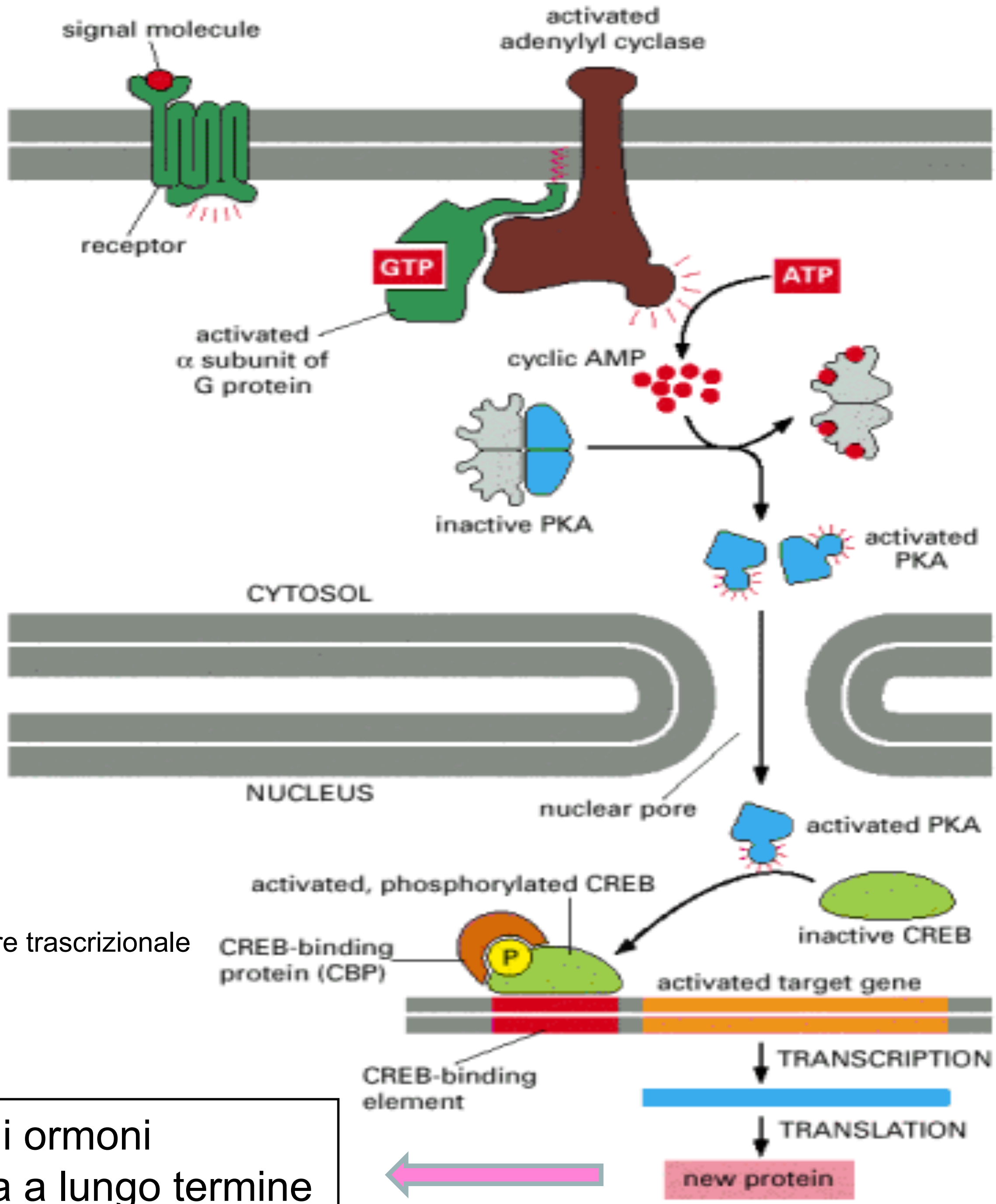
Risposte lente all'cAMP



Coattivatore trascrizionale

Sintesi di ormoni
Proteine memoria a lungo termine

G-proteins effectors:
Fosfolipasi C

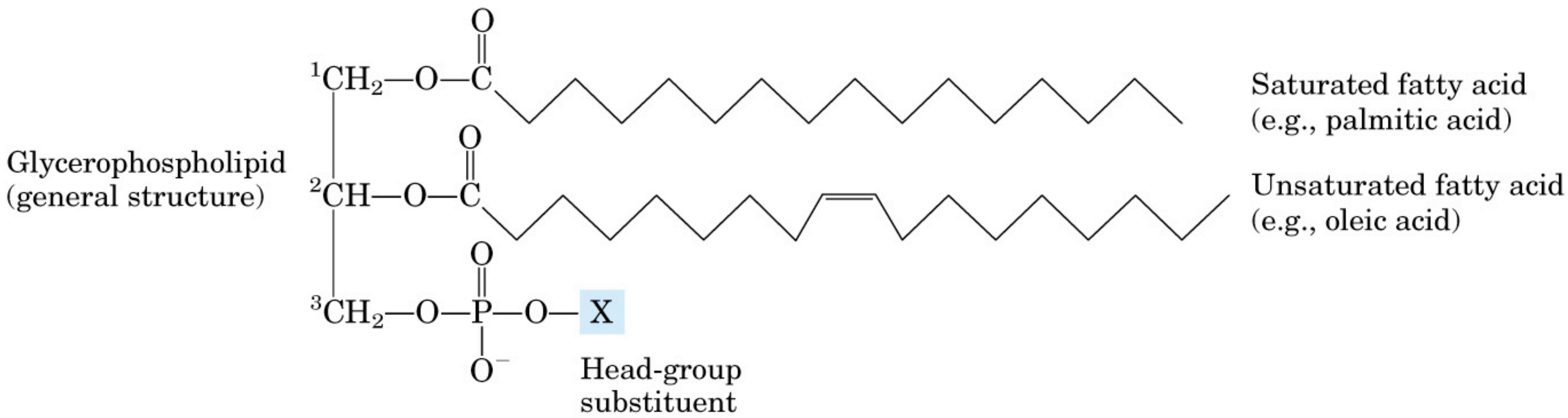
TABLE 4. *G protein effectors*

Class	Isotypes	G Protein Selectivity	Reference No.
Second messenger-generating and metabolizing enzymes			
Adenylyl cyclase	I	$\alpha_s + \beta\gamma - \alpha_o - \alpha_z -$	19, 95, 133, 159, 258, 313, 545–549, 551, 552, 554, 596–598, 604
	II	$\alpha_s + \beta\gamma +$	
	III	$\alpha_s +$	
	IV	$\alpha_s + \beta\gamma +$	
	V	$\alpha_s + \alpha_z -$	
	VI	$\alpha_s +$	
	VII	$\alpha_s +$	
	VIII	$\alpha_s +$	
	IX	$\alpha_s +$	
cGMP phosphodiesterase		$\alpha_t -$	214
Phospholipase C	β_1	$\alpha_q, \alpha_{11}, \alpha_{15}, \alpha_{16} + \beta\gamma +$	78, 229, 311, 328, 329, 393, 427, 429, 430, 512, 513, 555, 618
	β_2	$\alpha_q, \alpha_{11}, \alpha_{15}, \alpha_{16} + \beta\gamma +$	
	β_3	$\alpha_q, \alpha_{11}, \alpha_{15}, \alpha_{16} + \beta\gamma +$	
	β_4	$\alpha_q, \alpha_{11}, \alpha_{15}, \alpha_{16} + \beta\gamma +$	
Phosphoinositide 3-kinase	p120γ/p101	$\beta\gamma + \alpha_{o/i} +$	526
Phospholipase A_2		$\beta\gamma +$	262
Ion-selective channels			
K^+ channels			
	IK.ACH	$\alpha_i + \beta\gamma +$	106, 629
	IK.ATP	$\alpha_i +$	106
Ca^{2+} channels			
	Neuronal N type	$\alpha_{i1} - \alpha_{i2} - \beta_1 - \beta_3 -$	63, 104, 248, 497, 625
	Cardiac L type	$\alpha_s +$	
Na^+ channels			
	Cardiac	$\alpha_s +$	492
	Epithelial cell	$\alpha_{i3} -$	341
Cl^- channels			
	Cardiac	$\alpha_s +$	156
	Epithelial cell	$\alpha_{i/o} -$	156

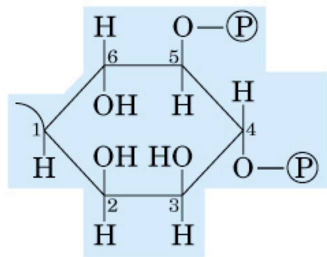
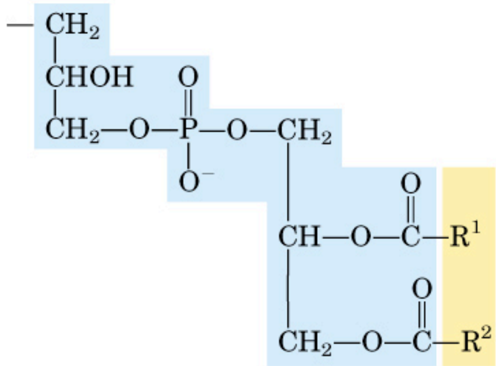
→ Cellule pace-maker del cuore

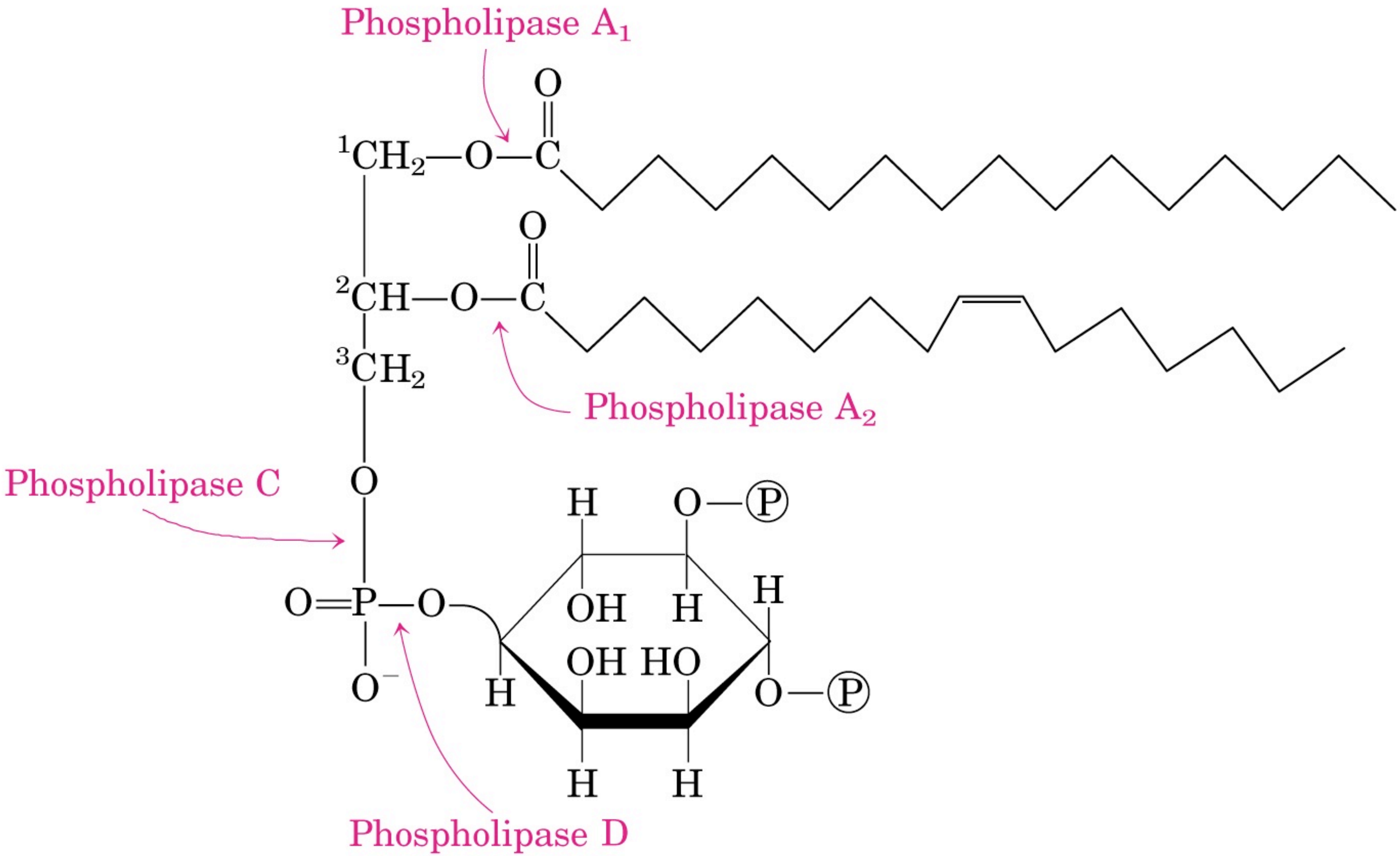
	SOME FAMILY MEMBERS	ACTION MEDIATED BY	FUNCTIONS
I	G_s	α	activates adenylyl cyclase; activates Ca^{2+} channels
	G_{olf}	α	activates adenylyl cyclase in olfactory sensory neurons
II	G_i	α	inhibits adenylyl cyclase
		$\beta\gamma$	activates K^+ channels
	G_o	$\beta\gamma$	activates K^+ channels; inactivates Ca^{2+} channels
		α and $\beta\gamma$	activates phospholipase C-β
	G_t (transducin)	α	activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
III	G_q	α	activates phospholipase C-β

