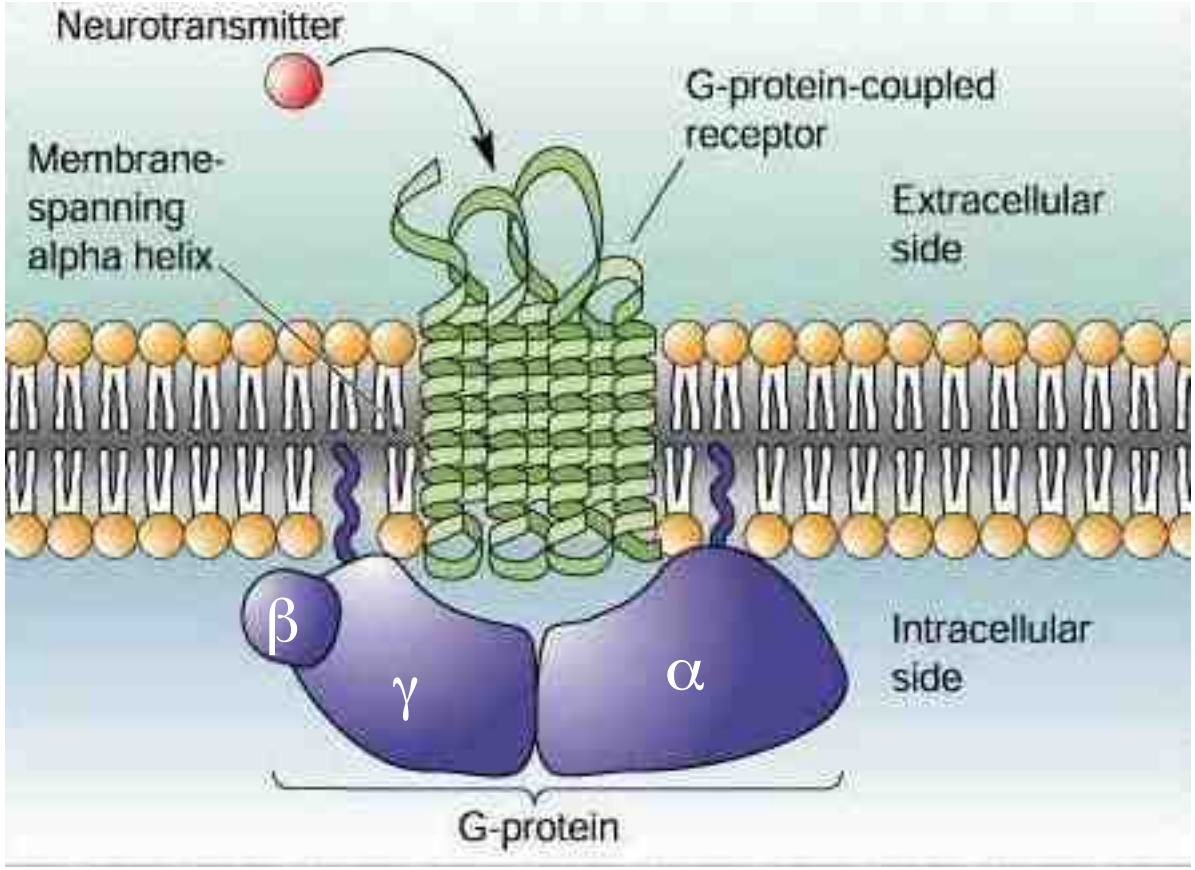
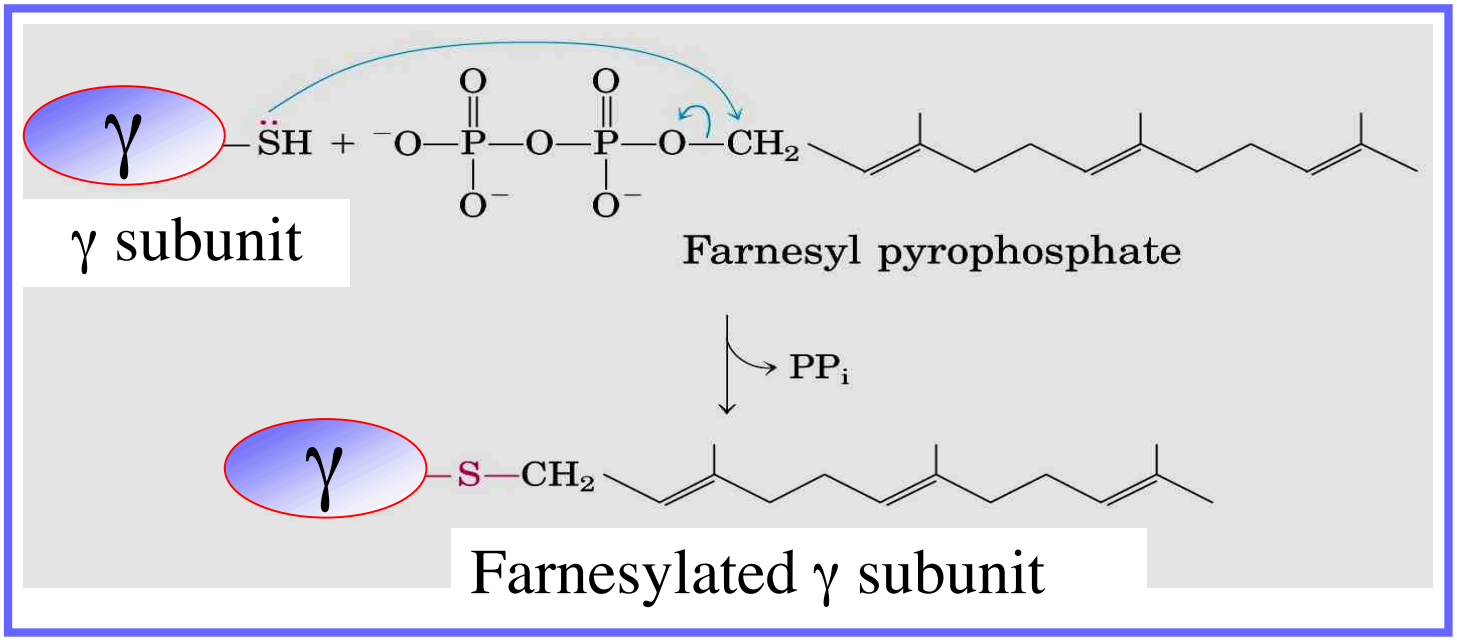
Membrane receptors

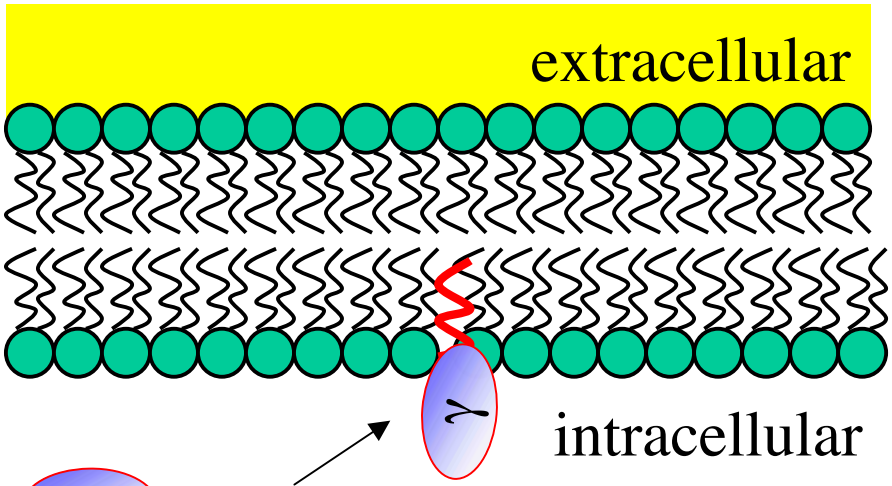
7 pass-transmembrane receptors: GPCR

7-pass transmembrane receptors
GPCR = G protein coupled receptors



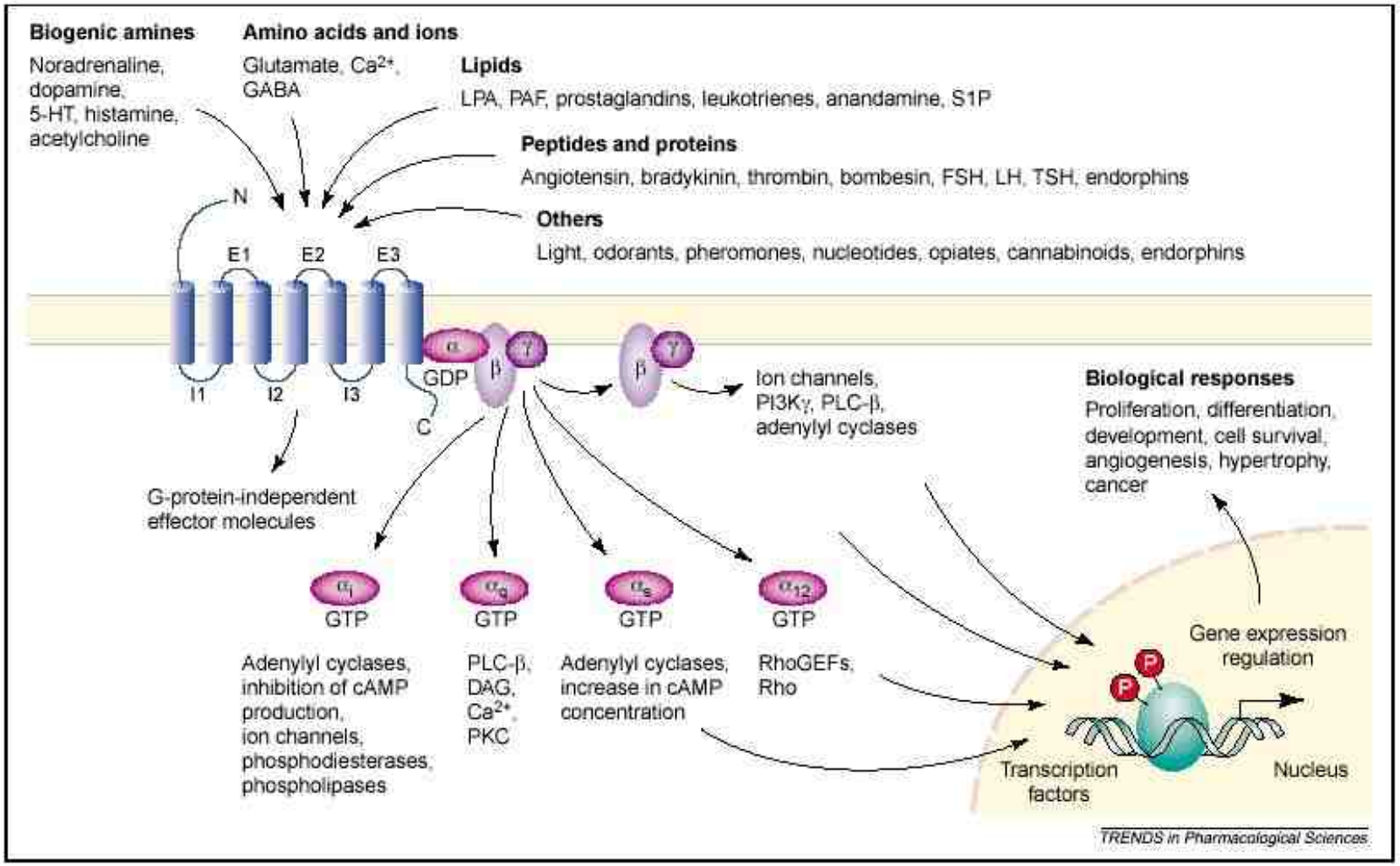
Post-translational modification

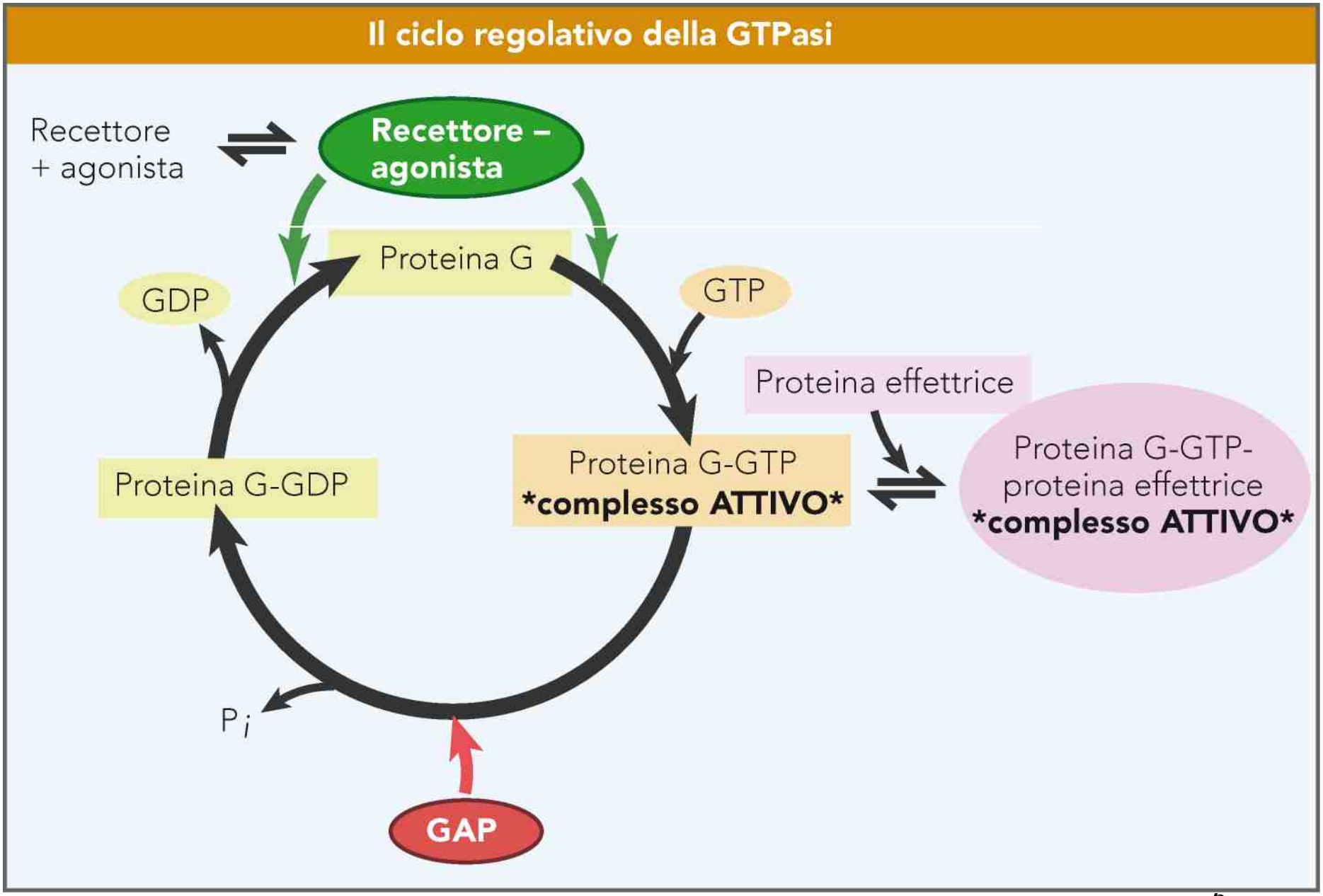




γ +

γ


Biogenic amines
Noradrenaline, dopamine, 5-HT, histamine, acetylcholine
Amino acids and ions
Glutamate, Ca2+, GABA
Lipids
LPA, PAF, prostaglandins, leukotrienes, anandamine, S1P
Peptides and proteins
Angiotensin, bradykinin, thrombin, bombesin, FSH, LH, TSH, endorphins
Others
Light, odorants, pheromones, nucleotides, opiates, cannabinoids, endorphins
N
E1
E2
E3
I1
I2
I3
C
GDP
α
β
γ
Ion channels, PI3Kγ, PLC-β, adenylyl cyclases
Biological responses
Proliferation, differentiation, development, cell survival, angiogenesis, hypertrophy, cancer
G-protein-independent effector molecules
αi GTP
αq GTP
αs GTP
α12 GTP
Adenylyl cyclases, inhibition of cAMP production, ion channels, phosphodiesterases, phospholipases
PLC-β, DAG, Ca2+, PKC
Adenylyl cyclases, increase in cAMP concentration
RhoGEFs, Rho
Gene expression regulation
P
Transcription factors
Nucleus
TRENDS in Pharmacological Sciences


Il ciclo regolativo della GTPasi
Recettore
+ agonista
Recettore –
agonista
Proteina G
GDP
GTP
Proteina effettrice
Proteina G-GTP
complesso ATTIVO
Proteina G-GTP-
proteina effettrice
complesso ATTIVO
Proteina G-GDP
Pi
GAP

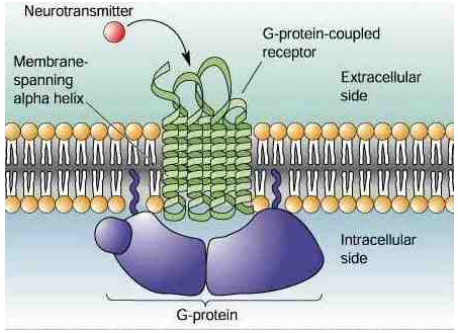
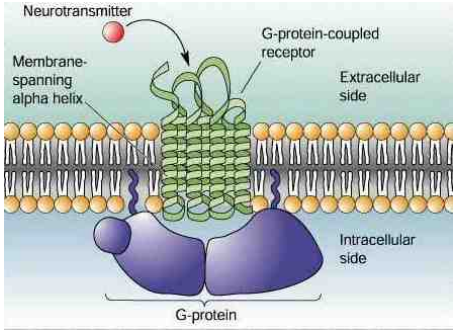
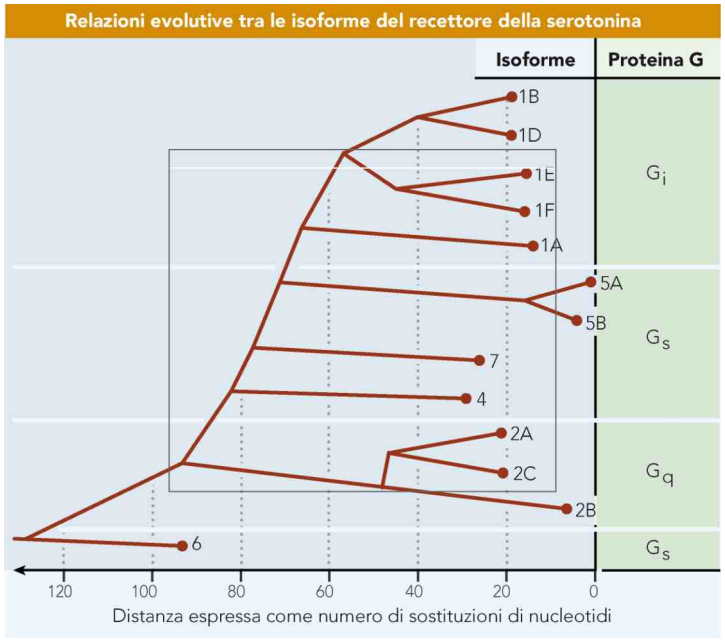


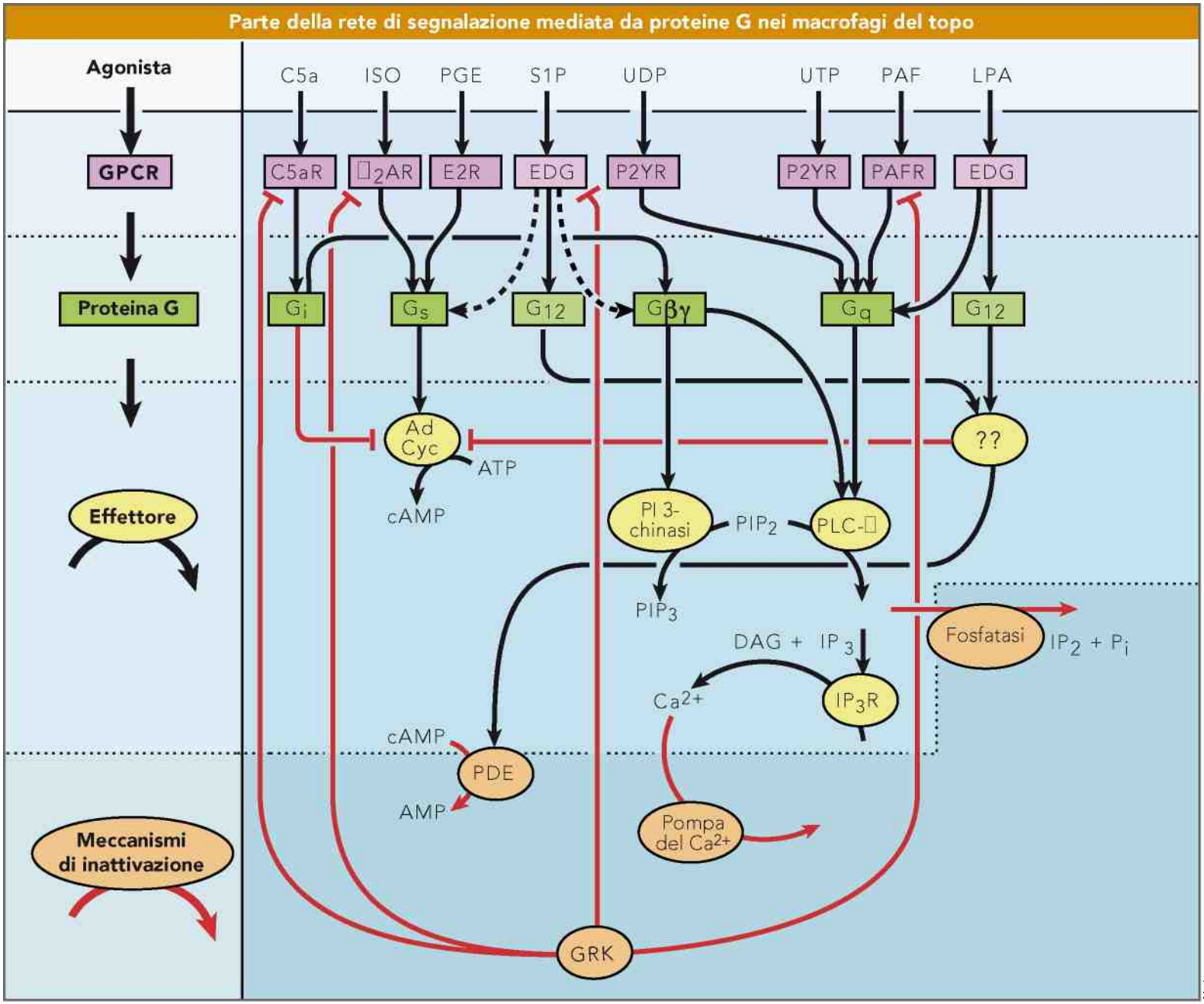
Table 7.1 Endogenous ligands for G-protein-coupled receptors

Small molecules	Glycoproteins	Peptides
Acetylcholine	Lutropin	Angiotensin
Adenosine	Thyrotropin	Bombesin
Adrenaline	FSH	Bradykinin
Cannabinoids		C5a
Dopamine		Calcitonin
Histamine		Cholecystokinin
Leukotrienes		Endothelin
Prostaglandins		f-MetLeuPhe
Retinal		Glucagon
Serotonin		Neurokinins
		Neuropeptide Y
		Neurotensin
		Opioids
		Oxytocin
		Parathyroid hormone
		Somatostatin
		Thrombin (amino-terminal cleavage peptide)
		Vasopressin



Bersagli delle proteine G		
	PROTEINA EFFETTRICE	
Proteina G	Stimolata	Inibita
G_s G_{olf}	Adenilato ciclasi	
G_i (3) G_o G_z	Canale per il K^+, PI 3-chinasi	Adenilato ciclasi
G_{gus}	Altri canali cationici	
G_t (2)	GMP ciclico fosfodiesterasi	
G_q (4)	Fosfolipasi-Cβ	
G_{12} G_{13}	Rho GEF	




Parte della rete di segnalazione mediata da proteine G nei macrofagi del topo
Agonista
C5a
ISO
PGE
S1P
UDP
UTP
PAF
LPA
GPCR
C5aR
2AR
E2R
EDG
P2YR
P2YR
PAFR
EDG
Proteina G
Gi
Gs
G12
Gβγ
Gq
G12
Ad Cyc
ATP
cAMP
??
Effettore
PI 3-chinasi
PIP2
PLC-
PIP3
DAG + IP3
Fosfatasi
IP2 + Pi
Ca2+
IP3R
cAMP
PDE
AMP
Pompa del Ca2+
Meccanismi di inattivazione
GRK

La via dell'AMPc

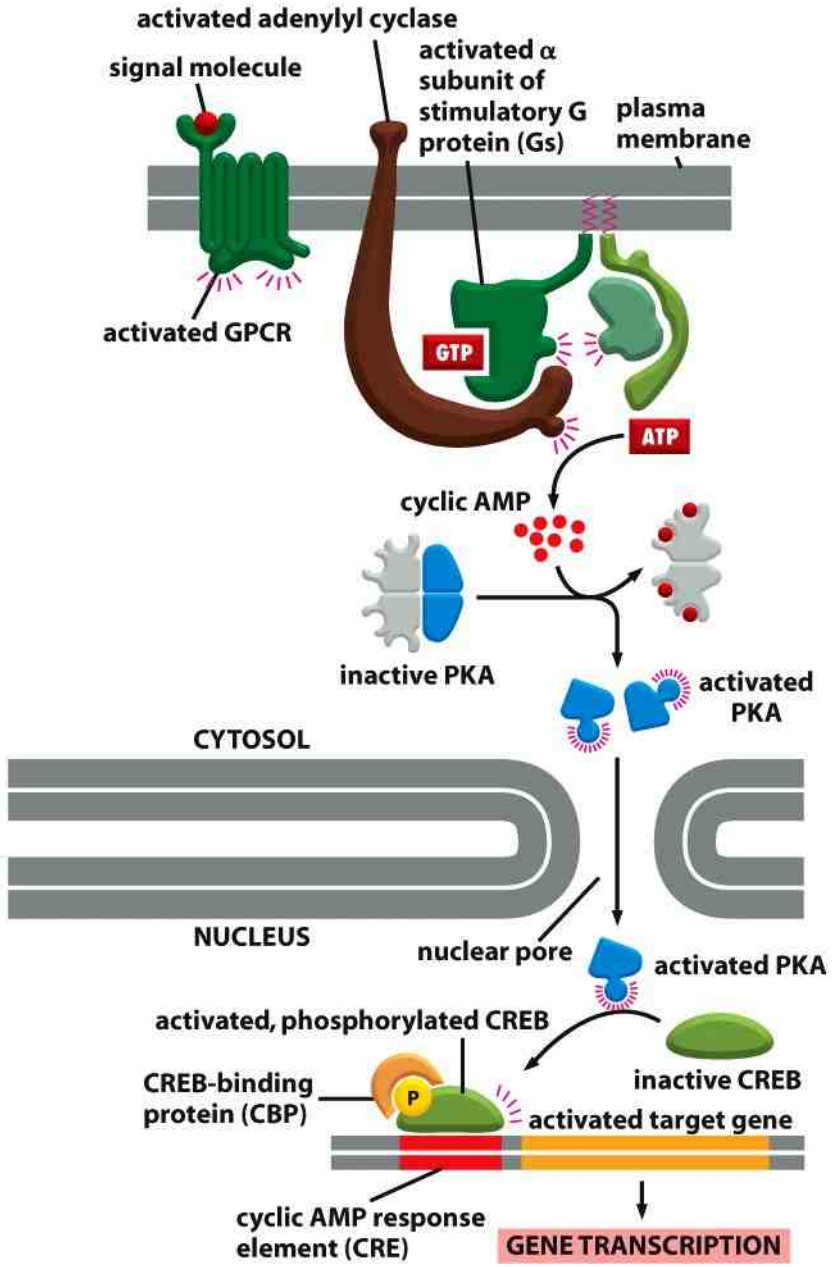
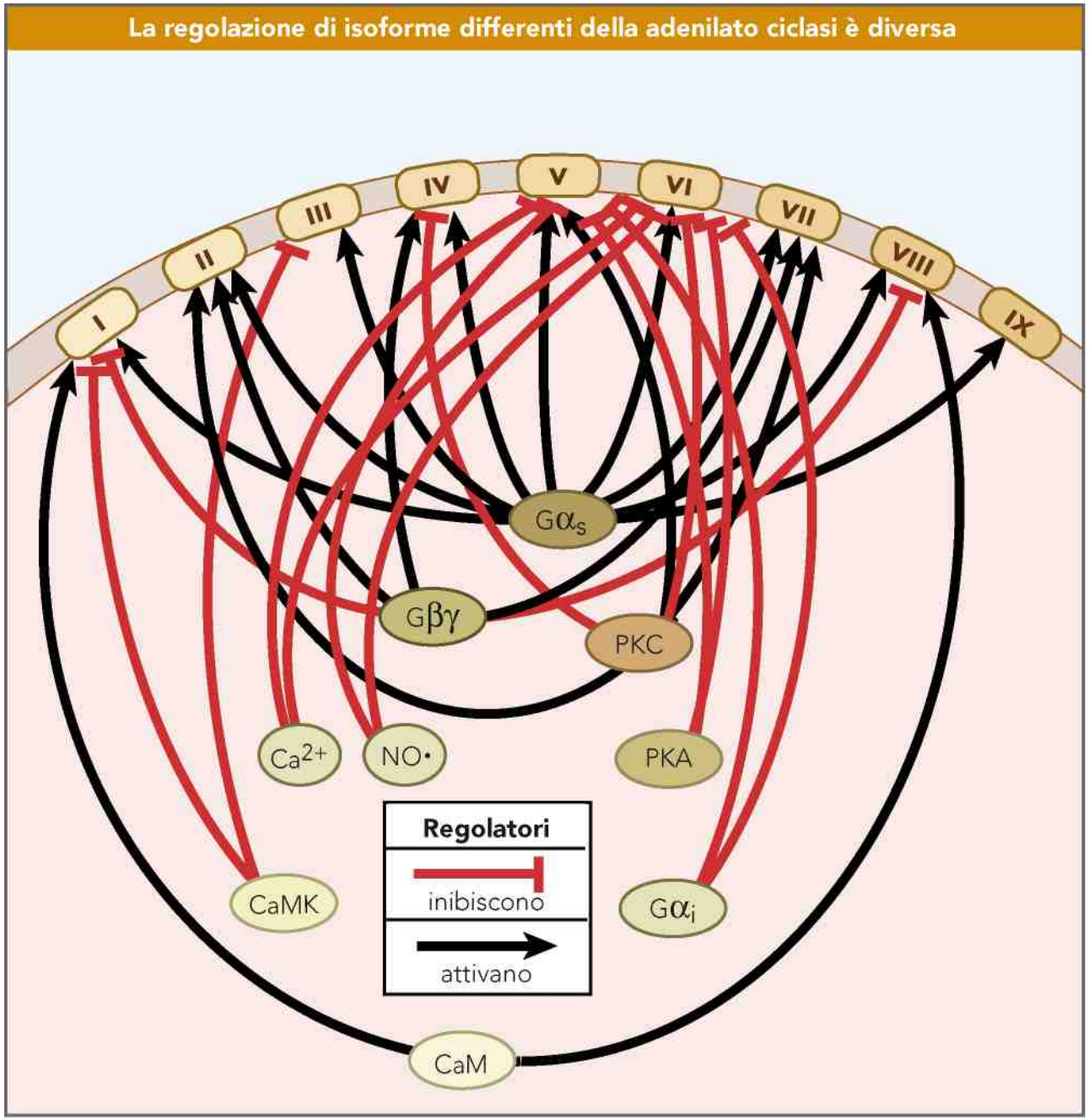
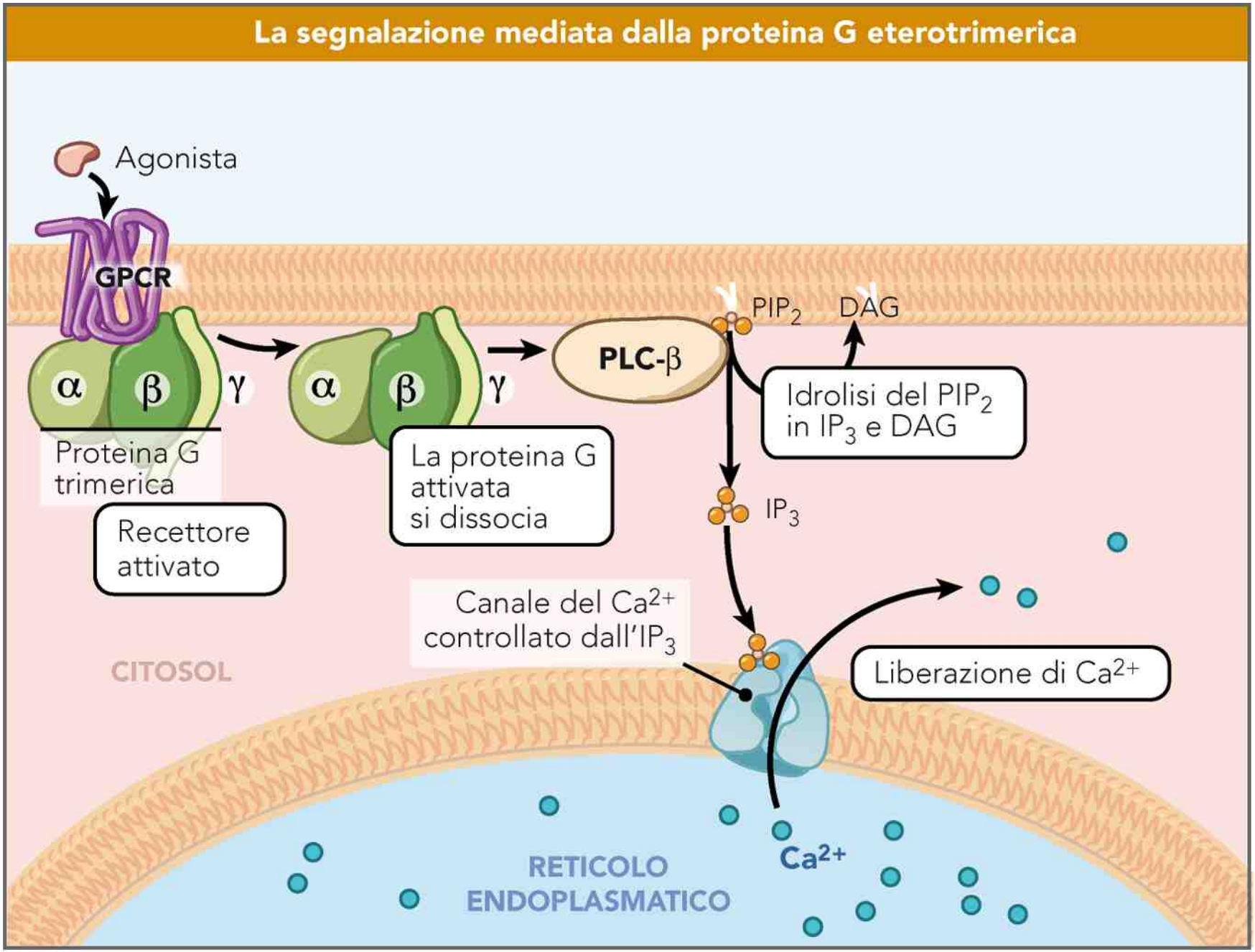


Figure 15-36 *Molecular Biology of the Cell* (© Garland Science 2008)


La regolazione di isoforme differenti della adenilato ciclasi è diversa
I
II
III
IV
V
VI
VII
VIII
IX
Gαs
Gβγ
PKC
Ca2+
NO•
PKA
CaMK
Gαi
Regolatori
inibiscono
attivano
CaM

La segnalazione mediata dalla proteina G eterotrimerica



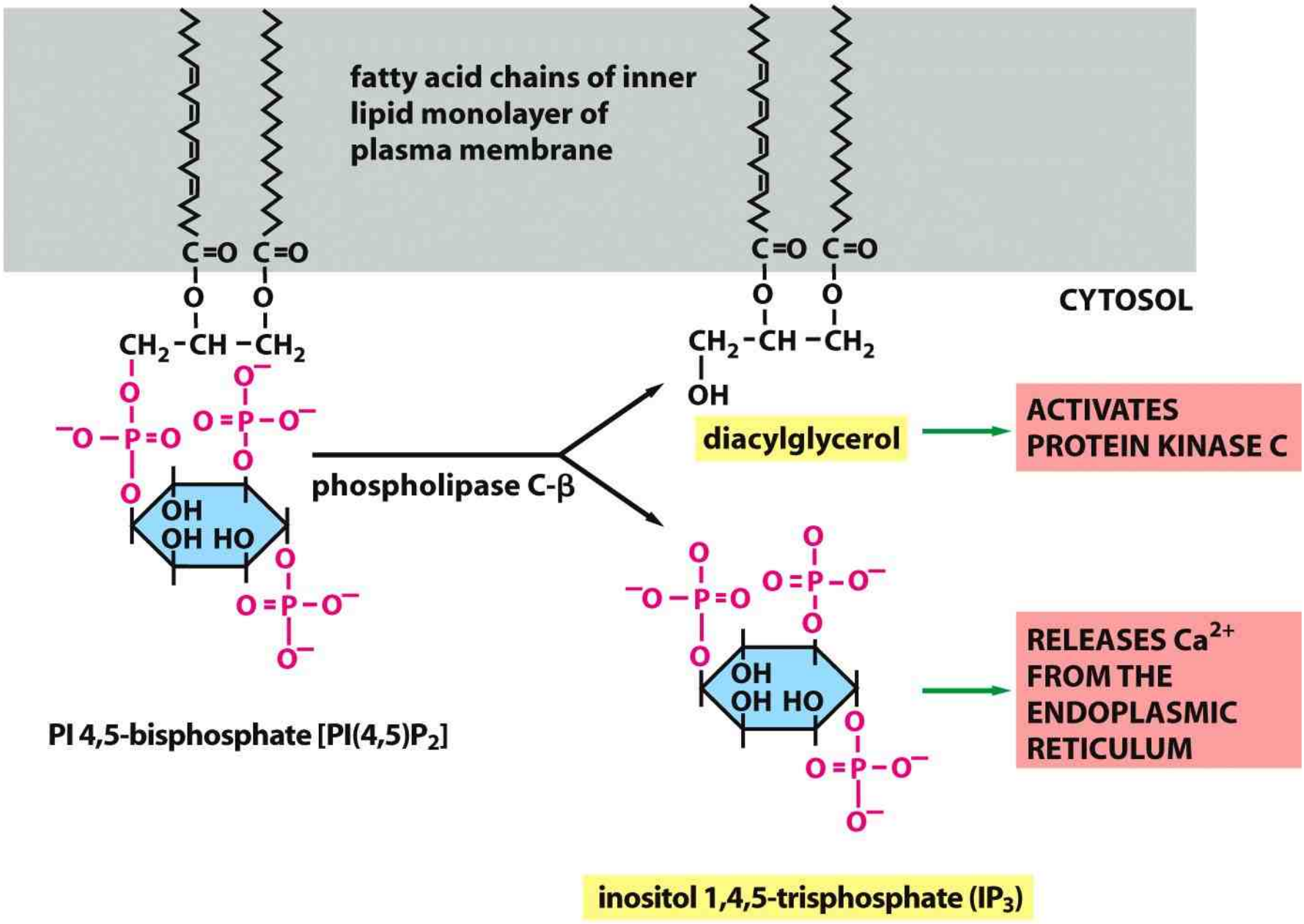


Figure 15-38 *Molecular Biology of the Cell* (© Garland Science 2008)

