Signal Transduction

SS 2018

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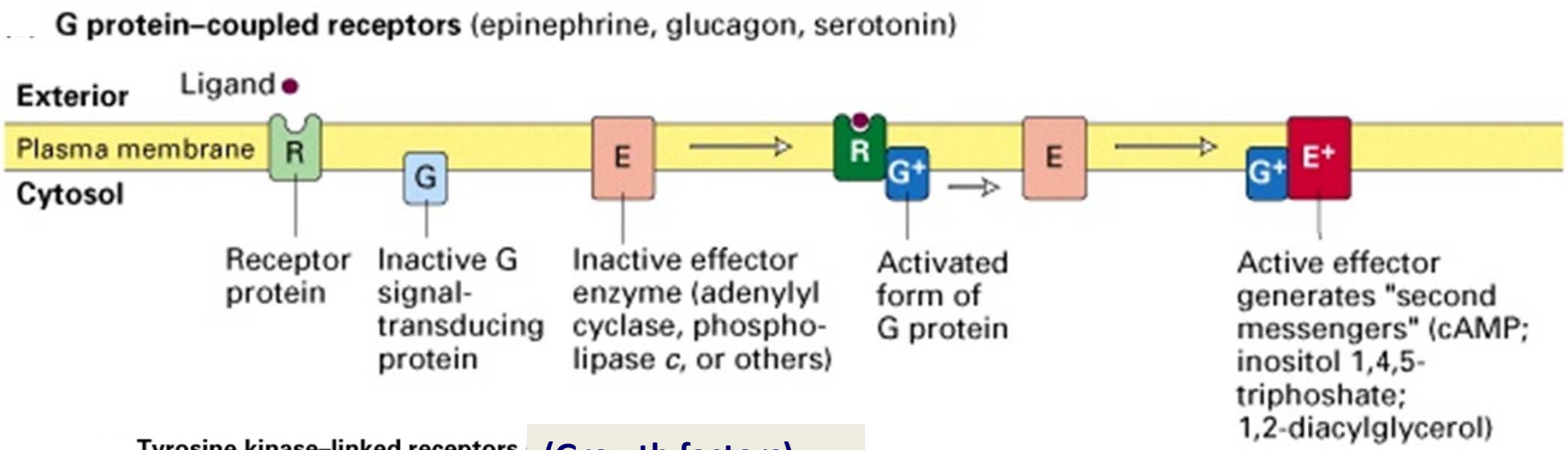
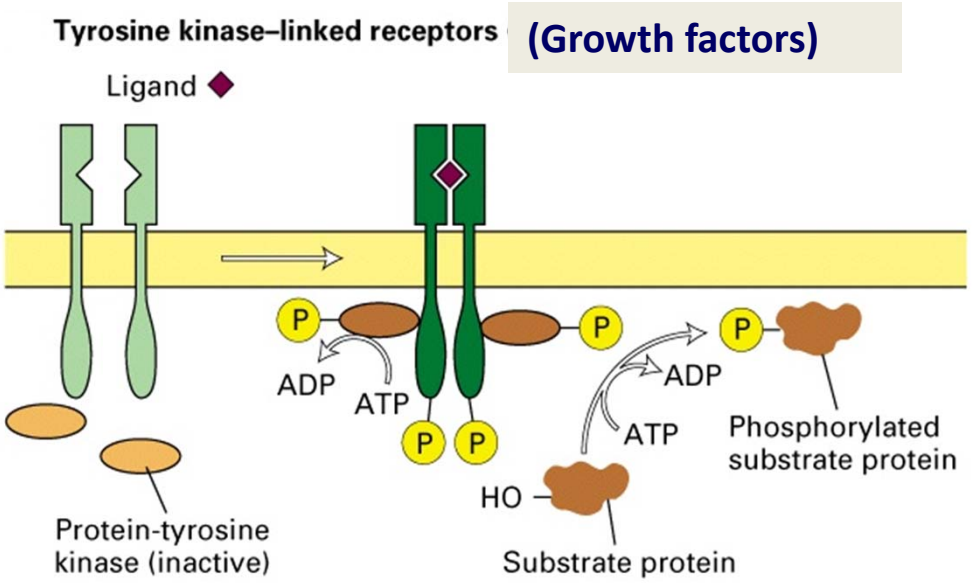
Focus on 2 classes of cell-surface receptors

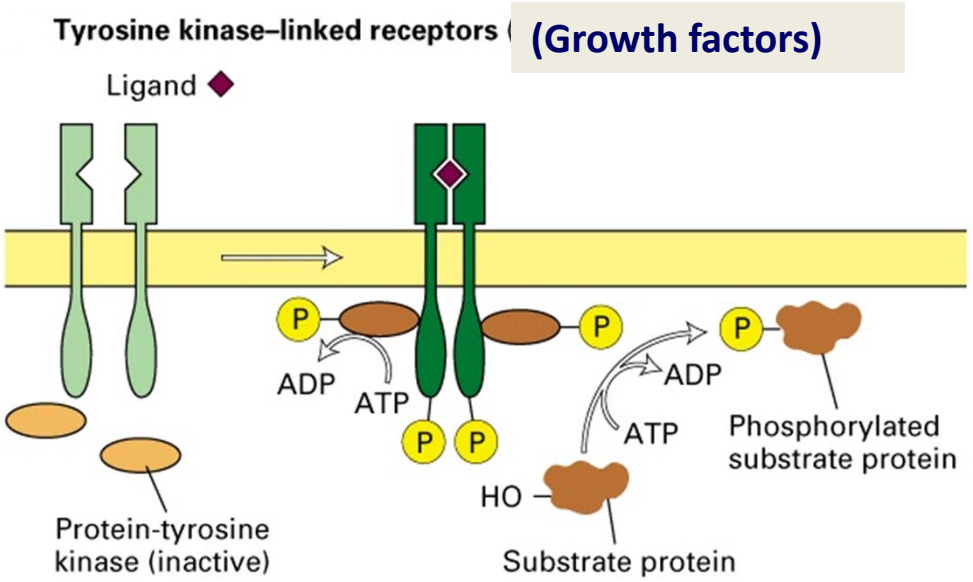




(Growth factors)

Lodish et al. Molecular Biology of the Cell

Elements of receptor tyrosine kinase systems



- a receptor with kinase activity in its cytosolic domain
- adaptor proteins recognizing phosphotyrosine residues
- a coupled monomeric G protein which functions as a switch
- protein kinases and phosphatases: propagation and amplification of signal

- down-regulation of RTK signalling by ligand-induced endocytosis and lysosomal degradation

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General structure and activation of RTKs

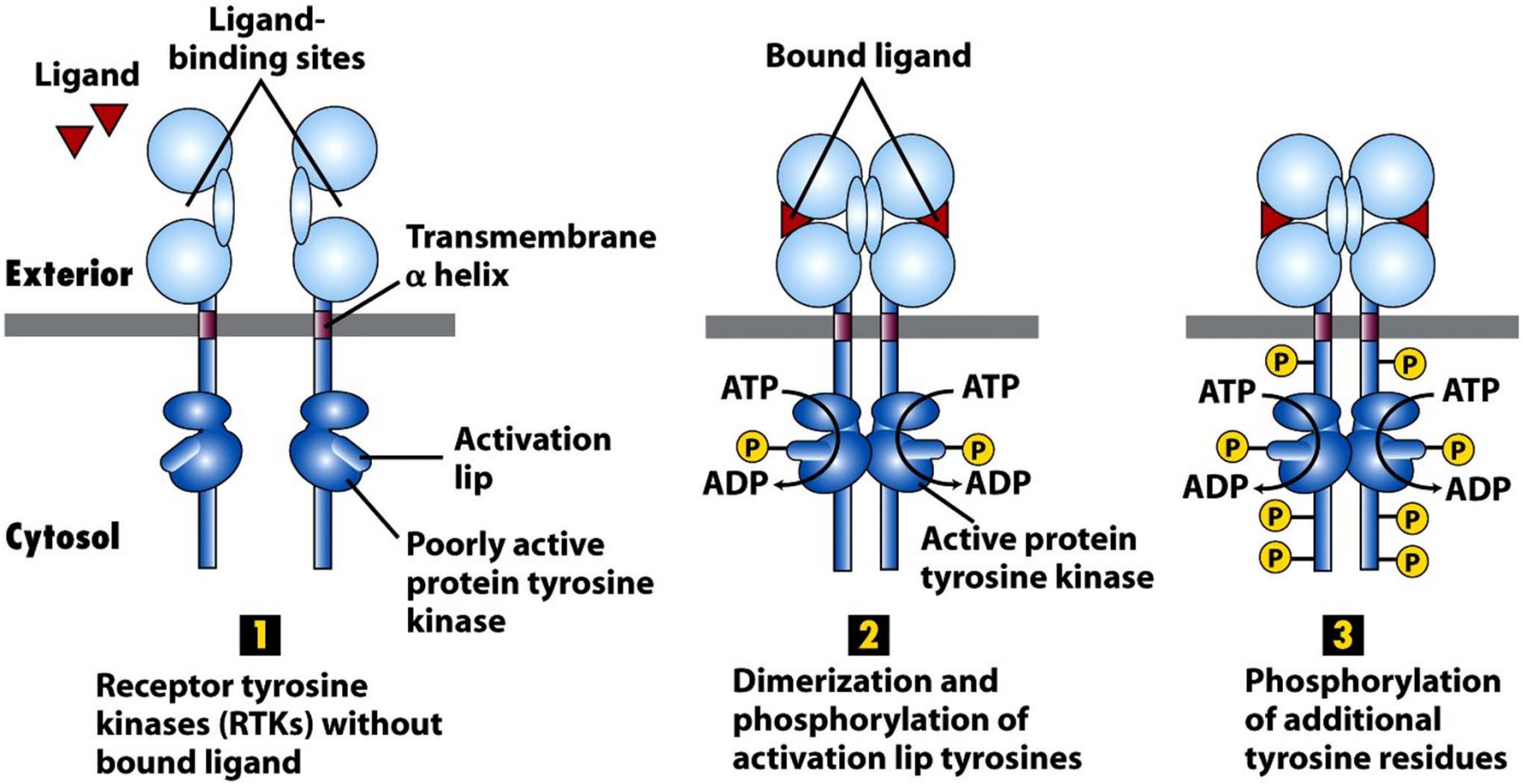

Figure 16-16
Molecular Cell Biology, Sixth Edition

Docking sites

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Phosphotyrosines are docking sites for adapter proteins with conserved PTB or SH2 domains

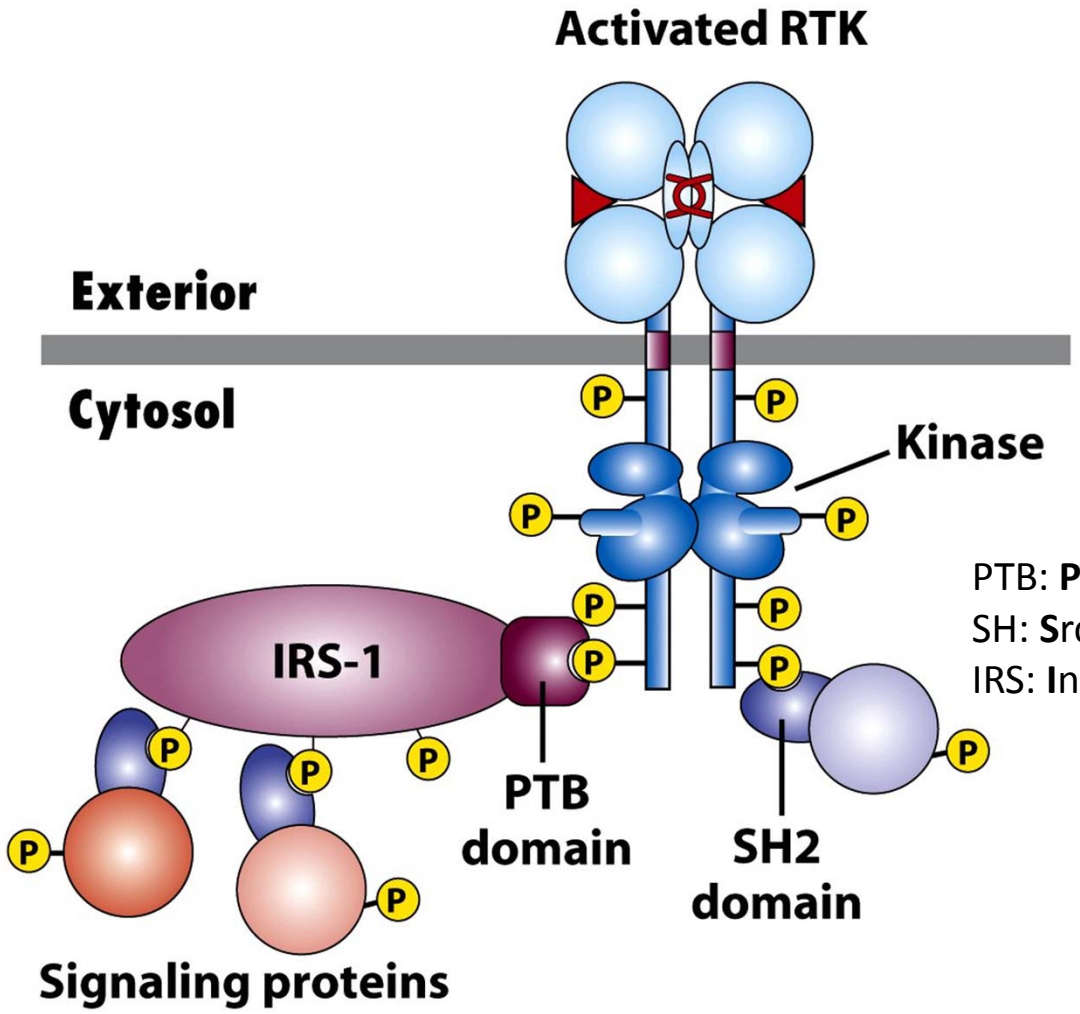

Figure 16-19
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PTB: **P**hospho**T**yrosine **B**inding
SH: **S**rc-**H**omology
IRS: **I**nsulin **R**eceptor **S**ubstrate

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Model of a SH2 domain bound to a phosphotyrosine-containing peptide