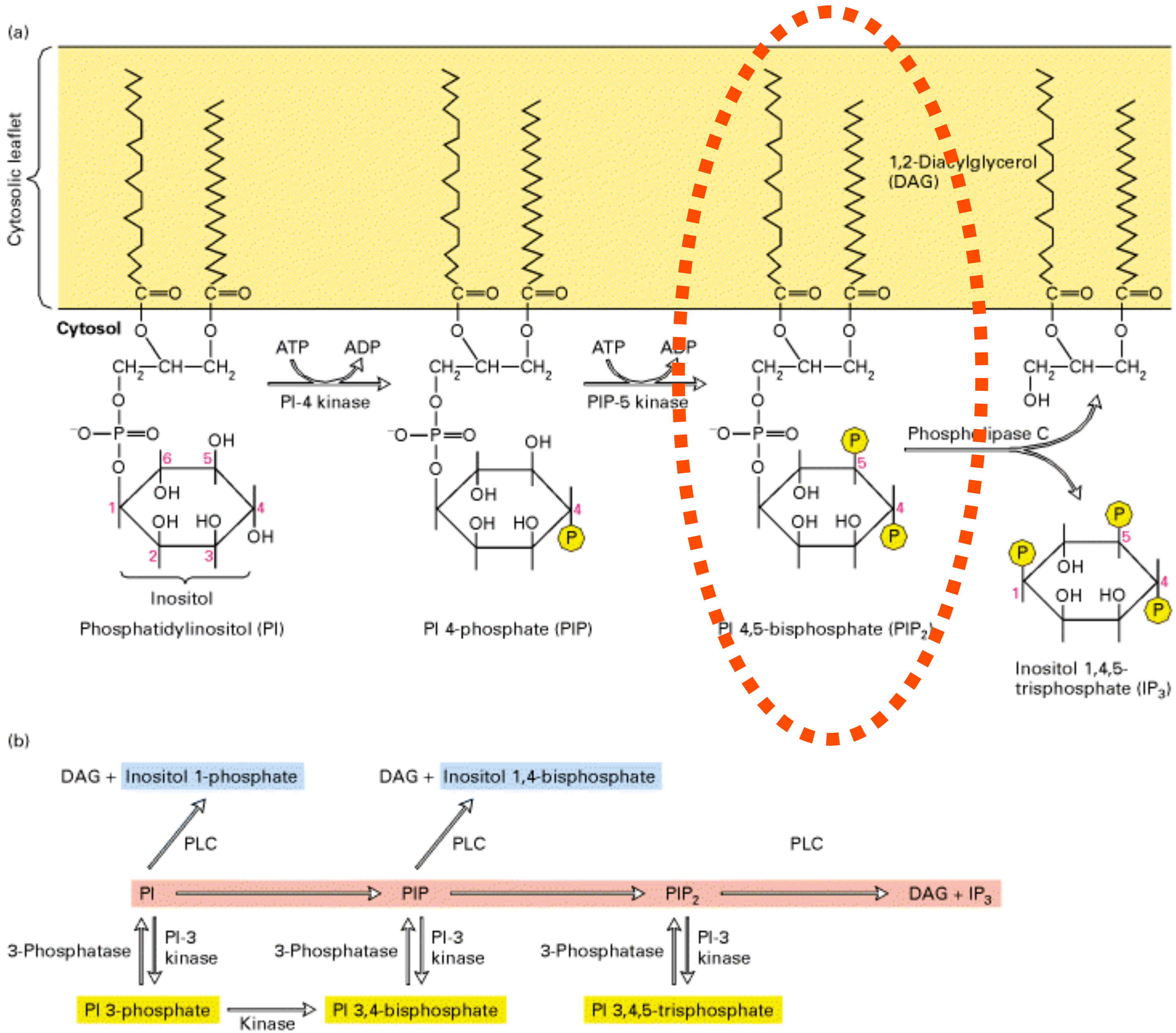
* Families are determined by amino acid sequence relatedness of the α subunits. Only selected examples are shown. About 20 α subunits and at least 4 β subunits and 7 γ subunits have been described in mammals.



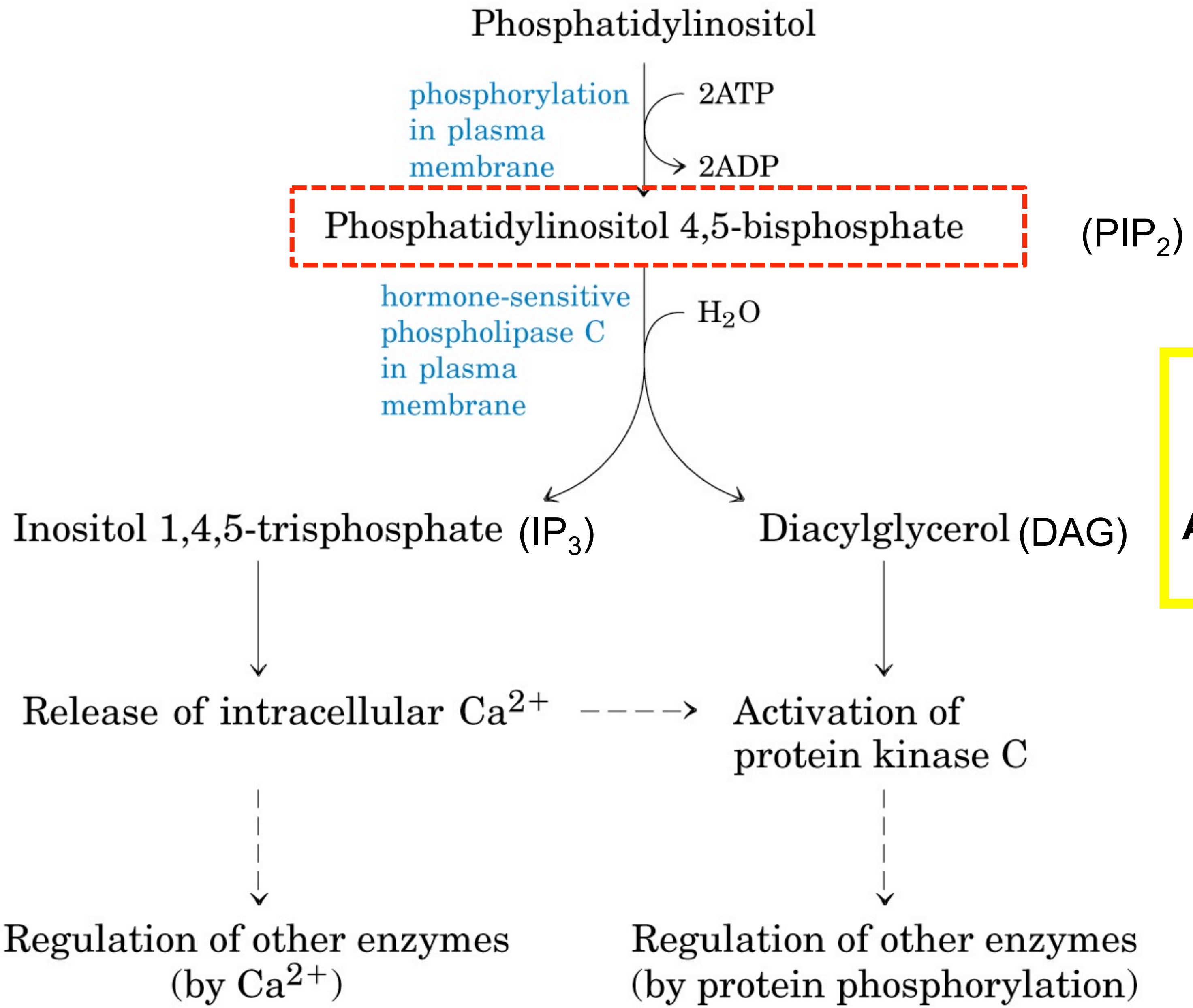
Glycerophospholipid
(general structure)
Saturated fatty acid
(e.g., palmitic acid)
Unsaturated fatty acid
(e.g., oleic acid)
X
Head-group
substituent

Name of glycerophospholipid	Name of X	Formula of X	Net charge (at pH 7)
Phosphatidic acid	—	$-H$	−1
Phosphatidylethanolamine	Ethanolamine	$-CH_2-CH_2-\overset{+}{N}H_3$	0
Phosphatidylcholine	Choline	$-CH_2-CH_2-\overset{+}{N}(CH_3)_3$	0
Phosphatidylserine	Serine	$-CH_2-\underset{\mid \atop COO^-}{CH}-\overset{+}{N}H_3$	−1
Phosphatidylglycerol	Glycerol	$-CH_2-\underset{\mid \atop OH}{CH}-CH_2-OH$	−1
Phosphatidylinositol 4,5-bisphosphate	*myo*-Inositol 4,5-bisphosphate		−4
Cardiolipin	Phosphatidyl-glycerol		−2

