Sec61β Regulates Barrier Functions of Tight Junction through Expression of Claudin-4 in Madin–Darby Canine Kidney Cells

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Sec61β is the β subunit of the Sec61 translocon and is responsible for expression of hydrophilic and macromolecular drugs.1,2) Paracellular permeability across epithelial cell monolayers is controlled principally by tight junctions (TJs), which are localized to most apical cell-cell contacts. Claudins with four transmembrane (TM) domains and two extracellular loops (ECL) are major constituents of TJs. The C-terminal YV motif of claudins associates with the PDZ1 domain of zonula occludens 1 (ZO-1) and binds acto-myosin filaments in the cytoskeleton. ZO-1 also associates with the TJ protein occludin through PDZ domains.3,4) Claudins polymerize through cis interactions and the ECL2 of claudins connects with that of adjacent cells through trans interactions to form TJ strand networks.5) These networks comprise continuous arrays of small particles with diameters of 5–10 nm.6) The interactions between claudin subtypes have been extensively studied in connection with TJ barrier functions.5–9) Claudin-4 has been reported to control TJ functions of epithelial cells, such as Madin–Darby canine kidney (MDCK) cells.10–12) The C-terminal fragments of Clostridium perfringens enterotoxin (C-CPE) bind specifically to the ECL2 of claudin-3 and -4 and has been used as a TJ modulator owing to its lower cytotoxicity. Treatments with C-CPE resulted in a decreased transepithelial electrical resistance (TER) and increased solute permeability, concomitant with selective removal of claudin-4 from TJ and decreased expression of claudin-4. However, claudin-1 expression and distribution was unresponsive to the presence or absence of C-CPE.6) Because structure and barrier functions are restored after removal of C-CPE,6) extensive research into C-CPE-enhanced drug delivery has been conducted.6,6)

Both TJ opening and reformation are critical to the precise control of drug intake through paracellular routes. In addition, disruption of TJ barrier functions for many hours can have undesirable side effects, such as inflammatory bowel disease;17) thus TJ reformation rate will be important. Hence, from the viewpoint of drug delivery systems (DDS), TJ opening and reformation rates should be considered. However, most studies have focused on the TJ opening for enhanced permeability of molecules, and few investigations of TJ modulation rates or TJ reformation have been reported. To enhance TJ modulation rate, upregulation of claudins synthesis and delivery is important. We selected Sec61β, which involves in co-translational translocation and delivery of basolateral membrane proteins, as a target molecule.

Sec61β is the β subunit of the Sec61 translocon, which comprises α, β, and γ subunits and is located on the endoplasmic reticulum (ER).18) In general, Sec61 participates in co-translational translocation of membrane proteins. These proteins are then transported to their final destinations. The functions of Sec61α (channel) and Sec61γ (stabilization) are well established, whereas those of Sec61β remains poorly understood. Several novel functions of Sec61β have been reported. First, Sec61β mediates associations of non-translating ribosomes with the ER in vitro and enhances protein synthesis.19) Another study showed binding of Sec61β to the 25-kDa subunits of signal peptidase complexes in ER and enhancement of the kinetics of co-translational translocation. However, no Sec61β-mediated binding of ribosomes to ER was apparent, implying the redundancy of ribosome association with ER.20) Sec61β co-localizes with epidermal growth factor receptors (EGFR) and promotes recruitment to inner nuclear membranes upon treatment with EGF.21) Involvement of Sec61β in delivery of the EGFR ligand gucken to the plasma membrane has also been reported.22) Finally, interactions of Sec61β with components of the exocyst complex, which is responsible for synthesis, delivery, and sorting of secretory and basolateral membrane proteins, have been reported.23) The resulting Sec61β/exocyst complexes upregulate post-transcriptional synthesis and delivery of secretory and basolateral membrane proteins.24–26) Given that claudins are basolateral membrane proteins, they may also be subjected to Sec61β-mediated upregulation.
Considering the roles of Sec61β, we hypothesized that Sec61β overexpression enhances TJ barrier functions and modulation rates through increased synthesis and delivery of claudin-4 upon addition or removal of a TJ modulator. We used MDCK cells as typical epithelial cells and C-CPE as a TJ modulator. The objective of the present investigation was to demonstrate the effect of Sec61β overexpression on TJ barrier functions and modulation rates.

