Biochimica et Biophysica Acta 1838 (2014) 1281–1287

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Structural and stoichiometric determinants of Ca^{2+} release-activated Ca^{2+} (CRAC) channel Ca^{2+}-dependent inactivation

Nathan R. Scrimgeour [a], David P. Wilson [a], Greg J. Barritt [b], Grigori Y. Rychkov [a,*]

[a] School of Medical Sciences, University of Adelaide, Adelaide, South Australia 5005, Australia
[b] Department of Medical Biochemistry, School of Medicine, Flinders University, Adelaide, South Australia 5001, Australia

ARTICLE INFO

Article history:
Received 18 October 2013
Received in revised form 3 January 2014
Accepted 14 January 2014
Available online 26 January 2014

Keywords:
STIM1
Orai1
Ca^{2+} channel
CRAC
Gating
Ca^{2+}-dependent inactivation

ABSTRACT

Depletion of intracellular Ca^{2+} stores in mammalian cells results in Ca^{2+} entry across the plasma membrane mediated primarily by Ca^{2+} release-activated Ca^{2+} (CRAC) channels. Ca^{2+} influx through these channels is required for the maintenance of homeostasis and Ca^{2+} signaling in most cell types. One of the main features of native CRAC channels is fast Ca^{2+}-dependent inactivation (FCDI), where Ca^{2+} entering through the channel binds to a site near its intracellular mouth and causes a conformational change, closing the channel and limiting further Ca^{2+} entry. Early studies suggested that FCDI of CRAC channels was mediated by calmodulin. However, since the discovery of STIM1 and Orai1 proteins as the basic molecular components of the CRAC channel, it has become apparent that FCDI is a more complex phenomenon. Data obtained using heterologous overexpression of STIM1 and Orai1 suggest that, in addition to calmodulin, several cytoplasmic domains of STIM1 and Orai1 and the selectivity filter within the channel pore are required for FCDI. The stoichiometry of STIM1 binding to Orai1 also has emerged as an important determinant of FCDI. Consequently, STIM1 protein expression levels have the potential to be an endogenous regulator of CRAC channel Ca^{2+} influx. This review discusses the current understanding of the molecular mechanisms governing the FCDI of CRAC channels, including an evaluation of further experiments that may delineate whether STIM1 and/or Orai1 protein expression is endogenously regulated to modulate CRAC channel function, or may be dysregulated in some pathophysiological states.

Contents

Abbreviations: BAPTA, 1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid; CAD, CRAC activation domain; CaM, calmodulin; CMD, CRAC modulation domain; CRAC, Ca^{2+} release-activated Ca^{2+}; EGTA, ethylene glycol tetraacetic acid; ER, endoplasmic reticulum; FCDI, Fast Ca^{2+}-dependent inactivation; I_{CRAC}, Ca^{2+} release-activated Ca^{2+}; SOC, store-operated Ca^{2+} channel; STIM1, Stromal Interacting Molecule 1

* Corresponding author. Tel.: +61 8 8313 3979; fax: +61 8 8313 3356.
E-mail address: Grigori.Rychkov@adelaide.edu.au (G.Y. Rychkov).

0005-2736/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamem.2014.01.019

1. Introduction

Store-operated Ca^{2+} channels (SOCs) are a class of plasma membrane channels activated upon depletion of intracellular endo/sarcoplasmic reticulum Ca^{2+} stores. The most extensively characterised SOC is the Ca^{2+} release-activated Ca^{2+} (CRAC) channel. The importance of CRAC channels has been demonstrated in many cell types. Physiologically, CRAC current (I_{CRAC}) is the critical Ca^{2+} entry mechanism that leads to activation of T lymphocytes, and genetic loss of function mutations manifest as severe combined immunodeficiency [7,13,43]. Given the ubiquitous importance of Ca^{2+} as a signalling molecule, I_{CRAC} has been identified as vital in many other processes, including maintenance of skeletal muscle tone [31,58], ectodermal development [13,31,43], and tumourigenesis [5,9,67]. Three defining characteristics of native CRAC channels include: exclusive activation by Ca^{2+} store depletion, high selectivity for Ca^{2+} over monovalent cations, and fast Ca^{2+}-dependent inactivation (FCDI). FCDI is typically studied in 10 mM or higher external Ca^{2+} and at hyperpolarised membrane potentials; however, it remains prominent at physiologically relevant concentrations and membrane potentials in both overexpressed channels and in native CRAC channels of Jurkat T cells and RBL-1 cells [8,21,51,52,75]. FCDI may play an important role in shaping Ca^{2+} signals and in limiting Ca^{2+} entry but due to its complex nature, involving numerous domains and residues in both STIM1 and Orai1, remains a less well-understood feature of the CRAC channel. This review discusses the structural elements of STIM1 and Orai1 relevant to FCDI and outlines the current understanding of the molecular mechanisms underlying Ca^{2+}-dependent gating of the CRAC channel.

2. Molecular components of CRAC channels

Although CRAC channels were first biophysically characterised twenty years ago [6,17,46,74], investigations into the molecular basis of CRAC channel activity were limited until the discovery of the two proteins that form the functional channel. Stromal Interacting Molecule 1 (STIM1) acts as the endoplasmic/sarcoplasmic reticulum (ER/SR) Ca^{2+} sensor [26,48], while Orai1 forms the Ca^{2+} permeable pore on the plasma membrane [34,44,63]. Following depletion of Ca^{2+} from ER/SR, STIM1 oligomerises, migrates towards regions of the ER/SR membrane in direct apposition to the plasma membrane, and interacts with Orai1. The STIM1/Orai1 complexes form functional CRAC channels that mediate I_{CRAC}. Ectopic co-expression of STIM1 and Orai1, or its pore-forming homologues Orai2/3, results in large I_{CRAC} activated by store depletion [27,34,53,62]. For a detailed review of the pathway of CRAC channel activation, see [23].

3. FCDI of CRAC channels

Fast Ca^{2+}-dependent inactivation (FCDI) is a negative feedback mechanism that was first described in voltage-gated Ca^{2+} channels [3]. In CRAC channels, FCDI can be observed during whole cell patch clamp recording by applying voltage steps to negative potentials from a holding potential of 0 mV after full activation of I_{CRAC} by store depletion [8,18,28,75]. The negative voltage step results in an instant increase in current through the open CRAC channels, but as Ca^{2+} passes through the channel pore, the current inactivates from its peak to a steady state with a biexponential time course, with time constants of ~10 ms and 100 ms (e.g. Fig. 1A) [8,18,28,75]. The evidence that this type of inactivation gating in the CRAC channel is a Ca^{2+}-dependent process comes from the extensive testing of the effects of different extracellular and intracellular [Ca^{2+}], and Ca^{2+} buffers on I_{CRAC} kinetics. FCDI is completely lost when current is carried by monovalent cations in the absence of external divalent cations [28,75], while increasing the external Ca^{2+} concentration results in an accelerated and increased extent of inactivation [8,75]. Furthermore, the fast Ca^{2+} buffer 1,2-bis(o-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid (BAPTA) in the patch pipette reduces the extent of inactivation compared to the slower Ca^{2+} buffer ethylene glycol tetraacetic acid (EGTA) [8,18,75].

