

PHSI3009: Calcium Signalling

Professor Philip Poronnik

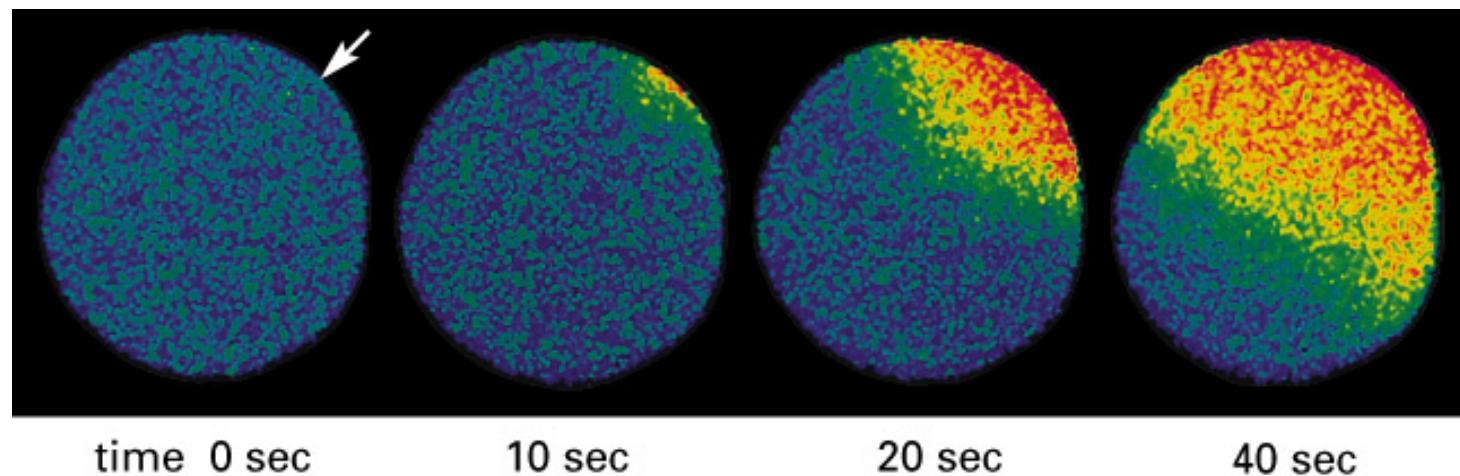


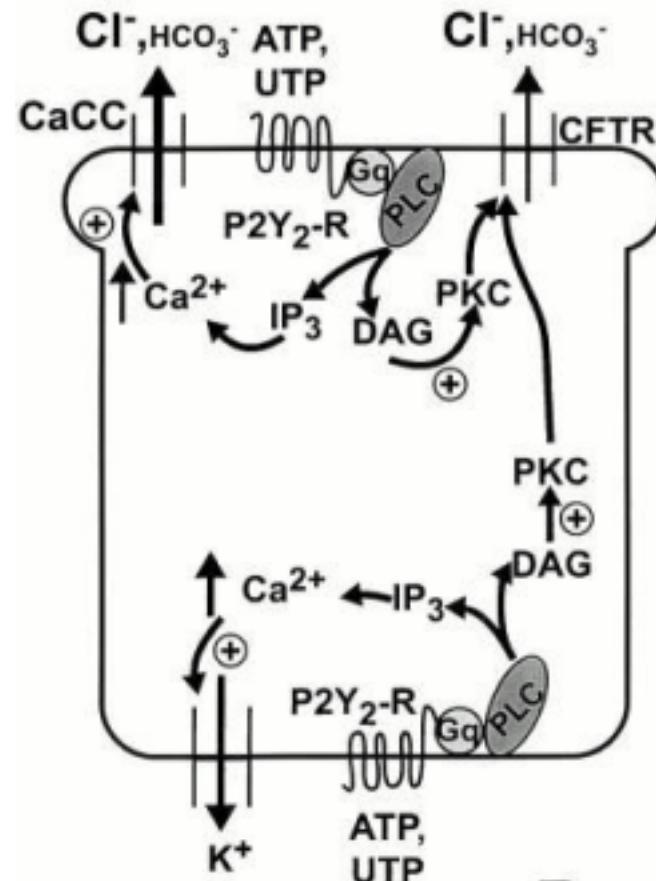
Figure 15–37. Molecular Biology of the Cell, 4th Edition.

Objectives

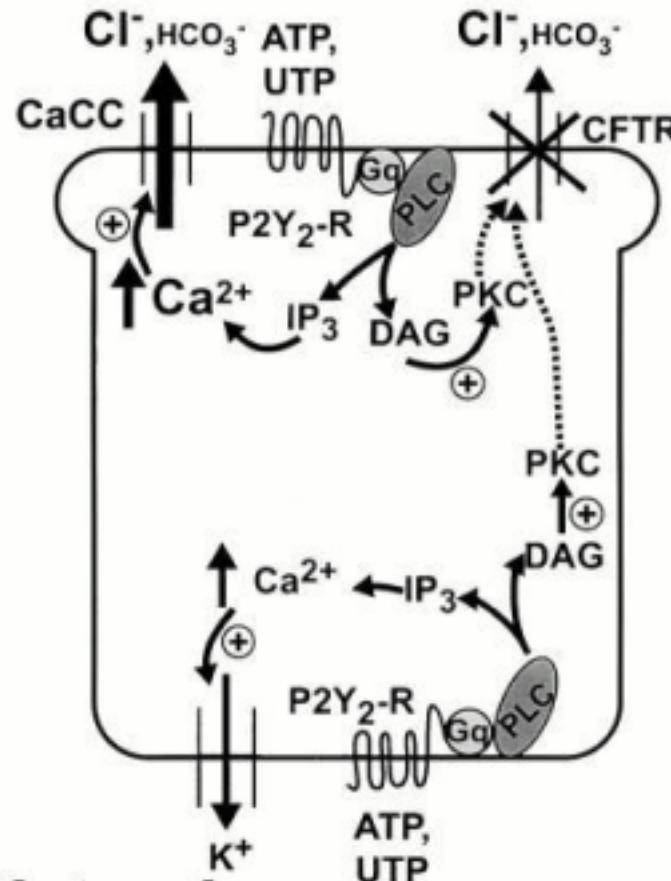
- Understand the role of calcium
- Explain how it is distributed in cells
- Describe the Ca signalling toolkit
- Describe the key components of the pathways
- Explain GPCR/Gq/IP3 pathway in detail
- *Understand how to measure Ca with dyes*

Apical

Normal



CF



Basolateral



Biochemical Society Award

Delivered at the Biochemical Society Centenary Event held at the Royal Society, London, on 16 December 2011

Sir Michael Berridge

Abstract

A wide range of Ca^{2+} signalling systems deliver the spatial and temporal Ca^{2+} signals necessary to control the specific functions of different cell types. Release of Ca^{2+} by InsP_3 (inositol 1,4,5-trisphosphate) plays a central role in many of these signalling systems. Ongoing transcriptional processes maintain the integrity and stability of these cell-specific signalling systems. However, these homoeostatic systems are highly plastic and can undergo a process of phenotypic remodelling, resulting in the Ca^{2+} signals being set either too high or too low. Such subtle dysregulation of Ca^{2+} signals have been linked to some of the major diseases in humans such as

cardiac disease, schizophrenia, bipolar disorder and Alzheimer's disease.

Introduction

Calcium (Ca^{2+}) is a highly versatile intracellular signal capable of regulating many different processes [1]. To achieve this versatility, the signalling system operates in many different modes, thus enabling it to function over a wide dynamic range. It can trigger exocytosis at synaptic endings within microseconds and muscle contraction in milliseconds, whereas, at the other end of the scale, it can operate over minutes to hours to drive processes such as gene transcription and cell proliferation. This versatility depends on each cell type having a specific Ca^{2+} signalling mechanism that is assembled from a very extensive Ca^{2+} signalling toolkit [1,2]. As cells differentiate, they express a unique set of toolkit components to create Ca^{2+} signalling systems with widely different spatial and temporal properties. Such Ca^{2+} signalling systems are not set in stone, but are constantly being remodelled to adapt to changing circumstances to ensure that each specific cell type continues to deliver the Ca^{2+} signals that characterizes its unique function. If the spatiotemporal properties of this output signal change due to a loss or defect of a key component, compensatory mechanisms come into play to restore the normal output signal. This remodelling

Keywords: Alzheimer's disease, bipolar disorder, calcium, heart, insitol, schizophrenia

Calcium signalling remodelling and disease. Berridge MJ.

Biochem Soc Trans. 2012 Apr;40(2):297-309

Calcium ‘selected’ by evolution as an intracellular messenger in preference to other monoatomic ions

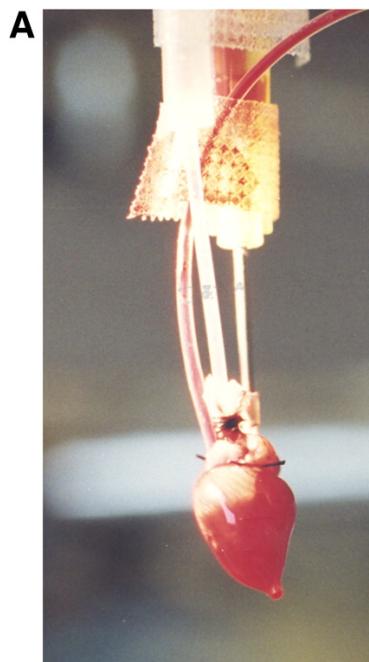
- Divalent - stronger protein binding than monovalent ions.
- More flexible than smaller divalent Mg²⁺ ions → more effective coordinate with protein-binding sites.
- Energetically favourable as 2nd messenger (large [Ca²⁺] gradient) (10^{-7} vs. 10^{-3} M) – rel small amt needed to enter cell to incr signaling → relatively little energy needed to pump it back out of the cell.

