



Trasduzione del segnale:

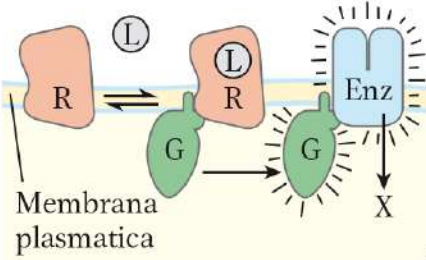
- canali ionici controllati
- recettori accoppiati alle proteine G (GPCRs)

Michele Sallese

Canali ionici controllati

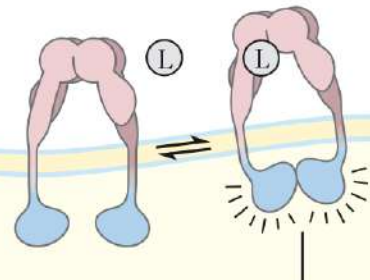
1. Recettori accoppiati alle proteine G

Il legame di un ligando esterno (L) al recettore (R) attiva una proteina intracellulare che lega il GTP (G); essa a sua volta regola l'attività di un enzima (Enz), che genera un secondo messaggero intracellulare (X).



2a. Recettore con attività tirosina chinasi

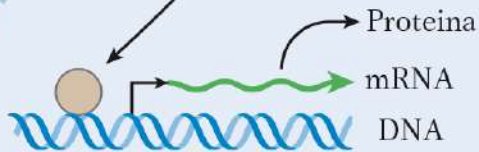
Il legame del ligando innesca l'attività tirosina chinasi mediante autofosforilazione.



2b. La chinasi attiva un fattore di trascrizione, alterando l'espressione genica.

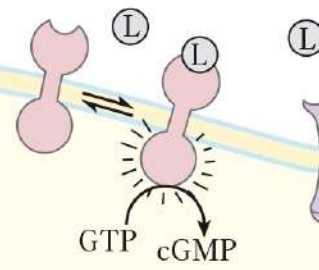
Cascata chinasi

Membrana nucleare



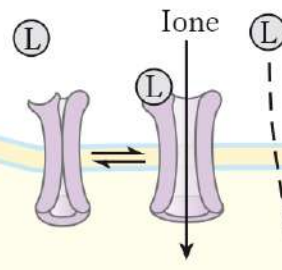
3. Recettore con attività guanilil ciclasica

Il legame del ligando al dominio extracellulare stimola la formazione del secondo messaggero, il GMP ciclico (cGMP).



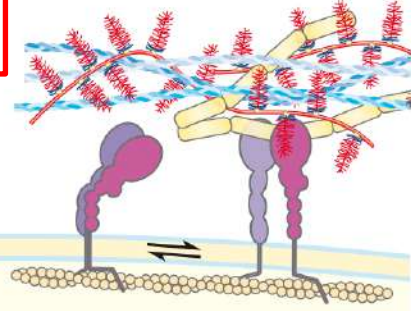
4. Canale ionico controllato

Si apre e si chiude in risposta alla concentrazione del ligando segnale o al potenziale di membrana.



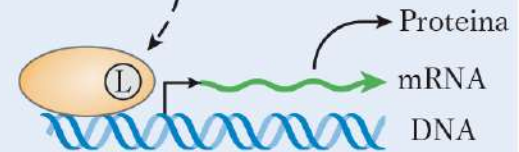
5. Recettore di adesione (integrina)

Legna molecole della matrice extracellulare, cambia la propria conformazione e altera l'interazione con il citoscheletro.



6. Recettore nucleare

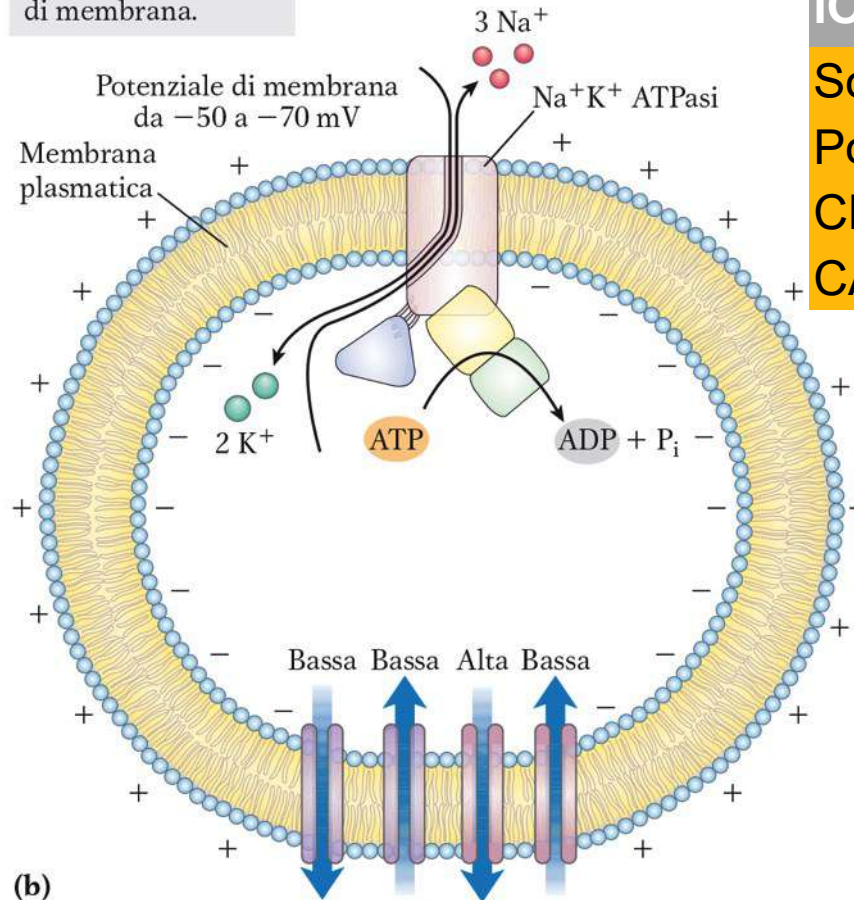
Il legame dell'ormone permette al recettore di regolare l'espressione di geni specifici.



Canali ionici controllati

(a)

La Na^+K^+ ATPasi elettrogenica genera un potenziale di membrana.



(b)

Gli ioni tendono a muoversi attraverso una membrana polarizzata in favore del gradiente elettrochimico.

IONE	CONCENTRAZIONE INTRACELLULARE	CONCENTRAZIONE EXTRACELLULARE
Sodio	15mM	145 mM
Potassio	150mM	4mM
CLORO	5mM	120 mM
CALCIO	10-7m	2mM

L'attivazione di canali ionici produce segnali elettrici cioè una variazione del potenziale di membrana

I CANALI IONICI

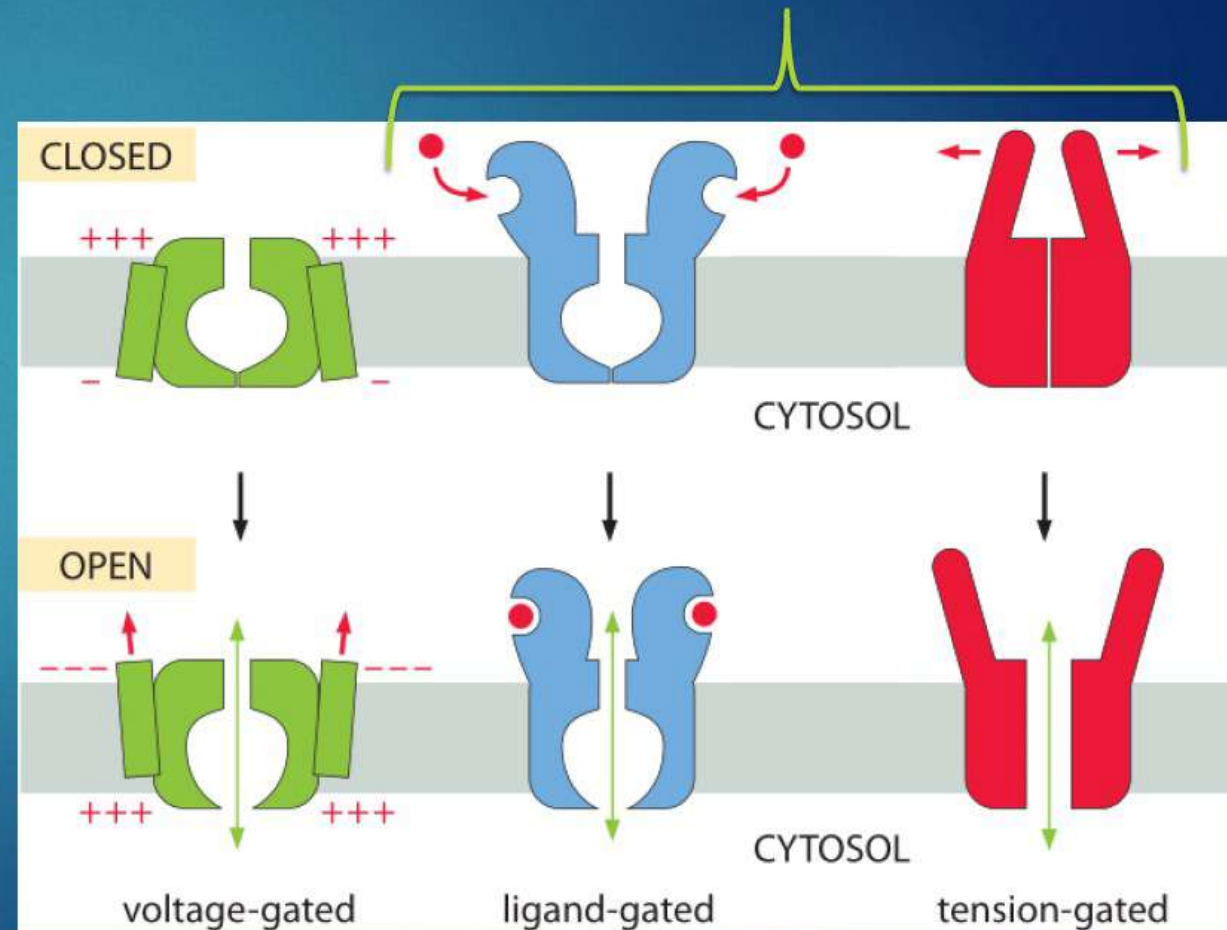
Poiché canali dedicati al passaggio dello stesso ione possono avere organizzazioni strutturali diverse, si preferisce di norma classificare i canali ionici in base alla modalità di regolazione della loro apertura.

Seguendo questi criteri i canali vengono raggruppati in **famiglie** di canali relativamente omogenei fra loro sia dal punto di vista funzionale che strutturale.

Possiamo avere:

- Canali regolati **dal potenziale di membrana**
- Canali regolati **da ligando**
- Canali regolati **da altre modalità (tensione meccanica)**

RECETTORI IONOTROPI



I RECETTORI-CANALE SONO SELETTIVI PER CARICA

In base alla carica che lasciano passare i recettori-canale si dividono in:

1- cationici: fanno passare **cariche positive** come sodio e

Calcio; esempio recettori per

l'acetilcolina,

il glutammato,

le purine,

la serotonina

i nucleotidi ciclici

2- anionici: fanno passare **cariche negative** come cloro;

Esempio recettore per

il GABA,

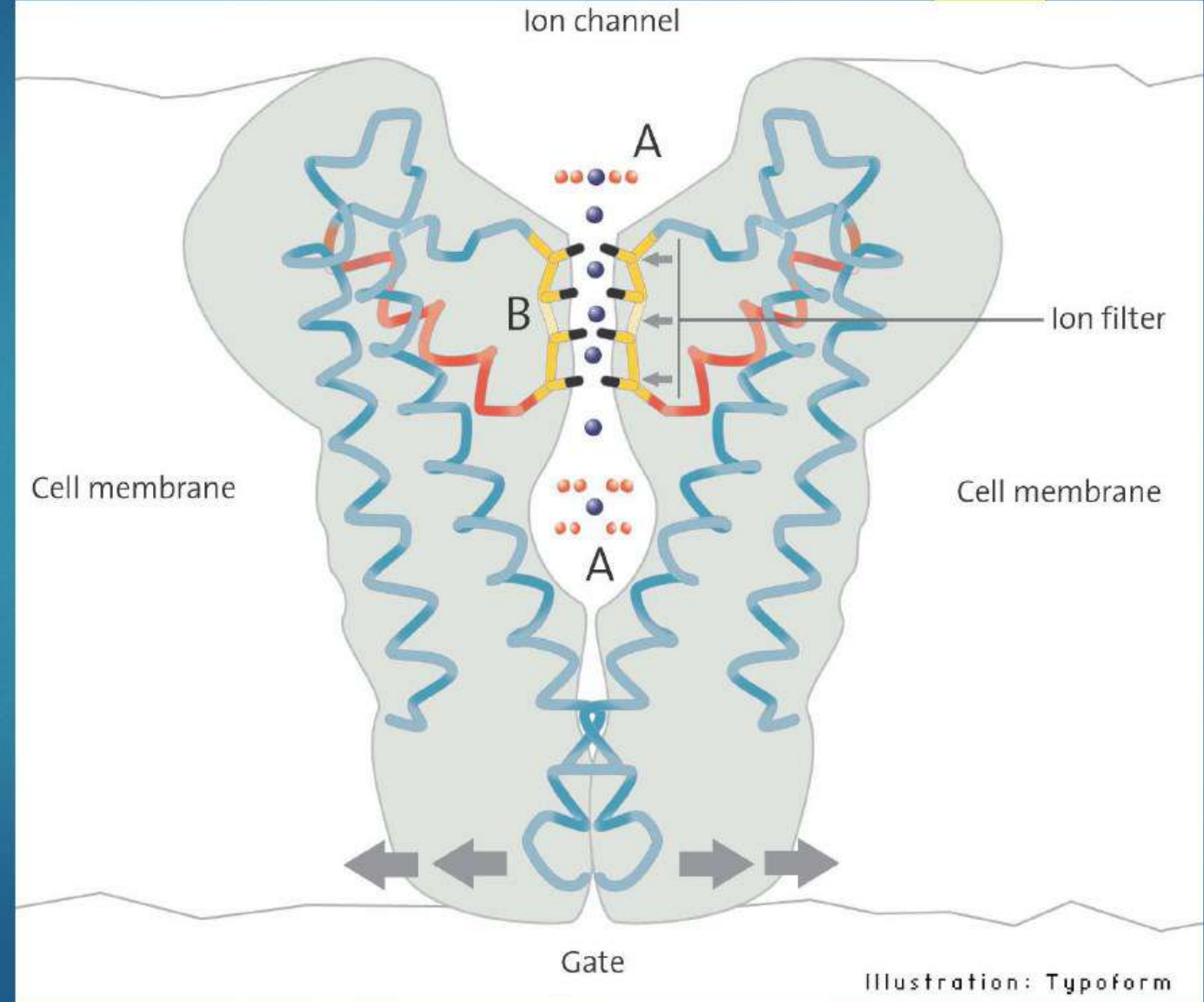
la glicina

I CANALI IONICI

Un'importante caratteristica dei canali ionici è la loro selettività per un dato ione o un dato tipo di ione (anioni, cationi).

Tale proprietà dipende dalle caratteristiche strutturali ed elettrostatiche del poro acquoso. Infatti l'imboccatura del canale è di norma dotata di un **filtro** (o **anello**) di **selettività**, composto da amminoacidi con carica elettrica positiva o negativa.

Altri fattori di selettività dipendono dalla dimensione e dalla struttura del poro acquoso, nonché nel meccanismo molecolare di trasferimento dello ione attraverso il poro (Ioni spogliati del guscio di idratazione)



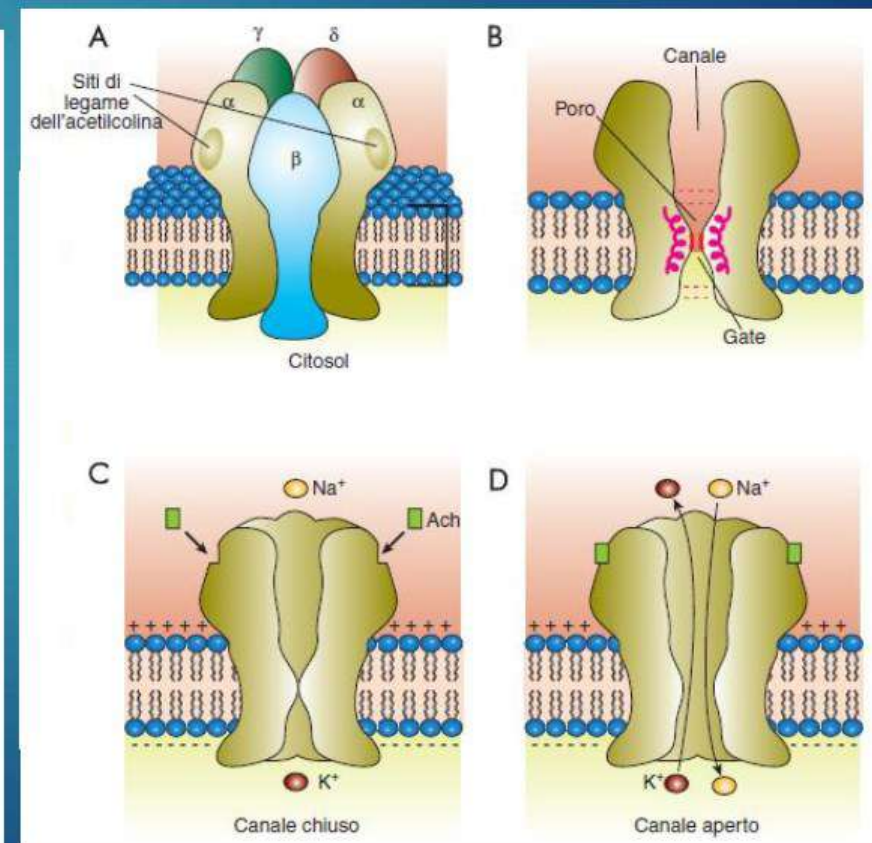
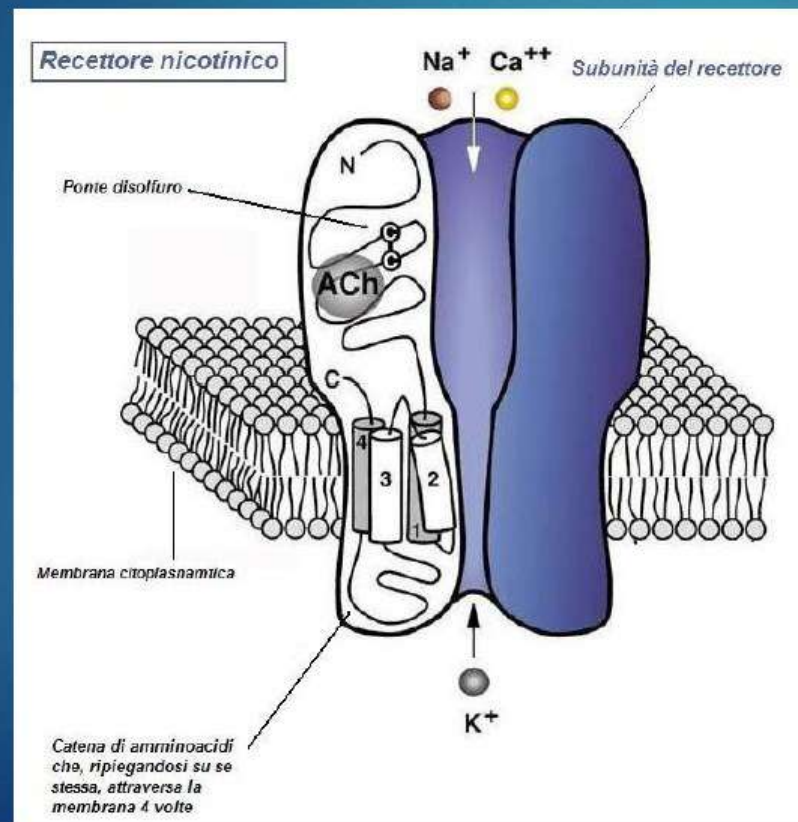
Recettori ionotropici

- I recettori ionotropici presentano una grande variabilità strutturale
- Sono costituiti da 3 a 5 subunità e possono essere suddivisi in classi strutturali:
 1. Recettori ionotropici della superfamiglia dei recettori nicotinici
 2. Recettori ionotropici per il glutammato
 3. Recettori ionotropici per i nucleotidi ciclici
 4. Recettori ionotropici purinergici

Recettore nicotino dell'Acetilcolina

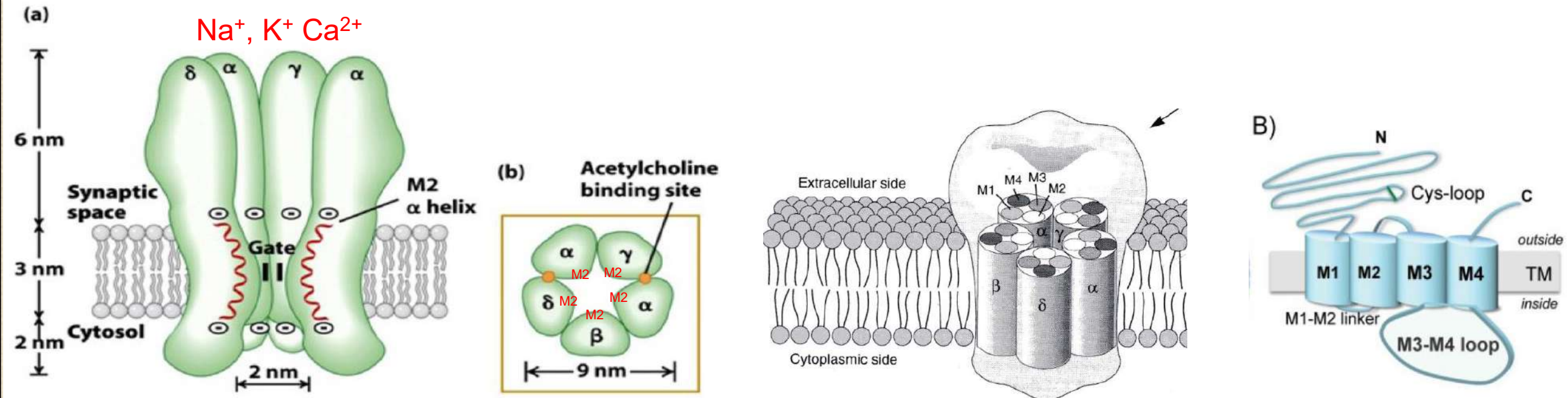
I recettori nicotinici dell'Acetilcolina (ACh), prendono il loro nome per via del loro potente agonista **nicotina**.

Essendo stati i primi recettori ionotropi ad essere studiati e classificati essi fungono da paradigma per tutti i recettori ionotropi pentamerici.



Recettore-canale attivato da ligando (acetil colina)

Il recettore muscolare è costituito da 5 subunità ($\alpha_2\beta\gamma\delta$)
Ciascuna con 4 eliche (M1-M4) transmembrana



Quando due molecole di acetilcolina si legano alla subunità alfa del recettore, si apre un canale ionico che permette il passaggio all'interno della cellula di ioni Na^+ e Ca^{++} con conseguente depolarizzazione ed eccitazione cellulare.

Recettore nicotino dell'Acetilcolina

La struttura e la funzionalità dei recettori per l'ACh sono diverse a seconda del tessuto in cui essi sono espressi.

È possibile infatti avere:

- Recettori muscolari (con sub-unità α , β , γ , δ ed ϵ)
- Recettori neuronali (con sub-unità α e β)

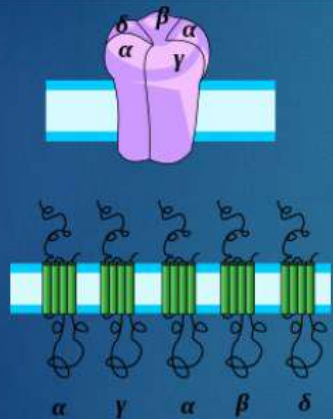
Tipo neuronale					Tipo muscolare
I	II	III			IV
$\alpha 9,$	$\alpha 7, \alpha 8$	1	2	3	$\alpha 1, \beta 1, \delta, \gamma, \epsilon$
		$\alpha 2, \alpha 3, \alpha 4, \alpha 6$	$\beta 2, \beta 4$	$\beta 3, \alpha 5$	

I sottotipi dei recettori nicotini.				
Sottotipi	Localizzazione sinaptica	Risposta fisiologica	Agonisti	Antagonisti
Muscolare ($\alpha 1$) ₂ $\beta 1 \delta \epsilon$	Giunzione neuro-muscolare: postsinaptico	Eccitatoria aumentano la permeabilità ai cationi (Na^+ , K^+)	ACh Carbacolo Suxametonio	Tubocurarina Pancuronio Esametonio α -Conotossina
Gangliare ($\alpha 4$) ₂ ($\beta 4$) ₃	Gangli autonomi: postsinaptico	Eccitatoria	ACh Carbacolo Nicotina DMPP Epibatidina	Mecamilamina Trimetafano
Neuronale ($\alpha 4$) ₂ ($\beta 2$) ₃	Cervello: pre e post-sinaptici	Eccitatoria – pre e post sinaptica, aumentano la permeabilità ai cationi (Na^+ , K^+)	Nicotina Epibatidina ACh Citosina	Mecamilamina Metilaconitina α -Conotossina
($\alpha 7$) ₅	Cervello: pre e post-sinaptici	Ca^{2+}	Epibatidina DMPP	Metilaconitina α -Bungarotossina

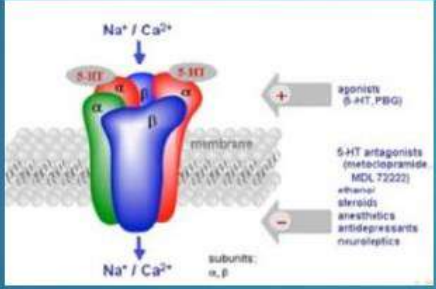
Recettori ionotropi della superfamiglia dei recettori nicotinici

Recettori nicotinici

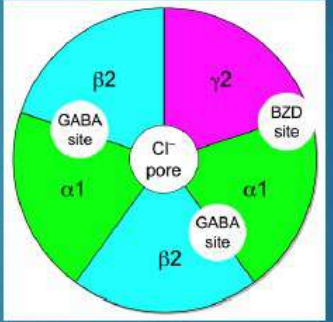
Acetilcolina



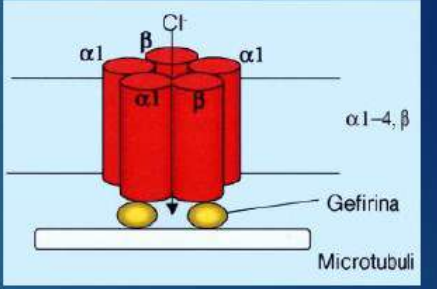
Serotonina



GABA



Glicina



Eccitatori - depolarizzazione



Inibitori - iperpolarizzazione

Recettore nicotinico della serotonina

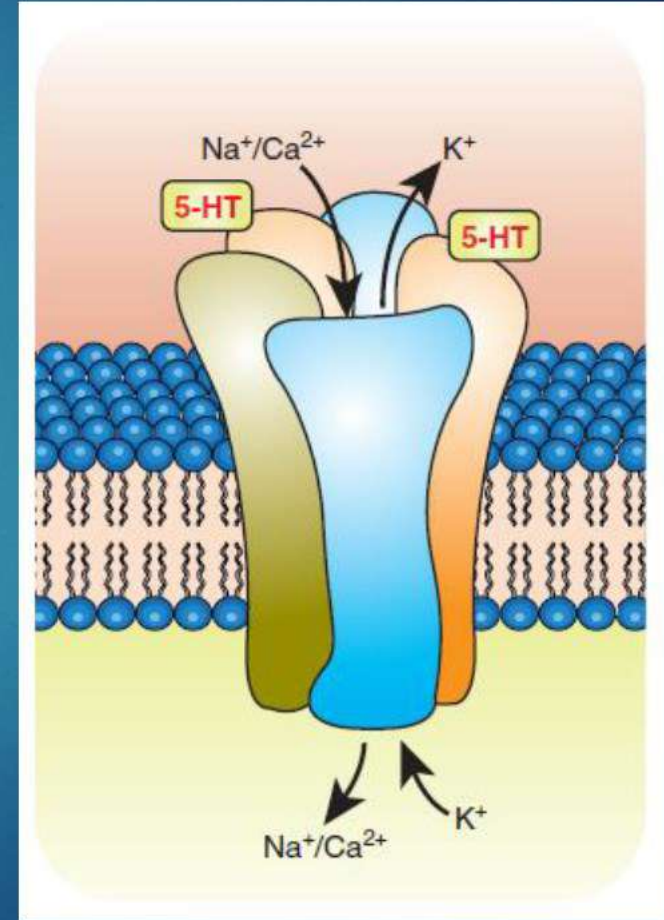
Il **recettore 5-HT₃** è un recettore-canale attivato dal ligando serotonina (5-idrossitriptamina) che permette il flusso di Na⁺ e K⁺; ha una struttura di membrana simile a quella del recettore colinergico nicotinico, con 5 sub-unità chiamate 5-HT_{3a-e}. La subunità 5-HT_{3a} è portatrice del sito di legame con la serotonina ed è quindi presente in ogni recettore in combinazione con le altre subunità.

Il legame della serotonina su due siti recettoriali determina l'apertura di un canale permeabile ai cationi Na⁺, K⁺ e Ca²⁺ con conseguente depolarizzazione desensibilizzante.

Questi recettori sono localizzati sulle terminazioni parasimpatiche nel tratto gastrointestinale e anche nelle afferenze vagali e splanchniche.

Nel sistema nervoso centrale (SNC), invece, vi è una elevata densità di recettori 5-HT₃ nel nucleo del tratto solitario e nell'area postrema (dove c'è il centro del vomito), ma anche nel nucleus accumbens, amigdala, ippocampo, corteccia entorinale e frontale)

I recettori 5-HT₃ presenti nel canale gastroenterico e nel SNC sono coinvolti nella risposta emetica e costituiscono la base anatomica per le proprietà antiemetiche degli antagonisti recettoriali 5-HT₃.

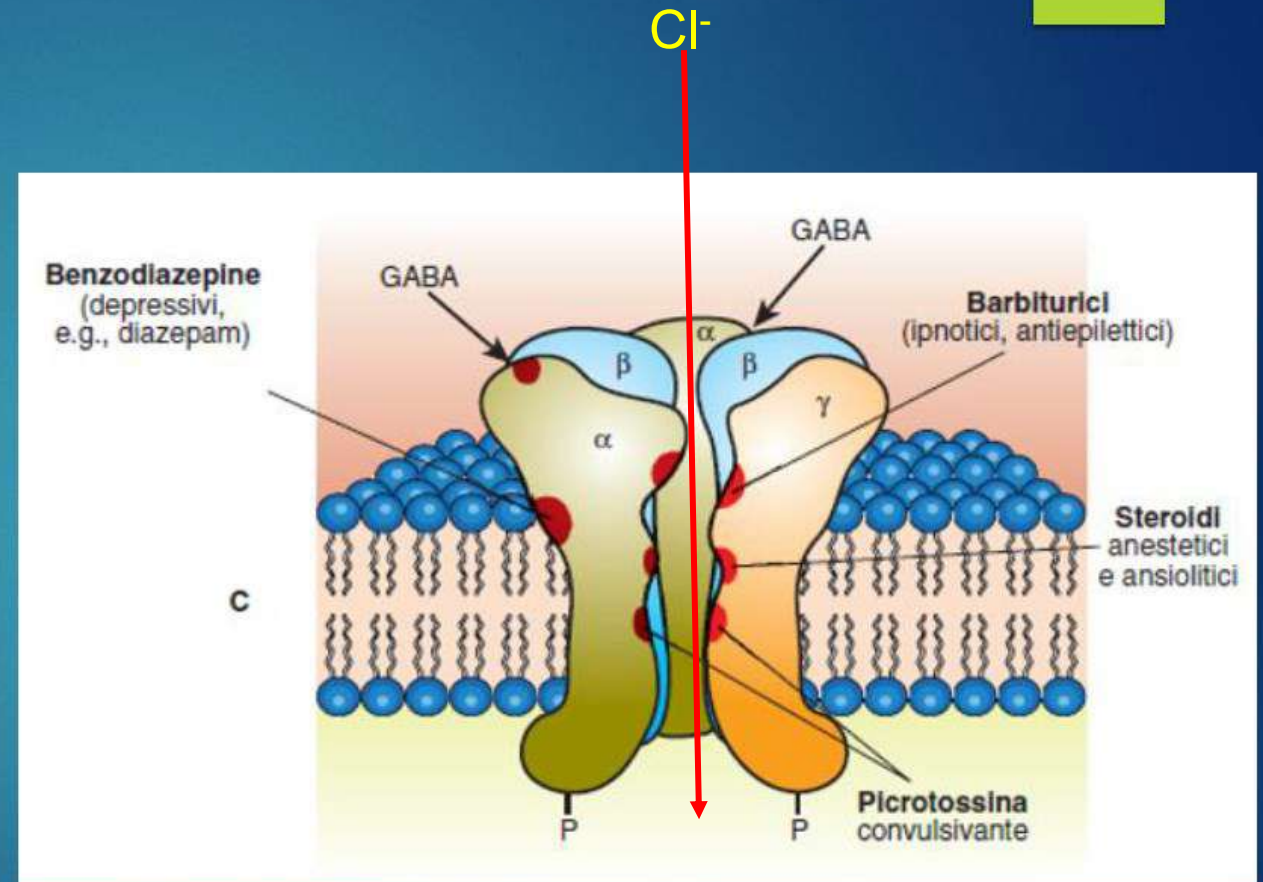


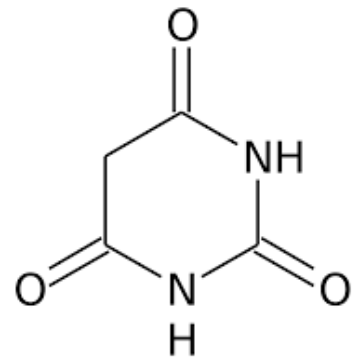
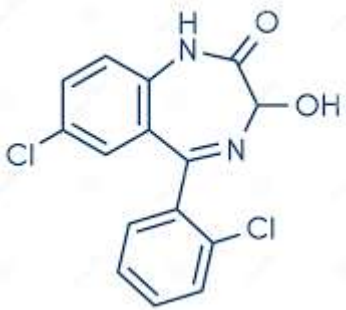
Recettore nicotinico del GABA

Il GABA-A è il recettore per l'acido gamma-amminobutirrico (GABA) che è il più importante neurotrasmettitore inibitorio del cervello. Il GABA è agonista endogeno del recettore e si lega sul sito di legame, mediando una modificazione allosterica che fa aprire il canale per gli anioni, principalmente Cl^- .

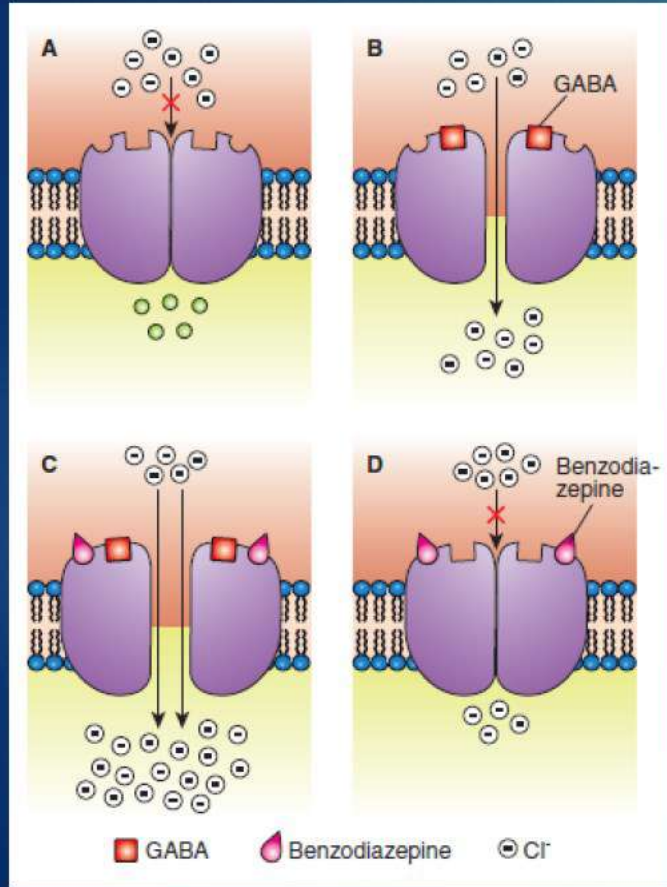
Il canale è formato da diverse subunità, leggermente diverse a seconda del distretto nervoso in cui si trova:

- 2 subunità α
- 2 subunità β
- 1 subunità γ





Recettore nicotinico del GABA



Esistono molti agonisti ed antagonisti del recettore GABA_A, che si legano alle diverse subunità su diversi siti di legame specifici, a loro dedicati, i più importanti comprendono:

- Benzodiazepine (ansiolitici)
- Barbiturici (sedativi, ipnotici)
- Steroidi (ormoni derivanti dal cortisolo)
- Etanolo (effetto simil-ansiolitico)
- Picrotossina (antagonista)

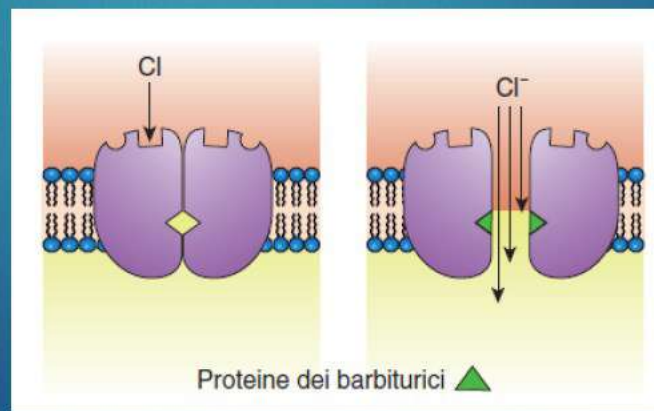


Fig. 5.21 - I barbiturici sono potenti agonisti del GABA_A. Legandosi a un sito posto all'interno del canale, i barbiturici lo aprono, ad alte dosi, in modo permanente causando un continuo influsso di Cl⁻, indipendentemente dalla presenza di GABA sul sito di legame.

Recettore nicotinico del GABA

Anche nel caso del GABA-A, le varie sub-unità possono presentare diverse isoforme.

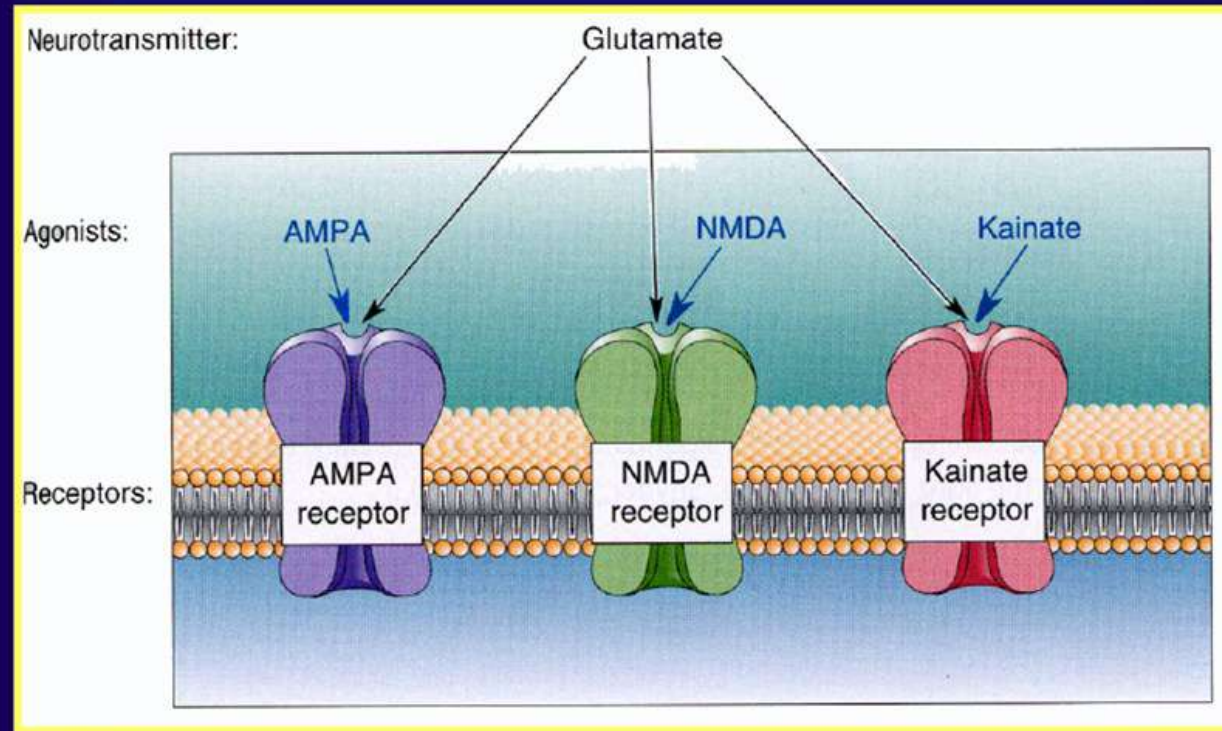
In particolare abbiamo 6 isoforme per l'unità alpha, 4 isoforme per la beta, e 4 isoforme per la gamma.

Queste isoforme si combinano fra loro in modo vario dando origine ad un ampio repertorio di sottotipi recettoriali espressi in modo specifico nei vari distretti del SNC

Tab. 5.2 - I principali sottotipi dei recettori GABA_A nel cervello del ratto

Sottotipo recettoriale	Abbondanza relativa nel cervello del ratto (%)	Localizzazione
$\alpha_1\beta_2\gamma_3$	43	Pressoché ubiquitario. In particolare abbondante negli interneuroni dell'ippocampo e della corteccia e nelle cellule di Purkinje del cervelletto
$\alpha_2\beta_{2/3}\gamma_2$	18	Motoneuroni del midollo spinale e cellule piramidali dell'ippocampo
$\alpha_3\beta_n\gamma_2/\gamma_3$	17	Neuroni colinergici e monoaminergici, ove regola il turnover dell'Acetilcolina e delle monoammine
$\alpha_2\beta_n\gamma_1$	8	Glia di Bergmann e nuclei del sistema limbico
$\alpha_5\beta_3\gamma_2/\gamma_3$	4	Cellule piramidali dell'ippocampo
$\alpha_6\beta\gamma_2$	2	Neuroni granulari del cervelletto
$\alpha_6\beta\delta$	2	Neuroni granulari del cervelletto; insensibile alle benzodiazepine
$\alpha_4\beta\delta$	3	Talamo e giro dentato dell'ippocampo; insensibile alle benzodiazepine
Altri sottotipi minori	3	Intero cervello

RECETTORI IONOTROPI PER IL GLUTAMMATO

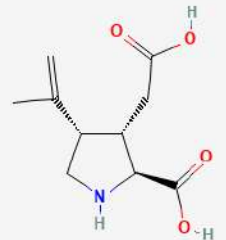


AMPA: acido α -amino-3-idrossi-5-metil-4-isoxazol-propionico

NMDA: N-metil-D-aspartato

La loro importanza deriva dal fatto che il glutammato è il neurotrasmettitore in assoluto più presente nel cervello e costituisce il principale agente della neurotrasmissione eccitatoria.

