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Essential role of the N-terminus of murine Orai1 in store-operated Ca^{2+} entry

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Abstract

Store-operated Ca^{2+} entry (SOCE) is a physiologically important process that is triggered by intracellular Ca^{2+} depletion. Recently, human Orai1 (the channel-forming subunit) and STIM1 (the calcium sensor) were identified as essential molecules for SOCE. Here, we report the cloning and functional analysis of three murine orthologs of Orai1, termed Orai1, 2, and 3. Among the genes identified, Orai1 contains a distinctive proline- and arginine-rich N-terminal cytoplasmic sequence. Co-expression of STIM1 with Orai1 produced a marked effect on SOCE, while co-expression with Orai2 or Orai3 had little effect. Expression of Orai1 without its N-terminal tail had a marginal effect on SOCE, while chimeric Orai2 containing the Orai1 N-terminus produced a marked increase in SOCE. In addition, a truncated version of Orai1 containing the N-terminus without the pore-forming transmembrane domain had a dominant negative effect on SOCE. These results reveal the essential role of Orai1 and its N-terminal sequence in SOCE.

Keywords: Ca^{2+}; Orai; STIM1

Ca^{2+} influx is essential for many cellular functions, including exocytosis, enzyme control, gene transcription, cell proliferation, and apoptosis [1–3]. One ubiquitous pathway of Ca^{2+} influx is store-operated Ca^{2+} entry (SOCE), or capacitative Ca^{2+} entry [4]. Although several biophysically distinct store-operated Ca^{2+} channels (SOCs) have been reported, the best characterized are the Ca^{2+} release-activated Ca^{2+} (CRAC) channels [4,5]. Recently, RNAi-based screening in *Drosophila* and mammalian cells identified two proteins that are essential components of store-operated Ca^{2+} influx: STIM1 [6,7] and Orai1 [8,9]. STIM1 senses the Ca^{2+} concentration in the endoplasmic reticulum (ER) with its EF-hand domain, while Orai1 is thought to form Ca^{2+} channels in the plasma membrane [10,11]. A recent study suggested that STIM1 interacts with Orai1 upon depletion of Ca^{2+} stores at sites of close apposition between the plasma membrane and the ER [12,13]. Based on these results, it has been suggested that Orai1 functions as the pore subunit of CRAC channels [10,11,14]. Although growing evidence shows the importance of Orai1, the biological interaction or relationship between Orai1 and STIM1 remains elusive [15,16]. Furthermore, except for the human Orais, other mammalian homologs have not been identified or characterized.

In this study, we identified three novel murine genes, Orai1, 2, and 3, which are expressed in a wide range of murine tissues. When co-expressed with STIM1, Orai1 produced a marked effect on SOCE. Chimeric protein expression analysis revealed the essential role of the proline- and arginine-rich Orai1 N-terminus in STIM1-mediated SOCE.

Abbreviations: CRAC, Ca^{2+} release-activated Ca^{2+} channel; ER, endoplasmic reticulum; SOC, store-operated Ca^{2+} channel; SOCE, store-operated Ca^{2+} entry.

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Materials and methods

RNA isolation and reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Total RNA was isolated from the whole brain of a C57/BL6 mouse using Isogen Reagent (Nippon Gene, Tokyo, Japan). Two micrograms of the template were reverse transcribed into cDNA using the SuperScript™ First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Murine Orai1, 2, and 3 cDNAs were obtained by RT-PCR using the following primers: mOrai1S (5′-ATGCATCCGGAGCCTGCCCC-3′), mOrai1AS (5′-GGCATAGTGGGTGCCCGGTG-3′), mOrai2S (5′-ATGAGTGCAGAGCTCAATGT-3′), mOrai2AS (5′-GGCATAGTG GGTGCCCGGTG-3′), mOrai3S (5′-ATGAAGGGCGGCGAGGGG GA-3′), and mOrai3AS (5′-CACAGCCTGCAGCTCCCCCT-3′). Each PCR product was cloned into pZero-2 (Invitrogen) and sequenced. Human Orai1, 2, and 3 were amplified over 35 cycles using custom primers (Fasmac, Kanagawa, Japan). To analyze the expression of each gene, murine Orai1, 2, and 3 were amplified using mixed primers for conserved sequences. The human and murine primer sequences were as follows: hOrai1S (5′-AGCAACGTGCACAATCTCAA-3′), hOrai1AS (5′-GTCT TATGGCTAACCAGTGA-3′), hOrai2S (5′-CGGCCATAAGGGCATG GATT-3′), hOrai2AS (5′-TTGTGGATGTTGCTCACGGC-3′), hOrai3S (5′-CTCTTCCTTGCTGAAGTTGT-3′), hOrai3AS (5′-CGATTCAGTT CCTCTAGTTC-3′), mOrai1, 2, and 3S (5′-CTTCGCCATGGTRGC SATGG-3′), and mOrai1, 2, and 3AS (5′-ACCARGGADCGGTAGAA RTG-3′) (R, A + G; S, C + G; D, A + G + T).

