Mitochondria have long been known to be the major site for aerobic production of intracellular ATP in eukaryotic cells; however, their role in intracellular Ca\(^{2+}\) signaling has had a much more checkered past. The view that mitochondrial Ca\(^{2+}\) transport was central to intracellular Ca\(^{2+}\) signaling, which was pervasive up until the late 1970s, was summarily dismissed in the 1980s when the ubiquitous Ca\(^{2+}\)-releasing messenger inositol 1,4,5-trisphosphate (InsP\(_3\)) was found to mobilize Ca\(^{2+}\) specifically from the endoplasmic reticulum. In addition, use of the newly available Ca\(^{2+}\)-sensitive fluorescent dyes revealed that mitochondria released very little Ca\(^{2+}\) into the cytosol. Furthermore, intracellular Ca\(^{2+}\) concentration could reach, at least globally, the micromolar range upon stimulation, levels considerably smaller than the apparent \(K_m\) for mitochondrial Ca\(^{2+}\) uptake (10\–20 \(\mu\)M). All of this changed in the mid-1990s, and mitochondria have now returned with a vengeance to occupy a key position in intracellular Ca\(^{2+}\) dynamics (17). Underpinning this mitochondrial renaissance was the localization of genetically targeted Ca\(^{2+}\)-selective probes (like the bioluminescent protein aequorin) or fluorescent dyes like rhod-2 to the mitochondrial matrix, which provided a direct and unambiguous readout of mitochondrial Ca\(^{2+}\) changes in living cells following receptor stimulation (17). In addition, following an increase in cytosolic Ca\(^{2+}\) to the micromolar range, careful single-cell analysis revealed that the rate of recovery of the Ca\(^{2+}\) signal to prestimulation values was sculpted by mitochondrial Ca\(^{2+}\) buffering (2).

### Mitochondrial Ca\(^{2+}\) transport

Mitochondria rapidly take up Ca\(^{2+}\) from the cytosol via a ruthenium red-sensitive uniporter spanning the inner mitochondrial membrane. This electrogenic passive uptake is driven mainly by the enormous negative potential across the inner mitochondrial membrane (\(-180\) mV), a consequence of proton extrusion along the electron transport chain. Inhibition of mitochondrial Ca\(^{2+}\) uptake, either by blocking the uniporter with ruthenium red and related compounds or by dissipating the mitochondrial membrane potential with protonophores like FCCP, results, for a given amount of Ca\(^{2+}\) entering the cytosol, in a larger-amplitude cytosolic Ca\(^{2+}\) transient and a slower rate of recovery (2, 17). The paradox of how mitochondria can buffer global cytosolic Ca\(^{2+}\) transients in the micromolar range despite a low-affinity uptake mechanism was neatly resolved by the finding that mitochondrial Ca\(^{2+}\) sensing local microdomains of elevated Ca\(^{2+}\) at the mouth of open Ca\(^{2+}\) channels, either in the endoplasmic reticulum or the plasma membrane (4, 17, 18). Although the Ca\(^{2+}\) concentration in such microdomains falls off steeply with distance from the open channel, they nevertheless reach estimated concentrations of several micromolar within a few tens of nanometers of the pore. Because mitochondria can be juxtaposed to both the stores and the plasma membrane, they are ideally situated for sensing such local Ca\(^{2+}\) domains (16). Recent evidence suggests that the voltage-dependent anion channel of the outer mitochondrial membrane might facilitate the transfer of such microdomains to the uniporter (16). Interestingly, Ca\(^{2+}\) itself has been found to reversibly promote close apposition between mitochondria and endoplasmic reticulum, a process that could contribute to the dynamics of mitochondrial Ca\(^{2+}\) signaling (20).

