Opposite Regulation of Transepithelial Electrical Resistance and Paracellular Permeability by Rho in Madin-Darby Canine Kidney Cells*

Small GTPase Rho has been thought to be important for the formation and the maintenance of tight junction in epithelial cells, but the role of Rho in the regulation of barrier function of tight junction is not well understood. We here examined whether Rho was involved in the barrier function of tight junction in Madin-Darby canine kidney (MDCK) cells. The activation of prostaglandin EP3β receptor, coupled to a Rho activation pathway, induced the increase in transepithelial electrical resistance (TER) but the increase in paracellular flux of mannitol in the preformed monolayer of the MDCK cells expressing the EP3β receptor. This effect of the EP3 receptor was mimicked by the expression of constitutively active RhoA but not by active Rac1 in MDCK cells, using an isopropyl-β-D-thiogalactoside-inducible expression system. On the other hand, the activation of EP3β receptor suppressed the elevation of TER and the decrease in paracellular mannitol flux during Ca2+ switch-induced tight junction formation, whereas the expression of active RhoA or Rac1 did not apparently affect the TER development in the Ca2+ switch. These results demonstrate that the EP3 receptor and active RhoA regulate permeabilities of ionic and nonionic molecules in opposite directions in the preformed monolayer, and the EP3 receptor suppresses the elevation of TER during the tight junction formation.

Epithelia form barriers and regulate vectorial transport of ions and solutes. Epithelial cells adhere to each other via three distinct adhesion systems, called tight junction, adherens junction, and desmosome. Of these, tight junction is the most apical component and is localized to the interface between apical and basolateral membrane domains (1–4). It forms not only a barrier to the diffusion of ions and solutes but also a barrier to the paracellular diffusion of ions and solutes. Recent studies have identified several proteins localized at tight junctions, including occludin (5), ZO-1 (6), ZO-2 (7, 8), ZO-3 (9), cingulin (10), TH6 antigen (11), symplekin (12), and claudin (13). Occludin has four transmembrane domains and has been shown to be the sealing protein of tight junctions (14–16). ZO-1 is a peripheral membrane protein with a molecular mass of 220 kDa and a member of the membrane-associated guanylate kinase-containing family proteins, most of which may play key roles in clustering of proteins at synaptic and septate junctions, and ZO-1 binds to the carboxyl-terminal cytoplasmic domain of occludin (17). ZO-2, which has been characterized as a binding partner of ZO-1, is also a member of the membrane-associated guanylate kinase-containing family (7). Many extracellular stimuli have been shown to regulate the barrier function of tight junctions. For example, stimulus reduced ZO-1 protein levels and decreased transepithelial electrical resistance (TER) in retinal epithelial cells (18), and glucocorticoid caused ZO-1 to localize to the cell-cell interaction site and increased TER in mammary epithelial cells (19). However, intracellular signaling pathways, which regulate the tight junction permeability, remain elusive.

Rho and Rac are members of a subfamily of small GTPase that are thought to involved in many cellular functions, including the regulation of actin filament reorganization, cell shape change, and gene expression (20, 21). In fibroblasts, Rho is responsible for regulating the assembly of focal adhesion and stress fiber formation, although Rac is involved in membrane ruffling and formation of lamellipodia (22, 23). In neuronal cells, the activation of Rho induces neurite retraction (24, 25), and Rac is involved in the neurite outgrowth (26). Besides, both Rho and Rac have been shown to be involved in serum response factor-mediated transcriptional activation (27). As for tight junction, C3 transferase, which ADP-ribosylates Rho on amino acid Asn-41 and is used as a specific inhibitor of Rho proteins (28, 29), was reported to induce the displacement of ZO-1 protein from tight junction and the increase in tight junction permeability, suggesting that Rho was involved in the regulation of tight junction integrity in epithelial cells (30).

Prostaglandin E2 exhibits a broad range of biological actions in diverse tissues through its binding to specific receptors on the plasma membrane (31). Prostaglandin E receptors are pharmacologically divided into four subtypes, EP1, EP2, EP3, and EP4, on the basis of their responses to various agonists and antagonists (32, 33). Among the four subtypes, the EP3 receptor has been most well characterized and is involved in such prostaglandin E2 actions as contraction of the uterus, modula-

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To whom correspondence should be addressed: Dept. of Molecular Neurobiology and Physiology, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyoku, Kyoto 606-8501, Japan.

