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TRPC channels as STIM1-regulated store-operated channels

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Abstract

Receptor-activated Ca^{2+} influx is mediated largely by store-operated channels (SOCs). TRPC channels mediate a significant portion of the receptor-activated Ca^{2+} influx. However, whether any of the TRPC channels function as a SOC remains controversial. Our understanding of the regulation of TRPC channels and their function as SOCs is being reshaped with the discovery of the role of STIM1 in the regulation of Ca^{2+} influx channels. The findings that STIM1 is an ER resident Ca^{2+} binding protein that regulates SOCs allow an expanded and molecular definition of SOCs. SOCs can be considered as channels that are regulated by STIM1 and require the clustering of STIM1 in response to depletion of the ER Ca^{2+} stores and its translocation towards the plasma membrane. TRPC1 and other TRPC channels fulfill these criteria. STIM1 binds to TRPC1, TRPC2, TRPC4 and TRPC5 but not to TRPC3, TRPC6 and TRPC7, and STIM1 regulates TRPC1 channel activity. Structure-function analysis reveals that the C-terminus of STIM1 contains the binding and gating function of STIM1. The ERM domain of STIM1 binds to TRPC channels and a lysine-rich region participates in the gating of SOCs and TRPC1. Knock-down of STIM1 by siRNA and prevention of its translocation to the plasma membrane inhibit the activity of native SOCs and TRPC1. These findings support the conclusion that TRPC1 is a SOC. Similar studies with other TRPC channels should further clarify their regulation by STIM1 and function as SOCs.

Introduction

The receptor-evoked Ca^{2+} signal entails Ca^{2+} release from the endoplasmic reticulum (ER) that is followed by activation of Ca^{2+} influx channels at the plasma membrane. Activation of Ca^{2+} influx in response to Ca^{2+} release from internal stores lead to the definition of these Ca^{2+} influx channels as store-operated channels or SOCs [1]. Ca^{2+} influx through SOCs is a key component of the receptor-evoked Ca^{2+} signal. SOCs-mediated Ca^{2+} influx controls

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numerous physiological functions on a time scale ranging from milliseconds to many hrs [2]. In the absence of Ca^{2+} influx the Ca^{2+}-mediated effects terminate within few minutes due to exhaustion of the finite intracellular Ca^{2+} stores. The molecular identity of the SOCs and how they sense the Ca^{2+} content in the ER has been a mystery for many years. Several recent breakthroughs that are the focus of this special issue lead to major inroads in addressing these questions. The best characterized SOC is the channel mediating the I_{crac} current. I_{crac} is activated by stimulation of receptors that signal through an increase in IP_3 and by passive depletion of ER Ca^{2+} [1,3]. A long awaited finding recently identifies the Orai proteins as the pore forming channels that mediate I_{crac} [4–9]. This topic is covered by several reviews in this issue. Another group of channels that function as SOCs are the TRPC channels. However, which of the TRPC channels function as SOC is still controversial, with evidence for and against for almost each of the channels. The important discovery of STIM1 as the sensor of ER Ca^{2+} content that conveys it to the SOCs [10,11] should greatly help in expanding the definition of SOCs and in clarifying which channel behaves as SOCs. Describing the regulation of TRPC1 channels by STIM1 and its relevance to the function of TRPC channels as SOCs is the aim of this short review.

