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IP_3 receptors and their intimate liaisons

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Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are intracellular Ca^{2+} channels. They allow cell-surface receptors that stimulate IP_3 formation to evoke rapid Ca^{2+} release from the endoplasmic reticulum (ER). IP_3Rs initiate local and global cytosolic Ca^{2+} signals, they deliver Ca^{2+} selectively to other organelles including mitochondria and lysosomes, and, by depleting the ER of Ca^{2+}, they control store-operated Ca^{2+} entry (SOCE). We consider two areas where recent work highlights the importance of liaisons between IP_3Rs and other intracellular membranes. Interactions between IP_3Rs and lysosomes illustrate striking parallels with the relationships between IP_3Rs and mitochondria. In each case, the ER concentrates Ca^{2+} from the cytosol and then delivers it through IP_3Rs to a low-affinity Ca^{2+}-uptake system in a juxtaposed organelle. Evidence that only immobile IP_3Rs parked alongside the sites where SOCE occurs are 'licensed' to evoke Ca^{2+} release suggests a mechanism whereby local depletion of the ER may activate SOCE without compromising other Ca^{2+}-dependent ER functions.

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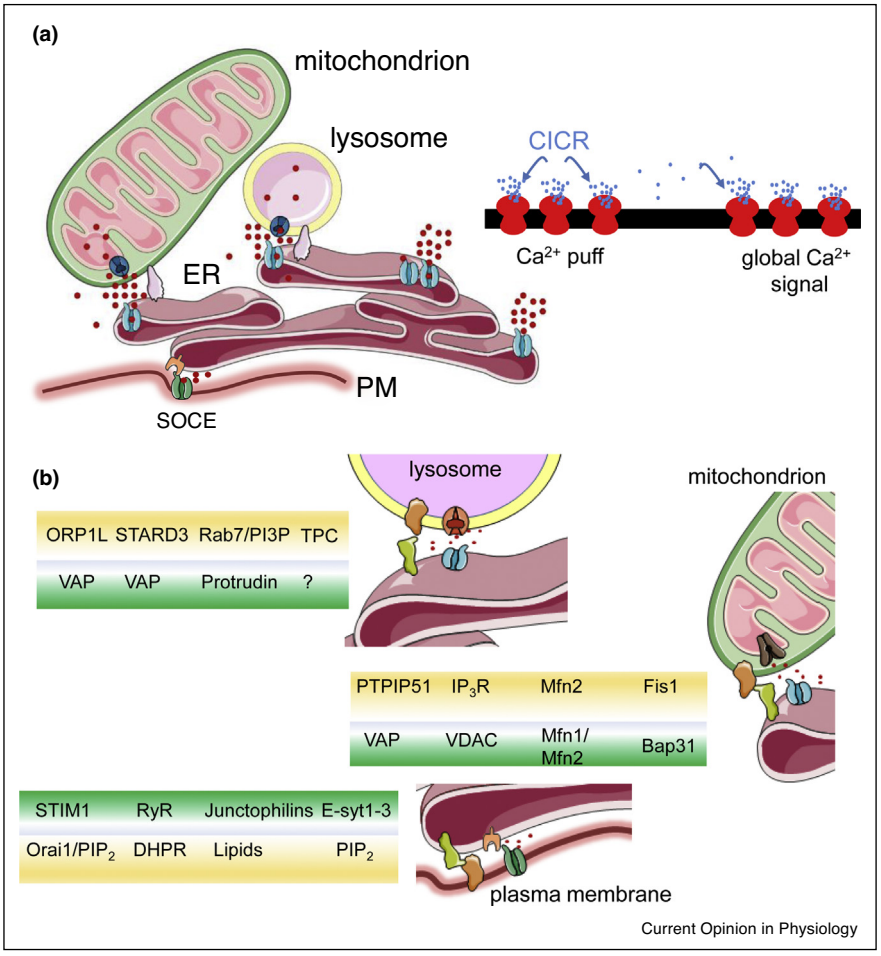
Introduction

