Distribution of FGF-5 in the Rhesus Macaque Retina

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Purpose. To determine the distribution of mRNA transcribed from the FGF-5 gene and the distribution of FGF-5 protein in the normal adult retina of the rhesus macaque.

Methods. Freshly enucleated globes from rhesus macaque were prepared for Northern blot analysis, in situ hybridization, and immunohistochemical studies. A 350 base pair sequence from the human FGF-5 was subcloned and used to prepare 35S-labeled cRNAs for Northern and in situ experiments. Antipeptide antibodies were raised against an aminoterminal sequence as well as an internal sequence and were affinity purified on recombinant FGF-5 coupled to AffiGel-10. The specificity of antibody probes was verified by dot blot and Western blot analyses.

Results. Ganglion cells and photoreceptors express the highest levels of FGF-5 mRNA, although all neurons and the retinal pigment epithelium (RPE) were clearly labeled. Immunohistochemical staining similarly revealed a widespread distribution of the protein, with prominent labeling observed in photoreceptor inner segments, especially in cone cells. Plexiform layers and RPE cells were also clearly labeled.

Conclusions. Clear evidence was established for the expression of FGF-5 in the retina, where it may play an important extracellular role as a secreted member of the FGF gene family. Invest Ophthalmol Vis Sci. 1994;35:3189-3198.

Interest in the biologic roles that fibroblast growth factors (FGFs) may play in the retina has dramatically increased during the past several years. Initial studies of the FGFs focused primarily on the angiogenic activities of FGFs during development and the pathogenesis of such fibrovascular diseases as proliferative diabetic retinopathy. It was, however, the observation that members of the FGF gene family could support the differentiation and viability of neurons, both in vitro and in vivo, that sparked dramatic new interest in these growth factors. The first reports concerning the trophic nature of the FGFs appeared in the literature in 1986, when Walicke et al showed that the viability of neurons from dorsal root ganglia in vitro was enhanced by basic fibroblast growth factor (bFGF).1 The first report of this type in the eye literature concerned the activity of acidic FGF (aFGF) on ganglion cells,2 but it was the observation of Faktorovich et al on the rescue activity of bFGF for photoreceptors in the dystrophic RCS rat that promoted widespread attention to this potential biologic and therapeutic role for the FGFs.3

At approximately the same time, an examination of the human and mouse genomes revealed the size and diversity of the FGF gene family. The ninth member of the FGF gene family has recently been described,4 and studies of the expression of these genes indicate that they play major roles during embryogenesis and wound repair. A subset of the FGF gene family, however, appears to be constitutively expressed in adult organisms, and in the central nervous system this subset includes bFGF, aFGF, and FGF-5. Our laboratory has previously demonstrated that these three genes are actively expressed by human retinal pigment epithelial (RPE) cells in vitro, both at the level of mRNA and protein.5,6

FGF-5 is one of the newer members of the FGF gene family. After the initial report of the cloning and characterization of FGF-5,7 a series of studies from the Goldfarb laboratory (College of Physicians and Surgeons of Columbia University, New York, NY) was published that reported the biochemistry and biology

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of this new growth factor. Two of the most critical observations concerning FGF-5 indicate that, first, FGF-5 is expressed in the adult central nervous system in a variety of neuronal populations.8,9 and, second, the FGF-5 protein contains a signal sequence and is clearly secreted by conventional cellular pathways.10 The latter observation stands in contrast to bFGF and aFGF, which bear no discernible signal sequence and must therefore be released from cells by a pathway different from normal secretion.

The observations that FGF-5 is both expressed and secreted in the adult central nervous system suggest that this member of the FGF gene family may play an important role as an extracellular mediator in the biology of the central nervous system and, more specifically, in the biology of the retina. Recent studies have shown that FGF-5 binds to FGF receptors 1 and 2 (R1 and R2)11 and, furthermore, that FGF-5 released from skeletal myocytes is a trophic factor for motor neurons.12 The studies presented here describe our initial results concerning the distribution of FGF-5 in the retina of the rhesus macaque and represent our initial efforts to study the biologic roles FGF-5 may play in this tissue.

