Molecular Analysis of a Store-operated and 2-Acetyl-sn-glycerol-sensitive Non-selective Cation Channel

HETEROMERIC ASSEMBLY OF TRPC1-TRPC3

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We have reported that internal Ca\(^{2+}\) store depletion in HSY cells stimulates a nonselective cation current which is distinct from \(I_{\text{CRAC}}\) in RBL cells and TRPC1-dependent \(I_{\text{SOC}}\) in HSG cells (Liu, X., Groschner, K., and Ambudkar, I. S. (2004) J. Membr. Biol. 200, 93–104). Here we have analyzed the molecular composition of this channel. Both thapsigargin (Tg) and 2-acetyl-sn-glycerol (OAG) stimulated similar nonselective cation currents and Ca\(^{2+}\) entry in HSY cells. The effects of Tg and OAG were not additive. HSY cells endogenously expressed TRPC1, TRPC3, and TRPC4 but not TRPC5 or TRPC6. Immunoprecipitation of TRPC1 pulled down TRPC3 but not TRPC4. Conversely, TRPC1 co-immunoprecipitated with TRPC3. Expression of antisense TRPC1 decreased (i) Tg- and OAG-stimulated currents and Ca\(^{2+}\) entry and (ii) the level of endogenous TRPC1 but not TRPC4. Antisense TRPC3 similarly reduced Ca\(^{2+}\) entry and endogenous TRPC3. Yeast two-hybrid analysis revealed an interaction between NTRPC1 and NTRPC3 (CTRPC1-CTRPC3, CTRPC3-CTRPC1, or CTRPC1-NTRPC3 did not interact), which was confirmed by glutathione S-transferase (GST) pull-down assays (GST-NTRPC3 pulled down TRPC1 and vice versa). Expression of NTRPC1 or NTRPC3 induced similar dominant suppression of Tg- and OAG-stimulated Ca\(^{2+}\) entry. NTRPC3 did not alter surface expression of TRPC1 or TRPC3 but disrupted TRPC1-TRPC3 association. In aggregate, our data demonstrate that TRPC1 and TRPC3 co-assemble, via N-terminal interactions, to form a heteromeric store-operated non-selective cation channel in HSY cells. Thus selective association between TRPCs generate distinct store-operated channels. Diversity of store-operated channels might be related to the physiology of the different cell types.

Mammalian canonical TRP\(^3\) channels (TRPCs) have been proposed as Ca\(^{2+}\)-permeable cation channels that are activated in response to stimulation of phosphatidylinositol 4,5-bisphosphate hydrolysis and internal Ca\(^{2+}\) release (1, 2). Some TRPC species are capable of forming highly Ca\(^{2+}\)-selective channels, while others form relatively nonselective cation channels that allow permeation of both mono- and divalent cations. In addition, the presently available data suggest that TRPCs can form two types of cation entry channels (3). Store-operated channels (SOCs) that are activated in response to depletion of internal Ca\(^{2+}\) stores. These channels can be activated by agonists, thapsigargin, and by intracellular increase in phosphatidylinositol 1,4,5-trisphosphate. In contrast receptor-operated TRPCs are activated by agonist-stimulated phosphatidylinositol 4,5-bisphosphate hydrolysis but not by thapsigargin. The latter TRPCs are also activated by OAG, an analogue of diacylglycerol, the proposed physiological ligand for these channels. TRPC3, TRPC6, and TRPC7 have been reported to form receptor-operated Ca\(^{2+}\) entry channels that can be activated by OAG (4), while TRPC1, TRPC4, and TRPC5 form components of store-operated Ca\(^{2+}\) channels in some cell types (5–7). The exact mechanisms regulating store-operated and receptor-operated Ca\(^{2+}\) channels or their molecular components have yet been conclusively identified.