Sidney Ringer



Sidney Ringer

1853 found that London tap water supported frog heart contraction while distilled water did not – Ringer's solutions!



Ca^{2+} signalling toolkit

- Receptors
- Ca^{2+} channels
- Ca^{2+} pumps and exchanges
- Ca^{2+} sensors
- Ca^{2+} sensitive cellular processes (insulin secretion, neurotransmitter release, actin remodelling etc !

Ca^{2+} as a signal

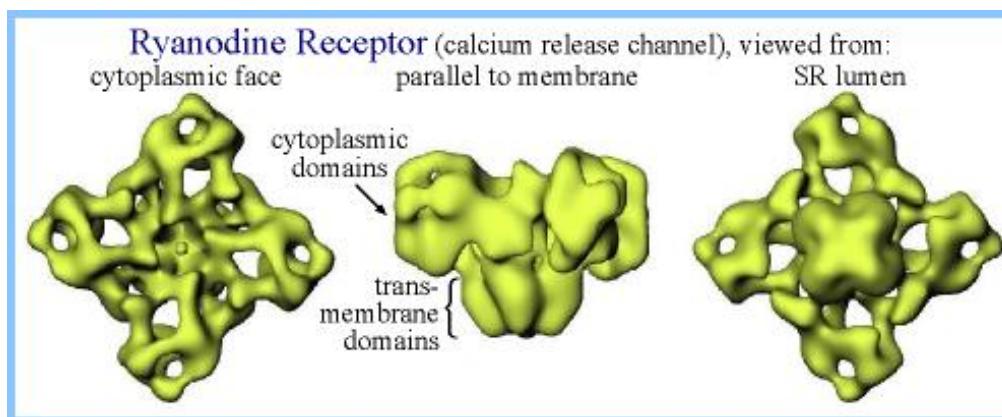
- Can come from outside or inside (rapidly mobilized).
- Ca^{2+} shut-off pathways (rapidly removed or sequestered)
- Voltage-gated Ca^{2+} channels inactivate
- IP_3 rapidly dephosphorylated
- Ca^{2+} rapidly pumped out (or taken up)

Ca²⁺ channels

- Voltage gated (N, T and L)
- Store operated (Crac) - Orai
- Non-selective cation channels (Trps) – stretch sensitive, capsaicin etc...
- Ligand gated- eg - P2X – ATP,

ER/SR main intracellular stores

- Bound to calsequestrin
- Ryanodine and IP_3 receptors (ns channels!)
- Lysosomes also important sources of Ca^{2+}
- Mito and nucleus – not so critical



Main transporters

- PMCA – plasma membrane Ca^{2+} -ATPase
- Na-Ca (NCX) exchanger (very important in muscle)
- SERCA – sarco-Endoplasmic reticulum Ca-ATPase (up to 80% of membrane protein in ER!)

Calcium uptake and deprivation

1. Na/Ca exchanger on plasma membrane, 2. Ca pump on ER membrane, 3. Ca binding molecules, 4. Ca pump on Mitochondria

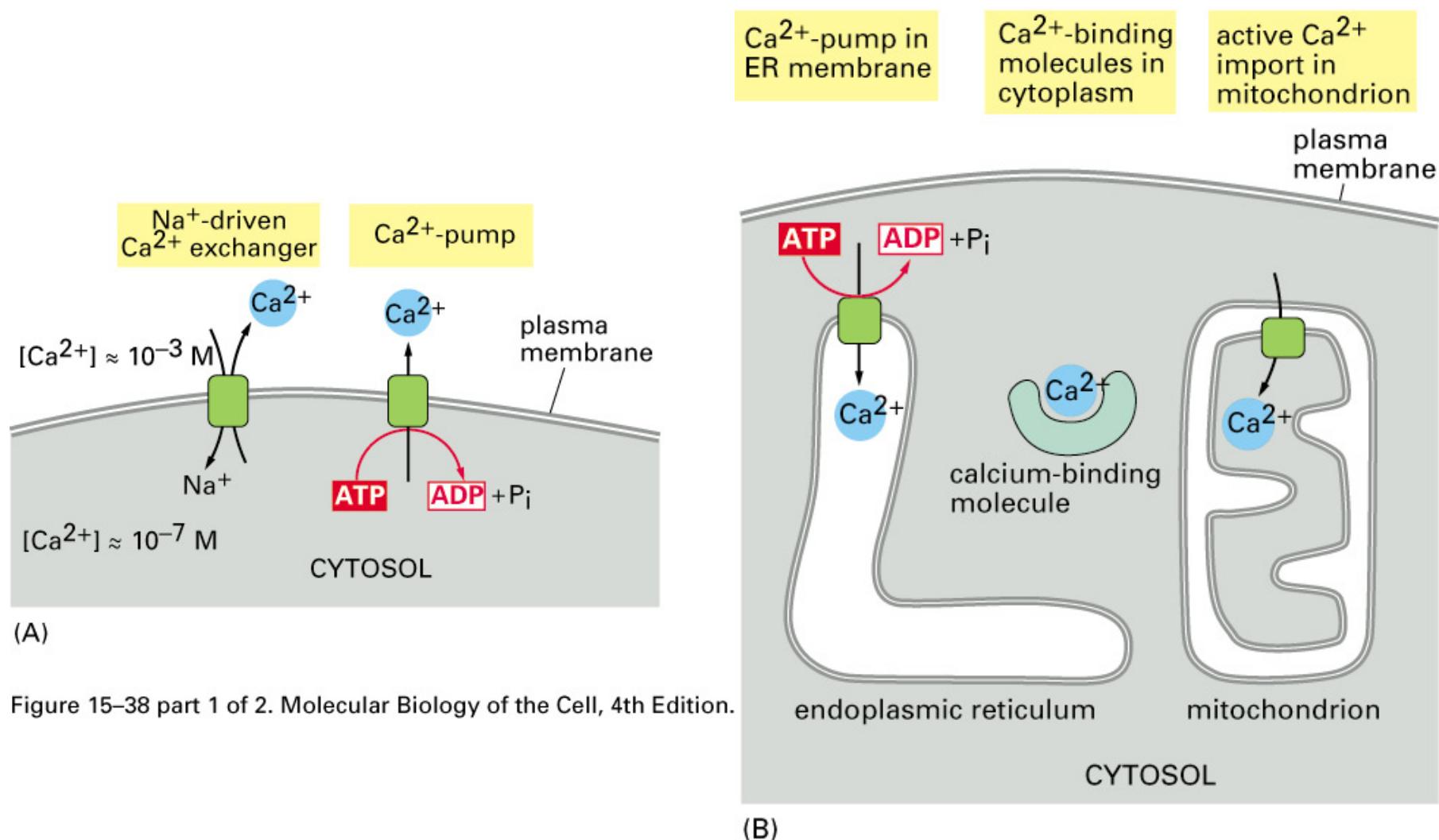
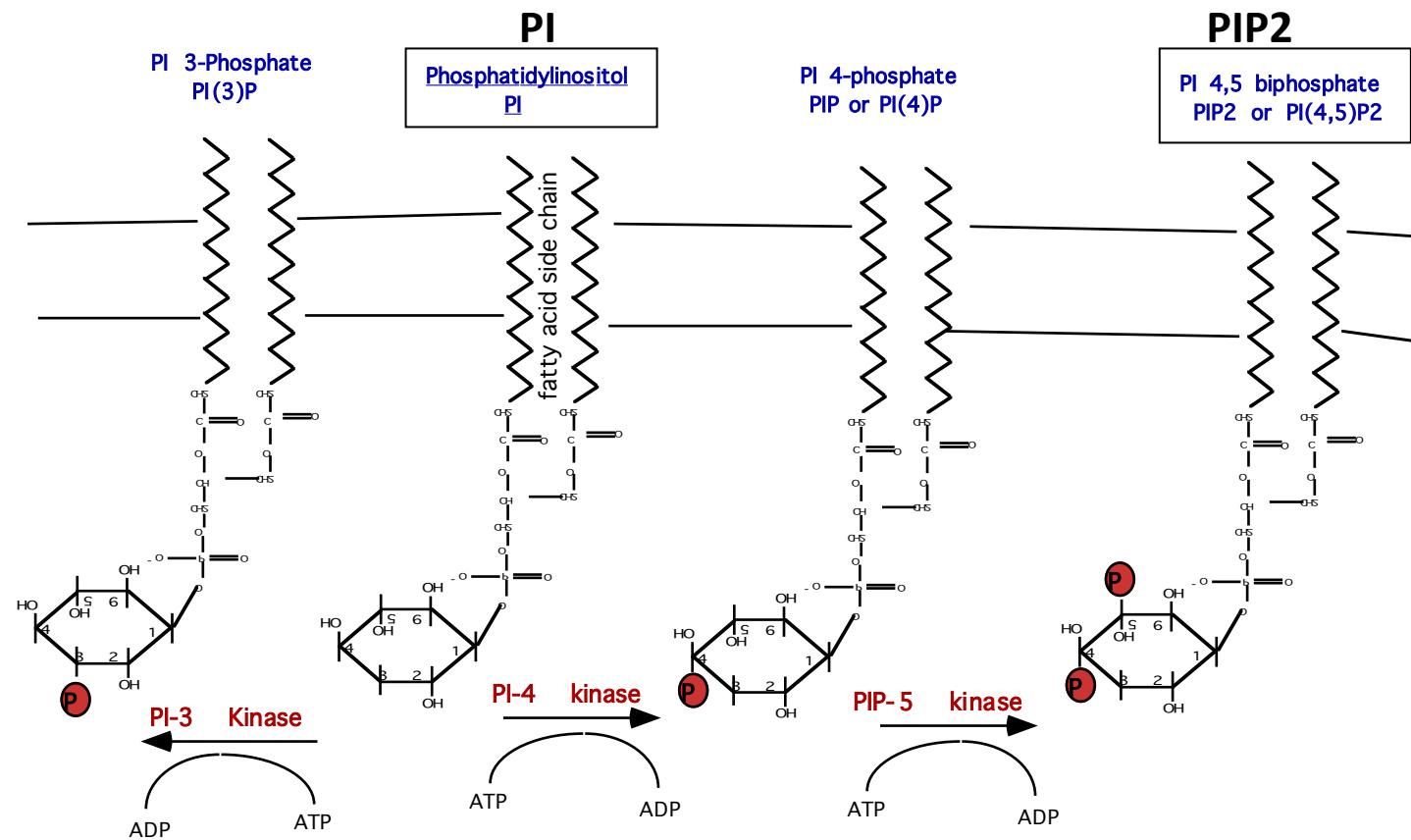


