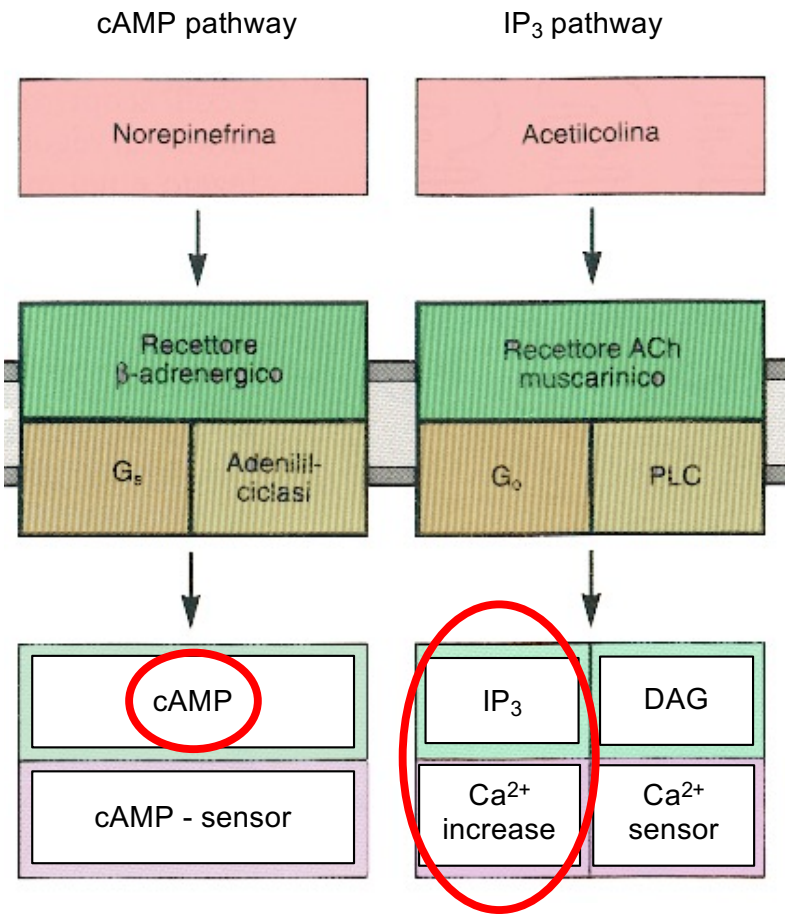
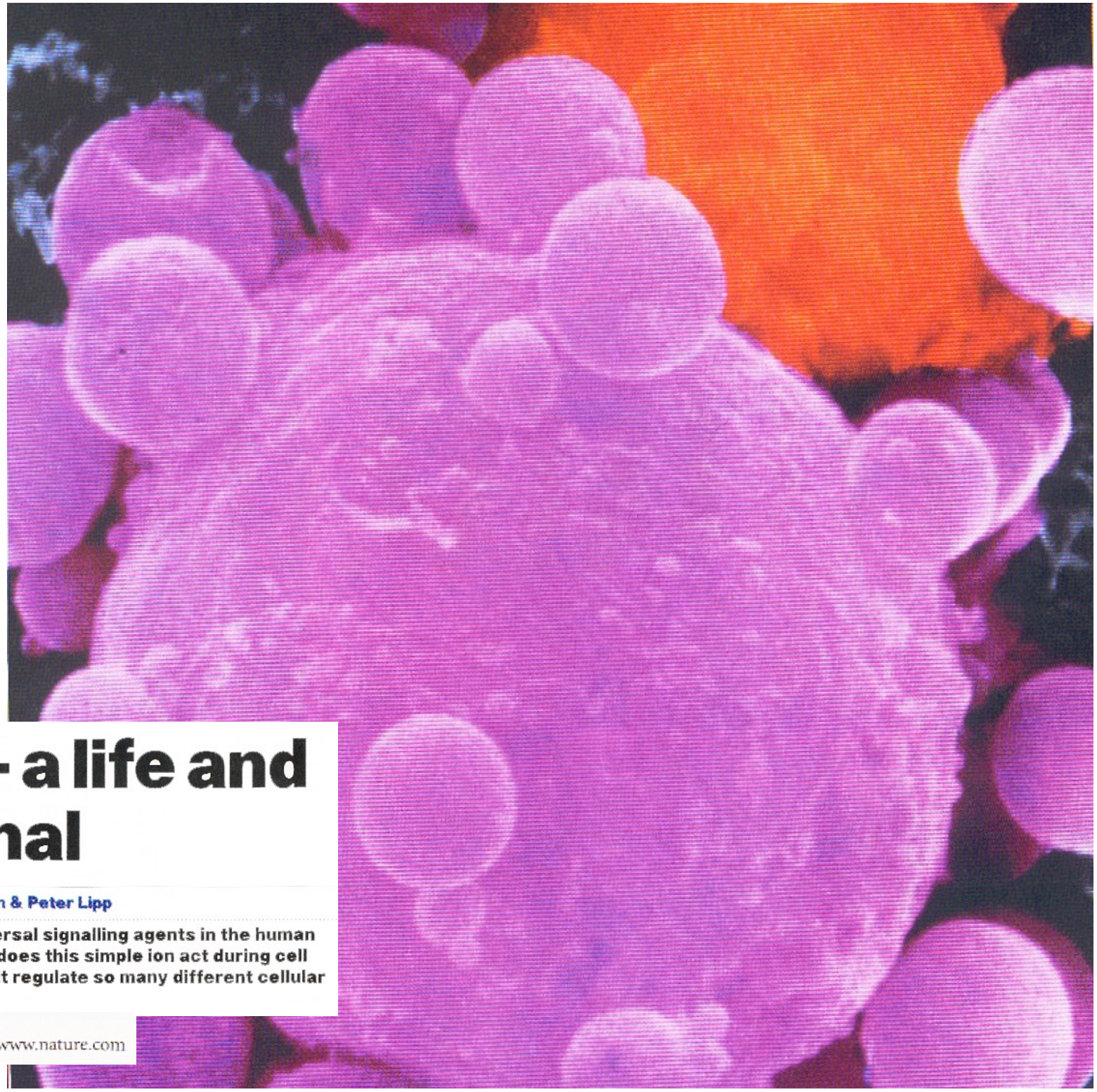


cAMP and calcium are pleiotropic messengers



Calcium — a life and death signal

Michael J. Berridge, Martin D. Bootman & Peter Lipp

One of the most versatile and universal signalling agents in the human body is the calcium ion, Ca^{2+}. How does this simple ion act during cell birth, life and death, and how does it regulate so many different cellular processes?

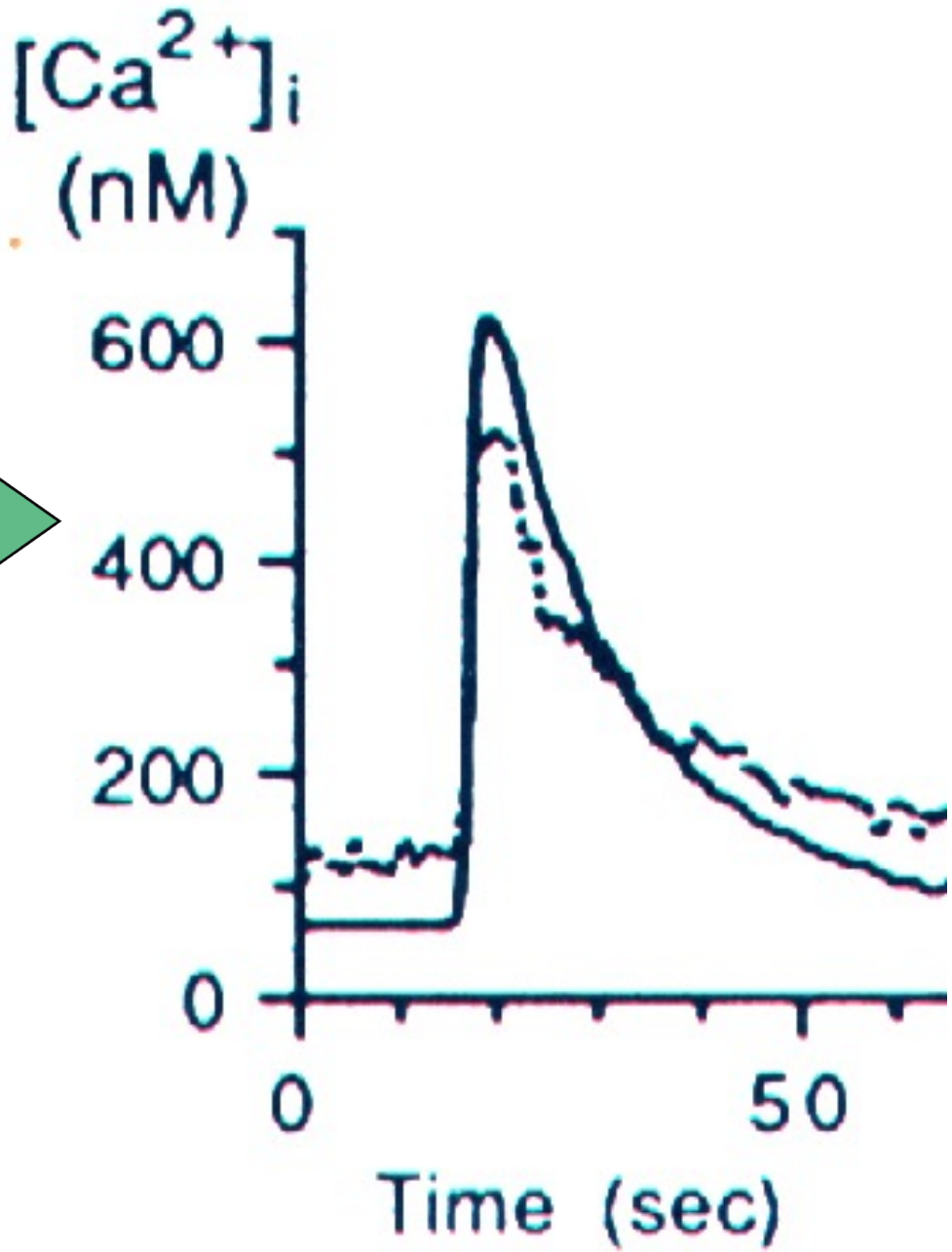
NATURE | VOL 395 | 15 OCTOBER 1998 | www.nature.com

Physiological conditions:

$[Ca^{2+}]_i$ changes
are transient events

Pathological conditions:

$[Ca^{2+}]_i$ changes
have higher amplitude
and/or longer duration



Protein kinases C and CAM kinases

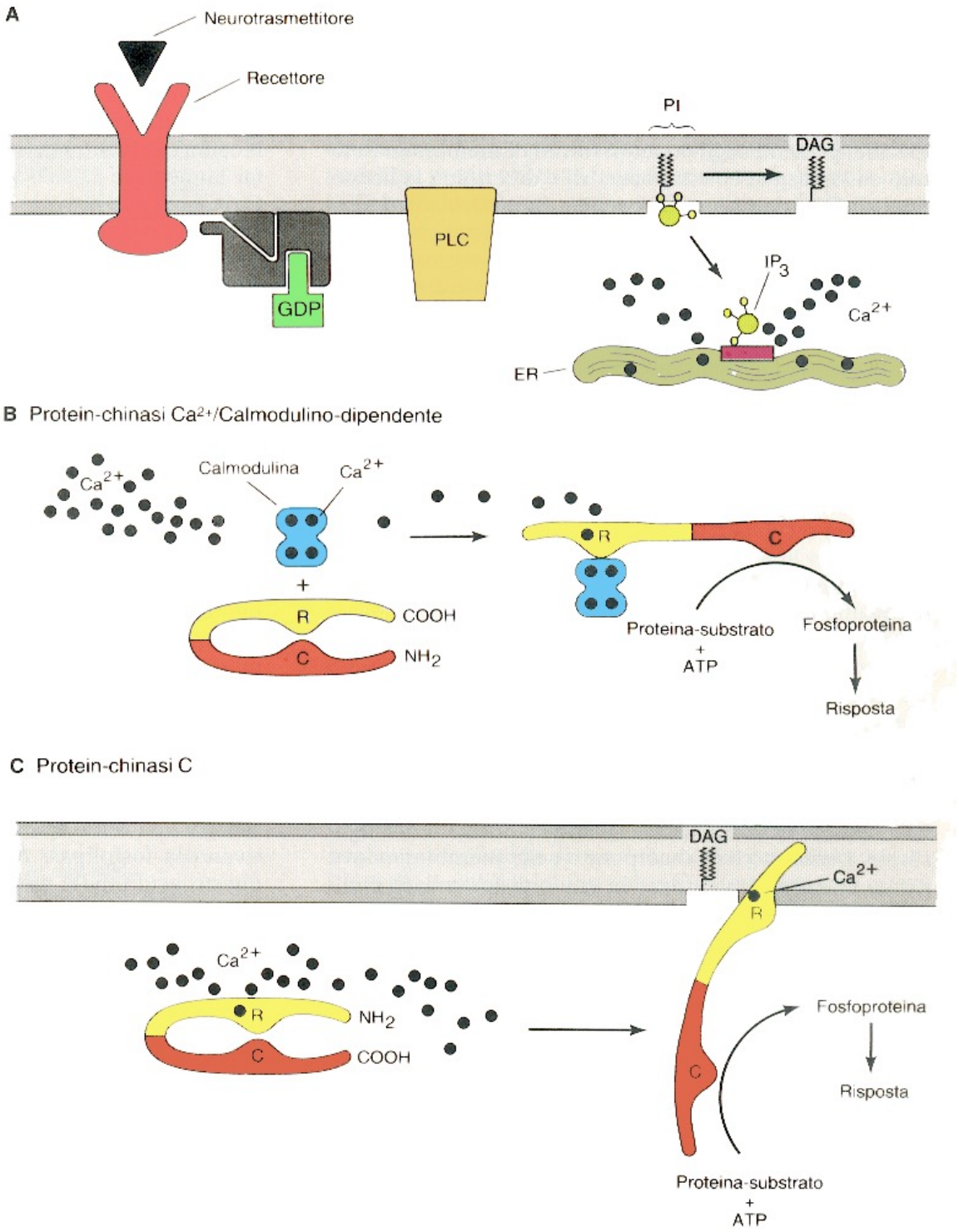


FIGURA 12-7
Attivazione di IP_3, della protein-chinasi Ca^{2+}/calmodulino-dipendente e della PKC.

A. Nella via inositolo-lipidi, il legame di un neurotrasmettitore con un recettore attiva una proteina-G che, a sua volta, attiva la fosfolipasi C. Questa fosfolipasi degrada il fosfatidil-inositolo (PI) PIP_2 in due secondi messaggeri, IP_3 e diacilglicerolo (DAG). L'IP_3 è un composto idrosolubile e può diffondere nel citoplasma dove si lega a un recettore localizzato sul reticolo endoplasmatico determinando la liberazione di Ca^{2+} dalle riserve interne.

B. I Ca^{2+} legati alla calmodulina attivano la protein-chinasi.

C. Il DAG, che è l'altro secondo messaggero prodotto dall'idrolisi del PIP_2, rimane nella membrana dove attiva la PKC; tale attivazione richiede la presenza dei fosfolipidi della membrana. Alcune isoforme di PKC non richiedono Ca^{2+} per venir attivate.

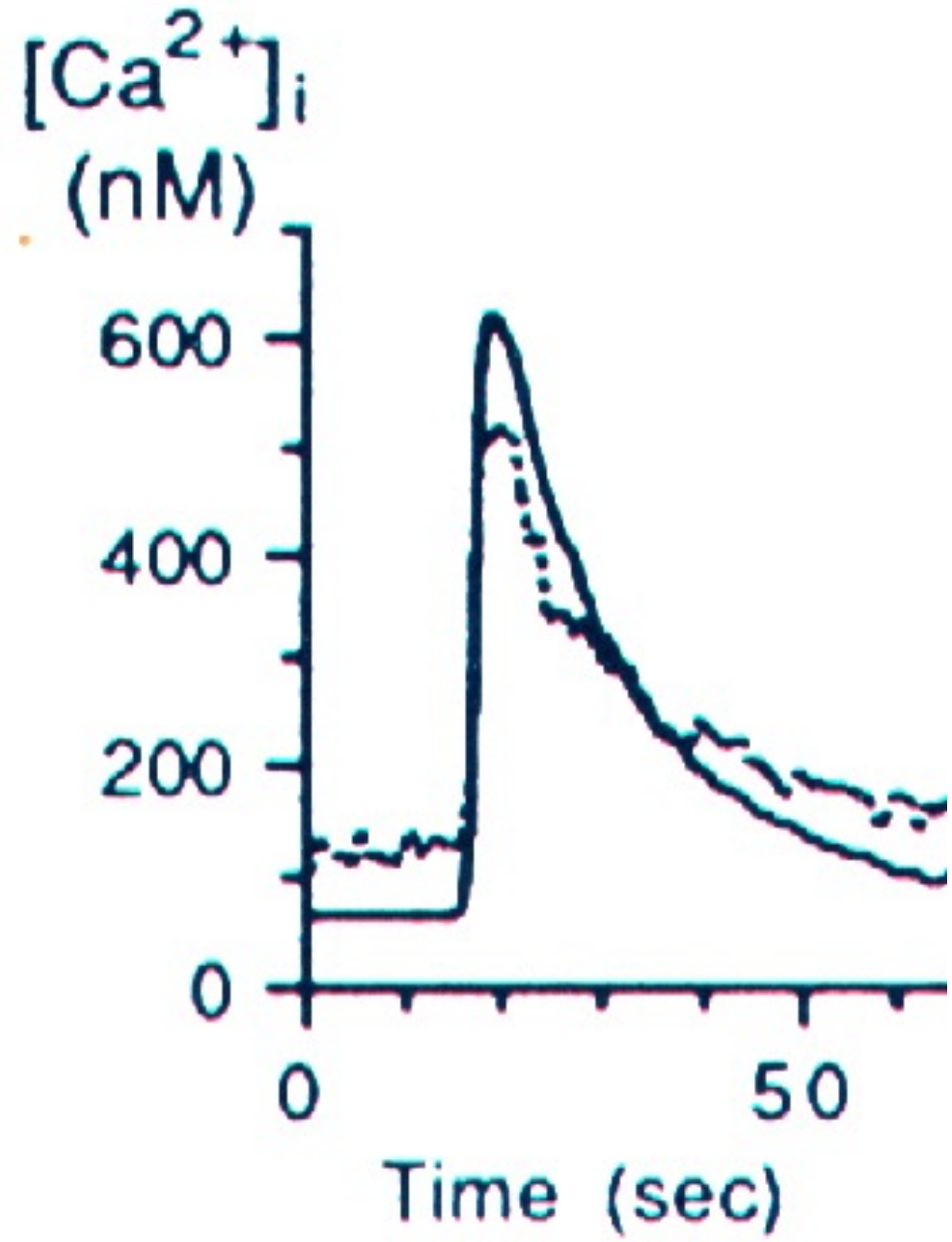
There are many Ca^{2+} sensors

Table 1. Examples of Mammalian Proteins Triggered by Ca^{2+}

Protein	Ca^{2+}-Binding Site	Protein Function
Troponin C	EF hand	Modulator of muscle contraction
Calmodulin	EF hand	Ubiquitous modulator of protein kinases and other enzymes (MLCK, CaM kinase II, adenylyl cyclase I)
Calretinin, retinin, visinin	EF hand	Activator of guanylyl cyclase
Calcineurin B	EF hand	Phosphatase
Calpain	EF hand	Protease
Inositol phospholipid-specific PLC	EF hand	Generator of $InsP_3$ and diacylglycerol
α-Actinin	EF hand	Actin-bundling protein
Annexin		Implicated in endo- and exocytosis, inhibition of PLA_2; ion channel?
Phospholipase A2		Producer of arachidonic acid
Protein kinase C		Ubiquitous protein kinase
Gelsolin		Actin-severing protein
Ca^{2+}-activated K^+ channel		Effector of membrane hyperpolarization
$InsP_3$ Receptor		Effector of intracellular Ca^{2+} release
Ryanodine receptor		Effector of intracellular Ca^{2+} release
Na^+/Ca^{2+} exchanger		Effector of the exchange of Ca^{2+} for Na^{2+} across the plasma membrane
Ca^{2+} ATPase		Pump of Ca^{2+} across membranes
Ca^{2+} antiporters		Exchanger of Ca^{2+} for monovalent ions
BoPCAR		G protein–linked Ca^{2+}-sensing receptor
Caldesmon		Regulator of muscle contraction
Villin		Actin organizer
Arrestin		Terminator of photoreceptor response
S100β		Unknown
Calreticulin		Ca^{2+} buffer/modulator of nuclear hormone receptor
Parvalbumin	EF hand	Ca^{2+} buffer
Calbindin	EF hand	Ca^{2+} buffer
Calsequestrin		Ca^{2+} buffer

