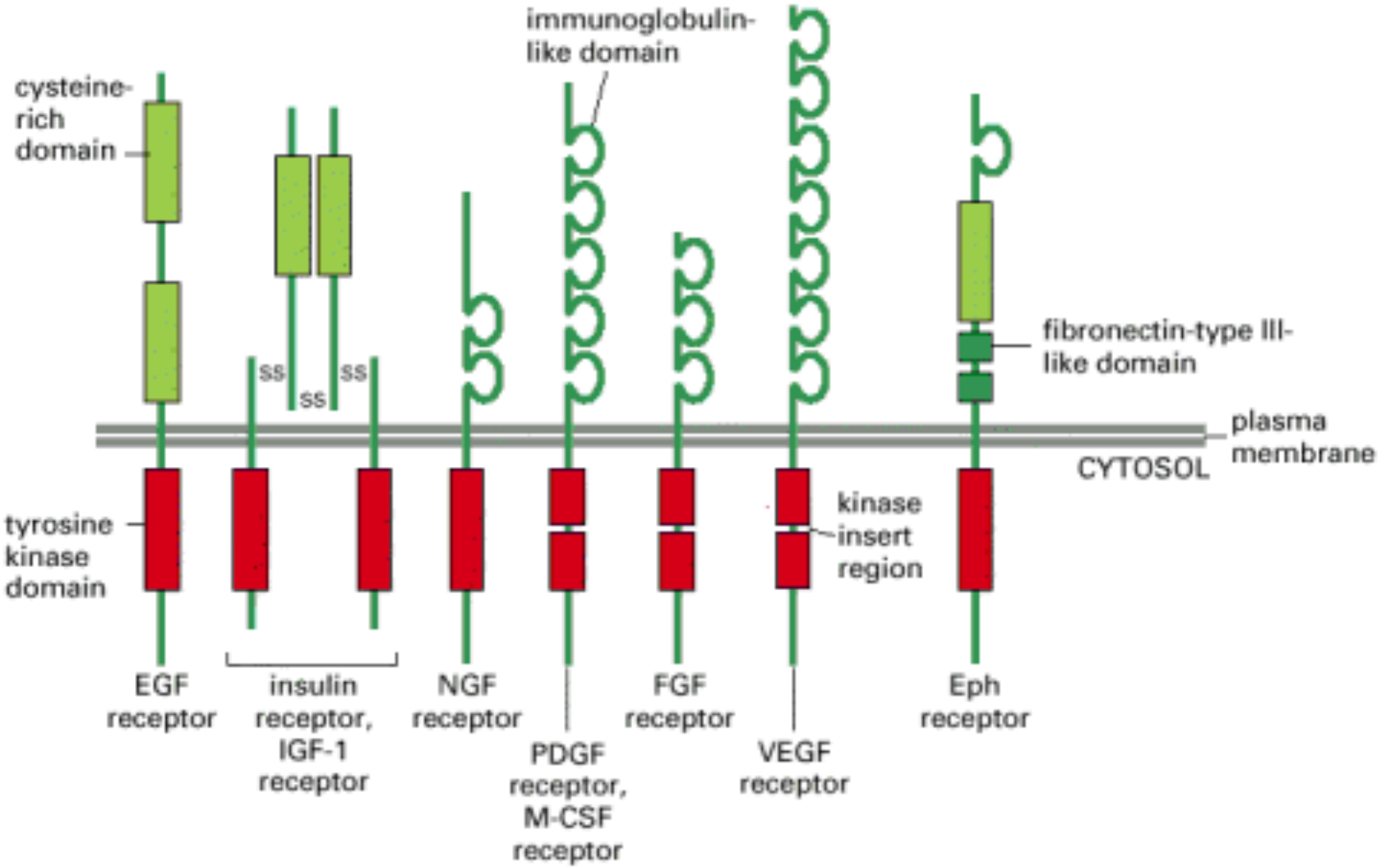
RECETTORI COLLEGATI AD ENZIMI

- ✓Recettori tirosina chinasi
- ✓ Recettori associati a tirosina chinasi
- ✓ Tirosina fosfatasi simili a recettori
- ✓ Serina\treonina chinasi recettoriali
- ✓ Recettori guanilico ciclasi

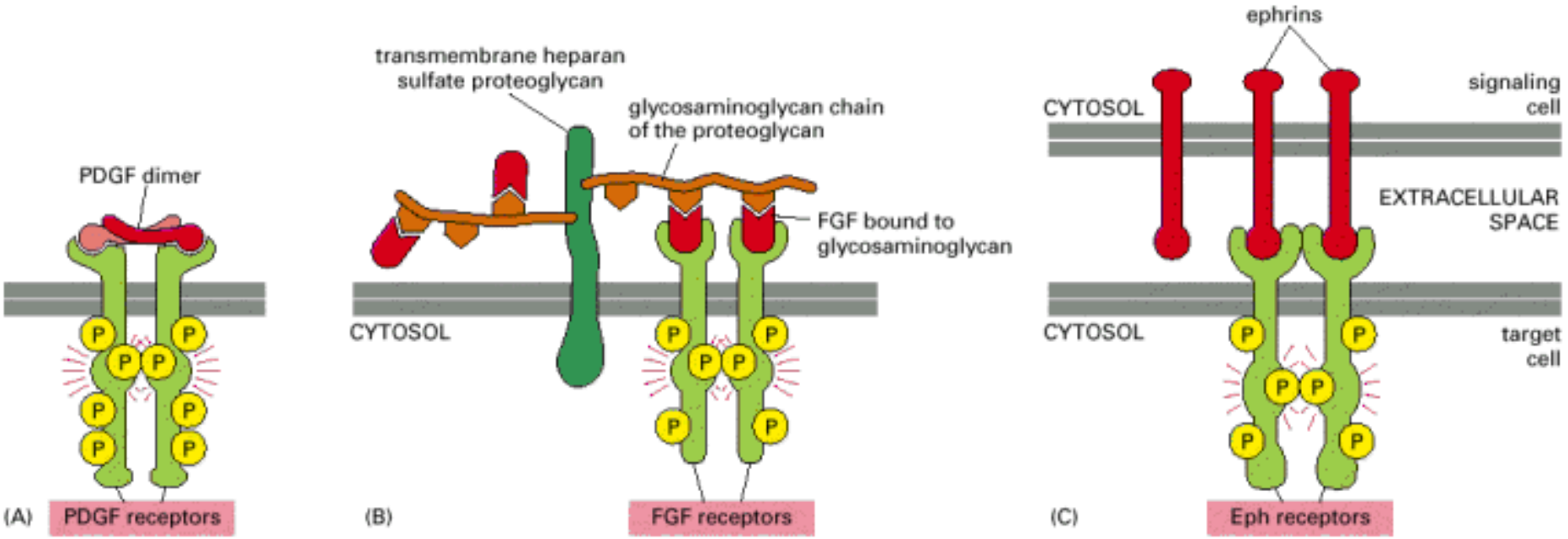
RECETTORI TIROSINA CHINASI

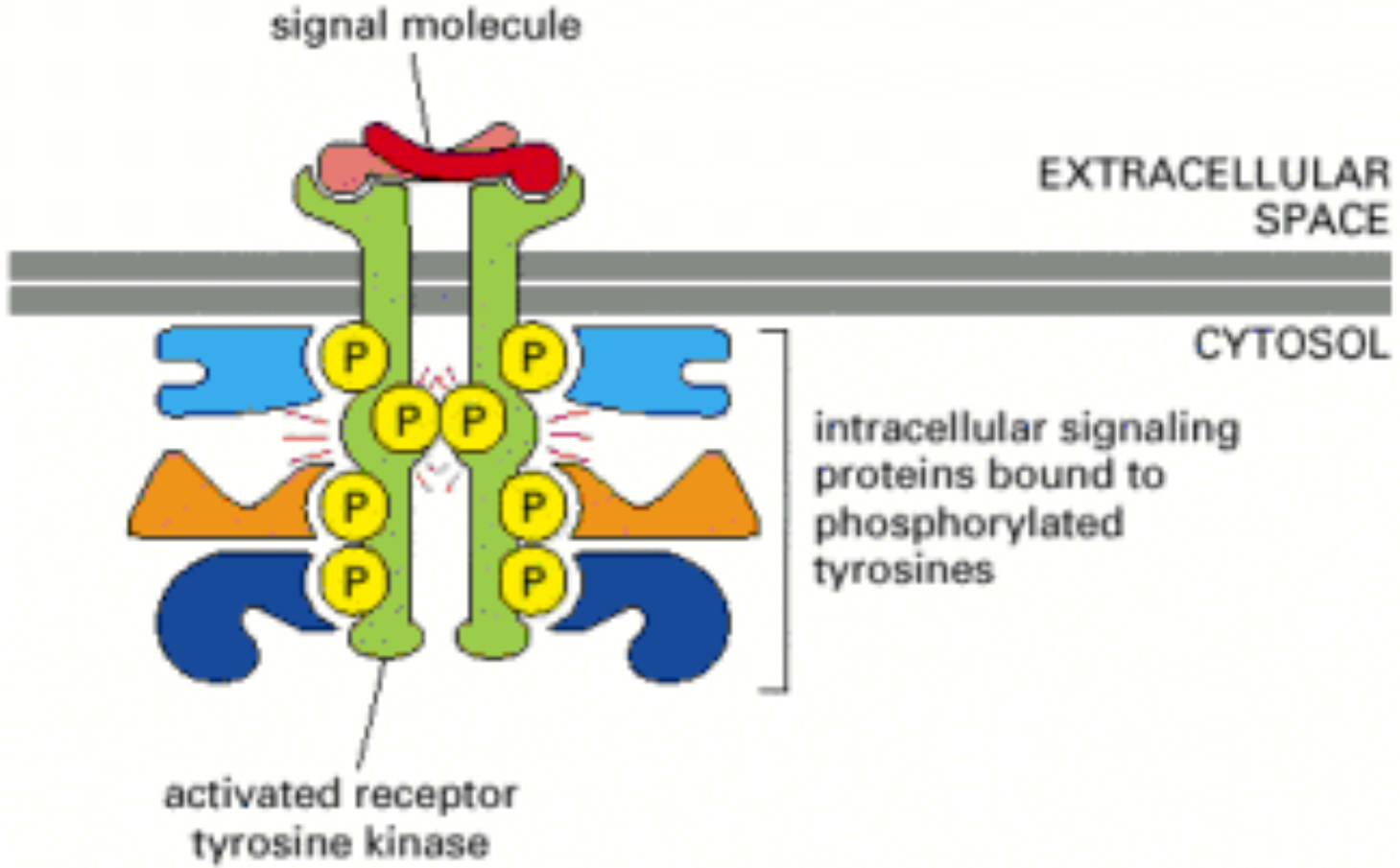
SIGNALING LIGAND	RECEPTORS	SOME RESPONSES
Epidermal growth factor (EGF)	EGF receptor	stimulates proliferation of various cell types
Insulin	insulin receptor	stimulates carbohydrate utilization and protein synthesis
Insulin-like growth factors (IGF-1 and IGF-2)	IGF receptor-1	stimulate cell growth and survival
Nerve growth factor (NGF)	Trk A	stimulates survival and growth of some neurons
Platelet-derived growth factors (PDGF AA, BB, AB)	PDGF receptors (α and β)	stimulate survival, growth, and proliferation of various cell types
Macrophage-colony-stimulating (M-CSF)	M-CSF receptor factor	stimulates monocyte/ macrophage proliferation and differentiation
Fibroblast growth factors (FGF-1 to FGF-24)	FGF receptors (FGF-R1-FGF- R4, plus multiple isoforms of each)	stimulate proliferation of various cell types; inhibit differentiation of some precursor cells; inductive signals in development
Vascular endothelial growth factor (VEGF)	VEGF receptor	stimulates angiogenesis
Ephrins (A and B types)	Eph receptors (A and B types)	stimulate angiogenesis; guide cell and axon migration

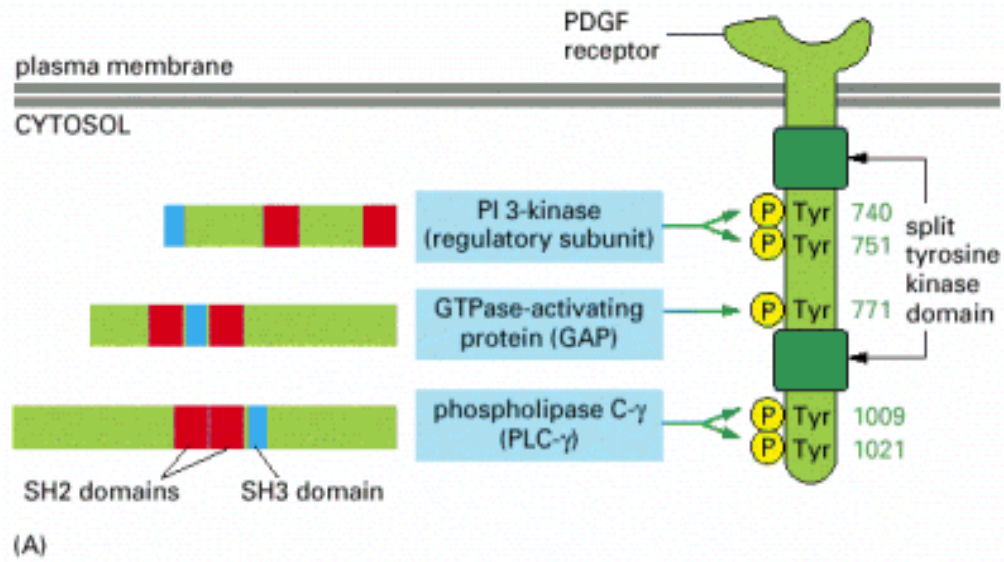
16 sottofamiglie

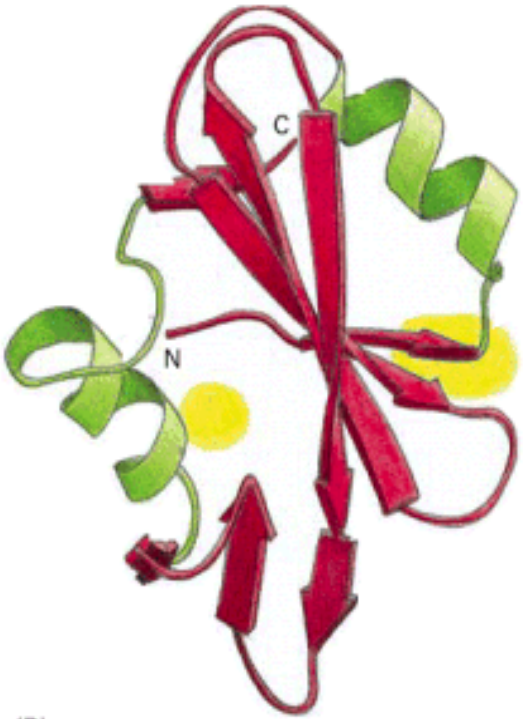


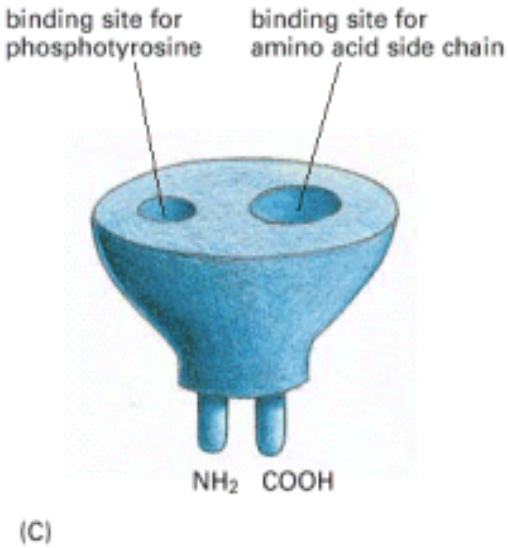
PDGF: Platelet Derived Growth Factor
FGF : Fibroblast Growth Factor




signal molecule
EXTRACELLULAR SPACE
CYTOSOL
P
intracellular signaling proteins bound to phosphorylated tyrosines
activated receptor tyrosine kinase