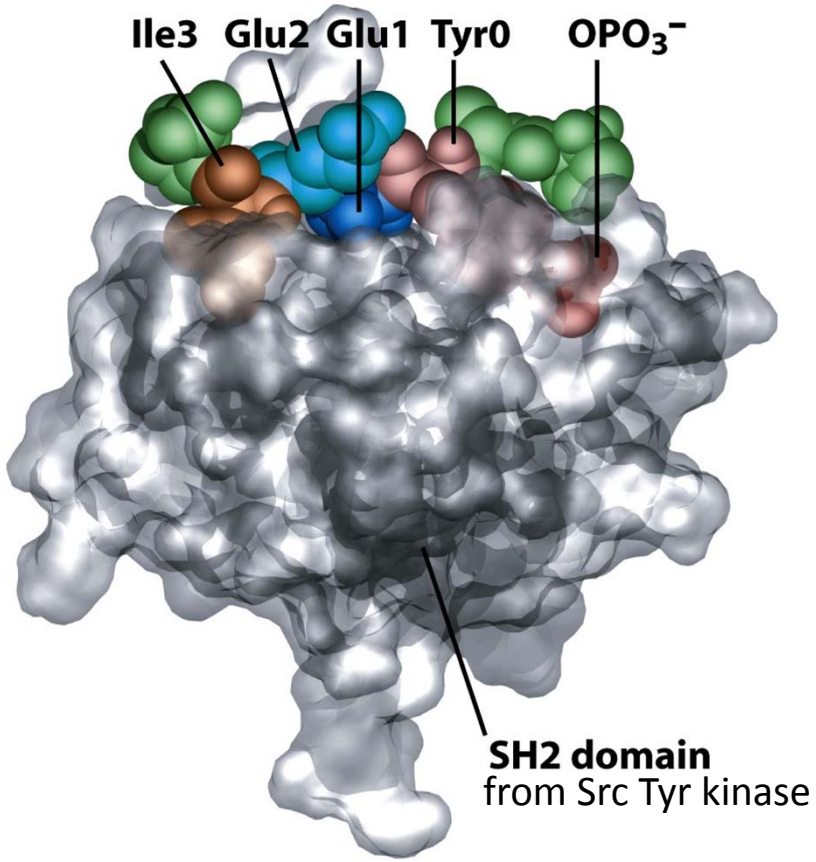

Figure 16-11
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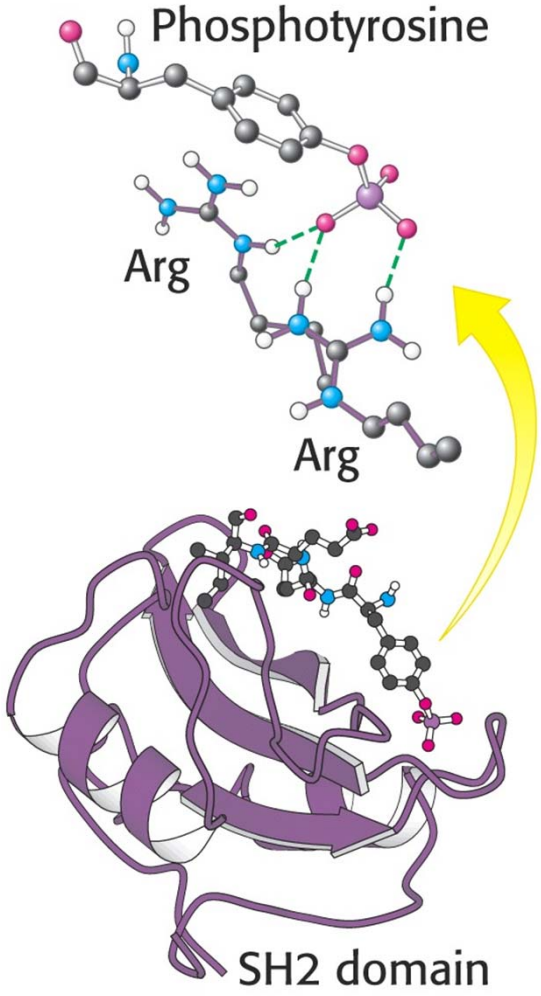
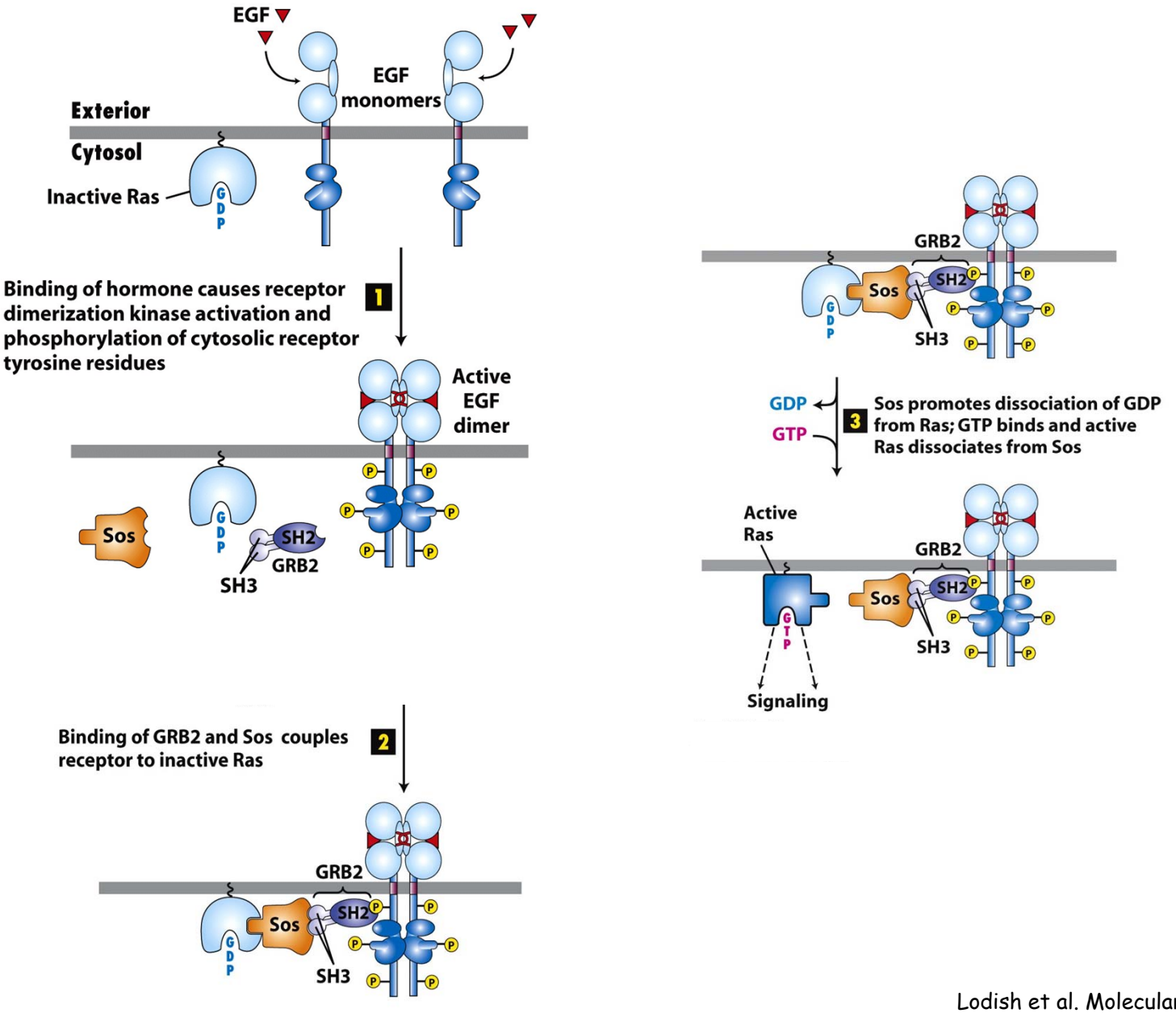


Berg, Tymoczko, Stryer: Biochemistry, 2002

Each SH2 domain binds to a distinct sequence of amino acids at the C-terminus of Tyr-P.

Signal transduction from RTKs to effector proteins



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Surface model of an SH3 domain bound to a target peptide

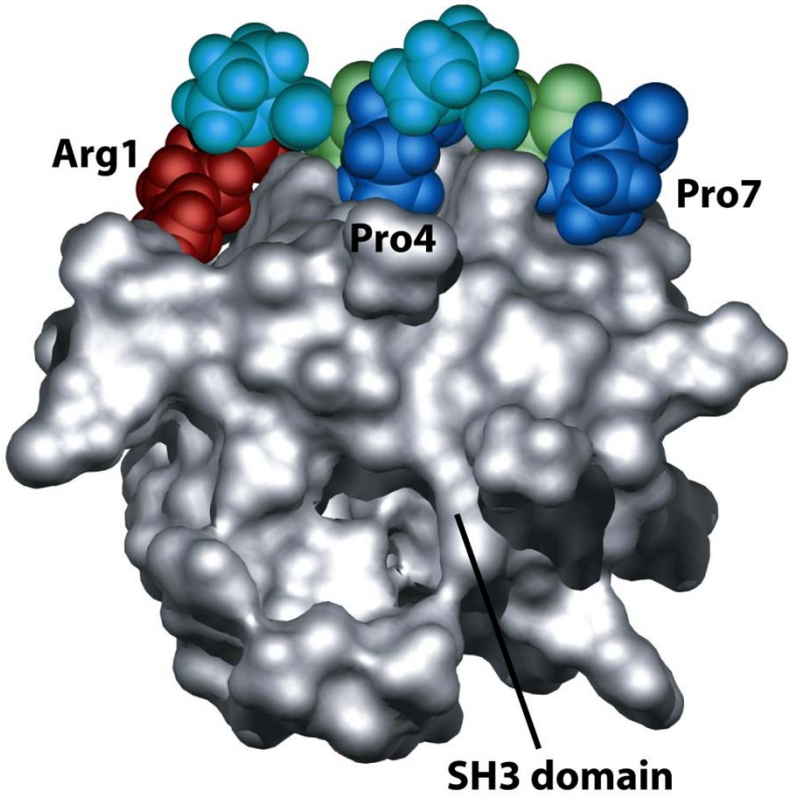

Figure 16-23
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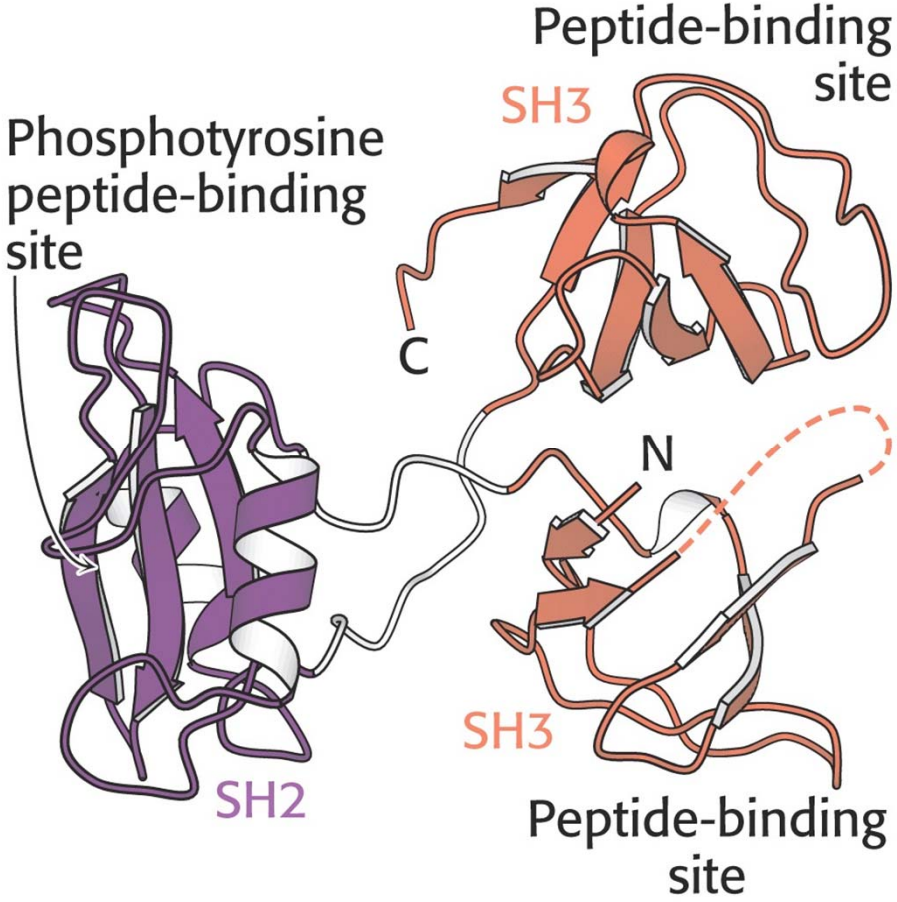
Structure of the adapter protein Grb

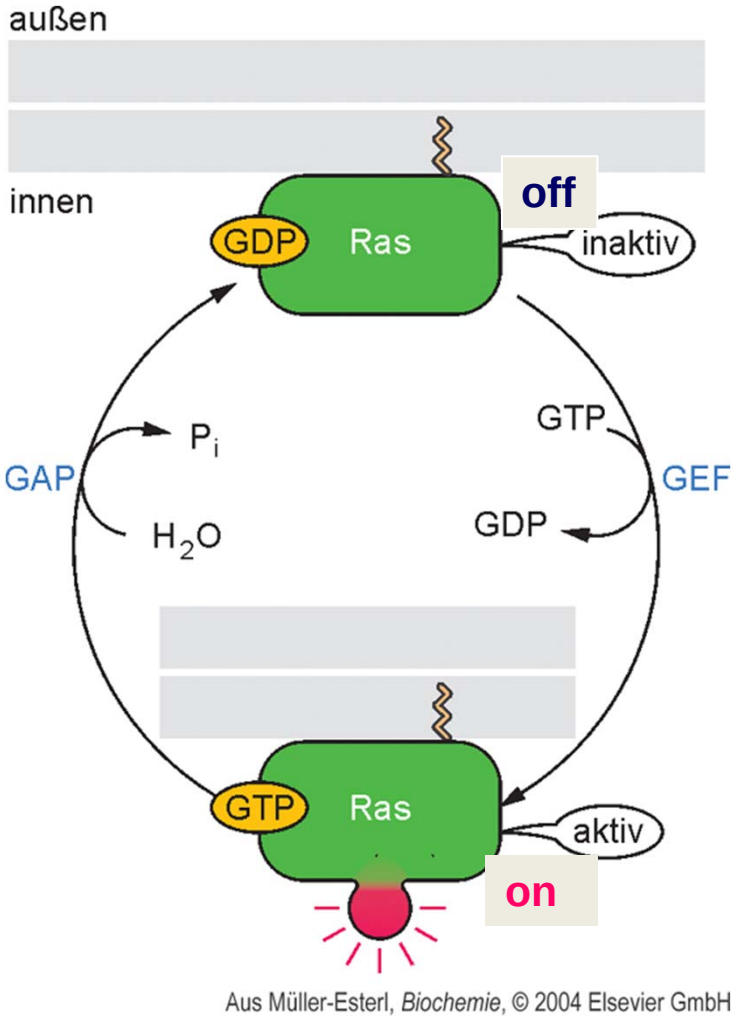
(***g****rowth factor* ***r****eceptor* ***b****inding protein)*



Berg, Tymoczko, Stryer: Biochemistry, 2002

SH2-domain recognizes distinct AA-sequence at C-terminus of P-Tyr on RTK
SH3-domain recognizes prolin-rich sequence of the GEF (Sos)

Regulation of the GTPase switch in the monomeric G-proteins (Ras)

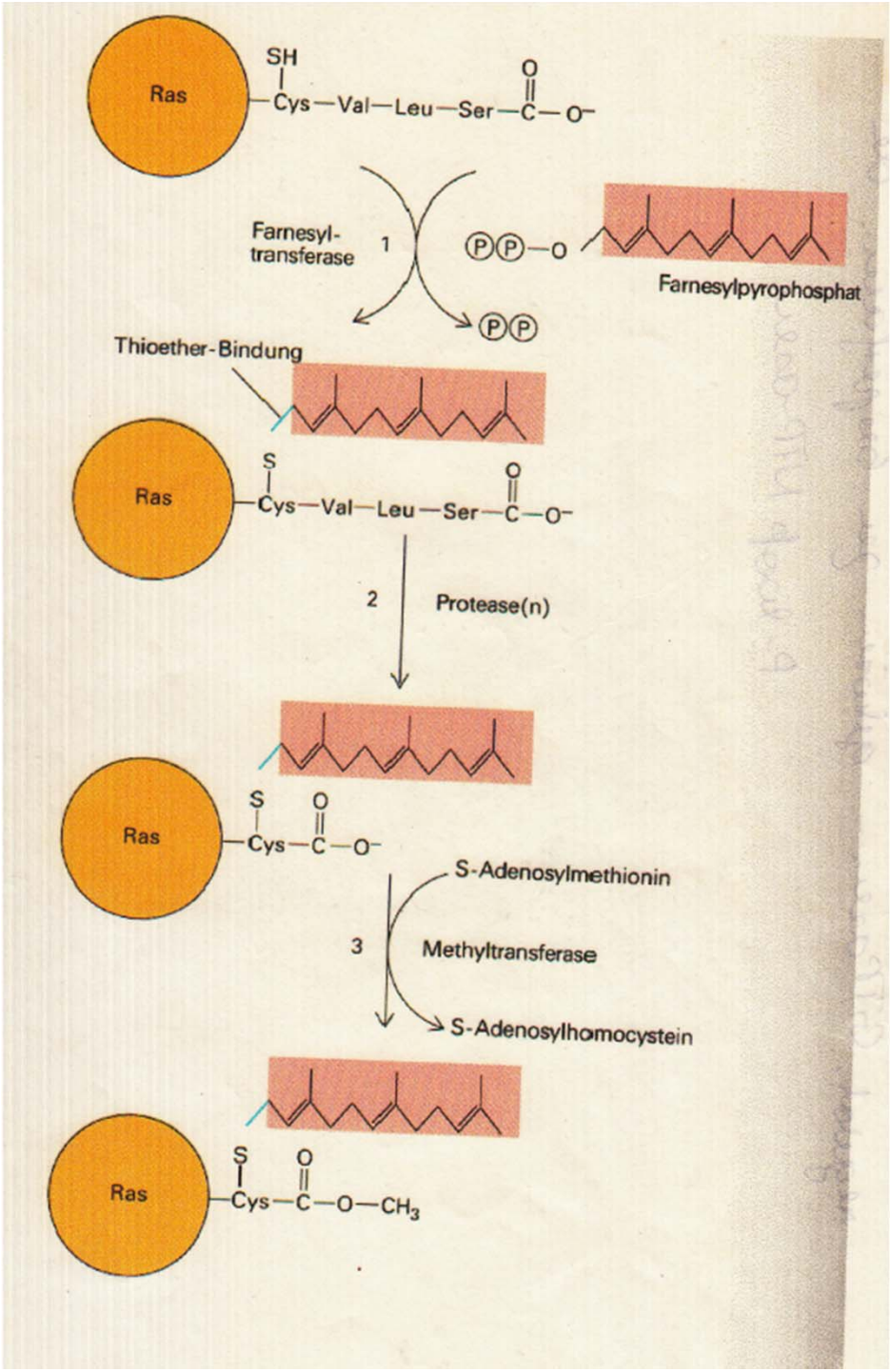


Aus Müller-Esterl, *Biochemie*, © 2004 Elsevier GmbH

Activation of Ras by replacement of GDP with GTP is promoted by GEF-proteins ;
Inactivation of Ras by hydrolysis of GTP is accelerated by GAP;
Inhibition of GAP blocks GTP-hydrolysis thus leading to a persisting activation of Ras.

