The Spatial Organization of Corneal Endothelial Cytoskeletal Proteins and Their Relationship to the Apical Junctional Complex

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Purpose. To determine the spatial organization of the major cytoskeletal proteins and their relationship to the apical junctional complex (AJC) in the normal rabbit corneal endothelium.

Methods. Normal endothelial cytoskeletal structure in three dimensions was studied in rabbit eyes by laser scanning confocal microscopy after en bloc immunocytochemical staining of whole corneal tissue with various antibodies and fluorescent probes; specificity of antibodies to rabbit corneal endothelial cell proteins was established by Western blot analysis.

Results. Normal actin microfilament network organization was seen predominantly as a complex apical array forming a circumferential bundle. The tight junction-associated protein ZO-1 was positive at the apical junctions, forming a hexagonal pattern that was localized between and just proximal to the circumferential actin microfilament bundles. The distribution of ZO-1 was discontinuous around the cell, with the largest gaps (1 μm in diameter) occurring at the Yjunction between adjacent endothelial cells; transmission electron microscopy of the apical face of the endothelium confirmed the existence of 1-μm diameter gaps in the adherens junctions located at the Yjunction. Antivimentin antibodies showed a ring of intermediate filaments located just below the circumferential actin microfilament band. This ring appeared to be continuous with a basal mat of filaments, which together formed a basketlike structure within endothelial cells. An intricate cytoplasmic, perinuclear network of microtubules was observed by antitubulin antibodies that appeared unrelated either to the apical circumferential actin microfilament bundle or to intermediate vimentin filament ring. Staining of endothelial cells with NBD-ceramide identified a prominent, perinuclear Golgi complex suggesting an association between microtubules and Golgi.

Conclusions. The organization of cytoskeletal elements and the tight junction-associated protein ZO-1 is similar to the classical AJC of transporting epithelia, comprised of a zonulae occludens (ZO) located apical to a zonulae adherens (ZA) and desmosomes. The organizational pattern seen in corneal endothelial cells, however, is distinct from transporting epithelia in that the ZO and ZA are discontinuous, with large gaps in the ZO-1 distribution at the Yjunction between adjacent endothelial cells. The authors propose that the structural differences in the AJC underlie the functional differences between classical transporting epithelia, which actively pump fluid from the lumen to the mucosa, and the corneal endothelium, which has a "pump-leak" fluid transport mechanism. Invest Ophthalmol Vis Sci. 1995;36:1115—1124.
corneal endothelium. Although reports by Gordon et al and others have shown the corneal endothelial microfilament system to be organized into a prominent, circumferential band, how the organization of this "band" is related to endothelial barrier either structurally or functionally is unknown.

In transporting epithelia, barrier function has been linked to the apical junctional complex (AJC) comprised of a zonulae occludens (ZO), zonulae adherens (ZA), and desmosomes (D) from proximal to distal, respectively. Actin-binding proteins, i.e., vinculin and a-actinin, have been localized by immunocytochemistry to the cytoplasmic region adjacent to ZO, suggesting a potential interaction with the cortical microfilament network. Furthermore, the formation of ZA has been associated with the development of a circumferential microfilament bundle, whereas the formation of D has been linked to the intermediate filament system in a keratinocyte cell line. Although the development of the AJC has been shown to involve calcium-dependent, cell-cell interactions mediated by E-cadherin or uvomorulin, the formation of ZO, ZA, and D after low calcium treatment is blocked by cytochalasin, indicating that microfilaments also play an important role in the initial formation of the AJC in transporting epithelia. Furthermore, cytochalasin treatment of intact epithelial monolayers has been shown in various systems to result in decreased resistance along the AJC whereas exposure to phallacidin increased resistance, suggesting that microfilaments might be involved in a dynamic regulation of the AJC barrier function.

Whether the cytoskeletal and the AJC interactions identified in transporting epithelia are similar to those of the "leaky" barrier system associated with the corneal endothelium is unknown. We have, therefore, sought to address this question by first identifying specific organizational interrelationships between the major cytoskeletal proteins—including microfilaments, intermediate filaments, and microtubules—and the AJC. Studies of hepatocyte, ZO-rich cell fractions have identified a monoclonal antibody against a 225-kd protein (ZO-1) that specifically localizes to the cytoplasmic region of the tight junction by immunoelectron microscopy. Using anti-ZO-1 as a marker for the tight junction in corneal endothelium, we determined the three-dimensional organization of the corneal endothelial cytoskeleton and its spatial relationship to the AJC. The data indicate that there are major differences in the AJC between the classical transporting epithelial barrier system and the leaky barrier system of the corneal endothelium. Specifically, the AJC in the leaky endothelial barrier is discontinuous with gaps in the ZO and ZA as much as 1 µm in diameter observed apically at the Y junction between adjacent cells. Furthermore, the circumferential microfilament band associated with ZA in the corneal endothelium appears to be more complex and extensive, suggesting that microfilaments have an active regulatory role in modulating barrier integrity and, hence, resistance to solute movements.

