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TRPC channels as STIM1-regulated SOCs

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Store-operated Ca^{2+} channels (SOCs) are Ca^{2+} influx channels at the plasma membrane whose opening is determined by the level of Ca^{2+} stored in the endoplasmic reticulum lumen. SOCs are activated in response to receptor-mediated or passive depletion of ER Ca^{2+} to regulate many Ca^{2+}-dependent cellular functions. Early work implicated the TRPC channels as SOCs. More recently, it was found that the Orai channels mediate the CRAC current and that the Ca^{2+} binding protein STIM1 functions as the ER Ca^{2+} sensor that mediates activation of the SOCs in response to depletion of ER Ca^{2+}. Key questions are whether both TRPC and Orai channels are opened by STIM1 and the molecular mechanism by which STIM1 opens the SOCs. Ample biochemical and functional evidence indicate interaction of the TRPC channels with STIM1. Furthermore, it was found that STIM1 gates TRPC channels by electrostatic interaction of STIM1(K684,K685) in the polybasic domain of STIM1 with two negative charges (aspartates or glutamates) that are conserved in all TRPC channels. Charge mutants of STIM1(K684,K685) and TRPC1(D639,D640) and TRPC3(D697,D698) were used to develop further direct evidence for the function of TRPC channels as SOCs. The evidence in favor of TRPC channels as SOCs are discussed.

Key words: TRPC channels, STIM1, SOCs, gating, electrostatic interaction

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Introduction

A central component of receptor-evoked Ca^{2+} signal is Ca^{2+} influx across the plasma membrane by store-operated Ca^{2+} influx channels (SOCs). SOCs are activated in response to Ca^{2+} release from the endoplasmic reticulum (ER) that is physiologically mediated by receptor-stimulated production of IP_3 and activation of the IP_3 receptors.[1] SOCs can also be conveniently activated by passive depletion of the ER Ca^{2+}, by inhibition of the SERCA Ca^{2+} pumps, or by Ca^{2+} ionophores.[2] SOCs are crucial for maintaining all cellular functions of Ca^{2+} after the first few seconds or minutes of cell stimulation. In addition, SOCs sustain Ca^{2+} oscillations by refilling the stores between Ca^{2+} spikes and at the end of cell stimulation.[3] When aberrant, the Ca^{2+} specifically entering the cells through SOCs is responsible for cell toxicity and cell death in several disease states.[4,5]

These diverse functions of the SOCs raised the question of their molecular identity and how SOCs at the plasma membrane are controlled by Ca^{2+} stored in another organelle, the ER. The answers to these questions remained elusive until the discovery first of STIM1,[6,7] and then of the Orai channels.[8-10] However, before the discovery of these proteins, multiple line of evidence implicated members of the Ca^{2+} permeable TRPC subfamily of the TRP channels as one form of SOCs. These are summarized in several refs.[2,11-15] and include knockdown by antisense and subsequently by siRNA, and finally knockout in mice. Most of these studies showed that reduction in expression of specific TRPC channels result in reduction in Ca^{2+} influx triggered by receptor stimulation and in some cases by passive store depletion. In addition, several studies expressed the channels in model systems

