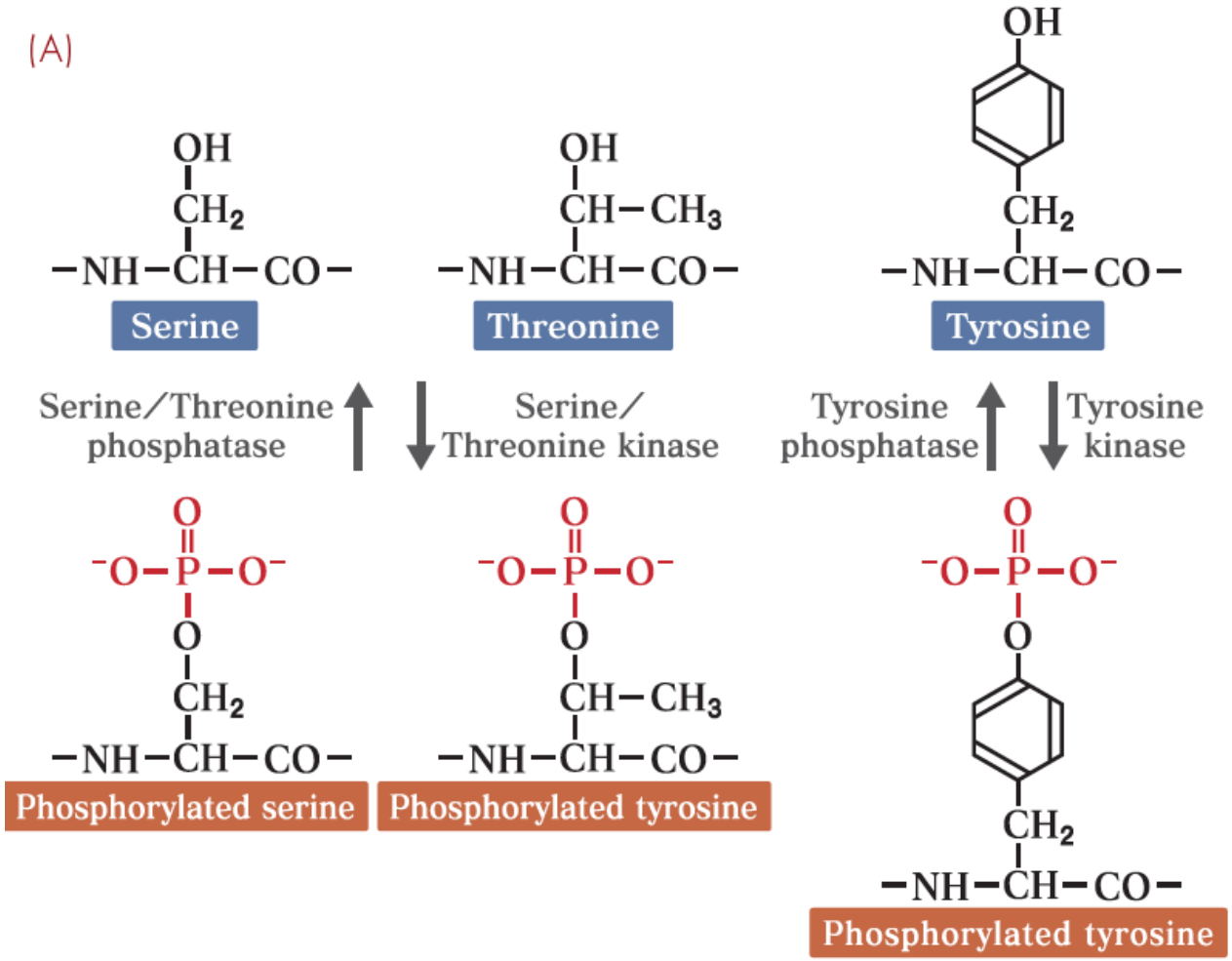
Key concepts



Phosphorylation and Dephosphorylation of Proteins

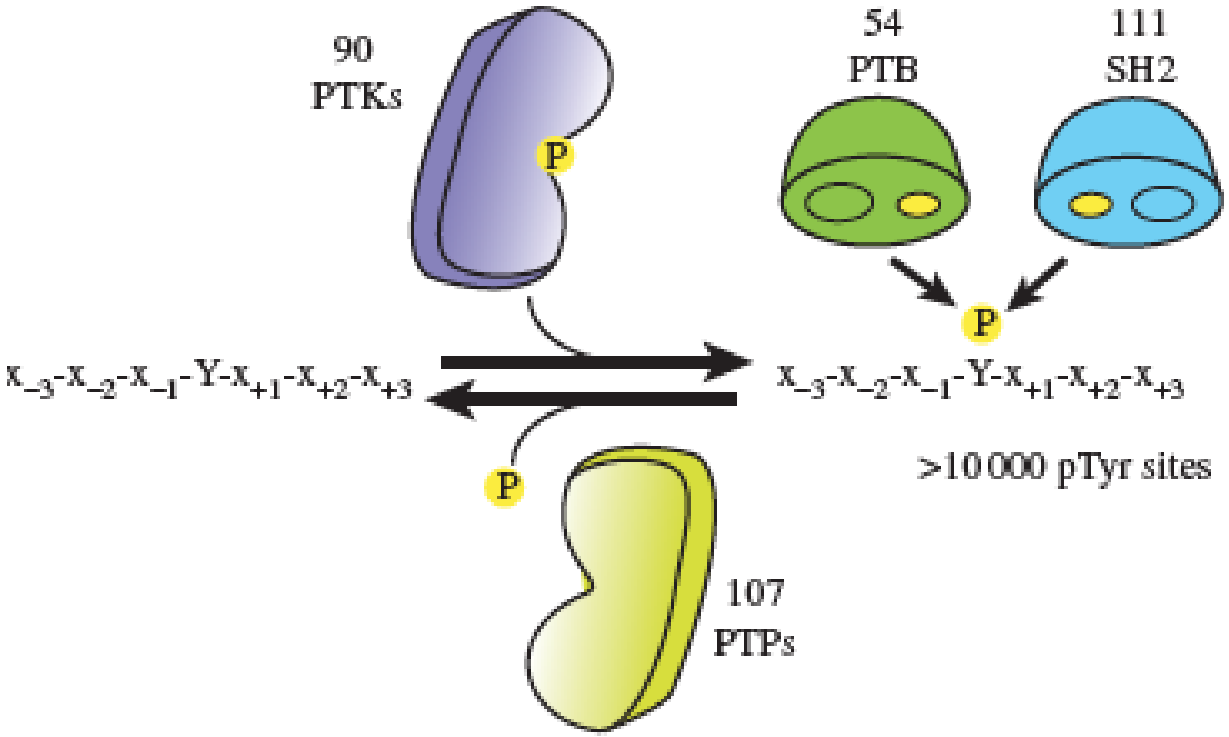
The most important chemical modification among the intracellular signal transduction mechanisms is the **phosphorylation** of the side chains of tyrosine, serine and threonine in proteins.

Phosphorylation is one of the most effective ways of **changing the structure of proteins** due to the large size and negative charge of the phosphate group; for the same reason, it is also effective as a **recognition marker** for other proteins.

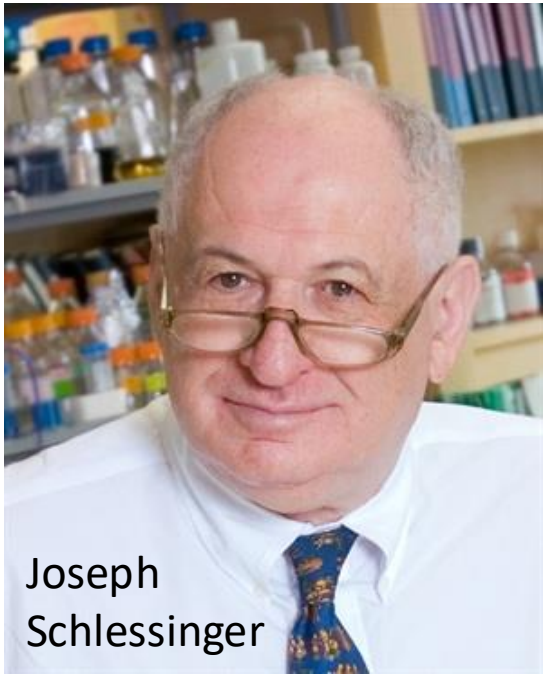


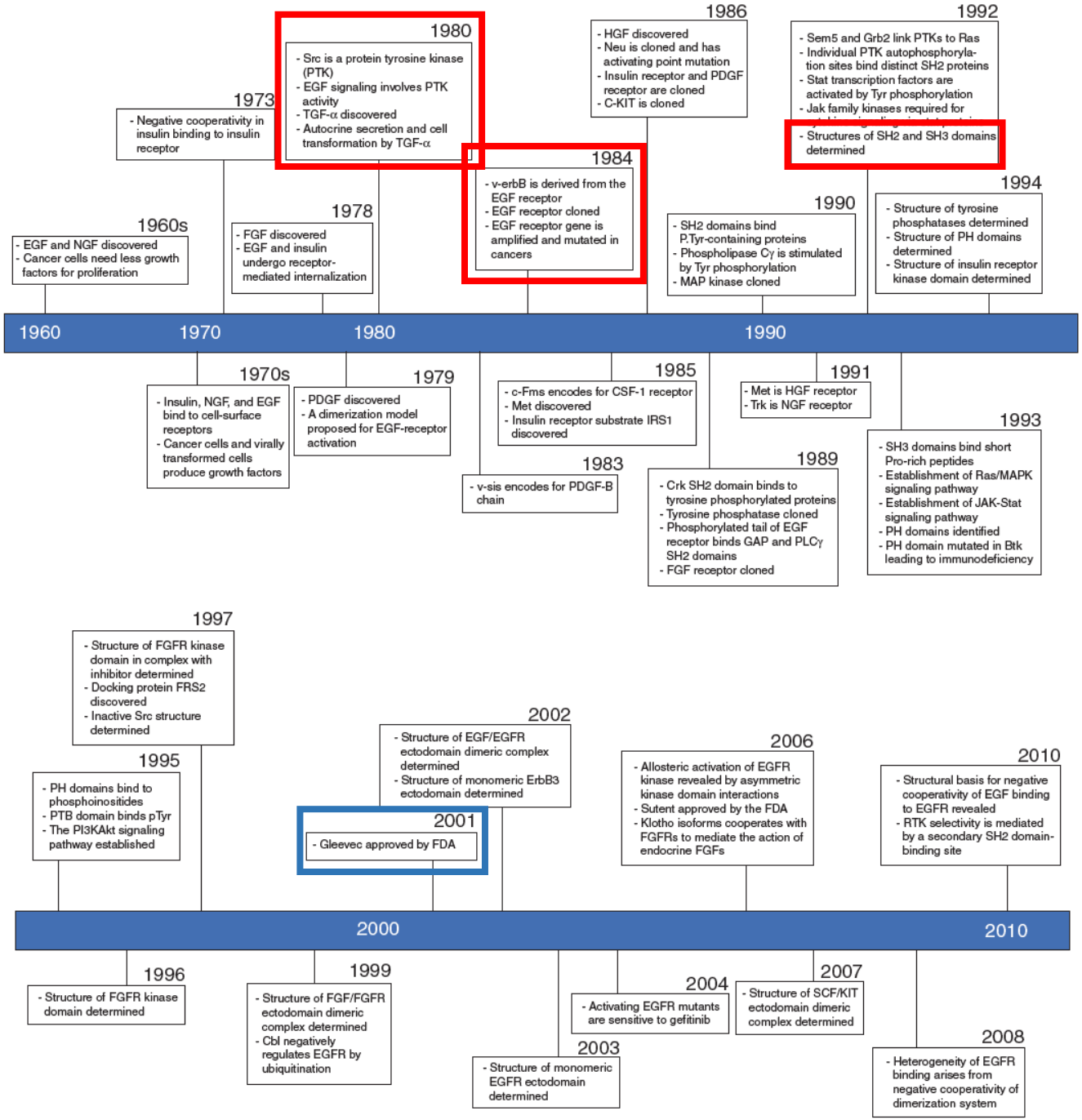
Key concepts

The eukaryotic phosphorylation-based network is operated by a modular kinase-phosphatase-interaction domain toolkit

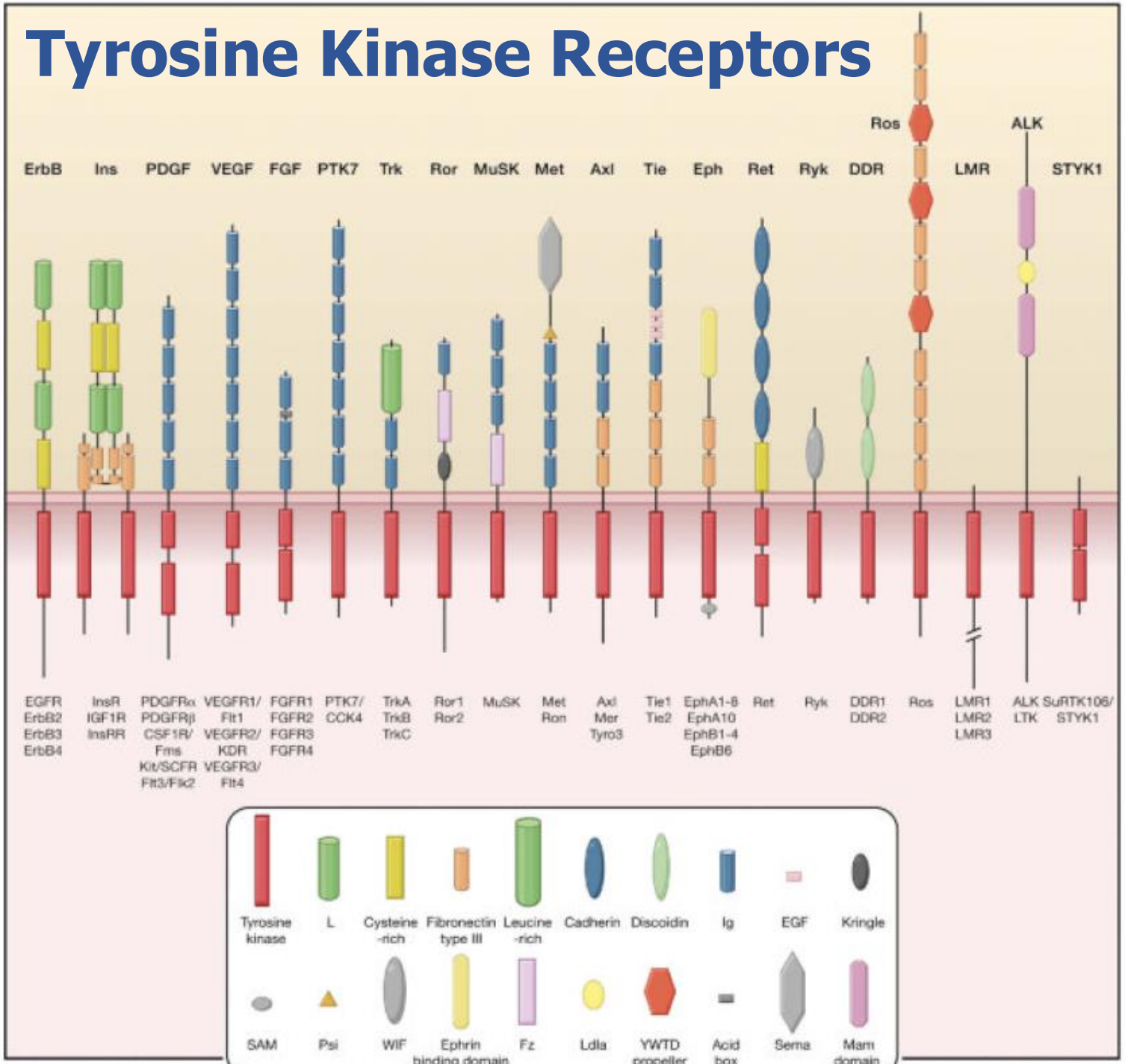


Key people




1960s
- EGF and NGF discovered
- Cancer cells need less growth factors for proliferation
1973
- Negative cooperativity in insulin binding to insulin receptor
1978
- FGF discovered
- EGF and insulin undergo receptor-mediated internalization
1980
- Src is a protein tyrosine kinase (PTK)
- EGF signaling involves PTK activity
- TGF-α discovered
- Autocrine secretion and cell transformation by TGF-α
1984
- v-erbB is derived from the EGF receptor
- EGF receptor cloned
- EGF receptor gene is amplified and mutated in cancers
1986
- HGF discovered
- Neu is cloned and has activating point mutation
- Insulin receptor and PDGF receptor are cloned
- C-KIT is cloned
1990
- SH2 domains bind P.Tyr-containing proteins
- Phospholipase Cγ is stimulated by Tyr phosphorylation
- MAP kinase cloned
1992
- Sem5 and Grb2 link PTKs to Ras
- Individual PTK autophosphorylation sites bind distinct SH2 proteins
- Stat transcription factors are activated by Tyr phosphorylation
- Jak family kinases required for
- Structures of SH2 and SH3 domains determined
1994
- Structure of tyrosine phosphatases determined
- Structure of PH domains determined
- Structure of insulin receptor kinase domain determined
1960
1970
1980
1990
1970s
- Insulin, NGF, and EGF bind to cell-surface receptors
- Cancer cells and virally transformed cells produce growth factors
1979
- PDGF discovered
- A dimerization model proposed for EGF-receptor activation
1983
- v-sis encodes for PDGF-B chain
1985
- c-Fms encodes for CSF-1 receptor
- Met discovered
- Insulin receptor substrate IRS1 discovered
1989
- Crk SH2 domain binds to tyrosine phosphorylated proteins
- Tyrosine phosphatase cloned
- Phosphorylated tail of EGF receptor binds GAP and PLCγ SH2 domains
- FGF receptor cloned
1991
- Met is HGF receptor
- Trk is NGF receptor
1993
- SH3 domains bind short Pro-rich peptides
- Establishment of Ras/MAPK signaling pathway
- Establishment of JAK-Stat signaling pathway
- PH domains identified
- PH domain mutated in Btk leading to immunodeficiency
1995
- PH domains bind to phosphoinositides
- PTB domain binds pTyr
- The PI3KAkt signaling pathway established
1997
- Structure of FGFR kinase domain in complex with inhibitor determined
- Docking protein FRS2 discovered
- Inactive Src structure determined
2001
- Gleevec approved by FDA
2002
- Structure of EGF/EGFR ectodomain dimeric complex determined
- Structure of monomeric ErbB3 ectodomain determined
2006
- Allosteric activation of EGFR kinase revealed by asymmetric kinase domain interactions
- Sutent approved by the FDA
- Klotho isoforms cooperates with FGFRs to mediate the action of endocrine FGFs
2010
- Structural basis for negative cooperativity of EGF binding to EGFR revealed
- RTK selectivity is mediated by a secondary SH2 domain-binding site
2000
2010
1996
- Structure of FGFR kinase domain determined
1999
- Structure of FGF/FGFR ectodomain dimeric complex determined
- Cbl negatively regulates EGFR by ubiquitination
2003
- Structure of monomeric EGFR ectodomain determined
2004
- Activating EGFR mutants are sensitive to gefitinib
2007
- Structure of SCF/KIT ectodomain dimeric complex determined
2008
- Heterogeneity of EGFR binding arises from negative cooperativity of dimerization system

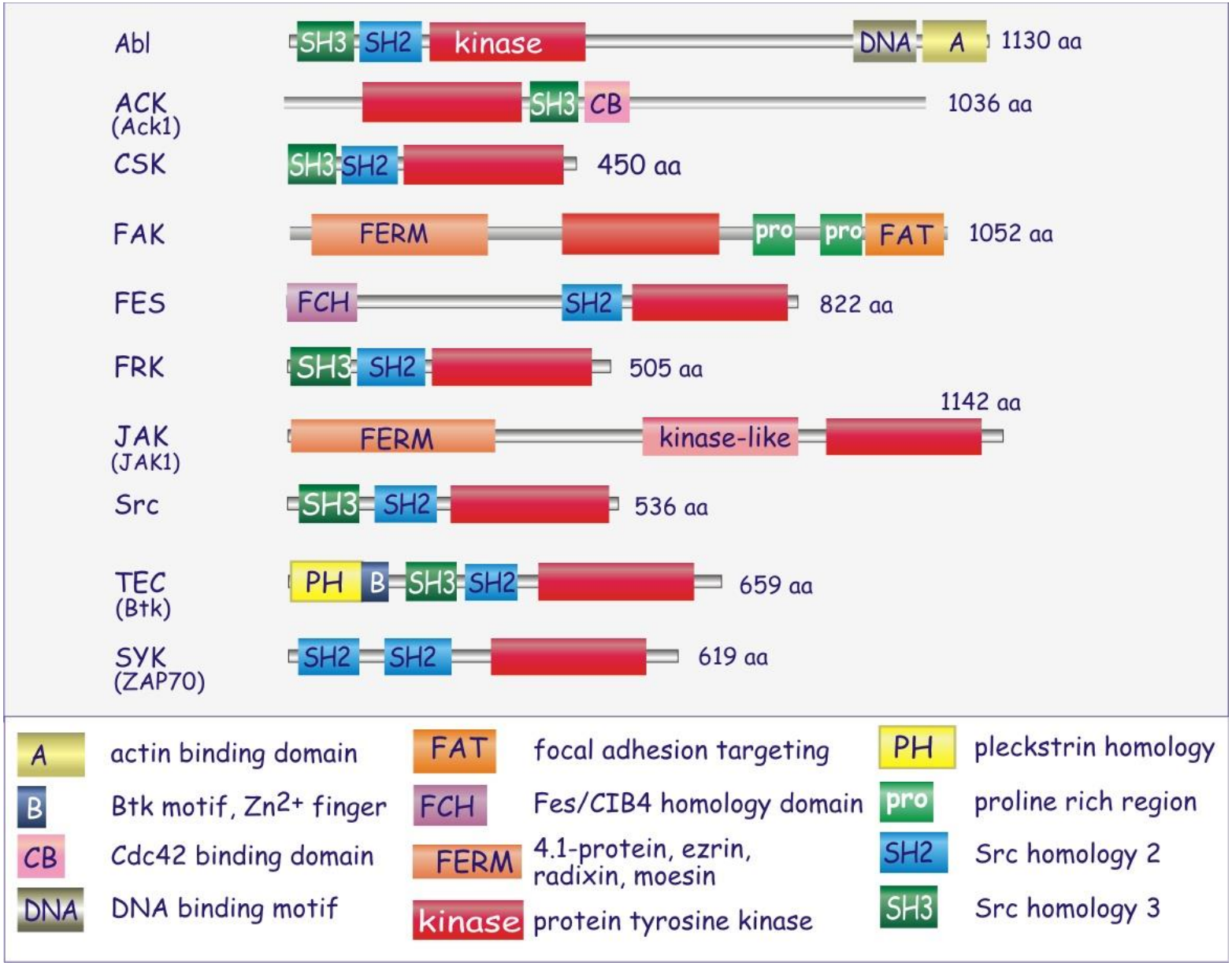
Tyrosine Kinase Receptors



Growth Factors

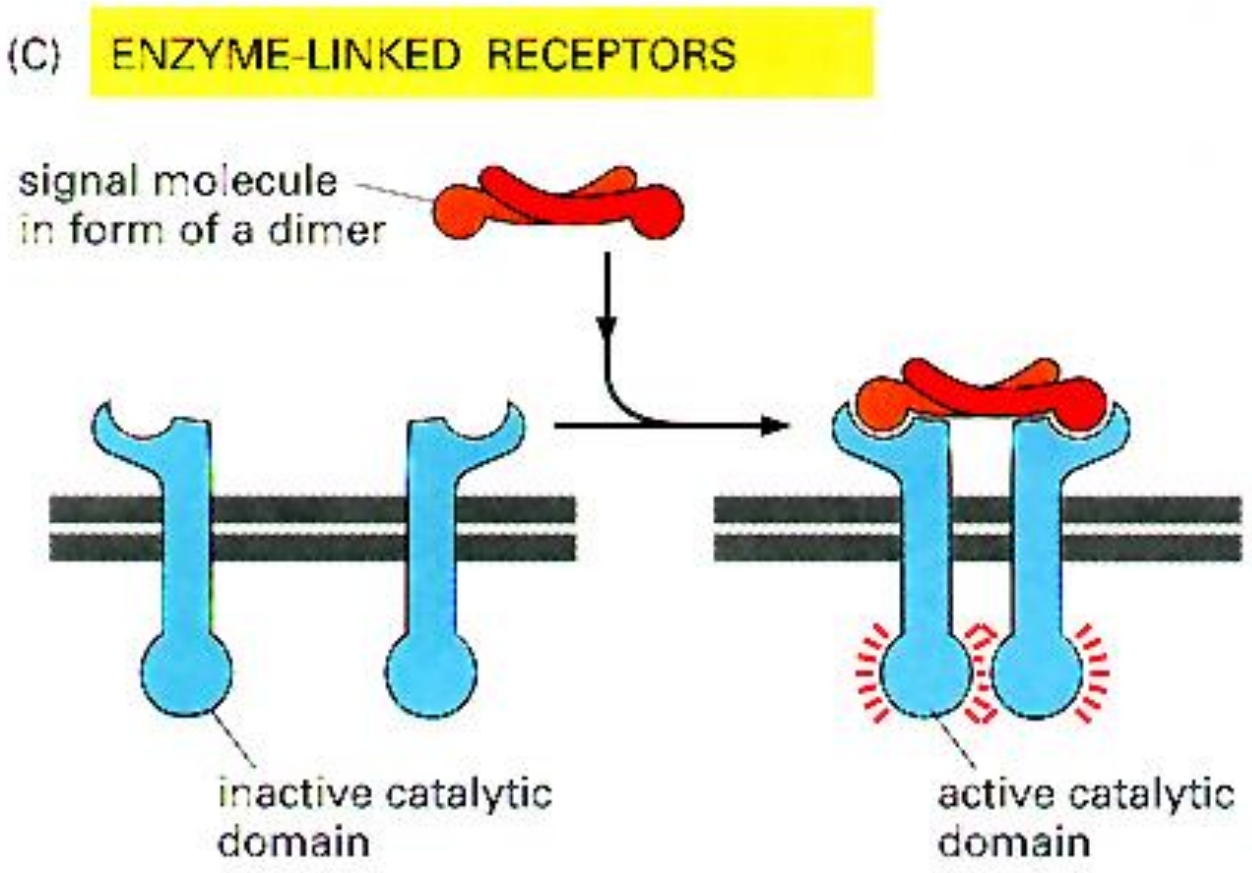
Factor	Principal Source	Primary Activity	Comments
PDGF	platelets, endothelial cells, placenta	promotes proliferation of connective tissue, glial and smooth muscle cells	two different protein chains form 3 distinct dimer forms; AA, AB and BB
EGF	submaxillary gland, Brunners gland	promotes proliferation of mesenchymal, glial and epithelial cells	
TGF-α	common in transformed cells	may be important for normal wound healing	related to EGF
FGF	wide range of cells; protein is associated with the ECM	promotes proliferation of many cells; inhibits some stem cells; induces mesoderm to form in early embryos	at least 19 family members, 4 distinct receptors
NGF		promotes neurite outgrowth and neural cell survival	several related proteins first identified as proto-oncogenes; trkA (***trackA***), trkB, trkC
Erythropoietin	kidney	promotes proliferation and differentiation of erythrocytes	
TGF-β	activated TH_1 cells (T-helper) and natural killer (NK) cells	anti-inflammatory (suppresses cytokine production and class II MHC expression), promotes wound healing, inhibits macrophage and lymphocyte proliferation	at least 100 different family members
IGF-I	primarily liver	promotes proliferation of many cell types	related to IGF-II and proinsulin, also called Somatomedin C
IGF-II	variety of cells	promotes proliferation of many cell types primarily of fetal origin	related to IGF-I and proinsulin

Non-receptor Tyrosine Kinases



Common activating mechanism:

The ligand induce a shape change in the receptor, activating its enzymatic activity in the intracellular portion of the molecule



Published December 1, 1986

1986

Mini-Review

Allosteric Regulation of the Epidermal Growth Factor Receptor Kinase

Joseph Schlessinger

Biotechnology Research Center, Meloy Laboratories, Rockville, Maryland 20850

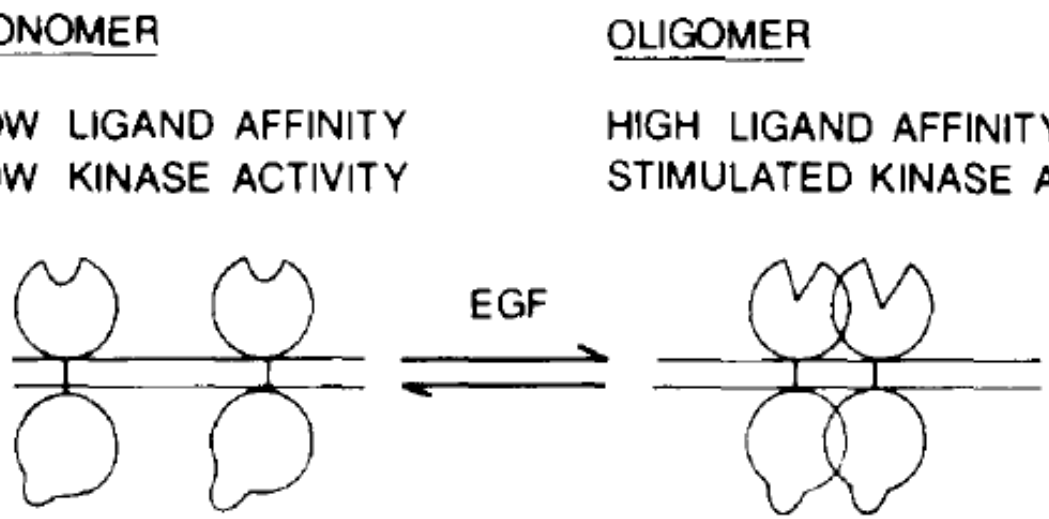
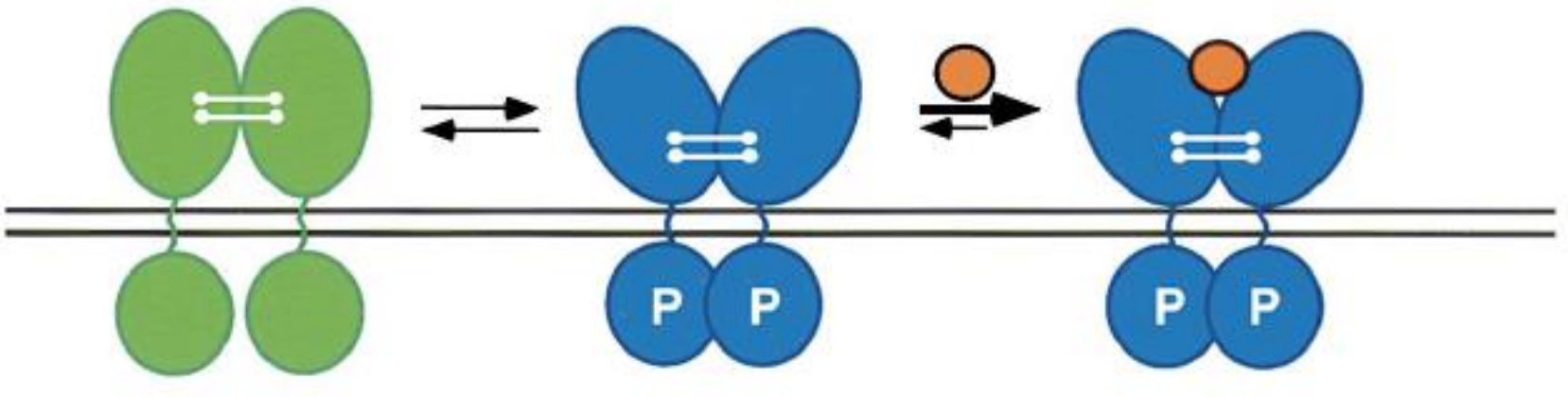


Figure 3. An allosteric oligomerization model for the activation o the EGF receptor kinase by EGF. EGF receptor is depicted as a biglobular transmembrane molecule as shown in Fig. 1. It is proposed that monomeric receptors exist in equilibrium with recepto oligomers. It is postulated that monomeric receptors possess low ligand affinity and reduced kinase activity and oligomeric receptors have high binding affinity and stimulated kinase activity. Hence EGF binding will drive the aggregation process and thus stimulate the protein tyrosine kinase activity.

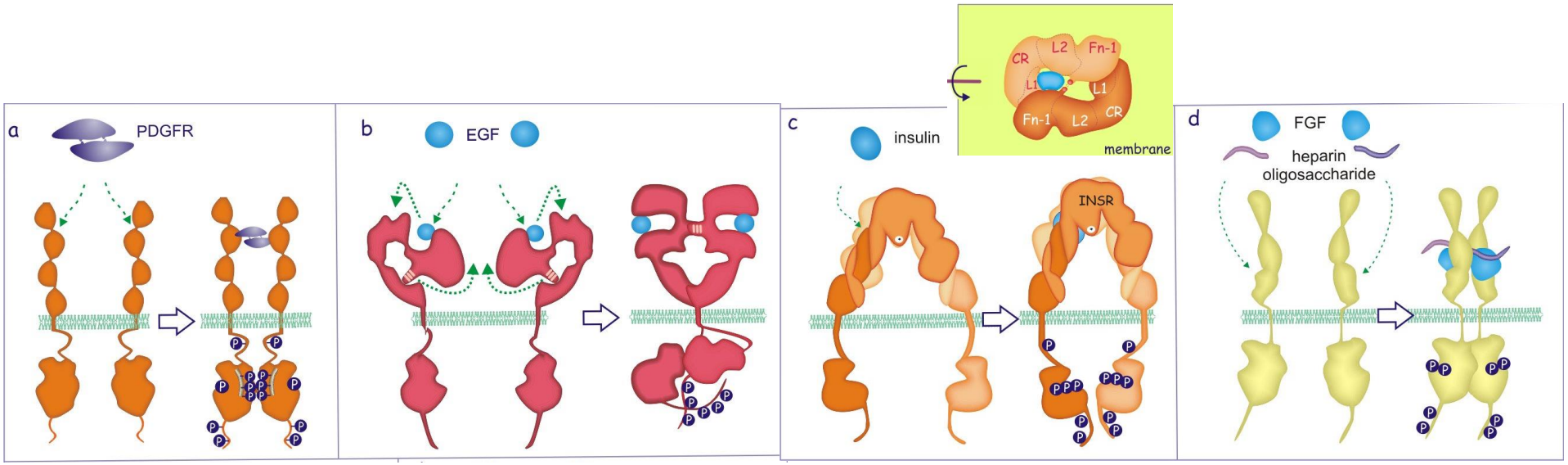
Ligand binding stabilizes the formation of activated receptors clusters

Inactive receptor monomers are in equilibrium with inactive or active receptor dimers. The active receptor dimers exist in a conformation compatible with trans-autophosphorylation. Ligand binding stabilizes active dimers formation and hence PTK activation.