MATERIALS AND METHODS

**Materials** Dulbecco’s modified Eagle’s medium (DMEM), phosphate-buffered saline (PBS), bovine serum albumin (BSA) and fetal bovine serum (FBS) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Penicillin–streptomycin–ciner mixed solution (PSS), non-essential amino acids solution (NEAA), and Triton X-100 were purchased from Nacalai Tesque (Kyoto, Japan). Nonidet® P40 was purchased from TaKaRa Bio Inc. (Otsu, Japan). Formaldehyde was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

** Antibodies** Primary polyclonal rabbit anti-claudin-1 (pAb) and monoclonal mouse anti-claudin-4 antibodies (mAb) were purchased from Invitrogen (Carlsbad, CA, U.S.A.). Rabbit anti-Sec61β pAb and mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mAbs were purchased from Millipore (Billerica, MA, U.S.A.). The ER marker mouse anti-calnexin mAb was purchased from BD Biosciences (San Jose, CA, U.S.A.). For immunoblotting, goat anti-mouse immunoglobulin G (IgG) from Cosmo Bio (Tokyo, Japan) was used as the secondary Ab for claudin-4 and GAPDH, and goat anti-rabbit IgG (Millipore) was used for claudin-1 and Sec61β. For immunofluorescence microscopy, conjugated goat anti-mouse IgG fluorescein isothiocyanate (FITC) from Cosmo Bio was used as the secondary Ab for claudin-4. Conjugated chicken anti-rabbit IgG Alexa594 and conjugated goat anti-mouse IgG Alexa680 from Invitrogen were used for Sec61β and calnexin, respectively.

**C-CPE Preparation** C-CPE comprising amino acid residues (184–319) of CPE was prepared and purified as described previously. The C-CPE plasmid was donated by Dr. Kondoh, Graduate School of Pharmaceutical Sciences, Osaka University.

**Vector and Sec61β Transfection** Human Sec61β cDNA was amplified by PCR and ligated into pcDNA3.1(−) with T4 DNA ligase (Invitrogen). The resulting plasmids with or without Sec61β were transfected into MDCK cells using FuGENE® 6 transfection reagent from Promega (Madison, WI) according to the manufacturer’s instructions. Cells were cultured on plates in media containing G418 (Millipore), and selected colonies were harvested. Transfected MDCK cells with or without Sec61β DNA were designated as MDCK(Sec61β+) and MDCK(Sec61β−), respectively. Human and canine Sec61β amino acid sequences are identical.

**Cell Culture** MDCK(Sec61β−) and MDCK(Sec61β+) cells were cultured in 24-well Transwell® dishes (Corning Inc., Corning, NY) at 37°C in DMEM supplemented with FBS, PSS, and NEAA at 500, 50, 5, and 5 v/v in an incubator at 95% relative humidity and 5% CO2. The media was replenished daily until TER reached a constant value.

**TER Assay** Resistance was monitored using a Millicell® ohmmeter (Millipore) every 24 h until the resistance became constant. TER of cell monolayers at time $t$ \([TER(t)]\) was calculated as $TER(t) = [R_{obs}(t) - R_{blank}] \times A$, where $R_{obs}(t)$, $R_{blank}$, and $A$ denote resistance observed at $t$, resistance of the blank (without cell monolayer), and the surface area of single wells, respectively. At constant TER, C-CPE (0.01 mg/mL) was added to the basal compartment. TER was then measured at the reported times. We concurrently performed TER time course experiments in the absence of C-CPE as a baseline for both cells. At least four independent experiments were performed in triplicate, and $t=0$ was defined as the point at which C-CPE was added. In both cell types, relative C-CPE-induced lactate dehydrogenase release rates$^{26}$ were <1% for 24 h after C-CPE treatment, indicating negligible cytotoxicity during the time-course experiments.

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblotting** After SDS-PAGE, proteins were electrophoretically transferred onto PVDF membranes, which were then immersed in 2% Block Ace® from Megmilk Snow Brand (Tokyo, Japan) in TBST (0.1 M Tris–HCl and 0.1 M NaCl) and incubated with primary Abs for 1 h at room temperature (RT). After the membranes were washed thrice with TBST, the respective secondary Abs were applied at RT for 1 h. After washing thrice in TBST, membranes were incubated with ECL prime from GE Healthcare Japan (Tokyo, Japan) for 5 min to visualize protein bands. Band intensities were quantified with a LAS-4000 mini instrument (ImageQuant®) from GE Healthcare Japan.

