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Signaling through Ca^{2+} Microdomains from Store-Operated CRAC Channels

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Calcium (Ca^{2+}) ion microdomains are subcellular regions of high Ca^{2+} concentration that develop rapidly near open Ca^{2+} channels in the plasma membrane or internal stores and generate local regions of high Ca^{2+} concentration. These microdomains are remarkably versatile in that they activate a range of responses that differ enormously in both their temporal and spatial profile. In this review, we describe how Ca^{2+} microdomains generated by store-operated calcium channels, a widespread and conserved Ca^{2+} entry pathway, stimulate different signaling pathways, and how the spatial extent of a Ca^{2+} microdomain can be influenced by Ca^{2+} ATPase pumps.

Cytosolic Ca^{2+} controls a plethora of important physiological processes within cells, including secretion, energy production, motility, growth, and differentiation (Clapham 2007). The versatility of Ca^{2+} as an intracellular messenger nevertheless belies its complexity. A fundamental challenge inherent to the use of the pleiotropic Ca^{2+} message is to maintain specificity and thus ensure that only the intended Ca^{2+}-dependent processes are activated, and not others, in response to a specific stimulus, but also to allow different stimuli that use the Ca^{2+} signal to evoke distinct responses.

DECODING THE Ca^{2+} SIGNAL: AMPLITUDE AND FREQUENCY MODULATION

Studies on many different cell types have established that both the amplitude and the temporal dynamics of the Ca^{2+} signal contribute to specificity (Berridge et al. 2003). Ca^{2+} sensors have different affinities for Ca^{2+} and, therefore, in a homogenous system, will be recruited in a sequential manner dictated by the magnitude or kinetics of the Ca^{2+} signal. Antigen-driven secretion in the RBL-2H3 mucosal mast cell line correlated well with the amplitude of the Ca^{2+} signal, suggesting an amplitude-driven response (Kim et al. 1997). Similarly, in B cells, the transcription factor nuclear factor (NF)-κB was stimulated by a high-amplitude Ca^{2+} spike, whereas another transcriptional regulator, nuclear factor of activated T cells (NFAT), was activated instead by a low-amplitude, but sustained, cytosolic Ca^{2+} signal (Dolmetsch et al. 1997). For a given Ca^{2+} increase, different Ca^{2+} sensors can be recruited in a manner dependent on the number of Ca^{2+}-binding sites present. Many Ca^{2+} sensors have multiple Ca^{2+}-binding sites. Perhaps the best-known example is

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calmodulin, which has two classical EF-hands on both the N- and C-lobes of the protein (Hoeflich and Ikura 2002). Increasing the number of identical Ca^{2+}-binding sites on a sensor shifts the Ca^{2+} dependence by altering the relationship between free Ca^{2+} concentration and fractional occupancy of the sensor (Parekh 2011). For a sensor bearing one Ca^{2+}-binding site with a K_D of 1 μM, 50% saturation will occur in the presence of 1 μM Ca^{2+}. In contrast, a sensor harboring four identical binding sites, each with a K_D of 1 μM, will show only 10% occupancy at 1 μM. Therefore, simply increasing the number of Ca^{2+}-binding sites on a sensor provides an effective mechanism through which different amplitudes of Ca^{2+} signal activate distinct Ca^{2+} sensors (Parekh 2011).

Specificity can also be imparted through the ability of different sensors to respond to different temporal patterns of Ca^{2+} signal. It has been shown that the duration of the Ca^{2+} signal is an important factor in contributing to specificity. In dog pancreatic duct epithelial cells, Ca^{2+} oscillations evoked by $P2Y_2$ receptor activation were considerably less effective in driving exocytosis than a nonoscillatory, but longer duration Ca^{2+} signal, but which had the same amplitude as that of the oscillatory response (Jung et al. 2006). However, most studies that have addressed the role of the temporal profile of the Ca^{2+} signal in driving specificity have focused on cytosolic Ca^{2+} oscillations.

Cytosolic Ca^{2+} signals in nonexcitable cells often oscillate over time, both spontaneously and following stimulation of Gq-coupled receptors (Thomas et al. 1996; Parekh 2011). Receptor-evoked cytosolic Ca^{2+} oscillations, which can have periodicities from <10 sec to >400 sec, have been implicated in a wide range of cellular responses, including stimulation of exocytosis (Tse et al. 1993), mitochondrial metabolism (Hajnóczky et al. 1995), activation of different Ca^{2+}-dependent transcription factors in a manner dependent on Ca^{2+} oscillation frequency (Dolmetsch et al. 1998), and neurite extension (Gu and Spitzer 1995). The frequency of the Ca^{2+} oscillations are decoded by sensors that change activity in response to the number of Ca^{2+} oscillations. Such sensors include conventional (Ca^{2+}-dependent) protein kinase Cα, βI, βII, γ, and Ca^{2+}/calmodulin-dependent protein kinase II (De Koninck and Schulman 1998; Oancea and Meyer 1998).

SPATIAL PROFILE: THE THIRD SOURCE OF INFORMATION IN A Ca^{2+} OSCILLATION

Cytosolic Ca^{2+} oscillations in response to receptor stimulation require activation of phospholipase Cβ by Gq-coupled receptors, or phospholipase Cγ by growth factor receptors (Berridge et al. 2003). Increased phospholipase C activity results in the hydrolysis of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2) in the inner leaflet of the plasma membrane (PM). PIP_2 hydrolysis generates the second messenger inositol 1,4,5-trisphosphate ($InsP_3$), which releases Ca^{2+} from the endoplasmic reticulum (ER) through opening of $InsP_3$-gated Ca^{2+} channels, and diacylglycerol, which activates protein kinase C isoforms (Berridge 1987). The mechanisms that generate Ca^{2+} oscillations might be both cell-type- and agonist-dependent (Parekh 2011). In some cell types, $InsP_3$ levels oscillate because of either feedback pathways that uncouple the receptor from phospholipase C (Woods et al. 1987; Kawabata et al. 1996) or because of the Ca^{2+} dependency of phospholipase C (Meyer and Stryer 1988). In other cell types, Ca^{2+} oscillations are thought to arise from coregulation of $InsP_3$ receptors by both $InsP_3$ and cytosolic Ca^{2+} (Bezprozvanny et al. 1991; Finch et al. 1991; Ivorra and Parker 1992). Low cytosolic Ca^{2+} concentrations (~200 nM) facilitate $InsP_3$-dependent Ca^{2+} release, whereas high concentrations (~1 μM) inhibit it (Bezprozvanny et al. 1991). Irrespective of the mode of generation, in most cell types, cytosolic Ca^{2+} oscillations can be maintained, if only for a few minutes, in the absence of external Ca^{2+}, showing that the primary mechanism for generating a Ca^{2+} oscillation is intracellular in origin (Thomas et al. 1996). However, Ca^{2+} oscillations do run down over time in Ca^{2+}-free solution. This is because a fraction of Ca^{2+} released from the ER is extruded from the cell by the PM Ca^{2+} ATPase (PMCA) pump. This was nicely shown in a study by Tepikin and

colleagues (1992), who measured simultaneously cytosolic and extracellular Ca^{2+} after placing pancreatic acinar cells in a small microdroplet. Stimulation with the physiological agonist cholecystokinin elicited oscillations in cytosolic Ca^{2+} and this was followed by pulsatile Ca^{2+} extrusion. The amount of Ca^{2+} extruded during the first Ca^{2+} oscillation was, on average, 39% of the total intracellular pool of mobilizable Ca^{2+} (Tepikin et al. 1992). Therefore, a sizeable fraction of Ca^{2+} released from the $InsP_3$-sensitive store is exported by the PMCA pumps. The cytosolic Ca^{2+} oscillations in Ca^{2+}-free solution run down over time because, after each spike, some Ca^{2+} is extruded from the cell and less Ca^{2+} is therefore available to refill the stores. To sustain oscillations requires Ca^{2+} influx and this is typically achieved through store-operated Ca^{2+} channels (Bird and Putney 2005).

Store-operated Ca^{2+} channels open following the loss of Ca^{2+} from within the ER, and the subsequent Ca^{2+} influx ensures the store is refilled with Ca^{2+} to support the next Ca^{2+} oscillation. The best understood store-operated channel is the Ca^{2+} release-activated Ca^{2+} (CRAC) channel (Hoth and Penner 1992; Parekh and Putney 2005). The molecular basis of the CRAC channel has been identified with the discoveries of STIM (Liou et al. 2005; Roos et al. 2005) and Orai proteins (Feske et al. 2006; Vig et al. 2006a,b; Zhang et al. 2006). STIM proteins are Ca^{2+} sensors that span the ER membrane (Lewis and Prakriya 2015). On store depletion, STIM proteins form multimers, which then migrate within the ER membrane to reach specialized ER–PM junctions located <20 nm below the PM (Wu et al. 2006). At these sites, STIM proteins capture and then gate open Orai proteins, which are the pore-forming subunit of the CRAC channel (Prakriya et al. 2006; Yeromin et al. 2006; Vig et al. 2006a).