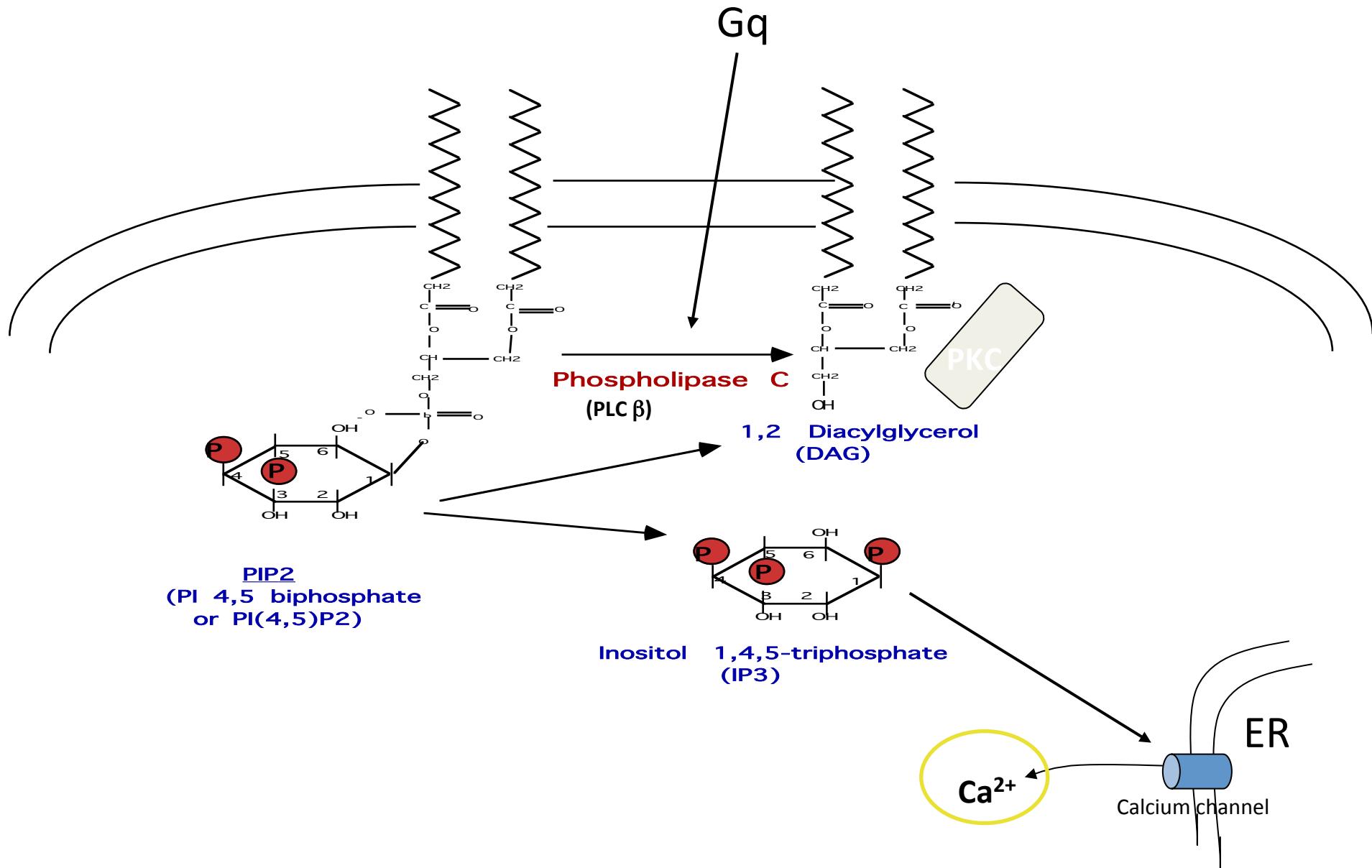
Figure 15–38 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Some G protein linked receptors linked to Gq activate Ca^{2+} signaling

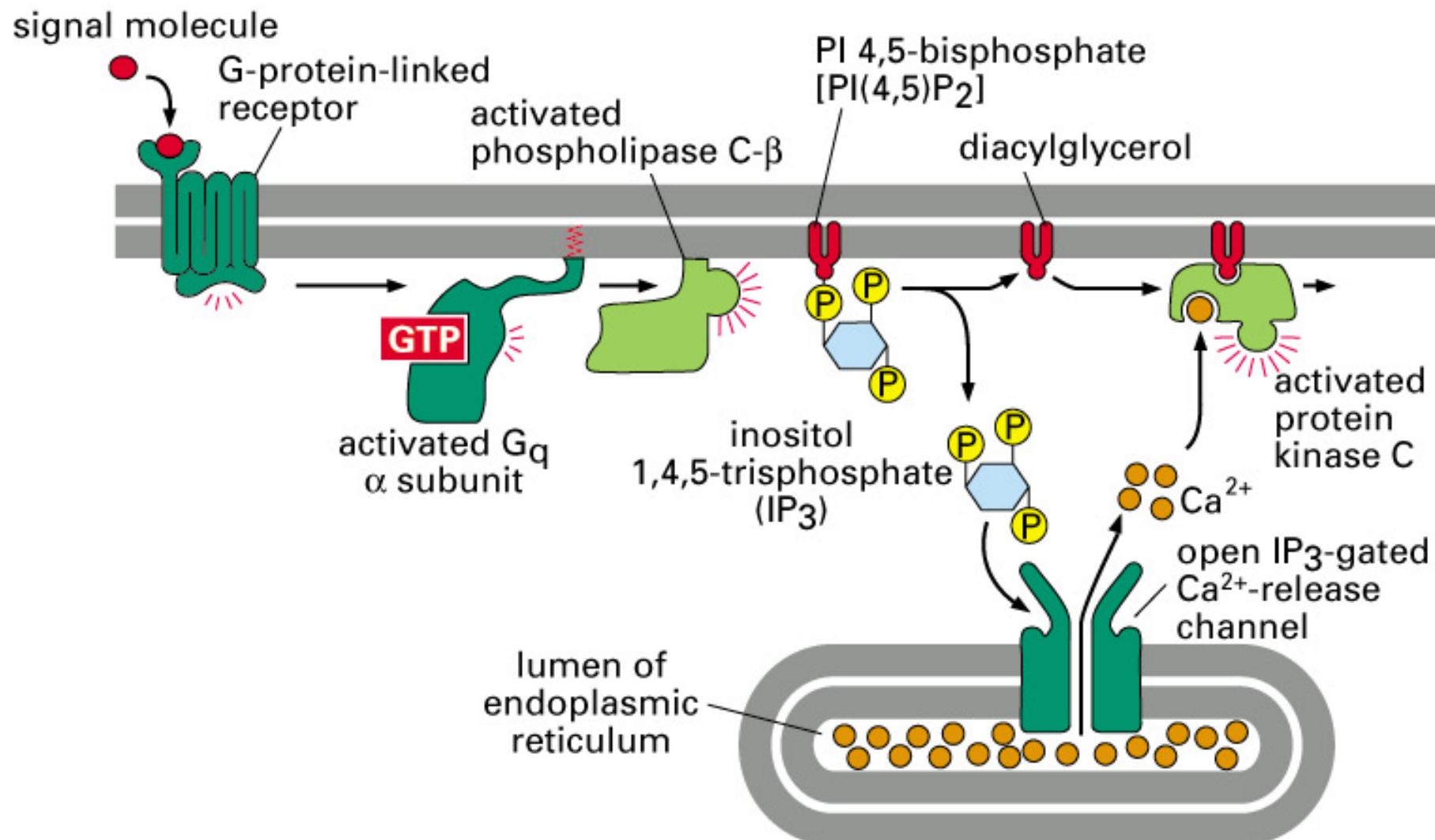
PIP2 plays an important role in Ca^{2+} signaling