MATERIALS AND METHODS

Tissues

All guidelines in the ARVO manual for the use and care of animals were strictly observed. Within 20 minutes of euthanasia, globes were removed from adult rhesus macaque monkeys and were differentially processed according to the requirements of experimental protocols. Six globes were obtained from three female rhesus macaque monkeys, ages 7, 14, and 15 years. For in situ hybridization studies, globes were immediately hemisected and the posterior poles were snap frozen in optimal cutting temperature compound (OCT, Miles, Elkhart, IN) and stored at -70°C. For immunohistochemical studies, globes were fixed for 5 minutes in cold 4% paraformaldehyde in phosphate buffer at pH 7.5, after which the globes were immersed for 1 hour in 4% paraformaldehyde in PBS, and then washed in cold PBS for 15 minutes. After washing in 0.1 M triethanolamine (TEA), sections were acetylated with acetic anhydride (2.5 μg/ml in 0.1 M TEA) and washed in 2 X SSC for 5 minutes at room temperature, followed by dehybridization with 70% ethanol, 95% ethanol, and 100% ethanol for 3 minutes each. Sections were dried under vacuum at room temperature for at least 2 hours. Riboprobes with specific activity of about 2 × 10^7 cpm/μg were synthesized using in vitro transcription with 35S UTP (NEN, DuPont). Each section received 50 μl of hybridization solution (50% formamide, 10 mM dextran sulfate, 0.3 M NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, 0.5 mg/ml tRNA, 10 mM dithiothreitol, and 5 × 10^6 cpm/ml 35S probe), which was then topped with a coverslip, and edges of the coverslip were then sealed with DPX mountant (BDH Ltd., Poole, UK). Sections were incubated at 55°C for 15 to 18 hours on a slide warmer. After peeling off the DPX and soaking off coverslips in 4 X SSC, sections were washed with four changes of 4 X SSC for 5 minutes each and then digested with RNase A solution (20 μg/ml) in 0.5 M NaCl, 10 mM Tris, pH 8.0, and 1 mM EDTA. Sections were washed and desalted through two changes of 2 X SSC for 5 minutes each, 1 X SSC and 0.5 X SSC for 10 minutes each at room temperature, and 0.1 X SSC for 30 minutes at 65°C. After briefly washing with 0.1 X SCC at room temperature, sections were dehydrated through graded ethanol (70%, 95%, two changes of 100% each for 3 minutes each), and dried under vacuum for 2 hours.

Preparation of Radiolabeled Single Stranded RNA Probes

For our studies, we digested a fragment from a cDNA clone of the human FGF-5 gene to make the radiolabeled riboprobes.7 The cDNA clone was a generous gift of Dr. M. Goldfarb. We generated a subclone that started at position 120 and extended to position 467 using an EcoRI-BamHI double restriction digest and ligated the 350 base pair piece into the pT7/T3a19 (Gibco BRL, Grand Island, NY) multiple cloning site. The plasmid was linearized as appropriate to generate sense or antisense cRNA using either the T3 or T7 RNA polymerase for in vitro transcription. Probes were labeled by the addition of carrier-free 35S UTP (NEN, DuPont, Boston, MA) to the in vitro transcription reaction, and reagents and protocols for in vitro transcription followed manufacturer’s instructions (Boehringer Mannheim, Indianapolis, IN). Labeled probes were separated from free nucleotide and reaction mixture using a spin column specifically designed for cRNA probes (5’-3’, Inc., Boulder, CO).

