Calcium — a life and death signal

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

Almost everything that we do is controlled by Ca^{2+} — how we move, how our hearts beat and how our brains process information and store memories. To do all of this, Ca^{2+} acts as an intracellular messenger, relaying information within cells to regulate their activity. For example, Ca^{2+} triggers life at fertilization, and controls the development and differentiation of cells into specialized types. It mediates the subsequent activity of these cells and, finally, is invariably involved in cell death. To coordinate all of these functions, Ca^{2+} signals need to be flexible yet precisely regulated. This incredible versatility arises through the use of a Ca^{2+}-signalling 'tool kit', whereby the ion can act in the various contexts of space, time and amplitude. Different cell types then select combinations of Ca^{2+} signals with the precise parameters to fit their physiology.

Space

At the cellular level, Ca^{2+} is derived from two sources — external and internal. It can enter from outside the cell by passing through channels that span the external barrier, plasma membrane. Or it can be released from internal Ca^{2+} stores, through channels in the endoplasmic or sarcoplasmic reticulum[1,2], membranous networks that are also the site of protein synthesis and transport.

Improvements in imaging technology mean that cell physiologists can now see how the Ca^{2+} signals are generated. When a Ca^{2+} channel opens, a highly concentrated plume of Ca^{2+} forms around its mouth and then dissipates rapidly by diffusion after the channel has closed. Such localized signals, which can originate from channels in the plasma membrane or on the internal stores, represent the elementary events — the basic building blocks of Ca^{2+} signalling (Fig. 1a)[3–5]. The spatio-temporal properties of these elementary events, such as Ca^{2+} sparks and Ca^{2+} puffs, differ depending on the nature and location of the channels. By characterizing these signals, we can discover how the Ca^{2+}-signalling repertoire is elaborated. Essentially, these elementary signals have two functions. They can either activate highly localized cellular processes in the immediate vicinity of the channels (Fig. 1a) or, by recruiting channels throughout the cell, they can activate processes at a global level (Fig. 1b, c).

The subcellular location of Ca^{2+} channels is crucial for targeting elementary signals to different cellular processes. In smooth muscle, for example, Ca^{2+} sparks that arise locally, near the plasma membrane, activate potassium (K^+) channels (Fig. 1a), causing the muscle to relax[6,7]. But when elementary-release events deeper in the cell are coordinated to create a global Ca^{2+} signal, the muscle contracts. This is a striking example of how spatial organization enables Ca^{2+} to activate opposing cellular responses in the same cell.

For sites of elementary Ca^{2+} release to produce global responses, the individual channels must communicate with each other, to set up Ca^{2+} waves (Fig. 1b). If cells are connected, such intracellular waves can spread into neighbouring cells and become intercellular waves to coordinate cellular responses within a tissue (Fig. 1c).

Time

One of the paradoxes surrounding Ca^{2+} is that it is a signal for both life and death — although elevations in Ca^{2+} are necessary for it to act as a signal, prolonged increases in the concentration of Ca^{2+} can be lethal. Cells avoid death either by using low-amplitude Ca^{2+} signals or, more usually, by delivering the signals as brief 'transients'. These principles apply to both elementary and global signals. Single transients are used to activate certain cellular processes, such as secretion of cellular material in membrane-