Inositol 1,4,5-trisphosphate receptors (IP_3Rs) are intracellular Ca^{2+} channels that are almost ubiquitously expressed in the endoplasmic reticulum (ER) of animal cells [1]. The discovery, in 1983, that IP_3 stimulates Ca^{2+} release from the ER [2] prompted analyses of both the mechanisms linking IP_3 binding to channel opening, and of the organization and functional significance of the resulting Ca^{2+} signals. The former established that IP_3Rs are large-conductance Ca^{2+}-permeable channels [3], opening of which allows Ca^{2+} to flow rapidly from the ER. The opening requires binding of IP_3 to all four subunits of an IP_3R [4•], which then primes the IP_3R to bind the Ca^{2+} that triggers channel opening [3,5]. Structural studies of IP_3R fragments [6–9] and, more recently, of the complete tetrameric IP_3R [10••,11••,12,13•] revealed the initial conformational changes evoked by IP_3 and they are beginning to suggest mechanisms by which they are transduced into rearranged Ca^{2+}-binding sites and thence channel opening.

In addressing the functional consequences of IP_3R activation, most attention focussed on the increase in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_c$). A recurrent theme has been that as stimulus intensities increase, IP_3-evoked cytosolic signals progress from local events, 'Ca^{2+} puffs' that report the coordinated opening of a few IP_3Rs within a cluster [14], to global increases in $[Ca^{2+}]_c$ that invade the entire cell [15]. It is assumed that co-regulation of IP_3Rs by IP_3 and Ca^{2+} allows this hierarchical recruitment, with the concentration of IP_3 and spacing of IP_3Rs tuning the sensitivity of an amplification mechanism (Ca^{2+}-induced Ca^{2+} release, CICR) [15]. Evidence that all three mammalian IP_3R subtypes can elicit rather similar Ca^{2+} puffs [16,17] has further fuelled speculation that the small clusters of IP_3Rs from which Ca^{2+} puffs originate may be the building blocks of all IP_3-evoked cytosolic Ca^{2+} signals. But that consensus was recently challenged by evidence that global Ca^{2+} signals appear not to be built from underlying Ca^{2+} puffs [18••]. Another puzzle is that while most IP_3Rs appear to be mobile [19–21], Ca^{2+} puffs occur repeatedly at relatively few fixed subcellular locations [22,23]. We address this enigma in the first part of this short review.

Two further observations establish the importance of IP_3Rs as Ca^{2+}-regulating hubs beyond their ability to evoke increases in $[Ca^{2+}]_c$ (Figure 1a). Firstly, IP_3Rs are the usual route through which physiological stimuli evoke the substantial decrease in ER luminal $[Ca^{2+}]$ that activates store-operated Ca^{2+} entry (SOCE) [24–28]. The core mechanisms are now clear, with store depletion causing stromal interaction molecule 1 (STIM1) to unfurl cytosolic domains that reach across a narrow gap between the ER and plasma membrane (PM) to capture Orai channels and trigger their opening [29]. It is not, however, clear how the substantial loss of ER Ca^{2+} needed to activate STIM1 occurs without compromising other Ca^{2+}-dependent ER functions. We speculate on a

Figure 1



IP_3Rs regulate different Ca^{2+} signals. **(a)** By controlling release of Ca^{2+} from the ER, IP_3Rs are gateways to different Ca^{2+} signals. They can selectively deliver Ca^{2+} from the ER to the cytosol, mitochondria or lysosomes, and by decreasing the $[Ca^{2+}]$ within the ER, they lead to activation of SOCE. Within the cytosol, co-regulation of IP_3Rs by IP_3 and Ca^{2+} allows regenerative recruitment of IP_3R activity by CICR. At low concentrations of IP_3, this CICR is restricted to small clusters of IP_3Rs which evoke Ca^{2+} puffs. At higher IP_3 concentrations, the regenerative activity propagates across the cell to give a global increase in $[Ca^{2+}]_c$. **(b)** Some of the candidates proposed to assemble the MCS wherein SOCE occurs, or where Ca^{2+} is selectively transferred from IP_3Rs in the ER to mitochondria or lysosomes. ORP1L, oxysterol-binding protein-related protein 1L [71]; STARD3, StAR-related lipid transfer domain-3 [72]; P13P, phosphatidylinositol 3-phosphate [65]; TPC, two-pore channel [70]; VAP, vesicle-associated membrane protein-associated proteins; PTPIP51, protein tyrosine phosphatase-interacting protein 51; Mfn1/2, mitofusin 1/2 [73,74]; Fis1, fission 1 homologue [75]; VDAC voltage-dependent anion channel [76]; Bap31, an ER integral membrane protein; RyR, ryanodine receptor; JP, junctophilins [77]; E-Syt, extended synaptotagmins [78]; PIP_2, phosphatidylinositol 4,5-bisphosphate; DHPR, dihydropyridine receptor.