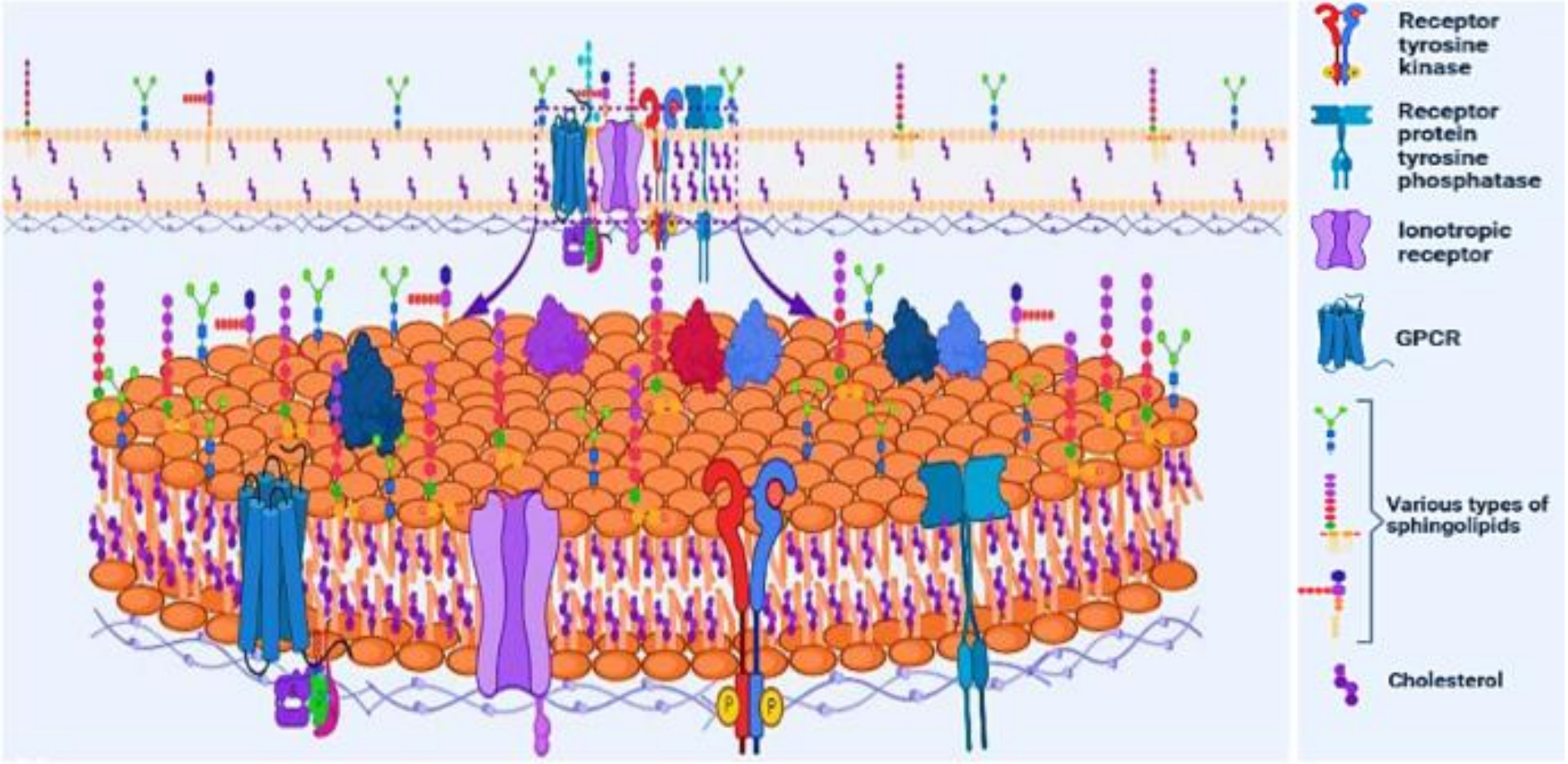
1. Inactive cluster
2. Active cluster
3. Ligand bound active cluster

RECEPTORS EMPLOY DIFFERENT CLUSTERIZATION STRATEGIES

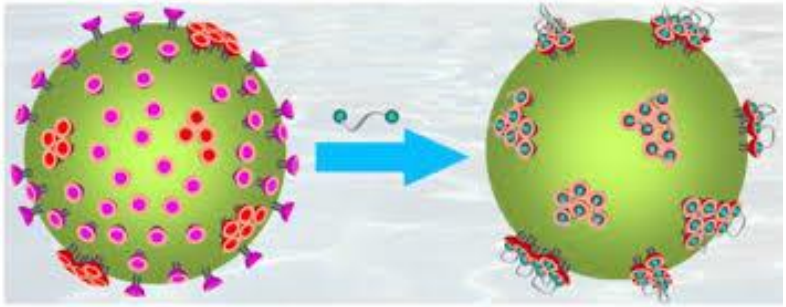


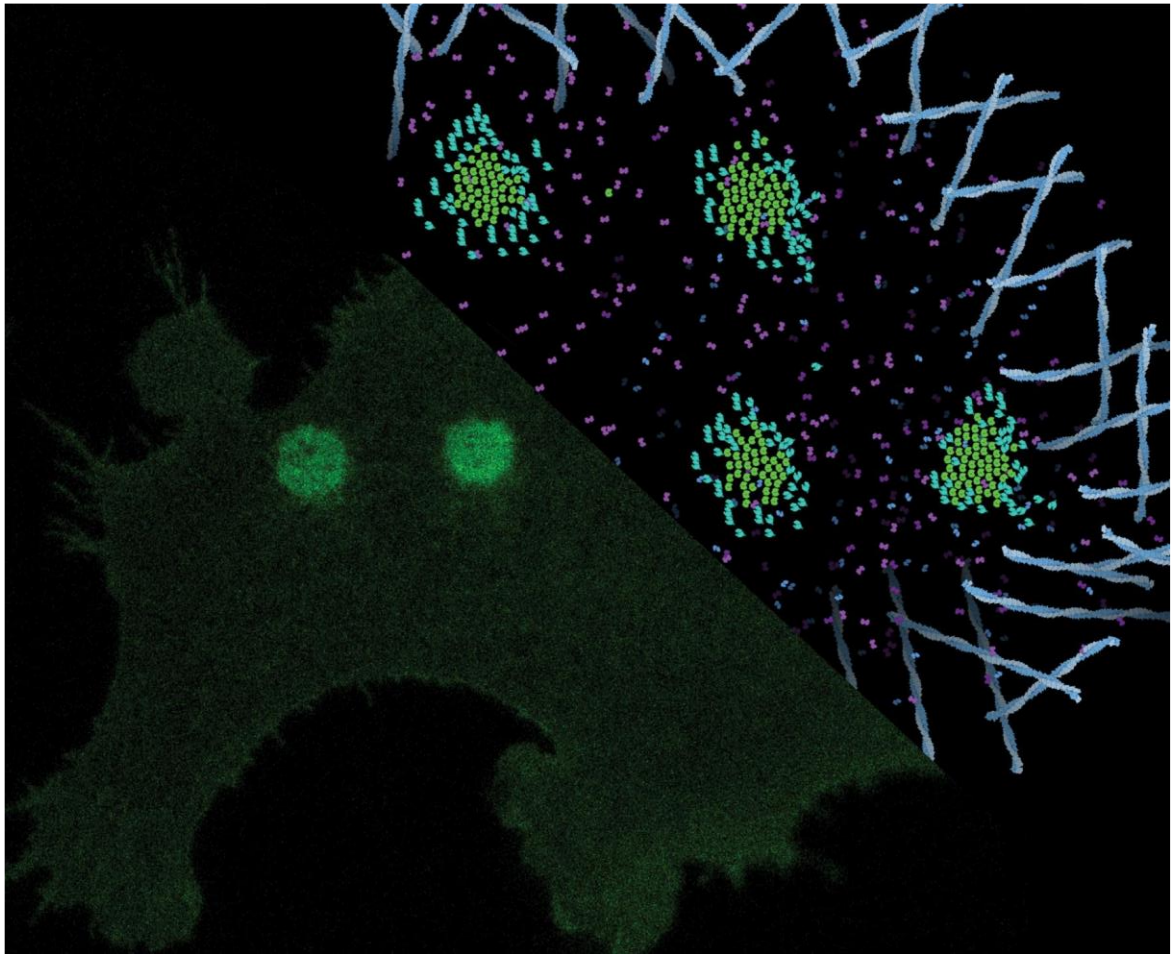
a) PDGF forms a ligand dimer of which each growth factor engages one receptor;
b) EGF has one binding site and its binding reveals a receptor dimerization motif;
c) insulin has two binding sites and its action somehow must change the conformation of an existing receptor dimer;
d) FGF has two binding sites but two ligands are needed to bring two receptors together. Stable dimers only form when two heparin sulphate oligosaccharides combine with receptor ligand complexes.

In a living cell…



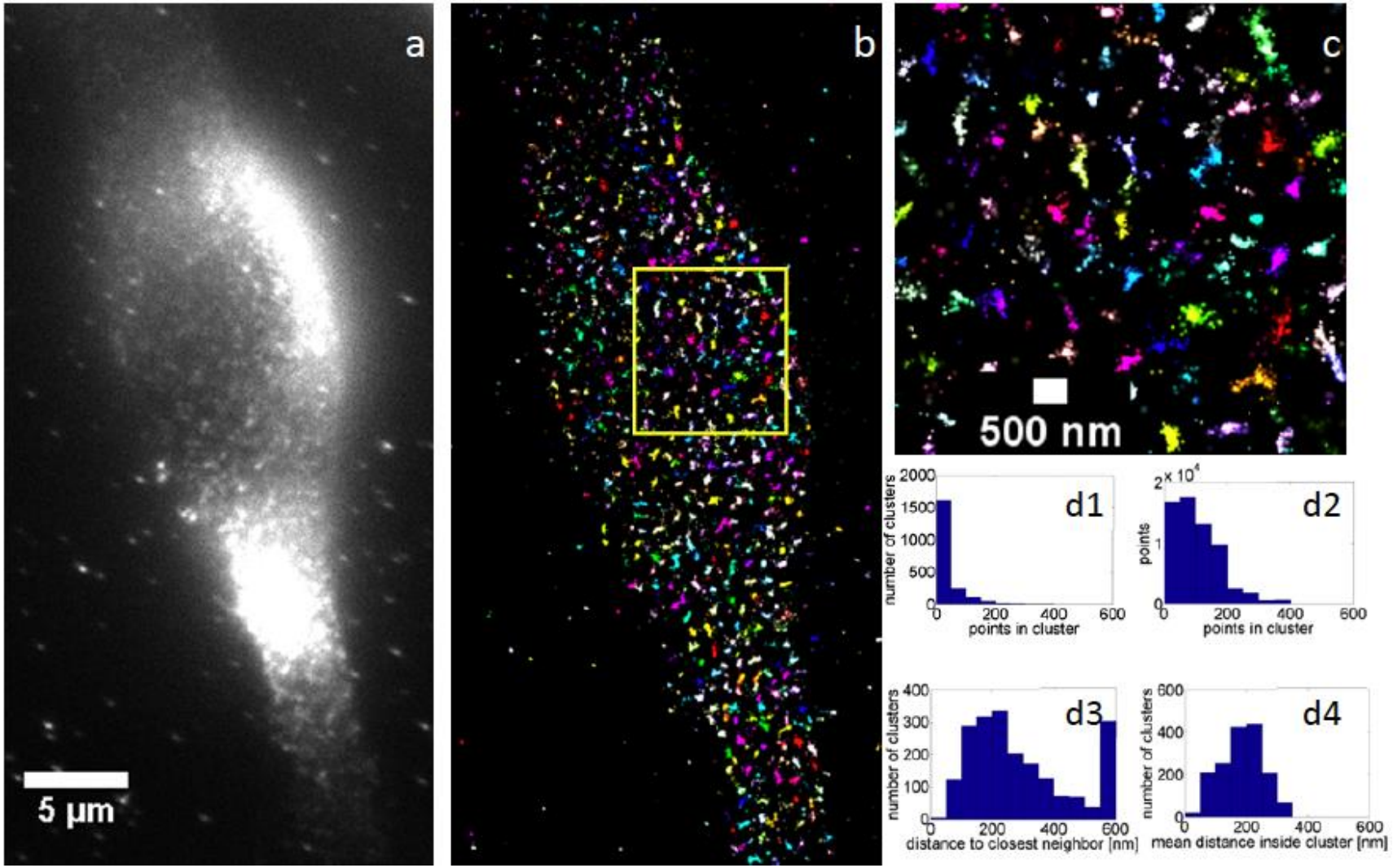
Ligand-receptor binding





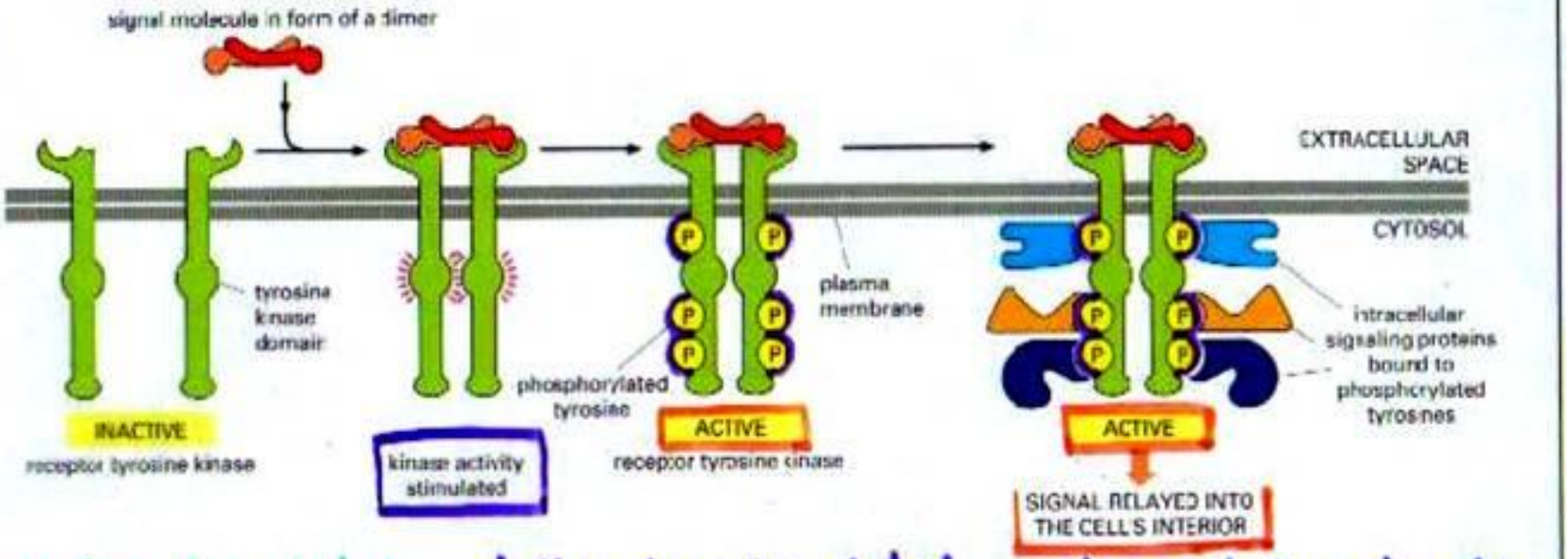
Imaging of insulin receptors in the plasma membrane of cells using super-resolution single molecule localization microscopy

Pavel Křížek[1], Peter W. Winter[2], Zdeněk Švindrych[1], Josef Borkovec[1], Martin Ovesný[1], Deborah A. Roess[3], B. George Barisas[4], and Guy M. Hagen[1,*]



Activation of a receptor kinase → signaling complex formation

(enzyme-linked receptor)



non-phosphorylated receptors → inactive

fully phosphorylated dimerized receptors → active

intracellular signaling proteins bound to phosphorylated residues → signaling to several pathways

Figure 15-28 The activation of a receptor tyrosine kinase results in the formation of an intracellular signaling complex.
Alberts et al.: *Essential Cell Biology*

Fig. 16-30

Nature Reviews Molecular Cell Biology | AOP, published online 30 September 2015; doi:10.1038/nrm4068

The discovery of modular binding domains: building blocks of cell signalling

Anthony James Pawson

(1952–2013)

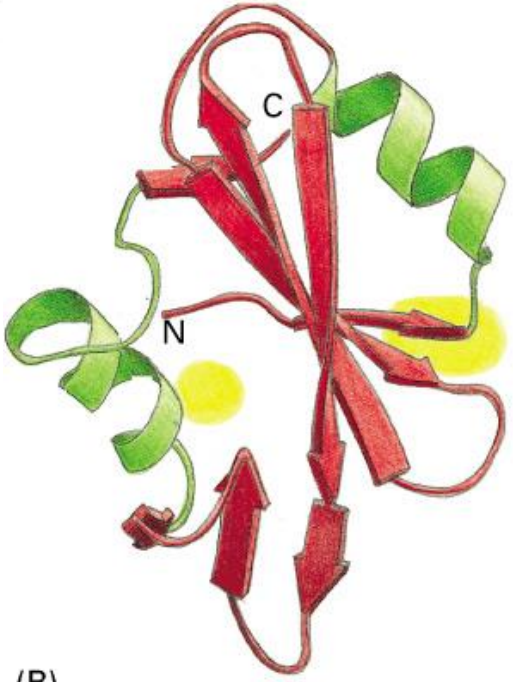
Biochemist whose vision of cell signalling transformed cancer research.

In the 1980s, early in his career, Pawson and his team discovered the Src homology region 2 (SH2). A subunit, or domain, of many proteins, SH2 directs how proteins interact and governs how cells respond to external cues. This finding set a path for all his future work.

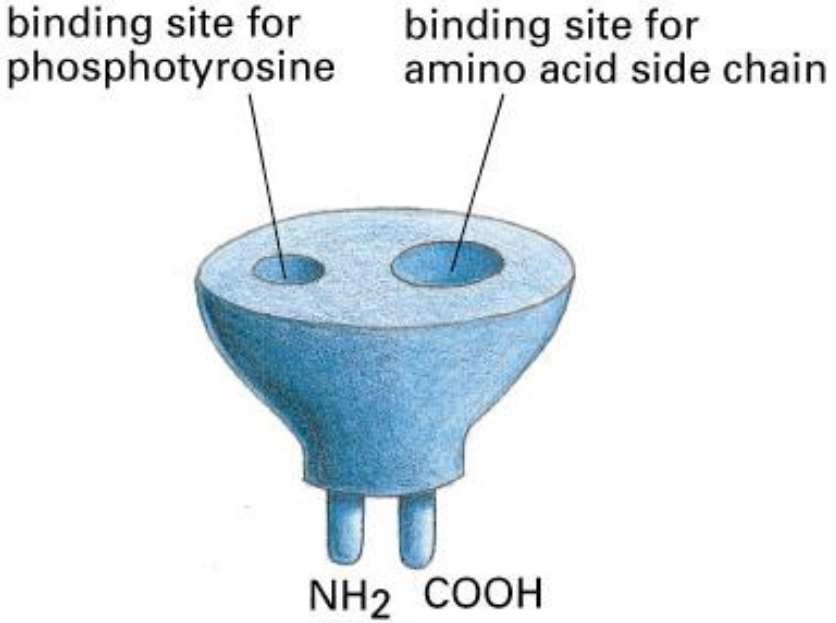
Pawson went on to show that combinations of a small number of domains could produce an enormous range of cellular responses. This 'modular' vision reshaped scientists' understanding of cellular regulation and paved the way for the development of drug classes that interfere with these protein interactions.

SH2 Domains: Properties

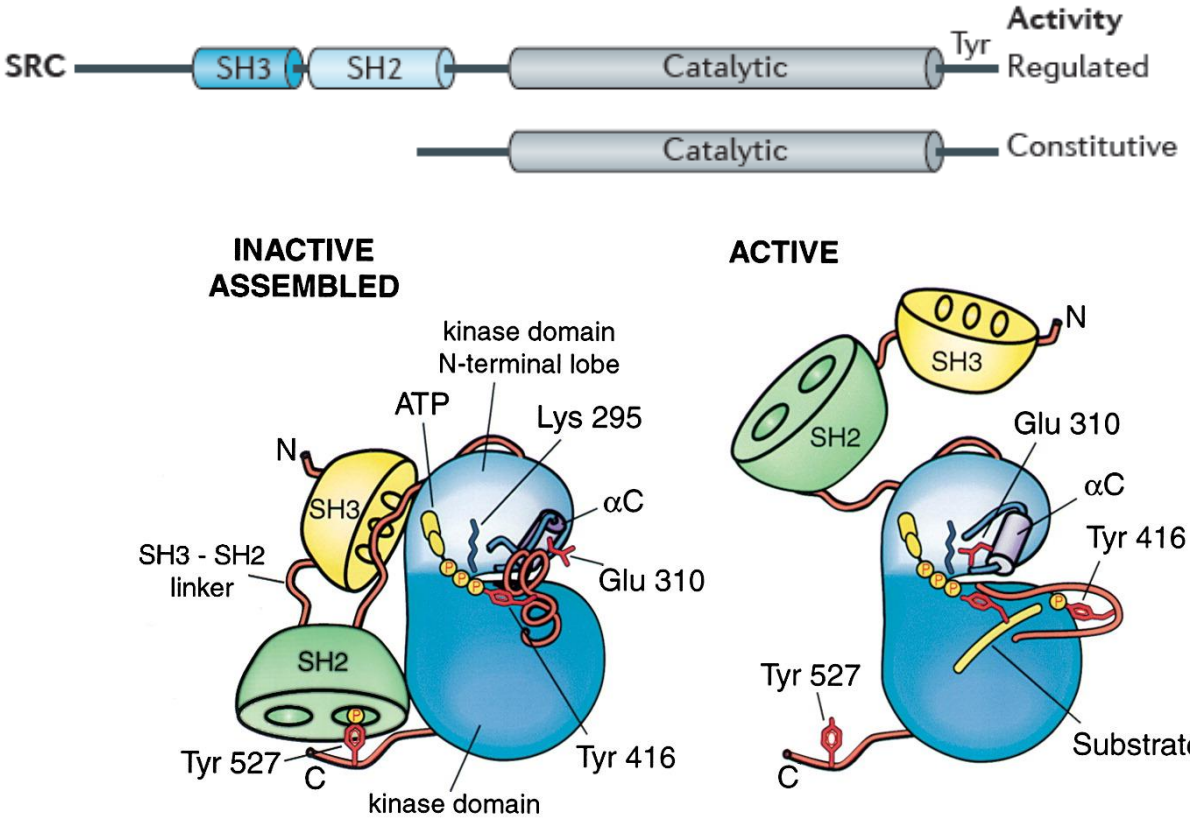
- Conserved regions of ~ 100 amino acids
- Bind tightly to tyrosine-phosphorylated peptides
- No binding in the absence of phosphorylation
- Mediate protein-protein interactions of effectors with activated growth factor and cytokine receptors
- Regulate non-receptor protein tyrosine kinase activity



(B)



Enzyme regulation by modular binding domains



SRC family non-receptor Tyr kinases contain an SH3, SH2 and catalytic domain, as well as a regulatory Tyr phosphorylation site at the carboxyl terminus.

The catalytic domain alone is unregulated and has high constitutive kinase activity.

The SH2 and SH3 domains bind intramolecularly to the catalytic domain, locking it in a catalytically inactive conformation. Dephosphorylation of Tyr527 destabilizes the repressed conformation, increasing the catalytic activity of SRC.

In the open, active conformation, the SH3 and SH2 domains of SRC can interact in *trans* with other proteins.

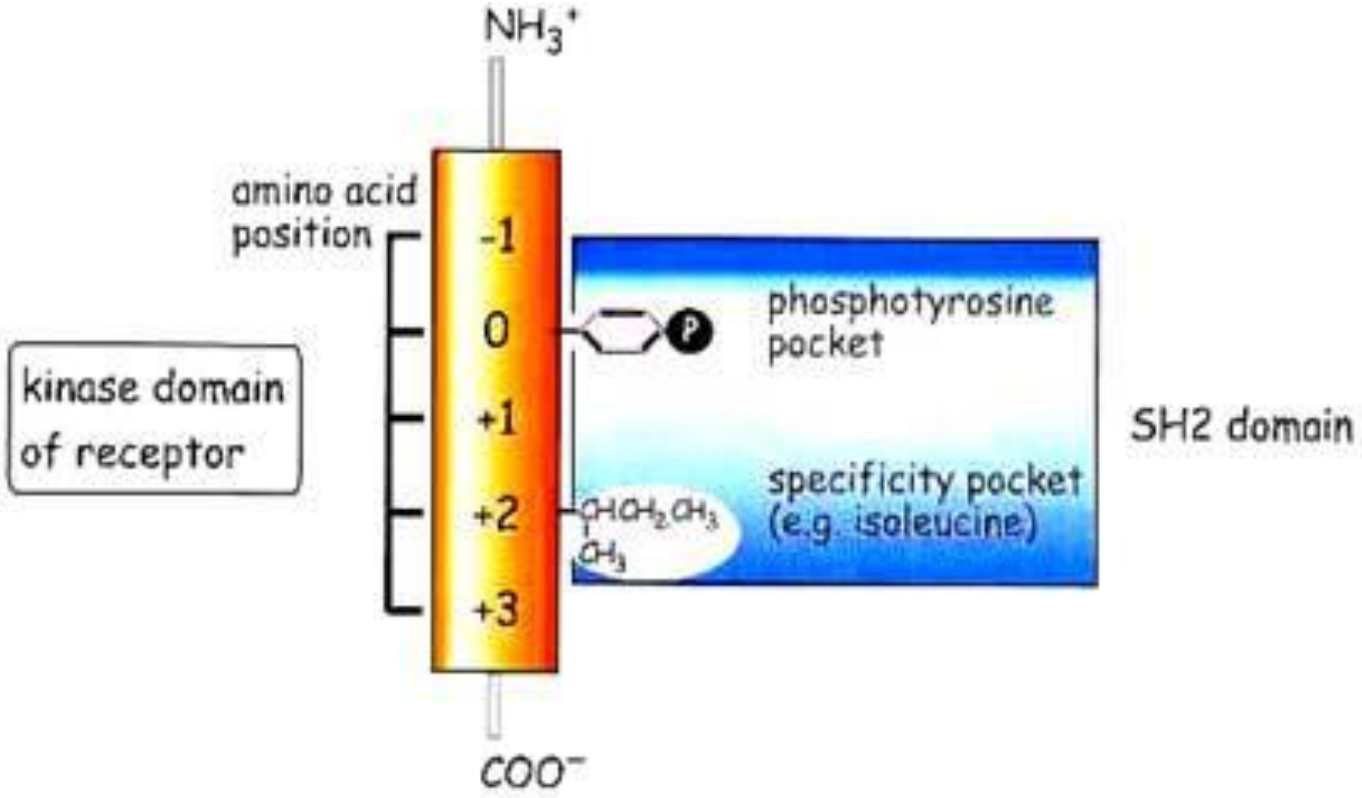
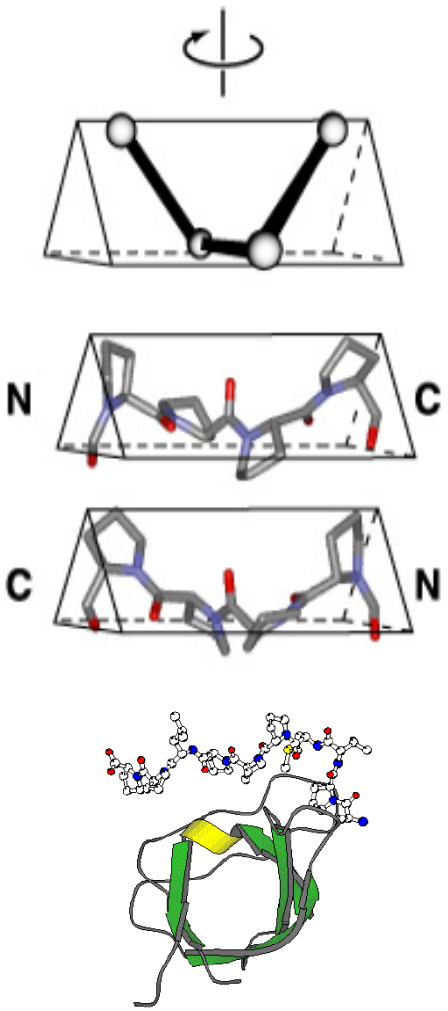


Figure 11.7 Recognition of phosphotyrosine and adjacent amino acids by the SH2 domain. Selectivity of recognition between different targets containing SH2 domains is conferred by the sequence of amino acids, particularly the third residue immediately adjacent on the C-terminal side of the phosphorylated tyrosine. As examples:

PI 3-kinase	-x-pY-x-x-M-
Grb2	-x-pY-x-N-x-
Src	-x-pY-x-x-I-.

SH3 Domains: Properties

- Compact: ~ 60 amino acids
- Signaling complex assembly and regulatory functions
- Bind proline-rich target sequences that form polyproline type II (PPII) helices:
  - Extended left-handed helix
  - 3 residues per turn
  - Conformationally rigid – provides stable docking site for SH3 binding
  - Rotationally symmetrical – bind in N ⇨ C or C ⇨ N orientation



Zarrinpar, et al. Science STKE 2003 re8, 2003

CellPress

Structure
Resource

Comprehensive Analysis of the Human SH3 Domain Family Reveals a Wide Variety of Non-canonical Specificities

Joan Teyra,[1,7] Haiming Huang,[1,2,7] Shobhit Jain,[1,3] Xinyu Guan,[4] Aiping Dong,[4] Yanli Liu,[4] Wolfram Tempel,[4] Jinrong Min,[4,5] Yufeng Tong,[4,6] Philip M. Kim,[1,2,3] Gary D. Bader,[1,2,3] and Sachdev S. Sidhu[1,2,8,*]

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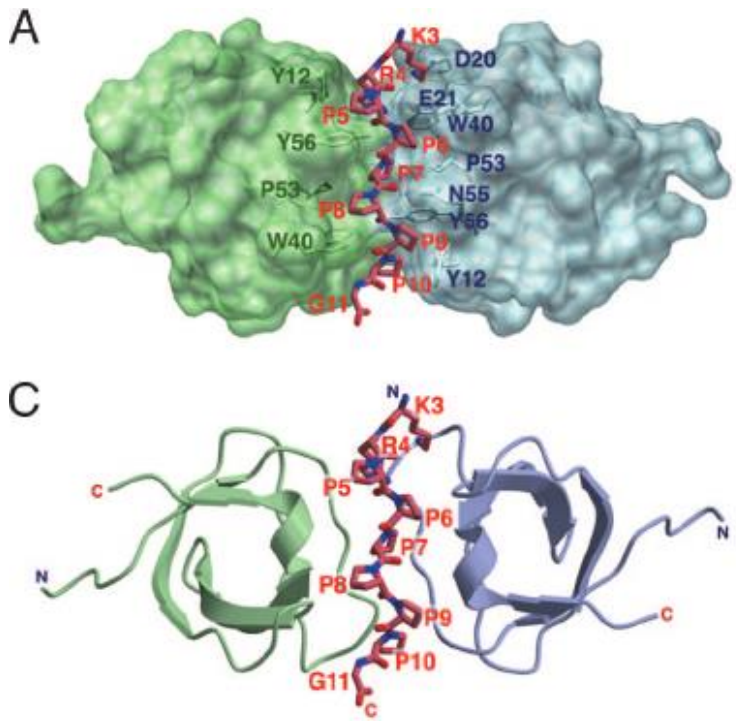
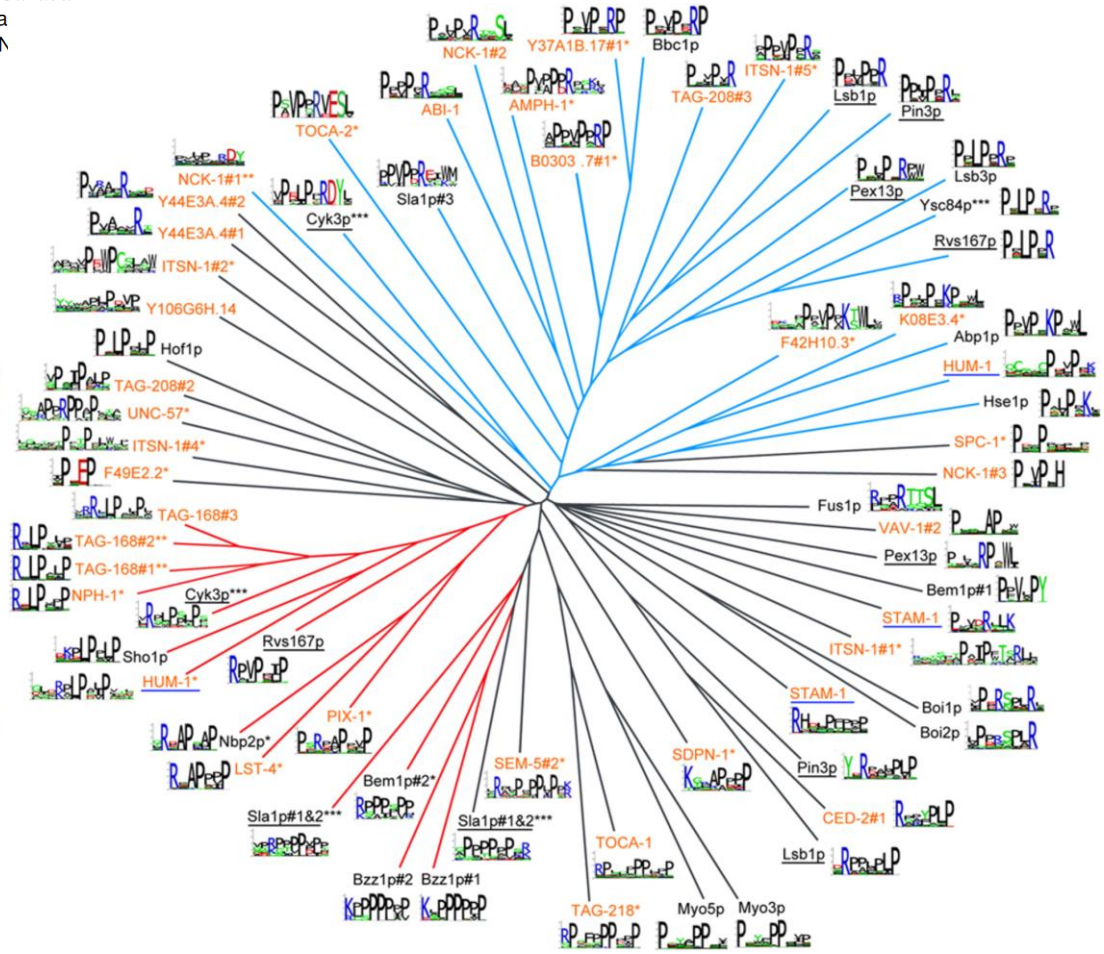
http://dx.doi.org/10.1016/j.str.2017.07.017





Key concept:

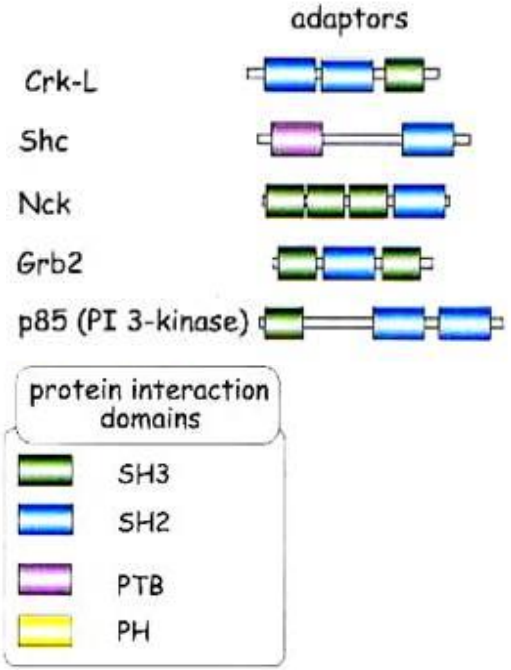
combinations of a small number of domains produce an enormous range of cellular responses

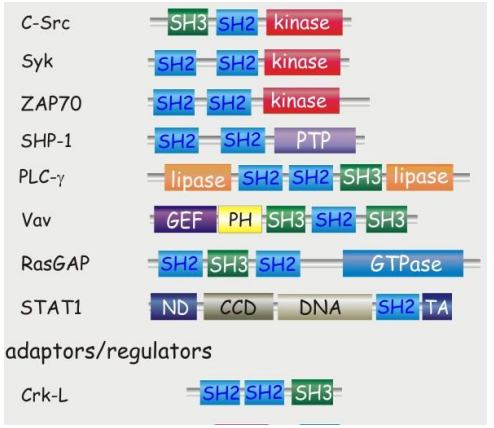
Which are the molecules binding to P-Y?

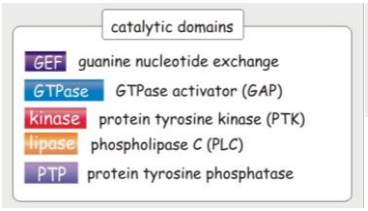
Adaptors

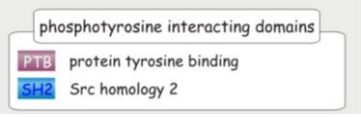
Adaptors lack intrinsic catalytic activity, but link phosphorylated receptors with other effector proteins.

