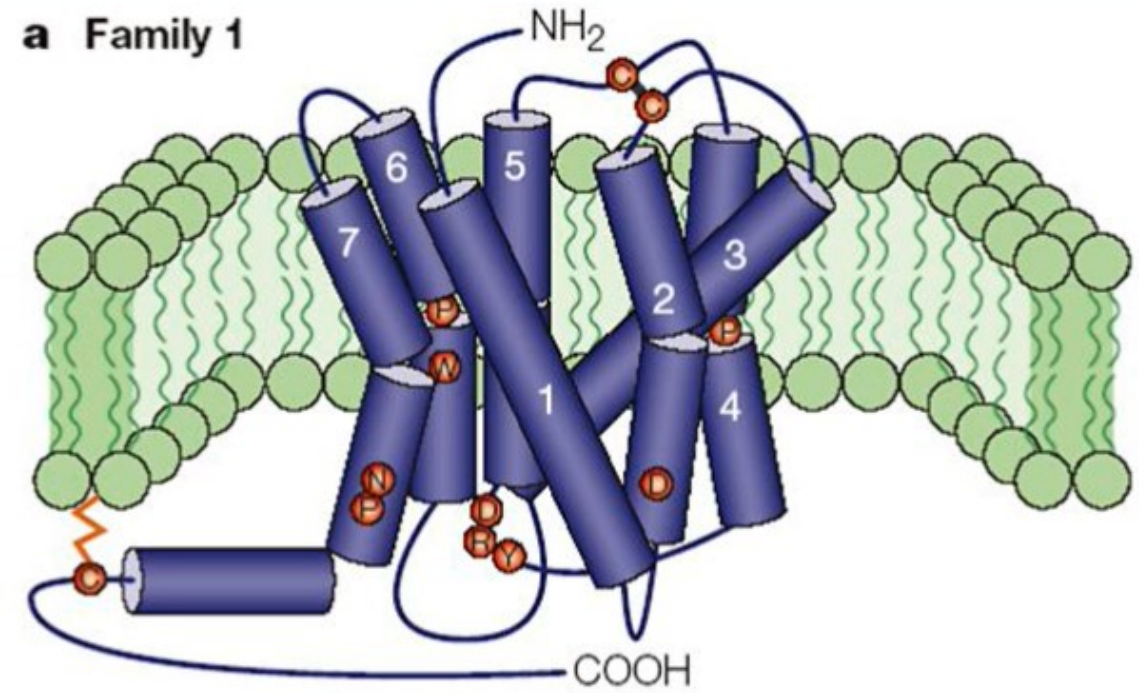
# Trasduzione del segnale:

-canali ionici controllati

-recettori accoppiati alle proteine G (GPCRs)

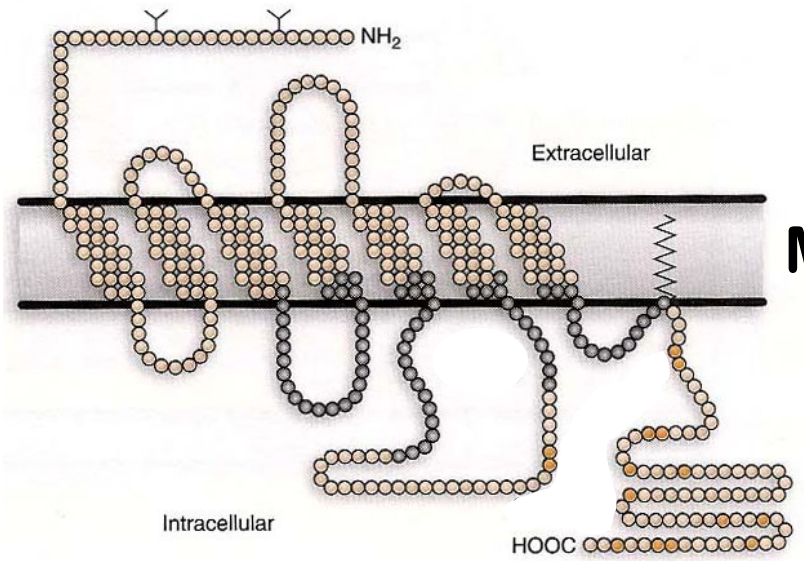
Michele Sallese

# GPCR signalling cascades



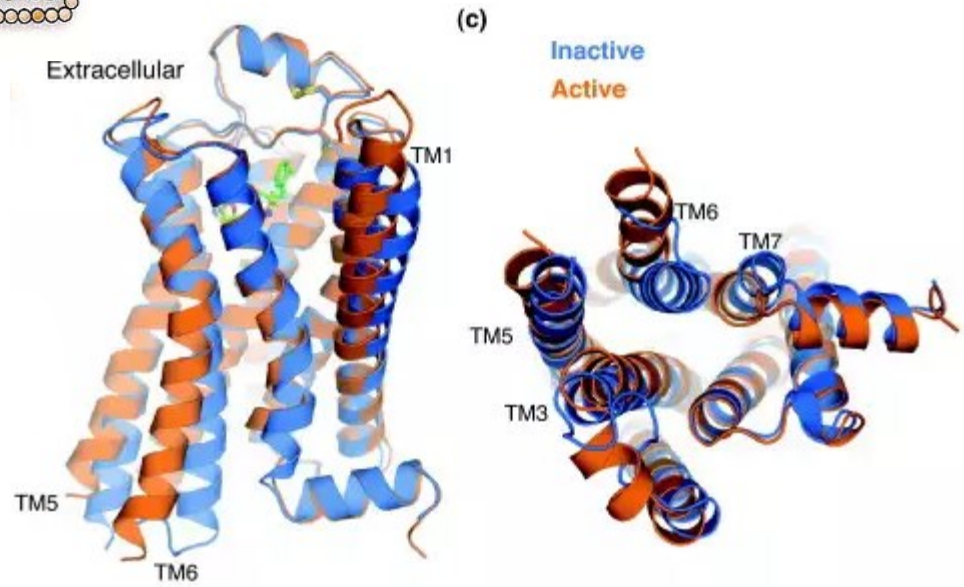

- Represent the predominant class of GPCRs;
- Several highly conserved amino acid (red circles);
- Disulfide bridge between first and second extracellular loops (ECLs), palmitoylated cys in C-tail;
- The binding of small molecule ligands occures within the TM region;
- Example: Rhodopsin, Dopamine receptor, Chemokine receptors;

# β2-Adrenergic receptor

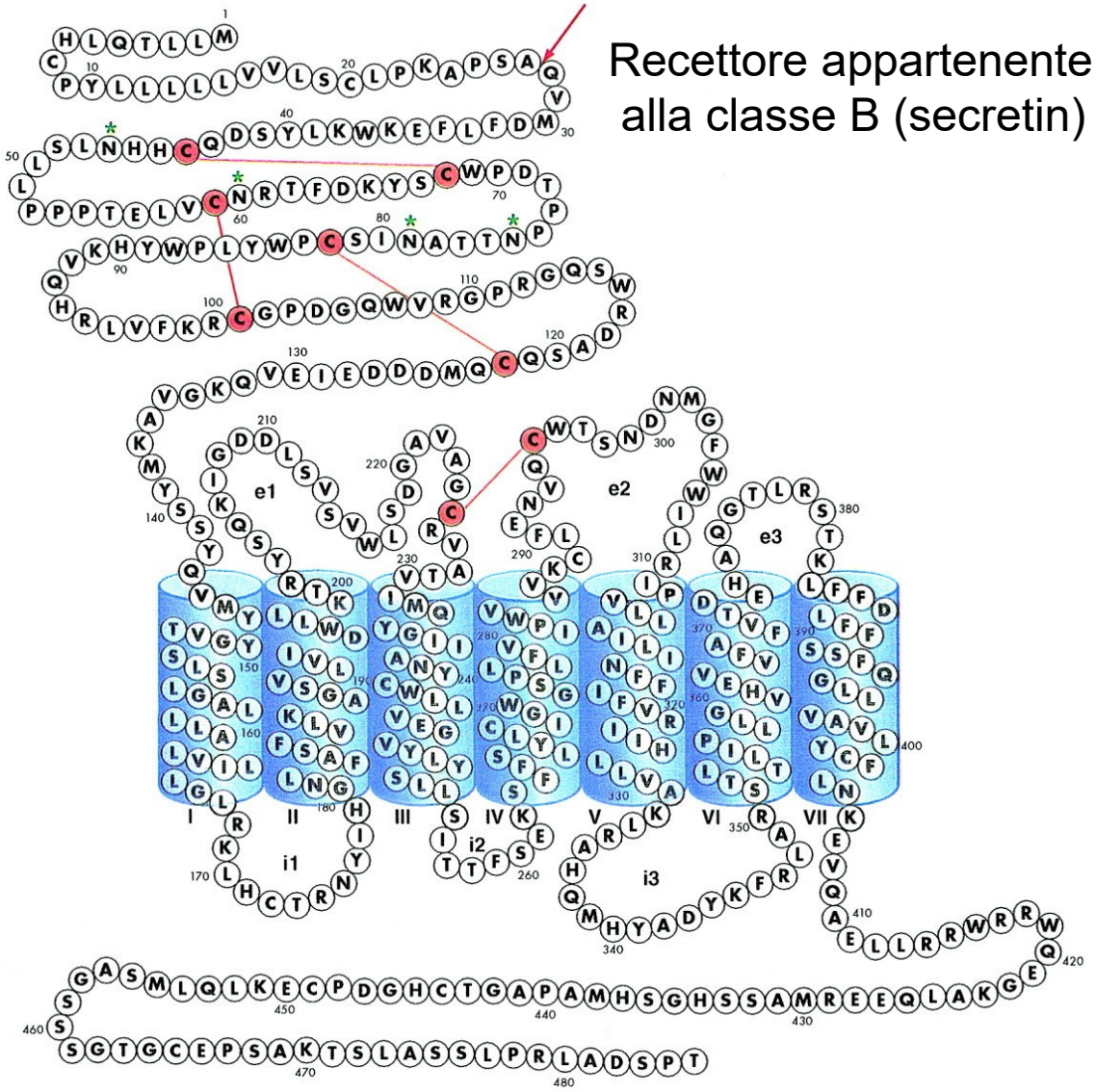



**Membrana plasmatica**

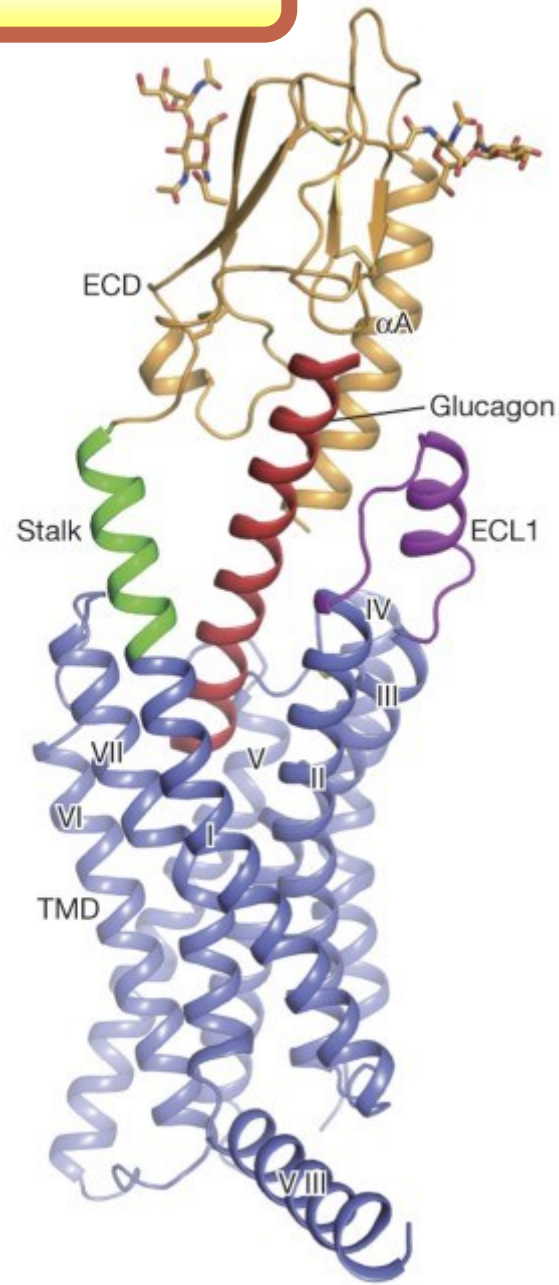
**413 amminoacidi**

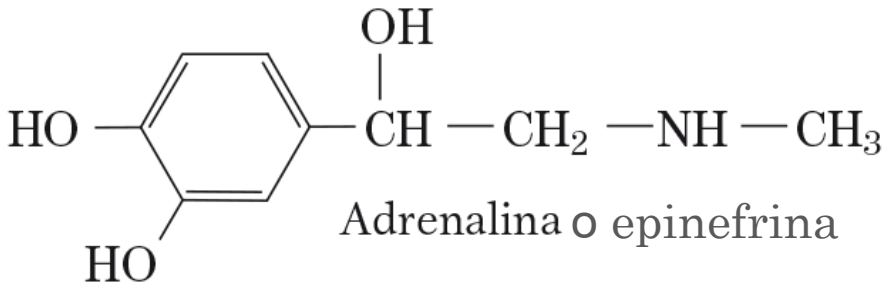


# Recettore per il glucagone




Recettore appartenente alla classe B (secretin)

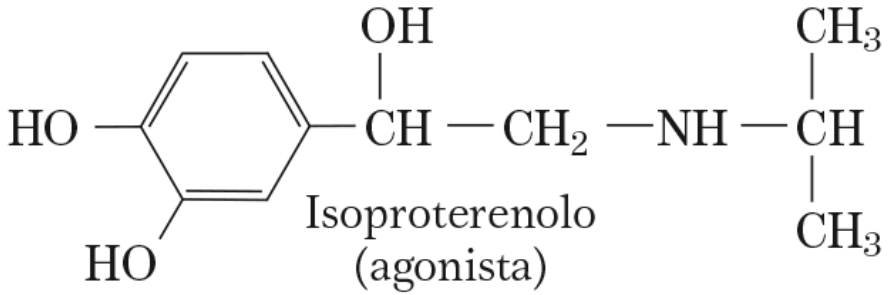





Adrenalina o epinefrina

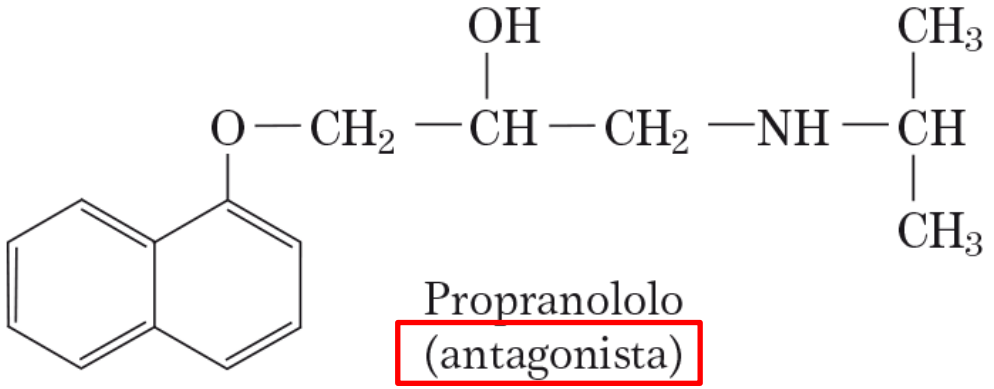
$k_d$ (μM)

5




Isoproterenolo
(agonista)

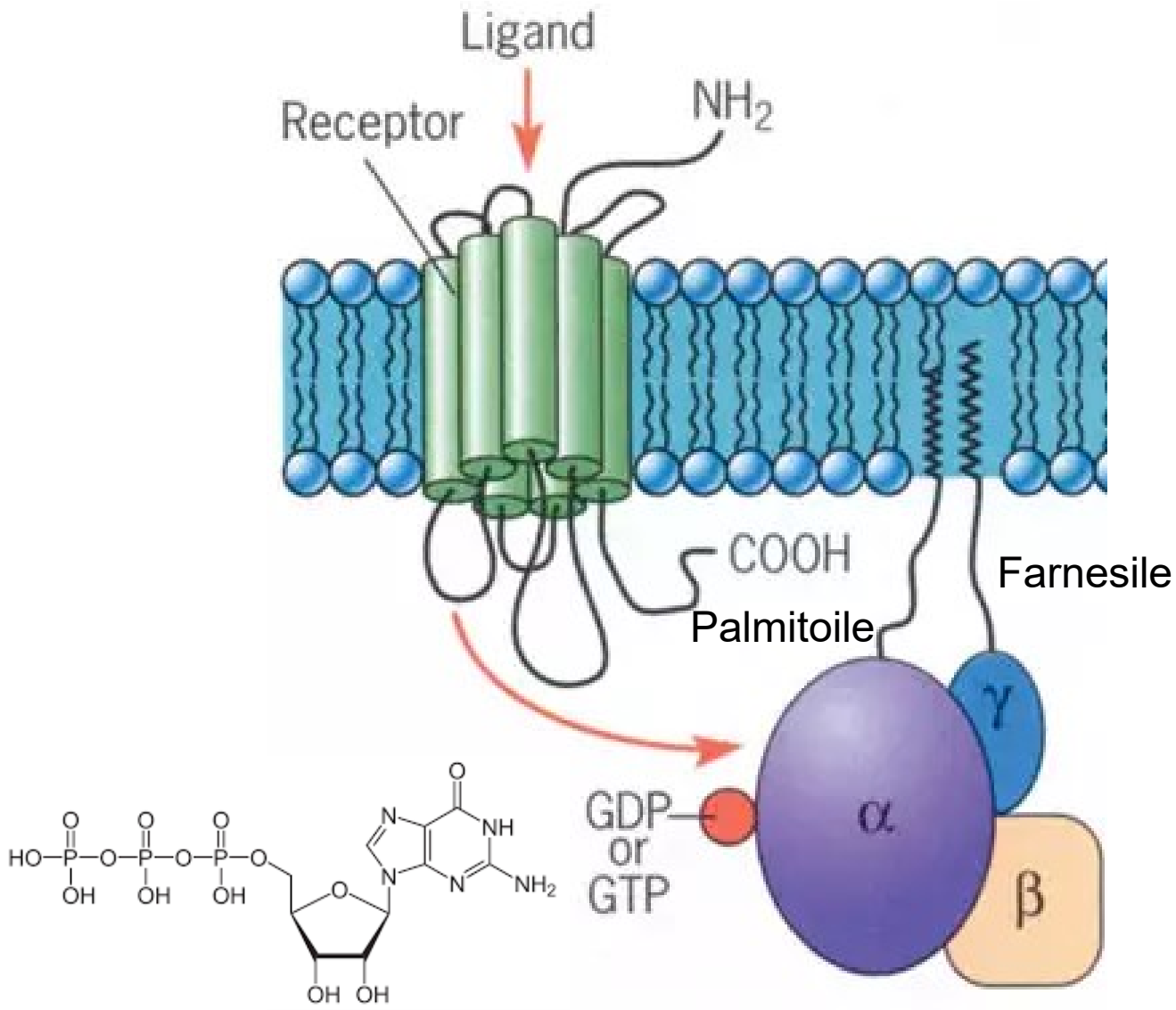
0,4




Propranololo
(antagonista)

0,0046

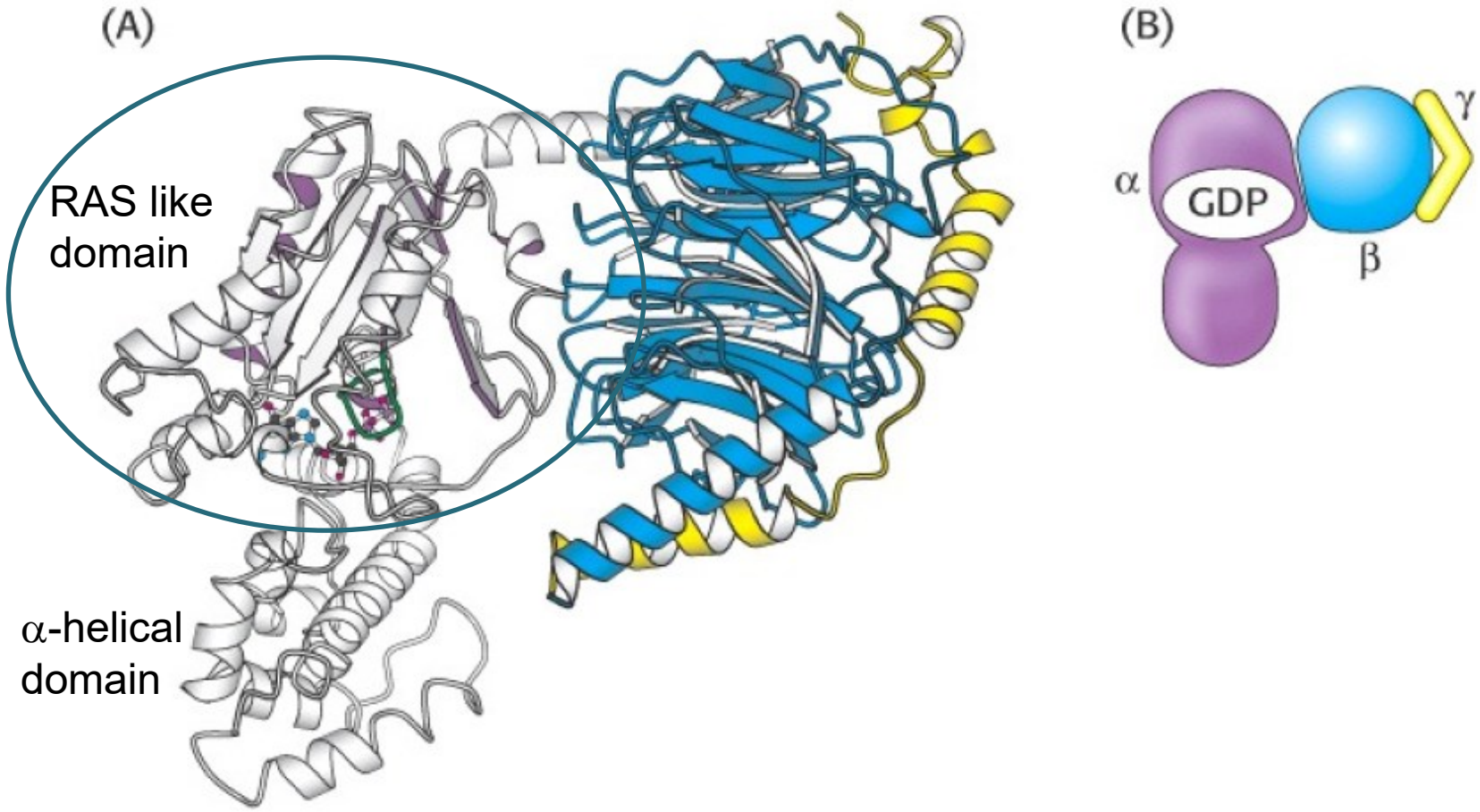
# Trasduzione del segnale βAR





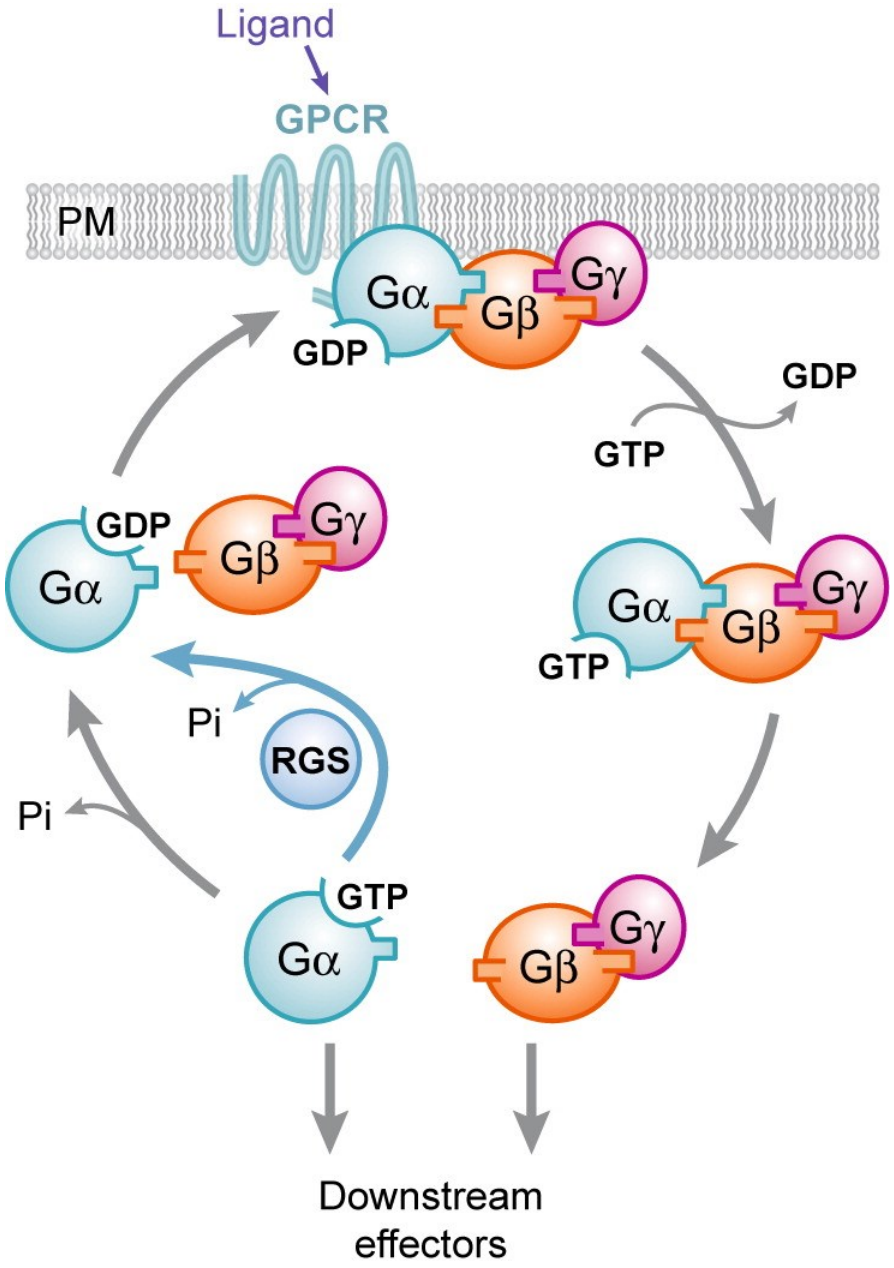

Guanosina trifosfato

# Struttura della proteina G

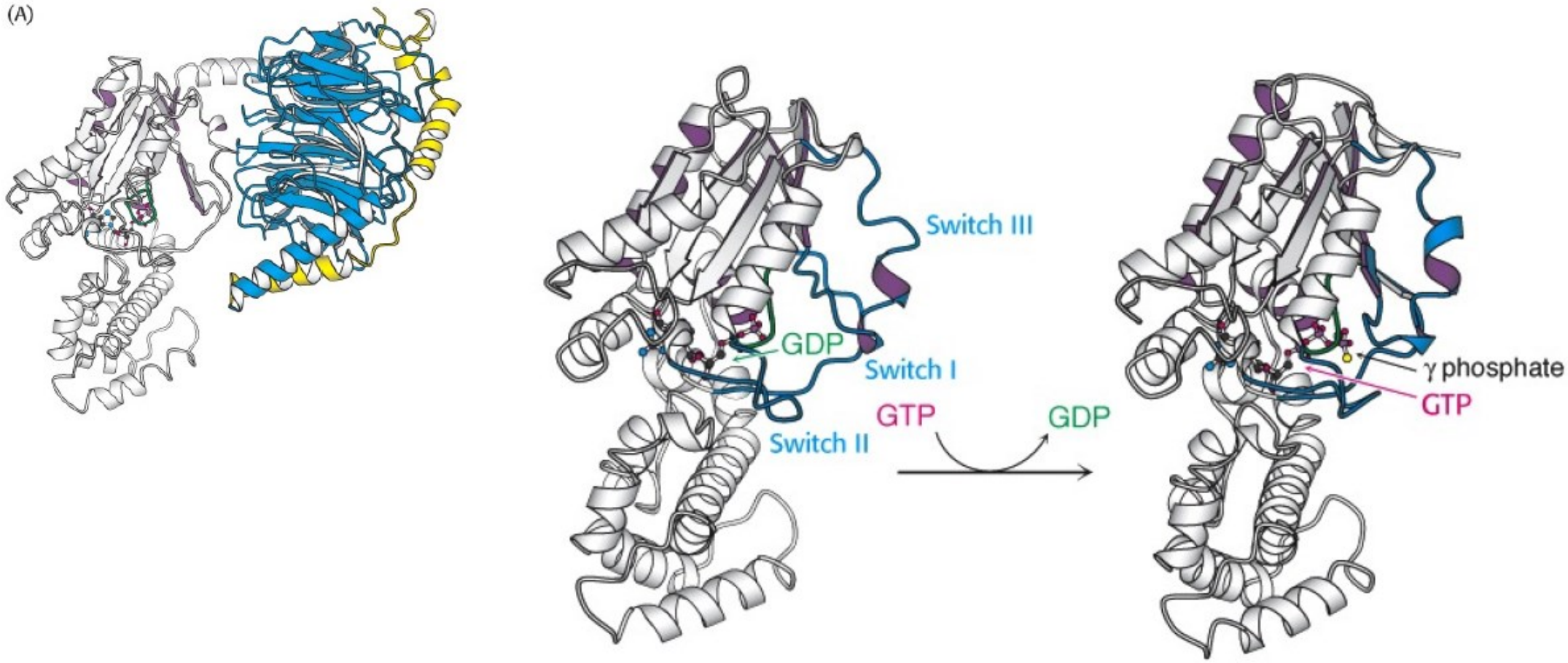



Le proteine G accoppiate ai recettori metabotropici sono **trimeri**, formati dalle subunità α (21 isoforme), β (6 isoforme) e γ (12 isoforme).

# G protein cycle



# Regioni della proteina G modificate durante l'attivazione/inattivazione

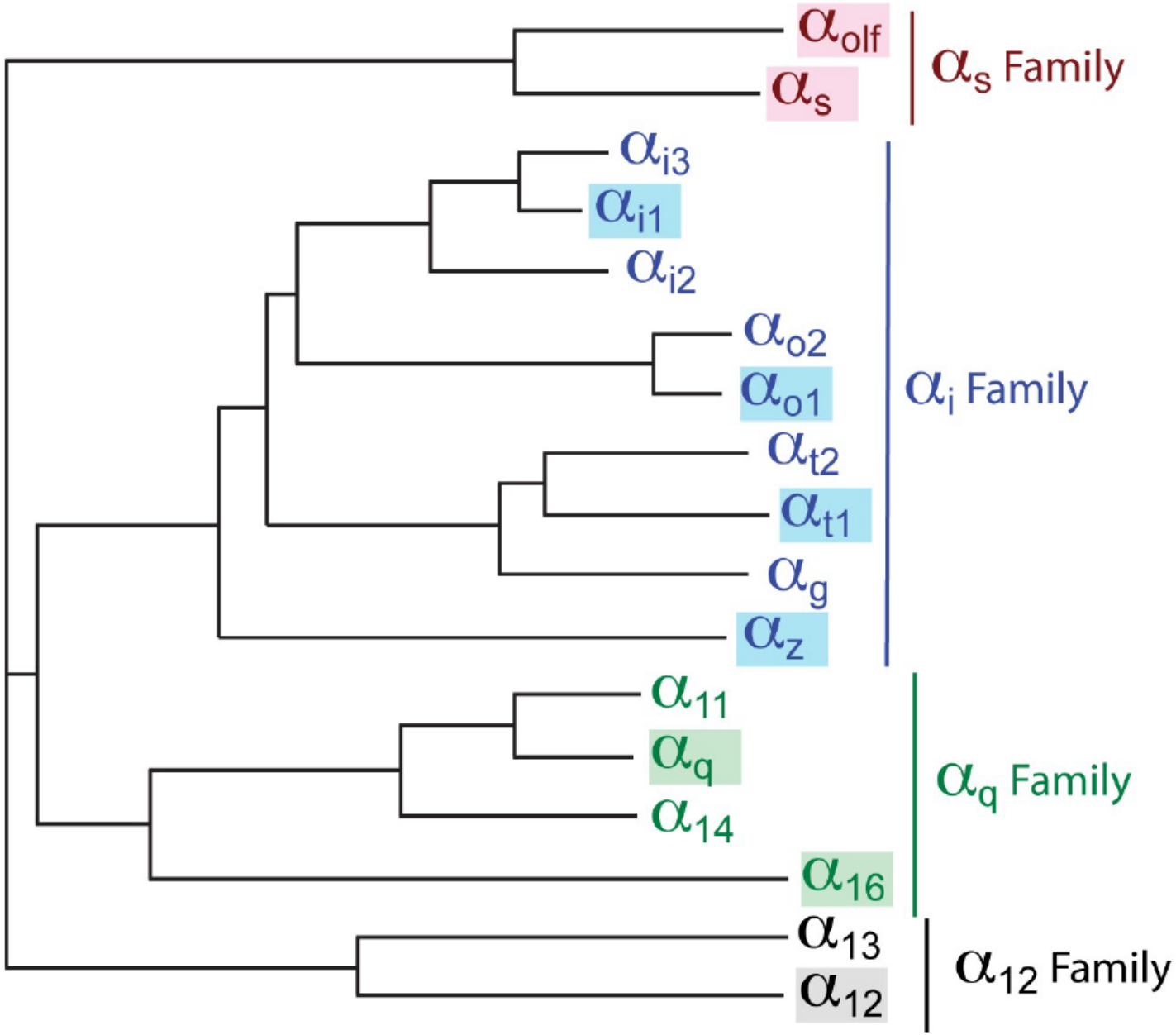


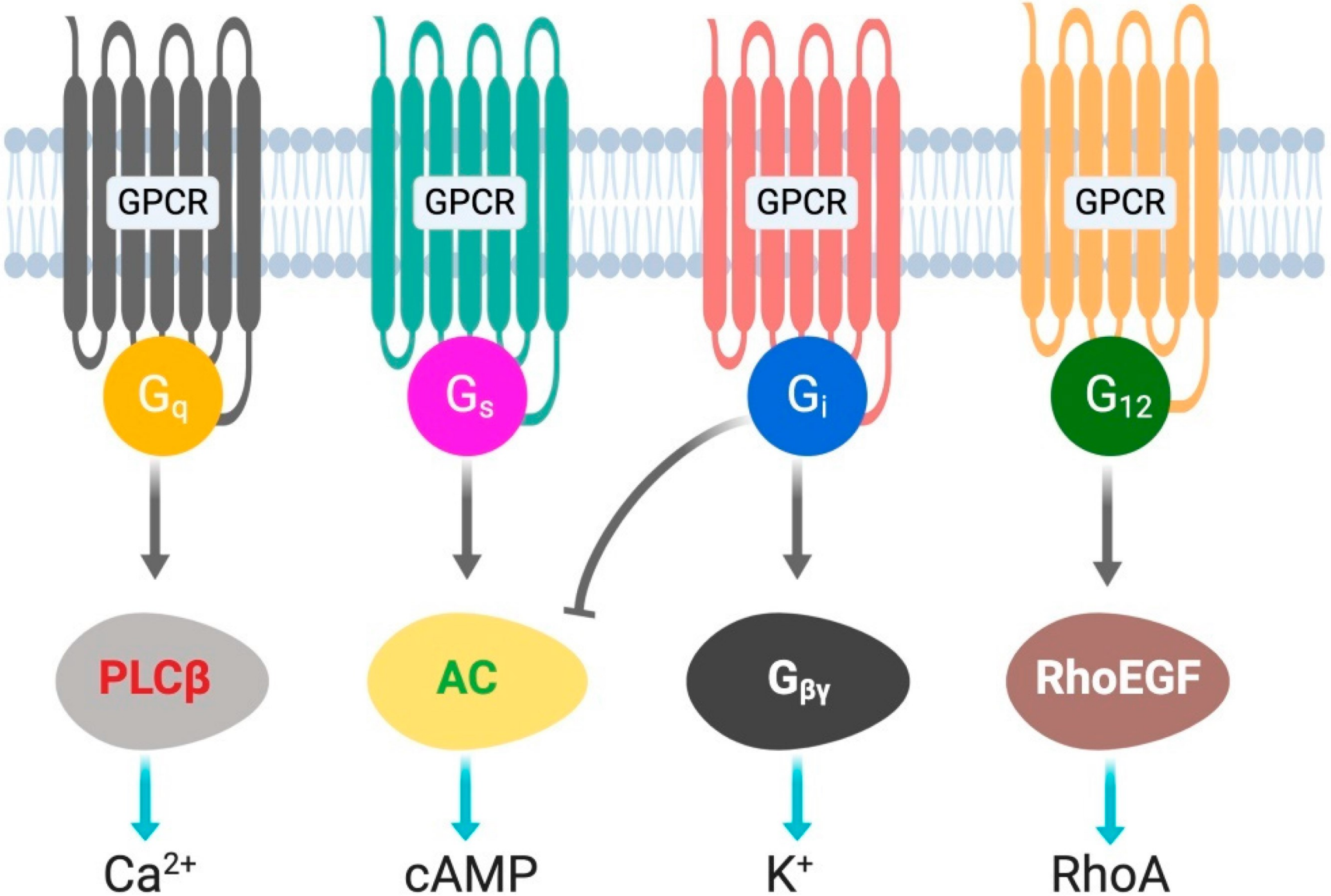

L'attivazione della proteina G consiste nella sostituzione di GDP con GTP che porta alla dissociazione del dimero βγ dalla subunità α-GTP.