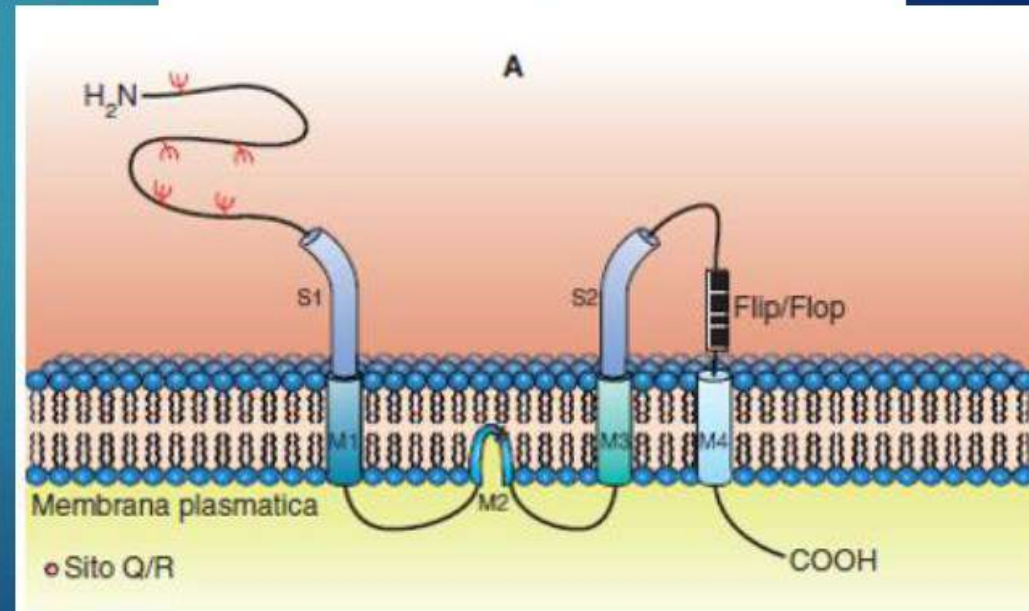
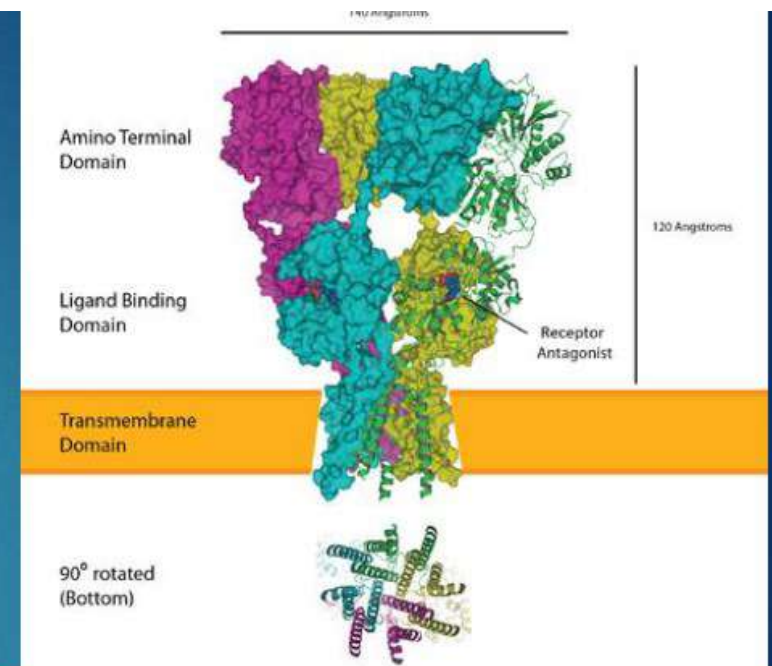
Questi recettori mediano importanti funzioni come la plasticità sinaptica (apprendimento e memoria), ma se stimolati eccessivamente (ictus, epilessia) possono dare il via a processi di neurotossicità che porta alla morte cellulare. Per tale motivo è possibile che questi recettori abbiano un ruolo alla base delle malattie neurodegenerative.



Recettori ionotropi del glutammato

Dal punto di vista strutturale, questi recettori sono composti da 4 o 5 sub-unità principali, caratterizzate da una comune organizzazione di base della catena polipeptidica, con l'estremità N-terminale in ambiente extracellulare e l'estremità C-terminale in ambiente intra-cellulare.

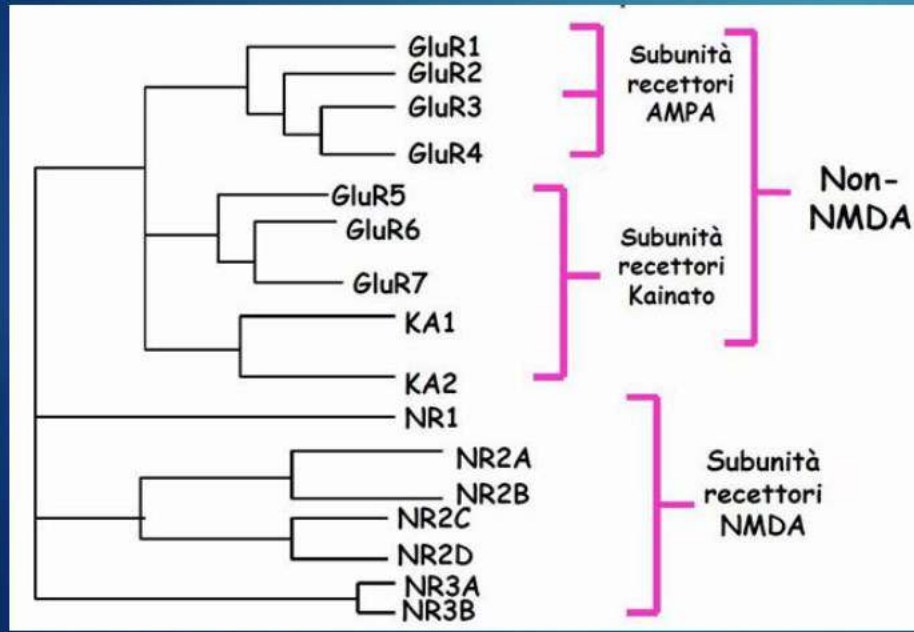
Ogni subunità contiene sempre 3 regioni trans-membrana (M1, M3 ed M4) ed un ansa (M2) situata nel versante intracellulare il cui scopo è controllare la permeabilità del poro acquoso.



Recettori ionotropi del glutammato

La specificità e l'importanza di questi recettori è indirettamente confermata dalla molteplicità dei tipi di sub-unità che li compongono.

Queste sub-unità possono essere divise in gruppi sulla base delle somiglianze nelle loro sequenze amminoacidiche



Tab. 5.3 - Le subunità dei recettori del glutammato nell'uomo

Gruppo	Recettore	Subunità	Gene	Localizzazione cromosomica
1	AMPA	GluR1	GRIA1	5q33
1	AMPA	GluR2	GRIA2	4q32-33
1	AMPA	GluR3	GRIA3	Xq25-26
1	AMPA	GluR4	GRIA4	11q22-23
2	Kainato	GluR5	GRIK1	21q21.1-22.1
2	Kainato	GluR6	GRIK2	6q16.3-q21
2	Kainato	GluR7	GRIK3	1p34-p33
3	Kainato	KA-1	GRIK4	11q22.3
3	Kainato	KA-2	GRIK5	19q13.2
4	NMDA	NR1	GRIN1	9q34.3
5	NMDA	NR2A	GRIN2A	16p13.2
5	NMDA	NR2B	GRIN2B	12p12
5	NMDA	NR2C	GRIN2C	17q24-q25
5	NMDA	NR2D	GRIN2D	19q13.1qter
6	NMDA	NR3A	GRIN3A	9q34
6	NMDA	NR3B	GRIN3B	19p13.3

Recettori ionotropi del glutammato

Recettori del glutammato

AMPA

GluR1
GluR3
GluR3
GluR4

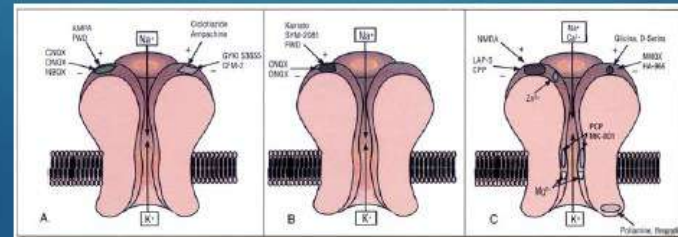
Kainato

KA1, 2
GluR5
GluR6
GluR7

NMDA

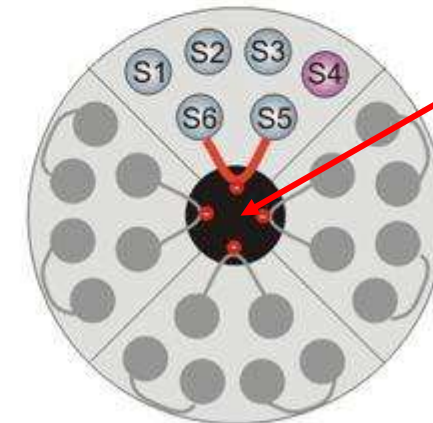
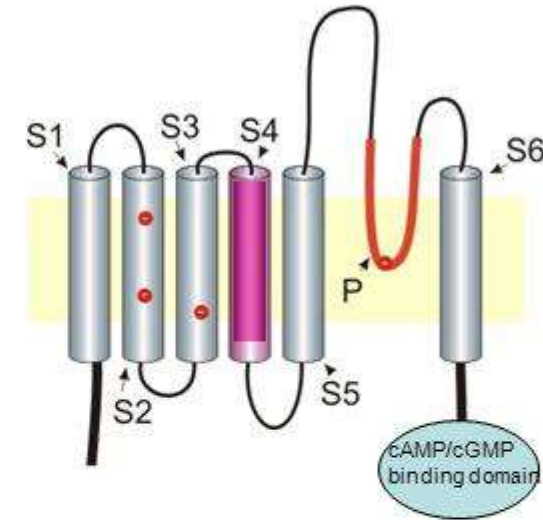
NR1
NR2A,B
C,D
NR3A,B

Eccitatori - depolarizzazione



I canali CNG

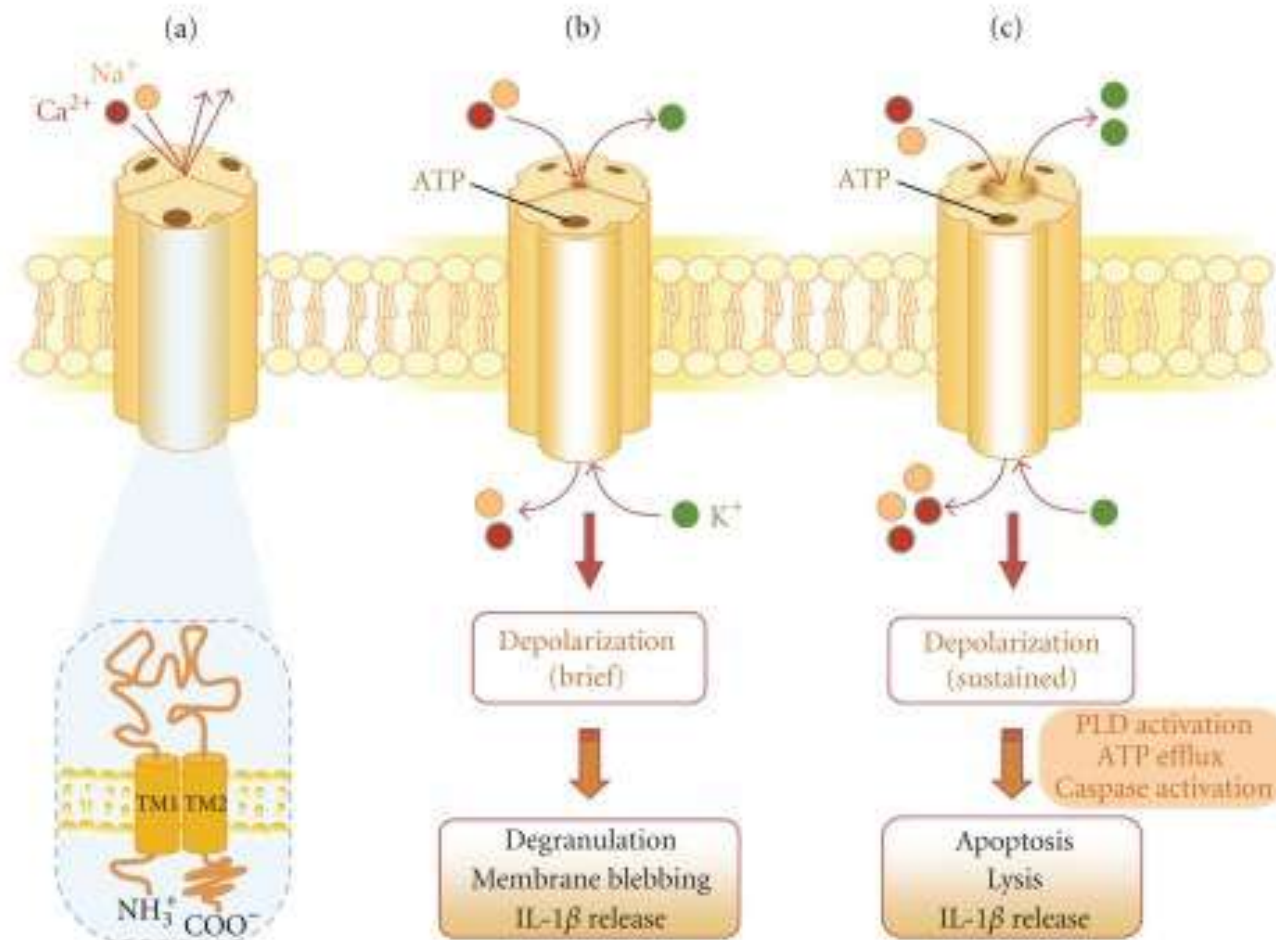
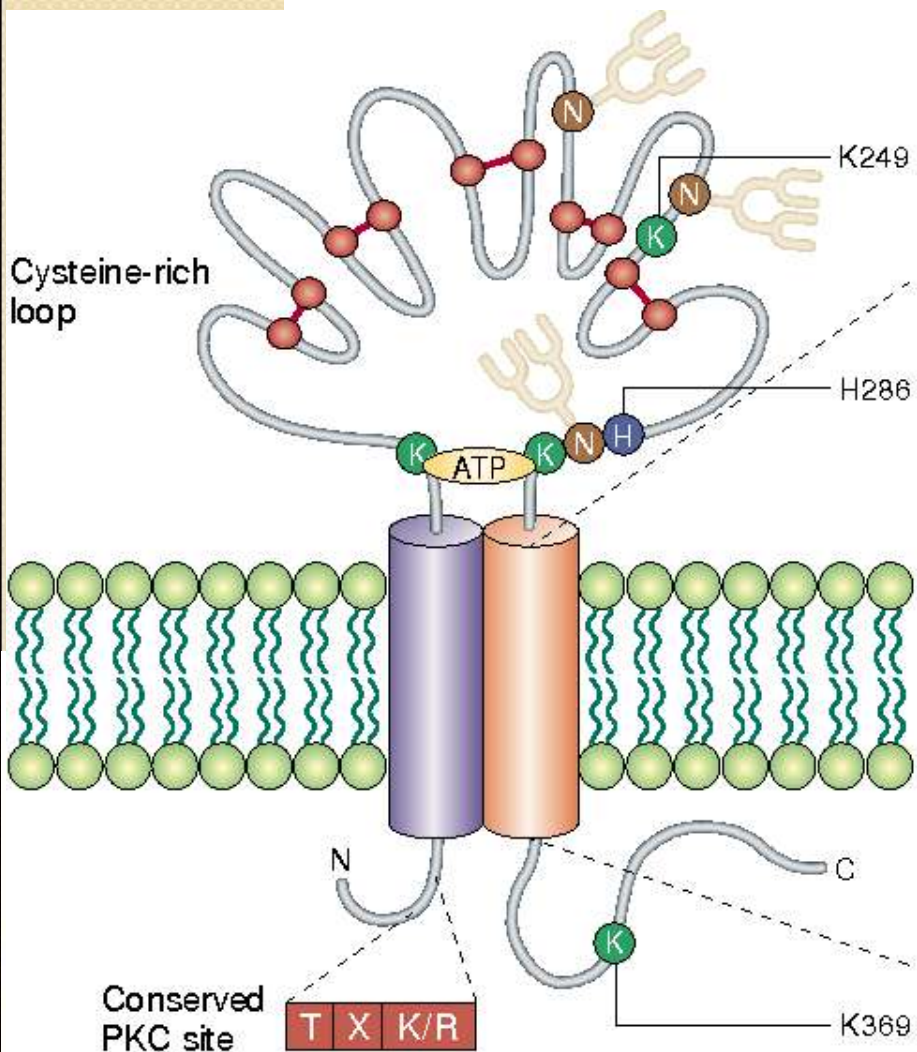
- Struttura omologa ai canali voltaggio-dipendenti per il K
- Tetramero, 6 eliche transmembrana, 1 regione P tra S5 e S6
- Differenze significative:
 - Poro senza filtro di selettività per il K
 - S4 senza cariche sensore del voltaggio
 - Dominio C-terminale per legame nucleotidi ciclici (cAMP, cGMP)
- Due sottofamiglie: alfa (CNGA1-4) e beta (CNGB1,3)



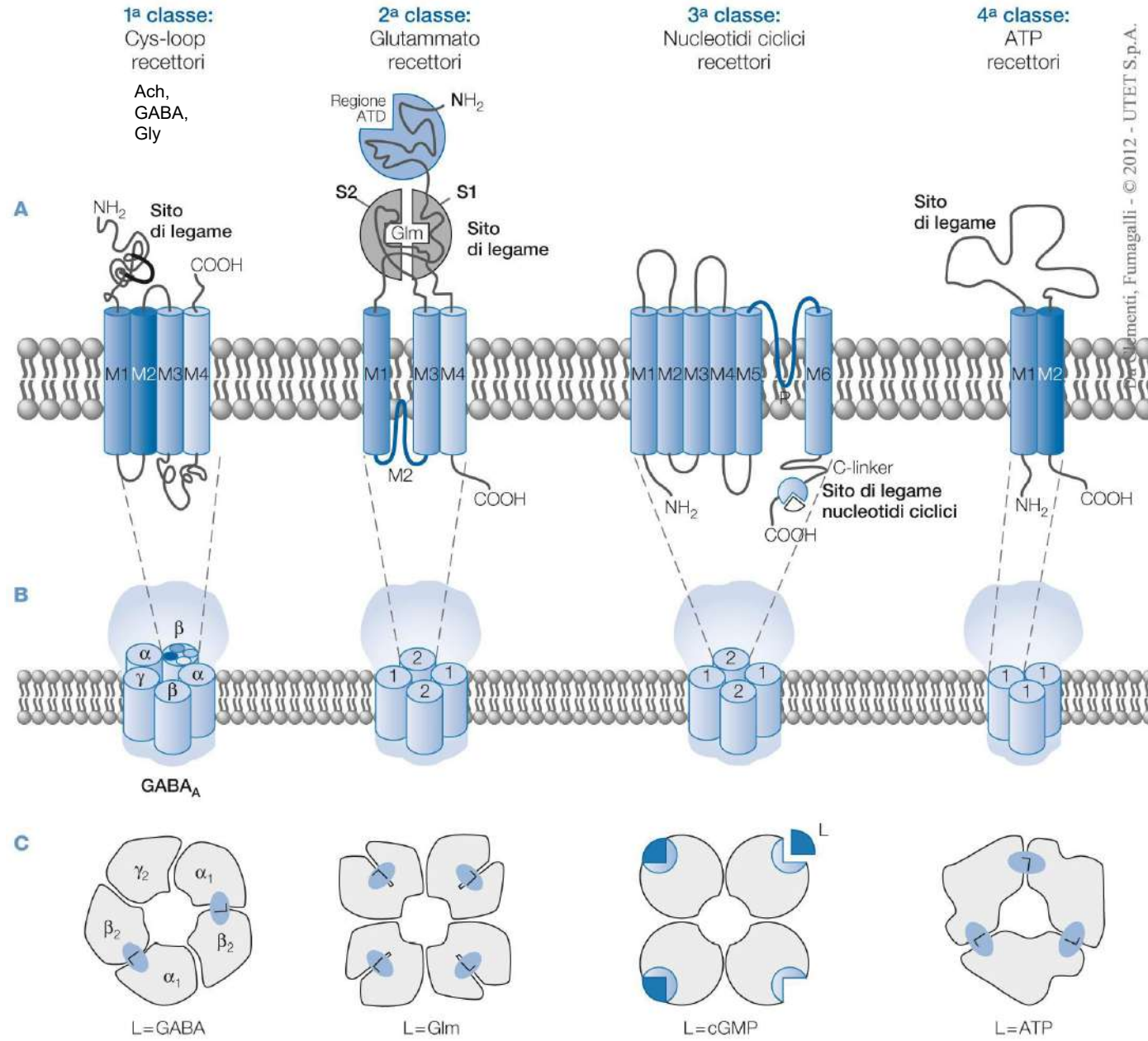
vista dall'alto

Recettori ionotropi per ATP

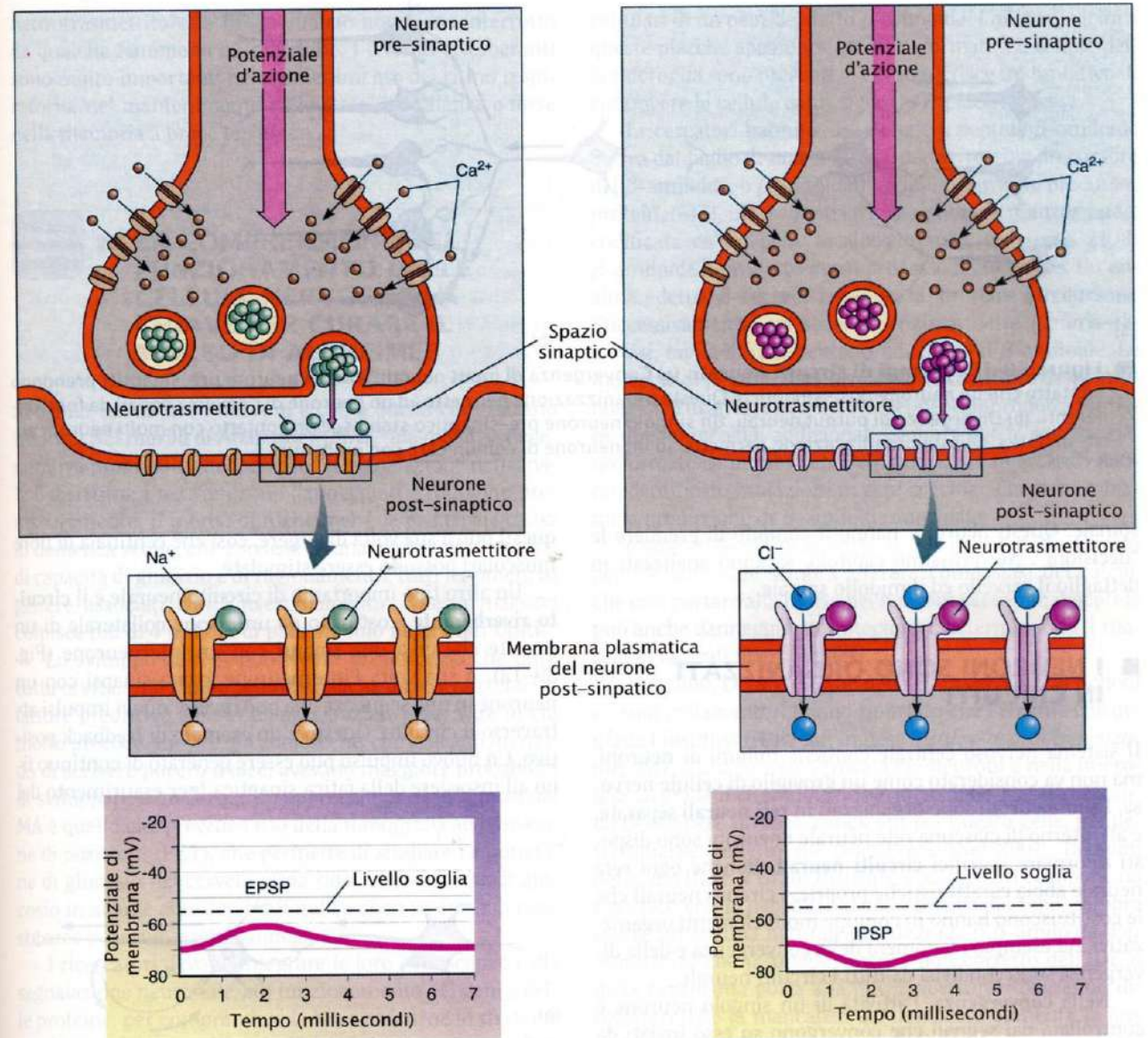
Omotrimeri di 7 differenti subunità (P2X1-7) formati da due segmenti transmembrana. Poro selettivo ai cationi (calcio +/- sodio)



Struttura molecolare dei recettori-canale



A seconda della carica permeante, la stimolazione recettoriale può portare a depolarizzazione o iperpolarizzazione



(a) Input eccitatorio

(b) Input inibitorio

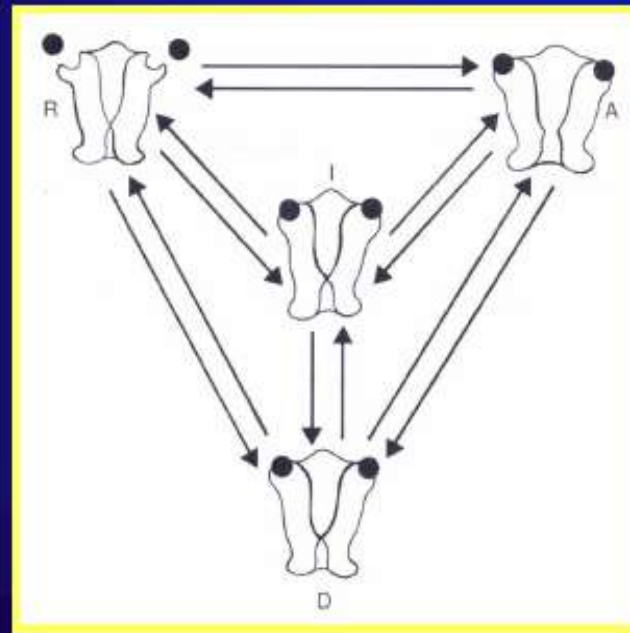
■ **Figura 39-11 Confronto tra (a) un potenziale post-sinaptico eccitatorio (EPSP) e (b) un potenziale post-sinaptico inibitorio (IPSP).** Da notare che un neurotrasmettitore, che apre i canali per il Na^+ genera EPSP. Questo porta alla genesi di un potenziale d'azione. Un neurotrasmettitore che apre i canali per il Cl^- genera IPSP e in questo modo iperpolarizza la membrana.

DESENSITIZZAZIONE DEI RECETTORI CANALE

Per questa superfamiglia di recettori la desensitizzazione equivale a una riduzione della capacità di andare incontro alle modificazioni conformazionali necessarie per produrre l'apertura del canale ionico.

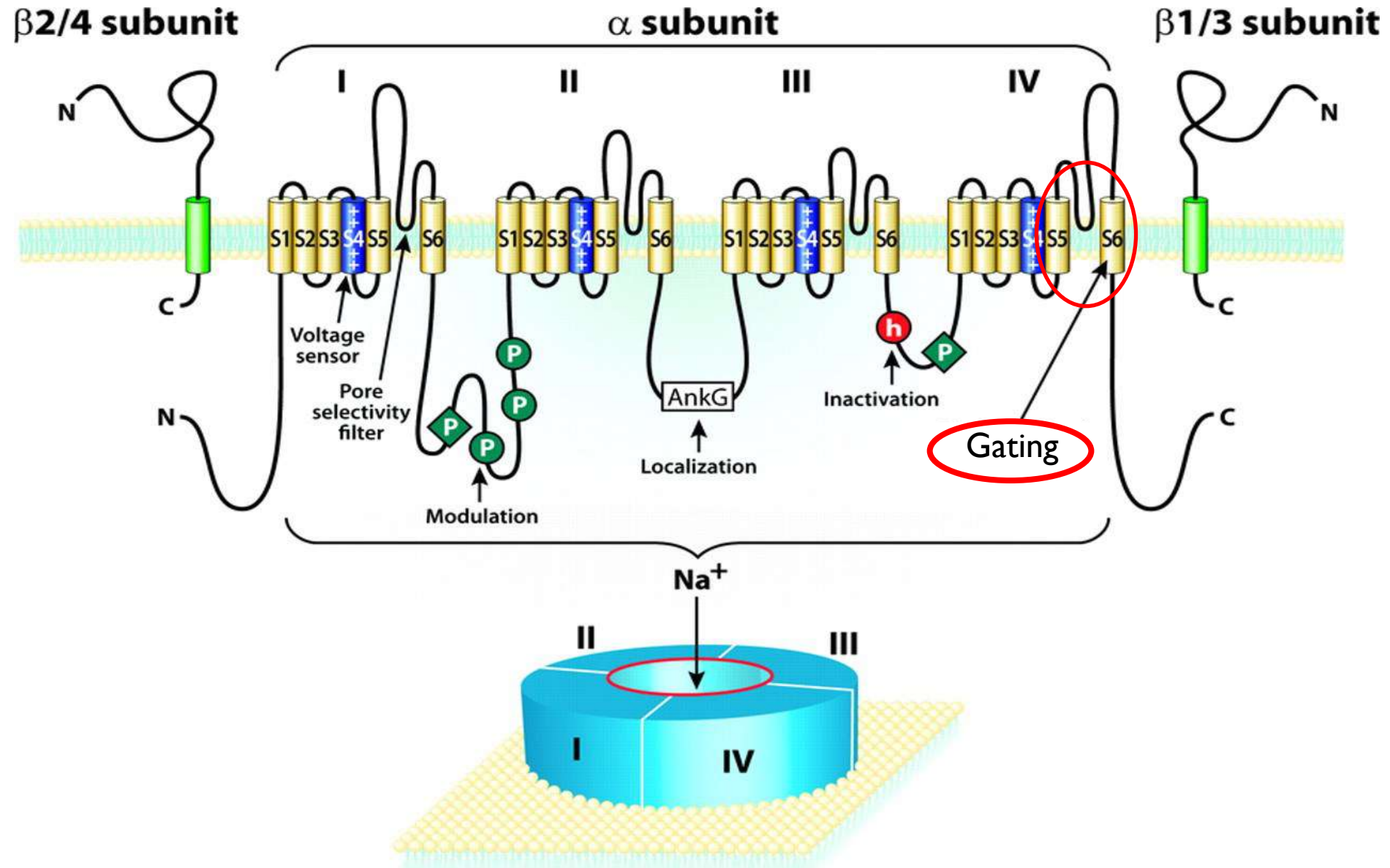
Applicazione AGONISTI e loro legame al recettore:

- 1) dopo pochi msec apertura del canale e flusso ionico (A)
- 2) successivamente transizione del R nello stato desensitizzato: il canale rimane chiuso nonostante l'affinità per l'agonista aumenti notevolmente (I e D)

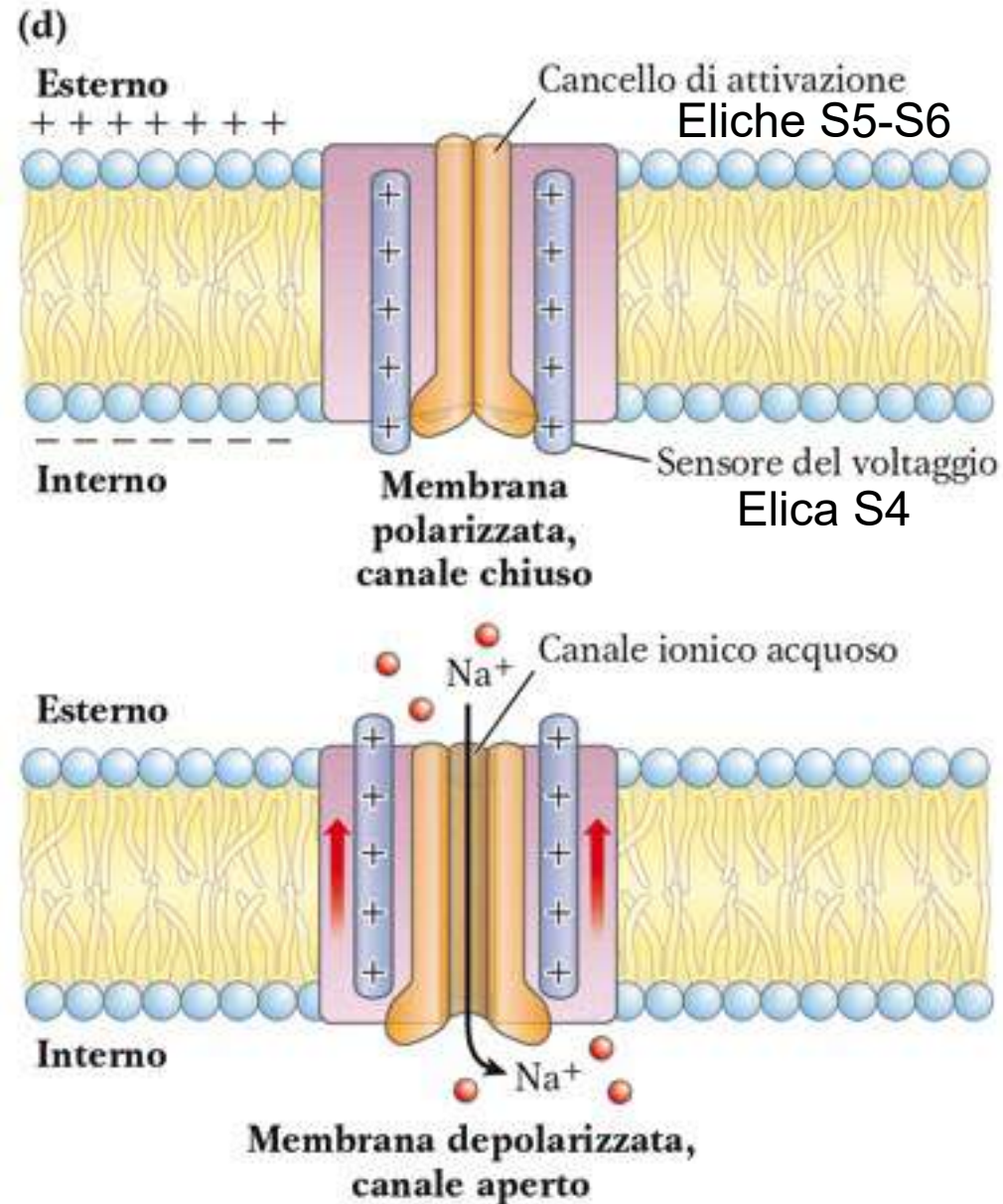


R= a riposo;
A= stato attivo;
I= componente rapida della desensitizzazione (stato desensitizzato);
D= componente lenta della desensitizzazione (stato refrattario)

Canale del Na⁺ **v**oltaggio dipendente

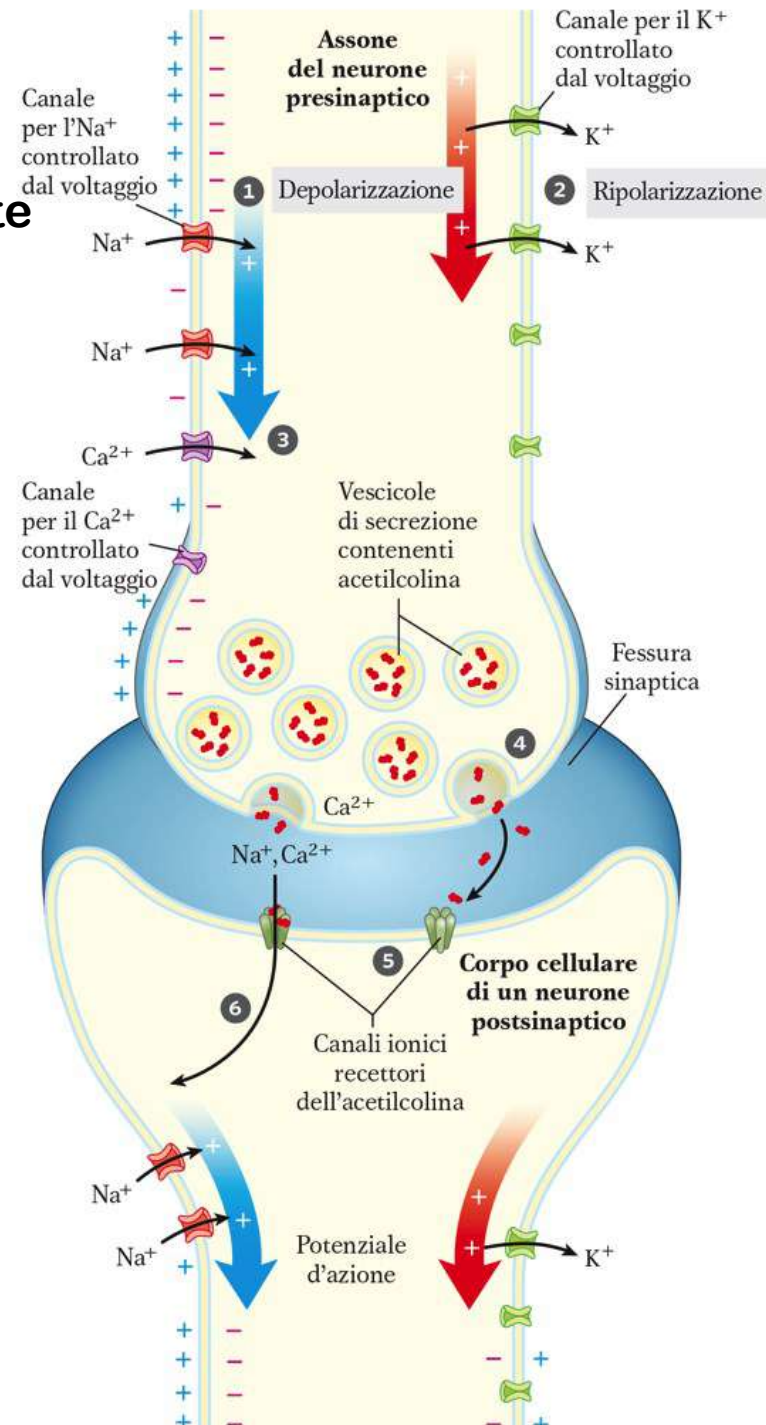


Canale del Na^+ voltaggio dipendente

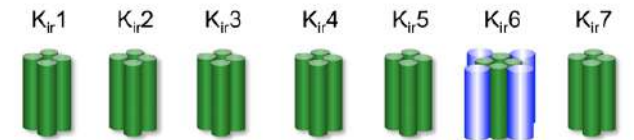
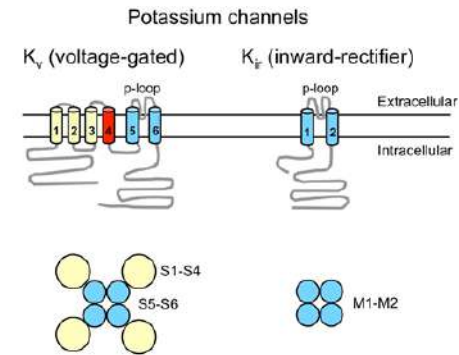


Si aprono quando la membrana si depolarizza localmente in risposta ad uno stimolo (neurotrasmettitore)

I canali per il Na^+ hanno un periodo refrattario che assicura la trasmissione unidirezionale dell'impulso elettrico



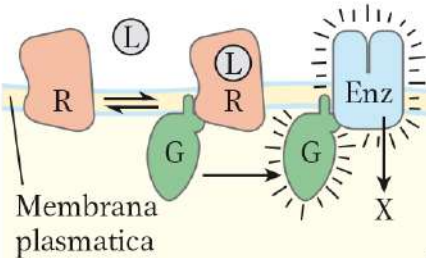
I canali K^+ si aprono una frazione di secondo dopo i canali per il Na^+ e contribuiscono a ripolarizzare la membrana



Principali tipi di recettori/trasduttori di segnale

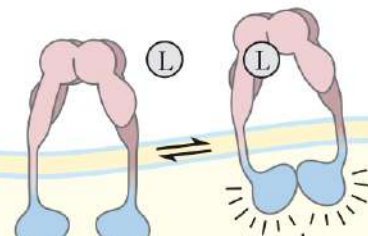
1. Recettori accoppiati alle proteine G

Il legame di un ligando esterno (L) al recettore (R) attiva una proteina intracellulare che lega il GTP (G); essa a sua volta regola l'attività di un enzima (Enz), che genera un secondo messaggero intracellulare (X).

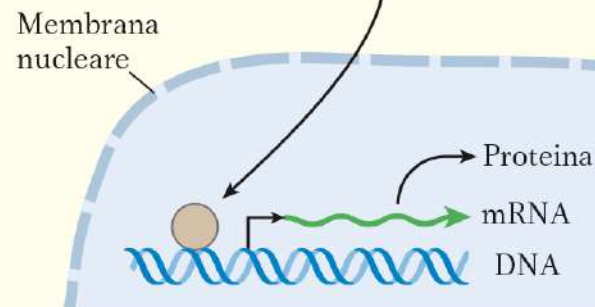


2a. Recettore con attività tirosina chinasi

Il legame del ligando innesca l'attività tirosina chinasi mediante autofosforilazione.

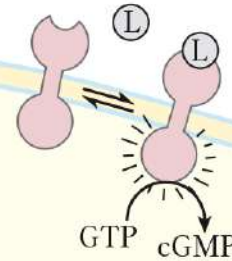


2b. La chinasi attiva un fattore di trascrizione, alterando l'espressione genica.



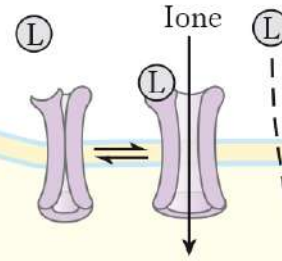
3. Recettore con attività guanilil ciclasica

Il legame del ligando al dominio extracellulare stimola la formazione del secondo messaggero, il GMP ciclico (cGMP).



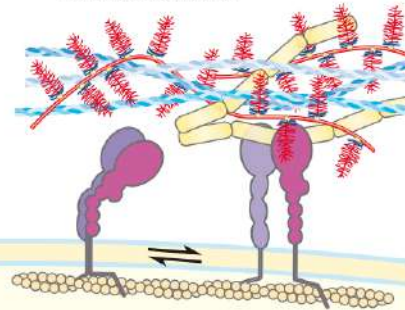
4. Canale ionico controllato

Si apre e si chiude in risposta alla concentrazione del ligando segnale o al potenziale di membrana.



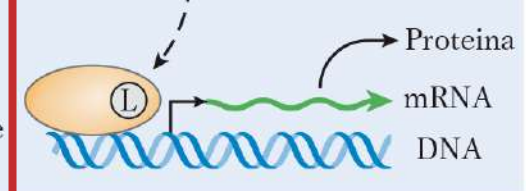
5. Recettore di adesione (integrina)

Lega molecole della matrice extracellulare, cambia la propria conformazione e altera l'interazione con il citoscheletro.



