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Sequestration of basic fibroblast growth factor in the primate retinal interphotoreceptor matrix

(cone matrix sheath/eye/proteoglycan/retina)

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ABSTRACT **The interphotoreceptor matrix (IPM) occupies the extracellular space between the photoreceptors of the retina and the apical surface of the retinal pigmented epithelium. A large proportion of the IPM is composed of aqueous-insoluble glycoconjugates, including chondroitin sulfate-containing proteoglycans, the distribution of which exhibits both apical–basal and photoreceptor cell type-specific heterogeneities. The precise function of most insoluble IPM constituents is unknown, although the available evidence suggests some may contribute to retinal adhesion or photoreceptor survival. We have now identified basic fibroblast growth factor (bFGF), or an immunologically related protein from the FGF family, within the IPM. The IPM is labeled on sections of primate retinas by a battery of polyclonal antibodies (Abs) directed against various peptide sequences of bFGF and by an Ab to bovine brain bFGF. bFGF Abs also bind to purified preparations of aqueous-insoluble IPM. All bFGF Abs utilized cross-react with equivalent low molecular mass components of 16.5–17.5 kDa on Western blots of insoluble IPM proteins, purified bFGF, and recombinant bFGF. The Abs do not bind any aqueous-soluble IPM components, suggesting that the bFGF is normally bound to an insoluble IPM constituent(s) *in situ*. The fact that bFGF is sequestered in the IPM and is located in such close proximity to the photoreceptors, the retinal pigmented epithelium, and Mueller's glia raises the strong possibility that it is synthesized by and regulates the activities of one or more of these three cell types *in vivo*.**

The fibroblast growth factors (FGFs) are a family of peptides that have been isolated from a broad range of cell types. FGFs share a number of structural, biochemical, and biological properties, the most characteristic being that they bind to heparin (1–4). The FGF family consists of acidic FGF (aFGF), basic FGF (bFGF), and a number of other related molecules that exhibit significant sequence homology to these two proteins. FGFs, like some other growth factors, are regarded as multifunctional in that they have a range of effects and activities *in vitro* that depend upon the target cell type (5). To date, FGFs have been shown: to influence neuronal differentiation, survival, and regeneration (6–9); to act as mitogens (10–13) and as inducing factors (14–17); to modulate protein synthesis (12, 18); and to induce cell motility and migration (19). Much more is known about the effects of FGFs *in vitro* than under *in vivo* conditions.

Although biochemical assays have shown that FGFs are present in a wide variety of cell types and tissues (for review, see refs. 3 and 4), only a few studies have sought to determine their specific cellular and/or extracellular distributions. Antibodies (Abs) have been used to localize bFGF to the cytoplasm of rat brain neurons (20–22) and chicken striated muscle myoblasts (23) and to the extracellular matrix of mouse hind-limb muscle (24). A recent comprehensive study of bFGF distribution in the 18-day rat fetus revealed intracellular labeling of a number of cell types, but immunoreactivity was most strongly associated with the extracellular matrix principally in the basement membranes underlying epithelia (25).

It has been recognized for some time that the retina, as well as other ocular tissues and fluids, contains substantial levels of aFGF and bFGF (26–31). aFGF immunoreactivity has been noted within the ganglion cell and inner nuclear layers of the retina and within the photoreceptor cell inner and outer segments (28, 32). bFGF immunoreactivity, however, has been reported only in association with the vasculature of fetal and adult bovine retinas (32, 33).

The data in this study demonstrate that bFGF, or an immunologically related protein, is present at a number of specific intracellular and extracellular locations in the normal adult primate retina. We report that bFGF is sequestered within and is apparently bound to the insoluble fraction of the interphotoreceptor matrix (IPM), the extracellular matrix entrapped between the neural retina and retinal pigmented epithelium (RPE). bFGF is especially prominent within domains that ensheath the cone photoreceptors, the "cone matrix sheaths" (CMSs) (34–37). Thus, these results suggest that bFGF may participate in the regulation of one or more aspects of RPE, photoreceptor, or Mueller cell metabolism *in vivo*.

MATERIALS AND METHODS

Animals. Adult cynomolgus macaques (*Macaca fascicularis*) were obtained from Charles River Laboratories and the National Institutes of Health. Animals were treated in conformity with National Institutes of Health guidelines (38). Monkey eyes were obtained immediately after euthanasia induced by an overdose of barbiturate. The eyecups were fixed in various primary fixatives or the retinas were removed for isolation of the IPM.