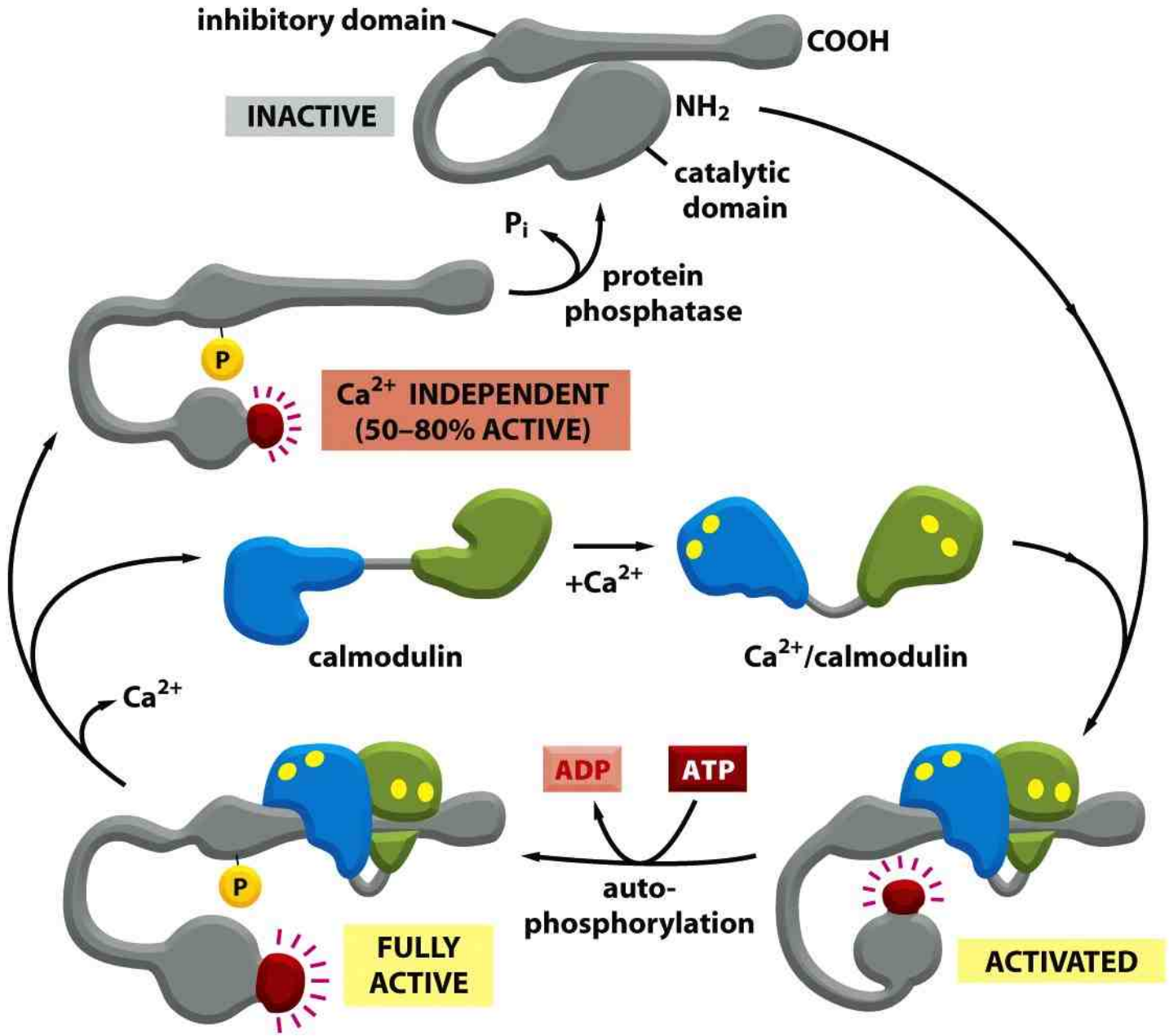
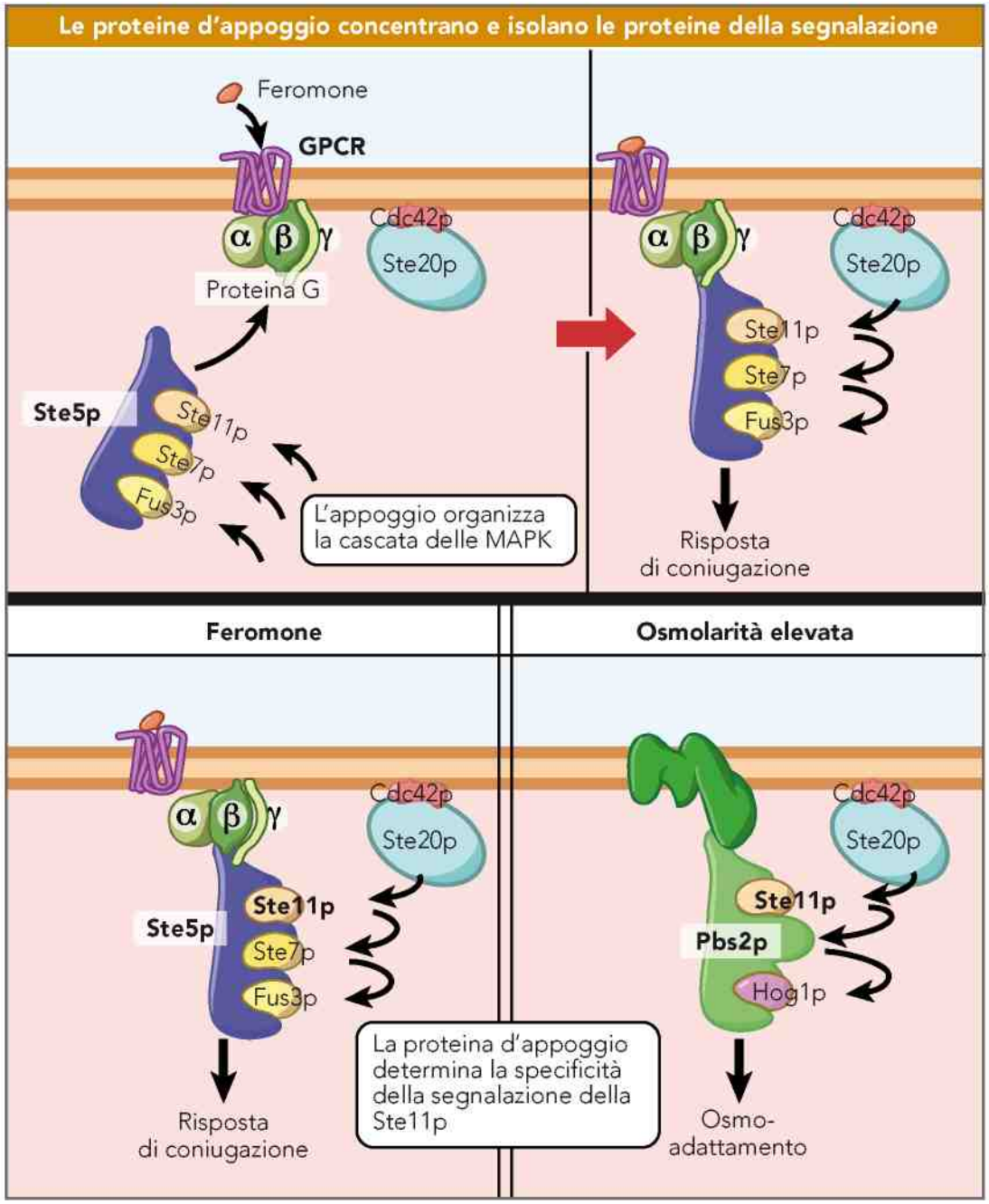
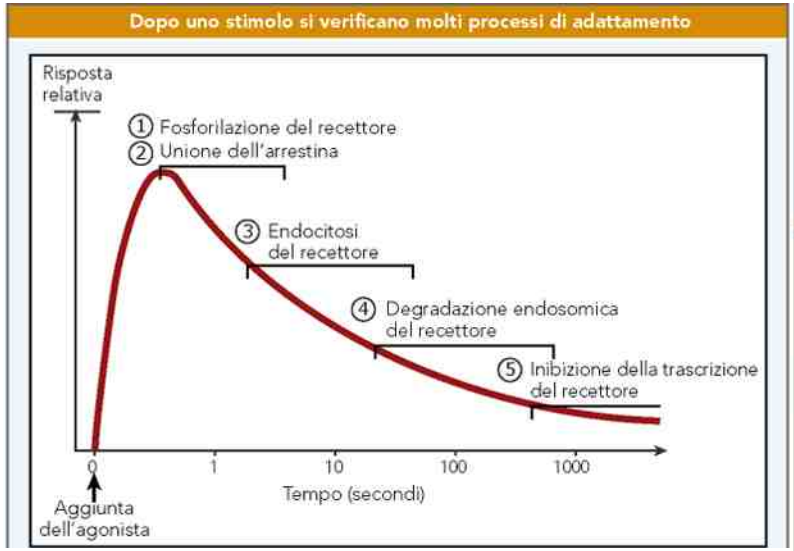
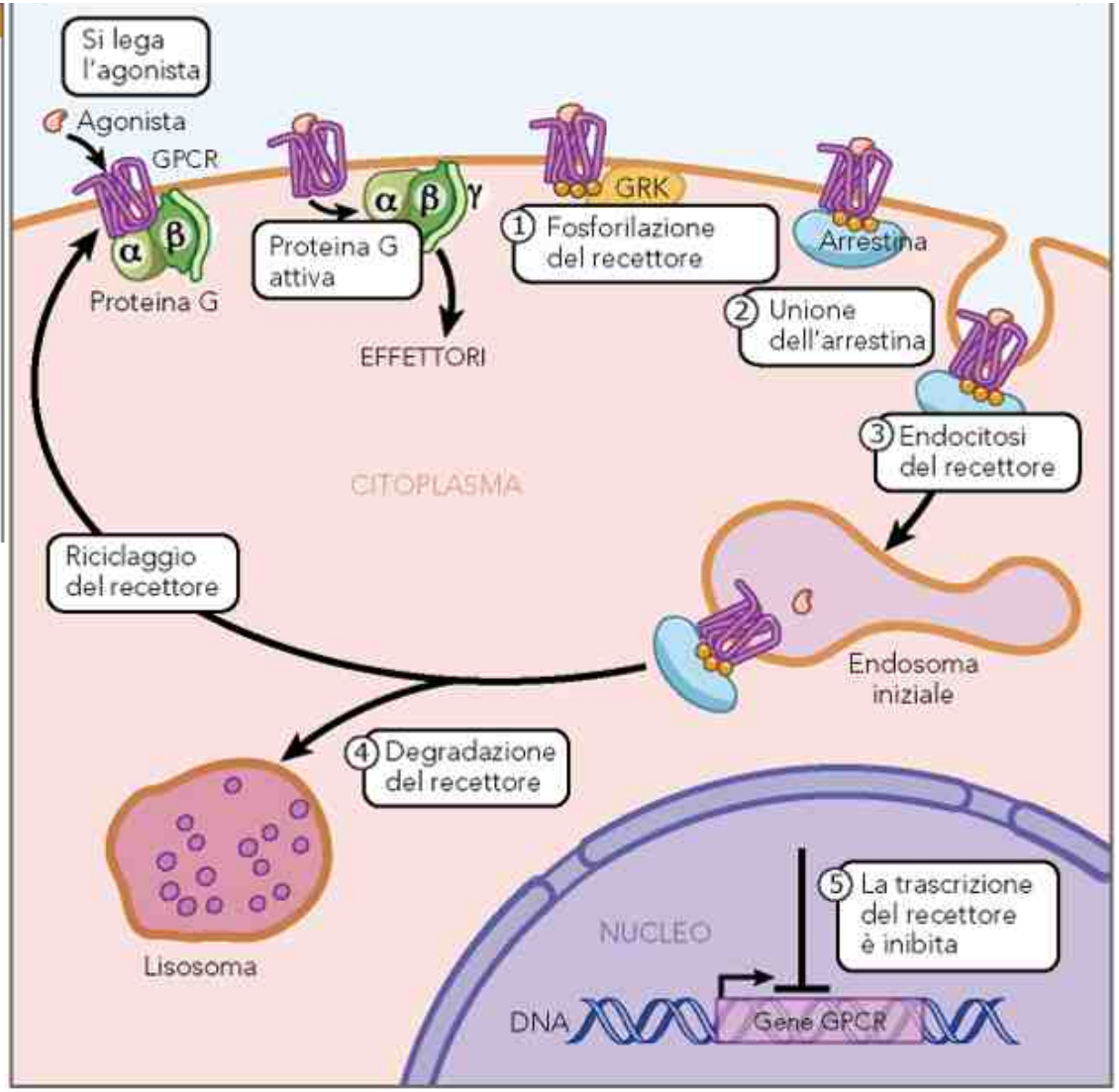


Figure 15-44 *Molecular Biology of the Cell* (© Garland Science 2008)


Le proteine d'appoggio concentrano e isolano le proteine della segnalazione
Feromone
GPCR
α β γ
Proteina G
Cdc42p
Ste20p
Ste5p
Ste11p
Ste7p
Fus3p
L'appoggio organizza la cascata delle MAPK
Risposta di coniugazione
Feromone
Osmolarità elevata
Pbs2p
Hog1p
La proteina d'appoggio determina la specificità della segnalazione della Ste11p
Osmo-adattamento

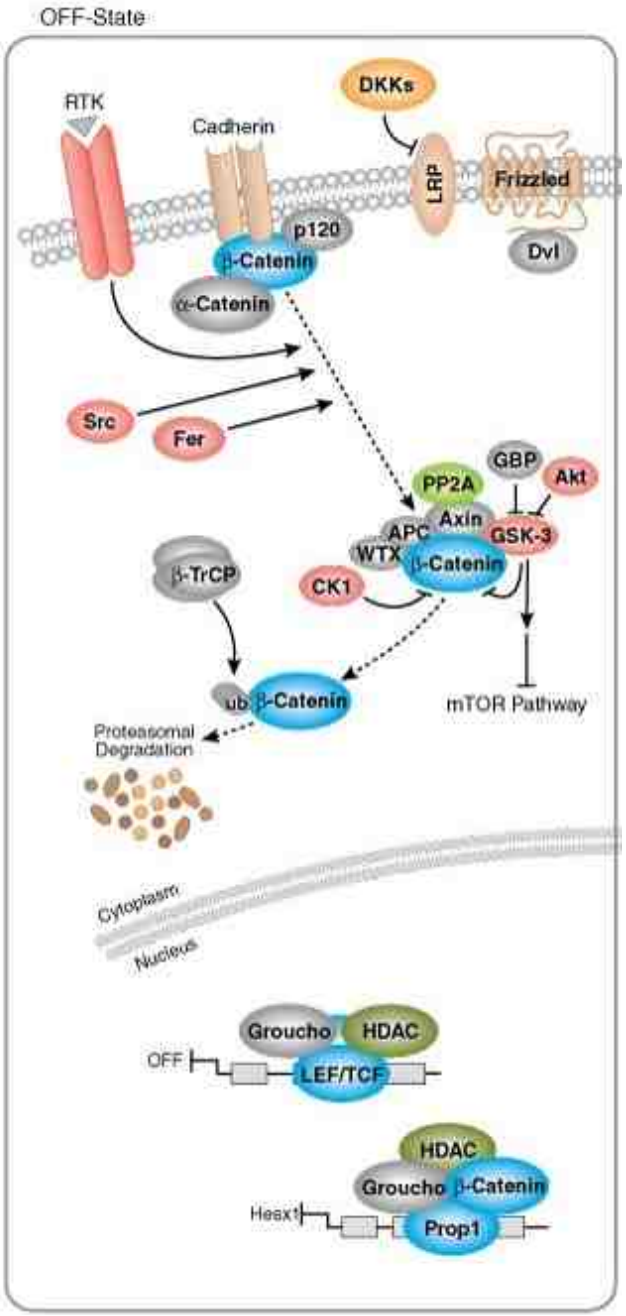

Dopo uno stimolo si verificano molti processi di adattamento
Risposta relativa
1 Fosforilazione del recettore
2 Unione dell'arrestina
3 Endocitosi del recettore
4 Degradazione endosomica del recettore
5 Inibizione della trascrizione del recettore
0
1
10
100
1000
Tempo (secondi)
Aggiunta dell'agonista


Si lega l'agonista
Agonista
GPCR
Proteina G
Proteina G attiva
EFFETTORI
GRK
1 Fosforilazione del recettore
Arrestina
2 Unione dell'arrestina
3 Endocitosi del recettore
CITOPLASMA
Riciclaggio del recettore
Endosoma iniziale
4 Degradazione del recettore
Lisosoma
NUCLEO
5 La trascrizione del recettore è inibita
DNA
Gene GPCR

Not all 7-pass transmembrane receptors are GPCR

Wnt/Frizzled: 7-pass transmembrane receptor and the activation of a transcription factor

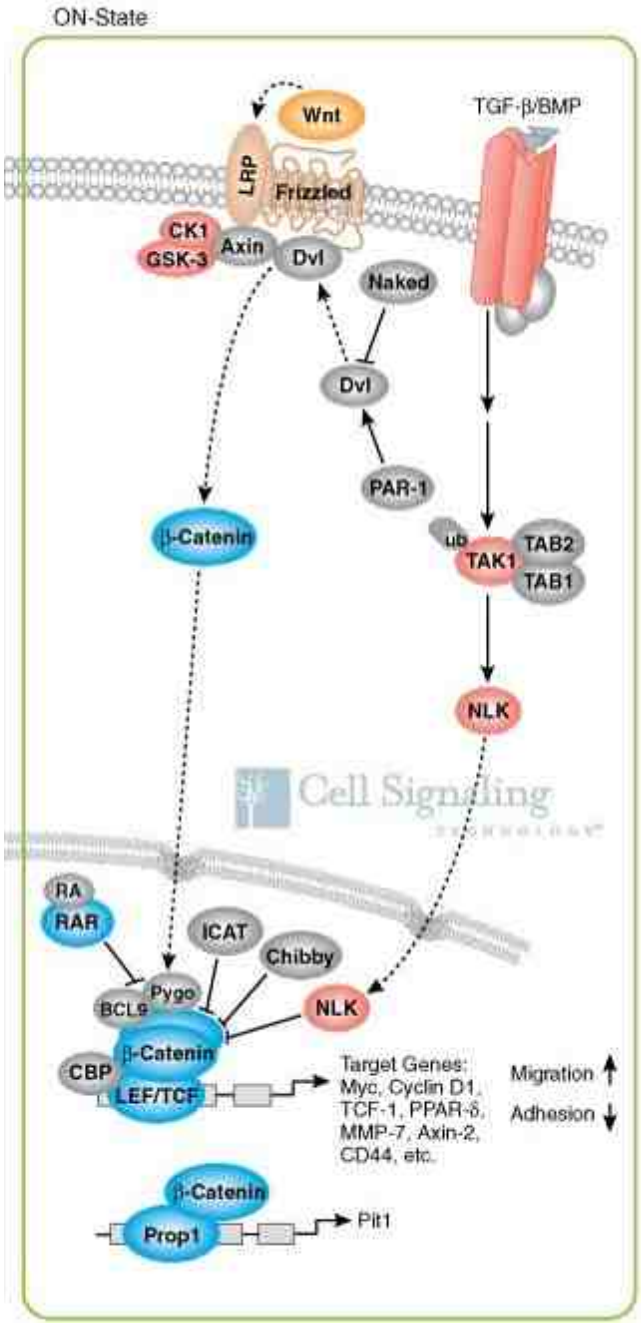
In assenza di segnale da parte di Wnt (**Off-state**), la beta-catenina non associata alle caderine è legata da diversi fattori tra cui APC (Adenomatous poliposis coli) che ne promuove l'ubiquitinazione e la degradazione proteosomale. Anche se viene trasportata nel nucleo, la beta-catenina non può promuovere la trascrizione a causa della presenza dei co-repressori Groucho e HDAC.



* In seguito al legame di Wnt (**On-state**) al suo recettore sono attivate diverse molecole che impediscono la degradazione della beta-catenina e ne promuovono il trasporto verso il nucleo.

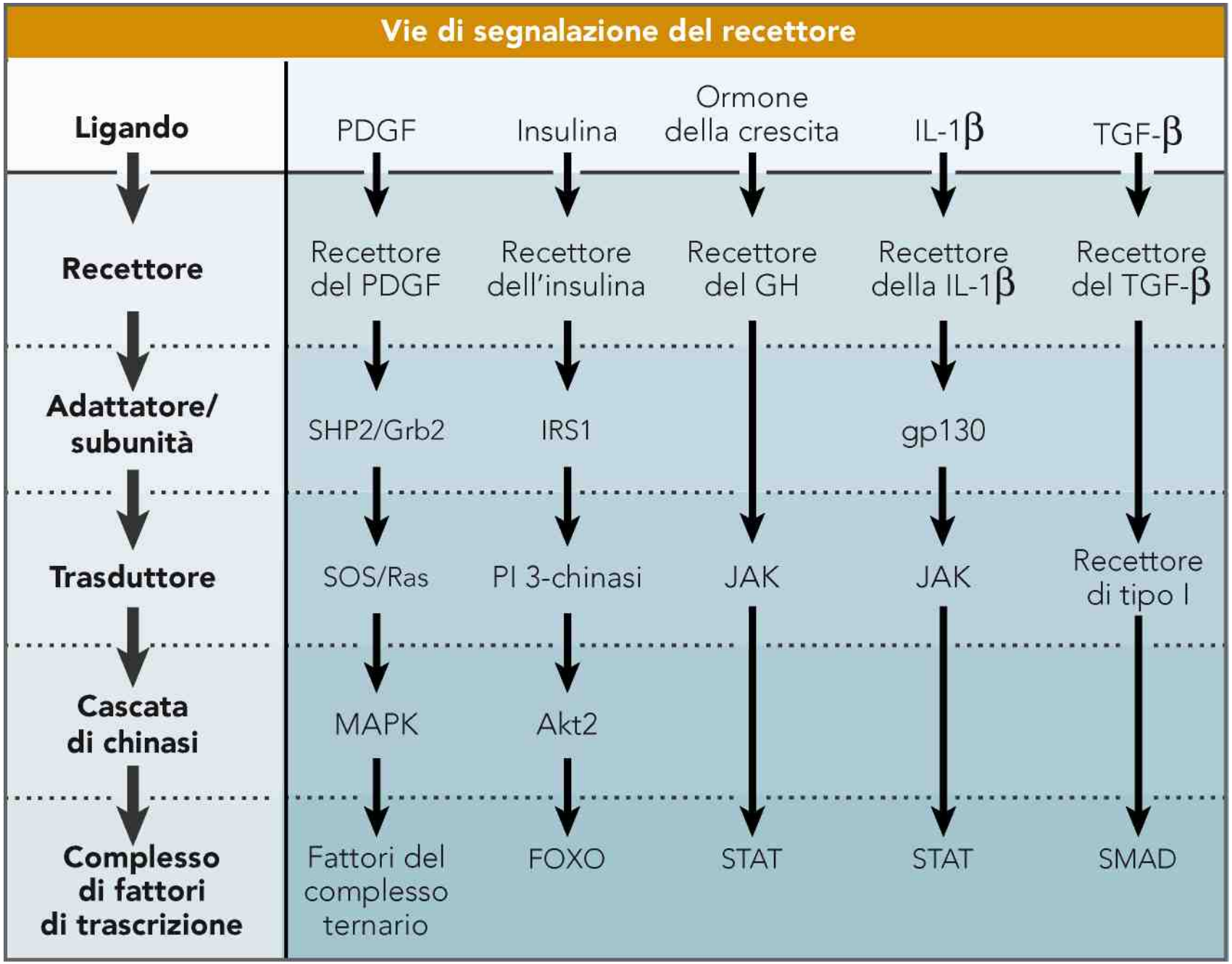
* Nel nucleo la beta-catenina partecipa alla complesso trascrizionale che promuove la trascrizione di geni che promuovono la proliferazione cellulare (Myc, ciclina D) e la migrazione e inibiscono l'adesione

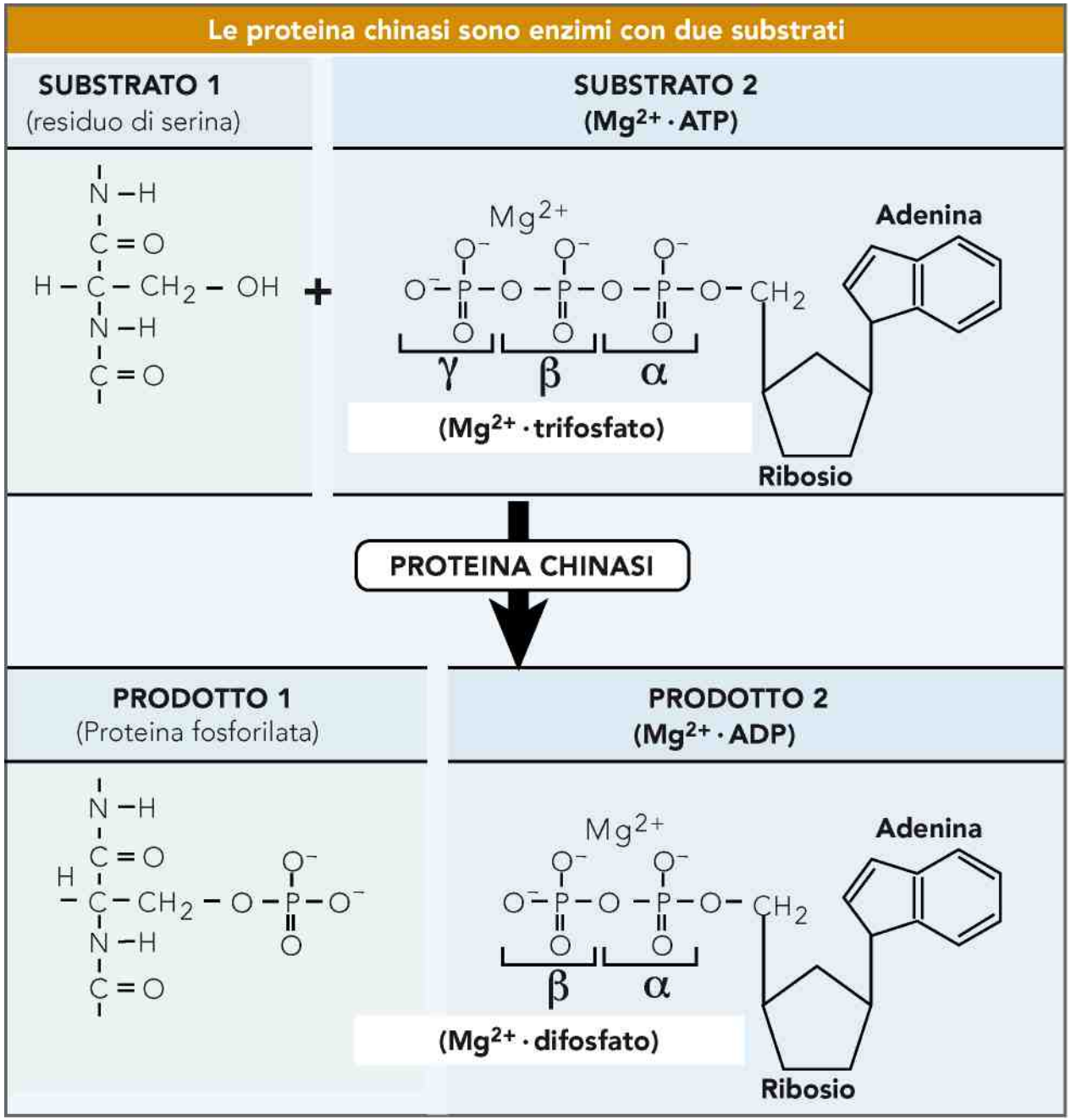
* Durante lo sviluppo embrionale la segnalazione Wnt/beta-catenina si integra con molte altre vie tra cui quelle dell'acido retinoico, FGF, BMP, TGF-beta….

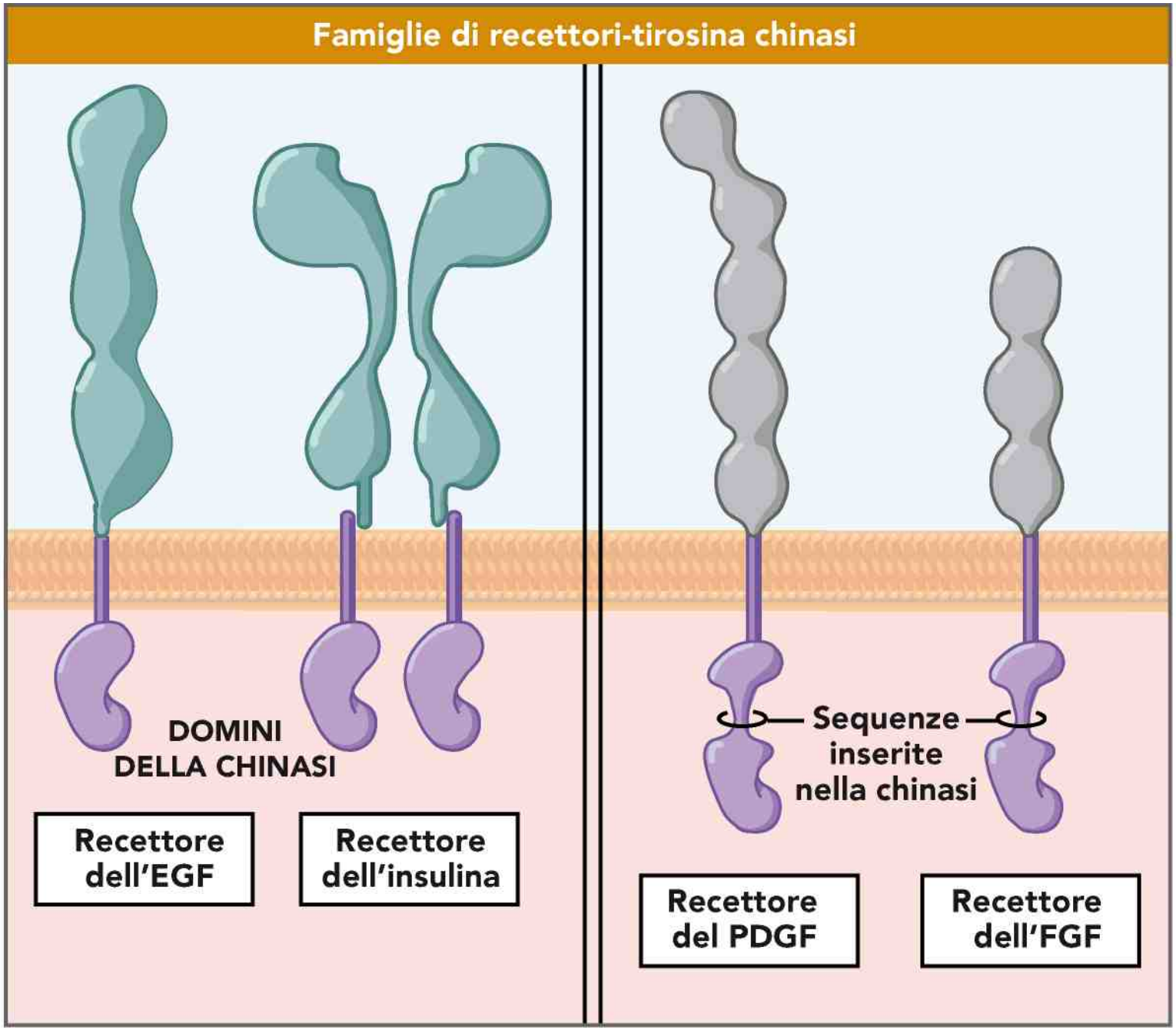


Tyrosine kinase receptors

Cytokine receptors


Vie di segnalazione del recettore
Ligando
PDGF
Insulina
Ormone della crescita
IL-1β
TGF-β
Recettore
Recettore del PDGF
Recettore dell'insulina
Recettore del GH
Recettore della IL-1β
Recettore del TGF-β
Adattatore/ subunità
SHP2/Grb2
IRS1
gp130
Trasduttore
SOS/Ras
PI 3-chinasi
JAK
JAK
Recettore di tipo I
Cascata di chinasi
MAPK
Akt2
Complesso di fattori di trascrizione
Fattori del complesso ternario
FOXO
STAT
STAT
SMAD


Le proteina chinasi sono enzimi con due substrati
SUBSTRATO 1
(residuo di serina)
SUBSTRATO 2
(Mg2+ · ATP)
Mg2+
Adenina
γ
β
α
(Mg2+ · trifosfato)
Ribosio
PROTEINA CHINASI
PRODOTTO 1
(Proteina fosforilata)
PRODOTTO 2
(Mg2+ · ADP)
Mg2+
Adenina
β
α
(Mg2+ · difosfato)
Ribosio


Famiglie di recettori-tirosina chinasi
DOMINI
DELLA CHINASI
Recettore
dell'EGF
Recettore
dell'insulina
Sequenze
inserite
nella chinasi
Recettore
del PDGF
Recettore
dell'FGF

Domini SH2 (o PTB) legano specifiche pTyr
Domini SH3 legano zone riche di proline

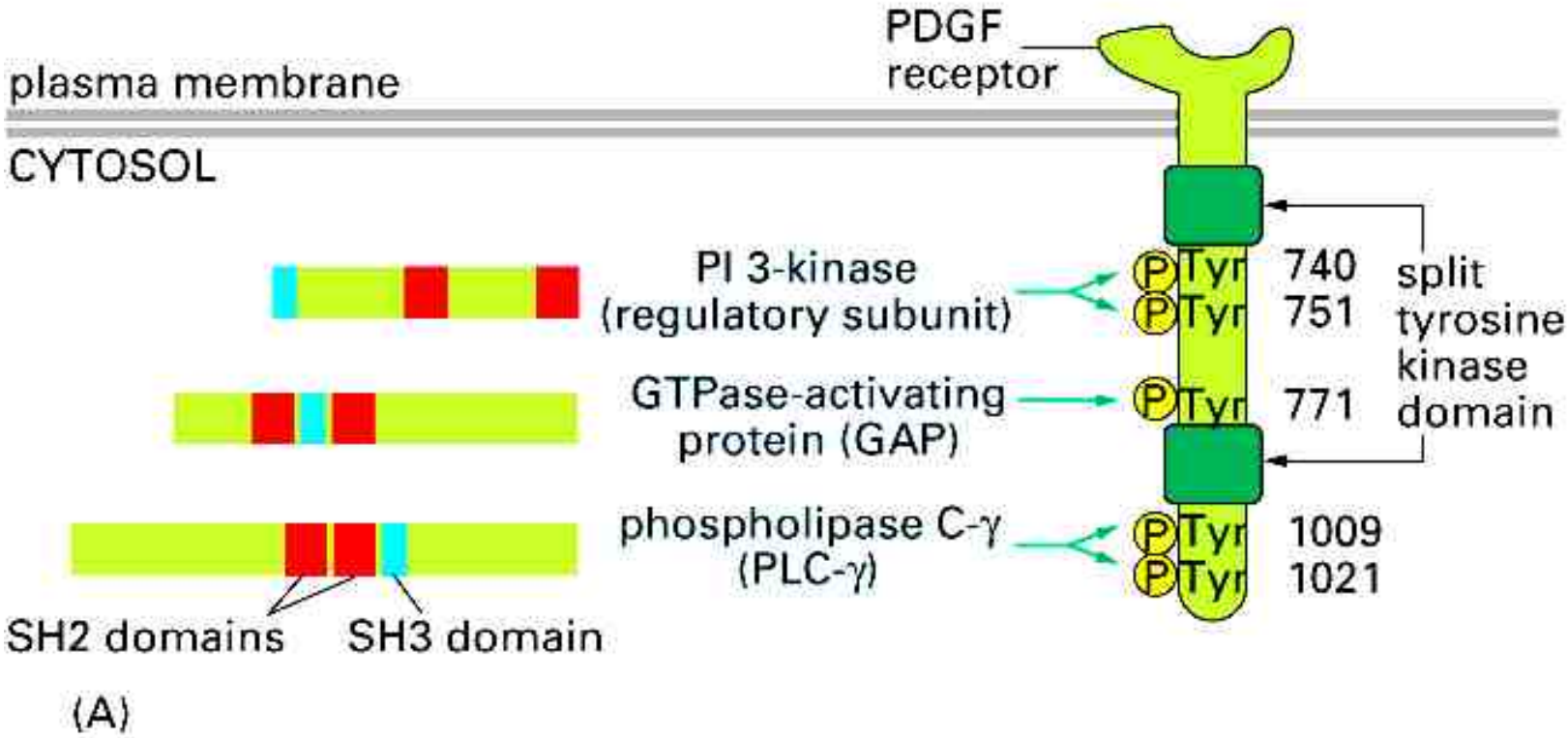


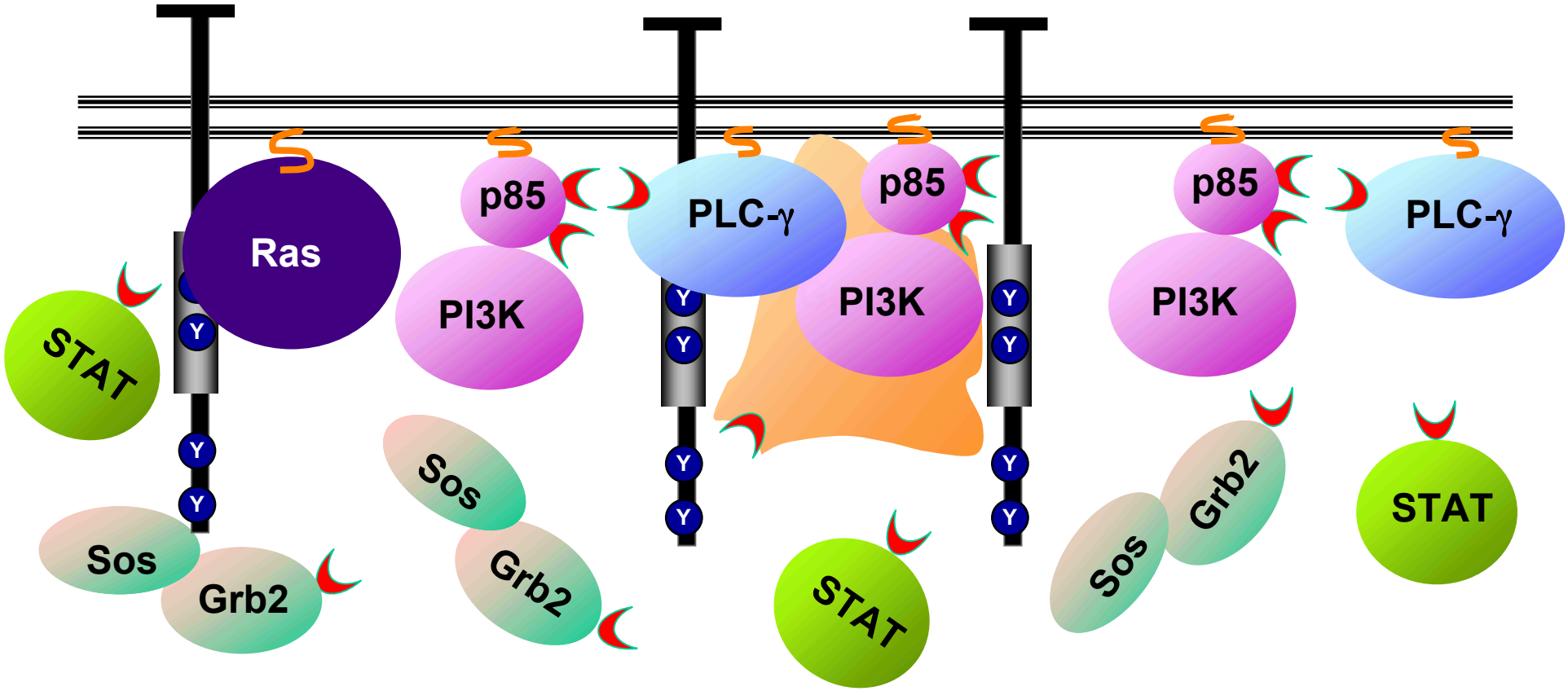
Figure 15–53 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

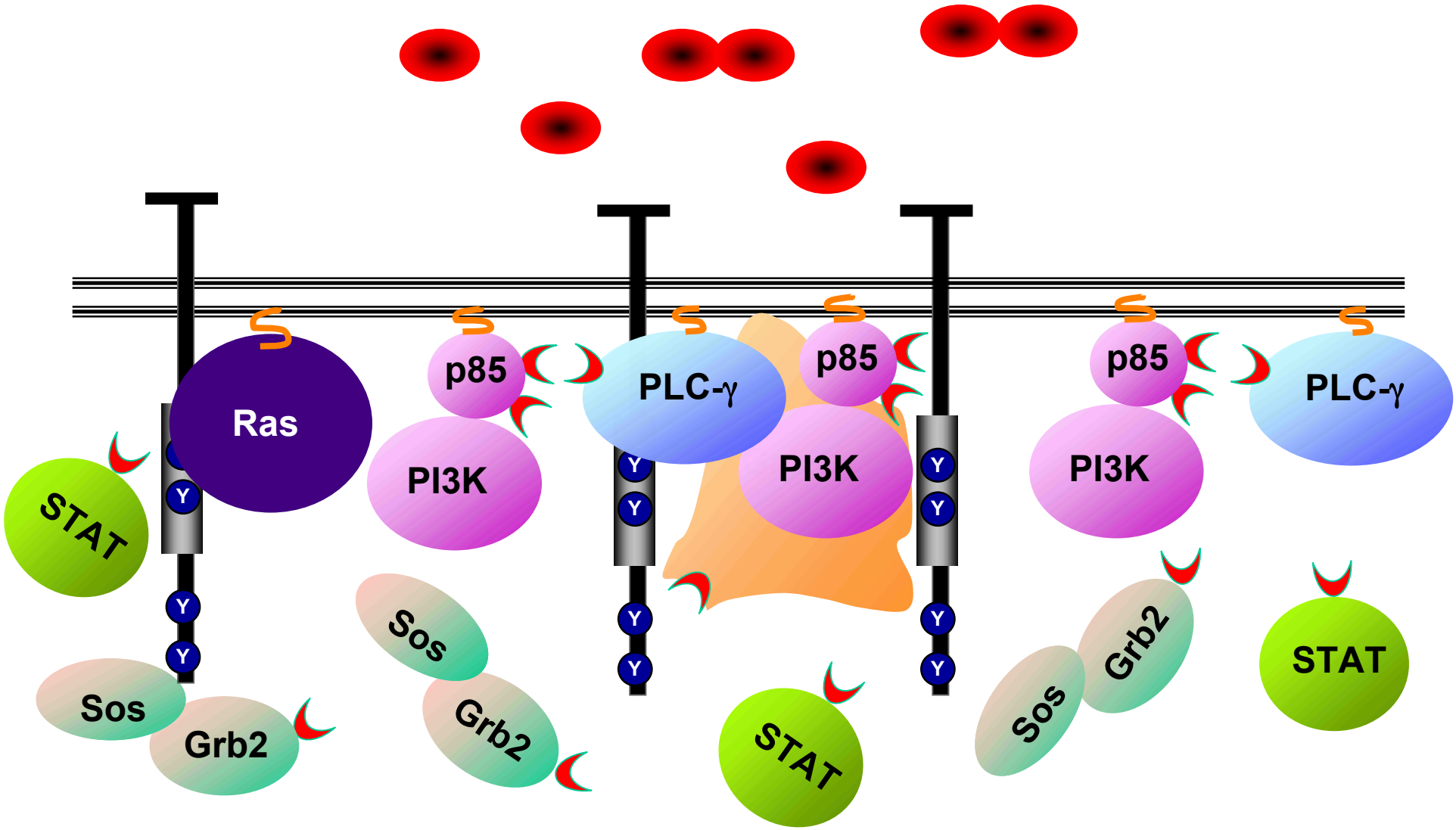
La specificità di riconoscimento dei domini SH2 è dovuta agli aa che seguono la pTyr