While FCDI has been consistently observed in recordings of endogenous I_{CRAC}, it has been reported to be reduced or absent in overexpressed STIM1/Orai1 I_{CRAC} [27,50,55,65]. Original investigations using overexpressed STIM1/Orai1 described I_{CRAC} Ca^{2+}-dependent inactivation as a complex behavior that included a phase in which current increased with time [22,27,50]. It was speculated that there may be three Ca^{2+}-dependent processes occurring: fast and slow exponential phases of FCDI and a third "reactivation" phase [27]. Several contemporary studies failed to identify a reactivation phase, with FCDI similar or only slightly weaker than that seen in endogenous I_{CRAC} [55,65].

Two mammalian homologues of Orai1, called Orai2, and Orai3 [7,63], co-expressed with STIM1 are able to reconstitute CRAC-like currents, albeit with some notable differences in their biophysical properties [22,27]. The Ca^{2+}-dependent kinetics of Orai2 and Orai3-mediated currents have been reasonably consistent, with Orai2 displaying moderate FCDI and no Ca^{2+}-dependent reactivation [22,27], and Orai3 showing prominent and rapid FCDI and no Ca^{2+}-dependent reactivation [22,27,50]. To date, the Ca^{2+} binding site for FCDI and the domain

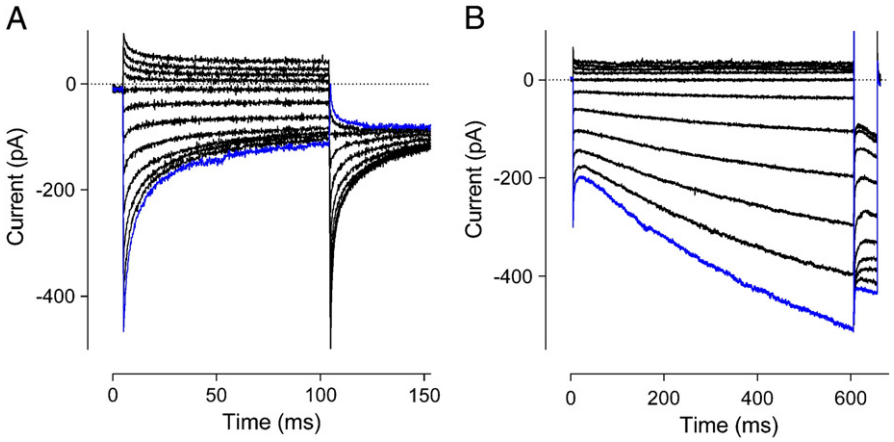

Fig. 1. Examples of FCDI and Ca^{2+}-dependent reactivation. Example I_{CRAC} from HEK293 cells with exogenously expressed STIM1 and Orai1. Traces are recorded in response to the initial steps ranging from +62 mV to −138 mV in 20-mV increments, the most negative step shown in blue, followed by a second step to −118 mV. (A) Strongly inactivating currents, similar to that which would be expected where an excess of STIM1 relative to Orai1 exists. (B) Strongly reactivating currents, similar to that which would be expected where an excess of Orai1 relative to STIM1 exists. The example here shows a longer initial step, demonstrating that the reactivation fails to approach a steady state even after 600 ms.

containing the fast inactivation gate have not been conclusively identified. Some likely candidates are discussed in this review.

4. Channel subunit stoichiometry

The disparity in the kinetics of Ca^{2+}-dependent gating of STIM1/Orai1-mediated current has led to the suggestion that Orai1 and STIM1 may form channels with variable stoichiometry. This idea was supported by experiments in which the expression levels of Orai1 and STIM1 were varied. Increasing the ratio of STIM1 to Orai1 produced stronger FCDI (Fig. 1A). In contrast, decreasing the ratio of STIM1 relative to Orai1 caused a loss of FCDI and introduction of Ca^{2+}-dependent reactivation of I_{CRAC} [51] (Fig. 1B). Associated with the change in the STIM1/Orai1 ratio were changes in apparent selectivity for Ba^{2+} and Sr^{2+} over Ca^{2+} and responsiveness to the nonspecific I_{CRAC} blocker 2-APB [51].

Overexpression of Orai1 without STIM1 overexpression does not result in any increase in I_{CRAC} and has in some instances been shown to reduce endogenous I_{CRAC} [34,55,63]. These findings led to the suggestion that a large excess of Orai1 results in an insufficient pool of STIM1 being able to bind to and activate each Orai1 channel pore, with the consequence of a reduced whole cell current.

This idea has been supported and extended through the use of fluorescence imaging techniques and concatemers of Orai1 and STIM1 to directly control or quantify the relative amounts of Orai1 and STIM1 expressed in an individual cell [15,24]. It was initially proposed that the Orai1 pore functions as a tetramer [20,36,42]. However, recent data from the crystal structure of the *Drosophila melanogaster* homologue of Orai suggest that Orai1 forms a hexameric pore [19]. Each Orai1 subunit can bind two STIM1 subunits as shown by yeast two-hybrid screens and coimmunoprecipitation experiments where the activation domain of STIM1 was capable of binding at both C- and N-termini of Orai1 [41]. The 1 Orai1:2 STIM1 stoichiometry produces an I_{CRAC} that is maximally activated and shows the greatest degree of FCDI. In contrast, in the 1 Orai1:1 STIM1 stoichiometry, whole cell currents are smaller, and Ca^{2+}-dependent reactivation of I_{CRAC} is exhibited [15,24].

5. Key domains and residues within STIM1

STIM1 was identified using high throughput RNAi screens in conjunction with Ca^{2+} imaging assays to find genes that regulate intracellular Ca^{2+} concentration [26,70]. STIM1 is a ubiquitously expressed 685 amino acid, single transmembrane domain, protein. Cell surface biotinylation shows that as much as 25% of immunoprecipitatable STIM1 is located on the plasma membrane [30]. However, STIM1 required for CRAC channel activation is located on the endoplasmic reticulum membrane [34]. Fig. 2 shows the schematic structure of STIM1, with key domains marked. The mature STIM1 protein is cleaved of its N-terminal 22 amino acid signal peptide during translation. The regions required for Ca^{2+} sensing and oligomerisation of STIM1 are found within the remaining ER/SR luminal N-terminal region (aa. 23–200). This region contains an ER/SR targeting peptide, a pair of EF-hand domains for ER/SR luminal Ca^{2+} sensing, and a sterile α-motif (SAM) domain required for oligomerisation of STIM1 (Fig. 2) [30,57,64]. Within the SAM domain are two N-glycosylation sites that modulate the rate of STIM1 translocation to junctional ER upon store depletion [21].

