Transdução de sinais: Sinalização por cálcio

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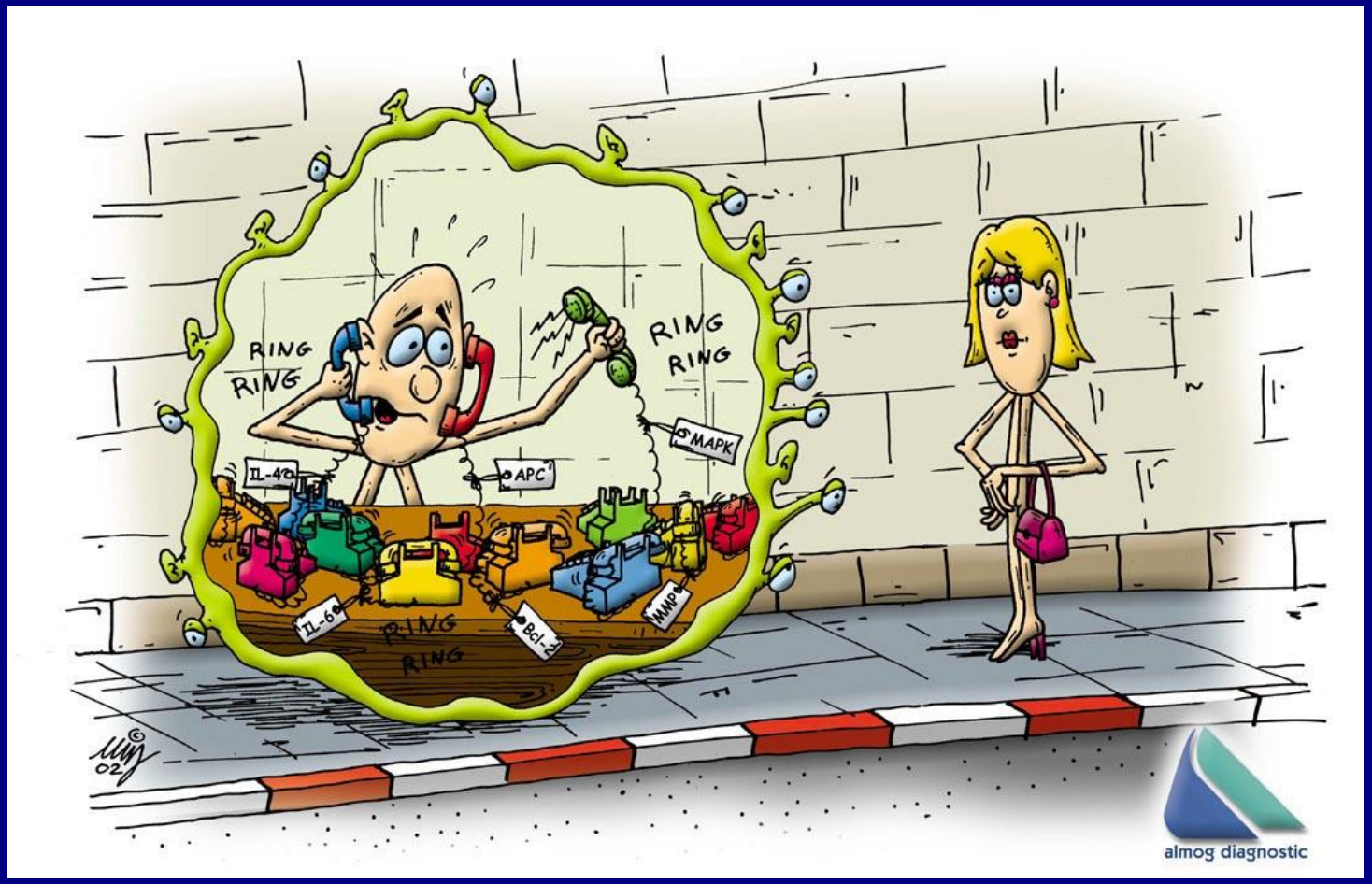
Transdução de Sinais

Tipos gerais de transdutores de sinais

- Canal iônico
- Receptor enzimático (fosforilação)
- Receptor serpentiante (proteína G)
- Receptor esteróide

Definição

Habilidade das células de receber e reagir a sinais vindos do outro lado da membrana. Estes sinais são detectados por um receptor específico e convertidos em uma resposta celular



Calcium is a vital second messenger

- Na^+, K^+ are the ions that are most important for the control of cell volume and the membrane potential.

- But Ca^{2+} plays an equally important role in practically every cell type.

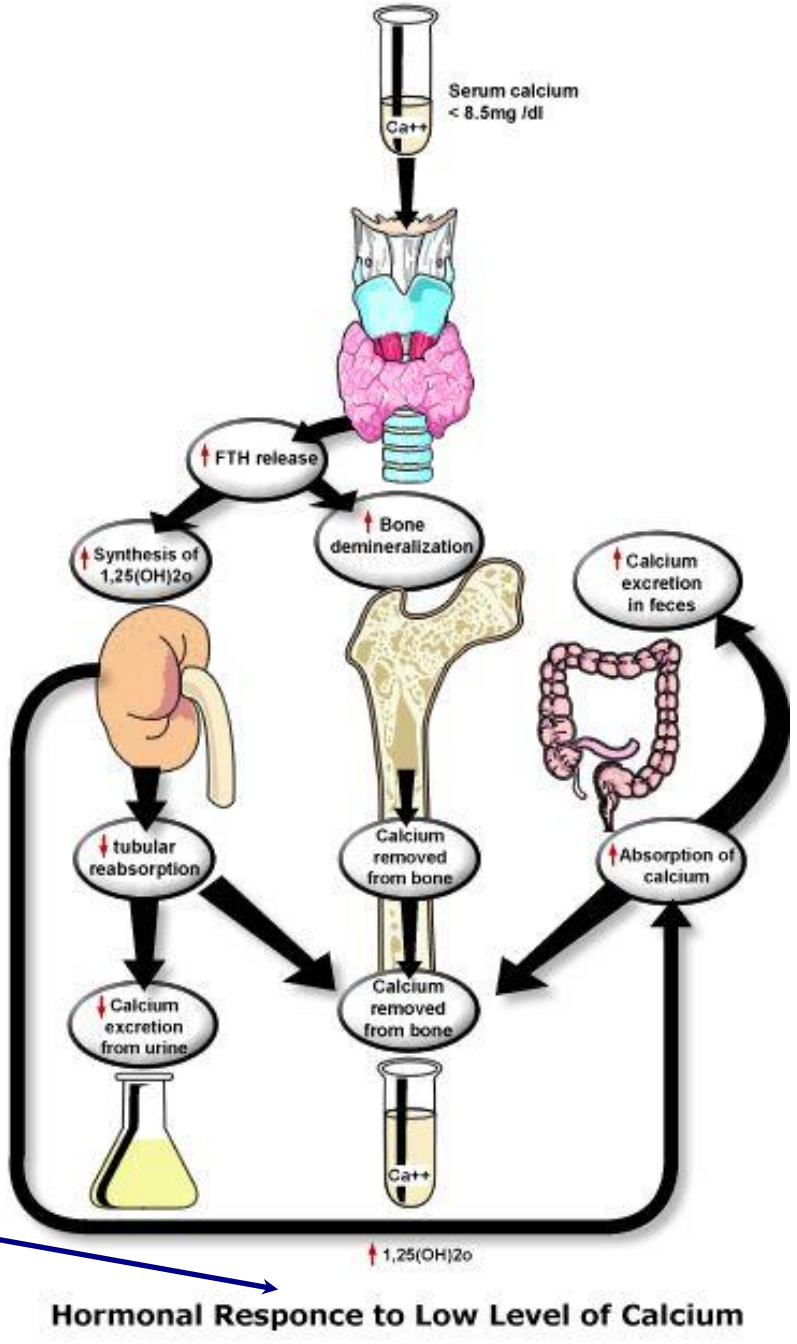
- Ca^{2+} controls secretion, cell movement, muscular contraction, cell differentiation and many other crucial events in metabolism

- Important in both excitable and non-excitable cells.

Whole-body control

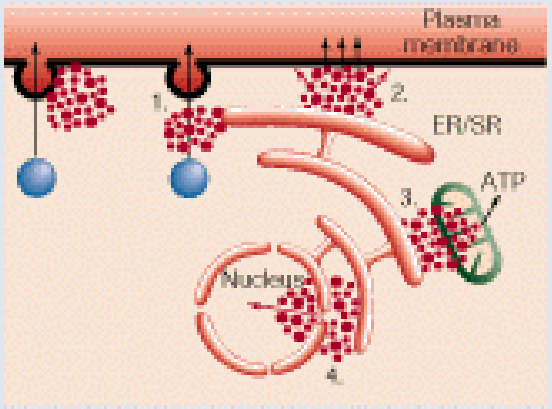
Maintained high levels of calcium in the blood

they really mean response



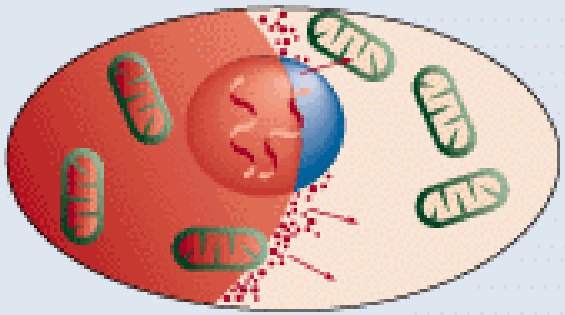
Spatial aspects of Ca^{2+} signalling

a Elementary events



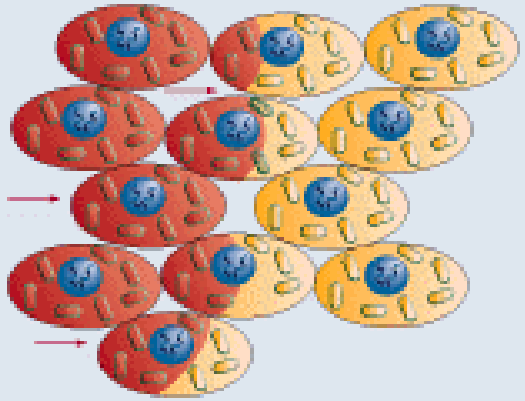
Growth-cone migration
Membrane excitability
Mitochondrial metabolism
Vesicle secretion
Smooth muscle relaxation
Mitosis
Synaptic plasticity

b Global Ca^{2+} wave (intracellular)



Fertilization
Smooth muscle contraction
Skeletal muscle contraction
Cardiac muscle contraction
Liver metabolism
Gene transcription
Cell proliferation

c Global Ca^{2+} wave (intercellular)



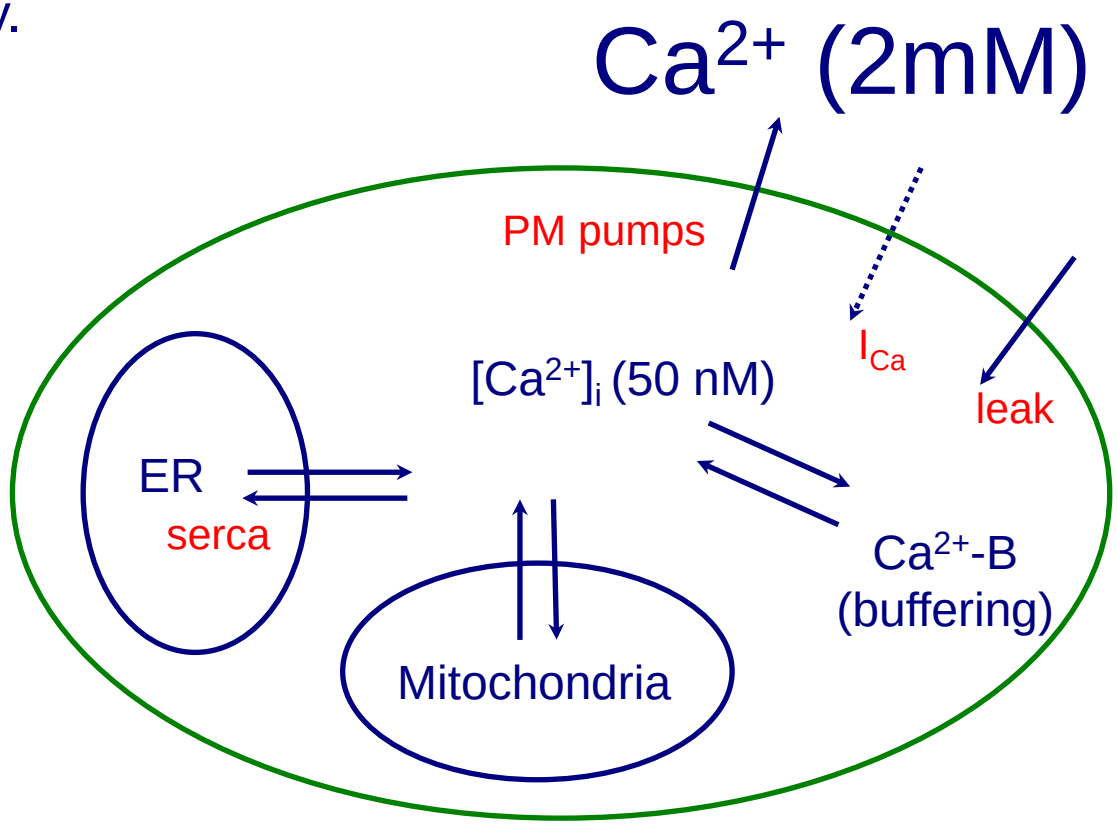
Wound healing
Ciliary beating
Glial cell function
Bile flow
Insulin secretion
Smooth muscle-induced nitric oxide synthesis in endothelium

Nature 395, 645 - 648 (1998)

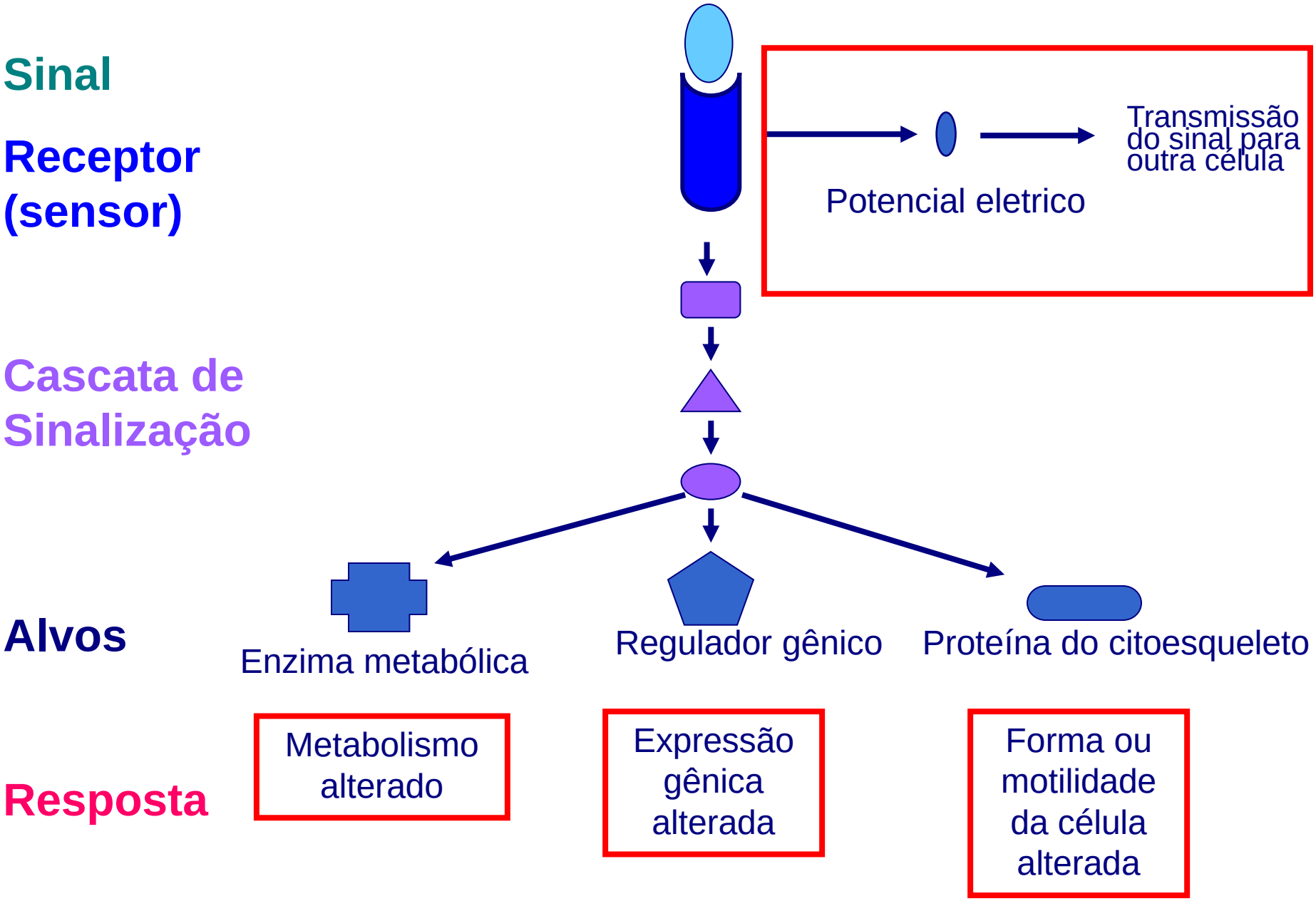
Calcium pressure

• Why? So cells can raise their internal Ca^{2+} quickly, and then decrease it quickly.

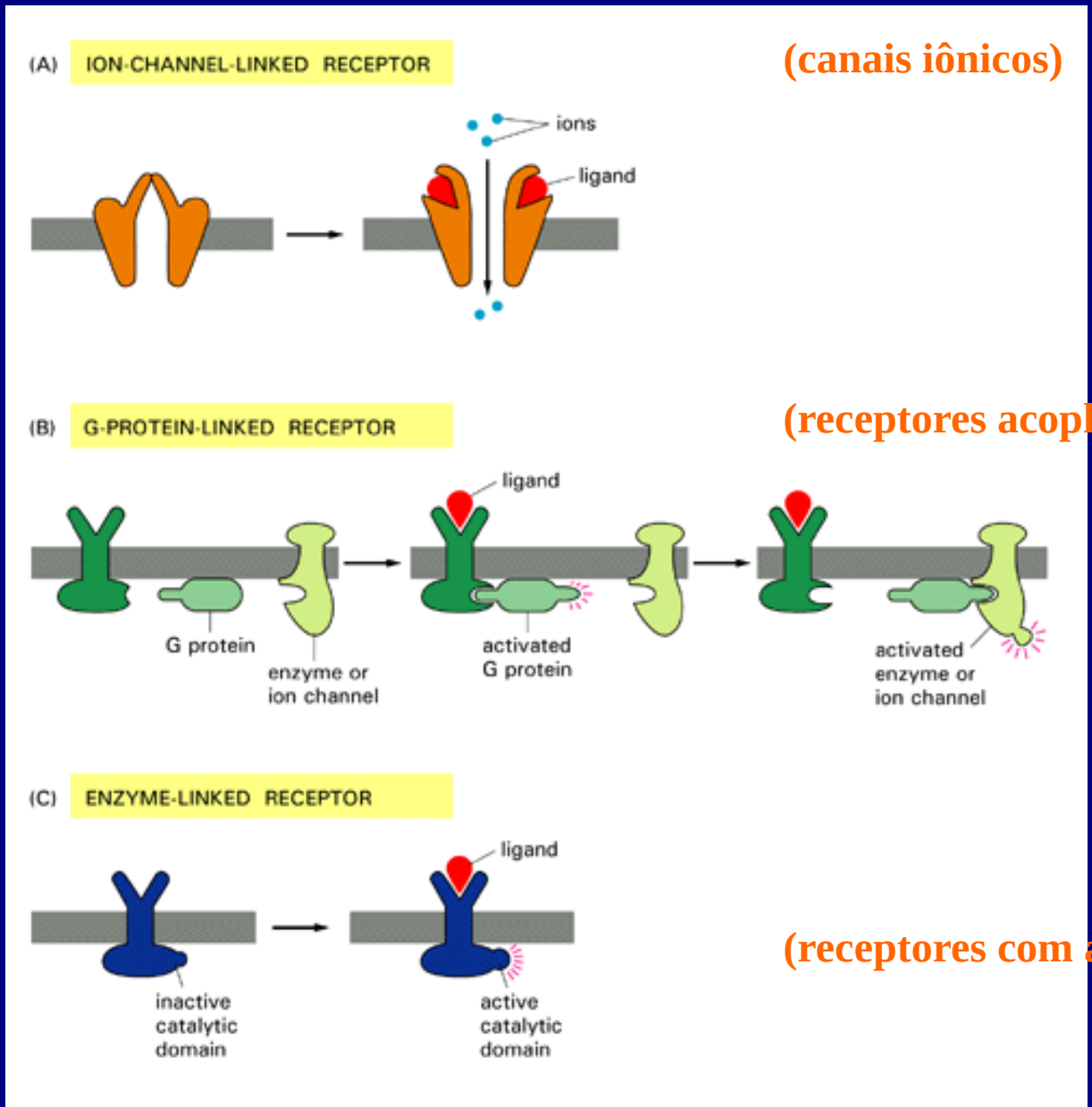
• Thus, can use Ca^{2+} as an intracellular signalling messenger, while avoiding toxic effects.



