

Tyrosine Kinase Network Signaling in Normal Physiology and Cancer

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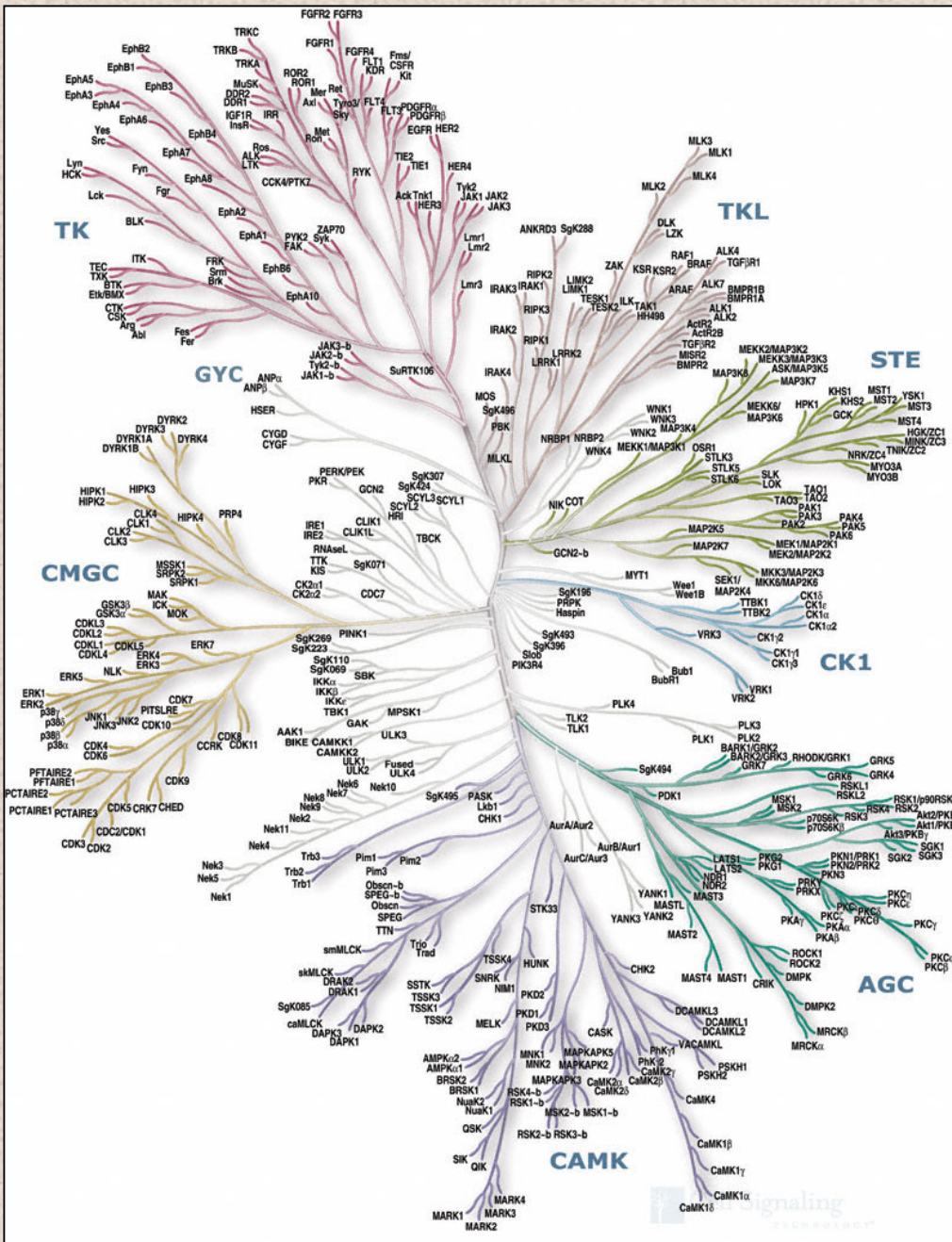
- Introduction of PTKs
- Src Family Kinases
- FAK/RAFTK(=PYK2) Family Kinases
- ErbB Family Kinases and Their Role in Breast Cancer: ErbB2 and EGFR

Protein Tyrosine Kinases (PTKs)

1. PTKs are enzymes that transfer gamma-phosphate groups from ATP to the hydroxyl group of tyrosine residues on signal transduction molecules
2. PTKs play an important role in the control of most fundamental cellular processes including the cell cycle, cell migration, cell metabolism and survival, as well as cell-cell proliferation and differentiation

PTKs = Protein Tyrosine Kinases

RTKs = Receptor Tyrosine Kinases

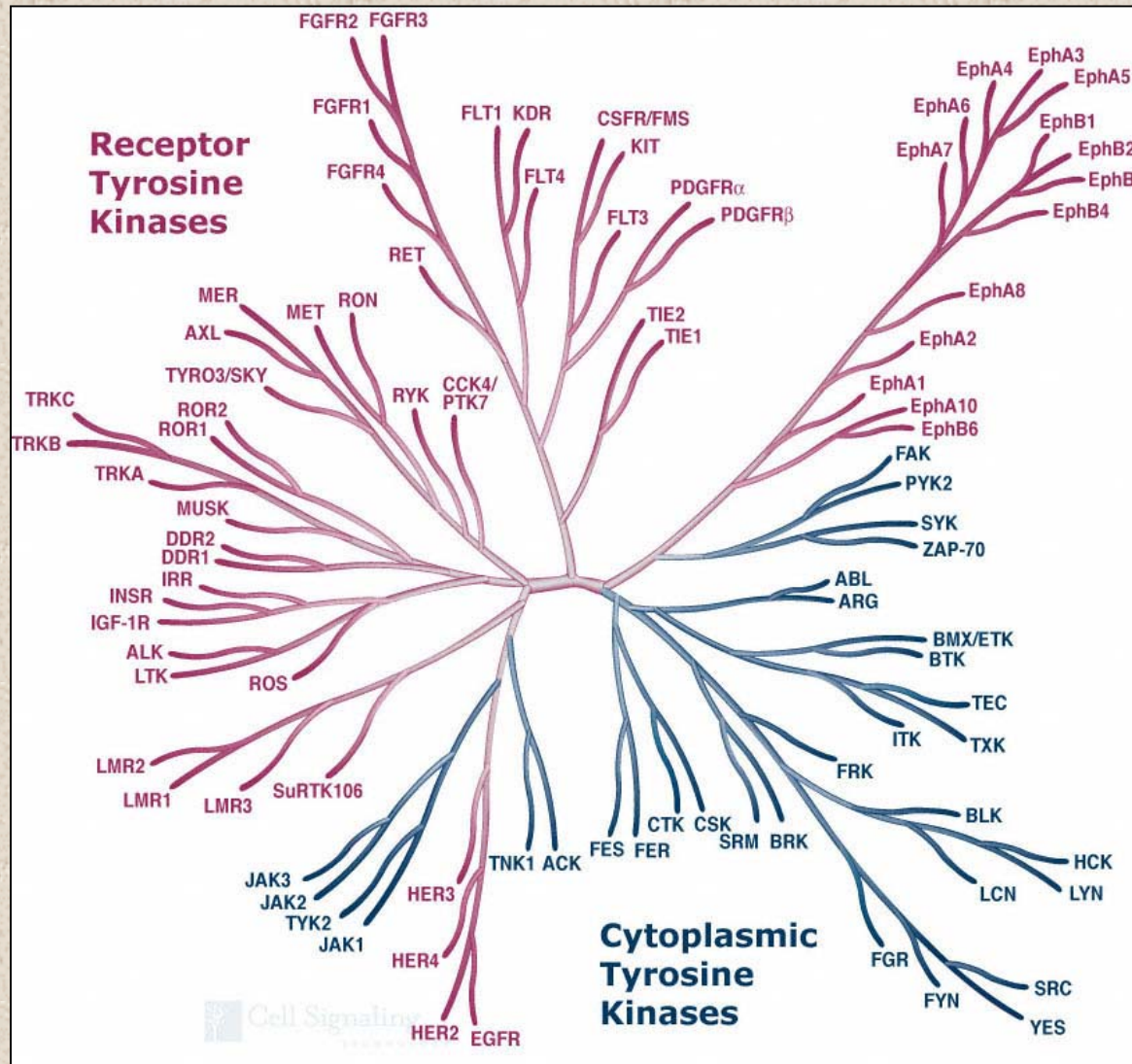


The human genome contains 518 protein kinase genes (~1.7% of total)

Manning et al. (2002)
Science 298, 1912-1934

Tyrosine kinases account for 17% of the total kinases

Two major divisions of tyrosine kinases are “receptors” and cytoplasmic “nonreceptors”



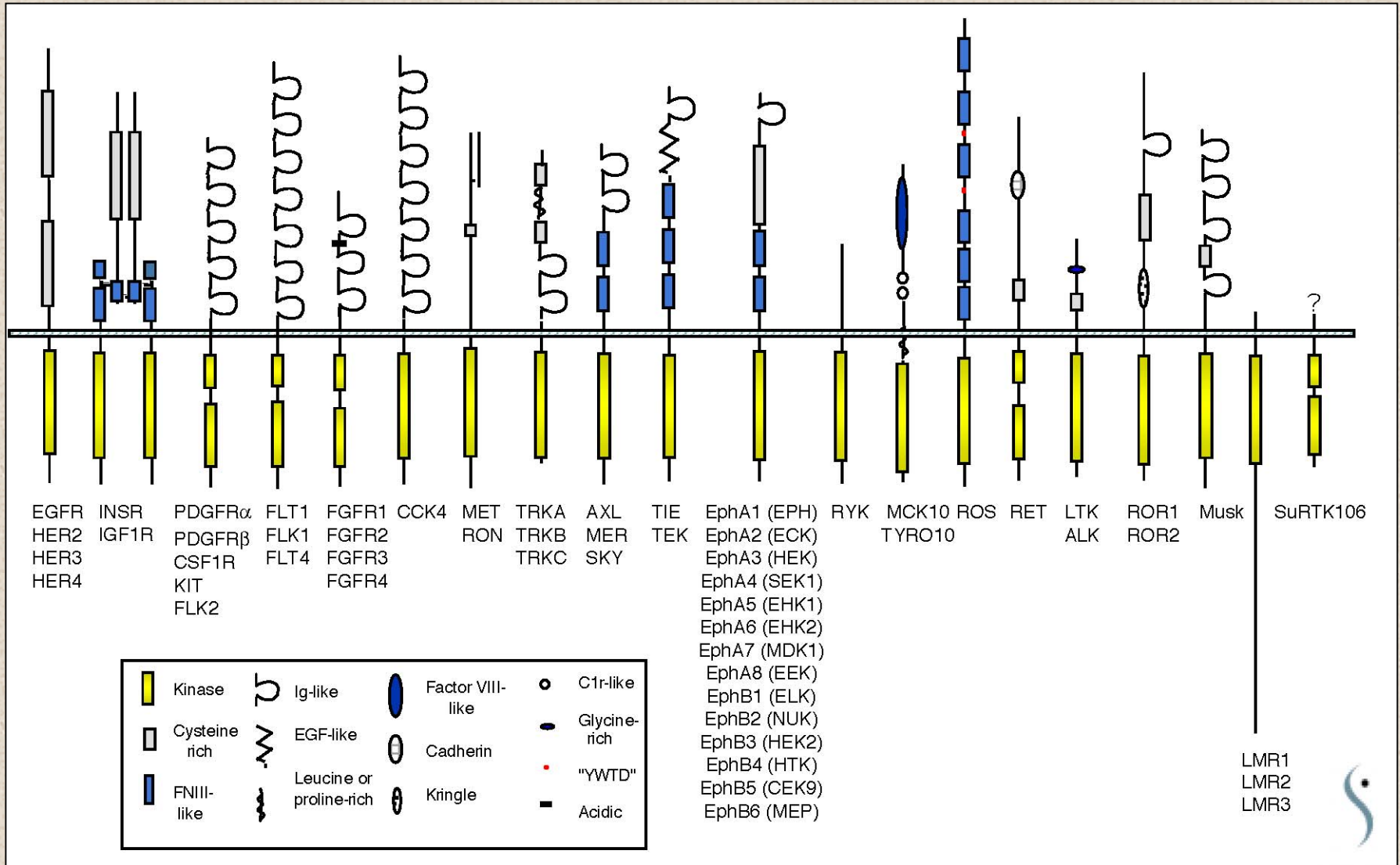
Protein Tyrosine Kinases (=PTKs)

- PTKs are important regulators of intracellular signal-transduction pathways mediating development and multicellular communication.
- PTK activity is normally tightly controlled and regulated.
- Perturbation of PTK signaling by mutations and other genetic alterations results in deregulated kinase activity and malignant transformation.

Cell Signaling by PTKs

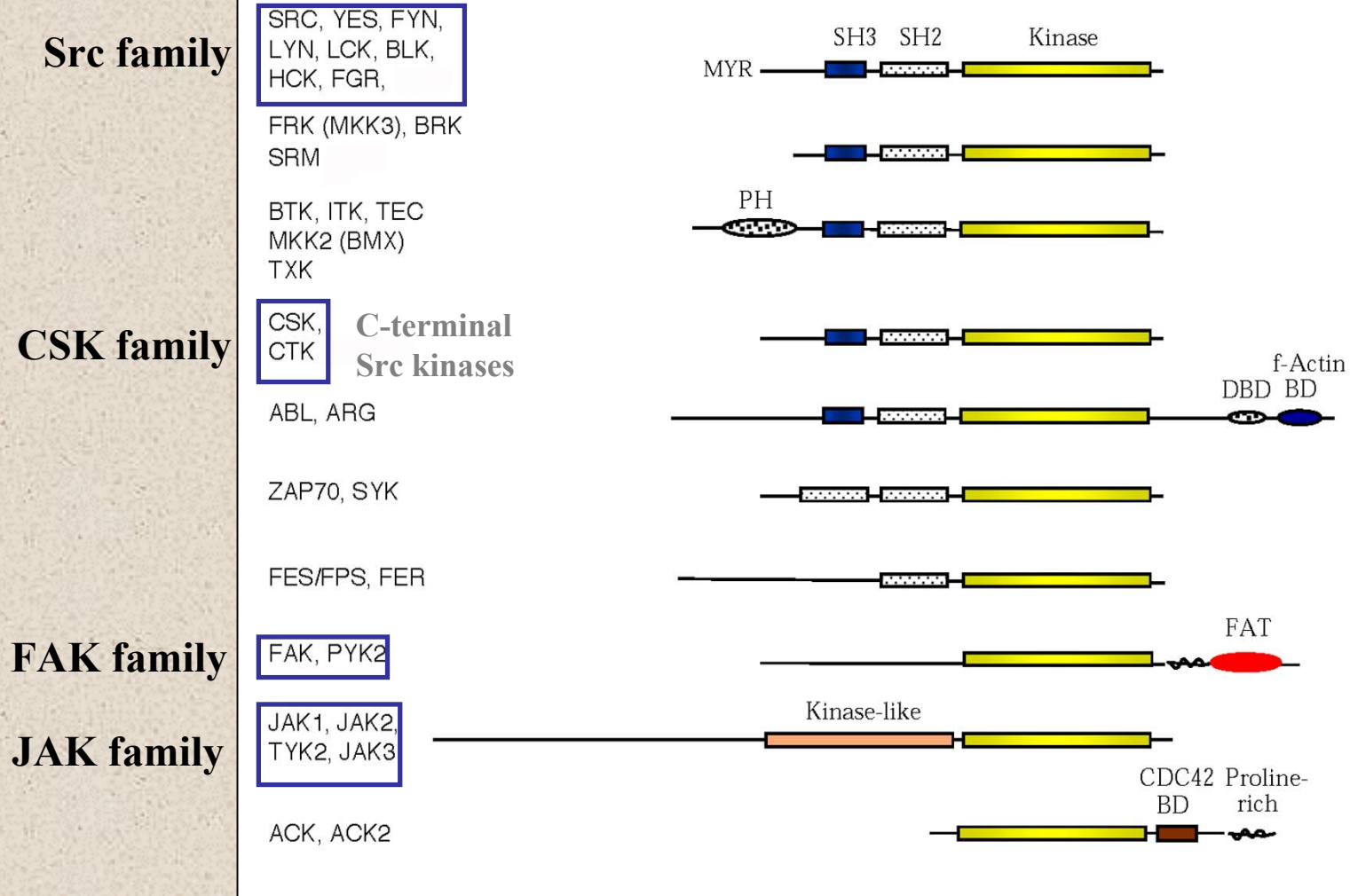
- Membrane receptors can be classified into distinct families based upon the ligands they recognize, the biological response they induce and their primary structures.

Receptor Tyrosine Kinases (20 families)



Nonreceptor tyrosine kinases

(32 total in 10 families)



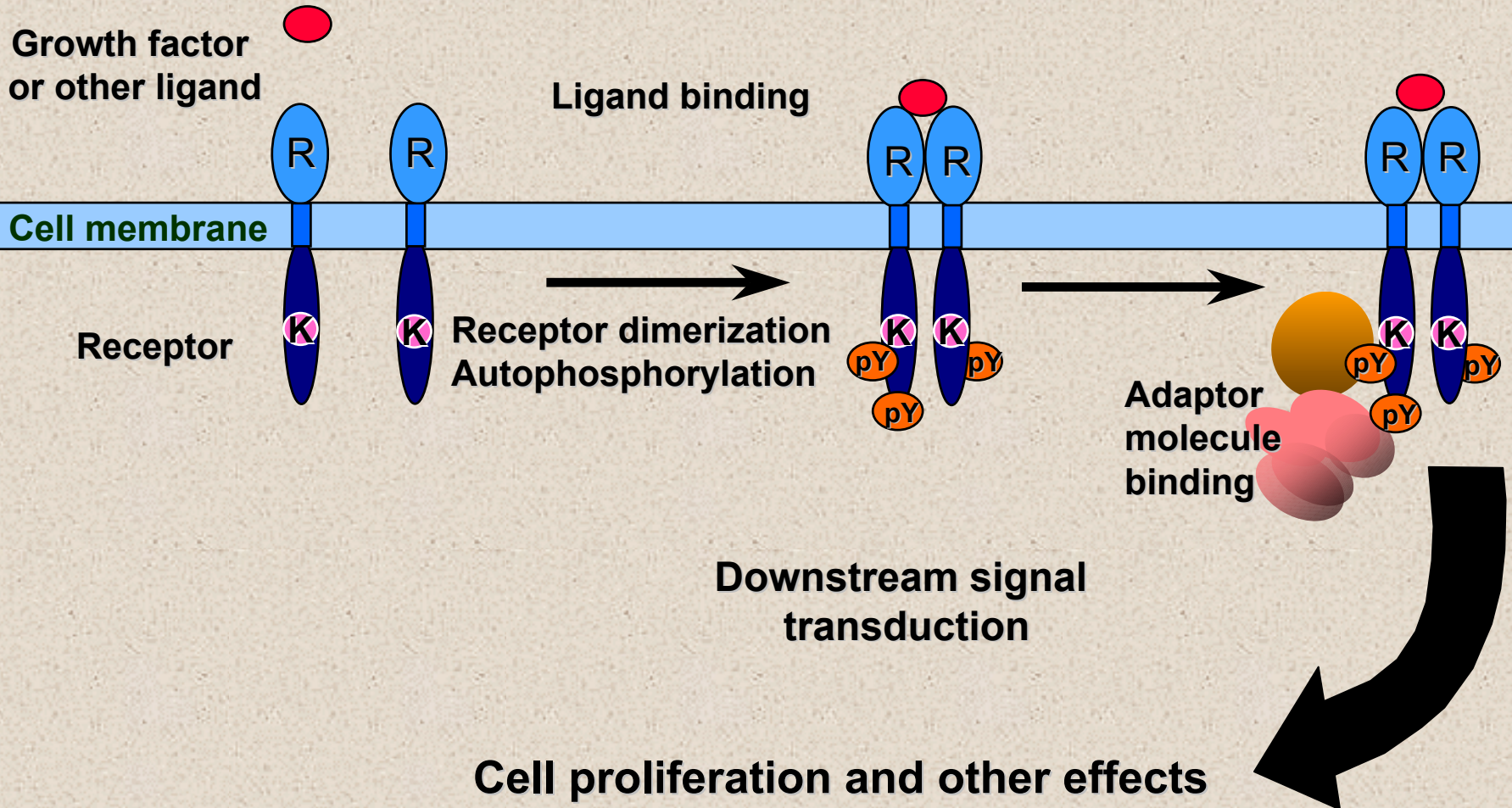
DBD: DNA binding domain

PH: Pleckstrin homology

FAT: Focal adhesion targeting

BD: Binding domain

Signal transduction by membrane-bound receptor tyrosine kinases (TKs)



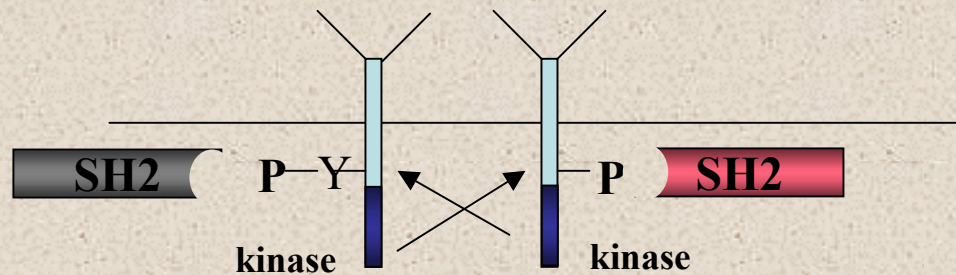
Receptor TKs as rational targets

Receptor TK	Example ligands	Example receptor TK-expressing cells
c-KIT	SCF	Wide range of tissues
EGFR	EGF, TGF α	Epithelial cells
FGFR	FGF	Fibroblasts, endothelial cells
PDGFR	PDGF	Fibroblasts, endothelial cells
VEGFR	VEGF	Vascular endothelial cells
HER2/ErbB-2	–	Wide range of tissues
BCR-ABL	–	Myeloid cells containing Philadelphia chromosome (e.g. chronic myeloid leukemia)

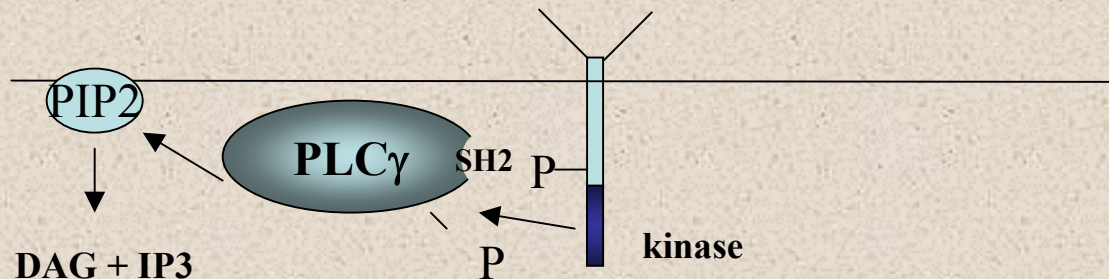
RTKs are considered not only as receptors with tyrosine kinase activity but also act as a platform for the recognition and recruitment of a specific complementary set of signaling proteins.

Two Functions for the Tyrosine Kinase Domain

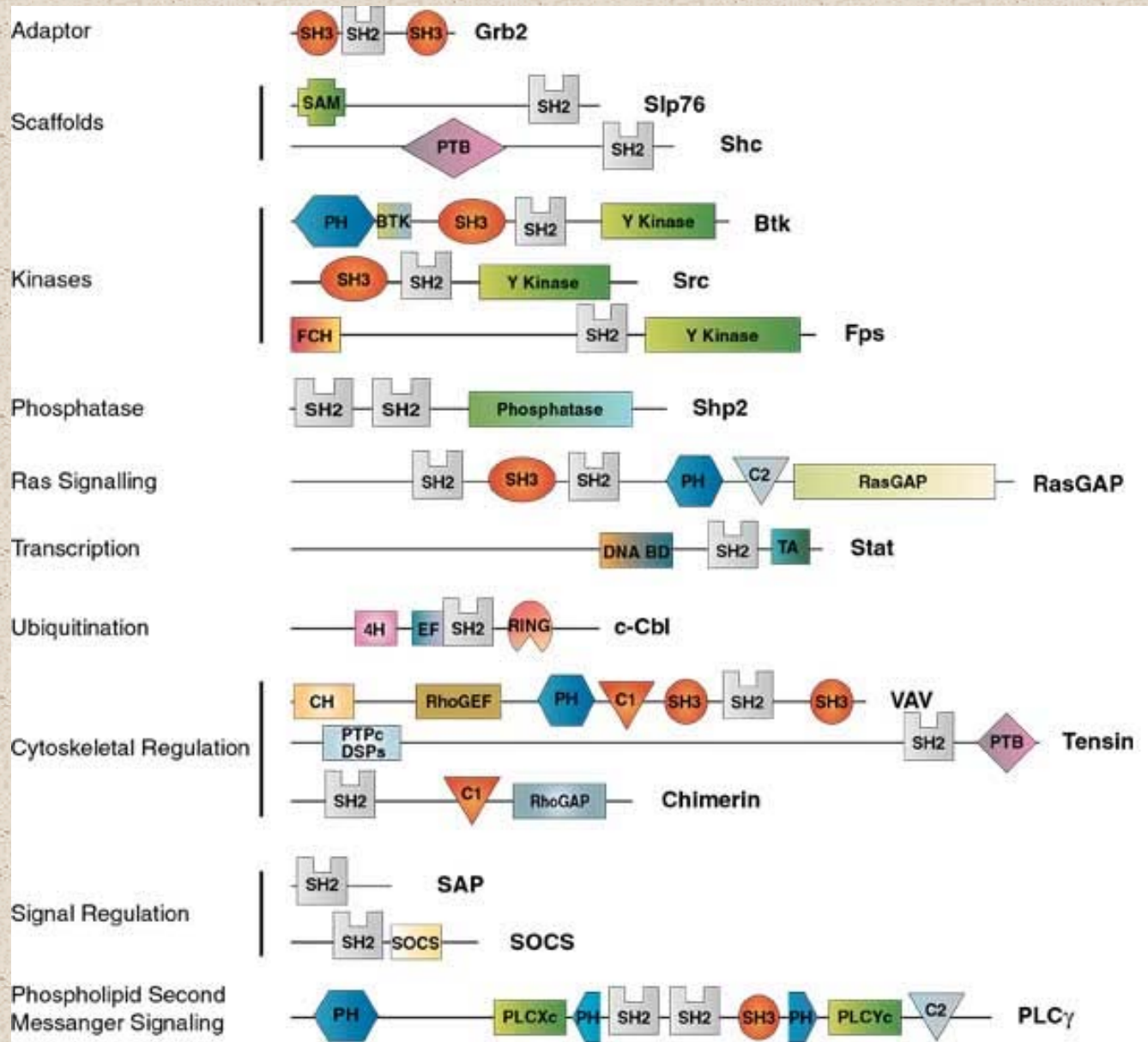
- 1) **Autophosphorylation (Transphosphorylation)- Generate docking sites for signaling molecules**



- 2) **Transphosphorylation- Activate an enzyme**



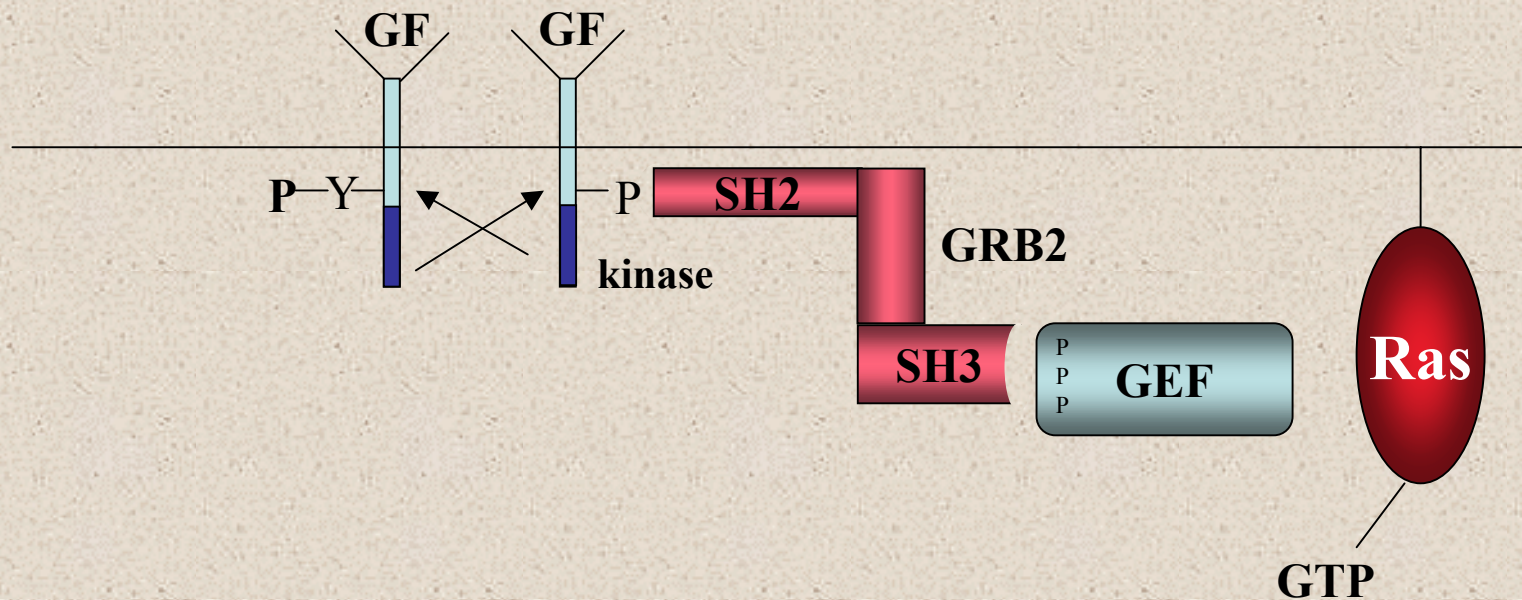
Protein Domains Used For Protein/Protein Interactions



