Microfilament-associated Protein 7 Increases the Membrane Expression of Transient Receptor Potential Vanilloid 4 (TRPV4)*

Makoto Suzuki‡, Atsushi Hirao, and Atsuko Mizuno
From the Department of Pharmacology, Jichi Medical School 3311-1, Yakushiji, Minamikawachi, Tochigi, 329-0049, Japan

The molecular mechanism of the transmission of changes in the shape of the cell surface to ion channels remains obscure. Ca2+ influx induced by cell deformation is inhibited by actin-freezing reagents, suggesting that the actin microfilament couples with an ion channel. Transient receptor potential vanilloid 4 (TRPV4) is a candidate in the calcium-permeable, swelling-activated mechanosensitive channel in heterogeneously expressed cells. To investigate the mechanosensitive molecular complex, we found that microtubule-associated protein 7 (MAP7) is the mouse TRPV4 C-terminal binding protein. MAP7 was coimmunoprecipitated with TRPV4. The results of a pull-down assay demonstrated that the alignment of amino acids 798–809 of TRPV4 was important in this interaction. TRPV4 and MAP7 colocalized in the lung and kidney. The coexpression of these two molecules resulted in the redistribution of TRPV4 toward the membrane and increased its functional expression. The alignment of amino acids 798–809 of TRPV4 was also important in the functional expression. The activated current was abolished by actin-freezing but not by microtubule-freezing reagents. We therefore believe that MAP7 may enhance the membrane expression of TRPV4 and possibly link cytoskeletal microfilaments.

Cells experience a wide variety of mechanical stresses and require a sensitive mechanism to regulate the deformity of the cell surface. The molecular structure that transmits cell surface deformities to ion channels (the mechanosensitive ion channel) has been shown by a number of findings to be in bacterial MscL (1), yeast Mid1 (2), mammalian DEG/MEC (3), and TREK (4) and their derivatives (5). These channels are monovalent cation channels that are permeable to sodium or potassium. However, the molecular structure of the mammalian calcium-permeable mechanosensitive channel remains obscure.

One of characteristics of this channel is its relation to the cytoskeleton. The current of MscL and TREK are increased or unaffected by the disruption of actin microfilaments (5), whereas the current of most calcium-permeable mechanosensitive channels is abolished by this manipulation, which has been recognized in neurons (6, 7), leukocytes (8), the gallbladder (9), hepatocytes (10), renal epithelia (11), and muscle (12, 13). Therefore, the relationship of the calcium-permeable ion channels to the cytoskeleton might differ from that of MscL and TREK.

On the other hand, the molecular structure of calcium-permeable mechanosensitive ion channels was not clarified. Great progress in understanding the molecular candidate was made with the cloning and identification of the vanilloid receptor (TRPV1), an ion channel of the transient receptor potential (TRP) ion channel family, which has been proposed to function at the transition step in the nociceptive pathway (14). Although mice lacking TRPV1 show a marked behavioral response to noxious heat, capsaicin, and acid, they do not show the difference in mechanical nociception (15). Mechanical nociception was suggested to be processed by other related genes. TRPV4 (SAC1 (16), TRP12 (17), OTRPC4 (18), VR-OAC (19), or VR2 (20, 21) is similar to TRPV1 and is reported to be a swelling-activated cation channel, a candidate for the mammalian mechano-gated calcium-permeable cation channel. The mice lacking this gene show a defect in the high-threshold mechanosensation, pressure, but maintain the low-threshold innocuous touch sensation (22). This result is supported in rat by using antisense methodology (23) or is proposed in a Drosophila mutant (24).

We had expressed TRPV4 in CHO cells but found a poor activity by swelling. We also found that TRPV4 was significantly activated by an inflation of the cell (22). We next expressed an N-terminal ankyrin or a C-terminal hydrophilic deletion mutant of TRPV4, resulting in a lack of activation. These findings brought us to a hypothesis that more proteins are needed for TRPV4 to be expressed on membrane and to be sensitive to cell deformation only when transfected in these cells. We, therefore, performed a binding study to search for a protein to reconstitute better mechanosensitive expression of TRPV4.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Screens—Approximately two million clones were screened from a mouse kidney cDNA library constructed in the Gal4 activation domain carrying vector pACTII (Clontech). The library was screened with bait that encoded the predicted intracellular domain of the mouse TRPV4 cloned in the Gal4 DNA-binding domain vector pAS2–1 (Clontech). The plasmids were transformed into yeast strain Y190, and transformants were selected on triple dropout media (Leu/His/Trp that contained 30 mM 3-amino-triazole) and assayed for β-galactosidase activity. Positive clones were cotransfected into yeast with either the bait vector or the pACTII vector backbone to confirm interactions. The activity of β-galactosidase was measured using the chlorophenol red-β-D-galactopyranoside assay according to the manufacturer’s protocol (Clontech). pACTII vectors with higher activity were recloned into DH5α and amplified for identification.