Transdução de sinais e comunicação intercelular



Tipos principais de receptores na superfície celular os quais induzem sinalização intracelular

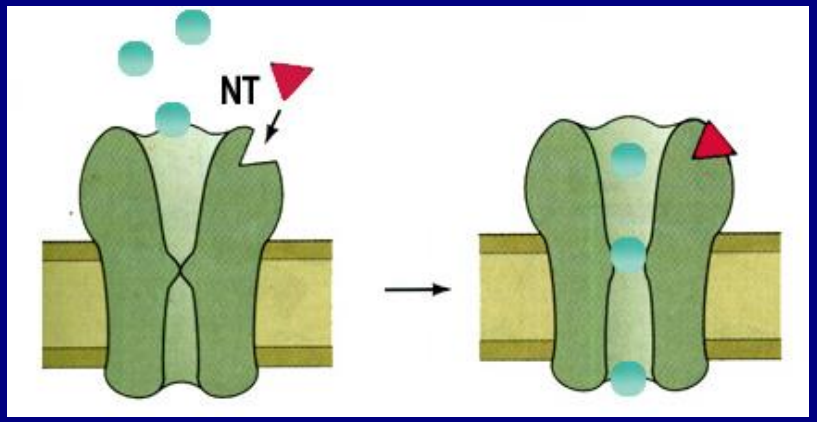


(canais iônicos)

(receptores acoplados a proteína G)

(receptores com atividade enzimática)

Ativação de fluxo de ions pela plasmamembrana

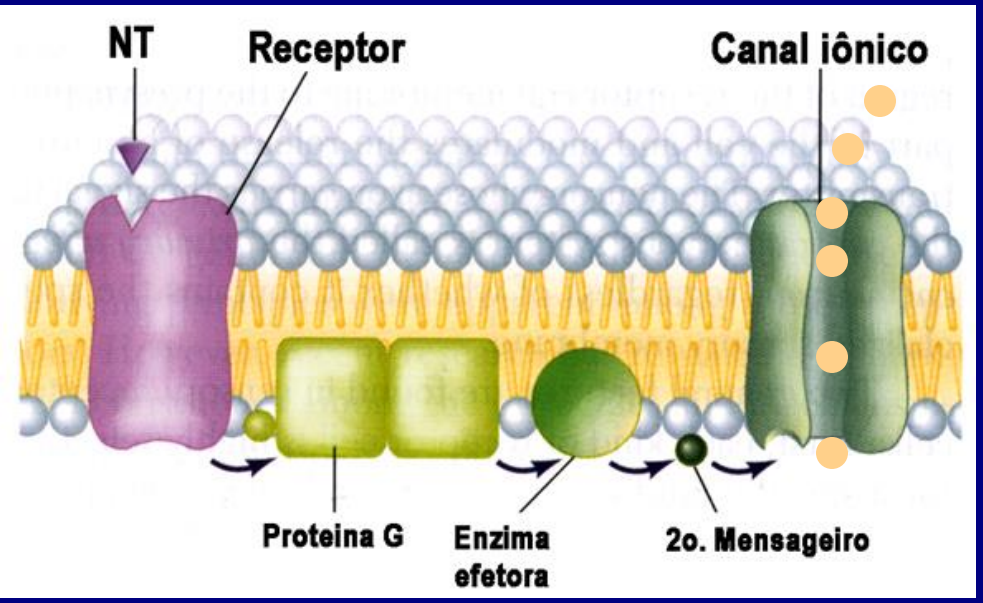


Há dois tipos de receptores pós-sinápticos

1) Receptor Ionotrópico

O NT abre o canal iônico DIRETAMENTE

Efeito rápido



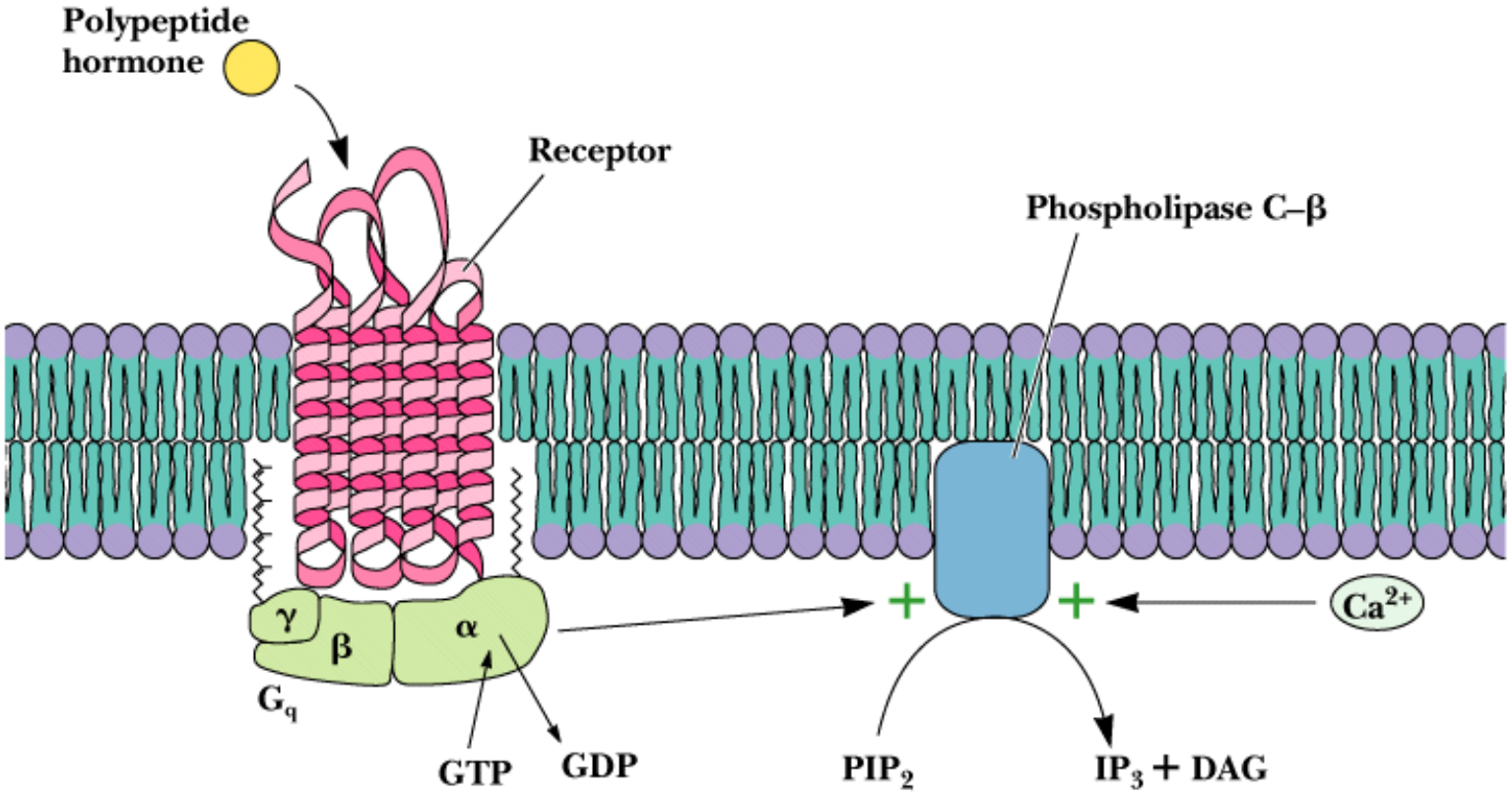
2) Receptor Metabotrópico

O NT abre o canal iônico INDIRETAMENTE
- freqüentemente, presença de 2º mensageiro para modificar a excitabilidade do neurônio pós-sináptico

Efeito mais demorado

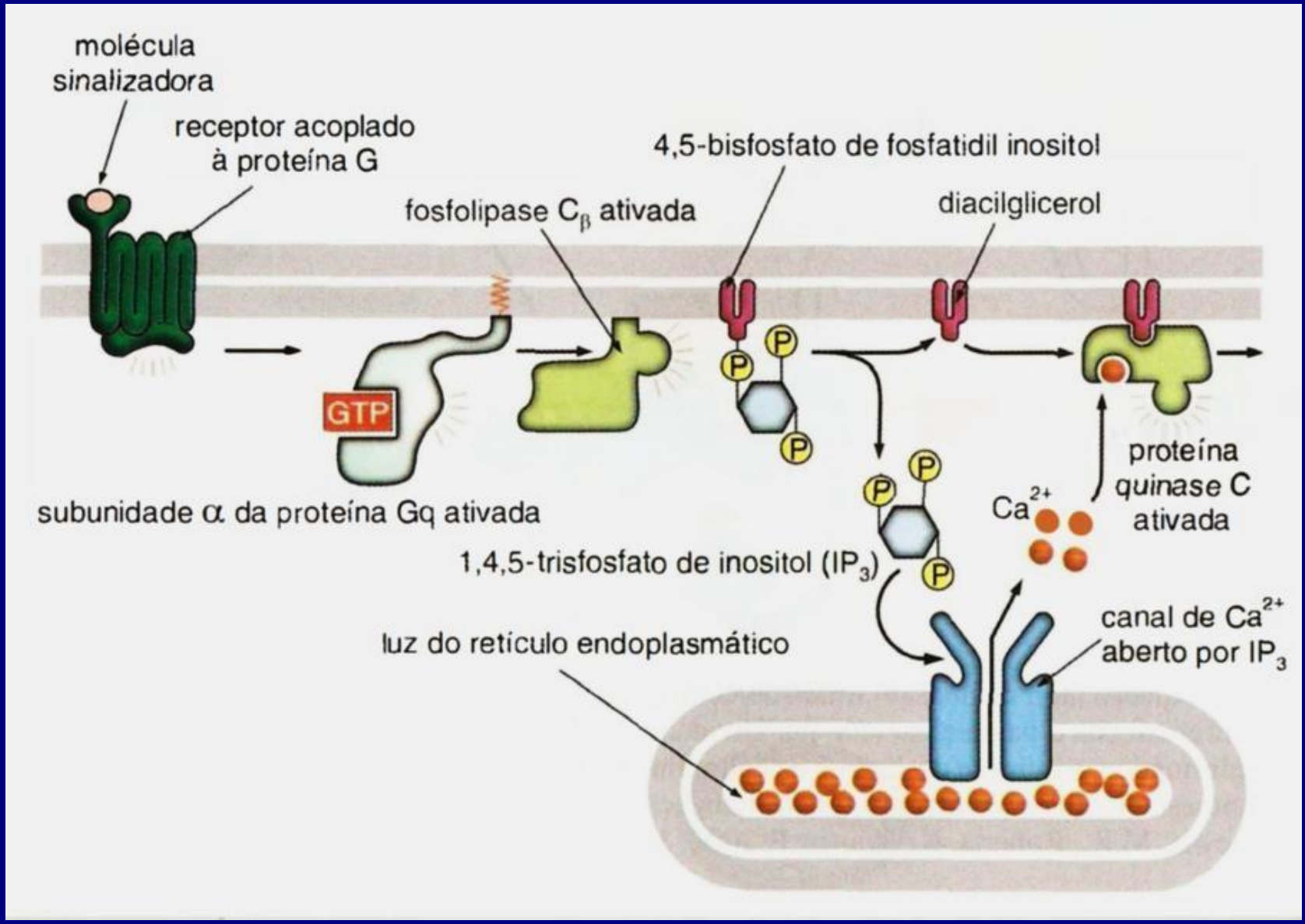
Inositol-3-fosfato como segundo messangeiro