Adaptors Link TKR to Ras

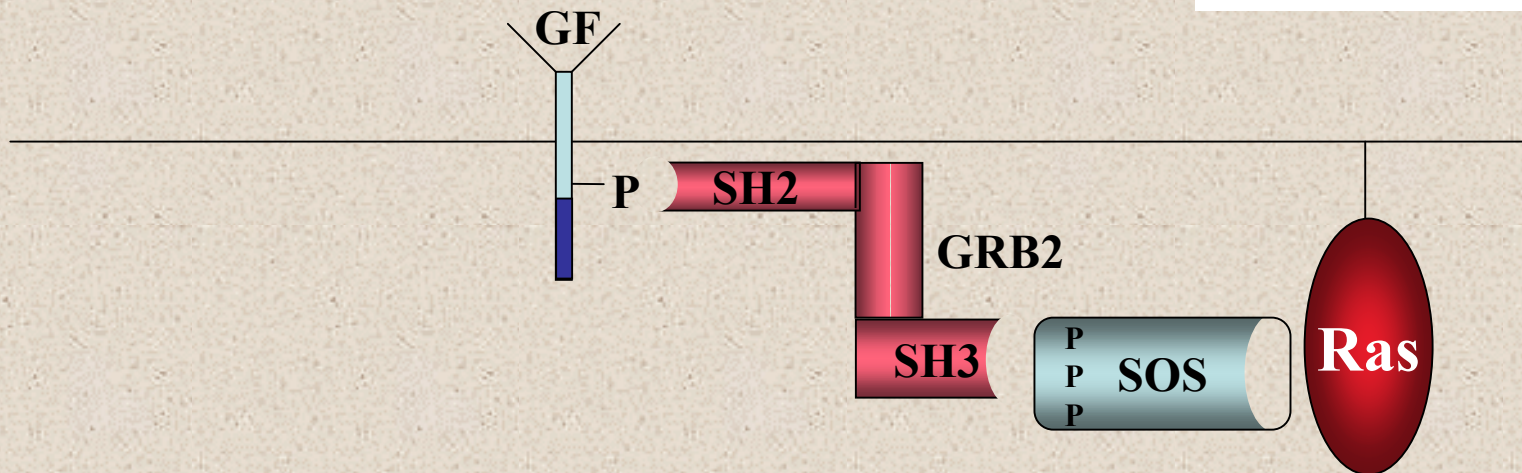
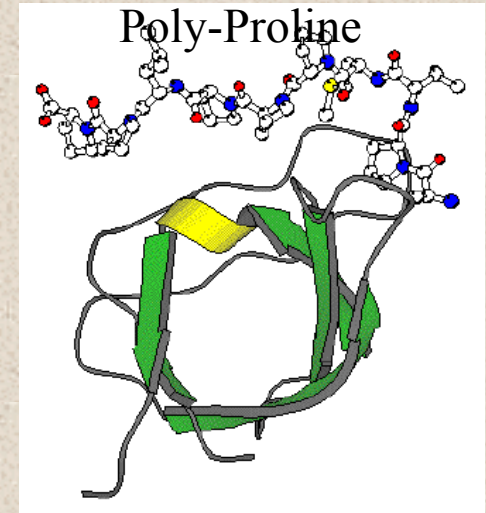
Function of SH2 Domains:

- 1) Found in many signaling molecules
- 2) Recognize p-Tyr

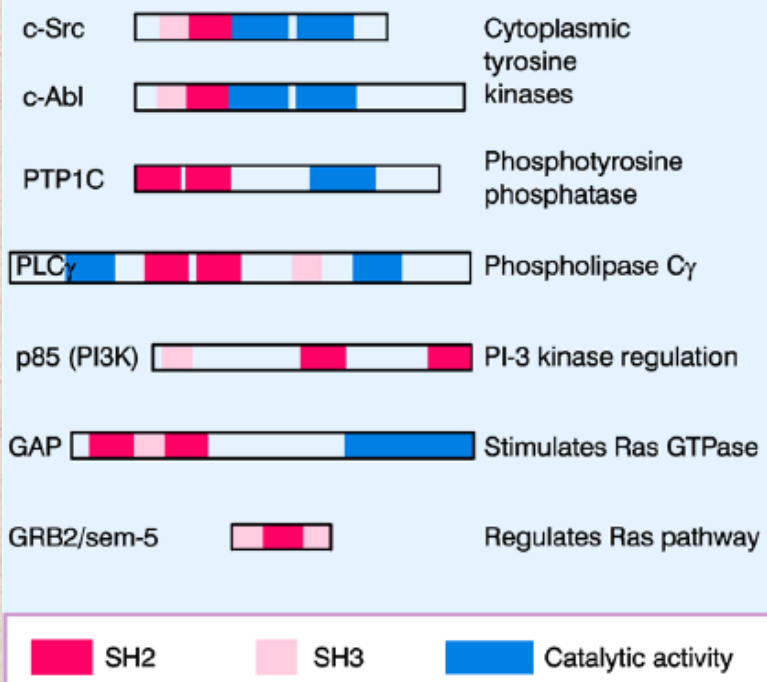


SH3 Domains

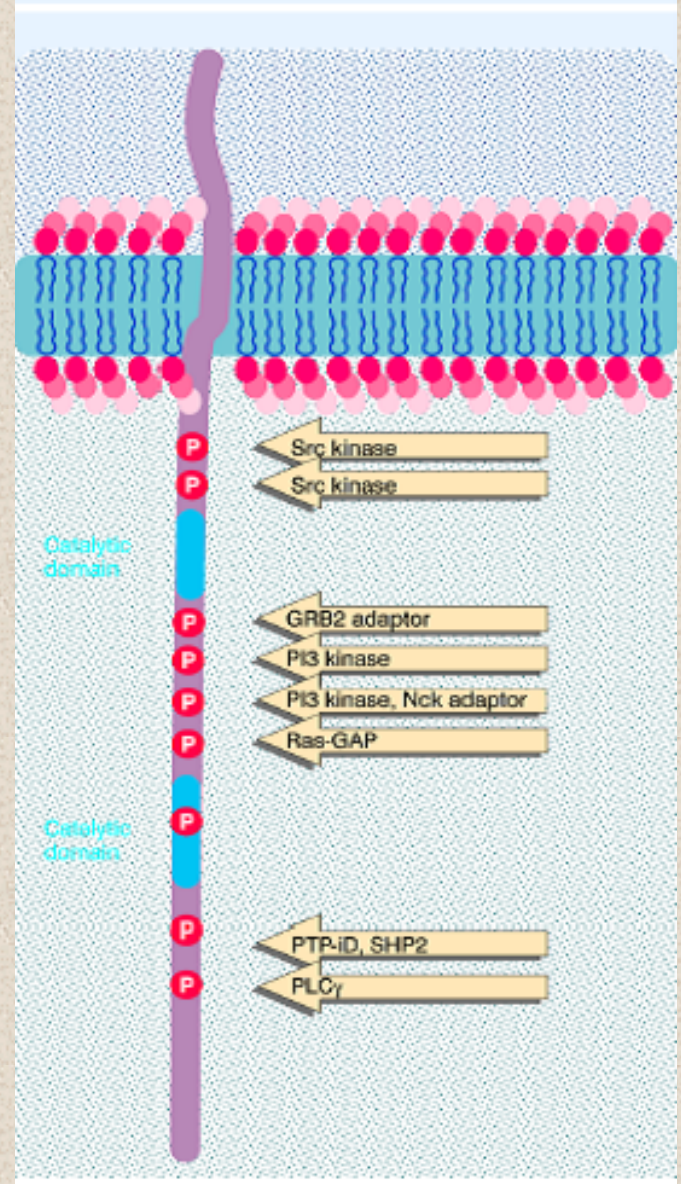
- Binding site is poly-proline
- SH3/PPP interaction can be constitutive (GRB2/SOS) or the SH3 domain only becomes exposed for binding to the poly-proline region after some stimulation



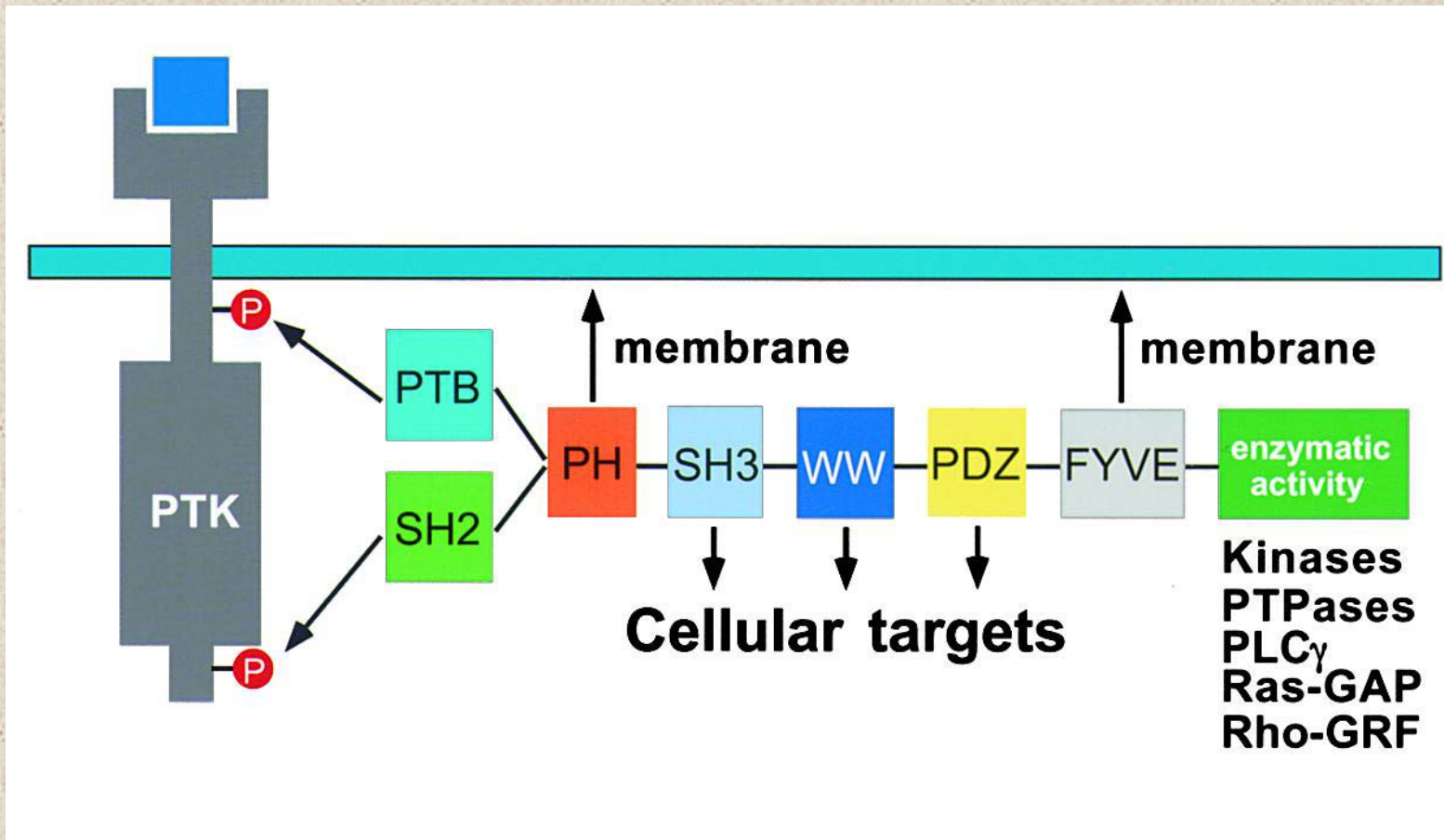
Several types of proteins involved in signaling have SH2 and SH3 domains.



Autophosphorylation of the cytosolic domain of the PDGF receptor creates SH2-binding sites for several proteins. Some sites can bind more than one type of SH2 domain. Some SH2-containing proteins can bind to more than one site. The kinase domain consists of two separated regions (shown in blue), and is activated by the phosphorylation site in it.



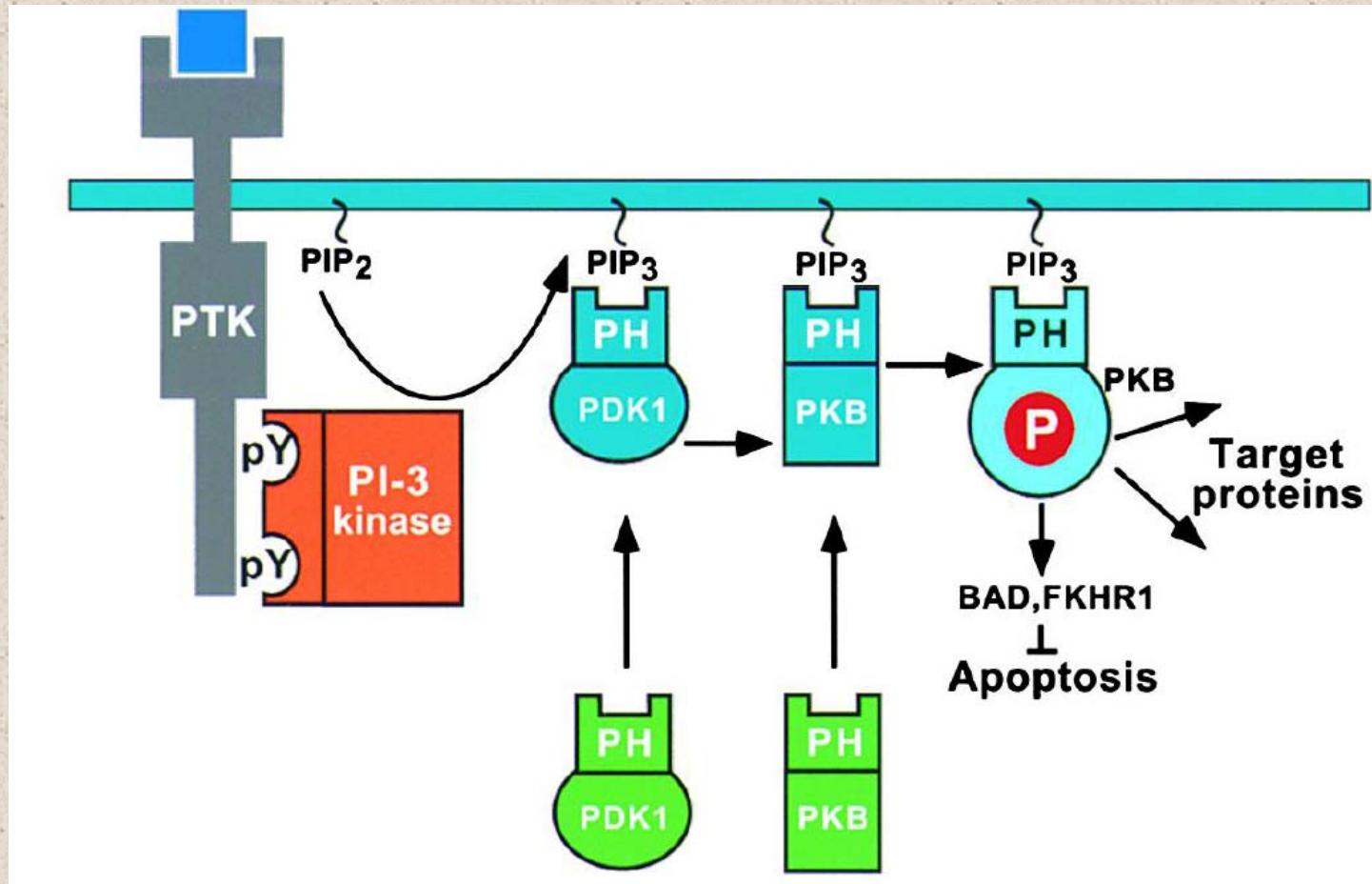
Protein Modules Implicated in the Control of Intercellular Signaling Pathways



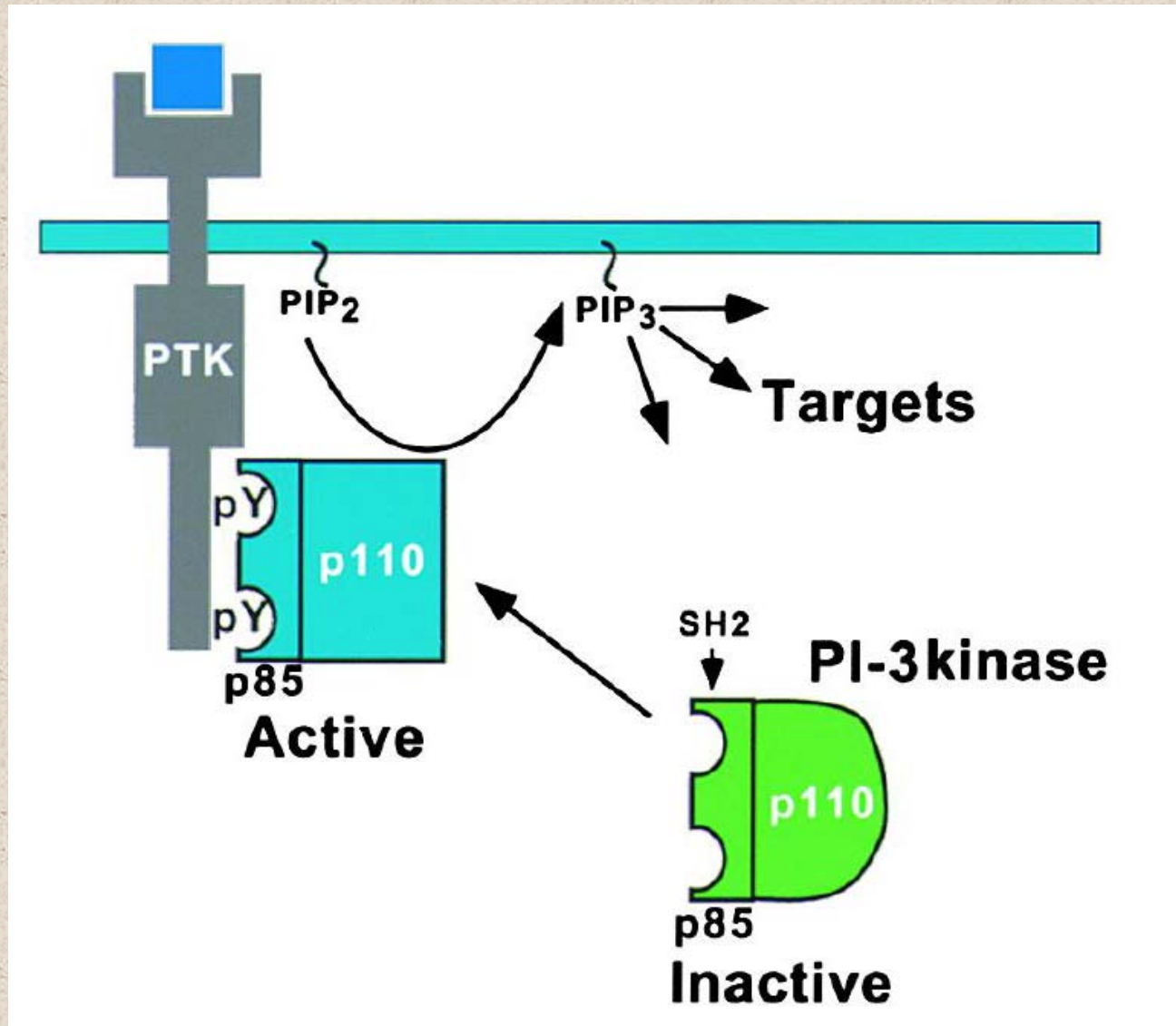
Paradigms for Activation of Signaling Proteins in Response to RTK Activation

- Activation by membrane translocation (PDGF R – PIP₂/PIP₃)
- Activation by a conformational change (PDGF R-Src-PI-3 kinase)
- Activation by tyrosine phosphorylation (EGF R-binding of PLC)

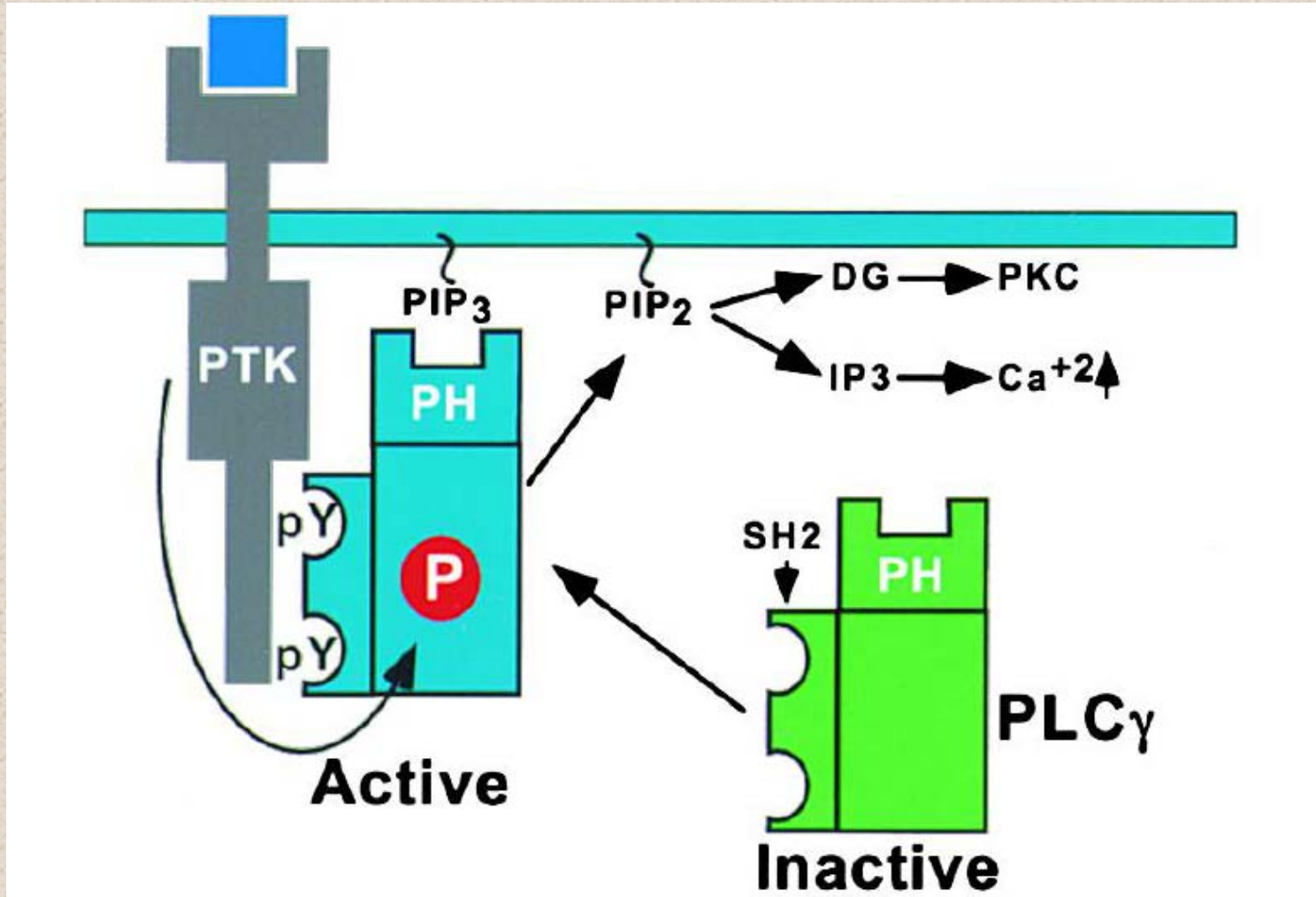
Activation of PKB by Membrane Translocation