The cytoplasmic region of STIM1 contains regions critical for interaction with Orai1. The minimum region of STIM1 necessary for CRAC channel activation is a ~100 amino acid long region known as the CRAC activation domain (CAD; aa. 342–448) (Fig. 2) [37,41,69]. Ectopic expression of a construct containing CAD but lacking all other regions of STIM1 (STIM1-CAD) with any of the Orai proteins is sufficient to cause a constitutive activation of CRAC current. In contrast, STIM1 constructs lacking the CAD are unable to activate current following store depletion [37,41,69]. An interesting property of I_{CRAC} activated by STIM1-CAD is that it fails to exhibit FCDI [40,69]. The CRAC modulatory domain

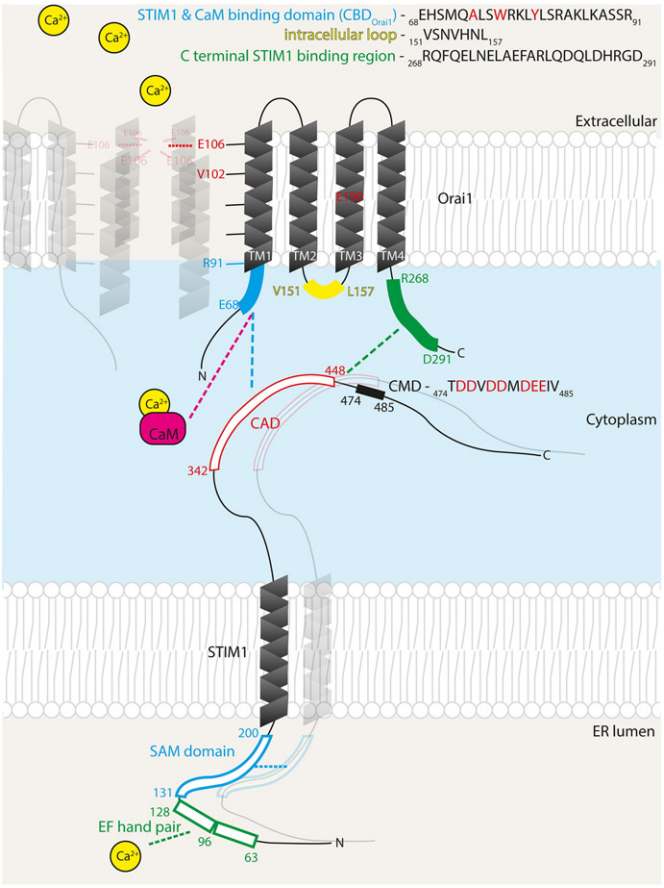

Fig. 2. Schematic diagram of Orai1/STIM1 organisation and key domains. Single Orai1 and STIM1 subunits are labelled, with associated subunits and relevant membranes unlabelled and faded for context. Intermolecular interactions are represented by dashed lines. STIM1 is a single transmembrane domain protein located on the endoplasmic reticulum membrane, and during store depletion migrates to regions of the membrane closely apposed to the plasma membrane. Key domains shown are the Ca^{2+} binding EF hand domain pair (green boxes), the SAM domain required for STIM1 oligomerisation (cyan box), CAD (red box), and CMD (black). Orai1 is located on the plasma membrane with 4 transmembrane domains and the N-terminus and C-terminus within in the cytoplasm. Transmembrane residues discussed are shown in red. Transmembrane domain 1 lines the pore, with the selectivity filter formed by a ring of six E106 residues in the functional hexameric channel. Key domains marked are CBDOrai1 (cyan), the intracellular loop (yellow) and the C terminal STIM1 binding region (green). Sequences for highlighted domains are given with specific residues discussed in this paper marked in red.

(CMD; aa. 474–485), C-terminal to CAD, is required for FCDI (Fig. 2) [4,22,40]. Expression of Orai1 with truncated STIM1 containing CAD and CMD produces current which retains FCDI. The CMD is rich in negatively charged glutamate and aspartate residues. The role of each of these negatively charged residues appears to be complex. Alanine neutralisation of D475/6A and D478/81A in the CMD reduces FCDI, while E482/3A causes increased and accelerated FCDI [40]. Neutralisation of six of these residues, including both E482 and E483, completely abolished FCDI of Orai1 [40].

6. Key domains and residues of Orai1

Orai1 is a 301 amino acid protein that is expressed on the plasma membrane of most cell types. Orai1 is predicted to have four transmembrane domains, with N and C termini as well as a single loop within the cytoplasmic space (Fig. 2).

6.1. N-terminus

The N-terminal residues 74–90 of Orai1 are highly conserved amongst species and are essential for channel activation; N-terminal

truncations of Orai1 up to aa. 74 allow for current to be activated upon store depletion [25]. The N-terminal Calmodulin (CaM) Binding Domain of Orai1 (CBD_{Orai1}, aa. 68–91) binds CaM in a Ca^{2+}-dependent manner [40]. Mutations of the CBD_{Orai1} that prevent CaM binding, e.g. A73E, W76A, W76E, W76S and Y80E, result in currents with reduced FCDI and prominent reactivation in response to hyperpolarising steps [40]. In contrast, mutations of the CBD_{Orai1} that retained CaM binding, Y80A and Y80S, enhance FCDI [40]. The notion of CaM involvement in FCDI is consistent with earlier observations that expression of a Ca^{2+}-insensitive CaM mutant in hepatocytes reduced FCDI of native I_{CRAC}. In this case, it was hypothesised that the mutant CaM would compete with wild-type CaM for Ca^{2+}-independent tethering sites on the channel, exerting a dominant negative effect on FCDI [28]. Consistent with immunoprecipitation and yeast two-hybrid data [41], the *Drosophila* Orai crystal structure reveals that the N-terminus forms a helix that extends into the cytoplasmic space and is also spatially available to bind STIM1 [19,49].The N-terminus of Orai1 also contains one arginine-rich and two proline-rich regions (aa. 1–47), which are either minimally conserved or completely absent in Orai2 and Orai3. The function of these regions remains poorly defined. Substitution of the Orai1 N-terminus into Orai3 causes a change in Ca^{2+}-dependent kinetics, introducing a reactivation phase similar to that of Orai1, but not associated with the usual strong inactivation of Orai3. While only a single proline-rich region is required for reactivation of Orai1, all three proline/arginine-rich regions are required for Orai3 reactivation, making it difficult to define an essential domain for reactivation and suggesting that other regions may be involved [11].

6.2. Intracellular loop

The Orai1 intracellular loop between transmembrane domains 2 and 3 is highly conserved between Orai1, 2 and 3, and between species, suggesting that it has an important function [10,56]. Consistent with this notion, co-transfection of STIM1 and Orai1 with residues 151–154 within the loop mutated to alanine results in an increase in whole cell current amplitude in response to store depletion [56]. Associated with this increase is a complete loss of FCDI. Interestingly, the transfection of a short peptide derived from the intracellular loop was able to restore FCDI, leading the investigators to the suggestion that the intracellular loop of Orai1 may form the inactivation gate [56]. Similarly, using chimeric Orai constructs, it has been demonstrated that the intracellular loop makes a substantial contribution to determining the inactivation profile of the construct. For example, an Orai1 intracellular loop substituted into an Orai3 construct displays inactivation similar to Orai1 [10].

6.3. C terminus

A region of the Orai1 C terminus, identified as aa. 268–291, containing a series of charged residues has shown by immunoprecipitation and yeast two-hybrid assays to interact with STIM1-CAD [39,41]. Deletion of this region results in a complete loss of I_{CRAC} [25,39,41]. The *Drosophila* Orai crystal structure confirmed the availability of this region to bind STIM1 [19,49].