Enzymes
Transcription factors

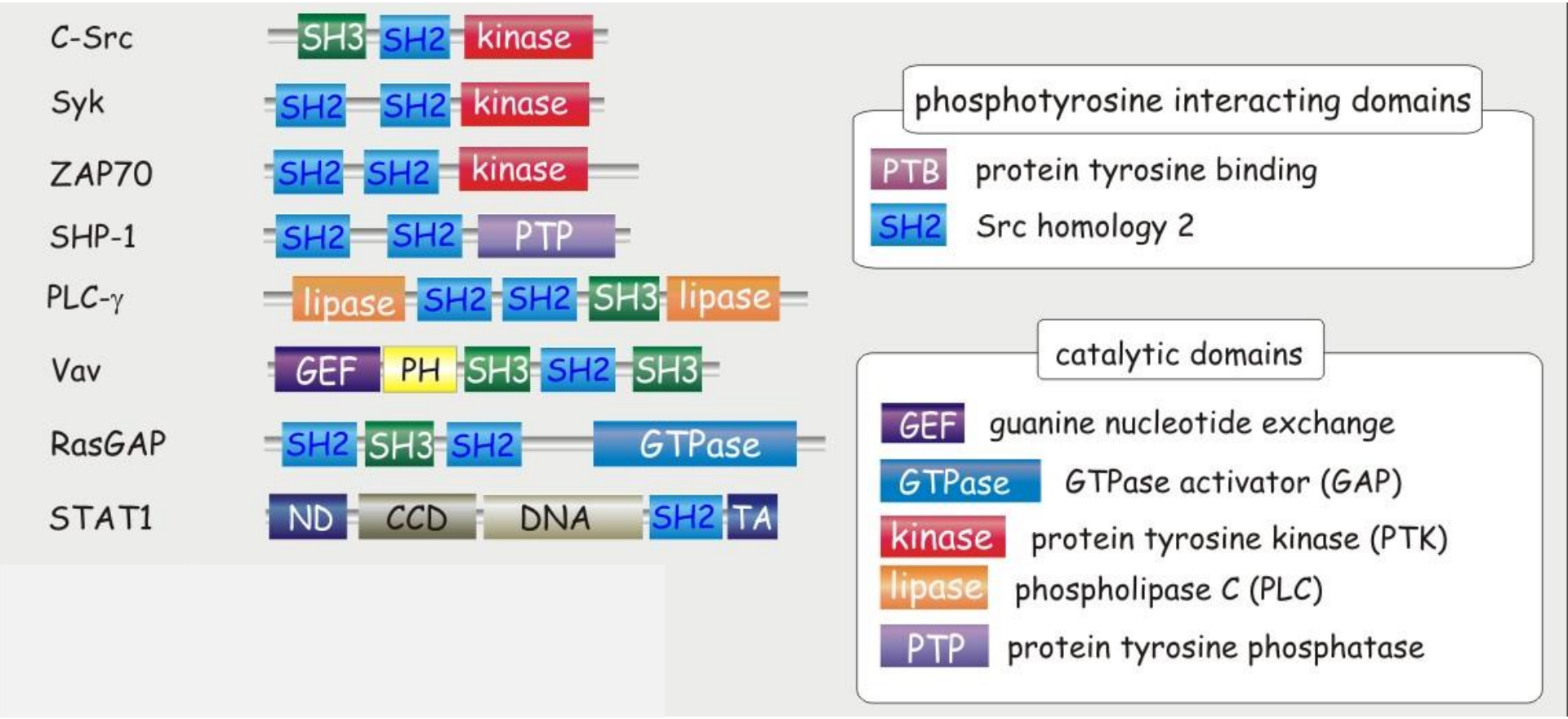




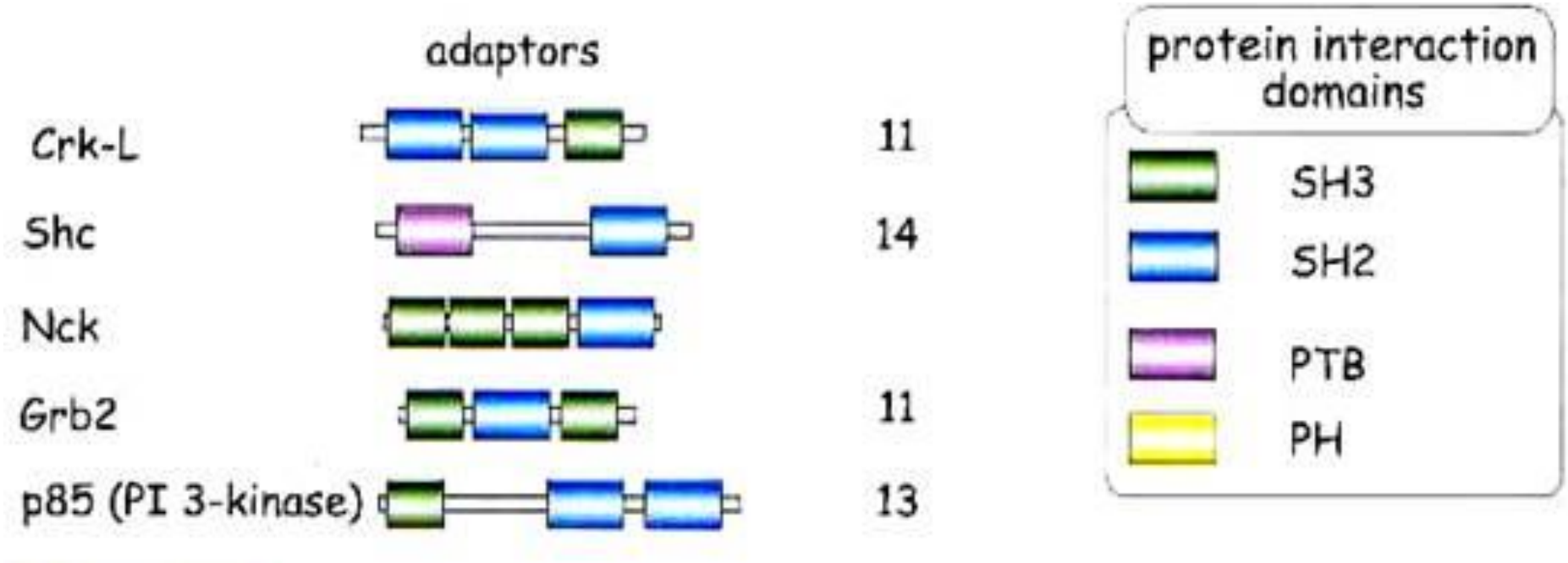




1) Enzymes/transcription factors



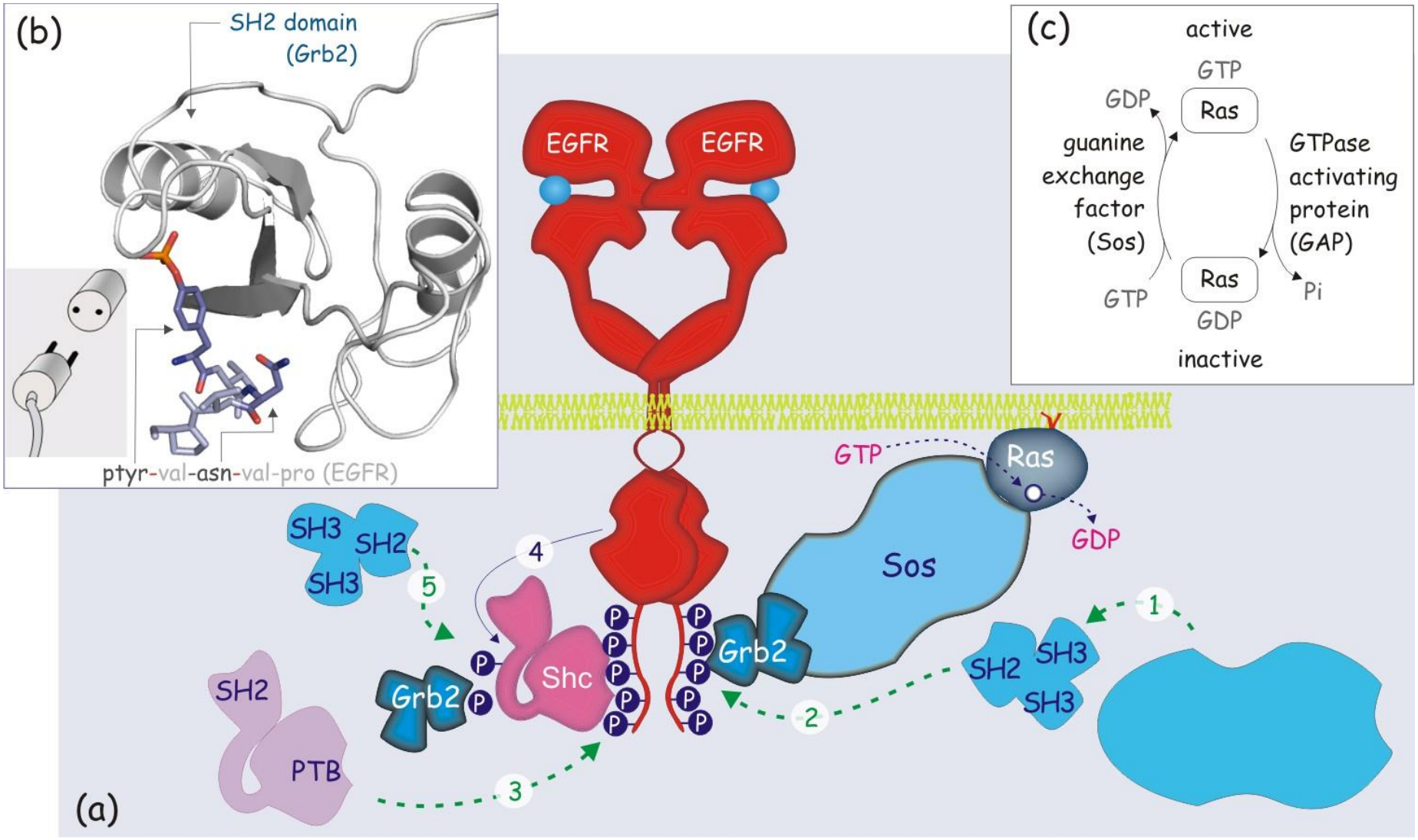
2) ***Adaptors*** lack intrinsic catalytic activity, but link phosphorylated receptors with other effector proteins.



Signaling Efficiency: Scaffolding Proteins and Signaling Complexes

- **Scaffolding proteins** are large relay proteins to which other relay proteins are attached
- Scaffolding proteins can increase the signal transduction efficiency by grouping together different proteins involved in the same pathway
- In some cases, scaffolding proteins may also help activate some of the relay proteins

An example of a signaling complex – a protein relay system using phosphorylation as a signal



Ras (RAT-Sarcoma)

- 1964: scoperta del virus oncogenico del sarcoma murino di Harvey (H-Ras)
- 1967: scoperta del virus oncogenico del sarcoma murino di Kirsten (K-Ras)
- 1973: Scolnick e colleghi, in un momento in cui gli strumenti per dimostrarlo mancavano, affermarono che le proprietà trasformanti di questi virus del sarcoma erano dovute alla trasduzione dei genomi virali nelle normali cellule e sequenze nucleotidiche del ratto.
- 1982: nella linea cellulare umana di carcinoma della vescica EJ/T24, attivazione molecolare del gene HRAS causata da una singola mutazione missenso nel codone 12, la stessa trovata anche in geni HRAS e KRAS virali.
- 1983: un terzo gene RAS è scoperto nel DNA di cellule di neuroblastoma umano ed è stato designato perciò NRAS.
- Oggi sappiamo che anche il genoma umano contiene tre geni RAS: *HRAS, KRAS, NRAS*.

CELLULAR ONCOGENES

- Present in cancer cells
- Contains introns characteristic of eukaryotic cells
- Encodes proteins triggering transformation of normal cells

VIRAL ONCOGENES

- Present in viruses
- Host cell origin
- Do not possesss introns
- Also called 'cancer genes'
- Encodes proteins triggering transformation of normal cells into cancer cells

VIRAL ONCOGENE	HUMAN ONCOGENE	ORIGIN	NATURE
V-src	C-src	Chicken	Sarcoma
V-ras	C-ras	Rat	Sarcoma
V-myc	C-myc	Chicken	Leukemia
V-fes	C-fes	Feline	Sarcoma
V-sis	C-sis	Simian	Sarcoma
V-mos	C-mos	Mouse	Sarcoma

Oncogenes vs proto-oncogenes

- An **oncogene** is a gene that has the potential to cause cancer.
- In tumor cells, they are often mutated or expressed at high levels.
- The first confirmed oncogene was discovered in 1970 and was termed src. Src was in fact first discovered as an oncogene in a chicken retrovirus.
- In 1976 Dominique Stehelin, J. Michael Bishop and Harold E. Varmus demonstrated that oncogenes were activated proto-oncogenes, found in many organisms including humans (for this discovery Bishop and Varmus were awarded the Nobel Prize in Physiology or Medicine in 1989).

- A **proto-oncogene** is a normal gene that becomes an oncogene due to mutations or increased expression.
- Proto-oncogenes code for proteins that regulate cell growth and differentiation. Proto-oncogenes are often involved in signal transduction and execution of mitogenic signals.
- Upon *activation*, a proto-oncogene becomes a tumor-inducing agent, an oncogene.

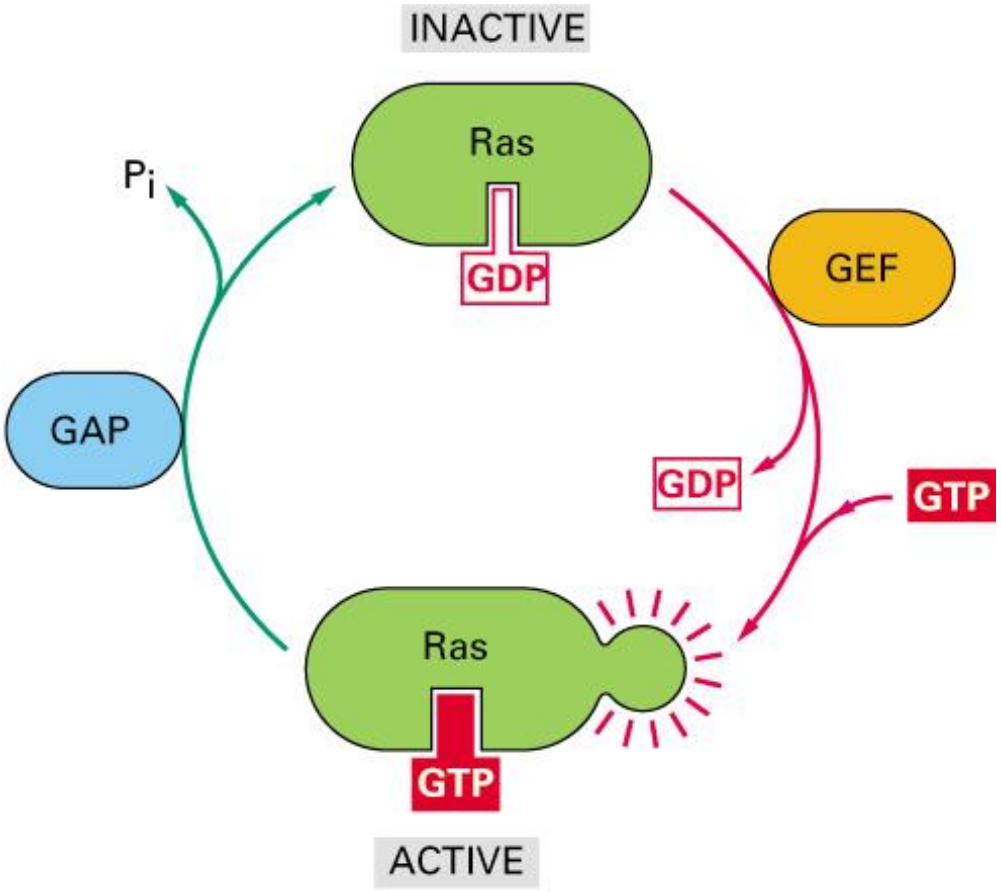
Table 2 | ***HRAS*, *KRAS*, *NRAS* and *BRAF* mutations in human cancer**

Cancer type	*HRAS*	*KRAS*	*NRAS*	*BRAF*
Biliary tract	0%	33%	1%	14%
Bladder	11%	4%	3%	0%
Breast	0%	4%	0%	2%
Cervix	9%	9%	1%	0%
Colon	0%	32%	3%	14%
Endometrial	1%	15%	0%	1%
Kidney	0%	1%	0%	0%
Liver	0%	8%	10%	3%
Lung	1%	19%	1%	2%
Melanoma	6%	2%	18%	43%
Myeloid leukaemia	0%	5%	14%	1%
Ovarian	0%	17%	4%	15%
Pancreas	0%	60%	2%	3%
Thyroid	5%	4%	7%	27%

The mutation data was obtained from the Sanger Institute Catalogue of Somatic Mutations in Cancer web site[148].

- Diversi tipi di cancro sembrano essere associati alla mutazione di una specifica isoforma RAS. Solitamente i carcinomi (in particolare quelli del colon e del pancreas) presentano mutazioni di KRAS, i tumori della vescica hanno mutazioni di HRAS e i tumori emopoietici presentano mutazioni di NRAS.

Ras is a monomeric GTPase

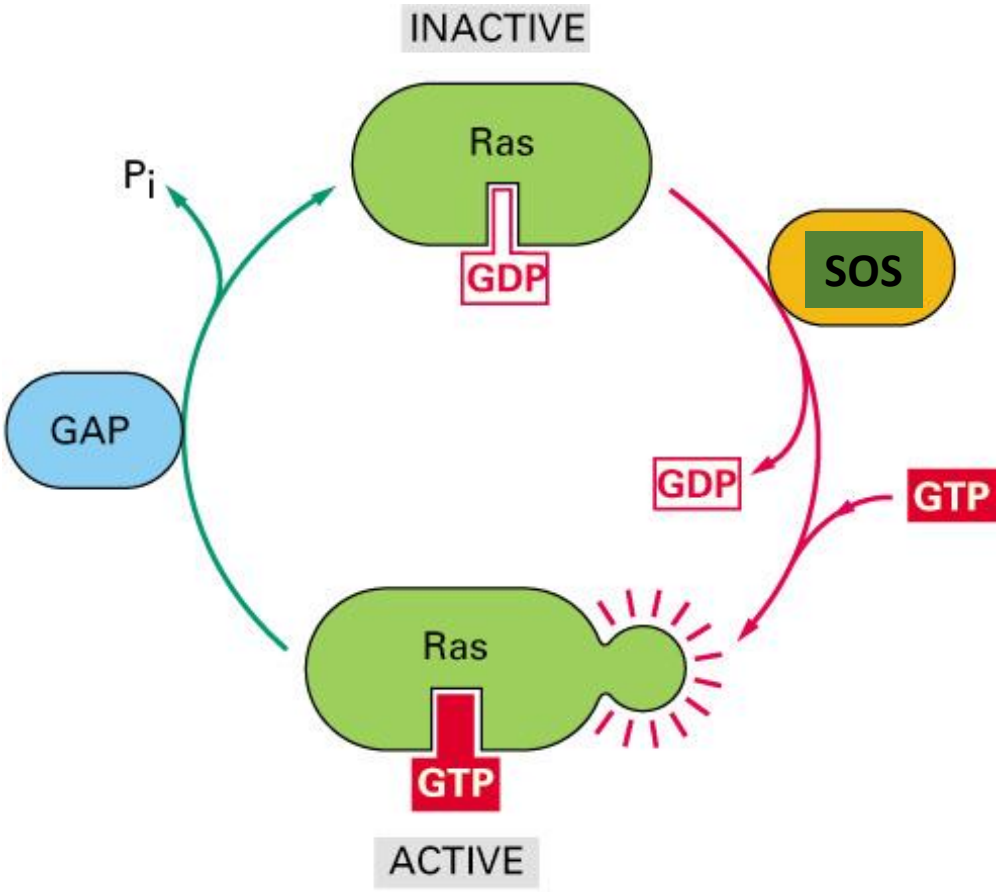


Ras has an intrinsic GTPase activity: the protein on its own will hydrolyze a bound GTP molecule into GDP. However this process is too slow for efficient function, and hence the GAP for Ras, *RasGAP*, may bind to and stabilize the catalytic machinery of Ras.

GEFs catalyze a "push and pull" reaction which releases GDP from Ras. Because intracellular GTP is abundant relative to GDP (approximately 10 fold more) GTP predominantly re-enters the nucleotide binding pocket of Ras and reloads the spring. Thus GEFs facilitate Ras activation.

The balance between GEF and GAP activity determines the guanine nucleotide status of Ras, thereby regulating Ras activity.

Ras is a monomeric GTPase



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The balance between GEF and GAP activity determines the guanine nucleotide status of Ras, thereby regulating Ras activity.

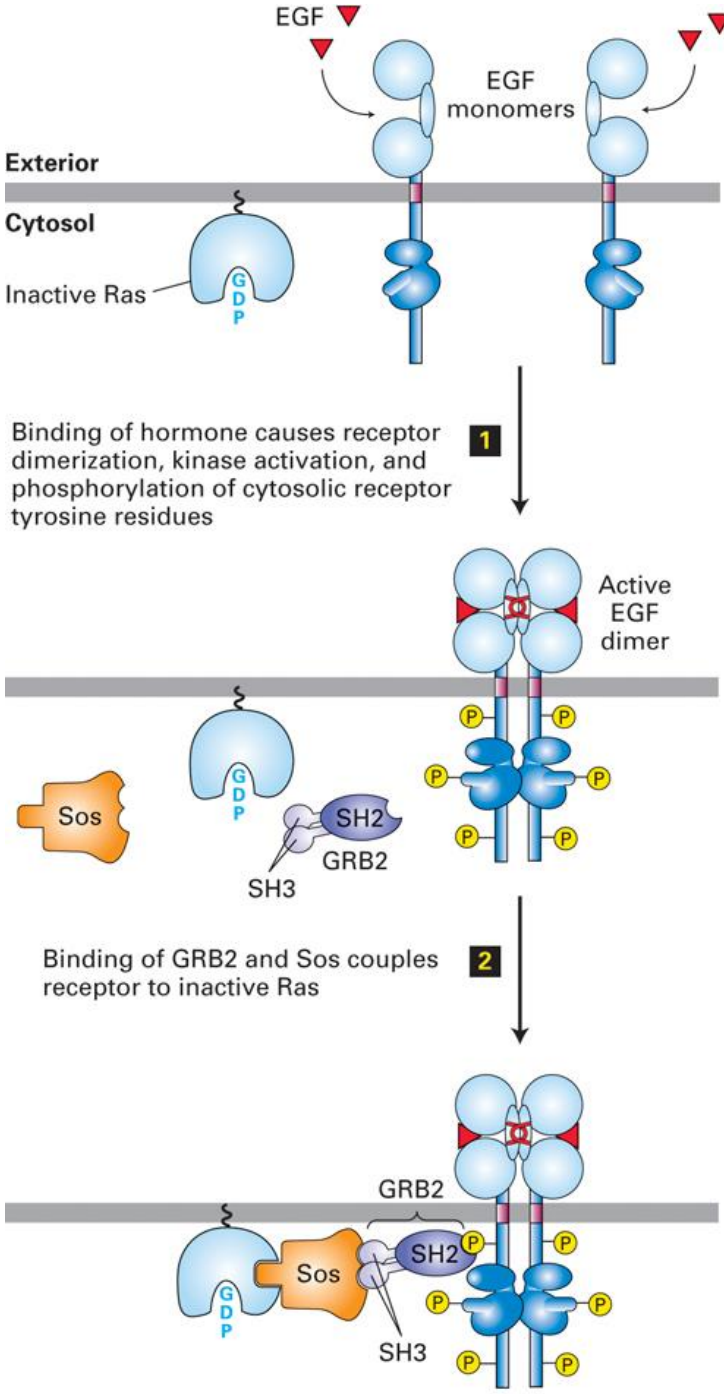
RTK Activation of Ras

EGF binding causes receptor clusterization and autophosphorylation on cytosolic tyrosines.

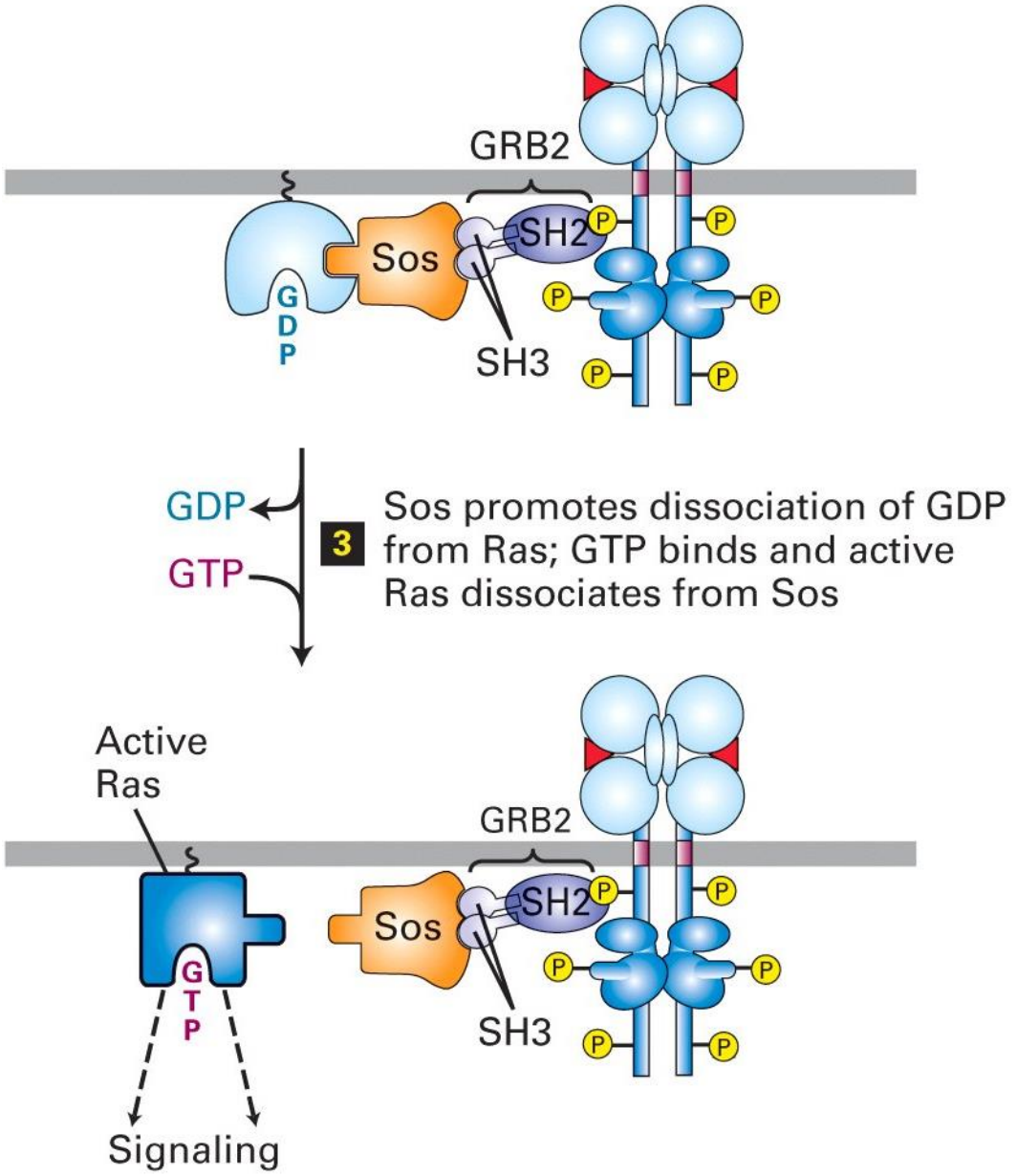
In Step 2, the adaptor protein GRB2 binds receptor phosphotyrosine residues via its SH2 domain. GRB2 contains SH3 domains that allow the GEF protein known as Sos to bind to the membrane complex.

The C-terminus of Sos inhibits its nucleotide exchange activity; binding of GRB2 relieves this inhibition

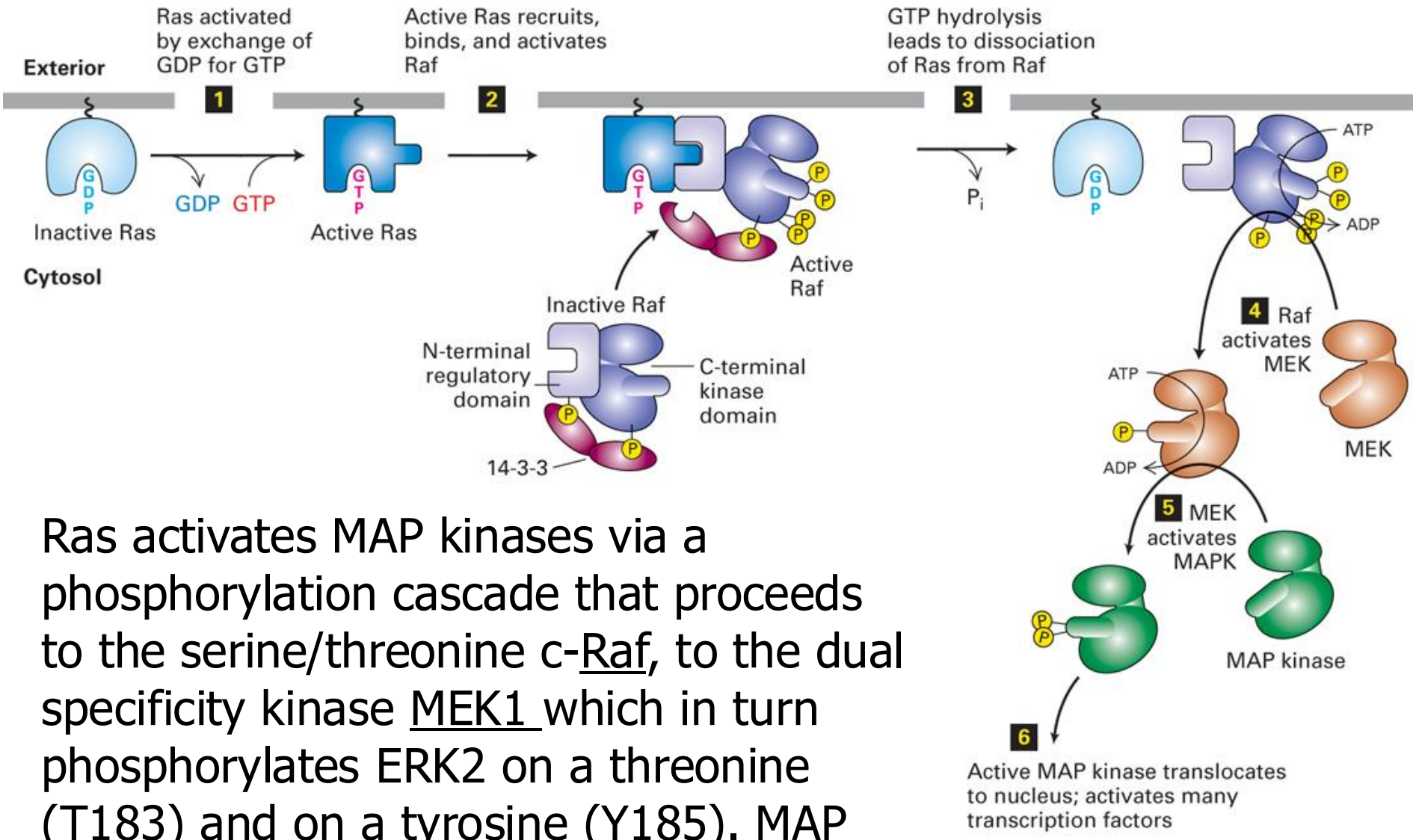
Sos converts inactive GDP-ras into active GTP-ras.



The activated Ras-GTP complex then dissociates from Sos, but remains tethered to the inner leaflet of the cytoplasmic membrane via a lipid anchor sequence. The active form of Ras then activates the MAP kinase portion of the signaling pathway.



Ras Activation of MAP Kinase

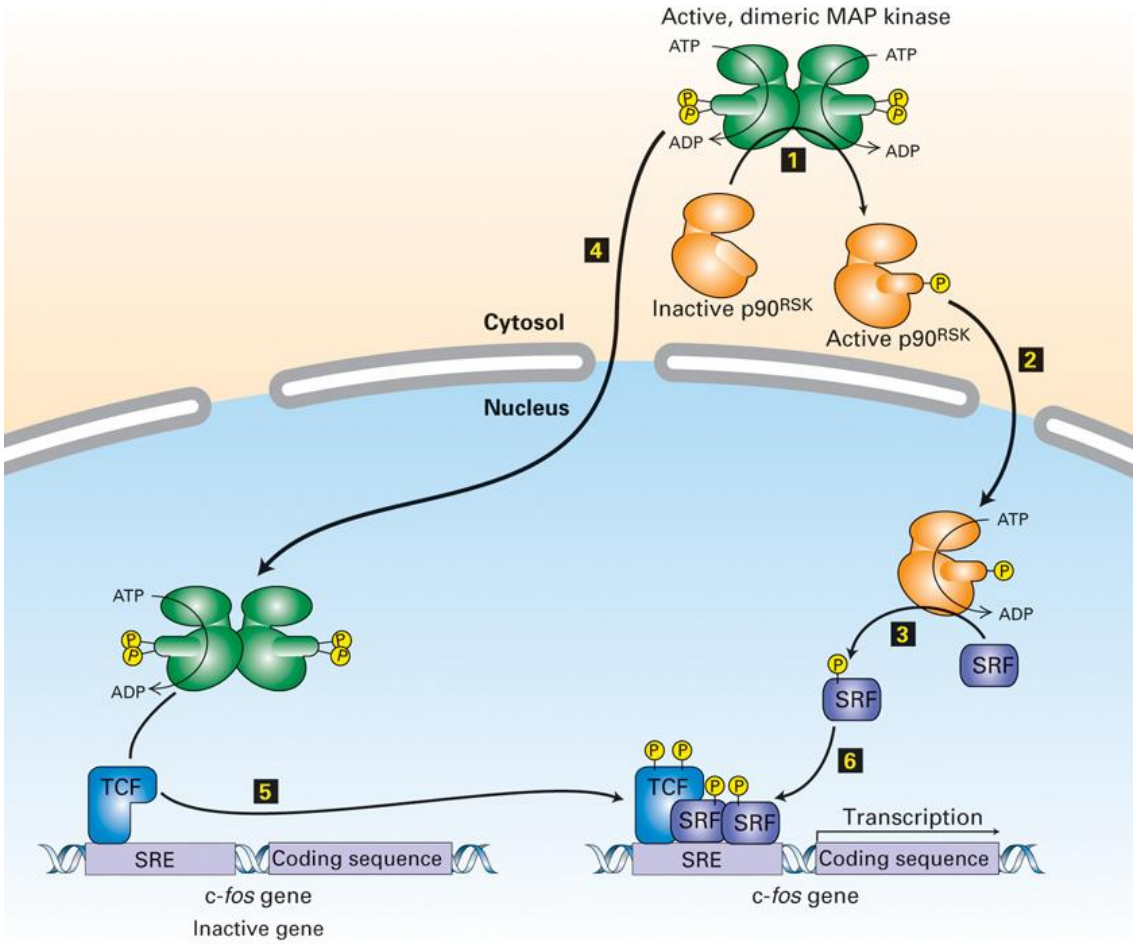


Ras activates MAP kinases via a phosphorylation cascade that proceeds to the serine/threonine c-Raf, to the dual specificity kinase MEK1 which in turn phosphorylates ERK2 on a threonine (T183) and on a tyrosine (Y185). MAP kinase then dimerizes and enters the nucleus.

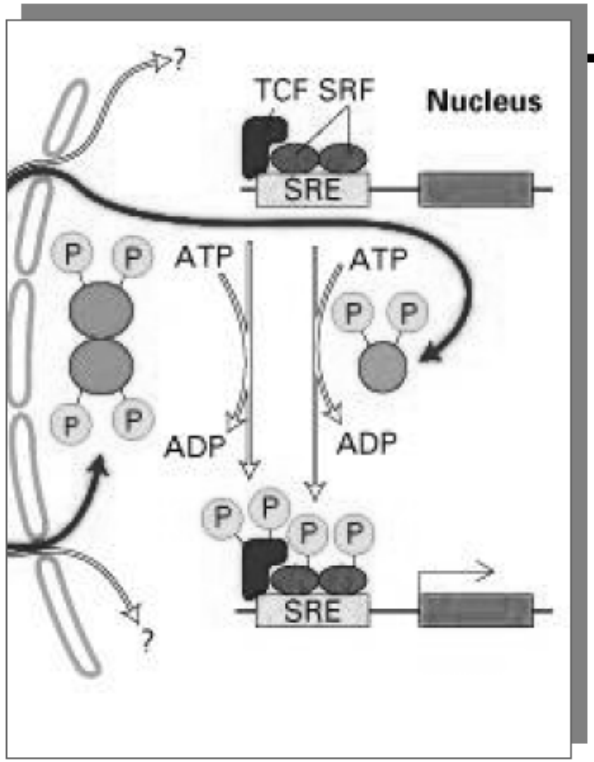
MAP Kinase Activation of Transcription

In the final steps of RTK-Ras/MAP kinase signaling, MAP kinase phosphorylates and activates the p90^{RSK} kinase in the cytoplasm. Both kinases enter the nucleus where they phosphorylate ternary complex factor (TCF) and serum response factor (SRF), respectively.

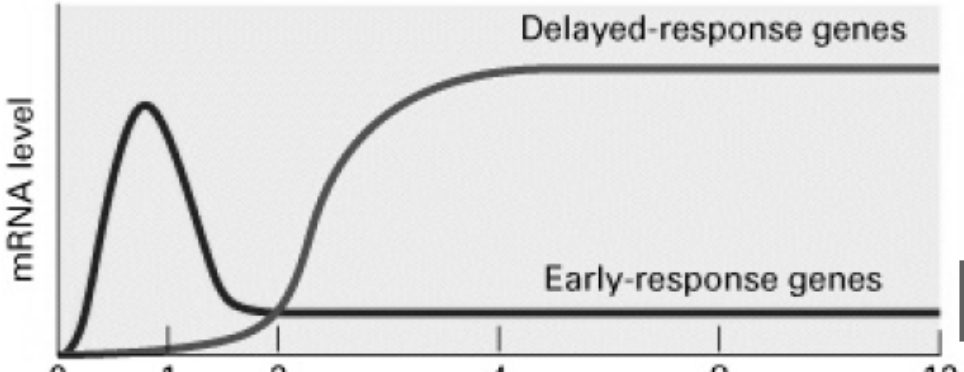
The phosphorylated forms of these TFs bind to serum response element (SRE) enhancer sequences that control genes regulated by growth factors present in serum (such as c-fos) and propel cells through the cell cycle.



Genes regulated by RTK/Ras pathway include early response genes.



