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# The interaction domains of transient receptor potential canonical (TRPC)1/4 and TRPC1/5 heteromultimeric channels

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ABSTRACT

Transient receptor potential canonical (TRPC) family contains a non-selective cation channel, and four TRPC subunits form a functional tetrameric channel. TRPC4/5 channels form not only the homotetrameric channel but also a heterotetrameric channel with TRPC1. We investigated the interaction domain required for TRPC1/4 or TRPC1/5 heteromultimeric channels using FRET and the patch-clamp technique. TRPC1 only localized at the plasma membrane (PM) when it was coexpressed with TRPC4 or TRPC5. The TRPC1/4 or TRPC1/5 heteromultimeric showed the typical outward rectifying I/V curve. When TRPC1 and TRPC4 form a heteromeric channel, the N-terminal coiled-coil domain (CCD) and C-terminal 725–745 region of TRPC1 interact with the N-terminal CCD and C-terminal 700–728 region of TRPC4. However, when TRPC1 and TRPC5 form a heteromeric channel, the N-terminal CCD and C-terminal 673–725 region of TRPC1 interact with the N-terminal CCD and C-terminal 707–735 region of TRPC5. In conclusion, the N-terminal CCD of TRPC channels is essential for the heteromultimeric structure of TRPC channels, whereas specific C-terminal regions are required for unique heteromerization between subgroups of TRPC channels.



## 1. Introduction

The TRP channel superfamily has been studied extensively due to its cellular function and its-disease associated characteristics [1,2]. The TRPC subfamily consists of 7 members. TRPC1, TRPC4, and TRPC5 channels are categorized into the same TRPC subfamily. TRPC1 was the first cloned mammalian TRPC channel. The membrane expression and function of TRPC1, unlike other TRPC channels, still remain controversial [1,3]. Four TRPC4 and TRPC5 subunits form a functional, tetrameric channel. These channels not only form the homotetrameric channels but also form a heterotetrameric channel with TRPC1. Given the observation that most organs have high levels of TRPC1 channel mRNA, a study of the TRPC1/4 or TRPC1/5 heteromultimeric channel rather than the TRPC4 or TRPC5 homomultimeric channels might have more pathophysiologic relevance. TRPC1 is also thought to form heterotetramers with TRPC3, TRPC6, and TRPC7 subunits or STIM1 and to play a role as an important linker or regulator subunit. A detailed database of TRPC1 channel protein–protein interactions [4] is available at http://www.trpchannel.org/. A recent study showed the existence of a TRPC1 with additional 78 amino acids (aa) at the N-terminus, and these newly discovered 78 aa are thought to play a key role in the channel function [5].

The I/V curves of TRPC4 and TRPC5 homomeric channels that are expressed in the PM independently show a double rectifying shape with a reversal potential at 0 mV. In contrary, in our hands, the TRPC1 channel was not expressed at the PM and did not show any current even when exposed to various stimuli or when mutated. Many researchers showed an outward rectifying I/V curve when TRPC1 and TRPC4 or TRPC5 were coexpressed to form

*Abbreviations:* TRPC, Transient receptor potential canonical; CCD, Coiled-coil domain; ARD, Ankyrin repeat domains; SESTD1, SEC14 and spectrin domains 1; aa, amino acid; PM, Plasma membrane; ER, Endoplasmic reticulum; FRET, Förster Resonance Energy Transfer; TM, Transmembrane domains.

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heteromultimeric TRPC1/4 or TRPC1/5 channels. Thus, we determined which domains involved in the interaction between TRPC1 and TRPC4 (or TRPC5) are required for the heteromultimeric TRPC1/4 (or TRPC1/5) channels.

## 2. Materials and methods

### 2.1. Cell culture and transient transfection

cDNA clones-Human embryonic kidney (HEK293) cells (ATCC, Manassas, VA) were maintained according to the supplier's recommendations. HEK293 cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated FBS and penicillin (100 U/ml), streptomycin (100 μg/ml) at 37 °C in 5% CO2 humidified incubator. Cells were seeded in confocal dish for recording FRET or 12 well plate for whole-cell patch clamp. The following day, transfection was performed with Fugene-6 according to the manufacturer's instructions. XFP (CFP or YFP) tagged TRPC4, TRPC5 and TRPC1 were transfection in this way. The next day we performed electrophysiology or FRET experiments.

### 2.2. Electrophysiology

The cells were transferred onto a solution chamber on the stage of an invert microscope (IX70, Olympus, Japan). The whole cell configuration was used to measure TRPC channel current in HEK cells as described previously [6,7]. Cells were left for 10–15 min to attach to coverslips. Whole cell current were recorded using an Axopatch 200B amplifier (Axon instruments). Patch pipettes were made from borosilicate glass and had resistances of 3–5 MΩ when filled with normal intracellular solutions. Bath solution was changed from Normal Tyrode (NT) to Cs + rich external solution after whole cell recording system established. The NT contained 135 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 10 mM glucose, and 10 mM HEPES with a pH that was adjusted to 7.4 using NaOH. The Cs + -rich external solution contained equimolar CsCl rather than NaCl and KCl. The internal solution contained 140 mM CsCl, 10 mM HEPES, 0.2 mM Tris-guanosine 5′-triphospate, 0.5 mM EGTA, and 3 mM Mg-adenosine 5′-triphosphate with a pH that was adjusted to 7.3 with CsOH. We used 0.2 mM guanosine 5′-O-[gamma-thio] triphosphate (GTPγS) that was purchased from Sigma. Voltage ramp pulse was applied from +100 mV to −100 mV for 500 ms at −60 mV holding potential. Experiments were performed at room temperature (18–22 °C). The recording chamber was continuously perfused at a flow rate of 1–2 ml/min.

