Calcium Signaling

What is a Calcium Signal?

Although it is almost never explicitly defined, a Ca^{2+} signal is generally understood to be a change in the concentration of *free* Ca^{2+} ions in some cellular compartment (e.g. cytosol, mitochondrion). Symbols: concentration of *free* Ca^{2+} ions (i.e., Ca^{2+} ions that are not bound to anything) in the cytosol is usually represented as $[Ca^{2+}]_i$.

Ubiquity of Calcium Signaling

Ca^{2+} ions may be the single most important signaling species in cells. It is probably safe to say that every biological process is directly or indirectly regulated by Ca^{2+}. Thus, Ca^{2+} has been determined, or inferred, to be important for cell division (mitosis), emergence of polarity in a developing embryo, motility (e.g. muscle contraction), vesicular secretion (including neurotransmitter release), control of electrical excitability in neurons, and activation of gene transcription. How can a simple ion like Ca^{2+} take on such a myriad of diverse roles? The answer that is emerging is that Ca^{2+} signaling at the cellular level is both temporally and spatially regulated (e.g. free Ca^{2+} concentration in the cytosol can change in an oscillatory fashion with well-defined periodicity, and Ca^{2+} release from intracellular stores can be highly localized). The Ca^{2+} signal transduction machinery, by being properly tuned to temporally varying signals (e.g. responding to only one oscillation frequency but not another), or by being optimally positioned at specific subcellular sites to sense localized Ca^{2+} signals, can generate a wide variety of downstream responses.

Why Ca^{2+}?

Biological information is encoded in the form of biopolymers (DNA/RNA). Such a system uses phosphate as the essential linker in the polymer backbone. This structural "decision" implies that the free phosphate concentration must be high in the cell. Indeed, soluble phosphate concentration is $[\text{Phosphate}]_{\text{Total}} \approx 0.01$ M. The problem with using phosphate is that calcium phosphate ($Ca_3(PO_4)_2$) is exceedingly insoluble. Its solubility product constant is $K_{sp} = [Ca^{2+}]^3[PO_4^{3-}]^2 = 2.07 \times 10^{-33}$. We can deduce the concentration of free Ca^{2+} ions that can be steadily present without precipitating $Ca_3(PO_4)_2$.

In order to estimate the tolerable $[Ca^{2+}]$, we need to calculate the concentration of free phosphate, $[PO_4^{3-}]$. Remember that phosphate can exists in 4 forms: phosphoric acid (H_3PO_4), dihydrogen phosphate ($H_2PO_4^-$), monohydrogen phosphate (HPO_4^{2-}), and phosphate (PO_4^{3-}). The fraction of total phosphate that is present in any form is expected to be dependent on $[H^+]$. The fraction of total phosphate actually in the form of PO_4^{3-} is f_3, which can be calculated as

$$f_3 = \frac{K_1 K_2 K_3}{[\mathrm{H}^+]^3 + [\mathrm{H}^+]^2 K_1 + [\mathrm{H}^+] K_1 K_2 + K_1 K_2 K_3}$$

where the *K*'s are the successive acid dissociation constants for phosphoric acid:

$K_1 = 7.1 \times 10^{-3}$; $K_2 = 6.2 \times 10^{-8}$; $K_3 = 4.4 \times 10^{-13}$

At pH = 7.2, $[H^+] = 6.310 \times 10^{-8}$ M, so the above expression gives $f_3 = 3.456 \times 10^{-6}$. Now, $[PO_4^{3-}] = f_3 \times [\text{Phosphate}]_{\text{Total}}$. This means that $[PO_4^{3-}] = 3.456 \times 10^{-6}$ M. Substituting this value back into the K_{sp} expression tells us that $[Ca^{2+}] = 5.6 \times 10^{-8}$ M, or about 56 nM. While this estimate is very "back-of-the-envelope", it does show that $[Ca^{2+}]$ cannot be elevated for prolonged periods in the cytosol. For this reason, intracellular Ca^{2+} signals are transient, with cytosolic $[Ca^{2+}]$ never staying high for extended periods of time. It is important to remember that resting cytosolic $[Ca^{2+}]$ is expected to be ~100 nM.