The uniporter itself is activated by a rise in Ca\(^{2+}\), and recent work demonstrates that channel activity can be enhanced by calmodulin (Csordas G and Hajnoczky G, personal communication). Even after cytosolic Ca\(^{2+}\) levels have returned to prestimulation concentrations, Ca\(^{2+}\)-calmodulin gating of the uniporter imparts a time-dependent enhancement of activity such that a subsequent increase in cytosolic Ca\(^{2+}\) results in a pronounced mitochondrial Ca\(^{2+}\) uptake. Sensing microdomains of both Ca\(^{2+}\) release from stores and Ca\(^{2+}\) influx across the plasma membrane enables the Ca\(^{2+}\) concentration within the mitochondrial matrix to reach up to 500 \(\mu\)M (12). In the adrenaline-secreting chromaffin cells of the adrenal medulla, such local Ca\(^{2+}\) buffering shapes the pattern of exocytosis (12). The ability of mitochondria to accumulate such large amounts of Ca\(^{2+}\), and to do this rapidly (within seconds), is due in part to the high Ca\(^{2+}\)-binding ratio within their matrix [estimated to be \(>2,000\) (2); corresponding values for the cytosol are typically 50–100]. Moreover, the Ca\(^{2+}\)-binding ratio may well be dynamic, in that it can be increased by uptake of buffer (e.g., phosphate), which then binds to and reversibly precipitates Ca\(^{2+}\) within the matrix (5). In this way, mitochondria may accumulate extraordinary amounts of Ca\(^{2+}\) yet maintain a disproportionately low free Ca\(^{2+}\) concentration within the matrix such that the permeability transition pore does not open.

Hence a large body of evidence demonstrates that mito-
Mitochondria can shape intracellular Ca\(^{2+}\) signals, both spatially and temporally, as a direct consequence of their rapid Ca\(^{2+}\) buffering. But mitochondria handle Ca\(^{2+}\) in a manner quite distinct from that of simple intracellular buffers. Rapid cytosolic Ca\(^{2+}\) buffers like ATP and calbindin release Ca\(^{2+}\) as cytosolic Ca\(^{2+}\) concentration falls, as dictated by the law of mass action. Mitochondria also release Ca\(^{2+}\) that has been accumulated following a cytosolic Ca\(^{2+}\) increase, but they do so in a kinetically complex manner that is dictated by the prevalent activities of the Ca\(^{2+}/\)Na\(^{+}\) and Ca\(^{2+}/\)H\(^{+}\) exchangers in the inner mitochondrial membrane. Mitochondrial Ca\(^{2+}\) release is probably both too slow and too small to be taken back up through the unipporter, and this slow Ca\(^{2+}\) release is particularly important because it represents a form of Ca\(^{2+}\) memory. It develops several seconds after the initial stimulus has been removed and is believed to account for presynaptic, posttetanic potentiation of synaptic transmission (19) as well as expediting the refilling of endoplasmic reticulum Ca\(^{2+}\) stores (1).

**Mitochondria spatially restrict Ca\(^{2+}\) signals**

Mitochondria can also function as fixed buffers, thereby partitioning the cell into discrete Ca\(^{2+}\) compartments. The most poignant example of this is seen in pancreatic acinar cells (15). In these highly polarized epithelia, moderate stimulation of receptors in the basolateral membrane results in Ca\(^{2+}\) oscillations restricted to the apical pole, where the zymogen granules are located. The Ca\(^{2+}\) signals are confined to the apical region by mitochondria, which form a three-dimensional belt or firewall that neatly separates the two poles of the cell. Only when mitochondrial Ca\(^{2+}\) buffering is compromised or excessive receptor stimulation is used does the apical Ca\(^{2+}\) signal invade the basolateral region. Mitochondria are normally mobile organelles, readily fusing with one another to form long tubular structures that undergo subsequent fission. Just how a subset of mitochondria in the acinar cell is removed from this dynamic pool and restricted to a role as fixed buffers is unclear at present, but unraveling the underlying mechanisms may well provide further insight into compartmentalization of intracellular Ca\(^{2+}\) signals.