**Reverse Transcription-Polymerase Chain Reaction (RT-PCR)** Total RNA was extracted from cell pellets at $t=0$, 24, 32, and 48 h using an RNeasy® kit from Qiagen (Tokyo, Japan) and reverse-transcribed into cDNA using a Supercript™ First-strand synthesis system from Invitrogen. The quantity of cDNA was measured using a Picogreen® kit from Invitrogen and used as template in PCR using a TaqMan® probe assay kit from Qiagen. Forward and reverse primers and the FAM/TAMRA probe for claudin-4 have the following sequences: 5′-gcctgcaaggtgta-3′, 5′-ctccattcctcagcatt-3′, and 5′-ctctcattcctcagcatt-3′, respectively. Mixtures of primers, probe, distilled water, and PCR Master Mix from Life Technologies (Tokyo, Japan) were incubated at 50°C for 2 min and 94°C for 10 min. Fluorescence intensity was then monitored up to 40 cycles of 94°C for 10 s and 55°C for 60 s using a 7300 Fast Real-Time PCR System from Life Technologies. Quantities of claudin-4 mRNA amplicons of 153 bp were normalized with that of cDNA.

**Immunoprecipitation** MDCK(Sec61β−) or MDCK (Sec61β+) monolayers were lysed at $t=0$ and 32 h in 1 mL of lysis buffer containing [50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Nonidet® P40, 0.5% sodium deoxycholate (Sigma-Aldrich), and protease inhibitor (Roche Diagnostics, Tokyo, Japan)] at 4°C for 2 h. Lysates were then precleared by incubation with protein G-agarose from Sigma-Aldrich. Cell pellets were lysed with 1 µg of claudin-4 mAb or 2.5 µg of Sec61β pAb at 4°C for 3 h, followed by precipitation with protein G-agarose for 3 h at 4°C. The resulting precipitates were repeatedly washed in lysis buffer, separated using SDS-PAGE, and immunoblotted. Immuno-co-precipitates and precleared lysates were referred to as IP and L, respectively, for the subsequent analysis. The $r$ statistic was calculated as $r = [I(claudin-4)_{IP}/I(claudin-4)_{L}] / [I(Sec61β)_{IP}/I(Sec61β)_{L}]$. The $r$ statistic indicates the proportion of total claudin-4 associat-
ed with Sec61β in the lysate and was calculated for subsequent comparisons: e.g., \( I(\text{claudin-4})_{\text{IP}} \) denotes the band intensity of claudin-4 in IP.

**Immunofluorescence Microscopy** Cell monolayers were fixed in 1% formaldehyde at 4°C for 3 h and incubated in 0.1% Triton X-100 for 15 min. After rinsing with PBS, fixed cells were blocked with 5% BSA in PBS for 45 min and incubated with claudin-4 and Sec61β primary Abs at RT for 1 h. After washing several times in PBS, cells were incubated with the respective secondary Abs at RT for 1 h. After washing again in PBS, cells were carefully mounted onto glass strips. Images of cells were generated using a confocal laser scanning...
Statistical Analyses. Data are presented as means±standard errors of the mean (S.E.M.). Comparisons between cell types MDCK(Sec61β−) and MDCK(Sec61β+) were made using Students’ t-test and differences were considered significant at p<0.05.

RESULTS

Effects of Sec61β Overexpression on C-CPE-Mediated Modulation of TER After confirming that relative Sec61β expression levels in MDCK(Sec61β+) cells (Sec61β overexpression) were higher than those in MDCK(Sec61β−) (control) and MDCK parent cells (Fig. 1), we measured the time course of TER.

TER(t) increased up to the initial time point (t=0), decreased upon addition of C-CPE, and increased again after removal of C-CPE (t=24 h) (Fig. 2A). Phase 1 (t<0), phase 2 (0≤t<24 h), and phase 3 (t≥24 h) corresponded to formation, opening, and reformation of TJs, respectively. TER(0) differed significantly between MDCK(Sec61β−) (1340±110 Ωcm²) and MDCK(Sec61β+) (2150±250 Ωcm²), indicating that Sec61β overexpression resulted in increased TER. Twenty-four hours after C-CPE addition, TER(24) decreased to approximately 10% of TER(0), and 24 h after removal of C-CPE, TER recovered in both cells (Fig. 2A).