Antibodies—A TRPV4-specific antibody (anti-TRPV4) was raised in a
rabbit against a C terminus, as reported elsewhere (21, 22). A MAP7-specific rabbit polyclonal antibody was raised against a synthetic peptide corresponding to eight C- or N-terminal amino acids (C-terminal TQQTAEVI and N-terminal MDQAKSAE). For coprecipitation and localization, an N-terminal antibody was used.

**In Vitro Cell-free Binding Assay**—The STP3™ (Novagen) was used to generate [35S]methionine-labeled MAP7 protein. TRPV4-His₅ fusion proteins were expressed in HEK293 cells and visualized using anti-TRPV4 antibody and detected by anti-His₅ antibody (21, 22). A MAP7 clone containing a penta-histidine (His5) at the C-terminal end or with FLAG at the N-terminal end. For the pull-down assay, extracts of subconfluent HEK293 cells (in a 60-mm dish) were washed three times in PBS and then lysed at 4 °C. The elution was boiled in sample buffer (2% SDS, 10% glycerol, and 62 mM Tris (pH 6.8), and bound proteins were resolved on SDS-PAGE.

**Immunoprecipitation and Affinity Purification (Pull-down Assay)**—Renal extracts were produced by homogenization in 300 mM sucrose, 25 mM imidazole, 1 mM EDTA, and protease inhibitors. TRPV4/MAP7 complexes were immunoprecipitated by using anti-TRPV4 coupled to Protein A-Sepharose. The precipitate and the supernatants were detected using the anti-FLAG antibody (anti-FLAG M2; Stratagene).

**Immunohistochemistry**—Tissues were removed from a C57BL/6 male and fixed in 4% paraformaldehyde. Cultured cells were fixed in 4% paraformaldehyde and then permeabilized by incubation with 0.1% Triton X-100, 5 mM EDTA, and protease inhibitors. Cell extracts were incubated with 100 μl of streptavidin-agarose (Pierce) at 4 °C overnight. The precipitated proteins were visualized by immunoblotting with anti-CD4 (Dako) or anti-His₅ antibody.

**RT-PCR**—Reverse transcriptase polymerase chain reaction (RT-PCR) was used to detect the interaction, TRPV4, its deletions, and mouse MAP7 cDNA were recloned into pCDNA3.1/V5-His (Intty) or pCMV-Tag2 (Stratagene), which fused them with penta-histidine (His₅) at the C-terminal end or with FLAG at the N-terminal end. For the pull-down assay, extracts of subconfluent HEK293 cells (in a 60-mm dish) were lysed by gentle sonication in 50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole, 0.05% Tween 20 (pH 8.0), and protease inhibitors. Ni-NTA magnetic beads (Qiagen) were used to pull the complex, which was washed in 50 mM NaH₂PO₄, 300 mM NaCl, and 20 mM imidazole (pH 8.0) and eluted in 50 mM NaH₂PO₄, 300 mM NaCl, 250 mM imidazole, and 20% glycerol (pH 8.0). Precipitates and supernatants were detected using the anti-FLAG antibody (anti-FLAG M2; Stratagene).

**RESULTS**

**Cloning of Binding Protein to TRPV4**—Using the C- or N-terminal intracellular domain of TRPV4 (the first 280 and the last 89 amino acids) as bait in a yeast two-hybrid screen of an adult mouse kidney cDNA library, we isolated one clone to the N terminus and 48 clones to the C terminus displaying homology to MAP7 (equal to E-MAP-115). To confirm the yeast two-hybrid results, we examined the interaction of TRPV4 with MAP7 using in vitro and in vivo methods. For in vitro cell-free binding, TRPV4 was expressed as a fusion protein with His₅, and MAP7 was produced and labeled with [35S]methionine by

---

2 The GenBank™ accession number of MAP7 is AB098611, which is identical to NM008635 or AA242501. Mouse TRPV4 has been reported to generate [35S]-labeled MAP7 protein for 1 hour at 45 °C and then washed three times with a buffer (50 mM NaH₂PO₄, 300 mM NaCl, and 20 mM imidazole). The solution was boiled in sample buffer (2% SDS, 10% glycerol, and 62 mM Tris (pH 6.8), and bound proteins were separated on SDS-PAGE.

