THE VERSATILITY AND UNIVERSALITY OF CALCIUM SIGNALLING

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The universality of calcium as an intracellular messenger depends on its enormous versatility. Cells have a calcium signalling toolkit with many components that can be mixed and matched to create a wide range of spatial and temporal signals. This versatility is exploited to control processes as diverse as fertilization, proliferation, development, learning and memory, contraction and secretion, and must be accomplished within the context of calcium being highly toxic. Exceeding its normal spatial and temporal boundaries can result in cell death through both necrosis and apoptosis.

Ca^{2+}-INDUCED Ca^{2+} RELEASE
An autocatalytic mechanism by which cytoplasmic Ca^{2+} activates the release of Ca^{2+} from internal stores through channels such as inositol-1,4,5-trisphosphate receptors or ryanodine receptors.

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Calcium (Ca^{2+}) is a ubiquitous intracellular signal responsible for controlling numerous cellular processes. At one level, its action is simple: cells at rest have a Ca^{2+}concentration of 100 nM but are activated when this level rises to roughly 1000 nM (FIG. 1). The immediate question is how can this elevation of Ca^{2+} regulate so many processes? The answer lies in the versatility of the Ca^{2+} signalling mechanism in terms of speed, amplitude and spatio-temporal patterning. This versatility emerges from the use of an extensive molecular repertoire of signalling components, which comprise a Ca^{2+} signalling toolkit (FIG. 2 and online poster) that can be assembled in combinations to create signals with widely different spatial and temporal profiles. More variations are achieved through the interactions that Ca^{2+} makes (crosstalk) with other signalling pathways. This versatility is exploited to regulate diverse cellular responses.

The Ca^{2+} signalling toolkit

The Ca^{2+} signalling network can be divided into four functional units (FIG. 1):

- Signalling is triggered by a stimulus that generates various Ca^{2+}-mobilizing signals.
- The latter activate the ON mechanisms that feed Ca^{2+} into the cytoplasm.
- Ca^{2+} functions as a messenger to stimulate numerous Ca^{2+}-sensitive processes.
- Finally, the OFF mechanisms, composed of pumps and exchangers, remove Ca^{2+} from the cytoplasm to restore the resting state.

The functional relationship between these units is illustrated in FIG. 2, which reveals that the signalling network is composed of many components (the Ca^{2+} signalling toolkit). Because many of the molecular components of this toolkit have several isoforms [online poster] with subtly different properties, each specific cell type can exploit this large repertoire to construct versatile Ca^{2+} signalling networks.

Generation of Ca^{2+}-mobilizing signals. Cells generate their Ca^{2+} signals by using both internal and external sources of Ca^{2+}. The internal stores are held within the membrane systems of the endoplasmic reticulum (ER) or the equivalent organelle, the sarcoplasmic reticulum (SR) of muscle cells. Release from these internal stores is controlled by various channels, of which the inositol-1,4,5-trisphosphate receptor ($InsP_3R$) and ryanodine receptor (RYR) families have been studied most extensively[1,2]. The principal activator of these channels is Ca^{2+} itself and this process of Ca^{2+}-INDUCED Ca^{2+} RELEASE is central to the mechanism of Ca^{2+} signalling (see below). Ca^{2+}-mobilizing second messengers that are generated when

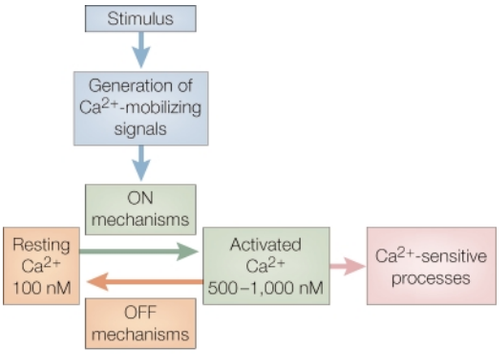


Figure 1 | **The four units of the Ca^{2+} signalling network.** Stimuli act by generating Ca^{2+}-mobilizing signals that act on various ON mechanisms to trigger an increase in the intracellular concentration of Ca^{2+}. The increased level of Ca^{2+} stimulates various Ca^{2+}-sensitive processes to trigger many different cellular pathways. The response is terminated by OFF mechanisms that restore Ca^{2+} to its resting level. Details of these four functional units, with the same colour coding, are revealed in FIG. 2.

stimuli bind to cell surface receptors (FIG. 2) determine whether Ca^{2+} can activate these channels. One is $Ins(1,4,5)P_3$ (REF. 1), which diffuses into the cell to engage the $InsP_3Rs$ and release Ca^{2+} from the ER (FIG. 2). The ability of Ca^{2+} to stimulate the RYRs is modulated by cyclic ADP ribose (cADPR)[3]. A related messenger, nicotinic acid dinucleotide phosphate (NAADP)[4], acts on a separate, as yet uncharacterized, channel. Sphingosine 1-phosphate (S1P) releases Ca^{2+} from the ER — possibly by binding to a sphingolipid Ca^{2+} release-mediating protein of the ER (SCaMPER)[5].

These different Ca^{2+}-mobilizing messengers can co-exist in cells, where they seem to be controlled by different receptors. For example, in the exocrine pancreas, muscarinic acetylcholine receptors act through $Ins(1,4,5)P_3$, whereas cholecystokinin receptors use cADPR[6]. Similarly, human SH-SY5Y cells have acetylcholine receptors linked through $Ins(1,4,5)P_3$ whereas lysophosphatidic acid acts through S1P[7]. So the versatility of the signalling network is enhanced by having different Ca^{2+}-mobilizing messengers linked to separate input signals.

ON mechanisms. The ON mechanisms depend on Ca^{2+} channels that control the entry of external Ca^{2+} or the release of Ca^{2+} from internal stores. For the first case, there are families of Ca^{2+} entry channels defined by the way in which they are activated. We know most about VOLTAGE-OPERATED CHANNELS (VOCs). In addition there are many channels that open in response to receptor activation. RECEPTOR-OPERATED CHANNELS (ROCs) open on binding external stimuli, usually transmitters such as glutamate, ATP or acetylcholine. Other channels are sensitive to various signals generated following receptor activation such as store emptying[8], diacylglycerol (DAG)[9] and arachidonic acid[10,11]. Most attention has focused on capacitative Ca^{2+} entry (FIG. 3a)where empty stores activate STORE-OPERATED CHANNELS (SOCs) in the plasma membrane through an unknown mechanism. Recent evidence[12,13] lends support to a conformational-coupling mechanism[14], which proposes that $InsP_3Rs$ in the ER are directly coupled to SOCs (FIG. 3a). There is considerable interest in SOCs because they provide the Ca^{2+} signals that control many cellular processes (see later).

