Histochemical Demonstration of Spatial Heterogeneity in the Interphotoreceptor Matrix of the Rat Retina

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The interphotoreceptor matrix (IPM) is a mixture of acidic mucosubstances, which can be demonstrated histochemically with cationic dyes, such as Alcian blue, and metal precipitates, such as colloidal iron. In the normal rat retina, staining of the IPM with these reagents is found predominantly at the apical surface of the retinal pigment epithelium (RPE) and, to a lesser extent, at the junction of the photoreceptor inner and outer segments (basal IS/OS zone). The authors have attempted to characterize the IPM in these two regions using histochemical staining of wax-embedded sections. Prior to staining, the following chemical treatments or enzymatic digestions were performed: 1) neuraminidase (NA), with or without prior deacetylation, 2) mild acid hydrolysis (MAH), 3) testicular hyaluronidase (TH), 4) Streptomyces hyaluronidase (SH), and 5) chondroitinase AC (ChAC). NA alone, deacetylation/NA, and MAH all result in great reduction or total loss of stainable IPM at the RPE apical surface, and only slight or no reduction of stainable IPM in the basal IS/OS zone. Since these procedures remove sialoglycoconjugates, the findings suggest that 1) the IPM concentrated at the apical surface of the RPE is composed in large part of sialoglycoconjugates, 2) that little sialoglycoconjugate is present in the basal zone, and 3) that the sialoglycoconjugates are of a neuraminidase-labile group. SH produces little or no reduction of stainable IPM in either the apical RPE or basal IS/OS zones. TH and ChAC also cause little or no reduction of stainable IPM at the RPE apical surface, but produce a great reduction of stainable IPM in the basal IS/OS zone, leaving a small amount of residual basal material. Since SH digests only hyaluronic acid, and TH and ChAC both digest hyaluronic acid and chondroitin SO₄ A and C, the findings suggest that 1) little or no hyaluronic acid is present in either the apical RPE or basal IS/OS zones, and 2) the IPM at the RPE apical surface contains little or no chondroitin SO₄ A and C, whereas the stainable IPM in the basal IS/OS zone is composed, at least in large part, of chondroitin SO₄ A and C. Predominant basal localization of chondroitin SO₄ is further suggested by the staining of this region with Alcian blue at low pH. Sequential digestion with TH/MAH or ChAC/NA produces a complete removal of all stainable IPM, including the TH-insensitive residual basal material. This residual material at the basal IS/OS zone, therefore, appears to be sialoglycoconjugate. Thus, our studies have demonstrated that a specific regional heterogeneity exists in the composition of the IPM in the normal rat. Invest Ophthalmol Vis Sci 27:1577-1586, 1986

The interphotoreceptor matrix (IPM) is the extracellular material that occupies the space between the photoreceptor cells and the retinal pigment epithelium (RPE). This mucoid matrix consists of a mixture of proteoglycans and glycoproteins that has been implicated in a variety of functions, such as retinal adhesion to the RPE, optical diffraction, transport of retinoids, outer segment recognition for phagocytosis, and transfer of nutrients to the photoreceptors from their choroidal vascular supply. Significant advances have been made in our understanding of the IPM with regard to its localization, composition, and synthesis/secretion. The IPM can be demonstrated histochemically with cationic dyes, such as Alcian blue or metal precipitates, such as colloidal iron, which stain acidic mucosubstances. We have previously reported that, if we apply any of these stains to the normal rat retina, staining of the IPM is predominantly at the apical surface of the RPE, with lesser staining of the interstitial space that surrounds the photoreceptor outer segments.† We use the term “interstitial zone” as that part of the interphotoreceptor space that surrounds primarily the photoreceptor outer segments to distinguish it from the apical and basal zone of the interphotoreceptor space, with which it is continuous.
More recently, with improved histological preparations and more consistent staining, we also regularly find a concentrated basal region of stain at the junction of the photoreceptor inner and outer segments, although this is not as concentrated as the apical band.27-29

Our attention has been focused on these two separate zones in the rat retina because of an unusual distribution and concentration of IPM in the Royal College of Surgeons (RCS) rat with inherited retinal dystrophy. In the RCS retina, the band of staining at the apical surface of the RPE is virtually absent, and there is an increased amount of stainable material in the basal region of the IPM.20 These changes in the distribution of the IPM are evident before overt photoreceptor cell death is observed. Thus it has been suggested that the IPM may play a role in the etiology of photoreceptor degeneration, but it is not yet clear which of the changes (i.e., in the apical or basal zone) is more significant.20

In both normal and mutant retinas, an additional issue is whether the apical and basal zones differ not only in distribution and concentration, but also in composition. If this were the case, then the two zones should be considered independently when investigating the normal physiological role of the IPM and its participation in retinal degenerations. Therefore, in this study, we have attempted to characterize the composition of various glycoconjugates in both regions of the IPM in the normal rat retina. In brief, analysis was carried out histochemically with specific enzymatic digestions and chemical treatments for hyaluronic acid, chondroitin SO4, and sialoglycoconjugates. Preliminary reports of this work have appeared elsewhere.27-29

Materials and Methods

Tissue Preparation

Normal rats of the RCS-\textit{rdy}\textsuperscript{+} strain (congenic with RCS\textsuperscript{30}) were anesthetized with ether at 25 days of age and perfused with modified Karnovsky fixative (2% glutaraldehyde, 2.5% paraformaldehyde, 0.1 M phosphate buffer, pH 7.3, 37°C) for 15 min. Eyes were then dissected out and immersed overnight at 4°C in fixative which contained 0.5% cetylpyridinium chloride, a mucosubstance precipitator. Some eyes were enucleated and immersion fixed. After bisection along the vertical meridian, eyes were rinsed in tap water, dehydrated in methanol-methylcellosolve, infiltrated, and, finally, embedded in polyester wax. Ten micron thick serial sections were cut and mounted on microscope slides with glycerol-albumin. For all histochemical treatments, sections were first dewaxed in xylene and hydrated in graded ethanol. All investigations involving animals reported in this study conform to the ARVO Resolution on the Use of Animals in Research.