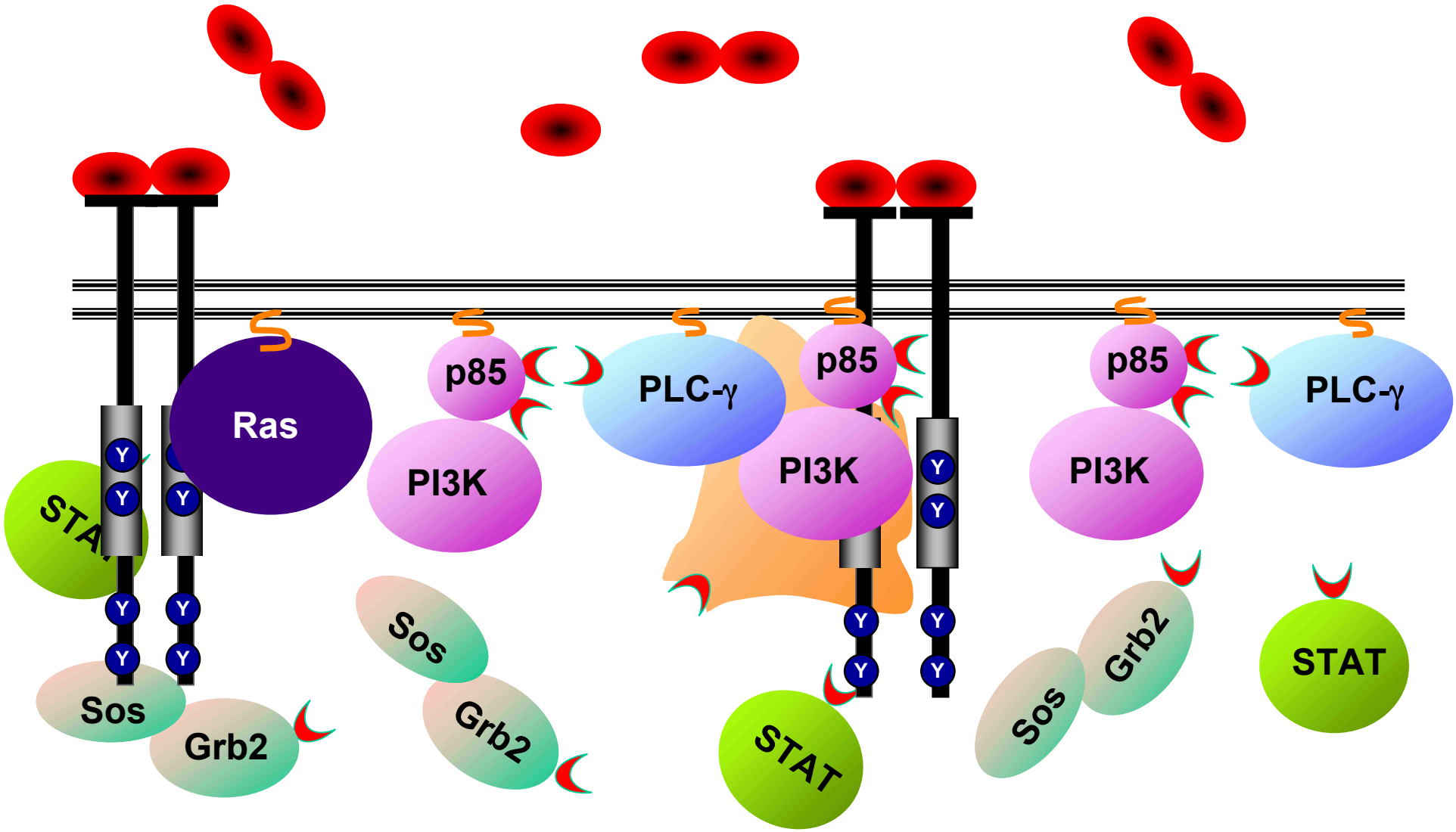
Table 1.1 Examples of Signaling Proteins Containing Modular Binding Domains

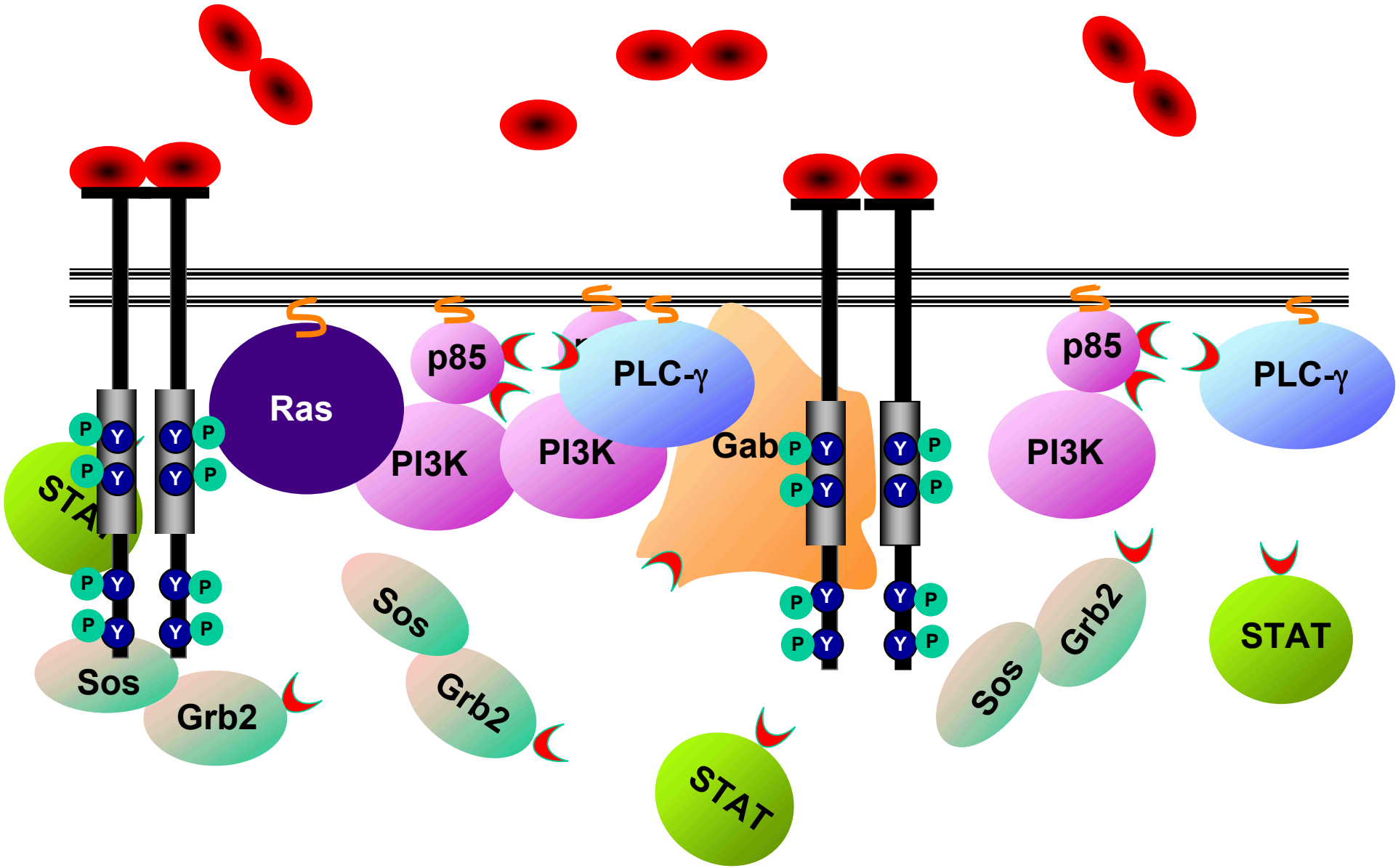
Protein	Structural organization[a]	Activity[a]
I. Proteins with known activities		
PLC-γ	PH–SH2–SH2–SH3–PH–catalytic domain	PtdIns(4,5)P_2 hydrolysis
GAP	SH2–SH3–SH2–PH–catalytic domain	Ras GTPase activator
SH-PTP1 and 2	SH2–SH2–catalytic domain	Tyrosine phosphatase
Src	SH3–SH2–catalytic domain	Tyrosine kinase
Fps	SH–2–catalytic domain	Tyrosine kinase
Syk	SH2–SH2–catalytic domain	Tyrosine kinase
VAV	PH–SH3–SH2–SH3	Ras GNEF
STAT proteins	Leucine repeats–SH3–SH2	Transcription factor
II. Proteins with no apparent intrinsic activity (adapters)		
p85	SH3–SH2–SH2	(Bound to PI3 kinase)
Grb2/Sem5	SH3–SH2–SH3	
Snc	PTB–SH2	
Nck	SH3–SH3–SH3–SH2	
Crk	SH2–SH3	
CrkII	SH2–SH3–SH3	

[a] Abbreviations: PH, pleckstrin homology domain; PtdIns(4,5)P2, phosphoinositol bisphosphate; GNEF, guanine nucleotide exchange factor; PTB, phosphotyrosine binding domain.

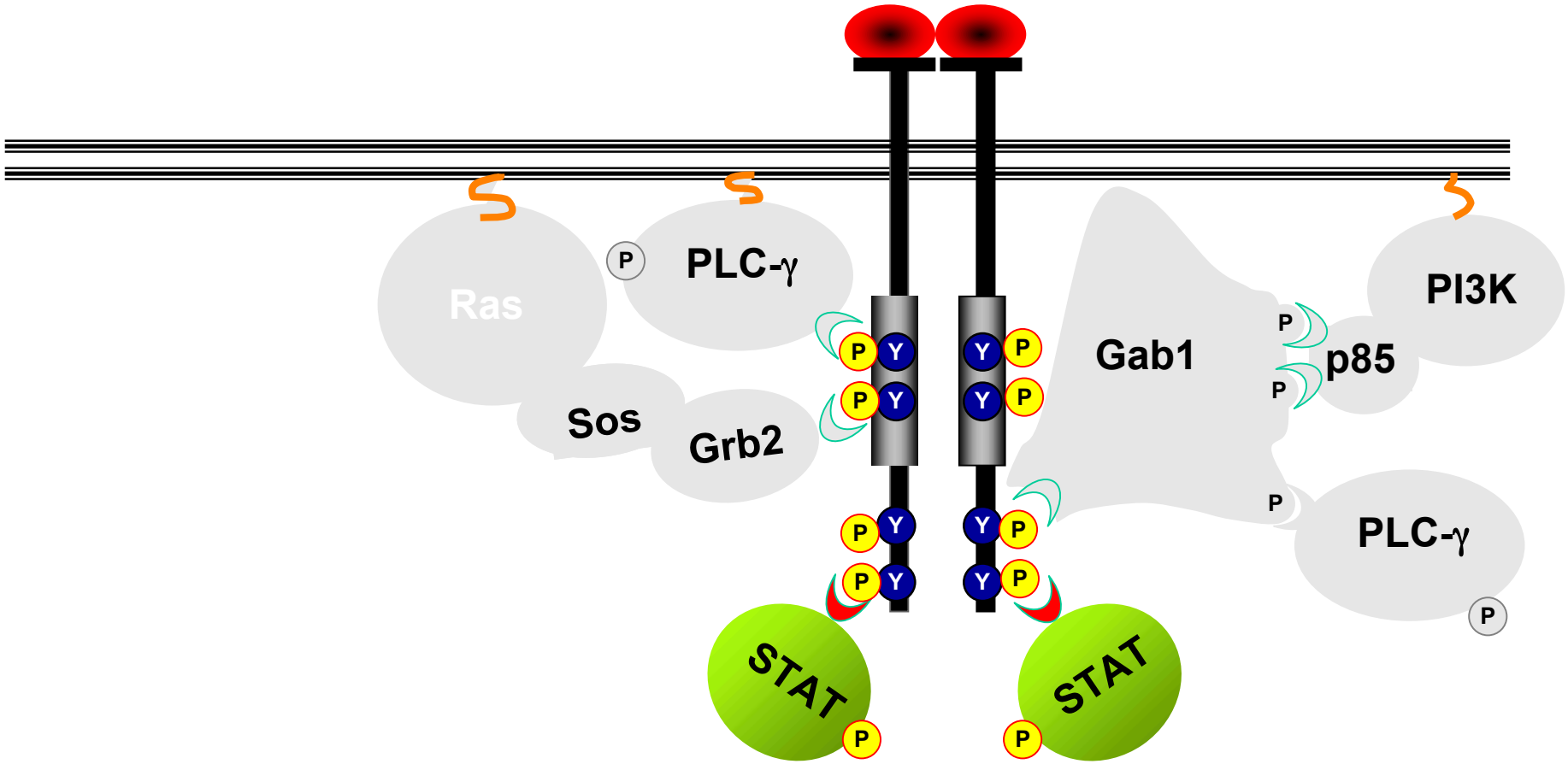

Ras
STAT
Sos
Grb2
p85
PI3K
Sos
Grb2
PLC-γ
p85
PI3K
STAT
p85
PI3K
Sos
Grb2
PLC-γ
STAT


Ras
STAT
p85
PI3K
PLC-γ
p85
PI3K
p85
PI3K
PLC-γ
Sos
Grb2
Sos
Grb2
STAT
Sos
Grb2
STAT
Y

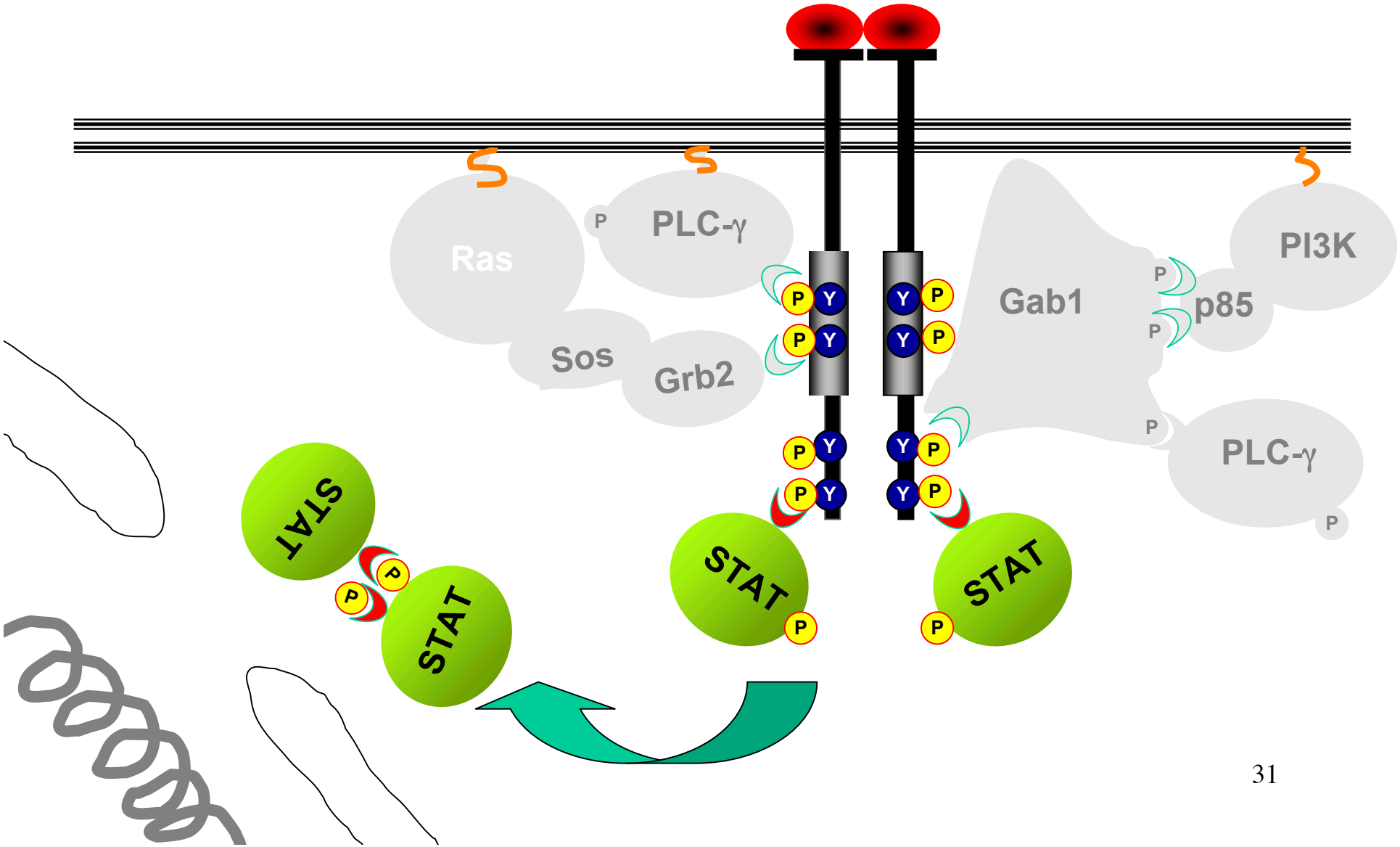

Ras
p85
PI3K
PLC-γ
p85
PI3K
p85
PI3K
PLC-γ
Y
Sos
Grb2
Sos
Grb2
STAT
Sos
Grb2
STAT


Ras
p85
PI3K
PLC-γ
PI3K
Gab
p85
PI3K
PLC-γ
STA
Sos
Grb2
Sos
Grb2
STAT
Sos
Grb2
STAT
P
Y

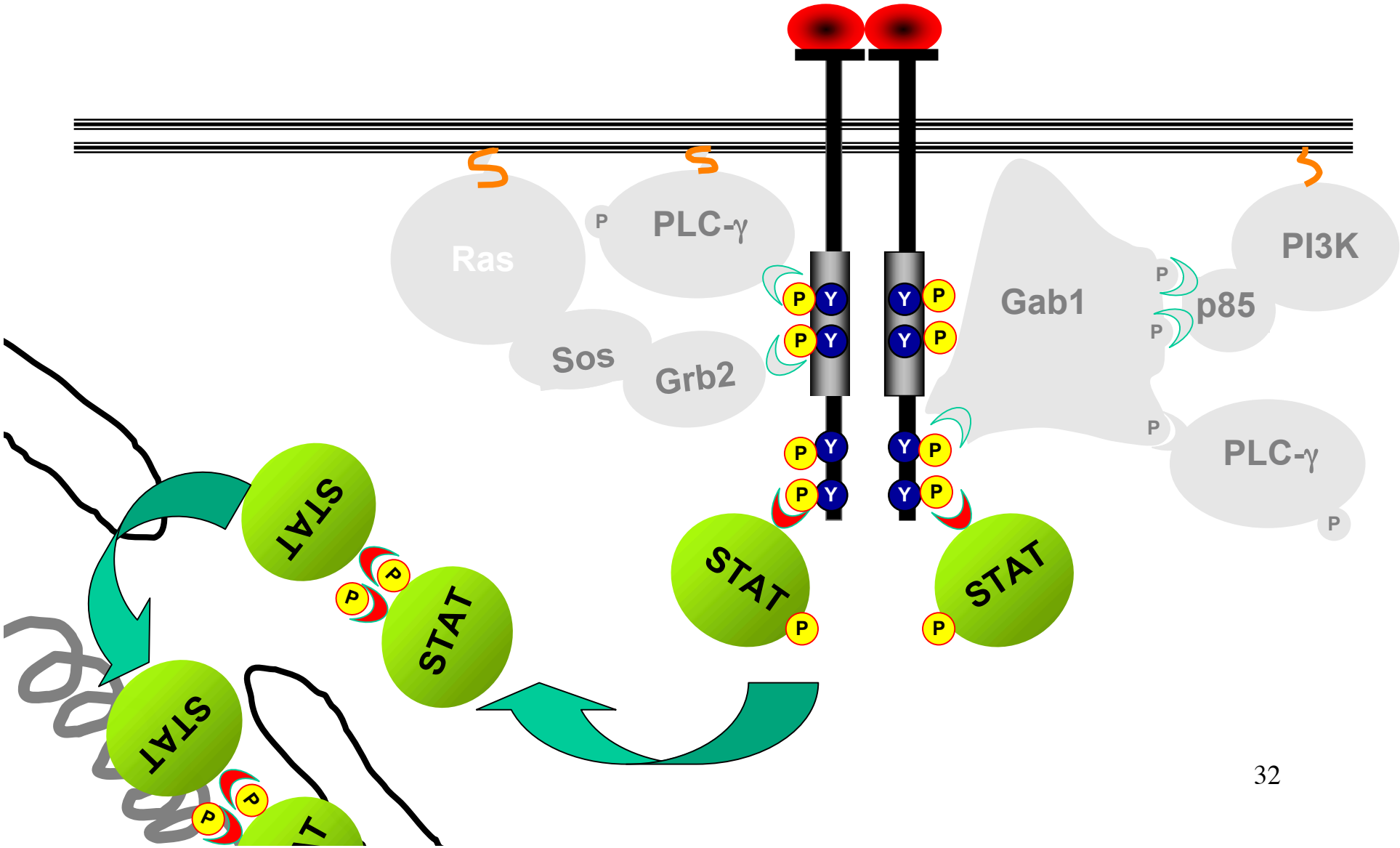
STAT=Signal Transducers and Activators of Transcription protein



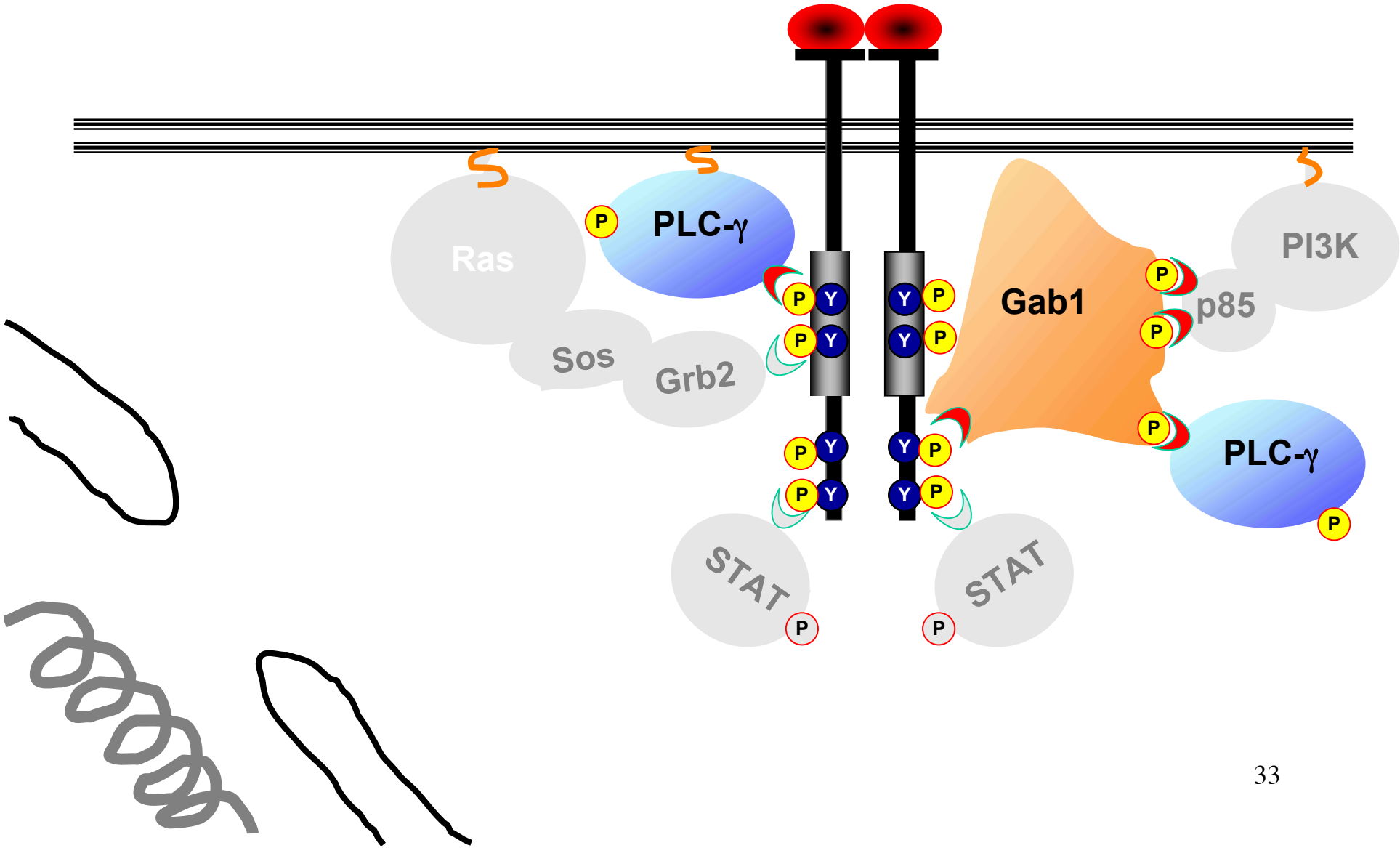
STAT=Signal Transducers and Activators of Transcription protein

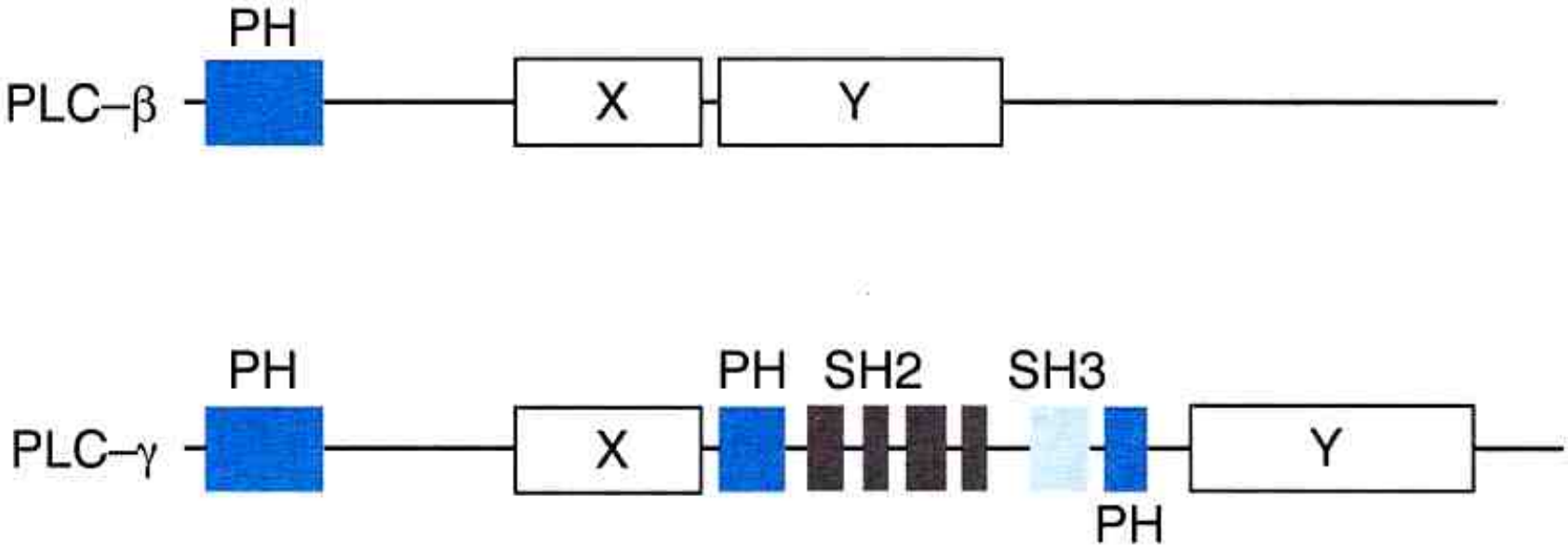


STAT=Signal Transducers and Activators of Transcription protein
STAT=Signal Transducers and Activators of Transcription protein



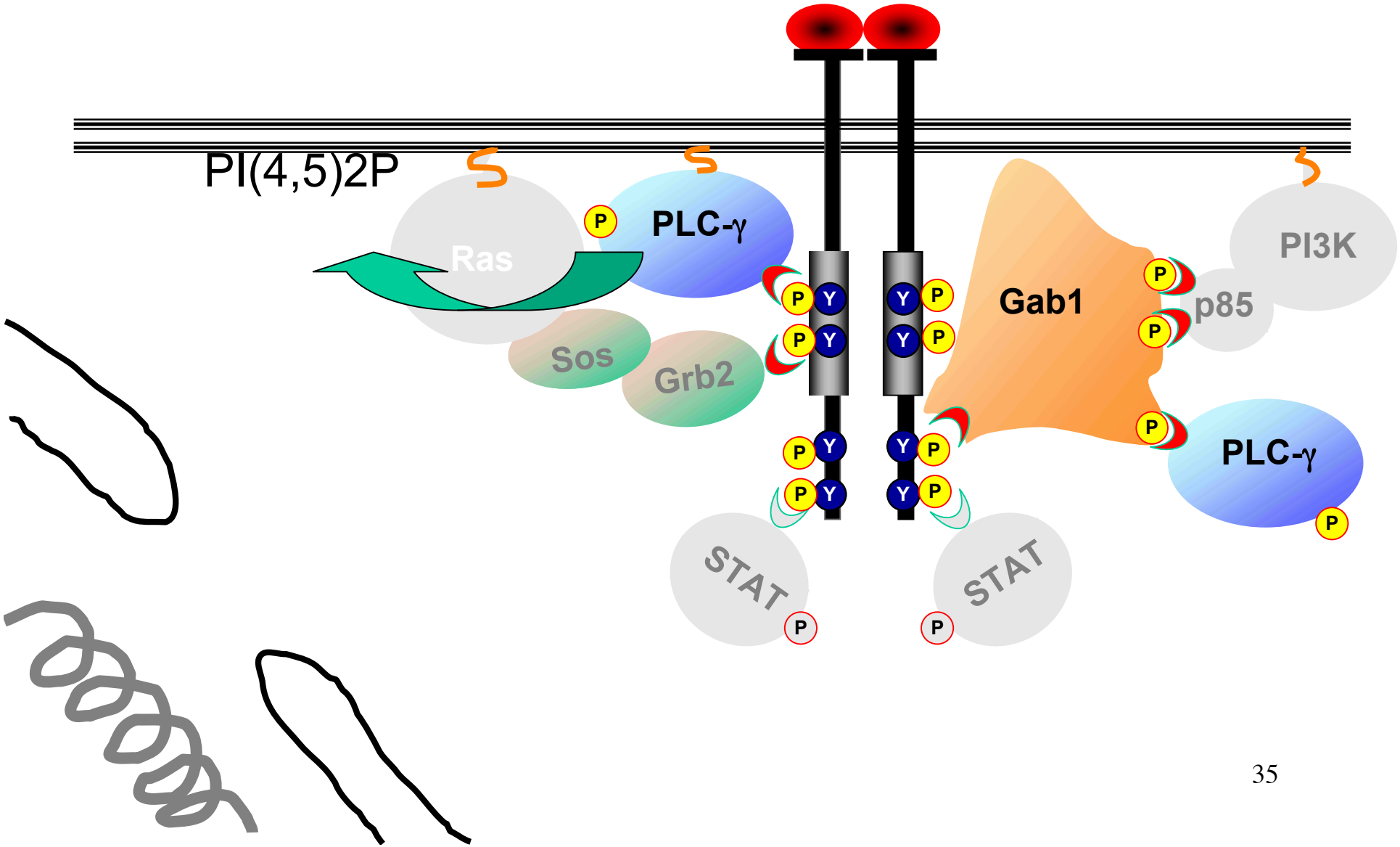
PLC-γ: Fosfolipasi C-gamma



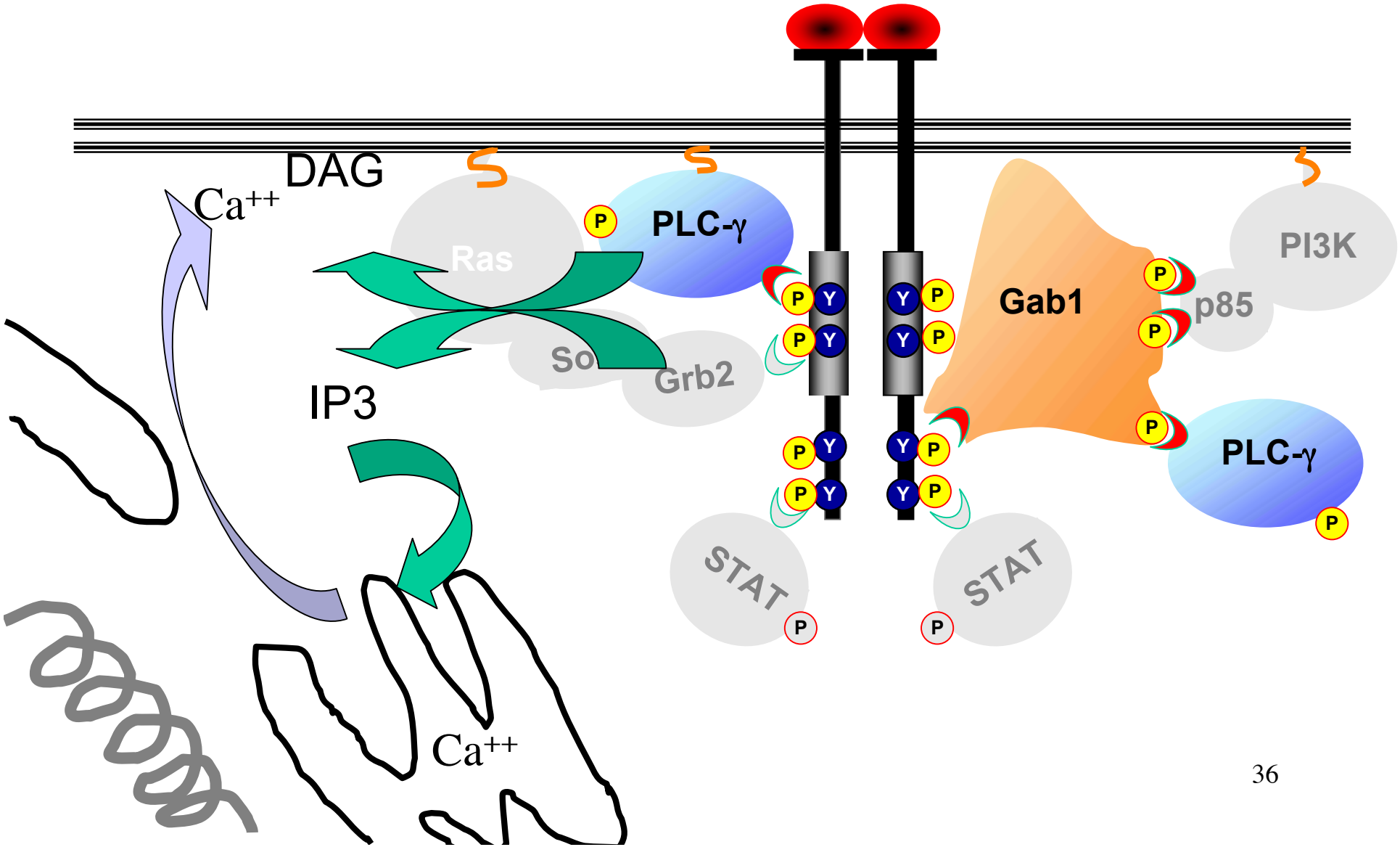


The hydrolysis of PIP2 is activated downstream of both G protein-coupled receptors and protein-tyrosine kinases. This occurs because one form of phospholipase C (PLC-β) is stimulated by G proteins, whereas a second (PLC-γ) contains SH2 domains that mediate its association with activated receptor protein-tyrosine kinases. This interaction localizes PLC-γ to the plasma membrane as well as leading to its tyrosine phosphorylation, which increases its catalytic activity.

