Research Paper

Store-Operated Orai1 and IP$_3$ Receptor-Operated TRPC1 Channel
Separation of the Siamese Twins

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Original manuscript submitted: 06/20/07
Revised manuscript submitted: 08/01/07
Manuscript accepted: 08/03/07
Previously published online as a Channels E-Publication:
http://www.landesbioscience.com/journals/channels/article/4835

KEY WORDS
store-operated channel, Orai1, TRPC1, iPLA$_2$

ACKNOWLEDGEMENTS
This study was supported by NIH (RO1HL71793 and RO1HL54150). Vladislav Zarayskiy is supported by NIH training grant (HL007224).

NOTE
Supplementary Material can be found at: http://landesbioscience.com/supplement/ZarayskiyCHAN1-4-sup.pdf

ABSTRACT

Store-operated channels (SOC) are known to be physiologically activated following agonist-induced IP$_3$ production and depletion of Ca$^{2+}$ stores. Here we present molecular, biophysical and mechanistic evidence that two ubiquitously expressed plasma membrane channels may be responsible for creating a complex and sometimes controversial SOC image: one being a real SOC encoded by Orai1 and activated exclusively upon depletion of Ca$^{2+}$ stores (via iPLA$_2$-dependent pathway), while the second one is an IP$_3$ receptor-operated channel (IP$_3$ROC) encoded by TRPC1 and activated via its conformational coupling with IP$_3$ receptor. In RBL-2H3 cells endogenously expressing Orai1 and TRPC1, we unmasked and characterized whole-cell current through IP$_3$ROC channels that was hiding behind some familiar fingerprints of I$_{CRAC}$, a current through the classical Ca$^{2+}$-selective SOC (CRAC) channels. We discriminated these currents by their molecular identity, selectivity and different requirements for store depletion, IP$_3$, iPLA$_2$ and conformational coupling to IP$_3$ receptor. New knowledge on the properties and coexistence of Orai1-encoded SOC and TRPC1-encoded IP$_3$ROC, and the use of experimental approaches introduced in this manuscript should help avoid further confusion about these channels, and open new exciting possibilities for their independent studies.

ABBREVIATIONS
SOC, store-operated channel; SOCE, store-operated Ca$^{2+}$ entry; IP$_3$ROC, IP$_3$ receptor-operated channel; IP$_3$R, IP$_3$ receptor; CRAC, Ca$^{2+}$ release-activated Ca$^{2+}$ channel; I$_{CRAC}$, whole-cell current mediated by CRAC; I$_{IP3ROC}$, whole-cell current mediated by IP$_3$ROC; iPLA$_2$, Ca$^{2+}$-independent phospholipase A$_2$ beta; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; IP$_3$, inositol 1,4,5-trisphosphate

INTRODUCTION

For over two decades store-operated Ca$^{2+}$ entry (SOCE) remains one of the most mysterious and highly controversial mechanisms in Ca$^{2+}$ signaling. Recent advancements in this field identified Orai1$^4$-7 as a new molecular candidate for SOC, STIM1$^8$-13 as a Ca$^{2+}$ sensor in the stores and activator of SOCE pathway, and Ca$^{2+}$-independent phospholipase A$_2$ beta (iPLA$_2$ or PLA2G6) as one of the crucial components for signal transduction from the stores to the plasma membrane channels. Alternatively, some investigators suggest that SOC may be encoded by TRPC1, and the conformational coupling of TRPC1 to IP$_3$ receptor (IP$_3$R), STIM1 or other components of the endoplasmic reticulum may be responsible for SOCE activation. To address these controversies, some models propose that the same, or different SOCs may be activated by multiple mechanisms, or that Orai1, TRPC1 and potentially other members of TRPC family may form subunits of the same SOC that is activated not only by store depletion, but also by numerous other mechanisms.