6. Recettore nucleare

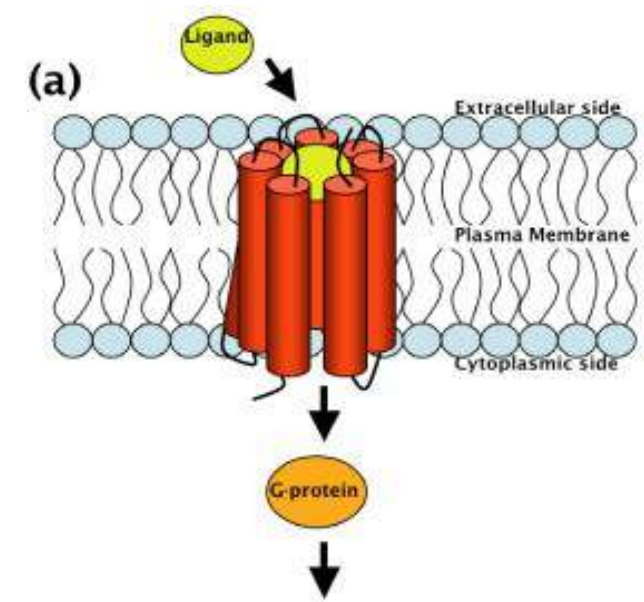
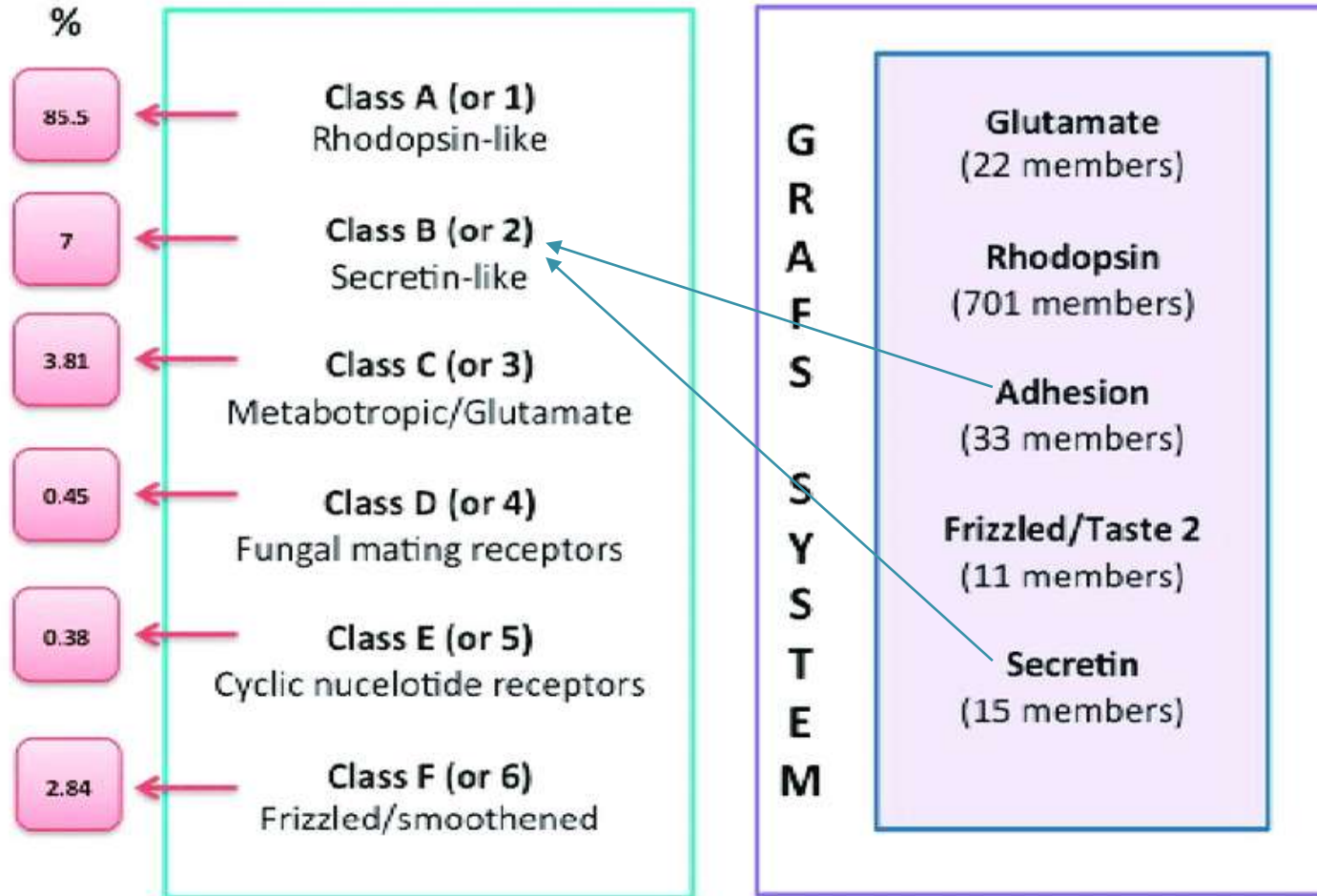
Il legame dell'ormone permette al recettore di regolare l'espressione di geni specifici.



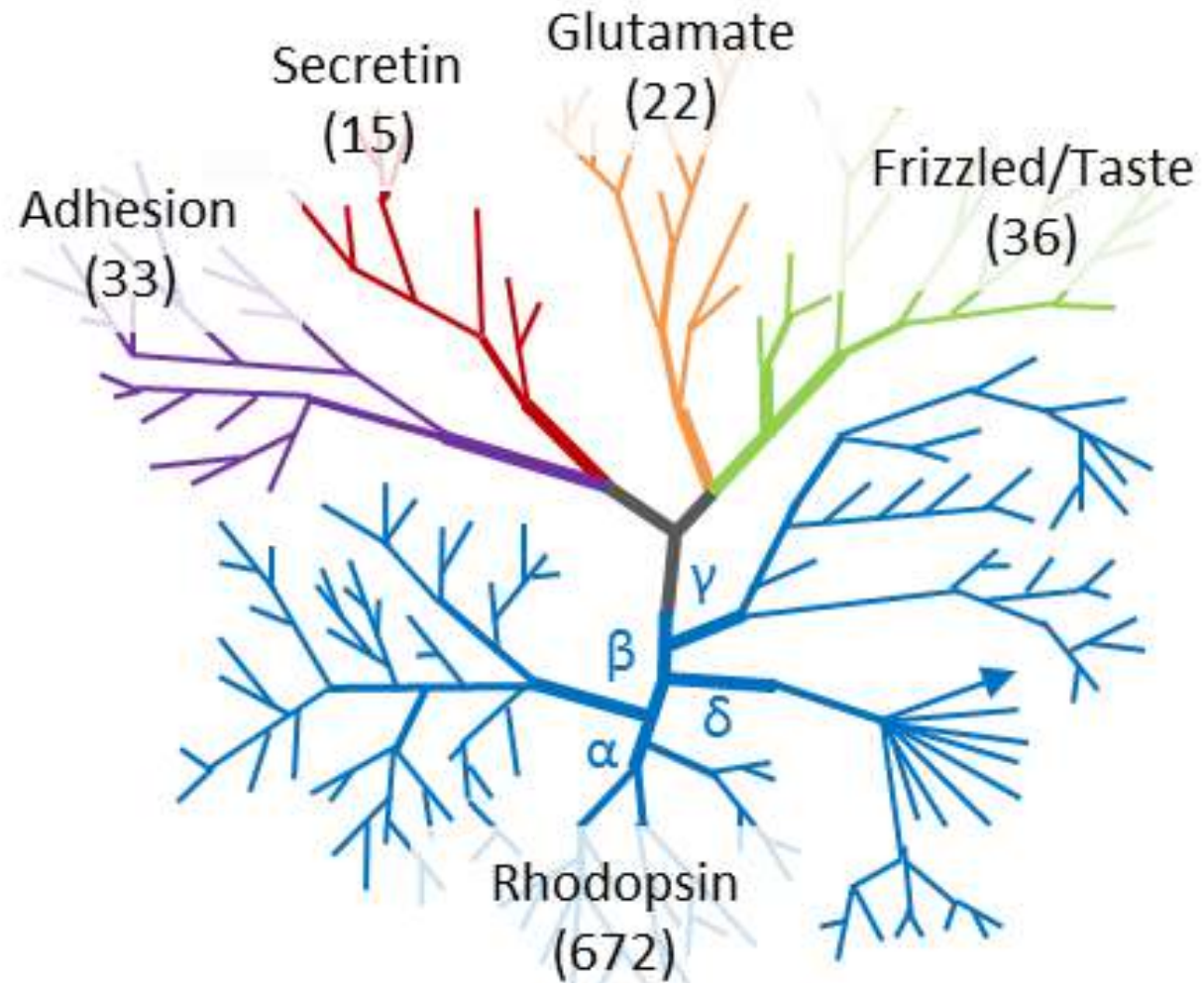
GPCRs classification

based on
sequence homology

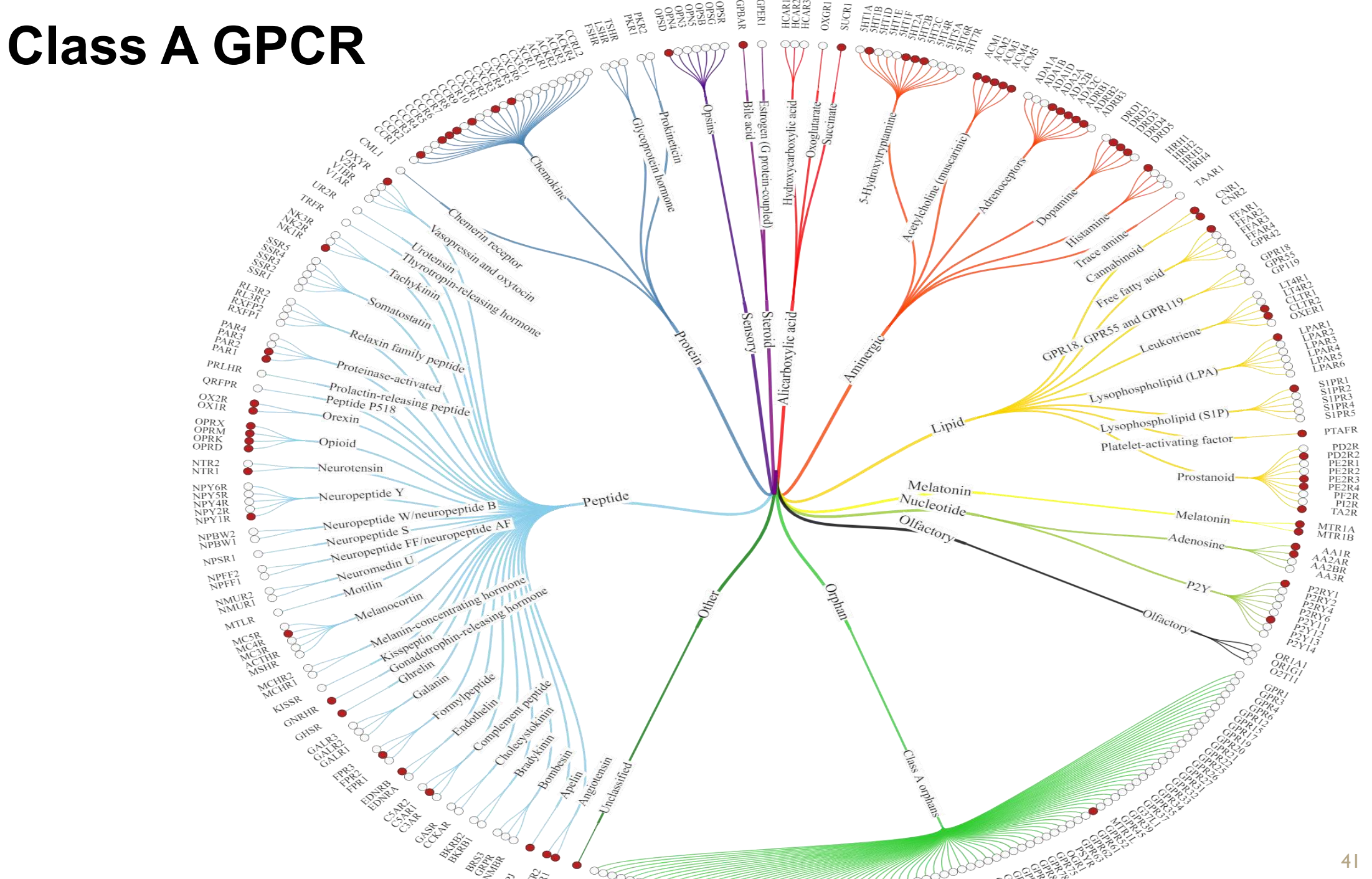
based on
phylogenetic origin



La superfamiglia dei recettori accoppiati alle proteine G (GPCR)

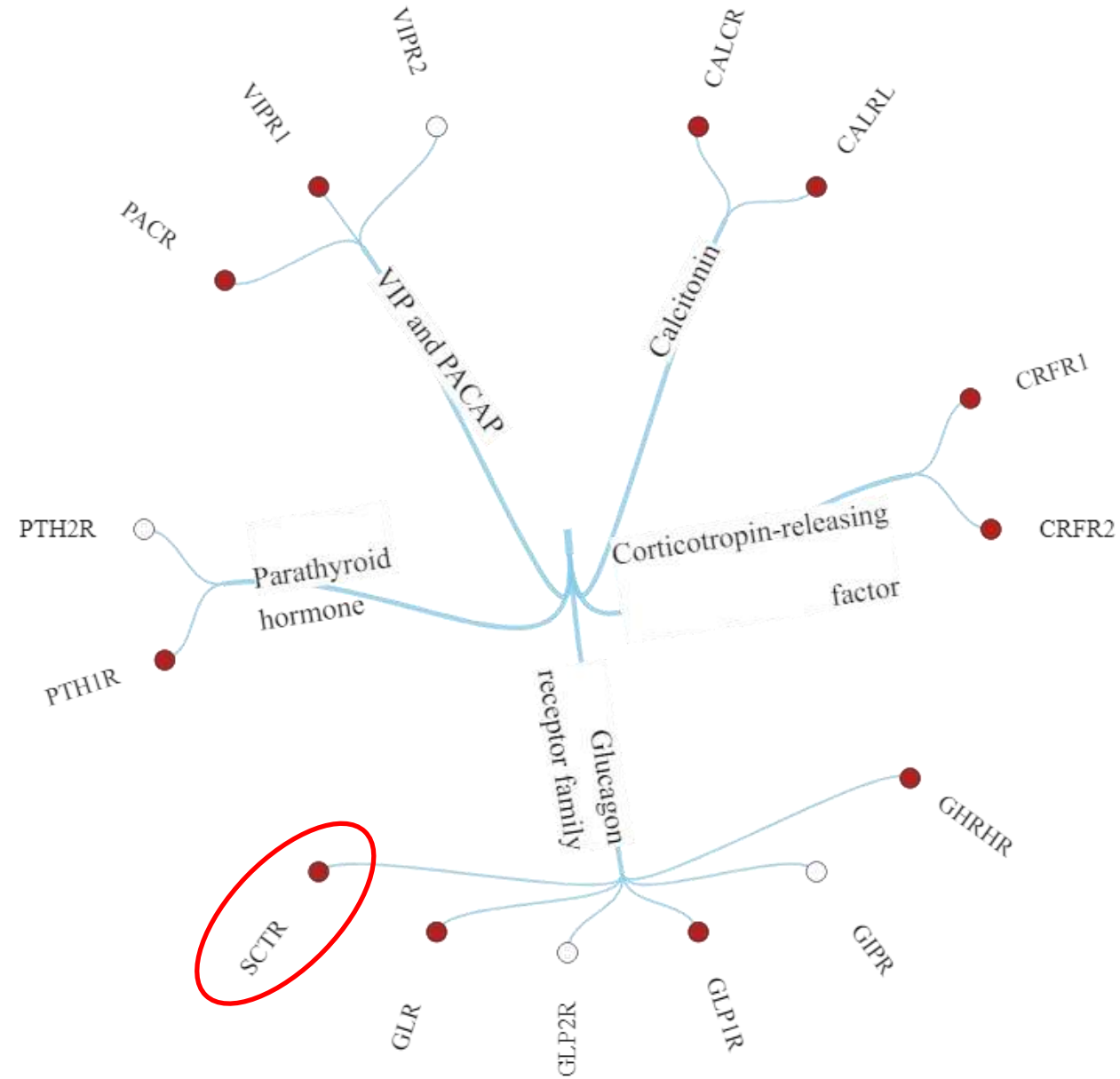


Class A GPCR

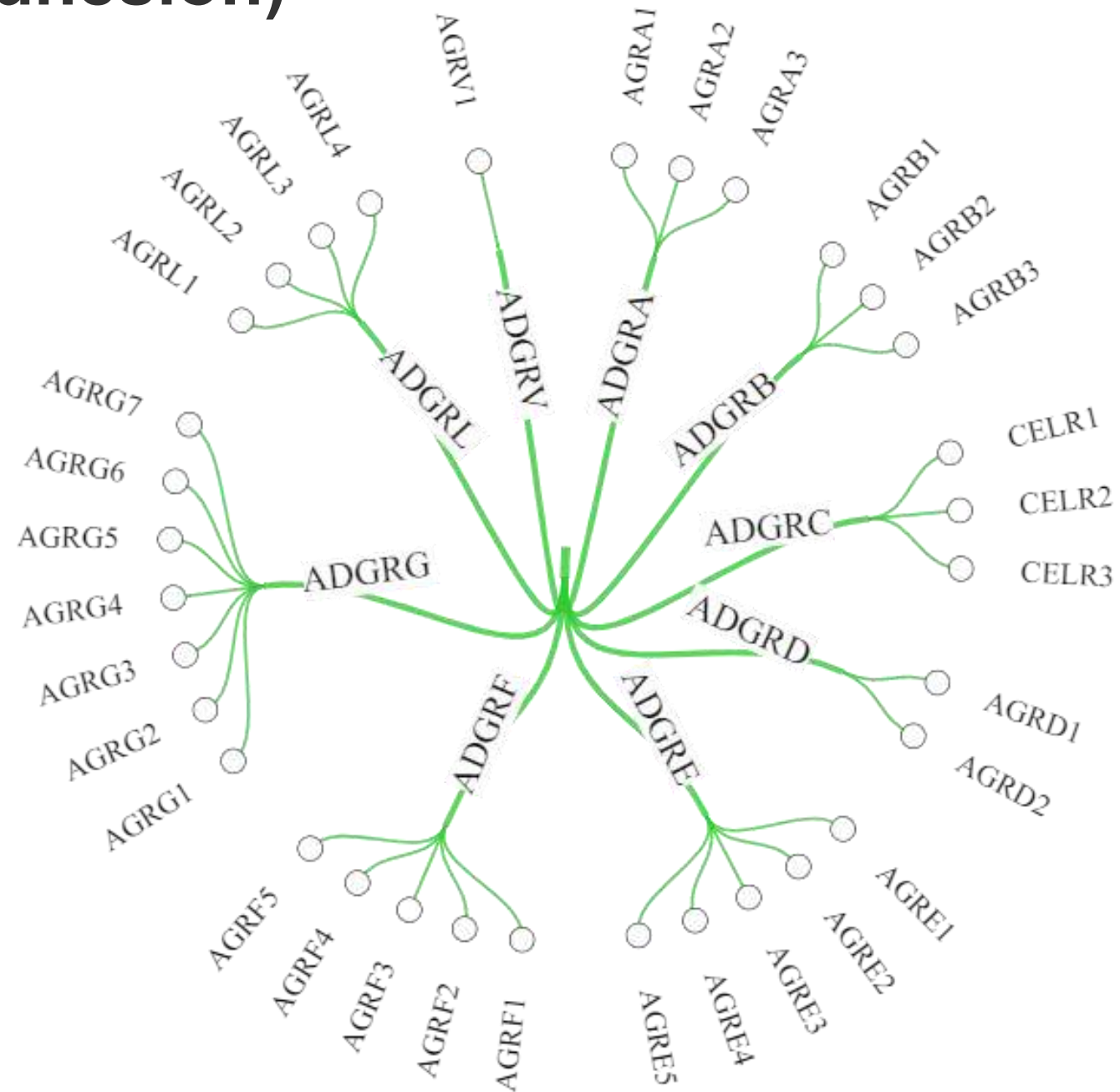


Class B1 (Secretin)

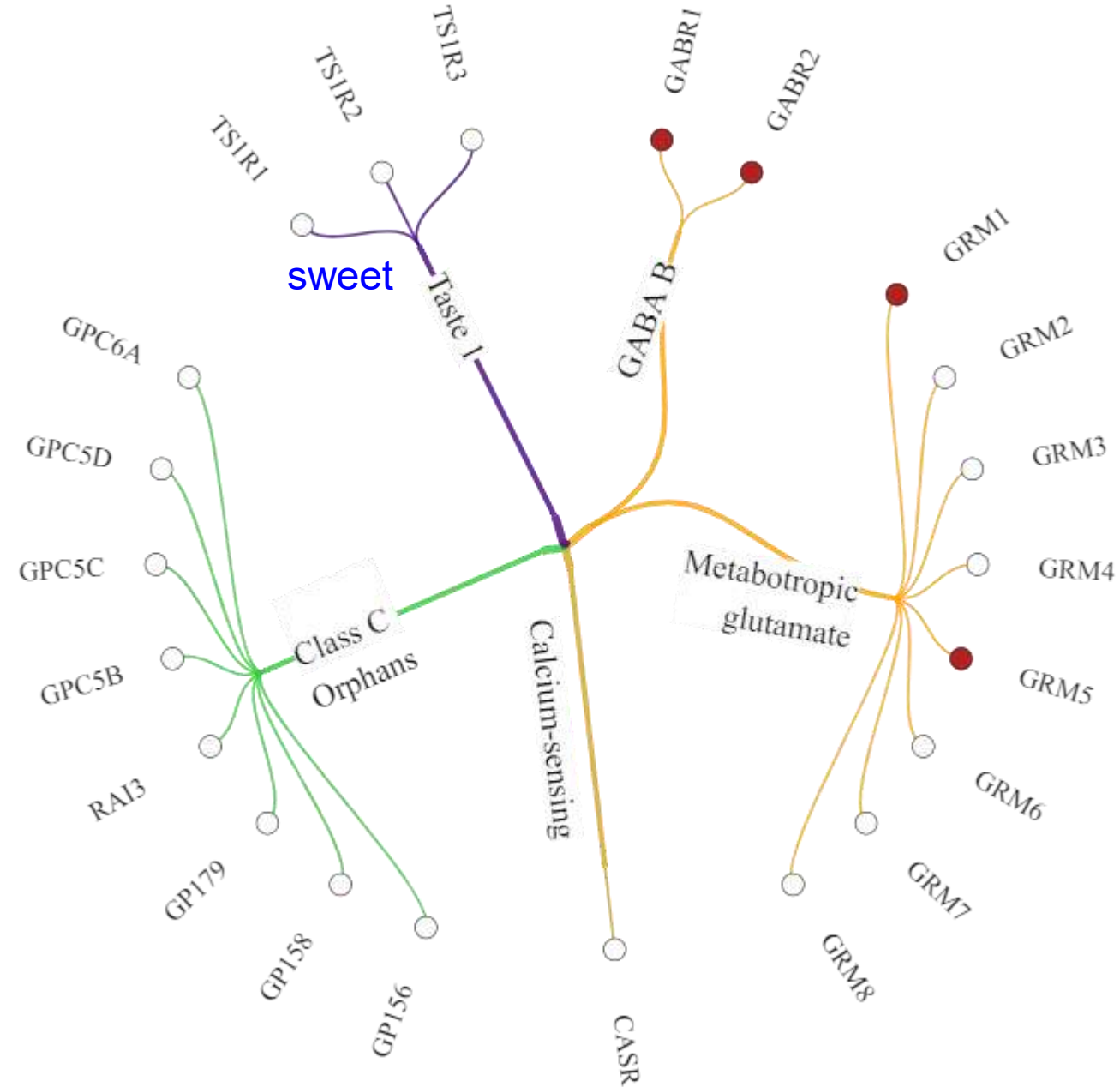
Ormone prodotto dal duodeno



Class B2 (Adhesion)

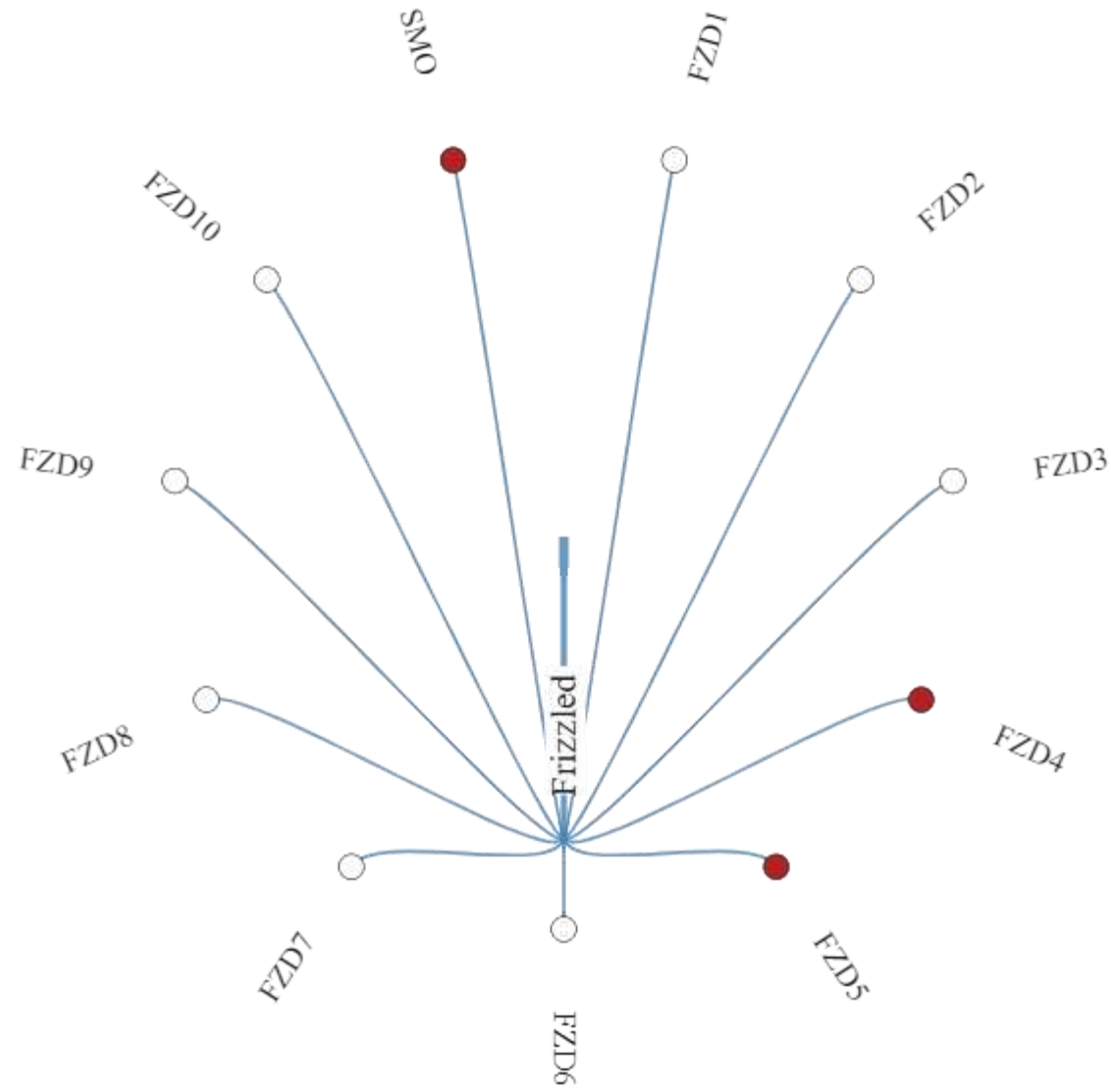


Class C (Glutamate)

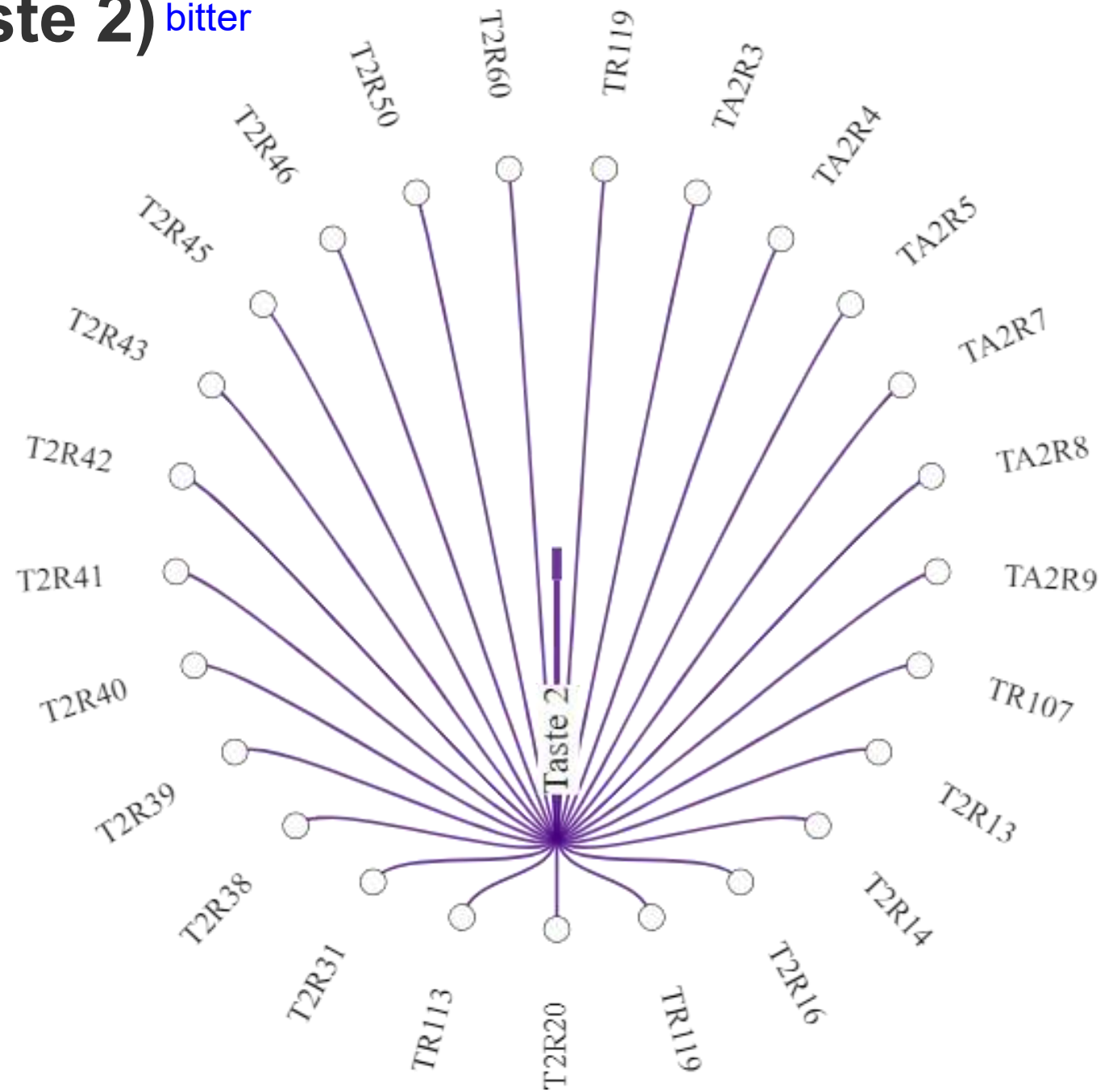


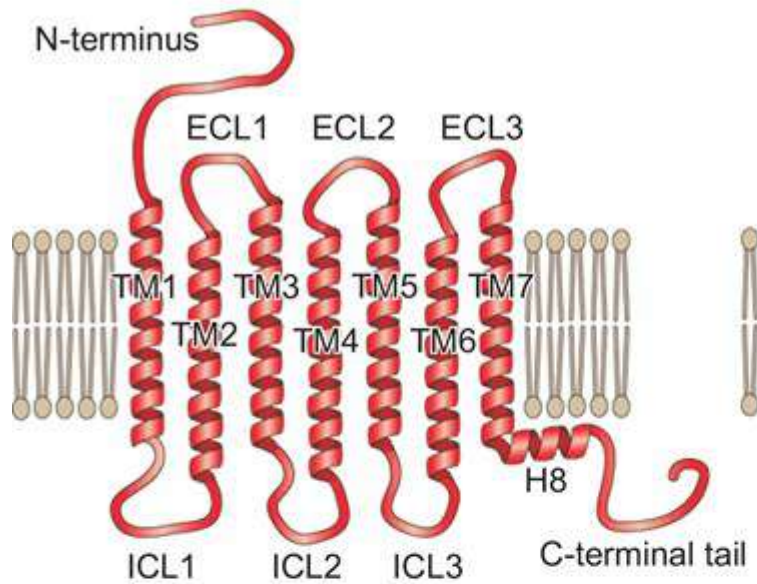
Class F (Frizzled)

Recettori per Wnt

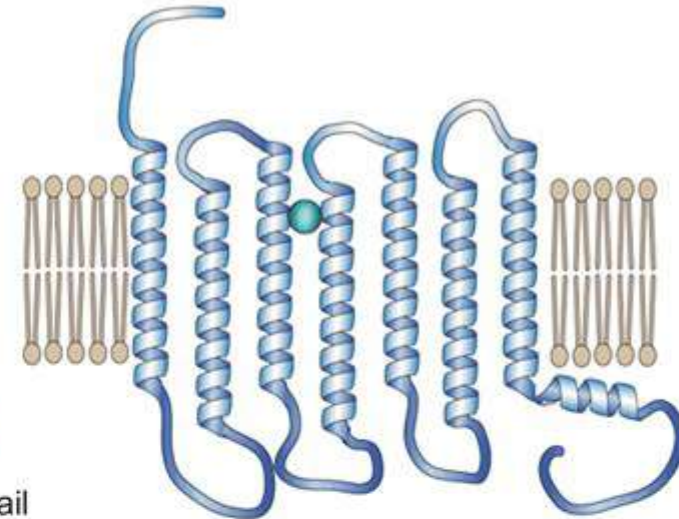


Class T (Taste 2) ^{bitter}

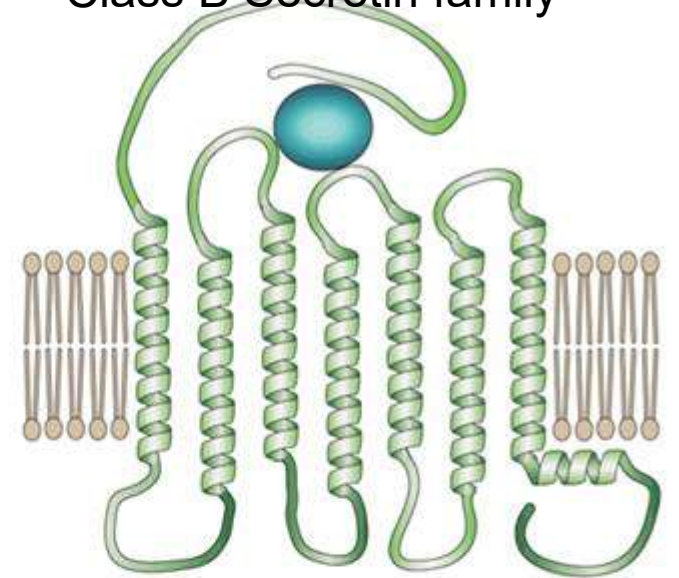




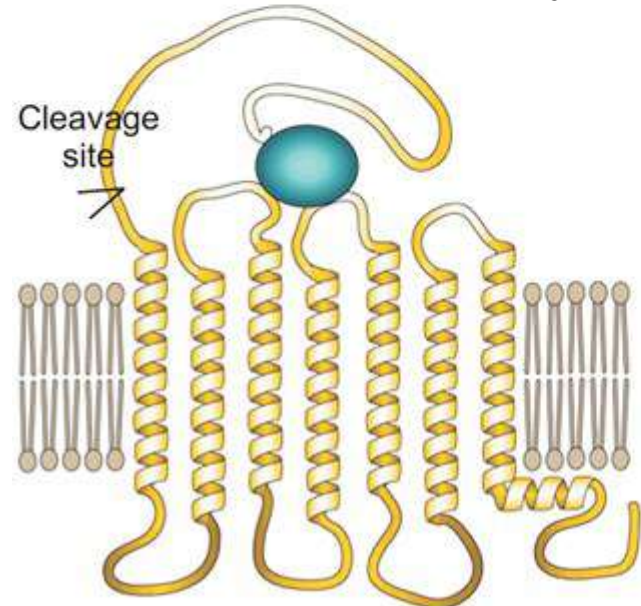
Class A Rhodopsin family



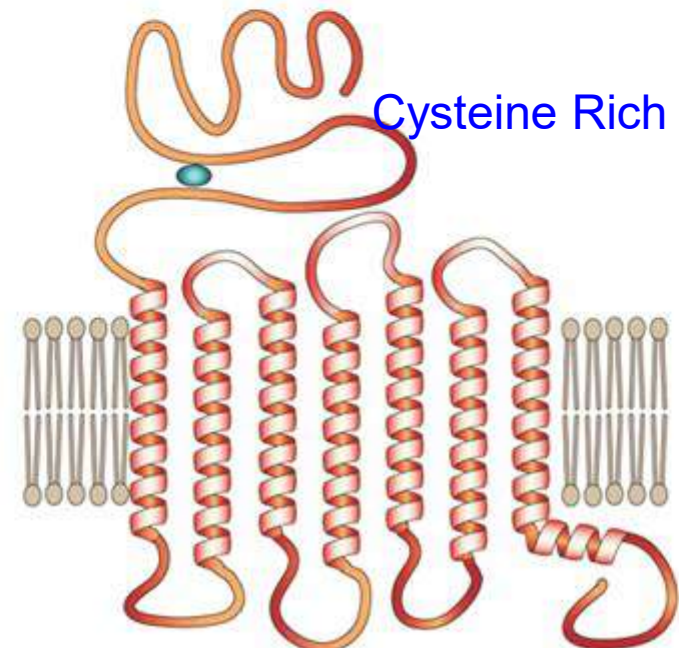
Class B Secretin family



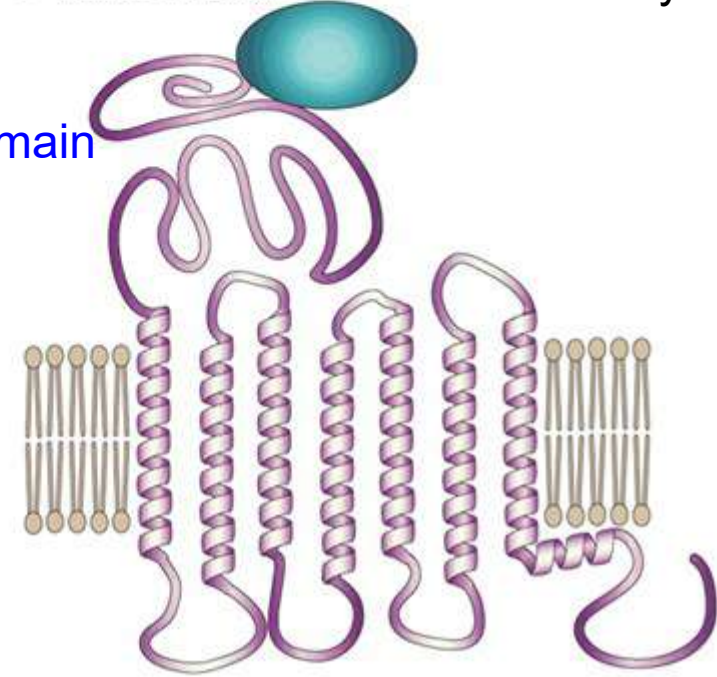
Class B Adhesion family



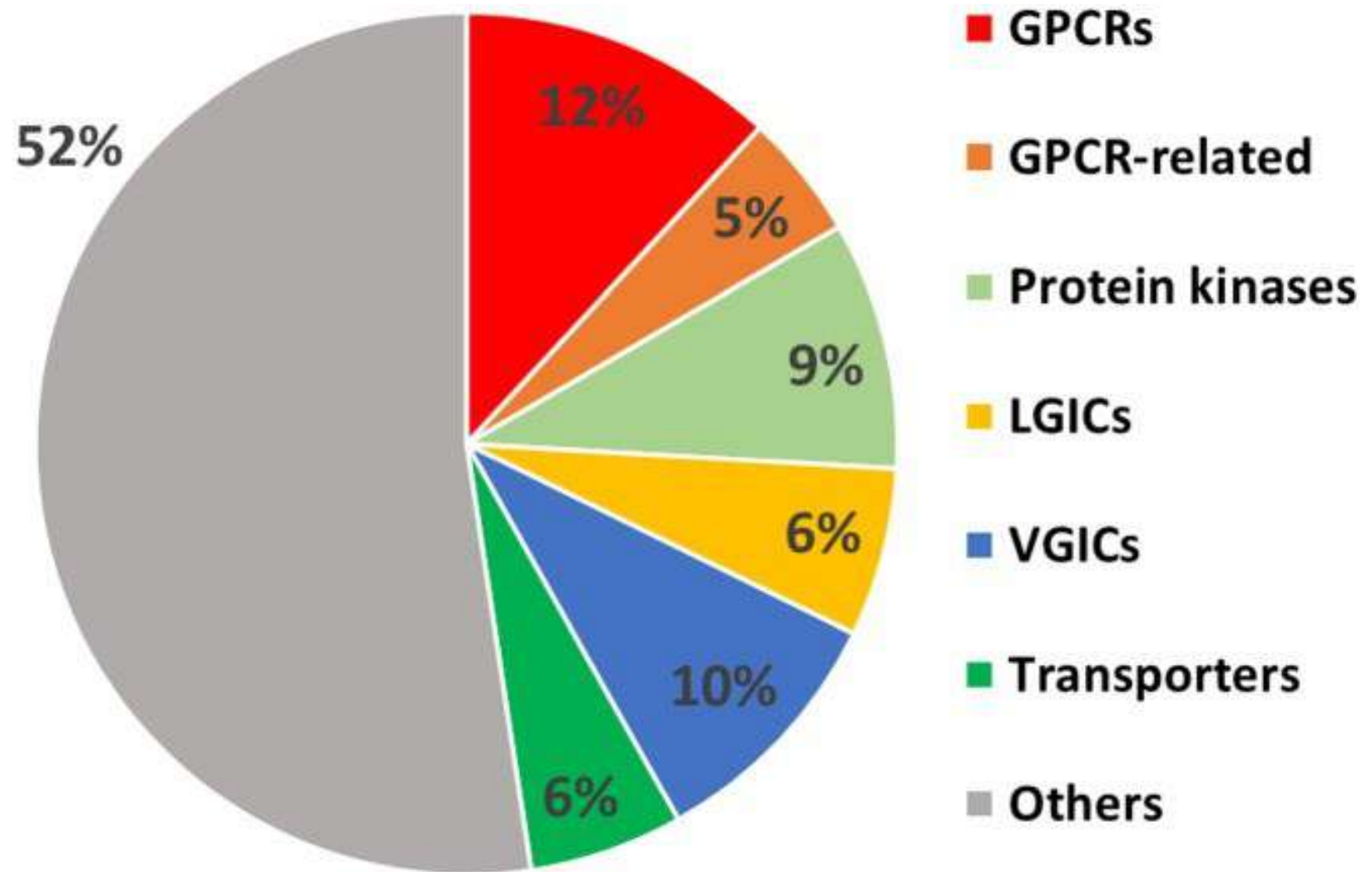
Class C Glutamate family



Class F Frizzled family



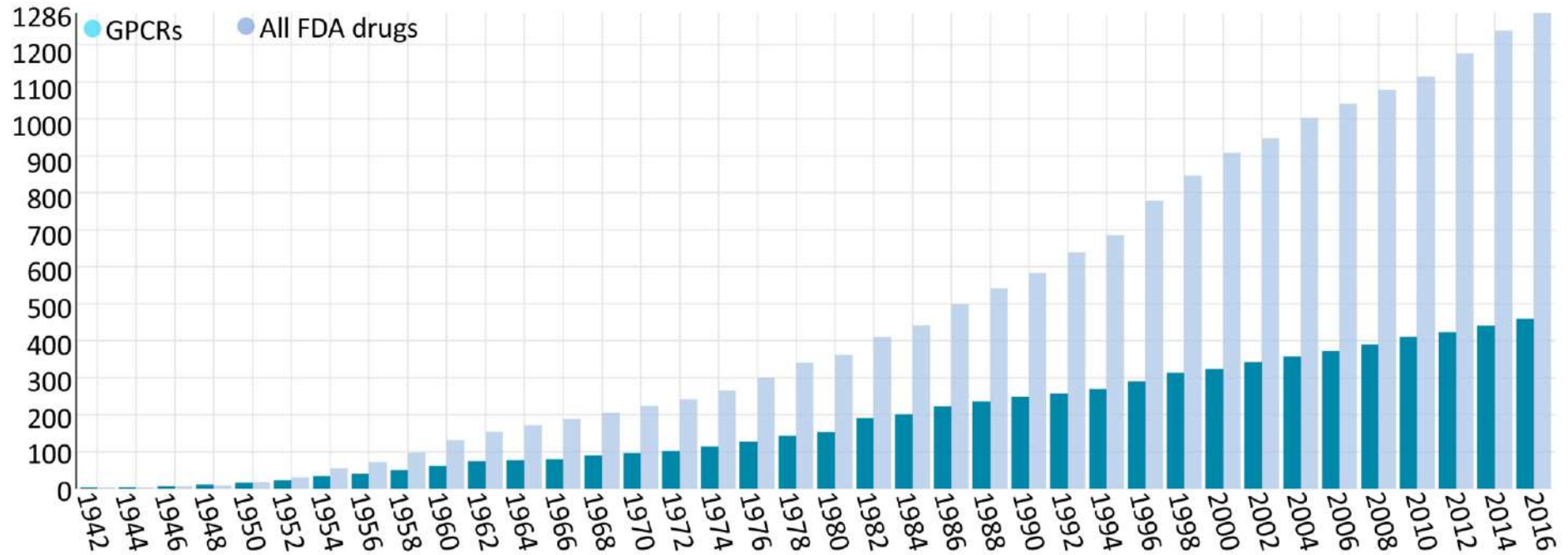
The estimated proportion of genes from different gene families that are targets for approved drugs.



