Intercellular Junctions of the Iris Epithelia
in Macaca Mulatta

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The intercellular junctions in the anterior myoepithelium and posterior pigmented epithelium of the rhesus monkey iris were examined using an ultrastructural tracer, conventional electron microscopy, and the freeze-fracture technique. Within the anterior myoepithelium the lateral cell margins were joined by puncta adhaerentia, desmosomes, and gap junctions. The distribution of the puncta adhaerentia and desmosomes was restricted to the apico-lateral region of these cells. Joining the apical surface of the anterior myoepithelium and posterior pigmented epithelium gap junctions, puncta adhaerentia, and desmosomes also were present. Adjacent posterior pigmented epithelial cells were joined by an apico-lateral junctional complex, which consisted of a zonula occludens, zonula adhaerens, and gap junction. These cells also were connected by one or more desmosomes. Intravenously injected horseradish peroxidase, which diffused from the ciliary body stroma, was prevented from reaching the posterior chamber by the presence of the zonulae occludentes between adjacent posterior pigmented epithelial cells. Their presence was confirmed using the double replica method of freeze-fracturing. The zonulae occludentes appeared as a continuous series of branching and anastomosing strands of particles on the P-fracture face, which were complemented on the E-fracture face by a series of shallow grooves. These junctions varied in complexity from one to eight or more strands indicating that they are analogous in both location and degree of permeability to the zonulae occludentes present in the nonpigmented ciliary epithelium. Invest Ophthalmol Vis Sci 25:1094–1104, 1984

The anterior and posterior epithelia of the iris represent direct continuations of the nonpigmented and pigmented ciliary epithelia, respectively. In both of these tissues, the two cell layers actually represent simple epithelia, which come to be joined at their apices by invagination of the optic cup during embryogenesis. In the case of the iris, the two layers are continuous at the pupillary margin.

The posterior epithelial cells of the iris are heavily pigmented and serve to prevent light from reaching the retina except through the pupil. The anterior epithelial cells also are pigmented but have modified basal processes, which represent the dilator muscle of the iris. For this reason, the anterior epithelium is sometimes referred to as a myoepithelium.1

An important function of the iridial epithelium is that, together with the ciliary epithelium, it represents one of the two morphologic components of the blood-aqueous barrier; the other component residing within the endothelium of the iris vasculature.7 All of these functions rely upon the presence of certain types of intercellular junctions.

The intercellular junctions of the ciliary epithelium have been described fully by Raviola and Raviola2 and those of the iris vasculature by Freddo and Raviola.3,4 Although several authors have discussed the morphology of the iris epithelium, a systematic study of the intercellular junctions present between these cells is lacking.5,6 Indeed, the results of the only previous freeze-fracture study of the iris epithelium denies the existence of tight junctions between these cells. This finding directly contradicts the observations of other investigators using conventional electron microscopy.8

As a means of resolving this matter, a systematic study was undertaken to determine the types and distribution of intercellular junctions within the epithelium of the rhesus monkey iris using both conventional electron microscopy of thin sections and the freeze-fracture technique.

Materials and Methods

A total of five rhesus monkeys (Macaca mulatta) of both sexes were used. These animals were from 6
months to 15 years old. All animals were sedated with an intramuscular injection of Ketamine-HCl (10 mg/kg; Ketalar, Parke Davis and Co.; Detroit, MI) and maintained under deep anesthesia with sodium pentobarbital (30 mg/kg, Nembutal; Abbott Laboratories; N. Chicago, IL). In three animals horseradish peroxidase (HRP, Type II; Sigma, St. Louis, MO) 500 mg/kg body weight, dissolved in 3–5 ml of phosphate buffered saline, pH 7.2) was injected slowly into the small saphenous vein. After 10–15 min, an overdose of anesthetic was administered intravenously, and the eyes were enucleated. These experiments were performed in strict accordance with the terms of the ARVO Resolution on the Use of Animals in Research.

In all five animals, enucleated eyes were opened at the equator and immediately immersed in fixative. The eyes of four monkeys were fixed with cacodylate buffered 2% paraformaldehyde, 2.5% glutaraldehyde, whereas the eyes of one animal were fixed in a mixture of 3% glutaraldehyde, 0.5% paraformaldehyde, 1.2% tannic acid in 0.1 M Sorenson phosphate buffer, pH 7.4. In all eyes, after 10–15 min, the lens was removed, and the anterior uvea was dissected from the corneoscleral tunic. The irises were fixed for 2–3 hr at room temperature and washed overnight in cold buffer. Subsequently, they were trimmed into radially oriented wedges from which 200 μm sections were cut with a DuPont TC-2 tissue chopper. These sections were oriented either parallel or perpendicular to the pupillary margin.