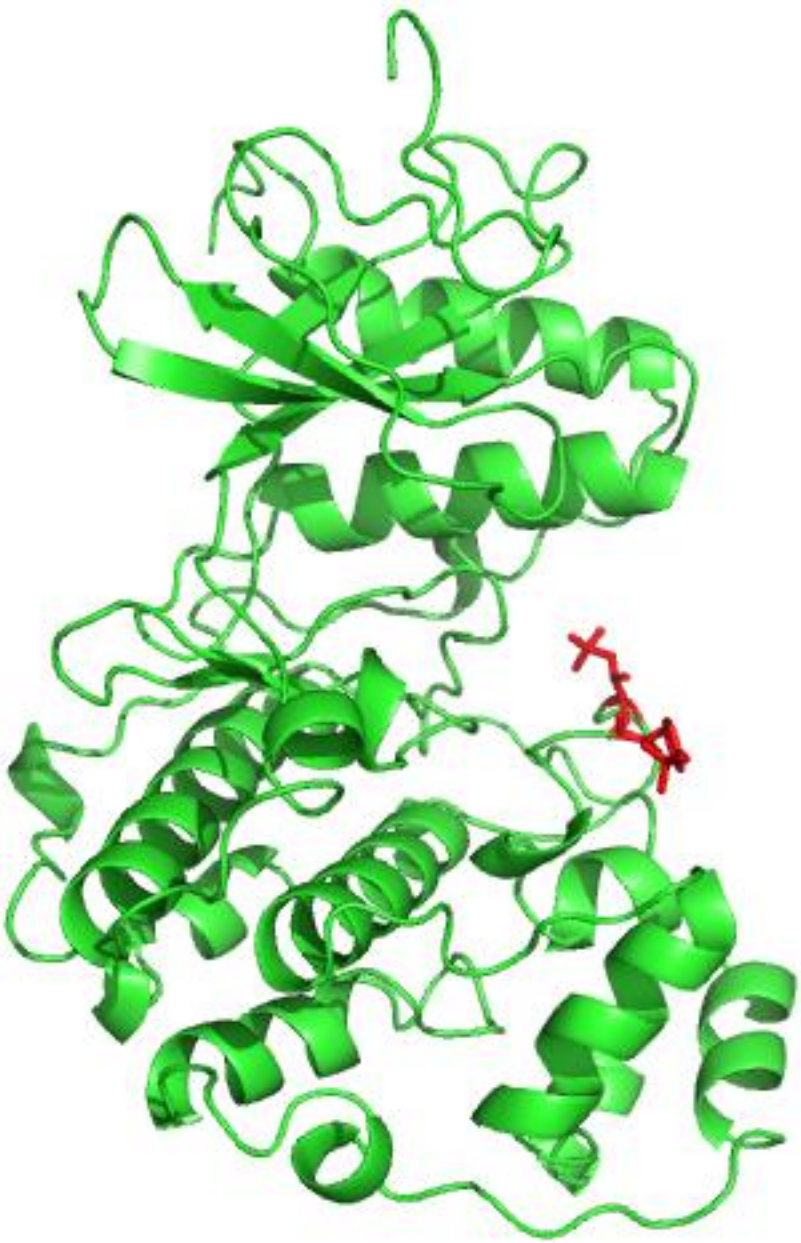
serum response elements;
-allows for activated transcription following growth factor (mitogen) stimulation
-found in genes involved in cellular proliferation.
Ex: c-fos gene
-c-fos is an early response gene
-required for the induction of delayed response genes including cyclin D.



D-type cyclins

C-fos transcription factor

MAP Kinases



- **Mitogen-activated protein kinases** are serine/threonine-protein kinases. They regulate proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis.

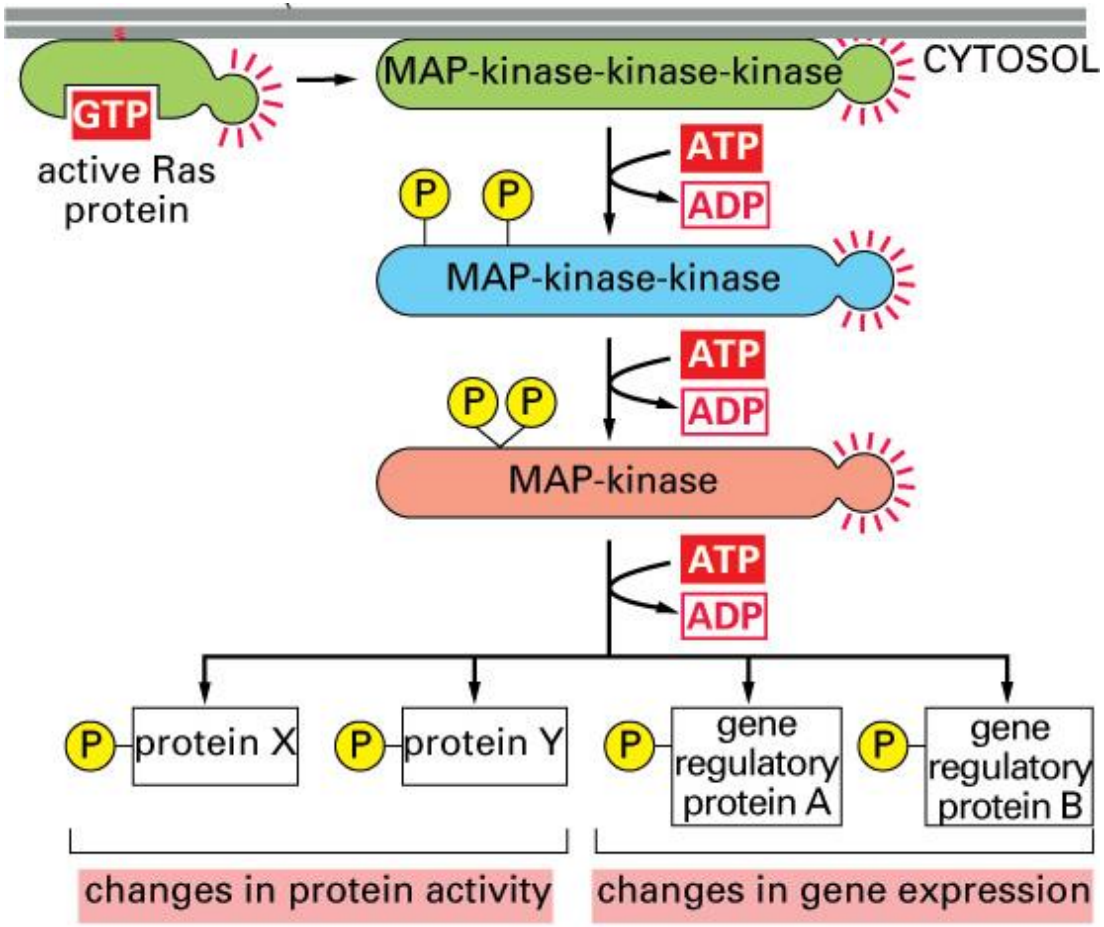
MAP kinases are found in eukaryotes only.

"Classical" MAPKs activation requires **two phosphorylation events**, both threonine and tyrosine residues, in order to lock the kinase domain in a catalytically competent conformation.

Inactivation of MAPKs is performed by a very conserved family of dedicated phosphatases is the so-called MAP kinase phosphatases (MKPs), dual-specificity phosphatases (DUSPs). They hydrolyze the phosphate from both phosphotyrosine and the phosphothreonine residues.

- Once activated, Ras propagates signaling further inside the cell via a kinase cascade that culminates in the activation of members of the MAP kinase family.
- MAP kinases phosphorylate TFs that regulate genes involved in the cell cycle, survival and in differentiation.

- As a result, mutations in *ras* genes can cause unintended and overactive signalling inside the cell and ultimately to cancer
- Ras is the most common oncogene in human cancer - mutations that permanently activate Ras are found in 20-25% of all human tumors and up to 90% in certain types of cancer (pancreatic cancer).



Visualizing Ras signalling in real-time

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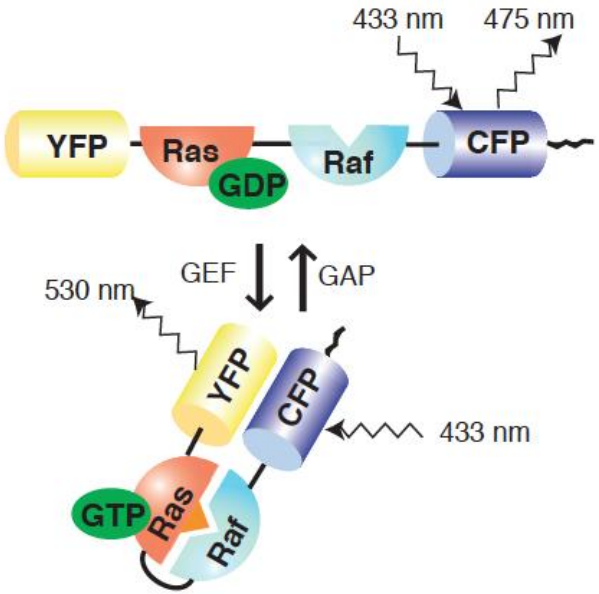
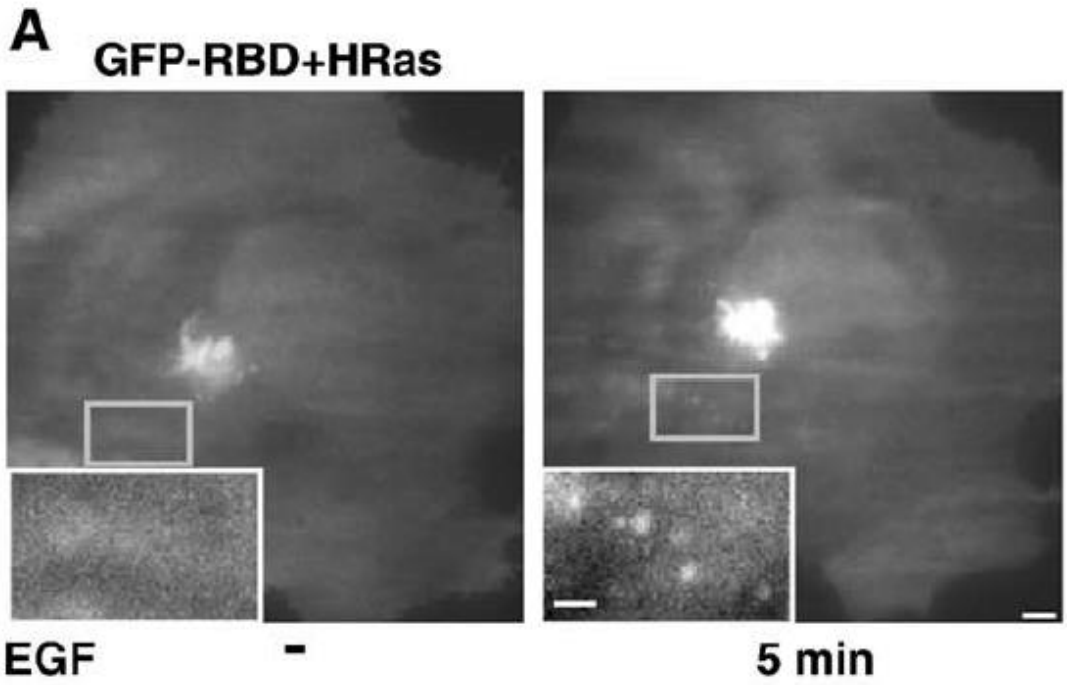
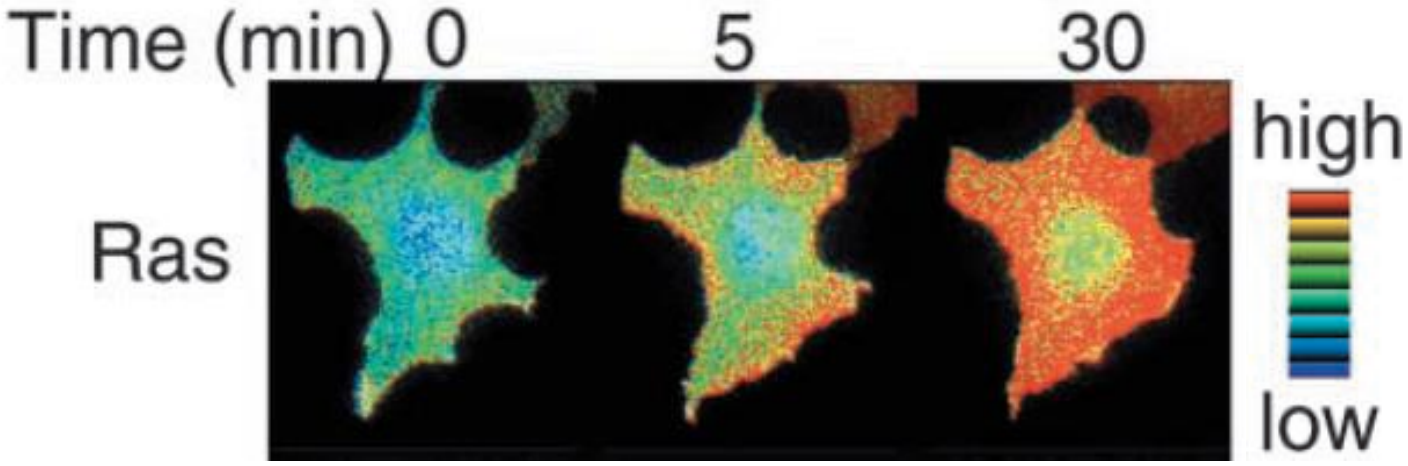
Journal of Cell Science 117, 2879-2886 Published by The Company of Biologists 2004
doi:10.1242/jcs.01285







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Rasosomes spread Ras signals from plasma membrane 'hotspots'

Merav Kofer-Geles, Irit Gottfried, Roni Haklai, Galit Elad-Zefadia, Yoel Kloog *, Uri Ashery *

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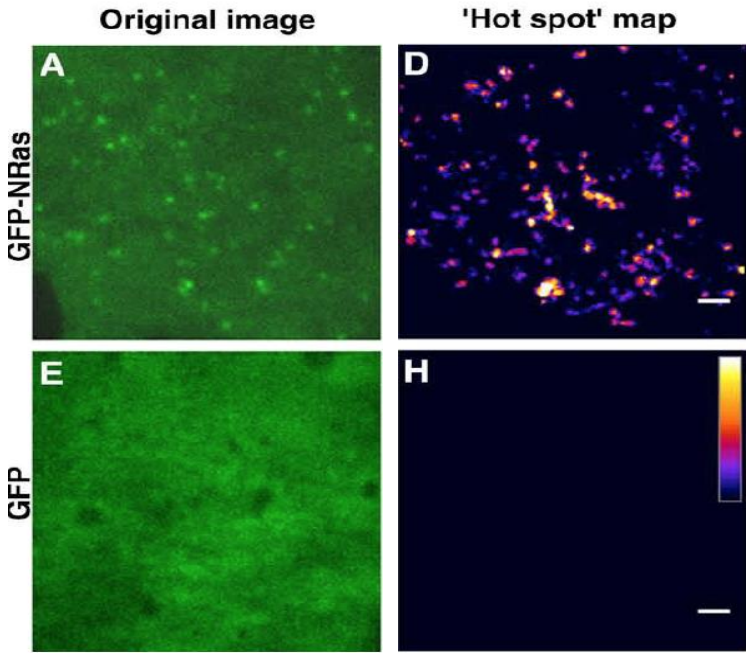
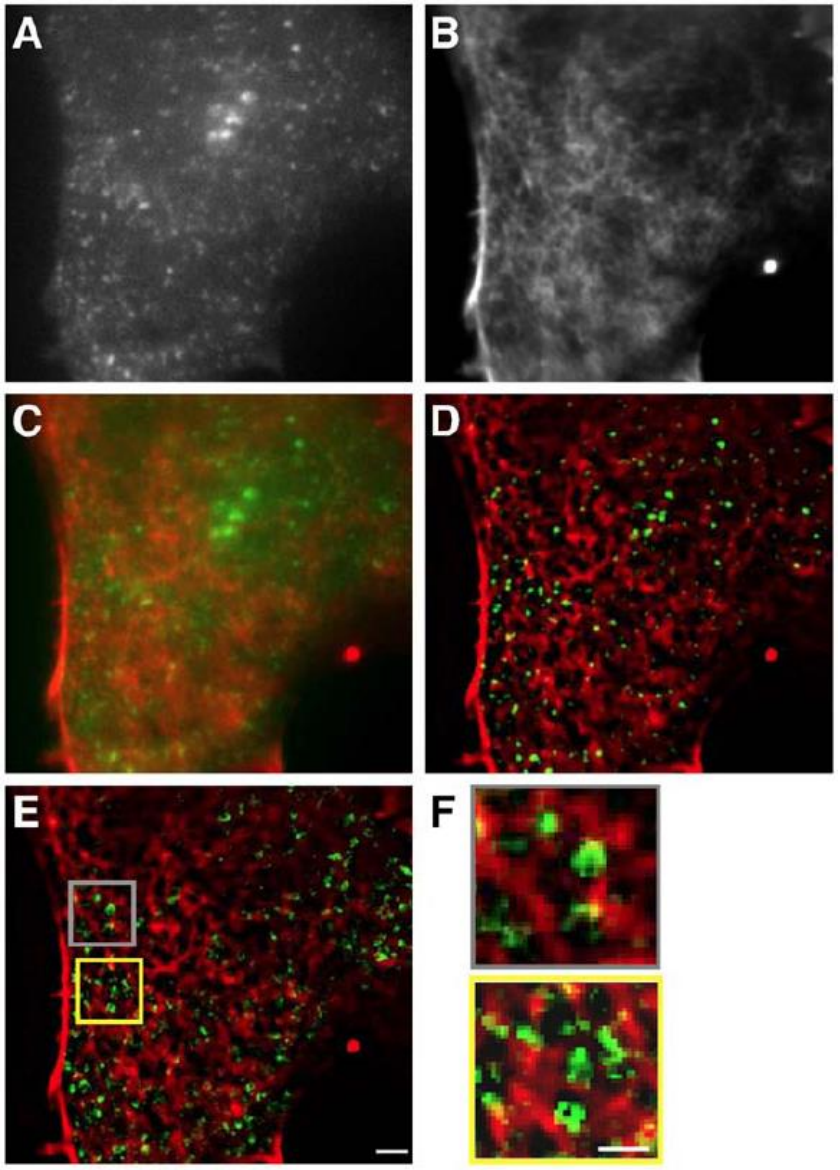
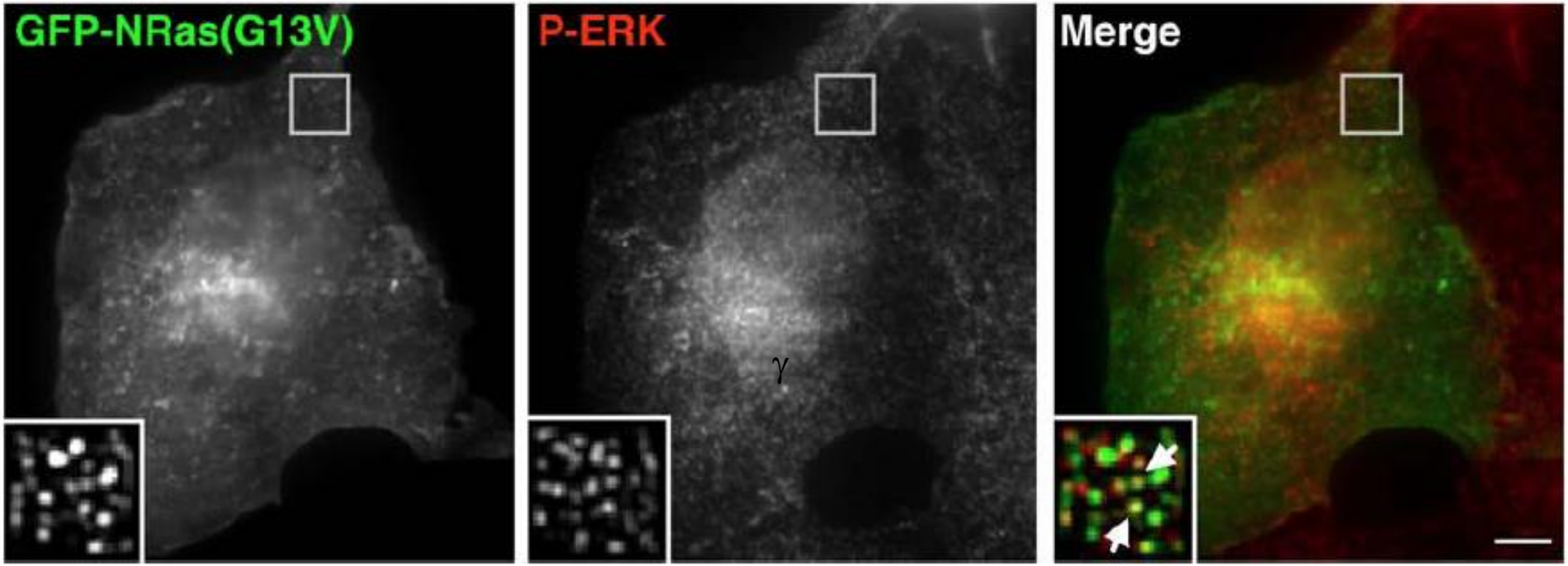


Ras-osomes move within distinct areas, rasosomal 'hotspots', near the PM.



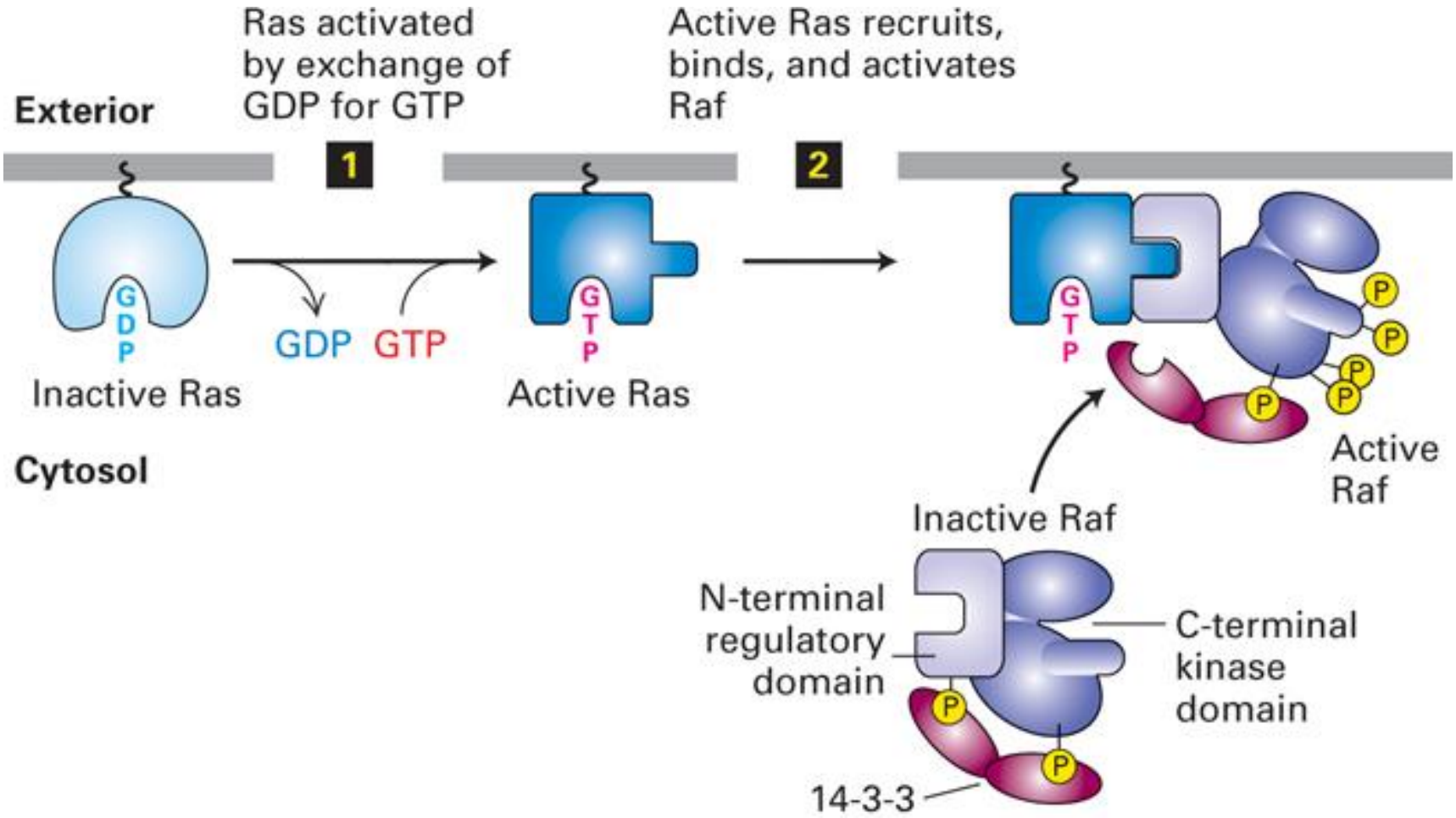
Rasosomes move within cortical actin cages.



GFP-NRas expressing cells were labeled with anti-phosphorylated-ERK Abs. Insets show filtered images of the boxed regions with arrows that indicate phospho-ERK positive GFP-NRas rasosomes

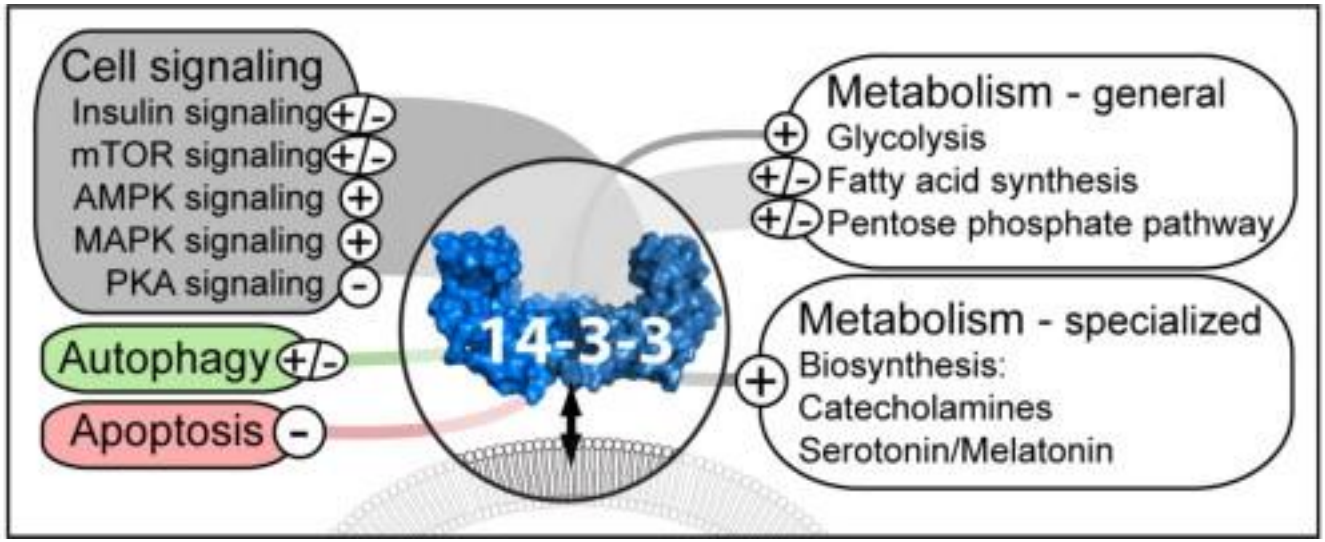
Regulation of RAF protein kinases in ERK signalling

Hugo Lavoie[1] and Marc Therrien[1,2]

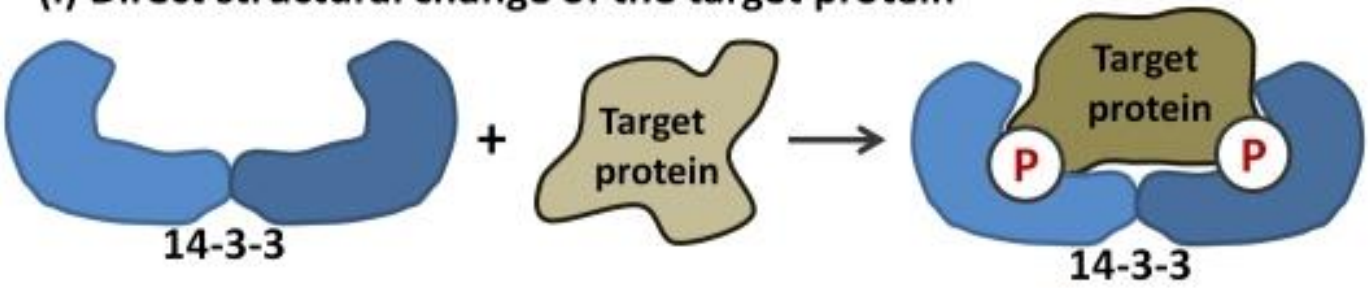


14-3-3 proteins

- Very well conserved in mammals, as well as in plants: they are among the very few signaling elements that are shared by both animals and plants.
- Family of acidic brain proteins. The name was given based on particular elution pattern on chromatography (14th fraction)
- They usually work as dimers
- They bind to peptides, usually containing a phosphorylated serine or threonine residue
- 14-3-3 proteins are a major class of molecular chaperones, with more than 200 proteins that have been shown to be targeted.

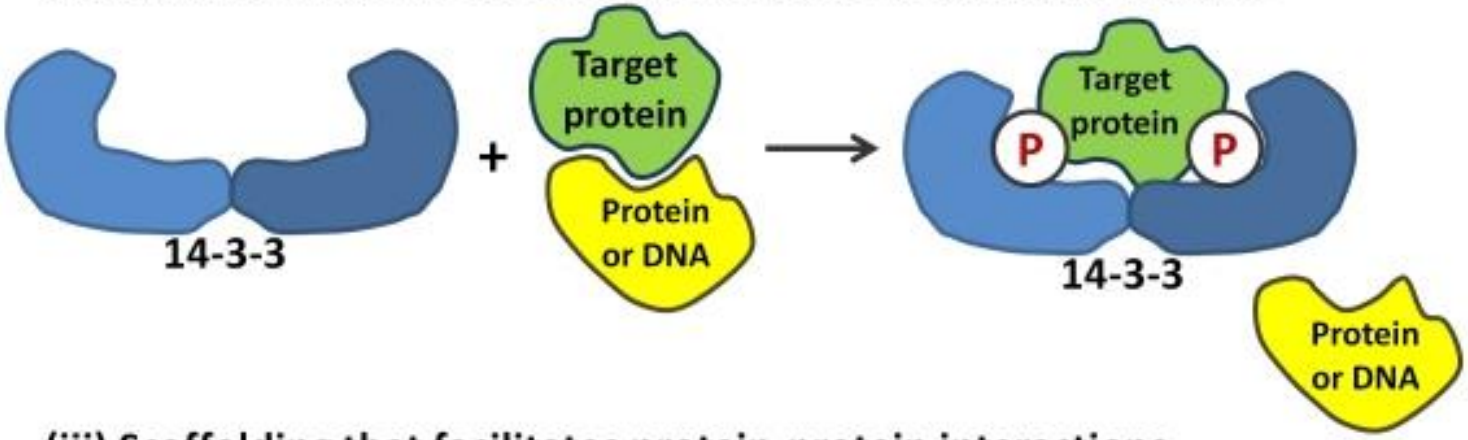


(i) Direct structural change of the target protein



Regulation of enzymatic activity

(ii) Physical occlusion of sequence-specific or structural features

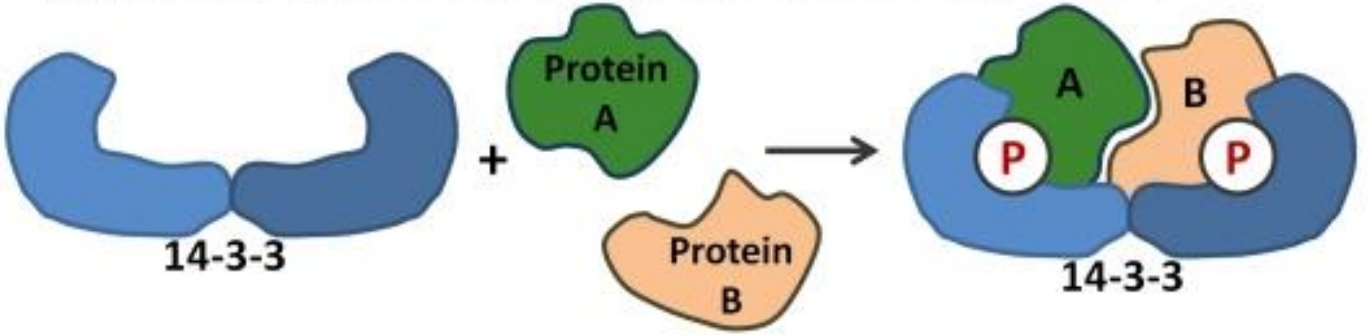


Regulation of subcellular localization

Inhibition of protein-protein or protein-DNA interactions

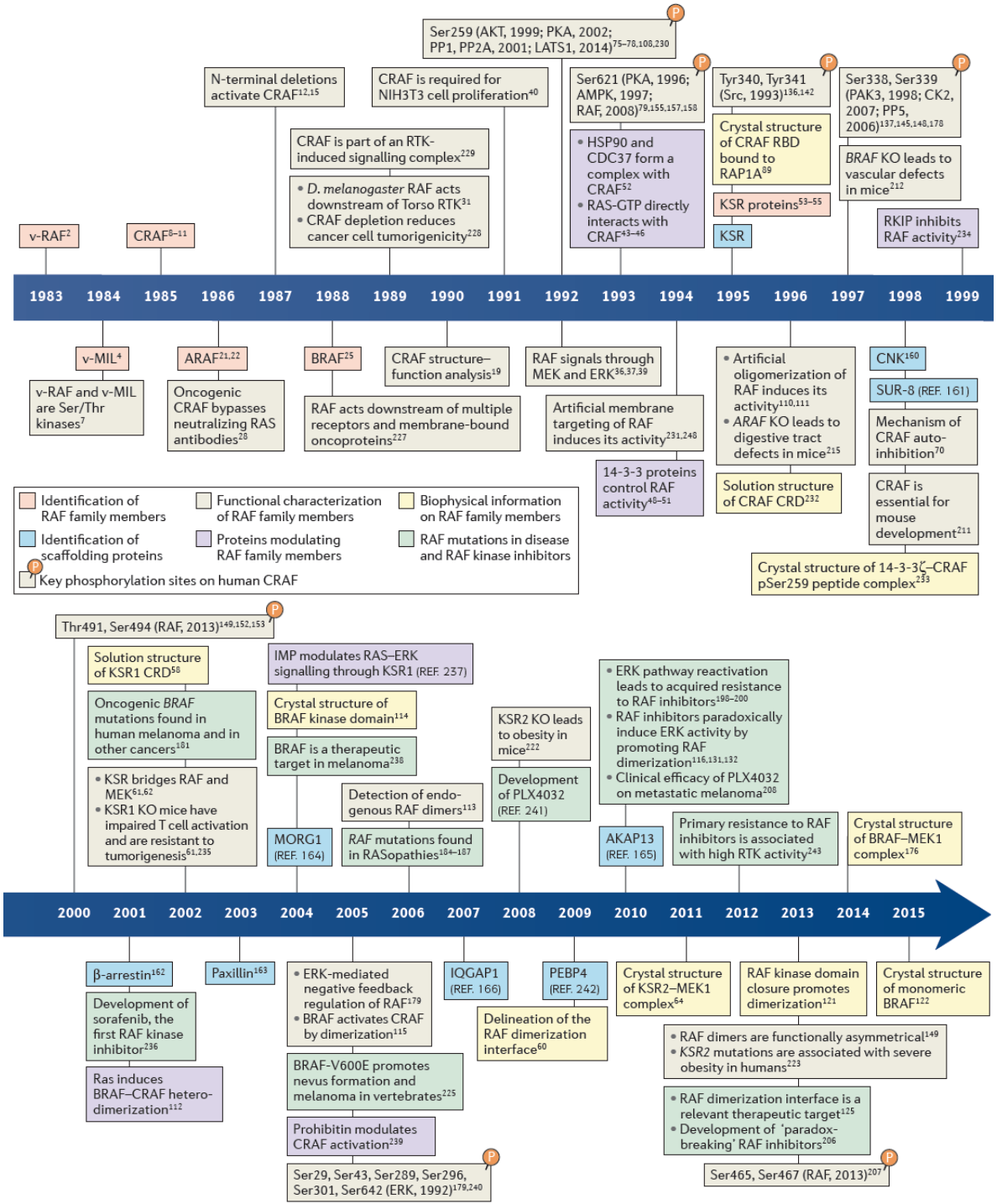
Protection against dephosphorylation or proteolytic degradation