Reagents. Polyclonal Abs raised in rabbits against various synthetic fragments of bovine pituitary bFGF were obtained from Andrew Baird (Whittier Institute of Diabetes and Endocrinology, La Jolla, CA). The amino acid sequence of the synthesized peptides corresponds to the published numbered sequences (39). The following bFGF antisera were utilized in

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Abbreviations: Ab, antibody; FGF, fibroblast growth factor; aFGF, acidic FGF; bFGF, basic FGF; CMS, cone matrix sheath; FITC, fluorescein isothiocyanate; IPM, interphotoreceptor matrix; PNA, peanut agglutinin; RPE, retinal pigmented epithelium.

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this study: Abs 729 [bFGF-(1–10)-peptide], 773 [bFGF-(1–24)-peptide], 781 [bFGF-(69–87)-peptide], and 810 [bFGF-(30–50)-peptide]. Abs 773 and 810 were purified further using protein A-Sepharose column chromatography. In addition, a neutralizing polyclonal Ab generated against bovine brain bFGF were purchased from R & D Systems (Minneapolis) as was purified bovine brain bFGF. Recombinant human bFGF was obtained from Upstate Biotechnology (Lake Placid, NY). Horseradish peroxidase- and fluorescein isothiocyanate (FITC)-conjugated secondary Abs were purchased from Sigma. FITC-conjugated peanut agglutinin (PNA) was obtained from Vector Laboratories.

Immunohistochemistry and Lectin Histochemistry. Some eyecups were fixed for 2–4 hr in 4.0% (vol/vol) formaldehyde in 100 mM sodium cacodylate (pH 7.2). After fixation, the eyecups were processed, embedded, and sectioned to a thickness of 5–6 μm on a cryostat by using published procedures (40). Unfixed retinas or retinas fixed 10–20 min in ice-cold (4°C) acetone or 70% ethyl alcohol were used in some cases.

Sections from each retina were incubated in FITC-conjugated PNA to confirm the presence of IPM by using a published procedure (35). Some sections or retinas, fixed and unfixed, were pretreated with type V hyaluronidase (1 mg/ml) (25) or 2 M NaCl prior to incubation in primary Ab. After blocking in 5% (vol/vol) globulin-free bovine serum albumin for 30 min, antisera or immunoglobulin fractions diluted in 10 mM sodium phosphate (pH 7.4) containing 1% bovine serum albumin were applied to sections at dilutions between 1:50 and 1:200 in a humidified chamber for 60 min at room temperature. Slides were rinsed in buffer and exposed to the appropriate affinity-purified secondary Ab in buffer in the dark for 30–60 min at room temperature. Sections were then rinsed once again, coverslipped, and examined using an epifluorescence microscope.

Controls included (*i*) incubation of sections with similar concentrations of normal or preimmune rabbit serum, other growth factor Abs (including aFGF, nerve growth factor, platelet-derived growth factor, or epidermal growth factor) in place of primary Ab, followed by incubation in secondary Ab, (*ii*) incubation in secondary Ab alone, and (*iii*) incubation in primary Ab preadsorbed for 8–12 hr at 4°C in buffer containing bFGF (5 μg/ml of anti-bFGF diluted 1:100).

Preparation of Insoluble IPM. Preparations of insoluble IPM were prepared as described (41). Neural retinas were separated from the RPE and subsequently rinsed for 7–10 min in 10 mM phosphate-buffered saline to remove all aqueous-soluble IPM constituents (42, 43). The retinas were then placed photoreceptor side down in a solution of 4 M urea, 0.5% Nonidet P-40, phosphate-buffered saline, and a mixture of protease inhibitors [2 mM phenylenethylsulfonyl fluoride/10 mM *N*-ethylmaleimide/5 mM benamide hydrochloride/10 mM 6-amino-*n*-caproic acid/pepstatin A (1 μg/ml)/leupeptin (1 μg/ml)/aprotinin (100 kallikrein inhibiting units/ml)/0.02% sodium azide/0.04% EDTA]. This procedure results in the separation of large "sheets" of insoluble IPM components from the neural retina. These sheets were pelleted and resuspended in five changes of buffer, pelleted again, and either fixed or utilized for biochemical analyses as described below.