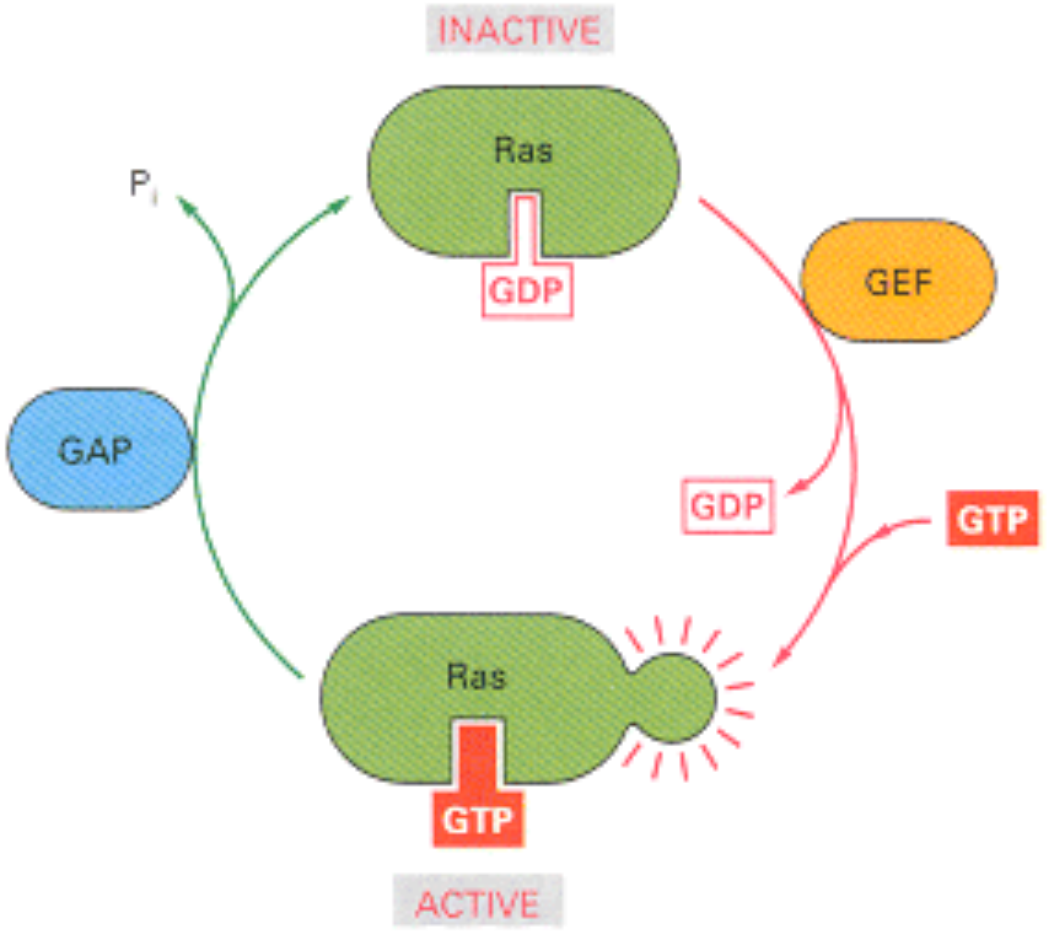




DOMINI SH2

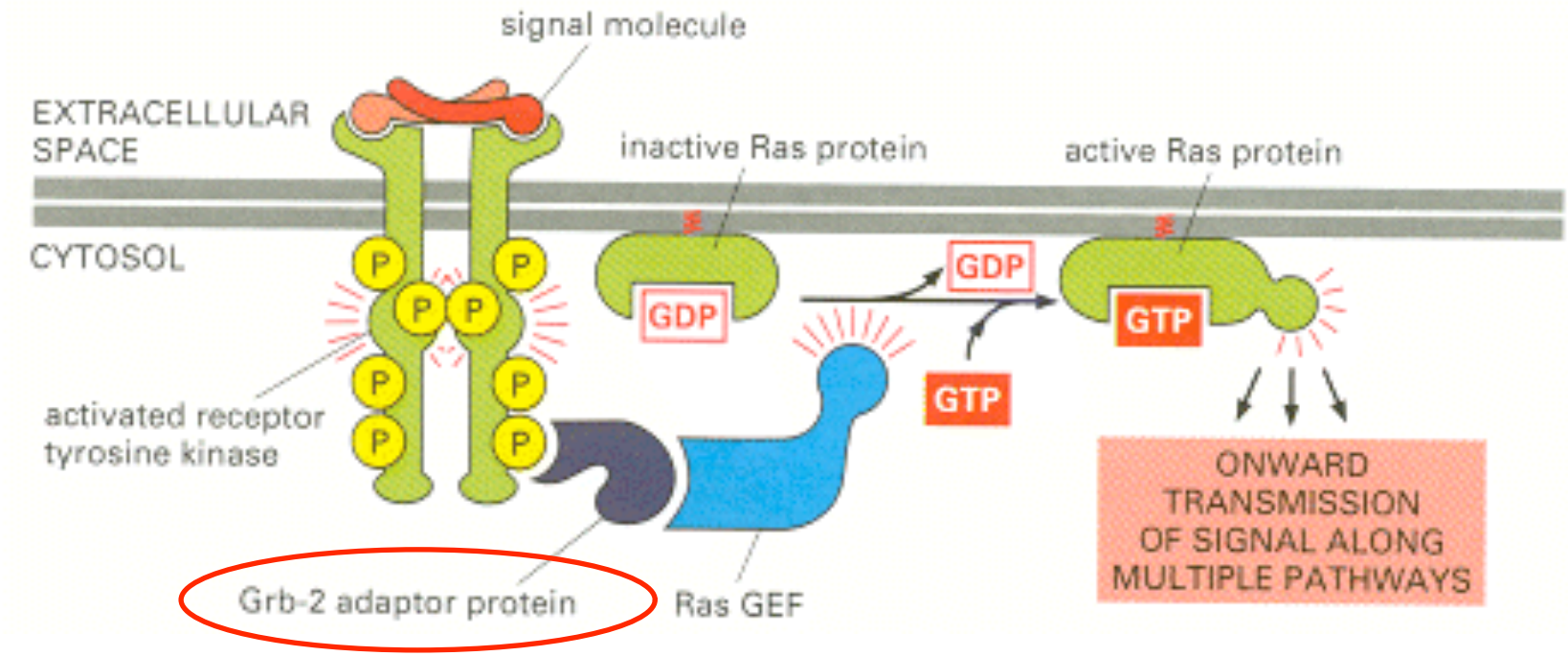
DOMINI SH3
Riconoscono e legano
Sequenze ricche in
prolina

Ras (GTPasi monomeriche)

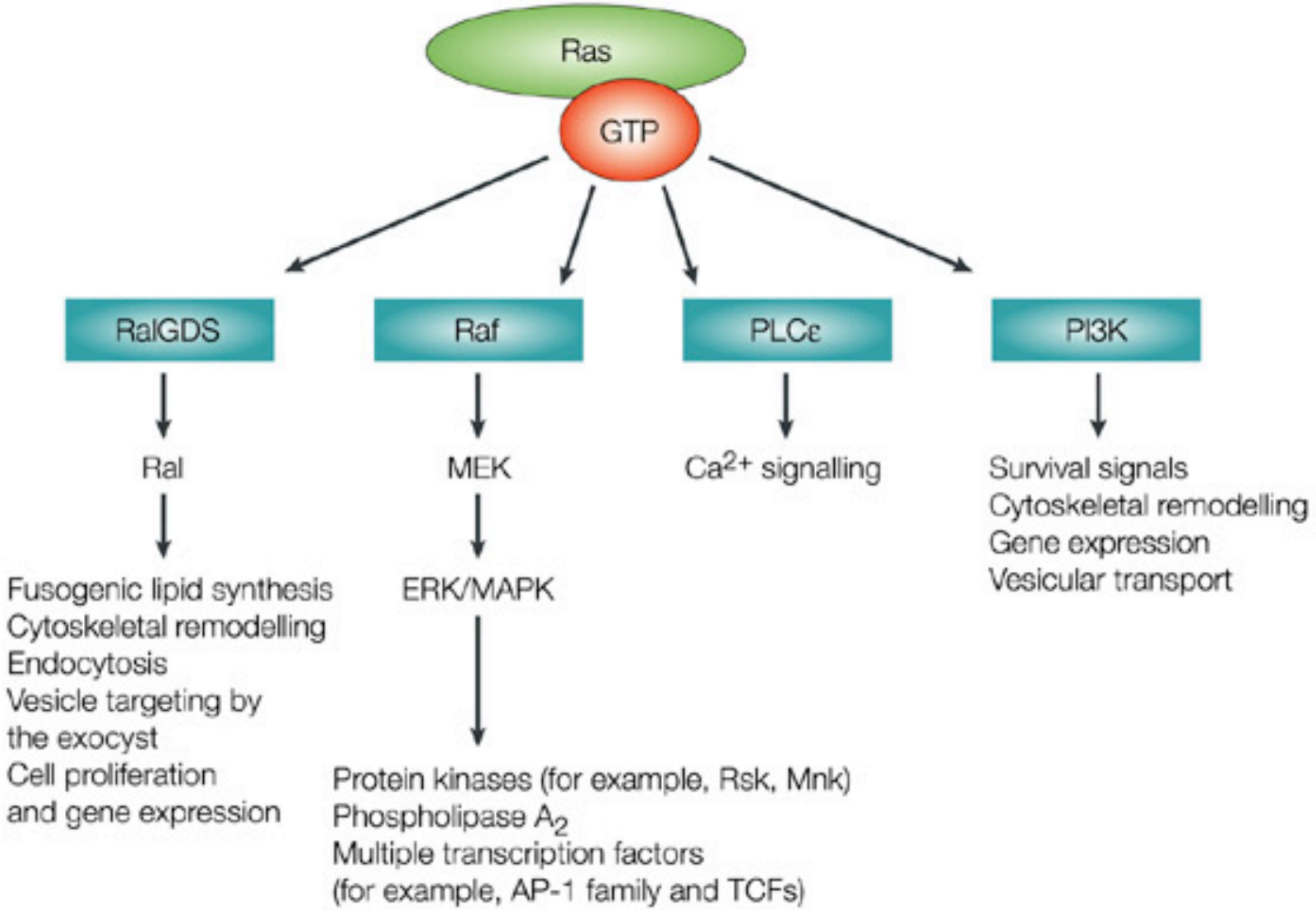


Rho: dai recettori al citoscheletro

Rab: trasporto intracellulare

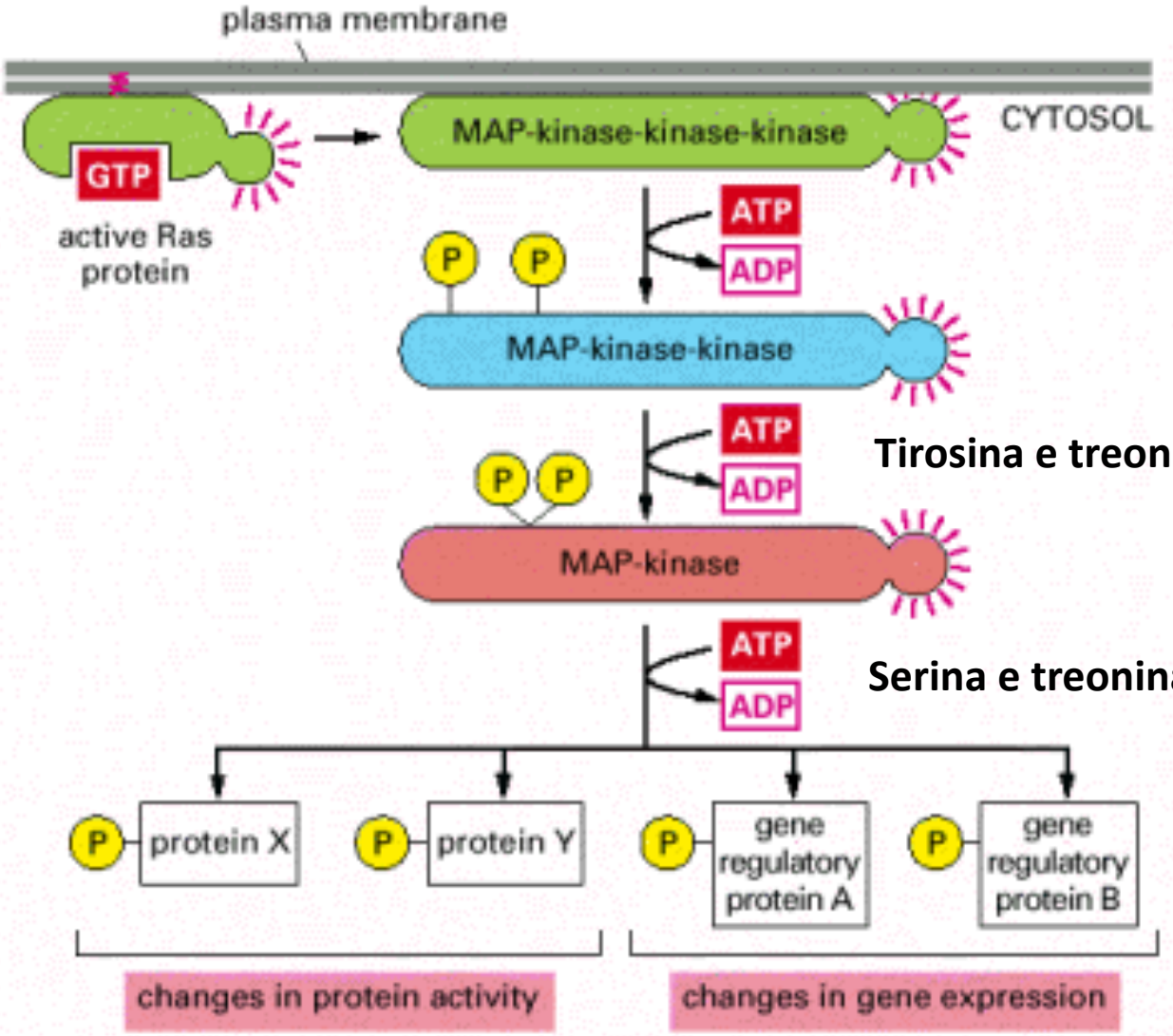

signal molecule
EXTRACELLULAR SPACE
CYTOSOL
inactive Ras protein
active Ras protein
GDP
GDP
GTP
GTP
P
activated receptor tyrosine kinase
Grb-2 adaptor protein
Ras GEF
ONWARD TRANSMISSION OF SIGNAL ALONG MULTIPLE PATHWAYS