1 The abbreviations used are: TER, transepithelial electrical resistance; PT, pertussis toxin; MDCK, Madin-Darby canine kidney; 8-bromo-cAMP, 8-bromo-cyclic AMP; HA, hemagglutinin; DMEM, Dulbecco’s modified Eagle’s medium; IPTG, isopropyl-β-D thiogalactoside; PBS, phosphate-buffered saline.
tion of neurotransmitter release, inhibition of gastric acid secretion, and sodium and water reabsorption in the kidney (34–37). We have cloned the mouse EP3 receptor and demonstrated that it is a G protein-coupled rhodopsin-type receptor that engages in inhibition of adenylate cyclase (38). Although the well known EP3 receptor-mediated actions, mentioned above, are believed to be mediated by Gs, coupling of EP3 receptor to other signal transduction pathways has been suggested (39, 40). We recently found that the EP3 receptor activated Rho via a pertussis toxin (PT)-insensitive heterotrimeric G protein, inducing neurite retraction in differentiated PC12 cells (25) and stress fiber formation in Madin-Darby canine kidney (MDCK) cells, an epithelial cell line derived from dog kidney, which are generally used as models of polarized cells (41). In the present study, we examined roles of the EP3 receptor and RhoA in the barrier function of tight junction in MDCK cells, and we demonstrate that the EP3 receptor and the constitutively active RhoA oppositely regulate TER and the paracellular flux rate of mannitol in the preformed monolayer of MDCK cells, and in addition the EP3 receptor suppresses the elevation of TER during the Ca2+−induced tight junction formation.

**EXPERIMENTAL PROCEDURES**

**Materials**—The MDCK strain II cell line and sulprostone were generous gifts from Drs. Keith E. Mostov (University of California, San Francisco, CA) and K.-H. Thierauc (Schering), respectively. Agents obtained and commercial sources were as follows: PT, Seikagaku Co., Japan; 5-hydroxy-cyclic AMP (8-h-cAMP), Research Biochemicals International, Na+; rhodamine-conjugated phalloidin, Molecular Probes, Inc.; rat anti-ZO-1 monoclonal antibody, Chemicon International Inc.; rabbit anti-hemagglutinin (HA) polyclonal antibody, MBL International Co.; rhodamine-conjugated goat F(ab′)2 anti-rat IgG, ICN Pharmaceuticals, Inc.; horseradish peroxidase-conjugated swine anti-rabbit IgG, DAKO; and cadmium binding ECL Western blotting system, Amersham Pharmacia Biotech.

**DNA Construction**—The cDNA for human RhoA was generated as described previously (42). The coding region of human Rac1 was generated by reverse transcription-polymmerase chain reaction from HL-60 cells used as primers 5′-CCCGCCTTCGGGAGAGAACAGGAGCTACAGAAATGTGTTG-TGCTG-3′ and 5′-CCGGAACTTCTTCAACAGCAGGATTCATCTTCTTCTT-3′. The polymerase chain reaction product with Boehringer and EcoRI, cloned into the plBluescript SK (+), and completely sequenced. cDNAs for RhoAV14 and Rac1V12 were generated by polymerase chain reaction-mediated mutagenesis (43) and subcloned into the BamHI/EcoRI sites of plBluescript SK(+) containing the HA epitope at the 5′ end. 0.75- or 0.65-kilobase pair NotI-NotI fragment containing full-length human RhoAV14 or Rac1V12 cDNA tagged at the amino terminus with HA epitope was introduced into the mammalian expression vector pOPRSVICAT (Stratagene) at the site of NotI (pOPRSVI/ HA-RhoAV14 or HA-Rac1V12).

**Cell Culture and Transfection**—MDCK cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum, 4 mM glutamine, 100 units/ml penicillin, and 0.1 mg/ml streptomycin under humidified air containing 5% CO2 at 37°C. The MDCK cell line expressing the EP3 receptor has been described previously (41). To establish the MDCK cell line, expressing RhoAV14 or Rac1V12 under the control of isopropyl-β-D thiogalactoside (IPTG), MDCK cells were first transfected with a Lac repressor vector, p3SS (Stratagene), by Cellfect Transfection Kit (Amersham Pharmacia Biotech). Stable transfectants were cloned by selection with hygromycin (Wako Corp., Osaka, Japan), and the expression of Lac repressor was confirmed by immunoblotting using a Lac repressor protein anti-serum. Lac repressor-positive cells were further transfected with the pOPRSV/HARhoAV14 or HA-Rac1V12 construct as described above. Stable transfectants were cloned by selection with hygromycin and G-418 (Life Technologies, Inc.). For the Ca2+ switch experiments, the cells were cultured in serum-free DMEM containing 1.9 mM EGTA (low Ca2+ medium; estimation of Ca2+ concentration, 1–2 μM) for 4 h. They were then transferred to serum-free DMEM containing 1.8 mM Ca2+ (normal Ca2+ medium).

