Histochem Cell Biol (1998) 110:543–551

ORIGINAL PAPER

Nomingerel Tserentsoodol · Bo-Chul Shin
Takeshi Suzuki · Kuniaki Takata

Colocalization of tight junction proteins, occludin and ZO-1, and glucose transporter GLUT1 in cells of the blood-ocular barrier in the mouse eye

Accepted: 7 September 1998

Abstract The facilitative glucose transporter GLUT1 is abundant in cells of the blood-ocular barrier and serves as a glucose transport mechanism in the barrier. To see the relationship between the glucose transfer function and junctional proteins in the barrier, we examined the localization of GLUT1 and the tight junction proteins, occludin and ZO-1, in the mouse eye. Their localization in the retina, ciliary body, and iris was visualized by double-immunofluorescence microscopy and immunogold electron microscopy. Occludin and ZO-1 were colocalized at tight junctions of the cells of the barrier: retinal pigment epithelial cells, non-pigmented epithelial cells of the ciliary body, and endothelial cells of GLUT1-positive blood vessels. Occludin was restricted to these cells of the barrier. ZO-1 was found, in addition, in sites not functioning as a barrier: the outer limiting membrane in the retina, in the cell border between pigmented and non-pigmented epithelial cells in the ciliary body, and GLUT1-negative blood vessels. These observations show that localization of occludin is restricted to tight junctions of cells of the barrier, whereas ZO-1 is more widely distributed.

Introduction

Epithelia and endothelia divide the mammalian body into different compartments having a variety of functions. Different milieus in such compartments are provided and maintained by the surrounding endothelial and epithelial cells connected by tight junctions. The eye is an organ highly specialized to detect light and transmit its signal to the brain. The blood-ocular barrier, composed of the blood-retinal and blood-aqueous barriers, effectively blocks the entry of blood constituents, such as blood cells, which may perturb the specialized optical and sensory characteristics of the eye (Raviola 1977; Henkind et al. 1979).

In the blood-aqueous barrier, thin-section and freeze-fracture replica electron microscopy revealed that tight junctions connect non-pigmented epithelial cells in the ciliary body (Raviola 1977; Raviola and Raviola 1978). Another part of the blood-aqueous barrier is the endothelial cells of the blood vessels in the iris, where tight junctions connect them (Raviola 1977). In the blood-retinal barrier, endothelial cells in the retina and the outermost retinal pigment epithelial cells, both connected by tight junctions to each other, serve as the structural basis of the barrier (Raviola 1977). The barrier function of epithelia and endothelia is considered to be mostly dependent on tight junctions, which block the passage of molecules as small as inorganic ions through the paracellular pathway (Citi 1993; Anderson and Van Itallie 1995; Denker and Nigam 1998). Several tight junction proteins have been identified in a variety of mammalian tissues and cell lines. ZO-1, a 210–225-kDa cytoplasmic protein, was the first to be shown as a tight junction constituent (Stevenson et al. 1986; Anderson et al. 1988). Occludin is a transmembrane protein specific to tight junctions (Furuse et al. 1993). It is a 65-kDa protein with four putative transmembrane domains and its cytoplasmic C-terminal domain binds ZO-1 (Furuse et al. 1993, 1994). ZO-2, a 160-kDa cytoplasmic protein, was identified by coimmunoprecipitation with ZO-1 (Gumbiner et al. 1991). Other proteins which were shown to be localized to tight junctions include cingulin (Citi et al. 1988), 7H6 antigen (Zhong et al. 1993; Satoh et al. 1996), rab13 (Zahraoui et al. 1994), rab3B (Weber et al. 1994), symplekin (Keon et al. 1996), AF-6 (Yamamoto 1997), and ZO-3 (Haskins et al. 1998). However, the molecular architecture of tight junctions and the molecules involved in the regulation of tight junctional barrier function have not yet been fully elucidated.

The mammalian retina is a specialized part of the central nervous system characterized by an extremely high

N. Tserentsoodol · B.-C.Shin · T.Suzuki · K.Takata (✉)
Laboratory of Molecular and Cellular Morphology,
Department of Cell Biology,
Institute for Molecular and Cellular Regulation,
Gunma University, Maebashi, Gunma 371–8512, Japan
e-mail: takata@akagi.sb.gunma-u.ac.jp
Tel. +81–27–220–8840; Fax +81–27–220–8844

energy requirement (Ames et al. 1992). Glucose is the primary source of metabolic energy for most mammalian cells and its transport across the plasma membrane is carried out by membrane proteins named glucose transporters (Wheeler and Hinkle 1985; Baldwin 1993; Bell et al. 1993; Takata et al. 1993). We showed previously that an isoform of the glucose transporter GLUT1 is abundant at the plasma membranes of cells of blood-tissue barriers and serves as a mechanism for transporting glucose across the barrier (Takata et al. 1990, 1997). In the eye, GLUT1 is concentrated at the critical plasma membrane of the cells of the blood-ocular barrier, enabling the transfer of glucose across the barrier (Harik et al. 1990; Takata et al. 1990, 1991a, 1992, 1997).

We suggested that the presence of tight junctions and the abundance of GLUT1 ensures the blockage of the non-specific entry of blood constituents and the selective and specific transport of glucose across the barrier cell layers (Takata et al. 1997). In the present study we analyzed the immunolocalization of occludin, ZO-1, and GLUT1 in the mouse retina, ciliary body, and iris by double-immunofluorescence microscopy and immunogold electron microscopy. We suggest a possible role of occludin, ZO-1, and GLUT1 in the blood-ocular barrier.

Material and methods

Tissue preparations

Male BALB/c mice of 4 weeks of age (supplied from the Animal Breeding Facility, Gunma University) were anesthetized and killed with an intraperitoneal injection of sodium pentobarbital and the eyes were removed. The ciliary body and retina specimens were isolated with sharp scissors under a dissecting microscope. For immunoblotting, specimens were rapidly frozen and stored in liquid nitrogen until use. For immunohistochemistry in cryostat sections, fresh specimens were embedded in Tissue Tek OCT compound (Sakura Finetechnical, Tokyo, Japan), rapidly frozen with liquid nitrogen, and stored at –80°C until use. For immunohistochemistry in cryostat sections, specimens were fixed in 1% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4, for 10–60 min at room temperature, washed with PBS, and infused with 20% sucrose in 0.1 M sodium phosphate buffer, pH 7.4. For semithin and ultrathin frozen sections, specimens were fixed in 1 or 3% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.4, for 3 h at 4°C, washed with PBS, and infused with 2.3 M sucrose in 0.1 M sodium phosphate buffer, pH 7.4, overnight (Takata and Singer 1988).