The Need for Buffering

Knowing that cell volume is typically about 1 to several picoliters (10^{-12} liter), if resting $[Ca^{2+}]_i$ in the cell is typically at ~50 nM, we might estimate that there are only ~5 × 10^{-20} moles of Ca^{2+} ions in the cytosol (or about 30,000 Ca^{2+} ions). It therefore appears that we can double the resting level just by adding a very small number of Ca^{2+} ions. Knowing that a low resting level of $[Ca^{2+}]_i$ is critically important for the survival of the cell, we suspect that there must be mechanisms that keep $[Ca^{2+}]_i$ *stably* low at rest, so that random fluctuations do not cause undesirable increases in $[Ca^{2+}]_i$. This suggests that $[Ca^{2+}]_i$ must be heavily buffered in the cell. Indeed, this is so—cytosolic Ca^{2+} buffering capacity can be in the hundreds of μM to even mM levels (for example, buffering capacity in cerebellar Purkinje neurons has been estimated to be ~40 mM!). An analogous situation is that of pH. Because most biochemical processes have strong pH dependence, cellular biochemistry cannot occur properly without maintaining a stable pH, which can be achieve through buffering (pH buffering in cells is estimated at mM levels). In view of the foregoing, it is not surprising that Ca^{2+} and H^+ ions are the two most heavily buffered ions in the cell. Ca^{2+} buffers in the cell are typically Ca^{2+}-binding proteins; these include calmodulin, parvalbumin, and calbindin.

Generation and Termination of Ca^{2+} Signals

Sources of Ca^{2+}

Influx pathways. Ca^{2+} can make its way from the extracellular fluid (where $[Ca^{2+}] \approx 2$ mM) into the cytosol (where $[Ca^{2+}]_i \approx 100$ nM—a 20,000-fold difference). Two types of routes are available for Ca^{2+} influx:

1) Ion channels that allow passage of Ca^{2+}:

Voltage-operated (or voltage-dependent) Ca^{2+} channels (VOCs or VDCCs):

Major categories with different characteristics include a) the L-type, b) the N-, P/Q-, R- type, and c) the T-type. These channels are highly selective for the passage of Ca^{2+}. These channels differ in pharmacology (L-type blocked by dihydropyridines such as nifedipine, nimodipine, etc.; N-type blocked by ω-Conotoxin GVIA); in kinetics (in response to depolarization, L-type opens more slowly than N- and T-type, but L-type also inactivates very slowly—over seconds, whereas N- and T-type inactivate rapidly, in tens of msec).

Receptor-operated channels (ROCs)

ROCs are cell-surface receptors that are themselves ion channels. Upon binding an extracellular ligand, the receptor-associated channel opens to allow ionic fluxes to occur. Examples of ROCs include certain P2X receptors (ATP is the ligand), the nicotinic acetylcholine receptor (nAChR), the NMDA-type of glutamate receptor (NMDA is *N*-methyl-D-aspartate, an artificial ligand that is specific for this type of glutamate receptor; the natural ligand is still glutamate), and the vanilloid receptor (VR1, a receptor that binds capsaicin, the "hot" essence of chili peppers; the endogenous ligand for VR1 has not been identified; VR1 may also be a sensor of heat—temperature elevation can turn the channel on). ROCs can be Ca^{2+}-selective or non-selective. Of the examples above, only the NMDA receptor is highly Ca^{2+}-selective. The others are non-selective; i.e., when they open, they have significant permeability to several common cations (Na^+, K^+, and Ca^{2+}).

Store-operated channels (SOCs)

When Ca^{2+} is released from intracellular Ca^{2+} stores (see below), a signal appears to reach the plasma membrane to allow a class of ion channels to open. These channels allow Ca^{2+} influx to occur. It has been presumed that the short-term function is to allow influx to replenish the Ca^{2+} in the intracellular stores. These ion channels can be very specific for Ca^{2+} (e.g. in the case of lymphocytes) or non-selective but Ca^{2+} permeant. Recent research has identified SOCs with members of the TRP family of channels. The mechanism whereby "depletion" of an intracellular Ca^{2+} store can signal a plasma membrane ion channel to open is still obscure.