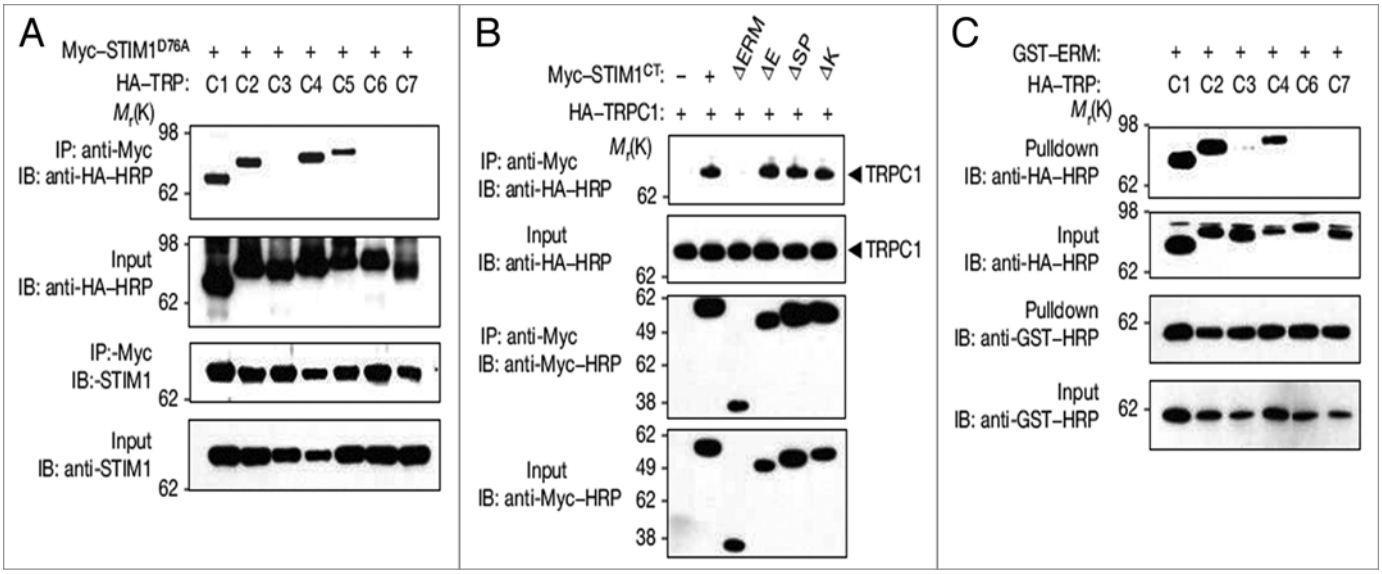

Figure 1. The STIM1 ERM domain interacts with the TRPC channels. (A) Co-immunoprecipitation of STIM1(D76A) with TRPC1, 2, 4 and 5, but not 3, 6 or 7 in HEK293 cells. (B) The ERM domain, but not glutamate-rich (E), serine-proline-rich (SP) or lysine-rich (K) sequences of STIM1, is necessary for association of STIM1 with TRPC1. (C) GST-ERM domain binds with TRPC1, 2 and 4. Data have been reproduced from ref. 22.

and obtained evidence that the expressed channels can function as SOCs, at least under specific expression conditions.[14,16-18] The expression studies generated the most controversy since the findings were not uniform, with several groups reporting that the expressed TRPC channels function as SOCs,[14,16-18] while others using the same expression system concluded that the same TRPC channels do not function as SOCs.[19,20] Several of these differences could be traced to expression level of the channels[16,21] and most likely because several of the TRPC channels can function both in SOC and non-SOC modes (see below).

Recently, others[24-33] and we[16,17,22,23] were able to obtain critical evidence for the function of the TRPC channels as SOCs by showing their interaction and gating by STIM1 and their communication with the Orai channels. STIM1 is a 685 residue, Ca^{2+} binding protein that resides in the ER and functions to transmit the ER Ca^{2+} load to the SOCs.[6,7] STIM1 has a single transmembrane domain that spans the ER membrane and places the STIM1 EF hand Ca^{2+} binding domain and a clustering SAM domain in the ER lumen. On the cytoplasmic side, STIM1 has several distinct regions, including an ERM domain that contains the sub-domain SOAR for gating Orai channels,[34-36] a serine/proline-rich domain and a polybasic lysine (K)-rich domain.[6,7] When the ER is filled with Ca^{2+}, the STIM1 EF hand binds the Ca^{2+} to keep STIM1 in a monomeric, non-clustered form and restricts its access to the SOCs. Depletion of ER Ca^{2+} leads to clustering of STIM1 that is aided by the SAM domain[37] and activation of SOCs.[11,38]