**METHODS**

**Animals**

Thirty New Zealand albino rabbits weighing 2.5 to 3.0 kg were used for this study. Animals were anesthetized with xylazine 2 mg/kg body weight and ketamine 30 mg/kg body weight. Animals were killed with pentobarbital 100 mg/kg body weight, and their corneas were removed immediately for in situ immunolabeling or electron microscopy. Rabbits were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**In Situ Immunolabeling**

Before removal, corneas used in immunolocalization studies were perfused through the anterior chamber with 1% paraformaldehyde in phosphate buffer for 5 minutes, except for specimens used in the localization of the Golgi and microtubules. The corneas were then removed and rinsed in phosphate buffer, pH 7.4, and permeabilized in acetone (−20°C) for 5 minutes. The tissues were rehydrated in buffer containing 1% dimethyl sulfoxide and 5% dextran 40. For Factin labeling, the tissues were incubated for 2 hours in fluorescein phalloidin, diluted 1:5 in phosphate buffer, and observed by laser scanning confocal microscopy. For antibody labeling, nonspecific staining was blocked by incubation with 0.1 mg/ml whole goat serum for 30 minutes. The serum was removed, and tissues were incubated for 2 hours in mouse monoclonal antivimentin IgG (Clone V9, ICN; Irvine, CA), mouse monoclonal anti-β-tubulin IgG (Clone DM-1B; ICN), or rat monoclonal anti-ZO-1 IgG (Clone MAB 1520; Chemicon, Temecula, CA), all at a 1:100 dilution in the above buffer. After removal of the primary antibodies and subsequent washes in buffer, the tissues were incubated for 1 hour in 10 µg/ml fluorescein or rhodamine conjugated affinity-purified goat anti-mouse IgG or goat anti-rat IgG (Organon Teknika, Durham, NC). The secondary antibody was removed, and the tissues were rinsed in buffer. For double labeling of Factin, the tissues were incubated in fluorescein phalloidin simultaneously with the rhodamine secondary antibodies.

To label the Golgi complex, fresh corneas were incubated in 10 µg/ml NBD ceramide (Molecular Probes, Eugene, OR) in minimal essential medium (MEM) for 10 minutes, then rinsed for 10 minutes in MEM alone (Gibco, Gaithersburg, MD) at 37°C. To double label the cell nuclei and Golgi, tissues were fixed and incubated with 10 µg/ml propidium iodide.
(Sigma, St. Louis, MO). Double labeling of Golgi and microtubules could not be performed because extraction of the cells with acetone was necessary to obtain penetration of the anti-β-tubulin antibodies, which caused concomitant release of NBD-ceramide from the Golgi membranes.

Electron Microscopy

Corneas were fixed with 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.4, for 10 minutes by anterior chamber perfusion. Corneas were removed and fixed in glutaraldehyde overnight. The specimens were postfixed for 1 hour with 1% osmium tetroxide in 0.2 M phosphate, pH 7.4, and dehydrated in graded ethanol solutions. Then graded ethanol dehydration tissues were embedded in spur and polymerized overnight in a 60°C oven. Ultramicrotome sections were cut and stained with uranyl acetate and lead acetate and examined on a JEOL (Peabody, MA) 1200 EX transmission electron microscope.

Laser Confocal Microscopy

Imaging of specimens was performed using the Leica confocal Laser scanning microscope (Leica, Deerfield, IL). The system consisted of a Leica Fluovert FU inverted epifluorescent microscope and an argon–krypton laser source that allowed dual wavelength excitation. Three-dimensional data sets (x-series) were obtained by collecting images in 0.5-μm steps beginning at the basal part of the cells and ending apically using a Zeiss (Thornwood, NY) 40X, NA = 0.9, or a Leitz (Deerfield, IL) 63X, NA = 1.4, objective. Image processing and analysis was performed on Silicon Graphics Personal IRIS workstations (Silicon Graphics, Mountain View, CA) using the ANALYZE software package (Mayo Medical Ventures, Rochester, MN) for general processing and analysis of two- and three-dimensional data sets. Copies of digital images were made using an AGFA–Matrix film recorder with Plus X, 125 ASA black-and-white, 4 × 5 sheet film (Eastman Kodak, Rochester, NY).