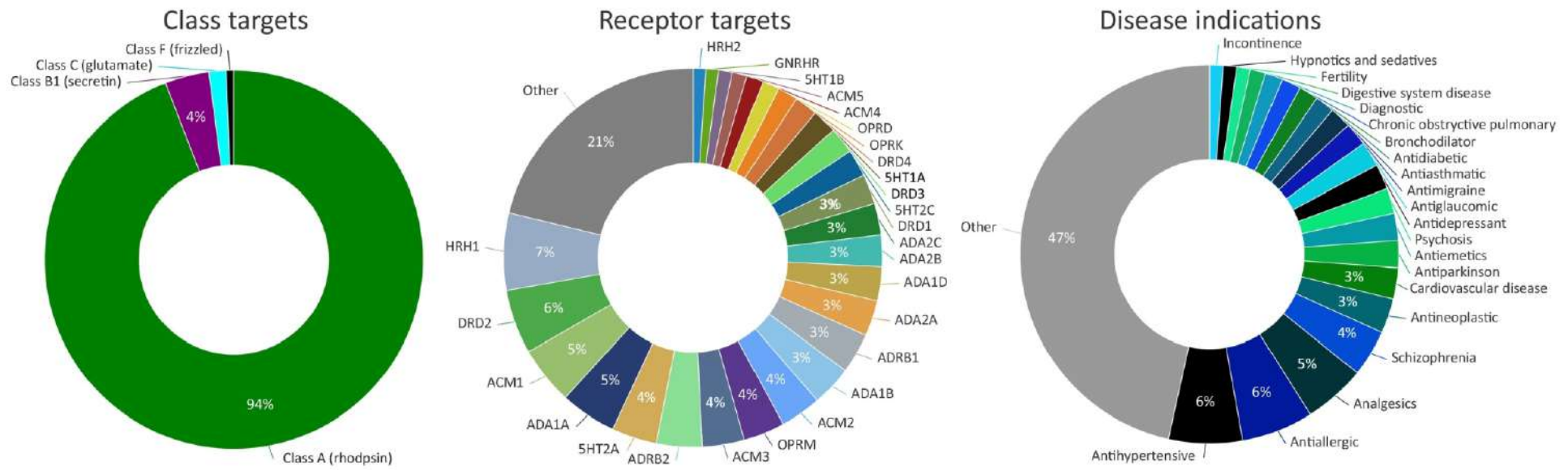
~1100 human gene/protein targets for approved drugs

There are 1286 drugs approved by the FDA, of which 460 drugs (36%) target GPCRs

(A)

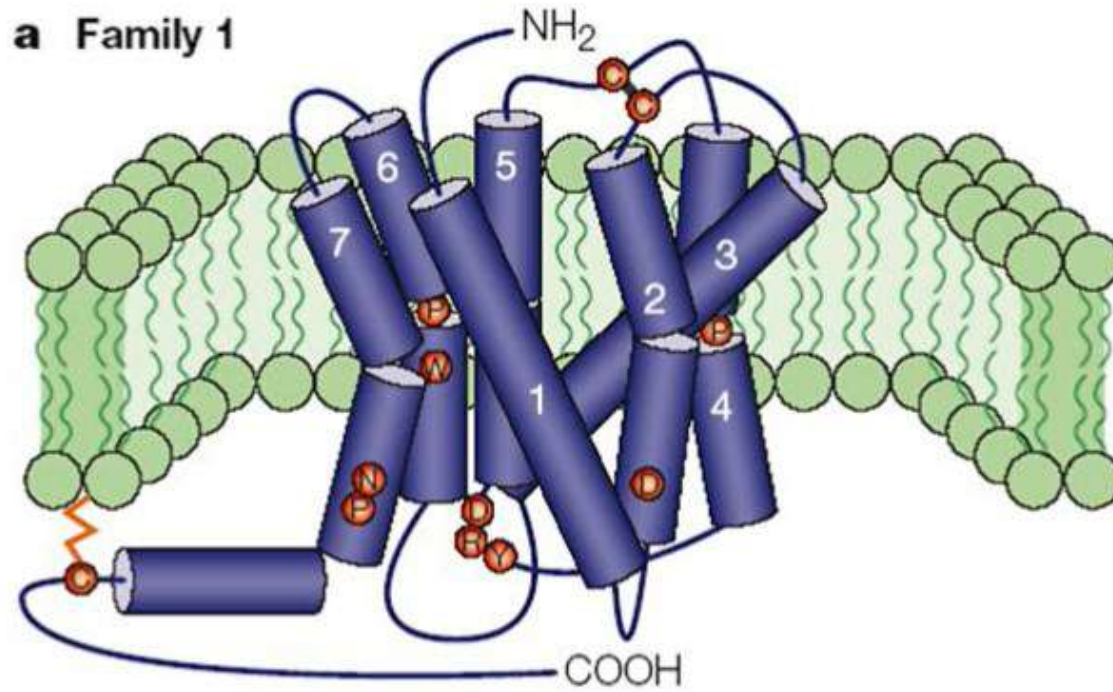


(B)



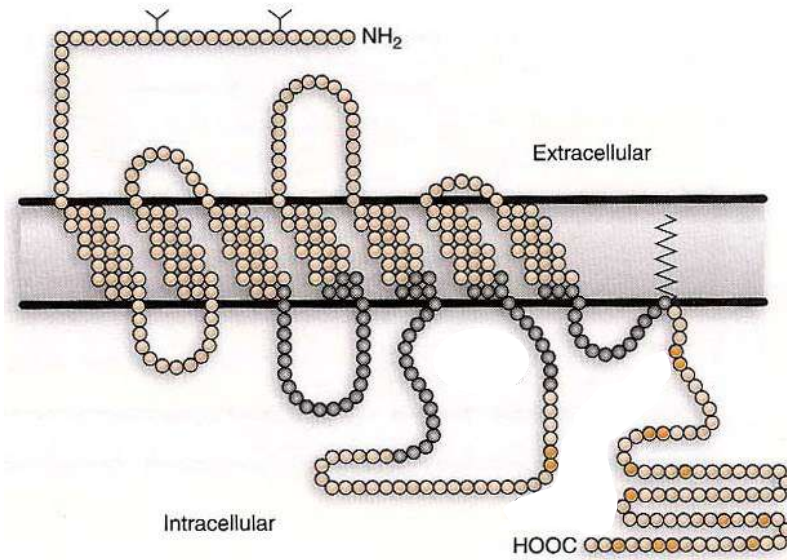
GPCR signalling cascades

a Family 1



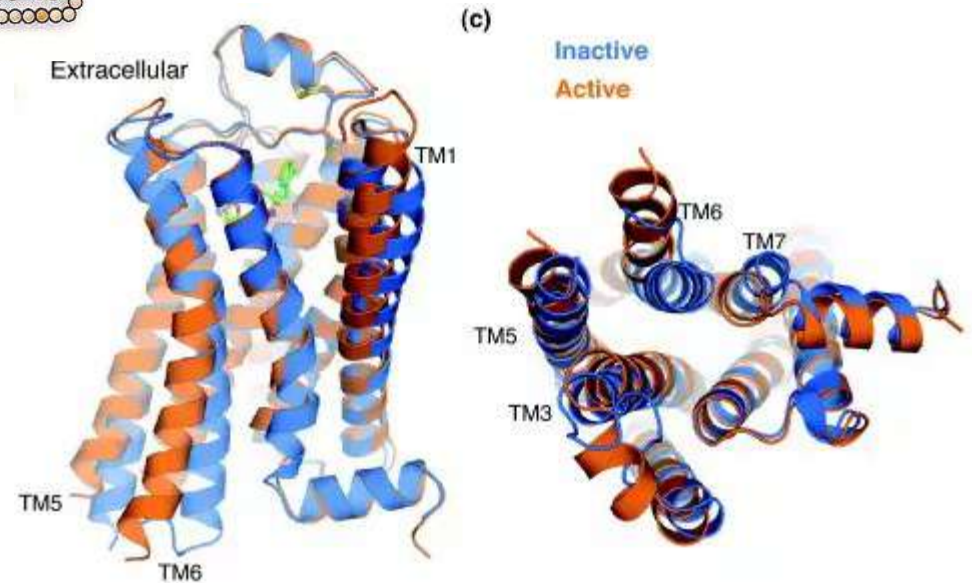
- ❖ Represent the predominant class of GPCRs;
- ❖ Several highly conserved amino acid (red circles);
- ❖ Disulfide bridge between first and second extracellular loops (ECLs), palmitoylated cys in C-tail;
- ❖ The binding of small molecule ligands occurs within the TM region;
- ❖ Example: Rhodopsin, Dopamine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

β 2-Adrenergic receptor



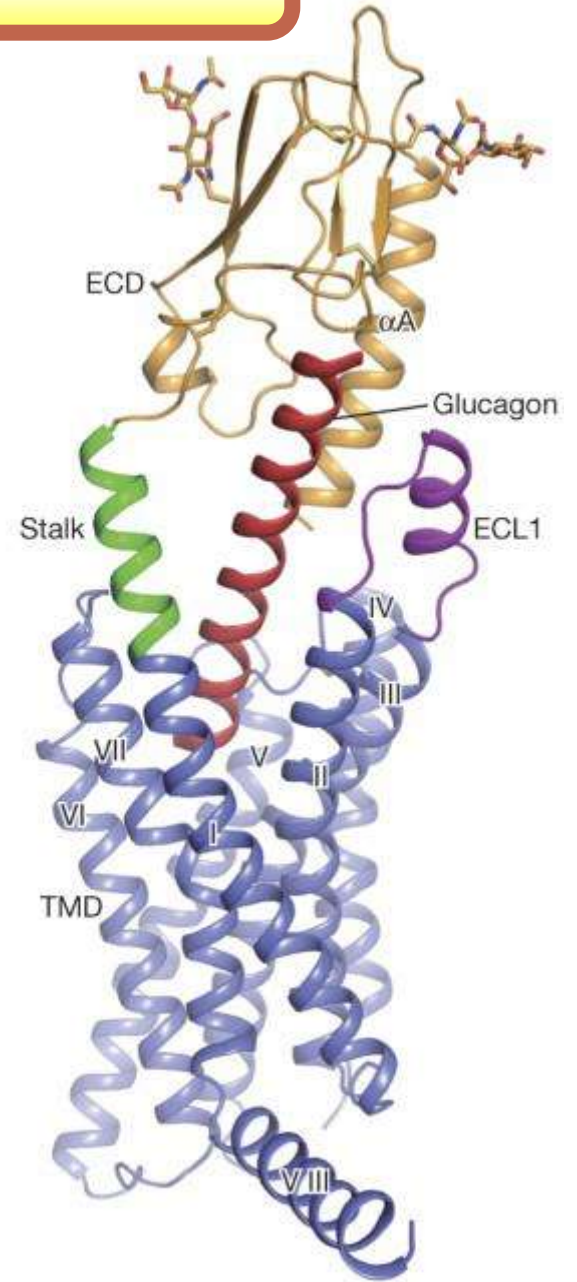
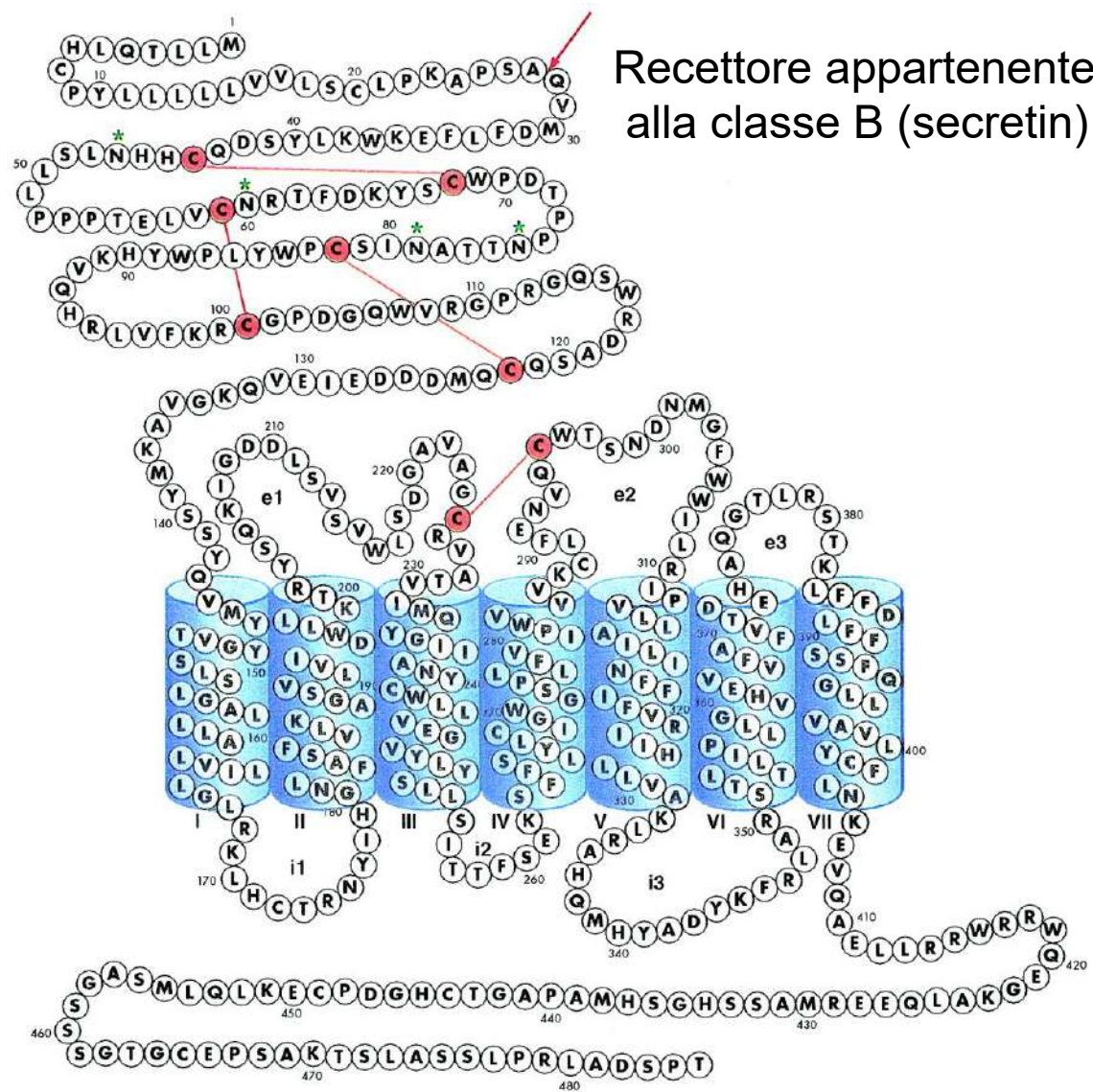
Membrana plasmatica

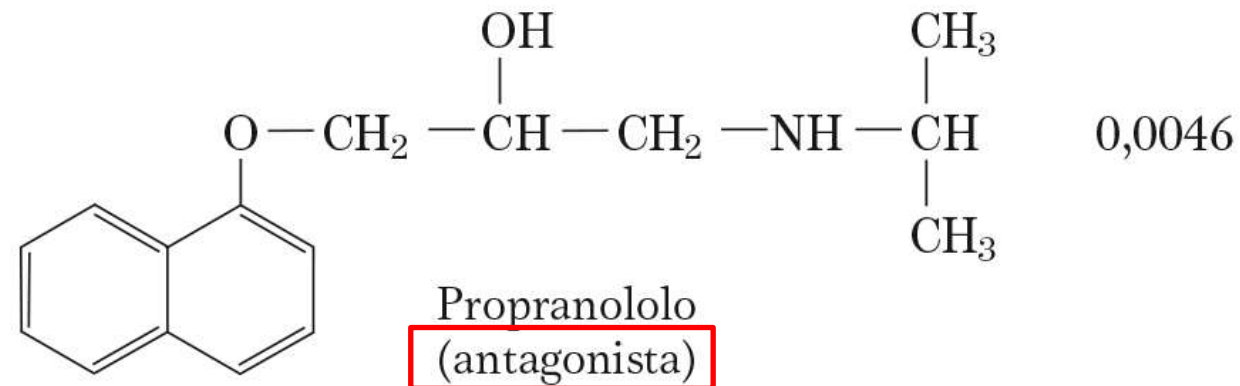
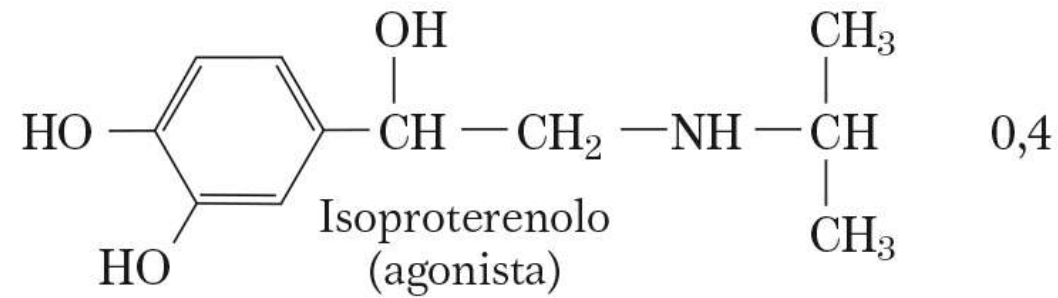
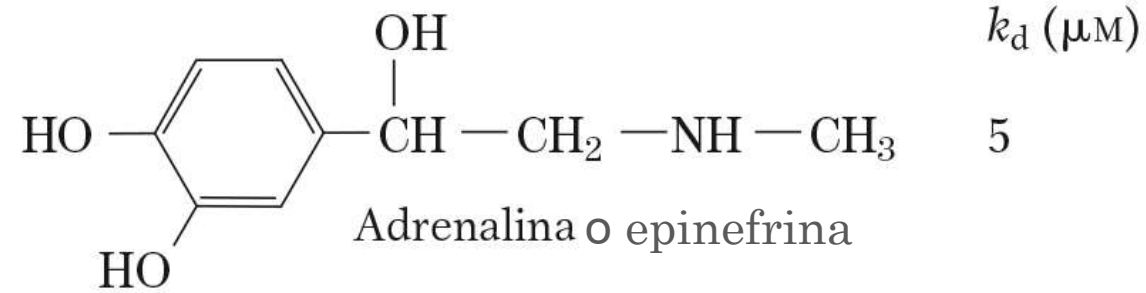
413 amminoacidi



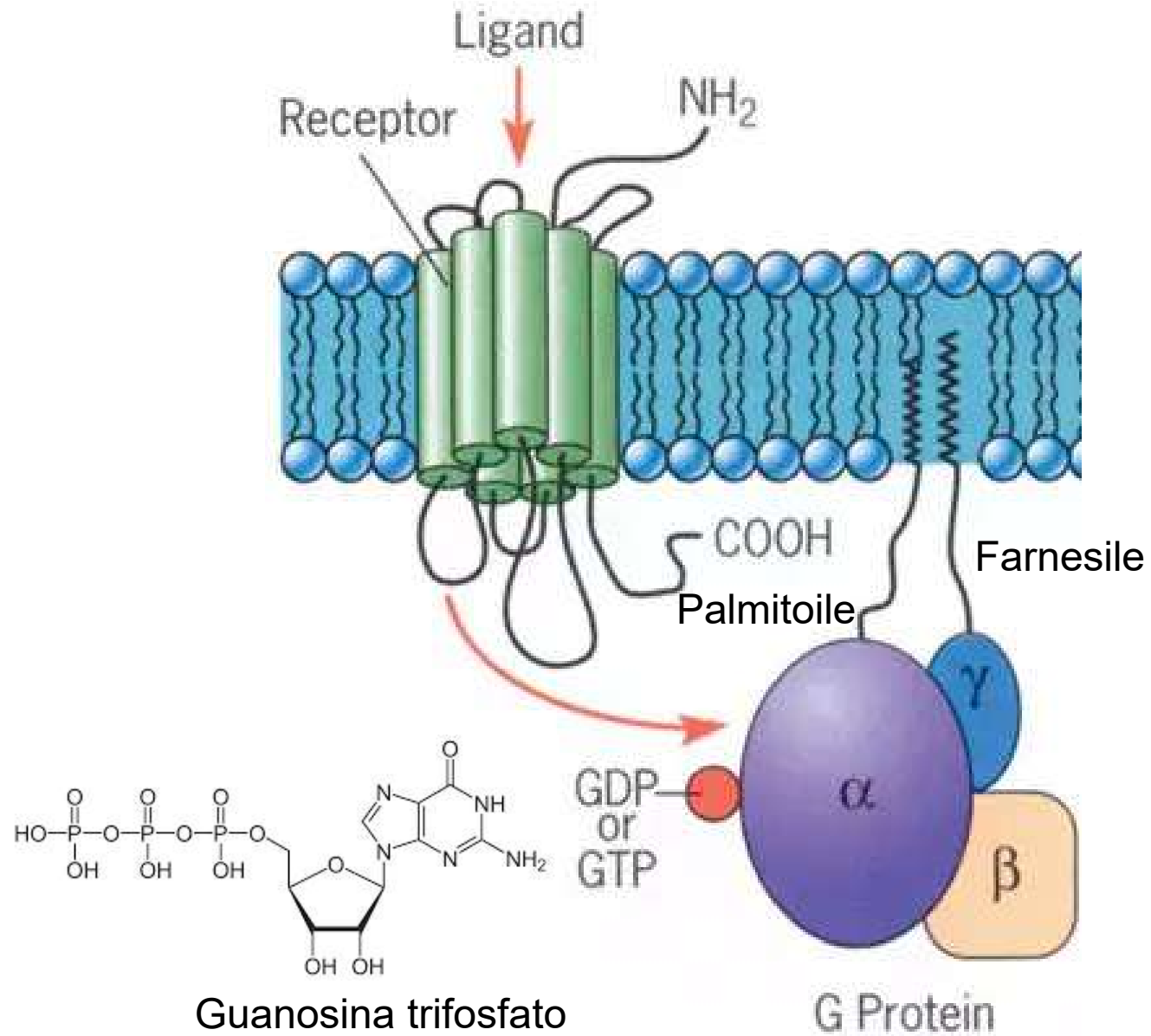
Recettore per il glucagone

t

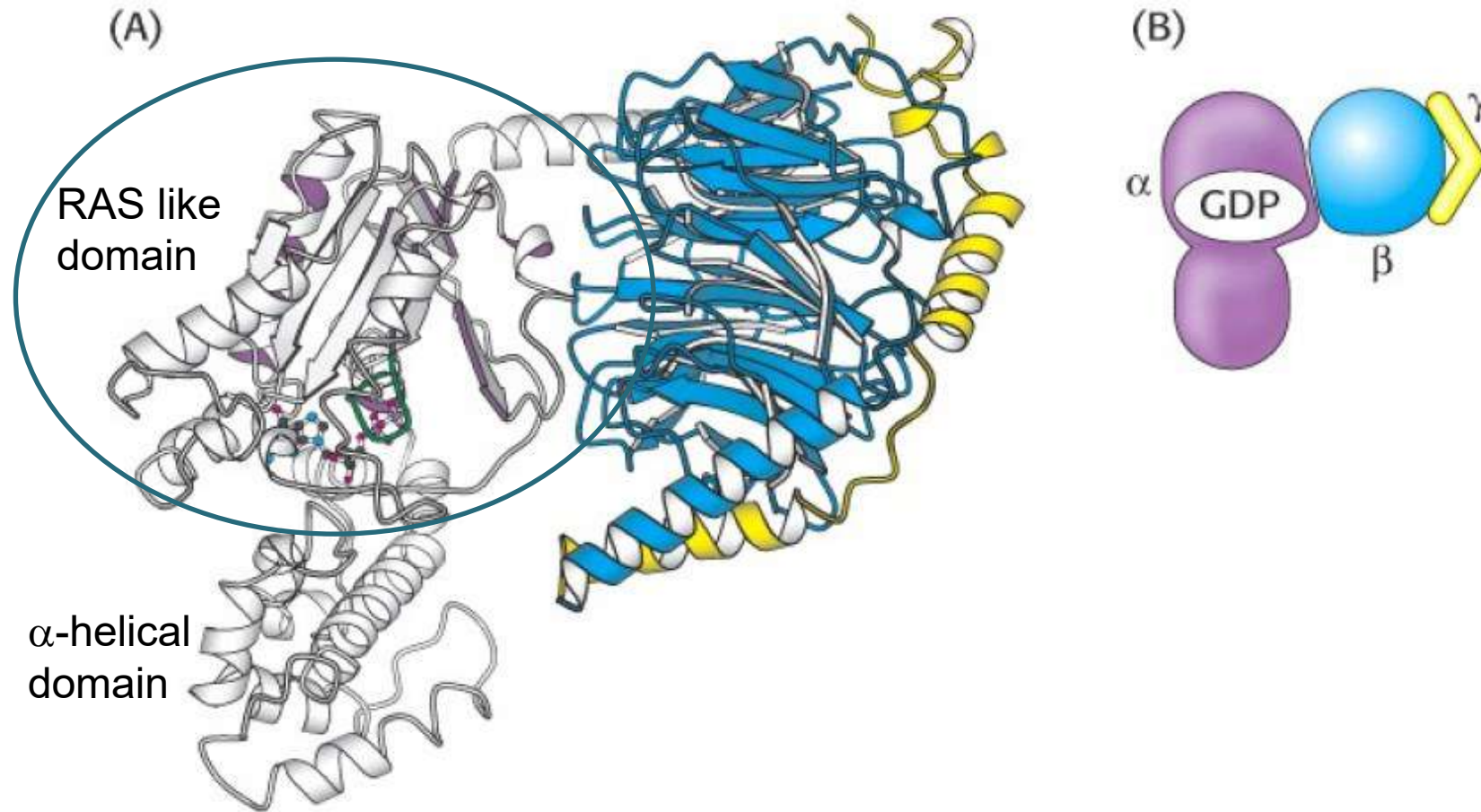




Trasduzione del segnale β AR

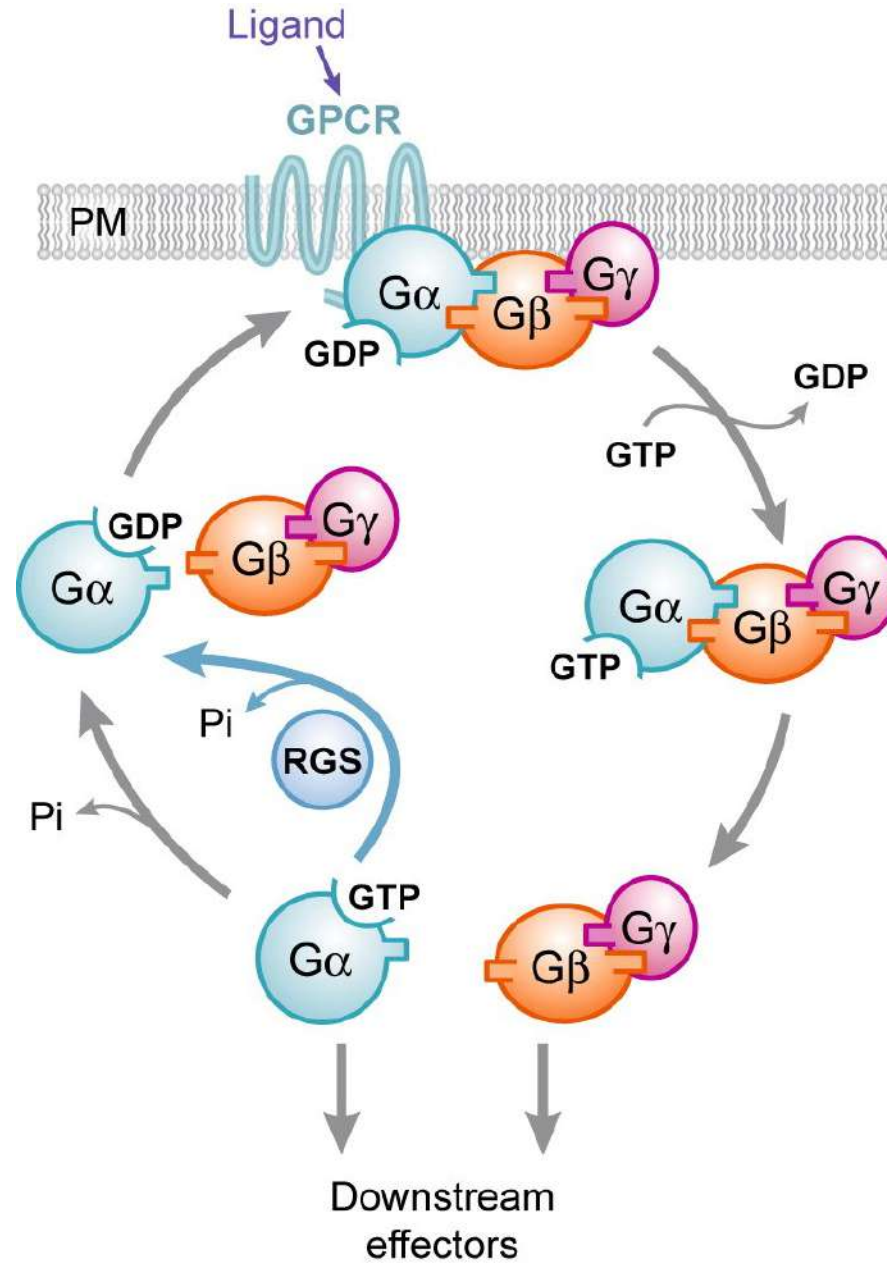


Struttura della proteina G

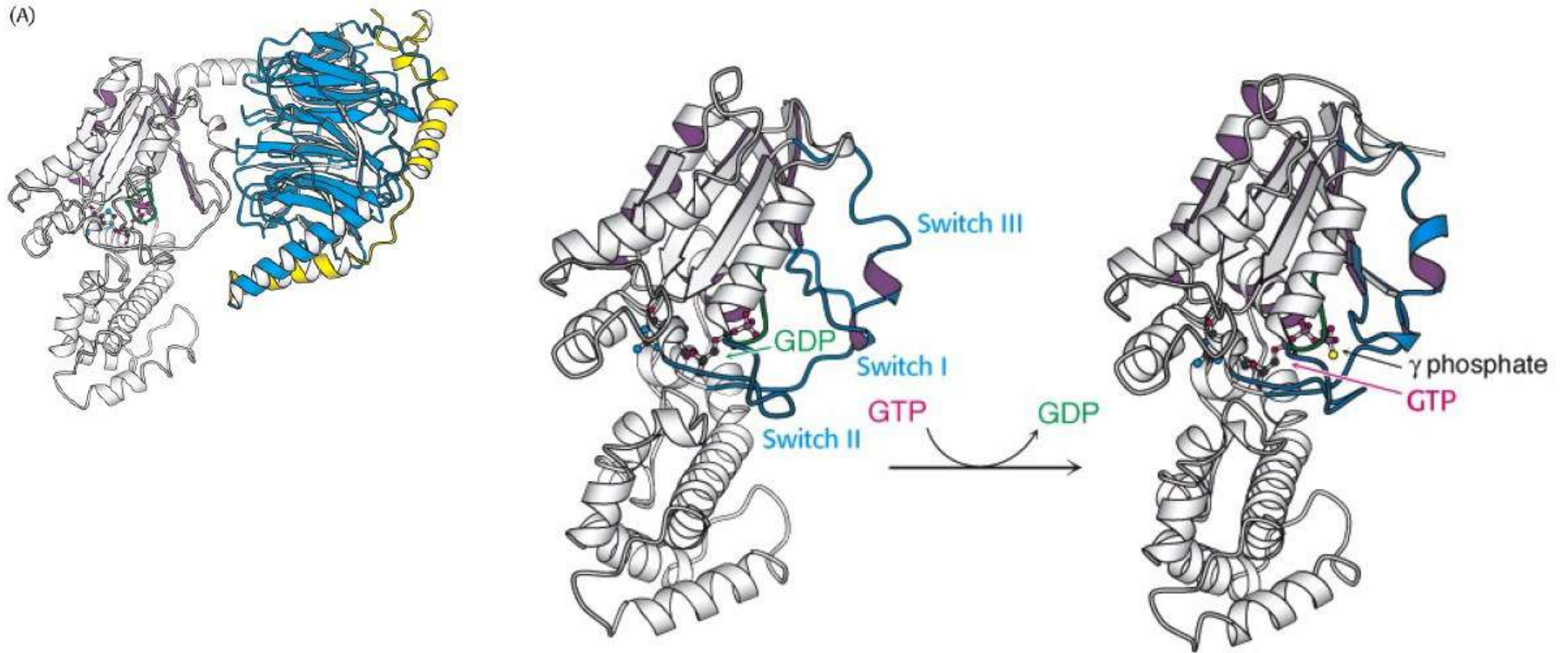


Le proteine G accoppiate ai recettori metabotropici sono **trimeri**, formati dalle subunità α (21 isoforme), β (6 isoforme) e γ (12 isoforme).

G protein cycle



Regioni della proteina G modificate durante l'attivazione/inattivazione

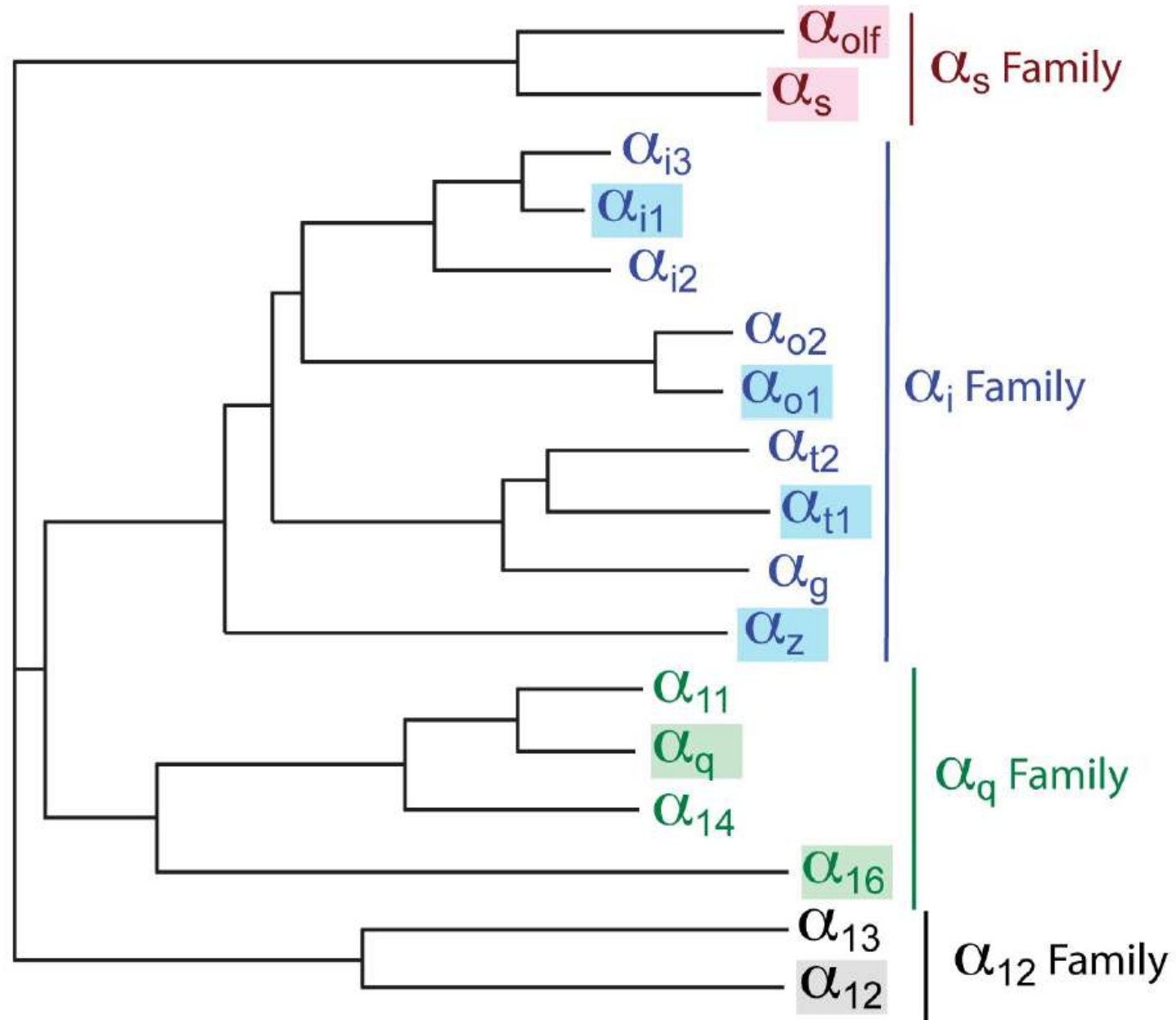


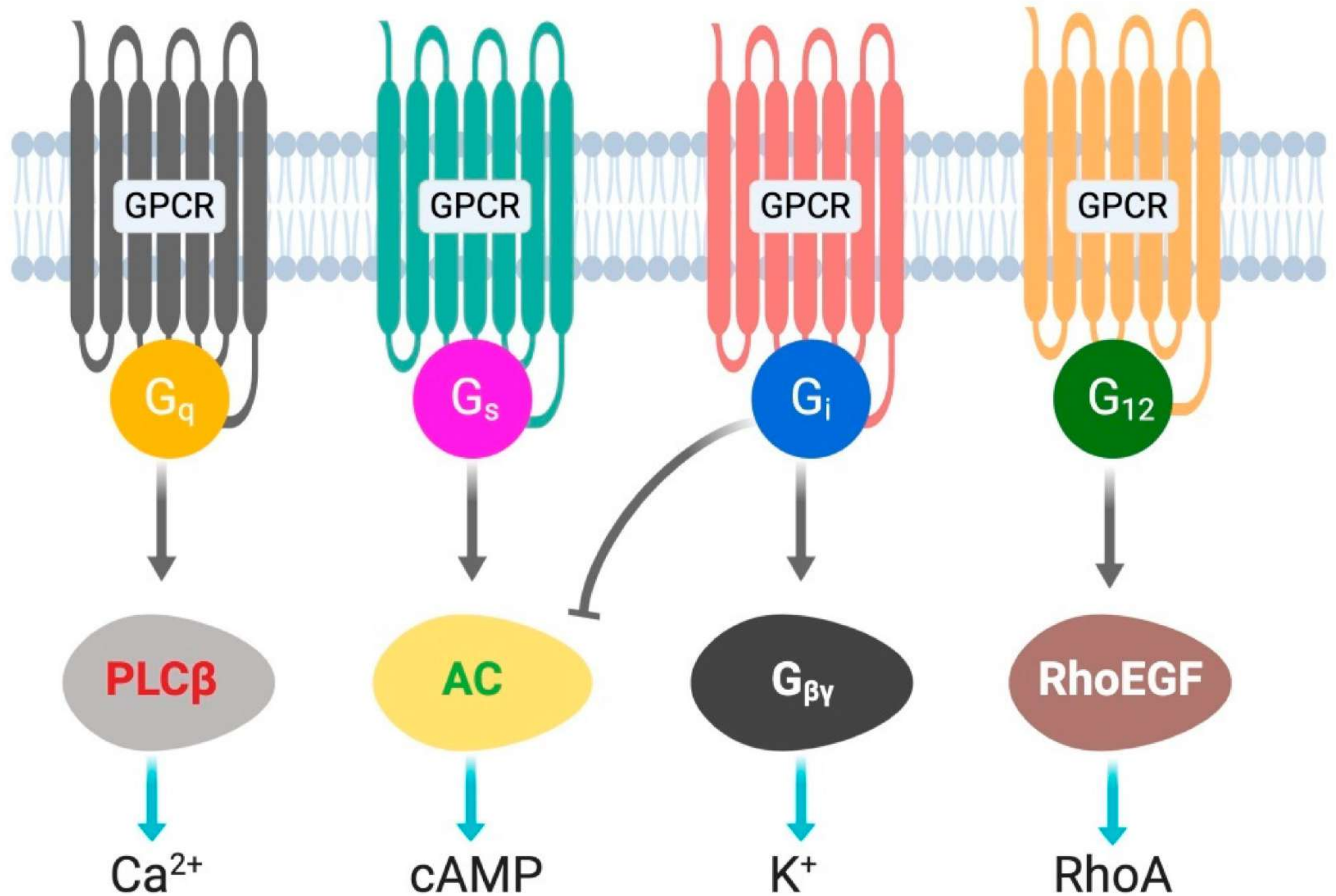
L'attivazione della proteina G consiste nella sostituzione di GDP con GTP che porta alla dissociazione del dimero $\beta\gamma$ dalla subunità α -GTP.