The C-terminus of Orai1-3 is also likely to regulate FCDI [11,22]. Chimeric Orai constructs where the C-terminus of Orai1, 2 and 3 were interchanged demonstrated that the characteristic inactivation properties of the different Orai homologues are influenced by the C-terminus. For example, a chimera of Orai1 with an Orai3 intracellular C-terminus displays robust FCDI similar to wild-type Orai3 [22]. The stronger FCDI associated with the Orai2 and Orai3 C-termini compared to Orai1 has led to the hypothesis that a conserved series of three glutamates found in Orai2 and Orai3, compared to the two present in Orai1, may be responsible for this phenomenon. Consistent with this, alanine neutralisation of all three glutamates strongly reduced the extent of FCDI [22]. In contrast, one report did not find any change in the inactivation profile following the swap of only C-termini between Orai1 and Orai3 but instead suggested that the C-terminus acts in cooperation together with the other intracellular domains of Orai3. For example, swapping both N- and C-termini had a greater effect than would be expected from the sum of single N- or C-terminus swaps [11].

6.4. Orai1 pore residues

The transmembrane domain 1 (TM1) of each of the six Orai1 subunits that constitute a CRAC channel is understood to line the channel pore. Mutation analysis has identified several residues within or near the TM1 as being important for permeation and therefore likely to be positioned within the domain's helical structure facing the centre of the pore [31]. In addition, E190Q mutation in TM3 has also been reported to interfere with permeation [44,62,65]. The transmembrane residues discussed below have also been reported to modulate Ca^{2+}-dependent kinetics of I_{CRAC}, suggesting that CRAC channel gating and permeation may be coupled processes.

Based on the understanding of voltage-gated Ca^{2+} channels, where transmembrane glutamates form the selectivity centre [59,66], E106 of Orai1 in TM1 was identified as the CRAC channel selectivity centre. Mutations of E106 of Orai1 to glutamine (E106Q) or alanine (E106A) have a dominant negative effect, suppressing any WT CRAC current [12,44,62]. A conservative mutation to aspartate (E106D) [44,62,65], or of the homologous residue in *Drosophila* Orai (E180D) [44,68], produces a channel that retains activation by store depletion when co-expressed with STIM1 but has altered permeation properties. E106D Orai1 shifts the reversal potential of STIM1/Orai1-mediated current towards 0 mV, indicating a loss of selectivity for Ca^{2+} over monovalent cations [44,62,68]. These data conclusively demonstrate that E106 of Orai1 is critical in determining the selectivity of CRAC channels.

In addition to its altered selectivity profile, E106D Orai1 has also been reported to display altered fast Ca^{2+}-dependent kinetics compared to WT Orai1 [52,65]. Initial investigations of E106D Orai1 showed that in response to steps to negative membrane potentials, the current undergoes a very rapid phase of current decay to a steady state in a bath solution containing 20 mM Ca^{2+} and 130 mM Na^{+}. This decay can be fitted with a single exponential with a time constant of approximately 1.5 ms, which is significantly faster than what is normally seen for FCDI [65]. Since E106D Orai1 is permeable to Na^{+}, and replacement of all Na^{+} in the extracellular solution with 110 mM Ca^{2+} eliminated this current decay, the investigators reasonably concluded that the observed phenomenon was due to the block of Na^{+} current by Ca^{2+} [65]. Consistent with these data, a reduction of extracellular [Ca^{2+}] to micromolar levels slowed down the kinetics of the block of Na^{+} current, while progressive increases of extracellular [Ca^{2+}] accelerated the kinetics of the block of Na^{+} current [65]. The loss of current inactivation in 110 mM Ca^{2+} was taken as evidence that the E106D mutant lacks FCDI and that the mechanism of FCDI may be linked to the selectivity filter [65]. Using a different approach, a subsequent study utilised impermeable $NMDG^{+}$ in place of Na^{+} in the extracellular solution and found that at lower Ca^{2+} concentrations, in response to steps to negative membrane potentials, inactivation of E106D Orai1 current could be observed and that this inactivation was dependent on extracellular Ca^{2+} [52]. The most striking difference between this inactivation and typical FCDI of WT Orai1 was that it was greatly accelerated and able to be fitted with a single exponential with a time constant of approximately 1 ms in 10 mM extracellular Ca^{2+}. The inactivation was found to be independent of relative STIM1 expression levels and maintained even if expressed with STIM1-CAD, indicating that the inactivation was independent of STIM1 [52]. Taken together with data showing that, unlike FCDI of WT Orai1, the inactivation of the E106D Orai1 could be supported by permeating Sr^{2+} and that changing the intracellular Ca^{2+} buffer from EGTA to BAPTA had no effect on the kinetics of the current, it was concluded that the binding site regulating inactivation of E106D mutant was different from that of typical FCDI [52]. Two

possible reasons for this are that E106D mutation affects either the interaction between Orai1 and STIM1, or the mechanism of inactivation within the pore. The clear effects of this mutation strongly suggest that Ca^{2+}-dependent inactivation is likely to be associated with the selectivity filter [52,65].

A V102I mutation of Orai1 was reported to potentiate I_{CRAC} in response to hyperpolarising steps both in the presence and absence of Ca^{2+} [54]. Since this potentiation was independent from the extracellular ion composition, it was suggested that this mutation introduces a slow voltage gate due to interaction of the V102I residue with a gating charge at the selectivity filter, presumably at E106 [54]. In contrast, wild-type STIM1/Orai1 channels have been found to show neither inactivation nor potentiation in the absence of external Ca^{2+}, no matter how strong inactivation or potentiation is with external Ca^{2+} [51,65]. It remains unclear as to whether a purely voltage-dependent gating process exists for this mutant, or if external Ca^{2+} is required for potentiation of I_{CRAC}. A later study found that current mediated by V102I Orai1 is influenced by the relative expression of STIM1/Orai1, and at high STIM1/Orai1 relative expression displays strong FCDI [52]. The discrepancies in these findings relating to the kinetics of V102I Orai1 remain unresolved.

A second study also suggests that V102I Orai1 is unlikely to be functionally different from WT Orai1; however, other mutations of this residue suggest that the V102 residue may influence gating. When mutated from hydrophobic valine to mildly hydrophobic or polar residues, for example V102C, Orai1 becomes constitutively active, independent of STIM1 or store depletion, poorly selective for Ca^{2+} and displays no FCDI [33]. However, when co-expressed with STIM1, upon store depletion V102C Orai1 associates with STIM1 and displays high Ca^{2+} selectivity and FCDI. These data confirm that interaction with STIM1 changes the properties of the Orai1 pore and that FCDI is not an intrinsic property of Orai1 alone [32].