Cell culture and transient expression analysis. Human embryonic kidney 293T (HEK293T) cells were cultured in Dulbecco's modified Eagle's medium with 5% fetal bovine serum. For expression analysis, HEK293T cells were transfected with expression vectors (pIRESpuro3) carrying the murine Orai1, 2, and 3 genes cloned into the N-terminal portion of enhanced green fluorescent protein (EGFP; Qbiogene Inc., Carlsbad, CA, USA). Transient expression was achieved using Effectane Reagent (Qiagen, Valencia, CA, USA). Two chimeras were produced by swapping the second and third transmembrane domains of Orai1 and Orai2 at their *Sph*I sites. Two additional chimeras were made by replacing the cytoplasmic N-terminal domain of Orai1 with the corresponding segment from Orai2 using a unique restriction site (*Hin*dIII). PCR was used to create truncations, deletions, and chimeras within the N-terminus of ntOrai1–2 and short sequences of the Orai1 N-terminus (Fig. 3A). The mutants were assembled in pZero-2 and verified by sequencing.

Measurement of intracellular free Ca^{2+} ($[Ca^{2+}]_i$). HEK293T cells were loaded with the calcium indicator fura-2AM (5 μM; Dojindo Laboratories, Kumamoto, Japan) in Hepes-buffered saline. Changes in $[Ca^{2+}]_i$ in individual cells were measured using the Aquacosmos System (Hamamatsu Photonics, Hamamatsu, Japan) with band-pass filters for 340 and 380 nm. We calculated the $[Ca^{2+}]_i$ from fura-2 fluorescence ratios (F340/F380) using linear regression between adjacent points on a calibration curve generated by measuring F340/F380 in at least seven calibration solutions with $[Ca^{2+}]$ between 0 and 854 nM. For Mn^{2+}-quenching analysis, an excitation wavelength of 360 nm was used. SOC-mediated influx of Ca^{2+} following stimulation with 1 μM Thapsigargin during the change from Ca^{2+}-free conditions to 2 mM Ca^{2+} was measured as previously described [17].

Western blot analysis. Membrane fractions of transfected cells were separated by SDS–PAGE on 6% gels and electrophoretically transferred to polyvinylidene fluoride membranes. Nonspecific binding was blocked with 1% bovine serum albumin in Tris-buffered saline and 0.1% Tween 20 for 1 h at room temperature. The membranes were then incubated with anti-EGFP polyclonal antibodies (Cell Signaling, Santa Cruz, CA, USA) at 4 °C for 4 h. Finally, the membranes were incubated with anti-rabbit horseradish peroxidase-conjugated IgG for 1 h. An enhanced chemiluminescence system (Promega Corporation, Madison, WI, USA) was used to detect the bound antibody.

Immunoprecipitation. Immunoprecipitation was performed using a Protein G Immunoprecipitation Kit (Sigma, St. Louis, MO, USA). The cell pellet was re-suspended in 1.0 ml of lysis buffer and 1 mg/ml Complete Protease Inhibitor Cocktail (Roche, Basel, Switzerland). The cleared lysate was incubated with monoclonal antibodies directed against the DsRed-tagged protein, which was inserted at the N-terminus of the STIM1 cDNA construct. Protein G–Sepharose was then added and the samples were incubated for 16 h at 4 °C. The eluted products were subjected to electrophoresis followed by Western blotting using anti-EGFP polyclonal antibodies.

siRNA. Orai1-, 2-, and 3-specific siRNA sequences and one scrambled sequence (i.e., negative control) were designed using vector-NTI. A BLAST search (National Center for Biotechnology Information) found no other mRNA species containing a similar sequence. The sequences used were: Orai1 siRNA (5′-CGTGCACAATCTCAACTCG-3′), Orai2 siRNA (5′-CCTGAACTCCATCAGCGAG-3′), Orai3 siRNA (5′-GCTGTGAG CAACATCCACA-3′), and scrambled siRNA (5′-CACTGCATACTCA AGTCAC-3′). The siRNAs were commercially prepared by Nippon EGT (Toyama, Japan). Cells transfected with the siRNAs were prepared following the manufacturer's protocol for Effectane.

Statistical analysis. The data are presented as the means ± SEM. Differences were evaluated using unpaired Student's *t*-tests and were considered statistically significant at $p < 0.05$.

Results and discussion

Identification and cloning of the full-length murine Orai genes

We identified three murine sequences that showed significant homology with the human Orai1 gene from an EST database. The homology was confirmed by sequencing the full-length cDNAs of the corresponding clones. The predicted full-length proteins displayed approximately 85% overall homology with the human Orai1 protein. The genes were subsequently named *mOrai1*, *mOrai2*, and *mOrai3*, according to their human homologs (Fig. 1A). Alignments with the human Orai1 protein indicated that the mOrai1 (912 bp), mOrai2 (750 bp), and mOrai3 (870 bp) sequences encoded full-length proteins. The mOrai1, mOrai2, and mOrai3 proteins were predicted to contain 304, 250, and 290 amino acids (aa), respectively. The murine Orais have four to seven putative protein kinase C (PKC) phosphorylation sites (Fig. 1A, #). Orai1 has one putative protein kinase A (PKA) phosphorylation site (Fig. 1A, residue 34 in Orai1). All three murine sequences are predicted to contain four transmembrane domains, similar to their human homologs (Fig. 1A, red characters). Feske et al. [8] reported a mutation in exon 1 of human Orai1 in a hereditary severe combined immune deficiency syndrome family. In this family, T lymphocytes are defective in SOCE and CRAC channel function. The mutation leads to the replacement of an arginine residue by a tryptophan at position 91 of the protein [8]. This arginine residue is conserved in murine Orai1 at position 93 and in the other Orai members (Fig. 1A, $).

