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Review

Store-operated calcium entry: Mechanisms and modulation

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ABSTRACT

Store-operated calcium entry is a central mechanism in cellular calcium signalling and in maintaining cellular calcium balance. This review traces the history of research on store-operated calcium entry, the discovery of STIM and ORAI as central players in calcium entry, and the role of STIM and ORAI in biology and human disease. It describes current knowledge of the basic mechanism of STIM-ORAI signalling and of the varied mechanisms by which STIM-ORAI signalling can be modulated.

1. Introduction

1.1. Store-operated calcium entry

The idea of store-operated calcium entry developed from studies of calcium signalling in the 1970s and 1980s. The field built on a considerable body of unsung work, but its progress can be traced in a few prominent papers [1–4]. Several conclusions had been firmly established by the early 1980s— that cells possess internal calcium stores, that calcium is released from internal stores in response to certain physiological agonists, that this mobilization of calcium leads to calcium efflux from the cell, and that therefore, over time, external calcium is needed to refill cellular calcium stores. Importantly, it was recognized that cells can be primed for uptake of calcium by the same physiological agonist that releases calcium from internal stores, and that continuing occupancy of the receptor is not needed for calcium uptake. The demonstration that the second messenger inositol 1,4,5-trisphosphate (IP3) releases calcium from ER stores [5] delineated the initial part of the calcium signalling pathway, from receptor through phospholipase C through production of IP3 and calcium release. Revisiting the earlier experiments with this pathway in mind, and with use of the newly available calcium indicator Fura-2, established that enhanced calcium uptake is independent not only of receptor occupancy but also of residual IP3, until internal calcium stores are refilled [6]. The accumulated evidence set the stage for wide acceptance of a model that sensing of store content controls a plasma membrane calcium influx mechanism [7,8].

1.2. CRAC current

The proposed store-operated calcium entry mechanism rapidly gained experimental support with the electrophysiological demonstration of the calcium release-activated calcium (CRAC) current in mast cells and T cells [9–11]. Further work reinforced the direct connection of CRAC current to Fcε receptor or T cell receptor engagement and to ER calcium store depletion [12–16]. The CRAC current is characterized *inter alia* by its responsiveness to store depletion, its small whole-cell current densities, its very small single-channel current, and its extreme selectivity for calcium under physiological conditions. CRAC current is not restricted to mast cells and T cells, though it was more readily detected there, in part because the cells have fewer interfering currents and in part because of dedicated experimentation. A current with the same characteristics has since been detected in many cell types. It is worth noting specifically the identification of CRAC current in *Drosophila* S2 cells [17] because of their role in RNA interference (RNAi) screens described below. Despite the distinctive electrophysiological fingerprint, the basis of the classical CRAC current in STIM and ORAI proteins was not identified for many years.

1.3. Less selective channels

This review focuses on the calcium-selective STIM-ORAI-dependent CRAC current. However, the STIM-ORAI pathway is not the only source of calcium for refilling of ER stores. It has long been

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recognized that other less selective calcium channels are activated upon depletion of ER calcium stores in some cell types [18], and particular attention has been given to the possible role of TRPC channels. This has been a controversial area, since it is clear that TRPC channels are not directly controlled by store depletion under many conditions of stimulation [19]. Nonetheless, there is now evidence that STIM controls the activation of certain TRPC channels [20,21] and that STIM-ORAI signalling controls insertion of TRPC1 channels into the plasma membrane in salivary gland cells [22]. The contribution of TRPC channels to store-operated calcium entry has been reviewed recently in Ref. [23].

2. Discovery of STIM and ORAI

2.1. RNAi screens

RNAi technology opened the way to the next major advances in the field. In 2005, two RNAi screens in *Drosophila* S2 cells and human HeLa cells focused on subsets of candidate genes and, using the cytoplasmic calcium signal as a readout, discovered the essential role of STIM proteins in store-operated calcium entry [24,25]. RNAi technology had developed at that time to the point that *Drosophila* cells could be used in genome-wide RNAi screening. In 2006, three genome-wide screens in *Drosophila* S2 cells, with either calcium entry or sustained signalling to the calcium-dependent transcription factor NFAT as readout, identified ORAI proteins as essential to store-operated calcium entry [26–28]. *Drosophila* has only a single Orai protein, whereas humans have three, and the NFAT-based screen in *Drosophila* cells was supported by parallel genetic mapping to human ORAI1 of a SCID mutation that impairs calcium influx in T cells [26].

