Cell Calcium 63 (2017) 14–19

Contents lists available at ScienceDirect

Cell Calcium

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

Review

Pore opening mechanism of CRAC channels

Priscilla S.-W. Yeung, Megumi Yamashita, Murali Prakriya *

Department of Pharmacology, Northwestern University, Feinberg School of Medicine, Chicago, IL 60611, United States

ARTICLE INFO

Article history:
Received 30 November 2016
Received in revised form
21 December 2016
Accepted 21 December 2016
Available online 23 December 2016

Keywords:
SOCE
Calcium
Orai1
STIM1
CRAC channel

ABSTRACT

Three decades ago, James W. Putney Jr. conceptualized the idea of store-operated calcium entry (SOCE) to explain how depletion of endoplasmic reticulum (ER) Ca^{2+} stores evokes Ca^{2+} influx across the plasma membrane. Since the publication of this highly influential idea, it is now established that SOCE is universal among non-excitable and probably even many types of excitable cells, and contributes to numerous effector functions impacting immunity, muscle contraction, and brain function. The molecules encoding SOCE, the STIM and Orai proteins, are now known and our understanding of how this pathway is activated in response to ER Ca^{2+} store depletion has advanced significantly. In this review, we summarize the current knowledge of how Orai1 channels are activated by STIM1, focusing on recent work supporting a hydrophobic gating mechanism for the opening of the Orai1 channel pore.

Contents

1. Introduction 14
2. Permeation, selectivity, and pore architecture of CRAC channels 15
3. Gating mechanism of CRAC channels 16
4. Future directions 18
Acknowledgements 18
References 18

1. Introduction

Of the several known mechanisms for eliciting Ca^{2+} signals in cells, store-operated calcium entry (SOCE) is one of the most widespread pathways. James W. Putney Jr. first described the idea of SOCE in 1986, noting that intracellular inositol triphosphate (IP_3) triggers a biphasic mobilization of Ca^{2+} in rat salivary and lacrimal glands that involves an initial release of Ca^{2+} from intracellular endoplasmic reticulum (ER) stores followed by a more sustained Ca^{2+} influx from the extracellular compartment [1]. He hypothesized that intracellular Ca^{2+} store depletion in these exocrine glands triggers activation of a plasma membrane Ca^{2+} influx pathway which refills depleted stores, naming it capacitative calcium entry (CCE) [1]. Later studies showed that this Ca^{2+} influx pathway elevates cytosolic [Ca^{2+}] in a wide variety of cells and was renamed SOCE to designate its dependence on the ER Ca^{2+} store content [2,3]. The best-studied SOCE channel is the "calcium release-activated calcium" (CRAC) channel, a highly Ca^{2+} selective channel with a very low unitary conductance [4]. CRAC channels are widespread, perhaps universally expressed among all animal cells, and are typically activated following stimulation of G-protein coupled receptors or receptor tyrosine kinases that produce IP_3 to deplete ER Ca^{2+} stores [4–6]. Apart from refilling ER Ca^{2+} stores, the opening of CRAC channels also causes a rise in intracellular Ca^{2+} that regulates a variety of effector cell responses including gene expression, cell proliferation, exocytosis, and motility [4]. Human studies have shown that loss of CRAC channel function leads to a devastating immunodeficiency along with additional symptoms of autoimmunity, ectodermal dysplasia, and muscle defects, highlighting the vital importance of CRAC channels for human health [7–9]. Conversely, constitutive channel activation from gain-of-function mutations in CRAC channel proteins are linked to pathologies such as tubular aggregate myopathy and thrombocytopenia [10–12]. In several instances, the molecu-

* Corresponding author.
E-mail address: m-prakriya@northwestern.edu (M. Prakriya).

http://dx.doi.org/10.1016/j.ceca.2016.12.006
0143-4160/

lar mechanisms of how these loss- and gain-of-function mutations disrupt CRAC channels remains unclear, underscoring the need for a better understanding of the operational mechanisms of CRAC channels including their gating mechanism.

