Calcium Signalling and Regulation of Cell Function

Secondary article

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The calcium ion (Ca^{2+}) is an almost universal intracellular messenger, controlling a diverse range of cellular processes such as gene transcription, muscle contraction and cell proliferation.

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Intracellular Ca^{2+} Homeostasis

The calcium ion (Ca^{2+}) is an almost universal intracellular messenger, controlling a diverse range of cellular processes, such as gene transcription, muscle contraction and cell proliferation (for reviews, see Berridge, 1993; Petersen *et al*., 1994; Clapham, 1995; Berridge *et al*., 1998). In most cells, Ca^{2+} has its major signalling function when it is elevated in the cytosolic compartment. From there it can also diffuse into organelles such as mitochondria and the nucleus. The Ca^{2+} concentration inside cells is regulated by the simultaneous interplay of several counteracting processes, which can be divided into Ca^{2+} 'on' and 'off' mechanisms depending on whether they serve to increase or decrease cytosolic Ca^{2+} (**Figure 1**).

The Ca^{2+} 'on' mechanisms include channels located at the plasma membrane (PM) which regulate the inexhaustible supply of Ca^{2+} from the extracellular space, and channels on the endoplasmic reticulum and sarcoplasmic reticulum (ER and SR, respectively) which release the finite intracellular Ca^{2+} stores. A more diverse set of 'off' mechanisms is employed by cells to remove Ca^{2+} from the cytoplasm. These include Ca^{2+} ATPases on the PM and ER/SR, in addition to exchangers that utilize gradients of other ions to provide the energy to transport Ca^{2+} out of the cell, e.g. Na^{+}/Ca^{2+} exchange. Occasionally, some of the 'off' mechanisms contribute to cytosolic Ca^{2+} increases, for example 'slippage' of Ca^{2+} through Ca^{2+}ATPases and reverse-mode Na^{+}/Ca^{2+} exchange. Organelles other than the ER and SR may also play important roles in Ca^{2+} homeostasis by sequestering or releasing Ca^{2+}. For example, mitochondria have been shown to limit the amplitude of cytosolic Ca^{2+} increases by rapidly sequestering Ca^{2+} and then more slowly return it to the cytoplasm.

When cells are at rest, the balance lies in favour of the 'off' mechanisms, thus yielding an intracellular Ca^{2+} concentration of $\sim 100\,\text{nmol}\,L^{-1}$. However, when cells are stimulated by various means (e.g. depolarization, mechanical deformation or hormones) the 'on' mechanisms are activated and the cytosolic Ca^{2+} concentration increases to levels of $1\,\mu\text{mol}\,L^{-1}$ or more. It is important to point out that not all cells employ each of the 'on' and 'off' mechanisms described in **Figure 1**. Instead, different cell types express various combinations of these channels, ATPases and exchangers to suit their physiology (Berridge, 1993). The diversity of Ca^{2+} 'on' and 'off' mechanisms underlies the huge variability in the characteristics of Ca^{2+} signals recorded in different cell types.

Ca^{2+} Channels

Ca^{2+} influx channels

Cells utilize several different types of Ca^{2+} influx channels, which can be grouped on the basis of their activation mechanisms.

Voltage-operated Ca^{2+} channels (VOCs) are employed largely by excitable cell types such as muscle and neuronal cells, where they are activated by depolarization of the PM. Mammalian VOCs generally comprise five protein subunits (α_1, α_2, β, γ, δ) with one member (the α_1 subunit) providing the Ca^{2+} channels, and the others serving to regulate channel gating. Multiple isoforms of these subunits have been detected, raising the possibility of myriad different combinations. Consistent with this, different types of VOCs, which are expressed in a tissue-specific manner, have been characterized on the basis of their gating characteristics and pharmacology.

Receptor-operated Ca^{2+} channels (ROCs) comprise a range of structurally and functionally diverse channels that are particularly prevalent on secretory cells and at nerve terminals. Well-known ROCs include the nicotinic acetylcholine receptor and the *N*-methyl-D-aspartate (NMDA) receptor. ROCs are activated by the binding of an agonist to the extracellular domain of the channel. The different ROCs are activated by a wide variety of agonists, e.g. ATP, serotonin, glutamate and acetylcholine, which may also bind to metabotropic receptors in the PM.

