Polarized Distribution of Integrin and Fibronectin in Retinal Pigment Epithelium

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We have examined the presence and distribution of integrin and fibronectin in the retinas of 21-day chick embryos and adult rats, with particular emphasis on the question of localization in the retinal pigment epithelium (RPE). Isolated sheets of RPE solubilized and separated by gel electrophoresis contain integrin, as indicated by immunoblotting with polyclonal and monoclonal antibodies to the complex. By the same technique, antibodies to fibronectin reacted with a single protein in the isolated RPE. In both chick and rat, integrin and fibronectin were localized by indirect immunofluorescence exclusively to the basement membrane of the RPE, the choriocapillaris and the retinal-vitreal border. When isolated RPE cells from chick retinas were examined, integrin was seen to be present along the basolateral surfaces of the cells as well. Similarly, in the intact rat retina, staining for integrin could be seen along the lateral surfaces of some of the RPE cells. Neither integrin nor fibronectin were present along the apical surfaces of the RPE in either rat or chick. The close similarity between the location of integrin and fibronectin supports the idea that the RPE adheres to the basal lamina at least in part via integrin-fibronectin linkages. A clear implication of our results is that the adhesion between RPE and retina requires a different set of linkage proteins. Invest Ophthalmol Vis Sci 28:1275–1280, 1987

The RPE cells comprise a polarized epithelial sheet with an apical surface of elaborate filopodia which interdigitate with and adhere to the outer segments of the photoreceptor cells. At their base the cells are adherent to the basal lamina, the so-called Bruch's membrane. The basal lamina of the RPE cells has been shown to be composed of type IV collagen, heparan sulfate, laminin and fibronectin.1-4 Recent studies have shown that cultured epithelial cells, myoblasts and fibroblasts adhere to the basal lamina or substratum via a complex of three integral membrane proteins which have been characterized and named integrin.5-7 This receptor complex has been shown to bind both fibronectin and laminin.8,9 Furthermore, integrin has been shown to interact with the cytoskeleton, thus forming a transmembrane linkage between the cytoskeleton and the extra-cellular matrix.10,11 This linkage may stabilize cell morphology and adhesion.

Based on these studies, one might expect that integrin would be found on the basal membrane of the RPE cells and absent from the apical surface. However, it was recently reported12 that in the rat, fibronectin was present all along the filopodia of the RPE cells. This would suggest a role for fibronectin both in cell-cell and cell-substratum adhesion and perhaps a nonpolarized distribution of integrin. To address this problem we examined the distribution of both integrin and fibronectin in the retinas of both chick and rat.

Materials and Methods

Animals

White Leghorn chick embryos used in these studies were procured from a local supplier and incubated in a forced draft incubator at 38.0° to 38.5°C. The chicks were used at embryonic day 21. Wistar rats were used in these studies and were maintained on a 12L:12D cycle. The animals were killed during the light period of the cycle. Rats were maintained and treated in accordance with the ARVO Resolution on the Use of Animals in Research.

Antibodies

A polyclonal antibody to the chick integrin complex was a gift of Dr. Caroline Damsky (University of California, San Francisco). A polyclonal antibody to rat integrin and monoclonal antibodies to the subunits were gifts of Dr. Clayton Buck (Wistar Institute, Philadelphia). Antibodies to human plasma fibronectin were purchased from BRL (Bethesda Research Institute).
Fig. 1. Immunoblot analysis of proteins from chick and rat RPE cells with antibodies to fibronectin (lanes A, B, C) and antibodies to integrin (G, H). Proteins were separated on 7.5% polyacrylamide gels and electrophoretically transferred to nitrocellulose paper. Lane A: fibronectin standard; lane B: chick RPE cells; lane C: rat RPE cells; all lanes crossreacted with anti-fibronectin. In D and E 10 μg of fibronectin standard (upper spots) and 10 μg of proteoglycans (dashed circle) were dot blotted on to GenScreen and reacted with antibody to fibronectin from BRL: lane D and from Cooper Biomedicals: lane E. Lane F is a Coomassie Blue-stained gel of chick RPE cells. Lane G: integrin standard; lane H: chick RPE cells; G and H reacted with the polyclonal antibody to chick integrin.

Laboratories, Bethesda, MD) and Cooper Labs (Malvern, PA).

