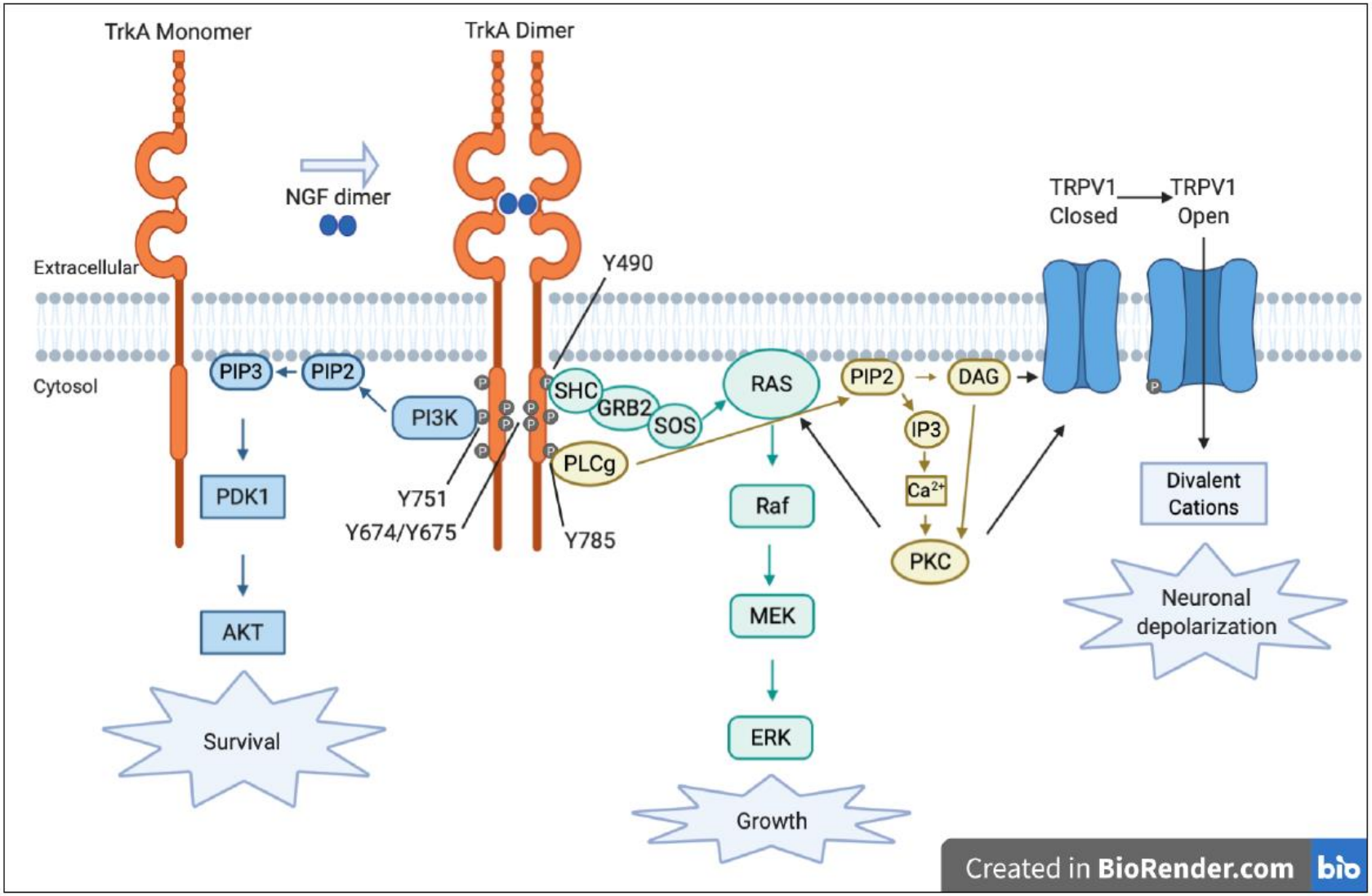
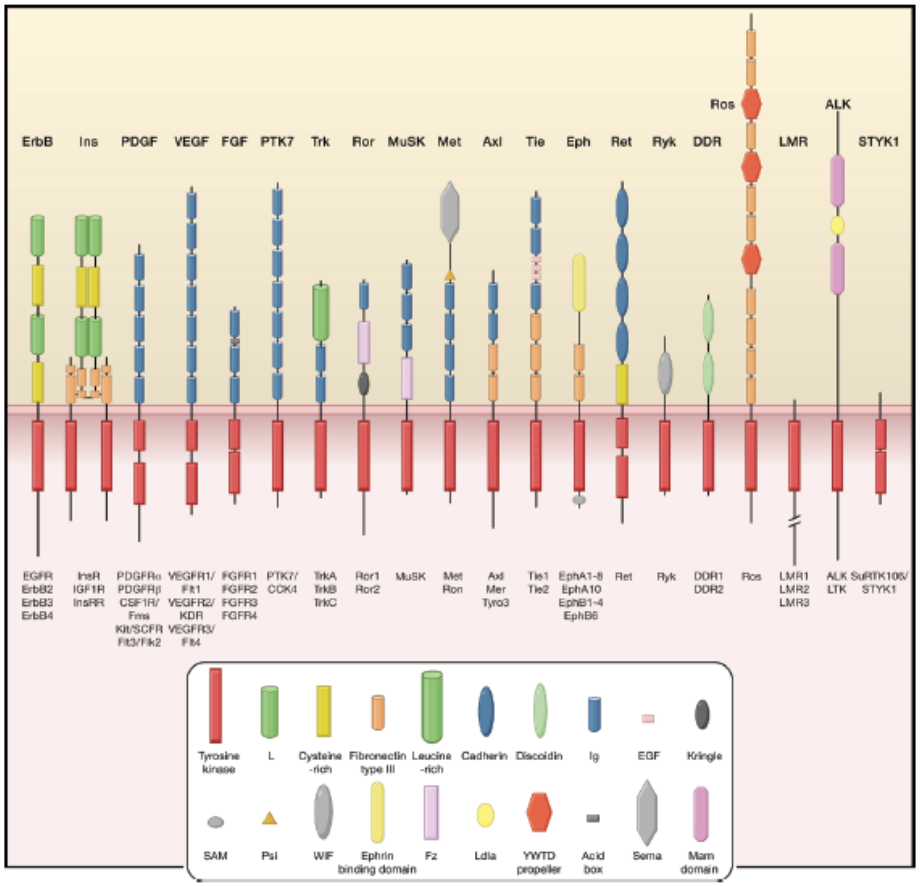
Tyrosine kinases

Receptor Tyrosine Kinases



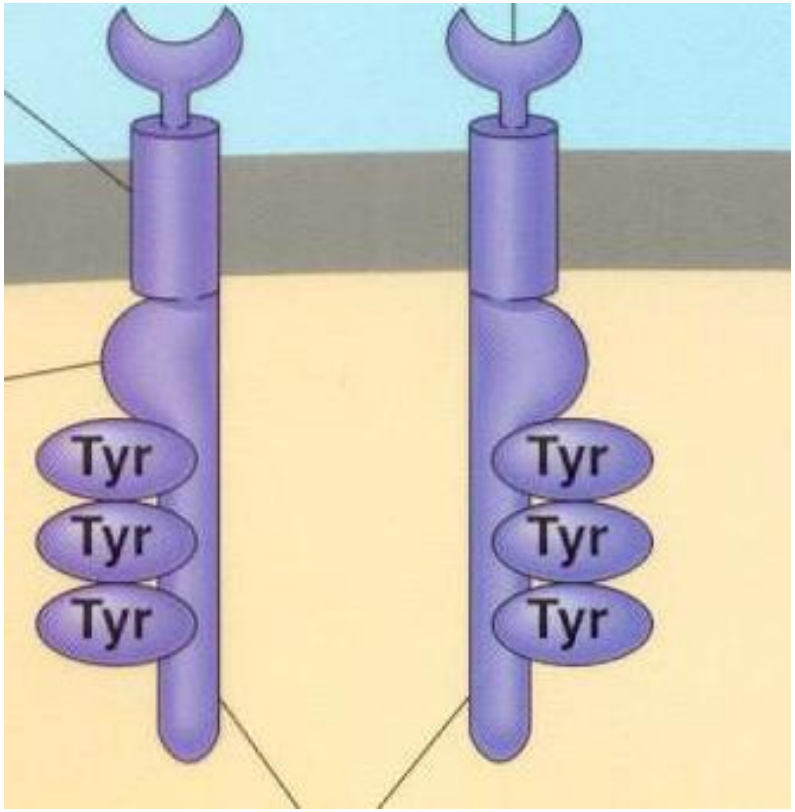
Humans have 58 known RTKs,20 subfamilies

"Receptor tyrosine kinases are a subclass of cell-surface growth-factor receptors with an intrinsic, ligand-controlled tyrosine-kinase activity".



Overall structure of RTK

Ligand binding domains in the extracellular region



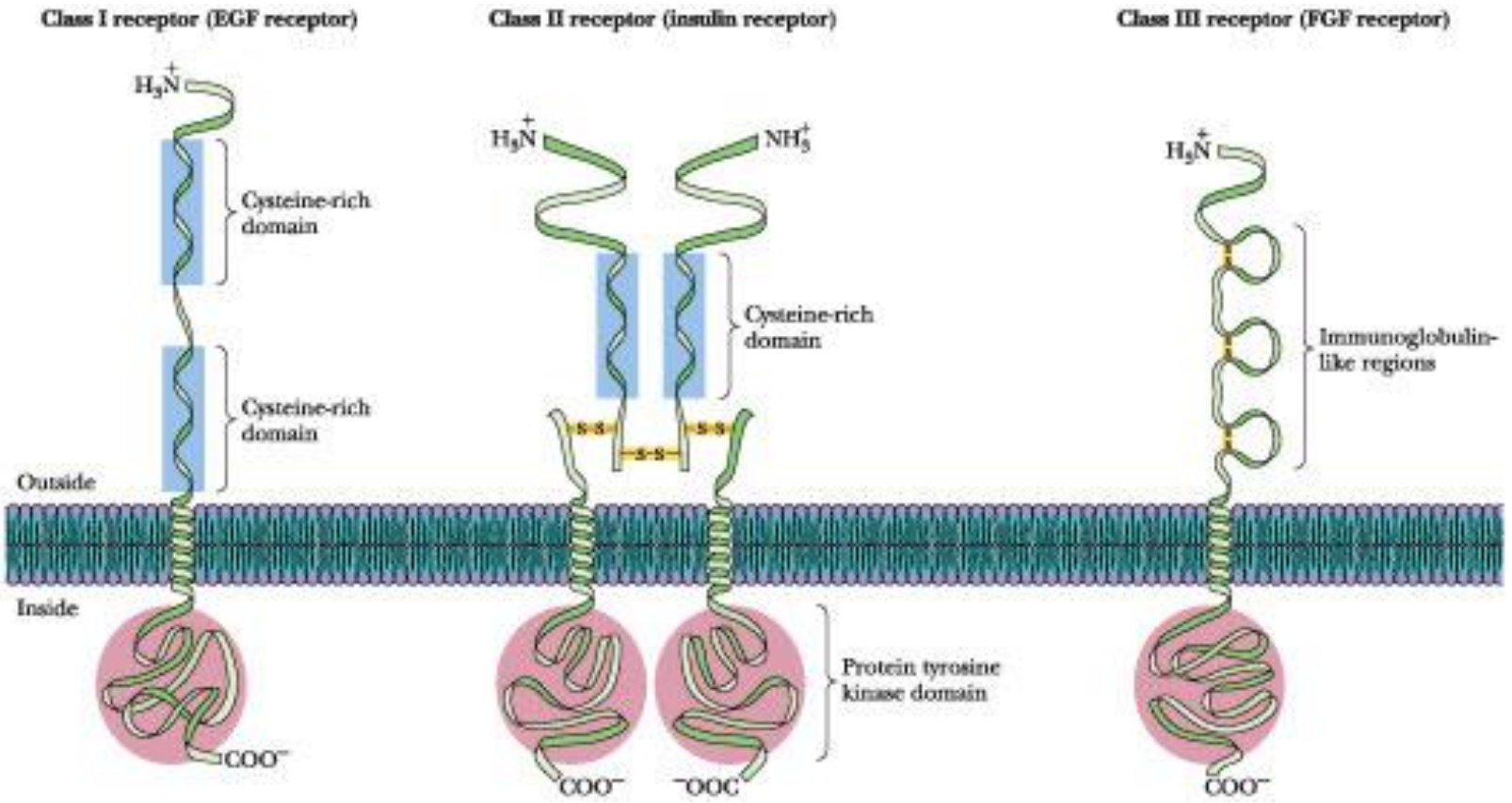
juxtamembrane regulatory regions

single transmembrane helix

cytoplasmic region proteintyrosine kinase (TK)