Under physiological conditions of external Ca^{2+}, Ca^{2+} entry through CRAC channels is required to counter PMCA pump-driven extrusion of cytosolic Ca^{2+} that has been released from the $InsP_3$-sensitive Ca^{2+} store. Store-operated Ca^{2+} channels therefore sustain cytosolic Ca^{2+} oscillations in response to continual agonist stimulation. Consistent with this, inhibition of the channels or knockdown of Orai1 accelerates the rundown of Ca^{2+} oscillations in the presence of external Ca^{2+} (Wedel et al. 2007; Kar et al. 2012). If PMCA pumps export Ca^{2+} released during Ca^{2+} oscillations, then inhibition of Ca^{2+} extrusion even in the absence of Ca^{2+} entry, should support sustained oscillatory Ca^{2+} signals because any released Ca^{2+} would no longer be extruded from the cell and therefore would now be taken back into the stores by the Ca^{2+} ATPase pumps in the ER membrane. Bird and Putney (2005) developed a neat approach to show this. Rare-earth metal ions, such as Gd^{3+} and La^{3+}, inhibit CRAC channels at low micromolar concentrations and block the PMCA ATPase pump at millimolar levels. In the presence of a low dose of Gd^{3+}, cytosolic Ca^{2+} oscillations following muscarinic receptor activation in HEK293 cells ran down quickly as Ca^{2+} entry was suppressed. However, when a high dose of Gd^{3+} was used to block additionally the PMCA pumps, Ca^{2+} oscillations were maintained despite the full inhibition of CRAC channels (Bird and Putney 2005). Under these conditions, the PM is "tight" to Ca^{2+} flux; Ca^{2+} cannot enter through CRAC channels nor be extruded by the PMCA pump. Therefore, Ca^{2+} released from the ER can no longer be exported from the cell and is therefore returned to the organelle, in readiness for the next oscillatory cycle.

Di Capite et al. (2009) exploited PMCA pump inhibition to address whether Ca^{2+} oscillations of the same amplitude and frequency, regardless of how they were generated, were equally effective in activating downstream gene expression. Stimulation of cysteinyl leukotriene type I receptors in RBL-1 mast cells with the agonist leukotriene C_4 (LTC_4) evoked cytosolic Ca^{2+} oscillations that ran down either in the absence of external Ca^{2+} or after block of CRAC channels. However, the Ca^{2+} oscillations were sustained in the absence of external Ca^{2+}, when the PMCA pump was inhibited by a high dose of La^{3+}. These latter Ca^{2+} oscillations involve Ca^{2+} release from the ER but without any accompanying Ca^{2+} influx, whereas the oscillations in the presence of functional CRAC channels are comprised of Ca^{2+} release followed by Ca^{2+} influx. Only the latter situation results in

elevation of the subplasmalemmal Ca^{2+} concentration, spatially restricted to the vicinity of the CRAC channels. A range of agonist concentrations was tested and it was found that, for any given concentration, the amplitude and frequency of the oscillations were indistinguishable between cells stimulated in the presence of external Ca^{2+} or in its absence (with PMCA pump inhibited to prevent loss of Ca^{2+} from the cell). However, Ca^{2+}-dependent expression of the immediate early gene c-fos occurred only in response to those Ca^{2+} oscillations in which Ca^{2+} entry through CRAC channels was active (Di Capite et al. 2009). Activation of the Ca^{2+}-dependent transcription factor NFAT also showed a similar dependency on CRAC channel activation during oscillatory Ca^{2+} signals evoked by LTC_4 (Kar et al. 2011). These results identified a major role for the spatial profile of the Ca^{2+} oscillation in excitation–transcription coupling driven by Ca^{2+} microdomains near open CRAC channels.

Very close to the mouth of an open Ca^{2+} channel, intracellular Ca^{2+} chelators are not fast enough to prevent the increase in the local Ca^{2+} concentration but reduce the lateral spread of the microdomain to an extent determined by the on-rate of the chelator for Ca^{2+} (Neher 1998) The chelator EGTA binds Ca^{2+} considerably more slowly than BAPTA. Although both reduce the bulk increase in Ca^{2+} similarly (as they have comparable equilibrium affinities for Ca^{2+}), only BAPTA is fast enough to reduce the extent of the Ca^{2+} microdomain (Neher 1998; Parekh 2008). Loading the cytosol of RBL cells with EGTA had no inhibitory effect on LTC_4-driven c-fos expression, whereas BAPTA suppressed c-fos induction (Di Capite et al. 2009). Because BAPTA is fast enough to constrain Ca^{2+} entry to within a few nanometers of the pore, these results support a central role for local Ca^{2+} entry, and not global Ca^{2+} oscillations, in activation of gene expression. An important factor in determining the size of a Ca^{2+} microdomain is the unitary current through the Ca^{2+} channel. The single channel flux depends on the electrochemical gradient for Ca^{2+}. Manipulation of either the electrical or chemical driving forces for Ca^{2+} entry through CRAC channels impaired c-fos expression, despite such maneuvers having little effect on the bulk cytosolic Ca^{2+} increase (Ng et al. 2009). In addition to c-fos and NFAT, several other pathways are activated by local Ca^{2+} entry through CRAC channels. These are described below.

In Madin–Darby canine kidney cells, bradykinin-induced oscillations in cytosolic Ca^{2+} concentration were dependent on Ca^{2+}-permeation through connexin 43 hemichannels expressed in the PM (De Bock et al. 2012). In contrast, ATP-evoked oscillations in cytosolic Ca^{2+} concentration were unaffected by manipulations that inhibited connexin function. Therefore, in some cell types, connexin hemichannels might contribute to Ca^{2+} oscillations in an agonist-specific context.

TARGETS FOR LOCAL Ca^{2+} ENTRY THROUGH CRAC CHANNELS

Adenylyl Cyclase

The first signaling pathway that was shown to be activated by local Ca^{2+} microdomains arising through store-operated Ca^{2+} channels was the adenylyl cyclase/cAMP (Fig. 1). Four isoforms of membrane-bound adenylyl cyclase are regulated by cytosolic Ca^{2+}; adenylyl cyclases 1 and 8 are activated by Ca^{2+}-calmodulin, whereas isoforms 5 and 6 are directly inhibited. In C2-2B glioma cells, Ca^{2+} entry was evoked following store-depletion with thapsigargin, a sesquiterpene lactone extracted from the plant *Thapsia garganica*, which potently inhibits the Ca^{2+} ATPase pump of the ER Ca^{2+} store. When the pump is inhibited, Ca^{2+} can no longer be taken back into the store and, in the presence of continuous flux of Ca^{2+} out of the ER through poorly characterized leak channels, the store gradually depletes of Ca^{2+} and this leads to the opening of CRAC channels. Application of thapsigargin in the glioma cells led to stimulation of type I adenylyl cyclase and inhibition of the type 6 isoform (Chiono et al. 1995; Fagan et al. 1996). In contrast, Ca^{2+} release evoked by thapsigargin, Gq-coupled receptor agonist or ionomycin all failed to alter adenylyl cyclase activity despite raising bulk Ca^{2+} to a higher level. Regulation of adenylyl cyclase activity by store-

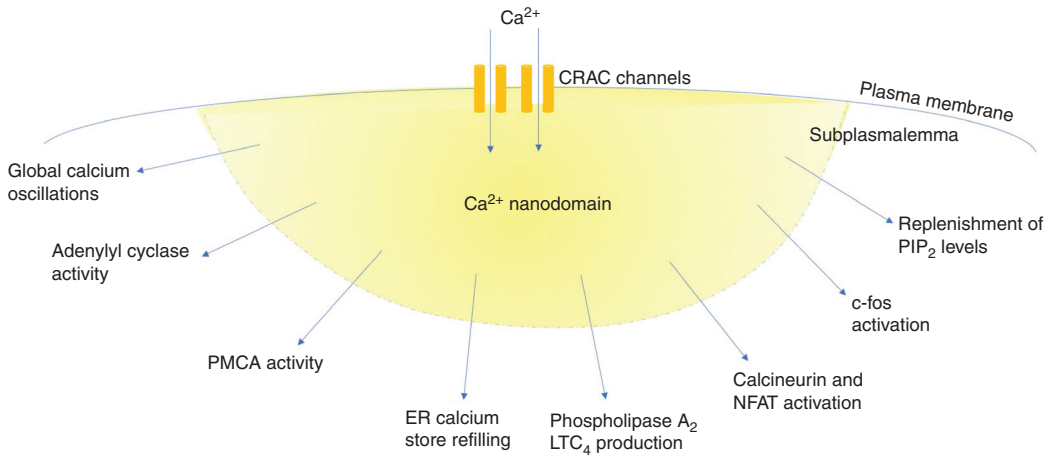

Figure 1. Cartoon summarizes various responses activated by local calcium (Ca^{2+}) entry through Ca^{2+} release-activated Ca^{2+} (CRAC) channels. PMCA, Plasma membrane Ca^{2+} ATPase; ER, endoplasmic reticulum; LTC_4, leukotriene C_4; NFAT, nuclear factor of activated T cells.

operated Ca^{2+} entry was suppressed by cytosolic BAPTA but not EGTA (Fagan et al. 1998), consistent with local regulation by Ca^{2+}. Opening of a different type of Ca^{2+}-permeable ion channel, a transient receptor potential canonical (TRPC) channel, increased subplasmalemmal Ca^{2+} and thereby bulk Ca^{2+} but failed to alter enzyme activity (Shuttleworth and Thompson 1999). Therefore, local Ca^{2+} entry through CRAC channels regulates adenylyl cyclase, suggesting the channel and enzyme colocalize. Strong evidence in support of this has been provided by a combination of pull-down and Förster resonance energy transfer (FRET) studies, which show close association between adenylyl cyclase 8 and Orai1 (Willoughby et al. 2012). Mutagenesis studies identified an arginine-rich sequence in the amino terminus of Orai1 (R28-R29, R31-R32-R33) that was required for binding to adenylyl cyclase 8 (Willoughby et al. 2012). The enzyme is therefore located close to the mouth of the channel, enabling it to respond rapidly and with high fidelity to the local Ca^{2+} increase.