Signal Ca^{2+} is also derived from the internal stores using the channels and Ca^{2+}-mobilizing messengers described above. As little is known about the channels opened by NAADP and S1P, we will focus on the $InsP_3Rs$ and the RYRs. These two channels are regulated by several factors, the most important of which is Ca^{2+} itself, which regulates Ca^{2+} release by acting from either the lumenal or cytoplasmic sides of the channel. Increasing the level of Ca^{2+} within the lumen of the ER/SR enhances the sensitivity of the RYRs and the same may apply to the $InsP_3Rs$. The cytosolic action of Ca^{2+} is more complex: it can be both stimulatory and inhibitory and can vary between the different $InsP_3R$ isoforms. In general, the $InsP_3Rs$ have a bell-shaped Ca^{2+} dependence when treated with low concentrations of $Ins(1,4,5)P_3$: low concentrations of Ca^{2+} (100–300 nM) are stimulatory but above 300 nM, Ca^{2+} becomes inhibitory and switches the channel off[15]. Emerging evidence indicates that $InsP_3Rs$ are sometimes not inhibited by high cytosolic Ca^{2+} concentrations. Instead of a bell-shape, the relationship between $InsP_3R$ activity and cytosolic Ca^{2+} is sigmoidal. This is particularly true in the presence of high $Ins(1,4,5)P_3$ levels, indicating that $Ins(1,4,5)P_3$ acts as a molecular switch and that once the receptor binds $Ins(1,4,5)P_3$, it becomes sensitive to the stimulatory, but not the inhibitory, action of Ca^{2+} (REF. 15). The function of cADPR is not so clear but it is known to increase the Ca^{2+} sensitivity of RYRs. The autocatalytic process of Ca^{2+}-induced Ca^{2+} release enables the $InsP_3Rs$ and RYRs to communicate with each other to establish coordinated Ca^{2+} signals, often organized into propagating waves[1,2]. The main function of the Ca^{2+}-mobilizing messengers, therefore, is to alter the sensitivity of the $InsP_3Rs$ and RYRs to this stimulatory action of Ca^{2+}. How do cells exploit the Ca^{2+} toolkit? Let us consider three tissues that generate Ca^{2+} signals in different ways (FIG. 4):

Muscle. Perhaps the most specialized mechanism is found in skeletal muscle, which has a L-type VOC (α_{1S}) located in the plasma membrane that interacts directly with the large cytoplasmic head of the RYR1 embedded in the SR (FIGS 3b, 4a). Membrane depolarization induces a conformational change in α_{1S} that is transmitted directly to RYR1, causing it to release Ca^{2+} from the SR. By contrast, cardiac cells use a related α_{1C} L-type channel to gate a small amount of trigger Ca^{2+} that then diffuses across the plasma membrane to activate RYR2 channels in the SR through Ca^{2+}-induced Ca^{2+} release.

Neurons. Neurons have numerous Ca^{2+} channels in different parts of the cell to carry out separate functions (FIG. 4b). N- and P/Q-type VOCs at synaptic endings trigger the release of neurotransmitters. The L-type VOCs on the cell body and proximal dendrites are ideally positioned to provide the Ca^{2+} signals that induce gene activation. They also function as 'kinetic filters', allowing

VOLTAGE-OPERATED CHANNELS
Plasma-membrane ion channels that are activated by membrane depolarization.

RECEPTOR-OPERATED CHANNELS
Plasma membrane ion channels that open in response to binding of an extracellular ligand.

STORE-OPERATED CHANNELS
Plasma membrane ion channels, of uncertain identity, that open in response to depletion of internal Ca^{2+} stores.

them to respond effectively to the small depolarizations that occur at synaptic spines[16]. The Ca^{2+} signals in spines, responsible for mediating the early synaptic modifications that are implicated in learning and memory, are provided by entry through such VOCs and also through ROCs (such as NMDA (*N*-methyl-D-aspartate) receptors) and by release from RYRs and $InsP_3Rs$ (reviewed in REF. 17). As $InsP_3Rs$ are sensitive to both $Ins(1,4,5)P_3$ and Ca^{2+}, they could act as coincidence detectors to correlate the activity of pre- and postsynaptic inputs, which is central to memory formation[17]. In hippocampal neurons, for example, electrical activity resulting in Ca^{2+} entry through VOCs acts together with $Ins(1,4,5)P_3$ produced by metabotropic glutamate receptors ($mGluR_1$) to produce a synergistic release of internal Ca^{2+} (REF. 18).

Pancreas. RYRs have also been described in non-excitable cells such as the pancreas, where they collaborate with $InsP_3Rs$ to control both fluid and enzyme secretion (FIG. 4c)[6]. Acetylcholine and cholecystokinin act through $Ins(1,4,5)P_3$. Cholecystokinin also acts through both NAADP and cADPR. NAADP might also initiate Ca^{2+} release from RYRs[19].

As a result of these ON mechanisms, Ca^{2+} flows into the cytoplasm to produce the increase in concentration that constitutes a Ca^{2+} signal (FIG. 1). However, the concentration that is measured in cells using various Ca^{2+} indicators (for example, aequorin or Ca^{2+}-sensitive dyes such as Fura2 or Fluo3) is only the tip of the iceberg because most of the Ca^{2+} that enters the cytoplasm is rapidly bound to various cytosolic buffers such as

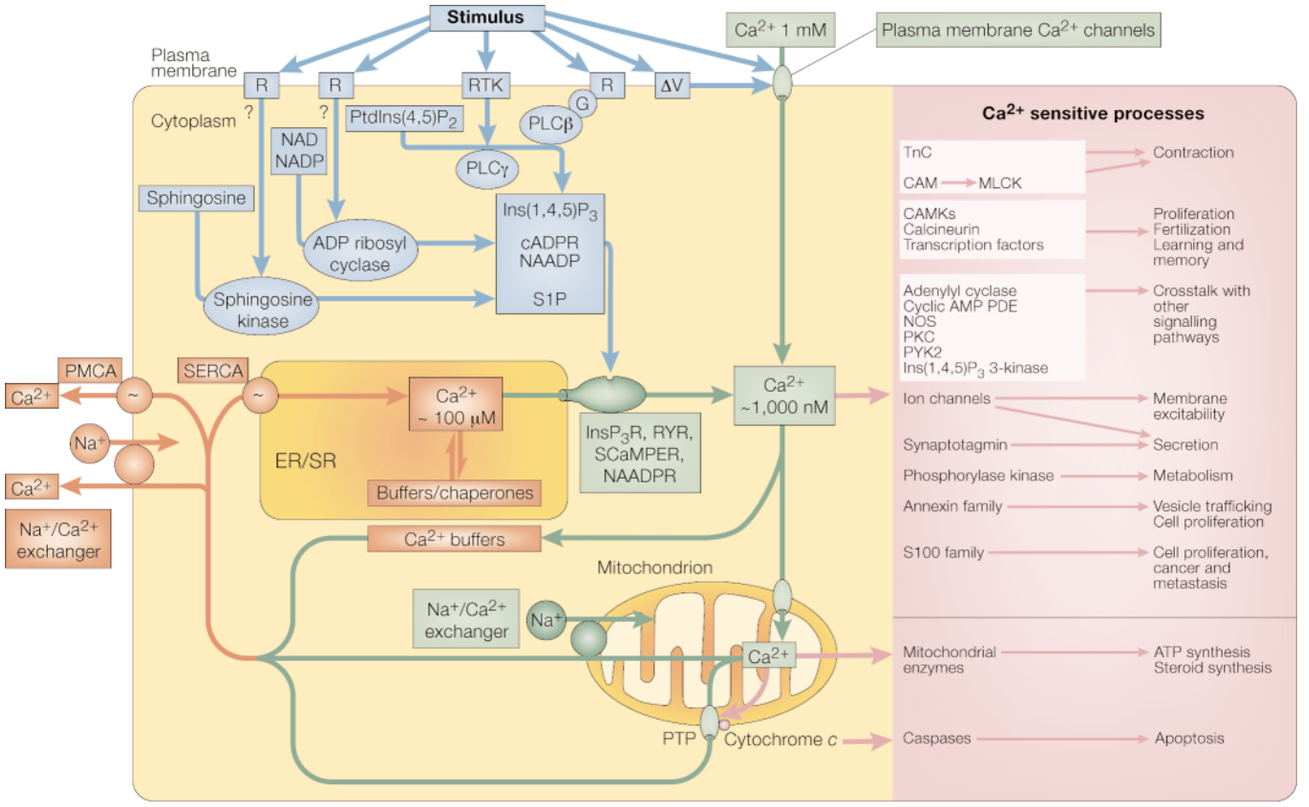


Figure 2 | **Elements of the Ca^{2+} signalling toolkit.** Cells have an extensive signalling toolkit that can be mixed and matched to create Ca^{2+} signals of widely different properties. Ca^{2+}-mobilizing signals (blue) are generated by stimuli acting through a variety of cell-surface receptors (R), 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.)