FUNZIONE DI RAS: I SUOI EFFETTORI



Nature Reviews | Molecular Cell Biology

MAP chinasi


plasma membrane
CYTOSOL
GTP
active Ras protein
MAP-kinase-kinase-kinase
ATP
ADP
P
P
MAP-kinase-kinase
ATP
ADP
P
P
MAP-kinase
ATP
ADP
Tirosina e treonina
Serina e treonina
P
protein X
P
protein Y
P
gene regulatory protein A
P
gene regulatory protein B
changes in protein activity
changes in gene expression

Ras superfamily

Ras
- H-Ras
- N-Ras
- K-Ras
- ...

- proliferazione
- sopravvivenza
- citoscheletro

Rho
- Rho
- Rac
- cdc42
- ...

- risposta a stress
- citoscheletro
- differenziamento

Ran
- Ran1
- ....

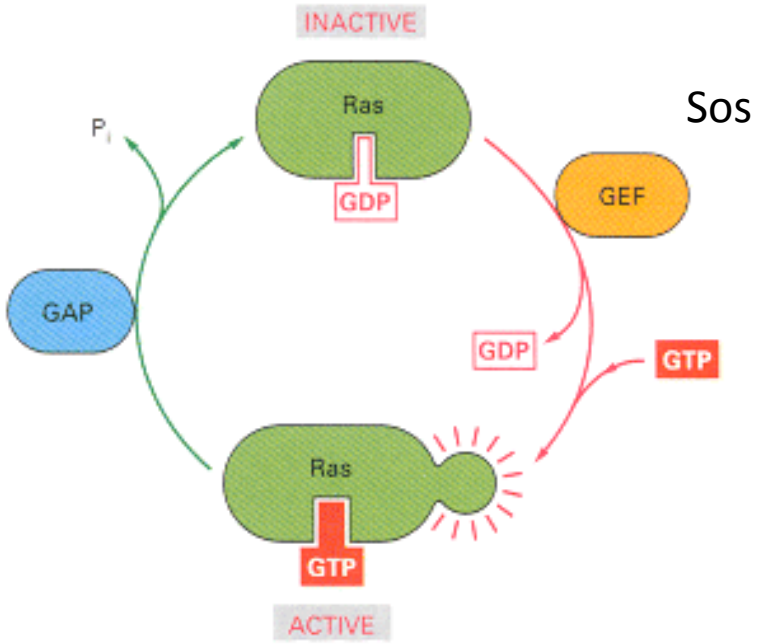
- trasporto nucleare

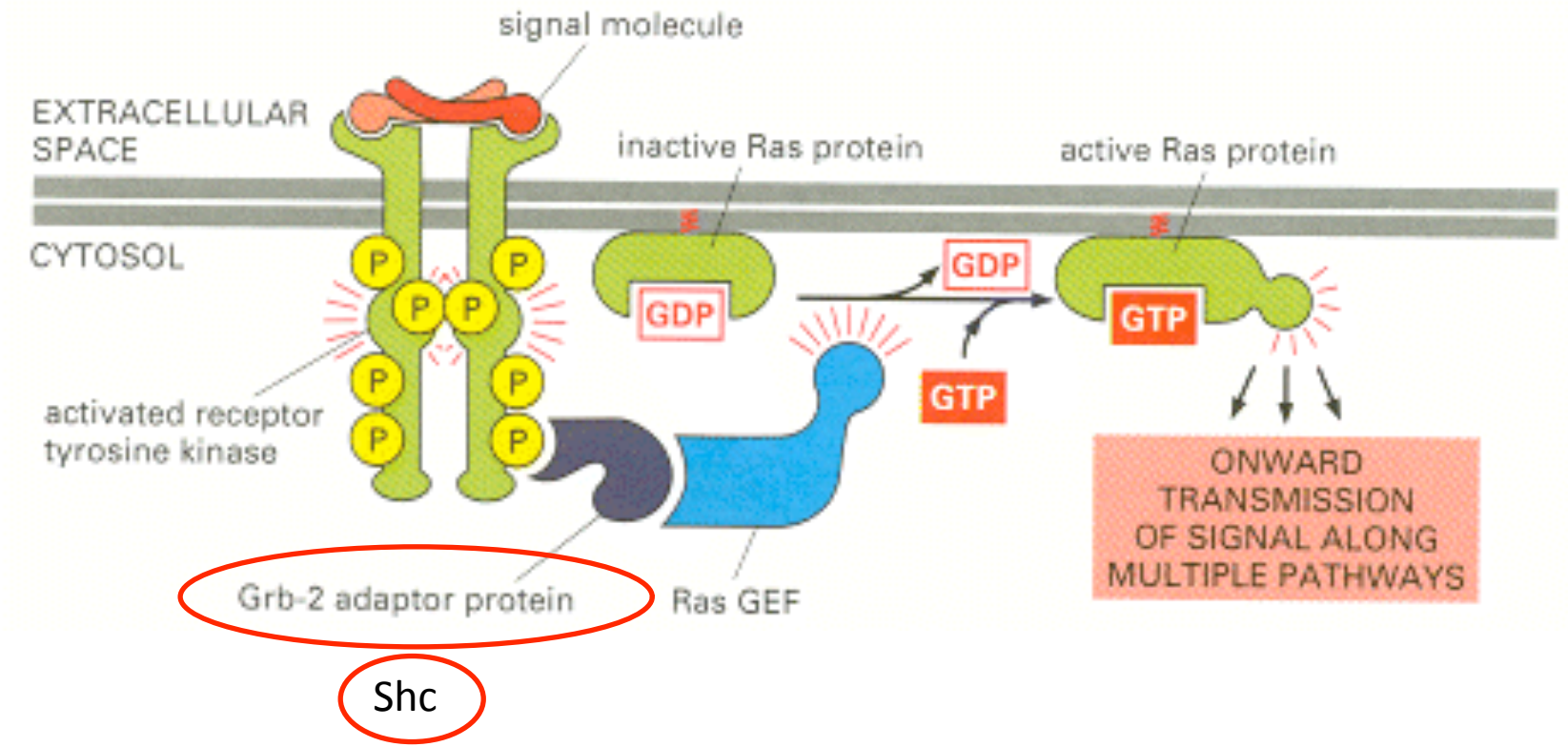
Rab
- Rab1
- ....

- traffico vescicolare

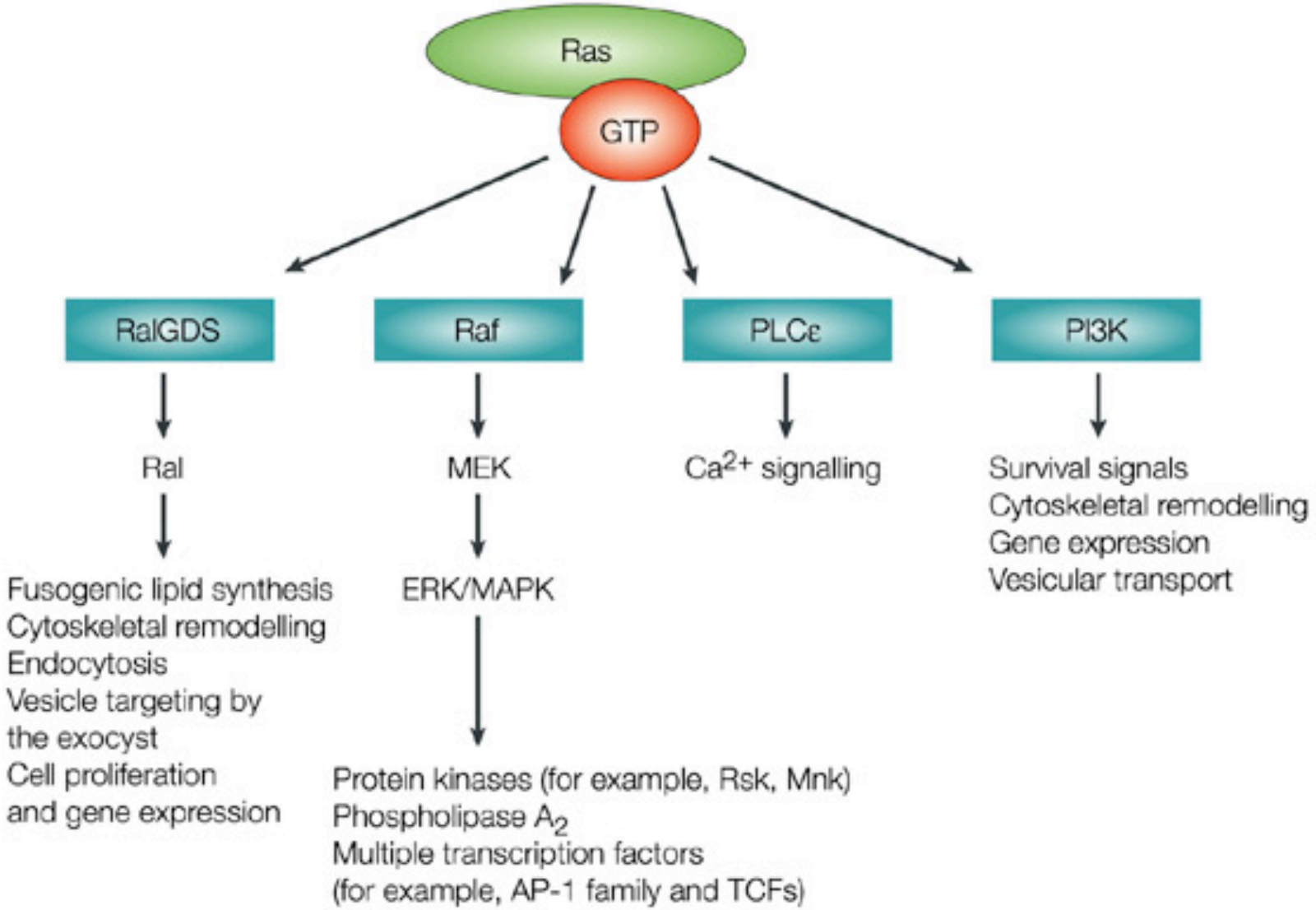
Piccole proteine G

- legano GDP/GTP
- diversa configurazione secondo il nucleotide legato
- hanno attività GTPasica

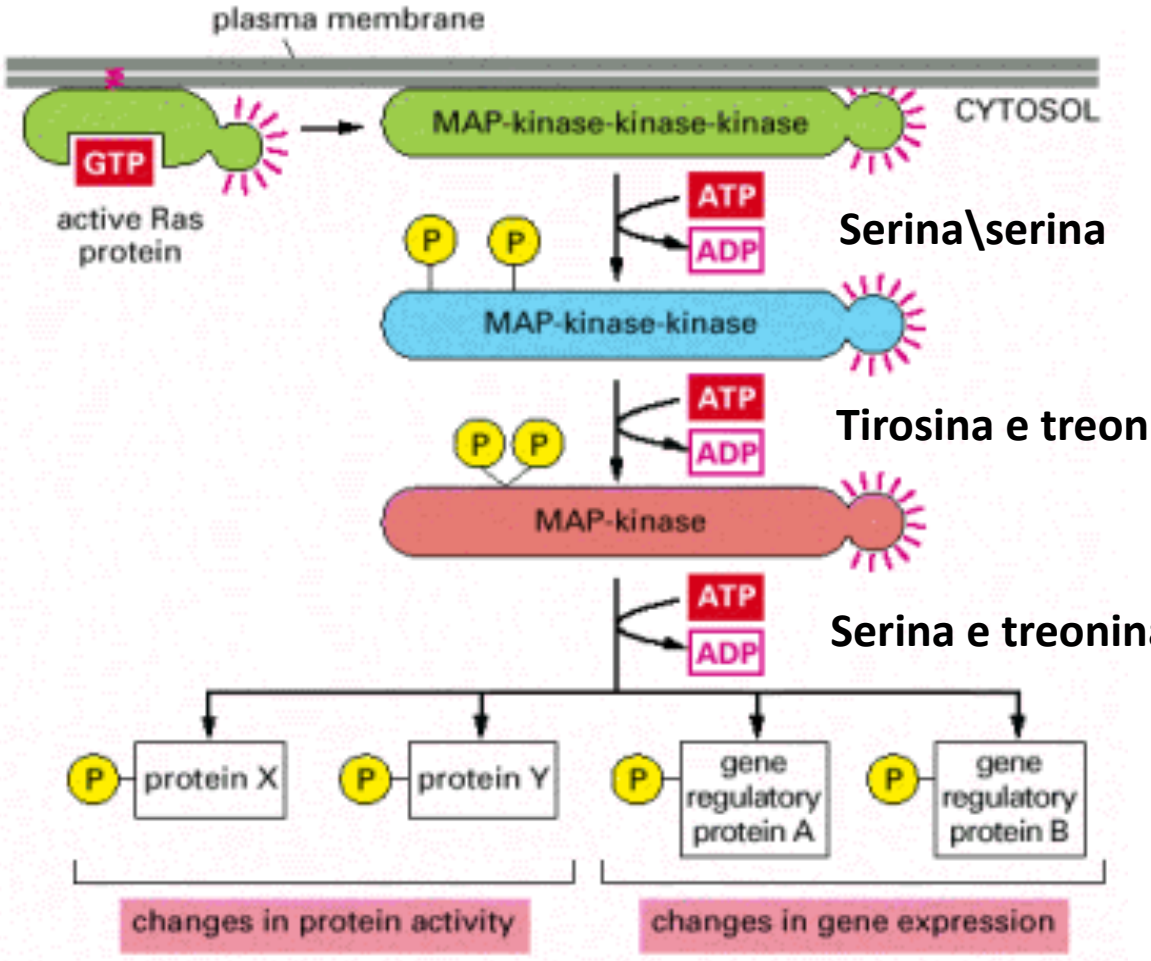



signal molecule
EXTRACELLULAR SPACE
CYTOSOL
inactive Ras protein
active Ras protein
GDP
GDP
GTP
GTP
P
activated receptor tyrosine kinase
Grb-2 adaptor protein
Ras GEF
ONWARD TRANSMISSION OF SIGNAL ALONG MULTIPLE PATHWAYS
Shc