Mutation of E190 in transmembrane domain 3 of Orai1 to aspartate or alanine has no influence on Orai1 current. Mutation of the same residue to glutamine (E190Q) has been reported to result in a channel which is activated by store depletion but displays reduced selectivity for Ca^{2+} [44,62,65]. The degree of loss of selectivity associated with E190Q has been inconsistent between studies [45,62,65] and evidence that E190C cannot be chemically induced to form disulfide bonds, nor bind permeating Cd^{2+}, implies that E190 is unlikely to line the pore [33,73]. It has also been suggested that E190Q Orai1 kinetics is characterised by a Ca^{2+}-dependent reactivation phase [65]. However, similar to early studies of V102I Orai1 that predated the understanding of the fundamental importance of the stoichiometry of Orai1/STIM1, it now seems unlikely that this residue is involved in regulating Ca^{2+}-dependent kinetics of I_{CRAC} [52].

7. Proposed mechanism for FCDI

The results discussed in this review indicate that FCDI is a complex process influenced by 1) the stoichiometry of binding between STIM1 and Orai1, 2) cytoplasmic domains of STIM1 and Orai1, and 3) the selectivity centre within the Orai1 pore. A plausible working model bringing together many of these concepts to explain the regulation of FCDI of CRAC channels is similar to that proposed for Ca_V 1.2, where Ca^{2+}-dependent CaM binding to the cytoplasmic site of the channel increases the affinity of the selectivity filter for Ca^{2+}, causing Ca^{2+} to "stick" in the pore and prevent further ion permeation [1]. Although this exact model may not necessarily directly apply to FCDI in CRAC channels, it highlights the possibility of the interaction between the selectivity centre in the pore and the CaM binding site in the cytoplasmic domain of the channel, where Ca^{2+} binding to one these sites causes a conformational change at another. Such interaction could explain why E106D mutation in the selectivity centre causes very similar changes in I_{CRAC} kinetics [52] as Y80A (or Y80S) mutation in CBD_{Orai1}[40]. Both mutants, E106D and Y80A, exhibited greatly accelerated FCDI, compared to WT Orai1, which followed a single exponential time course. It may be that the E106D mutation allosterically alters the Orai1 CaM binding domain, or that both the E106D pore mutant and the Y80A/Y80S mutants cause a similar change in the affinity or efficacy of Ca^{2+} binding to a site in the pore, which regulates FCDI.

8. Orai1/STIM1stoichiometry in light of new crystal structure data

The recent discovery of the hexameric crystal structure of *Drosophila* Orai allows the electrophysiological and molecular data regarding STIM1/Orai1 stoichiometry and FCDI to be analysed in a new light. Interestingly, while the transmembrane region of the channel has a six-fold symmetry, the cytoplasmic domains adopt two alternating conformations, conferring a three-fold symmetry where pairs of Orai subunits have closely interacting C-termini [19]. If a single STIM1 subunit bridges the N- and C-termini STIM1 binding sites, then this would only allow three binding sites per channel. STIM1 has been suggested to function as a dimer [38,69,72]. If this is the case, this would contradict the model in which two STIM1 subunits bind to each Orai1 subunit for maximal I_{CRAC} activation [49]. However, the level of oligomerisation of STIM1 subunits remains undefined, and it has also been suggested that STIM1 may function as a tetramer, given that STIM1-CAD forms tetramers in solution [41].

The conformation of the Orai1 C-termini in activated Orai1-STIM1 complexes remains unknown. It has been speculated that perhaps in the activated state, the initial STIM1 interaction may disrupt the Orai pairing, and instead the C-termini extend directly down into the cytoplasm to reveal six binding sites [19]. While these models can possibly explain how STIM1 can bind Orai1 in a 2:1 ratio, they do not explicitly account for how other possible stoichiometries may activate the channel and display weaker FCDI or reactivation.

One possibility is that in a hypothetical extended conformation, there exists enough space for an STIM1 subunit each to bind to both the N- and C-termini, giving twelve binding sites. As demonstrated by Park *et al.* (2009), STIM1-CAD binds to both the C-terminus and N-terminus of Orai1, with a higher affinity for the C-terminus [41]. It would therefore be reasonable to suggest that when the ER/SR is nearly full and STIM1 concentration at the junctional ER is low, available Ca^{2+} binding sites associated with STIM1-CAD would only be present at the C-terminus which would favour Ca^{2+}-dependent reactivation. In contrast, when ER/SR Ca^{2+} is fully depleted and the amounts of STIM1 are saturating, STIM1-CAD occupies both N- and C-terminal binding sites on Orai1, which may create a completely different set of Ca^{2+} binding sites, and/or different type of conformational changes in response to Ca^{2+} binding, favouring FCDI.

In a model where a single STIM1 subunit within a dimer bridges the N- and C-termini Orai1 binding sites, this gives six STIM1 binding sites. In this case, when two or fewer sites are occupied, the channel is closed, as at least one STIM1 subunit per Orai subunit is required for channel activation [15]. Once three sites are occupied, the channel opens, and with each subsequent STIM1 dimer binding, the open probability or single channel conductance increases, resulting in increased whole cell I_{CRAC}. In a CRAC channel where only a minimum required number, for a functional channel, of STIM1 binding sites on Orai1 are occupied, Ca^{2+} entering through the pore promotes reactivation, whereas in a channel with all or almost all STIM1 binding sites occupied, entering Ca^{2+} causes FCDI.

9. Future perspectives

There are currently no physiological examples of I_{CRAC} displaying FCDI associated with low STIM1 expression relative to Orai1, such as Ca^{2+}-dependent reactivation. One possible example in an experimental setting is the distinct I_{CRAC} Ba^{2+} conductances of mast cells, RBL and Jurkat cells [16]. We have previously found that differences in STIM1:Orai1 expression were also associated with differences in Ba^{2+} conductance [51].

It is plausible that variable stoichiometry of STIM1:Orai1 and subsequent variable I_{CRAC} properties may have functional significance. The activation of store-operated channels is graded depending on the level of intracellular store depletion [2,14,29], presumably by recruitment of additional activated STIM1 as stores are progressively depleted. Therefore, FCDI would be graded in a similar manner to I_{CRAC} amplitude, where during partial depletion with a relatively small amount of activated STIM1, small currents with little or no FCDI would be likely and during full depletion large currents with strong FCDI would be expected. The kinetics of I_{CRAC} activated in response to partial store depletion has not been thoroughly investigated, though one study did not report any changes in kinetics under these conditions [29]. In addition, little is known about the physiological regulation of Orai1 and STIM1 expression in many cell types, or if expression changes during development, or during stressful or pathophysiological conditions. There is some evidence that these may be the case, for example in the dynamic expression patterns of STIM1 throughout muscle development [58], oncogene or tumour suppressor-dependent expression of STIM1 in a model cancer cell line [47], and altered expression of STIM1 and Orai1 during T cell activation [60], or following vascular injury [71]. Future work is likely to focus on endogenous regulation mechanisms of STIM and Orai protein expression, given that these data have provided the framework to link *in vivo* examples of altered relative STIM1/Orai1 expression with the functional consequences of altered Ca^{2+} signaling. In addition, the formation of heteromeric STIM1/Orai channels is an intriguing concept that remains largely unexplored. There is conclusive evidence that heteromeric Orai channels are able to be formed [27,35,50,62] and, given the functional differences between Orai subunits, may be able to display a wide range of characteristics and be tuned for specific roles in certain tissues or under certain conditions.

