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Selective activation of distinct Orai channels by STIM1

Trevor J. Shuttleworth

Department of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, New York, 14642

Graphical abstract

Following on from Jim Putney's original demonstration that agonist-induced calcium entry (particularly in the “non-excitabile” cells that do not express voltage-gated calcium channels) was a consequence of the release of calcium from the endoplasmic reticulum (ER) stores - a process defined as store-operated calcium entry (SOCE) or capacitative calcium entry [1-2] - several studies demonstrated that such a pathway existed in a diverse variety of cell types. However, examination of this pathway revealed features that we felt raised some questions. For example, the absolute dependence of SOCE on the depletion of ER calcium stores could potentially be considered “dangerous”, given the importance of such ER calcium levels in the processing and “quality control” of cellular protein generation. Moreover, there was no clear indication of how such depletion of the ER calcium store could be translated into an appropriate signal for activation of a channel in the plasma membrane (PM). Of course, it was certainly possible to propose feasible models that could negate such concerns. For example, if the depletion involved only a minor subset of the total ER store, perhaps in a pool located close to the PM, then that could provide a valid explanation for the effectiveness of the SOCE process. Nevertheless, such “concerns” led us to explore the possibility of the presence of an alternative distinct pathway for agonist-induced calcium entry [3-6].

The first evidence suggesting the presence of such an alternative pathway came from an unexpected source, namely studies indicating the existence of a calcium entry pathway activated by the receptor-mediated generation of low levels of intracellular arachidonic acid (AA) [7-10]. Subsequent electrophysiological studies revealed that this AA-activated calcium entry pathway involved a conductance whose properties closely mirrored those of the previously identified store-operated CRAC channels. Specifically, small, inwardly-

rectifying, highly calcium-selective currents with markedly positive reversal potentials ($\sim +80$ mV), that were blocked by low concentrations of lanthanum and gadolinium. Critically however, currents through these two channels were strictly additive in the same cell, and activation of the ARC channels was entirely *independent* of ER calcium store-depletion [11-14].

Despite the early identification and characterization of the store-operated CRAC channels, identification of the key molecular “players” in this pathway remained unknown until 2005/6 which saw the discovery of STIM1 as the sensor of calcium-store (ER) depletion [15, 16], and the three Orai proteins (specifically Orai1) that formed the actual store-operated CRAC channel [17,18]. Based on the finding that STIM1 was the agent that sensed the depletion of the ER calcium store, we assumed that STIM1 would likely have no role in the activation of the store-*independent* ARC channels. Surprisingly however, siRNA-knockdown of endogenous STIM1 resulted in the complete loss of ARC channel activity. This unexpected finding was resolved when examination of the literature revealed that STIM1 had actually been originally identified some five years earlier, where it was described as a plasma membrane protein [19-20]. Consistent with this, we showed that some 15-25% of the total cellular STIM1 was typically resident in the plasma membrane (PM), and that preventing the delivery of STIM1 to the PM by introducing a glycosylation-mutant version, resulted in the specific loss of ARC channel currents without affecting the activity of co-existing CRAC channels [21]. Subsequent studies showed that, like the CRAC channels, the ARC channels were also formed by Orai proteins but, whilst Orai1 formed the CRAC channel, the ARC channel was a heteromeric assembly of Orai1 and Orai3 proteins [22-23].

A key puzzle resulting from these findings was that the essential initial step in the activation of STIM1, namely the sensing of depletion of ER free calcium levels (typically in the range of 100 to 600 μ M), involved the loss of calcium from a calcium-binding EF-hand domain located within the N-terminal portion of the protein that lay within the lumen of the ER [24]. For STIM1 located in the PM, this domain would be extracellular where it would be exposed to high, relatively stable calcium concentrations of around 2-3 mM. Consequently, for PM-located STIM1, this domain would never lose its bound calcium, indicating a clear distinction between the function of STIM1 in the activation of CRAC channels versus the ARC channels. To examine this we expressed a STIM1 construct lacking both the entire N-terminal and the transmembrane portions of the protein, leaving just the extensive cytosolic domain (~ 435 residues), to which we attached an N-terminal Lck sequence to enable its attachment to the inner cytosolic face of the PM. Expression of this construct (Lck-STIM1) resulted in AA-activated ARC channel currents that were indistinguishable from those seen with normal full-length STIM1, whilst the corresponding store-operated CRAC currents were essentially eliminated [25]. In contrast however, deletion of the so-called CAD (CRAC Activation Domain) or SOAR (STIM-Orai-Activating Region) domain (residues ~ 342 -448) within the cytosolic portion of the STIM1 protein and which had been shown to be essential for CRAC channel activation, was also shown to be essential for ARC channel activation [26]. Further studies showed that many of the domains within the cytosolic portions of STIM1 identified as essential for CRAC channel activation were similarly essential for activation of the ARC channels (Fig. 1). These included the C2A domain (W430, I433,

L436) essential for STIM1 dimerization, and the so-called Basic Region (BR) domain (K382/384-386) essential for CRAC activation [26].