2.2. Required for CRAC current

CRAC current had been defined originally in T cells and mast cells, and therefore it is important that several reports verified that STIM1 and ORAI1 were essential contributors to the classical CRAC current in T cells and mast cells. RNAi-mediated knockdown of STIM1 reduced store-operated calcium entry and CRAC current in human Jurkat T cells [24,25], and Stim1 deficiency impaired calcium influx in mouse mast cells and CRAC current in mouse T cells [29,30]. In the latter cases, calcium influx was restored by expression of Stim1 [29,30]. The strongest evidence linking ORAI1 to CRAC current was the absence of CRAC current in T cells of human SCID patients homozygous for the ORAI1(R91W) mutation, and restoration of the current by expression of wildtype ORAI1 in those cells [26]. Likewise, differentiated Orai1$^{-/-}$ T cells and Orai1$^{-/-}$ mast cells from mice had greatly reduced CRAC currents compared to wildtype controls [31,32], and normal T cell calcium influx was restored by expressing Orai1 [32]. Consistent with the broad expression of CRAC current, calcium signalling was also affected by Stim1 or Orai1 deficiency in other cell types in mice [32–36]. The fact that not all the cell types that have CRAC current showed a detectable impairment attests to the redundancy of STIM and ORAI proteins or to compensation by other calcium channels.

2.3. Pathology

Additional evidence on the role of STIM-ORAI calcium signalling in immunity has accumulated from a few human kindreds where STIM1 or ORAI1 loss-of-function mutations cause immunodeficiency [reviewed in Ref. [37]]. In one specific instance, a human patient with STIM1-related immunodeficiency also exhibited a detectable impairment of platelet function [38]. Other heritable alterations in STIM1 or ORAI1 present themselves as dominant, gain-of-function mutations. The first reported dominant mutation in STIM or ORAI— aside from mutations intentionally engineered into the proteins— was in a line of mice derived after chemical mutagenesis with N-ethyl-N-nitrosourea [39]. The heterozygous mice exhibited abnormal platelet function, interpreted as due to a partial constitutive activation of Stim1. Subsequently, various dominant mutations in human STIM1 and human ORAI1 have been shown to cause tubular aggregate myopathy, with one family having the same dominant STIM1 D84G mutation identified in the mice [40–43], and a separate dominant STIM1 R304W mutation has been shown to underlie Stormorken syndrome [41,44,45].

3. STIM and ORAI proteins

3.1. STIM proteins

There are two human STIM proteins, STIM1 and STIM2 [reviewed in [46]]. Both are predominantly located in the ER, though a minor amount of STIM1 is expressed at the cell surface [25,47–49]. ER-localized STIM controls CRAC channel gating [25,50–53]. STIM1 and STIM2 are similar in overall architecture, with an N-terminal domain in the ER lumen, a single transmembrane segment anchoring the protein in the ER, and a C-terminal cytoplasmic domain. Key subregions of the cytoplasmic domain— CC1, SOAR/CAD, and a polybasic segment at the C-terminus— are indicated in [Fig. 1] and discussed later. The isolated recombinant STIM1 cytoplasmic domain is a dimer [54], as are its functional fragments that include the SOAR/CAD domain [55–58], and full-length STIM is believed to exist as a dimer in unstimulated cells [46,59]. Some insight has been obtained into the structure of specific STIM regions. NMR structures have been reported for the isolated ER-luminal domains of both STIM1 and STIM2 with calcium bound to an EF-hand [60,61]. A crystal structure of the SOAR/

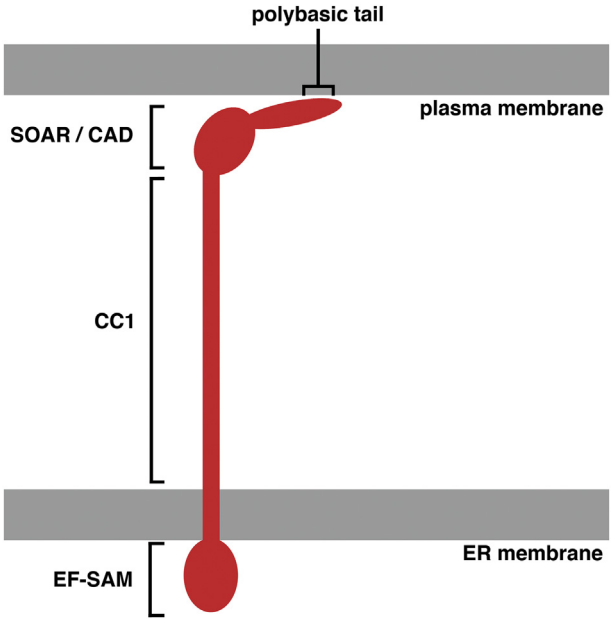

Fig. 1. Cartoon view of STIM1 in an extended conformation, bridging the distance from ER to plasma membrane. For clarity, a single STIM monomer is shown, but the active extended form in cells is oligomeric. Functional regions of STIM discussed in the text are the calcium-sensing EF-SAM domain in the ER lumen, the cytoplasmic CC1 region that both stabilizes inactive STIM and transmits the activating conformational change upon ER calcium store depletion, the SOAR/CAD domain that recruits and gates ORAI channels, and the polybasic tail that interacts with plasma membrane phosphoinositides.