Tissue distribution of the murine Orai gene family

Expression of the murine *Orai1*, *2*, and *3* genes was examined by RT-PCR using degenerate DNA primers, which enabled us to amplify specific products corresponding to mOrai1 (494 bp), mOrai2 (440 bp), and mOrai3

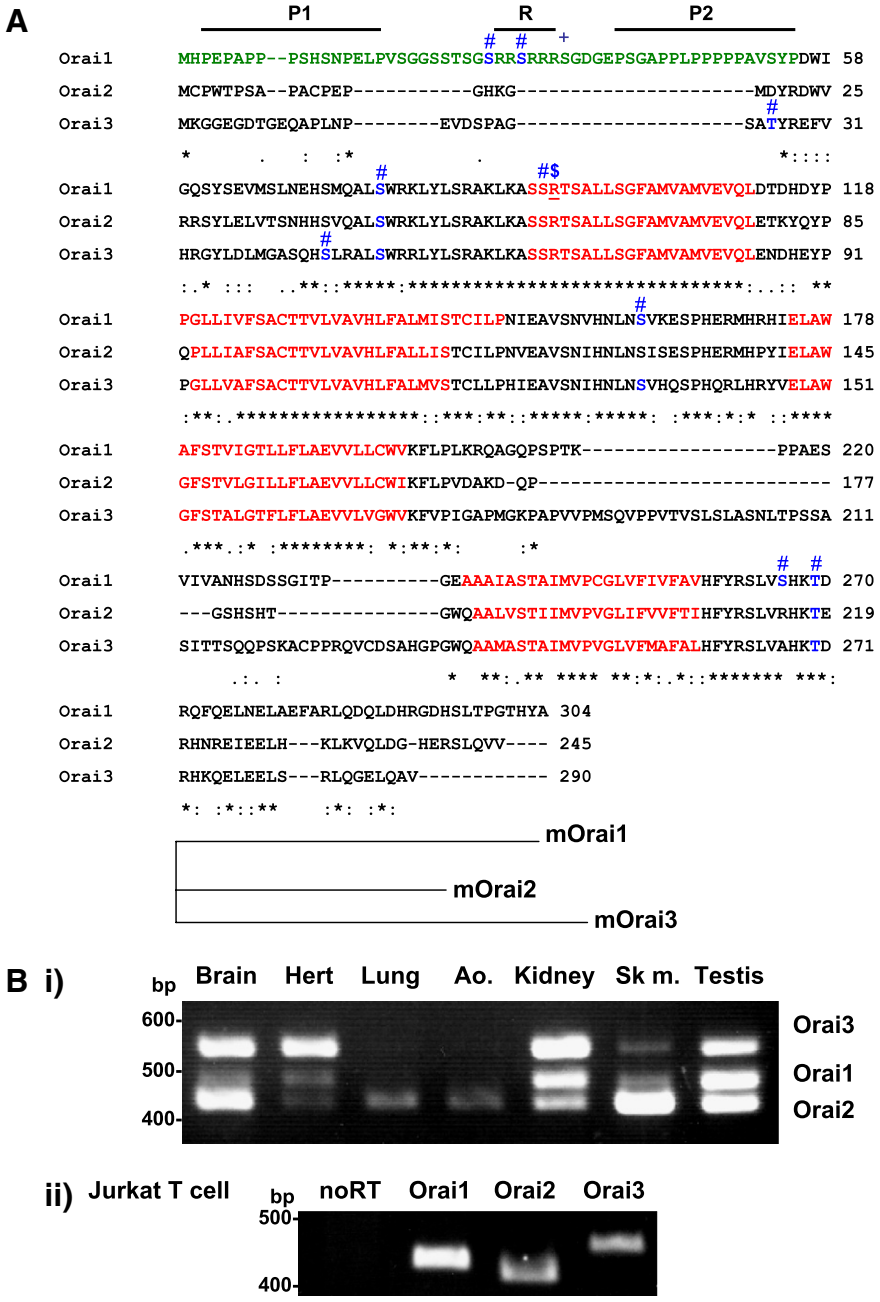

Fig. 1. Amino acid sequences of the mouse *Orai1*, *2*, and *3* genes and their tissue distributions. (A) Comparison of the derived amino acid sequences of the mouse *Orai1*, *2*, and *3* genes. The sequences are shown in the single-letter amino acid code. The amino acid positions are numbered on the right. The amino acids in the transmembrane segments are colored red. Asterisks below the sequences indicate three identical residues in the alignment, while dashes represent gaps in the sequences. The symbols above the amino acid sequences are as follows: putative PKC phosphorylation sites, #; putative PKA phosphorylation sites, +; arginine-rich domain, R; proline-rich domains, P1 and P2. The bottom panel shows a phylogenic tree of the murine Orais. (B) (i) Detection of murine Orai1 (494 bp), 2 (440 bp), and 3 (578 bp) transcripts in various tissues by RT-PCR. Ao, aorta; sk m, skeletal muscle. (ii) Human Orai1, 2, and 3 transcripts in Jurkat T cells by RT-PCR. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

(578 bp; Fig. 1B, i). Strong expression of the Orai genes was detected in the brain, kidney, and testis, while moderate expression was detected in the heart and skeletal muscle. Although the relative expression levels differed for each tissue, the *mOrai* genes were expressed in a wide range of murine tissues. We also examined the expression of Orai1, 2, and 3 in Jurkat T lymphocytes (Fig. 1B, ii). All three members of the Orai family were expressed in this cell line.

