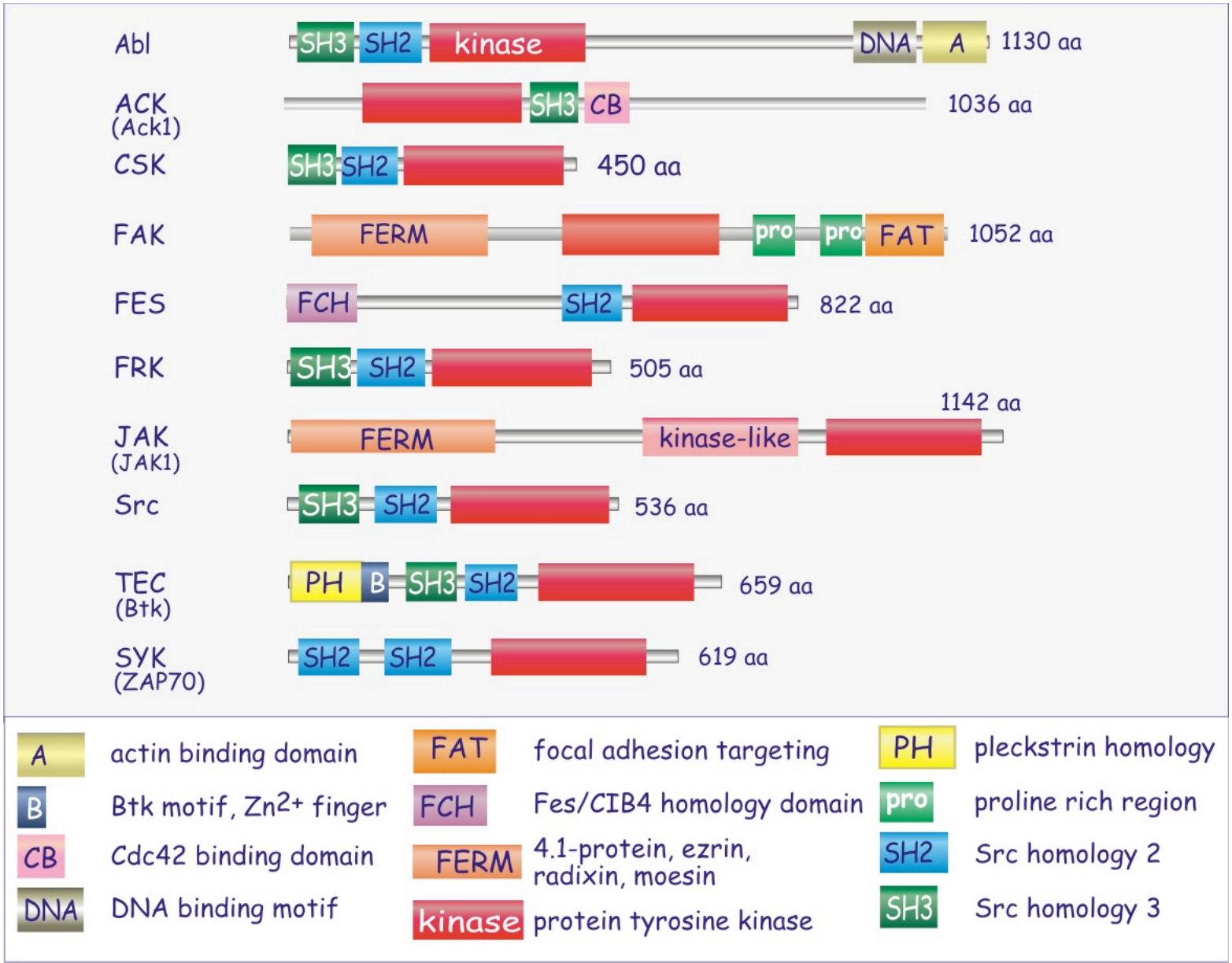
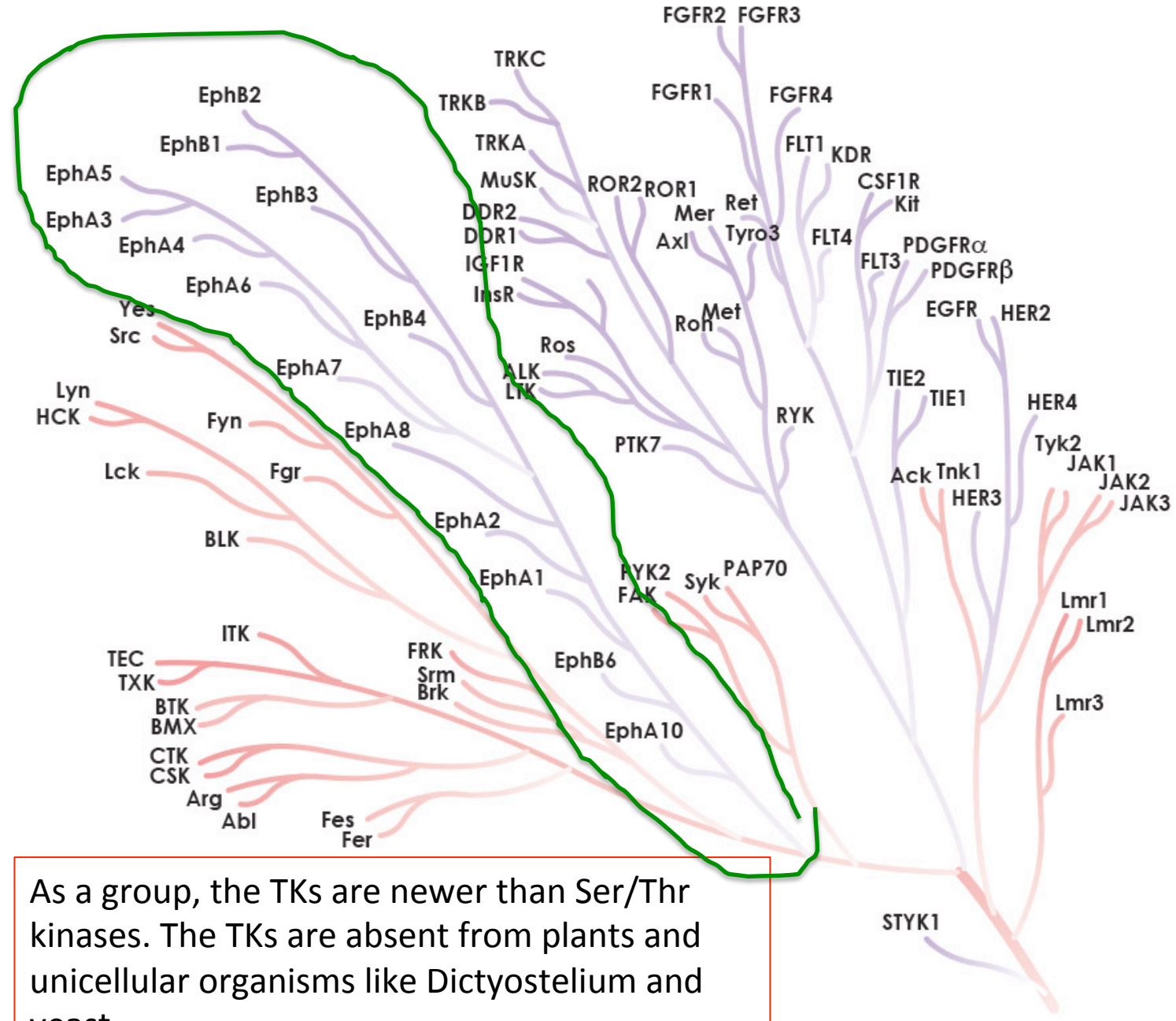
Growth Factors

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





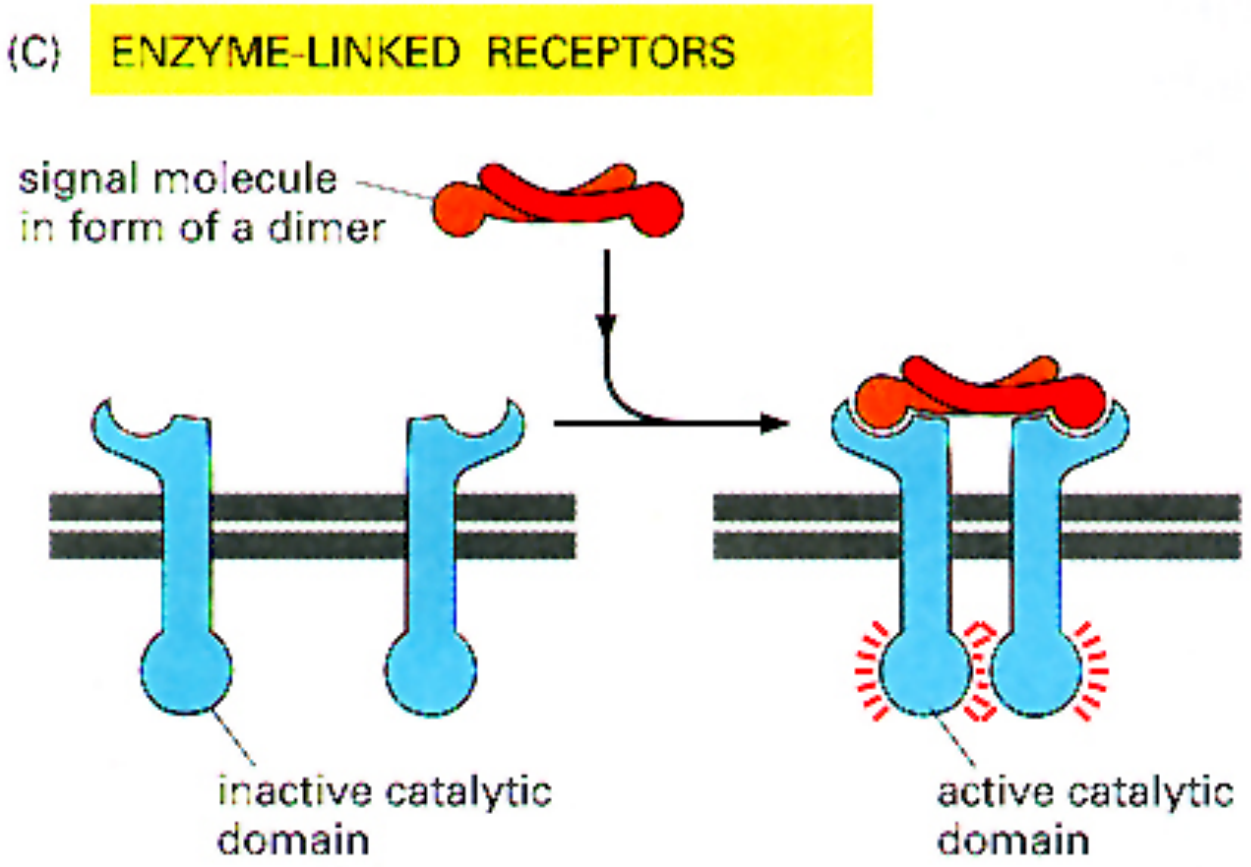
As a group, the TKs are newer than Ser/Thr kinases. The TKs are absent from plants and unicellular organisms like Dictyostelium and yeast.

Moreover, certain families have shown substantial expansion in humans: such expansion is thought to relate to a role for these RTKs in processes that are more advanced in humans, such as angiogenesis, hematopoiesis, and functioning of the nervous and immune systems.

modified from www.kinase.com/human/kinome

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

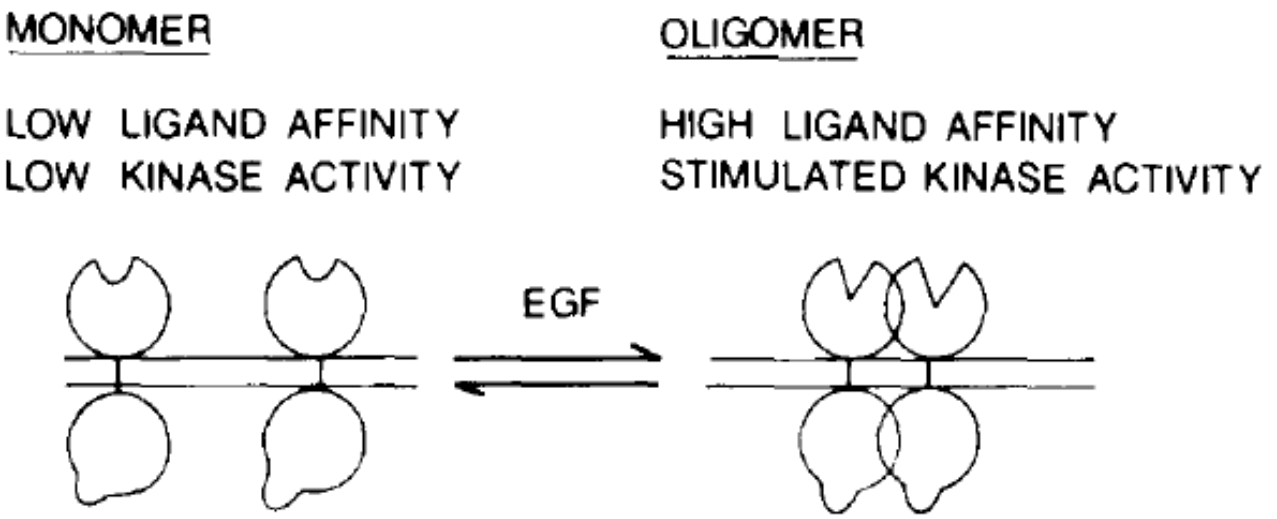
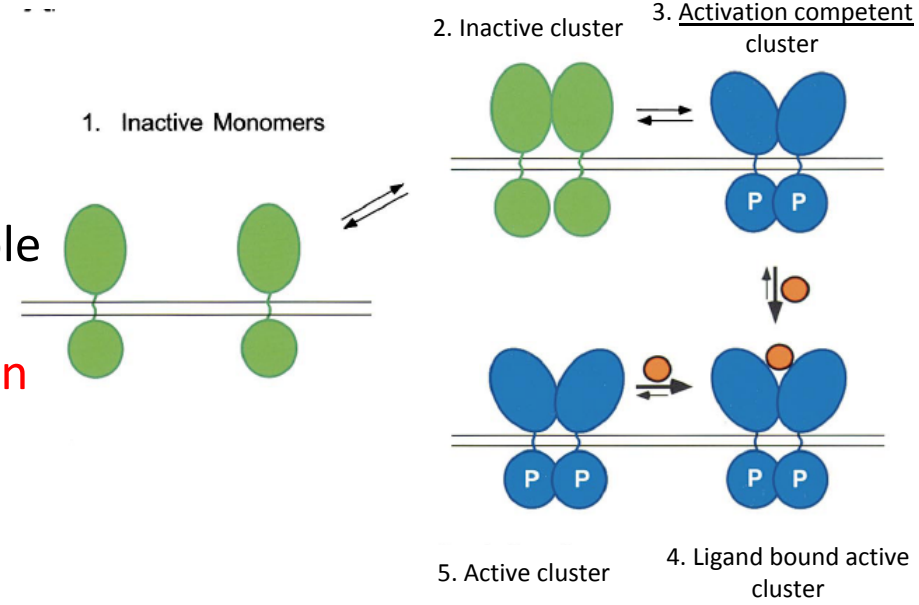
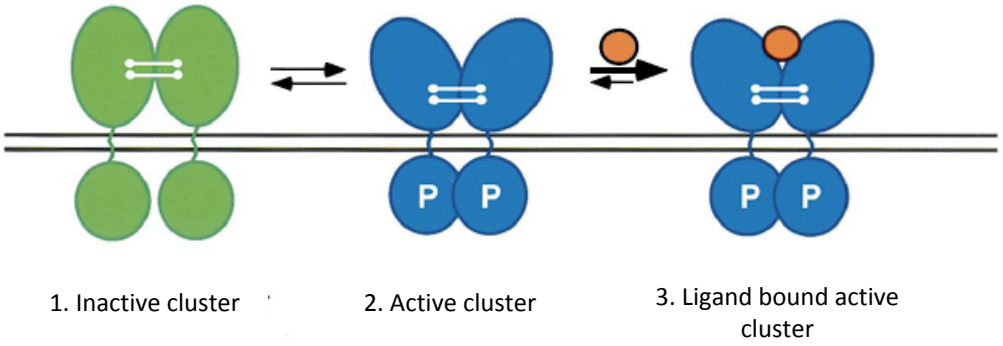


Figure 3. An allosteric oligomerization model for the activation o the EGF receptor kinase by EGF. EGF receptor is depicted as a biglobular transmembrane molecule as shown in Fig. 1. It is proposed that monomeric receptors exist in equilibrium with recepto oligomers. It is postulated that monomeric receptors 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 dimers

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.