10. Conclusions

The discovery of STIM1 and Orai1 has underpinned rapid advancements in the understanding of CRAC channel function over the past eight years. In that time, the pathway from store depletion to channel activation has been well defined, and the functional significance of many individual domains of Orai1 and STIM1 has been described, if not yet synthesised into a “whole channel” model. The recent publication of crystal structure data for *Drosophila* Orai offers a new set of challenges and opportunities. The finding of a hexameric pore may force a reevaluation of many results that presumed a tetrameric pore. However, recent electrophysiological data obtained using Orai1 concatamers suggest that classical I_{CRAC} is mediated by Orai1 tetramer, not hexamer [61]. Given the importance of the stoichiometry of STIM1:Orai1 binding and the functional significance of the cytoplasmic domains of both STIM1 and Orai1 on I_{CRAC} properties as discussed in this review, a full understanding of CRAC channel Ca^{2+}-dependent gating will require the additional knowledge of how Orai1 and STIM1 subunits are organised in the functional channel.

Conflicts of interest

The authors declare that they have no conflict of interest.

References

[1] O. Babich, V. Matveev, A.L. Harris, R. Shirokov, Ca^{2+}-dependent inactivation of $Ca_V1.2$ channels prevents Gd^{3+} block: does Ca^{2+} block the pore of inactivated channels? J. Gen. Physiol. 129 (2007) 477–483.

[2] O. Brandman, J. Liou, W.S. Park, T. Meyer, STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca^{2+} levels, Cell 131 (2007) 1327–1339.

[3] P. Brehm, R. Eckert, Calcium entry leads to inactivation of calcium channel in Paramecium, Science 202 (1978) 1203–1206.

[4] I. Derler, M. Fahrner, M. Muik, B. Lackner, R. Schindl, K. Groschner, C. Romanin, A Ca^{2+} release-activated Ca^{2+} (CRAC) modulatory domain (CMD) within STIM1 mediates fast Ca^{2+}-dependent inactivation of ORAI1 channels, J. Biol. Chem. 284 (2009) 24933–24938.

[5] M. Faouzi, F. Hague, M. Potier, A. Ahidouch, H. Sevestre, H. Ouadid-Ahidouch, Down-regulation of Orai3 arrests cell-cycle progression and induces apoptosis in breast cancer cells but not in normal breast epithelial cells, J. Cell. Physiol. 226 (2011) 542–551.

[6] C. Fasolato, M. Hoth, R. Penner, A GTP-dependent step in the activation mechanism of capacitative calcium influx, J. Biol. Chem. 268 (1993) 20737–20740.

[7] S. Feske, Y. Gwack, M. Prakriya, S. Srikanth, S.H. Puppel, B. Tanasa, P.G. Hogan, R.S. Lewis, M. Daly, A. Rao, A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function, Nature 441 (2006) 179–185.

[8] L. Fierro, A.B. Parekh, Fast calcium-dependent inactivation of calcium release-activated calcium current (CRAC) in RBL-1 cells, J. Membr. Biol. 168 (1999) 9–17.

[9] M. Flourakis, V. Lehen'kyi, B. Beck, M. Raphael, M. Vandenberghe, F.V. Abeele, M. Roudbaraki, G. Lepage, B. Mauroy, C. Romanin, Y. Shuba, R. Skryma, N. Prevarskaya, Orai1 contributes to the establishment of an apoptosis-resistant phenotype in prostate cancer cells, Cell Death Dis. 1 (2010) e75.

[10] I. Frischauf, M. Muik, I. Derler, J. Bergsmann, M. Fahrner, R. Schindl, K. Groschner, C. Romanin, Molecular determinants of the coupling between STIM1 and Orai channels: differential activation of Orai1-3 channels by a STIM1 coiled-coil mutant, J. Biol. Chem. 284 (2009) 21696–21706.

[11] I. Frischauf, R. Schindl, J. Bergsmann, I. Derler, M. Fahrner, M. Muik, R. Fritsch, B. Lackner, K. Groschner, C. Romanin, Cooperativeness of Orai cytosolic domains tunes subtype-specific gating, J. Biol. Chem. 286 (2011) 8577–8584.

[12] Y. Gwack, S. Srikanth, S. Feske, F. Cruz-Guilloty, M. Oh-hora, D.S. Neems, P.G. Hogan, A. Rao, Biochemical and functional characterization of Orai proteins, J. Biol. Chem. 282 (2007) 16232–16243.

[13] Y. Gwack, S. Srikanth, M. Oh-Hora, P.G. Hogan, E.D. Lamperti, M. Yamashita, C. Gelinas, D.S. Neems, Y. Sasaki, S. Feske, M. Prakriya, K. Rajewsky, A. Rao, Hair loss and defective T- and B-cell function in mice lacking ORAI1, Mol. Cell. Biol. 28 (2008) 5209–5222.

[14] A.M. Hofer, C. Fasolato, T. Pozzan, Capacitative Ca^{2+} entry is closely linked to the filling state of internal Ca^{2+} stores: a study using simultaneous measurements of I_{CRAC} and intraluminal $[Ca^{2+}]$, J. Cell Biol. 140 (1998) 325–334.

[15] P.J. Hoover, R.S. Lewis, Stoichiometric requirements for trapping and gating of Ca^{2+} release-activated Ca^{2+} (CRAC) channels by stromal interaction molecule 1 (STIM1), Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13299–13304.

[16] M. Hoth, Calcium and barium permeation through calcium release-activated calcium (CRAC) channels, Pflugers Arch. 430 (1995) 315–322.

[17] M. Hoth, R. Penner, Depletion of intracellular calcium stores activates a calcium current in mast cells, Nature 355 (1992) 353–356.

[18] M. Hoth, R. Penner, Calcium release-activated calcium current in rat mast cells, J. Physiol. 465 (1993) 359–386.

[19] X. Hou, L. Pedi, M.M. Diver, S.B. Long, Crystal structure of the calcium release-activated calcium channel Orai, Science 338 (2012) 1308–1313.

[20] W. Ji, P. Xu, Z. Li, J. Lu, L. Liu, Y. Zhan, Y. Chen, B. Hille, T. Xu, L. Chen, Functional stoichiometry of the unitary calcium-release-activated calcium channel, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 13668–13673.

[21] T. Kilch, D. Alansary, M. Peglow, K. Dorr, G. Rychkov, H. Rieger, C. Peinelt, B.A. Niemeyer, Mutations of the Ca^{2+}-sensing stromal interaction molecule STIM1 regulate Ca^{2+} influx by altered oligomerization of STIM1 and by destabilization of the Ca^{2+} channel Orai1, J. Biol. Chem. 288 (2013) 1653–1664.

[22] K.P. Lee, J.P. Yuan, W. Zeng, I. So, P.F. Worley, S. Muallem, Molecular determinants of fast Ca^{2+}-dependent inactivation and gating of the Orai channels, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 14687–14692.

[23] R.S. Lewis, Store-operated calcium channels: new perspectives on mechanism and function, Cold Spring Harb. Perspect. Biol. 3 (2011).