carboxy (C-) terminal regulatory regions

Diversity of RTK



FUNCTIONS

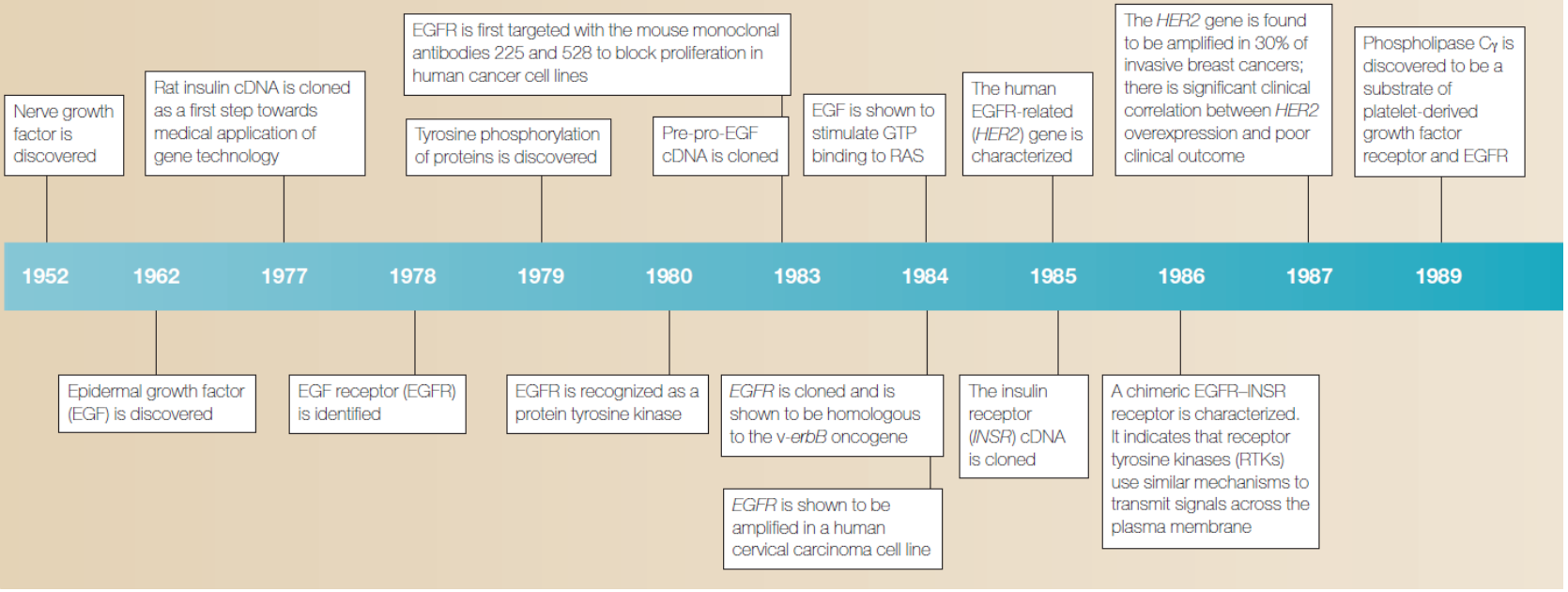
- Have a key role in cellular processes, such as proliferation, migration, metabolism, differentiation and survival, as well as those that regulate intercellular communication during development.

-They regulate diverse functions in normal cells and have a crucial role in oncogenesis

-RTK activity in resting, normal cells is tightly controlled. When they are mutated or structurally altered, however, RTKs become potent oncoproteins.

Timeline

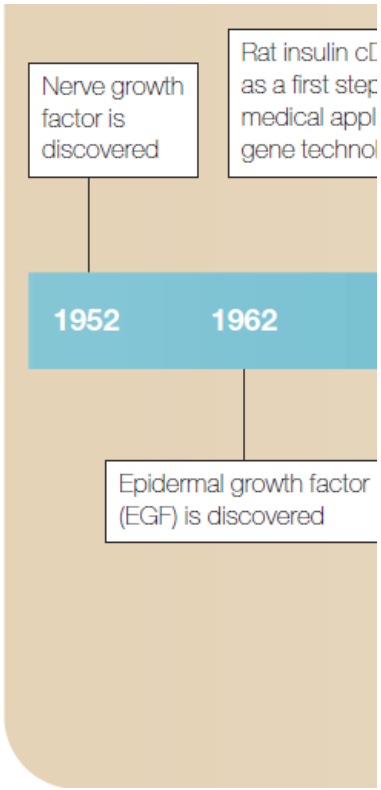
Timeline 1 | **Breakthrough discoveries on RTK signal transduction and RTK-based cancer therapy**



History, discovery of growth factors

Rita Levi-Montalcini in the laboratory of Viktor Hamburger discovered a secreted factor in mouse tumour cells that potently promoted neurite outgrowth in chicken embryos1

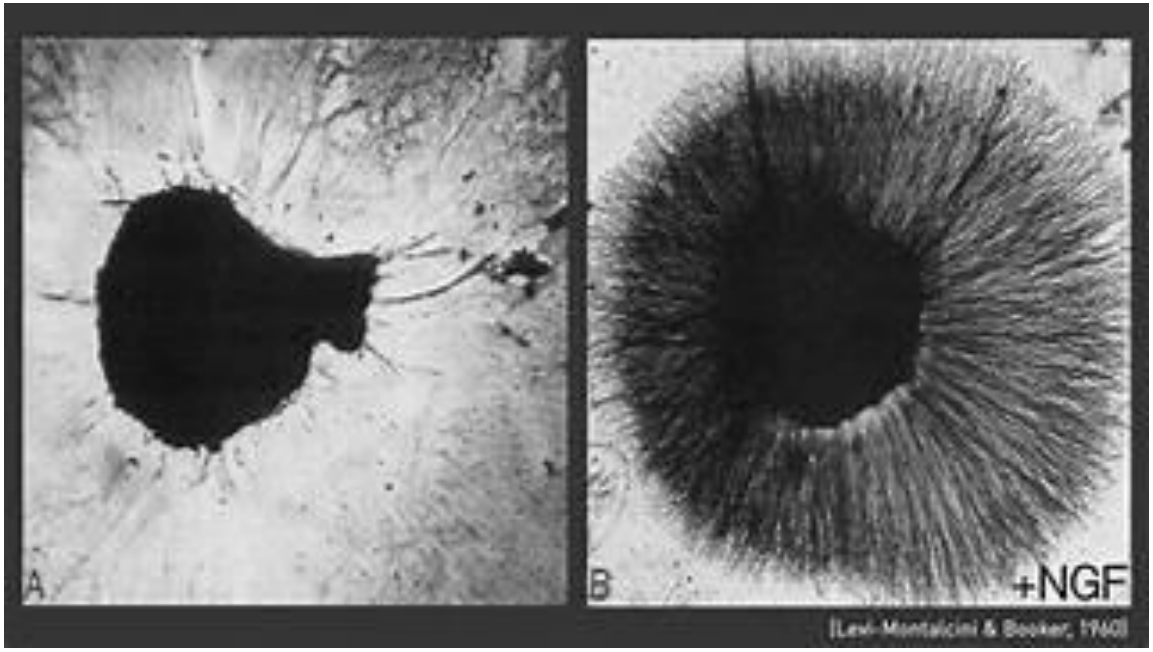
NGF) — was purified from snake venom and mouse salivary-gland extracts by Levi-Montalcini and Stanley Cohen in 1957.



In 1986, they won the Nobel Prize in Physiology or Medicine.

Cohen isolated and characterized another salivary-gland protein that induced precocious eyelid opening and tooth eruption when injected into newborn mice . Epidermal growth factor (EGF), stimulated the proliferation of epithelial cells.

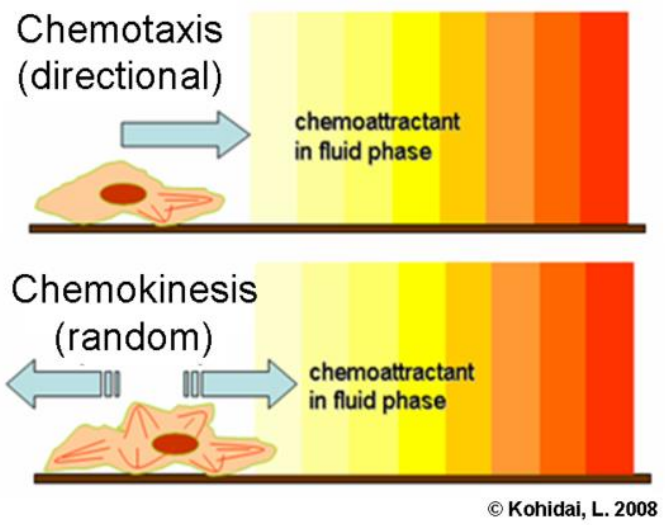
History, discovery of growth factors (NGF)



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	neuronal cells	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

GROWTH FACTOR FUNCTIONS *IN VITRO*

1. Proliferation
2. Differentiation
3. Chemo-attraction
4. Chemo-kinesis
5. Trophic action



GROWTH FACTOR FUNCTIONS *IN VIVO*

1. Early development

2. Tissue differentiation

3. Wound healing and tissue repair

4. Immune responses

5. Stromal mediators of sex and other hormones

77 1978

EGF receptor (EGFR) is identified

History, discovery of growth factor receptors and growth factor receptor phosphorylation

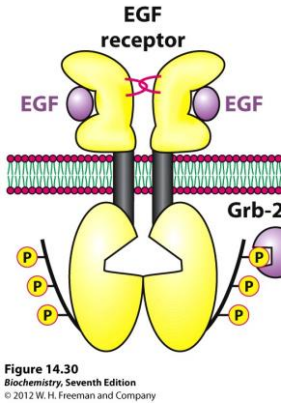


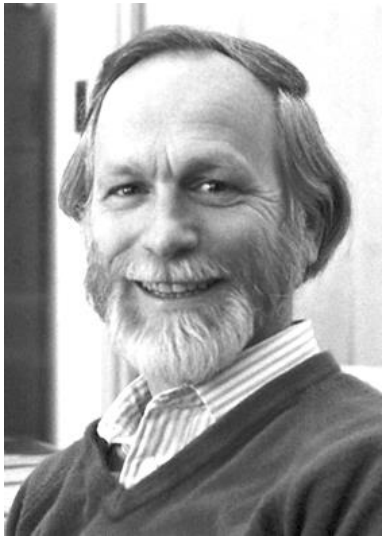
Figure 14.30
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

In 1975, using ^{125}I-labelled EGF and fibroblasts from different species, **Graham Carpenter** confirmed the presence of specific binding sites (receptors) for EGF on the surface of target cells.