2. Transporter-mediated Ca^{2+} influx

Na^+/Ca^{2+} exchanger

The Na^+/Ca^{2+} exchanger is a transporter protein that is normally thought of as a way to remove Ca^{2+} from the cytosol to the extracellular space (1 Ca^{2+} is extruded while 3 Na+ ions are brought into the cell). However, under certain conditions (e.g. membrane depolarization), the driving force on the Na^+/Ca^{2+} exchanger may change to allow Ca^{2+} to enter and Na^+ to exit the cell.

Ca^{2+} release from intracellular Ca^{2+} stores. Ca^{2+} is stored in membrane-enclosed organelles inside the cell. These intracellular storage compartments can release their Ca^{2+} content

rapidly into the cytosol to cause a rapid rise in $[Ca^{2+}]_i$. At least four different mechanisms have been identified:

1) The endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR, in muscle cells). These organelles contain high levels of Ca^{2+}. Free $[Ca^{2+}]$ in ER/SR has been estimated to be at least several hundred μM and up to approximately mM. This means that there is at least several thousand-fold (if not 10,000-fold) concentration difference for Ca^{2+} between the ER/SR lumen and the cytosol. Therefore, if there are efflux pathways that lead from the ER/SR lumen to the cytosol, the concentration gradient of Ca^{2+} would readily drive a large flux of Ca^{2+} into the cytosol to cause a sharp rise in $[Ca^{2+}]_i$. Three different mechanisms of Ca^{2+} release have been identified:

Inositol-1,4,5-trisphosphate receptors (IP_3Rs)

The IP_3Rs (three isoforms known) are large tetrameric protein channels in the ER membrane. When they bind the second messenger, IP_3, they are induced to open, and this allows efflux of Ca^{2+} from the ER into the cytosol. In "non-excitable" cells (i.e. not muscle or nerve), IP_3Rs appear to be the dominant intracellular Ca^{2+} release mechanism. IP_3Rs are pharmacologically inhibited by "intermediate molecular weight" heparin (e.g. average MW ~14,000), and by xestospongin C.

Ryanodine receptors (RyRs)

Three isoforms of RyRs are known. RyRs are also large tetrameric protein channels. The endogenous ligand for RyRs is Ca^{2+} ion itself: the binding of Ca^{2+} to sites in the cytoplasmic domain of the RyR causes the RyR channel to open, and thus allow Ca^{2+} efflux from the ER/SR into the cytosol. RyRs are the dominant Ca^{2+} release mechanism in muscle cells, and are also present in neurons. RyRs can bind, and are sensitive to, the plant alkaloid ryanodine (Ry; hence the name). Ry has complex pharmacological effect on RyRs: at low concentrations (nM), it appears to activate the RyR channels partially (by enabling a so-called "subconductance state"); at high concentrations (> μMs), it blocks the RyR channel.

Sphingosine-1-phosphate (S1P) dependent release

The lipid cermide can be cleaved by ceramidase to yield sphingosine, which can be phosphorylated to yield sphingosine-1-phosphate. There is evidence that when introduced into the cytosol, S1P can cause Ca^{2+} release (presumed to be from the ER). Beyond such phenomenological observation, the mechanism of S1P-mediated Ca^{2+} signaling is not well characterized. If S1P proves to be a true second messenger, it would be highly usual among signaling molecules, because there is a family of G-protein coupled receptors that can bind, and be activated by, S1P. Thus S1P may be both an extracellular and an intracellular messenger. (See Spiegel and Milstien 2002. J. Biol. Chem. 277:25851-4)

Cyclic-ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP)

These two molecules are relatively recent arrivals on the second messenger scene (discovered by Hon Cheung Lee, ~1990 and 1995 for cADPR and NAADP respectively). cADPR has been proposed as a modulator of the RyR. They were first identified as Ca^{2+}-mobilizing agonists in sea urchin egg extracts. Biochemical pathways that can lead to the production of these putative messengers have been at least partially characterized. While these molecules seem to work reliably in sea urchin eggs, experiments to probe their roles as Ca^{2+}-mobilizing second messengers in vertebrate cells has not given consistent results. It may be that these second messengers are not as widespread in occurrence in all cell types, or that their ability to mobilize Ca^{2+} is more subtly regulated by or dependent on cellular conditions. As more examples of the actions of these molecules emerge, their true roles in Ca^{2+} signaling will become better understood.