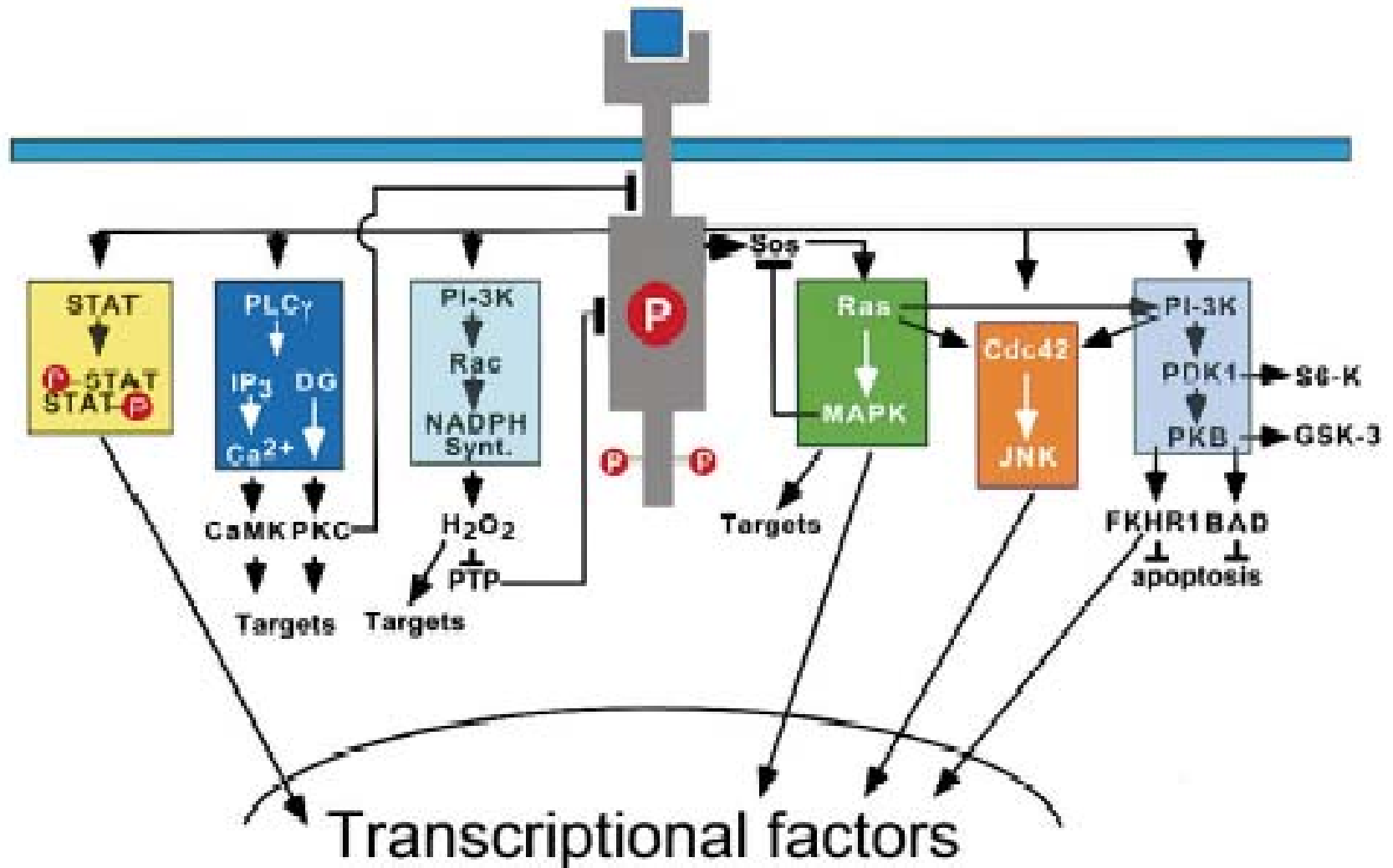
Activation by a Conformational Change



Activation by Tyrosine Phosphorylation

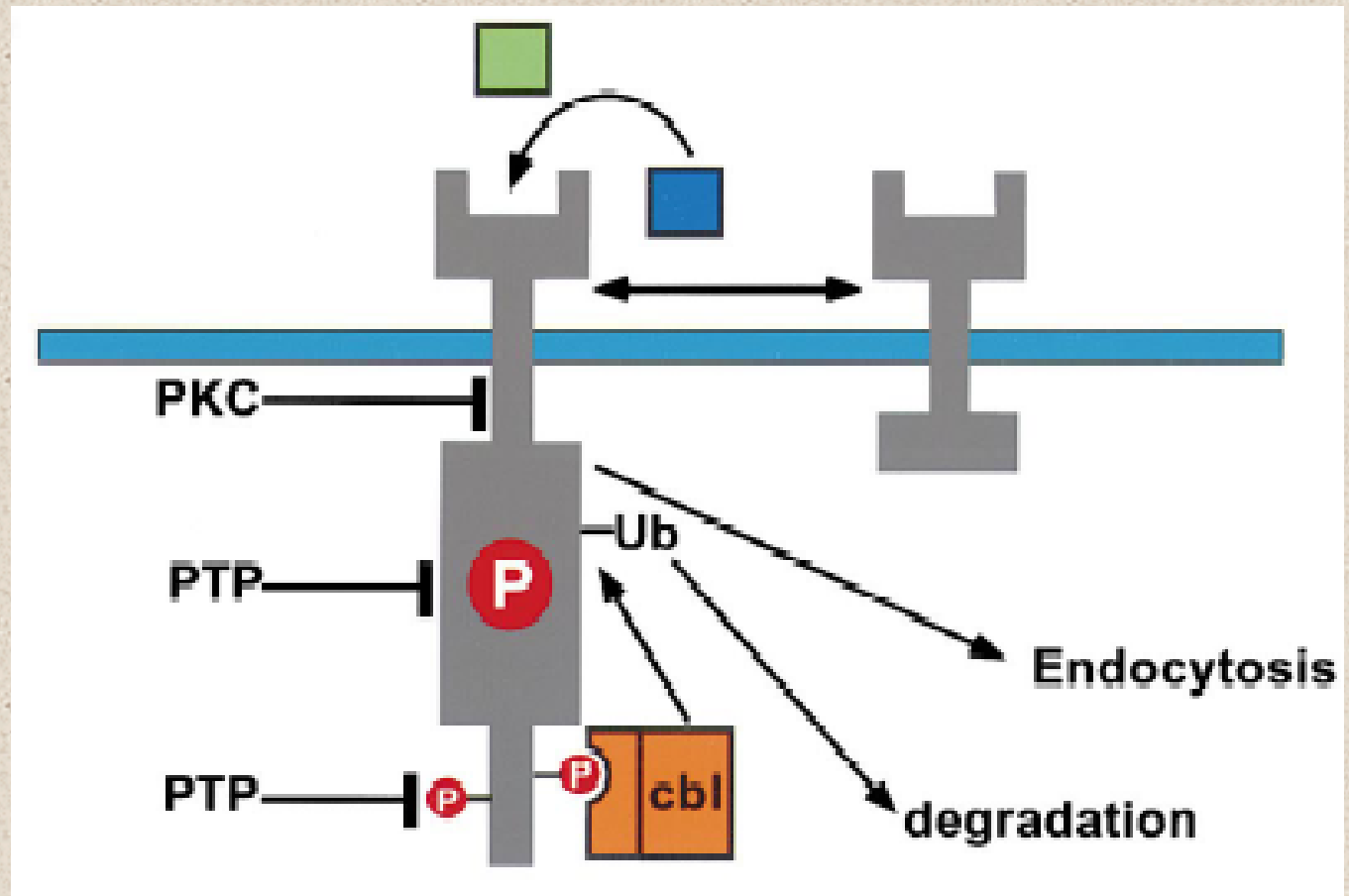


Activation of Different Signaling Pathways by PTKs



Mechanisms for Attenuation and Termination of RTK Activation

- Short-term phosphorylation
- Long-term phosphorylation
- Attenuation
- Termination



Factors that determine the Specificity of Signaling Pathways

Question: How are the myriad of extracellular cues transmitted to induce specific biological responses?

Several mechanisms have been proposed to control specificity in cell signaling:

1. **Combinational Control:** recruitment and activation of unique sets of signaling based in tyrosine autophosphorylation sites and by means of tyrosine phosphorylation sites on closely associated proteins (such as Gab1)
2. **Scaffold Proteins:** scaffolding proteins that bind simultaneously to several proteins are able to insulate key components of signaling pathways (MAP kinases)
3. **Cellular Compartmentalization:** membrane translocation, membrane rafts
4. **Signal Duration and Amplitude:** (EGFR->transient MAPK stimulation ->PC12 cell proliferation; PC12 cell differentiation. NGF R-> late and sustained MAPK activation)
5. **Cellular Context:** cell lineages, tissue specific, stage of differentiation, ligands.

Src family of non receptor protein tyrosine kinases

Gene	kDa	Expression
<i>c-Src</i>	60-61	NK cells, mast cells, monocytes, high in platelets
<i>c-Yes</i>	62	T cells, NK cells, mast cells, platelets
<i>Fyn</i>	59-60	T cells, B cells, NK cells, monocytes, platelets
<i>Lyn</i>	55-58	B cells, NK cells, mast cells, monocytes, platelets
<i>c-Fgr</i>	58	monocytes, granulocytes
<i>Lck</i>	56	T cells, NK cells, low in B cells
<i>Hck</i>	59-64	B cells, mast cells, monocytes, granulocytes, platelets
<i>Blk</i>	55-56	B cells
<i>Yrk</i>	60	monocytes, neurons

*: increased activities in human primary breast carcinoma

What Is the Cellular Function of c-Src?

- 1. Encoded by a proto-oncogene.**
 - deregulated activity promotes aberrant proliferation
- 2. Expressed ubiquitously.**
 - highest in blood platelets, neurons, and osteoclasts
 - suggests widespread function(s) not necessarily related to proliferation
- 3. *src* -/- mice are healthy, born and survive up to one year.**
 - no obvious defects in platelet and neuronal function
 - compensation by other Src-family kinases?

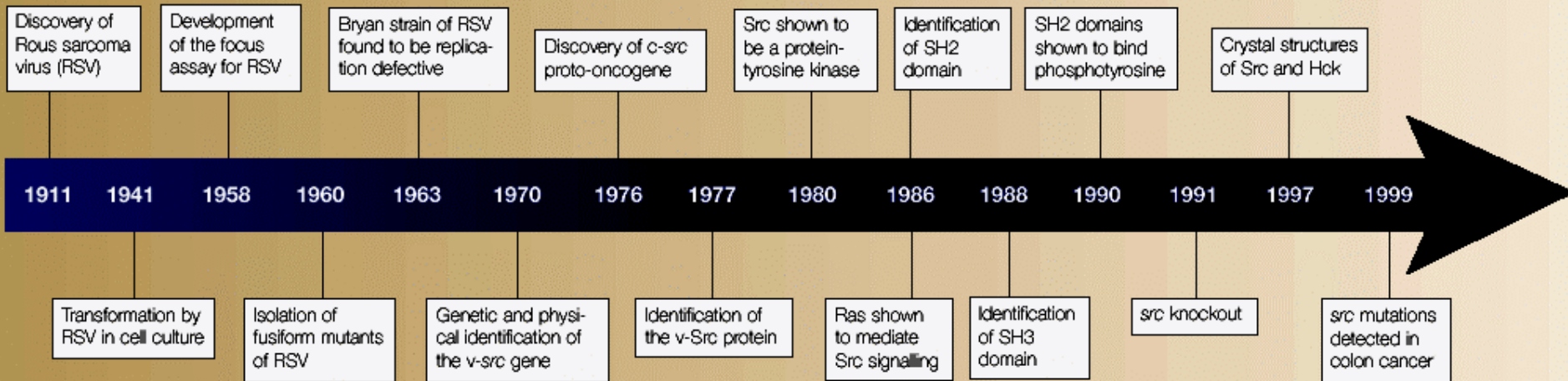
Expression of Src Family Kinases

Src	Ubiquitous
Fyn	Ubiquitous
Yes	Ubiquitous
Lyn	Brain, B-cells, myeloid cells
Hck	Myeloid cells
Fgr	Myeloid cells, B-cells
Blk	B-cells
Lck	T-cells, NK cells, brain

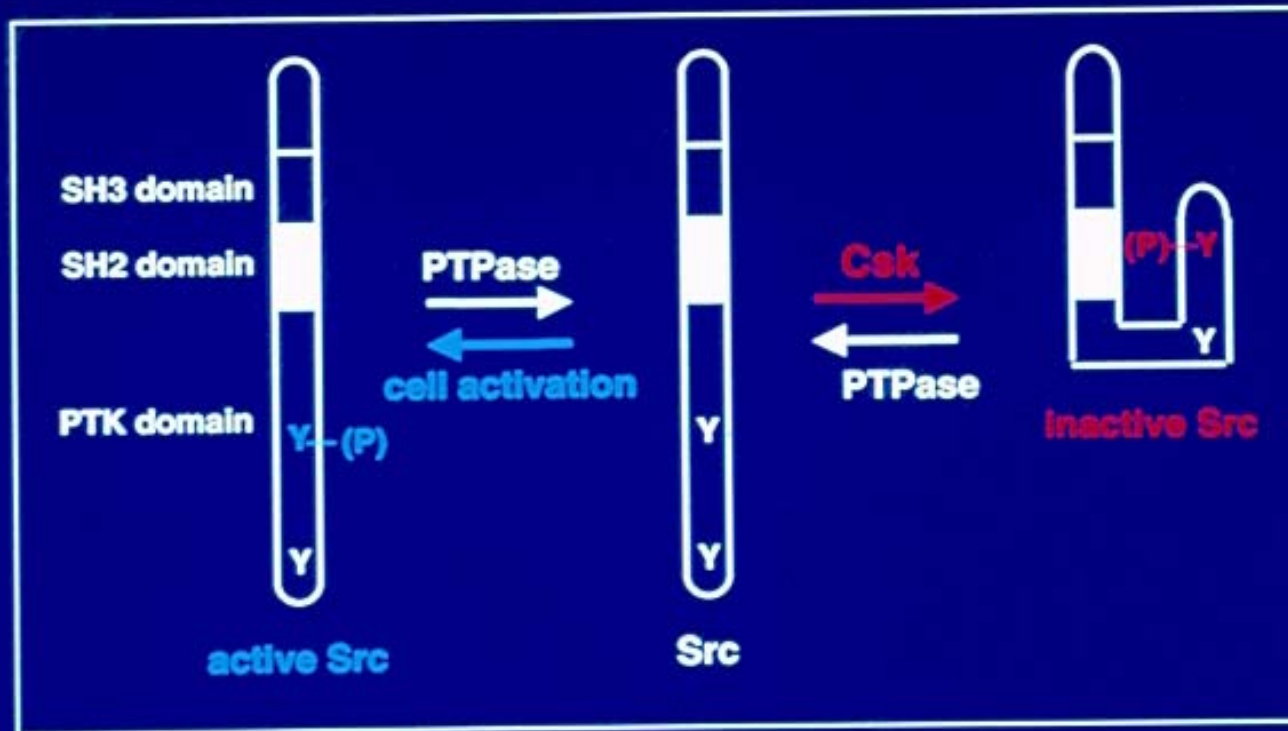
- 4. *src/fyn/yes* triple knockout is embryonically lethal at mid-gestation.**
 - cells have defects in motility but not proliferation
 - motility defects are rescued by reexpression of c-Src
- 5. c-Src and v-Src localize predominantly to cellular *focal adhesions*.**
 - consistent with their role in cell motility

Key Events

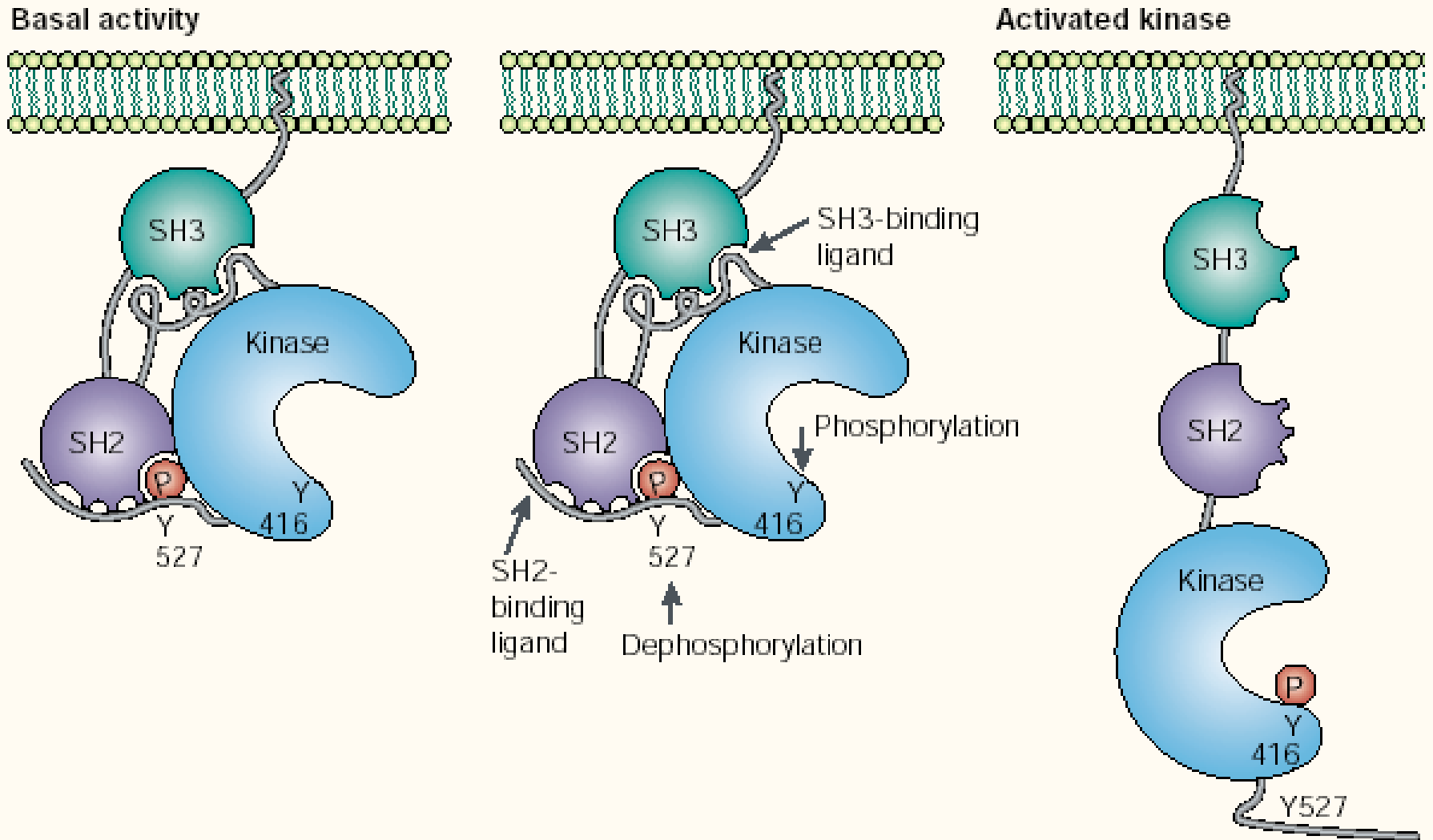
Timeline | Key events in hunting the Src



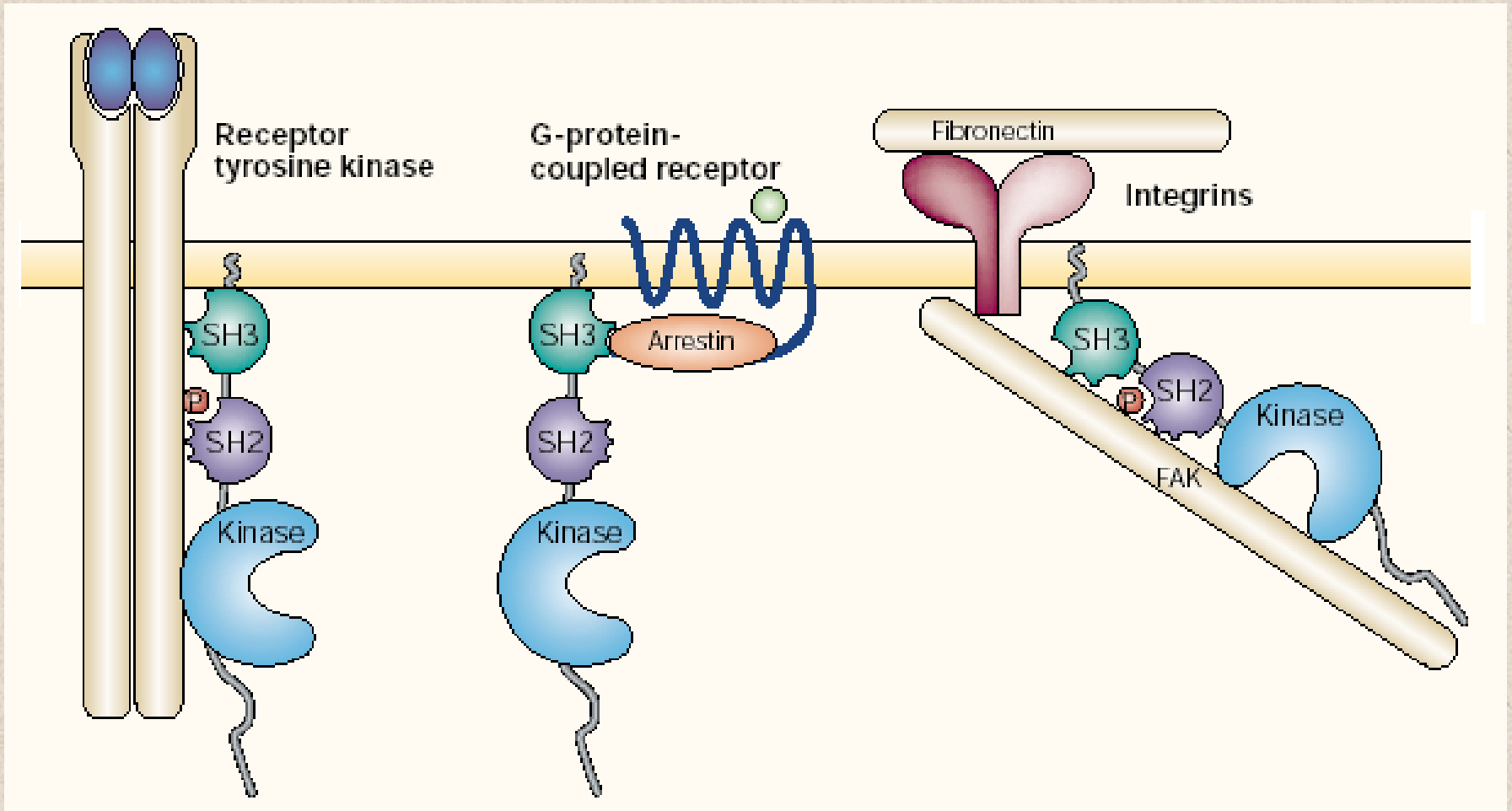
Regulation of Src family PTKs



Activation of c-Src

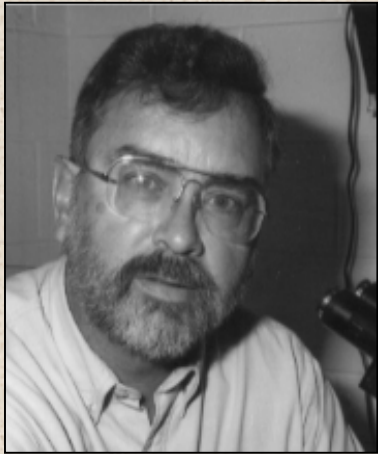


Regulation of c-Src by Surface Receptors



Identification of Src Substrates

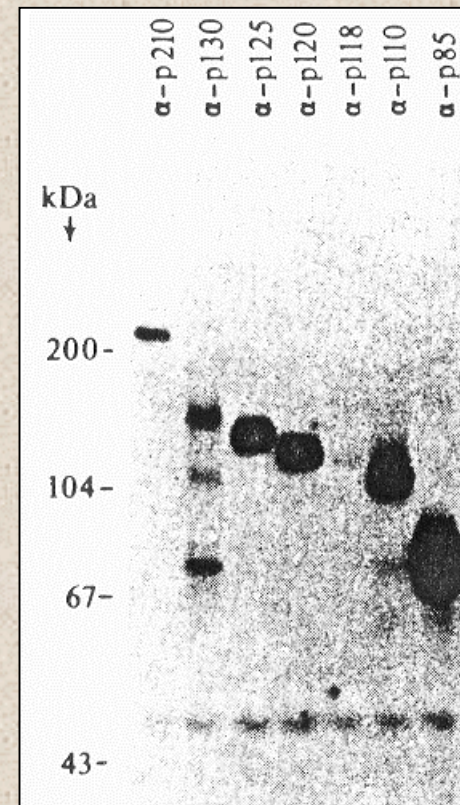
1990; **Tom Parsons** raised antibodies against tyrosine-phosphorylated proteins in v-Src transformed cells



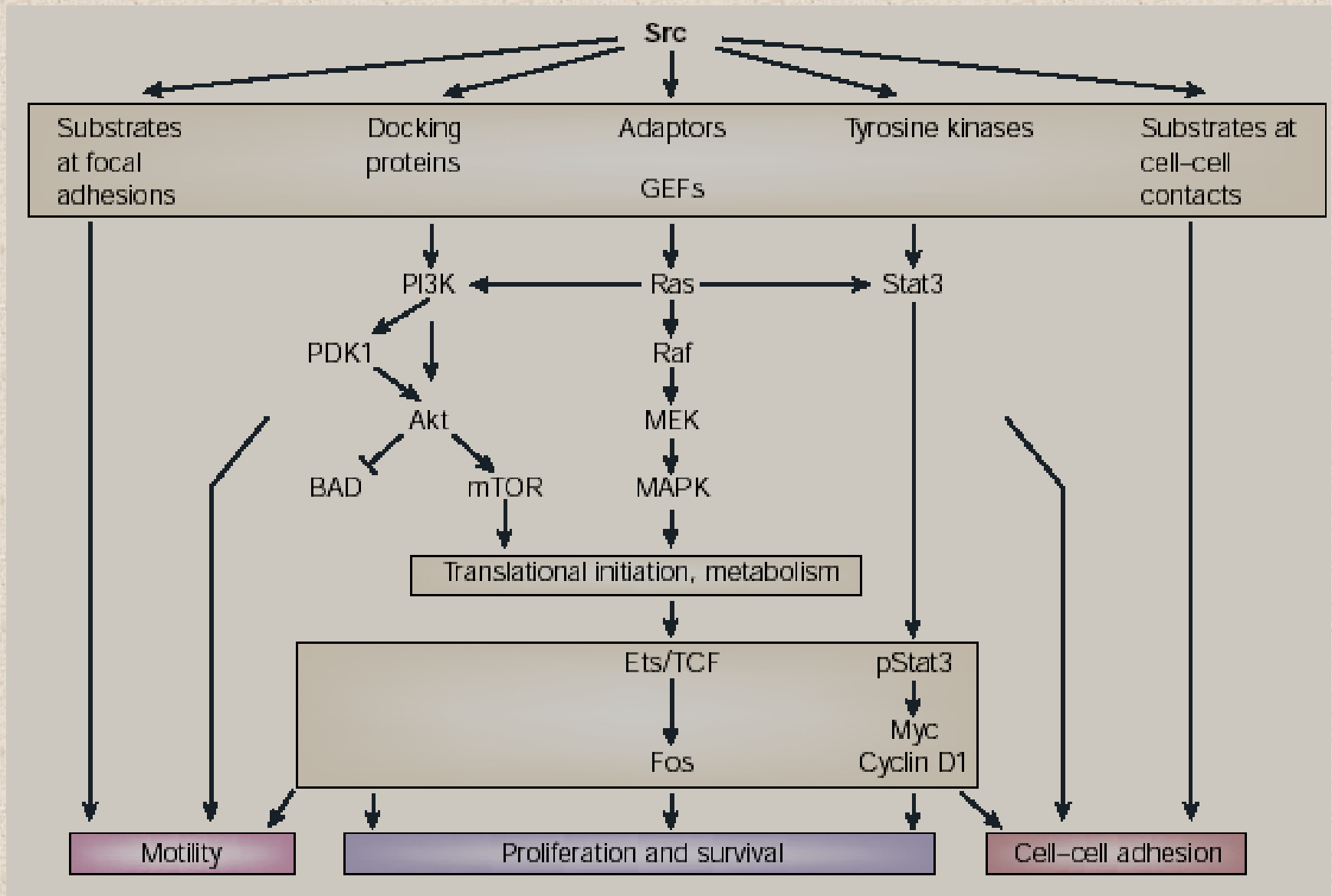
- *mice immunized with a mix of cellular pTyr-containing proteins purified using affinity-chromatography to anti-pTyr antibodies*
- *monoclonal antibodies recognizing several putative Src substrates were isolated*

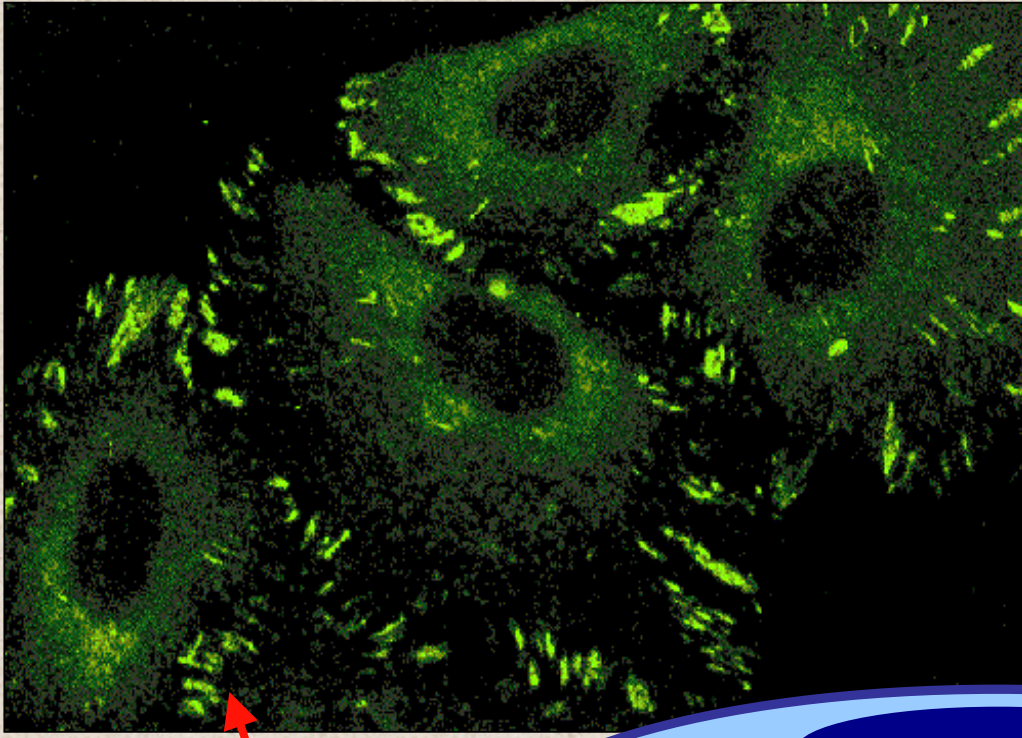
Figure: - immunoprecipitate with different mAbs,
- immunoblot with anti-pTyr antibody

What are p210, p130, p125, etc?