Adenylyl cyclases can also be activated following store depletion in a STIM1-dependent manner independent of Ca^{2+} influx (Lefkimmiatis et al. 2009). Store depletion with ionomycin or chelation of free Ca^{2+} within the store by TPEN both raised cAMP levels in the absence of external Ca^{2+} or after strongly buffering cytosolic Ca^{2+} with BAPTA. STIM1 has also been found to activate adenylyl cyclase in an Orai1-independent manner during melanogenesis (Motiani et al. 2018). In this study, STIM1 was found to interact with adenylyl cyclase 6 at ER–PM junctions after store depletion.

Ca^{2+}-Dependent Phospholipase A_2 and Metabolic Coupling

In several types of immune cell, Ca^{2+} influx stimulates de novo synthesis of the proinflammatory LTC_4, LTD_4, and LTE_4. Leukotrienes are produced from metabolism of arachidonic acid by 5-lipoxygenase enzyme. Ca^{2+} entry through CRAC channels increased arachidonic acid levels through stimulation of Ca^{2+}-dependent cytosolic phospholipase A_2 ($cPLA_2$) and this led to an increase in leukotriene production (Chang and Parekh 2004). Ca^{2+} release from the stores was unable to activate this pathway. Stimulating cells with thapsigargin in the absence of external Ca^{2+} and when PMCA pump activity was suppressed raised bulk cytosolic Ca^{2+} to levels higher than that achieved following store-operated Ca^{2+} entry, but the former was still unable to stimulate arachidonic acid production (Chang et al. 2008). Therefore, $cPLA_2$ is activated by

local Ca^{2+} entry. Manipulations that significantly reduced the unitary flux through CRAC channels, but which had little effect on bulk cytosolic Ca^{2+}, impaired arachidonic acid production, as predicted for a pathway stimulated by Ca^{2+} microdomains. Consistent with this, loading the cytosol with the slow Ca^{2+} chelator EGTA had little effect on $cPLA_2$ activated by CRAC channels (Chang et al. 2008). The underlying signal transduction pathway linking local Ca^{2+} entry to $cPLA_2$ and 5-lipoxygenase involved recruitment of Ca^{2+}-dependent protein kinases α and β1, which in turn activated the MAP kinase ERK (Chang et al. 2006). Activated ERK metabolically coupled the $cPLA_2$ and 5-lipoxygenase pathways. ERK phosphorylated serine 505 of $cPLA_2$, increasing its activity and thus generating more arachidonic acid. At the same time, ERK increased translocation of 5-lipoxygenase to the nuclear membrane, where it bound to, and was activated by, the nuclear membrane protein 5-lipoxygenase-activating protein. Through ERK activity, local Ca^{2+} entry through CRAC channels is therefore able to coordinate production of arachidonic acid with its subsequent metabolism to cysteinyl leukotrienes (Chang et al. 2006).

Regulation of PIP_5 Kinase

$InsP_3$ has a relatively short lifetime in the cytosol, as it is sequentially dephosphorylated within a few seconds via a series of inositol phosphatases to myoinositol monophosphate (Allbritton et al. 1992). The latter is metabolized to inositol by inositol monophosphatases and this step is inhibited by Li^+ (Berridge et al. 1982). Inositol then combines with cytidine diphosphate diacylglycerol to form phosphatidylinositol (PI), which is phosphorylated by PIP_4 and PIP_5 kinases to make PIP_2. Blocking inositol monophosphatases by elevating intracellular Li^+ depletes PIP_2 levels and therefore impairs the production of $InsP_3$, and this has been proposed as a possible mechanism to explain the mood-stabilizing effect of Li^+ treatment on bipolar disorder (Berridge et al. 1982). Ca^{2+} oscillations evoked by LTC_4 in RBL cells ran down in the presence of Li^+, indicating depletion of PIP_2 during continuous exposure to agonist (Alswied and Parekh 2015). In the blowfly salivary gland, a seminal study by Berridge and Fain (1979) showed that the decline in Ca^{2+} flux to continuous exposure to serotonin could be overcome by addition of exogenous inositol. In Li^+-treated RBL cells, exposure to inositol prevented rundown of Ca^{2+} oscillations to LTC_4 (Alswied and Parekh 2015). Application of exogenous phosphatidylinositol 4-phosphate (PI4P) also sustained the cytosolic Ca^{2+} oscillations in Li^+-treated cells, consistent with the need to replenish PIP_2 under these conditions. Sustained Ca^{2+} oscillations were evoked by LTC_4 in the absence of external Ca^{2+} provided PMCA pumps were blocked. These oscillations also ran down in the presence of Li^+ but could no longer be rescued by inositol or PI4P (Alswied and Parekh 2015). Therefore Ca^{2+} influx is required to replenish the PIP_2 pool used by LTC_4. In support of this, block of CRAC channels prevented exogenous inositol and PI4P from sustaining Ca^{2+} oscillations in Li^+-treated cells. PI4P is converted to PIP_2 by PIP_5 kinases. In cells in which PI5P kinase 1α or 1γ had been knocked down, Ca^{2+} oscillations ran down quickly and could not be rescued by PI4P (Alswied and Parekh 2015). Therefore, Ca^{2+} entry through CRAC channels replenishes PIP_2 levels by enhancing PIP_5 kinase activity. This was a local effect because oscillatory Ca^{2+} release in the absence of external Ca^{2+} was unable to replenish PIP_2 levels despite raising cytosolic Ca^{2+} to levels that matched those seen in the presence of external Ca^{2+}.

Ca^{2+}-Dependent Transcription Factors c-fos and NFAT

As described in an earlier section, local Ca^{2+} entry through CRAC channels during cytosolic Ca^{2+} oscillations leads to expression of c-fos and activates NFAT. The molecular mechanisms driving these responses have been teased apart in mast cells. For induction of c-fos expression, Ca^{2+} microdomains near open CRAC channels activate the nonreceptor tyrosine kinase Syk (Ng et al. 2008, 2009). Immunocytochemical and pull-down experiments have shown that Syk associates with Orai1 and remains so after acti-

vation of Ca^{2+} entry (Samanta et al. 2015). Syk phosphorylates, and thereby activates, the transcription factor Stat5 (Ng et al. 2009), mainly Stat5a (Yeh and Parekh 2015). Phosphorylated Stat5 dimerizes and then migrates into the nucleus to increase c-fos transcription.

A slightly different mechanism couples CRAC channel Ca^{2+} microdomains to NFAT1-4 activation. In resting cells, these NFAT proteins are extensively phosphorylated and trapped within the cytosol (Hogan et al. 2003). Dephosphorylation is mediated by the Ca^{2+}-dependent protein phosphatase 2B, calcineurin, the target for immunosuppressants cyclosporine and tacrolimus. Following a local increase in cytosolic Ca^{2+} near CRAC channels, Ca^{2+}-calmodulin binds to calcineurin to increase enzyme activity. Dephosphorylation of NFAT by active calcineurin exposes a nuclear localization sequence, which enables NFAT to migrate into the nucleus. As in some other cell types (Li et al. 2012), a fraction of the cellular pools of calcineurin and NFAT are associated with the PM through binding to the anchoring protein AKAP79 in RBL cells (Kar et al. 2014). Under nonstimulated conditions, Orai1 and AKAP79 do not coimmunoprecipitate but do so after store depletion (Kar et al. 2014). In this way, calcineurin is brought close to the Ca^{2+} microdomain and, once activated, has immediate access to its target NFAT.

CLUSTERING OF CRAC CHANNELS ENHANCES SIGNALING STRENGTH OF LOCAL Ca^{2+}

As the preceding examples show, Ca^{2+} microdomains near open CRAC channels activate a diverse range of downstream signaling pathways. In resting cells, the channel pore-forming subunit Orai1 is distributed throughout the PM. However, after store depletion, STIM1 proteins oligomerize and migrate to peripheral ER located just below the PM. At these specialized ER–PM junctions, STIM1 proteins bind to and gate Orai1, resulting not only in channel activation but also a clustered distribution of the channels (Lewis and Prakriya 2015). This spatial rearrangement of the CRAC channels raises further questions; first, what is the signaling advantage conferred by such clustering of CRAC channels? Second, how high can local Ca^{2+} reach at these regions? Finally, what is the role of Ca^{2+} extrusion in controlling the size of the local Ca^{2+} signal?