**Immunofluorescence**—MDCK cells were seeded onto poly-l-lysine-coated glass coverslips in 12-well plates at a density of 2 × 105 cells/well, and cultured for 2 days. For localization of actin filaments or ZO-1, and the membrane flux rate of mannitol in the preformed monolayer of MDCK cells, an epithelial cell line derived from dog kidney, which are generally used as models of polarized cells (41). Briefly, the cells were fixed in 0.1% phosphate buffer (pH 7.4) containing 4% paraformaldehyde and 3% sucrose for 1 h at 4°C. They were then permeabilized with TBS/HS (10 mM Tris-HCl, pH 7.4, containing 0.1% NaCl and 0.1% Tween 20), containing 0.1% Triton X-100 at room temperature for 10 min and washed with TBS/HS. They were then cotransfected with 0.5% bovine serum albumin in TBS/LS (PBS containing 0.05% Tween 20) for 1 h at room temperature and rinsed with TBS/HS. To identify the polymerized actin, they were incubated with 1:1000 dilution of rhodamine-conjugated phalloidin in TBS/LS for 1 h at room temperature. Then, they were washed with TBS/HS three times and mounted onto a slide glass in PBS/glycerol containing p-phenylenediamine dihydrochloride. Cells were photographed on the confocal microscope under the control of isopropyl-β-D thiogalactoside (IPTG) in the barrier function of tight junction in MDCK cells, and we demonstrate that the EP3 receptor and the constitutively active RhoA oppositely regulate TER and the paracellular flux rate of mannitol in the preformed monolayer of MDCK cells, and in addition the EP3 receptor suppresses the elevation of TER during the Ca2+−induced tight junction formation.

**RESULTS**

**Effect of EP3 Receptor on the Barrier Function of Tight Junction in the Preformed Monolayer of MDCK Cells**—We previously established MDCK cell line expressing mouse EP3 receptor, which is coupled to Rho activation via a PT-insensitive heterotrimeric G protein (41). To examine a role of the EP3 receptor in the barrier function of tight junction in MDCK cells, we first investigated the effect of sulprostone, an EP3 agonist, on TER and the paracellular flux rate of mannitol. TER is thought to be a parameter of tight junction ionic permeability in MDCK strain II cells (44). Fig. 1 shows the time course of the effect of sulprostone on TER in the preformed monolayer of the EP3-expressing MDCK cells. Sulprostone at 1 μM induced the increase in TER with a lag period of 6 h, reaching a maximum level by 18 h. The sulprostone-induced TER increase was not observed in wild-type MDCK cells (data not shown). We previously showed that the EP3 receptor was linked to two signal transduction pathways, Gi-mediated adenylate cyclase inhibition and Rho activation pathway via a PT-insensitive G protein (41, 45). We then examined the effect of PT on the EP3 recep-
tor-mediated increase in TER. Sulprostone markedly induced the increase in TER in the PT-pretreated cells, the maximum level being slightly lower than that in the untreated cells, indicating that the EP3β receptor-induced increase in TER is mediated by the PT-insensitive pathway.

We next examined the other parameter of tight junction permeability, the paracellular flux rate of mannitol. As shown in Fig. 2, sulprostone stimulated the paracellular flux of mannitol in a time-dependent manner in the preformed monolayer of EP3β-expressing cells, and this increase in the flux was not affected by the PT treatment. Thus, the EP3β receptor oppositely regulated TER and paracellular mannitol flux via the PT-insensitive pathway. Sulprostone failed to affect the amount of occludin, as assessed by immunoblot and Northern blot analyses, indicating that the effect of the EP3 receptor on the barrier function is not due to fluctuation of cellular content of occludin (data not shown).