It has been proposed that the seven TRPC proteins (TRPC1–7) can assemble to form homomers or heteromers (8). Heteromeric interactions between TRPCs can potentially generate a wide variety of different channels and there are convincing data to substantiate these suggestions. Heterologous expression of different combinations of TRPC channels generate distinct channels (9, 10). Furthermore, fluorescence resonance energy transfer measurements have confirmed interactions between heterologously expressed TRPCs (11). Immunoprecipitation experiments have demonstrated association of both endogenous and exogenously expressed TRPCs (9, 10, 12, 13). In aggregate, these previous studies suggest that heteromeric interactions occur between members of two groups of TRPCs: TRPC1/TRPC4/TRPC5 and TRPC3/TRPC6/TRPC7. Interestingly, these two groups also represent receptor-operated and store-operated channels, respectively. The latter group is also suggested to be activated by OAG (4). Exceptions to these findings have also been reported. Montell and co-workers (14) reported that exogenously expressed TRPC1 and TRPC3 co-assemble to form a heteromeric complex. Novel heteromeric associations between endogenous TRPCs were reported by thapsigargin; SOC, store-operated channel; GFP, green fluorescent protein; IP, immunoprecipitation (or immunoprecipitated); IB, immunoblotting (or immunoblotted); HA, hemagglutinin.
Strubing et al. (10) in embryonic brain. Lintschinger et al. (15) reported that exogenously expressed TRPC1 and TRPC3 share the property of activation by diacylglycerols and co-assemble to form channels regulated by phospholipase C and Ca\(^{2+}\) but not by Tg. However, there is little information regarding the contribution of endogenous TRPCs, and specifically of heteromeric TRPC channels, to store-operated Ca\(^{2+}\) entry seen in different cell types. Furthermore, the physiological implication of such diverse SOCs is not yet understood. Downstream events regulated by Ca\(^{2+}\) entry likely determine the type of channel that is formed in different cells (1, 2, 16).

We have reported earlier that the salivary gland cells lines HSG (human submandibular gland ductal cells) and HSY (human parotid gland ductal cells) display distinct carbachol-stimulated Ca\(^{2+}\) oscillations (17). Furthermore, internal Ca\(^{2+}\) store depletion activates a non-selective cation current in HSY cells, which is distinct from the relatively Ca\(^{2+}\)-selective cation current \((I_{SOC})\) activated in HSG cells. \(pCa/pNa\) was 40 and 4.6 for HSG and HSY cells, respectively. Evidence for anomalous mole fraction behavior of Ca\(^{2+}/Na^+\) permeation was obtained with HSG cells but not HSY cells (18). Importantly, our studies have suggested that TRPC1, but not TRPC3, is an integral component of the store-operated channel in HSG cells (7, 19). In this study we have analyzed the molecular components of the non-selective, store-operated channel in HSY cells and present data to demonstrate that it is formed by selective heteromeric interactions between endogenously expressed TRPC1 and TRPC3.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—HSY cells were cultured in Earle’s minimal essential medium supplemented with 10% fetal calf serum, 2 mM glutamine, 1% penicillin/streptomycin at 37 °C in 5% CO\(_2\). For transfection, 1 µg of DNA was combined with 2.4 µg of Lipofectamine 2000 (Invitrogen) in serum-free medium and incubated at room temperature for 20 min. The mixture was then added to the cells. 5 h after the start of transfection, the medium was replaced with fresh medium containing serum and antibiotics. For functional studies, cells were plated onto glass coverslips or MatTek tissue culture dishes. Cells were transiently transfected with 5 µg of plasmids encoding antisense

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**Fig. 1. Thapsigargin and OAG stimulate the same Ca\(^{2+}\) entry pathway in HSY cells.** Perfusion of cells with media containing 1 µM Tg, 50 µM OAG, or both together increased membrane currents (A–C). Currents detected at −80 mV are plotted in the traces shown in A–E. \(I-V\) relationships of the currents at the times indicated by arrows in the traces are shown next to the trace. Currents induced by Tg and OAG were blocked by 1 µM Gd\(^{3+}\) (D and E). Fura2 fluorescence measurements in HSY cells treated with Tg (F), OAG (G), and Tg + OAG (H). \([Ca^{2+}]_i\) in the external medium is indicated below the traces. Average current densities and peak fluorescence value during Ca\(^{2+}\) entry are given in the text. Ca\(^{2+}\) entry was blocked by 1 µM Gd\(^{3+}\) (data not shown).

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**Assembly of Heteromeric TRPC1-TRPC3 Store-operated Channels**
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TRPC1, antisense TRPC3, NTRPC1, or NTRPC3 and 1 µg GFP-encoding plasmid. GFP-positive cells were selected for the experiment 24 h after transfection.