Garrett & Grisham: Biochemistry, 2/e
Figure 34.13

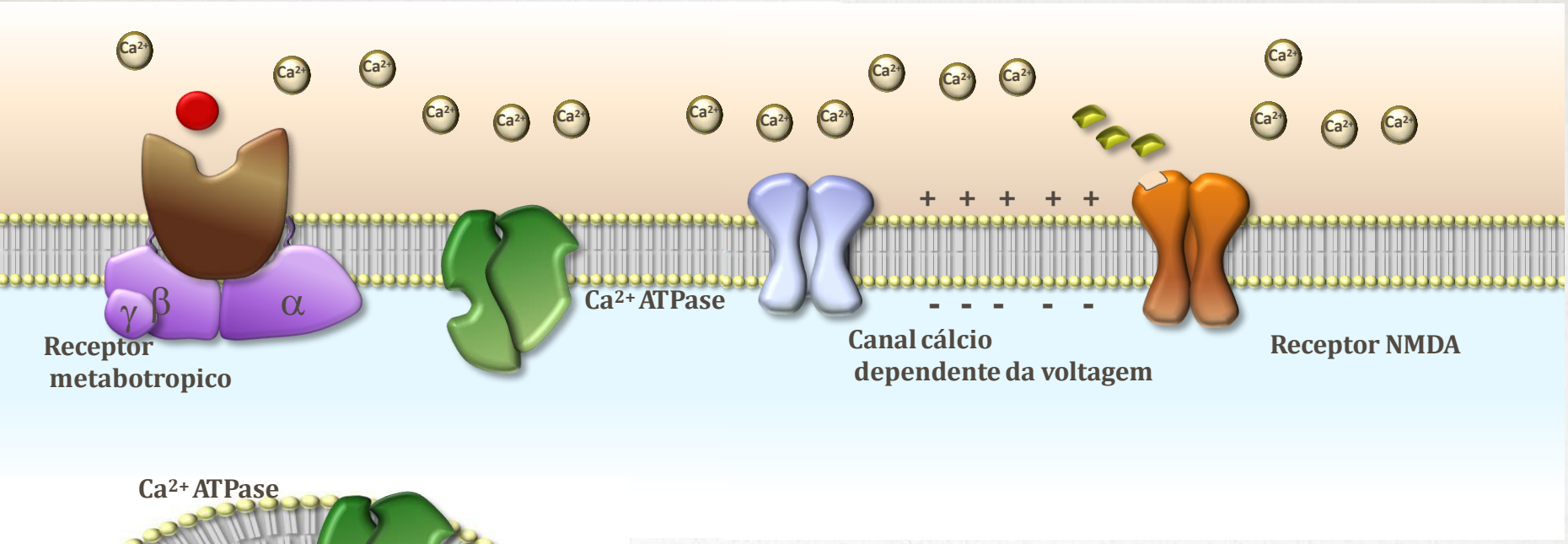


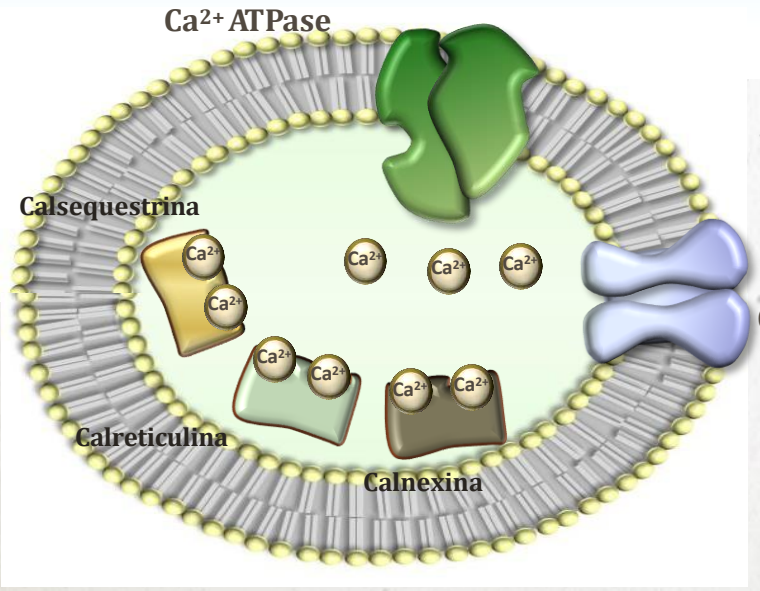
Saunders College Publishing

RECEPTORES ACOPLADOS A IP_3 E DAG



SINALIZAÇÃO DO CÁLCIO



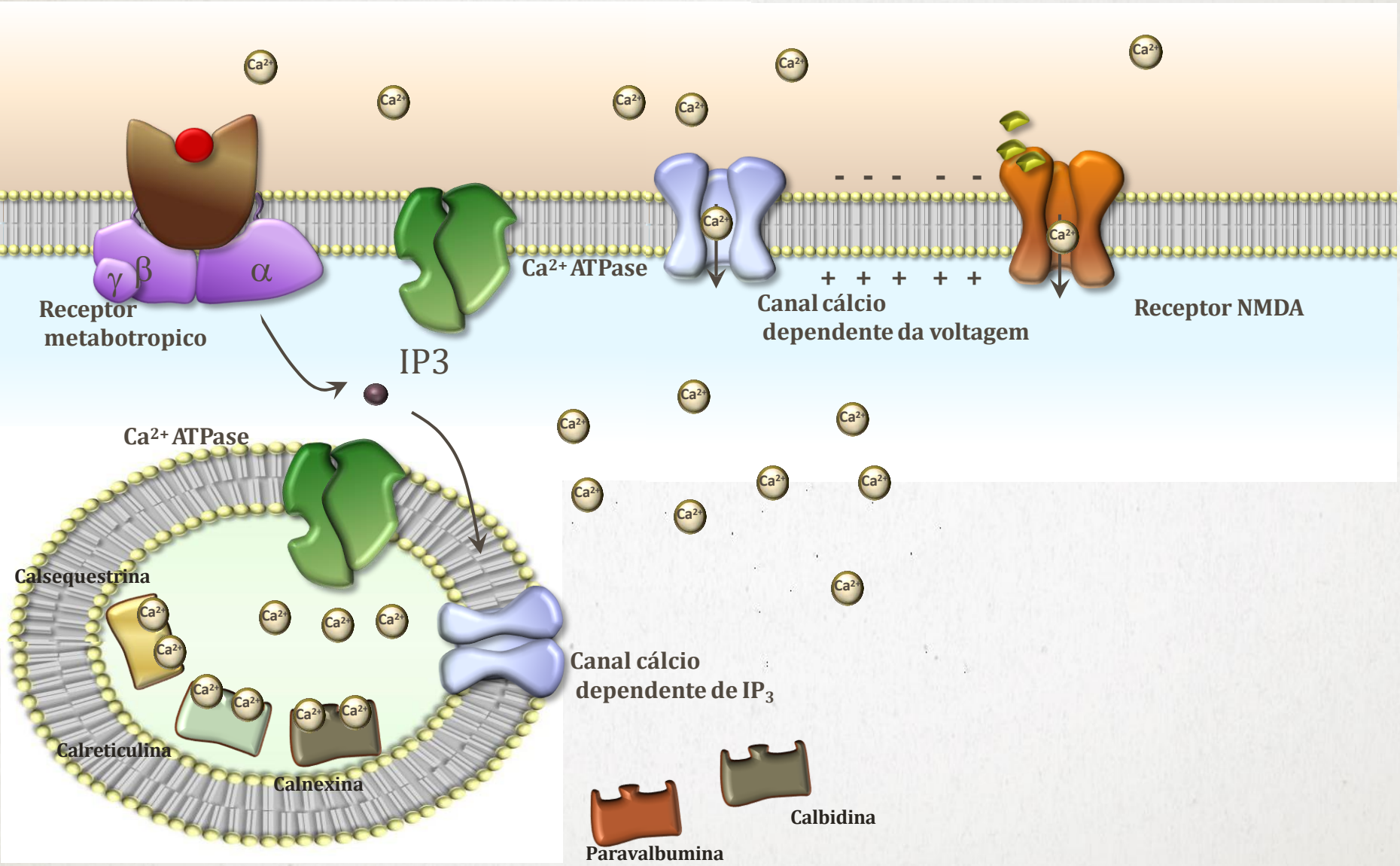


Canal cálcio dependente de IP_3

Calbidina

Paravalbumina

SINALIZAÇÃO DO CÁLCIO



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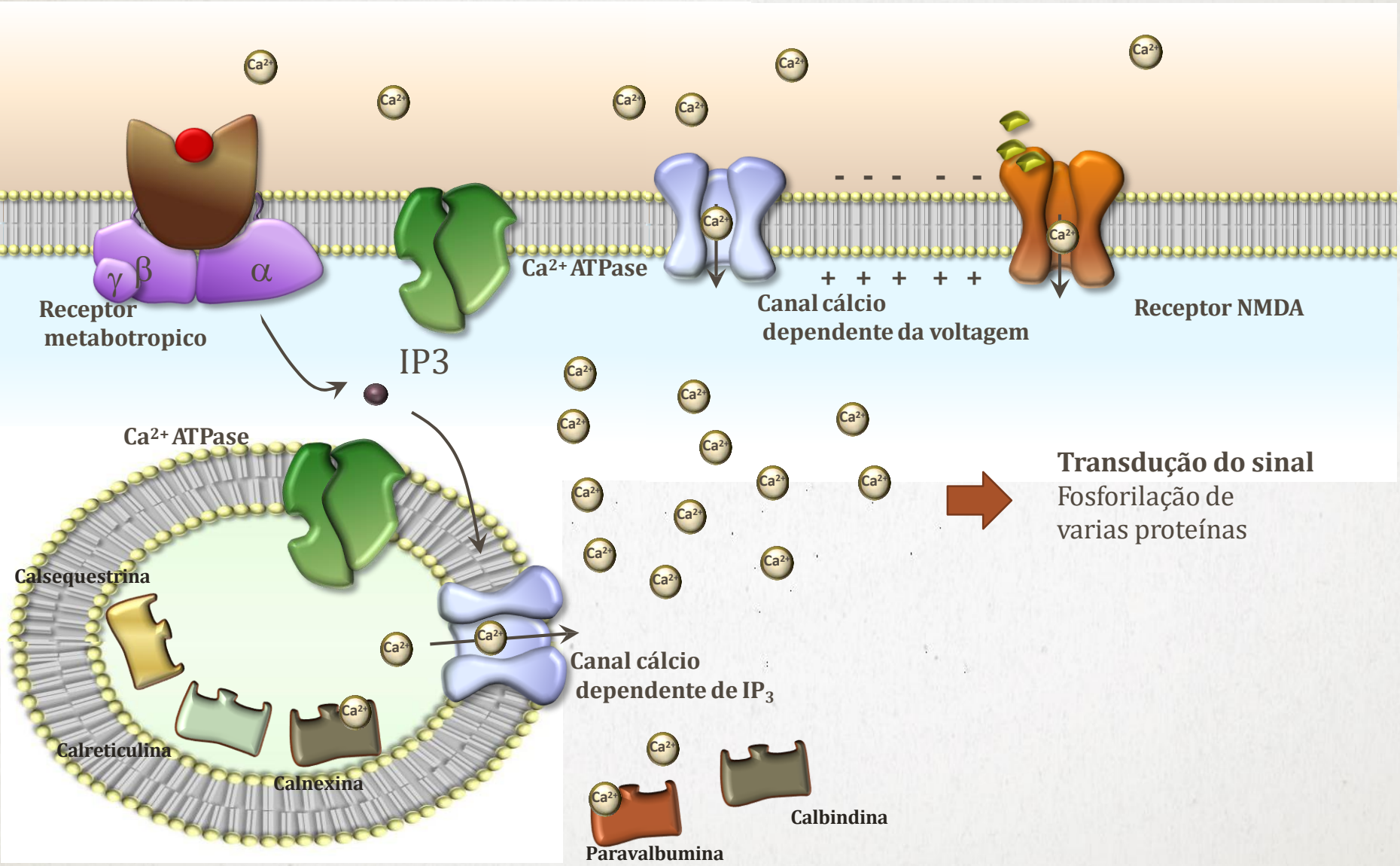

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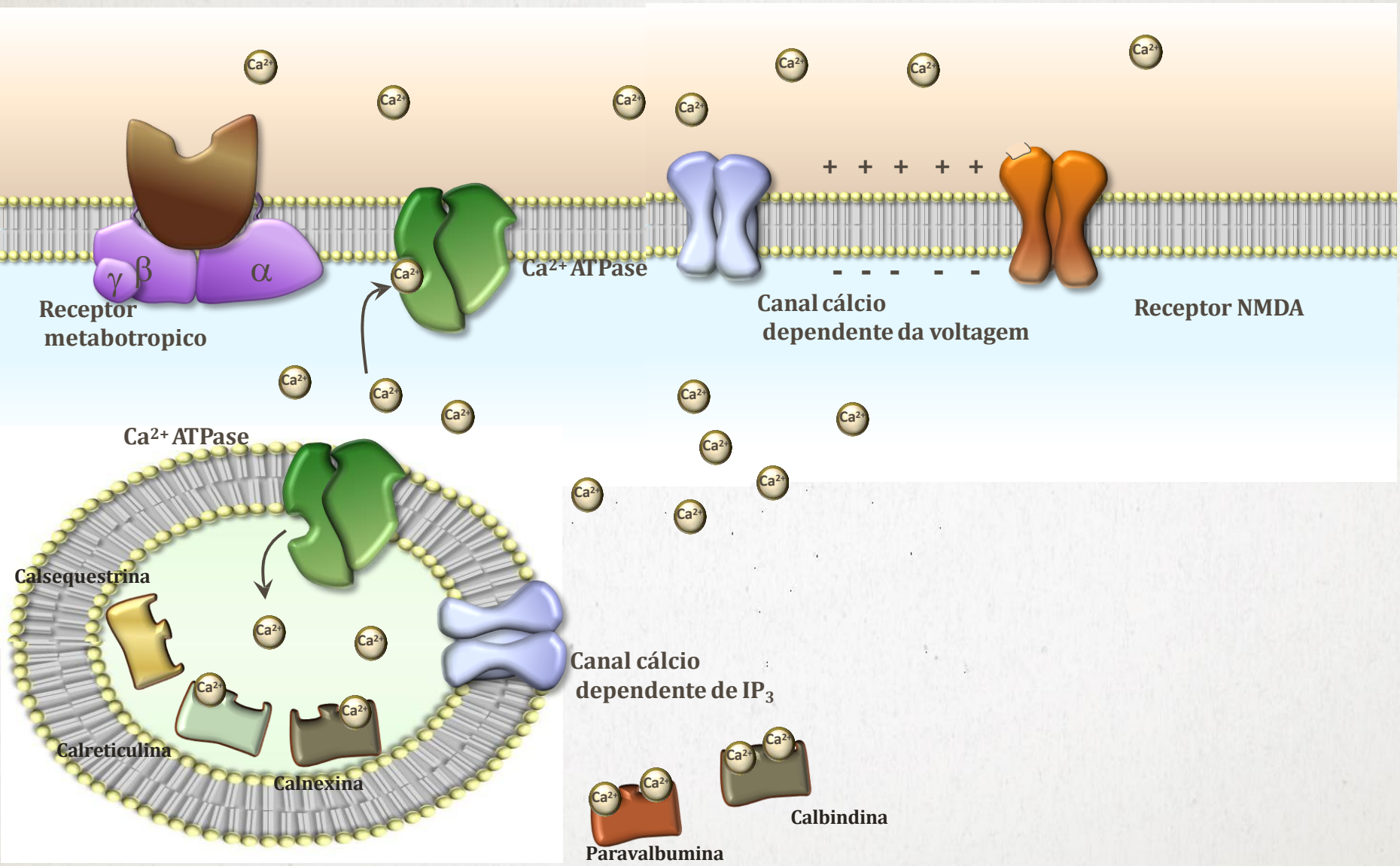

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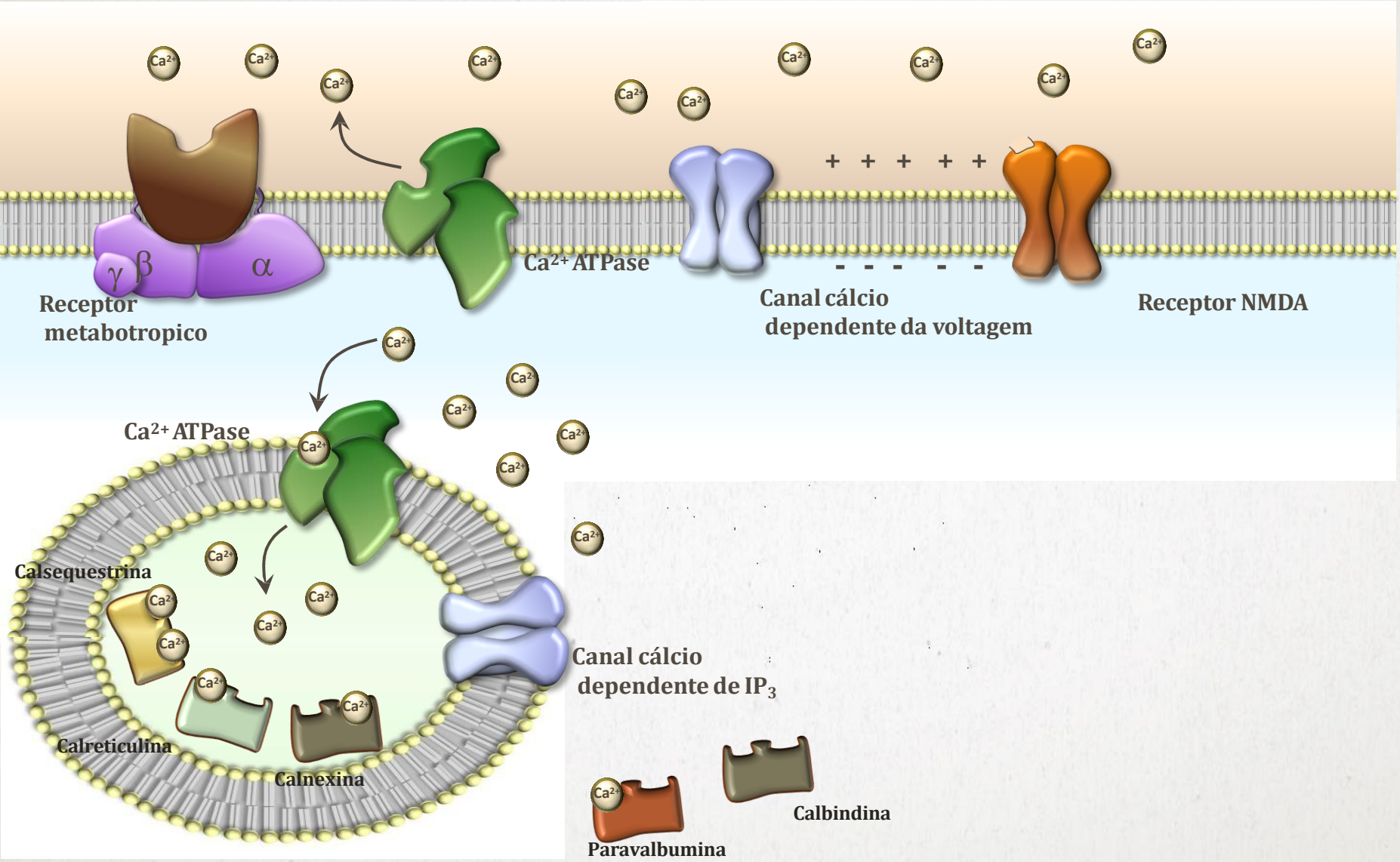

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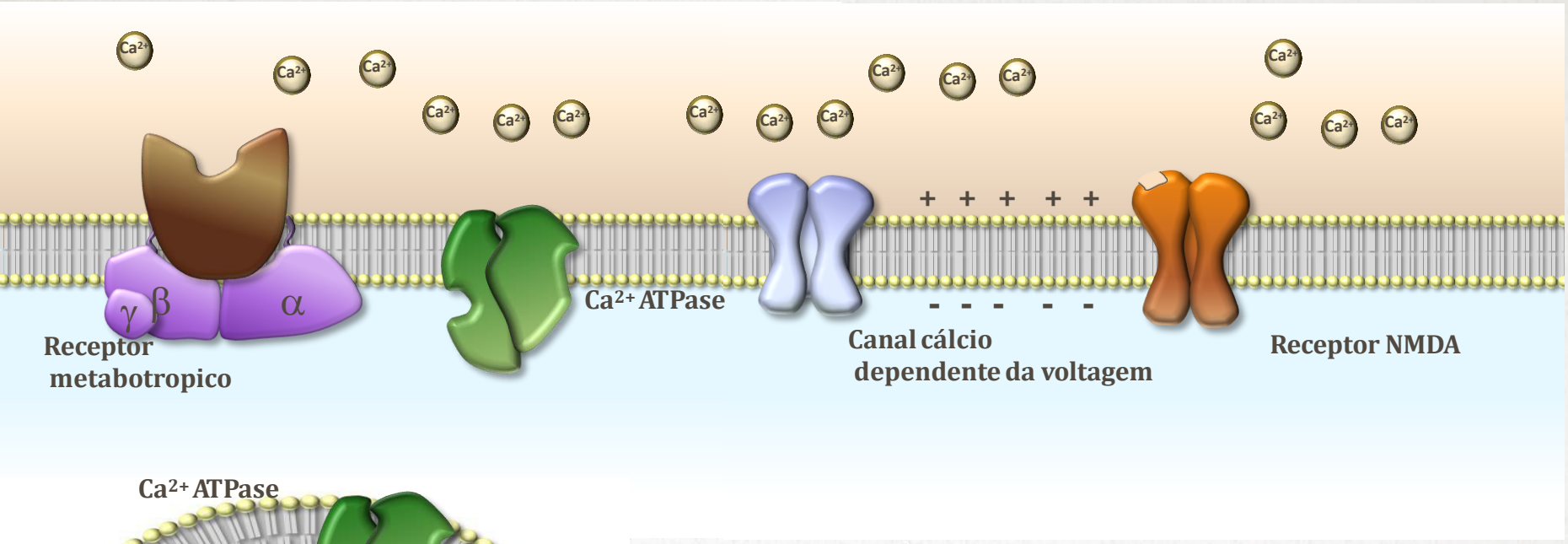
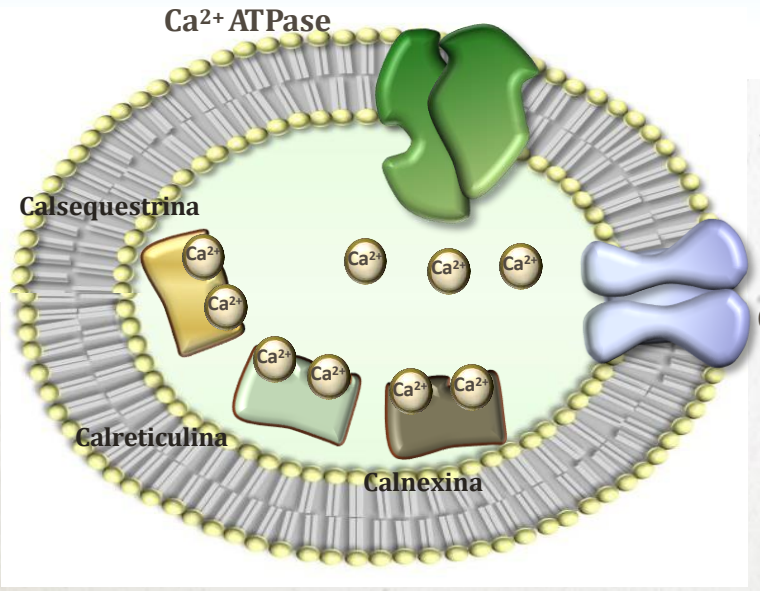