Trimeric G-proteins

SUBUNIT	SUB-TYPES	EXPRESSION (CELLS)	PRIMARY EFFECT	OTHER EFFECTS	RECEPTORS	TOXIN
G_s - family						
→ α_s - generic form	4	ubiquitous, including neurons	↑ AC (subtypes 1-10)	↑ cardiac Ca ²⁺ channels ↑ K _{Ca} channels ↓ Na ⁺ channels	NE, EPI, histamine, LH, FSH	CTX
α_{olf}	1	olfactory epithelium; some neuronal ganglia	↑ AC (subtypes 1-6)		odorant chemicals	CTX
G_i - family						
α_o	≥ 2	neurons; neuroendocrine cells; astroglia; cardiac muscle	↑ PLC-β	↓ AC ↓ L- & N-type Ca ²⁺ channels ↑ neuronal K ⁺ channels	ACh, NE, opiates, All, many peptides	PTX
α_i	13	ubiquitous, including neurons	↓ AC (subtypes 1-10)	↑ K _{ATP} channels ↑ K _v channels ↓ L- & N-type Ca ²⁺ channels	same as above	PTX
α_z	≥ 1	platelets; adrenals	↓ AC	↑ PLC	same as above	none
G_q - family						
→ α_q	≥ 5	ubiquitous	↑ PLC-β		ACh, NE	none
$\alpha_{11,14}$?	ubiquitous	↑ PLC-β		ACh, NE	none
Unrelated?						
α_t (transducin)	≥ 2	outer segments of retinal rods & cones	↑ γ PDE (cGMP-PDE)		light	CTX, PTX
α_{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 $G\alpha$ subunits

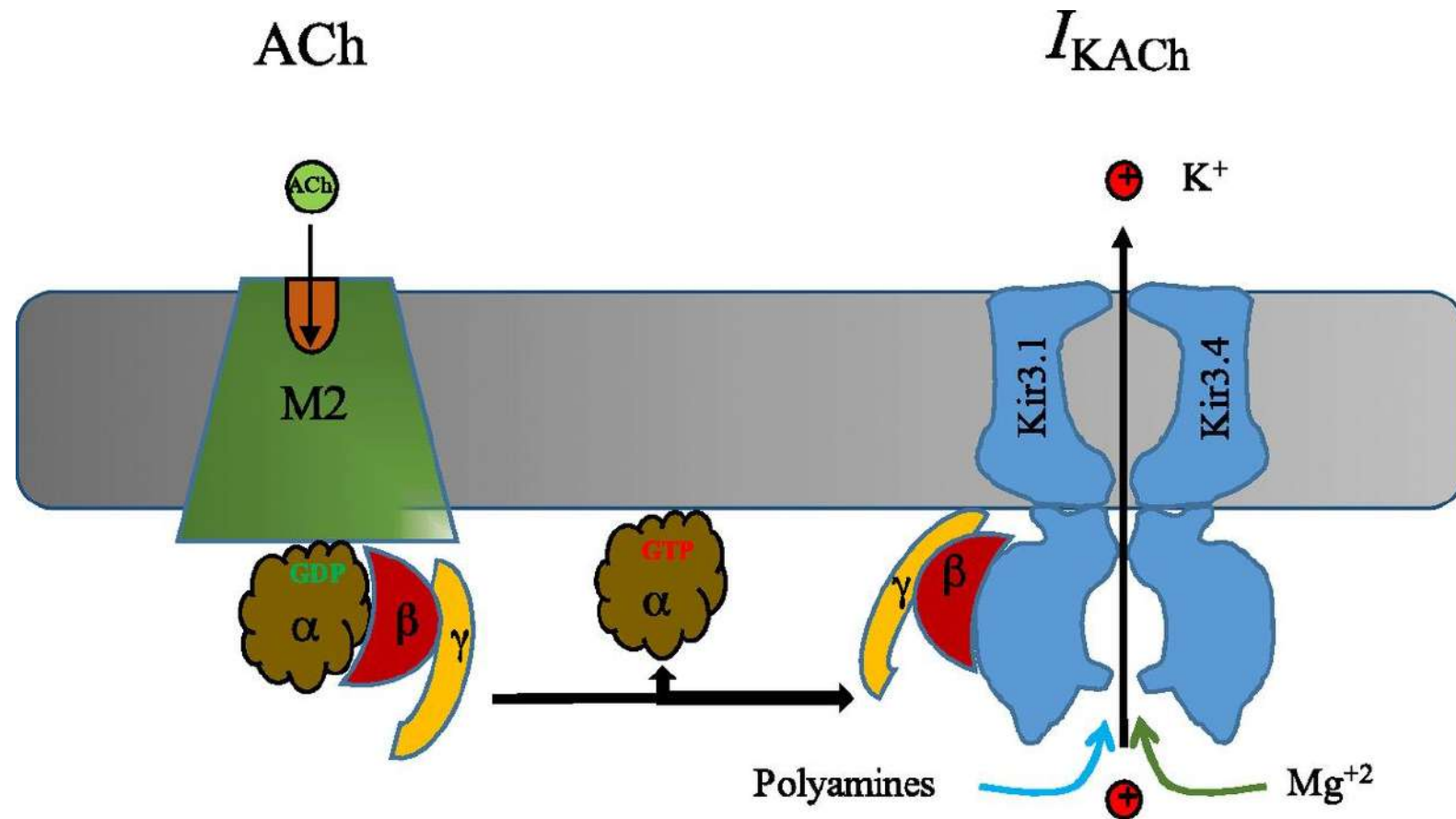




$\beta\gamma$ effectors

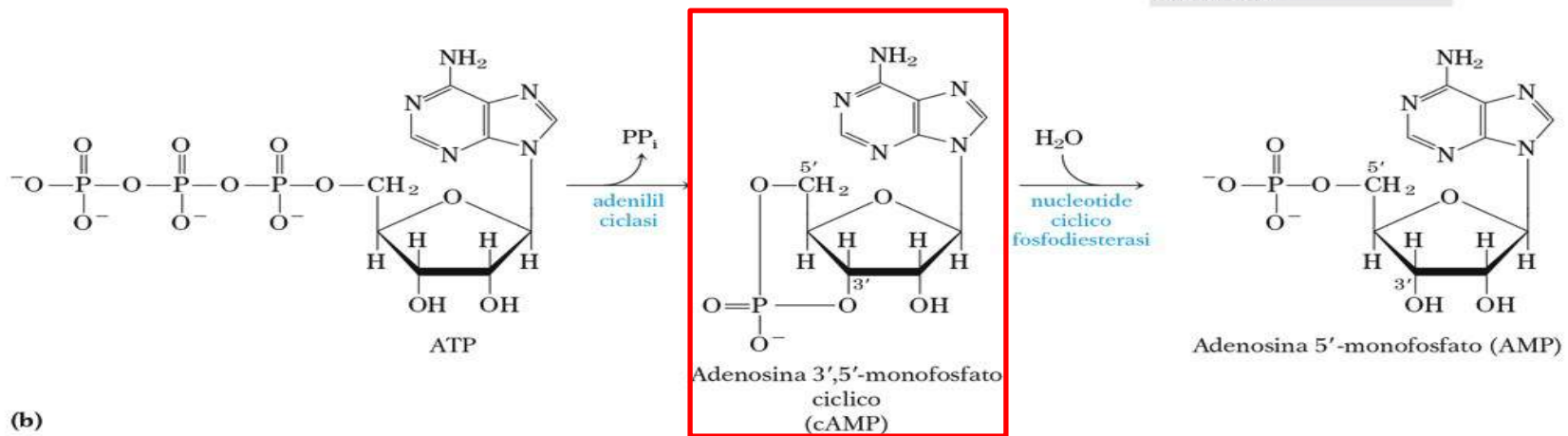
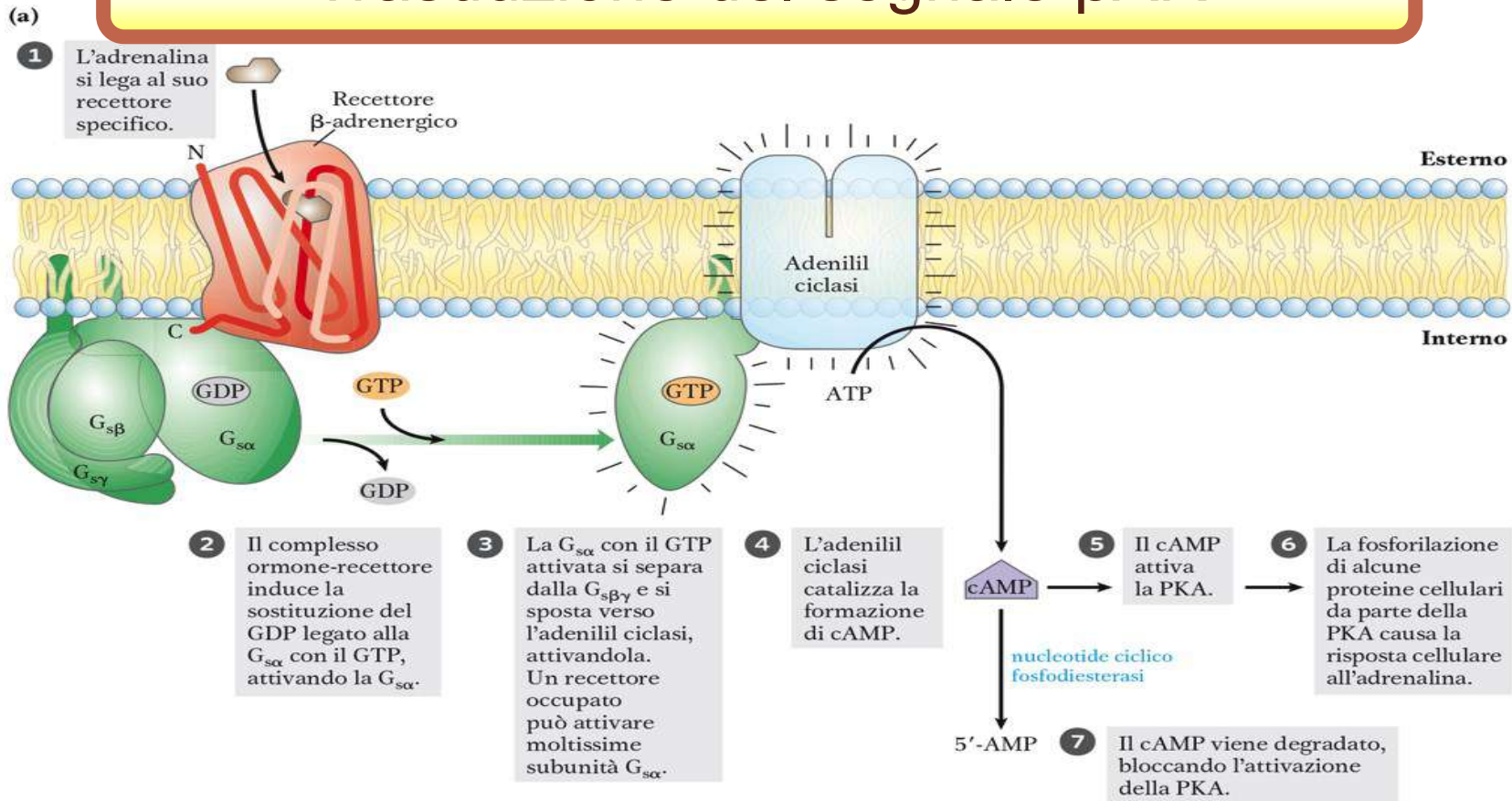
	Effector	Signalling effect
→	<u>GIRK2</u>	activation
	<u>GIRK4</u>	activation
	<u>N-type calcium channel</u>	inhibition
	<u>P/Q-type calcium channels</u>	inhibition
	<u>Phospholipase A</u>	activation
→	<u>PLCβ1</u>	activation
	<u>PLCβ2</u>	activation
	<u>PLCβ3</u>	activation
→	<u>Adenylyl cyclase</u>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

Parasympathetic signaling pathway in cardiac cells

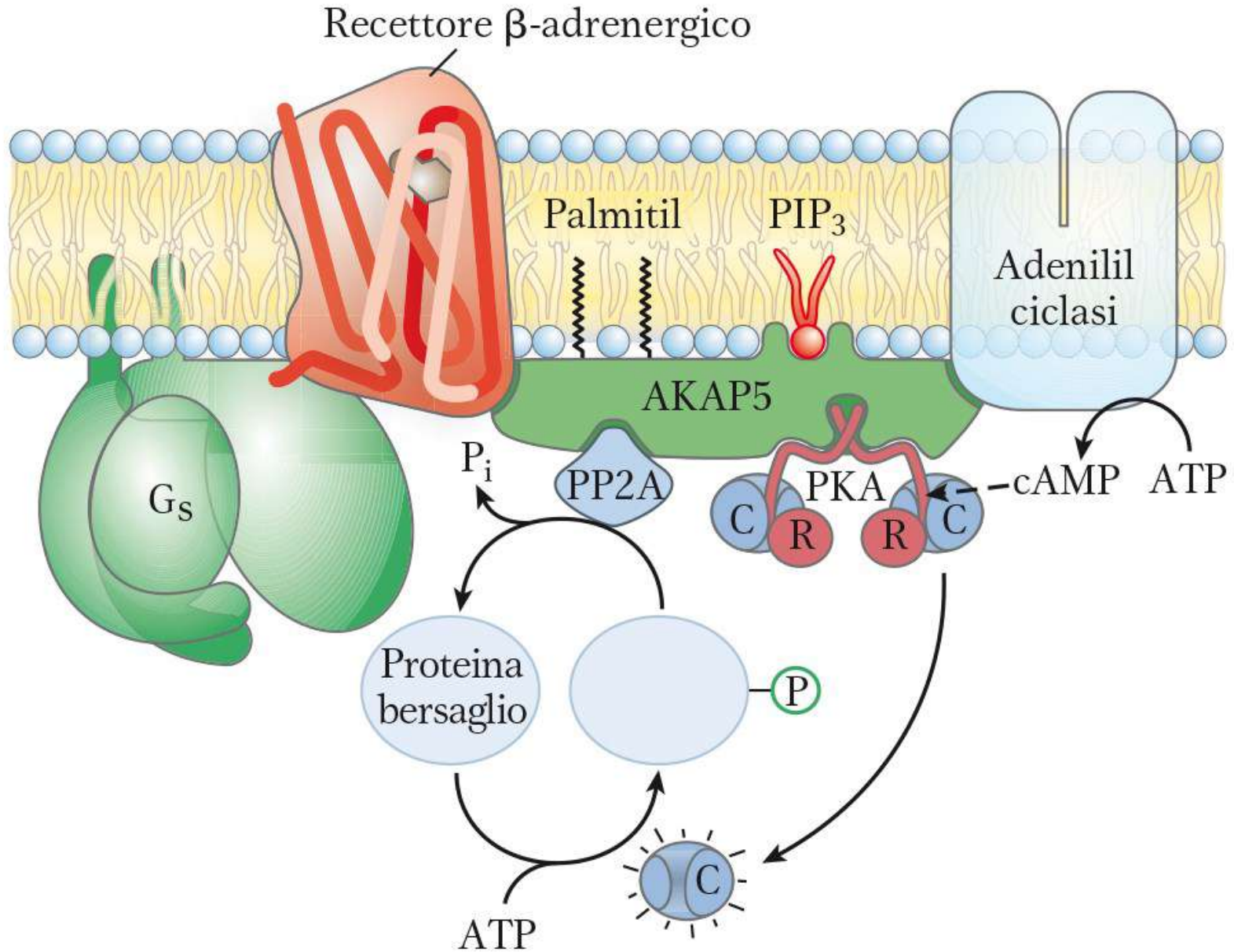


The binding of ACh to M2R induces dissociation of the $\beta\gamma$ -complex (from Gi/o proteins) to directly interact with the K_{ACH} channel and then decreases cardiac excitability.

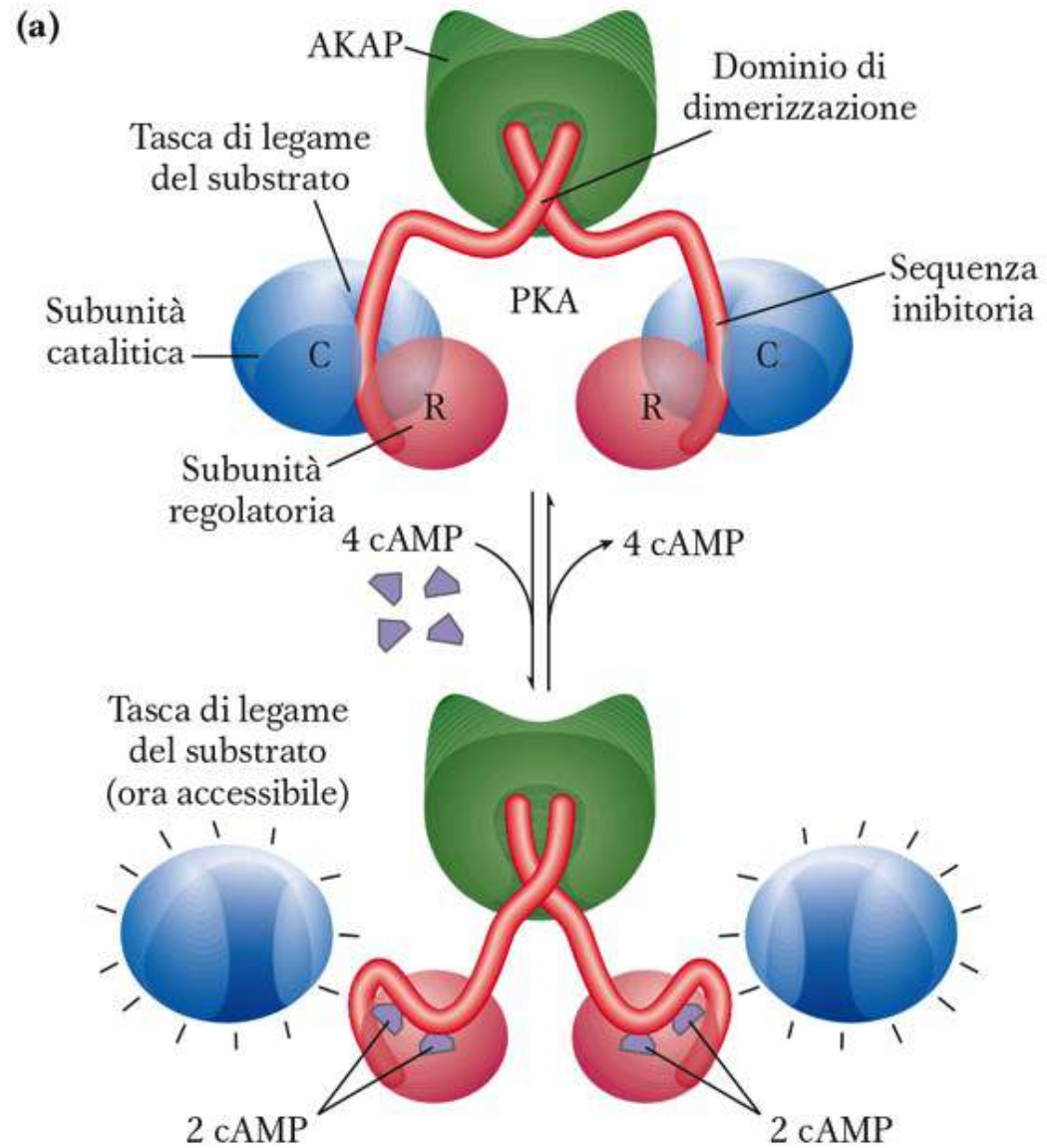
Trasduzione del segnale β AR



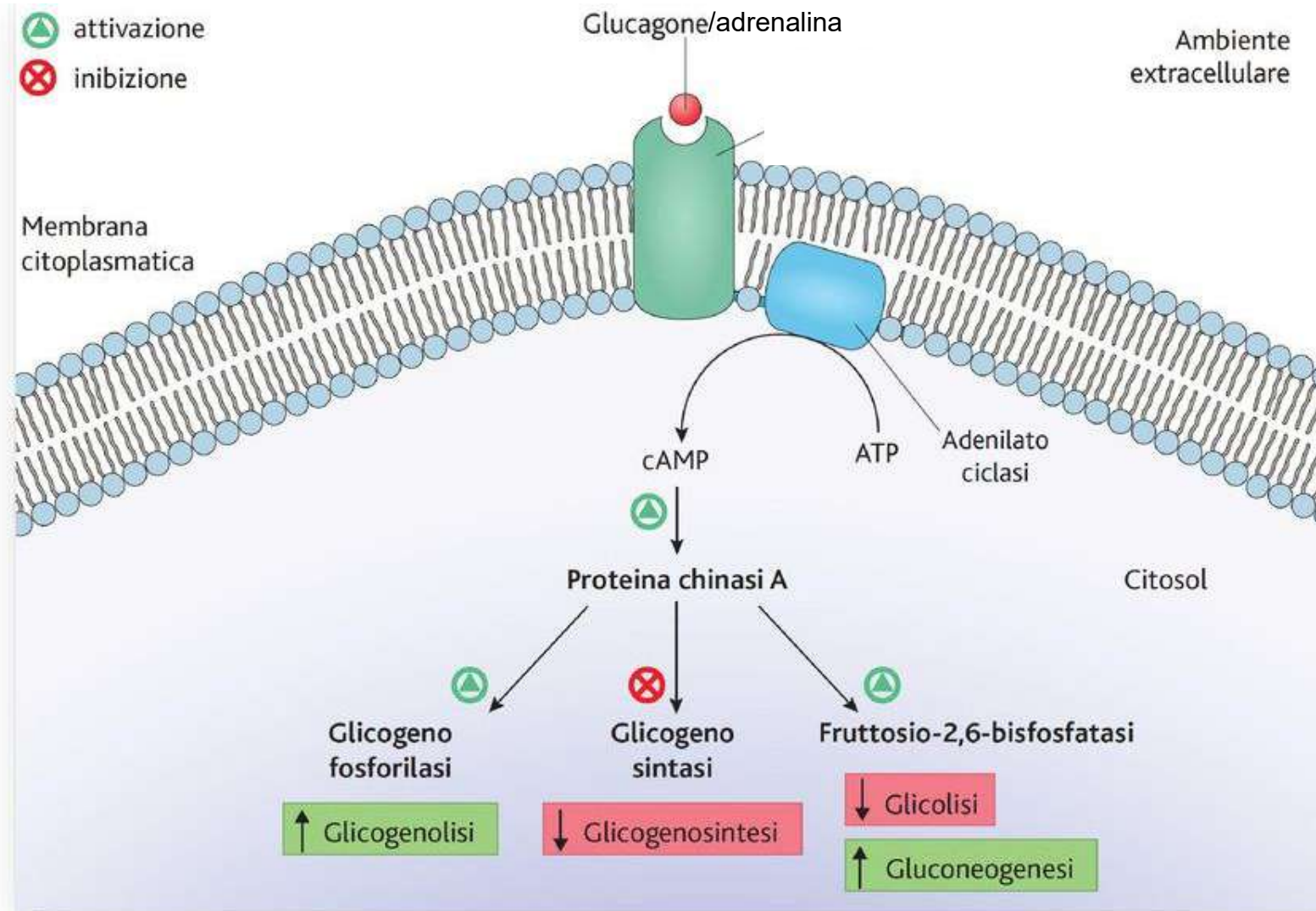
Restrizione spaziale della biosegnalazione



Chinasi dipendente da cAMP (PKA)



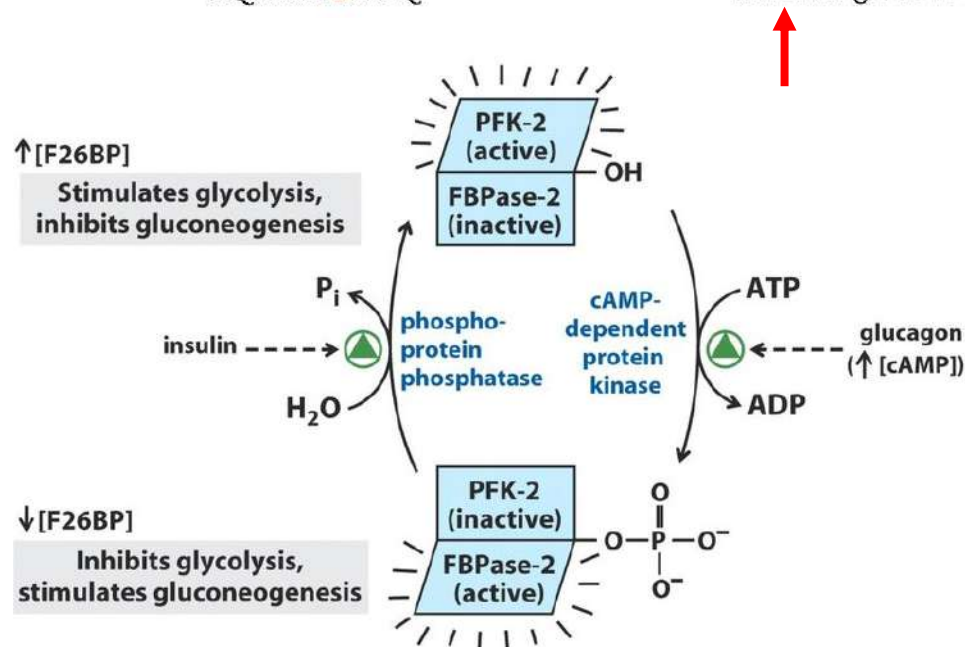
Effetti sul metabolismo del glucosio di adrenalina e glucagone



PKA e metabolismo del glucosio

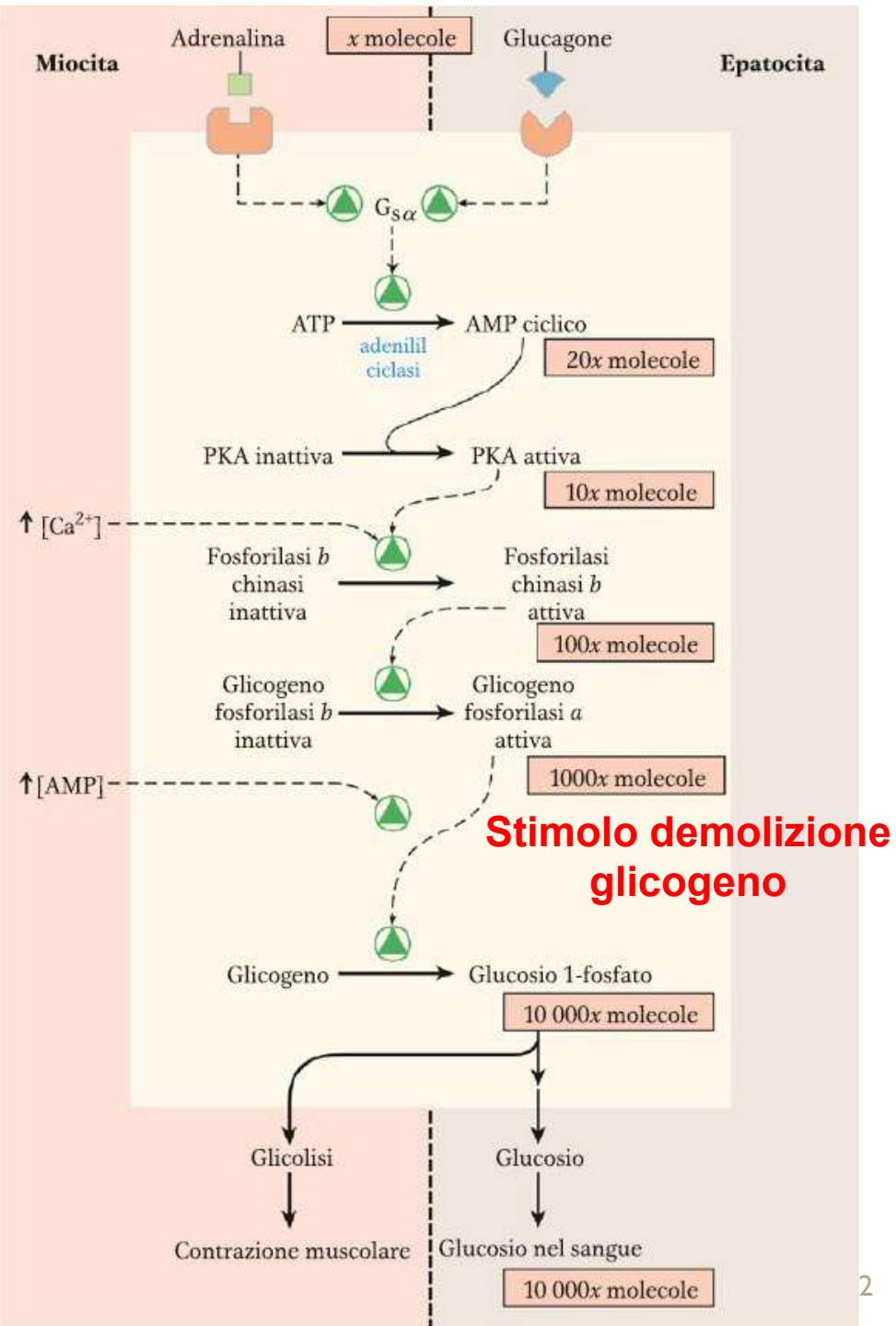
TABELLA 12.2 Alcuni enzimi e altre proteine regolati dalla fosforilazione cAMP-dipendente (da PKA)

Enzima/proteina	Sequenza fosforilata ^a	Via metabolica/processo regolato
Glicogeno sintasi	RASCTSSS inibizione	Sintesi del glicogeno
Fosforilasi b chinasi subunità α subunità β	VEFRRLSI } attivazione RTKRSGSV }	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

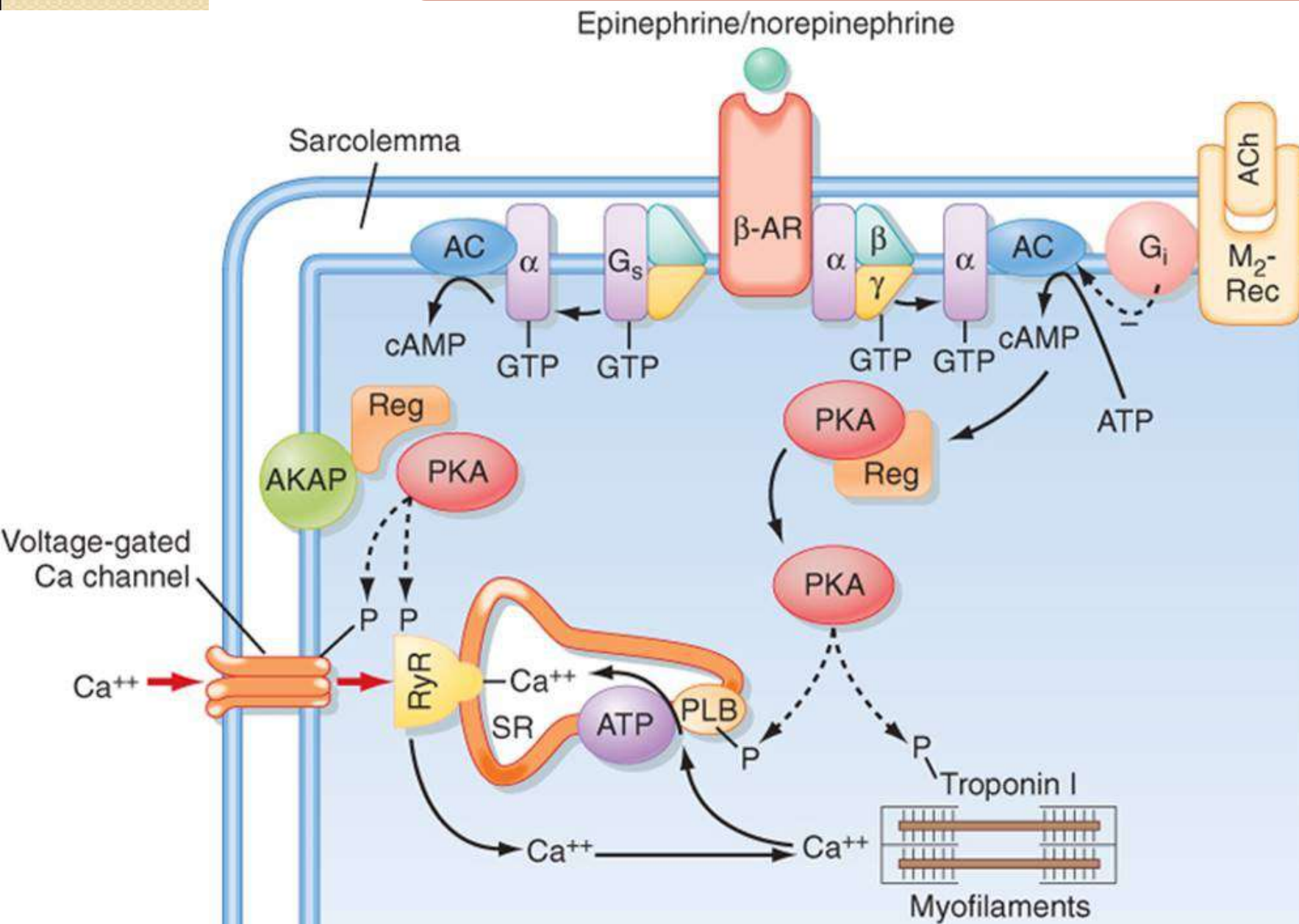


Substrati della PKA

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 <i>b</i> chinasi subunità α subunità β	VEFRRLSI } 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

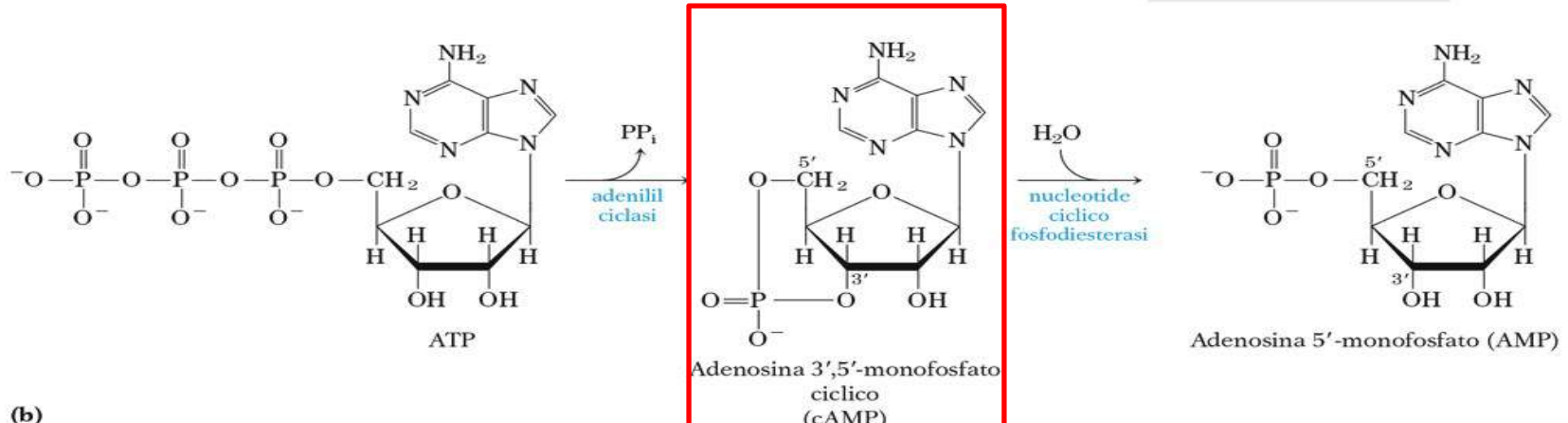
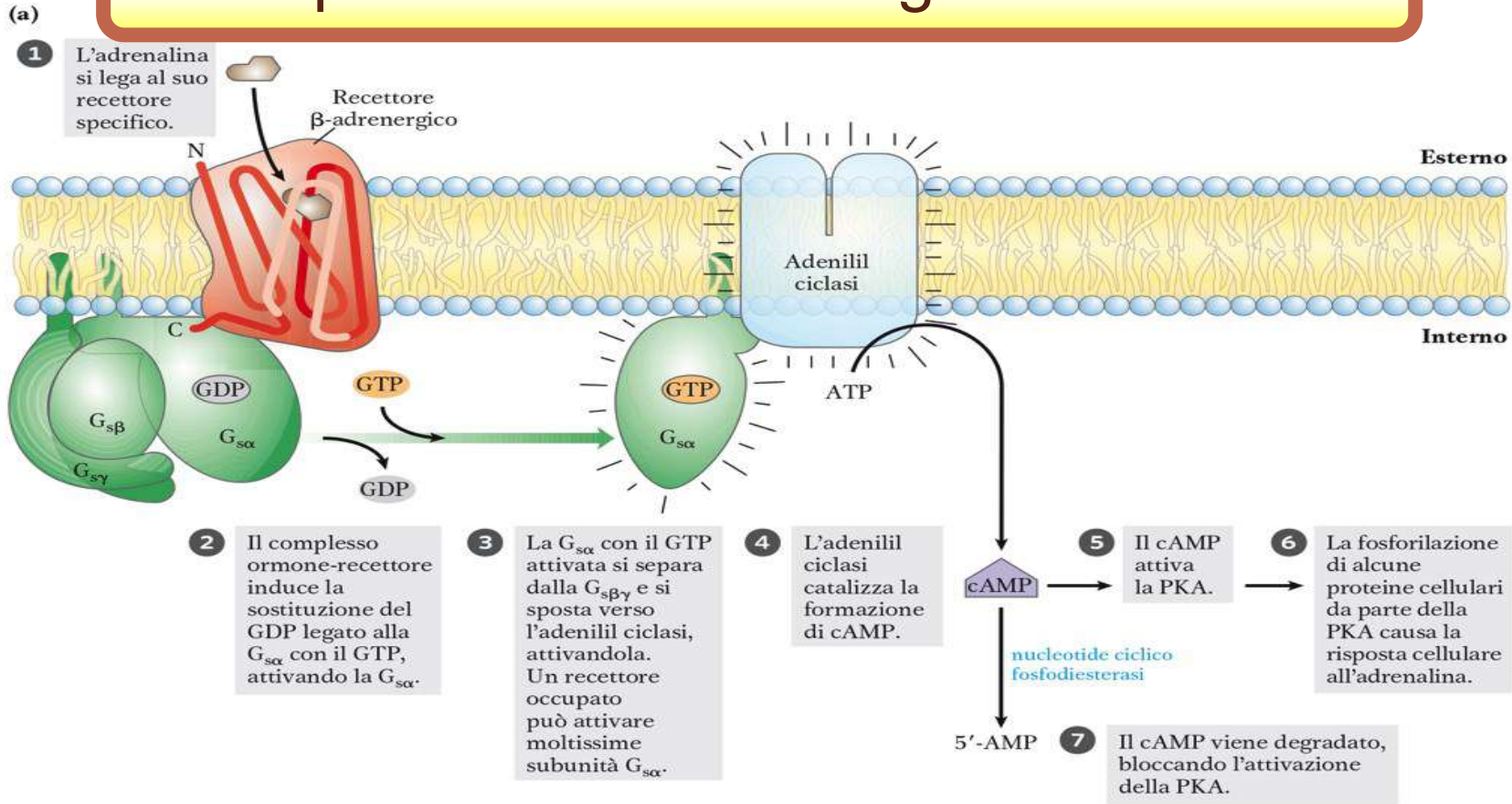
Effetti del signalling adrenergico sul cuore



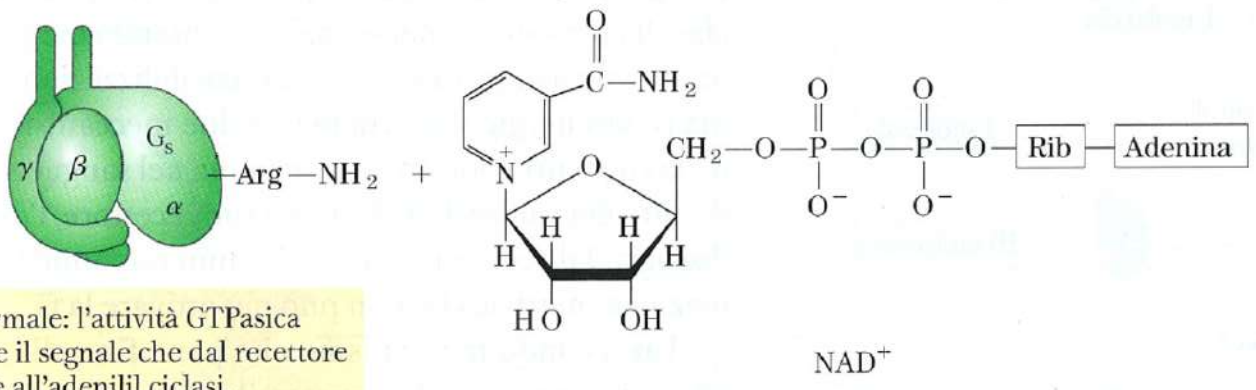
Catecholamine induces calcium influx into the sarcoplasmic reticulum

therefore increases both inotropy and lusitropy.