STIM1 activates the Orai channels, which function as SOCs and mediate the Ca^{2+}-release activated Ca^{2+} current, known as the CRAC current.[8-10] In a recent work, others[34,35] and we[36] reported that a highly conserved, 98 amino-acid domain of STIM1 that we named SOAR (for STIM1 Orais Activating Region) is necessary and sufficient to fully activate Orai channels. Other domains in STIM1 likely regulate activation of Orai1 but are not necessary to open the Orai channels. Indeed, the polybasic K-domain appears to regulate access of STIM1 to Orai channels to affect the voltage dependence of Orai1.[36]

Interaction of STIM1 with the TRPCs

Here, we discuss the evidence for regulation of TRPC channels by STIM1 and, thus, the function of TRPC channels as SOCs. The earlier studies showed that TRPC1, TRPC2, TRPC4 and TRPC5 can be co-immunoprecipitated with STIM1 when co-expressed in model systems.[22] Deletion analysis and pulldown assays showed that the STIM1 ERM domain mediates the interaction with the TRPC channels (**Fig. 1**). The finding that SOAR within the ERM domain gates the Orai channels[36] raises the question of whether SOAR mediates the interaction between STIM1 and the TRPC channels. Inhibition of TRPC1 activity by SOAR,[17] suggests that SOAR may interact with TRPC channels and that SOAR is the domain mediating interaction of STIM1 with the two types of Ca^{2+} influx channels, TRPCs and the Orai channels.

STIM1 interacts with TRPC1, TRPC4 and TRPC5, but not with TRPC3 and TRPC6.17 Interaction of STIM1 with TRPC1,25,26 and TRPC4,31 was also reported by others in other cell types. Therefore, not surprisingly, regulation of TRPC1, TRPC4 and TRPC5 by STIM1 could be demonstrated by inhibition of channel activity by the dominant negative STIM1(ΔERM) and by knockdown of STIM1 with siRNA.16 Similar inhibition of TRPC1,25,26 TRPC4,31 and TRPC5,39 was found by knockdown of STIM1 in other cell types. Since TRPC3 and TRPC6 do not interact directly with STIM1, it was expected that they should not be regulated by STIM1. However,

