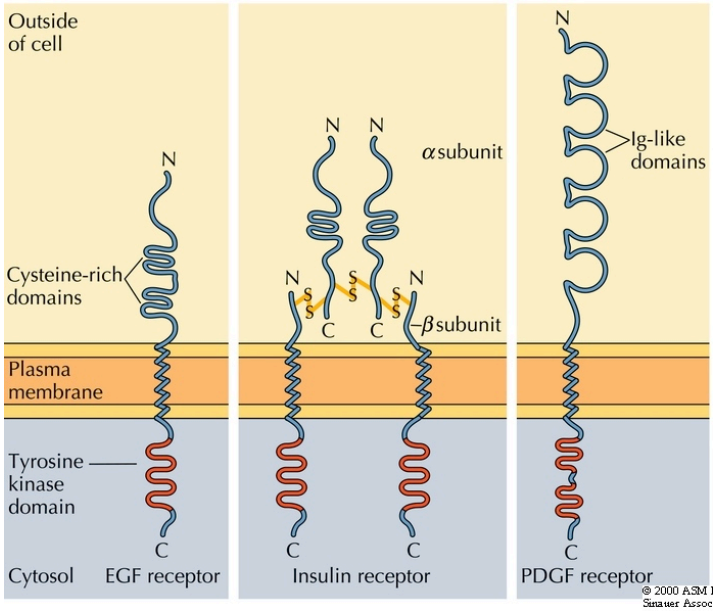
Enzyme-linked receptors fall into 3 categories:

1- Tyrosine Kinase Receptors

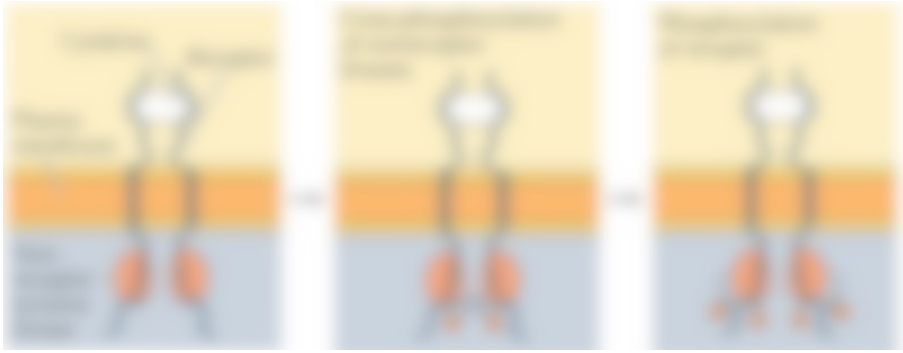
- Not only a receptor
- Also an enzyme: Tyrosine kinase