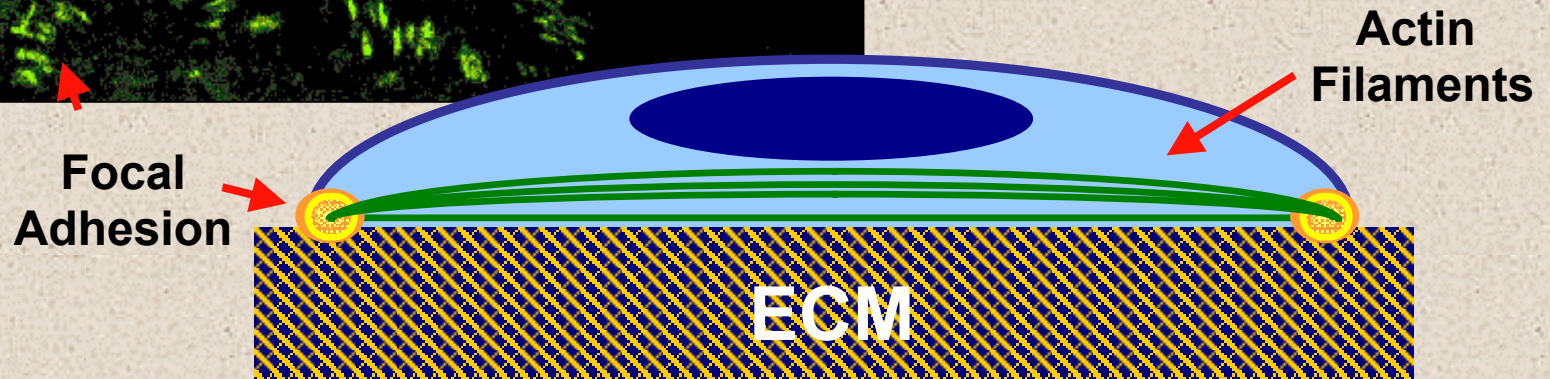
Signaling by Src





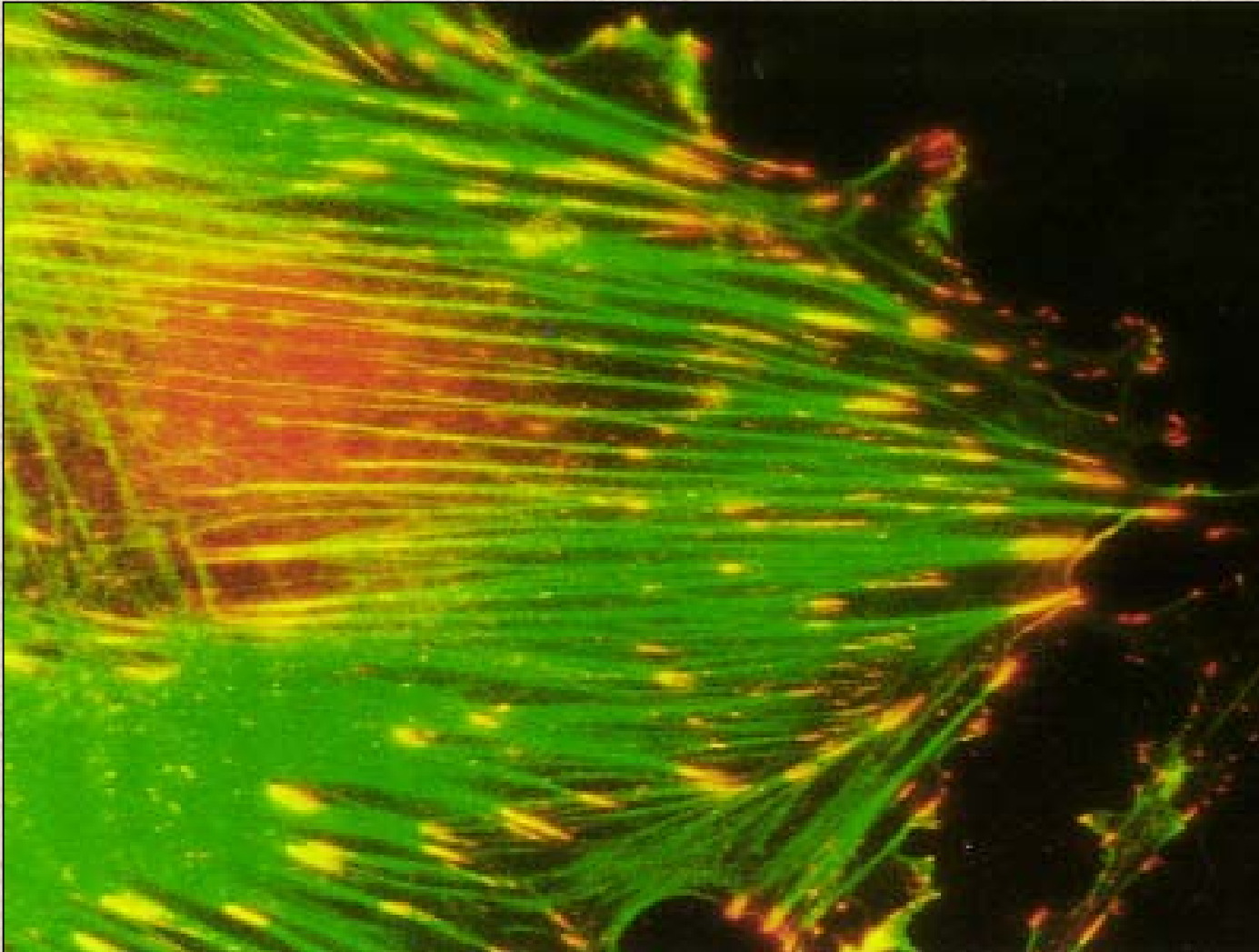
Focal Adhesions:

Specialized regions of the plasma membrane formed at sites where cultured cells adhere tightly to the underlying ECM substratum



- sites of clustering of integrin receptors for ECM adhesive glycoproteins
- site of anchorage of actin filaments >> cell spreading and locomotion

Tyrosine-Phosphorylated Proteins Are Enriched in Focal Adhesions



Green = actin

Red = pTyr

**Yellow = costain
(focal adhesions)**

Review of Key Concepts

Tyrosine kinases make up a large family of receptor and cytoplasmic protein kinases that function to regulate cellular responses to extracellular stimuli associated with metazoan complexity (*ECM, cytokines and growth factors, antigens, etc*).

Cellular phosphotyrosine is rare, compared to phosphoserine and phosphothreonine, indicating that tyrosine phosphorylation of proteins is transient and highly regulated.

Tyrosine phosphorylation can be assessed by phospho-amino acid analysis or, more commonly, using phosphotyrosine-specific antibodies.

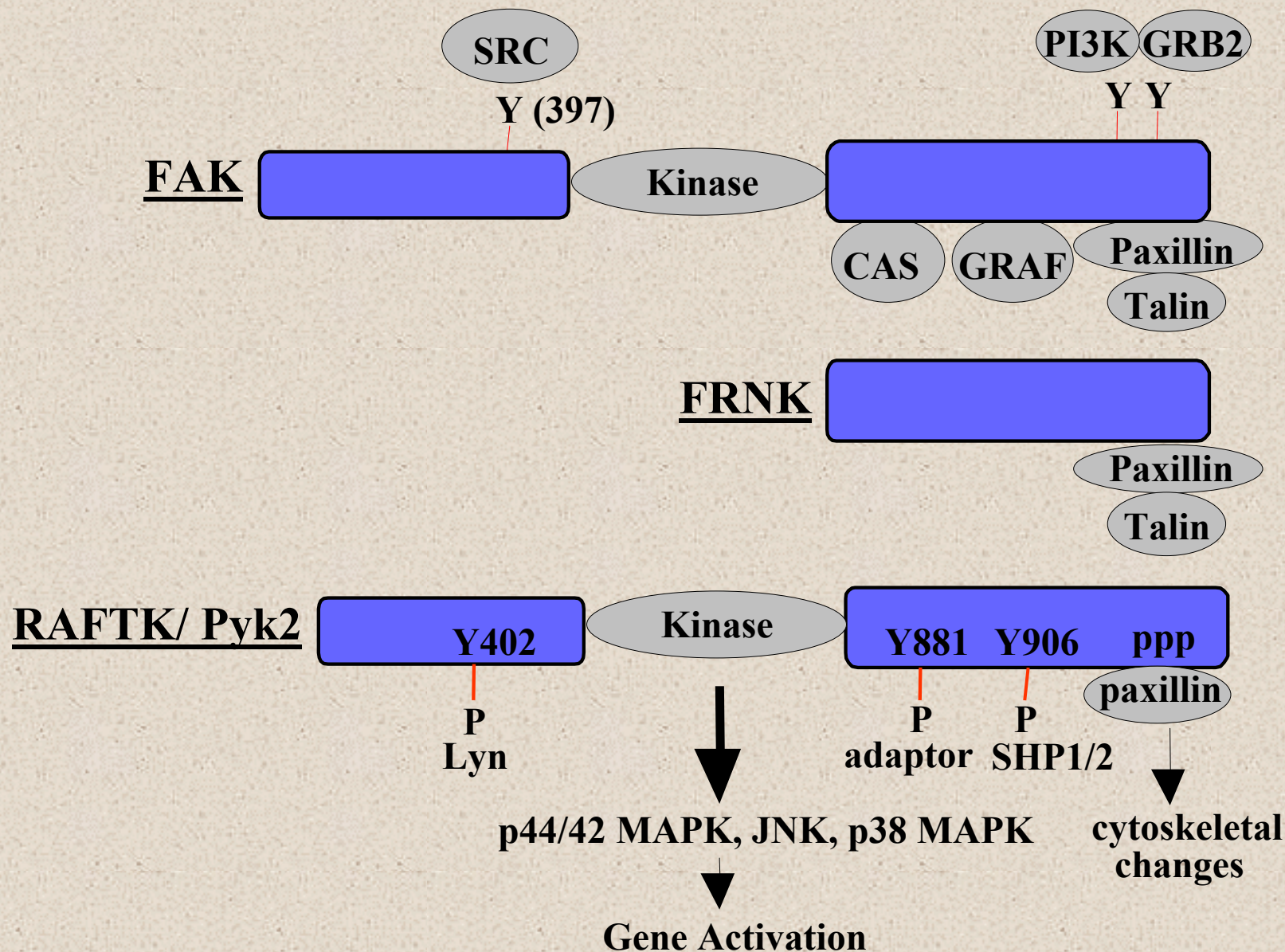
Mutations that result in deregulated tyrosine kinase signaling often lead to uncontrolled cell proliferation associated with cancer.

Tyrosine kinase signaling is frequently involved in the recruitment of SH2-containing effector proteins to phosphorylated tyrosine sites.

Different SH2 domains exhibit different binding preferences based on amino acid residues close to the C-terminal of the phosphotyrosine.

FAK/RAFTK (=Pyk2)

Schematic Presentation of FAK and RAFTK/Pyk2 and their Respective Binding Partners

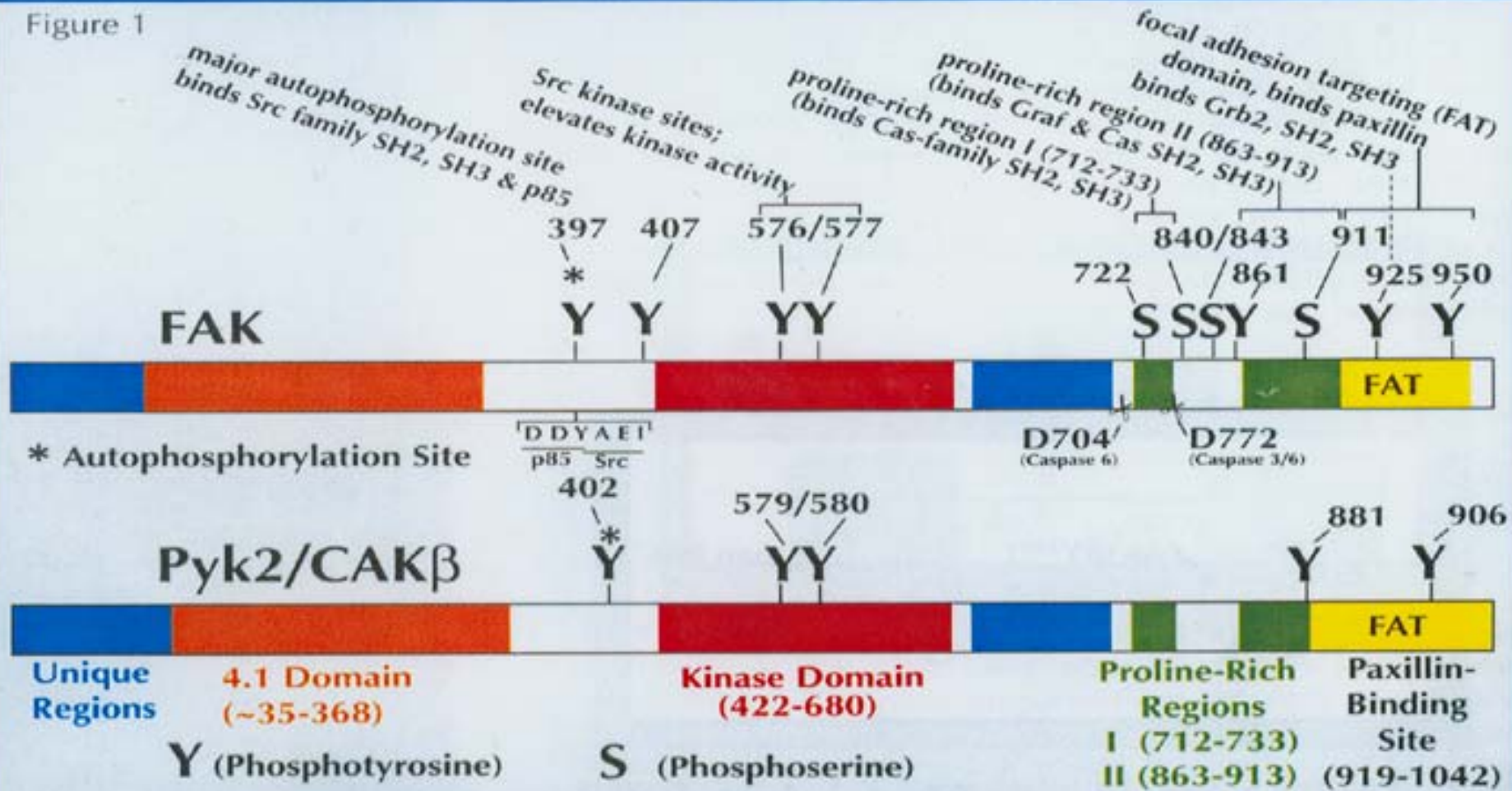


Focal Adhesion Protein Tyrosine Kinase Family

- 1. This family consists of FAK and RAFTK/Pyk2 kinase (also designated FAK2, CAK- β or CADTK).**
- 2. Both kinases have a molecular mass between 110-125 KDa and are closely related in their overall structures.**
- 3. Neither kinase contains SH2 and SH3 domains.**
- 4. Analyses of FAK and RAFTK/Pyk2 activation events have shown that phosphorylation occurs at several sites in vivo (next table).**
- 5. The presence of two proline-rich motifs within the C-terminal domains of RAFTK/Pyk2 and FAK is also conserved. The proline-rich motifs are sites for SH3-mediated protein-protein interactions.**
- 6. FAK is expressed in almost all tissues while RAFTK/Pyk2 is expressed mainly in the central nervous system and in hematopoietic cells.**
- 7. FAK is localized to focal adhesion sites in adherent cells, while RAFTK/Pyk2 is mainly diffused throughout the cytoplasm and is concentrated in the perinuclear region.**

FAK and RAFTK/Pyk2

Figure 1

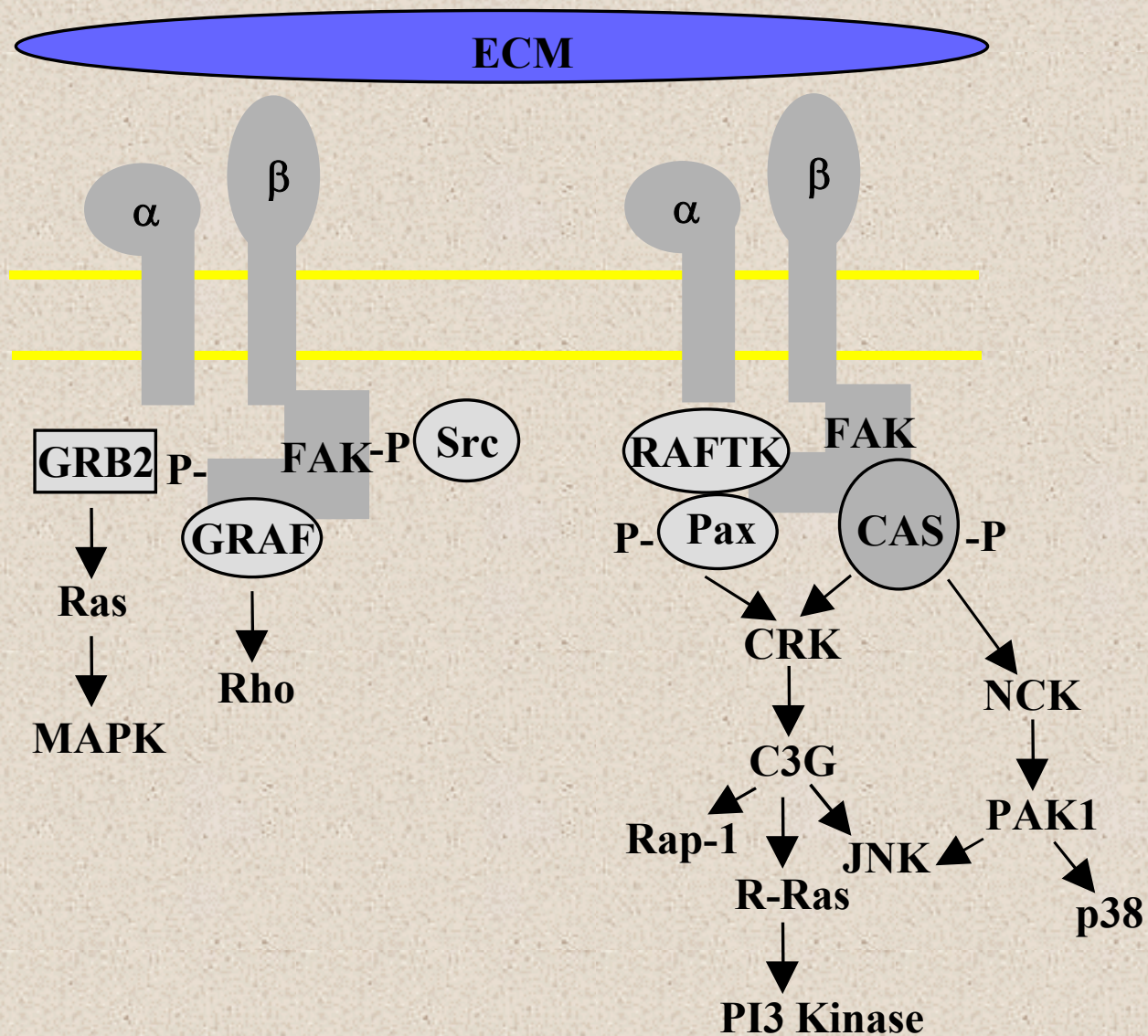


Modified from: Karkkainen et al., Nature Cell Biol. (2002)

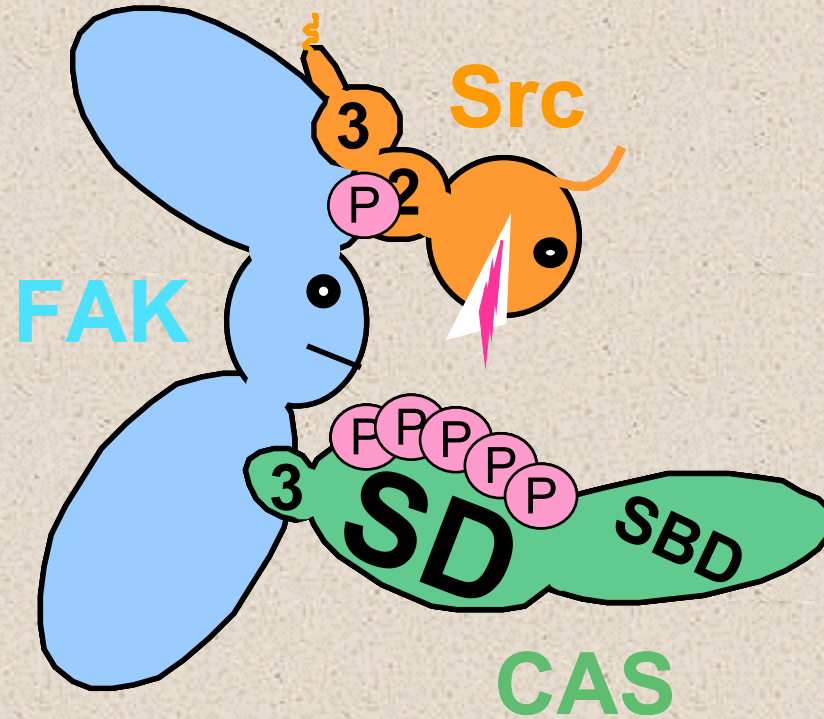
Table 1: Phosphorylation binding motifs in human FAK and RAFTK/Pyk2

Site in FAK	FAK motif	Site in RAFTK/Pyk2	RAFTK/Pyk2 motif	Function
Tyr³⁹⁷	YAE1	Tyr⁴⁰²	YAE1	Src-family SH2 binding
Tyr⁴⁰⁷	YTMP	—	—	Unknown
Tyr^{576/577}	STYYKAS	Tyr^{579/580}	EDYYKAS	Regulatory-kinase activation loop
Tyr⁸⁶¹	YQPV	—	—	Unknown
Tyr⁹²⁵	YENV	Tyr⁸⁸¹	YLVN	Grb2-SH2 binding site
Pro^{712/713}	<u>APPKPSPG</u>	Pro^{714/715}	<u>PPPKPSPK</u>	p130^{Cas} SH3-binding site
Pro^{876/877}	<u>PPKKPPRPG</u>	Pro^{859/860}	<u>PPQKPPRLG</u>	p130^{Cas}/Graf SH3-binding site

Schematic of Signaling Cascades Downstream of FAK and RAFTK/Pyk2

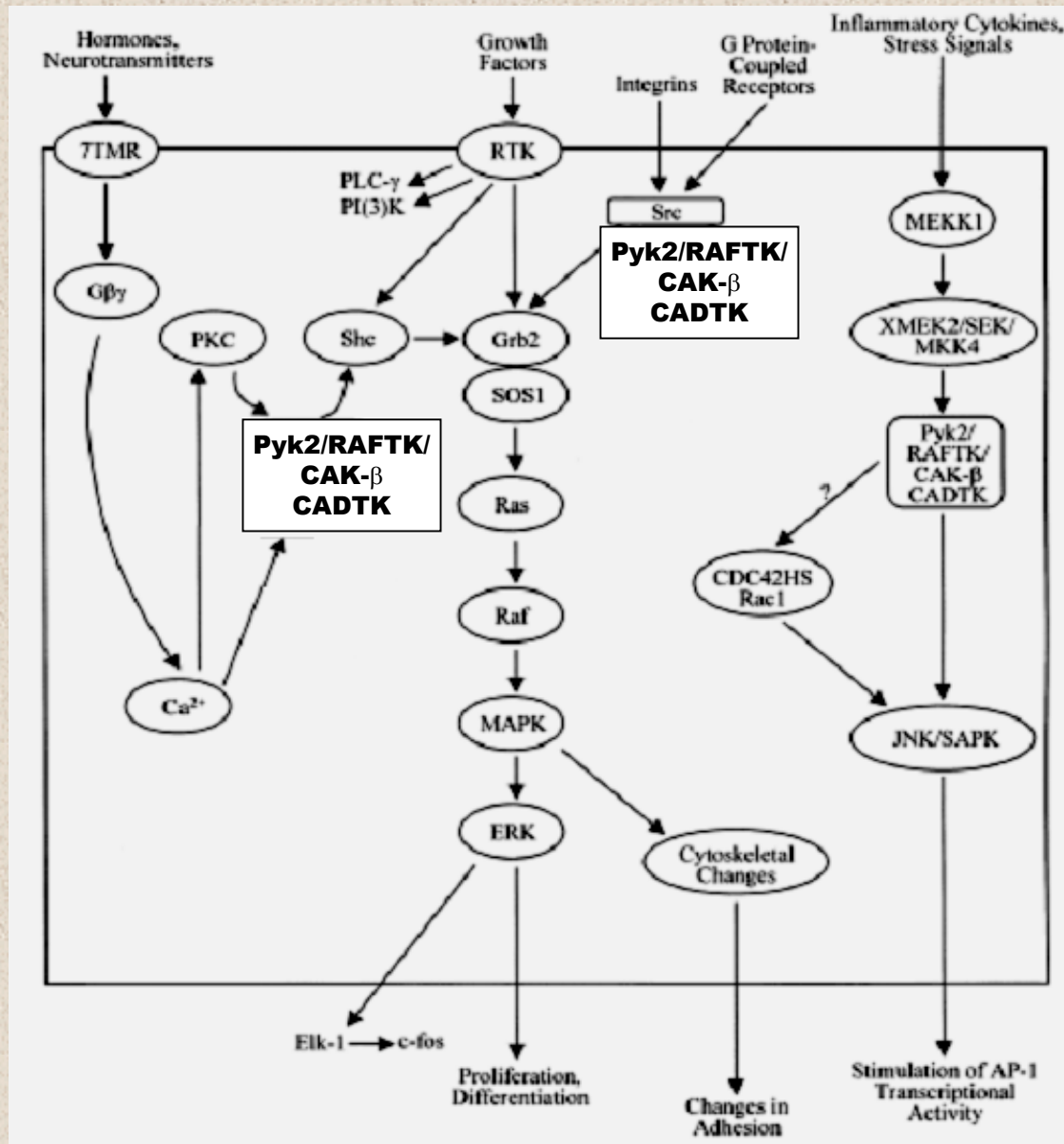


Integrin Signaling Through the FAK/Src/CAS Complex



FAK autophosphorylates Tyr-397, then acts as a scaffold to recruit and activate Src to phosphorylate the CAS “substrate domain”

Potential RAFTK/Pyk2/CAK- β /CADTK Mediated Signaling Pathways



VEGF Regulates Focal Adhesion Assembly Through FAK & RAFTK/PYK2

Focal Adhesion Assembly

- * is a complex comprised of scaffolding and signaling proteins organized by adhesion to the ECM**
- * links the actin cytoskeleton to ECM via the integrin receptor complex**
- * includes actin-binding proteins (such as talin, vinculin and tensin) that co-localize with integrins**

VEGF & Focal Adhesion Assembly

VEGF-induced migration of ECs: is a key step in angiogenic response & is mediated by an accelerated rate of focal adhesion complex assembly & disassembly