Co-expression of Orai1 and STIM1 causes an increase in SOCE

To study the function of the Orai genes via heterologous expression, their cDNAs were subcloned into expression vectors to form EGFP-fusion proteins. Using anti-EGFP polyclonal antibodies, roughly equal amounts of the Orai1, 2, and 3 proteins were detected in membrane fractions of

transiently transfected HEK293T cells (Fig. 2A, left panel). The apparent molecular mass of the recombinant proteins was 60 kDa, which is in good agreement with their predicted molecular masses (Orai1, 33.1 kDa; Orai2, 27.7 kDa; Orai3, 31.4 kDa; and EGFP, 26.9 kDa).

We then measured SOCE in the Orai-overexpressing cells (Fig. 2A, right panel). Expression of Orai1, 2, or 3 alone had no significant effect on SOCE, which is consistent with previous reports showing no additional increase in SOCE with Orai overexpression, although Orai1 has been identified as a plasma membrane protein essential for SOCE [8–12,18,19].

Mercer et al. [20] reported a synergistic effect of Orai1 and STIM1 on SOCE; therefore, we next studied the effect of expressing STIM1 with Orai1 (Fig. 2B and C). Expression of STIM1 alone did not affect SOCE (data not

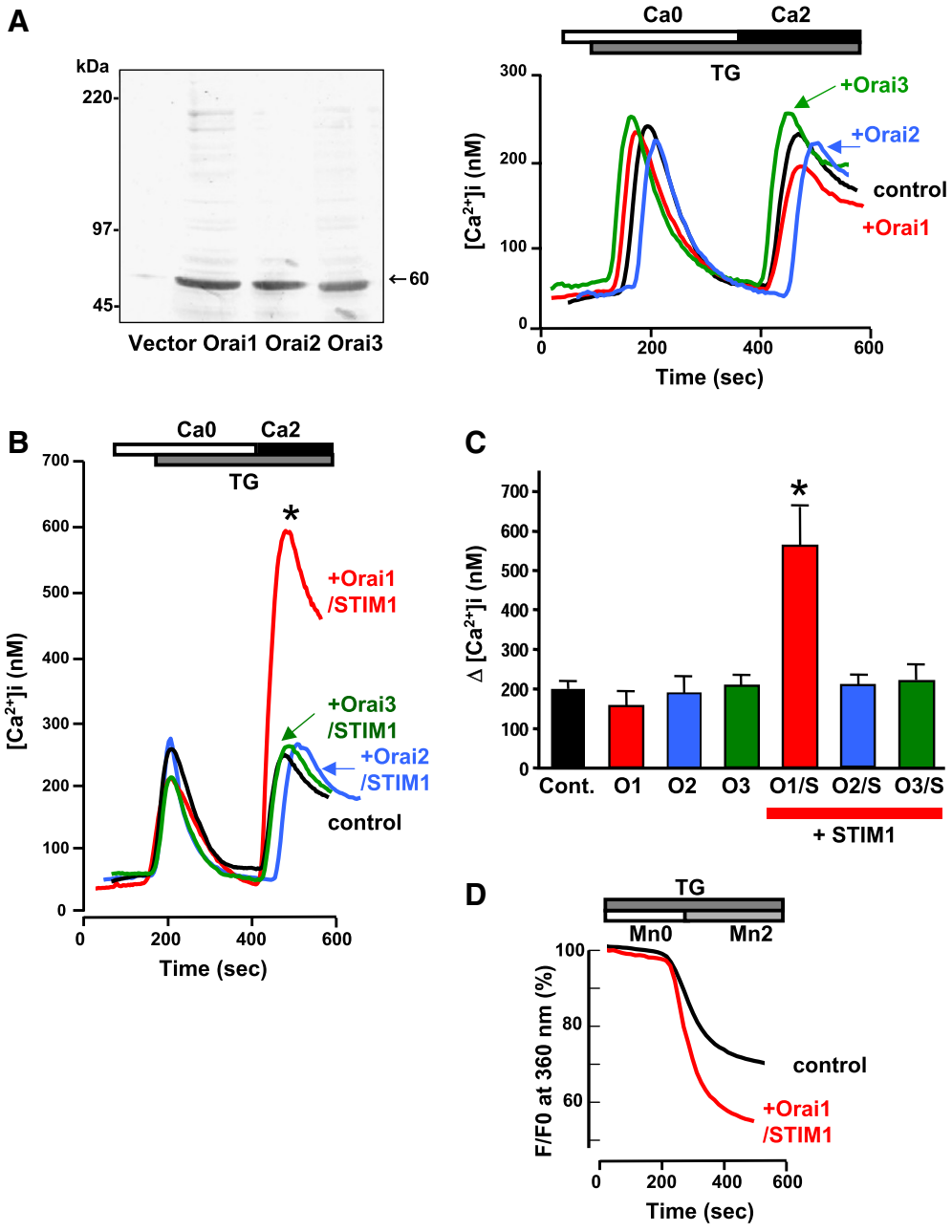

Fig. 2. SOCE in cells expressing the Orai gene family and STIM1. (A) Left panel, immunodetection of Orai protein. Western blots of membrane proteins from EGFP-tagged Orai1-, 2-, and 3-transfected HEK293T cells are shown. Right panel, overexpression of Orai1, 2, and 3 failed to increase SOCE. The results are based on 51 Orai1-, 49 Orai2-, and 46 Orai3-transfected HEK293T cells. (B) Co-expression of Orai1 and STIM1 significantly increased SOCE. The results are based on 32 Orai1-, 40 Orai2-, and 36 Orai3-transfected HEK293T cells. (C) Statistical analysis of SOCE in control cells and cells co-expressing Orai1, 2, or 3 with STIM1. The statistical significance of the differences between the control cells and the HEK293T cells expressing Orai1 is indicated by $^{*}p < 0.01$. (D) Mn^{2+}-quenching analysis of cells transfected with Orai1 and STIM1. Orai1/STIM1-co-expressing cells showed an accelerated decrease in fura-2 fluorescence compared to control cells.