**Electrophysiology**—TRPV4 and MAP7 were ligated to pCMVSPORT1 and transfected into CHO cells with pEGFPN1 (Clontech) as the marker of the expression. Patch clamp recordings were carried out after 2 days according to methods described elsewhere (25). Currents were recorded at room temperature with an EPC-9 patch clamp amplifier (HEKA). Capacitance of the individual cell (density). The bath solution contained 140 mM NaCl, 1.0 mM MgCl₂, and 3 mM HEPES (300 mosmol). The hypo-osmotic solution (200 mosmol) was produced by the addition of water. The whole-cell patch pipette contained a filtered solution of 150 mM CsCl, 1.0 mM MgCl₂, and 1 mM ATP (pH 7.2; pCa 7.6).

---

2 The GenBank™ accession number of MAP7 is AB098611, which is identical to NM008635 or AA242501. Mouse TRPV4 has been reported to generate [35S]-labeled MAP7 protein for 1 hour at 45 °C and then washed three times with a buffer (50 mM NaH₂PO₄, 300 mM NaCl, and 20 mM imidazole). The solution was boiled in sample buffer (2% SDS, 10% glycerol, and 62 mM Tris (pH 6.8), and bound proteins were separated on SDS-PAGE.

**Electrophysiology**—TRPV4 and MAP7 were ligated to pCMVSPORT1 and transfected into CHO cells with pEGFPN1 (Clontech) as the marker of the expression. Patch clamp recordings were carried out after 2 days according to methods described elsewhere (25). Currents were recorded at room temperature with an EPC-9 patch clamp amplifier (HEKA). Capacitance of the individual cell (density). The bath solution contained 140 mM NaCl, 1.0 mM MgCl₂, and 3 mM HEPES (300 mosmol). The hypo-osmotic solution (200 mosmol) was produced by the addition of water. The whole-cell patch pipette contained a filtered solution of 150 mM CsCl, 1.0 mM MgCl₂, and 1 mM ATP (pH 7.2; pCa 7.6).
in vitro transcription and translation. TRPV4-His<sub>6</sub> fusion proteins immobilized on beads were incubated with [³⁵S]methionine-labeled MAP7 protein. MAP7 bound specifically to immobilized TRPV4-His<sub>6</sub> (Fig. 1a) but not to His<sub>6</sub> alone, which indicates that they interacted in vitro.

We carried out an immunoprecipitation experiment to test whether TRPV4 and MAP7 interact in vivo. Anti-MAP7 and anti-TRPV4 recognized major bands of 85 and 98 kDa, respectively, in the kidney. Renal extracts were immunoprecipitated using anti-TRPV4 and then probed with anti-MAP7. The MAP7 was precipitated with anti-TRPV4 but not with control sera or with an excess of antigen (Fig. 1b). Thus, the anti-MAP7 antibody specifically detected the endogenous MAP7. MAP7 and TRPV4 interacted in vitro and in vivo.

Interaction was also analyzed by affinity purification using a pull-down assay in which MAP7 was fused with the FLAG protein and expressed in HEK293 cells. The binding site of TRPV4 on MAP7 was also analyzed using deletion mutants. Amino acids downstream of amino acid 809 of TRPV4 were required for the pull-down. Thus, amino acids 785–808 were found to be critical for the binding of MAP7 (Fig. 1c). The amino acid alignment appeared well conserved among TRPV1–6 family members.

Localization of MAP7—The expression of MAP7 mRNA was examined using RT-PCR (Fig. 2a). The distribution of MAP7 mRNA in tissue was similar to that of TRPV4, i.e. it occurred in the lung, kidney, brain, and fat, but it was also detected in the liver and the heart, where TRPV4 is absent (data not shown). This distribution of TRPV4 is similar to that reported by others (17–20). We next compared the distribution of MAP7 and TRPV4 in sections of mouse tissues. Colocalization was suggested by results in continuous sections in ependymal cells in the choroid plexus, the luminal membrane of renal tubule, and the basolateral membrane of alveolar epithelial cells (Fig. 2b). Colocalization was evident in bronchial (Fig. 2b, yellow) and renal cortical tubular cells (Fig. 2c). The bright staining in glomeruli in the renal cortex was an artifact caused by our method. Colocalization was less marked in the other cells, such as some neurons, which may be a reflection that MAP7 does not bind to TRPV4 or, alternatively, that MAP7 may perform a different function in this region.