(iii) Scaffolding that facilitates protein-protein interactions

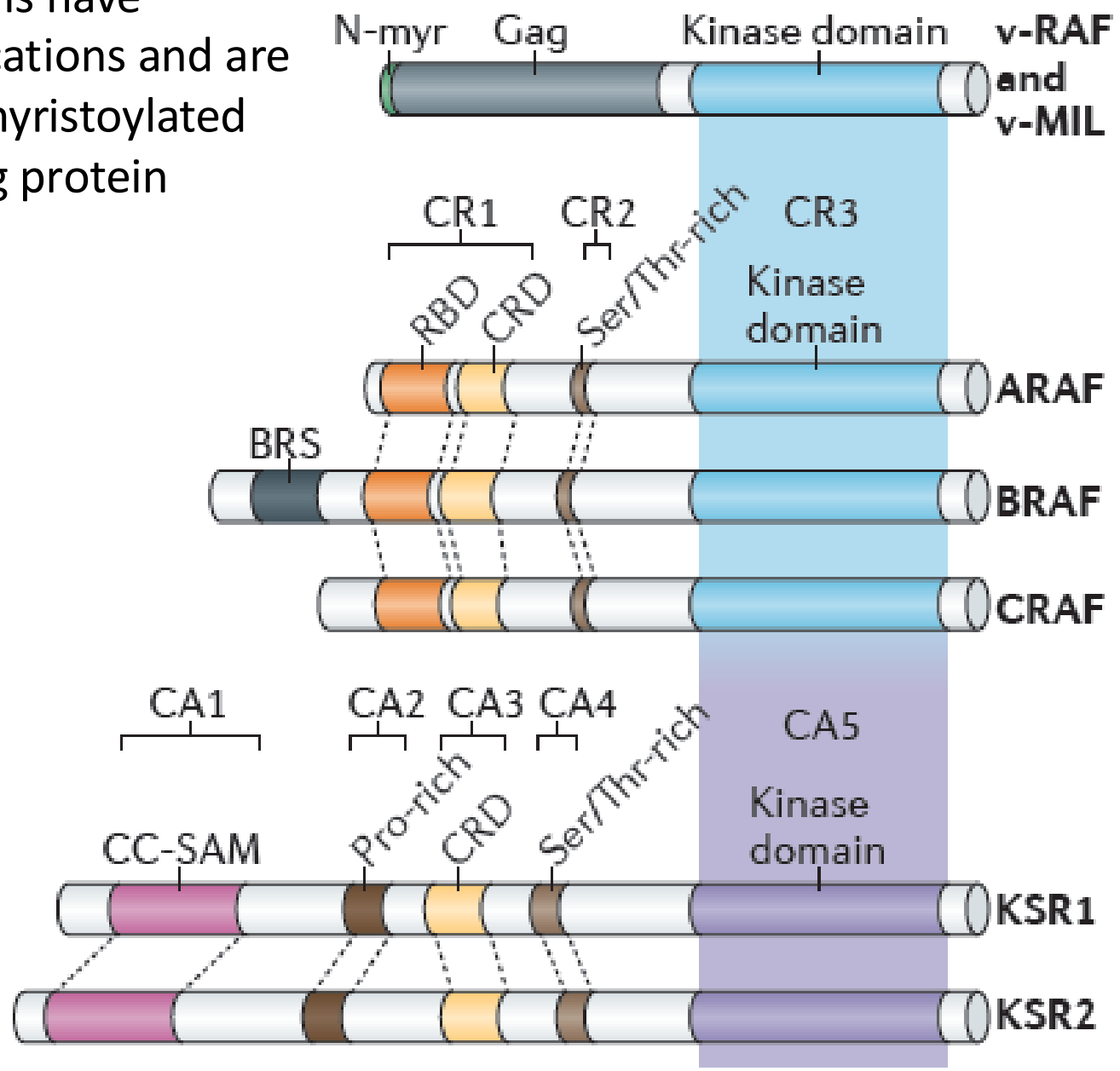


Stabilization of multiprotein complexes

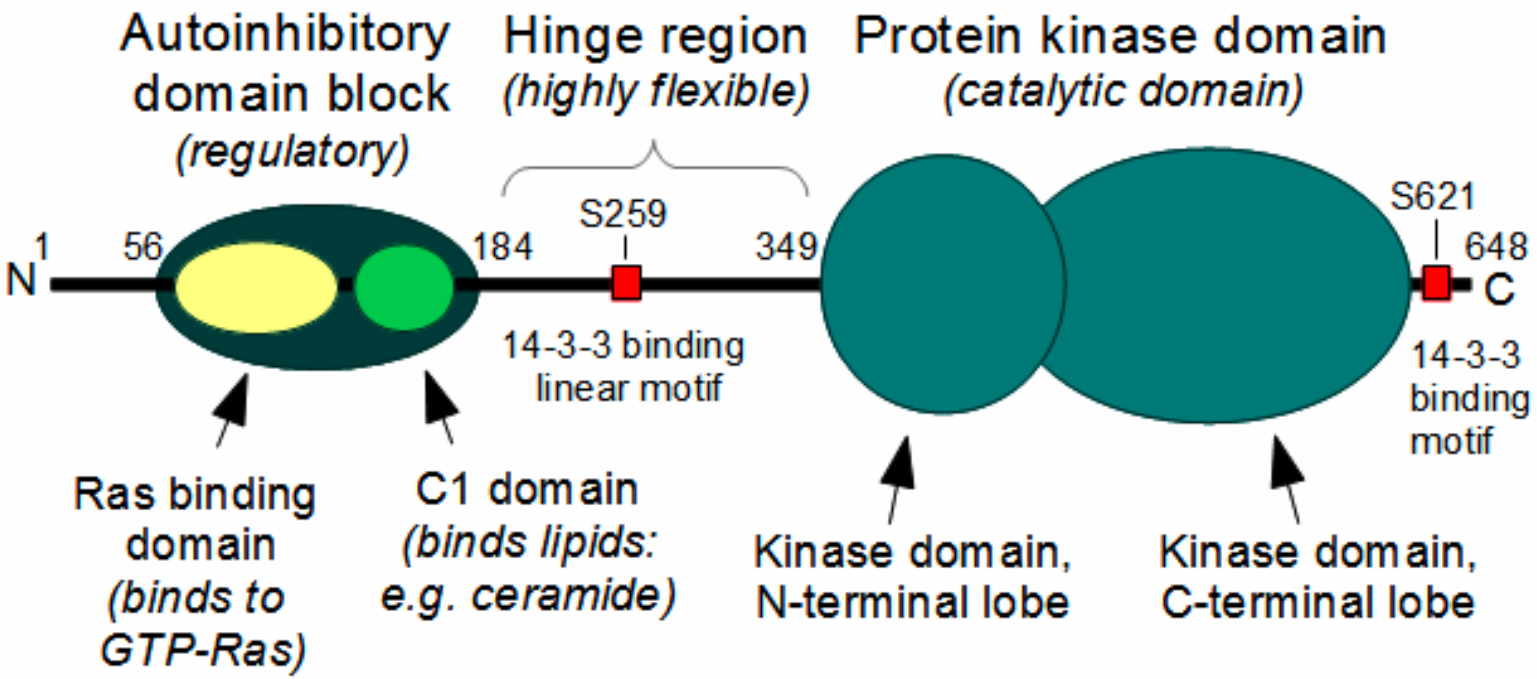
c-Raf


Ser259 (AKT, 1999; PKA, 2002; PP1, PP2A, 2001; LATS1, 2014)[75–78,108,230]
N-terminal deletions activate CRAF[12,15]
CRAF is required for NIH3T3 cell proliferation[40]
Ser621 (PKA, 1996; AMPK, 1997; RAF, 2008)[79,155,157,158]
Tyr340, Tyr341 (Src, 1993)[136,142]
Ser338, Ser339 (PAK3, 1998; CK2, 2007; PP5, 2006)[137,145,148,178]
CRAF is part of an RTK-induced signalling complex[229]
• D. melanogaster RAF acts downstream of Torso RTK[31]
• CRAF depletion reduces cancer cell tumorigenicity[228]
• HSP90 and CDC37 form a complex with CRAF[52]
• RAS-GTP directly interacts with CRAF[43–46]
Crystal structure of CRAF RBD bound to RAP1A[89]
BRAF KO leads to vascular defects in mice[212]
v-RAF[2]
CRAF[8–11]
KSR proteins[53–55]
KSR
RKIP inhibits RAF activity[234]
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
v-MIL[4]
ARAF[21,22]
BRAF[25]
CRAF structure–function analysis[19]
RAF signals through MEK and ERK[36,37,39]
• Artificial oligomerization of RAF induces its activity[110,111]
• ARAF KO leads to digestive tract defects in mice[215]
CNK[160]
v-RAF and v-MIL are Ser/Thr kinases[7]
Oncogenic CRAF bypasses neutralizing RAS antibodies[28]
RAF acts downstream of multiple receptors and membrane-bound oncoproteins[227]
Artificial membrane targeting of RAF induces its activity[231,248]
14-3-3 proteins control RAF activity[48–51]
Solution structure of CRAF CRD[232]
SUR-8 (REF. 161)
Mechanism of CRAF auto-inhibition[70]
CRAF is essential for mouse development[211]
Crystal structure of 14-3-3ζ–CRAF pSer259 peptide complex[233]
Identification of RAF family members
Functional characterization of RAF family members
Biophysical information on RAF family members
Identification of scaffolding proteins
Proteins modulating RAF family members
RAF mutations in disease and RAF kinase inhibitors
Key phosphorylation sites on human CRAF
Thr491, Ser494 (RAF, 2013)[149,152,153]
Solution structure of KSR1 CRD[58]
IMP modulates RAS–ERK signalling through KSR1 (REF. 237)
Oncogenic BRAF mutations found in human melanoma and in other cancers[181]
Crystal structure of BRAF kinase domain[114]
KSR2 KO leads to obesity in mice[222]
• ERK pathway reactivation leads to acquired resistance to RAF inhibitors[198–200]
• RAF inhibitors paradoxically induce ERK activity by promoting RAF dimerization[116,131,132]
• Clinical efficacy of PLX4032 on metastatic melanoma[208]
BRAF is a therapeutic target in melanoma[238]
• KSR bridges RAF and MEK[61,62]
• KSR1 KO mice have impaired T cell activation and are resistant to tumorigenesis[61,235]
Detection of endogenous RAF dimers[113]
Development of PLX4032 (REF. 241)
MORG1 (REF. 164)
RAF mutations found in RASopathies[184–187]
AKAP13 (REF. 165)
Primary resistance to RAF inhibitors is associated with high RTK activity[243]
Crystal structure of BRAF–MEK1 complex[176]
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
β-arrestin[162]
Paxillin[163]
• ERK-mediated negative feedback regulation of RAF[179]
• BRAF activates CRAF by dimerization[115]
IQGAP1 (REF. 166)
PEBP4 (REF. 242)
Crystal structure of KSR2–MEK1 complex[64]
RAF kinase domain closure promotes dimerization[121]
Crystal structure of monomeric BRAF[122]
Development of sorafenib, the first RAF kinase inhibitor[236]
Delineation of the RAF dimerization interface[60]
• RAF dimers are functionally asymmetrical[149]
• KSR2 mutations are associated with severe obesity in humans[223]
Ras induces BRAF–CRAF heterodimerization[112]
BRAF-V600E promotes nevus formation and melanoma in vertebrates[225]
• RAF dimerization interface is a relevant therapeutic target[125]
• Development of 'paradox-breaking' RAF inhibitors[206]
Prohibitin modulates CRAF activation[239]
Ser29, Ser43, Ser289, Ser296, Ser301, Ser642 (ERK, 1992)[179,240]
Ser465, Ser467 (RAF, 2013)[207]

Viral oncoproteins have N-terminal truncations and are fused to the *N*-myristoylated (N-myr) viral Gag protein



Kinase suppressor of RAS

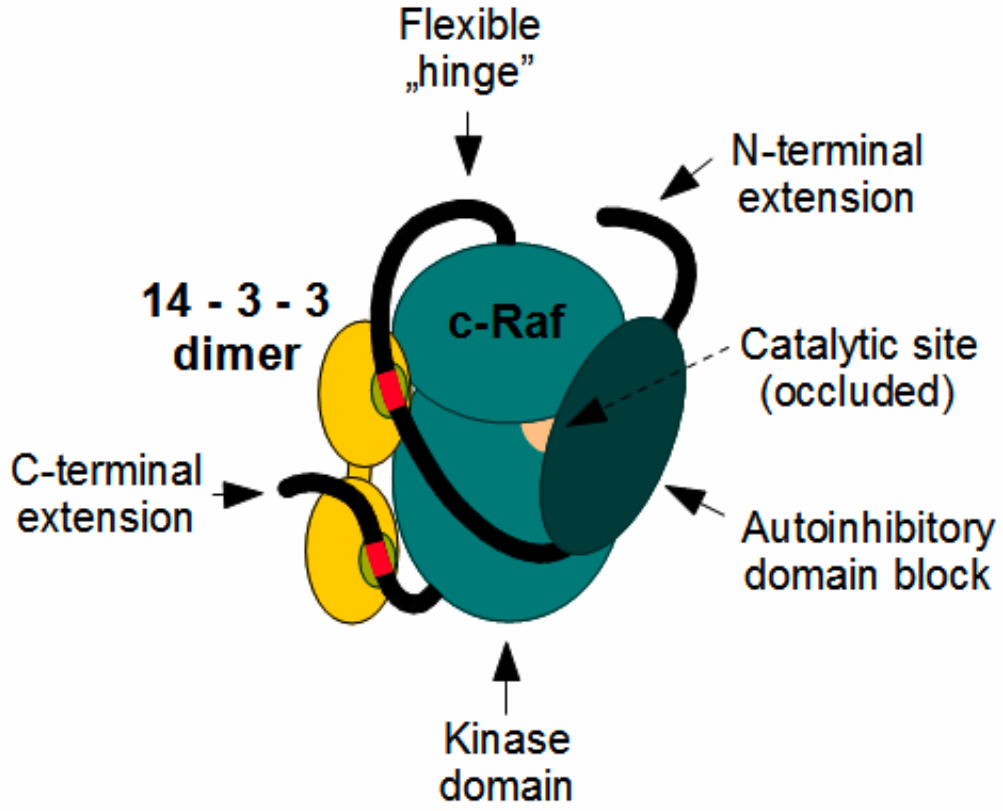


- Ras-binding domain: it binds GTP-Ras
- C1 domain: it is a special zinc finger, rich in cysteines and stabilized by two zinc ions. It interacts with lipids and aids in the recognition of GTP-Ras. The close proximity of these two domains allows them to act as a single unit to negatively regulate the activity of the protein kinase domain, by direct physical interaction.

Between the auto-inhibitory domain block and the catalytic kinase domain, a long and very flexible region acts as a natural "hinge" between the rigidly folded autoinhibitory and catalytic domains.

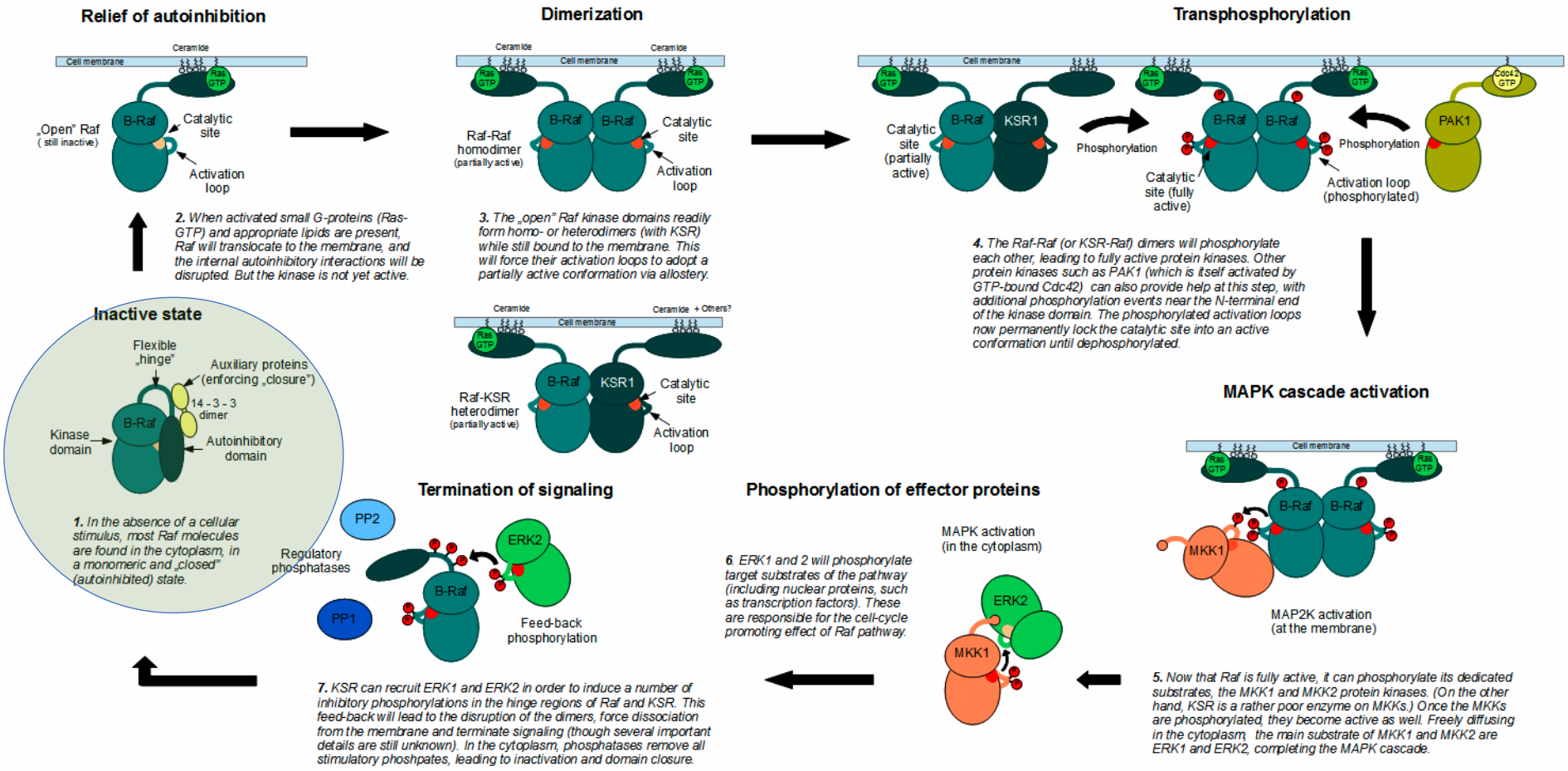
- The C-terminal half of c-Raf folds into a single protein domain, responsible for catalytic activity.

Regulation of c-Raf activity



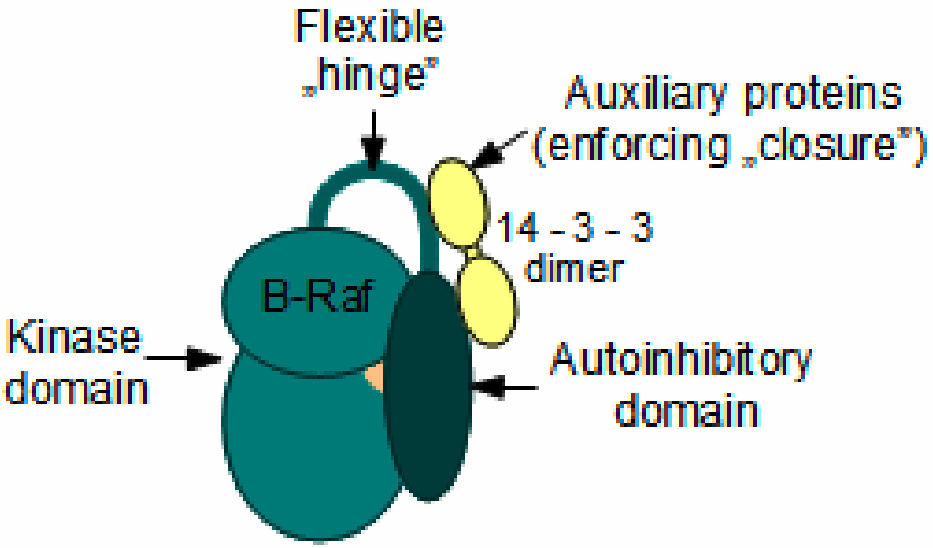
The most important regulatory mechanism involves the direct, physical association of the N-terminal autoinhibitory block to the kinase domain of c-Raf. It results in the occlusion of the catalytic site and full shutdown of kinase activity. This "closed" state can only be relieved if the autoinhibitory domain of Raf engages GTP-bound Ras.

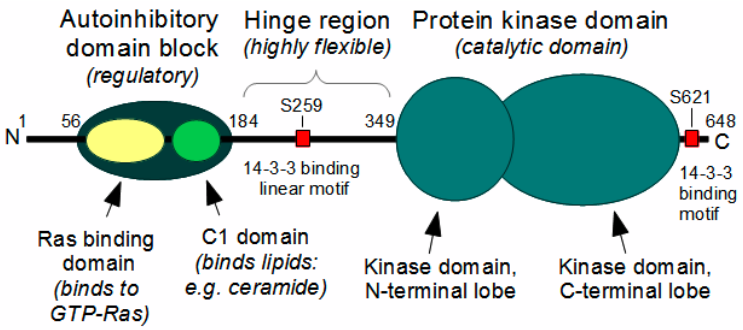
The activation cycle of mammalian c-Raf



The activation cycle of mammalian c-Raf

Inactive state



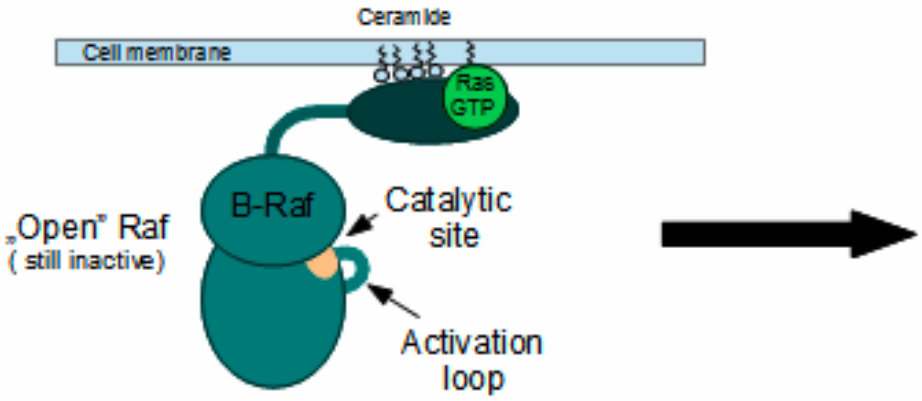


***1.** In the absence of a cellular stimulus, most Raf molecules are found in the cytoplasm, in a monomeric and „closed" (autoinhibited) state.*

In quiescent cells, Raf-1 is phosphorylated on both 14-3-3 binding sites (by PKA?) and 14-3-3 maintains the Raf closed inactive conformation

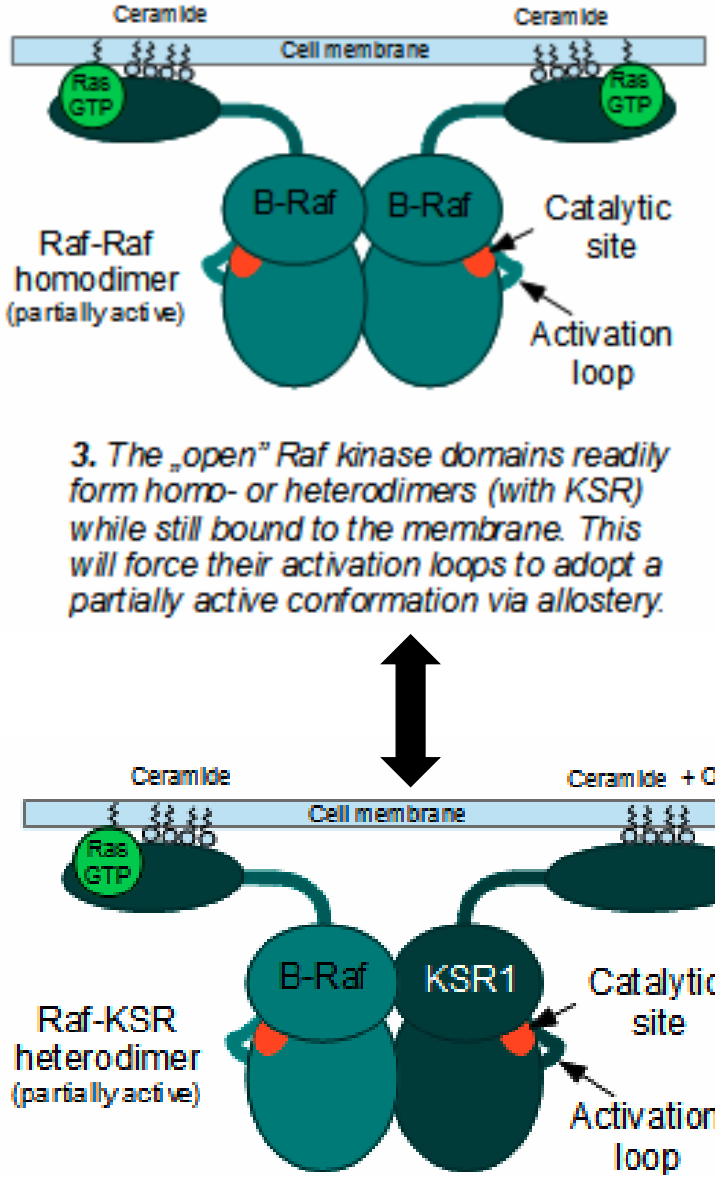
Regulation and function of Raf kinases / *Matallanas et al.* *Genes & Cancer* / vol 2 no 3 (2011)

Relief of autoinhibition



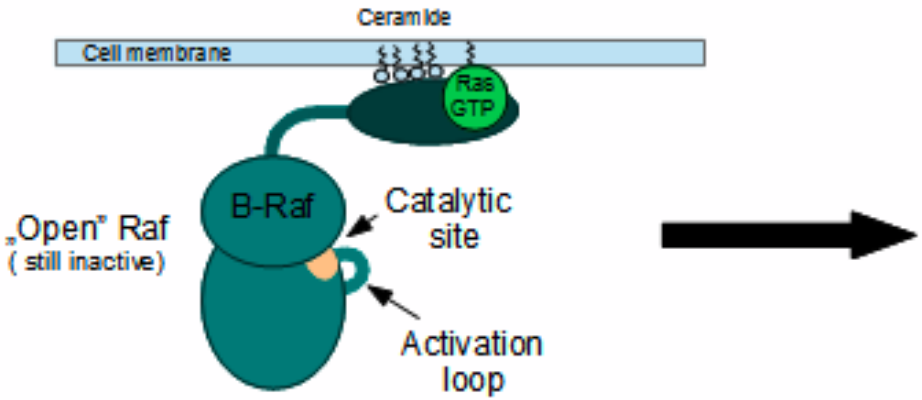
2. *When activated small G-proteins (Ras-GTP) and appropriate lipids are present, Raf will translocate to the membrane, and the internal autoinhibitory interactions will be disrupted. But the kinase is not yet active. Phosphatases PP1 or PP2A are co-recruited to the plasma membrane and de-phosphorylate the inhibitory residues; 14-3-3 proteins are released in the cytoplasm.*

Dimerization



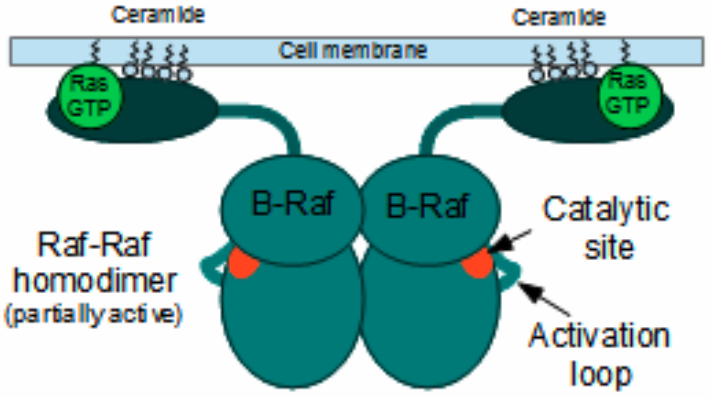
3. *The „open" Raf kinase domains readily form homo- or heterodimers (with KSR) while still bound to the membrane. This will force their activation loops to adopt a partially active conformation via allostery.*

Relief of autoinhibition



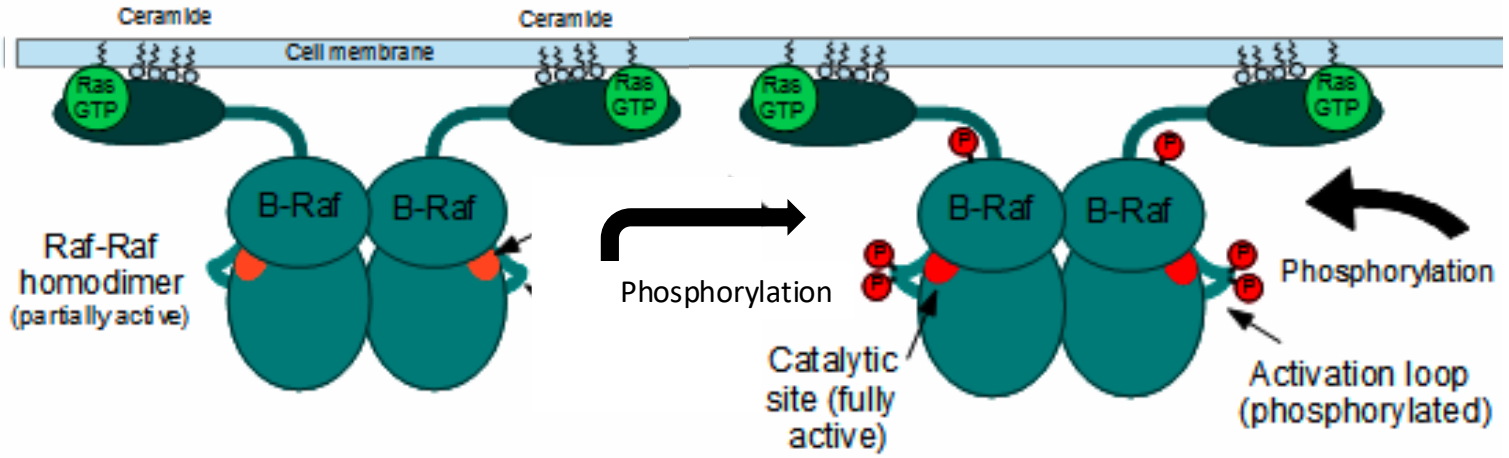
2. *When activated small G-proteins (Ras-GTP) and appropriate lipids are present, Raf will translocate to the membrane, and the internal autoinhibitory interactions will be disrupted. But the kinase is not yet active.*

Dimerization



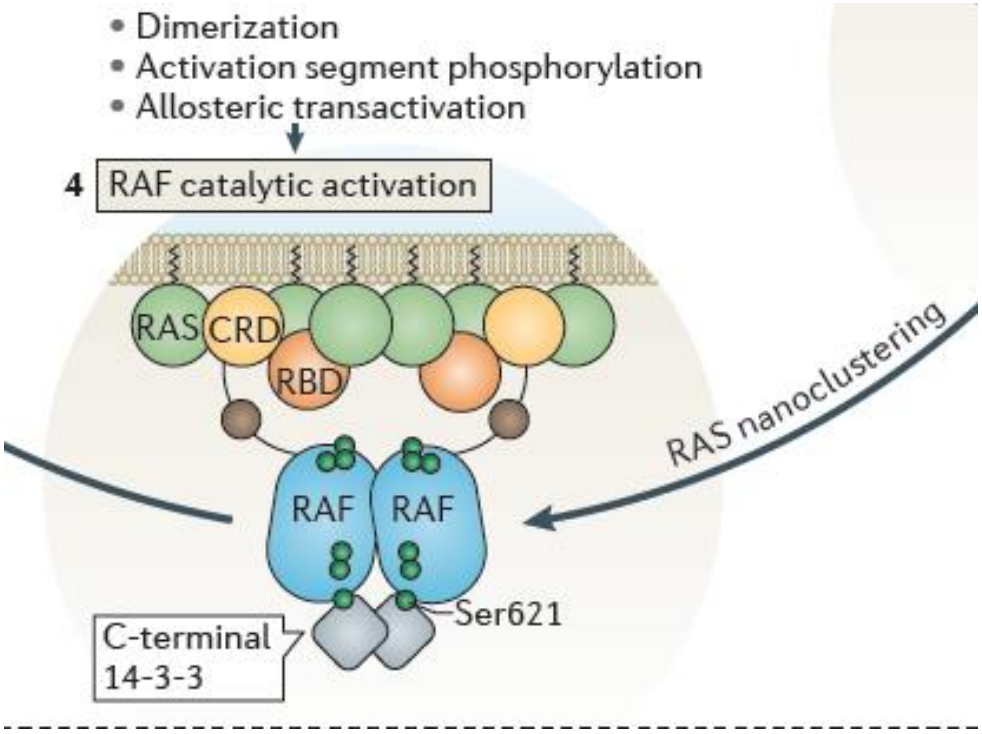
3. *The „open" Raf kinase domains readily form homo- or heterodimers (with KSR) while still bound to the membrane. This will force their activation loops to adopt a partially active conformation via allostery.*

Transphosphorylation



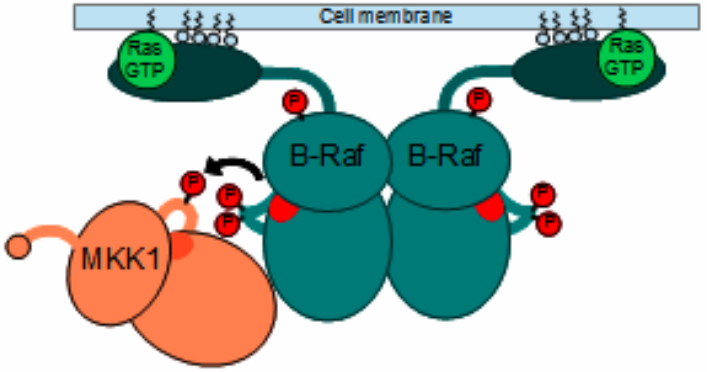
4. *The Raf-Raf (or KSR-Raf) dimers will phosphorylate each other, leading to fully active protein kinases.*

The phosphorylated activation loops now permanently lock the catalytic site into an active conformation until dephosphorylated.



Membrane binding and RAS nanoclustering augment the effective concentration of RAF and thereby contribute to RAF dimerization.

MAPK cascade activation

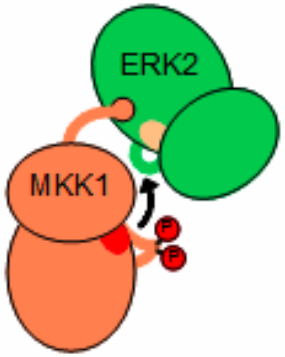


MAP2K activation
(at the membrane)

5. *Now that Raf is fully active, it can phosphorylate its dedicated substrates, the MKK1 and MKK2 protein kinases. (On the other hand, KSR is a rather poor enzyme on MKKs.) Once the MKKs are phosphorylated, they become active as well. Freely diffusing in the cytoplasm, the main substrate of MKK1 and MKK2 are ERK1 and ERK2, completing the MAPK cascade.*

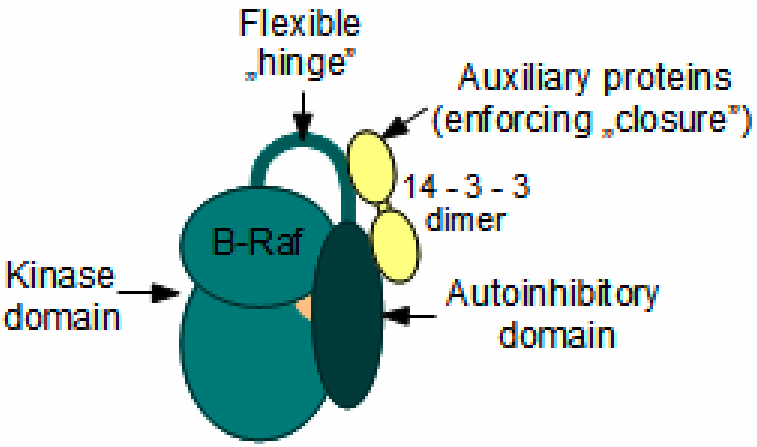
Phosphorylation of effector proteins

MAPK activation
(in the cytoplasm)



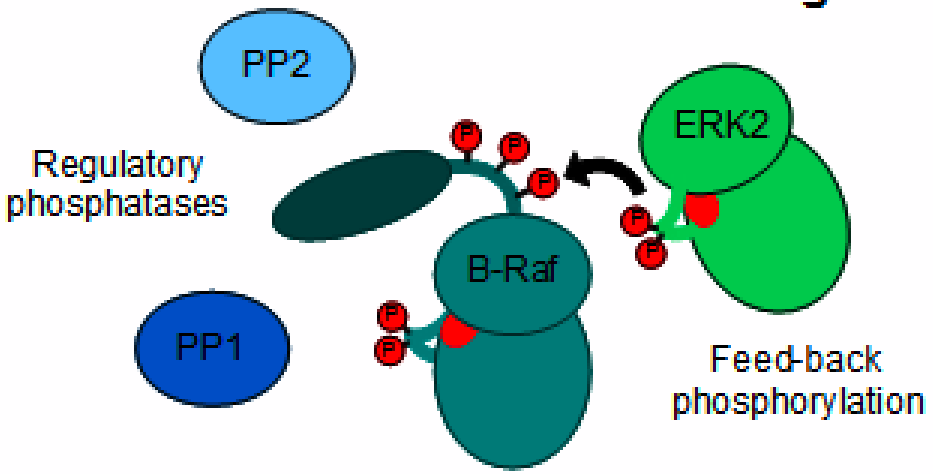
6*. ERK1 and 2 will phosphorylate target substrates of the pathway (including nuclear proteins, such as transcription factors). These are responsible for the cell-cycle promoting effect of Raf pathway.*

Inactive state



***1.** In the absence of a cellular stimulus, most Raf molecules are found in the cytoplasm, in a monomeric and „closed" (autoinhibited) state.*

Termination of signaling



ERK signalling implements a **negative feedback loop** in which ERK phosphorylates several inhibitory sites in distinct regions of activated RAF, causing a release from activated RAS and the disruption of RAF dimers. In the cytoplasm, phosphatases remove stimulatory phosphates leading to inactivation and domain closure

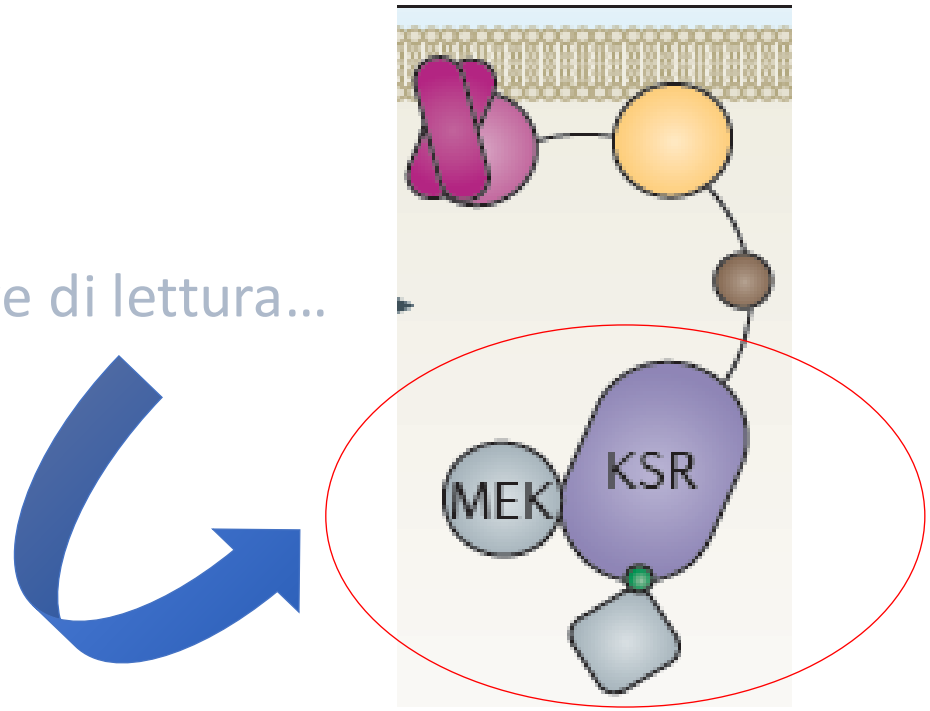
Come e' possibile che le MAPKinasi, solubili nel citoplasma, riescano a trovarsi esattamente nei paraggi di Raf attivato in membrana e subirne la fosforilazione?