SDS–PAGE and Western Blot Analysis

Rabbit corneal endothelial cells were cultured to obtain sufficient protein material for Western blot analysis and to confirm the specificity of antibodies used in immunolabeling. Descemet's membranes with attached endothelium were peeled from rabbit corneas obtained from Pel Freeze (Rogers, AR). Descemet's membrane was digested for 4 hours in 0.5 mg/ml hyaluronidase (Worthington Biochemical, Freehold, NJ) and 2.0 mg/ml collagenase (Gibco Life Technologies, Grand Island, NY) in MEM (Gibco) at 37°C. Endothelial cells were centrifuged, and the pellet was washed with MEM. Cells were plated at high density in 25 cm² tissue culture flasks (Becton–Dickinson, Oxford, CA) and grown in MEM supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO) and 1% pen/strep (Gibco) for 14 days.

Cells were washed with phosphate-buffered saline, pH 7.4, and solubilized in buffer containing 25 mM Tris–HCl (pH 7.4), 1% sodium dodecyl sulfate, 1 mM EDTA, 1 mM EGTA, 10 mM dithiothreitol, 5 μg/ml antipain, 5 μg/ml pepstatin A, and 1 mM phenylmethylsulfonyl fluoride. Cells were scraped from the tissue culture flask, transferred to test tubes, and sonicated for 5 minutes. Proteins were quantified using the Bradford technique. Samples were made 1% in β-mercaptoethanol, boiled 5 minutes, and run on 10% gels using standard techniques. Proteins were transferred to nitrocellulose paper (BioRad Laboratories, Hercules, CA), nonspecific binding was blocked with 1% ovalbumin (Sigma) in Tris saline (pH 7.4), and paper strips were washed and incubated overnight with various antibodies diluted 1:1000. Control strips were incubated without first antibody. Papers were washed and incubated for 2 hours with appropriate horseradish peroxidase conjugated secondary antibodies (diluted 1:500), and color was developed by diaminobenzidine reaction.

RESULTS

Antibody Specificity

Cellular proteins collected from tissue-cultured rabbit corneal endothelial cells and analyzed by Western blotting (Fig. 1) showed staining of single-protein bands by anti-human ZO-1 (lane 2), anti-human vimentin (lane 3) and anti-human β-tubulin (lane 4) with molecular weights of 225 kd, 58 kd, and 55 kd, respectively. These molecular weights are consistent with the published molecular weights for the human protein species and confirm that these antibodies cross-react with the appropriate corresponding rabbit ZO-1, vimentin, and β-tubulin proteins.

F-Actin and the Apical Junctional Complex

Fluorescein phalloidin staining of normal corneal endothelium shows the presence of two distinct and spatially separated actin microfilament populations, one located basally and the other apically (Fig. 2). In the basal region of the cell, microfilaments are organized into short, randomly arranged bundles that might be associated with cell–matrix interactions between the endothelium and Descemet's membrane (z = 2 μm). Apically, microfilaments are organized into a distinct circumferential band, as has been noted by Gordon and others (z = 6 μm). Note that in the normal endothelium, the circumferential band of microfilaments in individual cells appears extensive yet is separated from those in adjacent cells (arrows). This double-band appearance is distinctly different from that reported for transporting epithelia that show a simple

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FIGURE 1. Western blot analysis of endothelial proteins run on 10% reducing SDS–PAGE gel. Lane 1: Coomassie blue stain of protein preparation; lane 2: immunoblot of anti-ZO-1; lane 3: anti-vimentin; lane 4: anti-β-tubulin.

outlining and an apparent fusion of the microfilament bundles between cells.13,14

Colocalization of the apical microfilament bundles and apical tight junctions in the corneal endothelium was identified by dual fluorescent staining of f-actin (Fig. 3, left side) with rhodamine phalloidin and the tight junction-associated protein ZO-1 (Fig. 3, right side) with monoclonal anti-ZO-1 labeled by fluorescein isothiocyanate goat anti-rat IgG. ZO-1 was associated with only the apical circumferential (z = 6 μm), not basal (z = 2 μm), microfilament bundles.