Mechanically-activated Ca^{2+} channels are present on many cell types and respond to cell deformation. Such channels convey information into the cell concerning the

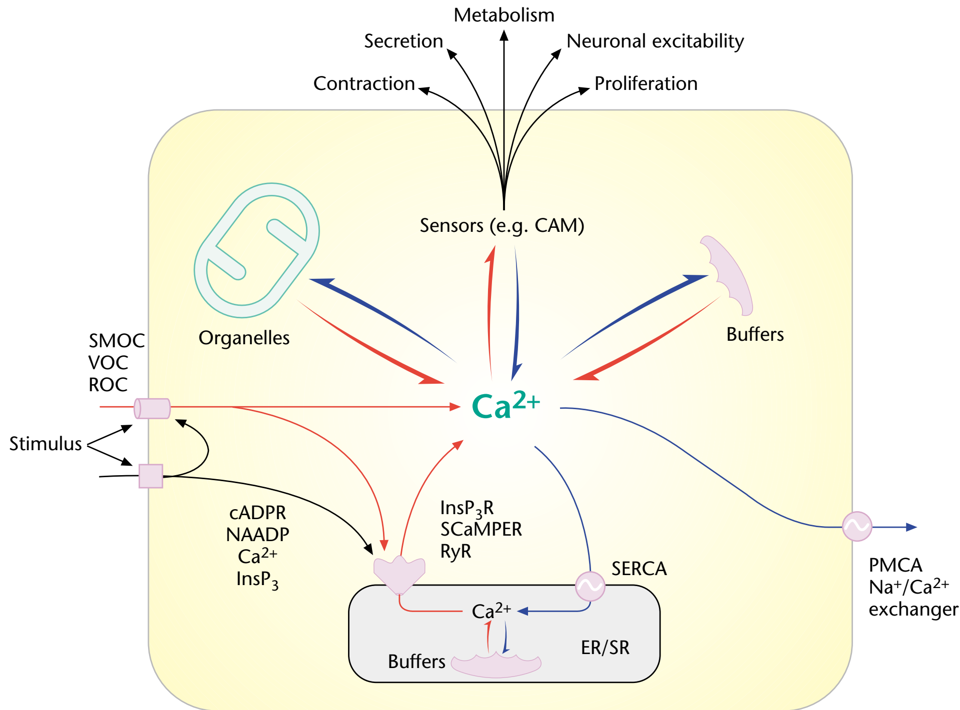

Figure 1 Ca^{2+} 'on' and 'off' mechanisms. This figure represents a summary of the processes that modulate cytoplasmic Ca^{2+} levels. The 'on' mechanisms responsible for increasing cytosolic Ca^{2+} are marked by red arrows, and the Ca^{2+} 'off' mechanisms are shown in blue. A dynamic interplay of these processes determines the spatiotemporal characteristics of the Ca^{2+} signal. See the text for details. PMCA, plasma membrane Ca^{2+} ATPase; SERCA, sarcoplamic/endoplasmic reticulum Ca^{2+} ATPase; CAM, calmodulin; other abbreviations as defined in the text.

stress/shape changes that a cell is experiencing. A particularly nice example of mechanically-induced Ca^{2+} signalling was observed in epithelial cells from the trachea, where deformation of a single cell led to a radial Ca^{2+} wave that synchronized the Ca^{2+}-sensitive beating of cilia on many neighbouring cells (Boitano *et al*., 1992). It is likely that such a mechanism is employed to aid clearance of mucus or particles from the lungs.

Store-operated Ca^{2+} channels (SOCs) are activated in response to depletion of the intracellular Ca^{2+} store, either by physiological Ca^{2+}-mobilizing messengers or by pharmacological agents. The mechanism by which the SOCs 'sense' the filling status of the intracellular pool is unknown. Since many different types of cells have been shown to have an enhanced Ca^{2+} entry following Ca^{2+} pool depletion, SOCs may be one of the most ubiquitous PM Ca^{2+} channels. Electrophysiological recordings of SOC activity have been made, and it appears that the characteristics of the current through these channels can differ significantly between cell types, suggesting that alternative types of SOCs are expressed by cells. At present, the best candidate for the molecular identity of SOCs are homologues of a protein named TRP (*t*ransient *r*eceptor *p*otential) that functions in *Drosophila* photoreception. Several mammalian TRP homologues have been identified, and found to be expressed in almost all tissues.

Ca^{2+} release channels

Release of Ca^{2+} stored within the ER or SR is mediated by several types of channels (**Figure 2**). In some cell types, these channels have an overlapping expression and localization. The consequence of this is that the opening of one channel type can activate the others so that they all participate in the generation of Ca^{2+} signals. In other cells, however, the different channels mediate spatially or temporally distinct Ca^{2+} signals.

Inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$)

The binding of many hormones and growth factors to specific receptors on the PM leads to the activation of an enzyme that catalyses the hydrolysis of phospholipids to produce the intracellular messenger inositol 1,4,5-trisphosphate ($InsP_3$). Although derived from a lipid, $InsP_3$ is water soluble and diffuses into the cell interior where it can encounter $InsP_3Rs$ on the ER/SR. The binding of $InsP_3$ changes the conformation of $InsP_3Rs$ such that an integral channel is opened, thus allowing the Ca^{2+} stored at high

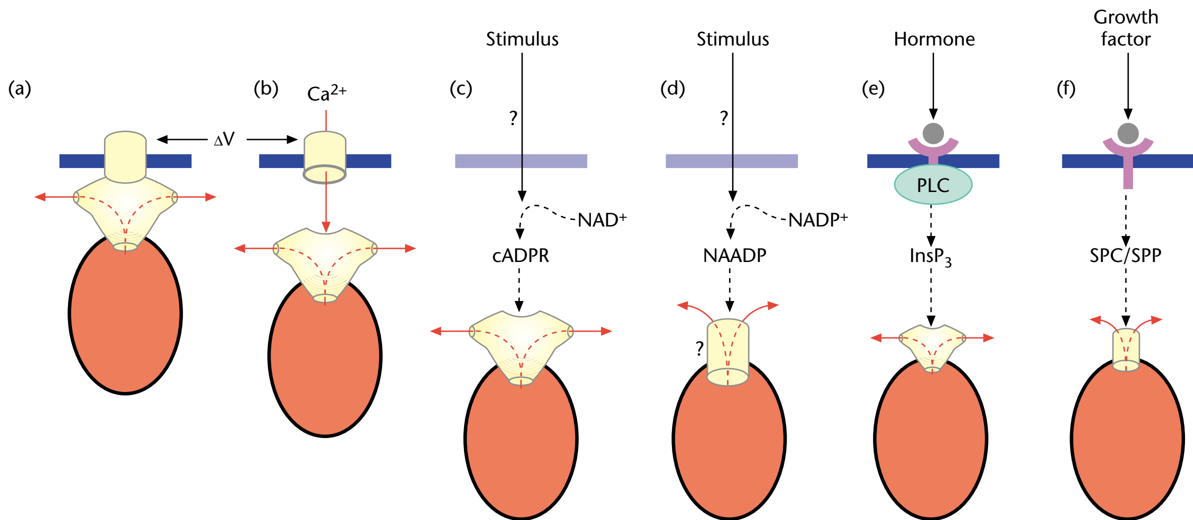

Figure 2 Mechanisms of Ca^{2+} release. Panels (a) to (f) depict the known mechanisms underlying Ca^{2+} release from the ER/SR. (a), (b) and (c) illustrate the activation of RyRs in skeletal muscle, cardiac muscle and in cells utilizing cADPR, respectively. The question marks in (c) and (d) indicate that the way in which stimulation of cells leads to increases in either cADPR or NAADP is not yet clear. Panel (d) shows release of Ca^{2+} by NAADP from as yet unknown channels. Ca^{2+} release via $InsP_3Rs$ or SCaMPER is depicted in (e) and (f), respectively. RyRs and $InsP_3Rs$ are drawn with a similar shape, since they are structurally homologous. The structure of SCaMPER has not yet been fully resolved, although it is much smaller than RyRs or $InsP_3Rs$. See the text for details. SPC denotes sphingosylphosphocholine.

concentrations in the ER/SR to enter the cytoplasm. $InsP_3Rs$ are large structures composed of four subunits (total molecular mass ~1200 kDa), and three different genes encoding these subunits have been discovered. The $InsP_3Rs$ encoded by these genes appear to differ subtly in their characteristics, such as affinity for $InsP_3$. Furthermore, analysis of $InsP_3R$ expression has shown that both homomeric and heteromeric $InsP_3Rs$ exist, raising the possibility that a range of functionally diverse $InsP_3Rs$ is utilized by cells.

Although $InsP_3Rs$ absolutely require $InsP_3$ to open, their activation is regulated by the Ca^{2+} concentration at their cytosolic surface. In fact, $InsP_3R$ opening is enhanced by modest increases in Ca^{2+} (0.5–1 μmol L^{-1}), whereas higher Ca^{2+} concentrations (> 1 μmol L^{-1}) inhibit their opening. This dependence of $InsP_3R$ activity on cytosolic Ca^{2+} is crucial in the generation of the complex patterns of Ca^{2+} signals seen in many cells. Although it is well established that $InsP_3R$ opening can be modulated by both $InsP_3$ and Ca^{2+}, recent evidence has suggested that high concentrations of $InsP_3$ may prevent Ca^{2+}-dependent inactivation of $InsP_3Rs$.