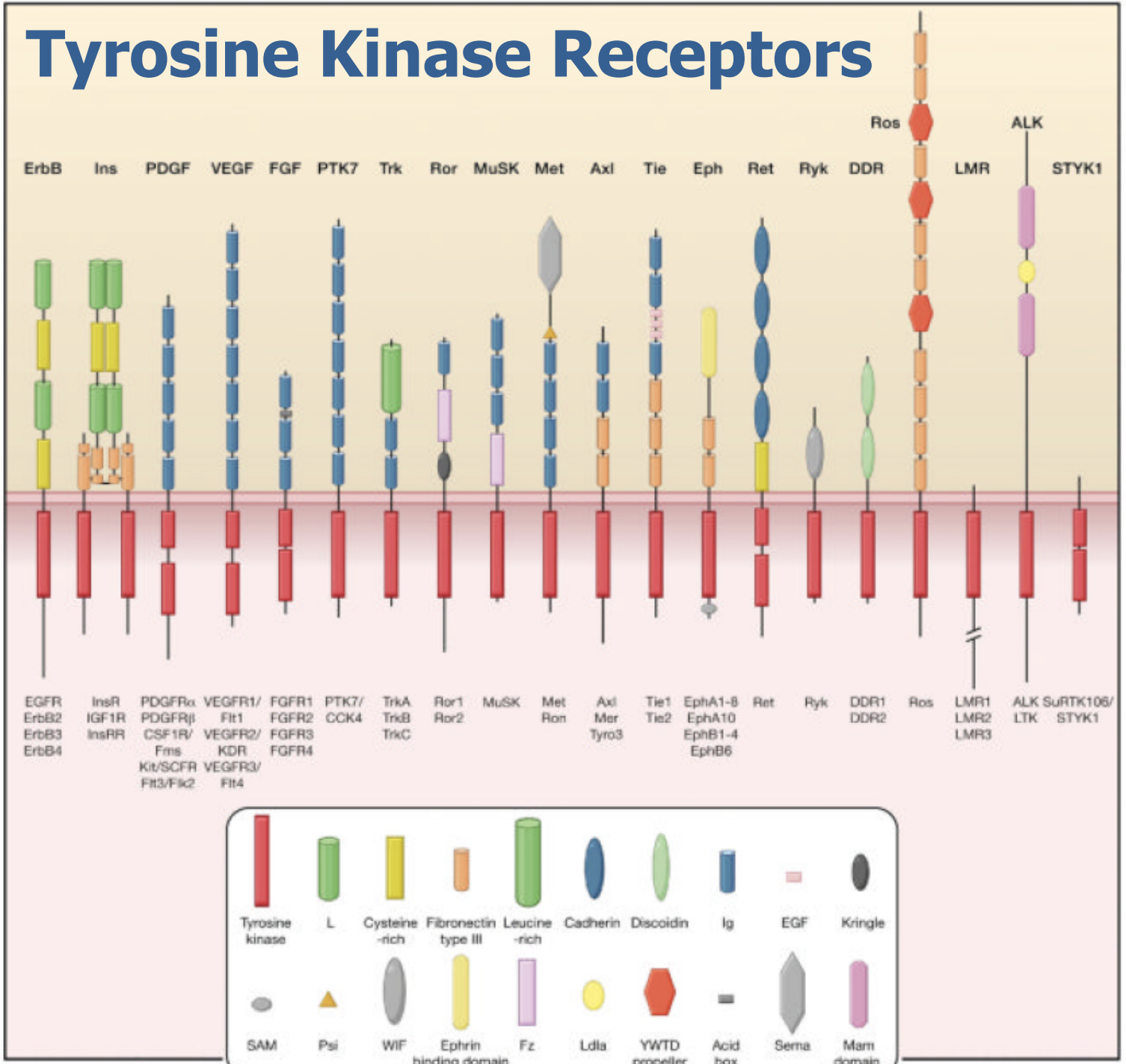
2- TGF-β receptors

3- Cytokine superfamily receptors

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



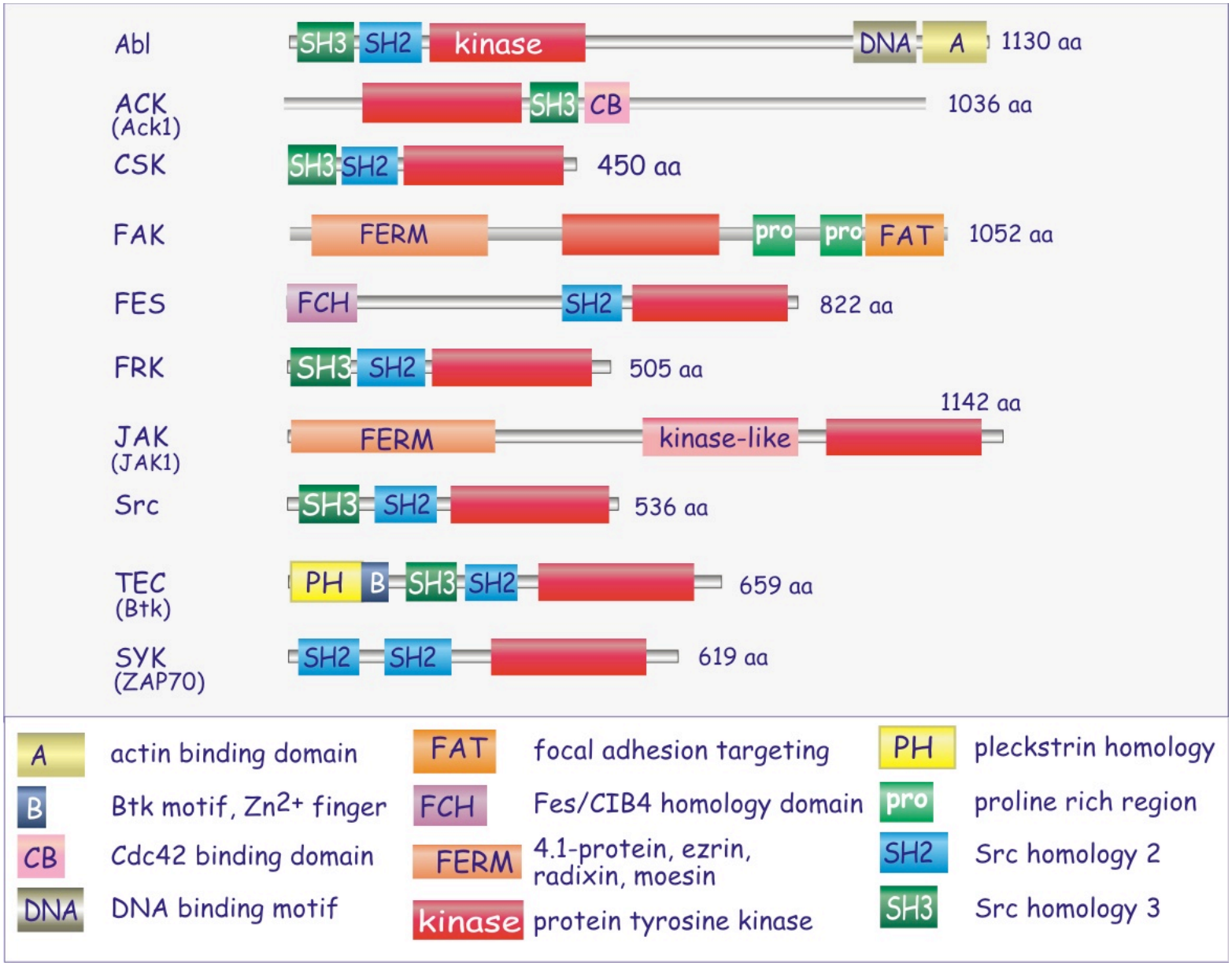
Tyrosine Kinase Receptors

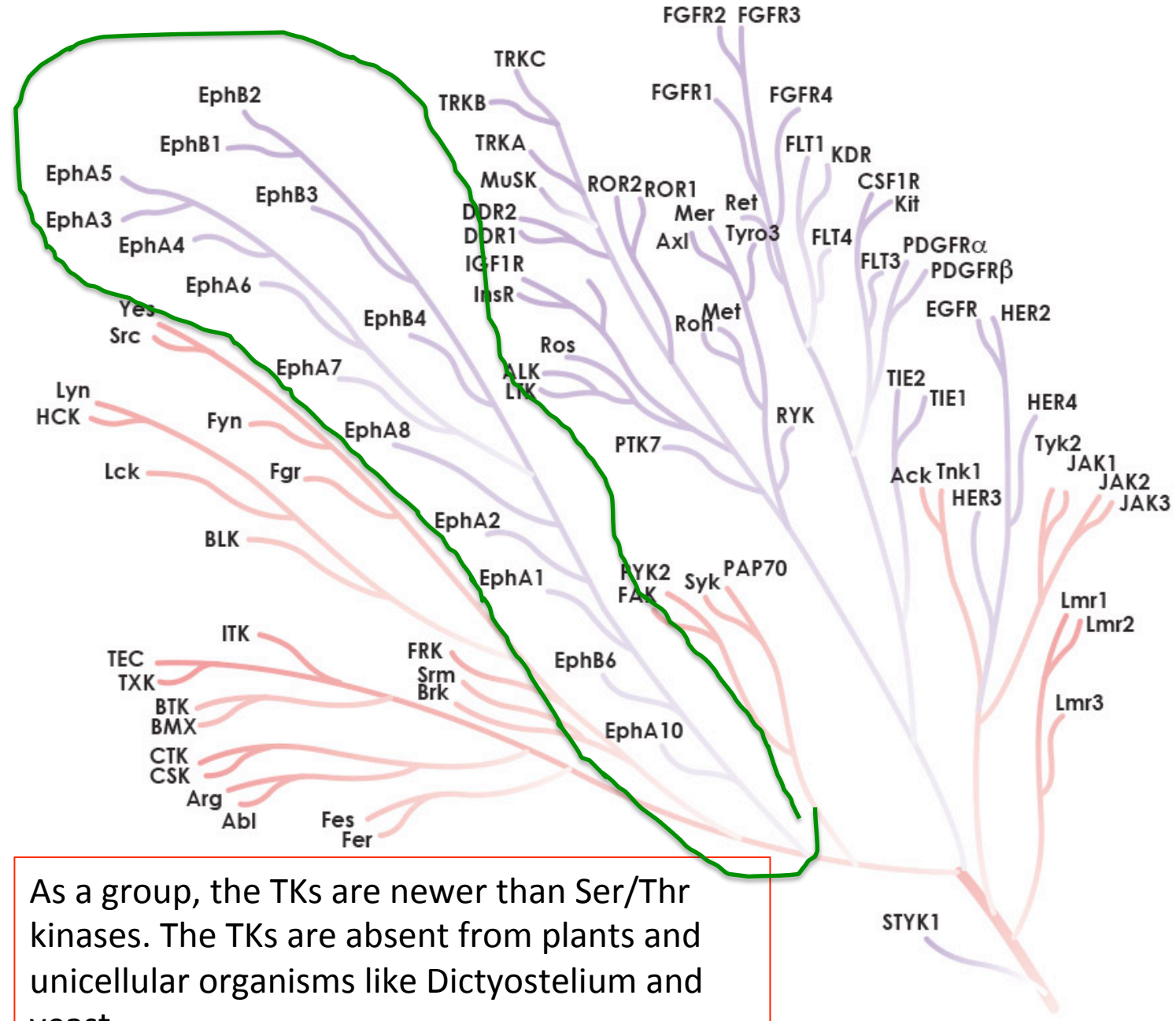


Growth Factors

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





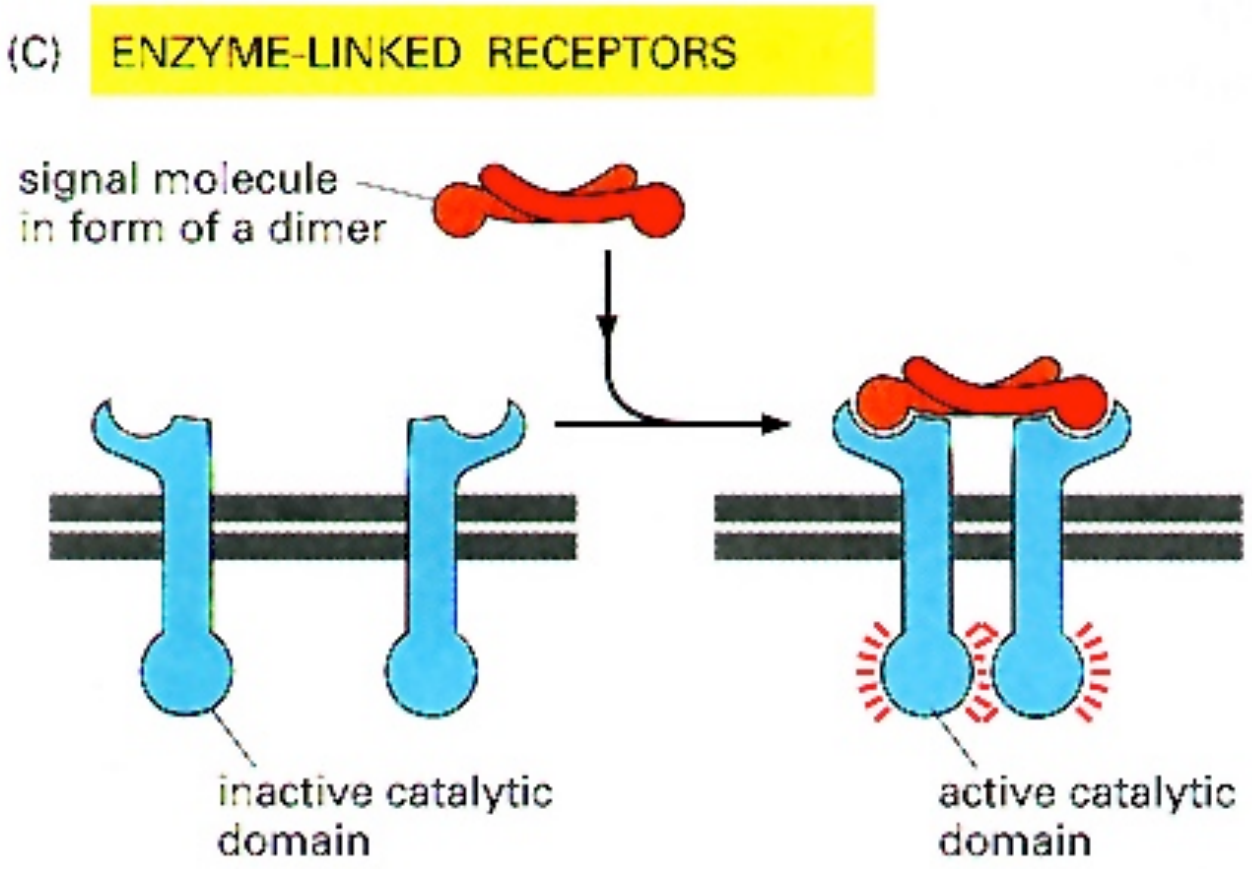
As a group, the TKs are newer than Ser/Thr kinases. The TKs are absent from plants and unicellular organisms like Dictyostelium and yeast.

