Desmosomal–Mitochondrial Complexes in Human Nonpigmented Ciliary and Retinal Pigment Epithelia

U. Schlörzer-Schrehardt,* H.-G. Müller,† P. M. Wirz,† and G. O. H. Naumann*

In the normal human nonpigmented ciliary epithelium structural associations between mitochondria and desmosomes are described electron-microscopically: either a single desmosome or a linear array of several desmosomes joined by filamentos bundles is associated with one or two mitochondria (19.6 ± 5.4%; n = 29). The frequency of occurrence of these complexes was studied in five different regions of the ciliary body; analysis of covariance revealed a significantly increased number of associations in the ciliary processes. Further, the age dependence of their occurrence was examined in 29 different age classes (15 to 86 years); correlation analysis revealed no correlation between age and number of associations. Similar complexes occur, in addition, in the retinal pigment epithelium, but have not been observed in ciliary pigmented, lens, iris and corneal epithelia. Desmosomal–mitochondrial complexes are considered to be a characteristic feature of basic physiological significance of certain epithelia only. The cytochemical demonstration of calcium in the associated mitochondria provides support for the hypothesis that the mitochondria may serve as buffers for intracellular calcium by controlling the local calcium concentration, thus increasing the stability and functional integrity of desmosomal junctions in secretory or actively transporting epithelia with high endogenous calcium levels. Invest Ophthalmol Vis Sci 31:664–669, 1990

Structural associations between desmosomes and cytoplasmic organelles, particularly mitochondria, have been reported in a variety of developing and adult animal and human tissues under both physiological and pathological conditions1–7; the functional significance of this relationship, however, has not yet been established.

Recently, Freddo8 demonstrated the existence of desmosomal–mitochondrial complexes in the ciliary epithelia of human, monkey and rabbit eyes. We extended this study by documenting their additional occurrence in the human retinal pigment epithelium, and by adding some new ultrastructural, ultrahistochemical and quantitative information on these associations in the normal human nonpigmented ciliary epithelium. A semiquantitative method has been used to measure the frequency of occurrence of the complexes both in different regions of the ciliary body and in different age groups. In view of the established hypothesis that the associated mitochondria are capable of calcium ion accumulation and regulation,8 the antimonate precipitation method9 has been applied to visualize the calcium distribution in the ciliary and retinal pigment epithelia at the ultrastructural level.

Materials and Methods

Twenty-seven normal human autopsy eyes, enucleated and fixed less than 5 hr postmortem, and two eyes enucleated because of intraocular tumors were studied. The ages ranged from 15 to 86 years. Small meridional sectors of the ciliary body were fixed in 2.5% glutaraldehyde in 0.15 M Soerensen buffer (pH 7.2), postfixed in 2.0% buffered OsO4, and dehydrated and embedded in Epon according to standard fashion. Ultrathin transverse sections were made on a Reichert-Ultracut, stained with uranyl acetate and lead citrate, and examined with a Zeiss (Oberkochen, West Germany) EM 9A electron microscope.

In each of the specimens, four different regions of the ciliary body were investigated electron-microscopically for semiquantitative evaluation: I: transition zone between iris and ciliary body; II: middle portion of pars plicata—(a) ciliary processes, (b) ciliary valleys; III: transition zone between pars plicata and pars plana; and IV: middle portion of pars plana. In each zone a total of 500 consecutive desmo-
somes between adjacent nonpigmented epithelial cells was counted, and the percentage of desmosomal-mitochondrial complexes was determined. Counts were recorded only on mitochondria obviously attached to the desmosomal filaments; those observed close, but not attached to the desmosomes were not counted.

Additionally, a survey was made of several ocular epithelia (i.e., corneal epithelium, lens epithelium, iris epithelium, and retinal pigment epithelium) to see if similar complexes were present.

Statistics

The methods used were simple linear regression analysis and analysis of covariance with a repeated measurements factor. Data were checked for normality by using normal probability plots. The methods were applied by using the BMDP2V-program and the SPSS/PC+ procedure “regression.”