Online poster

parvalbumin, calbindin-D_{28K} and calretinin. The buffer capacity — the number of Ca^{2+} ions that are bound for each free ion — varies considerably between cells[20]. Cytosolic buffers are involved in shaping both the amplitude and duration of Ca^{2+} signals. During each spike, they act as a halfway house for Ca^{2+} by loading it up during the ON mechanisms and then unloading it during the OFF mechanisms described later (FIG. 2). Buffers also limit the spatial spreading of local Ca^{2+} signals. This is particularly important in neurons that contain high concentrations of buffers such as parvalbumin and calbindin, which ensure that Ca^{2+} signals are largely confined to synapses.

Ca^{2+}-sensitive processes. Once the ON mechanisms have generated a Ca^{2+} signal, various Ca^{2+}-sensitive processes translate this into a cellular response (FIGS 2 AND 4). The Ca^{2+} signalling toolkit has numerous Ca^{2+}-binding proteins, which can be divided into Ca^{2+} buffers (described above) and Ca^{2+} sensors, on the basis of their main functions [online poster]. The latter respond to an increase in Ca^{2+} by activating diverse processes (FIG. 2). The classical sensors are troponin C (TnC) and calmodulin (CAM), which have four EF hands that bind Ca^{2+} and undergo a pronounced conformational change to activate various downstream effectors. TnC has a limited function to control the interaction of actin and myosin during the contraction cycle of cardiac and skeletal muscle (FIG. 4a). CAM is used more generally to regulate many processes such as the contraction of smooth muscle, crosstalk between signalling pathways, gene transcription, ion channel modulation and metabolism. The same cell can use different sensors to regulate separate processes. In skeletal muscle, for example, TnC regulates contraction whereas CAM stimulates phosphorylase kinase to ensure a parallel increase in ATP production (FIG. 4a). In addition to the above proteins, which act generally, there are numerous Ca^{2+}-binding proteins designed for more specific functions. For example, synaptotagmin is associated with membrane vesicles and is a Ca^{2+} sensor for exocytosis.

The versatility of Ca^{2+} signalling is greatly enhanced by some of the Ca^{2+}-sensitive processes linking into other signalling pathways (BOX 1). The ability of Ca^{2+} to recruit the control elements of other signalling pathways (for example, cyclic AMP and mitogen-activated protein kinase pathways) is particularly evident in the control of gene transcription in neurons (FIG. 4b).

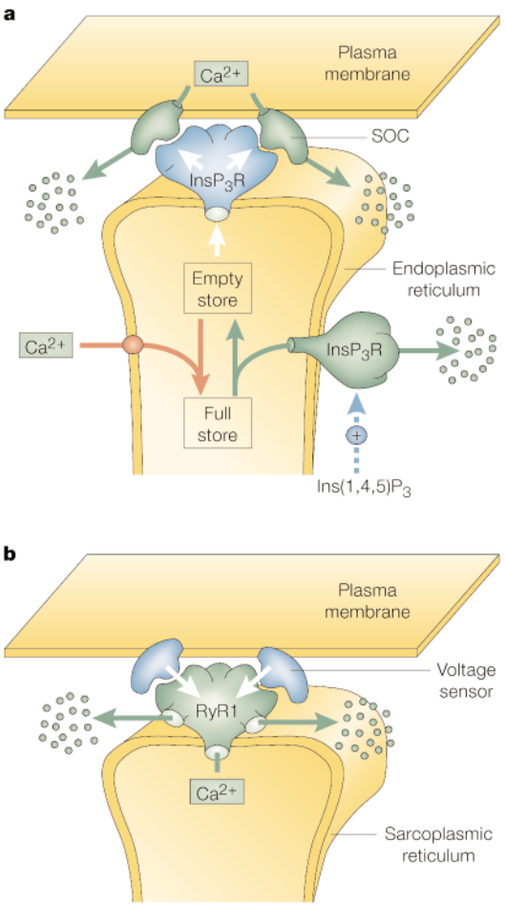


Figure 3 | **Ca^{2+} signalling by conformational coupling using macromolecular complexes. a** | Capacitative Ca^{2+} entry. In response to a Ca^{2+}-mobilizing signal such as inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$), Ca^{2+} is released from the endoplasmic reticulum. Emptying of the store is detected by a protein, most probably an inositol-1,4,5-trisphosphate receptor ($InsP_3R$) or a ryanodine receptor (RYR), which undergoes a conformational change (white arrows) that is transmitted to the store-operated channel (SOC) to induce Ca^{2+} entry across the plasma membrane. **b** | Ca^{2+} release in skeletal muscle. Voltage sensors (α_{1S} subunit of an L-type Ca^{2+} channel) located in the plasma membrane induce a conformational change in the RYR1 channels (open arrows) that then release Ca^{2+} from the sarcoplasmic reticulum.

OFF mechanisms. Once Ca^{2+} has carried out its signalling functions, it is rapidly removed from the cytoplasm by various pumps[21] and exchangers[22] (FIG. 2). The plasma membrane Ca^{2+}-ATPase (PMCA) pumps and Na^+/Ca^{2+} exchangers extrude Ca^{2+} to the outside whereas the sarco-endoplasmic reticulum ATPase (SERCA) pumps return Ca^{2+} to the internal stores.

The mitochondrion is another important component of the OFF mechanism in that it sequesters Ca^{2+} rapidly during the development of the Ca^{2+} signal and then releases it back slowly during the recovery phase (FIG. 2). This uptake of Ca^{2+} by the mitochondrion is important in shaping both the amplitude[23] and the spatio-temporal patterns of Ca^{2+} signals[24–26]. Mitochondria extrude protons to create the electrochemical gradient that allows ATP synthesis. The same gradient is used to drive Ca^{2+} uptake through a uniporter that has a low sensitivity to Ca^{2+} (half-maximal activation around 15 μM). This low sensitivity means that mitochondria accumulate Ca^{2+} more effectively when they are close to Ca^{2+}-releasing channels[27]. Here, they may form a 'quasi-synapse', allowing them to directly sense the high local Ca^{2+} concentration that builds up in the vicinity of open Ca^{2+} channels, such as the $InsP_3Rs$ and RYRs[28]. There seem to be reciprocal interactions between the two organelles in that the ER/SR provides the Ca^{2+} that enters the mitochondria, which in turn modifies the Ca^{2+} feedback mechanisms that regulate Ca^{2+} release from the ER/SR.