In an attempt to find some easy explanation for contradicting findings, we hypothesized that not one, but two distinct plasma membrane channels may be involved in creating a rather controversial image of a store-operated channel, and here we provide strict biophysical, molecular and mechanistic evidence that endogenous Orai1 and TRPC1 may indeed comprise two distinct channels that coexist in the same cells. Orai1 encoding a true SOC (with Ca$^{2+}$ store depletion being required and sufficient for its activation),
and TRPC1-encoded channel (IP₃ROC) being independent of store depletion, but requiring IP₃ and conformational coupling with IP₃,R.

RESULTS

In search for a best possible model for these studies, we found a specific RBL-2H3 cells line that endogenously express both, Orai1 and TRPC1. RBL is a widely used model for investigation of Ca²⁺ selective SOC (historically called Ca²⁺ release-activated Ca²⁺ channel, or CRAC²,37,38), which could be easily recognized and studied in these cells. Whole-cell current through CRAC channels (ICRAC) was recorded in the cells dialyzed with strong Ca²⁺ chelator (10 mM BAPTA), which prevents Ca²⁺ back sequestration and passively depletes the stores. As a second commonly used approach, IP₃ was included in the dialyzing pipette (along with BAPTA), which promote active Ca²⁺ release and accelerate store depletion. Careful comparison of the whole-cell currents that develop in RBL-2H3 cells under these two widely used experimental conditions brought rather unexpected results.

Figure 1A demonstrates the time course of development of an inward current (at -80 mV) during cell dialysis with 10 mM BAPTA, and Figure 1C shows its current-voltage (I/V) relationship. This current had all established fingerprints of ICRAC: it developed slowly reaching maximum within 3 minutes, had a reversal potential >+50 mV, and showed a pronounced inward rectification. Importantly, in total agreement with previously described role of iPLA₂β in its activation,⁵⁰,³⁹ ICRAC did not develop in iPLA₂β-deficient cells (Fig. 1C): maximum current density in RBL cells transfected with antisense to iPLA₂β was -0.27 ± 0.06 pA/pF (n = 8), while RBL cells treated with sense oligonucleotides showed current that was not significantly different from the control (-1.61 ± 0.40 pA/pF, n = 4).

When IP₃ (10nM-100 µM) was included in dialyzing pipette (in addition to 10 mM BAPTA), an inward current developed (Fig. 1B and D) that looked very similar to ICRAC (Fig. 1C), though with significantly larger amplitude (-2.34 ± 0.21 pA/pF, n = 10 in IP₃ vs. -1.42 ± 0.16 pA/pF, n = 18 in BAPTA alone). This current showed strong inward rectification, had reversal potential >+50 mV, and was totally blocked by two known inhibitors of ICRAC, diethylstilbestrol (DES, 10 µM)⁴⁰ and 2-aminoethoxydiphenyl borate (2-APB, 100 µM, not shown).⁴¹

In spite of some striking similarities, further studies of the currents activated in RBL cells in the absence and presence of IP₃ revealed numerous hidden, but very important differences. First, when IP₃ was included in the pipette (Fig. 1D), a significant amount of the inwardly rectifying current remained present in iPLA₂β-deficient cells, in which ICRAC was abolished (Fig. 1C). Being clearly iPLA₂β-independent, this current (Fig. 1) looked surprisingly similar to ICRAC: the same inward rectification, reversal potential >+50 mV, and inhibition by DES (10 µM). However, contrary to ICRAC this iPLA₂β-independent current was not activated by simple store depletion (with 10 mM BAPTA).