Antibodies

Anti-GLUT1 antibodies raised in a guinea pig and rabbits were as previously described (Takata et al. 1990; Shin et al. 1996a). Monoclonal rat anti-mouse occludin (Ando-Akatsuka et al. 1996) was a kind gift from Dr. S. Tsukita (Kyoto University). Rabbit anti-ZO-1 was purchased from Zymed (San Francisco, Calif., USA).

Immunoblotting

Lenses were removed from the eyes and the remaining eye specimens were homogenized in phosphate-buffered saline (PBS) containing protease inhibitors (Takata et al. 1992). The protein content was measured by the BCA protein assay reagent (Pierce, Rockford, Ill., USA). The homogenate (10 μg protein) was electrophoresed through SDS-polyacrylamide gels, and transferred to membrane filters (Immobilon-PSQ; Millipore, Bedford, Mass., USA). The blotted membranes were sequentially incubated with 3% bovine serum albumin, anti-occludin, or anti-ZO-1 antibody, and [^{125}I]-protein G (New England Nuclear, Wilmington, Del., USA) or [^{125}I]-protein A (New England Nuclear). Autoradiography was performed with imaging plates, and the plates were processed with a BAS2000 bioimage analyzer (Fuji Film, Tokyo, Japan).

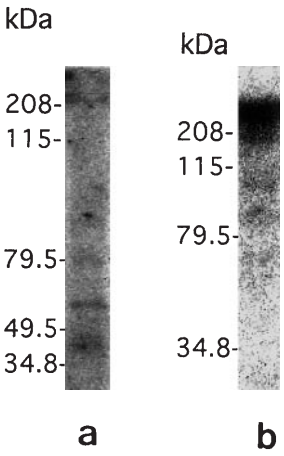

Fig. 1 Immunoblotting of the eye with anti-occludin (**a**) and anti-ZO-1 (**b**). Eye homogenate (10 μg each) was applied to SDS-polyacrylamide gel electrophoresis, and subjected to immunoblotting with rat anti-occludin (**a**) or rabbit anti-ZO-1 antibodies (**b**)

Immunofluorescence microscopy

Cryostat sections (4 μm thick) were cut from fresh frozen specimens and mounted on poly-L-lysine-coated glass slides. For occludin staining, sections were fixed in acetone at –20°C for 30 min and then in 95% ethanol for 1 min at room temperature (Kimura et al. 1996). For ZO-1 staining, sections were immediately fixed in 1% paraformaldehyde at room temperature for 20–30 min or in acetone and ethanol and washed with PBS. Semithin frozen sections (1 μm thick) were cut with an Ultracut S UCT ultramicrotome, equipped with an FCS cryokit (Leica, Vienna, Austria) and glass knives, and mounted on glass slides. Indirect immunofluorescence staining was carried out essentially as described previously (Shin et al. 1996a). Rat anti-occludin, rabbit anti-ZO-1, rabbit anti-GLUT1, and guinea pig anti-GLUT1 were used at dilutions of 1:20, 1:200, 1:500, and 1:500, respectively. Fluorescence-labeled secondary antibodies used were Cy3 (indocarbocyanine)-labeled donkey anti-rat IgG (Jackson Immunoresearch, West Grove, Pa., USA), dichlorotriazinylaminofluorescein (DTAF)-labeled donkey anti-rabbit IgG (Jackson Immunoresearch) Cy3-labeled donkey anti-rabbit IgG (Jackson Immunoresearch), and DTAF-labeled donkey anti-guinea pig IgG (Jackson Immunoresearch). For nuclear counterstaining, 2 μg/ml 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Boehringer Mannheim, Mannheim, Germany) was included in the secondary antibody solution (Takata et al. 1991b). Double-immunofluorescence staining was carried out by the sequential incubation of a mixture of the primary antibodies raised in different animal species and of a mixture of the corresponding species-specific secondary antibodies as described (Shin et al. 1996a). All of the incubation procedures were done at room temperature. Sections were mounted in 22% polyvinyl alcohol in 56 mM TRIS-HCl buffer, pH 9, 11% glycerol containing 5% 1,4-diazabicyclo[2,2,2]octane as an anti-bleaching reagent (Shin et al. 1996a). Specimens were examined with a BX-50 or an AX-70 microscope equipped with Nomarski differential interference-contrast and epifluorescence optics (Olympus, Tokyo, Japan).