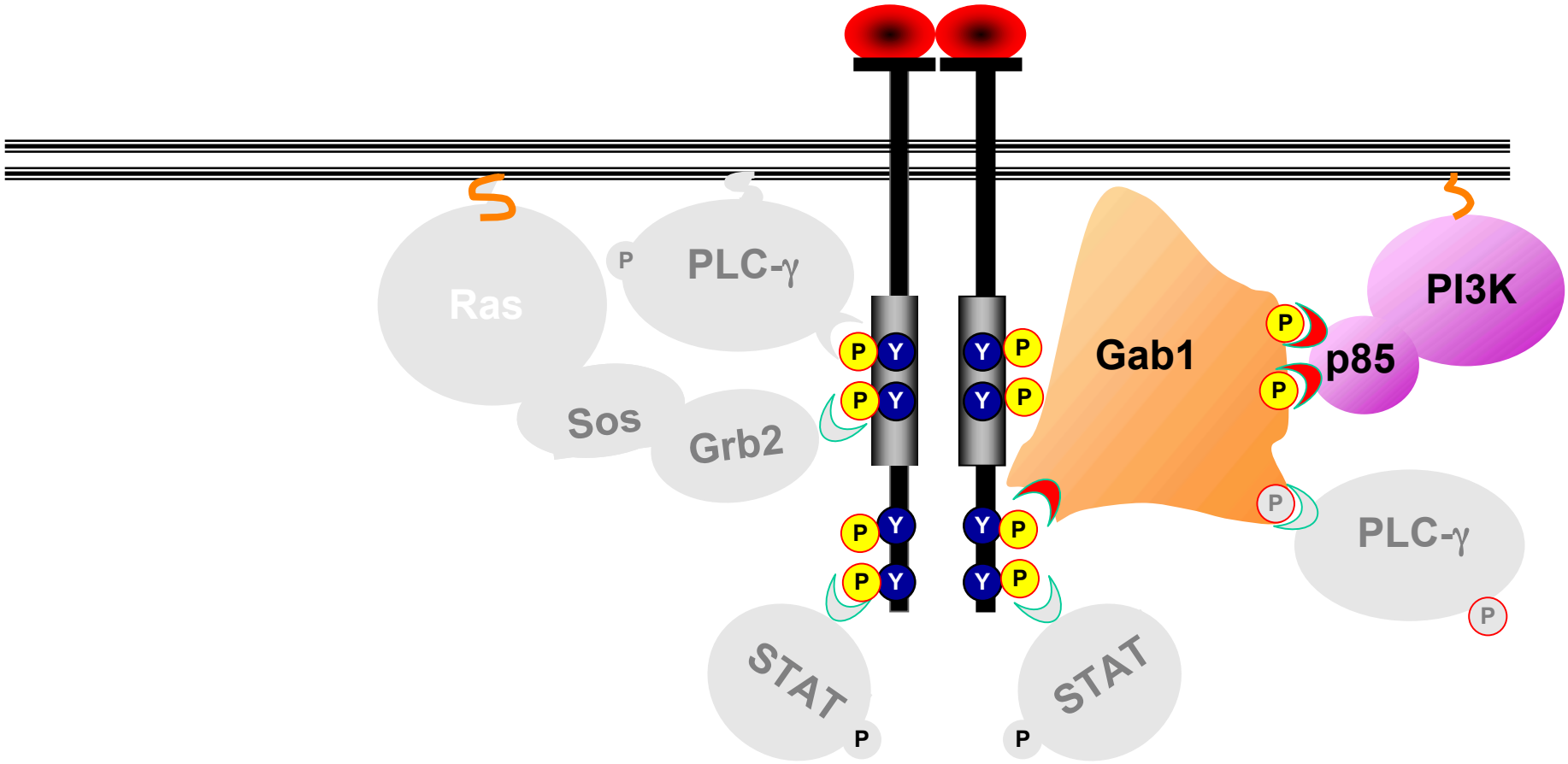
PLC-γ: Fosfolipasi C-gamma



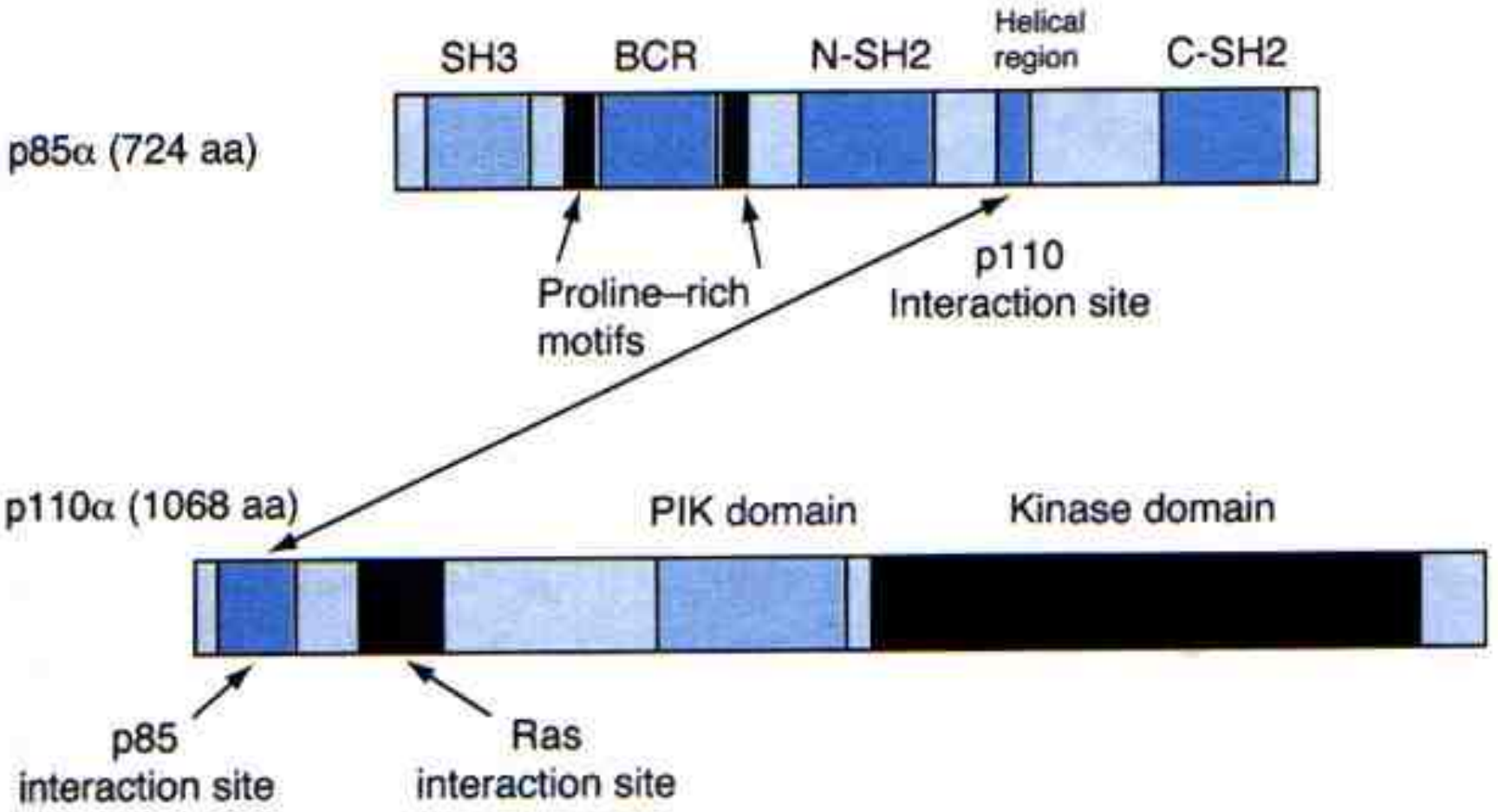
PLC-γ: Fosfolipasi C-gamma
PLC-γ: Stessa funzione di PLC-B (vedere sezione GPCR)

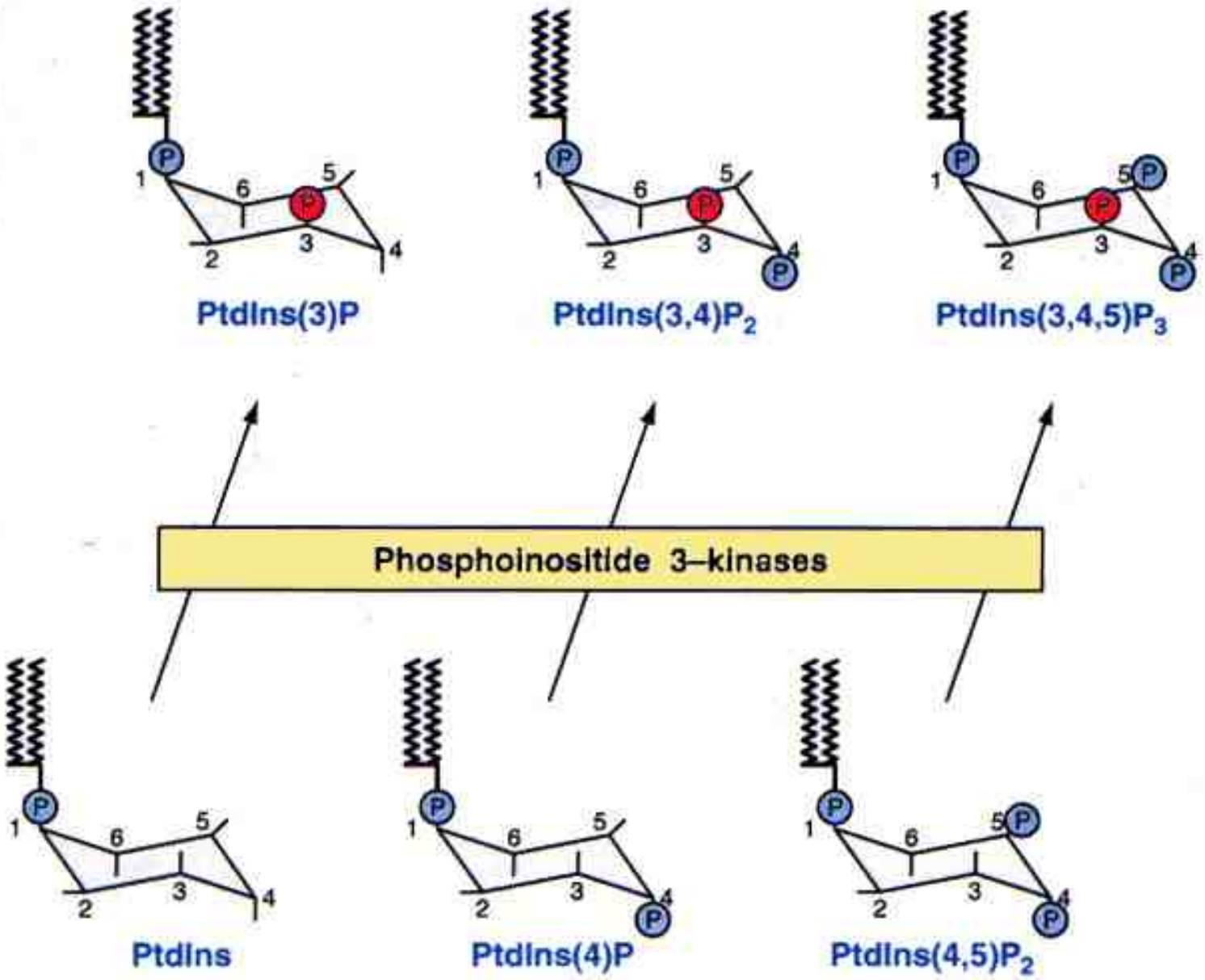


Fosfatidil inositolo 3 chinasi (PI3K)



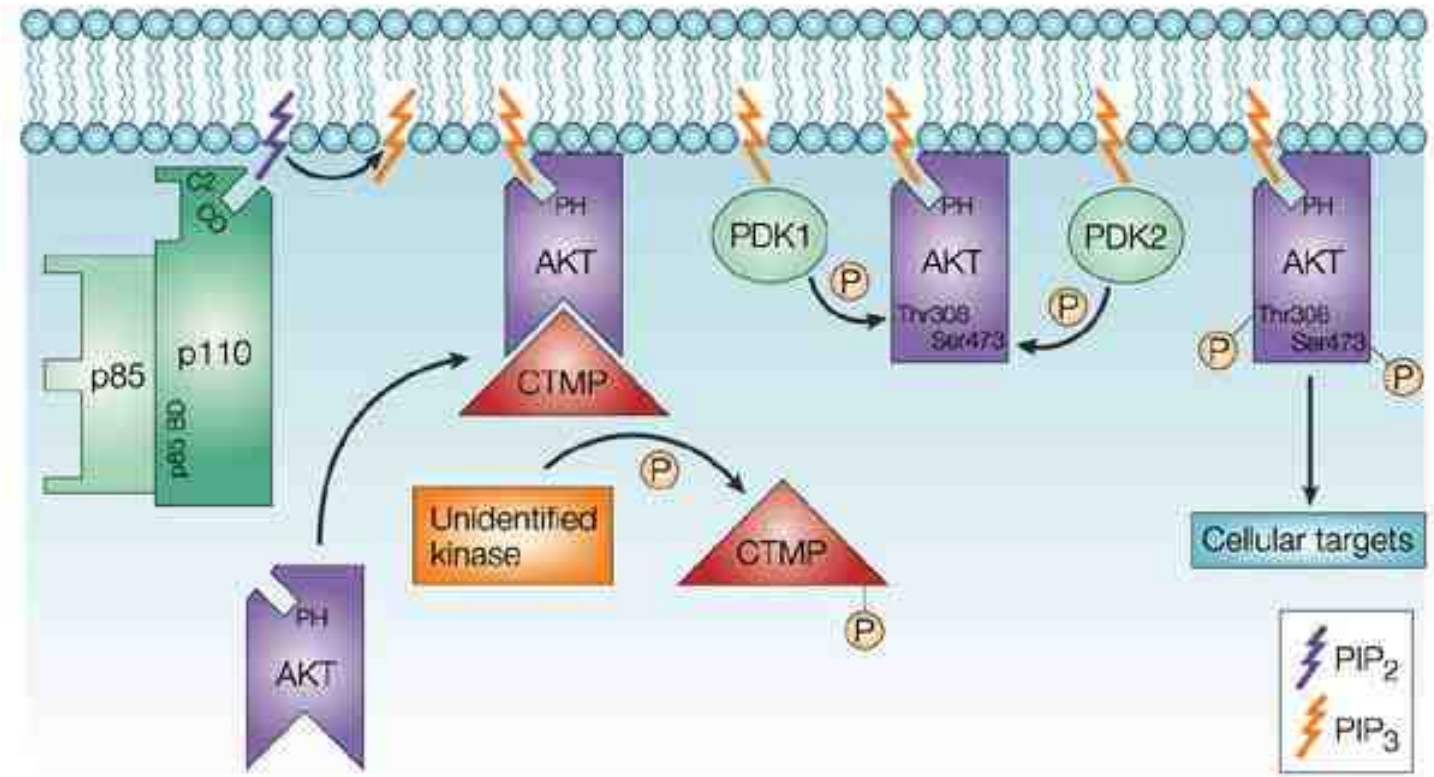
PI3K: un dimero composto da una subunità regolativa (p85) e una subunità catalitica (p110)




PtdIns(3)P
PtdIns(3,4)P2
PtdIns(3,4,5)P3
Phosphoinositide 3–kinases
PtdIns
PtdIns(4)P
PtdIns(4,5)P2

Fosfatidil inositidi fosforilati in posizione 3 servono da sito di aggancio per proteine che contengono domini PH (plextrin homology).

PI3K ---> PIP3 ---> aggancio di PKD1 e AKT/PKB ---> PKD1 fosforila e attiva AKT/PKB (vedi effetto antiapoptotico di AKT/PKB)



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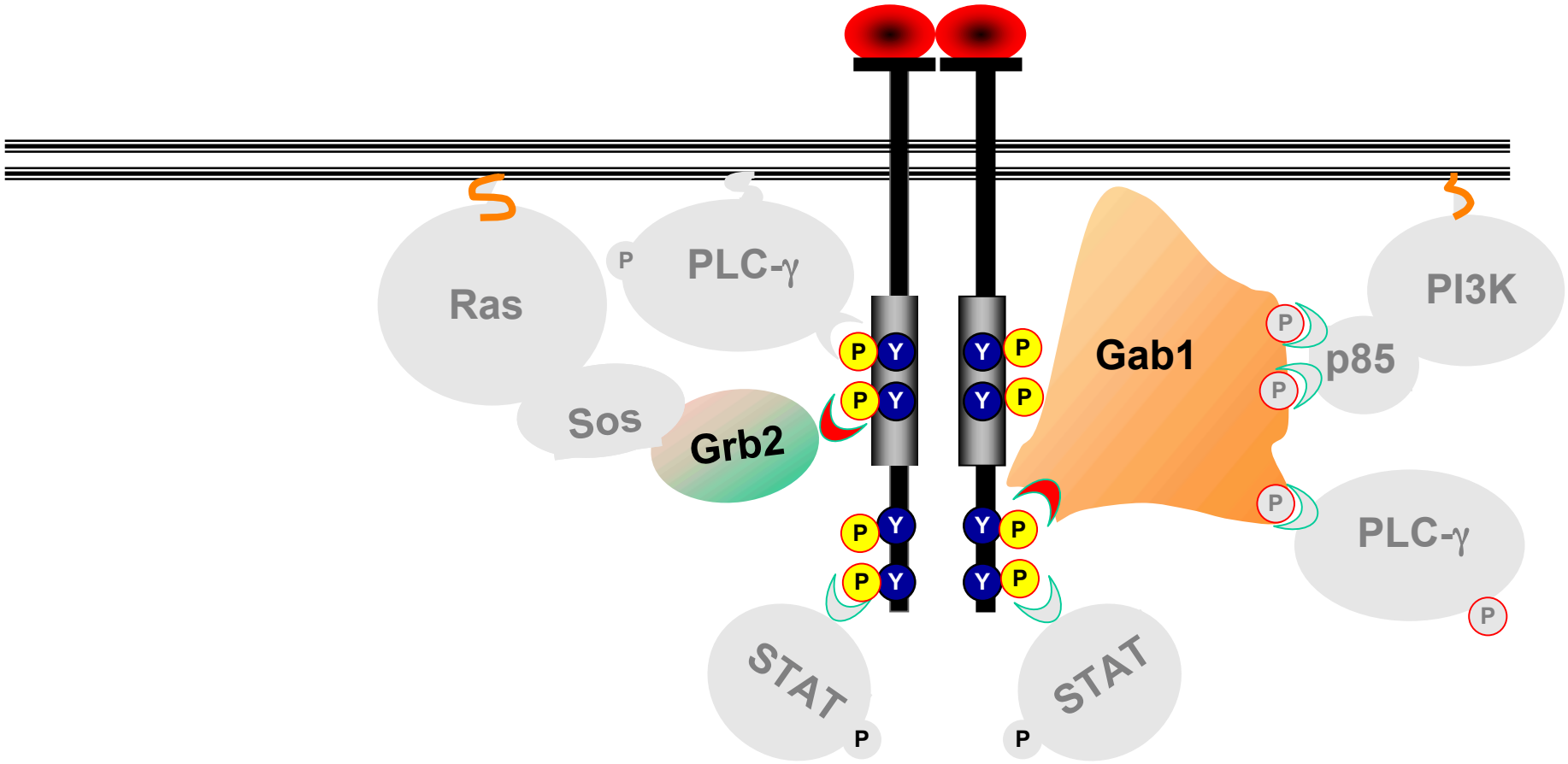
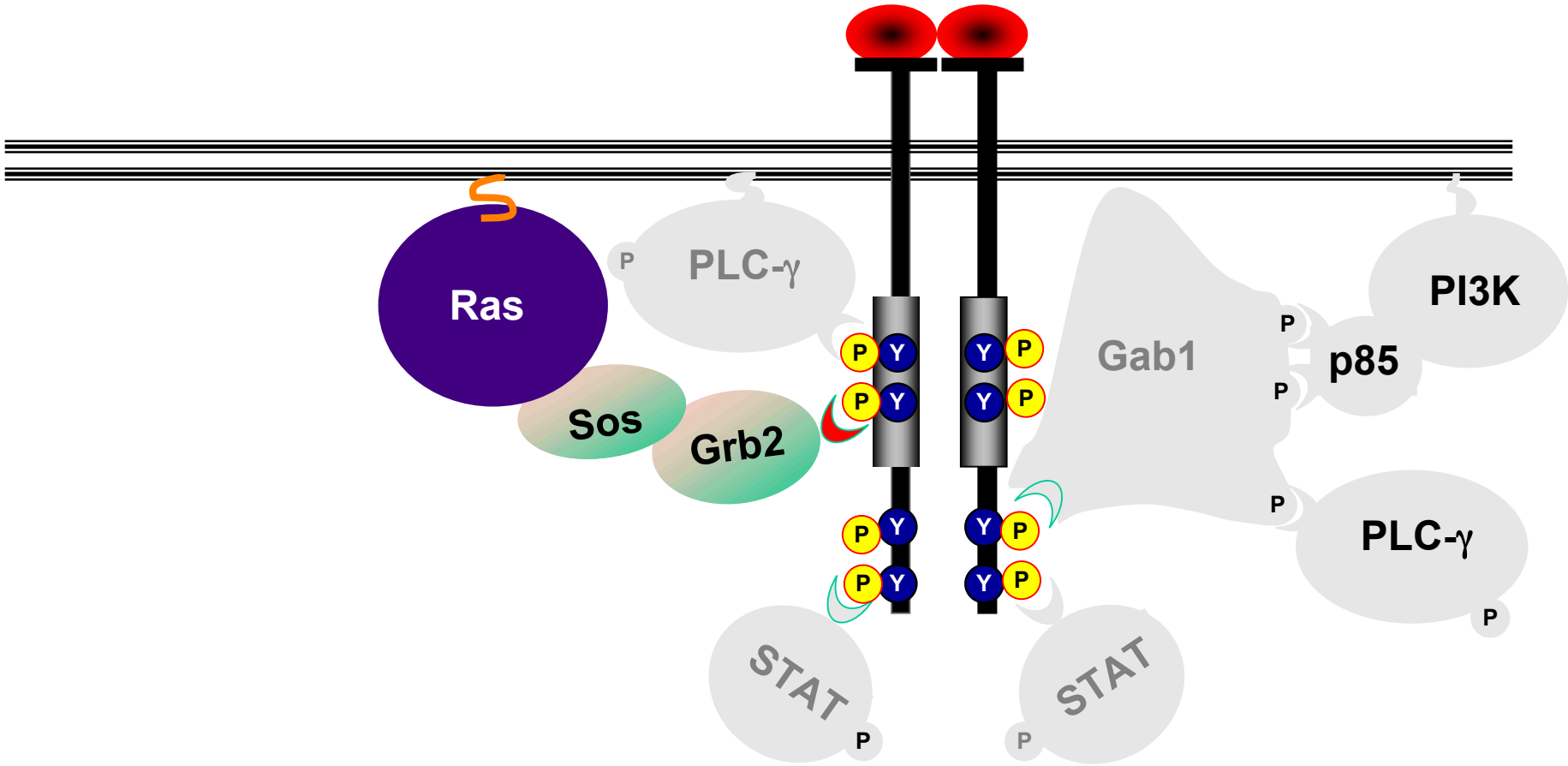
Grb2 e Gab1 sono degli adattatori



Attivazione di Ras: Grb2-Sos ------> Ras



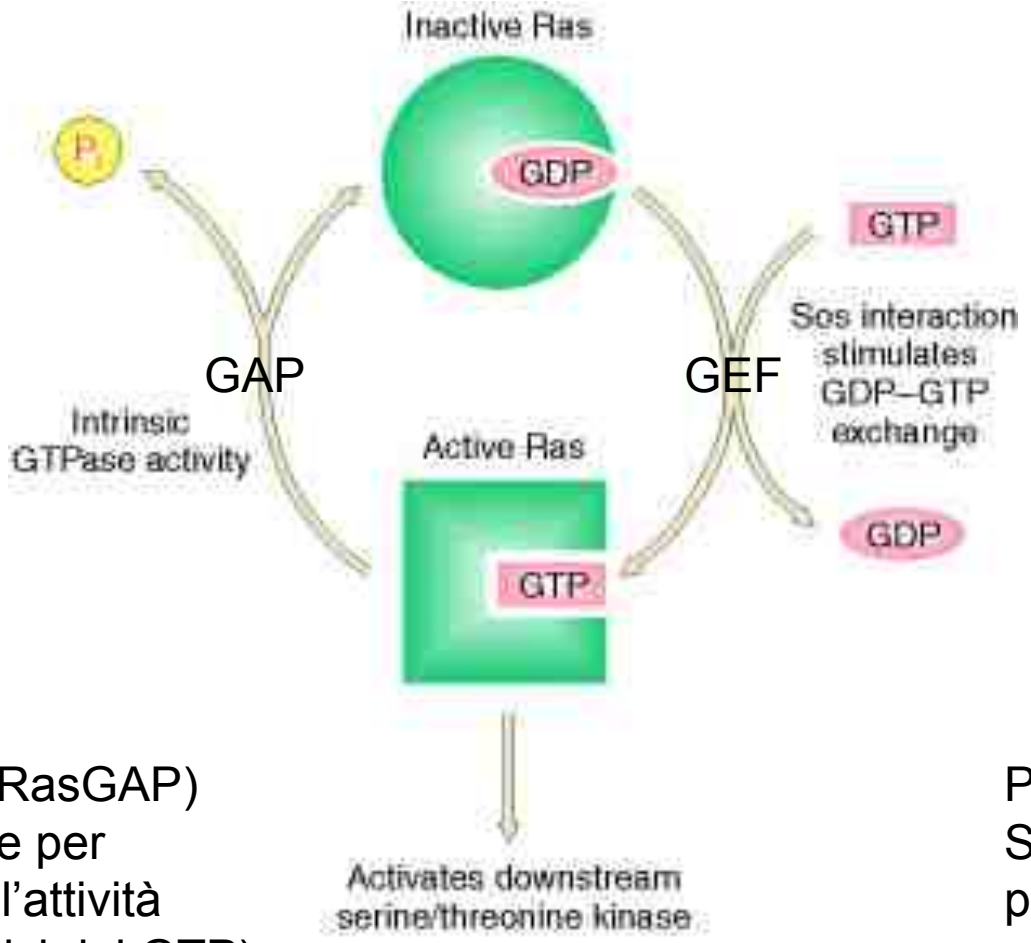
Ras: proteina G monomerica

Disattivazione

Attivazione

G-protein Activating Protein

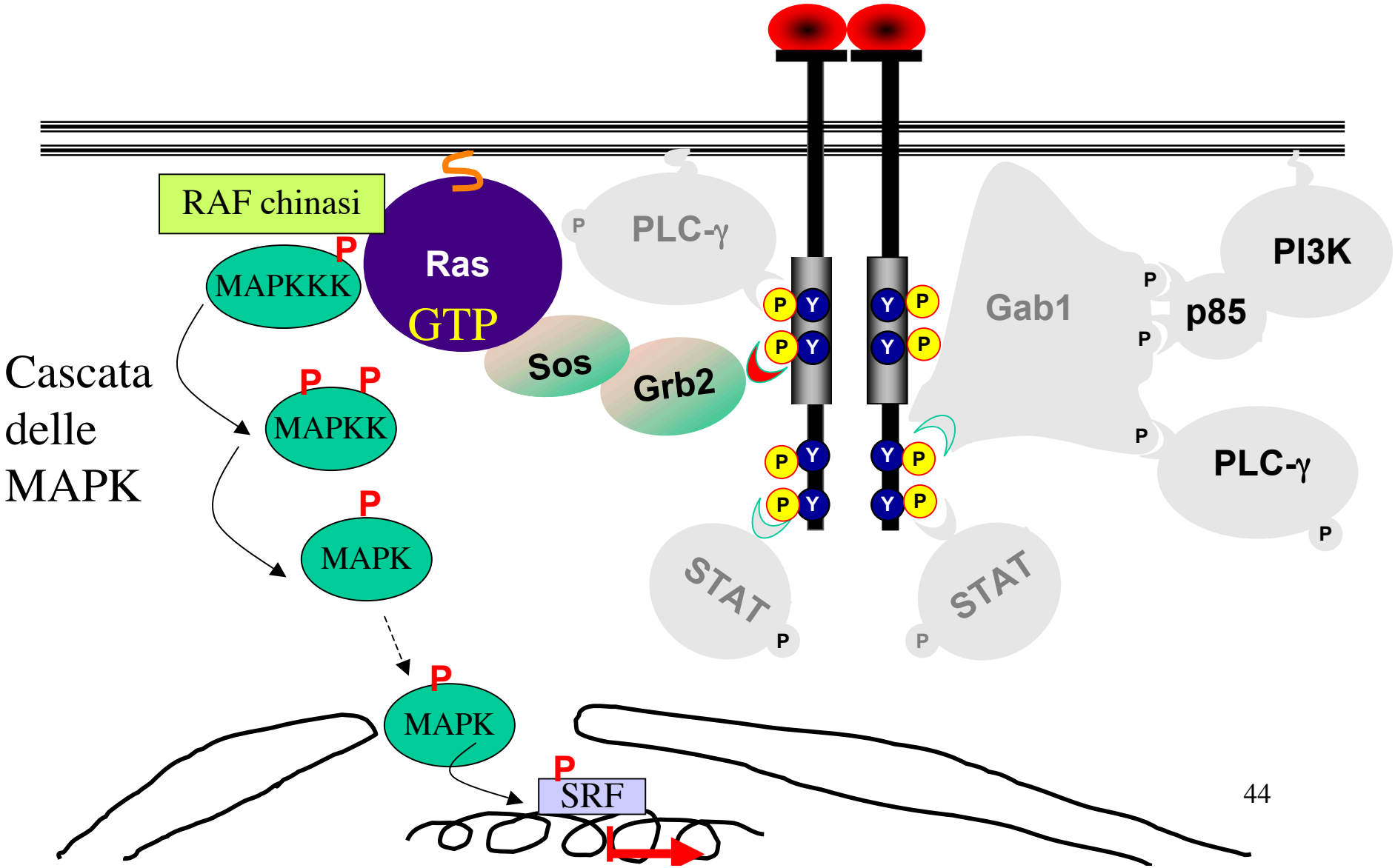
Guanine Exchange Factor

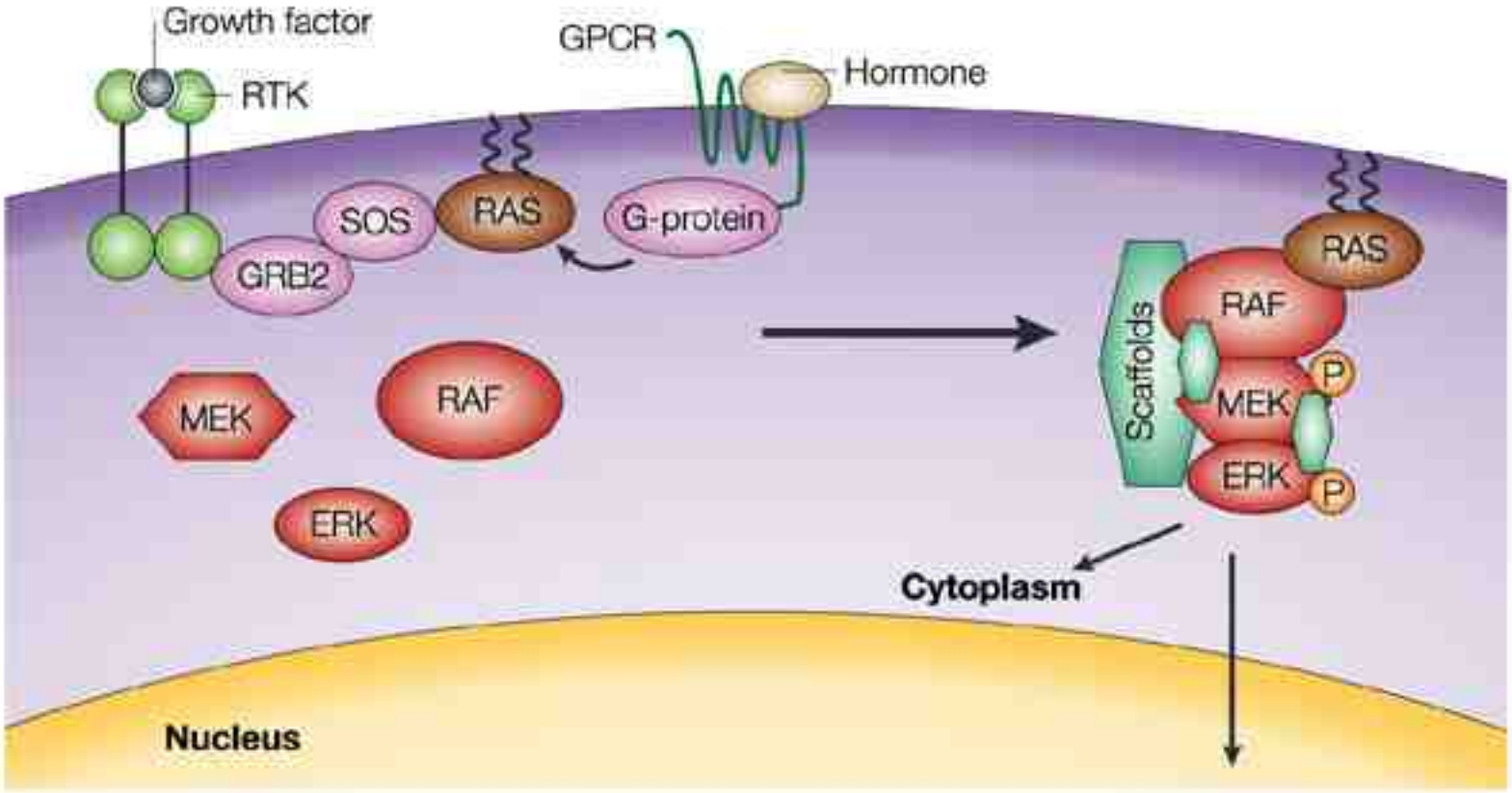


Proteine GAP (RasGAP) sono necessarie per l'attivazione dell'attività GTPasica (idrolisi del GTP) di Ras

Proteine GEF (come Sos) sono necessarie per lo scambio di nucleotide da GDP a GTP