shown), but co-expression of Orai1 and STIM1 resulted in a marked increase in SOCE (Fig. 2B and C). In particular, the initial rise in Ca^{2+} in cells overexpressing Orai1 and STIM1 was much greater than in control cells. On the other hand, co-expression of Orai2 or 3 with STIM1 produced only a marginal effect on SOCE (Fig. 2B and C). These findings are inconsistent with a previous study [20]. There are several possible explanations for this discrepancy, including racial differences in humans or mice or differences in the amount of membrane expression between the molecules. As shown in the left panel of Fig. 2A, the level of expression in each experiment was roughly equal; therefore, the latter possibility is less likely, although the subcellular localization of the overexpressed Orai molecules was not determined.

To confirm that the increase in $[Ca^{2+}]_i$ following Thapsigargin stimulation was due to a Ca^{2+} influx, we performed a Mn^{2+}-quenching assay. Mn^{2+} entry was significantly greater in the cells co-expressing Orai1 and STIM1 than in the control cells (Fig. 2D). Taken together, these results indicate that the combined expression of mOrai1, but not the other Orais, with STIM1 amplifies SOC function and enhances SOCE, suggesting a specific role for Orai1 in SOC function.

Essential role of the Orai1 N-terminus in SOCE

As only Orai1 significantly enhanced SOCE with STIM1, we compared the predicted amino acid sequences of the murine Orais. Although each of the predicted Orai proteins has four transmembrane domains (Fig. 1A, red characters), Orai1 has unique features in its cytosolic N-terminus, with two proline-rich domains and one arginine-rich domain (Fig. 1A, green characters).

To clarify whether the Orai1 N-terminus is essential for SOCE, we made two chimeric Orai1 and Orai2 constructs (Orai1–2 and Orai2–1) by swapping their transmembrane domains at their *Sph*I sites (Fig. 3A, iii and iv). Fig. 3B shows the SOCE mediated by these two chimeras. Co-expression of the Orai1–2 chimera containing the Orai1 N-terminus and STIM1 increased SOCE to nearly the level seen by co-expression of Orai1 and STIM1, while co-expression of Orai2–1 and STIM1 produced only a marginal increase in SOCE (Fig. 3B). We then made additional chimeras focusing on the cytoplasmic N-terminal domain of Orai1. The first construct, named ntOrai2–1, contained the cytoplasmic N-terminal portion of Orai2 followed by the transmembrane sequences from Orai1 (Fig. 3A, v). The second construct, named ntOrai1–2, contained the cytoplasmic N-terminal portion of Orai1 followed by the transmembrane sequences from Orai2 (Fig. 3A, vi). The third construct, named ntOrai1, contained only the cytoplasmic sequences from the Orai1 N-terminus without a transmembrane domain. This third construct was expected to affect SOC in a dominant-negative manner (Fig. 3A, vii). As expected, ntOrai1–2 significantly increased SOCE compared to the control (Fig. 3C, asterisk), while co-expression of the ntOrai2–1 chimera with STIM1 produced only a small effect on SOCE (Fig. 3C). In contrast, ntOrai1 decreased SOCE below the control level (Fig. 3C, arrow), suggesting a dominant-negative effect. These results strongly suggest that the Orai1 N-terminus is important for STIM1-mediated activation of SOCE. Fig. 3D shows the results of a statistical analysis of our SOCE experiments.

Which region of the Orai1 N-terminus participates in SOCE? The N-terminal cytosolic domain of Orai1 has several unique features, including five positively charged arginine residues (Fig. 1A, R) and two proline-rich domains (Fig. 1A, P1 and P2). Huang et al. [21] showed that the STIM1 C-terminus, especially the ERM domain, activates native SOC. ERM domains bind with positively charged amino acid clusters in the juxta-membrane cytoplasmic domains of CD44, CD43, and ICAM-2 [22]. Moreover, proline-rich domains serve as binding sites in various types of proteins. For example, the proline-rich domain in group 1 metabotropic glutamate receptors mediates the interaction with Homer proteins [23].

To further evaluate the structural determinants of the STIM1–Orai interaction, DsRed-tagged STIM1 and EGFP-tagged Orai constructs were co-expressed in HEK293T cells, and the interaction between STIM1 and the Orais was examined by immunoprecipitation using anti-DsRed antibody. The immunoprecipitated products were detected by Western blotting with anti-EGFP antibody. As shown in Fig. 3E, STIM1 co-immunoprecipitated not only with Orai1 but also with the Orai1–2 chimera. In contrast, the Orai2–1 chimera (Fig. 3E) and Orai2 showed only weak interactions with STIM1 (data not shown). These results emphasize the importance of the Orai1 N-terminus in its interaction with STIM1. Therefore, upon store depletion, activated STIM1 probably binds to the N-terminal cytosolic domain of Orai1 to elicit SOCE. Further studies will be needed to identify the underlying mechanism and amino acids involved in this physiologically important event.