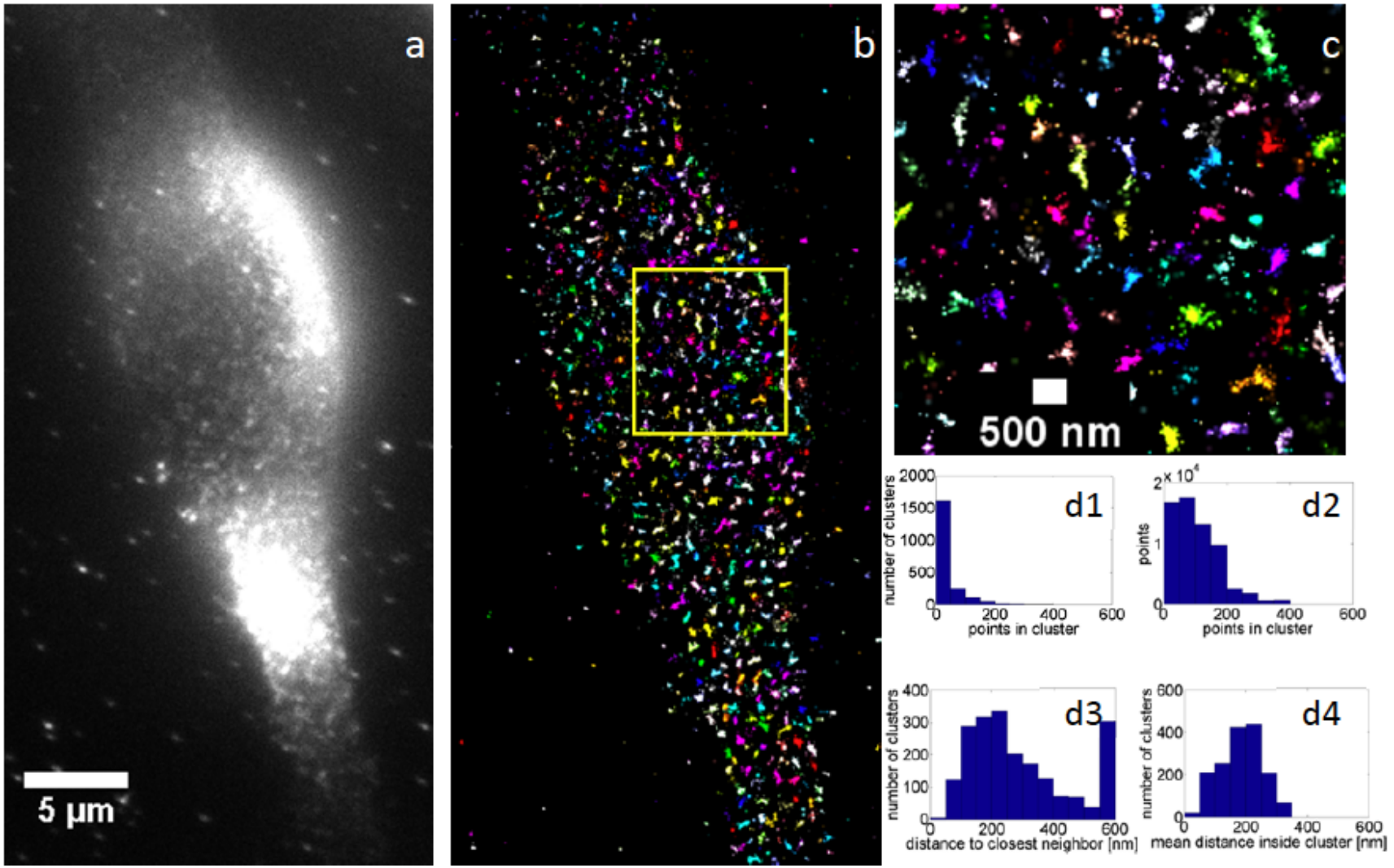


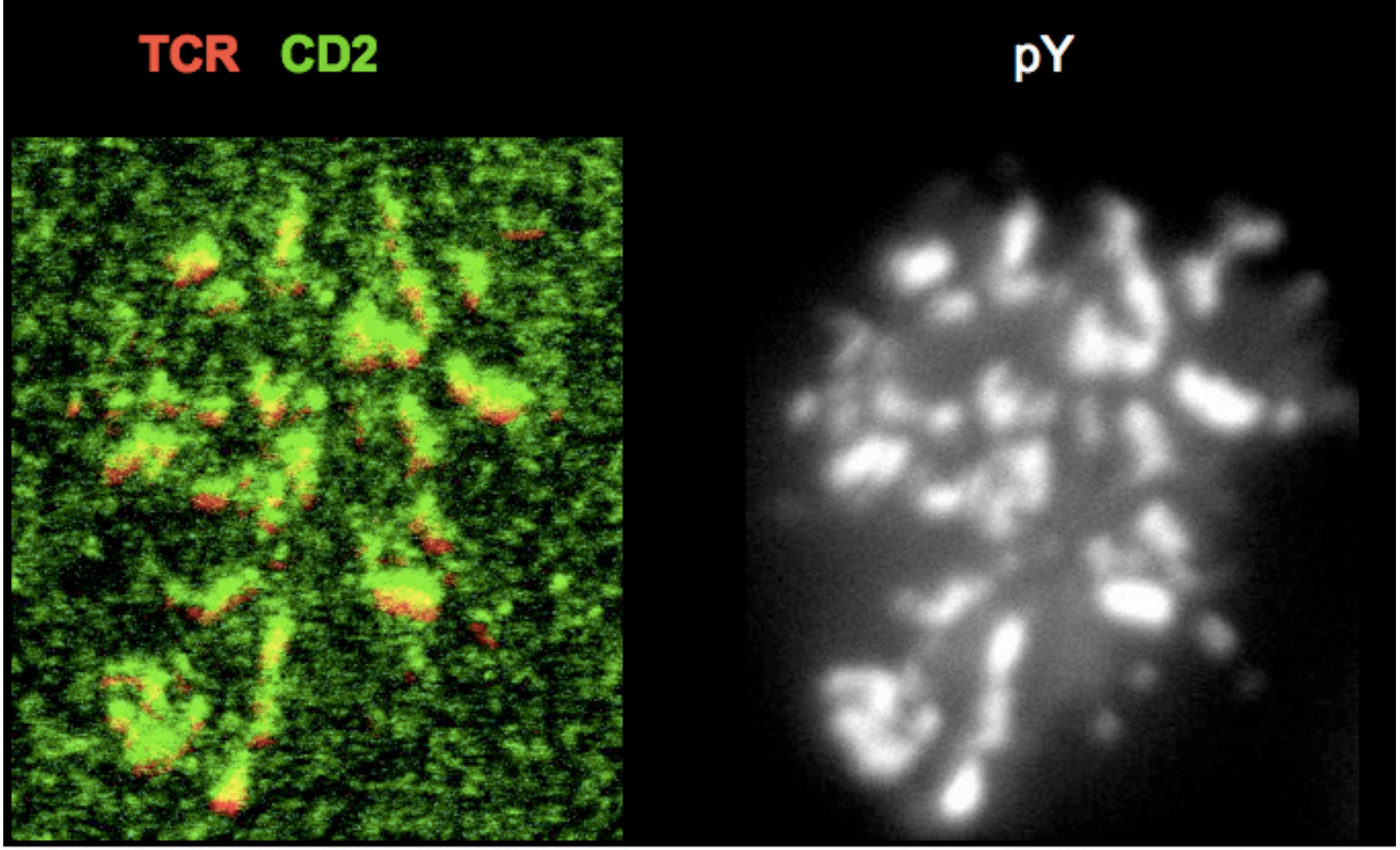
Inactive disulphide bridged insulin-receptor dimers are in equilibrium with active dimers. Insulin binding stabilizes the active dimeric state, leading to PTK activation.

Microscopy: advances in scientific research and education (A. Méndez-Vilas, Ed.)

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,*]





Jurkat T cells placed on planar lipid bilayers with anti-TCR antibodies and CD58 (ligand for CD2) results in the co-clustering of TCR and CD2. Signaling is active in these clusters as evidenced by enriched phosphotyrosine staining. Kaizuka, Y., Douglass, A.D., Vardhana, S., Dustin, M.L. and Vale, R.D. (2009) The coreceptor CD2 uses plasma membrane microdomains to transduce signals in T cells. J Cell Biol 185: 521-534.

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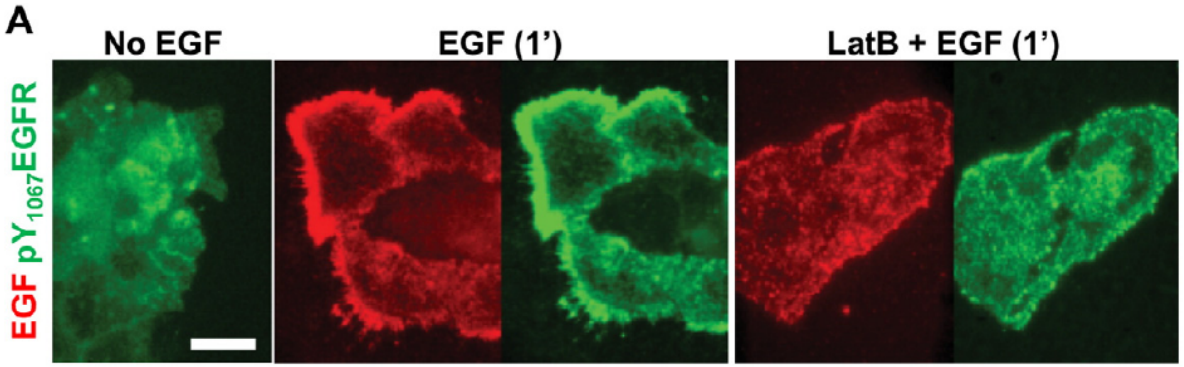
journal homepage: www.elsevier.com/locate/bbamem

Review

Optical measurement of receptor tyrosine kinase oligomerization on live cells☆

Inhee Chung

Janelia Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, VA 20147, USA



NATURE COMMUNICATIONS | DOI: 10.1038/ncomms13307

ARTICLE

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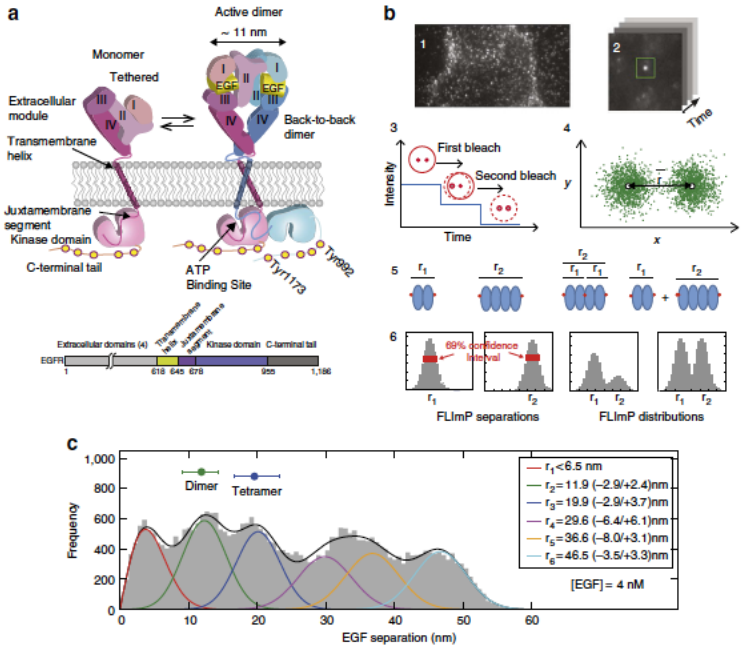
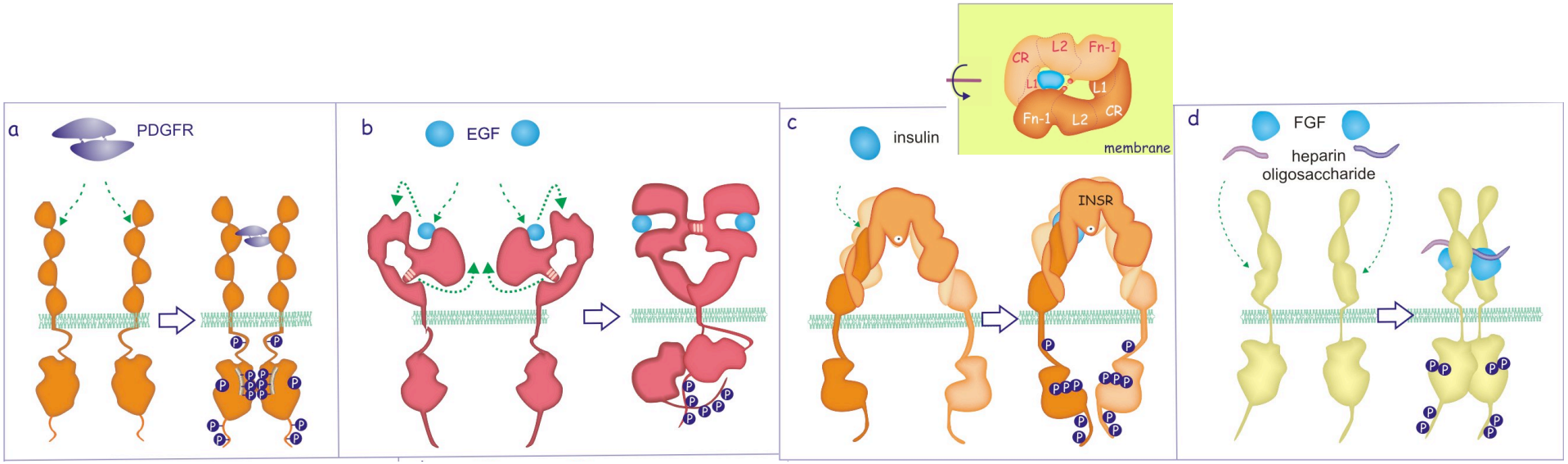
DOI: 10.1038/ncomms13307 OP

EGFR oligomerization organizes kinase-activ dimers into competent signalling platforms

Sarah R. Needham[1], Selene K. Roberts[1], Anton Arkhipov[2], Venkatesh P. Mysore[2], Christopher J. Laura C. Zanetti-Domingues[1], Eric T. Kim[2], Valeria Losasso[3], Dimitrios Korovesis[1], Michael Hirs Daniel J. Rolfe[1], David T. Clarke[1], Martyn D. Winn[3], Alireza Lajevardipour[4], Andrew H.A. Clayton[4] Michela Perani[6], Peter J. Parker[6,7], Yibing Shan[2], David E. Shaw[2,8] & Marisa L. Martin-Fernande



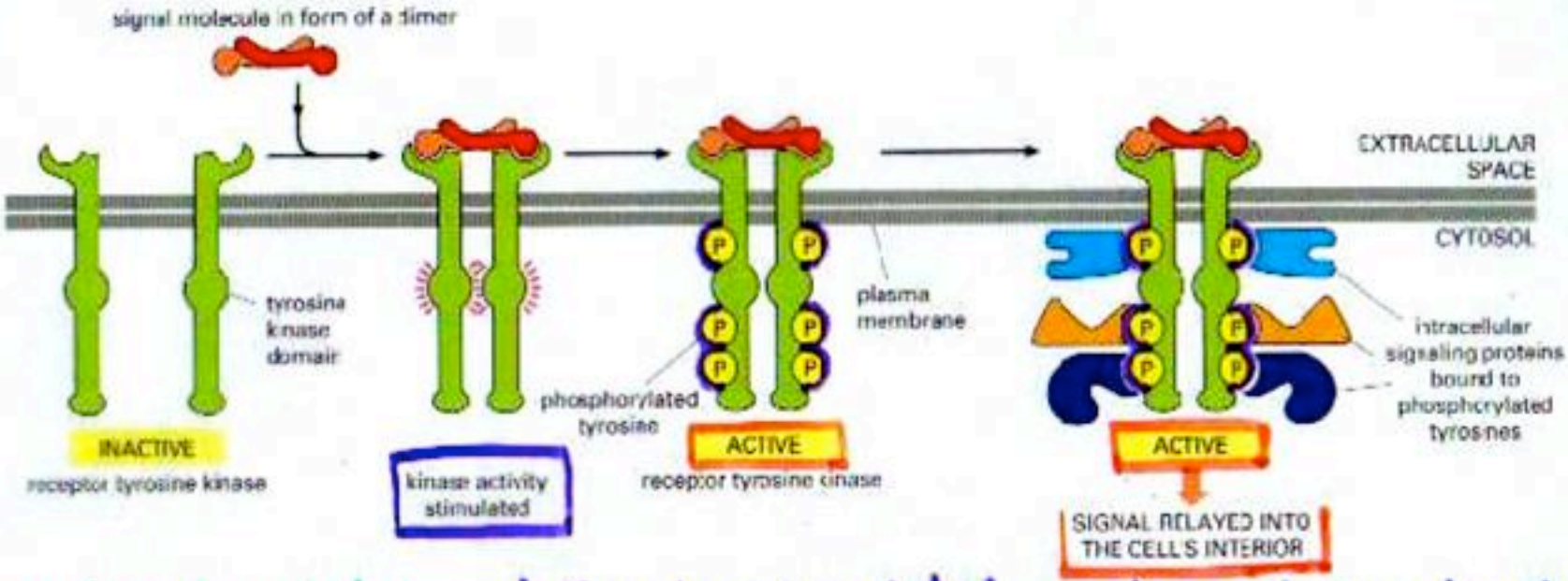
RECEPTORS EMPLOY DIFFERENT CLUSTERIZATION STRATEGIES



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.

Activation of a receptor kinase → signaling complex formation

(enzyme-linked receptor)



non-phosphorylated receptors → inactive

fully phosphorylated dimerized receptors → active

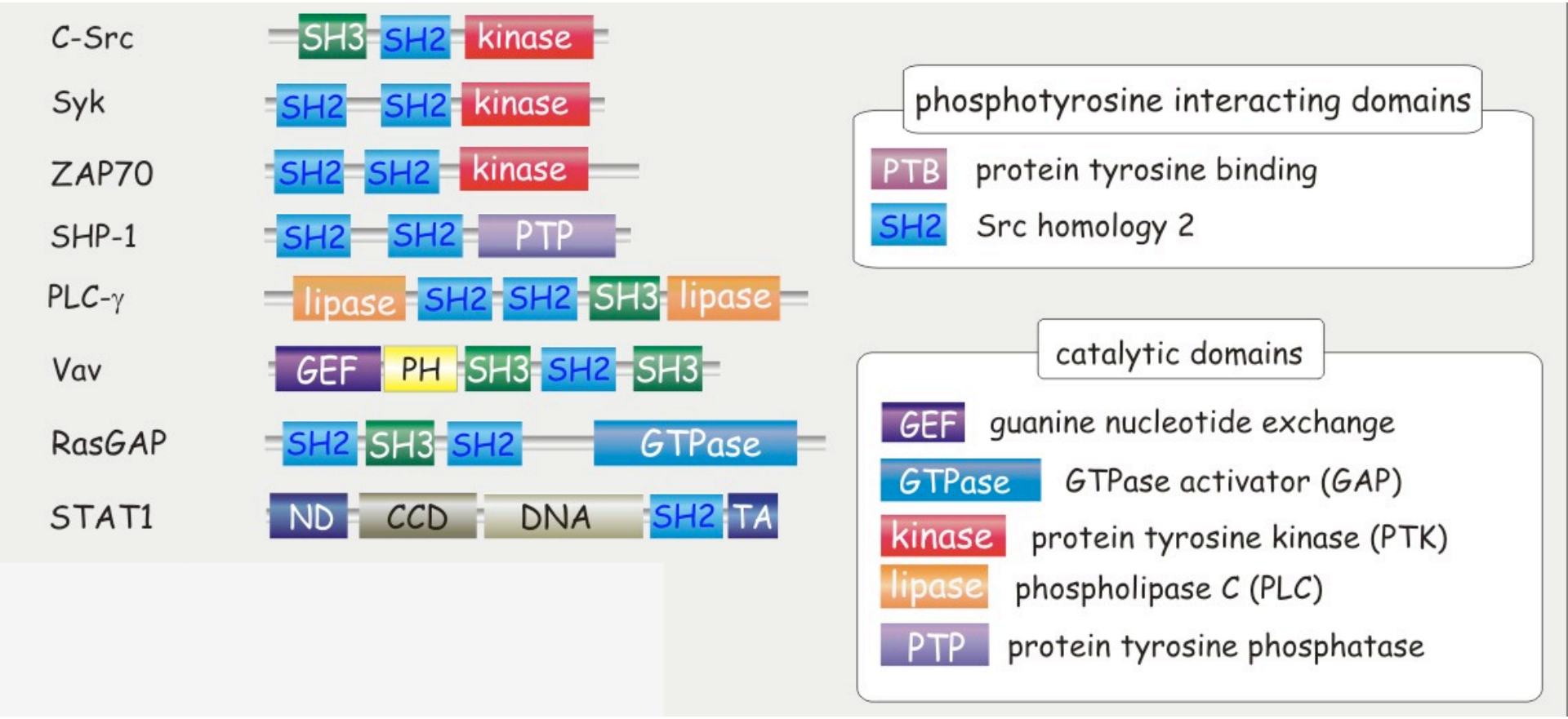
intracellular signaling proteins bound to phosphorylated residues → signaling to several pathways

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*

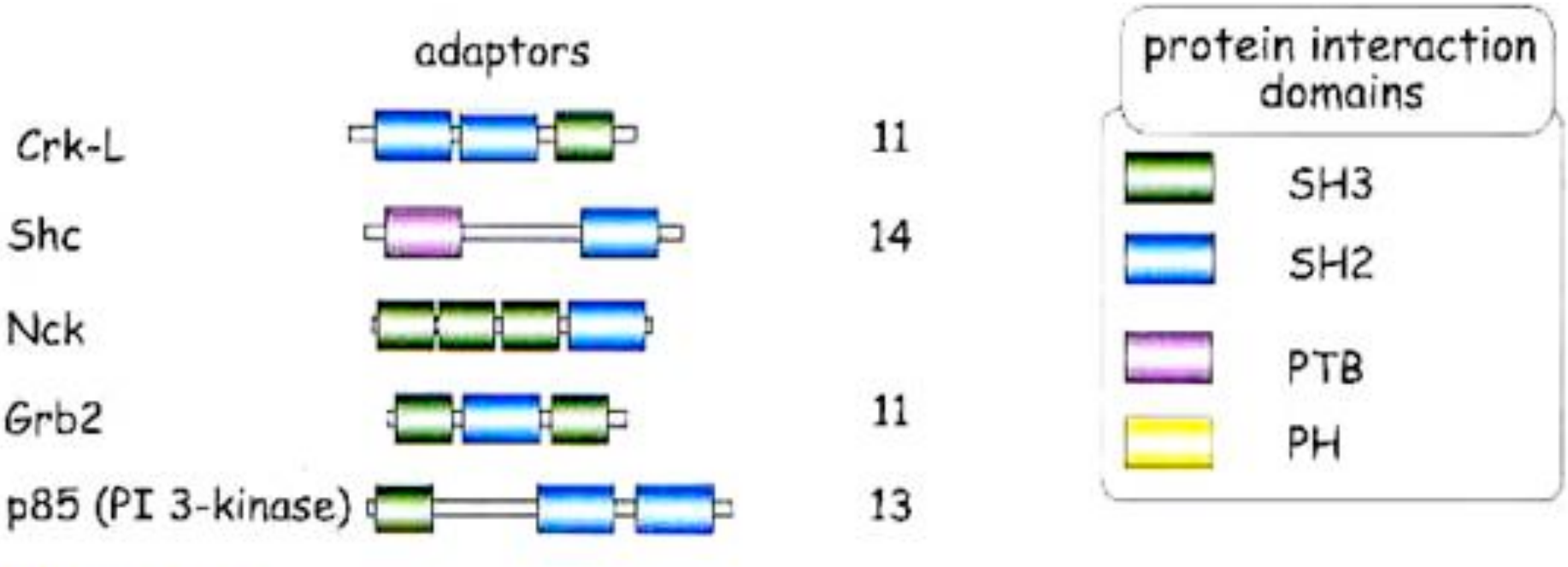
Fig. 16-30

COSA SI LEGA AL RECETTORE FOSFORILATO?