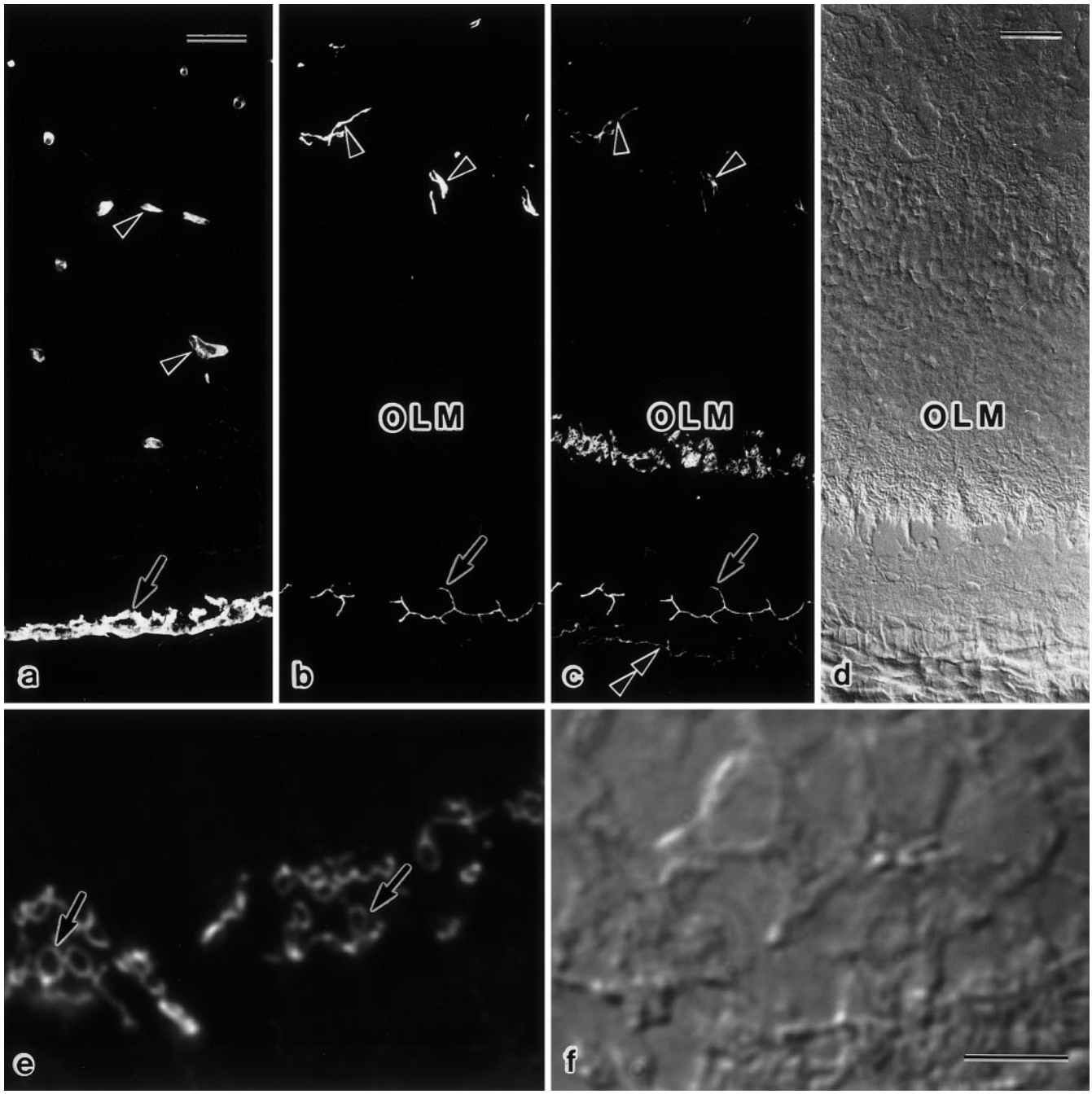

Fig. 2 Immunofluorescence localization of GLUT1 (**a**), occludin (**b**), and ZO-1 (**c**) in the retina. **a** GLUT1 is concentrated in the retinal pigment epithelium (*arrow*) and blood vessels (*arrowheads*) in the retina. *Bar* 25 μm. **b–d** Double-immunofluorescence localization for occludin (**b**) and ZO-1 (**c**), and the corresponding Nomarski differential interference-contrast image (**d**). Occludin and ZO-1 are colocalized in the retinal pigment epithelium (*arrows*) and blood vessels in the retina (*arrowheads*). ZO-1 is positive along the outer limiting membrane (*OLM*) and blood vessels in the choroid (*double arrowhead*), both of which are negative for occludin. *Bar* 25 μm. **e, f** ZO-1 (**e**) and the corresponding Nomarski differential interference-contrast image (**f**) in the outer limiting membrane. In the oblique section of the retina, positive sites for ZO-1 appear as a cluster of circles (*arrows*). *Bar* 5 μm

Immunogold electron microscopy

Ultrathin frozen sections were cut, mounted on grids coated with Formvar and carbon, and immunolabeled essentially as previously described (Takata et al. 1992; Shin at al. 1996b). In short, grids were floated on PBS containing 5% normal goat serum and 1% gelatin for 10 min followed by incubation with rabbit anti-ZO-1 antibody for 1.5 h, and affinity-purified goat anti-rabbit IgG (Jackson Immunoresearch)-colloidal gold (diameter 10 nm) conjugate [prepared according to DeMey (1984) and Slot and Geuze (1985)] for 1 h. They were subsequently washed with PBS and refixed with 2% glutaraldehyde, after which the specimens were treated with uranyl acetate and embedded in 1.8% methylcellulose-0.5% uranyl acetate (Liou et al. 1996). Specimens were observed with a JEM-1010 transmission electron microscope (JEOL, Tokyo, Japan).