Ca^{2+}-ON mechanisms
(rise phase)

Ca^{2+}-OFF mechanisms
(decay phase)



Control of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)

Ca^{2+}-ON mechanisms
(rise phase)

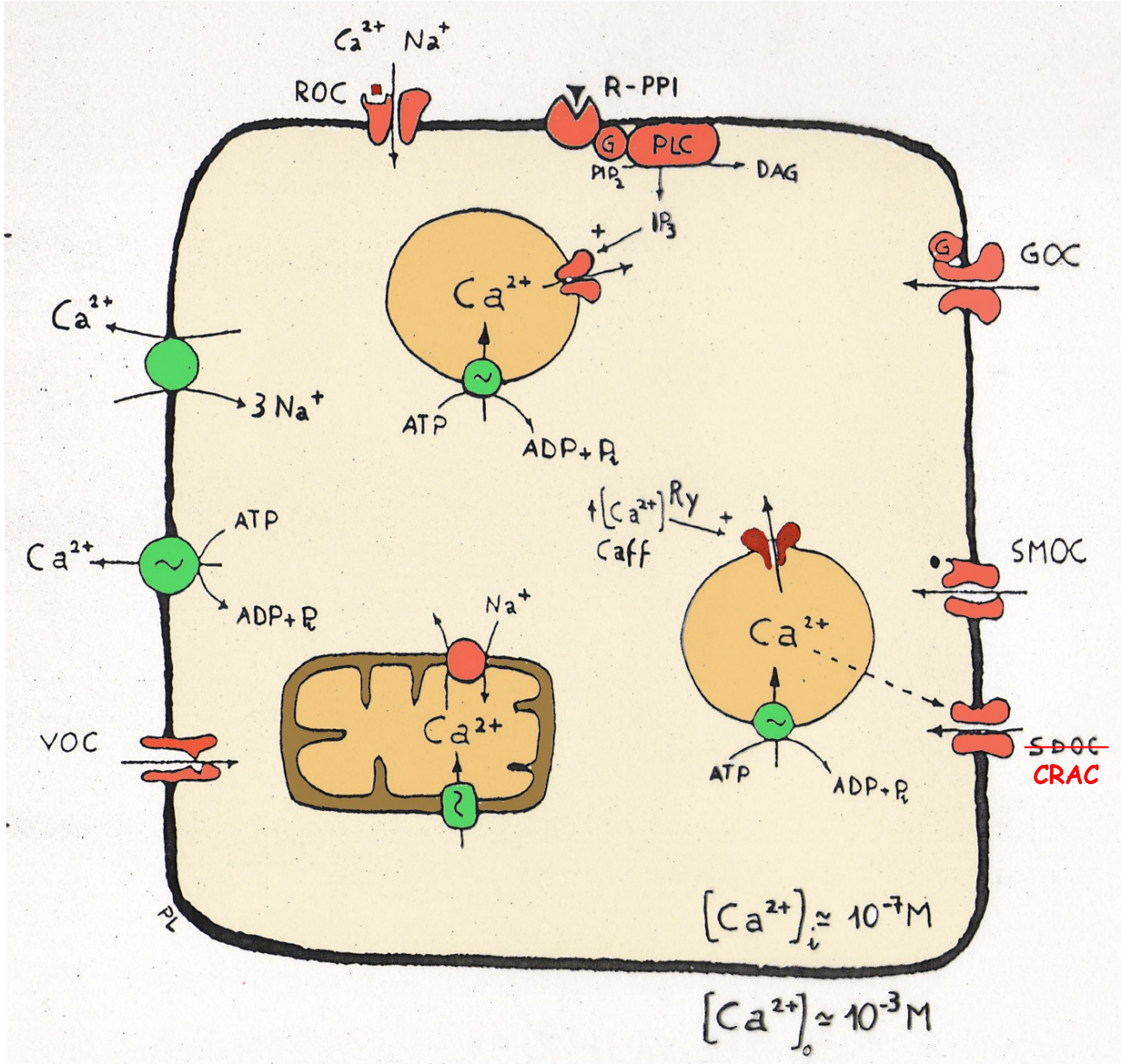
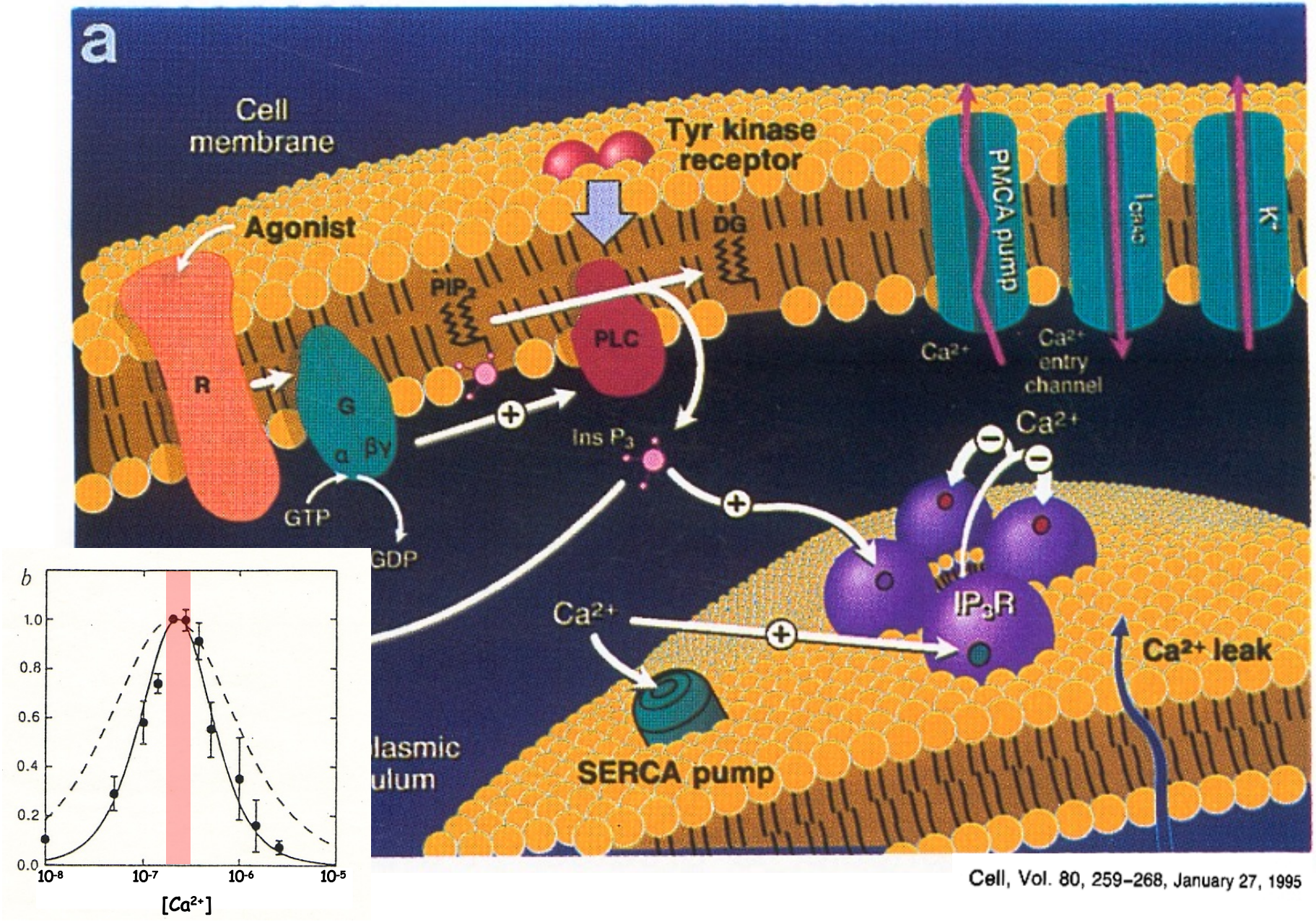


Fig 1. Overall picture of the control of Ca^{2+} homeostasis in nerve cells. A large number of mechanisms operate coordinately to keep $[Ca^{2+}]_i$ about four orders of magnitude lower than the concentration of free Ca^{2+} in the extracellular space, $[Ca^{2+}]_0$. At the plasma membrane Ca^{2+} influx can occur through a variety of channel types, some of which voltage gated (VOC, lower right side), others ligand- or receptor-gated such as the nicotinic cholinergic and the NMDA glutamate receptors (ROC, upper side). In addition, activation of receptors coupled to the hydrolysis of polyphosphoinosidides (R-PPI, upper side) causes the stimulation of Ca^{2+} influx apparently mediated by various types of channels: activated by G proteins (GOC), by second messengers (SMOC) and by the discharge of intracellular stores (SDOC), all depicted on the right side. Efflux across the plasmalemma is sustained by both the Ca^{2+} pump and the Ca^{2+}/Na^+ exchanger (left side). Within the cell only three membrane-bounded structures are depicted. Mitochondria accumulate Ca^{2+} at the expenses of their membrane potential, while the organelles sensitive to IP_3 (upper cytoplasm) and ryanodine (Ry, lower right cytoplasm), here shown separate from each other, are endowed with Ca^{2+} pumps. In some cell types (*eg* PC12 cells) Ca^{2+} stores exist sensitive to both IP_3 and Ry, suggesting colocalization of the two receptors in their limiting membrane. The intracellular release channels are depicted white for the IP_3 receptor, black for the Ry receptor. For the latter is indicated the sensitivity to both changes in $[Ca^{2+}]_i$ (calcium-induced-Ca^{2+}-release) and caffeine (Caff, a drug that lowers the Ca^{2+} threshold of the latter process).

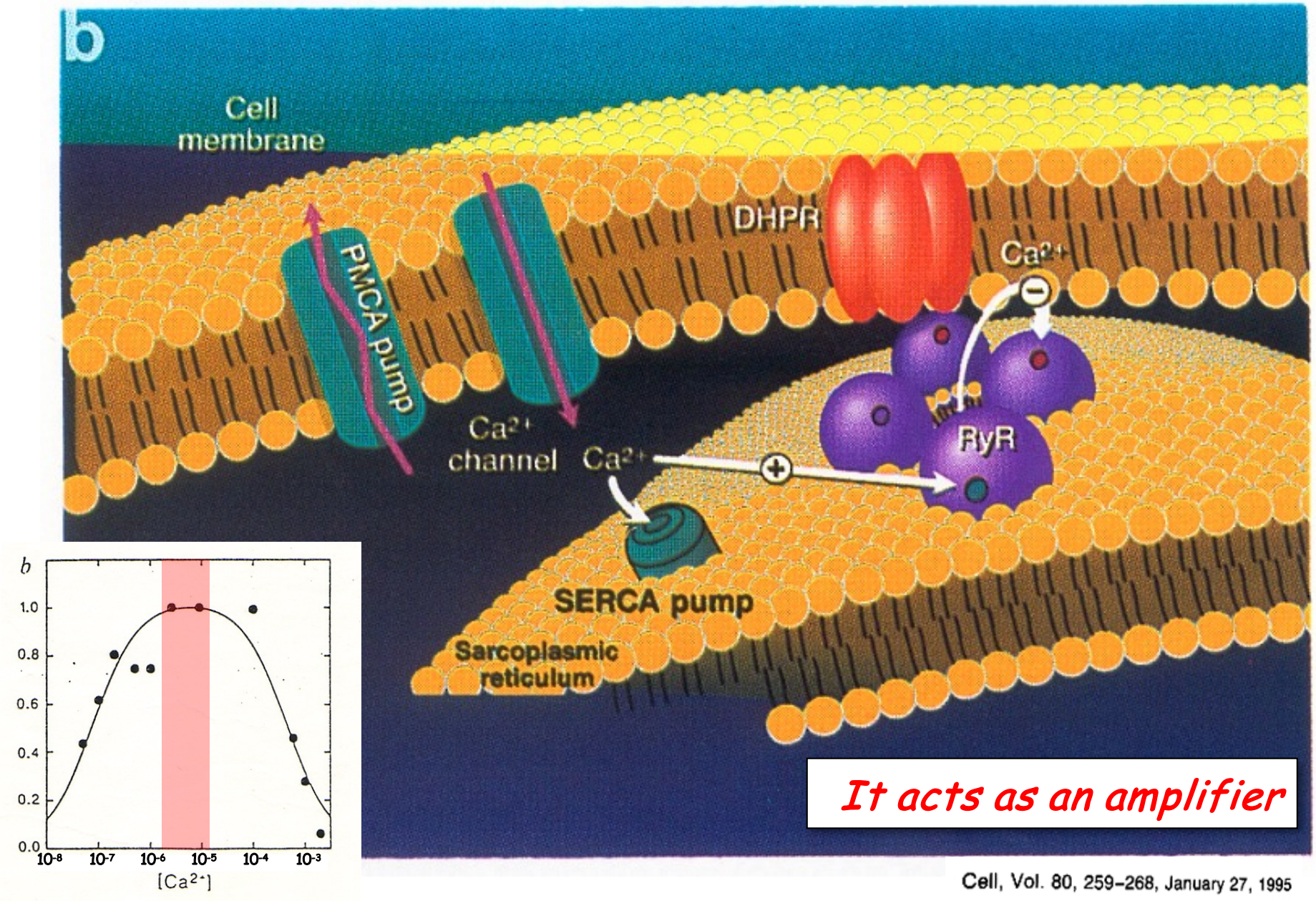
CRAC: calcium release activated calcium channels

IP_3 receptors and IP_3-sensitive store



Cell, Vol. 80, 259–268, January 27, 1995

Ca^{2+} receptors and Ca^{2+}-sensitive store



It acts as an amplifier

Cell, Vol. 80, 259–268, January 27, 1995

A new ligand for a new receptor

NAADP

nicotinic acid adenine dinucleotide phosphate

NAADP releases Ca^{2+} from acidic endolysosomal Ca^{2+} stores

TPC1 and TPC2 ion channels are responsible for NAADP-mediated Ca^{2+} release

(Pitt *et al* (2016) J Physiol 594: 4171-4179)

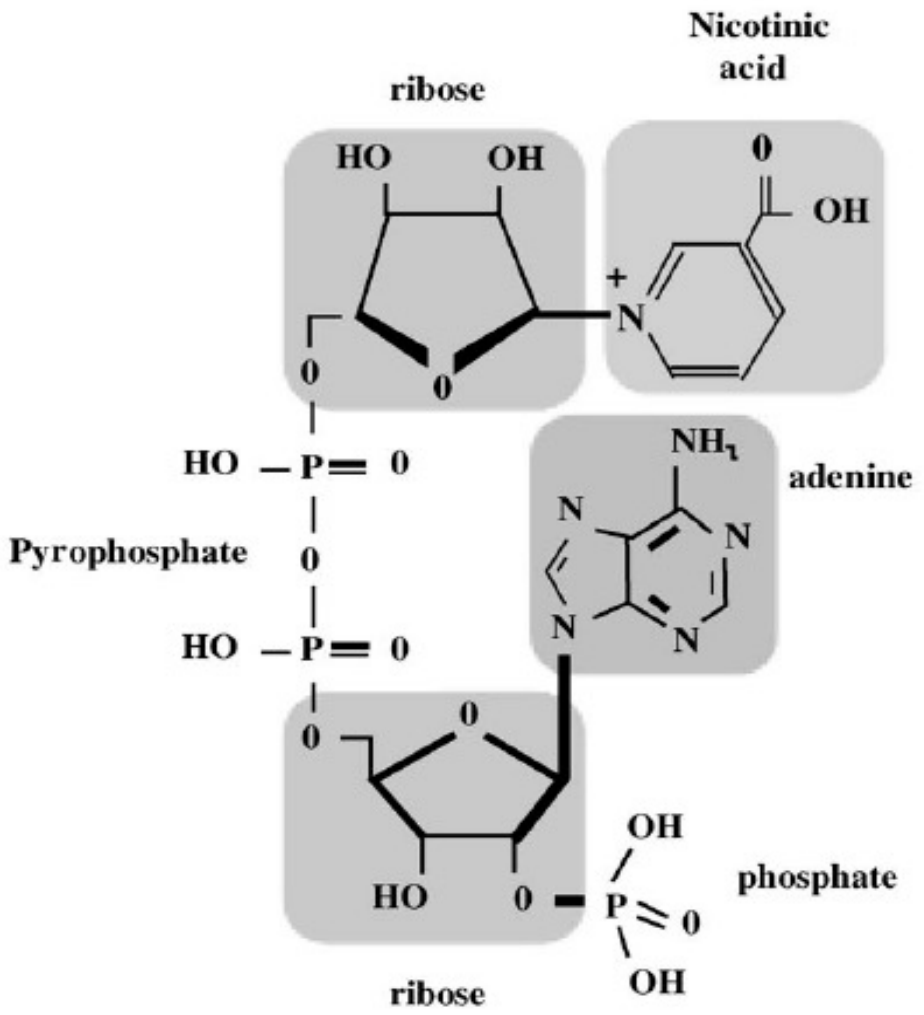


Fig. 1. **Structure of NAADP.** Constituent chemical groups are labelled.