1) Enzymes/transcription factors



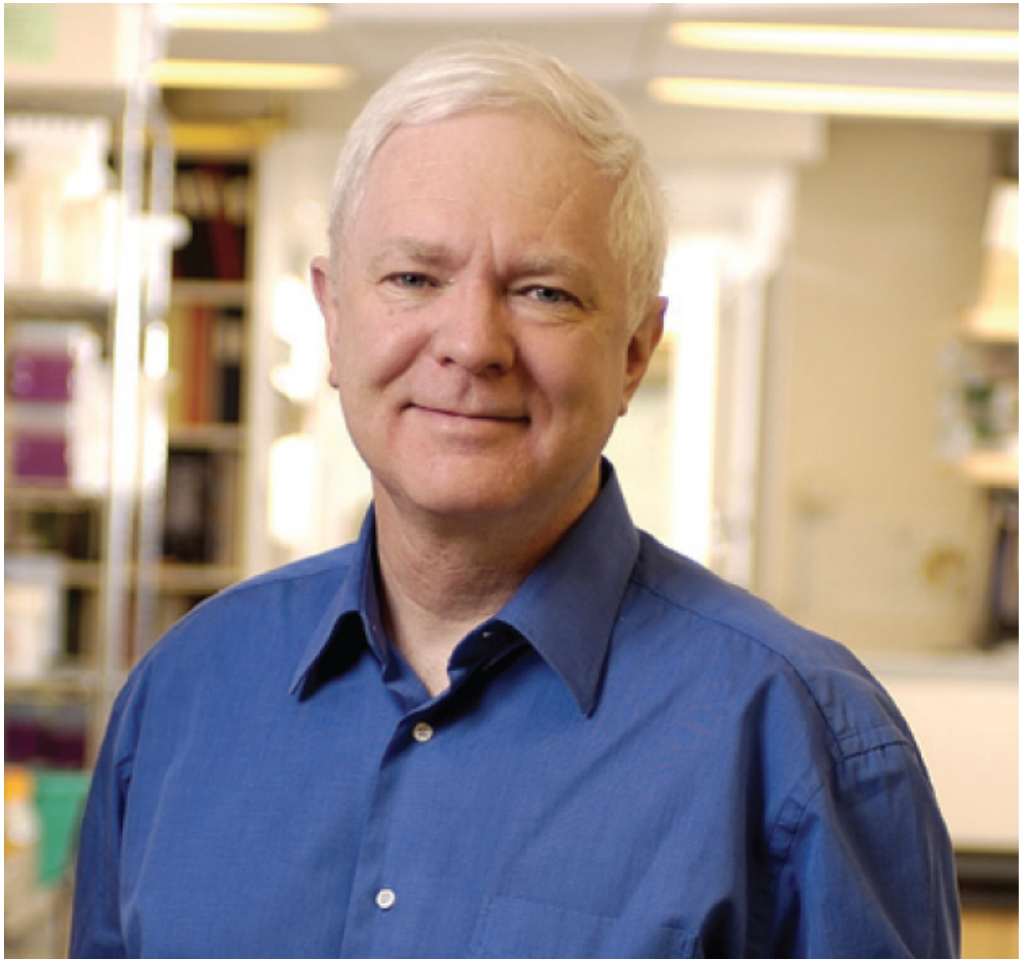
2) ***Adaptors*** lack intrinsic catalytic activity, but link phosphorylated receptors with other effector proteins.



Anthony James Pawson

(1952–2013)

Biochemist whose vision of cell signalling transformed cancer research.

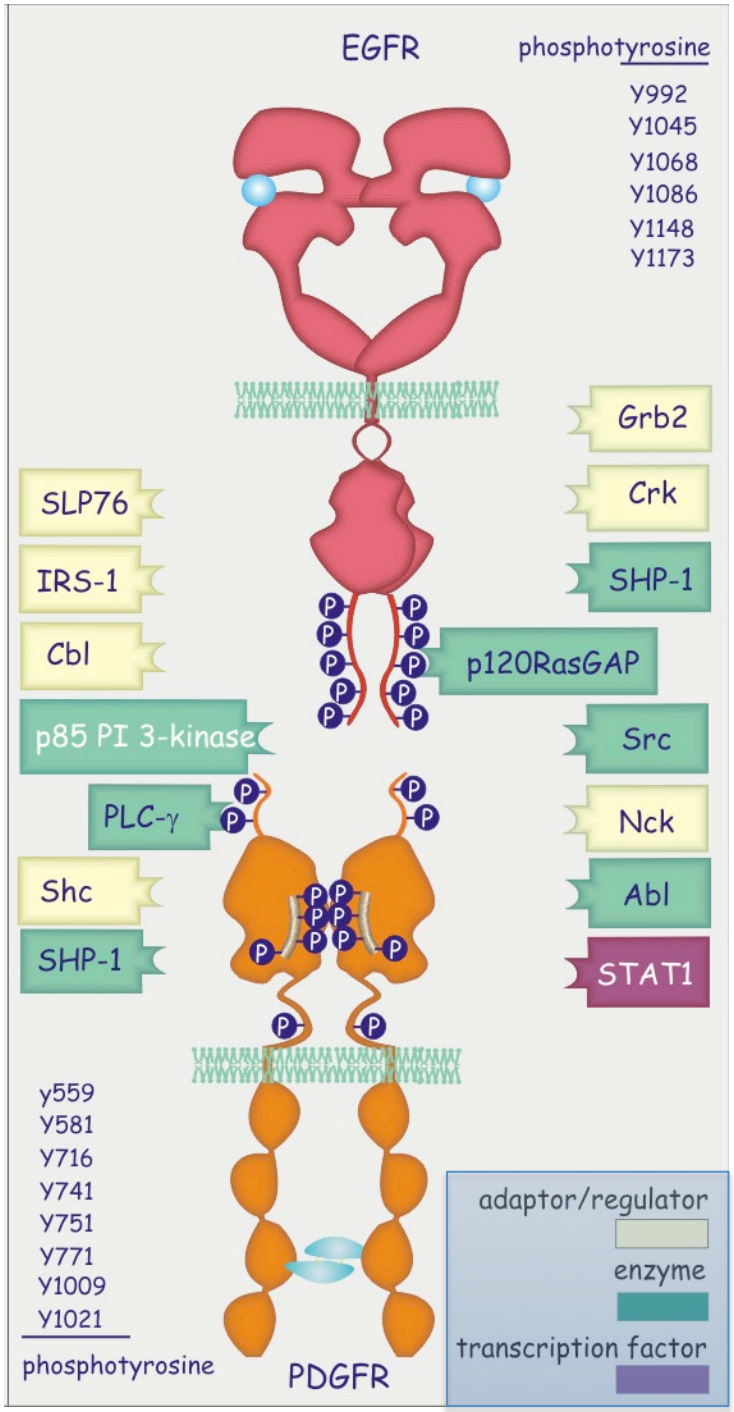


In the 1980s, early in his career, Pawson and his team discovered the Src homology region 2 (SH2). A subunit, or domain, of many proteins, SH2 directs how proteins interact and governs how cells respond to external cues. This finding set a path for all his future work.

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.

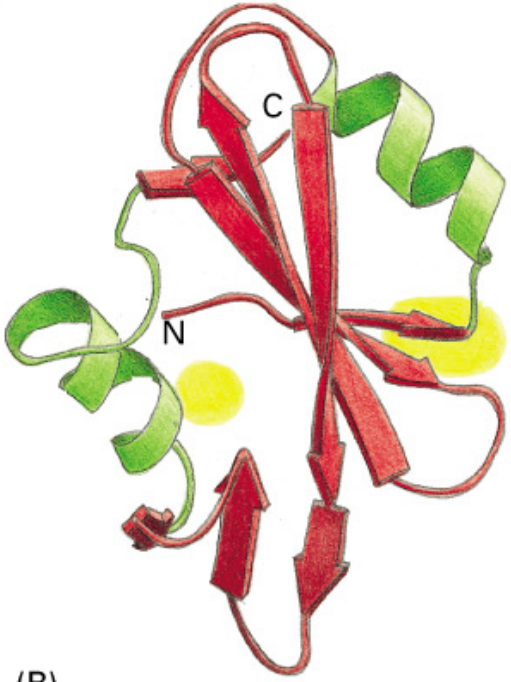
Key concept:

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

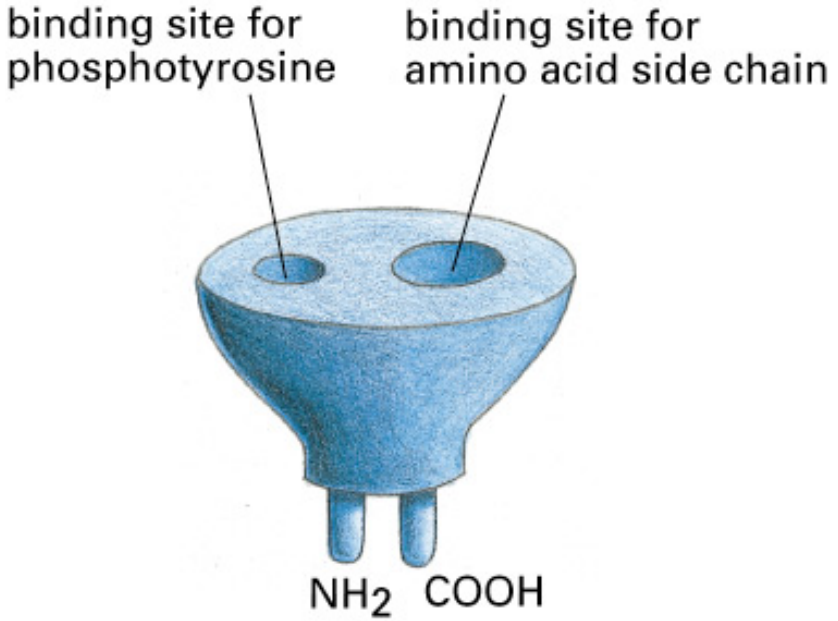

EGFR
phosphotyrosine
Y992
Y1045
Y1068
Y1086
Y1148
Y1173
Grb2
Crk
SHP-1
p120RasGAP
Src
Nck
Abl
STAT1
SLP76
IRS-1
Cbl
p85 PI 3-kinase
PLC-γ
Shc
SHP-1
y559
Y581
Y716
Y741
Y751
Y771
Y1009
Y1021
phosphotyrosine
PDGFR
adaptor/regulator
enzyme
transcription factor

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)



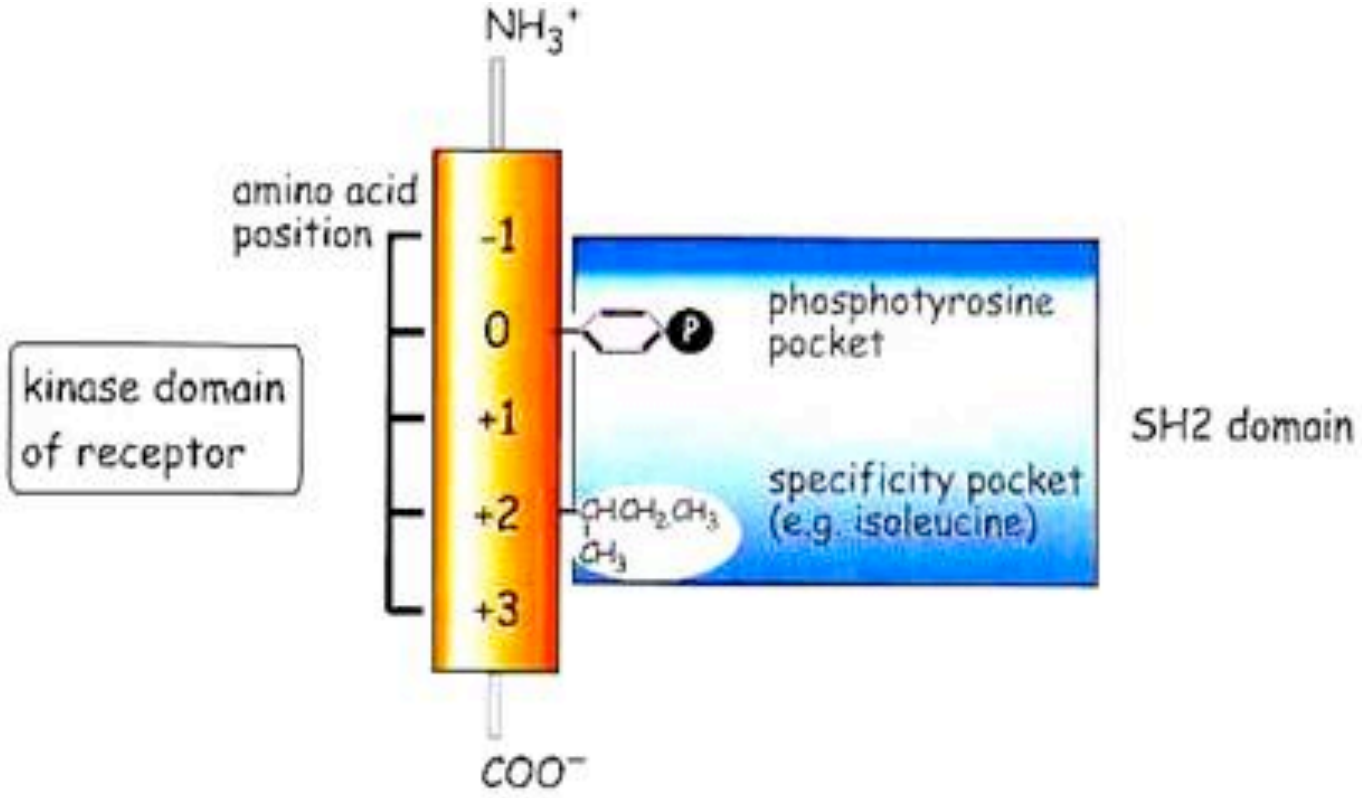
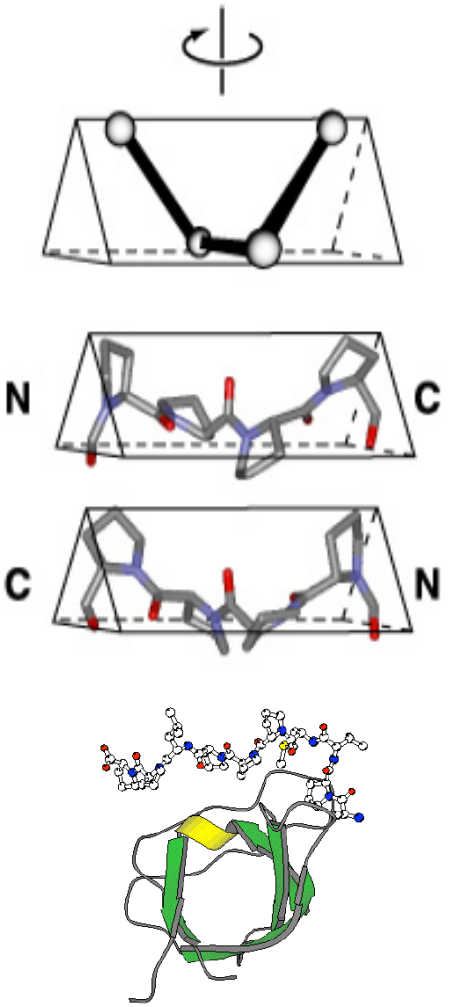


Figure 11.7 Recognition of phosphotyrosine and adjacent amino acids by the SH2 domain. Selectivity of recognition between different targets containing SH2 domains is conferred by the sequence of amino acids, particularly the third residue immediately adjacent on the C-terminal side of the phosphorylated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

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

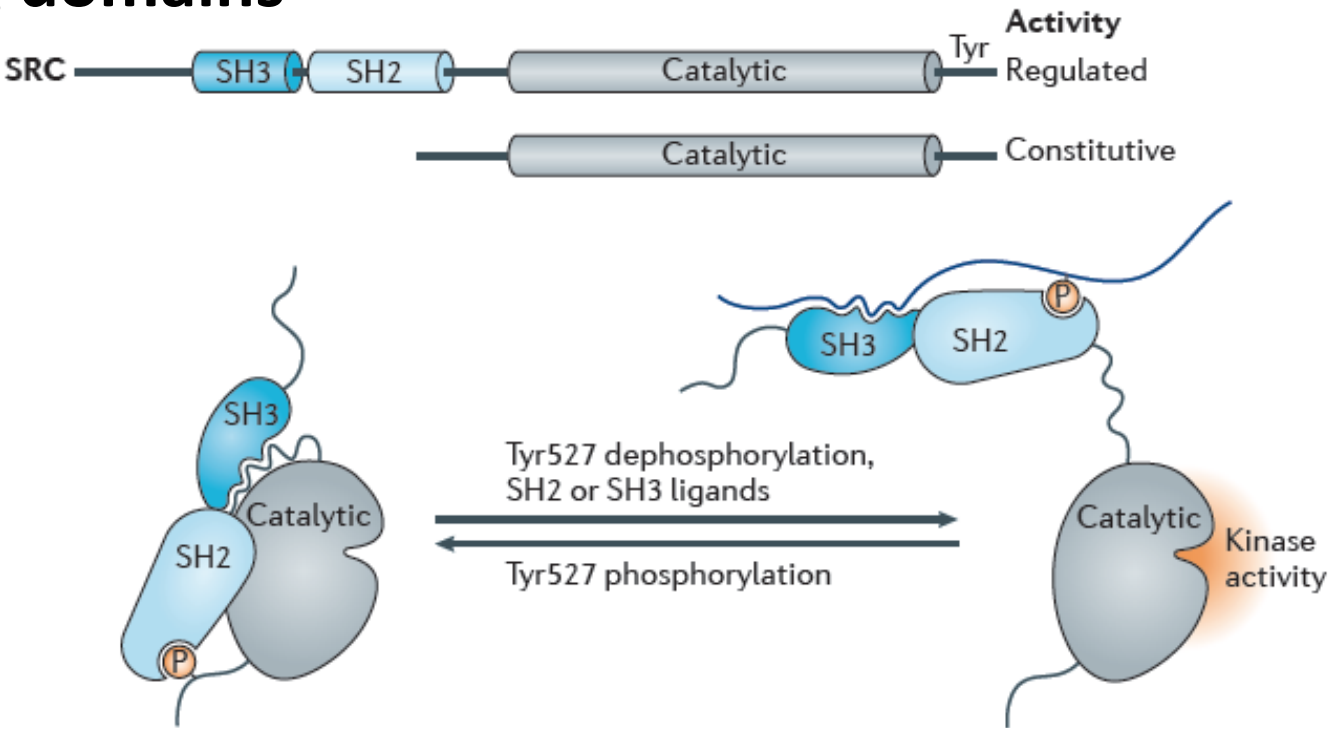
SH3 Domains: Properties

- 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

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, which potentially targets them for phosphorylation by the SRC catalytic domain and leads to changes in the subcellular localization of SRC.