Ultrahistochemistry

Ciliary body and retinal specimens were fixed in a solution of 2.0% glutaraldehyde containing 4.0% potassium pyroantimonate (pH 7.2), postfixed in 1.0% OsO4 containing 4.0% potassium pyroantimonate, and embedded for electron microscopy. To investigate the role of calcium in precipitate formation, control experiments included precipitate removal by incubation of ultrathin sections in 5 mM EGTA at 60°C for 1 hr.

Results

In the normal human ciliary epithelium, desmosomal junctions were regularly found connecting the lateral faces of nonpigmented epithelial cells without showing any preferential locations. Some of these desmosomes were structurally associated with mitochondria; in these cases one mitochondrion or two mitochondria from two adjacent nonpigmented cells were attached to the cytoplasmic surfaces of the desmosomal junction (Fig. 1). Desmosomes had associated mitochondria in 19.6 ± 5.4% (n = 29; per count of 2500 desmosomes) of desmosomes observed. Serial sections revealed that some of the mitochondria not obviously associated with desmosomes in one particular profile were attached to a junction in another plane of section. Therefore, the actual percentage probably is somewhat higher.

The intimate relationship between desmosomes and mitochondria was demonstrated by the flattening of the mitochondrial membrane in the area of contact, usually running parallel to the plasma membrane. The ultrastructural organization of the desmosomes conformed to the pattern typical of other epithelia. Intermediate filaments, paralleling the plasma membrane, filled the 63 to 85 nm wide space between junction and mitochondria. The tonofilaments did not appear to blend with the outer mitochondrial membrane, because no point of contact was evident. Instead, a constant gap of approximately 5 nm could be demonstrated between both structures.

![Fig. 1. Electron micrograph of the nonpigmented ciliary epithelium (zone III; 42 years) showing mitochondria (M), desmosomes (D), and a desmosomal-mitochondrial association (arrow). LM, inner limiting membrane; N, nucleus; P, pigment granulum; R, rough endoplasmic reticulum.](image)
Desmosomal–mitochondrial associations occurred as two appearances of different complexity: either a single desmosome (Fig. 2A) or a linear array of several desmosomes, usually two to three (Fig. 2B), was involved. In the latter case the serially arranged junctions, spaced 50 to 350 nm, were always joined by additional filamentous bundles clinging to the outer mitochondrial membrane. Filaments coming from the desmosomal plaques appeared to enter the 20 to 30 nm thick bundles directly. The average percentages of associations between mitochondria and such series of desmosomes in the different regions of the ciliary body (zone I: 2.2%; zone IIa: 2.3%; zone IIb: 4.5%; zone III: 3.4%; zone IV: 1.5%; per count of 500 desmosomes; n = 29) revealed an increased frequency of occurrence in zones IIb and III.

Organelle-junctional associations of both types involving one or two mitochondria could be found in the nonpigmented epithelium in all regions of the ciliary body, as well as in all age classes examined. Serial sections did not reveal any preferential distribution of these complexes within the cells. The frequency of occurrence of associations in the different zones is given as average percentages along with the corresponding 95% confidence intervals in Table 1, as well as graphically visualized in Figure 3.

Further, the age dependence of their occurrence was investigated for the different zones separately by correlation analysis: zone I: $r = 0.06$, $P = 0.75$; zone IIa: $r = 0.16$, $P = 0.39$; zone IIb: $r = -0.03$, $P = 0.86$; zone III: $r = 0.14$, $P = 0.44$; zone IV: $r = -0.13$, $P = 0.48$; n = 29. Obviously, there was no correlation between age and number of complexes within the different zones.

Analysis of covariance with covariate age of subject and zone as repeated measurements factor yielded the following results: the effect of the zone was highly significant.