possible mechanism in light of recent evidence that the IP_3Rs that evoke Ca^{2+} puffs sit alongside the sites where SOCE occurs [30,31••]. Secondly, IP_3Rs can selectively deliver Ca^{2+} at high concentrations to the mitochondrial surface [32]. This can both regulate mitochondrial mobility [33] and allow Ca^{2+} uptake by the low-affinity mitochondrial Ca^{2+} uniporter (MCU) [34], with many functional consequences including stimulation of oxidative phosphorylation [35]. The intimate contact is maintained by tether proteins that hold the ER and the outer mitochondrial membrane less than 25 nm apart [36••], scarcely any further than the cytosolic dimensions of an IP_3R [10••]. Among the fifty or so proteins associated with these membrane contact sites (MCS) [35], the relative importance of candidate tethers is unclear, perhaps reflecting some redundancy (Figure 1b). But a key observation is that IP_3Rs may themselves form tethers independent of their ability to release Ca^{2+} [36••]. There is a parallel between SOCE and these synapse-like contacts between ER and mitochondria, in that Ca^{2+} channels (Orai or IP_3R) contribute not only to the Ca^{2+} flux within the MCS, but also to its assembly (Figure 1b). Many reviews describe the architecture and function of ER-mitochondrial contacts [34,35,37]. Here, we consider

evidence that there may be a similar association of lysosomes with IP_3Rs in the ER.

Licensed IP_3Rs liaise with SOCE

Ca^{2+} puffs repeatedly initiate at rather few fixed sites within a cell [22,23], yet most IP_3Rs are mobile, at least when overexpressed for live-cell imaging of tagged proteins [19–21]. In cells with endogenous IP_3R tagged with EGFP using gene editing, most IP_3Rs form loose clusters, typically comprising about eight IP_3Rs [31••]. Most of these clusters (~70%) are mobile, but Ca^{2+} puffs, whether evoked by IP_3 provided by endogenous pathways or uniformly throughout the cytosol by photolysis of caged-IP_3, invariably occur at immobile clusters and only those located within ER immediately beneath the PM. Hence, only a tiny fraction of the several thousand IP_3Rs within a cell are 'licensed' to evoke Ca^{2+} puffs. The licensing mechanism has yet to be defined. It is, however, noteworthy that the licensed IP_3Rs are parked immediately alongside the MCS where SOCE occurs, although IP_3Rs are not themselves required to assemble the SOCE MCS [31••]. This additional level of IP_3R regulation, licensing, suggests possible explanations for the enigmas described earlier.

Firstly, 'licensing' suggests a regulatory mechanism that both immobilizes some IP_3Rs and permits them to evoke Ca^{2+} puffs. Mobile IP_3Rs do not evoke Ca^{2+} puffs. Since the mobile and immobile pools of IP_3Rs do not freely mix, at least over tens of minutes [31••], the function of most IP_3Rs, the mobile ones and those remote from the PM, remains mysterious. They may provide a pool from which IP_3Rs can be drawn for licensing; they may have different roles in Ca^{2+} signalling, for example in selectively delivering Ca^{2+} to mitochondria or lysosomes (many of which do not lie immediately beneath the PM); or they may have roles unrelated to Ca^{2+} signalling. The latter would be consistent with many different proteins, not all obviously related to Ca^{2+} signalling, associating with IP_3Rs [38].