Ca^{2+}- binding proteins in the lumen of Ca^{2+} stores

Table 1. Examples of Mammalian Proteins Triggered by Ca^{2+}

Protein	Ca^{2+}-Binding Site	Protein Function
Troponin C	EF hand	Modulator of muscle contraction
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Calbindin	EF hand	Ca^{2+} buffer
Calsequestrin		Ca^{2+} buffer

Dynamic distribution of Ca^{2+} stores

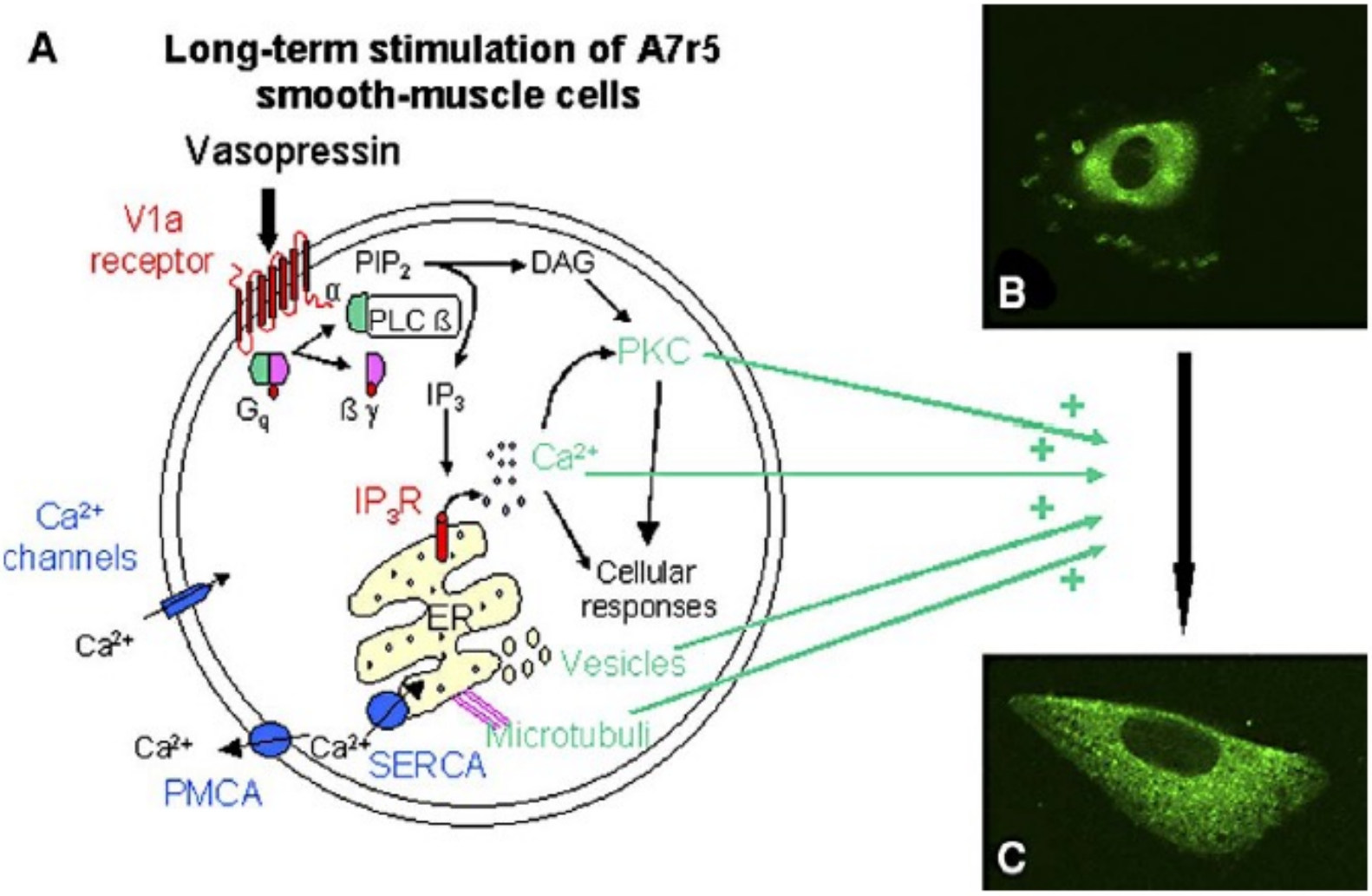
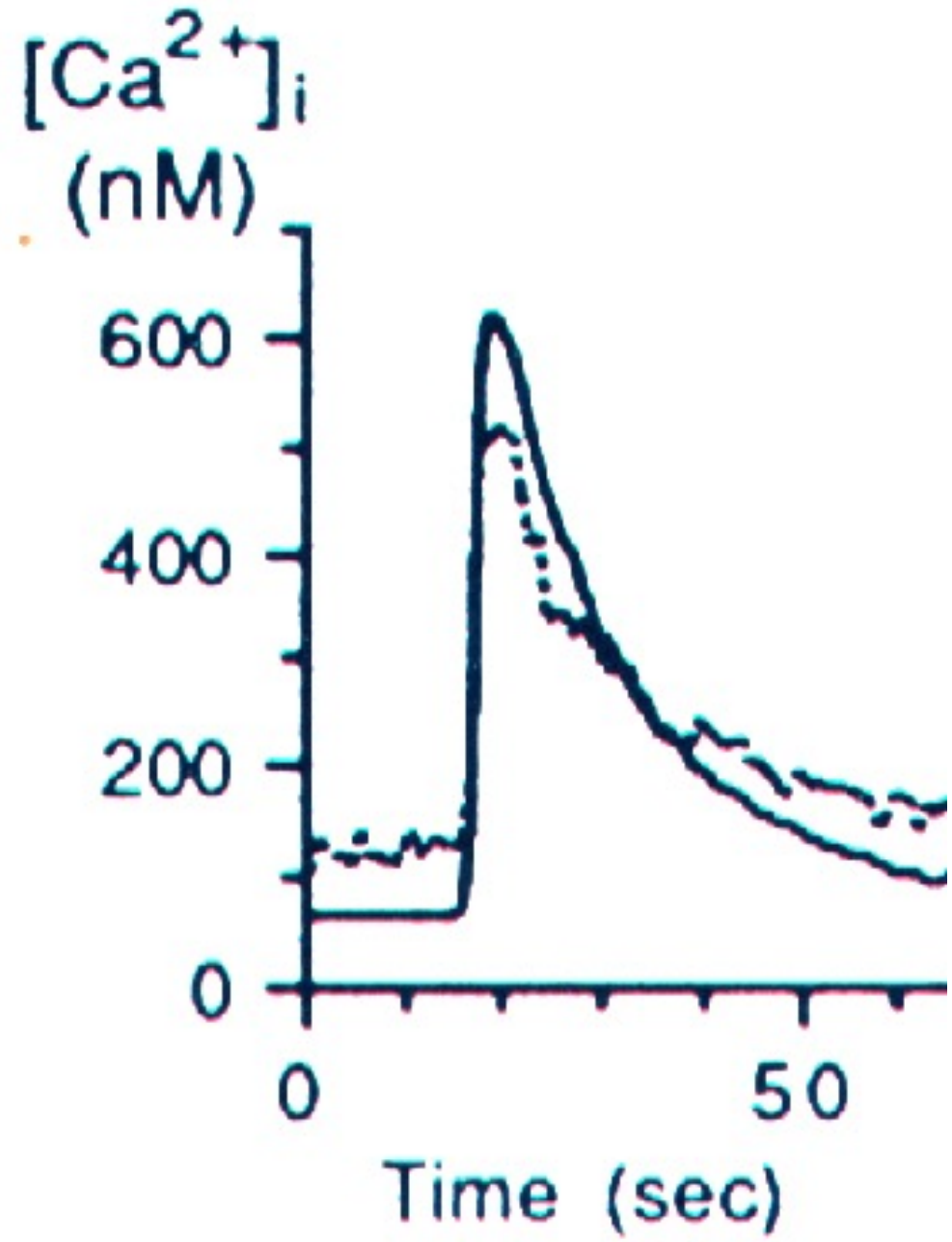


Fig. 2. Redistribution of IP_3Rs during long-term agonist stimulation of A7r5 smooth-muscle cells. **A.** Activation of the cells by binding of vasopressin to the V1a receptors leads to phospholipase C activation. The subsequent production of IP_3 and diacylglycerol leads to IP_3-induced Ca^{2+} release from the intracellular Ca^{2+} stores and to PKC activation. **B-C.** IP_3R1 was visualized using an isoform-specific antibody. The increase in cytosolic Ca^{2+} concentration and the activation of PKC lead, in a process involving the microtubular network and vesicle trafficking, to a redistribution of IP_3R1 from a predominantly perinuclear position **(B)** to a more homogeneous distribution over the cytoplasm **(C)**.

Ca^{2+}-ON mechanisms
(rise phase)

Ca^{2+}-OFF mechanisms
(decay phase)



Control of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)

Ca^{2+}-OFF mechanisms
(decay phase)

PMCA, plasma membrane Ca^{2+} ATPase
SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase

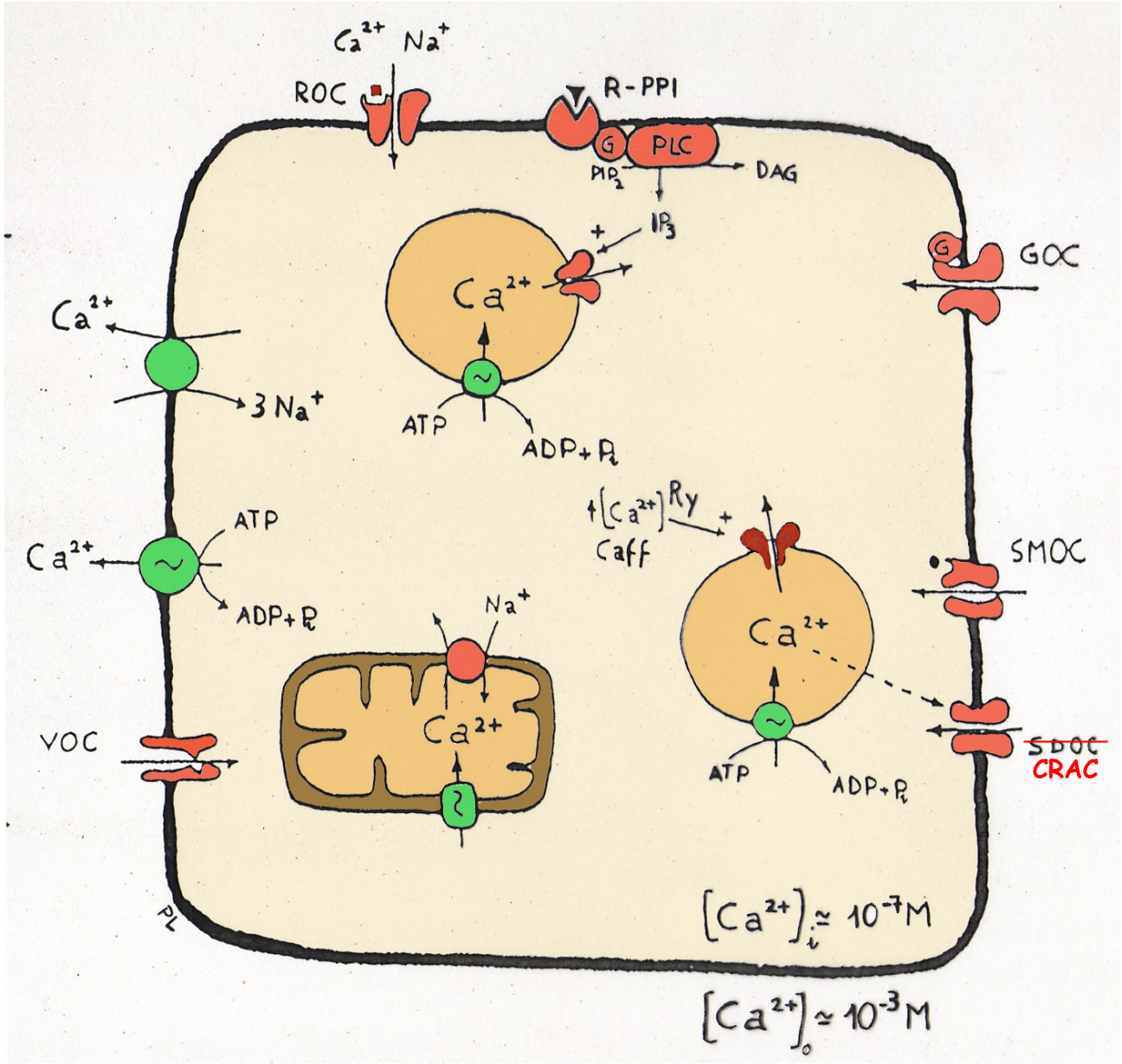


Fig 1. Overall picture of the control of Ca^{2+} homeostasis in nerve cells. A large number of mechanisms operate coordinately to keep $[Ca^{2+}]_i$ about four orders of magnitude lower than the concentration of free Ca^{2+} in the extracellular space, $[Ca^{2+}]_0$. At the plasma membrane Ca^{2+} influx can occur through a variety of channel types, some of which voltage gated (VOC, lower right side), others ligand- or receptor-gated such as the nicotinic cholinergic and the NMDA glutamate receptors (ROC, upper side). In addition, activation of receptors coupled to the hydrolysis of polyphosphoinosidides (R-PPI, upper side) causes the stimulation of Ca^{2+} influx apparently mediated by various types of channels: activated by G proteins (GOC), by second messengers (SMOC) and by the discharge of intracellular stores (SDOC), all depicted on the right side. Efflux across the plasmalemma is sustained by both the Ca^{2+} pump and the Ca^{2+}/Na^+ exchanger (left side). Within the cell only three membrane-bounded structures are depicted. Mitochondria accumulate Ca^{2+} at the expenses of their membrane potential, while the organelles sensitive to IP_3 (upper cytoplasm) and ryanodine (Ry, lower right cytoplasm), here shown separate from each other, are endowed with Ca^{2+} pumps. In some cell types (*eg* PC12 cells) Ca^{2+} stores exist sensitive to both IP_3 and Ry, suggesting colocalization of the two receptors in their limiting membrane. The intracellular release channels are depicted white for the IP_3 receptor, black for the Ry receptor. For the latter is indicated the sensitivity to both changes in $[Ca^{2+}]_i$ (calcium-induced-Ca^{2+}-release) and caffeine (Caff, a drug that lowers the Ca^{2+} threshold of the latter process).

Store-operated calcium entry (SOCE)

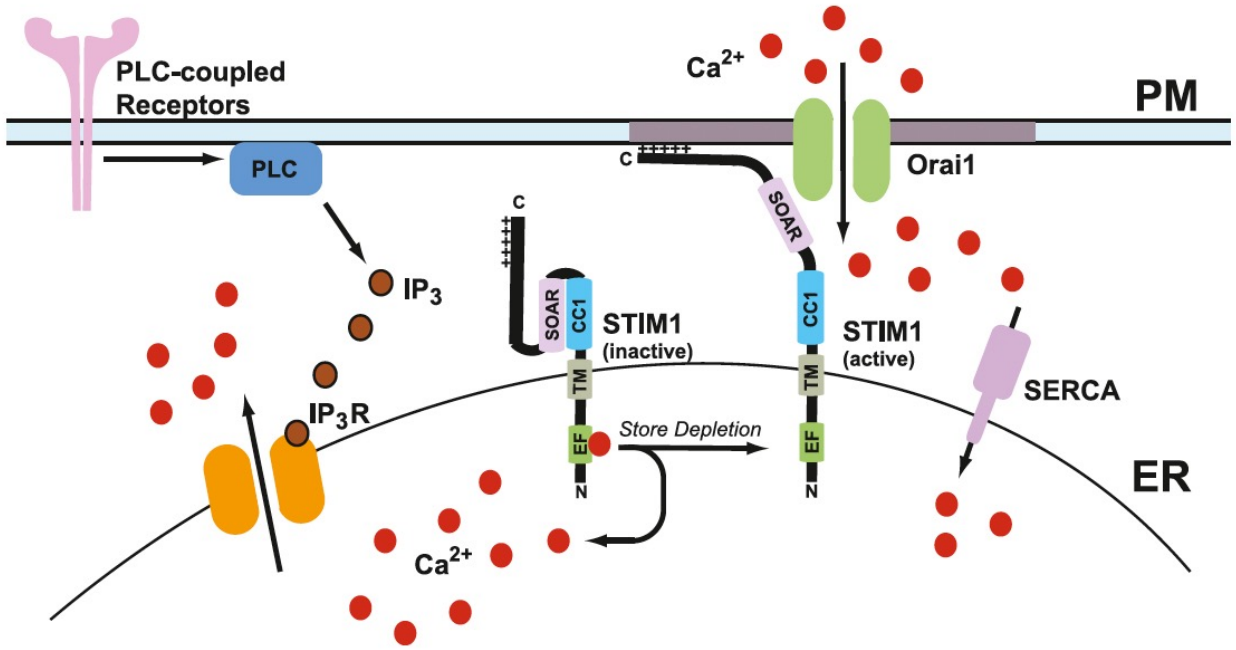
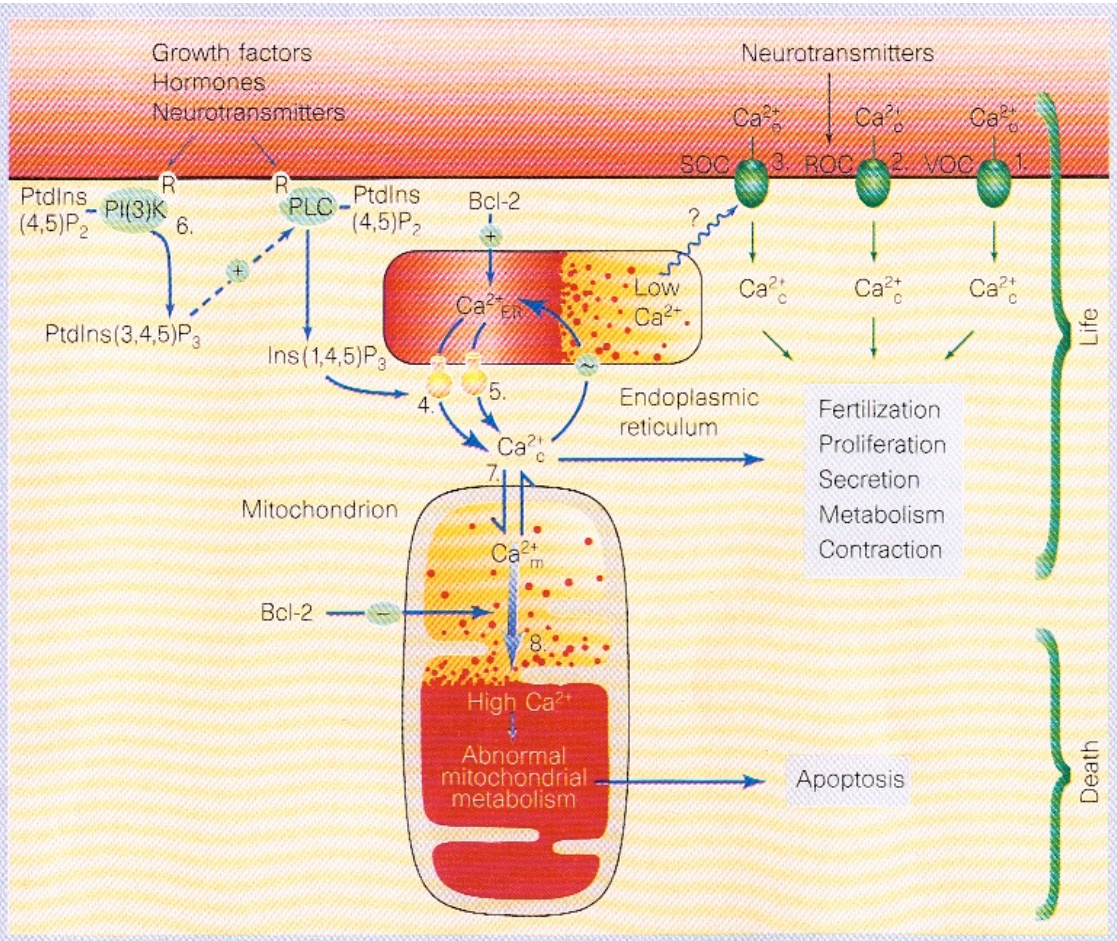


FIGURE 1
General overview of store-operated calcium entry (SOCE). Agonist stimulation through the PLC-coupled receptors leads to IP_3-mediated calcium release which leads to ER calcium store depletion. As a result, calcium dissociates from the EF-hand of STIM1 and leads to its activation. This leads to a conformation change in STIM1 which extends its C-terminus toward plasma membrane where it binds to Orai1 channels and form calcium release-activated calcium (CRAC) channels. CRAC channels promote calcium entry from extracellular milieu into the cells. One subunit of STIM1 dimer is shown here for simplicity.

Kodakandla et al. (2023), *Front. Physiol*. 14:1330259

STIM1: Stromal Interaction Molecule 1 (calcium sensor)
Orai1: Calcium channel
STIM1+Orai1 = calcium release-activated calcium (**CRAC**) channel
SOCE: Store-operated calcium entry (calcium influx driven by Orai1)

Role of the mitochondria



Ca^{2+} signalling depends on increased levels of intracellular Ca^{2+} (Ca^{2+}_c), derived either from sources outside the cell (Ca^{2+}_o) or from stores within the endoplasmic reticulum (Ca^{2+}_{ER}). Ca^{2+}_o may enter through (1) voltage-operated Ca^{2+} channels (VOCs) in excitable cells such as neurons or muscle cells, or (2) receptor-operated Ca^{2+} channels (ROCs) in response to neurotransmitters. Store-operated Ca^{2+} channels (SOCs; 3), which open when the internal stores are emptied of Ca^{2+}, are mainly found in non-excitable cells.