To compare the localizations of Factin and ZO-1, both z-series were reconstructed by stacking the images, selecting a two-dimensional slice through the series (obtained from the area marked by the dotted line in the 6-μm image), and rotating the slices 90° to view in cross-sectional orientation (X–Z plane). The resulting X–Z reconstructions (Fig. 3, bottom) are displayed so that the top is apical, the bottom is basal, and the line at the bottom of the image represents the approximate location of Descemet’s membrane. When corresponding areas on both images are compared (Fig. 3, bottom, arrows), the ZO-1 appears to be associated closely with and located between and just apical to the f-actin microfilament bands. This pattern is consistent with the structural organization of a tight barrier AJC comprised of a ZO proximal to a ZA-associated microfilament bundle.

Examination of the ZO-1 distribution under higher magnification revealed the presence of gaps in staining along the lateral cell border, demonstrating that the corneal endothelial ZO was not circumferentially continuous around the cells (Fig. 4A, arrows). Although this seems consistent with a leaky barrier, the gaps appear to occur uniformly at the Yjunctional area between three adjacent endothelial cells (Fig. 4, window). At these locations, the separation in ZO-1 staining was as much as 1 to 2 μm (Fig. 4, inset); furthermore, these gaps in ZO appear to correlate with gaps in ZA as identified by transmission electron microscopy of the corneal endothelial cells (Fig. 4B). Transmission electron microscopic sections cut through the apical face of the endothelium showed that the lateral cell–cell borders are lined by characteristic ZA with interdigitating, electron-dense cell junctions (arrows) and circumferential microfilament bundles (CF). Note, however, that there appear to be no adherens junctions at the intersection between three adjacent endothelial cells or the Yjunction (curved arrow). Because the separation in ZA at the Y-junction measures approximately 1 μm, similar to that for ZO-1, it appears that there is a consistent major discontinuity in the AJC at this region.

Interestingly, a distinct, apical central spot of positive ZO-1 staining not associated with F-actin also was observed in each cell (Fig. 3, X–Z, asterisk; Fig. 4A, curved arrow). It is unclear what this staining indicates, though it may be that ZO-1 is associated with the attachment site for endothelial cilia.

F-Actin and Intermediate Filament Organization
Double labeling of the corneal endothelium with phalloidin (Fig. 5, left column) and antibodies to vimentin (Fig. 5, right column) revealed the presence of a prominent network of intermediate filaments located in the base of the cells, which showed no correlation with the basal microfilament bundles (z = 2 μm). Apically, the intermediate filament network formed a ring that appeared to be continuous with adjacent endothelial cells (z = 6 μm, curved arrows). The apical intermediate filament ring appeared to overlap the microfilament network near the apical cell–cell junctions (z = 6 μm, arrows). However, slicing of serial reconstructions in the X–Z plane showed that the most intense staining of the apical intermediate filament network was located slightly below the apical circumferential bands (data not shown). Overall, the intermediate filaments appeared to form a basketlike structure that was continuous from the basal mat to the apical ring.

Microtubules
Anti-β-tubulin staining of microtubules showed a fine perinuclear microtubule network with no apical distri-
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bution (Fig. 6, left column). The position of the micro-
tubule bundle was confirmed by double labeling cell 
nuclei with propidium iodide (Fig. 6, right column). 
The microtubule bundle appeared to surround the 
nuclei and, in some instances, was observed crossing 
the nuclei (arrow). This perinuclear location is similar 
to that of the Golgi in endothelial cells. Cells stained 
with propidium iodide (Fig. 7A) and NBD-ceramide, 
a marker for the Golgi complex (Fig. 7B), showed a 
large Golgi complex located adjacent to nuclei, similar 
to the microtubule network.

**DISCUSSION**

As indicated by the localization of anti-ZO-1 antibody 
staining proximal to the apical f-actin circumferential 
bundle, three-dimensional immunocytochemical data 
suggest that the AJC of the corneal endothelium is 
similar spatially to that of classical transporting epithe-
ilia in that there is a ZO proximal to the ZA.7 Although 
markers for D junctions were not used, the presence of 
an intermediate filament ring just distal to the apical 
microfilament-associated ZA is consistent with the clas-
sical AJC comprised of ZO, ZA, and D. The endothe-
lium also has a prominent microtubular network that 
does not appear to have any particular localization to 
the AJC apically and seems to be closely associated 
with the large Golgi complex. The association of the 
microtubules with the Golgi complex might be indica-
tive of their function in the intracellular transport of 
proteins.