Grb2-Sos ---> Ras ---> cascata delle MAPK





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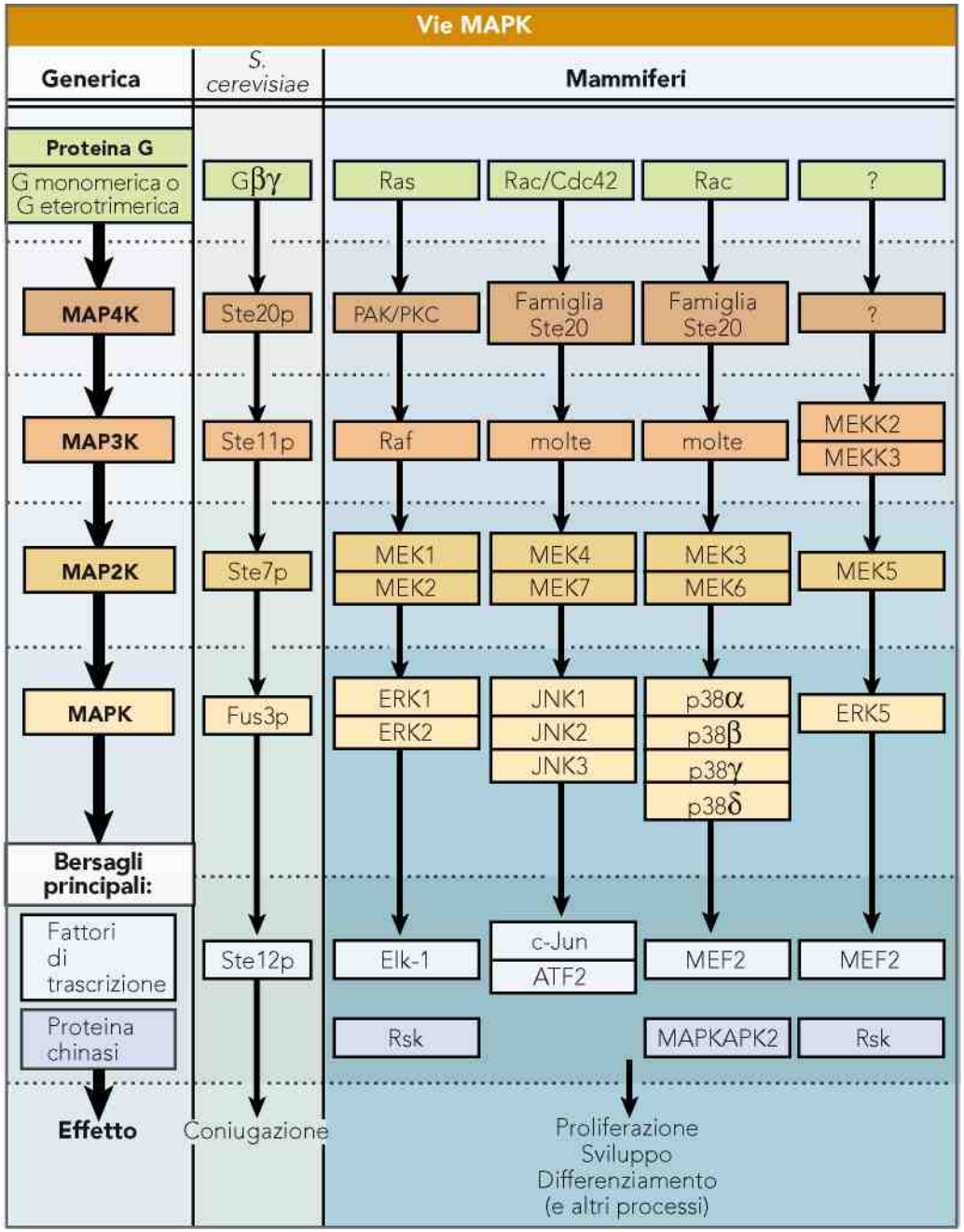
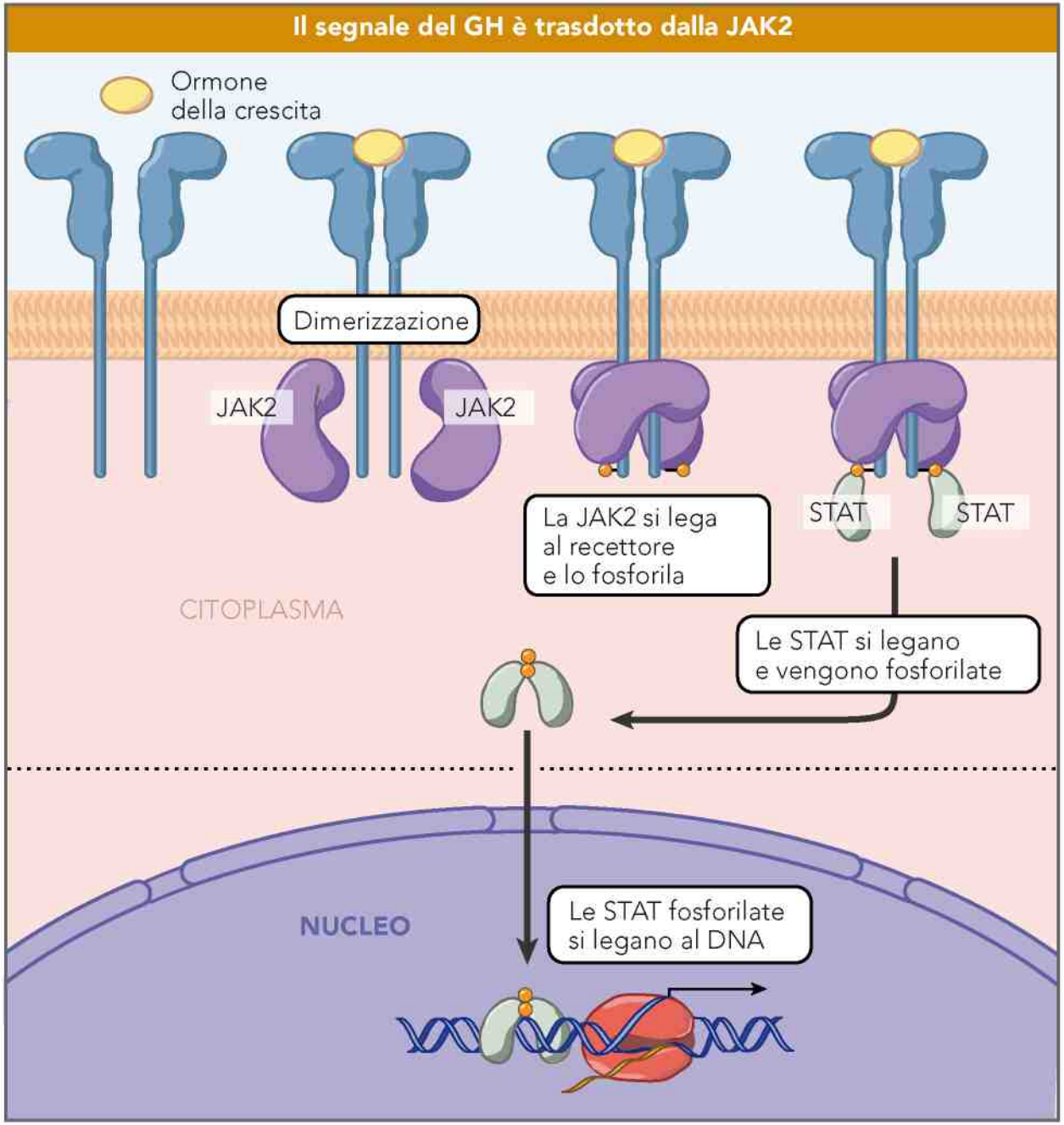
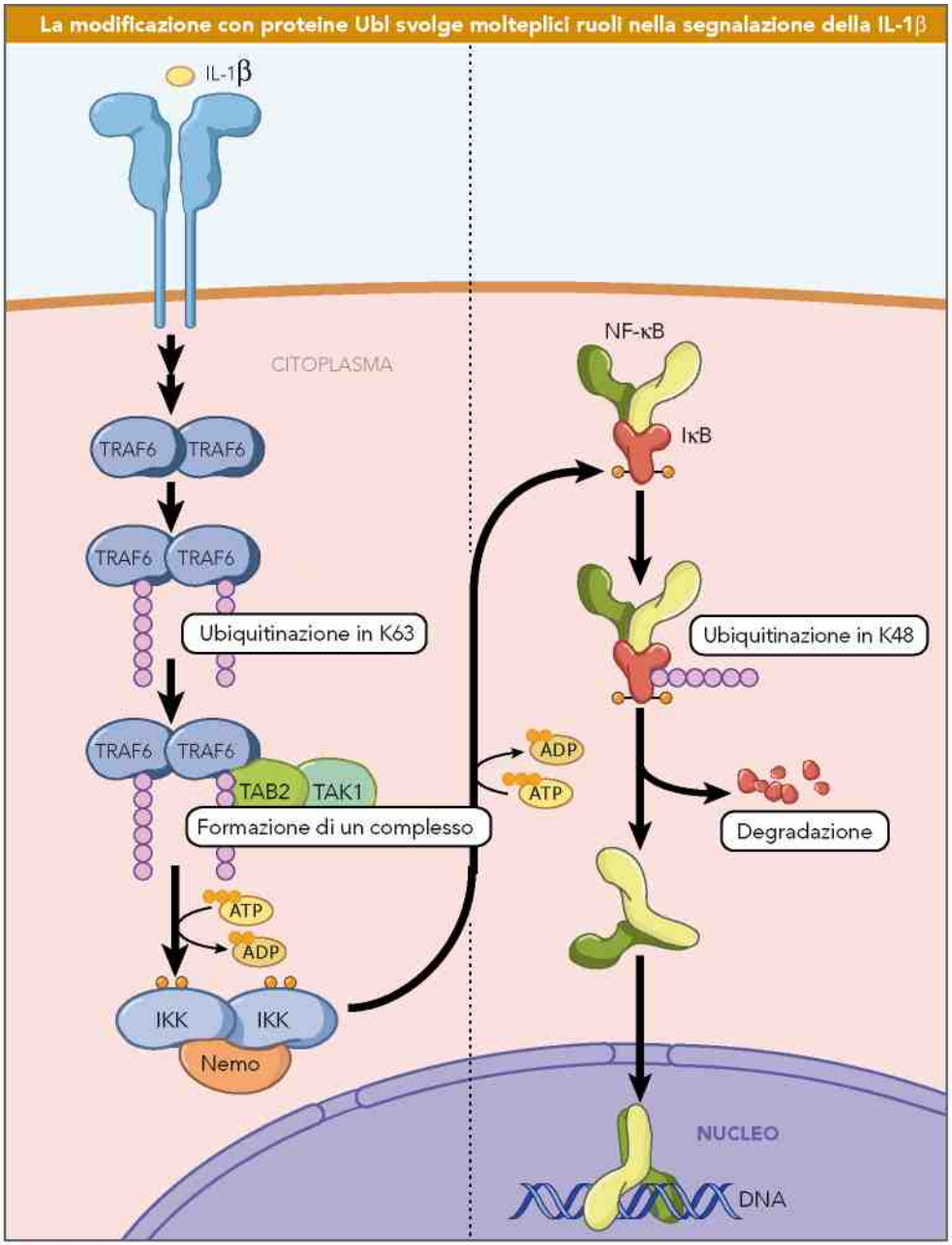
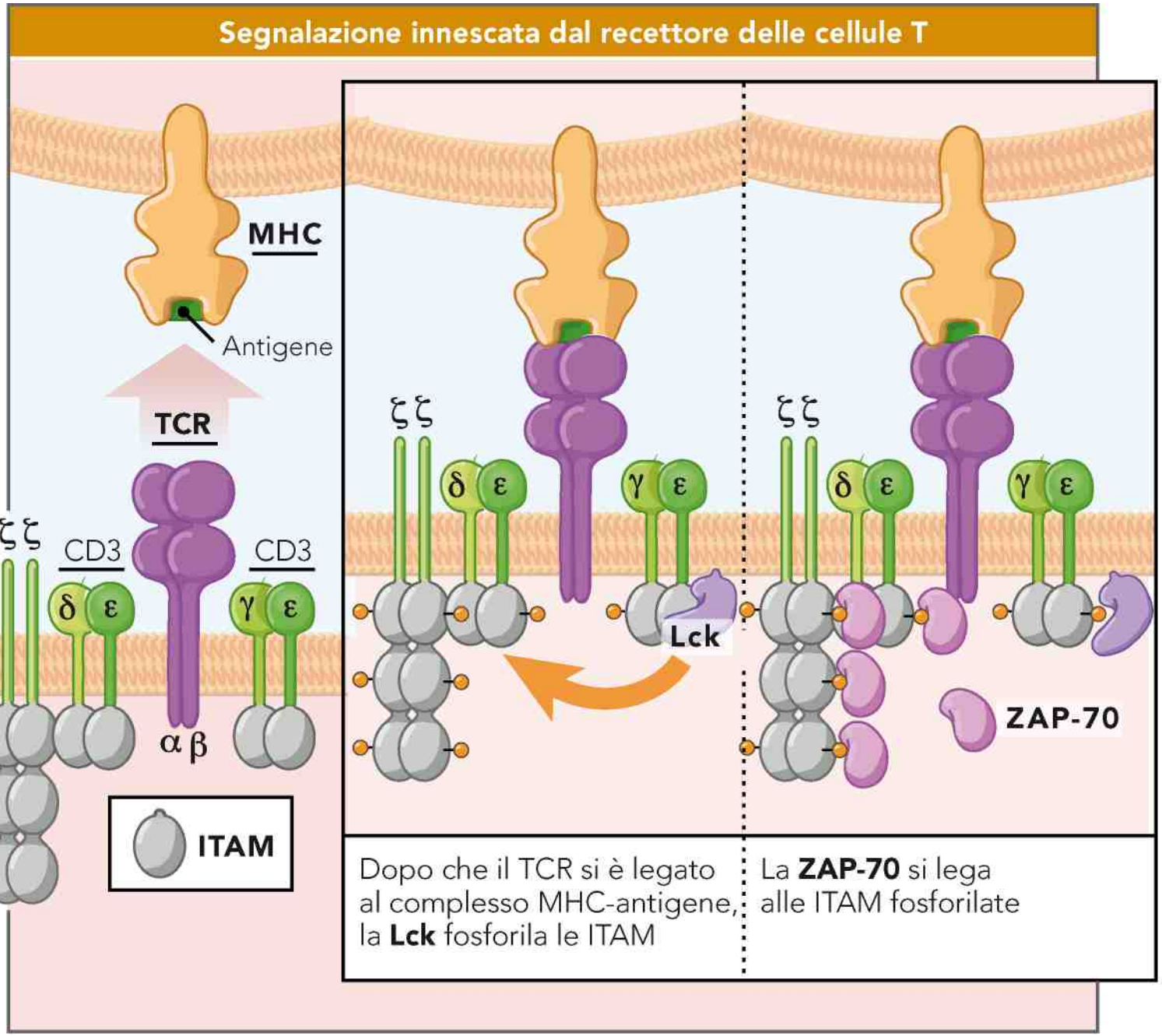
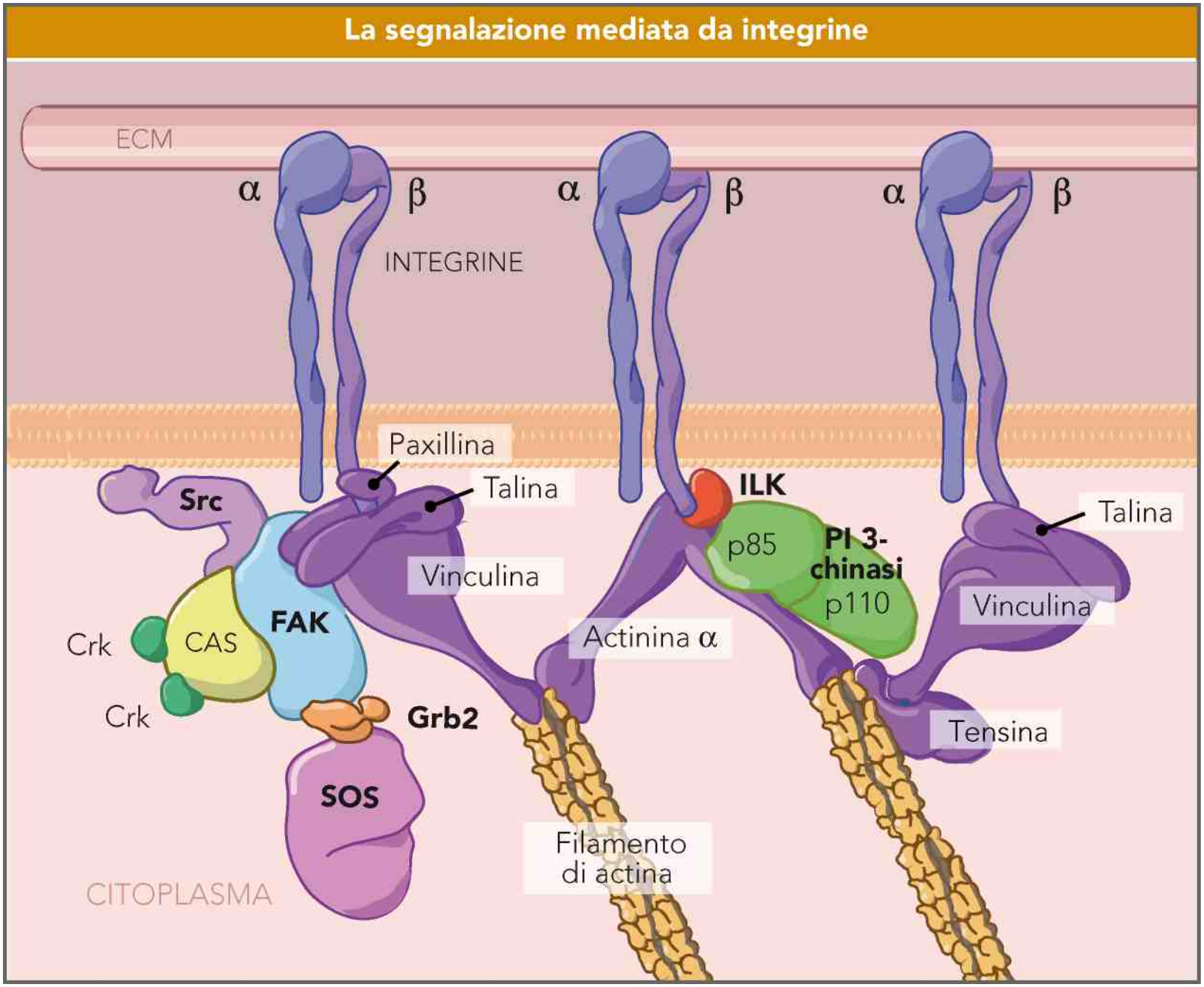
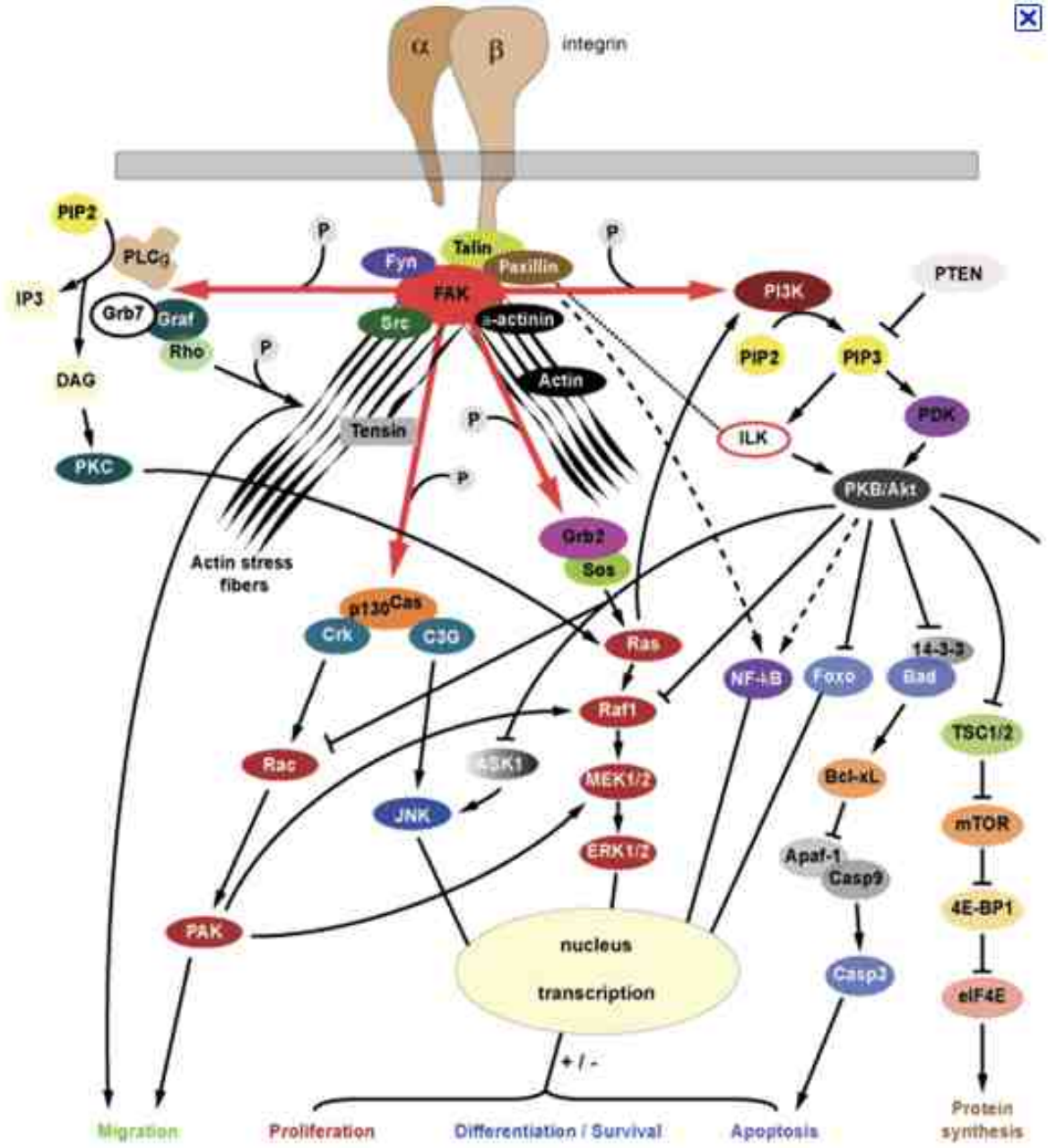

Vie MAPK
Generica
S. cerevisiae
Mammiferi
Proteina G
G monomerica o G eterotrimerica
Gβγ
Ras
Rac/Cdc42
Rac
?
MAP4K
Ste20p
PAK/PKC
Famiglia Ste20
Famiglia Ste20
?
MAP3K
Ste11p
Raf
molte
molte
MEKK2
MEKK3
MAP2K
Ste7p
MEK1
MEK2
MEK4
MEK7
MEK3
MEK6
MEK5
MAPK
Fus3p
ERK1
ERK2
JNK1
JNK2
JNK3
p38α
p38β
p38γ
p38δ
ERK5
Bersagli principali:
Fattori di trascrizione
Proteina chinasi
Ste12p
Elk-1
c-Jun
ATF2
MEF2
MEF2
Rsk
MAPKAPK2
Rsk
Effetto
Coniugazione
Proliferazione
Sviluppo
Differenziamento
(e altri processi)


Il segnale del GH è trasdotto dalla JAK2
Ormone della crescita
Dimerizzazione
JAK2
JAK2
La JAK2 si lega al recettore e lo fosforila
STAT
STAT
CITOPLASMA
Le STAT si legano e vengono fosforilate
NUCLEO
Le STAT fosforilate si legano al DNA


La modificazione con proteine Ubl svolge molteplici ruoli nella segnalazione della IL-1β
IL-1β
CITOPLASMA
TRAF6
TRAF6
TRAF6
TRAF6
Ubiquitinazione in K63
TRAF6
TRAF6
TAB2
TAK1
Formazione di un complesso
ATP
ADP
IKK
IKK
Nemo
ADP
ATP
NF-κB
IκB
Ubiquitinazione in K48
Degradazione
NUCLEO
DNA


Segnalazione innescata dal recettore delle cellule T
MHC
Antigene
TCR
ζ ζ
CD3
CD3
δ ε
γ ε
α β
ITAM
Lck
ZAP-70
Dopo che il TCR si è legato al complesso MHC-antigene, la **Lck** fosforila le ITAM
La **ZAP-70** si lega alle ITAM fosforilate


La segnalazione mediata da integrine
ECM
α
β
α
β
α
β
INTEGRINE
Paxillina
Talina
Src
ILK
p85
PI 3-chinasi
p110
Talina
Vinculina
Vinculina
FAK
CAS
Crk
Crk
Actinina α
Grb2
Tensina
SOS
Filamento di actina
CITOPLASMA


α
β
integrin
PIP2
PLCγ
IP3
DAG
PKC
Grb7
Graf
Rho
P
Fyn
Talin
Paxillin
FAK
Src
α-actinin
Actin
Tensin
Actin stress fibers
PI3K
PTEN
PIP2
PIP3
PDK
ILK
PKB/Akt
Grb2
Sos
p130Cas
Crk
C3G
Ras
Raf1
Rac
JNK
MEK1/2
ERK1/2
PAK
NF-kB
Foxo
14-3-3
Bad
Bcl-xL
Apaf-1
Casp9
Casp3
TSC1/2
mTOR
4E-BP1
eIF4E
Protein synthesis
nucleus
transcription
+ / -
Migration
Proliferation
Differentiation / Survival
Apoptosis

NOTCH

* La segnalazione dovuta all'attivazione di Notch è fondamentale durante lo sviluppo embrionale.

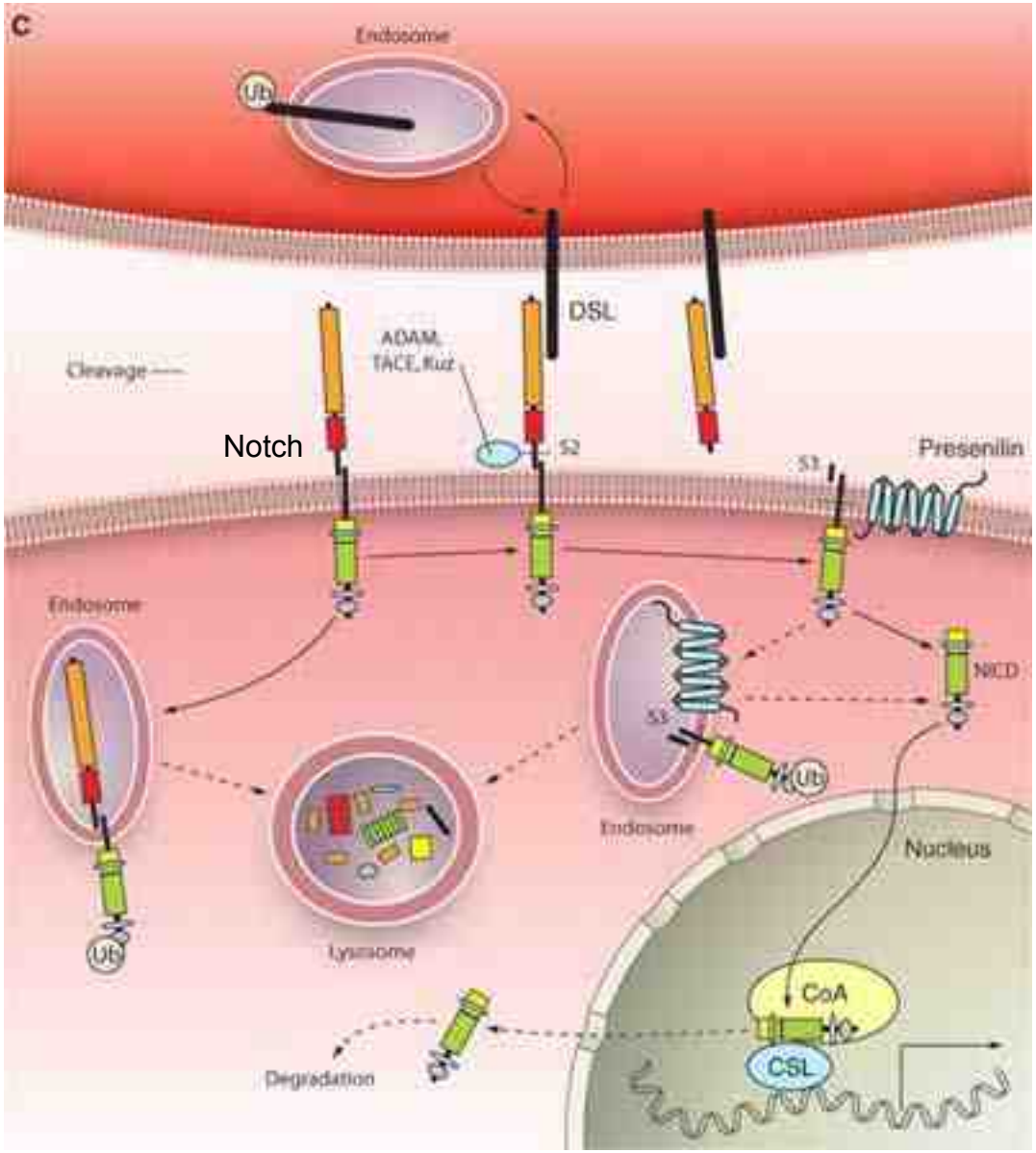
* Il precurosre del recettore Notch è una proteina di 300kD che subisce già un taglio proteolitico (S1) durante la sua maturazione nel Golgi. Le due subunità rimangono associate e trasportate in membrana.

* Notch è attivato da Delta (DSL) mediante comunicazione juxtacrina (interazione cellula-cellula).

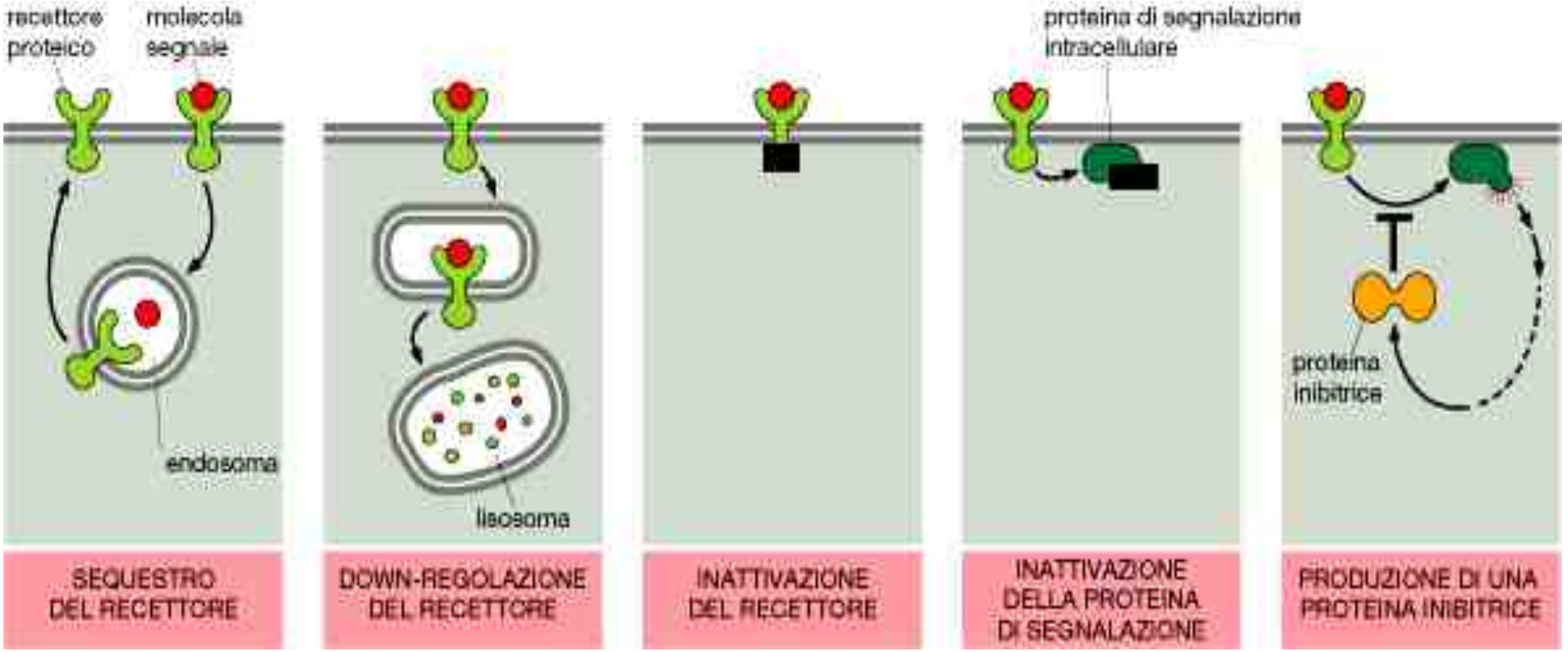
* Il legame di Delta induce il taglio proteolitico di Notch nella regione extracellulare (S2) da parte della metalloprotease ADAM/TACE. Il dominio extracellulare rimane associato a Delta e viene endocitato e degradato dalla cellula che esprime Delta.

* Il dominio transmembrana di Notch interagisce con la beta-secretase (protease) chiamata presenilina e viene tagliato (S3) e/o internalizzato assieme alla presenilina.

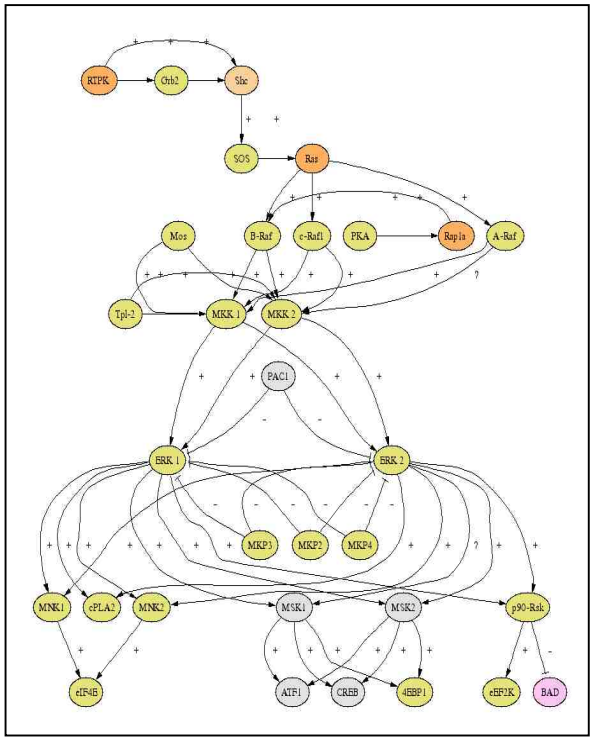
* Il risultato del taglio S3 libera il dominio citoplasmatico (NICD) che è trasportato nel nucleo dove si associa e regola fattori trascrizionali.

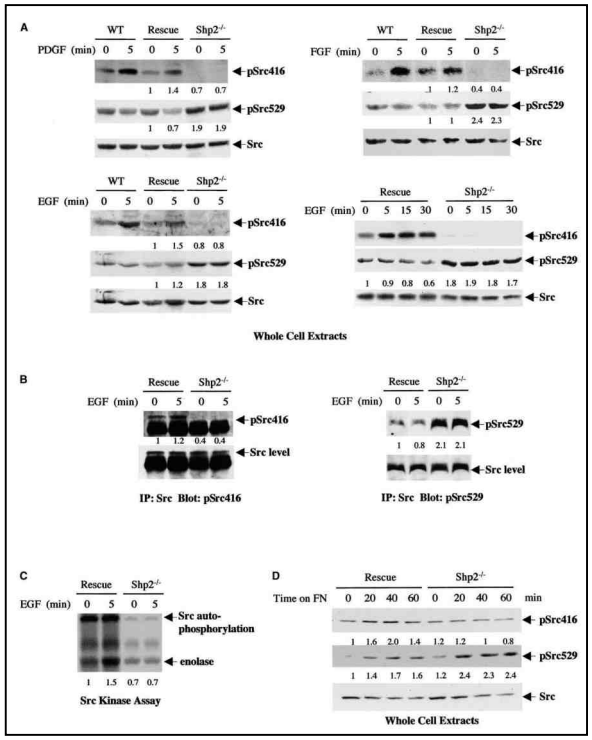


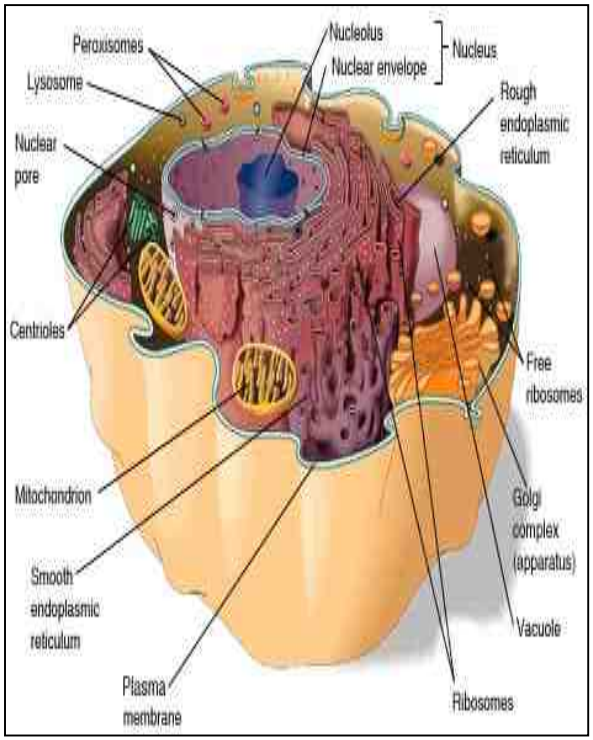
diversi modi di desensibilizzazione ad una molecola segnale



Signal Transduction





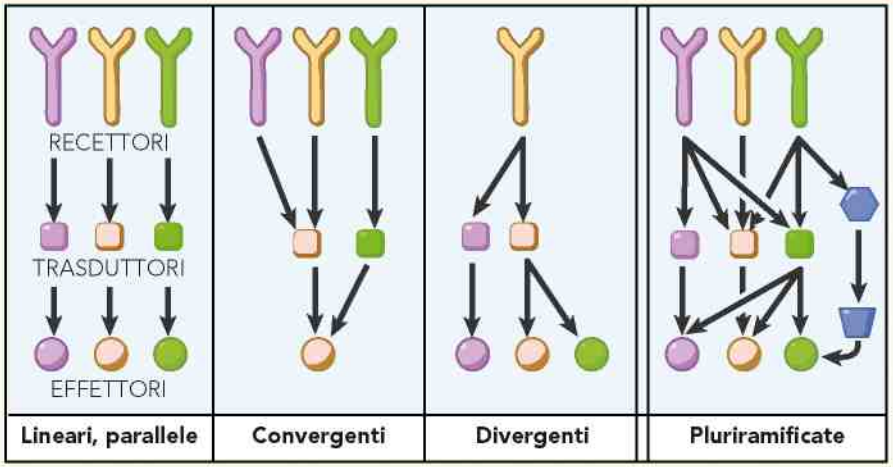


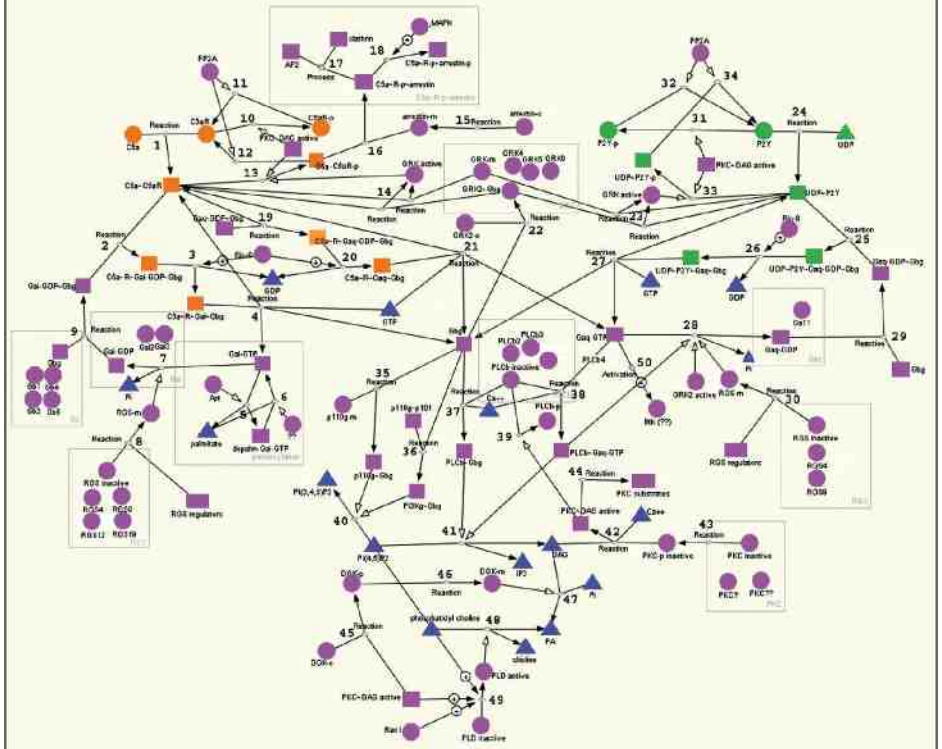
What?

When?

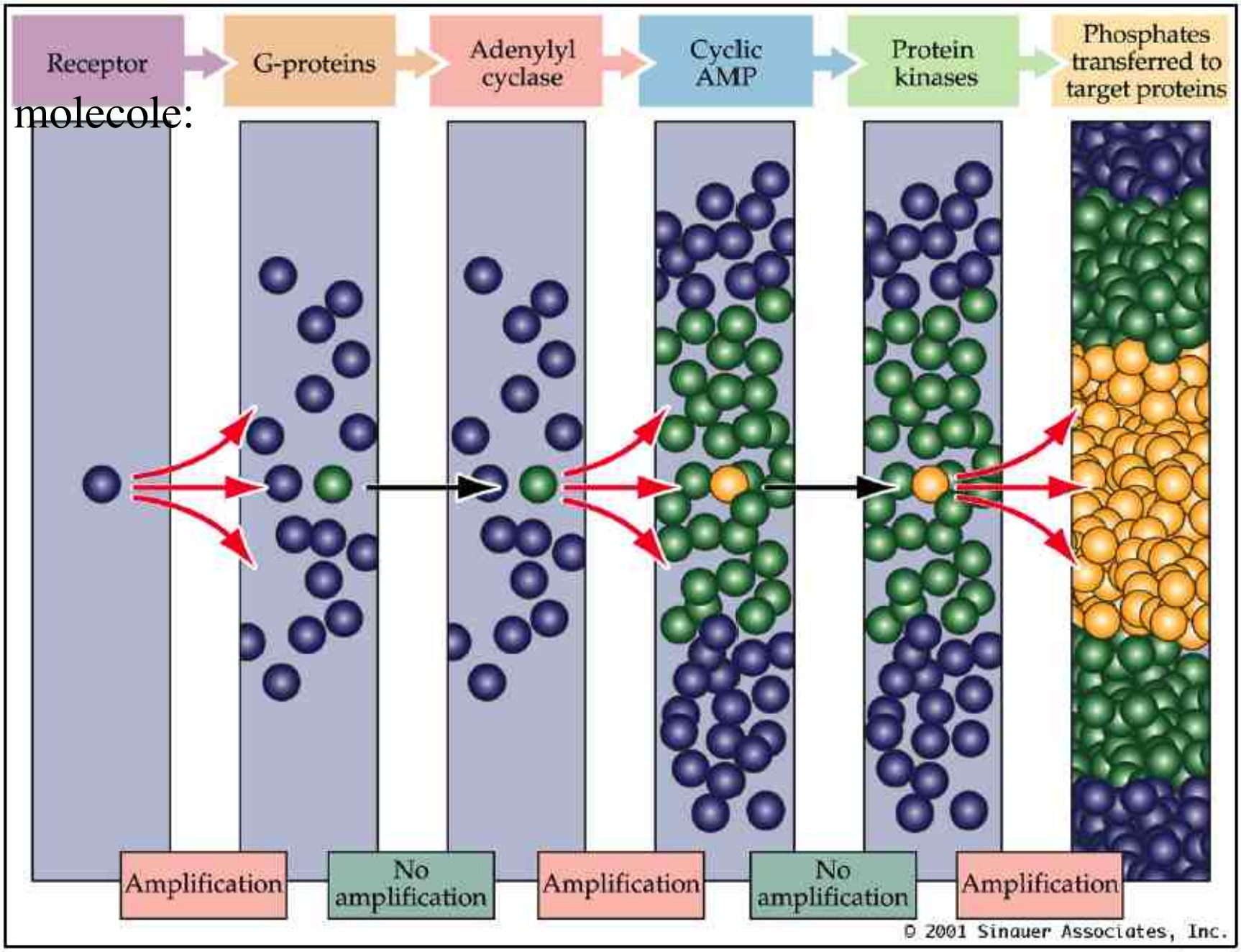
Where?

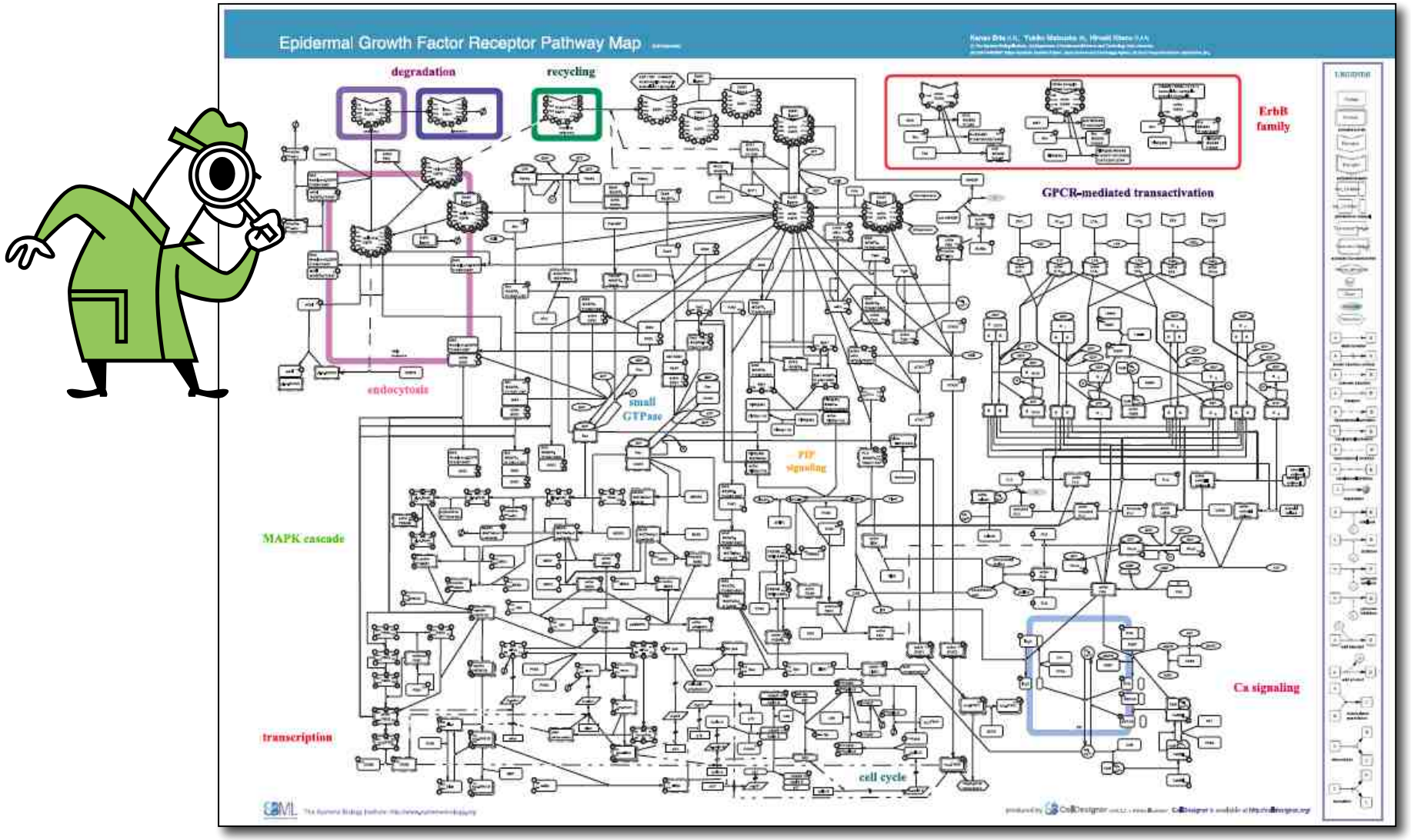
Vie di segnalazione convergenti e divergenti





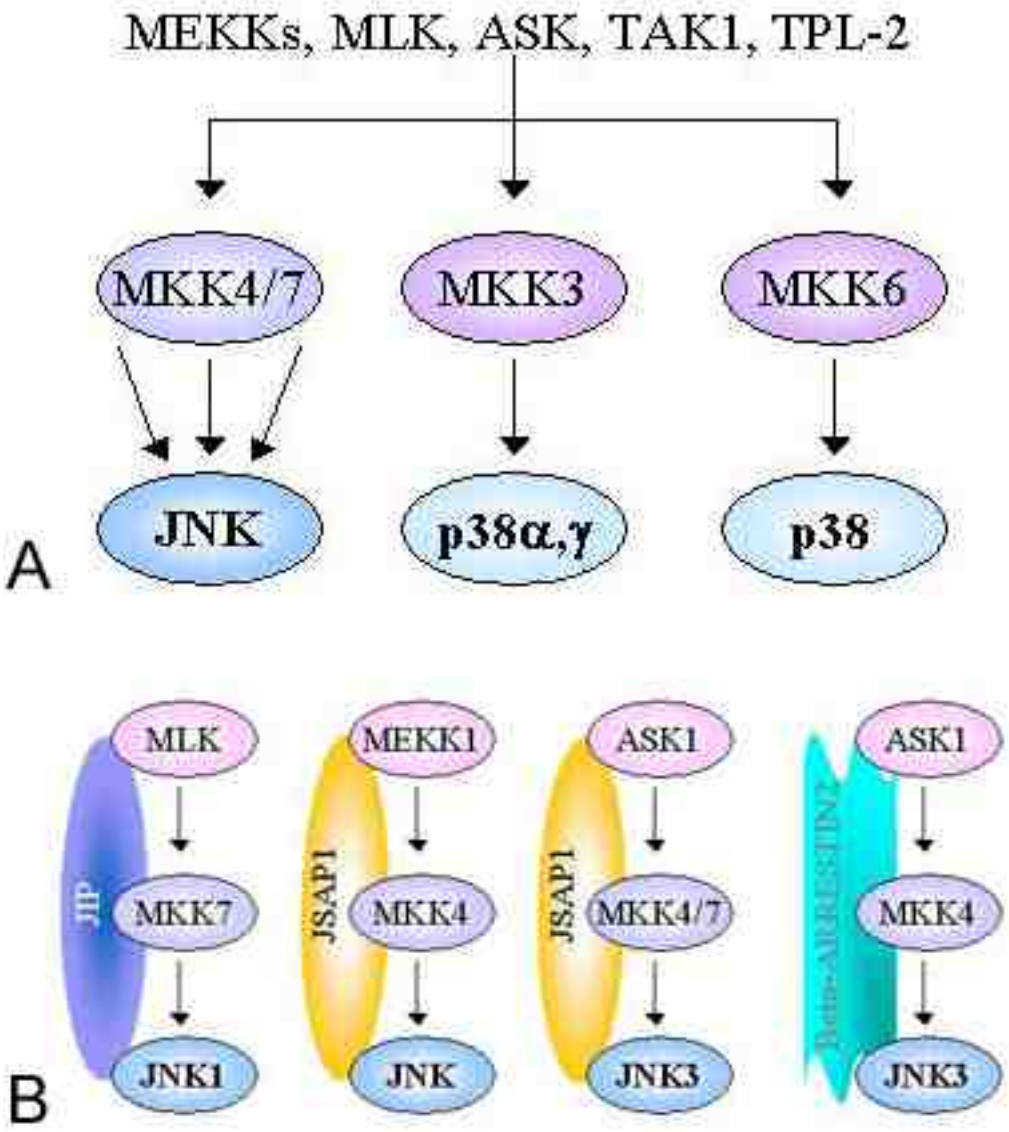
1 10^2 10^2 10^4 10^4 10^5 ..... 10^8





protein-protein interaction:

Experimental approach

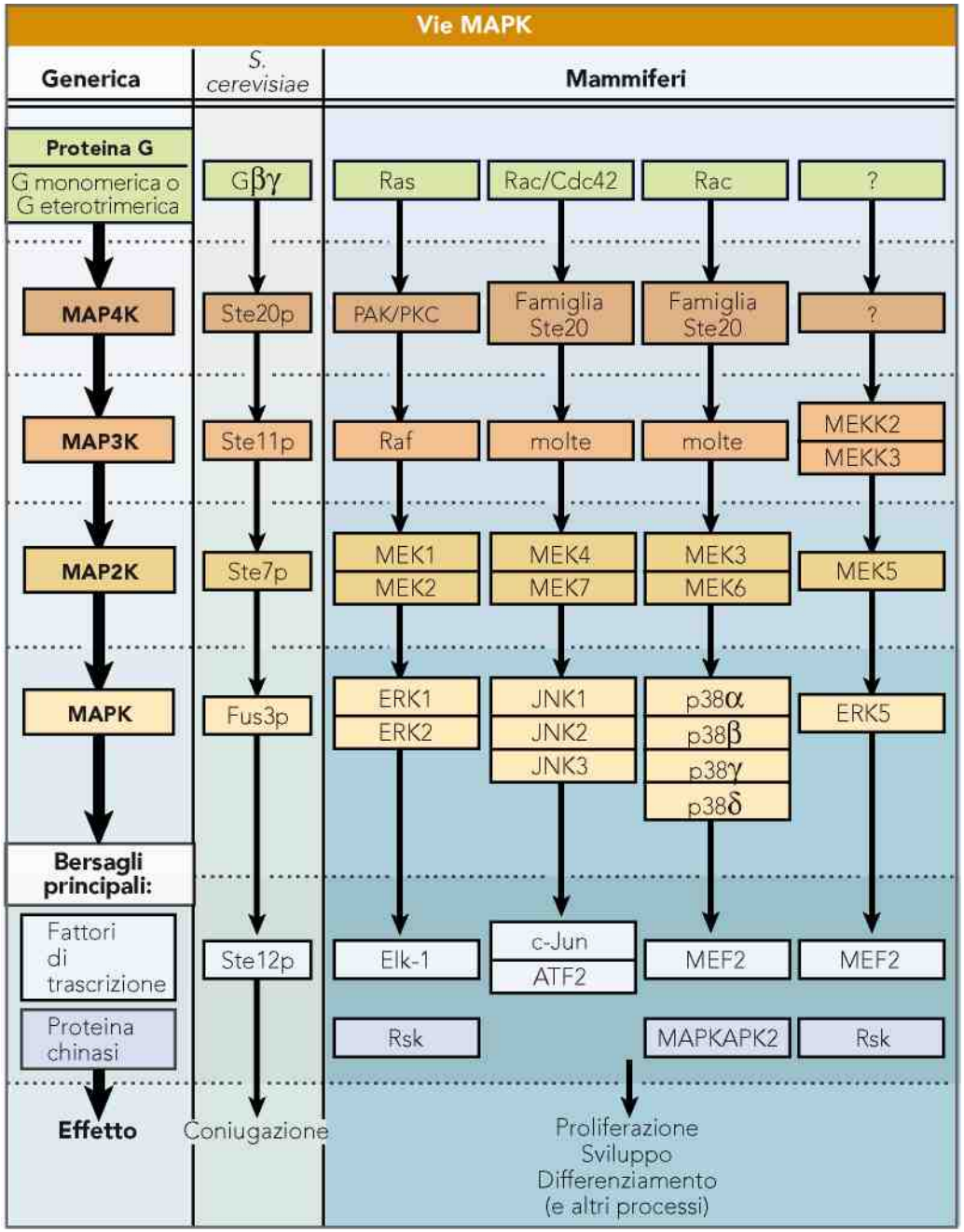

MEKKs, MLK, ASK, TAK1, TPL-2
MKK4/7
MKK3
MKK6
JNK
p38α,γ
p38
A
MLK
MEKK1
ASK1
ASK1
JIP
MKK7
JSAP1
MKK4
JSAP1
MKK4/7
Arrestin
MKK4
JNK1
JNK
JNK3
JNK3
B

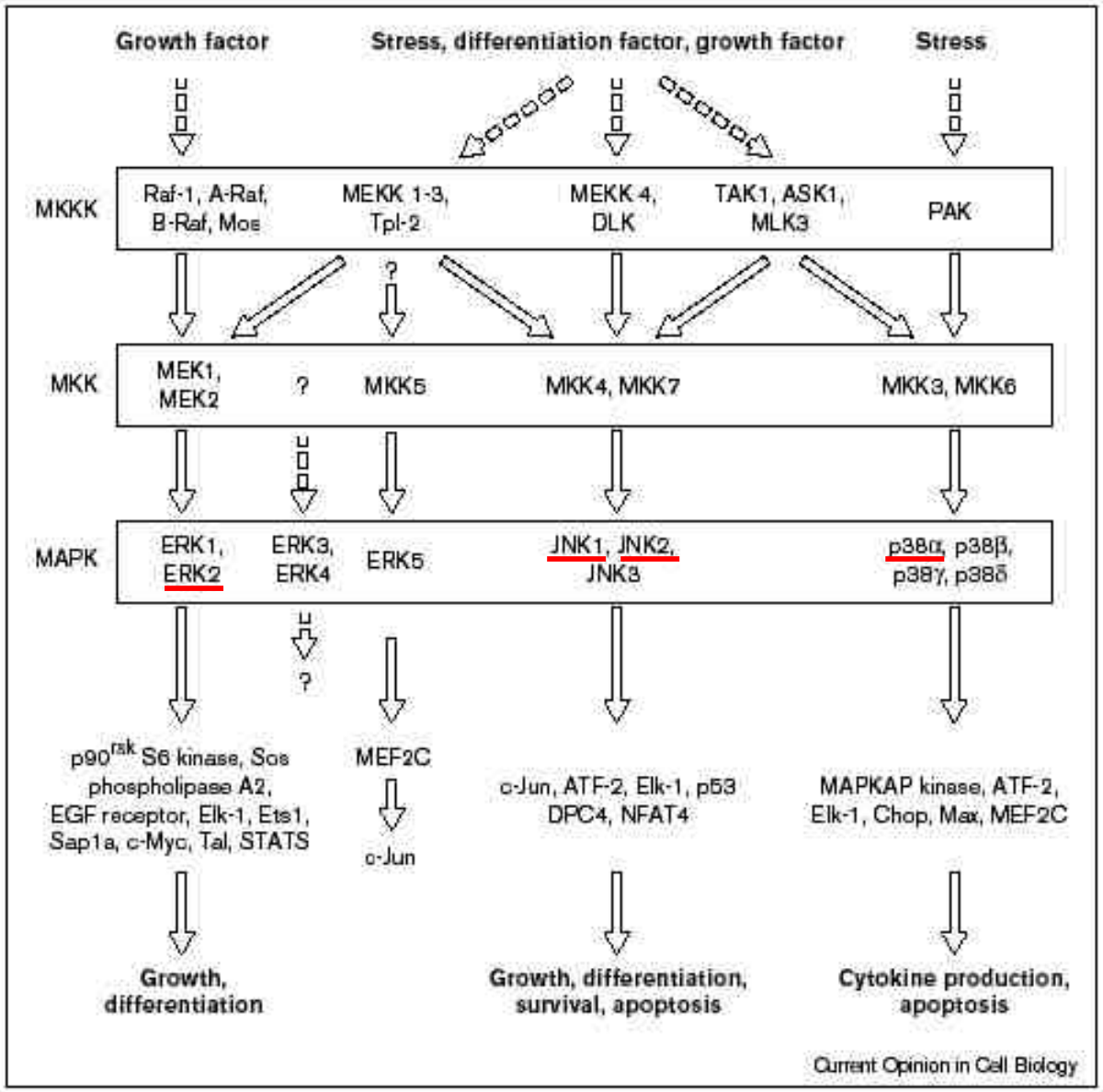
A Mammalian Scaffold Complex That Selectively Mediates MAP Kinase Activation

Alan J. Whitmarsh, Julie Cavanagh, Cathy Tournier, Jun Yasuda, Roger J. Davis*

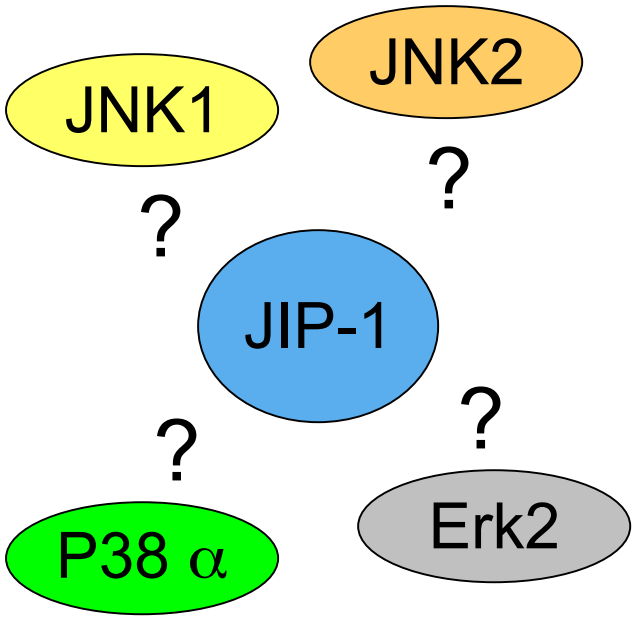
The c-Jun NH_2-terminal kinase (JNK) group of mitogen-activated protein (MAP) kinases is activated by the exposure of cells to multiple forms of stress. A putative scaffold protein was identified that interacts with multiple components of the JNK signaling pathway, including the mixed-lineage group of MAP kinase kinase kinases (MLK), the MAP kinase kinase MKK7, and the MAP kinase JNK. This scaffold protein selectively enhanced JNK activation by the MLK signaling pathway. These data establish that a mammalian scaffold protein can mediate activation of a MAP kinase signaling pathway.

Science. 1998 Sep 11;281(5383):1671-4.


Vie MAPK
Generica
S. cerevisiae
Mammiferi
Proteina G
G monomerica o G eterotrimerica
Gβγ
Ras
Rac/Cdc42
Rac
?
MAP4K
Ste20p
PAK/PKC
Famiglia Ste20
Famiglia Ste20
?
MAP3K
Ste11p
Raf
molte
molte
MEKK2
MEKK3
MAP2K
Ste7p
MEK1
MEK2
MEK4
MEK7
MEK3
MEK6
MEK5
MAPK
Fus3p
ERK1
ERK2
JNK1
JNK2
JNK3
p38α
p38β
p38γ
p38δ
ERK5
Bersagli principali:
Fattori di trascrizione
Proteina chinasi
Ste12p
Elk-1
c-Jun
ATF2
MEF2
MEF2
Rsk
MAPKAPK2
Rsk
Effetto
Coniugazione
Proliferazione
Sviluppo
Differenziamento
(e altri processi)



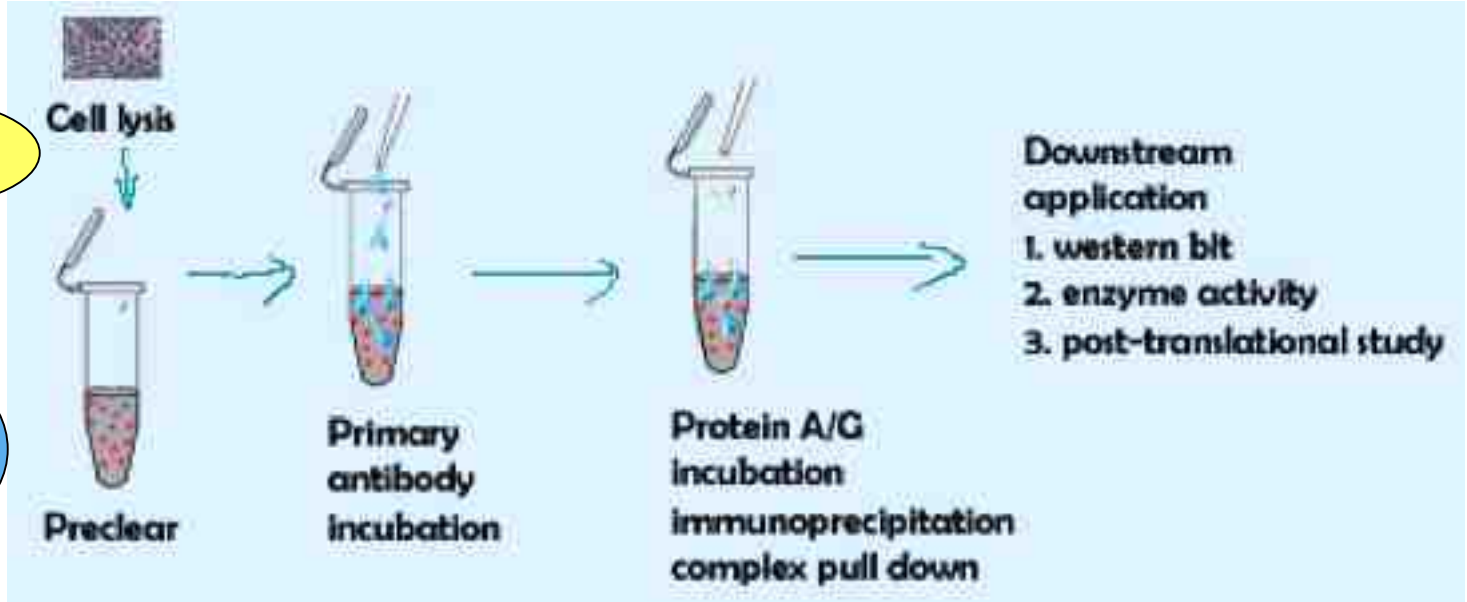
Which MAPK interact with JIP-1?



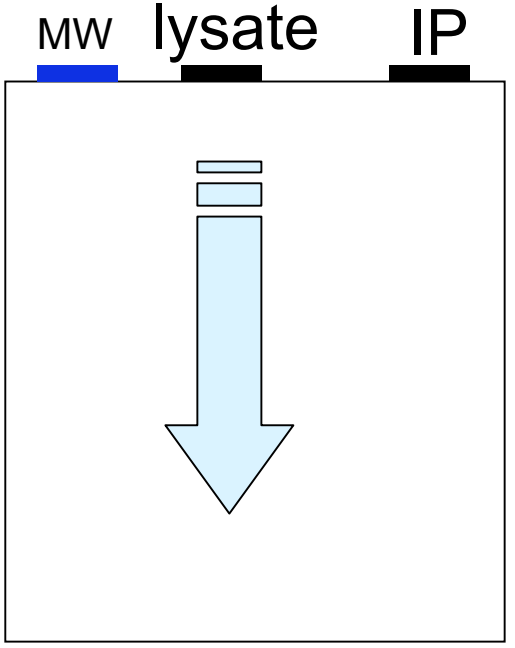
JNK1

?

JIP-1



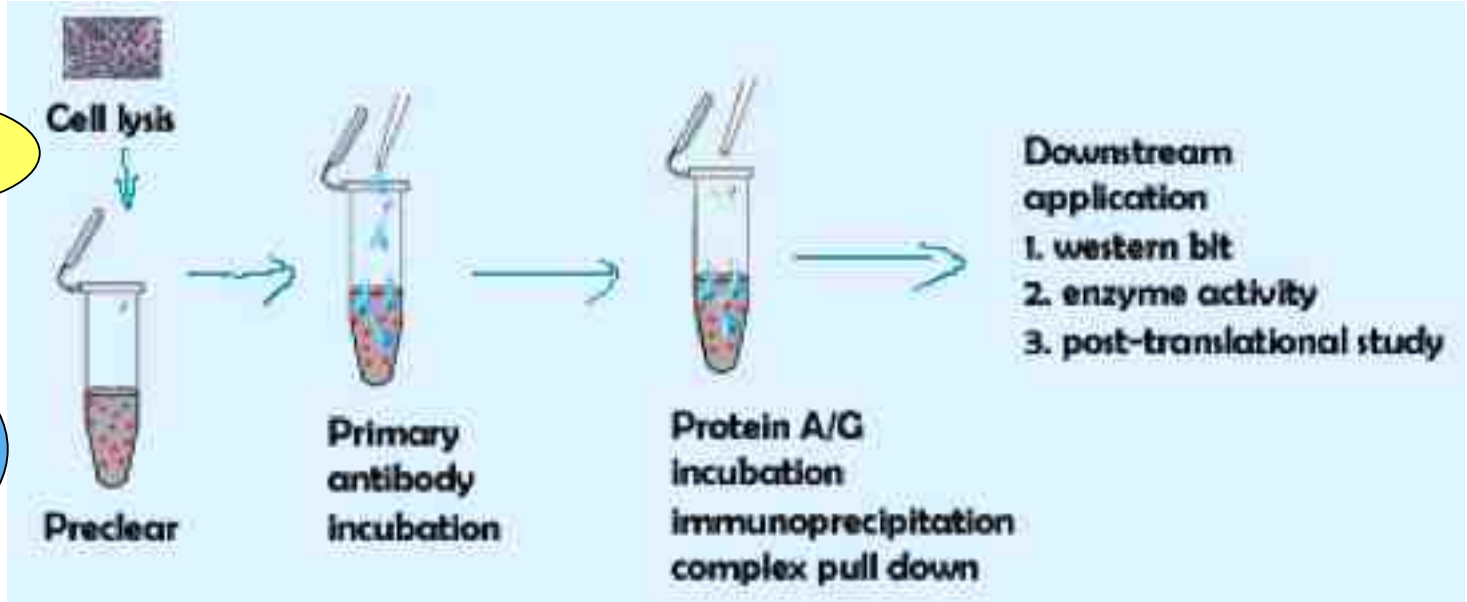
IP anti JNK1



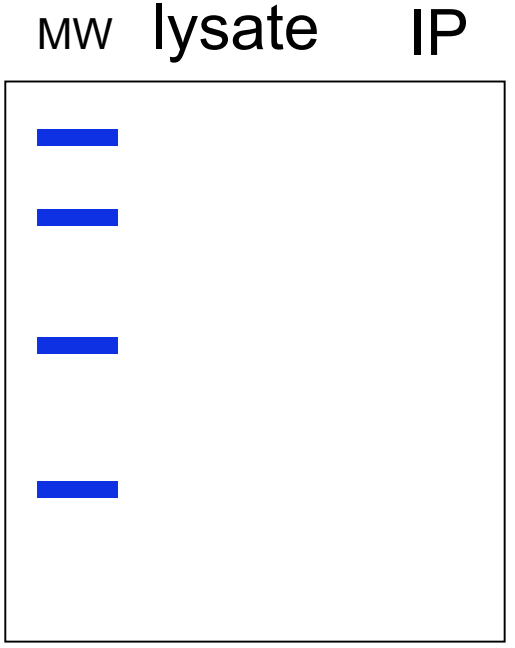
JNK1

?

JIP-1



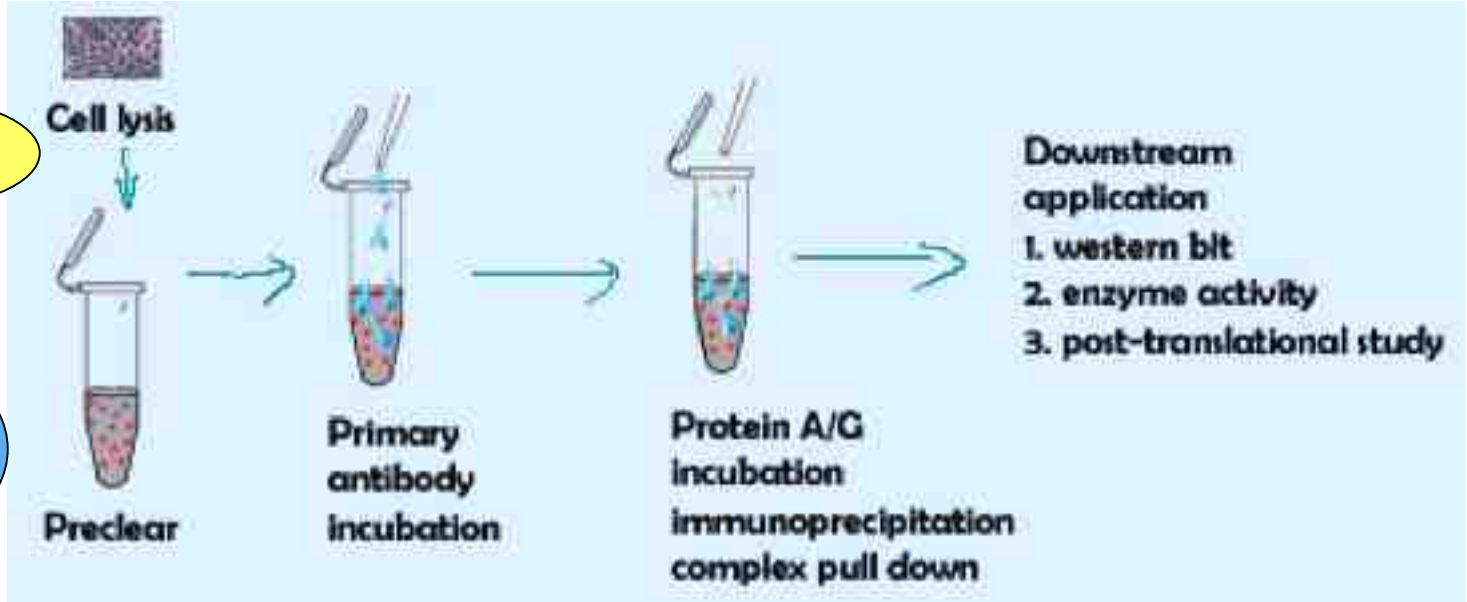
IP anti JNK1



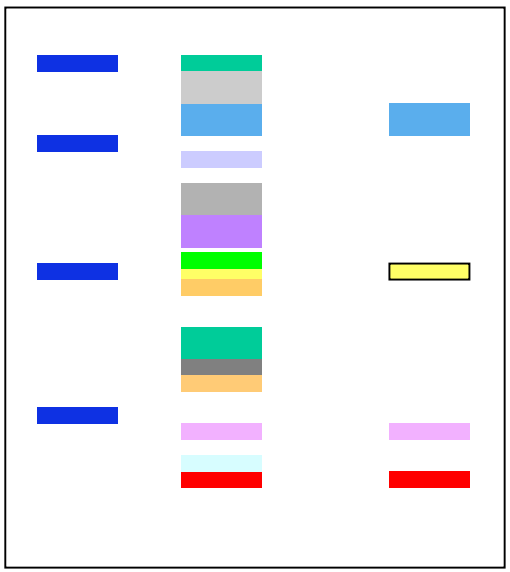
JNK1

?

JIP-1



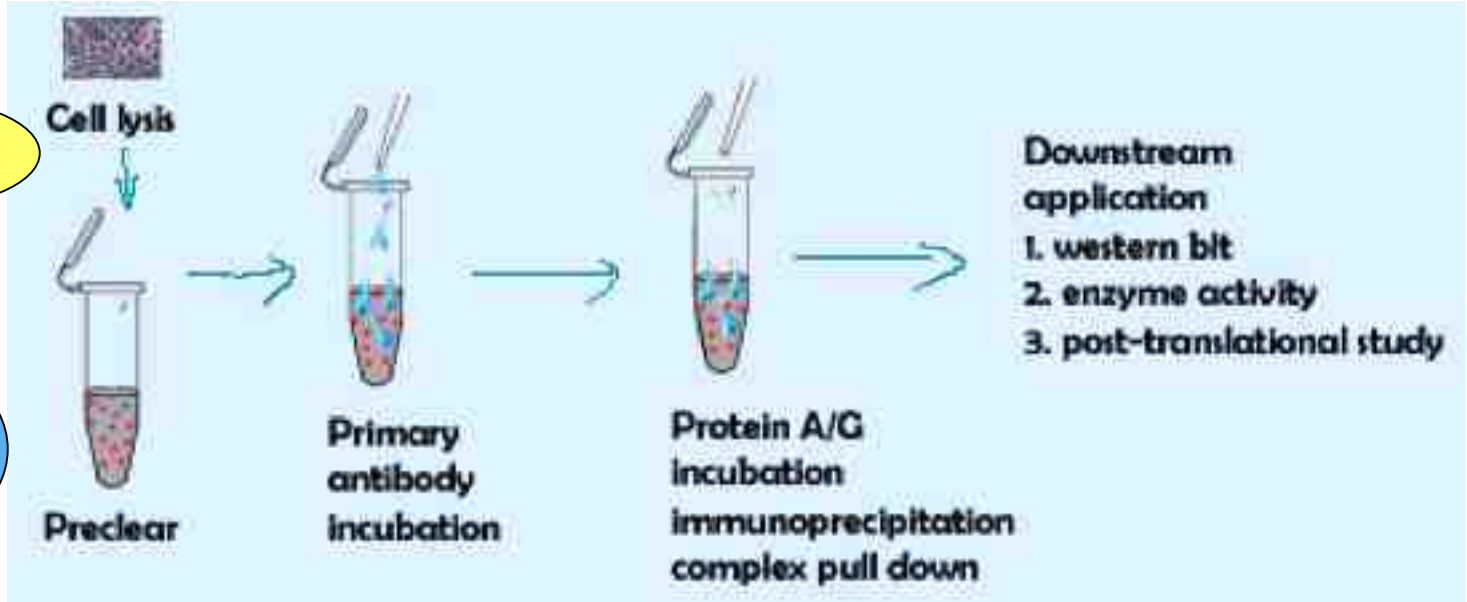
IP anti JNK1



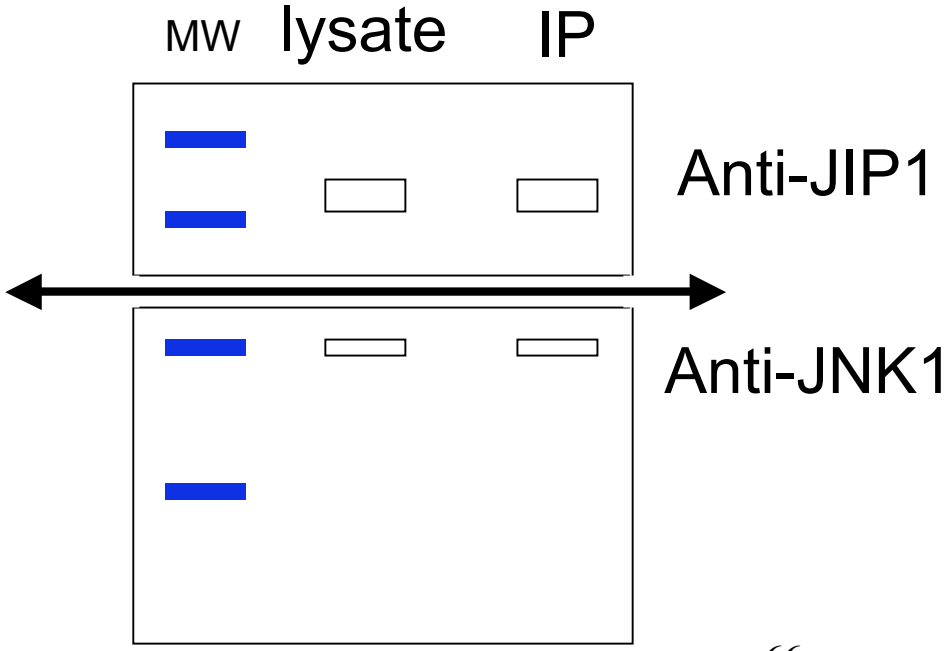
JNK1

?

JIP-1



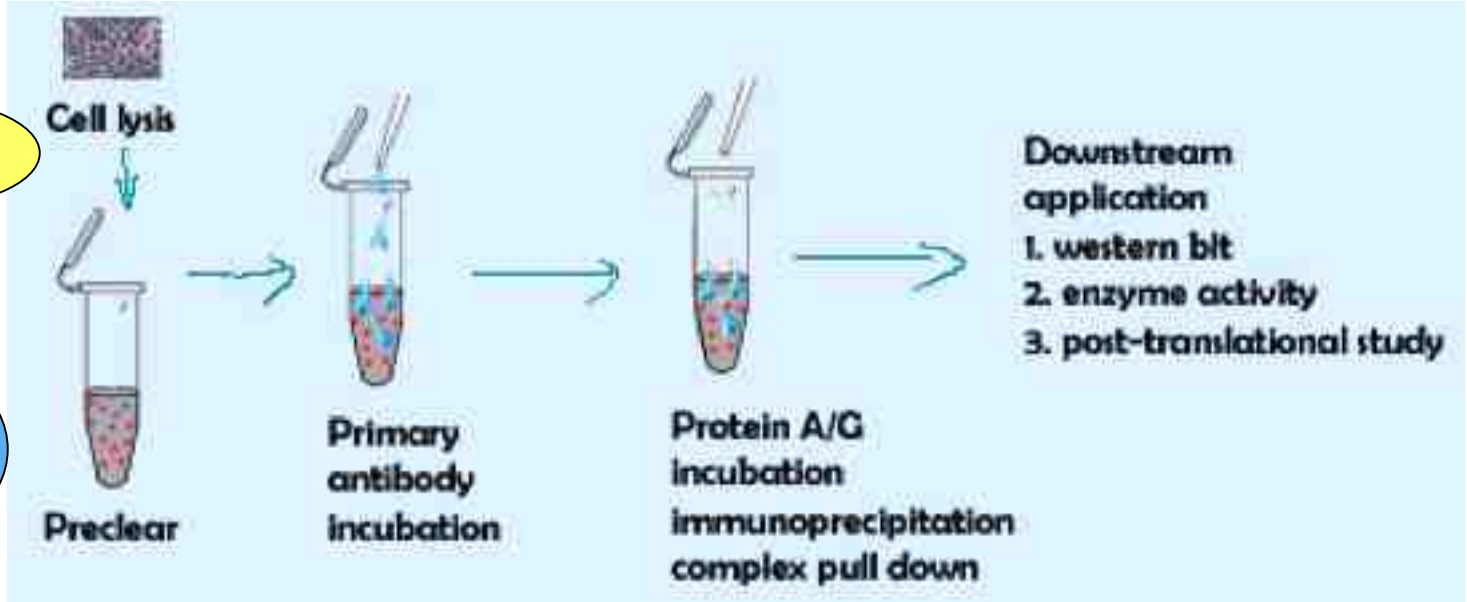
IP anti JNK1

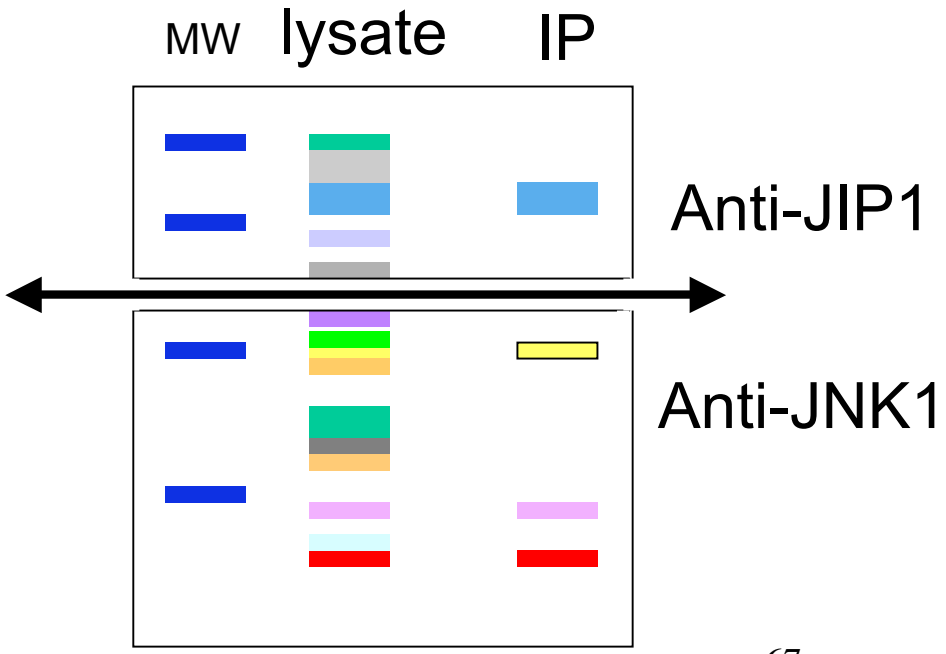


JNK1

?

JIP-1

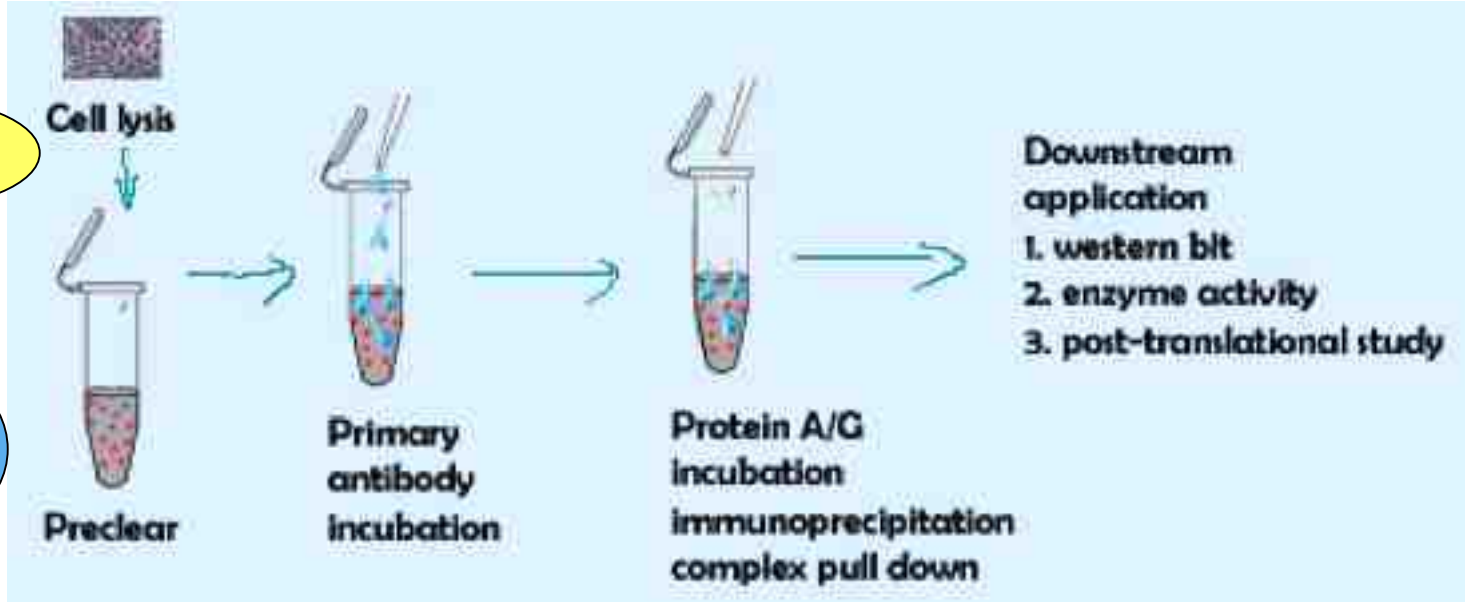

Cell lysis
Preclear
Primary antibody incubation
Protein A/G incubation immunoprecipitation complex pull down
Downstream application
1. western blt
2. enzyme activity
3. post-translational study


MW
lysate
IP
Anti-JIP1
Anti-JNK1

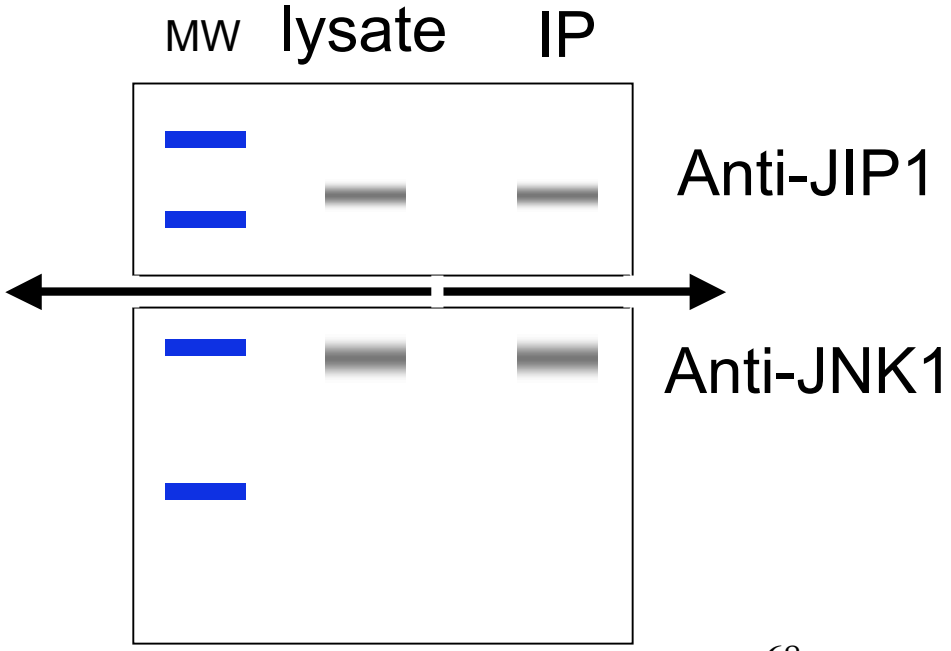
JNK1

?

JIP-1



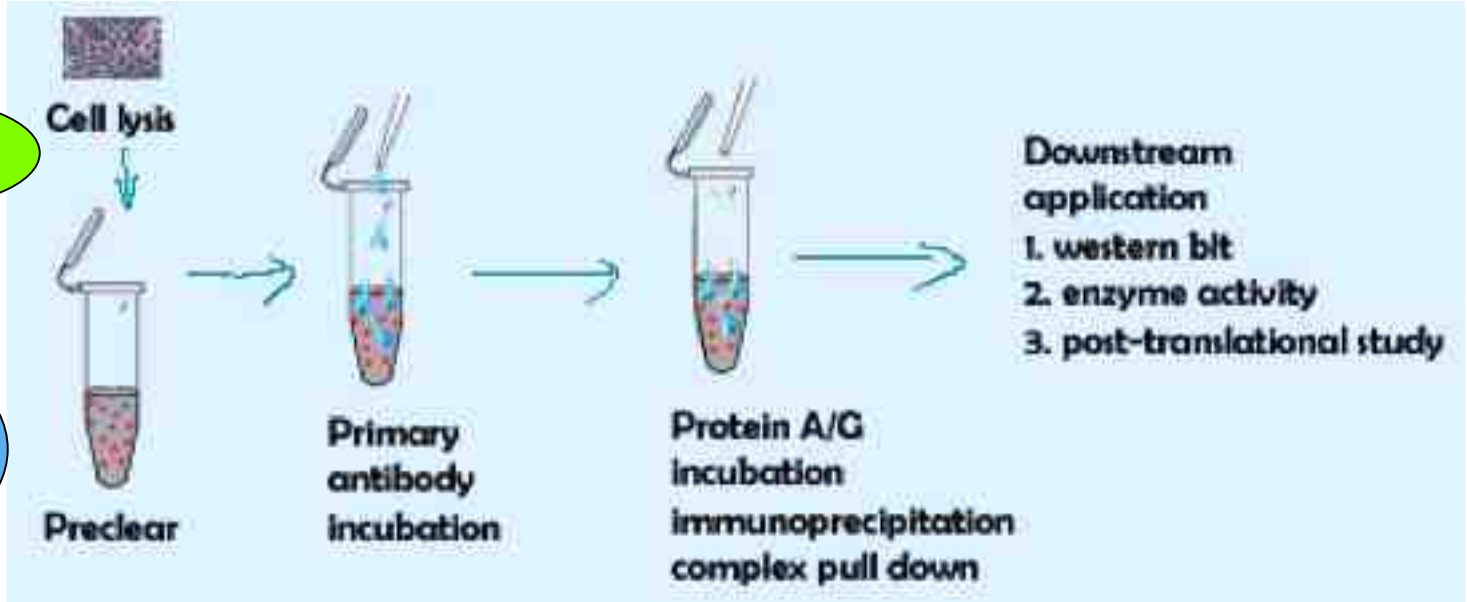
IP anti JNK1



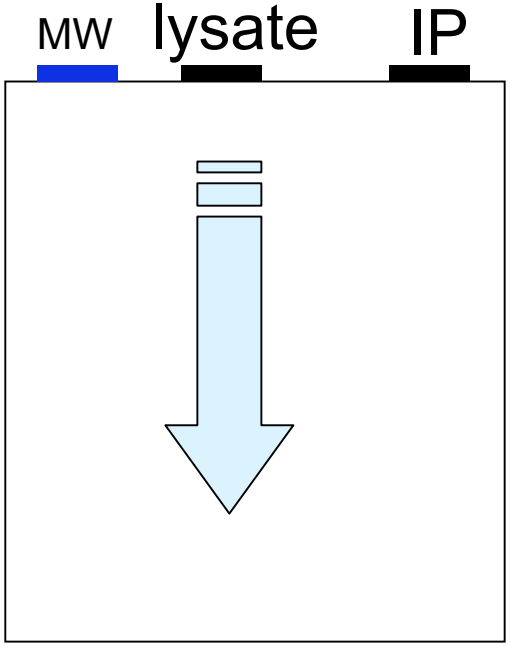
P38α

?