GEF: Guanine Nucleotide Exchange Factor
GAP: GTPase Activating Protein

Ras wird mittels Farnesylierung in der Plasmamembran verankert



The compound eye of *Drosophila melanogaster*

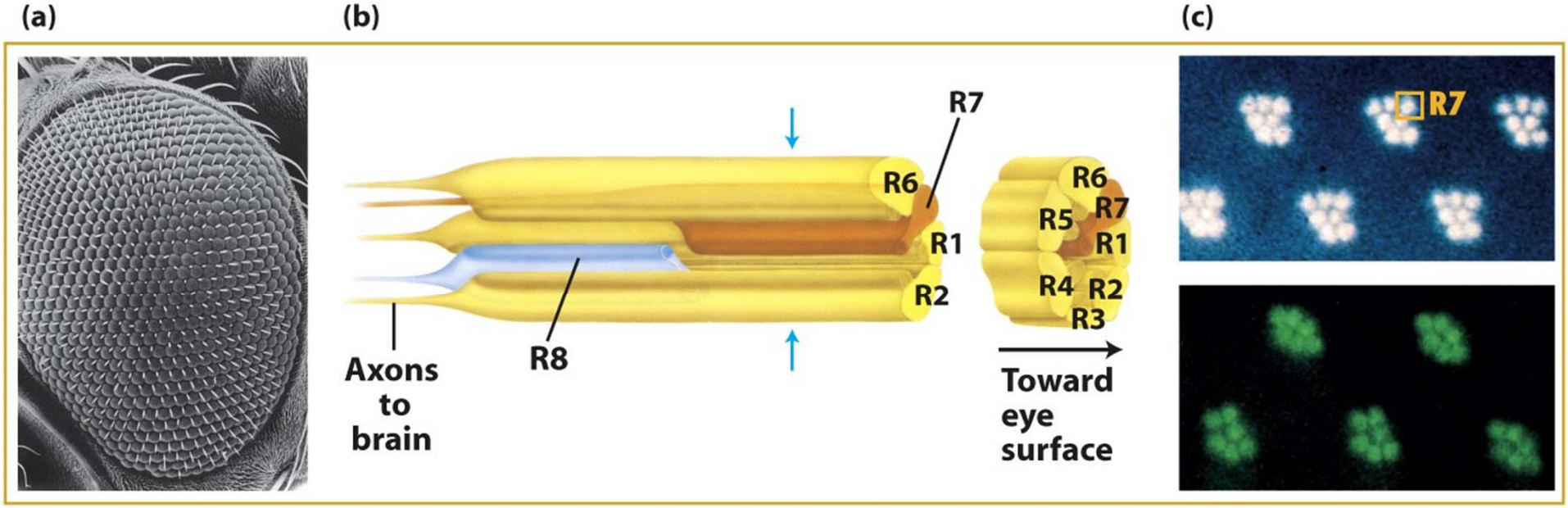


Figure 16-21
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(a) Komplexauge aus ca 800 Ommatidien
im Rasterelektronenmikroskop

(b) Längsschnitt bzw. Ausschnitt eines Ommatidiums

(c) Vergleich der Augen vom Wildtyp und der *sevenless* Mutante
(Schnittstelle ist in (b) durch blaue Pfeile angezeigt)

Genetic studies reveal that activation of Ras induces development of R7 photoreceptor in the *Drosophila* eye

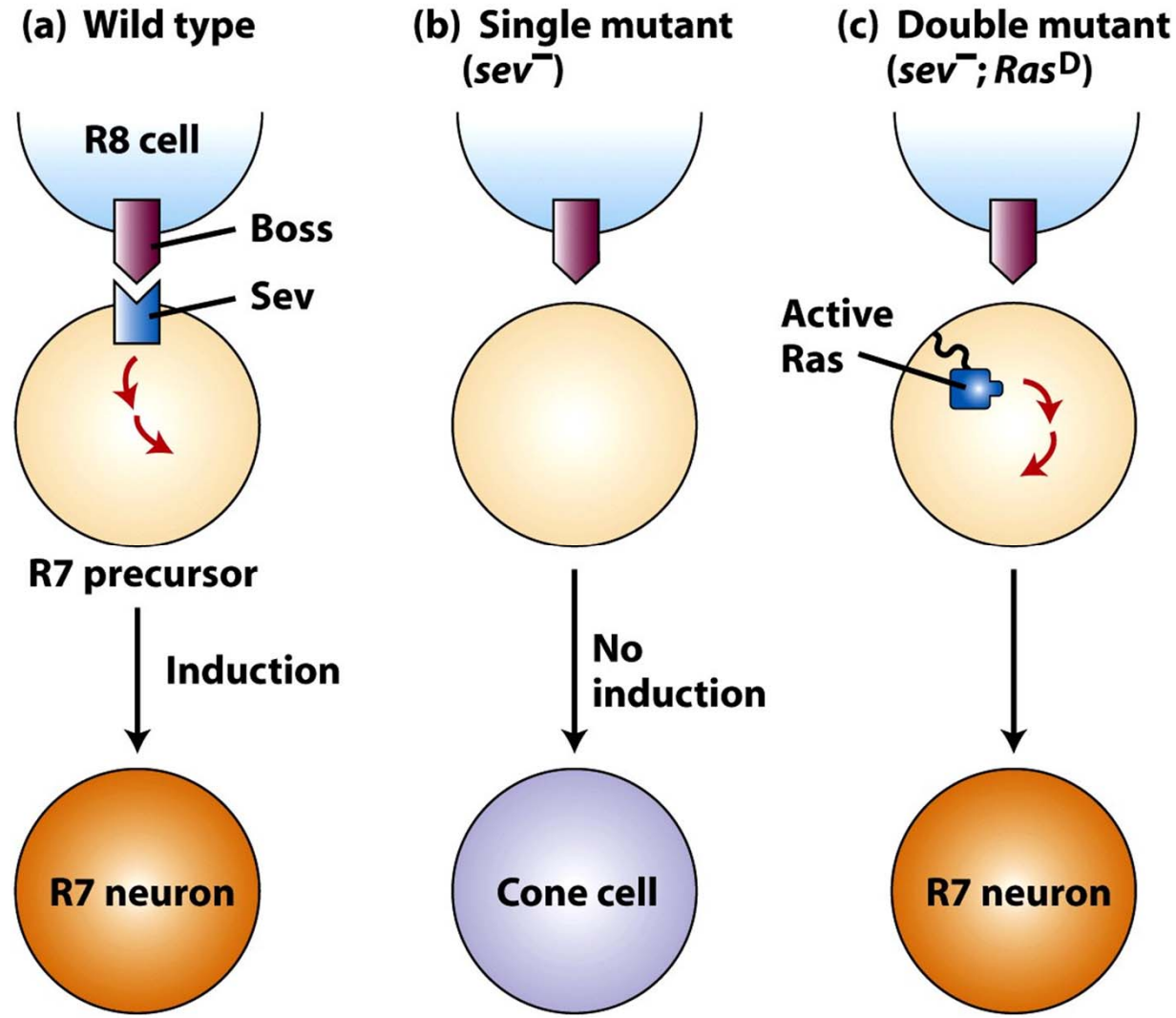

Figure 16-22
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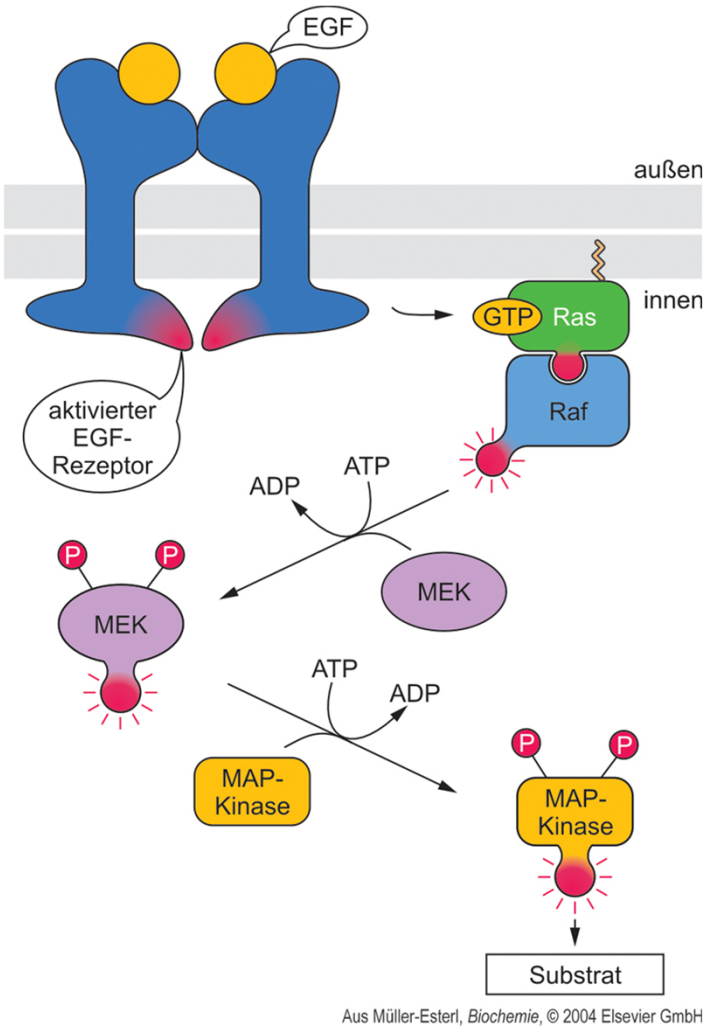
GTP-Ras triggers the MAP-Kinase-Signaling Pathway



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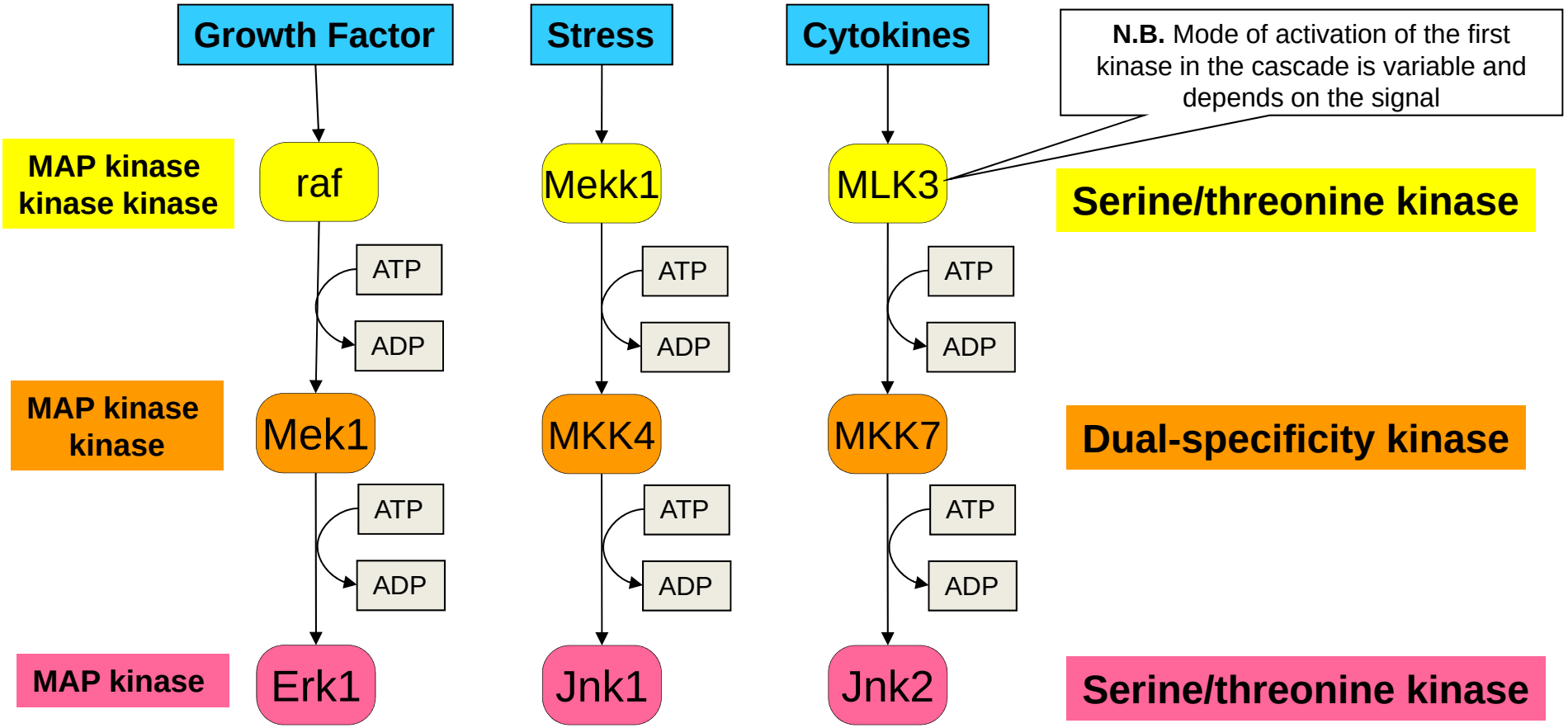
GTP-Ras triggers the MAP-Kinase cascade via three enzymatic steps:

1. **Raf** (Ras-activated factor)
2. **MEK** (MAP/ERK-Kinase, also MAP-Kinase-Kinase) and
3. **MAP**-Kinase (Mitogen-activated Protein-Kinase; synonym with ERK, *extracellular signal regulated kinase*).

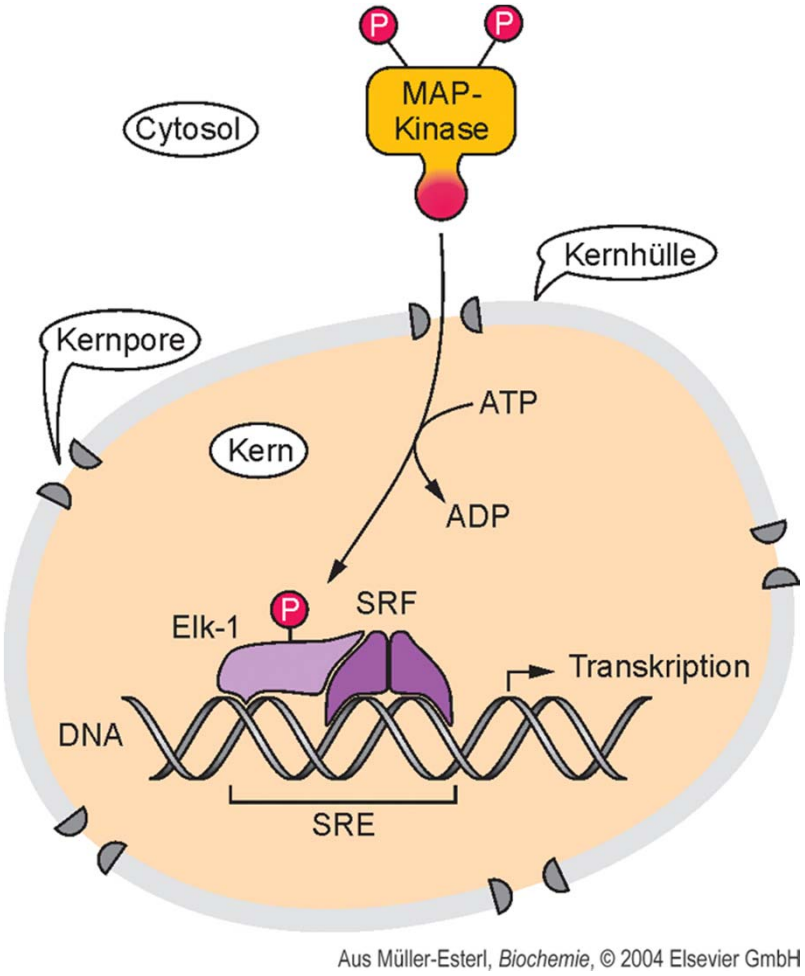
These kinases are successively phosphorylated and thus activated; thereby each downstream kinase represents the specific substrate for the upstream enzyme.
The most common MAP-kinases are ERK-1 und ERK-2.