# Trimeric G-proteins

| SUBUNIT | SUB-TYPES | EXPRESSION (CELLS) | PRIMARY EFFECT | OTHER EFFECTS | RECEPTORS | TOXIN |
|---|---|---|---|---|---|---|
| **$G_s$ - family** | | | | | | |
| $\alpha_{s}$ - generic form | 4 | ubiquitous, including neurons | ↑ AC (subtypes 1-10) | ↑ cardiac $Ca^{2+}$ channels<br>↑ $K_{Ca}$ channels<br>↓ $Na^{+}$ channels | NE, EPI, histamine, LH, FSH | CTX |
| $\alpha_{olf}$ | 1 | olfactory epithelium; some neuronal ganglia | ↑ AC (subtypes 1-6) | | odorant chemicals | CTX |
| **$G_i$ - family** | | | | | | |
| $\alpha_{o}$ | ≥ 2 | neurons; neuroendocrine cells; astroglia; cardiac muscle | ↑ PLC-β | ↓ AC<br>↓ L- & N-type $Ca^{2+}$ channels<br>↑ neuronal $K^{+}$ channels | ACh, NE, opiates, All, many peptides | PTX |
| $\alpha_{i}$ | 13 | ubiquitous, including neurons | ↓ AC (subtypes 1-10) | ↑ $K_{ATP}$ channels<br>↑ $K_{+}$ channels<br>↓ L- & N-type $Ca^{2+}$ channels | same as above | PTX |
| $\alpha_{z}$ | ≥ 1 | platelets; adrenals | ↓ AC | ↑ PLC | same as above | none |
| **$G_q$ - family** | | | | | | |
| $\alpha_{q}$ | ≥ 5 | ubiquitous | ↑ PLC-β | | ACh, NE | none |
| $\alpha_{11,14}$ | ? | ubiquitous | ↑ PLC-β | | ACh, NE | none |
| **Unrelated?** | | | | | | |
| $\alpha_{t}$ (transducin) | ≥ 2 | outer segments of retinal rods & cones | ↑ γPDE (cGMP-PDE ) | | light | CTX, PTX |
| $\alpha_{gust}$ (gustducin) | ≥ 2 | taste bud receptors | ↑ PLC-β | ↑ cAMP-PDE | chemical stimuli | CTX, PTX |
| $\alpha_{12,13}$ | ≥ 2 | ubiquitous | ↑ GTPases | ↑ nucleotide exchange | mechanical stimuli | ? |

# Phylogenetic relationship of human Gα subunits

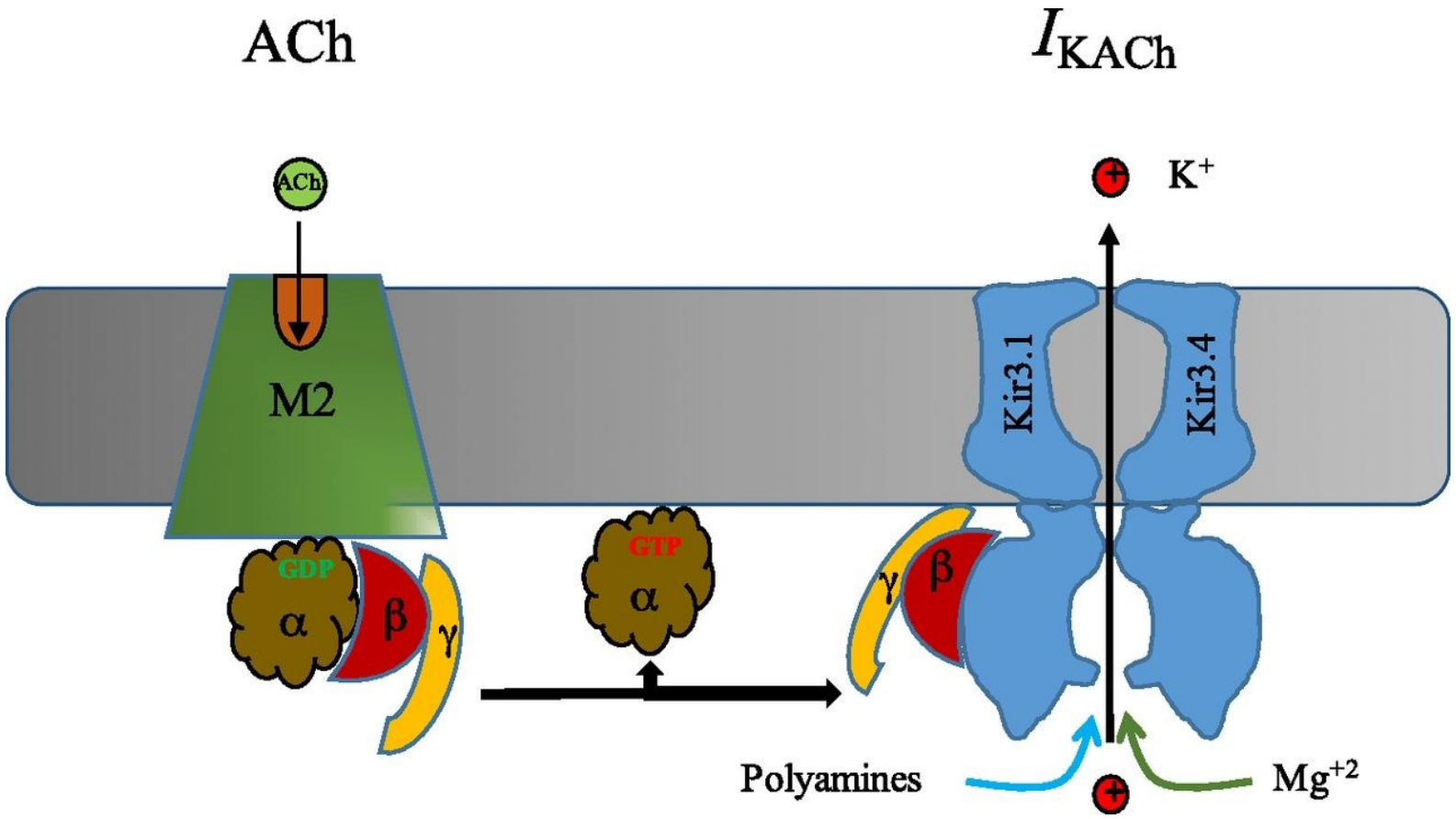



GPCR
GPCR
GPCR
GPCR
$G_q$
$G_s$
$G_i$
$G_{12}$
PLCβ
AC
$G_{\beta\gamma}$
RhoEGF
$Ca^{2+}$
cAMP
$K^+$
RhoA

# βγ effectors

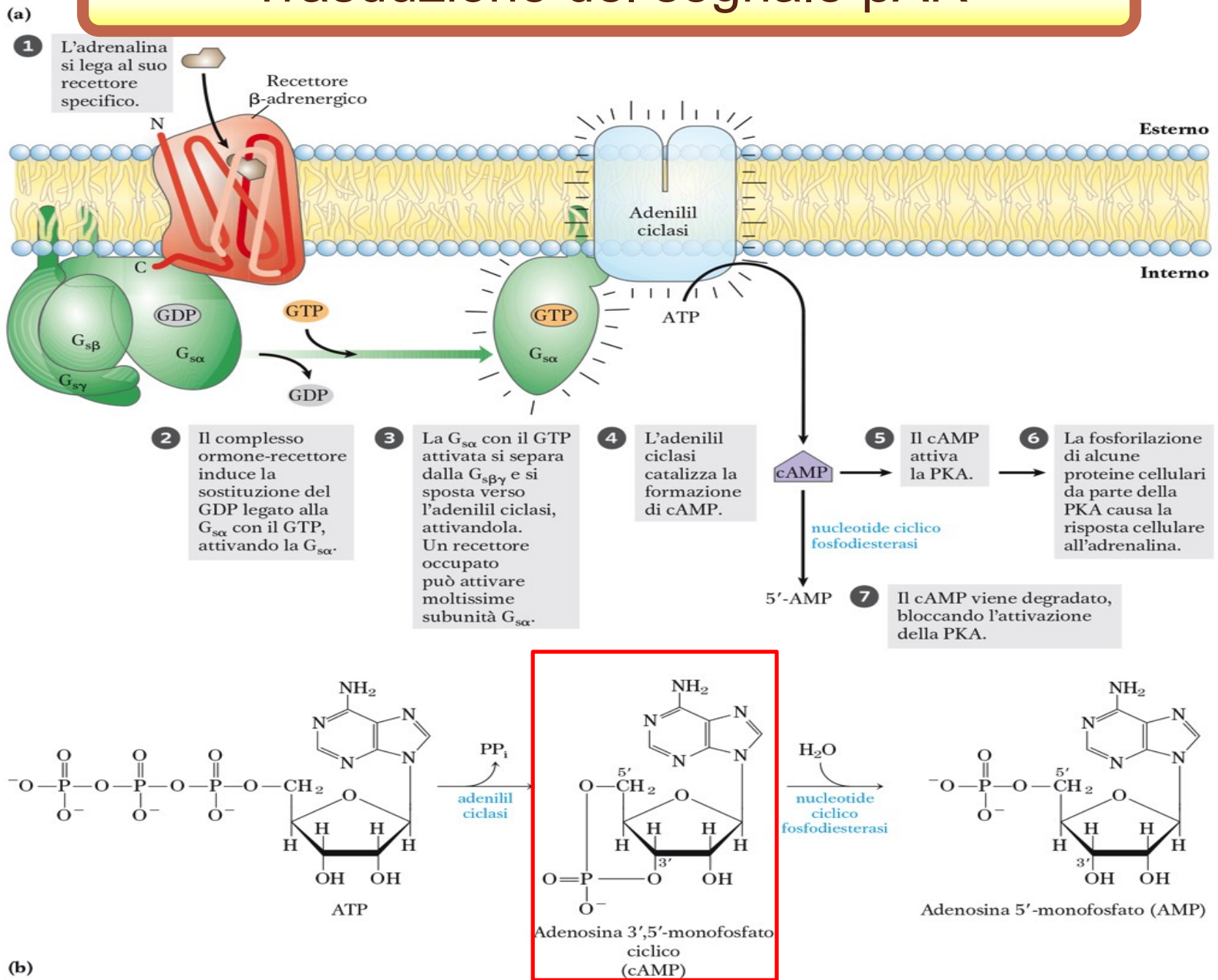
| Effector | Signalling effect |
|---|---|
| GIRK2 | activation |
| GIRK4 | activation |
| N-type calcium channel | inhibition |
| P/Q-type calcium channels | inhibition |
| Phospholipase A | activation |
| PLCβ1 | activation |
| PLCβ2 | activation |
| PLCβ3 | activation |
| Adenylyl cyclase Type I, III, V, VI, VII | inhibition |
| Adenylyl cyclase Type II, IV | activation |
| PI3K | inhibition |
| βARK1 | activation |
| βARK2 | activation |
| Raf-1 | activation |
| Ras exchange factor | activation |
| Bruton's tyrosine kinase | activation |

# Parasympathetic signaling pathway in cardiac cells

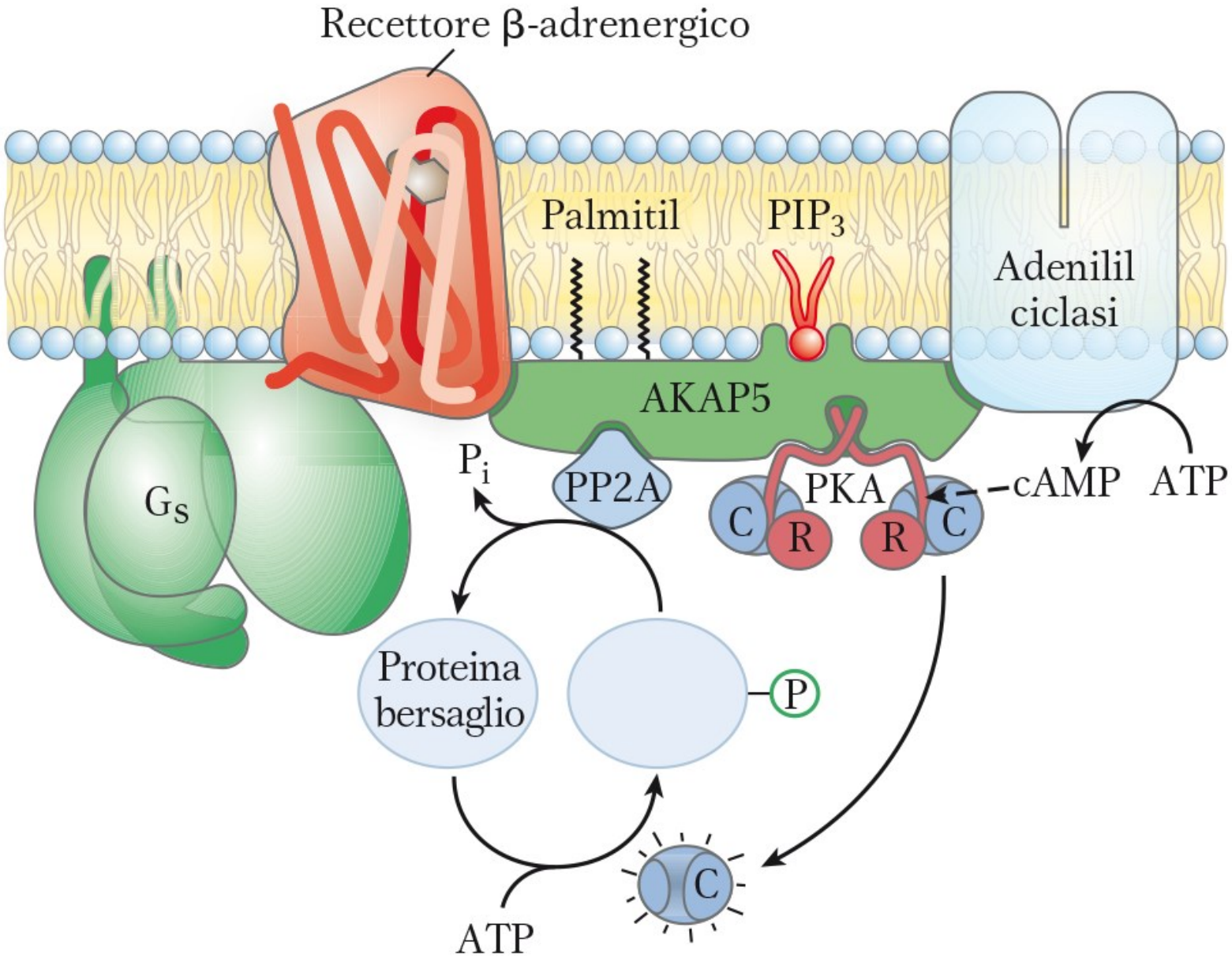



The binding of ACh to M2R induces dissociation of the *βγ*-complex (from Gi/o proteins) to directly interact with the $K_{ACh}$ channel and then decreases cardiac excitability.

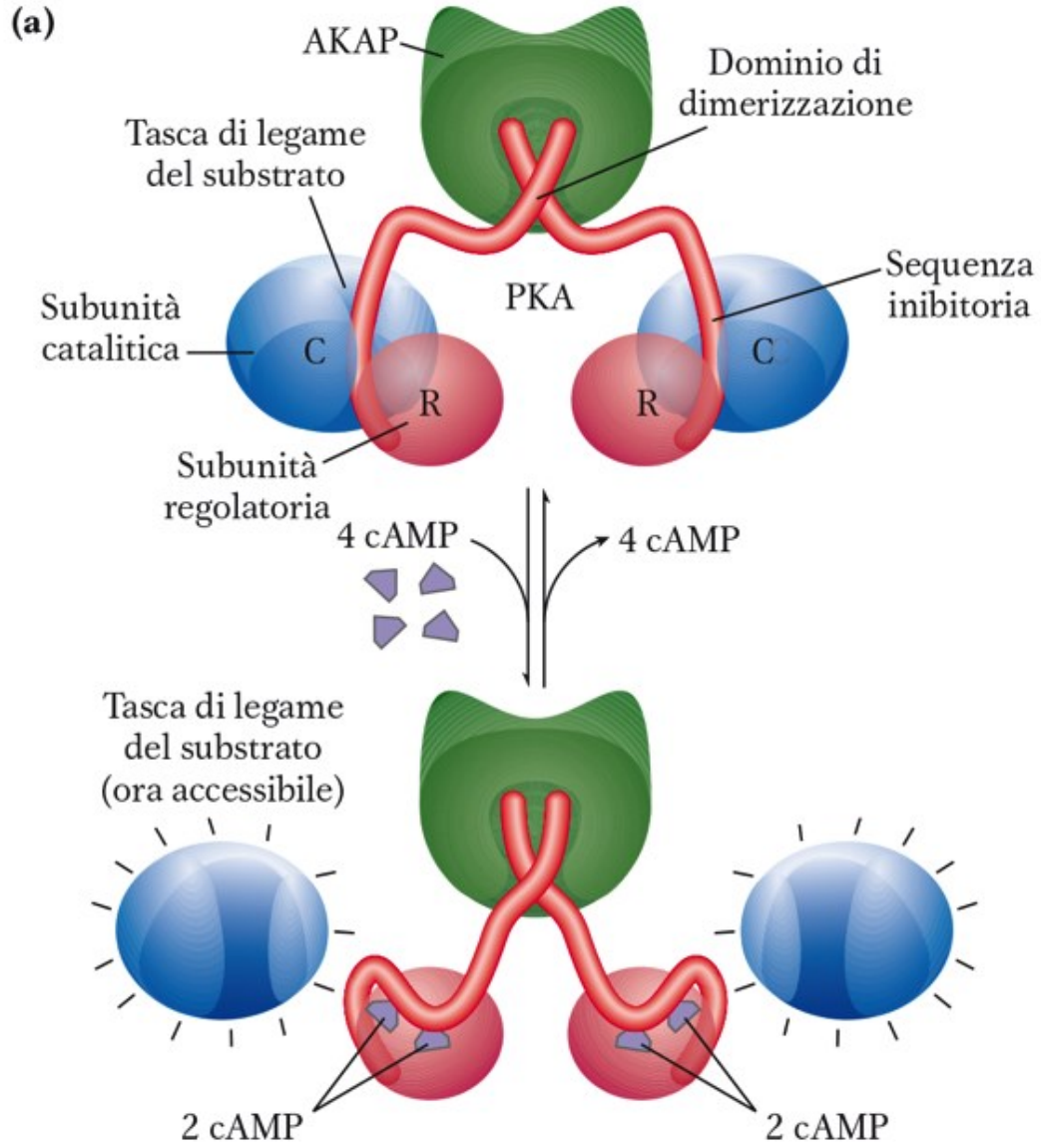
# Trasduzione del segnale βAR



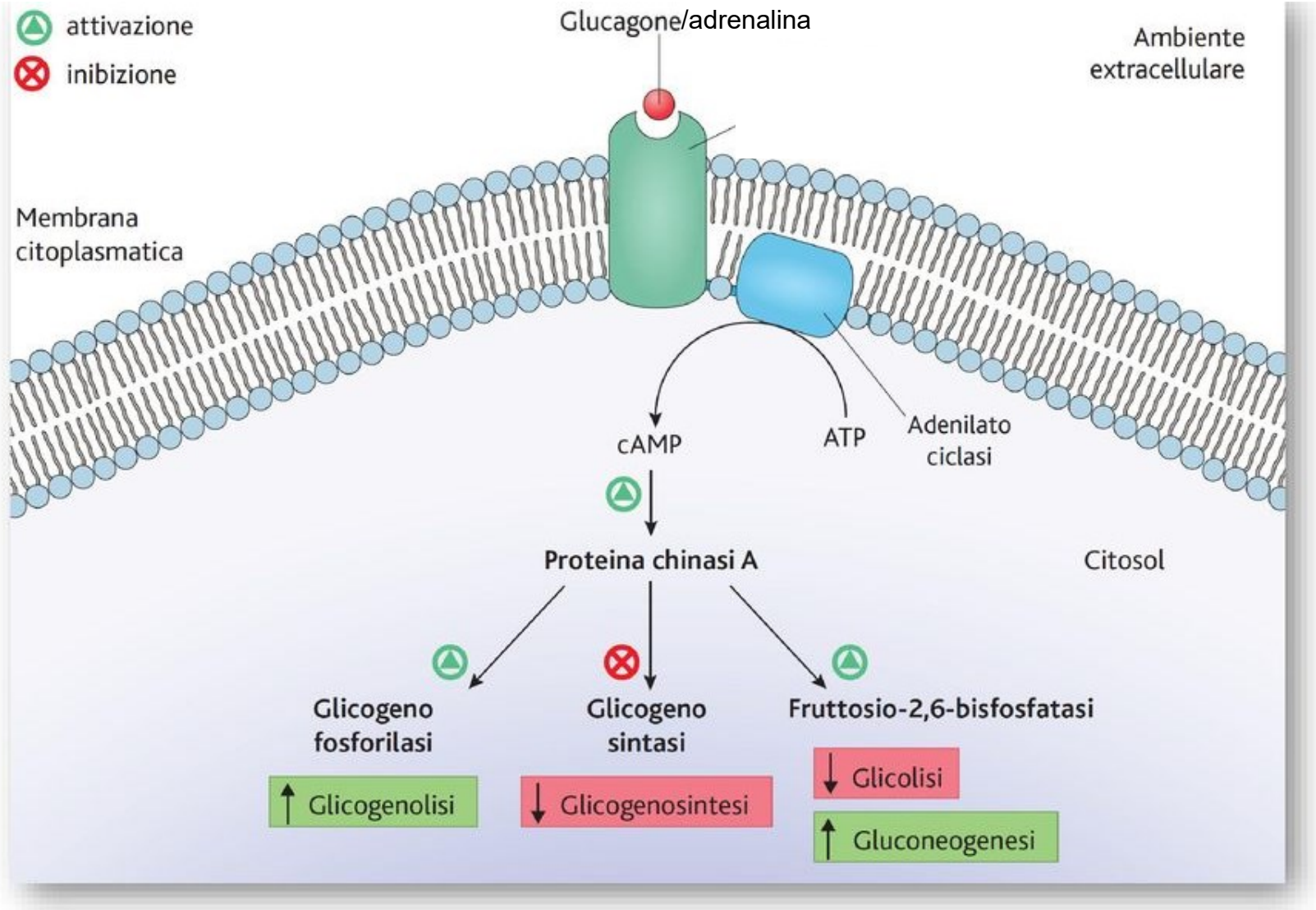
# Restrizione spaziale della biosegnalazione



# Chinasi dipendente da cAMP (PKA)



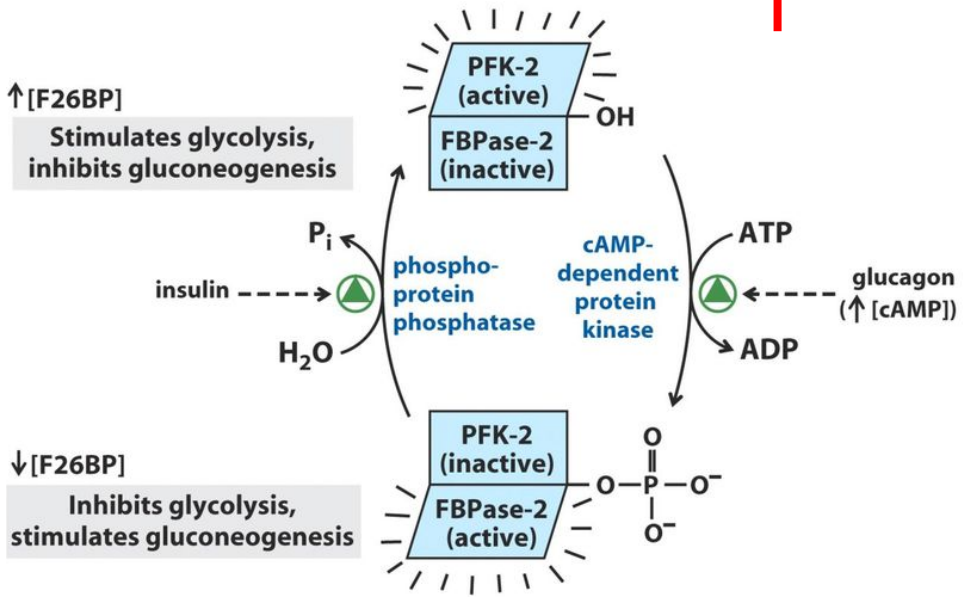
# Effetti sul metabolismo del glucosio di adrenalina e glucagone



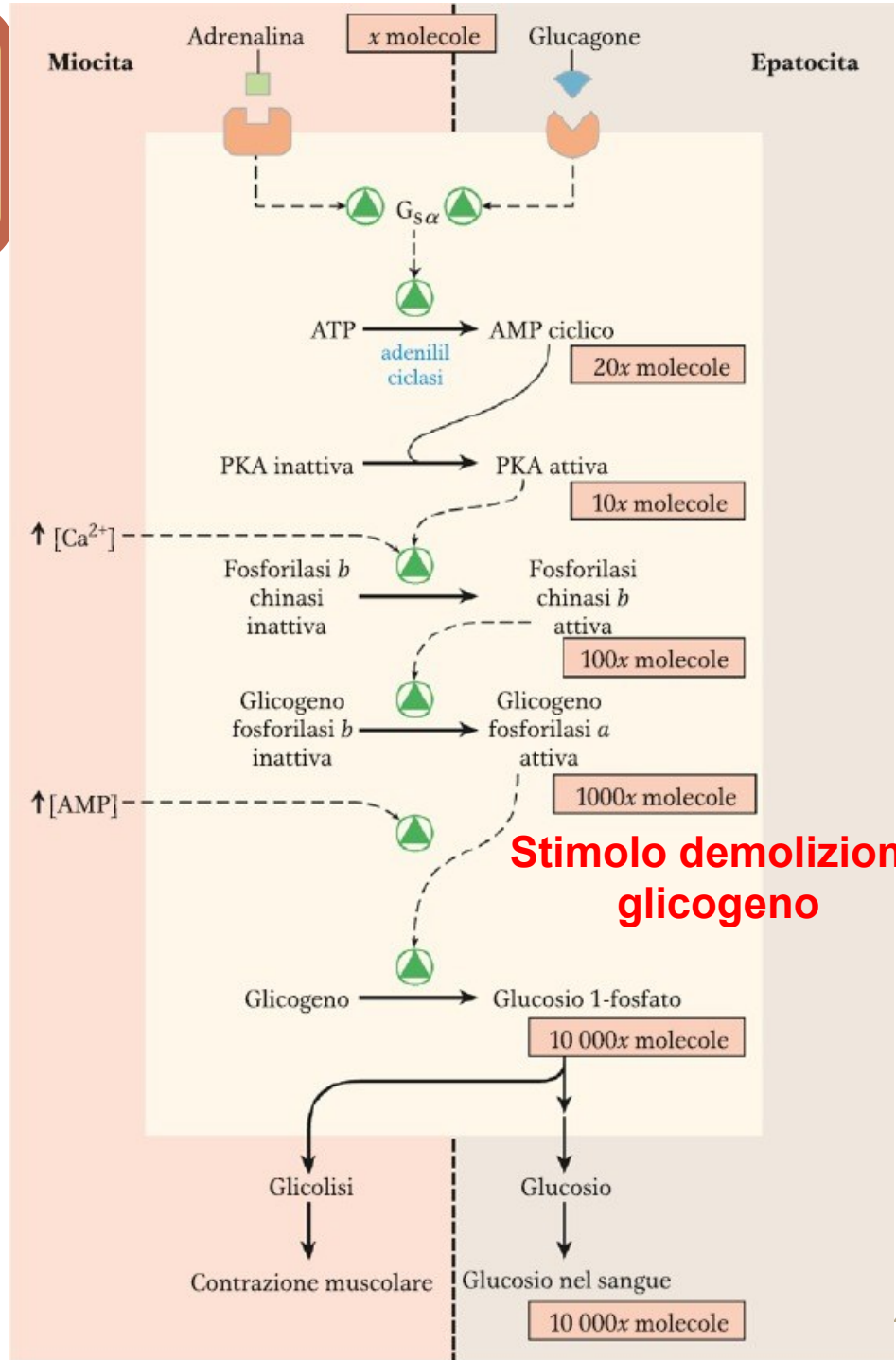
# PKA e metabolismo del glucosio

**TABELLA 12.2** Alcuni enzimi e altre proteine regolati dalla fosforilazione cAMP-dipendente (da PKA)

| Enzima/proteina | Sequenza fosforilata[a] | Via metabolica/processo regolato |
|---|---|---|
| Glicogeno sintasi | RASCTSSS inibizione | Sintesi del glicogeno |
| Fosforilasi *b* chinasi<br>subunità α<br>subunità β | VEFRRLSI<br>RTKRSGSV } attivazione | Demolizione del glicogeno |
| Piruvato chinasi (fegato di ratto) | GVLRRASVAZL inibizione | Glicolisi |
| Complesso della piruvato deidrogenasi (tipo L) | GYLRRASV inibizione | Da piruvato ad acetil-CoA |
| Lipasi ormone-sensibile | PMRRSV attivazione | Mobilizzazione dei triacilgliceroli e ossidazione degli acidi grassi |
| Fosfofruttochinasi-2/fruttosio 2,6-bisfosfatasi | LQRRRGSSIPQ | Glicolisi/gluconeogenesi |



# Effetti metabolici dell'adrenalina/glucagone
# Amplificazione del segnale

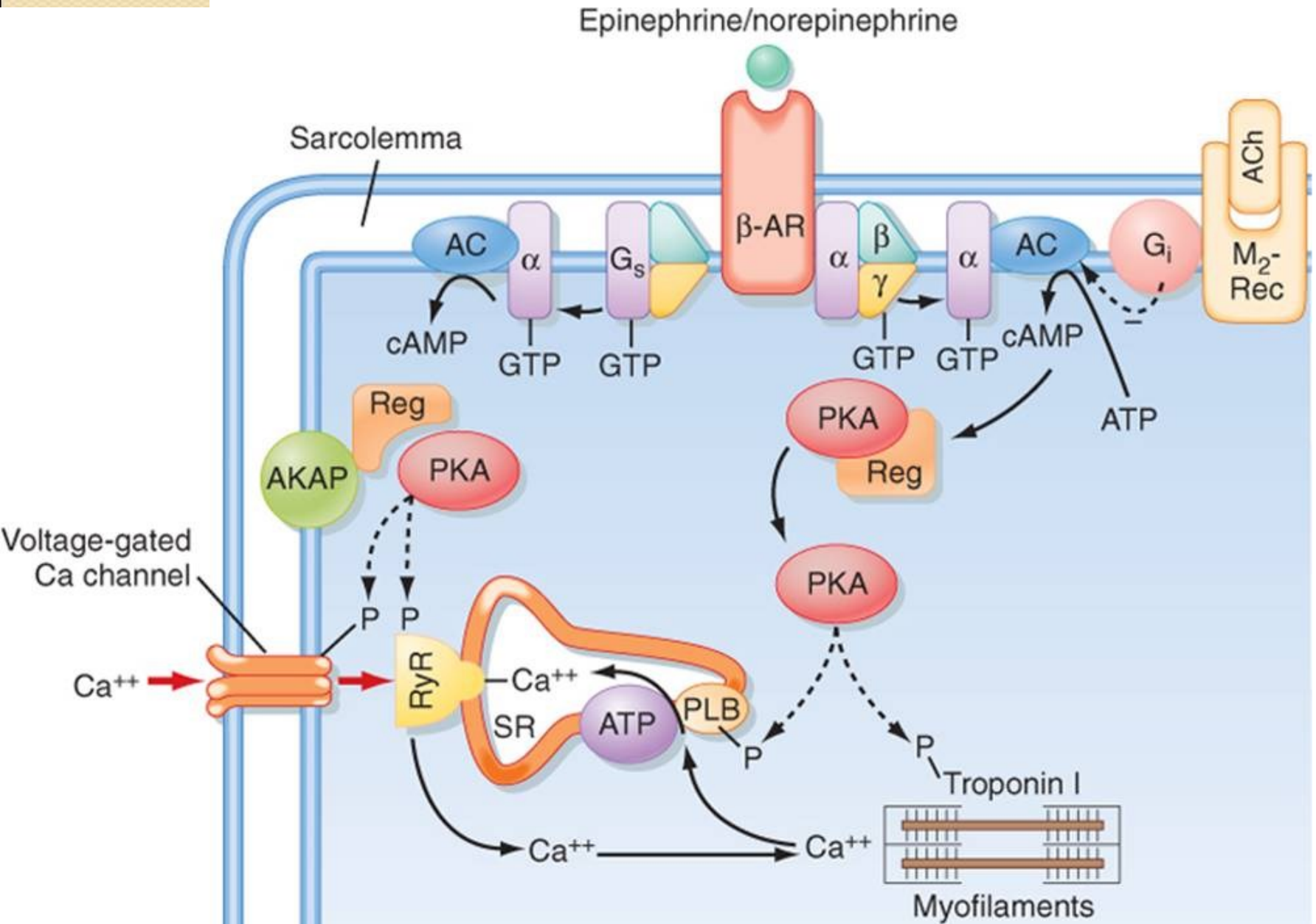


# Substrati della PKA

**TABELLA 12.2** Alcuni enzimi e altre proteine regolati dalla fosforilazione cAMP-dipendente (da PKA)

| Enzima/proteina | Sequenza fosforilata[a] | Via metabolica/processo regolato |
|---|---|---|
| Glicogeno sintasi | RASCTSSS inibizione | Sintesi del glicogeno |
| Fosforilasi *b* chinasi<br>subunità α<br>subunità β | VEFRRLSI<br>RTKRSGSV } attivazione | Demolizione del glicogeno |
| Piruvato chinasi (fegato di ratto) | GVLRRASVAZL | Glicolisi |
| Complesso della piruvato deidrogenasi (tipo L) | GYLRRASV | Da piruvato ad acetil-CoA |
| Lipasi ormone-sensibile | PMRRSV | Mobilizzazione dei triacilgliceroli e ossidazione degli acidi grassi |
| Fosfofruttochinasi-2/fruttosio 2,6-bisfosfatasi | LQRRRGSSIPQ | Glicolisi/gluconeogenesi |
| Tirosina idrossilasi | FIGRRQSL | Sintesi di L-dopa, dopamina, noradrenalina e adrenalina |
| Istone H1 | AKRKASGPPVS | Condensazione del DNA |
| Istone H2B | KKAKASRKESYSVYVYK | Condensazione del DNA |
| Fosfolambano cardiaco (una pompa regolatrice cardiaca) | AIRRAST | $[Ca^{2+}]$ intracellulare |
| Inibitore-1 della proteina fosfatasi-1 | IRRRRPTP | Defosforilazione delle proteine |
| Sequenza consenso della PKA[b] | xR[RK]x[ST]B | Molte |

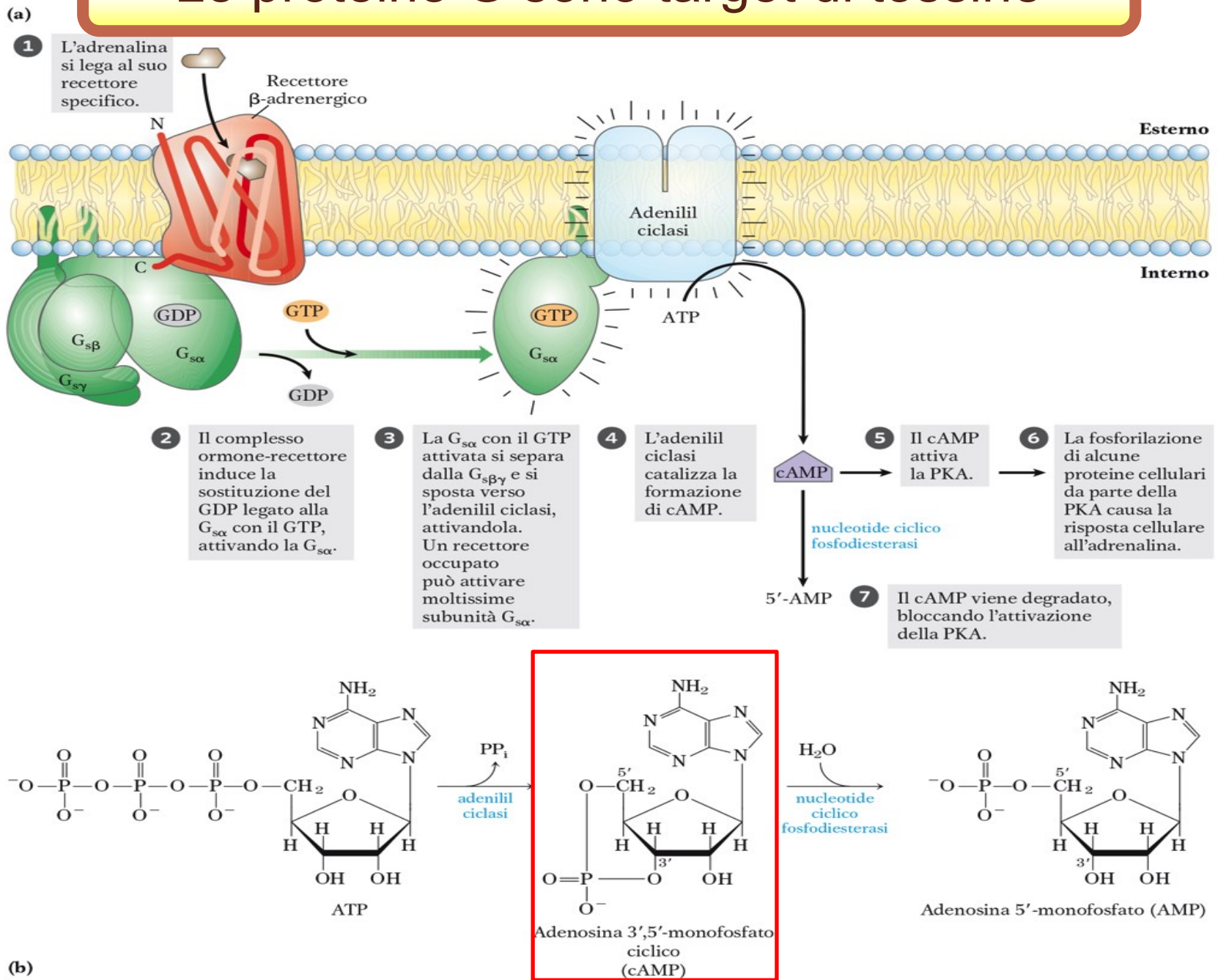
# Effetti del signalling adrenergico sul cuore



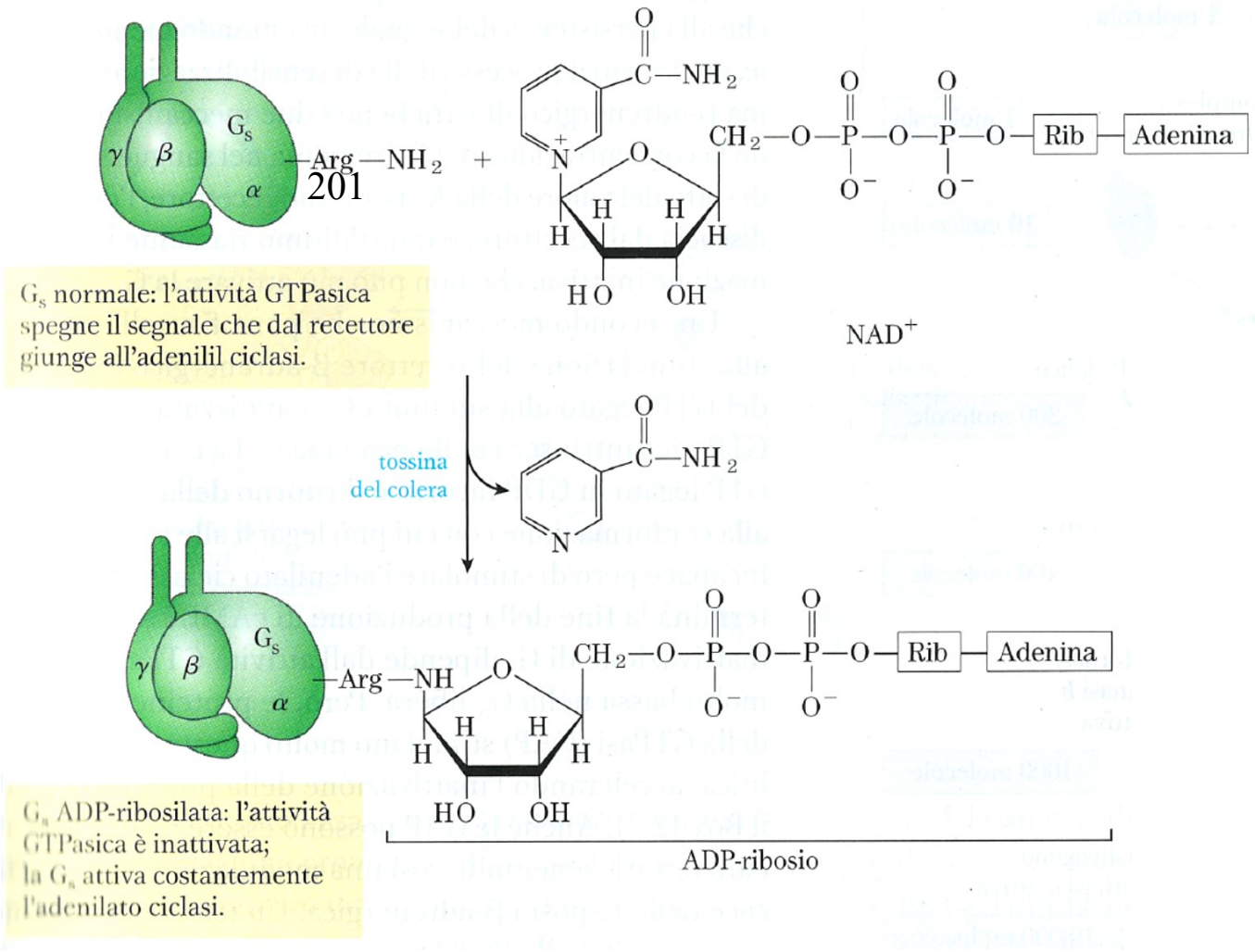

Catecholamine induces calcium influx into the sarcoplasmic reticulum

and increases both inotropy and lusitropy.