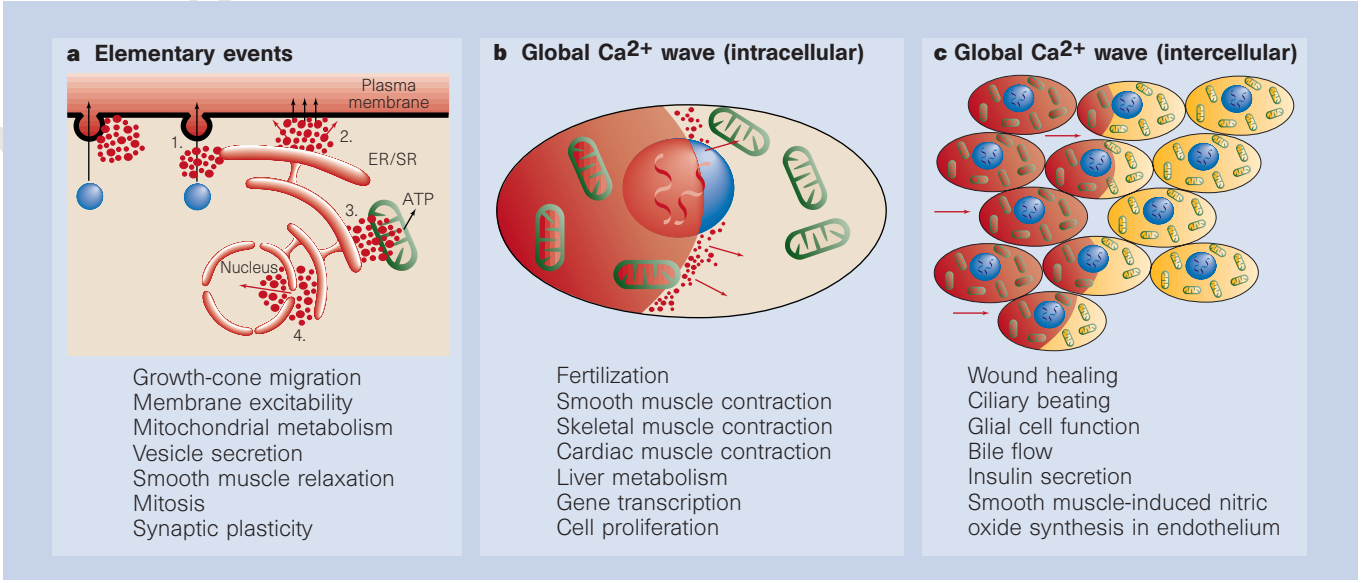


Figure 1 **Spatial aspects of Ca^{2+} signalling.** a, **Elementary events (red) result from the entry of external Ca^{2+} across the plasma membrane or release from internal stores in the endoplasmic or sarcoplasmic reticulum (ER/SR). They generate localized concentrations of Ca^{2+} that can activate many processes, including export of cellular material (1), opening of K^+ channels (2) and metabolism in the mitochondria (3). The Ca^{2+} signals can also enter the nucleus (4). All of these processes respond to the very high concentrations of Ca^{2+} that build up within the sub-domain of the elementary events.** b, **Global Ca^{2+} signals are produced by coordinating the activity of elementary events to produce a Ca^{2+} wave that spreads throughout the cell.** c, **The activity of neighbouring cells within a tissue can be coordinated by an intercellular wave that spreads from one cell to the next.**

bound vesicles, or muscle contraction. However, when information has to be relayed over longer time periods, cells use repetitive signals known as Ca^{2+} oscillations. Both the elementary events and the global signals can oscillate, but they have widely different periods. For example, whereas the period of elementary Ca^{2+} sparks in arterial smooth muscle is 0.1–0.5 seconds, it is 10–60 seconds for global waves in liver cells, 1–35 minutes for Ca^{2+} waves in human eggs after fertilization, and 10–20 hours for the spontaneous Ca^{2+} transients that control cell division.

Cells use frequency modulation (FM) to vary the intensity and nature of the physiological output. For instance, arteries can be made to dilate by increasing the frequency of Ca^{2+} sparks, which cause the smooth muscle lining the arteries to relax[6,7]. And by varying the frequency of global Ca^{2+} signals, different genes can be activated[8]. To use FM signalling, cells have developed decoders that respond to the frequency and longevity of the Ca^{2+} signals. Probably the best-known example is an enzyme called calmodulin-dependent protein kinase II, which is found in both animal and plant cells and which regulates other enzymes that rely on Ca^{2+}. It works by 'counting' Ca^{2+} transients[9] and varying its activity accordingly. The enzyme is composed of many identical subunits, and these are activated to varying degrees depending on the frequency of the Ca^{2+} oscillations.

Amplitude

Information can also be encoded in the amplitude of Ca^{2+} signals. Such amplitude-modulated (AM) signalling is generally considered to be less reliable than that based on frequency, owing to the difficulties of detecting small Ca^{2+} changes above the background level. However, it has been shown that cells can interpret modest changes in the concentration of Ca^{2+}. For example, different genes can be activated by varying the amplitude of Ca^{2+} signals[10].