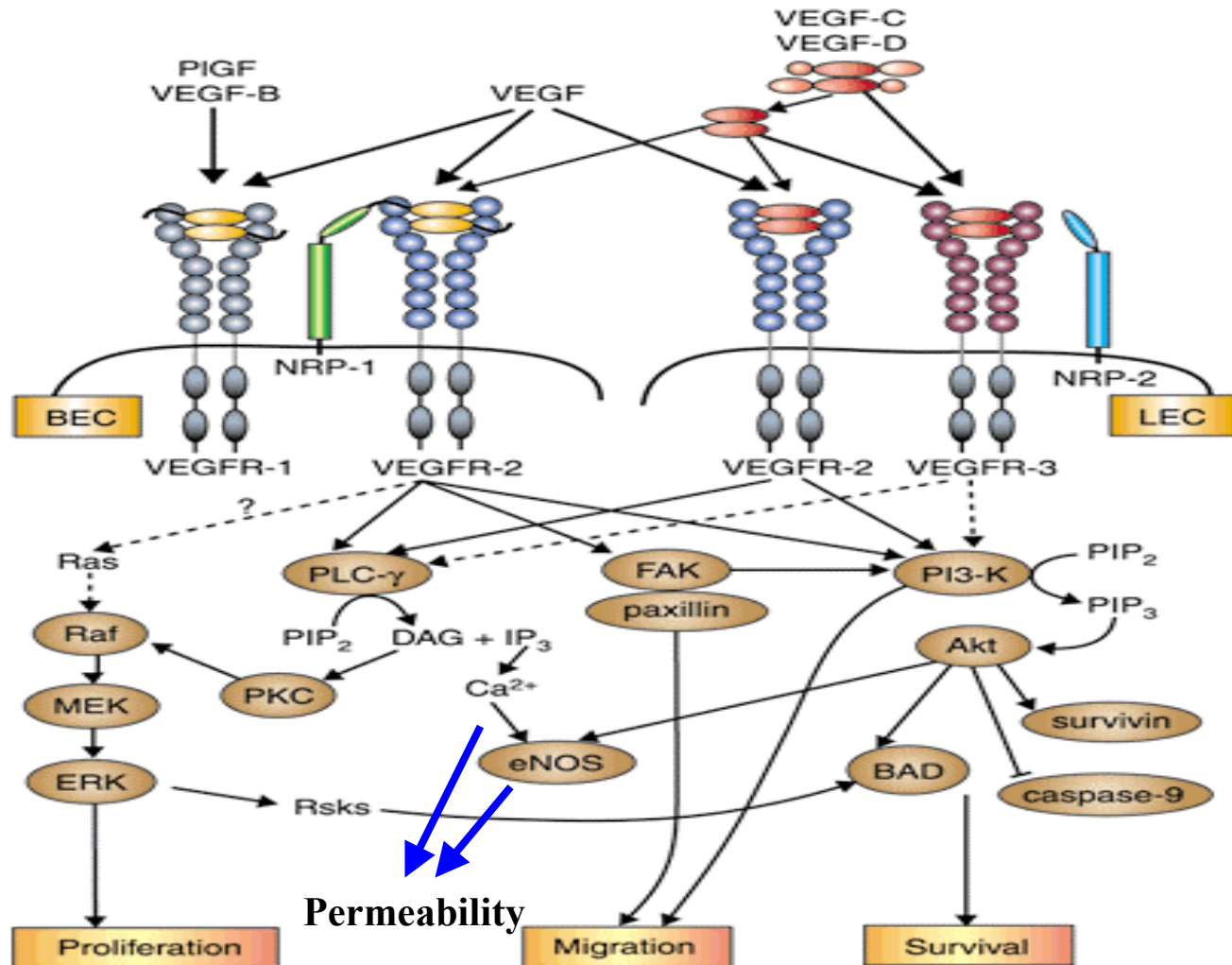
VEGF

- * a basic, heparin-binding, homodimeric glycoprotein of 45kD
- * VEGF₁₂₁, VEGF₁₆₅, VEGF₁₈₉, VEGF₂₀₆ (alternative exon splicing of a single VEGF gene)

Functions

- * Potent vascular permeable activity
- * Induction of plasma protein leakage in BBB
- * Induction of fenestrations in endothelial cells

VEGF Signaling Pathways in ECs

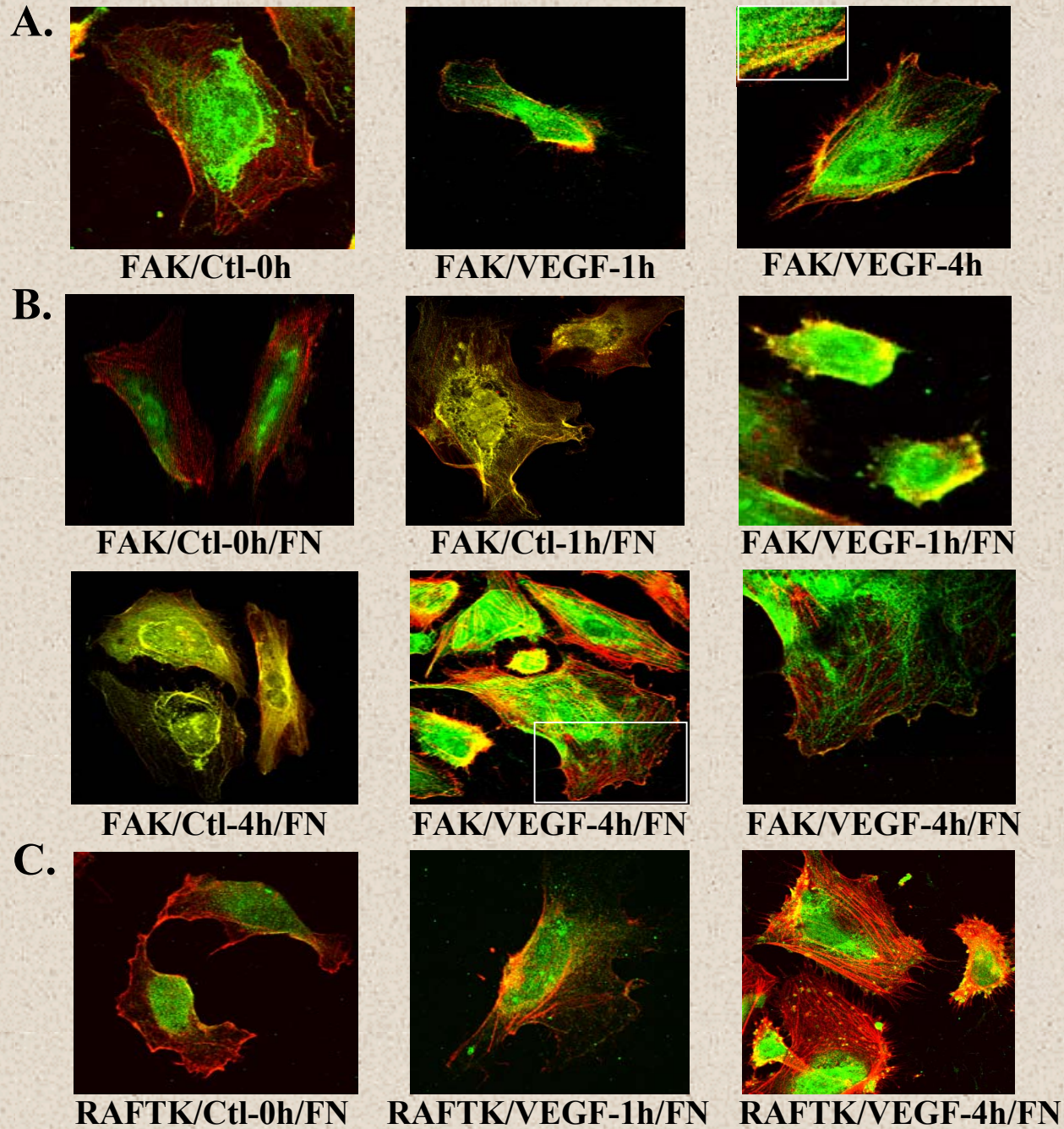


BEC: blood EC

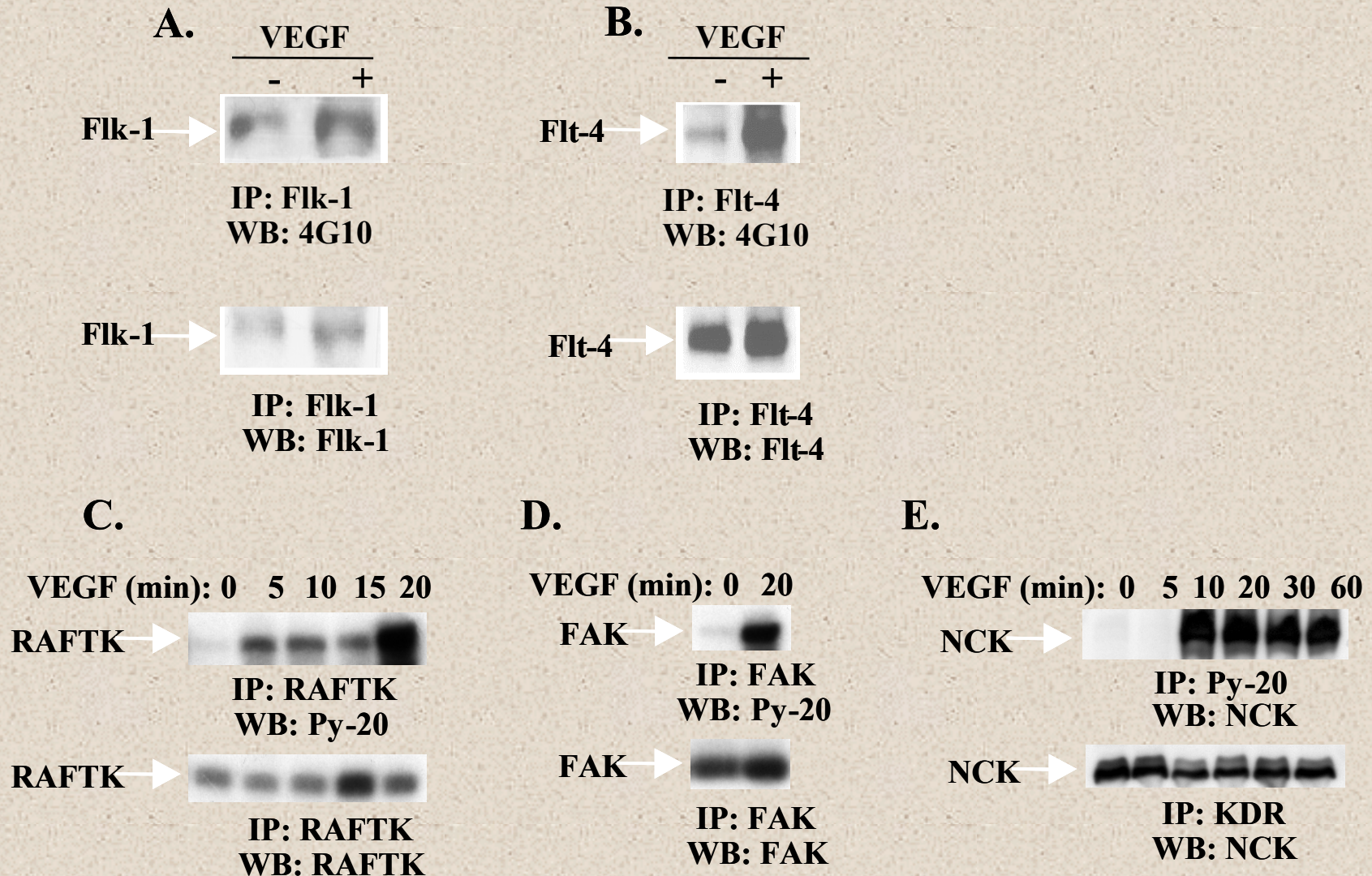
LEC: lymphatic EC

Modified from: Karkkainen et al.,
Nature Cell Biol. (2002)

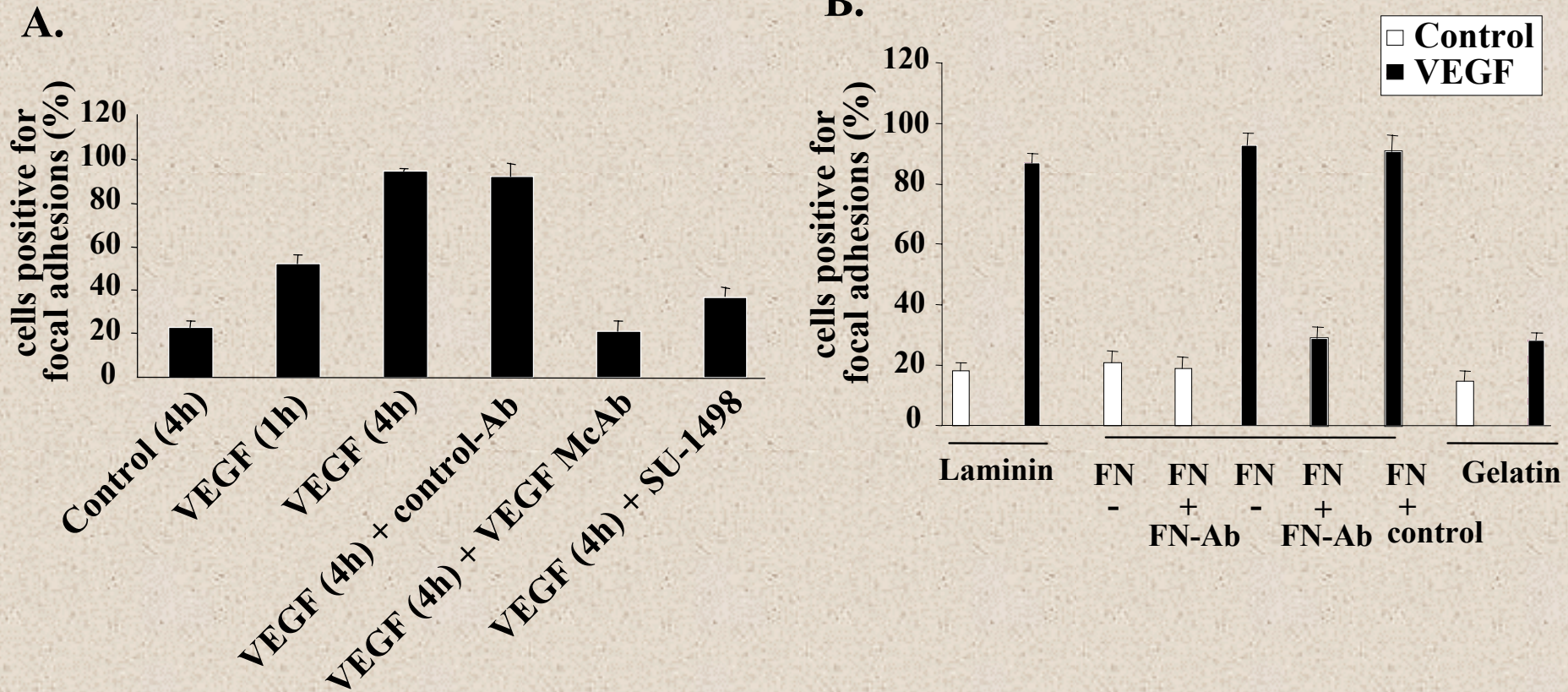
Focal Adhesion Assembly in HBMECs



Tyrosine Phosphorylation of Flk-1/KDR, Flt-4, FAK and RAFTK/Pyk2 upon VEGF Treatment in HBMECs

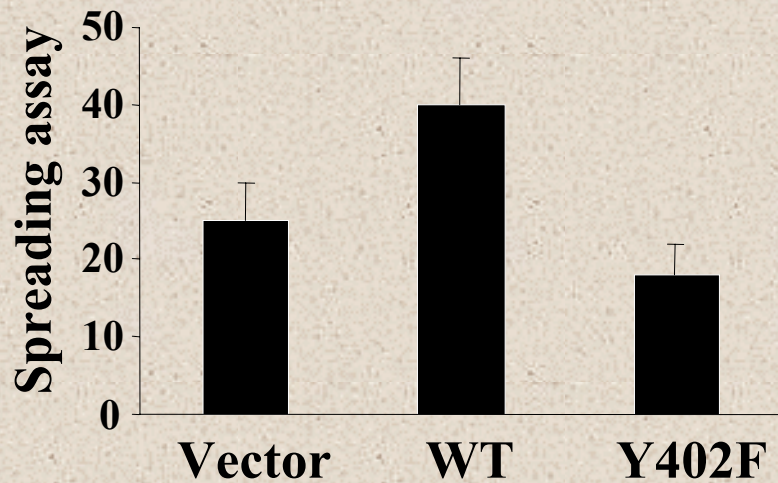


Effects of VEGF and Integrins on Focal Adhesion Assembly in HBMECs

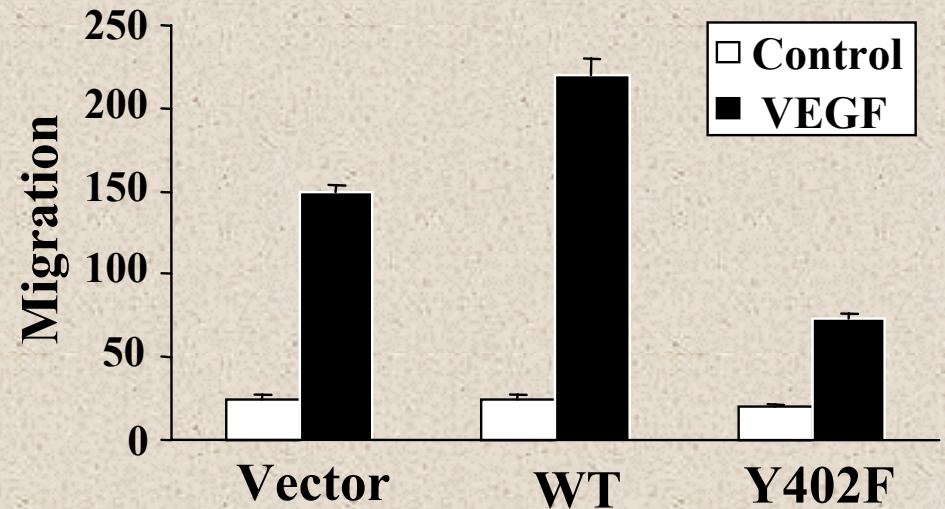


RAFTK/Pyk2 Kinase Activity is Critical for HBMEC Spreading and Migration

A.

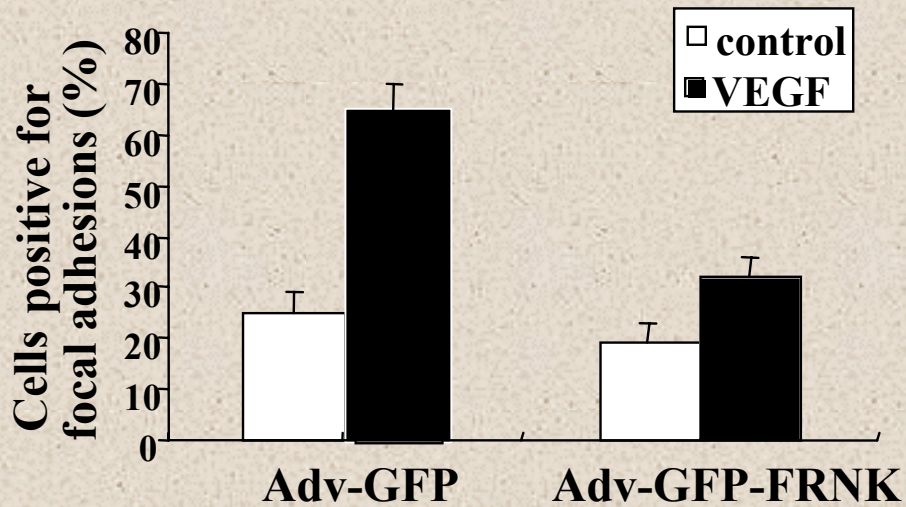


B.

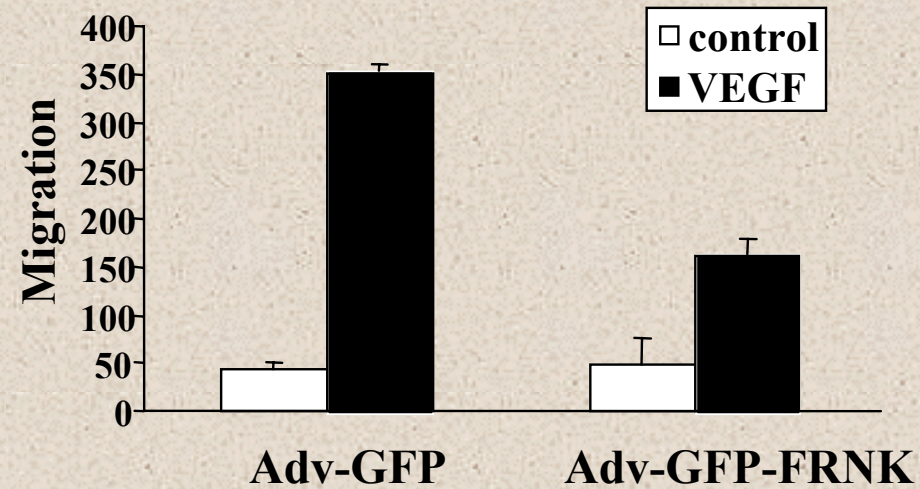


Effects of FRNK Overexpression on VEGF-Induced Focal Adhesion Assembly and Migration

A.



B.

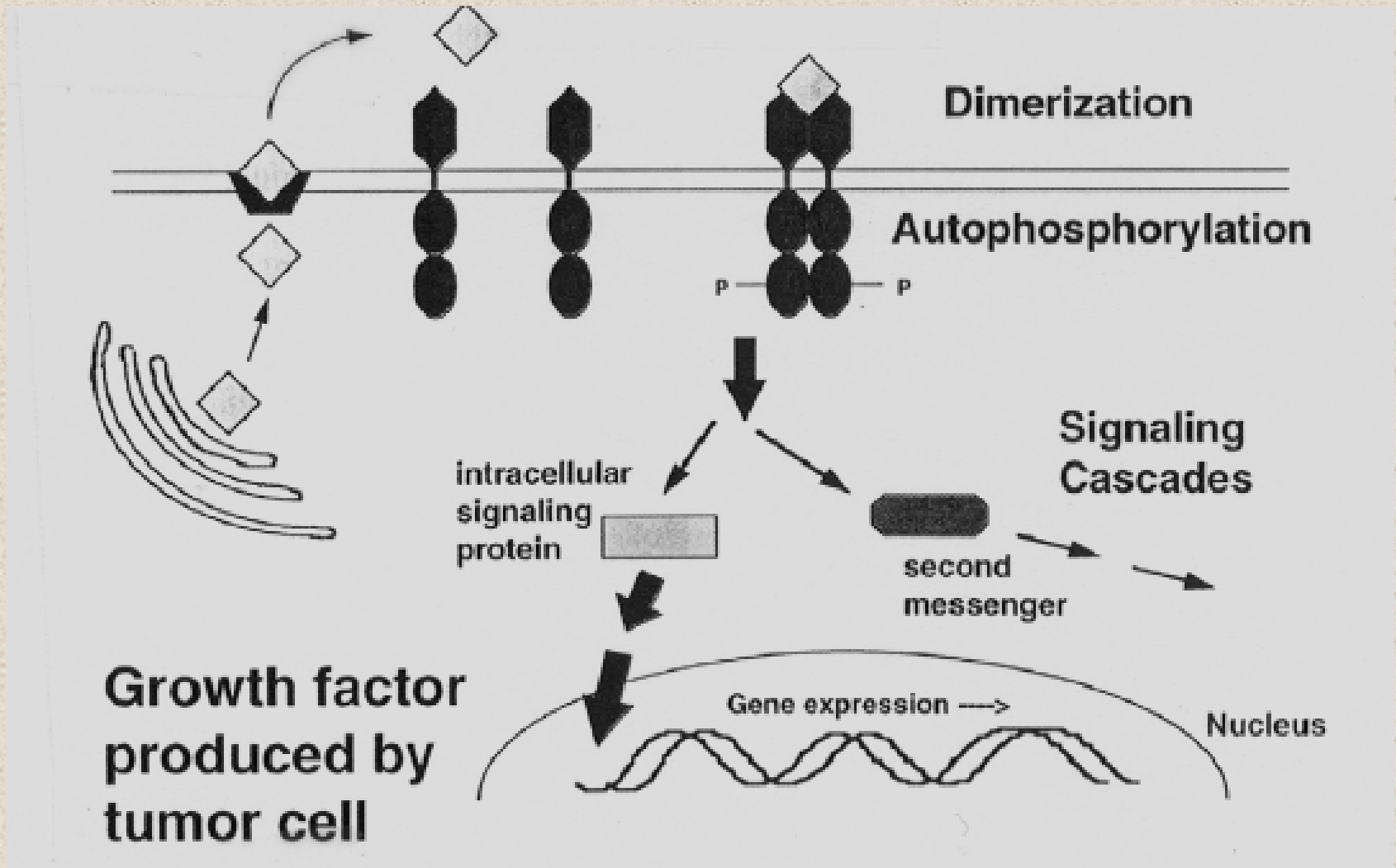


*** Conclusions ***

These studies define a mechanism for the regulatory role of VEGF in focal adhesion complex assembly in HBMECs, via activation of FAK & RAFTK/Pyk2

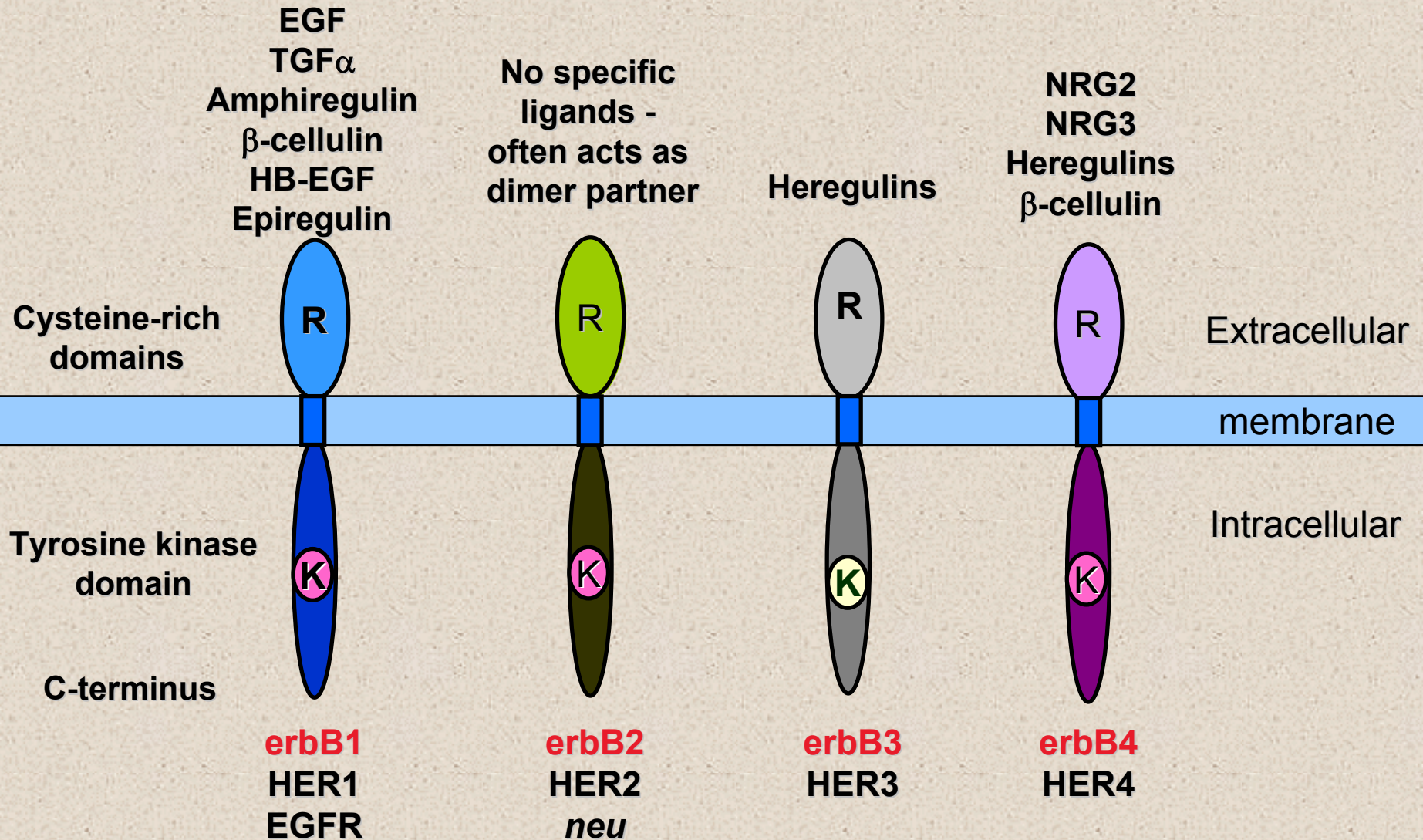
- 1. VEGF treatment of HBMECs plated on laminin or fibronectin stimulated cytoskeletal organization and increased focal adhesion sites.**
- 2. VEGF induced formation of focal adhesions in HBMECs when cells were seeded on fibronectin.**
- 3. FAK and RAFTK/Pyk2 kinases were tyrosine-phosphorylated by VEGF.**
- 4. Overexpression of wild-type RAFTK/Pyk2 increased cell spreading and the migration of HBMECs.**
- 5. Overexpression of catalytically-inactive mutant RAFTK/Pyk2 markedly suppressed HBMEC spreading (~70%), adhesion (~82%) & migration (~65%).**
- 6. Inhibition of FAK by dominant-interfering mutant FRNK (FAK-related non-kinase) significantly inhibited HBMEC spreading and migration as well as disrupted focal adhesions.**

Receptors and Growth Factors



- Activation by an autocrine loop
- Constitutive ligand production leading to tyrosine kinase activation

The EGF Receptor (ErbB) Family and Its Ligands



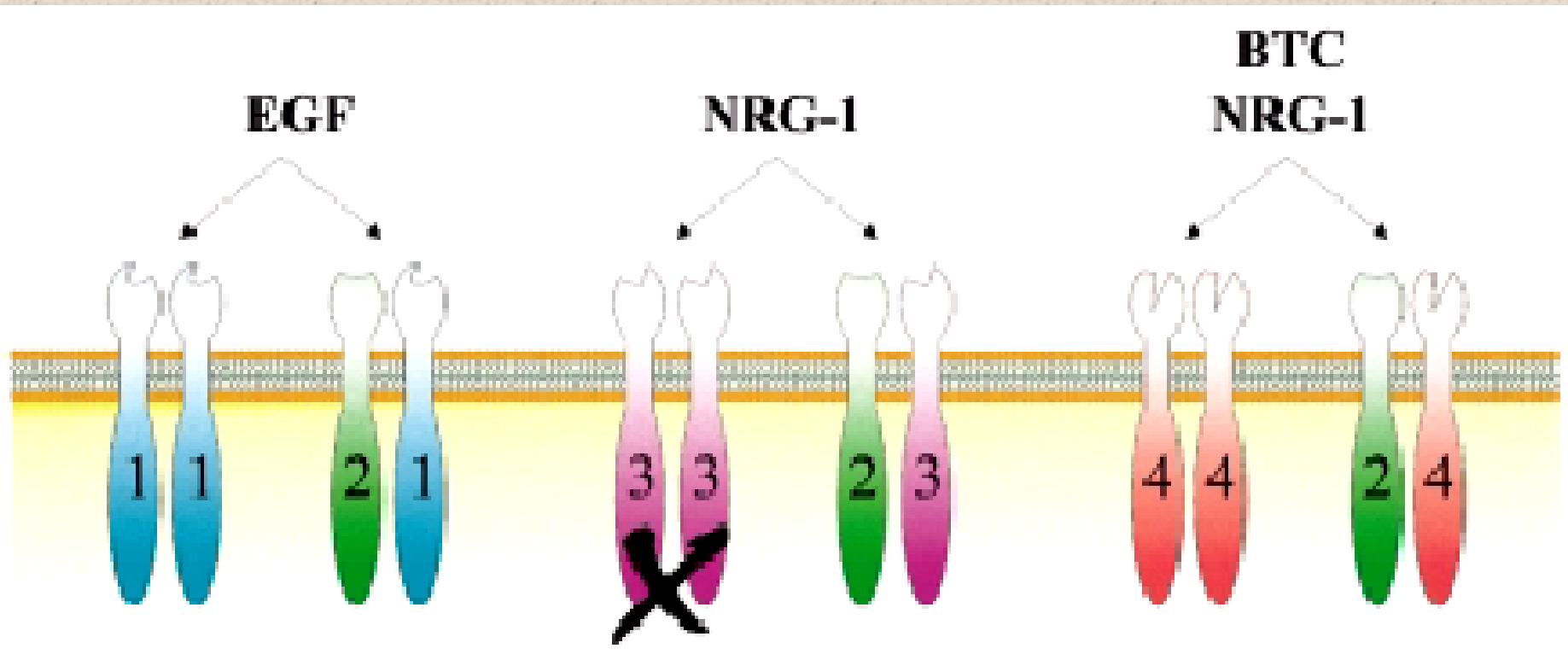
The ErbB Signaling Network

- 1) There are four members of the ErbB family:
 - Epidermal growth factor-EGF receptor (=EGFR); also termed ErbB1/HER1
 - ErbB2/Neu/HER2
 - ErbB3/HER3
 - ErbB4/HER4
- 2) A family of ligands, the EGF-related peptide growth factors bind the extracellular domain of ErbB receptors leading to the formation of both homo and heterodimers.
- 3) Dimerization consequently stimulates the intrinsic tyrosine kinase activity of the receptors and triggers autophosphorylation of specific tyrosine residues with the cytoplasmic domain.
- 4) These phosphorylated residues serve as docking sites for signaling molecules involved in the regulation of intracellular signaling cascades.
- 5) The downstream effects on gene expression determine the biological response to receptor activation.