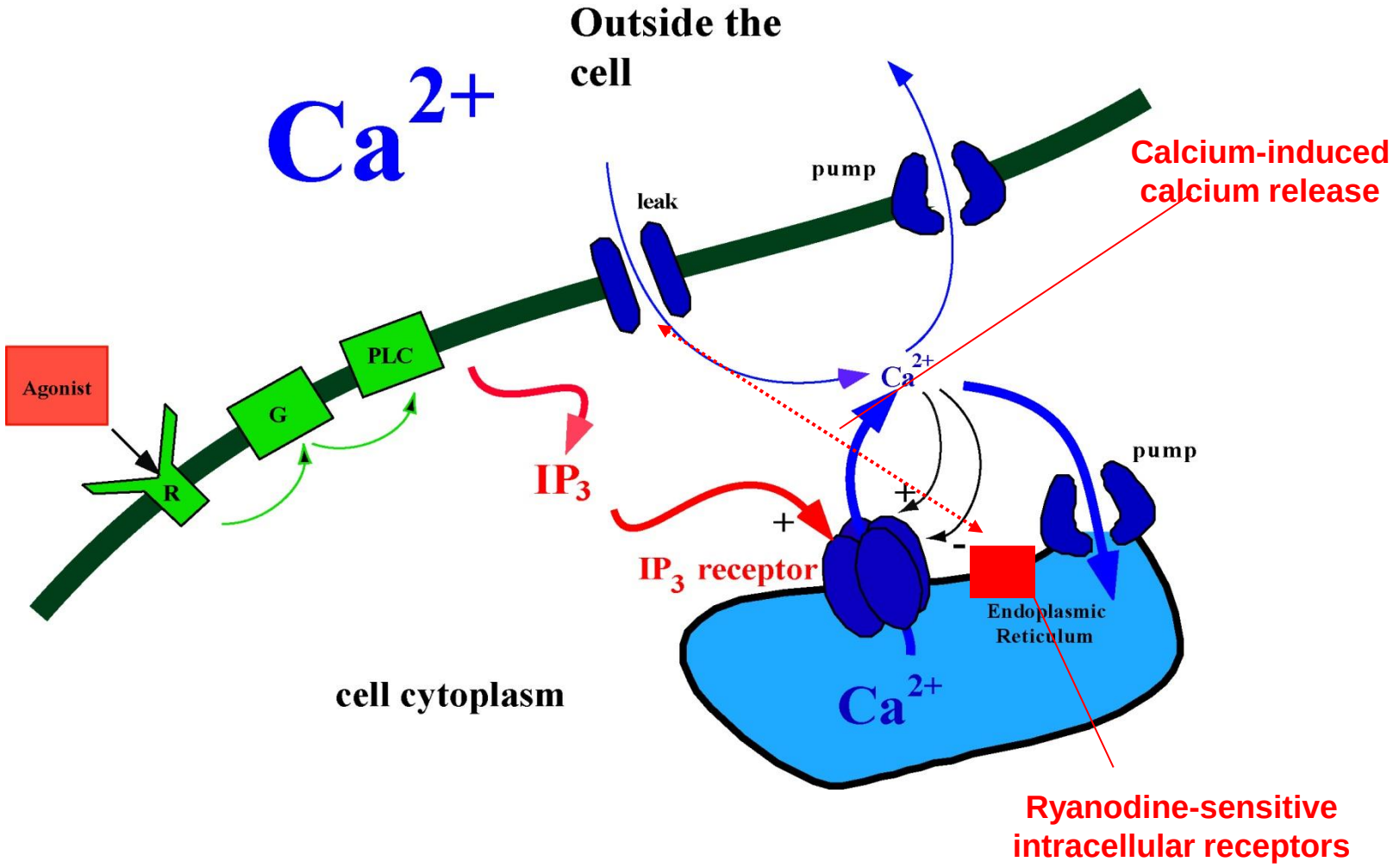


Canal cálcio dependente de IP_3

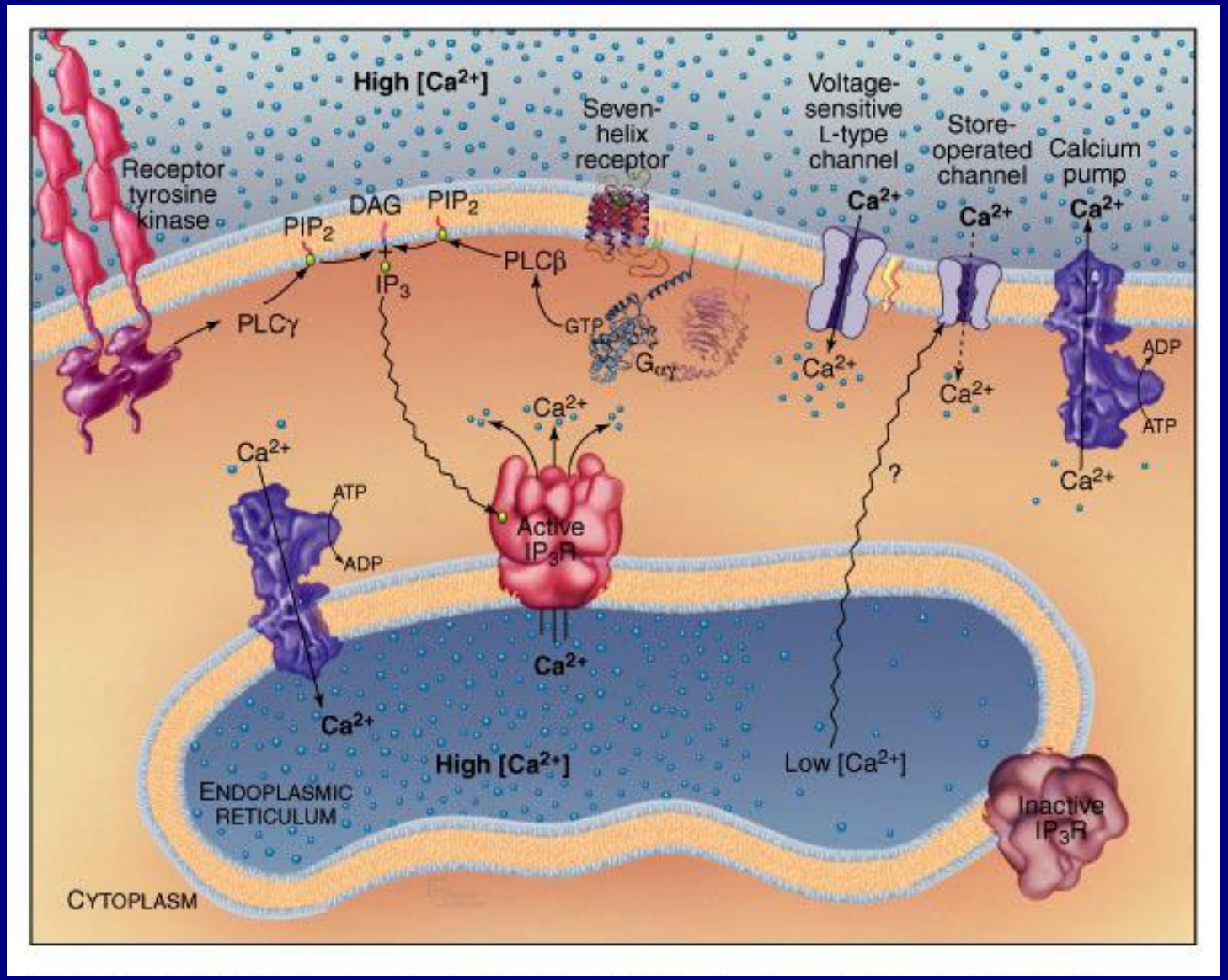
Calbindina

Paravalbumina

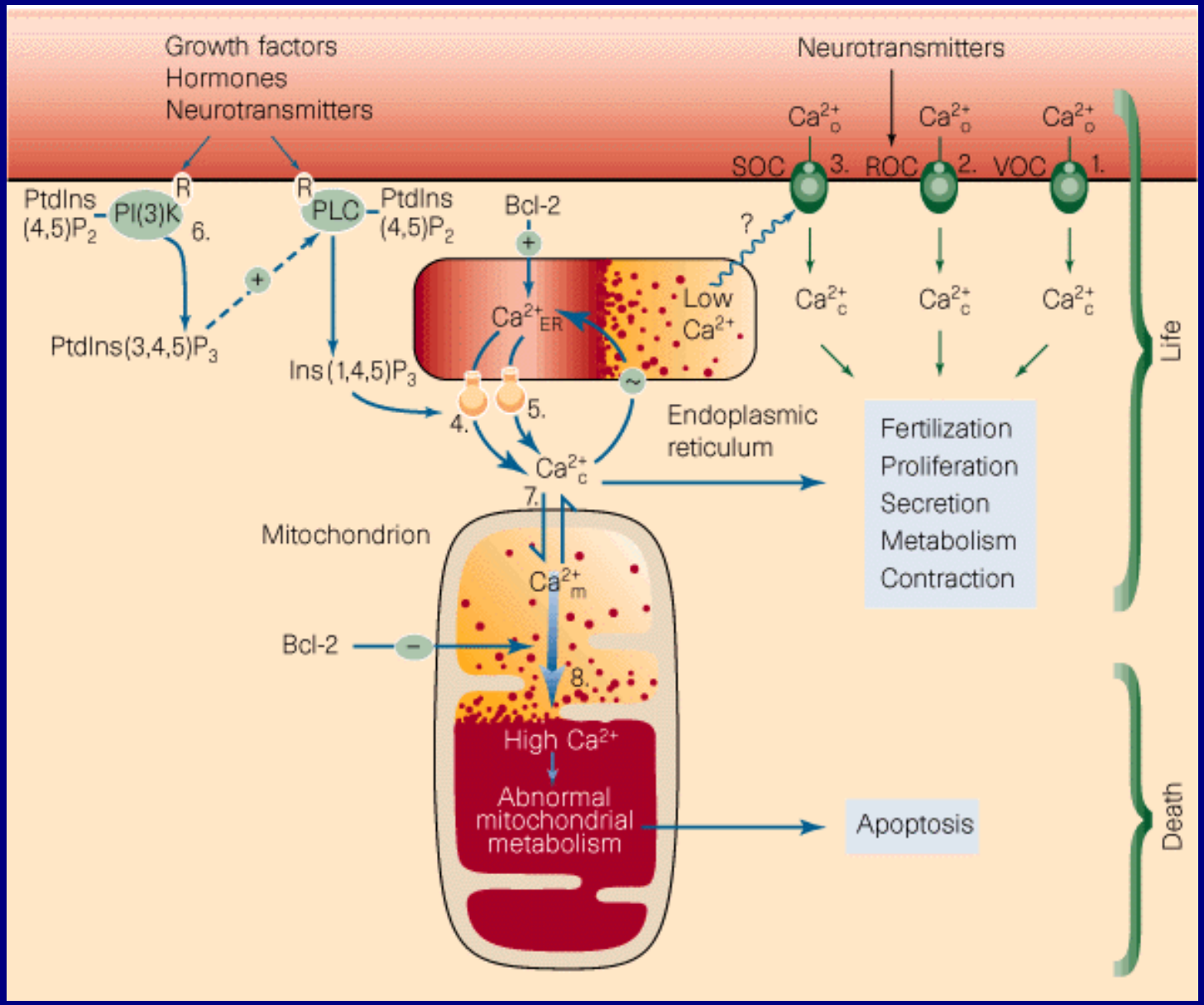
IP_3 Receptor pathway



Intracellular Ca^{2+} levels are highly regulated

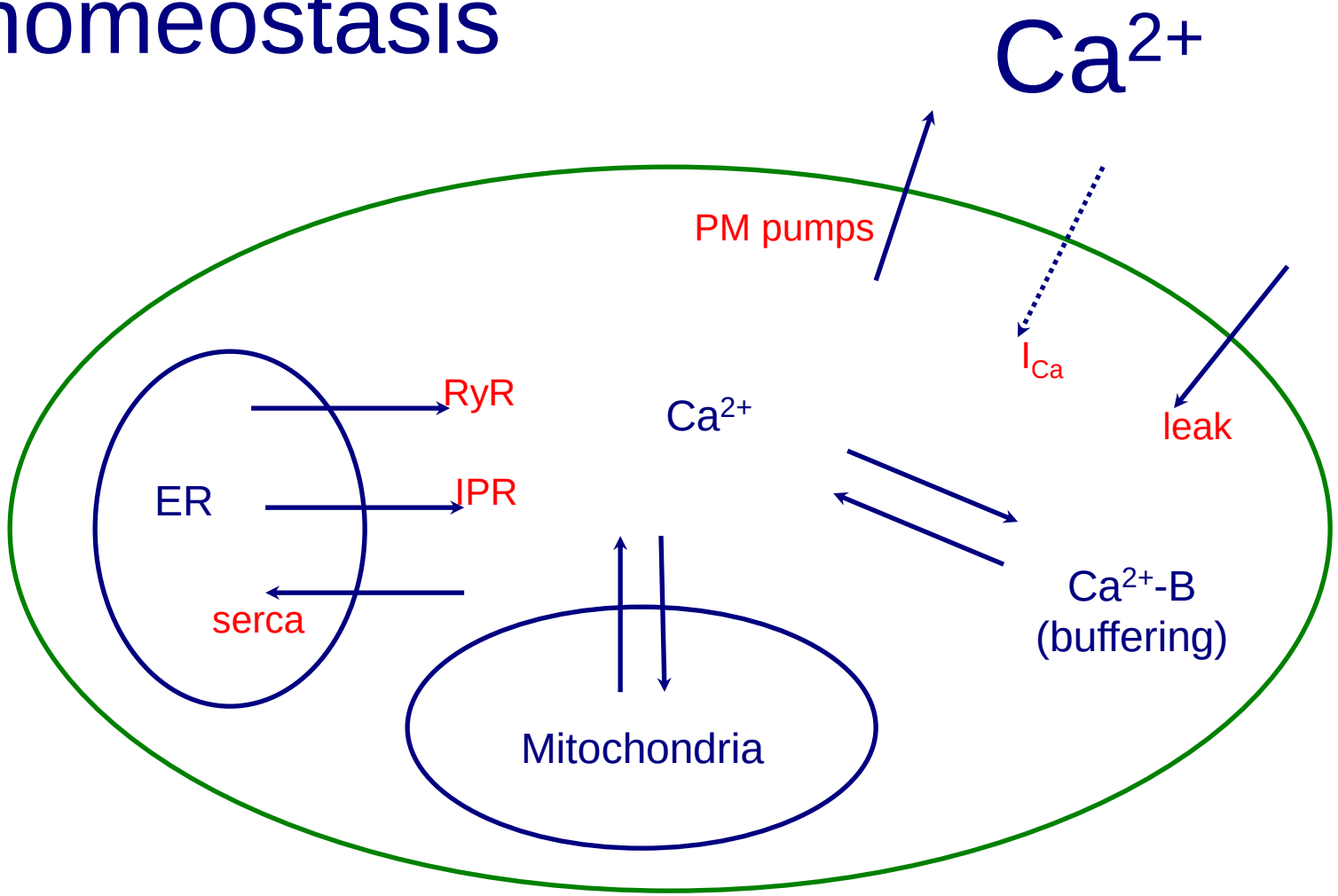


Basic Mechanisms of Ca^{2+} Signaling

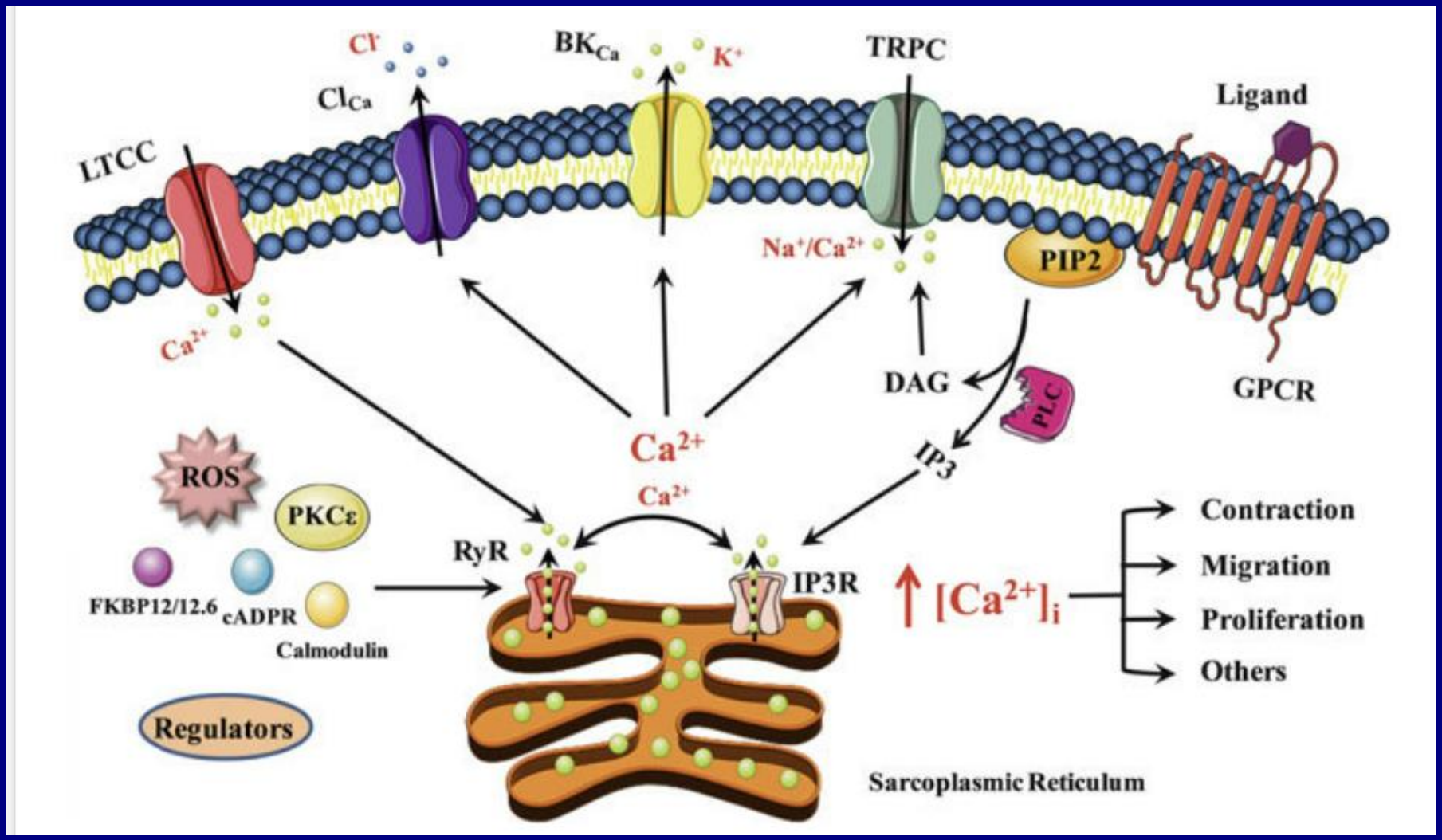


Nature 395, 645 - 648 (1998)

Summary of calcium homeostasis



Intracellular Ca^{2+} Levels Affect Overall Channel Activity (example: Airway Smooth Muscle Cells)



Calcium excitability

• Both IPR and RyR release calcium in an excitable manner. They both respond to a calcium challenge by the release of even more calcium.

• The precise mechanisms are not known for sure (although, as we shall see, detailed models can be constructed).

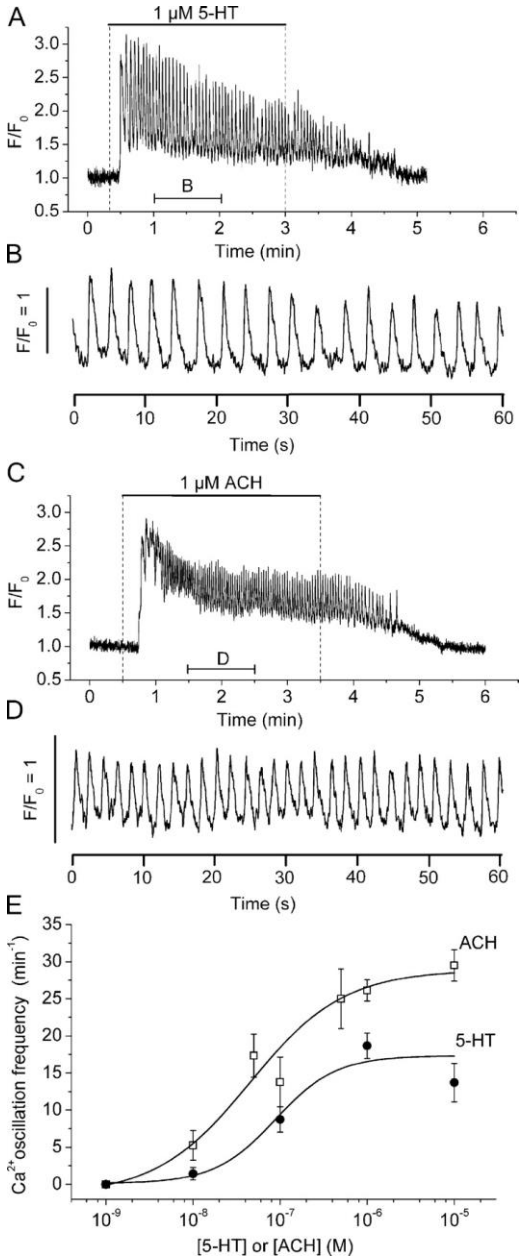
• An IPR behaves very like a Na^+ channel (in some ways). In response to an increase in $[Ca^{2+}]$ it first activates quickly, and then inactivates slowly, resulting in the short-term release of a large amount of calcium.