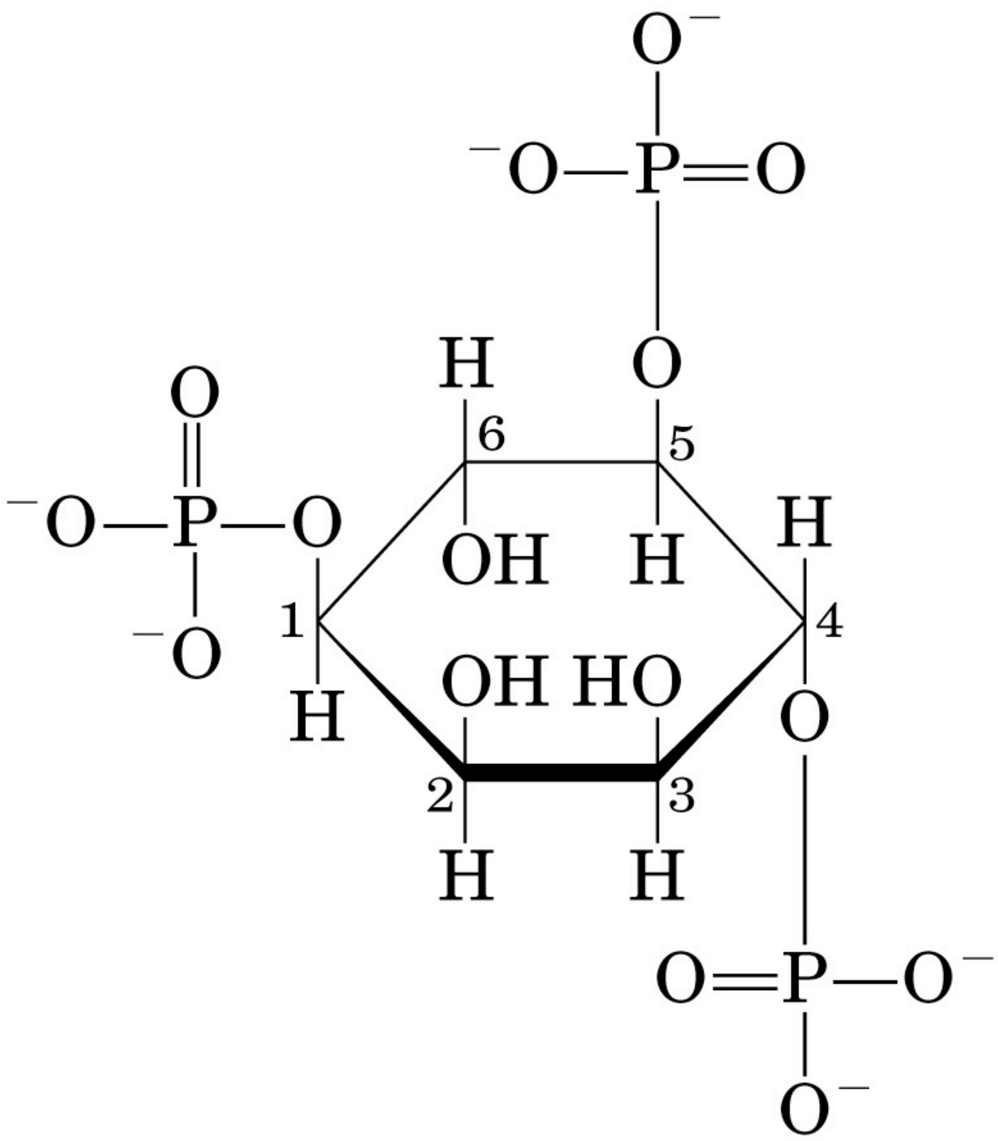

Phospholipase A1
Phospholipase A2
Phospholipase C
Phospholipase D



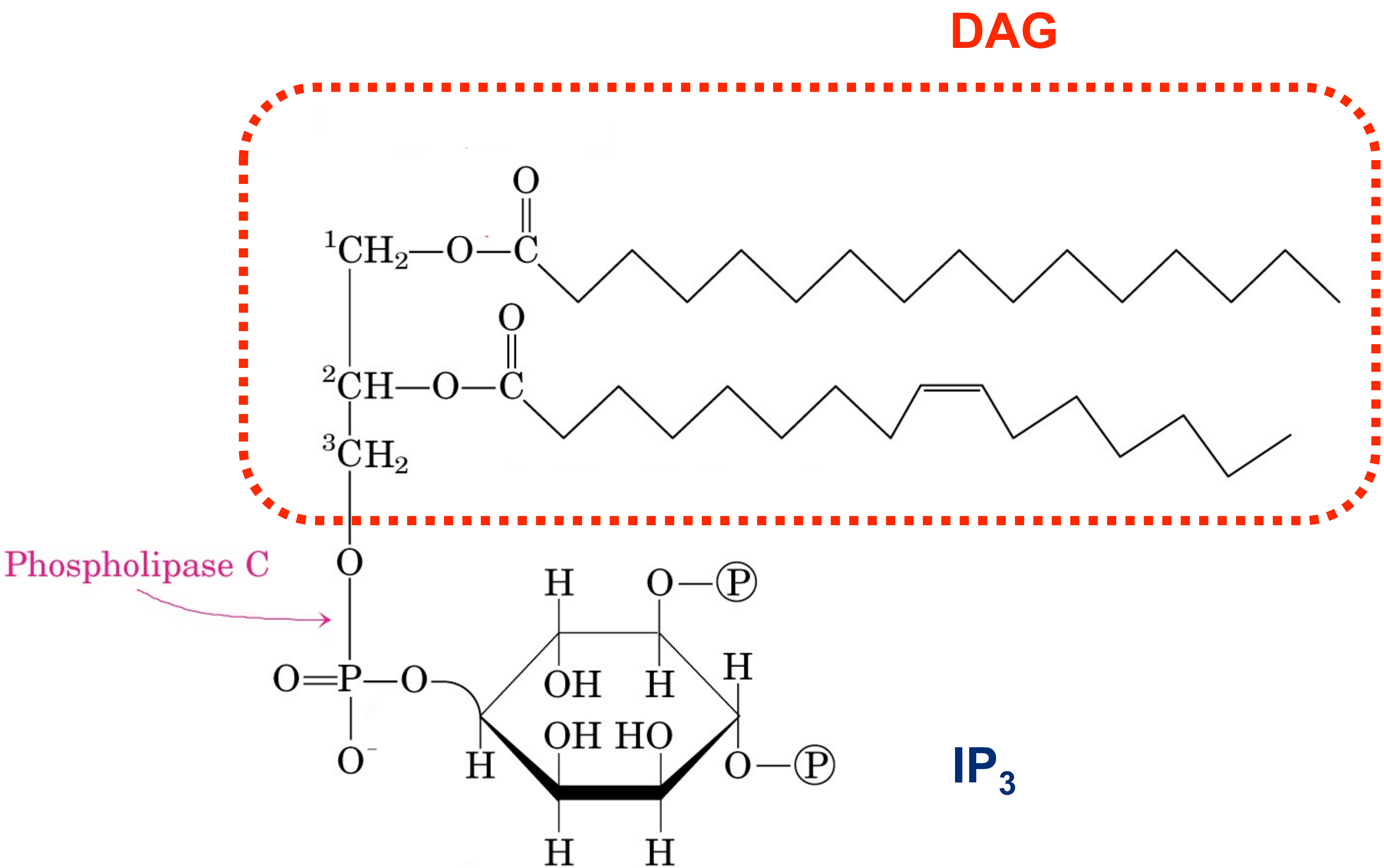
(a)
Cytosolic leaflet
Cytosol
ATP
ADP
PI-4 kinase
PIP-5 kinase
1,2-Diacylglycerol (DAG)
Phospholipase C
Inositol
Phosphatidylinositol (PI)
PI 4-phosphate (PIP)
PI 4,5-bisphosphate (PIP2)
Inositol 1,4,5-trisphosphate (IP3)
(b)
DAG + Inositol 1-phosphate
DAG + Inositol 1,4-bisphosphate
PLC
PI
PIP
PIP2
DAG + IP3
3-Phosphatase
PI-3 kinase
PI 3-phosphate
Kinase
PI 3,4-bisphosphate
PI 3,4,5-trisphosphate



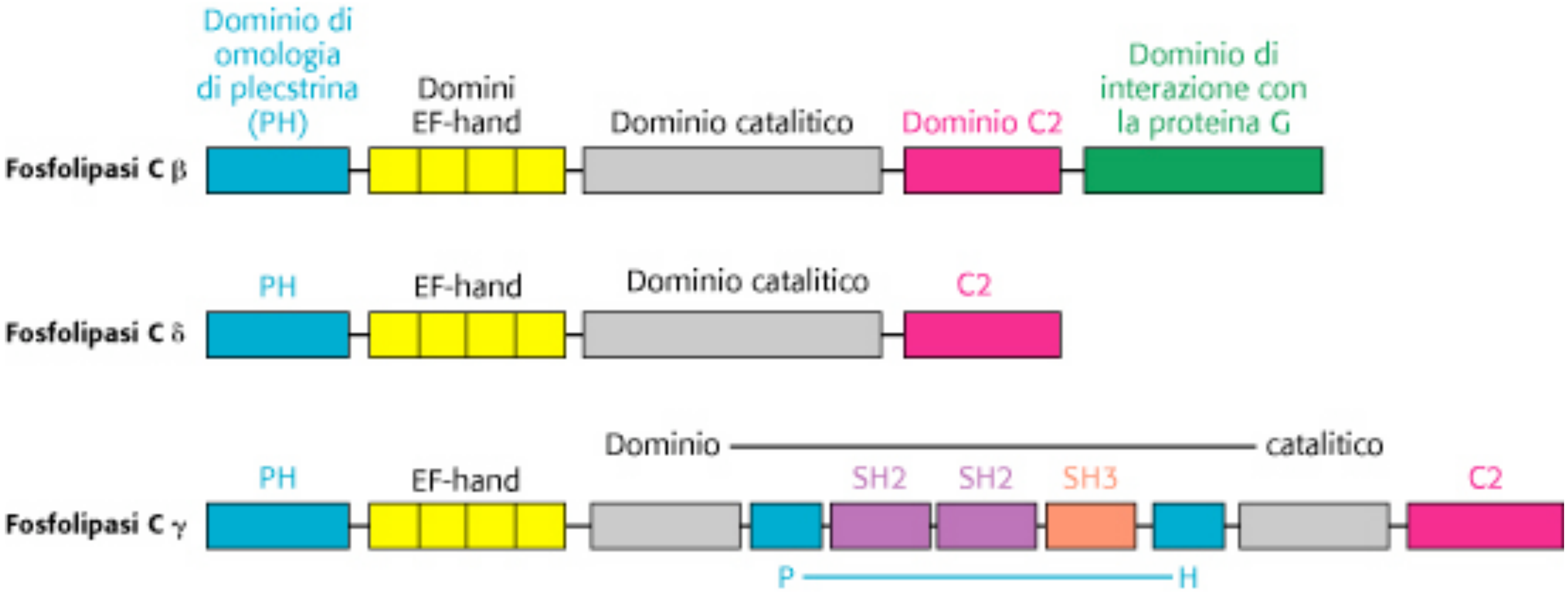
Metabolismo
Secrezione
Contrazione
Attività neuronale
Proliferazione



Inositol 1,4,5-trisphosphate


DAG
$^1CH_2—O—C$
O
$^2CH—O—C$
O
$^3CH_2$
Phospholipase C
O
O=P—O
O⁻
H
O—P
OH
H
H
OH HO
H
O—P
H
H
IP_3

PLC



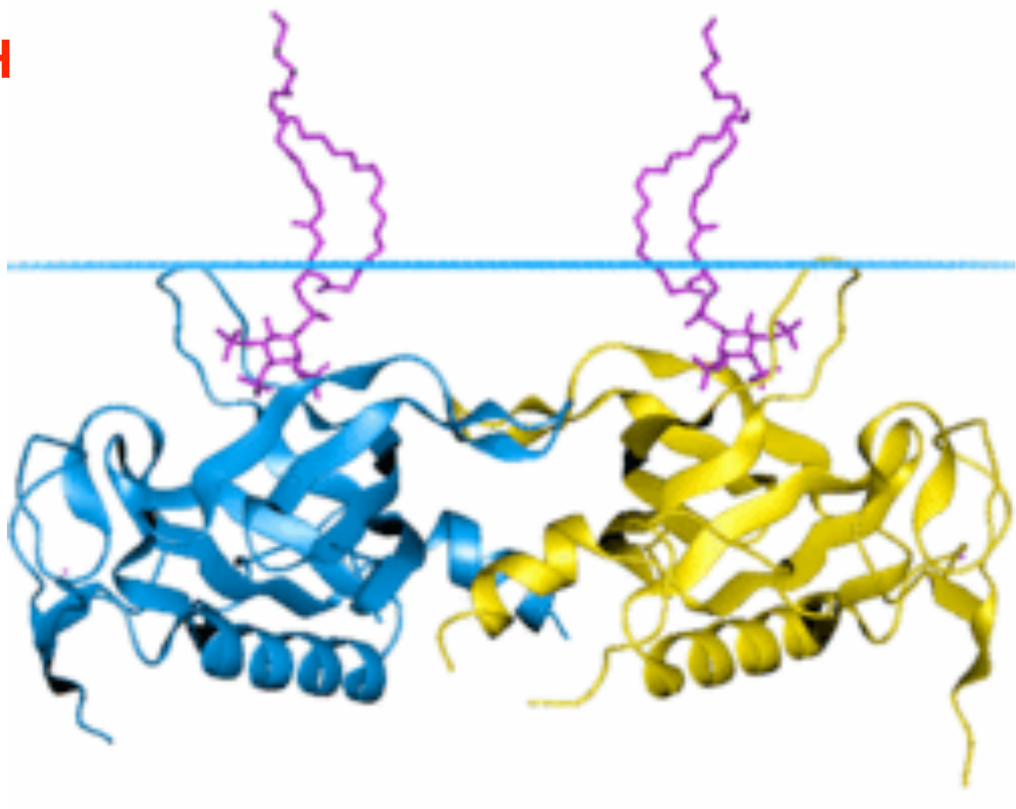
✓Peso molecolare

✓Sequenza aa

Attività calcio-dipendente

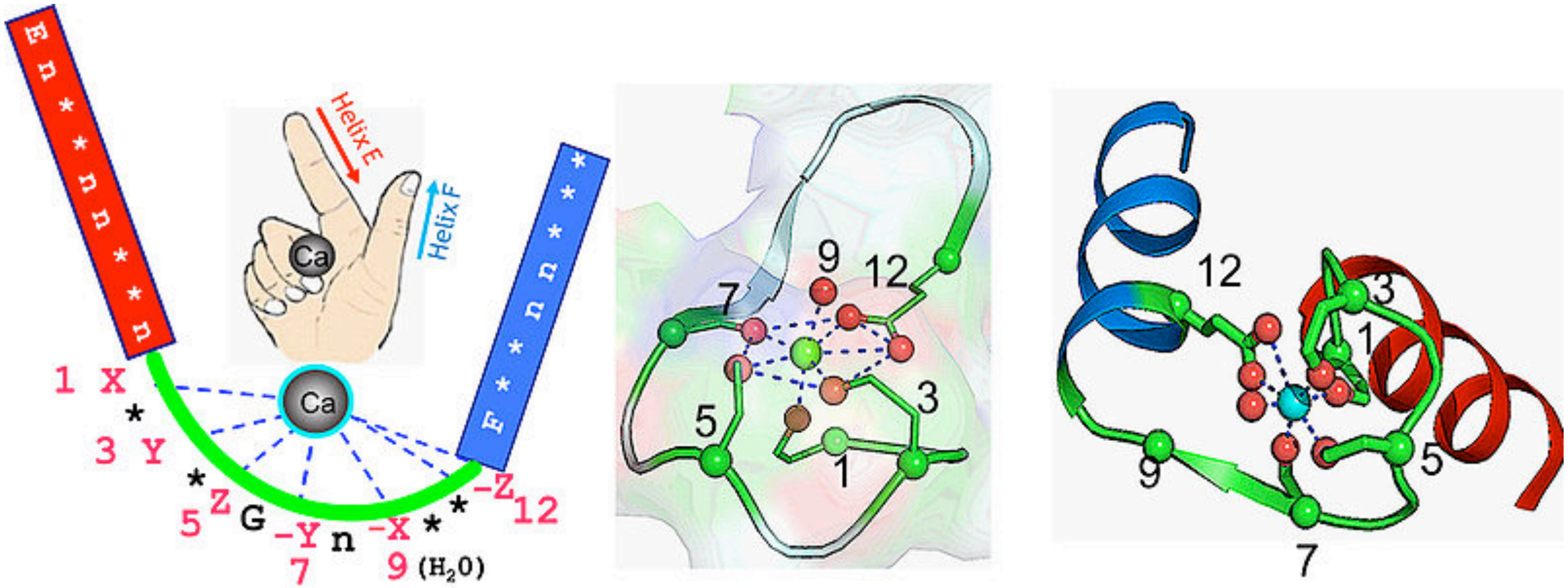
Localizzazione citoplasmatica

DOMINIO PH



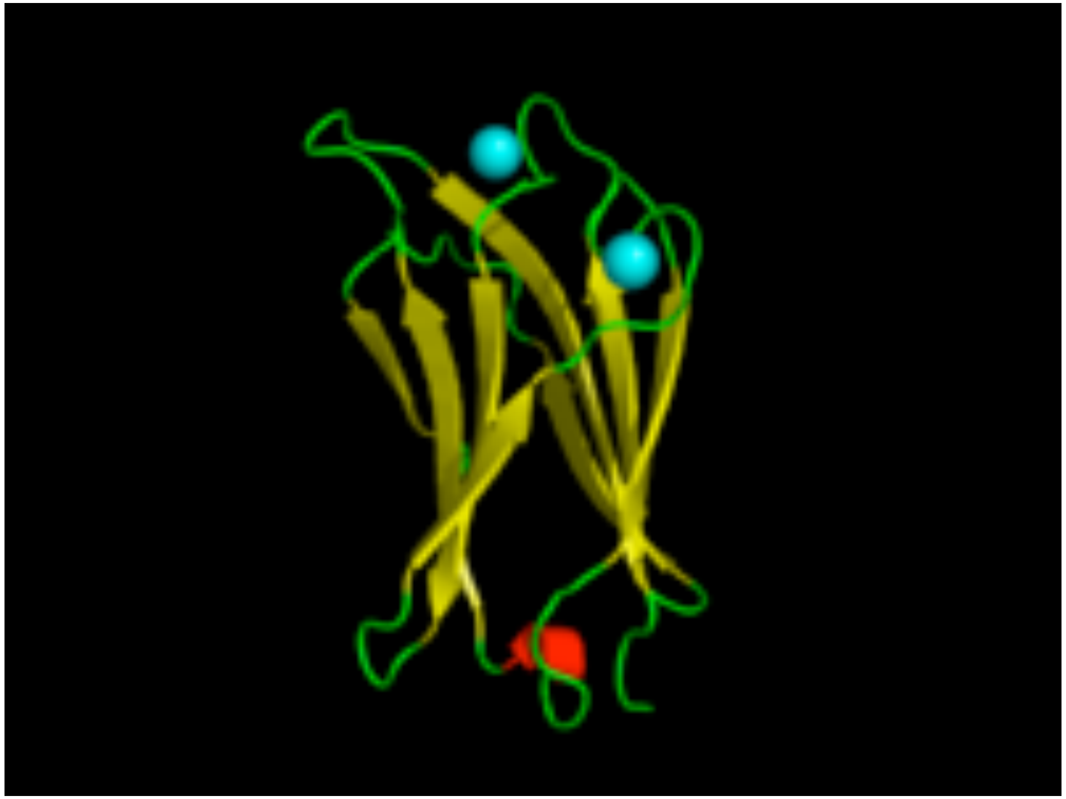
E’ costituito da circa 120 aa. Esistono diversi PH domains, caratterizzati da diversa affinita’ nei confronti di fosfatidilinositoli a diverso grado di fosforilazione. Questo Tipo di dominio puo’ anche legare le subunita’ βγ

