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Heteromeric TRPC3 with TRPC1 formed via its ankyrin repeats regulates the resting cytosolic Ca^{2+} levels in skeletal muscle

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ABSTRACT

The main tasks of skeletal muscle are muscle contraction and relaxation, which are mediated by changes in cytosolic Ca^{2+} levels. Canonical-type transient receptor potential 3 (TRPC3) contains an ankyrin repeat (AR) region at the N-terminus (38–188 amino acids) and forms extracellular Ca^{2+}-entry channels by homo or heteromerization with other TRP subtypes in various cells including skeletal myotubes. However, previous research has not determined which region(s) of TRPC3 is responsible for the heteromerization, whether the AR region participates in the heteromerizations, or what is the role of heteromeric TRPC3s in skeletal muscle. In the present study, the heteromerization of TRPC3 with TRPC1 was first examined by GST pull-down assays of TRPC3 portions with TRPC1. The portion containing the AR region of TRPC3 was bound to the TRPC1, but the binding was inhibited by the very end sub-region of the TRPC3 (1–37 amino acids). In-silico studies have suggested that the very end sub-region possibly induces a structural change in the AR region. Second, the very end sub-region of TRPC3 was expressed in mouse primary skeletal myotubes, resulting in a dominant-negative inhibition of heteromeric TRPC3/1 formation. In addition, the skeletal myotubes expressing the very end sub-region showed a decrease in resting cytosolic Ca^{2+} levels. These results suggest that the AR region of TRPC3 could mediate the heteromeric TRPC3/1 formation, and the heteromeric TRPC3/1 could participate in regulating the resting cytosolic Ca^{2+} levels in skeletal muscle.

1. Introduction

The transient receptor potential (TRP) super-family consists of a diverse group of Ca^{2+}-permeable non-selective cation channels on the plasma membrane, and plays important roles in extracellular Ca^{2+}-entry into various cells [1]. Tetrameric complexes of TRPs function as the channels, and the formation of tetrameric complexes is accomplished by homo or heteromerization of TRPs of either the same or different subfamilies [2]. Canonical-type TRP (TRPC) is a sub-family of the TRP super-family, and seven TRPC3 subtypes have been known [1]. Each TRPC subtype has six transmembrane domains, the N-terminus containing an ankyrin repeat (AR) region, and the C-terminus containing a TRP domain and a proline-rich motif [1,3].

Ca^{2+} stored in the sarcoplasmic reticulum is the major Ca^{2+} source for the excitation–contraction coupling (ECC) of skeletal muscle [4,5]. By allowing extracellular Ca^{2+}-entry, TRPC3 in skeletal myotubes maintains a sustained high Ca^{2+} level in the cytosol to allow a full gain during ECC [4,6]. TRPC3 binds to ECC-mediating proteins in skeletal myotubes, such as ryanodine receptor 1 (RyR1) [7,8], and plays an important role in the proliferation and differentiation of skeletal myoblasts to myotubes [6,8,9]. TRPC3 is also closely related to skeletal muscle disease. Patients with myasthenia gravis show fluctuating muscle weakness and fatigue due to the circulation of auto-antibodies against TRPC3 [10]. TRPC3-overexpressing transgenic mice show a phenotype of Duchenne muscular dystrophy (DMD) involving muscle weakness, wasting, and premature death [11]. A mouse model of atrophy shows a decrease in TRPC3 expression [12].

Ankyrin repeat (AR) is an interface for protein–protein interactions and is involved in a wide variety of physiological and pathophysiological processes via the mediation of local and short-range protein–protein interactions [13]. AR consists of 30–34 amino acids, and each repeat contains an anti-parallel helix-turn-helix

Abbreviations: TRP, transient receptor potential; TRPC, canonical-type TRP; AR, ankyrin repeats; ECC, excitation–contraction coupling; DMD, Duchenne muscular dystrophy; 3D, three-dimensional.

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structure followed by a flexible loop leading to the turn of the next repeat, all of which are stacked together to form a single domain [13,14]. An AR region consisting of four ARs exists in the N-terminus of TRPC3, and is required for the correct targeting of TRPC3 to the plasma membrane [15]. On the other hand, TRPC3 is heteromerized with other TRPC subtypes to form functional channels [2]. Among them, the heteromerization of TRPC3 with TRPC1 is found in both heterologous expressions and bona-fide systems including skeletal myotubes [2,16–18]. Through yeast two-hybrid analysis, an interaction between the N-termini from TRPC3 and TRPC1 has been reported [16]. However, there have been no studies to determine more specific region(s) in the N-terminus of TRPC3 that could be responsible for the heteromerization with TRPC1. In addition, no study has addressed whether the AR region participates in the heteromerization or what is the role of heteromeric TRPC3s in skeletal muscle, although heteromeric TRPC3s are known to exist in skeletal muscle [18], and the presence of the AR region is a characteristic of the TRPC family [1,3]. Therefore, in the present study, we examined the role of the AR region of TRPC3 in the heteromeric TRPC3/1 formation and the role of the heteromeric TRPC3/1 in skeletal myotubes.