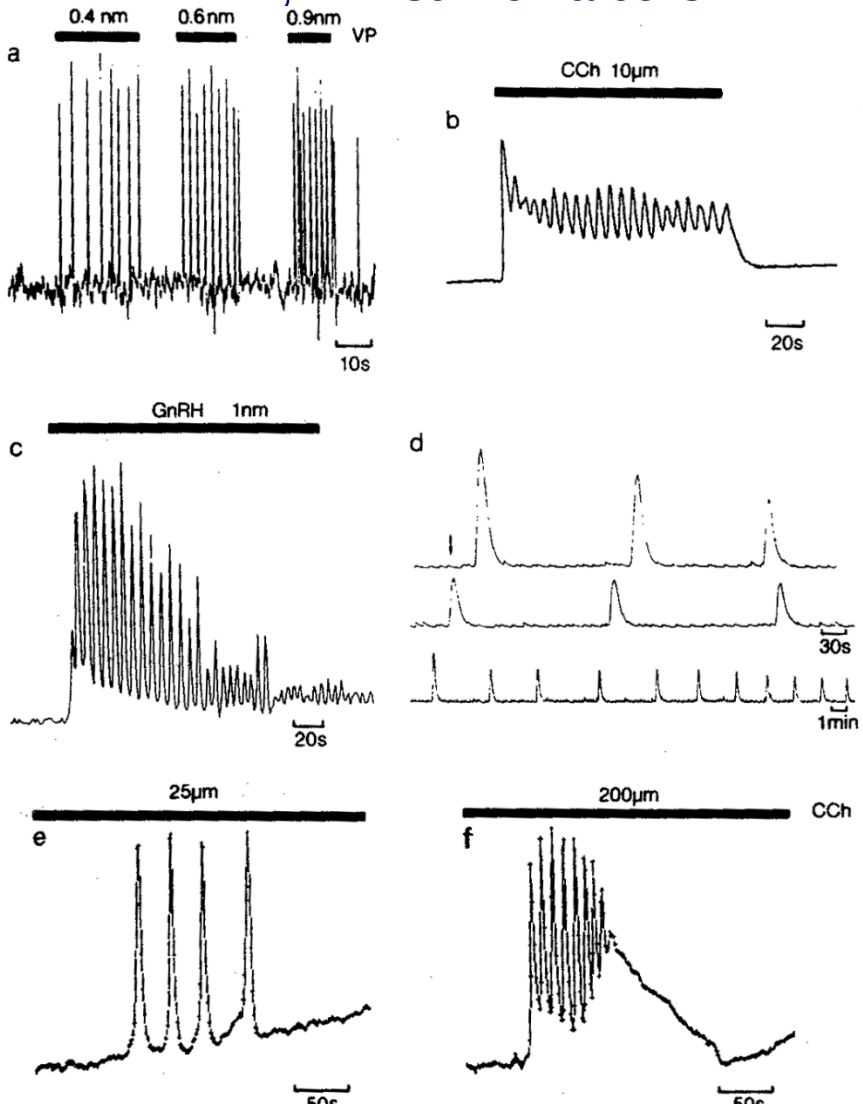
• A lot of attention has been focused on IPR and RyR. Less on pumping. But the dynamics of pumping is (obviously) equally important.

Typical Calcium oscillations

A: Hepatocytes
B: Rat parotid gland
C: Gonadotropes
D: Hamster eggs (post-fertilisation)
E, F: Insulinoma cells

Neurons



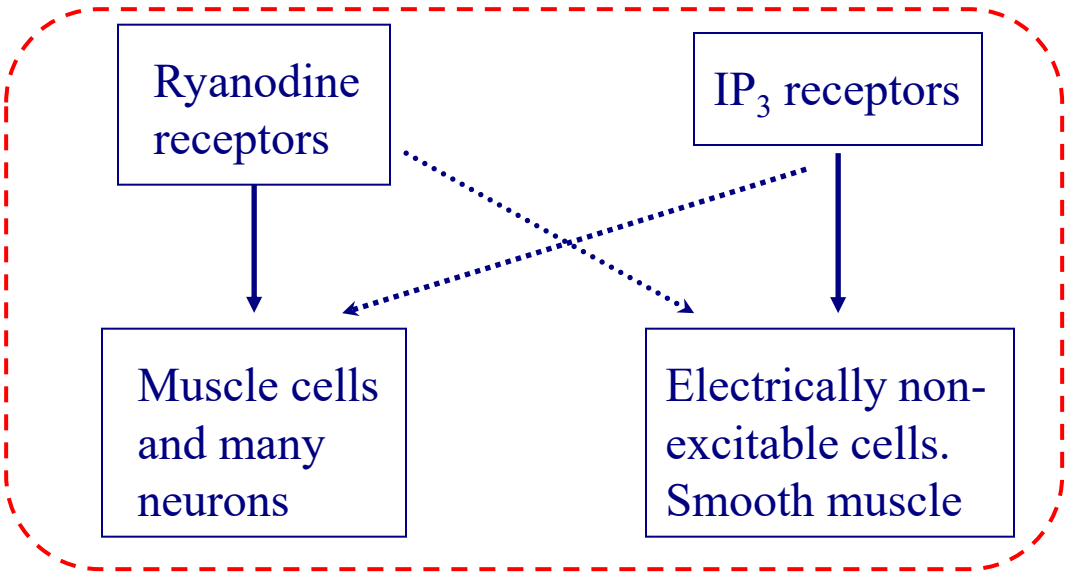


Three principal mechanisms

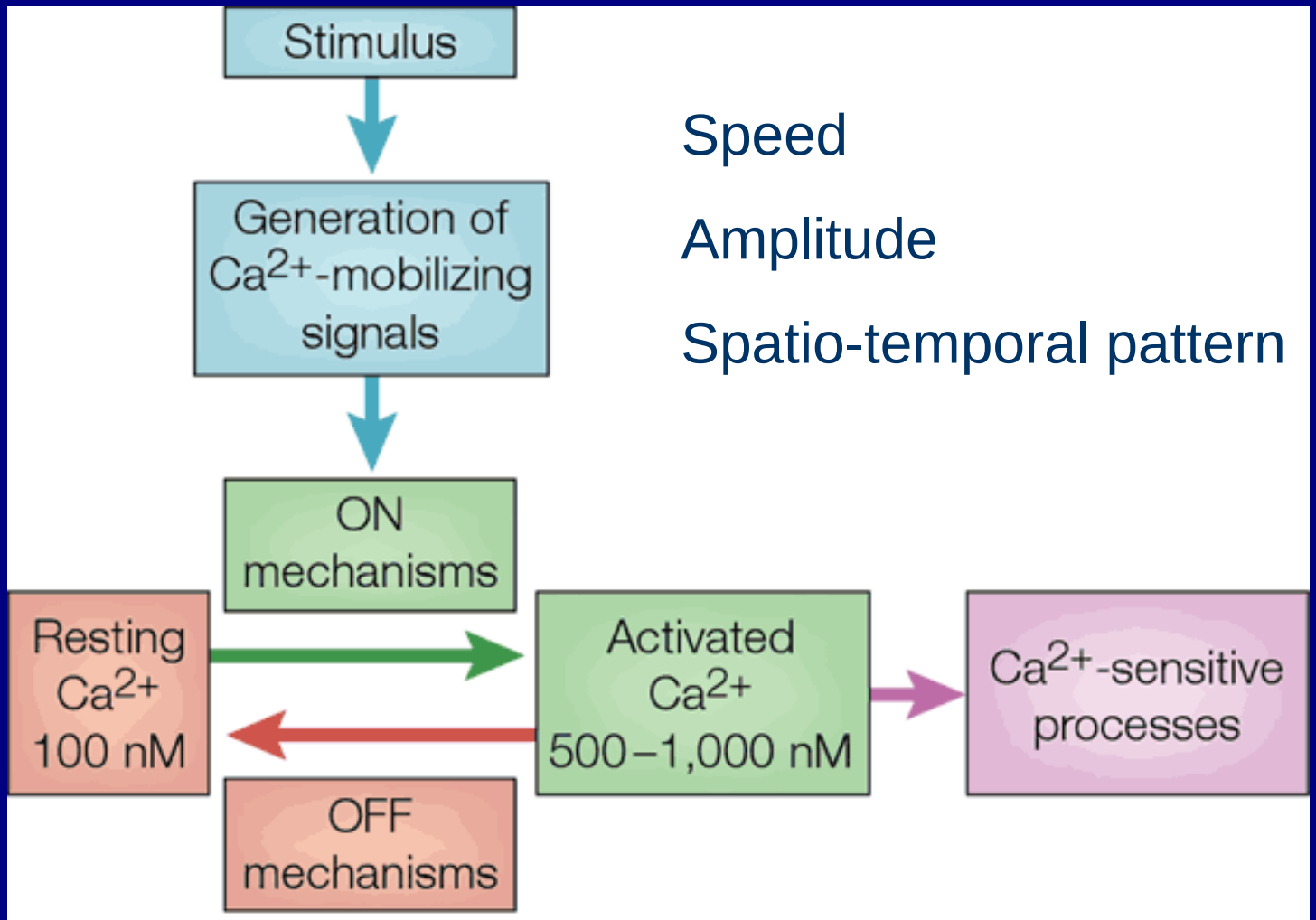
Inward flux of calcium through voltage-gated calcium channels. Dependent on fluctuations of the membrane potential.

Often seen in electrically excitable cells such as neurosecretory cells

Not dependent on membrane potential. Oscillations arise from recycling of calcium to and from internal stores (ER and mitochondria)

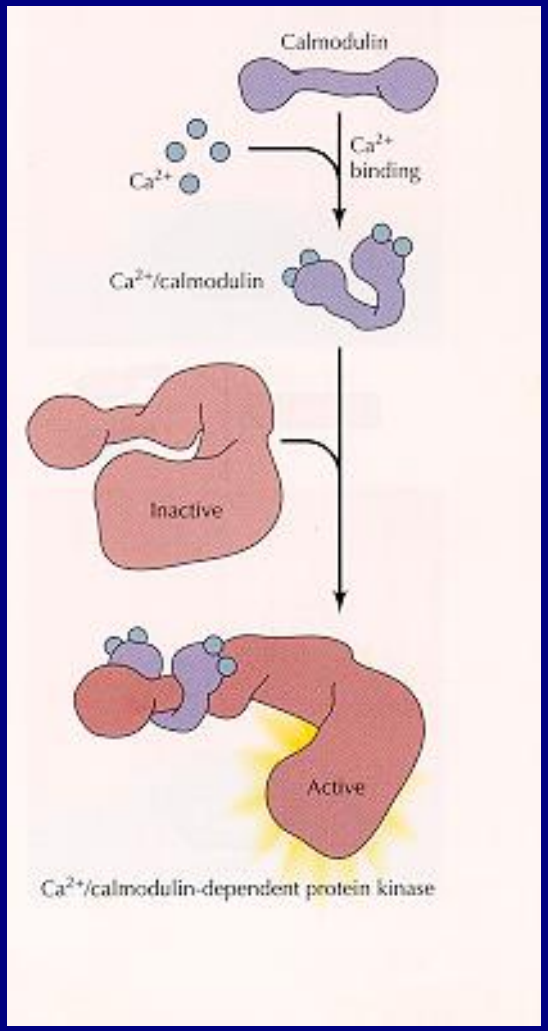


The four units of the Ca^{2+} signalling network



Nature Rev. Mol. Cell Biol. 1, 11-21 (2000)

Calmodulin as calcium buffer and inducer of calcium-induced phosphorylation cascades



Calcium cellular concentration is maintained low by pumps that transport calcium across the plasma membrane and from the cytosol inside the endoplasmic reticulum (ER). High concentrations of calcium activate the functions of proteins including protein **kinase** and **phosphatases.**

Many of the effects of calcium are mediated by the Ca++-binding protein **calmodulin,** which is activated by calcium binding when the concentration of cytosolic calcium increases from 0.1 to 0.5 micromolar.

Calmodulin, in turn, binds to a variety of target proteins including **protein kinases (CaM).**

One of the proteins activated by Ca/calmodulin is a kinase called CaM

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

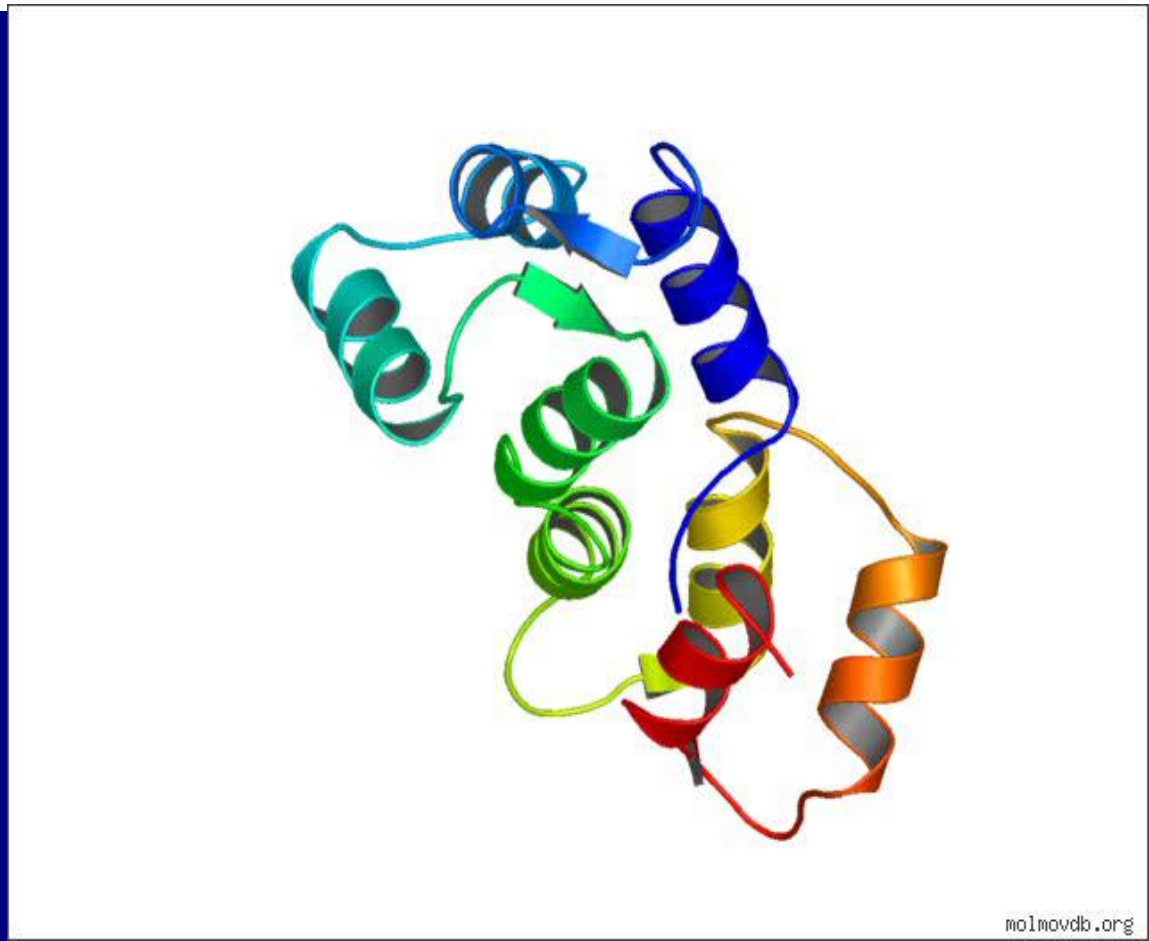
Plasma membrane Ca^{2+} ATPase (Ca^{2+} pump)

RNA helicase (p68)

Calcium is used for many signaling purposes in the cell - Why?

2. The binding of calcium to a protein can induce large conformational changes.

Calcium binds tightly to proteins. both negatively charged oxygen from glutamate and aspartate and uncharged oxygen from glutamine and asparagine bind well to calcium.

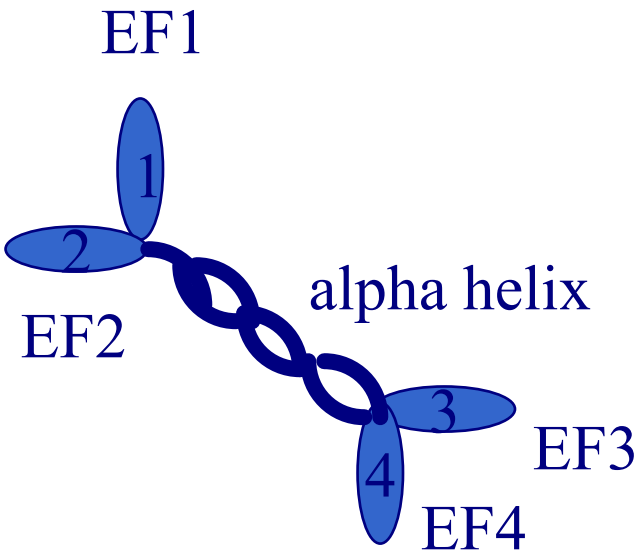


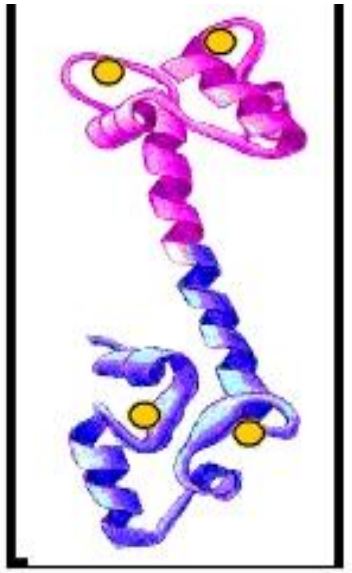
What is calmodulin and what does it do?

calmodulin is an EF hand protein that serves as a calcium sensor.

Calmodulin

- Multi-functional calcium receptor
- member of the EF hand family of proteins.
- consists of two globular lobes joined by a long alpha helix.
- Each lobe contains two EF hands. There are 4 calcium binding sites (shown as 1, 2, 3, and 4.)



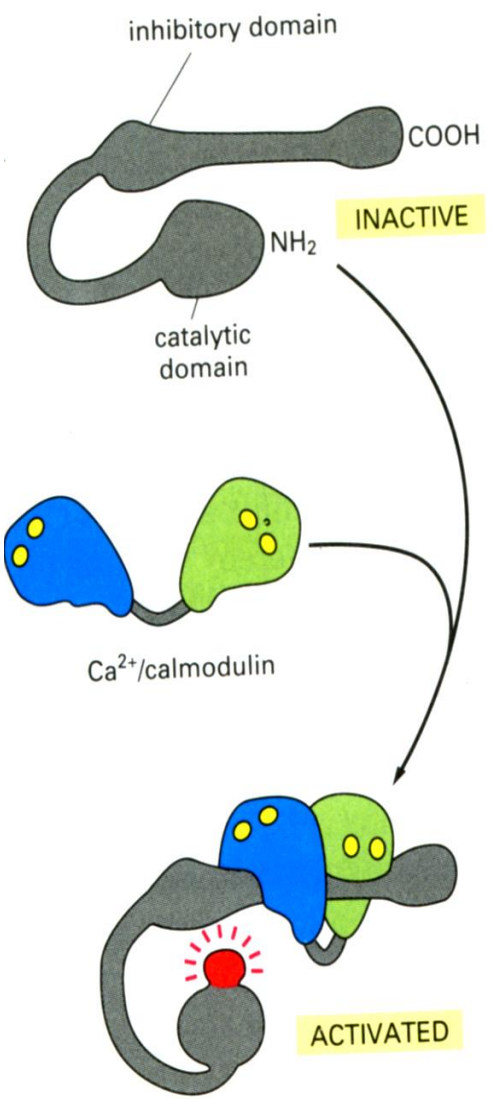


calmodulin

Calmodulin activates CaM-kinases

CaM-kinase functions as a molecular memory device, "remembering" Ca^{2+}/calmodulin activation.