Signaling Efficiency: Scaffolding Proteins and Signaling Complexes

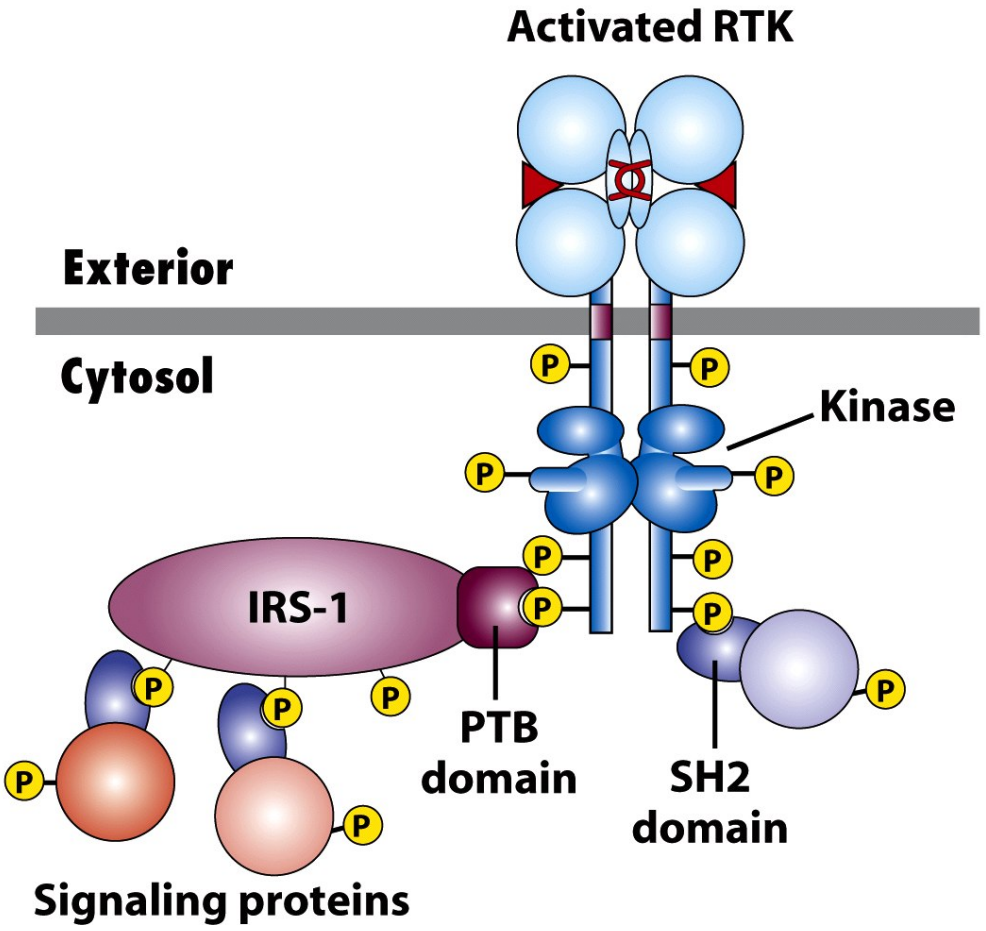
- **Scaffolding proteins** are large relay proteins to which other relay proteins are attached
- Scaffolding proteins can increase the signal transduction efficiency by grouping together different proteins involved in the same pathway
- In some cases, scaffolding proteins may also help activate some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

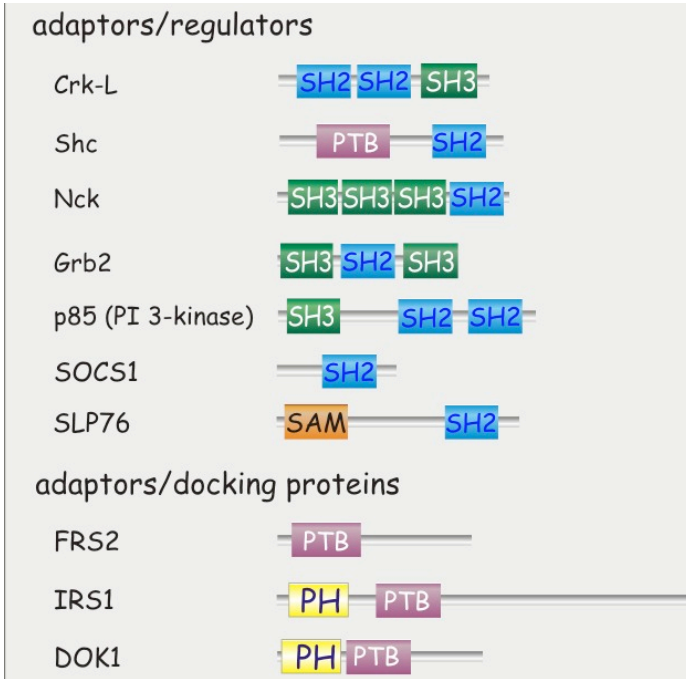
Recruitment of Signal Transduction Proteins to Activated Receptors

RTKs downstream effectors interact with phosphorylated RTKs via phosphotyrosine binding domains.

Two main binding domains PTB and SH2 are involved in the recruitment of adaptors such as the multi-docking protein known as the insulin receptor substrate-1 (IRS-1).

The binding of signaling proteins allows them to be phosphorylated by the receptor.

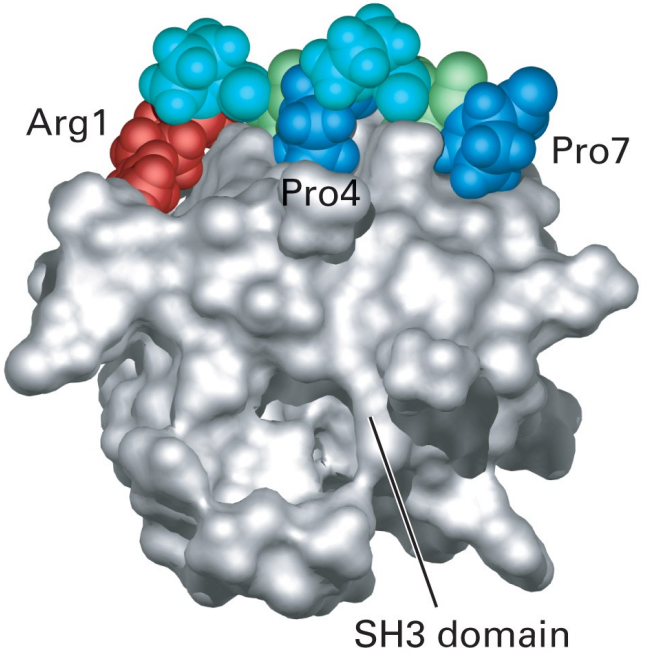


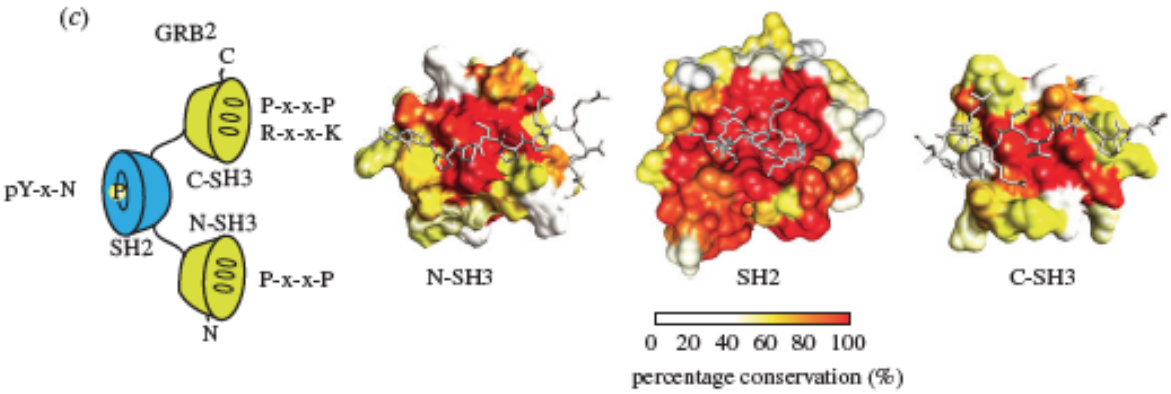


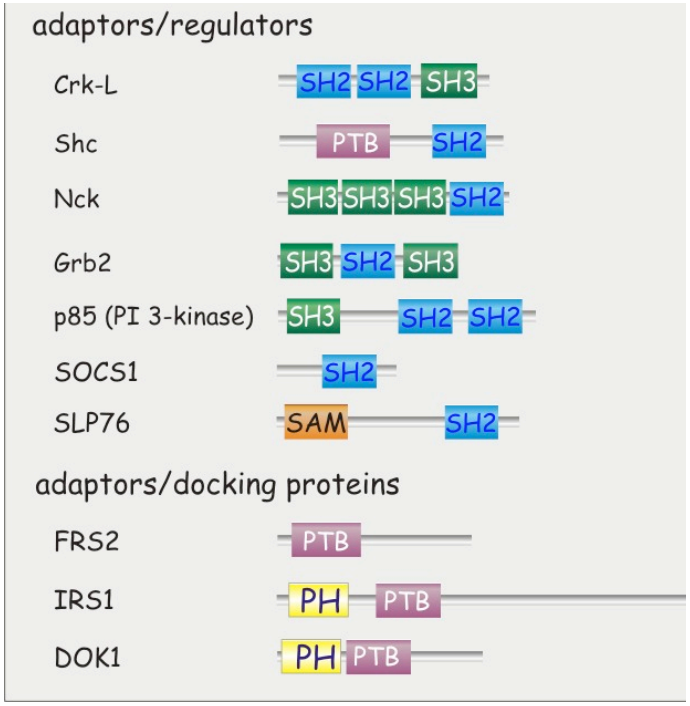
ADAPTORS PROTEINS: GRB-2

Protein domains of GRB2

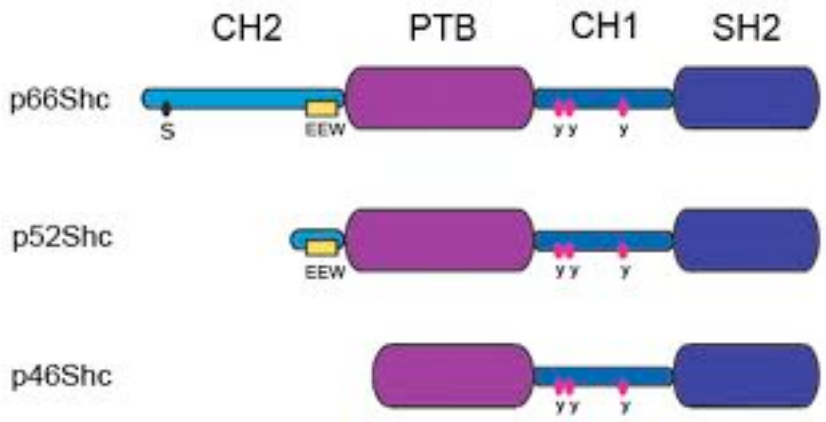
SH2: binds to phosphotyrosine residues
SH3: binds to proline rich sequences

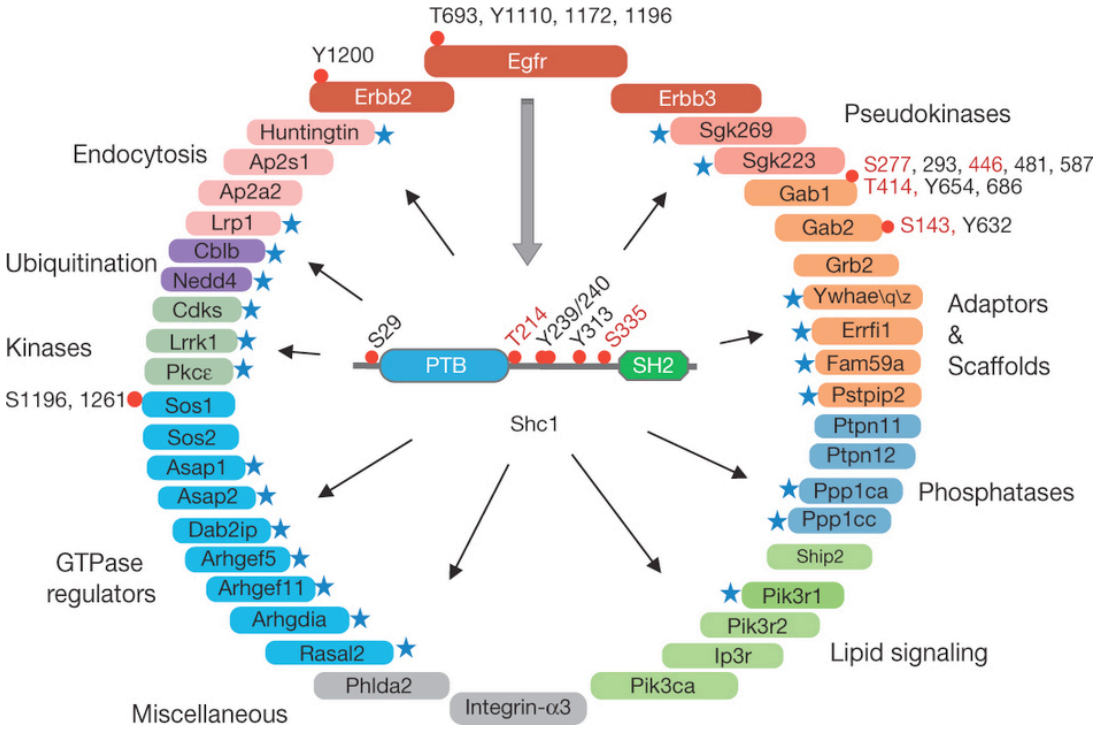






ADAPTORS PROTEINS: Shc

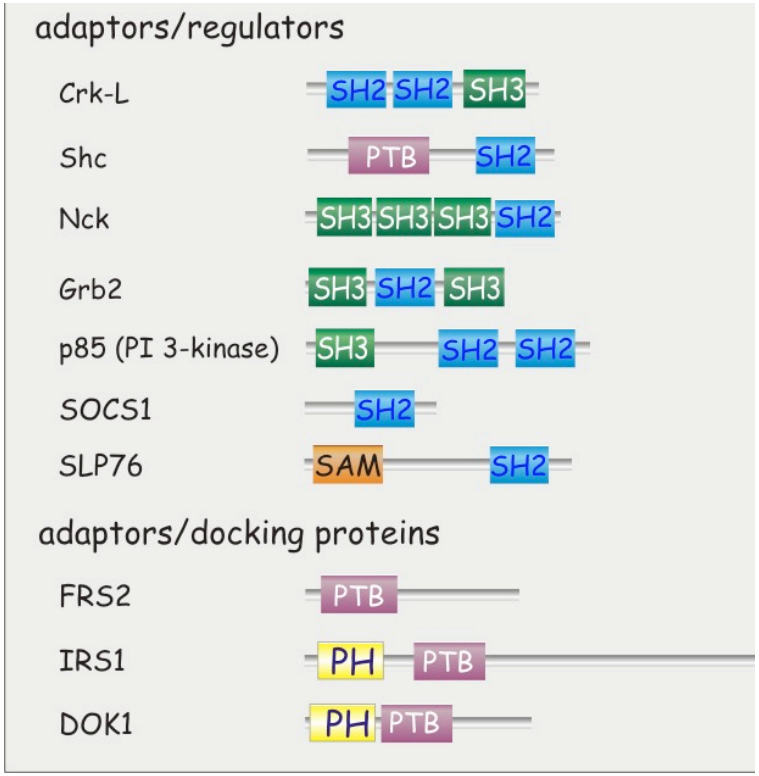




Protein domains of Shc

SH2: binds to phosphotyrosine residues

PTB: binds to phosphotyrosine rich sequences

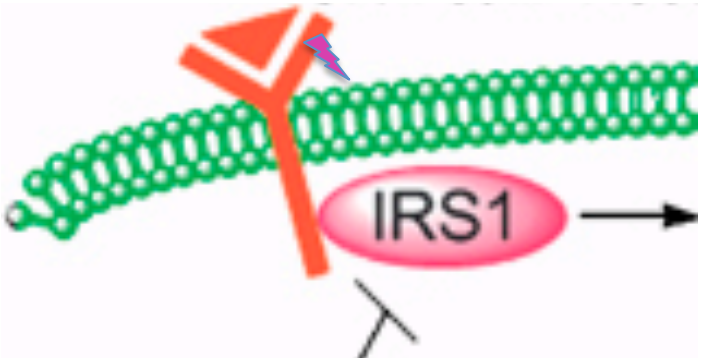


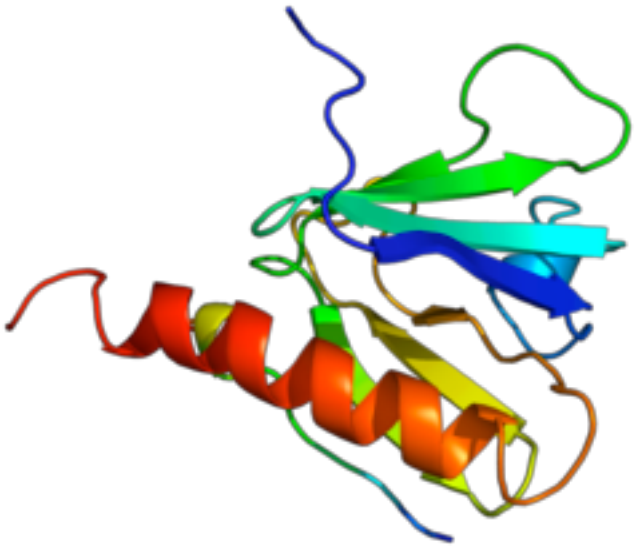
ADAPTORS PROTEINS: IRS-1

Protein domains of IRS-1:

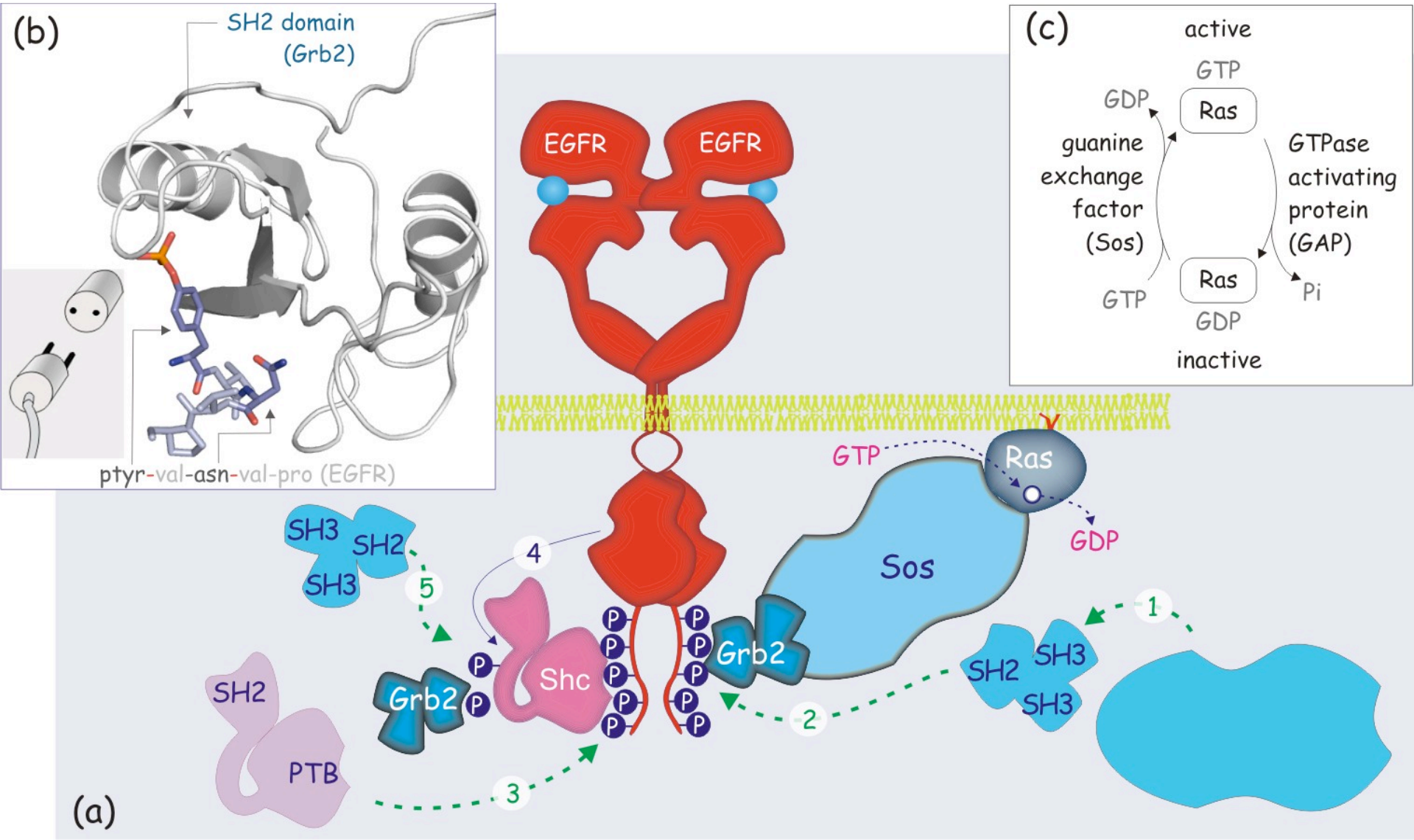
PH: binds to bind PI lipids within biological membranes (PIP2-PIP3) and proteins such as the βγ-subunits of G proteins and PKc

PTB: bind to phosphotyrosine residues





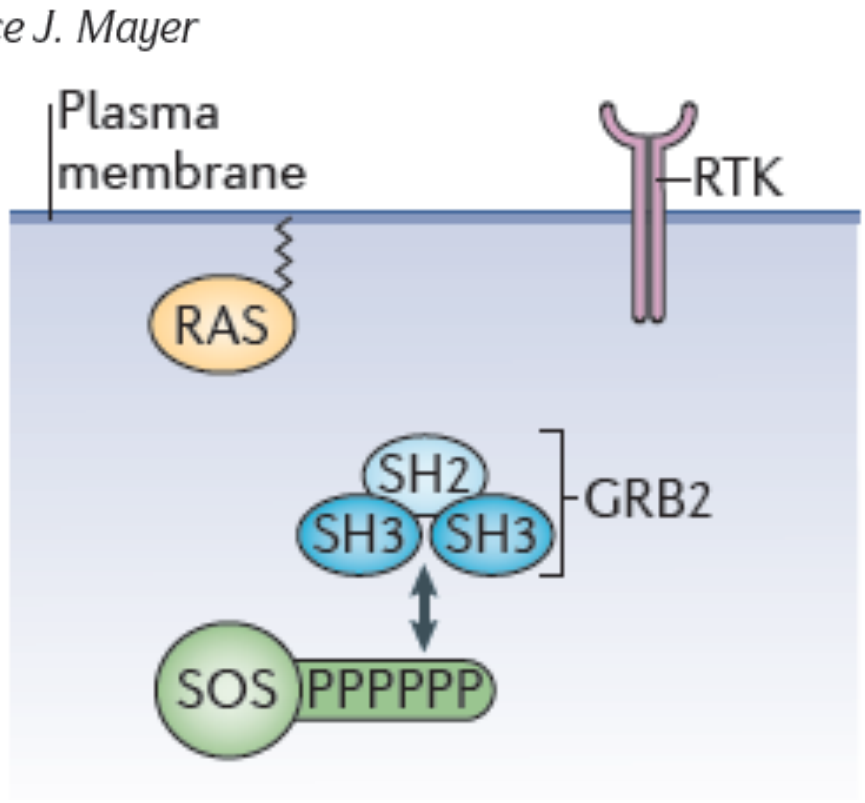
An example of a signaling complex – a protein relay system using phosphorylation as a signal

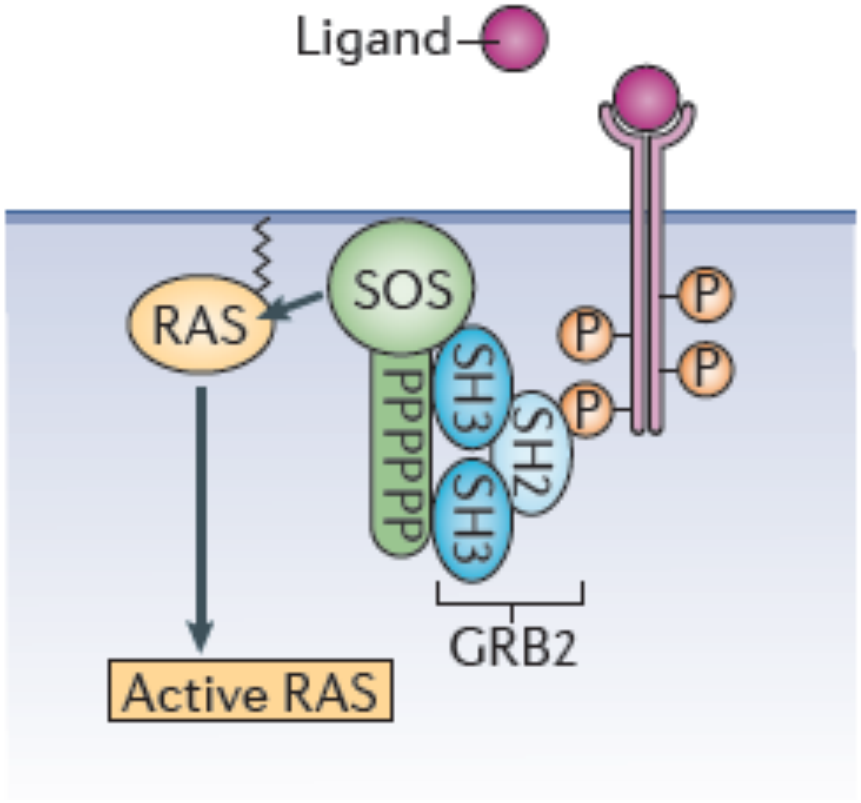


Nature Reviews Molecular Cell Biology | AOP, published online 30 September 2015; doi:10.1038/nrm4068

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

Bruce J. Mayer





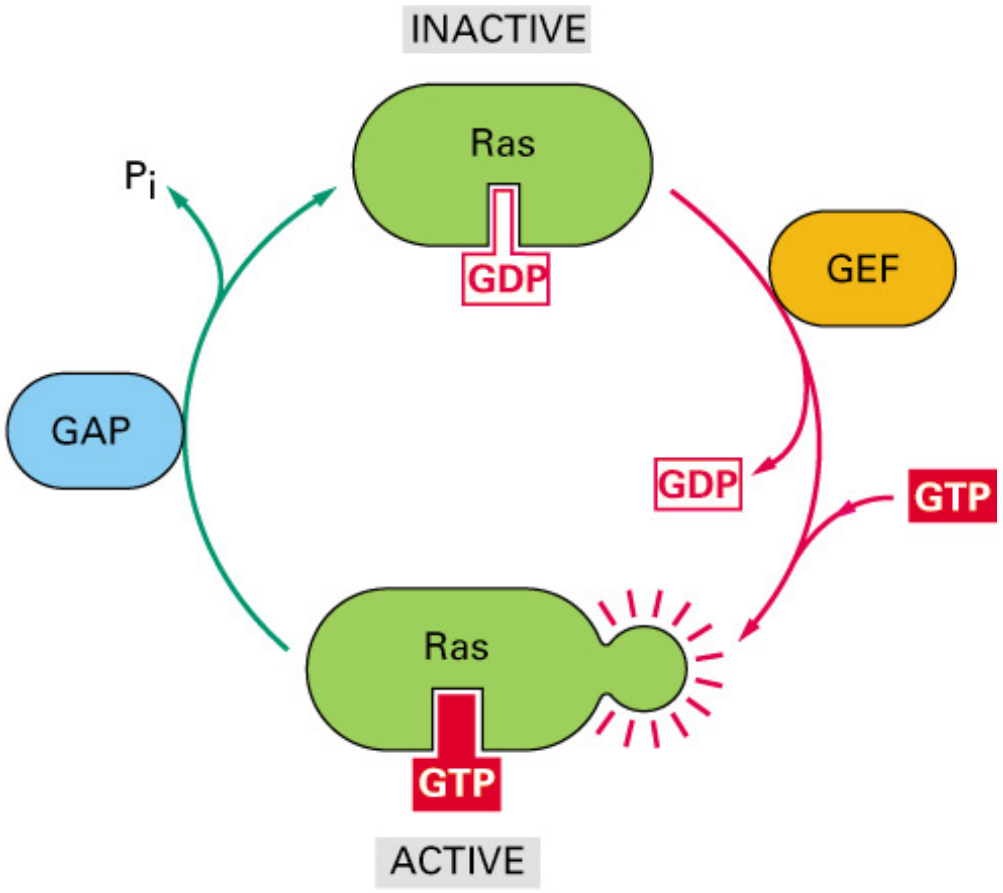
Paradigms for activation of RTK signaling cascade:

Translocation to the plasmalemma is essential for activation of most effector proteins

Ras (RAT-sarcoma) **is a monomeric GTPase**

- Ras is a G protein (guanosine-nucleotide-binding protein), a small GTPase
- The first two *ras* genes, *HRAS* and *KRAS*, were first identified from the Harvey sarcoma virus and Kirsten sarcoma virus, by Scolnick and colleagues at (NIH) in 1982. In 1982, activated and transforming human *ras* genes were discovered in human cancer cells. A third *ras* gene was subsequently discovered and named *NRAS*, for its initial identification in human neuroblastoma cells.
- Anchored at the plasma membrane owing to its prenylation and palmitoylation (HRAS and NRAS) or the combination of prenylation and a polybasic sequence adjacent to the prenylation site (KRAS).
- The C-terminal region of Ras first gets farnesylated at its Cys residue in the cytosol, allowing Ras to loosely insert into the membrane of the endoplasmatic reticulum and other cellular membranes.
- The three human *ras* genes encode extremely similar proteins made up of chains of 188 to 189 amino acids, designated H-Ras, N-Ras and K-Ras4A and K-Ras4B (from alternative splicing).

Ras is a monomeric GTPase

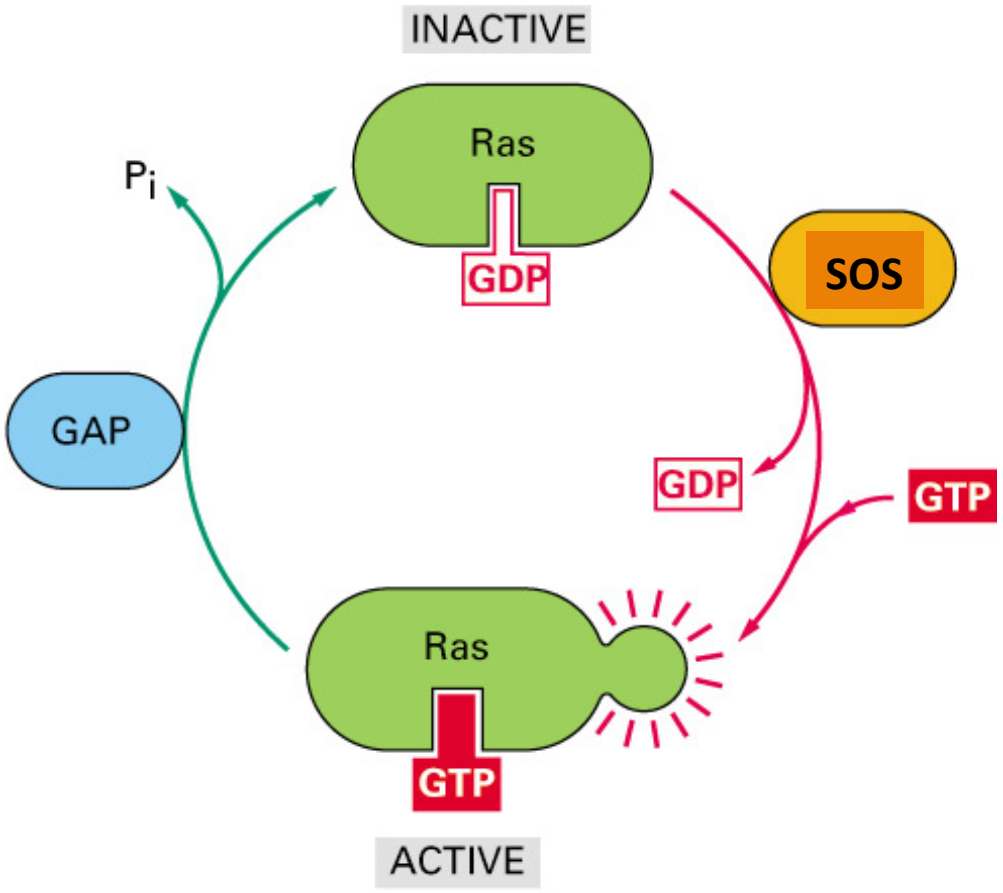


Ras has an intrinsic GTPase activity: the protein on its own will hydrolyze a bound GTP molecule into GDP. However this process is too slow for efficient function, and hence the GAP for Ras, *RasGAP*, may bind to and stabilize the catalytic machinery of Ras.

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 activation of Ras by RTKs

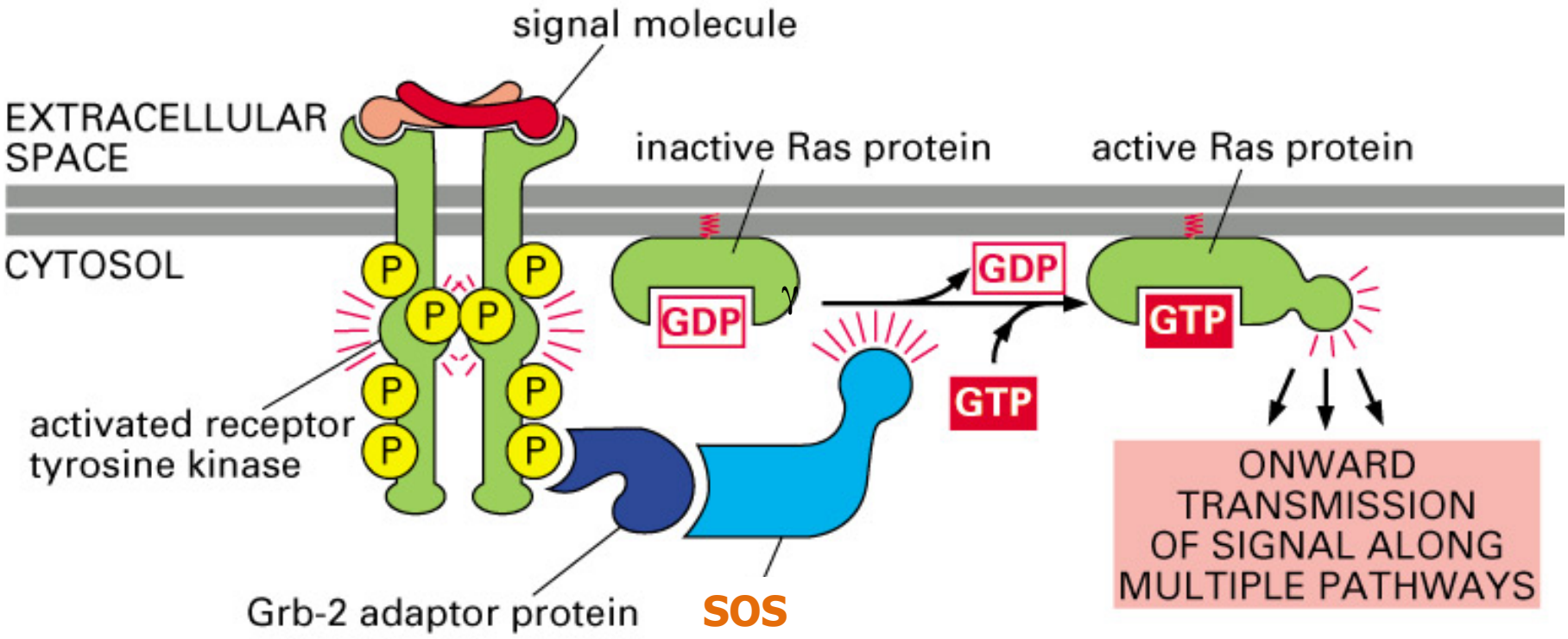


Figure 15–55. Molecular Biology of the Cell, 4th Edition.