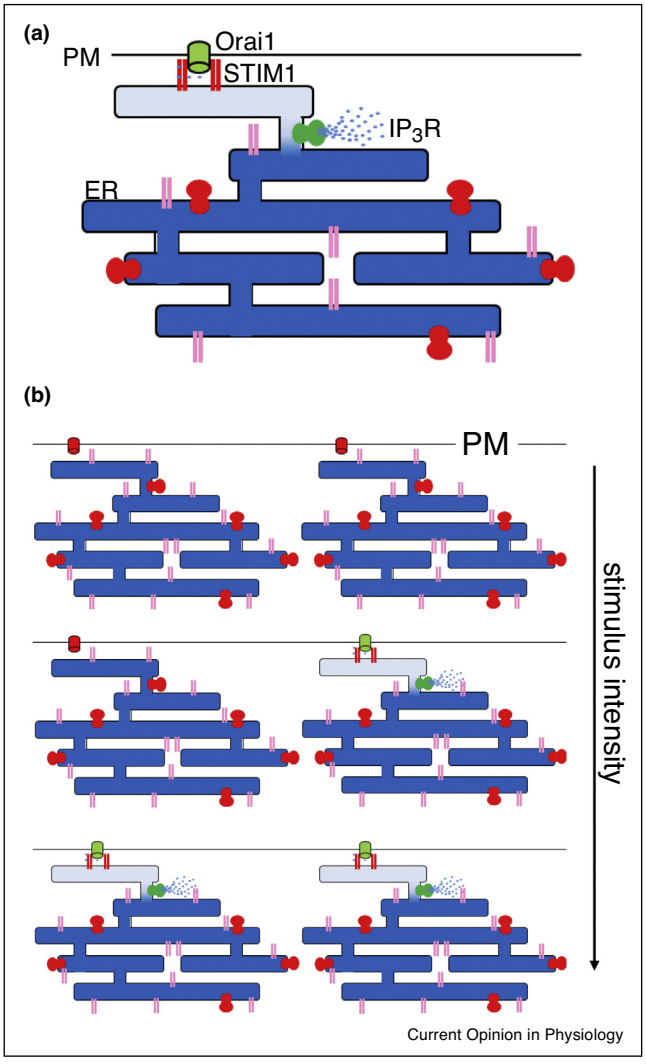
Secondly, the location of licensed IP_3Rs alongside the MCS where STIM1 accumulates after store depletion [31••] suggests a mechanism whereby activation of SOCE by physiological stimuli, namely those that evoke IP_3 formation, might activate STIM1, and thereby SOCE, without substantially depleting the entire ER of Ca^{2+} [30,39]. We speculate that an ER structure with a short neck and flattened head, somewhat reminiscent of a dendritic spine, might allow licensed IP_3Rs to locally and substantially deplete ER within a SOCE MCS of Ca^{2+} (Figure 2). Each of these junctions might then function as a 'digital switch', such that STIM1 within the depleted ER would be fully active after stimulation of associated IP_3Rs. Graded activation of SOCE within a cell would come from recruitment of each MCS, and without global depletion of ER Ca^{2+} (Figure 2b). Additional mechanisms, notably hetero-oligomerization of STIM2 with STIM1, might tune the Ca^{2+} sensitivity of Orai1 activation to respond to less than complete store emptying by combining the greater Ca^{2+} sensitivity of STIM2 [25] and its enhanced affinity for phosphatidylinositol 4,5-bisphosphate (PIP_2) [40], with the greater efficacy of STIM1 in activating Orai1 [41••].

IP_3R and lysosomes – more intimate liaisons

Lysosomes, with their luminal cocktail of hydrolytic enzymes and acidic pH, are best known as degradative organelles [42,43], but there is also evidence that they make important contributions to intracellular Ca^{2+} handling [44]. Experimental analyses of the latter are compromised by the hostile luminal environment of the lysosome, which constrains use of protein-based indicators and demands meticulous pH-corrections to the properties of classical Ca^{2+} indicators. An ingenious construct that tethers conventional pH and Ca^{2+} indicators to a DNA backbone may provide the much-needed tool to effectively measure [Ca^{2+}] within lysosomes [45••]. At present, reliable measures of luminal free [Ca^{2+}] for lysosomes are scarce, but they suggest a concentration comparable (350–600 μM) to that of the ER [45••,46,47], although lysosomes enclose a much smaller intracellular volume (perhaps 500-times less than that of the ER) [48]. There is, however, persuasive evidence that lysosomes express Ca^{2+}-permeable channels, including two-pore channels (TPC1-3), transient receptor potential mucolipin (TRPML1-3) and type 2 purinoceptors ($P2X_4$) [44,49], and that Ca^{2+} released from lysosomes regulates membrane fission and fusion within the endocytic pathway [42,50–52]. Indeed, Ca^{2+} release through TPCs is required for trafficking of Ebola virus through late stages of the endocytic pathway [53]. In addition to this local Ca^{2+} signalling within the endocytic pathway, lysosomes may also initiate larger cytosolic Ca^{2+} signals when Ca^{2+} released through lysosomal channels, triggers Ca^{2+}-induced Ca^{2+} release from the ER through IP_3Rs or ryanodine receptors [54,55].