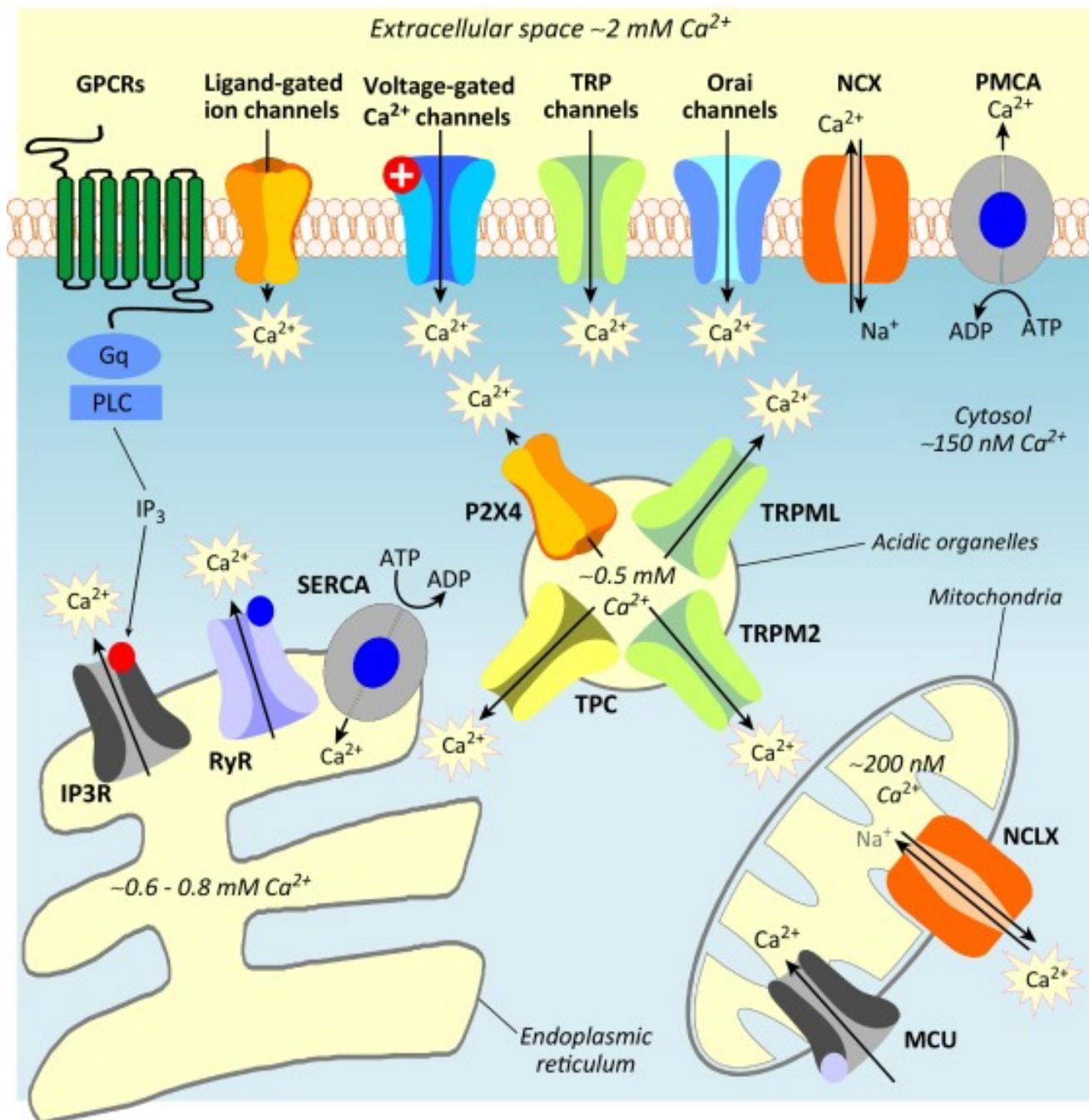


Cleavage of PIP2 gives rise to Ca influx



Gq signaling pathways and Calcium





Ca^{2+} sensors

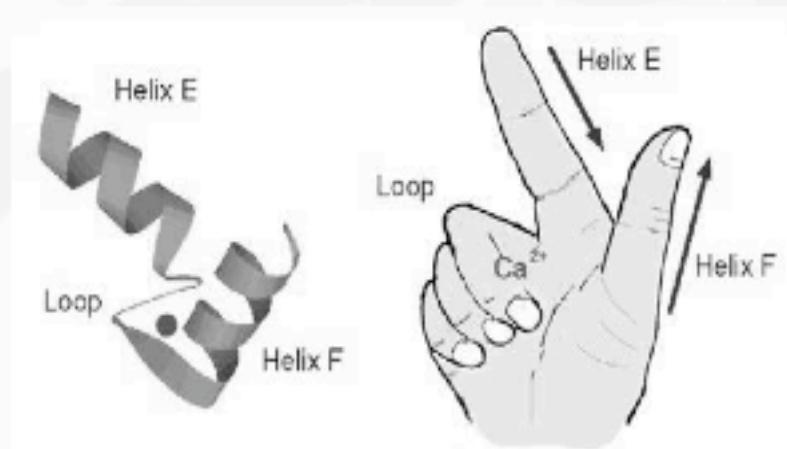
Ca^{2+} Sensors:

- Annexins:

Family of proteins w/ common feature that they interact w/ membranes in a Ca^{2+} - dependent manner.
Low affinity for Ca^{2+} -ions restricts action to membrane proximity (high local Ca^{2+} conc.);
implicated in the regulation of PLA2, cytoskeletal (re)organization and vesicle movement

- EF-hand proteins:

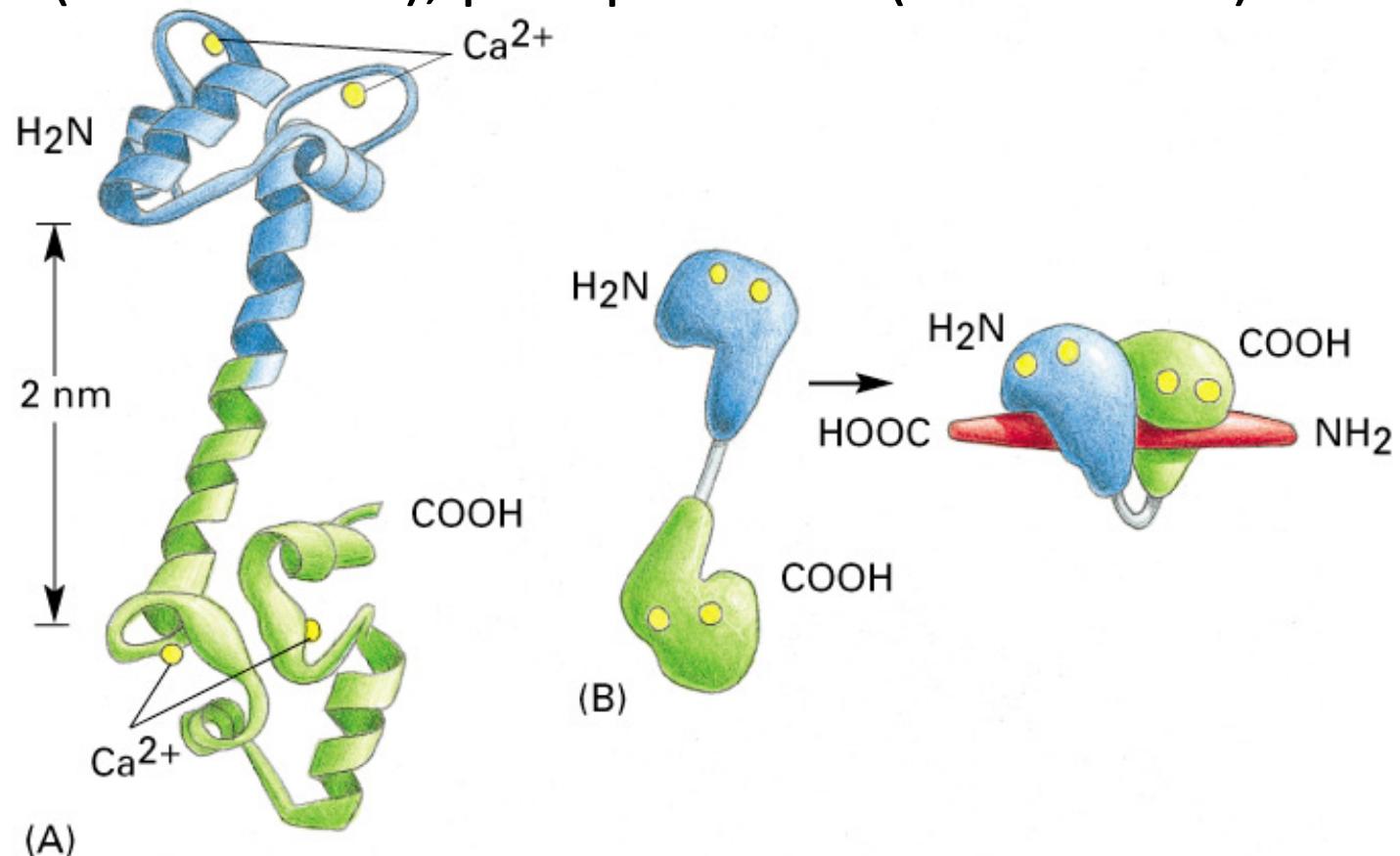
named after the shape created by the E and F α -helices of the Ca^{2+} -binding domain; high affinity



