# The where and when of intracellular $Ca^{2+}$ signalling

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$Ca^{2+}$ is essential for cellular functions. The ability of this ion to act as an intracellular signal, common to almost all eukaryotic cells, allows it to orchestrate an enormous repertoire of cellular activities, from initiating egg development after fertilization to synaptic transmission between neurons.

The first indication that $Ca^{2+}$ has a role as a dynamic physiological signal, beyond its role as a structural component of teeth and bones, was in an experiment performed by British cardiovascular physiologist Sydney Ringer in 1883, just 14 years after Dmitri Mendeleev proposed his periodic table of the elements. In the absence of $Ca^{2+}$ salts in a circulating saline solution, Ringer found that contractions of isolated frog hearts progressively weakened and then ceased. The contractions resumed when $Ca^{2+}$ was restored. We know now that these serendipitous observations reflect the need, during each cycle of contraction and relaxation, for $Ca^{2+}$ release and re-uptake from intracellular stores. The need for extracellular $Ca^{2+}$ arises because the ryanodine receptors (RyR), through which the $Ca^{2+}$ is released, open when they bind $Ca^{2+}$. This trigger $Ca^{2+}$ is provided via the opening of voltage-gated $Ca^{2+}$ channels in the plasma membrane (PM).

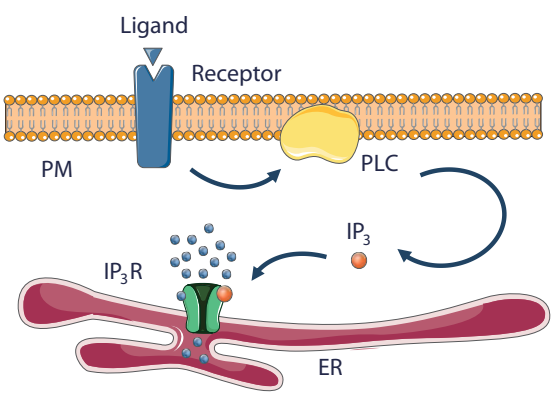


**Figure 1.** The path from extracellular stimulus to intracellular $Ca^{2+}$ release.
Many extracellular stimuli activate receptors that signal through phospholipase C (PLC). PLC generates inositol 1,4,5-trisphosphate ($IP_3$), a diffusible intracellular messenger molecule that opens $IP_3$ receptors ($IP_3Rs$), which are $Ca^{2+}$-permeable channels in the endoplasmic reticulum (ER) membrane. $IP_3Rs$ are the signalling hubs that control the redistribution of $Ca^{2+}$ from internal stores to the cytosol, other organelles and the flow of $Ca^{2+}$ across the plasma membrane through store-operated $Ca^{2+}$ entry.
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Nearly 150 years later, the role of $Ca^{2+}$ as an intracellular messenger is established in processes as diverse as muscle contraction, neurotransmitter secretion, gene transcription, cell division and apoptosis. $Ca^{2+}$ is unusual among intracellular messengers in that it is an ion, rather than a more complex component that might be made or degraded, and so changes in the free cytosolic $Ca^{2+}$ concentration (abbreviated to $[Ca^{2+}]_c$) can only occur by moving $Ca^{2+}$ across membranes. Ion channels allow $Ca^{2+}$ to move quickly down its electrochemical gradient, while ATP-dependent pumps or exchange proteins can move $Ca^{2+}$ against its electrochemical gradient to extrude it from the cell or return it to stores. The complex sequence of conformational changes associated with active $Ca^{2+}$ transport means that $Ca^{2+}$ pumps typically transport $Ca^{2+}$ thousands of times more slowly than ion channels. Hence, most acute increases in $[Ca^{2+}]_c$ are brought about by the regulated opening of $Ca^{2+}$-permeable channels. One of the most widely expressed of these channels is the inositol 1,4,5-trisphosphate receptor ($IP_3R$), a cousin of RyR. Similar to RyRs, $IP_3Rs$ are stimulated to open by $Ca^{2+}$, but for $IP_3Rs$ this only occurs after they have bound $IP_3$.