# Le proteine G sono target di tossine

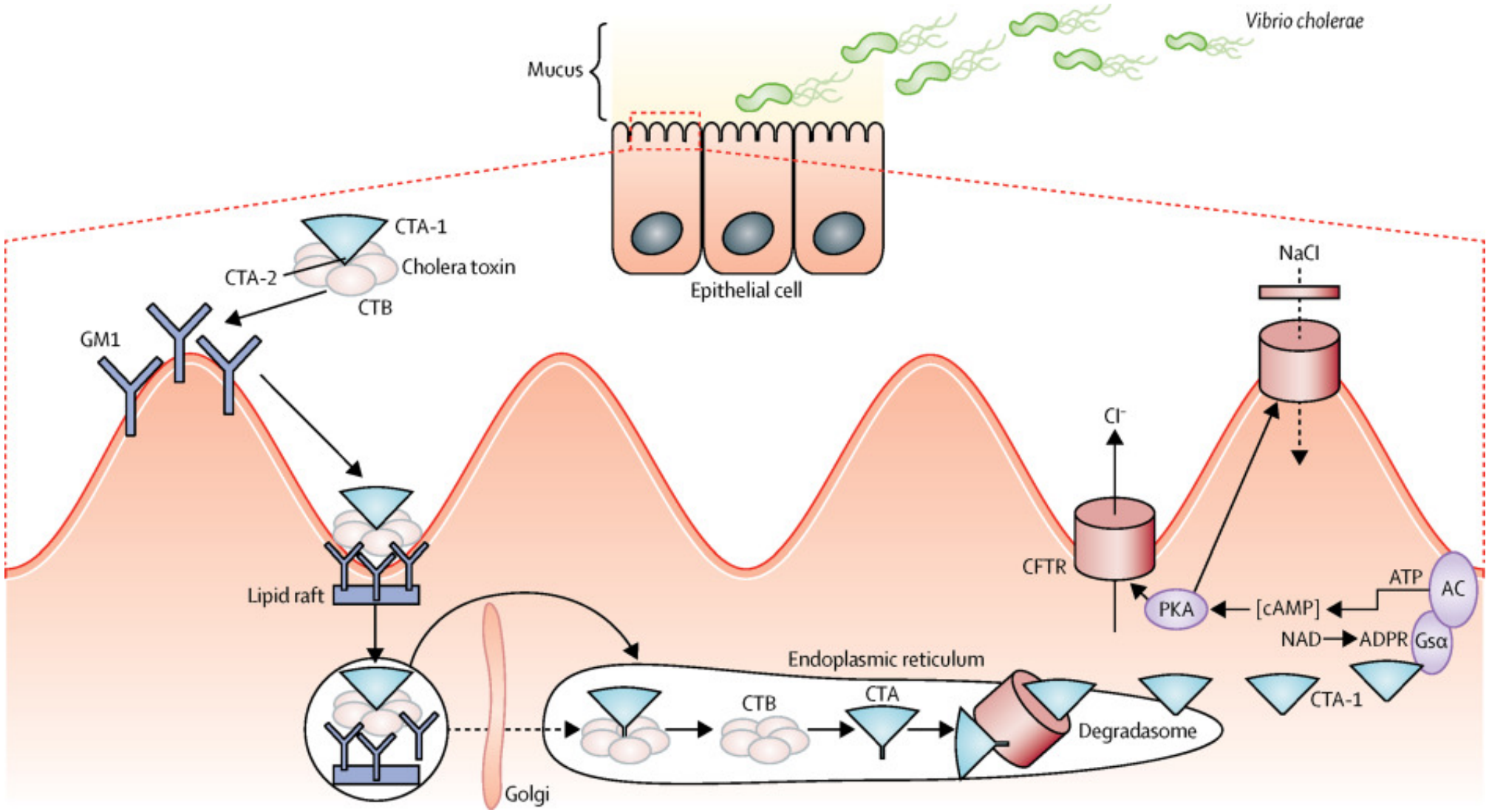


# Effetti della tossina colerica




INATTIVAZIONE DELL'ATTIVITÀ GTPasica

# Effetti della tossina colerica



# Proteine G e tossine

| G-protein targets | Modulating toxins | Modification | Effect on G protein | Ref. |
|---|---|---|---|---|
| ***Small GTPases*** | | | | |
| RhoA, Rac1,Cdc42 | TcdA, TcdB, TcsH, TcsL, Tcnα | Monoglucosylation | Inactivation | [59,60] |
| Rap1, Rap2 | TcdA, TcsL | Monoglucosylation | Inactivation | [61,62] |
| RhoA, RhoB, RhoC | clostridial C3 | ADP-ribosylation | Inactivation | [51] |
| Ras | ExoS | ADP-ribosylation | Inactivation | [79–82] |
| RhoA, Rac1,Cdc42 | ExoS, ExoT, SptP, YopE | GAP-like activity | Inactivation | [72–74] |
| Rab1 | LepB | GAP-like activity | Inactivation | [86] |
| RhoA, Rac1 | YpkA, YopO | GDI-like activity | Inactivation | [85] |
| RhoA, Rac1,Cdc42 | YopT, Avr/PhpB | Proteolysis | Inactivation | [61] |
| RhoA, RhoB, RhoC, Rac1, Cdc42 | MARTX | Unknown | Inactivation | [92] |
| RhoA, Rac1, Cdc42 | SopE, SifA, SifB, lpgB1,lpgB2, Map, EspM, EspT | GEF-like activity | Activation | [69] |
| Arf | RalF | GEF-like activity | Activation | [71] |
| Rab1 | DrrA/SidM | GEF-like activity | Activation | [86–88] |
| Rab1 | DrrA/SidM | GDF-like activity | Activation | [86–88] |
| RhoA, Rac1, Cdc42 | CNF1,CNF2, CNF3 (Cytotoxic Necrotizing Factor) | Deamidation[ǂ] | Activation | [19] |
| RhoA | CNFY | Deamidation[ǂ] | Activation | [100] |
| RhoA, Rac1, Cdc42 | DNT | Transglutamination[ǂ] | Activation | [19] |
| ***Heterotrimeric Gα subunits*** | | | | |
| $G_i$, $G_o$ $G_t$ | PT | ADP ribosylation | Inactivation[§] | [45,48] |
| $G_q$ | YpkA | Phosphorylation | Inactivation | [84] |
| $G_s$, $G_{olf}$, $G_t$ | CT, HLT | ADP ribosylation | Activation[§] | [45,48] |
| $G_q$, $G_{13}$, $G_i$ | PMT | Deamidation | Activation[§] | [9,21,32,112,113,135] |
| ***Large, multidomain GTPases*** | | | | |
| EF-2 | DT, ExoA | ADP ribosylation | nactivation | [41] |

# GTPase cycle of heterotrimeric G proteins and points of toxin interactions

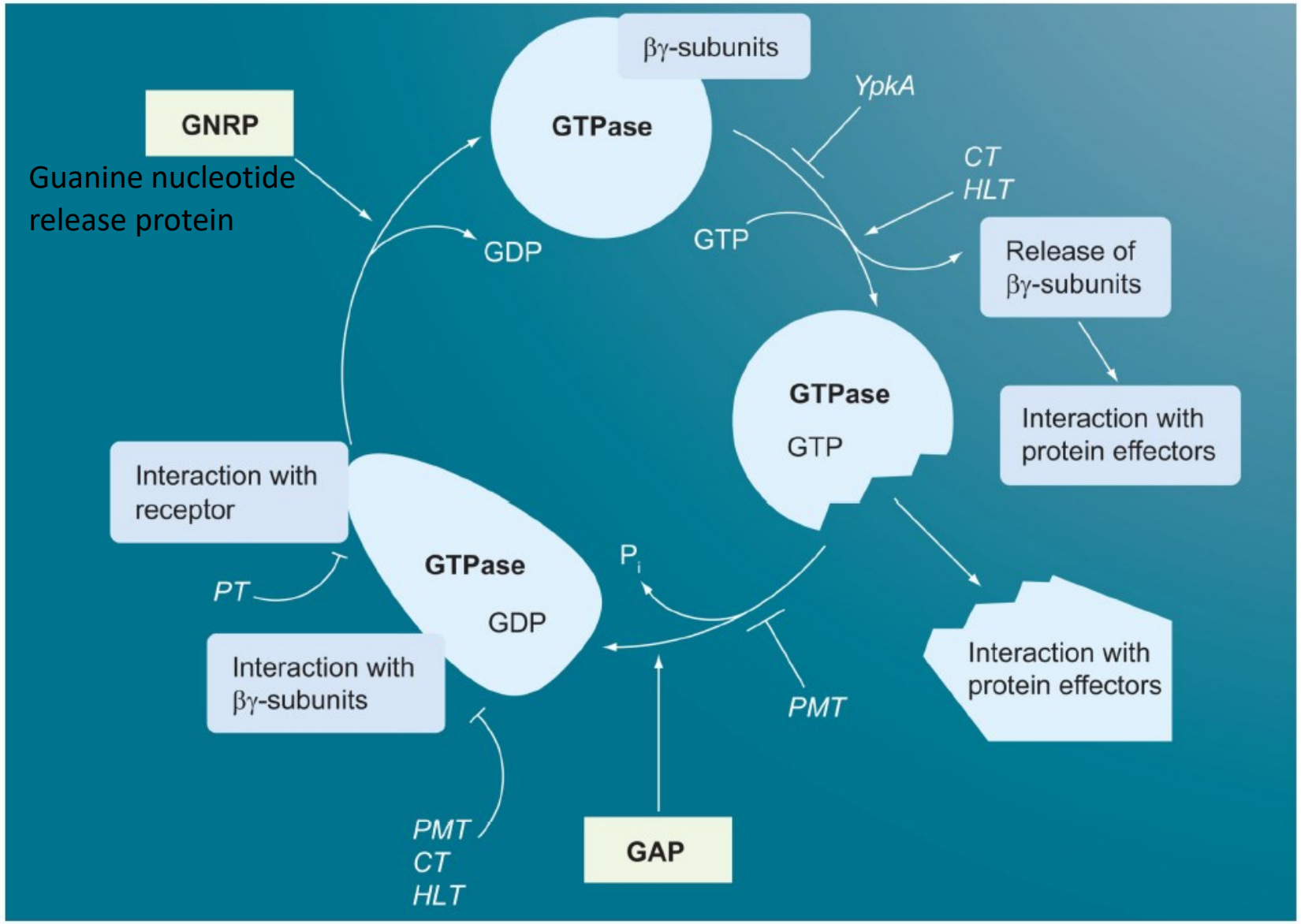



Figure 2. GTPase cycle of heterotrimeric G proteins and points of toxin interactions

YpKA: Yersinia pseudotuberculosis serotype I (phosphorylation);

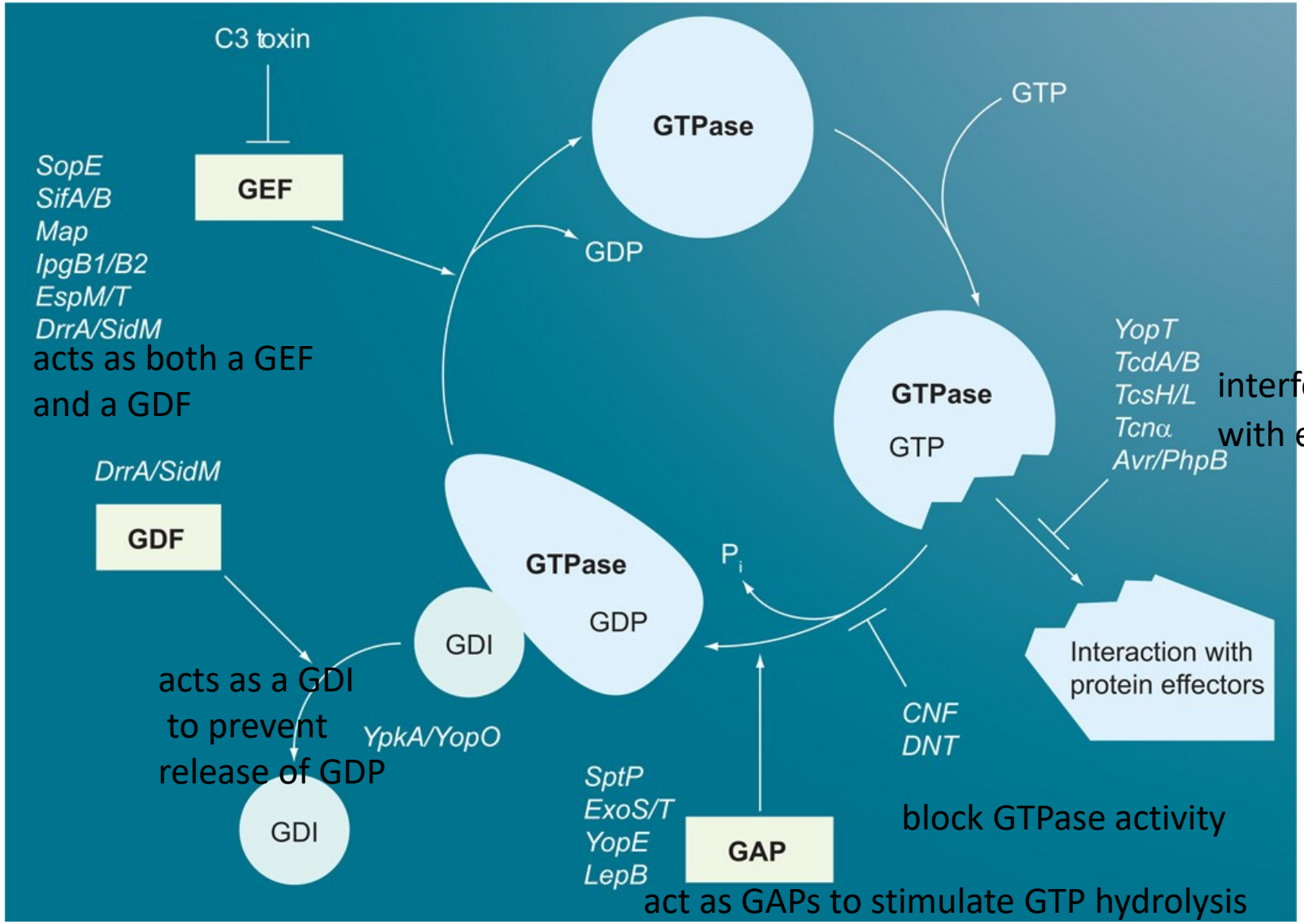
CT: Cholera toxin;

HLT: Heat-labile enterotoxins;

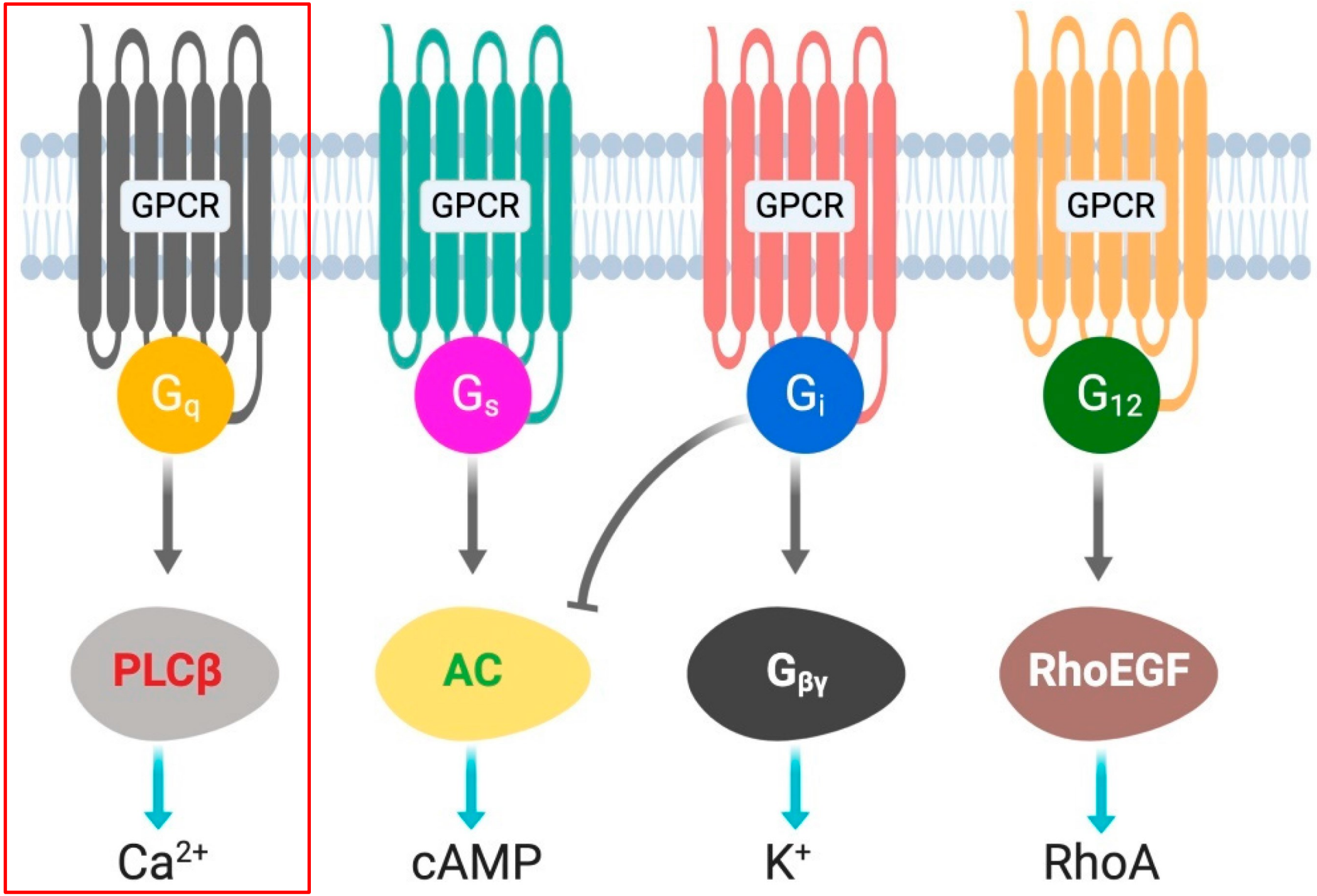
PMT: Pasteurella multocida toxin;

PT: Pertussis toxin.

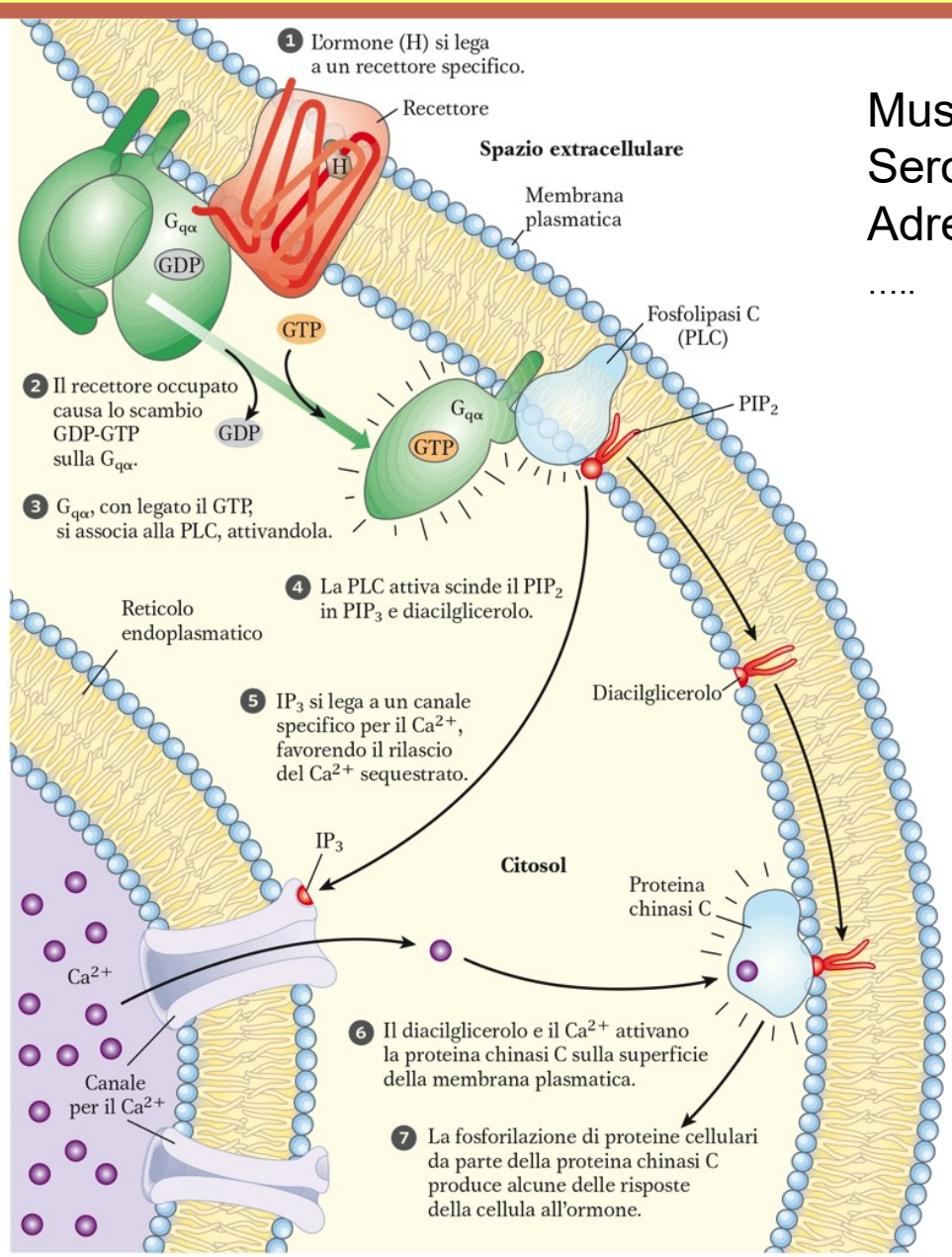
# GTPase cycle of small G proteins and points of toxin interactions




interfere with the GTPase interaction with effectors.

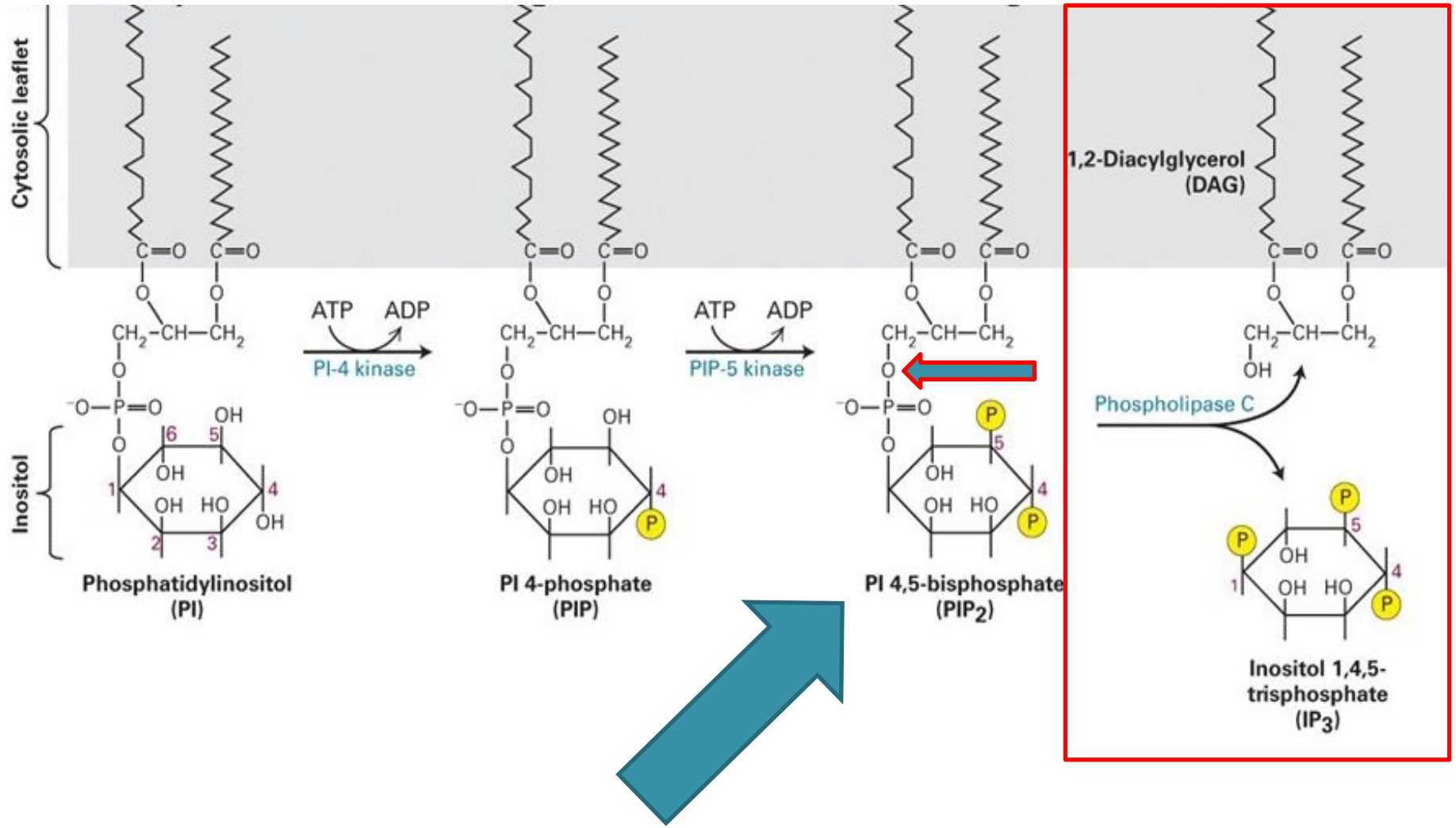

GPCR
GPCR
GPCR
GPCR
$G_q$
$G_s$
$G_i$
$G_{12}$
PLCβ
AC
$G_{\beta\gamma}$
RhoEGF
$Ca^{2+}$
cAMP
$K^+$
RhoA

# Recettori accoppiati a Gq

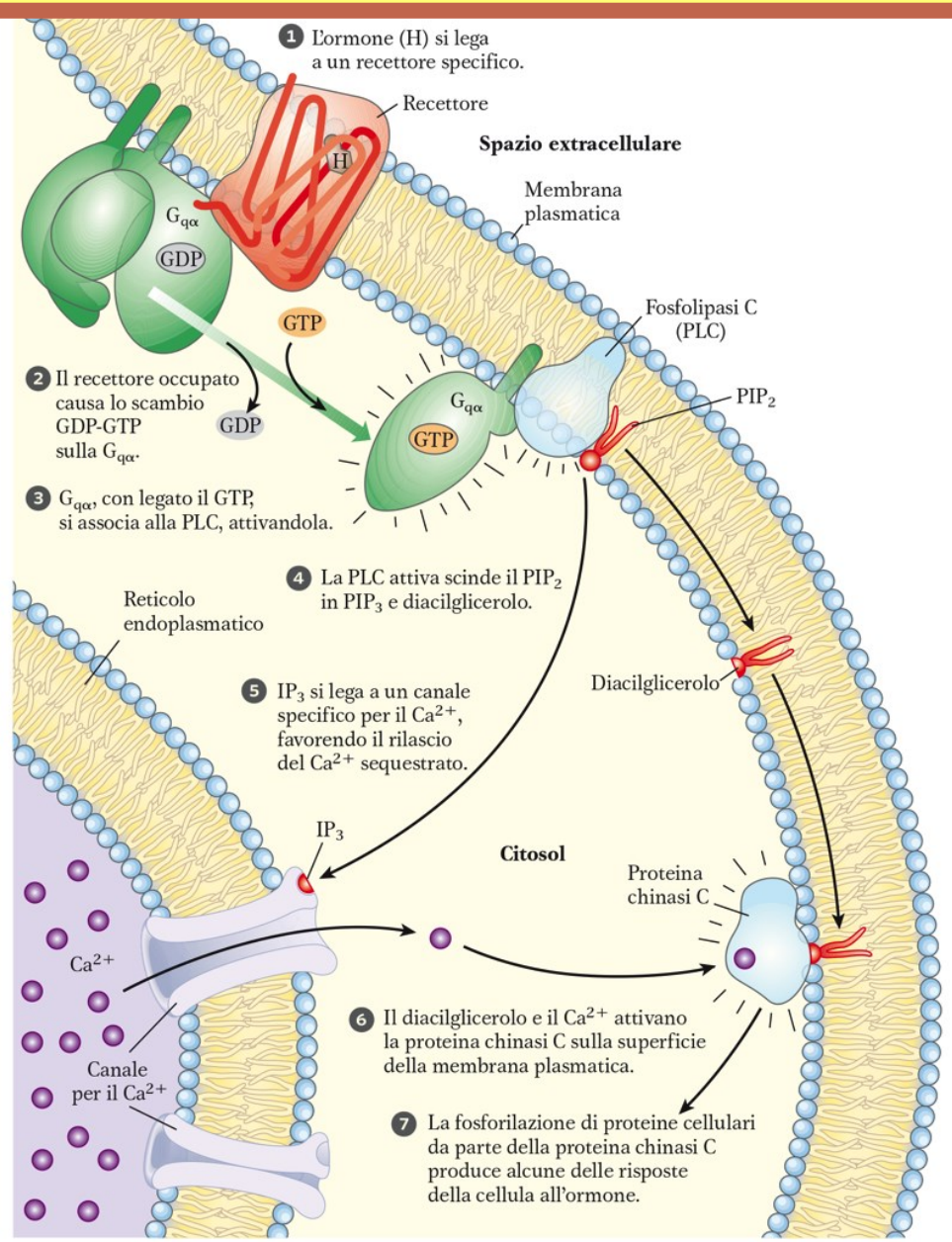



Muscarinico M3
Serotoninergici 5-$HT_{2a,b,c}$
Adrenergico Alpha1
…..

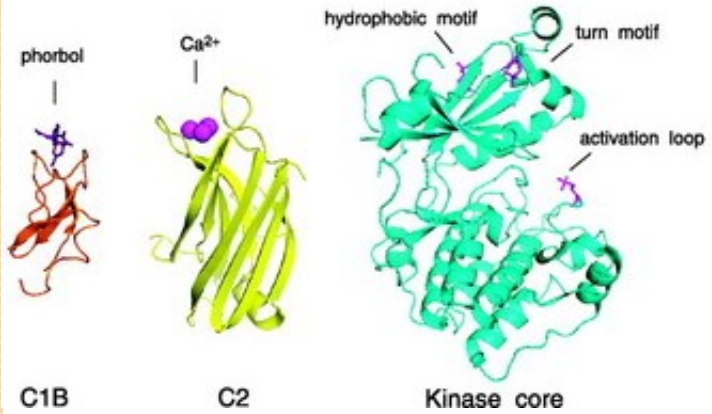
# Produzione di IP3

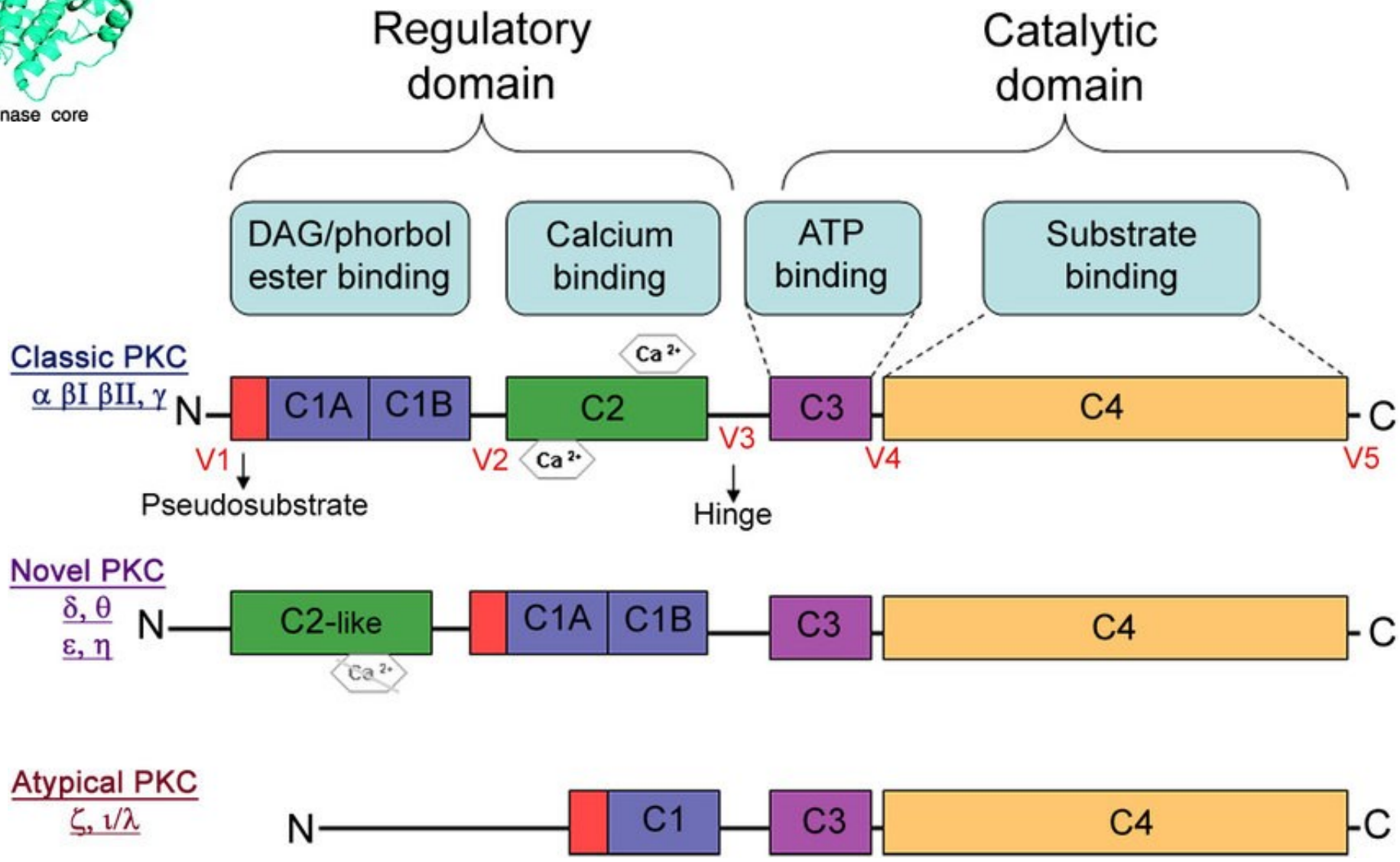


# Recettori accoppiati a Gq

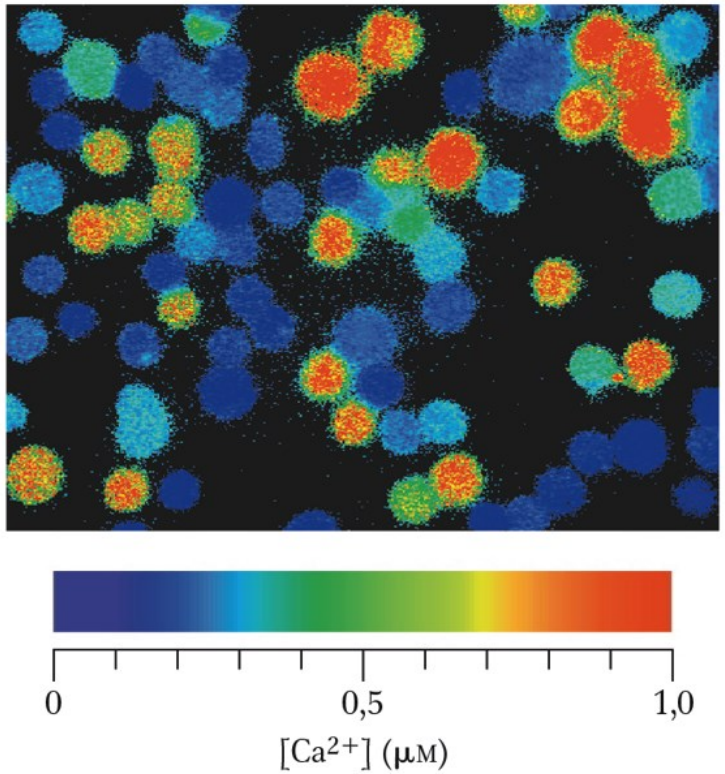


# PKC domains

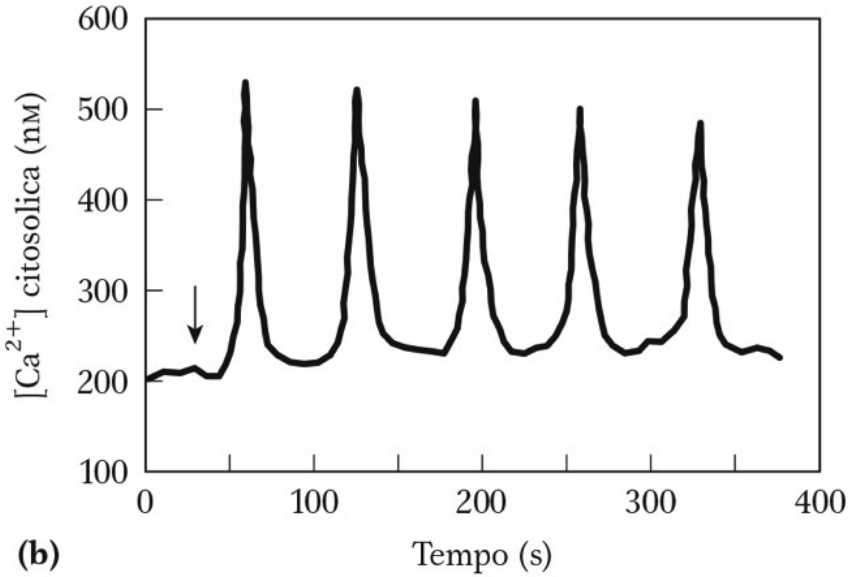

phorbol
Ca2+
hydrophobic motif
turn motif
activation loop
C1B
C2
Kinase core


Regulatory domain
Catalytic domain
DAG/phorbol ester binding
Calcium binding
ATP binding
Substrate binding
Classic PKC
α βI βII, γ
N
C1A
C1B
C2
C3
C4
C
Ca 2+
V1
V2
V3
V4
V5
Pseudosubstrate
Hinge
Novel PKC
δ, θ
ε, η
C2-like
Atypical PKC
ζ, ι/λ
C1

# Peculiarità del rilascio di calcio
# Ca-oscillations

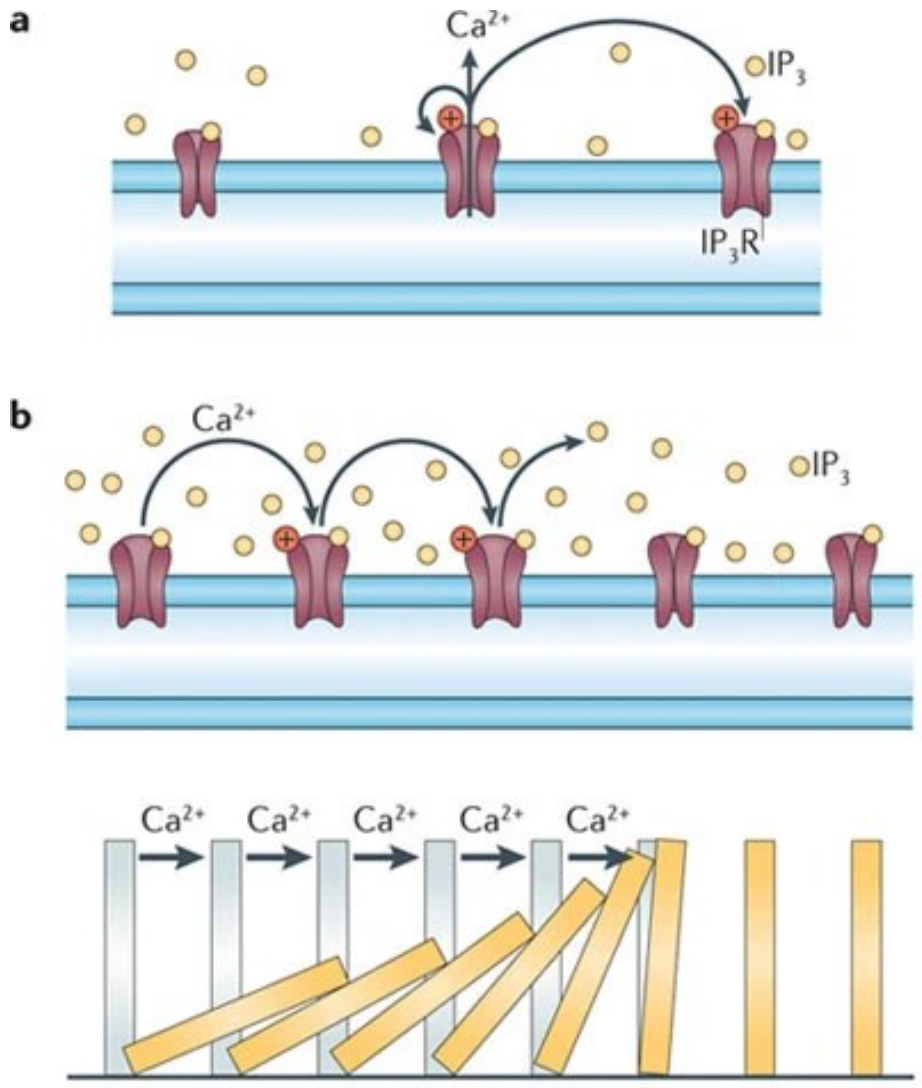



(a)