Ca^{2+} Removal Mechanisms (sometimes called "sinks")

1. Plasma membrane Ca^{2+} ATPases (PMCAs)

These Ca^{2+} pumps extrude Ca^{2+} from the cytosol into the extracellular fluid. There are no good selective blockers of these enzymes.

2. SR/ER Ca^{2+} ATPase (SERCA pumps)

These are ATPases on the ER/SR membranes that are responsible for re-uptake of Ca^{2+} back into the ER/SR. Three major isoforms are known. SERCA pumps are irreversibly blocked by the plant derivative thapsigargin, and reversibly by the microbial metabolite, cyclopiazonic acid (CPA). For review, see Hussain and Inesi 1999. J. Membr. Biol. 172:91-9.

3. Na^+/Ca^{2+} exchanger (NCX)

As mentioned earlier, the Na^+/Ca^{2+} exchanger can extrude Ca^{2+} by counter-transporting Na^+ ions into the cytosol (i.e. it uses the electrochemical potential energy stored in the Na^+ gradient and the membrane potential to transport Ca^{2+} out of the cell, against a very strong electrochemical gradient). The physiology of the NCX has been reviewed by Blaustein and Lederer (Physiol. Rev. 1999. 79763-854).

A note on the relative kinetics of transport processes: It should be noted that there is a significant difference in the kinetics of transport between the pumps and the exchangers. The pumps, being enzymes, have a turnover rate of just ~100/sec, while the "secondary active transporters," like NCX, have turnover rates of $\gtrsim$1,000/sec.

4. The mitochondria

In biochemical studies, mitochondrial have the ability to take up Ca^{2+}, with EC_{50} being in the 10 µM range. Because this level of $[Ca^{2+}]$ is so high relative to the normal $[Ca^{2+}]_i$ of a healthy cell, it had been assumed that mitochondria participate in Ca^{2+}

sequestration only under cyto-pathological conditions, when the cytosolic [Ca^{2+}] reaches extraordinarily high levels. Recent evidence (Rizzuto et al. 1998. Science 280:1763-6) suggests that mitochondria that are closely apposed to sites of Ca^{2+} influx into the cytosol (e.g. near IP_3Rs or plasma membrane VOCs) can take up substantial amounts of Ca^{2+}, presumably because locally, at the site of release, [Ca^{2+}] can be transiently very high (at least many μM). One suggested role of Ca^{2+} uptake by mitochondria is that oxidative phosphorylation through the electron transport chain is stimulated by Ca^{2+}. Thus, the coupling between Ca^{2+} entry into the cytosol and mitochondria may be a way for Ca^{2+} to signal the need for increased energy production. (For review of the Ca^{2+} physiology of mitochondria, see Rizzuto et al. 2000. J. Physiol. 529:37-47.)

Signaling pathways: How are the Ca^{2+} signals generated

Metabotropic mechanisms

This term is used to denote a sequential process by which activation of cell-surface receptors leads to the production of second messengers. The most prominent mechanisms for Ca^{2+} signaling depend on the activation of phospholipase C (PLC; Rebecchi and Pentyala 2000. Physiol. Rev. 80:1291-335), which, when activated, catalyzes the conversion of phosphatidylinositol bisphosphate (PIP_2) into inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG or DAG). Activation of PLC may occur in two ways: 1) activation of receptor tyrosine kinases (e.g. the epidermal growth factor receptor, EGFR) leads to phosphorylation of PLC-γ, which becomes active and able to cleave PIP_2, or 2) binding of ligand to a receptor (e.g. ACh to mAChR or Glu to mGluR) activates the receptor, which catalyzes the exchange of a bound GDP for a GTP on the α-subunit of a heterotrimeric G-protein. GTP-bound G_α can in turn activate a G-protein-coupled PLC-β, which then can cleave PIP_2 to yield IP_3 and DAG.

Of the two messengers generated by PLC action, IP_3 is a water-soluble diffusible second messenger, which can bind and open IP_3R channels. The DAG is lipophilic, stays within the plasma membrane, and can bind the Ca^{2+}-dependent protein kinase (PKC or C-kinase). DAG-bound PKC has increased affinity for Ca^{2+}; i.e. DAG and Ca^{2+} are synergistic activators of PKC.