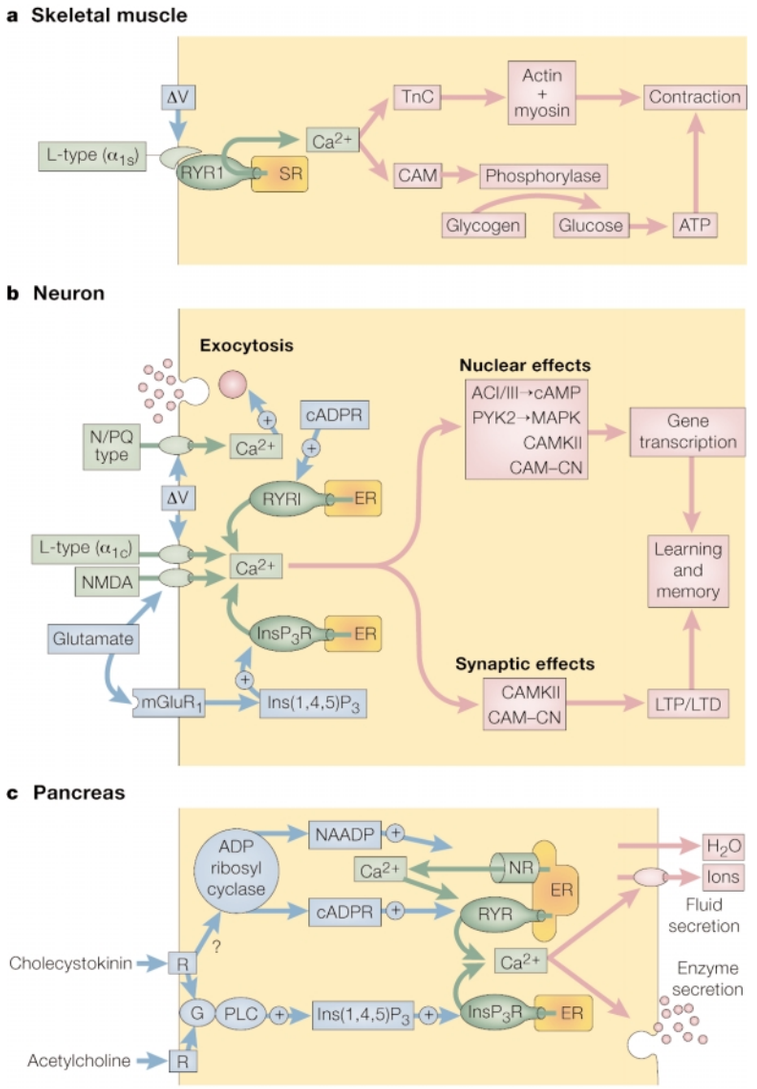


Figure 4 | **Application of the Ca^{2+} signalling toolkit to regulate different cellular processes. a** | In skeletal muscle, an L-type Ca^{2+} channel (α_{1S}) senses membrane depolarization (ΔV) and undergoes a conformational change that is transmitted to the ryanodine receptor 1 (RYR1) (FIG. 3b). Ca^{2+} released from the sarcoplasmic reticulum (SR) interacts with two sensors, troponin C (TnC), which triggers contraction, and calmodulin (CAM), which activates glycogen metabolism to synthesize ATP. **b** | Neurons have several Ca^{2+}-sensitive processes located in different regions. Membrane depolarization (ΔV) is sensed by N- or P/Q-type channels at the synaptic endings to produce a localized pulse of Ca^{2+} that triggers exocytosis. In the cell body and dendrites, L-type channels sense the same depolarization and induce the entry of Ca^{2+} which has a number of targets: adenylyl cyclase I or III (AC I/III) leading to cyclic AMP production, proline-rich tyrosine kinase (PYK2), mitogen-activated protein kinase (MAPK), Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII) and calmodulin–calcineurin (CAM–CN). Some of these targets induce gene transcription. The neurotransmitter glutamate can also generate Ca^{2+} signals either by activating receptor-operated channels such as NMDA (*N*-methyl-D-aspartate) receptors, or by stimulating the metabotropic glutamate receptor $mGluR_1$ to produce inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$) to mobilize internal Ca^{2+} from the endoplasmic reticulum (ER). These glutamate-induced Ca^{2+} signals are localized to synaptic endings, where they contribute to processes such as long-term potentiation (LTP) and long-term depression (LTD), which have been implicated in learning and memory. **c** | The exocrine pancreas uses two signalling systems regulated by separate receptors. Acetylcholine uses $Ins(1,4,5)P_3$ to release internal Ca^{2+}. As well as stimulating $Ins(1,4,5)P_3$ formation, cholycystokinin also acts through both cyclic ADP ribose (cADPR) and nicotinic acid dinucleotide phosphate (NAADP). The latter seems to act by releasing a small amount of trigger Ca^{2+} through the NAADP receptor (NR) that then acts together with cADPR to release further Ca^{2+} through RYRs.

Various proteins such as presenilins and apoptosis regulatory proteins (such as Bcl-2 described later) modulate the way these two organelles handle Ca^{2+}. The presenilins, located in the ER membrane, not only function to process the β-amyloid precursor protein but also modulate Ca^{2+} signalling. Mutations of presenilin result in overfilling of the ER leading to larger Ca^{2+} signals and a decrease in capacitative Ca^{2+} entry[29].

The mitochondrion has an enormous capacity to accumulate Ca^{2+} and the mitochondrial matrix contains buffers that prevent the concentration from rising too high. Once the cytosolic Ca^{2+} has returned to its resting level, a mitochondrial Na^+/Ca^{2+} exchanger pumps the large load of Ca^{2+} back into the cytoplasm, from which it is either returned to the ER or removed from the cell (FIG. 2). Ca^{2+} can also leave the mitochondrion through a permeability transition pore (PTP)[26,30], which has all the elements of Ca^{2+}-induced Ca^{2+} release because its formation is activated by the build up of Ca^{2+} within the mitochondrial matrix[31]. This PTP may have two functional states. A low conductance state of the pore can act reversibly, allowing mitochondria to become excitable, and this may contribute to the generation of Ca^{2+} waves[31]. On the other hand, an irreversible high conductance state of the PTP has a marked effect on the mitochondrion in that it collapses the transmembrane potential and leads to the release of cyctochrome *c* and the initiation of apoptosis (see later).