TRPC channels as SOCs

TRPC channels are non-selective, Ca^{2+}-permeable cation channels that are activated by stimulation of G proteins-coupled and Tyrosine phosphorylated receptors. There is evidence indicating that several TRPC channels function as SOCs. However, the majority of the evidence relies on deletion of specific TRPC isoforms or their silencing by antisense or siRNA, rather than demonstrating directly their function as SOCs. For example, HEK cells express native TRPC1, TRPC3, TRPC4, TRPC6 and TRPC7. Treatment with antisense to specifically inhibit transcription of the endogenous TRPC1 [12–15] and TRPC3 [12,13] reduces Ca^{2+} influx when activated either by receptor stimulation or passive store depletion. Knockdown (KD) of the same channels by siRNA [16] also inhibits SOC activity. An important finding was that comparable inhibition of Ca^{2+} influx occurs by KD of several individual TRPC channels. KD of TRPC1, TRPC3, or TRPC7 by siRNA markedly inhibits SOCs activated by passive depletion of ER Ca^{2+}, whereas suppression of TRPC4 or TRPC6 has no effect. Moreover, the concomitant KD of TRPC1+TRPC3, TRPC1+TRPC7, TRPC3+TRPC7, or TRPC1+TRPC3 +TRPC7 reduces SOCs similar to the KD of TRPC1 alone [16]. Interestingly, the same study showed that agonist-stimulated Ca^{2+} influx was inhibited by suppression of any of the TRPC channels expressed in HEK cells [16]. The implication of these findings is that the complement of cellular TRPC channels is assembled into a single or limited number of complexes that form at least part of the SOCs.

Additional evidence for the function of TRPC channels as SOCs 1s provided by the finding that KD of TRPC1 reduces I_{soc} current in the smooth muscle cell line A7r5 cell [17] and reduces SOC activity in human keratinocytes [18]. KD of TRPC3 reduces SOC activity in rat prostate smooth muscle cells [19]. Disruption of the *trpc4* gene in mice revealed that TRPC4 functions as SOC in endothelial cells [20,21]. KD of TRPC4 reduces SOCs activity in mouse mesangial cells [22] and human corneal epithelial cells [23]. Disruption of the interaction between protein 4.1 and TRPC4 inhibits I_{soc} current in endothelial cells [24]. An interesting behavior was observed with TRPC7. When it is expressed at high levels, TRPC7 functions as a store-independent channel that is inhibited by high concentration of Gd^{3+}. By contrast, at low expression level TRPC7 behaves as a SOC, its activity depends on the presence of IP_3 receptors and it is highly sensitive to inhibition by Gd^{3+} [25–27]. Hence, the function of TRPC7 as SOCs depends on its expression level. TRPC3 function is also affected by its expression level. When it is expressed at low levels, it can be partially activated by passive store depletion [28]. However, at high expression levels TRPC3 behaves as store- and IP_3 receptors-independent Ca^{2+} influx channel [29,30]. Altered TRPC channels behavior based on their expression level

may account for some of the variable results reported in the literature. The combined findings from the knockout and knockdown approaches indicate that TRPC channels form one type of SOCs and mediate a large fraction of the receptor-stimulated Ca^{2+} influx in many cell types and for many receptors.

For a long time, SOCs were equated only with I_{crac}. That the properties of native and topically expressed TRPC channels are different from those of I_{crac} forms the major objection in accepting the TRPC channels as SOCs. For example, an I_{crac}-like current that is highly selective for Ca^{2+} and is activated by passive store depletion can be found in several cell types. However, when TRPC3 is overexpressed in HEK 293 cells, it behaves as a non-selective monovalent cation permeable channel with a large conductance [28,31,32]. Additional uncertainty was raised by contradictory reports on the activation of TRPC3 by store depletion [28–30,32]. The dependence of TRPC channels behavior on their expression level discussed above and the discovery of the Orais and STIM1 may provide an explanation to some of these observations. It is now clear that Orai1 [4–7,9,33–35], and perhaps Orai2 and Orai3 [34,36] are the proteins that mediate I_{crac}. However, recently it was shown that Orai1 forms a complex with STIM1 and TRPC1 [37]. Of particular significance is the finding that KD of TRPC1 partially inhibited SOCs-mediated Ca^{2+} influx and I_{soc}, whereas KD of Orai1 completely inhibited Ca^{2+} influx and I_{soc} [37]. These findings are consistent with multiple forms of SOCs that are related to Orai1, TRPC channels and I_{crac}. Indeed, KD with siRNA and western blot show that both Orai1 and several TRPC channels are expressed in HEK cells and both channel types are regulated by STIM1 [9,33–35,38]. The properties of channel regulation by STIM1 lead to an expanded and a molecular definition of SOCs and allow further examination of the SOC properties of TRPC channels.