### 2.3. Imaging and FRET measurements

3-cube FRET image (cube setting for CFP, YFP, and Raw FRET) were gained from a pE-1 Main Unit to three FRET cubes (excitation, dichroic mirror, filter) through a fixed collimator: CFP (ET435/20 nm, ET CFP/YFP/mCherry beamsplitter, ET470 nm, Chroma), YFP (ET500/20 nm, ET CFP/YFP/mCherry beamsplitter, ET535/30 nm, Chroma), and Raw FRET (435/20 nm, ET CFP/YFP/mCherry beamsplitter, ET535/30 nm, Chroma). Each image was captured on cooled 10 MHZ (14bit) CCD camera under the control of MetaMorph 7.6 software (Molecular devices, Japan). To obtain the FRET efficiency of a cell, we utilized a microscope (IX70, Olympus, Japan) with a 60 x oil objective and the three-cube FRET method calculation [8].

### 2.4. Statistical analysis

Results are expressed as the mean ± SEM. Results were compared using Student's *t* test two groups or using ANOVA followed by post hoc test among three groups or more. $p < 0.05$ was considered statically significant.

## 3. Results

### 3.1. The distribution of TRPC1, TRPC4 and TRPC5 channel and the channel function

The TRPC channel consists of 6 transmembrane domains (TM) and cytosolic N- and C-termini. There are specific domains, such as the ankyrin repeat domain (ARD) at the N-terminus, the TRP box and the PH-like domain at the C-terminus, the CCDs at N- and C-termini, and the pore region between TM 5 and TM 6 (Supplementary Fig. 1A). To map the putative CCDs of TRPC channels, we used Marcoil 1.0 software. The TRPC1, TRPC4, and TRPC5 subunits have high homology, and 4 subunits act as a functional channel, which forms either a homotetrameric or a heterotetrameric structure [9,10].

First, we generated a fluorescence-tagged TRPC channel construct and determined the cellular distribution, the folding and the electrophysical channel activity. The fluorescence-tagged TRPC4 and TRPC5 were located at the PM with punctate distribution, but the fluorescence-tagged TRPC1 was retained at the endoplasmic reticulum (ER) (Supplementary Fig. 1B). FRET is a sensitive reporter of the proximity between an acceptor and donor, and it provides a noninvasive method of monitoring the subunit assembly of ion channels and other proteins. FRET was measured at the PM for TRPC4 and TRPC5 and in the cytoplasm for TRPC1. No FRET signal was detected for the co-transfection of the CFP-tagged TRPC channel and empty YFP. CFP- and YFP-tagged TRPC1α (YFP/CFP-TRPC1α) (14.1 ± 1.0%), TRPC1β (YFP/CFP-TRPC1β) (9.7 ± 1.2%), TRPC4β (TRPC4β-YFP/CFP) (29.7 ± 2.2%) and TRPC5 (YFP/CFP-TRPC5) (29.3 ± 0.9%) showed high FRET efficiency (Fig. 1A). These results suggest that the fluorescence-tagged TRPC constructs properly folded into the homotetrameric channel structure at each expression site. Patch-clamp experiments in the whole-cell mode using TRPC4-CFP and CFP-TRPC5 provided a double rectifying I/V curve (Fig. 1B). The current densities of TRPC4 and TRPC5 at +100 mV and −60 mV were respectively 248.3 ± 79.9 and −171.2 ± 33.6 pA/pF (TRPC4) and 315.6 ± 69.3 and −226.0 ± 104.1 pA/pF (TRPC5) in response to GTPγS stimulation in $Cs^+$ rich solution (Fig. 1B). However, the amplitude of the CFP-TRPC1α current was not different from the currents measured from untransfected cells. These results suggest that homotetrameric TRPC4 and TRPC5 channels were expressed at the PM with as functional channels, but homotetrameric TRPC1 could neither translocate to the PM nor function.

Next, we constructed deletion mutants of TRPC1, TRPC4, and TRPC5 to investigate the domains of the TRPC channel required for heteromultimeric structures (Supplementary Fig. 1C). The deletion mutants were based on the important domain for the function of each channel. For the TRPC1 channel, TRPC1(Δ1–187), TRPC1(Δ187–278) and TRPC1(Δ1–278) were deletions of the entire ARD, the CCD, and the ARD and CCD at the N-terminus, respectively. TRPC1(1–673), TRPC1(1–725) and TRPC1(1–745) were deletions of the entire C-terminus and of some C-terminal regions. For the TRPC4 channel, similar deletion mutants, TRPC4(Δ1–20), TRPC4(Δ1–97), TRPC4(Δ228–257), TRPC4(1–700), TRPC4(1–720), and TRPC4(Δ700–728), were made. For TRPC5, the deletion mutants TRPC5(Δ1–20), TRPC5(Δ1–97), TRPC5(Δ229–250), TRPC5(1–624), TRPC5(1–843), and TRPC5(Δ707–735) were generated. Deletion mutants were expressed in HEK293 cells with WGA to mark the PM (Fig. 1C and Supplementary Fig. 1D). To quantify the surface expression of the mutants, we used the fluorescence intensity of the PM in the image divided by the total intensity of the whole cell (Fig. 1D). In our