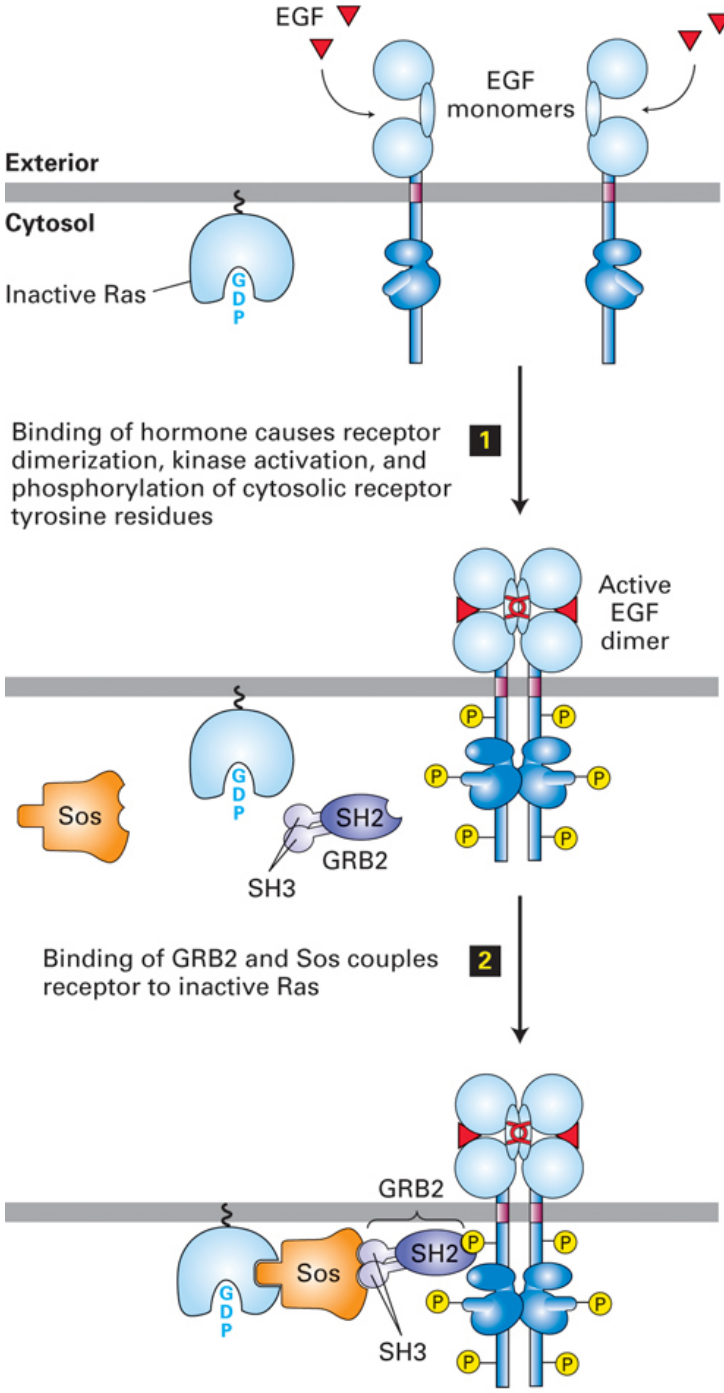
RTK Activation of Ras

EGF binding causes receptor clusterization and autophosphorylation on cytosolic tyrosines.

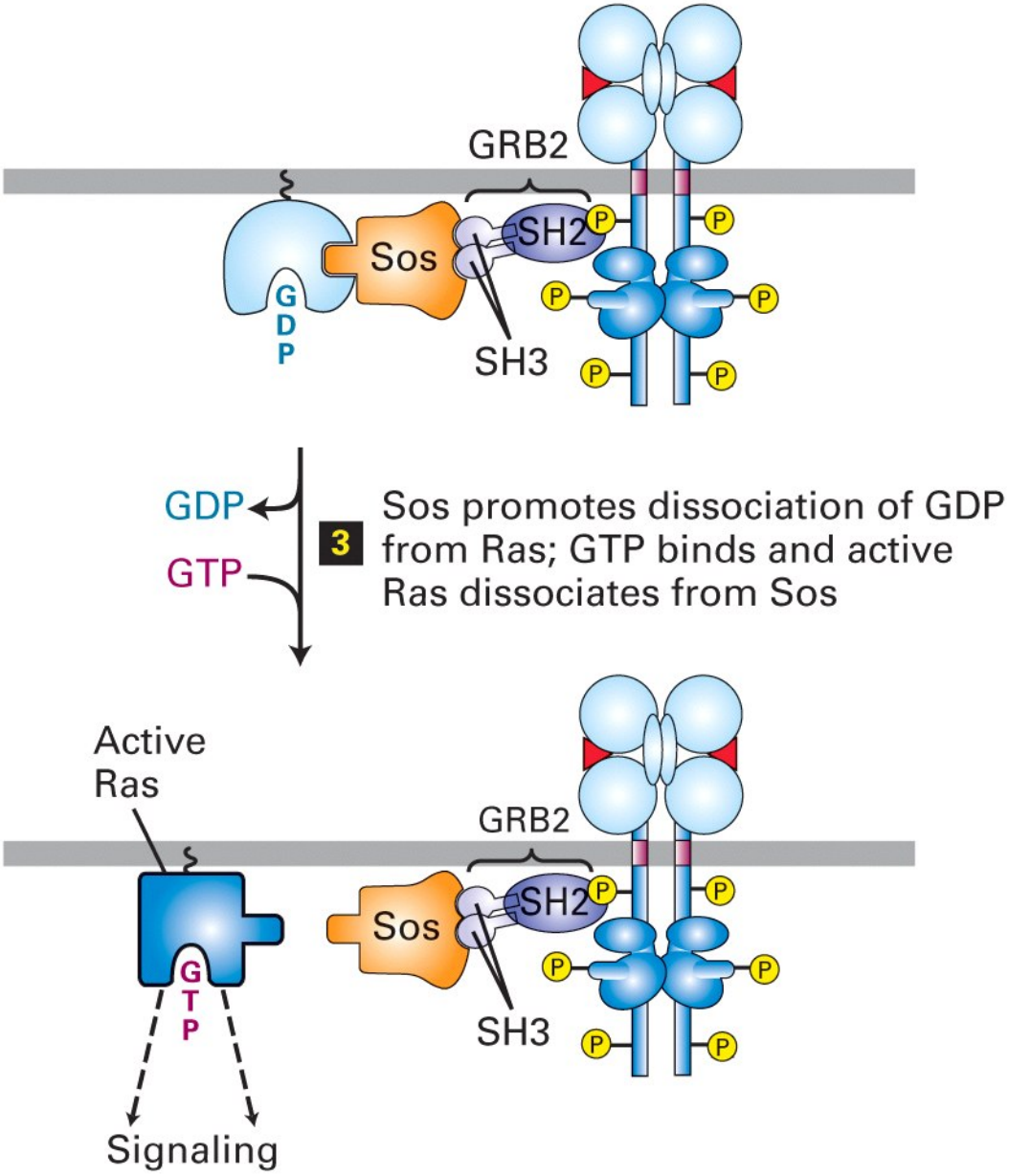
In Step 2, the adaptor protein GRB2 binds receptor phosphotyrosine residues via its SH2 domain. GRB2 contains SH3 domains that allow the GEF protein known as Sos to bind to the membrane complex.

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

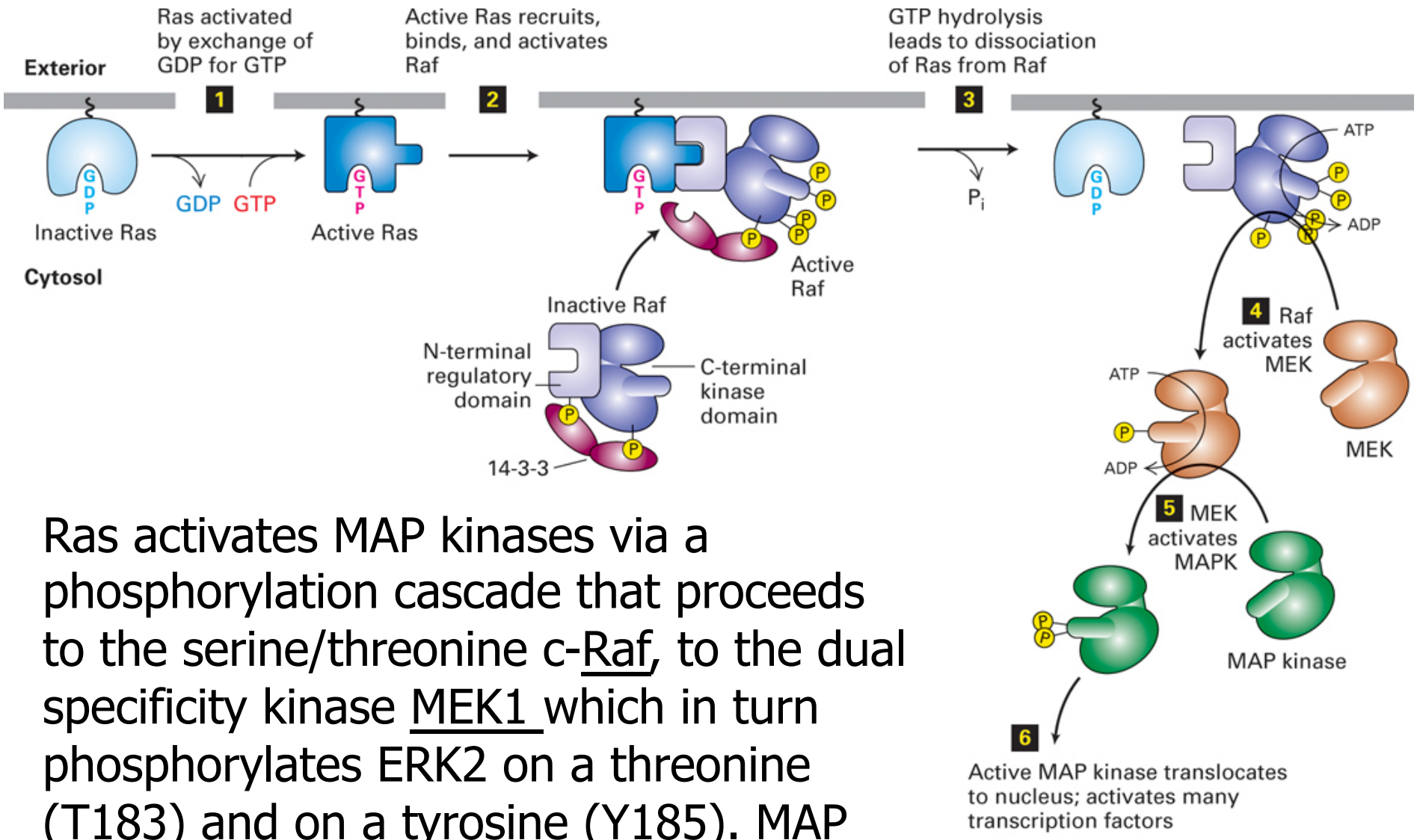
Sos converts inactive GDP-ras into active GTP-ras.



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

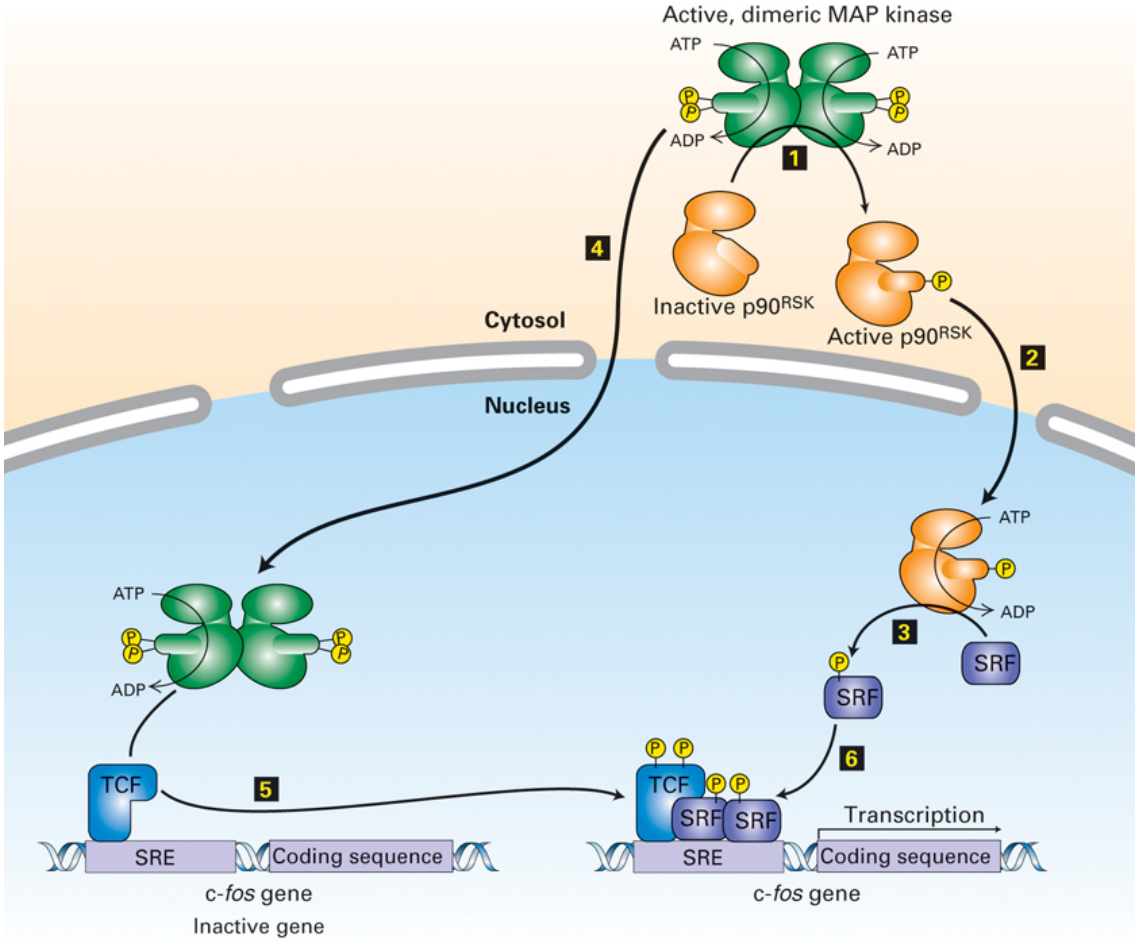


Ras activates MAP kinases via a phosphorylation cascade that proceeds to the serine/threonine c-Raf, to the dual specificity kinase MEK1 which in turn phosphorylates ERK2 on a threonine (T183) and on a tyrosine (Y185). MAP kinase then dimerizes and enters the nucleus.

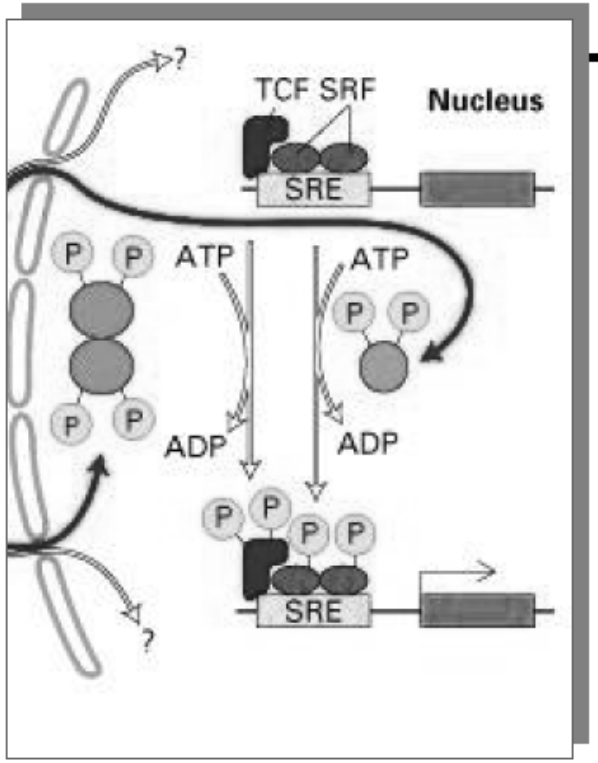
MAP Kinase Activation of Transcription

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.

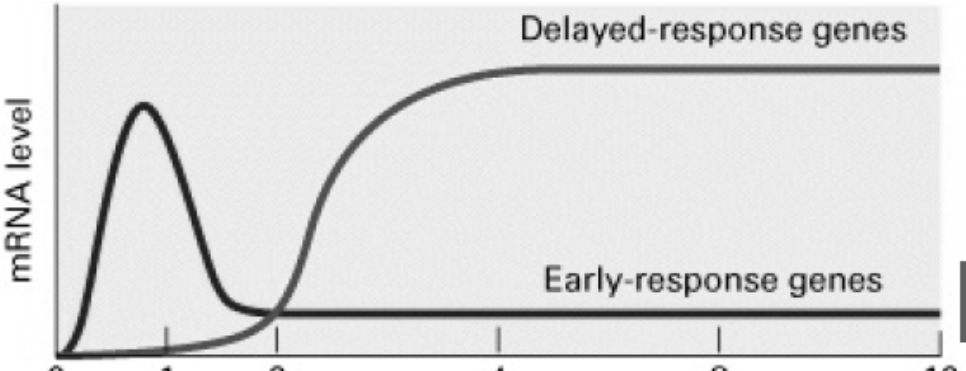
The phosphorylated forms of these TFs bind to serum response element (SRE) enhancer sequences that control genes regulated by growth factors present in serum (such as c-fos) and propel cells through the cell cycle.