JIP-1



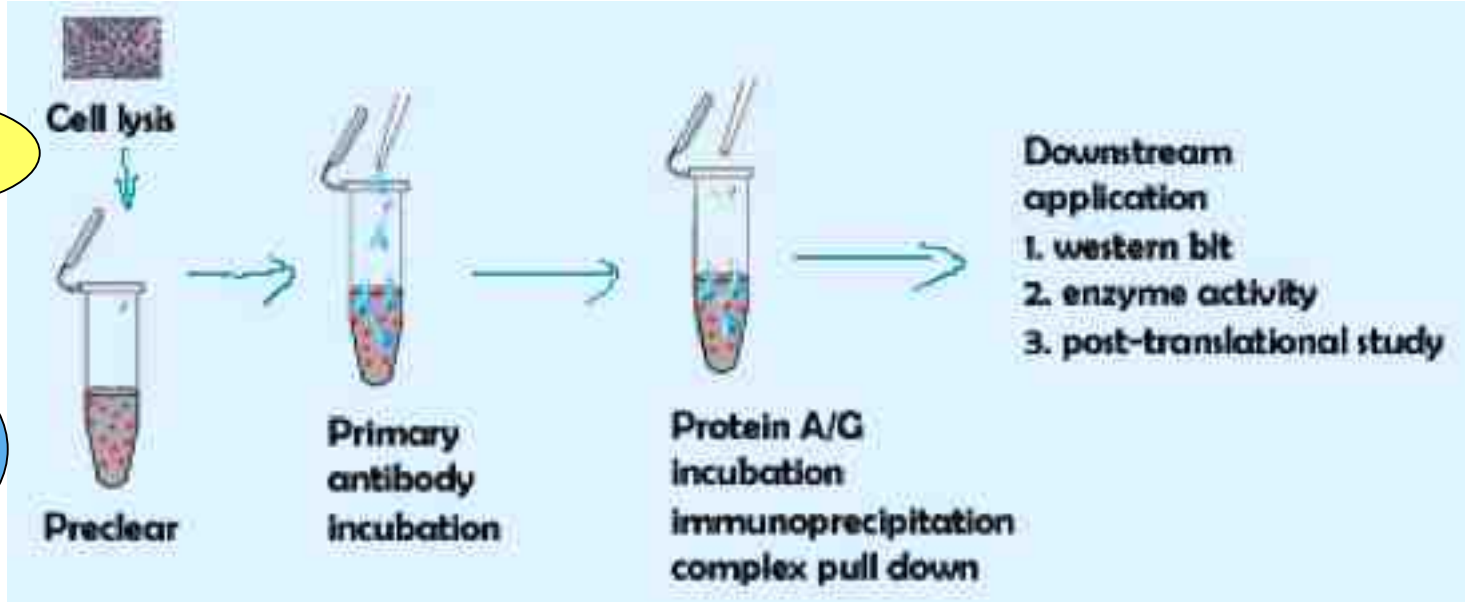
IP anti P38α



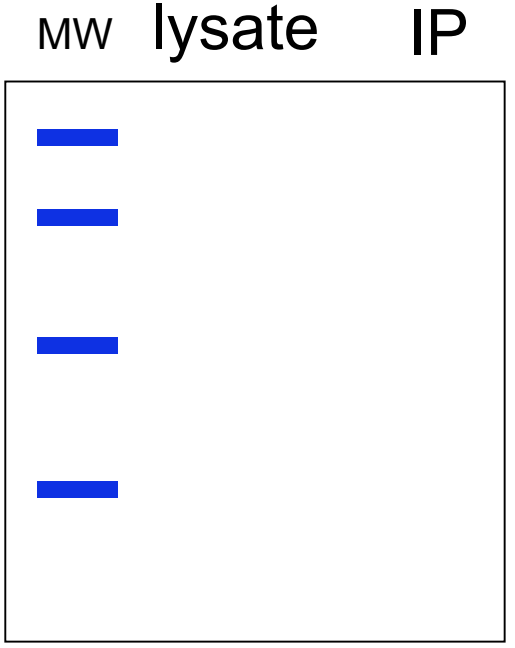
JNK1

?

JIP-1



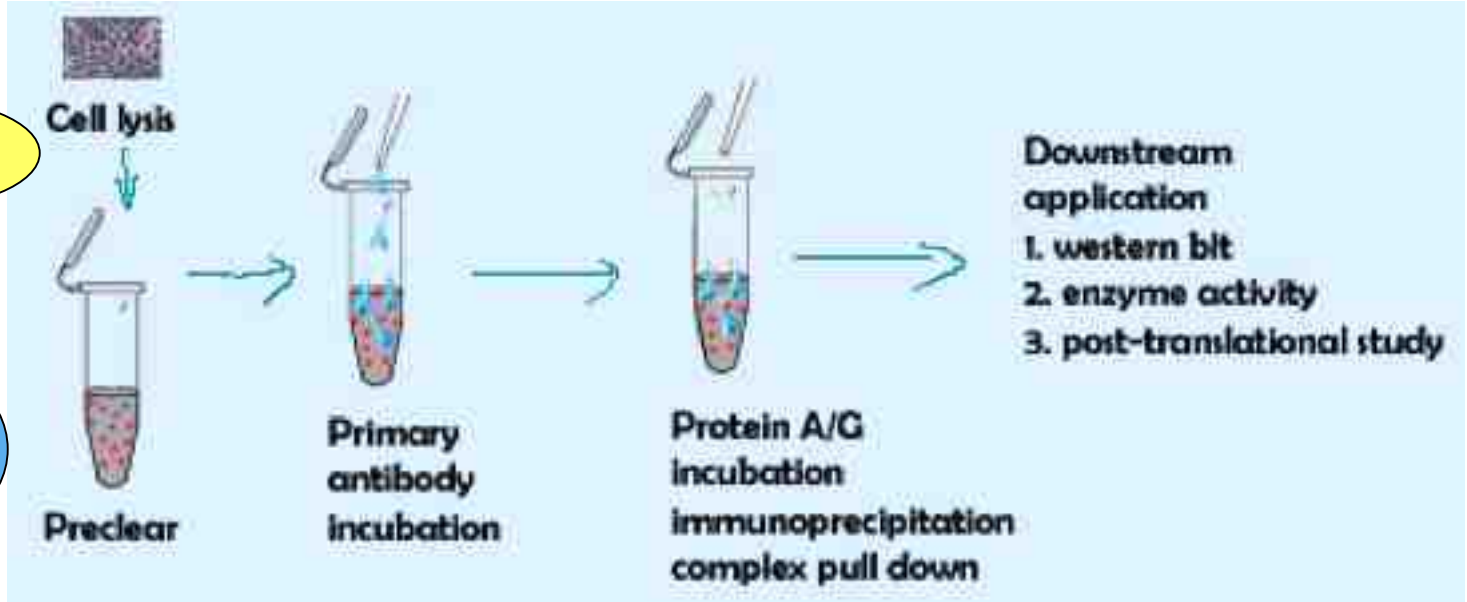
IP anti JNK1



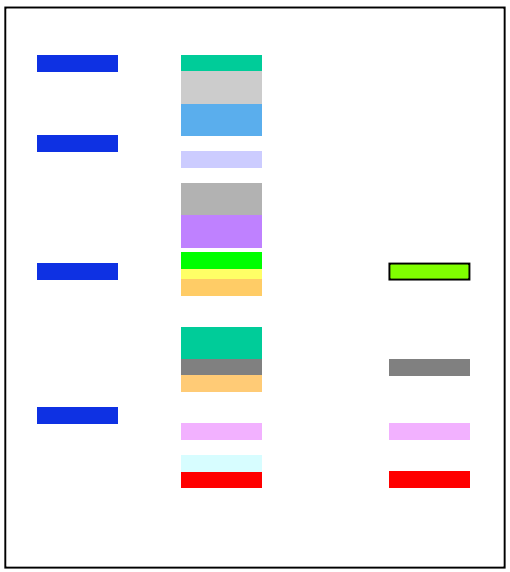
P38α

?

JIP-1



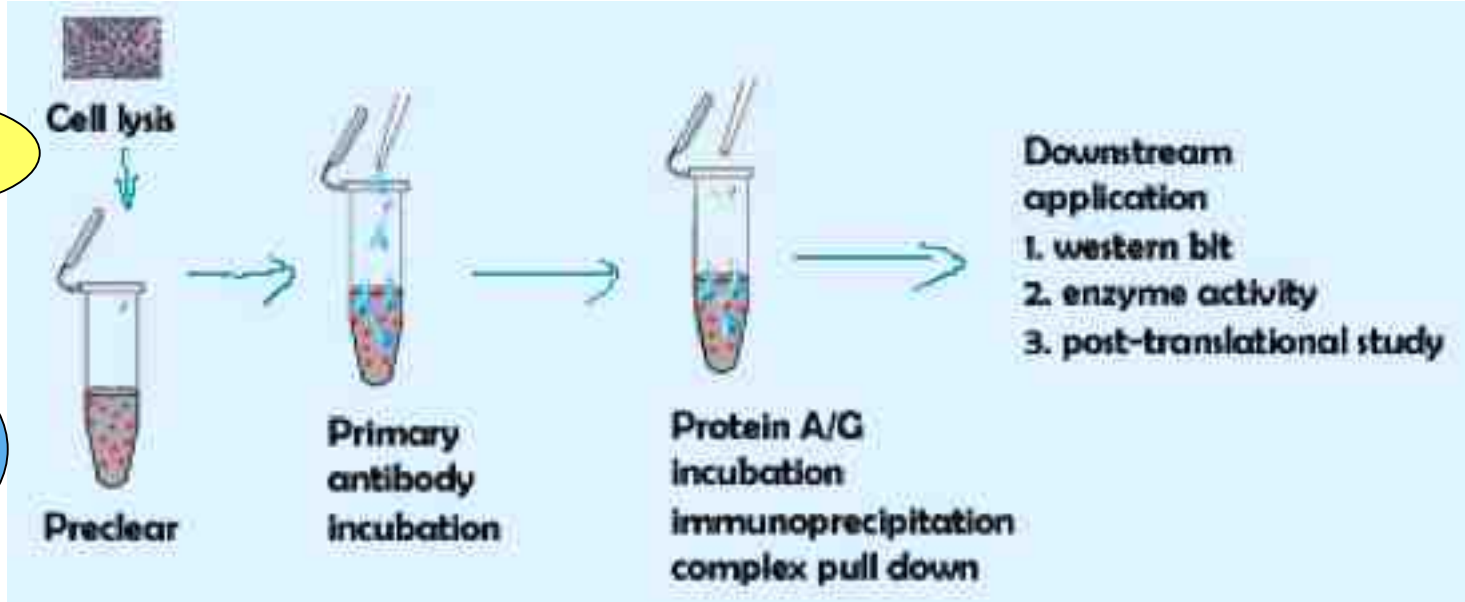
IP anti P38 α



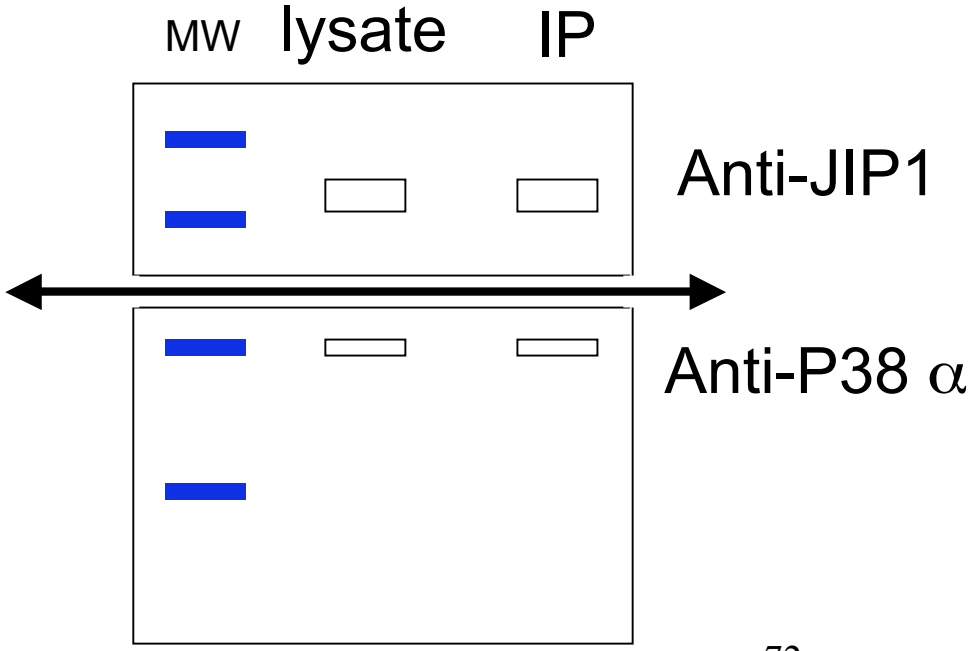
P38 α

?

JIP-1



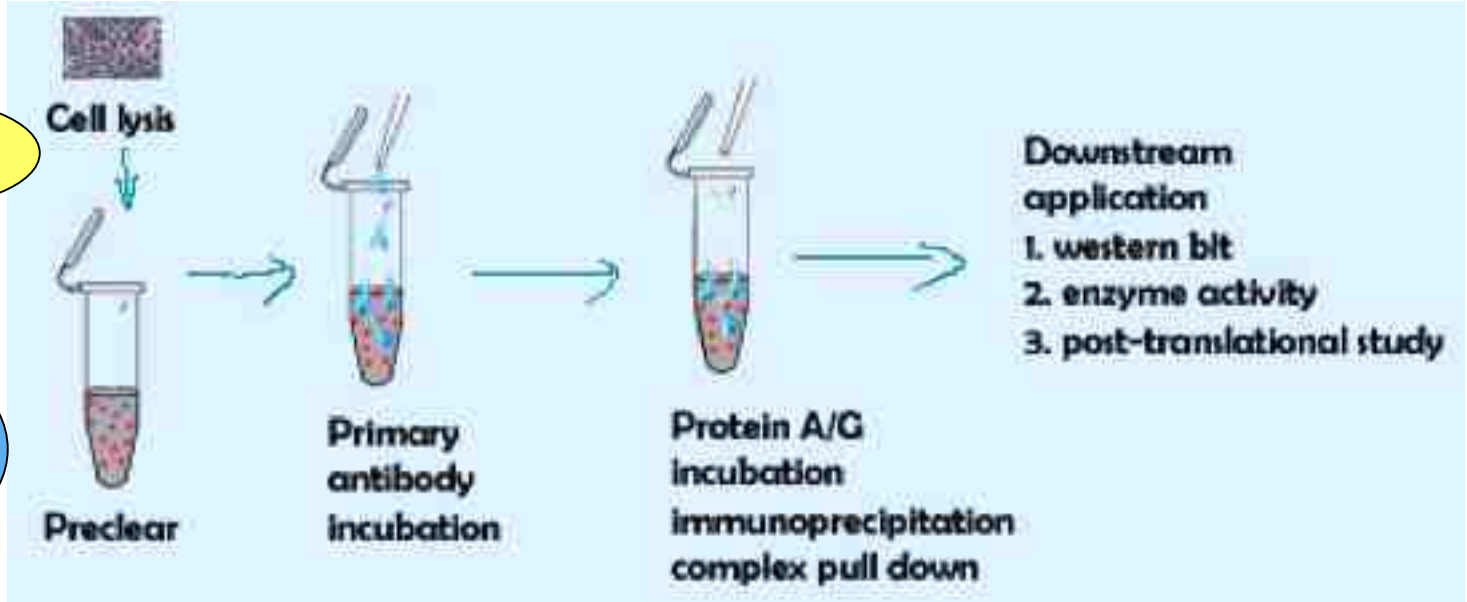
IP anti P38 α

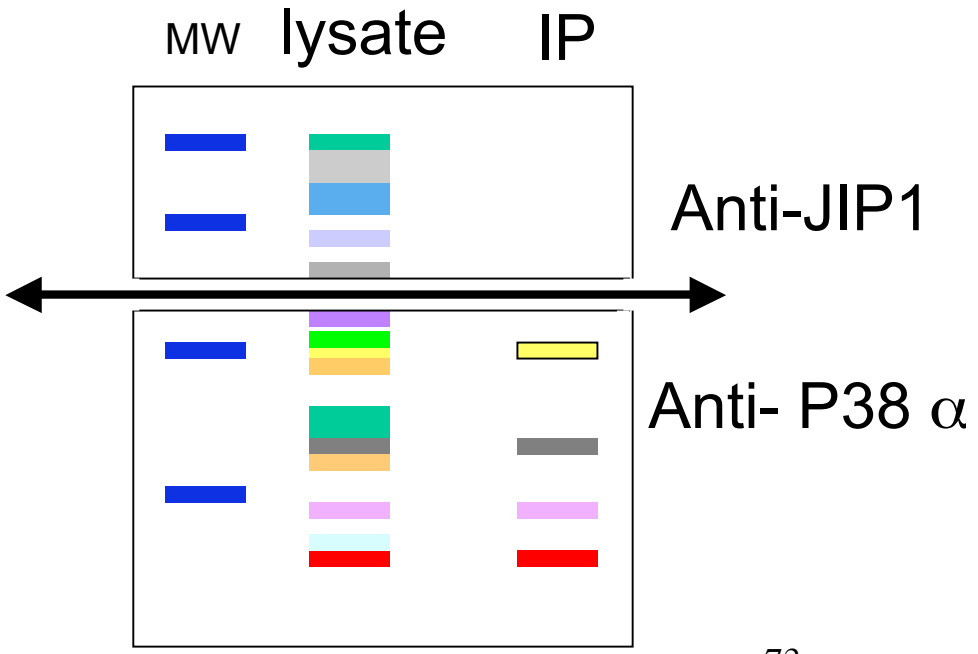


P38 α

?

JIP-1

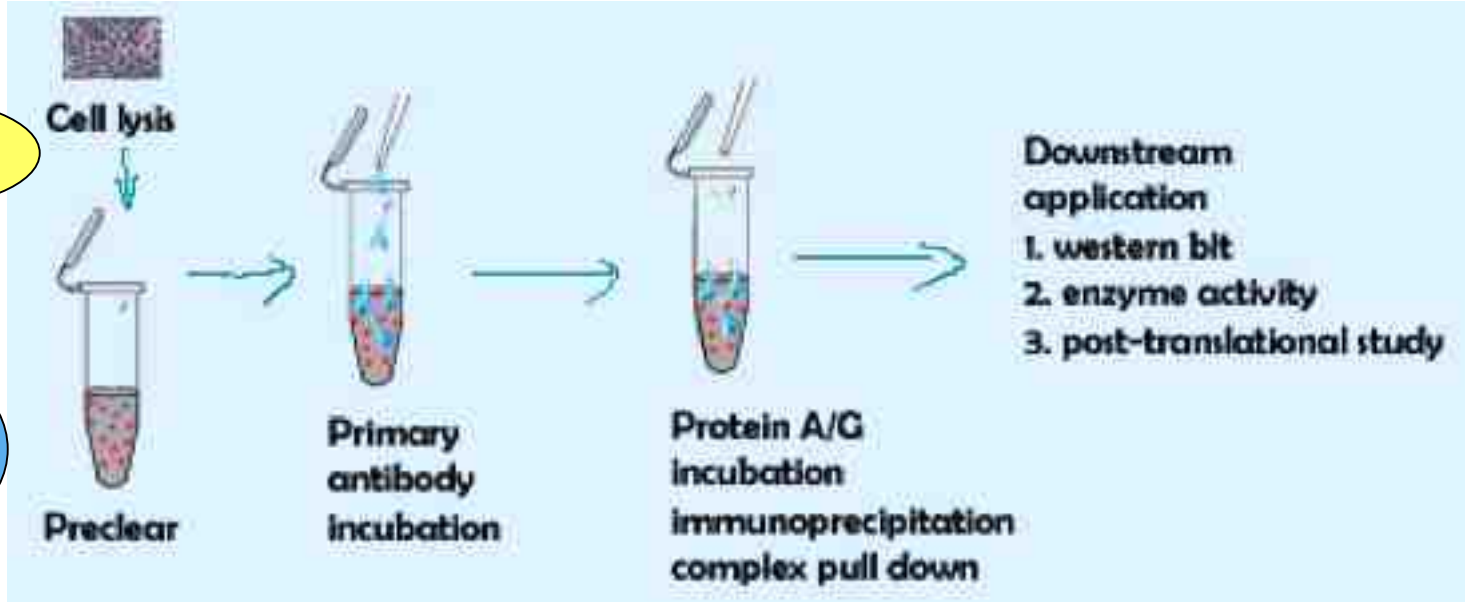

Cell lysis
Preclear
Primary antibody incubation
Protein A/G incubation immunoprecipitation complex pull down
Downstream application
1. western blt
2. enzyme activity
3. post-translational study


MW
lysate
IP
Anti-JIP1
Anti- P38 α

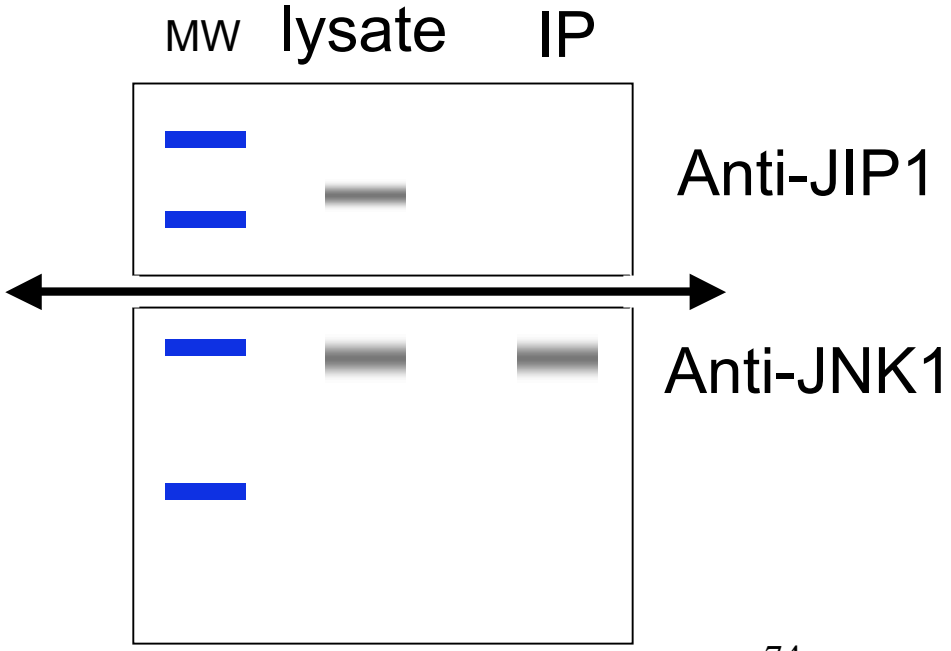
JNK1

?

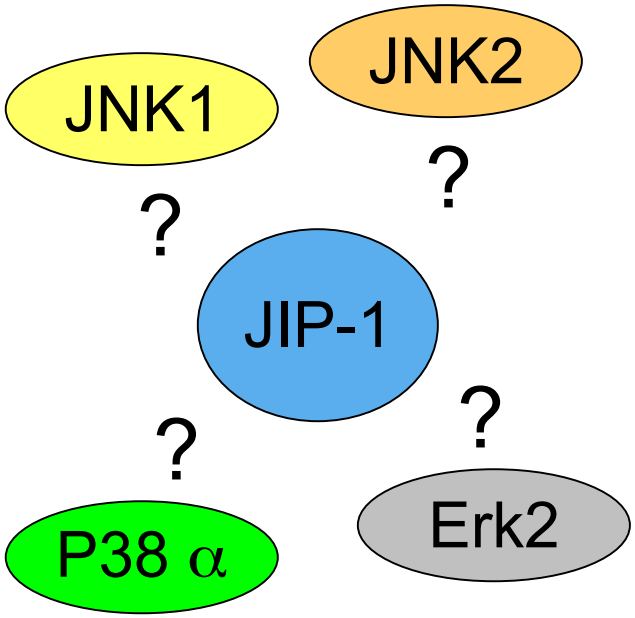
JIP-1

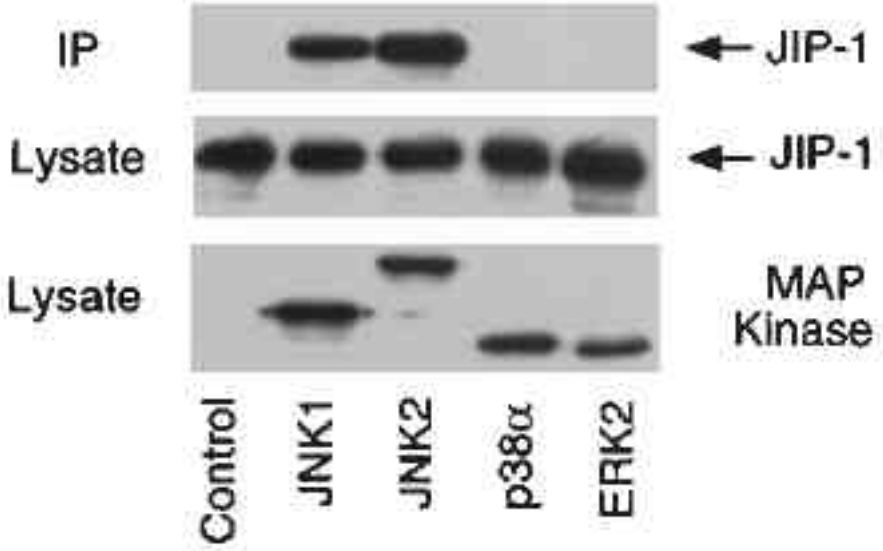


IP anti JNK1



Selective binding of JIP-1 to the MAP kinase JNK





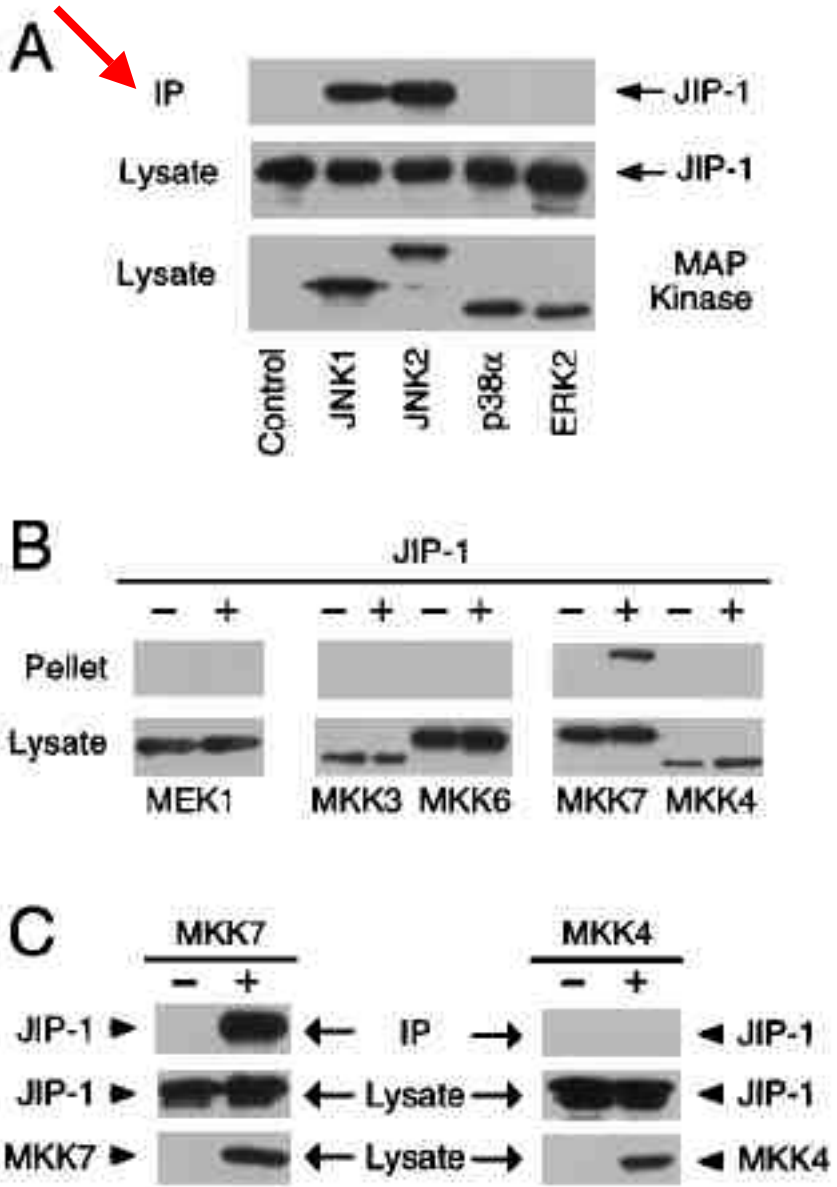
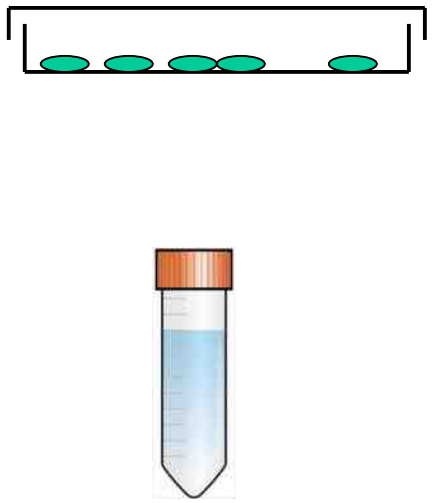


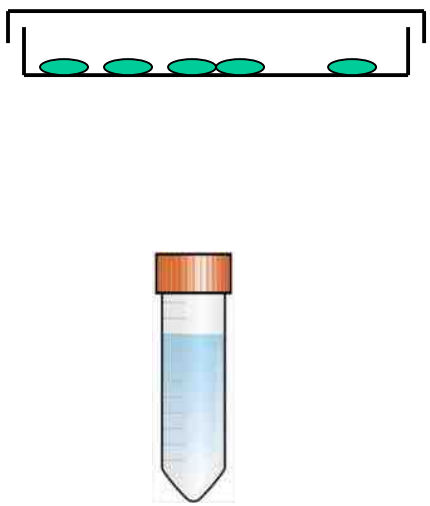
Fig. 1. Selective binding of JIP-1 to the MAP kinase JNK and the MAP kinase kinase MKK7. (**A**) Epitope-tagged JIP-1 (T7-Tag) was expressed in cells with the HA-tagged MAP kinases ERK2, p38α, JNK1, and JNK2 (*15*, *16*). The MAP kinases were immunoprecipitated with an antibody to HA. The presence of JIP-1 in the immunoprecipitates (IP) was detected on immunoblots probed with an antibody to T7-Tag. The amount of JIP-1 and MAP kinases in the cell lysates was examined by protein immunoblot analysis. (**B**) JIP-1 was expressed in cells as a GST fusion protein together with epitope-tagged MEK1, MKK3, MKK4, MKK6, or MKK7 (*15*, *16*). JIP-1 was precipitated from cell lysates with glutathione-agarose, and the MAPKKs present in the pellet were detected by protein immunoblot analysis. The amount of the MAPKKs in the cell lysates was examined by protein immunoblot analysis. (**C**) Epitope-tagged JIP-1 (T7-Tag) was expressed in cells with Flag-tagged MKK4 or MKK7 (*15*, *16*). The presence of JIP-1 in Flag IP was detected by protein immunoblot analysis with an antibody to T7-Tag. The amount of the MAPKKs in the cell lysates was examined by protein immunoblot analysis.



IP:
anticorpo anti-proteina
d'interesse

Costrutto "taggato"

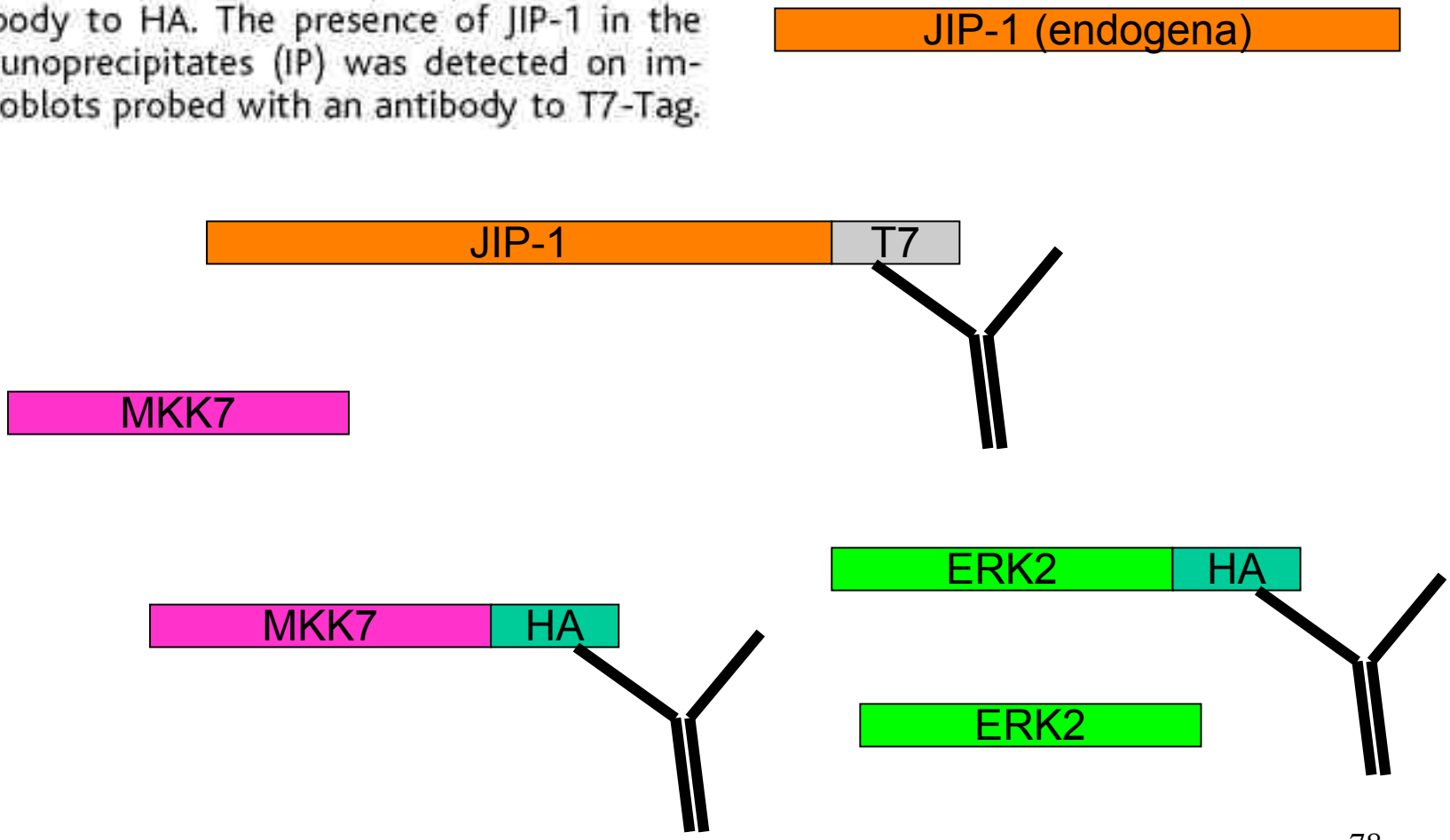
Trasfezione
transiente



IP:
anticorpo anti-Tag

Differenza?

Fig. 1. Selective binding of JIP-1 to the MAP kinase JNK and the MAP kinase kinase MKK7. (**A**) Epitope-tagged JIP-1 (T7-Tag) was expressed in cells with the HA-tagged MAP kinases ERK2, p38α, JNK1, and JNK2 (*15*, *16*). The MAP kinases were immunoprecipitated with an antibody to HA. The presence of JIP-1 in the immunoprecipitates (IP) was detected on immunoblots probed with an antibody to T7-Tag.