**Thin Sections**

Chopper sections to be used for conventional electron microscopy were postfixed in 1% OsO₄ and 1.5% potassium ferrocyanide in distilled water, dehydrated, and embedded in an Epon-Araldite mixture. Specimens from animals that had received HRP were reacted to demonstrate peroxidase activity as previously described.⁹

**Freeze-Fracture**

Specimens were processed for freeze-fracturing as previously described except that tissues were mounted between two specimen carriers to permit use of the double-replica technique of freeze-fracturing. Unlike conventional freeze-fracturing in which a single replica is produced, the double replica technique produces a pair of complementary replicas of the fractured surfaces permitting examination of both the P-face and E-face of the same cell.

All thin-sectioned or freeze-fractured specimens were examined with either an RCA-3G and AEI-6B or a JEOL-100 CX electron microscope.

**Results**

**Thin Sections**

The posterior pigmented epithelium of the iris (PPE) is distinguished from the anterior myoepithelium (AME) by several features including its lighter cytoplasm (Fig. 1). The AME cells are distinguished primarily, however, by the polarization of their cytoplasmic constituents; the nucleus and virtually all of the melanosomes present in these cells are confined to the apical region. The basal portions of these cells, which are essentially devoid of melanosomes, more closely resemble smooth muscle and, in fact, represent the dilator muscle of the iris.

Intravenously injected HRP diffuses from the normally leaky vessels of the ciliary body stroma. After 10–15 min, the tracer reaches the root of the iris, permeating the intercellular clefts between adjacent AME cells and filling the intercellular spaces between the apices of the anterior myoepithelium and posterior pigmented epithelium (Fig. 2). The tracer is excluded from the intercellular clefts between adjacent PPE cells, however, by the presence of a junctional complex including a zonula occludens, zonula adhaerens, and often a gap junction (Fig. 2).

Specifically, the passage of the tracer through the intercellular spaces is prevented by the zonulae occludentes, which are seen at high magnification to represent points of fusion between the outer leaflets of the adjacent cell membranes (Fig. 2, inset). In addition to a junctional complex, adjacent PPE cells also are joined by typical desmosomes (Fig. 3). At these junctional sites the adjoining cell membranes are separated by a very regular intercellular space, 17 nm wide, which is bisected by a dense line. The cytoplasmic aspects of the membranes are reinforced by a thin plaque into which a bundle of 9–10 nm tonofilaments is inserted. A smaller number of well-developed desmosomes also is found to join the apices of the AME and PPE cells (Fig. 3). These junctions are encountered rarely, however, between adjacent AME cells. Where present, their distribution is entirely limited to the epithelial (ie, apical) portions of these cells.

The same restrictive distribution between adjacent AME cells is found for another intercellular junction—the punctum adhaerens. This junction is characterized by the presence of a mat of fine filaments on the cytoplasmic aspect of each junctional membrane and an intercellular cleft, which varies in width from 12–17 nm. The largest complement of puncta adhaerenets, however, is found joining the apical surfaces of the AME and PPE cells. Among these are several puncta...
Fig. 1. Electron micrograph of iridial epithelia in M. mulatta. The posterior pigmented epithelium (PPE) is distinguished from the anterior myoepithelium (AME) by its lighter cytoplasm. The anterior myoepithelial cells give rise to processes of the dilator muscle of the iris (arrows) (X10,000).
Fig. 2. Electron micrograph of the iridal epithelium in *M. mulatta*. Black HRP reaction product fills the intercellular cleft between adjacent anterior myoepithelial cells (AME) and between the apices of the anterior myoepithelial and posterior pigmented epithelial cells (PPE). Passage of tracer between adjacent posterior pigmented epithelial cells is blocked by the presence of a zonula occludens (curved arrow). za, zonula adhaerens; d, desmosome (×25,500). Inset, A zonula occludens is identified as a point of fusion (arrows) between the outer membrane leaflets of adjacent posterior pigmented epithelial cells (×69,200).

adhaerentes, which occupy positions coterminus with gap junctions (Fig. 4).

Gap junctions are ubiquitous within both the AME and PPE. They are most abundant between the apical surfaces of the two epithelia but also are observed commonly joining the lateral surfaces of both PPE and AME cells. Within the AME, gap junctions are evident between both the apical, epithelial and basal, muscular portions of adjacent cells.