In Situ Hybridization

In situ hybridization studies generally followed the methods of Simmons et al,13 developed for examining the central nervous system using single stranded cRNA probes. Briefly, 10 to 15 μm sections were cut on a Reichert Jung Cryocut 1800 (Cambridge Instruments, Heidelberg, Germany) and mounted on glass slides coated with Vectabond (Vector, Burlingame, CA) according to the manufacturer’s protocol. Sections were fixed for 5 minutes in cold 4% paraformaldehyde in PBS, and then washed in cold PBS for 15 minutes. After washing in 0.1 M triethanolamine (TEA), sections were acetylated with acetic anhydride (2.5 μg/ml in 0.1 M TEA) and washed in 2 X SSC for 5 minutes at room temperature, followed by dehybridization with 70% ethanol, 95% ethanol, and 100% ethanol for 3 minutes each. Sections were dried under desiccant under vacuum at room temperature for at least 2 hours. Riboprobes with specific activity of about 2 × 10^7 cpm/μg were synthesized using in vitro transcription with 35S UTP (NEN, DuPont). Each section received 50 μl of hybridization solution (50% formamide, 10% dextran sulfate, 0.3 M NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, 1 X Denhardt’s solution, 0.5 mg/ml tRNA, 10 mM dithiothreitol, and 5 × 10^6 cpm/ml 35S probe), which was then topped with a coverslip, and edges of the coverslip were then sealed with DPX mountant (BDH Ltd., Poole, UK). Sections were incubated at 55°C for 15 to 18 hours on a slide warmer. After peeling off the DPX and soaking off coverslips in 4 X SSC, sections were washed with four changes of 4 X SSC for 5 minutes each and then digested with RNase A solution (20 μg/ml) in 0.5 M NaCl, 10 mM Tris, pH 8.0, and 1 mM EDTA. Sections were washed and desalted through two changes of 2 X SSC for 5 minutes each, 1 X SSC and 0.5 X SSC for 10 minutes each at room temperature, and 0.1 X SSC for 30 minutes at 65°C. After briefly washing with 0.1 X SCC at room temperature, sections were dehydrated through graded ethanol (70%, 95%, two changes of 100% each for 3 minutes each), and dried under vacuum for 2 hours.
Autoradiography
Sections were delipidized with xylene for 30 minutes, washed with 100% ethanol, and air dried. Sections were then dipped in Ilford K.5D (Ilford Ltd., Cheshire, UK) nuclear emulsion at 40°C and kept in a dark box with desiccant at 4°C for exposure. After exposure for 1 to 4 weeks, slides were developed with Kodak D-19 (Eastman Kodak, Rochester, NY) for 4 minutes and fixed with Kodak fixer for 5 minutes.

Generation and Affinity Purification of Antibodies
Antibodies to peptide sequences from human FGF-5 were raised in the rabbit and affinity purified according to our previously published procedures.14 Two peptide sequences were chosen on the basis of apparent antigenicity and lack of homology with other members of the FGF gene family. The first peptide was a 25mer, of which 24 amino acids were from the amino-terminus residues 22 to 45 of the protein with a cysteine added and having the sequence GEKRALPGQPAPATDRNPRGSSC. The second peptide was a 14mer, of which 11 amino acids were from the middle of the coding sequence from residues 175 and 185 with three amino acid linkers added to make the sequence CGGASAIIHRTEKTG. This peptide was previously used by Dr. M. Goldfarb to raise an anti-peptide antibody against human FGF-5. Both peptides were conjugated to keyhole limpet hemocyanin (KLH) using sulfo-MBS (Pierce, Rockford, IL) as previously described. For each KLH conjugate, four adult female New Zealand White rabbits were immunized via the intradermal route, each with 2 ml of a 1:1 mixture of PBS and Freund's complete adjuvant containing 100 µg of peptide-KLH conjugate. Titers were assessed by dot blot analysis, and, when these values began to fall after initial immunization (a period of several months), animals were boosted with 50 µg of conjugate in 2 ml of a 1:1 mixture of PBS and Freund's incomplete adjuvant by a combination of intradermal and intramuscular injections. When titers reached peak values, rabbits were exsanguinated, and the serum was collected for storage at −80°C.

Affinity purification was accomplished by selective absorption and elution from recombinant human FGF-5 coupled to AffiGel-10. The expression of FGF-5 in *Escherichia coli* and its subsequent purification were previously described, as were procedures for coupling to AffiGel-10.15 Aliquots of immune serum (5 to 20 ml, depending on titer) were diluted 1:1 with 50mm Tris, pH 7.6, 150 mM NaCl (TBS) and incubated overnight with affinity resin at 4°C, after which the resin was washed extensively and then eluted with 0.1 M glycine HCl, pH 2.5, containing 4 M urea and 1 mg/1 ml bovine serum albumin (BSA). Eluates were immediately dialyzed against three changes of TBS and stored at −80°C. IgG content of affinity fractions was assessed by slot blot analysis against purified rabbit IgG standards.