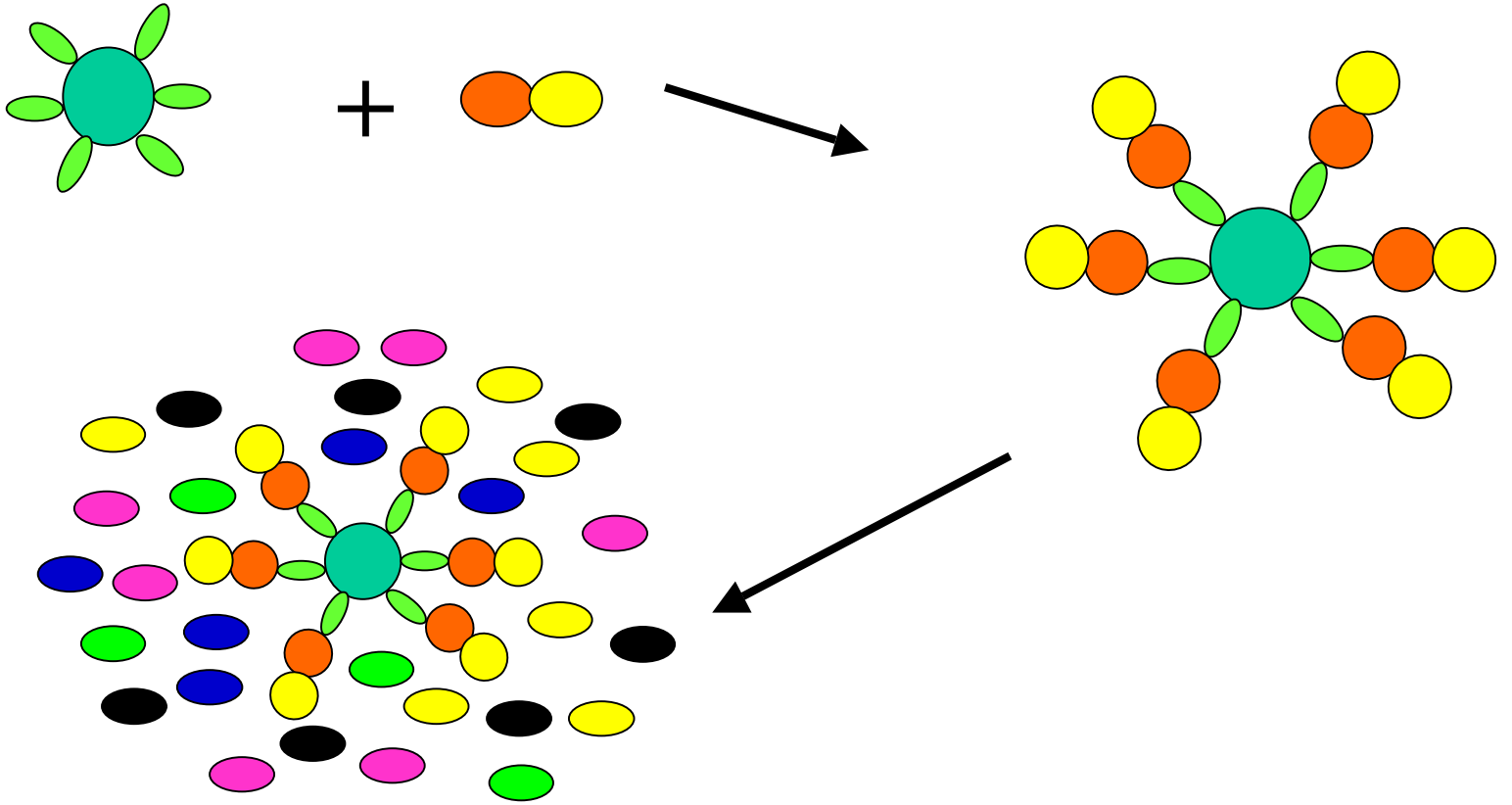


PULL-DOWN

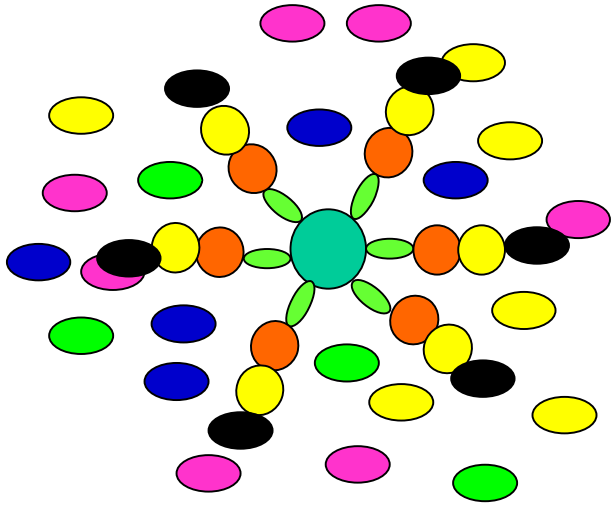
sferette di sepharose- glutatione

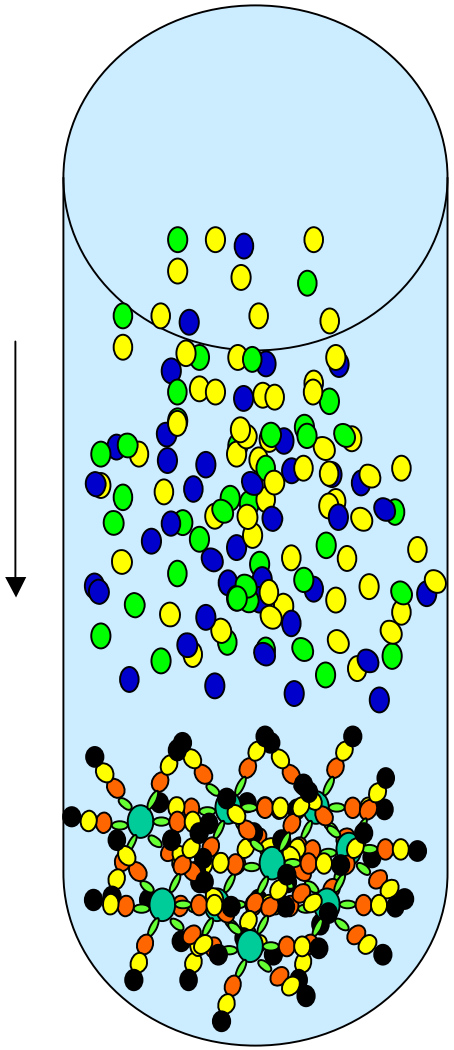
\+

GLUTATIONE TRANSFERASI | PROTEINA ESCA



• metto in agitazione (rotazione) a 4°C 2h o O/N affinché la proteina “esca”, (fusa con la glutatione transferasi) vada in contatto con tutte le proteine presenti nell’estratto





• centrifugo a bassa velocità (3000 rpm, 1 min, 4°C). Sul fondo della provetta si depositano:sferette di sepharose-glutatione--glutationetransferasi-proteina esca; in soluzione restano:proteine non legate alla proteina “esca”

•procedo come per l’immunoprecipitazione

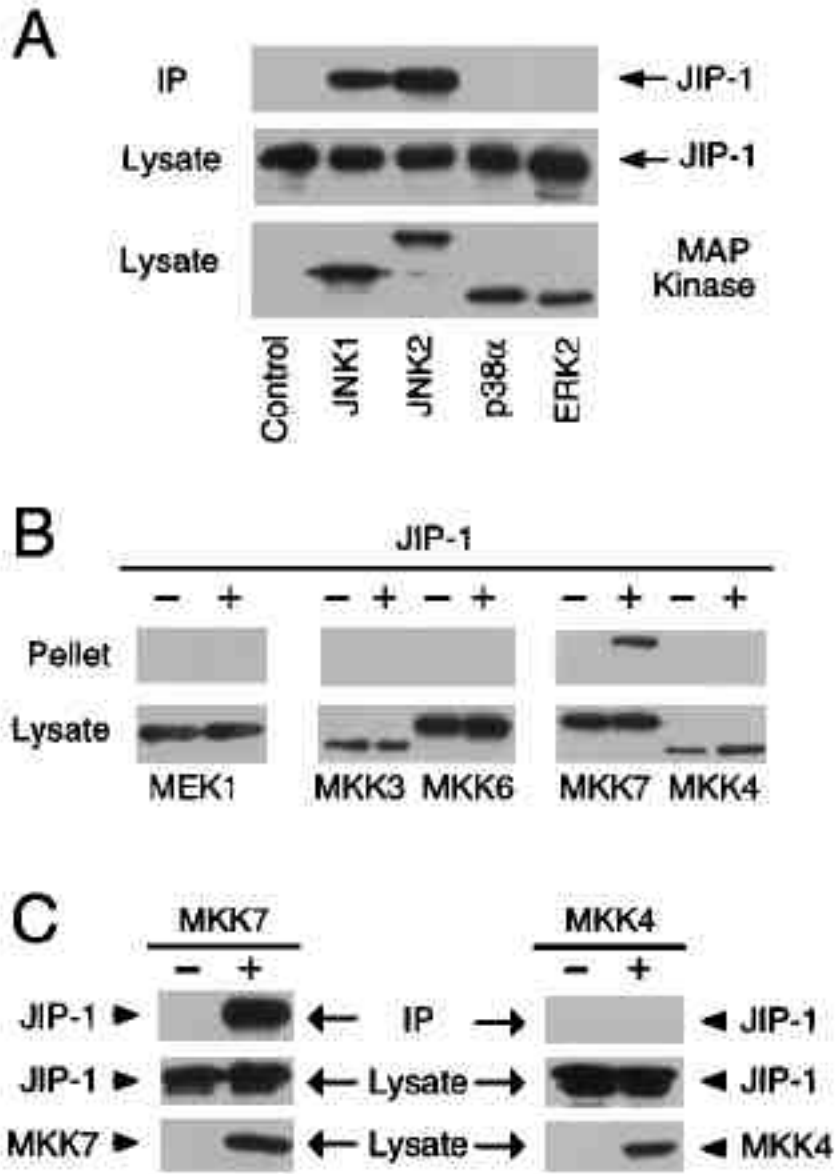


Fig. 1. Selective binding of JIP-1 to the MAP kinase JNK and the MAP kinase kinase MKK7. (**A**) Epitope-tagged JIP-1 (T7-Tag) was expressed in cells with the HA-tagged MAP kinases ERK2, p38α, JNK1, and JNK2 (*15*, *16*). The MAP kinases were immunoprecipitated with an antibody to HA. The presence of JIP-1 in the immunoprecipitates (IP) was detected on immunoblots probed with an antibody to T7-Tag. The amount of JIP-1 and MAP kinases in the cell lysates was examined by protein immunoblot analysis. (**B**) JIP-1 was expressed in cells as a GST fusion protein together with epitope-tagged MEK1, MKK3, MKK4, MKK6, or MKK7 (*15*, *16*). JIP-1 was precipitated from cell lysates with glutathione-agarose, and the MAPKKs present in the pellet were detected by protein immunoblot analysis. The amount of the MAPKKs in the cell lysates was examined by protein immunoblot analysis. (**C**) Epitope-tagged JIP-1 (T7-Tag) was expressed in cells with Flag-tagged MKK4 or MKK7 (*15*, *16*). The presence of JIP-1 in Flag IP was detected by protein immunoblot analysis with an antibody to T7-Tag. The amount of the MAPKKs in the cell lysates was examined by protein immunoblot analysis.

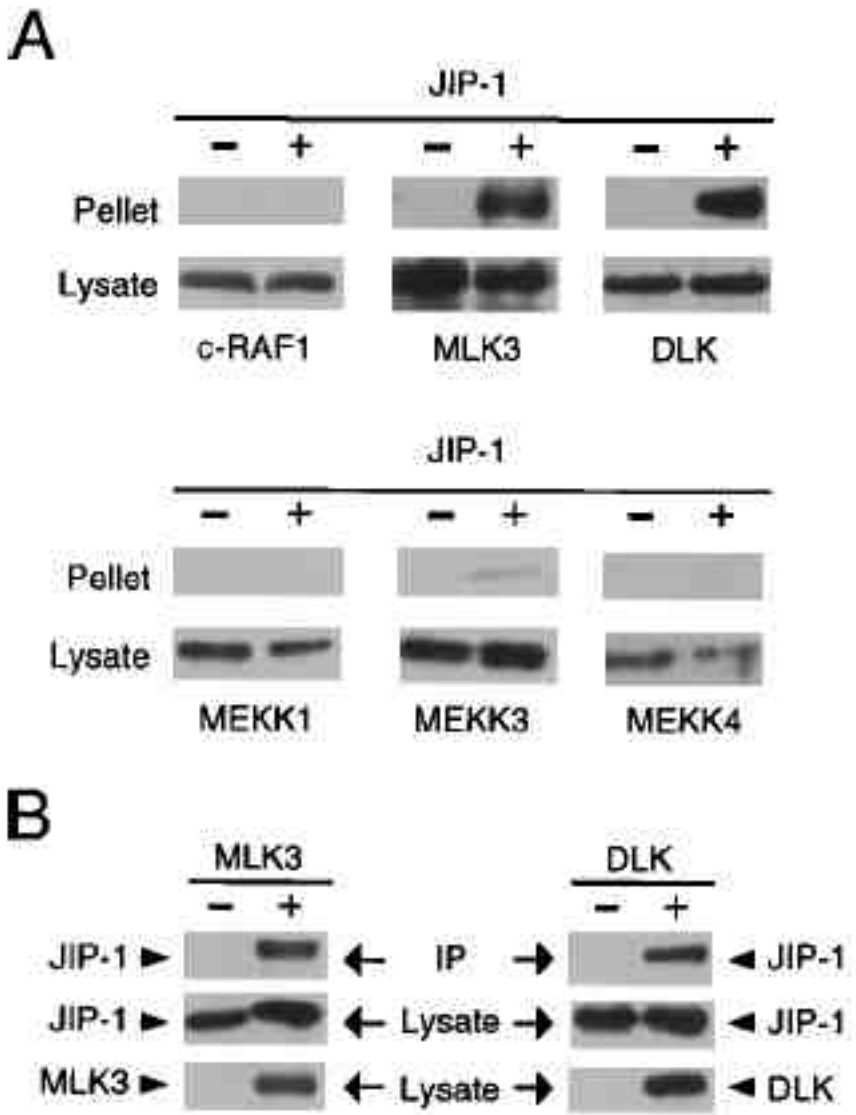
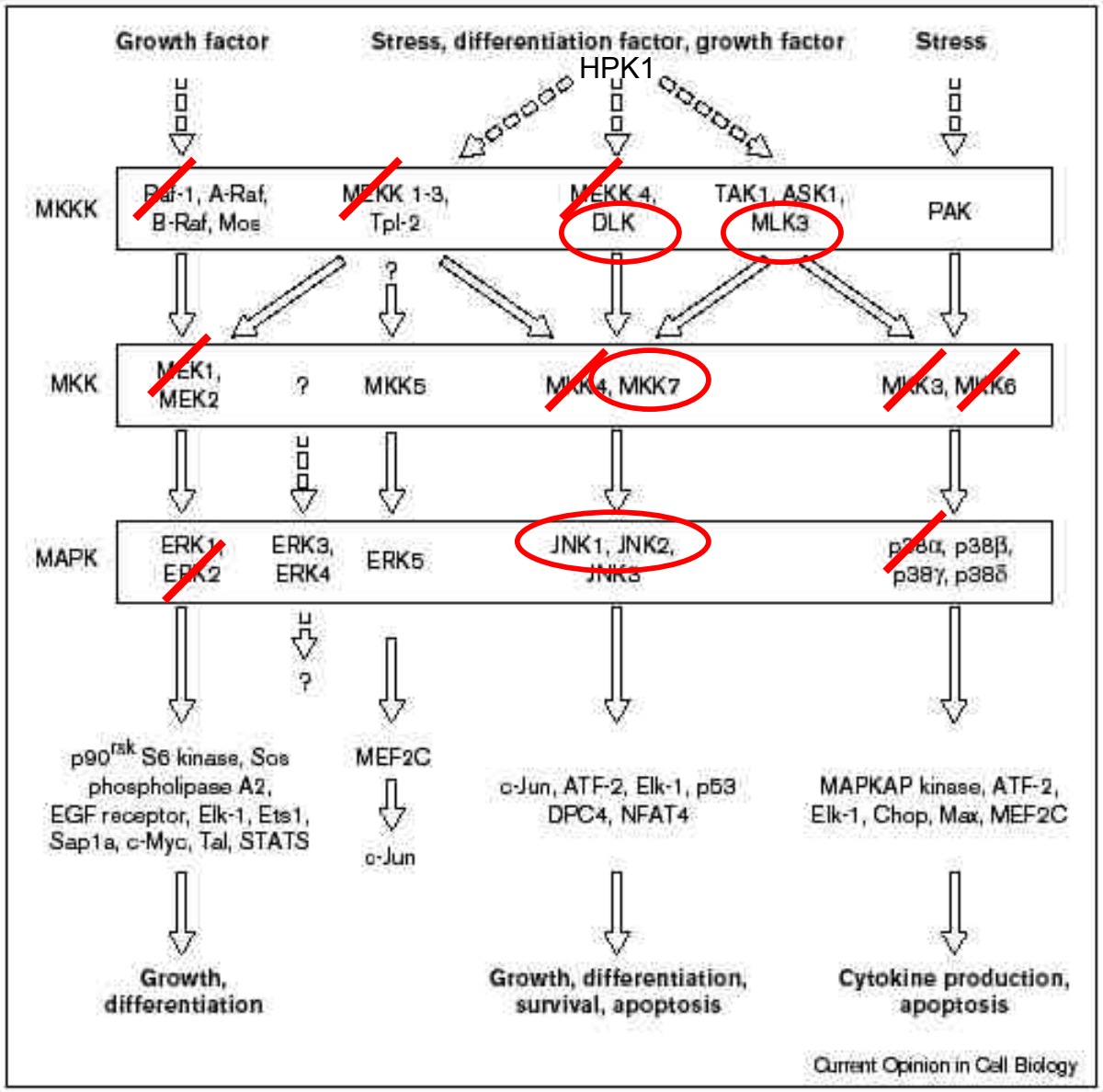
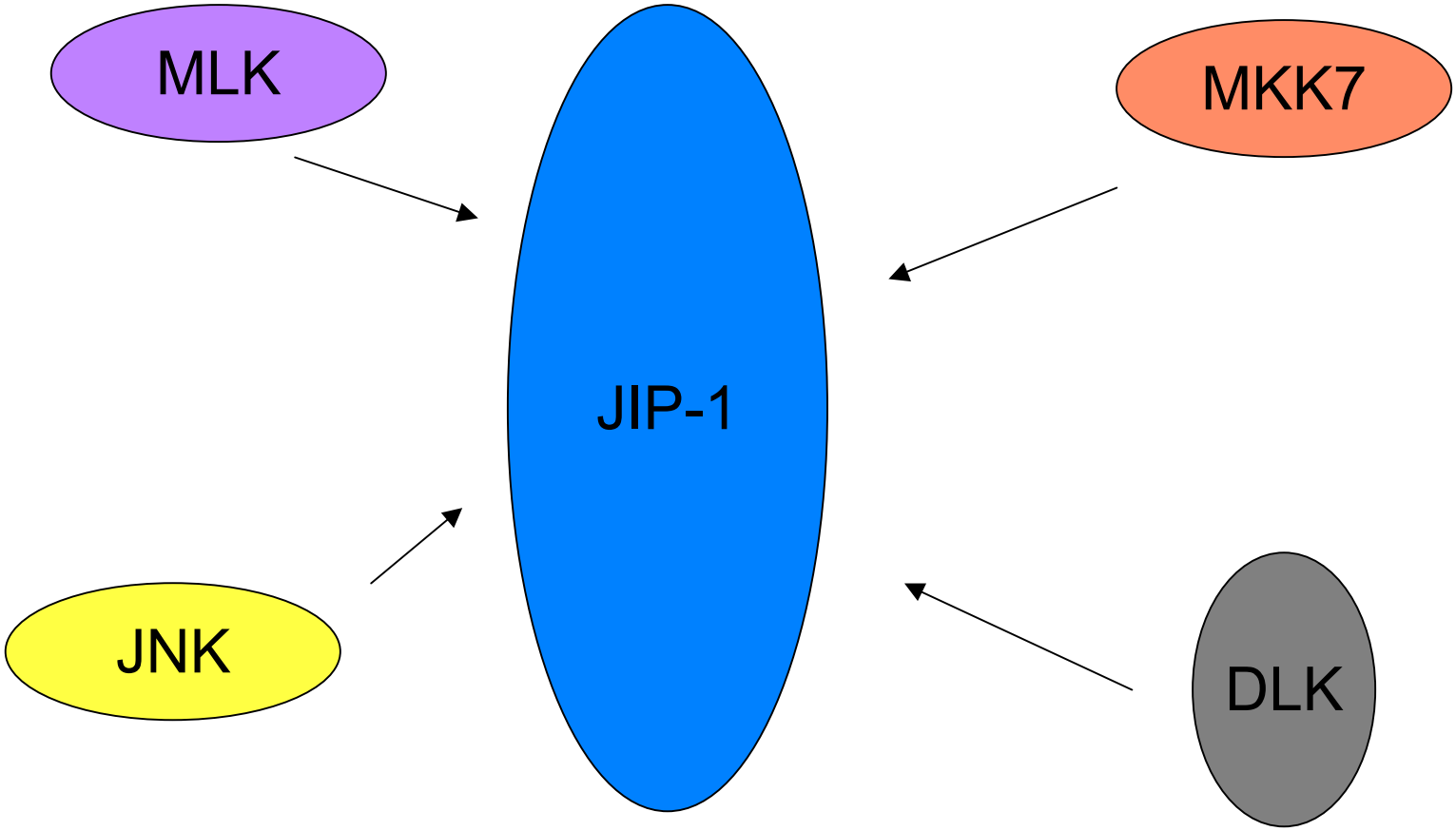


Fig. 2. Selective binding of JIP-1 to the mixed-lineage group of MAPKKKs. (**A**) JIP-1 was expressed in cells as a GST fusion protein together with the epitope-tagged MAPKKKs (*15, 16*). The presence of MAPKKKs in glutathione-agarose precipitates (pellet) was assayed by protein immunoblot analysis. The amount of the MAPKKKs in the cell lysates was examined by protein immunoblot analysis. (**B**) Epitope-tagged JIP-1 was coexpressed in cells with epitope-tagged MLK3 or DLK (*15, 16*). The presence of JIP-1 in the MLK3 and DLK immunoprecipitates (IP) was examined by protein immunoblot analysis. The amount of the MAPKKKs in the cell lysates was examined by protein immunoblot analysis.



Mitogen-activated protein kinase modules. The MAPK module consists of an MKKK, an MKK, and a MAPK. MKKKs respond to a variety of extracellular signals, including growth factors, differentiation factors and stress. The activated MKKKs can then activate one or several MKKs. In contrast, the MKKs are relatively specific for their target MAPKs. Once activated, MAPKs can then phosphorylate transcription factors (for example ATF-2, Chop, c-Jun, c-Myc, DPC4, Elk-1, Ets1, Max, MEF2C, NFAT4, Sap1a, STATs, Tal, p53), other kinases (MAPKAP kinase, p90^{rsk} S6 kinase), upstream regulators (EGF receptor, son of sevenless [Sos] Ras exchange factor), and other regulatory enzymes such as phospholipase A2. These downstream targets then control cellular responses including growth, differentiation, and apoptosis [1••,2,3].



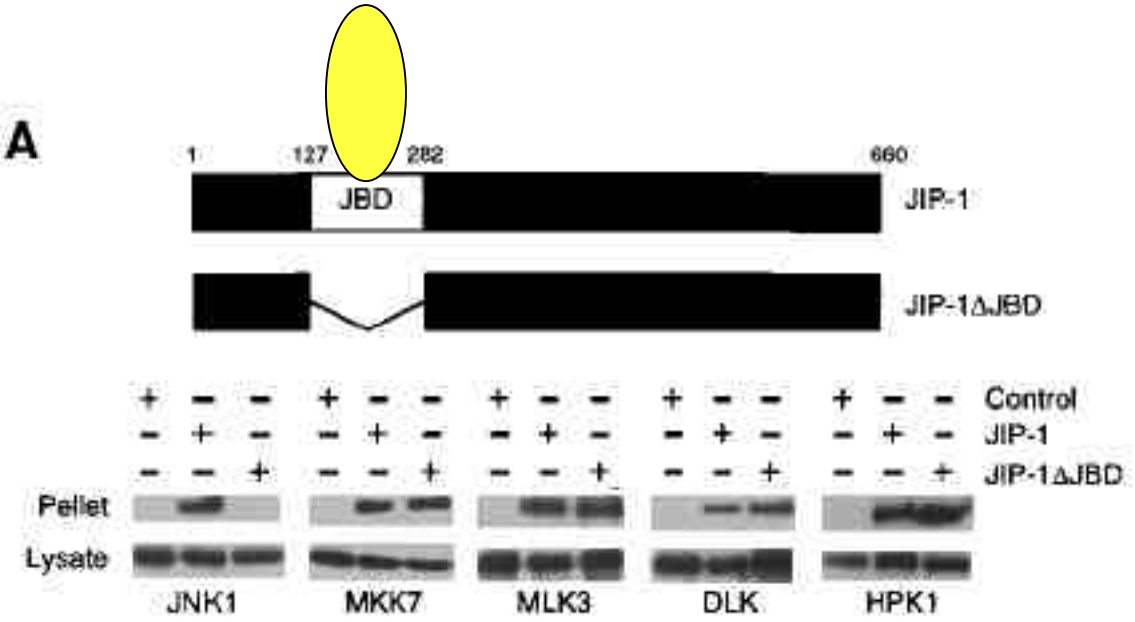
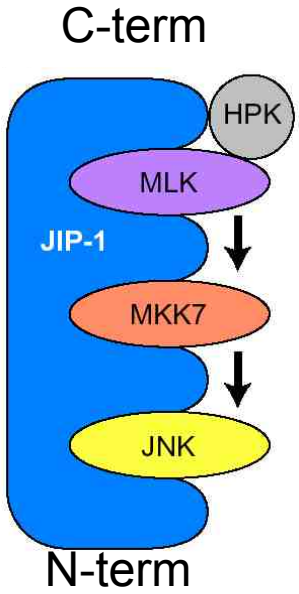
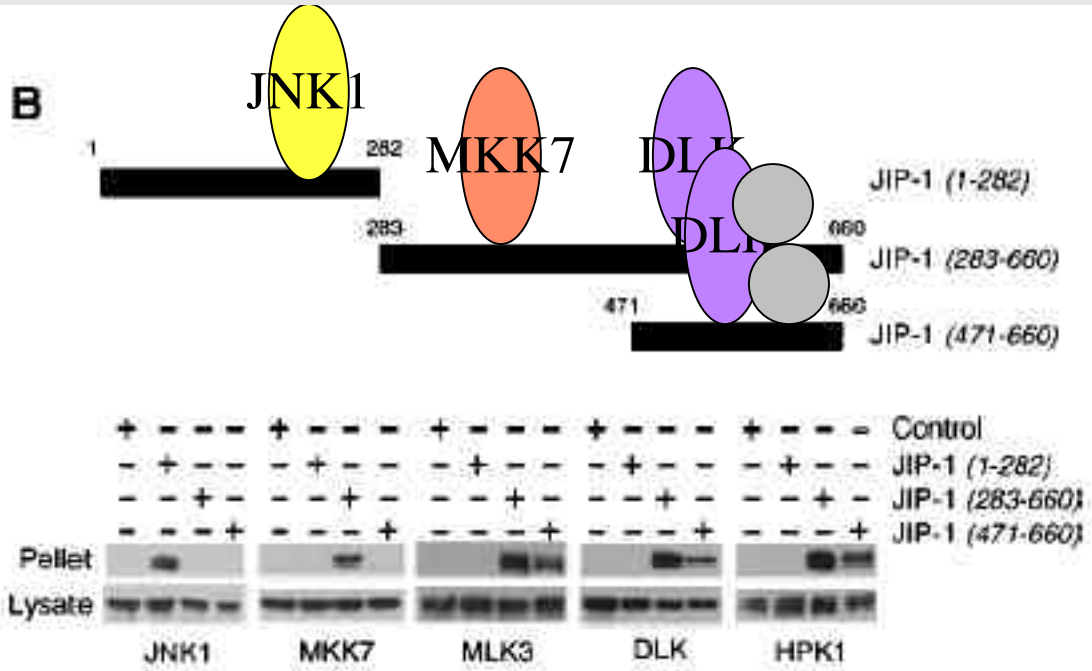


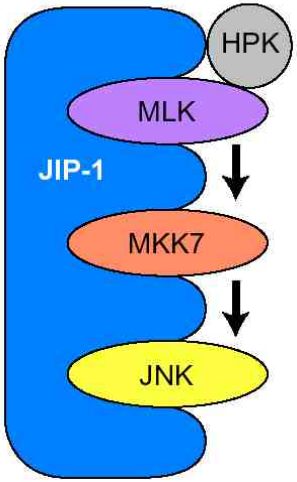
Fig. 3. Binding of JIP-1 to MKK7, MLK, and HPK1 independently of JNK. (**A**) The binding of JIP-1 to HPK1, MLK3, DLK, and MKK7 is independent of JNK. GST (Control), GST-tagged JIP-1, and a GST-tagged JIP-1 mutant with an in-frame deletion of the JNK binding domain (JIP-1ΔJBD) were coexpressed in cells together with HPK1 and epitope-tagged JNK1, MKK7, MLK3, and DLK (*15*, *16*). The presence of kinases in the reduced glutathione-agarose precipitates (pellets) and cell lysates was examined by protein immunoblot analysis. (**B**) Deletion analysis of the

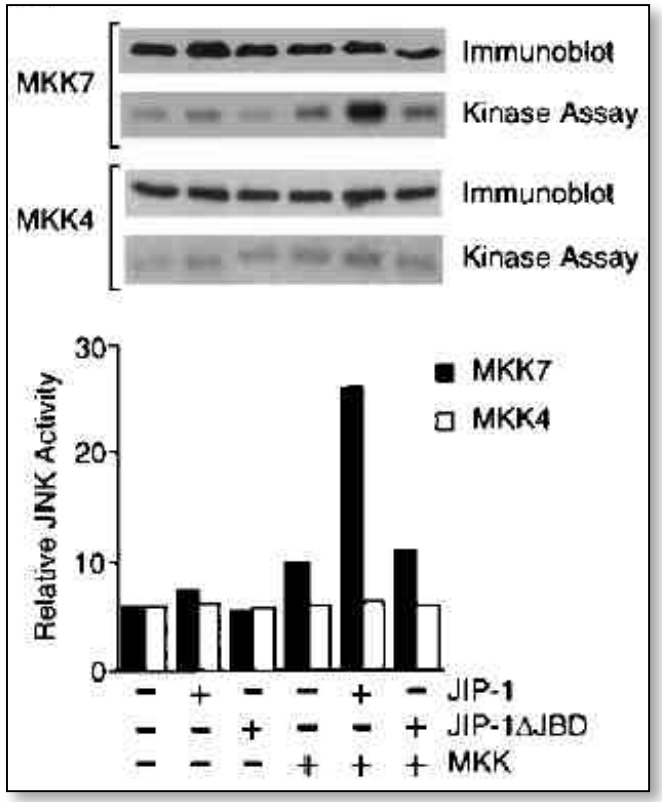




(**B**) Deletion analysis of the binding of JIP-1 to JNK1, MKK7, MLK3, and DLK. JIP-1 was expressed in cells as a GST fusion protein together with HPK1 or epitope-tagged JNK1, MKK7, MLK3, and DLK (*15*, *16*). The presence of these kinases in glutathione-agarose precipitates was examined by protein immunoblot analysis. (**C**) The subcellular distribution of MKK7 and MKK4 was examined in

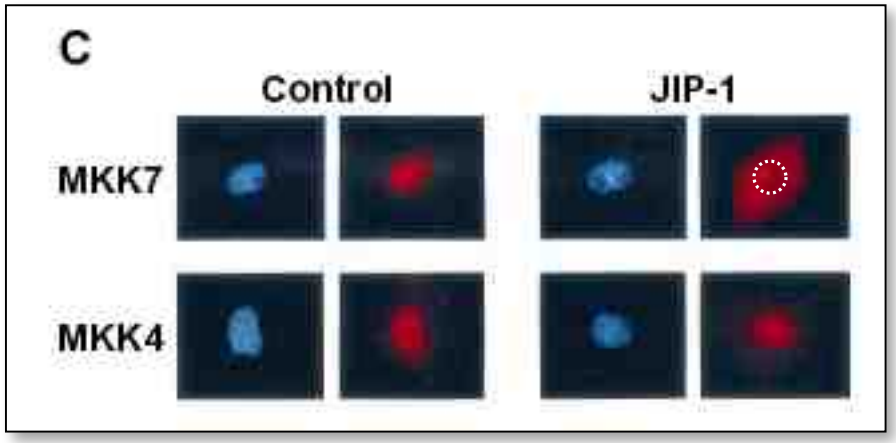
JIP1 enhances the activation of JNK by MKK7 and MLK



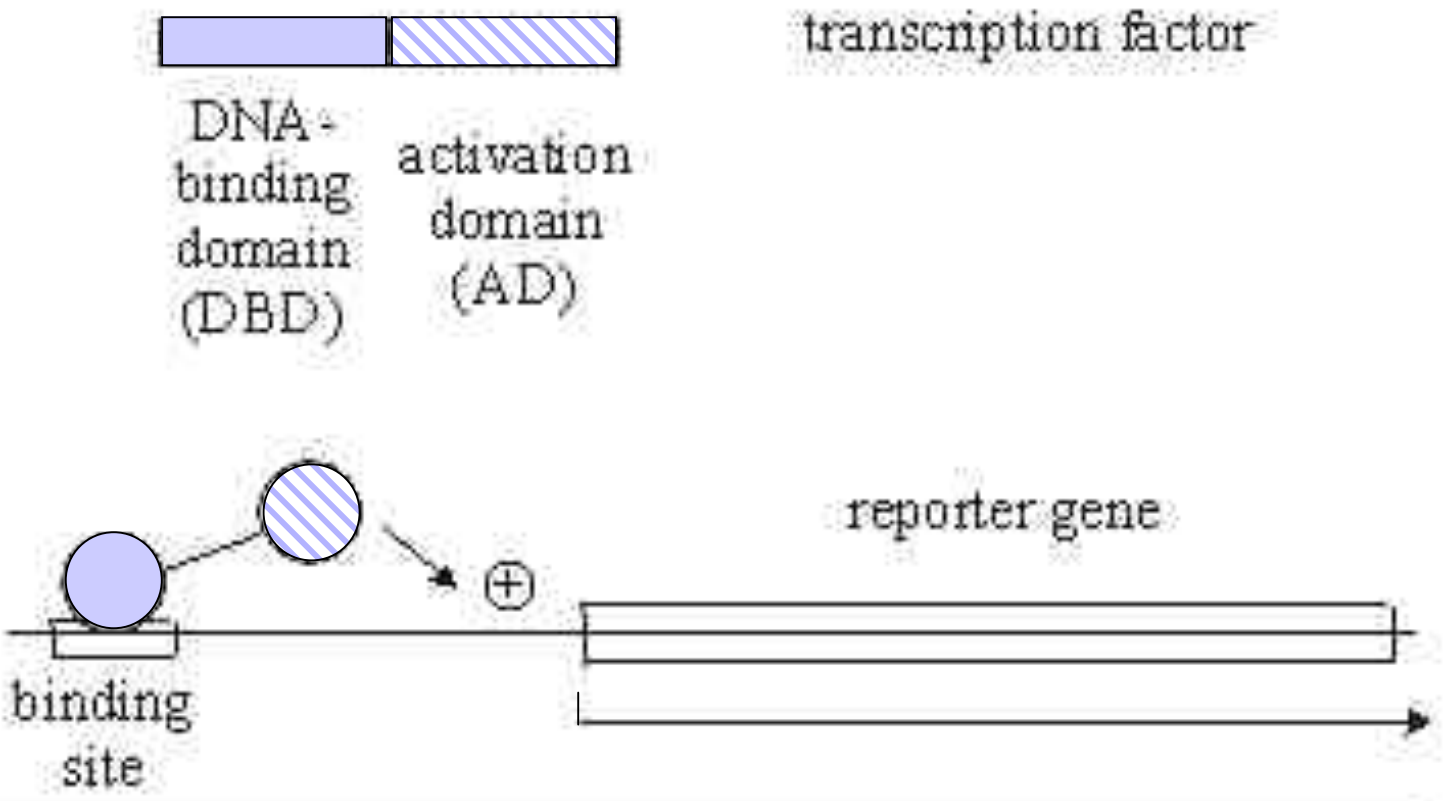


JIP=JNK inhibiting protein, why?

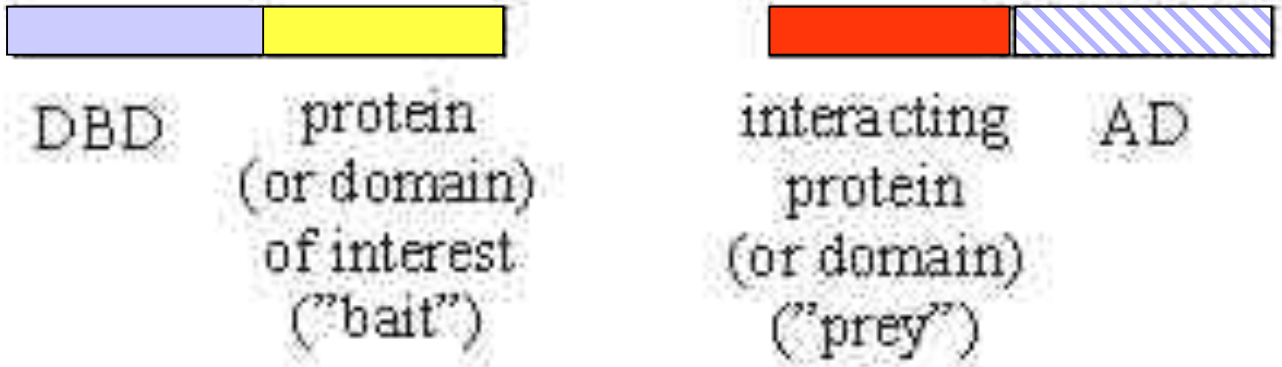
Overexpression of JIP1 inhibits JNK traslocation to the nucleus and in vivo activity

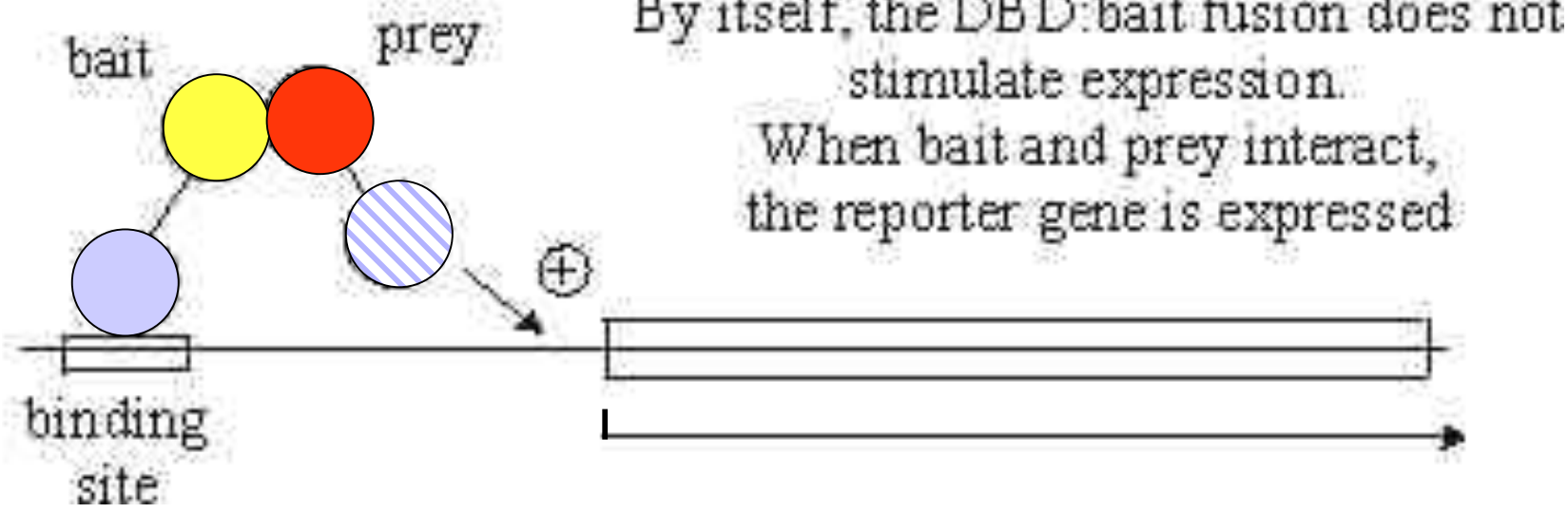


Structure-function properties of a typical **transcription factor**

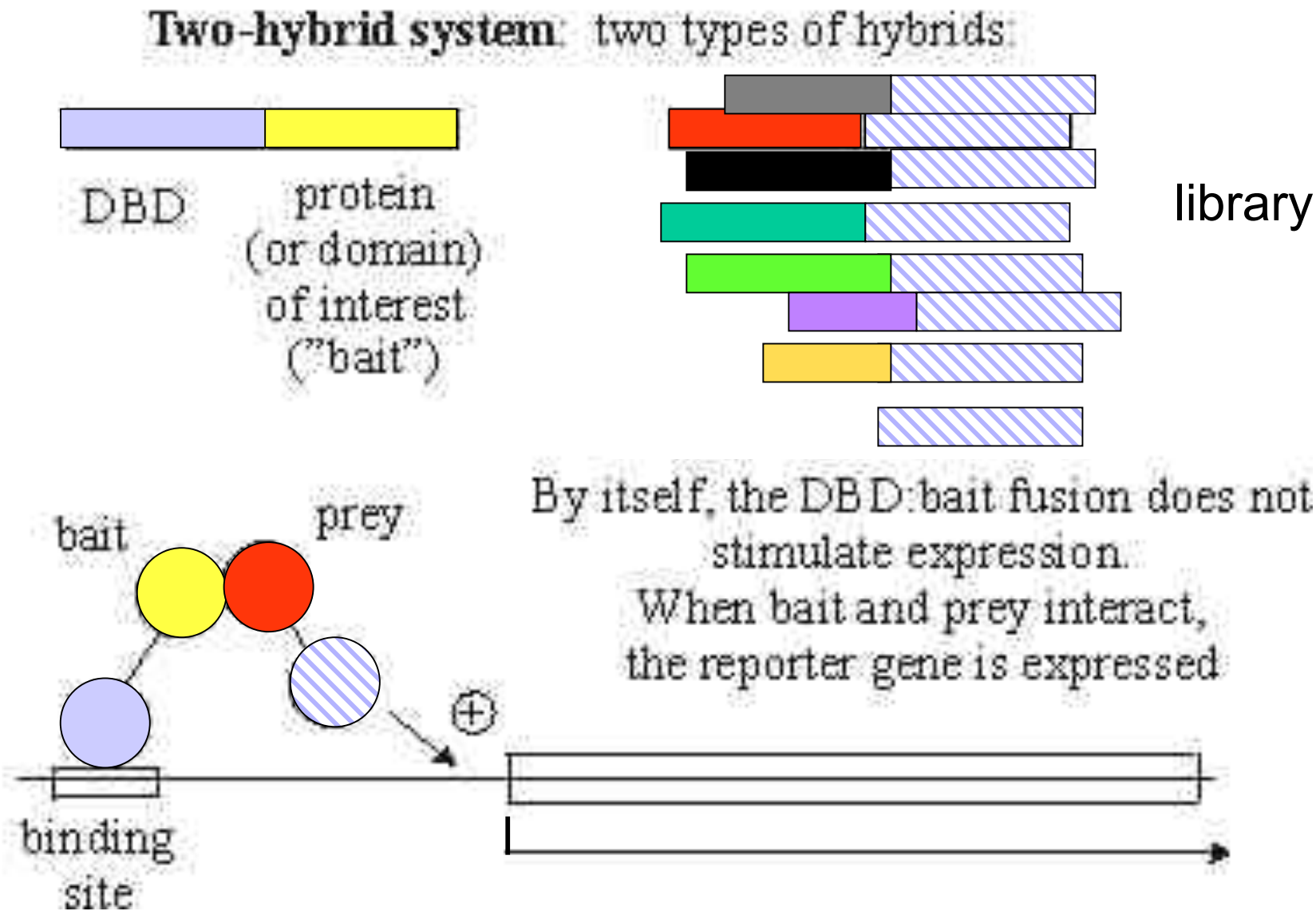


Two-hybrid system: two types of hybrids:





By itself, the DBD:bait fusion does not stimulate expression.
When bait and prey interact, the reporter gene is expressed



Wikipedia