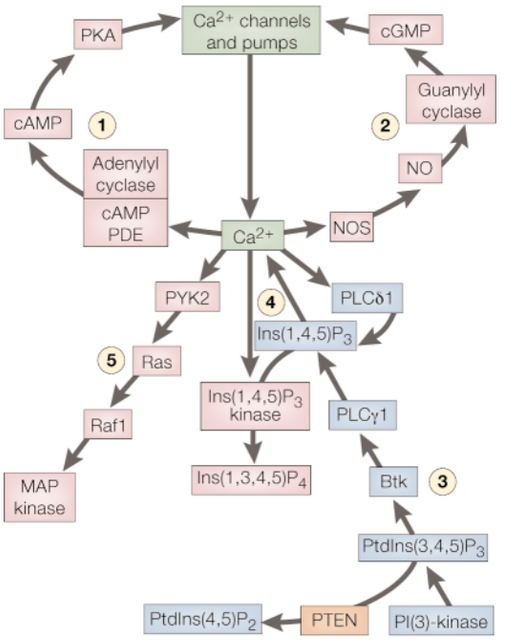
Global aspects of Ca^{2+} signalling

Elementary events. Further versatility is achieved by varying the spatial and temporal aspects of Ca^{2+} signalling[32,33]. The different types of Ca^{2+} signals shown in FIG. 5 result from the $InsP_3Rs$ and/or RYRs having different degrees of excitability depending on the levels of the appropriate Ca^{2+}-mobilizing messenger. At low levels of stimulation, the degree of excitability is such that individual RYRs or $InsP_3Rs$ open and these single-channel events have been recorded as quarks[34] or blips[35], respectively (FIG. 5b). These may be considered as the fundamental events that are the building blocks from which more complex Ca^{2+} signals are constructed. These single-channel events are rare and the more usual event is larger, resulting from the coordinated opening of clusters of $InsP_3Rs$ or RYRs, known as puffs or sparks, respectively (FIG. 5c). Sparks were first described in cardiac cells[36] where they represent Ca^{2+} signals from a group of RYR2 channels opening in concert. The puffs recorded in either *Xenopus* oocytes[37,38] or HeLa cells[39] have diverse amplitudes indicating that there are either variable numbers of $InsP_3Rs$ within each cluster or variable numbers of channels open within an individual cluster.

Ca^{2+} waves. Sparks and puffs contribute to intracellular Ca^{2+} signals, such as the Ca^{2+} waves that sweep through cells (FIG. 5d). For waves to occur, most of the $InsP_3Rs$ and the RYRs must be sufficiently sensitive to Ca^{2+} to respond to each other through the process of Ca^{2+}-induced Ca^{2+} release. One group of channels releases Ca^{2+}, which then diffuses to neighbouring receptors to

Box 1 | A Ca^{2+} nexus — crosstalk between signalling pathways

The Ca^{2+} signalling toolkit interacts with many other signalling pathways. The interactions are reciprocal in nature in that information flows to and from the other signalling pathways. It is difficult to make any generalizations and each set of interactions has to be treated separately:



Ca^{2+}–cyclic AMP interactions (1)
The function of the cyclic AMP and Ca^{2+} signalling systems are intimately linked. Some of the adenylyl cyclase isomers are activated by Ca^{2+} whereas others are inhibited. Ca^{2+} can also stimulate some of the cAMP phosphodiesterases (cAMP PDE). Changes in the level of cAMP can feed back to influence the level of Ca^{2+} by acting on both Ca^{2+} channels and pumps. In cardiac and skeletal muscle, the activity of the L-type Ca^{2+} channel is enhanced by cAMP.

Ca^{2+}–NO interactions (2)
An important function of Ca^{2+} is to activate nitric oxide (NO) synthase to generate NO, which functions as a local hormone to regulate the activity of neighbouring cells. The NO activates guanylyl cyclase to produce cyclic GMP, which feeds back to influence the activity of Ca^{2+} channels and pumps. For example, smooth muscle cells relax when cGMP phosphorylates an inositol-1,4,5-trisphosphate receptor ($InsP_3R$)-associated cGMP kinase substrate that reduces inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$)-induced Ca^{2+} release[108].

Ca^{2+}–phosphatidylinositol-3-OH kinase interaction (3)
The ubiquitous phosphatidylinositol-3-OH-kinase (PI(3)K) signalling pathway has many functions in cells, which are mediated by the lipid second messenger phosphatidylinositol-3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$). One function of $PtdIns(3,4,5)P_3$ is to activate the non-receptor tyrosine kinase Btk that then phosphorylates and activates phospholipase Cγ1 (PLCγ1)[81]. The tumour suppressor PTEN, a 3-phosphatase that lowers the level of $PtdIns(3,4,5)P_3$, reduces both the level of $Ins(1,4,5)P_3$ and the influx of external Ca^{2+} (REF. 109).

Ca^{2+} feedback interactions (4)
There are numerous feedback interactions within the Ca^{2+} signalling pathway whereby Ca^{2+} can modulate its own activity. For example, Ca^{2+} can activate phospholipase Cδ1 (PLCδ1) to increase the level of $Ins(1,4,5)P_3$. Conversely, it can lower the level of this second messenger by stimulating the $Ins(1,4,5)P_3$ kinase to produce $Ins(1,3,4,5)P_4$. Finally, Ca^{2+} can exert profound effects on the Ca^{2+} channels and pumps.

Ca^{2+}–mitogen-activated protein kinase interaction (5)
Ca^{2+} can interact with the mitogen-activated protein kinase (MAPK) signalling pathway by activating a proline-rich tyrosine kinase 2 (PYK2), which then acts through the small GTPase Ras to induce the MAPK cascade[110]. For example, the growth of smooth muscle cells may depend on the Ca^{2+}-dependent activation of the MAPK pathway[111]. A more indirect method may depend on Ca^{2+} stimulating a metalloproteinase to release epidermal growth factor (EGF) from a precursor, as seems to occur in prostate carcinoma cells[112].

excite further release, therefore setting up the regenerative process. When gap junctions connect cells, these intracellular waves can spread to neighbouring cells, to create intercellular waves (FIG. 5e) capable of coordinating the activity of many cells[40] (see Sanderson lab page). For example, intercellular Ca^{2+} waves in the lung epithelium stimulate the beat frequency of the cilia that expel inhaled contaminants from the airways[40]. Intercellular waves have also been recorded in the intact liver[41] and in insect salivary glands[42]. Just how the wave traverses the gap junction is a matter of considerable debate. For the two examples given above, Ca^{2+} seems to be the signal that crosses the gap junction[41,42] (FIG. 5e). In the case of the lung epithelium[40], the messenger seems to be $Ins(1,4,5)P_3$ and there is also evidence for the presence of extracellular messengers such as ATP in other cell types.

In addition to creating global responses, these elementary events have signalling functions within highly localized cellular domains. A classic example is the process of exocytosis at synaptic endings where N- or P/Q-type VOCs create a local pulse of Ca^{2+} to activate synaptotagmin and trigger vesicle release (FIG. 4b). Ca^{2+} released through $InsP_3Rs$[43] can stimulate exocytosis in various secretory cells. In adrenal glomerulosa cells, T-type Ca^{2+} channels in the plasma membrane seem to have a 'Ca^{2+} pipeline', enabling them to feed Ca^{2+} directly into the mitochondria to stimulate steroidogenesis[44]. Sparks that activate Ca^{2+}-sensitive K^+ channels to trigger membrane hyperpolarization control the excitability of neurons and smooth muscle cells. In HeLa cells, Ca^{2+} puffs are concen-

trated around the nucleus where they feed Ca^{2+} directly into the nucleoplasm[45]. Finally, as mentioned earlier, the mitochondria located near the sites of elementary events take up Ca^{2+} rapidly and this stimulates mitochondrial metabolism to increase ATP formation (FIG. 2).