Major differences between the AJC of the corneal 
endothelium and the classical AJC of transporting epi-
theilia were also observed. First, the ZO and ZA identi-
fied by immunocytochemical staining and transmis-
sion electron microscopy are not continuous around 
the endothelial cells. Rather, there are major disconti-
uities of as much as 1 μm in the ZO and ZA that 
appear to occur preferentially at the Yjunctions. 
These gaps in ZO-1 staining might correspond to 
“free ends” in the tight junctional strands observed 
by Stiemke et al9 in freeze-fracture studies of neonatal 
and mature rabbit corneal endothelium. These find-
ings are distinct from classical transporting epithelia 
that have continuous ZO and ZA around the cell, with 
more intense anti-ZO-1 staining at the Yjunctions.13 
Additionally, the organization of the ZA-associated cir-
cumferential microfilament band is more complex in 
the corneal endothelium and appears to be separated 
from adjacent endothelial cells. This is in contrast to 
a simple circumferential microfilament band that ap-
pears fused and continuous between cells in trans-

**FIGURE 2.** F-actin distribu-
tion. Corneal endothelium 
stained with fluorescein 
phalloidin. Images were 
taken from a three-dimen-
sional data set (z-series) ob-
tained by imaging every 0.5 
μm from the basal part 
of cells to the apical surface. 
Two distinct microfilament 
populations are observed— 
short, random bundles bas-
ally (z = 2 μm) and a double 
hexagonal outline of individ-
ual cells (z = 6 μm, 
arrows)—that appear to be 
continuous between cells at 
Yjunctions apically.
porting epithelia. Overall, these cytoskeletal variances indicate differences in the barrier structure that might underlie the functional differences in net fluid transport between these two cell types.

In transporting epithelia, fluid moves unidirectionally along an osmotic gradient from the luminal surface to the mucosal surface (lumen-to-mucosa transport) established by a tight, high-resistance, apical junction and a basolateral Na+K+-ATPase pump. This unidirectional transcellular movement of fluid is reversed in some secretory epithelia in which fluid transport from the mucosal to the luminal surface (i.e., lumen-to-mucosa transport) involves an apical Na+K+-ATPase pump and a basolateral tight junctional complex (e.g., choroid plexus). By contrast, fluid movement in the corneal endothelium is thought to be related to a “pump-leak” transport mechanism wherein fluid from the anterior chamber
FIGURE 4. (A) High magnification of corneal endothelium stained by anti-ZO-1 antibodies. "Gaps" in ZO-1 staining are observed around the cells (arrows), particularly at Y junctions (window, inset). Again a centrally located spot of ZO-1 is observed (curved arrow). (B) Transmission electron microscopic section through the apical face of the corneal endothelium shows the association of adherens junctions (arrow) with the circumferential, microfilament band (CF). Microfilaments are observed extending from the zonulae adherens (arrowheads). Note the absence of adherens junctions at the Y junctional region where three endothelial cells meet (curved arrow). Bar = 500 nm.
"leaks" into the stroma across a "leaky," low-resistance apical junction (passive lumen-to-mucosa transport) while fluid is actively "pumped" from the stroma into the anterior chamber (active mucosa-to-lumen transport). Deturgescence in the cornea is, therefore, thought to be regulated by a balance between these two fluid transport pathways.

Although the route of active fluid movement (i.e., transcellular or paracellular), is not known, current models are based on an ion-coupled transport mechanism that establishes a sodium activity gradient across the endothelial layer, as has been verified recently by Stiemke et al.21 Ion-coupled transport appears to be critically dependent on the ouabain-sensitive basolateral Na+K+-ATPase and the endothelial apical junctions,2223 which together are thought to establish a basal-to-apical sodium current through the high conductance "leaky" apical junctions, as proposed earlier by Fischbarg et al.24 Both passive "leak" and active "pumping" were thought to occur through the same paracellular route because it was assumed that the apical junctions are organized uniformly around the endothelial cell. The finding in this study that there are regional differences in the distribution of ZO and ZA contradicts this assumption and might have important implications regarding endothelial function and the "pump-leak" fluid transport pathways.

First, the discontinuous distribution of ZO-1 staining suggests that around an individual endothelial cell there are regions of high and low resistance, with areas of low resistance occurring preferentially at the Y-junctions. Therefore, the Y-junctions might represent major breaks in the barrier function of the endothelium and, hence, might be sites of enhanced permeability and fluid "leak" into the stroma. This conclusion is

FIGURE 5. Z-series of double-labeling of cells for F-actin (left column) and vimentin intermediate filaments (right column). Proteins appear to be closely associated apically (z = 6 μm, arrow), and the intermediate filaments appear to be continuous between cells (curved arrow). Basally (z = 2 μm), vimentin filaments are more abundant than F-actin and do not appear to be colocalized to F-actin.