Knockdown effect of Orais in HEK293T cells

Our overexpression analyses revealed a fair amount of background SOCE in HEK293T cells (Figs. 2B and 3B, C). To clarify which Orai molecule is responsible for the background SOCE, we used RNA-interference against Orai1, 2, and 3 in HEK293T cells. First, we examined whether each siRNA affected the expression of its corresponding Orai. Endogenous expression of Orai1 was clearly suppressed by siRNA against Orai1 (Fig. 4A). The suppression of SOCE by the Orai1 siRNA could be titrated as a function of the siRNA concentration (Fig. 4B, red lines), suggesting that most of the background SOCE was due to channels formed by endogenous Orai1. In contrast, Orai2 siRNA had no effect on SOCE (Fig. 4C, blue line). Unexpectedly, Orai3 siRNA significantly reduced SOCE (Fig. 4C, green line), suggesting that

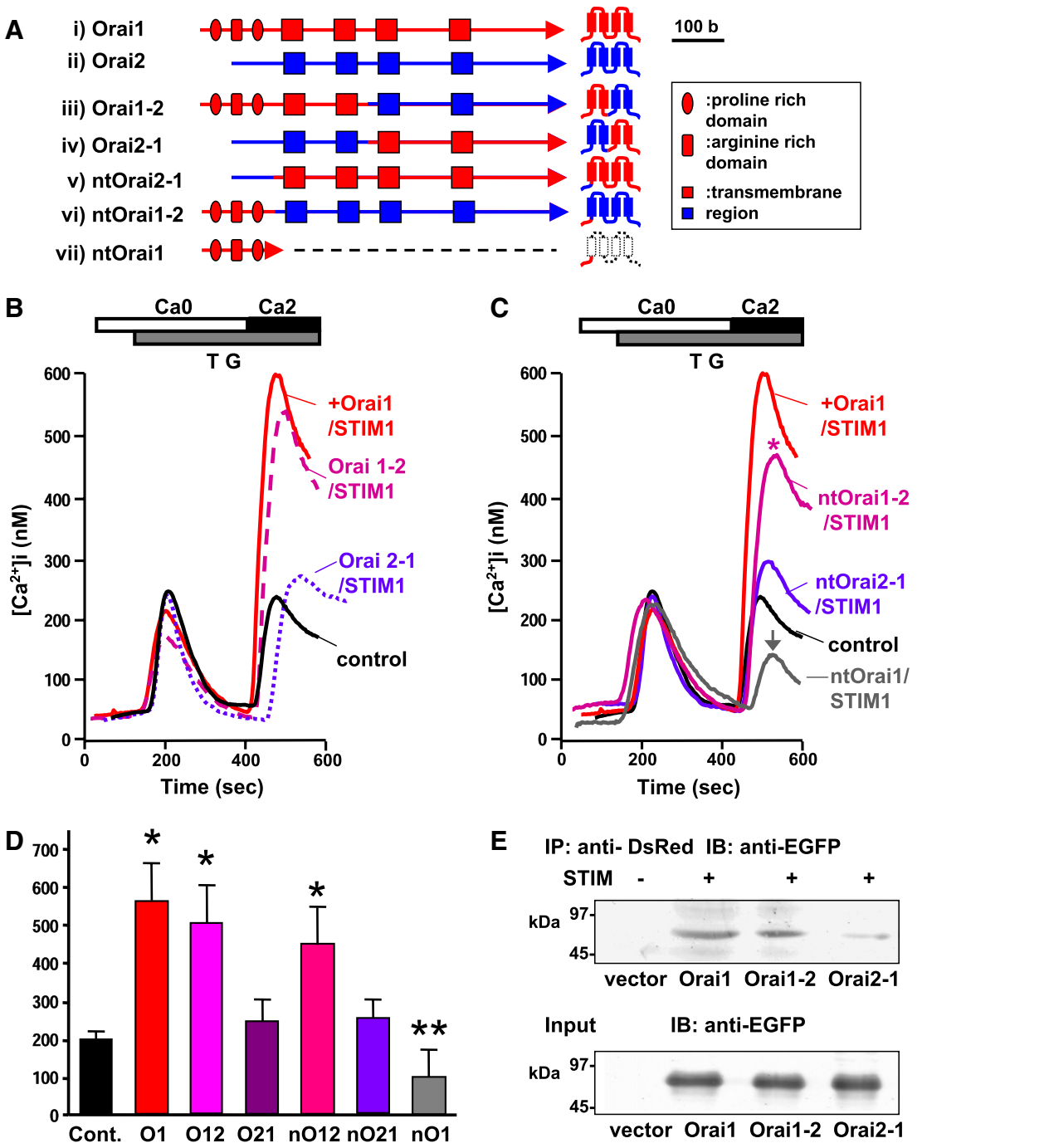

Fig. 3. Effect of chimeric Orai1/Orai2 on SOCE. (A) Schematic figures of the chimeras with red for Orai1 and blue for Orai2. (B,C) HEK293T cells cotransfected with STIM1 and Orai1 or the Orai1–Orai2 chimeras were used to measure SOCE. (D) Statistical analysis of SOCE in control cells and cells co-expressing the Orai1–Orai2 chimera with STIM1. The statistical significance of the differences between the control cells is indicated by $^{*}p < 0.01$. (E) STIM1 coimmunoprecipitates with Orai1 and the Orai1–2 chimera in HEK293T cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

this molecule participates in SOC formation. The suppression of SOCE by Orai3 siRNA is inconsistent with our overexpression analysis, which indicated no significant effect of STIM1 and Orai3 co-expression on SOCE (Figs. 2B and C). The exact reason for these discrepant results is unclear, but heteromultimeric channel formation by the Orai molecules is one possible explanation [24,25]. Our preliminary results indicated that the mOrais are capable of binding with each other to form homo- and hetero-multimers. Therefore, overexpression of Orai3 alone may lead to the formation of homomultimers without Orai1, and homomultimeric Orai3 channels may not function as SOCs. Nevertheless, future studies will be needed to clarify Orai-related SOC channel assembly.