Retinal Pigment Epithelial Cell Culture and Conditioned Medium
The primary culture and routine maintenance of human RPE cells were described previously.15 Cultured human RPE cells were plated at 2 × 10⁴ cells/cm² and incubated at 37°C, 10% CO₂ for 3 days. To prepare conditioned medium, the cells were washed three times in Hank's balanced salt solution (Gibco BRL) and serum-free DMEM/F12 (Bio Whitaker, Walkersville, MD) medium supplemented with 100 µg/ml BSA fraction V (Boehringer Mannheim), 5 µg/ml porcine heparin (Sigma, St. Louis, MO), and 10 µM N⁶-2'-O-dibutyryladenosine-3',5'-cyclic monophosphate (dibutyryl cAMP) (Sigma) was added to 10 ml/75 cm² tissue culture flask. After 48 hours, the cells were dosed with added dibutyl cAMP (final total 20 µM). After 24 hours, the medium was harvested free of cells and incubated overnight with 10 µl heparin sepharose (Pharmacia, Piscataway, NJ) for every 10 ml conditioned medium. The heparin sepharose was then collected and used in Western blot analysis.

Dot Blot and Western Blot Analyses
Immunoblot analyses were performed according to our previously published procedures.14,15 For dot blot analyses, samples of recombinant human bFGF, aFGF, and FGF-5 were dotted directly on filter paper, after which individual sets of lanes were blocked with 10% nonfat dry milk and incubated in affinity-purified antibody, an equivalent concentration of nonimmune IgG, or affinity-purified antibody absorbed against one of the FGF gene family members coupled to AffiGel-10. Alkaline phosphatase conjugated anti-rabbit IgG antibodies were applied in a second step, and the results were subsequently visualized with standard reagents.

For Western blot analysis, we looked for the secreted protein in cultured human RPE cells. FGF-5 was purified from conditioned media using heparin sepharose. After three washes with 0.5 M NaCl, 10 mM Tris, pH 7.5, and two washes with 10 mM Tris, pH 7.5, the FGF-5 on heparin sepharose from conditioned media was eluted with equal volume of 2X sample buffer. The eluted material was run on 15% T SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) at 10 µl/lane and then transferred to nitrocellulose as previously described.14 The blotted membrane was blocked with 10% nonfat dry milk and processed for visualization of bands with alkaline phosphatase immunochemistry as described.14
Immunohistochemistry

Blocks of fixed tissue were dissected from the posterior pole and embedded in Immunobed (Polysciences) according to the manufacturer's instructions. Sections were cut at 2 to 3 μm on a Sorvall MT-2 ultra-microtome (DuPont, Wilmington, DE) with a glass knife and mounted on glass slides for immunohistochemistry. After blocking for 30 minutes in PBS with 1% BSA and 5% normal goat serum, slides were washed twice in PBS and then incubated with primary antibody over night at 4°C. All antibody dilutions were made in PBS with 1% BSA. Colloidal gold conjugated secondary antibody was then applied for 1 hour at room temperature, and, after two washes for 5 minutes each in PBS and two washes for 5 minutes each in distilled water, antibody binding was visualized by silver enhancement of colloidal gold conjugated secondary antibodies, as suggested by the manufacturer (Amersham, Arlington Heights, IL).

Microscopy

Routine observations of slides at low magnification were made on a Zeiss Photonic II (Carl Zeiss, Thornwood, NY). Higher magnification epipolarization light microscopy was performed on a Zeiss Photonic II adapted for epipolarization microscopy in the laboratory of M. LaVail at the University of California at San Francisco. Photomicrographs of both bright field and dark field or epipolarization images were taken with Technical Pan film (Eastman Kodak) and printed on Ilfosped RC paper (Ilford Ltd.).

RESULTS

Localization of FGF-5 mRNA in the Rhesus Macaque Retina by In Situ Hybridization

To begin our investigation of the distribution of FGF-5 in the rhesus macaque retina, we performed in situ hybridization. Frozen sections were briefly postfixed in cold buffered paraformaldehyde, hybridized with ^35S single stranded cRNA probes for 24 hours, and then dipped for autoradiography and maintained at 4°C in the dark for 1 month before development. Antisense probe was used for (A) (bright field) and (B) (dark field). Sense probe was used for (C) (bright field) and (D) (dark field). Magnification bar equals 100 μm and refers to all panels.
hybridization studies using single-stranded cRNA probes. A 350 base pair sequence of the human FGF-5 sequence was subcloned into the pT7/T3α18 and α19 transcription vectors to prepare sense and antisense RNAs of appropriate length for in situ studies.