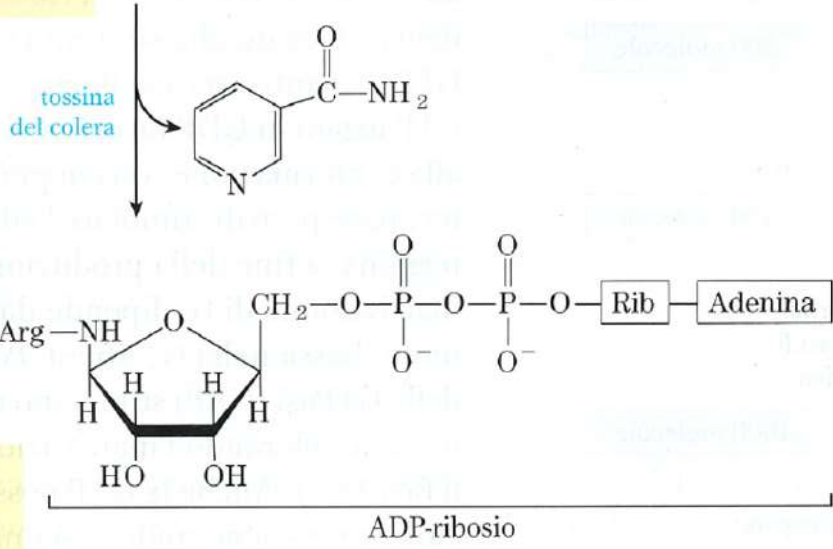
Le proteine G sono target di tossine



Effetti della tossina colerica



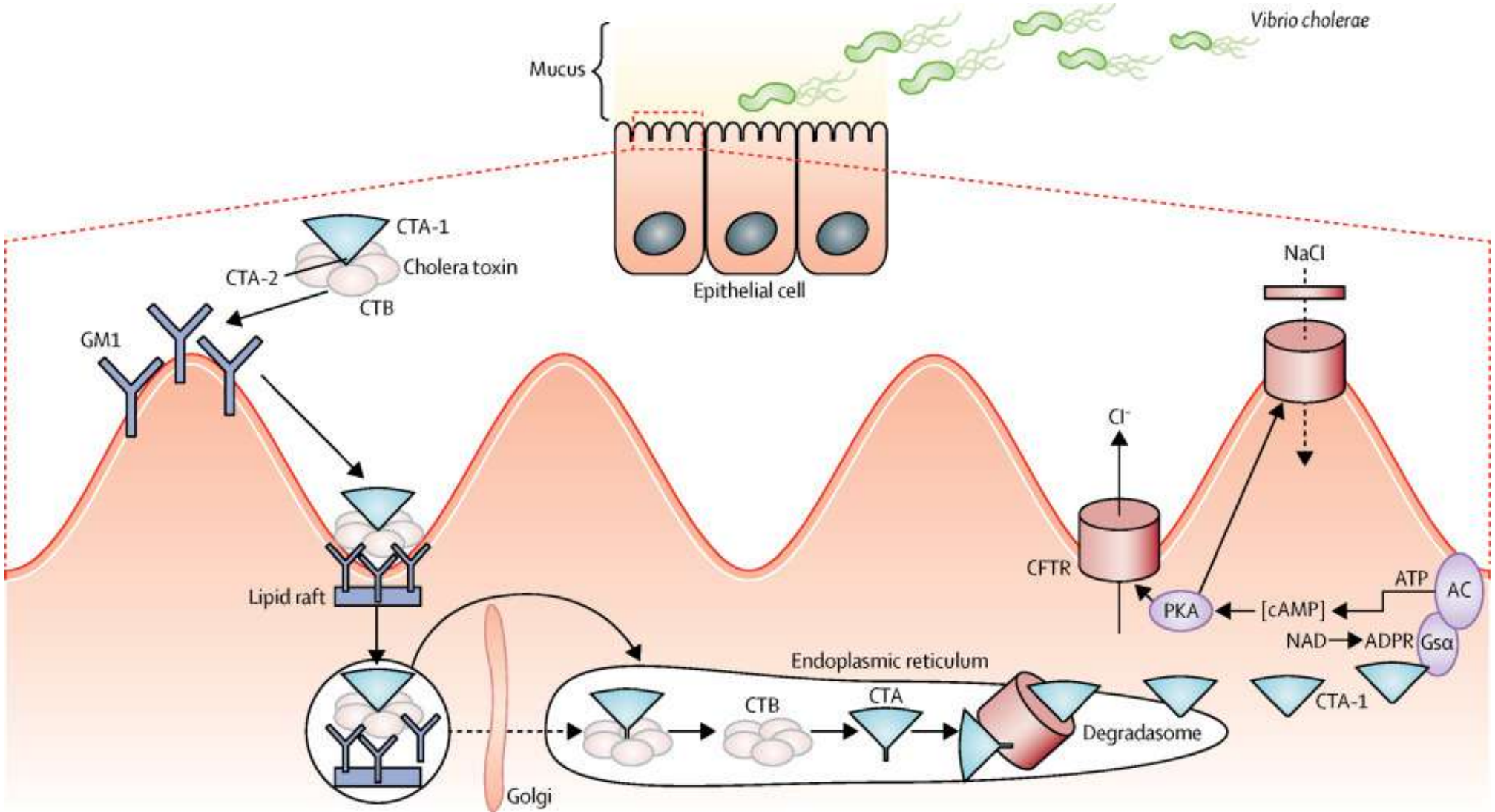
G_s normale: l'attività GTPasica spegne il segnale che dal recettore giunge all'adenilil ciclasi.



G_s ADP-ribosilata: l'attività GTPasica è inattivata; la G_s attiva costantemente l'adenilato ciclasi.

INATTIVAZIONE DELL'ATTIVITÀ GTPasica

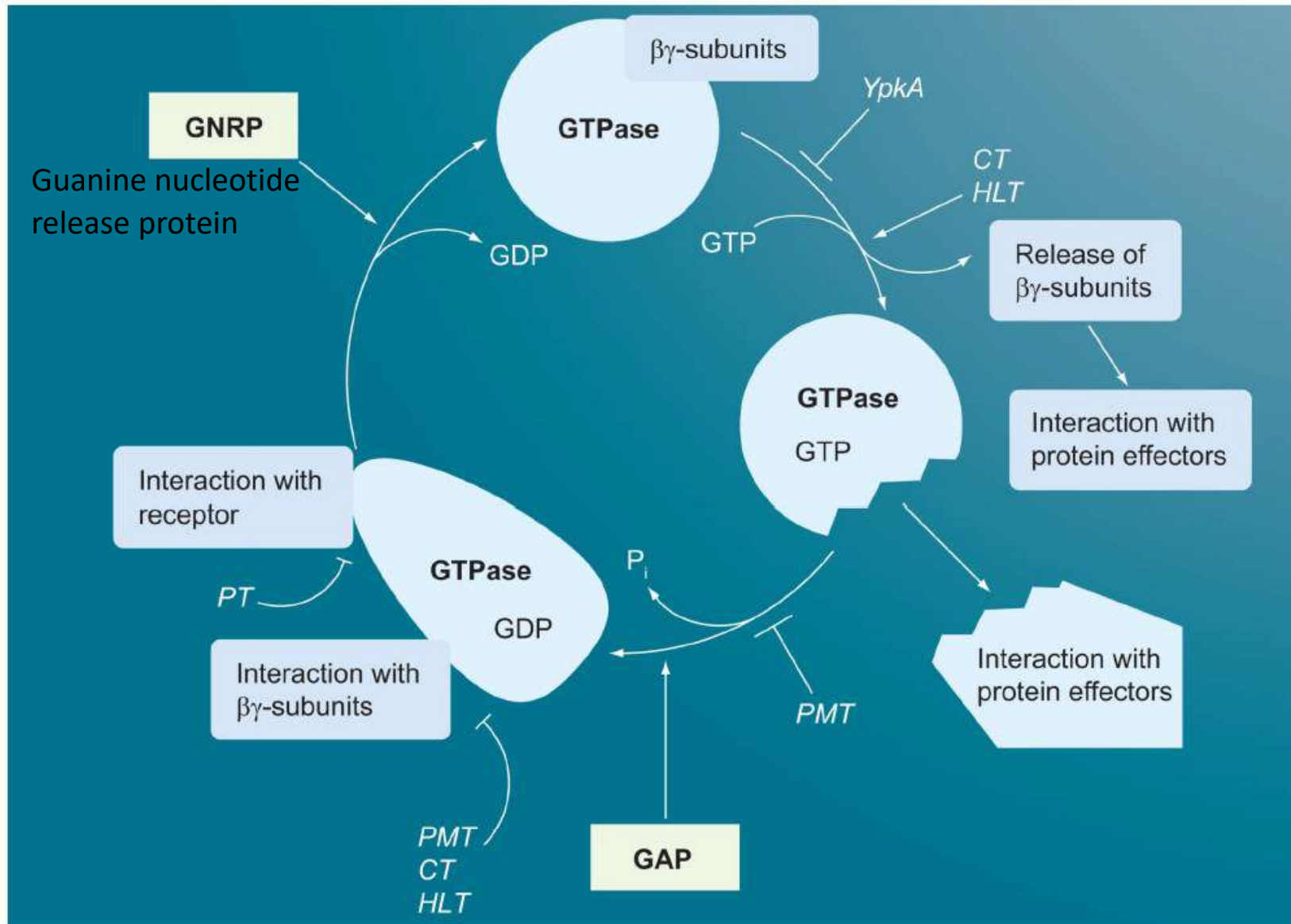
Effetti della tossina colerica



Proteine G e tossine

G-protein targets	Modulating toxins	Modification	Effect on G protein	Ref.
<i>Small GTPases</i>				
RhoA, Rac1, Cdc42	TcdA, TcdB, TcsH, TcsL, Tcna	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/PhxB	Proteolysis	Inactivation	[61]
RhoA, RhoB, RhoC, Rac1, Cdc42	MARTX	Unknown	Inactivation	[92]
RhoA, Rac1, Cdc42	SopE, SifA, SifB, IpgB1, IpgB2, Map, EspM, EspT	GEF-like activity	Activation	[69]
Arf	RaF	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	Deamidation [†]	Activation	[19]
RhoA	CNFY	Deamidation [†]	Activation	[100]
RhoA, Rac1, Cdc42	DNT	Transglutamination [‡]	Activation	[19]
<i>Heterotrimeric Gα subunits</i>				
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]
<i>Large, multidomain GTPases</i>				
EF-2	DT, ExoA	ADP ribosylation	nactivation	[41]

GTPase cycle of heterotrimeric G proteins and points of toxin interactions



YpKA: *Yersinia pseudotuberculosis* serotype I;

CT: Cholera toxin;

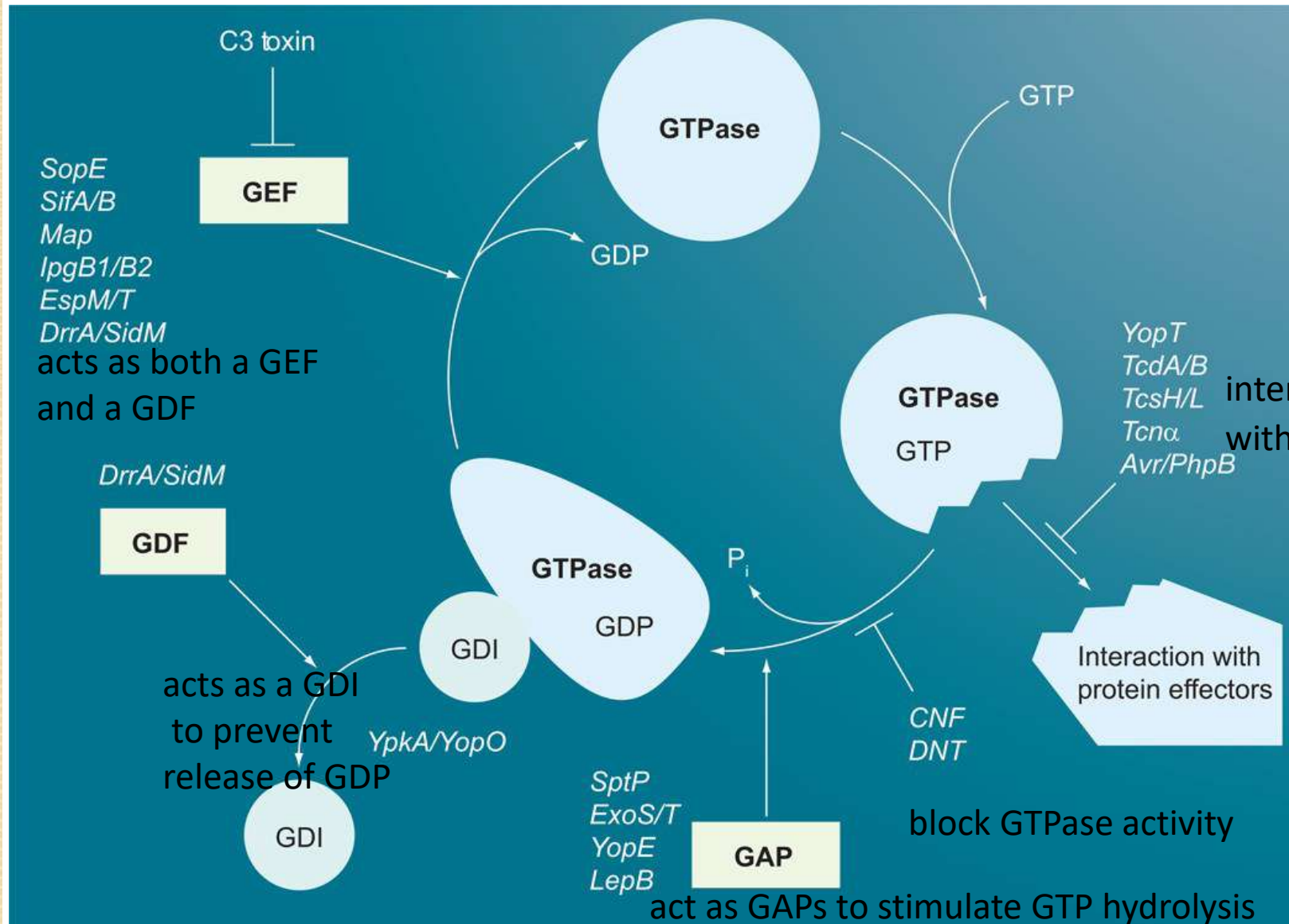
HLT: Heat-labile enterotoxins;

PMT: *Pasteurella multocida* toxin;

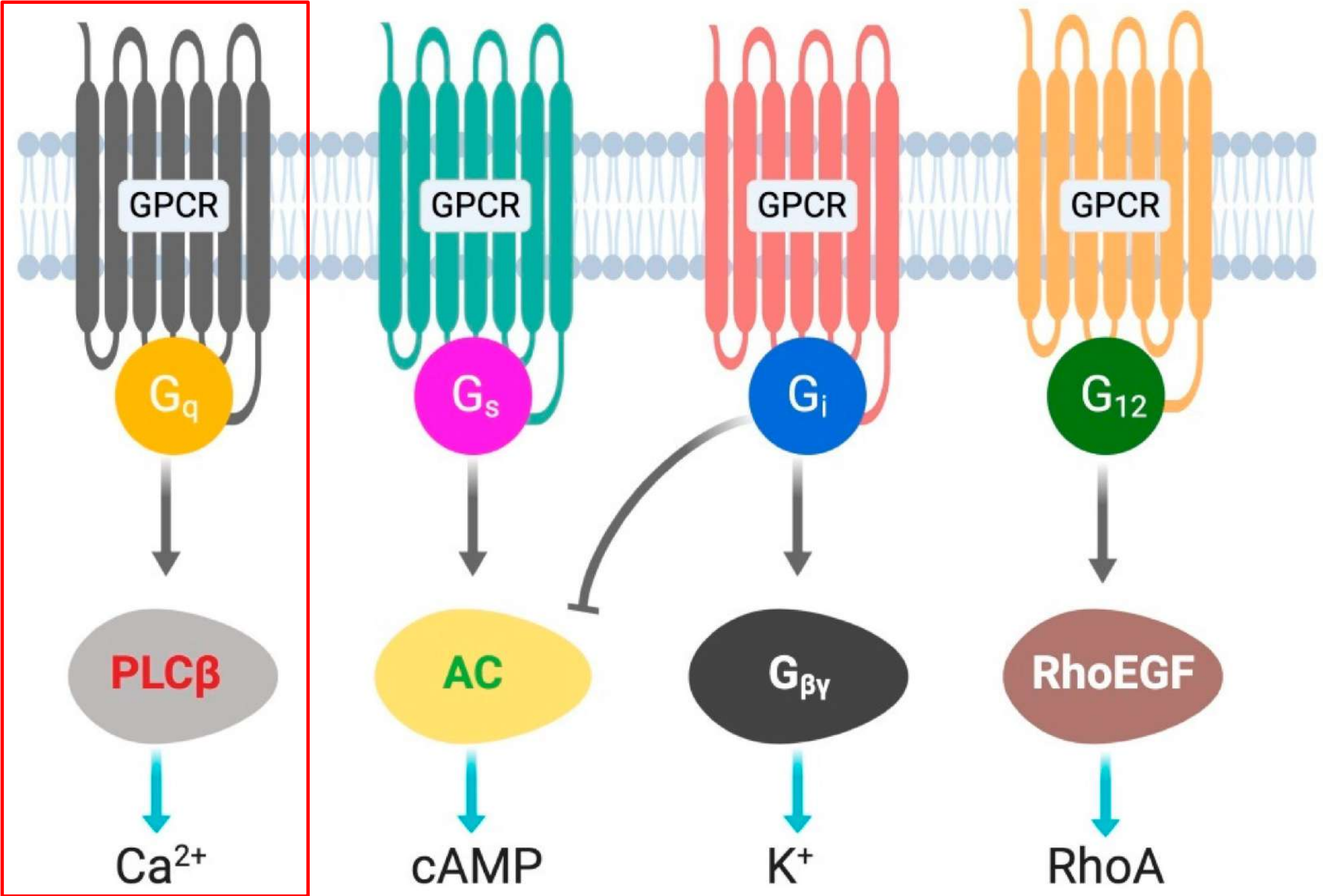
PT: Pertussis toxin.

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

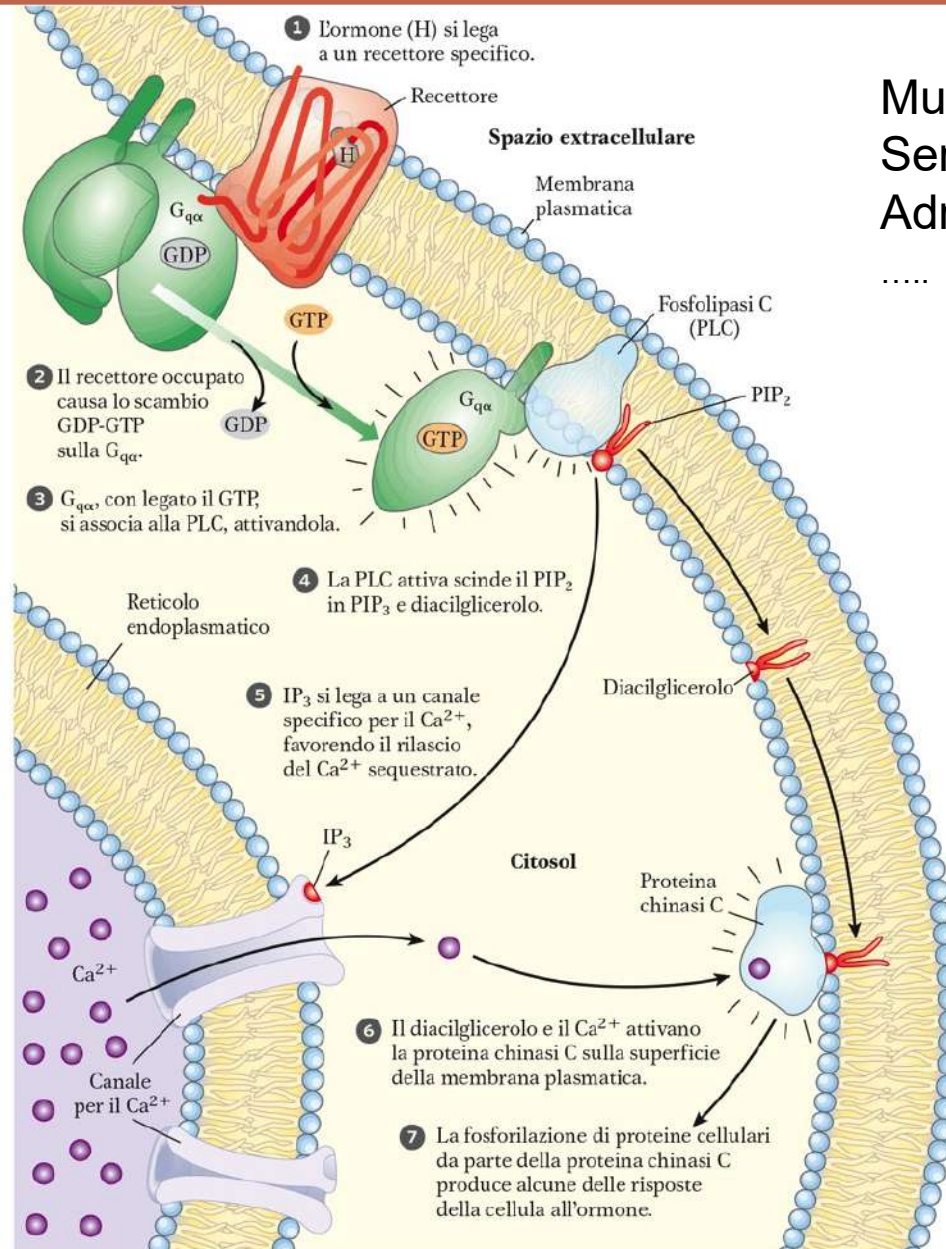
GTPase cycle of small G proteins and points of toxin interactions



interfere with the GTPase interaction with effectors.

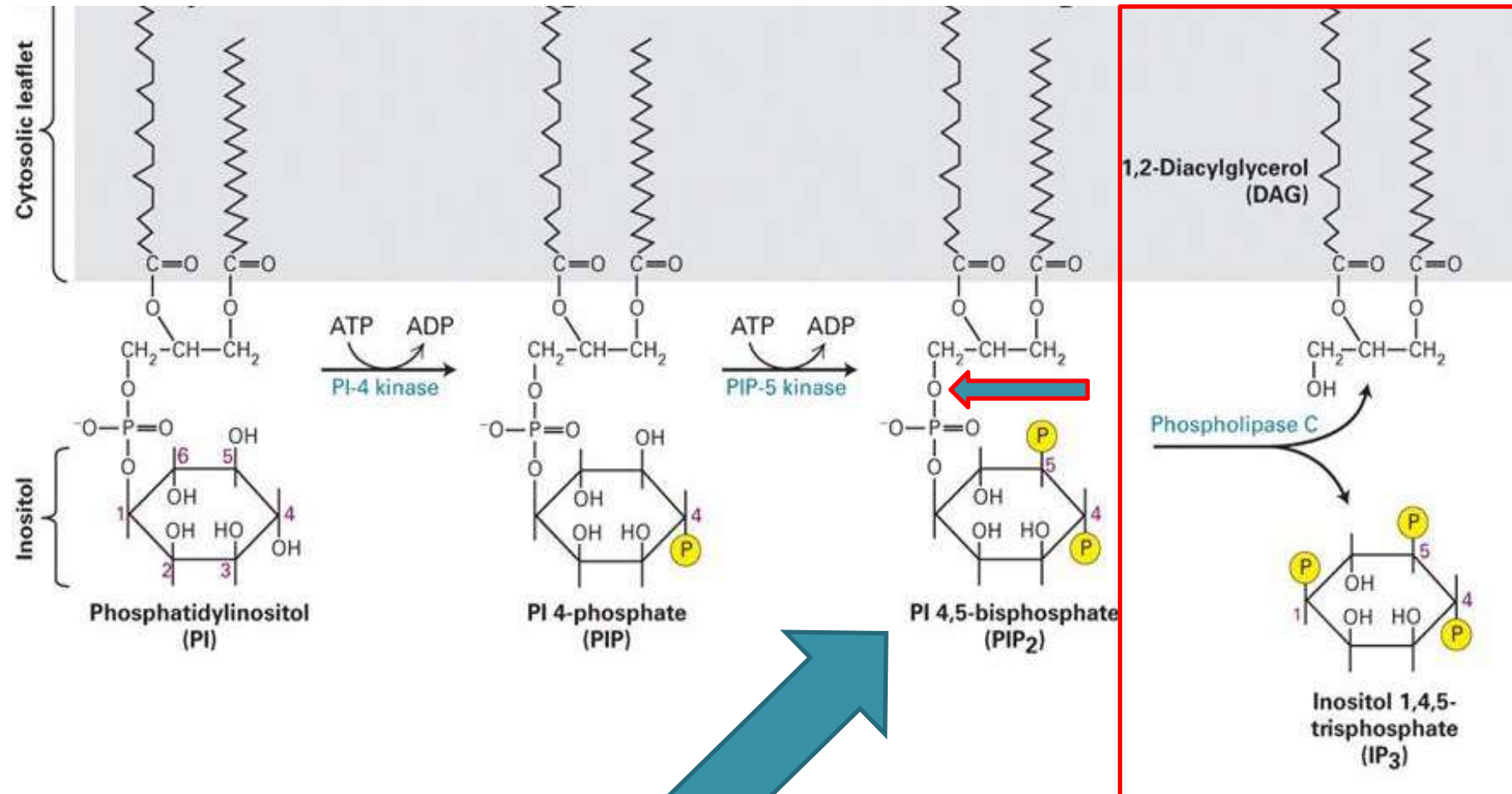


Recettori accoppiati a Gq

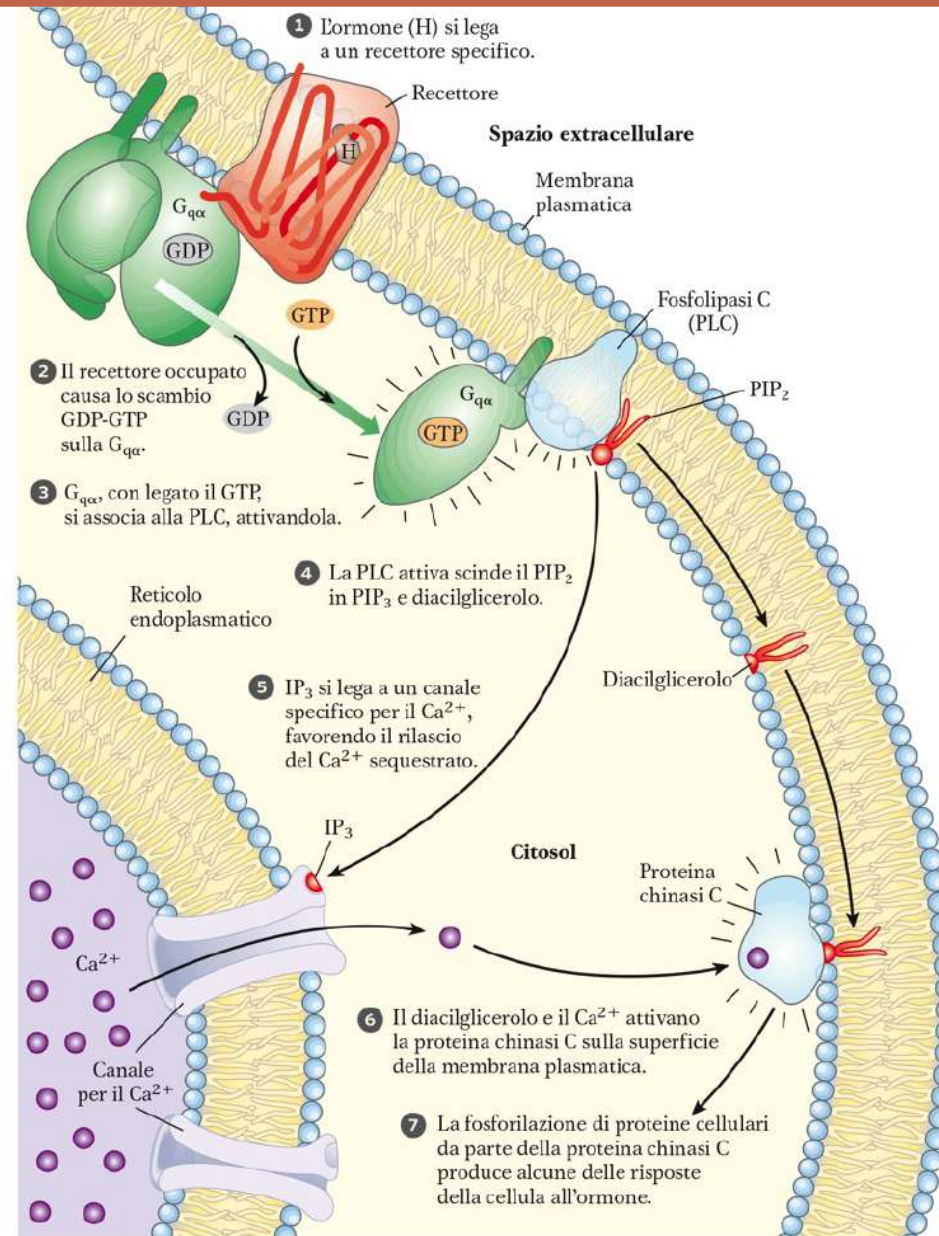


Muscarinico M3
Serotonergici 5-HT_{2a,b,c}
Adrenergico Alpha1

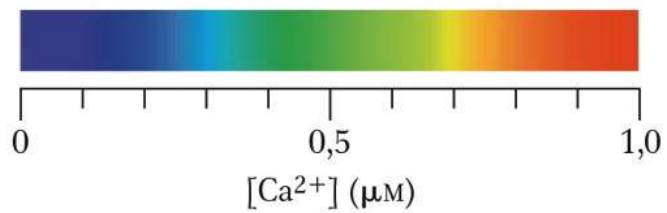
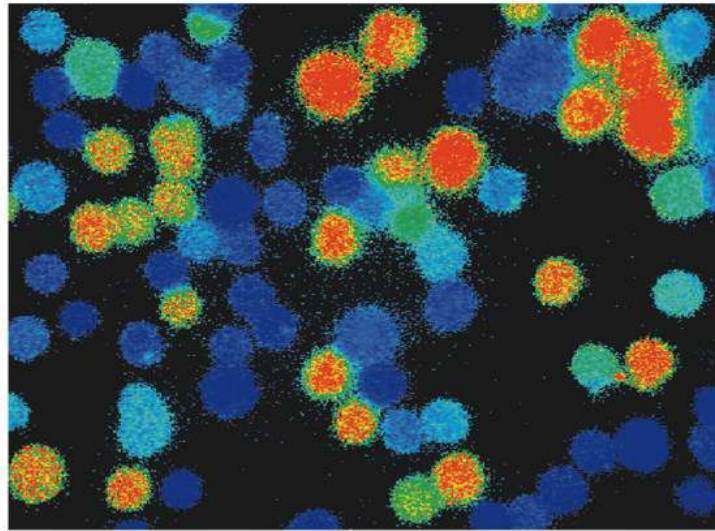
Produzione di IP3



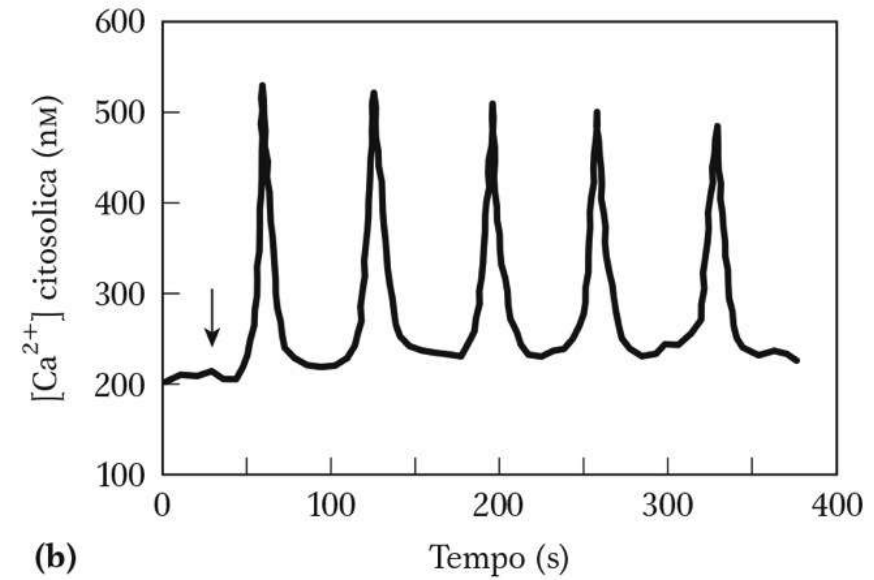
Recettori accoppiati a Gq



Peculiarità del rilascio di calcio

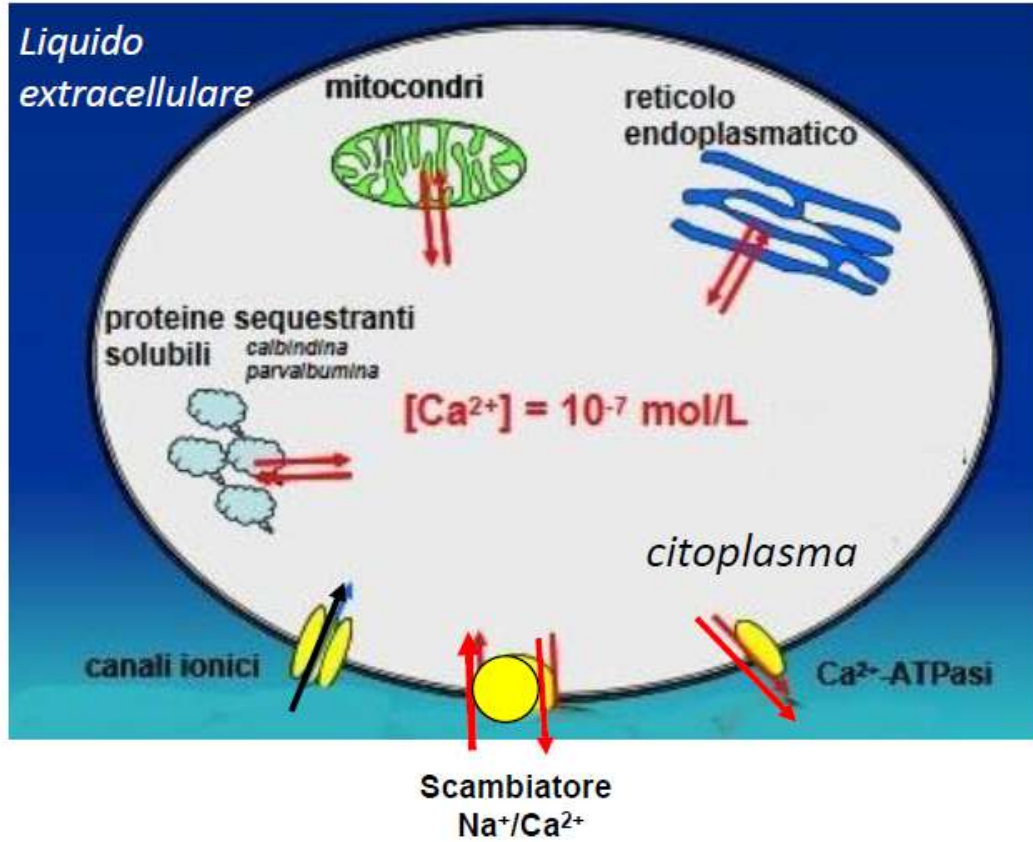


(a)



(b)

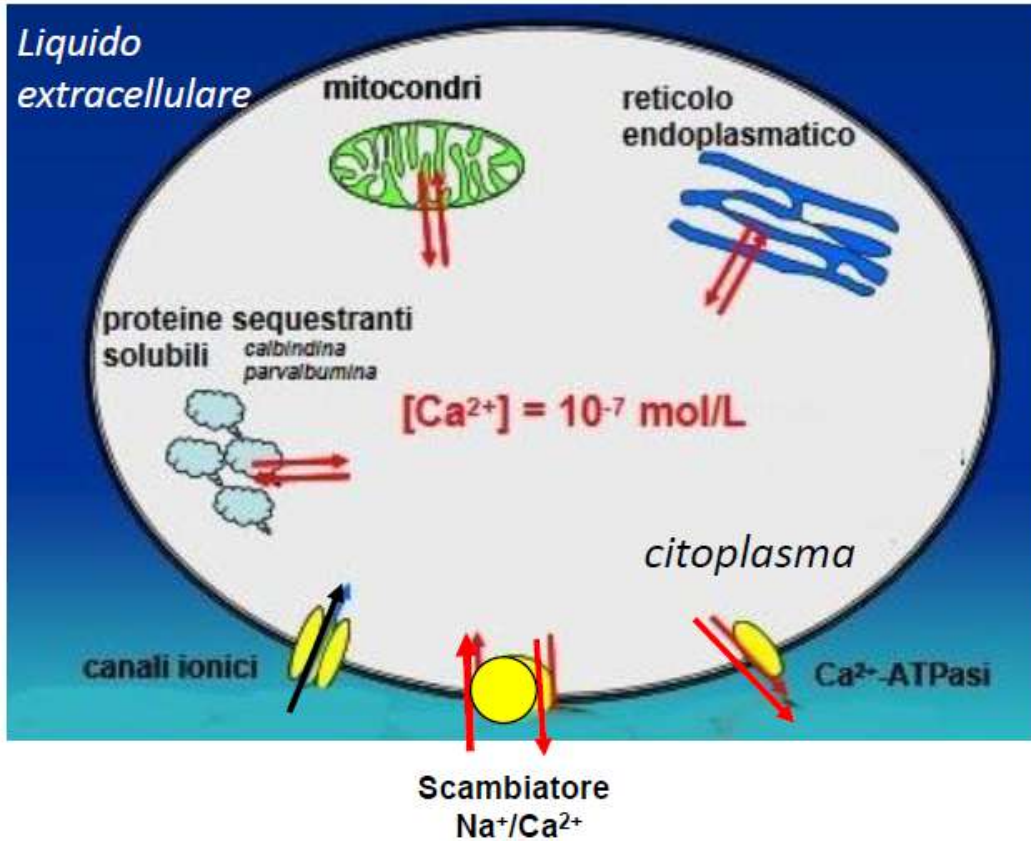
Gli ioni Ca^{2+} come secondi (o terzi messaggeri)



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)



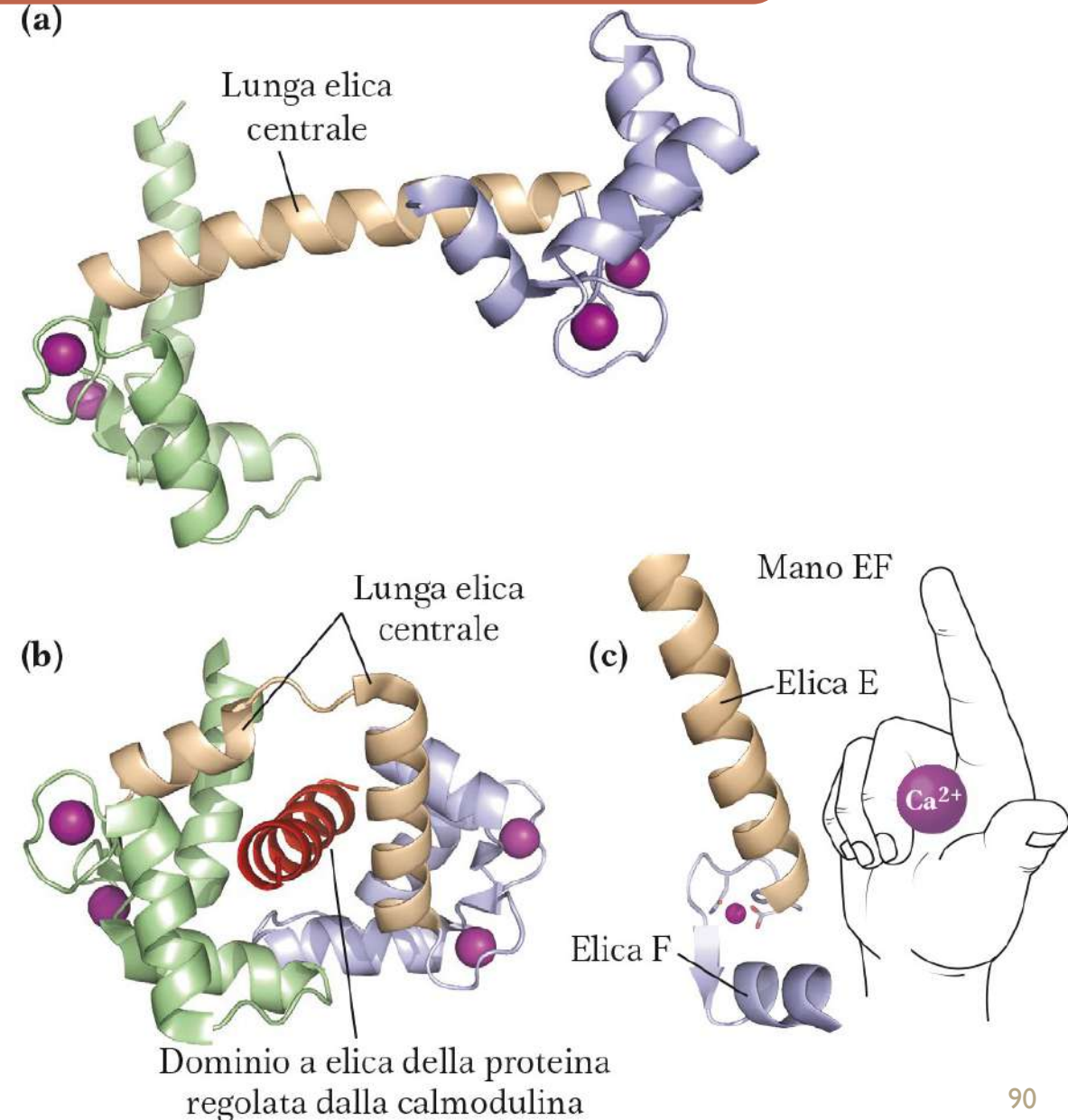
La concentrazione citoplasmatica di calcio della cellula eucariotica a riposo si aggira intorno a 10^{-7}M nonostante una concentrazione extracellulare di 1-2 mM.