Expanded and molecular definition of SOCs

The classical definition of SOCs is channels that are similarly activated by agonist-mediated and agonist-independent Ca^{2+} release from the ER [1]. The discovery of STIM1 and its mode of action allow a more general and molecular definition of the SOCs. The most fundamental property of STIM1 is that it is a Ca^{2+} binding protein that migrates within the ER membrane to regions that are very close to the plasma membrane and reorganizes in punctae that associate with Ca^{2+} influx channels and activate them [10,11,34,38–42]. This is also illustrated in Fig. 1, which shows a Total Internal Fluorescence (TIRP) images of cells expressing YFP-mGluR1 (A, B) or YFP-(D76A)STIM1 (C, D) and stained with anti-GFP. In the case of YFP-mGluR1 the intrinsic (A) and surface fluorescence (B) are similar, indicating the insertion of YFP-mGluR1 into the plasma membrane. By contrast, the punctate intrinsic fluorescence of the constitutively active YFP-(D76A)STIM1 is not observed on the cell surface, indicating the (D76A)STIM1 is not inserted into the plasma membrane, although it activates TRPC1. Since depletion of Ca^{2+} from the ER is obligatory for the translocation of STIM1 and for the activation of SOCs and I_{crac} [11,38,39,41,42], an expanded and molecular definition of SOCs became possible. SOCs are channels that are regulated by STIM1 and require the translocation of STIM1 from the ER towards the plasma membrane to form punctae in response to depletion of ER Ca^{2+}. We consider all channels that fulfill these criteria to be SOCs. These criteria are sufficient to exclude STIM1-regulated channels that are not SOCs. For example, the arachidonate-regulated Ca^{2+}-selective channel (ARC) is regulated by STIM1 [43]. However, this channel is regulated by plasma membrane resident STIM1 and does not require translocation of STIM1 from the ER to the plasma membrane [43].

The STIM1 domains and their potential functions

STIM1 is a Ca^{2+} binding protein with several functional domains (Fig. 2).

STIM1 was originally identified as a tumor suppressor [44] that affects proliferation of B-cells [45] and several tumor cell lines [44,46]. STIM1 asumed center stage in Ca^{2+} signaling research when two independent screens for regulators of Ca^{2+} influx channels, one in drosophila S2 cells [10] and one in HEK cells [11], identified STIM1 as a regulator of Ca^{2+} influx channels. Domain analysis revealed that STIM1 has an N-terminal EF hand Ca^{2+}-binding domain, a sterile α motif (SAM) domain, a single transmembrane domain (TM), an ezrin–radixin–moesin (ERM) domain, a serine-proline-rich region (S/Pregion) and a lysine-rich region. Part of the ERM domain is predicted to have two coiledcoil domains and to contain a glutamate-rich region (E-region). The orientation of STIM1 places the EF hand and the SAM domains in the ER lumen when STIM1 is in the ER and in the extracellular space when STIM1 is at the plasma membrane.

Recent structure-function studies revealed some of the role of these domains. The Ca^{2+} binding EF hand and SAM domains regulate aggregation of STIM1 and its translocation from the ER to the plasma membrane [42,47]. Thus, Ca^{2+} release from the ER and mutations in the Ca^{2+} binding pocket of the EF hand to prevent Ca^{2+} binding results in translocation of STIM1 towards the plasma membrane, formation of punctae and activation of SOCs [10,11,38,40–42,48]. Accordingly, the EF hand resident domain has relatively low Kd for Ca^{2+} of about 0.2–0.6 mM [47]. When bound with Ca^{2+} the EF hand and SAM domains exist as monomers, whereas the Ca^{2+} free domains form multimeric aggregates [47]. In addition, the SAM domain is required for coalescing STIM1 into punctae upon Ca^{2+} release from the ER [42]. Interestingly, the C terminal domain of STIM1 was sufficient for activation of SOCs, although it was not as active as full length STIM1 [38]. This would suggest that the translocation of STIM1 from the ER towards the plasma membrane and gating of SOCs by STIM1 are mediated by two different sectors of STIM1.