FUNZIONE DI RAS: I SUOI EFFETTORI



MAP chinasi (mitogen-activated protein kinases)



Raf-1

Serina\serina

MEK

Tirosina e treonina

ERK1\2

Serina e treonina

Fosforilazione precoce e tempo-dip

⇩

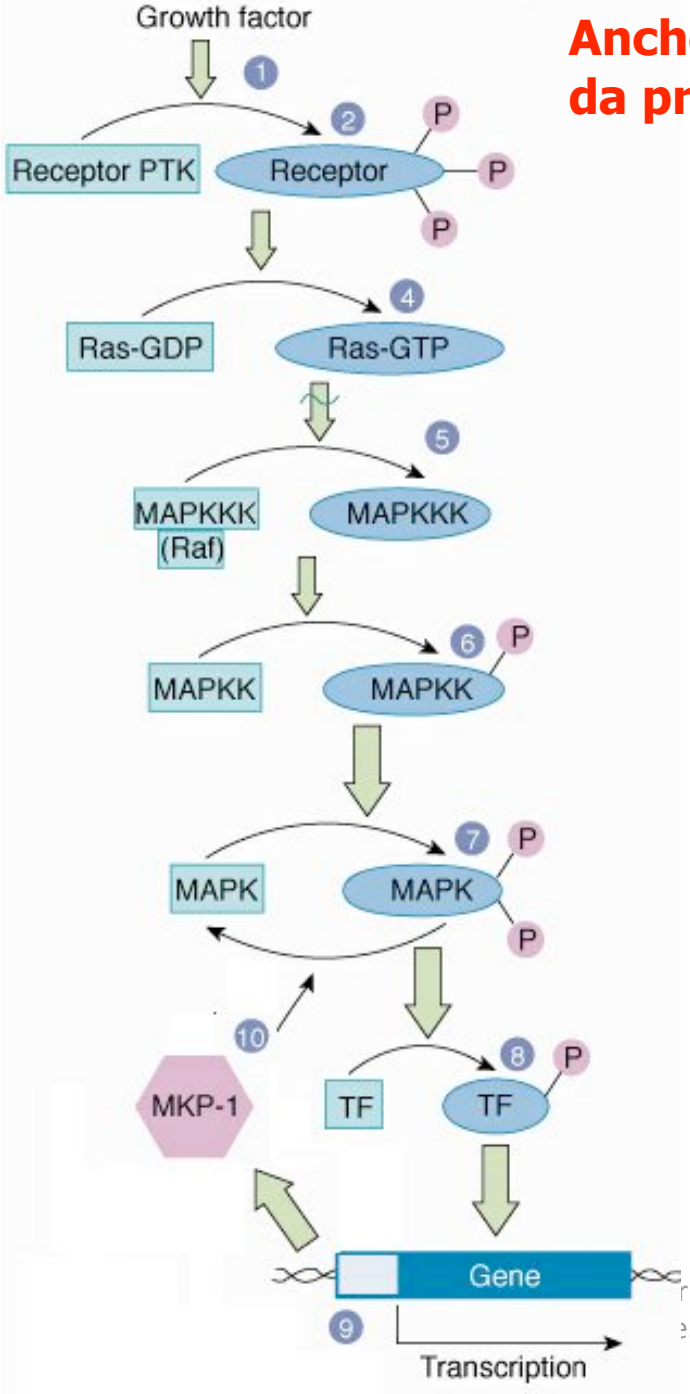
Proliferazione

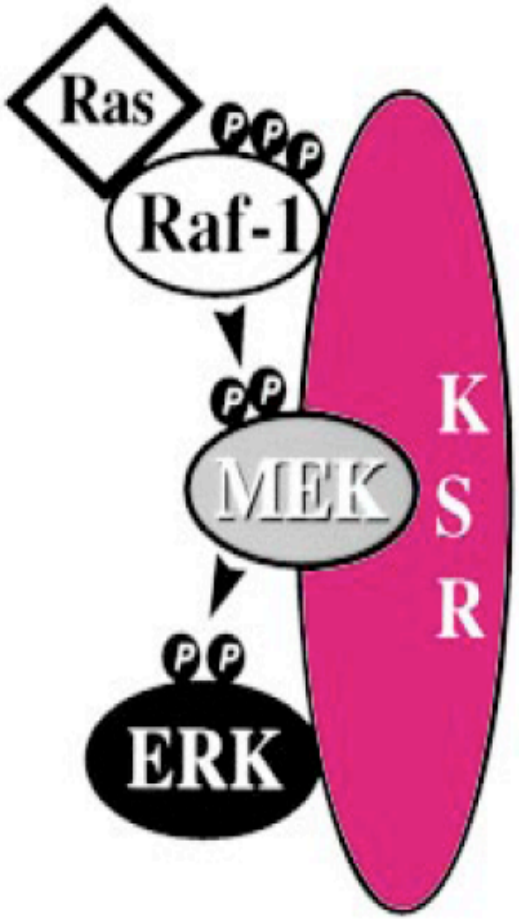
Fosforilazione sostenuta

⇩

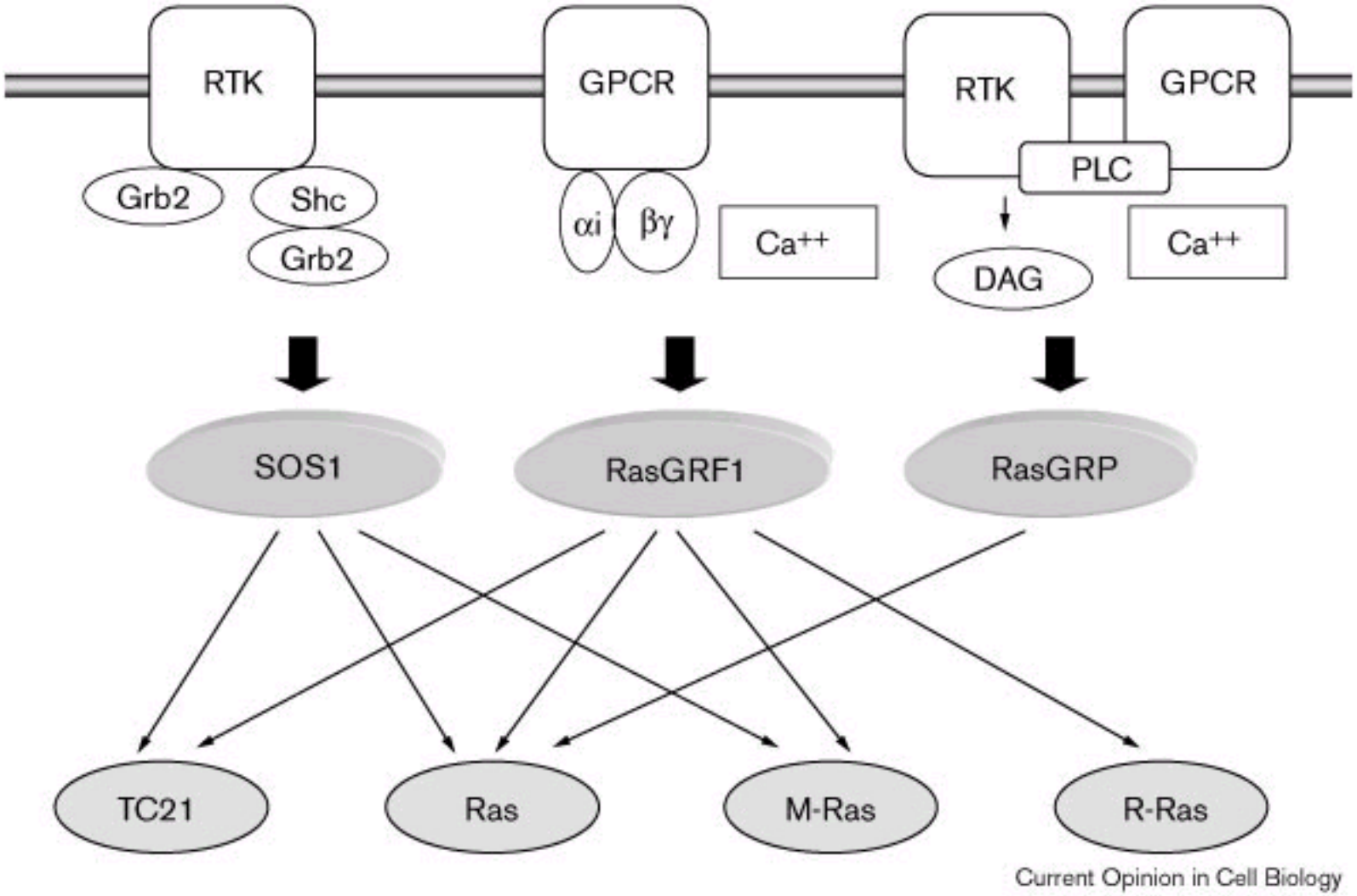
Differenziamento

Anche la via Ras/Raf-1/MEK/ERK è "guidata" da proteine impalcatura ⇨ SPECIFICITA'





Ras\MAPK può essere attivato anche da GPCR via altri GEF-Ca^{++} e DG-dipendenti



PI 3-CHINASI (PI3-K)

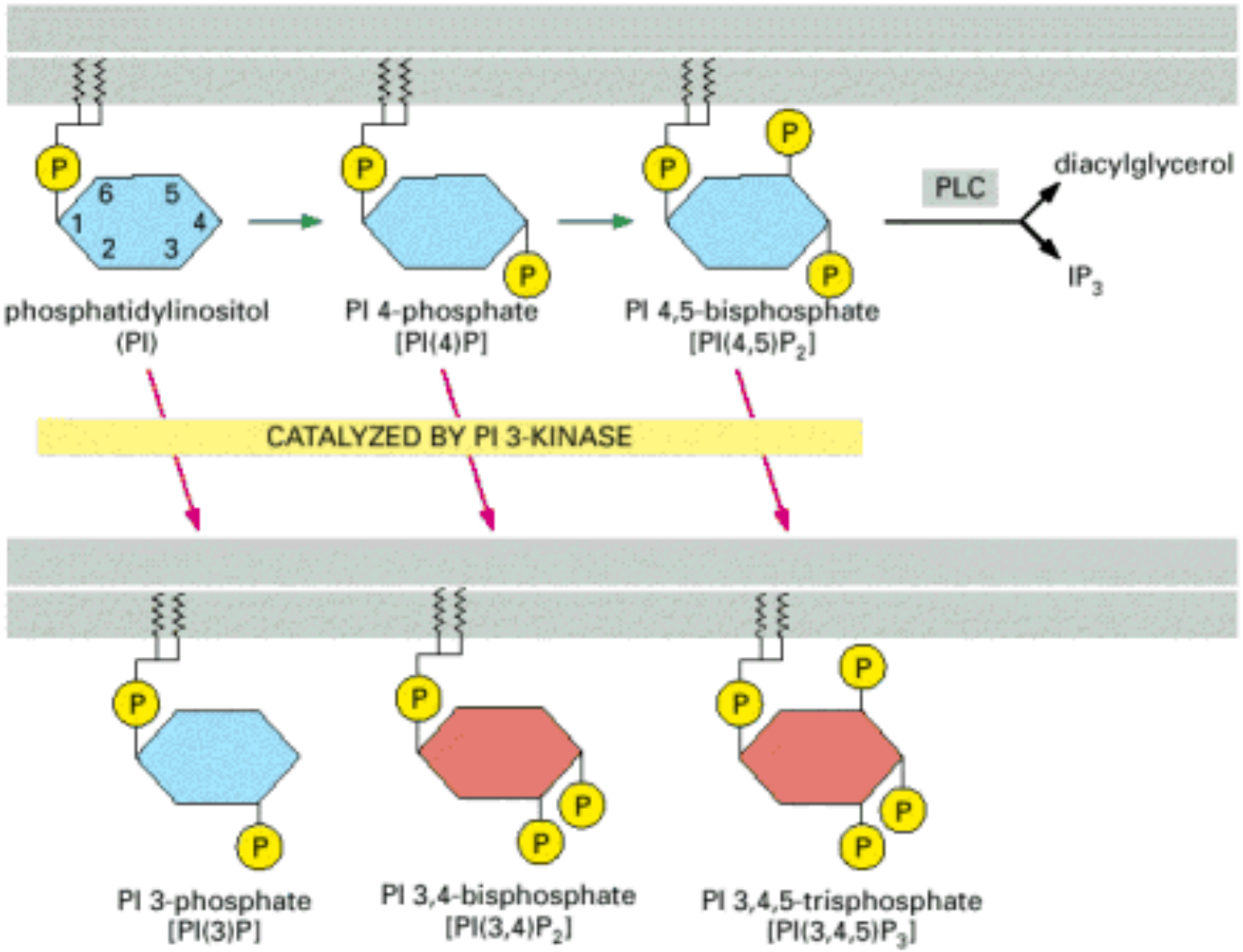
CATALIZZA LA FOSFORILAZIONE DI INOSITOLO FOSFOLIPIDI SULLA POSIZIONE 3 DELL'ANELLO DELL'INOSITOLO PER GENERARE LIPIDI QUALI