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:

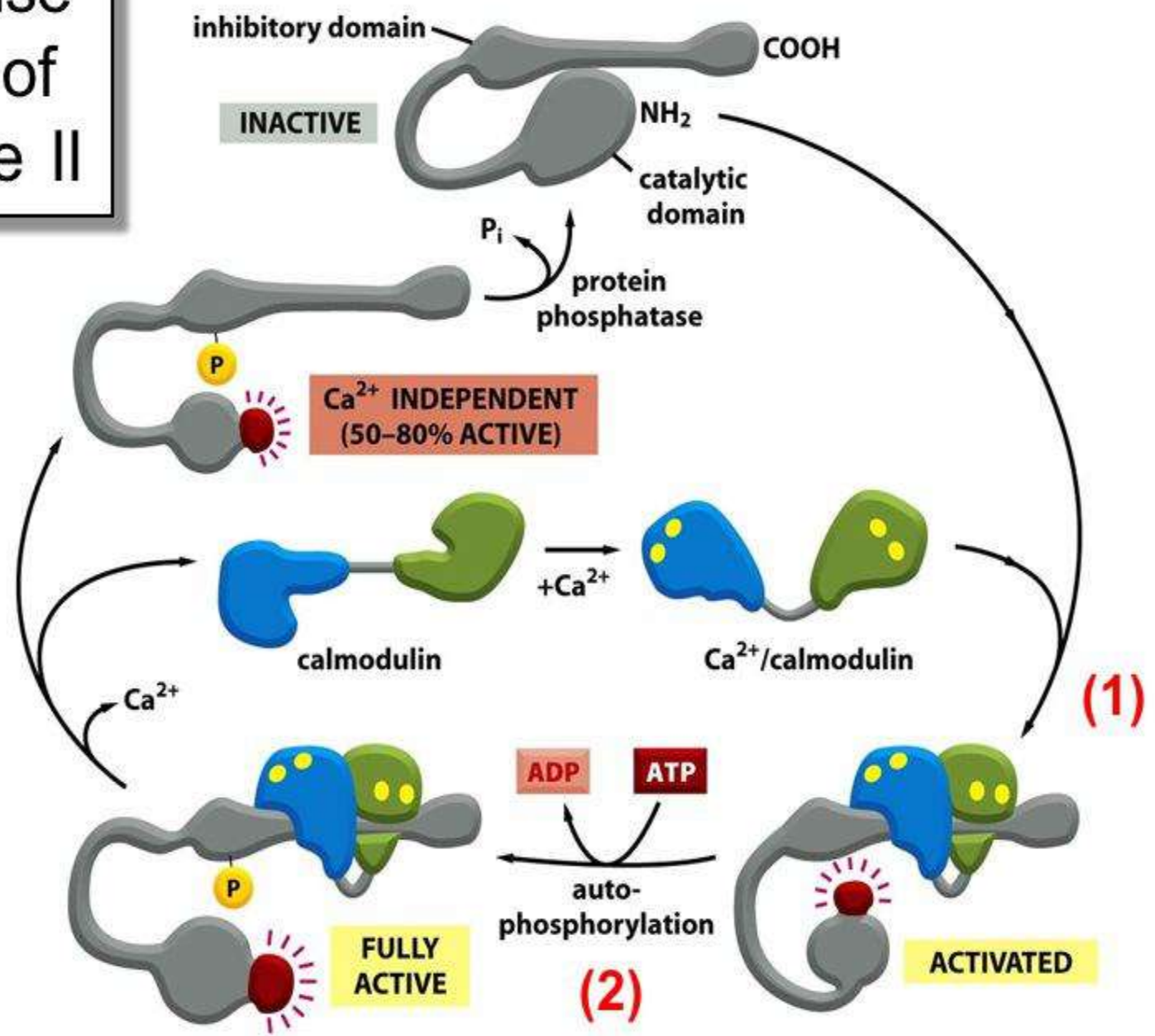
- trasporti attivi primari e secondari che provvedono ad estrudere il Ca^{2+} dalla cellula e ad accumularlo in depositi intracellulari (reticolo endoplasmico, mitocondri).
- sistemi tampone della concentrazione di Ca^{2+} rappresentati da proteine leganti il Ca^{2+} (*Ca^{2+} binding proteins*), di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

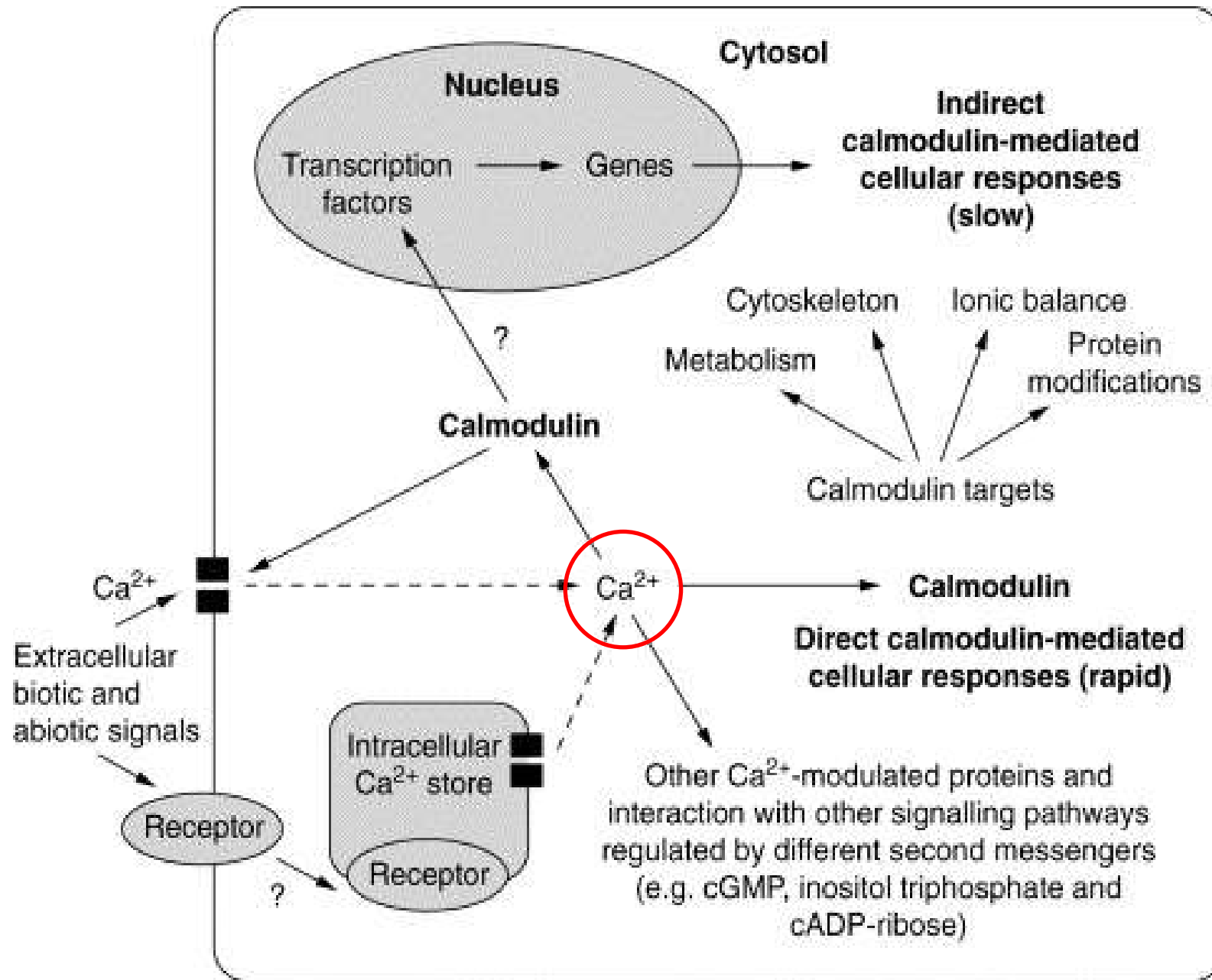
La calmodulina un effettore del Ca^{2+}

- 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.



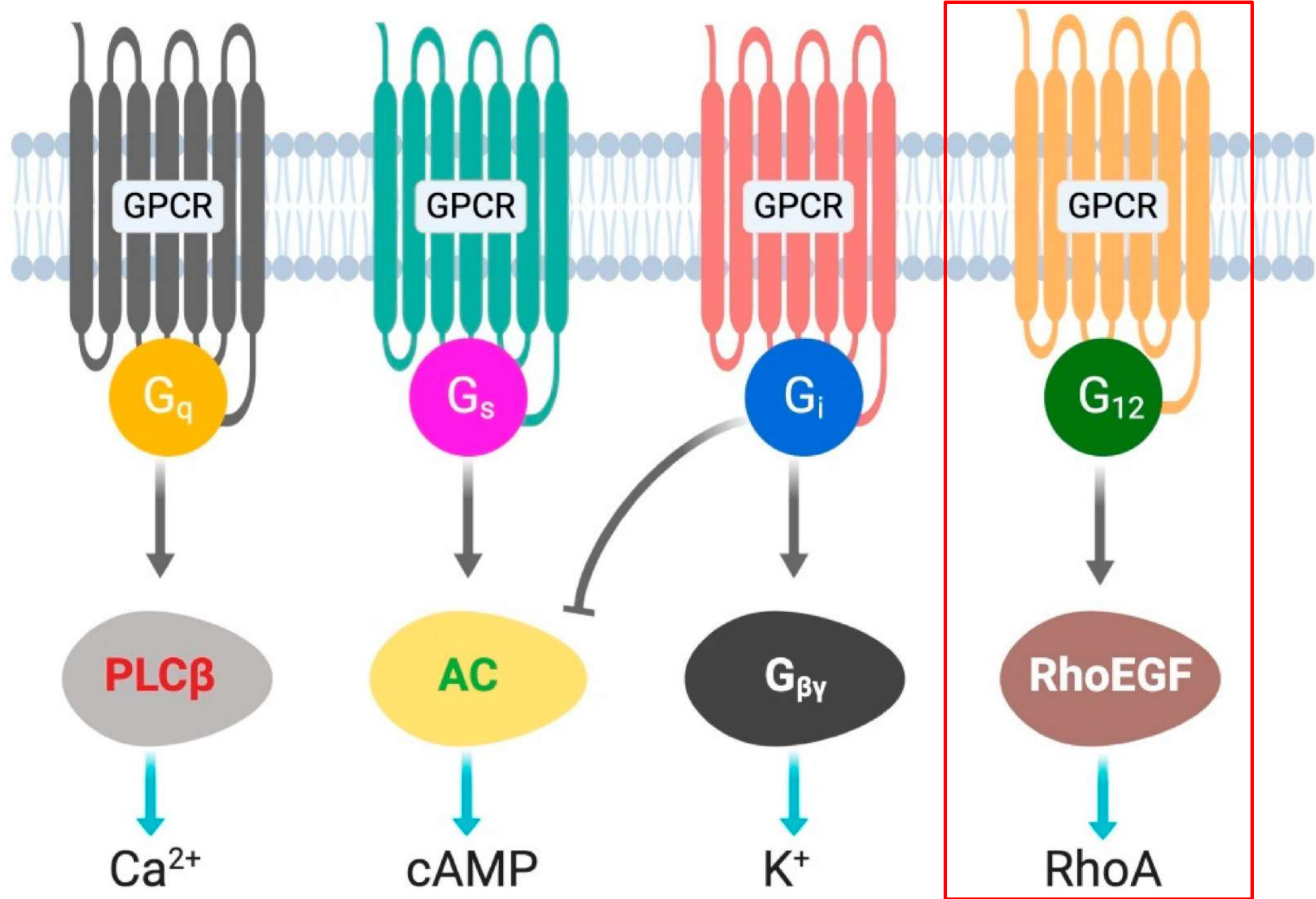
The stepwise activation of CaM-kinase II









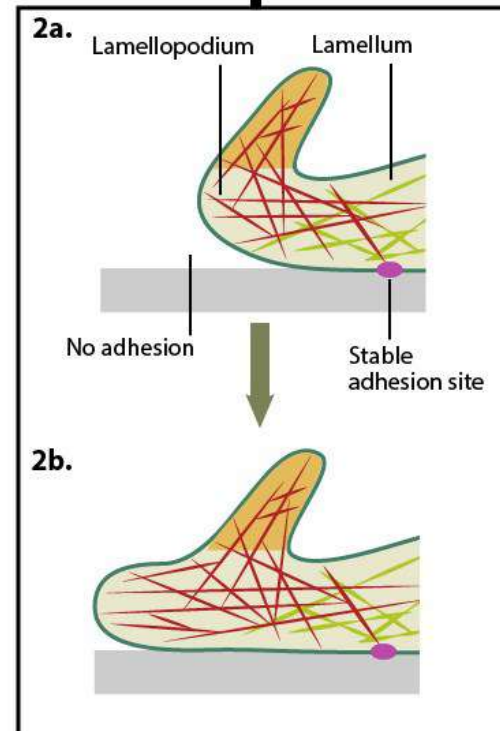
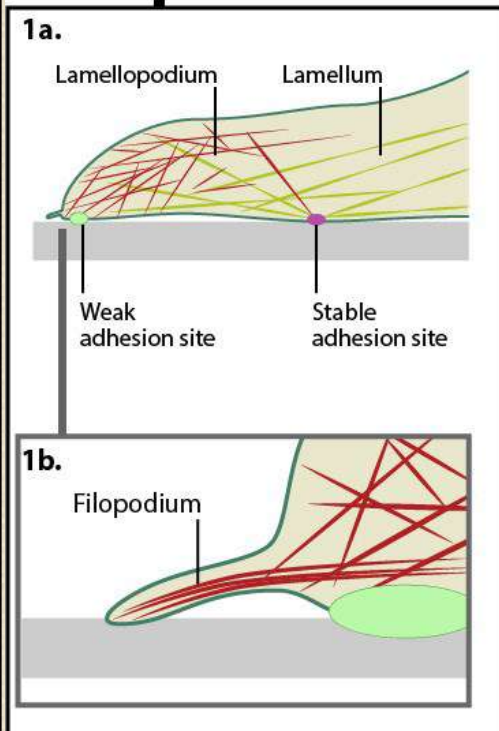
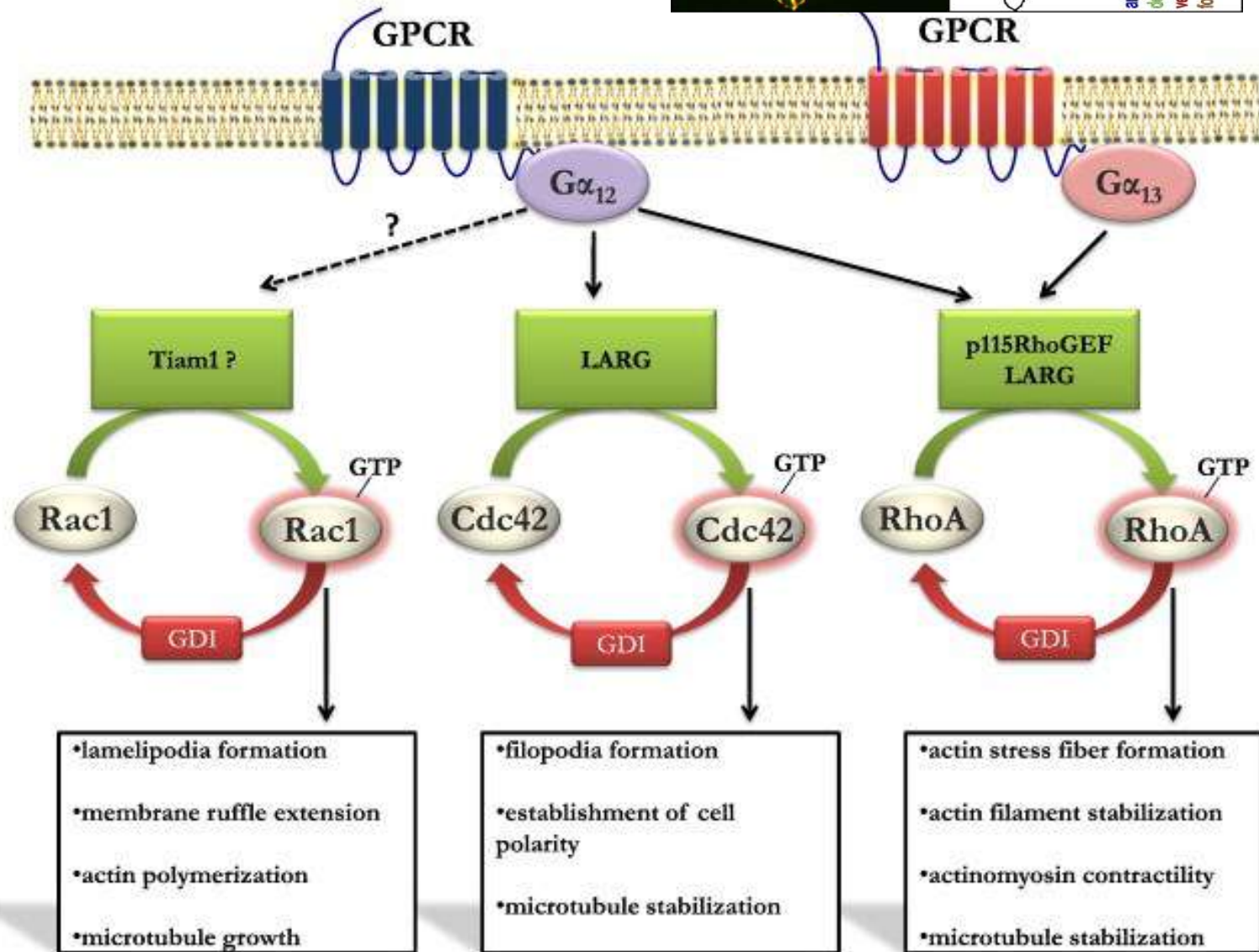
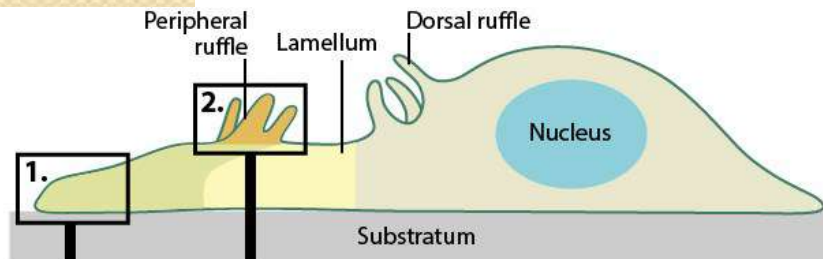
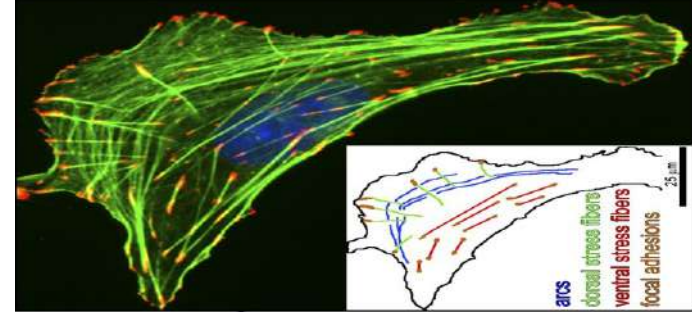


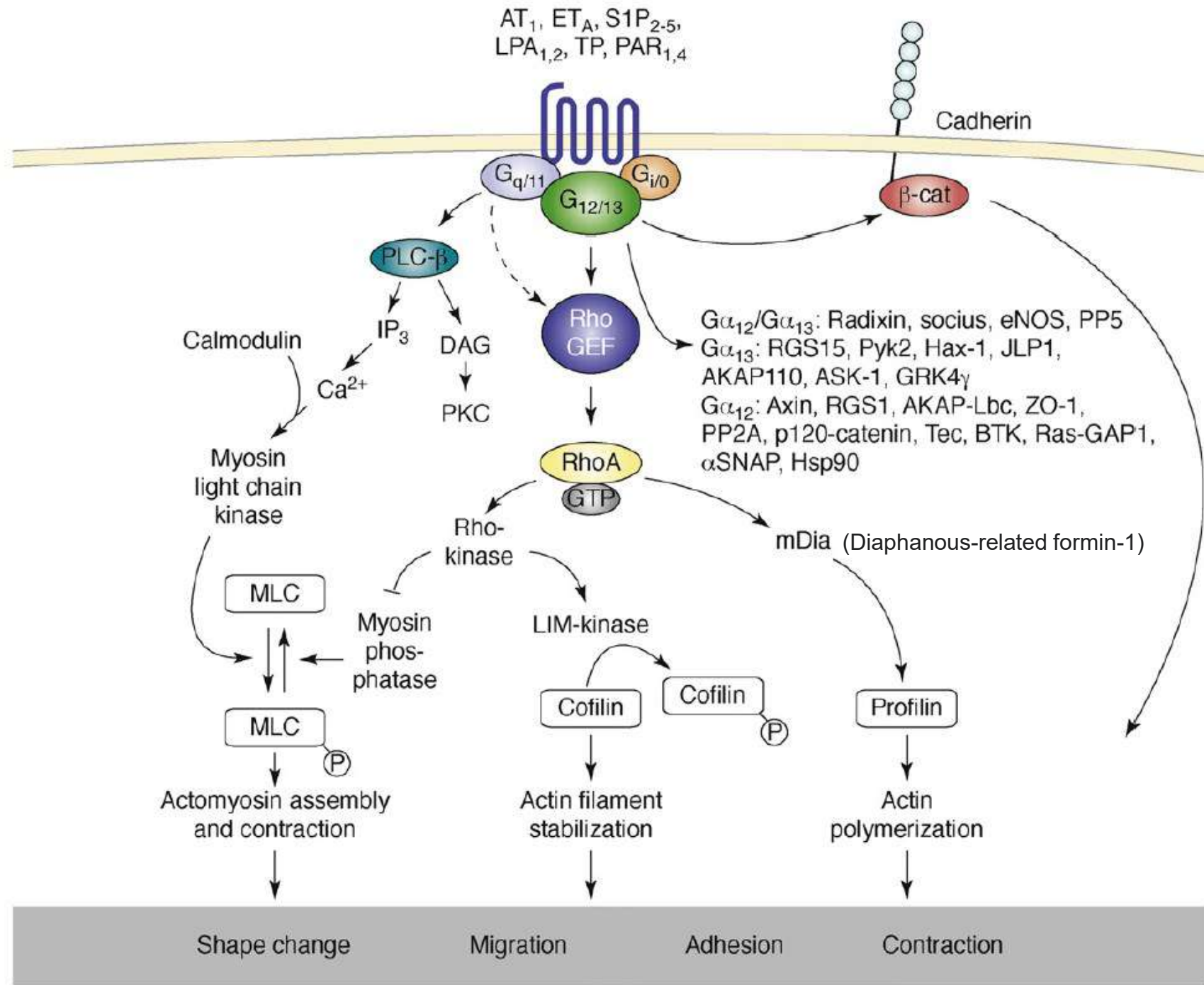
Recettori accoppiati a G12/13

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

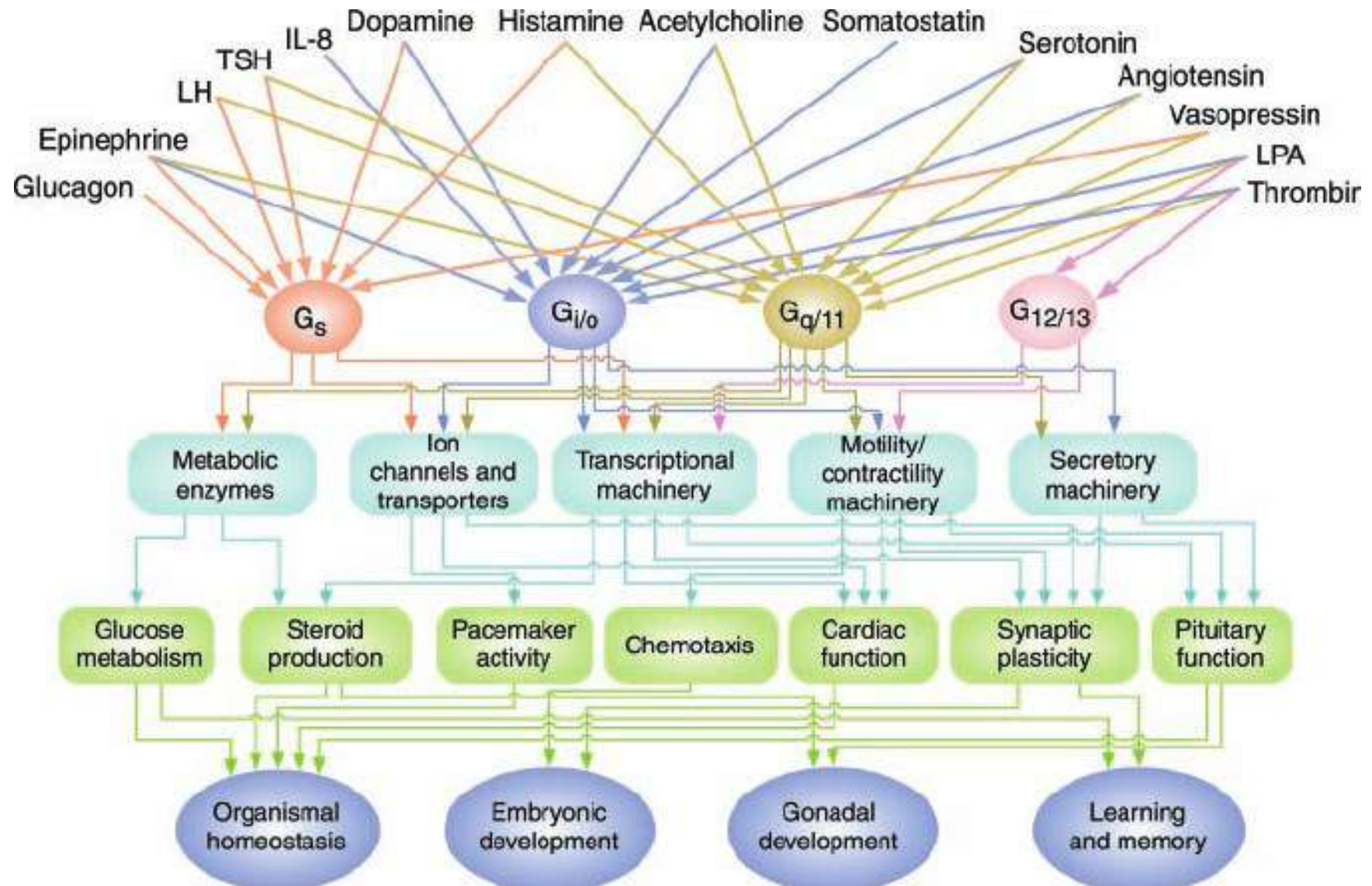
Receptors	G proteins	Functions	References
Sphingosine 1-phosphate			
S1P2/S1P3	G $\alpha_{12/13}$	Stress fiber formation	133
S1P1/S1P3/S1P5	G α_{12}	Inflammation	62
S1P3	G $\alpha_{12/13}$	Inflammation	134
S1P2	G $\alpha_{12/13}$	Myofibroblast contraction	135
S1P3	G α_{13}	Cardioprotection	136
S1P receptor	G α_{12}	Hepatic stellate cell activation	45
Thrombin			
PAR1	G α_{12}	Monocyte migration	63
PAR1	G α_{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 α_{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 α_{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 α_{12}	Stress fiber accumulation	140
ATP			
P2Y6	G $\alpha_{12/13}$	Cardiac fibrosis	131
Adenosine			
A1/A2a/A2b/A3	G α_{12}	Fatty acid oxidation	38
Bradykinin			
B2	G α_{13}	Stress fiber formation	140
Serotonin			
5-HT2C	G α_{13}	Stress fiber formation	140
Vasopressin			
V1A	G α_{12}	Stress fiber accumulation	140

Recettori accoppiati a $G_{12/13}$

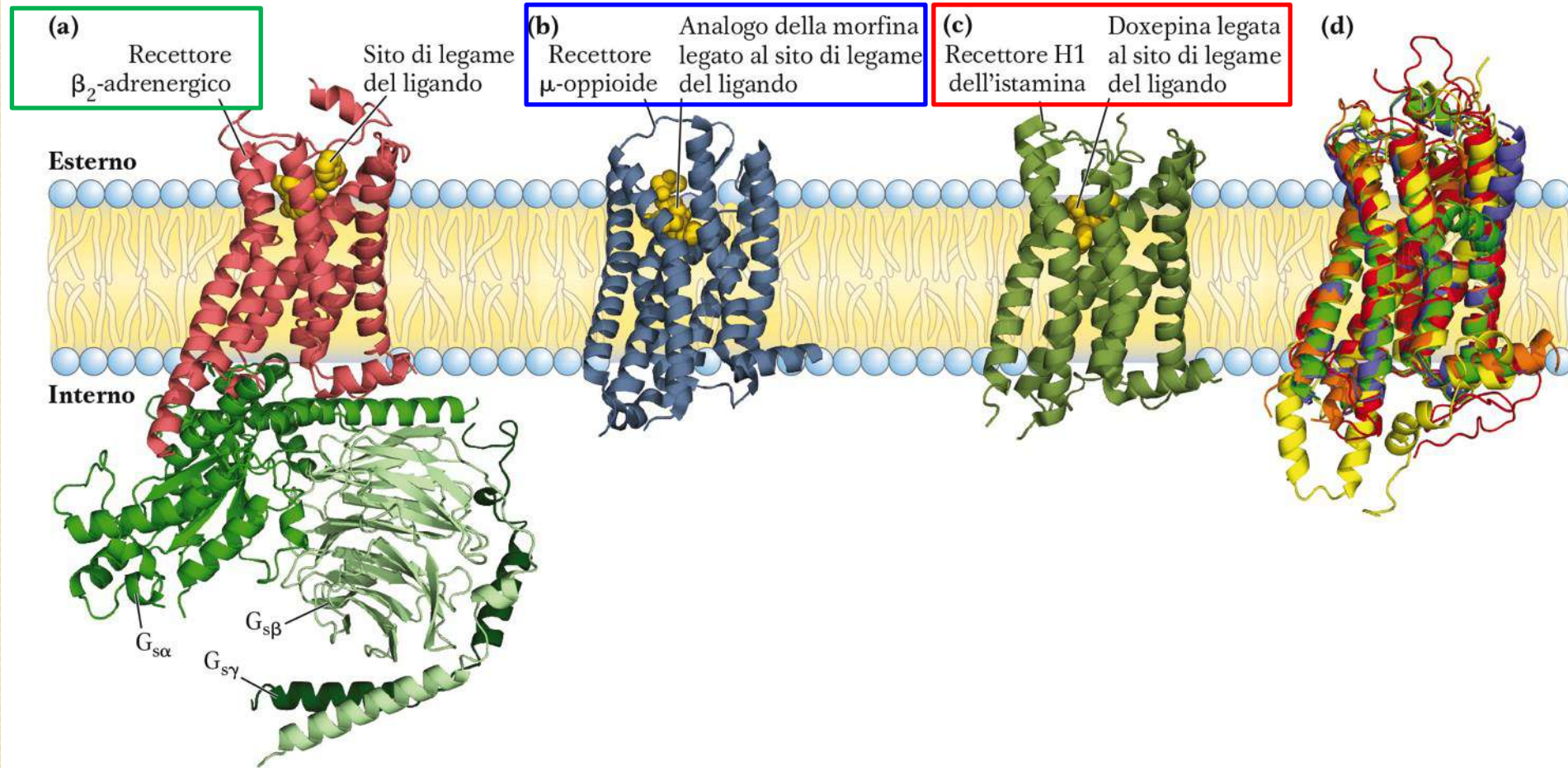




La biosegnalazione via GPCR



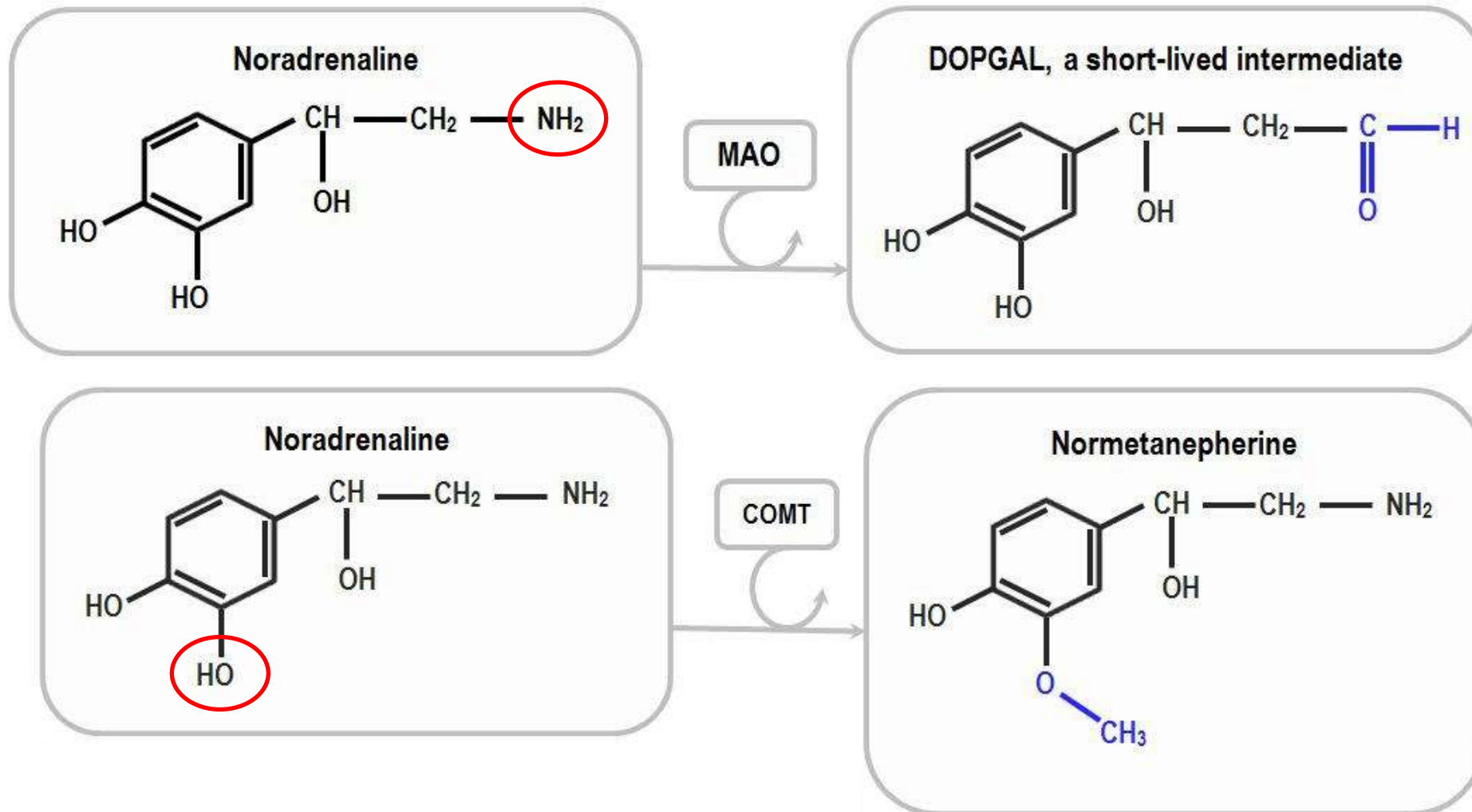
Somiglianze di struttura nei GPCR



Terminazione della risposta

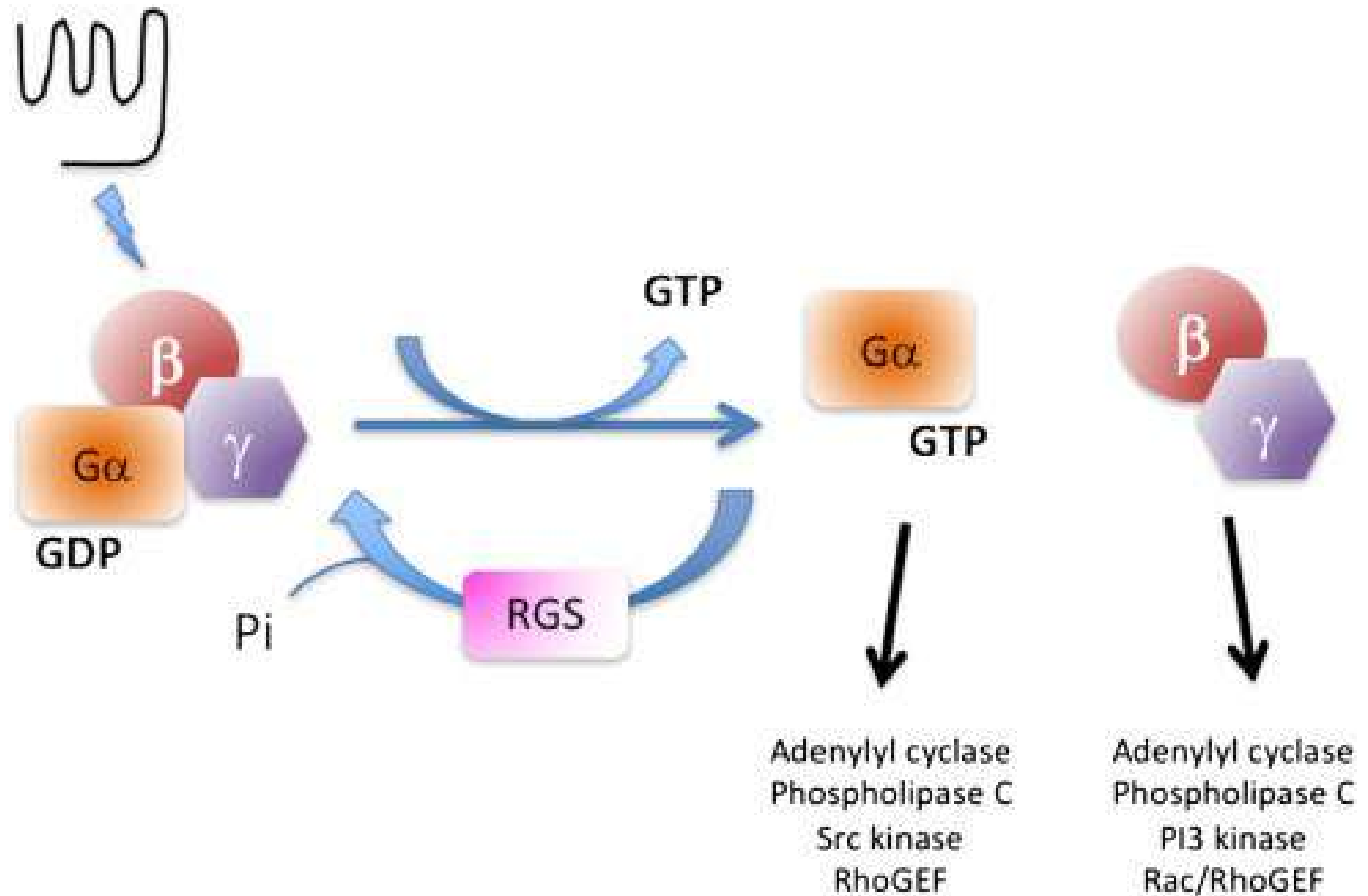
- Re-uptake e degradazione: Monoamino ossidasi MAO e catecol-O-metiltrasferasi (COMT)
- Attività GTPasi intrinseca delle G protein e GAP accessorie (RGS)
- G protein-coupled receptor kinases (GRK), PKA, etc.....
- Arrestins
- Fosfodiesterasi (PDE)/ pompa del Ca²⁺

Azione delle MAO e COMT

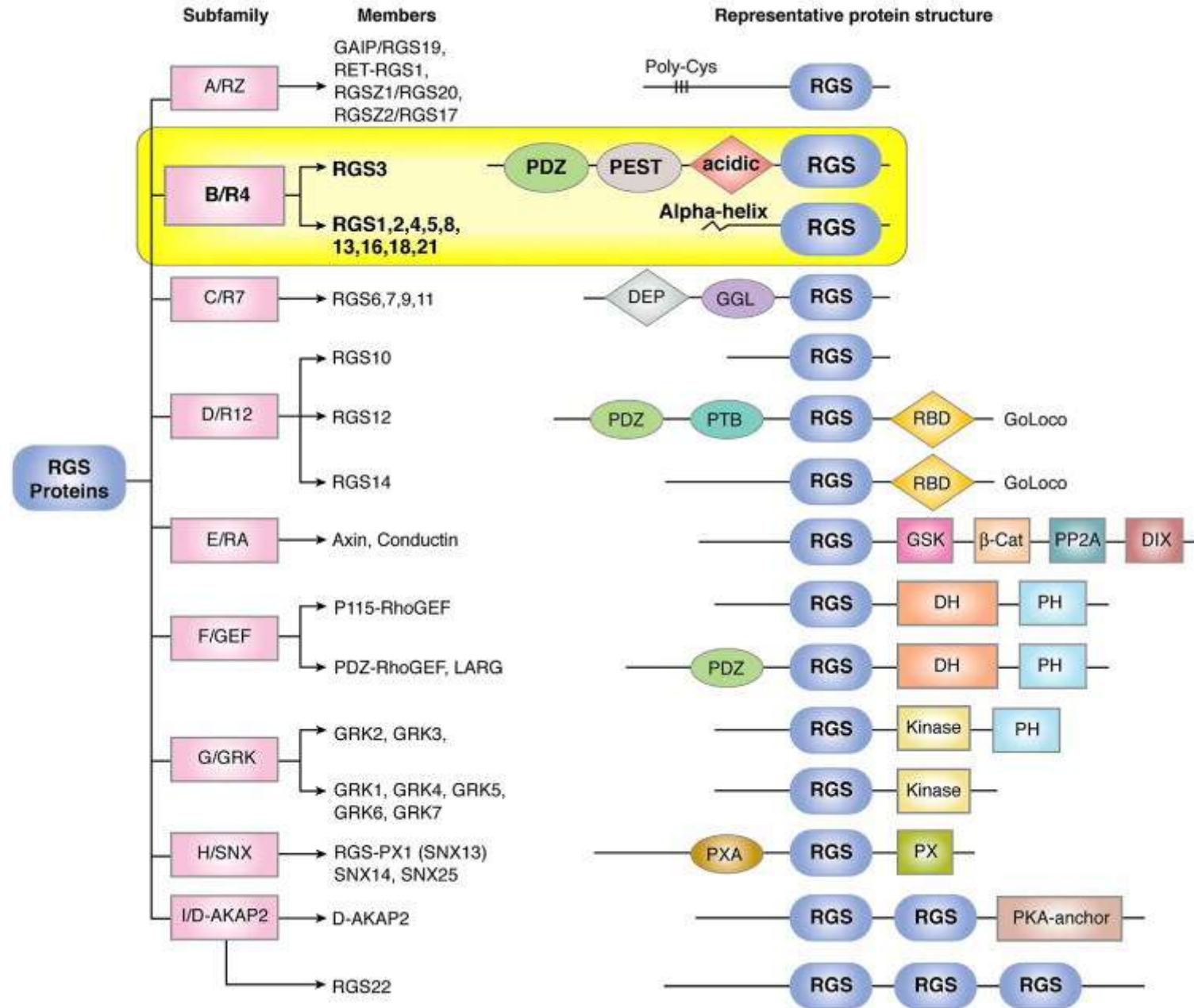


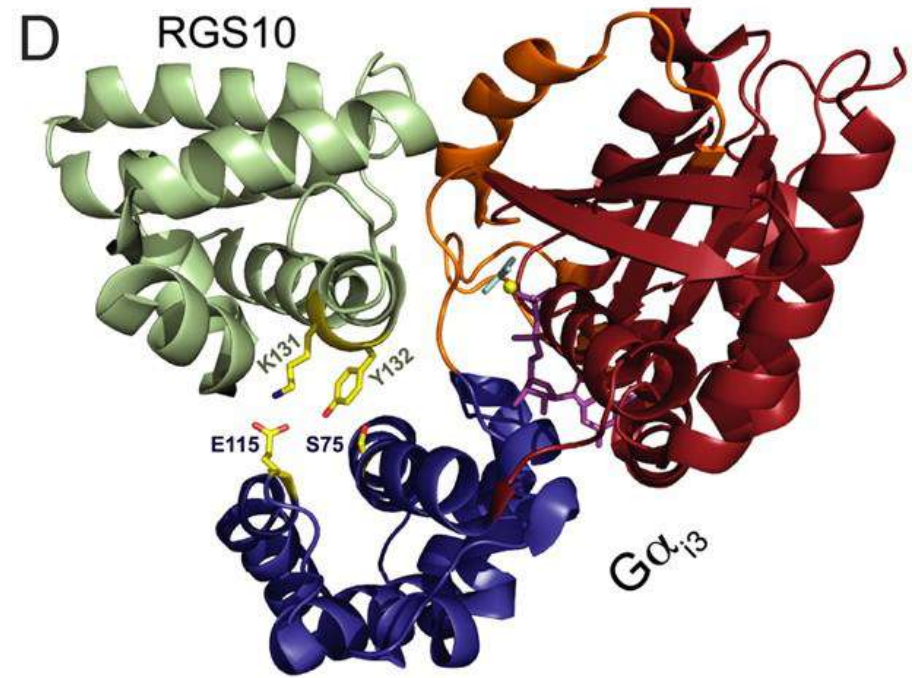
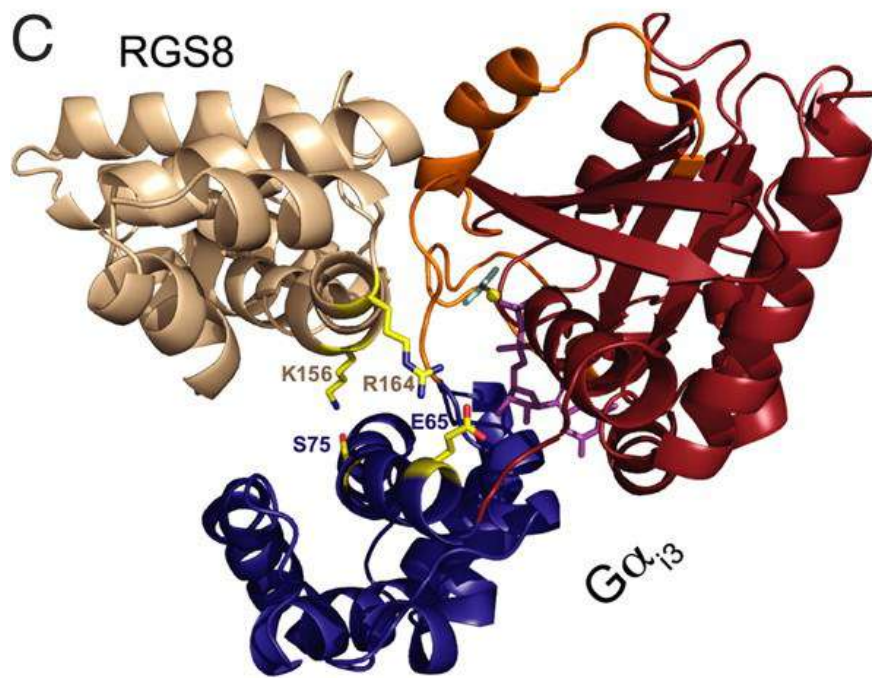
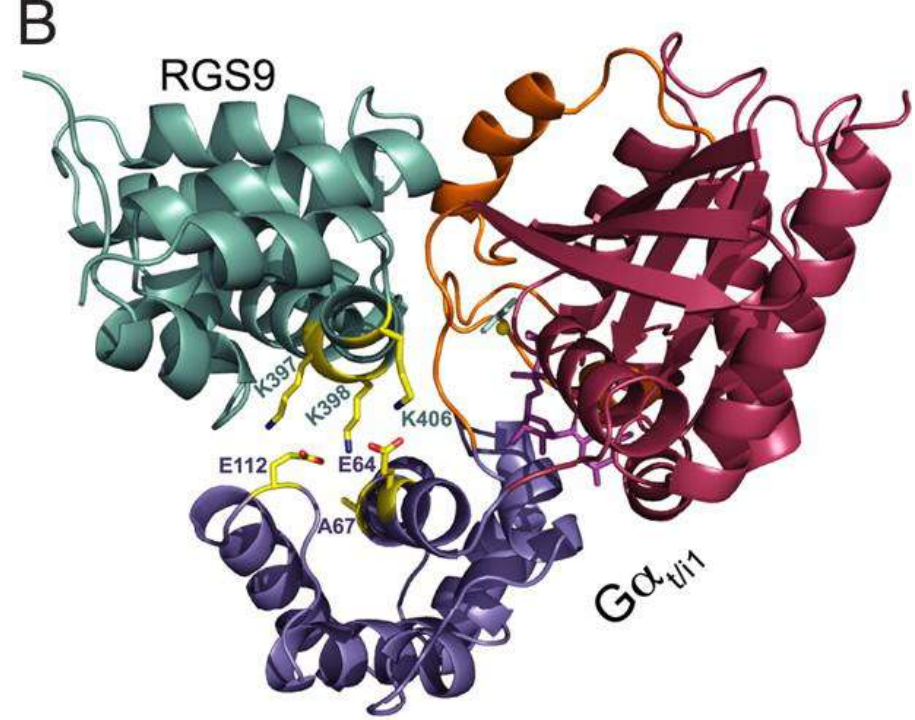
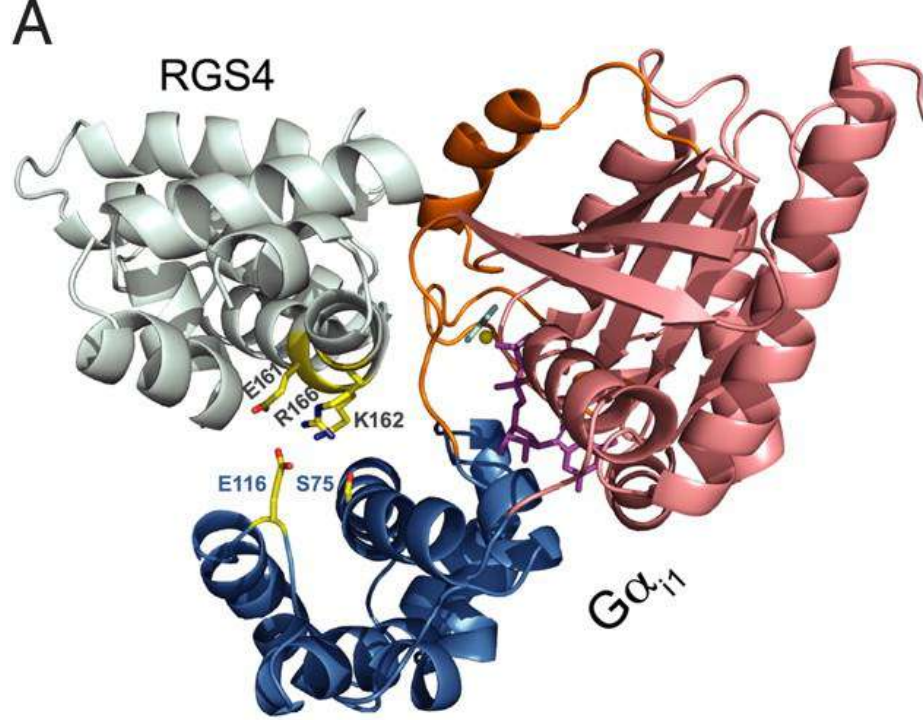
Terminazione della risposta