Based on the effect of deletion of the SAM together with the coiled-coil domains and of the S/P-region Baba et al concluded that these domains are required for activation of SOCs by STIM1 [42]. However, we reported that deletion of the E-region that forms part of the first coiled-coil domain and of the S/P-region failed to prevent activation of SOCs by the C-terminal half of STIM1 [38]. There are at least two explanations for the contradictory findings. First, we and Baba et al deleted different sequences. For deletion of part or the entire coiled-coil domain we deleted amino acids 270–336 and Baba et al deleted amino acids 249–390. It is possible that different deletions results in different effects. However, it is more likely that the different results are due to the different assays used to evaluate SOC activity. Baba et al used direct measurement of Ca^{2+} influx [42], whereas we used the NFAT nuclear translocation assay that requires long incubation of the cells with the constructs [38]. If the deletion constructs of the constitutively active C-terminus STIM1 retain even small activity it would be sufficient to drive NFAT into the nucleus, resulting in an apparent lack of effect of the deletion. Measurement of Ca^{2+} influx is more likely to reflect the function of a particular domain. Therefore, it is likely that the coiled-coil domain and S/P-region are required for activation of SOCs and perhaps TRPC channels by STIM1.

Another important domain is the lysine-rich region at the C terminal tail of STIM1. Fig. 3 shows that the 14 amino acids of the lysine-rich region are predicted to form a two-turn helix with the three terminal lysines on one surface. Deletion of this lysine-rich region prevents gating of the native SOCs and TRPC1 by STIM1 [38]. The potential role of the lysine-rich region in the function of STIM1 is discussed further below in relation to activation of TRPC channels by STIM1.

Regulation of TRPC channels by STIM1

The participation of TRPC channels in receptor-mediated Ca^{2+} influx and SOCs prompted examination of whether they interact and are regulated by STIM1. We have examined the

interaction of all mammalian TRPC channels with topically expressed STIM1 and found that STIM1 binds TRPC1, TRPC2, TRPC4 and TRPC5, but not TRPC3, TRPC6 and TRPC7 [38]. In addition, it was shown that STIM1 binds to the native TRPC1 in platelets [49] and is present in a complex with TRPC1-STIM1-Orai1 [37].

This is illustrated in Fig. 4a, which shows that TRPC channels interact with STIM1. Deletion analysis revealed that the STIM1-ERM domain mediates the binding of STIM1 to the TRPC channels. Fig. 4b and [38] show that the STIM1-ERM domain binds TRPC1, TRPC2 and TRPC4, but not TRPC3, TRPC6 and TRPC7, similar to the findings with the full-length STIM1, suggesting that the STIM1-ERM domain binds to a sequence or structural motif that is similar in TRPC1, TRPC2, TRPC4 and TRPC5.

Regulation of TRPC channels activity by STIM1 has been demonstrated only for TRPC1 so far [38,49]. KD of STIM1 or over-expression of the dominant negative ΔERM-STIM1(D76A) inhibit TRPC1 channel activity. Expression of the EF hand mutant (D76A)STIM1, that constitutively assembles into punctae at near plasma membrane domains, activates TRPC1 and increases its spontaneous activity [38]. A critical question in the mechanism by which STIM1 regulates SOCs is whether STIM1 is a channel subunit. This question is highlighted in the finding that STIM1 is obligatory for the function of Orai1 [9,33–35,42] and Orai2 [34] as I_{crac} channels. Hence, expression of Orai1 alone does not result in I_{crac} current and suppresses the native SOC activity, whereas co-expression of Orai1 and STIM1 results in a large I_{crac} current. STIM1 does not play a similar permissive role with TRPC channels. Thus, expression of high levels of WT-STIM1 and the constitutively active (D76A)STIM1 only marginally increase or have no effect of TRPC1 current density [38]. That STIM1 is unlikely to function as a channel subunit is further suggested by the finding that the cytoplasmic C terminal domain of STIM1 (CT-STIM1), which lacks the transmembrane domain, is sufficient to activate TRPC1 even when native STIM1 was effectively KD [38].