Cohen and co-workers identified EGFR as a 170-kDa membrane component that showed increased 32P incorporation in response to EGF treatment in A-431 epidermoid carcinoma cells.

REGULATION OF GROWTH FACTOR/RECEPTOR INTERACTIONS

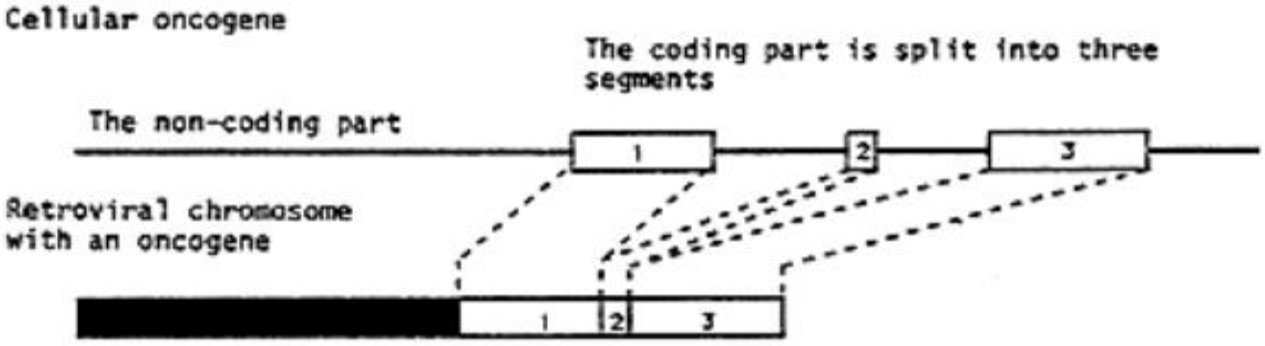
1. Determined by growth factor availability and receptor expression levels.
2. Different modes of growth factor action - autocrine, paracrine, endocrine, intracrine and juxtacrine.
3. Secretory properties - secretory signal; proteoglycan or serum protein binding.
4. More than one member of same growth factor gene family may act on the same receptor.
5. Same growth factor may cluster more than one receptor member of the same receptor family; homo- vs hetero-dimers.
6. Interactions regulated by alternative growth factor/receptor products.



History, oncogenes that initiate signalling pathways through tyrosine phosphorylation could also induce human cancer.

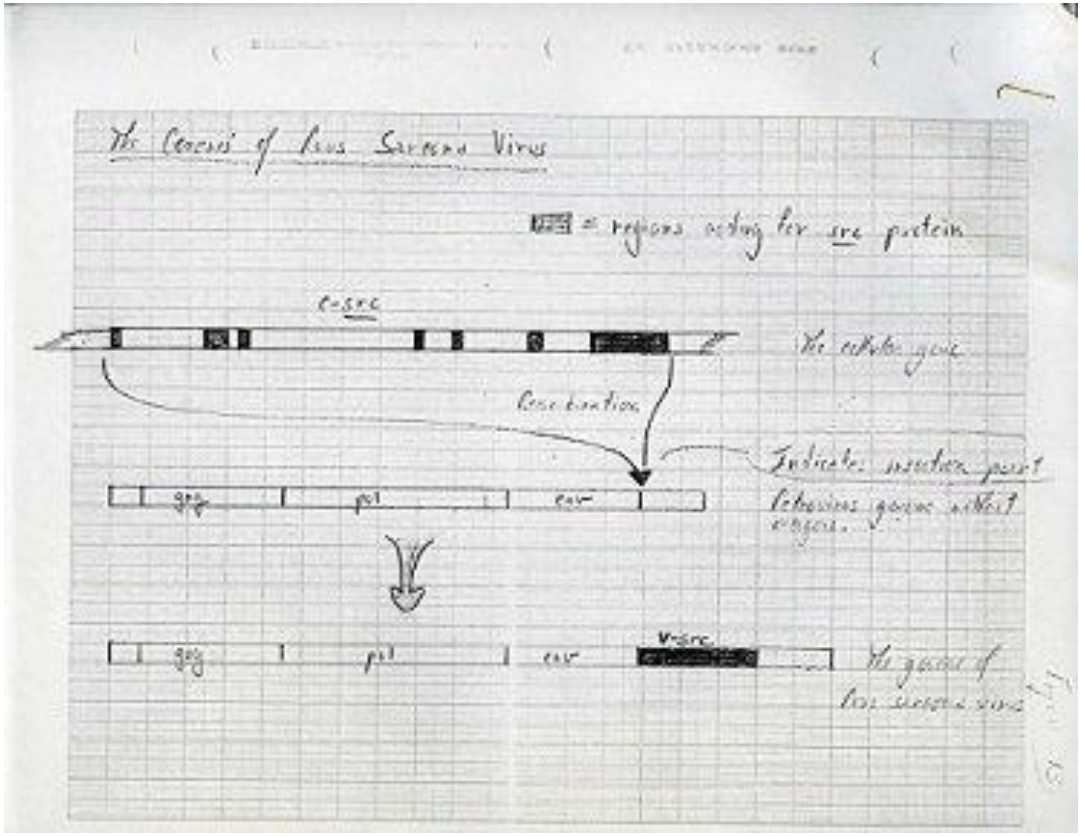
Noble Prize in 1989 for proving that viruses contain a cancer-causing gene derived from the genome of the organism they infect. Specifically, they showed that chicken Rous Sarcoma Virus (RSV) carried an oncogene called *v-src* and this gene was an intronless version of a normal chicken gene called *c-src* .

Michael Bishop and
Harold Varmus

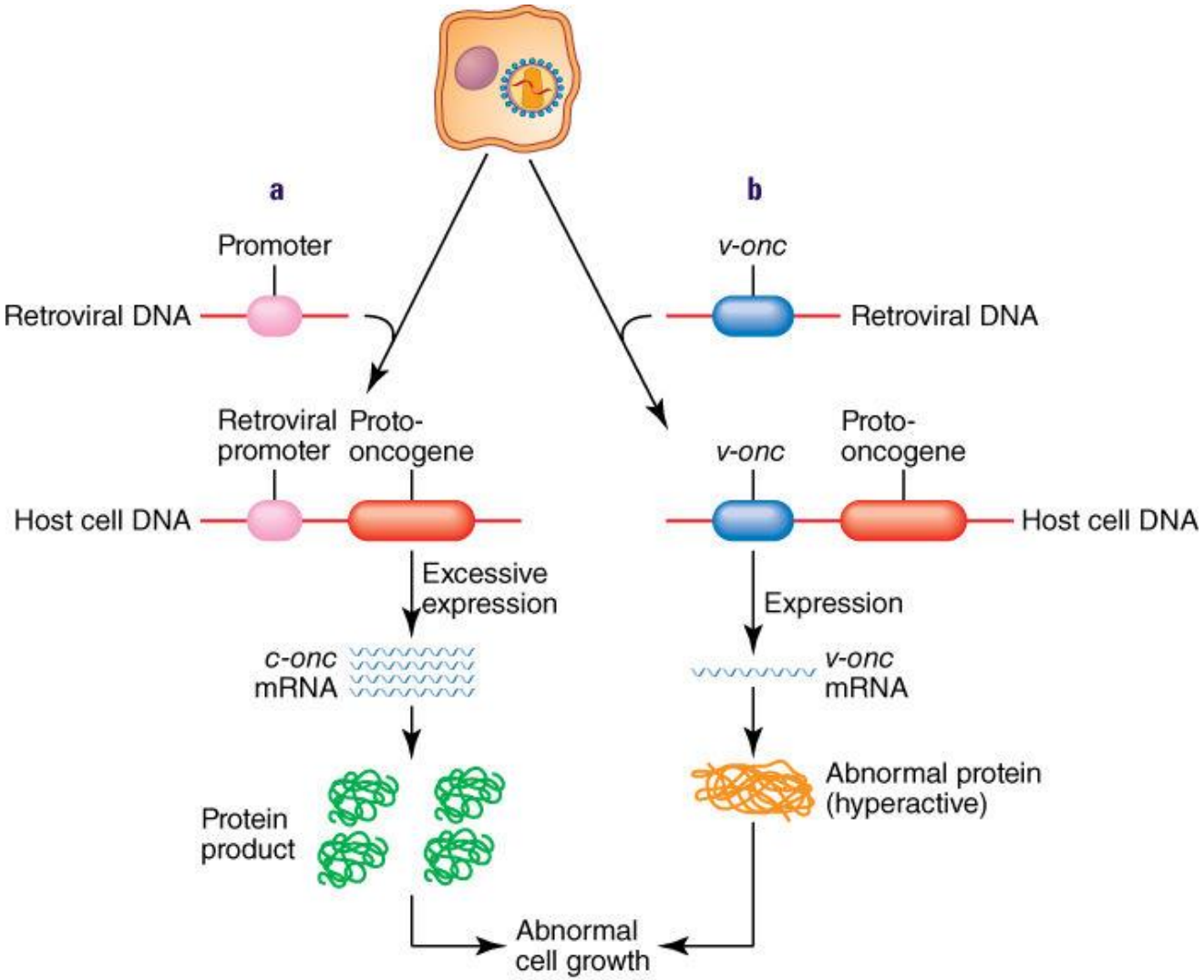


The *c-src* gene encodes a tyrosine kinase family of enzymes belonging to pathways triggered by specific signals leading to the regulation of growth and cell division.

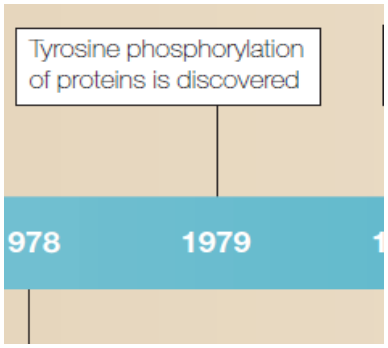
Finally, the work of Bishop and Varmus helped change our view of the genome. Not only do retrovirus sequences pop in and out of the genome but they can also capture cellular genes by converting them to retrogenes. The work confirmed the idea that genomes were dynamic—a idea that began with transposons. Not only that, the discovery of the retrogene, *v-src*, showed us that introns were almost certainly not an essential component of a gene.