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

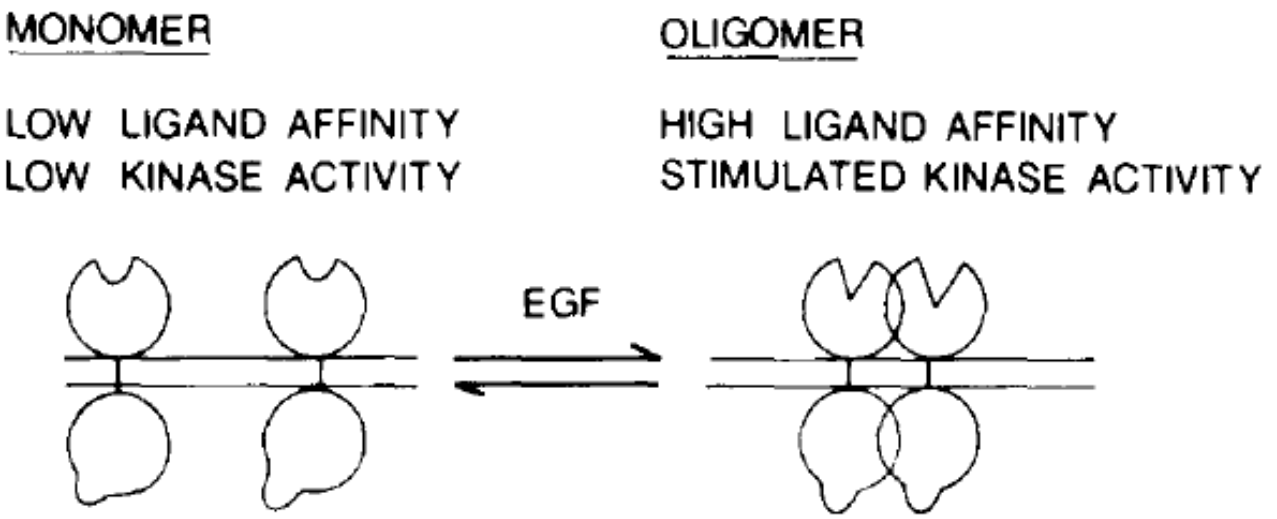
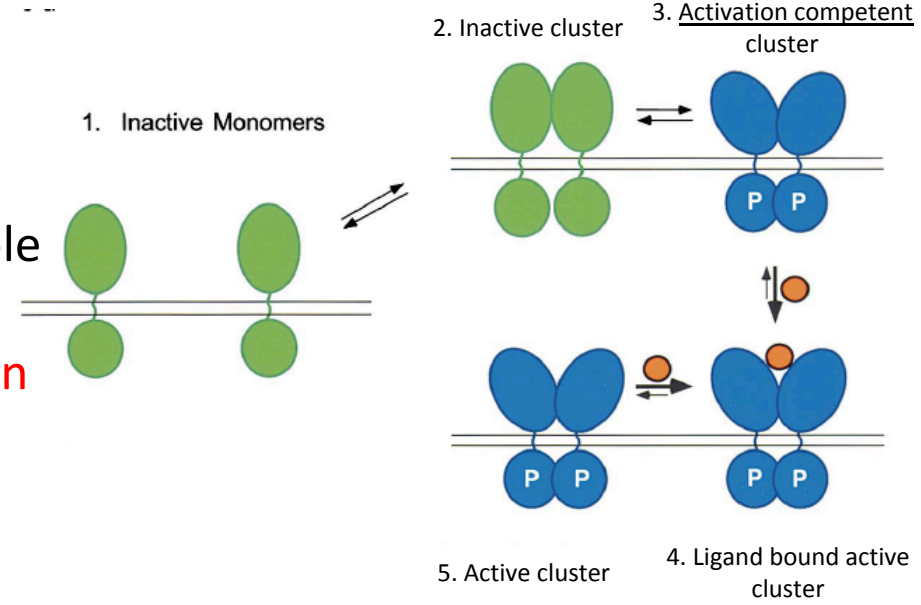
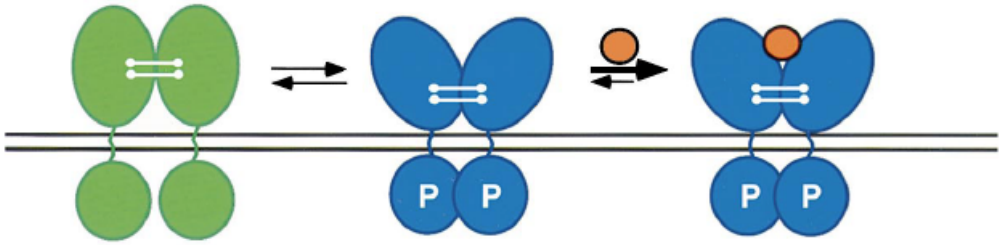


Figure 3. An allosteric oligomerization model for the activation o the EGF receptor kinase by EGF. EGF receptor is depicted as a biglobular transmembrane molecule as shown in Fig. 1. It is proposed that monomeric receptors exist in equilibrium with recepto oligomers. It is postulated that monomeric receptors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