An interesting finding is that deletion of part of the first coiled-coil domain that includes the glutamate-rich region and deletion of the S/P-rich region of the CT-STIM1 do not prevent binding of CT-STIM1 to TRPC1 [38]. However, similar deletions inhibits activation of SOCs by STIM1 [42]. Moreover, deletion of these domains impairs the translocation of STIM1 towards the plasma membrane [42], but they are not required for activation of TRPC1 by CT-STIM1 [38]. The combined findings suggest that the first coiled-coil domain and the S/P-rich region do not participate in the gating of the SOCs. Rather, they mediate the translocation of STIM1 from the ER towards the plasma membrane in response to depletion of ER Ca^{2+}.

A region of STIM1 that is important in gating of SOCs is the lysine-rich domain. Deletion of the lysine-rich region (ΔK-STIM1) or K/E and K/A substitution of all lysines in this region inhibits the ability of STIM1 to activate TRPC1 and the native SOCs [38]. Importantly, the ΔK-STIM1 and the K/E mutants act as dominant negative constructs and inhibit the function of the native STIM1. On the other hand, the lysine-rich region is not required for binding of STIM1 to TRPC1 [38]. Together, these findings implicate the lysine-rich region of STIM1 in gating of TRPC channels and other SOCs. How the lysine-rich region participates in the action of STIM1 to gate the channels remains to be elucidated.

Although it has not been examined thoroughly, it will be important to test whether other TRPC channels are regulated by STIM1 and to examine their mode of regulation by STIM1. Since TRPC2, TRPC4 and TRPC5 bind to the ERM domain of STIM1, it is expected that STIM1 does regulate the activity of these channels, and the regulation is in a mechanism analogous to that of the regulation of TRPC1 by STIM1 [38]. Surprisingly, a recent study reported that a combined KD of TRPC1, 3, 4, 5, 6 channels in HEK cells did not affect agonist-stimulated Ca^{2+} influx [50]. However, the actual KD of the TRPCs and the extent of the KD were not

examined in this work. The lack of binding of STIM1 to TRPC3, TRPC6 and TRPC7 predicts that STIM1 does not directly regulate these channels. Rescue of Ca^{2+} oscillations by TRPC3 in HEK cells in which STIM1 was KD was taken to suggest that TRPC3 is not regulated by STIM1 [50]. However, since cells express multiple TRPC channels [16,22,23,51], and several TRPC channels interact with each other or are present in the same Ca^{2+} signaling complexes [44–46,52–58], it is necessary to examine the role of STIM1 in the activity of each of the TRPC channels before it is known which of these channels are regulated by STIM1 and how STIM1 gates each of the channels.