DOMINIO EF-HAND



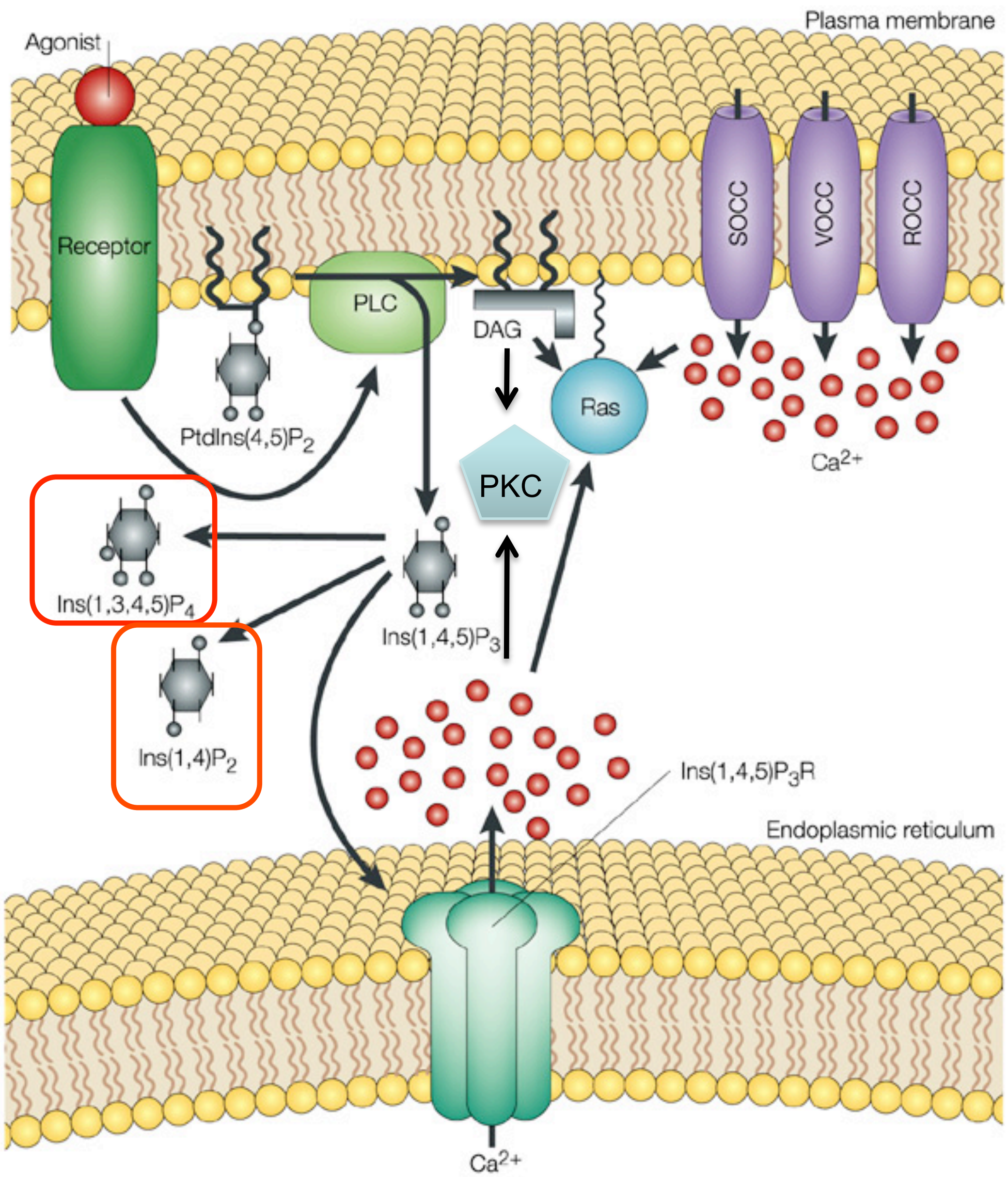
The Ca^{2+} binding pocket is localized between E and F α-helix segments and adopts a pentagonal bipyramidal geometry

DOMINIO C2



Dominio con struttura a β-sandwich costituito da circa 116 amminoacidi.
E' coinvolto nel targeting delle proteine alla membrana, potendo interagire con diversi PL, in particolare con fosfatidilserina e con fosfatidilcolina.
Puo' legare 2/3 ioni calcio.

INOSITOLO 1,4,5-TRIFOSFATO (IP_3) CALCIO



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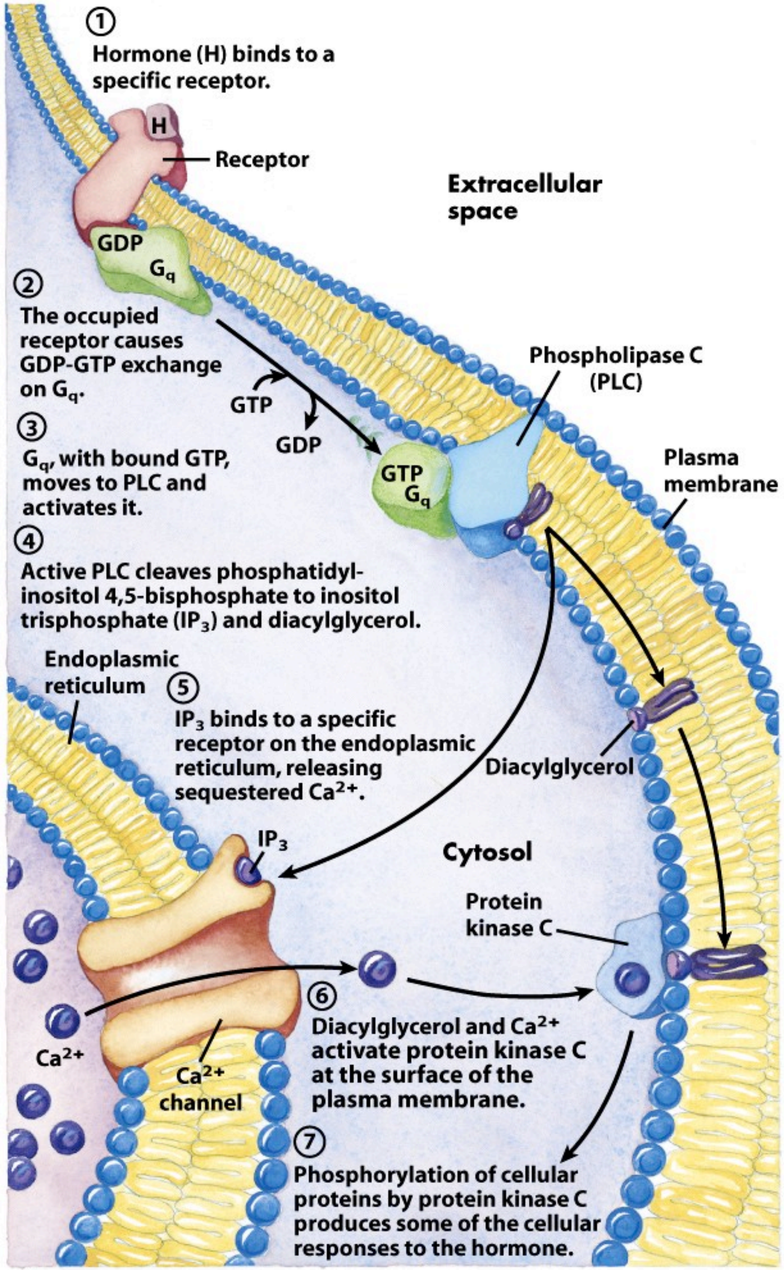


Figure 12-10

Lehninger Principles of Biochemistry, Fifth Edition

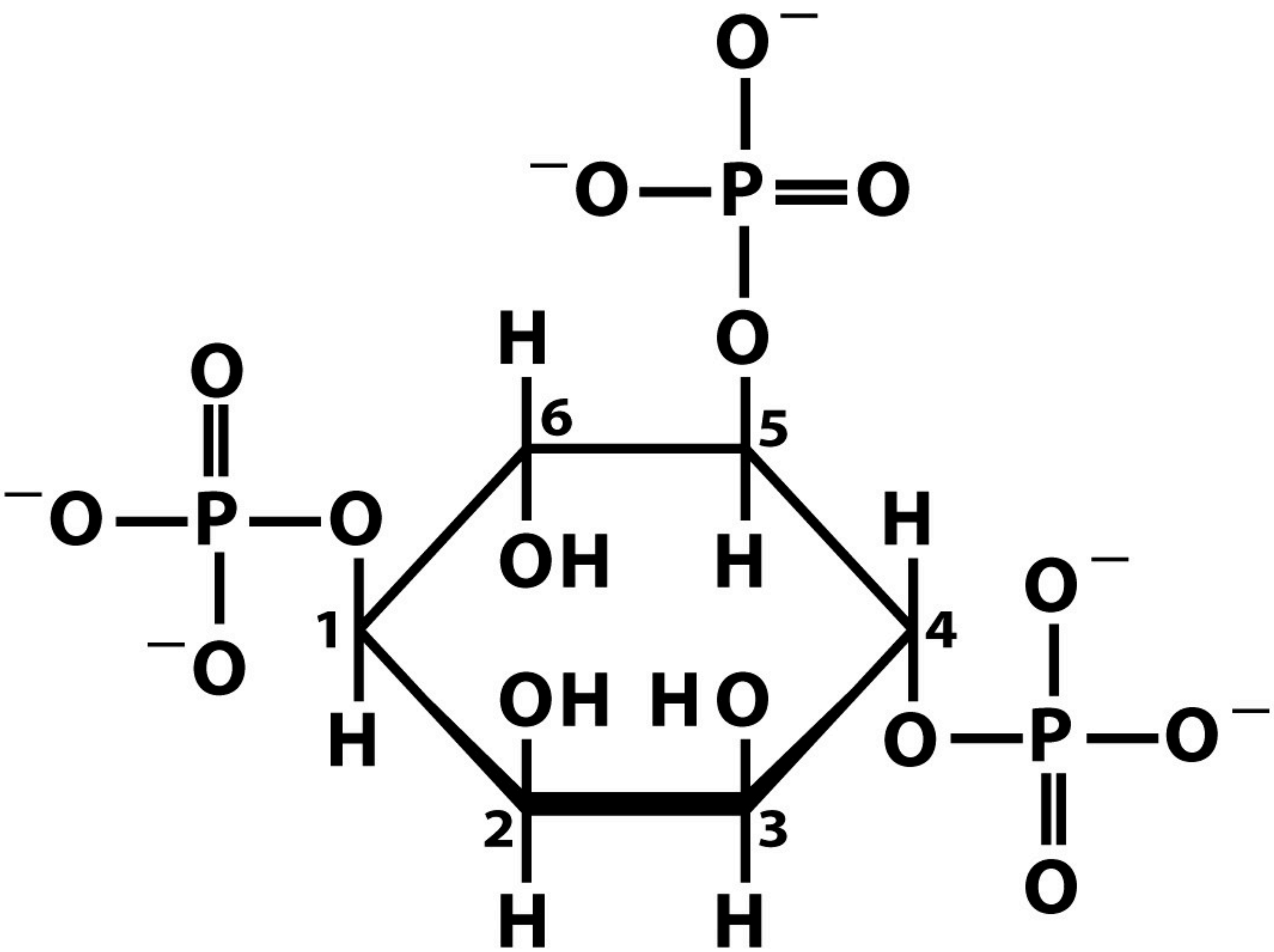
table 13–5

Some Signals That Act through Phospholipase C and IP_3

Acetylcholine [muscarinic M_1]
α_1-Adrenergic agonists
Angiogenin
Angiotensin II
ATP [P_{2x} and P_{2y}]*
Auxin
Gastrin-releasing peptide
Glutamate
Gonadotropin-releasing hormone (GRH)
Histamine [H_1]*
Light (*Drosophila*)
Oxytocin
Platelet-derived growth factor (PDGF)
Serotonin [5-HT-1c]*
Thyrotropin-releasing hormone (TRH)
Vasopressin