Samanta et al. (2015) compared the ability of mutant CRAC channels that did not redistribute to ER–PM junctions, with a similar number of wild-type channels that did relocate, to activate c-fos and NFAT. Both mutant and wild-type channels evoked similar increases in bulk cytosolic Ca^{2+} (Samanta et al. 2015). Channel relocalization to ER–PM junctions led to more robust transcription factor activation and subsequent gene expression, identifying a significant benefit to CRAC channel clustering.

Calculations suggest that about five endogenous CRAC channels occupy each ER–PM junction in a T lymphocyte or RBL cell after store depletion, assuming all the junctions are occupied and contain a similar number of channels (Hogan 2015; Samanta et al. 2015). Assuming the channels are noncoupled, the mean distance between any one CRAC channel picked at random and its nearest neighbor in a junction is ~47 nm. Simulations suggest, for channels spaced 47 nm apart, that the spatial profile of local Ca^{2+} is elevated in the mid-range of the junction and shows two peaks; a large one of ~6 μm corresponding to a single channel and a second, smaller peak that represents spillover from a couple of proximal channels (Samanta et al. 2015). If CRAC channels colocalize at a junction then the minimum distance between two adjacent pores would be ~6.3 nm, based on a lineal measure from juxtaposition of the crystal structures of the hexameric channel. The spatial profile for five such colocalized channels was considerably altered; now, local Ca^{2+} concentration increased to ~13 μM, almost 30-fold higher than the corresponding bulk cytosolic Ca^{2+} concentration (Samanta et al. 2015).

The narrow gap between peripheral ER and the PM excludes mitochondria from being localized adjacent to CRAC channels at the junctions. Therefore, other Ca^{2+} clearance pathways have the potential to regulate directly the size and expanse of the local Ca^{2+} signal. In many nonexcitable cells that rely heavily on store-op-

erated Ca^{2+} entry such as lymphocytes and mast cells, PM Na^{+}-Ca^{2+} exchangers are either absent or weak. Therefore, the PMCA pump would be a potentially important candidate for shaping local Ca^{2+} signals that arise from open CRAC channels.

CROSS TALK BETWEEN CRAC CHANNELS AND PMCA PUMPS

Studies on Ca^{2+} clearance pathways in Jurkat T lymphocytes identified important functional interaction between Ca^{2+} entry through CRAC channels and PMCA pump activity (Bautista et al. 2002). In cells kept in Ca^{2+}-free solution in the presence of thapsigargin, readmission of external Ca^{2+} led to a cytosolic Ca^{2+} increase caused by Ca^{2+} entry through CRAC channels. Subsequent removal of external Ca^{2+} resulted in a bi-exponential decay of the Ca^{2+} signal. Information on Ca^{2+} clearance rates could be extracted from the decay phase of the Ca^{2+} by applying linear fits to the initial phases or exponential fits to the whole process. Following exposure to the combination of antimycin A and oligomycin (to depolarize mitochondria and thus suppress mitochondrial Ca^{2+} buffering), brief readmission of external Ca^{2+} to thapsigargin-treated cells led to an increase in cytosolic Ca^{2+}, which then fell mono-exponentially caused by PMCA pump activity with a time constant of ~40 sec for small Ca^{2+} increases less than ~0.5 μM and this decreased to ~25 sec when cytosolic Ca^{2+} rose to >1.5 μM. The rate of Ca^{2+} clearance rose as the duration of the Ca^{2+} pulse was prolonged, increasing approximately fivefold when Ca^{2+} pulses lasted >60 sec, a process that was termed modulation (Bautista et al. 2002). Recovery from modulation was considerably slower than its onset, with a time constant of ~240 sec, thereby imparting a form of memory to the PMCA pump of the previous Ca^{2+} elevation. By applying a Michaelis–Menten type model, it was found that modulation increased both the maximal rate of the PMCA pump and reduced the K_M. Subsequent experiments established a major role for local Ca^{2+} influx through CRAC channels and not an increase in bulk Ca^{2+} in driving development of the modulation process (Bautista and Lewis 2004). Modulation therefore increases Ca^{2+} efflux rate to counter prolonged Ca^{2+} influx through CRAC channels and thus plays an important role in shaping the spatiotemporal profile of the Ca^{2+} signal.

The mechanism underlying modulation of the PMCA pump in T cells was not identified but two possibilities were considered (Bautista and Lewis 2004). One involved physical coupling between PMCA pumps and CRAC channels perhaps involving the PDZ domain of the PMCA4b (the major isoform expressed in T cells) and Dlg proteins. Physical tethering of the pump to the CRAC channel would expose the pump directly to the Ca^{2+} microdomains that enhance modulation. The second possibility was that coupling between the channels and the pumps was indirect, mediated by a diffusible molecule. In this scenario, a Ca^{2+} sensor would detect the local Ca^{2+} near CRAC channels and then diffuse to more remote PMCA pumps to induce modulation.

Subsequent studies in T cells do not support physical coupling between the PMCA pump and CRAC channels, at least under physiological conditions (Quintana et al. 2011). Stimulation of T cells by engaging the T-cell receptor induces the formation of the immunological synapse, which reflects tight apposition of a T cell with an antigen-presenting cell. The synapse is the site where the T-cell receptor is triggered by its antigen ligand and where Orai1 channels relocalize and cluster. Analysis of the distribution of Orai1 and PMCA4b in T cells using total internal reflection microscopy revealed that the two proteins did not colocalize (Quintana et al. 2011). Orai1 was found within the immunological synapse, whereas PMCA4b was confined to the periphery of the synapse. Despite this spatial separation, Ca^{2+} entry though CRAC channels was still able to induce pump modulation (Quintana et al. 2011).

A plausible diffusible signal linking CRAC channels to PMCA pumps is calmodulin. Several PMCA pump isoforms including PMCA4b bind calmodulin, and Ca^{2+}-calmodulin reduces K_M and increases V_{Max}, mirroring the changes observed in modulation. Studies with a fluorescent calmodulin protein showed that cal-

modulin binding to PMCA pump was slow, developing over several tens of seconds (Penheiter et al. 2003). This is consistent with the observed temporal induction of modulation in T cells, where modulation developed over tens of seconds (Bautista et al. 2002).

Can calmodulin regulation account for modulation of PMCA pumps by CRAC channels? Interestingly, an earlier study by Caride et al. (2001) reported Ca^{2+}-dependent memory to PMCA4b and that this modulation involved calmodulin. In their study, they expressed PMCA pump isoforms in Sf9 insect cells and measured ATPase activity in microsomal membranes. These investigators used a two-pulse protocol to show induction of Ca^{2+}-dependent memory. Microsomes were first exposed to 500 nM Ca^{2+} for 300 sec and then rapidly perfused with 50 nM Ca^{2+}. PMCA2b showed prolonged memory of the high Ca^{2+} pulse in that pump activity declined in low Ca^{2+} with a time constant of ~80 sec. In contrast, PMCA4b activity declined considerably more quickly, with a time constant of ~30 sec (Caride et al. 2001). Studies with a calmodulin-binding peptide suggested that the memory process was a consequence of calmodulin remaining bound to the PMCA pump for several seconds after switching from high (500 nM) to low (50 nM) Ca^{2+}. However, the kinetics of calmodulin regulation of PMCA4b in microsomes do not match the time course of modulation in T lymphocytes. Although both have a broadly similar activation time course, reversal of modulation because of calmodulin dissociation is almost an order of magnitude faster in microsomes (Caride et al. 2001) than in T cells (Bautista et al. 2002). One possible explanation for this mismatch is that interaction between PMCA pumps and the cytoskeleton or other regulatory proteins is lost in the microsomal system and such interactions might affect calmodulin dissociation from PMCA pumps. Another possibility is that in intact cells calmodulin binding might render the pump susceptible to some form of posttranslational modification, trapping it in place. Finally, modulation might not involve calmodulin but another protein that regulates PMCA pump activity. One possible candidate is neuroplastin, an immunoglobulin superfamily protein. In T cells, neuroplastin was found to associate with both PMCA1 and PMCA4 isoforms as well as regulate the levels of pump expression (Korthals et al. 2017). Using cryo-electron microscopy, the structure of PMCA1 in complex with neuroplastin was reported to 4.1 Å resolution (Gong et al. 2018). The transmembrane domain of neuroplastin interacted with transmembrane domain 10 of PMCA1 as well as the linker region between transmembrane domains 8 and 9. Neuroplastin binding induces a marked structural rearrangement of the pump, exposing the cytosolic Ca^{2+}-binding site. An interesting hypothesis has been put forward that suggests binding of PMCA interacting proteins such as STIM1 and POST might alter the association between neuroplastin and the pump (Go and Soboloff 2018). Such a rearrangement could mask or impair access to the cytosolic Ca^{2+}-binding site, reducing pump activity. Whether the PMCA modulation seen in T cells involves neuroplastin is at present unclear.

