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PLCδ: Johnny-come-lately to ORAI and the ups and downs of calcium signalling

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Gene mapping and RNA interference screens identified the calcium (Ca^{2+}) sensor STIM1 and the Ca^{2+} channel Orai1 as central components of store-operated Ca^{2+} entry (SOCE) and its corresponding Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}) in leukocytes and HEK293 cells (for reviews see Vig & Kinet, 2007; Potier & Trebak, 2008; Hogan *et al.* 2010). SOCE is activated physiologically through receptor-mediated production of inositol 1,4,5-trisphosphate (IP_3) and subsequent IP_3-induced store depletion. A quite natural inclination is to expect Orai1 homologues, Orai2 and Orai3, to fulfill a similar role by mediating SOCE and I_{CRAC} in different mammalian tissues and cell types. While a general consensus among investigators is that Orai2 and Orai3 mediate SOCE and I_{CRAC} in overexpression systems when co-expressed with STIM1, the situation in native systems is less clear. A large number of mammalian cell types and tissues studied to date exclusively signal through Orai1 in response to Ca^{2+} store depletion despite concomitant expression of Orai2 and Orai3 proteins in these systems. So far, the exception to this rule are two reported instances where Orai2 and Orai3 mediate native SOCE and I_{CRAC} under quite unusual circumstances: Orai2 supports SOCE in T cells from Orai1$^{-/-}$ mice (Gwack *et al.* 2008; Vig *et al.* 2008) and a subset of breast epithelial cancer cell lines that expresses the oestrogen receptor uses Orai3 as its SOCE channel (Motiani *et al.* 2010). An attractive possibility would entail that under native conditions Orai isoforms form diverse groups of homomultimeric and heteromultimeric receptor-activated channels, some of which activated by alternative store-independent means, including mechanisms involving second messengers, reactive oxygen and nitrogen species and lipid metabolites. A large number of molecularly distinct store-operated and store-independent Orai channels would serve to enhance the diversity of Ca^{2+} selective conductances for the purpose of selective cell signalling in response to different membrane receptors and different strengths of agonist stimulation. Viewed from this angle, it is thus not surprising that the Ca^{2+} selective store-independent arachidonate-regulated Ca^{2+} (ARC) channel turned out to be regulated by STIM1 and encoded by heteromultimers of Orai1 and Orai3 (Shuttleworth, 2009). I_{ARC} is likely to end up representing the archetypical store-independent current for novel native Orai-mediated store-independent currents that future research might uncover.

Mechanisms of Ca^{2+} oscillations are diverse and vary between excitable and non-excitable cells. Depending on the cell type considered, Ca^{2+} oscillations may be entirely due to oscillatory responses of plasma membrane channels, entirely to oscillations of Ca^{2+} release channels, or to a combination of both. The mechanisms regulating Ca^{2+} oscillations in response to stimulation of membrane receptors that couple to IP_3 production remain incompletely understood. Such intracellular Ca^{2+} oscillations arise in response to low physiological concentrations of agonists. Proposed models for regenerative Ca^{2+} oscillations in response to receptor stimulation include either spiking of cellular IP_3 levels (Woods *et al.* 1987) or fluctuations of cytosolic Ca^{2+} concentrations while cellular IP_3 levels remain constant (Berridge, 1990) (for review see Putney & Bird, 2008). Although these Ca^{2+} oscillations often result from cycles of discharge and re-uptake of Ca^{2+} by intracellular stores, in most cases Ca^{2+} influx across plasma membrane channels is essential for maintenance of Ca^{2+} oscillations; in the absence of extracellular Ca^{2+}, Ca^{2+} spiking will cease after a few cycles (Dupont *et al.* 2011). Ca^{2+} oscillations are proposed to provide digitally encoded signals in terms of amplitude and frequency that ensure efficiency and specificity of coupling to downstream effectors (Dupont *et al.* 2011). In a recent issue of *The Journal of Physiology*, Thompson & Shuttleworth (2011) provide significant new insights on a novel means by which the frequency of Ca^{2+} oscillations is modulated by the rate of Ca^{2+} influx through ARC channels in a HEK293 cell line stably expressing the muscarinic m3 receptor (m3-HEK cells). Using the rate of decay of the PIP_2-dependent inwardly rectifying K^+ channel (Kir2.1) as a read out for PIP_2 depletion, Thompson and Shuttleworth show that the Ca^{2+} entry signal through I_{ARC} causes PIP_2 decay. Significantly, they show that this ability to deplete PIP_2 is specific to I_{ARC} and could not be achieved under maximal activation of I_{CRAC}, suggesting that global increases in cytosolic Ca^{2+} have no effect on PIP_2 depletion and that this effect is mediated specifically by highly localized Ca^{2+} signals through I_{ARC}. In fact, they show that transfection into cells of an Orai3 dominant negative construct (E81Q; shown previously to abrogate I_{ARC} activity with no effect on I_{CRAC}; (Mignen *et al.* 2008) essentially inhibited the PIP_2 depletion seen in response to I_{ARC} activation. How does Ca^{2+} entry through I_{ARC} specifically couple to PIP_2 decay to modulate the frequency of Ca^{2+} oscillations? Thompson and Shuttleworth show that an isoform of the archetypical phospholipase C (PLC), the Ca^{2+}-activated PLCδ3, is involved in sensing Ca^{2+} entry through I_{ARC}. Incorporation of high concentrations of IP_3 (50 μM, known to inhibit PLCδ activity) in the patch pipette, specific RNAi against PLCδ3 and transfection of a catalytically impaired form of PLCδ3 (H352A) all inhibit I_{ARC}-mediated decay of Kir2.1 activity. Finally, transfection of the E81Q-Orai3 or H352A-PLCδ3 dominant negative constructs into m3-HEK cells significantly reduced the frequency of Ca^{2+} oscillations in response to very low concentrations of agonist (0.5 μM carbachol) by one-third.