Fertilization and development

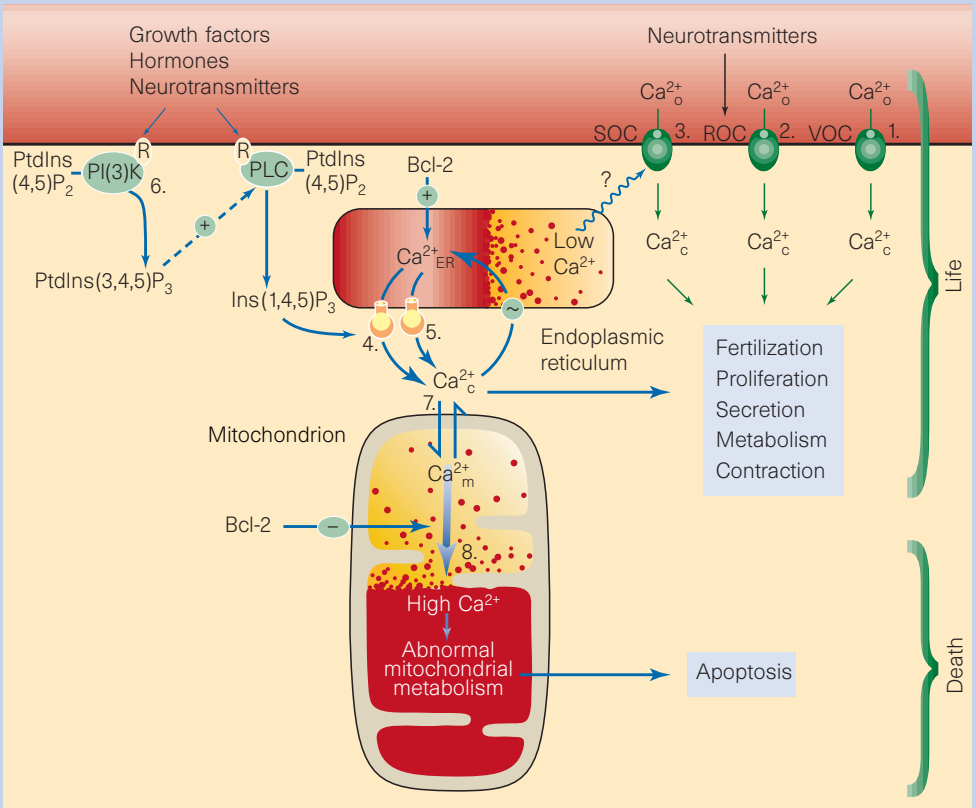
In mammals, life begins at fertilization when the sperm interacts with the egg to trigger a Ca^{2+} oscillation that persists for several hours. This prolonged period of repetitive Ca^{2+} pulses triggers the developmental programme by stimulating the enzymatic machinery involved in the cell-division cycle. There are no further changes in Ca^{2+} until the one-cell embryo is ready to divide, when a spontaneous Ca^{2+} transient triggers cleavage to form two daughter cells. There are indications that this orderly programme may be controlled by two distinct oscillators — Ca^{2+} signals and oscillating levels of proteins involved in the cell-division cycle. The Ca^{2+} oscillator seems to be the main mechanism, because it persists when dissociated from the cell-cycle oscillator[11]. Just what drives this Ca^{2+} oscillator, with a period of 10–20 hours, is a mystery, but it may depend on periodic increases in the level of a diffusible intracellular messenger called inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$)

Box 1 Basic mechanisms of Ca^{2+} signalling

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

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

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



with each division[12]. In many cells, hormones and growth factors activate the enzyme phospholipase C, which catalyses the production of $Ins(1,4,5)P_3$ from the membrane lipid phosphatidylinositol 4,5-bisphosphate (Box 1).

As embryos grow and groups of cells differentiate to perform specialized functions, Ca^{2+} signalling contributes to body polarity and pattern formation. For instance, in amphibians and zebrafish it is thought to help specify which cells will form structures at the top (dorsal) or the bottom (ventral) part of the embryo. When this dorso-ventral axis is being specified, there is a spontaneous increase in the level of $Ins(1,4,5)P_3$, possibly in the form of a gradient ranging from low levels on the dorsal side to high levels on the ventral side[13]. Indeed, if an antibody is added to prevent the cells from detecting $Ins(1,4,5)P_3$, ventral cells are converted to dorsal cells[14]. The increase in $Ins(1,4,5)P_3$, in zebrafish at least, coincides with the appearance of Ca^{2+} spikes restricted to a small group of cells. These spikes may be involved in dorso-ventral specification, because their frequencies are sensitive to the expression of developmental genes[15].