Cascading Kinases

The raf → Mek-1 → Erk-1 cascade is one example of a MAP kinase cascade. Although these cascades utilise specific kinases, the pathways are very similar.



Phosphorylierung von Elk-1 durch MAP-Kinase



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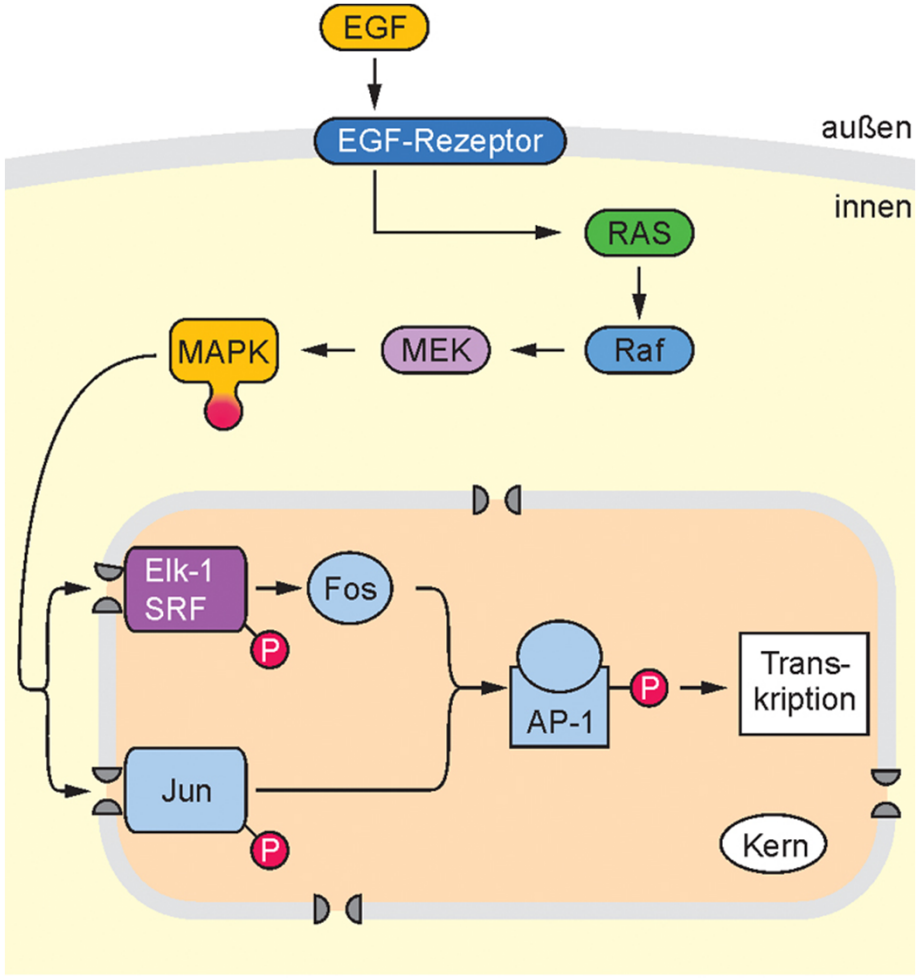
In den Kern translozierte MAP-Kinase phosphoryliert Elk-1 (engl. *ets-like protein*), das im Komplex mit SRF (engl. *serum responsive factor*) an die regulatorische Promotorsequenz SRE (engl. *serum responsive element*) bindet.

Der aktivierte Komplex aus Elk-1 und SRF stimuliert die Expression zahlreicher Zielgene.

Ras-abhängige Regulation der Genexpression



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Der über den MAP-Kinasen-Weg aktivierte Komplex aus Elk-1 und SRF aktiviert das *fos*-Gen.

Sein Genprodukt Fos bildet mit Jun, das wiederum von MAP-Kinase phosphoryliert wird, den Komplex AP-1, der dann weitere Gene aktiviert.

Ras/MAP kinase pathway

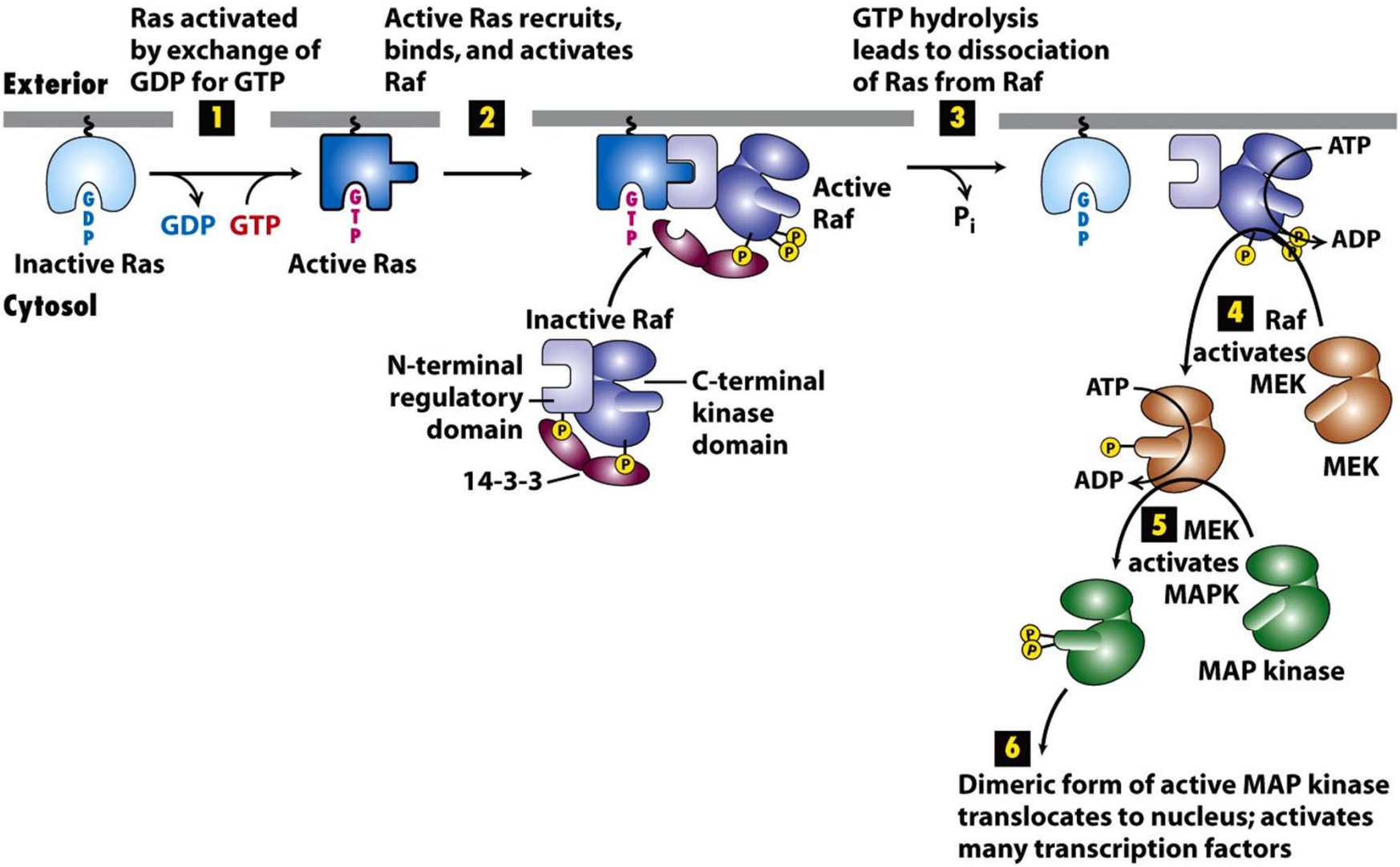

Figure 16-25
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MAP kinase regulates the activity of many transcription factors

TCF, ternary complex factor

p90^{RSK}, ribosomal S6 kinase

SRE, serum response elements

SRF, serum response factor

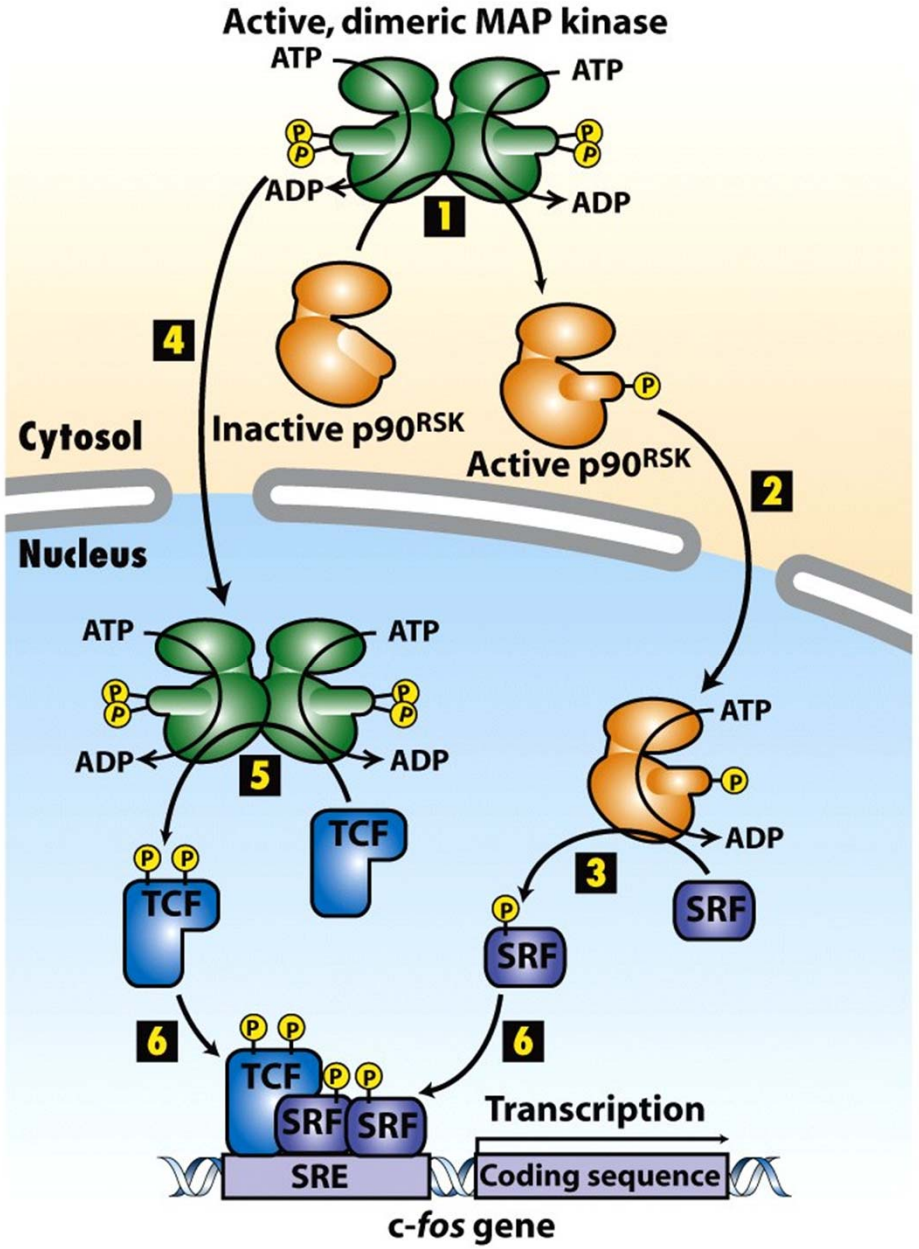

Figure 16-27
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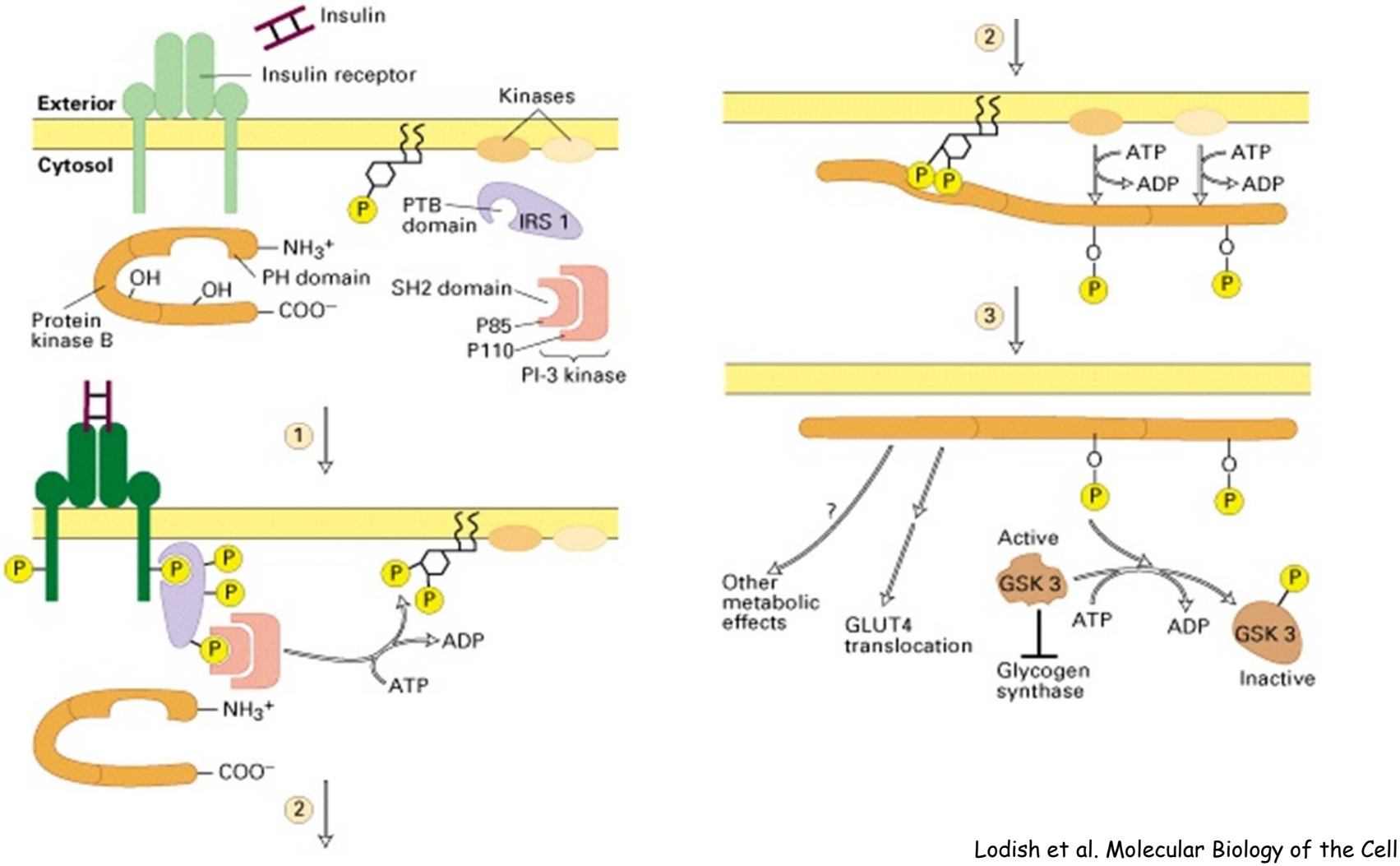
Insulin activates PKB via PI-3 kinase

A Ras independent pathway activates Protein Kinase B



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Ras Proteins

- Related proteins with similar structure, function
  - H-*ras* (Harvey-ras murine sarcoma virus)
  - K-*ras* (Kirsten-ras murine sarcoma virus)
  - N-*ras* (Neuroblastoma)
- 21 kDa proteins ($p21^{ras}$)
- Ras proteins relay signals from receptor tyrosine kinases to nucleus to stimulate cell proliferation or differentiation
- Ras proteins active with GTP bound, inactive with GDP bound
- Receptor tyrosine kinases can activate
  - GTPase-activating protein (GAP → inactive Ras)
  - or Guanine-nucleotide Releasing Protein (GEF, Sos → active Ras)