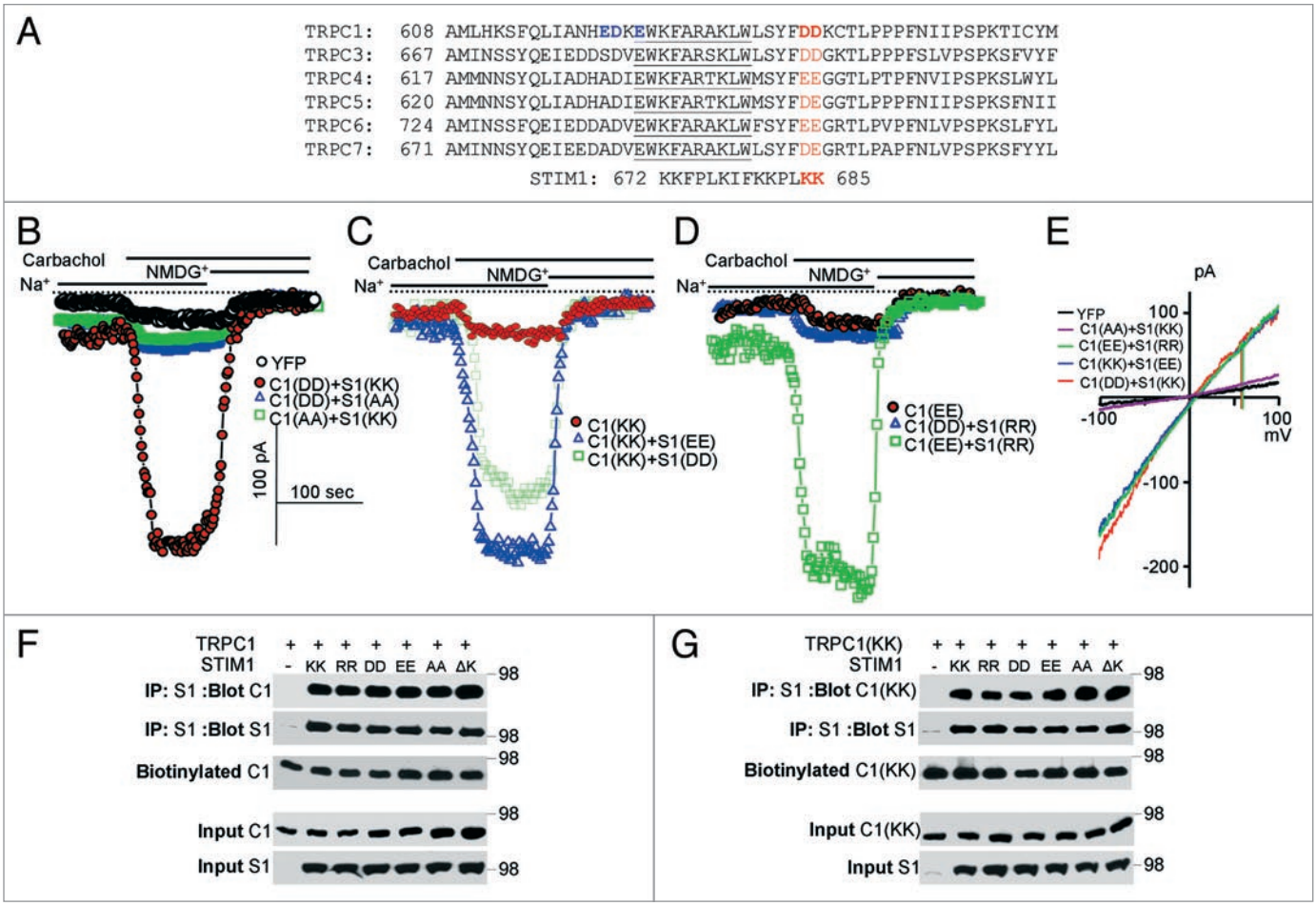

Figure 2. Gating of TRPC1 by STIM1 is mediated by electrostatic interaction of TRPC1(D639D640) with STIM1(K684K685). (A) shows alignment of the indicated C-terminal sequences of TRPC channels. Highlighted in red are the conserved negative charges (DD, EE or DE) in TRPCs. Also shown is the STIM1 K-rich domain. (B–E) HEK cells were transfected with HA-TRPC1 and myc-STIM1 constructs. Cells incubated in Na^+-containing media, were stimulated with 100 μM carbachol, and then incubated in Na^+-free media to determine the zero current. (B) The current of control cell transfected with YFP (black) and cells transfected with wild-type TRPC1(D639,D640) and STIM1(K684,K685) (red); inhibition of TRPC1(D639,D640) by STIM1(K684A,K685A) (blue), and a lack of current by TRPC1(D639A,D640A) (green). (C) Lack of current of TRPC1(D639K,D640K) (red) and its rescue by STIM1(K684E,K685E) (blue) and STIM1(K684D,K685D) (green). (D) The lack of current of TRPC1(D639E,D640E) (red), inhibition of wild-type TRPC1(D639,D640) by STIM1(K684R,K685R) (blue), and rescue of TRPC1(D639E,D640E) by STIM1(K684R,K685R) (green). (E) Representative I/Vs of the indicated TRPC1 + STIM1 combinations. (F) HEK cells were co-transfected with wild-type HA-TRPC1 and the indicated myc-STIM1 mutants, biotinylated, and used to determine effect of the mutants on co-IP of STIM1 and TRPC1 and total and surface expression of TRPC1. (G) The same experiment as in (E) except that effect of the STIM1 mutants was measured on expression of TRPC1(D639K,D640K). Data have been reproduced from ref. 17.