[24] Z. Li, L. Liu, Y. Deng, W. Ji, W. Du, P. Xu, L. Chen, T. Xu, Graded activation of CRAC channel by binding of different numbers of STIM1 to Orai1 subunits, Cell Res. 21 (2011) 305–315.

[25] Z. Li, J. Lu, P. Xu, X. Xie, L. Chen, T. Xu, Mapping the interacting domains of STIM1 and Orai1 in Ca^{2+} release-activated Ca^{2+} channel activation, J. Biol. Chem. 282 (2007) 29448–29456.

[26] J. Liou, M.L. Kim, W.D. Heo, J.T. Jones, J.W. Myers, J.E. Ferrell Jr., T. Meyer, STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx, Curr. Biol. 15 (2005) 1235–1241.

[27] A. Lis, C. Peinelt, A. Beck, S. Parvez, M. Monteilh-Zoller, A. Fleig, R. Penner, CRACM1, CRACM2, and CRACM3 are store-operated Ca^{2+} channels with distinct functional properties, Curr. Biol. 17 (2007) 794–800.

[28] T. Litjens, T. Nguyen, J. Castro, E.C. Aromataris, L. Jones, G.J. Barritt, G.Y. Rychkov, Phospholipase C-gamma1 is required for the activation of store-operated Ca^{2+} channels in liver cells, Biochem. J. 405 (2007) 269–276.

[29] R.M. Luik, B. Wang, M. Prakriya, M.M. Wu, R.S. Lewis, Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation, Nature 454 (2008) 538–542.

[30] S.S. Manji, N.J. Parker, R.T. Williams, L. van Stekelenburg, R.B. Pearson, M. Dziadek, P.J. Smith, STIM1: a novel phosphoprotein located at the cell surface, Biochim. Biophys. Acta 1481 (2000) 147–155.

[31] C.A. McCarl, C. Picard, S. Khalil, T. Kawasaki, J. Rother, A. Papolos, J. Kutok, C. Hivroz, F. Ledeist, K. Plogmann, S. Ehl, G. Notheis, M.H. Albert, B.H. Belohradsky, J. Kirschner, A. Rao, A. Fischer, S. Feske, ORAI1 deficiency and lack of store-operated Ca^{2+} entry cause immunodeficiency, myopathy, and ectodermal dysplasia, J. Allergy Clin. Immunol. 124 (2009)(1311–8 e7).

[32] B.A. McNally, A. Somasundaram, M. Yamashita, M. Prakriya, Gated regulation of CRAC channel ion selectivity by STIM1, Nature 482 (2012) 241–245.

[33] B.A. McNally, M. Yamashita, A. Engh, M. Prakriya, Structural determinants of ion permeation in CRAC channels, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 22516–22521.

[34] J.C. Mercer, W.I. Dehaven, J.T. Smyth, B. Wedel, R.R. Boyles, G.S. Bird, J.W. Putney Jr., Large store-operated calcium selective currents due to co-expression of Orai1 or