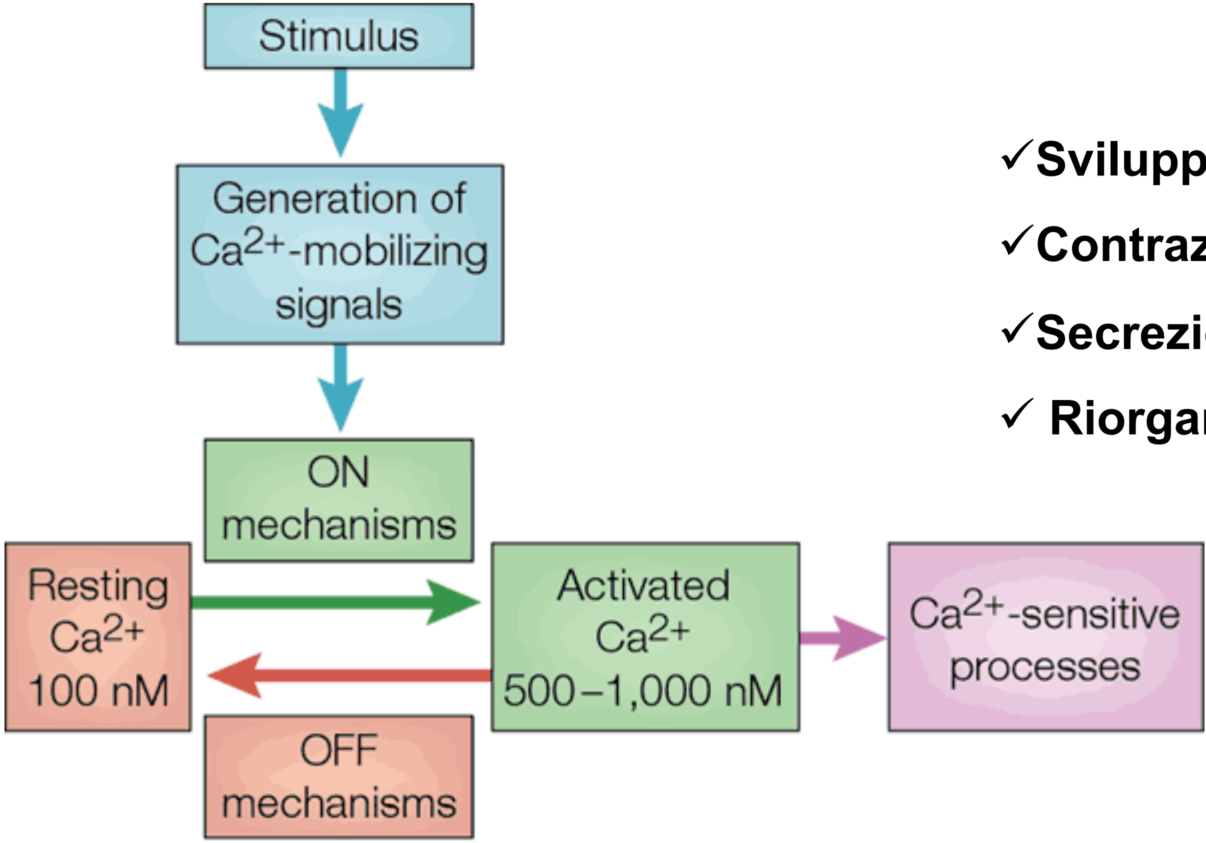
*Receptor subtypes are in square brackets; see footnote to Table 13–4.

Calcium signaling



Inositol 1,4,5-trisphosphate (IP$_3$)

Unnumbered 12 p432
Lehninger Principles of Biochemistry, Fifth Edition



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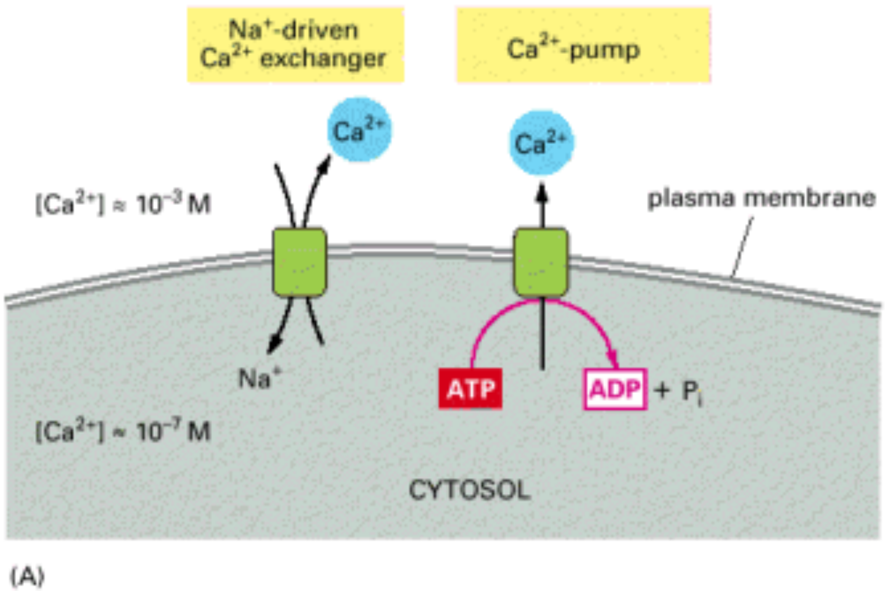
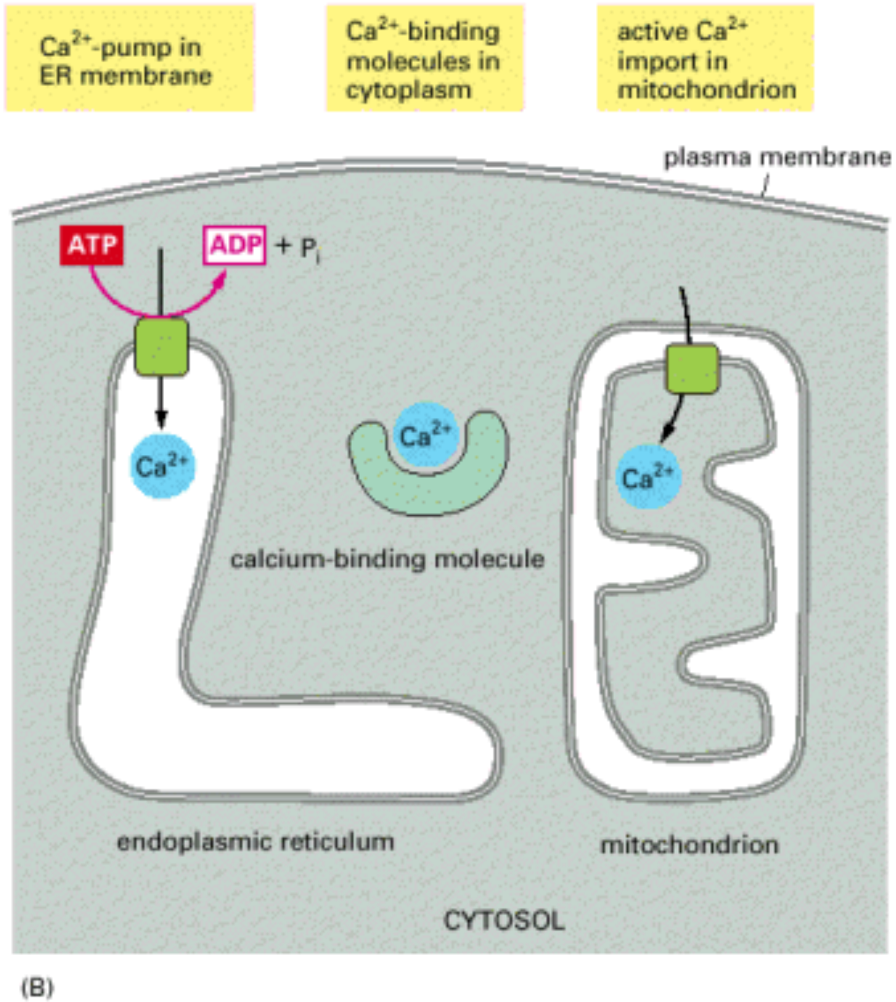
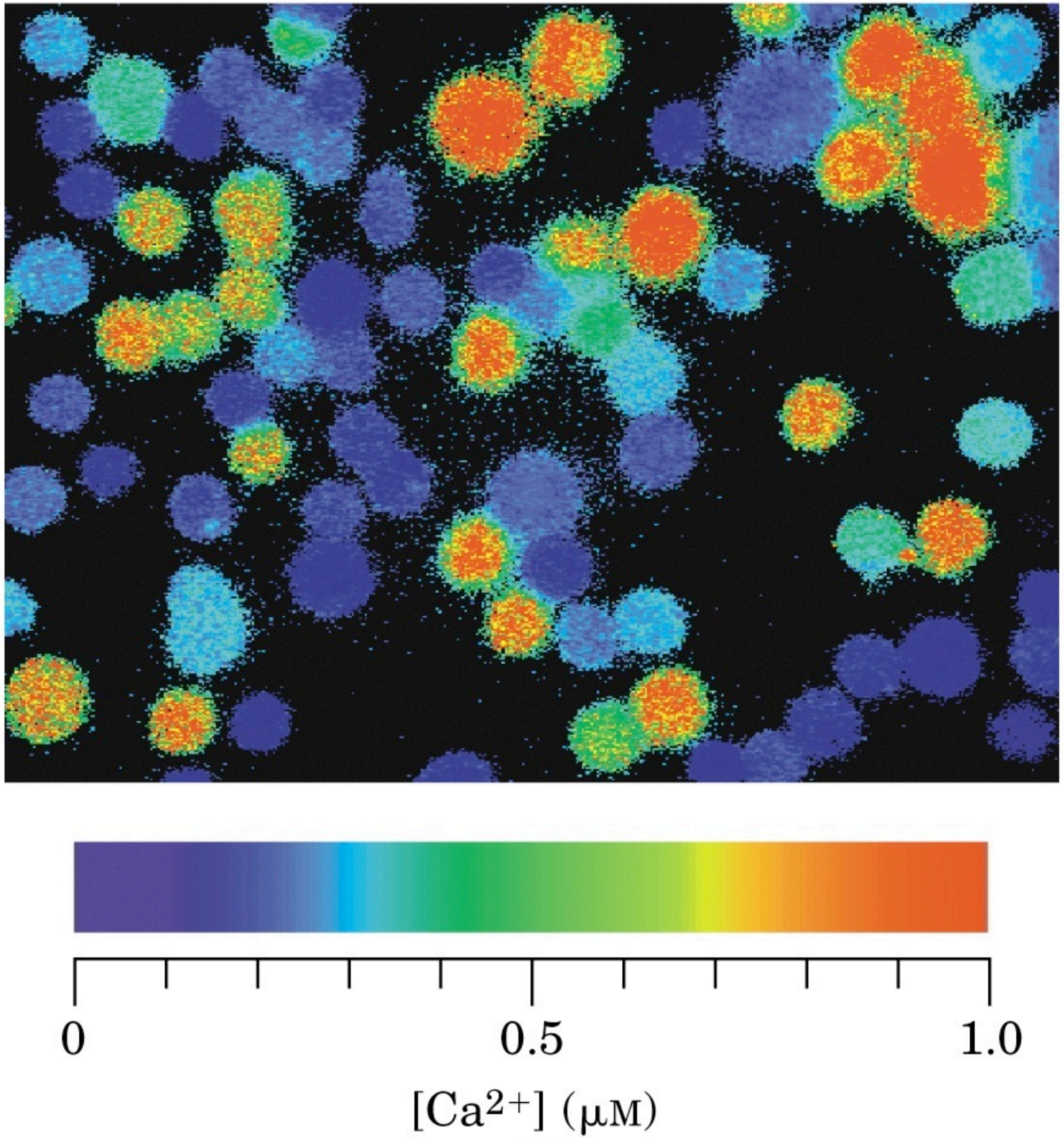
- ✓**Sviluppo embrionale**
- ✓**Contrazione muscolare**
- ✓**Secrezione ormonale e nervosa**
- ✓ **Riorganizzazione del citoscheletro**

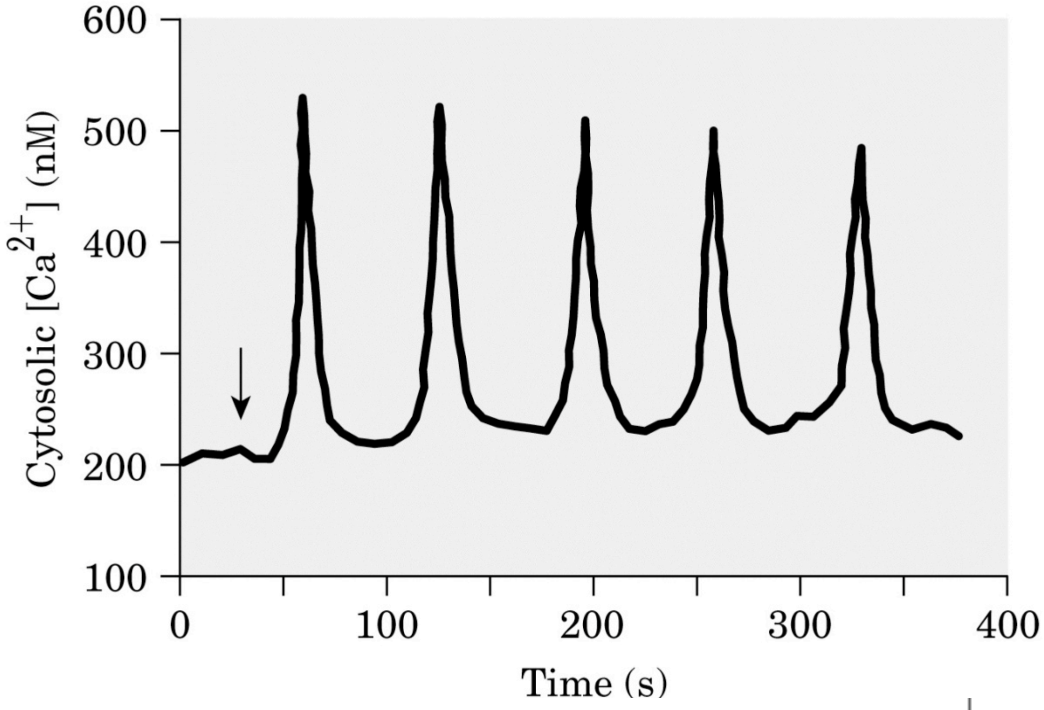
Vie principali tramite le quali cellule eucariotiche mantengono bassa la $[Ca^{2+}]$ libero nel citosol