Later in development, when the final form of the embryo is emerging, Ca^{2+} signals are again used to control the differentiation of specific cell types. For example, spontaneous Ca^{2+} transients control assembly of the contractile machinery during the formation of muscle[16]. In development of the heart, the Ca^{2+}-dependent 'nuclear factor of activated T cells' (NF-AT) helps to form the cardiac septum and valves[17]. Ca^{2+} is also involved in development of the nervous system. Spontaneous Ca^{2+} transients with different frequencies are responsible for various aspects of differentiation in developing amphibian neurons[18]. In the main body of the neuron, the natural frequency of two to four Ca^{2+} transients per hour is optimal for maturation of K^+ channels. But growth of projections called neurites, out in the extremities of the neuron, is tuned to higher Ca^{2+} signal frequencies. Ca^{2+} signals also help to control the migration of neuronal cells[19], and the initial wiring-up processes that produce the complex neuronal circuits of the developing brain[20].

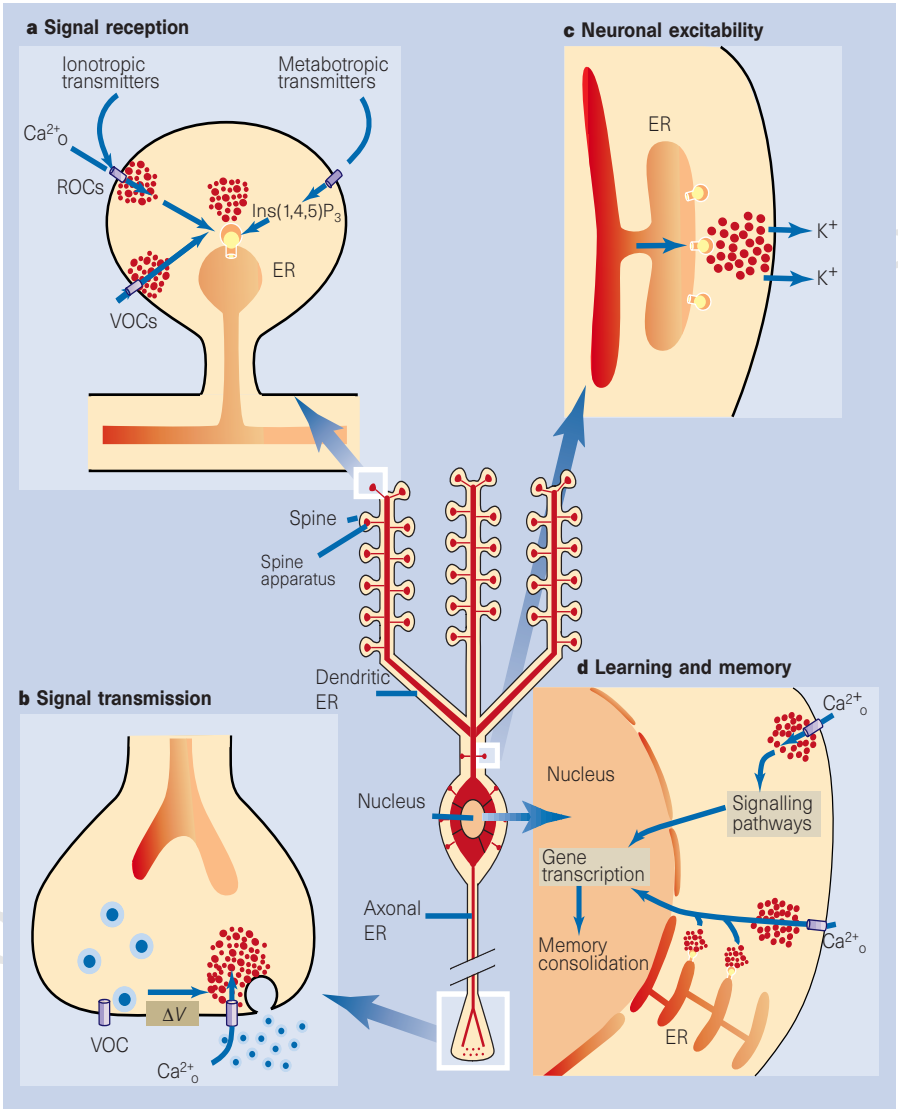


Figure 2 **Compartmentalization of Ca^{2+} signals in neurons.** a, **External Ca^{2+} enters through receptor-operated channels (ROCs) or voltage-operated channels (VOCs), and the signal may be amplified by activating receptors for $Ins(1,4,5)P_3$ on the spine apparatus. By integrating separate input signals, these receptors could act as coincidence detectors.** b, **In response to electrical signals (ΔV), the entry of Ca^{2+} through VOCs stimulates neurotransmitter release. Such signals, arising through Ca^{2+} entry, are usually very rapid in onset and decline.** c, **Localized Ca^{2+} release from the endoplasmic reticulum (ER) opens K^+ channels to modulate neuronal excitability.** d, **Proposed role of Ca^{2+} in memory consolidation. Entry of external Ca^{2+} can act locally by co-opting other signalling pathways, or it can act globally by flooding directly into the nucleus. Such a global signal might be amplified by release of Ca^{2+} from the internal stores.**

Cell proliferation

A prolonged period of Ca^{2+} signalling — similar to that in fertilization — is an important growth signal for many cells[21]. Alterations in Ca^{2+} signalling can underlie defects in cell growth and are implicated in some cancers. Conversely, cell proliferation can be reduced by interfering with the generation or action of Ca^{2+} signals. Because internal Ca^{2+} stores are finite, prolonged bouts of signalling depend on the influx of external Ca^{2+} through so-called store-operated Ca^{2+} channels (SOCs) in the plasma membrane (Box 1). These SOCs might be the target of a Ca^{2+}-influx inhibitor, which has been found, in clinical trials, to slow down the growth of certain aggressive cancer cells[22].