(b)

# Ca waves: Ca-induced calcium release

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# Calcium-induced calcium release

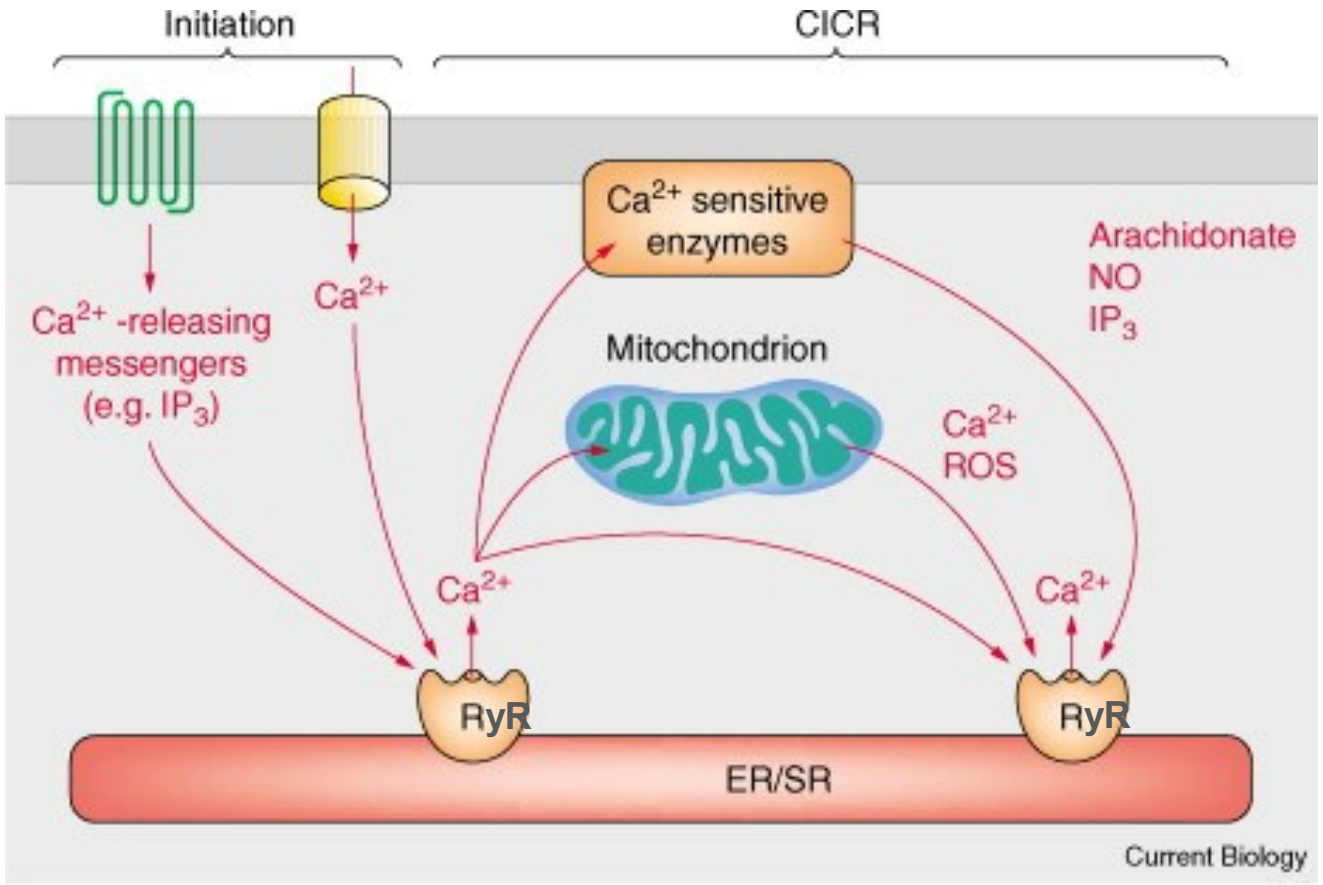
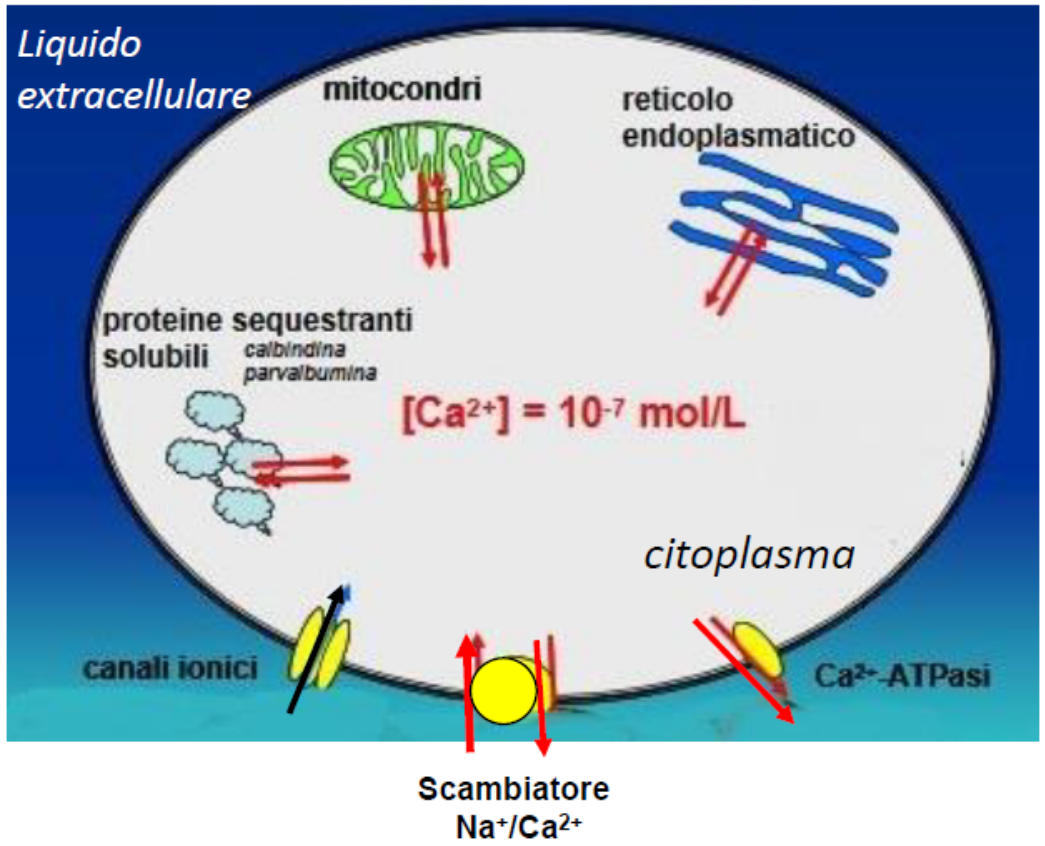



Figure 1.
This cartoon depicts how CICR mechanisms amplify calcium signals. Starting from the left-hand side, the figure illustrates the initiation of a calcium signal via an intracellular channel ('R''). This leads to direct release of calcium from neighbouring channels or production of calcium-releasing messengers.

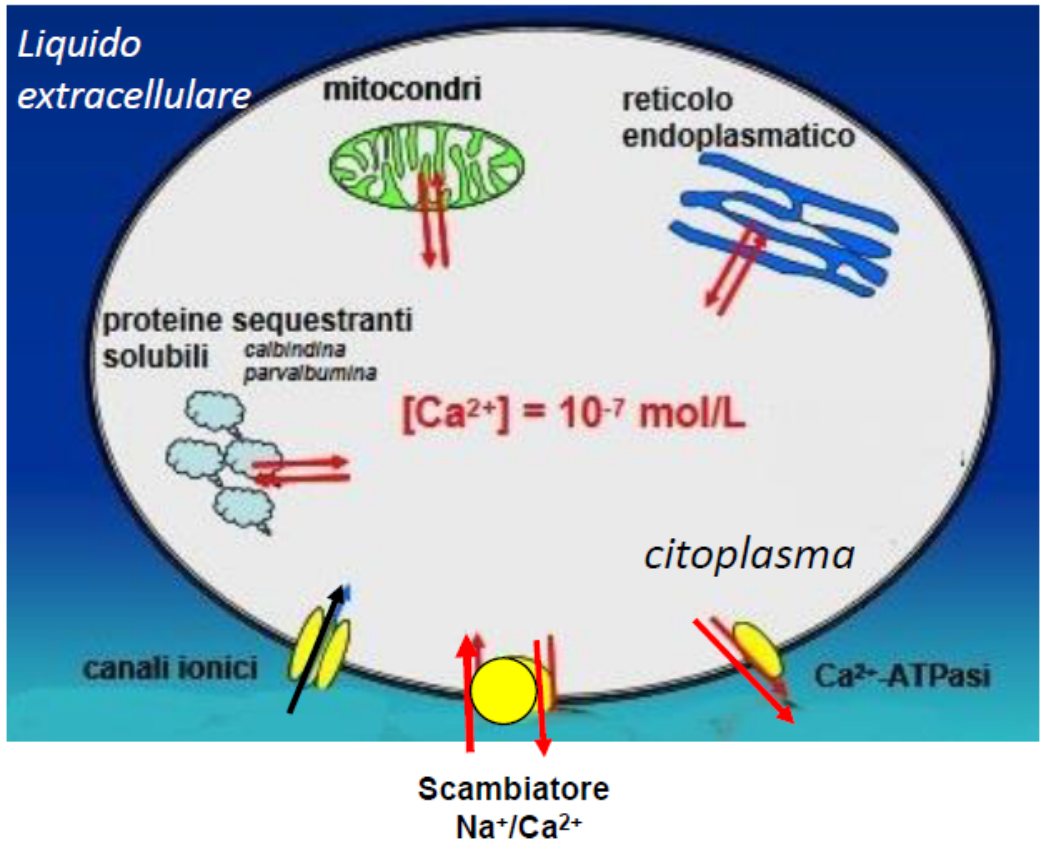
# Gli ioni $Ca^{2+}$ come secondi (o terzi messaggeri)




La concentrazione intracellulare di $Ca^{2+}$ è finemente regolata in quanto il $Ca^{2+}$ rappresenta un messaggero intracellulare. Infatti, transitori cambiamenti della sua concentrazione intracellulare rappresentano segnali per la cellula per l'attivazione di risposte quali: l'esocitosi, la contrazione muscolare, l'apertura o chiusura di canali ionici, ecc.

Pertanto, il mantenimento di una bassa concentrazione citoplasmatica di $Ca^{2+}$ fa sì che anche piccoli flussi di $Ca^{2+}$ in ingresso rappresentino significativi segnali intracellulari.

# Gli ioni $Ca^{2+}$ come secondi (o terzi messaggeri)



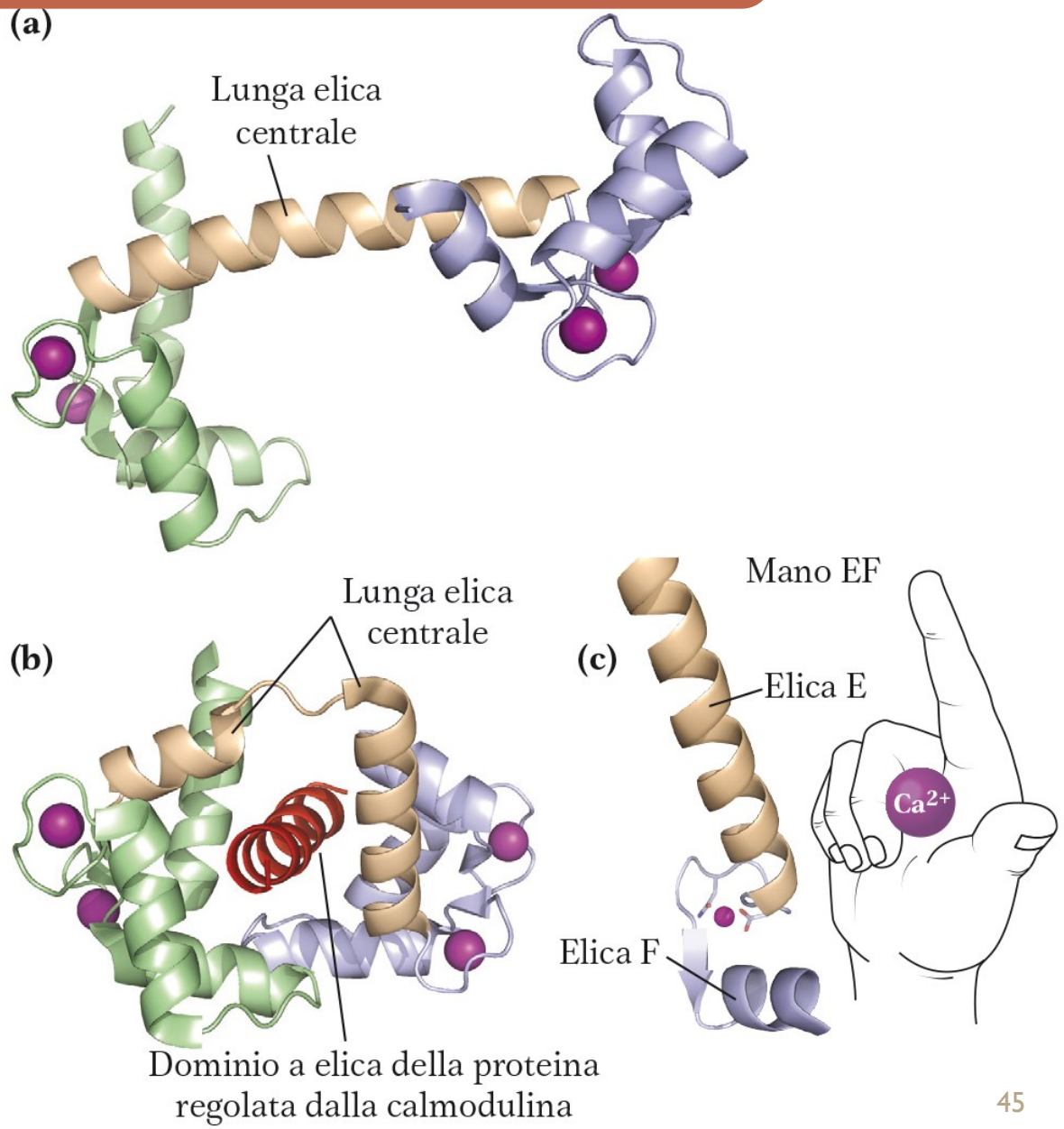

La concentrazione citoplasmatica di calcio della cellula eucariotica a riposo si aggira intoro a $10^{-7}$M nonostante una concentrazione extracellulare di 1-2 mM.

Il mantenimento di una tale differenza di concentrazione tra l'interno e l'esterno della cellula è il risultato dell'azione coordinata di differenti sistemi che comprendono:

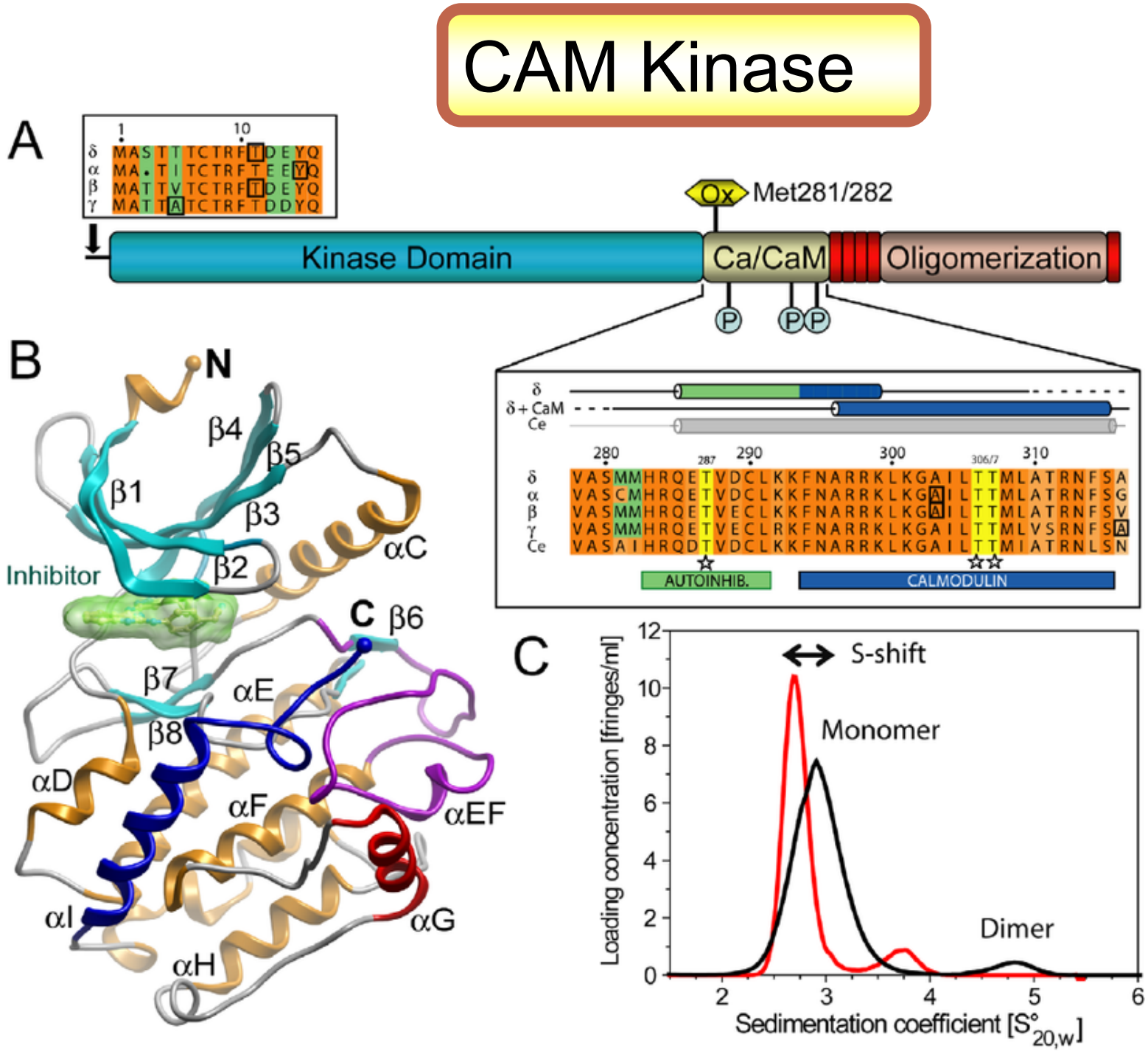
- trasporti attivi primari e secondari che provvedono ad estrudere il $Ca^{2+}$ dalla cellula e ad accumularlo in depositi intracellulari (reticolo endoplasmico, mitocondri).
- sistemi tampone della concentrazione di $Ca^{2+}$ rappresentati da proteine leganti il $Ca^{2+}$ (*$Ca^{2+}$ bindig proteins*), di cui la più nota è la calmodulina.

# La calmodulina un effettore del $Ca^{2+}$

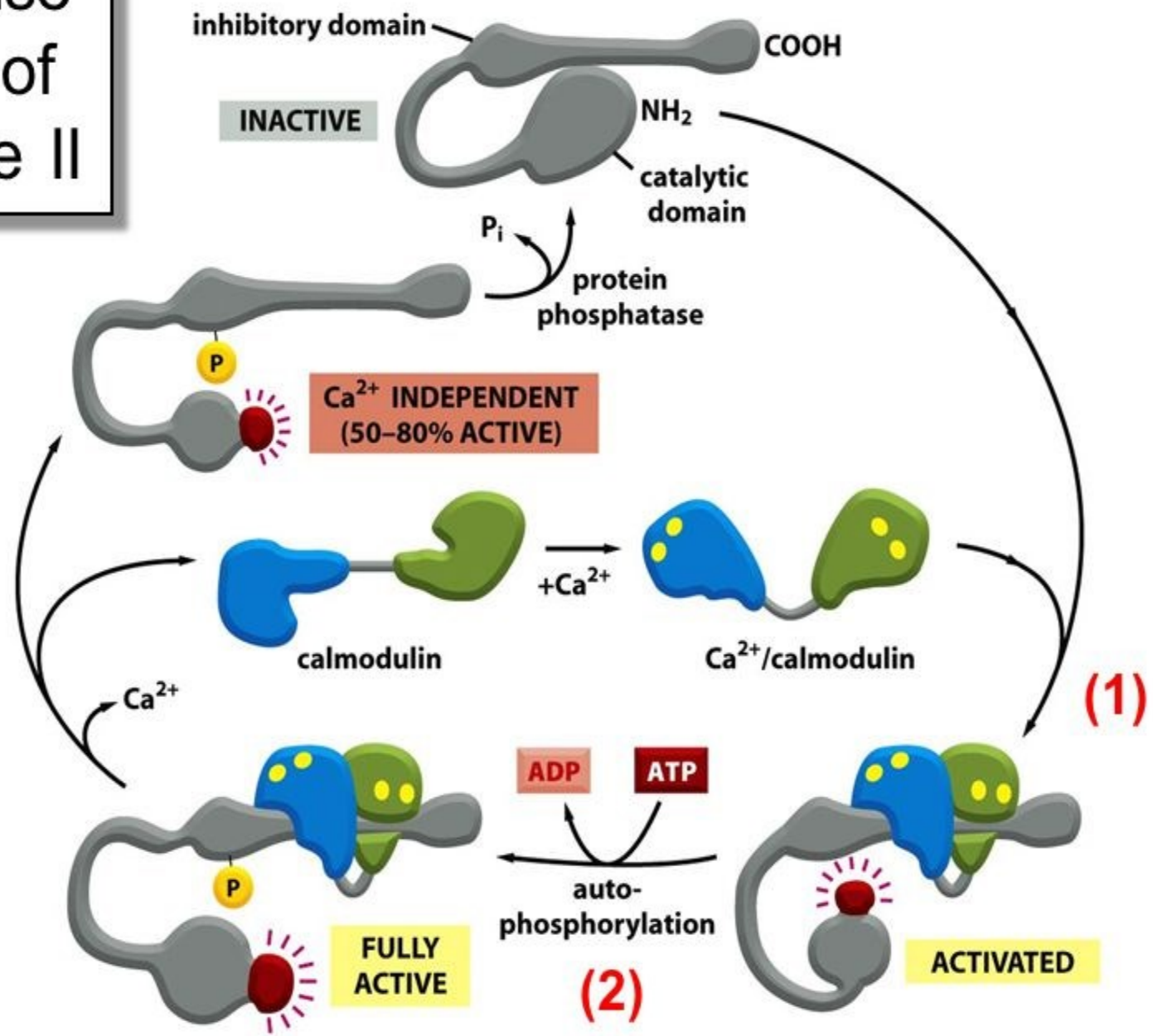
- In numerosissime vie di segnalazione l'azione del calcio è mediata dalla calmodulina.
- La calmodulina è una piccola proteina regolatrice attivata dal calcio presente in tutte le cellule eucariotiche.
- È costituita da due domini globulari, ciascuno dei quali è in grado di legare due ioni $Ca^{2+}$; ciò conferisce alla calmodulina la capacità di legare complessivamente 4 ioni $Ca^{2+}$.
- L'interazione con il calcio provoca una notevole modificazione conformazionale in seguito alla quale la proteina viene convertita nella sua forma attiva, in cui è capace di interagire con diversi tipi di proteine bersaglio.
- In alcuni casi il bersaglio è costituito da protein-chinasi che, a loro volta, attivano o disattivano altri bersagli mediante aggiunta di gruppi fosforici. Altri bersagli, come la pompa $Ca^{2+}$-ATPasi e alcune proteine che regolano l'assemblaggio dei microtubuli, sono sottoposte a regolazione per interazione diretta col complesso $Ca^{2+}$/calmodulina.

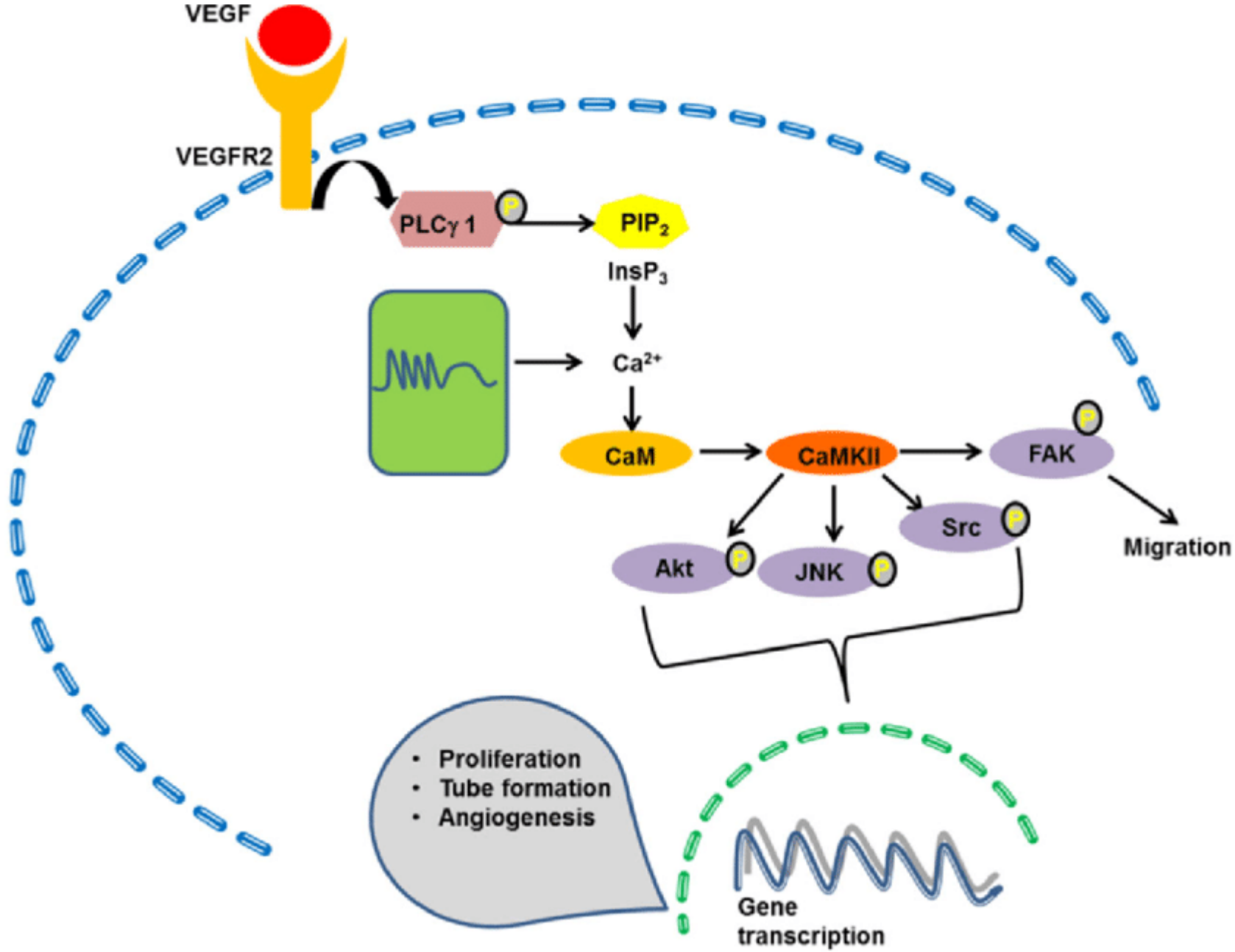


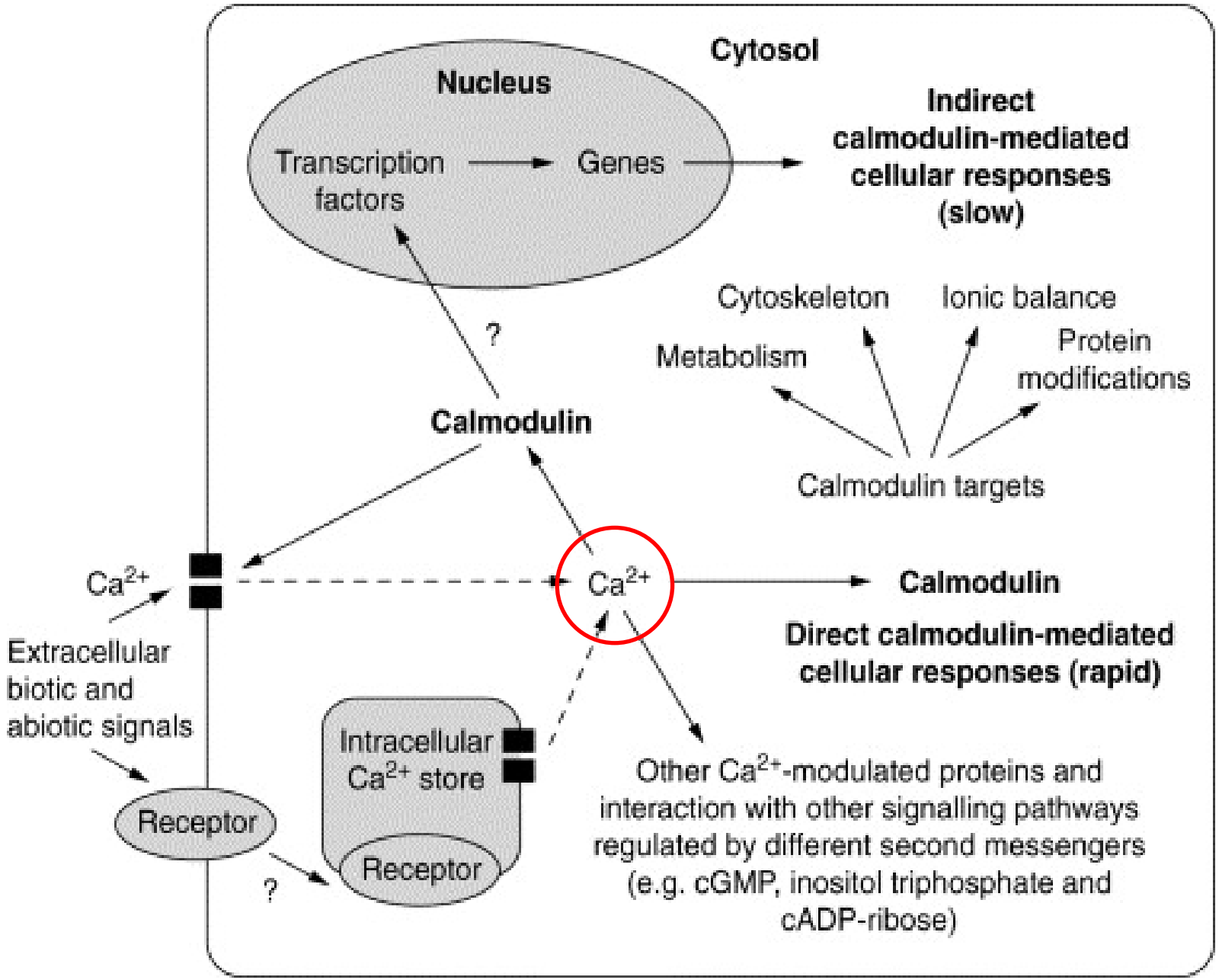
# CAM Kinase

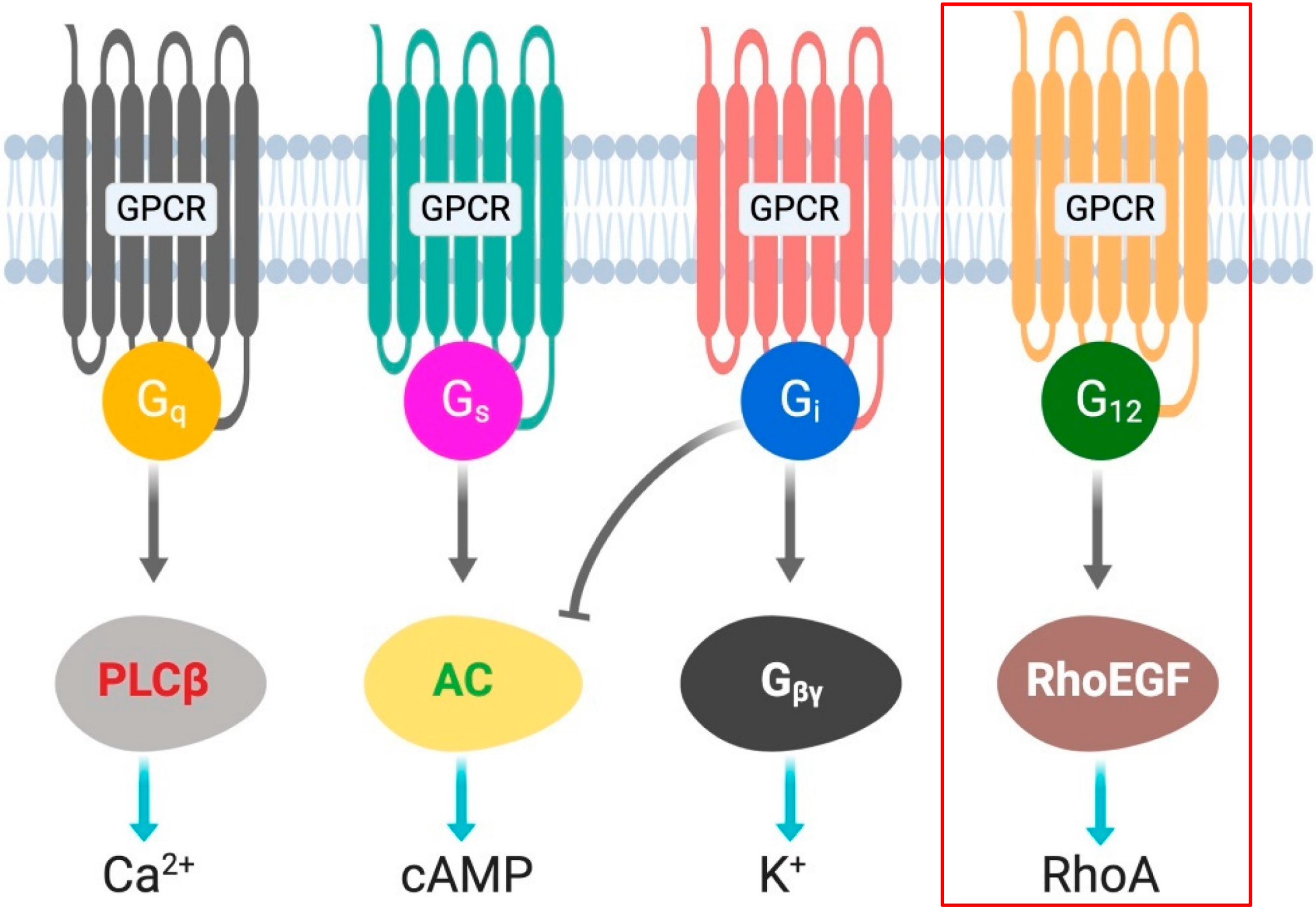

A
Ox Met281/282
Kinase Domain
Ca/CaM
Oligomerization
P
P P
δ
δ+CaM
Ce
AUTOINHIB.
CALMODULIN
B
N
C
Inhibitor
β1
β2
β3
β4
β5
β6
β7
β8
αC
αD
αE
αF
αG
αH
αI
αEF
C
S-shift
Monomer
Dimer
Loading concentration [fringes/ml]
Sedimentation coefficient [S°20,w]

# The stepwise activation of CaM-kinase II




VEGF
VEGFR2
PLCγ 1
P
PIP2
InsP3
Ca2+
CaM
CaMKII
FAK
P
Src
P
Akt
P
JNK
P
Migration
• Proliferation
• Tube formation
• Angiogenesis
Gene
transcription

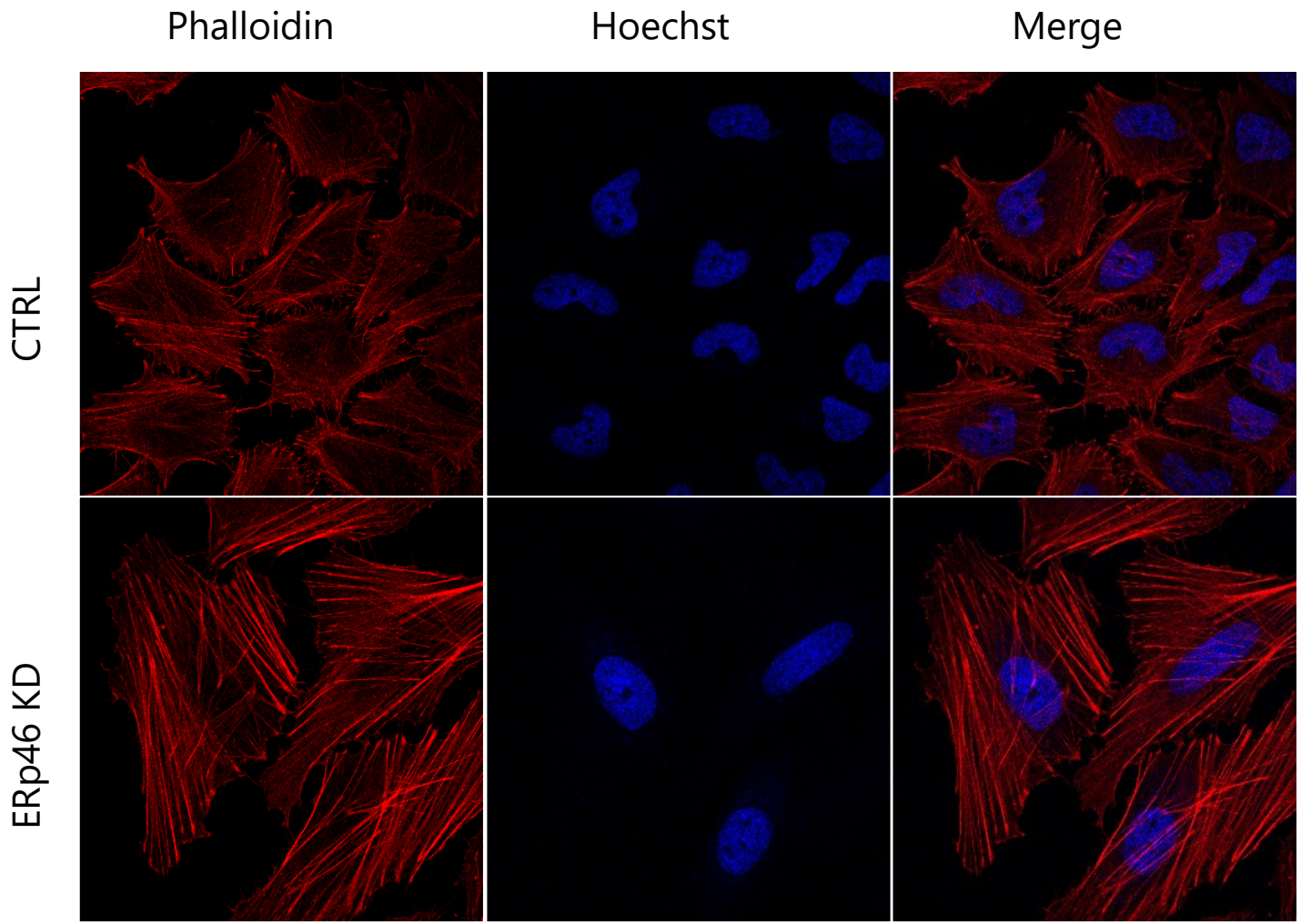

Cytosol
Nucleus
Transcription factors
Genes
Indirect calmodulin-mediated cellular responses (slow)
?
Cytoskeleton
Ionic balance
Metabolism
Protein modifications
Calmodulin
Calmodulin targets
Ca2+
Ca2+
Calmodulin
Direct calmodulin-mediated cellular responses (rapid)
Extracellular biotic and abiotic signals
Intracellular Ca2+ store
Receptor
Receptor
?
Other Ca2+-modulated proteins and interaction with other signalling pathways regulated by different second messengers (e.g. cGMP, inositol triphosphate and cADP-ribose)