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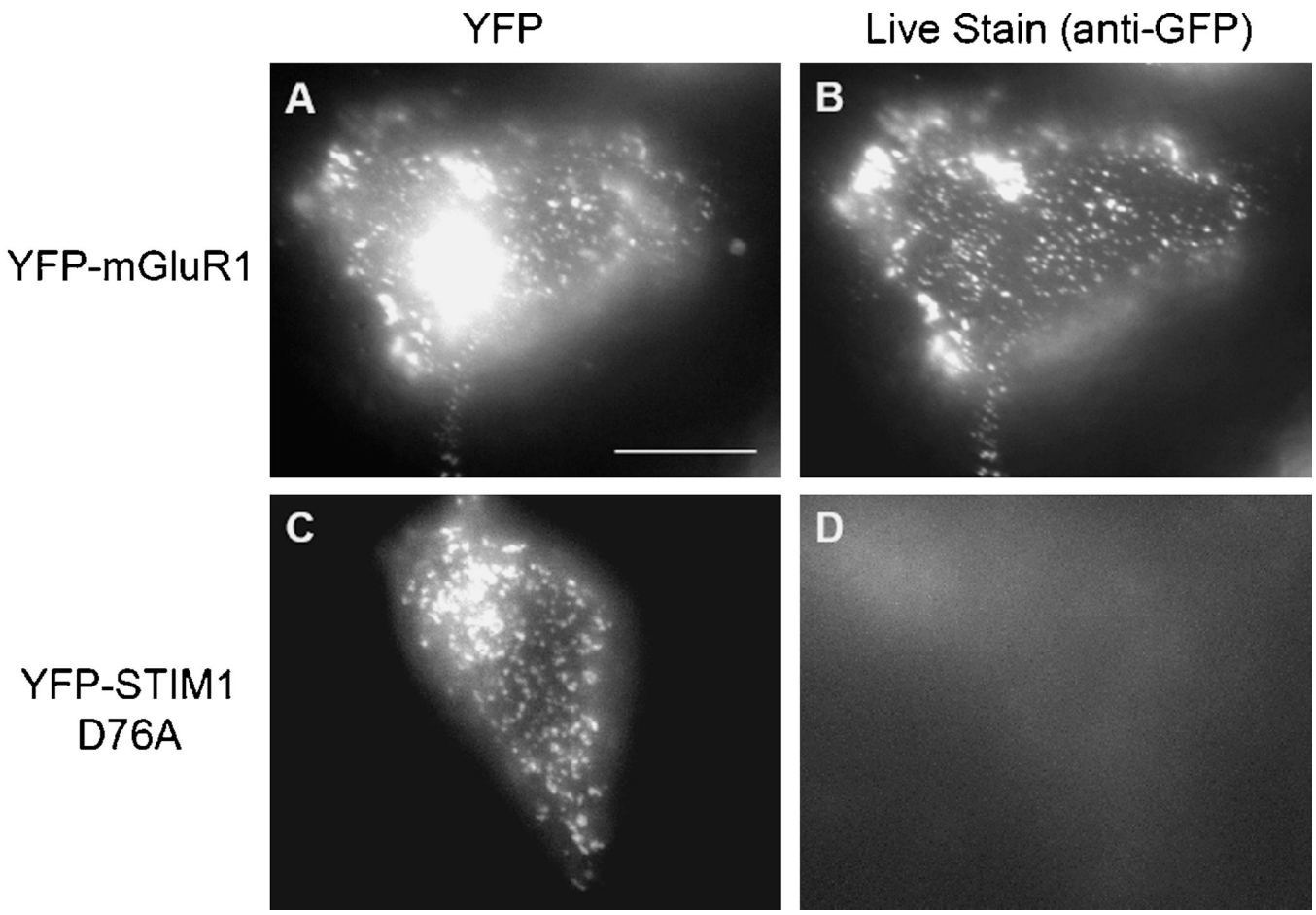

Fig. 1.

Majority of the YFP-STIM1 D76A punctae are not on the plasma membrane. HEK293 cells were transfected with YFPmGluR1 together with Homer1 and Shank3 to get the YFP-mGluR1 to the plasma membrane (A, B) or YFP-STIM1 D76A (C, D). A and C are intrinsic YFP signals. B and D are images of surface live staining with an anti- GFP rabbit polyclonal antibody. The intense signal in A is from Golgi. Scale bar, 10 μm.