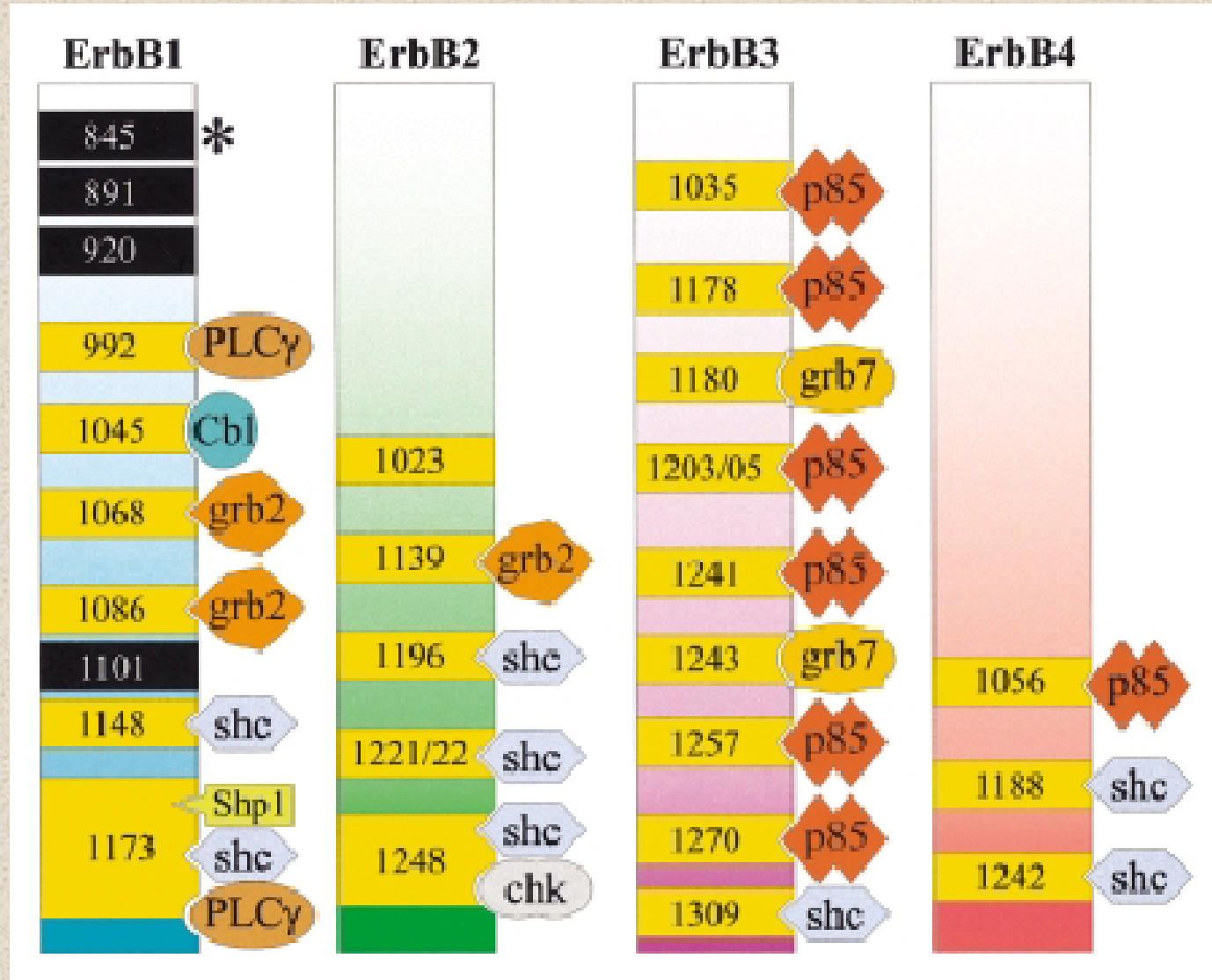
Expression of ErbB receptors

- 1) ErbB receptors are expressed in a variety of tissues of epithelial, mesenchymal and neuronal origin.**
- 2) ErbB receptors play fundamental roles in development, proliferation, and differentiation.**
- 3) Deregulated expression of ErbB receptors, in particular ErbB1 and ErbB2, has been implicated in the development and malignancy of several types of cancer.**
- 4) The primary function of ErbB2 is as a coreceptor. ErbB2 is the preferred heterodimerization partner for all other ErbB family members and plays a role in the potentiation of ErbB receptor signaling.**
- 5) ErbB heterodimers provide signal diversification.**

ErbB-2 Is the Preferred Dimerization Partner for the Other ErbB Receptors



Specific Phosphotyrosine Residues and Binding of Signaling Molecules to the ErbB Receptors



The Power of ErbB Receptor Heterodimerization

- 1. ErbB heterodimerization provides for signal amplification**
- 2. ErbB heterodimerization provides for signal diversification**
- 3. The interplay of the ErbB family and their ligands in the context of the entire signaling network as present in live cells:**
 - Cross-talk between different pathways**
 - ErbB receptors as signal integrators**

Ductal carcinoma *in situ* (DCIS)



- **90% express EGFR**
- **70% express c-erbB2**
- **Comedo DCIS is estrogen independent**
- **Role of adjuvant treatment unclear**

Human Breast Cancer

- ↳ **Leading cause of death in women 30 to 70 years of age**
- ↳ **Acquisition of several successive distinct genetic changes**
 - **inherited: BRCA1, BRCA2 mutations**
 - **acquired: overexpression of oncogenes**
 - **HER-2/neu**
 - **Src family**
 - **mutations of tumor suppressor genes**
 - **p53**
 - **PTEN**
 - **...**

HER (ErbB) family in cancer

HER-1 ?

HER-2 overexpression in - breast carcinomas (25-30%)

- ovarian carcinomas
- lung carcinomas
- gastric carcinomas
- oral carcinomas

HER-3 overexpression in non small cell lung carcinomas (NSCLC)

HER-4 overexpression in papillary thyroid carcinomas

HER (ErbB) family of protein tyrosine kinase receptor

HER-1 ligands = - epithelial growth Factor (EGF)

- betacellulin (BTC)
- epiregulin (EPR)
- amphiregulin (AR)
- transforming growth factor alpha (TGF- α)

HER-2 no ligand

HER-3 ligands = heregulin (HRG) = neu differentiation factor (NDF) = neuregulin (NRG)
kinase-dead

HER-4 ligands = - HRG (NDF)(NRG)

- EGF
- BTC
- EPR

HER-2/neu (c-erbB2) protein tyrosine kinase receptor

♦ HER-2/neu activation

normal cells = heterodimers:

HER-2 / EGF-R \leftarrow EGF

HER-2 / HER-3 \leftarrow HRG (NDF) (NRG)

cancer cells = homodimers:

HER-2 / HER-2 \leftarrow constitutively active

♦ HER-2/neu signaling

transmembrane protein tyrosine kinase receptor

activation \rightarrow auto(P)

\rightarrow binding to SH2 containing proteins

= Shc, PLC- γ , Ras-GAP, PI-3K, c-Src, ...

Regulation of ErbB2 by CHK and CSK Kinase

Csk Homologous Kinase (CHK)

◆ Cloning

Matk : human megakaryocytes (1994)

Ctk : mouse brain (1994)

Hyl : human megakaryocytes (1994)

Ntk : mouse fetal thymus (1994)

Lsk : PHA-stimulated human peripheral T cells (1994)

Batk : rat hippocampus (1994)

>> CHK (1997)

◆ 2 Isoforms

alternative splicing

- p57 Chk (human)

- p52 Chk (mouse) lacks 40 N-terminal residues

CHK / Csk similarities

♦ Homology

- 53% amino acid identity overall
- 59 % amino acid identity within the catalytic domain

♦ Structure

- SH3 domain - SH2 domain - Catalytic domain
- no amino-terminal myristoylation site >> cytoplasmic
- no autophosphorylation site, no negative regulatory site >> constitutively active

♦ Function

- phosphorylation and downregulation of Src family PTKs

CHK → in vitro phosphorylation of Lck, Fyn, c-Src, Lyn

CHK / Csk differences

♦ Expression

Csk = ubiquitous

constitutive

CHK = restricted to neuronal and hematopoietic cells

induced - by IL-2 in NK cells

- by PHA in T lymphocytes

- by SCF in megakaryocytes

- by IL-4 and IL-13 in monocytes

♦ Activity

Csk = constitutive

CHK = ?

CHK in Breast Cancer

♦ **CHK expression**

- **no expression in human normal breast tissues (0/19)**
- **expression in human primary breast cancer tissues (70/80)**
- **co-localization CHK / HER-2 in human primary breast cancer tissues (6/6)**

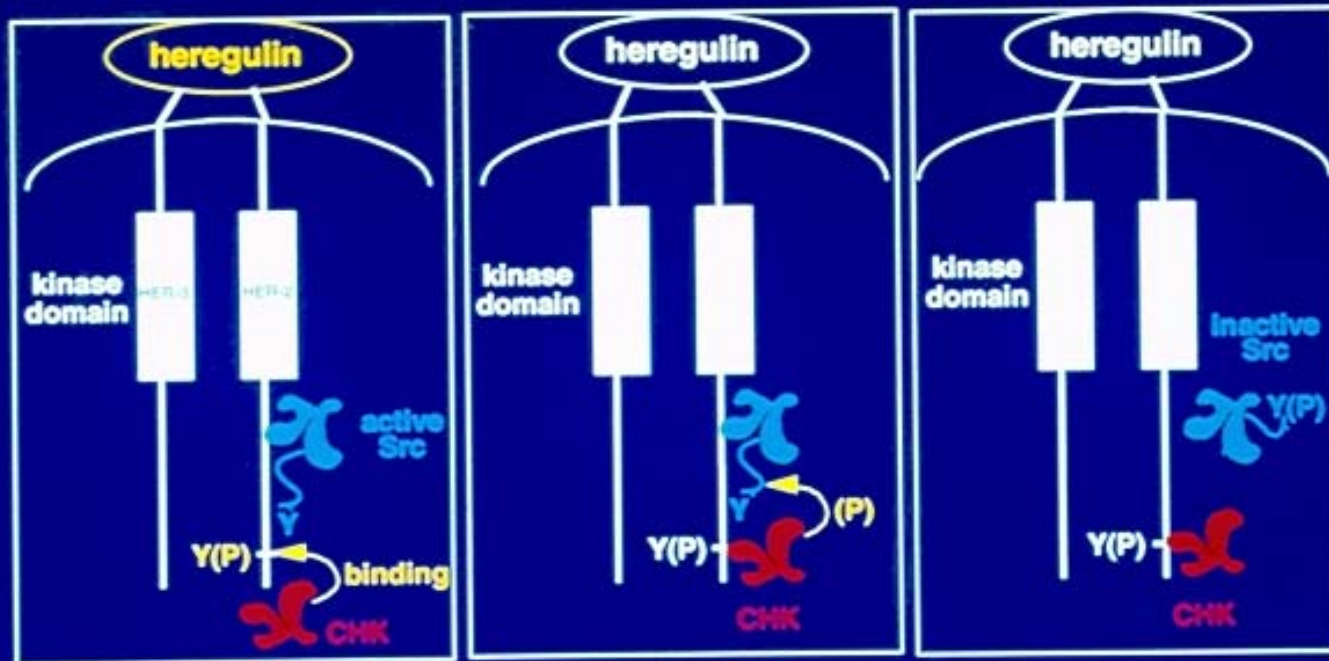
♦ **Role in HER-2/neu signaling**

upon HRG stimulation (not upon EGF stimulation)

>> binding of CHK to auto(P)-Tyr¹²⁵³ of HER-2/neu via CHK SH2 domain

>> downregulation of HER-2/neu-activated Src kinases

CHK in Breast Cancer

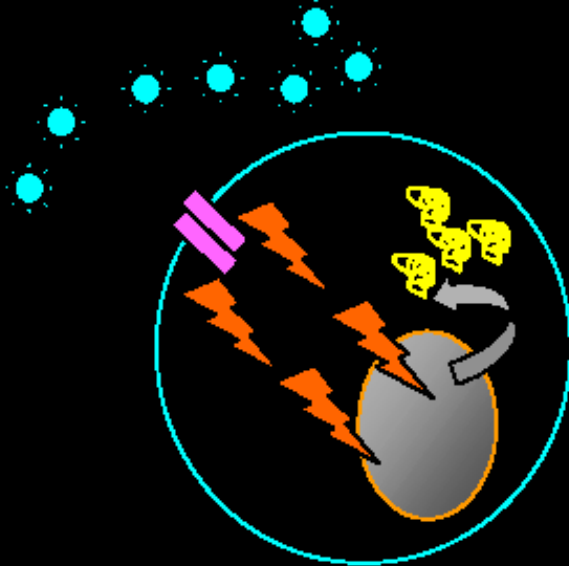


Model for the regulation of HER-2/neu-activated Src kinases by CHK

S. Zrihan-Licht et al. (1998) *J. Biol. Chem.* 273: 4065

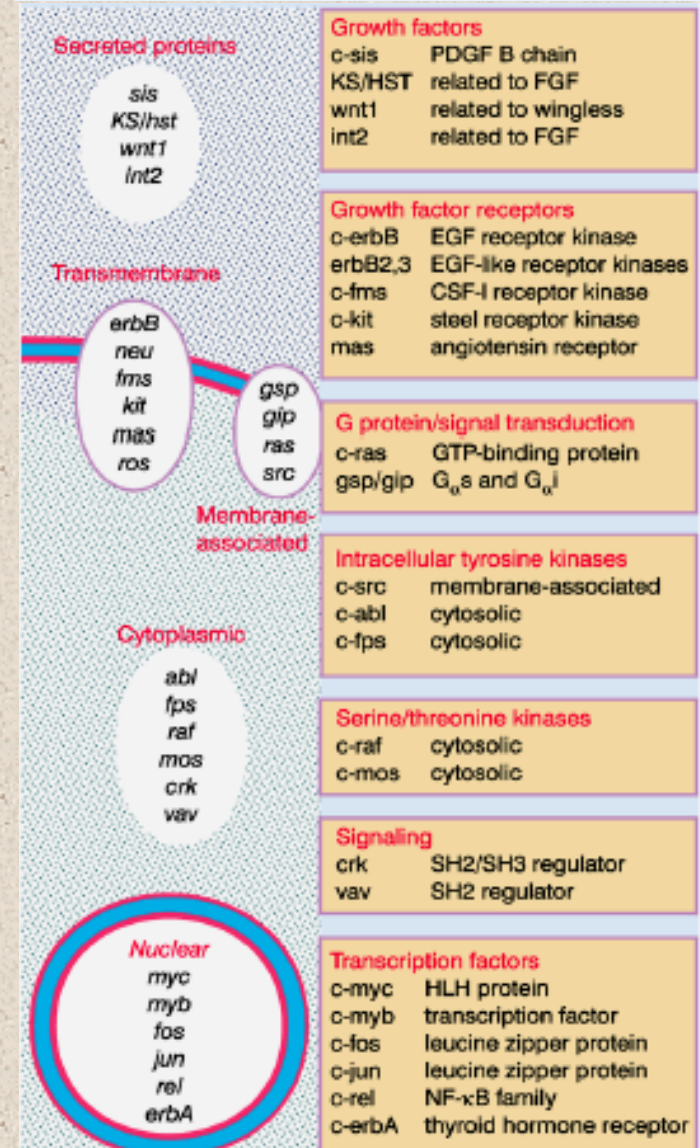
Growth Factors

e.g. c-erbB encoding EGF tyrosine kinase

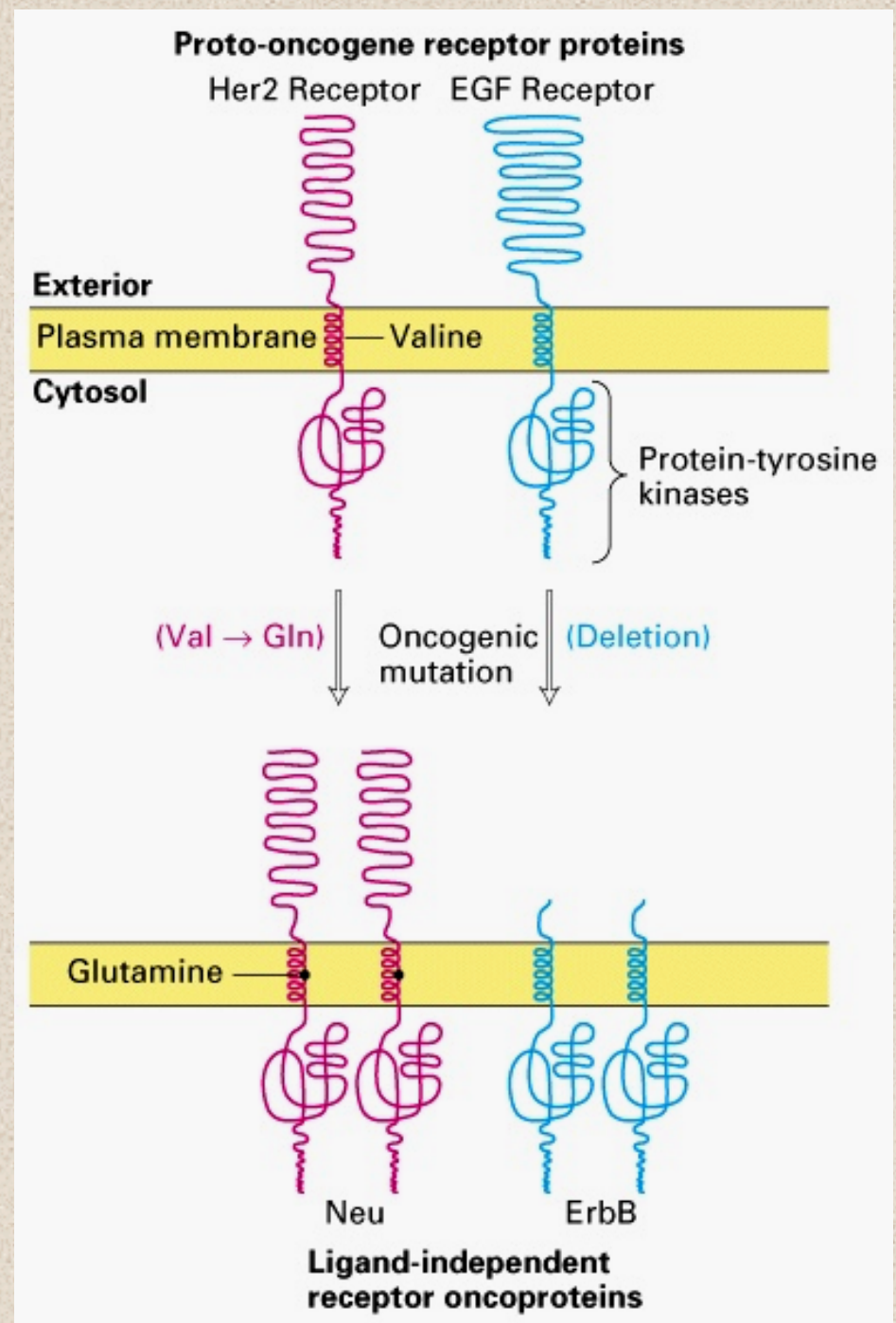


Express mutant or un-regulatable forms of receptors which are constantly active in breast, lung, bladder and brain tumours

Oncogenes may code for secreted proteins, transmembrane proteins, cytoplasmic proteins, or nuclear proteins.

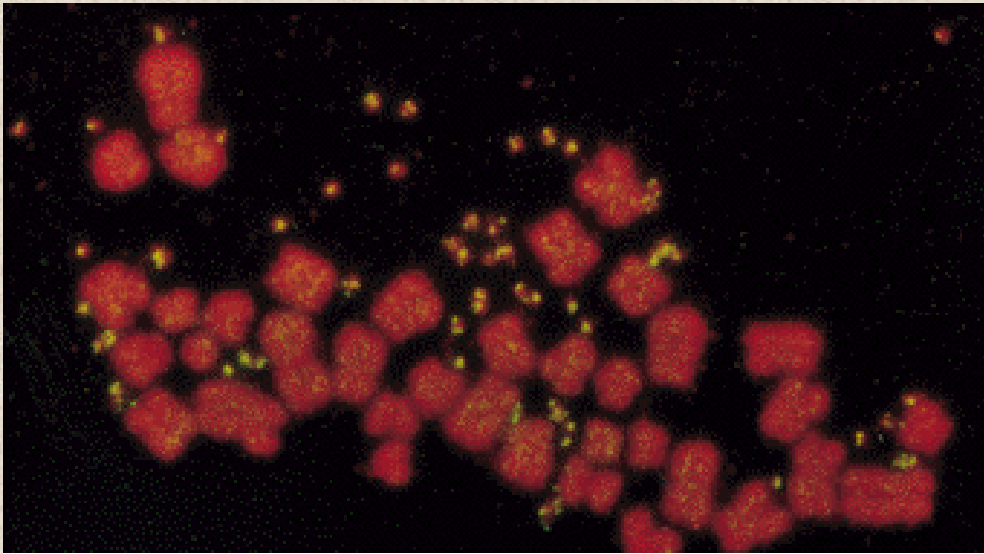


Activating mutations in RTKs takes several forms but all lead to ligand-independent dimerization and thus activation



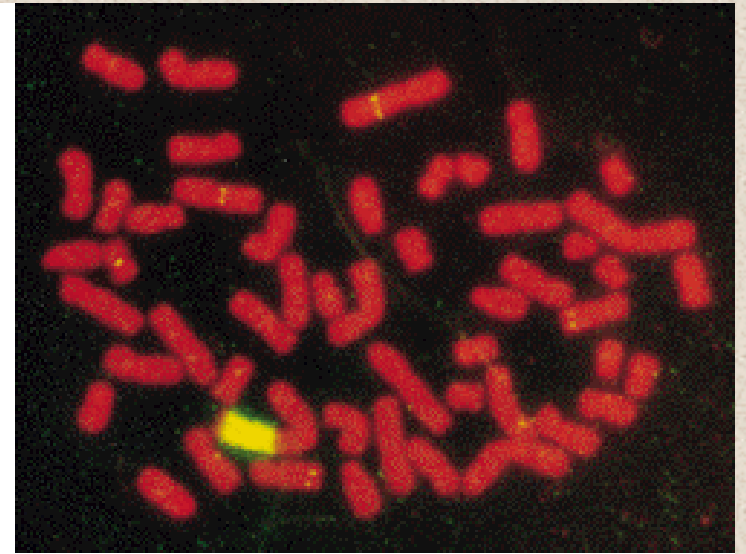
Gene Amplification is also a Common Mechanism of Inappropriate Gene Activation in Human Tumors

Double minute chromosomes



(A)

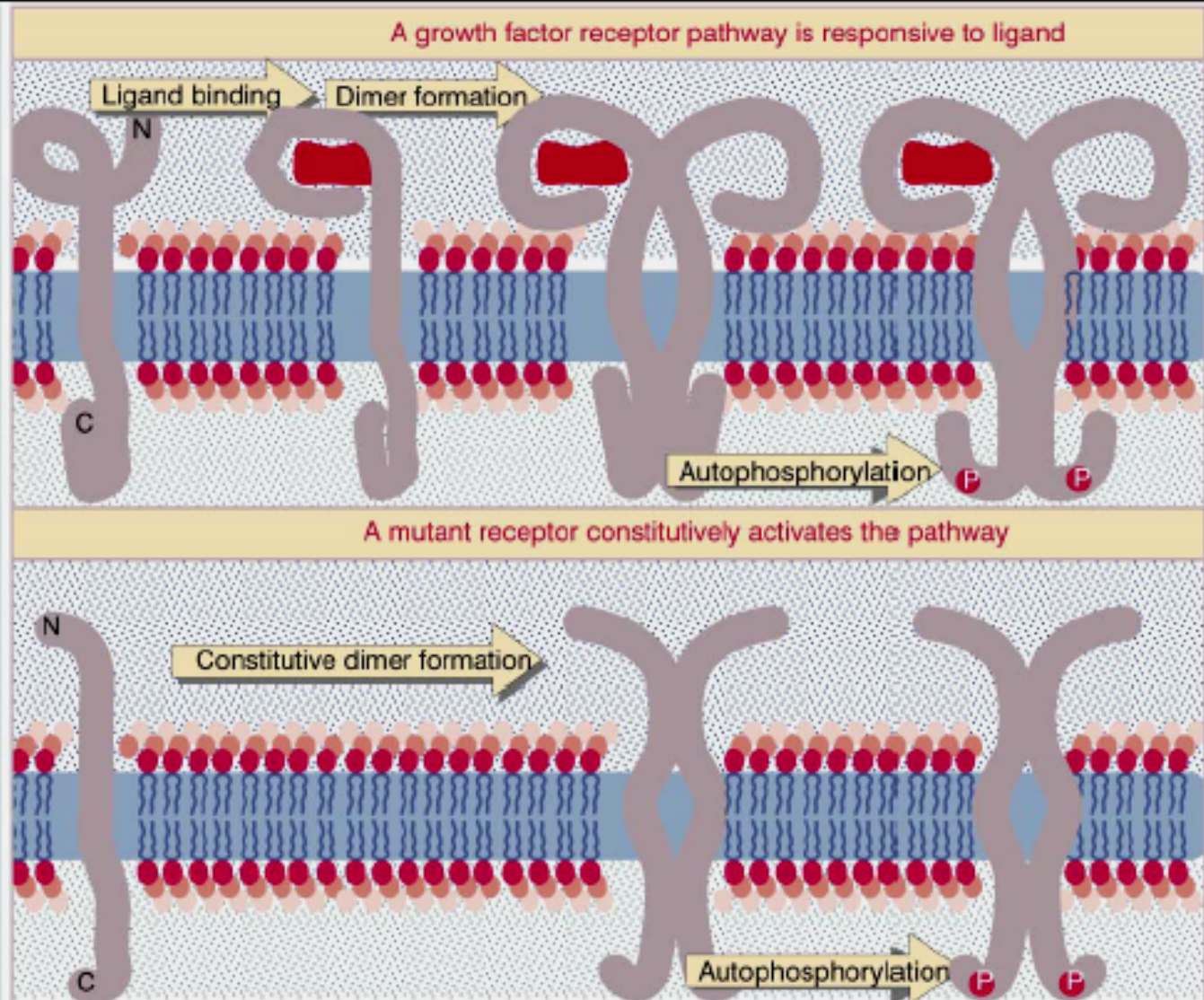
Tandem duplications



(B)

Mutant Receptor May Lack the Ligand Binding Domain (e.g. c-ErbB and Breast Cancer)

Activation of a growth factor receptor involves ligand binding, dimerization, and autophosphorylation. A truncated oncogenic receptor that lacks the ligand-binding region is constitutively active because it is not repressed by the N-terminal domain.