The different role that iPLA₂β played in activation of these currents allowed us to separate and carefully compare them in RBL-2H3 cells. Classic ICRAC could be recorded in control cells dialyzed with 10 mM BAPTA (without IP₃). The current through IP₃-activated channels (which we will call IP₃ receptor-operated channel, or IP₃ROC, as will be clarified by experiments below) did not require iPLA₂β, and could be easily observed in iPLA₂β-deficient cells (in which ICRAC was absent). Comparison of ICRAC (Fig. 1) and IP₃ROC (Fig. 1) showed that under standard experimental conditions (with 20 mM Ca²⁺ and
Figure 2. Effects of extracellular and intracellular monovalent cation substitution on I_{CRAC} (A and C) and I_{IP3ROC} (B and D). I_{CRAC} was recorded in control cells dialyzed with 10 mM BAPTA (A and C), and I_{IP3ROC} was unmasked in iPLA_2-deficient cells (transfected with a/s to iPLA_2β) dialyzed with 10 μM IP_3 + 10 mM BAPTA. (A and B) Summary data showing the effects of extracellular Na^+ substitution by NMDG^+ or Cs^+ on the inward currents recorded at -80 mV. Ca^{2+} (20 mM) remains present in all extracellular solutions. Each bar summarizes results from 5–6 cells (as specified) and represents the currents normalized to their control level in extracellular Na^+. (C and F) I/V relationships of the maximum currents that developed during dialysis with Cs^+ (open symbols), representative of 4 experiments. The average peak current ± SE is shown at 12 s and 100 s (n = 4).

Figure 3. The dependence of I_{IP3ROC} on concentrations of IP_3 and BAPTA in dialyzing solutions. (A) Summary data showing the maximum inward current that develops in iPLA_2-deficient cells upon their dialysis with increasing concentration of IP_3. BAPTA (10 mM) is present in all dialyzing solutions. Each bar summarizes results from 3–8 cells. (B) The time-course of the outward I_{IP3ROC} development (at +80 mV) in cells dialyzed with 10 μM IP_3 and 1 mM BAPTA. The trace is representative of 4 experiments. The average peak current ± SE is shown at 12 s and 100 s (n = 4).

130 mM Na^+ in the bath), their I/V relationships are identical, both demonstrating strong inward rectification, typical for high selectivity of I_{CRAC} to Ca^{2+}, which is the sole cation permeating this channel when Ca^{2+} and Na^+ are both present in the bath. Consistent with Ca^{2+} being a sole ion permeating CRAC channels (in the presence of 20 mM Ca^{2+} in the bath), substitution of extracellular Na^+ with N-methyl-D-glucamine (NMDG^+) had no effect on I_{CRAC} (Fig. 2). Surprisingly, substitution of Na^+ with NMDG^+ reduced inward I_{IP3ROC} by more than 75% (Fig. 2), suggesting that it has a very significant Na^+ permeability, and only a fraction of the total inward current (the residual current in NMDG^+ conditions) may be carried by Ca^{2+} (20 mM) that was a second cation present in the bath. Interestingly, I_{IP3ROC} appeared to be poorly permeable for Cs^+, which ironically had been used by us, and is still used by many other investigators as the sole monovalent cation in dialyzing pipette solutions. Figure 2B shows that substitution of Na^+ by Cs^+ in the bath did not affect Ca^{2+}-selective I_{CRAC} while I_{IP3ROC} virtually disappeared. When Cs^+, a major cation in dialyzing pipette solutions in experiments shown in Figure 1 was changed to symmetrical Na^+, I_{CRAC} was not altered (Fig. 2), but I_{IP3ROC} lost its inward rectification and a significant outward current emerged (Fig. 2) with a reversal potential of +5.0 ± 2.4 mV (n = 9). This current was inhibited by 1 μM La^3+ (Supplemental Fig. 1). In contrast to the high Ca^{2+} permeability of I_{CRAC} (P_{Ca}:P_{Na} > 1000), we estimated the relative Na^+/Ca^{2+} selectivity of I_{IP3ROC} to be P_{Ca}:P_{Na} < 0.4. Poor permeability of I_{IP3ROC} to Cs^+ is rather unusual for non-selective cation channels, but may easily explain why in routine experiments (with Cs^+ as a main monovalent cation in dialyzing pipettes) I_{IP3ROC} did not show any outward current, creating an inward rectification and hiding behind the familiar fingerprints of I_{CRAC}. To better study I_{IP3ROC} one may use symmetrical Na^+ conditions, which did not affect the inwardly rectifying I_{CRAC} (Fig. 2), but unmasked the outward component of I_{IP3ROC} (Fig. 2), making it easy to detect its presence. Under these conditions, the whole-cell current in control cells now showed the presence of both, inwardly rectifying and outward components (Fig. 5), and had a reversal potential of +16.4 ± 3.3 mV (n = 15).