- 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 Ca²⁺

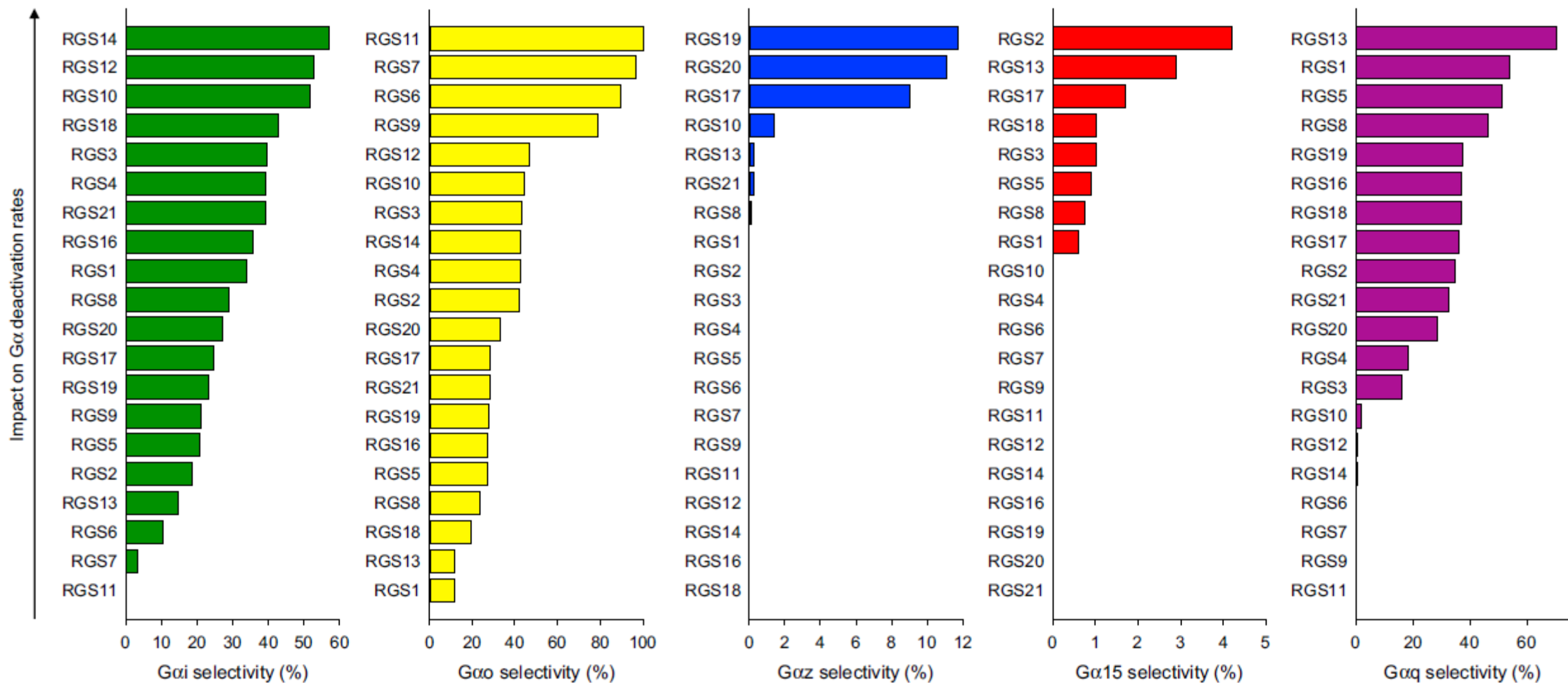


Classification of Mammalian RGS Proteins





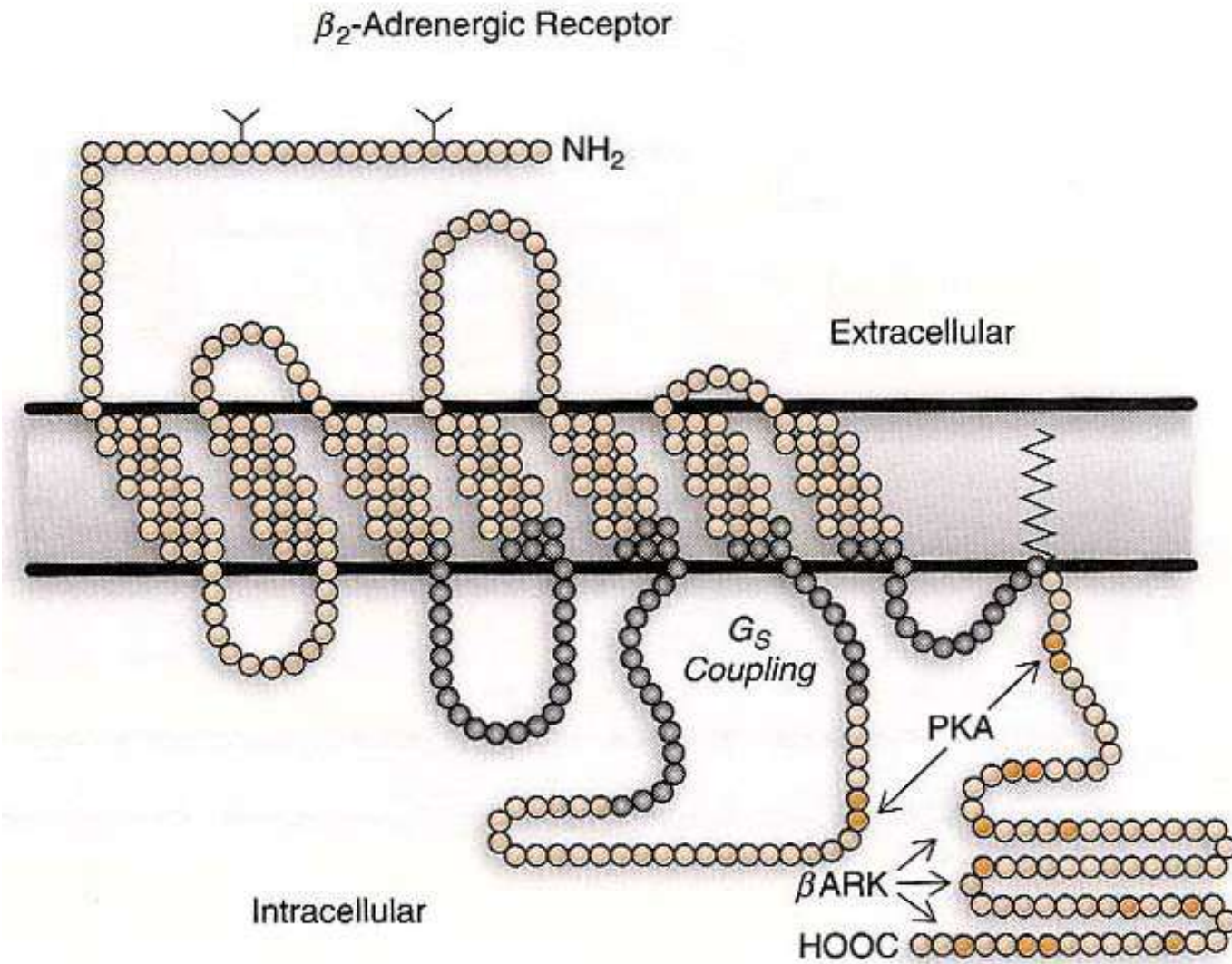
RGS selectivity of G α subunits



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 Ca²⁺

GPCR desensitization



G protein coupled receptor kinase: GRK

GRK1/GRK7



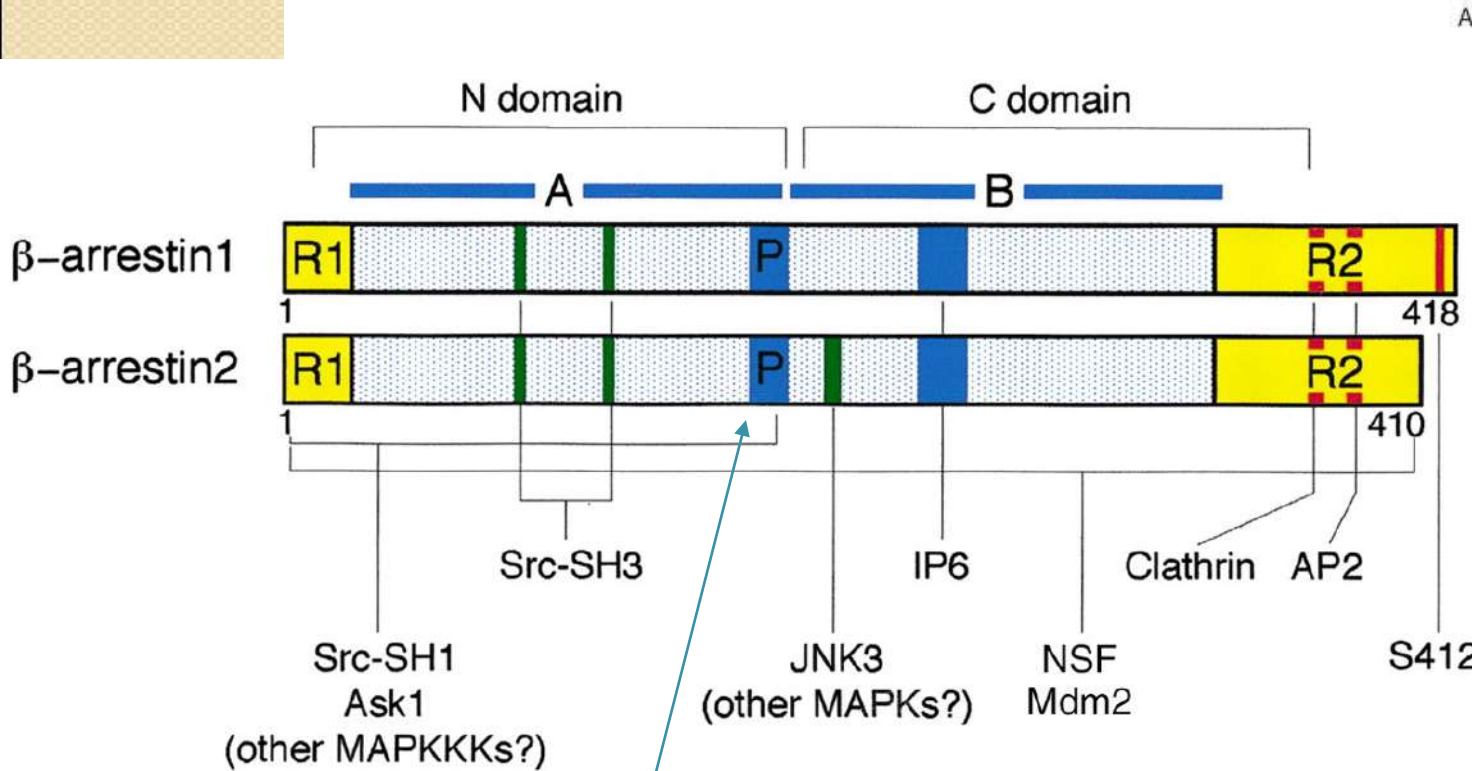
GRK2/GRK3



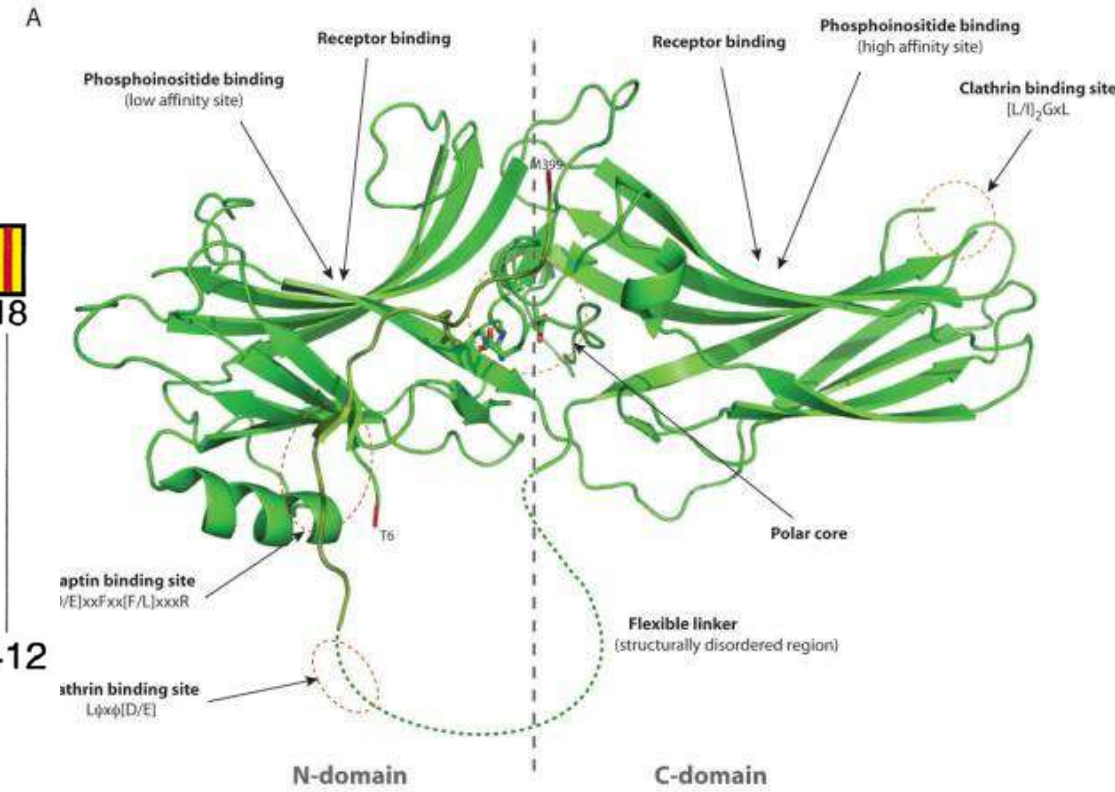
GRK4/GRK5/GRK6



Beta-arrestins



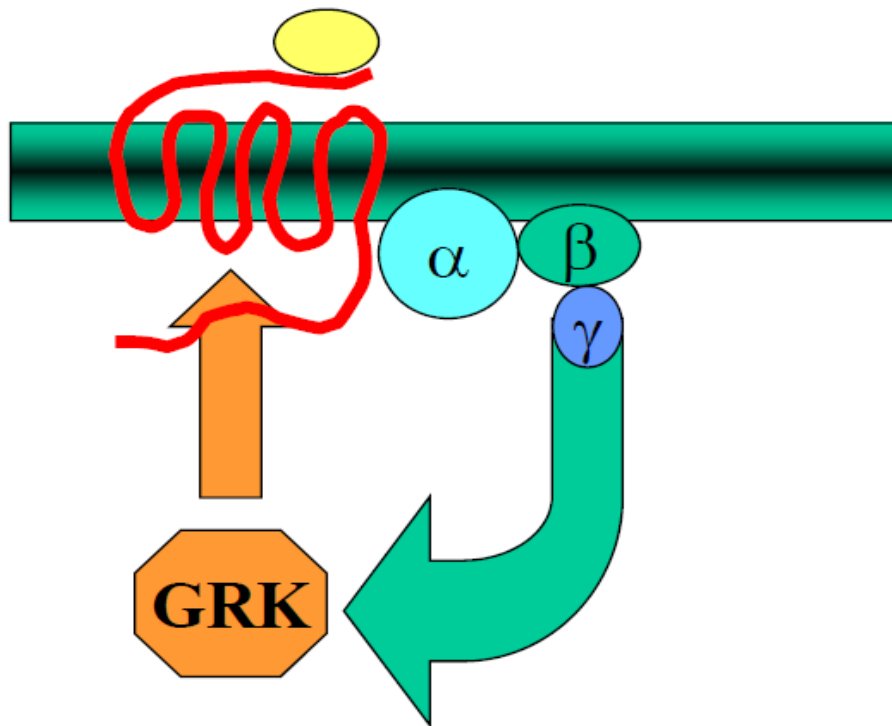
Phosphate sensor domain



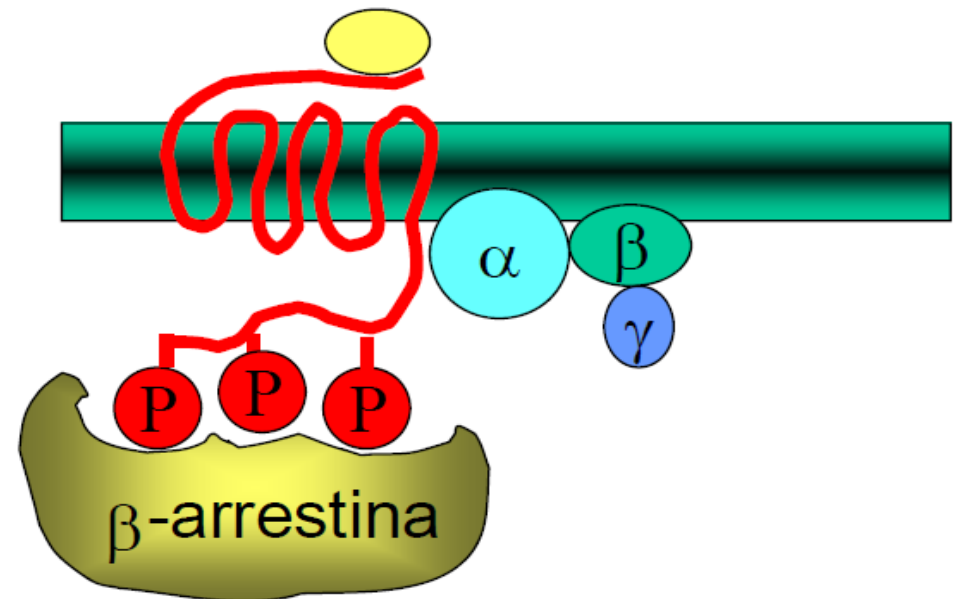
Seven-stranded β sandwich

Desensibilizzazione omologa

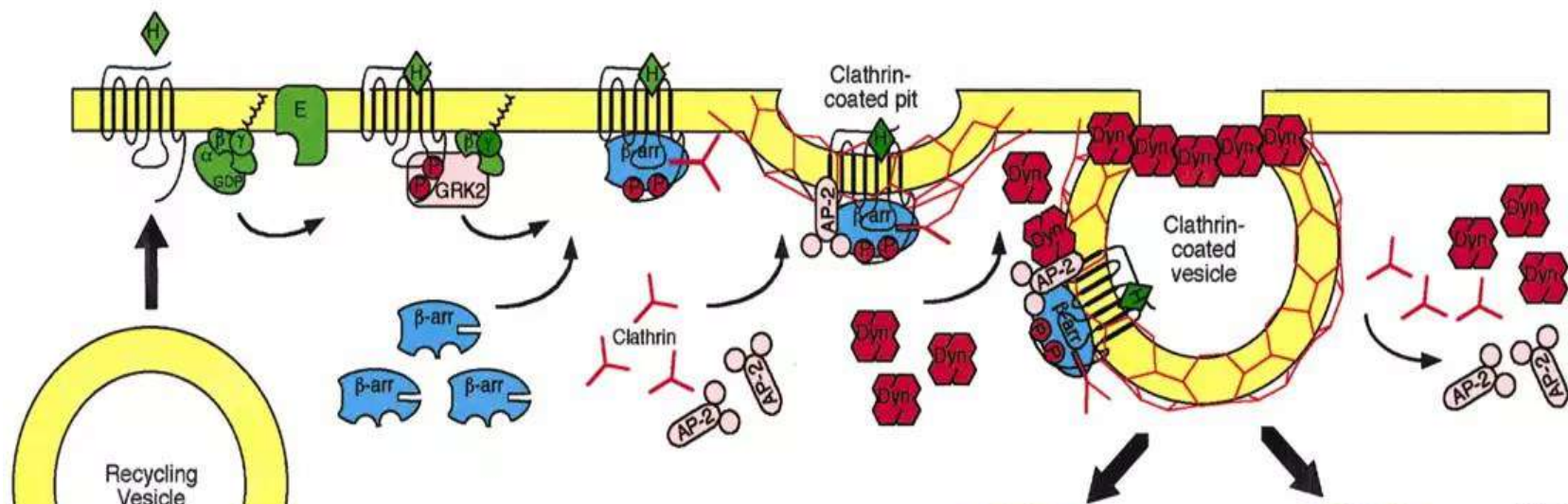
Disaccoppiamento recettore-proteine G in risposta alla **fosforilazione del recettore tramite le GRK** (G protein-coupled receptor kinase) promossa dal dimero $\beta\gamma$



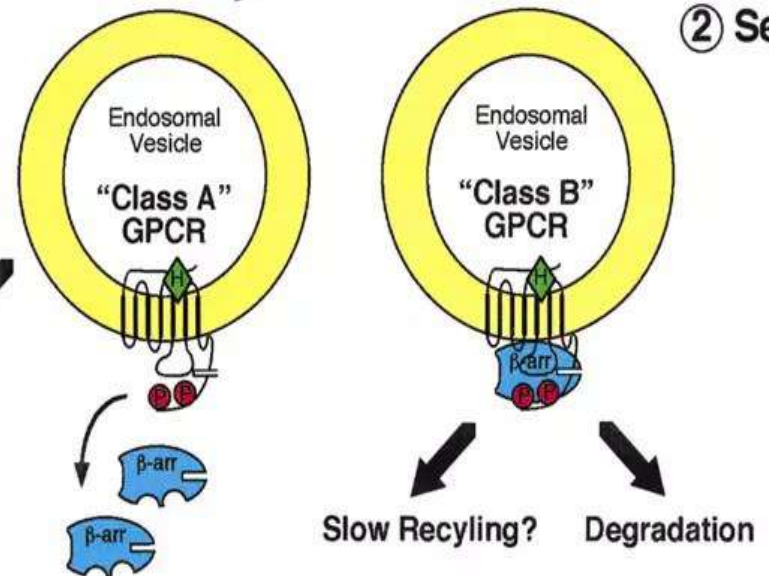
La fosforilazione tramite GRK spesso non è sufficiente per inattivare completamente i recettori; la completa inattivazione richiede un componente aggiuntivo, **l'arrestina**



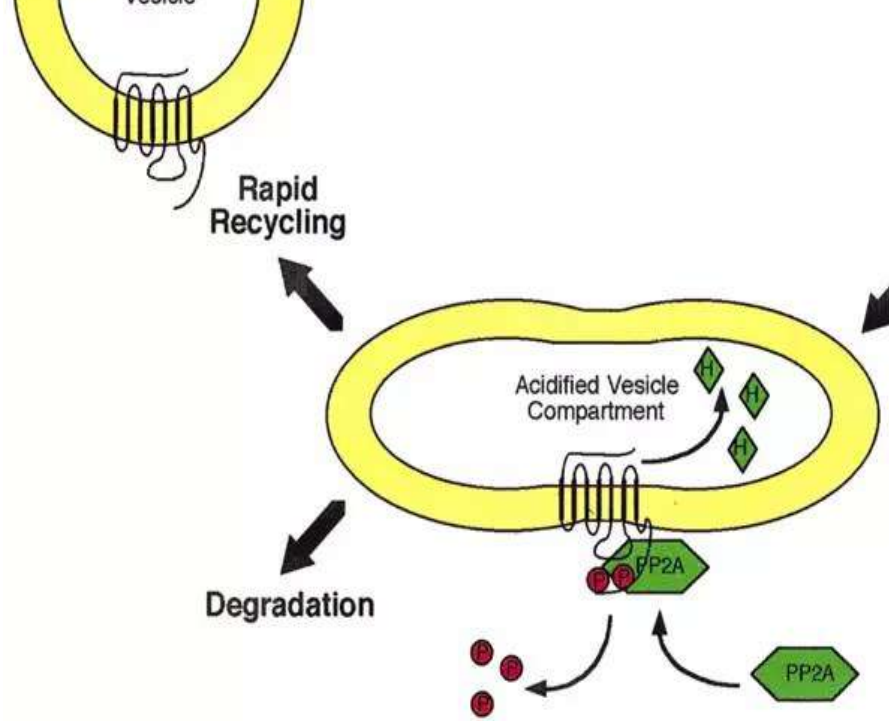
① Desensitization

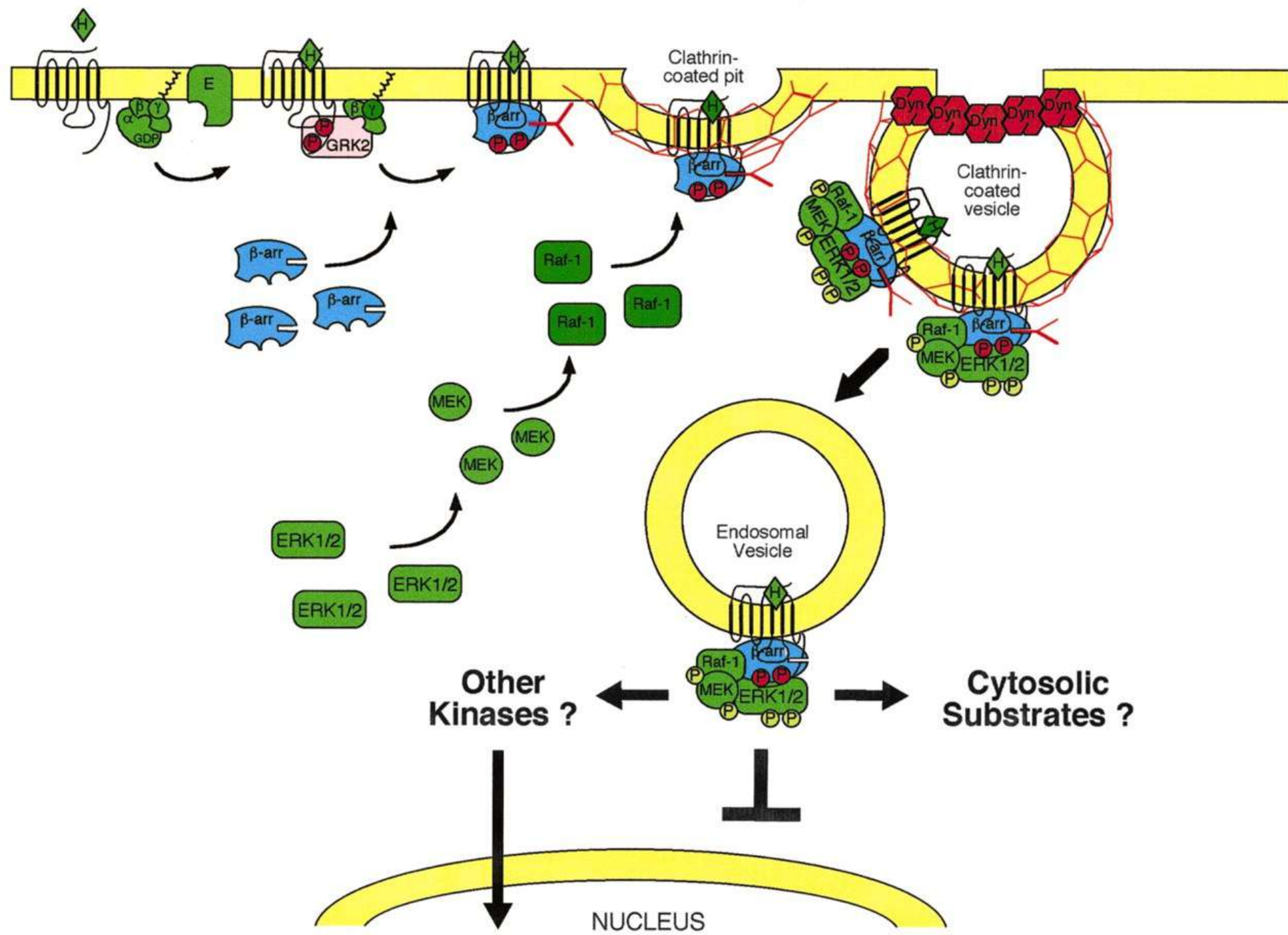


② Sequestration



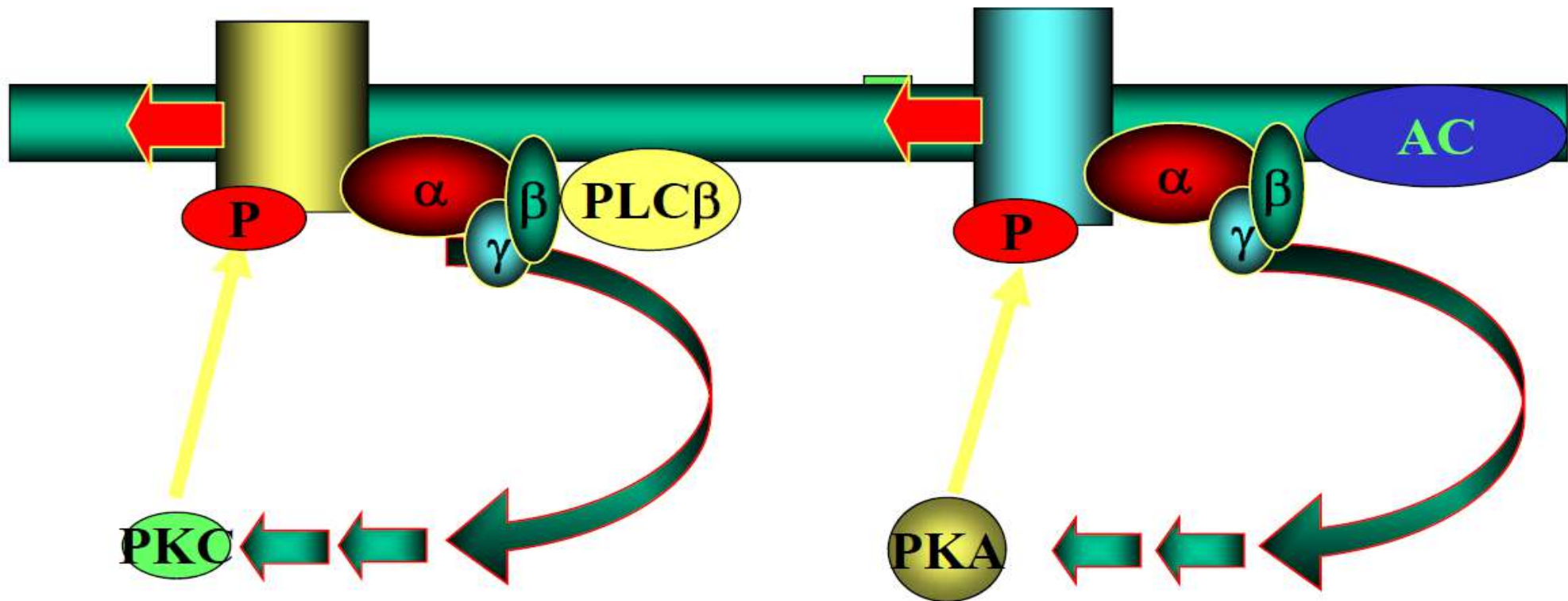
③ Recycling / Downregulation





Desensibilizzazione eterologa

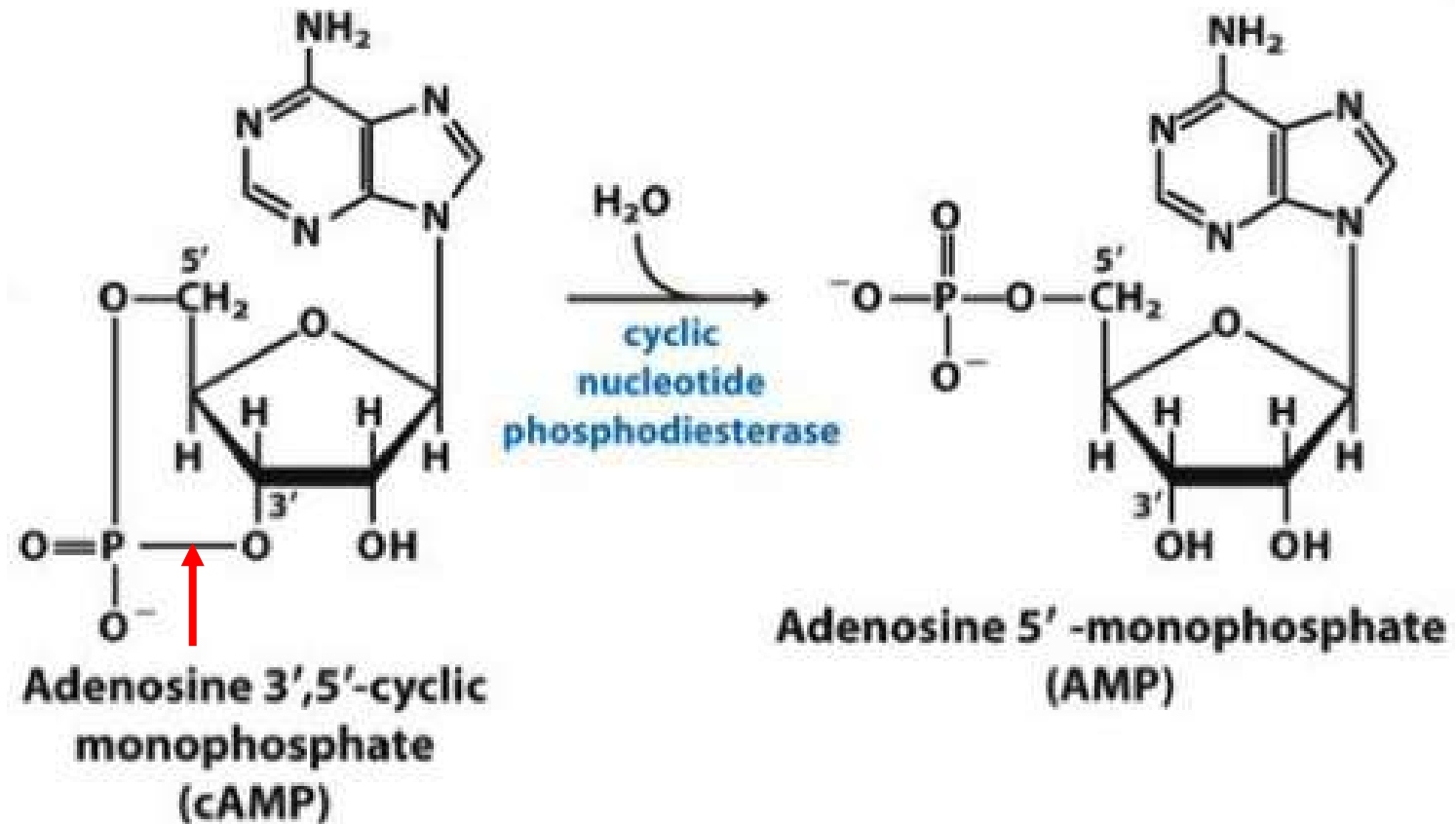
Desensibilizzazione attraverso disaccoppiamento recettore-proteine *G* in risposta alla **fosforilazione del recettore tramite PKA e/o PKC**



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

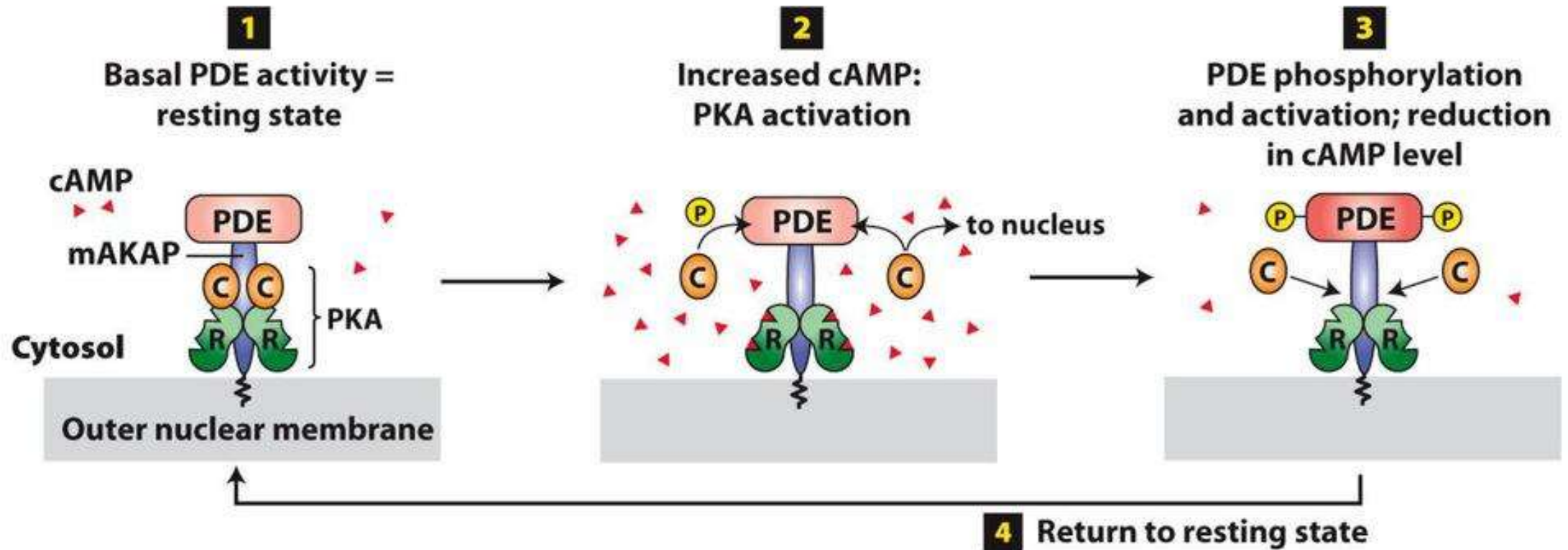


Figure 15-31
Molecular Cell Biology, Eighth Edition
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Manoscritti per presentazione studenti

Illuminating G-Protein-Coupling Selectivity of GPCRs

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<https://doi.org/10.1016/j.cell.2019.04.044>

SUMMARY

Heterotrimeric 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 $G\alpha$ 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 $G\alpha$ subunit C termini. For each receptor, we probed chimeric $G\alpha$ 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 $G\alpha$, $G\beta$, and $G\gamma$ subunits, where distinct $G\alpha$ subunits specify both GPCR interactions and the transduction of particular downstream signaling events (Wettschureck and Offermanns, 2005). The human genome encodes 16 $G\alpha$ 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 $G\alpha$ 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

A Global Map of G Protein Signaling Regulation by RGS Proteins

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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 ($G\alpha$). Mammalian genomes encode 20 canonical RGS and 16 $G\alpha$ genes with key roles in physiology and disease. To understand the principles governing the selectivity of $G\alpha$ regulation by RGS, we examine the catalytic activity of all canonical human RGS proteins and their selectivity for a complete set of $G\alpha$ substrates using real-time kinetic measurements in living cells. The data reveal rules governing RGS- $G\alpha$ 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- $G\alpha$ 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.

Receptor Quaternary Organization Explains G Protein-Coupled Receptor Family Structure

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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

EGFR Ligands Differentially Stabilize Receptor Dimers to Specify Signaling Kinetics

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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- α (TGF α), 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 α 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

Bias Factor and Therapeutic Window Correlate to Predict Safer Opioid Analgesics

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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 β arrestin 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 β arrestin-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 β arrestin2 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

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

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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 coatamer 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 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

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

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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