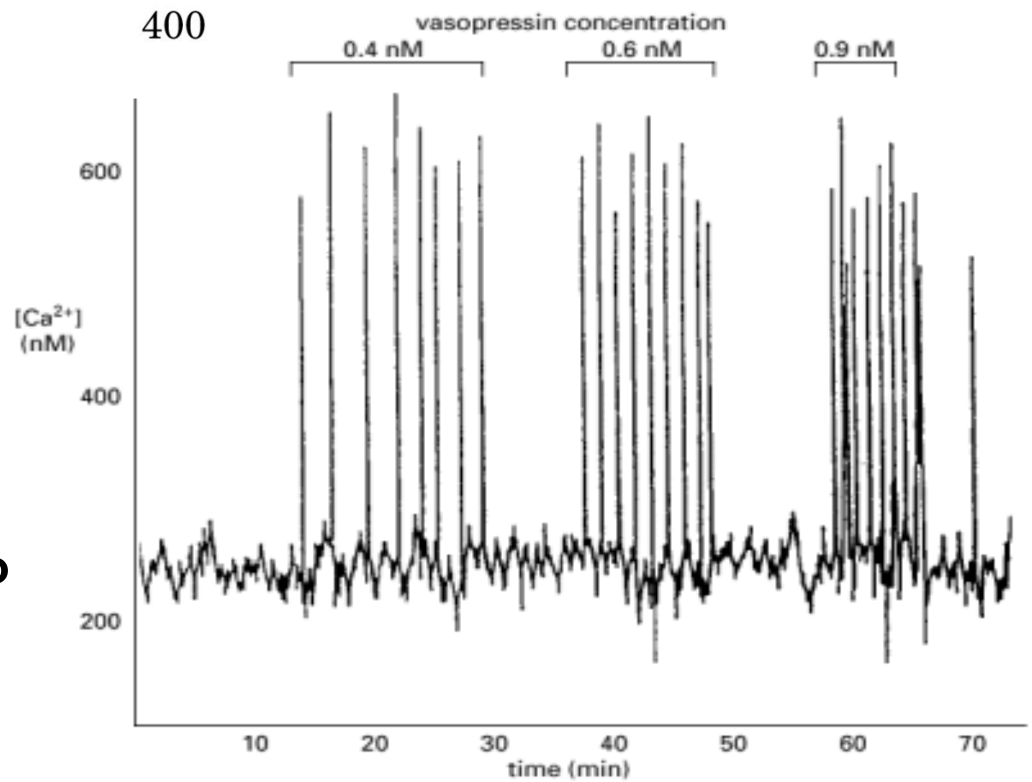




(a)



La frequenza delle oscillazioni riflette la forza dello stimolo



Controllo a feedback dovuto a:

- **calcio**
- **IP3**
- **canali ionici che rilasciano calcio**

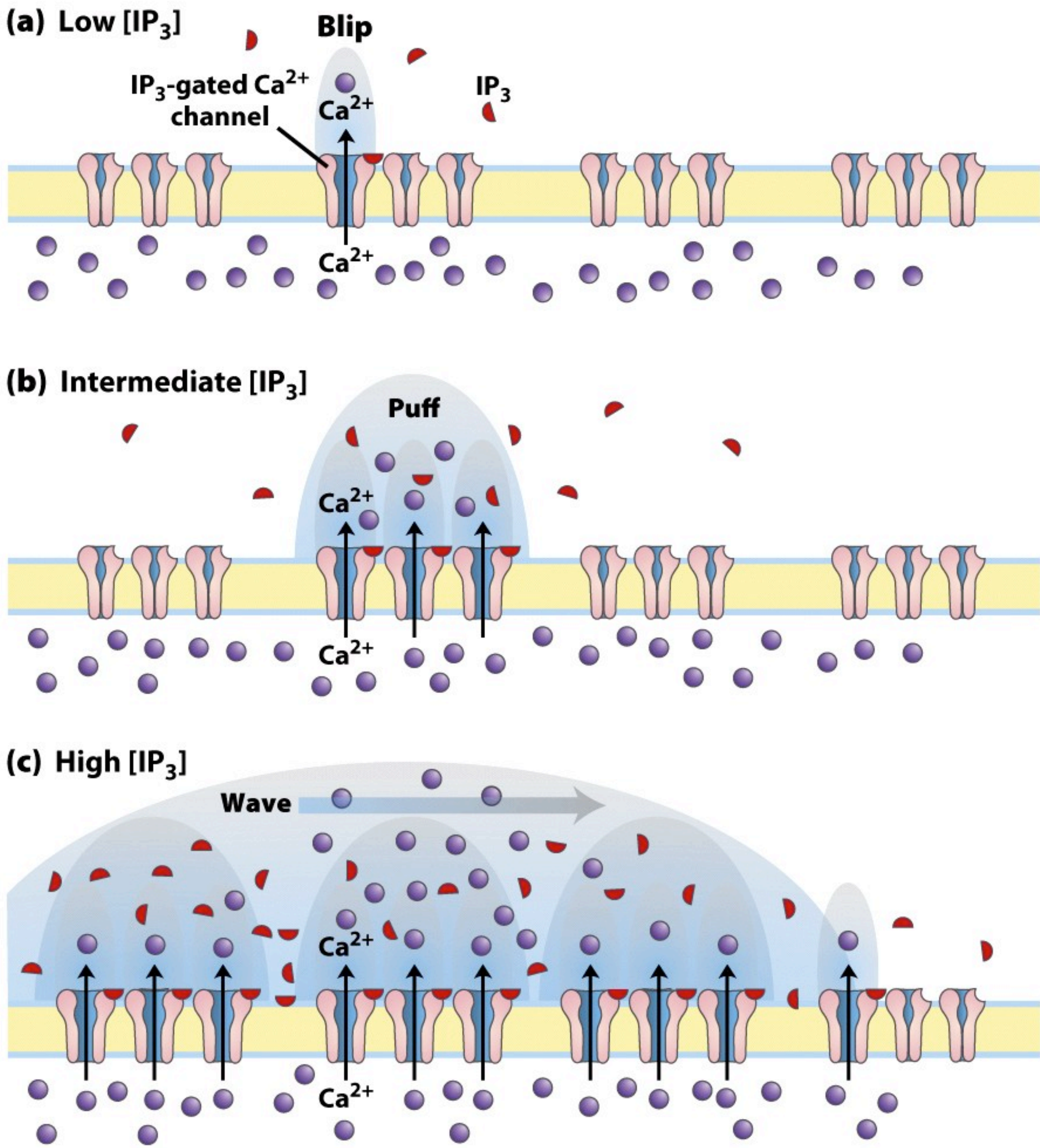
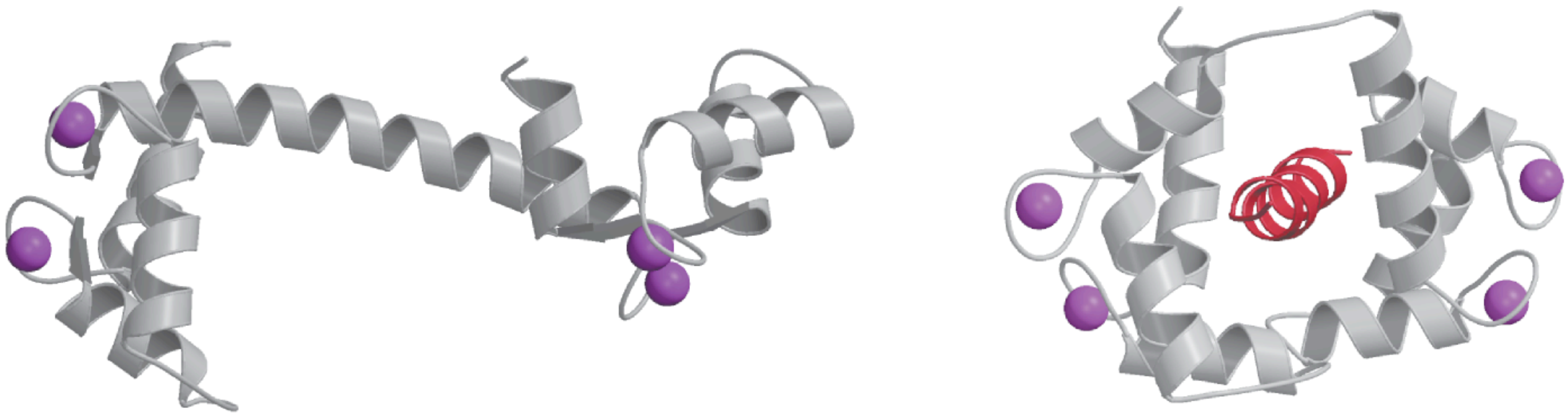


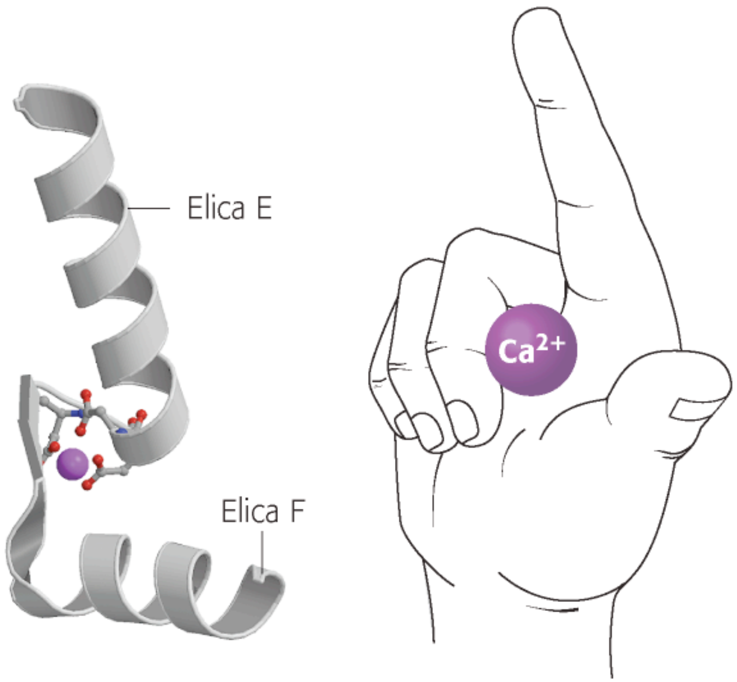
Figure 12-13
Lehninger Principles of Biochemistry, Fifth Edition



(a) (b)

Struttura della CALMODULINA, sensore del calcio.