Putney and Bird have proposed that I_{CRAC} is the Ca^{2+} influx channel necessary to maintain Ca^{2+} oscillations and as of this writing the relative contributions of I_{ARC} and I_{CRAC} to agonist-mediated Ca^{2+} oscillations in HEK293 cells is still a matter of debate (Shuttleworth, 2004; Putney & Bird, 2008). One reason for the discrepancy between the results of these two groups might lie in the cell type used; while Thompson and Shuttleworth use m3-HEK cells, Putney and Bird have conducted their studies in a HEK293 cell line with

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endogenous levels of muscarinic receptors. Alternatively, both I_{ARC} and I_{CRAC} might contribute, to varying degrees, to the maintenance of Ca^{2+} oscillations in the same cell and could compensate for each other when the function of one conductance is abrogated. In fact, Bird and Putney showed that ectopic expression of the non-selective transient receptor potential canonical 3 (TRPC3) channel can also support Ca^{2+} oscillations in HEK293 cells (Bird & Putney, 2005). In the same study, Bird and Putney used a protocol where HEK293 cells are incubated with 1 mM Gd^{3+} to block both Ca^{2+} entry and Ca^{2+} extrusion (called lanthanide insulation) and showed that under these conditions Ca^{2+} oscillations are maintained in the absence of extracellular Ca^{2+} (Bird & Putney, 2005). Although not statistically significant under the experimental conditions used, an intriguing finding in this study is the decrease in the frequency of Ca^{2+} oscillations during lanthanide insulation by approximately 30–40% that could be explained by a role of Ca^{2+} influx through I_{ARC} in regulating the frequency of Ca^{2+} oscillations. Likewise, the molecular interventions used by Thompson and Shuttleworth did not completely prevent oscillations, allowing for a significant contribution of I_{CRAC} in their system.

The study of Thompson and Shuttleworth provides insights into how a discrete and spatially restricted Ca^{2+} signal can couple to an IP_3-producing enzyme to modulate oscillatory Ca^{2+} signals. Because I_{ARC} does not produce enough IP_3 to cause any perceptible Ca^{2+} release, Thompson and Shuttleworth propose that PLCδ acts as a signal amplifier rather than an initiator, a proposition that is in line with earlier clues deduced from the unique biochemical features of PLCδ (Rebecchi & Pentyala, 2000). The work of Thompson and Shuttleworth prompts a number of important questions: what is the nature of the specific coupling between PLCδ and I_{ARC}? What mediators, scaffolding proteins or membrane lipids, if any, are involved in this coupling, since the authors failed to detect direct interactions between STIM1, Orai1, Orai3 and PLCδ using co-immunoprecipitations and bimolecular fluorescence complementation? Are there other downstream effectors that are targeted specifically by I_{ARC}-generated Ca^{2+} microdomains, which would couple to transcription of specific I_{ARC}-associated genes? Does Ca^{2+} influx through I_{ARC} control a specific set of cell functions, distinct from those mediated by I_{CRAC}? Earlier work has proposed that subcellular Ca^{2+} influx through CRAC channels can couple to specific isoforms of adenylate cyclase to modulate spatially restricted cAMP-dependent signalling (Willoughby & Cooper, 2007), and more recent findings suggest that the specificity of the information conveyed by CRAC-mediated Ca^{2+} oscillations in RBL mast cells is encoded within the Ca^{2+} influx site during the Ca^{2+} spikes; the coupling of Ca^{2+} oscillations to *c-fos* gene expression is lost when Ca^{2+} oscillations are stimulated under lanthanide insulation (Parekh, 2011). The study by Thompson and Shuttleworth introduces PLCδ as another player in the game of Ca^{2+} oscillations and provides the first example of a protein specifically activated by I_{ARC}-generated Ca^{2+} signals. Future insights into the transcriptional programmes and cell functions specifically controlled by I_{ARC}-mediated Ca^{2+} signals will likely generate novel means for specific control of physiological and pathophysiological functions during disease.

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