Subcellular Localization of TRPV4 by MAP7—Before the coexpression study, we searched cultured cells without the endogenous interactive molecules. MAP7 mRNA was widely detected in excitable neuronal or vascular cells as well as in epithelial cells, including HEK293, but not in CHO cells (Fig. 3a).

To investigate the possibility that MAP7 controls the subcellular localization of TRPV4, we expressed TRPV4 and MAP7 in CHO cells. Representative cells with the TRPV4 antibody (Fig. 3b, left panel, green) or MAP7 (Fig. 3b, center panel, red) are shown along with the merge image (Fig. 3b, right panel). There are two cells in each panel of Fig. 3b. TRPV4 alone was expressed in the cell on the right in each panel of Fig. 3b, whereas TRPV4 and MAP7 were coexpressed in the cell shown on the left in each panel of Fig. 3b. The expression the TRPV4 by itself produced diffuse surface staining (Fig. 3b, green, cell on the right in each panel). Coexpression of TRPV4 with MAP7 (Fig. 3b, red, cell on the left in each panel) resulted in a remarkable change in the distribution of TRPV4. TRPV4, co-localized with MAP7, was brought toward the membrane surface (white in the merged image, Fig. 3b).

Fig. 2. Localization of MAP7 and TRPV4 in mouse tissues. a, RT-PCR-based localization of MAP7 mRNA. Mouse tissues were used as the samples for the detection of mRNA of MAP7 by conventional RT-PCR. b, colocalization of TRPV4 (left column) and MAP7 (right column) visualized in continuous sections of brain (upper), renal cells (middle), and lung (bottom). TRPV4 was detected by amino ethylcarbazole (red), and MAP7 was detected by 3,3′-diaminobenzidine (brown). Arrows indicate positive cells. They were viewed through a microscope (BX-50; Olympus). c, colocalization of TRPV4 (green) and MAP7 (red) visualized by immunofluorescence in lung (upper) or renal (lower) sections. Merge image was constructed on the screen. The arrow indicates the colocalization of TRPV4 and MAP7, showing as yellow cells in the merged image. Kd, kilodalton.
To clarify surface expression, we collected membrane protein by biotinylation. Sulfosuccinimidyl-6-(biotinamido) hexanoate is a molecule that binds to membrane surface amino acids and was collected as a streptavidin conjugate. Compared with membrane protein CD4, TRPV4 is poorly expressed on the membrane when it is transfected alone. TRPV4 colocalized with MAP7 revealed an increase in the TRPV4 signal of the membrane fraction. Thus, MAP7 bound to TRPV4 and regulated TRPV4 surface expression (Fig. 3c).

**Electrophysiological Analysis of TRPV4 with MAP7**—In addition to the expression of TRPV4 toward the membrane surface, we speculated that MAP7 contributed mechanical transmission through actin microfilaments. The electrophysiological measurement of the activity of TRPV4 was designed to investigate its functional consequence and relevance to actin by coexpression. The osmolality of the bath solution was changed from 300 to 200 and then to 400 mosmol. The basal currents of TRPV4 with MAP7 and TRPV4 alone were not much different. However, the current evoked by hypo-osmolality (from 300 to 200 mosmol) was altered dramatically (Fig. 4a). The current produced by TRPV4 with MAP7 became steeply sensitive to the hypo-osmolality. A state of hyper-osmolality (to 400 mosmol) diminished the activated current, but no less than did basal current. Currents evoked by hypo-osmolality in TRPV4 alone or TRPV4 with MAP7 showed that the two molecules induced a remarkable outward rectified conductance (Fig. 4b).
tivated current was blocked by 0.1 μM ruthenium red (26). The ruthenium red-responsive current of TRPV4 deletion mutants with MAP7 met the results of protein binding study by swelling (Fig. 4c). To elucidate the interaction of cytoskeletal fibers, the evoked current of TRPV4 with MAP7 was examined with reagents in pipette fill. The actin-modifying reagents cytochalasin B ($10^{-6}$ M), taxol ($10^{-5}$ M), and phalloidin ($10^{-6}$ M) abolished the activation, whereas the microtubule-freezing reagents colchicine ($10^{-5}$ M) and vincristin ($10^{-6}$ M) did not influence it. Therefore, the activation of TRPV4 with MAP7 by swelling requires actin microfilaments but not microtubules (Fig. 4c).