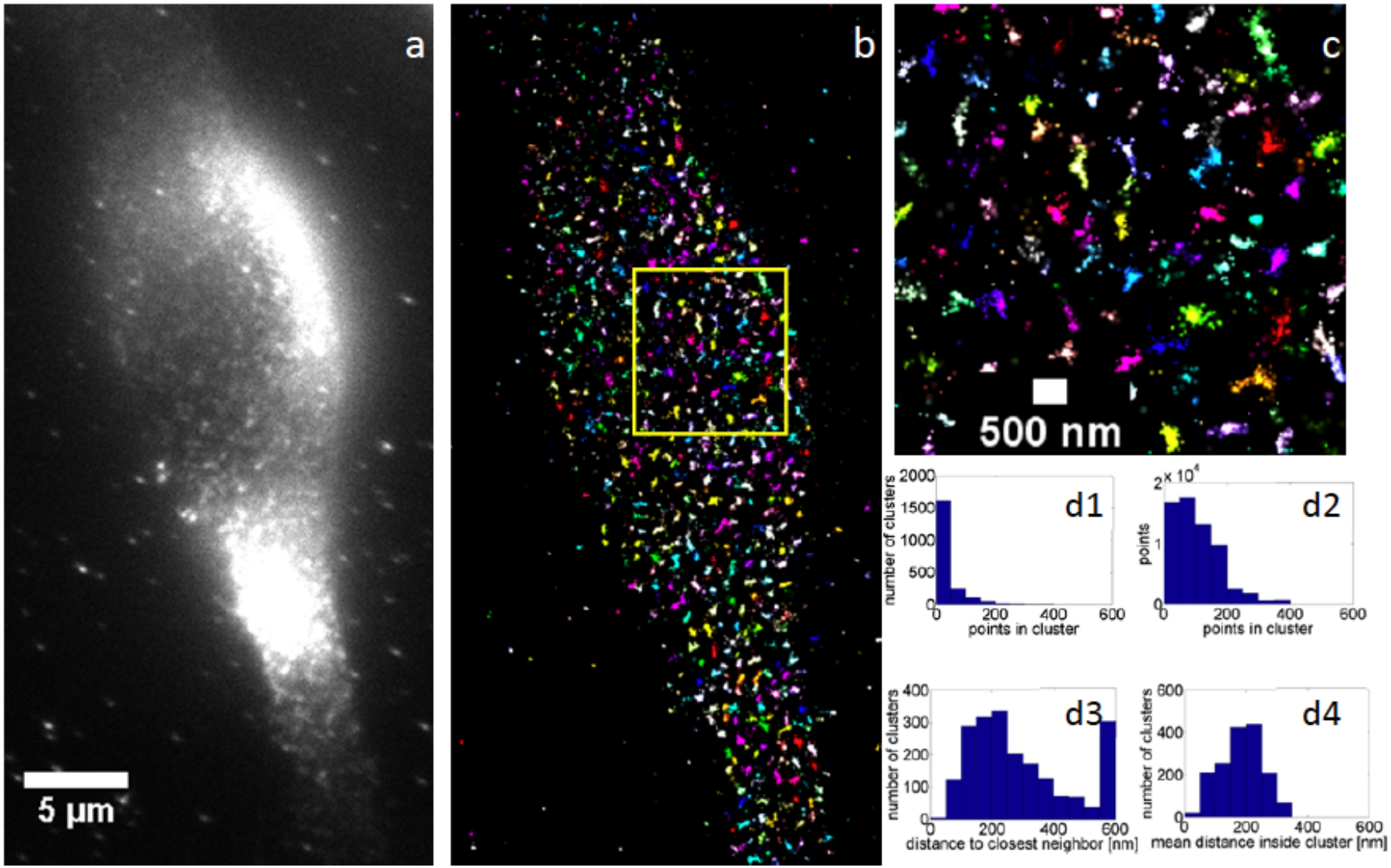


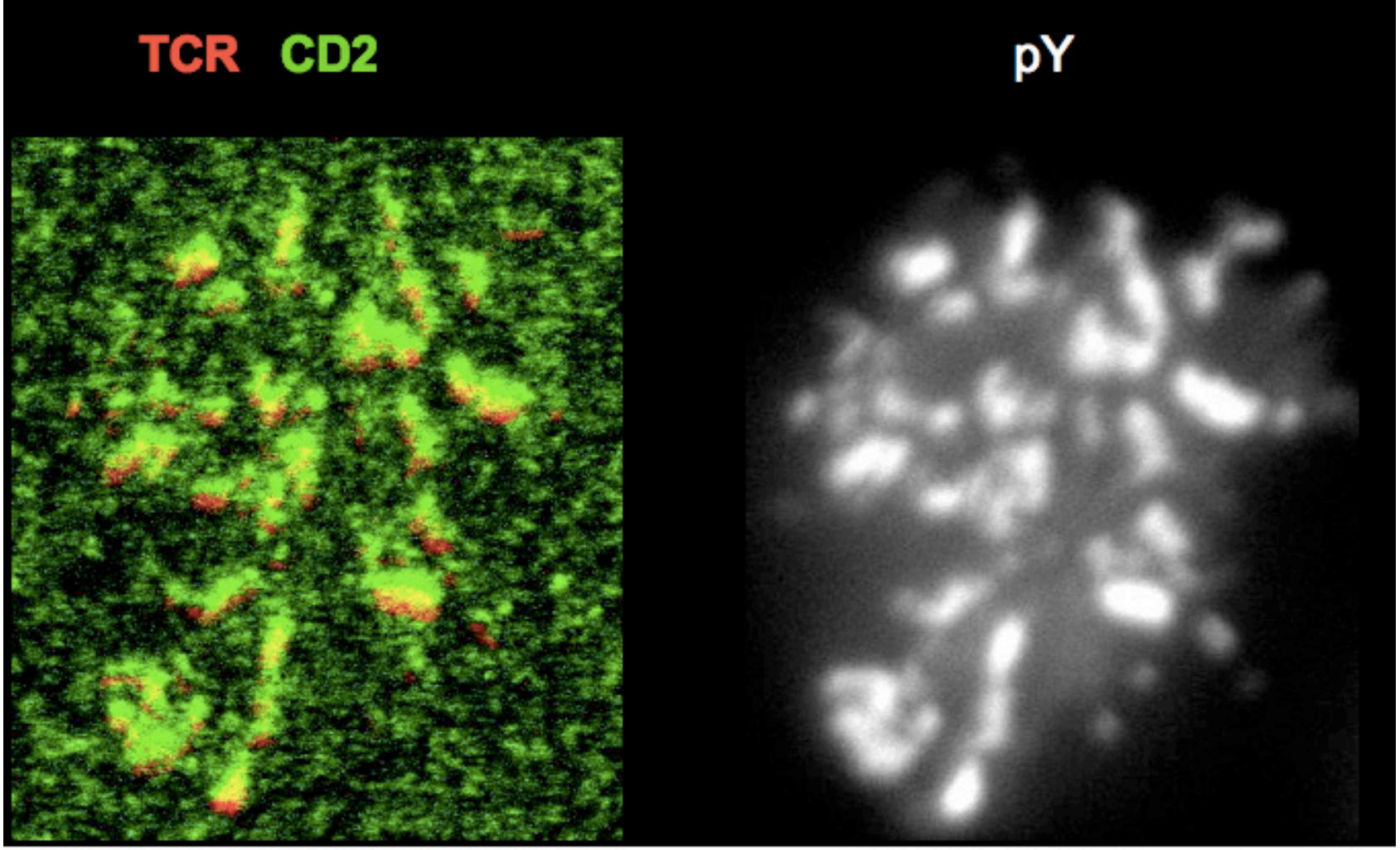
Inactive disulphide bridged insulin-receptor dimers are in equilibrium with active dimers. Insulin binding stabilizes the active dimeric state, leading to PTK activation.

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

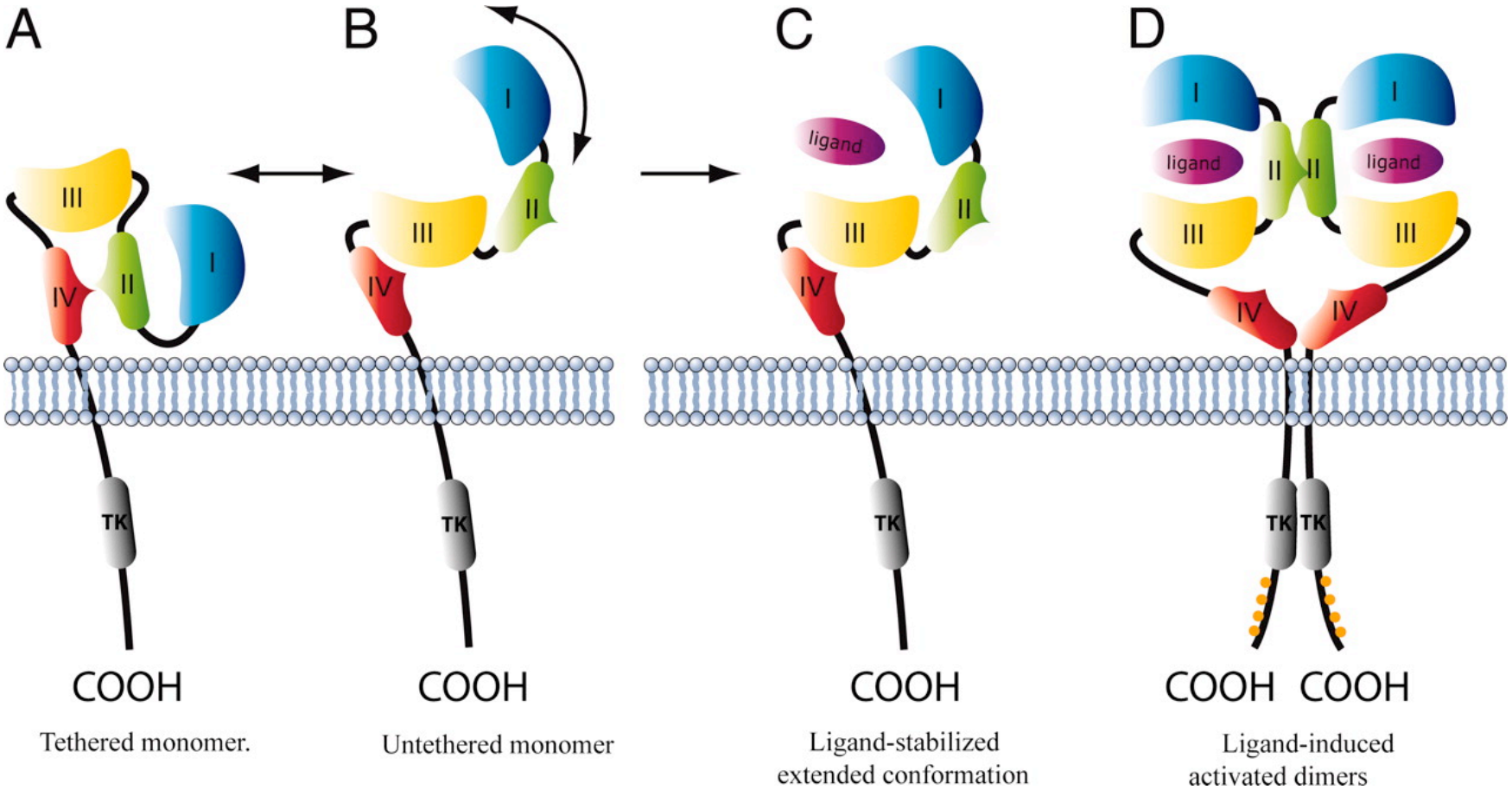
Pavel Křížek[1], Peter W. Winter[2], Zdeněk Švindrych[1], Josef Borkovec[1], Martin Ovesný[1], Deborah A. Roess[3], B. George Barisas[4], and Guy M. Hagen[1,*]