Gel Electrophoresis and Immunoblot Analysis. Approximately 100 μg of purified insoluble IPM and 1–5 μg of recombinant human bFGF or purified bovine brain bFGF were diluted in sample buffer, boiled for 5 min, and centrifuged for 5 min at 10,000 × *g*. After centrifugation, 100–150 μg of IPM per well was loaded onto 15% polyacrylamide slab gels containing SDS. Separated proteins were transferred electrophoretically to nitrocellulose membranes and, after incubation for 1 hr in the bFGF Abs diluted 1:100 or 1:200 in TBMC (50 mM Tris·HCl, pH 7.04/1 mM $MgCl_2$/1 mM $CaCl_2$), were processed as described (35) to visualize the bFGF antigen. The absence of contaminants from the aqueous-soluble IPM and from photoreceptor outer segments was verified by Western blot analysis of insoluble IPM by using Abs to interphotoreceptor retinoid binding protein (the major soluble IPM protein) and opsin (the major photoreceptor outer segment membrane protein). Controls for Ab binding specificity were similar to those outlined above.

RESULTS

Immunohistochemical Localization of bFGF. All bFGF Abs utilized in this study except 729, the Ab generated against bFGF-(1–10)-peptide, bound intensely to the IPM on retinal sections (Fig. 1 *A–D* and *G–I*). No specific binding was observed under any of the control conditions (Fig. 1*J*). Intense labeling of the IPM, including the CMSs, was also observed when isolated pellets of IPM material were fixed, sectioned, and then incubated with specific bFGF peptide Abs (Fig. 1 *L* and *M*). At higher magnifications, positive labeling of IPM constituents associated with rod photoreceptor cells was also apparent in such preparations (Fig. 1*L*). In general, anti-bFGF binding to the IPM was not fixation dependent, although only weak binding to the IPM was obtained under some fixation conditions using the polyclonal Ab to bovine brain bFGF. Anti-bFGF labeling associated with the IPM was also detected in tissues fixed solely in dehydrating agents such as acetone or ethanol (Fig. 1 *C* and *D*). Under such conditions, IPM constituents collapse toward the photoreceptor outer segment plasma membranes.

Binding of bFGF Abs to other regions of the neural retina and RPE was dependent upon the fixative, the embedding medium, and/or the specific Abs employed. Labeling throughout the retina, possibly associated with Mueller's glia (Fig. 1 *G–I*), and labeling of basement membranes at several retinal locations demonstrate this finding. For example, binding associated with the basement membrane at the interface between the vitreous humor and the neural retina (i.e., the inner limiting membrane) was demonstrable with acetone (Fig. 1*C*) but not 4% (wt/vol) paraformaldehyde (Fig. 1*B*) as the fixative. In contrast, bFGF peptide Ab 781 bound to the basement membranes associated with the retinal vasculature (Fig. 1*H*) irrespective of the fixation regimen employed.

All binding patterns outlined above were specific, based on observation of controls (Fig. 1*J*). Pretreatment of sections with type V hyaluronidase, reported to enhance labeling in rat fetal tissues (25), did not alter the retinal labeling patterns. In contrast preincubation of unfixed sections or freshly isolated retinas in 2 M NaCl, a treatment known to release bFGF from extracellular matrices (44), reduced the intensity of anti-bFGF labeling dramatically (Fig. 1*K*).

Immunoblot Analysis. All bFGF peptide Abs reacted strongly with two to three bands between 16.5 and 17.5 kDa on Western blots of insoluble IPM proteins separated by SDS/PAGE (see Fig. 2). All of the bFGF Abs utilized also bound to purified bovine brain bFGF (Fig. 2, lanes E and F) and to recombinant human bFGF (Fig. 2, lanes G and H). The former ran as either a doublet or broad band at 17–17.5 kDa; the latter ran as a single band of 17.5 kDa. In both cases, the purified bFGFs and the immunoreactive bands present in the IPM preparations comigrated at the same molecular mass position(s) (Fig. 2, lanes B and C). Dimers of the purified bFGFs (Fig. 2, lane E) comigrated with the higher of two 33- to 35-kDa IPM components that also cross-reacted with bFGF Abs (Fig. 2, lanes B and C). These two components appeared to be aggregates of the lower molecular mass doublet since the addition of a higher concentration of glycerol to the SDS/PAGE buffer prevented their appearance on gels. In some cases, the bFGF Abs bound to an