Can PMCA pump modulation itself be regulated? An elegant study in T cells showed that PMCA pump modulation was suppressed by mitochondrial Ca^{2+} buffering under physiological conditions (Quintana et al. 2011). Following induction of the immunological synapse, Orai1 accumulated at the synapse, as observed by others (Barr et al. 2008; Lioudyno et al. 2008), whereas mitochondria and PMCA pumps were confined to the fringes. There was little colocalization between Orai1 channels and either mitochondria or PMCA pumps, but considerably more overlap between mitochondria and PMCA protein (Quintana et al. 2011). In contrast, following stimulation with thapsigargin, which does not induce synapse formation, PMCA pumps showed a homogeneous distribution through the PM and areas of colocalization between the pumps and Orai1 clusters were observed (Quintana et al. 2011). This might reflect the fact that both proteins are abundant in T cells and so some colocalization might occur by chance owing to random positioning of the proteins in the membrane. Collectively, these data show that PMCA pumps redistribute away from Orai1 after synapse formation and instead are

positioned close to mitochondria. Mitochondria are rapid and high-capacity Ca^{2+} buffers and their juxtaposition with PMCA proteins raises the possibility that mitochondrial Ca^{2+} uptake might siphon Ca^{2+} away from the PMCA pump, suppressing the development of modulation. This turned out to be the case; no modulation occurred following Ca^{2+} entry through Orai1 channels, provided mitochondria were able to take up local Ca^{2+}(Quintana et al. 2011). Mitochondrial depolarization, which inhibits Ca^{2+} uptake into the organelle, enabled PMCA pump modulation to occur. In contrast, in thapsigargin-treated cells, modulation developed normally. Therefore, the rearrangement of Orai1 channels, PMCA pumps, and mitochondria at the immunological synapse enables mitochondria to remove Ca^{2+} from the vicinity of the pumps and thereby suppress the development of modulation.

A further twist to how CRAC channels and PMCA pumps interact has been provided by the report that STIM1 suppresses pump activity in T cells (Ritchie et al. 2012). Activation of Jurkat T cells with the agonist PHA led to strong upregulation of STIM1 and PMCA4 proteins. These investigators adopted the protocol for measuring PMCA pump activity previously described (Bautista et al. 2002), wherein CRAC channels were activated by exposure to thapsigargin and pump activity was measured following removal of external Ca^{2+}, and found that Ca^{2+} decay was a double exponential process, as also shown in previous reports from T cells (Ritchie et al. 2012). Ca^{2+} clearance was initially fast, with a half-time of ~12 sec and this was followed by a much slower clearance phase, with a half-time of ~80 sec. Increased levels of STIM1, either by PHA activation or simply by transfection of STIM1 plasmid, slowed Ca^{2+} clearance with a pronounced effect on the second phase. Whereas the half-time of the first phase increased slightly from 12 sec to 18 sec after PHA treatment, the second phase increased from 80 sec to 142 sec. Studies with truncated constructs identified the carboxy-terminal domain of STIM1 close to the poly lysine tail as being responsible for this inhibitory action (Ritchie et al. 2012). It is unclear whether STIM1 directly binds to the PMCA pump but immunoprecipitation experiments in the absence of PHA revealed significant interaction of STIM1 and PMCA under resting conditions, and this was not altered much by PHA stimulation (Ritchie et al. 2012). The amount of colocalization between STIM1 and PMCA pump was measured using fluorescence microscopy and only minimal overlap was observed in cells under resting conditions, but strong colocalization was seen after PHA-induced activation. Thapsigargin had no effect on STIM1/PMCA colocalization, suggesting STIM1 regulation of PMCA pump was independent of store depletion. Collectively, these results suggest that STIM1 impairs PMCA pump activity and therefore serves to sustain Ca^{2+} entry through CRAC channels independent of store depletion.

Interestingly, in the study by Ritchie et al. (2012), Ca^{2+} clearance kinetics was biphasic even after inhibition of mitochondrial Ca^{2+} uptake. This is different from previous reports in T cells that showed inhibition of mitochondrial Ca^{2+} uptake by depolarization with antimycin A and oligomycin to impair flux through the uniporter channel switched the biphasic decay to a monophasic event (Bautista et al. 2002; Bautista and Lewis 2004; Quintana et al. 2011). The slow second-phase reflected slow release of Ca^{2+} from mitochondria after the organelle had been loaded with Ca^{2+} during store-operated Ca^{2+} influx (Bautista et al. 2002). In contrast, RU360, considered a membrane permeable inhibitor of the uniporter channel, had no effect on Ca^{2+} clearance kinetics compared with control cells (Ritchie et al. 2012).

In T cells, there are therefore two models for how PMCA pumps impact on local Ca^{2+} concentration at the immunological synapse (Fig. 2). The models yield the same output, namely, PMCA activity is reduced after CRAC channel activation, but differ mechanistically in how this is accomplished. The Quintana et al. model (Fig. 2A) proposes that PMCA pump activity is low because mitochondria buffer Ca^{2+} at the edges of the synapse, in which the pumps are located, and the reduction in local Ca^{2+} both directly reduces PMCA pump activity and suppresses modulation from developing (Quintana et al.

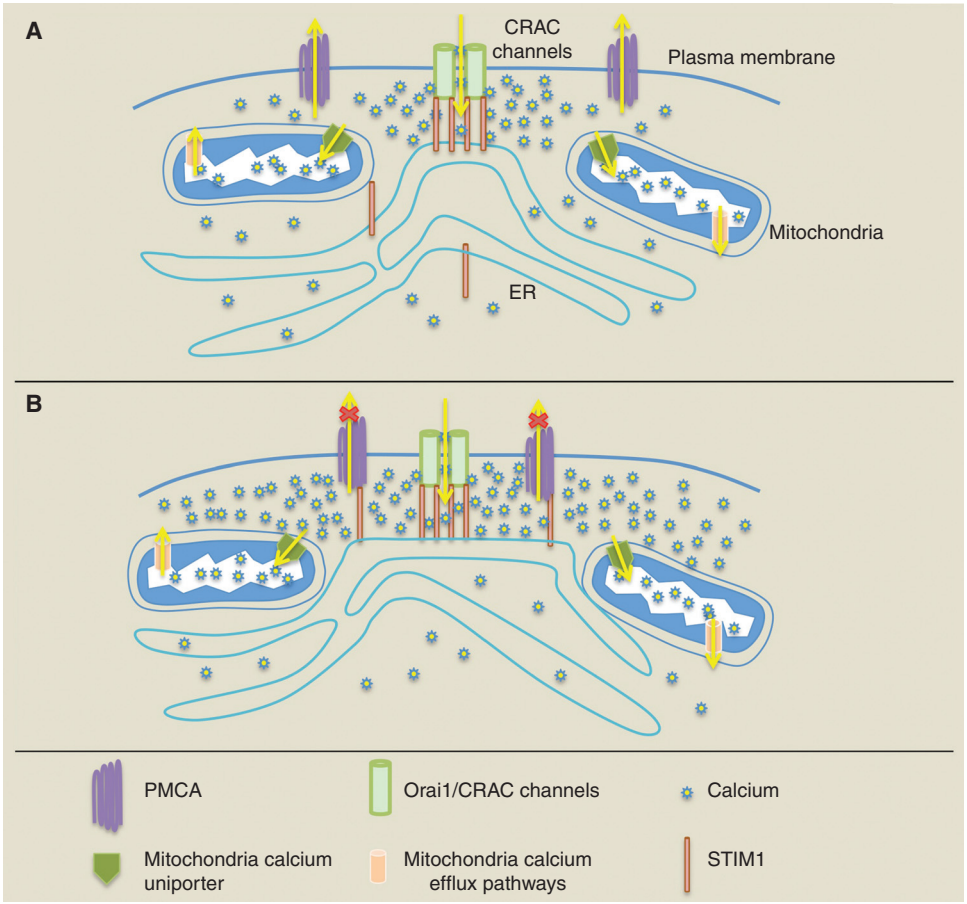

Figure 2. The cartoon summarizes two models (*A*,*B*) for how the plasma membrane Ca^{2+} ATPase (PMCA) pump activity is regulated at the immunological synapse in T cells (see text for further details). CRAC, Ca^{2+} release-activated Ca^{2+}; ER, endoplasmic reticulum.

2011). The Ritchie et al. model (Fig. 2B) posits that PMCA pump activity is low because of STIM1 inhibition of the transporter (Ritchie et al. 2012). The STIM1 block is presumably constitutive because the coimmunoprecipitation data suggested PMCA pump and STIM1 already interacted at rest and no further association was seen following stimulation with thapsigargin (Ritchie et al. 2012). If PMCA pumps are blocked by STIM1 at the synapse, then they should not be able to develop modulation. However, the data from Quintana et al. (2011) show robust modulation at the immunological synapse after mitochondrial depolarization. Now, local Ca^{2+} can increase sufficiently to stimulate the modulation process, an observation that is not predicted by model 2.

The models could be reconciled by postulating a Ca^{2+}-dependence to STIM1 block of the PMCA pump. During the fast phase of cytosolic Ca^{2+} clearance, cytosolic Ca^{2+} levels are high and this might prevent STIM1 from inhibiting PMCA pump activity. However, during the slow phase of Ca^{2+} clearance cytosolic Ca^{2+} is considerably reduced, and this could lead to STIM1-mediated PMCA inhibition. Such a mechanism could also explain why modulation occurs following mitochondrial depolarization (Figs. 1 and 2A); the elevated local Ca^{2+} concentration will no longer enable STIM1 to block PMCA

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pumps. It will be interesting to see in the future whether there is a Ca^{2+} dependence to STIM1 block of the PMCA pump or whether other factors account for the differences between the two models. Regardless, the PMCA pump has emerged as an important factor that can help shape local Ca^{2+} signals near CRAC channels and thus impact on their signaling strength. How CRAC channel-dependent Ca^{2+} microdomains communicate with PMCA pumps and whether such functional coupling can be regulated by stimulation of cell-surface receptors are interesting questions for the future.