Ca^{2+}_{ER} is released by two types of channel. Inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) is generated by the action of the enzyme phospholipase C (PLC) on phosphatidylinositol 4,5-bisphosphate ($PtdIns(4,5)P_2$) at the plasma membrane, in response to the action of growth factors, hormones or neurotransmitters at receptors (R). $Ins(1,4,5)P_3$ acts on receptors in the endoplasmic reticulum (4), which cause the release of Ca^{2+}_{ER} from the store. Ryanodine receptors also cause the release of Ca^{2+}_{ER}, especially in excitable cells (5).

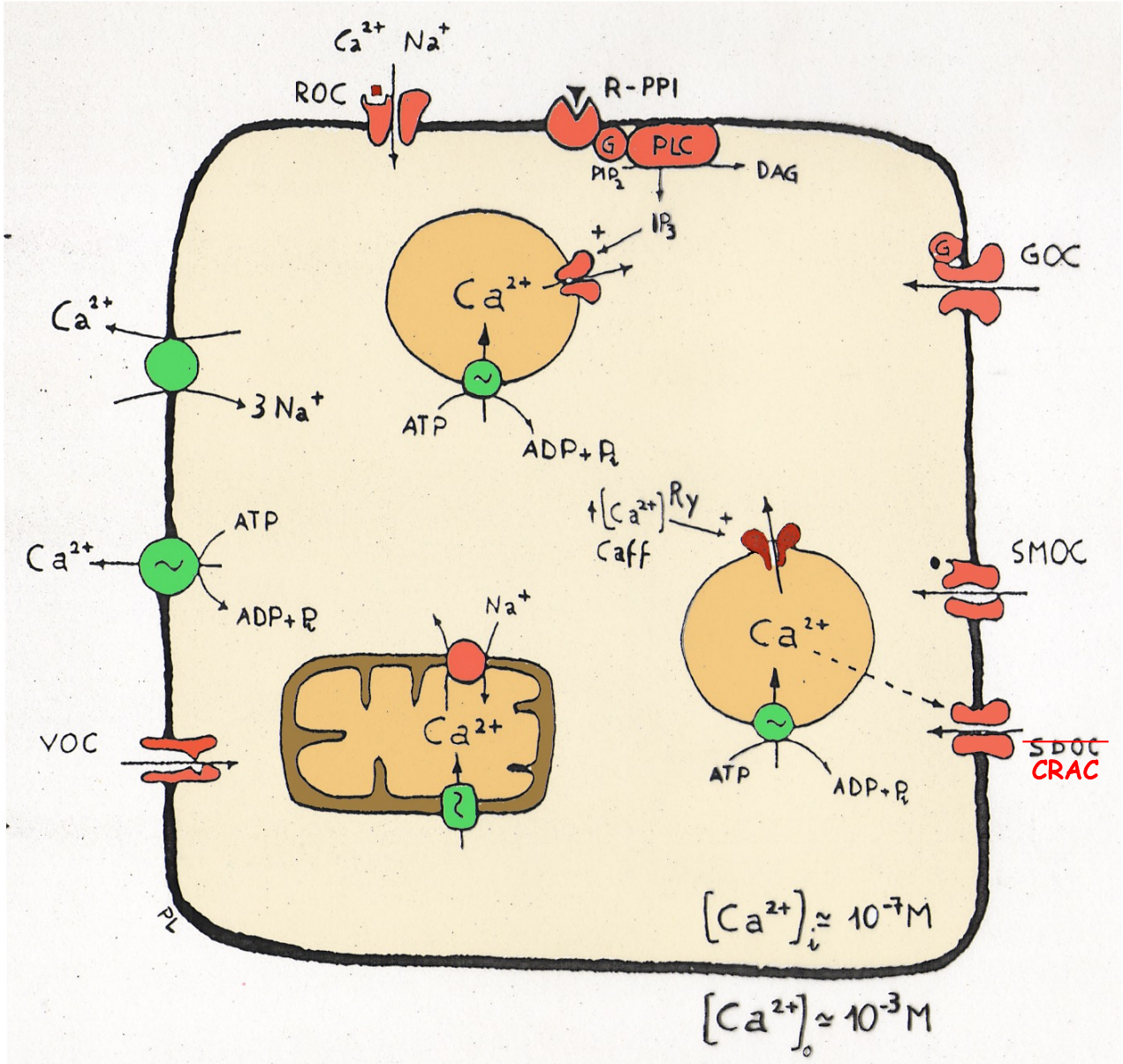
In some cells, such as lymphocytes, the production of $Ins(1,4,5)P_3$ is modulated by the phosphatidylinositol 3-OH kinase, PI(3)K, signalling pathway, which uses $PtdIns(4,5)P_2$ to produce the $PtdIns(3,4,5)P_3$ that acts as a messenger to maintain the activity of PLC. Some of the Ca^{2+}_{ER} is rapidly taken up by the mitochondria (Ca^{2+}_m) and is then returned to the endoplasmic reticulum (7), although most of the stored Ca^{2+}_{ER} resides in the lumen of the endoplasmic reticulum. But if the mitochondria become overloaded with Ca^{2+}_m, the result is abnormal mitochondrial metabolism (8), which may activate programmed cell death.

M.J.B., M.D.B. & P.L.

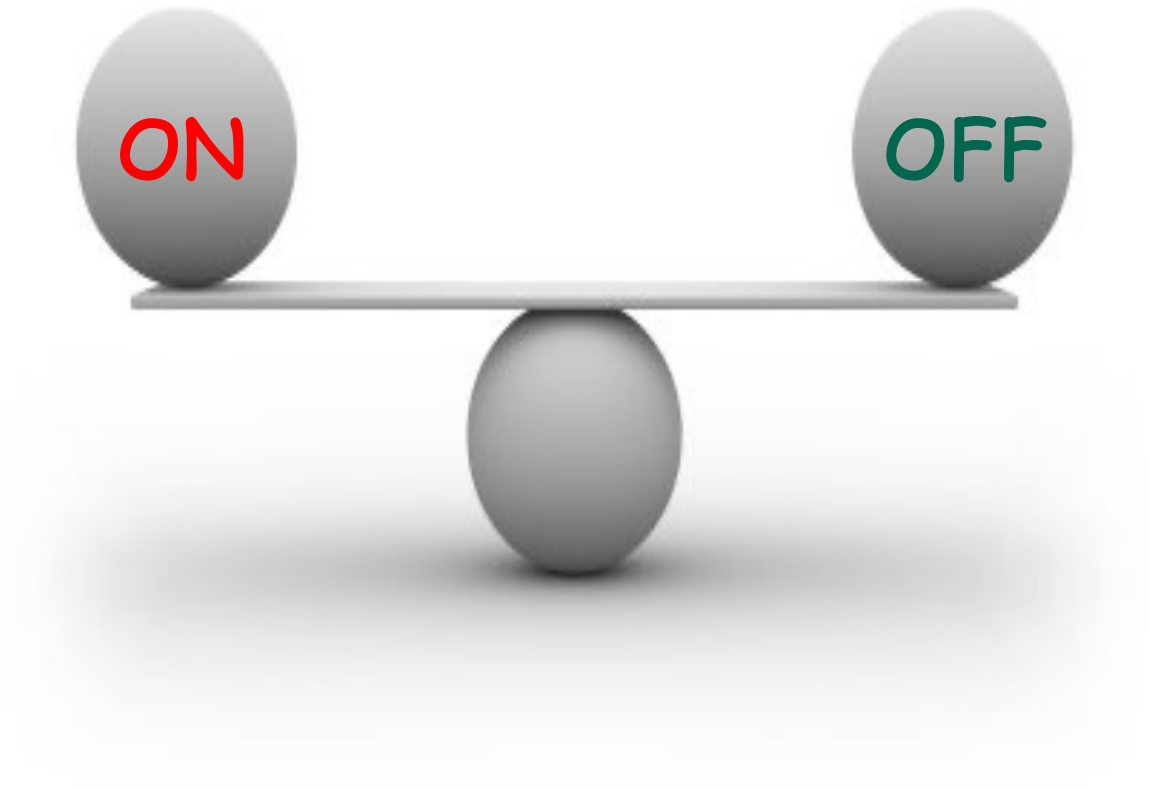
Control of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)

Ca^{2+}- ON mechanisms

Ca^{2+}- OFF mechanisms



Interplay between ON and OFF mechanisms



$[Ca^{2+}]_i$ and ageing

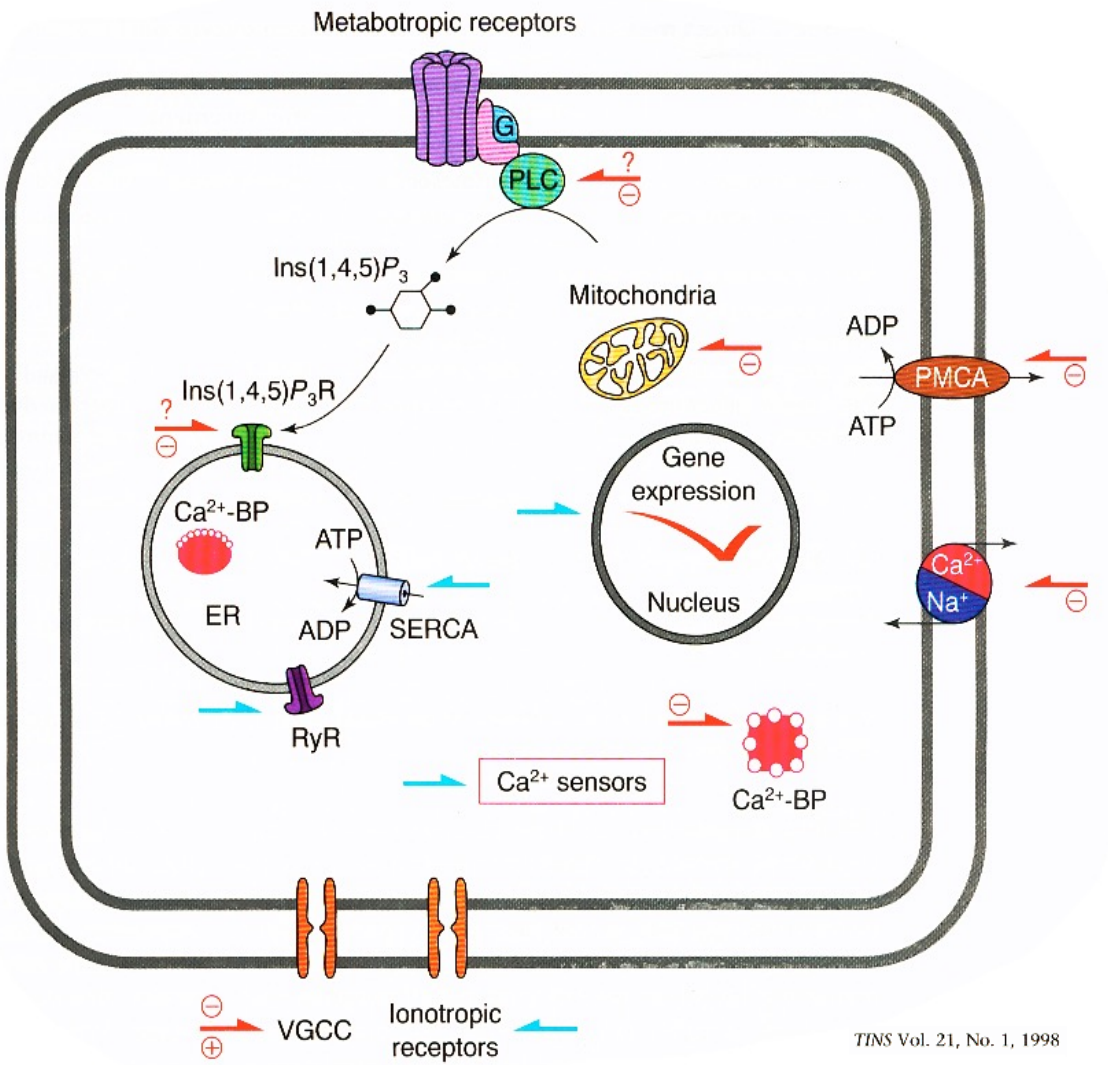


Fig. 1. ***Age-dependent alterations of the homeostatic mechanisms of intracellular Ca^{2+} concentration.*** *The various systems responsible for: the maintenance of low $[Ca^{2+}]_i$ level; Ca^{2+} clearance [PMCA, plasmalemmal Ca^{2+}-ATPases; Na^+/Ca^{2+} exchanger; Ca^{2+}-BP, Ca^{2+}-binding protein; SERCA, endo(sarco)plasmic-reticulum (ER) Ca^{2+} ATPases; mitochondria]; and Ca^{2+} delivery into the cytoplasm [VGCC, voltage-gated Ca^{2+} channels; ionotropic receptors, metabotropic receptors that control Ins(1,4,5)P_3R, inositol (1,4,5)-trisphosphate-gated intracellular Ca^{2+} channels; RyRs, Ca^{2+}-gated ryanodine-sensitive intracellular Ca^{2+} channels] could be altered in aged neurones (G, G-protein; PLC, phospholipase C). The red arrows with '+' or '–' signs show the changes found experimentally, and the question marks indicate where results were inconclusive; the blue arrows show the systems that might be altered, but have not been experimentally assessed. It would be interesting to explore the age-dependent alterations that occur in the intracellular Ca^{2+} sensors: for example, the ras–raf system and the Ca^{2+}–calmodulin system are involved in the transduction of the Ca^{2+} signal into gene expression (alternatively, the Ca^{2+} signal might propagate to the nucleus and affect the genome directly).*

$[Ca^{2+}]_i$ in aged neurons

TABLE 2. Direct measurements of the concentration of cytosolic free Ca^{2+} in neurones from aged rodents

Source	Preparation	Method of measurement	Resting $[Ca^{2+}]_i$	Ca^{2+} signals	Refs
Rat: whole brain	Synaptosomes	Quin2, Fura-2	Increased	Increased	23
Rat: cortex cerebrum	Synaptosomes	Quin2	Increased (NS)	Increased and prolonged decay	24
Rat: hippocampus and cerebrum	Synaptosomes	Fluo-3	Increased*	Increased	60
Rat: hippocampus and cerebral cortex	Synaptosomes and cell suspension	Fluo-3	Increased*	Increased and prolonged decay	60
Rat, mouse: whole brain	Cell suspension	Fura-2	Decreased†	Decreased	61
Rat: cortex, hippocampus, striatum, cerebellum	Cell suspension	Fura-2	Decreased in hippocampus and cortex†	Decreased	62
Rat: DRG	Culture	Fura-2	Increased	Decreased and prolonged decay	63
Rat: DRG, hippocampus, neocortex	Freshly isolated	Indo-1	Increased	Decreased and prolonged decay	57
Mouse: cerebellar granule neurones	Slice	Fura-2	Increased	Decreased and prolonged decay	56

*Fluo-3 does not permit accurate estimation of the concentration of intracellular Ca^{2+}. †Unusually high resting concentration of intracellular Ca^{2+} (300–600) nM. Abbreviation: NS, not significant.

Decoding of $[Ca^{2+}]_i$ signals generated by diverse inputs

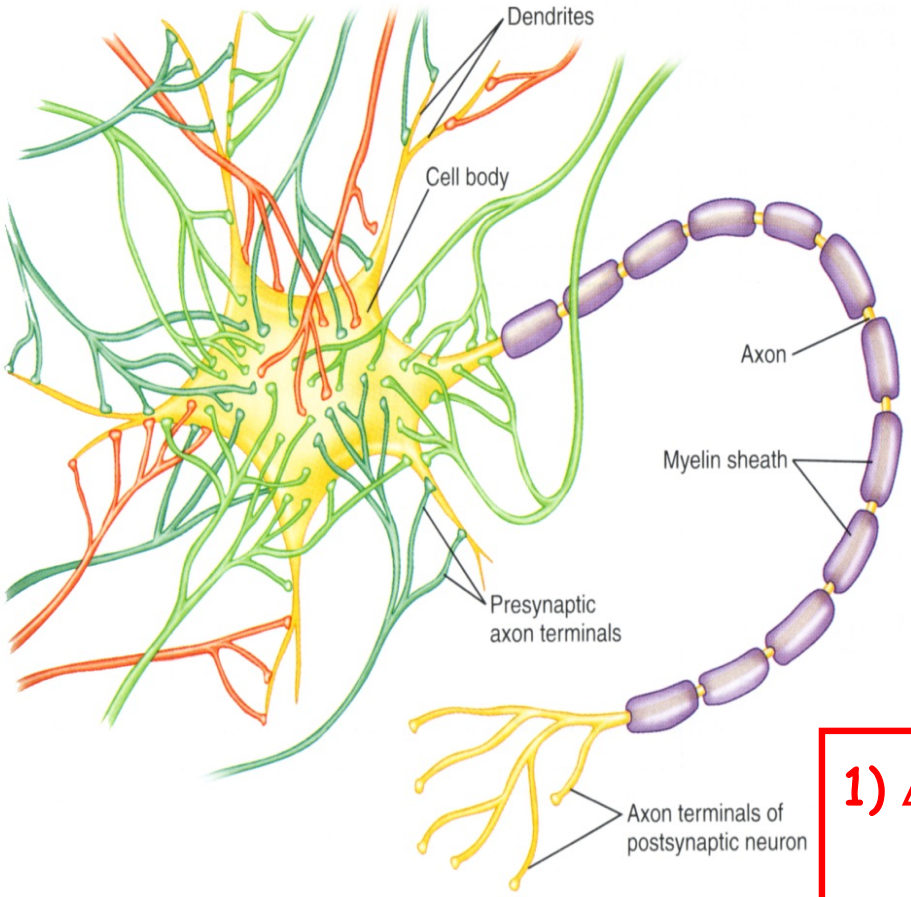


FIGURE 7.8 Convergence, in which many presynaptic cells synapse on one postsynaptic cell. *Most synapses occur on the cell body and dendrites.*

1) $\Delta[Ca^{2+}]_i$ kinetics/affinity of the sensors

2) spatial organisation of Ca^{2+} signals

3) temporal organisation of Ca^{2+} signals

Some cytosolic Ca^{2+}- binding proteins are not effectors (cytosolic Ca^{2+} buffers)

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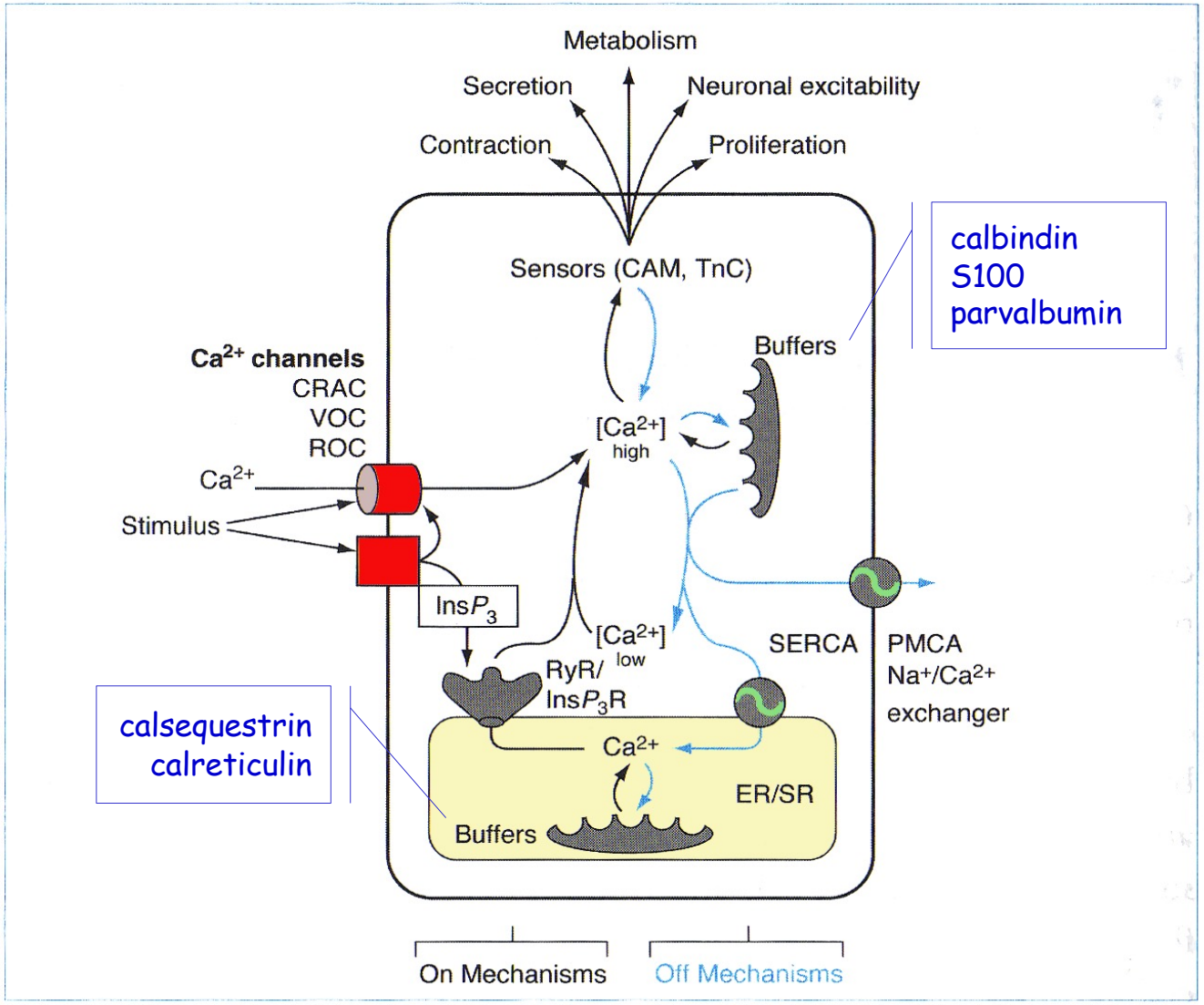
Parvalbumin	EF hand	Ca^{2+} buffer
Calbindin	EF hand	Ca^{2+} buffer
Calsequestrin		Ca^{2+} buffer

The importance of the intracellular Ca^{2+} buffers

Figure 14.1 Summary of the major ON and OFF mechanisms responsible for regulating the concentration of intracellular Ca^{2+}. Stimuli raise the level of Ca^{2+} by activating the ON mechanisms, which promote either the entry of external Ca^{2+}, or the release of Ca^{2+} from intracellular stores (ER/SR). Changes in Ca^{2+} are damped by buffers located both in the cytoplasm and in the ER/SR compartments. The OFF mechanisms restore the low resting level of Ca^{2+} by either pumping it out of the cell or back into the stores. The effects of an elevated Ca^{2+} concentration are mediated by sensors such as calmodulin (CAM) or troponin C (TnC), to regulate a wide range of cellular activities (see Figure 14.4 for details).