We examined the swelling-activated current in TRPV4- or vehicle-transfected CHO and HEK293 cells in the same settings. The entire cellular current was measured in a bath solution from 300 to 200 mosmol, through 240 mosmol, and then returning to 320 mosmol. The current density of TRPV4 in HEK293 was significantly activated by 200 mosmol and decreased by 320 mosmol. In contrast, the density was not significantly altered in CHO cells (Fig. 4d).

**DISCUSSION**

**Biochemical Interaction of MAP7 and TRPV4**—Although the MAP family was first cloned as a protein associated with microtubules (27), a subsequent study suggested its ability to interact with actin microfilaments (28). MAP7, which is identical to E-MAP-115 (29), is a novel member that is widely expressed in differentiated epithelial cells. MAP7 may play a role in mature rather than immature epithelial cells and may contribute to epithelial polarity (30). The MAP family has been linked to microfilament channels as well. MAP1-B is an example of this, because it promotes GABA<sub>A</sub> receptors in the cytoskeleton in retinal synapse (31, 32). Although the colocalization of MAP7 and TRPV4 in the same cell was suggested by our results (Fig. 3b), we did not detect any intracellular colocalization of the two molecules in situ. Because MAP7 is rich in microtubules (27), the detection of cell surface interaction required monoclonal or equivalent antibodies. Thus, the visualization of both molecules in the same section awaits further study.

The results of our series of immunoprecipitation, pull-down assay, and histological findings indicated that MAP7 is an associated protein of TRPV4. But MAP7 occurred in the liver (Fig. 2), whereas TRPV4 did not. Thus, MAP7 is considered to be distributed more widely than TRPV4 and to play other roles. This is the case with MAP1-B, which is distributed more widely than GABA<sub>A</sub> (31, 32). The coexpression of MAP7 and TRPV4 resulted in the surface expression of TRPV4 (Fig. 3). This is also the case with MAP1-B. The coexpression of GABA<sub>A</sub> and MAP1-B promoted their appearance at the cell surface (31, 32). The surface expression that we saw was suggested by fluorescence images. However, these results did not fully indicate the colocalization of two molecules. On the other hand, membrane expression was enhanced by cotransfection of MAP7 and TRPV4 and collecting the membrane fraction using biotinylation. This method is more reliable than ultracentrifugation, as the entire cellular current was measured in a bath containing 300 to 200 mosmol, through 240 mosmol, and then returning to 320 mosmol. The current density of TRPV4 in HEK293 Cells was significantly activated by 200 mosmol and decreased by 320 mosmol. In contrast, the density was not significantly altered in CHO cells (Fig. 4d).

MAP7 interacts with the C terminus of TRPV4. Our pull-down assay and functional study suggested the importance of amino acids 789−809. However, the motif of the binding domain was not predicted by the BLOCK program and is still unknown. We hope to search for the motif by using mutants in a future study.

**Functional Interaction of MAP7 and TRPV4**—The MAP family is phosphorylated on various sites (34). The phosphorylation of MAP7 was involved in the activation of TRPV4 by swelling, because the current density was significantly reduced when there was no ATP in the pipette (data not shown). However, the current of TRPV4 in HEK293 (18) or CHO (19) cells was not significantly changed by the removal of ATP from the pipette. Nonetheless, TRPV4 was also activated by a reaction using tyrosine phosphorylation (34), which is a protein kinase C analogue in HEK293 cells (26). Thus, the direct phosphorylation of MAP7 or its indirect modification by another kinase is involved in the mechanism of mechanogating, although the process remains unclear.