Single Nucleotide Change→ H-*ras* Oncogene

	1	2	3	4	5	6	7	8	9	10	11	12	13		188	189
Normal	Met	Thr	Glu	Tyr	Lys	Leu	Val	Val	Val	Gly	Ala	Gly	Gly		Leu	Ser
Human *ras*H	ATG	ACG	GAA	TAT	AAG	CTG	GTG	GTG	GTG	GGC	GCC	GGC	GGT	.....	CTC	TCC
Activated EJ *ras*H	Met	Thr	Glu	Tyr	Lys	Leu	Val	Val	Val	Gly	Ala	GTC Val	Gly	.....	Leu	Ser

- Mutations in codon 12 → reduced GTPase activity
- Ras remains active longer than appropriate
- Overstimulation of MAP-kinase pathway

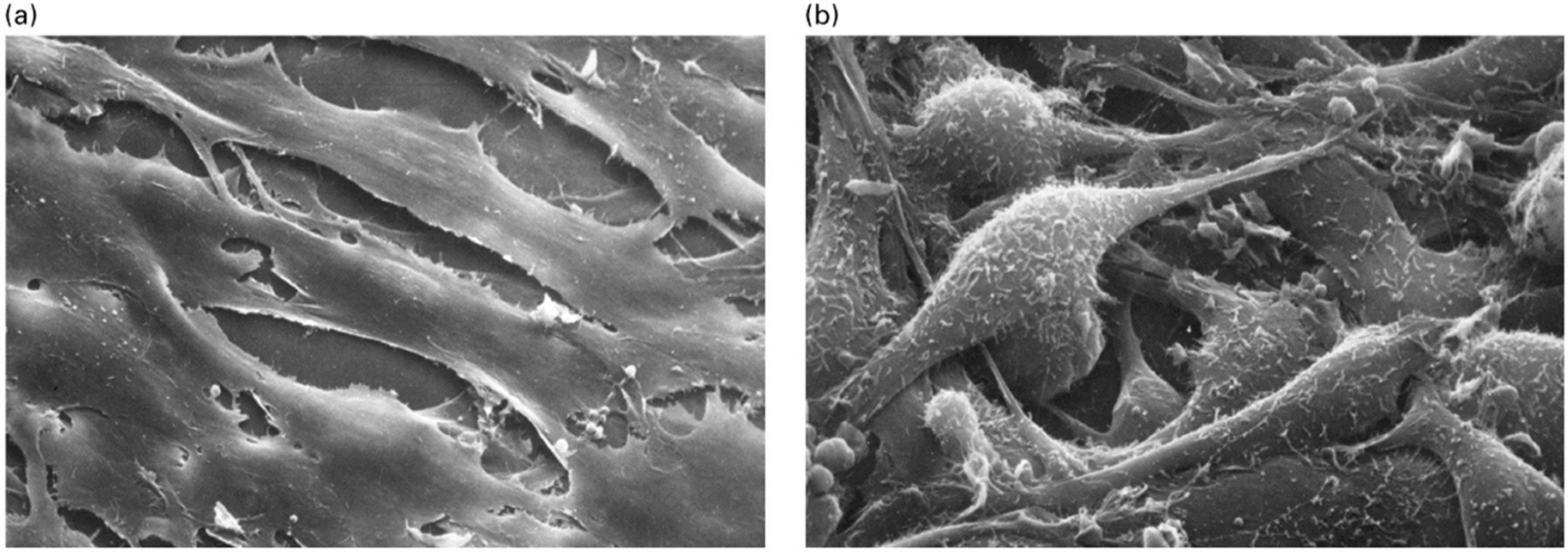
About 25% of <u>all</u> tumors have mutations inactivating Ras GTPase

Oncogenes (Cooper)

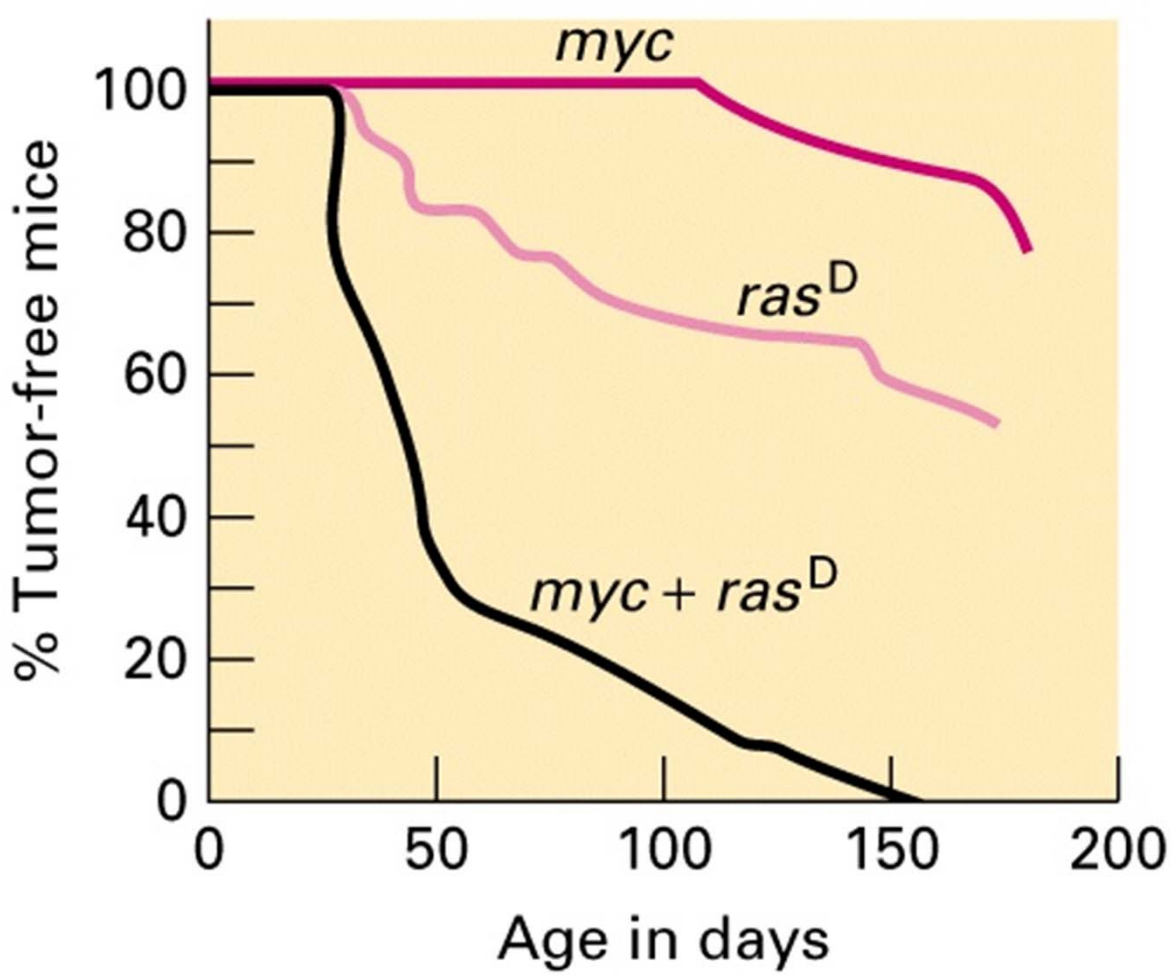
Cells that continue to grow when normal cells have become quiescent are said to be transformed

Transformed cells may exhibit many of the properties of malignant tumor cells

normal **transformed**



Overexpression of multiple oncogenes increases tumor formation



The HER family of RTKs and their ligands

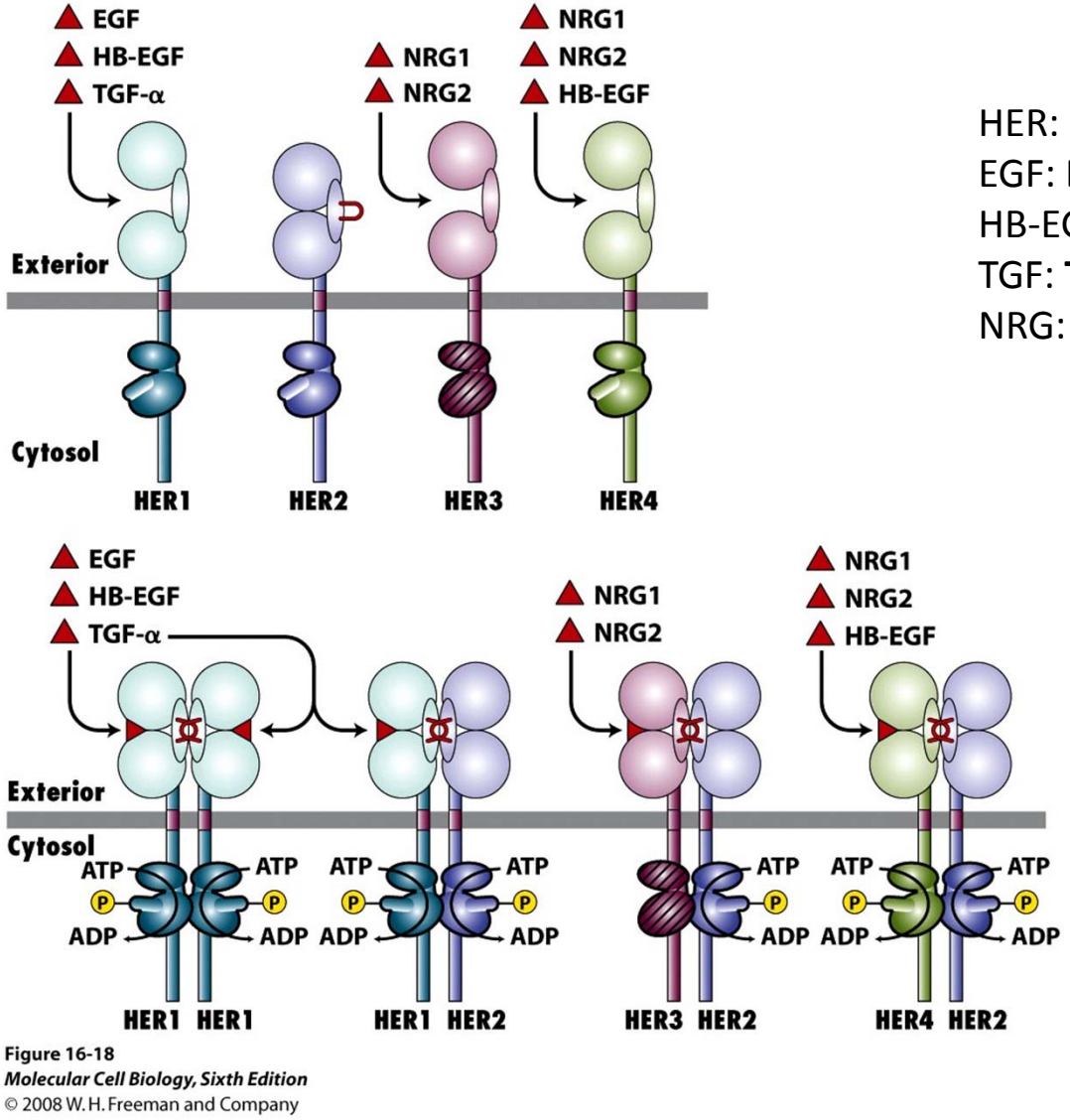


Figure 16-18
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HER: **H**uman **E**GF-**R**eceptor
EGF: **E**pidermal **G**rowth **F**actor
HB-EGF: **H**eparin **B**inding-EGF
TGF: **T**umor-Derived **G**rowth **F**actor
NRG: **N**euro**reg**ulins

Amplification of *Her2* gene occurs in ~ 25% of breast cancer patients.

Tyrosine Kinase gekoppelte Membranrezeptoren

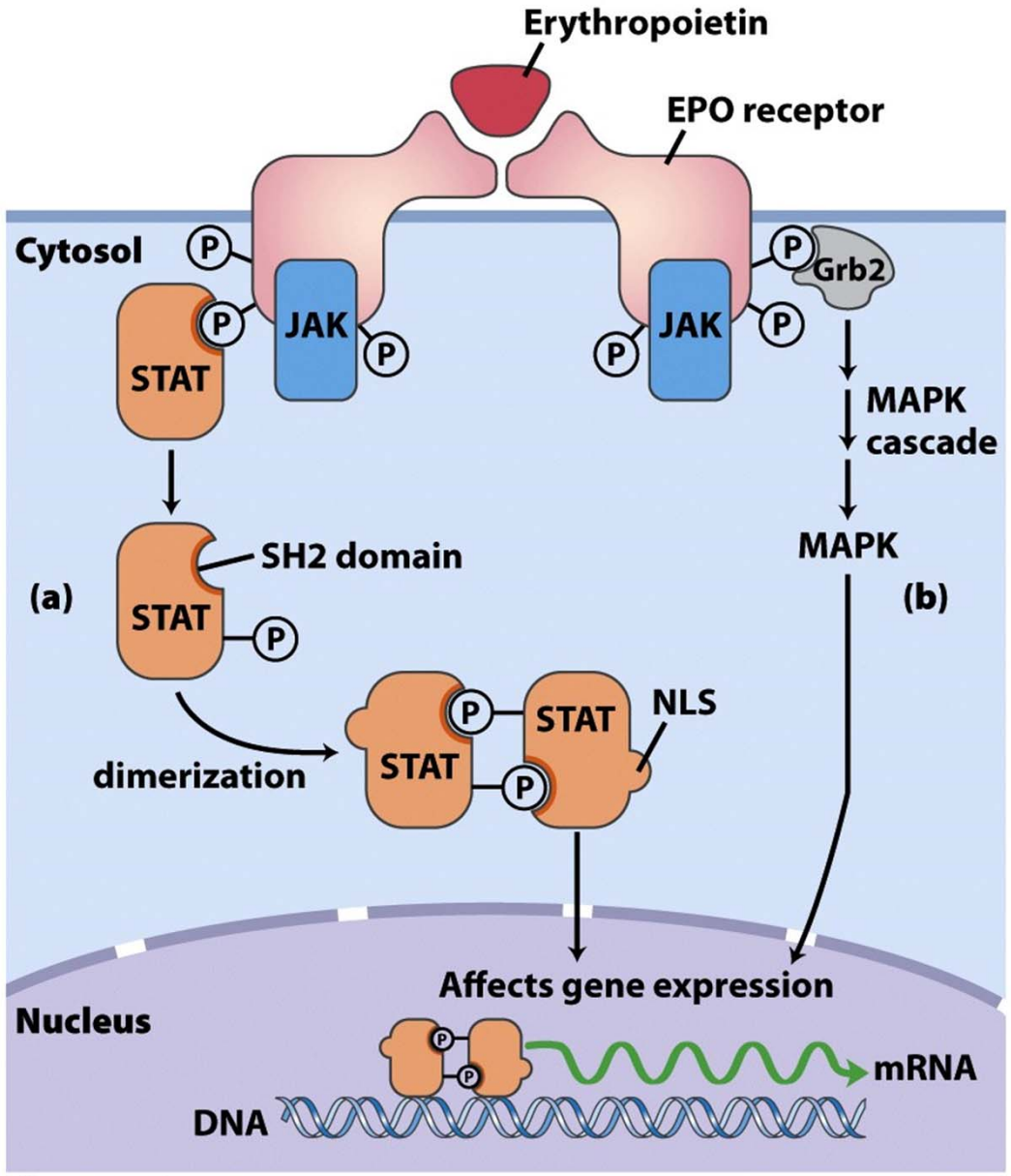
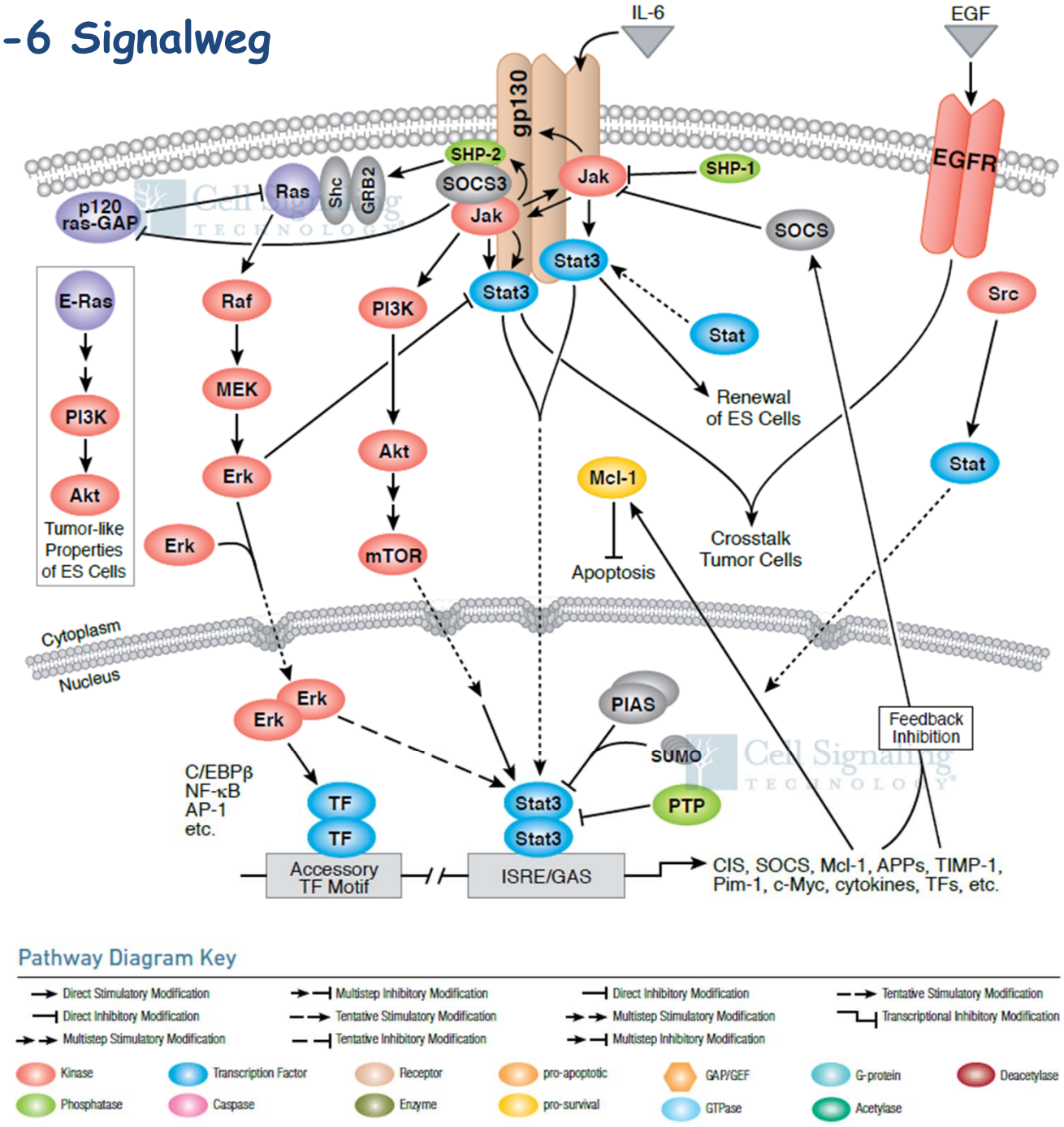