Relatively early in our studies of the AA-activated ARC channel currents we became aware that they were significantly inhibited by elevated levels of cytosolic calcium - specifically within the range of ~250-400 nM [27]. Subsequent studies showed that this was a result of a calcineurin-mediated dephosphorylation, and could be readily reversed by a PKA-mediated phosphorylation [28]. To examine the molecular basis for this response, we examined potential PKA phosphorylation sites using an *in silico* analysis of the STIM1 cytosolic domain [29]. Although this revealed several such potential sites, we could eliminate most of these as they lay beyond residue H448, and we had shown that termination of STIM1 at this site had no effect on ARC channel activity. The only clear potential PKA-site in the remaining STIM1 cytosolic sequence was a threonine at position 389, and *in vitro* hyperphosphorylation analysis confirmed that this residue was a genuine PKA target site [29] (Fig. 2A). To examine the physiological effect of phosphorylation of the T389 residue, we expressed STIM1 constructs in which the T389 residue was replaced by either a “phospho-mutant” T389A residue, or a “phosphomimetic” T389E residue. Expression of these constructs revealed that, whilst the T389A phospho-mutant displayed normal CRAC channel currents, ARC channel currents were reduced to negligible levels. Correspondingly, expression of the T389E phosphomimetic mutant showed normal ARC channel currents, whilst CRAC channel currents were essentially eliminated [29] (Fig. 2B). This PKA-mediated phosphorylation-dependent “switch”, was shown to involve an AKAP, namely AKAP79, as expression of an AKAP79 construct specifically lacking the PKA binding site resulted in the loss of ARC channel currents - an effect that could be completely restored by expression of the T389E phospho-mutant STIM1 in the presence of the same PKA-mutant AKAP79 [27]. In addition, the previously demonstrated calcineurin-dependent inhibition of ARC channel currents at elevated cytosolic calcium levels could be abolished by expression of the T389E phospho-mutant STIM1. Finally, we were able to demonstrate significant FRET signals between STIM1 and AKAP79, STIM1 and Orai3, and AKAP79 and Orai3. Critically, these signals were seen under resting conditions, suggesting that AKAP79, plasma membrane STIM1, and the ARC channel exist in a constitutive complex [29].

In summary, these findings indicate that phosphorylation of the T389 residue in STIM1 is an essential feature determining the activity of store-independent ARC channels, and is mediated by an AKAP79/PKA-dependent process (Fig. 3). Moreover, because phosphorylation of T389 in STIM1 “inhibits” CRAC channel activation, this AKAP/PKA mediated STIM1-dependent process can act to modulate the relative activities of these two co-existing calcium entry pathways in a manner appropriate to induce their distinct roles in cellular calcium signaling.

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Highlights

- CRAC and ARC channels are distinct calcium entry channels formed by Orai proteins
- Activation of both these channels involves distinct pools of STIM1 (ER versus PM)
- Selective activation of these two channels is determined by the phosphorylation status of a single threonine residue within the STIM1 cytosolic domain

Figure 1.
Diagram of the different domains within the STIM1 molecule (top), with the key regions responsible for Orai channel interaction and activation (below).

Image from RCSB Protein Data Bank

Figure 2.

A) Diagram illustrating the currently proposed conformation of the CC2 and CC3 domains of a STIM1 dimer, and indicating the corresponding predicted location of the critical T389 residue.

B) CRAC (grey) and ARC (red) whole-cell currents measured at -80 mV in cells treated with an siRNA against endogenous STIM1, and transfected with either the wild-type STIM1 (WT), the phospho-mutant STIM1/T389A construct, or the phosphomimetic STIM1/T389E construct.

Figure 3.

A model illustrating the sequence of events leading to the activation of ARC currents and their subsequent deactivation. Shown is the constitutive translocation of a pool of STIM1 to the plasma membrane (PM), where it undergoes an AKAP79-mediated, PKA-induced phosphorylation of the T389 residue (STIM1-P*). The presence of an appropriate agonist results in the local generation of arachidonic acid (AA), and the STIM1-P*-mediated activation of the ARC channels (**RED ARROW**), and calcium entry. Also shown is the subsequent deactivation of the ARC channels as a result of an AKAP79-mediated calcineurin-induced dephosphorylation of the T389 residue of STIM1.