Non puo' essere un evento regolato dal caso!!

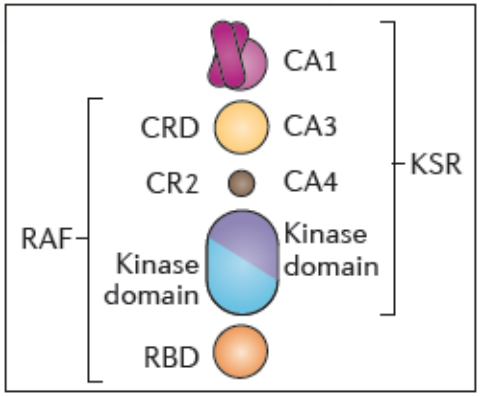
Come e' possibile che le MAPKinasi, solubili nel citoplasma, riescano a trovarsi esattamente nei paraggi di Raf attivato in membrana e subirne la fosforilazione?

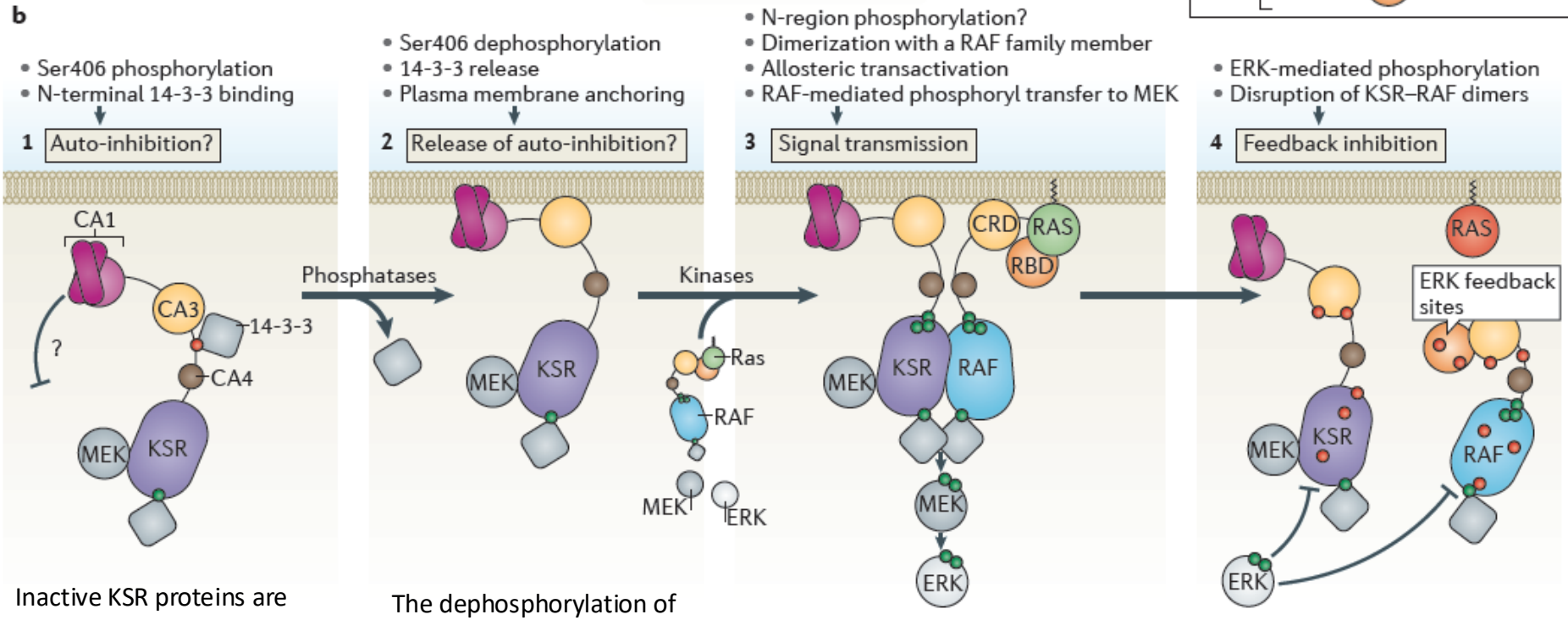
Non puo' essere un evento regolato dal caso!!

Chiave di lettura…



Steps involved in KSR regulation often parallel those defined for RAF proteins





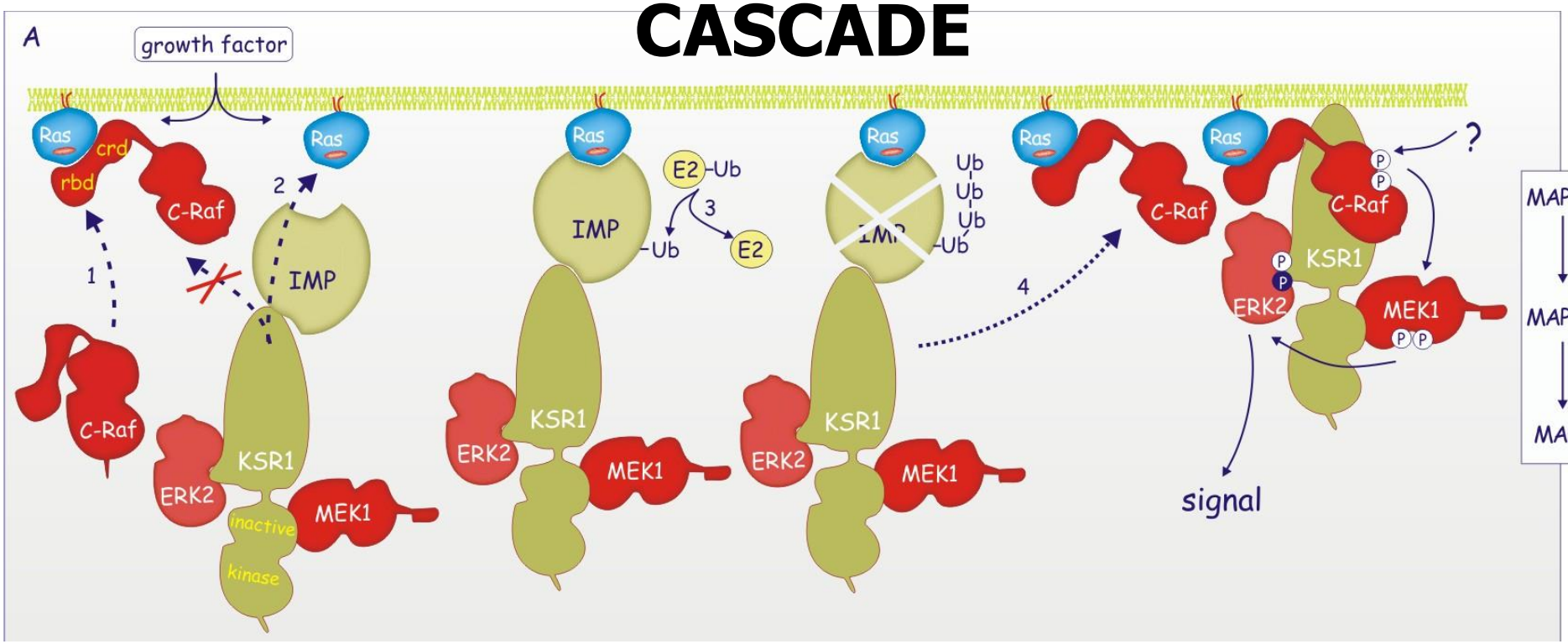
Inactive KSR proteins are kept in the cytosol through interaction with inhibitory 14-3-3 proteins in their N-terminal region. KSR and MEK proteins form constitutive complexes

The dephosphorylation of Ser406 allows 14-3-3 release and plasma membrane anchoring of KSR proteins via conserved area 1 (CA1) and CA3.

KSR proteins heterodimerize with other RAF proteins, leading to RAF transactivation and MEK–ERK signalling.

ERK-mediated negative feedback phosphorylation of several sites in RAF and KSR disrupts RAF–KSR dimers, leading to signal attenuation.

REGULATION OF THE MAPK SIGNALING CASCADE

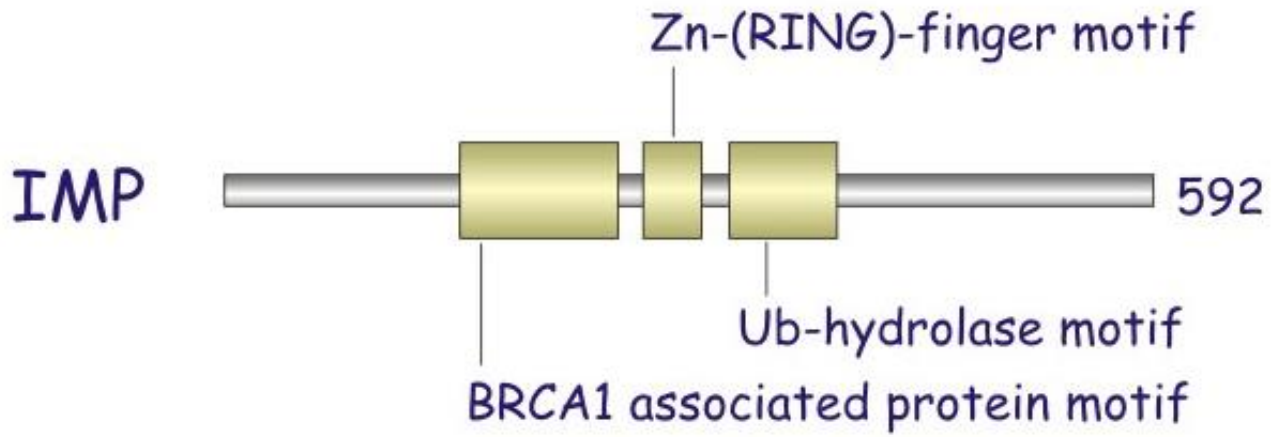


In order to activate the ERK pathway, Ras has not only to recruit C-Raf, but also to remove **IMP**, the inhibitor which prevents formation of the Raf-MEK-ERK signalling cassette.

RasGTP binds IMP and this initiates a series of autoubiquitylations that mark the protein for destruction by the proteasome.

MEK1 and ERK2, linked to the scaffold protein KSR1, are now able to join C-Raf, enabling the signal to pass from one kinase to another

REGULATION OF THE MAPK SIGNALING CASCADE



IMP (impedes mitogenic signal propagation) is an E3-ligase and binds E2-ubiquitin.
The E3-ligase activity is activated by binding to RasGTP and this results in the auto-ubiquitylation of IMP, followed by its destruction.

NATURE | VOL 427 | 15 JANUARY 2004 | www.nature.com/nature

Ras regulates assembly of mitogenic signalling complexes through the effector protein IMP

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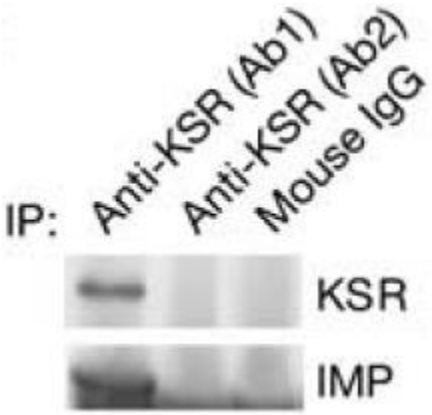
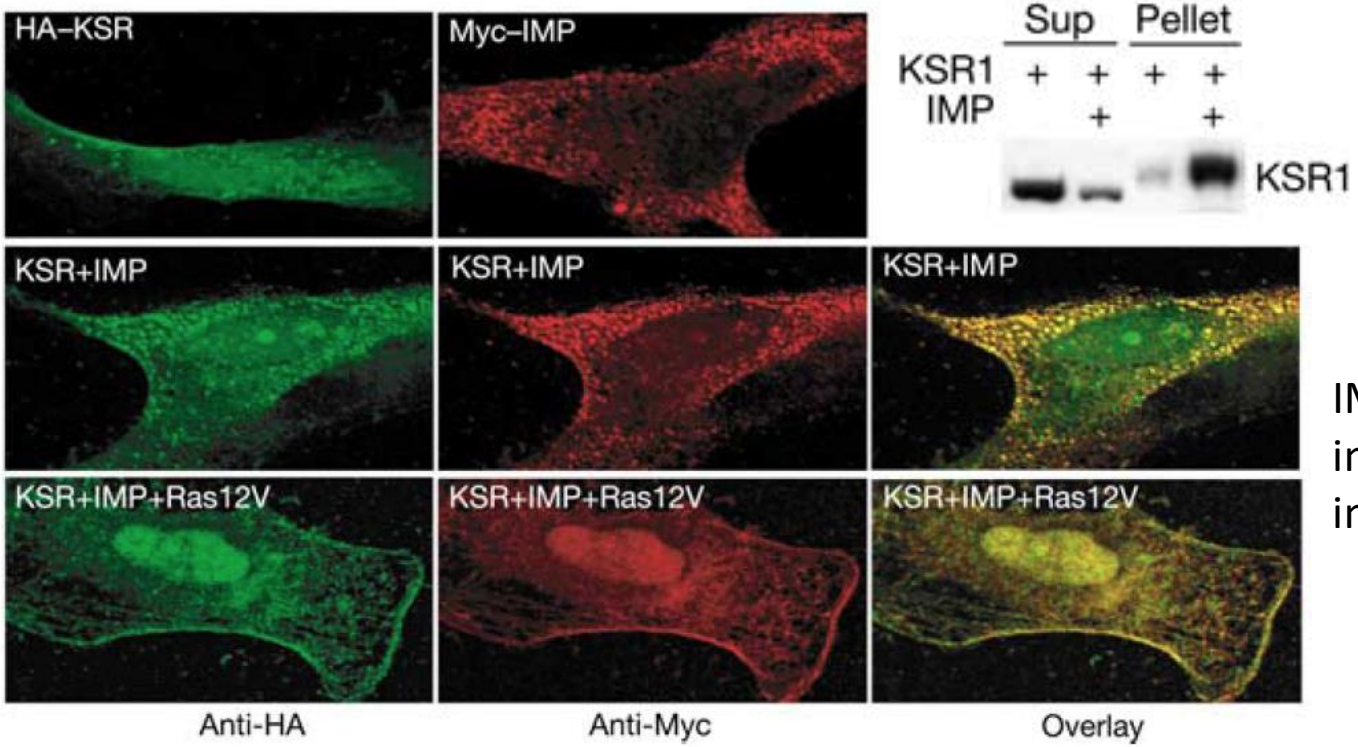
[1]*Department of Cell Biology, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, Texas 75390-9039, USA*
[2]*Eppley Institute for Research in Cancer and Allied Diseases, Department of Pathology, University of Nebraska Medical Center, Omaha, Nebraska 68198-6805, USA*



IMP co-immunoprecipitates with KSR

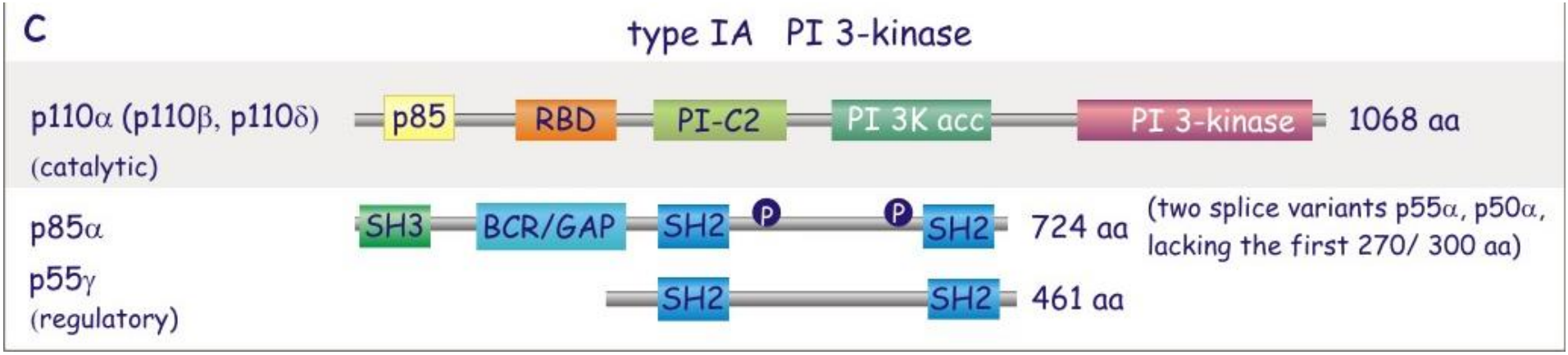


IMP and KSR1 colocalize in Ras-sensitive Triton-insoluble structures

Paradigms for activation of RTKs signaling cascade

As many protein targets of RTKs are located at the cell membrane, translocation to the plasmalemma is essential for activation of many effector proteins

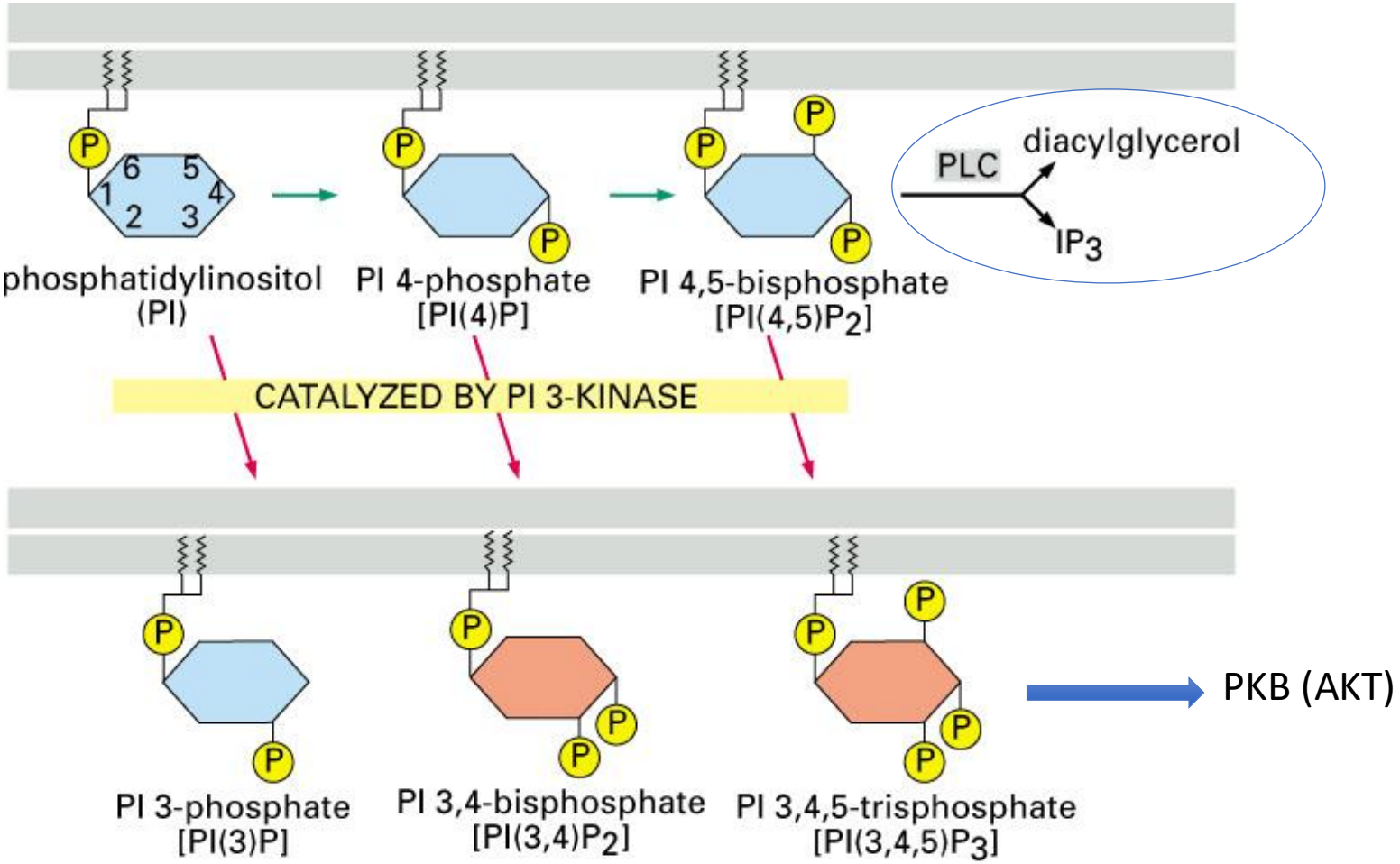
PI3-KINASES



- The catalytic subunits all possess a p85- and Ras-binding site.
- They also have a PI-C2 domain to interact with phospholipids.
- The PI 3K accessory domain serves as a spine on which the other domains are fastened.

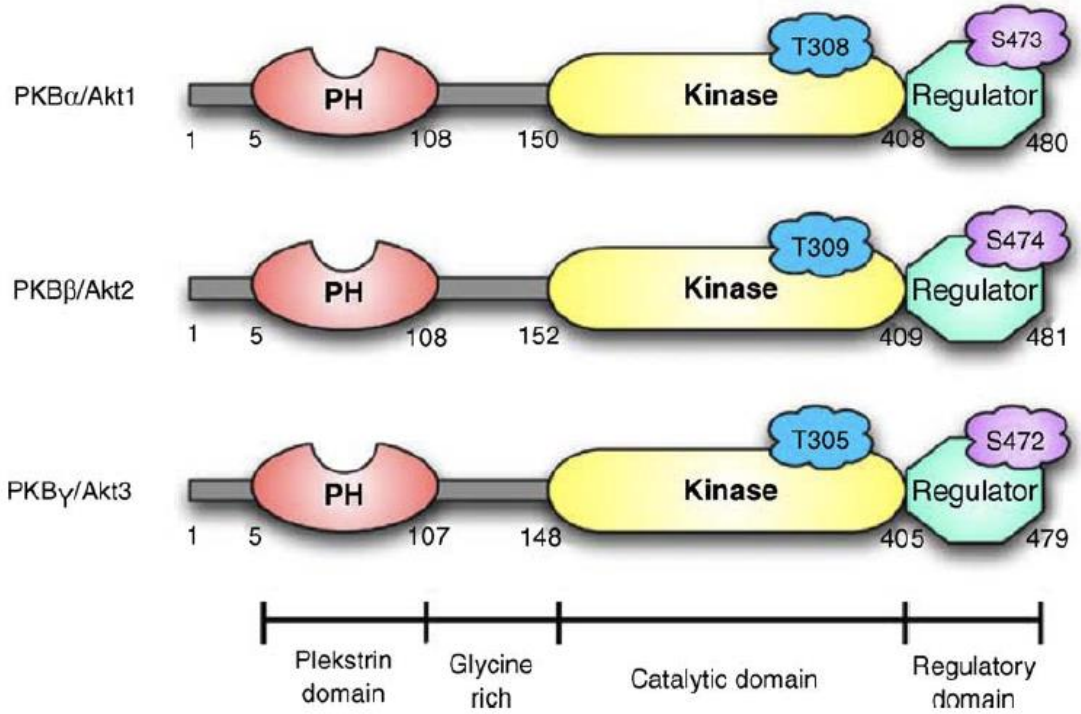
- The regulatory subunits, p85α is particularly versatile: its SH3 domain interacts with proline rich sequences, its BCR/GAP domain interacts with monomeric GTPases of the Rho family (Cdc42 and Rac), whereas its SH2 domain interacts with phosphotyrosines.

Lipids formed by PI3Kinase



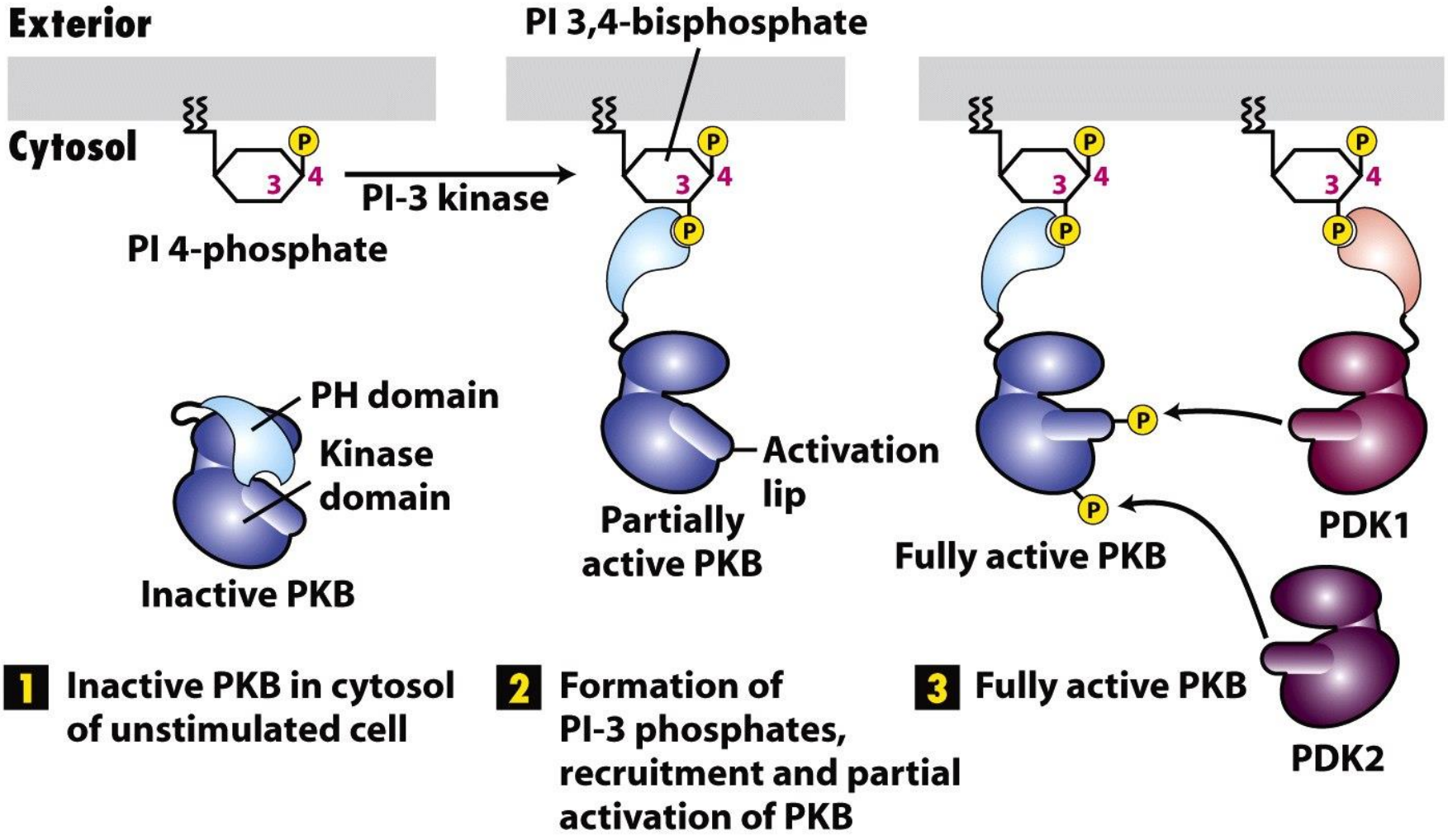
- The PI 3-kinases phosphorylate the 3-OH-position in the inositol ring of the phosphatidylinositol lipids.
- The PI 3-phosphate compounds synthesized by PI-3 kinase activate protein kinase B (PKB).

PKB (AKT)



- Akt/PKB, the cellular homologue of the viral oncogene v-Akt/PKB.
- Akt1 and 2 are ubiquitously expressed; Akt3 is mainly expressed in the brain and testis
- Akt/PKB is an ~57-kDa serine/threonine kinase containing an N-terminal pleckstrin homology
- (PH) domain that mediates binding to phosphatidylinositol (3,4,5) P3 phosphate (PIP3) and a catalytic domain containing a threonine residue (T308 for Akt1/PKB) whose phosphorylation is necessary for activation of Akt/PKB. Next to the kinase domain there is a hydrophobic C-terminal tail containing a second regulatory phosphorylation site (S473 in Akt1/PKB).
- Both phosphorylation events are required for the full activation of Akt/PKB.

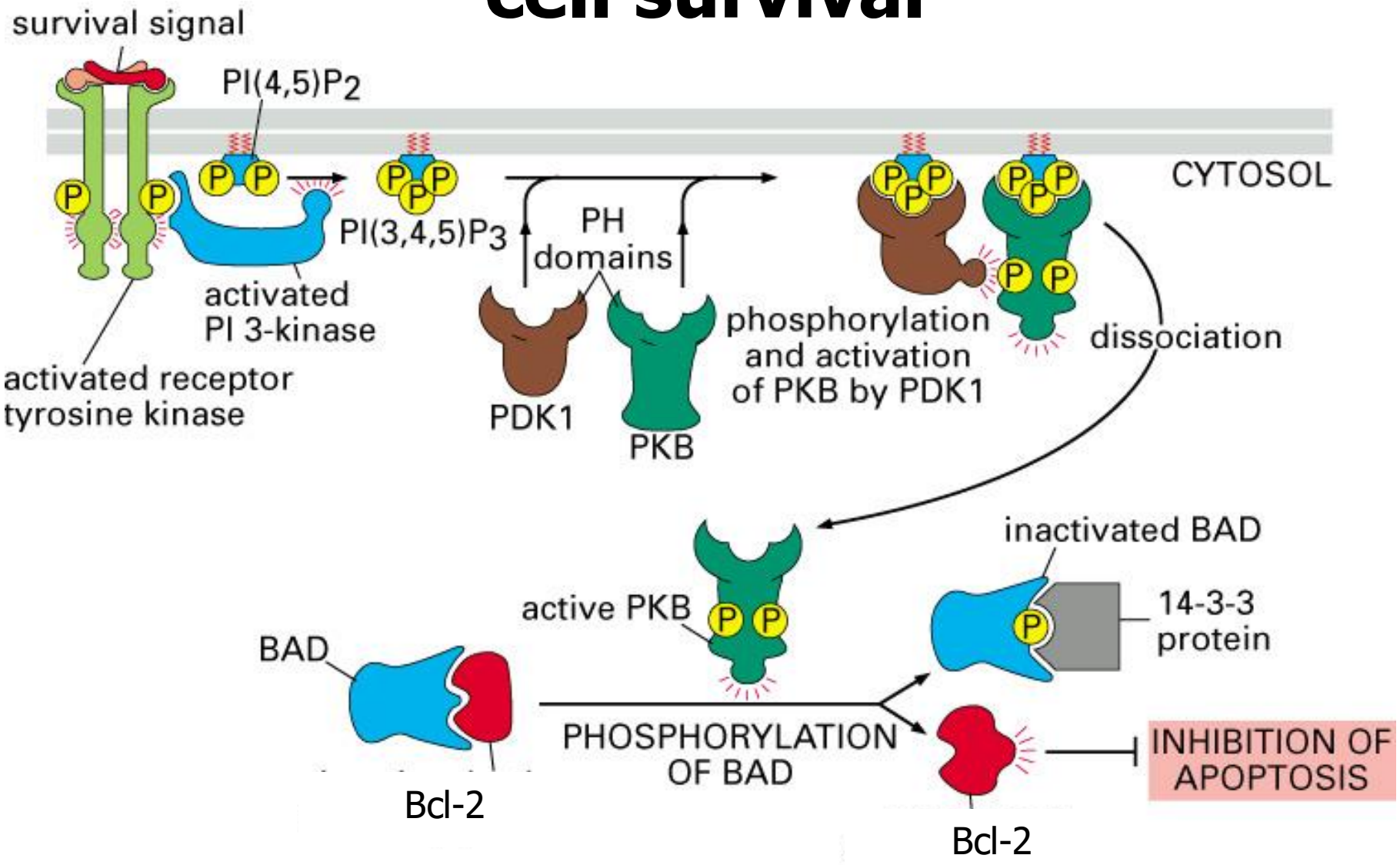
Activation of Protein S/T Kinase B (Akt)



Signaling downstream of PI 3-phosphates is driven by PKB.

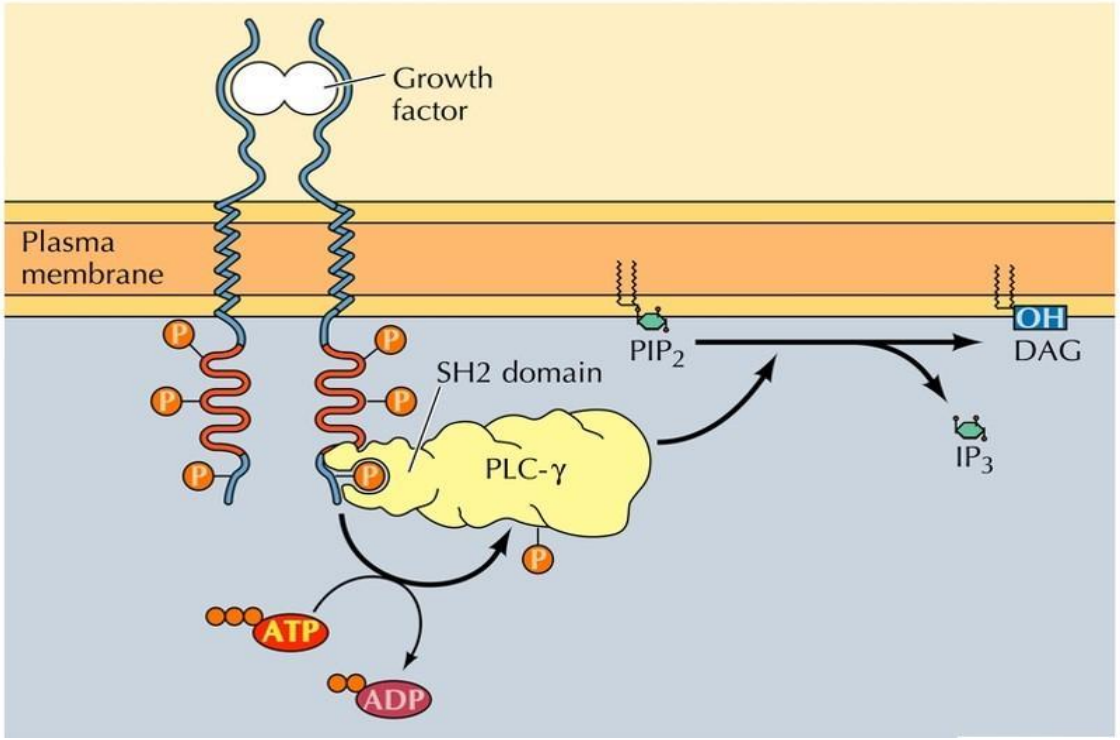
- Generation of 3-phosphoinositides (PIP3) by PI3K recruits Akt/PKB and PDK1 to the membrane. Akt/PKB is subsequently phosphorylated at Thr 308 and at Ser 473 by PDK1 and PDK2, respectively.
- Akt/PKB translocation to the nucleus results in phosphorylation of many substrates that control various biological signaling cascades.

The PI3K pathway to regulate cell survival



PLC$_\gamma$

- PLC-gamma is recruited to receptor tyrosine kinases or to adaptor proteins through the interaction between its SH2 domain
- PLC-gamma has two SH2 domains, and the one in the NH2-terminal side is responsible for the recruitment.
- Then, the PLC is phosphorylated on Tyr residues by the RTK, or by a non-receptor tyrosine kinase associated with the adaptor protein and the phosphorylated protein becomes active.