Temporal aspects of Ca^{2+} signalling

Ca^{2+} signals are usually presented as brief spikes. In some cases, individual spikes are sufficient to trigger a cellular response such as the contraction of skeletal muscle (FIG. 4a) or neurotransmitter release(FIG. 4b). When longer periods of signalling are necessary, spikes are repeated to give waves with different frequencies, ranging from 1–60 seconds (in pancreas and liver) to 24 hours (in the Ca^{2+} oscillator that initiates mitosis during the cell cycle).

Cells often respond to changes in stimulus intensity by varying the frequency of Ca^{2+} waves. To use such a frequency-modulated signalling system, cells have evolved sophisticated 'molecular machines' for decoding frequency-encoded Ca^{2+} signals. The two Ca^{2+}-sensitive proteins that seem to decode wave frequency are Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII)[46] and protein kinase C[47]. Frequency coding is used to control processes such as liver metabolism, smooth muscle contractility and differential gene transcription, especially in developing systems. For example, Ca^{2+} spikes can initiate gene expression more effectively than a steadily maintained level of the same average $[Ca^{2+}]$[48]. A low frequency of spiking activates the transcription factor NF-κB, whereas higher frequencies are necessary to switch on the transcription factor NF-AT[49].

Ca^{2+} may also be important in entraining the circadian clock in the suprachiasmatic nucleus. This can be reset by releasing Ca^{2+} from either the RYR-sensitive[50] or the $InsP_3R$-sensitive[51] stores.

The universality of Ca^{2+} signalling

Ca^{2+} signalling is used throughout the life history of an organism. Life begins with a surge of Ca^{2+} at fertilization and this versatile system is then used repeatedly to control many processes during development and in adult life. One of the fascinating aspects of Ca^{2+} is that it plays a direct role in controlling the transcriptional events that select out the types of Ca^{2+} signalling systems that are expressed in specific cell types. Such a role for Ca^{2+} in differential gene transcription is still in its infancy but is rapidly developing into an active area of research.

Fertilization. During fertilization, mammalian eggs generate regular Ca^{2+} spikes that persist for about two hours and initiate development. Each spike is a global signal that sweeps through the egg, driven by Ca^{2+} release from $InsP_3Rs$[52]. The increase in $Ins(1,4,5)P_3$ necessary to support such waves may be generated by a unique phospholipase C that is transferred into the egg by the sperm at fertilization[53]. This regular pattern of Ca^{2+} spiking stimulates CAMKII, which then acts through CDC25 to dephosphorylate the enzyme cyclin-dependent kinase 1 (CDK1), resulting in cyclin B activation and the completion of meiosis. The male and female nuclei now fuse, marking the end of the fertilization-induced Ca^{2+} spikes.

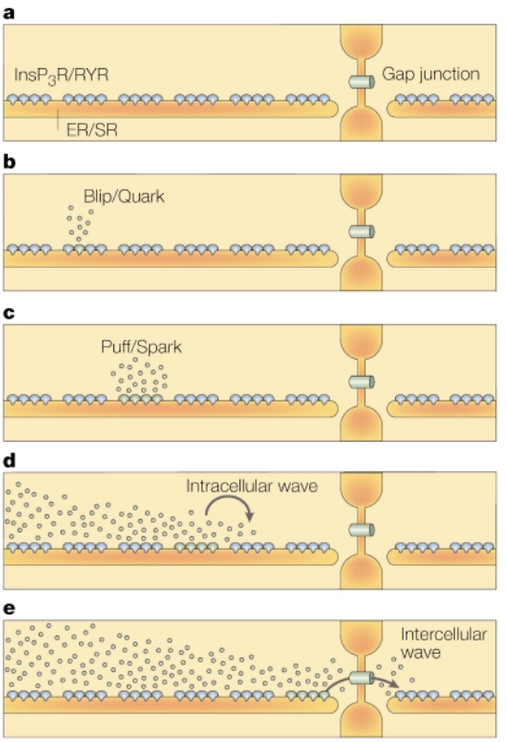


Figure 5 | **The spatial organization of Ca^{2+} release from internal stores. a** | Inositol-1,4,5-trisphosphate receptors ($InsP_3Rs$) and ryanodine receptors (RYRs) are distributed over the surface of the endoplasmic and/or sarcoplasmic reticulum (ER/SR). **b** | In response to weak stimuli, individual channels open to give either blips ($InsP_3Rs$) or quarks (RYRs). **c** | At higher levels of stimulation, groups of $InsP_3Rs$ or RYRs open together to produce puffs or sparks, respectively. **d** | When cells are fully excitable, the elementary events depicted in (c) can excite neighbouring receptors through a process of Ca^{2+}-induced Ca^{2+} release to set up an intracellular wave. **e** | When gap junctions connect cells, waves can travel from one cell to the next to set up an intercellular wave.

Animated online

The Ca^{2+} signalling system has completed its first task in the young embryo but it is soon called into play again to trigger the mitotic events at the end of the first cell cycle.

The cell cycle consists of an orderly programme of events controlled by two-linked oscillators — a cell cycle oscillator and a Ca^{2+} oscillator[54]. The former depends on the synthesis and periodic proteolysis of various cyclins at specific points during the cell cycle. The Ca^{2+} oscillator, based on the periodic release of stored Ca^{2+}, is responsible for initiating specific events associated with mitosis. As the embryo approaches mitosis, a series of spontaneous Ca^{2+} transients trigger specific events such as nuclear envelope breakdown[55] and cell cleavage[56]. In the case of *Xenopus* oocytes, the Ca^{2+} oscillator persists for at least 5 hours with a periodicity of 30 minutes, which exactly coincides with the length of each cell cycle[57,58]. Just what drives the Ca^{2+} oscillator is a mystery but it seems to depend on the periodic elevation of $Ins(1,4,5)P_3$ (REFS 59–61).

CDC25
A dual-specificity threonine/tyrosine phosphatase required for progression of the cell cycle. It dephosphorylates and activates cyclin–CDK complexes.

SOMITES
A series of paired blocks of cells that form during early vertebrate development and give rise to the backbone and body muscle.

Embryonic pattern formation. During the next stage of development, the zygote proliferates rapidly to produce large groups of cells and the Ca^{2+} signalling system controls the specification processes responsible for pattern formation and cell differentiation. The orchestrated sequences of Ca^{2+} signals that occur during developmental processes (for example, gastrulation, formation of SOMITES and neural induction) have been documented in the zebrafish embryo[62–64]. There are indications that $Ins(1,4,5)P_3$ and Ca^{2+} act during specification of the dorsoventral axis. The concentration of $Ins(1,4,5)P_3$ increases significantly during development of the dorsoventral axis in both *Xenopus*[65,66] and zebrafish[67]. Imaging studies have revealed a standing gradient with a prolonged elevation of Ca^{2+} in the ventral region of early zebrafish embryos[62]. Dorsoventral gradients of Ca^{2+} have also been recorded in early *Drosophila* embryos[68]. The proposed gradient of $Ins(1,4,5)P_3/Ca^{2+}$, being high in the ventral region and low in the dorsal region, may specify pattern formation in the developing embryo. Consistent with the existence of such a gradient, the activity of CAMKII is higher in the ventral region[69]. Procedures designed to disrupt this $Ins(1,4,5)P_3/Ca^{2+}$ gradient, such as the injection of an antibody that inhibits $Ins(1,4,5)P_3$-induced Ca^{2+} release, can respecify the axis in *Xenopus* embryos[70] — as can altering the CAMKII activity gradient[69]. The dorsoventral axis may therefore be determined by a gradient in the activity of the Ca^{2+} signalling pathway.