PI 3,4-BIFOSFATO E PI 3,4-TRIFOSFATO (sono siti di attacco per proteine con domini PH)

CRESCITA CELLULARE

SOPRAVVIVENZA

TRAFFICO VESCICOLARE


P
6
5
1
4
2
3
phosphatidylinositol
(PI)
PI 4-phosphate
[PI(4)P]
PI 4,5-bisphosphate
[PI(4,5)P2]
PLC
diacylglycerol
IP3
CATALYZED BY PI 3-KINASE
PI 3-phosphate
[PI(3)P]
PI 3,4-bisphosphate
[PI(3,4)P2]
PI 3,4,5-trisphosphate
[PI(3,4,5)P3]

FAK

Cdc42

Src

Ras

Attivatori di PI3-K

Rho

Recettori

AKT\PKB

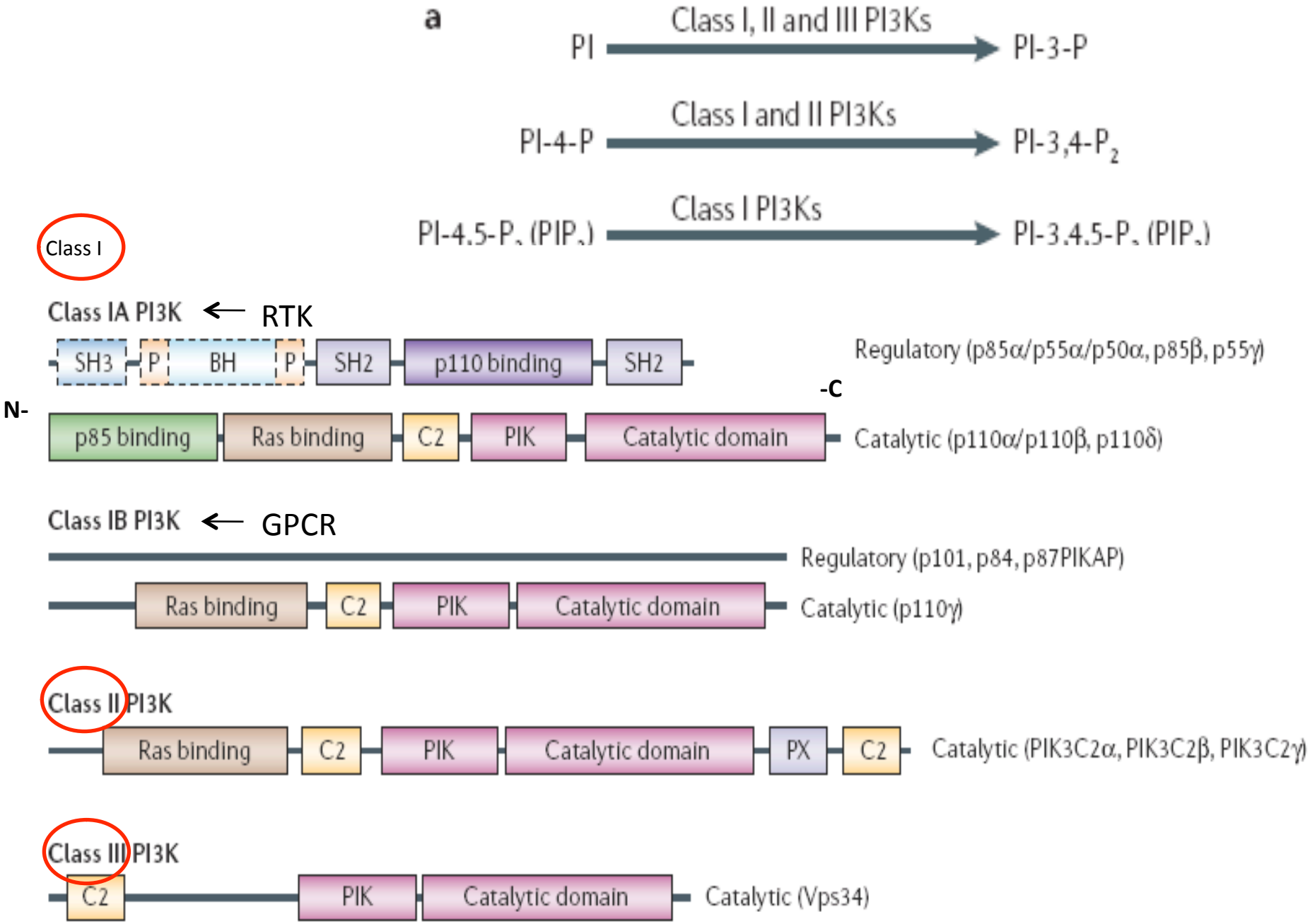
Downstream targets

Rac

PKC

PLC

JNK

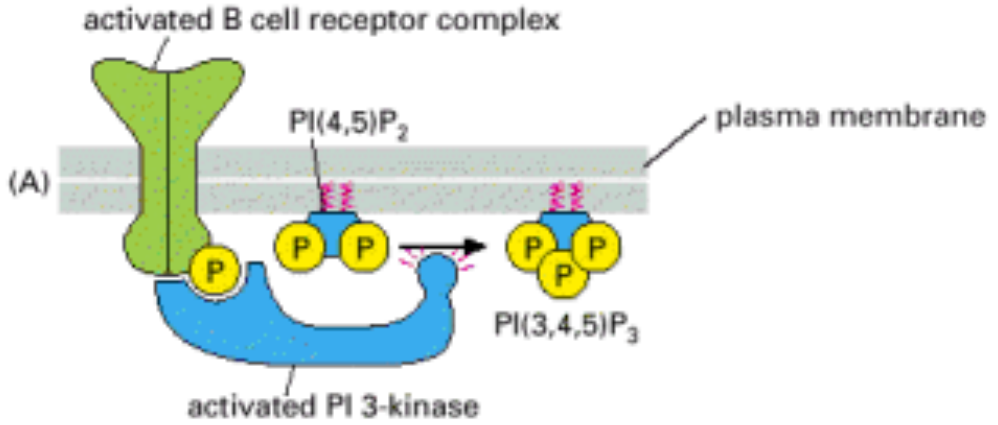

a
PI
Class I, II and III PI3Ks
PI-3-P
PI-4-P
Class I and II PI3Ks
PI-3,4-P2
PI-4,5-P2 (PIP2)
Class I PI3Ks
PI-3,4,5-P3 (PIP3)
Class I
Class IA PI3K
RTK
SH3
P
BH
P
SH2
p110 binding
SH2
Regulatory (p85α/p55α/p50α, p85β, p55γ)
N-
-C
p85 binding
Ras binding
C2
PIK
Catalytic domain
Catalytic (p110α/p110β, p110δ)
Class IB PI3K
GPCR
Regulatory (p101, p84, p87PIKAP)
Ras binding
C2
PIK
Catalytic domain
Catalytic (p110γ)
Class II PI3K
Ras binding
C2
PIK
Catalytic domain
PX
C2
Catalytic (PIK3C2α, PIK3C2β, PIK3C2γ)
Class III PI3K
C2
PIK
Catalytic domain
Catalytic (Vps34)

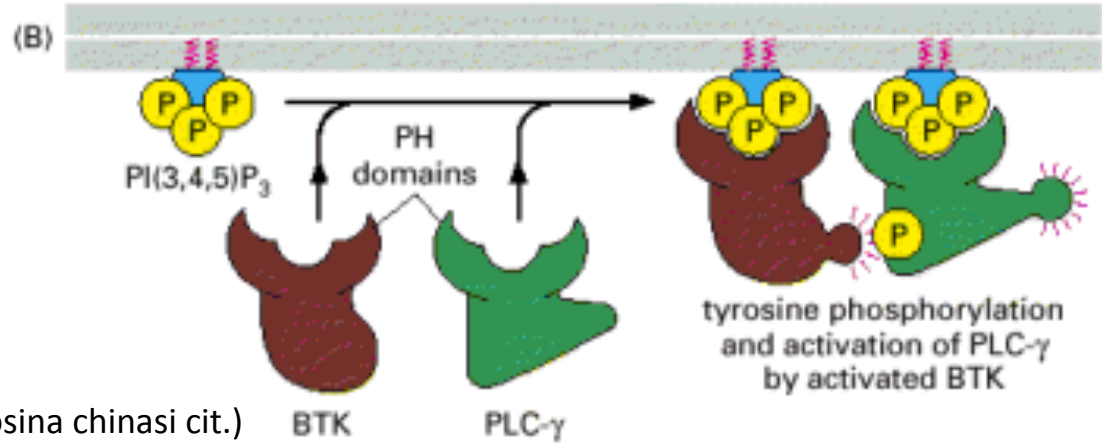
Esempio dell'importanza dei domini

PH:

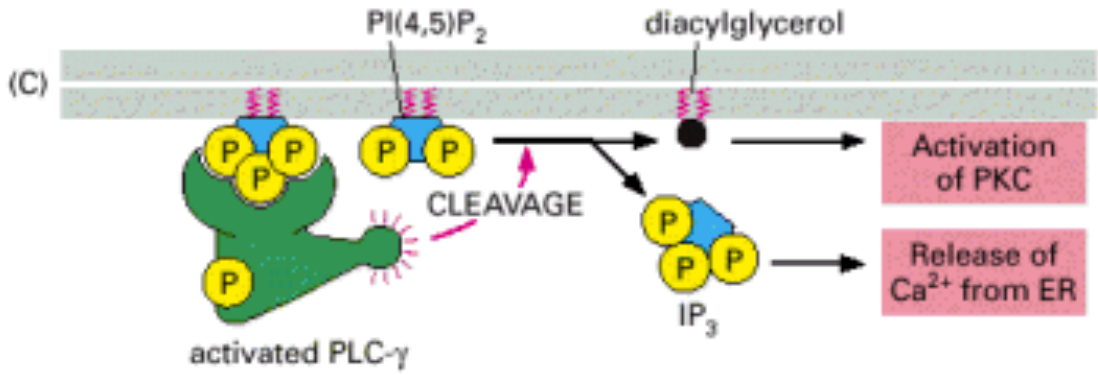
MANCATA PRODUZIONE DI

ANTICORPI



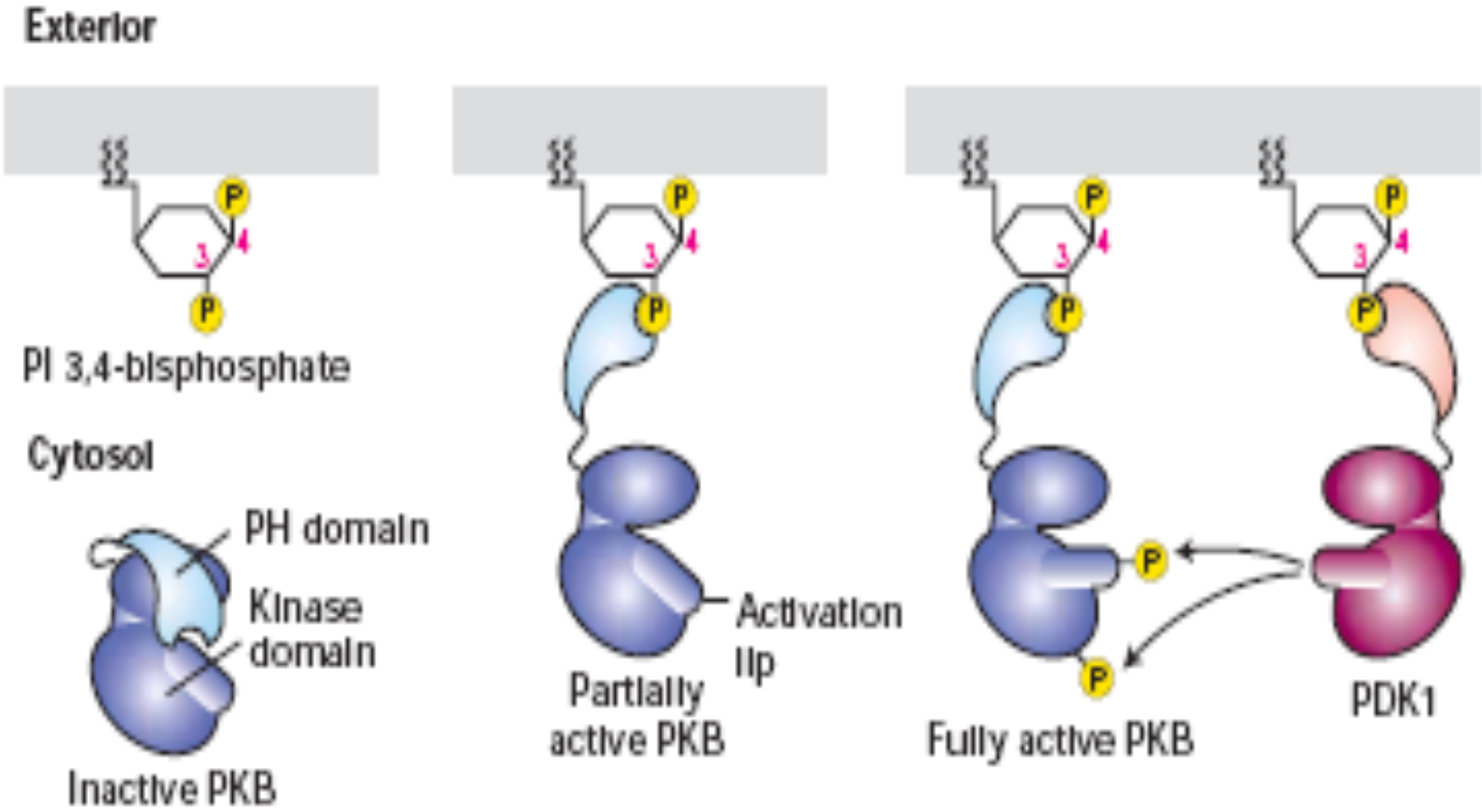


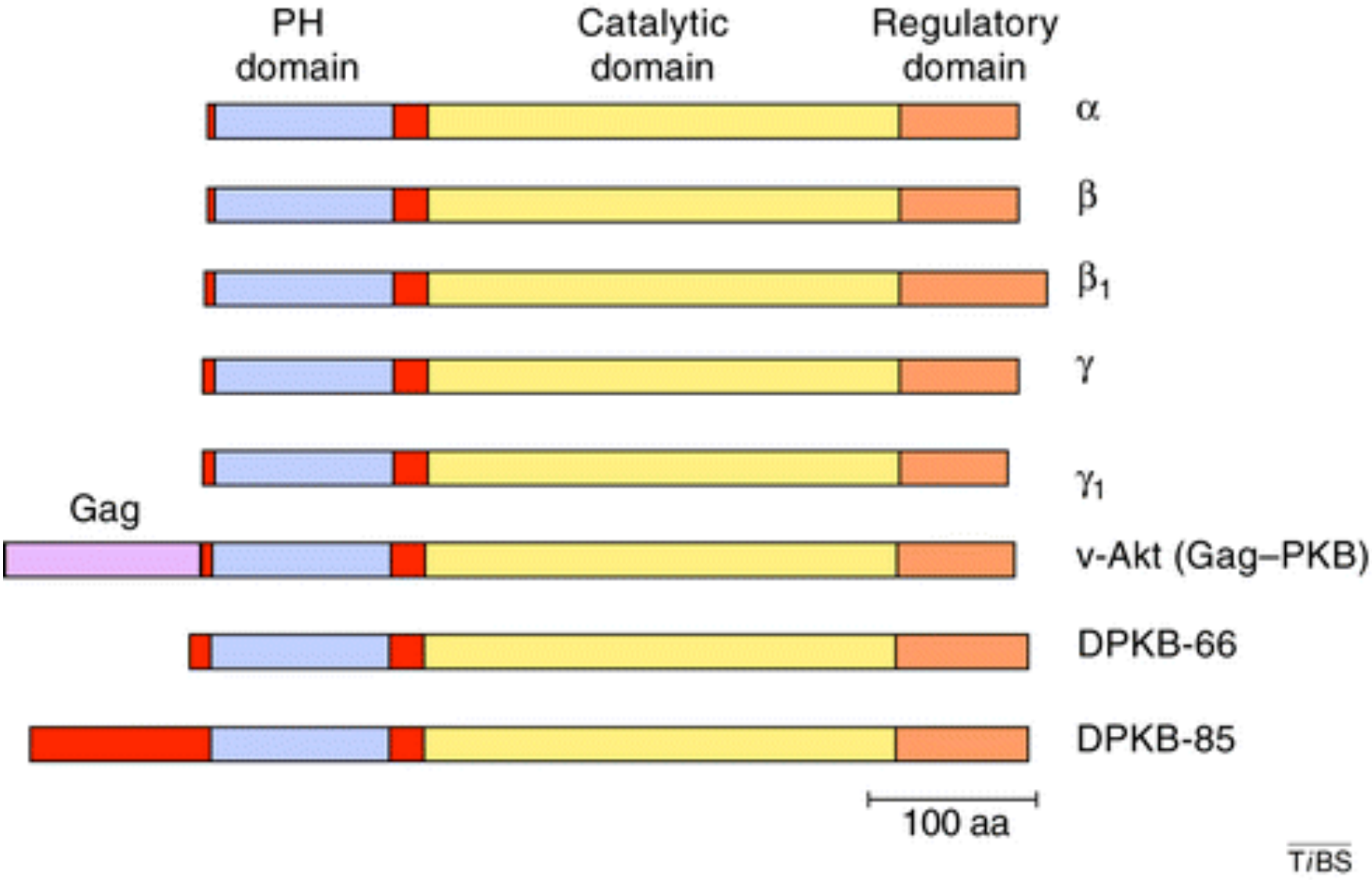
(Tirosina chinasi cit.)



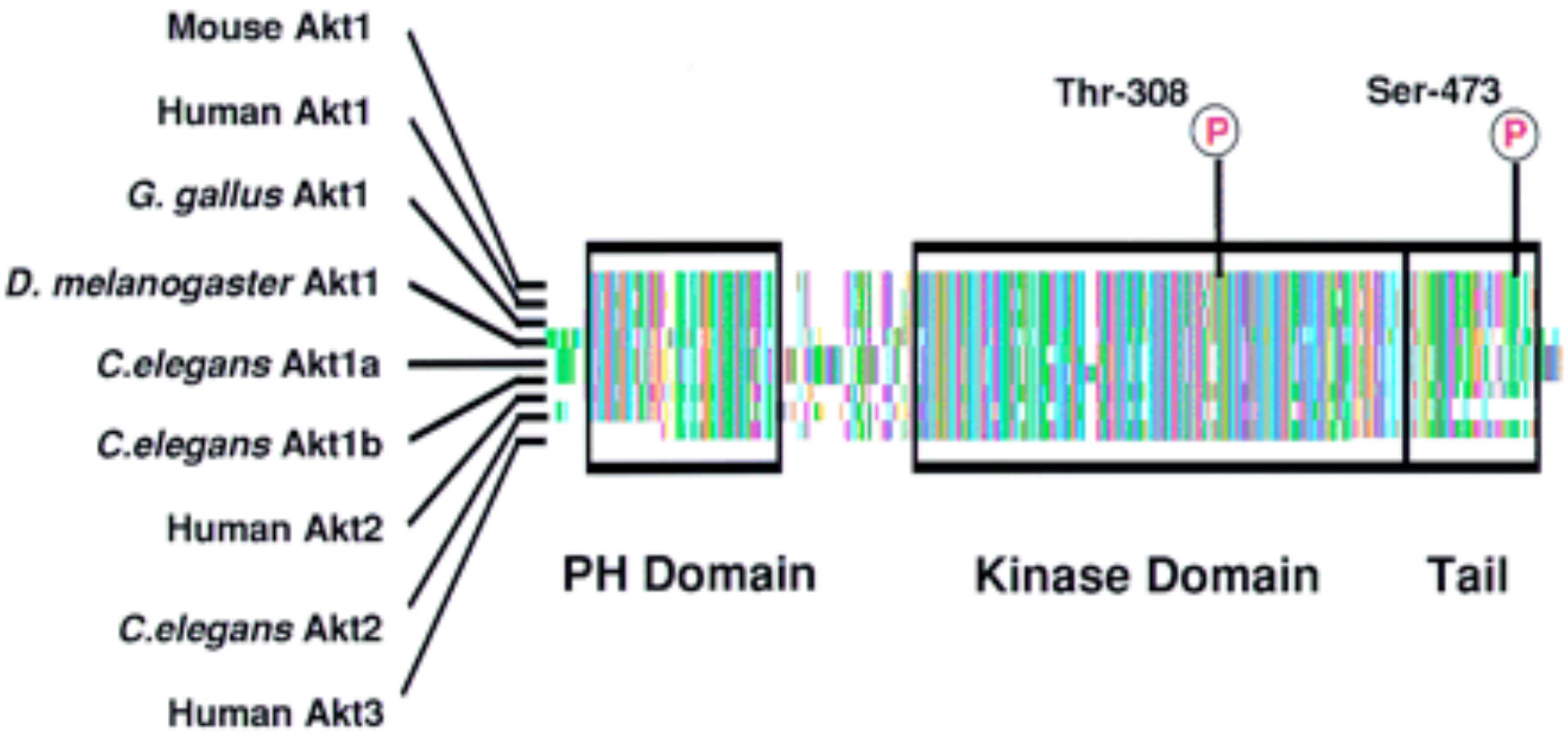
Akt/PKB

AKT/PKB ‘E UNA SERINA\TREONINA CHINASI ATTIVATA MEDIANTE RECLUTAMENTO ALLA MEBRANA DA PARTE DI PIP_3 E FOSFORILAZIONE IN SERINA




PH domain
Catalytic domain
Regulatory domain
α
β
β1
γ
γ1
Gag
v-Akt (Gag–PKB)
DPKB-66
DPKB-85
100 aa
TiBS

La seq aa di Akt/PKB è estremamente conservata durante l'evoluzione, in particolare nei domini PH, chinasico e nella coda



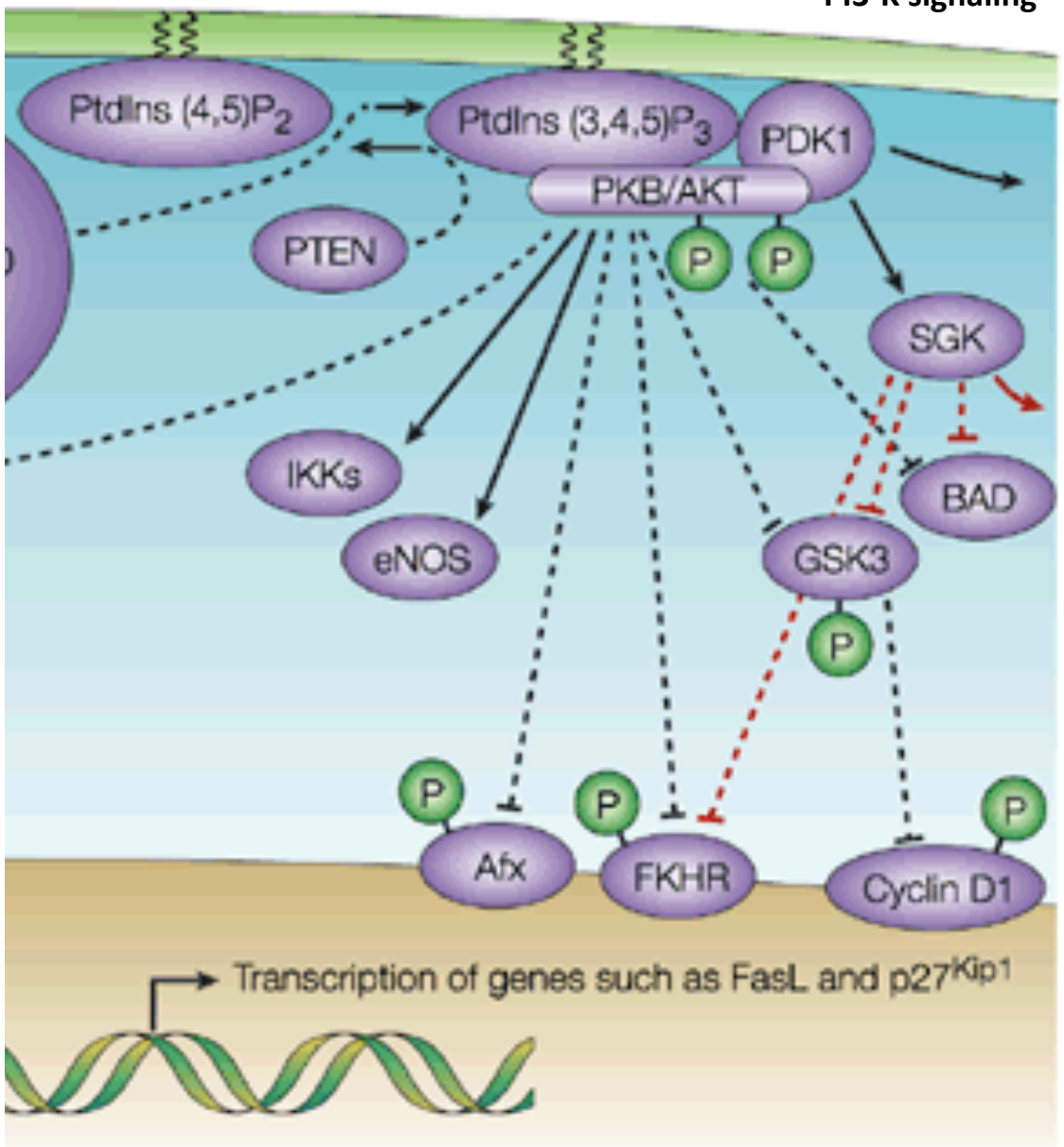
Akt fosforila proteine coinvolte nel:

-Metabolismo

-regolazione NO

-inibizione apoptosi

PI3-K signaling



AKT e BAD

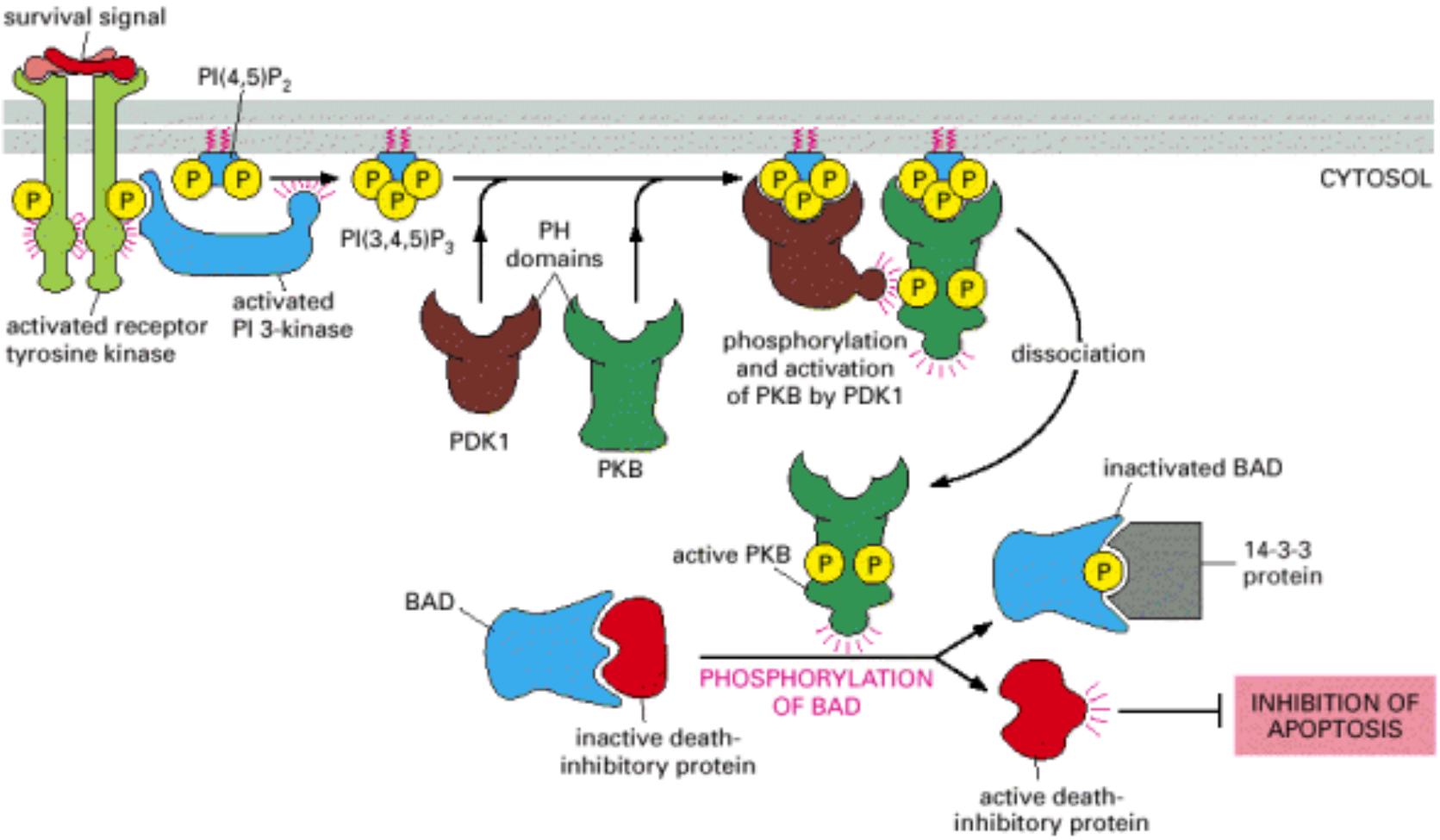

survival signal
PI(4,5)P2
PI(3,4,5)P3
PH domains
CYTOSOL
activated receptor tyrosine kinase
activated PI 3-kinase
PDK1
PKB
phosphorylation and activation of PKB by PDK1
dissociation
inactivated BAD
active PKB
14-3-3 protein
BAD
PHOSPHORYLATION OF BAD
INHIBITION OF APOPTOSIS
inactive death-inhibitory protein
active death-inhibitory protein