Ca^{2+} has a short-range action

Range of Messenger Action of Calcium Ion and Inositol 1,4,5-Trisphosphate

Nancy L. Allbritton, Tobias Meyer, Lubert Stryer

The range of messenger action of a point source of Ca^{2+} or inositol 1,4,5-trisphosphate (IP_3) was determined from measurements of their diffusion coefficients in a cytosolic extract from *Xenopus laevis* oocytes. The diffusion coefficient (*D*) of [^{3}H]IP_3 injected into an extract was 283 $\mu m^2/s$. *D* for Ca^{2+} increased from 13 to 65 $\mu m^2/s$ when the free calcium concentration was raised from about 90 nM to 1 μM. The slow diffusion of Ca^{2+} in the physiologic concentration range results from its binding to slowly mobile or immobile buffers. The calculated effective ranges of free Ca^{2+} before it is buffered, buffered Ca^{2+}, and IP_3 determined from their diffusion coefficients and lifetimes were 0.1 μm, 5 μm, and 24 μm, respectively. Thus, for a transient point source of messenger in cells smaller than 20 μm, IP_3 is a global messenger, whereas Ca^{2+} acts in restricted domains.

Table 1. Estimated range and time scale of messenger action of Ca^{2+} and inositol 1,4,5-trisphosphate.

Messenger	Diffusion coefficient ($\mu m^2/s$)	Time scale (s)	Range (μm)
Calcium			
Free ion	223	0.00003	0.1
Buffered	13	1	5
Inositol 1,4,5-trisphosphate	280	1	24

Fluorescent Ca^{2+} dyes

THE JOURNAL OF BIOLOGICAL CHEMISTRY
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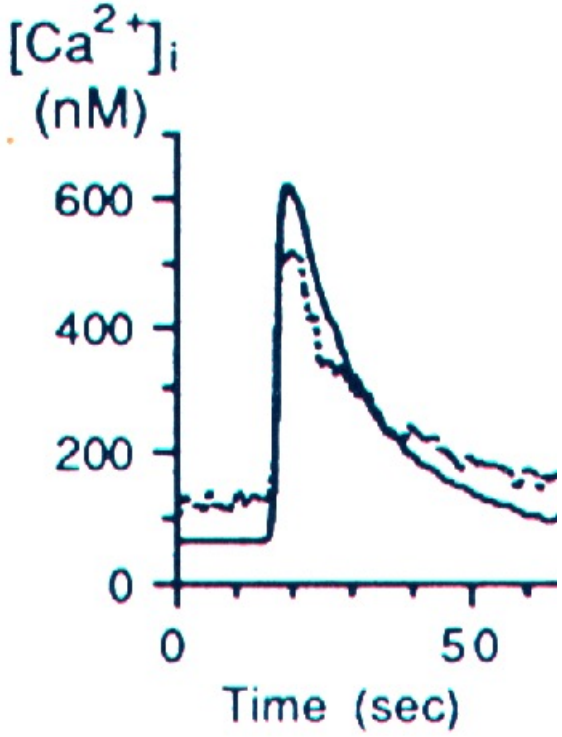
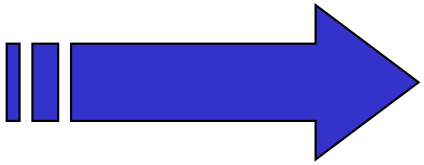
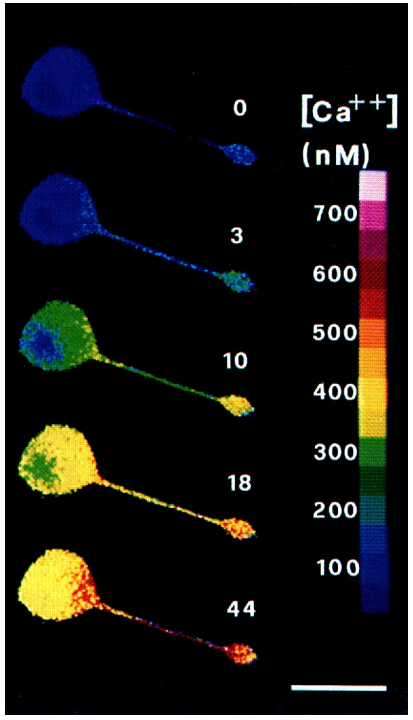
A New Generation of Ca^{2+} Indicators with Greatly Improved Fluorescence Properties*

(Received for publication, August 23, 1984)

Grzegorz Grynkiewicz‡, Martin Poenie, and Roger Y. Tsien§
From the Department of Physiology-Anatomy, University of California, Berkeley, California 94720

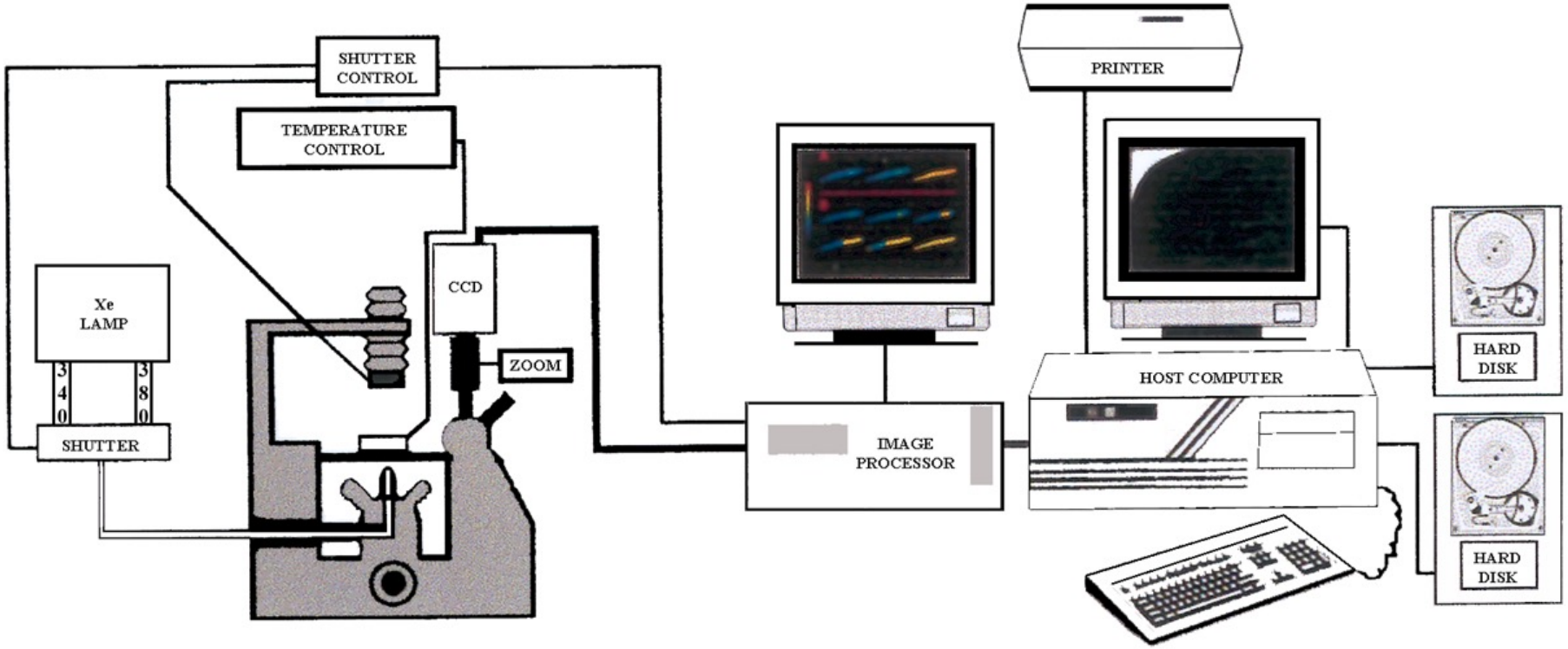






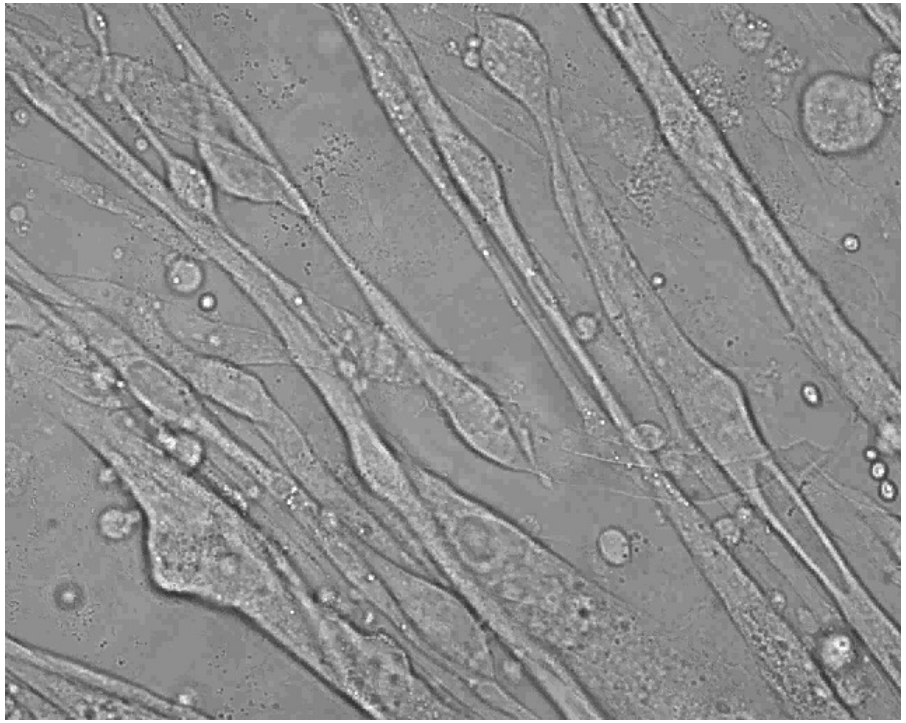
Ca^{2+} imaging

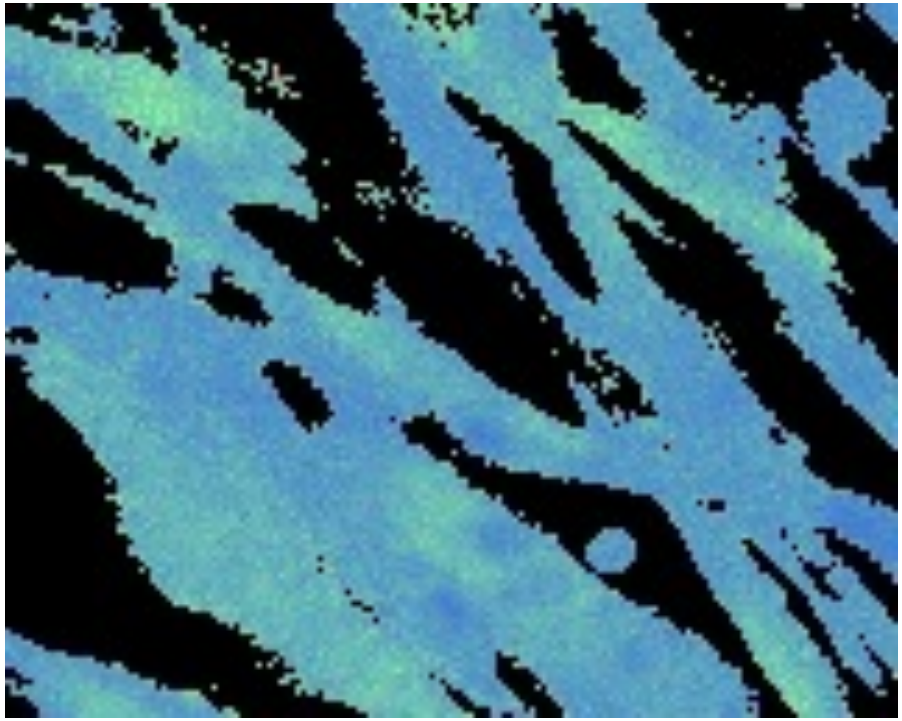
The set-up



An example

https://youtu.be/80Cnyp6HUll?si=7bttMXQFQ48WVtG9





Nobel prize in Chemistry 2008

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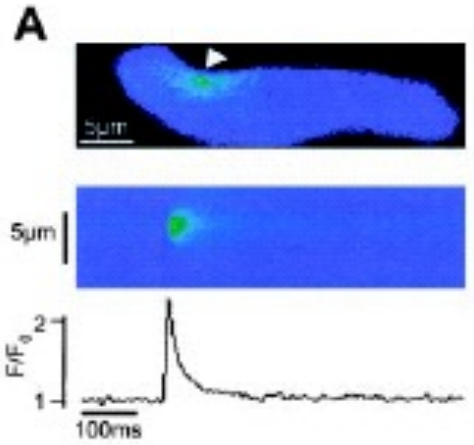
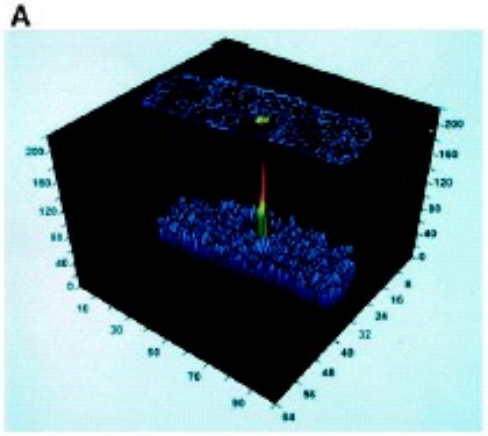
(1952 – 2016)

Elementary Ca^{2+} release events

CRU	A Ca^{2+} release unit (CRU) refers to a group of Ca^{2+} release channels (RyRs or IP3Rs or both) clustered in the ER/SR membrane[82]
Ca^{2+} sparks	Event or the optical image of Ca^{2+} release from a single CRU[6]
Ca^{2+} sparklet	Event or the optical image of Ca^{2+} fluxing through a single Ca^{2+}-permeable channel[75]
Compound Ca^{2+} sparks	Event or the optical image of near-synchronous activation of multiple adjacent CRUs[48]
Ca^{2+} puff	Synonym to Ca^{2+} spark or compound Ca^{2+} spark when a single or multiple CRUs of IP_3R are involved[49]
Ca^{2+} quark	Synonym to RyR Ca^{2+} sparklet[83]
Ca^{2+} blip	Synonym to IP_3R Ca^{2+} sparklet[58]
Ca^{2+} spike	(1) Spatially averaged Ca^{2+} transient measured in the presence of excessive Ca^{2+} buffers. In cardiac myocytes, it mainly reflects SR Ca^{2+} release function.[81]
	(2) Local Ca^{2+} transient measured in the presence of excessive Ca^{2+} buffers. In cardiac myocytes, it mainly reflects Ca^{2+} release function underlying a single or compound Ca^{2+} spark at a Z-line/TT site.[81]
Ca^{2+} scraps	Spatially averaged SR Ca^{2+} depletion transients that mirror cytosolic Ca^{2+} transients ("sparks" spelled backward)[110]
Ca^{2+} mark	Ca^{2+} transient in a single mitochondrion[113]