To quantify TJ modulation rates, we calculated $T_{1/2}$ as the time required for $TER_{N}(t)=|TER(t)/TER(0)|/|TER(0)/TER(0)|=0.5$ (Fig. 2B) in phase 2. Superscripts + and −, indicate with and without C-CPE treatment, respectively. The denominator $TER(t)/TER(0)$ was used to correct for baseline TER. In phase 3, $TER_{N}(t)$ was extrapolated to $TER_{N}(0)=0$, and the time was calculated from this point (Fig. 2B) to correct for non-zero TER(N,24). The correction was approximately 12% of $T_{1/2}$ in both cell types. $T_{1/2}$ corresponded approximately to the time required for the paracellular resistance to become half of that at $t=0$. Of note is that $T_{1/2}$ is commonly used as a measure for degradation and recovery rates of certain chemical species, including claudins.9,29)

$T_{1/2}$ during phase 2 was significantly smaller than that during phase 3 in both cell types, indicating that TJ opening was more rapid than TJ reformation. In addition, $T_{1/2}$ in MDCK(Sec61β+) cells was smaller in phase 2 and markedly smaller in phase 3 than that in MDCK(Sec61β−) cells, indicating that TJ opening and reformation in MDCK(Sec61β+) was more rapid than those in MDCK(Sec61β−) cells (Fig. 2C). These observations suggest that the overexpression of Sec61β

![Fig. 3. Effects of Sec61β on C-CPE-Mediated Modulation in Claudins Protein Expression](image_url)

(A) Immunoblotting for claudin-1, claudin-4, Sec61β, and GAPDH at t=0 (just before C-CPE addition), 24 h (C-CPE removal), 32 h (8 h after C-CPE removal), and 48 h (24 h after C-CPE removal) in MDCK(Sec61β−) and MDCK(Sec61β+) cells. Molecular weights of claudin-1 and claudin-4 are approximately 22 kDa, and those of Sec61β and GAPDH are 11 and 36 kDa, respectively. (B) Relative claudin-4 protein expression is shown (top). Protein band intensities are normalized with respect to the loading control GAPDH. Error bars represent standard errors of the mean of at least four independent experiments. Relative claudin-4 mRNA expression is shown (bottom). The quantity of claudin-4 mRNA is normalized with respect to loading control cDNA. Normalized quantity for MDCK(Sec61β−) cells at t=0 is defined as 1.0 in both protein and mRNA expression. Open and filled columns denote MDCK(Sec61β−) and MDCK(Sec61β+) cells, respectively. Data are presented as means of duplicate experiments.
increased TJ reformation rates.

**Effects of Sec61β Overexpression on C-CPE-Mediated Claudin Expression** Figure 3A shows the immunoblotting of claudin-1, -4, Sec61β, and GAPDH in MDCK(Sec61β−) and MDCK(Sec61β+) cells. Band intensity was normalized with respect to the loading standard GAPDH. Sec61β expression levels in MDCK(Sec61β+) cells were 1.3–1.5-fold higher than those in MDCK(Sec61β−) cells for the entire time course, indicating that C-CPE did not alter the effect of Sec61β. In claudin proteins, claudin-1 expression in MDCK(Sec61β−) cells remained comparable with that in MDCK(Sec61β+) cells and was not affected by the presence or absence of C-CPE. In contrast, claudin-4 expression in MDCK(Sec61β+) cells was higher than that in MDCK(Sec61β−) cells over the entire time course. Moreover, claudin-4 expression was significantly attenuated (t=24h) and restored (t=48h) upon the addition or removal of C-CPE, respectively, in both cell types (Fig. 3B top). Of note is that claudin-3 expression was negligibly small in both cell types over the entire time course (data not shown). Reportedly, claudin-4 principally regulates TER in MDCK confluent cell monolayers.10–12 Accordingly, hereafter we focus on claudin-4.

Claudin-4 mRNA expression was determined with the aim of assessing whether upregulation of claudin-4 protein by Sec61β overexpression was transcriptional or post-transcriptional. Claudin-4 mRNA level was equal in the two cell types at t=0, 24, 32, and 48 h (Fig. 3B bottom), indicating post-transcriptional upregulation of claudin-4 at t=0. At t=32 h, the claudin-4 mRNA level returned to the initial value, then remained. Although mRNA expression was increased by ap-

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**Fig. 4. Effects of Sec61β on C-CPE-Mediated Modulation of Claudin-4 Protein Distribution**

(A) Confocal laser scanning microscopic images for claudin-4 (green) and Sec61β (red) at t=0, 24, 32, and 48 h. Claudin-4 and Sec61β were double stained: the scale bar represents 20 μm. (B) Enlarged merge images of claudin-4 and Sec61β in MDCK(Sec61β−) and MDCK(Sec61β+) cells at t=0 and 32 h. Yellow particles represent co-distribution (arrow head) of claudin-4 and Sec61β: the scale bar represents 20 μm. (C) Distribution of Sec61β (left) and calnexin (right) in MDCK(Sec61β−) cells at t=0: images were obtained from independent experiments. Arrow and arrow heads represent ER and outside ER, respectively: the scale bar represents 10 μm.
proximately 2.5-fold in both cell types upon the addition of C-CPE, protein expression decreased.