Effects of RhoAV14 and Rac1V12 on the Barrier Function of Tight Junction in the Preformed Monolayer of MDCK Cells—

E3 receptor is coupled to Rho activation pathway via a PT-insensitive G protein (25, 41). C3 transferase is a very useful tool to verify the involvement of Rho in cellular functions, but C3 transferase is inadequate for application onto the regulation of permeability by the EP3 receptor, because microinjected C3 transferase and cell permeable C3 transferase have been shown to disrupt cell-cell adhesion and tight junction, respectively (30, 46). We also observed that the microinjection of C3 transferase induced the complete disruption of the cell-cell adhesion in MDCK cells (data not shown). Therefore, we next investigated the effect of constitutively active RhoA on the permeability of tight junction in the preformed monolayer of MDCK cells. We introduced HA-tagged GTPase-deficient constitutively active RhoA (RhoAV14) and Rac1 (Rac1V12) into MDCK cells using a Lac-inducible vector, and established MDCK cell lines, which express RhoAV14 and Rac1V12 under the control of IPTG (henceforth referred to as “RhoAV14” or “Rac1V12” inducible MDCK cells,” respectively). HA-RhoAV14 or HA-Rac1V12 was expressed by the incubation with 5 mM IPTG for 3–6 h in RhoAV14, or Rac1V12 inducible cells (Fig. 3). To verify the effects of RhoAV14 and Rac1V12 on the distribution of F-actin and ZO-1, we stained the cells by rhodamine-conjugated phalloidin or anti-ZO-1 antibody. In parental MDCK cells expressing Lac repressor alone, stress fiber was not apparently found. IPTG did not show any alteration in F-actin distribution in these cells (Fig. 4, A–C). In RhoAV14-inducible cells, although almost no stress fiber was seen in the unstimulated cells, IPTG induced clear stress fiber formation at 12 or 24 h (Fig. 4, D–F). In Rac1V12-inducible cells, the incubation with IPTG induced the marked increase in F-actin staining at the cell-cell adhesion sites (Fig. 4, D–F). Therefore, RhoAV14 and Rac1V12 expressed in the cells were functional. ZO-1 showed discrete and continuous patterns of distribution in the unstimulated parental and RhoAV14- and Rac1V12-inducible cells. Although IPTG treatment did not affect the ZO-1 distribution in parental and Rac1V12-inducible cells, this treatment induced the redistribution of ZO-1, and ZO-1 showed the discontinuous and fragmented staining pattern in RhoAV14-inducible cells (Fig. 5).

We next investigated the effects of constitutively active RhoA and active Rac1 on the barrier function of tight junction (Fig. 6). The induction of Rac1V12 affected neither TER nor paracellular flux in the preformed monolayer of Rac1V12-inducible cells. On the other hand, the induction of RhoAV14 by IPTG progressively elevated the level of TER up to 24 h with a lag period of 12 h in the preformed monolayer of RhoAV14-inducible cells. RhoAV14 induction also stimulated the mannitol flux. The
incubation with IPTG affected neither TER nor the paracellular flux of mannitol in parental MDCK cells (data not shown). Thus, constitutively active RhoA oppositely regulated TER and the flux rate of mannitol in the preformed monolayer of MDCK cells, whereas constitutively active Rac1 had no ability to modulate the barrier function of tight junction. The RhoAV14 induction did not affect the amount of occludin, as assessed by immunoblot and Northern blot analyses, indicating that the effect of RhoAV14 on the barrier function is not due to fluctuation of cellular content of occludin (data not shown).

Effect of cAMP on the EP3 Receptor- and RhoAV14-induced Elevation of TER—Cyclic AMP plays important roles in many aspects of cellular functions, and protein kinase A has been shown to modulate Rho-mediated function (47). We then examined the effect of cAMP on the EP3 receptor- and active RhoA-induced regulation of barrier function. As shown in Fig. 7A, the pretreatment with 8-bromo-cAMP, a stable cAMP analogue, suppressed by 72% the TER increase induced by sulprostone without any change in the basal TER level in the preformed monolayer of EP3β-expressing cells. This treatment also suppressed the RhoAV14-induced TER increase by 65% in the preformed monolayer of RhoAV14-expressing cells (Fig. 7B). These results indicate that protein kinase A is a negative regulator for the RhoA-induced TER increase.