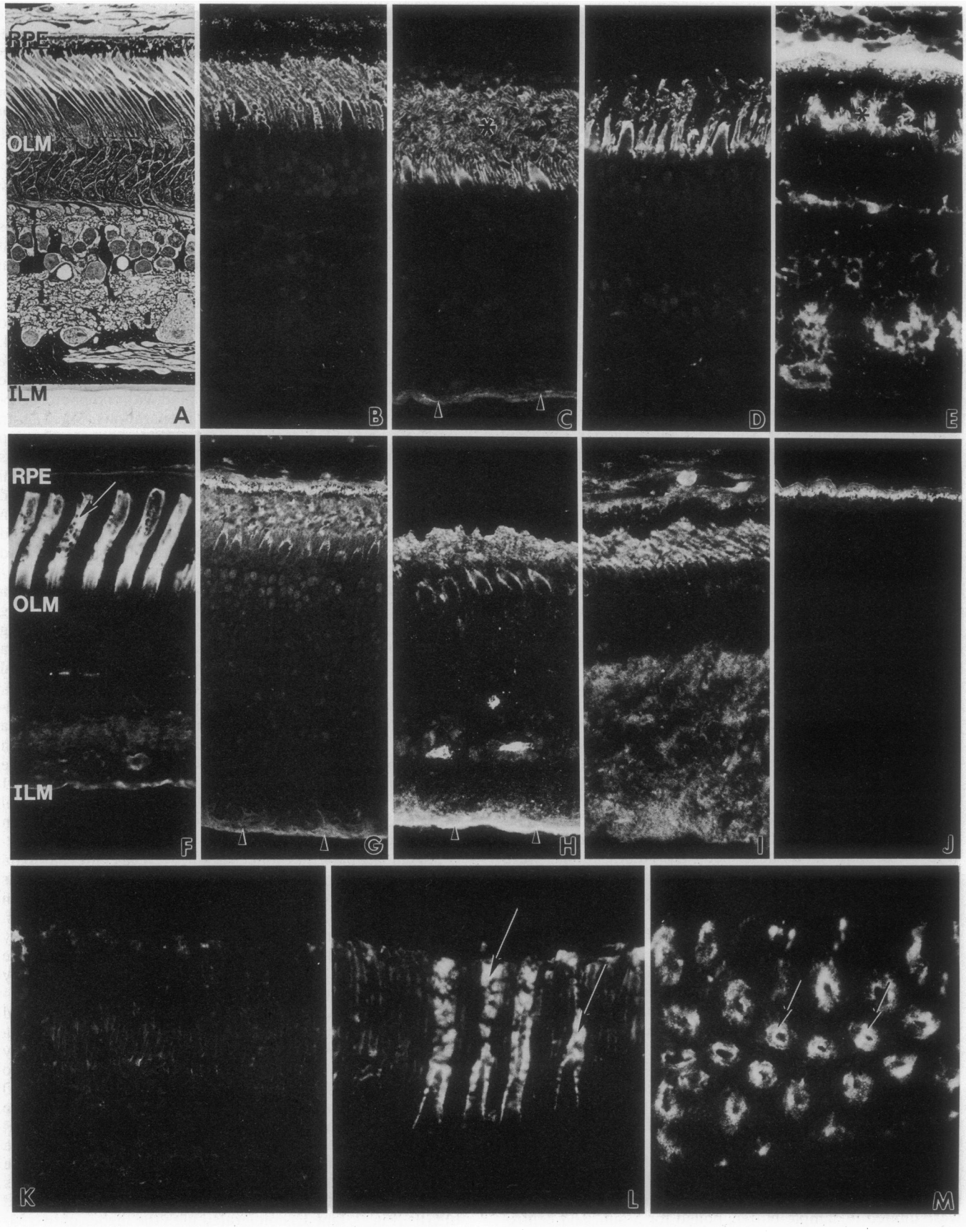

FIG. 1. Fluorescence light micrographs (*B–K*) of retina and isolated IPM (*L* and *M*) from the monkey eye. Specimens were fixed in various fixatives and incubated with a battery of bFGF Abs. (*A*) Silver-stained section (5 μm) of retina. (*F*) Adjacent section stained with FITC-conjugated peanut agglutinin (PNA). The IPM is defined as the extracellular compartment between the RPE and the outer limiting membrane of the neural retina. CMSs, distinct domains of the IPM associated with cone photoreceptor inner and outer segments, are stained selectively using PNA (arrow). Retinas were fixed in 4% paraformaldehyde (*B*), ethanol (*C*), acetone (*D*), or frozen directly without fixation (*E*). Sections were incubated with Ab generated from a synthetic peptide corresponding to bFGF-(30–50)-peptide-amide (Ab 810). Note that in *B–E*