knockdown of STIM1 and the use of STIM1(ΔERM) showed that, when expressed at low levels, both channels are regulated by STIM1 due to STIM1-mediated heteromultimerization of TRPC3 with TRPC1 and of TRPC6 with TRPC4.16

The heteromultimerization of TRPC1-TRPC3 and TRPC4-TRPC6 was required for regulation of TRPC3 and TRPC6 by STIM1. Yet, knockdown of TRPC1 and TRPC4 did not prevent the function of TRPC3 and TRPC6, respectively, but rather converted them to STIM1-independent channels. Moreover, when expressed at high levels, both channels showed spontaneous activity that was independent of STIM1.[16] This implies that TRPC3 and TRPC6 can function in two modes. When expressed at high levels or in the absence of TRPC1 and TRPC4, they function as STIM1-independent channels. In this mode, TRPC3 and TRPC6 do not function as SOCs, but rather function as receptor-operated channels (ROCs). On the other hand, when expressed at low physiological levels and in the presence of TRPC1 and TRPC4, TRPC3 and TRPC6 function as STIM1-regulated SOCs. This also implies that the function of TRPC3 and TRPC6 as SOCs is critically dependent on the TRPC1/TRPC3 and TRPC4/TRPC6 expression ratio and that deviation from the ratio that stabilizes the SOC mode results in a ROC mode. This may explain a recent report claiming that TRPC3 and TRPC6 expressed in HEK cells do not function as SOCs[19] and that knockdown of TRPC6 does not affect SOC activity in proliferative vascular smooth muscle cells.[20]

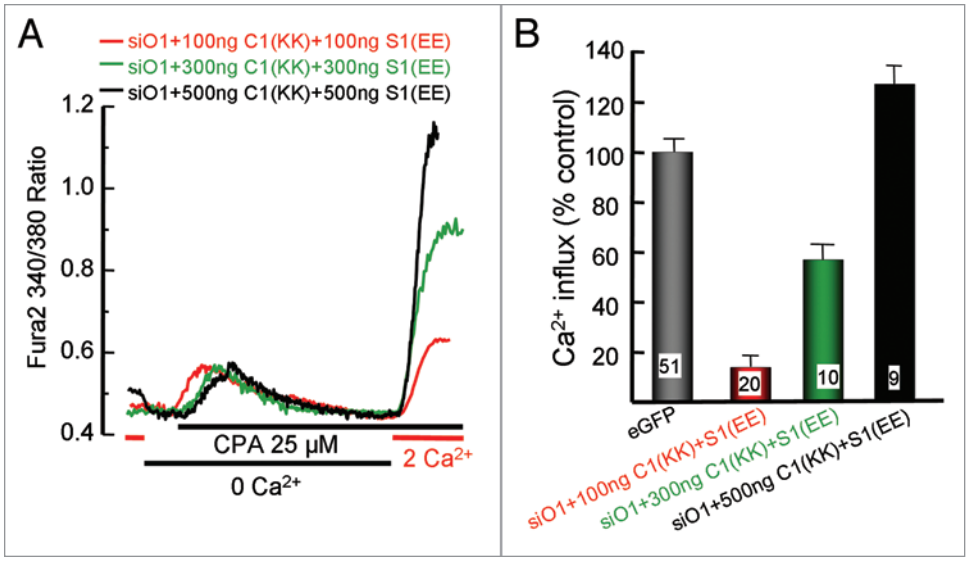

Figure 3. TRPC1 functions as SOC. In (A), HEK cells treated with siOrai1 for 48 hrs were transfected with the combination of the mutants TRPC1(D639K,D640K) + STIM1(K684E,K685E) at 100 ng (red), 300 ng (green), or 500 ng (black). After 24 hrs, the transfected cells were used to measure SOC activity. (B) shows the mean ± s.e.m. of the indicated number of cells. Data have been reproduced from ref. 23.