Retroviruses



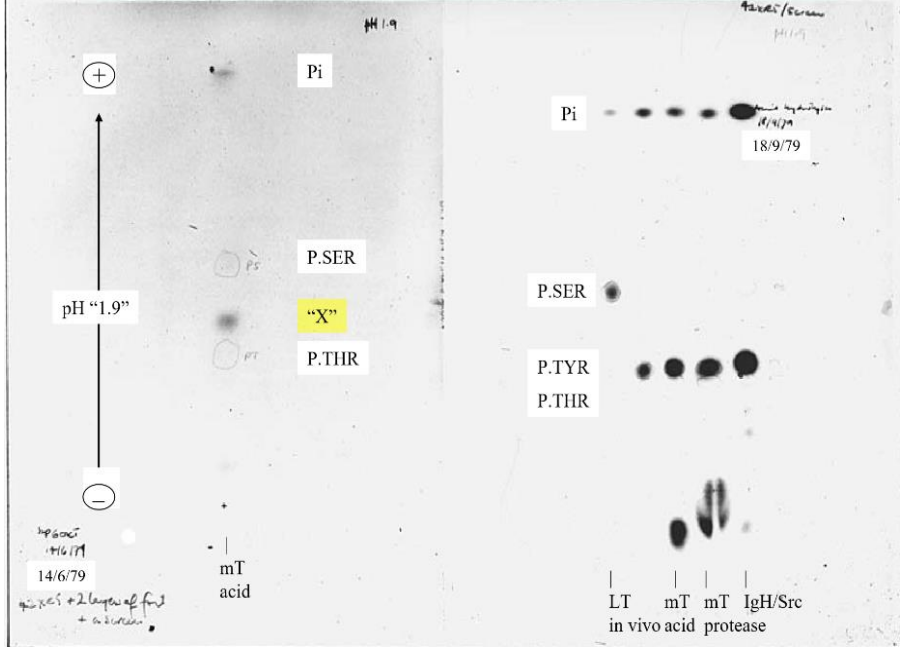
Information from Marks, D. B., Marks, A. D., and Smith, C. M. *Basic Medical Biochemistry: A Clinical Approach*. Lippincott Williams & Wilkins, 1996.

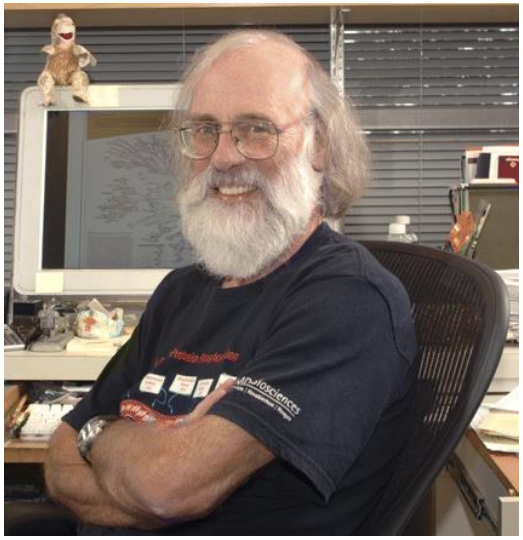


History, discovery of tyrosine phosphorylation and of a possible connection between tyrosine phosphorylation and tumorigenesis

Hunter and Sefton in 1980 found that the transforming protein of Rous sarcoma tumour virus, v-SRC, has tyrosine phosphorylation activity.

This indicated that deregulated protein tyrosine phosphorylation might be important in tumorigenesis.





"As is widely known, it turned out that the reason that pTyr had resolved from pThr was that I had been too lazy to make up fresh pH 1.9 buffer, which we routinely reused. Upon repeated reuse the pH of the buffer dropped from 1.9 to ~1.7, and this small pH difference was responsible for the resolution of pTyr and pThr, which comigrate at pH 1.9."

PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, U.S.A.

Request for opinion on manuscript by **Tony Hunter and Bartholomew M. Sefton**

Title **The transforming gene product of rous sarcoma virus phosphorylates tyrosine**

The *Proceedings of the National Academy of Sciences, U.S.A.*, an interdisciplinary journal, intends to publish brief reports of original research of exceptional importance or novelty. I am writing to ask your opinion on the following points, together with any other comments you may offer.

1. Does the evidence justify the conclusions drawn? Yes ☒ No ☐
2. Are the procedures used sufficiently documented so that other competent investigators can repeat the work? Yes ☒ No ☐
3. Is this a paper of particular broad interest to diverse groups of scientists? Yes ☒ No ☐
4. If this is primarily a "method" paper, does the method described markedly increase available sensitivity, specificity, or convenience when compared to existing techniques? Yes ☐ No ☐
5. Is the paper clearly written? Yes ☒ No ☐
6. Is the overall quality of this paper in the top 10th percentile of papers in its field? Yes ☒ No ☐

What is novel or significant about this paper?

Reports a new protein kinase specificity (for tyrosine).

Comments (use additional pages if necessary; send original and two copies).

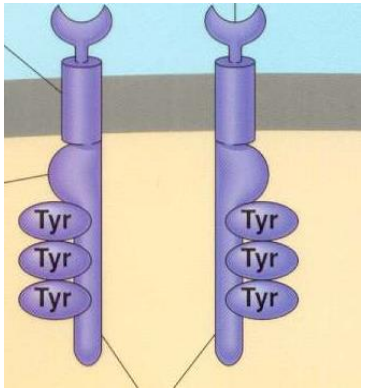
This is a clear well-written MS of great significance. This unusual specificity for tyrosine will greatly improve chances for identification of the core kinase targets.

No revision is necessary and immediate publication is strongly recommended. This is very nice work.

History , growth factor receptors are tyrosine kinases

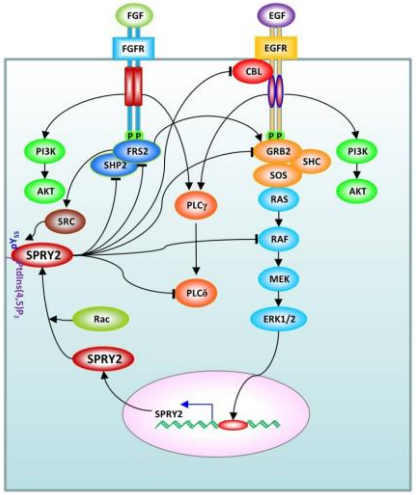
Discovery that EGFR, the insulin receptor (INSR) and the platelet-derived growth factor receptor (PDGFR) are protein tyrosine kinases that can be activated by their respective ligands.

.



History, intracellular proteins are tyrosine phosphorylated upon growth factor receptor activation

Subsequently, Hunter and co-workers showed that the stimulation of A431 cells by EGF, and that of NIH-3T3 cells by PDGF, leads to rapid tyrosine phosphorylation of intracellular proteins downstream of the activated growth-factor receptors.



History, animal oncogenes are similar to growth factor receptors.

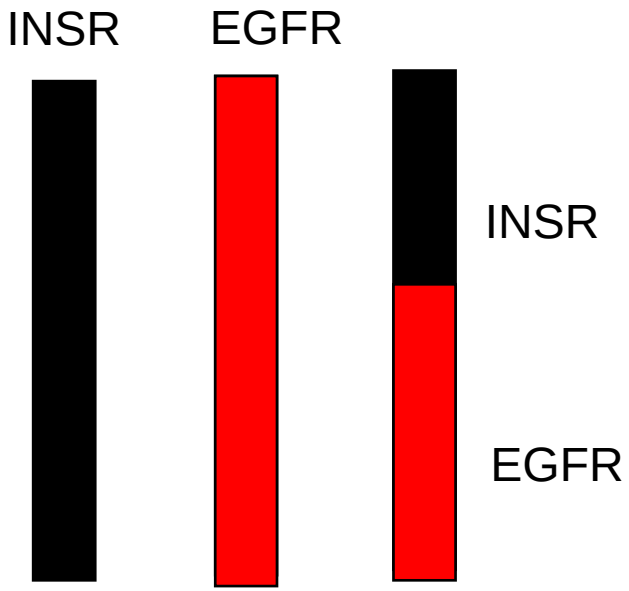
Julian Downward in Michael Waterfield's laboratory found a high level of similarity between the EGFR peptides and sequences of an avian oncogene, v-*erbB* (Avian erythroblastosis vírus).

This discovery connected, for the first time, an animal oncogene with a human gene that encoded a cell-growth-controlling membrane protein.

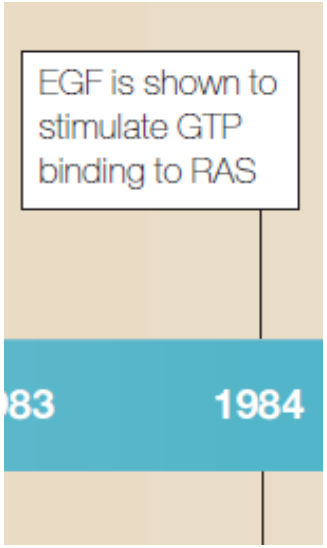
Closely related mechanisms of signal transduction amongst RTKs

Ullrich and co-workers designed a chimeric receptor molecule that comprised the extracellular region of INSR joined to the transmembrane and intracellular domains of EGFR.

Found that the EGFR kinase domain of the chimeric protein was activated by insulin binding, indicating that individual RTKs use closely related mechanisms for signal transduction across the plasma membrane.