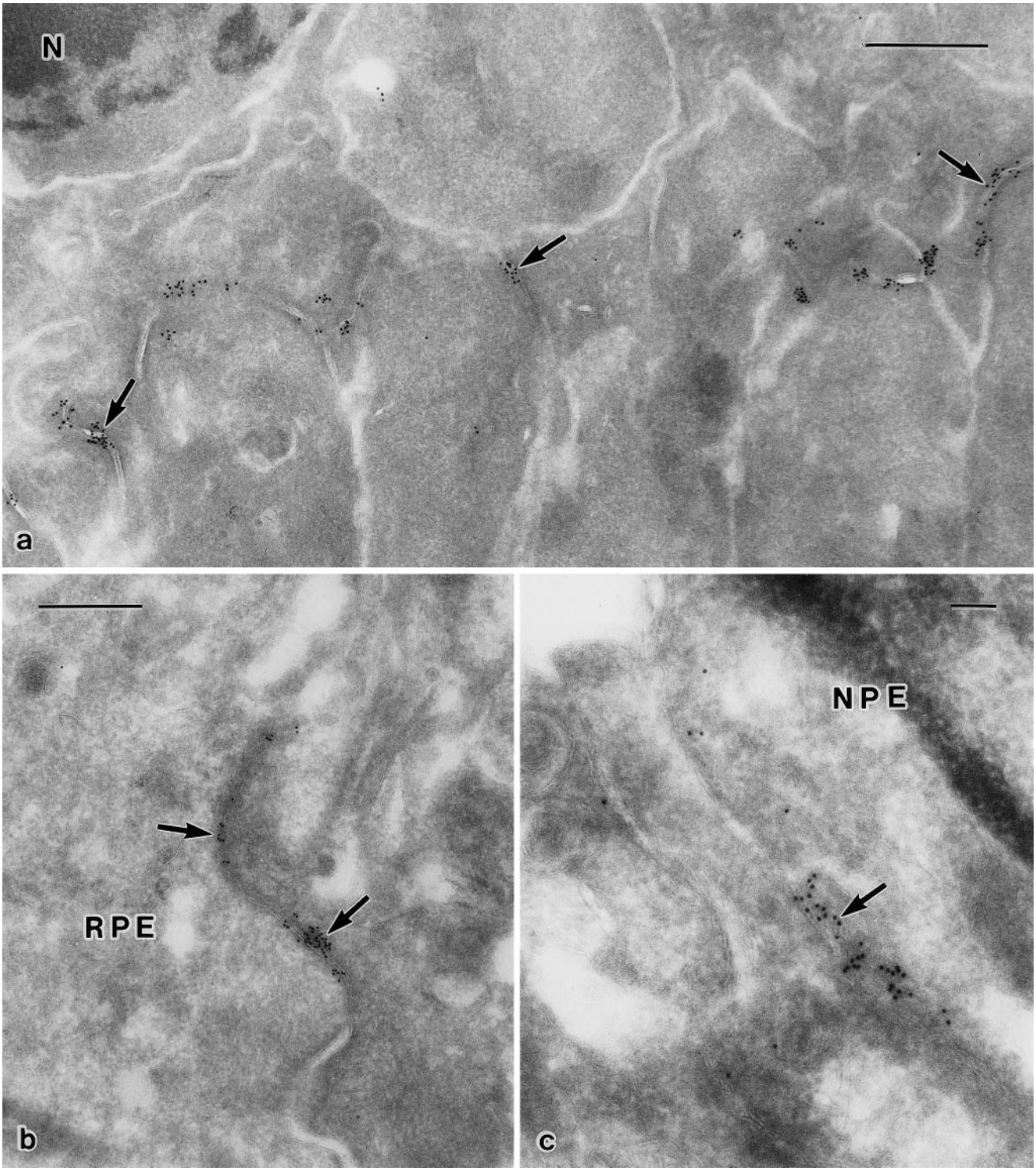

Fig. 3 Immunogold detection of ZO-1 in the retina (**a, b**) and ciliary body (**c**). **a** ZO-1 is localized at the junction regions in the outer limiting membrane (*arrows*). *N* Nucleus of the photoreceptor cell. *Bar* 0.5 µm. **b** ZO-1 is localized in the tight junction (*arrows*) between retinal pigment epithelial (*RPE*) cells. *Bar* 0.5 µm. **c** ZO-1 is localized in the tight junction between non-pigmented epithelial (*NPE*) cells (*arrow*). *Bar* 0.1 µm

Immunohistochemical controls

As histochemical controls for immunofluorescence and immunogold staining, primary antibodies were replaced with normal serum or normal immunoglobulin, none of which gave positive labeling. In addition, the results of double-immunofluorescence staining were compared with those of single-immunofluorescence staining to exclude possible crossreactions. These histochemical controls confirmed the specificity of the staining.

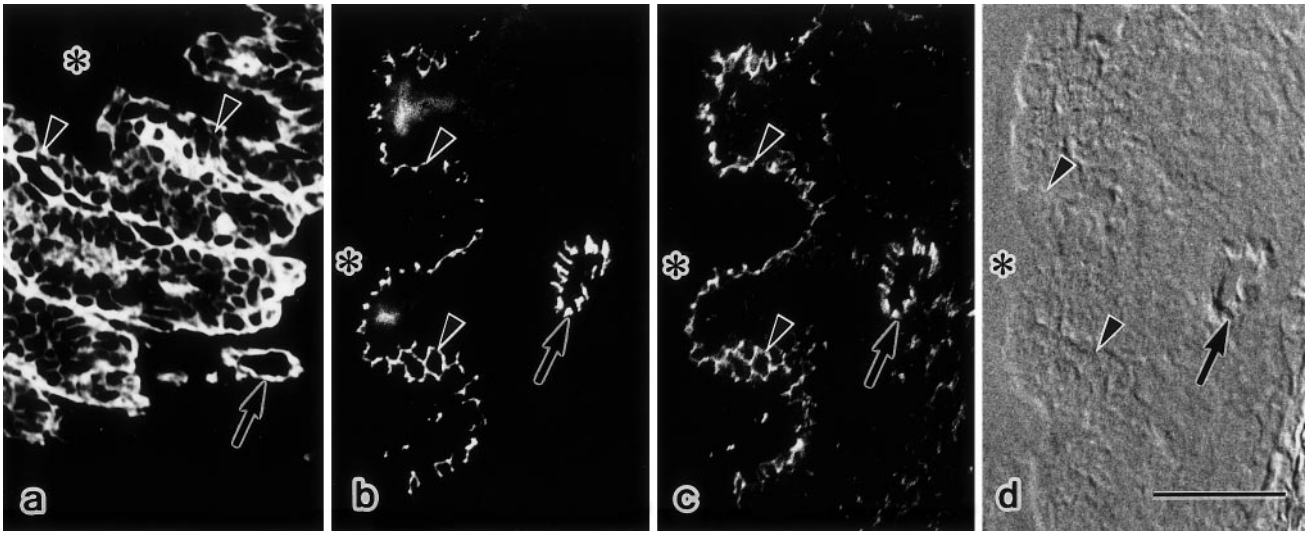

Fig. 4 Immunofluorescence localization of GLUT1 (**a**), occludin (**b**), and ZO-1 (**c**) in the ciliary body. **a** GLUT1 is abundant in the epithelium of the ciliary body (*arrowheads*) and blood vessels at the base of the ciliary process (*arrow*). **b–d** Double-immunofluorescence localization for occludin (**b**) and ZO-1 (**c**), and the corresponding Nomarski differential interference-contrast image (**d**). Occludin and ZO-1 are colocalized in the epithelium of the ciliary body (*arrowheads*) and a blood vessel at the base of the ciliary process (*arrows*). *Asterisks* Posterior chamber. *Bar* 50 μm

Results

Immunoblotting

By immunoblotting of the homogenate of the eyes with anti-occludin antibody, a 64-kDa protein was detected (Fig. 1a). With anti-ZO-1 antibody a 218-kDa protein was detected (Fig. 1b). The apparent Mr. of these proteins detected were within the range of occludin (Saitou et al. 1997) and ZO-1 (Stevenson et al. 1986).