2. Materials and methods

2.1. Ethics statement

All surgical interventions and all pre- and post-surgical animal care were provided in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Guidelines and Policies for Rodent Survival Surgery provided by the Institutional Animal Care and Use Committee of the College of Medicine at The Catholic University of Korea.

2.2. cDNA construction and protein expression of GST-fused TRPC3 portions

Using full-length human TRPC3 cDNA (GenBank Accession No. NM_003305.2) as a template, the N-terminal portions of TRPC3 were synthesized by PCR using the primers presented in Supplementary data 1. The PCR products were inserted into pGEX-4T-1 vector at the EcoRI and SalI sites (GST-fused NF1–NF4, or NF5) or into pEGFP-C1 mammalian expression vector at the XhoI and SalI sites (GFP-NF4). The sequences of all constructs were confirmed by sequencing both strands using an ABI Prism 3700 DNA Sequencer (Applied Biosystems, Foster City, CA). Each GST-fused protein was expressed in *Escherichia coli* (DH5α), as previously described [19].

2.3. Preparation of the triad sample and the GST pull-down assay of TRPC3 portions with TRPC1

The triad vesicles containing TRPC1 were prepared from rabbit fast-twitch skeletal muscle and were solubilized, as previously described [7,20]. GST pull-down assays were performed as previously described [19]. Briefly, affinity beads were prepared by immobilizing each GST-fused TRPC3 portion on GST beads (Amersham Biosciences, Pittsburgh, PA). The affinity beads were then incubated with 150 μg of the solubilized triad sample for 8 h at 4 °C. The proteins that were pulled down were separated on SDS–PAGE gels and subjected to immunoblot assay.

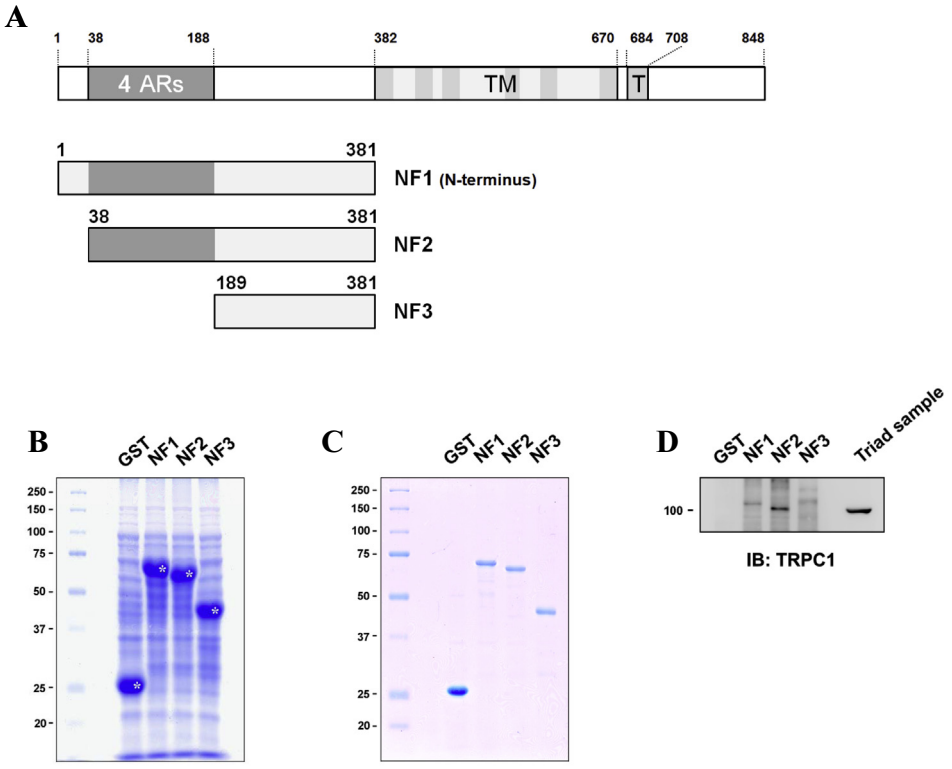

Fig. 1. Schematic primary sequences of TRPC3 and TRPC3 portions, and GST pull-down assays of the TRPC3 portions with TRPC1. (A) Schematic diagrams of full-length TRPC3 and N-terminal TRPC3 portions. Numbers indicate the sequence of amino acids. AR, ankyrin repeat; TM, transmembrane domain; T, TRP domain. (B) Bacterial lysate expressing each GST-fused TRPC3 portion was separated on SDS–PAGE gel, and the gel was stained with Coomassie Blue. GST-fused TRPC3 portions are indicated by asterisks. (C) Immobilized GST-fused TRPC3 portions on GST beads were separated on SDS–PAGE gel, and the gel was stained with Coomassie Blue. (D) Proteins obtained from the GST pull-down assays of GST-fused TRPC3 portions with TRPC1 were separated on SDS–PAGE gel, and the gel was subjected to immunoblot assay with anti-TRPC1 antibody. GST was used as a negative control. Three independent experiments were conducted and a representative result is presented. Only NF2 would bind to TRPC1.