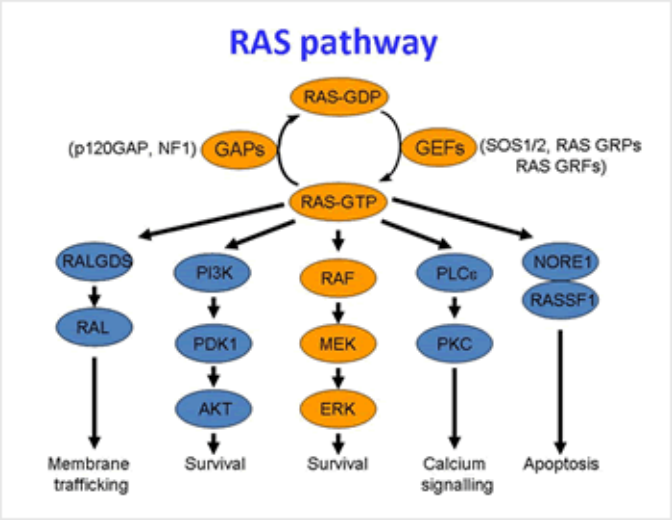


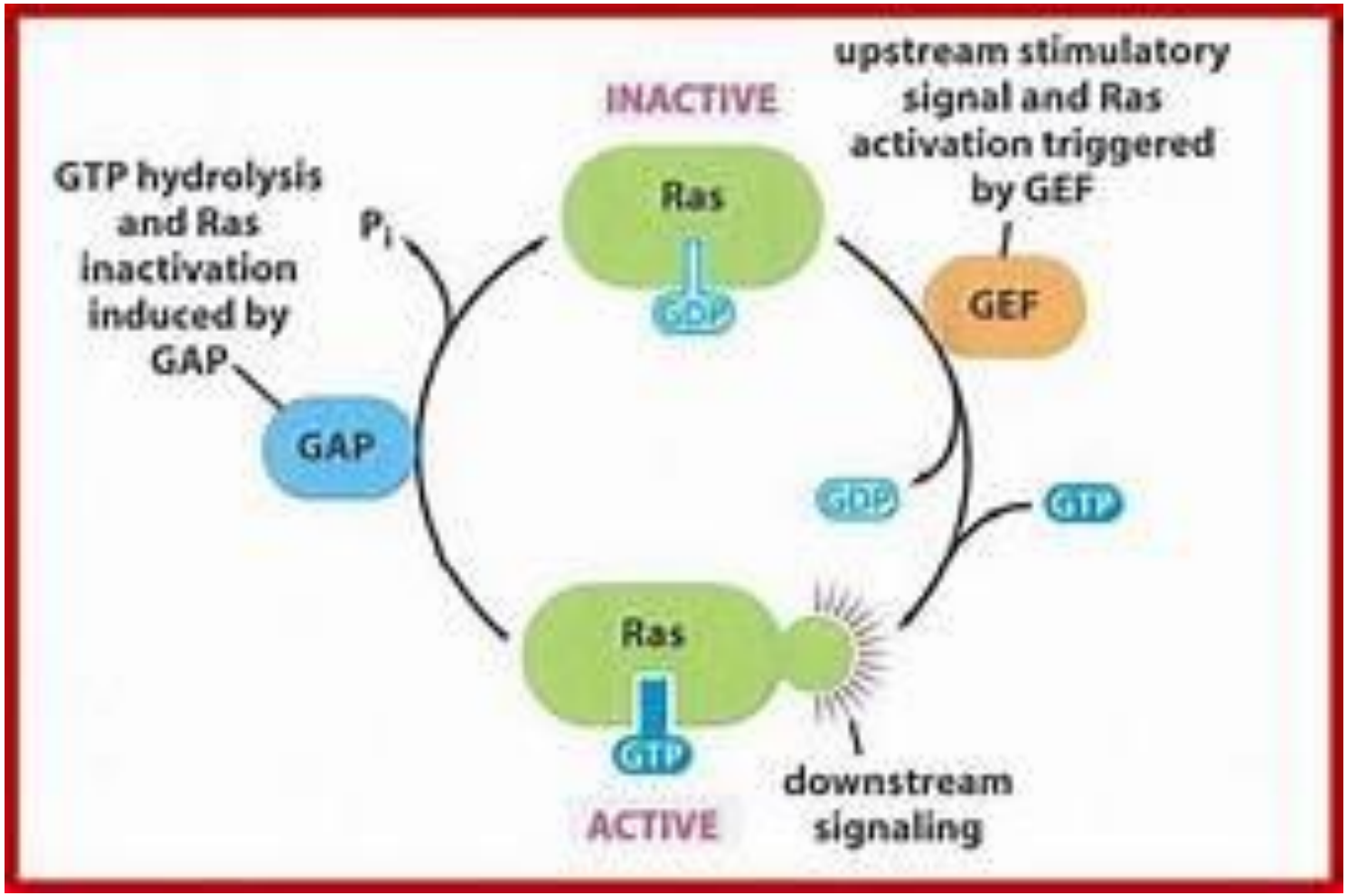
Downstream signaling



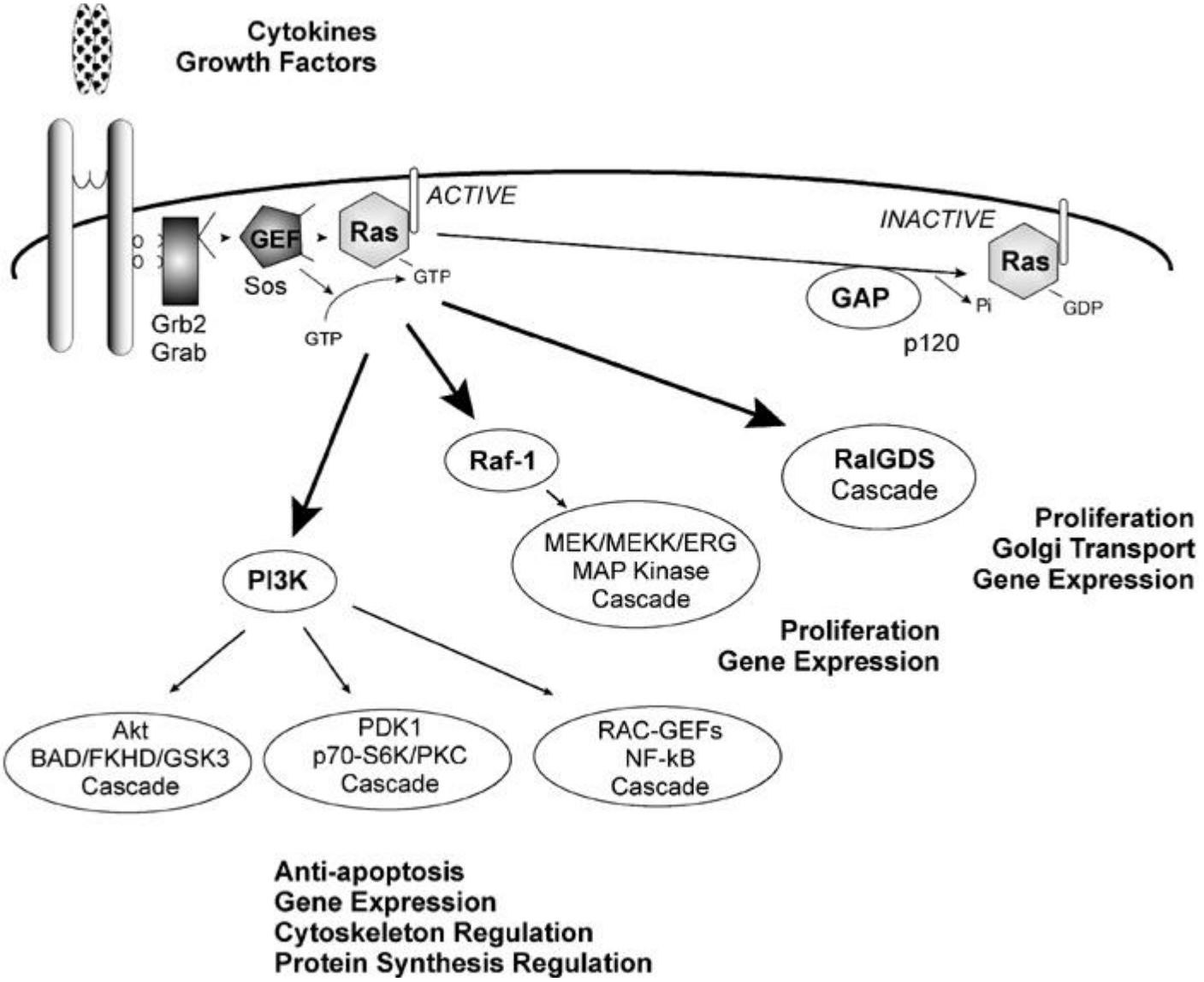
1984 Kamata and Feramisco showed that EGF stimulates the RAS oncoprotein to switch from its inactive GDP-bound form to its active GTP-bound form.

Two years later, Stacey and co-workers showed that RAS is essential for cell transformation by RTK-derived oncoproteins, indicating that RAS is a downstream mediator of activated RTKs.

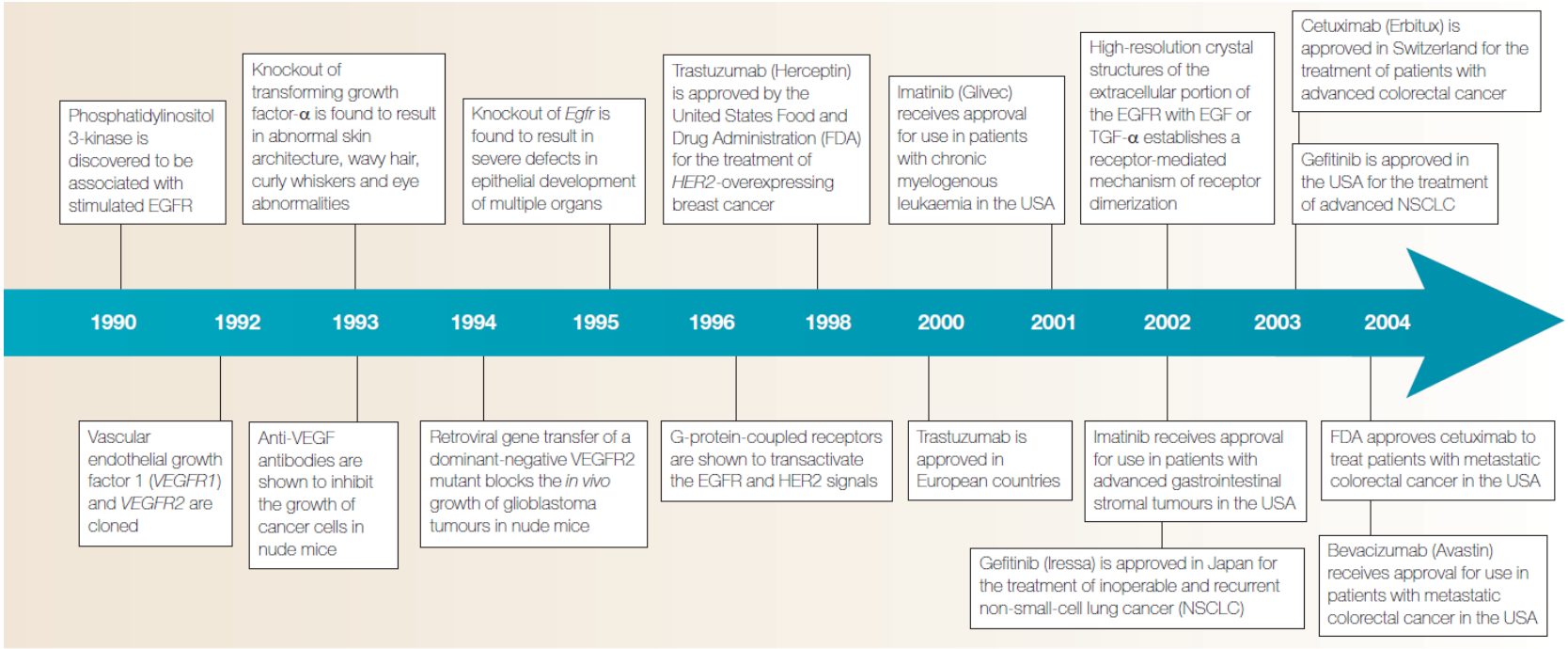



INACTIVE
upstream stimulatory signal and Ras activation triggered by GEF
GTP hydrolysis and Ras inactivation induced by GAP
Ras
GDP
P_i
GEF
GAP
GDP
GTP
Ras
GTP
ACTIVE
downstream signaling