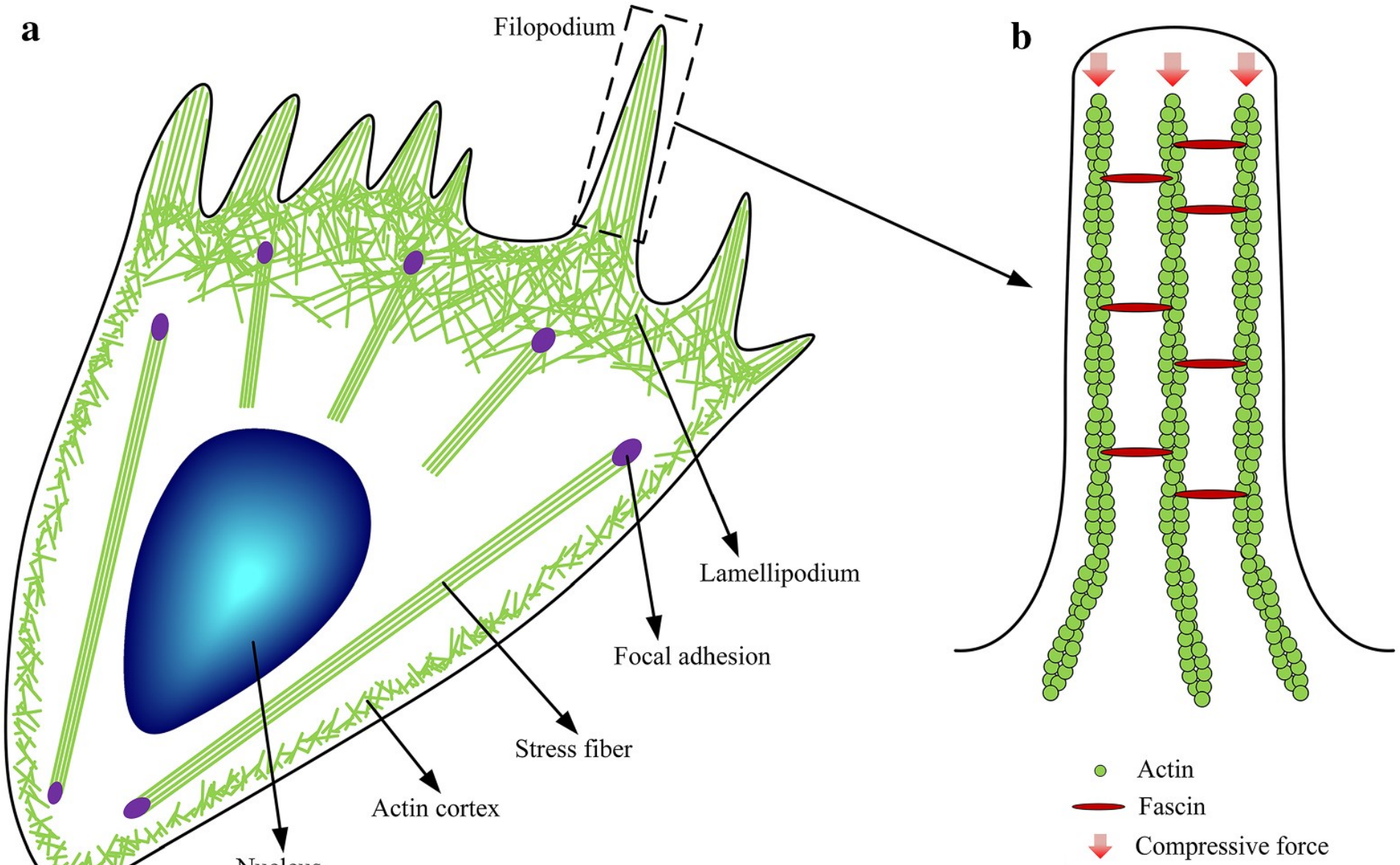

GPCR
GPCR
GPCR
GPCR
$G_q$
$G_s$
$G_i$
$G_{12}$
PLCβ
AC
$G_{\beta\gamma}$
RhoEGF
$Ca^{2+}$
cAMP
$K^+$
RhoA

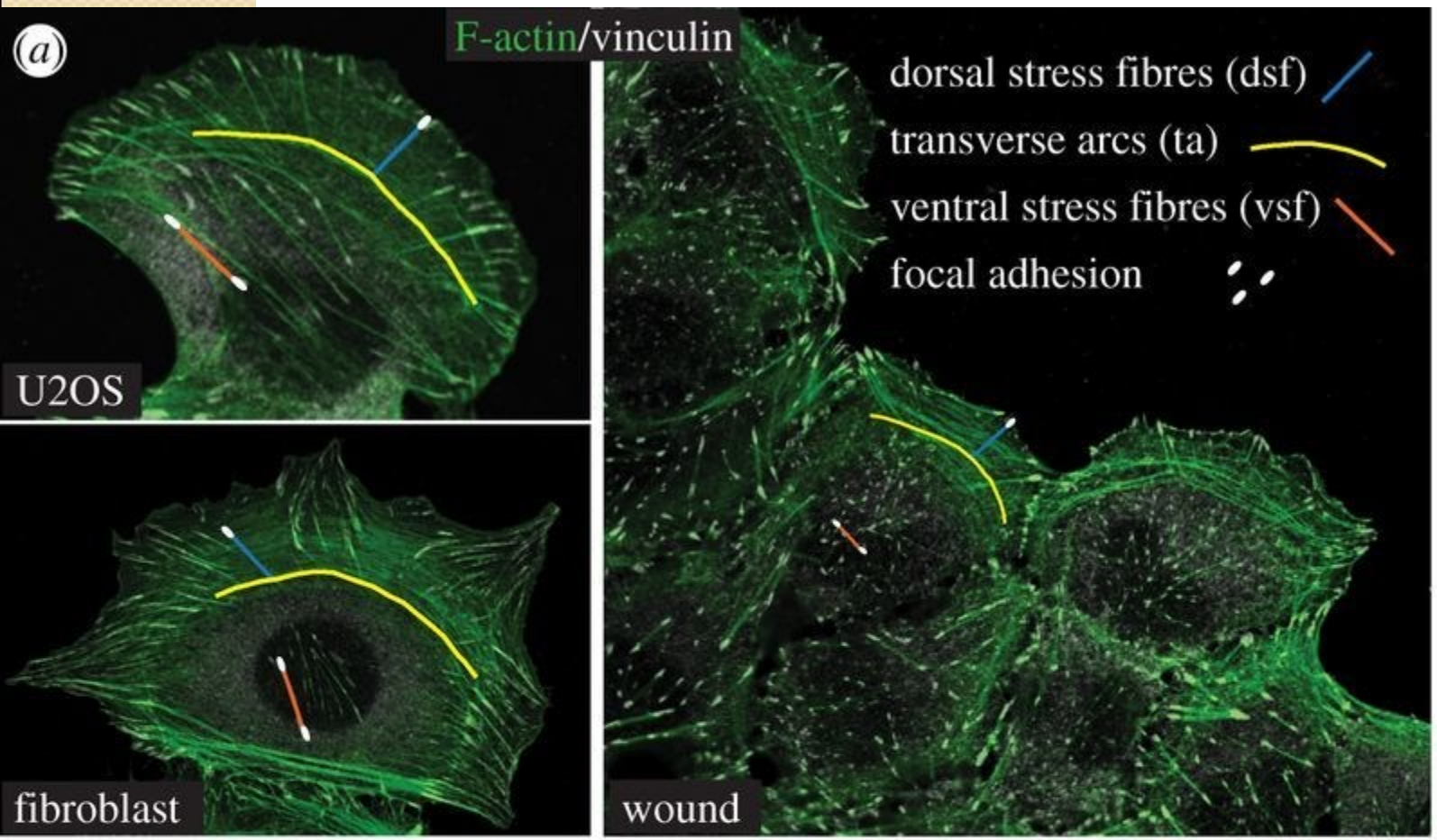
# Recettori accoppiati a G12/13

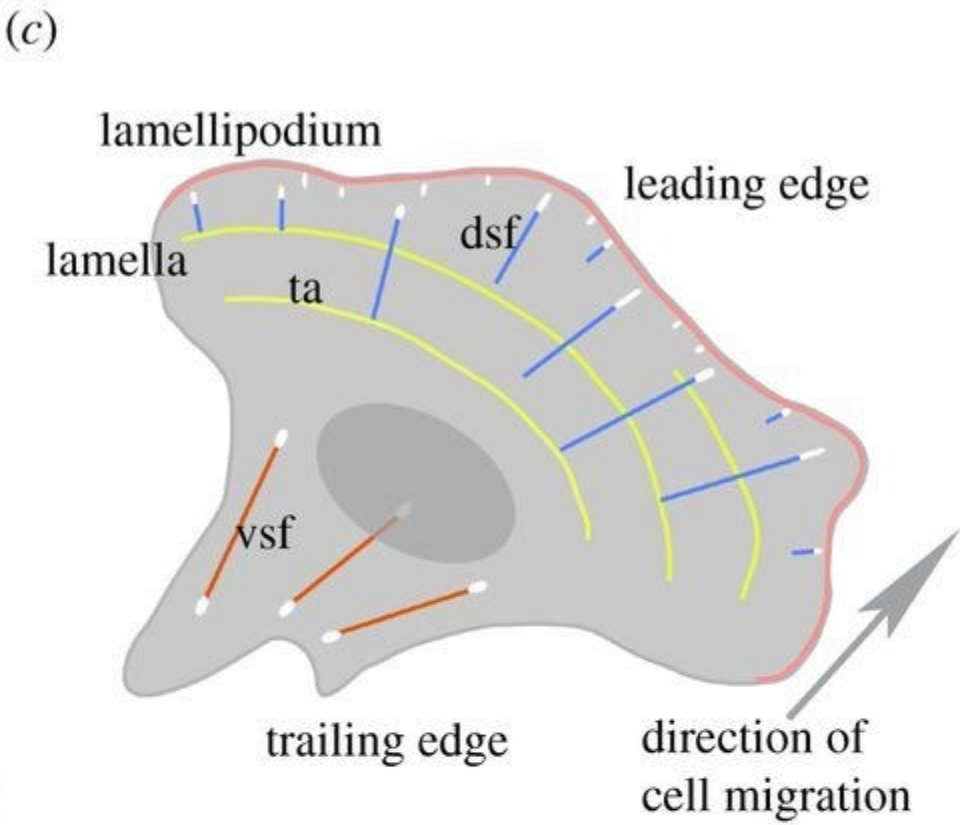
**Table 1 $G\alpha_{12/13}$-associated GPCRs and physiological functions.**

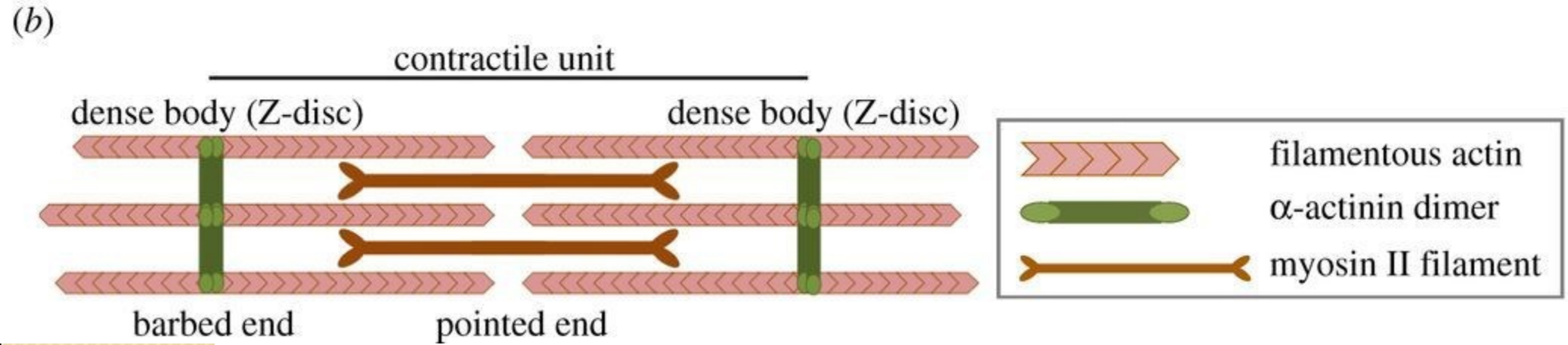
| Receptors | G proteins | Functions | References |
|---|---|---|---|
| Sphingosine 1-phosphate | | | |
| S1P2/S1P3 | $G\alpha_{12/13}$ | Stress fiber formation | 133 |
| S1P1/S1P3/S1P5 | $G\alpha_{12}$ | Inflammation | 62 |
| S1P3 | $G\alpha_{12/13}$ | Inflammation | 134 |
| S1P2 | $G\alpha_{12/13}$ | Myofibroblast contraction | 135 |
| S1P3 | $G\alpha_{13}$ | Cardioprotection | 136 |
| S1P receptor | $G\alpha_{12}$ | Hepatic stellate cell activation | 45 |
| Thrombin | | | |
| PAR1 | $G\alpha_{12}$ | Monocyte migration | 63 |
| PAR1 | $G\alpha_{13}$ | Cell transformation | 137 |
| PAR1 | $G\alpha_{12/13}$ | Endothelial cell permeability | 138 |
| Thrombin receptor | $G\alpha_{12/13}$ | NO production in macrophage | 139 |
| Thrombin receptor | $G\alpha_{12}$ | Stress fiber accumulation | 140 |
| Thrombin and thromboxane A2 | $G\alpha_{12/13}$ | Platelet activation | 25 |
| Lysophosphatidic acid | | | |
| LPA4 | $G\alpha_{12/13}$ | Limits proper adipose tissue expansion and remodeling in diet-induced obesity | 141 |
| LPA4 | $G\alpha_{12/13}$ | Hypertensive response | 122 |
| LPA4/LPA6 | $G\alpha_{12/13}$ | Angiogenesis | 142 |
| LPA receptor | $G\alpha_{13}$ | Stress fiber formation | 140 |
| Angiotensin | | | |
| AT1R | $G\alpha_{12/13}$ | Hyperplasia of cardiac fibroblasts | 143 |
| AT1R | $G\alpha_{12/13}$ | Vascular endothelial dysfunction | 144 |
| Endothelin | | | |
| $ET_A$ | $G\alpha_{12}$ | Stress fiber accumulation | 140 |
| ATP | | | |
| P2Y6 | $G\alpha_{12/13}$ | Cardiac fibrosis | 131 |
| Adenosine | | | |
| A1/A2a/A2b/A3 | $G\alpha_{12}$ | Fatty acid oxidation | 38 |
| Bradykinin | | | |
| B2 | $G\alpha_{13}$ | Stress fiber formation | 140 |
| Serotonin | | | |
| 5-HT2C | $G\alpha_{13}$ | Stress fiber formation | 140 |
| Vasopressin | | | |
| V1A | $G\alpha_{12}$ | Stress fiber accumulation | 140 |


Phalloidin
Hoechst
Merge
CTRL
ERp46 KD


a
Filopodium
Lamellipodium
Focal adhesion
Stress fiber
Actin cortex
Nucleus
b
Actin
Fascin
Compressive force


(a)
F-actin/vinculin
dorsal stress fibres (dsf)
transverse arcs (ta)
ventral stress fibres (vsf)
focal adhesion
U2OS
fibroblast
wound


(c)
lamellipodium
leading edge
lamella
dsf
ta
vsf
trailing edge
direction of
cell migration


(b)
contractile unit
dense body (Z-disc)
dense body (Z-disc)
barbed end
pointed end
filamentous actin
α-actinin dimer
myosin II filament

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| Thrombin | | | |
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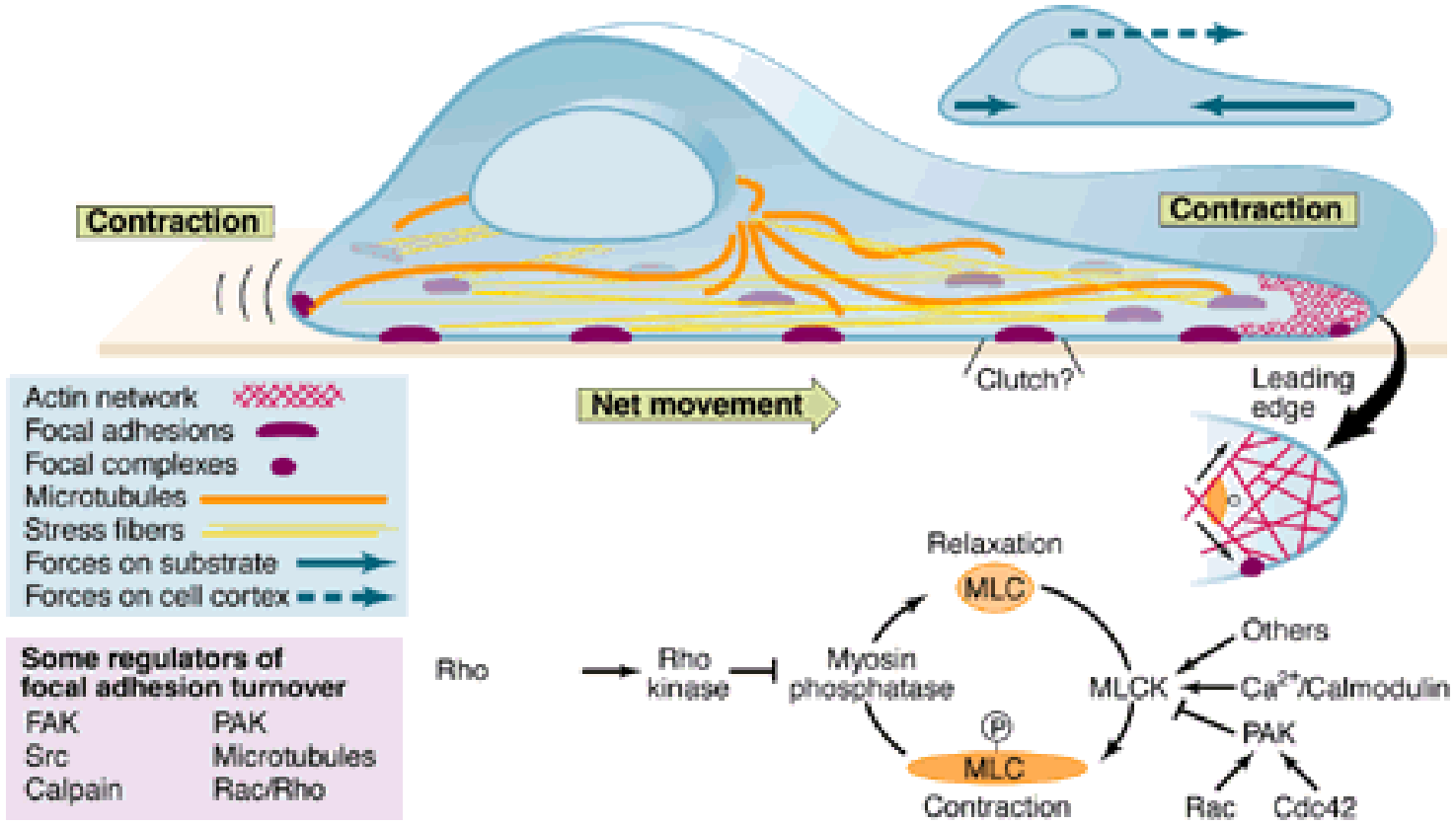
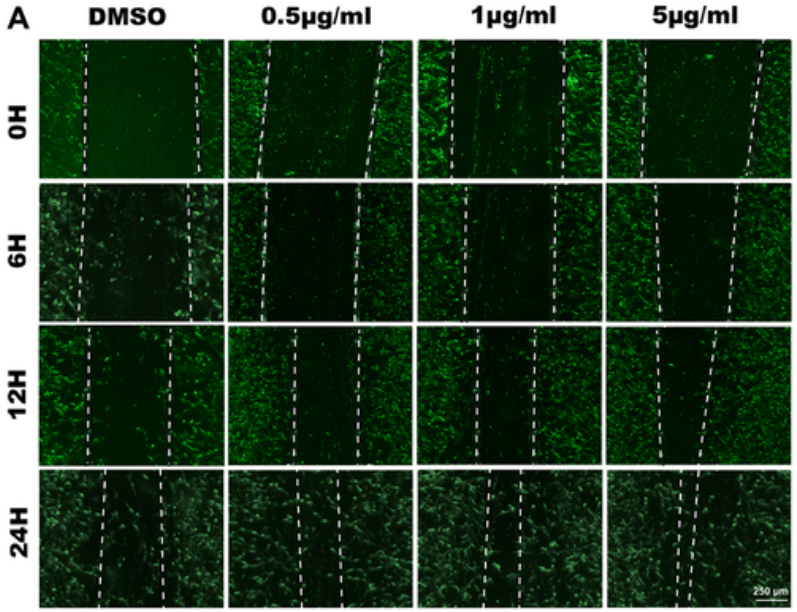
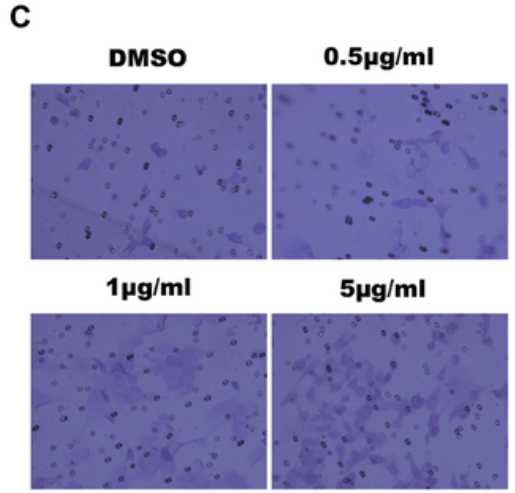
| Adenosine | | | |
| A1/A2a/A2b/A3 | $G\alpha_{12}$ | Fatty acid oxidation | 38 |
| Bradykinin | | | |
| B2 | $G\alpha_{13}$ | Stress fiber formation | 140 |
| Serotonin | | | |
| 5-HT2C | $G\alpha_{13}$ | Stress fiber formation | 140 |
| Vasopressin | | | |
| V1A | $G\alpha_{12}$ | Stress fiber | 140 |

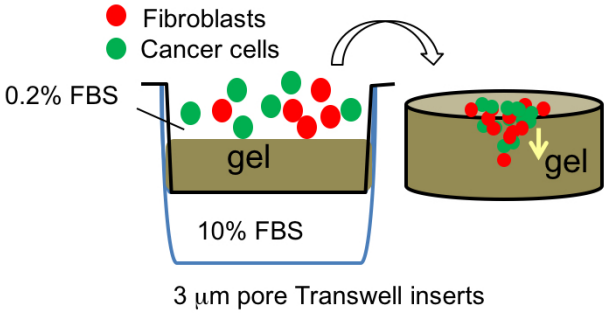
# Migration

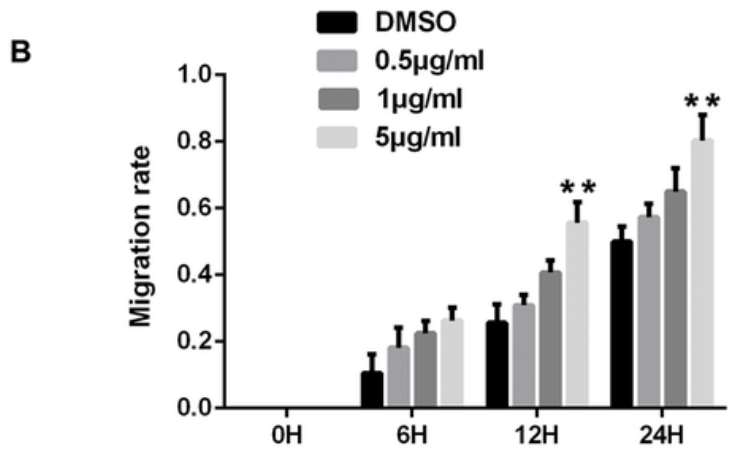

Contraction
Contraction
Clutch?
Leading edge
Net movement
Actin network
Focal adhesions
Focal complexes
Microtubules
Stress fibers
Forces on substrate
Forces on cell cortex
Some regulators of focal adhesion turnover
FAK
Src
Calpain
PAK
Microtubules
Rac/Rho
Rho
Rho kinase
Myosin phosphatase
Relaxation
MLC
MLCK
Others
Ca2+/Calmodulin
PAK
Rac
Cdc42
P
MLC
Contraction