The means by which lysosomes accumulate Ca^{2+} is unknown. Considerable evidence, including an increase in lysosomal pH after addition of Ca^{2+} [56], and loss of lysosomal Ca^{2+} after dissipation of the lysosomal pH gradient [46,47,57,58], suggest an important role for luminal H^+, but there is no evidence that placental mammals express a lysosomal Ca^{2+}-H^+ exchanger (CAX) [59]. Several reviews consider alternative lysosomal pH-dependent mechanisms for Ca^{2+} uptake [44,45••,49], and a recent report suggests a possible role for a P-type ATPase (ATP13A2), distinct from those that mediate Ca^{2+} transport across other biological membranes, in mediating lysosomal Ca^{2+} uptake [45••]. This is intriguing because mutations in ATP13A2 are associated with a juvenile form of Parkinson's disease [60] and defective autophagy [61], but there is presently no direct evidence that

Figure 2



Local regulation of SOCE by licensed IP_3Rs. **(a)** Licensed clusters of IP_3Rs (green) parked alongside SOCE-MCS may allow substantial loss of Ca^{2+} from ER close to the PM, while negligibly affecting the much larger volume of ER deeper in the cell. The large local decrease in ER $[Ca^{2+}]$ may then locally activate STIM1, and thereby the Ca^{2+} channel, Orai1, that mediates SOCE. Active and inactive STIM1 dimers are shown in red and pink, respectively. **(b)** Digital recruitment of all-or-nothing SOCE-MCS may allow graded activation of SOCE in cells.

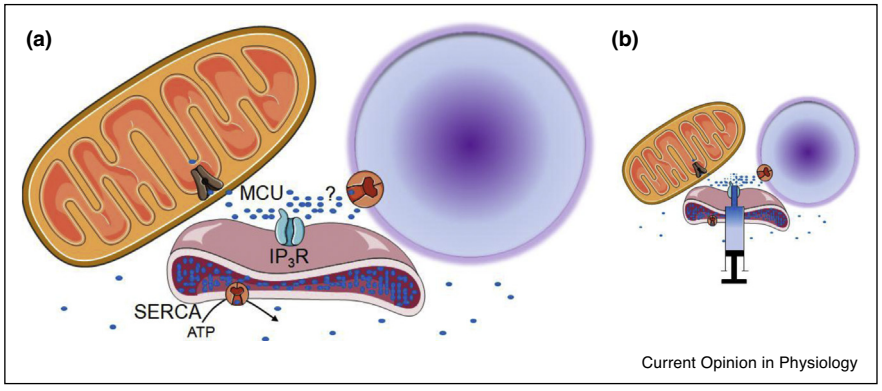
ATP13A2 is a Ca^{2+} pump. The limited evidence available suggests that whatever the lysosomal Ca^{2+} uptake mechanism, it probably has relatively low affinity for Ca^{2+} [62]. There is, therefore, a problem similar to that faced by mitochondria and their MCU, namely the organelles acquire Ca^{2+} from the cytosol, where the global $[Ca^{2+}]_c$ is too low to fuel the low-affinity uptake systems. For mitochondria, the solution is provided by having the ER, with its high-affinity Ca^{2+} pump (the SR/ER Ca^{2+}-ATPase, SERCA), concentrate Ca^{2+} from the cytosol and then squirt it back at MCU through large-conductance IP_3Rs (Figures 1b and 3 a). Recent evidence suggests a similar scheme for lysosomes.