Downstream signaling RAS



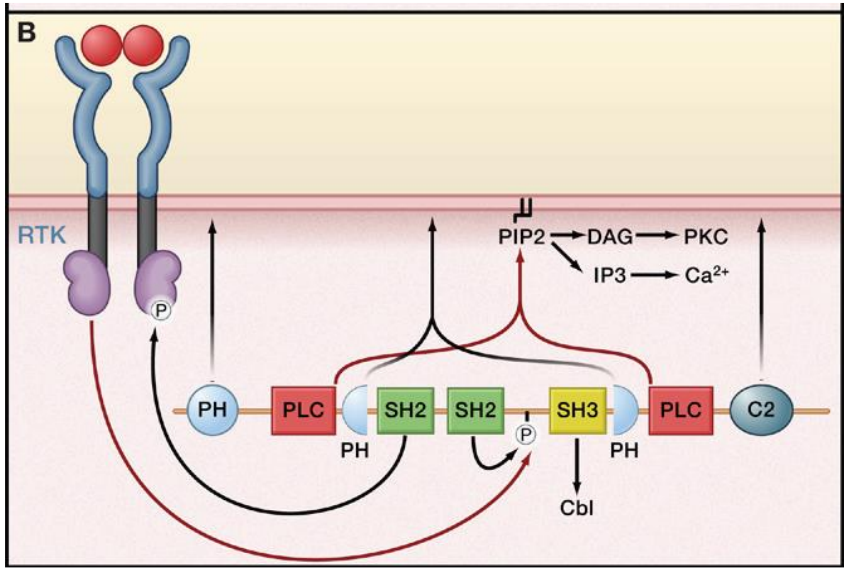
Timeline

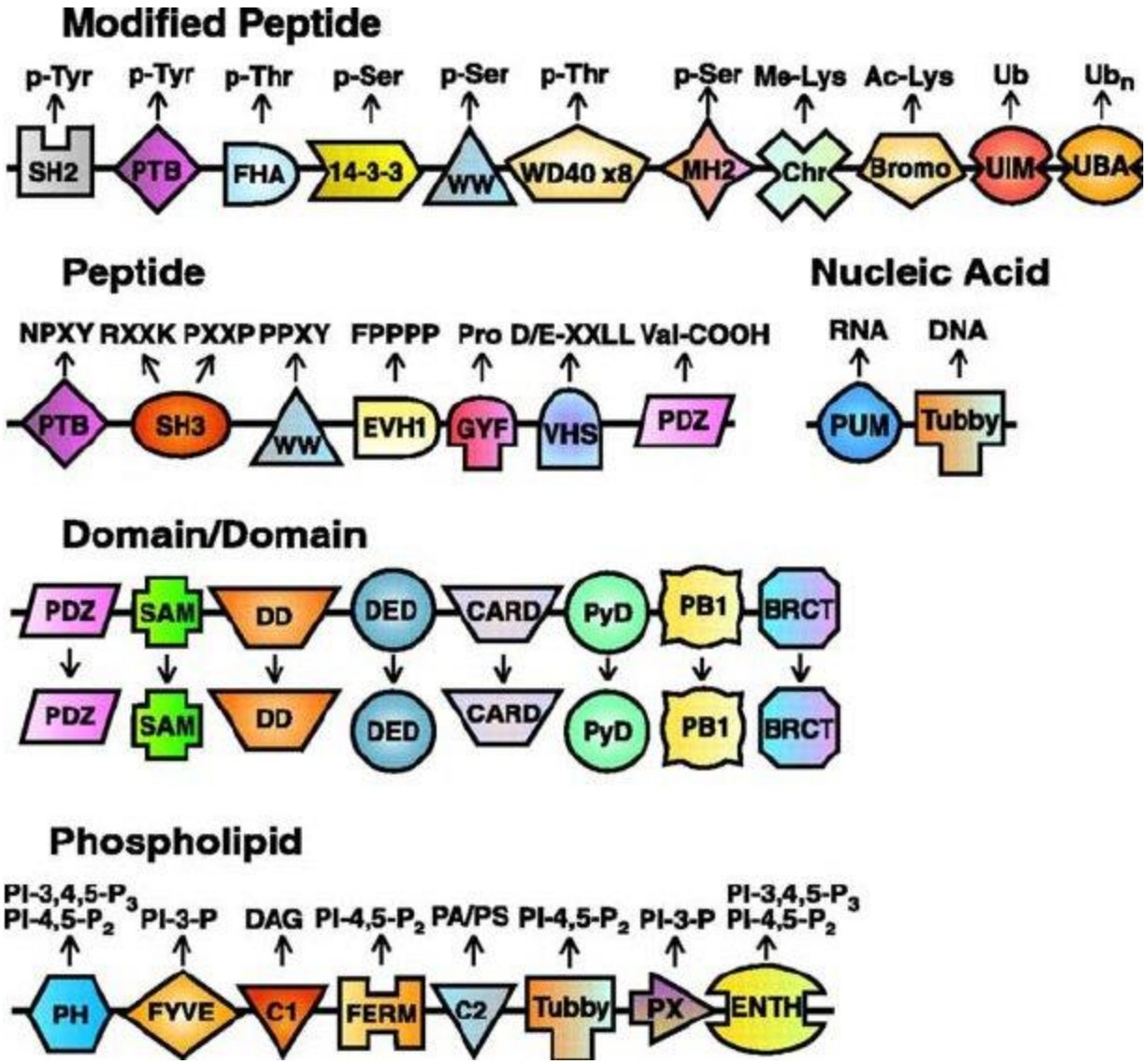

1990
Phosphatidylinositol 3-kinase is discovered to be associated with stimulated EGFR
1992
Vascular endothelial growth factor 1 (*VEGFR1*) and *VEGFR2* are cloned
1993
Knockout of transforming growth factor-α is found to result in abnormal skin architecture, wavy hair, curly whiskers and eye abnormalities
Anti-VEGF antibodies are shown to inhibit the growth of cancer cells in nude mice
1994
Knockout of *Egfr* is found to result in severe defects in epithelial development of multiple organs
Retroviral gene transfer of a dominant-negative VEGFR2 mutant blocks the *in vivo* growth of glioblastoma tumours in nude mice
1995
1996
Trastuzumab (Herceptin) is approved by the United States Food and Drug Administration (FDA) for the treatment of *HER2*-overexpressing breast cancer
G-protein-coupled receptors are shown to transactivate the EGFR and HER2 signals
1998
2000
Imatinib (Glivec) receives approval for use in patients with chronic myelogenous leukaemia in the USA
Trastuzumab is approved in European countries
2001
High-resolution crystal structures of the extracellular portion of the EGFR with EGF or TGF-α establishes a receptor-mediated mechanism of receptor dimerization
2002
Imatinib receives approval for use in patients with advanced gastrointestinal stromal tumours in the USA
Gefitinib (Iressa) is approved in Japan for the treatment of inoperable and recurrent non-small-cell lung cancer (NSCLC)
2003
Cetuximab (Erbitux) is approved in Switzerland for the treatment of patients with advanced colorectal cancer
Gefitinib is approved in the USA for the treatment of advanced NSCLC
2004
FDA approves cetuximab to treat patients with metastatic colorectal cancer in the USA
Bevacizumab (Avastin) receives approval for use in patients with metastatic colorectal cancer in the USA

History, identification of direct TKR substrates

In 1989, phospholipase Cγ1 (PLCγ1) was identified as the first downstream substrate that physically interacts with activated EGFR, demonstrating a connection between EGFR stimulation, phosphatidylinositol turnover, intracellular Ca2+ mobilization and activation of protein kinase C.

PLCγ1 structure led to the identification of **SRC-homology 2** (SH2) domains and led to some key discoveries by the groups of Pawson and Hanafus. They found that SH2 domains bind tyrosinephosphorylated peptides *in vitro*, and that phosphotyrosine residues in the cytoplasmic regions of RTKs are recognized as docking sites by signalling factors such as PLCγ1 through their **SH2 domains**.

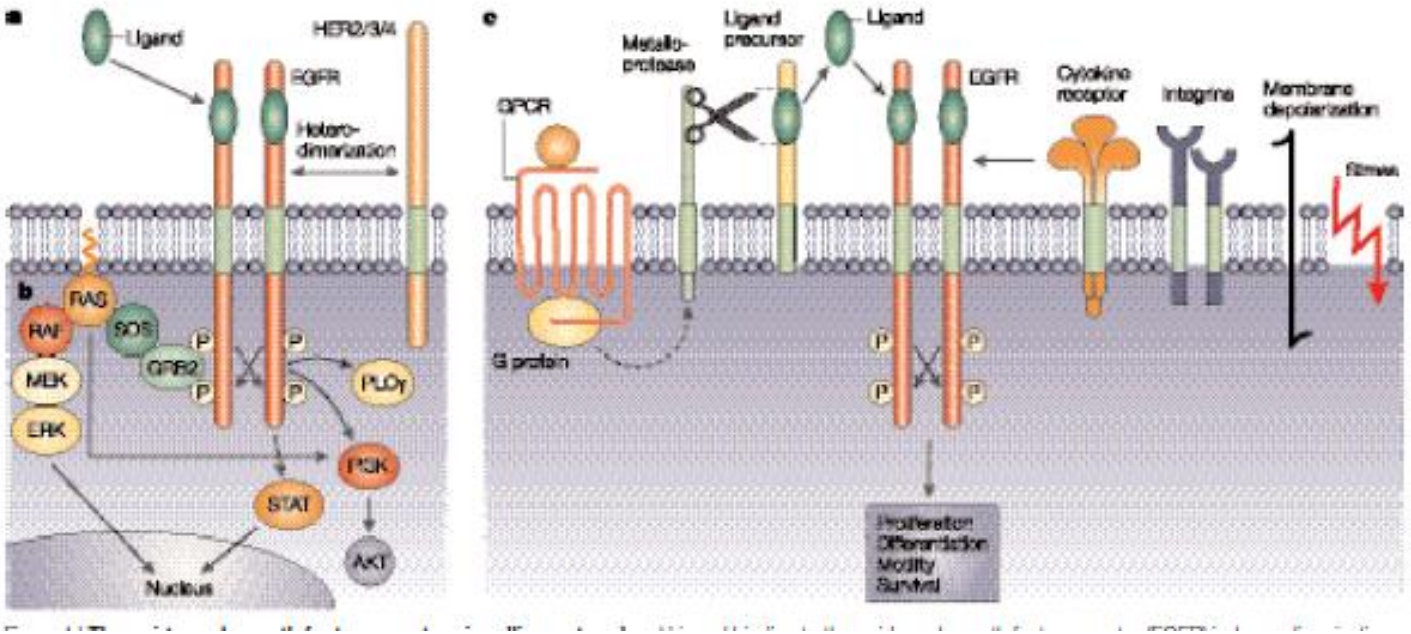



Modified Peptide
p-Tyr
p-Tyr
p-Thr
p-Ser
p-Ser
p-Thr
p-Ser
Me-Lys
Ac-Lys
Ub
Ub$_n$
SH2
PTB
FHA
14-3-3
WW
WD40 x8
MH2
Chr
Bromo
UIM
UBA
Peptide
NPXY
RXXK
PXXP
PPXY
FPPPP
Pro
D/E-XXLL
Val-COOH
PTB
SH3
WW
EVH1
GYF
VHS
PDZ
Nucleic Acid
RNA
DNA
PUM
Tubby
Domain/Domain
PDZ
SAM
DD
DED
CARD
PyD
PB1
BRCT
PDZ
SAM
DD
DED
CARD
PyD
PB1
BRCT
Phospholipid
PI-3,4,5-P$_3$
PI-4,5-P$_2$
PI-3-P
DAG
PI-4,5-P$_2$
PA/PS
PI-4,5-P$_2$
PI-3-P
PI-3,4,5-P$_3$
PI-4,5-P$_2$
PH
FYVE
C1
FERM
C2
Tubby
PX
ENTH

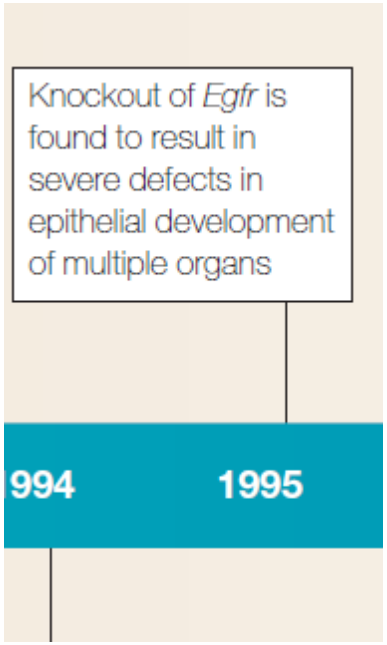
History, identification of other signal transuction components

Julian Downward, as well as Wolfman and Macara, identified one of the missing links between RTKs and RAS. They identified several mammalian cytosolic factors that catalyse the exchange of RAS-bound GDP for GTP **GEF**

Identification of additional downstream signalling pathways of activated RTKs. **Phosphatidylinositol 3-kinase (PI3K),** the survival mediator **AKT** (also known as protein kinase B) and the signal transducer and activator of transcription (STAT) proteins58 were found to be activated by RTKs and to be involved in cellular responses such as anti-apoptotic signalling, motility and invasiveness.