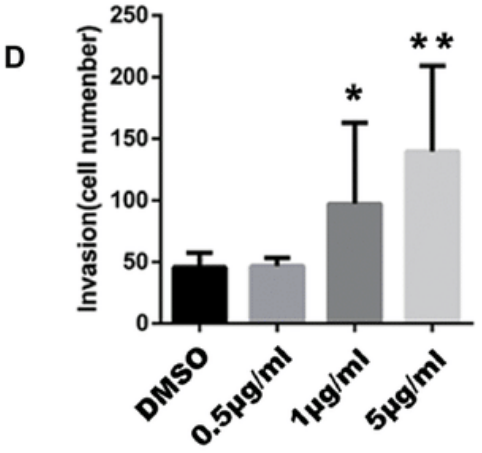
# Wound-healing and invasion assays



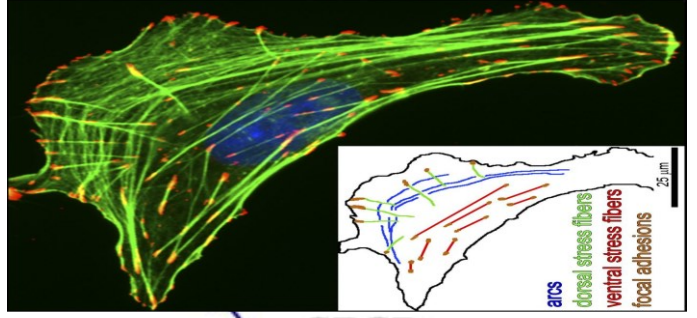



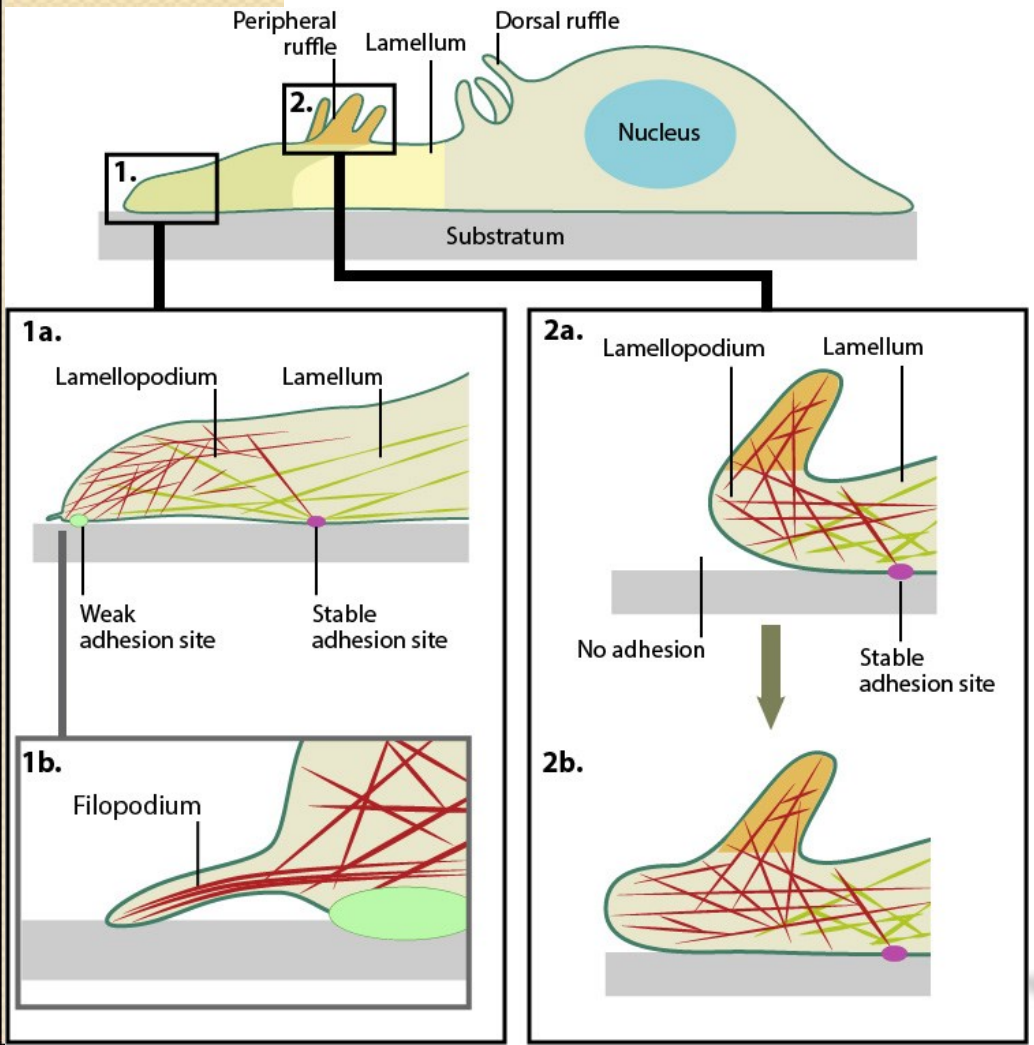



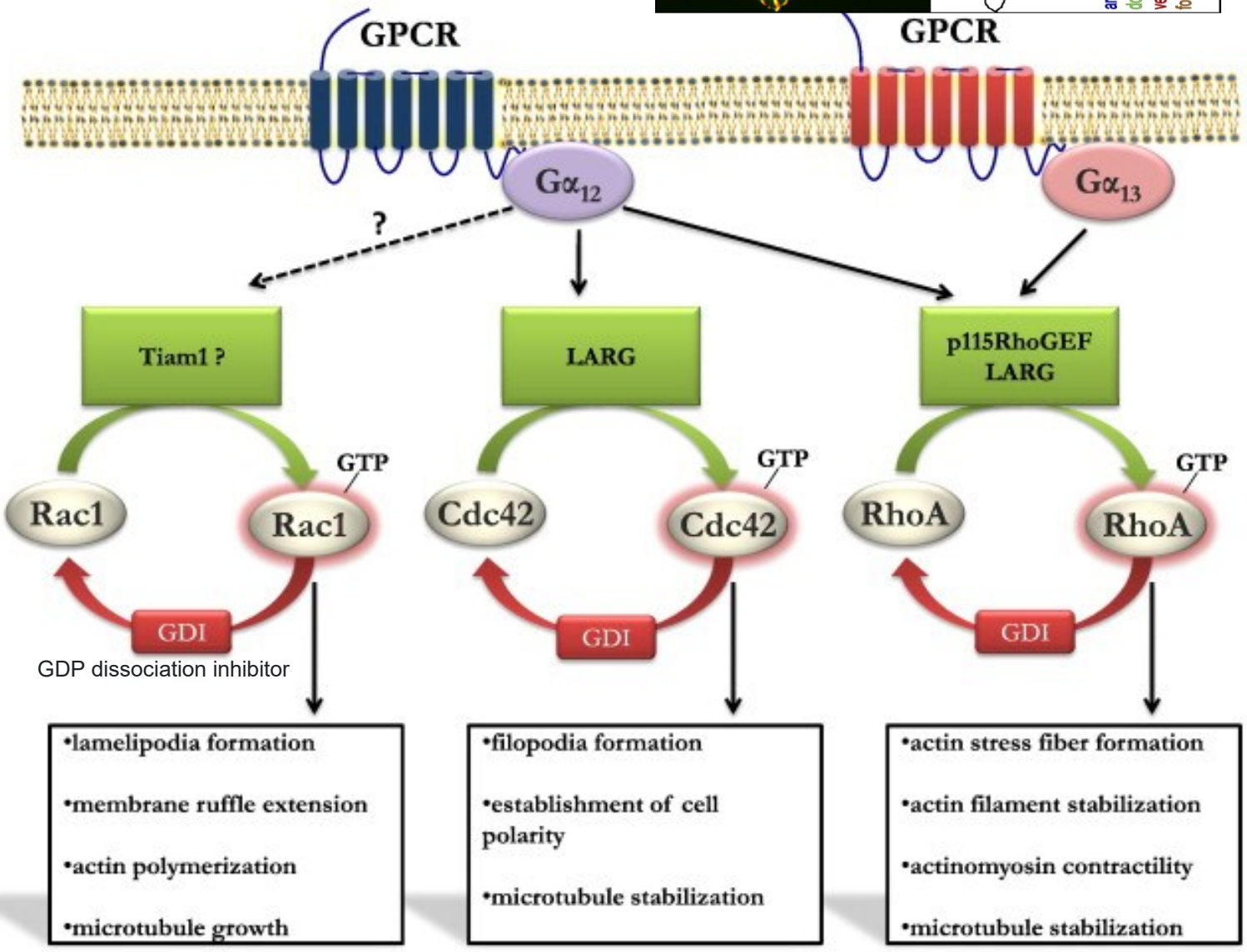



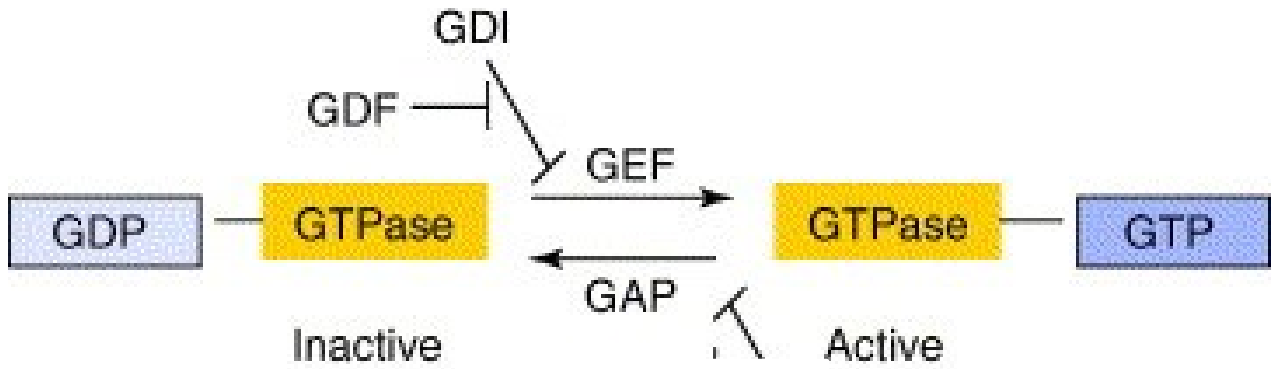
# Recettori accoppiati a $G_{12/13}$



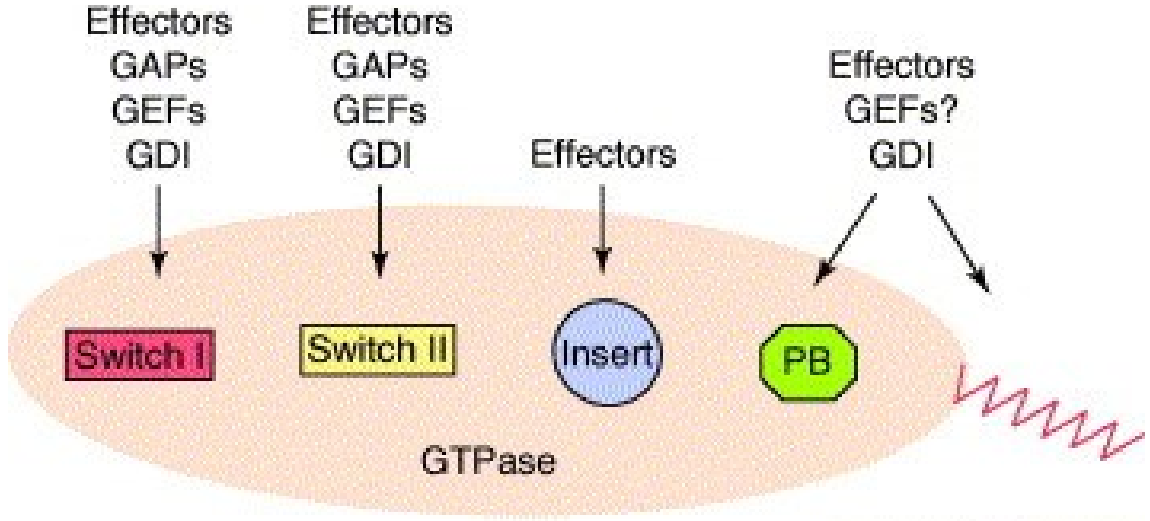



# Small GTPase cycle

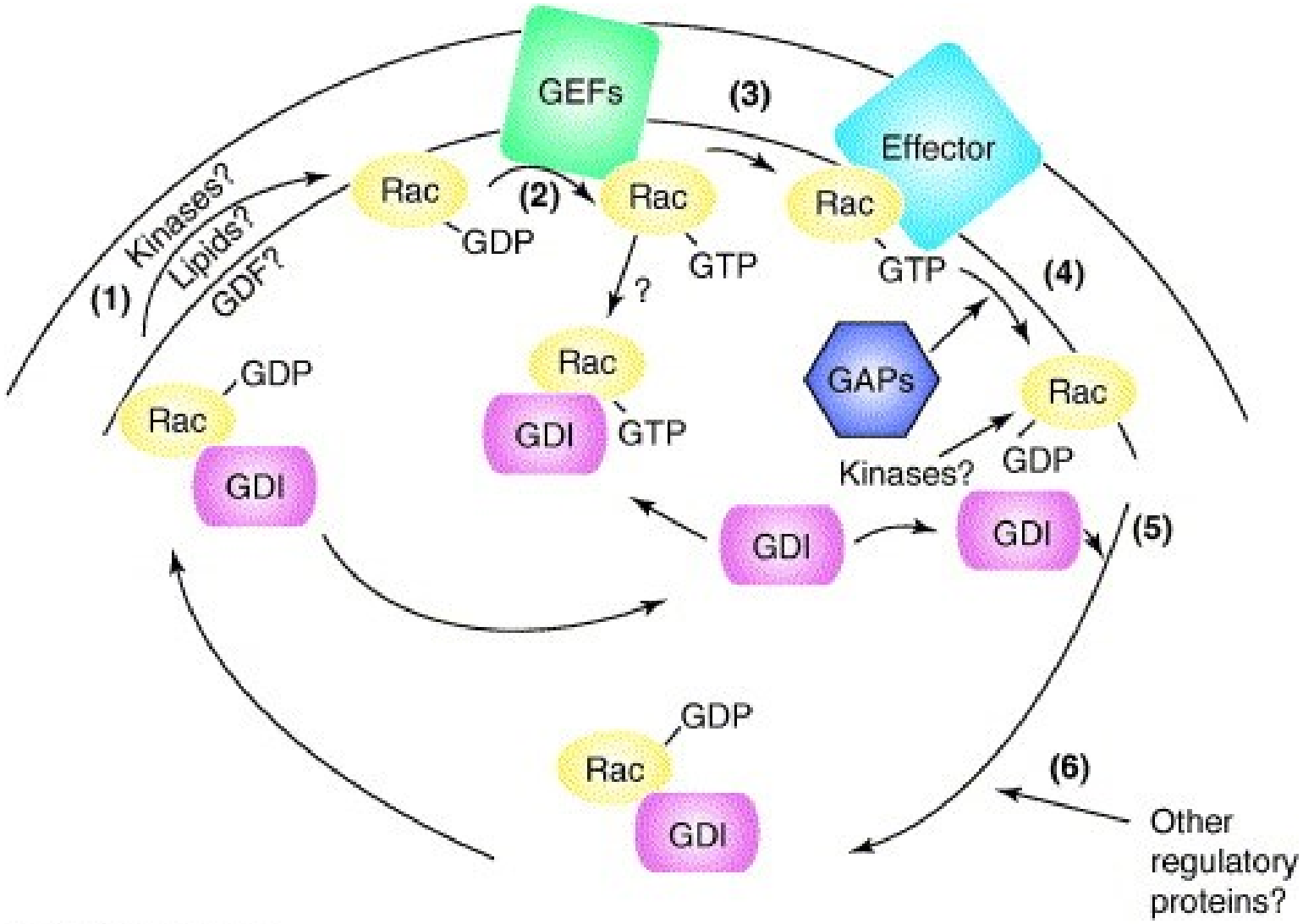



TRENDS in Cell Biology

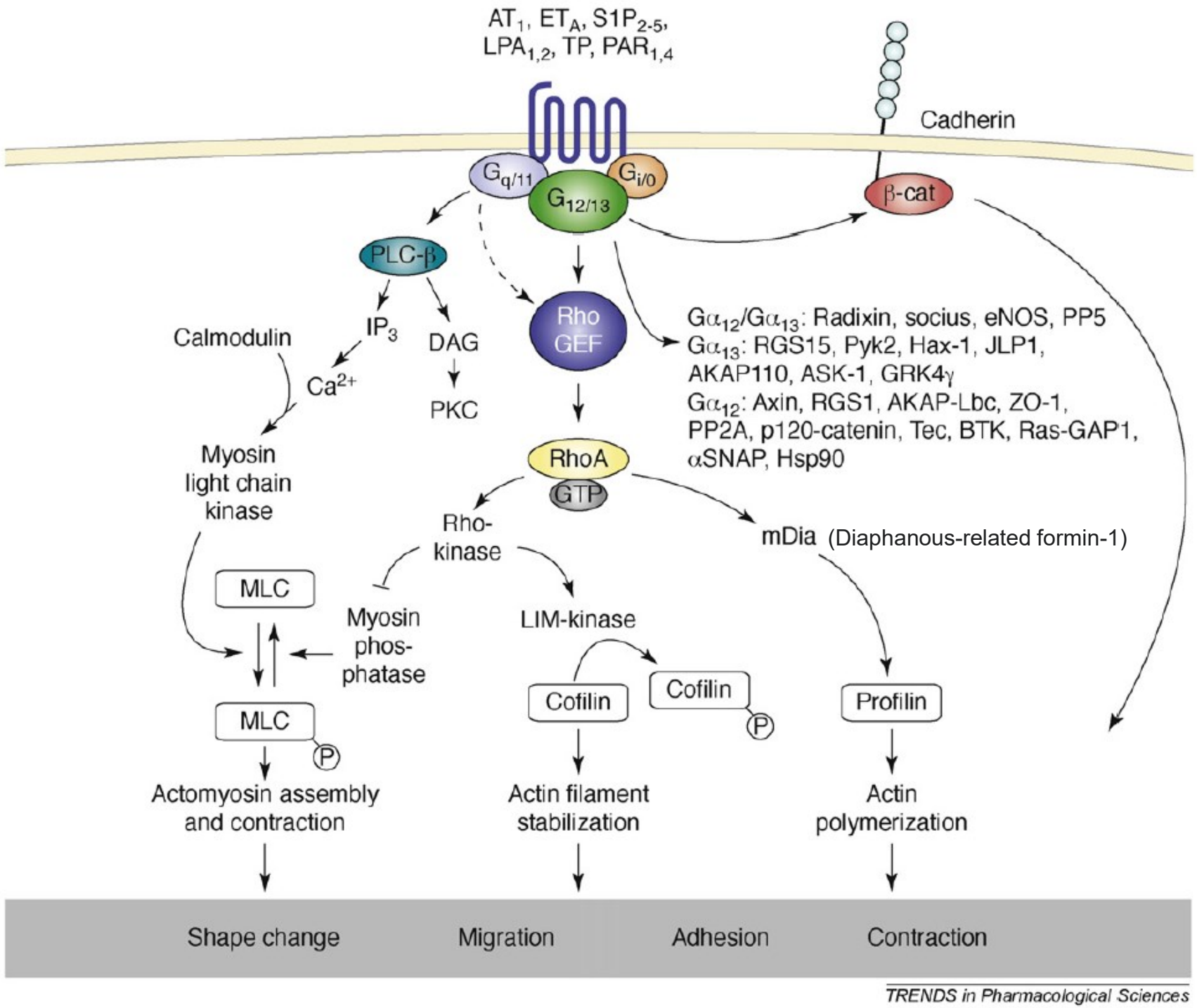


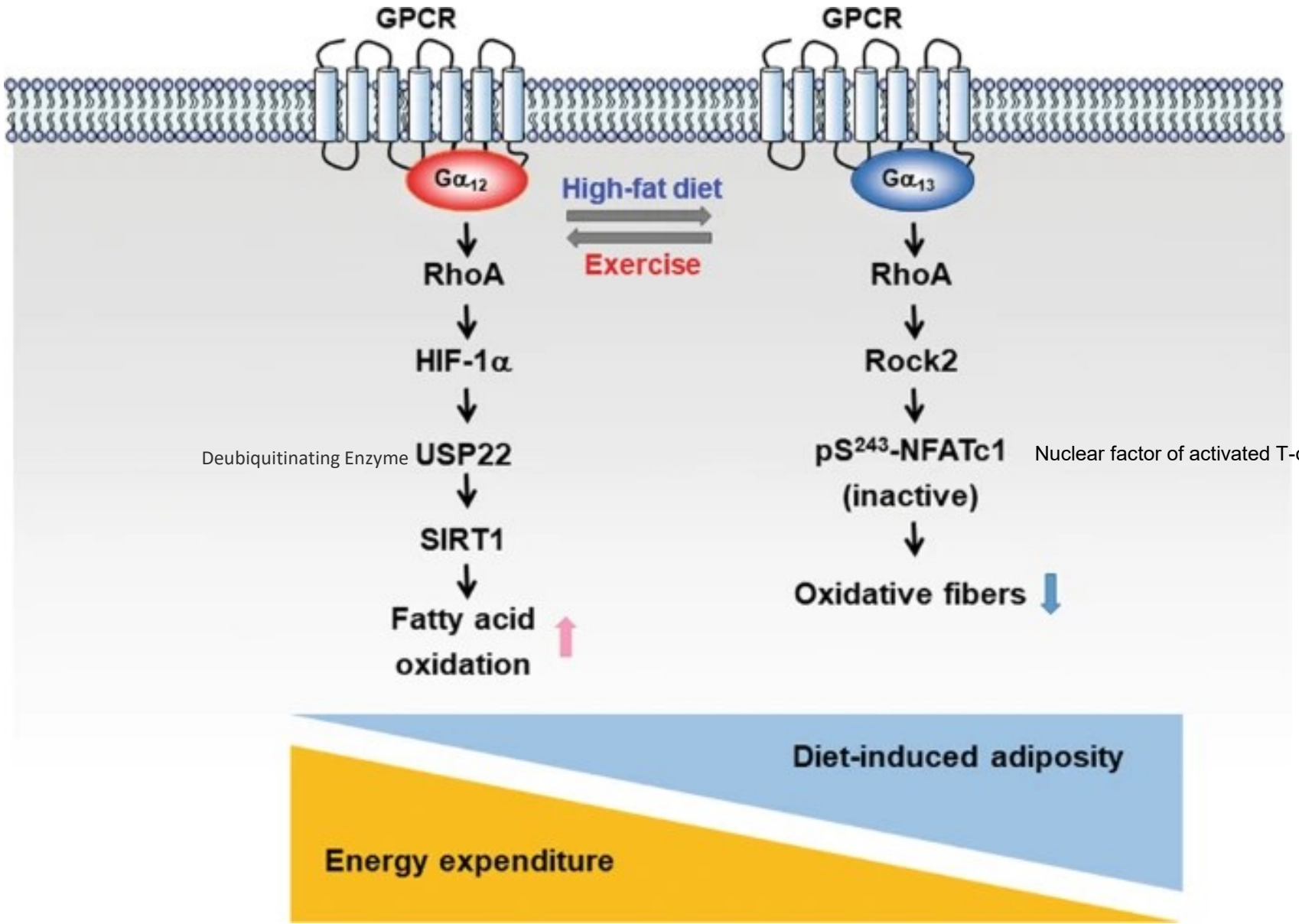

TRENDS in Cell Biology

# GDP dissociation inhibitor

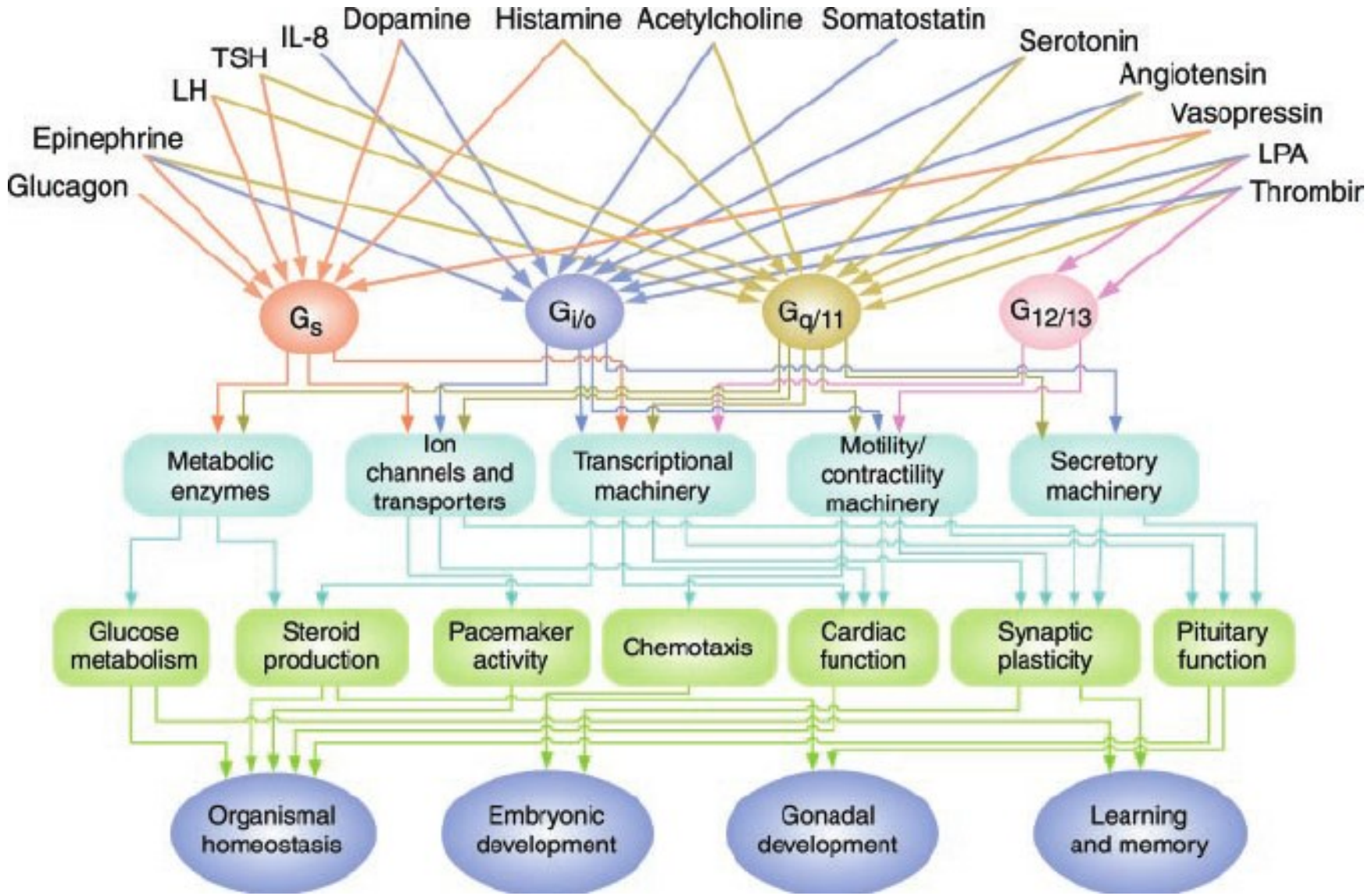



TRENDS in Cell Biology

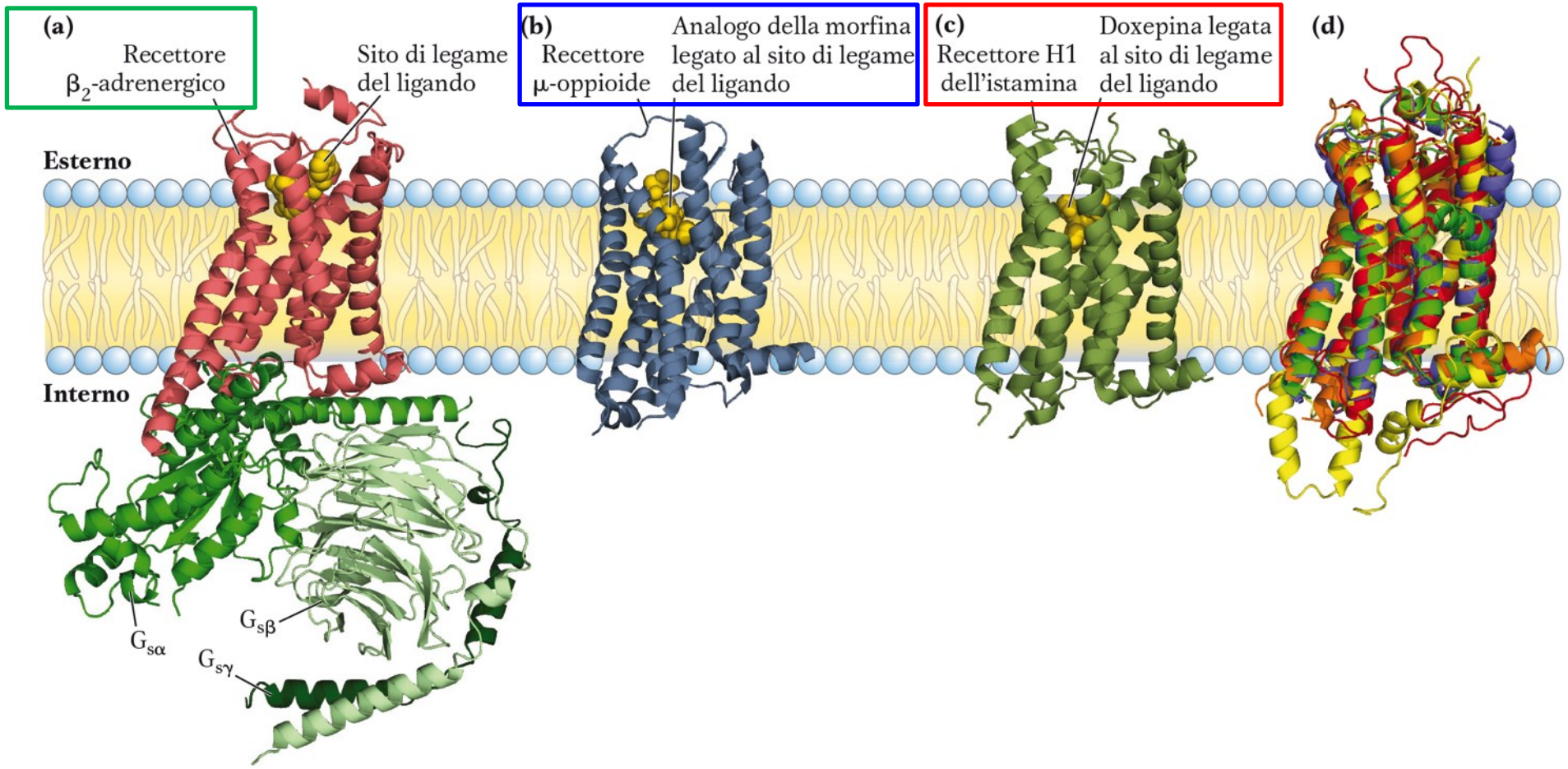

AT1, ETA, S1P2-5,
LPA1,2, TP, PAR1,4
Cadherin
Gq/11
Gi/0
G12/13
β-cat
PLC-β
IP3
DAG
Calmodulin
Ca2+
PKC
Rho GEF
Gα12/Gα13: Radixin, socius, eNOS, PP5
Gα13: RGS15, Pyk2, Hax-1, JLP1,
AKAP110, ASK-1, GRK4γ
Gα12: Axin, RGS1, AKAP-Lbc, ZO-1,
PP2A, p120-catenin, Tec, BTK, Ras-GAP1,
αSNAP, Hsp90
Myosin
light chain
kinase
RhoA
GTP
Rho-
kinase
mDia (Diaphanous-related formin-1)
MLC
Myosin
phos-
phatase
LIM-kinase
Cofilin
Cofilin
P
Profilin
MLC
P
Actomyosin assembly
and contraction
Actin filament
stabilization
Actin
polymerization
Shape change
Migration
Adhesion
Contraction
TRENDS in Pharmacological Sciences


GPCR
GPCR
Gα12
Gα13
High-fat diet
Exercise
RhoA
HIF-1α
Deubiquitinating Enzyme USP22
SIRT1
Fatty acid oxidation
RhoA
Rock2
pS243-NFATc1 (inactive)
Nuclear factor of activated T-cells, cytoplasmic 1
Oxidative fibers
Diet-induced adiposity
Energy expenditure

# La biosegnalazione via GPCR



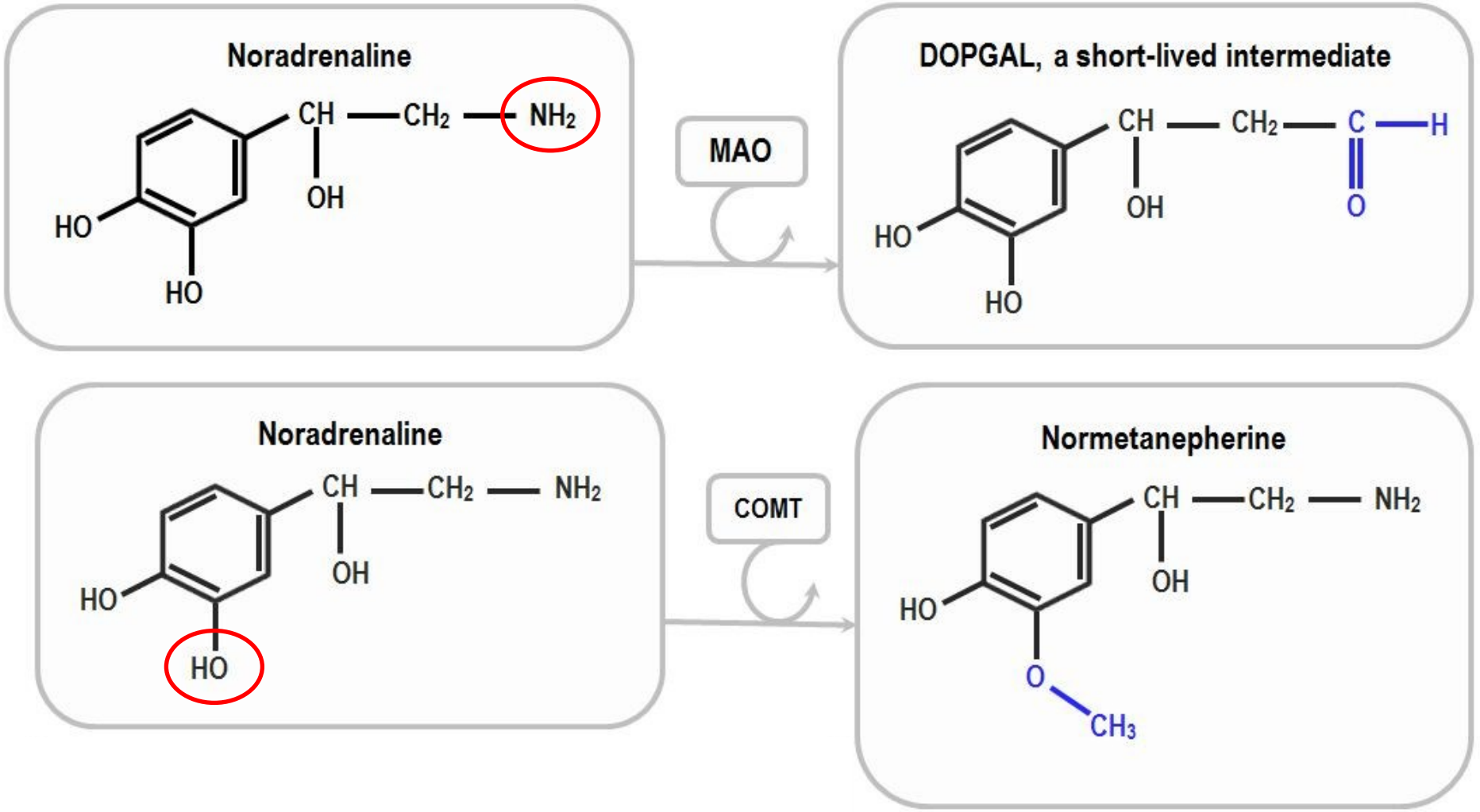
# Somiglianze di struttura nei GPCR



# Terminazione della risposta

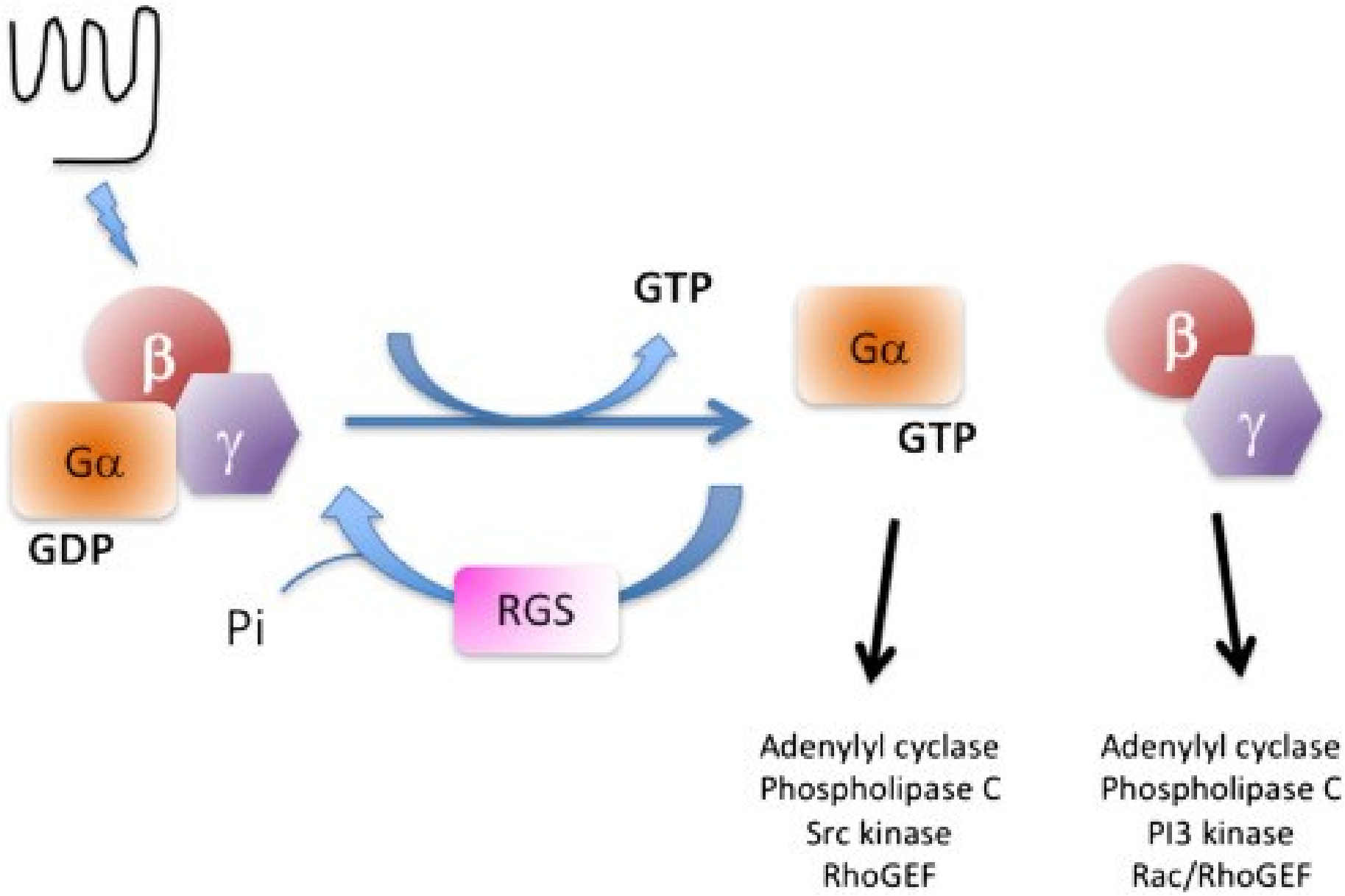
- *Re-uptake* e degradazione: Monoamino ossidasi MAO e catecol-O-metiltrasferasi (COMT)
- Attività GTPasi intrinseca delle G protein e GAP accessorie (RGS)
- G protein-coupled receptor kinases (GRK), PKA, etc…..
- Arrestins
- Fosfodiesterasi (PDE)/ pompa del Ca+2

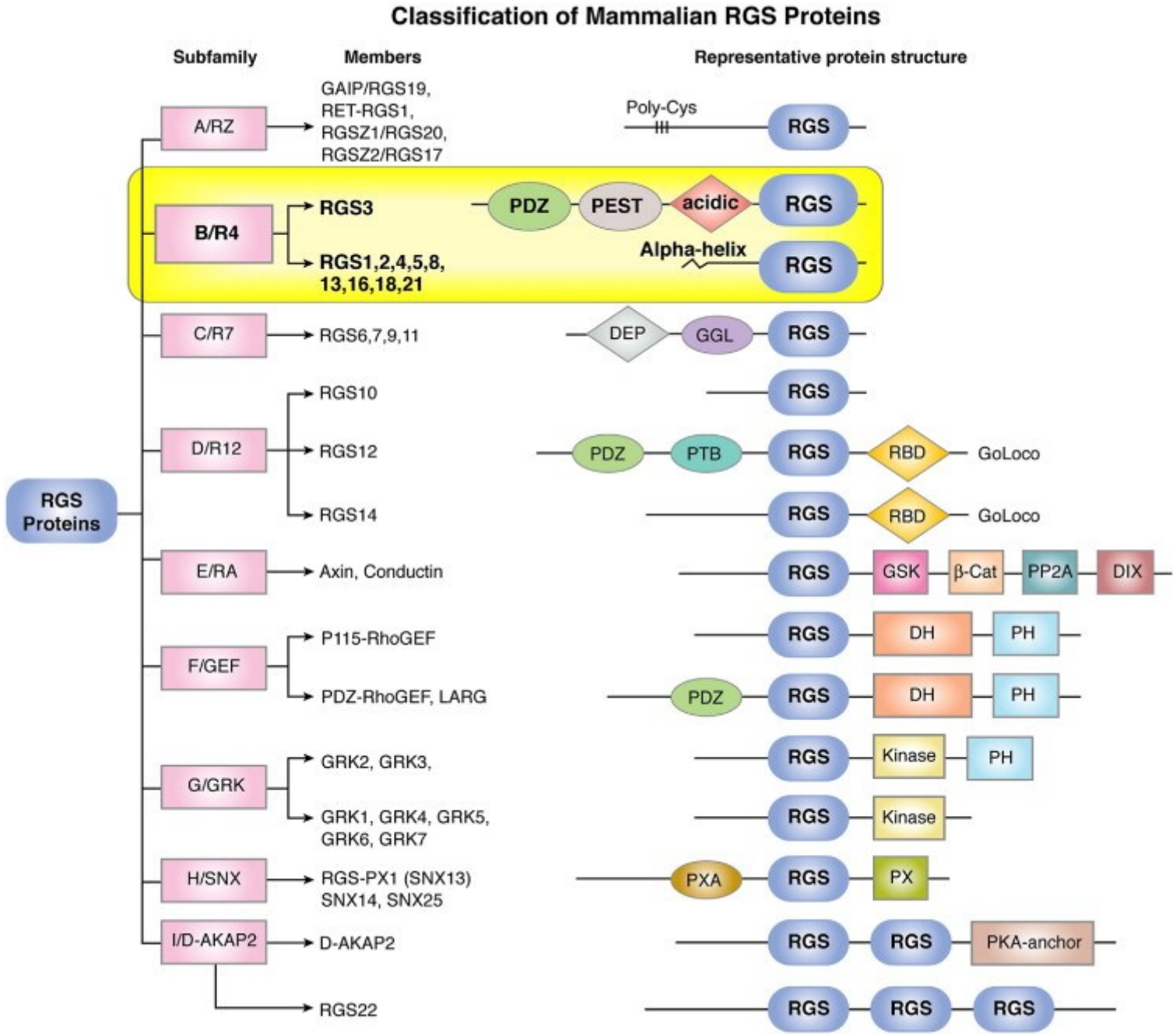
# Azione delle MAO e COMT

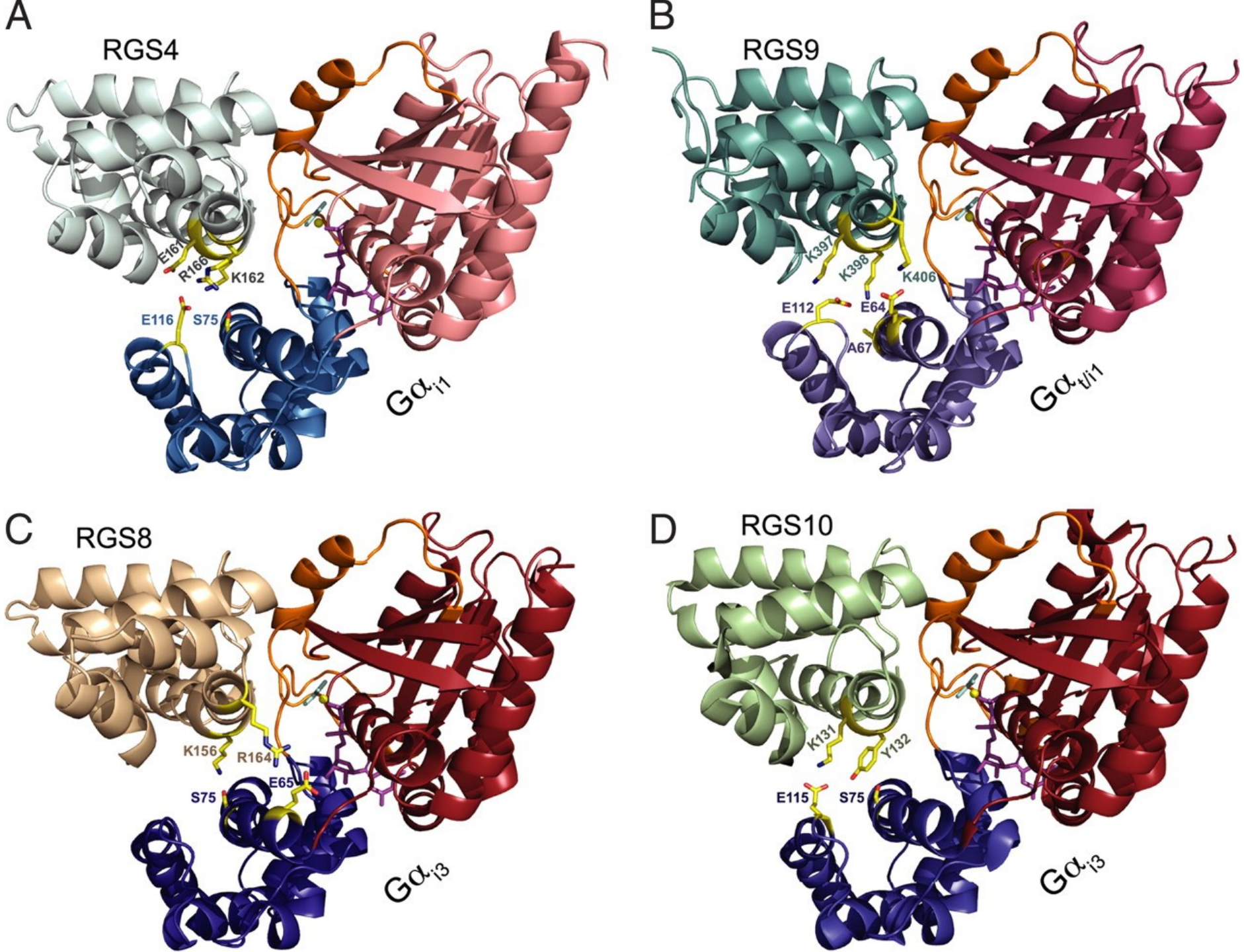


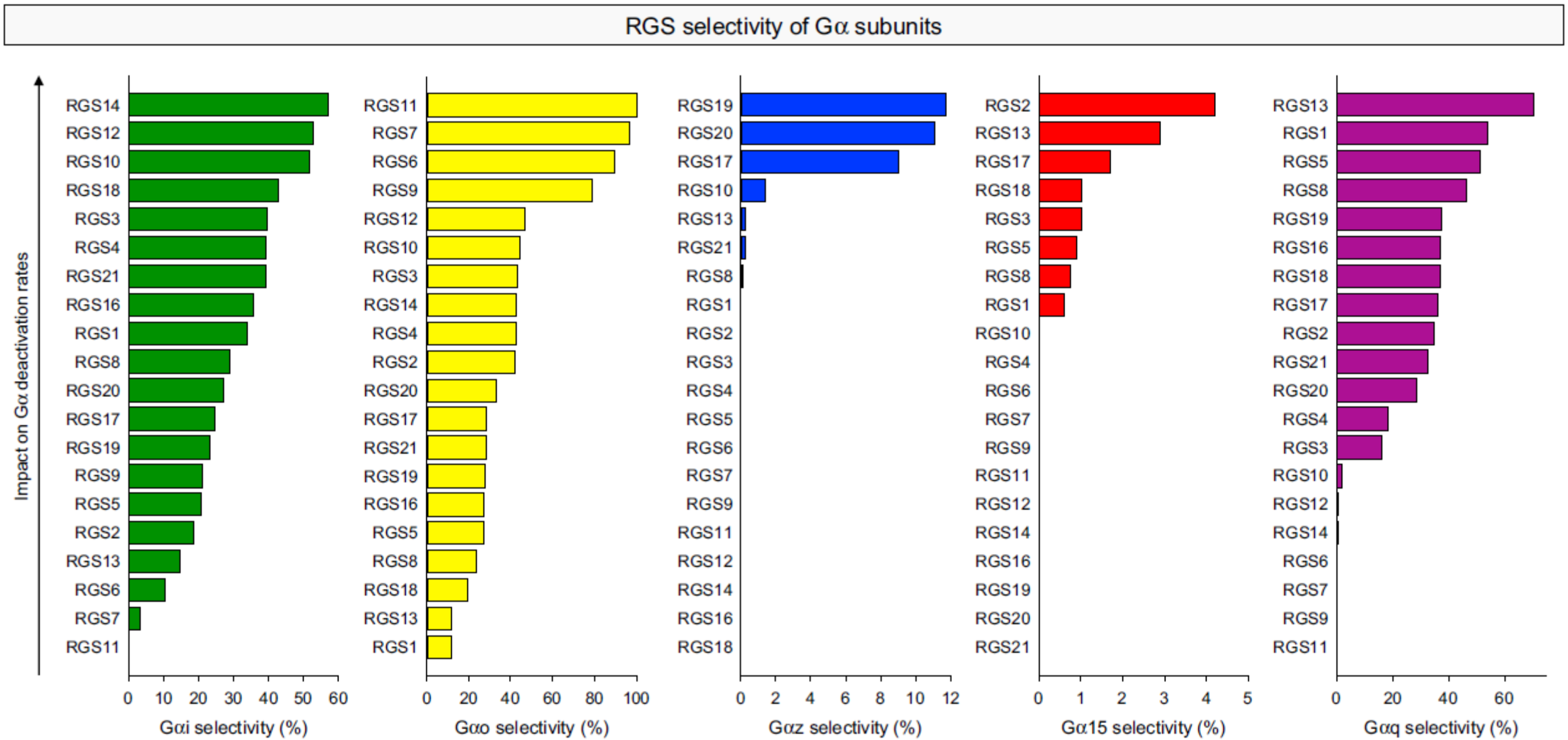
# Terminazione della risposta

- Monoamino ossidasi MAO e catecol-O-metiltrasferasi (COMT)
- Attività GTPasi intrinseca alle G protein e GAP accessorie (RGS)
- G protein coupled receptor kinases (GRK), PKA, etc
- Arrestin
- Fosfodiesterasi (PDE)/ pompa del Ca+2


β
γ
Gα
GDP
GTP
Gα
GTP
β
γ
Pi
RGS
Adenylyl cyclase
Phospholipase C
Src kinase
RhoGEF
Adenylyl cyclase
Phospholipase C
PI3 kinase
Rac/RhoGEF


Classification of Mammalian RGS Proteins
Subfamily
Members
Representative protein structure
RGS Proteins
A/RZ
GAIP/RGS19, RET-RGS1, RGSZ1/RGS20, RGSZ2/RGS17
Poly-Cys
RGS
B/R4
RGS3
RGS1,2,4,5,8, 13,16,18,21
PDZ
PEST
acidic
Alpha-helix
C/R7
RGS6,7,9,11
DEP
GGL
D/R12
RGS10
RGS12
RGS14
PTB
RBD
GoLoco
E/RA
Axin, Conductin
GSK
β-Cat
PP2A
DIX
F/GEF
P115-RhoGEF
PDZ-RhoGEF, LARG
DH
PH
G/GRK
GRK2, GRK3,
GRK1, GRK4, GRK5, GRK6, GRK7
Kinase
H/SNX
RGS-PX1 (SNX13) SNX14, SNX25
PXA
PX
I/D-AKAP2
D-AKAP2
PKA-anchor
RGS22

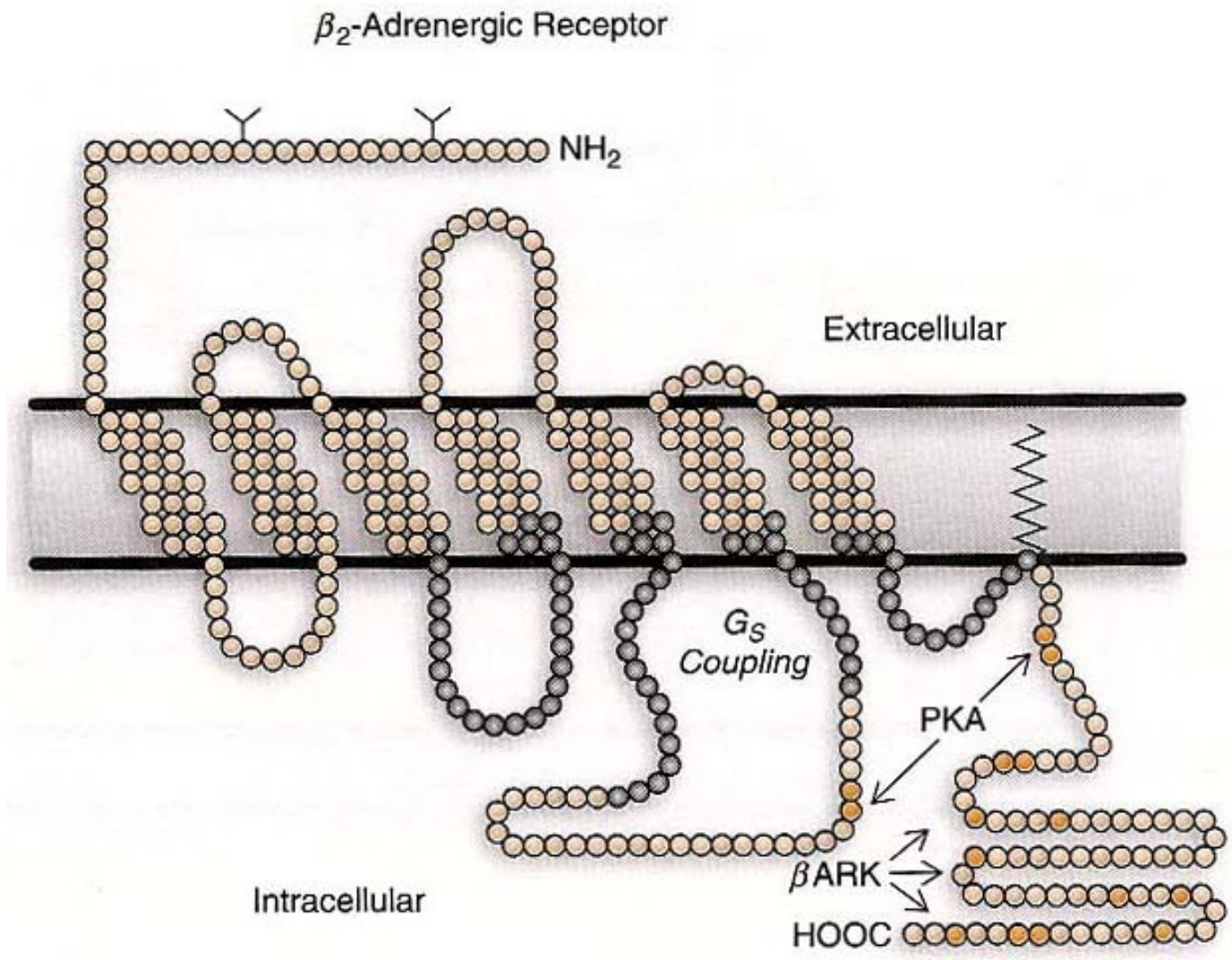

A
RGS4
E161
R166
K162
E116
S75
Gαi1
B
RGS9
K397
K398
K406
E112
E64
A67
Gαt/i1
C
RGS8
K156
R164
E65
S75
Gαi3
D
RGS10
K131
Y132
E115
S75
Gαi3