Effects of Sec61β Overexpression on C-CPE-Mediated Claudin Distribution

We evaluated the effects of Sec61β overexpression on the distribution of claudin-4 after treatments with C-CPE (Fig. 4A). Sec61β expression and distribution were unaffected by C-CPE treatments in both cell types, so that Sec61β remained stable during the experimental time course. Claudin-4 strongly segregated at plasma membranes at \( t=0 \) in both cell types and then became delocalized into the cytoplasm upon addition of C-CPE. Only weak segregation of claudin-4 in the plasma membrane was observed as a diffuse pattern at \( t=2 \) (not shown) and 24h, and again segregated toward the plasma membrane at \( t=32 \) and 48h. Such C-CPE-mediated claudin-4 translocation from and segregation toward plasma membranes has been reported in MDCK cells.6) The discontinuity of claudin-4 at plasma membrane at \( t=32h \) in MDCK(Sec61β-) cells was more marked than that in MDCK(Sec61β+) cells. Furthermore, at \( t=48h \), claudin-4 was still discontinuous in MDCK(Sec61β−), while was continuous in MDCK(Sec61β+) (Fig. 4A). These observations indicated that C-CPE-mediated claudin-4 segregation toward plasma membrane in MDCK(Sec61β+) cells occurred substantially faster than that in MDCK(Sec61β−) cells.

We evaluated the degree to which claudin-4 and Sec61β are co-distributed. At \( t=0 \) co-distribution near plasma membranes was weak in both cell types, and at \( t=24h \), co-distribution was not detectable owing to attenuated claudin-4 signals in both cell types. At \( t=48h \) co-distribution was similar in both cell types (Fig. 4A). At \( t=32h \), co-distribution near plasma membranes in MDCK(Sec61β+) cells was stronger than that in MDCK(Sec61β−) cells (Fig. 4B). Optical slicing experiments indicated simultaneous distribution of claudin-4 and Sec61β near plasma membranes. The ER marker calnexin was distributed in perinuclear regions, whereas Sec61β was distributed both at (dark) and outside (bright) the ER (Fig. 4C). These observations indicate that the co-distribution of Sec61β with claudin-4 occurred principally outside the ER and near plasma membranes.

Claudin-4 Association with Sec61β

To determine whether claudin-4 associated with Sec61β in the cytoplasm of MDCK(Sec61β+) and MDCK(Sec61β−) cells at \( t=0 \) and 32h, we performed immunoprecipitation (Fig. 5). At \( t=0 \), claudin-4 association with Sec61β in both cell types was weak. At \( t=32h \), claudin-4 association with Sec61β in MDCK(Sec61β−) cells was weak, while that in MDCK(Sec61β+) was detectable. These observations were in agreement with immunofluorescence microscopic observations. A similar tendency of claudin-1 was observed in both cell types. In Drosophila megatracea, the invertebrate homolog of claudin was also shown to associate with Sec61β.30 Importantly, the r values (Materials and Methods: Immunoprecipitation) were found to be less than 0.05 in all cases. Further, extraction with Triton X-100 rather than NP-40 resulted in weaker claudin-4 (\( r<0.01 \)) and claudin-1 (\( r=0.019 \)) association with Sec61β (Fig. 5, 3rd panel); Note that Triton X-100 gives less damage on plasma membrane (and hence less extraction of molecules in cytoplasm) than NP-40.31

These observations indicated that only a small proportion of claudin was associated with Sec61β due to limited complex yields during the experimental procedure. Transport vesicles containing claudins associate with the same exocytosis complex as Sec61β.31 NaCl and NP-40 treatments reportedly lead to extraction of proteins,31 implying indirect binding between Sec61β and the claudins.