Phospholipase C (PLC) isozymes

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Advan. Enzyme Regul. 47 (2007) 104–116
ADVANCES IN ENZYME REGULATION
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On/Off-regulation of phospholipase C-γ1-mediated signal transduction

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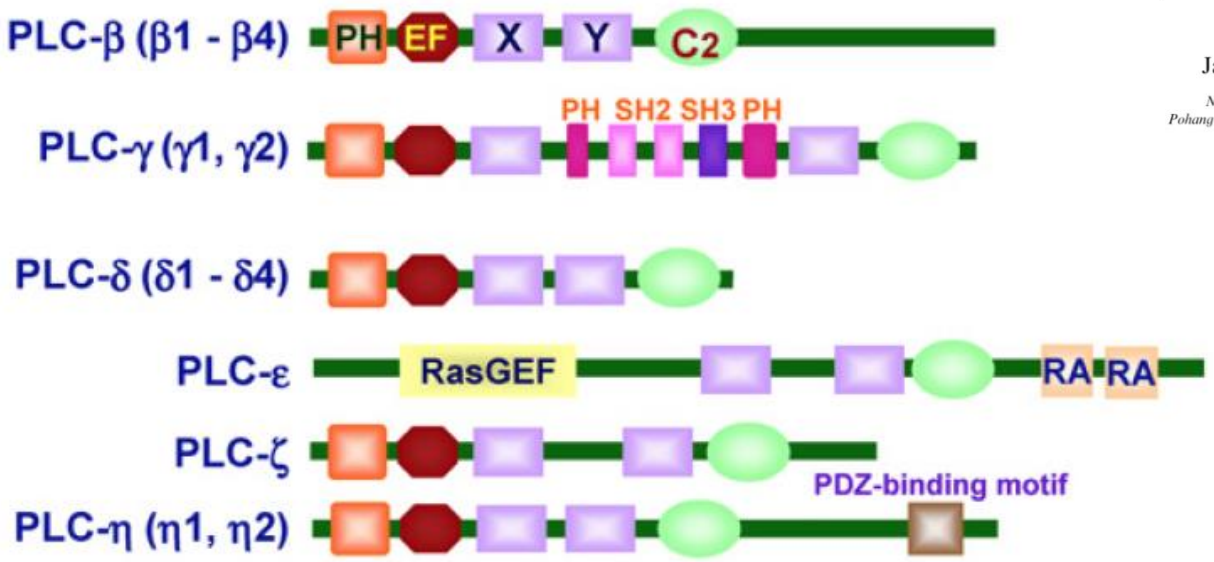
National Research Laboratory of Signaling Network, Department of Life Science, Pohang University of Science and Technology, Pohang, Kyungbuk 790-784, Republic of Korea



Dominio PH (*Pleckstrin Homology*): in grado di legarsi a diversi polifosfoinositidi.

Dominio "a mano EF": in grado di legare il Ca2+, generalmente in misura di uno ione per dominio (Tutte le fosfolipasi infatti necessitano del Ca2+ come cofattore per la loro attività catalitica).

Dominio catalitico: è costituito dall'associazione dei domini X ed Y, ognuno dei quali va a costituire una metà di una struttura simile ad un "*TIM-barrel*" distorto e chiuso.

Dominio C2: rappresenta un modulo proteico, costituito da circa 120 aminoacidi, presente in copia singola o multipla in numerose proteine, molte delle quali sono coinvolte nella trasduzione del segnale o nell'interazione con le membrane lipidiche.

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Phospholipase C-γ: diverse roles in receptor-mediated calcium signaling

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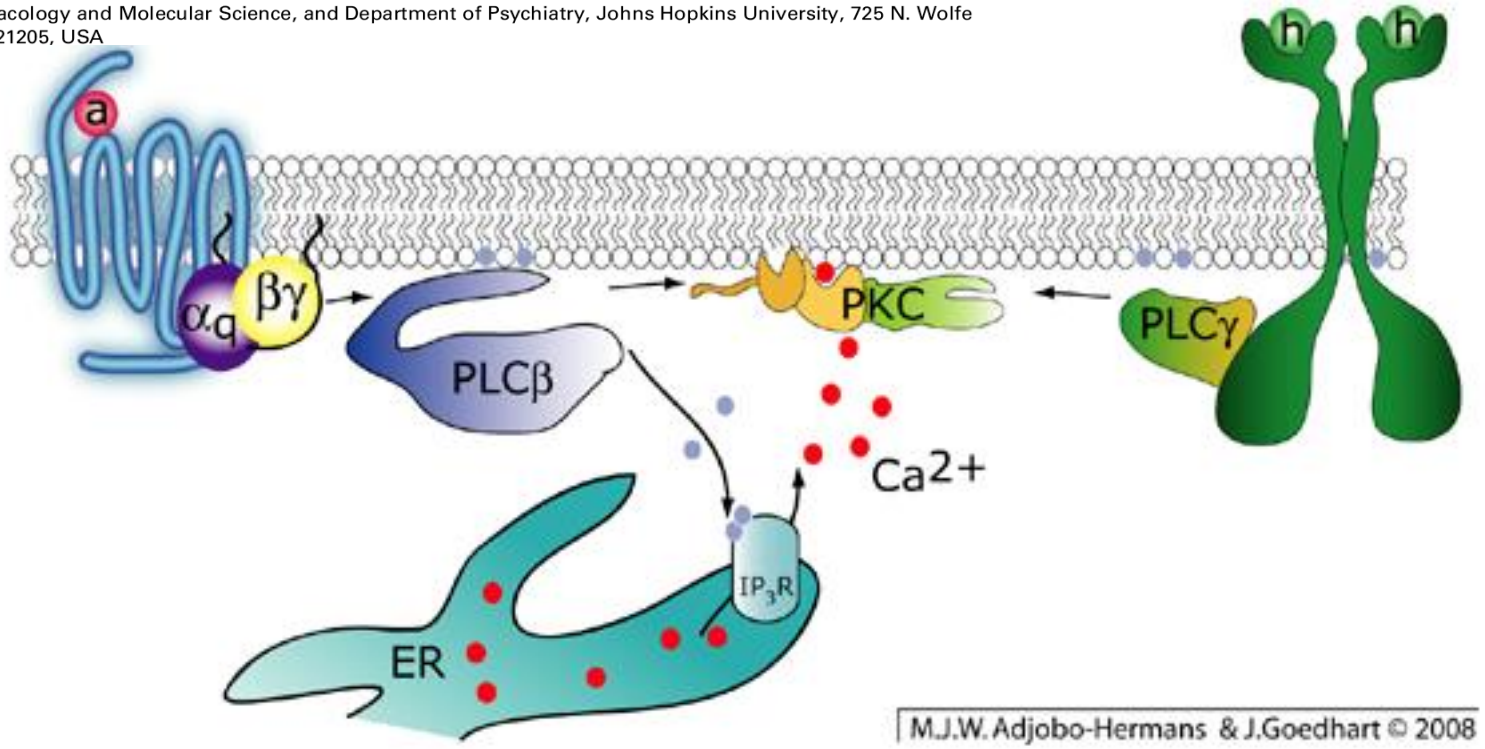


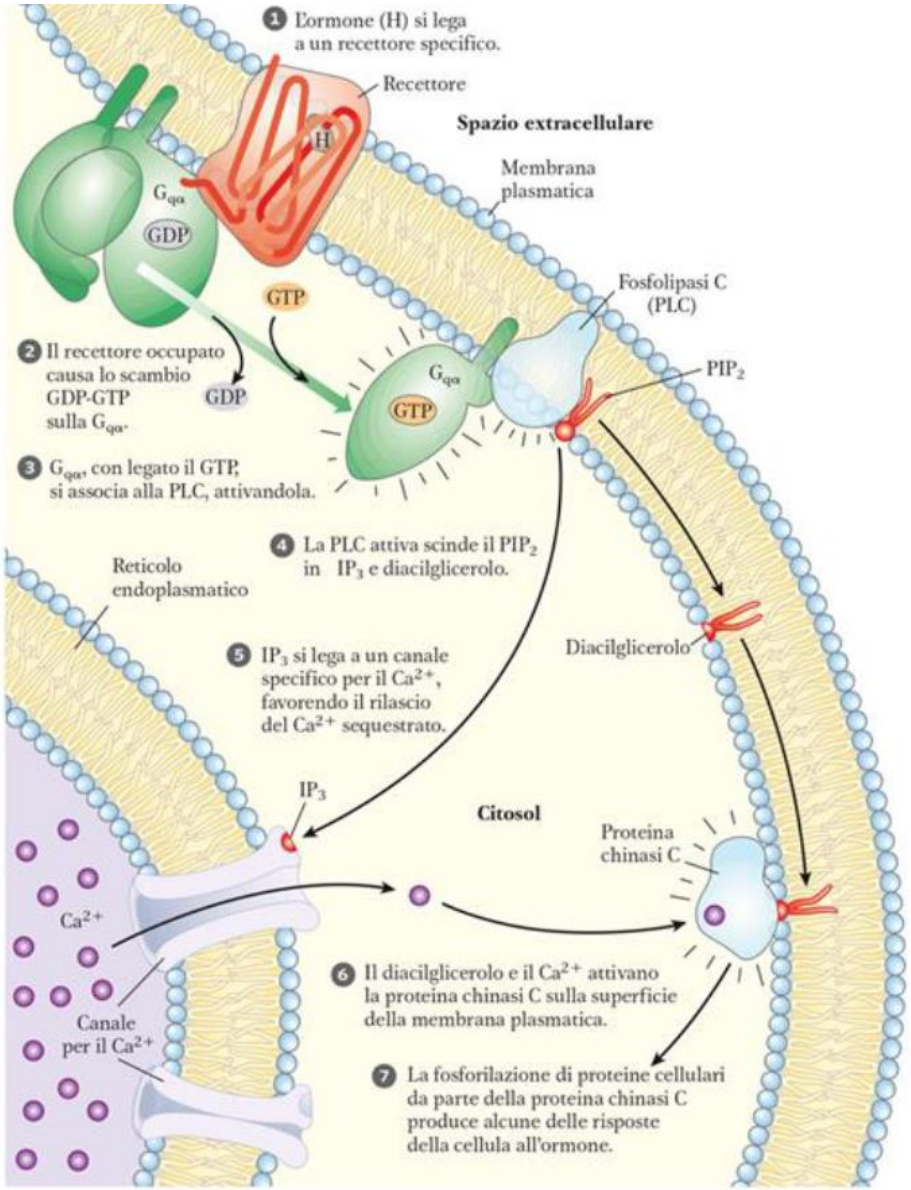
G-protein-coupled receptors (GPCRs) signal to **PLC-β** via activation of G proteins.
PLCs transform PIP2 to DAG and inositol (1,4,5)-triphosphate. IP3 activates the IP3R to cause Ca^{2+} release and Ca^{2+} entry.

FOSFOLIPASI C ATTIVATA DALL'ORMONE E PRODUZIONE DI IP_3

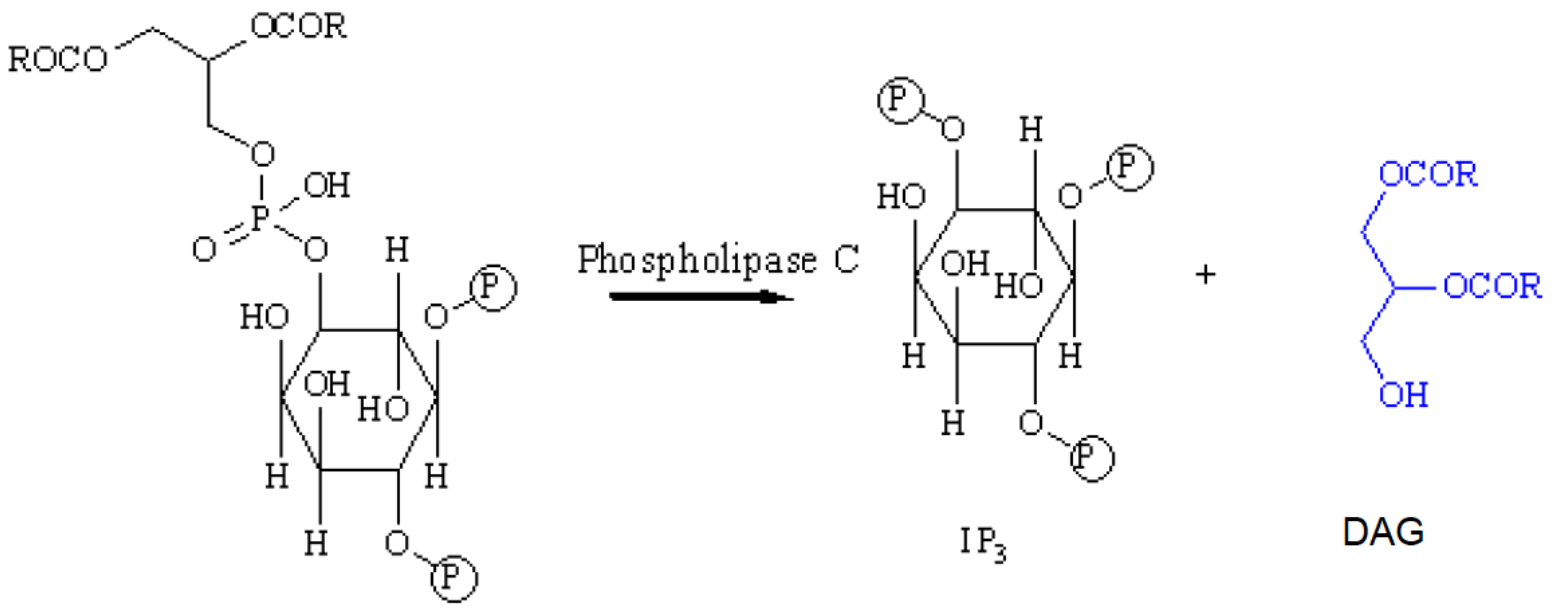
Più isozimi PKC

Proteine bersaglio della PKC: Proteine del citoscheletro, enzimi e proteine nucleari.

Ampio spettro di azioni cellulari: funzioni neuronali e immunitarie, regolazione della divisione cellulare



Formazione di DAG e IP3



fosfatidilinositolo 4,5-bisfosfato

PIP2

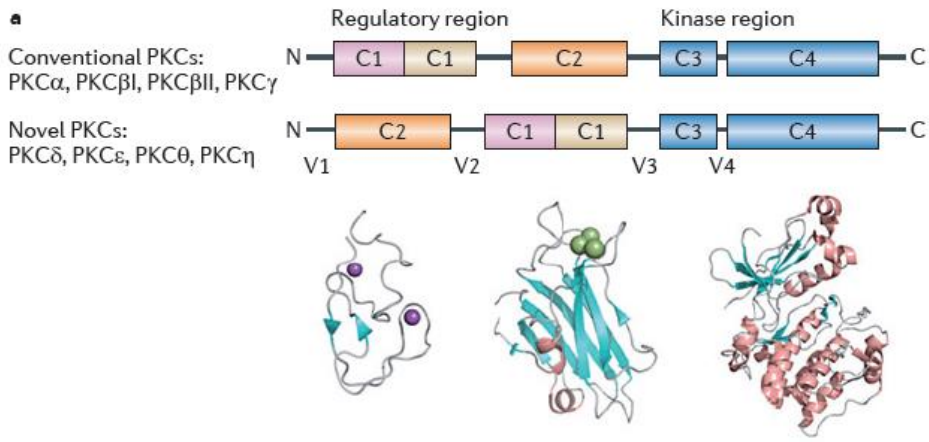
IP_3

DAG

NATURE REVIEWS | DRUG DISCOVERY
VOLUME 11 | DECEMBER 2012 |

Protein kinase C and other diacylglycerol effectors in cancer

Erin M. Griner and Marcelo G. Kazanietz



Isozyme	Overall homology (%)	C1 domain (%)	C2 domain (%)	Kinase domain (%)
PKCβ	38	51	8	65
PKCε	41	44	13	62
PKCθ	64	85	52	67

At a glance

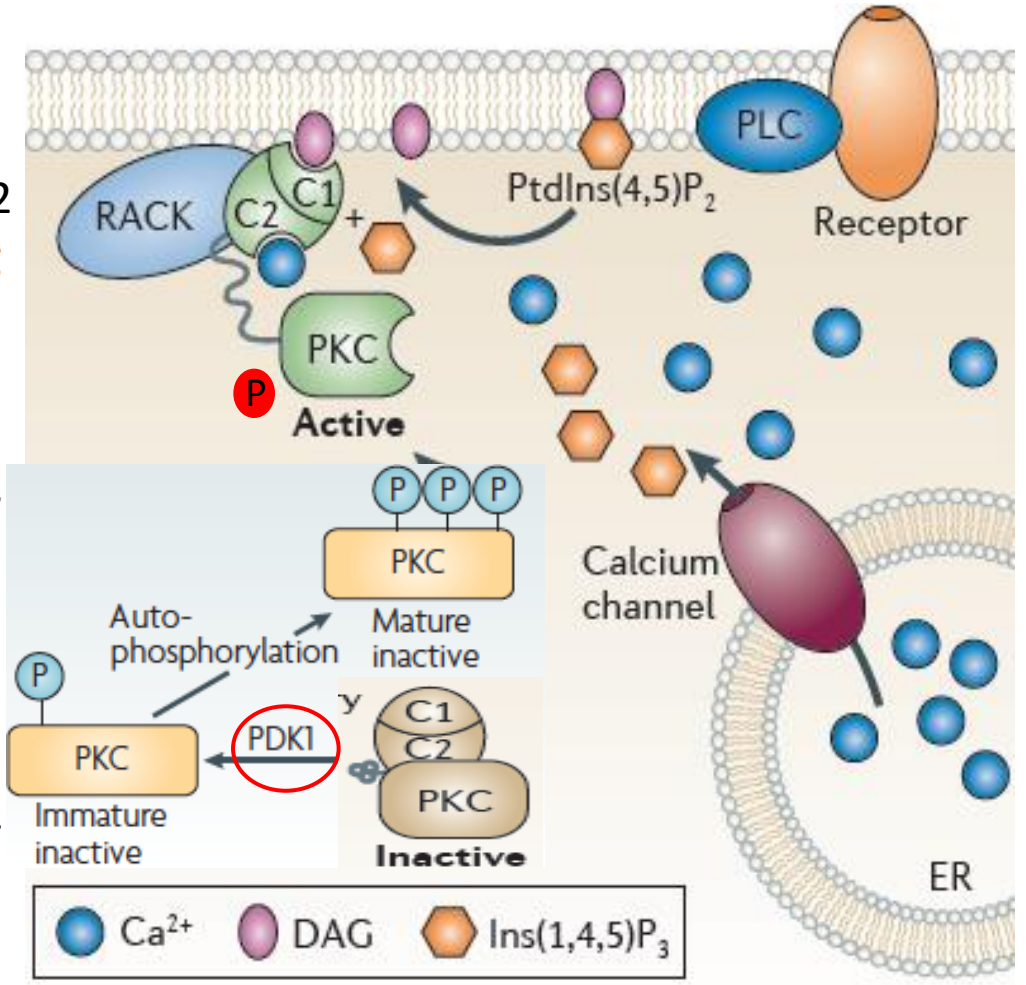
- Protein kinase C (PKC) is a family of serine/threonine kinases that regulates a diverse set of cellular processes including proliferation, apoptosis, cell survival and migration, and there is a substantial amount of evidence linking PKC to tumorigenesis. Studying PKC regulation of these processes and how misregulation might contribute to tumorigenesis is complicated by the fact that each individual PKC isozyme has a distinct role in these processes in a cell-type-dependent manner.
- There is a limited number of instances in which mutation of PKCs in humans is linked to a cancer phenotype; however, altered levels of PKC isoforms can be found in many types of human cancers. In many cases, altered expression of PKC can also be linked to disease progression.
- PKCs were originally thought to be pro-mitogenic kinases, but this effect seems to be PKC-isozyme-dependent and cell-type-dependent, as many PKCs can also inhibit cell-cycle progression. Several PKCs have been shown to be anti-proliferative in various cell types, generally through upregulation of cell-cycle inhibitors.
- PKCε promotes cell survival in many cell types through increased activation of the Akt pathway and upregulation of pro-survival factors. Furthermore, PKCε overexpression has been linked to chemotherapeutic resistance in various cell types.
- PKCδ is generally considered a growth inhibitory or pro-apoptotic PKC, and many types of apoptotic stimuli can induce PKCδ translocation to mitochondria, leading to cytochrome *c* release, caspase-3 cleavage and generation of a constitutively active PKCδ catalytic fragment that is important for phosphorylation of nuclear PKC substrates. Activation of PKCδ can also trigger the autocrine secretion of death factors and kill cells through the activation of the extrinsic apoptotic pathway.
- Several PKCs have been implicated in invasion and metastasis of cancer cells; however, knowledge of the molecular mechanisms through which PKC might contribute to these processes is still vague.
- Emerging evidence indicates that PKC, specifically PKCβII, might be an important mediator of vascular endothelial growth factor (VEGF)-induced angiogenesis and have a role in VEGF-induced endothelial-cell proliferation.
- Several other classes of proteins can be activated by phorbol esters or DAG, including protein kinase D, Ras guanyl nucleotide-releasing proteins, chimaerins, diacylglycerol kinases and Munc13s. Several of these proteins have also been implicated in cancer progression.

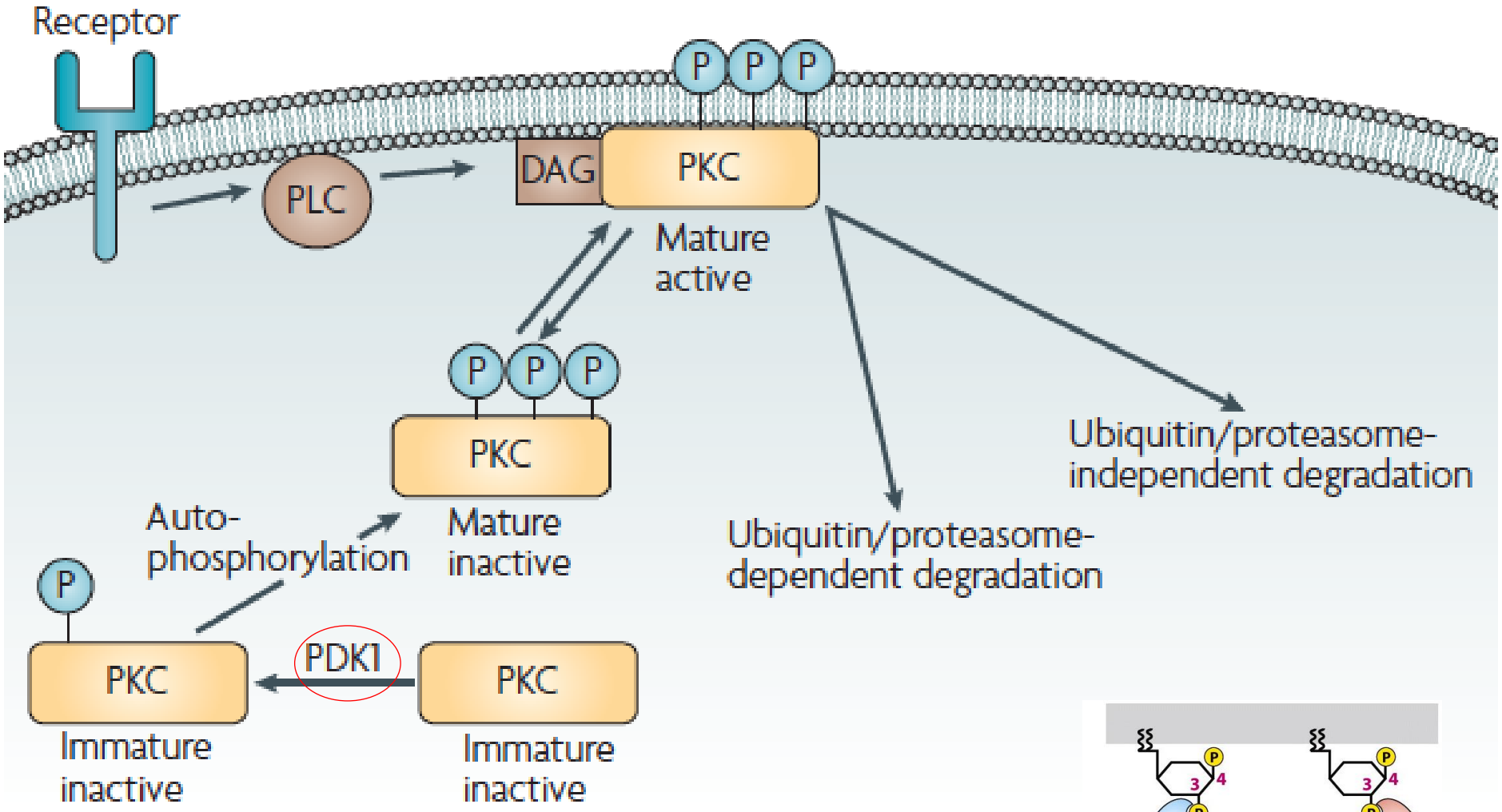
- PDK1 phosphorylates the activation-loop
- Autophosphorylation leads to stabilization of the enzyme.
- PKC, 'primed' for activation by DAG and calcium, is released into the cytosol and kept in an inactive conformation by intramolecular interactions between the N-terminal region and the kinase domain.
-On RTK activation, PKC is tethered to the membrane through calcium binding to the C2 domain, where it interacts with its **anchoring protein**, receptor of activated C-kinase (**RACK**).
-DAG binding confers a high-affinity interaction between PKC and the membrane, leading to a massive conformational change, allowing for substrate binding, phosphorylation and the activation of downstream signalling effectors.

-The short half-life of DAG is probably key for reversing the activation of PKC, down-regulated through internalization (caveolae or ubiquitin–proteasome-dependent)

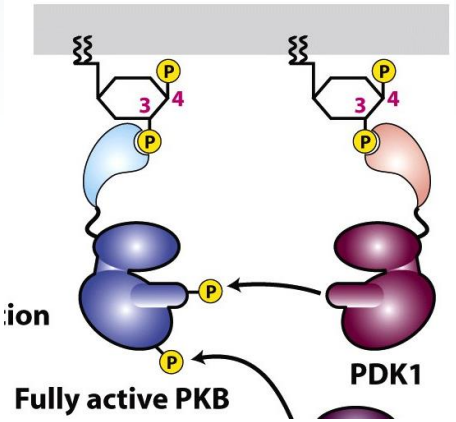
Protein kinase C, an elusive therapeutic target?

Daria Mochly-Rosen[1,2], Kanad Das[2] and Kevin V. Grimes[1,2]

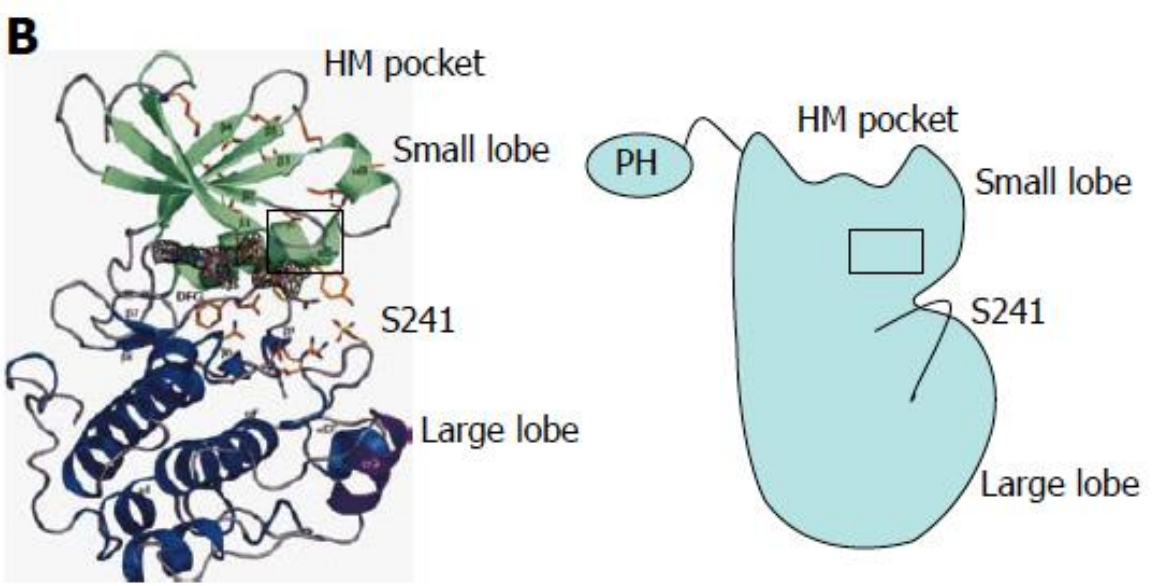




Inactive conformation by intramolecular interactions between the N-terminal region and the kinase domain



Phosphoinositide-dependent kinase-1 (PDK1)



- PDK1 is a **master kinase**, crucial for the activation of AKT/PKB and many other kinases including PKC, S6K, SGK.
- Mice lacking PDK1 die during early embryonic development, indicating that this enzyme is critical for transmitting the growth-promoting signals necessary for normal mammalian development.
- The structure of PDK1 can be divided into two domains; the kinase or catalytic domain and the PH domain.
- The PH domain functions mainly in the interaction of PDK1 with phosphatidylinositol (3,4)-bisphosphate and phosphatidylinositol (3,4,5)-trisphosphate.
- The kinase domain has crucial binding sites: the substrate binding site, the ATP binding site.
- **PDK1 is constitutively active and at present, there is no known inhibitor for PDK1**.

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Cell Signaling by Receptor Tyrosine Kinases

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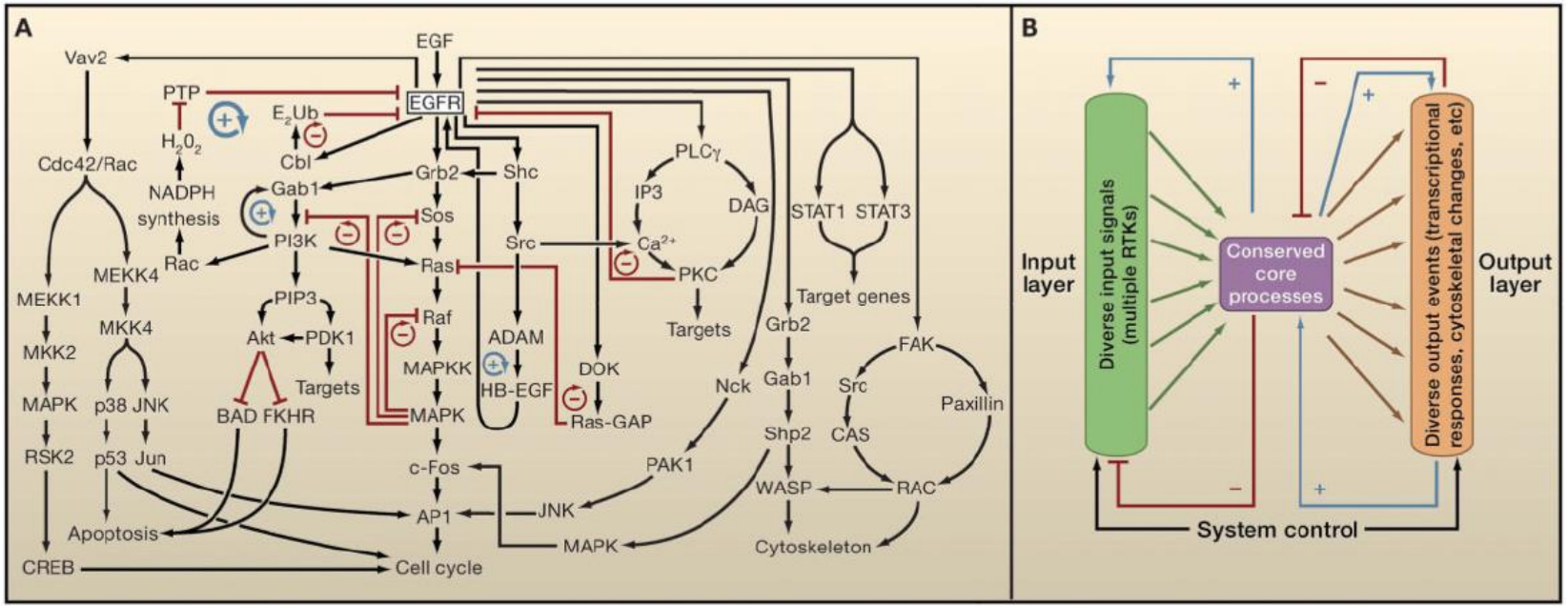
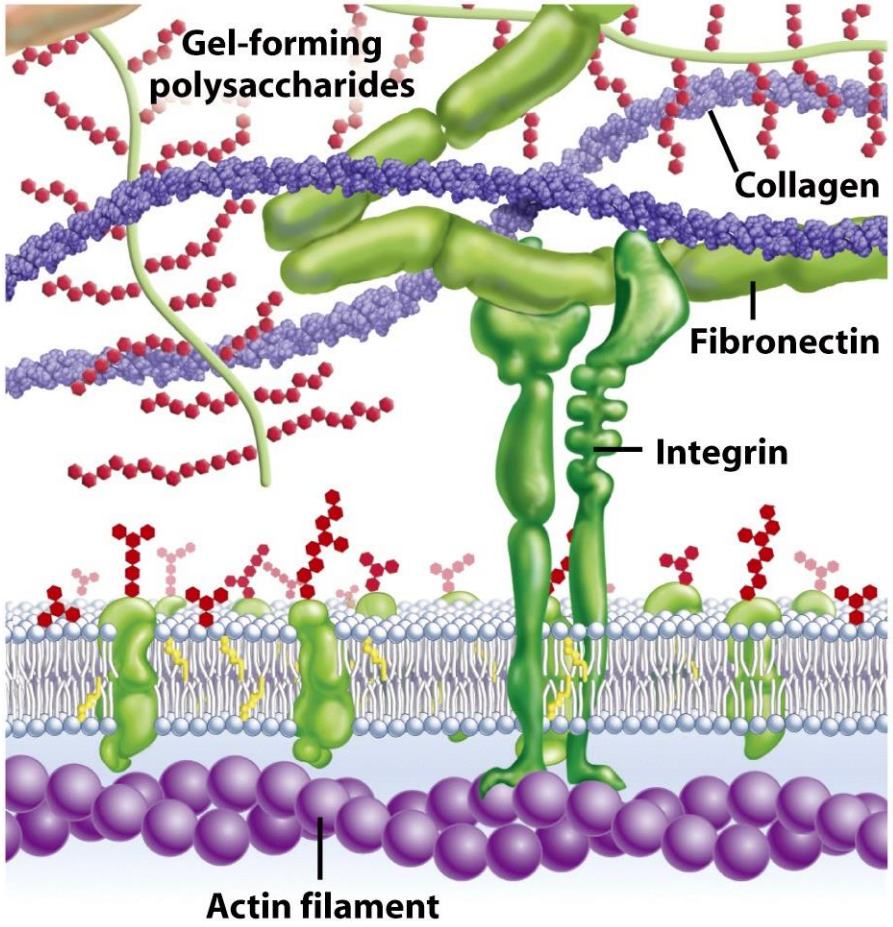


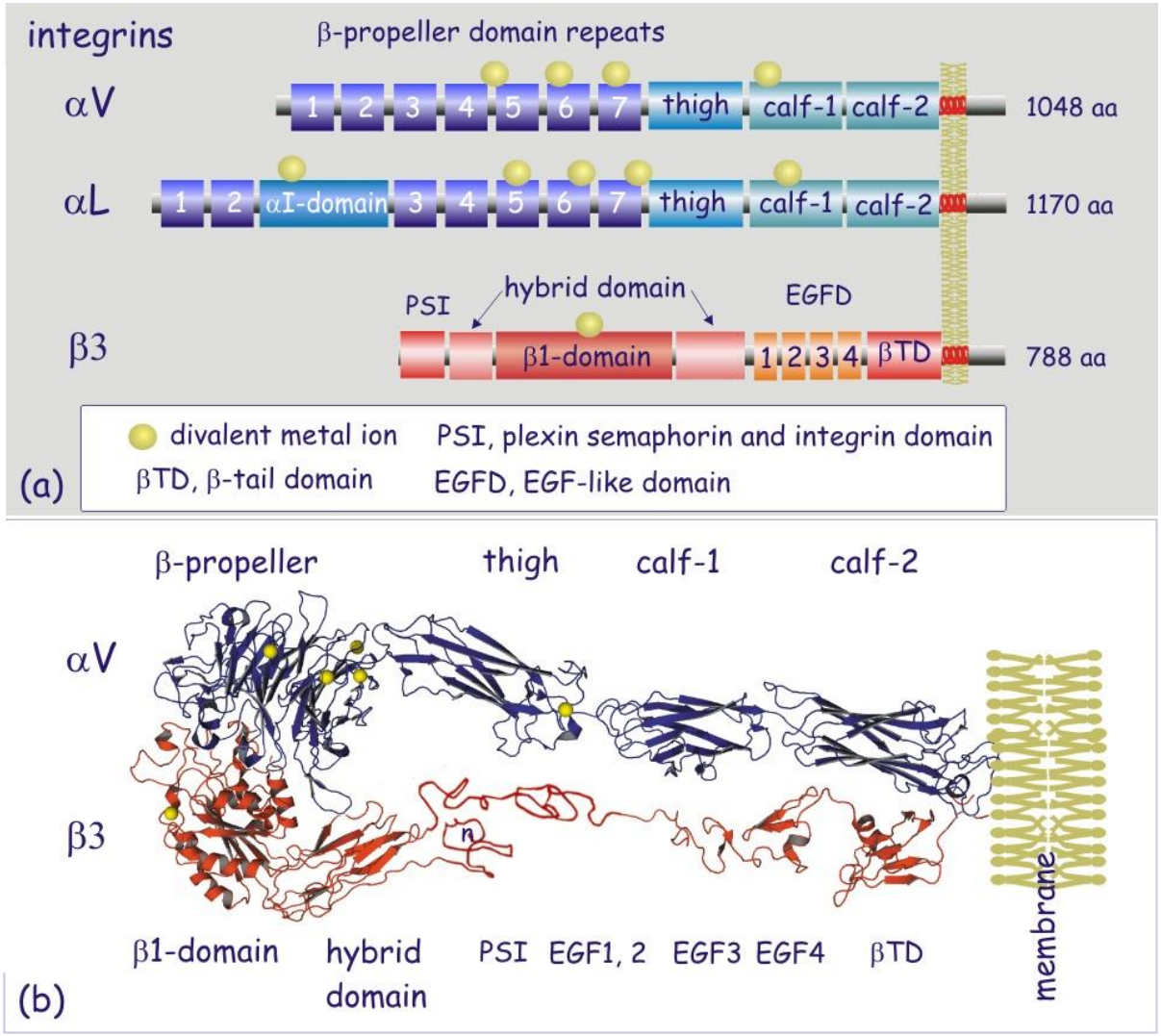
Integrins



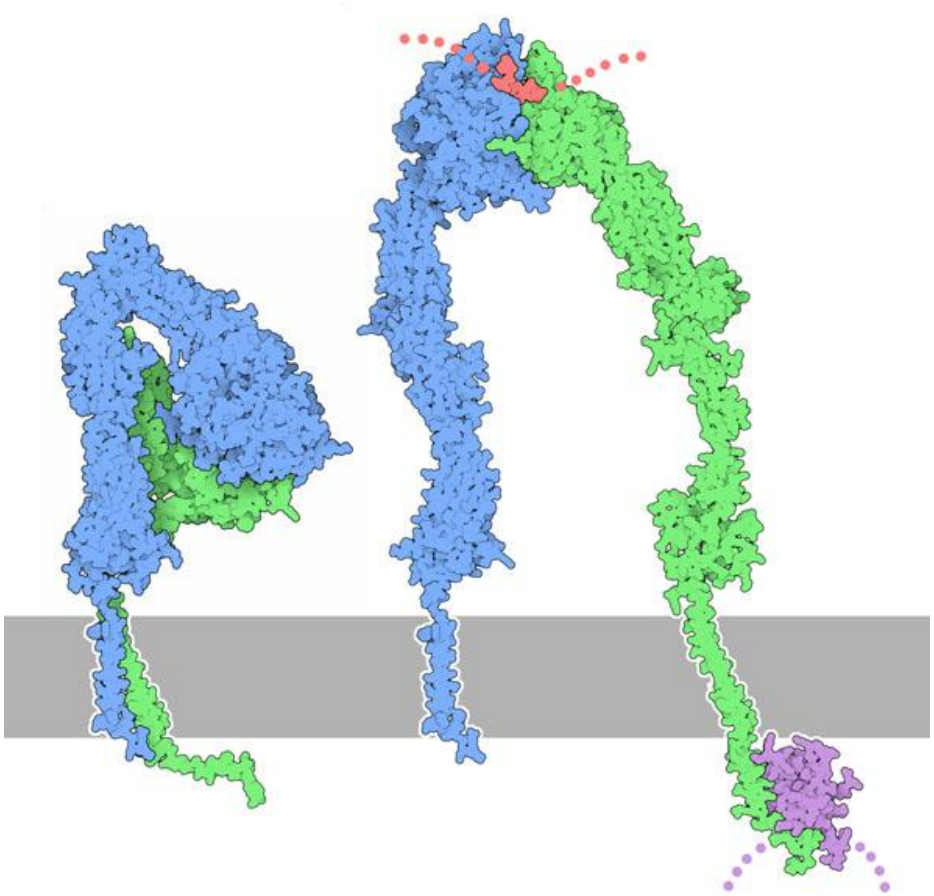
Integrins are transmembrane receptors that mediate the attachment between a cell and other cells or the extracellular matrix (ECM) components such as fibronectin, vitronectin, collagen, and laminin. In addition to transmitting mechanical forces across otherwise vulnerable membranes, they are involved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

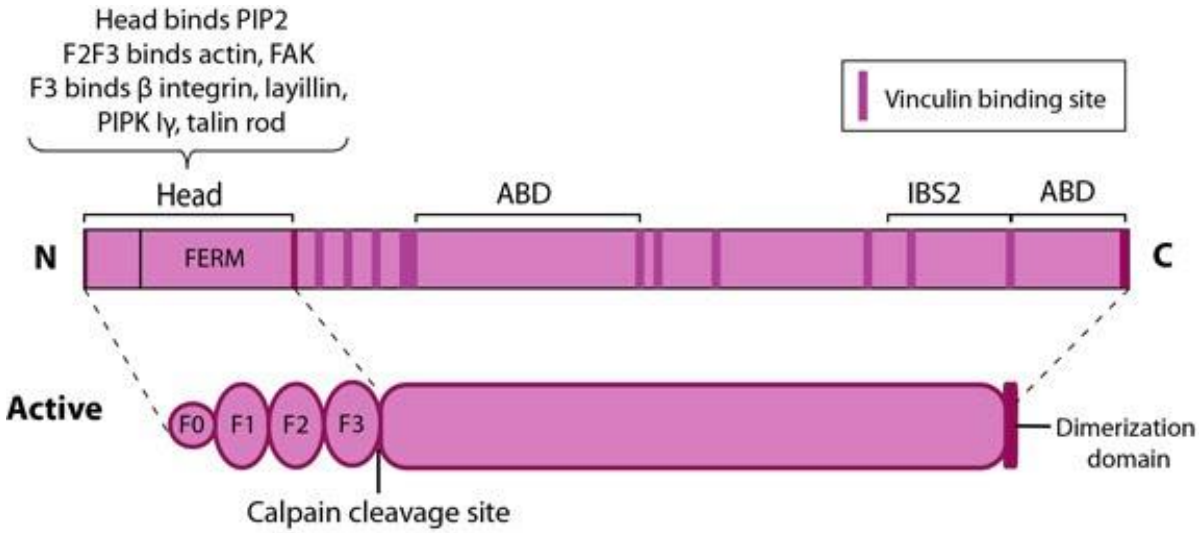
In cells, the priming is accomplished by **talin**, which binds to the β tail of the integrin dimer and changes its conformation.