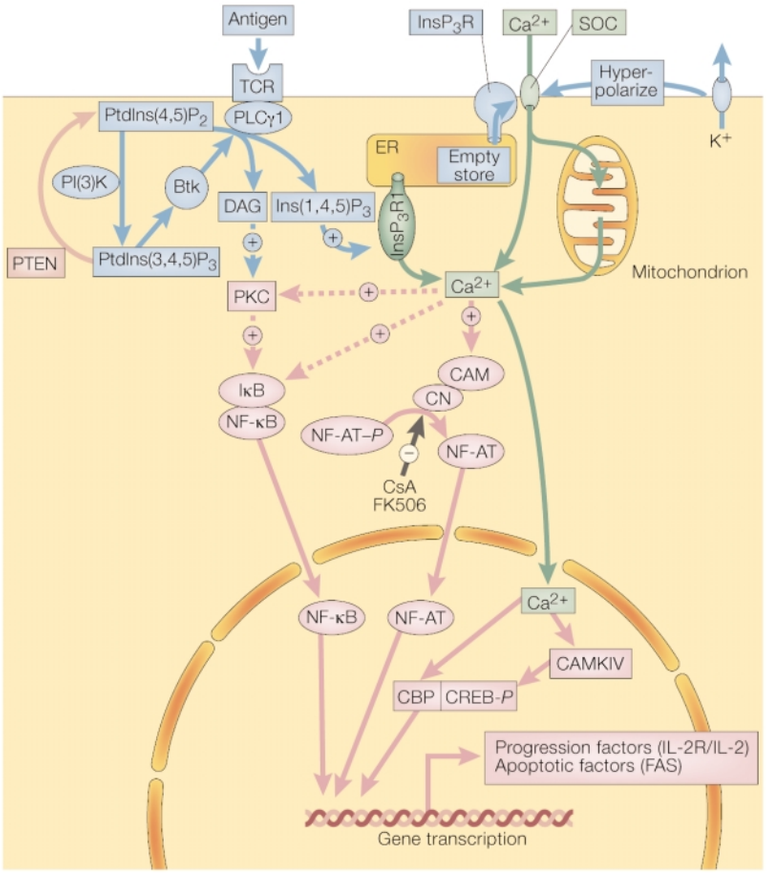


Figure 6 | **Ca^{2+} function during lymphocyte proliferation.** Antigen interacts with the T-cell receptor (TCR) to recruit phospholipase Cγ1 (PLCγ1) to generate both diacylglycerol (DAG) and inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$). The production of $Ins(1,4,5)P_3$ is maintained by the phosphatidylinositol-3-OH kinase (PI(3)K) pathway, which generates phosphatidylinositol-3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$). This stimulates the non-receptor tyrosine kinase Btk which, in turn, phosphorylates and activates phospholipase Cγ1 (PLCγ1). $Ins(1,4,5)P_3$ releases Ca^{2+} from the endoplasmic reticulum (ER) through the type 1 $Ins(1,4,5)P_3$ receptor ($InsP_3R1$). Emptying of this store activates store-operated channels (SOCs) (FIG. 3b). The latter are kept open by potassium channels, which hyperpolarize the membrane, and by mitochondria, which reduce the negative feedback effect of Ca^{2+} on the SOCs. Ca^{2+} initiates the proliferative response by stimulating various transcription factors such as NF-κB, NF-AT and CREB. The stimulatory action of Ca^{2+} on the calmodulin (CAM)–calcineurin (CN) complex that dephosphorylates NF-AT is inhibited by the immunosuppressants cyclosporin A (CsA) and FK506. ($PtdIns(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate; PKC, protein kinase C; IκB, inhibitor of NF-κB; *P*, phosphate)

Cell differentiation. Later in development, Ca^{2+} is involved in inducing the differentiation of individual cells. In contrast to the standing gradient of Ca^{2+} responsible for axis specification, Ca^{2+} spiking induces cell differentiation, at least in neural and muscle cells[71]. In *Xenopus*, spontaneous Ca^{2+} spikes produced by RYRs during a narrow developmental window[72] drive the differentiation of myocytes into somites. The development of neurons is also regulated by Ca^{2+} spikes that control processes such as the expression of specific neurotransmitters and channels[73,74], the behaviour of growth cones[75] and the establishment of the specific connections within neural circuits[76].

Differentiation culminates with the emergence of different cell types specialized for specific functions, some of which were described earlier (FIG. 4). A key element of the differentiation process, therefore, is to install those components of the Ca^{2+} signalling toolkit that each specialized cell needs to fulfil its particular function.

Cell proliferation. Once cells have been assigned specific jobs, they usually stop proliferating. In many cases, however, such differentiated cells maintain the option of re-entering the cell cycle and this usually occurs in response to growth factors. Ca^{2+} is one of the key regulators of cell proliferation, functioning in conjunction with other signalling pathways such as those regulated through MAPK and phosphatidylinositol-3-OH kinase (PI(3)K) (BOX 1)[77,78]. The function of Ca^{2+} is well illustrated in lymphocytes responding to antigen (FIG. 6). Here, the 'growth factor' is the antigen that binds to the T-cell receptor to initiate the assembly of a supramolecular activation cluster[79] containing scaffolding and signal transducing elements. One of the latter is phospholipase Cγ1 (PLCγ1), which produces both DAG and $Ins(1,4,5)P_3$ for a period of at least two hours to activate proliferation. The need for such prolonged periods of signalling to initiate proliferation is not unique to lymphocytes. For example, stimulation of Chinese hamster ovary cell proliferation by gastrin correlated with its ability to maintain an oscillatory Ca^{2+} signal for at least two hours. Conversely, carbachol, acting through muscarinic M3 receptors, gave a short-lived Ca^{2+} response and failed to stimulate proliferation[80].

As Ca^{2+} stores have a limited capacity, such a prolonged period of Ins(1,4,5)P_3-induced Ca^{2+} signalling depends on the influx of external Ca^{2+} through SOCs (FIG. 6), controlled by several modulatory mechanisms. The first is an example of the crosstalk between signalling pathways (BOX 1) and concerns the ability of the PI(3)K signalling pathway to stimulate PLCγ1 to maintain the supply of Ins(1,4,5)P_3 (REF. 81). The second is the activation of K^+ channels that serve to hyperpolarize the membrane to enhance the entry of external Ca^{2+} (FIG. 6)[82]. Finally, SOCs are prone to Ca^{2+}-induced inhibition but this negative feedback pathway is reduced by mitochondria, which soak up the Ca^{2+} entering through the channels[83]. One possibility is that the mitochondria may then redistribute the Ca^{2+} by releasing it deeper within the cell[83].