Electrophysiological Measurements—Measurements were made as described earlier (18). For patch clamp experiments, coverlips with cells were transferred to the recording chamber and perfused with the standard intracellular solution that contained the following (mM): cesium sulfate, 145; NaCl, 8; MgCl2, 10; Hepes, 10; Glucose, 10; pH 7.4 (NaOH). The patch pipette had resistances between 3 and 5 MΩ after filling with the standard intracellular solution that contained the following (mM): cesium sulfate, 145; NaCl, 8; MgCl2, 10; Hepes, 10; EGTA, pH 7.2 (CsOH). Osmolarity for all the solutions was adjusted with mannose to 300–315 mmol/kg using a vapor pressure osmometer (Wescor, Inc.).

Patch clamp experiments were performed in the tight-seal whole cell configuration at room temperature (22–25 °C) using an Axopatch 200B amplifier (Axon Instruments, Inc.). Development of the current was assessed by measuring the current amplitudes at a potential of −80 mV, taken from high resolution currents in response to voltage ramps ranging from −90 to 90 mV over a period of 1 s imposed every 4 s (holding potential was 0 mV) and digitized at a rate of 1 KH. Liquid-junction potentials were less than 8 mV and were not corrected. Capacitative currents and series resistance were determined and minimized. For analysis, current recorded during the first ramp was used for leak subtraction of the subsequent current records. Tg and OAG were purchased from Calbiochem.

Cell Surface Biotinylation—Control and NTRPC3-expressing cells were washed with ice-cold phosphate-buffered saline and incubated for 20 min with 0.5 mg/ml Sulfo-NHS-Biotin (Pierce) on ice. Cells were then washed three times with buffer containing 0.1 M glycine and solubilized with 2 ml of immunoprecipitation buffer. Biotinylated proteins were pulled down with NeutrAvidin-linked beads (Pierce). Bound fraction was washed and released with SDS-PAGE sample buffer and analyzed by Western blotting using anti-TRPC3 or anti-TRPC1 antibodies as described above.

**RESULTS AND DISCUSSION**

Thapsigargin and OAG Stimulate Similar Currents in HSY Cells—In whole cell patch clamp experiments perfusion of HSY cells with 1 µM Tg in the external medium increased membrane currents (Fig. 1A). The Tg-induced cation currents inactivated relatively fast. Consistent with our previous report (18) the I-V relationship was linear with a reversal potential of +5 mV. Perfusion of cells with OAG also generated a current that had similar characteristics (pattern of I-V curve and Frev, see Fig. 1B) as that induced by Tg. Furthermore, when cells were perfused with OAG + Tg-containing medium the current generated was similar in characteristics and magnitude to that seen with Tg alone (Fig. 1C). Maximum current densities (picoamperes/picofarad) measured in the three conditions were Tg, 6.3 ± 0.7 (n = 5); OAG, 4.5 ± 0.5 (n = 4); OAG + Tg, 6.4 ± 0.9 (n = 6). Thus, the effects of OAG and Tg on the current were not additive. In addition, both Tg- and OAG-stimulated currents were completely blocked by 1 µM Gd²⁺ (Fig. 1, D and E).
FIG. 3. Antisense TRPC1 and antisense TRPC3 attenuate Tg- and OAG-stimulated membrane currents in HSY cells. Tg-, OAG-, and Tg + OAG-stimulated membrane currents in cells treated with TRPC1-as (A) and TRPC3-as (B). Currents obtained at −80 mV (see the legend to Fig. 1 for details) and I-V plots are shown in each case. Average data comparing the current densities with that in control cells (see Fig. 1) are shown in C. TRPC1-as and TRPC3-as induce a similar decrease in Tg-, OAG-, and Tg + OAG-stimulated currents. Values marked are significantly different (p < 0.05; n ≥ 7) from unmarked values in the same group but not from each other.