Ca^{2+}/calmodulin



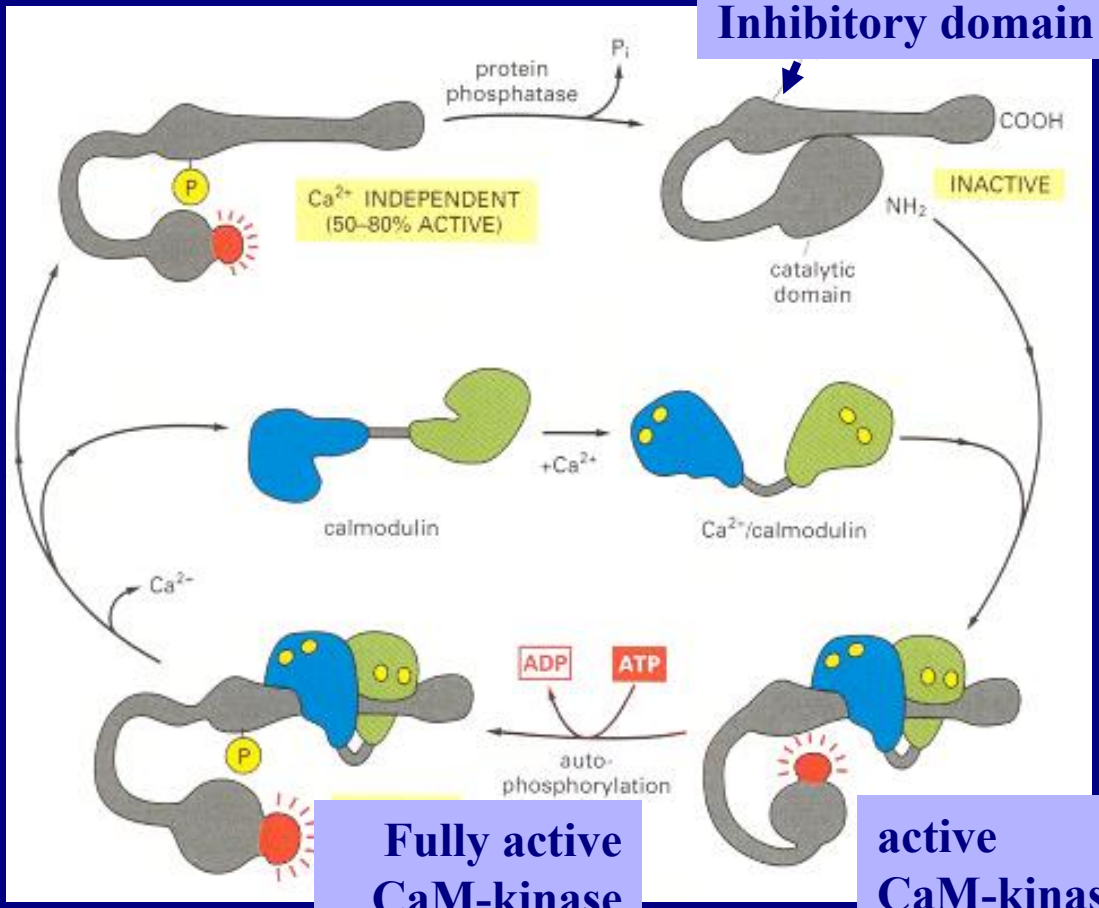
Inactive CaM-kinase

active CaM-kinase

Why is CaM-kinase II said to have a "memory"?

How?

CaM-kinase becomes activated when calmodulin binds to it and remains active after calcium withdrawal.

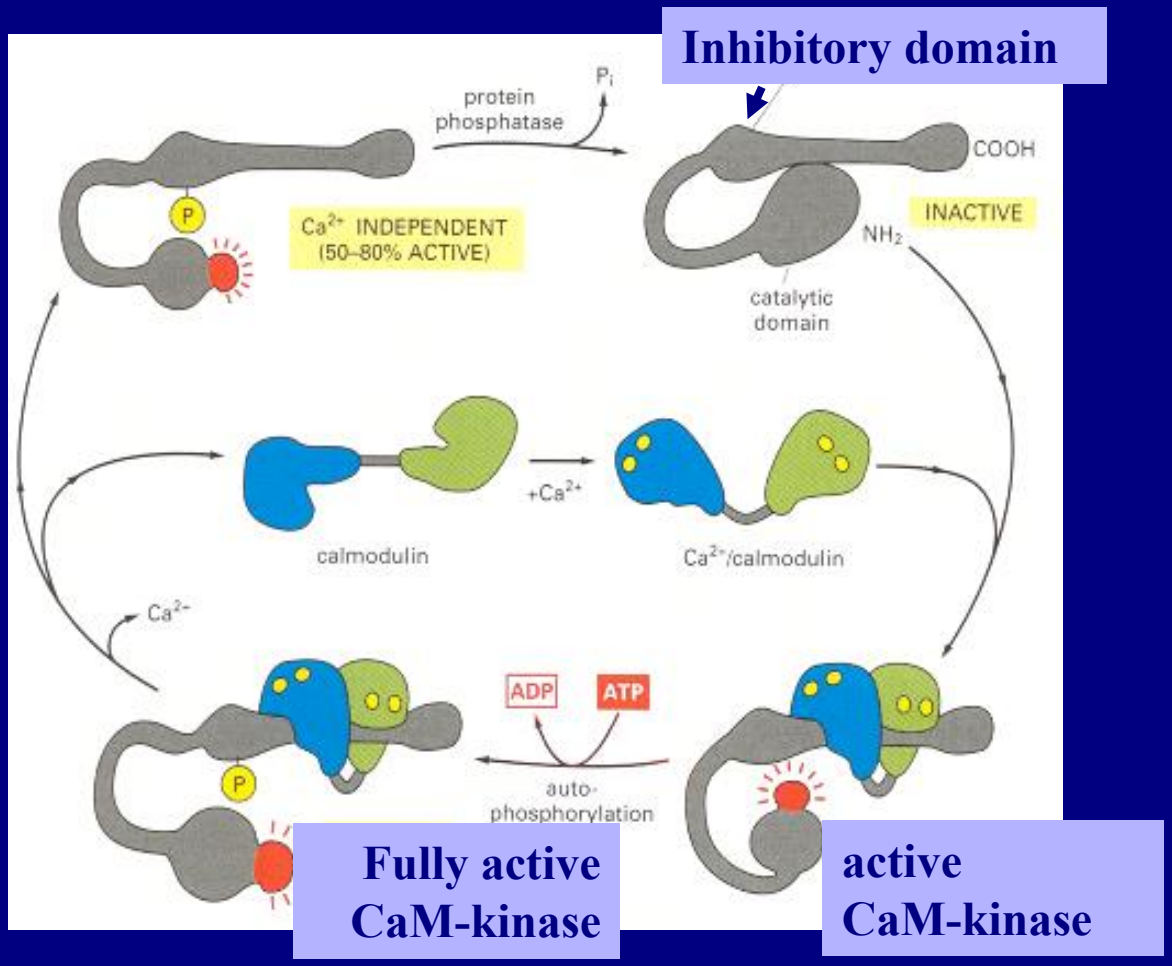


CaM-kinase has a memory

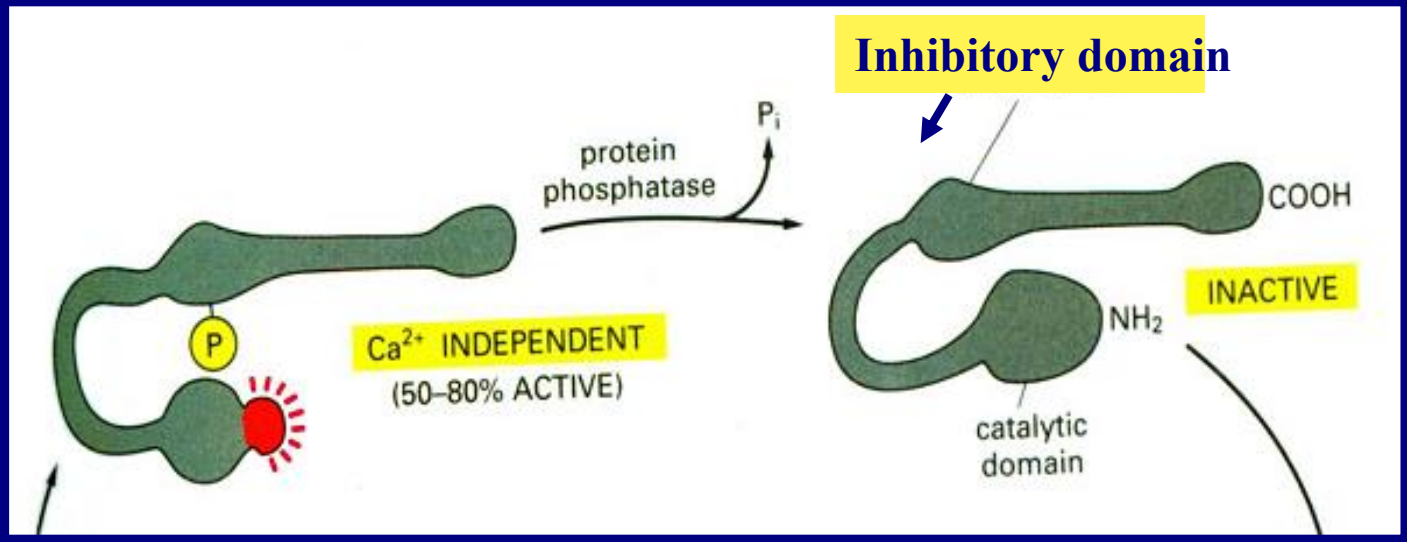
Autophosphorylation:

-traps calmodulin so that it does not dissociate from the enzyme until cytosolic calcium levels remain at base line for 10 seconds.

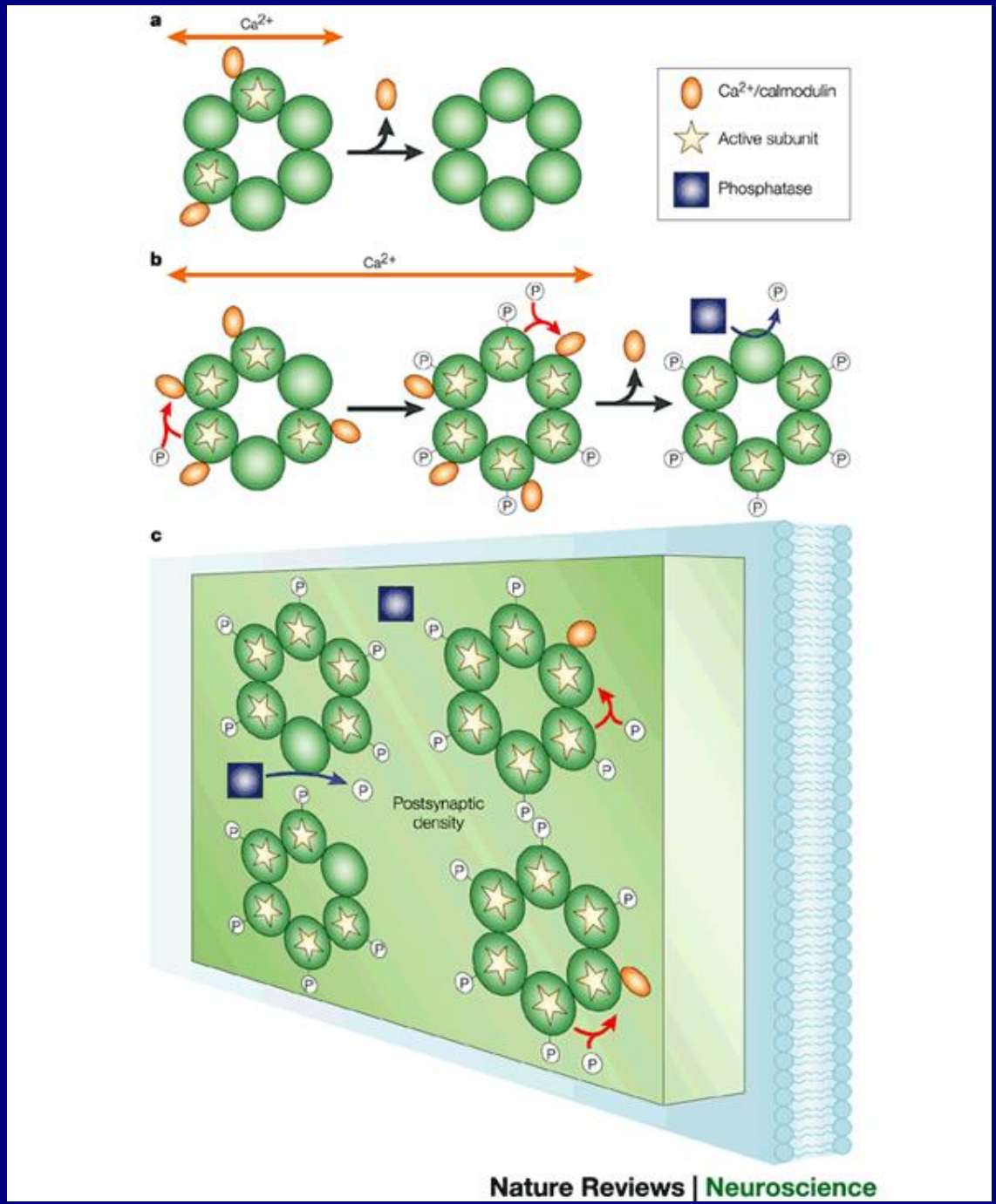
-converts the enzyme to a calcium-independent form so that the enzyme remains partially active even after calmodulin dissociates from it.



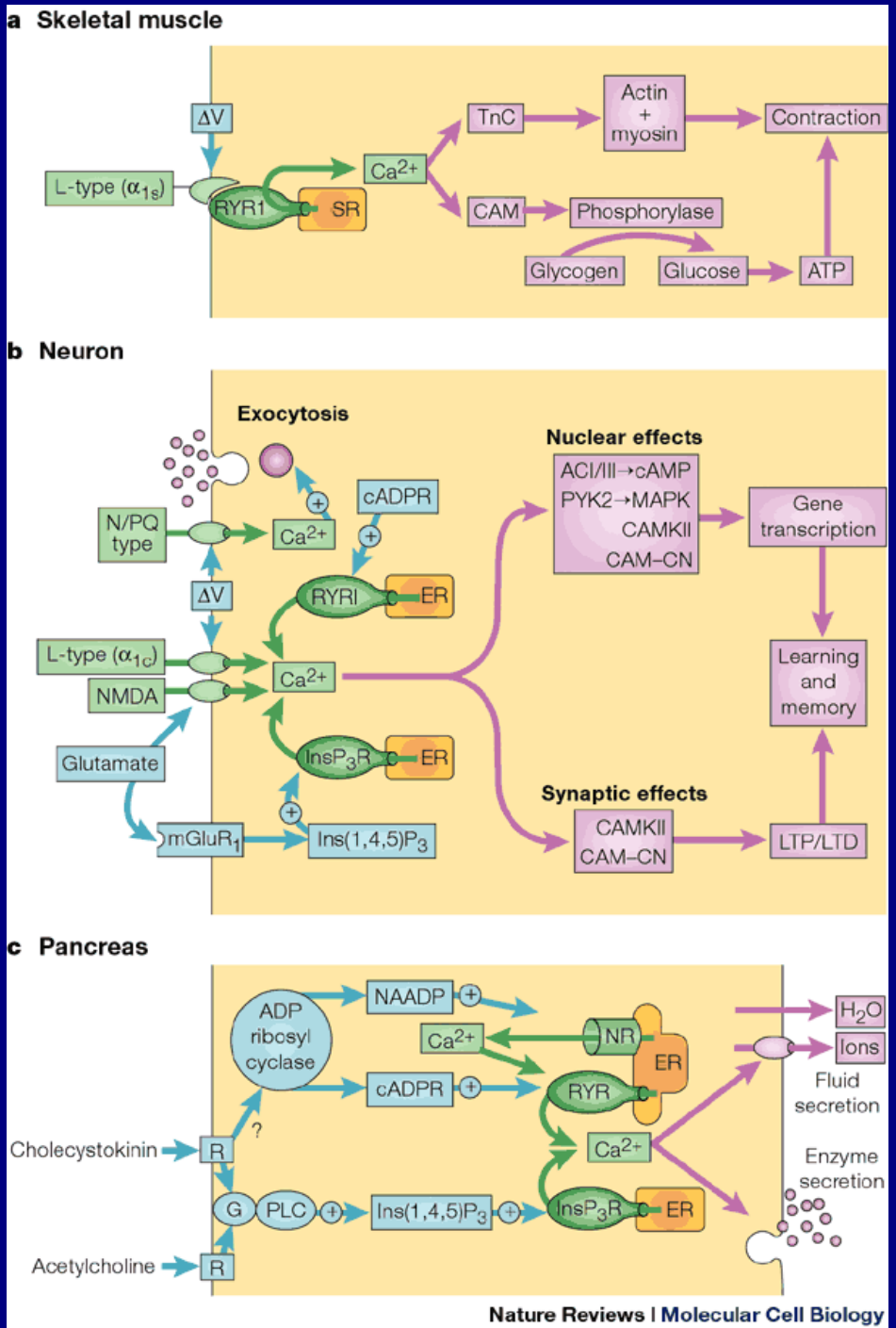
A protein phosphatase inactivates CaM-kinase



CaM-kinase remains partially active until a protein phosphatase removes the phosphate modification

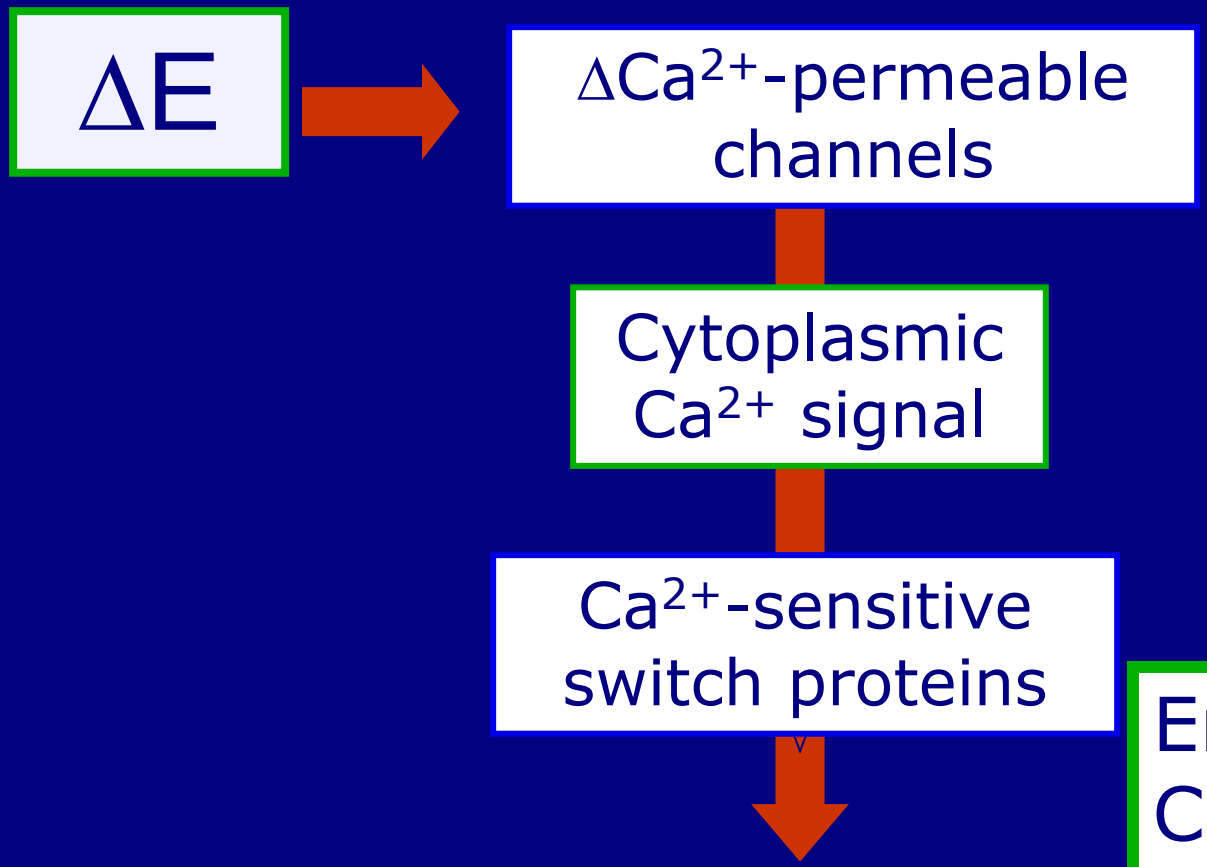

a
Ca2+
Ca2+/calmodulin
Active subunit
Phosphatase
b
Ca2+
c
Postsynaptic density
Nature Reviews | Neuroscience

Application of the Ca^{2+} signaling toolkit to regulate different cellular processes