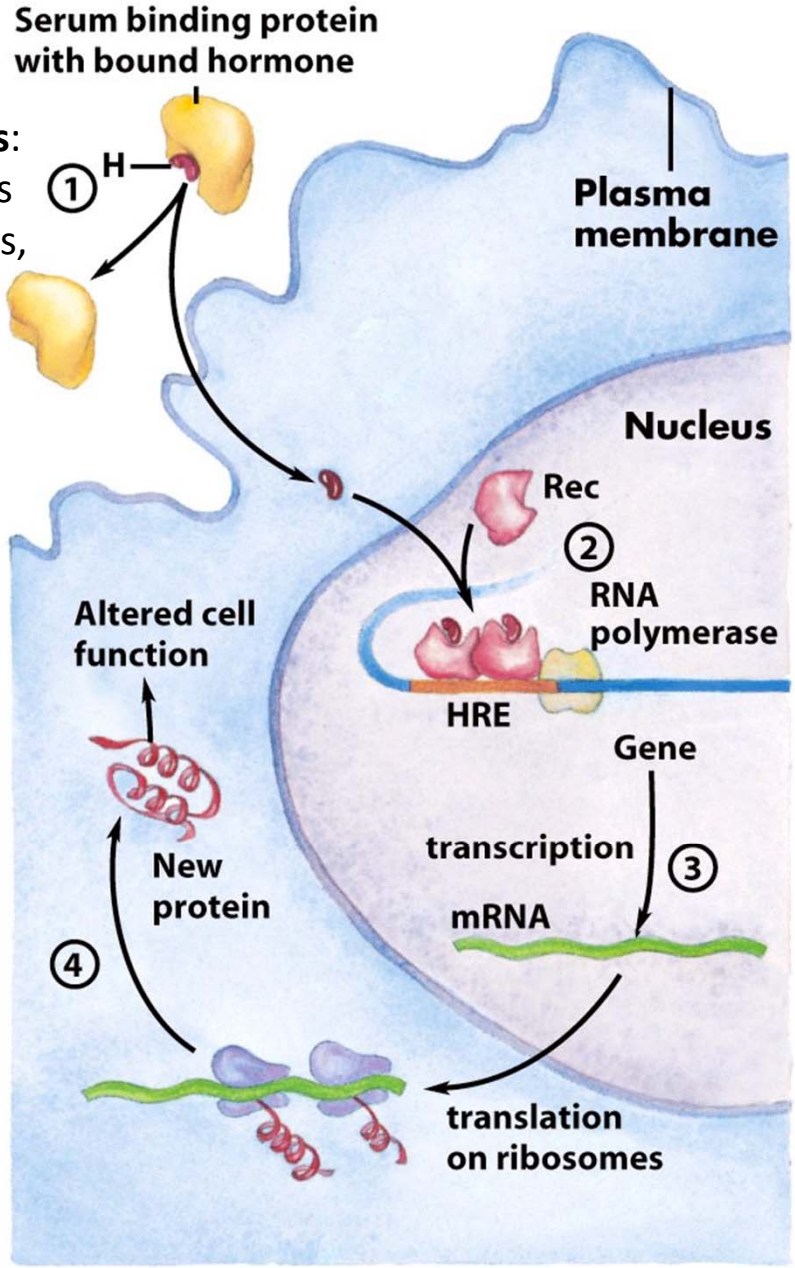
Figure 12-18
Lehninger Principles of Biochemistry, Fifth Edition

Interleukin-6 Signalweg



General mechanism by which lipophilic compounds regulate gene expression

Lipophilic ligands:
steroid hormones
thyroid hormones,
retinoids (vit. A)
vitamin D



1 Hormone (H), carried to the target tissue on serum binding proteins, diffuses across the plasma membrane and binds to its specific receptor protein (Rec) in the nucleus.

2 Hormone binding changes the conformation of Rec; it forms homo- or heterodimers with other hormone-receptor complexes and binds to specific regulatory regions called hormone response elements (HREs) in the DNA adjacent to specific genes.

3 Binding regulates transcription of the adjacent gene(s), increasing or decreasing the rate of mRNA formation.

4 Altered levels of the hormone-regulated gene product produce the cellular response to the hormone.

Nelson, Cox: Lehninger Biochemistry,

Phosphoinositides as Signal Transducers

- Phospholipase C: different isoforms are activated by different signals that bind either GPCR or RTK

- PI-3 kinase pathway

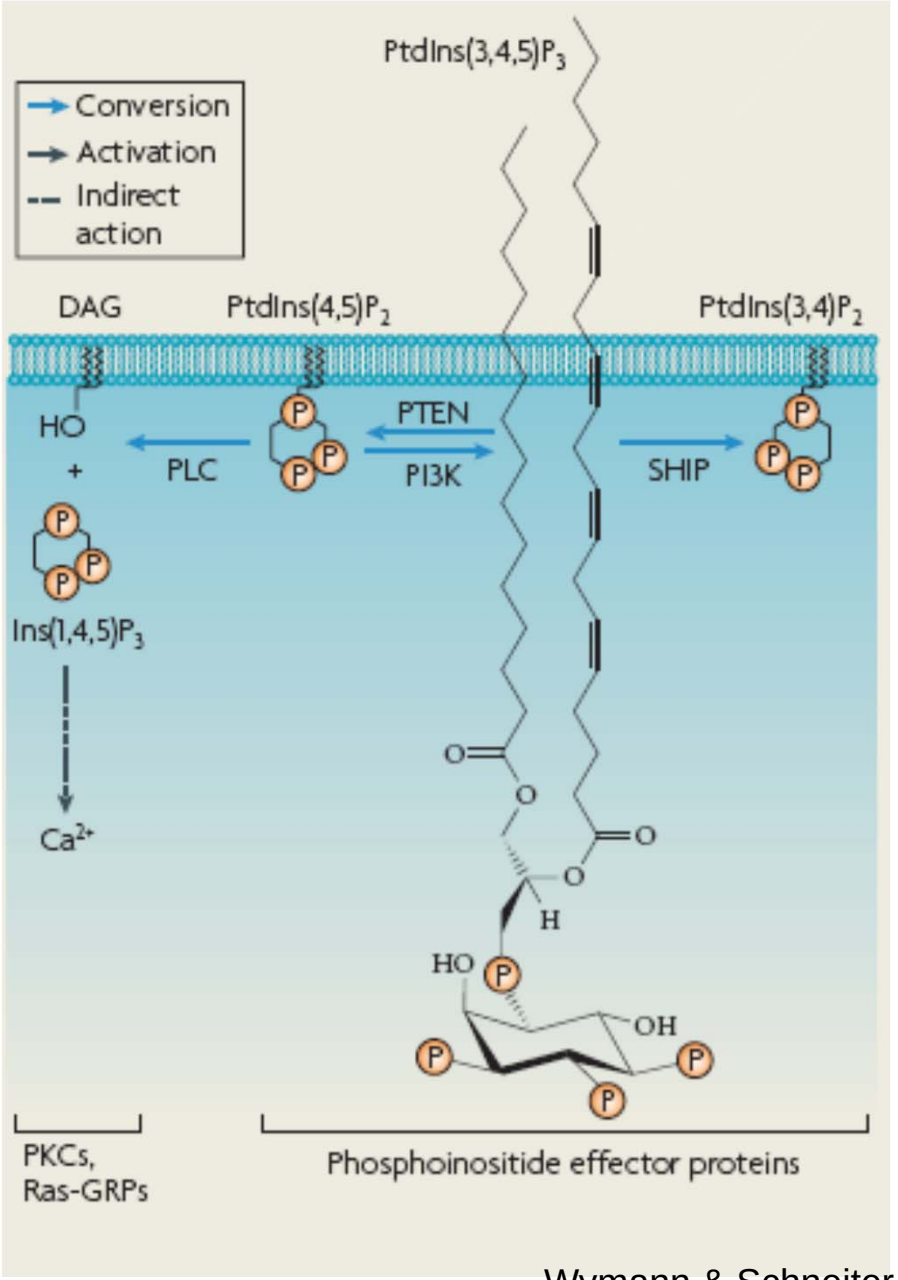
Intracellular signalling by phosphoinositids

Nature has used several possible versions of the inositol head group to create highly specific docking sites for lipid binding proteins

PTEN: phosphatase and tensin homologue deleted on chromosome ten

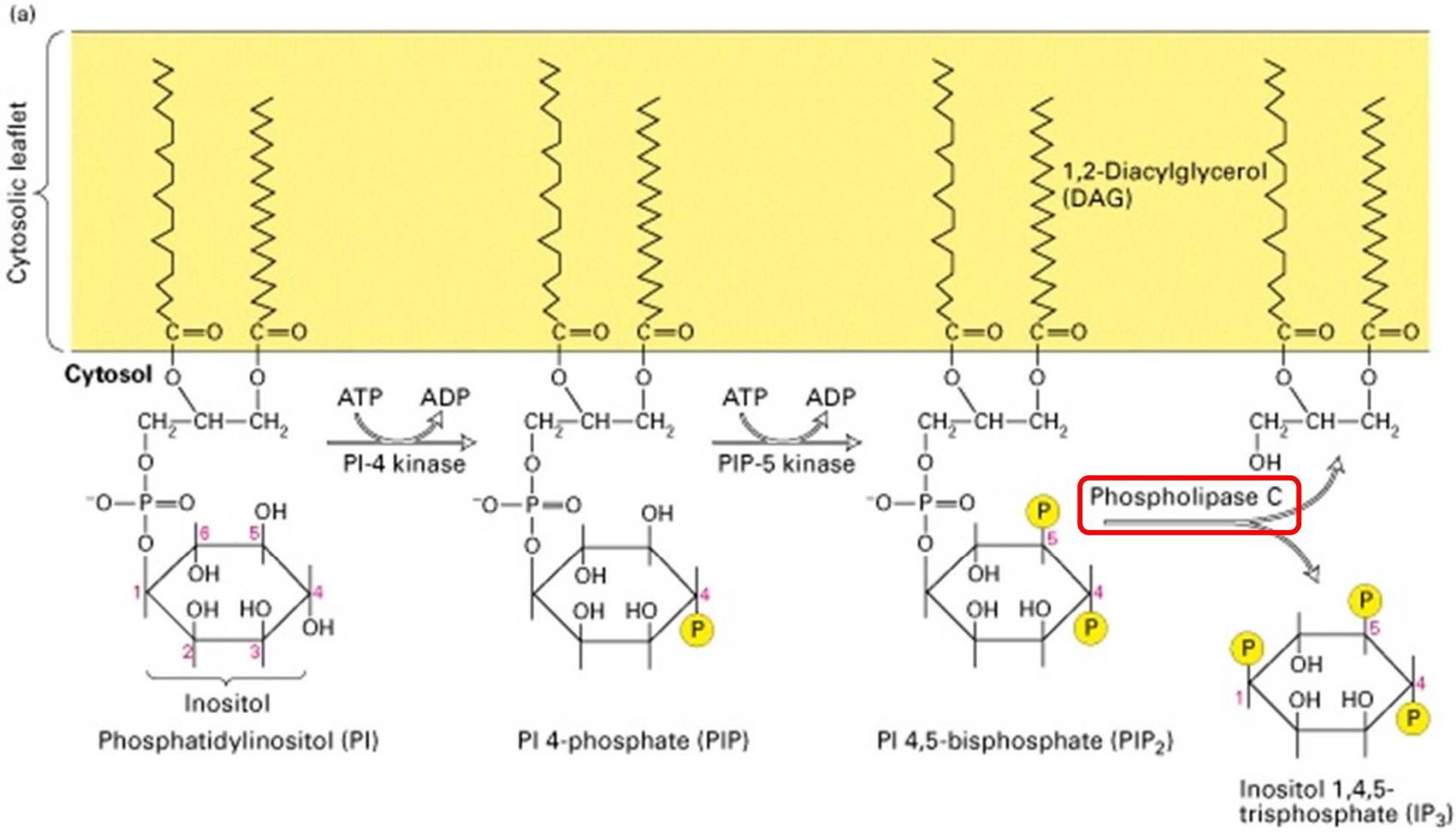
SHIP: Src-homology-2 (SH2) domain-containing inositol 5-phosphatase

Ras-GRP: Ras guanyl nucleotide-releasing protein



Wymann & Schneiter, Nature, 2008

Modification of a common phospholipid precursor generates several second messengers: DAG and IP_3