The ER is the major intracellular $Ca^{2+}$ store, and since most $IP_3Rs$ are expressed within ER membranes, $IP_3Rs$ allow $Ca^{2+}$to be redistributed from the ER to the cytosol. The link with extracellular signals, like hormones or growth factors, is provided by receptors in the PM that can stimulate phospholipase C (PLC). The $IP_3$ produced by PLC binds to $IP_3Rs$ causing them to open and release $Ca^{2+}$ from the ER, with several important consequences (Figure 1). This action increases $[Ca^{2+}]_c$, which can



 

then regulate cellular activities often through the ubiquitous $Ca^{2+}$-binding protein, calmodulin. As $IP_3Rs$ are stimulated by $Ca^{2+}$ once they have bound $IP_3$, they can propagate cytosolic $Ca^{2+}$ signals regeneratively. $IP_3Rs$ held close to other organelles, mitochondria for example, can deliver $Ca^{2+}$ to them and regulate their activities. Finally, loss of $Ca^{2+}$ from the ER lumen stimulates store-operated $Ca^{2+}$ entry (SOCE) across the PM. SOCE occurs when the luminal $Ca^{2+}$-binding site of another ER protein, STIM1, senses the loss of $Ca^{2+}$, causing it to unfold the cytosolic domain through which it binds to a $Ca^{2+}$ channel in the PM, Orai1. This direct interaction between STIM1 and Orai1 opens the $Ca^{2+}$ channel allowing $Ca^{2+}$ to flow into the cell across the PM. Hence, it is clear that $IP_3Rs$ serve as hubs that control the redistribution of $Ca^{2+}$ from the ER to the cytosol or other organelles, and ultimately the flow of $Ca^{2+}$ across the PM.

Cells are bombarded with extracellular stimuli that control every cellular activity, and much of this communication passes through $Ca^{2+}$ signals; so how is specificity maintained? The answer seems to lie largely with the spatial and temporal organization of $Ca^{2+}$ signals. Where and when $Ca^{2+}$ signals are generated is just as important as how they are generated.

## Location, location, location

Cytosolic $Ca^{2+}$ buffers are so abundant that only one of every 100 $Ca^{2+}$ ions to flow into the cytosol remains unbound. This buffering slows $Ca^{2+}$ diffusion and allows local $Ca^{2+}$ signals to persist around the mouths of open $Ca^{2+}$ channels. These microdomains of increased $[Ca^{2+}]_c$ can selectively regulate $Ca^{2+}$-binding proteins situated within them. Many of these $Ca^{2+}$-binding proteins can ignore small changes in $[Ca^{2+}]_c$ and tune-in to the much larger changes within a microdomain because they have a relatively low affinity for $Ca^{2+}$. Within neurons, for example, such microdomains allow $Ca^{2+}$ signals to selectively regulate neurotransmitter release or gene transcription, and in many cells they allow the $Ca^{2+}$ signals evoked by SOCE or $IP_3Rs$ to control different events. A now classic example of the importance of spatial organization is provided by the smooth muscle of blood vessels, where global increases in $[Ca^{2+}]_c$ trigger contraction by activating, via calmodulin, myosin light-chain kinase. But local $Ca^{2+}$ signals provided by RyRs beneath the PM deliver their $Ca^{2+}$ to $Ca^{2+}$-activated $K^+$ channels, causing them to open and allow $K^+$ to leave the cell. The resulting hyperpolarization of the PM prevents activation of voltage-gated $Ca^{2+}$ channels, and by attenuating global increases in $[Ca^{2+}]_c$, causes relaxation. Hence, $Ca^{2+}$ signals can, according to their location, cause relaxation or contraction.