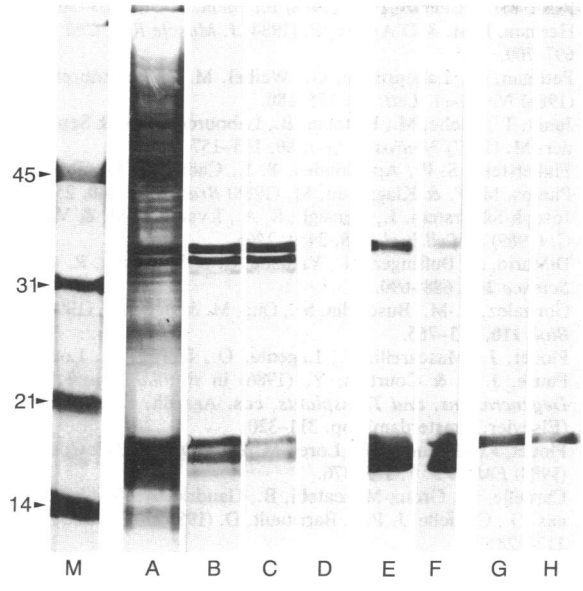

FIG. 2. Western blot analysis of aqueous-insoluble IPM (lanes A–D), purified bovine brain bFGF (lanes E and F), and recombinant human bFGF (lanes G and H) separated on one-dimensional SDS/polyacrylamide gels. Insoluble IPM preparation was stained for total protein with India ink (lane A). A broad band of insoluble material migrates at ≈17.5 kDa. bFGF Abs cross-react with either a doublet or a triplet of 16.5–17.5 kDa in the IPM material (Ab 810, lane B, and R & D Ab, lane C). Both of these Abs cross-react with 17- to 17.5-kDa components on blots of bovine brain bFGF (lanes E and F) and recombinant human bFGF (lanes G and H). Both Abs also recognize two 35-kDa doublets in the IPM preparation (lanes B and C) and a single 35-kDa band on blots of bovine brain bFGF (lanes E and F) that appear to be aggregates of the 16.5- to 17.5-kDa components. Such aggregates are absent on blots of recombinant human bFGF (lanes G and H). No IPM components were recognized when nonimmune serum (lane D), preimmune serum, or other growth factor Abs at similar concentrations were used in place of the primary bFGF Abs. Lane M contains molecular mass markers; masses are in kDa.

additional higher molecular mass IPM component at ≈150 kDa that is known to be a chondroitin 6-sulfate-containing proteoglycan (data not shown; see *Discussion*). Anti-bFGF binding was not observed on blots when preimmune serum, normal rabbit serum (Fig. 2, lane D), or antisera to other growth factors were used in place of bFGF primary Abs.

DISCUSSION

The results from this study show that a battery of Abs directed against bovine brain bFGF and various synthetic peptides corresponding to bFGF sequences bind to various intracellular and extracellular regions of primate retina and to isolated preparations of insoluble IPM. Immunolabeling at certain locations is found with multiple bFGF peptide Abs under a variety of fixation conditions, whereas labeling at other locations is observed only when specific Ab(s) and/or fixation conditions are employed. Hanneken *et al.* (32, 33) have reported labeling of vascular cells only when an Ab to bFGF-(1–15)-peptide-amide was used on frozen sections of developing and mature bovine retinas. No binding to the IPM was identified. This apparent discrepancy may be attributable to our general finding that anti-bFGF labeling at certain locations is demonstrable only under conditions where specific fixatives, embedding media, and/or Abs are used.

bFGF is sequestered in close proximity to the surfaces of three cell types that border the IPM: the photoreceptor inner and outer segments and the apices of RPE and Mueller's glia. Therefore, it is logical to assume that it is synthesized by and plays some role in regulating the activities of one or more of these three cell types. The apparent cytoplasmic labeling of Mueller's glia (Fig. 1 *G–I*) raises the distinct possibility that this cell type may synthesize and release bFGF into the IPM; but neither the photoreceptors nor the RPE can be excluded from such a role. RPE cells have the capacity to synthesize bFGF under *in vitro* conditions (45), and recent *in situ* hybridization studies using ^{35}S-labeled antisense probes to detect bFGF mRNA have found evidence of photoreceptor inner segment labeling in the rat (46). Moreover, ^{125}I-labeled aFGF and bFGF bind to isolated preparations of bovine rod outer segments and disc membranes (27, 29). Abs to putative bFGF receptor molecules (*flg* gene product) have been used to identify FGF cellular targets in retina. Thus far, however, only diffuse immunolabeling of the outer plexiform and outer nuclear layers has been reported (47).