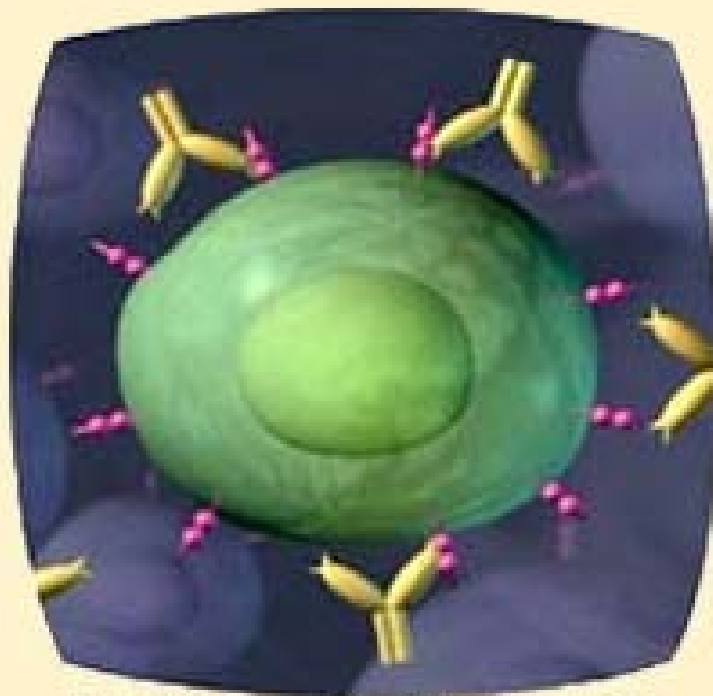
ErbB-Directed Therapies

- 1) ErbB2 is a target for cancer therapy**
- 2) A mAb that targets the extracellular domain of ErbB2 specifically inhibits in-vitro growth of ErbB2 overexpressing tumor cells.**
- 3) The humanized version of this antibody (Herceptin) has been validated in the clinic as an ErbB2-directed therapeutic approach.**
- 4) However, although all patients treated with Herceptin do have tumors exhibiting ErbB2 overexpression, not all respond to treatment.**

How Herceptin Works

What is a monoclonal antibody?

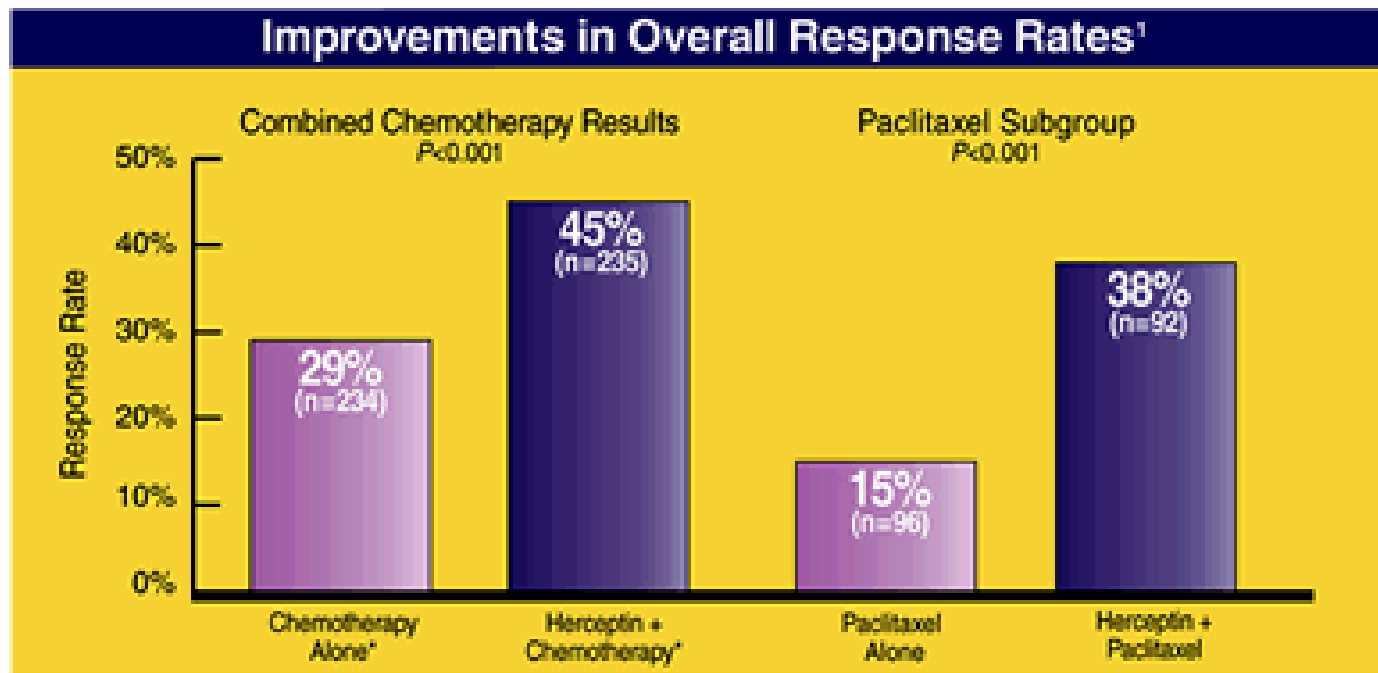
An *antibody* is a protein made by the body's own natural immune system. They are directed against foreign and infectious agents, called antigens. *Monoclonal antibodies* engineered through biotechnology are produced to provide specific anti-tumor action within the human body.



Monoclonal antibodies targeting a HER2 protein overexpressing cell

Data From Phase III Clinical Trials of Herceptin

In first-line combination use with paclitaxel in this landmark Phase III clinical trial, Herceptin achieved a 38% response rate vs 15% for paclitaxel alone ($P < 0.001$). [1] In addition, overall response rates (ORR) (complete response + partial response) increased by 55%. Forty-five percent of patients with Herceptin plus chemotherapy responded, vs 29% of patients receiving chemotherapy alone ($P < 0.001$). [1]

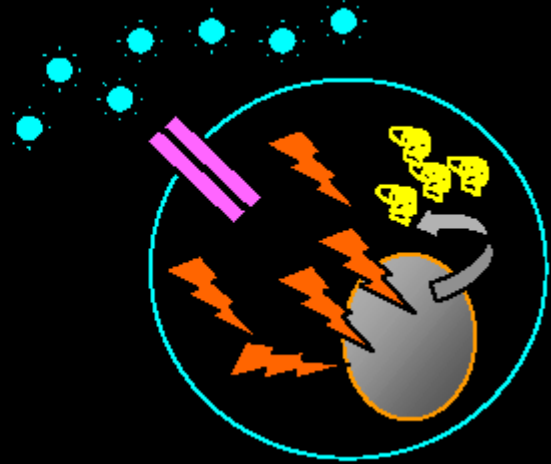


*Chemotherapy = either doxorubicin or epirubicin plus cyclophosphamide, or paclitaxel.

Intracellular Transducer

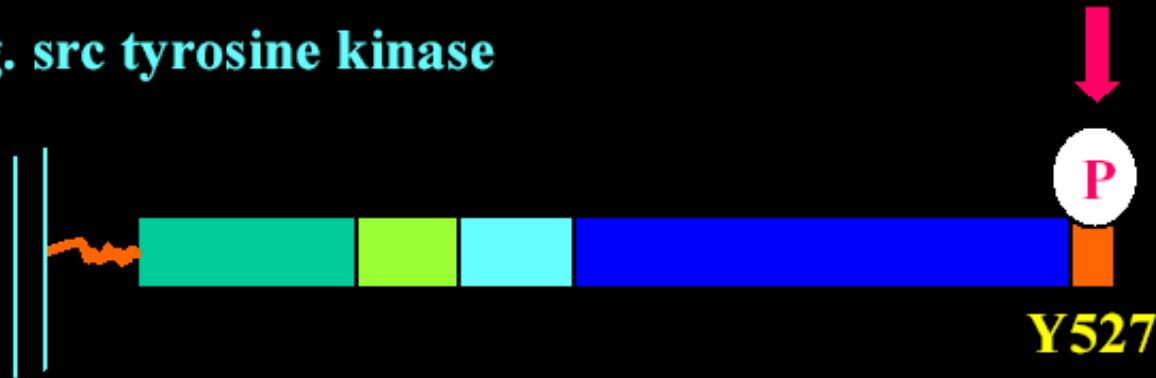
Member of the expanding family of closely related tyrosine kinases
Functions in relaying information to the nucleus
Non-receptor tyrosine kinase with at least 9 members expressed in a variety of cells.

Loss of regulation of c-src is closely associated with colon carcinomas



Intracellular Transducer

e.g. src tyrosine kinase



Multi-domain protein

Myristate anchors protein to plasma membrane

Unique domain

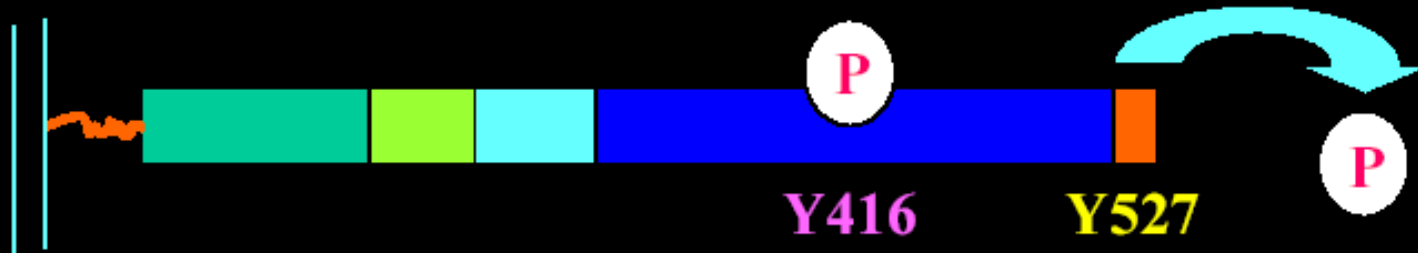
SH-2 domain for tyrosine phosphate binding

SH-3 domain for protein:protein interaction

Catalytic domain

Regulatory domain basally phosphorylated at Y527

Model for the activation of c-src in response to growth factors



For activation of src, the protein requires:

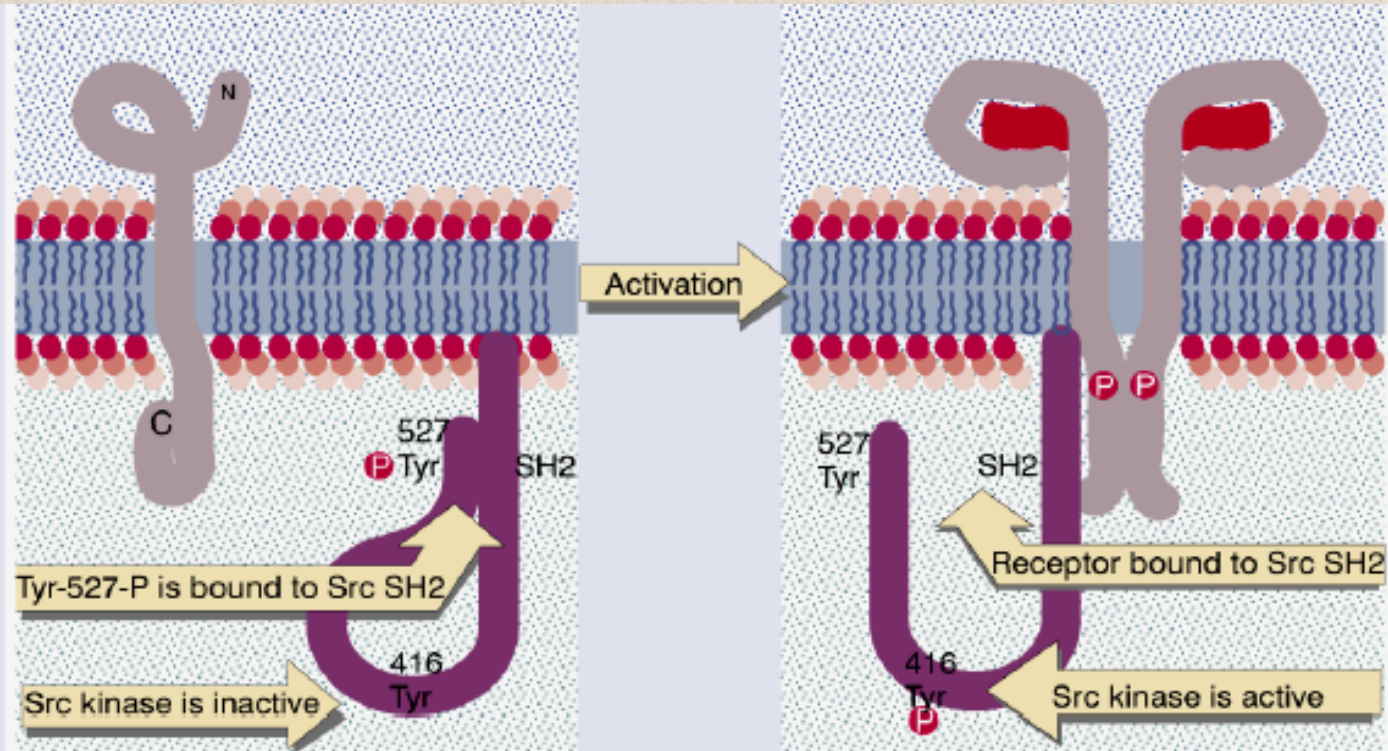
Localisation to the plasma membrane

De-phosphorylation of tyrosine 527

Autophosphorylation of tyrosine 416

Model for the activation of c-src in response to growth factors

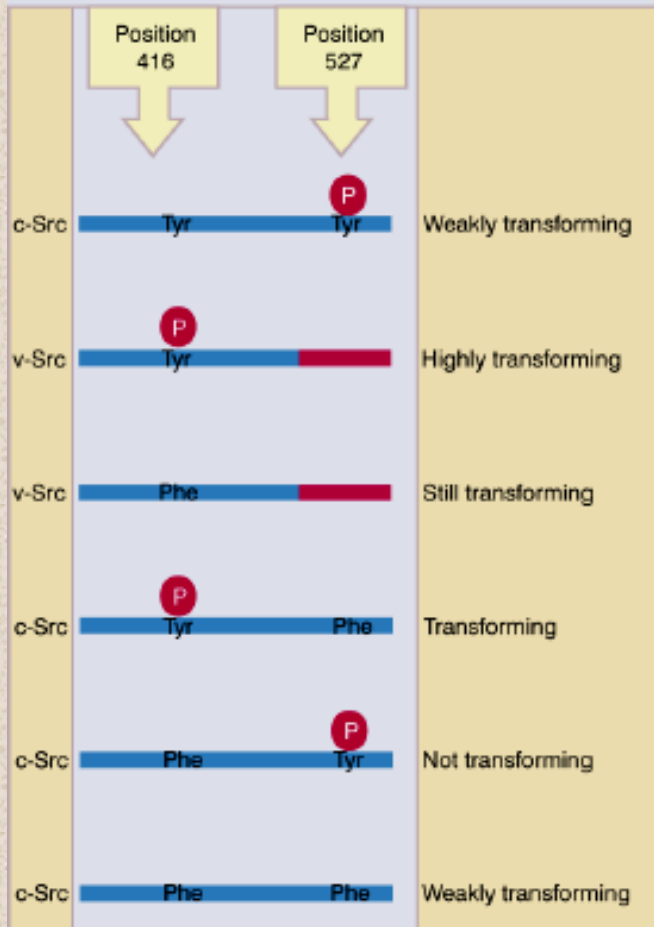
When a receptor tyrosine kinase is activated, autophosphorylation generates a binding site for the Src SH2 domain, Tyr-527 is released and dephosphorylated, Tyr-416 becomes phosphorylated, and Src kinase is activated.



Y527 interacts with src SH-2 domain in a low-affinity interaction
Growth factor receptor activation creates a high affinity binding site
Src associates with the activated receptor and changes conformation
Y527 is de-phosphorylated by phosphatases recruited to the receptor
Y416 is phosphorylated and src is activated

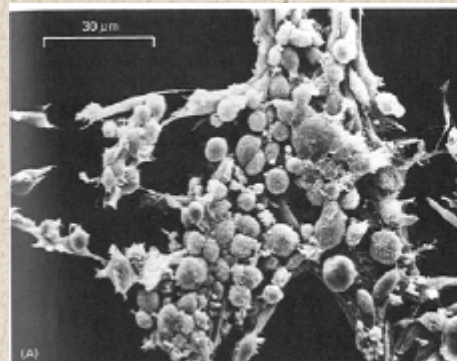
Tyrosine phosphorylation is important for the activation of c-src

Two tyrosine residues are targets for phosphorylation in Src proteins. Phosphorylation at Tyr-527 of c-Src suppresses autophosphorylation at Tyr-416, which is associated with transforming activity. Only Tyr-416 is present in v-Src. Transforming potential of c-Src may be activated by removing Tyr-527 or repressed by removing Tyr-416.

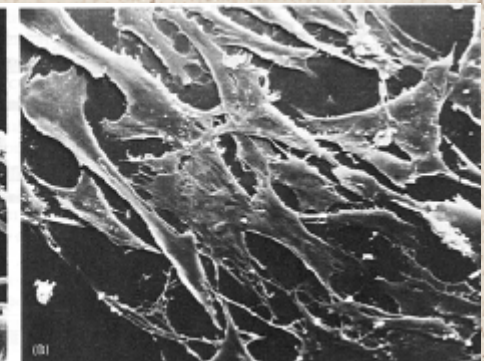


Y527P suppresses phosphorylation at Y416

v-src has lost the last 19 amino acids so that regulation *via* Y527 is lost



v-Src

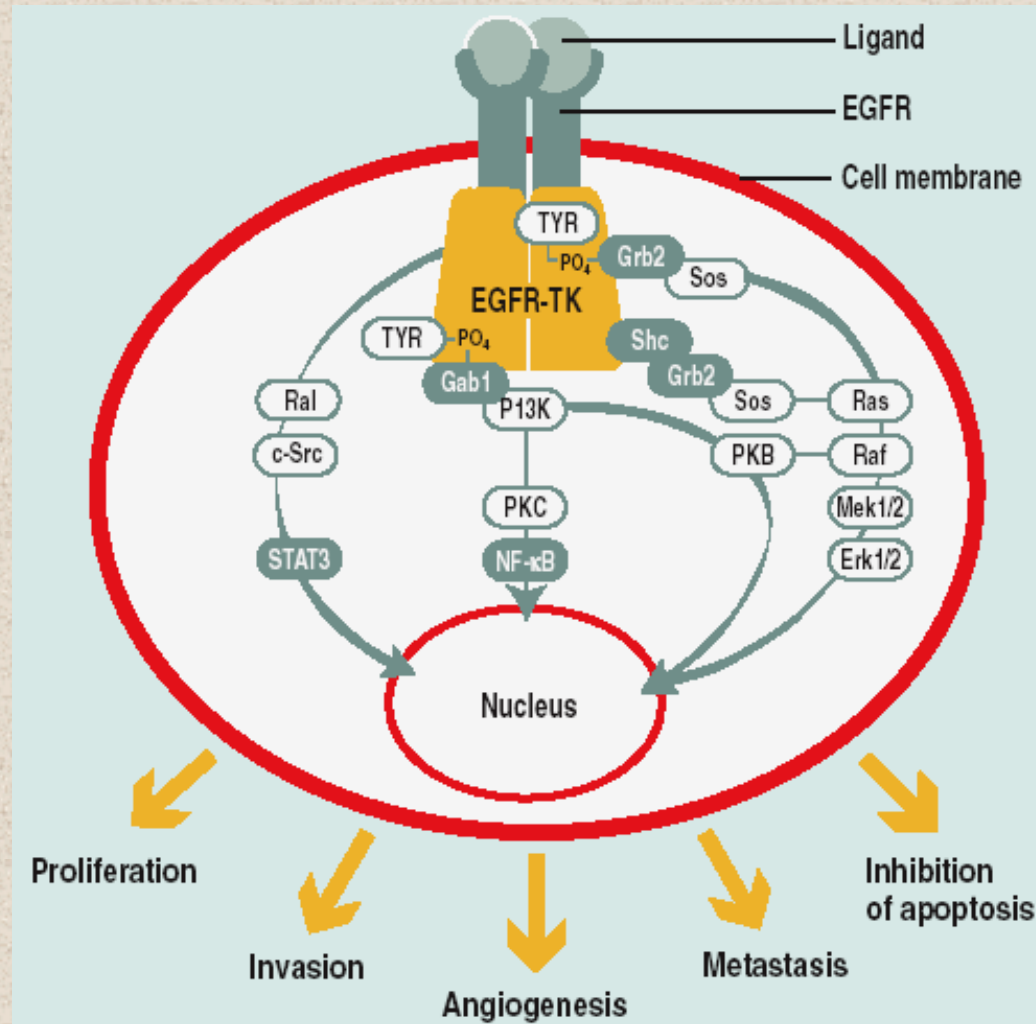


c-Src

Tumors Showing High EGFR Expression

● NSCLC	40-80%
● Prostate	40-80%
● Gastric	33-74%
● Breast	14-91%
● Head & Neck	90-100%
● Colorectal	25-77%
● Pancreatic	30-50%
● Ovarian	35-70%

Signal Transduction Through EGFR-TK



Ligand binding induces receptor dimerization and autophosphorylation, creating docking sites for adaptor molecules leading to the activation of downstream effector molecules. A variety of signaling pathways results in pleiotropic effects, including cell proliferation, control of the cell cycle, regulation of apoptosis and survival, and alterations in cell migration and invasiveness.

EGFR Expression in Human Tumors

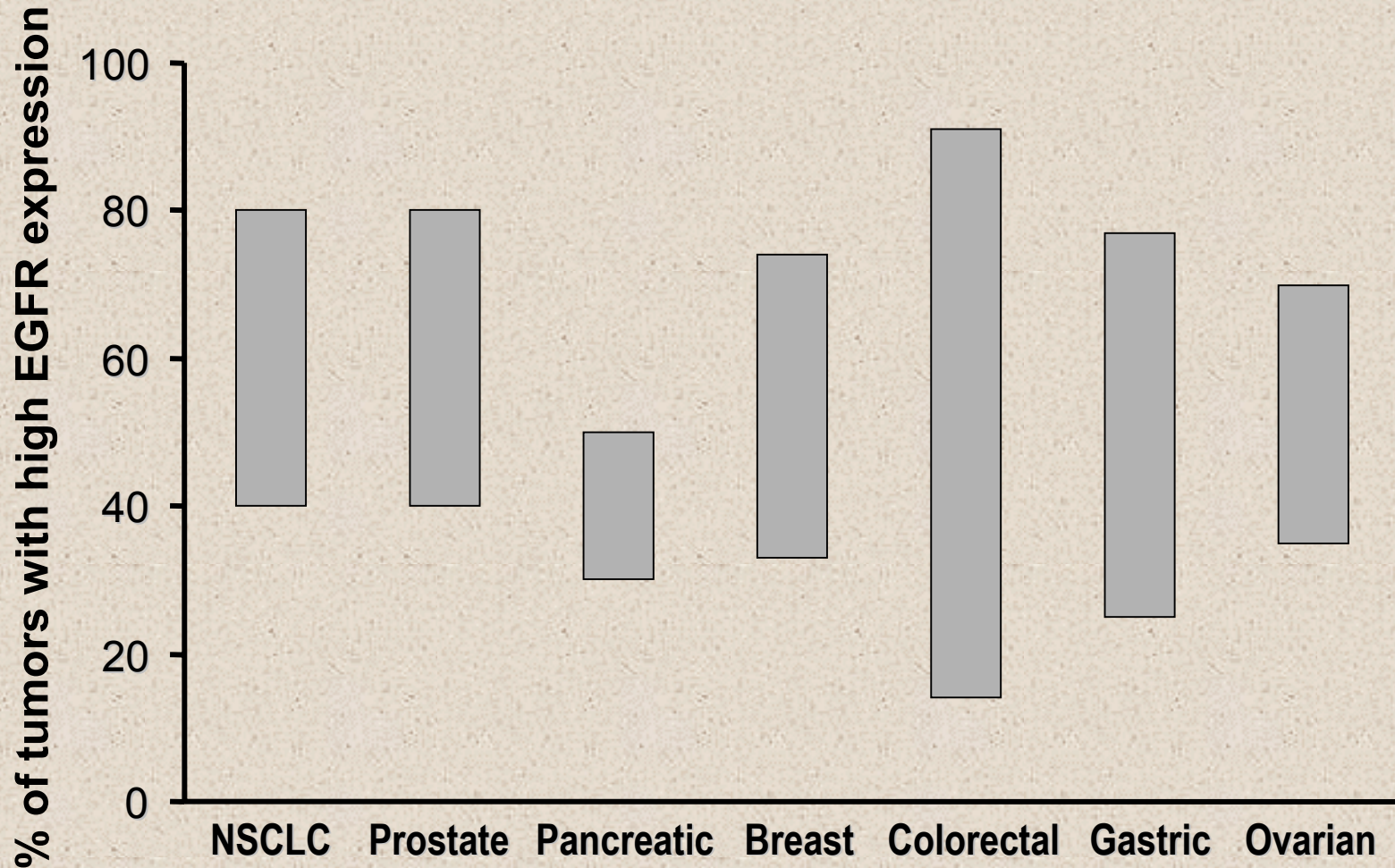
High expression is generally associated with

- Invasion
- Metastasis
- Late-stage disease
- Chemotherapy resistance
- Hormonal therapy resistance
- Poor outcome

EGFR and Cancer

- 1) EGFR is overexpressed or aberrantly activated in the most common solid tumors including non-small cell lung cancer (NSCLC) and cancers of the breast, prostate and colon.**
- 2) There are several small molecule EGFR-TK inhibitors approved in clinical development (Table 2).**
- 3) Randomized clinical trials of the EGFR-TK inhibitor gefitinib have demonstrated clinical benefits in patients with advanced non-small cell lung cancer (NSCLC).**
- 4) As TK inhibitors become available for clinical use, new challenges include predicting which patients are most likely to respond to these targeted TK inhibitors.**