**Table 1.** Average percentages of desmosomal–mitochondrial associations in the different zones of the ciliary body along with the corresponding 95% confidence intervals (n = 29; counts per zone/subject: 500)

<table>
<thead>
<tr>
<th>Zone</th>
<th>Mean percentage</th>
<th>95% Confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower bound</td>
</tr>
<tr>
<td>I</td>
<td>18.803</td>
<td>18.794</td>
</tr>
<tr>
<td>IIa</td>
<td>22.393</td>
<td>22.383</td>
</tr>
<tr>
<td>IIb</td>
<td>18.903</td>
<td>18.894</td>
</tr>
<tr>
<td>III</td>
<td>18.772</td>
<td>18.763</td>
</tr>
<tr>
<td>IV</td>
<td>18.393</td>
<td>18.384</td>
</tr>
</tbody>
</table>
significant, in particular the mean number of associations for zone Ila was significantly larger than that for the other zones ($P = 0.0002$), whereas age of subject had no significant influence ($P = 0.76$).

Connections between desmosomes and other organelles could not be observed.

Desmosomal junctions connecting both ciliary epithelial layers at the apical surfaces of the cells occasionally were attached to only one mitochondrion on the nonpigmented epithelial aspect. The pigmented epithelial cells of the ciliary body, in contrast, did not show any structural relationship between desmosomes and mitochondria.

An additional survey of several ocular epithelia (i.e., corneal epithelium, lens epithelium, iris epithelium) failed to show evidence of similar complexes. Comparable relationships between mitochondria and single desmosomes, however, could be found, on occasion, along the lateral faces of retinal pigment epithelial cells (Fig. 4); associations with serially arranged junctions, however, were equally lacking.

The ultrahistochemical findings revealed that coarse, electron-dense deposits of antimony in the ciliary nonpigmented and retinal pigment epithelial cells were present in the cytoplasm, nuclei and intercellular spaces, but were localized in abundance on the plasmalemma and both in associated and nonassociated mitochondria (Fig. 5). With the technique used, calcium is indicated by the antimonate precipi-
tates. Furthermore, control experiments with EGTA incubation of sections resulted in the almost complete removal of the deposits, suggesting that the precipitate visualized was preferentially due to calcium in the tissue.

Discussion

In accordance with Freddo's recent documentation of desmosomal–mitochondrial complexes (DMCs) in the ciliary epithelia of human, monkey and rabbit eyes, the present findings revealed DMCs to be a characteristic ultrastructural feature of human nonpigmented, but not the pigmented ciliary epithelium, a differentiation which was not made in above-mentioned study. Additionally, the present report demonstrates a comparable topographic association between desmosomes and mitochondria in the human retinal pigment epithelium, thus refuting Freddo's statement that DMCs are unique in the ciliary epithelium among ocular tissues.

Although similar structural associations have been reported in a wide range of animal and human tissues, their functional significance remains obscure. Existing speculations regard them as sites of intercellular communication, sites of intensified mechanical stability, pathologic alterations or purely fortuitous attachments. The present investigation of 29 unselected normal eyes demonstrates the existence of DMCs in the nonpigmented ciliary and retinal pigment epithelia of all subjects examined. The associations, therefore, obviously represent a constant, age-independent feature of certain epithelia only, reflecting a condition of physiological significance rather than pathologic alterations. Furthermore, the relatively even frequency of DMCs throughout all regions of the ciliary body, as well as their complete absence in other epithelia, argue against a relationship due to random chance. The mechanism of anchoring between desmosomes and mitochondria is not known. A gap of constant width between mitochondrial membrane and tonofilaments suggests not only close apposition, but specific adhesion. A real blending of the filaments with the outer mitochondrial membrane, as found by Freddo, could, however, not be observed.

Why do DMCs selectively occur in certain epithelia only, of which are obviously united by a secretary function? The ciliary epithelium generally is accepted to be involved in aqueous humor secretion and other synthetic capacities. Experimental studies on aqueous formation indicate that the active transport system is located in the nonpigmented cells. The retinal pigment epithelial cells have an equally high metabolic rate due to many complex functions, including the secretion of an acid–mucopolysaccharide complex and active transport of numerous metabolites, including calcium. The findings of the present report, therefore, provide additional support for the hypothesis that DMCs may play a role in maintaining the functional integrity and stability of secretary or, in general, actively transporting epithelia with a high endogenous level of calcium.

Key words: desmosomal–mitochondrial complexes, ciliary epithelium, retinal pigment epithelium
Acknowledgment

The authors are indebted to Professor M. Yanoff, MD for helpful discussion.

References