DISCUSSION

We discovered that Sec61β overexpression enhanced TER and upregulated claudin-4 expression (Figs. 2A, 3B top). Sec61β overexpression mediated post-transcriptional upregulation of claudin-4 expression (Fig. 3B) as reported for other basolateral membrane proteins19,20 resulting in enhanced TER. TER in MDCK(Sec61β+) cells was approximately 1.5-fold greater than that in MDCK(Sec61β−) cells at \( t=0 \), and the ratio was comparable to that of claudin-4 protein expression (approximately 1.6-fold), in accord with the correlation between TER and claudin-4 in MDCK cell monolayers.10 In contrast, Sec61β overexpression did not upregulate claudin-1 expression. Difference in amino acid sequence of the two claudins may cause such contrasted behavior. Detailed studies are remained for future works.

We further demonstrated that \( T_{1/2} \) was shorter in MDCK(Sec61β+) cells than in MDCK(Sec61β−) cells, par-

Fig. 5. Immuno-Co-precipitation of Claudins and Sec61β in MDCK(Sec61β−) and MDCK(Sec61β+) Cells

The 1st panel shows immuno-co-precipitation with Sec61β pAb and subsequent IB with Sec61β, claudin-1 or claudin-4 antibodies at \( t=0 \) and 32h in MDCK(Sec61β−) cells. “IP,” “Ab,” and “L” denote immuno-co-precipitate, Sec61β pAb, and lysate, respectively. The 2nd panel shows immuno-co-precipitation with Sec61β pAb and subsequent IB with Sec61β, claudin-1 or claudin-4 antibodies at \( t=32 \) in MDCK(Sec61β+) cells. Sample dilutions were used for subsequent calculation of r.
particularly in phase 3 (Fig. 2C). Claudin-4 synthesis and subsequent delivery to TJ were involved in TJ reformation, as evidenced by increased claudin-4 expression and segregation toward plasma membranes (Figs. 3B top, 4B). Claudin-4 segregation occurred faster, and co-distribution and association of Sec61β with claudin-4 was more marked in MDCK(Sec61β+) cells compared with those in MDCK(Sec61β−) cells during phase 3 (Figs. 4, 5). On the other hand, the increments in claudin-4 expression during phase 3 were not systematic: MDCK(Sec61β+) cells, 26% (t=24–32h) and 68% (t=24–48h), MDCK(Sec61β−) cells, 13% (t=24–32h) and 116% (t=24–48h), implying no clear evidence of enhanced claudin-4 synthesis rate. These observations indicate that Sec61β overexpression increases TJ reformation rates by increasing the delivery of claudin-4 to TJ after C-CPE removal, in accord with Sec61β overexpression-mediated upregulation in delivery of other secretory and basolateral membrane proteins, where Sec61β/exocyst complexes are involved.23,25,26

In contrast, in phase 2, C-CPE associates with the ECL2 domain of claudin-4 and forms sequestering complexes at the basolateral portion of plasma membranes.6 Most of the resulting complexes undergo degradation, leading to disruption of TJ strands as evidenced by the reduction in claudin-4 expression (Fig. 3B) and removal of claudin-4 from the plasma membrane (Fig. 4A). Furthermore, concomitant upregulation of mRNA with downregulation of protein suggested claudin-4 degradation. \(T_{1/2}\) in phase 2 would thus reflect the degradation rate of claudin-4 in TJ. Given that Sec61β enhances protein delivery from trans-Golgi network to plasma membranes,22 the effect of Sec61β overexpression on \(T_{1/2}\) in phase 2 is limited.

Both precise control of TJ opening and reformation are critical to drug delivery systems (DDSs) when the paracellular route is utilized, given that slow TJ reformation can have undesirable side effects. Our observations showing that Sec61β overexpression upregulates TJ barrier functions predominately through upregulated delivery of claudin-4 suggest a novel strategy for DDS. In particular, precise knowledge of the relationship between TJ modulation and protein trafficking between subcellular compartments is required for better DDS and awaits future investigation.

**CONCLUSION**

The effects of Sec61β overexpression on TJ modulation upon the addition and removal of C-CPE in MDCK cells were investigated by monitoring the time course of TER and claudin expression and distribution. Overexpression of Sec61β led to post-transcriptional upregulation of claudin-4 and increased TER. Sec61β overexpression also caused enhanced TJ opening and reformation in response to C-CPE treatments by increasing claudin-4 removal from and segregation toward plasma membranes, respectively. Sec61β was found to associate indirectly with claudin-4, contributing to the enhanced delivery of claudin-4. Importantly, these effects appeared through exogenously overexpressed Sec61β despite substantial amount of endogenous Sec61β expression in MDCK cells. We concluded that Sec61β may be a novel target for TJ modulation, including barrier functions and modulation rates, providing a novel strategy for drug delivery.

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