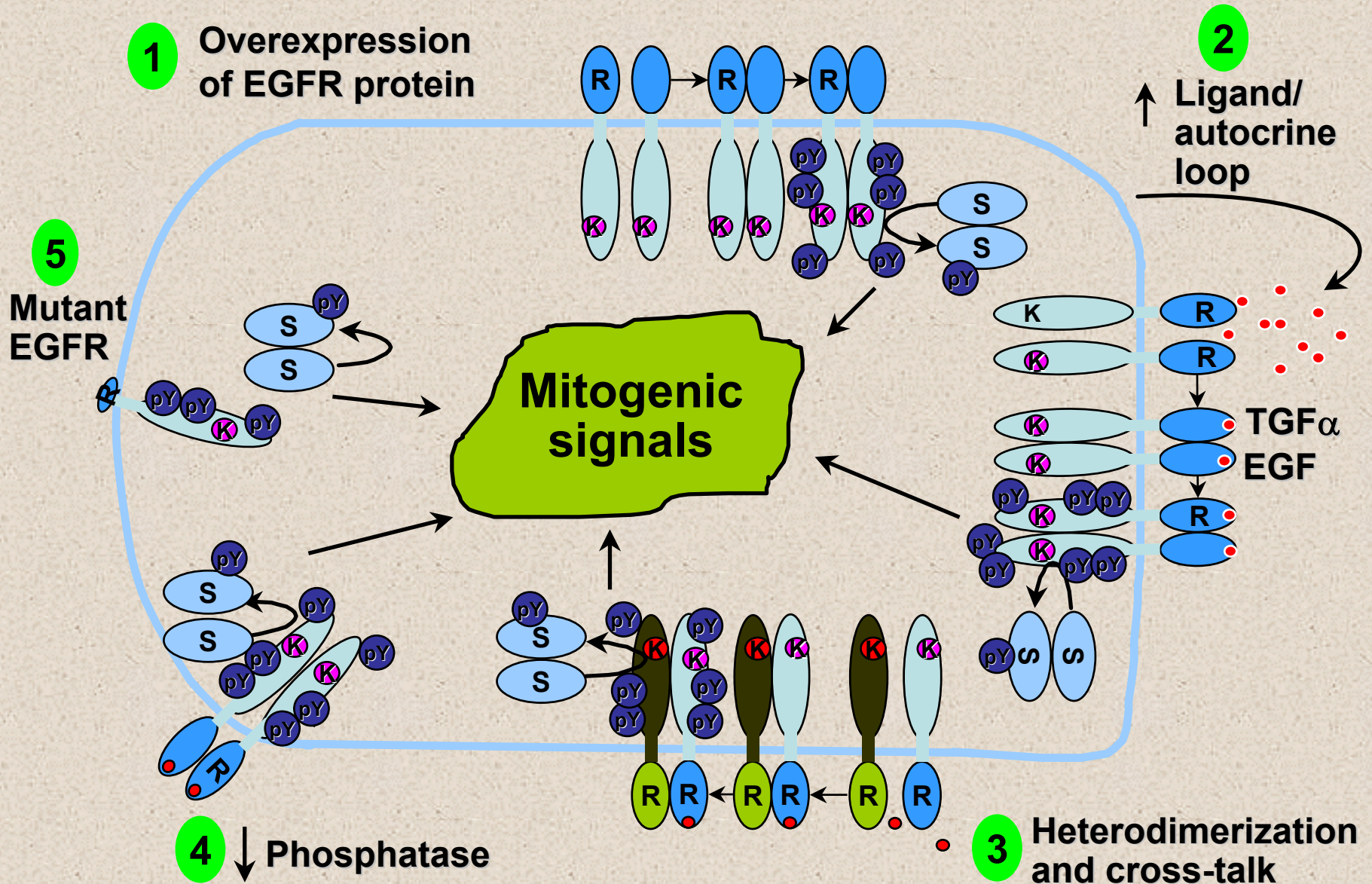
EGFR Expression or Overexpression in Human Tumors



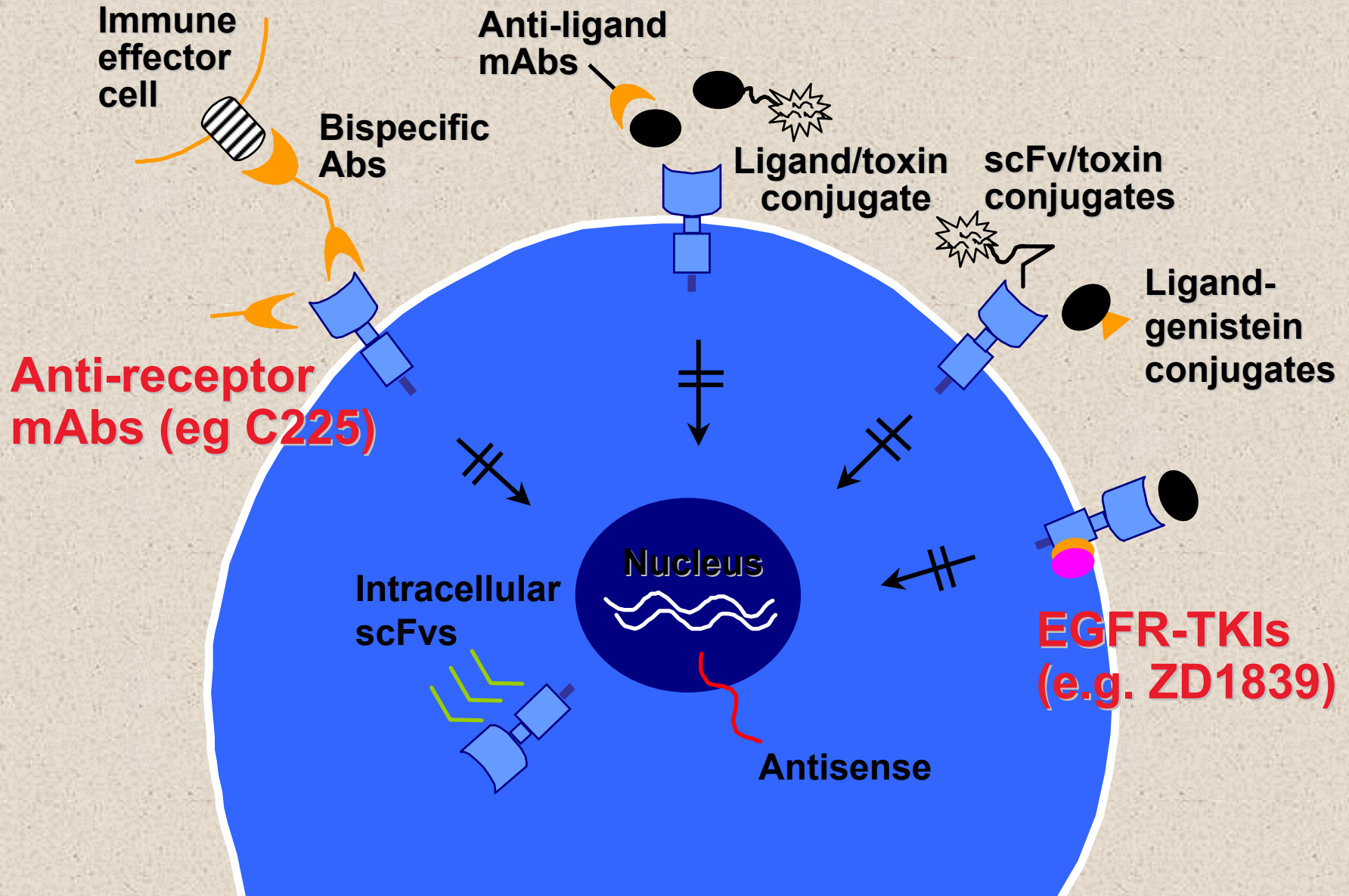
Measurement of EGFR Expression

- **Immunohistochemistry**
 - **EGFR autophosphorylation**
- **RT-PCR, ELISA, labelled ligand**
- **Downstream markers of EGF signaling**
 - **proliferation markers**
 - **maturation markers**

Mechanisms of Increased EGFR Activation



Strategies for EGF Signaling Inhibition

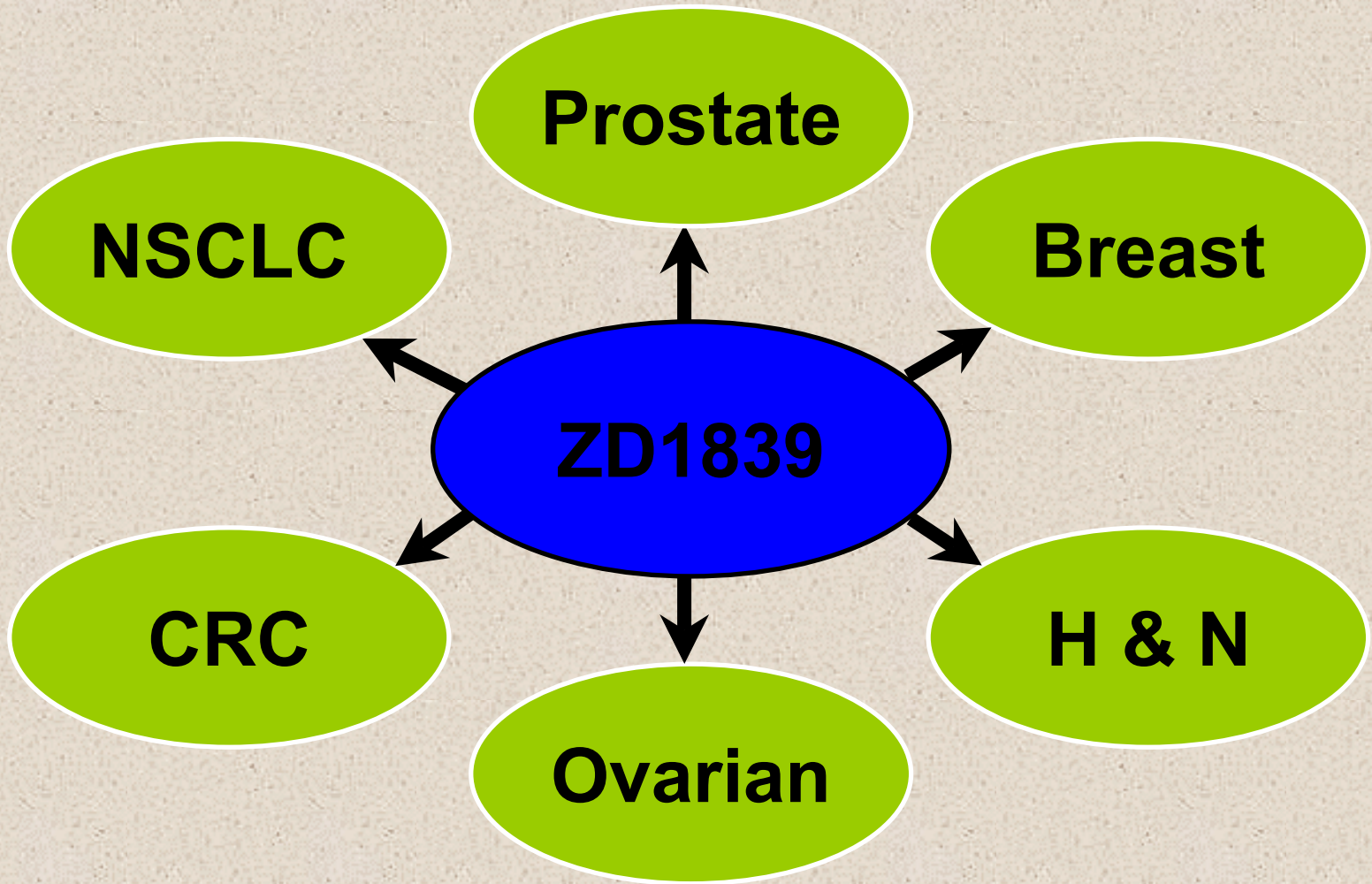


Protein Kinase Inhibitors

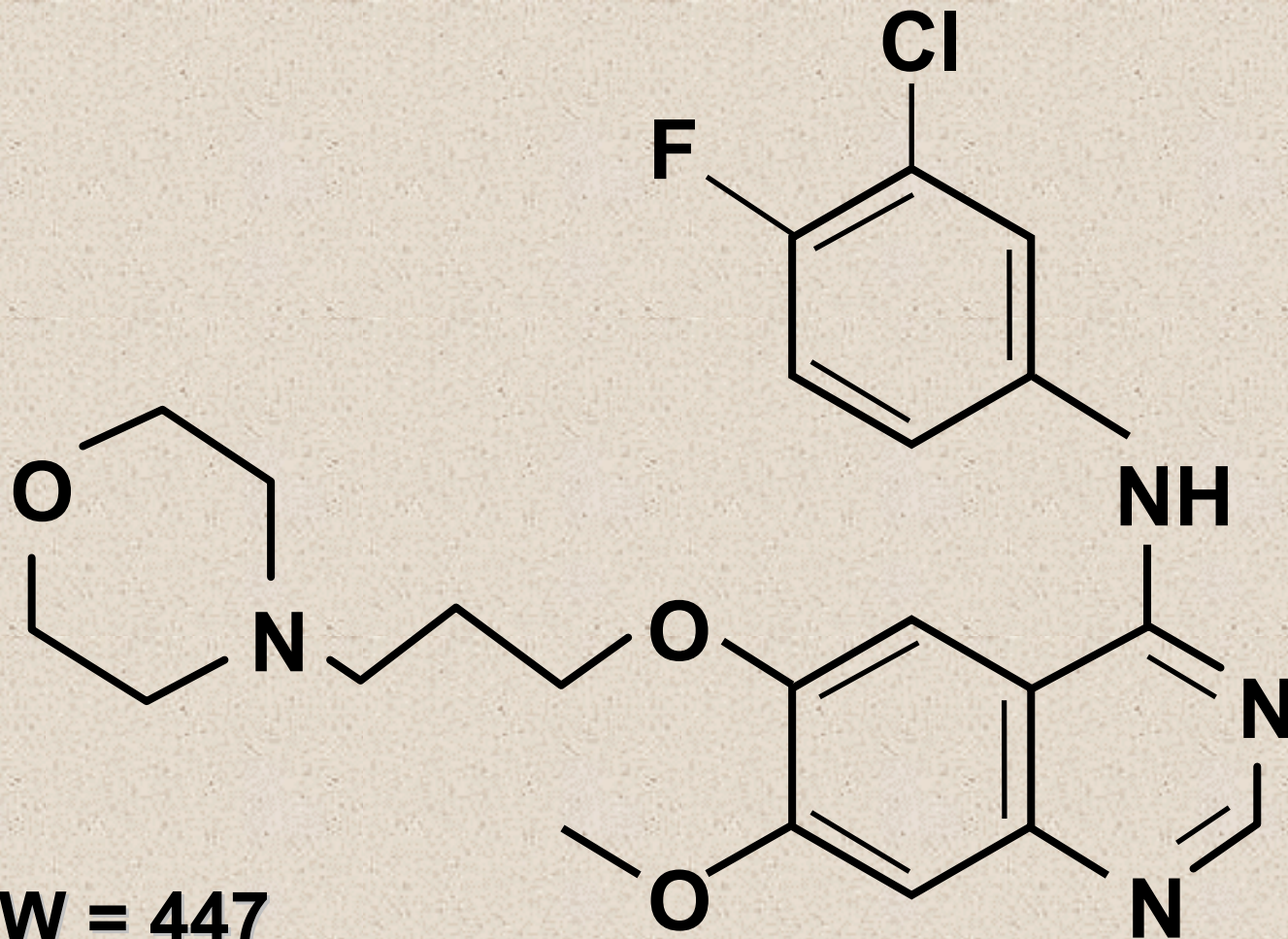
- 1) Protein kinases are targets for treatment of a number of diseases.**
- 2) Structures of some PTKs have provided insights into drug design aimed at the inactive or active form of the kinase targeted and mechanisms of inhibition.**
- 3) The specific PTKs include: EGFR, VEGFR, and FGFR.**
- 4) Nonreceptor tyrosine kinase Bcr-Abl.**
- 5) Cancer therapeutic agents include herceptin and Gleevec.**
- 6) Among the serine-threonine kinases, p38, Rho-kinase, and cyclin-dependent kinase have been targeted for inflammation and cancer**

EGFR-TK Inhibitors Approved or in Development

Agent	Molecular target	Tumor types	Development
gefitinib	EGFR-TK selective	NSCLC, breast, head & neck	Approved (NSCLC)
OSI-774	EGFR-TK selective	NSCLC, head & neck, ovarian	Phase II, III
CI-1033	Pan-ErbB-TK	SCLC, solid tumors	Phase I
PKI-166	EGFR-TK selective	Solid tumors	Phase I
GW-572016	Pan-ErbB-TK	Healthy volunteers	Phase I

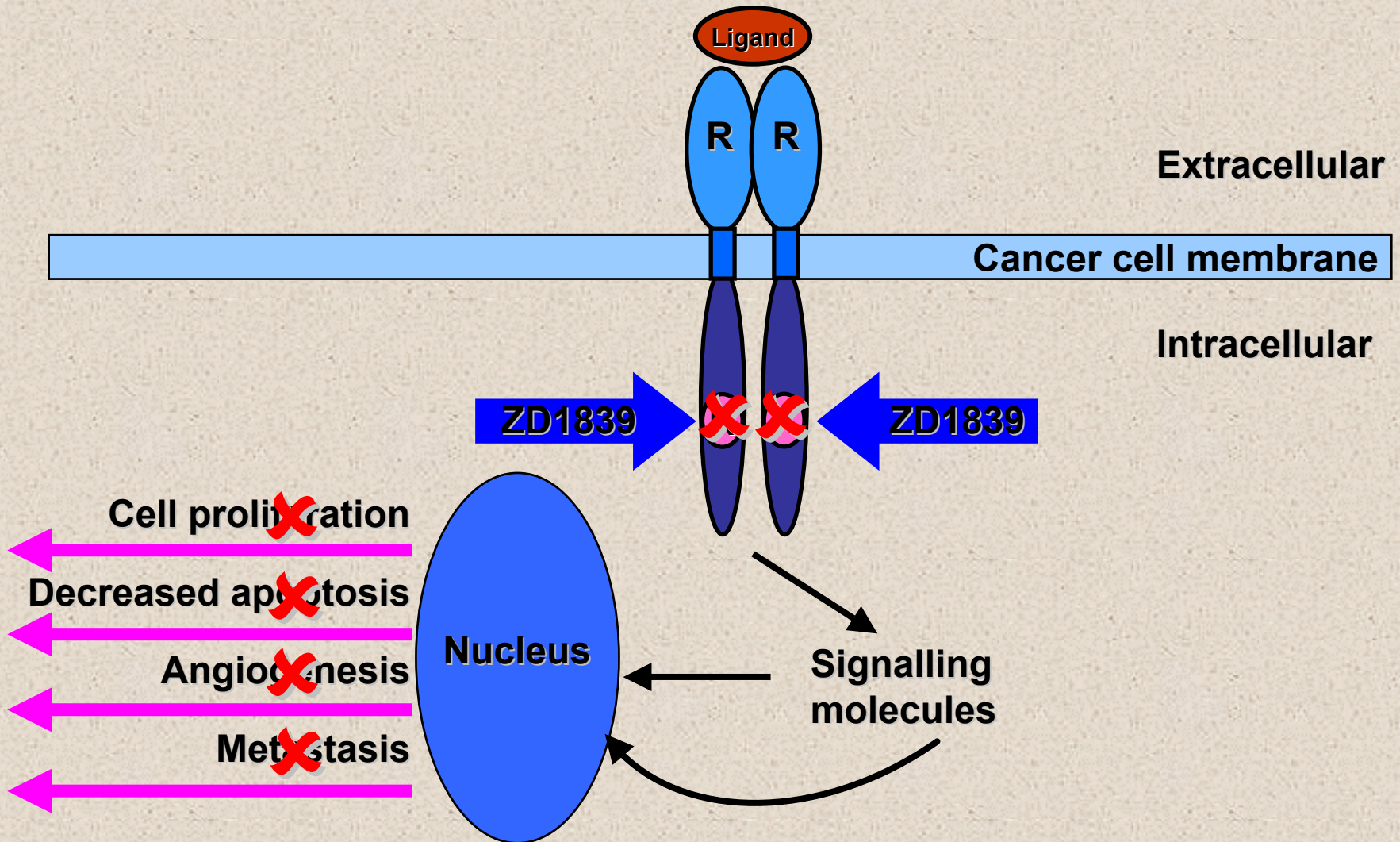


Structure of ZD1839 ('Iressa'), an EGFR-Tyrosine Kinase Inhibitor



MW = 447

ZD1839 Mode of Action

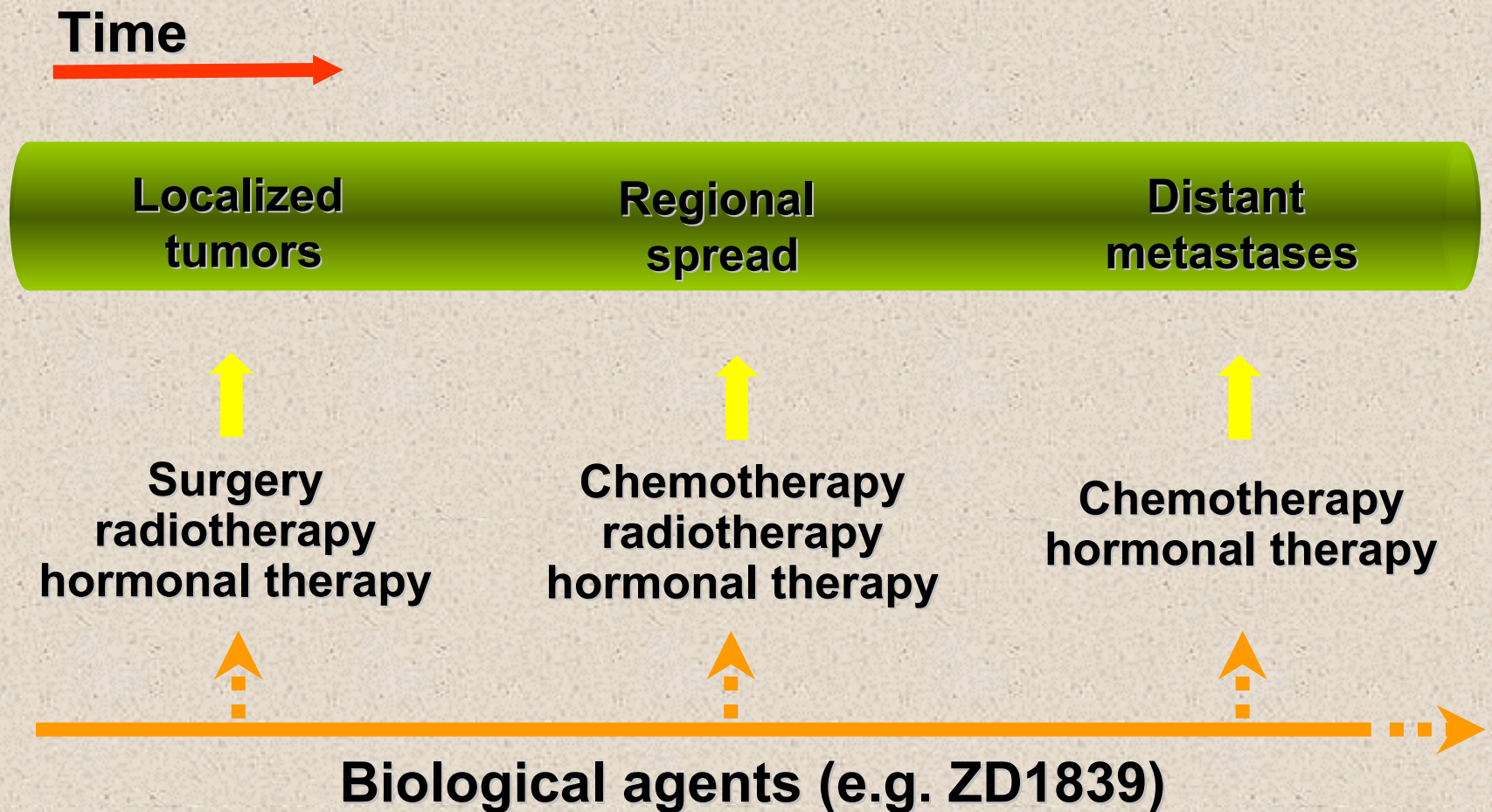


ZD1839: a Selective and Potent Inhibitor of EGFR Tyrosine Kinase

- *In vitro* cell growth inhibition $IC_{50} = 0.08 \mu\text{M}$
- Selective *in vitro* tyrosine kinase enzyme inhibition

Kinase	ZD1839 IC_{50} (μM)
Tyrosine kinases	
erbB1 (EGFR)	0.033
erbB2	1.2-3.7
KDR	3.7-33
c-flt	>100
Serine/threonine kinases	
PKC	>100
raf	~100
MEK-1	23-30
ERK 2	>100

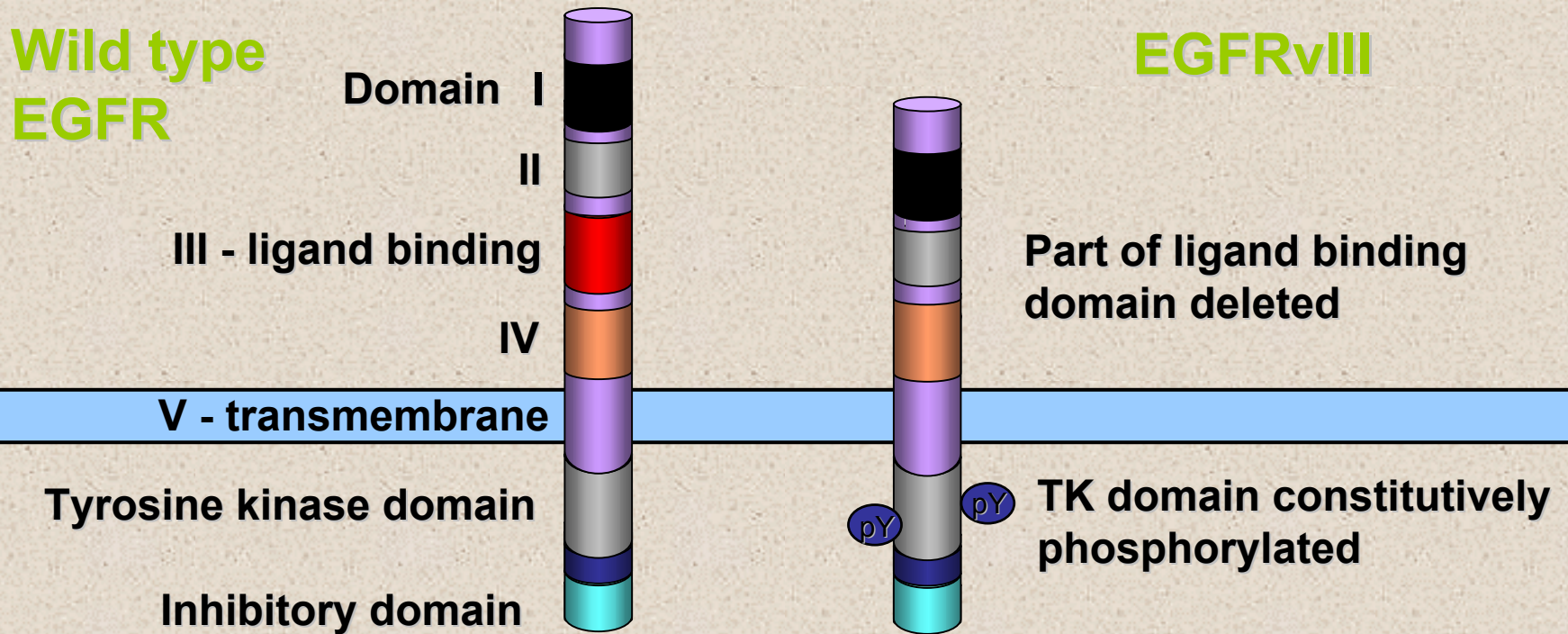
Potential Treatment Options for Solid Tumors



ZD1839 Inhibitor

Available data from preclinical, pharmacokinetic, and pharmacodynamic studies provide strong support for clinical trials of ZD1839, and other therapies targeting EGFR-TK, in a range of cancers.

Type III EGFR Mutation



- TK sites constitutively active
- May provide a tumor-cell-specific target for selective small-molecule tyrosine kinase inhibitors

EGFR: Mutation and Modes of Resistance to Therapy

- **Type III EGFR mutation (EGFRvIII) is common in solid tumors**
 - breast (77%), ovarian (75%), NSCLC (16%)
- **EGFRvIII is often amplified in genome, resulting in overexpression**
- **EGFRvIII can also arise by differential mRNA splicing**
- **EGFRvIII heterodimer formation may have significant effects on EGFR signaling**
- **Mutation may lead to reduced activity of EGFR inhibitors**

Summary

- **Protein kinases are targets for treatment of a number of diseases.**
- **PTKs are important for cell development, differentiation and proliferation.**
- **PTKs are important components of signaling cascades, via protein networks that are subjected to multiple positive and negative feedback mechanisms.**
- **New development will be in the measurement of the kinetics of cellular reactions in the context of living cells and live animals, to gain insights into signaling networks and in-vivo cell signaling.**