RGS selectivity of Gα subunits
Impact on Gα deactivation rates
RGS14
RGS12
RGS10
RGS18
RGS3
RGS4
RGS21
RGS16
RGS1
RGS8
RGS20
RGS17
RGS19
RGS9
RGS5
RGS2
RGS13
RGS6
RGS7
RGS11
0 10 20 30 40 50 60
Gαi selectivity (%)
RGS11
RGS7
RGS6
RGS9
RGS12
RGS10
RGS3
RGS14
RGS4
RGS2
RGS20
RGS17
RGS21
RGS19
RGS16
RGS5
RGS8
RGS18
RGS13
RGS1
0 20 40 60 80 100
Gαo selectivity (%)
RGS19
RGS20
RGS17
RGS10
RGS13
RGS21
RGS8
RGS1
RGS2
RGS3
RGS4
RGS5
RGS6
RGS7
RGS9
RGS11
RGS12
RGS14
RGS16
RGS18
0 2 4 6 8 10 12
Gαz selectivity (%)
RGS2
RGS13
RGS17
RGS18
RGS3
RGS5
RGS8
RGS1
RGS10
RGS4
RGS6
RGS7
RGS9
RGS11
RGS12
RGS14
RGS16
RGS19
RGS20
RGS21
0 1 2 3 4 5
Gα15 selectivity (%)
RGS13
RGS1
RGS5
RGS8
RGS19
RGS16
RGS18
RGS17
RGS2
RGS21
RGS20
RGS4
RGS3
RGS10
RGS12
RGS14
RGS6
RGS7
RGS9
RGS11
0 20 40 60
Gαq selectivity (%)

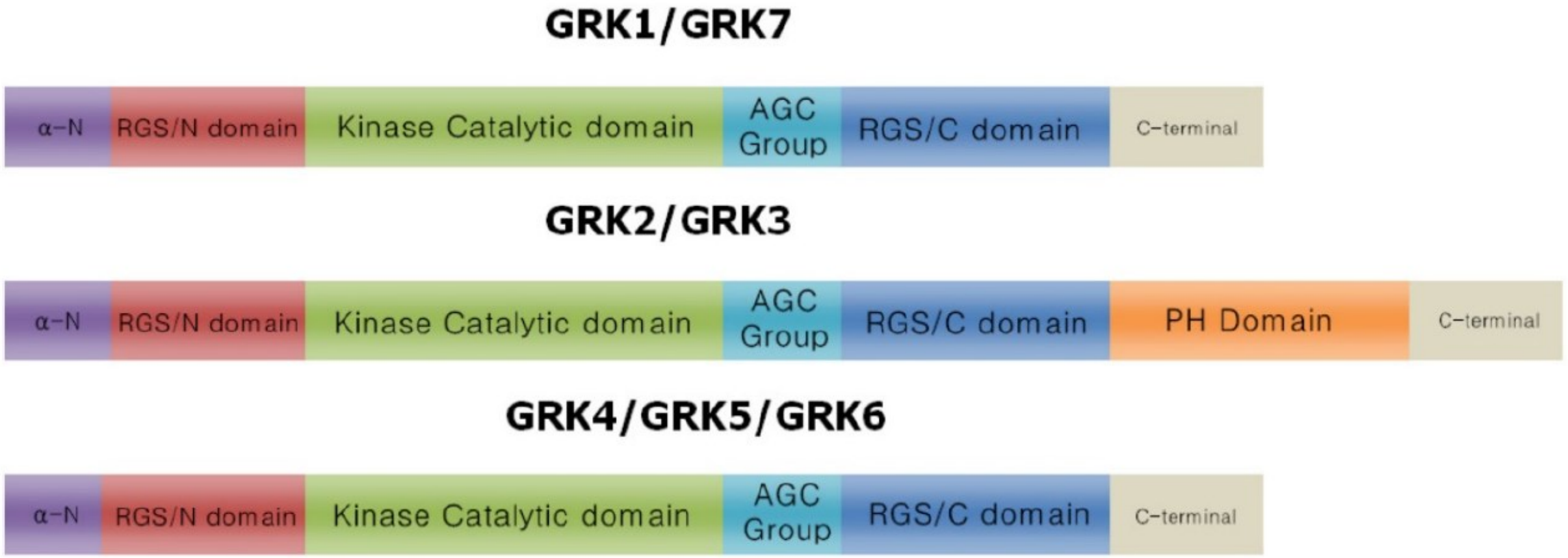
# Terminazione della risposta

- Monoamino ossidasi MAO e catecol-O-metiltrasferasi (COMT)
- Attività GTPasi intrinseca alle G protein e GAP accessorie (RGS)
- G protein coupled receptor kinases (GRK), PKA, etc
- Arrestin
- Fosfodiesterasi (PDE)/ pompa del Ca+2

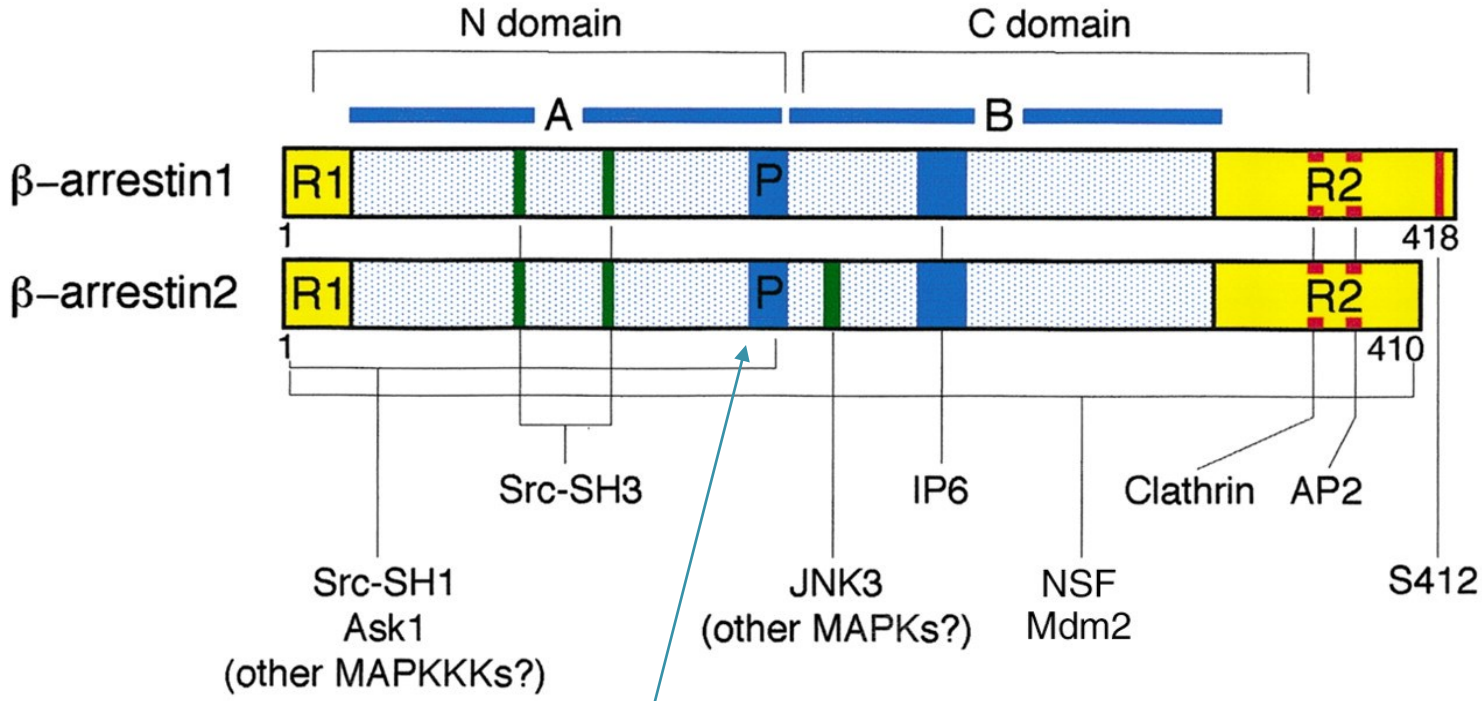
# GPCR desensitization



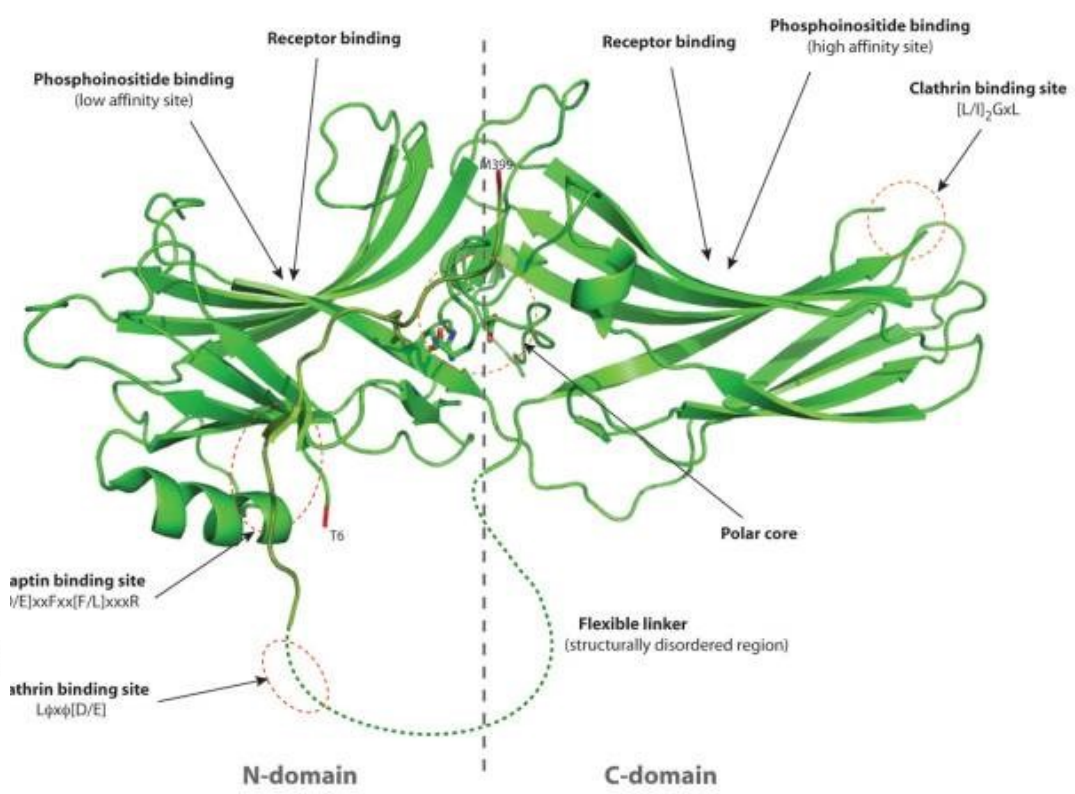
# G protein coupled receptor kinase: GRK



# Beta-arrestins



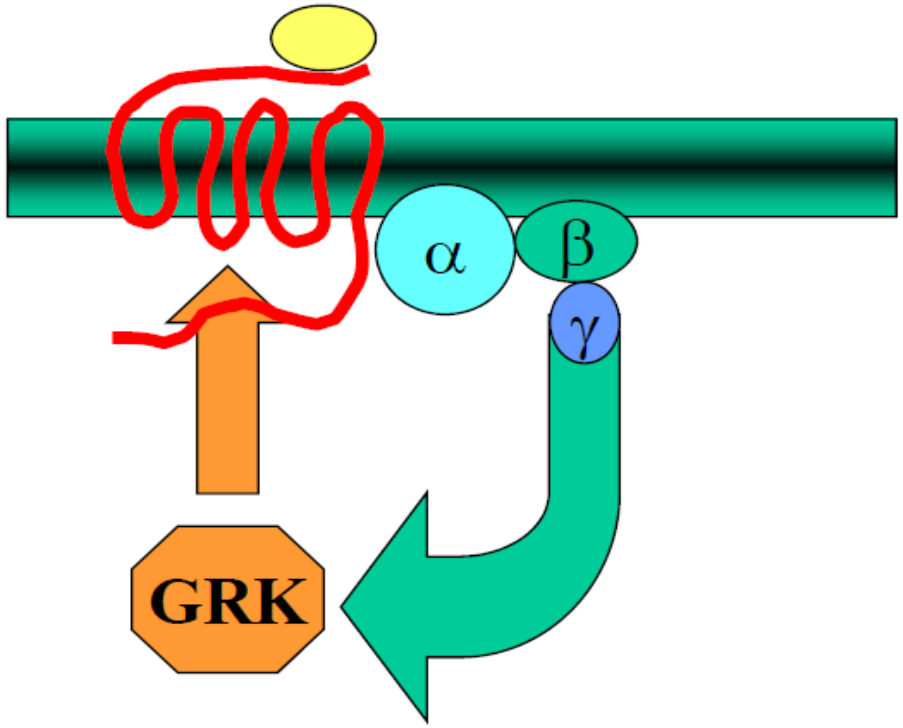

Phosphate sensor domain



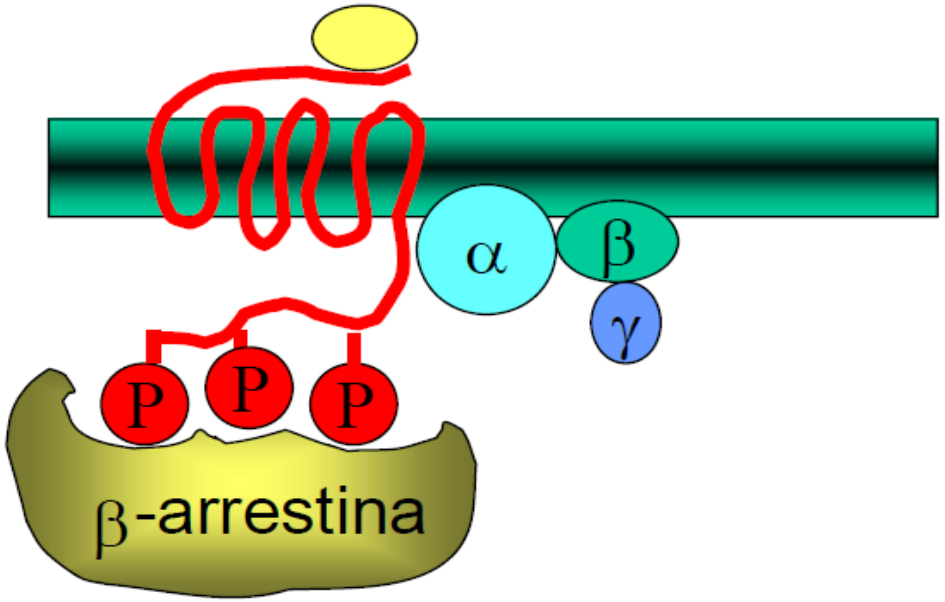

Seven-stranded β sandwich

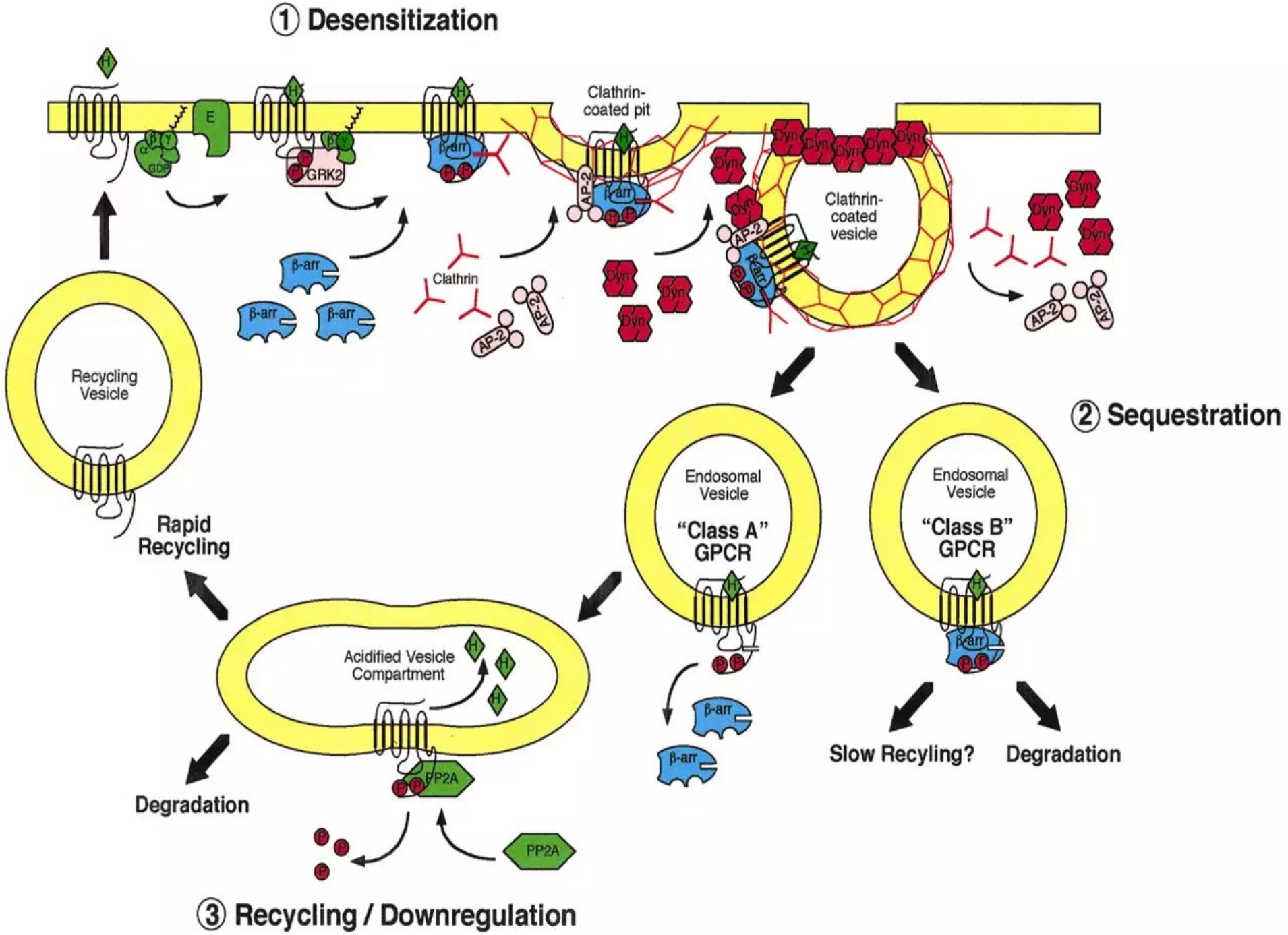
# Desensibilizzazione omologa

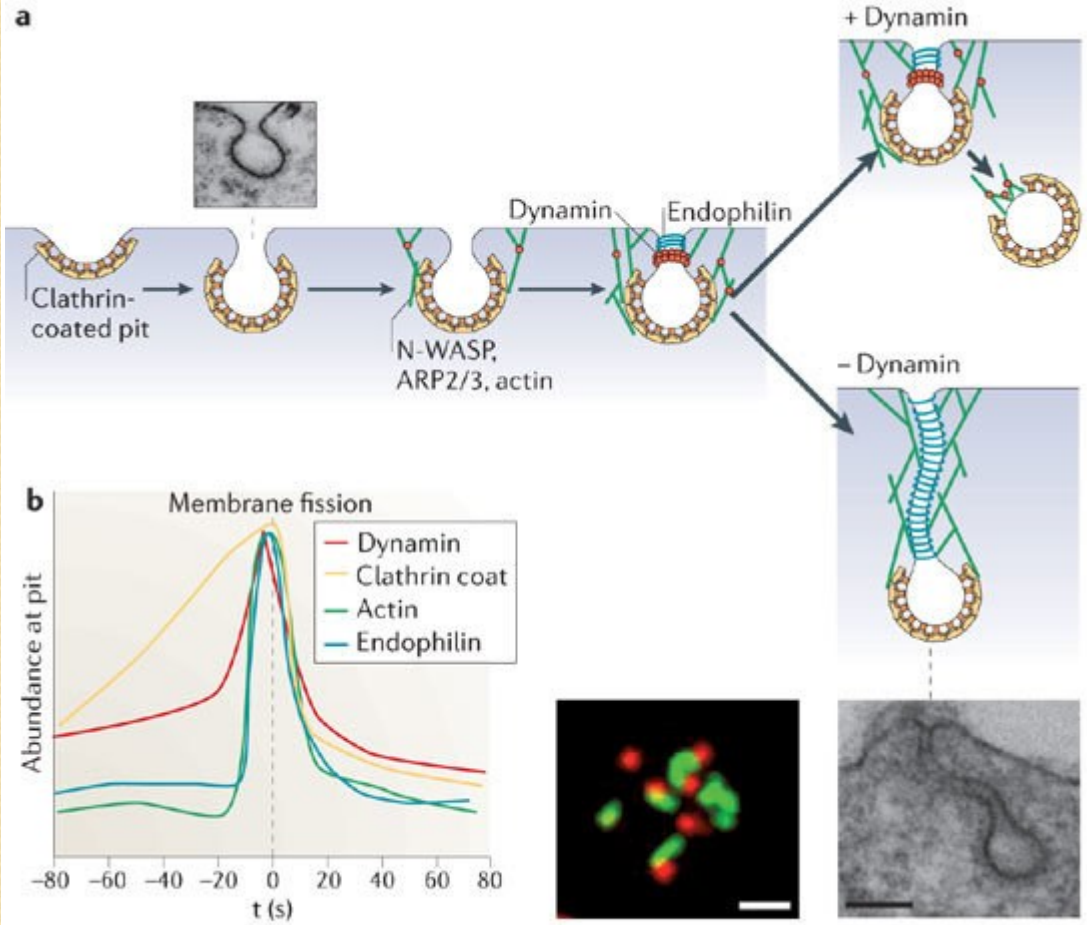
Disaccoppiamento recettore-proteine G in risposta alla **fosforilazione del recettore tramite le GRK** (**G protein-coupled receptor kinase**) promossa dal dimero βγ



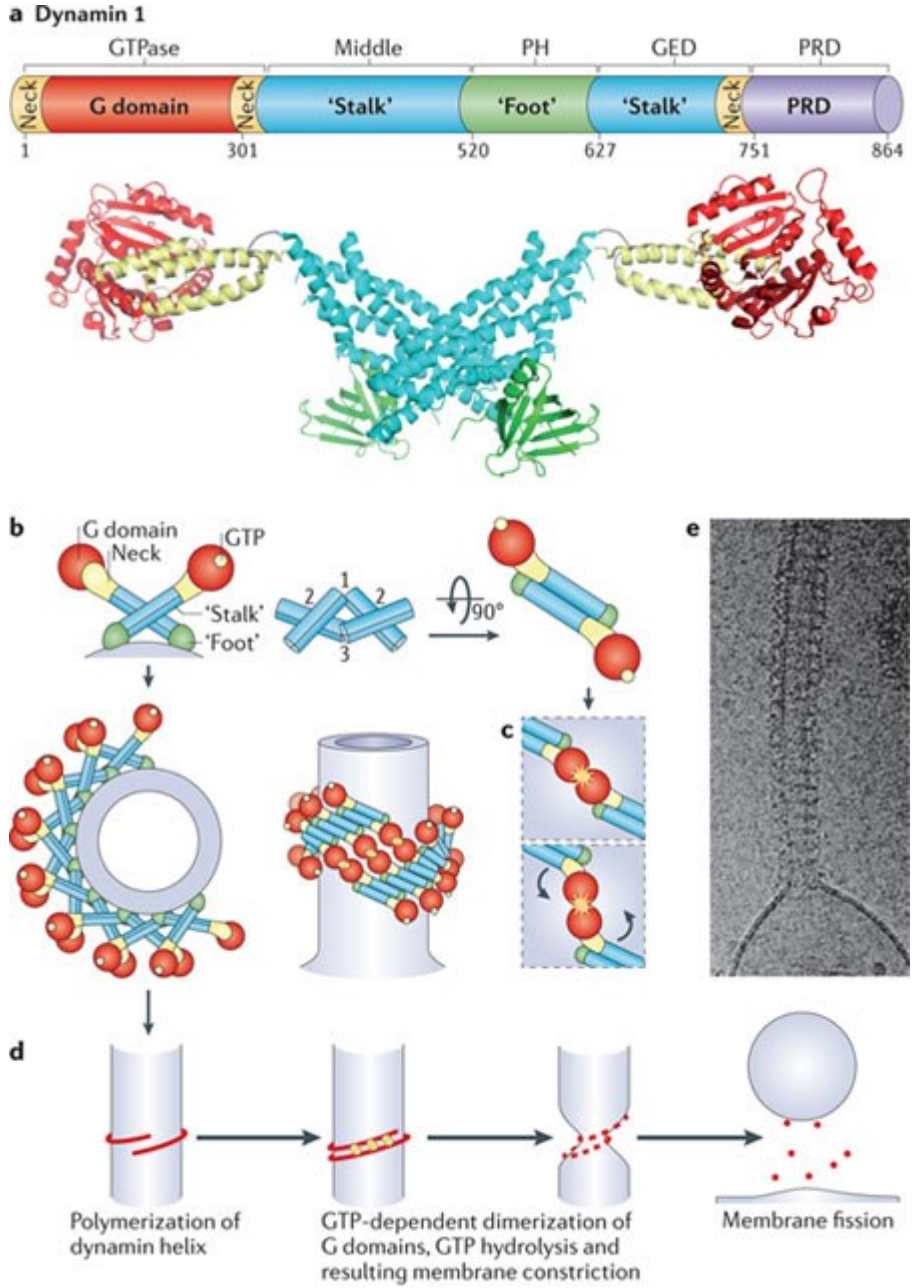

La fosforilazione tramite GRK spesso non è sufficiente per inattivare completamente i recettori; la completa inattivazione richiede un componente addizionale, l'**arrestina**



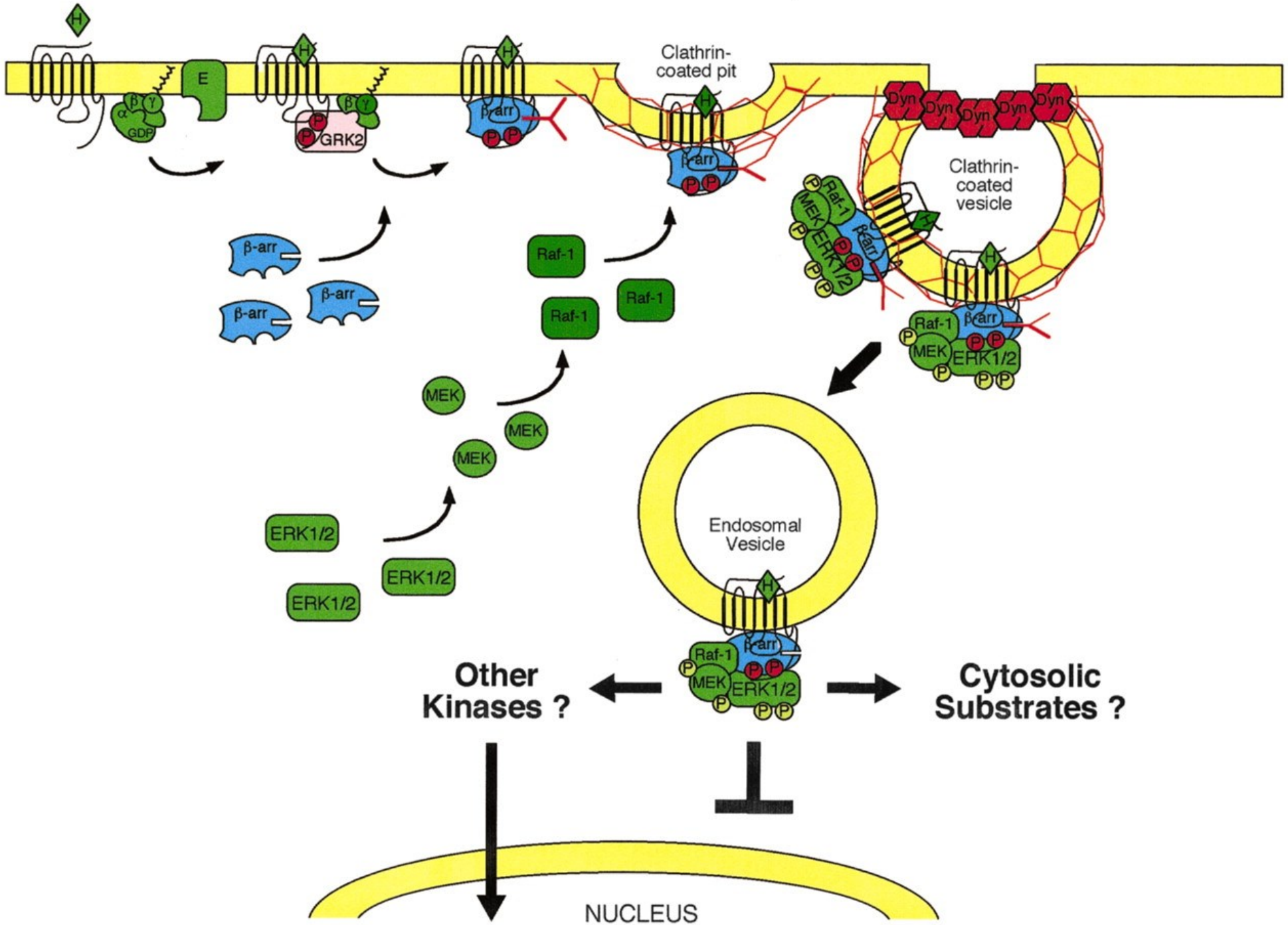

① Desensitization
Clathrin-coated pit
Clathrin-coated vesicle
Recycling Vesicle
Rapid Recycling
② Sequestration
Endosomal Vesicle
"Class A" GPCR
Endosomal Vesicle
"Class B" GPCR
Slow Recyling?
Degradation
Acidified Vesicle Compartment
Degradation
③ Recycling / Downregulation
GRK2
β-arr
Clathrin
AP-2
Dyn
PP2A
GDP




Nature Reviews | Molecular Cell Biology

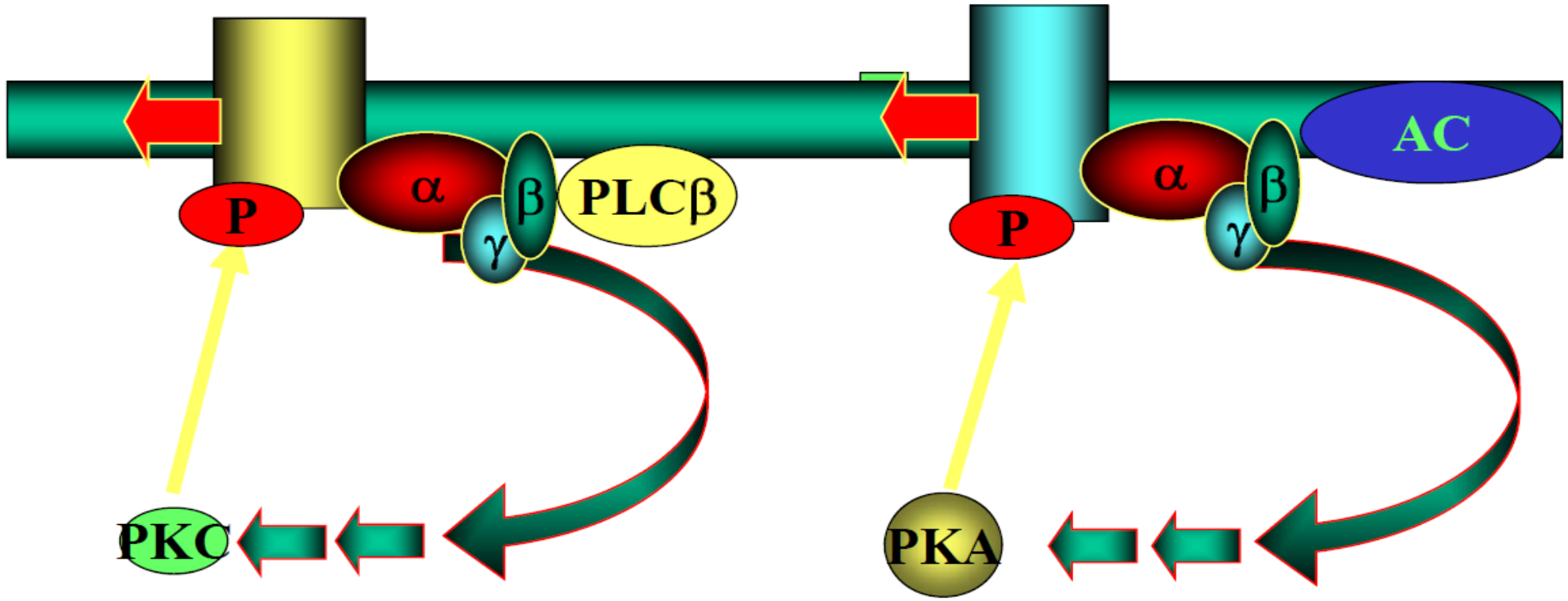



Nature Reviews | Molecular Cell Biology


H
E
GDP
GRK2
β-arr
Clathrin-coated pit
Dyn
Clathrin-coated vesicle
Raf-1
MEK
ERK1/2
Endosomal Vesicle
Other Kinases ?
Cytosolic Substrates ?
NUCLEUS

# Desensibilizzazione eterologa

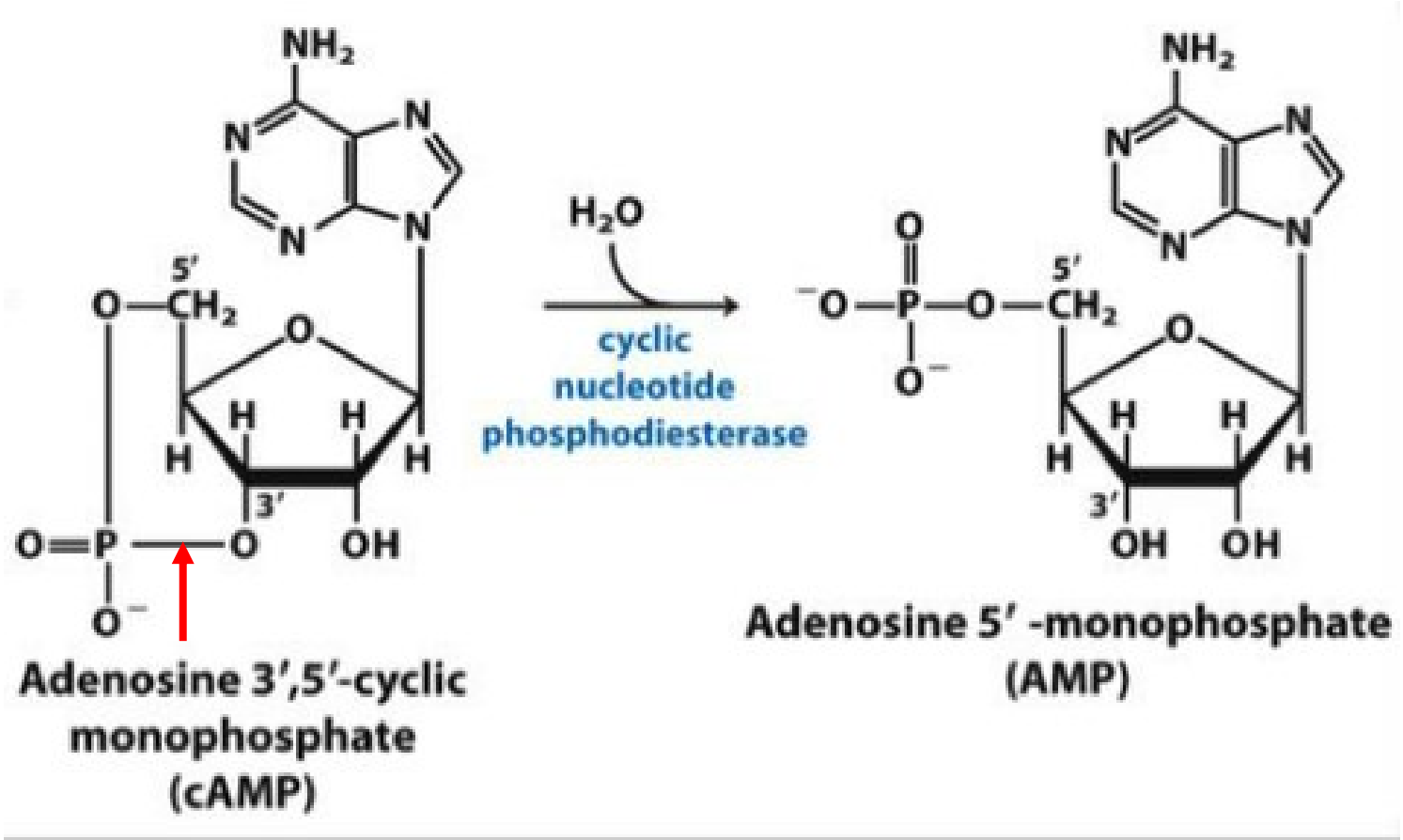
Desensibilizzazione attraverso disaccoppiamento recettore-proteine G in risposta alla **fosforilazione del recettore tramite PKA e/o PKC**



# Terminazione della risposta

- Monoamino ossidasi MAO e catecol-O-metiltrasferasi (COMT)
- Attività GTPasi intrinseca alle G protein e GAP accessorie (RGS)
- G protein coupled receptor kinases (GRK), PKA, etc
- Arrestin
- Fosfodiesterasi (PDE)/ pompa del $Ca^{+2}$

# Fosfodiesterasi



# Feed back negativo via fosfodiesterasi

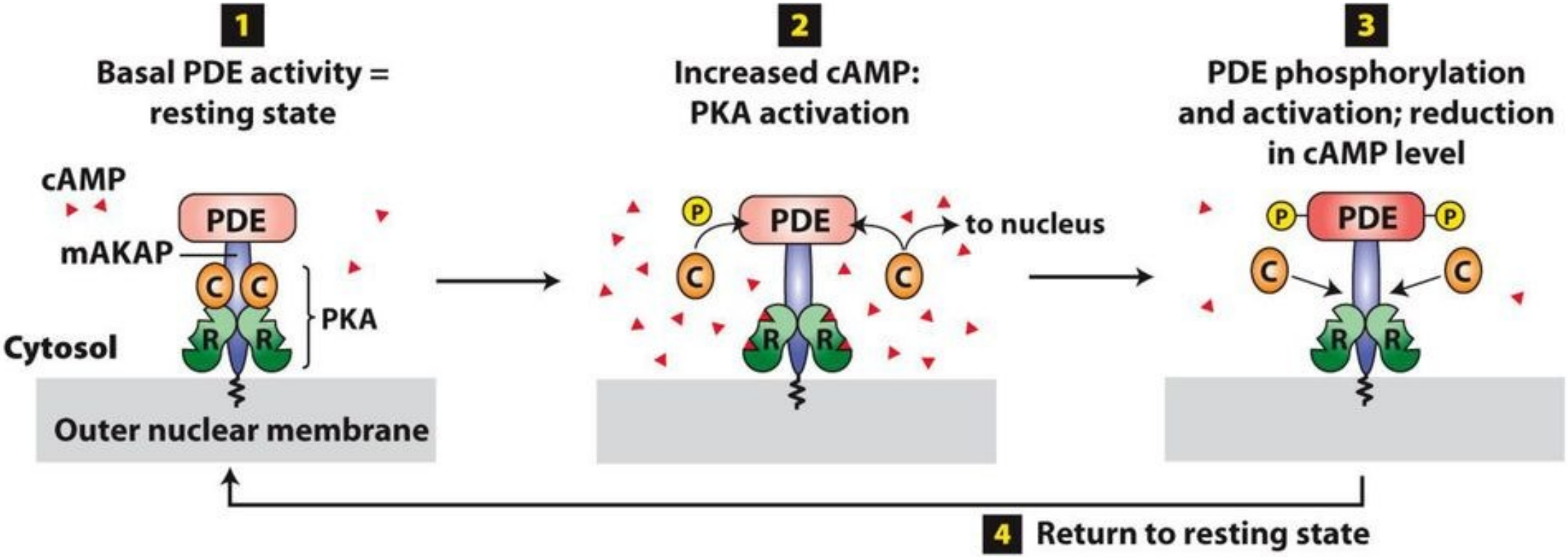



**Figure 15-31**
*Molecular Cell Biology*, Eighth Edition

Manoscritti per
presentazione studenti

29 aprile 2022

# Illuminating G-Protein-Coupling Selectivity of GPCRs

Asuka Inoue,[1,2,3,7,8,*] Francesco Raimondi,[4,5,7,*] Francois Marie Ngako Kadji,[1] Gurdeep Singh,[4,5] Takayuki Kishi,[1] Akiharu Uwamizu,[1] Yuki Ono,[1] Yuji Shinjo,[1] Satoru Ishida,[1] Nadia Arang,[6] Kouki Kawakami,[1] J. Silvio Gutkind,[6] Junken Aoki,[1,3] and Robert B. Russell[4,5,*]