**Mitochondrial regulation of InsP\(_3\) receptors**

Although mitochondria do not seem to release Ca\(^{2+}\) directly in response to cell-surface receptor stimulation, they nevertheless play a pivotal role in controlling Ca\(^{2+}\) release patterns through effects on InsP\(_3\)-evoked Ca\(^{2+}\) mobilization. The ubiquitous second messenger InsP\(_3\) releases Ca\(^{2+}\) from internal stores by binding to tetrameric ligand-gated Ca\(^{2+}\)-permeable channels that span the endoplasmic reticulum membrane. Molecular cloning studies have revealed at least three types of InsP\(_3\) receptor (InsP\(_3\)R1, InsP\(_3\)R2, and InsP\(_3\)R3). InsP\(_3\)R1 is strongly modulated by cytosolic Ca\(^{2+}\) concentration. Channel activity in the presence of a submaximal concentration of InsP\(_3\) is increased substantially as cytosolic Ca\(^{2+}\) increases. Ca\(^{2+}\) therefore acts as a coagonist of the InsP\(_3\) receptor, and this positive feedback is thought to underlie the explosive nature of the Ca\(^{2+}\) release transient. As cytosolic Ca\(^{2+}\) concentration increases further, however, Ca\(^{2+}\) ions feed back to inactivate the InsP\(_3\) receptor, thereby curtailing further Ca\(^{2+}\) release. Such inactivation is probably mediated by calmodulin. This results in a bell-shaped Ca\(^{2+}\) dependence of InsP\(_3\)-mediated Ca\(^{2+}\) release. By virtue of their low Ca\(^{2+}\) affinity and very close apposition to Ca\(^{2+}\) release channels, mitochondrial unipporters are ideally designed to modulate InsP\(_3\) receptor activity through effects on the Ca\(^{2+}\) dependence of Ca\(^{2+}\) release. In Xenopus oocytes, InsP\(_3\)-mediated Ca\(^{2+}\) waves were synchronized and increased in both amplitude and velocity following the addition of substrates that increased mitochondrial respiration (9). Such energized mitochondria have a more hyperpolarized membrane potential, which increases the electrical driving force for Ca\(^{2+}\) uptake through the unipporter. This enhanced uptake was proposed to lower cytosolic Ca\(^{2+}\) concentration in the vicinity of open InsP\(_3\) receptors to a level below that required to induce Ca\(^{2+}\)-dependent inactivation. In this scheme, mitochondria are promoting more extensive Ca\(^{2+}\) mobilization by preventing inactivation of the Ca\(^{2+}\) release channels. In other systems, however, the converse is observed. In permeabilized hepatocytes, impairment of mitochondrial Ca\(^{2+}\) uptake results in more extensive Ca\(^{2+}\) release to submaximal concentrations of InsP\(_3\) and regions devoid of mitochondria are more sensitive to ambient InsP\(_3\) levels (8). By rapidly buffering cytosolic Ca\(^{2+}\), mitochondria are able to reduce the positive feedback effect of Ca\(^{2+}\) on Ca\(^{2+}\) release, thereby resulting in less effective Ca\(^{2+}\) mobilization by InsP\(_3\) receptors. A similar mechanism seems to underlie the reduced speed of propagating Ca\(^{2+}\) waves in astrocytes following mitochondrial depolarization (3). Hence mitochondria can result in either greater or lesser Ca\(^{2+}\) release to InsP\(_3\), depending on their precise location and hence whether they affect the ascending or descending limbs of the bell-shaped curve for Ca\(^{2+}\) release via InsP\(_3\)R1. Whether InsP\(_3\)R2 and InsP\(_3\)R3 also exhibit a bell-shaped dependence on Ca\(^{2+}\) release is more controversial. But both of these receptors can and do form heteromultimers with InsP\(_3\)R1, and in such complexes the properties of InsP\(_3\)R1 seem to dominate. Hence mitochondria are likely to affect Ca\(^{2+}\) release profiles in heteromultimers if at least one subunit is InsP\(_3\)R1.