Ryanodine receptors (RyRs)

Ryanodine receptors are structurally and functionally analogous to $InsP_3Rs$, although they have approximately twice the conductance and molecular mass of $InsP_3Rs$. Another property that RyRs share with $InsP_3Rs$ is their sensitivity to cytosolic Ca^{2+} concentrations, although they are generally activated and inhibited by higher concentrations (activation at 1–10 μmol L^{-1}; inhibition at > 10 μmol L^{-1}). In contrast to $InsP_3Rs$, which are almost ubiquitously expressed in mammalian tissues, RyRs are largely present in excitable cell types, such as muscle and neurons. As with $InsP_3Rs$, RyR subunits are encoded by three genes. However, these genes do not appear to have the same functional redundancy as observed with the $InsP_3R$ isoforms. Instead, the different RyR proteins are often used for specific functions. For example, only 'type 1' RyRs are activated during contraction of skeletal muscle, whereas only 'type 2' RyRs fulfil this role in cardiac muscle.

Ryanodine receptors are so called because of their high affinity for the plant alkaloid ryanodine, which has proved to be an invaluable investigative tool. The binding of ryanodine to RyRs is 'use-dependent', meaning that the channels have to be in the activated state. At low concentrations (1–10 μmol L^{-1}), ryanodine binding locks the RyRs into a long-lived subconductance state, while higher concentrations (~100 μmol L^{-1}) irreversibly inhibit channel opening. RyRs are also activated by millimolar caffeine concentrations. This is due to a pronounced increase in the sensitivity of RyRs to Ca^{2+} in the presence of caffeine, such that basal Ca^{2+} concentrations become activatory.

$InsP_3Rs$ and RyRs are focal points for the convergence of many different signalling pathways. The opening of these channels has been shown to be modulated by numerous factors, including phosphorylation, adenine

nucleotides, thiol-reactive compounds, pH and the Ca^{2+} load of the ER/SR.

Sphingolipid Ca^{2+}-release mediating protein of endoplasmic reticulum (SCaMPER)

SCaMPER is a Ca^{2+} release channel that responds to the lipid sphingosylphosphocholine, which can increase inside cells stimulated with growth factors such as platelet-derived growth factor (PDGF). SCaMPER bears no structural resemblance to either $InsP_3$Rs or RyRs, and in fact is much smaller (approximately 20 kDa). Although it appears to be expressed in many different types of tissue, including cardiac muscle, pancreas and liver, the properties and physiological function of this channel are not yet known.

Intracellular Ca^{2+}-releasing Messengers

Inositol 1,4,5-trisphosphate ($InsP_3$)

$InsP_3$ is produced by the enzyme phospholipase C (PLC), which becomes activated when hormones or growth factors bind to their receptors on the surface of cells. PLC actually catalyses the hydrolysis of a lipid on the inner leaflet of the PM, giving rise to both $InsP_3$ and diacylglycerol, which can also act as an intracellular messenger. Although it is produced at the PM, $InsP_3$ is highly diffusible, and can rapidly traverse the cytoplasm of cells. However, an $InsP_3$ molecule has a lifetime only on the order of seconds inside cells, since it is metabolized by enzymes that either add or remove a phosphate group to terminate the Ca^{2+}-releasing potential of $InsP_3$. The rapid metabolism of $InsP_3$ by these enzymes means that $InsP_3$ may have a spatially-restricted action even though it can freely diffuse inside cells.

The addition of a phosphate group by a Ca^{2+}-dependent kinase that phosphorylates $InsP_3$ to produce inositol 1,3,4,5-tetrakisphosphate ($InsP_4$) may actually produce another intracellular messenger. Although its role in cell physiology is not entirely clear, $InsP_4$ binds to a specific small GTPase-activating protein of the Ras family that has been shown to modulate Ca^{2+} release (Cullen, 1998).

Cyclic ADP ribose (cADPR)

cADPR releases Ca^{2+} via activation of RyRs. The Ca^{2+}-releasing action of cADPR was discovered during studies looking at the effects of NAD^+ derivatives on Ca^{2+} signalling in sea urchin eggs. cADPR is synthesized from β-NAD^+ by enzymes called ADP-ribosyl cyclases, which appear to be ubiquitous although they have not yet been well described. One such ADP-ribosyl cyclase enzyme is the ectopically expressed glycoprotein CD38 which is present on many cell types. This enzyme also catalyses the formation of cADPR from β-NAD^+, intriguingly this activity is extracellular. It has been suggested that CD38 also transports cADPR into cells.