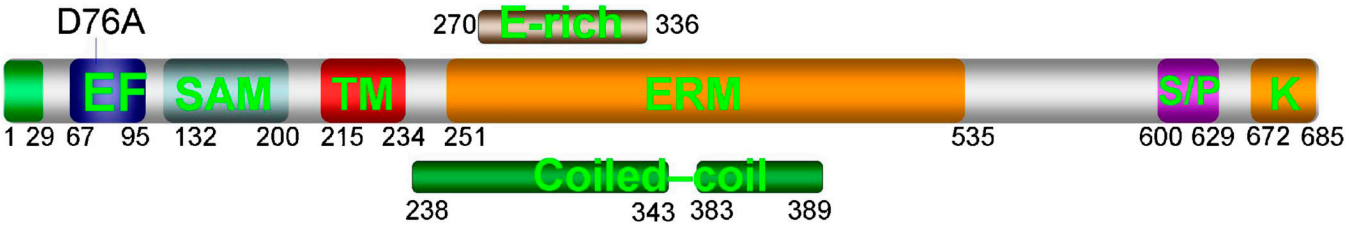

Fig. 2.
STIM1 and its domains

DSSPGR KKFPLKIFKKPLKK

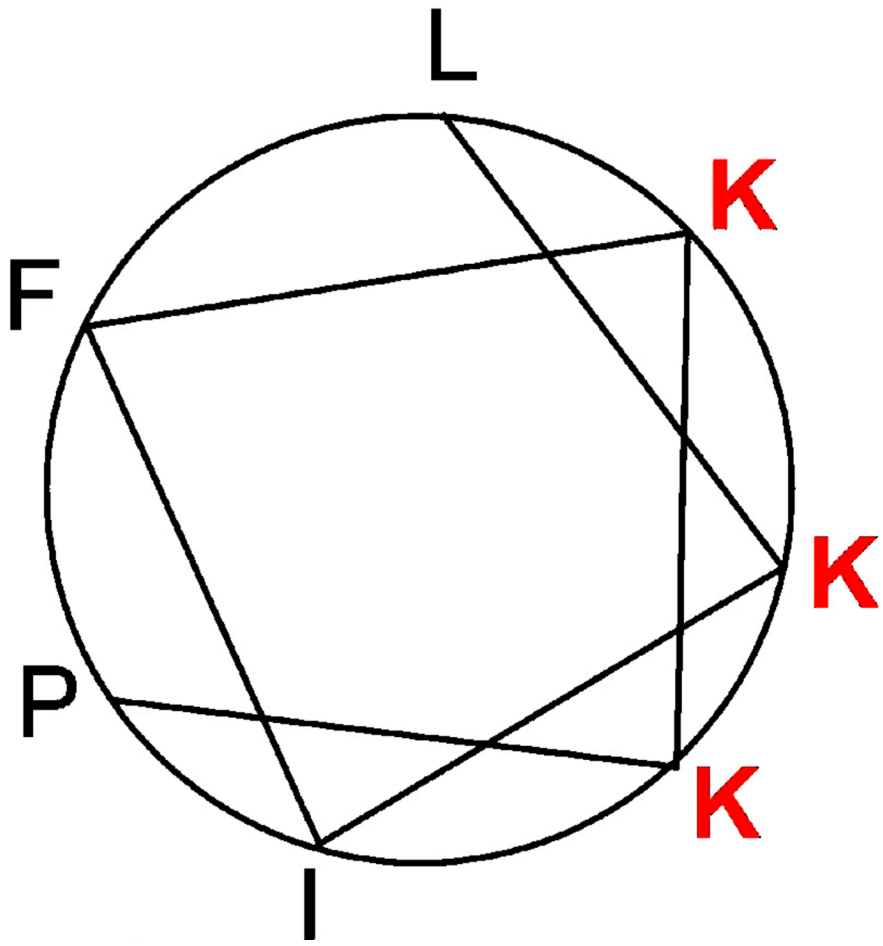

Fig. 3.
The predicted two-turn helix of the C-terminal tail of STIM1.