Effects of EP3 Receptor, RhoAV14, and Rac1V12 on the Newly Formed Tight Junction in Ca2+-Switch Experiment—We next examined the effects of the EP3 receptor, active RhoA, and active Rac1 on the Ca2+-induced formation of tight junction using a Ca2+-switch experiment. The elimination of Ca2+ in the medium induced the disruption of cell-cell adhesion, and the following addition of Ca2+ induced the formation of tight junctions (48). The 4-h incubation with a low Ca2+ medium decreased the level of TER to almost zero. As shown in Fig. 8A, the addition of Ca2+ induced the marked increase in TER in the unstimulated EP3β-expressing MDCK cells, the level reaching the maximum at 6 h. The stimulation with sulprostone suppressed this Ca2+-induced increase in TER, the maximum level decreasing by 60% (Fig. 8A). We next examined the PT sensitivity of the inhibition by EP3 receptor. The pretreatment with PT by itself reinforced the Ca2+-induced elevation of TER, as reported previously (48), but this pretreatment did not inhibit the suppression by sulprostone of Ca2+-induced TER elevation (Fig. 8B). At this condition, sulprostone-induced stress fiber formation was observed, indicating that the EP3 receptor was linked to Rho activation pathway (data not shown). We further investigated the effect of EP3 receptor on the Ca2+-induced decrease in paracellular flux of mannitol. The Ca2+ switch induced the tight junction formation and eventually decreased the paracellular flux of mannitol to the low level of flux (30.1 ± 6.3 nmol/h/cm2). Sulprostone elevated this value decreased by the Ca2+ switch (Fig. 8C). These results indicate that the activation of EP3 receptor suppressed the Ca2+-induced tight junction formation and that this inhibitory action of EP3 receptor was mediated by a PT-insensitive G protein.

We next examined the effects of active RhoA and Rac1 on the formation of tight junction induced by Ca2+-switch. After constitutively active RhoAV14 and Rac1V12 had been expressed by the 8-h incubation with IPTG, we analyzed the pattern of TER development after the Ca2+-switch. The IPTG treatment clearly induced RhoAV14 and Rac1V12 proteins, the expression levels being maintained during the Ca2+-switch experiment. After conformation of RhoAV14 in the preformed monolayer of MDCK cells. Although we could not directly indicate the involvement of Rho in this action of the EP3 receptor due to inadequacy of application of C3 transferase for analysis of barrier function of preformed tight junction, the opposite regulation of barrier function by the EP3 receptor appears to be mediated by Rho activation because this regulation was insensitive to PT treatment and mimicked by the
constitutively active RhoA.

The barrier function of tight junctions is assessed by measuring the TER and paracellular permeability of nonionic molecules, such as mannitol. The EP3 receptor and constitutively active RhoA induced the increase in TER, viz. the decrease in ionic permeability, while they oppositely promoted the perme-

Fig. 6. Effects of Rac1V12 and RhoAV14 on TER and the paracellular flux of mannitol in the preformed monolayer of MDCK cells. A and B, serum-starved Rac1V12-inducible (A) or RhoAV14-inducible (B) MDCK cells were incubated in the presence (●) or absence (○) of 5 mM IPTG throughout the 24-h time course. The TER was monitored at the indicated times, and the ohm × cm² was calculated as described under “Experimental Procedures.” C, after serum-starved cells had been incubated with (closed column) or without (open column) 5 mM IPTG for 24 h, the paracellular flux of mannitol for 1 h was determined as described under “Experimental Procedures.” The results shown are the means ± S.E. for triplicate determinations and are expressed as a percentage of control obtained with the Rac1V12-inducible cells in the absence of IPTG (13.7 ± 1.1 nmol/h/cm²).

Fig. 7. Effect of 8-br-cAMP on the EP3β receptor- and RhoAV14-induced elevation of TER. A, after serum-starved MDCK cells expressing the EP3β receptor had been pretreated with or without 500 μM 8-br-cAMP for 30 min, they were incubated in the presence or absence of 1 μM sulprostone for 24 h. TER was monitored, and the ohm × cm² was calculated as described under “Experimental Procedures.” B, RhoAV14-inducible MDCK cells were incubated for 24 h with vehicle, 500 μM 8-br-cAMP, 5 mM IPTG, or both. After the 24-h incubation, TER was monitored, and the ohm × cm² was calculated as described under “Experimental Procedures.” The results shown are the means ± S.E. for triplicate determinations.