Nature Reviews | Molecular Cell Biology

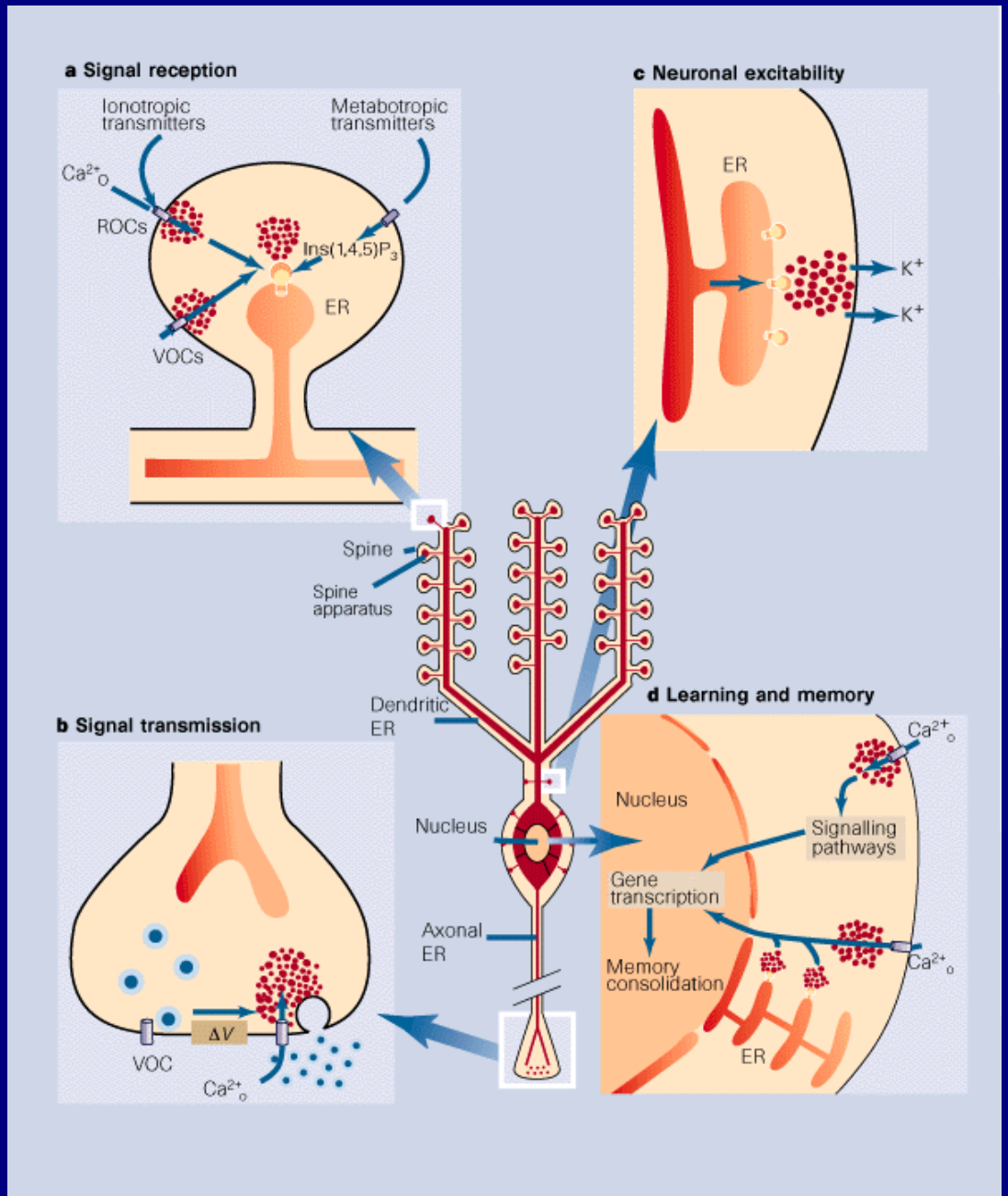
The electrical call to action



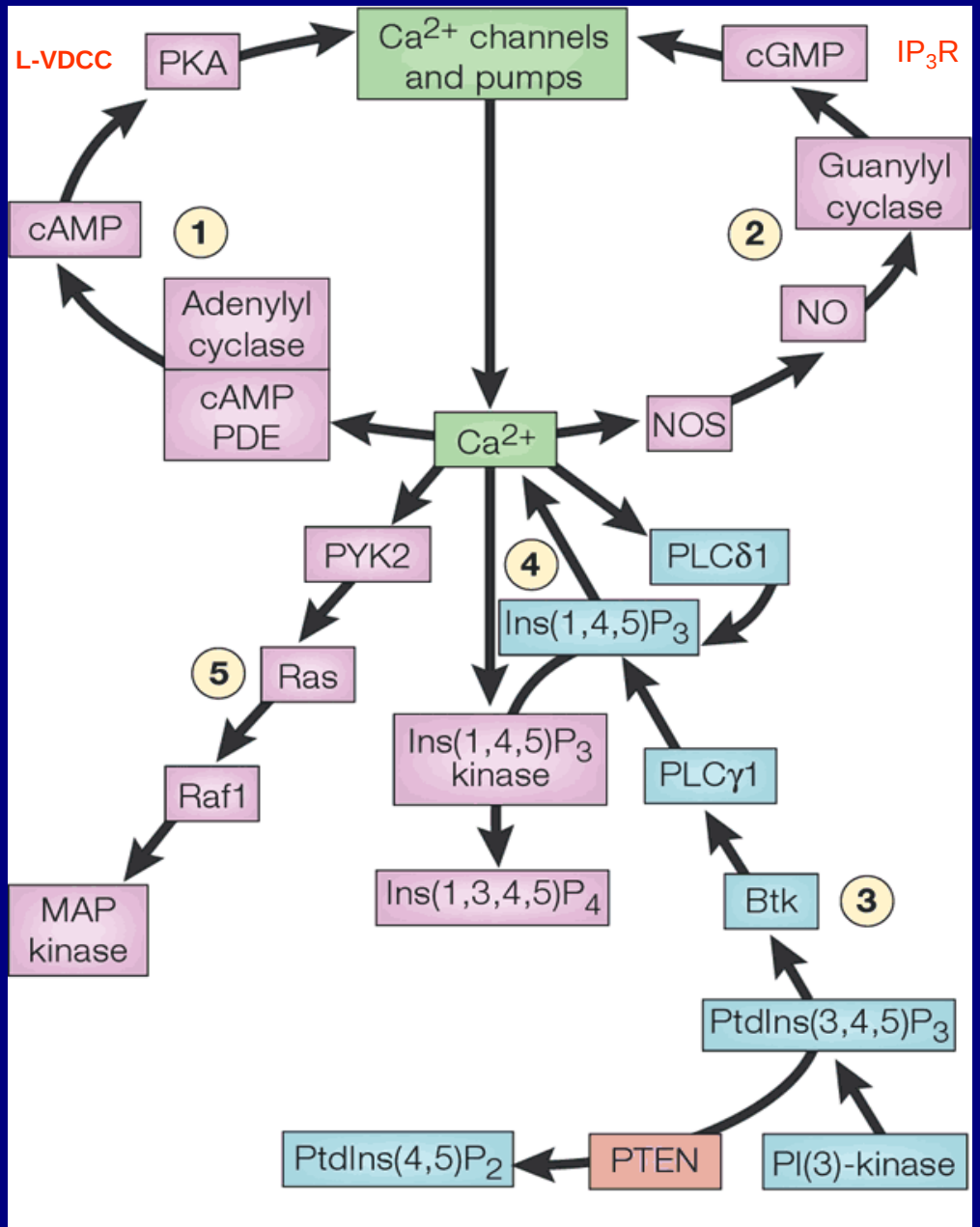
Enzyme activities
Contraction
Secretion
Channel gating
Gene expression

This is the only way that electrical signals of excitable cells are turned into biological actions.

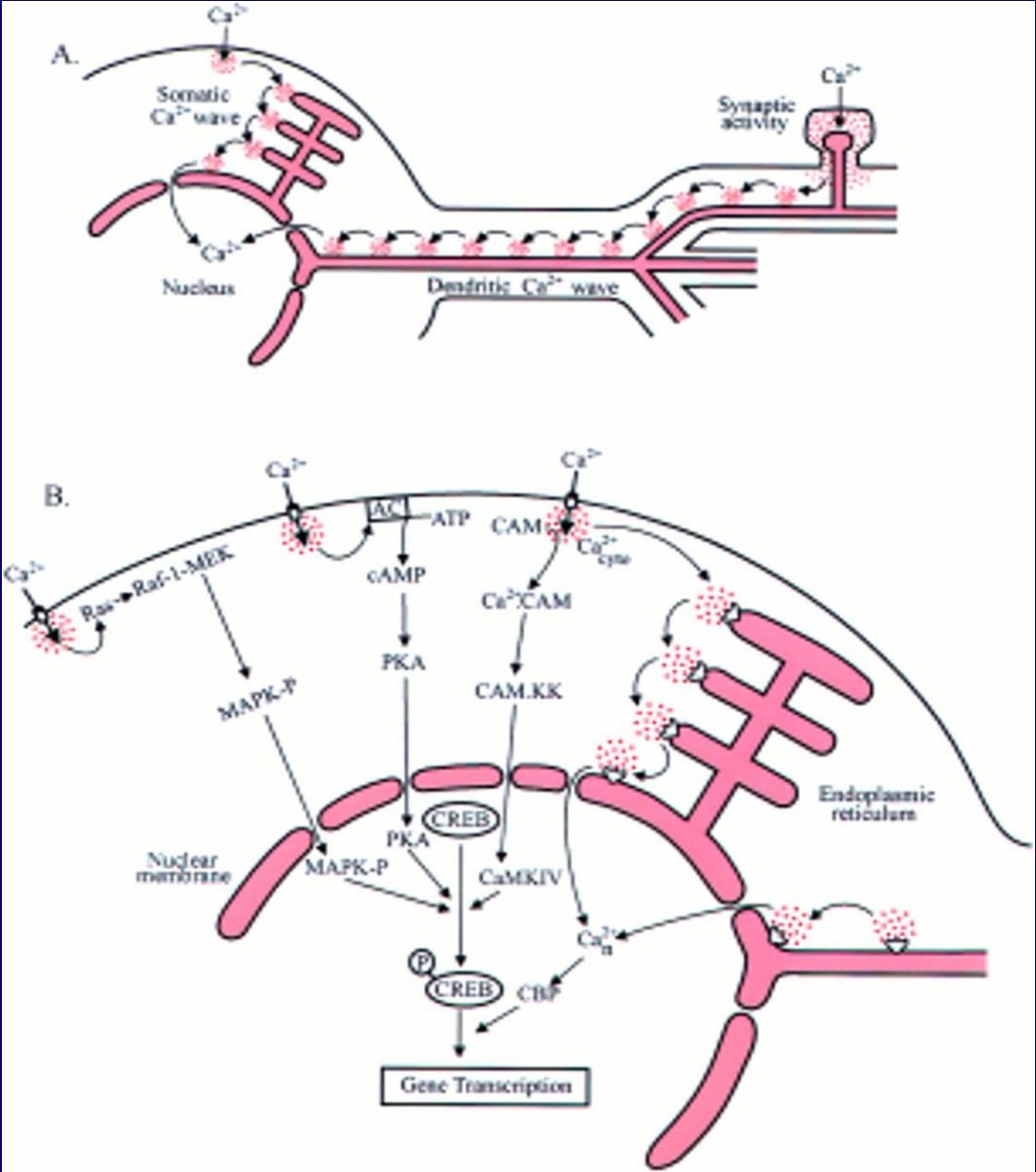
Compartmentalization of Ca^{2+} signals in neurons



A Ca^{2+} nexus — crosstalk between signalling pathways



Proposed Role of Ca^{2+} Signaling in Neuronal Gene Transcription



Ca^{2+} is a versatile intracellular second messenger

Take-home messages:

Ca controls many cellular functions

Cytoplasmic concentration is normally very low

Transporters constantly clearing Ca away

Delivered locally and quickly to cytoplasm by ion channels

May come from outside or from "Ca stores" (ER/SR)

Often acts locally

Required for translation of electrical signaling

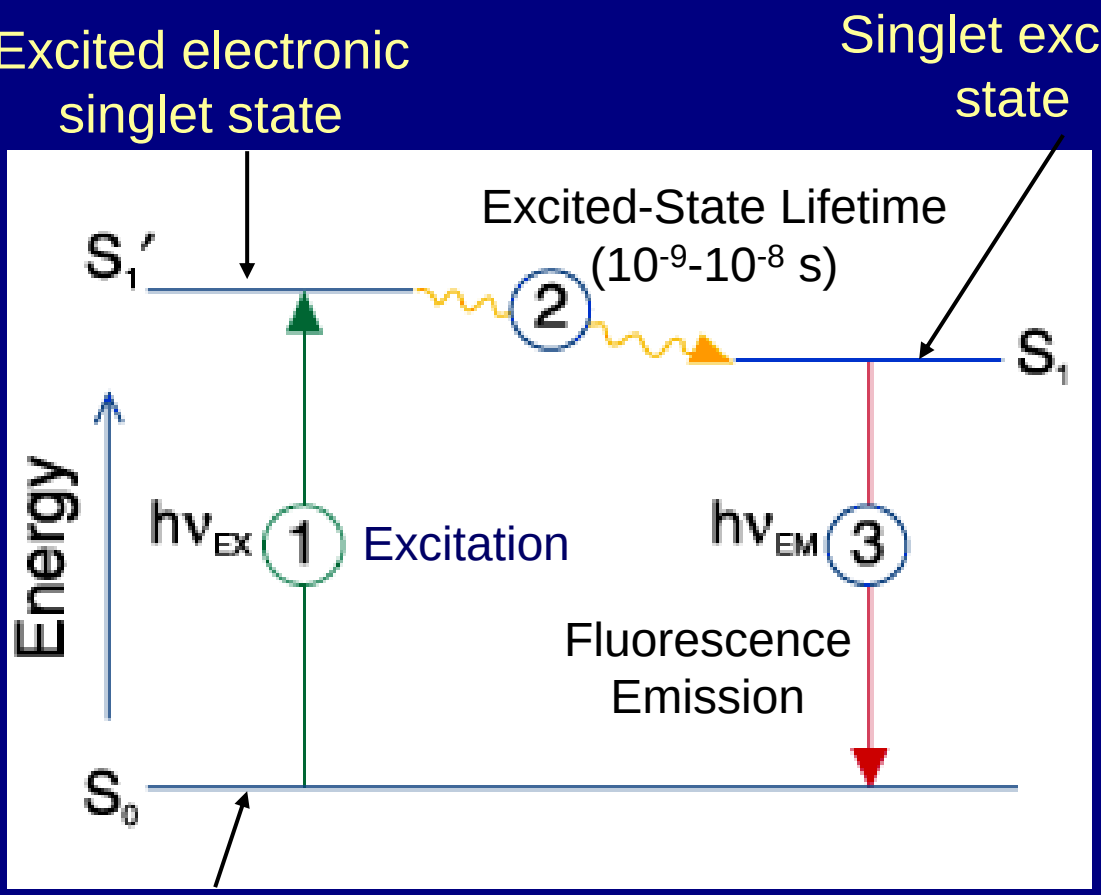
Diversity of strategies

Sensed by a diversity of Ca^{2+}-sensitive switch proteins

All eukaryotes do it -- in all cells

Techniques for measurements of variations of intracellular free calcium concentration $[Ca^{2+}]_i$

JABLONSKI DIAGRAM

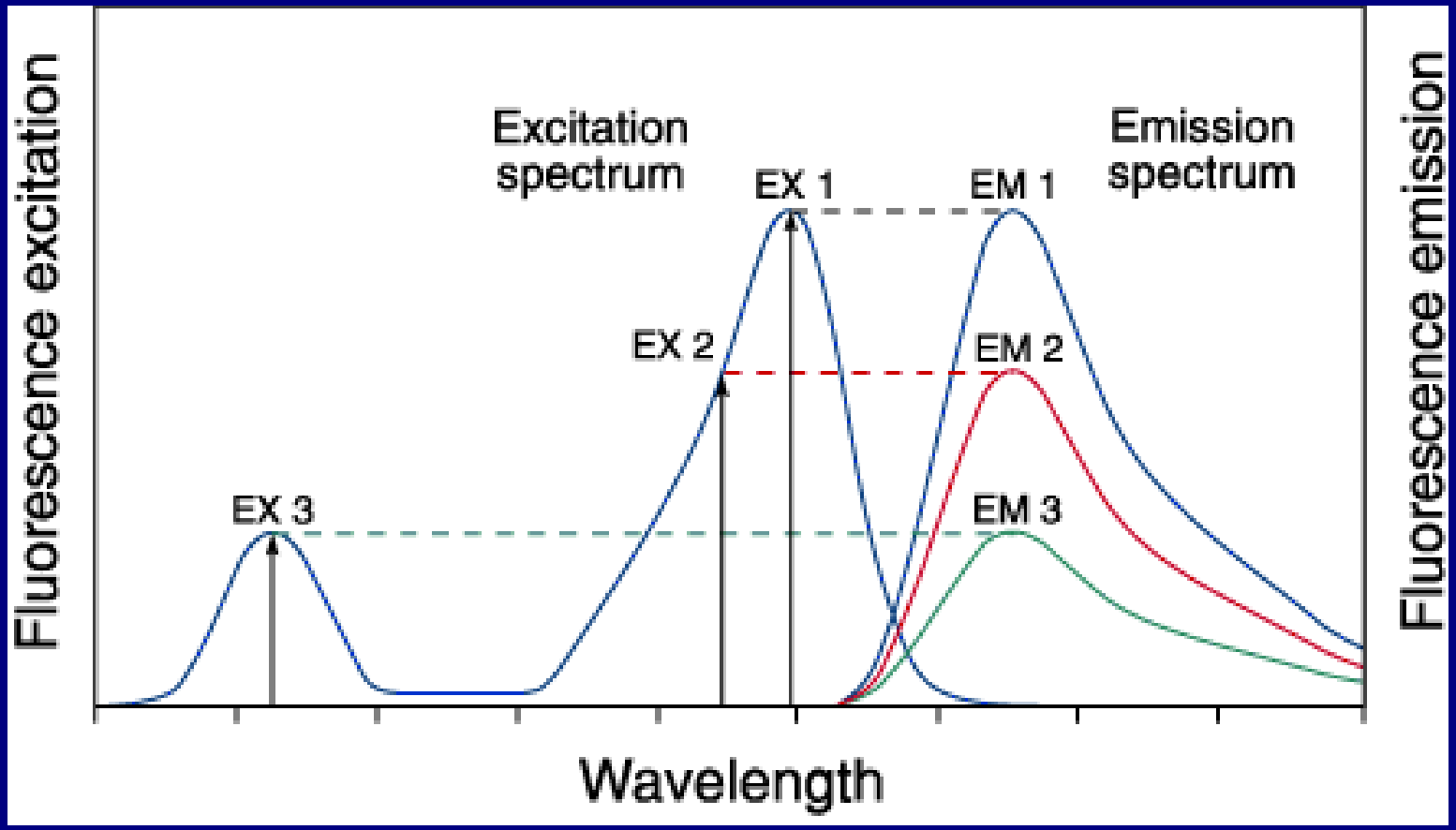


Stokes shift
$h\upsilon_{EX} - h\upsilon_{EM}$

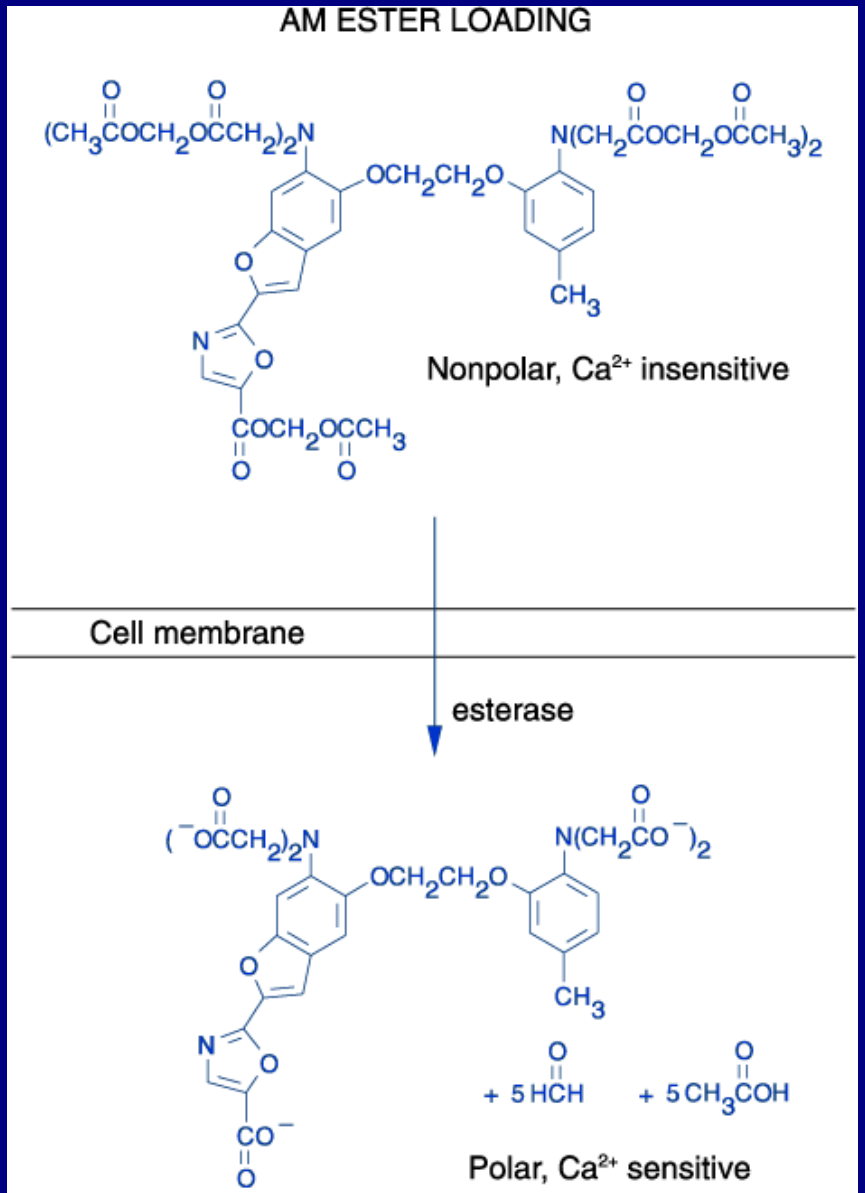
FLUORESCENCE QUANTUM YIELD

$$\frac{\#\text{ fluorescence photons emitted (Stage 3)}}{\#\text{ fluorescence photons absorbed (Stage 1)}}$$