Figure 1 presents the results of a typical in situ experiment. In panel A, antisense probe can be seen to hybridize to several layers of the retina as evidenced by the presence of silver grains formed during autoradiography. The inner and outer nuclear layers, as well as ganglion cells, appear to be labeled. Photoreceptor inner segments are possibly labeled as well because the band of silver grains in Figure 1A is broader than the stained nuclei seen in Figure 1A. Panel B is a conventional dark-field view of the image found in panel A. Control studies (panels C and D, Figure 1) with sense probe exhibit almost no labeling, as indicated by the very sparse distribution of silver grains. Comparison of Figures 1B and 1D suggests that the RPE layer is also labeled for FGF-5.

Although Figures 1B and 1D suggest the expression of FGF-5 in the RPE, we sought to confirm this observation using a second microscopic technique. We examined our specimens with the aid of epipolarization microscopy in the laboratory of Dr. M. LaVail. This technique makes use of the anisotropic property of light reflected from silver grains relative to the isotropic nature of light reflected from pigment granules to enhance selectively the appearance of the silver grains.11 Figure 2, Panel A, shows a higher magnification bright-field image of the outer retina after an in situ study with antisense probe. In panel B, an epipolarization image of the same field is shown. Note the silver grains above the RPE, which can now be clearly visualized. Panels C and D of Figure 2 are corresponding bright-field and epipolarization images of a sense probe or control study. These images, once again, exhibit little or no signal, as indicated by the very sparse presence of silver grains.

**Production of Antipeptide Antibodies to Human FGF-5**

To localize the FGF-5 protein in the retina specifically, it was necessary first to produce multiple independent antibodies and to characterize each with respect to cross-reactivity to other members of the FGF gene family. Our previous studies have indicated that several antipeptide antibodies raised against specific sequences in bFGF cross-react with FGF-5, and that polyclonal antibodies raised against aFGF also cross-react with bFGF. With these studies in mind, we chose two peptides for the production of antipeptide antibodies. The first is a 24-amino acid sequence from the aminoterminus of FGF-5, representing a region with no homology to other members of the FGF gene family. The second peptide was previously demonstrated by Goldfarb to yield antibodies that lacked cross-reactivity with other members of the gene family. Antisera were raised to both peptides after conjugation to KLH, and individual antisera were affinity purified on recombinant human FGF-5 coupled to AffiGel-10. Two of the resulting antibody preparations were char-
characterized for cross-reactivity with aFGF and bFGF by dot blot analysis. These results are given in Figure 3. The three columns on the left exhibit the reactivity of antibody 4532 against the three members of the FGF gene family known to be expressed in retina and RPE, and the middle three columns show similar results for antibody 4539. Controls represent primary antibody deletion or absorption of primary antibodies with solid-phase antigens. Both antibodies exhibit little, if any, cross-reactivity with aFGF and bFGF.

Next we characterized the specificity of our antibody preparations for FGF-5, which was synthesized, fully glycosylated, and secreted by human cells. Our laboratory had previously documented the expression of FGF-5 at the level of mRNA and protein by human RPE cells in vitro, and these cultures were used in our current study to examine the species of FGF-5 recognized by our new antibody preparations. Figure 4 presents a Western blot analysis of media conditioned by human RPE cells. Samples of FGFs released or secreted into the culture medium were collected on heparin agarose and then directly electrophoresed and transferred to nitrocellulose. Both the aminoterminal antibody (4539) and the antibody directed against an internal peptide (4532) recognize three primary bands ranging between 35 and 40 kD (lanes 2 and 6). These bands were previously identified in RPE-conditioned media using a sample of antibody donated from Dr. M. Goldfarb. Our controls indicate that equivalent concentrations of nonimmune IgG (lanes 5 and 9) or antibody preparations absorbed with recombinant human FGF-5 coupled to AffiGel-10 (lanes 3 and 7) fail to react with any heparin-binding proteins. However, when absorbed with human bFGF coupled to AffiGel-10 (lanes 4 and 8), the recognition does not change.