Fig. 8. Effect of sulprostone on the Ca²⁺-induced formation of tight junction in EP3β-expressing MDCK cells. A and B, after MDCK cells expressing the EP3β receptor had been treated with (B) or without (A) 10 ng/ml PT for 5 h, they were incubated with low Ca²⁺ medium for 4 h. They were then switched to normal Ca²⁺ medium and incubated in the presence (●) or absence (○) of 1 μM sulprostone throughout the following 8-h time course. The TER was monitored at the indicated times, and the ohm × cm² was calculated as described under “Experimental Procedures.” C, after MDCK cells expressing the EP3β receptor had been incubated with low Ca²⁺ medium for 4 h, they were switched to normal Ca²⁺ medium and incubated for 9 h in the presence (closed column) or absence (open column) of 1 μM sulprostone. After the 9-h incubation, the paracellular flux of mannitol for 1 h was monitored as described under “Experimental Procedures.” The results shown are the means ± S.E. for triplicate determinations and are expressed as a percentage of control obtained with the cells switched to normal Ca²⁺ medium in the absence of agonist (30.1 ± 6.3 nmol/h/cm²).
Rho and Rac are believed to be important regulators in cell-cell adhesion (53). Constitutively active Rac1 has been shown to accumulate actin filaments, E-cadherin, and β-catenin at the cell-cell adhesion sites without any effect on the distribution of ZO-1 in MDCK cells, suggesting that Rac regulates the formation of the cadherin-based cell-cell adhesion (46). In contrast, constitutively active RhoA was shown to induce stress fiber formation on the basal membrane and discontinuous redistribution of ZO-1 in tight junction without any change in E-cadherin distribution in MDCK cells (54, 55), and we also observed the active RhoA-induced discontinuous redistribution of ZO-1. As for tight junction permeability, active RhoA but not Rac1 induced the changes in the permeability in the preformed monolayer cells. Thus, Rho is a key regulator for the barrier function of tight junction. Protein kinase A has been reported to modulate Rho-mediated function (47). We examined the sensitivity to 8-bromo-cAMP of regulation of the permeability by Rho and revealed that 8-bromo-cAMP inhibited the regulation of the permeability by activation of Rho, indicating that protein kinase A is a negative regulator for the Rho activity in the barrier function. However, 8-bromo-cAMP treatment caused partial inhibition. There might be cAMP-independent mechanisms of Rho regulation.

Furthermore, we analyzed the effect of Rho on tight junction biogenesis induced by Ca$^{2+}$ switch. In contrast to the preformed monolayer cells, the expression of active RhoA and Rac1 did not apparently affect the TER elevation during the formation of tight junction induced by Ca$^{2+}$ switch, even if active RhoA induced stress fiber formation in this condition. However, recent studies demonstrated that the treatment with cell-permeable C3 transferase or microinjection of C3 transferase displaced ZO-1 from tight junction and induced disruption of the cell-cell adhesion, suggesting that Rho is involved in maintenance of cell-cell interaction (30, 46). In addition, C3 transferase has been reported to inhibit the Ca$^{2+}$- and 12-O-tetradecanoylphorbol-13-acetate-induced formation of tight junction, indicating that Rho is necessary for the formation of tight junction (46). Considering these results, the activity of endogenous Rho seems strong enough for formation and maintenance of tight junction, and additional expression of constitutively active RhoA may show no apparent effect on the formation. On the other hand, the EP3 receptor suppressed the Ca$^{2+}$ switch-induced decrease in permeability of both ionic and nonionic molecules, indicating that the EP3 receptor negatively regulates the formation of tight junction. In view of the lack of the ability of active RhoA to suppress the TER elevation, Rho may not be involved in the EP3 receptor-induced suppression. However, the EP3 receptor was linked to the Rho activation pathway in the Ca$^{2+}$ switch-induced tight junction formation, and we cannot exclude the possibility that the EP3 receptor suppresses the TER elevation through Rho activation. The inhibitory effect of the EP3 receptor was insensitive to the PT treatment, suggesting that the receptor induced the inhibition of the tight junction formation through a PT-insensitive heterotrimeric G protein. The involvement of heterotrimeric G proteins in tight junction formation has been suggested. Confocal studies have shown that Go$_{aq}$, Go$_{ar}$, and Go$_{ap}$ were localized in the vicinity of the tight junction (56–58). Recently, it was reported that the constitutively active forms of PT-insensitive G proteins, Go$_{aq}$ and Go$_{ar}$, were localized to tight junction and accelerated tight junction formation, indicating that PT-sensitive G proteins act as an accelerator for tight junction biogenesis (54, 59). Although involvement of PT-insensitive G proteins in tight junction biogenesis remains unclear, the EP3 receptor may modulate the tight junction formation via a PT-insensitive G protein. In summary, we showed here that the
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EP3 receptor and active RhoA oppositely regulated TER and the paracellular flux in the preformed monolayer MDCK cells.

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