Ca^{2+} binding molecules

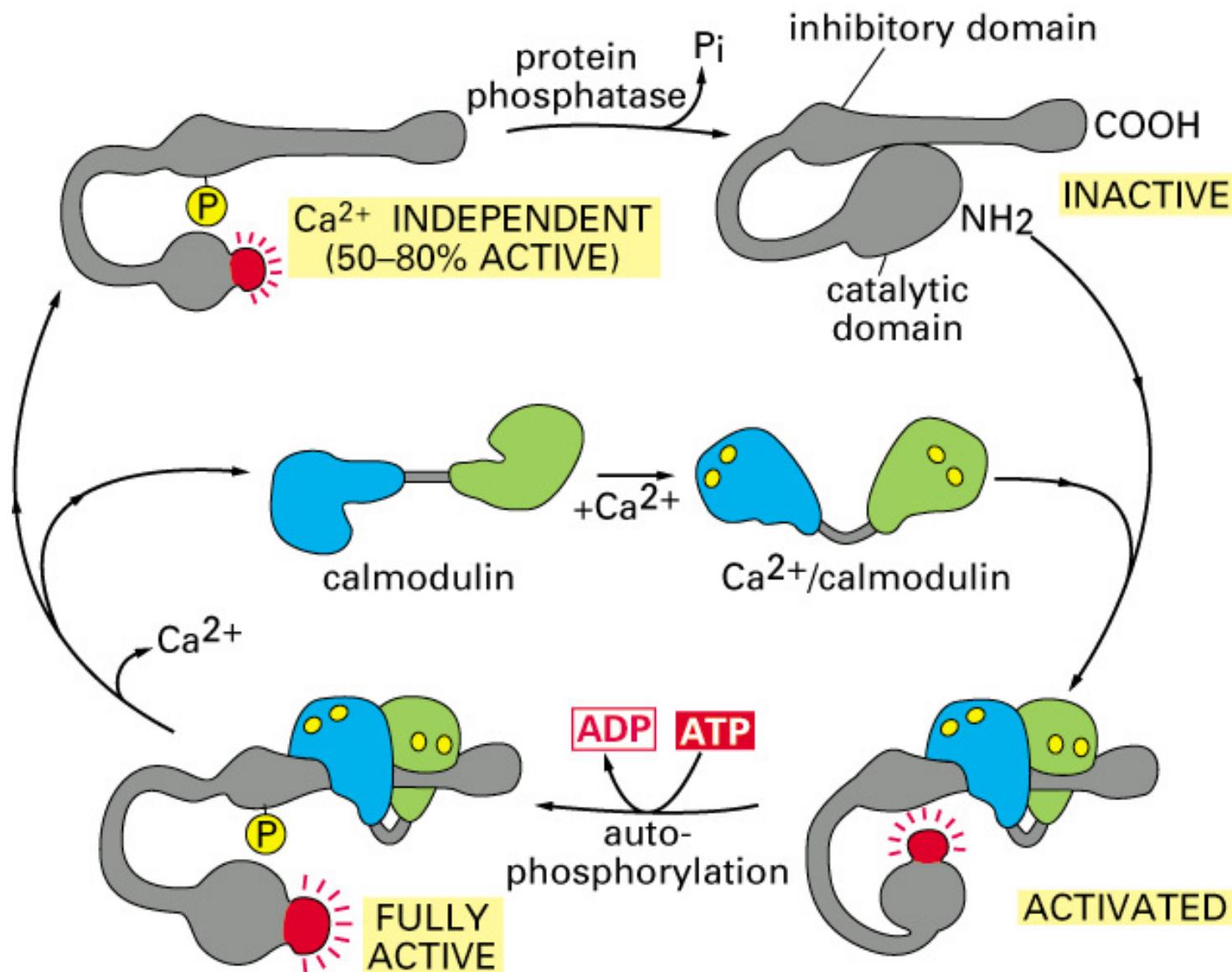
Calmodulin is a Ca^{2+} receptor that mediates many Ca^{2+} responses

Calcium binding protein Calmodulin – ubiquitous – targets other kinases (CAMkinase), phosphatases (calcineurin)

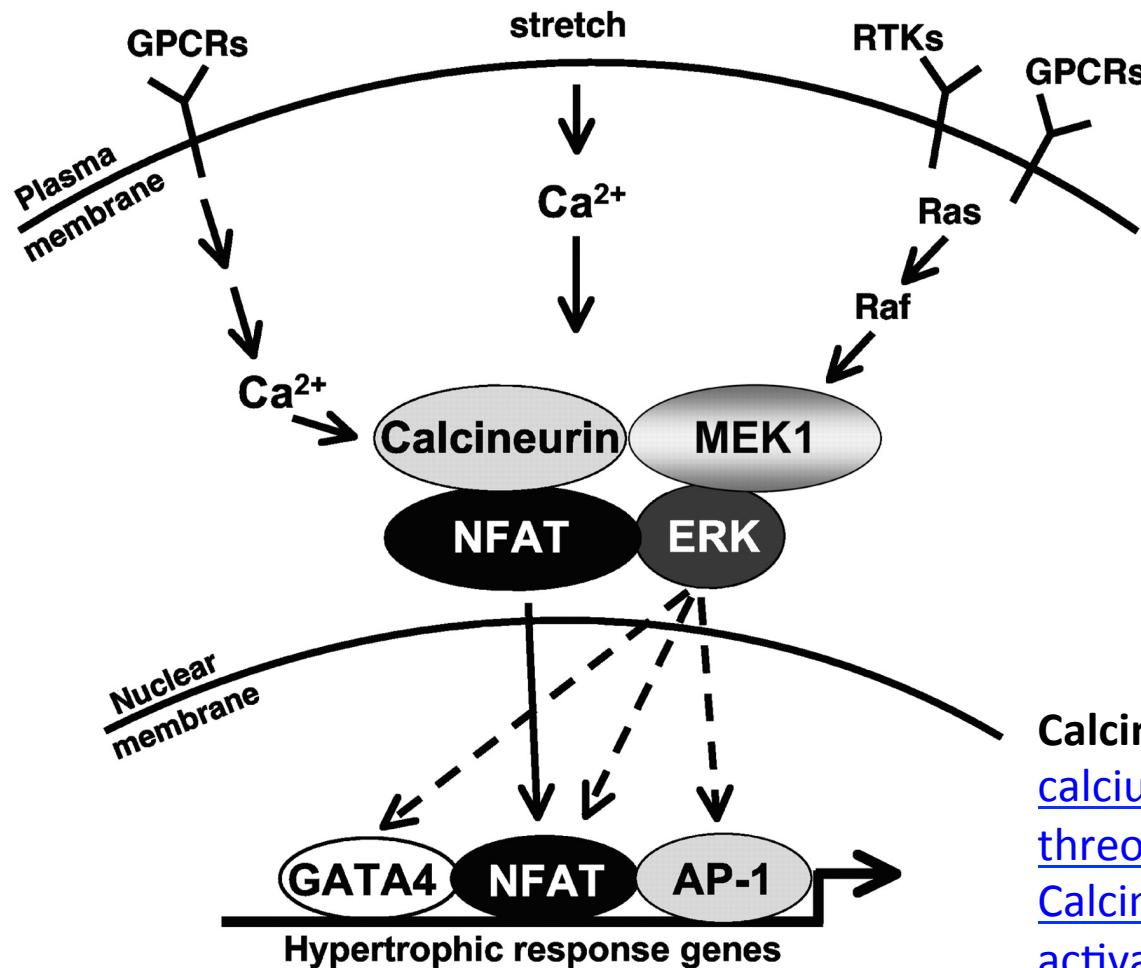


Ca^{2+} /calmodulin dependent protein kinase (CaM-kinase)

Memory function: 1. calmodulin dissociate after 10 sec of low calcium level; 2. remain active after calmodulin dissociation