Orai2 with the intracellular calcium sensor, Stim1, J. Biol. Chem. 281 (2006) 24979–24990.
[35] O. Mignen, J.L. Thompson, T.J. Shuttleworth, Both Orai1 and Orai3 are essential components of the arachidonate-regulated Ca^{2+}-selective ARC channels, J. Physiol. 586 (2007) 185–195.
[36] O. Mignen, J.L. Thompson, T.J. Shuttleworth, Orai1 subunit stoichiometry of the mammalian CRAC channel pore, J. Physiol. 586 (2008) 419–425.
[37] M. Muik, M. Fahrner, I. Derler, R. Schindl, J. Bergsmann, I. Frischauf, K. Groschner, C. Romanin, A cytosolic homomerization and a modulatory domain within STIM1 C terminus determine coupling to ORAI1 channels, J. Biol. Chem. 284 (2009) 8421–8426.
[38] M. Muik, M. Fahrner, R. Schindl, P. Stathopulos, I. Frischauf, I. Derler, P. Plenk, B. Lackner, K. Groschner, M. Ikura, C. Romanin, STIM1 couples to ORAI1 via an intramolecular transition into an extended conformation, EMBO J. 30 (2011) 1678–1689.
[39] M. Muik, I. Frischauf, I. Derler, M. Fahrner, J. Bergsmann, P. Eder, R. Schindl, C. Hesch, B. Polzinger, R. Fritsch, H. Kahr, J. Madl, H. Gruber, K. Groschner, C. Romanin, Dynamic coupling of the putative coiled-coil domain of ORAI1 with STIM1 mediates ORAI1 channel activation, J. Biol. Chem. 283 (2008) 8014–8022.
[40] F.M. Mullins, C.Y. Park, R.E. Dolmetsch, R.S. Lewis, STIM1 and calmodulin interact with Orai1 to induce Ca^{2+}-dependent inactivation of CRAC channels, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 15495–15500.
[41] C.Y. Park, P.J. Hoover, F.M. Mullins, P. Bachhawat, E.D. Covington, S. Raunser, T. Walz, K.C. Garcia, R.E. Dolmetsch, R.S. Lewis, STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1, Cell 136 (2009) 876–890.
[42] A. Penna, A. Demuro, A.V. Yeromin, S.L. Zhang, O. Safrina, I. Parker, M.D. Cahalan, The CRAC channel consists of a tetramer formed by Stim-induced dimerization of Orai dimers, Nature 456 (2008) 116–120.
[43] C. Picard, C.A. McCarl, A. Papolos, S. Khalil, K. Luthy, C. Hivroz, F. LeDeist, F. Rieux-Laucat, G. Rechavi, A. Rao, A. Fischer, S. Feske, STIM1 mutation associated with a syndrome of immunodeficiency and autoimmunity, N. Engl. J. Med. 360 (2009) 1971–1980.
[44] M. Prakriya, S. Feske, Y. Gwack, S. Srikanth, A. Rao, P.G. Hogan, Orai1 is an essential pore subunit of the CRAC channel, Nature 443 (2006) 230–233.
[45] M. Prakriya, R.S. Lewis, Regulation of CRAC channel activity by recruitment of silent channels to a high open-probability gating mode, J. Gen. Physiol. 128 (2006) 373–386.
[46] C. Randriamampita, R.Y. Tsien, Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx, Nature 364 (1993) 809–814.
[47] M.F. Ritchie, C. Yue, Y. Zhou, P.J. Houghton, J. Soboloff, Wilms tumor suppressor 1 (WT1) and early growth response 1 (EGR1) are regulators of STIM1 expression, J. Biol. Chem. 285 (2010) 10591–10596.
[48] J. Roos, P.J. DiGregorio, A.V. Yeromin, K. Ohlsen, M. Lioudyno, S. Zhang, O. Safrina, J.A. Kozak, S.L. Wagner, M.D. Cahalan, G. Velicelebi, K.A. Stauderman, STIM1, an essential and conserved component of store-operated Ca^{2+} channel function, J. Cell Biol. 169 (2005) 435–445.
[49] B.S. Rothberg, Y. Wang, D.L. Gill, Orai channel pore properties and gating by STIM: implications from the Orai crystal structure, Sci. Signal. 6 (2013) pe9.
[50] R. Schindl, I. Frischauf, J. Bergsmann, M. Muik, I. Derler, B. Lackner, K. Groschner, C. Romanin, Plasticity in Ca^{2+} selectivity of Orai1/Orai3 heteromeric channel, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 19623–19628.
[51] N. Scrimgeour, T. Litjens, L. Ma, G.J. Barritt, G.Y. Rychkov, Properties of Orai1 mediated store-operated current depend on the expression levels of STIM1 and Orai1 proteins, J. Physiol. 587 (2009) 2903–2918.
[52] N.R. Scrimgeour, D.P. Wilson, G.Y. Rychkov, Glutamate 106 in the Orai1 pore contributes to fast Ca^{2+}-dependent inactivation and pH dependence of Ca^{2+} release-activated Ca^{2+} (CRAC) current, Biochem. J. 441 (2012) 743–753.
[53] J. Soboloff, M.A. Spassova, X.D. Tang, T. Hewavitharana, W. Xu, D.L. Gill, Orai1 and STIM reconstitute store-operated calcium channel function, J. Biol. Chem. 281 (2006) 20661–20665.
[54] M.A. Spassova, T. Hewavitharana, R.A. Fandino, A. Kaya, J. Tanaka, D.L. Gill, Voltage gating at the selectivity filter of the Ca^{2+} release-activated Ca^{2+} channel induced by mutation of the Orai1 protein, J. Biol. Chem. 283 (2008) 14938–14945.
[55] M.A. Spassova, J. Soboloff, L.P. He, W. Xu, M.A. Dziadek, D.L. Gill, STIM1 has a plasma membrane role in the activation of store-operated $Ca(^{2+})$ channels, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 4040–4045.
[56] S. Srikanth, H.J. Jung, B. Ribalet, Y. Gwack, The intracellular loop of Orai1 plays a central role in fast inactivation of Ca^{2+} release-activated Ca^{2+} channels, J. Biol. Chem. 285 (2009) 5066–5075.
[57] P.B. Stathopulos, L. Zheng, G.Y. Li, M.J. Plevin, M. Ikura, Structural and mechanistic insights into STIM1-mediated initiation of store-operated calcium entry, Cell 135 (2008) 110–122.
[58] J. Stiber, A. Hawkins, Z.S. Zhang, S. Wang, J. Burch, V. Graham, C.C. Ward, M. Seth, E. Finch, N. Malouf, R.S. Williams, J.P. Eu, P. Rosenberg, STIM1 signalling controls store-operated calcium entry required for development and contractile function in skeletal muscle, Nat. Cell Biol. 10 (2008) 688–697.
[59] S. Tang, G. Mikala, A. Bahinski, A. Yatani, G. Varadi, A. Schwartz, Molecular localization of ion selectivity sites within the pore of a human L-type cardiac calcium channel, J. Biol. Chem. 268 (1993) 13026–13029.
[60] P. Thakur, A.F. Fomina, Density of functional Ca^{2+} release-activated Ca^{2+} (CRAC) channels declines after T-cell activation, Channels 5 (2011) 510–517.
[61] J.L. Thompson, T.J. Shuttleworth, How many Orai's does it take to make a CRAC channel? Sci. Rep. 3 (2013) 1961.
[62] M. Vig, A. Beck, J.M. Billingsley, A. Lis, S. Parvez, C. Peinelt, D.L. Koomoa, J. Soboloff, D.L. Gill, A. Fleig, J.P. Kinet, R. Penner, CRACM1 multimers form the ion-selective pore of the CRAC channel, Curr. Biol. 16 (2006) 2073–2079.
[63] M. Vig, C. Peinelt, A. Beck, D.L. Koomoa, D. Rabah, M. Koblan-Huberson, S. Kraft, H. Turner, A. Fleig, R. Penner, J.P. Kinet, CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry, Science 312 (2006) 1220–1223.
[64] R.T. Williams, S.S. Manji, N.J. Parker, M.S. Hancock, L. Van Stekelenburg, J.P. Eid, P.V. Senior, J.S. Kazenwadel, T. Shandala, R. Saint, P.J. Smith, M.A. Dziadek, Identification and characterization of the STIM (stromal interaction molecule) gene family: coding for a novel class of transmembrane proteins, Biochem. J. 357 (2001) 673–685.
[65] M. Yamashita, L. Navarro-Borelly, B.A. McNally, M. Prakriya, Orai1 mutations alter ion permeation and Ca^{2+}-dependent fast inactivation of CRAC channels: evidence for coupling of permeation and gating, J. Gen. Physiol. 130 (2007) 525–540.
[66] J. Yang, P.T. Ellinor, W.A. Sather, J.F. Zhang, R.W. Tsien, Molecular determinants of Ca^{2+} selectivity and ion permeation in L-type Ca^{2+} channels, Nature 366 (1993) 158–161.
[67] S. Yang, J.J. Zhang, X.Y. Huang, Orai1 and STIM1 are critical for breast tumor cell migration and metastasis, Cancer Cell 15 (2009) 124–134.
[68] A.V. Yeromin, S.L. Zhang, W. Jiang, Y. Yu, O. Safrina, M.D. Cahalan, Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai, Nature 443 (2006) 226–229.
[69] J.P. Yuan, W. Zeng, M.R. Dorwart, Y.J. Choi, P.F. Worley, S. Muallem, SOAR and the polybasic STIM1 domains gate and regulate Orai channels, Nat. Cell Biol. 11 (2009) 337–343.
[70] S.L. Zhang, Y. Yu, J. Roos, J.A. Kozak, T.J. Deerinck, M.H. Ellisman, K.A. Stauderman, M.D. Cahalan, STIM1 is a Ca^{2+} sensor that activates CRAC channels and migrates from the Ca^{2+} store to the plasma membrane, Nature 437 (2005) 902–905.
[71] W. Zhang, K.E. Halligan, X. Zhang, J.M. Bisaillon, J.C. Gonzalez-Cobos, R.K. Motiani, G. Hu, P.A. Vincent, J. Zhou, M. Barroso, H.A. Singer, K. Matrougui, M. Trebak, Orai1-mediated I (CRAC) is essential for neointima formation after vascular injury, Circ. Res. 109 (2011) 534–542.
[72] Y. Zhou, P. Meraner, H.T. Kwon, D. Machnes, M. Oh-hora, J. Zimmer, Y. Huang, A. Stura, A. Rao, P.G. Hogan, STIM1 gates the store-operated calcium channel ORAI1 in vitro, Nat. Struct. Mol. Biol. 17 (2010) 112–116.
[73] Y. Zhou, S. Ramachandran, M. Oh-Hora, A. Rao, P.G. Hogan, Pore architecture of the ORAI1 store-operated calcium channel, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 4896–4901.
[74] A. Zweifach, R.S. Lewis, Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} stores, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 6295–6299.
[75] A. Zweifach, R.S. Lewis, Rapid inactivation of depletion-activated calcium current (I_{CRAC}) due to local calcium feedback, J. Gen. Physiol. 105 (1995) 209–226.