INTRACELLULAR Orai1 CHANNELS

Although the majority of Orai channels are in the PM, a fraction is found on intracellular organelles. That these Orai proteins function as intracellular Ca^{2+} release channels was elegantly shown by Hille and colleagues (Dickson et al. 2012). Secretory granules bud from the *trans*-Golgi network and after maturation carry cargo to the PM, which is released by exocytosis. Free Ca^{2+} concentration within a secretory granule is ~70 μM (Dickson et al. 2012), around three orders of magnitude larger than resting cytosolic free Ca^{2+}. Using a FRET cameleon probe targeted to the secretory granule lumen, Dickson and colleagues found that stimulation of cell-surface Gq-coupled P2Y receptors led to slow release of Ca^{2+} from secretory granules into the cytosol. Three pieces of evidence suggested this form of Ca^{2+} release was mediated by Orai1 channels (Dickson et al. 2012). First, Ca^{2+} mobilization from the granules was prevented by stimulating P2Y receptors in the absence of external Ca^{2+}. Second, the CRAC channel blocker BTP2 prevented Ca^{2+} release from the granules and third, expression of a nonconducting dominant-negative Orai1 channel (Orai1-E106A) prevented Ca^{2+} release from the granules during Gq-coupled P2Y receptor stimulation (using ATP). Confocal colocalization analysis using Orai1–GFP and tPA-mcherry (a marker for secretory granules) revealed a fraction of Orai1 channels resided on secretory granules. In contrast, there was little colocalization between STIM1 and secretory granules at rest. However, P2Y receptor stimulation increased colocalization between STIM1 and secretory granules and this was prevented by removal of external Ca^{2+}. To explain these findings, these investigators proposed a very interesting model. Following stimulation, STIM1–Orai1 clusters formed below the PM. Subsequent store-operated Ca^{2+} entry raised local Ca^{2+} and this led to disaggregation of some STIM1 molecules from Orai1. The released STIM1 proteins were able, after a delay, to bind to and activate low-density Orai1 channels on secretory granules. Because the Ca^{2+} concentration within a granule is considerably lower than extracellular Ca^{2+} concentration (~70 μM compared with 1–2 mM), the local Ca^{2+} near open Orai channels in the granule membrane will be much lower than the corresponding level at the PM and so STIM1 will presumably remain associated with granule Orai1 for longer.

What could be the physiological role of Orai1-dependent Ca^{2+} release from secretory granules? Because these Orai1 channels are activated by STIM1 resident in the ER membrane, Ca^{2+} release from the granules could contribute to effective refilling of the ER with Ca^{2+}.

Another nice example of local Ca^{2+} signals generated by intracellular Orai1 stems from studies in neutrophils (Nunes et al. 2012). These phagocytes ingest foreign particles and degrade them within phagocytic vesicles. STIM1 was found to sustain phagocytosis by bringing thin ER cisternae to phagosomes, which then formed tight ER–phagosomal junctions. Local periphagosomal Ca^{2+} signals were seen in intact cells following particle ingestion and the frequency of these signals was significantly reduced by STIM1 ablation (Nunes et al. 2012). The local Ca^{2+} signals arose from phagosomal store-operated Orai1 channels that were activated by STIM1. These findings provide new potential targets for the treatment of infections caused by intracellular pathogens.

TUNNELING OF LOCAL Ca^{2+} ENTRY NEAR Orai1 CHANNELS THROUGH THE ER

Local Ca^{2+} entry through CRAC channels can be delivered to distant targets through a mechanism that bypasses diffusion through the cytosol. This

pathway involves Ca^{2+} tunneling through the ER and was first described in pancreatic acinar cells (Mogami et al. 1997). In these polarized epithelial cells, store-operated Ca^{2+} entry occurs at the basolateral membrane. Local Ca^{2+} influx is then rapidly pumped into basolateral ER by SERCA pumps. The ER in acinar cells is a contiguous organelle, protruding into the apical pole where trypsin-containing secretory granules are confined (Park et al. 2000). $InsP_3$ receptors are found at high density in the apical pole. The ER has a low Ca^{2+}-buffering capacity compared with the cytosol (Mogami et al. 1999), and therefore Ca^{2+} that is taken up into the ER at the basolateral pole diffuses rapidly to the apical portion where it can be released into the cytosol through $InsP_3$ receptors. In this way, local Ca^{2+} influx at the basolateral region can drive Ca^{2+}-dependent exocytosis at the apical end. Ca^{2+} tunneling also couples store-operated Ca^{2+} entry to Ca^{2+}-activated Cl^- channels in *Xenopus* oocytes (Courjaret and Machaca 2014). In this system, the Ca^{2+} channels and Ca^{2+}-activated Cl^- channels are located apart but local Ca^{2+} influx is taken up into the ER by SERCA pumps and then released by $InsP_3$ receptors positioned close to the Cl^- channels, reminiscent of tunneling in acinar cells (Petersen et al. 2017). In mast cells, stimulation of Gq-coupled cysteinyl leukotriene type 1 receptors with the agonist LTC_4 generates oscillations in nuclear Ca^{2+} that arise from opening of $InsP_3$ receptors in the inner nuclear membrane and maintains NFAT4 transcription factor activity and subsequent cytokine gene expression (Kar et al. 2016). The oscillations run down rapidly in the presence of the CRAC channel blocker BTP2 or when external Ca^{2+} is removed. Ca^{2+} influx contributes little to the amplitude or decay rate of each cytoplasmic Ca^{2+} oscillation evoked by LTC_4 (Di Capite et al. 2009). That the nuclear store, which is contiguous with the ER, is replenished by Ca^{2+} flux through CRAC channels at the cell surface is therefore another example of long-range Ca^{2+} tunneling.

CONCLUSION

Ca^{2+} microdomains near open Ca^{2+}-permeable ion channels that populate the PM or internal Ca^{2+} stores regulate a range of cellular responses over a broad temporal bandwidth. Recent work has led to a remarkable wave of progress in understanding how these local Ca^{2+} signals are decoded by cells and how different types of Ca^{2+} channel tap into different intracellular signaling pathways. Recent advances in superresolution microscopy combined with increasingly sophisticated structural and biochemical approaches should provide more detailed insight into the properties of local Ca^{2+} signals and how they might be manipulated in the treatment of human disease.

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REFERENCES

Allbritton NL, Meyer T, Stryer L. 1992. Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. *Science* **258:** 1812–1815. doi:10.1126/science.1465619

Alswied A, Parekh AB. 2015. Ca^{2+} influx through store-operated calcium channels replenishes the functional phosphatidylinositol 4,5-bisphosphate pool used by cysteinyl leukotriene type I receptors. *J Biol Chem* **290:** 29555–29566. doi:10.1074/jbc.M115.678292

Barr VA, Bernot KM, Srikanth S, Gwack Y, Balagopalan L, Regan CK, Helman DJ CL, Oh-Hora M S, Rao A, et al. 2008. Dynamic movement of the calcium sensor STIM1 and the calcium channel Orai1 in activated T-cells: Puncta and distal caps. *Mol Biol Cell* **19:** 2802–2817. doi:10.1091/mbc.e08-02-0146

Bautista DM, Lewis RS. 2004. Modulation of plasma membrane calcium-ATPase activity by local calcium microdomains near CRAC channels in human T cells. *J Physiol* **556:** 805–817. doi:10.1113/jphysiol.2003.060004

Bautista DM, Hoth M, Lewis RS. 2002. Enhancement of calcium signalling dynamics and stability by delayed modulation of the plasma-membrane calcium-ATPase in human T cells. *J Physiol* **541:** 877–894. doi:10.1113/jphysiol.2001.016154

Berridge MJ. 1987. Inositol trisphosphate and diacylglycerol: Two interacting second messengers. *Annu Rev Biochem* **56:** 159–193. doi:10.1146/annurev.bi.56.070187.001111

Berridge MJ, Fain JN. 1979. Inhibition of phosphatidylinositol synthesis and the inactivation of calcium entry after prolonged exposure of the blowfly salivary gland to 5-hydroxytryptamine. *Biochem J* **178:** 59–69.