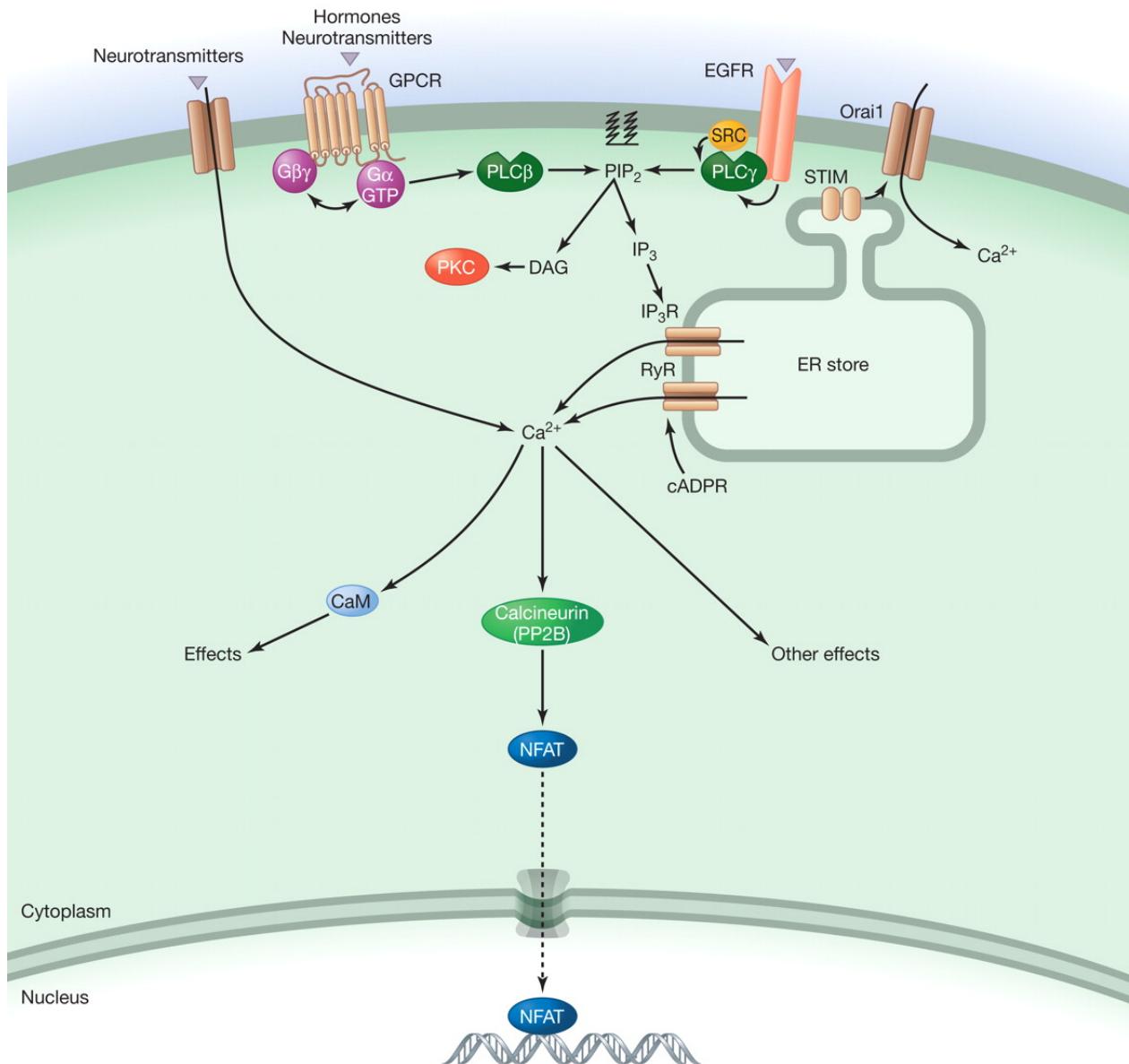


Calcineurin



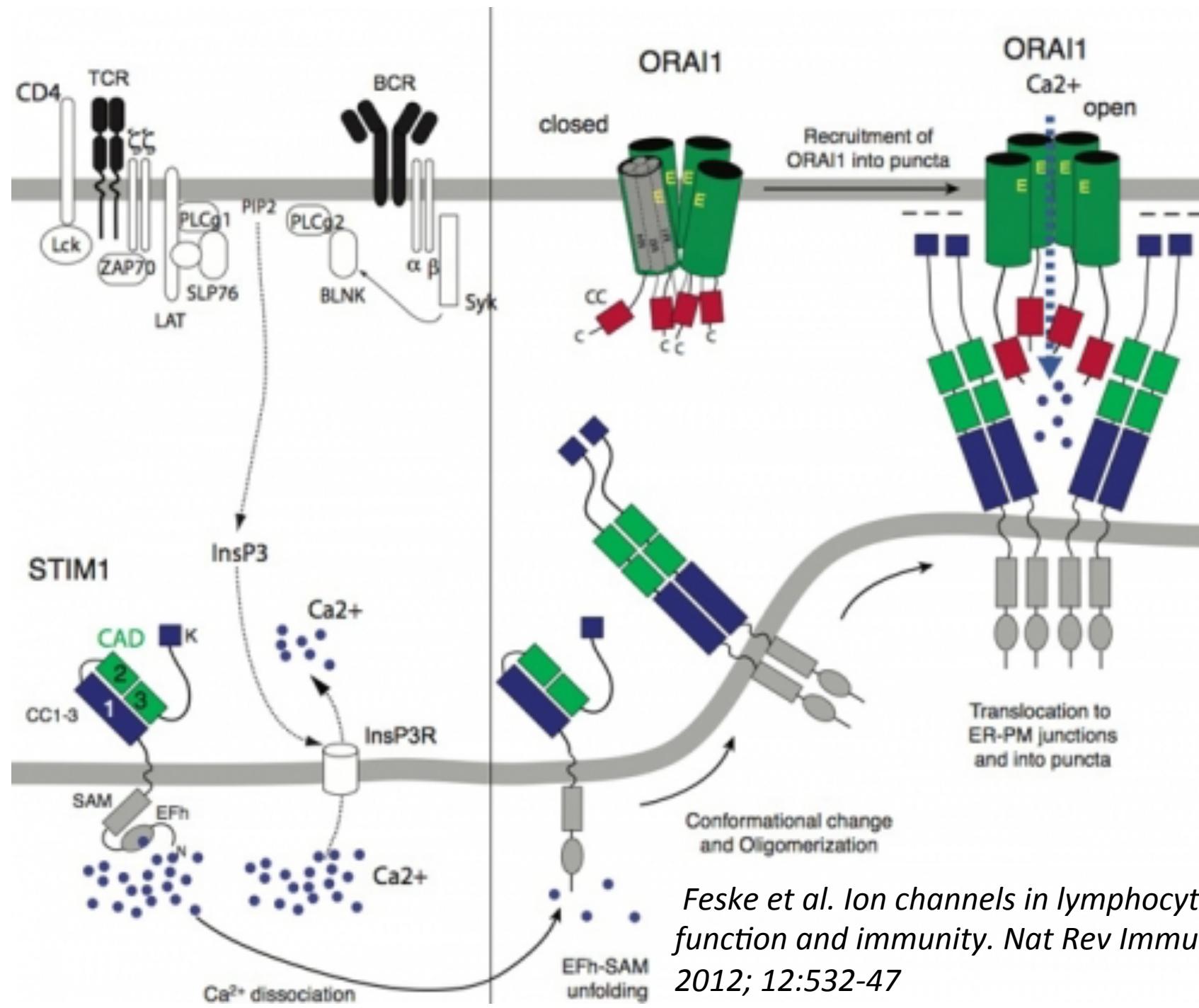
Calcineurin (CN) is a [calcium and calmodulin dependent serine/threonine](#) protein phosphatase. [Calcineurin activates nuclear factor of activated T cell, cytoplasmic \(NFATc\)](#), a transcription factor, by dephosphorylating it.

Calcium signaling (simplified view !!)



Lots of things to see !!!!

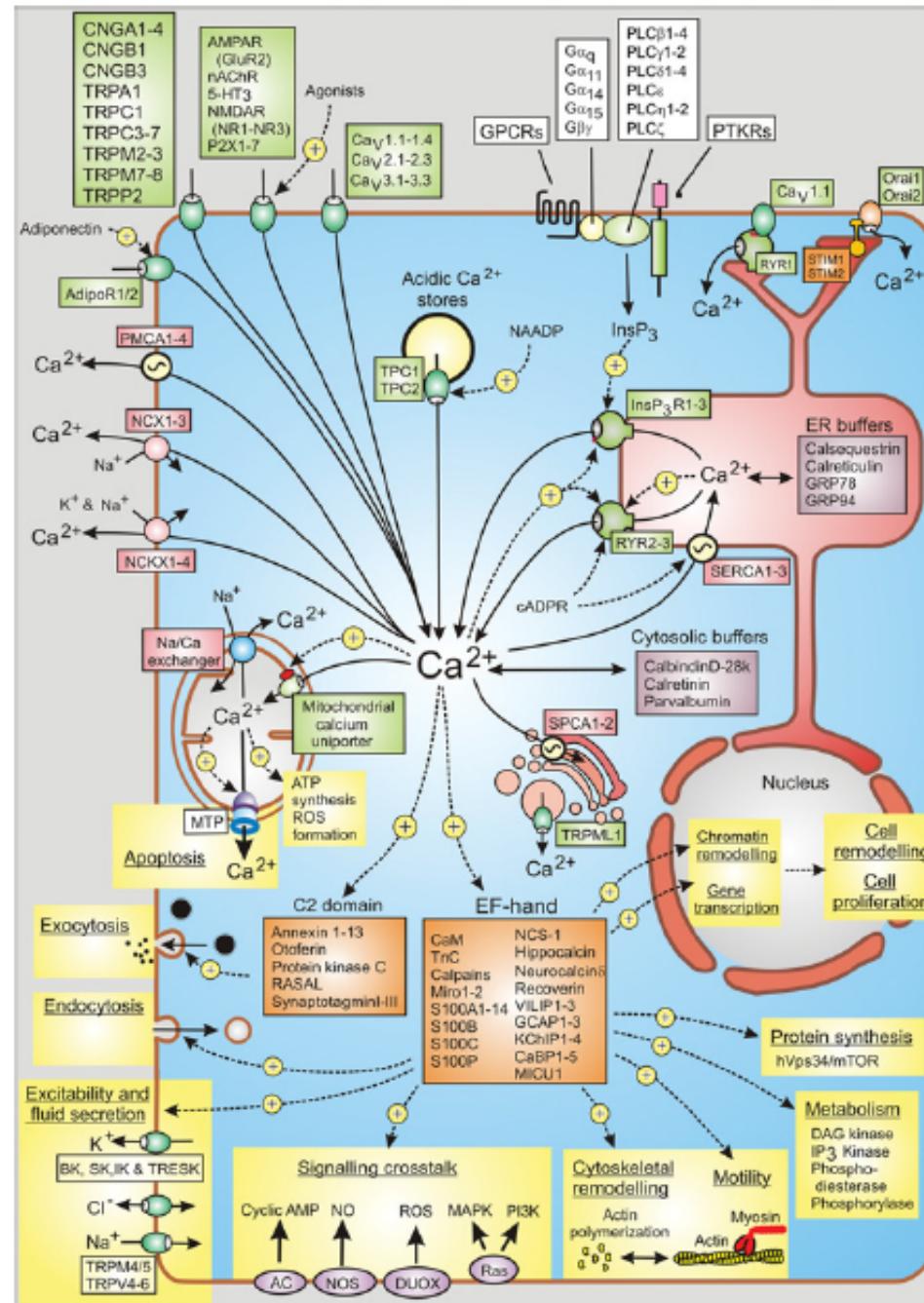




Feske et al. Ion channels in lymphocyte function and immunity. Nat Rev Immunol. 2012; 12:532-47