The newly discovered ability to discriminate I_{IP3ROC} allowed us to look more carefully at its IP_3 dependence and other important properties. First, I_{IP3ROC} showed a clear dose-dependence on IP_3 in the pipette (Fig. 3). Remarkably, this current started to develop at ~10 nM, a concentration far below the threshold for IP_3-induced Ca^{2+} release. Second, we found that I_{IP3ROC} could be activated by IP_3 not only under high Ca^{2+} buffering condition (with 10 mM BAPTA), but also when buffering was significantly reduced and 1 mM BAPTA was used in the dialyzing pipettes. The maximum outward current was identical under the low and high Ca^{2+} buffering conditions: 1.02 ± 0.25 pA/pF (n = 5) in 1 mM BAPTA and 0.92 ± 0.26 pA/pF (n = 5) in 10 mM BAPTA. However, contrary to non-inactivating current in 10 mM BAPTA, I_{IP3ROC} showed a fast inactivation when the buffering capacity was decreased (Fig. 3). With 1 mM BAPTA in dialyzing solutions the outward current peaked at 11.4 ± 2.7 s after...
break-in (n = 5), and by 100 s declined to 16.6 ± 0.7% (n = 5) of its maximum amplitude. Thus, the use of 10 mM BAPTA in the pipette was not required for \( I_{\text{IP3ROC}} \) activation, but was important to prevent Ca\(^{2+}\)-induced inactivation of this IP3-dependent current. To further test the idea that IP3 binding to IP3R rather than store depletion may be a stimulus for \( I_{\text{IP3ROC}} \) activation, we used 10 mM BAPTA and high concentration of IP3 in the pipette to deplete the stores, but also added heparin (500 µg/ml) to prevent IP3 binding to IP3R. Under these conditions we indeed found activation of \( I_{\text{IP3ROC}} \) to be highly impaired (Supplemental Fig. 1).