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[2]Advanced Research & Development Programs for Medical Innovation (PRIME), Japan Agency for Medical Research and Development (AMED), Chiyoda-ku, Tokyo 100-0004, Japan
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[4]CellNetworks, Bioquant, Heidelberg University, Im Neuenheimer Feld 267, 69120 Heidelberg, Germany
[5]Biochemie Zentrum Heidelberg (BZH), Heidelberg University, Im Neuenheimer Feld 328, 69120 Heidelberg, Germany
[6]Department of Pharmacology and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA
[7]These authors contributed equally
[8]Lead Contact
*Correspondence: iaska@tohoku.ac.jp (A.I.), francesco.raimondi@bioquant.uni-heidelberg.de (F.R.), robert.russell@bioquant.uni-heidelberg.de (R.B.R.)
https://doi.org/10.1016/j.cell.2019.04.044

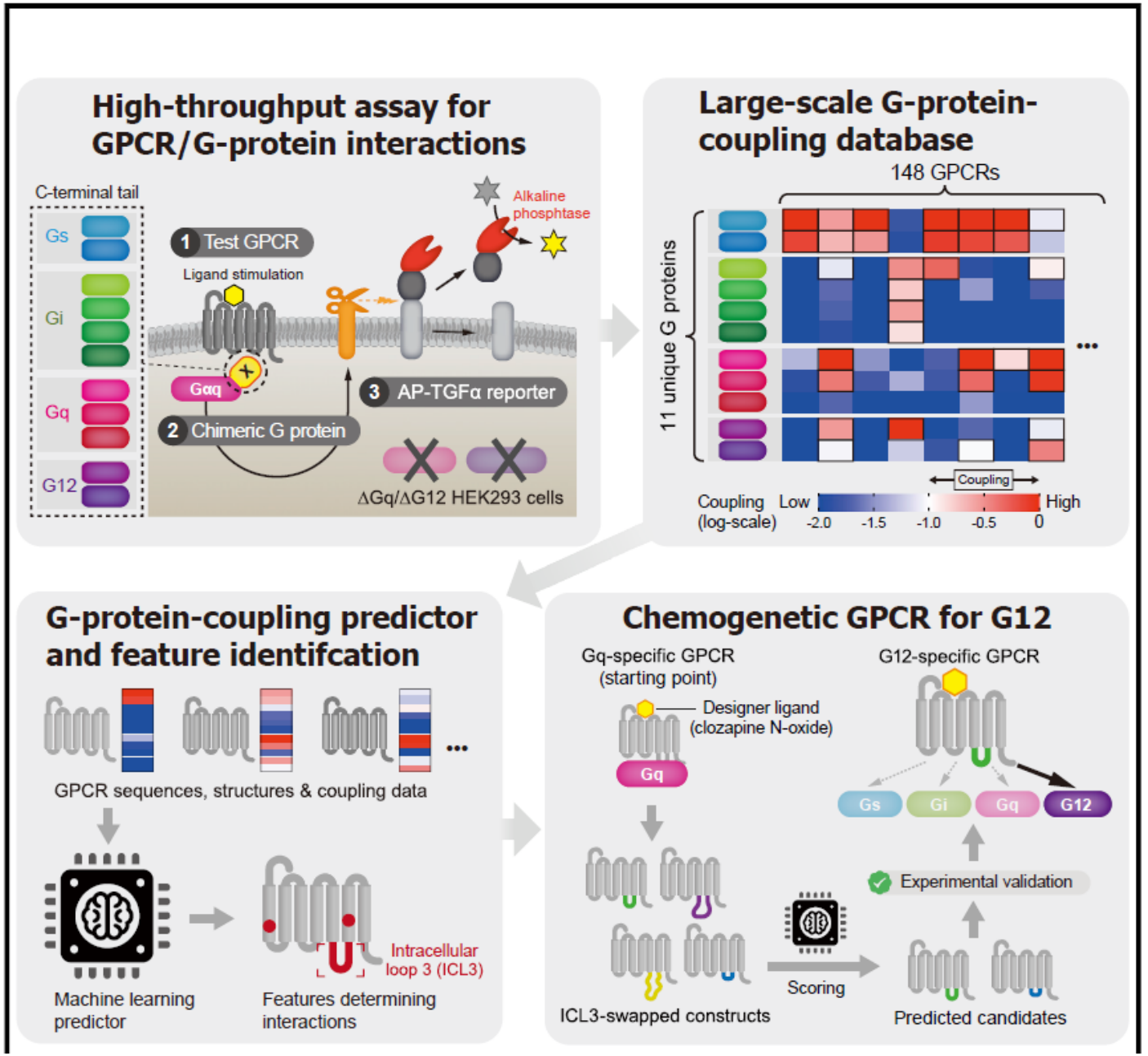
## SUMMARY

Heterotrimetic G proteins consist of four subfamilies ($G_s$, $G_{i/o}$, $G_{q/11}$, and $G_{12/13}$) that mediate signaling via G-protein-coupled receptors (GPCRs), principally by receptors binding Gα C termini. G-protein-coupling profiles govern GPCR-induced cellular responses, yet receptor sequence selectivity determinants remain elusive. Here, we systematically quantified ligand-induced interactions between 148 GPCRs and all 11 unique Gα subunit C termini. For each receptor, we probed chimeric Gα subunit activation via a transforming growth factor-α (TGF-α) shedding response in HEK293 cells lacking endogenous $G_{q/11}$ and $G_{12/13}$ proteins, and complemented G-protein-coupling profiles through a NanoBiT-G-protein dissociation assay. Interrogation of the dataset identified sequence-based coupling specificity features, inside and outside the transmembrane domain, which we used to develop a coupling predictor that outperforms previous methods. We used the predictor to engineer designer GPCRs selectively coupled to $G_{12}$. This dataset of fine-tuned signaling mechanisms for diverse GPCRs is a valuable resource for research in GPCR signaling.

complexes, each consisting of Gα, Gβ, and Gγ subunits, where distinct Gα subunits specify both GPCR interactions and the transduction of particular downstream signaling events (Wettschureck and Offermanns, 2005). The human genome encodes 16 Gα genes that are grouped into four subfamilies $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$ that capture broad properties of downstream signaling (e.g., adenylyl cyclase activation by $G\alpha_s$) (Wettschureck and Offermanns, 2005). In general, each of the hundreds of mammalian GPCRs couple with more than one G protein giving each a distinct coupling profile (Harding et al., 2018), or signature, which evokes a unique cellular response. Determining these GPCR profiles is critical to understanding their biology and pharmacology.

Pharmaceutical interest in GPCRs has prompted many efforts during the last decades to determine both their ligands and signaling (Hauser et al., 2018). Among approximately 360 non-sensory GPCR genes encoded in the human genome, one-third are still labeled as *orphans* to reflect the fact that either ligands and/or signaling are unknown (Harding et al., 2018). Previous efforts to uncover signaling profiles have been laborious and not standardized yet tended to identify only the subfamily-level signaling outcome (e.g., $Ca^{2+}$, cAMP, inositol phosphate, Rho activation), rather than the specific Gα subunit binding event (Thomsen et al., 2005). Although this has led a collection of data on GPCR ligands and signaling exemplified in the IUPHAR/BPS Guide to Pharmacology (GtoPdb) (Harding et al., 2018), these databases have issues

# Graphical Abstract



# A Global Map of G Protein Signaling Regulation by RGS Proteins

Ikuo Masuho,[1] Santhanam Balaji,[2,3] Brian S. Muntean,[1] Nickolas K. Skamangas,[1] Sreenivas Chavali,[2,4] John J.G. Tesmer,[5] M. Madan Babu,[2,3] and Kirill A. Martemyanov[1,6,*]

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[2]MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, UK

[3]Departments of Structural Biology and Center for Data Driven Discovery, St. Jude Children's Research Hospital, Memphis, TN 38105, USA

[4]Department of Biology, Indian Institute of Science Education and Research (IISER) Tirupati, Karakambadi Road, Tirupati 517 507, India

[5]Departments of Biological Sciences and Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN 47907-2054, USA

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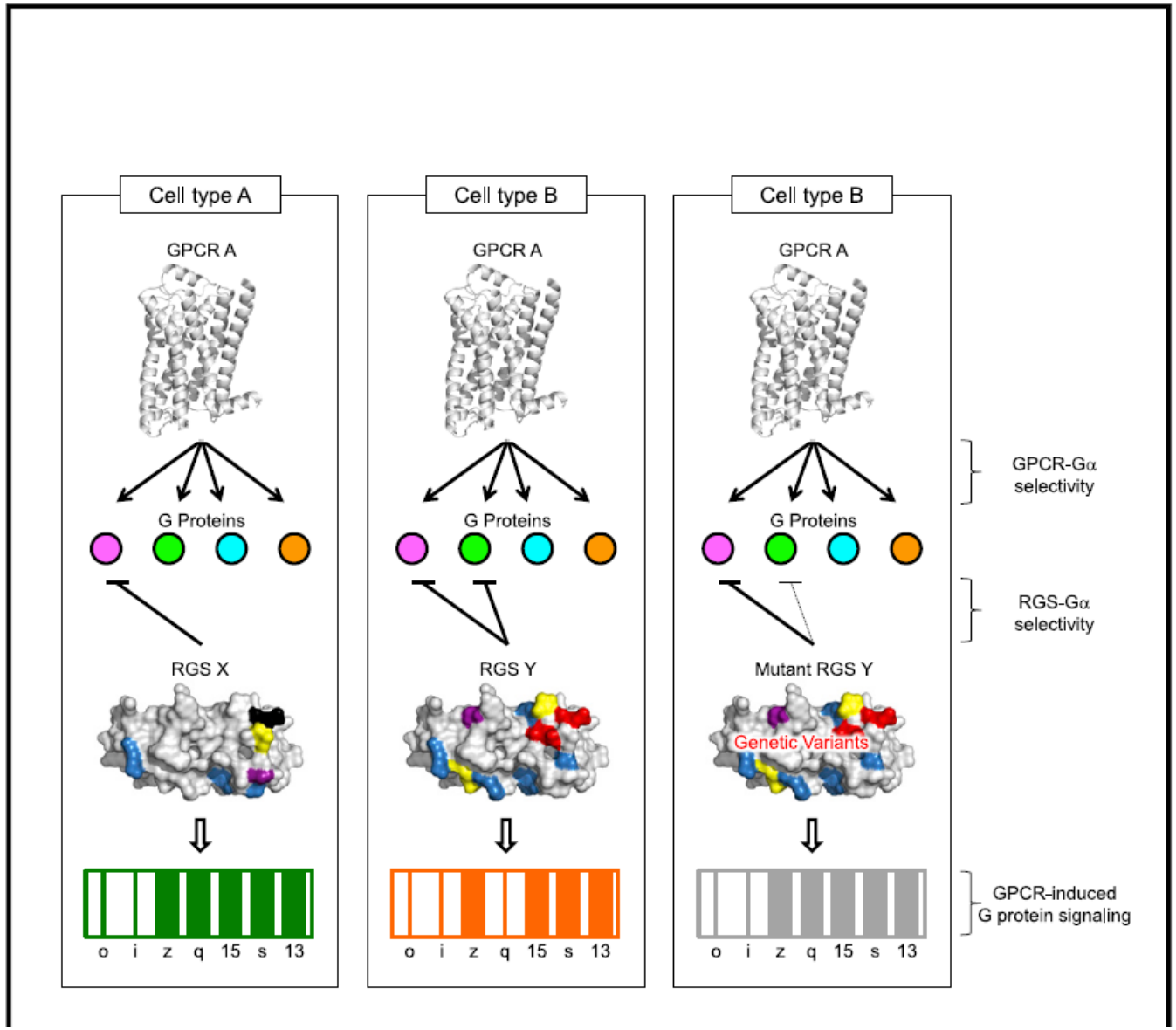
https://doi.org/10.1016/j.cell.2020.08.052

## SUMMARY

The control over the extent and timing of G protein signaling is provided by the regulator of G protein signaling (RGS) proteins that deactivate G protein α subunits (Gα). Mammalian genomes encode 20 canonical RGS and 16 Gα genes with key roles in physiology and disease. To understand the principles governing the selectivity of Gα regulation by RGS, we examine the catalytic activity of all canonical human RGS proteins and their selectivity for a complete set of Gα substrates using real-time kinetic measurements in living cells. The data reveal rules governing RGS-Gα recognition, the structural basis of its selectivity, and provide principles for engineering RGS proteins with defined selectivity. The study also explores the evolution of RGS-Gα selectivity through ancestral reconstruction and demonstrates how naturally occurring non-synonymous variants in RGS alter signaling. These results provide a blueprint for decoding signaling selectivity and advance our understanding of molecular recognition principles.

# Graphical Abstract



# Receptor Quaternary Organization Explains G Protein-Coupled Receptor Family Structure

James H. Felce,[1] Sarah L. Latty,[2] Rachel G. Knox,[1] Susan R. Mattick,[1] Yuan Lui,[1] Steven F. Lee,[2] David Klenerman,[2,*] and Simon J. Davis[1,3,*]

[1]Radcliffe Department of Medicine and Medical Research Council Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford OX3 9DS, UK

[2]Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, UK

[3]Lead Contact

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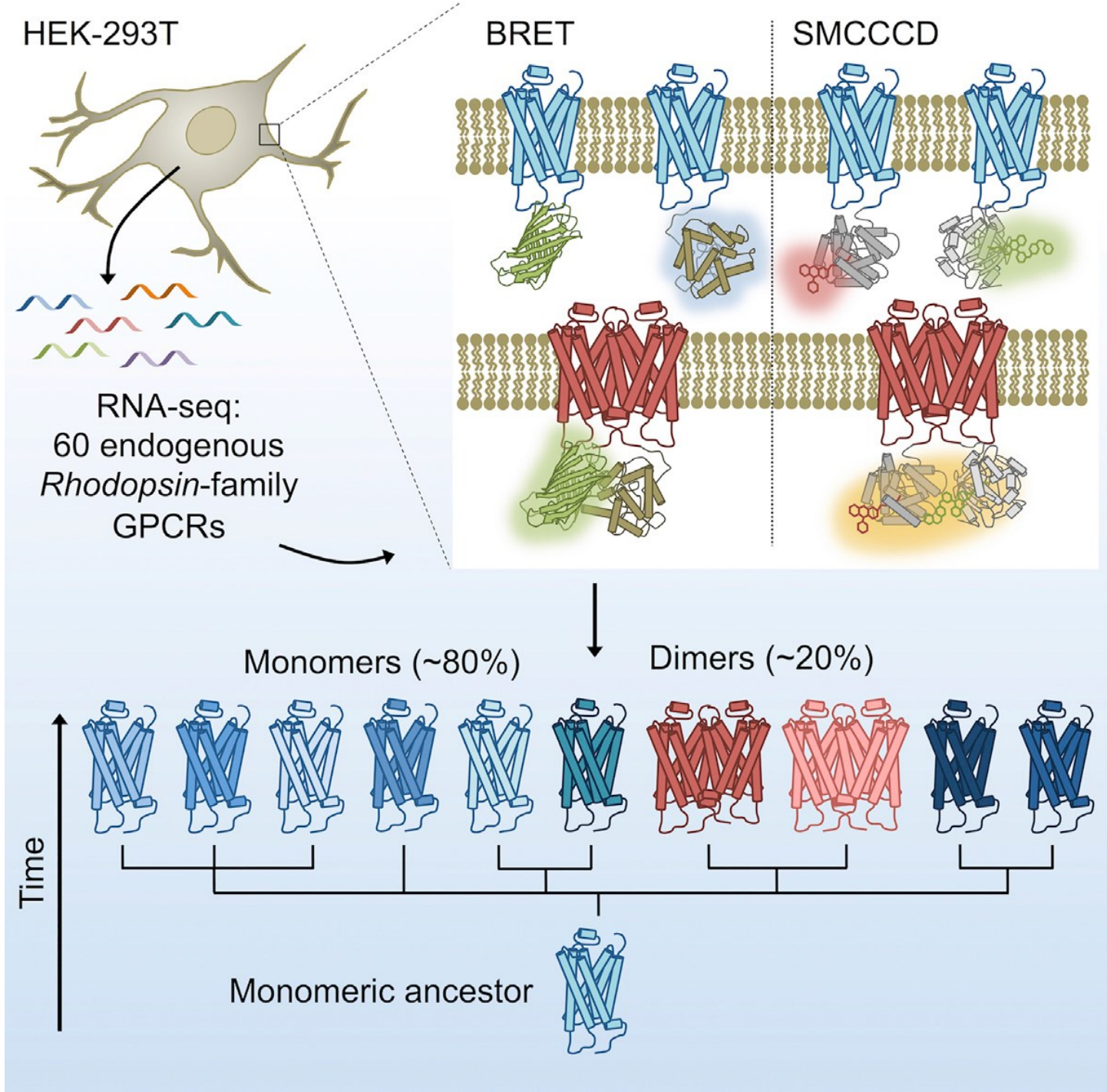
http://dx.doi.org/10.1016/j.celrep.2017.08.072

## SUMMARY

The organization of *Rhodopsin*-family G protein-coupled receptors (GPCRs) at the cell surface is controversial. Support both for and against the existence of dimers has been obtained in studies of mostly individual receptors. Here, we use a large-scale comparative study to examine the stoichiometric signatures of 60 receptors expressed by a single human cell line. Using bioluminescence resonance energy transfer- and single-molecule microscopy-based assays, we found that a relatively small fraction of *Rhodopsin*-family GPCRs behaved as dimers and that these receptors otherwise appear to be monomeric. Overall, the analysis predicted that fewer than 20% of ~700 *Rhodopsin*-family receptors form dimers. The clustered distribution of the dimers in our sample and a striking correlation between receptor organization and GPCR family size that we also uncover each suggest that receptor stoichiometry might have profoundly influenced GPCR expansion and diversification.

cant structural variation between GPCRs is restricted to the ligand-binding regions, and the parts of the receptors involved in signal transduction are typically much more highly conserved (Katritch et al., 2012), allowing similar conformational changes to accompany receptor activation (Deupi and Standfuss, 2011). Several studies of isolated GPCRs (Bayburt et al., 2007; Ernst et al., 2007; Kuszak et al., 2009; Leitz et al., 2006; Whorton et al., 2007) convincingly show that signal transduction can occur on the scale of single, autonomous receptors, consistent with GPCRs forming 1:1 complexes with G proteins (Rasmussen et al., 2011).

Without question, the most contentious aspect of GPCR biology concerns their quaternary structures. This is not an insignificant issue, as homo- or hetero-oligomer formation offers, e.g., a simple explanation for a wealth of pharmacological data implying that receptors engage in "cross-talk" (although other explanations are possible; Chabre et al., 2009; Tubio et al., 2010) and new opportunities for pharmacological intervention. Whereas several small families of GPCRs comprise receptors whose large N- and C-terminal domains are known to effect dimerization, e.g., the *Glutamate* (class C) receptors (Gurevich and Gurevich, 2008b), there is no consensus regarding the "typical" quaternary structure of the largest group of GPCRs, i.e., the *Rhodopsin* family. It was initially thought that


HEK-293T
BRET
SMCCCD
RNA-seq:
60 endogenous
*Rhodopsin*-family
GPCRs
Monomers (~80%)
Dimers (~20%)
Time
Monomeric ancestor

# Bias Factor and Therapeutic Window Correlate to Predict Safer Opioid Analgesics

Cullen L. Schmid,[1] Nicole M. Kennedy,[2] Nicolette C. Ross,[1,2] Kimberly M. Lovell,[1,2] Zhizhou Yue,[2] Jenny Morgenweck,[1] Michael D. Cameron,[1] Thomas D. Bannister,[2] and Laura M. Bohn[1,3,*]

[1]Departments of Molecular Medicine and Neuroscience

[2]Department of Chemistry

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[3]Lead Contact

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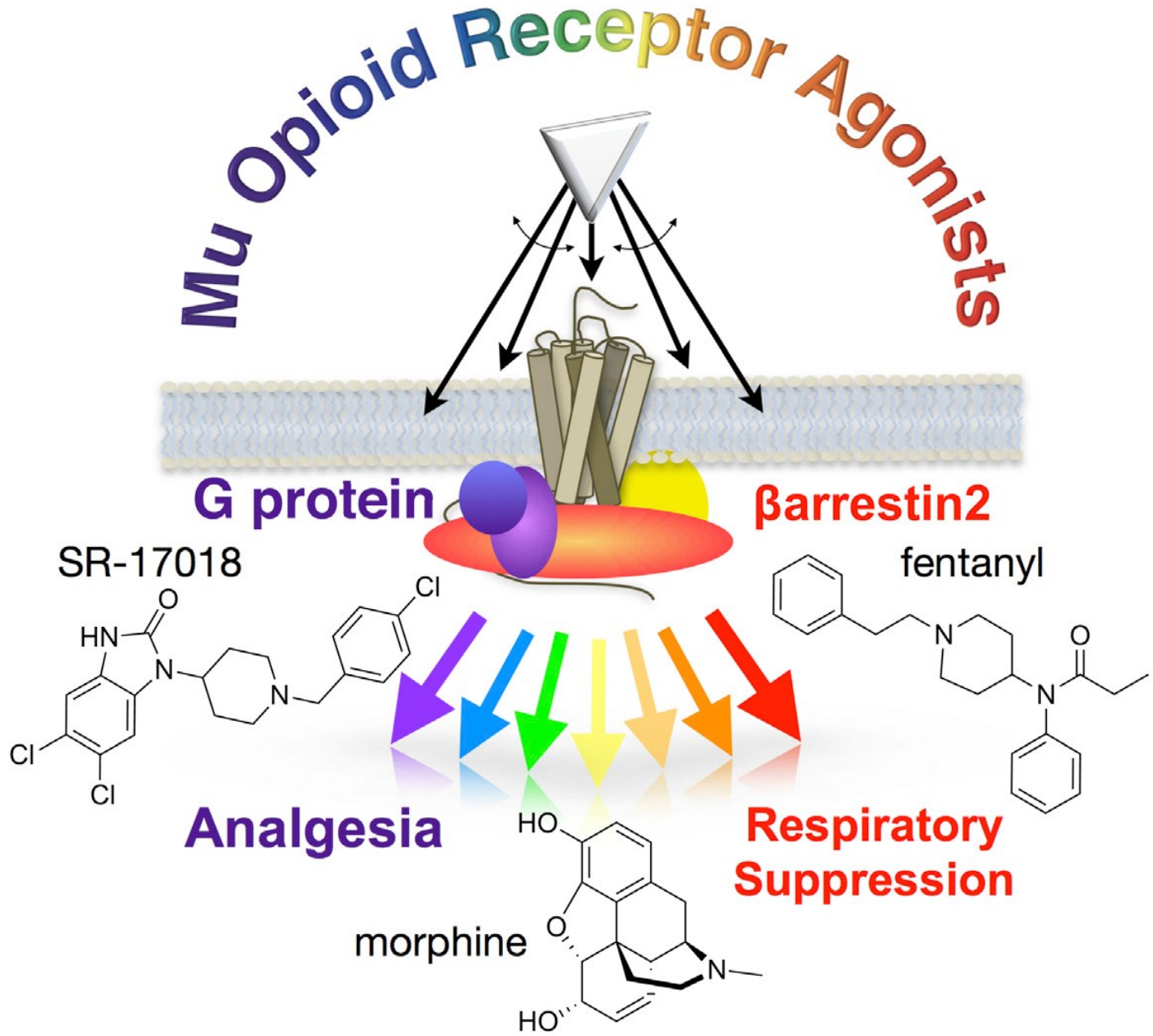
https://doi.org/10.1016/j.cell.2017.10.035

## SUMMARY

Biased agonism has been proposed as a means to separate desirable and adverse drug responses downstream of G protein-coupled receptor (GPCR) targets. Herein, we describe structural features of a series of mu-opioid-receptor (MOR)-selective agonists that preferentially activate receptors to couple to G proteins or to recruit βarrestin proteins. By comparing relative bias for MOR-mediated signaling in each pathway, we demonstrate a strong correlation between the respiratory suppression/antinociception therapeutic window in a series of compounds spanning a wide range of signaling bias. We find that βarrestin-biased compounds, such as fentanyl, are more likely to induce respiratory suppression at weak analgesic doses, while G protein signaling bias broadens the therapeutic window, allowing for antinociception in the absence of respiratory suppression.

induced respiratory suppression (Bohn et al., 1999; Raehal et al., 2005). These findings suggest that activating the MOR without engaging βarrestin2 regulation may be critically important for developing safer opioid analgesics.

A recent development in pharmacological theory and practice is the concept that the structure of a GPCR ligand may be systematically modified to confer alternative receptor conformations upon binding, with each displaying a unique pattern of activation of intracellular signaling cascades (Rankovic et al., 2016; Urban et al., 2007). This concept of functional selectivity, or biased agonism, can be quantified by comparing drug potency and efficacy in cell-based signaling assays to the performance of a reference agonist (a compound that fully activates the system and thus defines the full potential of what one might hope to measure in the assay). Application of the operational model, described by Black and Leff (1983), allows one to simultaneously compare the relative potency and efficacy of a test agonist to the reference agonist, allowing for normalization within an assay (derivation of the parameter: $\Delta\text{Log } \tau/K_A$). After normalization, the performance of the compound can then be compared to its performance within another assay. The result of the comparison can be calculated


Mu Opioid Receptor Agonists
G protein
βarrestin2
SR-17018
fentanyl
Analgesia
Respiratory Suppression
morphine

# EGFR Ligands Differentially Stabilize Receptor Dimers to Specify Signaling Kinetics

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https://doi.org/10.1016/j.cell.2017.09.017

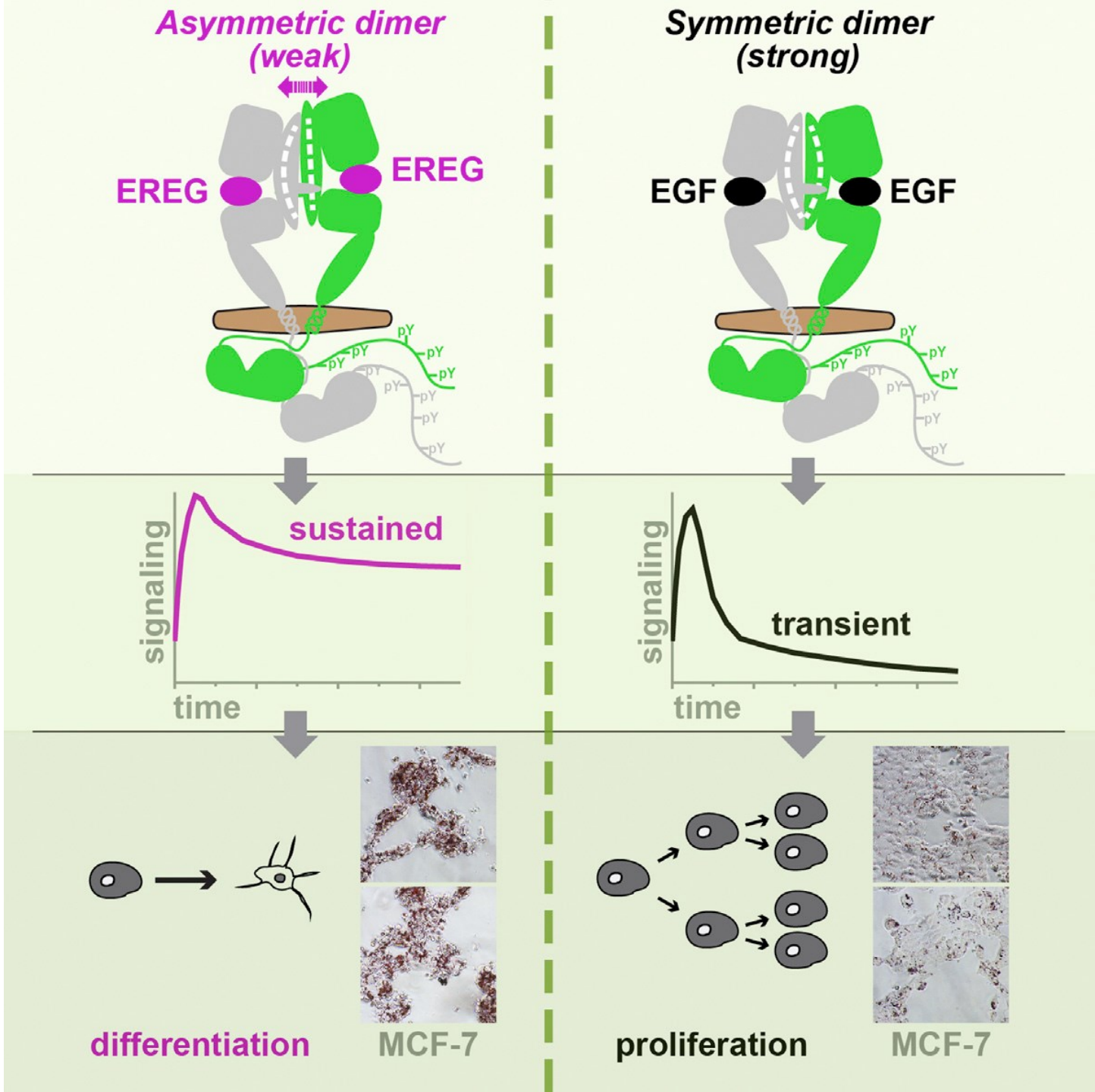
## SUMMARY

Epidermal growth factor receptor (EGFR) regulates many crucial cellular programs, with seven different activating ligands shaping cell signaling in distinct ways. Using crystallography and other approaches, we show how the EGFR ligands epiregulin (EREG) and epigen (EPGN) stabilize different dimeric conformations of the EGFR extracellular region. As a consequence, EREG or EPGN induce less stable EGFR dimers than EGF—making them partial agonists of EGFR dimerization. Unexpectedly, this weakened dimerization elicits more sustained EGFR signaling than seen with EGF, provoking responses in breast cancer cells associated with differentiation rather than proliferation. Our results reveal how responses to different EGFR ligands are defined by receptor dimerization strength and signaling dynamics. These findings have broad implications for understanding receptor tyrosine kinase (RTK) signaling specificity. Our results also suggest parallels between partial and/or biased agonism in RTKs and G-protein-coupled receptors, as well as new therapeutic opportunities for correcting RTK signaling output.

to explain how EGFR family RTKs signal differently in response to their multiple cognate ligands (Sweeney and Carraway, 2000; Wilson et al., 2009).

EGFR is activated by seven different growth factors (Harris et al., 2003), which fall into two groups based on receptor-binding affinity. The high-affinity ligands are EGF, transforming growth factor-$\alpha$ (TGF$\alpha$), betacellulin (BTC), and heparin binding EGF-like growth factor (HB-EGF), which bind cell-surface EGFR with apparent $K_d$ of 0.1–1 nM. The low-affinity ligands are epiregulin (EREG), epigen (EPGN), and amphiregulin (AREG), which bind 10- to 100-fold more weakly. Numerous studies report distinct EGFR-dependent cellular responses to the different ligands (Wilson et al., 2009), with a given cell line responding differently to individual EGFR ligands in terms of cell proliferation (Wilson et al., 2012), differentiation (Kochupurakkal et al., 2005; Rizzi et al., 2013), and/or motility (Willmarth and Ethier, 2006). Individual EGFR ligands also induce qualitatively and quantitatively different downstream signals (Knudsen et al., 2014; Ronan et al., 2016; Wilson et al., 2012) and are linked to unique phenotypes in vivo (Wilson et al., 2009).

It remains unclear from current mechanistic understanding how different ligands could promote distinct cellular signaling responses through the same RTK. Crystal structures have described how EGF or TGF$\alpha$ induce formation of activated EGFR dimers (Ferguson et al., 2003; Garrett et al., 2002; Kovacs et al., 2015; Ogiso et al., 2002). Our more recent work (Bessman


Asymmetric dimer
(weak)
EREG
EREG
Symmetric dimer
(strong)
EGF
EGF
pY
signaling
sustained
time
signaling
transient
time
differentiation
MCF-7
proliferation
MCF-7

# Auto-regulation of Secretory Flux by Sensing and Responding to the Folded Cargo Protein Load in the Endoplasmic Reticulum

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https://doi.org/10.1016/j.cell.2019.01.035

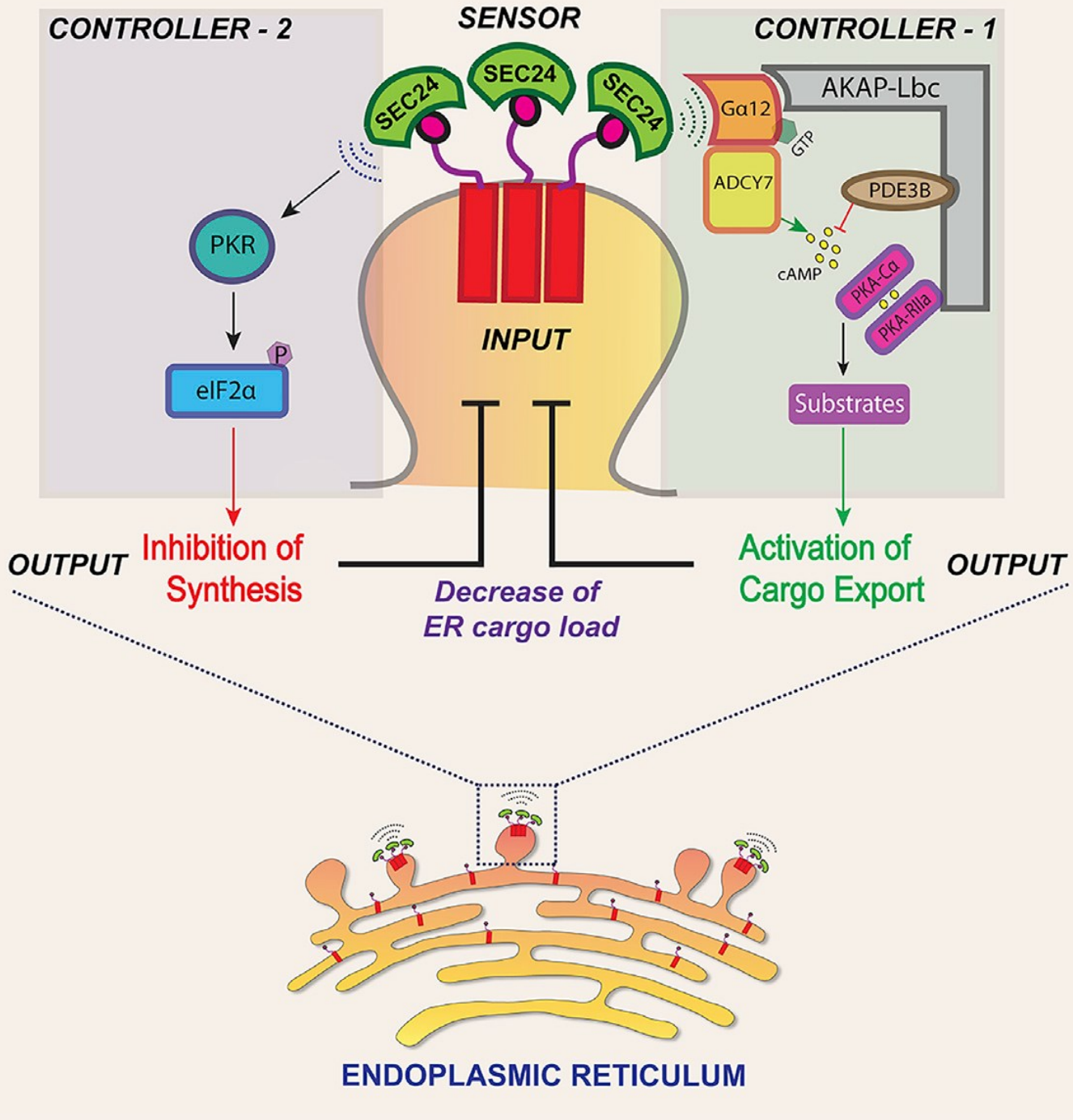
## SUMMARY

Maintaining the optimal performance of cell processes and organelles is the task of auto-regulatory systems. Here we describe an auto-regulatory device that helps to maintain homeostasis of the endoplasmic reticulum (ER) by adjusting the secretory flux to the cargo load. The cargo-recruiting subunit of the coatomer protein II (COPII) coat, Sec24, doubles as a sensor of folded cargo and, upon cargo binding, acts as a guanine nucleotide exchange factor to activate the signaling protein Gα12 at the ER exit sites (ERESs). This step, in turn, activates a complex signaling network that activates and coordinates the ER export machinery and attenuates proteins synthesis, thus preventing large fluctuations of folded and potentially active cargo that could be harmful to the cell or the organism. We call this mechanism AREX (autoregulation of ER export) and expect that its identification will aid our understanding of human physiology and diseases that develop from secretory dysfunction.

optimally despite the physiological and pathological perturbations to which it is exposed. Therefore, it must maintain tight coordination between the transport and processing activities of its main stations—the endoplasmic reticulum (ER), Golgi complex, and endo-lysosomal system—at all times. To date, the cellular mechanisms responsible for this type of coordination remain poorly understood.

In this study, we focus on the first steps in the pathway; namely, the folding and export of secretory cargo from the ER. Here, the fluctuations in protein synthesis that may occur in many cell types (Dolfi et al., 2013; Ron and Harding, 2012; van Anken et al., 2003) could lead to aberrant accumulation of folded and potentially active cargo in the lumen of this this organelle, with harmful consequences for the cell or organism. Examples of cargo mislocalization and anomalous activation at the ER, leading to diseases such as cancer, have been reported (Choudhary et al., 2009). Thus, the production of folded cargo proteins in the ER must be coupled with the export process by a mechanism that senses folded cargo and regulates cargo export and synthesis accordingly.

To unravel the expected coordinating mechanism, we developed a strategy based on exposing the ER to a suitable (specific and non-toxic) artificial perturbation designed to induce a synchronous increase of folded cargo in the ER lumen (or "folding


CONTROLLER - 2
SENSOR
CONTROLLER - 1
SEC24
SEC24
SEC24
Gα12
GTP
AKAP-Lbc
ADCY7
PDE3B
cAMP
PKA-Cα
PKA-RIIa
PKR
P
eIF2α
INPUT
Substrates
OUTPUT
Inhibition of Synthesis
Decrease of ER cargo load
Activation of Cargo Export
OUTPUT
ENDOPLASMIC RETICULUM

Grazie per l’attenzione

# Mechanisms of signalling and biased agonism in G protein-coupled receptors

*Denise Wootten[1,2]*, Arthur Christopoulos[1], Maria Marti-Solano[3], M. Madan Babu[3] and Patrick M. Sexton[1,2]**

Abstract | G protein-coupled receptors (GPCRs) are the largest group of cell surface receptors in humans that signal in response to diverse inputs and regulate a plethora of cellular processes. Hence, they constitute one of the primary drug target classes. Progress in our understanding of GPCR dynamics, activation and signalling has opened new possibilities for selective drug development. A key advancement has been provided by the concept of biased agonism, which describes the ability of ligands acting at the same GPCR to elicit distinct cellular signalling profiles by preferentially stabilizing different active conformational states of the receptor. Application of this concept raises the prospect of 'designer' biased agonists as optimized therapeutics with improved efficacy and/or reduced side-effect profiles. However, this application will require a detailed understanding of the spectrum of drug actions and a structural understanding of the drug–receptor interactions that drive distinct pharmacologies. The recent revolution in GPCR structural biology provides unprecedented insights into ligand binding, conformational dynamics and the control of signalling outcomes. These insights, together with new approaches to multi-dimensional analysis of drug action, are allowing refined classification of drugs according to their pharmacodynamic profiles, which can be linked to receptor structure and predictions of preclinical drug efficacy.

# Duttilità dei recettori accoppiati alle proteine G