The first evidence that lysosomes selectively sequester Ca^{2+} released by IP_3Rs came from cells in which cytosolic Ca^{2+} signals evoked by receptors that stimulate IP_3 formation were potentiated when lysosomes were perturbed. SOCE-evoked signals were unaffected [63,64••]. Furthermore, a low-affinity Ca^{2+}-sensor expressed on the cytosolic surface of lysosomes, reported large Ca^{2+} signals in response to IP_3-evoked Ca^{2+} release, but not in response to SOCE [64••]. Close contacts (MCS) between ER and lysosomes are well documented [65–67], and recent evidence suggests that these contacts form preferentially at ER populated with IP_3Rs [64••]. Another study, which used cytosolic Ca^{2+} sensors targeted to a lysosomal Ca^{2+} channel to determine lysosomal Ca^{2+} content, concluded that Ca^{2+} released from the ER through IP_3Rs is required to fuel lysosomal Ca^{2+} uptake [68•]. There are, however, some concerns in that the sensor used had an affinity for Ca^{2+} that was too high to selectively report local Ca^{2+} signals, and there was some reliance on glycyl-L-phenylalanine 2-naphthylamide (GPN) to release Ca^{2+} from lysosomes, when recent work shows that GPN evokes Ca^{2+} release from the ER [69•]. The studies concur, however, in suggesting that Ca^{2+} uptake by lysosomes is fuelled by Ca^{2+} release from the ER [63,64••,68•], and in suggesting that the lysosomal pH gradient is not required for that Ca^{2+} uptake [64••,68•], although it is required to maintain the ER-lysosome MCS [64••].

Hence, intimate liaisons between ER and lysosomes allow a two-way traffic of Ca^{2+} between them. Ca^{2+} release through lysosomal Ca^{2+} channels is amplified by CICR from ER channels [54,55]; and the ER, through IP_3Rs [64••,68•] and other leak channels [64••] provides Ca^{2+} for a low-affinity lysosomal Ca^{2+}-uptake system. The latter provides a close analogy with MCU in mitochondria. In both cases, the ER works like a piston, concentrating Ca^{2+} from the cytosol into the ER lumen and then delivering it rapidly through a large-conductance channel, the IP_3R, at sufficient local concentration to allow the organelle to sequester it with a low-affinity uptake system (Figure 3b). An unresolved question is whether the mechanisms that license IP_3Rs to evoke cytosolic Ca^{2+} puffs serve also to license the IP_3Rs that deliver Ca^{2+} to lysosomes and mitochondria.

We noted earlier that amongst the many proteins that may contribute to MCS between ER and mitochondria, IP_3R fulfilled a structural role in assembling the junction [36••]. At SOCE MCS too, the Orai Ca^{2+} channel may contribute to assembly of the junction. This 'hijacking' of Ca^{2+} channels to assemble the MCS within which they function may extend also to the ER-lysosome MCS, because although IP_3Rs are not required for their assembly, there is evidence that TPC1 regulates assembly of the MCS between ER and endosomes [70].

Figure 3



Delivering cytosolic Ca^{2+} to other organelles via the ER. **(a)** High-affinity Ca^{2+} pumps (SERCA) allow the ER to concentrate Ca^{2+} from the cytosol, where its concentration (~100 nM) is too low to allow sequestration by low-affinity uptake systems in mitochondria (MCU) or lysosomes (unknown ,?). When they open, IP_3Rs, with their large single-channel conductances, allow Ca^{2+} to be delivered from the ER to the organellar uptake systems at sufficient concentration to support their activity. **(b)** We can regard the ER as a piston that concentrates Ca^{2+} from the cytosol before squirting it at organelles within enclosed MCS.

Concluding remarks

IP_3Rs provide an essential link between receptors in the PM that transduce extracellular stimuli into an increase in intracellular IP_3 concentration, and the redistribution of Ca^{2+} across biological membranes. By rapidly releasing Ca^{2+} from the ER, IP_3Rs selectively deliver Ca^{2+} to the cytosol or other organelles, and they activate SOCE (Figure 1). 'Licensed' IP_3Rs parked alongside SOCE-MCS may allow local regulation of SOCE without compromising other ER functions (Figure 2). Comparisons of ER contacts with lysosomes and mitochondria suggest a general scheme, wherein the ER behaves like a piston, concentrating Ca^{2+} from the cytosol, where its concentration is very low, and then rapidly releasing it through large-conductance IP_3Rs into enclosed MCS, where the high local $[Ca^{2+}]$ is sufficient to fuel Ca^{2+} uptake by the low-affinity Ca^{2+} uptake systems of the organelles (Figure 3).

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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