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.

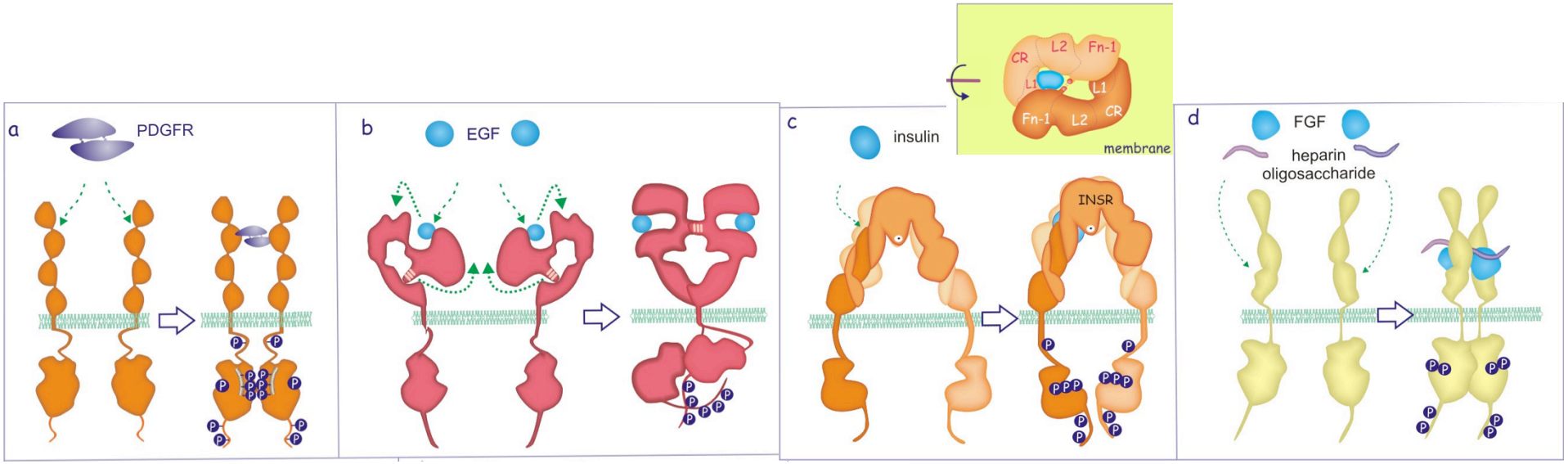
How ligand binding to the extracellular region stimulates the tyrosine kinase activity in the RTK cytoplasmic region?



Both tyrosine kinase activation and tyrosine autophosphorylation are mediated in trans by an intermolecular process.

(Kovacs et al., 2015a; Lemmon et al., 2014)

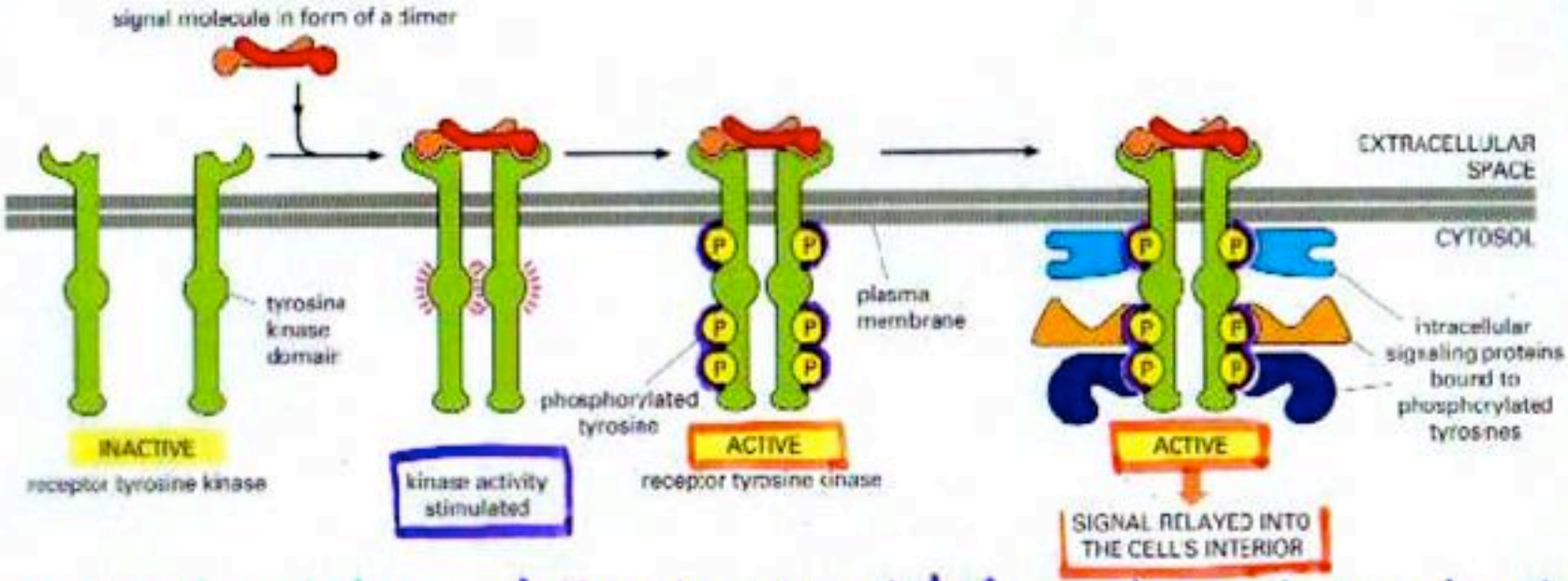
RECEPTORS EMPLOY DIFFERENT CLUSTERIZATION STRATEGIES