Ca^{2+} is also involved in the proliferation of immune cells (lymphocytes) in response to foreign antigens. Both T and B lymphocytes detect antigens through complex receptors on their surface. When a foreign molecule binds to an antigen receptor, $Ins(1,4,5)P_3$ is produced, stimulating the release of Ca^{2+} from internal stores. Once these stores are empty, entry of external Ca^{2+} is activated through the SOCs (Box 1)[21], allowing lymphocytes to maintain a prolonged increase of Ca^{2+}. This increase, which often occurs as a series of regular Ca^{2+} oscillations, activates factors such as NF-AT, which enter the nucleus and cause genes to be turned on. The importance of Ca^{2+} signalling in lymphocyte activation is highlighted by the fact that immunosuppressant drugs, such as cyclosporin, work by inhibiting the Ca^{2+}-dependent activation of NF-AT.

Neurons

Neurons provide an excellent example of how a combination of elementary and global Ca^{2+} signals have been adapted to regulate a range of processes in a single cell[23]. For example, Ca^{2+} is pivotal in receiving and transmitting neuronal signals, as well as in regulating

excitability and the changes that underlie learning and memory (Fig. 2). Ca^{2+} influx into the neuron has long been regarded as the main source of Ca^{2+} signals, but the significance of Ca^{2+} release from internal stores such as the endoplasmic reticulum is now becoming apparent. This continuous network extends throughout the neuron, and can be considered as a neuron within a neuron — it has properties that resemble those of the plasma membrane[23]. There are many examples of how the plasma membrane and endoplasmic reticulum interact to produce spatially restricted signals in some regions, whereas in others they create global Ca^{2+} signals over large parts of the neuron.

Neurons have four main regions — a dendritic tree containing the spines that receive inputs from other neurons, a cell body that contains the nucleus, a long axon that conveys electrical signals, and the synaptic ending, which transmits these signals to target cells such as other neurons, muscle cells or gland cells (Fig. 2). The neuronal spines act as microprocessors within the brain and, although they have a volume of only around 0.1 μm^3, they can function as autonomous compartments. There is increasing evidence that Ca^{2+} signals within spines may be responsible for the 'synaptic plasticity' that leads to short-term memory.

When a signal is received, Ca^{2+} either enters the spines from the outside or is released from the spine apparatus, a continuation of the endoplasmic reticulum that bears channels such as those activated by $Ins(1,4,5)P_3$. Because these receptors can be stimulated by both $Ins(1,4,5)P_3$ and Ca^{2+}, they could act as coincidence detectors to integrate information from separate neural signals (Fig. 2a)[23]. For example, signals that promote the entry of Ca^{2+} will cooperate with those that generate $Ins(1,4,5)P_3$. Such concurrent stimuli will promote the explosive release of stored Ca^{2+}, bringing about the modifications that are thought to be responsible for learning and memory.