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



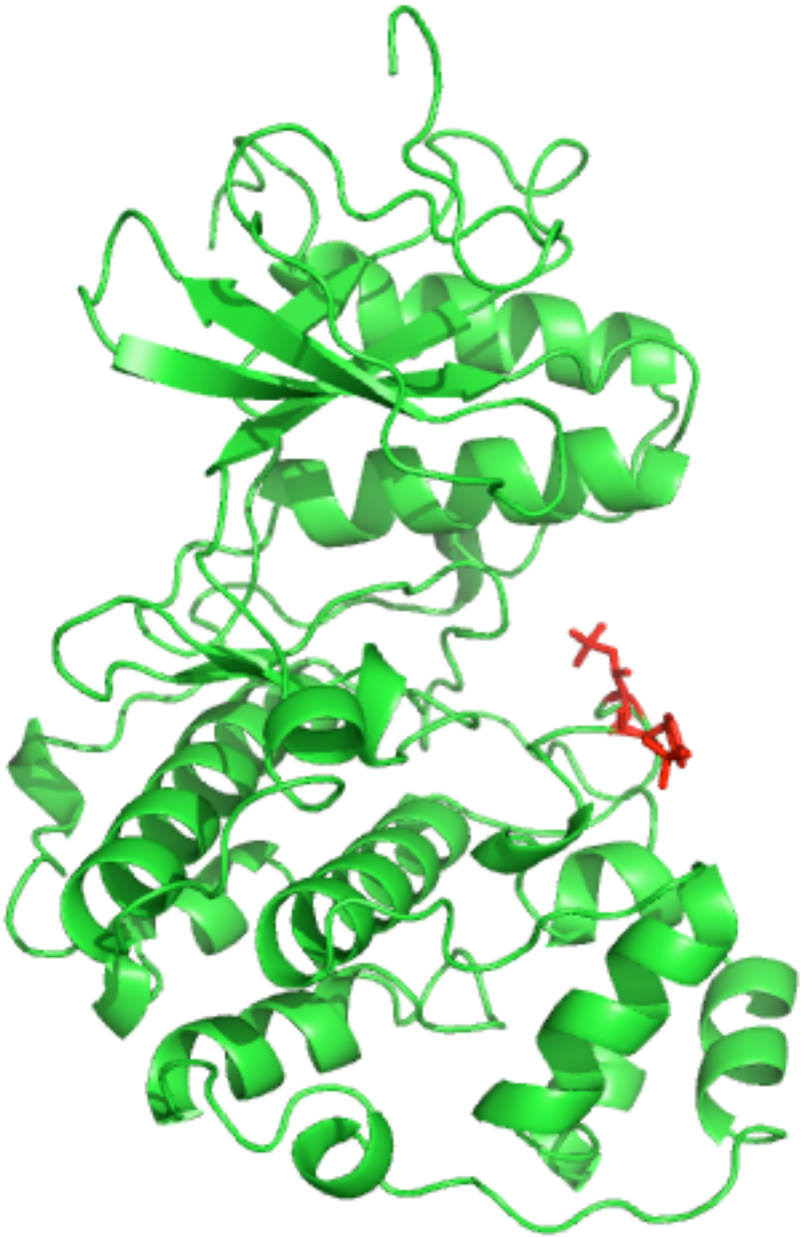
serum response elements;
-allows for activated transcription following growth factor (mitogen) stimulation
-found in genes involved in cellular proliferation.
Ex: c-fos gene
-c-fos is an early response gene
-required for the induction of delayed response genes including cyclin D.



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).

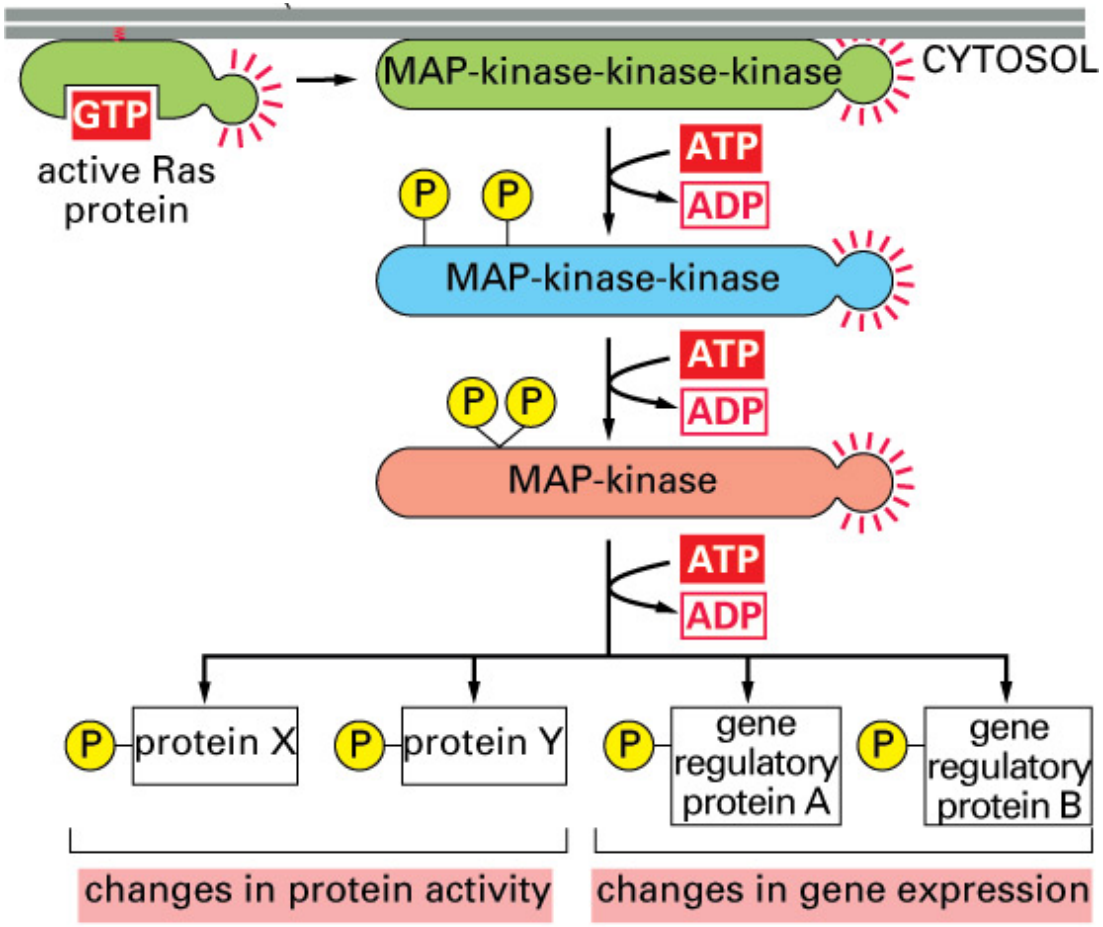


Table 2 | ***HRAS*, *KRAS*, *NRAS* and *BRAF* mutations in human cancer**

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.

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.

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

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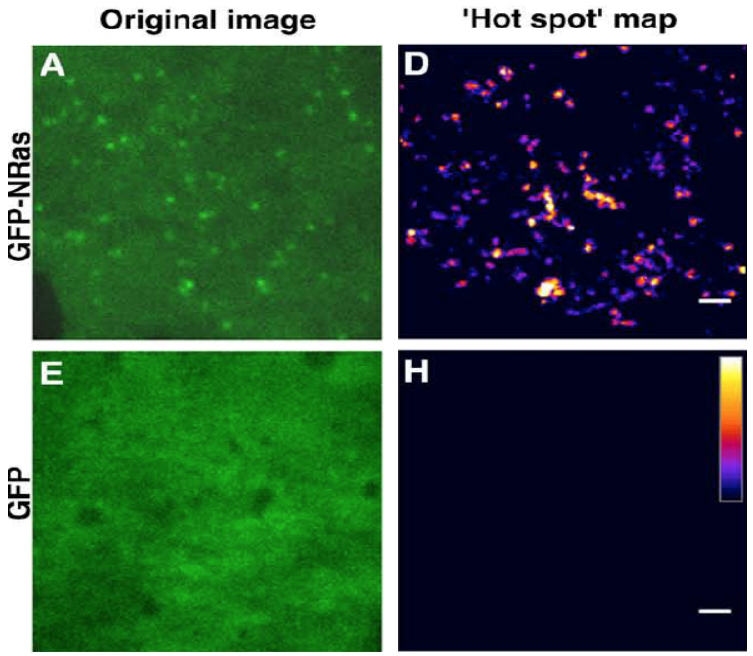
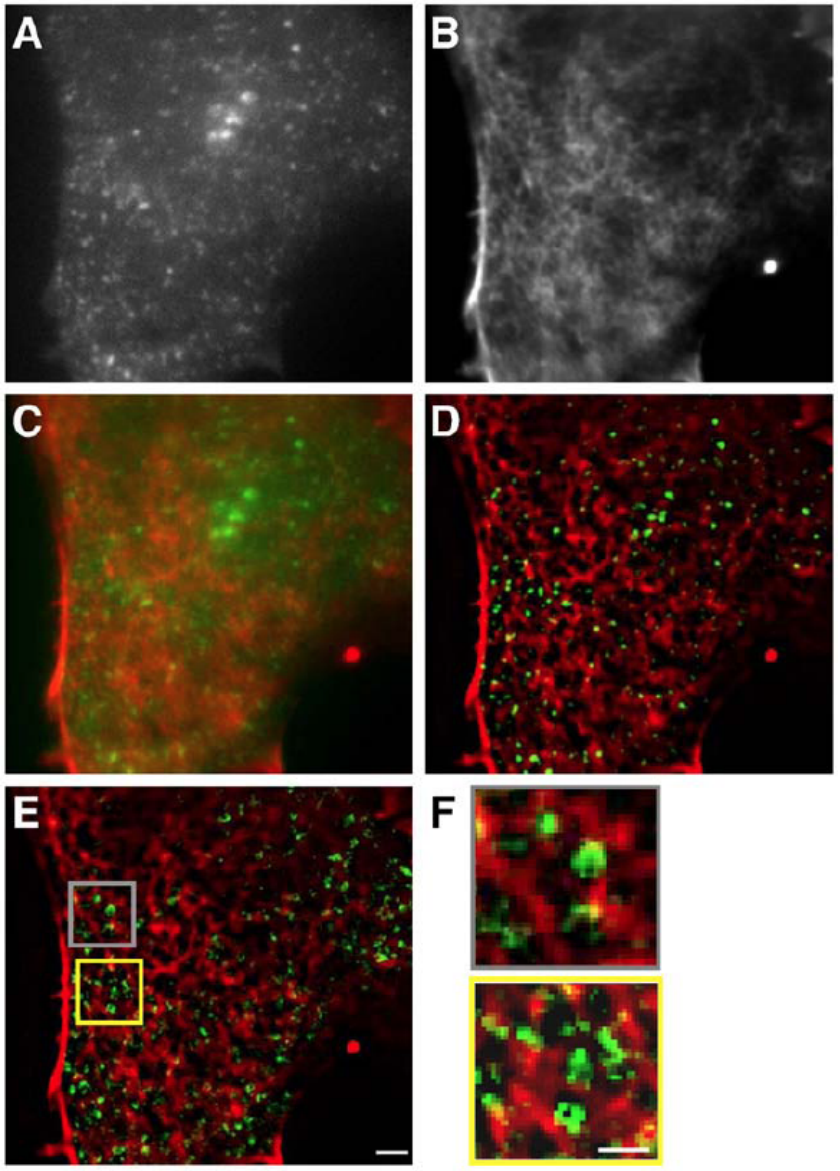
Rasosomes spread Ras signals from plasma membrane 'hotspots'

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

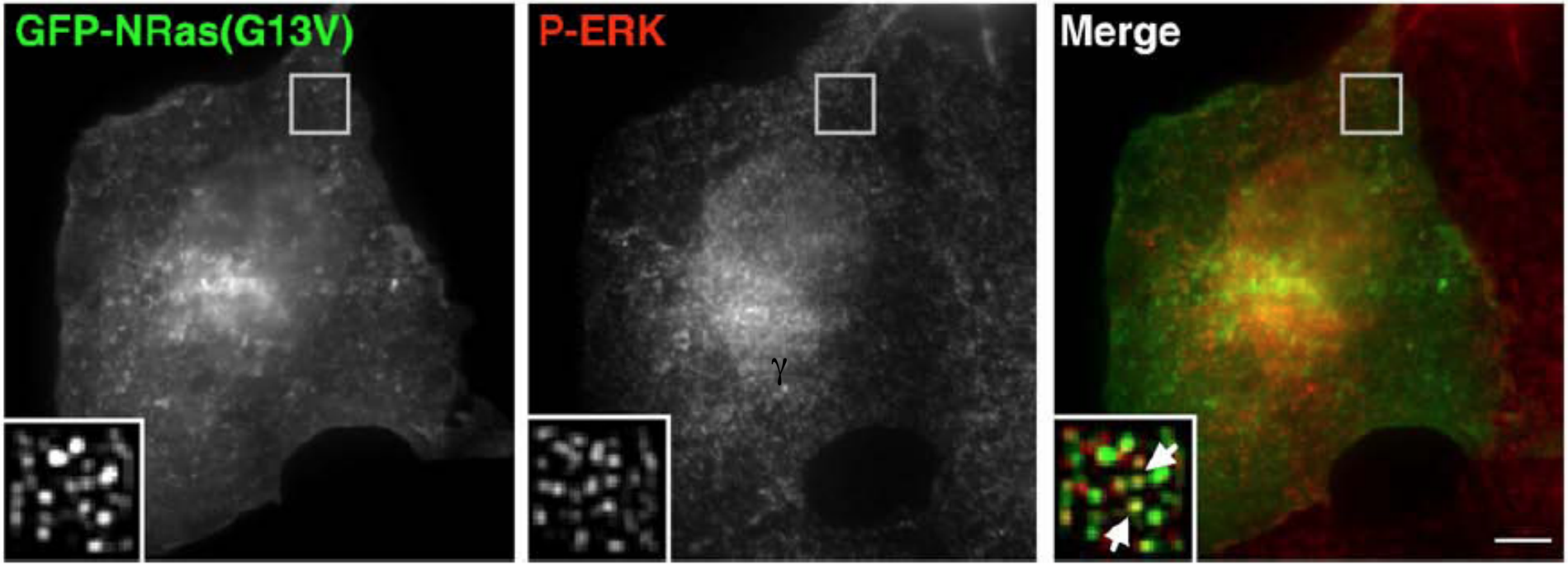
Department of Neurobiology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, 69978 Tel Aviv, Israel



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



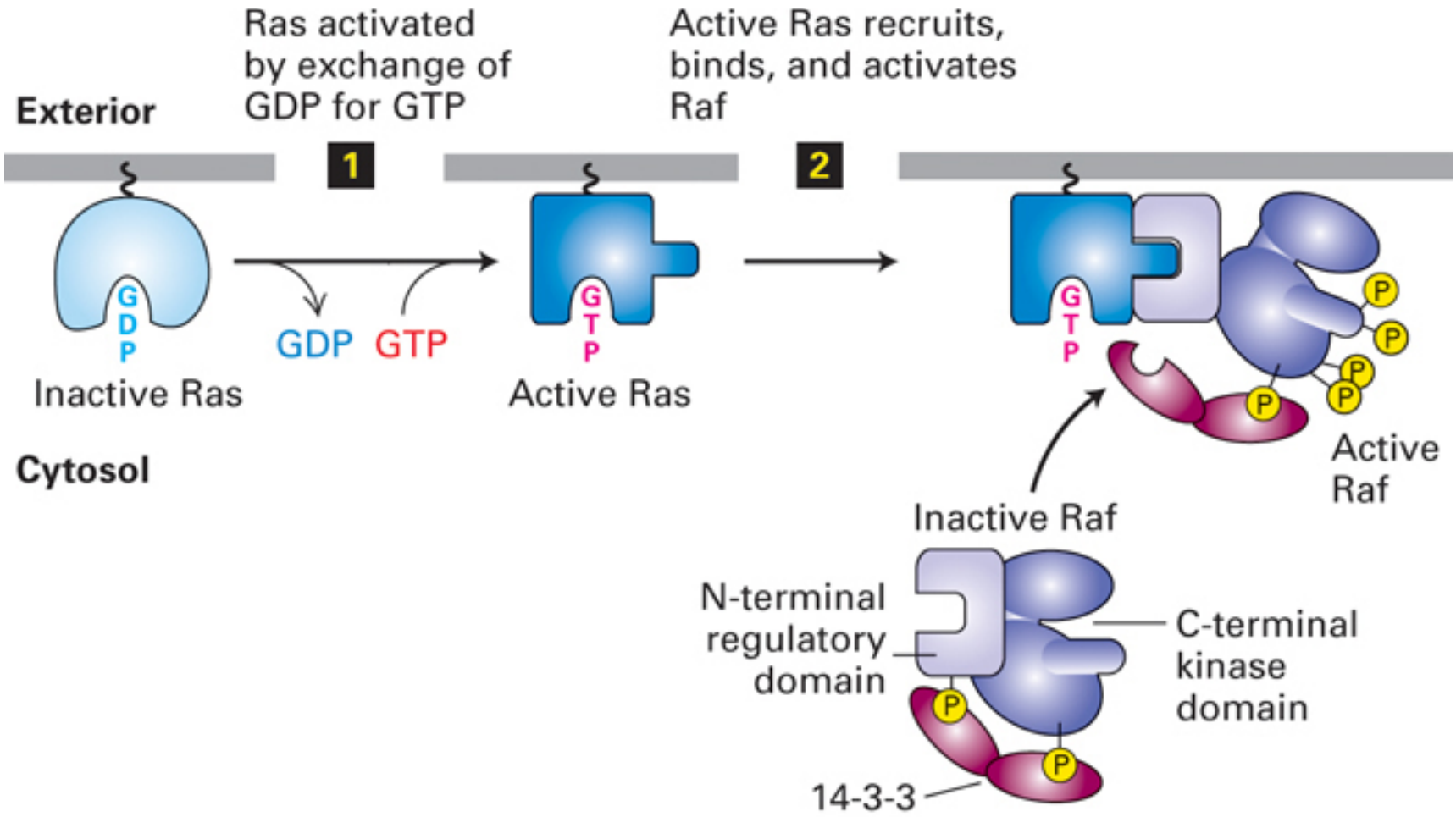
Rasosomes move within cortical actin cages.