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

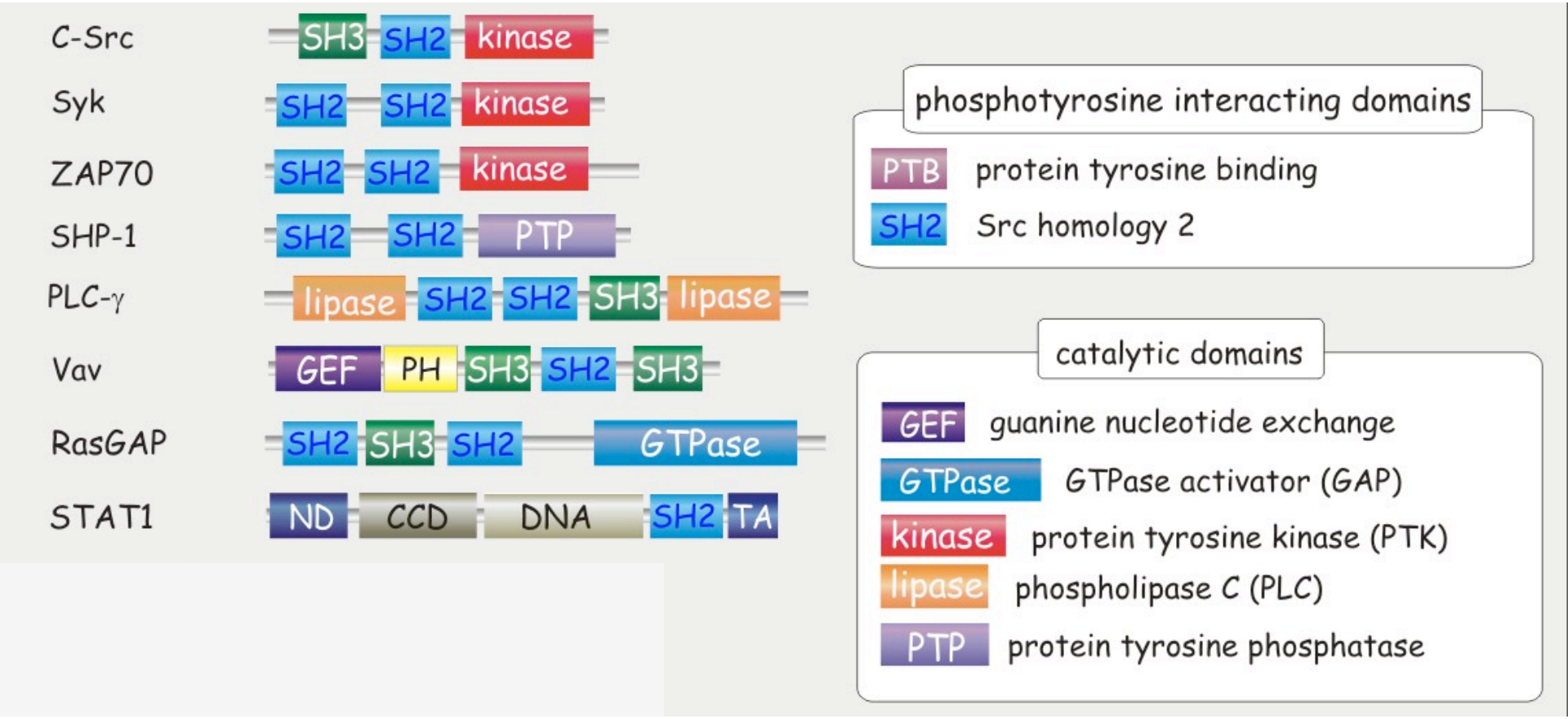
intracellular signaling proteins bound to phosphorylated residues → signaling to several pathways

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*

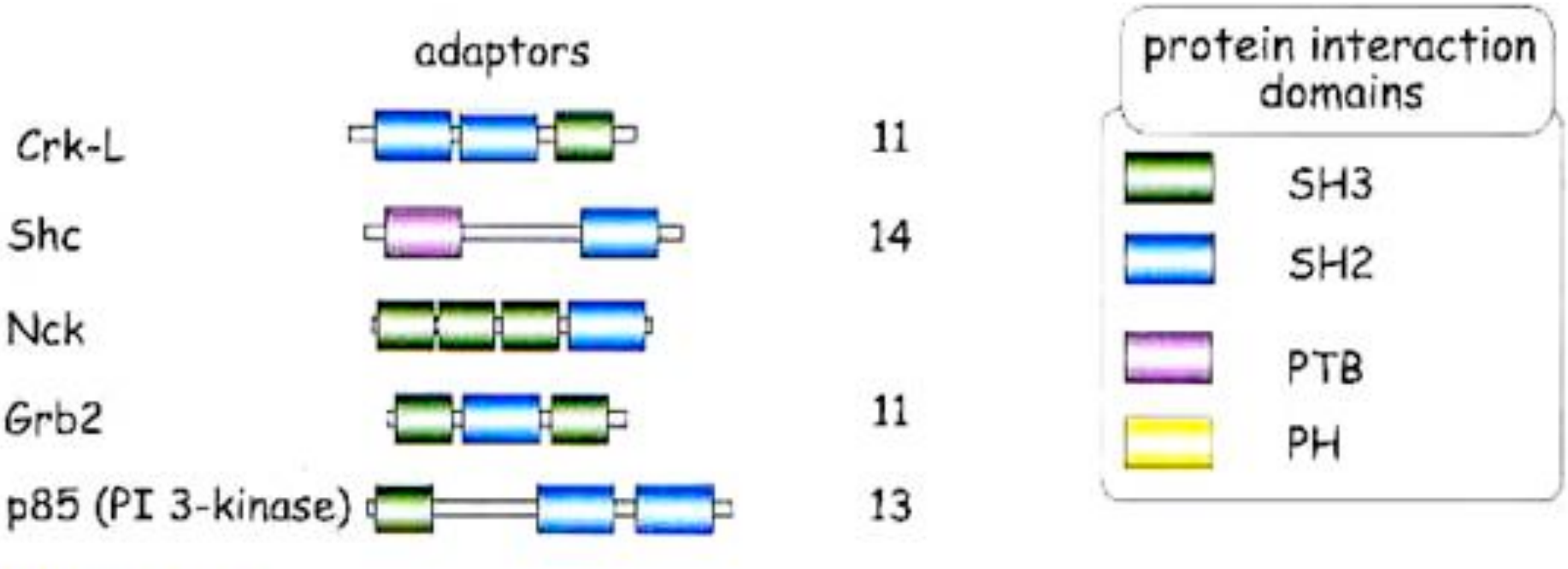
Fig. 16-30

COSA SI LEGA AL RECETTORE FOSFORILATO?

1) Enzymes/transcription factors



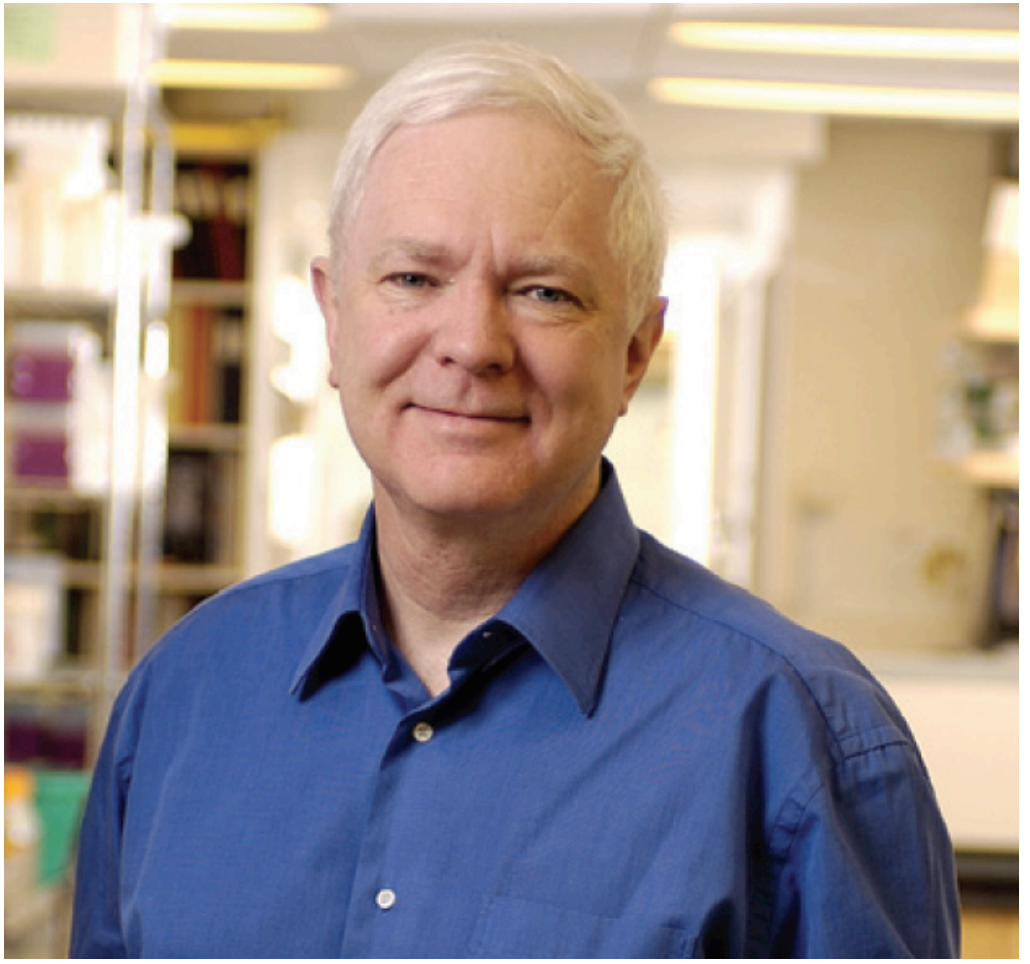
2) ***Adaptors*** lack intrinsic catalytic activity, but link phosphorylated receptors with other effector proteins.