Occludin, ZO-1, and GLUT1 in the retina

Localization of occludin, ZO-1, and GLUT1 was examined by immunofluorescence (Fig. 2) and immunogold electron (Fig. 3) microscopy. GLUT1 was abundant in the cells of the blood-retinal barrier: retinal pigment epithelial cells and endothelial cells of blood vessels (Fig. 2a). Occludin and ZO-1 were present in these GLUT1-positive cells of the barrier (Fig. 2a–d). ZO-1 and occludin colocalized in the retinal pigment epithelium in a honeycomb-like or continuous belt-like array along the epithelium, suggesting their presence in the epithelial cell-to-cell junctions. Immunogold electron microscopy revealed that ZO-1 was localized in the tight junctions between retinal pigment epithelial cells (Fig. 3b). Occludin-positive cells precisely matched with GLUT1-positive cells, the site of the barrier (Fig. 2a,b), whereas ZO-1 was found, in addition to the site of the barrier, along the outer limiting membrane and chorio-capillaries (Fig. 2c,d). In the outer limiting membrane, positive labeling for ZO-1 was seen as a belt-like cluster of small circles at higher magnification (Fig. 2c–f), suggesting that ZO-1 is localized in the Müller cell junctions. Immunogold electron microscopy showed that ZO-1 was localized in the junctions between Müller cells and the inner segments of photoreceptor cells (Fig. 3a).

Occludin, ZO-1, and GLUT1 in the ciliary body

GLUT1 was abundant in the epithelium of the ciliary body, which serves as a barrier between the blood and the aqueous humor (blood-aqueous barrier; Fig. 4a). Both occludin and ZO-1 were present along the epithelium of the ciliary body (Fig. 4b–d). They were concentrated in the junctional regions between non-pigmented epithelial cells (Fig. 5a,b). Immunogold electron microscopy showed that ZO-1 was localized to the tight junctions of non-pigmented epithelial cells (Fig. 3c). Localization of occludin was restricted to these junctional regions. ZO-1, in addition, was localized in the cell border between pigmented and non-pigmented epithelial cells (Fig. 5b). Blood vessels in the core stroma of the ciliary process were negative for GLUT1 and occludin, and were positive for ZO-1 (Fig. 5a,b, Table 1). GLUT1-positive blood vessels located at the base of the ciliary process, on the other hand, were positive for occludin and ZO-1 (Fig. 4, Table 1). These observations showed that the presence of occludin was restricted to cells of the barrier, which were strongly positive for GLUT1.

Occludin, ZO-1, and GLUT1 in the iris

The iris is another site of the blood-aqueous barrier. GLUT1 was concentrated in the endothelial cells of the blood vessels in the iridial stroma (Fig. 5c). The iridial epithelium, which is developmentally the outermost extension of the epithelium of the ciliary body, was also

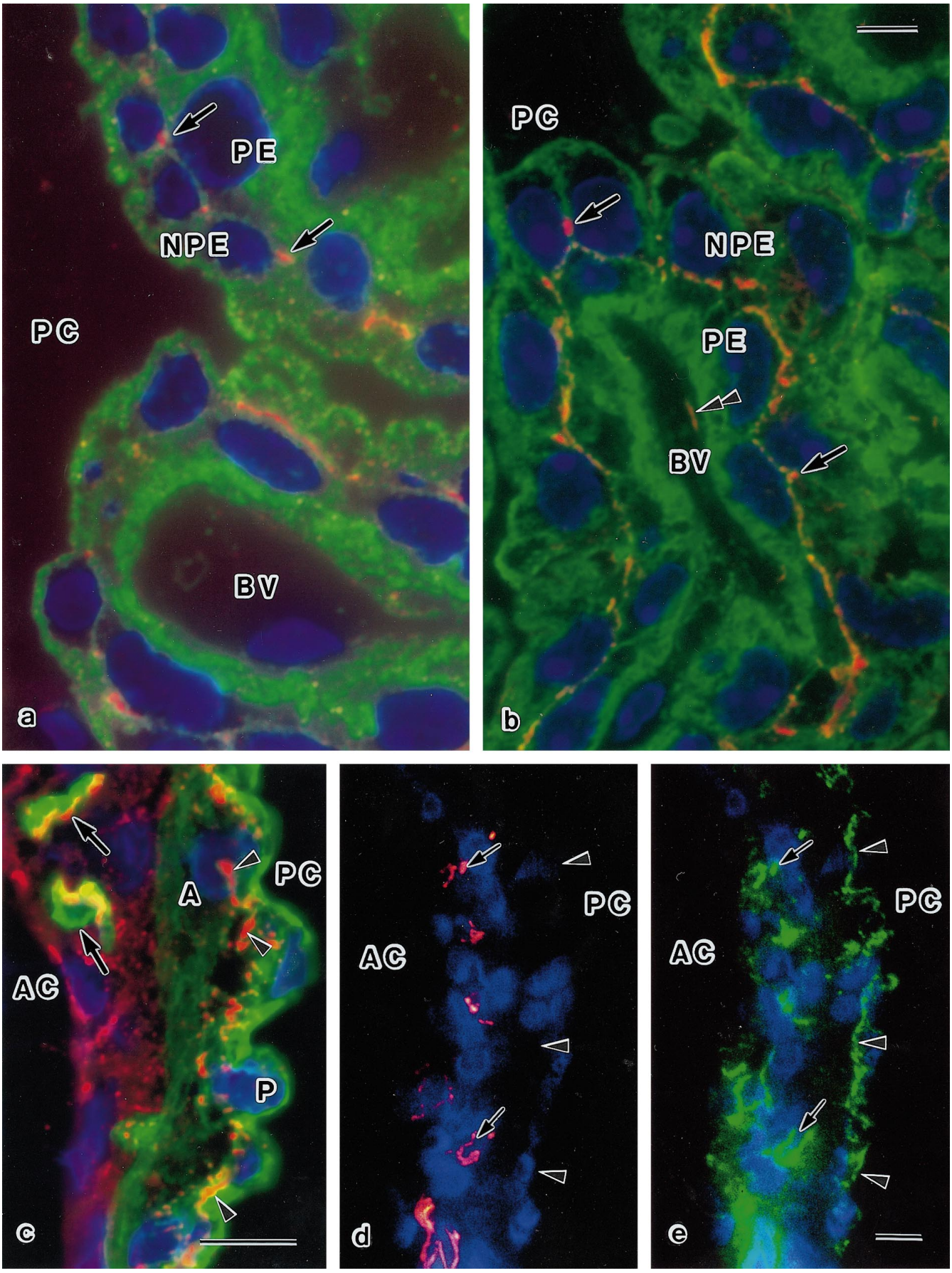
PE
NPE
PC
BV
a
PC
NPE
PE
BV
b
A
PC
AC
P
c
PC
AC
d
PC
AC
e