Ryanodine receptor-mediated Ca\(^{2+}\) signals are also efficiently propagated into mitochondria, and this transfer seems to involve both local microdomains of elevated Ca\(^{2+}\) around the mouth of each ryanodine receptor as well as close apposition between sarcoplasmic reticulum and mitochondria (17).

**Mitochondrial regulation of store-operated Ca\(^{2+}\) channels**

Mitochondria are also able to buffer Ca\(^{2+}\) that enters through both voltage-operated and store-operated Ca\(^{2+}\) chan-
nely, in nonexcitable cells, store-operated Ca\(^{2+}\) channels, which are activated by the emptying of intracellular Ca\(^{2+}\) stores, provide a major route for Ca\(^{2+}\) influx. Ca\(^{2+}\) entry through these channels is required for controlling a host of Ca\(^{2+}\)-dependent processes ranging from exocytosis to cell growth and proliferation. In many cell types, store-operated Ca\(^{2+}\) entry is manifest as a non-voltage-gated Ca\(^{2+}\) current called the Ca\(^{2+}\)-release-activated Ca\(^{2+}\) current (I\(_{\text{CRAC}}\)). Under physiological conditions of weak intracellular Ca\(^{2+}\) buffering, the second messenger InsP\(_3\) fails to activate any macroscopic I\(_{\text{CRAC}}\) despite substantial Ca\(^{2+}\) release. Hence the inability of InsP\(_3\) to activate macroscopic I\(_{\text{CRAC}}\) in weak Ca\(^{2+}\) buffer does not reflect a failure to mobilize Ca\(^{2+}\) from the stores. It turns out that the sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) pumps, which refill the stores with Ca\(^{2+}\) following InsP\(_3\)-evoked Ca\(^{2+}\) release, are very powerful and can prevent InsP\(_3\) from depleting the stores sufficiently or long enough for macroscopic I\(_{\text{CRAC}}\) to activate (14). If the SERCA pumps are blocked, then InsP\(_3\) can activate a robust I\(_{\text{CRAC}}\) in weak buffer. SERCA pump activity therefore appears to be one of the main determinants of whether I\(_{\text{CRAC}}\) activates or not under physiological conditions (14). How might SERCA pump activity be reduced under physiological conditions of weak Ca\(^{2+}\) buffering? Clearly, an increase in the rate of Ca\(^{2+}\) removal from the cytosol by another clearance mechanism would be rather effective, because this would compete with SERCA pumps for Ca\(^{2+}\) and hence reduce the rate and extent of store refilling. Moreover, enhanced Ca\(^{2+}\) clearance away from the endoplasmic reticulum might also reduce Ca\(^{2+}\)-dependent inactivation of InsP\(_3\) receptors. Combined, this would empty the stores to a greater extent and I\(_{\text{CRAC}}\) should activate. An unexpected discovery was that mitochondrial Ca\(^{2+}\) uptake is key (6). In the presence of energized mitochondria, InsP\(_3\) is able to routinely activate I\(_{\text{CRAC}}\) even in the presence of active SERCA pumps (6, 14). The size of the current can be increased further by inhibiting SERCA pumps, consistent with the idea that competition between these two major Ca\(^{2+}\)-removal mechanisms dictates the extent of store depletion and hence amplitude of I\(_{\text{CRAC}}\) (14). Moreover, mitochondrial Ca\(^{2+}\) buffering shifts the relationship between InsP\(_3\) concentration and extent of I\(_{\text{CRAC}}\) to the left (14). A subthreshold concentration of InsP\(_3\) becomes capable of activating I\(_{\text{CRAC}}\) when mitochondria are energized. Mitochondrial Ca\(^{2+}\) uptake therefore lowers the threshold concentration of InsP\(_3\) that is required to activate I\(_{\text{CRAC}}\), thereby increasing the dynamic range of concentrations over which InsP\(_3\) is able to function as the physiological messenger that triggers activation of store-operated Ca\(^{2+}\) influx.