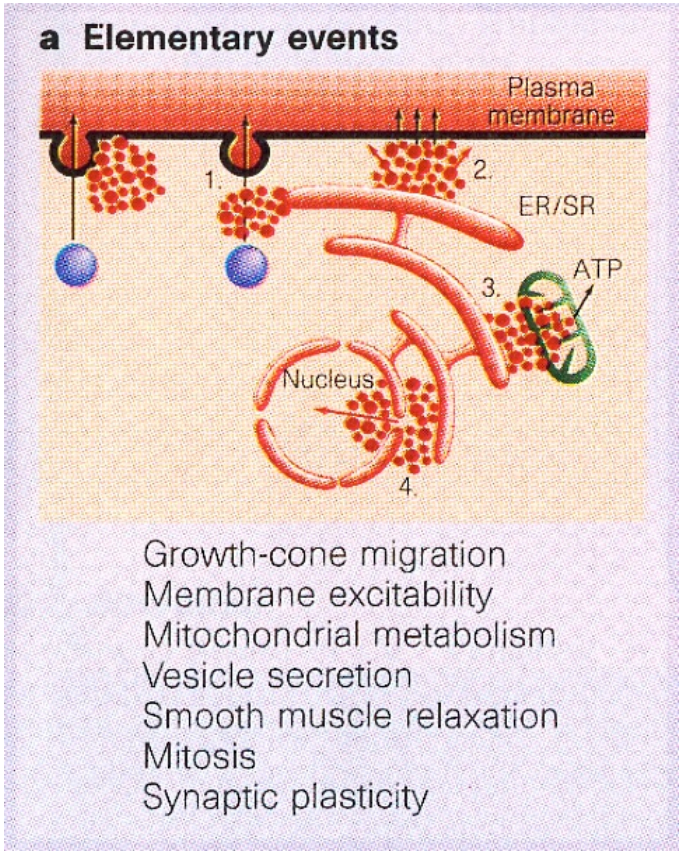


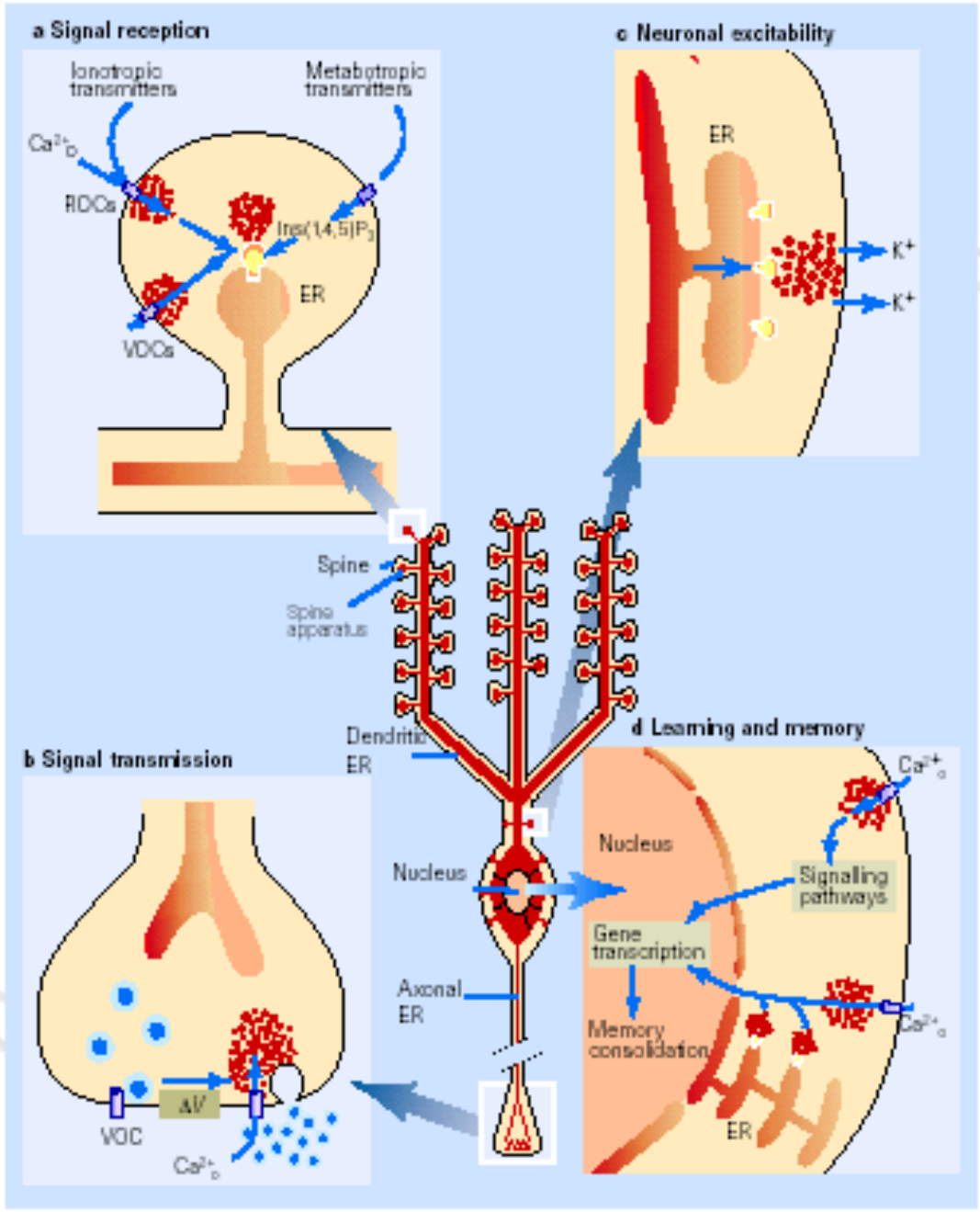
Circulation Research April 30, 2004

Spatial aspects of Ca^{2+} signalling

1. Localised $[Ca^{2+}]_i$ transients

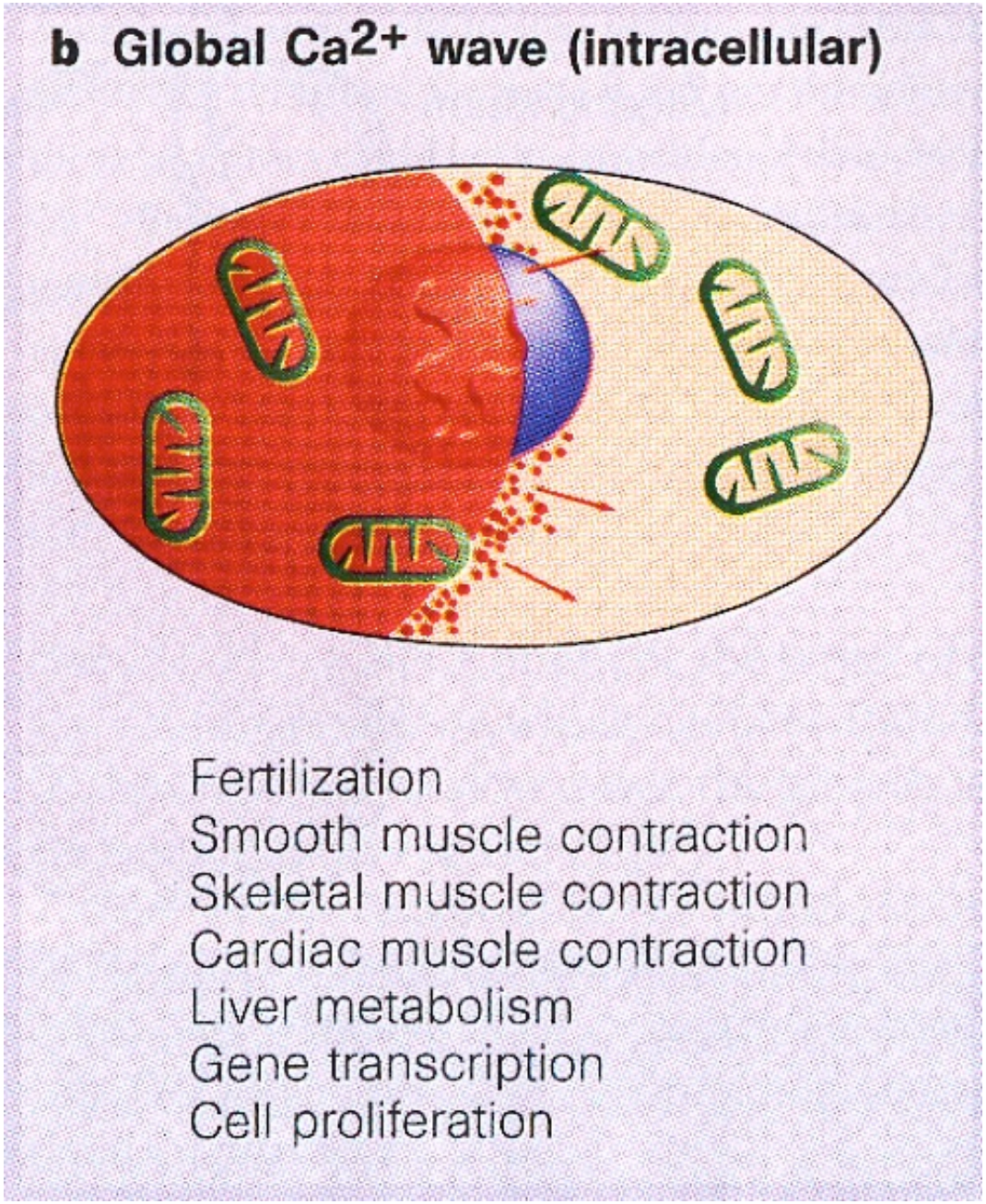
(microdomains/nanodomains)





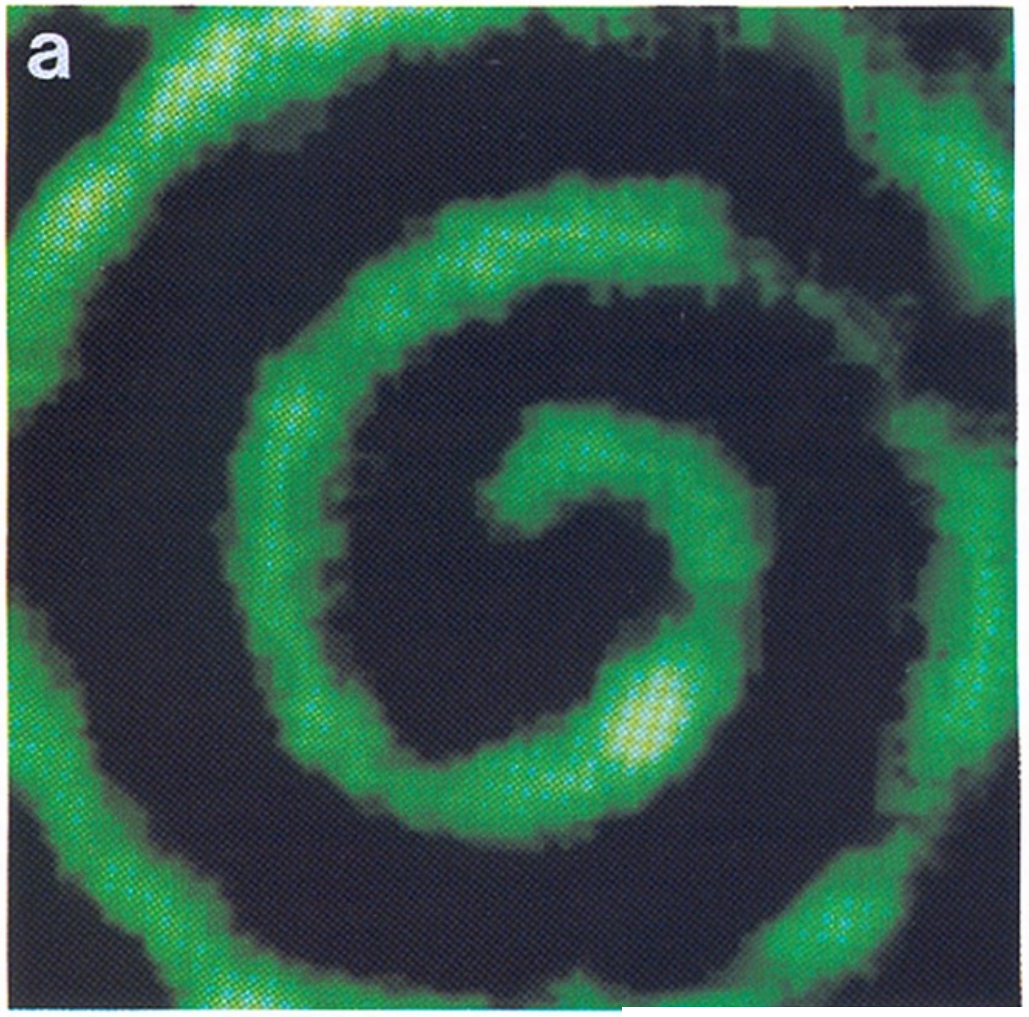
Spatial aspects of Ca^{2+} signalling

2. $[Ca^{2+}]_i$ waves



$[Ca^{2+}]_i$ waves in oocytes after fertilisation

spiral orientation

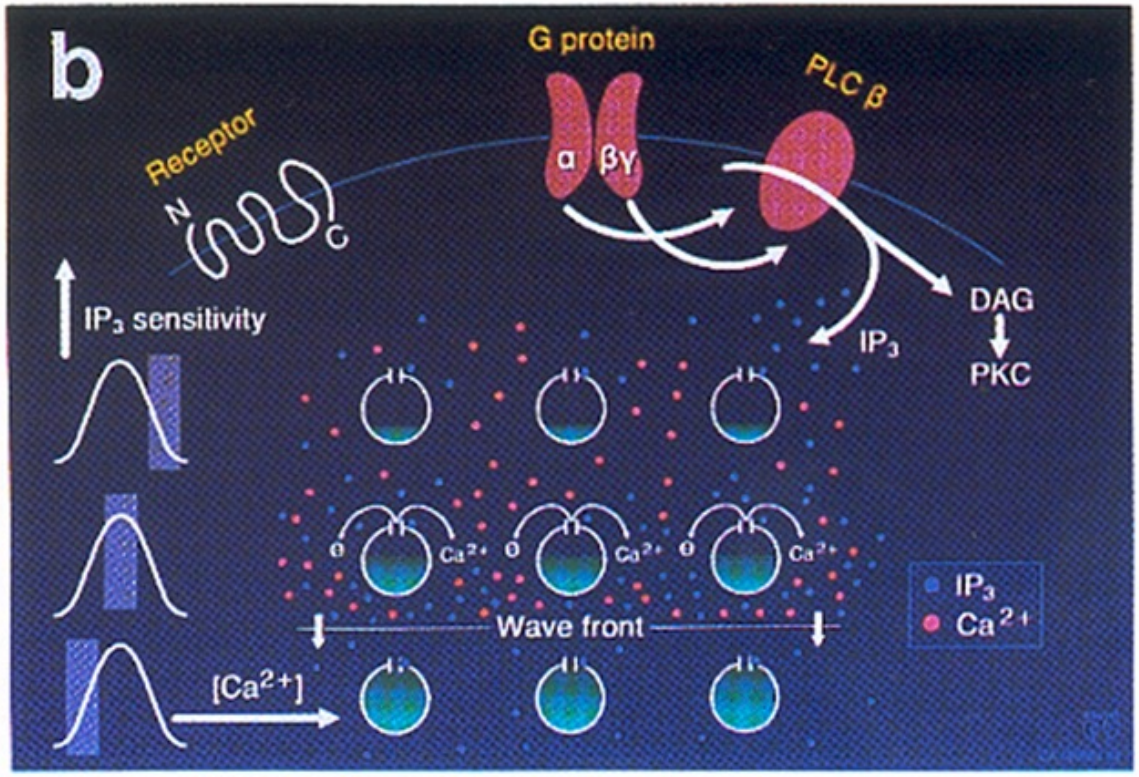


Cell, Vol. 80, 259–268, January 27, 1995

(A) Ca^{2+} wave observed in an intact Xenopus oocyte. Green indicates high $[Ca^{2+}]$ (peak level, approximately 0.5 μM). An oocyte was loaded with $InsP_3$ (10 μM) to evoke Ca^{2+} release and with the Ca^{2+}-sensitive fluorescent dye Fluo3 (15 μM) prior to confocal imaging. The distance between expanding wavefronts, or wavelength, is 250 μm, and waves travel 15–30 μm/s. Many other complex patterns, as well as simple planar waves, can be observed within the same oocyte.

One of the mechanisms generating $[Ca^{2+}]_i$ waves

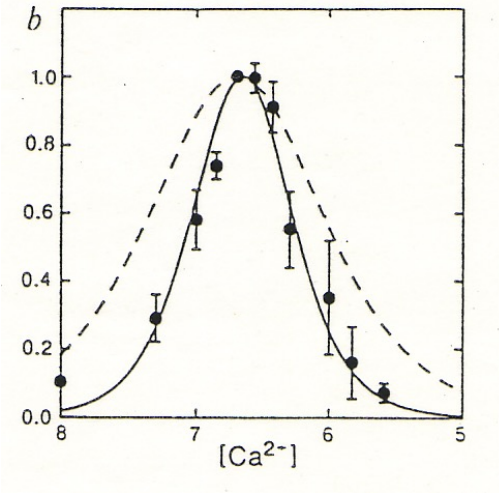
Cell, Vol. 80, 259–268, January 27, 1995

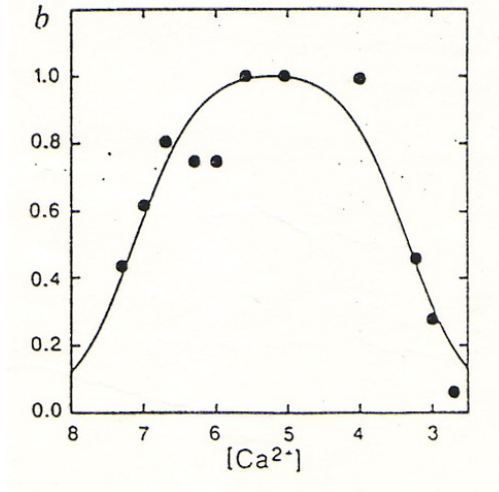


An alternative:
CICR based on RyR - activation
(Calcium-Induced Calcium Release)

Bell-shaped calcium-response curves of Ins(1,4,5)P_3- and calcium-gated channels from endoplasmic reticulum of cerebellum

NATURE · VOL 351 · 27 JUNE 1991





Ca^{2+} waves can propagate with opposite orientation

+BK

+ATP

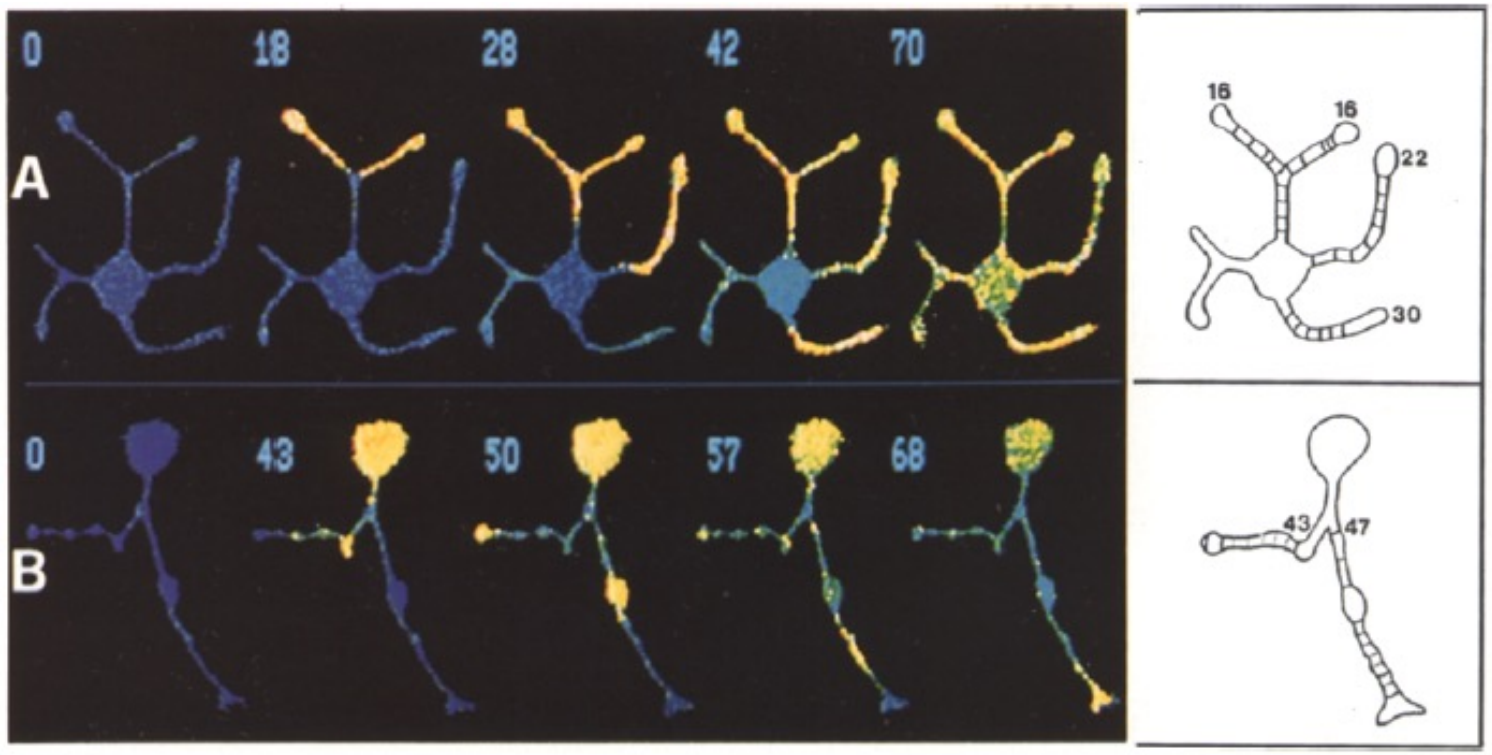


Figure 3. Ca^{2+} waves with opposite orientation induced in two differentiated PC12 cells by BK and ATP administered at 18°C in the Ca^{2+}-free medium. Application of the agonists in the Ca^{2+}-free medium activated Ca^{2+} release from intracellular stores. With 100 nM BK, after various and marked delays, $[Ca^{2+}]_i$ rose at the tip of three neurites and then developed into slow, autoregenerative waves (tides) directed towards the cell body (*A*). In the cell stimulated with 100 μM ATP the signal appeared in the cell body and propagation of the Ca^{2+} waves was oriented centrifugally, appearing however more as bands (*B*). The drawings to the right summarize the $[Ca^{2+}]_i$ events elicited in the neurites of the two cells. Numbers indicate the time when waves first appeared in each neurite. Transversal lines (one second apart) mark the progression of the waves as revealed by the whole set of images. Sharp boundaries (always occurring with the BK tides) are marked by continuous lines; confused boundaries (often appearing with the ATP-induced bands) by dotted lines.