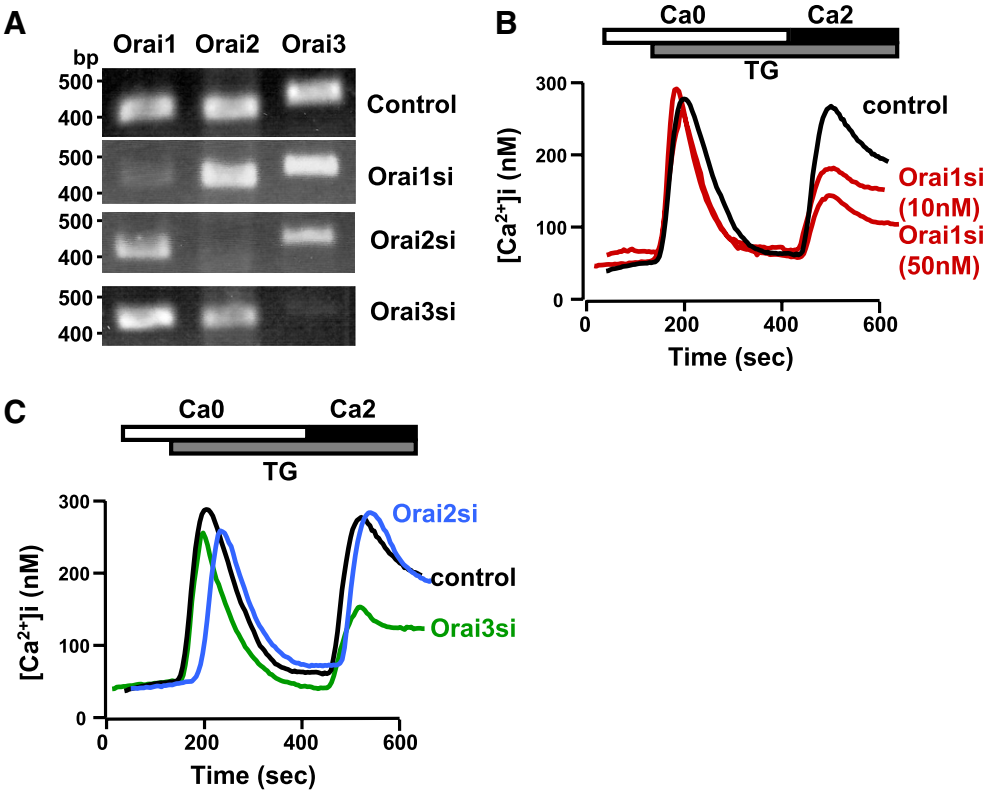

Fig. 4. Knockdown effect of Orai1, 2, and 3 on SOCE. (A) Detection of Orai1, 2, and 3 transcripts in HEK293T cells. siRNA knockdown effect of Orais. Orai1, 2, and 3 siRNA (50 nM) selectively decreased the expression of each target mRNA. (B) Dose-dependent suppression of SOCE by Orai1–siRNA (10 and 50 nM). (C) Effect of Oari2 siRNA and Orai3 siRNA on SOCE. Orai3 siRNA suppressed SOCE, while no effect was observed with Orai2 siRNA. Changes in the $[Ca^{2+}]_i$ in individual cells were measured with fura-2, a calcium indicator. The results are based on 40 Orai1-, 38 Orai2-, and 49 Orai3-siRNA-treated HEK293T cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

In summary, we have identified the first murine homologs of Orai1, 2, and 3. Co-expression of Orai1 and STIM1 markedly enhanced SOCE, while Orai2 and 3 showed no such effect. In chimeric cDNA expression analysis, the cytoplasmic N-terminal portion of Orai1, which has two proline-rich domains and an arginine-rich domain, was found to be essential for the enhancement of SOCE by the co-expression of Orai1 and STIM1.