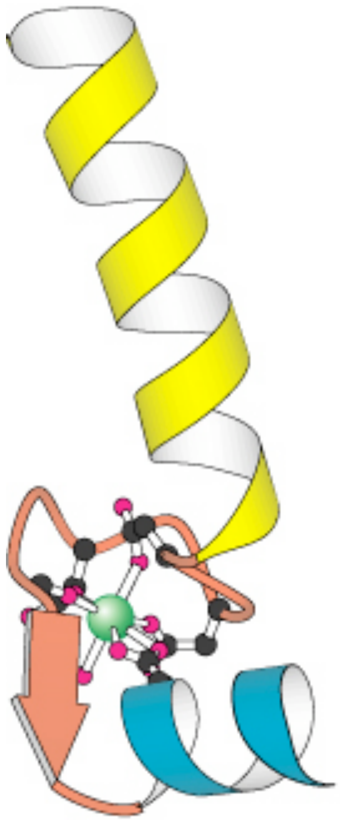
Contiene 4 unità simili in una stessa catena polipeptidica EF hand

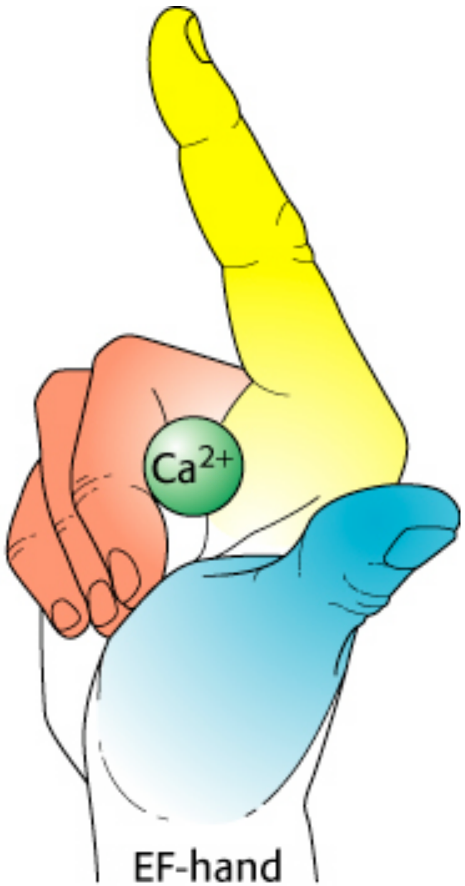


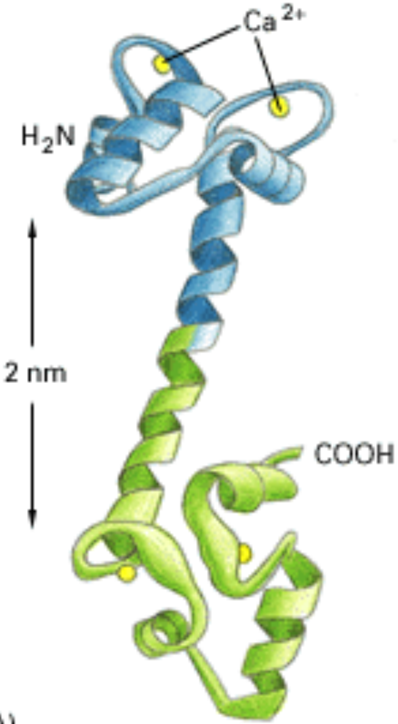
EF hand (mano EF)

(c)

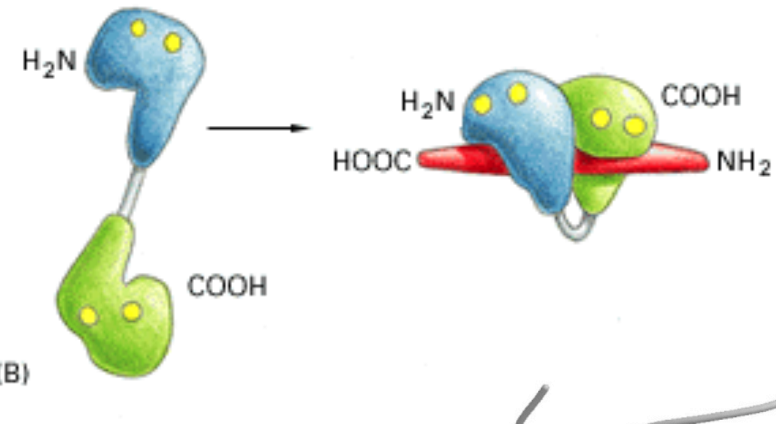
IL Ca^{++} AGISCE COME REGOLATORE ALLOSTERICO, LEGANDOSI A DOMINI SPECIFICI (EF-hand), E PROMUOVENDONE UN CAMBIO CONFORMAZIONALE



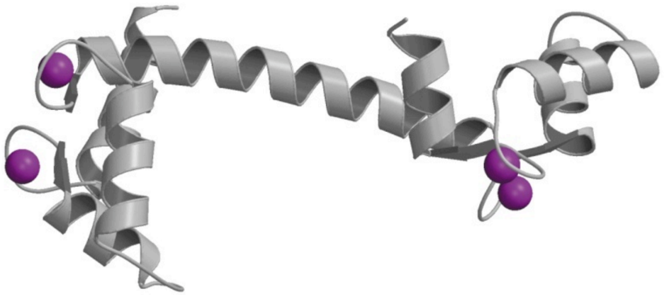




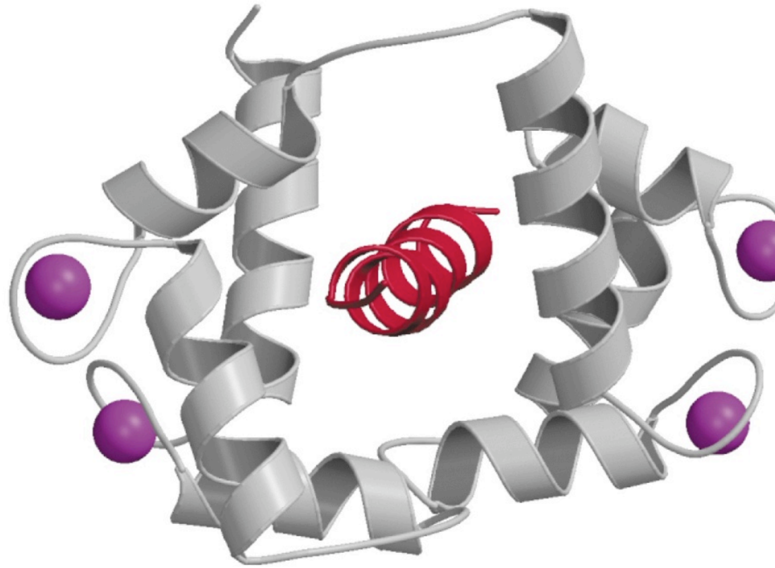
(A)



(B)

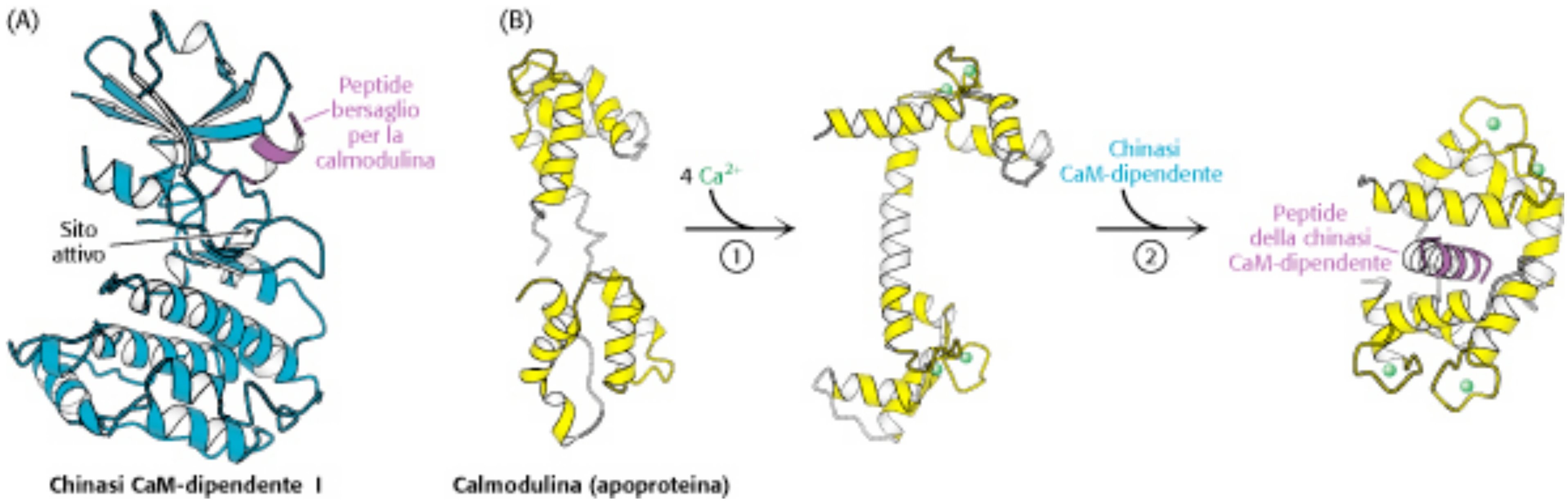


(a)



(b)

La calmodulina si lega a sequenze ad α-elica di proteine bersaglio



CaM-Ca^{++} è la subunità regolatoria di una famiglia di serina chinasi (CaMK)

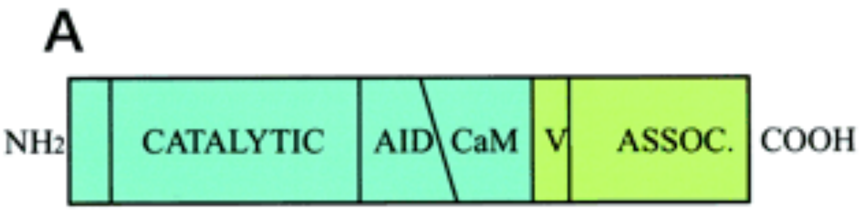
MOLTE PROTEINE CONTENGONO DOMINI E-F HAND CALMODULIN-LIKE E SONO QUINDI REGOLATE DA Ca++

table 13–6

Some Proteins Regulated by Ca^{2+} and Calmodulin

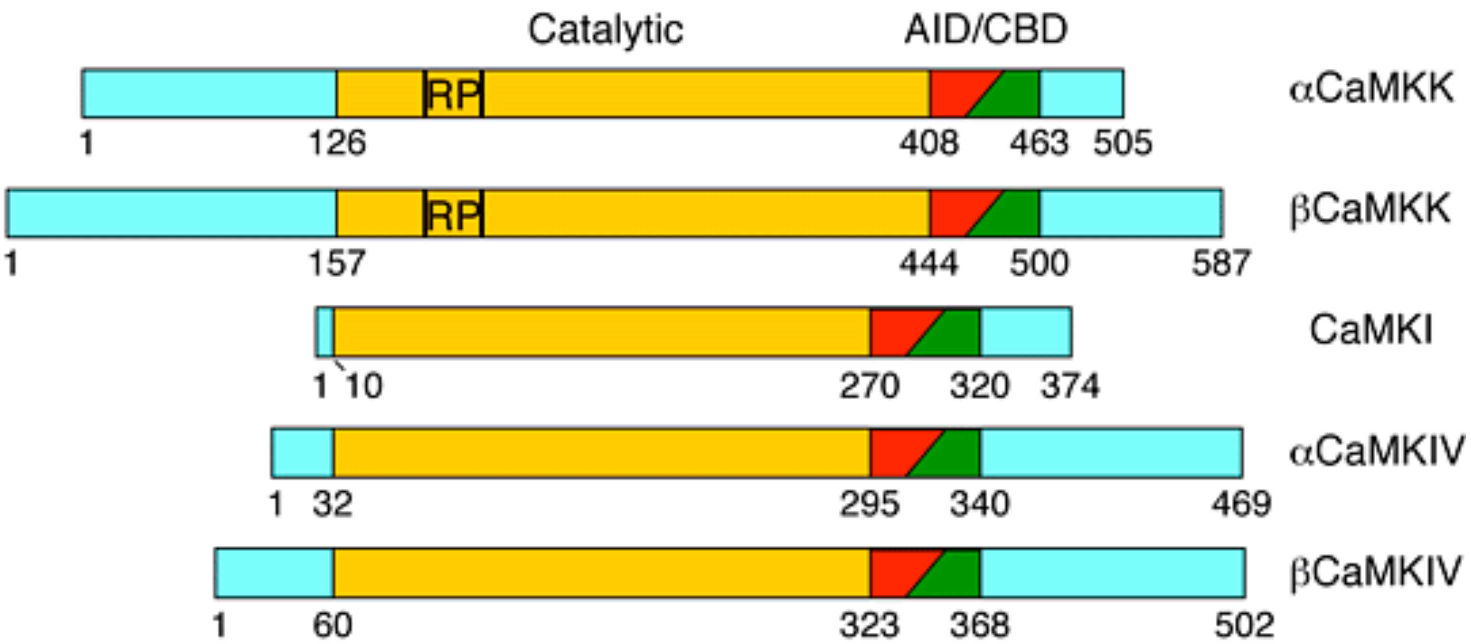
Adenylyl cyclase (brain)
Ca^{2+}/calmodulin-dependent protein kinases
Ca^{2+}-dependent Na^{+} channel (*Paramecium*)
Ca^{2+} release channel of sarcoplasmic reticulum
Calcineurin (phosphoprotein phosphatase 2B)
cAMP phosphodiesterase
cAMP-gated olfactory channel
cGMP-gated Na^{+}, Ca^{2+} channels (rod and cone cells)
Myosin light chain kinases
NADH kinase
Nitric oxide synthase
PI-3 kinase
Plasma membrane Ca^{2+} ATPase (Ca^{2+} pump)
RNA helicase (p68)

CAM KINASE FAMILY (Proteina chinasi dipendente da Ca^{2+}\calmodulina) Serina\treonina chinasi