FLUORESCENCE SPECTRA

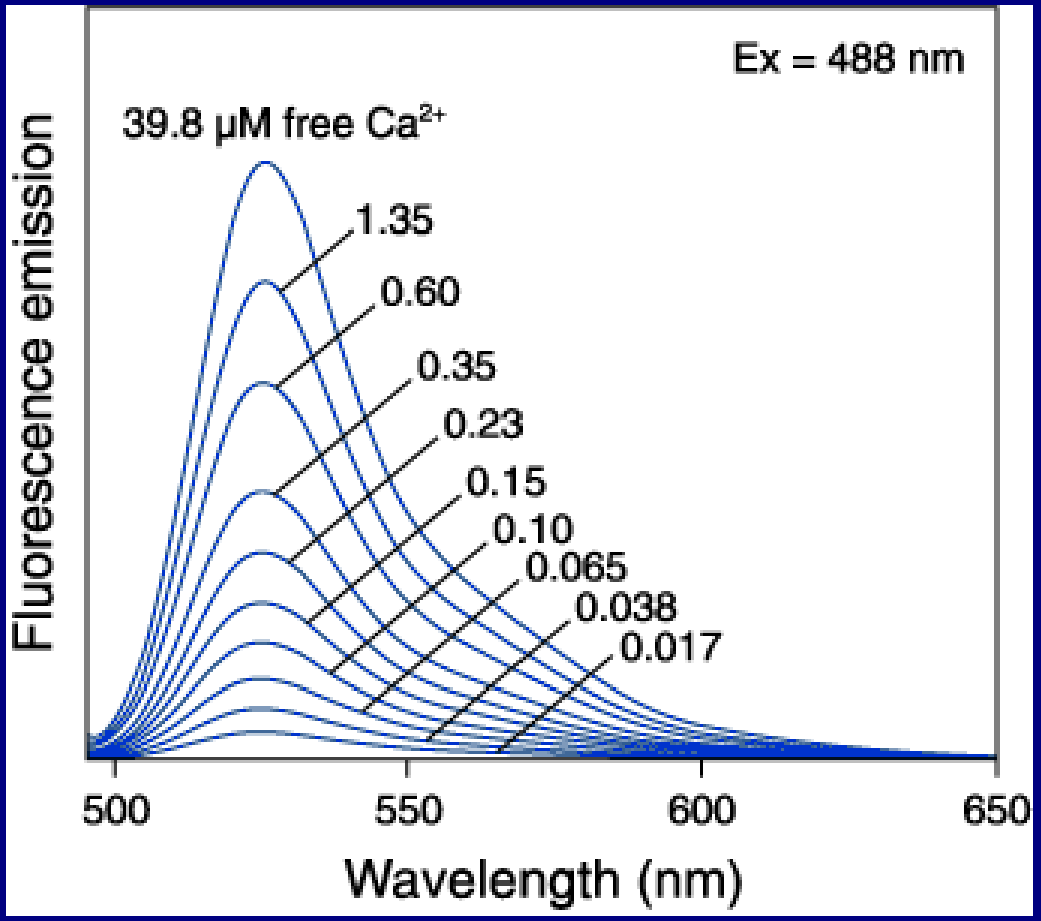


SCHEMATIC DIAGRAM OF LOADING THE CELLS USING ACETOXYMETHYL (AM) ESTER DERIVATIVE FURA-2/AM

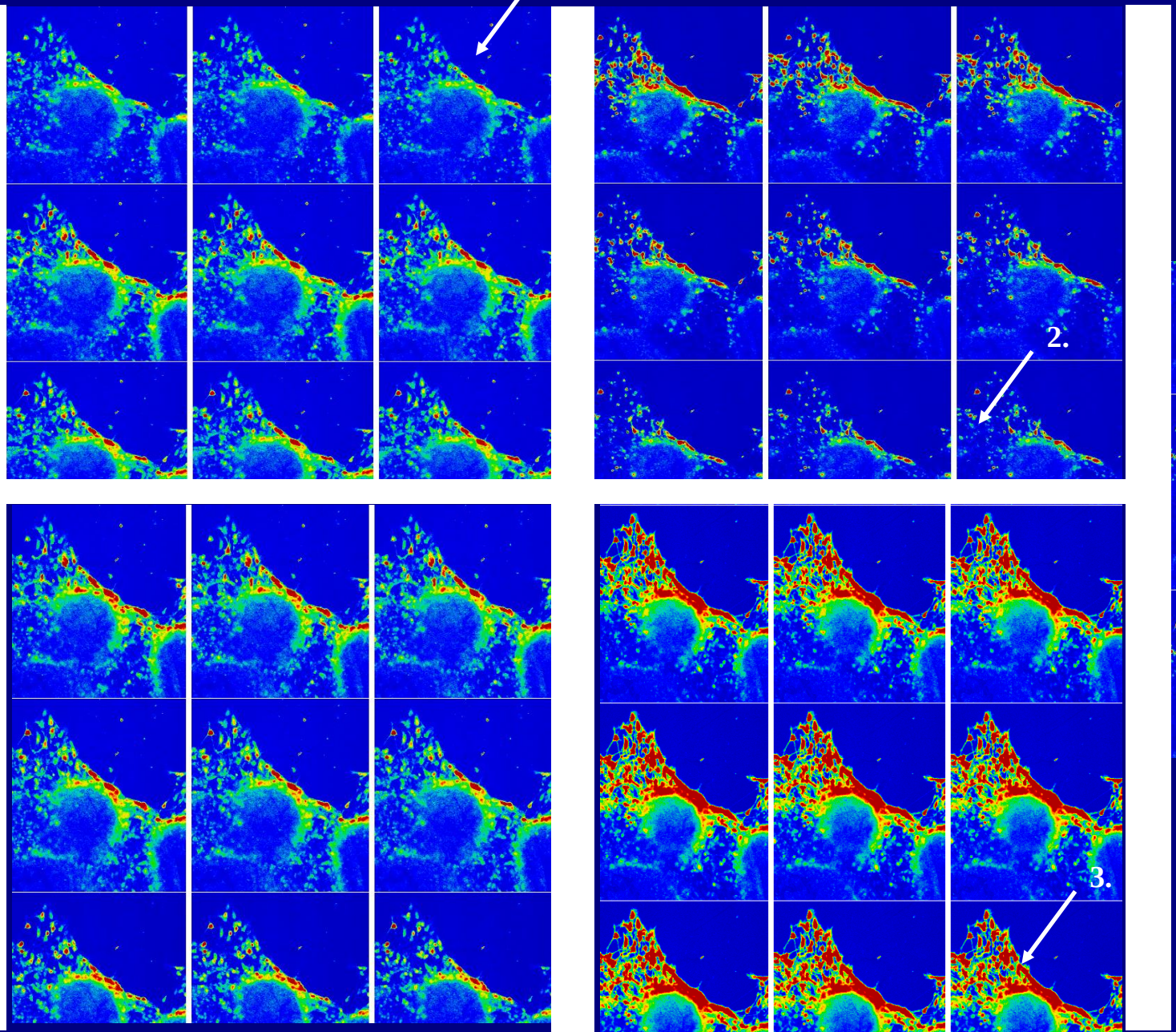


PROBLEMS:
Compartmentalization
Incomplete AM ester hydrolysis
Leakage

Ca^{2+}-DEPENDENT FLUORESCENCE EMISSION SPECTRA OF FLUO-3

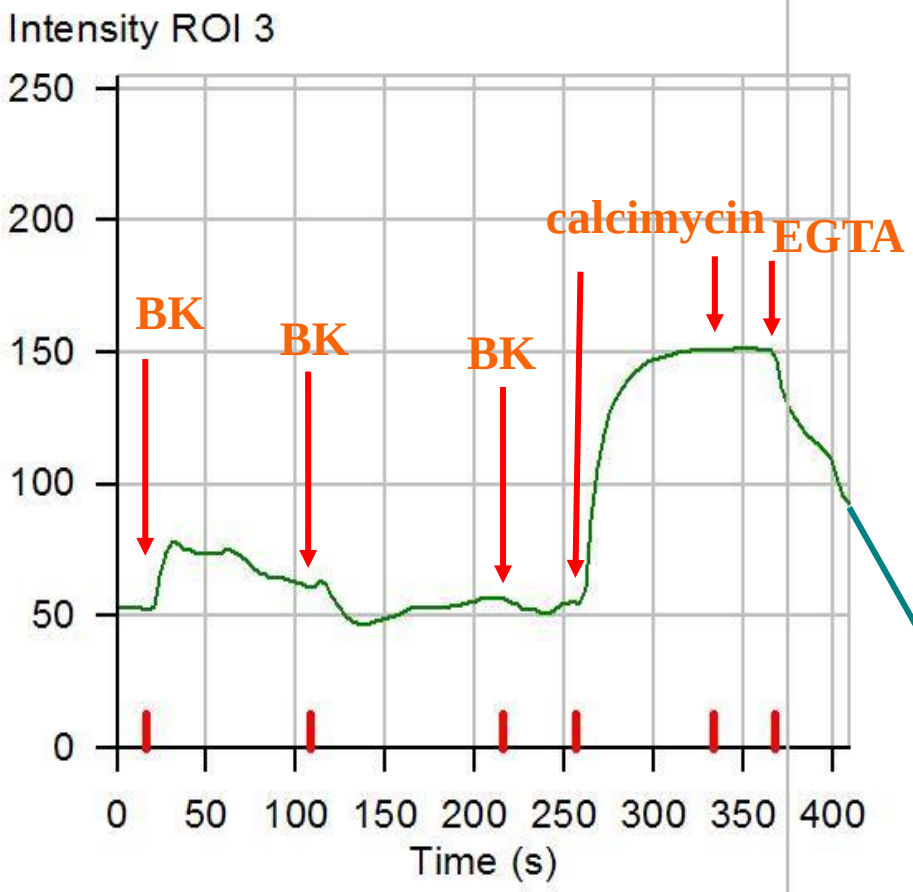


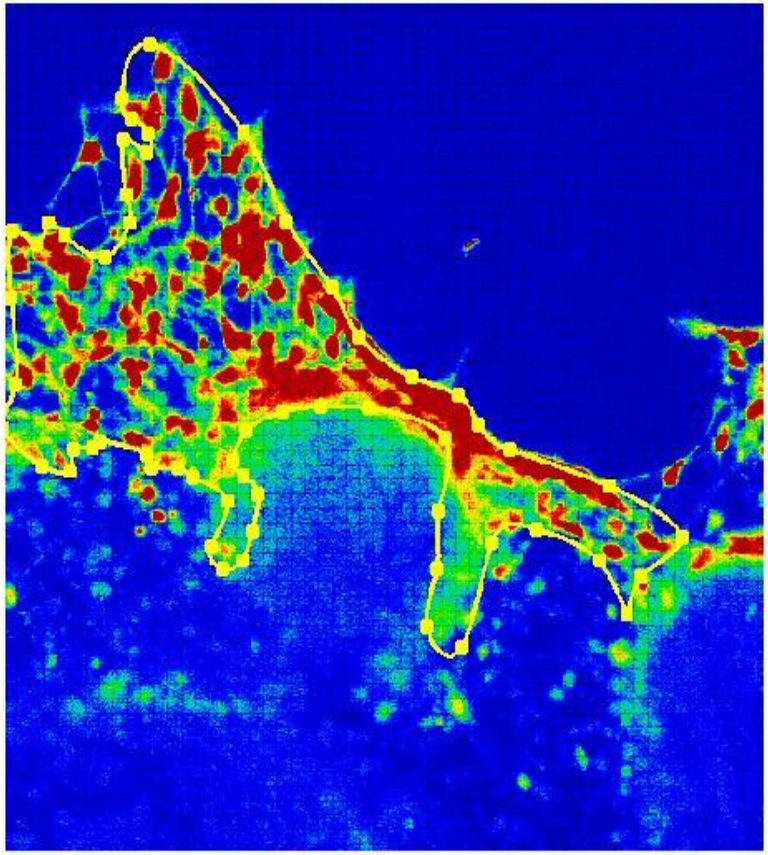
Stimulation of Iransient Increase in Cytosolic Calcium in Neural Progenitor Cells



1. Bradykinin (B
2. Ionophore
3. EGTA

Analysis of a Calcium Imaging Experiment





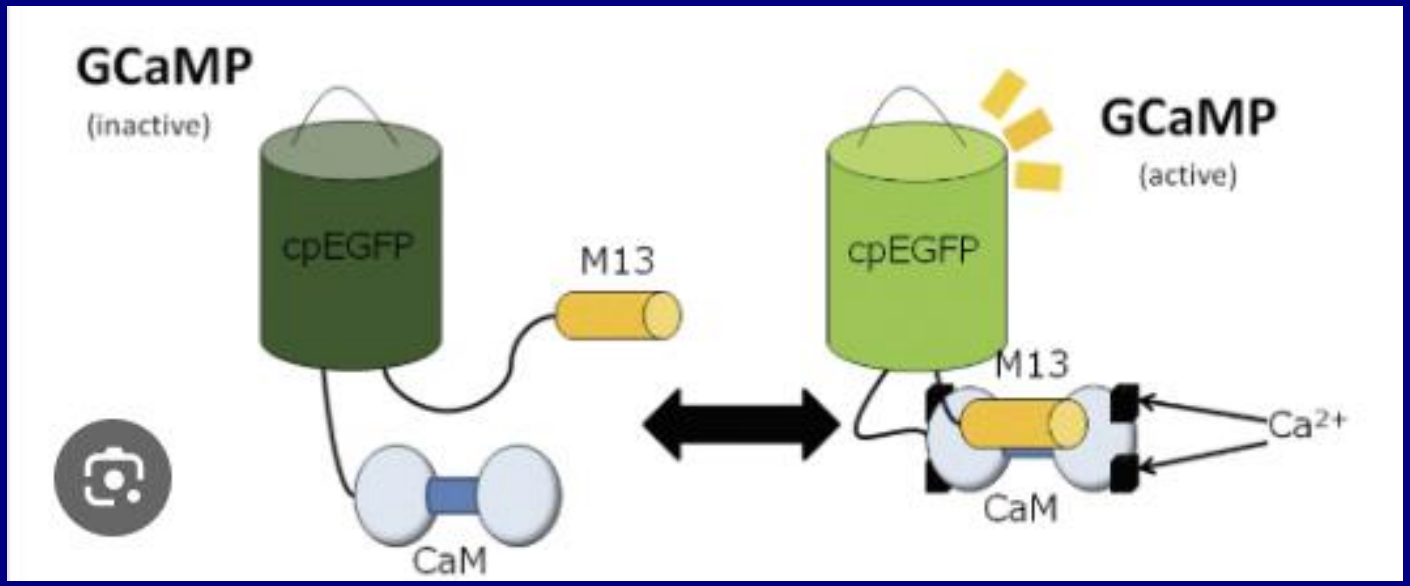
$$[Ca^{2+}]_i = K_d(F-F_{min})/(F_{max}-F)$$

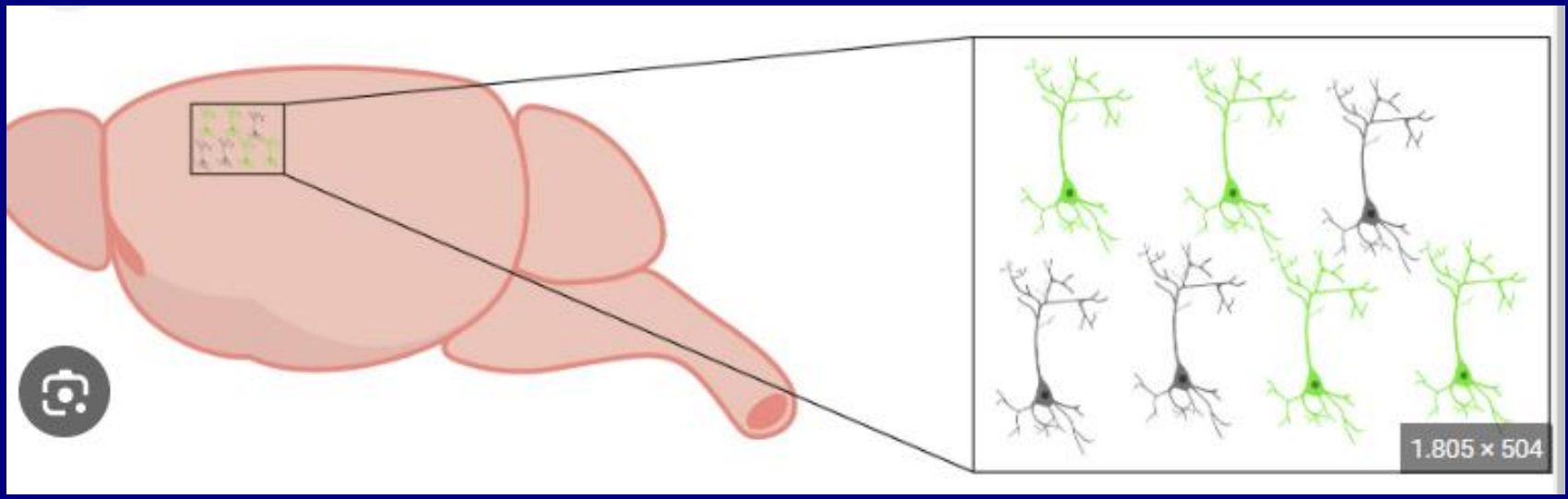
Genetically Encoded Calcium Indicators (GECIs) like GCaMP are molecules designed to monitor the concentration of calcium ions within cells

. GCaMP is widely used to study the dynamics of calcium signaling in living cells, particularly neurons.

GCaMP consists of a fluorescent protein, a calcium-binding protein (calmodulin), and a peptide sequence that undergoes conformational changes in response to calcium binding.

When calcium ions bind to calmodulin, the conformational change results in an increase in fluorescence, allowing researchers to detect changes in calcium levels within cells.


GCaMP
(inactive)
cpEGFP
M13
CaM
GCaMP
(active)
cpEGFP
M13
Ca2+
CaM


1.805 × 504