One puzzle is that Ca^{2+} has been implicated in both stimulating and depressing the transmission of nervous signals. Subtle modifications in the amplitude, or spatial and temporal presentation of Ca^{2+} signals, as described earlier, may account for this. Spatially restricted Ca^{2+} signals also control neuronal excitability (Fig. 2c). When neurons fire, information is relayed down the axon to the synaptic ending, activating the secretion of neurotransmitters — chemicals that excite neighbouring neurons. Elementary Ca^{2+} signals are used to produce brief, highly localized transients that trigger release of vesicles containing the neurotransmitter (Fig. 2b)[24]. In the cell body itself, elementary Ca^{2+} signals can modulate neuronal excitability by activating Ca^{2+}-dependent K^+ channels. These channels allow an efflux of K^+ ions through the plasma membrane, inhibiting subsequent electrical activity (Fig. 2c).

To create more permanent memories, the short-term modifications described above have to be consolidated by information from the nucleus. Once again, Ca^{2+} is involved (Fig. 2d)[25,26]. But how can the information arriving at the synaptic ending trigger gene transcription far away in the nucleus? To do this, Ca^{2+} seems to recruit additional signalling components that migrate into the nucleus and activate genes there[25]. In addition, the Ca^{2+} signals themselves, derived from either the entry or release of Ca^{2+}, can also activate genes in the nucleus[26].

Cell death

Very high concentrations of Ca^{2+} can lead to the disintegration of cells (necrosis) through the activity of Ca^{2+}-sensitive protein-digesting enzymes. Calcium has also been implicated in the more orderly programme of cell death known as apoptosis. Apoptosis is important during both normal development (the formation of tissue patterns, for example) and pathological conditions such as AIDS, Alzheimer's disease and cancer. A protein that is mutated in cancerous cells, called Bcl-2, prevents the cell death that would normally limit the survival and proliferation of cancer cells. Bcl-2 mediates some of its anti-apoptotic action by modifying the way in which organelles such as the endoplasmic reticulum and mitochondria (where respiration occurs) handle Ca^{2+} (Box 1).

But what is the relationship between Bcl-2, Ca^{2+} signalling and apoptosis? In many cells, mitochondria participate in the recovery phase of normal Ca^{2+} transients — they sequester some of the Ca^{2+} signal, which is later returned to the endoplasmic reticulum. So, during normal Ca^{2+} signalling, there is a continuous shuttling of Ca^{2+} between these two organelles. Normally, most of the Ca^{2+} resides within the lumen of the endoplasmic reticulum, with very little in the mitochondria. These high levels of Ca^{2+} are essential. Not only do they form a reservoir of signal Ca^{2+} in the endoplasmic reticulum, but they are also essential for the synthesis and processing of proteins there.

If the Ca^{2+} stored within the endoplasmic reticulum was depleted, the mitochondria would become overloaded and there would be two main consequences for the cells. First, the decline in levels of Ca^{2+} in the endoplasmic reticulum would lead to the activation of stress signals, which switch on the genes associated with cell death. Interestingly, some of these genes also specify proteins that bind Ca^{2+} in the endoplasmic reticulum, and this could be a desperate attempt by the cell to restore the correct balance of Ca^{2+} between the endoplasmic reticulum and the mitochondria. Second, the build-up of mitochondrial Ca^{2+} initiates a programme of events that leads to cell death (Box 1). In normal cells, Bcl-2 may modify the Ca^{2+}-handling properties of the endoplasmic reticulum[27] and the mitochondria[28](Box 1), to restore the correct Ca^{2+} balance.

Conclusion

This brief — and all too simplistic — journey through some of the processes controlled by Ca^{2+} illustrates the universality of this signalling mechanism, which triggers a new life at fertilization and is then re-used over and over again to regulate the developmental programme as it unfolds to produce a new organism. As cells differentiate to perform different functions, they select out those components of the Ca^{2+}-signalling tool kit that best fit their remit. This versatility is emphasized by growing evidence that Ca^{2+} controls cellular processes as diverse as cell proliferation and the neuronal plasticity that is responsible for learning and memory. But at any moment, any of these orderly signalling events can be switched to activate a programme that leads to cell death — a big challenge for the future is to understand how Ca^{2+} suddenly transforms from a signal for life to a signal of death. □

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