The Molecular Mechanism of Gating of TRPCs by STIM1

To understand how STIM1 gates TRPC channels, we examined the role of several STIM1 domains, including the polylysine-rich domain. Polybasic domains, such as the polylysine domain, commonly function as membrane anchoring modules. However, mutational analysis revealed that the STIM1 polylysine domain directly gates TRPC channels by interacting with two conserved negative charges in the C terminus of the TRPCs.17 Neutralization or reversing the negative charges in TRPC channels (for example, D639D640 in TRPC1 and D697D698 in TRPC3) resulted in inactive channels. Similarly, neutralizing or reversing the positive charge in any of the seven lysines in the STIM1 polybasic domain resulted in mutants that inhibited the activity of TRPC1. However, the key STIM1 lysines were found to be K864 and K865 since these lysines interact with the conserved negative charges in TRPC1. Indeed, in key experiments, switching charged residues in STIM1 and TRPCs, channel activity could be rescued. In fact, all negative-positive pairs in STIM1-TRPC, independent of the location of charges, resulted in functional channels.17 This is illustrated in **Figure 2**. These findings provide unequivocal evidence that (a) STIM1 directly gates TRPC channels, (b) the STIM1 polylysine domain opens TRPC channels, and (c) the polylysine STIM1 domain gates TRPC channels by electrostatic interaction rather than regulating the channel pore.

TRPC Channels as SOCs

Gating by the STIM1 polylysine domain is specific for TRPC channels. Activation of the Orai channels by STIM1 does not require the polylysine domain.[17] Moreover, the 98 amino acid SOAR domain within STIM1 is necessary and sufficient for full activation of the Orais.[34-36] To the extent tested, Orai1 activated by STIM1 and by SOAR show the same kinetic properties.[36] Although not required for opening the Orai channels, the polylysine domain regulates Orai1 by interacting with a proline-rich domain at the N terminus of Orai1.[36] Hence, the polylysine domain of STIM1 has different functions in the two channel types; it only regulates Orai1 activity, while it opens TRPC channels by electrostatic interaction with the conserved DD/DE residues at the C terminus of the TRPC channels. The finding that the polylysine domain is dispensable for activation of the Orais but is essential for activation of the TRPCs was used to develop direct evidence for the function of TRPC1 as a bona fide SOC.[23] This is illustrated in **Figure 3**. Cells treated with Orai1 siRNA are transfected with the reverse charge mutants TRPC1(D639K,D640K) and STIM1(K864E,K865E). TRPC1(D639K,D640K) by itself is not active, and STIM1(K864E,K865E) sequesters the native STIM1 to inhibit all remaining STIM1-dependent activity. STIM1(K864E,K865E) can activate TRPC1(D639K,D640K), and thus, the Ca^{2+} influx in **Figure 3** can result only from activation of TRPC1(D639K,D640K) by STIM1(K864E,K865E). This Ca^{2+} influx is activated by passive store depletion, indicating that TRPC1 functions as SOC.

Perspective and Future Questions

The results described above provide multiple independent lines of evidence for the regulation of TRPC channels by STIM1 and their function as SOCs. Yet, this topic is not completely resolved in that TRPC channels appear not to function as SOCs in all cell types and under all conditions.[2] It remains to be resolved why this is the case. A major step forward will be the identification of the STIM1 binding sites in the TRPC channels and how the binding is regulated. Several studies have reported biochemical assembly of STIM1-TRPCs-Orai1 complexes[12,23,26-28,40] and functional interaction between TRPC

channels and Orai1, particularly when expressed at low, physiological levels.[12,16,17,23,26-28,31,36,40] Moreover, the channel function of both TRPC and Orai1 channels appears to be required for their function,[23,25] although when expressed at high levels in the same cells, both channels can function independently of each other.[17] An important future goal will be to determine the relationship between the two types of channels and their contribution to cell-specific SOCs activity.

Acknowledgements

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