Table 2 | **Frequency of mutations in the PI3K–AKT pathway in cancers**

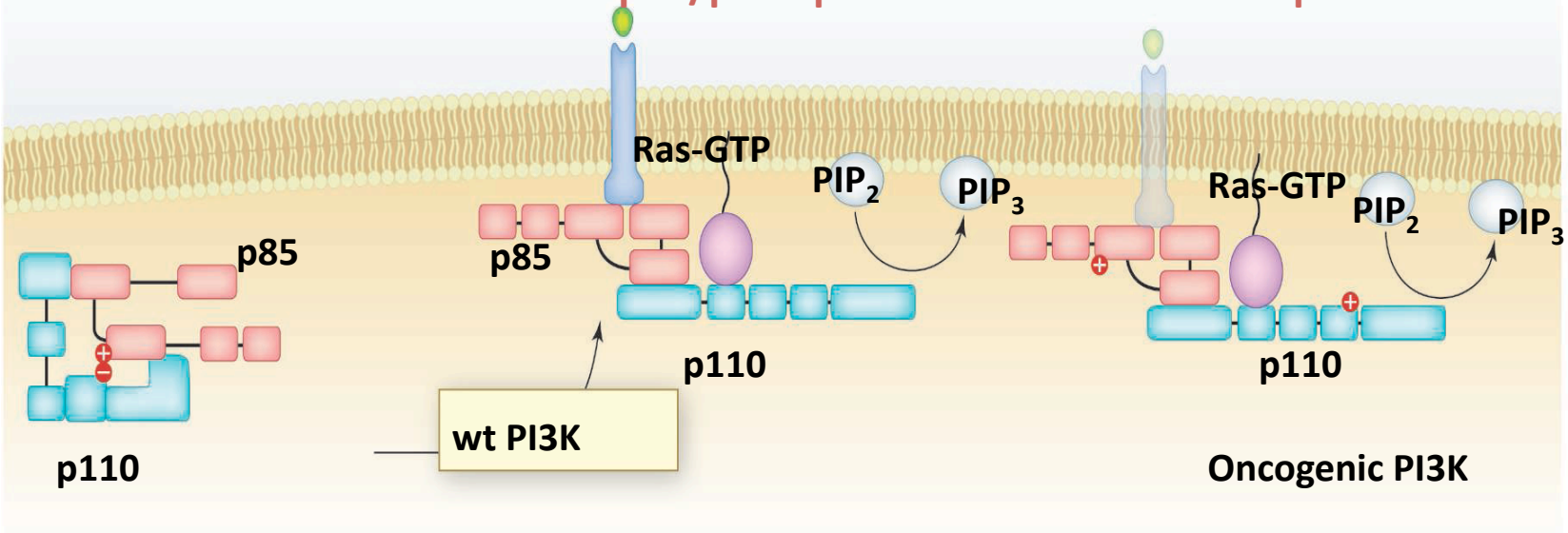
Genetic mutations	Cancer type	Percentage frequency*	References
PIK3CA (p110α)			
Mutations	Breast	26% (176/684)	78, 126–131
	Colon	26% (88/337)	78,132
	Glioma	8% (14/182)	78,133, 134
	Hepatocellular	36% (26/73)	128
	Ovarian	10% (35/365)	127,130
	Lung	2% (4/253)	78,128
	Gastric	7% (24/338)	78,128, 132,135
Amplifications	Head and neck	42% (54/128)	136,137
	Thyroid	9% (12/128)	138
	Lung:		139,140
	Squamous cell	66% (46/70)	
	Adenocarcinoma	5% (4/86)	
	Breast	9% (8/92)	126
	Gastric	36% (20/55)	141
	Oesophageal adenocarcinoma	6% (5/87)	142
	Cervical	69% (11/16)	143

STUDIANDO LE MUTAZIONI DI p110α NEI TUMORI E IDENTIFICANDO LA STRUTTURA 3D DELL'ETERODIMERO p85/p110, 'E STATO IDENTIFICATO UN NUOVO MECCANISMO DI ATTIVAZIONE DI PI 3-KINASE:

- la p85 lega p110a costringendola in una configurazione inattiva

- in seguito a reclutamento di p85 al recettore fosforilato, l'interazione SH2-recettore fosforilato, induce un cambio di configurazione di p85 che permette la rimozione dell'interazione inibitoria su p110

- il cambiamento dell'interazione p85/p110 permette l'interazione di p110 con Ras-GTP



Miled et al. Science, July 2007

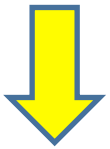
Le proteine tirosina chinasi citosoliche della famiglia Src

La famiglia Src comprende 10 membri:

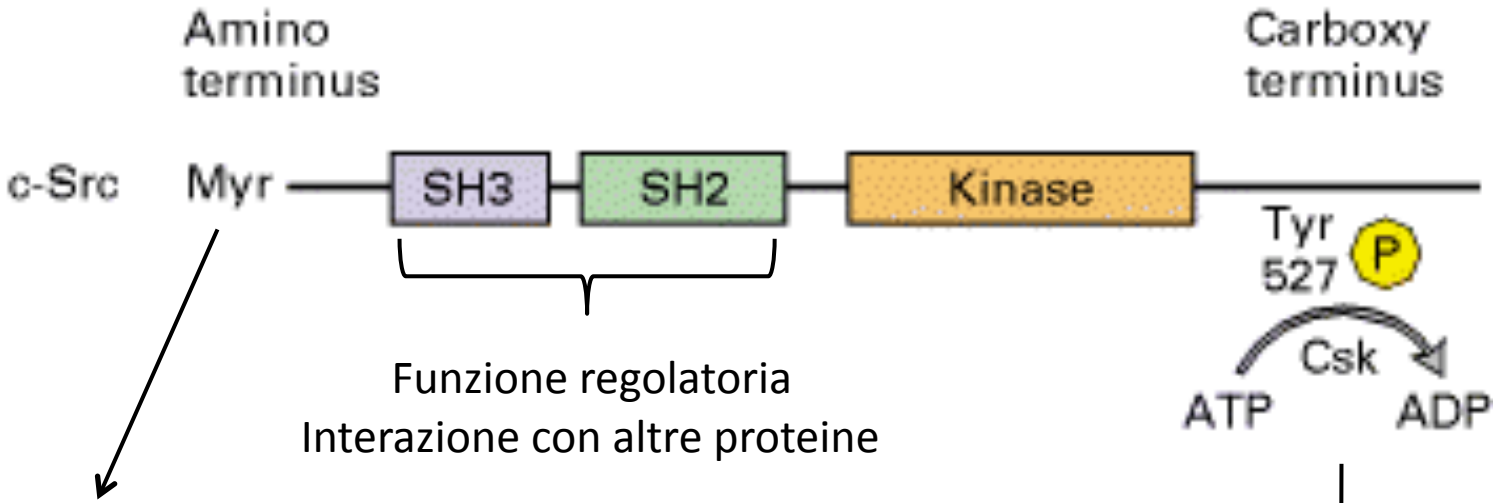
Src, Yes, Fyn → espresse ubiquitariamente

Blk, Fgr, Hck, Lck, Lyn → sistema ematopoietico

Frk, IyK → cellule epiteliali



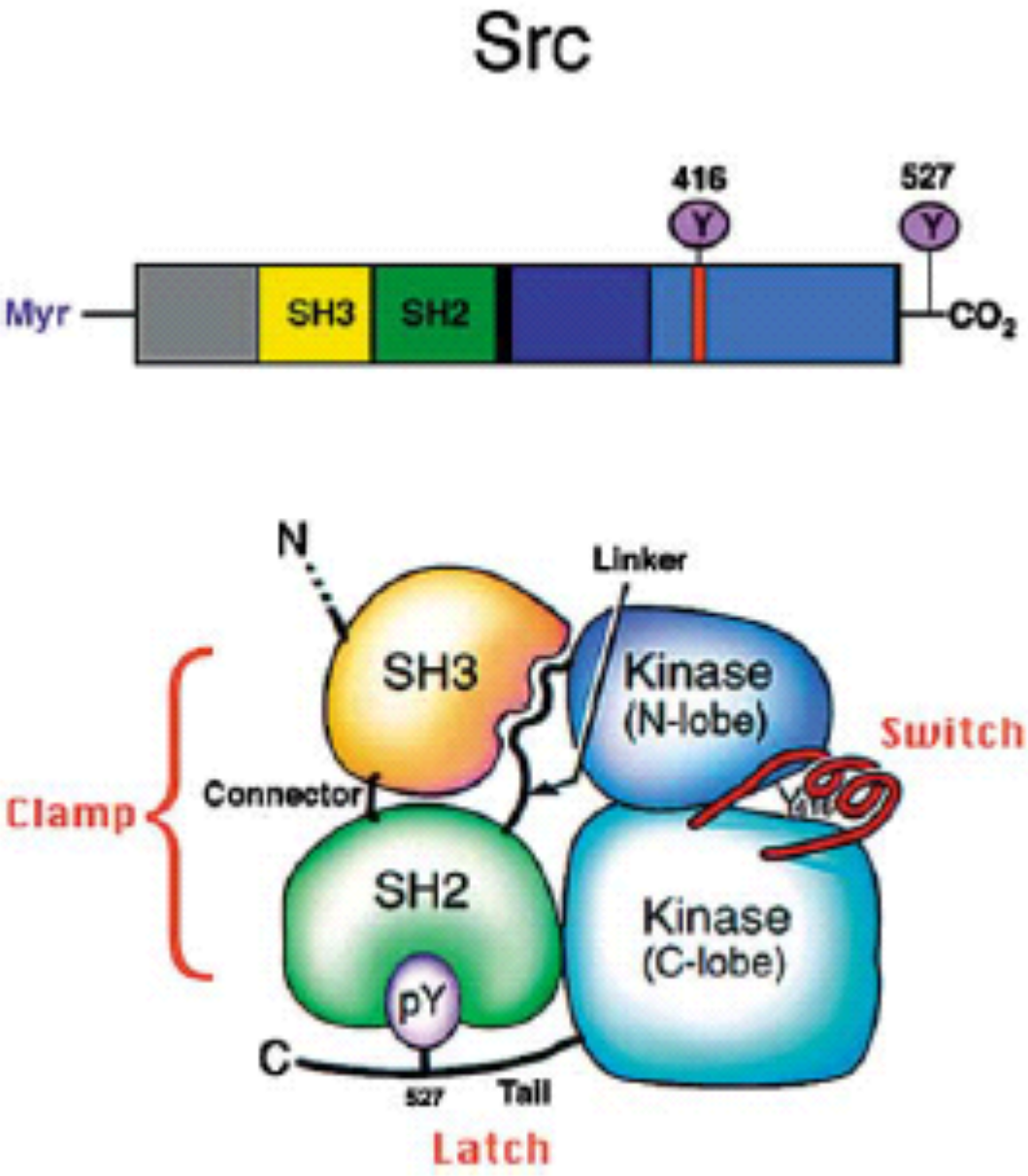
Proliferazione
Adesione
Movimento
Differenziamento
Sopravvivenza