The next important step was to determine the molecular identity of \( I_{\text{CRAC}} \) and \( I_{\text{IP3ROC}} \). First, we tested the role of Orai1 in both currents. Figure 4 shows that molecular down-regulation of Orai1 (using siRNA) abolished the current activated by 10 mM BAPTA, consistent with Orai1 being fully responsible for \( I_{\text{CRAC}} \). In contrast, Orai1-deficient cells had a normal \( I_{\text{IP3ROC}} \) (Figs. 4, 5B and 6B) with a reversal potential close to 0 mV. The next set of experiments revealed the core component of \( I_{\text{IP3ROC}} \). Transfection of RBL-2H3 cells with antisense to TRPC1 did not affect inwardly rectifying \( I_{\text{CRAC}} \) (Fig. 4), but the linear component of the total current (that develops in control cells in the presence of IP3) disappeared, leaving only pure \( I_{\text{CRAC}} \) intact in cells deficient of TRPC1 (Figs. 4B, 5C and 6A). Figure 5 shows representative examples of the whole cell currents in RBL-2H3 cells dialyzed with IP3 + BAPTA. Control cells had both currents present (a), while in the cells in which either Orai1 (b) or TRPC1 (c) were knocked down, only \( I_{\text{IP3ROC}} \) (b) or \( I_{\text{CRAC}} \) (c) remained, as summarized in Figure 4. Thus, while Orai1 was essential for \( I_{\text{CRAC}} \), TRPC1 appeared to be crucial for \( I_{\text{IP3ROC}} \). Interestingly, in some earlier studies in RBL-1 (a cell line that is very close to RBL-2H3), only \( I_{\text{CRAC}} \) seemed to be activated, even when IP3 was present in the dialyzing pipette. \( I_{\text{IP3ROC}} \) could not be unmasked by differential cation selectivity, and inhibition of iPLA2 abolished the whole-cell current, leaving no trace of \( I_{\text{IP3ROC}} \) in these cells. It looked like these cells simply did not express TRPC1, and Western blot analysis (Supplemental Fig. 2) indeed confirmed that contrary
After finding TRPC1 playing a crucial role in I\textsubscript{IP3ROC}, we further tested the idea that IP\textsubscript{3}ROC (rather than Orai1-encoded SOC) may in fact be the channel that is activated by a conformational coupling with IP\textsubscript{3}R. To test this possibility, we dialyzed the cells with a synthetic 30-amino-acids peptide that corresponds to the C-terminus of TRPC1 (that may not even cause Ca\textsuperscript{2+} release from the stores). Upon Ca\textsuperscript{2+} release (which by itself may produce Ca\textsuperscript{2+} dependent inactivation of IP\textsubscript{3}ROC, as shown by our studies), store depletion will activate SOC (activated specifically upon depletion of the stores via iPLA\textsubscript{2}-dependent pathway), and TRPC1-encoded IP\textsubscript{3}ROC (activated by IP\textsubscript{3} via conformational coupling with IP\textsubscript{3} receptor). In the cells in which Orai1 and TRPC1 are expressed endogenously, they form two distinct channels that may account for the complex cellular response. Coexistence of these endogenously expressed channels may be responsible for some mutually exclusive findings in SOCE field (review in refs. 2, 3), and could easily explains why SOCE is absent in the cells in which either Orai1 or iPLA\textsubscript{2} were down-regulated, while it remains intact and fully functional in the cells in which either TRPC1\textsubscript{16} or IP\textsubscript{3}R\textsubscript{41,47,48} are absent or knocked-down. At the same time it explains why TRPC1 channels and conformational coupling to IP\textsubscript{3}R were rightly found to play a significant role in agonist-induced IP\textsubscript{3}-mediated Ca\textsuperscript{2+} signaling, while their share in TG-induced SOCE varied significantly in different cell types. IP\textsubscript{3}-operated plasma membrane channels may mirror a well established presence and physiological role of the rather ubiquitous TRPC1 channel (reviewed in refs. 21–24), as well as may show some properties of the channels encoded by TRPC6,49 and other TRPC’s that are known to associate with TRPC1 to form endogenous heteromeric channels (reviewed in refs. 50–53). In view of our findings, one may expect IP\textsubscript{3}ROC to play a major role in processes that involve IP\textsubscript{3}, especially when it is produced at sub-threshold levels (that may not even cause Ca\textsuperscript{2+} release from the stores). Upon Ca\textsuperscript{2+} release (which by itself may produce Ca\textsuperscript{2+} dependent inactivation of IP\textsubscript{3}ROC, as shown by our studies), store depletion will activate SOC, which may take over and sustain Ca\textsuperscript{2+} entry after IP\textsubscript{3}ROC is inactivated. In this way both channels may be involved and barely distinguishable in a wide variety of signaling processes. Our new findings emphasize that cellular responses to agonist-induced IP\textsubscript{3}-mediated stimulation, and even to TG, should be studied and interpreted with more caution and expectations than one may expect IP\textsubscript{3}ROC to play a major role in processes that involve IP\textsubscript{3}, especially when it is produced at sub-threshold levels (that may not even cause Ca\textsuperscript{2+} release from the stores). Upon Ca\textsuperscript{2+} release (which by itself may produce Ca\textsuperscript{2+} dependent inactivation of IP\textsubscript{3}ROC, as shown by our studies), store depletion will activate SOC, which may take over and sustain Ca\textsuperscript{2+} entry after IP\textsubscript{3}ROC is inactivated. In this way both channels may be involved and barely distinguishable in a wide variety of signaling processes.