This mechanism may underlie the differing TRPV4 response of expressed cells to hypo-osmolality. TRPV4 was more highly activated in HEK293 cells than in CHO cells, possibly because HEK293 cells contain endogenous MAP7 (Fig. 4d). The difference observed in the present study is not opposed to the findings of Liedtke et al. (19), who used CHO cells and saw an activated current of 400 pA, which corresponds to a density of ~10 pA/pF. However, recent studies have used HEK293 cells, which exhibit a larger current. The discrepancy of the activation by hypotonicity is explained by the endogenous MAP7 in HEK293 cells. The dependence of functional expression in the cells is also observed in the heat activation study. Neither we nor Liedtke et al. (19) observed any heat activation of rodent TRPV4, although it was observed in HEK293 cells (18). HEK293 is an epithelial cell line that was isolated from the kidney, where TRPV4 mRNA is abundant. It is therefore possible that HEK293 cells contain a larger volume of the components necessary to support TRPV4 expression. In contrast, CHO cells are cells derived from ovary cells, which exhibit small channel currents. This hypothesis also supports the results regarding the reconstitution of the molecules. Endothelial cells, which showed a swelling-activated cation current (36), contained TRPV4 but not MAP7 mRNA (Fig. 3). Given that a difference in mechanosensitivity was indicated in different cells, the combination of TRPVs with molecules of the MAP subfamily may contribute the mechanosensitive calcium-permeable cation channel complex.

The mechanosensitive molecular complex could be compared with the known channel complex, MEC/DEG/EnaC. MEC-4 and MEC-10 produce an amiloride-sensitive stretch-activated ion channel, which is connected to microtubules via MEC-2. In the presence of MEC-2, the activity of the MEC-4/MEC-10 channel increases 40-fold (37). Likewise, MAP7 links TRPV4 to microfilaments, resulting in an increase in activity by, at least, lowering the threshold of mechanosensation to membrane surface deflection. Thus, the mechanosensitive complex may generally require both the channel and the linking molecule to play a physiological role.

However, TRPV4 with MAP7 did not appear in the stretch-activated channel. We obtained a single channel recording on TRPV4 and MAP7-transfected CHO cells. Although we were able to find a susceptible cation channel, it was not similar to the stretch-activated channels in situ. This result is compatible with that observed in HEK cells (18).

The mechanosensitivity of TRPV4 has been suggested in vivo (22, 23) and in vitro. Recently, investigations of the mechanism under the swelling-induced activation of TRPV4 were developed that explain how swelling of the cell-activated tyrosine kinase (35) or prostaglandin (26, 38) cascade results in the direct activation of the TRPV4 channel. There can be variable mechanisms under the signal transduction of cell swelling in variable tissues (39). Therefore, deformity in the cell may transmit the signal to actin and then activate TRPV4 through MAP7 in certain cells. This possibility should be clarified in future studies.

Ours is the first article to report an associated protein of...
TRPV4. By linkage with MAP7, TRPV4 becomes functionally active by an increase in the membrane expression. Because MAP7 is a protein that is associated with cytoskeletal filaments, this linkage may underlie the mechanism of mechanosensitivity to cell deformation.

Acknowledgments—We thank the RIKEN Genomic Center for the gift of the clone (BB870222), and we also thank Y. Oyama and Y. Waranabe for technical assistance.