An on-going problem with the designation of cADPR as an intracellular messenger is that there are few convincing examples of a link between stimulation of cells and an increase in cADPR concentration. Furthermore, appreciable levels of cADPR seem to exist in cells that do not express RyRs. These data may indicate that cADPR is a relatively constant endogenous regulator of Ca^{2+} signalling or that it has cellular functions distinct from Ca^{2+} release.

The breakdown product of cADPR is ADP ribose (ADPR), which does not appear to mobilize Ca^{2+}. However, ADPR has been suggested to modulate events that occur during fertilization and also to affect ion channels in smooth-muscle cells. In this respect, cADPR resembles $InsP_3$, in that a metabolite of the original messenger has additional functions.

Nicotinic acid–adenine dinucleotide phosphate (NAADP)

This has only recently emerged as a likely candidate for an intracellular messenger (for review, see Genazzani and Galione, 1997) although its Ca^{2+}-releasing potential was first observed in the study that originally pinpointed the action of cADPR. NAADP is formed from β-$NADP^+$, potentially by the same ribosyl cyclases that produce cADPR (NB: These enzyme can also produce cyclic ADP ribose phosphate from β-$NADP^+$ and cyclic GDP ribose from nicotinamide–guanine nucleotide. The signalling functions of these compounds have not been rigorously explored as yet.)

Although NAADP is produced by the same enzyme as cADPR, it does not activate RyRs. Indeed, the receptor for NAADP is not yet known, but it has been shown to have some unique characteristics that distinguish it from both $InsP_3$Rs and RyRs. In particular, the NAADP response can be completely inactivated for prolonged periods by concentrations of NAADP below the threshold for Ca^{2+} release. This peculiar property suggests that for NAADP to be an intracellular messenger it must be almost absent in unstimulated cells, and must be rapidly produced upon activation. The NAADP response can also be distinguished from that of $InsP_3$ and cADPR by its sensitivity to L-type Ca^{2+} channel antagonists such as verapamil, and also by the fact that the NAADP-sensitive Ca^{2+} stores appear to be insensitive to the Ca^{2+} ATPase inhibitor thapsigargin.

The expression of different combinations of Ca^{2+} influx and Ca^{2+} release channels, coupled with the use of various Ca^{2+}-releasing stimuli gives rise to a huge range of

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mechanisms for increasing Ca^{2+} that can be considered to comprise a Ca^{2+} signalling 'toolkit' (Berridge *et al.*, 1998). Some cell types employ only a few of these 'tools', while others seem to utilize many of the possible combinations. This diversity of Ca^{2+} signalling mechanisms leads to the stunning array of spatially and temporally complex Ca^{2+} signals that can been seen during stimulation of intact cells.

Diversity of Intracellular Ca^{2+} Signals

Calcium ions are a stimulus for a wide range of cellular processes, including secretion, muscle contraction, gene transcription and cell proliferation. However, although these different cellular activities require Ca^{2+}, they are not necessarily activated by the same signals. Instead, cells employ different components selected from the Ca^{2+} signalling tool kit, as befits their physiology. The following sections briefly discuss a few examples of cell-specific Ca^{2+} signalling.

Muscle

In the case of muscle contraction, for example, the nature of the Ca^{2+} signal differs markedly between cardiac, skeletal and smooth muscle (Lipp and Niggli, 1996). In cardiac muscle, type 2 RyRs are activated following depolarization of the sarcolemma. The Ca^{2+} that enters the cell following VOC activation triggers release of Ca^{2+} from the RyRs, by a process known as Ca^{2+}-induced Ca^{2+} release (CICR). This Ca^{2+} can then diffuse to the myofibrils and promote the interaction between actin and myosin that leads to contraction. In skeletal muscle, a two-step process is responsible for generating the contraction-activating Ca^{2+} signal. The depolarization of the sarcolemma leads to the activation of type 1 RyRs by a direct physical connection with the voltage-sensing L-type Ca^{2+} channels. The conformational change in the VOCs is transmitted directly to the RyRs, causing them to open and release the Ca^{2+}. This initial Ca^{2+} release is then greatly amplified by CICR from surrounding RyRs. Smooth muscle is yet further complicated in that it may display CICR similar to that seen in cardiac myocytes, but it can also respond to $InsP_3$-generating hormones.

Apart from the different mechanisms for introducing Ca^{2+} into the cytoplasm, these different kinds of muscle serve to illustrate that the Ca^{2+} homeostatic properties of cells are also cell type-dependent. For example, cardiac muscle has an oscillating influx and efflux of Ca^{2+} during each heartbeat. This is necessary to refill the SR after depletion, and to remove Ca^{2+} from the cytoplasm at the end of the contraction. In contrast, skeletal muscle is an almost closed system, with the released Ca^{2+} being efficiently resequestered by Ca^{2+}ATPases on the SR following each contraction.