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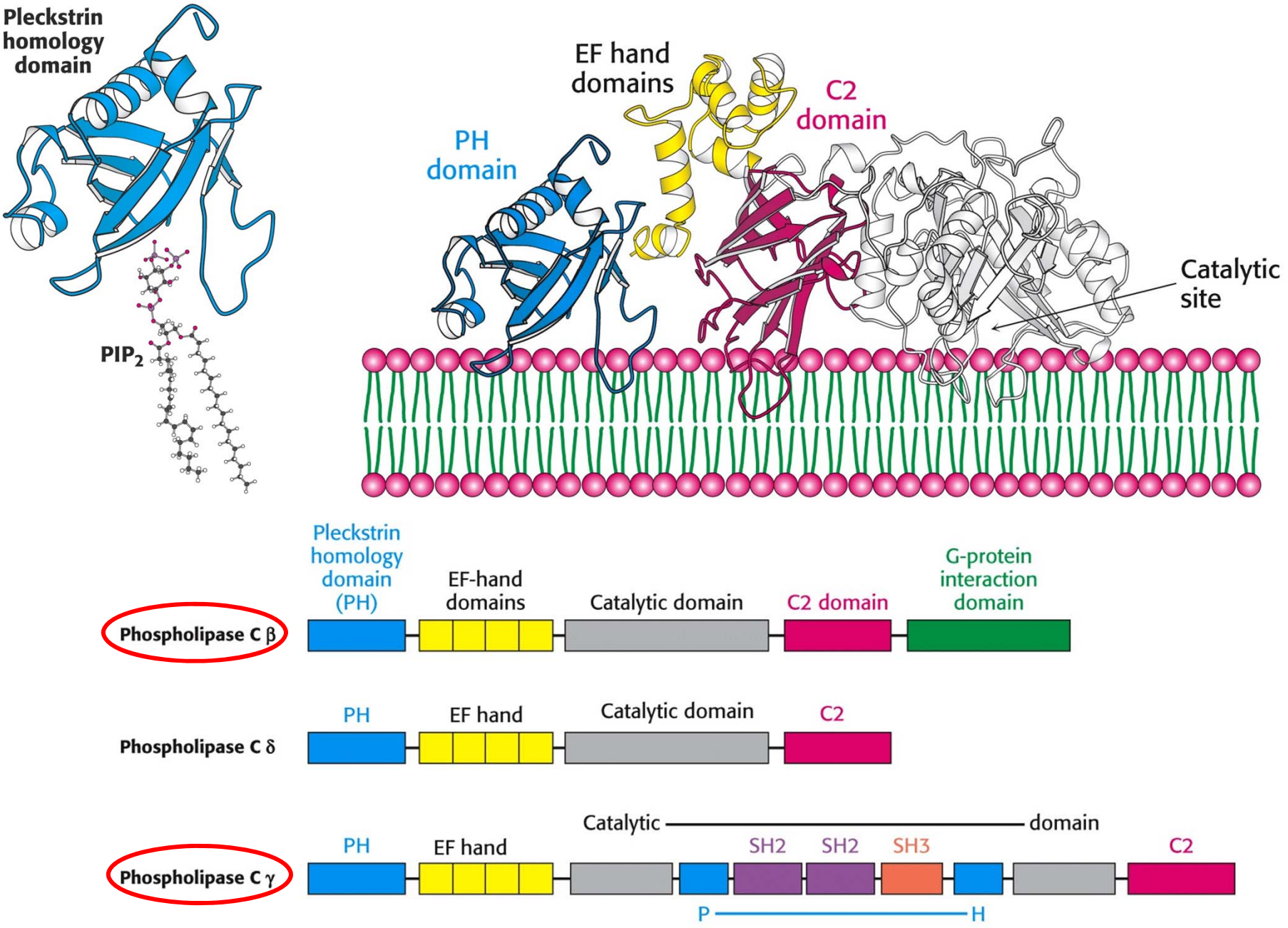
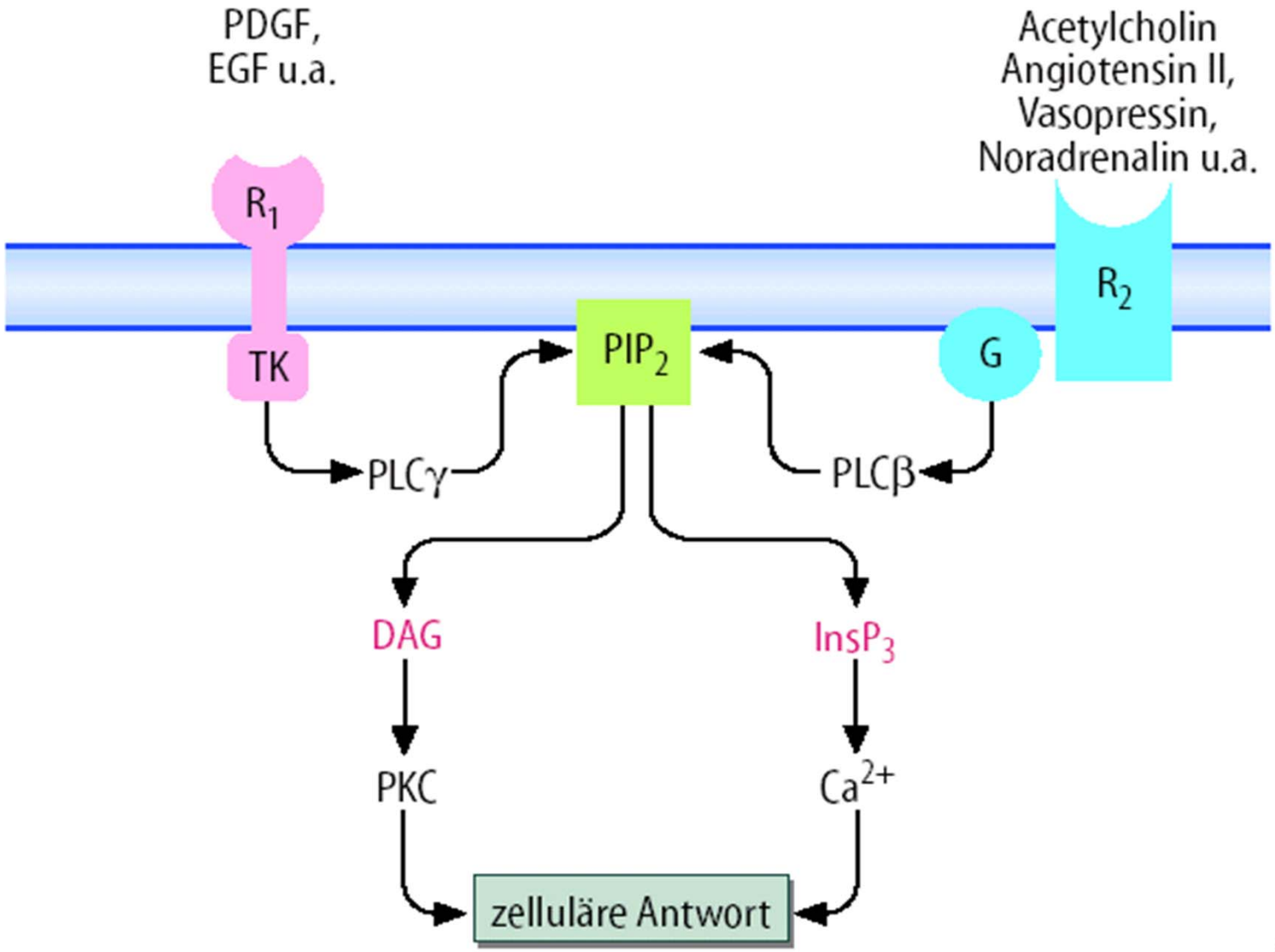
Phospholipase C Isoforms



Berg, Tymoczko, Stryer: Biochemistry, 2002

PLC-induced release of Ca^{2+} from the ER is mediated by IP_3



Löffler, Petrides, Biochemie und Pathobiochemie

PLCβ is an effector targeted by GPCRs

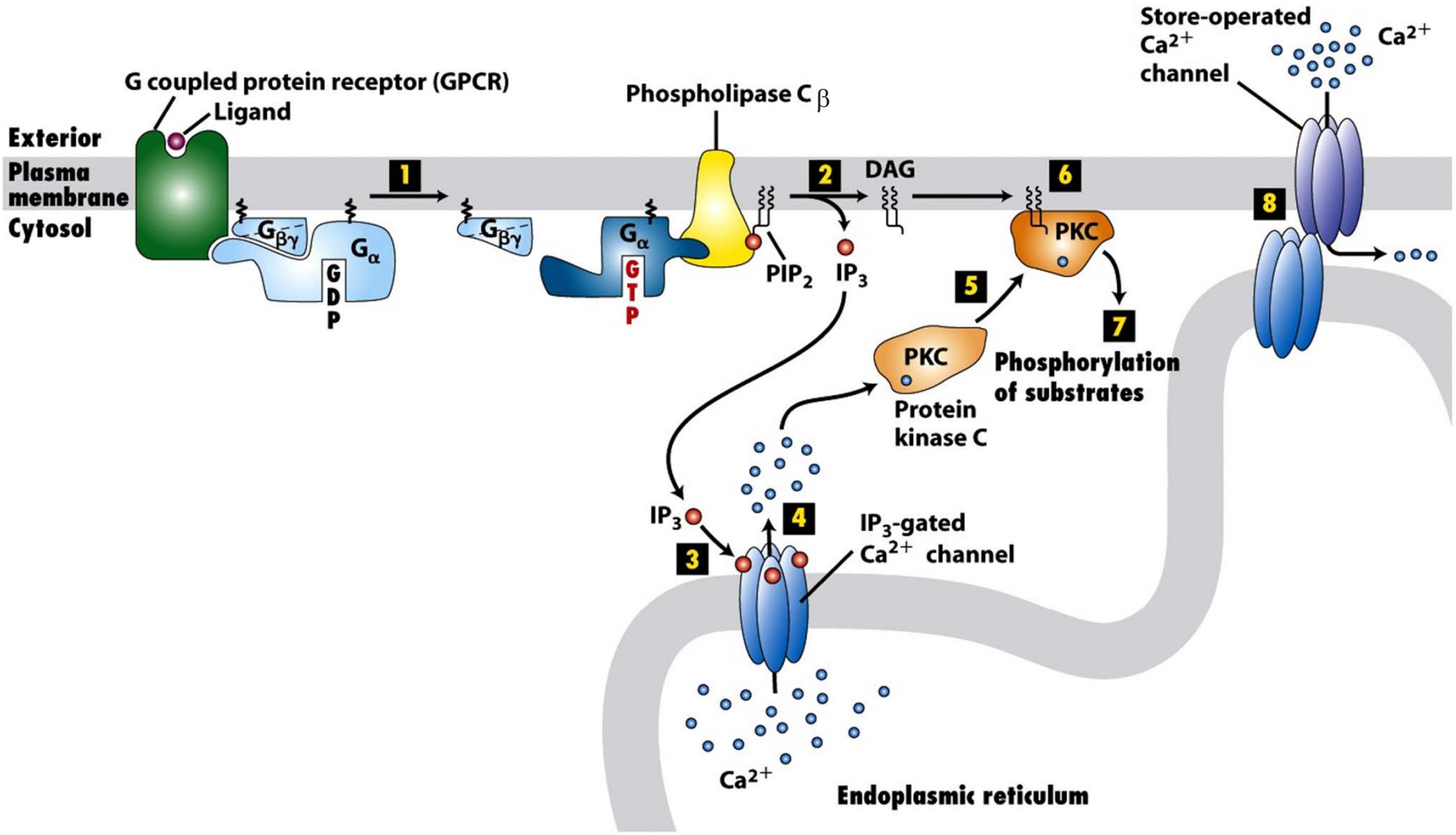
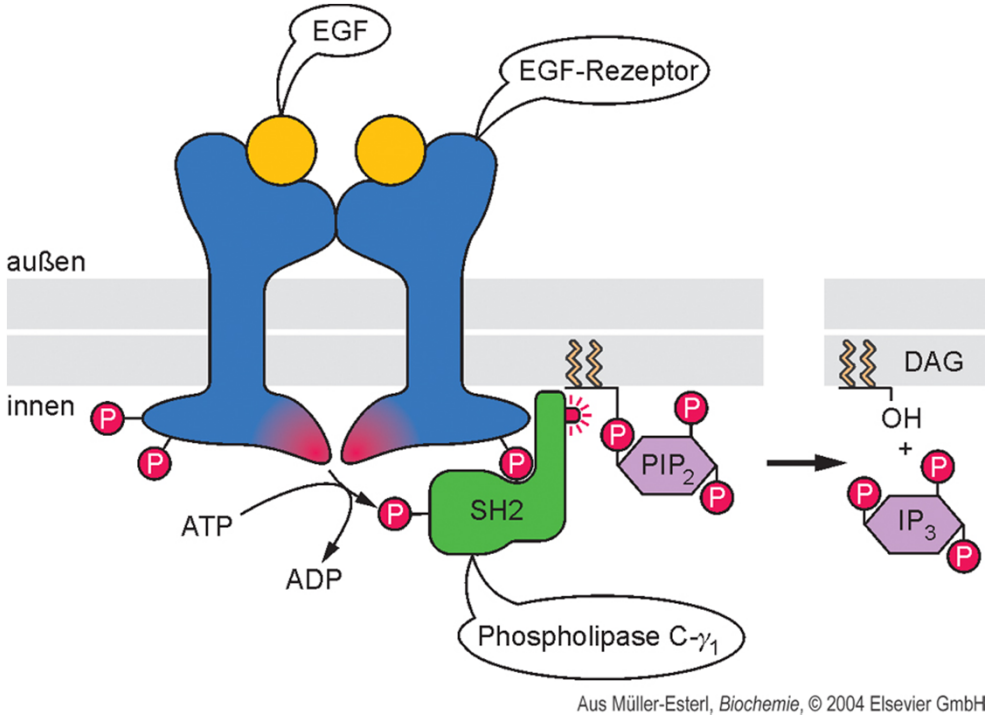


Figure 15-30
Molecular Cell Biology, Sixth Edition

Short term effects on cell metabolism and movement
Long term effects on gene expression

PLCγ_1 is an effector targeted by RTKs

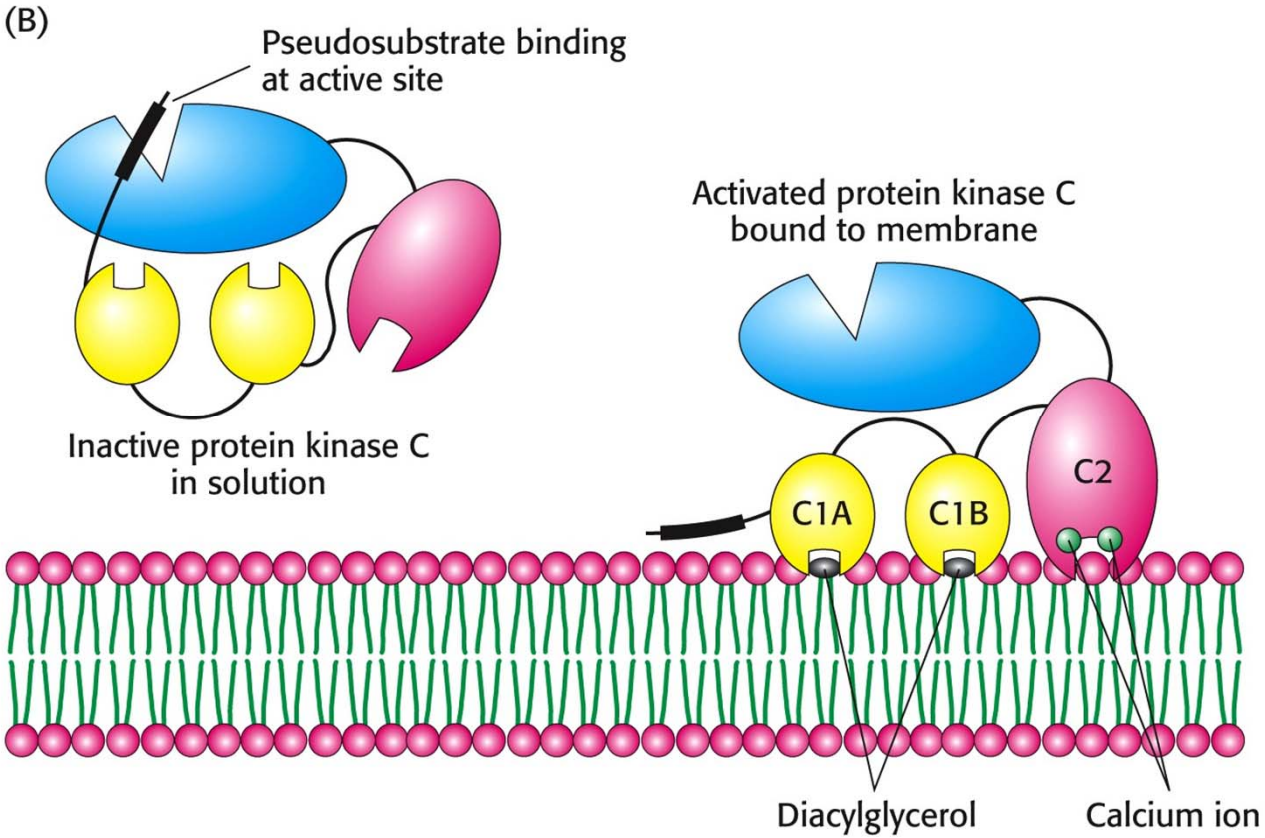


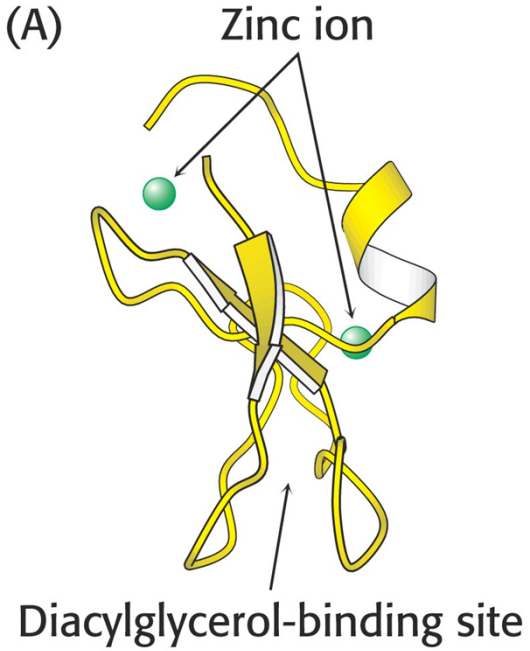
Aus Müller-Esterl, *Biochemie*, © 2004 Elsevier GmbH