The patch clamp data were substantiated with Fura2 measurements. 1 μM Tg rapidly increased intracellular Ca2⁺ concentration in cells incubated in Ca²⁺-free external medium and addition of 1 mM Ca²⁺ to the bath induced a rapid significant increase in [Ca²⁺], (Fig. 1F, note that basal Ca²⁺ entry is minimal in these cells). Peak Fura2 fluorescence (340/380 ratio) was 1.2 ± 0.3 (n = 118). OAG alone did not induce increase in [Ca²⁺], until external Ca²⁺ was replenished (Fig. 1G). Peak 340/380 fluorescence ratio was 1.0 ± 0.3 (n = 121). These data demonstrate that Tg induces internal Ca²⁺ release and Ca²⁺ entry, while OAG induces only Ca²⁺ entry. This is consistent with previous reports showing that OAG directly activates some TRPC channels such as TRPC3, -6, and -7 (4, 22–24). Importantly, and consistent with the current measurements, addition of Tg + OAG induced internal Ca²⁺ release and Ca²⁺ entry that were similar to that induced by Tg alone (Fig. 1H). Peak 340/380 fluorescence ratio was 1.2 ± 0.4 (n = 135). Importantly, and consistent with the current measurements, addition of 1 mM Ca²⁺ to the bath induced a rapid significant increase in [Ca²⁺], (Fig. 1F, note that basal Ca²⁺ entry is minimal in these cells). Peak Fura2 fluorescence (340/380 ratio) was 1.2 ± 0.3 (n = 118). OAG alone did not induce increase in [Ca²⁺], until external Ca²⁺ was replenished (Fig. 1G). Peak 340/380 fluorescence ratio was 1.0 ± 0.3 (n = 121). These data demonstrate that Tg induces internal Ca²⁺ release and Ca²⁺ entry, while OAG induces only Ca²⁺ entry. This is consistent with previous reports showing that OAG directly activates some TRPC channels such as TRPC3, -6, and -7 (4, 22–24). Importantly, and consistent with the current measurements, addition of Tg + OAG induced internal Ca²⁺ release and Ca²⁺ entry that were similar to that induced by Tg alone (Fig. 1H). Peak 340/380 fluorescence ratio was 1.2 ± 0.4 (n = 135). In aggregate, the data in Fig. 1 suggest that OAG and Tg stimulate the same Ca²⁺ entry mechanism in HSY cells.

Expression and Heteromeric Interaction between Endogenous TRPC1 and TRPC3 in HSY Cells—To identify the channels activated by Tg and OAG, we examined the endogenous expression of TRPC proteins in HSY cells. TRPC1, TRPC3, and TRPC4 were detected by Western blotting, while TRPC5 and TRPC6 were not detected (Fig. 2A, proteins detected using the respective antibodies are indicated below each blot; TRPC2, which is a pseudogene in human cells, and TRPC7 were not examined). Lane 1 in each blot was loaded with a sample containing crude membrane preparation from cells expressing exogenous TRPCs, as indicated (the expressed proteins were further confirmed using the anti-epitope tag antibodies; data not shown). Lane 2 was loaded with a sample containing crude membrane preparation from HSY cells and demonstrates the presence of endogenous TRPCs. Thus, HSY cells appear to express three different TRPC proteins, TRPC1, TRPC3, and TRPC4. Although distinct store-operated currents have been detected in different cell types, there is little information regarding the molecular composition of the endogenous channels mediating these currents or the role of endogenous TRPC proteins in the generation of these channels.

Both exogenously expressed and endogenous TRPCs have been shown to form homo- and heteromultimers. Furthermore, heterologous expression of TRPCs has demonstrated that different combinations of TRPCs generate channels with distinct characteristics. These heteromeric TRPC complexes have been shown to form both store-operated and store-independent cation channels (9, 10, 15, 19, 20, 23–25). Thus we examined possible heteromerization between the endogenously present TRPCs in HSY cells. When anti-TRPC3 was used to pull down TRPC3, TRPC1 was co-immunoprecipitated (Fig. 2B). Reverse IP using anti-TRPC1 pulled down TRPC1 and TRPC3. Furthermore, IP of TRPC1 did not pull down TRPC4 (Fig. 2B). These data suggest a selective interaction between TRPC1 and TRPC3 in HSY cells.

Antisense TRPC1 and Antisense TRPC3 Decrease Store-operated Ca²⁺ Entry in HSY Cells—To examine the contribution of TRPC1 and TRPC3 to the OAG and Tg-stimulated currents described above, cells were transiently transfected with TRPC1-as (C1-as) or TRPC3-as (C3-as), and expression of TRPC1 and TRPC3 was assessed. TRPC3-as induced a decrease in endogenous TRPC3 but did not affect expression of TRPC1 or TRPC4 (Fig. 2C). Similarly, TRPC1-as induced a
decrease in TRPC1 but not TRPC3 or TRPC4. These data demonstrate the specificity of the antisense treatments.