Berridge MJ, Downes CP, Hanley MR. 1982. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem J* **206:** 587–595. doi:10.1042/bj2060587

Berridge MJ, Bootman MD, Roderick HL. 2003. Calcium signalling: Dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* **4:** 517–529. doi:10.1038/nrm1155

Bezprozvanny I, Watras J, Ehrlich BE. 1991. Bell-shaped calcium-response curves of Ins(1,4,5)P_3– and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature* **351:** 751–754. doi:10.1038/351751a0

Bird GS, Putney JWJ. 2005. Capacitative calcium entry supports calcium oscillations in human embryonic kidney cells. *J Physiol* **562:** 697–706. doi:10.1113/jphysiol.2004.077289

Caride AJ, Penheiter AR, Filoteo AG, Bajzer Z, Enyedi A, Penniston JT. 2001. The plasma membrane calcium pump displays memory of past calcium spikes. *J Biol Chem* **276:** 39797–39804. doi:10.1074/jbc.M104380200

Chang WC, Parekh AB. 2004. Close functional coupling between Ca^{2+} release-activated Ca^{2+} channels, arachidonic acid release and leukotriene secretion. *J Biol Chem* **279:** 29994–29999. doi:10.1074/jbc.M403969200

Chang WC, Nelson C, Parekh AB. 2006. Ca^{2+} influx through CRAC channels activates cytosolic phospholipase A_2, leukotriene C_4 secretion and expression of c-fos through ERK-dependent and independent pathways in mast cells. *FASEB J* **20:** 2381–2383. doi:10.1096/fj.06-6016fje

Chang WC, Di Capite J, Singaravelu K, Nelson C, Halse V, Parekh AB. 2008. Local Ca^{2+} influx through Ca^{2+} release-activated Ca^{2+} (CRAC) channels stimulates production of an intracellular messenger and an intercellular pro-inflammatory signal. *J Biol Chem* **283:** 4622–4631. doi:10.1074/jbc.M705002200

Chiono M, Mahey R, Tate G, Cooper DMF. 1995. Capacitative Ca^{2+} entry exclusively inhibits cAMP synthesis in C6-2B glioma cells. Evidence that physiologically evoked Ca^{2+} entry regulates Ca^{2+}-inhibitable adenylyl cyclase in non-excitable cells. *J Biol Chem* **270:** 1149–1155. doi:10.1074/jbc.270.3.1149

Clapham DE. 2007. Calcium signaling. *Cell* **131:** 1047–1058. doi:10.1016/j.cell.2007.11.028

Courjaret R, Machaca K. 2014. Mid-range Ca^{2+} signalling mediated by functional coupling between store-operated Ca^{2+} entry and IP3-dependent Ca^{2+} release. *Nat Commun* **5:** 3916. doi:10.1038/ncomms4916

De Bock M, Wang N, Bol M, Decrock E, Ponsaerts R, Bultynck G, Dupont G, Leybaert L. 2012. Connexin 43 hemichannels contribute to cytoplasmic Ca^{2+} oscillations by providing a bimodal Ca^{2+}-dependent Ca^{2+} entry pathway. *J Biol Chem* **287:** 12250–12266. doi:10.1074/jbc.M111.299610

De Koninck P, Schulman H. 1998. Sensitivity of CaM kinase II to the frequency of Ca^{2+} oscillations. *Science* **279:** 227–230. doi:10.1126/science.279.5348.227

Di Capite J, Ng SW, Parekh AB. 2009. Decoding of cytoplasmic Ca^{2+} oscillations through the spatial signature drives gene expression. *Curr Biol* **19:** 853–858. doi:10.1016/j.cub.2009.03.063

Dickson EJ, Duman JG, Moody MW LC, Hille B. 2012. Orai-STIM–mediated Ca^{2+} release from secretory granules revealed by a targeted Ca^{2+} and pH probe. *Proc Natl Acad Sci* **109:** E3539–E3548. doi:10.1073/pnas.1218247109

Dolmetsch RE, Lewis RS, Goodnow CC, Healy JI. 1997. Differential activation of transcription factors induced by Ca^{2+} response amplitude and duration. *Nature* **386:** 855–858. doi:10.1038/386855a0

Dolmetsch RE, Xu K, Lewis RS. 1998. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature* **392:** 933–936. doi:10.1038/31960

Fagan KA, Mahey R, Cooper DMF. 1996. Functional co-localization of transfected Ca^{2+}-stimulable adenylyl cyclases with capacitative Ca^{2+} entry sites. *J Biol Chem* **271:** 12438–12444. doi:10.1074/jbc.271.21.12438

Fagan KA, Mons N, Cooper DMF. 1998. Dependence of the Ca^{2+}-inhibitable adenylyl cyclase of C6–2B glioma cells on capacitative Ca^{2+} entry. *J Biol Chem* **273:** 9297–9305. doi:10.1074/jbc.273.15.9297

Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SV, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A. 2006. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* **441:** 179–185. doi:10.1038/nature04702

Finch EA, Turner TJ, Goldin SM. 1991. Calcium as a co-agonist of inositol 1,4,5-trisphosphate-induced calcium release. *Science* **252:** 443–446. doi:10.1126/science.2017683

Go CK, Soboloff J. 2018. Hold the door: hPMCA1/neuroplastin interactions regulate Ca^{2+} binding site accessibility. *Cell Calcium* **76:** 135–136. doi:10.1016/j.ceca.2018.10.003

Gong D, Chi X, Ren K, Huang G, Zhou G, Yan N, Lei J, Zhou Q. 2018. Structure of the human plasma membrane Ca^{2+} ATPase 1 in complex with its obligatory subunit neuroplastin. *Nat Commun* **9:** 3623. doi:10.1038/s41467-018-06075-7

Gu X, Spitzer NC. 1995. Distinct aspects of neuronal differentiation encoded by frequency of spontaneous Ca^{2+} transients. *Nature* **375:** 784–787. doi:10.1038/375784a0

Hajnóczky G, Robb-Gaspers LD, Seitz MB, Thomas AP. 1995. Decoding of cytosolic calcium oscillations in the mitochondria. *Cell* **82:** 415–424. doi:10.1016/0092-8674(95)90430-1

Hoeflich KP, Ikura M. 2002. Calmodulin in action: Diversity in target recognition and activation mechanisms. *Cell* **108:** 739–742. doi:10.1016/S0092-8674(02)00682-7

Hogan PG. 2015. The STIM1–ORAI1 microdomain. *Cell Calcium* **58:** 357–367. doi:10.1016/j.ceca.2015.07.001

Hogan PG, Chen L, Nardone J, Rao A. 2003. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev* **17:** 2205–2232. doi:10.1101/gad.1102703

Hoth M, Penner R. 1992. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature* **355:** 353–356. doi:10.1038/355353a0

Ivorra I, Parker I. 1992. Inhibition by Ca^{2+} of inositol trisphosphate-mediated Ca^{2+} liberation: A possible mechanism for oscillatory release of Ca^{2+}. *Proc Natl Acad Sci* **87:** 260–264. doi:10.1073/pnas.87.1.260

Jung SR, Kim K, Hille B, Nguyen TD, Koh DS. 2006. Pattern of Ca^{2+} increase determines the type of secretory mechanism activated in dog pancreatic duct epithelial cells. *J Physiol* 163–178. doi:10.1113/jphysiol.2006.114876

Kar P, Nelson C, Parekh AB. 2011. Selective activation of the transcription factor NFAT1 by calcium microdomains near Ca^{2+} release-activated Ca^{2+} (CRAC) channels. *J Biol Chem* **286:** 14795–14803. doi:10.1074/jbc.M111.220582

Kar P, Bakowski D, Di Capite J, Nelson C, Parekh AB. 2012. Different agonists recruit different stromal interaction molecule proteins to support cytoplasmic Ca^{2+} oscillations and gene expression. *Proc Natl Acad Sci* **109:** 6969–6974. doi:10.1073/pnas.1201204109

Kar P, Samanta K, Kramer H, Morris O, Bakowski D, Parekh AB. 2014. Dynamic assembly of a membrane signaling complex enables selective activation of NFAT by Orai1. *Curr Biol* **24:** 1361–1368. doi:10.1016/j.cub.2014.04.046

Kar P, Mirams GR, Christian HC, Parekh AB. 2016. Control of NFAT isoform activation and NFAT-dependent gene expression through two coincident and spatially segregated intracellular Ca^{2+} signals. *Mol Cell* **64:** 746–759. doi:10.1016/j.molcel.2016.11.011

Kawabata S, Tsutsumi R, Kohara A, Yamaguchi T, Nakanishi S, Okada M. 1996. Control of calcium oscillations by phosphorylation of metabotropic glutamate receptors. *Nature* **383:** 89–92. doi:10.1038/383089a0

Kim TD, Eddlestone GT, Mahmoud SF, Kuchtey J, Fewtrell C. 1997. Correlating Ca^{2+} responses and secretion in individual RBL-2H3 mucosal mast cells. *J Biol Chem* **272:** 31225–31229. doi:10.1074/jbc.272.50.31225

Korthals M, Langnaese K, Smalla KH, Kaehne T, Herrera-Molina R, Handschuh J, Lehmann AC, Mamula D, Naumann M, Seidenbecher C, et al. 2017. A complex of neuroplastin and plasma membrane Ca^{2+} ATPase controls T cell activation. *Sci Rep* **7:** 8358. doi:10.1038/s41598-017-08519-4

Lefkimmiatis K, Srikanthan M, Maiellaro I, Moyer MP, Curci S, Hofer AM. 2009. Store-operated cyclic AMP signalling mediated by STIM1. *Nat Cell Biol* **11:** 433–442. doi:10.1038/ncb1850

Lewis RS, Prakriya M. 2015. Store-operated calcium channels. *Physiol Rev* **95:** 1383–1436. doi:10.1152/physrev.00020.2014