The Journal of Cell Biology, Volume 129, Number 3, May 1995 797–804

Decoding of $[Ca^{2+}]_i$ signals generated by diverse inputs

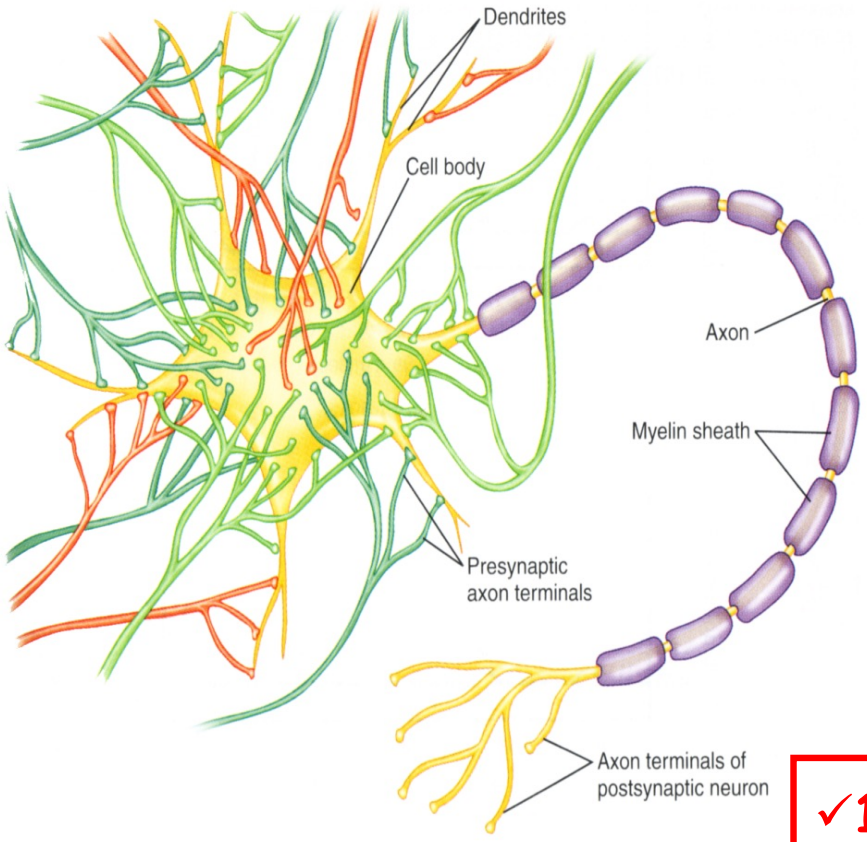


FIGURE 7.8 Convergence, in which many presynaptic cells synapse on one postsynaptic cell. *Most synapses occur on the cell body and dendrites.*

✓1) $\Delta[Ca^{2+}]_i$ kinetics/affinity of the sensors

✓2) spatial organisation of Ca^{2+} signals

3) temporal organisation of Ca^{2+} signals

Temporal aspects of Ca^{2+} signalling

Induced $[Ca^{2+}]_i$ oscillations

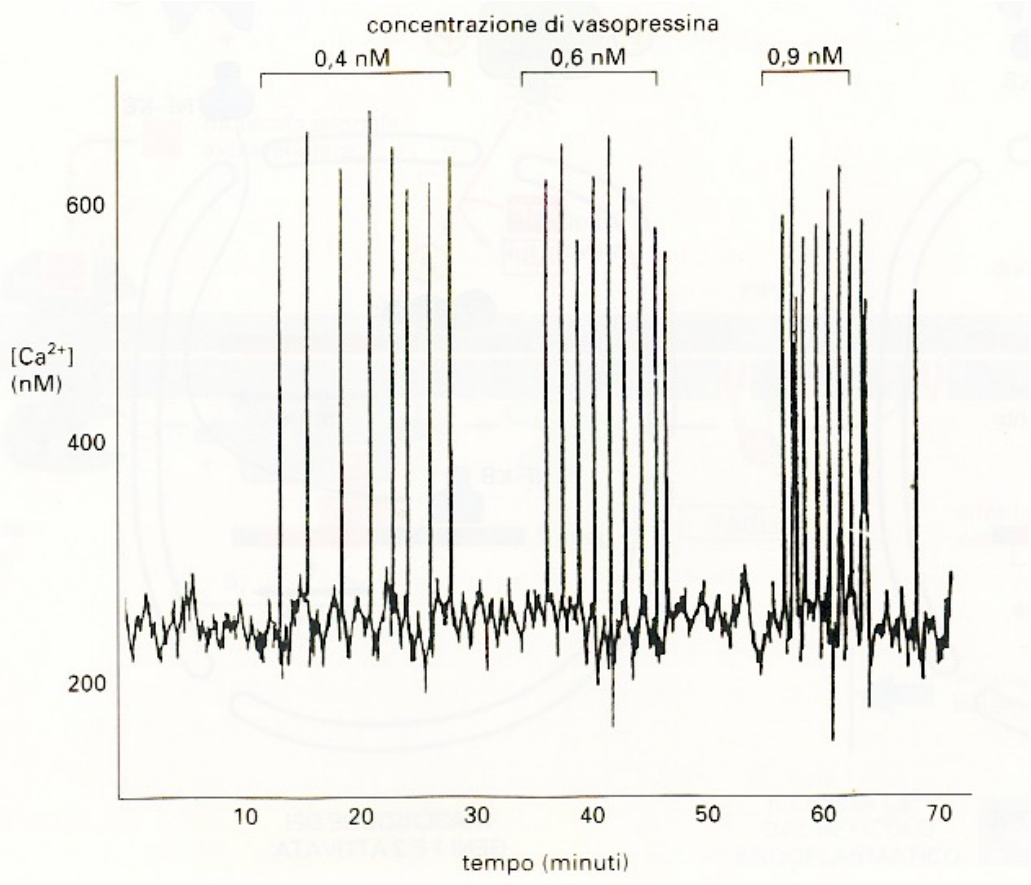
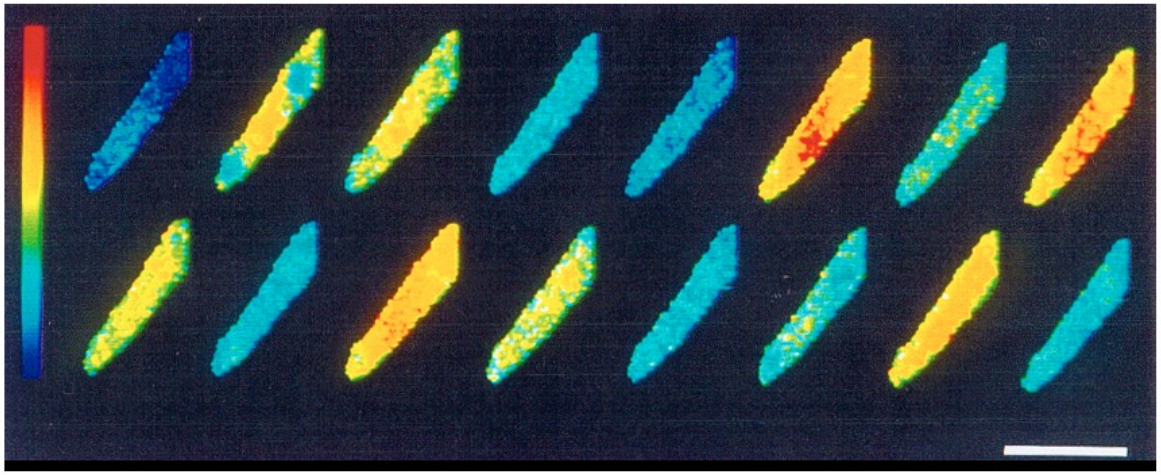


Figura 15.31 Oscillazioni del Ca^{2+} in una cellula di fegato indotte da vasopressina. La cellula è stata caricata con la proteina sensibile al Ca^{2+} equorina e quindi esposta a concentrazioni crescenti di vasopressina. Si noti che la frequenza dei picchi di Ca^{2+} aumenta con l'aumentare della concentrazione della vasopressina ma che la loro ampiezza non viene modificata. (Adattata da N.M. Woods, K.S.R. Cuthbertson e P.H. Cobbold, *Nature* 319:600-602, 1986. © 1986 Macmillan Magazines Ltd.)

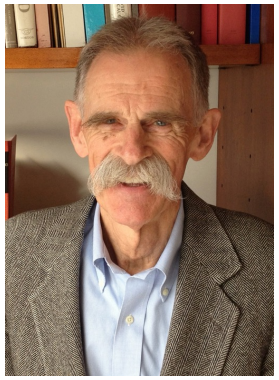
Temporal aspects of Ca^{2+} signalling

spontaneous $[Ca^{2+}]_i$ oscillations

Myogenesis



Lorenzon *et al.* (1997) *Eur. J. Neurosci.* 9; 800-808



Temporal aspects of Ca^{2+} signalling

Spontaneous $[Ca^{2+}]_i$ oscillations

NATURE · VOL 375 · 29 JUNE 1995

Distinct aspects of neuronal differentiation encoded by frequency of spontaneous Ca^{2+} transients

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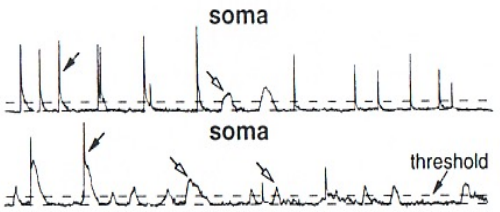
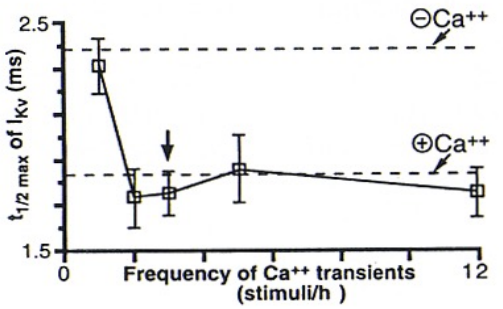
STIMULATION of transient increases in intracellular calcium (Ca_i^{2+}) activates protein kinases[1–3], regulates transcription[4–9] and influences motility and morphology[10–12]. Developing neurons generate spontaneous Ca_i^{2+} transients, but their role in directing neuronal differentiation and the way in which they encode information are unknown. Here we image Ca^{2+} in spinal neurons throughout an extended period of early development, and find that two types of spontaneous events, spikes and waves, are expressed at distinct frequencies. Neuronal differentiation is altered when they are eliminated by preventing Ca^{2+} influx. Reimposing different frequency patterns of Ca^{2+} elevation demonstrates that natural spike activity is sufficient to promote normal neurotransmitter expression and channel maturation, whereas wave activity is sufficient to regulate neurite extension. Suppression of spontaneous Ca^{2+} elevations by BAPTA loaded intracellularly indicates that they are also necessary for differentiation. Ca^{2+} transients appear to encode information in their frequency, like action potentials, although they are 10^4 times longer in duration and less frequent, and implement an intrinsic development programme.

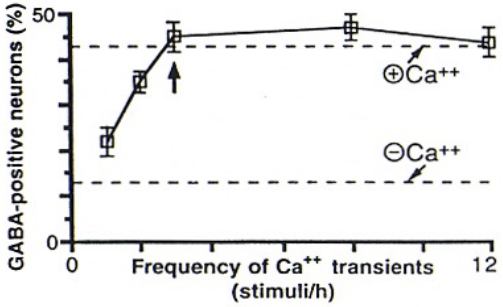
Neurogenesis

gene expression regulation

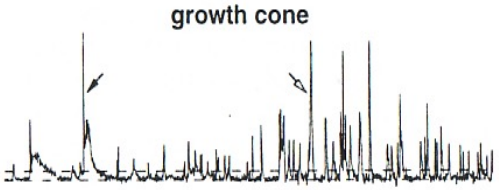


low frequency

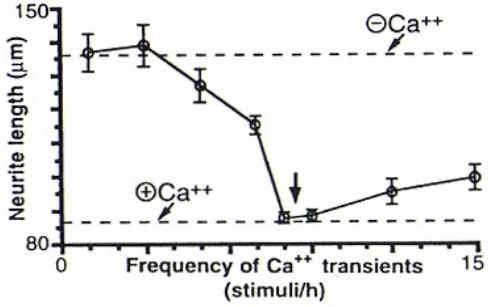




neurite extension

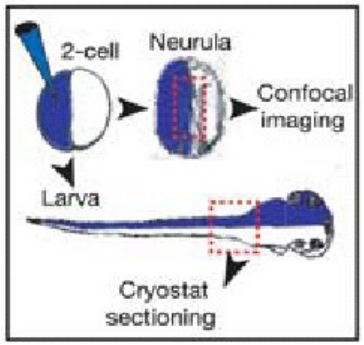


high frequency



Temporal aspects of Ca^{2+} signalling

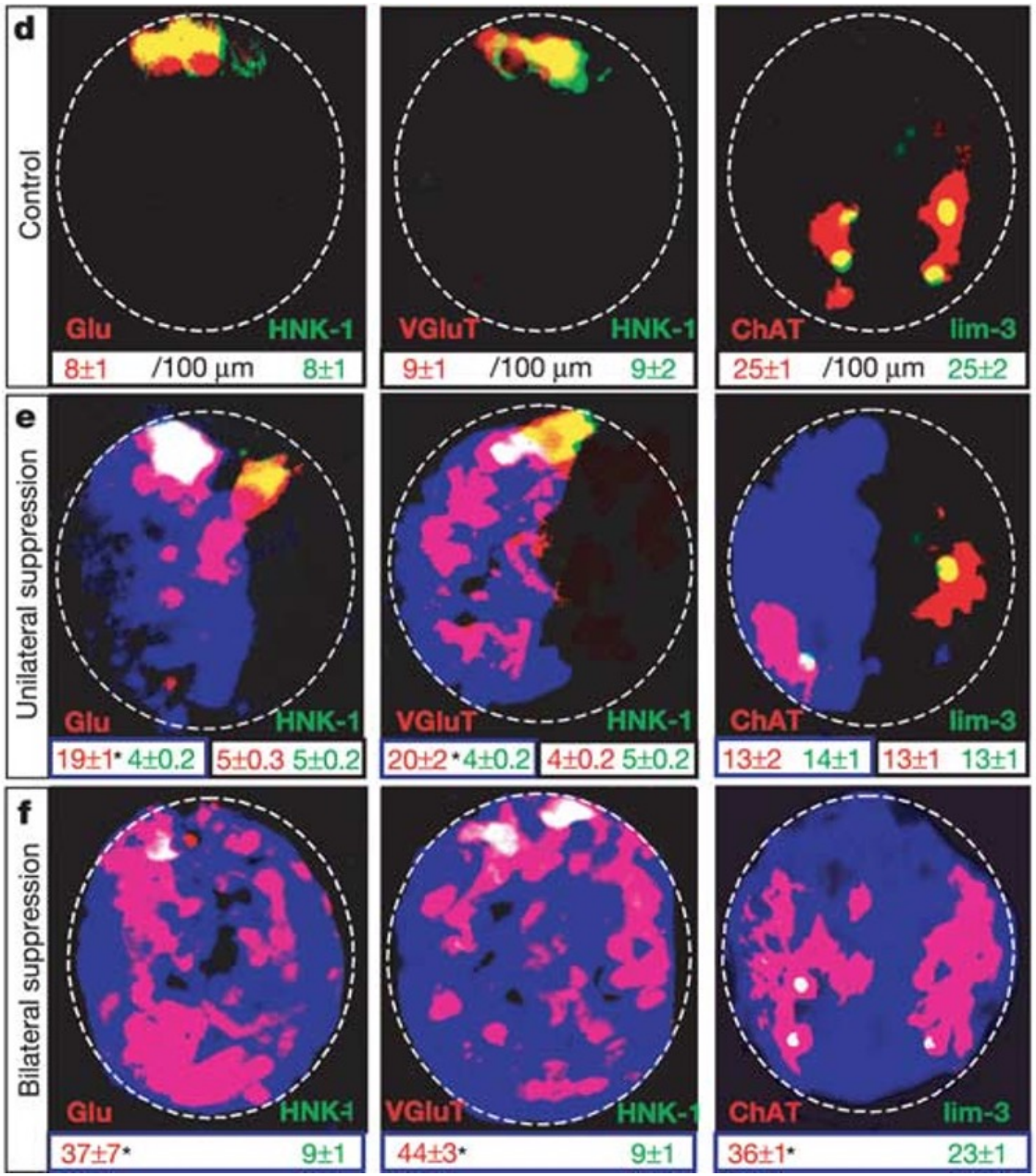
spontaneous $[Ca^{2+}]_i$ oscillations



injection of transcripts
in one or both blastomers

Figure 2 Suppression of spike activity *in vivo* by overexpression of inward rectifier K^+ channels increases the incidence of expression of glutamatergic and cholinergic phenotypes. **a**, Experimental design. hKir2.1 transcripts and fluorescent tracer were injected together into one or both blastomeres at the two-cell stage. Ca^{2+} imaging was performed on stage 22–26 neural-tube embryos (boxed region) and stage 40 larvae were sectioned for immunocytochemistry. **b**, Neural tube resulting from unilateral injection of transcripts plus tracer (left), loaded with bisoxonol (Bisox) to image membrane potential (right), reveals that dorsolateral neurons containing transcripts are hyperpolarized (cells are pseudocoloured blue; $n = 7$ neural tubes). White dashed lines indicate margins of the neural tube. **c**, Neural tube resulting from unilateral injection of transcripts plus tracer (left), loaded with fluo-4 acetoxymethyl ester to image spikes (middle), reveals that spikes in dorsal neurons are suppressed on the side containing transcripts (active cells are circled). The incidence of spiking is reduced in both dorsal and ventral neurons marked with tracer ($n = 15$ dorsal and ventral neural tubes). Dotted columns, control; hatched columns, Kir2.1 unilateral; solid columns, Kir2.1 bilateral. **d**, Neural-tube sections from control embryos stained for glutamate (Glu) or the vesicular glutamate transporter (VGluT) in combination with HNK-1, and choline acetyltransferase (ChAT) in combination with lim-3. **e**, Embryos unilaterally silenced; **f**, bilaterally silenced and stained as in **d**. White dashed ovals indicate neural-tube perimeters in this and subsequent figures. Numbers of immunoreactive neurons per 100 μm of neural tube are indicated beneath panels. For unilaterally silenced embryos these numbers are tabulated separately for each side of the neural tube. In **c**–**f**, values are means ± s.e.m. and asterisks indicate significantly different from control.

Neurogenesis in vivo



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Borodinsky et al.