FIGURE 6. Images from a three-dimensional data set of normal microtubule (anti-β-tubulin) distribution (left column) colocalized with propidium iodide staining of nuclei (right column). The perinuclear localization of the microtubule bundle is evident in each cell, appears to surround the nuclei, and at times crosses over nuclei (arrow). There does not appear to be any distinct difference in microtubule localization from basal to apical.
supported by the recent report of Andrews et al, which showed that water appears to move preferentially along channels located at the Y junctions under extreme physiologic, hyperosmolar conditions. The presence of a heterogeneous population of apical junctions also is consistent with structural and functional studies of occluding junctions in Madin-Darby canine kidney cells, which show “tight” and “leaky” regions of electrical conductivity that appear to correlate with a high (five to seven) or a low (zero to two) number of junctional strands, respectively. Whether other low-resistance, “leaky” epithelia (e.g., intestinal mucosa and gallbladder) have a Y-junctional organization similar to that observed in the endothelium is unknown; however, based on the work of Madora and Dharmasathaphorn, it is clear that in a heterogeneous population of junctions there is a wide range of “tight” and “leaky” apical junction configurations that might lead to a similar net resistance.

Second, the complex organization of the circumferential microfilament bundle and its close, spatial relationship to ZO-1 staining of apical tight junctions suggest that interactions between F-actin and ZO-1 might be important in modulating endothelial barrier function, perhaps by controlling the channel size of the Y junction. This possibility is supported by the observation that cytochalasin D, which disrupts the apical microfilament bundle, increases endothelial permeability to carboxyfluorescein. Additionally, cytochalasins have been shown to decrease resistance in Madin-Darby canine kidney cells and Necturus gallbladder by presumed microfilament interactions with tight junction-associated proteins leading to changes in complexity of tight junctional strands. Regulation of junctional permeability by interactions with F-actin are suggested also by studies showing that phalloidin and cytokinins, F-actin-stabilizing drugs, induce rapid and reversible increases in epithelial resistance. Overall, the possible regulation of endothelial permeability and barrier function by F-actin is contrary to the present view that the barrier does not change and therefore needs additional study.

Third, the presence of regulated, Y-junctional channels might influence active fluid transport. As discussed by Fischbarg, the active fluid “pump” of the corneal endothelium is thought to reside at sites of passive fluid “leak,” which represent areas of high electrical conductance. The Y junctions, as sites of very low resistance, represent regions of high electrical conductance that are consistent with an ion-coupled, paracellular fluid transport mechanism. If such is the case, changes in the size of the Y junction channel would be expected to have significant effects on the active “pump” because small changes in the diameter of the channel would affect hydraulic and ionic permeability. This might explain the finding that cytochalasin D, which increases endothelial permeability, has no effect on corneal deturgescence, presumably because of increased fluid “pump.” Alternatively, localization of low-resistance regions to the Y junction and regions of higher resistance along the borders of the cell could provide a means of segregating the two transport pathways (i.e., passive paracellular “leak”) through the Y junctions and active paracellular or transcellular “pump” through the lateral junctions.

In conclusion, the corneal endothelium appears to exhibit a unique apical junctional complex comprised, in part, of a discontinuous apical zonulae occludens and zonulae adherens. These discontinuities appear to occur preferentially at the Y junctions between three adjacent endothelial cells and might underlie the structural basis for the “leaky barrier” function exhibited by the endothelium. Based on previous studies of transporting epithelium and the findings presented here, we propose that the observed discontinuities in the AJC, predominantly at the Y junctions, represent sites of low resistance and increased hydraulic and ionic permeability, as proposed by Fischbarg et al and Stiemke et al. Furthermore, the prominence of the apical, circumferential, F-actin bundle and the known sensitivity of the corneal endothelium to microfilament-disrupting agents suggests that the “leaky” barrier, the active “pump” function, or both, might be regulated specifically by organizational changes in the F-actin network. Further studies of the state of actin organization during active endothelial fluid transport clearly are necessary to test this novel hypothesis.

**Key Words**
endothelium, cornea, microfilaments, intermediate filaments, microtubules, ZO-1, confocal microscopy, fluid transport

**References**