In the 18-day rat fetus, bFGF is widely distributed in many tissues. At extracellular locations it is strongly associated with basement membranes at the interface between epithelia and mesenchyme (25, 44). These findings are consistent with those reported here. Our results show that anti-bFGF labeling is associated with the basement membranes at two retinal locations: those associated with retinal blood vessels (Fig. 1 *E* and *H*) and at the inner limiting membrane between the neural retina and vitreous humor (i.e., the inner limiting membrane; Fig. 1*C*). In addition, we find intense anti-bFGF labeling in the IPM: the extracellular compartment that occupies the space between the neural retina and the RPE (Fig. 1 *B–E, G–I, L,* and *M*). The IPM is distinct embryologically from the extracellular matrices of mesodermally derived connective tissue. As such, many matrix constituents prominent in such matrices (e.g., collagens, laminin, and fibronectin) have not been detected within the IPM (37, 43, 48). There is no precedent for the occurrence of bFGF on the apical surface of an epithelium derived from neuroectoderm. For example, there is no apparent labeling of the apical (luminal) surface of the choroid plexus epithelium, a tissue related embryologically to the RPE, using one of the same Abs (773) employed in this study (25). Thus, the presence of bFGF in the IPM may constitute an extracellular compartment for bFGF sequestration and, as such, it raises questions about its function(s) in the retina.

The IPM contains a variety of aqueous-soluble and -insoluble components (for reviews, see refs. 37, 48, and 49), most of which remain uncharacterized. Information regarding IPM composition has originated from several histochemical and lectin cytochemical studies (35, 50–52). The CMS, identified initially on the basis of its PNA-binding properties (34, 50), was later shown to contain chondroitin 6-sulfate (36). Both PNA and anti-chondroitin 6-sulfate bind to 150- and 200-kDa components on Western blots of insoluble IPM (37). The 150-kDa CMS component cross-reacts, but to a lesser degree, with the bFGF Abs. This implies, but does not prove, that

the IPM is labeled intensely. In *C*, labeling is also associated with the internal limiting membrane (arrowheads). In *E* profiles of retinal vessels are labeled; there is also focal labeling throughout the retina. Sections were fixed in 4% paraformaldehyde and incubated with Ab 773 (*G*), Ab 781 (*H*), anti-bovine brain bFGF (*I*), or rabbit nonimmune serum (*J*). In addition to the IPM, there is labeling of the internal limiting membrane, Mueller's glia endfeet (*G* and *H*, arrowheads), and possibly throughout the Mueller cell cytoplasm (*I*). Control sections incubated with nonimmune serum show only nonspecific fluorescence due to lipofuscin pigment within the RPE cytoplasm (*J*). (*K*) High magnification of a retinal section pretreated with 2 M NaCl prior to incubation in bFGF Ab 810. Longitudinal (*L*) and tangential (*M*) sections of the insoluble IPM are shown. The extracellular nature of the bFGF immunolabeling associated with the CMSs (arrows) is clearly evident. A comparison of *K* and *L* illustrates the reduction in labeling intensity produced by preincubation of retinal sections in 2 M NaCl. (*A–J*, ×900; *K–M*, ×1440.)

bFGF is bound to a larger IPM proteoglycan *in vivo*. Whether such binding is to chondroitin sulfate, the proteoglycan core protein, or some unidentified heparan sulfate-containing component of the IPM remains to be determined. This conclusion is supported by findings showing that bFGF is not detected in the aqueous-soluble fraction of the IPM even after mild detergent extraction and extensive rinsing of retinas in buffer and that bFGF can be released by exposure to a 2 M salt solution (Fig. 1*K*).

Collectively, these results suggest that the insoluble IPM serves as a depot for bFGF. Both heparin and heparan sulfate bind to bFGF and are capable of altering its activity *in vitro* (53, 54). These glycosaminoglycans have also been shown to protect bFGF from inactivation (11). The binding of exogenous bFGF (55) and the localization of native bFGF to basement membranes *in vivo* (25, 47) provide added support for this view. By analogy, then, we speculate that bFGF is normally bound to a proteoglycan(s) in the aqueous-insoluble portion of the IPM and that such binding may modulate its biological activity.