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

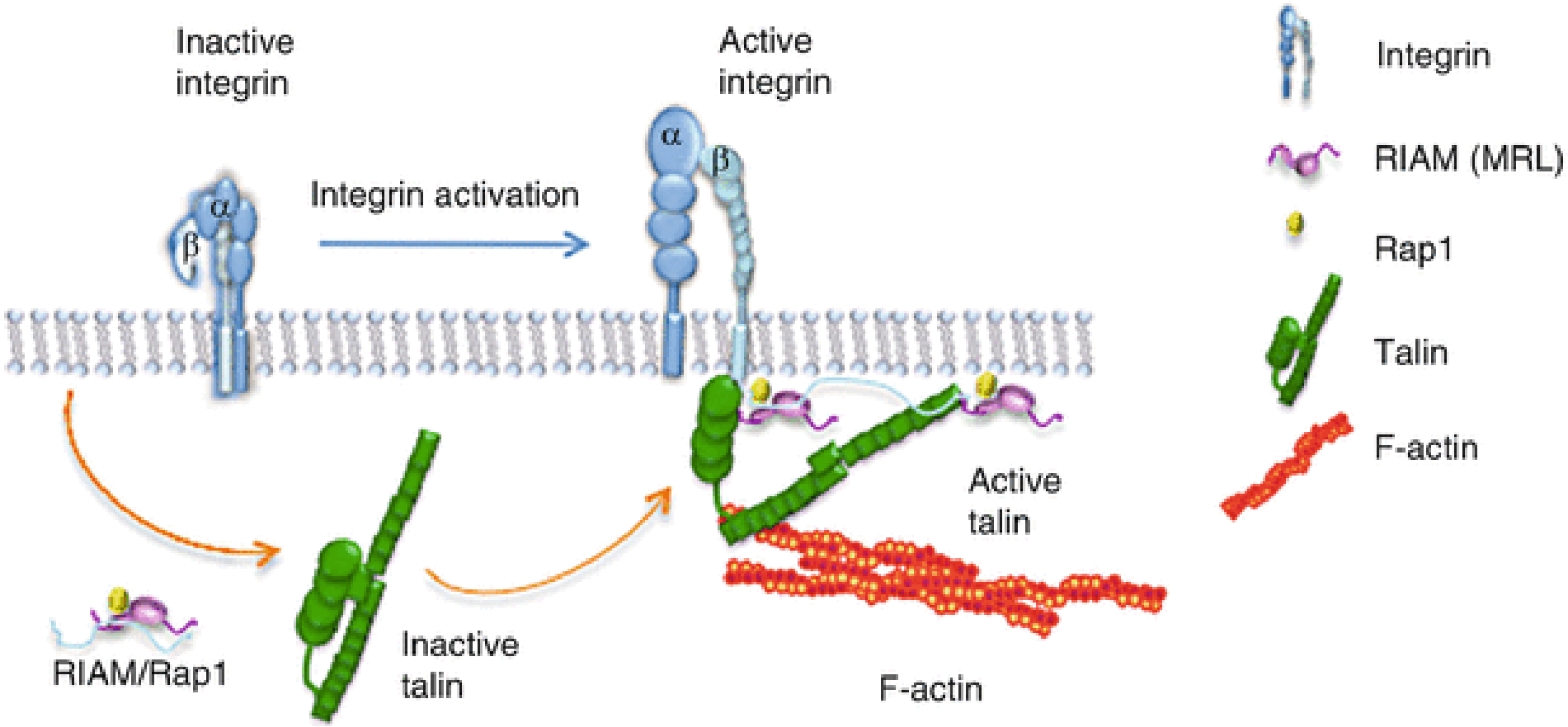
Talin is a 270kDa cytoskeletal protein concentrated at regions of cell–substratum contact and, in lymphocytes, at cell–cell contacts. It is a structural platform that is required for the initial linkage between the contractile cytoskeleton and sites of integrin/fibronectin adhesion



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 membrane localization and activation by RIAM

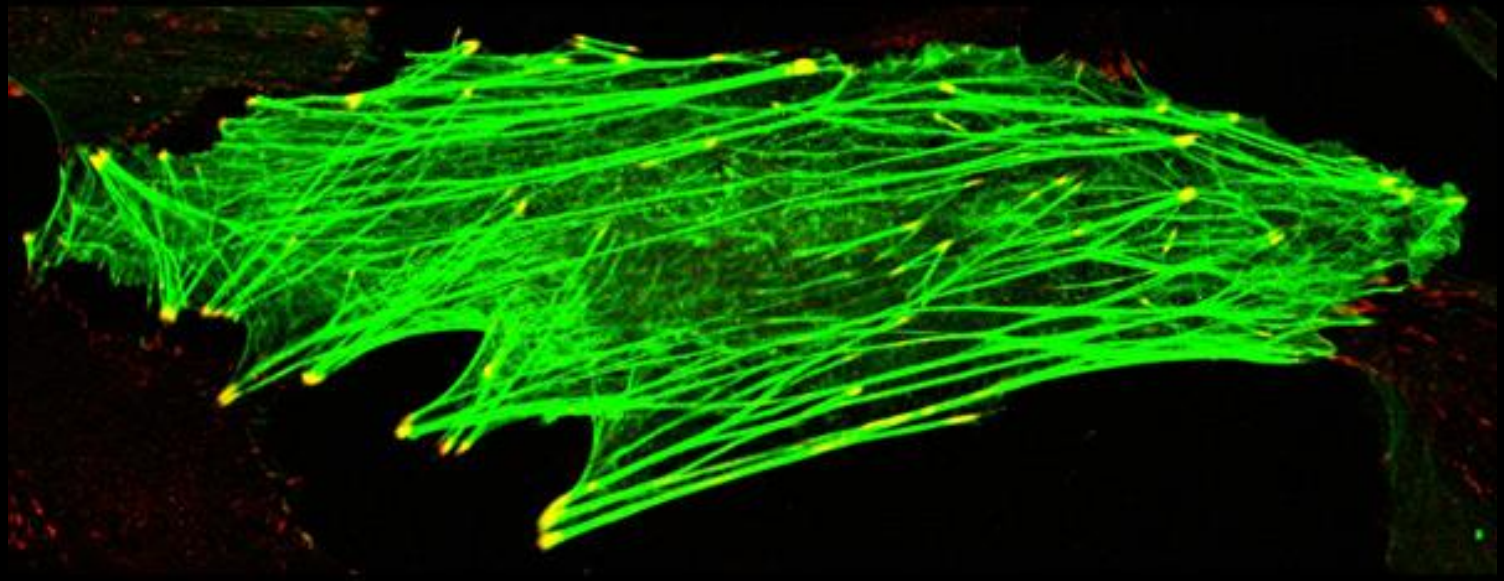


In resting cells, most integrins are kept inactive, possibly owing to conformational constraints in the cytoplasmic tails. A small proportion of the integrin dimers display the thermodynamically unfavourable, active conformation and can bind their ligand.

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.

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

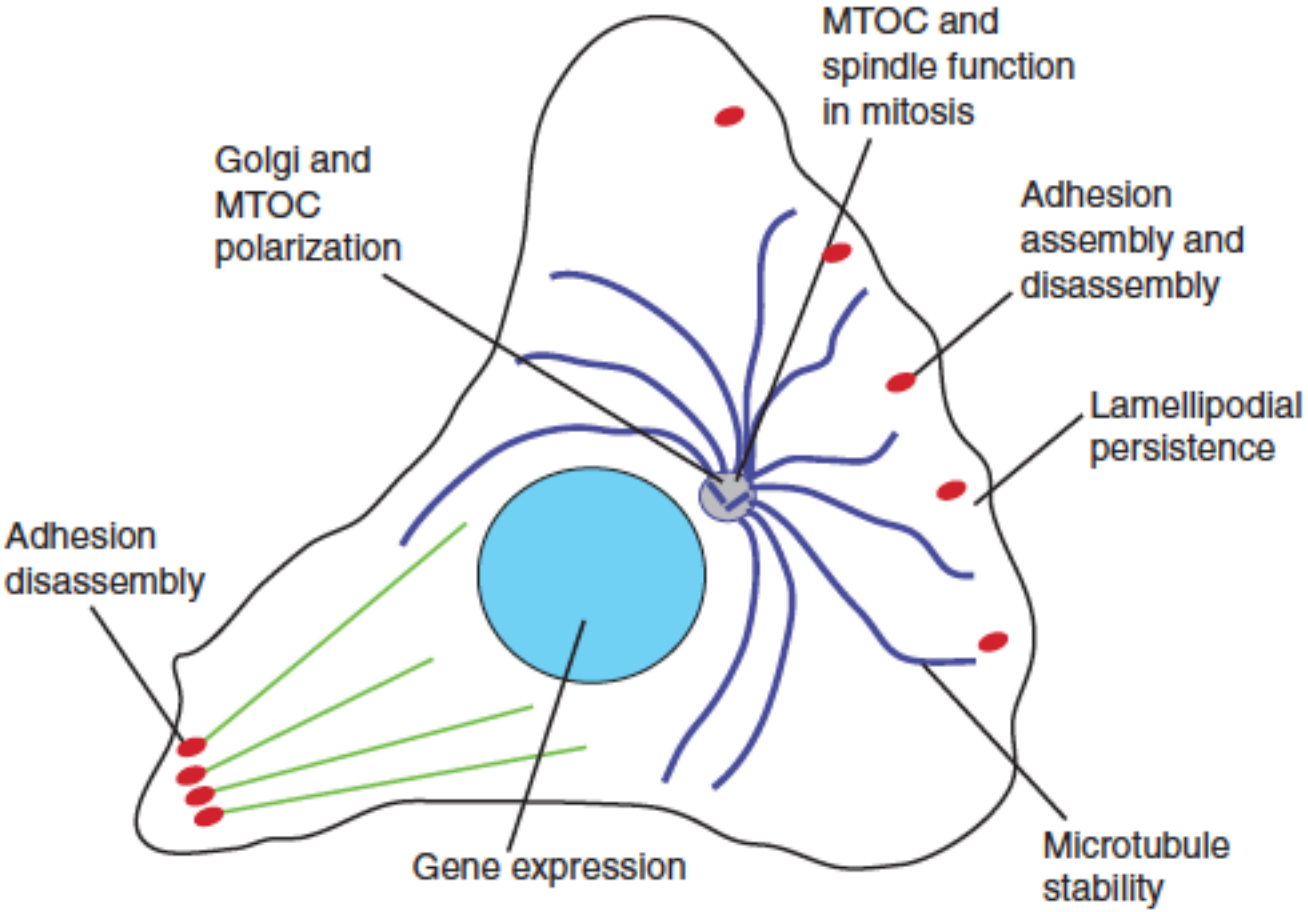


Fig. 2. Major cellular functions of FAK. A migrating cell with the leading edge (right) and trailing edge (left) is shown. Cell-ECM adhesions (red), stress fibers (green), microtubules (dark blue), the MTOC (grey) and nucleus (blue) are illustrated. Black lines denote cellular targets of FAK signaling.

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

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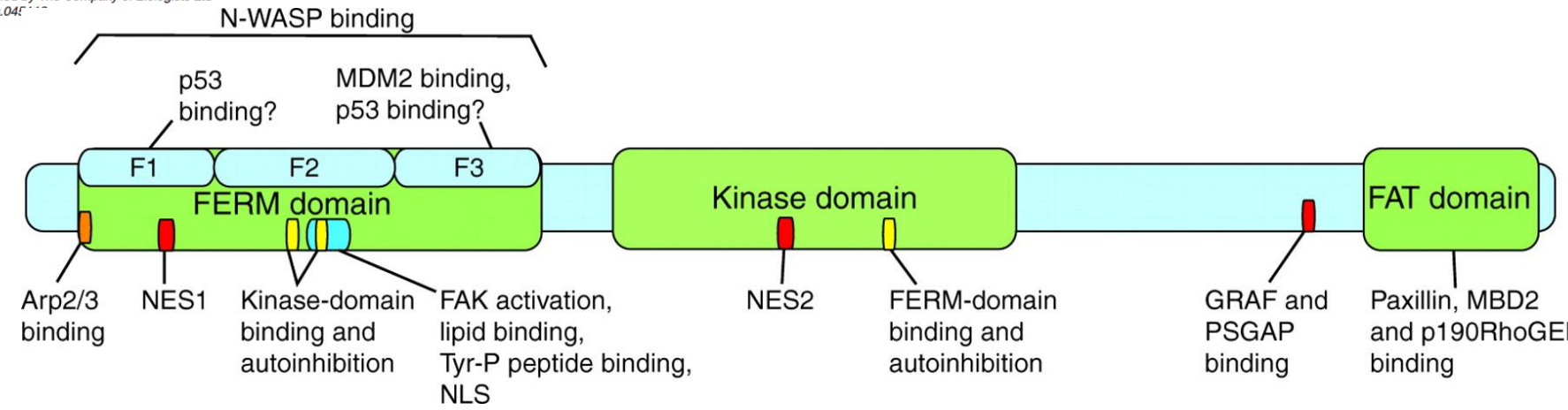
doi:10.1242/jcs.045



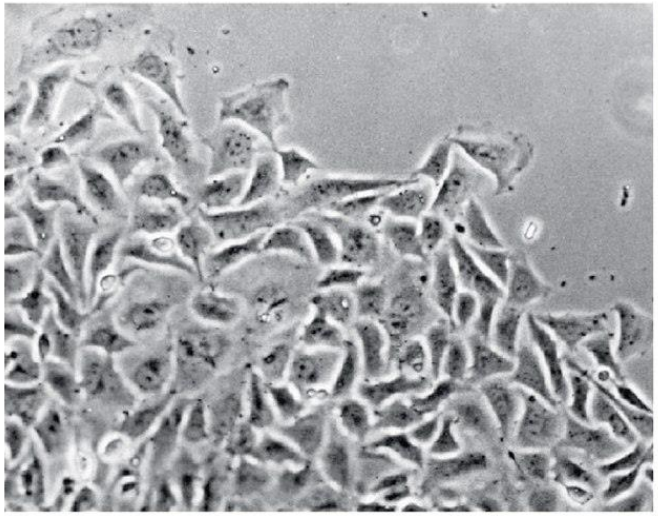
Sequence and structural analysis reveals 4 distinct domains:

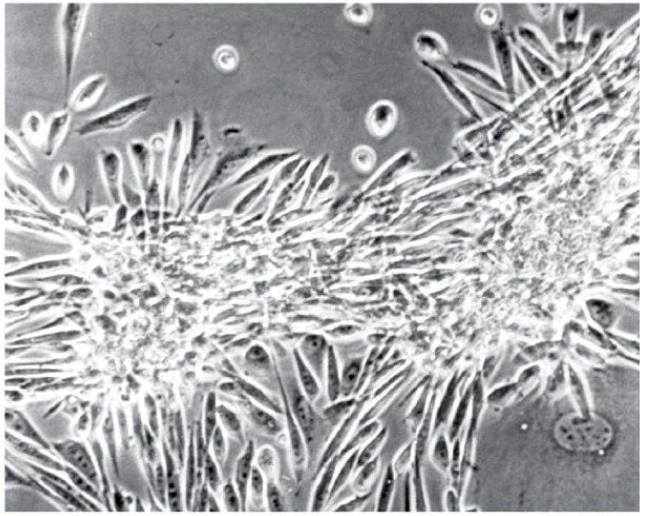
① an N-terminal FERM domain;
② a centrally located catalytic tyrosine kinase domain;
③ a C-terminal focal-adhesion targeting (FAT) domain (a four-helix bundle);
④ and an unstructured proline-rich region between the catalytic and FAT domains

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.

No contact inhibition of cell division

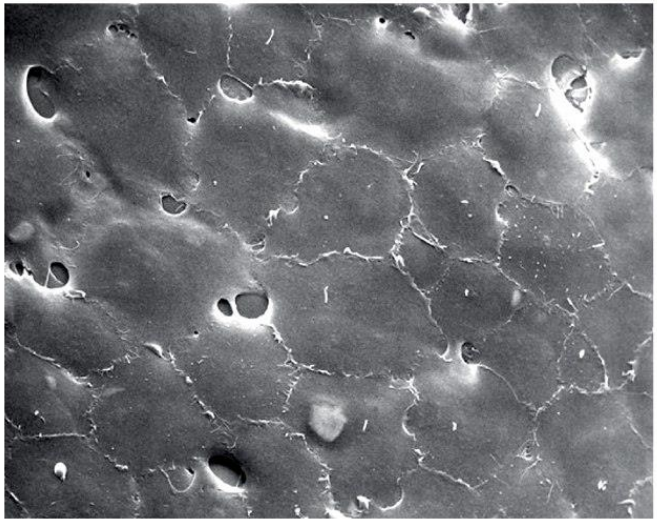
Normal

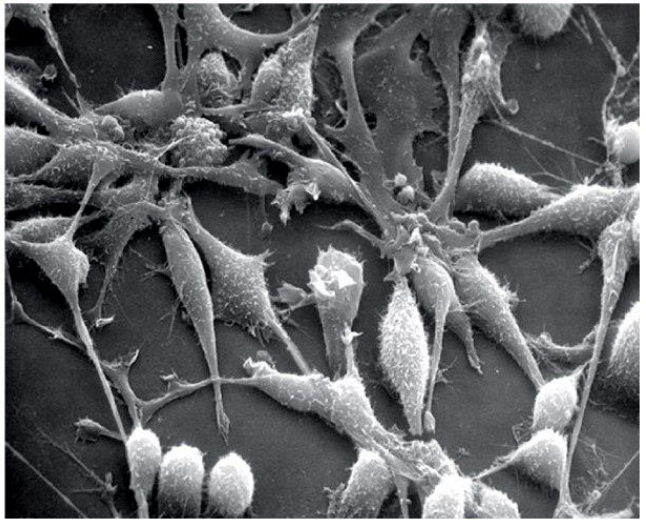




Cancer

Normal





Cancer

MOLECULAR AND CELLULAR BIOLOGY, Feb. 2002, p. 1203–1217 Vol. 22, No. 4
0270-7306/02/$04.00+0 DOI: 10.1128/MCB.22.4.1203–1217.2002

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

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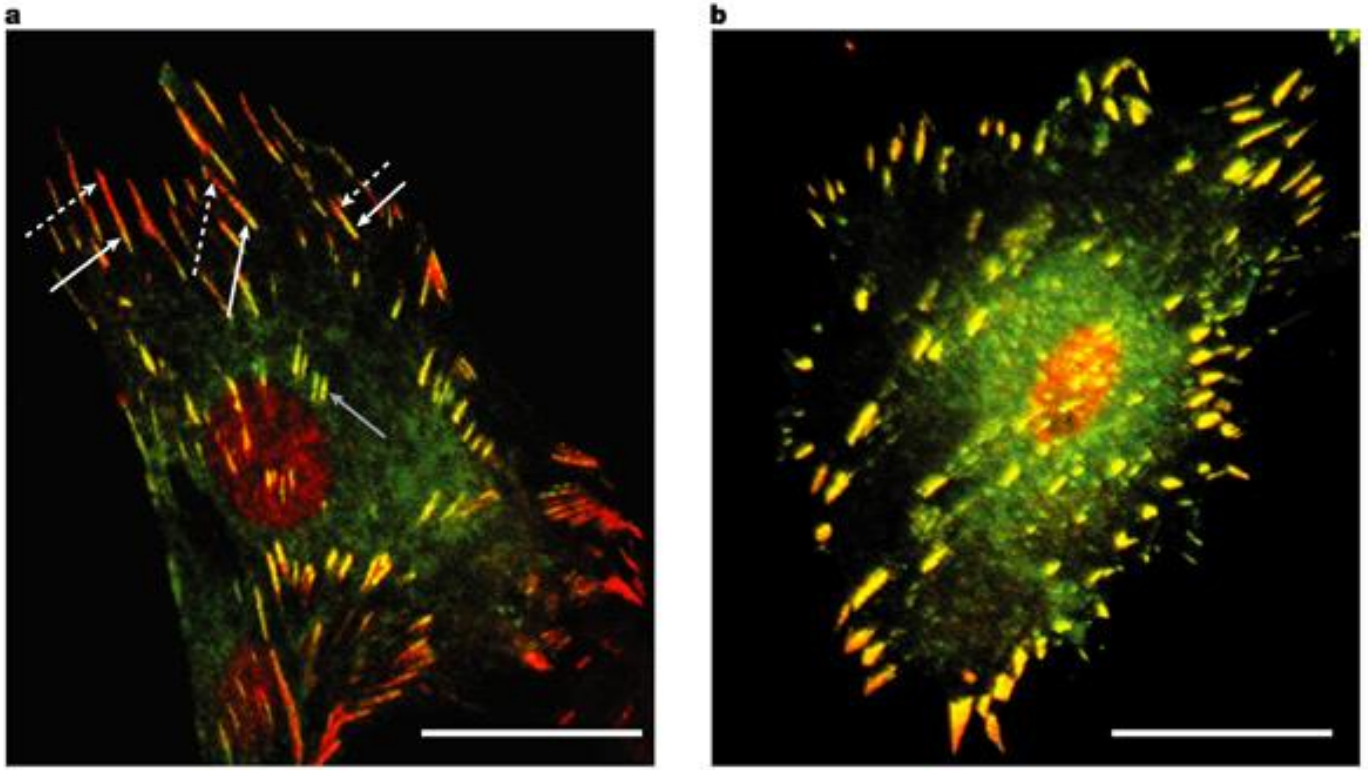
Identifying the targets of Src

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

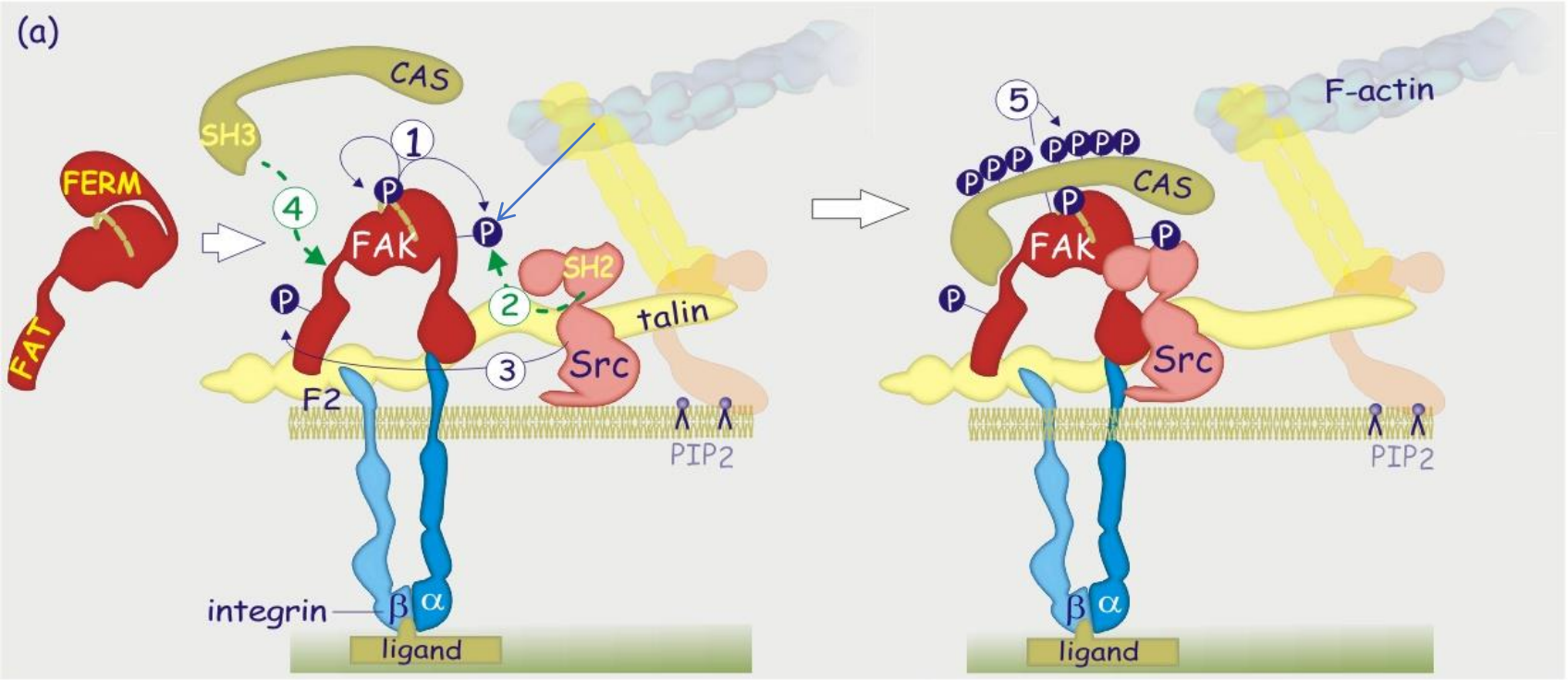
Mike Schaller, ex-UNC

(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.



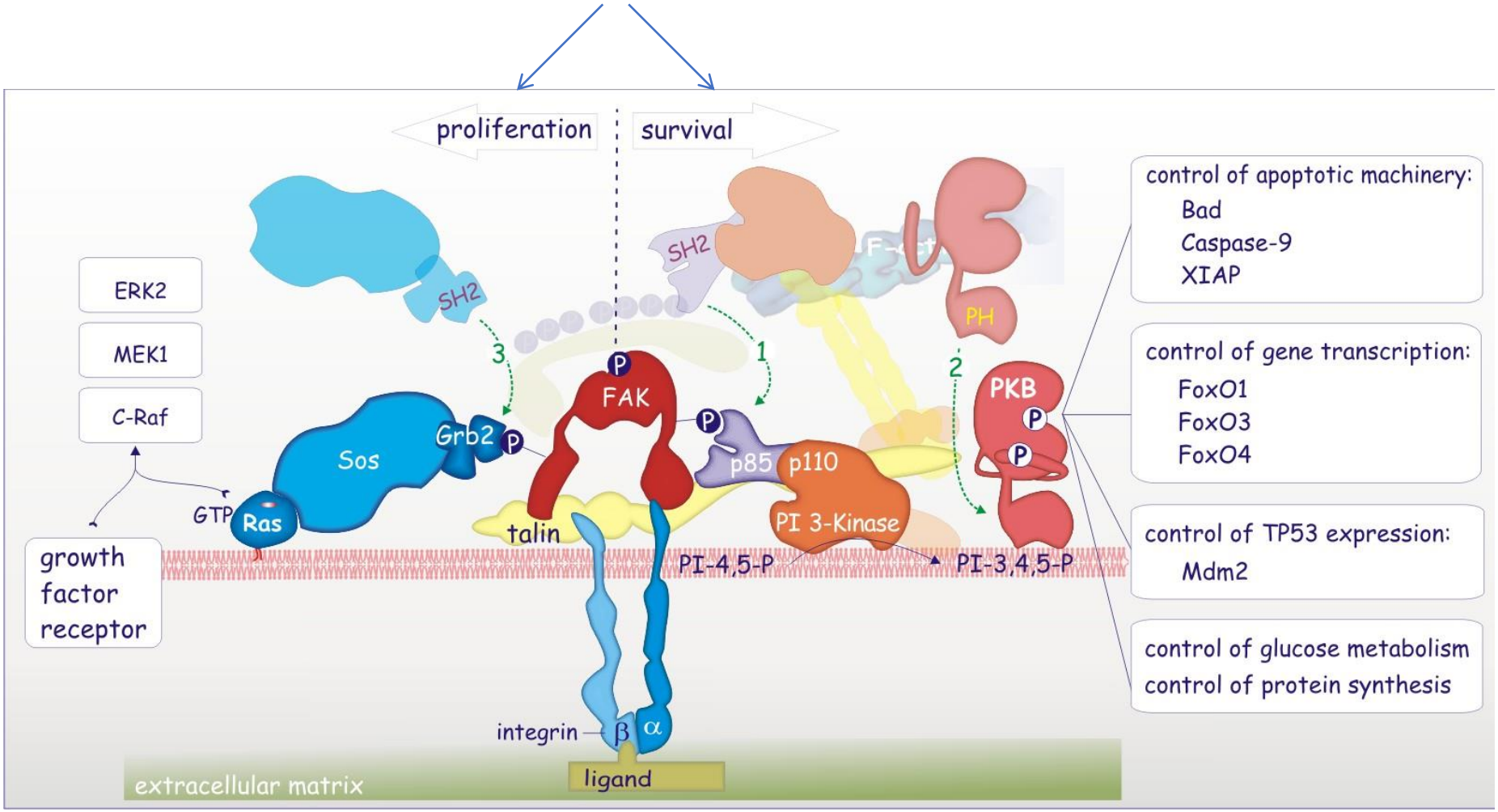
(B) FAK (red) and v-Src (green) co-localize in smaller adhesion structures at the cell periphery when v-Src is active, and focal adhesions (and the associated actin filaments) are dynamically regulated. These adhesion characteristics indicate that v-Src-KD impairs adhesion turnover and that focal-adhesion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

Integrin signaling complex

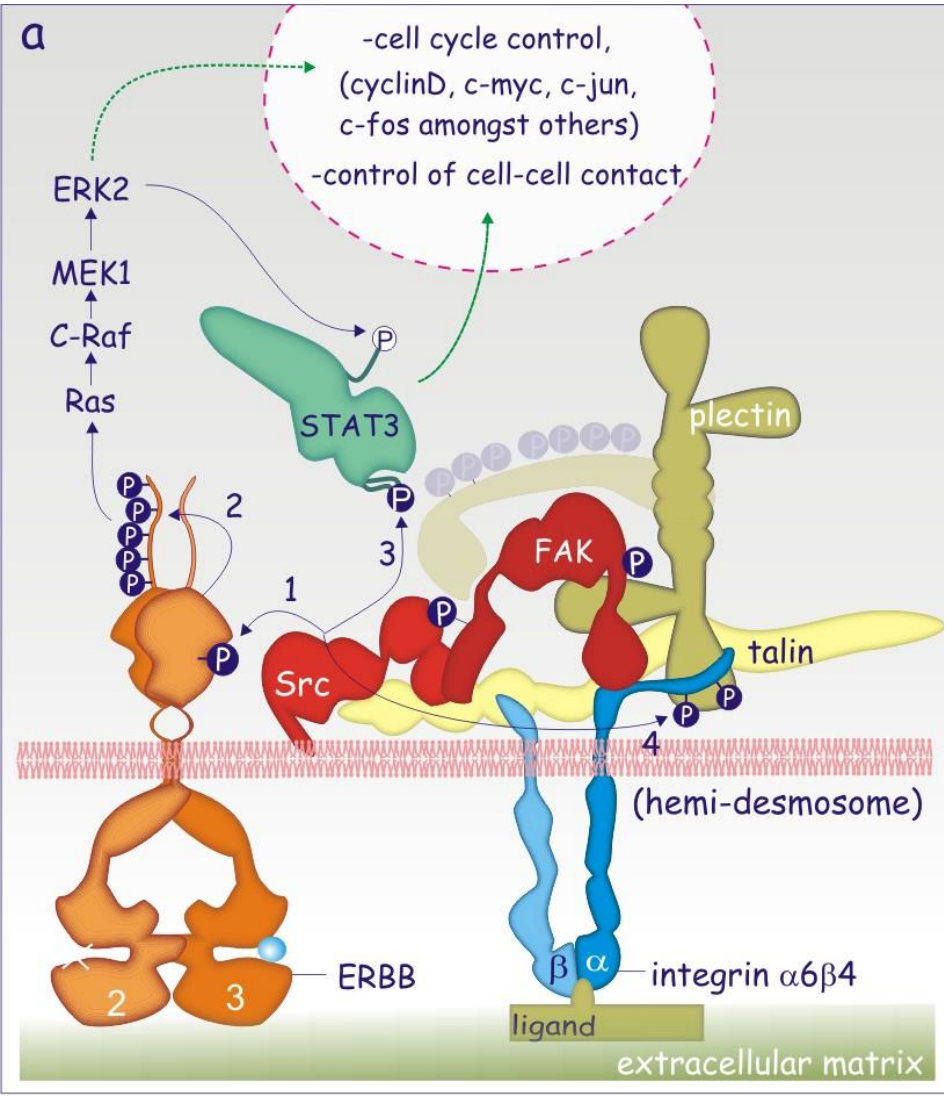


The focal adhesion kinase FAK associates with talin. Autophosphorylation of FAK then generates a docking site for the SH2 domain of Src which phosphorylates FAK at Y925. Src and FAK next phosphorylate the FAK-associated docking protein CAS at multiple sites.
An integrin-signalling complex is formed that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

Integrin signaling



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.