Following activation of I\(_{\text{CRAC}}\), the subsequent rise in cytosolic Ca\(^{2+}\) concentration can feed back to inactivate the CRAC channels. Three independent mechanisms have been described whereby a rise in cytosolic Ca\(^{2+}\) can inhibit CRAC channel activity (see Ref. 14 and references therein). Ca\(^{2+}\)-dependent fast inactivation occurs when permeating Ca\(^{2+}\) ions feed back to partially inactivate the channel through which they permeated. Inactivation occurs over tens of milliseconds and is largely independent of the macroscopic current (consistent with local feedback), and is more effectively reduced by BAPTA than EGTA, suggesting that the intracellular Ca\(^{2+}\) binding site is within a few nanometers of the pore. Two slower Ca\(^{2+}\)-dependent regulatory pathways also exist, operating over a time frame of several tens of seconds: Ca\(^{2+}\)-dependent store refilling and Ca\(^{2+}\) entry-dependent but store-independent inactivation (14). Either energizing or depolarizing mitochondria has little impact on the rate or extent of fast inactivation, suggesting that mitochondria do not modulate this form of inactivation (6, 7). However, through their competition with SERCA pumps for Ca\(^{2+}\) uptake, mitochondria Ca\(^{2+}\) buffering also slows down the rate of store refilling. Strikingly, mitochondria Ca\(^{2+}\) buffering also slows down the rate and extent of Ca\(^{2+}\)-dependent but store-independent slow inactivation (14). Hence once I\(_{\text{CRAC}}\) has activated, mitochondria prolong the duration of Ca\(^{2+}\) entry. Indeed, buffering of store-operated Ca\(^{2+}\) entry by mitochondria has been observed in nonexcitable cells, and this might be a ubiquitous way to activate Ca\(^{2+}\)-dependent inactivation of the entry channels.