It has been reported that intraocular injection of bFGF can arrest inherited photoreceptor degeneration in the Royal College of Surgeons rat for up to 2 months (55). Exogenous bFGF can also prolong ganglion cell survival after optic nerve transection (8). Such results have led to its characterization as a potential neuronotrophic agent in retina. Further characterization of the role of bFGF in the IPM should help to clarify its function in the retina and to define the entire range of its biological activities elsewhere in the central nervous system.

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1. Maciag, T., Mehlman, T., Friesel, R. & Schreiber, A. B. (1984) *Science* **255**, 932–935.
2. Shing, Y., Folkman, J., Sullivan, R., Butterfield, C., Murray, J. & Klagsbrun, M. (1984) *Science* **223**, 1296–1299.
3. Burgess, W. H. & Maciag, T. (1989) *Annu. Rev. Biochem.* **58**, 575–606.
4. Baird, A. & Bohlen, P. (1990) in *Peptide Growth Factors and Their Receptors I*, eds. Sporn, M. B. & Roberts, A. B. (Springer, Berlin), pp. 369–418.
5. Sporn, M. B. & Roberts, A. B. (1988) *Nature (London)* **332**, 217–219.
6. Morrison, R. S., Sharma, A., DeVellis, J. & Bradshaw, R. A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7537–7541.
7. Schubert, D., Ling, N. & Baird, A. (1987) *J. Cell Biol.* **104**, 635–643.
8. Sievers, J., Hausmann, B., Unsickner, K. & Berry, M. (1987) *Neurosci. Lett.* **76**, 157.
9. Anderson, K. J., Dam, D., Lee, S. & Cotman, C. W. (1988) *Nature (London)* **332**, 360–361.
10. Baird, A., Esch, F., Mormede, P., Ueno, N., Ling, N., Bohlen, P., Ying, S. Y., Wehrenberg, W. & Guillemin, R. (1986) *Recent Prog. Horm. Res.* **42**, 143–205.
11. Gospodarowicz, D. & Cheng, J. (1986) *J. Cell Physiol.* **128**, 475–484.
12. Gospodarowicz, D., Neufeld, G. & Schweigerer, L. (1986) *Mol. Cell Endocrinol.* **46**, 187–204.
13. Gospodarowicz, D., Ferrara, N., Schweigerer, L. & Neufeld, G. (1987) *Endocr. Rev.* **8**, 95–114.
14. Risau, W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3855–3859.
15. Risau, W. & Ekblom, P. (1986) *J. Cell Biol.* **103**, 1101–1107.
16. Liu, L. & Nicoll, C. S. (1988) *Endocrinology* **123**, 2027–2031.
17. Seed, J., Olwin, B. B. & Hauschka, S. D. (1988) *Dev. Biol.* **128**, 50–57.
18. Ross, M. & Ballard, F. J. (1988) *Biochem. J.* **249**, 363–368.
19. Herman, I. M. & D'Amore, P. (1984) *J. Muscle Res. Cell Motil.* **5**, 697–709.
20. Pettman, B., Labourdette, G., Weibel, M. & Sensenbrenner, M. (1986) *Neurosci. Lett.* **68**, 175–180.
21. Janet, T., Miehe, M., Pettman, B., Labourdette, G. & Sensenbrenner, M. (1987) *Neurosci. Lett.* **80**, 153–157.
22. Finkelstein, S. P., Apostolides, P. J., Caday, C. G., Prosser, J., Philips, M. F. & Klagsbrun, M. (1988) *Brain Res.* **460**, 253–259.
23. Joseph-Silverstein, J., Consigli, S. A., Lyser, K. M. & Ver Pault, C. (1989) *J. Cell Biol.* **108**, 2459–2466.
24. DiMario, J., Buffinger, N., Yamada, S. & Strohman, R. C. (1989) *Science* **244**, 688–690.
25. Gonzalez, A.-M., Buscaglia, M., Ong, M. & Baird, A. (1990) *J. Cell Biol.* **110**, 753–765.
26. Plouet, J., Mascarelli, F., Lagente, O., Dorey, C., Lorans, G., Faure, J. P. & Courtois, Y. (1986) in *Retinal Signal Systems, Degenerations, and Transplants*, eds. Agardh, E. & Ehinger, B. (Elsevier, Amsterdam), pp. 311–320.