Li H, Pink MD, Murphy JG, Stein A, Dell'Acqua ML, Hogan PG. 2012. Balanced interactions of calcineurin with AKAP79 regulate Ca^{2+}-calcineurin-NFAT signaling. *Nat Struct Mol Biol* **19:** 337–345. doi:10.1038/nsmb.2238

Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell Jr JE, Meyer T. 2005. STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx. *Curr Biol* **15:** 1235–1241. doi:10.1016/j.cub.2005.05.055

Lioudyno MI, Kozak JA, Penna A, Safrina O, Zhang SL, Sen D, Roos J, Stauderman KA, Cahalan MD. 2008. Orai1 and STIM1 move to the immunological synapse and are upregulated during T cell activation. *Proc Natl Acad Sci* **105:** 2011–2016. doi:10.1073/pnas.0706122105

Meyer T, Stryer L. 1988. Molecular model for receptor-stimulated calcium spiking. *Proc Natl Acad Sci* **85:** 5051–5055. doi:10.1073/pnas.85.14.5051

Mogami H, Nakano K, Tepikin AV, Petersen OH. 1997. Ca^{2+} flow via tunnels in polarized cells: Recharging of apical Ca^{2+} stores by focal Ca^{2+} entry through basal membrane patch. *Cell* **88:** 49–55. doi:10.1016/S0092-8674(00)81857-7

Mogami H, Gardner J, Gerasimenko OV, Camello P, Petersen OH, Tepikin AV. 1999. Calcium binding capacity of the cytosol and endoplasmic reticulum of mouse pancreatic acinar cells. *J Physiol* **518:** 463–467. doi:10.1111/j.1469-7793.1999.0463p.x

Motiani RK, Tanwar J, Raja DA, Vashisht A, Khanna S, Sharma S, Srivastava S, Sivasubbu S, Natarajan VT, Gokhale RS. 2018. STIM1 activation of adenylyl cyclase 6 connects Ca^{2+} and cAMP signaling during melanogenesis. *EMBO J* **37:** e97597. doi:10.15252/embj.201797597

Neher E. 1998. Vesicle pools and Ca^{2+} microdomains: New tools for understanding their roles in neurotransmitter release. *Neuron* **20:** 389–399. doi:10.1016/S0896-6273(00)80983-6

Ng SW, DiCapite JL, Singaravelu K, Parekh AB. 2008. Sustained activation of the tyrosine kinase Syk by antigen in mast cells requires local Ca^{2+} influx through Ca^{2+} release-activated Ca^{2+} channels. *J Biol Chem* **283:** 31348–31355. doi:10.1074/jbc.M804942200

Ng SW, Nelson C, Parekh AB. 2009. Coupling of Ca^{2+} microdomains to spatially and temporally distinct cellular responses by the tyrosine kinase Syk. *J Biol Chem* **284:** 24767–24772. doi:10.1074/jbc.M109.011692

Nunes P, Cornut D, Bochet V, Hasler U, Oh-Hora M, Waldburger JM, Demaurex N. 2012. STIM1 juxtaposes ER to phagosomes, generating Ca^{2+} hotspots that boost phagocytosis. *Curr Biol* **22:** 1990–1997. doi:10.1016/j.cub.2012.08.049

Oancea E, Meyer T. 1998. Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. *Cell* **95:** 307–318. doi:10.1016/S0092-8674(00)81763-8

Parekh AB. 2008. Ca^{2+} microdomains near plasma membrane Ca^{2+} channels: Impact on cell function. *J Physiol* **586:** 3043–3054. doi:10.1113/jphysiol.2008.153460

Parekh AB. 2011. Decoding cytosolic Ca^{2+} oscillations. *Trends Biochem Sci* **36:** 78–87. doi:10.1016/j.tibs.2010.07.013

Parekh AB, Putney JWJ. 2005. Store-operated calcium channels. *Physiol Rev* **85:** 757–810. doi:10.1152/physrev.00057.2003

Park MK, Petersen OH, Tepikin AV. 2000. The endoplasmic reticulum as one continuous Ca^{2+} pool: Visualization of rapid Ca^{2+} movements and equilibration. *EMBO J* **19:** 5729–5739. doi:10.1093/emboj/19.21.5729

Penheiter AR, Bajzer Z, Filoteo AG, Thorogate R, Torok K, Caride AJ. 2003. A model for the activation of plasma membrane calcium pump isoform 4b by calmodulin. *Biochemistry* **42:** 12115–12124. doi:10.1021/bi027098+

Petersen OH, Courjaret R, Machaca K. 2017. Ca^{2+} tunnelling through the ER lumen as a mechanism for delivering Ca^{2+} entering via store-operated Ca^{2+} channels to specific target sites. *J Physiol* **595:** 2999–3014. doi:10.1113/JP272772

Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. 2006. Orai1 is an essential pore subunit of the CRAC channel. *Nature* **443:** 230–233. doi:10.1038/nature05122

Quintana A, Pasche M, Junker C, Al-Ansary D, Rieger H, Kummerow C, Nunez L, Villalobos C, Meraner P, Becherer U, et al. 2011. Calcium microdomains at the immunological synapse: How Orai channels, mitochondria and calcium pumps generate local calcium signals for efficient T-cell activation. *EMBO J* **30:** 3895–3912. doi:10.1038/emboj.2011.289

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Ritchie MF, Samakai E, Soboloff J. 2012. STIM1 is required for attenuation of PMCA-mediated Ca^{2+} clearance during T-cell activation. *EMBO J* **31:** 1123–1133. doi:10.1038/emboj.2011.495

Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, et al. 2005. STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *J Cell Biol* **169:** 435–445. doi:10.1083/jcb.200502019

Samanta K, Kar P, Mirams GR, Parekh AB. 2015. Ca^{2+} channel re-localization to plasma-membrane microdomains strengthens activation of Ca^{2+}-dependent nuclear gene expression. *Cell Rep* **12:** 203–216. doi:10.1016/j.celrep.2015.06.018

Shuttleworth TJ, Thompson JL. 1999. Discriminating between capacitative and arachidonate-activated Ca^{2+} entry pathways in HEK293 cells. *J Biol Chem* **274:** 31174–31178. doi:10.1074/jbc.274.44.31174

Tepikin AV, Voronina SG, Gallacher DV, Petersen OH. 1992. Pulsatile Ca^{2+} extrusion from single pancreatic acinar cells during receptor-activated cytosolic Ca^{2+} spiking. *J Biol Chem* **267:** 14073–14076.

Thomas AP, Bird GS, Hajnóczky G, Robb-Gaspers LD, Putney JWJ. 1996. Spatial and temporal aspects of cellular calcium signaling. *FASEB J* **10:** 1505–1517. doi:10.1096/fasebj.10.13.8940296

Tse A, Tse FW, Almers W, Hille B. 1993. Rhythmic exocytosis stimulated by GnRH-induced Ca^{2+} oscillations in rat gonadotropes. *Science* **260:** 82–84. doi:10.1126/science.8385366

Vig M, Beck A, Billingsley JM, Lis A, Parvez S, Peinelt C, Koomoa DL, Soboloff J, Gill DL, Fleig A, et al. 2006a. CRACM1 multimers form the ion-selective pore of the CRAC channel. *Curr Biol* **16:** 2073–2079. doi:10.1016/j.cub.2006.08.085

Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, et al. 2006b. CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry. *Science* **312:** 1220–1223. doi:10.1126/science.1127883

Wedel B, Boyles RR, Putney JW, Bird GS. 2007. Role of the store-operated calcium entry proteins Stim1 and Orai1 in muscarinic cholinergic receptor-stimulated calcium oscillations in human embryonic kidney cells. *J Physiol* **579:** 679–689. doi:10.1113/jphysiol.2006.125641

Willoughby D, Everett KL, Halls ML, Pacheco J, Skroblin P, Vaca L, Klussmann E, Cooper DM. 2012. Direct binding between Orai1 and AC8 mediates dynamic interplay between Ca^{2+} and cAMP signaling. *Sci Signal* **5:** ra29. doi:10.1126/scisignal.2002299

Woods NM, Cuthbertson KS, Cobbold PH. 1987. Phorbol-ester-induced alterations of free calcium ion transients in single rat hepatocytes. *Biochem J* **246:** 619–623. doi:10.1042/bj2460619

Wu MM, Buchanan J, Luik RM, Lewis RS. 2006. Ca^{2+} store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane. *J Cell Biol* **174:** 803–813. doi:10.1083/jcb.200604014

Yeh YC, Parekh AB. 2015. Distinct structural domains of caveolin-1 independently regulate Ca^{2+} release-activated Ca^{2+} channels and Ca^{2+} microdomain-dependent gene expression. *Mol Cell Biol* **35:** 1341–1349. doi:10.1128/MCB.01068-14

Yeromin AV, Zhang SL, Jiang W, Yu Y, Safrina O, Cahalan MD. 2006. Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai. *Nature* **443:** 226–229. doi:10.1038/nature05108

Zhang SL, Yeromin AV, Zhang XHF, Yu Y, Safrina O, Penna A, Roos J, Stauderman KA, Cahalan MD. 2006. Genome-wide RNAi screen of Ca^{2+} influx identifies genes that regulate Ca^{2+} release-activated Ca^{2+} channel activity. *Proc Natl Acad Sci* **103:** 9357–9362. doi:10.1073/pnas.0603161103

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