**Multiple pools of mitochondria**

So far, I have focussed on how mitochondrial Ca\(^{2+}\) buffering can help shape the pattern of Ca\(^{2+}\) release and Ca\(^{2+}\) influx, as well as confine Ca\(^{2+}\) signals to subcellular regions. It is entirely clear if mitochondria are a relatively homogenous population and that their effects on Ca\(^{2+}\) signaling are similar in a consequence of those mitochondria that just happen to be in the local vicinity or whether specific subpopulations of mitochondria exist with different transport properties that enable them to carry out their local tasks effectively. The latter scenario seems more realistic. In pancreatic acinar cells, the different populations of mitochondria were found (15). One group, in the basolateral area close to the plasma membrane, preferentially buffered incoming Ca\(^{2+}\) through store-operated channels. The second pool of mitochondria circled the nucleus and neatly separated nuclear and cytosolic Ca\(^{2+}\) signals. A third pool formed the belt in the lower apical area that was described earlier. This pool preferentially buffered Ca\(^{2+}\) oscillations originating in the granule-rich apical section. The different pools of mitochondria were distinct entities, because local photobleaching studies revealed that they did not share a connected lumen. It would be very interesting to see whether these different pools differ in key properties like Ca\(^{2+}\) transport activities, membrane potential (and hence driving force for electrogenic Ca\(^{2+}\) uptake), and so on.
One form of signaling is mitochondrial release of ATP. Three rate-limiting enzymes of the Krebs cycle within the matrix (pyruvate dehydrogenase, NAD+-isocitrate dehydrogenase, and 2-oxoglutarate dehydrogenase) are activated by a rise in Ca\(^{2+}\) concentration to the micromolar range, and, in the presence of metabolic substrates, this results in an increased production of ATP, which is subsequently transported into the cytosol. ATP can act as a local messenger between mitochondria and juxtaposed endoplasmic reticulum (10). For example, InsP\(_3\)-dependent Ca\(^{2+}\) release is modulated by ATP levels, an effect thought to reflect direct binding of the nucleotide to the release channels. Inhibition of local mitochondrial ATP production significantly reduces the rate of InsP\(_3\)-mediated Ca\(^{2+}\) release in fibroblasts (10). More intriguingly, SERCA pumps, which utilize the free energy that is released from ATP hydrolysis to drive active transport of Ca\(^{2+}\) into the stores, seem to preferentially favor ATP produced locally by mitochondria within a subcellular metabolic microdomain (10). Inhibition of local mitochondrial ATP production significantly reduces the rate of InsP\(_3\)-mediated Ca\(^{2+}\) release in fibroblasts (10). More intriguingly, SERCA pumps, which utilize the free energy that is released from ATP hydrolysis to drive active transport of Ca\(^{2+}\) into the stores, seem to preferentially favor ATP produced locally by mitochondria within a subcellular metabolic microdomain (10). Short-range signals emanating from mitochondria can therefore involve ATP acting as a diffusible messenger. Could mitochondria release other messengers too? In the insulin-secreting \(\beta\)-cells of the pancreas, mitochondria have been reported to release small molecules like glutamate, which then prime secretory vesicles such that their sensitivity to cytosolic Ca\(^{2+}\) increases (11), although this is finding is controversial. We have speculated that a related mechanism might be operating for mitochondrial regulation of CRAC channels in mast cells under physiological conditions of weak Ca\(^{2+}\) buffering (7). Mitochondrial depolarization after store depletion impairs both store-operated Ca\(^{2+}\) influx and the extent of \(I_{\text{Ca,CRAC}}\). Since the depolarization occurs after store depletion, mitochondria are unlikely to be modifying Ca\(^{2+}\) release. Moreover, because the Ca\(^{2+}\) current is suppressed without initially developing at all, it is difficult to envisage how this can be explained entirely by Ca\(^{2+}\) feedback inactivation mechanisms. One possible explanation is that mitochondria release factors in a Ca\(^{2+}\)-dependent manner, which subsequently modulate (but do not activate) CRAC channels in the mast cell membrane. Interestingly, a novel signal involving an as yet undefined chemical messenger is thought to be generated by mitochondria in skeletal muscle, where it then diffuses to the plasma membrane to activate Na\(^+\) channels (13). Mitochondria may integrate Ca\(^{2+}\) signals following entry into the matrix and then release messengers that help coordinate an appropriate response.

The multifarious effects of mitochondrial Ca\(^{2+}\) uptake are summarized in Fig. 1. Given that mitochondria are a source, among other things, of reactive oxygen species, can alter cytosolic pH, and release an array of messengers like ATP and glutamate as well as cytochrome C, which is associated with apoptosis, it is likely that their role in Ca\(^{2+}\) signaling will be extended from just a role, albeit an important one, in Ca\(^{2+}\) buffering.

I would like to thank Daniel Bakowski for help with the figure. Because of strict space constraints, it has not been possible to cite all relevant papers, and I apologize to colleagues for this.

Research in my laboratory is supported by a Medical Research Council Programme Grant and the Lister Institute.

FIGURE 1. Summary of processes that can be regulated by mitochondrial Ca\(^{2+}\) uptake. See text for further details. Note that, for display purposes, the channels and transporters of the inner mitochondrial membrane are shown to span both membranes. The voltage-dependent anion channels, which facilitate transfer of microdomains from inositol 1,4,5-trisphosphate (InsP\(_{3}\)) receptors (InsP\(_{3}\)R) on the endoplasmic reticulum (ER) to the mitochondrial uniporter (see text), have been omitted for clarity. CRAC, Ca\(^{2+}\) release-activated Ca\(^{2+}\) current; RyR, ryanodine receptor; SERCA, sarco(endoplasmic reticulum Ca\(^{2+}\)-ATPase; SR, sarcoplasmic reticulum; VOCC, voltage-operated Ca\(^{2+}\) channel.
References