SDS Polyacrylamide Gel Electrophoresis and Western Blots

RPE cells were isolated from chick retinas as previously described.13 Rat RPE cells were obtained by removing the neural retina from the posterior eye cup and blotting a disc of nitrocellulose paper onto the RPE. This method resulted in a crude preparation of RPE cells which contained both intact and broken cells as well as some choroidal contamination. The discs were placed in Laemmli sample buffer to extract the cellular proteins from the nitrocellulose paper. Samples from both the chick and the rat preparations were electrophoresed on microslab gels14 using the buffer system of Laemmli.15 Gels were run at a constant voltage of 150 V, and the proteins were then electrophoretically transferred to nitrocellulose paper at 30 V and 0.1 A overnight using a TransBlot apparatus (Biorad Laboratories, Richmond, CA). The nitrocellulose paper was washed for 1 hr in buffer containing 50 mM Tris pH 7.8, 0.5% Nonidet P-40, 0.15 M NaCl and 3% bovine serum albumin, followed by exposure to the primary antibodies for 60 min. Antibody to fibronectin obtained from BRL was used at a dilution of 1:100 in a PBS buffer containing 1% BSA, 0.05% Tween and 0.02% azide. The sections were washed in the same buffer and incubated with rhodamine conjugated goat anti-rabbit diluted 1:100 (Jackson Laboratories, Avondale, PA), then washed again and mounted onto slides. Isolated RPE cells prepared as previously described13 were processed as above. Slides were examined on a Zeiss Research microscope (Carl Zeiss, West Germany) equipped with a 63X phase objective with a N.A. of 1.4. Photographs were taken with Kodak Tri-X film (Rochester, NY).

Results

Characterization of Antibody Specificity

The preparations of chick and rat RPE cells were tested for cross-reactivity with antibodies to fibronectin, using the procedures and antibody concentrations described above.

Immunocytochemistry

Eyes from chick embryos and adult rats were fixed for 30 min in 2% paraformaldehyde in PBS containing 1 mM MgCl2 and 3% sucrose, rinsed in PBS, then washed overnight at 4°C in PBS containing 30% sucrose. The eyes were embedded in Tissue Tek II (Lab Tek, Naperville, IL) and frozen in liquid nitrogen. Six micrometer cryostat sections were cut and mounted on gelatin-coated coverslips. The sections were incubated with primary antibodies which were diluted 1:100 in a PBS buffer containing 1% BSA, 0.05% Tween and 0.02% azide. The sections were washed in the same buffer and incubated with rhodamine conjugated goat anti-rabbit diluted 1:100 (Jackson Laboratories, Avondale, PA), then washed again and mounted onto slides. Isolated RPE cells prepared as previously described13 were processed as above. Slides were examined on a Zeiss Research microscope (Carl Zeiss, West Germany) equipped with a 63X phase objective with a N.A. of 1.4. Photographs were taken with Kodak Tri-X film (Rochester, NY).
Fig. 2. Immunofluorescence-phase pairs of sections of chick retina (a–d) or of isolated chick RPE cells (e, f). Frames a, b, e, f are treated with anti-chick integrin at 1:100; frames c and d are with antifibronectin at 1:100. Notice the staining of the choriocapillaris and along the basement membrane with both antibodies. There is no staining of the apical processes with either antibody. This is especially clear in the isolated cells, where the small arrows in (e) and (f) point to apical processes which are not stained by anti-integrin. During isolation, fibronectin is released so that diffuse staining is seen (not shown). Note that the staining with anti-integrin extends about half way along the lateral borders of the isolated cells. Bar is 10 μm.

Immunofluorescent Localizations

Indirect immunofluorescence was used to localize fibronectin and integrin in the retinas of the chick and of the rat. In both the chick and the rat, staining with BRL anti-fibronectin gave a fluorescent band along the basal surface of the RPE cells in Bruch’s membrane as well as brilliant staining of the underlying capillaries and choroid (Fig. 2c, d and Fig. 3c, d). 

boronectin standard but not with proteoglycans purified from nasal septum. The anti-fibronectin from Cooper laboratories reacted more weakly with the fibronectin standard than the antibody from BRL (Fig. 1D, E). Additionally, the anti-fibronectin from Cooper showed nonspecific binding to RPE cell proteins on Western blots (data not shown).

Antibodies to the integrin complex were also tested on Western blots of whole RPE cells. The polyclonal antibody to integrin crossreacted primarily with the 120 kD subunit of the integrin complex (Fig. 1G, H). There was also some crossreaction with a higher molecular weight component which probably represents aggregates of the integrin molecule, since this is often seen with the purified complex (C. Buck, personal communication). The lower molecular weight component probably represents degradation products.

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A thin line of staining was also observed in both species at the inner limiting membrane (ILM) (Fig. 4b, c). No staining with antibronectin was observed in the region of the apical processes or in the interphotoreceptor space in either the chick or the rat.