Ionotropic mechanisms

Direct influx of Ca^{2+} ions into the cytosol can occur via one of two major pathways. Membrane depolarization (e.g., by an action potential) can activate VOCs and thus allow Ca^{2+} influx. Alternatively, extracellular ligands (e.g. neurotransmitters) can bind to ionotropic receptors (e.g. the NMDAR or the nAChR) and open the associated channels. Thus ligand gating of such Ca^{2+}-permeable receptor-associated channels enables Ca^{2+} influx.

The accompanying figure from the review by Berridge et al. summarizes the Ca^{2+} signaling pathways discussed above.

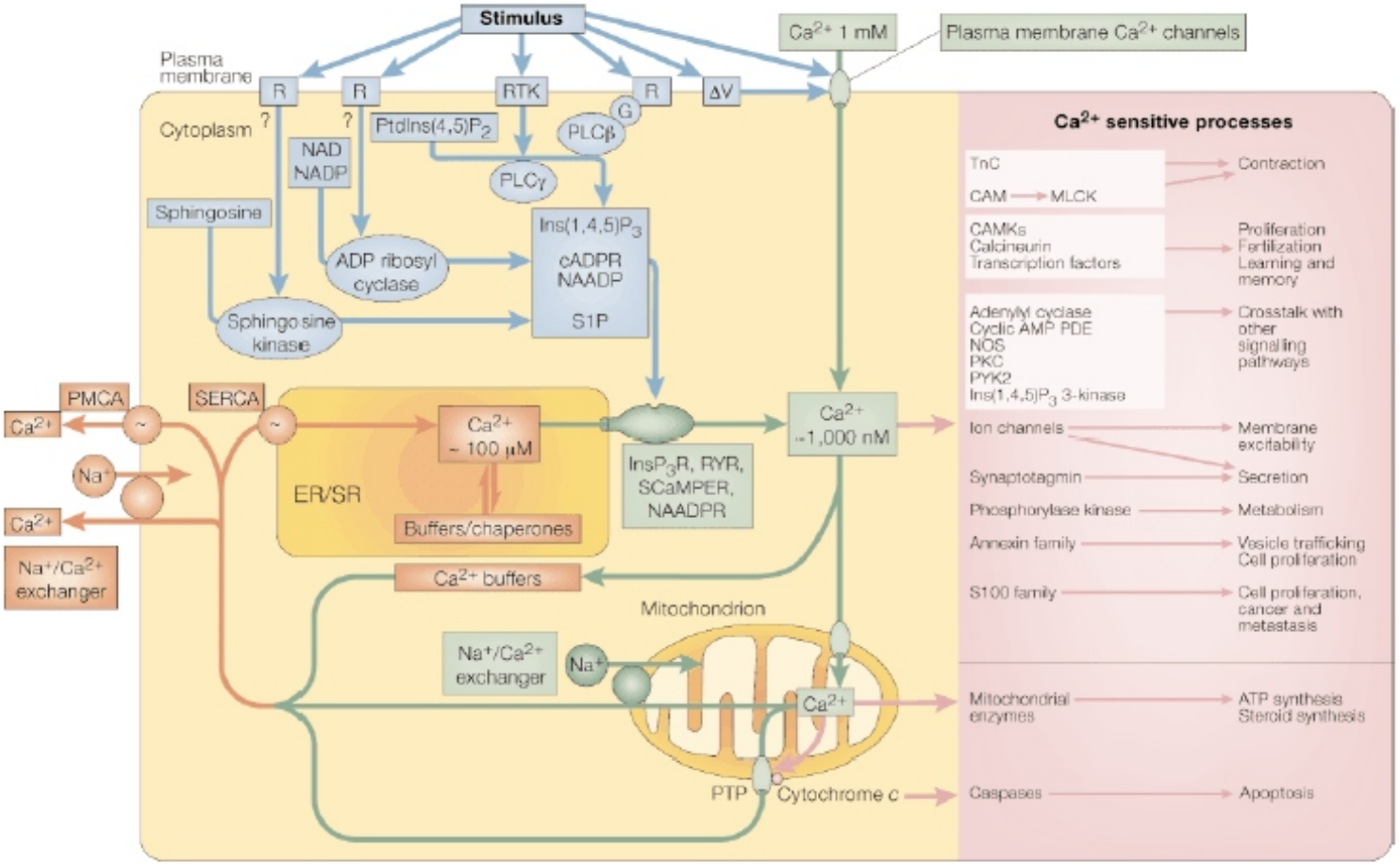
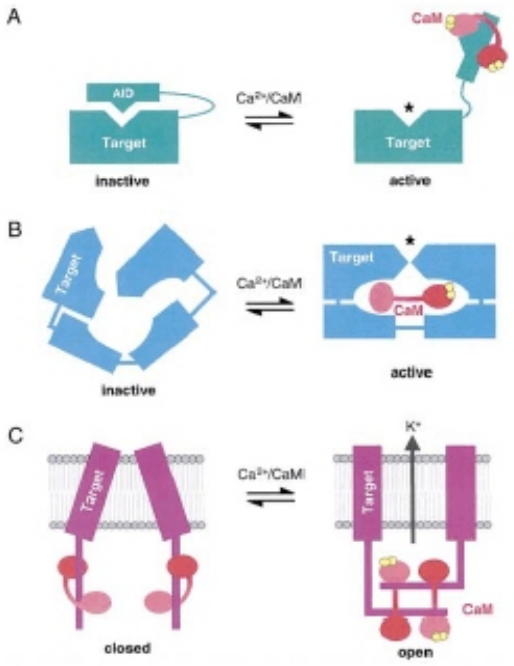