Consistent with the decrease in TRPC1 expression, TRPC1-as induced a significant reduction in both Tg and OAG-stimulated membrane currents (Fig. 3, A and C). The current seen with OAG, Tg, or OAG + Tg were all similarly decreased. Importantly, the remaining currents displayed similar I-V relationships as seen in control HSY cells. Similar effects on Tg- and OAG-stimulated currents were seen with TRPC3-as-treated cells (Fig. 3, B and C). The average current densities, relative to that in control cells, are shown in Fig. 3 C. Thus, antisense-induced decrease in specific TRPC proteins does not alter channel characteristics indicating that both TRPC1 and TRPC3 contribute to the OAG- and Tg-stimulated currents. This also suggests that the remaining TRPCs do not reassemble to generate channels with different compositions.

The effect of antisense treatment on the store-operated current was further confirmed by Fura2 measurements. Expression of TRPC1-as or TRPC3-as induced similar decreases in Tg-, OAG-, and Tg + OAG-stimulated \( \text{Ca}^{2+} \) entry (Fig. 4, A). Additions of the agents and \( \text{Ca}^{2+} \) concentration in the external medium are indicated in each trace. Average data comparing peak Fura2 fluorescence after \( \text{Ca}^{2+} \) addition (representing \( \text{Ca}^{2+} \) entry) under different conditions in control and as-treated cells are shown in cells. D. TRPC1-as and TRPC3-as induced similar decreases in Tg- as well as OAG-stimulated \( \text{Ca}^{2+} \) entry. Values marked are significantly different (\( p < 0.05; n > 100 \) cells) from unmarked values in the same group but not from each other.

**FIG. 4.** Antisense TRPC1 and antisense TRPC3 attenuate Tg- and OAG-stimulated \( \text{Ca}^{2+} \) entry in HSY cells. Tg-, OAG-, and Tg + OAG-stimulated \( \text{Ca}^{2+} \) entry was measured in Fura2-loaded control HSY cells (A) and cells treated with TRPC1-as (B) and TRPC3-as (C). Additions of the agents and \( \text{Ca}^{2+} \) concentration in the external medium are indicated in each trace. Average data comparing peak Fura2 fluorescence after \( \text{Ca}^{2+} \) addition (representing \( \text{Ca}^{2+} \) entry) under different conditions in control and as-treated cells are shown in cells. D. TRPC1-as and TRPC3-as induced similar decreases in Tg- as well as OAG-stimulated \( \text{Ca}^{2+} \) entry. Values marked are significantly different (\( p < 0.05; n > 100 \) cells) from unmarked values in the same group but not from each other.
form homomultimers, and (ii) that TRPC3 and TRPC1 can interact to form TRPC1-TRPC3 heteromultimers. Additionally, we found that the NTRPC1-TRPC3 and NTRPC3-TRPC1 interactions were relatively stable and did not dissociate even in the presence of 0.5% SDS (data not shown). These results are consistent with reports showing that Drosophila TRP and TRPL co-assembly is mediated through their N terminus (14). Homomultimeric TRPC3 has not been assessed in this study, and its role, if any, in HSY cell Ca\(_{2+}\) entry is presently not known.

**NTRPC1 and NTRPC3 Suppress Store-operated Channel Activation in HSY Cells**—The data discussed above suggest that the N-terminal domains of TRPC1 and TRPC3 are involved in assembly of the TRPC1-TRPC3 heteromeric channel in HSY cells. This was further examined by expressing either NTRPC1 or NTRPC3 in these cells. Significantly, both NTRPC1 and NTRPC3 induced similar dominant suppression of Tg- and OAG-stimulated currents in these cells. Furthermore, the characteristics of the remaining currents were similar to that seen in control cells (Fig. 5, C and D). Importantly, expression of or NTRPC3 in HSY cells (i) did not alter the expression of TRPC1 or TRPC3 and (ii) disrupted TRPC1-TRPC3 association (Fig. 5E). Immunoprecipitation of TRPC1 from lysates of control cells (Con) and cells transfected with NTRPC3 (NTRPC3) using anti-TRPC1 antibody (upper panels). Lower panels show input protein. Blots were probed for TRPC3 or TRPC1 as indicated (IB: TRPC3 and IB TRPC1, respectively). F, surface expression of TRPC1 and TRPC3 in control and NTRPC3-expressing cells. Cells were biotinylated as described under “Experimental Procedures.” Upper blots show levels of TRPC1 (left) or TRPC3 (right) in the biotinylated fraction pulled down from cell lysates using avidin-linked beads (IP-avidin; IB was done using anti-TRPC1 or anti-TRPC3 antibodies, respectively). Lower blots show the level of proteins in the input (cell lysate). IP, immunoprecipitated; IB, immunoblotted.