- FIGURE. The molecular choreography of CRAC channel activation. In resting lymphocytes, ER Ca^{2+} stores are filled with Ca^{2+} bound to the EF hand Ca^{2+} binding domain in the N-terminus of STIM1. Antigen receptor stimulation causes the activation TCR/BCR-proximal signalling cascades and the production of InsP3, resulting in the release of Ca^{2+} from the ER through InsP3 receptors, which are non-selective ion channels. The fall in ER Ca^{2+} concentration leads to the dissociation of Ca^{2+} from the EF hand domain in STIM1, unfolding of the STIM1 N-terminus and the multimerization of STIM1 proteins 6. STIM1 multimers translocate to junctional ER sites in which the ER membrane is juxtaposed to the plasma membrane. STIM1 multimers form large clusters (or puncta) into which they recruit ORAI1 CRAC channels. A minimal CRAC channel activation domain (variously referred to as the CAD, SOAR, OASF or CCB9 domain) in the C terminus of STIM1 (green boxes) is necessary and sufficient for ORAI1 binding, CRAC channel activation, and SOCE 29, 185, 187, 188. This domain contains two coiled (CC) domains, which interact with a CC domain in the C-terminus (red boxes) and additional domains in the N-terminus (not shown) of ORAI1 27. Abbreviations: SAM, sterile-alpha motif. From: Feske et al. *Ion channels in lymphocyte function and immunity*. Nat Rev Immunol. 2012; 12:532-47

TRPV, vanilloid TRP channel; VILIP, visinin-like protein. Adapted with permission from Berridge, M.J. (2012) Cell Signalling Biology; doi:10.1042/csb0001002. © 2012 Portland Press Limited.



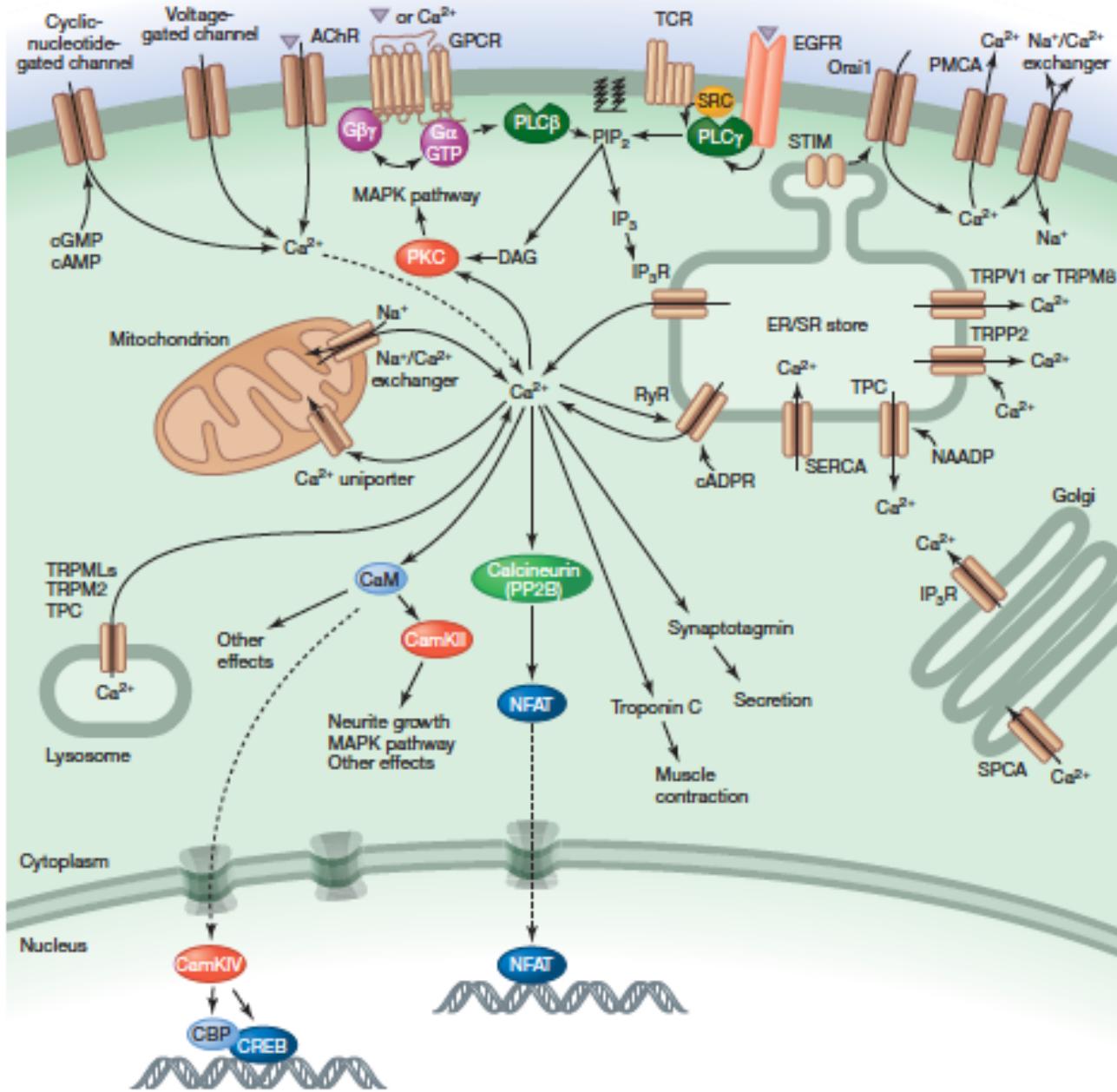
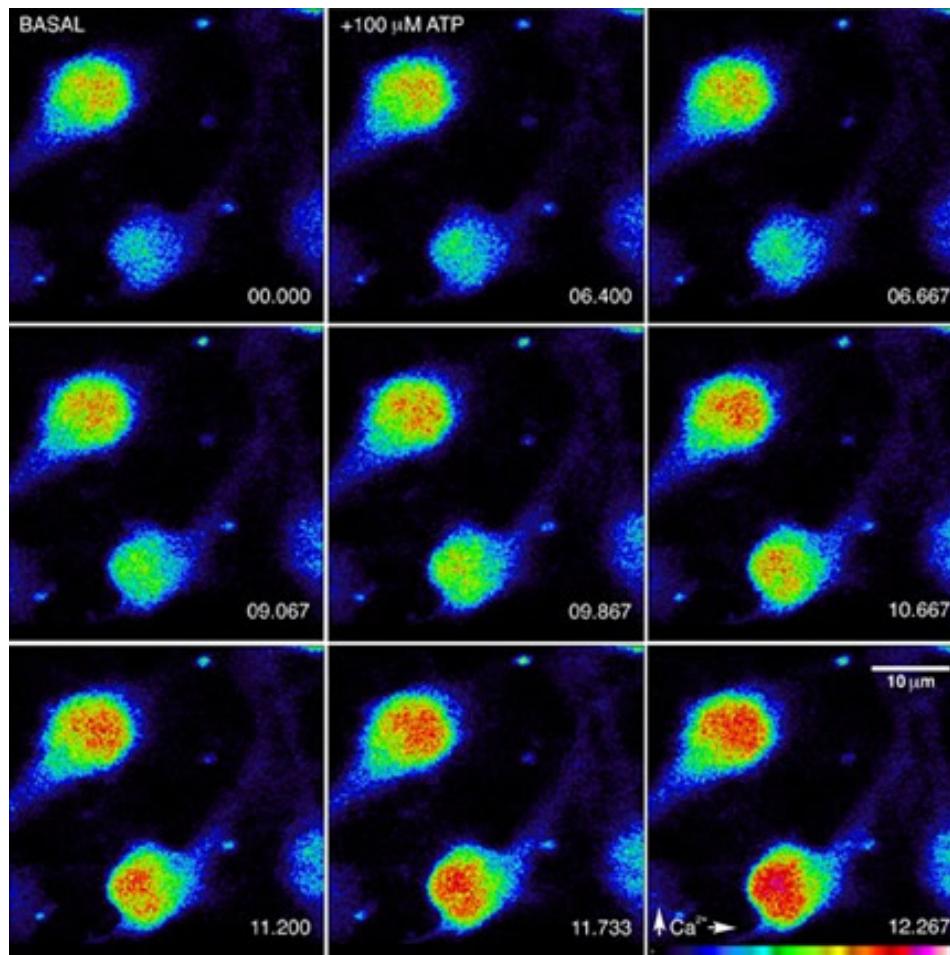
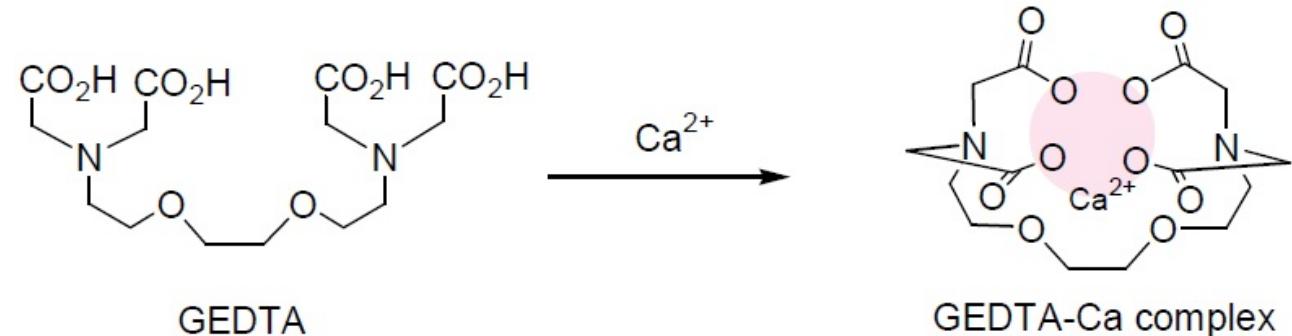