Table 1 GLUT1, occludin, and ZO-1 in the blood vessels of the mouse eye. The results are expressed as +(positive) or –(negative)

Location of blood vessels	GLUT1	Occludin	ZO-1
Retina	+	+	+
Choroid	–	–	+
Core stroma of ciliary process	–	–	+
Base of ciliary process	+	+	+
Iridial stroma	+	+	+

positive for GLUT1 (Fig. 5c). Occludin was positive only in the endothelial cells of the iridial blood vessels (Fig. 5d) and ZO-1 colocalized with occludin in these cells (Fig. 5c,e, Table 1). ZO-1, but not occludin, was found in the iridial epithelium, where it was concentrated between two epithelial layers, namely the anterior and posterior epithelial cell layers (Fig. 5c,e).

Discussion

We have shown in this study that the tight junction proteins, occludin and ZO-1, are colocalized in the endothelial and epithelial cells of the blood-ocular barrier in the mouse eye. Colocalization was restricted to the tight junctions connecting the cells of the barrier. In the blood-tissue barriers, including the blood-ocular barrier, an isoform of the facilitated-diffusion glucose transporter GLUT1 is abundant at the critical plasma membrane of the cells of the barrier (Takata et al. 1997). GLUT1 plays a pivotal role in the specific transfer of glucose across the barrier. The blood-ocular barrier consists of the blood-retinal barrier and the blood-aqueous barrier (Raviola 1977). In the retina, GLUT1 is concentrated in the retinal pigment epithelium and the retinal blood vessels, sites of outer and inner blood-retinal barriers, respectively (Takata et al. 1992). Immunogold electron microscopy revealed that GLUT1 was present at the sites of both entry into and exit from the barrier cell layer, thereby enabling transfer of glucose across the barrier (Takata et al. 1991a, 1992; Shin et al. 1996a, b). In addition to GLUT1, expression of GLUT3 was reported in the retina (Watanabe et al. 1996). GLUT3 was localized in the plexiform layers, probably in the neuronal processes, serving in the uptake of glucose into neuronal cells once glucose passes the barrier via GLUT1. We observed that the presence of occludin exactly coincided at these sites of the blood-retinal barrier with a high level of expression of the glucose transporter GLUT1. ZO-1 was always found in the occludin-positive tight junctions, which is in accordance with previous observations (Konari et al. 1995; Hirase et al. 1997; Saitou et al. 1997). In fact, ZO-1 was shown to bind the carboxyl terminus of occludin (Furuse et al. 1994). In the ciliary body, colocalization of occludin, ZO-1, and GLUT1 was observed in the tight junctions of non-pigmented epithelial cells, the site of the blood-aqueous barrier. Such colocalization of occludin, ZO-1, and GLUT1 was also seen in the endothelial cells of blood vessels in the base of the ciliary process. The presence of occludin, ZO-1, and GLUT1, therefore, seems to be closely related to the properties of the blood-ocular barrier: occludin and ZO-1 for barrier function and GLUT1 for the selective transfer of glucose across it.

Immunofluorescence examination showed that strong labeling for ZO-1, but not occludin or GLUT1, was seen along the outer limiting membrane which is in accordance with observations in the guinea pig retina (Saitou et al. 1997). By immunogold electron microscopy, we found that ZO-1 was localized in the adherens junctions between the Müller cells and the outer segments of photoreceptor cells. Junctions of the outer limiting membrane are composed of zonulae adherentes (Uga and Smelser 1973). Therefore, the outer limiting membrane does not serve as a barrier in the retina. In fact, peroxidase administered in the vitreous rapidly diffused all over the retina until finally stopped by the retinal pigment epithelium (Peyman et al. 1971). The abundance of ZO-1 in the Müller cell junctions may be important in maintaining retinal architecture, probably by stabilizing the arrangement of photoreceptor cells. Such ZO-1-positive, occludin-negative, and GLUT1-negative labeling was also seen in the blood vessels in the choroid and in the core stroma of the ciliary process. By freeze-fracture electron microscopy, ridges or strands typical of tight junctions are absent in the endothelial cells of the choriocapillary and blood vessels beneath the ciliary body epithelium (Raviola 1977). These blood vessels are highly

Fig. 5 Immunofluorescence localization of GLUT1, occludin, and ZO-1 in the ciliary body (**a, b**) and iris (**c–e**). **a, b** Double-immunofluorescence localization for occludin (*red*) and GLUT1 (*green*) (**a**), and ZO-1 (*red*) and GLUT1 (*green*) (**b**) in the ciliary body. Cell nuclear DNA is labeled with DAPI in *blue*. GLUT1 (*green* in **a, b**) is abundant in both non-pigmented epithelial (*NPE*) cells and pigmented epithelial (*PE*) cells. Occludin (*red* in **a**) is localized in the junctions between NPE cells (*arrows* in **a**). ZO-1 is concentrated in the junctions between NPE cells as well (*arrows* in **b**). ZO-1 is also seen along the border between PE and NPE cells, and in the blood vessel of the ciliary body (*double arrowhead*). *PC* Posterior chamber, *BV* blood vessels. *Bar* 5 μm. **c** Double-immunofluorescence localization for GLUT1 (*green*) and ZO-1 (*red*) in the iris. Cell nuclear DNA is labeled with DAPI in *blue*. GLUT1 is concentrated in the blood vessels in the stroma (*arrows*) and posterior epithelium (*P*). GLUT1 is also weakly positive in the anterior epithelium (*A*). ZO-1 is colocalized in GLUT1-positive blood vessels (*arrows*) and in between GLUT1-positive anterior and posterior epithelial cells (*arrowheads*). *AC* Anterior chamber, *PC* posterior chamber. *Bar* 10 μm. **d, e** Double-immunofluorescence localization for occludin (*red* in **d**) and ZO-1 (*green* in **e**) in the iris. Cell nuclear DNA is labeled with DAPI in *blue*. Occludin is restricted to the blood vessels (*arrows*) in the stroma. ZO-1 is seen in the blood vessels (*arrows*) and the iridial epithelium (*arrowheads*). Iridial epithelium is negative for occludin (*arrowhead*). *AC* Anterior chamber, *PC* posterior chamber. *Bar* 10 μm

permeable due to the fenestration in their endothelial walls and do not serve as barriers. ZO-1 in these cells may not serve as a part of the barrier, but may be important in maintaining cell-to-cell contact.