### DISCUSSION

A model in Figure 7 distinguishes Orai1-encoded SOC (activated specifically upon depletion of the stores via iPLA\textsubscript{2}-dependent pathway), and TRPC1-encoded IP\textsubscript{3}ROC (activated by IP\textsubscript{3} via conformational coupling with IP\textsubscript{3} receptor). In the cells in which Orai1 and TRPC1 are expressed endogenously, they form two distinct channels that may account for the complex cellular response. Coexistence of these endogenously expressed channels may be responsible for some mutually exclusive findings in SOCE field (review in refs. 2 and 3), and could easily explains why SOCE is absent in the cells in which either Orai1 or iPLA\textsubscript{2} were down-regulated, while it remains intact and fully functional in the cells in which either TRPC1\textsubscript{16} or IP\textsubscript{3}R\textsubscript{41,47,48} are absent or knocked-down. At the same time it explains why TRPC1 channels and conformational coupling to IP\textsubscript{3}R were rightly found to play a significant role in agonist-induced IP\textsubscript{3}-mediated Ca\textsuperscript{2+} signaling, while their share in TG-induced SOCE varied significantly in different cell types. IP\textsubscript{3}-operated plasma membrane channels may mirror a well established presence and physiological role of the rather ubiquitous TRPC1 channel (reviewed in refs. 21–24), as well as may show some properties of the channels encoded by TRPC6,49 and other TRPC’s that are known to associate with TRPC1 to form endogenous heteromeric channels (reviewed in refs. 50–53). In view of our findings, one may expect IP\textsubscript{3}ROC to play a major role in processes that involve IP\textsubscript{3}, especially when it is produced at sub-threshold levels (that may not even cause Ca\textsuperscript{2+} release from the stores). Upon Ca\textsuperscript{2+} release (which by itself may produce Ca\textsuperscript{2+} dependent inactivation of IP\textsubscript{3}ROC, as shown by our studies), store depletion will activate SOC, which may take over and sustain Ca\textsuperscript{2+} entry after IP\textsubscript{3}ROC is inactivated. In this way both channels may be involved and barely distinguishable in a wide variety of signaling processes.

Table 1 summarizes the main properties of I\textsubscript{CRAC} and I\textsubscript{IP3ROC}, and shows that under certain conditions they may share a common appearance, and can both respond to IP\textsubscript{3}-mediated stimulation, but they have totally different biophysical properties, molecular identity and mechanism of activation.

Table 1. Biophysical and molecular discrimination of I\textsubscript{CRAC} and I\textsubscript{IP3ROC}.

| Requirement of IP\textsubscript{3} | I\textsubscript{CRAC} | I\textsubscript{IP3ROC} |
|----------------------------------|---------------------|---------------------|
| Requirement of IP\textsubscript{3} | Yes                 | No                  |
| Reversal potential: |                     |                     |
| (Na\textsuperscript{+},Ca\textsuperscript{2+})\textsubscript{out}/(Na\textsuperscript{+},Ca\textsuperscript{2+})\textsubscript{in} | > +50 mV > +50 mV | > +50 mV > +50 mV |
| Selectivity | P\textsubscript{Ca2+}/P\textsubscript{Na+} > 1000 | P\textsubscript{Ca2+}/P\textsubscript{Na+} < 0.4 |
| iPLA\textsubscript{2}-deficient cells | Absent              | Present             |
| Orai1-deficient cells | Present            | Absent              |
| TRPC1-deficient cells | Present            | Absent              |
| Inhibition by DES | Yes                | Yes                 |
| Inhibition by 2-APB | Yes                | Yes                 |
| TRPC1/IP\textsubscript{3} peptide | No effect           | Inhibition          |
| Calyculin A | No effect           | Inhibition          |