The prototypical CRAC channel is formed by two subunits: Orai1, the pore-forming protein in the plasma membrane [13–16], and STIM1, the Ca^{2+} sensor in the ER [17,18]. Orai1 contains four transmembrane domains (TMs) with cytosolic N- and C-termini that serve as interaction sites for STIM1 (Fig. 1A). STIM1 makes a single pass through the ER membrane and contains several functional domains including a luminal EF-hand motif that acts as the ER Ca^{2+} sensor [17–19] and two coiled-coil domains in the cytoplasmic side that comprise the CRAC activation domain (CAD) [20] or STIM1-Orai1 activating region (SOAR) [21]. CAD/SOAR is the catalytic region of STIM1 that binds to and activates Orai1 channels [20–22]. In response to store depletion, STIM1, which is diffusely distributed in the bulk ER membrane at rest, exhibits a complex molecular choreography in which it first oligomerizes and subsequently migrates to ER-PM junctions [19,23,24], where it then physically interacts with the cytoplasmic tails of Orai1 to gate Orai1 channels [20,25–30]. This physical interaction causes the two previously diffusely localized proteins to accumulate into distinct puncta at ER-PM junctions to form active CRAC channels [20,31].

2. Permeation, selectivity, and pore architecture of CRAC channels

CRAC channels are characterized by two biophysical features which provide a relatively easy way to identify them in native cells: high Ca^{2+} selectivity and low unitary conductance. As measured by the proportion of current carried by Ca^{2+} in a physiological mix of Na^+ and Ca^{2+} ions, CRAC channels select for Ca^{2+} over the more prevalent Na^+ ions by a ratio >1000 ($P_{Ca}/P_{Na} > 1000$) [14,32,33], making them amongst the most Ca^{2+}-selective channels known. Early studies using mutational analysis and measurements of binding kinetics and voltage-dependence revealed that a ring of glutamates (E106) at the external mouth of the pore function as the selectivity filter [14–16,34]. These glutamates form a Ca^{2+} binding site to block permeation of Na^+ ions through the pore ($K_i \approx 20\,\mu M$ [34–36]), analogous to the mechanism of Ca^{2+} selectivity described for voltage-gated Ca^{2+} (Ca_V) channels [37]. More recently, this explanation for the CRAC channel's high Ca^{2+} selectivity has been modified based on the observation that Ca^{2+} selectivity in Orai3 channels is controlled not only by the binding of Ca^{2+} ions to the selectivity filter (formed by E81), but also by the overall rates of ion entry and exit from the selectivity filter, with slower ion flux rates yielding higher selectivity [38]. This latter feature likely explains why CRAC channels exhibit comparable levels of Ca^{2+} selectivity as Ca_V channels despite 20-fold weaker Ca^{2+} binding in the pore ($K_i < 1\,\mu M$ in Ca_V channels [37] vs. 20 μM in CRAC channels [4]). In effect, a slow ionic flux rate in CRAC channels essentially eliminates the need for tight Ca^{2+} binding to achieve high Ca^{2+} selectivity. Interestingly, studies in constitutively active Orai1 channels indicate that the Ca^{2+} selectivity of CRAC channels is strongly regulated by STIM1 binding [39,40], suggesting that the selectivity of the CRAC channel is not a fixed property of the open state structure, but a dynamic property coupled to channel gating. The details of how this occurs remain to be formally determined, but as discussed below, one possibility is that Ca^{2+} binding to the selectivity filter is altered by the conformational motions in the pore triggered by STIM1 binding.

The second functional characteristic of CRAC channels, their low unitary conductance, is less well understood. Estimates from analysis of the whole-cell current noise indicate that the unitary conductance is only 10–30 fS in 2–110 mM Ca^{2+}-containing solutions [35,38,41], which suggests the pore contains significant energy barriers for ion conduction in the active state.