So-called invaginated gap junctions also are observed commonly along the apical and lateral surfaces of PPE cells (Fig. 5). Each of them appears as a vesicular outpouching from the surface of one cell, which is outlined by a complementary invagination in the surface of the adjacent cell. The contents of each vesicle remain in continuity with the cytoplasm of the cell of origin via a narrow inlet and over the circumference of this vesicle the two cells are joined by a gap junction (Fig. 5). In specimens reacted for demonstration of HRP activity, both invaginated and linear gap junctions exhibit a
series of cross-striations, which are known to be unrelated to the presence of exogenous HRP.²

Freeze-Fracture

In freeze-fracture replicas of the iris epithelia, the fracture plane commonly traverses the melanosome-filled cytoplasm of these cells revealing only small regions of cell membrane. In several specimens fractured using the double-replica technique, however, advantageous views of the intercellular junctions were obtained.

Freeze-fracture replicas confirm the presence of gap junctions joining the apical and lateral surfaces of the
cells in both epithelial layers. The largest complement of gap junctions is observed joining the apices of the two epithelial layers. These junctions appear as aggregates of 8–9 nm particles on the inner leaflet of the plasma membrane (P-face), which are complemented by an array of pits on the outer leaflet of the adjoining plasma membrane. At the edge of the gap junction, a punctum adherens (pa) is present (×84,750).

Fig. 5. An invaginated gap junction is seen as a vesicular outpouching of one cell into the domain of its neighbor. The contents of the vesicle remain in continuity with the cytoplasm of the cell of origin via a narrow inlet (curved arrow) and over the circumference of the vesicle the two cells are joined by a gap junction in which numerous cross-striations are evident (small arrows). A curved gap junction is also present (arrowhead) (×76,000).
cell’s membrane (E-face) (Fig. 6). The gap junctions vary in size from just a few particles to large plaques 1.0 μm in diameter. Within these arrays of particles, linear or polygonal areas of aparticulate membrane are observed commonly. In addition, some of the gap junctions are seen to be associated with discontinuous tight junctional strands (Fig. 6, inset).

Use of the double replica technique permits visualization of both the E-face and P-face of the same cell and is of value in demonstrating the continuity of zonular tight junctions and their complementarity within the membrane.

Replicas obtained using this technique confirm the presence of continuous zonulai occludentes joining the apico-lateral surfaces of adjacent PPE cells (Fig. 7). These junctions appear as a continuous series of branching and anastomosing single or double strands of particles on the P-face, which are complemented on the E-face by an identical series of shallow grooves with discontinuous rows of particles at their bases. The basal margin of the junctional network is distinguished by the presence of several junctional strands, which do not participate in anastomoses but instead end as free spurs (Fig. 7).

Inserted within the junctional network, small polygonally shaped gap junctions are evident, which have an appearance similar to those found elsewhere in the iris epithelia (Fig. 7).

The tight junctional network on the inner and outer leaflets of the plasma membranes complement each other when both replica pairs, obtained using the double replica technique, are compared. In several locations, the fracture plane can be seen to abruptly shift from the interior of the plasma membrane of one cell to that of its adjoined neighbor. At these sites, the P-face ridges of one cell are found in perfect register with the E-face grooves of the other, indicating that the tight junctional strands represent linear foci of membrane fusion, which effectively occlude the intercellular cleft (Fig. 7).

The number of tight junctional strands occluding the intercellular cleft was found to vary from as few as one to eight or more (Fig. 8). Among these, a number of parallel double strands were observed (Fig. 8).

Morphometric data on the complexity of the zonulai occludentes of the PPE is shown in Table 1. In a total junctional length of 35 μm, more than two-thirds of the zonulai occludentes (67.0%) consisted of 2–4 strands. Approximately one-third of the length was occupied by five or more strands. Only 1% of the entire junctional length consisted of a single strand.

**Discussion**

The types and distribution of intercellular junctions in the iridial epithelia of the rhesus monkey iris are summarized as follows. In the apical portion of the iris, the lateral cell margins are joined by puncta adherentes, desmosomes, and gap junctions. Only the latter of these three junctions are evident between basal, dilator, muscle processes of these cells. Joining the apico-lateral surfaces of the PPE cells a junctional complex is evident, which consists of a continuous zonula occludens, zonula adhaerens, and gap junction. The lateral margins of these cells also are joined by one or more desmosomes. The apical surfaces of the AME and PPE are joined by desmosomes, puncta adhaerentes, and gap junctions; the latter two of these occasionally appear to be continuous with one another.