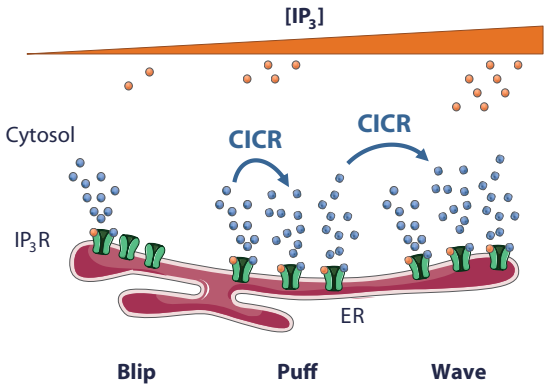


**Figure 2.** Local $Ca^{2+}$ signals are the building blocks of global $Ca^{2+}$ signals. $IP_3Rs$ require the binding of both $IP_3$ and $Ca^{2+}$ to open. This $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) is the basis for a hierarchical model of how local $Ca^{2+}$ release events, such as blips (the result of a single $IP_3R$ opening) and puffs (the concerted opening of $IP_3R$ clusters), contribute to global signals, such as waves and spikes in response to increasing $IP_3$ concentration.

For $IP_3Rs$, the local $Ca^{2+}$ signals evoked by the opening of single channels can grow into larger ones because $IP_3Rs$ are stimulated by both $IP_3$ and $Ca^{2+}$, so that $Ca^{2+}$ released by one $IP_3R$ can ignite the activity of its neighbours to generate a '$Ca^{2+}$ puff' as a small cluster of $IP_3Rs$ open together (Figure 2). The 'gain' on this regenerative $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) mechanism is set by the concentration of $IP_3$ (which primes $IP_3Rs$ to respond to $Ca^{2+}$) and by the distance between clustered $IP_3Rs$. $Ca^{2+}$ puffs become more frequent as the $IP_3$ concentration increases and the $Ca^{2+}$ signal can then spread, like a bushfire, across the entire cell as a regenerative wave. Recent work suggests that although $Ca^{2+}$ puffs may be the basic building blocks of $IP_3$-evoked $Ca^{2+}$ signals, only a tiny fraction of the $IP_3Rs$ in a cell are responsible for generating them. These 'licensed' $IP_3Rs$ are strategically placed alongside the sites where STIM1 regulates SOCE, suggesting that the spatial organization of $IP_3Rs$ may also contribute to regulating $Ca^{2+}$ entry across the PM.

In textbooks, you might see organelles—such as the ER, mitochondria and lysosomes—represented as separate compartments. In reality, organelles form intimate contacts with each other, where their membranes are held just a few nanometres apart by scaffold proteins. These membrane contact sites mediate many exchanges between organelles, including the transfer of $Ca^{2+}$ from

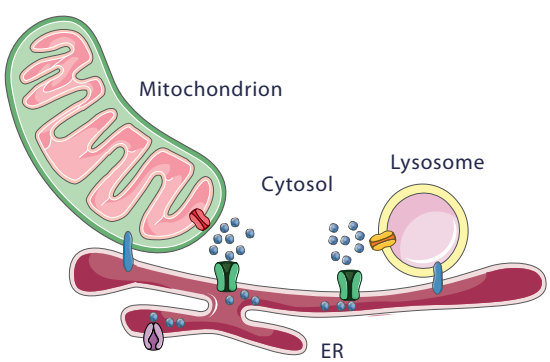


**Figure 3.** Intimate contact between organelles. Organelles form membrane contact sites that are held together by scaffolding proteins (blue). $Ca^{2+}$-uptake systems in mitochondria and lysosomes (red and orange) have low affinity for $Ca^{2+}$. The high-affinity uptake system of the ER, the sarco/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA, purple), actively accumulates $Ca^{2+}$ within the ER lumen. $IP_3Rs$ (green) sat next to membrane contact sites can then selectively deliver high concentrations of $Ca^{2+}$ directly to organelles, allowing $Ca^{2+}$ to mediate organelle-specific functions such as ATP synthesis by oxidative phosphorylation in mitochondria. Created with BioRender.com

one organelle to another, notably between the ER and mitochondria. $Ca^{2+}$ uptake into mitochondria occurs via a channel, the mitochondrial uniporter (MCU), that only opens when it is exposed to much higher $[Ca^{2+}]_c$ than typically prevails in a healthy cell. Hence, mitochondria accumulate $Ca^{2+}$ only when it is delivered to them by closely apposed $Ca^{2+}$ channels—for example, $IP_3Rs$ in the ER (Figure 3). The link with $IP_3Rs$ is apt because $IP_3$-evoked cytosolic $Ca^{2+}$ signals will trigger cellular activity, for example smooth muscle contraction, while local delivery of $Ca^{2+}$ to the outer mitochondrial membrane both parks mitochondria in regions where $[Ca^{2+}]_c$ is elevated and by driving $Ca^{2+}$ uptake through MCU, it stimulates oxidative phosphorylation. $IP_3$-evoked release of $Ca^{2+}$ from the ER thereby both stimulates cellular activity and ensures delivery of the ATP required to sustain it.