Neurons

Neurons provide an excellent example of how different combinations of Ca^{2+} signals have been adapted to regulate a wide range of processes in a single cell type (Berridge, 1998). For example, Ca^{2+} plays a pivotal role in the reception of signals (input), signal transmission (output), the regulation of neuronal excitability as well as the cellular changes that underlie learning and memory. Neurons use a wide combination of elements from the Ca^{2+} signalling tool kit, which are expressed at varying levels by different neurons. Furthermore, we are just beginning to understand that interaction between the signalling systems in the PM and ER controls many neuronal activities. Of particular interest is that the nature of this interaction is dependent upon the stimulus and the immediate history of the cell.

Neurons can generate Ca^{2+} signals that are restricted to the tiny volumes ($\sim 0.1\ \mu m^3$) of dendritic spines, or larger signals that spread over many dendrites, perhaps reaching the soma and axon. There is increasing evidence that Ca^{2+} signals within dendritic spines, which function as microprocessors within the brain, may be responsible for the 'synaptic plasticity' that leads to short-term memory. During synaptic input, Ca^{2+} enters either from the outside through VOCs or ROCs, or it can be released from the spine apparatus; a continuation of the ER that extends into many spines and bears both RyRs and $InsP_3$Rs. One of the puzzling aspects of synaptic plasticity is that Ca^{2+} has been implicated in both potentiation and depression of synaptic transmission. Subtle modifications in the amplitude or spatial and temporal presentation of Ca^{2+} signals may account for how it can regulate these diametrically opposite events.

Hepatocytes

Since elevated cytoplasmic Ca^{2+} levels can be toxic (see Intracellular Ca^{2+} and cellular pathology, below), most cells do not usually respond with sustained Ca^{2+} signals. Rather, Ca^{2+} is commonly presented in a pulsatile manner (Bootman and Berridge, 1995). A well-known example of such repetitive Ca^{2+} increases is the response of hepatocytes to stimulation with various hormones that, for example, regulate glucose metabolism. The Ca^{2+} increases that occur in hepatocytes during such stimulations are transient spikes, which arise from a steady basal Ca^{2+} concentration. The frequency of the Ca^{2+} spikes is directly proportional to the concentration of hormone applied to the cells, and they can persist for the duration of agonist application. An intriguing observation is that the time course of the Ca^{2+} spikes (particularly the recovery phase) varies between different agonists. Adrenergic stimuli usually give rise to the briefest (< 20 s) spikes, whereas those stimulated by purinergic agonists can be significantly longer (> 40 s). These Ca^{2+} spikes are essentially a digital

read-out of cell stimulation. It has been suggested that the frequency of Ca^{2+} spikes relays information regarding the concentration of stimulus, while the shape of the spike conveys the identity of the agonist.

Pulsatile increases in Ca^{2+}, such as those observed in hepatocytes, are generally considered to have a much higher fidelity of information transfer than simple tonic changes in Ca^{2+} concentrations, since they are much less prone to noisy fluctuations. The sensors for these Ca^{2+} spikes are Ca^{2+}-binding proteins such as calmodulin. This ubiquitous protein is one of a family of proteins bearing structural Ca^{2+}-binding motifs known as 'EF-hands'. The binding of Ca^{2+} to calmodulin is highly cooperative with a K_d around 1 μmol L^{-1}, making it an ideal receiver for the rapid transient Ca^{2+} increases seen with each spike. One of the best-known enzymes that uses calmodulin to help it 'count' Ca^{2+} spikes is calmodulin-dependent protein kinase II, which can activate other proteins via phosphorylation. This enzyme is composed of many identical subunits that undergo variable degrees of activation depending on the frequency of Ca^{2+} spikes. Essentially, high frequency Ca^{2+} spikes maintain this enzyme in an active state. The same is true for other enzymes that utilize calmodulin as a sensor, such as the kinase that phosphorylates $InsP_3$ to give $InsP_4$ (see above).