REFERENCES

1. Martinec, B., Buechner, M., Delcour, A. H., Adler, J., and Kung, C. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 2297–2301
2. Isla, H., Nakamura, H., Ono, T., Okamura, M., and Anraku, Y. (1994) Mol. Cell. Biol. 14, 8259–8271
3. Overnos, J. G., and Corey, D. P. (1997) Annu. Rev. Neurosci. 20, 567–594
4. Patel, A., Honore, E., MainGret, F., Lesage, F., Fink, M., Duprat, F., and Lazdunski, M. (1998) EMBO J. 17, 4289–4296
5. Hamill, O. P., and Martinac, B. (2003) Physiol. Rev. 81, 687–740
6. Cornet, M., Delpire, E., and Gilles, R. (1987) Pfluegers Arch. Eur. J. Physiol. 410, 223–225
7. Small, D. L., and Morris, C. E. (1994) Am. J. Physiol. 267, C598–C606
8. Downey, G. P., Grinstein, S., Sue-A-Quan, A., Czabam, B., and Chan, C. K. (1995) J. Cell. Physiol. 163, 96–104
9. Foskett, J. K., and Spring, K. E. (1985) Am. J. Physiol. 248, C27–C36
10. Kuhlbusch, W. E., and Wondergem, R. (1991) Hepatology 13, 962–969
11. Linshaw, M. A., Fogwl, C. A., Downey, G. P., Koo, E. W., and Gotlieb, A. I. (1992) Am. J. Physiol. 262, F144–F150
12. Katz, B. (1950) J. Physiol. 111, 261–282
13. Gucharny, F., and Sacha, F. (1984) J. Biol. Chem. 352, 685–701
14. Caterina, M. J., Rosen, T. A., Tominaga, T., Brake, A. J., and Julius, D. (1999) Nature 398, 436–441
15. Caterina, M. J., Esteller, A., Malinberg, A. B., Martin, W. J., Trafton, J., Petersen-Hein, K. R., Kolzenburg, M., Basbaum, A. I., and Julius, D. (2000) Science 288, 306–312
16. Suzuki, M., Ishibashi, K., and Imai, M. (1999) J. Am. Soc. Nephrol. 10, pp44
17. Wissenbach, U., Bodding, M., Freuchel, M., and Plocke, V. (2000) FEBS Lett. 485, 127–134
18. Strotmann, R., Harteneck, C., Nunnemacher, K., Schultz, G., and Plant, T. D. (2000) Nat. Cell Biol. 2, 695–702
19. Liedtke, W., Choe, Y., Marti-Benom, M. A., Bell, A. M., Denis, C. S., Sali, A., Hudspeth, A. J., Friedman, J. M., and Heller, S. (2000) Cell 103, 525–535
20. Delany, L. S., Hurle, M., Facer, P., Almadif, T., Plumpdon, C., Kinghorn, I., See, C. G., Custigan, M., Anand, P., Woolf, C. J., Crowther, D., Sanseau, P., and Tate, S. N. (2001) Physiol. Genomics 4, 165–174
21. Suzuki, M., Ohki, G., Mochizuki, T., Sumio, S., Ishibashi, K., and Imai, M. (2002) FEBS Lett. 517, 219–224
22. Suzuki, M., Mizuno, A., Koidaira, K., and Imai, M. (2003) J. Biol. Chem. 278, 22664–22668
23. Alessandri-Haber, N., Yeh, J. J., Boyd, A. E., Parada, C. A., Chen, X., Reichling, D. B., and Levine, J. D. (2003) Neuron 31, 497–511
24. Tracey, W. D., Wilson, R. L., Laurent, G., and Benzer, S. (2003) Cell, 113, 261–273
25. Suzuki, M., Sato, J., Kutsuwada, K., Ooki, G., and Imai, M. (1999) J. Biol. Chem. 274, 6330–6335
26. Watanabe, H., Kousa, S., Suh, S. H., Benham, C. D., Droogmans, G., and Nilius, B. (2002) J. Biol. Chem. 277, 47044–47051
27. Kuznetsov, S. A., Rodionov, V. I., and Rosenblat, V. A. (1981) FEBS Lett. 135, 237–240
28. Arakawa, T., and Frieden, C. (1984) J. Biol. Chem. 259, 11730–11734
29. Masson, D., and Kreis, T. E. (1993) J. Biol. Chem. 268, 357–371
30. Fabre-Jonca, N., Allaman, J. M., Radlgruber, G., Meda, P., Kiss, J. Z., French, L. E, and Masson, D. (1998) Differentiation 63, 186–190
31. Jonathan G. H., Koulou, P., Bedford, F., Gordon-Weeks, P. R., and Moss, S. J. (1999) Nature 397, 66–69
32. Wang, H., Fiuma, K., Bedford, N., Branden, J., Moss, S. J., and Olsen, R. W. (1999) Nature 397, 69–73
33. Lin, D. H., Sterling, H., Lerea, K. M., Giebisch, G., and Wang, W. H. (2002)
34. Mayer, H. E., Mandelkow, E., and Mandelkow, E. (1995) J. Biol. Chem. 270, 44278–44284
35. Mayer, H. E., Mandelkow, E., and Mandelkow, E. (1995) J. Biol. Chem. 270, 7679–7688
36. Nilius, B., and Droogmans, G. (2001) Physiol. Rev. 18, 1415–1459
37. Goodman, M. B., Ernstrom, G. G., Chelur, D. S., O7', Hagen, R., Yao, A. A., and Chalfie, M. (2002) Nature, 415, 1039–1042
38. Watanabe, H., Krenn, J., Droogmans, G., Voets, T., and Nilius, B. (2003) Nature, 424, 434–438
39. Sarkadi, B., and Parker, J. C. (1991) Biochim. Biophys Acta 1071, 407–427