27. Plouet, J., Mascarelli, F., Loret, M. D., Faure, J. P. & Courtois, Y. (1988) *EMBO J.* **7**, 373–376.
28. Caruelle, D., Groux-Muscatelli, B., Gaudric, A., Sestier, C., Coscas, G., Caruelle, J. P. & Barritault, D. (1989) *J. Cell Biochem.* **39**, 117–128.
29. Mascarelli, F., Raulais, D. & Courtois, Y. (1989) *EMBO J.* **8**, 2265–2273.
30. Glaser, B. M., D'Amore, P. A., Michels, R. S., Patz, A. & Fenselau, A. (1980) *J. Cell Biol.* **84**, 298–304.
31. Baird, A., Esch, F., Gospodarowicz, D. & Guillemin, R. (1985) *Biochemistry* **24**, 7855–7860.
32. Hanneken, A., de Juan, E., Lutty, G. & Hjelmelaud, L. (1990) *Invest. Ophthalmol. Vis. Sci. Suppl.* **31**, 193a (abstr.).
33. Hanneken, A., Lutty, G. A., McLeod, D. S., Robey, F., Harvey, A. K. & Hjelmeland, L. M. (1989) *J. Cell. Physiol.* **138**, 115–120.
34. Johnson, L. V., Hageman, G. S. & Blanks, J. C. (1985) in *The Interphotoreceptor Matrix in Health and Disease*, eds. Bridges, C. D. B. & Adler, A. J. (Liss, New York), pp. 36–46.
35. Hageman, G. S. & Johnson, L. V. (1986) *J. Comp. Neurol.* **249**, 499–510.
36. Hageman, G. S. & Johnson, L. V. (1987) *Curr. Eye Res.* **6**, 639–646.
37. Hageman, G. S. & Johnson, L. V. (1990) in *Progress in Retinal Research*, eds. Osborne, N. & Chader, J. (Pergamon, New York), Vol. 10, pp. 207–249.
38. Committee on Care and Use of Laboratory Animals (1986) *Guide for the Care and Use of Laboratory Animals* (Natl. Inst. Health, Bethesda, MD), DHHS Publ. No. (NIH) 86-23.
39. Baird, A., Schubert, D., Ling, N. & Guillemin, R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2324–2328.
40. Johnson, L. V. & Blanks, J. (1984) *Curr. Eye Res.* **3**, 969–974.
41. Hageman, G. S., Hewitt, A. T., Kirchoff, M. A. & Johnson, L. V. (1989) *Invest. Ophthalmol. Vis. Sci. Suppl.* **28**, 341a (abstr.).
42. Adler, A. J. & Klucznik, K. M. (1982) *Exp. Eye Res.* **34**, 423–434.
43. Adler, A. J. & Severin, K. M. (1981) *Exp. Eye Res.* **32**, 755–769.
44. Folkman, J., Klagsbrun, M., Sasse, J., Wadzinski, M., Ingber, D. & Vlodavsky, I. (1988) *Am. J. Pathol.* **130**, 393–400.
45. Schweigerer, L., Malerstein, B., Neufeld, G. & Gospodarowicz, D. (1987) *Biochem. Biophys. Res. Commun.* **143**, 934–940.
46. Noji, S., Matsuo, T., Koyama, E., Yamaai, T., Nohno, T., Matsuo, N. & Taniguchi, S. (1990) *Biochem. Biophys. Res. Commun.* **168**, 343–349.
47. Nakatake, J., Ohira, A., de Juan, E. & Wong, F. (1990) *Invest. Ophthalmol. Vis. Sci. Suppl.* **31**, 199a (abstr.).
48. Hewitt, A. T. & Adler, R. (1989) in *Retina*, eds. Ogden, T. E. (Mosby, St. Louis), Vol. 1, pp. 57–64.
49. Chader, G. J. (1989) *Invest. Ophthalmol. Vis Sci.* **30**, 7–22.
50. Johnson, L. V., Hageman, G. S. & Blanks, J. C. (1986) *Invest. Ophthalmol. Vis. Sci.* **27**, 129–135.
51. Uehara, F., Sameshina, M., Murametsu, T. & Ohba, N. (1983) *Exp. Eye Res.* **36**, 113–123.
52. LaVail, M. M., Pinto, L. H. & Yashamura, D. (1981) *Invest. Ophthalmol. Vis. Sci.* **21**, 658–668.
53. Walicke, P. A. (1988) *Exp. Neurol.* **102**, 144–148.
54. Damon, D. H., D'Amore, P. A. & Wagner, J. A. (1988) *J. Cell. Physiol.* **135**, 293–300.
55. Faktorovich, E. G., Steinberg, R. H., Yasumura, D., Matthes, M. T. & LaVail, M. M. (1990) *Nature (London)* **347**, 83–86.