Figure 2 | **Elements of the Ca^{2+} signalling toolkit.** Cells have an extensive signalling toolkit that can be mixed and matched to create Ca^{2+} signals of widely different properties. Ca^{2+}-mobilizing signals (blue) are generated by stimuli acting through a variety of cell-surface receptors (R), including G-protein (G)-linked receptors and receptor tyrosine kinases (RTK). The signals generated include: inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$), generated by the hydrolysis of phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$) by a family of phospholipase C enzymes (PLCβ, PLCγ); cyclic ADP ribose (cADPR) and nicotinic acid dinucleotide phosphate (NAADP), both generated from nicotinamide-adenine dinucleotide (NAD) and its phosphorylated derivative NADP by ADP ribosyl cyclase; and sphingosine 1-phosphate (S1P), generated from sphingosine by a sphingosine kinase. ON mechanisms (green) include plasma membrane Ca^{2+} channels, which respond to transmitters or to membrane depolarization (ΔV), and intracellular Ca^{2+} channels — the $Ins(1,4,5)P_3$ receptor ($InsP_3R$), ryanodine receptor (RYR), NAADP receptor and sphingolipid Ca^{2+} release-mediating protein of the ER (SCaMPER). The Ca^{2+} released into the cytoplasm by these ON mechanisms activates different Ca^{2+} sensors (purple), which augment a wide range of Ca^{2+}-sensitive processes (purple), depending on cell type and context. OFF mechanisms (red) pump Ca^{2+} out of the cytoplasm: the Na^+/Ca^{2+} exchanger and the plasma membrane Ca^{2+} ATPase (PMCA) pumps Ca^{2+} out of the cell and the sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps it back into the ER/SR. (TnC, troponin C; CAM, calmodulin; MLCK, myosin light chain kinase; CAMK, Ca^{2+}/calmodulin-dependent protein kinase; cyclic AMP PDE, cyclic AMP phosphodiesterase; NOS, nitric oxide synthase; PKC, protein kinase C; PYK2, proline-rich kinase 2; PTP, permeability transition pore.)

From: Berridge et al. 2000. Nature Reviews of Molecular Cell Biology. 1:11-21.