History, RTK knockout



Egfr knockout in mice resulted in **embryonic lethality** or severe failure of epithelial development in several organs — including skin, lung and gastrointestinal tract.

History, correlation between RTK expression and,mutagenesis with tumorigenesis

In the 1980s, numerous reports described the **overexpression of EGFR in various epithelial tumours** and substantiated the view that deregulated EGFR signaling has an important role in human cancers.

Many laboratories embarked on a massive search for *EGFR* mutations in human cancers and several deletions and point mutations were described that result in **increased catalytic tyrosine-kinase activity of the receptor**.

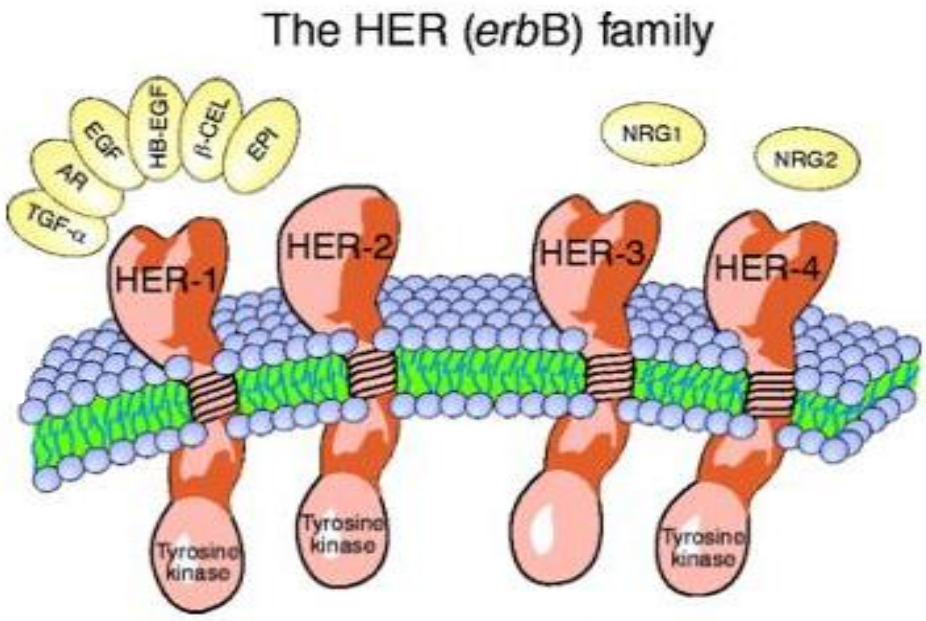
Recently, impaired receptor downregulation has been recognized as another mechanism of RTK deregulation. In particular, oncogenic forms of the **ubiquitin ligase CBL** were shown to function as dominant-negative mutants that prevent CBL from negatively regulating RTKs.

http://www.youtube.com/watch?v=3nODx3cT1RU

History, RTK and tumorigenesis

In 1985, as a by-product of the *EGFR* cloning project, Axel Ullrich's group at Genentech described the complete primary structure of a putative RTK that showed a high level of homology to human EGFR and was therefore
named human **EGFR-related 2 (HER2)**.

The chromosomal localization of *HER2* is identical to that of **the rat *neu* oncogene**.



History, Her2 , breast and ovarian cancer

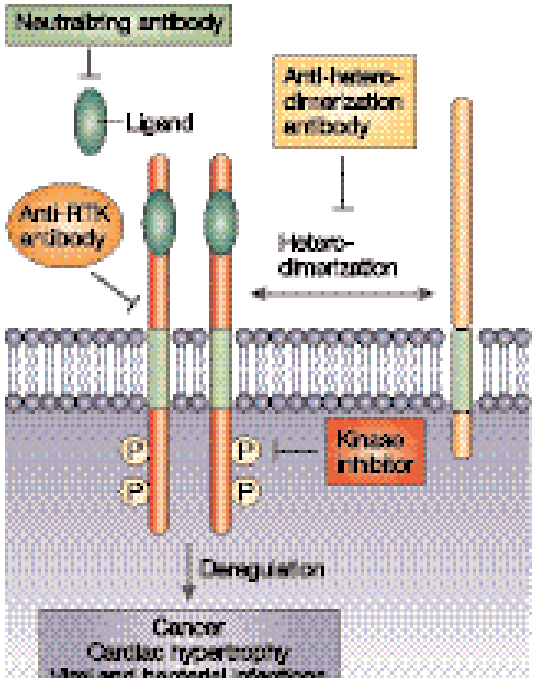
Ullrich laboratory and Dennis Slamon, an oncologist at the University of California, Los Angeles. Slamon had assembled a collection of primary breast tumours and was ready to use Ullrich's gene probes to search for abnormalities in tumour DNA.

Two years later, this collaborative team reported that the *HER2* gene is **amplified in 30% of invasive breast cancers** and, for the first time, showed a significant correlation between *HER2* overexpression in tumours and reduced patient survival and time to relapse.

These findings established *HER2* as a prognostic factor and indicated a crucial role of *HER2* overexpression in the pathogenesis of breast and ovarian cancers.

Therapeutic interventions (HER2)

The oncogenic significance of *neu* was further substantiated when Robert Weinberg and co-workers showed that monoclonal antibodies (mAbs) against the *neu* oncogene reverted its transforming effects.



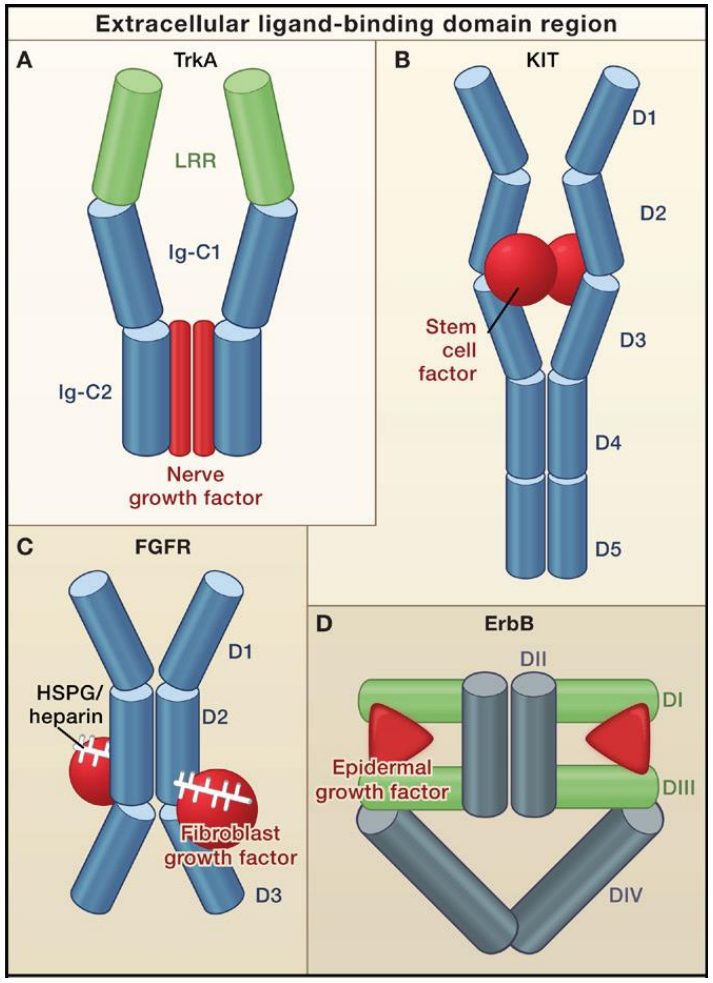
http://www.youtube.com/watch?NR=1&v=48VSU4AZ-L0

Table 2 | **Cancer therapies targeted to receptor tyrosine kinases**

Names	Targets	Status	Description	Company
Trastuzumab, Herceptin	HER2	Approved for metastatic breast cancer	Humanized anti-HER2 IgG1κ	Genentech
Imatinib, Glivec, STI571	BCR–ABL, KIT, PDGFR	Approved for CML and GIST	2-Phenylaminopyrimidine	Novartis
Gefitinib, Iressa, ZD1839	EGFR	Approved for NSCLC	Quinazoline	AstraZeneca
Cetuximab, Erbitux	EGFR	Approved for colorectal cancer	Chimeric anti-EGFR IgG1	ImClone/Merck
Bevacizumab, Avastin	VEGF	Approved for colorectal cancer	Humanized anti-VEGF (rhu mAb-VEGF)	Genentech
OSI-774, Tarceva	EGFR	Clinical development	Quinazoline	Genentech/ Roche/OSI
CI-1033	EGFR, HER2	Clinical development	4-Anilinoquinazoline, irreversible inhibitor	Pfizer
EKB-569	EGFR, HER2	Clinical development	4-Anilinoquinoline-3-carbonitrile, irreversible inhibitor	Wyeth
CDP860	PDGFR	Clinical development	Anti-PDGFβ-receptor antibody fragment	Celltech
Pertuzumab, Omnitarg, 2C4	HER2	Clinical development	Humanized anti-HER2 (heterodimerization inhibitor)	Genentech
SU6668	VEGFR2, PDGFR, FGFR	Clinical development	Indoline-2-one	Sugen/Pfizer
SU11248	VEGFR2, KIT, PDGFR, FLT3	Clinical development	Indoline-2-one	Sugen/Pfizer
ZD6474	VEGFR2	Clinical development	Quinazoline	AstraZeneca
PTK-787/ZK222584	VEGFR1/2, PDGFR	Clinical development	Anilinophthalazine	Novartis/Schering
AG013736	VEGFR2, PDGFR	Clinical development	–	Pfizer
CP549, 632	VEGFR2, FGFR1, TIE2	Clinical development	–	Pfizer
PKC-412, midostaurin	PKC, VEGFR2, PDGFR, FLT3, KIT	Clinical development	*N*-Benzoylstaurosporine	Novartis
CEP-701	FLT3, TRK kinases	Clinical development	Indolocarbazole alkaloid	Cephalon
MLN-518, CT53518	PDGFR, KIT, FLT3	Clinical development	Quinazoline	Millennium