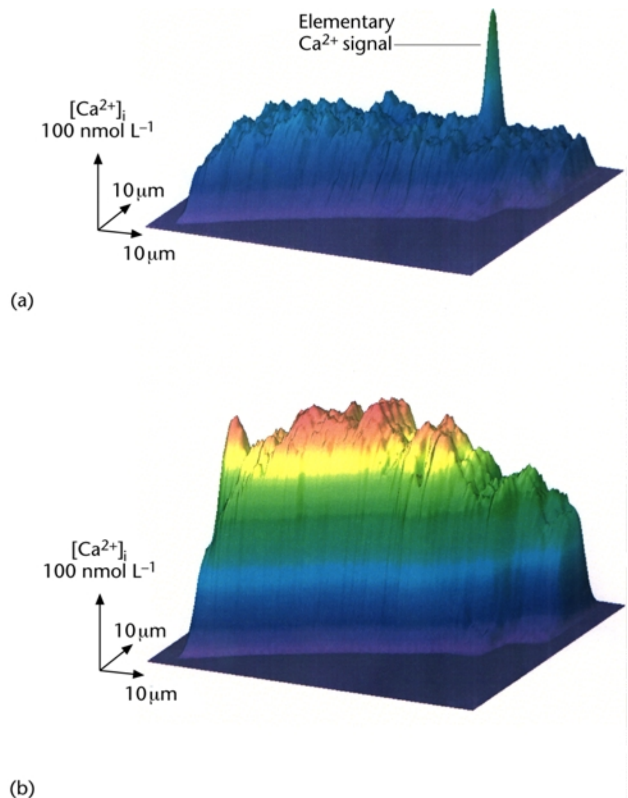

Figure 3 Elementary and global Ca^{2+} signals in a single cardiac myocyte. The figure depicts Ca^{2+} signals recorded using laser scanning confocal microscopy of a single cardiac myocyte. Panel (a) illustrates the global increase in Ca^{2+} that occurs after an action potential. This global Ca^{2+} signal is constructed by the recruitment and spatial summation of many elementary Ca^{2+} release signals. A single elementary Ca^{2+} signal, which occurred spontaneously in the same myocyte when the cell was not electrically stimulated, is depicted in (b).

Constructing Ca^{2+} Signals

The versatility of calcium signalling arises via the utilization of the Ca^{2+} signalling toolkit, as described above. At the lowest level, this toolkit comprises elementary Ca^{2+} signals that reflect the opening of one or a few Ca^{2+} channels. At the other end of the scale, these signals can sum to construct larger global Ca^{2+} signals that pervade the entirety of cells (**Figure 3**).

Elementary Ca^{2+} signals

When activated, both Ca^{2+}-entry and Ca^{2+}-release channels introduce Ca^{2+} into the cytoplasm. However, since these channels have short open-times, they introduce only brief pulses of Ca^{2+} that form a small plume around the mouth of the channel before diffusing into the cytoplasm. For some of the Ca^{2+} entry and release channels, these localized plumes of Ca^{2+} have been visualized using confocal microscopy of living cells. Such Ca^{2+} increases have been recorded in vastly different cell types, prompting the realization that these 'elementary' Ca^{2+} signals represent the basic building blocks of Ca^{2+} signalling (Bootman and Berridge, 1995; Lipp and Niggli, 1996; Berridge *et al.*, 1998). The identification and characterization of these elementary Ca^{2+} signals have greatly enhanced our understanding of Ca^{2+} signalling at two levels. First, the elementary Ca^{2+} signals themselves have been shown to provide local control of many physiological functions. Second, they have enabled us to see that complex global Ca^{2+} signals, such as Ca^{2+} oscillations and waves, are constructed by recruiting these basic building blocks.

Some examples of processes that are directly controlled by elementary Ca^{2+} signals include the release of synaptic and secretory vesicles, the activation of ion channels, mitochondrial energy metabolism and the generation of nuclear-specific Ca^{2+} signals. The advantages of using elementary Ca^{2+} signals, rather than global increases in Ca^{2+}, to control such processes are several-fold. For example, since the elementary Ca^{2+} signals have only a limited spatial spread (usually 1–6 μm), and the Ca^{2+} concentration declines sharply with distance from the site of origin, regulation of cellular activities relies on close localization of the Ca^{2+} channels and their targets, thus allowing Ca^{2+} to have a highly specific effect. In addition, elementary Ca^{2+} signals can have a rapid action at

relatively low energy cost to the cells, in contrast to global Ca^{2+} changes. The rapidity of signalling through elementary events is evident, for example, from its use in synaptic transmission, where VOCs located next to synaptic vesicles trigger exocytosis by providing high intensity pulses of Ca^{2+}. For example, the elementary Ca^{2+} entry signals recorded in squid giant axons last for ~1.25 ms, during which time the concentration can rise to 200–300 μmol L^{-1}. The limited number of Ca^{2+} ions used to generate such signals can be removed from the cytoplasm both more rapidly and without consuming as much ATP as would be necessary to reverse global Ca^{2+} signals.