Immunohistochemical Localization of FGF-5 in the Rhesus Macaque Retina

Having raised and characterized multiple antibodies to human FGF-5, we next turned to immunohistochemical studies to determine the distribution of FGF-5 in the retina. Silver-enhanced colloidal gold labeling of second antibodies was chosen for visualization to take advantage once again of epipolarization microscopy for observations over the RPE and choroid. We first evaluated several fixation protocols for our two antibody preparations (4532 and 4539), including 4% cold buffered paraformaldehyde, 4% cold buffered paraformaldehyde containing 0.1% glutaraldehyde, and a mixture of 2% paraformaldehyde and 2% glutaraldehyde. Inclusion of any glutaraldehyde in the fixative completely eliminated immunostaining for FGF-5 (data not shown), and, as a consequence, tissue fixed with cold buffered paraformaldehyde was used in all our studies.
Figure 5 presents immunohistochemical results using antibody 4532, the internal sequence. The amino-terminus sequence antibody 4539 yielded identical but vastly weaker immunostaining patterns (data not shown). Panels A and B are bright-field and dark-field images of the full retina. Labeling can be seen in multiple areas of the retina, including the two plexiform layers and especially the photoreceptor inner segments. In these micrographs, the outer plexiform layer appears more heavily labeled than the inner plexiform layer, and the labeling of photoreceptor inner segments has some variability that can be noted as irregular clumping. The dark-field image (panel B) adequately demonstrates the distribution of silver grains over the neural retina but is inadequate for observations over the RPE and choroid due to the background caused by pigment granules present in these layers. Labeling over the thin margin of adherent vitreous body, as well as the zone of photoreceptor outer segments, is low, and these regions are useful for internal background comparisons.

Control studies were performed by substituting primary antibody with an equivalent concentration of nonimmune rabbit IgG (panels C and D) or absorbing antibody 4532 against recombinant human FGF-5 coupled to AffiGel-10. These controls demonstrate low nonspecific binding of IgG to our tissue sections and the removal of specific staining upon absorption with recombinant human FGF-5.

To clarify the distribution of FGF-5 in the outer retina and RPE, we examined our sections by epipolarization microscopy at higher magnification. Figure 6 presents composite bright-field images (left) of the entire retina stained with 4532, as well as the corresponding epipolarization images (right) of the same microscopic fields. Again, specific labeling can be seen in the two plexiform layers. In these micrographs, the irregular or discontinuous nature of the labeling in the photoreceptor inner segments can now be seen as the result of higher labeling of cone inner segments when compared with rod inner segments. In addition, labeling in the RPE can now be clearly appreciated against the distinctive pattern of pigment granules.

To provide a direct comparison for the distribution of FGF-5 with the distributions of bFGF and aFGF, we used our previously characterized antibodies 388 (against bFGF) and 1613 (against aFGF) to label similar sections of rhesus macaque retina. The results are presented in Figure 7 (bFGF) and Figure 8 (aFGF). Once again, composite bright-field images are presented on the left and composite epipolarized light images are presented on the right. The distribution of bFGF is similar to previously published results for paraformaldehyde-fixed tissues. Nearly all nuclei are labeled, including the nuclei of the RPE, and the labeling in the inner retina seems more pronounced. Labeling of blood vessels and basement membranes is ab-

**Figure 5. Immunohistochemical localization of FGF-5 in the rhesus macaque retina.** Posterior poles were fixed in cold buffered paraformaldehyde and embedded in Immuno
ded. Affinity-purified antibody 4532 (0.8 µg/ml) was incubated with sections overnight at 4°C and then visualized by silver enhancement of colloidal gold-labeled second antibody reactions [(A), bright field; (B), dark field. Control studies were performed by replacing primary antibody with an equivalent concentration of nonimmune IgG (C), bright field; (D), dark field and absorption of primary antibody with recombinant human FGF-5 coupled to AffiGel-10 (E), bright field; (F), dark field]. Magnification bar equals 100 µm and refers to all panels.
FIGURE 6. Immunohistochemical localization of FGF-5 in the rhesus macaque retina. Sections were prepared for immunohistochemistry as in Figure 5. Affinity-purified antibody 4532 at 0.8 μg/ml was used as the primary antibody. Left panels are a composite of bright-field images of the entire retina. Right panels are a composite of epipolarized light images. Magnification bar equals 50 μm and refers to all panels.

sent, presumably due to the use of antibody 38815 in combination with aldehyde fixation. The distribution of αFGF as revealed by antibody 16136 is nearly uniform throughout the retina and RPE. Again, nearly all nuclei are labeled. Figure 9 presents an IgG control study for Figures 6, 7, and 8. The choice of nonimmune IgG concentration was based on the highest IgG concentration used among the three antibodies for Figures 6 to 9. This figure demonstrates a detectable but low nonspecific labeling of sections with nonimmune IgG.