An important breakthrough in our understanding of the molecular basis of ion conduction in CRAC channels came from identification of the pore-lining residues [42,43]. In one approach, the substituted cysteine accessibility method (SCAM) was used to examine the extent of current inhibition after thiol-reactive reagents were applied to the extracellular side of Orai1 channels bearing cysteine mutations [42]. In a second approach, the fraction of inter-subunit disulfide cross-linking of Orai1 cysteine mutants was quantified based on the prediction that cysteine residues introduced at pore-lining positions should be able to form disulfide bonds across the pore upon protein oxidation [43]. The results of these studies, which were consistent with one another, indicated that residues in TM1 flank the pore and ruled out a contribution for TM3 and, specifically, E190, a residue in TM3 that was previously thought to regulate Ca^{2+} selectivity [14,15]. In the TM1 domain, the key pore-lining residues found from these studies included E106, V102, G98, L95 and R91 (Fig. 1B). The SCAM study used Cd^{2+} ions to form metal ion bridges between introduced cysteine residues across the pore [42]. Because the length of Cd^{2+}-S bridges is short (~2.5 Å) [44,45], the strong reactivity to Cd^{2+} at several pore-lining residues indicated that TM1 helices are close to one another and therefore line a narrow pore, a feature that has been proposed to account for the CRAC channel's low permeability to large cations (>3.8 Å) and its low unitary conductance. Moreover, differences in the accessibility of probes of different sizes showed that the pore

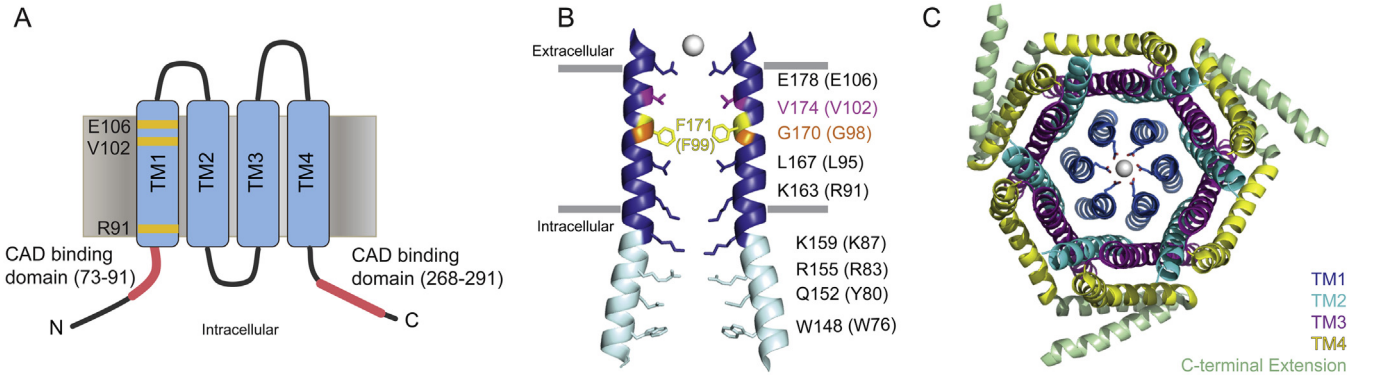

Fig. 1. (A) Topology diagram of Orai1 showing four transmembrane domains and cytosolic N- and C- termini that serve as interaction sites for STIM1. TM1 lines the channel pore with the selectivity filter formed by E106. Potential inner and outer channel gates are proposed to be near V102 and R91, respectively. Residue numbering corresponds to human Orai1. (B) Two diagonally facing subunits in the crystal structure of *Drosophila* Orai (PDB code ID 4HKR; ref 46) showing predicted pore-lining TM1 residues. dOrai residue numbering is indicated with equivalent hOrai1 residues shown in parentheses. (C) Top-down view of the complete crystal structure of dOrai. Each channel is formed by six Orai proteins arranged in concentric rings surrounding the narrow pore flanked by TM1 [46].