Global Ca^{2+} signals

In addition to controlling local functions of cells, elementary Ca^{2+} signals are responsible for the generation of global Ca^{2+} signals such as waves and spikes. Essentially, global Ca^{2+} signals arise via the coordinated recruitment of multiple elementary Ca^{2+} release and entry channels. The mechanisms by which this is achieved and the balance between Ca^{2+} influx and release are cell specific. For example, in many nonexcitable cell types, Ca^{2+} release provides the major signal component, while excitable cells often rely on both Ca^{2+} entry and release to varying degrees (see Neurons, above).

Such global Ca^{2+} signals can also pass between coupled cells via gap junctions to coordinate the activities of whole tissues or organs. Such intercellular Ca^{2+} waves are responsible for spreading the activation of cilia on tracheal epithelial cells, as mentioned earlier (Boitano *et al.*, 1992). Ca^{2+} does not necessarily only pass between the same types of cell but can also pass between different cells types, such as smooth muscle/endothelial cells and neurons/glial cells.

Intracellular Ca^{2+} and Cellular Pathology

There are numerous examples of Ca^{2+} being involved in cellular pathology and even cell death. By using Ca^{2+} as an intracellular messenger, cells walk a delicate tightrope between life and death. When the homeostatic mechanisms responsible for regulating cellular Ca^{2+} are compromised, cells will die. This can be either in a disordered manner by necrosis, or by the more deliberate apoptotic mechanism (Berridge *et al.*, 1998).

It is possible that only subtle changes in Ca^{2+} signalling are necessary to have a dramatic pathological effect on cells. For example, the effect of many $InsP_3$-generating hormones on cardiac myocytes appears to be to activate elementary Ca^{2+} signals that do not coincide with the electrical pacing of the heart. Individually, such 'spontaneous' elementary Ca^{2+} signals do not pose a problem. However, over time they have an integrated effect and can begin to interfere with gene transcription, possibly leading to hypertrophy of the heart. In addition, at sufficient frequencies, the elementary Ca^{2+} signals may be able to generate electrical signals by altering the activity of electrogenic processes, e.g. Na^{+}/Ca^{2+} exchange, at the PM. Such spontaneous electrical activity can lead to cardiac arrhythmias and sudden heart death.

Another well-known example of Ca^{2+} signalling exceeding physiological requirements and becoming pathological is glutamate-induced excitotoxicty in the brain. This arises owing to excessive activation of NMDA receptors, and is a major cause of neuronal death associated with stroke and ischaemia.

Summary

The universality and versatility of Ca^{2+} as an intracellular messenger is illustrated. It is striking that a simple ion such as Ca^{2+} can regulate so many different cellular processes. The central role of Ca^{2+} in cell biology essentially arises owing to the utilization of a Ca^{2+} signalling toolkit, whereby cells employ specific Ca^{2+} 'on' and 'off' mechanisms selected from a diverse array of channels, pumps and exchangers. Subtle modulation of the amplitude or the temporal/spatial presentation of Ca^{2+} signals can differentially regulate Ca^{2+}-sensitive processes within the same cell. However, cells must utilize Ca^{2+} with care, since it can also trigger deleterious processes that culminate in cell death.

References

Berridge MJ (1993) Inositol trisphosphate and calcium signalling. *Nature* **361**: 315–325.

Berridge MJ (1998) Neuronal calcium signalling. *Neuron* **21**: 13–26.

Berridge MJ, Bootman MD and Lipp P (1998) Calcium – a life and death signal. *Nature* **395**: 645–648.

Boitano S, Dirksen ER and Sanderson MJ (1992) Intercellular propagation of calcium waves mediated by inositol trisphosphate. *Science* **258**: 292–295.

Bootman MD and Berridge MJ (1995) The elemental principles of calcium signalling. *Cell* **83**: 675–678.

Clapham DE (1995) Calcium signalling. *Cell* **80**: 259–268.

Cullen PJ (1998) Bridging the GAP in inositol 1,3,4,5-tetrakisphosphate signalling. *Biochimica et Biophysica Acta* **1436**: 35–47.

Genazzani AA and Galione A (1997) A Ca^{2+} release mechanism gated by the novel pyridine nucleotide, NAADP. *TiPS* **18**: 108–110.

Lipp P and Niggli E (1996) A hierarchical concept of cellular and subcellular Ca^{2+} signalling. *Progress in Molecular Biology* **65**: 265–296.

Petersen OH, Petersen CCH and Kasai H (1994) Calcium and hormone action. *Annual Review of Physiology* **56**: 297–319.