Integrin also showed a polarized distribution in the RPE cells. Staining with anti-integrin was localized mainly to the basal surface of the RPE cells, (Fig. 2a, b, and Fig. 3a, b) but in some instances, lateral staining was also observed. This was seen more often in sections of rat tissue (arrows in Fig. 3a, b) and very clearly in isolated chick RPE cells as shown in Figure 2e, f. As was shown with fibronectin, the antibody to integrin brightly stains basement membranes of capillaries and tissues of the choroid and sclera. Staining was also observed with anti-integrin at the ILM, but unlike the localization of fibronectin, the staining extended into the nerve fiber layer (Fig. 4a).

A polarized distribution of integrin was also observed in the nonpigmented layer of the ciliary epithelium, which is oriented with polarity reversed with respect to the RPE. The inner, apical surface of these cells showed little or no staining with anti-integrin while the basal and lateral surfaces exhibited bright staining (Fig. 4d, e). A similar staining pattern for fibronectin has previously been reported.

**Discussion**

It has been known for many years that the constituents of the extracellular matrix are important regulators of morphogenesis and cell differentiation. Recently, plasma membrane proteins have been identified which link cells directly to the components of the extracellular matrix. Integrin is an integral membrane glycoprotein complex that has been shown to have binding affinities for both laminin and fibronectin. Additionally, integrin has an intracellular binding site for talin, a cytoskeletal linking protein. Since it provides a transmembrane linkage...
between the extracellular matrix and the cytoskeleton, integrin could affect cell adhesion and migration. This was demonstrated in experiments where antibody to the integrin complex was shown to disrupt cell-substrate adhesion and to reduce cell migration.

Adherence of the RPE cells to the basement membrane and to the neural retina is critical for maintaining the nonproliferative, differentiated state of these cells. In the present study we have shown that integrin exhibits a polarized distribution in RPE cells. Its localization is restricted to the basolateral membranes of the RPE cells both in the chick and in the rat. This finding agrees with a recent report which found integrin localized to the basement membranes of many epithelial tissues. We have also found that talin co-localizes with integrin in the RPE cells (unpublished observation). The distribution of integrin is similar to the distribution of fibronectin (Figs. 2 and 3) and the previously reported distribution of laminin, suggesting a role for this protein in adherence of the RPE cells to the basement membrane. The lack of expression of integrin on the apical surface of the RPE cells shows that different mechanisms regulate cell-cell (at least, RPE-neural retina) and cell-substratum interactions.

In addition to the localization of integrin to the basal region of the RPE cells, we also find staining with antibody to integrin at the ILM of the retina (Fig. 4). Staining with the antibody to integrin is not limited to the ILM but extends into the nerve fiber layer, while staining with anti-fibronectin is seen as a thin line restricted to the ILM. A similar localization of fibronectin has been reported for the bovine eye. The presence at the ILM of both integrin and fibronectin suggests a role for integrin in vitreal-retinal ad-
hesion. Studies at the EM level are needed to determine which cell type is expressing integrin; its distribution suggests that Müller cells are likely candidates. While under normal conditions integrin may function to maintain adhesion of the RPE to the substratum, after ocular trauma it may play a direct role in the migration of the RPE cells toward the vitreous. Fibronectin is not normally present in the interphotoreceptor space (Fig. 2 and 3, and refs. 1, 3), but an insult to the eye can cause a retinal tear resulting in the leakage of plasma fibronectin into the interphotoreceptor space. Studies from several laboratories have shown that RPE cells are found in the vitreal cavity after retinal detachment or cryotherapy.20,21 The RPE cells apparently migrate to the vitreous cavity where they proliferate and attach to the vitreous or to the ILM, and participate in the formation of cellular membranes. Recent in vitro work has shown that RPE cells will migrate along a fibronectin gradient.22 Support for integrin-fibronectin interactions in RPE cell migration is demonstrated by studies which show that the fibronectin tetrapeptide (arg/gly/asp/ser) inhibits the attachment of RPE cells to fibronectin and inhibits the migration of RPE cells along a fibronectin gradient.23 The tetrapeptide is the cell binding domain of fibronectin recognized by the integrin complex. In conclusion, we have demonstrated the presence of the integrin molecule in the basolateral membrane of the RPE, suggesting a role for this protein in adherence of the RPE cells to the basement membrane. Since neither integrin nor fibronectin are present on the apical surface of the RPE, this demonstrates that fibronectin is not involved in retinal adhesion.

Acknowledgments

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Key words: integrin, fibronectin, retinal pigment epithelium, retina, immunocytochemistry

References