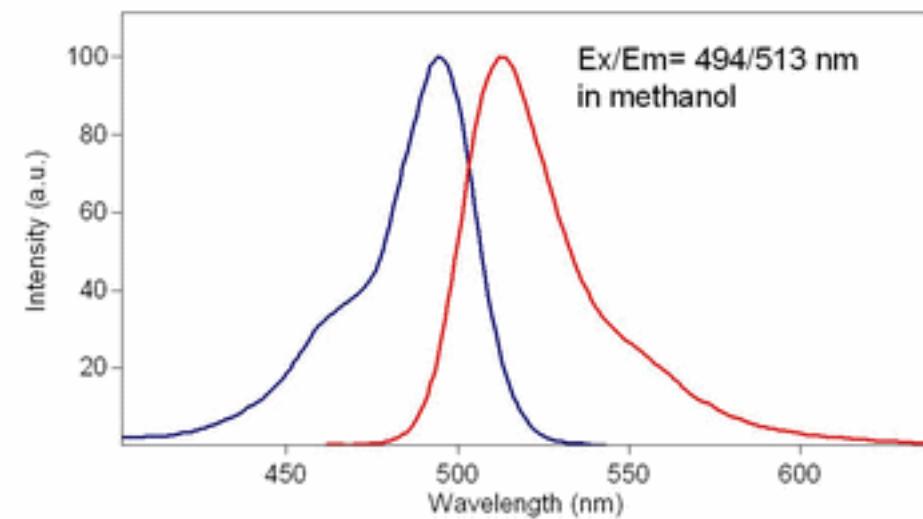
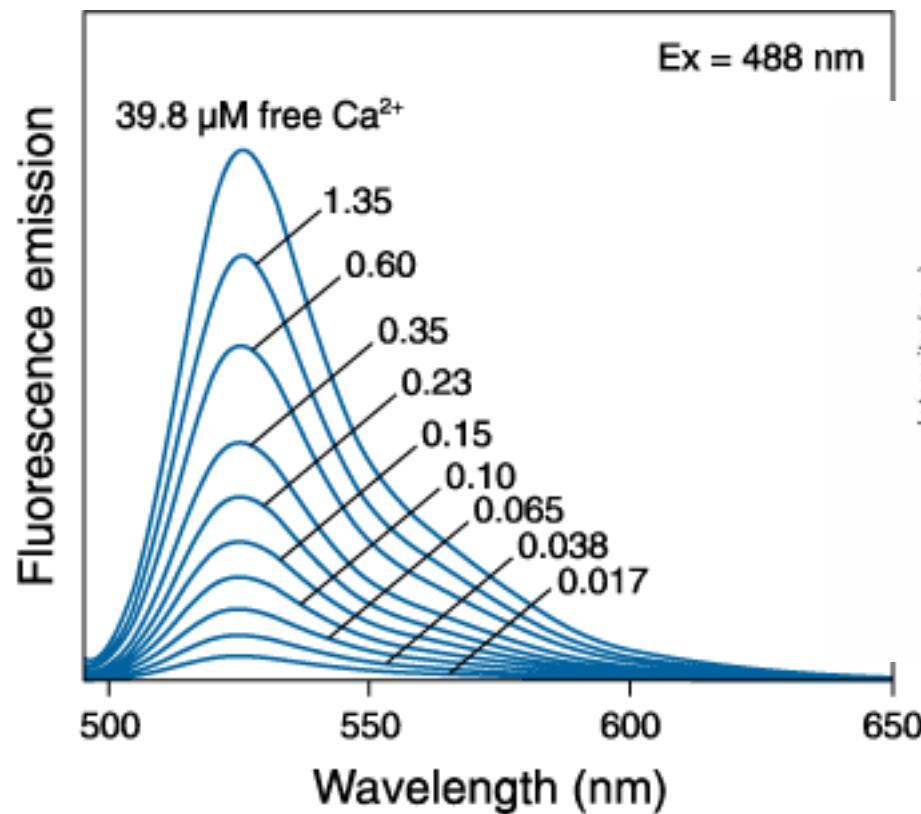
Figure 2. Calcium signaling
Martin D. Bootman Cold Spring Harb Perspect Biol
2012;4:a011171

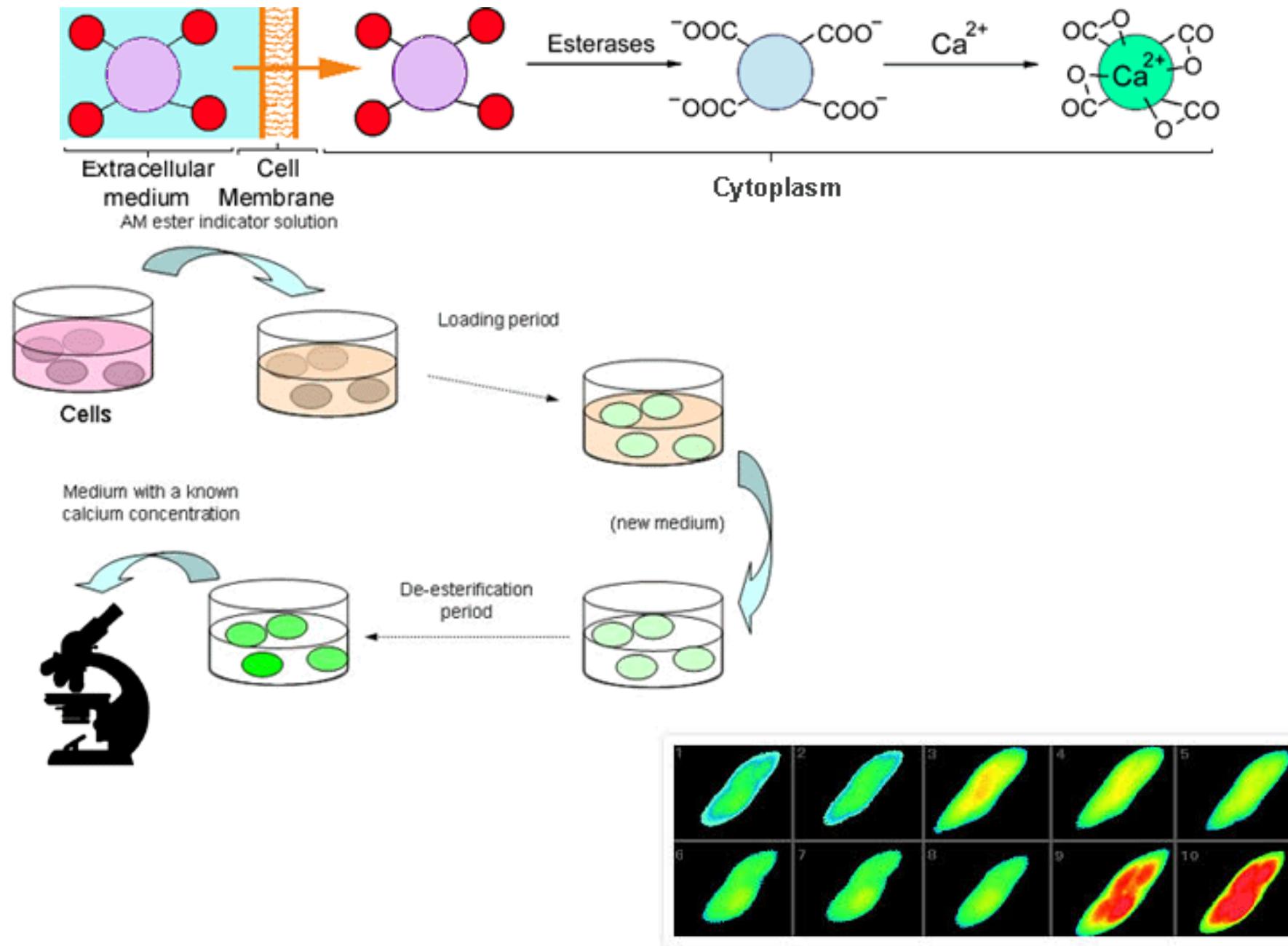
So how can we see this ?





From Ca^{2+} chelators we get Ca^{2+} sensitive fluorescent dyes





Insulin secretion