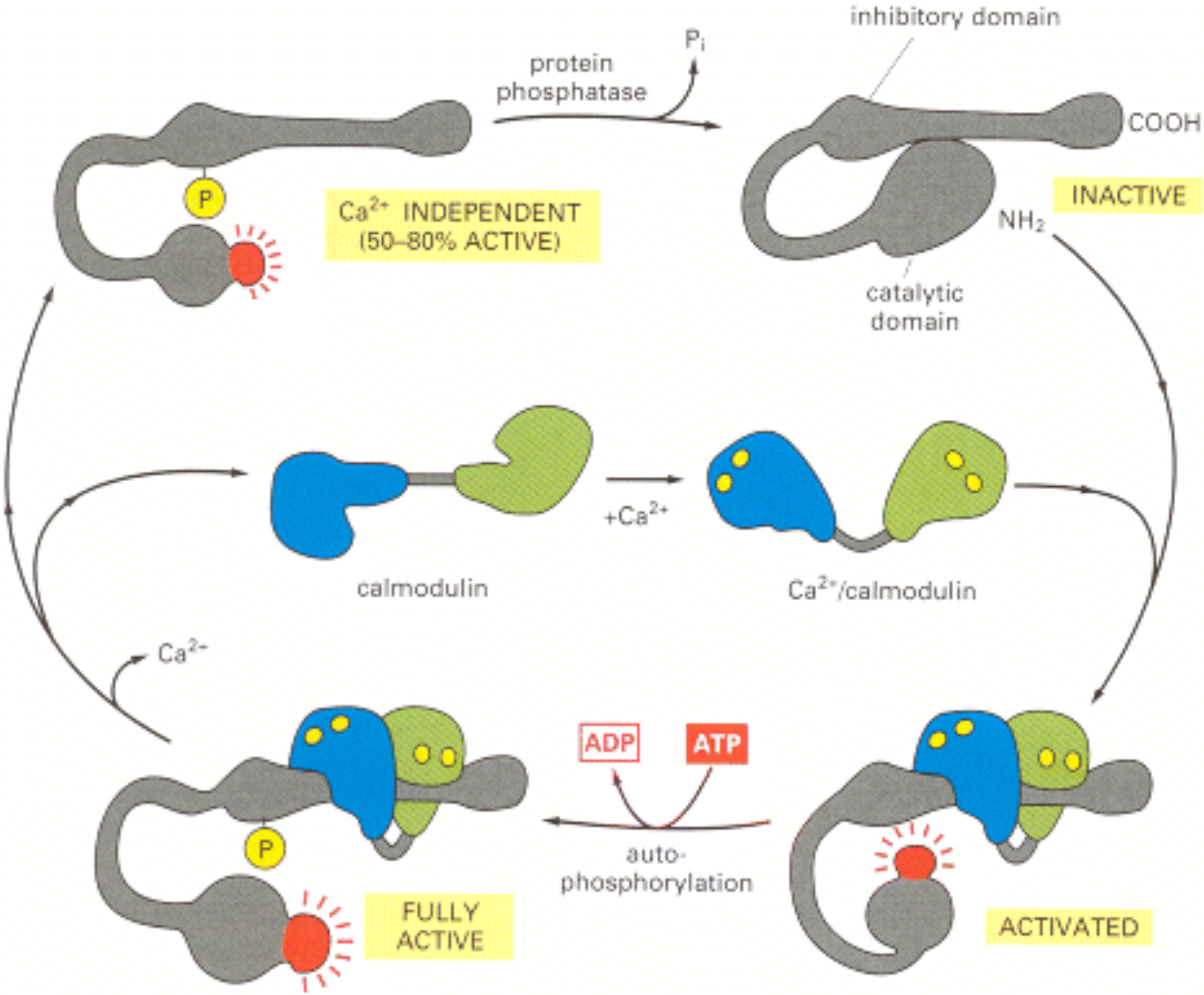


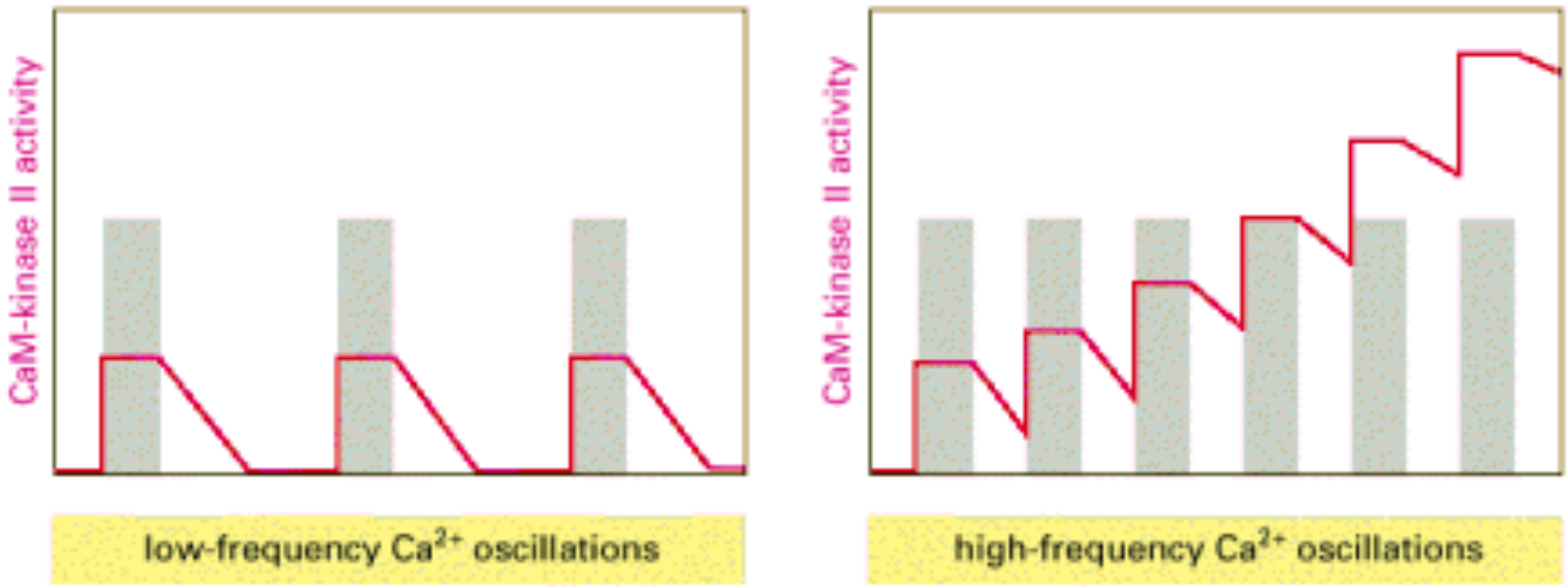
- ❖Chinasi della catena leggera della miosina
- ❖ Fosforilasi chinasi
- ❖ CaM-chinasi multifunzionali (CaM-chinasi II)

AID: autoinhibitory domain
CBD: calmodulin binding domain



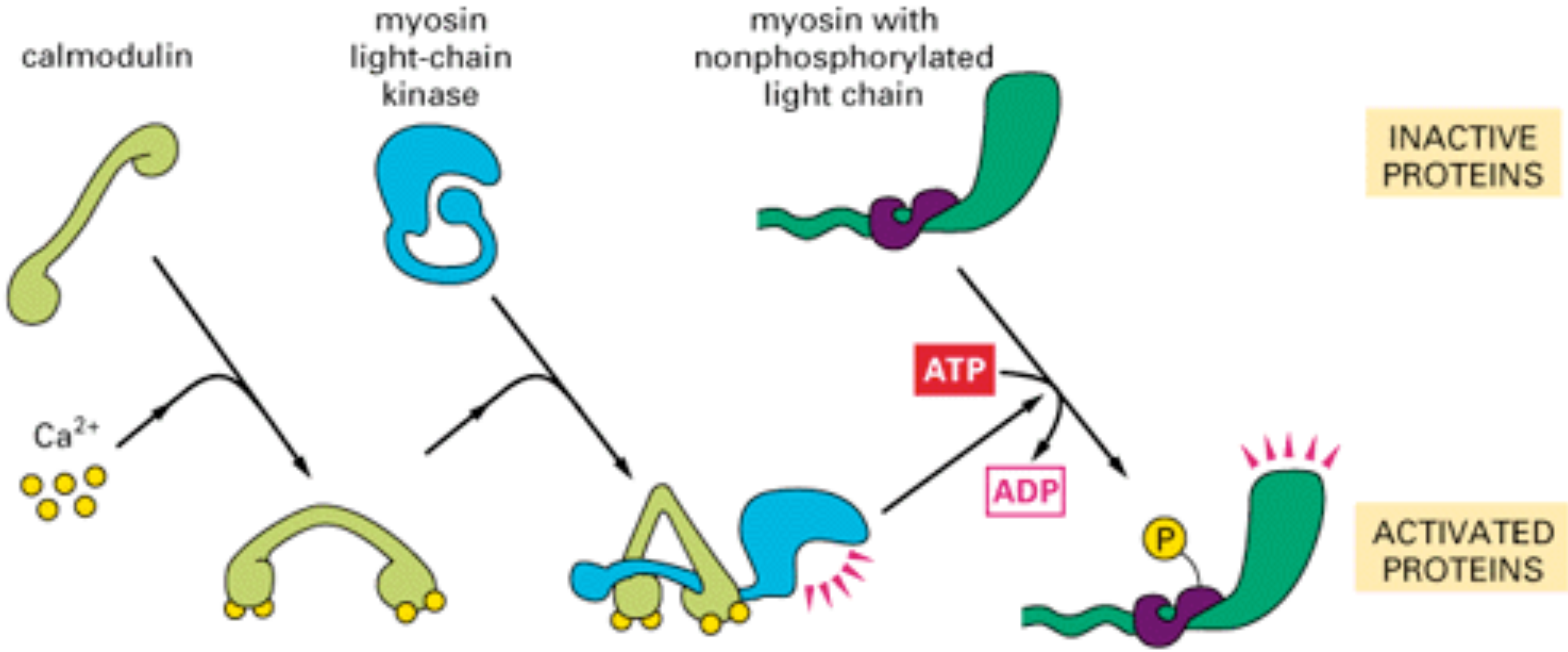
Attivazione della CaM-chinasi ⇨ Attiva tirosina idrossilasi (controlla velocità di sintesi delle catecolammine)





Il meccanismo di attivazione delle CaM kinases, è sensibile alle variazioni di frequenza delle oscillazioni di [Ca++], piuttosto che alle variazioni di [Ca++]

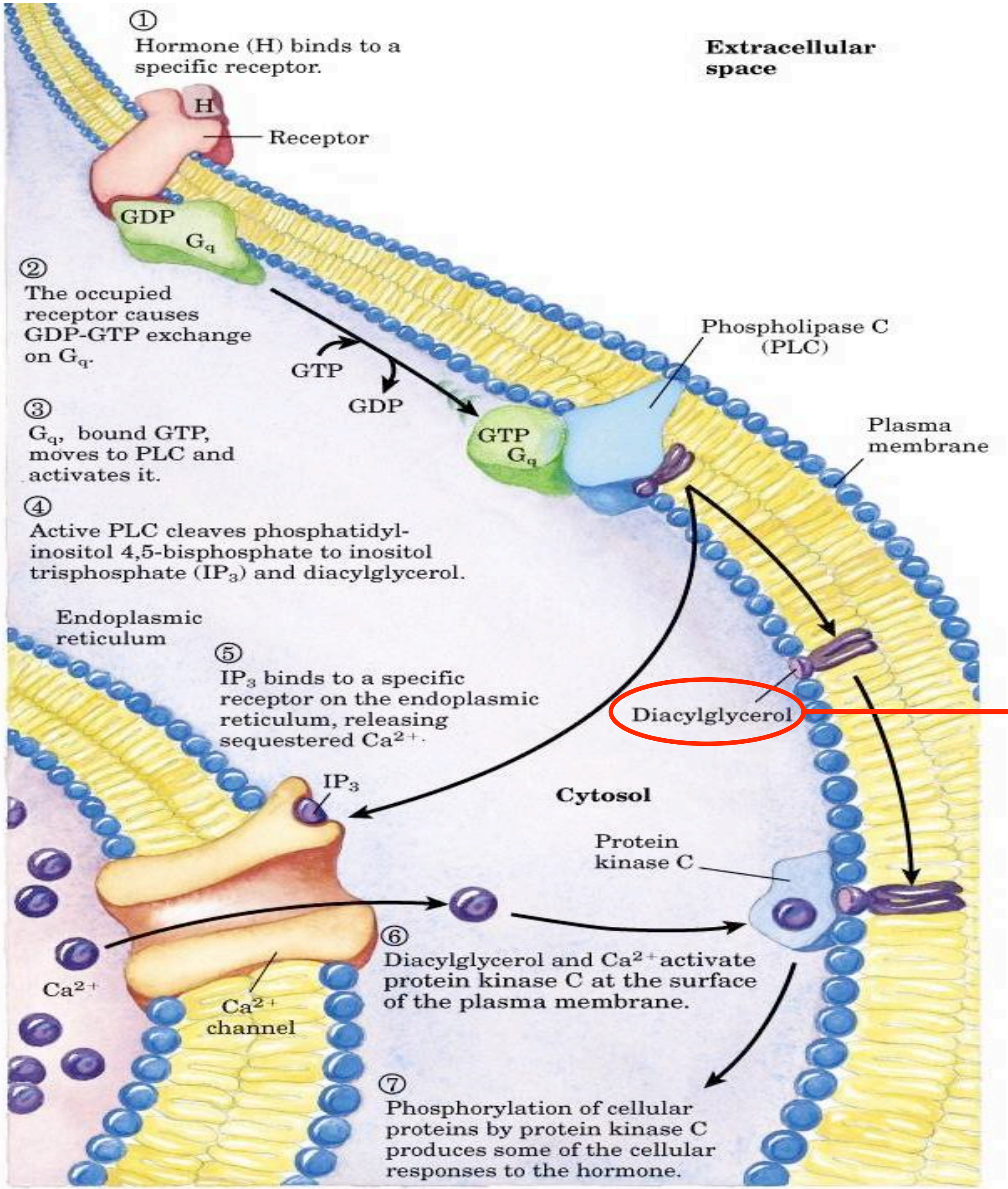
CaM è la subunità attivatoria di Myosin Light Chain Kinase (MLCK), prinicipale regolatore della <u>contrazione del muscolo liscio</u>



La <u>contrazione del muscolo scheletrico</u> è promossa dai cambiamenti conformazionali e dalle interazioni proteina-proteina di Troponina, una proteina contenente domini EF hand, CaM-like

Calcineurina: una serina fosfatasi contenente un dominio EF-hand CaM-like

Inibita dal complesso ciclofillina/ciclosporina (meccanismo della forte attività immunosoppressiva della ciclosporina, farmaco che ha "reso possibili" i trapianti)



-Nuova sintesi di fosfoinositidi

- Ac. Arachidonico

↑ DAGL/MAGL/DGK

DAG

↓ DAGL

2-Arachidonoil glicerolo (2-AG)
endocannabinoide

↓ MAGL

Acido arachidonico