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Review

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

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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

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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

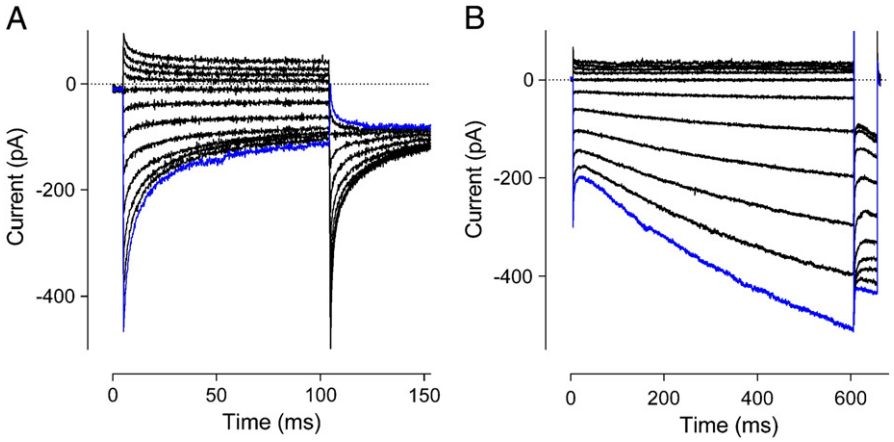

Fig. 1. Examples of FCDI and Ca^{2+}-dependent reactivation. Example I_{CRAC} from HEK293 cells with exogenously expressed STIM1 and Orai1. Traces are recorded in response to the initial steps ranging from +62 mV to −138 mV in 20-mV increments, the most negative step shown in blue, followed by a second 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.