Funzione regolatoria
Interazione con altre proteine

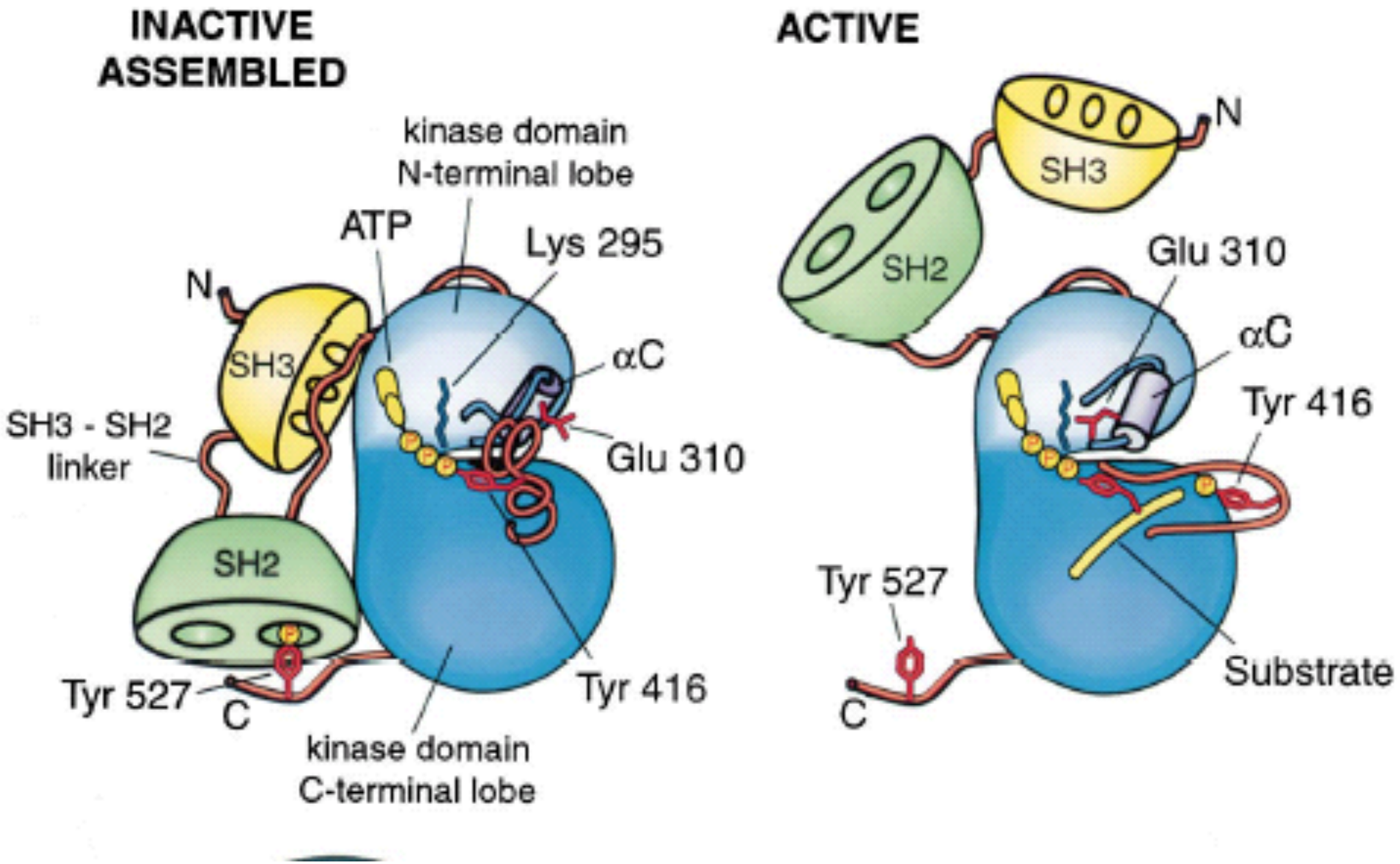
Ancora Src alla membrana plasmatica

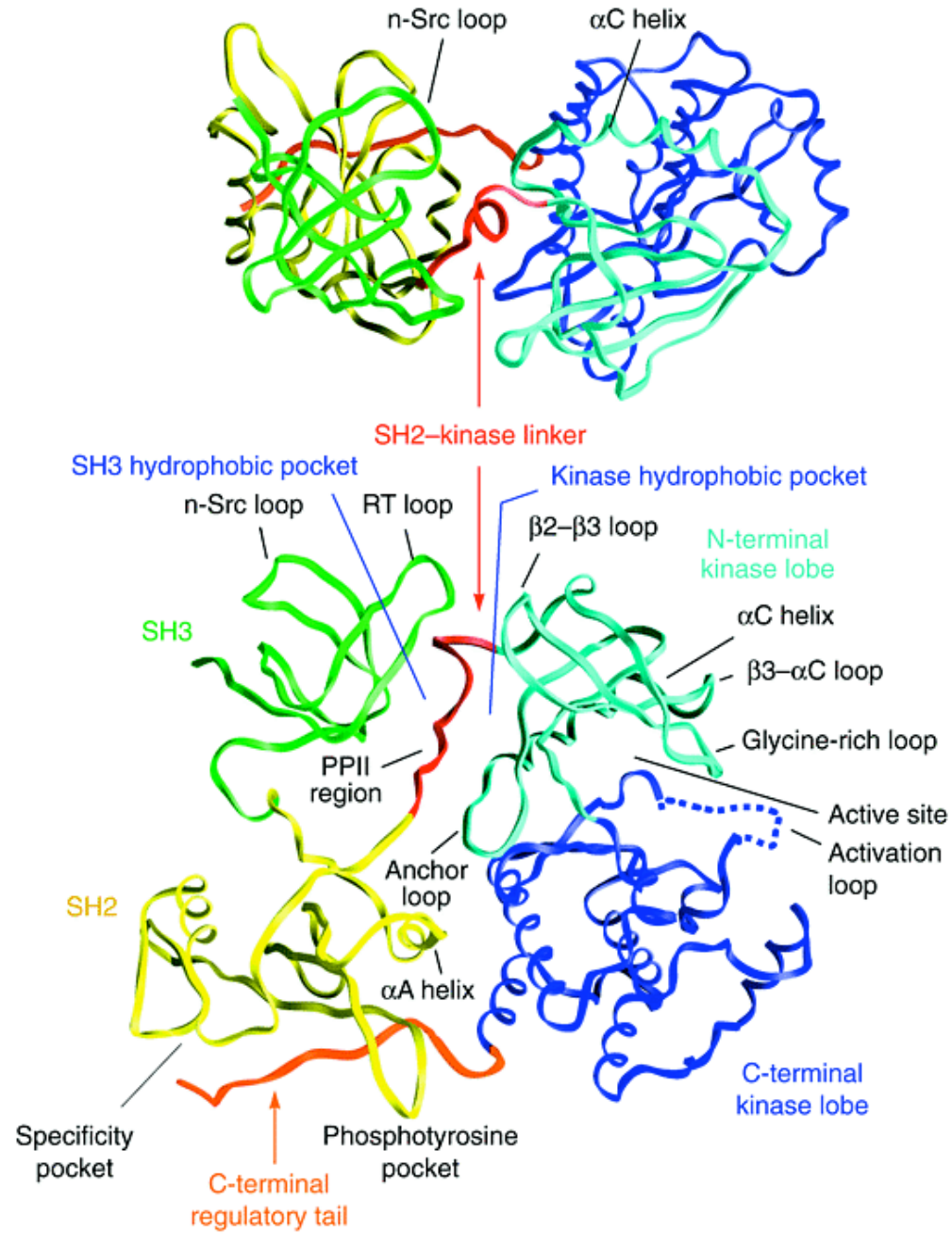
Funzione inibitoria

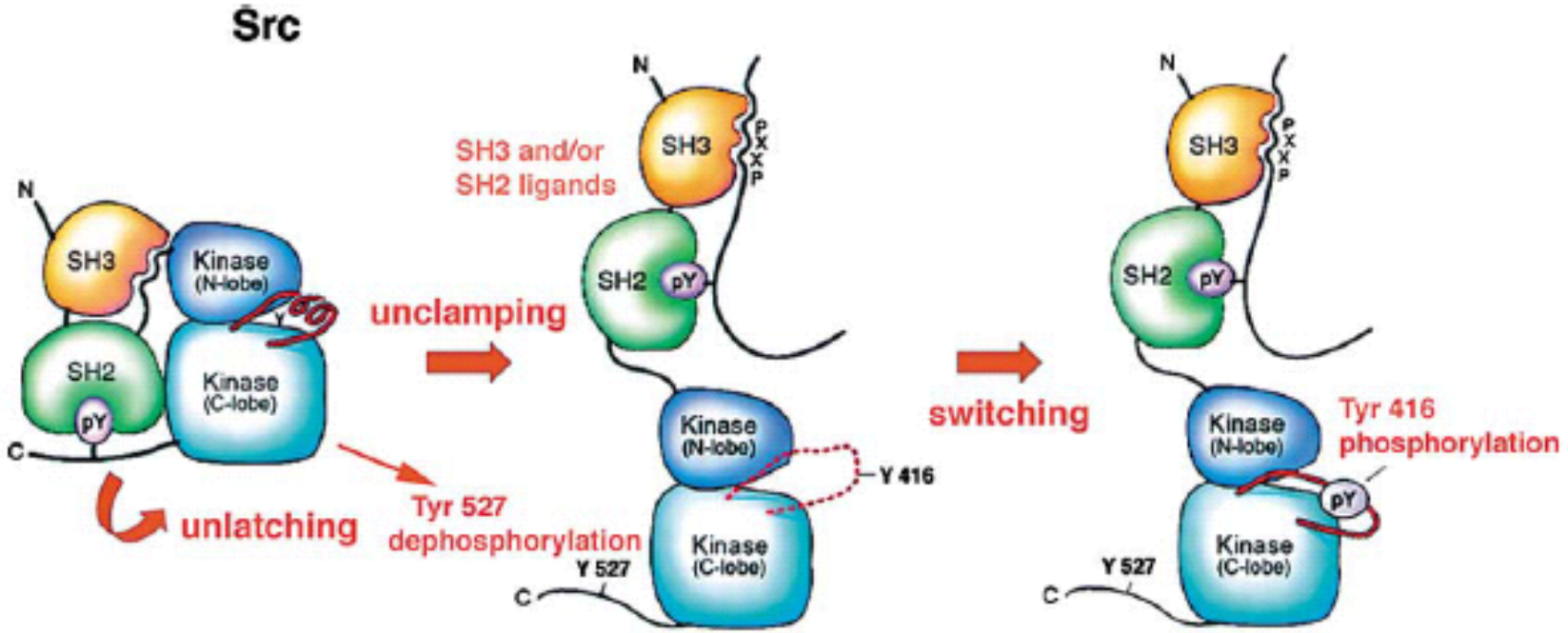


Conformazione CHIUSA: Src **inattiva** e fosforilata sulla Tyr 527

Conformazione APERTA: Src **attiva** e fosforilata sulla Tyr 416




n-Src loop
αC helix
SH2–kinase linker
SH3 hydrophobic pocket
Kinase hydrophobic pocket
n-Src loop
RT loop
β2–β3 loop
N-terminal kinase lobe
αC helix
SH3
β3–αC loop
Glycine-rich loop
PPII region
Active site
Activation loop
Anchor loop
SH2
αA helix
C-terminal kinase lobe
Specificity pocket
Phosphotyrosine pocket
C-terminal regulatory tail

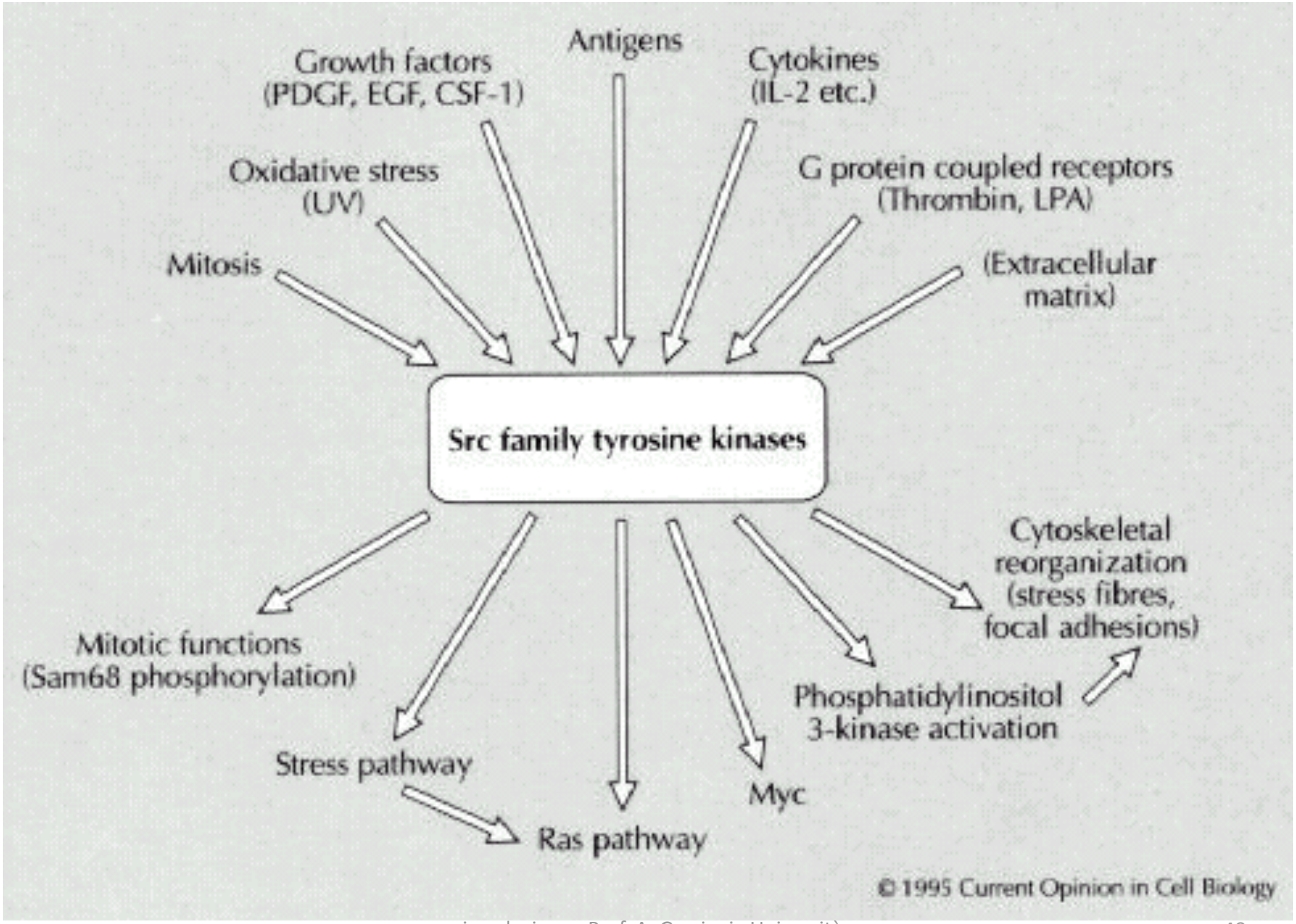


Src is activated by:

- **SH2 domain agonists, i.e.receptor docking sites, other proteins.**

- **SH3 domain agonists, i.e. binding of Src SH3 domain to specific PXXP sequence (Nef of HIV, ...)**

- **other proteins which destabilize the inhibitory intramolecular interactions (Gαs).**


Growth factors
(PDGF, EGF, CSF-1)
Antigens
Cytokines
(IL-2 etc.)
Oxidative stress
(UV)
G protein coupled receptors
(Thrombin, LPA)
Mitosis
(Extracellular
matrix)
Src family tyrosine kinases
Cytoskeletal
reorganization
(stress fibres,
focal adhesions)
Mitotic functions
(Sam68 phosphorylation)
Phosphatidylinositol
3-kinase activation
Stress pathway
Myc
Ras pathway
© 1995 Current Opinion in Cell Biology