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References

[1] M.J. Berridge, Inositol trisphosphate and calcium signaling, Nature 361 (1993) 315–325.
[2] E. Carafoli, Calcium signalling: a tale for all seasons, Proc. Natl. Acad. Sci. USA 99 (2002) 1115–1122.
[3] L. Lipskaia, A.M. Lompre, Alteration in temporal kinetics of Ca^{2+} signaling and control of growth and proliferation, Biol. Cell 96 (2004) 55–68.
[4] A.B. Parekh, J.W. Putney Jr., Store-operated calcium channels, Physiol. Rev. 85 (2005) 757–810.
[5] M. Hoth, R. Penner, Depletion of intracellular calcium stores activates a calcium current in mast cells, Nature 355 (1992) 353–356.
[6] J. Roos, P.J. DiGregorio, A.V. Yeromin, K. Ohlsen, M. Lioudyno, S. Zhang, O. Safrina, J.A. Kozak, S.L. Wagner, M.D. Cahalan, G. Velicelebi, K.A. Stauderman, STIM1, an essential and conserved component of store-operated Ca^{2+} channel function, J. Cell Biol. 169 (2005) 169435–169445.
[7] J. Liou, M.L. Kim, W.D. Heo, J.T. Jones, J.W. Myers, J.E. Ferrell Jr., T. Meyer, STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx, Curr. Biol. 15 (2005) 1235–1241.
[8] S. Feske, Y. Gwack, M. Prakriya, S. Srikanth, S.H. Puppel, B. Tanasa, P.G. Hogan, R.S. Lewis, M. Daly, A. Rao, A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function, Nature 441 (2006) 179–185.
[9] M. Vig, C. Peinelt, A. Beck, D.L. Koomoa, D. Rabah, M. Koblan-Huberson, S. Kraft, H. Turner, A. Fleig, R. Penner, J.P. Kinet, CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry, Science 312 (2006) 1220–1223.
[10] M. Prakriya, S. Feske, Y. Gwack, S. Srikanth, A. Rao, P.G. Hogan, Orai1 is an essential pore subunit of the CRAC channel, Nature 443 (2006) 230–233.
[11] A.V. Yeromin, S.L. Zhang, W. Jiang, Y. Yu, O. Safrina, M.D. Cahalan, Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai, Nature 443 (2006) 226–229.
[12] M.M. Wu, J. Buchanan, R.M. Luik, R.S. Lewis, Ca^{2+} store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane, J. Cell Biol. 174 (2006) 803–813.
[13] R.M. Luik, M.M. Wu, J. Buchanan, R.S. Lewis, The elementary unit of store-operated Ca^{2+} entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions, J. Cell Biol. 174 (2006) 815–825.

[14] M. Vig, A. Beck, J.M. Billingsley, A. Lis, S. Parvez, C. Peinelt, D.L. Koomoa, J. Soboloff, D.L. Gill, A. Fleig, J.P. Kinet, R. Penner, CRACM1 multimers form the ion-selective pore of the CRAC channel, Curr. Biol. 16 (2006) 2073–2079.

[15] P. Xu, J. Lu, Z. Li, X. Yu, L. Chen, T. Xu, Aggregation of STIM1 underneath the plasma membrane induces clustering of Orai1, Biochem. Biophys. Res. Commun. 350 (2006) 969–976.

[16] H.L. Ong, K.T. Cheng, X. Li, B.C. Bandyopadhyay, B.C. Paria, J. Soboloff, B. Pani, Y. Gwack, S. Srikanth, B.B. Singh, D. Gill, I.S. Ambudkar, Dynamic assembly of TRPC1/STIM1/Orai1 ternary complex is involved in store operated calcium influx: evidence for similarities in SOC and CRAC channel components, J. Biol. Chem. (2007), [Epub ahead of print].

[17] M. Murakami, F. Xu, I. Miyoshi, E. Sato, K. Ono, T. Iijima, Identification and characterization of the murine TRPM4 channel, Biochem. Biophys. Res. Commun. 307 (2003) 522–528.

[18] C. Peinelt, M. Vig, D.L. Koomoa, A. Beck, M.J. Nadler, M. Koblan-Huberson, A. Lis, A. Fleig, R. Penner, J.P. Kinet, Amplification of CRAC current by STIM1 and CRACM1 (Orai1), Nat. Cell Biol. 8 (2006) 771–773.

[19] J. Soboloff, M.A. Spassova, X.D. Tang, T. Hewavitharana, W. Xu, D.L. Gill, Orai1 and STIM reconstitute store-operated calcium channel function, J. Biol. Chem. 281 (2006) 20661–20665.

[20] J.C. Mercer, W.I. Dehaven, J.T. Smyth, B. Wedel, R.R. Boyles, G.S. Bird, J.W. Putney Jr., Large store-operated calcium selective currents due to co-expression of Orai1 or Orai2 with the intracellular calcium sensor, Stim1, J. Biol. Chem. 281 (2006) 24979–24990.

[21] G.N. Huang, W. Zeng, J.Y. Kim, J.P. Yuan, L. Han, S. Muallem, P.F. Worley, STIM1 carboxyl-terminus activates native SOC, I (crac) and TRPC1 channels, Nat. Cell Biol. 8 (2006) 1003–1010.

[22] S. Yonemura, M. Hirao, Y. Doi, N. Takahashi, T. Kondo, S. Tsukita, Ezrin/radixin/moesin (ERM) proteins bind to a positively charged amino acid cluster in the juxta-membrane cytoplasmic domain of CD44, CD43, and ICAM-2, J. Cell Biol. 140 (1998) 885–895.

[23] J.C. Tu, B. Xiao, J.P. Yuan, A.A. Lanahan, K. Leoffert, M. Li, D.J. Linden, P.F. Worley, Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors, Neuron 4 (1998) 717–726.

[24] M. Goel, W.G. Sinkins, W.P. Schilling, Selective association of TRPC channel subunits in rat brain synaptosomes, J. Biol. Chem. 277 (2002) 48303–48310.

[25] T. Hofmann, M. Schaefer, G. Schultz, T. Gudermann, Subunit composition of mammalian transient receptor potential channels in living cells, Proc. Natl. Acad. Sci. USA 99 (2002) 7461–7466.