Hirase et al. (1997) showed that occludin is present in the brain endothelial cells at high level. In contrast, non-neural tissues have a much lower expression of occludin. Blood vessels serving as barriers in the eye were positive for occludin, ZO-1, and GLUT1. On the other hand, blood vessels of a non-barrier property were negative for occludin and GLUT1. These differences in the endothelial cells were typically observed in the ciliary body. Endothelial cells beneath the epithelium in the core stroma of the ciliary process were negative for occludin and GLUT1, whereas nearby endothelial cells at the base of the ciliary process were positive for occludin and GLUT1 (Table 1). These observations suggest that the properties of the endothelial cells may be regulated by the microenvironment of the blood vessels.

Iridial epithelium covering the posterior surface of the iris is a continuation of the ciliary body epithelium (Raviola 1977). It consists of two layers, the posterior and anterior epithelial layers. The posterior epithelium is continuous with the non-pigmented epithelium of the ciliary body, and the anterior epithelium with the pigmented epithelium. Thin-section and freeze-fracture electron microscopic examination showed that cells of the posterior epithelium are connected by tight junctions with continuous series of branching and anastomosing strands of intramembranous particles (Freddo 1984). Tracer experiments showed that horseradish peroxidase in the iridial stroma was blocked by tight junctions between posterior epithelial cells (Freddo 1984). These observations indicate that the posterior epithelium serves as a barrier between posterior and anterior chambers (Freddo 1984). Occludin was not detected in the iridial epithelium. The precise molecular architecture of the tight junctions of the iridial epithelium remains to be solved. Recently, occludin-deficient cells were shown to differentiate into polarized epithelial cells bearing tight junctions (Saitou et al. 1998). Claudins (Furuse et al. 1998), newly found tight junction membrane proteins, might be present in the iridial epithelium.

ZO-1 was concentrated in the borders of the anterior and posterior epithelial cell layers in the iris, which represent the direct continuation of the pigmented and non-pigmented epithelial cell layers, respectively (Freddo 1984). Similar localization of ZO-1 was obtained in the epithelium of the ciliary body. The role of ZO-1 in these regions, where two simple epithelia are joined at their apical surfaces, is not clear. ZO-1 in these regions may be important in stabilizing and maintaining the contact of rarely found apical-to-apical cell surfaces.

In summary, expression of occludin was restricted to epithelial and endothelial cells of the GLUT1-positive cells forming the blood-ocular barrier. ZO-1 was much more widely distributed, as compared to occludin, and seems to play roles in cell-to-cell contacts other than those of barriers in the eye.

Acknowledgements We thank S. Tsukui for secretarial assistance. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

References

Ames A III, Li Y-Y, Heher EC, Kimble CR (1992) Energy metabolism of rabbit retina as related to function: high cost of Na^+ transport. J Neurosci 12:840–853

Anderson JM, Van Itallie CM (1995) Tight junctions and the molecular basis for regulation of paracellular permeability. Am J Physiol 269:G467–G475

Anderson JM, Stevenson BR, Jesaitis LA, Goodenough DA, Mooseker MS (1988) Characterization of ZO-1, a protein component of the tight junction from mouse liver and Madin-Darby canine kidney cells. J Cell Biol 106:1141–1149

Ando-Akatsuka Y, Saitou M, Hirase T, Kishi M, Sakakibara A, Itoh M, Yonemura S, Furuse M, Tsukita S (1996) Interspecies diversity of the occludin sequence: cDNA cloning of human, mouse, dog, and rat-kangaroo homologues. J Cell Biol 133: 43–47

Baldwin SA (1993) Mammalian passive glucose transporters: members of an ubiquitous family of active and passive transport proteins. Biochim Biophys Acta 1154:17–49

Bell GI, Burant CF, Takeda J, Gould GW (1993) Structure and function of mammalian facilitative sugar transporters. J Biol Chem 268:19161–19164

Citi S (1993) The molecular organization of tight junctions. J Cell Biol 121:485–489

Citi S, Sabanay H, Jakes R, Geiger B, Kendrick-Jones J (1988) Cingulin, a new peripheral component of tight junctions. Nature 333:272–276

DeMey J (1984) Colloidal gold as marker and tracer in light and electron microscopy. EMSA Bull 14:54–66

Denker BM, Nigam SK (1998) Molecular structure and assembly of the tight junction. Am J Pysiol 274:F1–F9

Freddo TF (1984) Intercellular junctions of the iris epithelia in *Macaca mulatta*. Invest Ophthalmol Vis Sci 25:1094–1104

Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S (1993) Occludin: a novel integral membrane protein localizing at tight junctions. J Cell Biol 123:1777–1788

Furuse M, Itoh M, Hirase T, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S (1994) Direct association of occludin with ZO-1 and its possible involvement in the localization of occludin at tight junctions. J Cell Biol 127:1617–1626

Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S (1998) Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Biol 141:1539–1550

Gumbiner B, Lowenkopf T, Apatira D (1991) Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1. Proc Natl Acad Sci USA 88:3460–3464

Harik SI, Kalaria RN, Whitney PM, Andersson L, Lundahl P, Ledbetter SR, Perry G (1990) Glucose transporters are abundant in cells with "occluding" junctions at the blood-eye barriers. Proc Natl Acad Sci USA 87:4261–4264

Haskins J, Gu L, Wittchen ES, Hibbard J, Stevenson BR (1998) ZO-3, a novel member of the MAGUK protein family found at the tight junction, interacts with ZO-1 and occludin. J Cell Biol 141:199–208

Henkind P, Hansen RI, Szalay J (1979) Ocular circulation. In: Records RE (ed) Physiology of the human eye and visual system. Harper and Row, Hagerstown, pp 98–155

Hirase T, Staddon JM, Saitou M, Ando-Akatsuka Y, Itoh M, Furuse M, Fujimoto K, Tsukita S, Rubin LL (1997) Occludin as a possible determinant of tight junction permeability in endothelial cells. J Cell Sci 110:1603–1613