**Fig. 5.** TRPC1 and TRPC3 interact via N-terminal regions to generate store-operated cation channel in HSY cells. A, yeast two-hybrid interactions between NTRPC1 and NTRPC3 (interaction was confirmed by \(\beta\)-galactosidase assays; data not shown). B, GST pull-down assays. GST proteins are indicated on top of the blots. Lysates of control non-transfected cells (panel 1) and cells expressing either HA-TRPC1 (panel 2) or HA-TRPC3 (panel 3) were used (indicated below the blot). IB antibody was anti-HA for all the blots. GST was used as a control (shown in panel 3, first lane). C, Tg- and OAG-activated membrane currents (trace shows currents measured at -80 mV, see the legend to Fig. 1 for details) and respective I-V relationships in control and NTRPC3- and NTRPC1-expressing cells. D, average current densities obtained in each case (n = at least 6 cells in each experiment). Significant difference from respective controls is indicated by **; marked values are not different from each other. E, effect of NTRPC3 expression on co-IP of TRPC1 and TRPC3. TRPC1 was immunoprecipitated from lysates of control cells (Con) and cells transfected with NTRPC3 (NTRPC3) using anti-TRPC1 antibody (upper panels). Lower panels show input protein. Blots were probed for TRPC3 or TRPC1 as indicated (IB: TRPC3 and IB TRPC1, respectively). F, surface expression of TRPC1 and TRPC3 in control and NTRPC3-expressing cells. Cells were biotinylated as described under “Experimental Procedures.” Upper blots show levels of TRPC1 (left) or TRPC3 (right) in the biotinylated fraction pulled down from cell lysates using avidin-linked beads (IP-avidin; IB was done using anti-TRPC1 or anti-TRPC3 antibodies, respectively). Lower blots show the level of proteins in the input (cell lysate). IP, immunoprecipitated; IB, immunoblotted.
plasma membrane expression of either endogenous TRPC1 or TRPC3 in HSY cells. The upper blots show the level of the proteins in the biotinylated fraction (pull-down with avidin) obtained from cells expressing NTRPC3 and control cells (blots on the left show TRPC1, and blots on the right show TRPC3; input protein is shown in the lower blots). These data provide strong evidence that the store-operated channel in HSY cells is assembled via N-terminal interactions between TRPC1 and TRPC3. Consistent with these findings, it has been reported previously that expression of NTRPC3 suppresses a Tg-stimulated TRPC3-dependent conductance in human umbilical vein endothelial cells (26).

Above, we have identified the molecular components of a store-operated, non-selective cation channel in HSY cells. We show that this channel is activated by thapsigargin and more uniquely also by OAG. The effect of OAG and Tg are non-additive demonstrating that both activate the same channel. Importantly, our data show that endogenous TRPC1 and TRPC3 co-assemble heteromerically to form this store-operated cation channel in HSY cells and that channel assembly involves interactions between their N-terminal domains. This channel is distinct from the TRPC1-dependent SOCs in HSG cells (7, 19, 20) and Ca$^{2+}$ release-activated channel in RBL cells. The molecular components of the latter, relatively well studied channel, are presently unknown (27, 28). Notably, assembly of TRPC1 and TRPC3 in HSY cells generates a channel that is relatively non-selective for Ca$^{2+}$ when compared with the channels in HSG and RBL cells (pCa/pNa are 4, 40, and >400 in HSY, HSG, and RBL cells, respectively). This channel also does not exhibit anomalous mole fraction behavior of Ca$^{2+}$/Na$^+$ permeation, unlike the channels in RBL and HSG cells. Typically, TRPC3 has been suggested to interact with TRPC6/ TRPC7 and form non-store-operated Ca$^{2+}$ entry channels (4).

In conclusion, we report here the presence of a Tg- and OAG-sensitive, store-operated non-selective cation channel in HSY cells. We demonstrate that TRPC1 and TRPC3 co-assemble, via N-terminal domain interactions, to form this novel channel. Thus, selective assembly of endogenous TRPC proteins in cells can generate distinct cation channels that are activated by agonist stimulation of the cells, internal Ca$^{2+}$ store-depletion, or both. Further studies are required to understand the physiological relevance of such distinct store-operated calcium channels in different cell types.

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