Anthony James Pawson

(1952–2013)

Biochemist whose vision of cell signalling transformed cancer research.

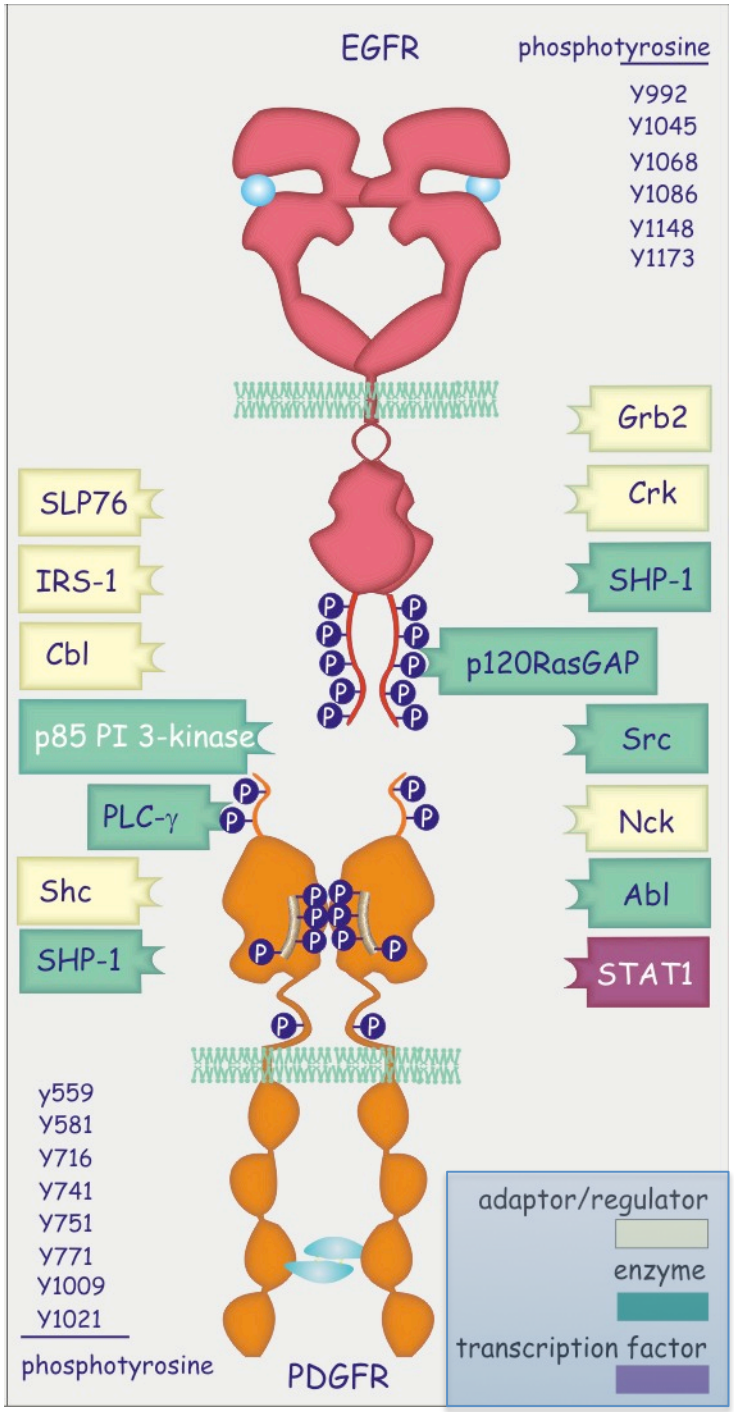


In the 1980s, early in his career, Pawson and his team discovered the Src homology region 2 (SH2). A subunit, or domain, of many proteins, SH2 directs how proteins interact and governs how cells respond to external cues. This finding set a path for all his future work.

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.

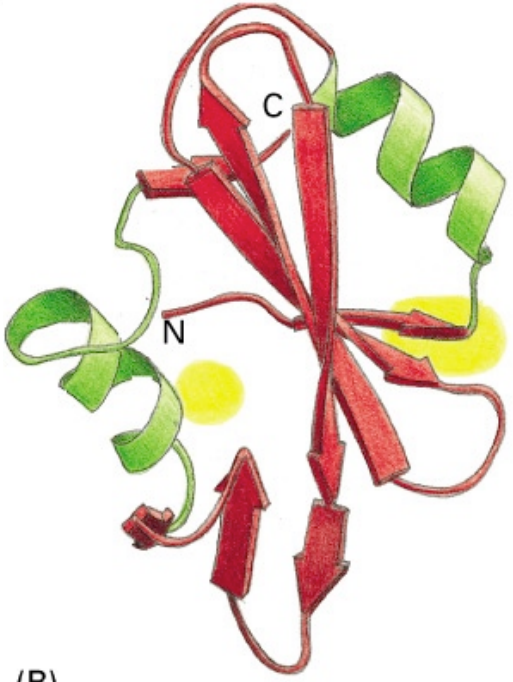
Key concept:

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

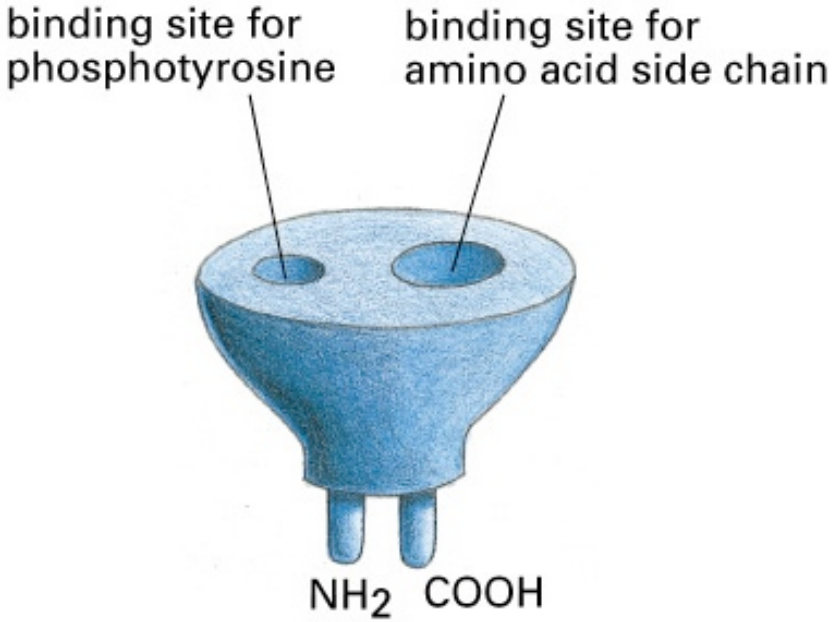

EGFR
phosphotyrosine
Y992
Y1045
Y1068
Y1086
Y1148
Y1173
Grb2
Crk
SHP-1
p120RasGAP
Src
Nck
Abl
STAT1
SLP76
IRS-1
Cbl
p85 PI 3-kinase
PLC-γ
Shc
SHP-1
y559
Y581
Y716
Y741
Y751
Y771
Y1009
Y1021
phosphotyrosine
PDGFR
adaptor/regulator
enzyme
transcription factor

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)