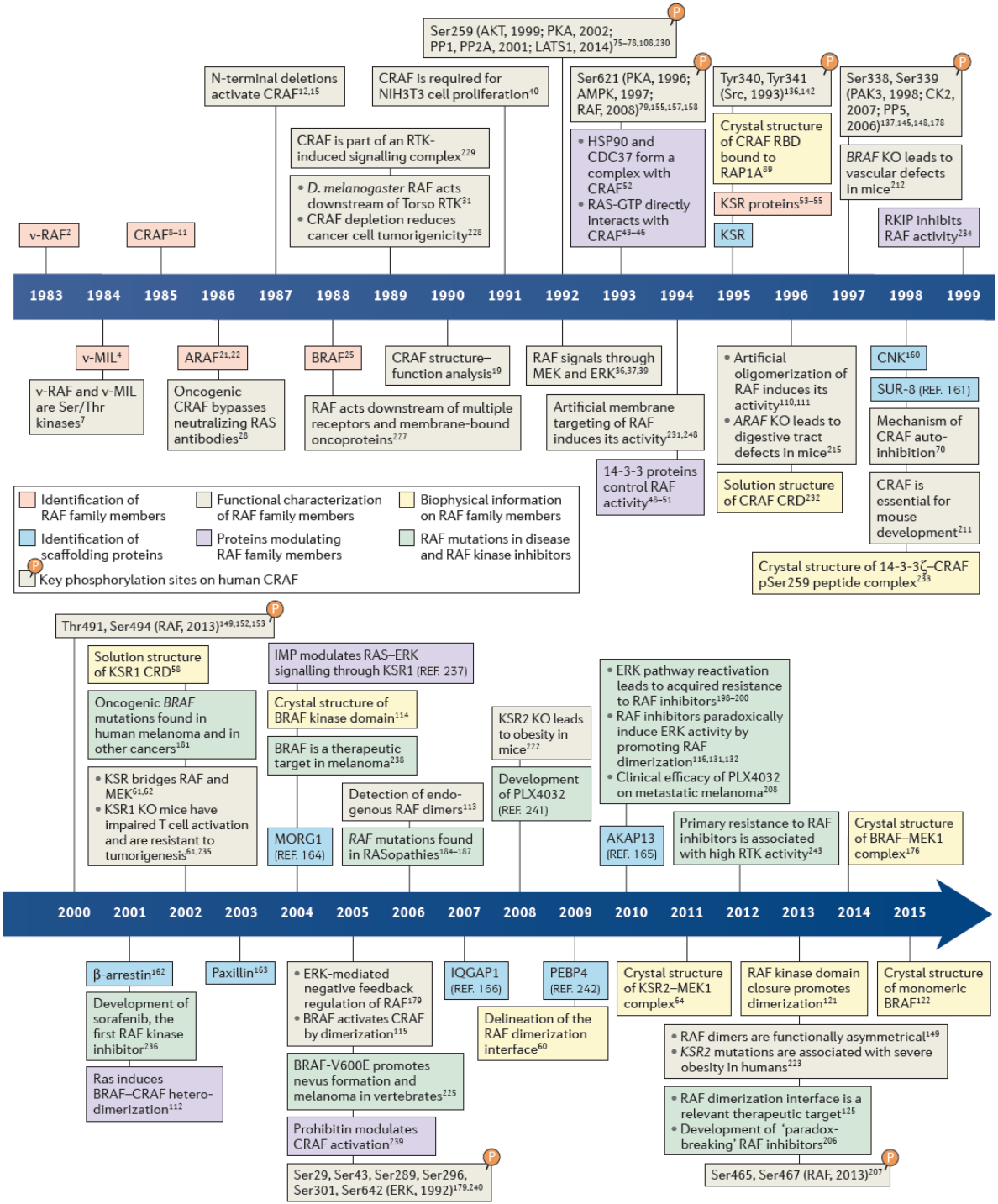
GFP-NRas expressing cells were labeled with anti-phosphorylated-ERK Abs. Insets show filtered images of the boxed regions with arrows that indicate phospho-ERK positive GFP-NRas rasosomes

Regulation of RAF protein kinases in ERK signalling

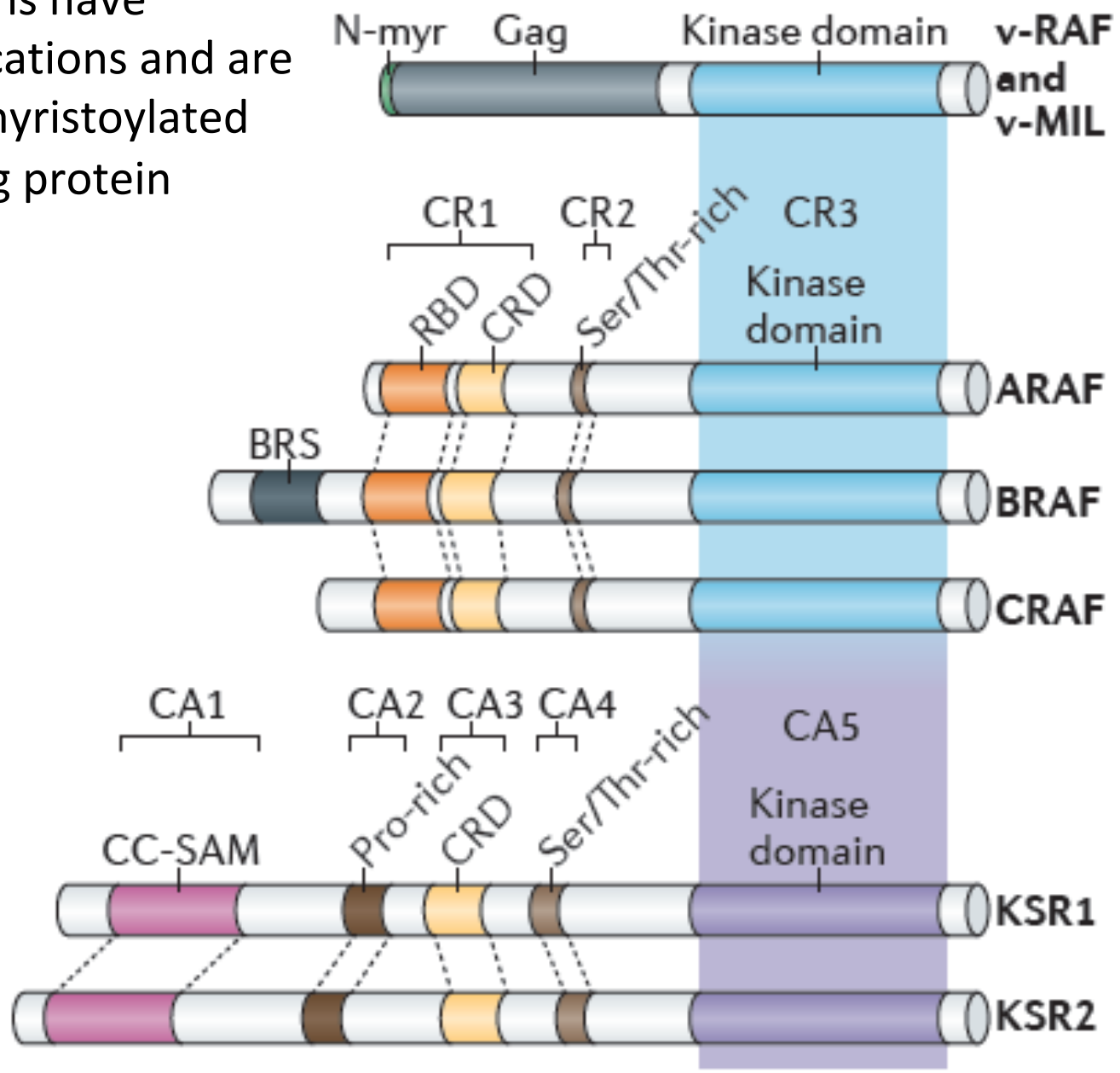
Hugo Lavoie[1] and Marc Therrien[1,2]



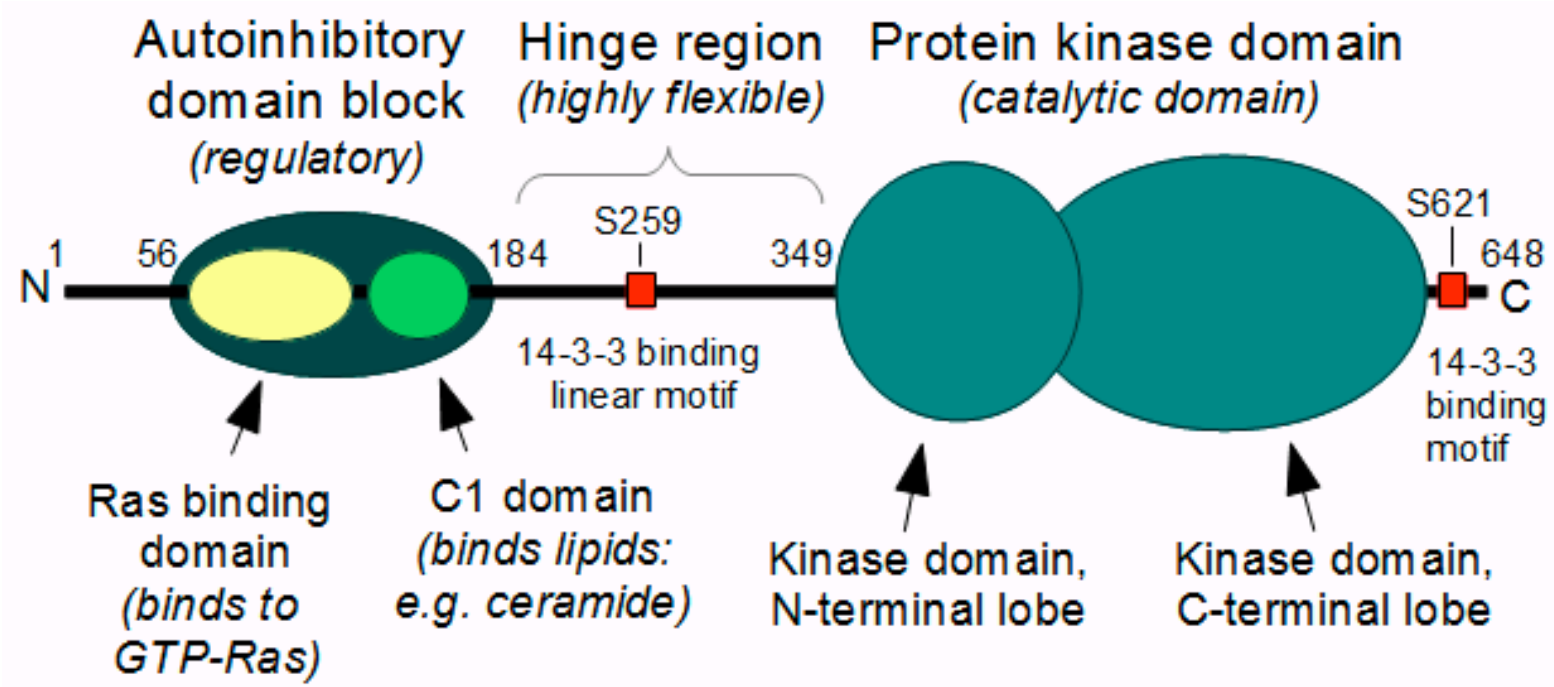
c-Raf


Ser259 (AKT, 1999; PKA, 2002; PP1, PP2A, 2001; LATS1, 2014)[75–76,108,230]
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[196–200]
• 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]
Development of PLX4032 (REF. 241)
• 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]
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

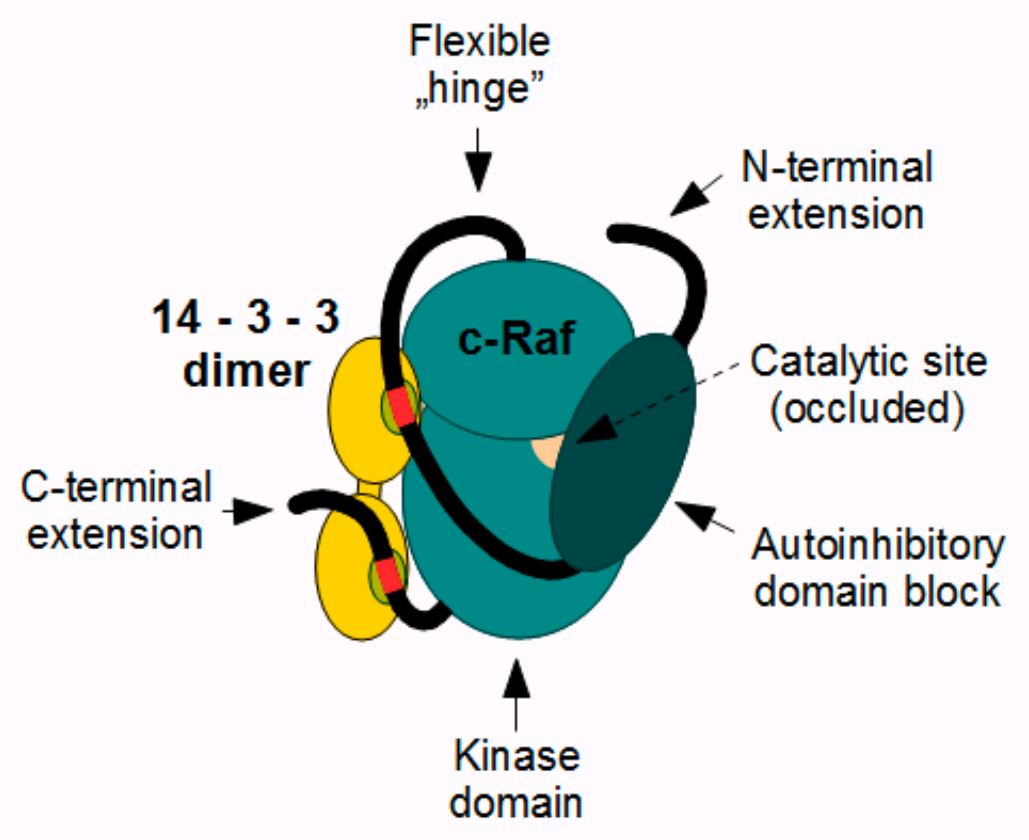


- Ras-binding domain: it binds GTP-Ras
- C1 domain: it is a special zinc finger, rich in cysteines and stabilized by two zinc ions. It interacts with lipids and aids in the recognition of GTP-Ras. The close proximity of these two domains allows them to act as a single unit to negatively regulate the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

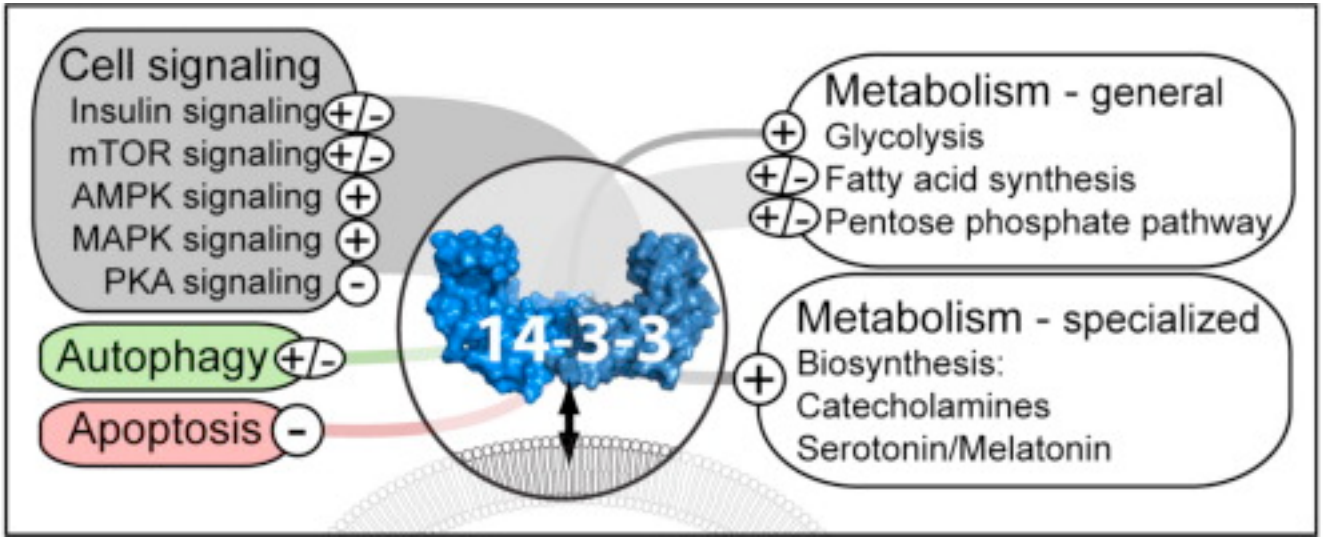
Regulation of c-Raf activity



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.

14-3-3 proteins

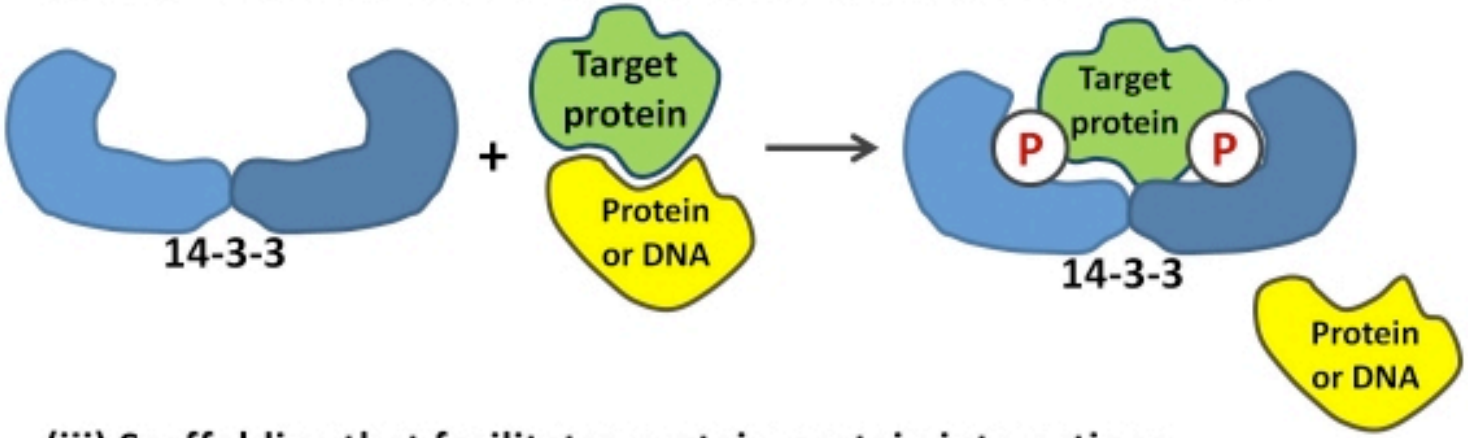
- Very well conserved in mammals, as well as in plants: they are among the very few signaling elements that are shared by both animals and plants.
- Family of acidic brain proteins. The name was given based on particular elution pattern on chromatography (14th fraction)
- They usually work as dimers
- 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

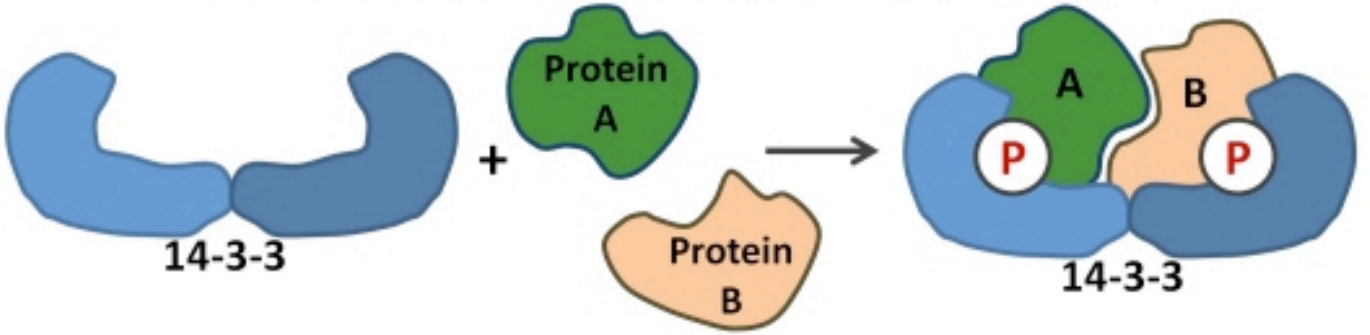


Regulation of subcellular localization

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