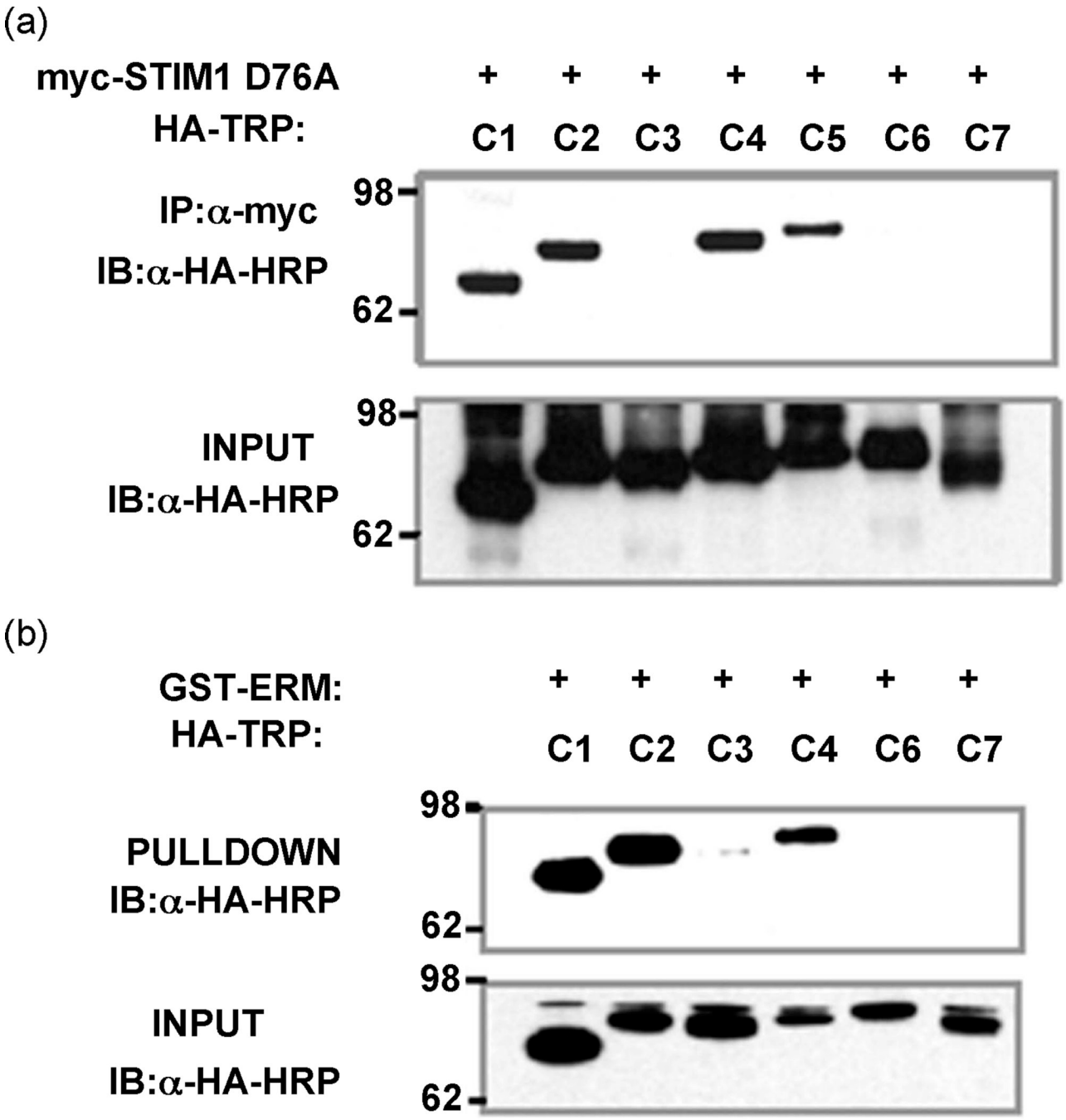

Fig. 4. Interaction of STIM1 with TRPC channels

Panel (a) shows to co-IP of STIM1 and the indicated TRPC channels expressed in HEK cells. Panel (b) shows the pull-down of the indicated TRPC channels by GST-ERM domain of STIM1.