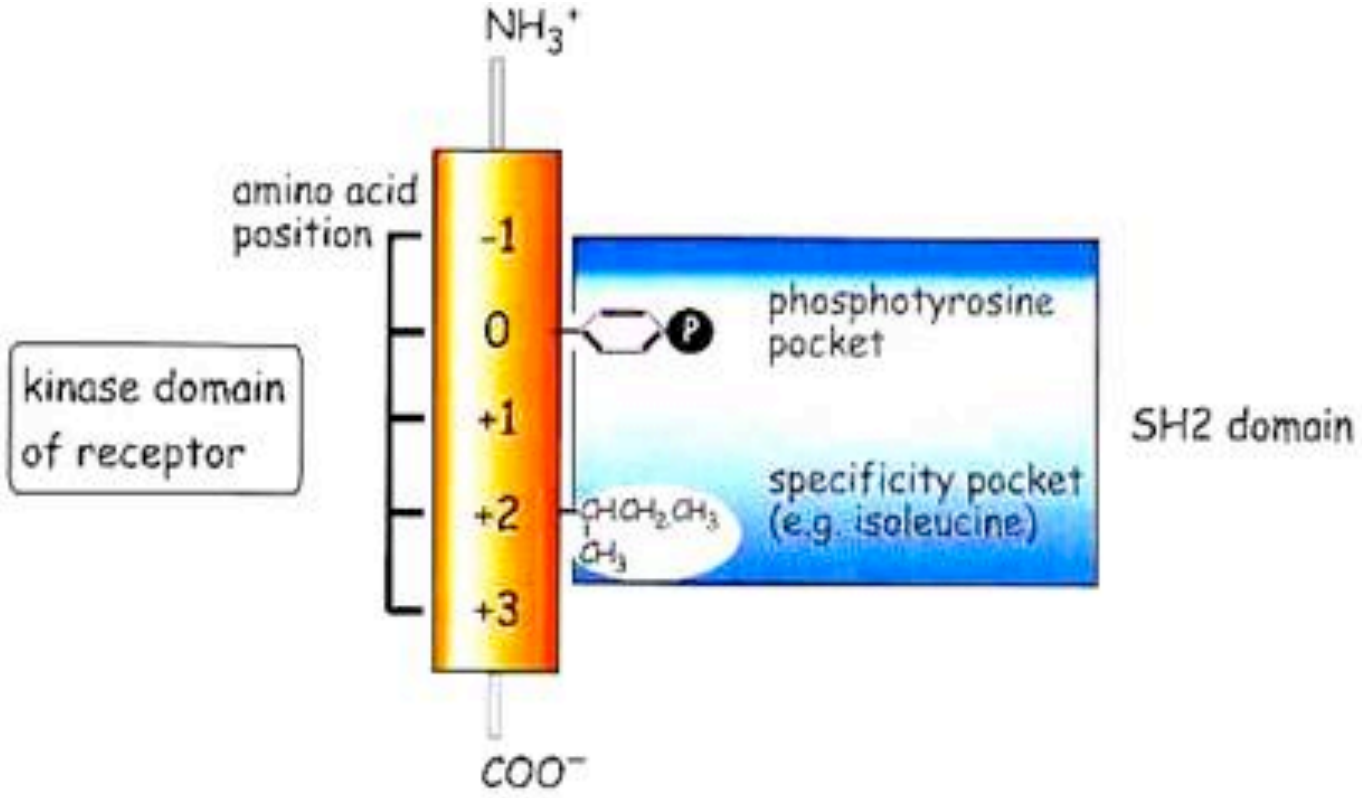
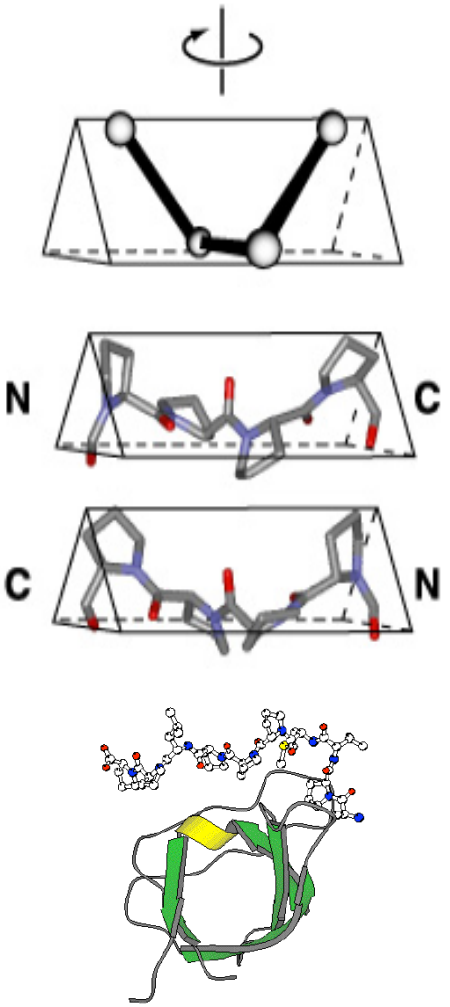


Figure 11.7 Recognition of phosphotyrosine and adjacent amino acids by the SH2 domain. Selectivity of recognition between different targets containing SH2 domains is conferred by the sequence of amino acids, particularly the third residue immediately adjacent on the C-terminal side of the phosphorylated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

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

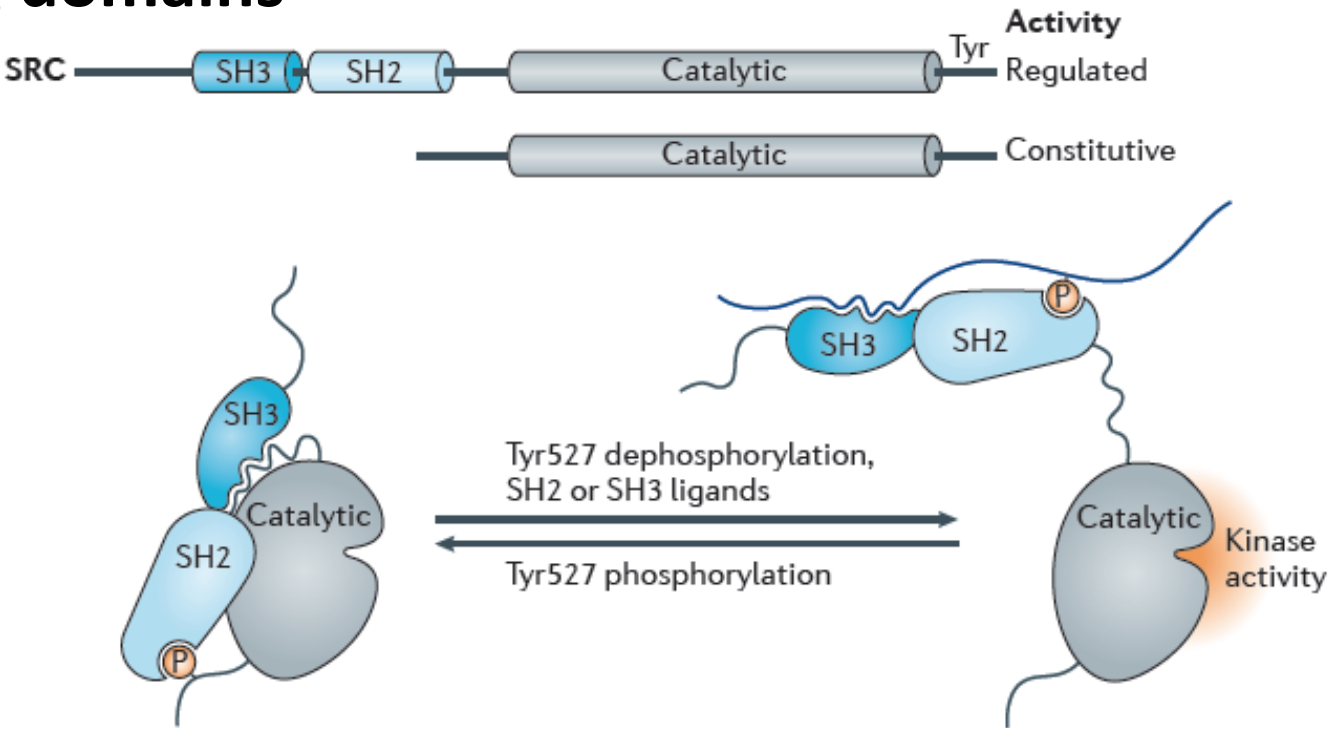
SH3 Domains: Properties

- 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.