Figure 6. Differential effects of IP\textsubscript{3}R-binding peptide on I\textsubscript{CRAC} (A) and I\textsubscript{IP3ROC} (B). I\textsubscript{CRAC} was recorded in control cells dialyzed with 10 mM BAPTA (A). I\textsubscript{IP3ROC} alone or in combination with I\textsubscript{CRAC} was recorded in cells dialyzed with 10 mM IP\textsubscript{3} + 10 mM BAPTA (B). Extracellular and intracellular solutions contained symmetrical 130 mM Na\textsuperscript{+}. Extracellular solution contained 20 mM Ca\textsuperscript{2+}. (A and B) I/V relationships of maximum current in cells transfected with either TRPC1 a/s (A), or Orai1 siRNA (B). Peptide corresponding to TRPC1 domain responsible for its binding to IP\textsubscript{3}R (20 ng/ul) was either absent (open symbols) or present (filled symbols) in intracellular solution. Each trace is an average ± SE from 4–6 cells.
Figure 7. Two Ca²⁺ entry pathways mediated by two distinct channels, Orai1-encoded SOC and TRPC1-dependent IP₃ROC. Both pathways may be activated upon receptor (R) and G-protein (G)-mediated stimulation of phospholipase C (PLC) that lead to IP₃ production. Store-operated pathway (on the right) is activated upon depletion of Ca²⁺ stores that leads to oligomerization of STIM1 in ER membrane in close vicinity to plasma membrane and triggers production of calcium influx factor (CIF) that displaces inhibitory calmodulin (CaM) from a plasma membrane bound Ca²⁺-independent phospholipase A₂ (iPLA₂), which in turn activates Orai1-encoded SOC channels and allow store-operated Ca²⁺ entry (SOCE). IP₃ receptor-operated pathway (on the left) does not require store depletion, and is activated directly by IP₃ binding to IP₃ receptor (IP₃R) and conformational coupling of IP₃R to TRPC1-dependent plasma membrane channel (IP₃ROC).

activation of phospholipase C (PLC)⁵⁴ resulting in sub-threshold IP₃ production and activation of IP₃ROC. Thus, TG-induced Ca²⁺ entry may be a complex phenomenon, which in some cells may be mediated not only by SOC, but may also involve an IP₃ROC component, leading to controversial results and conflicting conclusions.

To summarize, new molecular and biophysical approaches allowed us to distinguish two plasma membrane channels that may be endogenously present and may respond to the same physiological (or experimental) stimuli in the cells. Like Siamese twins, these channels may share some similarities in appearance, but they have different molecular identities, biophysical properties and mechanisms of activation. New knowledge on the potential coexistence of different molecular identities, biophysical properties and mechanisms may share some similarities in appearance, but they have (or experimental) stimuli in the cells. Like Siamese twins, these endogenously present and may respond to the same physiological us to distinguish two plasma membrane channels that may be leading to controversial results and conflicting conclusions.

Electrophysiology. Whole-cell currents were recorded in RBL cells using standard whole-cell (dialysis) patch clamp technique as we (previously described in ref. 39). Axopatch 200B amplifier was used; data were digitized at 5 kHz and filtered at 1 kHz. Pipettes were used with tip resistance of 2–4 MΩ. Amplitude of the current was expressed in pA/pf. The time course of current development was analyzed at -80 mV for each individual cell, and summary data for 5–10 cells are shown in the figures. Average I/V relationships are shown during ramp depolarization (from -100 to +100 mV, 150 ms; divided into every 3 s) after the current reached its maximum (right before application of DES, which inhibits the currents, as in experiments shown in Figure 1. Passive leakage current with zero reversal potential (at the moment of breaking into the cell, or after current inhibition with 10 μM DES⁴⁰) was subtracted. Please, notice that CRAC current amplitude could vary in different sets of experiments due to variations related to different cell passages and cell cycle.⁵⁵ Standard extracellular solution was (in mM): 130 NaCl, 3 CsCl, 20 CaCl₂, 1 MgCl₂, 10 HEPES (pH 7.4). Standard intracellular (pipette) solution was (in mM): 145 glutamic acid, 165 CsOH, 3 MgCl₂, 10 BAPTA, 10 HEPES, (pH 7.2). Na⁺ pipette solution was (in mM): 145 glutamic acid, 130 NaOH, 40 CsOH, 3 MgCl₂, 10 BAPTA, 10 HEPES, (pH 7.2). BAPTA (1 or 10 mM) was present in all intracellular solutions, and IP₃ (10 nM to 100 μM) was added in some experiments, as indicated in the text. Peptide corresponding to TRPC1 binding domain to IP₃R (Ac-VRNLKQRDNYQKVCMCCIVHRYLTSRQKOH) was added at 20 ng/μl into the pipette solutions in specified experiments. Experiments were performed at 20–22°C.

Statistical analysis. Summary data are presented as mean ± SE. Student’s t-test was used to determine the statistical significance of the obtained data. Data were considered significant at p < 0.01.

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