DISCUSSION

The data presented here concerning the distribution of mRNA for FGF-5 in the retina are reminiscent of recently published studies in the brain.8,9 Both in the rat and the mouse, mRNA for FGF-5 was found to be widely distributed in several populations of neurons, but regional differences were found to exist with respect to intensity of signal. Overall, FGF-5 transcripts are not abundant, as evidenced by the requirement of 3 to 4 weeks to adequately expose photographic emulsions for in situ procedures. It was especially interesting to note that RPE cells express easily observable levels of FGF-5 transcripts in vivo because transcripts for bFGF, which are easily observed in vitro, are not easily observed in vivo17 even with species-specific probes. Studies of the rat and mouse brain did not comment on the expression of FGF-5 by the choroid plexus, a cell layer that has many similarities in structure and function to the RPE.

Our immunohistochemical studies of the distribution of FGF-5 protein yielded results that agree largely, but not completely, with our in situ studies. Again, many populations of cells were labeled by our antibody preparations, but the strongest signals for each of the two techniques appeared in different cell layers. Although FGF-5 transcripts seem to be most abundant in ganglion cells and photoreceptors, the FGF-5 protein appears to be most abundant in photoreceptors and RPE cells, but the differences be-
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Overall, the distribution of FGF-5 in the retina appears to be different from the distribution of aFGF and bFGF. In our study, for example, FGF-5 was not found in vascular basement membranes, nor was it localized to the nucleus, as was reported for bFGF in the retina.14

Perhaps the one striking observation in our immunohistochemical studies was the apparent abundance of FGF-5 in a subset of cone photoreceptors compared with the rod photoreceptors or other types of neurons. The data presented in our study are not quantitative and do not provide a detailed regional survey of the retina that would allow a firm conclusion to be drawn, but our initial results suggest that a more thorough investigation would be of some interest.

Finally, the results presented here raise some interesting questions concerning the roles of the FGFs in the biology of the retina and, more specifically, the roles FGF-5 may play. The distribution and developmental expression of aFGF and bFGF in the vertebrate retina have now been studied,14,19 although a final consensus on these issues has not yet been reached in the literature. The proposed biologic roles for these two factors include vascular development and the differentiation and trophic support of a variety of neurons, and the proposals concerning trophic roles have gained considerable attention due to observations by Faktorovich et al3 concerning the ability of bFGF and aFGF to rescue photoreceptors in the dystrophic RCS rat retina. The subsequent claim that bFGF was specifically localized to the interphotoreceptor matrix (IPM)20 implied a simple model that could account for trophic support of photoreceptors.
and perhaps the polarity of retinal development. The conjectured presence of bFGF in the IPM could be either from the RPE cell, the photoreceptor cell, the Müller cell, or any combination of these. Our own results in the bovine retina do not support the presence of bFGF in the IPM.14

FGF-5 must now also be included as a possible trophic factor in the biology of the outer retina. It is clear from our data that FGF-5 is expressed at the level of mRNA and protein in both photoreceptor and RPE cells. Because FGF-5 possesses a signal peptide and is clearly a secreted protein,10 it is probable that both cell types actively secrete FGF-5 into the extracellular environment. It is also reasonable to speculate that FGF-5 may be secreted from the apical surface of RPE cells because most, but not all, actively secreted proteins are apically released from polarized epithelia. No clear speculation can be made with respect to secretion are apically released from polarized epithelia. No clear speculation can be made with respect to secretion.

Therefore, it should be of some interest to determine in future studies if FGF-5 is released in a polarized fashion from RPE or photoreceptor cells and if FGF-5 can provide direct trophic support for photoreceptors. Finally, it will be critical to explore the developmental expression of FGF-5 in the retina and to provide convincing evidence concerning the presence or absence of various members of the FGF gene family in the IPM.

Key Words
rhesus macaque retina, FGF-5, immunohistochemistry, in situ hybridization

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