Transcription factor activation. The main function of Ca^{2+} in controlling cell proliferation is to activate transcription factors either in the cytoplasm (NF-AT, NF-κB) or within the nucleus (CREB) (FIG. 6). The function of Ca^{2+} during stimulation of gene transcription in lymphocytes (FIG. 6) is similar to that in neurons during learning (FIG. 4b). One action of Ca^{2+} is to stimulate the Ca^{2+}-sensitive protein phosphatase calcineurin to dephosphorylate NF-AT, which then enters the nucleus[84]. As soon as Ca^{2+} signalling stops, kinases in the nucleus rapidly phosphorylate NF-AT, which then leaves the nucleus, and transcription of NF-AT-responsive genes ceases. The prolonged period of Ca^{2+} signalling that is required to induce proliferation is therefore necessary to maintain NF-AT in its active form. Interrupting this signalling cascade at various points decreases gene transcription and cell proliferation. Transcription is inhibited in mutants with defective SOCs that cannot sustain Ca^{2+} signalling[85]. Likewise, the immunosuppressants cyclosporin A and FK506 prevent transcription by inhibiting the action of calcineurin (FIG. 6). An increase in Ca^{2+} is one of the signals that can trigger the proteolysis of the inhibitory IκB subunit, allowing the active NF-κB subunit to enter the nucleus.

CREB, in contrast to the factors discussed above, is a nuclear Ca^{2+}-responsive transcription factor, which is phosphorylated by CAMKII and CAMKIV. In addition, Ca^{2+} acting within the nucleus is also responsible for stimulating the Ca^{2+}sensitive transcriptional co-activator CREB-binding protein (CBP)[86,87]. A CAM inhibitory peptide targeted to the nucleus can block DNA synthesis and cell-cycle progression, emphasizing the importance of a nuclear Ca^{2+} signal for cell proliferation[88]. These Ca^{2+}-sensitive transcription factors activate numerous target genes; some code for progression factors such as the interleukin 2 system that is responsible for switching on DNA synthesis, whereas others produce components such as Fas and the Fas ligand that trigger apoptosis. So Ca^{2+} is central in setting up the signalling systems that enable cells to decide whether to grow or to die.

Ca^{2+} disregulation and cancer. Phospholipase C has been referred to as a malignancy-linked signal transducing enzyme[89] and its overexpression will promote transformation and tumorigenesis in NIH3T3 cells[90]. The activity of the enzyme phosphatidylinositol-4-OH kinase, which catalyses production of the precursor that is hydrolysed to form Ins(1,4,5)P_3, is greatly enhanced in certain cancer cells[91]. Several drugs that block Ca^{2+} entry can retard the growth of human melanoma, lung and colon carcinoma cells[92], vascular smooth muscle cells[93] and human prostate cancer cells[94]. One inhibitor, carboxy-amidotriazole, has been used in clinical trials to control refractory cancers[95]. Finally, the auxiliary subunit of a voltage-dependent Ca^{2+} channel $\alpha_2\delta$ is a potential tumour suppressor for several cancers[96].

Calcium and apoptosis. The function of Ca^{2+} in apoptosis is an enormously complex subject involving interplay between many systems including the SPHINGOMYELIN SIGNALLING PATHWAY, the redox system, the STRESS-ACTIVATED PROTEIN KINASE cascade and the Ca^{2+} signalling pathway. In the last case, one function of Ca^{2+} is to control the expression of the apoptotic signalling components such as the Fas system described above. In addition, Ca^{2+} can also induce apoptosis in response to various pathological conditions and this often depends on an interplay between the mitochondria and the ER[97]. As described earlier (FIG. 2), there is a continuous ebb and flow of Ca^{2+} between these two organelles. There are indications that pro-apoptotic stimuli such as ceramide can influence how mitochondria respond to this periodic flux of Ca^{2+}. The Ca^{2+} signals produced by Ins(1,4,5)P_3 are handled normally, but when superimposed on a background of ceramide they induce apoptosis through formation of the PTP[98]. The latter usually forms when the mitochondria become overloaded with Ca^{2+} and so release cytochrome *c* (see above).

The apoptosis regulatory proteins that function either as death antagonists (Bcl-2 and Bcl-X_L) or death agonists (Bax, Bak and Bad), may exert some of their actions by interfering with the Ca^{2+} dynamics of these two organelles. For example, Bcl-2 is located both in the ER and in mitochondria. Both Bax and Bad accelerate opening of the voltage-dependent anion channel, which is part of the permeability transition pore (PTP), and so contribute to the release of cytochrome *c*[99].Conversely, Bcl-2 and Bcl-X_L seem to block Ca^{2+}-induced apoptosis[100–101]. They enable the mitochondria to cope with large loads of Ca^{2+} (REFS 102–104). The function of Bcl-2 on the ER is uncertain. There are reports that Bcl-2 enhances the store of Ca^{2+} (REF. 104), perhaps by upregulating SERCA gene expression[105]. However, other reports indicate that it increases membrane permeability, thereby reducing the concentration of Ca^{2+} in the ER[106,107]. An important consequence of having less Ca^{2+} in the ER is that the amount of Ca^{2+} being released during signalling is reduced[106,107], which also decreases the amount taken up by the mitochondria. The anti-apoptotic action of Ca^{2+} may therefore depend on this reduction of the amount of Ca^{2+} circulating within the ER/mitochondrial system.

From universality to individuality

Cells have access to an extensive Ca^{2+} signalling toolkit,

SPHINGOMYELIN SIGNALLING
Several metabolites of sphingomyelin affect apoptosis through poorly undertood mechanisms: ceramide and sphingomyelin are generally proapoptotic whereas sphingosine 1-phosphate is generally antiapoptotic.

STRESS-ACTIVATED PROTEIN KINASES
Members of the mitogen-activated protein kinase (MAPK) family that are activated by stress, including c-Jun N-terminal kinase (JNK) and p38 MAPK.

from which they can assemble signalling systems with variable spatial and temporal properties. Imaging techniques have enabled us to characterize a physiological toolkit in the form of the elementary events that are used to create either localized signals, or the coordinated activity of these events to produce global signals in the form of intracellular and intercellular waves. Now that the molecular and physiological mechanisms have been identified, the new challenge is to determine how this versatile Ca^{2+} signalling system functions in specific cellular processes. The universality of this signalling system is evident in its emerging function during various developmental processes such as axis specification, pattern formation and cellular differentiation. In the developing nervous system, for example, patterns of Ca^{2+} spikes regulate axonal growth and neuronal connectivity. The process of differentiation is particularly interesting because Ca^{2+} acts directly in setting up these Ca^{2+} signalling pathways. An important problem for the future, therefore, is to understand how Ca^{2+} functions to select out those components of the molecular toolkit that are uniquely expressed in each cell type.

Links

DATABASE LINKS

α_{1S} | α_{1C} | $\alpha_2\delta$ | Bad | Bak1 | β-amyloid precursor protein| Bax | Bcl-2 | Bcl-X_L | calbindin-D_{28K} | Calcineurin | calmodulin | calretinin | CAMKII | CAMKIV | CBP | cholecystokinin receptors | CREB | Cytochrome *c* | EF hands | FAS | FAS ligand | gastrin | IκB | Inositol-1,4,5-trisphosphate receptors | interleukin-2 | MAPK | muscarinic acetylcholine receptors | muscarinic M3 receptors | Na^+/Ca^{2+} exchanger | NF-AT | NF-κB | parvalbumin | phosphorylase kinase | PKC | presenilins | PI(3)K | PLCγ1 | Phosphatidylinositol-4-OH kinase | ryanodine receptors | synaptotagmin | troponin C

FURTHER INFORMATION

Inositol signalling | M. J. Sanderson's lab page

ELS LINKS

Calcium signalling and regulation of cell function| Calcium and neurotransmitter release | Calcium channel diversity

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