Keon BH, Schäfer S, Kuhn C, Grund C, Franke WW (1996) Symplekin, a novel type of tight junction plaque protein. J Cell Biol 134:1003–1018

Kimura M, Sawada N, Kimura H, Isomura H, Hirata K, Mori M (1996) Comparison between the distribution of 7H6 tight junction-associated antigen and occludin during the development of chick intestine. Cell Struct Funct 21:91–96

Konari K, Sawada N, Zhong Y, Isomura H, Nakagawa T, Mori M (1995) Development of the blood-retinal barrier in vitro: formation of tight junctions as revealed by occludin and ZO-1 correlates with the barrier function of chick retinal pigment epithelial cells. Exp Eye Res 61:99–108

Liou W, Geuze HJ, Slot JW (1996) Improving structural integrity of cryosections for immunogold labeling. Histochem Cell Biol 106:41–58

Peyman GA, Spitznas M, Straatsma BR (1971) Peroxidase diffusion in the normal and photocoagulated retina. Invest Ophthalmol Vis Sci 10:181–189

Raviola G (1977) The structural basis of the blood-ocular barriers. Exp Eye Res [Suppl] 25:27–63

Raviola G, Raviola E (1978) Intercellular junctions in the ciliary epithelium. Invest Ophthalmol Vis Sci 17:958–981

Saitou M, Ando-Akatsuka Y, Itoh M, Furuse M, Inazawa J, Fujimoto K, Tsukita S (1997) Mammalian occludin in epithelial cells: its expression and subcellular distribution. Eur J Cell Biol 73:222–231

Saitou M, Fujimoto K, Doi Y, Itoh M, Fujimoto T, Furuse M, Takano H, Noda T, Tsukita S (1998) Occuludin-deficient embryonic stem cells can differentiate into polarized epithelial cells bearing tight junctions. J Cell Biol 141:397–408

Satoh H, Zhong Y, Isomura H, Saitoh M, Enomoto K, Sawada N, Mori M (1996) Localization of 7H6 tight junction-associated antigen along the cell border of vascular endothelial cells correlates with paracellular barrier function against ions, large molecules, and cancer cells. Exp Cell Res 222:269–274

Shin B-C, Suzuki T, Tanaka S, Kuraoka A, Shibata Y, Takata K (1996a) Connexin 43 and the glucose transporter, GLUT1, in the ciliary body of the rat. Histochem Cell Biol 106:209–214

Shin B-C, Suzuki T, Matsuzaki T, Tanaka S, Kuraoka A, Shibata Y, Takata K (1996b) Immunolocalization of GLUT1 and connexin 26 in the rat placenta. Cell Tissue Res 285:83–89

Slot JW, Geuze HJ (1985) A new method of preparing gold probes for multiple-labeling cytochemistry. Eur J Cell Biol 38:87–93

Stevenson BR, Siliciano JD, Mooseker MS, Goodenough DA (1986) Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. J Cell Biol 103:755–766

Takata K, Singer SJ (1988) Phosphotyrosine-modified proteins are concentrated at the membranes of epithelial and endothelial cells during tissue development in chick embryos. J Cell Biol 106:1757–1764

Takata K, Kasahara T, Kasahara M, Ezaki O, Hirano H (1990) Erythrocyte/HepG2-type glucose transporter is concentrated in cells of blood-tissue barriers. Biochem Biophys Res Commun 173:67–73

Takata K, Kasahara T, Kasahara M, Ezaki O, Hirano H (1991a) Ultracytochemical localization of the erythrocyte/HepG2-type glucose transporter (GLUT1) in the ciliary body and iris of the rat eye. Invest Ophthalmol Vis Sci 32:1659–1666

Takata K, Kasahara T, Kasahara M, Ezaki O, Hirano H (1991b) Localization of Na^+-dependent active type and erythrocyte/HepG2-type glucose transporters in rat kidney: immunofluorescence and immunogold study. J Histochem Cytochem 39:287–298

Takata K, Kasahara T, Kasahara M, Ezaki O, Hirano H (1992) Ultracytochemical localization of the erythrocyte/HepG2-type glucose transporter (GLUT1) in cells of the blood-retinal barrier in the rat. Invest Ophthalmol Vis Sci 33:377–383

Takata K, Kasahara M, Oka Y, Hirano H (1993) Mammalian sugar transporters: their localization and link to cellular functions. Acta Histochem Cytochem 26:165–178

Takata K, Hirano H, Kasahara M (1997) Transport of glucose across the blood-tissue barriers. Int Rev Cytol 172:1–53

Uga S, Smelser GK (1973) Comparative study of the fine structure of retinal Müller cells in various vertebrates. Invest Ophthalmol Vis Sci 12:434–448

Watanabe T, Matsushima S, Okazaki M, Nagamatsu S, Hirosawa K, Uchimura H, Nakahara K (1996) Localization and ontogeny of GLUT3 expression in the retina. Dev Brain Res 94:60–66

Weber E, Berta G, Tousson A, St. John P, Green MW, Gopalokrishnan U, Jilling T, Sorscher EJ, Elton TS, Abrahamson DR, Kirk KL (1994) Expression and polarized targeting of a rab3 isoform in epithelial cells. J Cell Biol 125:583–594

Wheeler TJ, Hinkle PC (1985) The glucose transporter of mammalian cells. Annu Rev Physiol 47:503–517

Yamamoto T, Harada N, Kano K, Taya S, Canaani E, Matsuura Y, Mizoguchi A, Ide C, Kaibuchi K (1997) The ras target AF-6 interacts with ZO-1 and serves as a peripheral component of tight junctions in epithelial cells. J Cell Biol 139:785–795

Zahraoui A, Joberty G, Arpin M, Fontaine JJ, Hellio R, Tavitian A, Louvard D (1994) A small rab GTPase is distributed in cytoplasmic vesicles in non-polarized cells but colocalizes with the tight junction marker ZO-1 in polarized epithelial cells. J Cell Biol 124:101–115

Zhong Y, Saitoh T, Minase T, Sawada N, Enomoto K, Mori M (1993) Monoclonal antibody 7H6 reacts with a novel tight junction-associated protein distinct from ZO-1, cingulin and ZO-2. J Cell Biol 120:477–483