CML, chronic myelogenous leukaemia; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; FLT, FMS-related tyrosine kinase; GIST, gastrointestinal stromal tumour; HER, human EGFR-related; Ig, immunoglobulin; NSCLC, non-small-cell lung carcinoma; PDGFR, platelet-derived growth factor receptor; PKC, protein kinase C; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

Diversity of RTK

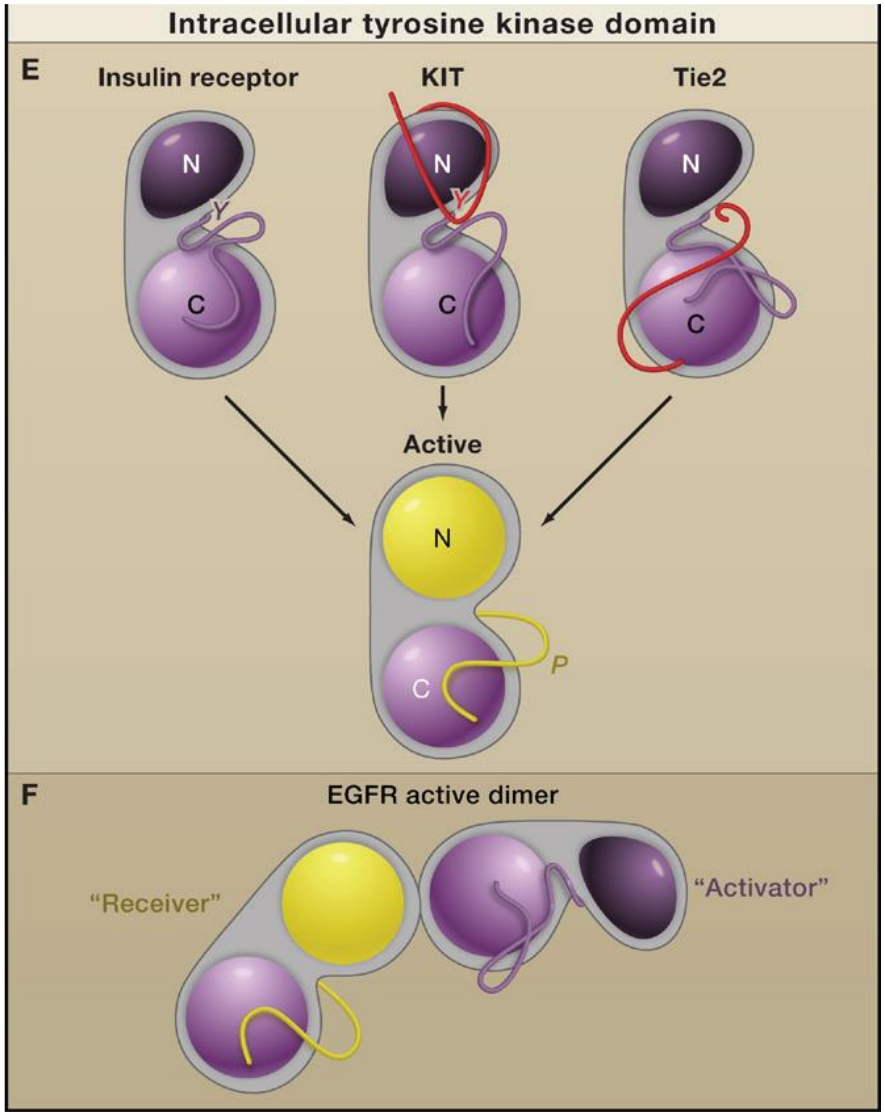


Ligand binding and dimerization

Bringing two TKDs together in a specific stable dimer increases their respective local concentrations and may also promote allosteric effects.

These influences dramatically increase the probability that a transiently active TKD will encounter another TKD that can be phosphorylated in a way that promotes activation. *Trans*-autophosphorylation ensues, and the receptors become activated.

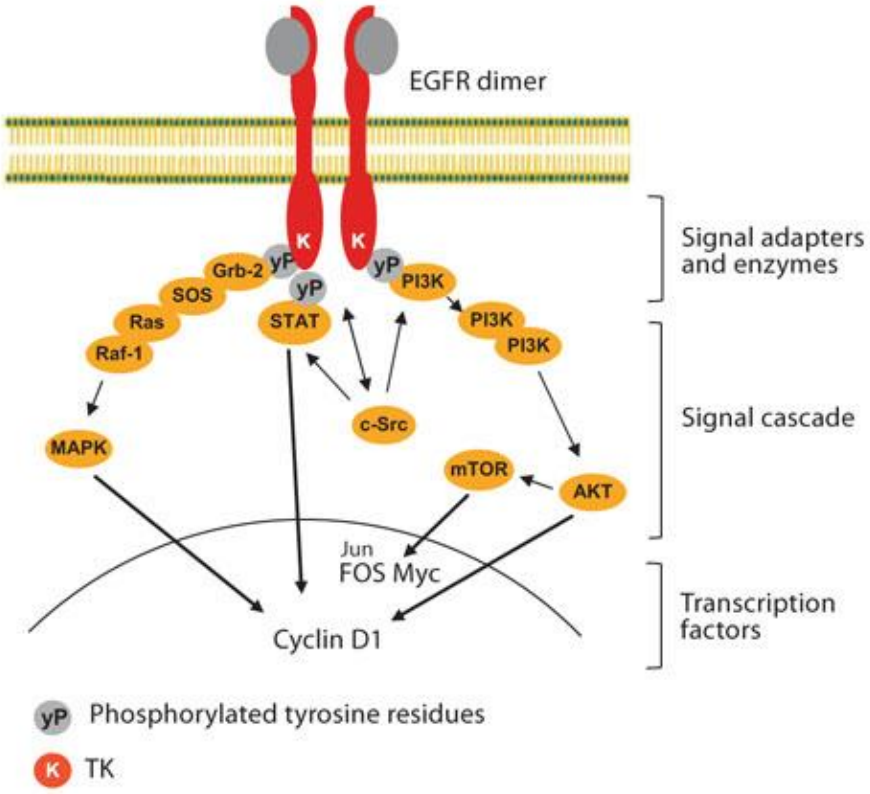
Diversity of RTK, auto-inhibition



Mutations that disrupt the autoinhibitory juxtamembrane interactions in the KIT/PDGFR family constitutively activate these RTKs and are frequently found in cancers.

Signal transduction specificity ?

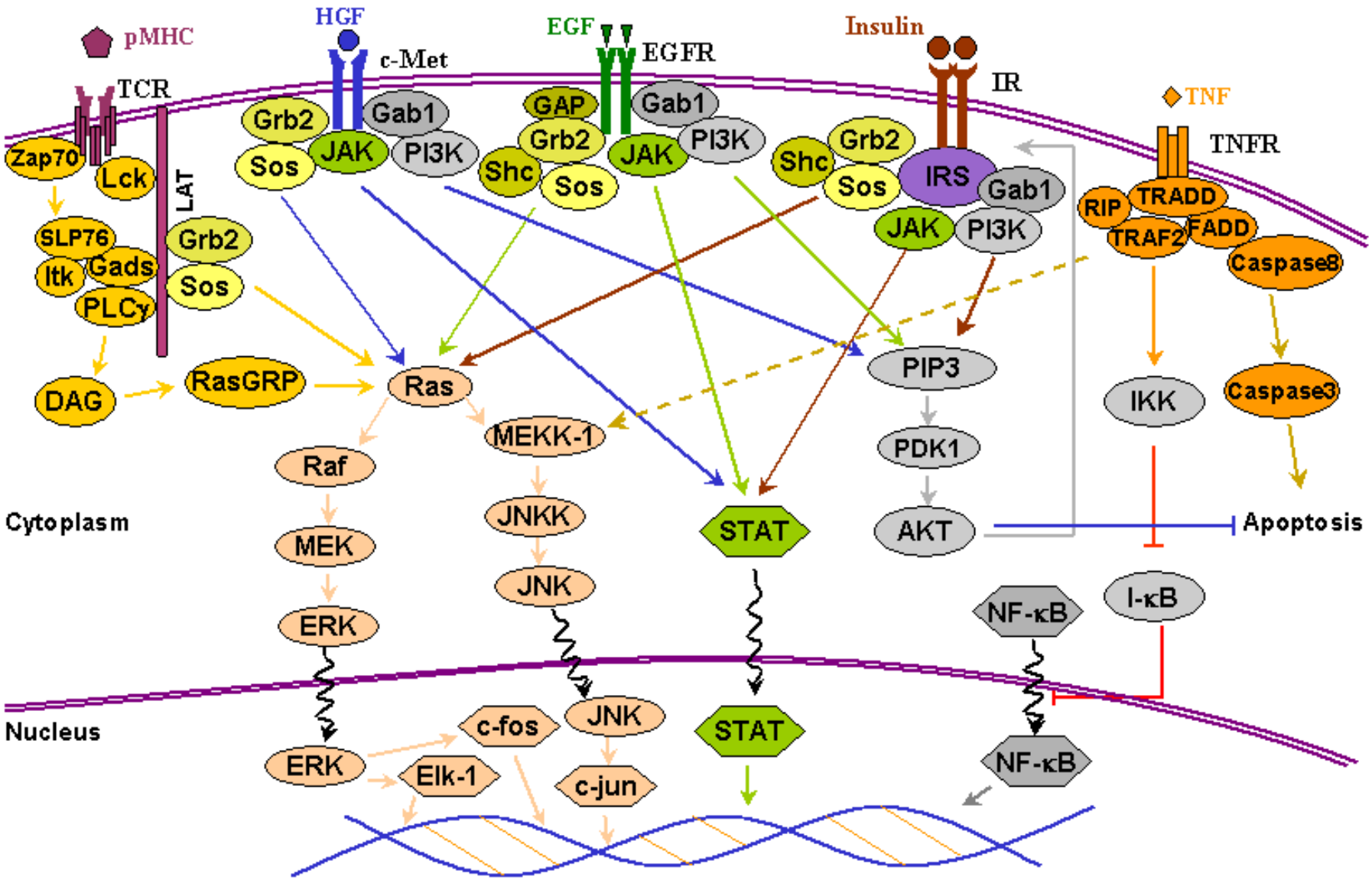
First, RTKs such as EGFR have multiple (5–12) autophosphorylation sites that can each recruit different SH2 and PTB domain-containing proteins; second, a given adaptor or scaffold protein can interact with multiple signaling molecules (Pawson, 1995; Schlessinger, 2000).



Signal transduction specificity ?

If both the EGF and NGF receptors activate similar sets of RTK-proximal downstream signaling molecules, how can activating the EGFR lead to cell proliferation whereas activating the NGF receptor promotes neurite outgrowth and differentiation in the same cell (Marshall, 1995)?

…may be activated simultaneously by different GF/Receptor and transduction systems



MECHANISMS OF RTK REGULATION:
attenuation and termination of signaling