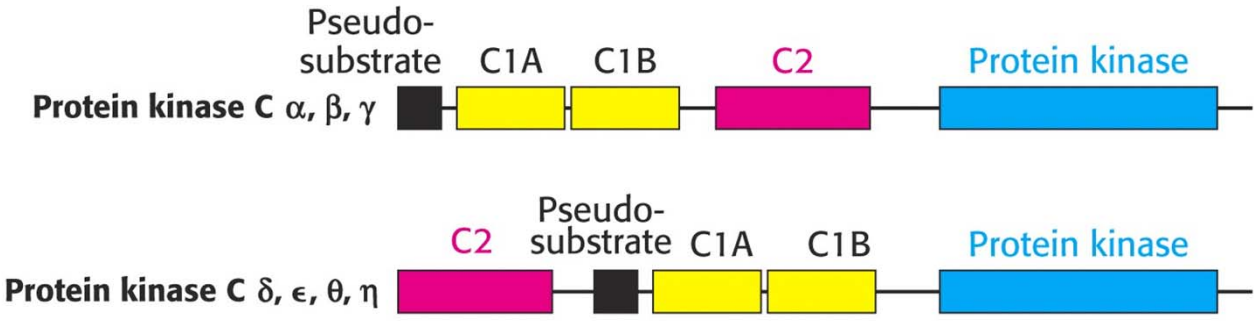
The activated EGF-receptor recruits the cytosolic phospholipase C-γ_1 (substrate PIP_2) via its **SH2-domain** and activates the enzyme by phosphorylation.

Phosphatases terminate this process.

Proteinkinase C







Berg, Tymoczko, Stryer: Biochemistry,

PI 3-phosphates recruit and activate protein kinase B (PKB)

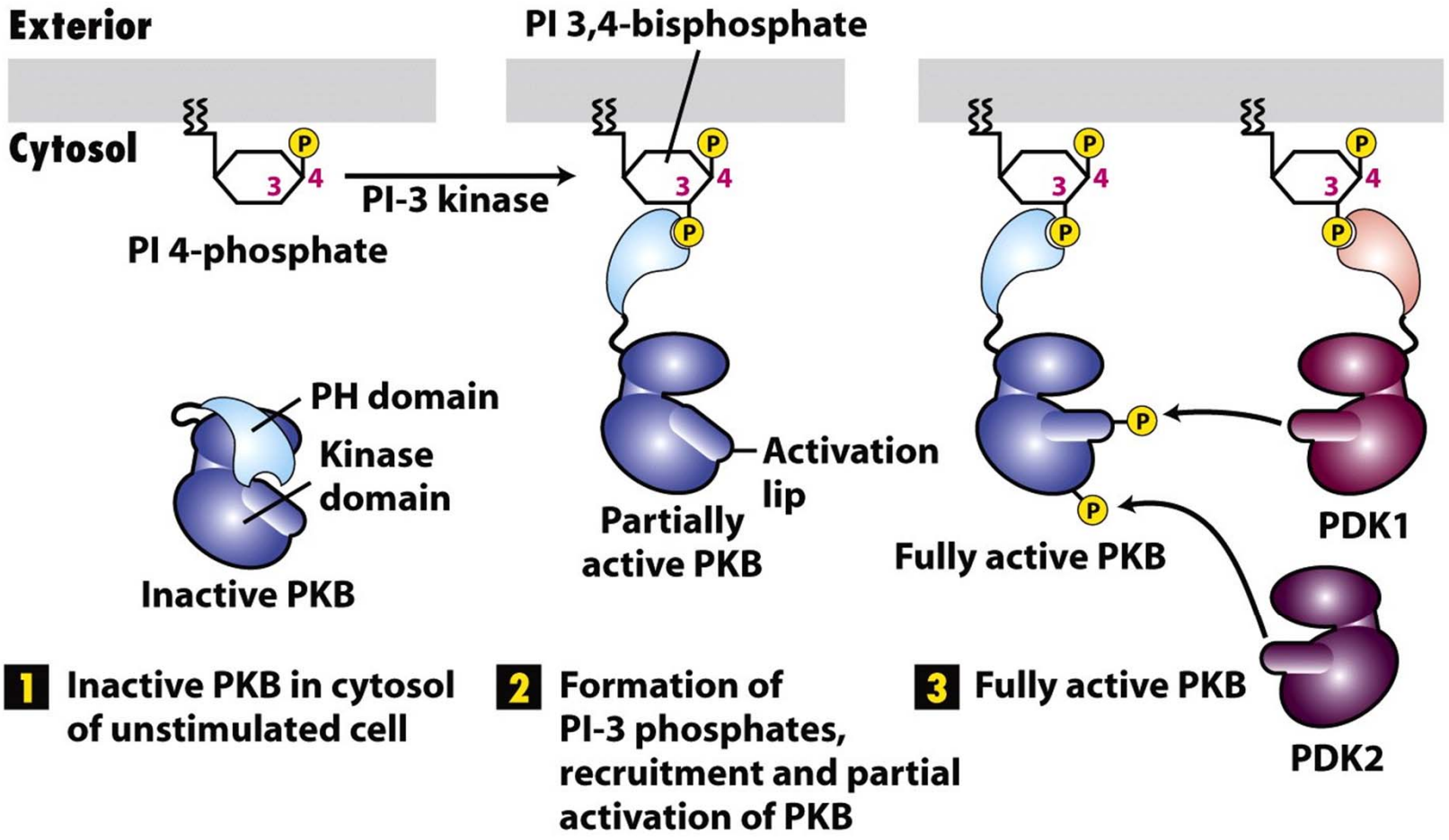


Figure 16-30
Molecular Cell Biology, Sixth Edition

PH: **p**leckstrin **h**omology
PDK: **P**I-**d**ependent **k**inase

PI-3 kinase generates phosphatidylinositol 3-phosphates, which are binding sites for various signal-transduction proteins, usually triggering survival.

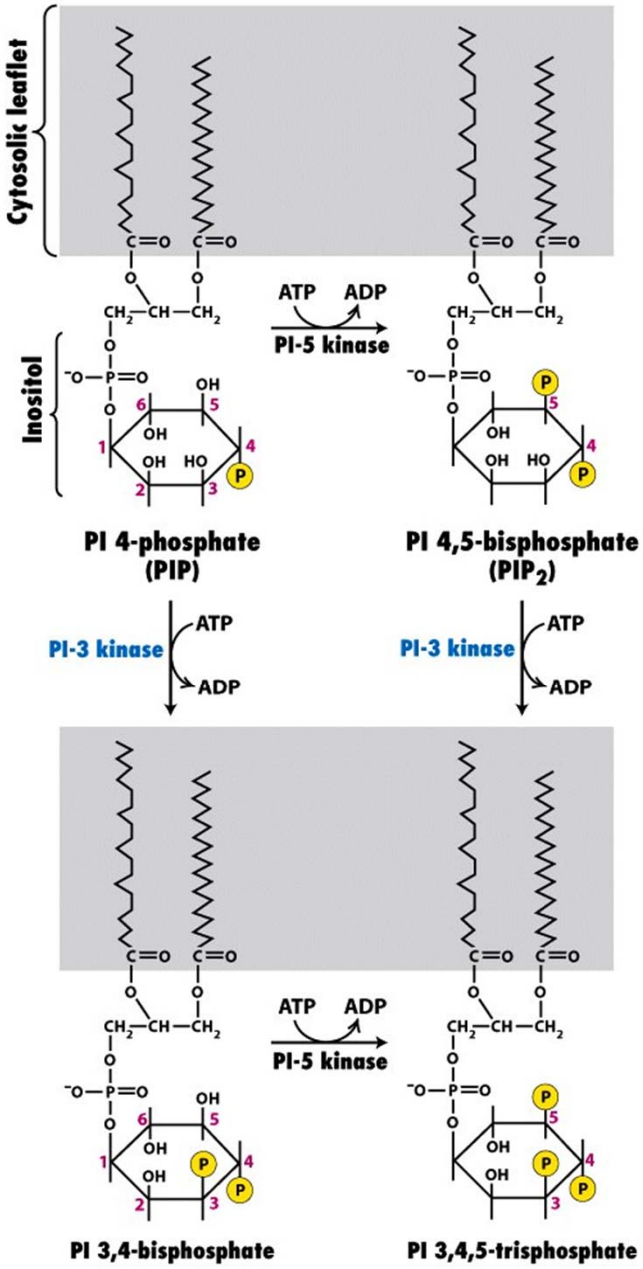


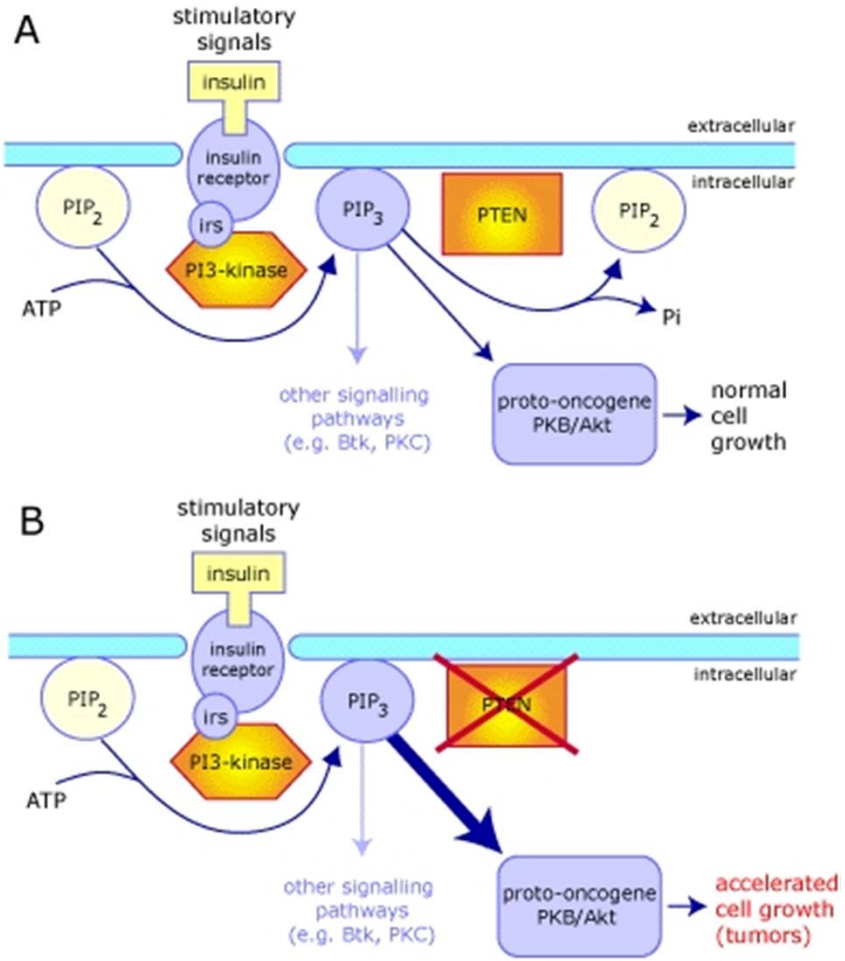
Figure 16-29
Molecular Cell Biology, Sixth Edition

PTEN: the first tumour suppressor with phosphatase activity

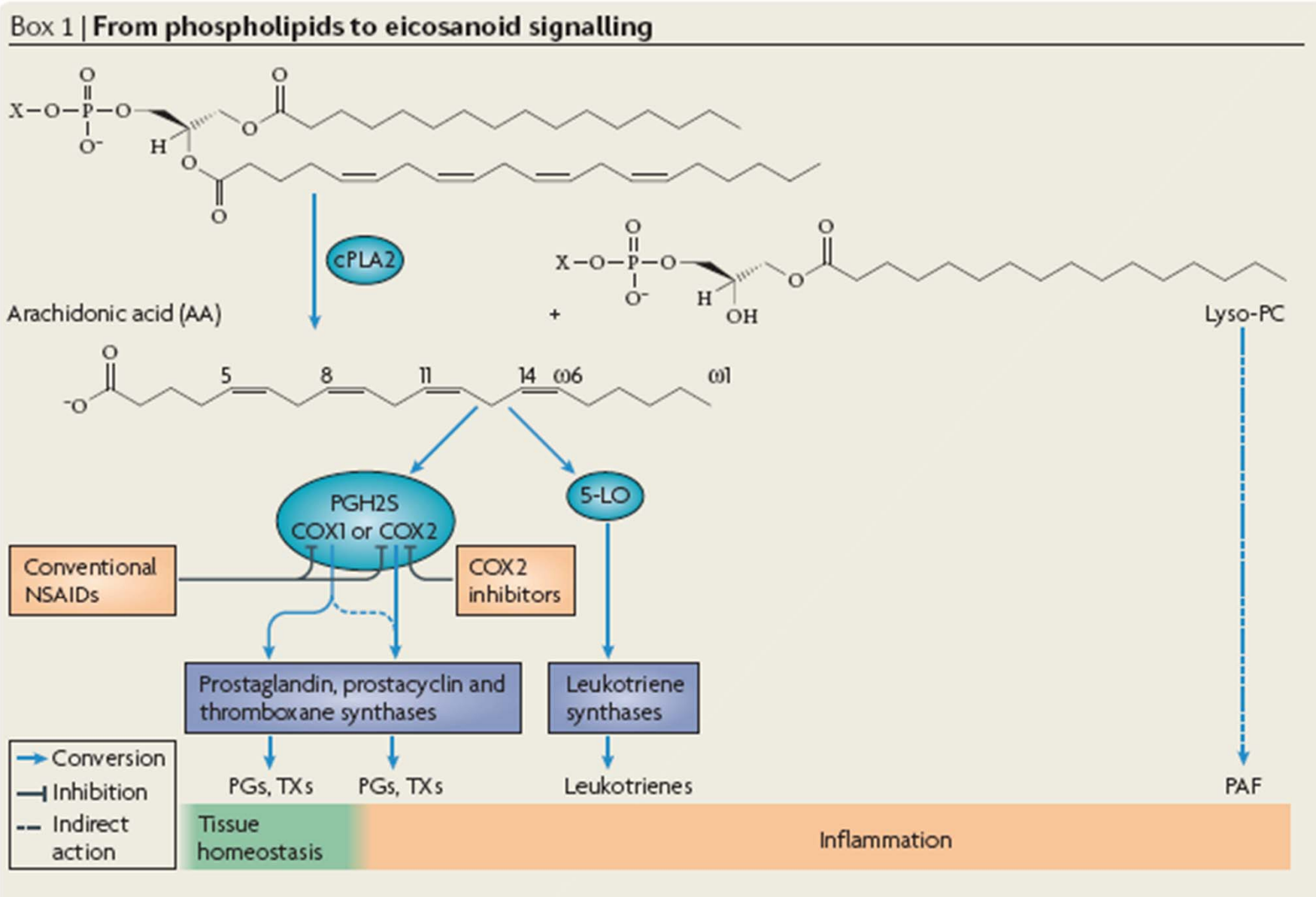
PTEN phosphatase has a broad specificity but its major function in cells is to reverse the PI-3 kinase catalyzed reaction.

PTEN is deleted or mutated in multiple types of human cancer (glioblastoma, prostate cancer, endometrial tumour).

Overexpression of PTEN promotes apoptosis in cultured mammalian cells.



www.ncbi.nlm.nih.gov/bookshelf/picrender.fcgi...



Wymann & Schneiter, Nature, 2008