Receiving Ca^{2+}signals: Calcium-binding proteins



Because a Ca^{2+} signal is simply a rise in $[Ca^{2+}]_i$, the signal can only be transduced if Ca^{2+} ions can interact with other cellular components. For this reason, Ca^{2+}-binding proteins (CaBPs) are abundant in the cell. Known CaBPs fall into two classes: 1) Proteins that merely bind Ca^{2+} and act as Ca^{2+} buffers (e.g. calbindin, parvalbumin). 2) Proteins that bind Ca^{2+} and then interact with other downstream targets (this class is often referred to as "Ca^{2+} sensors"). The best-known and most abundant Ca^{2+} sensor is calmodulin (CaM) (reviewed by Chin and Means 2000. Trends Cell Biol. 10:322-8). Intracellular concentration of CaM has been estimated to range from a few μM to a few tens μM. CaM has two domains (the N- and C-terminal "lobes") connected by a short flexible linker. Each lobe contains a pair of EF-hand motifs (each EF-hand is capable of binding one Ca^{2+} ion). The Ca^{2+} K_d's of the EF-hands range from sub-μM to a few μM, with the C-terminal EF-hands having higher binding affinity for Ca^{2+}. CaM can interact with a diverse range of proteins. The interactions are of three types, as shown in the figure taken from Hoeflich and Ikura (2002. Cell 108:739-42): 1) Ca^{2+}-bound CaM (Ca/CaM) can bind a peptide segment (the "auto-inhibitory peptide" or AIP) on a target protein that normally blocks the function of that protein. Ca/CaM binding of the sequence relieves inhibition and the target protein becomes activated. Multiple examples of this mode of action are known, notably the activation of Ca/CaM-dependent kinases (CaMKs), myosin light chain kinase (MLCK), and calcineurin (a Ca/CaM-dependent phosphatase). 2) Ca/CaM can bind to a protein, causing major conformational rearrangements that lead to appearance of an active site. An example is the activation of Ca/CaM-dependent adenylyl cyclase. 3) CaM may be constitutively bound to a target protein, even at low resting $[Ca^{2+}]_i$. Upon activation by Ca^{2+}, the still-protein-bound Ca/CaM may bind to a different part of the protein and alter its function, or it may bind and recruit another,separate, protein molecule. An example of the former is Ca^{2+}-dependent inactivation (CDI) of L-type Ca^{2+} channels; an example of the latter is the activation of small-conductance Ca^{2+}-activated K^+ channels (SK channels).

Besides CaM, numerous EF-hand proteins have been identified. Prominent examples include troponin C (the Ca^{2+}-sensitive switch in skeletal and cardiac muscle contraction), recoverin (which mediates restoration of photoreceptor sensitivity in the eye after light exposure), and frequenin (a neuronal Ca^{2+} sensor (NCS) protein that regulates certain voltage-gated K^+ channels).

A note about Ca^{2+} binding proteins:

Ca^{2+} binding proteins, by binding to Ca^{2+} ions, would tend to buffer the [Ca^{2+}]. But they can also modify the spatial extent and kinetics of Ca^{2+} signal spread.

Short- and long-term effects of Ca^{2+} signals

Because Ca^{2+} can only bind to protein sensors by equilibrium binding, its ability to influence the state of a proteins sensor must be limited (being not much longer than the duration of a Ca^{2+} transient). So we can easily imagine a transient elevation of [Ca^{2+}] being able to trigger a fast change. The prime example of this is the case of skeletal and cardiac muscle contraction. However, for longer term effects (e.g. down stream effects such as turning on gene transcription), one expects that covalent modification of protein sensor or their downstream targets must be necessary. Thus Ca^{2+} sensitive enzymes (kinases and phosphatases) are important in long-term Ca^{2+} dependent effects. Examples are CaMK II and CaMK IV, and the Ca-sensitive phosphatase, calcineurin.

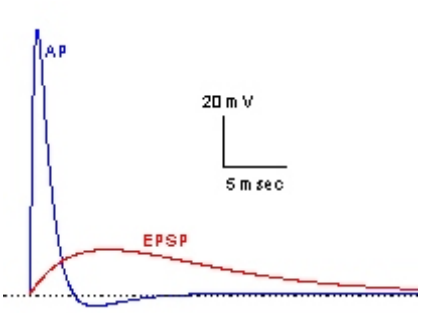
Deciphering temporal and spatially distinct Ca^{2+} signals

Two examples of temporal signals:

Ca^{2+}-activated gene expression in lymphocytes is dependent on the frequency of occurrence of cytosolic Ca^{2+} spikes or "Ca^{2+} oscillations" (Dolmetsch et al. 1998. Nature 392:933-6; Li et al. 1998. Nature 392:936-41). The activation of transcription factors NF-AT, Oct/OAP, and NF-κB 9 was dependent on the frequency of Ca^{2+} oscillations: fast oscillations turn on all 3, slow oscillations turn on only genes under NF-κB control. Thus, the interleukin IL-2, whose transcription is controlled by NF-AT and Oct/OAP, cannot be expressed at low Ca^{2+} oscillation frequencies (as slow as 1 Ca^{2+} spike every 400 sec), while IL-8, which is under the control of NF-κB, can be expressed when cells are stimulated with high or low frequency Ca^{2+} oscillations. When activated, the transcription factors are recruited from the cytosol into the nucleus, where they turn on transcription. The recruitment processes depends on dephosphorylation by calcineurin, a Ca/CaM-dependent phosphatase. The rate at which the transcriptional factors are re-phosphorylated by kinases in the nucleus determines the rate at which the factors are turned off and return to the cytosol. Calcium, by controlling the balance of dephosphorylation/phosphorylation, controls which transcription factors are recruited to, and retained in, the nucleus.

Ca^{2+}-dependent phosphorylation of the cAMP-response element binding protein (CREB) in hippocampal neurons is preferentially activated by synaptic stimulation that generates excitatory post-synaptic potentials (EPSPs) and not by action potentials (APs) (Mermelstein et al. 2000. J. Neurosci. 20:266-73). It had been shown that CREB phosphorylation depends on the translocation of Ca/CaM (presumably complexed with a Ca/CaM-binding protein, possibly a kinase) to the nucleus (Deisseroth et al. 1998. Nature 393:198-202; Mermelstein et al. 2001. Proc. Natl. Acad. Sci. 98:15342-7). How might a nerve cell distinguish APs from EPSPs, and allow Ca^{2+}-dependent gene expression to be

preferentially activated only by EPSPs? The answer appears to lie in the quantitative and temporal differences between APs and EPSPs. Approximate relative amplitudes and time courses for an AP and an EPSP are shown in the accompanying figure. It can be seen that whereas the AP reaches much more positive voltages, the depolarization achieved by an EPSP is much less. Furthermore, whereas the rise and decay kinetics of the AP are very fast, those of the EPSP are comparatively slow. Given the characteristics of L-type and the N/P/Q-type Ca^{2+} channels (L-type activates more slowly, and at more negative voltages, than the N and P/Q types), Ca^{2+} influx through L-type Ca^{2+} channels is favored by EPSPs, while APs favor activation of the N and P/Q types of channels. This is consistent with earlier findings that translocation of Ca/CaM into the nucleus to activate CREB phosphorylation occurred when L-type channels were activated, but not when N- and P/Q-type channels were activated (Deisseroth et al. 1998. Nature 393:198-202).



An example of spatially organized Ca^{2+} signals

In hippocampal neurons, there are two major type of Ca^{2+} activated K^+ channels: the BK type has large conductance, while the SK type has small conductance. The BK channels underlie the repolarization of action potentials and the "fast after-hyperpolarization" (the fAHP: following an AP, the dip to potentials more negative than the resting membrane potential; see the previous figure for the AP wave form). The SK channels underlie a more long-lasting afterhyperpolarization that can last several hundred milliseconds. The BK channels activated by Ca^{2+} influx through only N-type Ca^{2+} channels, while the SK channels are activated by Ca^{2+} influx through only L-type channels. In experiments where the electrophysiology of microscopic patches of cell membrane was examined by patch-clamp methods, Marrion and Tavalin determined that N-type Ca^{2+} channels are co-localized with BK channels, while L-type Ca^{2+} channels are co-localized with SK channels (Marrion and Tavalin 1998. Nature 395:900-5). Indeed, activation of N-type Ca^{2+} channels did not lead to opening of SK channels, and conversely, BK channels could not be activated by opening of L-type Ca^{2+} channels. These studies demonstrated that on the subcellular scale, sources of Ca^{2+} signals and their target effectors can be organized into stable, spatially distinct cyto-architectural complexes.

More elaborate examples of the microscopic spatial organization of Ca^{2+} signal transduction machinery are explored in the assigned research paper:

Delmas et al. 2002. Signaling microdomains define the specificity of receptor-mediated $InsP_3$ pathways in neurons. *Neuron* 14:209-220.