The association between puncta adhaerentes and gap junctions is not unique to the iris epithelia and has been observed in other tissues including mouse and rabbit blastocysts and in granulosa cells during folliculogenesis. The same relationship has been observed between gap junctions and puncta adhaerentia joining the apices of the pigmented and nonpigmented ciliary epithelia in the adult rhesus monkey eye. Furthermore, in a comprehensive study of the formation and distribution of intercellular junctions in the developing optic cup of the rhesus monkey, Townes-Anderson and Raviola also noted the relationship of these junctional types and concluded that the presence of “intermediate” junctions may be a prerequisite for gap junction formation. This concept is supported further by morphometric studies of ovarian tissue, which demonstrated that the amount of “adhaeren” junctional membrane decreases as the amount of gap junctional membrane increases.

Gap junctions, such as those present throughout the iris epithelia, are known to mediate both metabolic and electronic coupling, thus permitting these two cell layers to function as a syncytium. This capability is essential for the iris to perform the rapid, precisely coordinated movements required to adjust the pupillary diameter for changes in light level and accommodative posture.

The invaginated gap junctions present in the iridial epithelium are identical to those reported in other tis-
Fig. 7. A complementary pair of freeze-fracture replicas exhibit continuous zonulae occludentes in the posterior pigmented epithelium of *M. mulatta*. Presented as mirror images, zonulae occludentes are represented by a network of branching and anastomosing strands on the P-face complemented by grooves on the E-face. At the bases of these grooves, particles dislodged from the P-face in fracturing are seen. Gap junctions are present within the tight junctional matrix (arrow) and free spurs are present at the basal margin of the junctions (arrow head). In locations where the fracture plane shifts from the interior of the plasma membrane of one cell to that of its neighbor the E-face groove and P-face ridge are in perfect register (open arrow) (x52,000).

The contraction of the smooth muscle elements within the AME creates mechanical stresses, which are shared by both epithelial layers. The well-developed desmosomes joining the lateral and apical cells surfaces of these epithelia likely represent the major morphologic feature resisting these forces.

Results of the ultrastructural tracer studies confirm the presence of a barrier to the passage of macromolecules between adjacent PPE cells. This location
is entirely analogous to that reported in the nonpigmented epithelium of the ciliary body. The present studies confirm that the morphologic equivalents of this barrier are the tight junctions, which occlude the intercellular cleft between the PPE cells.

An unusual feature of the zonulæ occludentes in the iris epithelium is the presence of adjacent double strands of particles within the junctional network. This arrangement of tight junctional strands is not unique to the iris epithelium, however. Double strands have been demonstrated in the tight junctions of several ocular and nonocular tissues including human and rat nephrons, chameleon brain capillaries, human pneumocytes, frog retinal pigment epithelium, and rhesus monkey iris blood vessels.

A correlation is known to exist between the complexity of tight junctions and the functional barrier properties of the junctional network. In the iris epithelium, the complexity of the zonulæ occludentes is quantified in Table 1.

Table 1. Complexity of tight junctions in the posterior pigmented epithelium of M. mulatta

<table>
<thead>
<tr>
<th>No. of strands in Zonulæ Occludentes</th>
<th>Percent of total junctional length</th>
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<tbody>
<tr>
<td>1 strand</td>
<td>1.0%</td>
</tr>
<tr>
<td>2-4 strands</td>
<td>67.0%</td>
</tr>
<tr>
<td>5-8 strands</td>
<td>27.0%</td>
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<tr>
<td>&gt;8 strands</td>
<td>5.0%</td>
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Total junctional length measured: 35 µm.
plexity of tight junctions, as revealed by freeze-fracturing, and physiologic properties such as hydraulic conductivity and electrical resistance, which express the leakiness of a cell layer.21,22 Comparing the morphometric data in Table 1 with that of Claude and Goodenough in which the degree of permeability in various simple epithelia was correlated with tight junctional complexity, the PPE of the iris is similar in junctional complexity to that reported for rabbit gallbladder epithelium.22 Not surprisingly, it is also similar to that observed in the non-pigmented epithelium of the rhesus monkey ciliary body.2

In conclusion, it appears that the types and distribution of intercellular junctions present within the epithelia of the rhesus monkey iris are entirely appropriate to their known functions and are most similar to those found in the epithelia of the ciliary body. Together, these epithelia protect the posterior chamber from circulating macromolecules.

Key words: Macaca mulatta, iris, intercellular junctions, electron microscopy, freeze-fracturing

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References