Ca^{2+} as decoder of the electrical activity

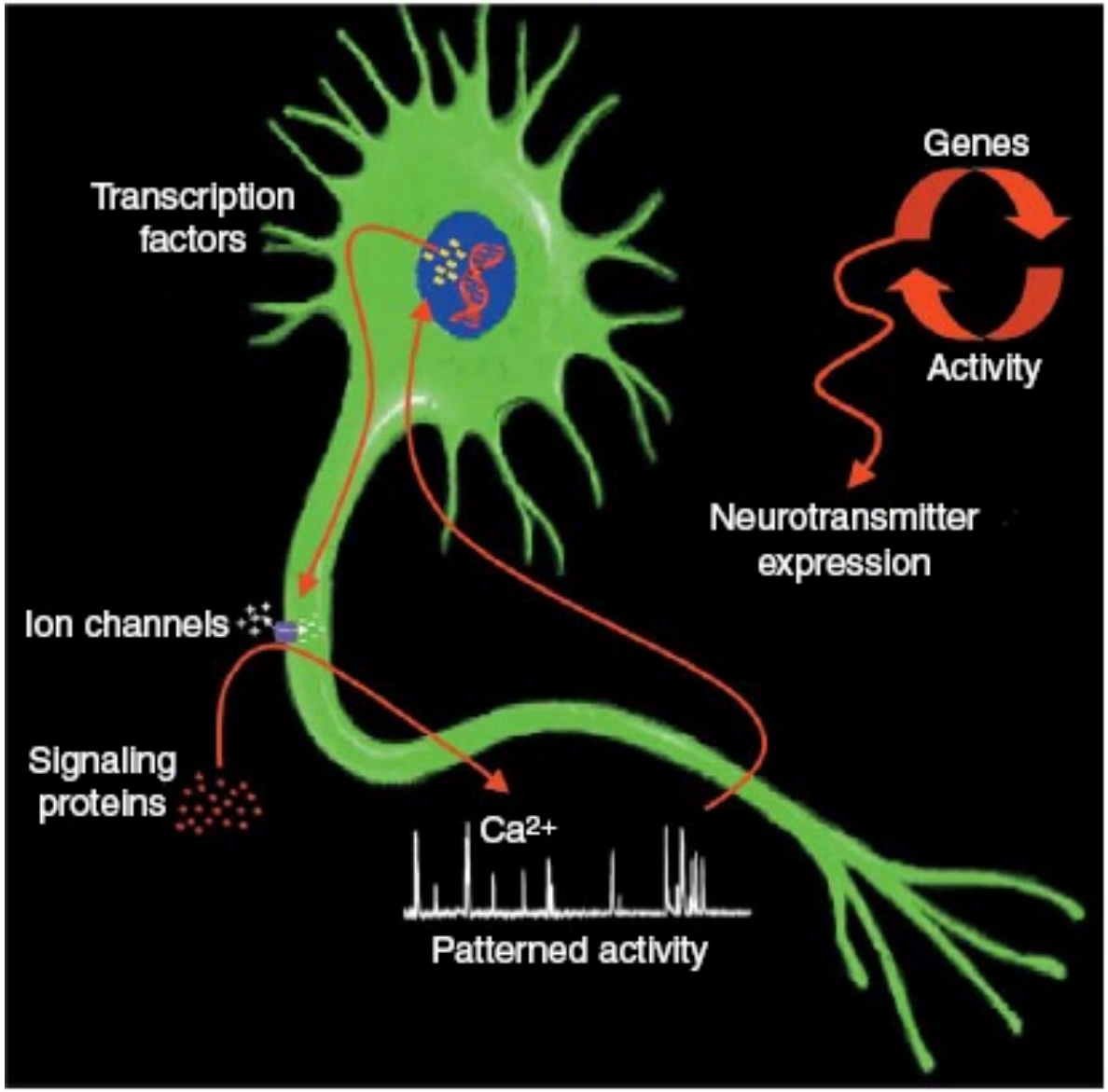


Figure 5. Model for neurotransmitter specification based on studies of the *Xenopus* spinal cord. Expression of transcription factors defines groups of neurons that express constellations of ion channels. These channels produce patterns of Ca^{2+} spike activity that are modulated by signaling proteins. Patterns of spike activity, acting via Ca^{2+}-dependent transcription factors, regulate expression of transcripts encoding enzymes that synthesize, and transporters that store, specific transmitters. Thus, genes and activity collaborate to specify the choice of neurotransmitter.

Ca^{2+} pleiotropy is based on:

1) $\Delta[Ca^{2+}]_i$ kinetics/affinity of sensors
2) spatial organisation of Ca^{2+} signals
3) temporal organisation of Ca^{2+} signals

during differentiation:

frequency depends on intrinsic membrane electrical properties

in adulthood:

frequency depends on network electrical properties

Protein kinases C and CAM kinases

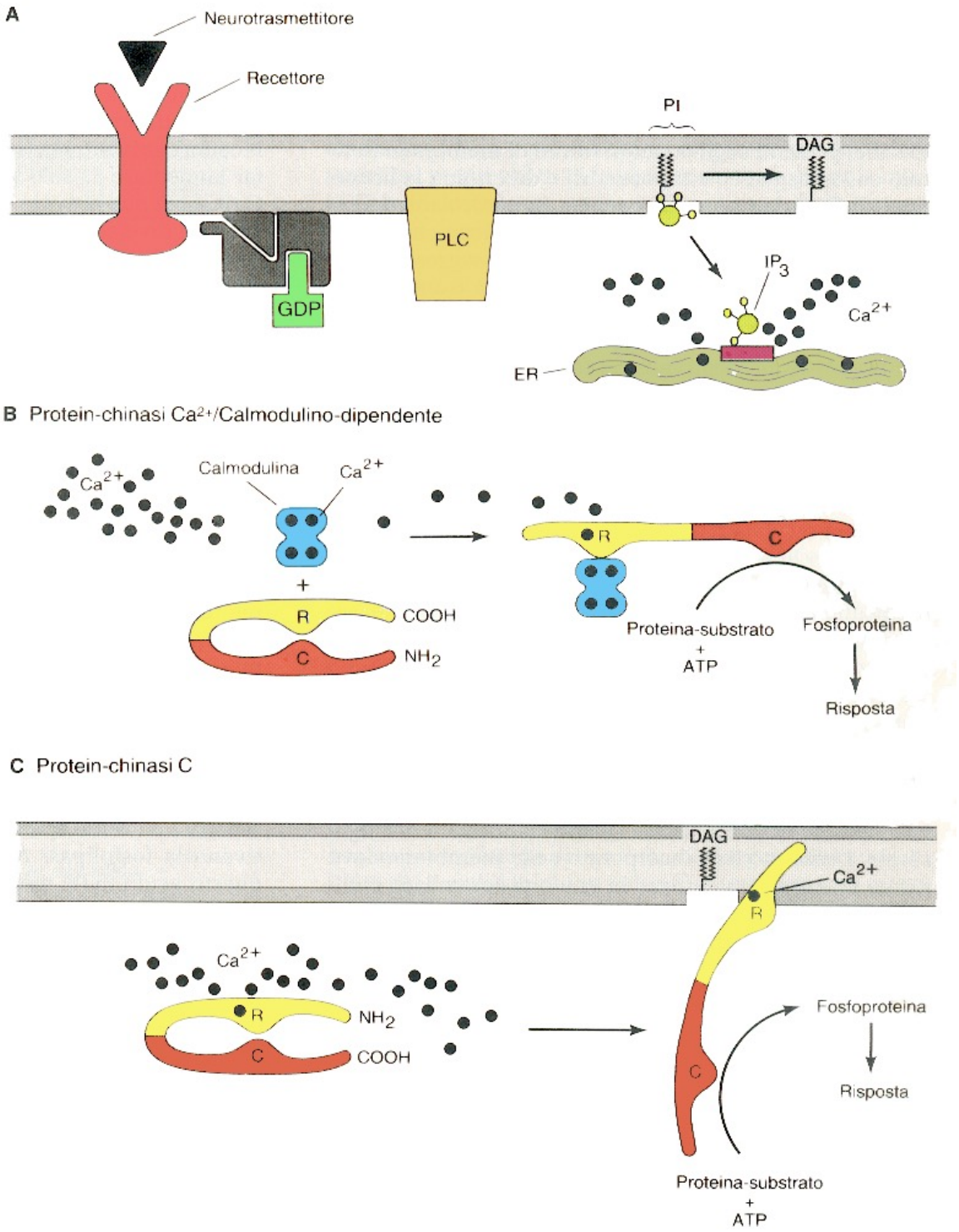


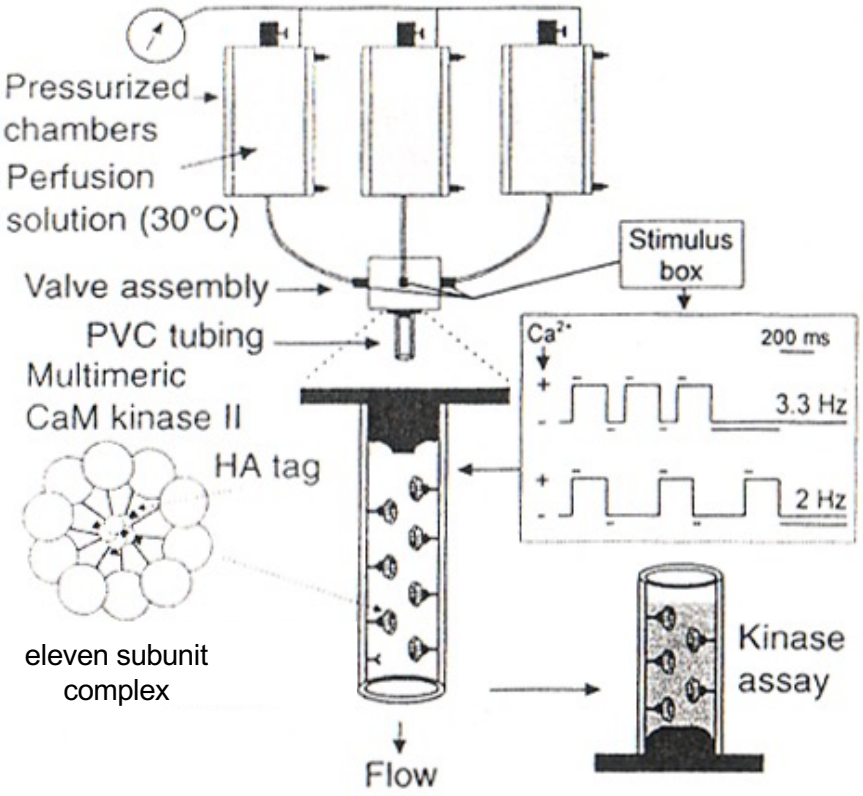
FIGURA 12-7
Attivazione di IP_3, della protein-chinasi Ca^{2+}/calmodulino-dipendente e della PKC.

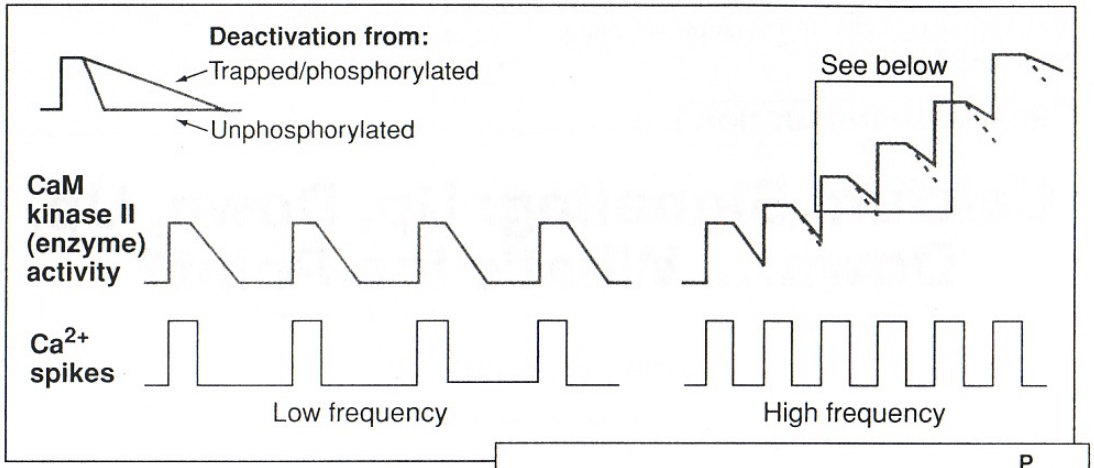
A. Nella via inositolo-lipidi, il legame di un neurotrasmettitore con un recettore attiva una proteina-G che, a sua volta, attiva la fosfolipasi C. Questa fosfolipasi degrada il fosfatidil-inositolo (PI) PIP_2 in due secondi messaggeri, IP_3 e diacilglicerolo (DAG). L'IP_3 è un composto idrosolubile e può diffondere nel citoplasma dove si lega a un recettore localizzato sul reticolo endoplasmatico determinando la liberazione di Ca^{2+} dalle riserve interne.

B. I Ca^{2+} legati alla calmodulina attivano la protein-chinasi.

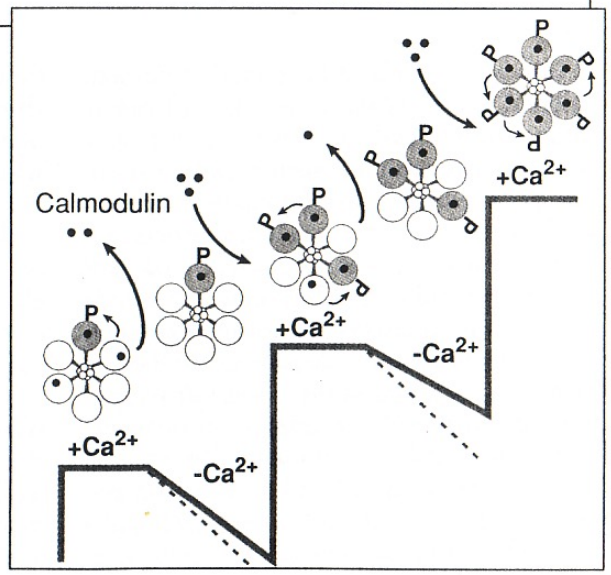
C. Il DAG, che è l'altro secondo messaggero prodotto dall'idrolisi del PIP_2, rimane nella membrana dove attiva la PKC; tale attivazione richiede la presenza dei fosfolipidi della membrana. Alcune isoforme di PKC non richiedono Ca^{2+} per venir attivate.

CAM kinase II is one of the decoders of $[Ca^{2+}]_i$ oscillation frequencies





Calcium rollercoaster. *(Top)* At low frequency, there is no incremental rise in enzyme activity because the kinase fully deactivates between spikes. At high frequency, the kinase cannot fully deactivate which ratchets up the activity. Inset: a CaM kinase II subunit either deactivates slowly if autophosphorylated, or quickly if unphosphorylated. *(Right)* After a series of high frequency Ca^{2+} spikes, the kinase (shown as a hexamer) is autophosphorylated (P on dark gray subunit). As the Ca^{2+} declines, calmodulin (small dots) dissociates but the subunit remains active (light gray). Additional phosphorylation occurs at the next Ca^{2+} pulse, but more readily because the calmodulin binds to a subunit that is already active. This continues until the enzyme is maximally phosphorylated.



Sensitivity of CaM Kinase II to the Frequency of Ca^{2+} Oscillations
Paul De Koninck and Howard Schulman
Science **279**, 227 (1998);

CAM kinase II domains and regulation

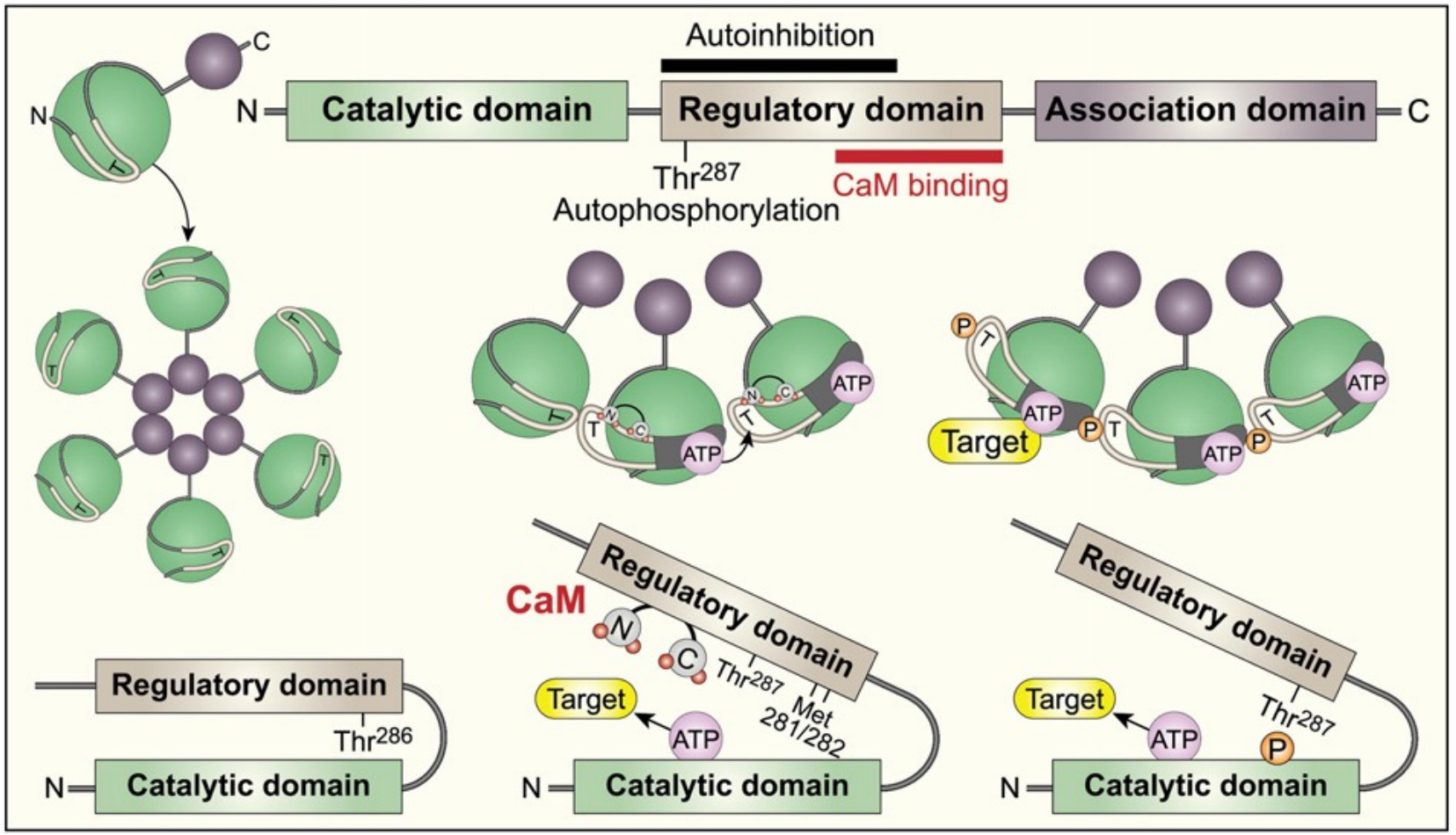


Fig. 1. CaMKII structural domains and regulation. CaMKII monomers consist of an N terminal catalytic domain and a C terminal association domain that bound a regulatory domain (top). The association domains (maroon circles) are required for assembly of the CaMKII monomers into the holoenzyme (middle panels). Under resting conditions the catalytic domain is constrained by the regulatory domain (left middle and bottom panels). After intracellular Ca^{2+} rises and complexes with calmodulin (CaM) the Ca^{2+}/CaM binds to the C terminal portion of the CaMKII regulatory domain (mid portion of the top, middle and bottom panels) to prevent autoinhibition of the regulatory domain on the catalytic domain, activating CaMKII. With sustained Ca^{2+}/CaM or increased oxidation, CaMKII transitions into a Ca^{2+}/CaM-autonomous active enzyme after autophosphorylation (at Thr 287) or oxidation (at Met281/282) of amino acids in the regulatory domain.

Journal of Molecular and Cellular Cardiology 51 (2011) 468–473