## Frequency codes

There is, however, a problem with the toxicity of $Ca^{2+}$; sustained or excessive increase in $[Ca^{2+}]_c$ kill cells. However, natural selection has provided defences: most $Ca^{2+}$ channels are inhibited by increases in $[Ca^{2+}]_c$, constraining their ability to poison the cell; and many $Ca^{2+}$ extrusion mechanisms are stimulated by increased $[Ca^{2+}]_c$. So how can $Ca^{2+}$ evoke sustained responses when powerful homeostatic mechanisms are engaged to prevent sustained increases in $[Ca^{2+}]_c$? $Ca^{2+}$ oscillations or spikes provide an answer and, by encoding stimulus intensity in the frequency of $Ca^{2+}$ spikes—just as nerves encode activity in the frequency of action potentials—they provide a robust means of communication. It is easier to distinguish a doubling of signal frequency than it is to distinguish a doubling of its amplitude, as the switch from AM (amplitude-modulated) to digital FM (frequency-modulated) radio attests.

To make the most of these digital $Ca^{2+}$ signals, cells must decode them into graded and sustained responses. $Ca^{2+}$-calmodulin-dependent protein kinase II (CaMKII) provides a classic example of how a digital frequency-modulated signal can be transduced into an amplitude-modulated signal. CaMKII forms a complex structure with 12 subunits arranged around the spokes of 2 wheels with a shared axle. Binding of $Ca^{2+}$-calmodulin to a subunit activates its protein kinase activity, but it also primes phosphorylation of the subunit. Phosphorylation is important because it prolongs the kinase activity beyond the duration of the $Ca^{2+}$ signal (it creates a memory of a preceding $Ca^{2+}$ signal), but phosphorylation only occurs when two adjacent subunits have both bound $Ca^{2+}$-calmodulin: one subunit to provide the enzyme, and the other the substrate. Hence, appreciable autophosphorylation occurs only when several of the 12 subunits are occupied. This tunes the initiation of the molecular memory to large $Ca^{2+}$ signals, such as occur at the peak of $Ca^{2+}$ spikes. The greater the frequency of the $Ca^{2+}$ spikes, the less opportunity there is to reverse the phosphorylation between spikes and the greater the amplitude of the sustained CaMKII activity. Hence, CaMKII reads the frequency of $Ca^{2+}$ spikes—indeed different forms of CaMKII are tuned to read different frequencies—and transduces them into graded increases in kinase activity.

However, as with any biological system that relies on relatively small numbers of molecules, there is an element of stochasticity to $Ca^{2+}$ spikes. The interval between one spike and the next can vary randomly, and spiking frequencies show heterogeneity even between cells within the same population. So how can frequency reliably encode changes in stimulus intensity, given this randomness? In response to $Ca^{2+}$-mobilizing agonists, cells with variable spiking frequencies show a consistent *fold* change in the average interspike interval that scales with increasing stimulus strength. Drawing a musical analogy, although there are variations in 'pitch' (the absolute frequency of spikes), the change from one 'note' to another (the fold-change in frequency) is conserved. In this way, information about the initial stimulus intensity can be encoded in the temporal profile of $Ca^{2+}$ transients.

## Concluding remarks

Unlike most intracellular messengers, which can be made or degraded, $Ca^{2+}$ is an element, and can only be redistributed from one cellular compartment to another. $IP_3Rs$ are often responsible for this redistribution, delivering $Ca^{2+}$ from the ER to the cytosol or other organelles, and initiating the events that stimulate $Ca^{2+}$ entry across the PM. Hence, $IP_3Rs$ are the hubs that coordinate the spatially and temporally organized $Ca^{2+}$ signals that regulate so many cellular activities. ■



 

## Further reading


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