Histochemical Methods

Analysis for Hyaluronic Acid and Chondroitin SO4

Hyaluronic acid content was determined by digestion with \textit{Streptomyces} hyaluronidase, which is a specific hyaluronic acid-degrading enzyme that cleaves susceptible hexosaminidic bonds between glucosamine and glucuronic acid moieties.31,32 Chondroitin SO4 content was determined using either testicular hyaluronidase or chondroitinase AC. Both of these enzymes digest Chondroitin-4-SO4 (Type A), Chondroitin-6-SO4 (Type C), and hyaluronic acid by cleavage of hexosaminidic bonds between hexosamine and glucuronic acid.33-35 Sections were incubated in 0.1 M Na Acetate buffer, pH 5.0 alone (control), or in buffer containing either 50 TRU/ml \textit{Streptomyces} hyaluronidase (Calbiochem-Behring, San Diego, CA) or 1 mg/ml testicular hyaluronidase (bovine testes, Type I-S; Sigma Chemical Co., St. Louis, MO) for 2 hr at 37°C. Some sections were also incubated in either 0.1 M Tris-HCl buffer, pH 7.3 alone (control), or in buffer containing 1 unit/ml chondroitinase AC (from \textit{Arthrobacter aurescens}; Sigma Chemical Co., St. Louis, MO) for 2 hr at 37°C. Additional controls for the digestions included staining unincubated sections and using human umbilical cord as a positive control tissue.

Analysis for Sialoglycoconjugates

The presence of sialic acid-containing glycoconjugates was determined using two methods: enzymatic digestion with neuraminidase, and chemical treatment with mild acid hydrolysis. Neuraminidase digestion removes N-acetyl sialic acid residues of glycoproteins.36-38 Some sialic acid-containing glycoproteins are resistant to this digestion because they contain O-acetyl groups which are, in some way, responsible for this inhibition. Deacetylation of these O-acetyl groups with an alkaline aqueous alcohol solution (NH\textsubscript{3}, ethanol, H2O) removes this inhibition, so that subsequent treatment with neuraminidase will bring about digestion of all sialic acid residues.36 Mild acid hydrolysis (0.1 N H\textsubscript{2}SO\textsubscript{4}) breaks alpha-2-glycosidic acid linkages by which sialic acid residues are attached to adjacent sugars in oligosaccharide chains of glycoproteins and, therefore, removes all sialic acid residues of glycoproteins, including those that are resistant to digestion by neuraminidase.36-38

Sections of eyes were incubated in either 0.1 M Na Acetate buffer pH 5.0 alone (control) or in buffer containing 20–25 units/ml neuraminidase (\textit{Clostridium
Fig. 1. Sections of normal RCS-rdy rat retinas at postnatal (P) day 25 that have been incubated in either acetate buffer alone (control) (a), Streptomyces hyaluronidase (b), or testicular hyaluronidase (c). In control sections (a), the most intense staining of the IPM occurs at the apical surface of the retinal pigment epithelium (RPE) and at the basal junction of photoreceptor outer segments (OS) and inner segments (IS). Streptomyces hyaluronidase (b) removed little or no stainable IPM, whereas testicular hyaluronidase (c) removed most of the IPM material from the basal and interstitial zones, leaving the apical band and a small amount of residual material at the basal inner and outer segment junction (arrow). Note that neither treatment eliminated staining of Bruch's membrane (BM). Colloidal iron/PAS counterstain; posterior retina. (×1275)

perfringens. Type V; Sigma Chemical Co., St. Louis, MO) for 16 hr at 37°C. Some sections were first deacetylated for 24 hr at 37°C and then digested with neuraminidase as described above. For mild acid hydrolysis, sections were incubated in either distilled H2O (control) or in 0.1 N H2SO4 for 1 hr at 80°C. Additional controls for mild acid hydrolysis and neuraminidase digestion included staining unincubated sections and using rat salivary gland as a positive control tissue.

Sequential Digestions

Sequential digestions were also performed in which retinal sections were first treated with either testicular hyaluronidase or chondroitinase AC, followed by treatment with mild acid hydrolysis or neuraminidase. This procedure was carried out for two reasons: 1) to fully analyze the stainable components of any residual IPM material, and 2) to circumvent the possible problem that heavily stained IPM material would "mask" the loss of a minor constituent after a single digestion.

Stains

Alcian blue 8GX (C.I. 74240; Sigma Chemical Co., St. Louis, MO) is a cationic dye that specifically stains acidic mucosubstances. A tissue component is more intensely stained if Alcian blue is used at the pH at which reacting groups (carboxyl, sulfate) are fully ionized.36 Strongly sulfated mucosubstances will stain most consistently at pH levels below 1.0. Weakly sulfated mucosubstances, hyaluronic acid, and neuraminidase-labile sialoglycoconjugates stain well at pH levels between 1.0 and 3.2. Since there is considerable overlap in the reactions of various types of acidic mucosubstances, a pH 2.5 solution of Alcian blue is used for general demonstration of these tissue components.36

To achieve comprehensive staining of the IPM components, we, therefore, used a pH 2.5 solution of 1% Alcian blue made with 3% glacial acetic acid. Strongly sulfated mucosubstances were demonstrated with a 1% Alcian blue solution at pH 1.0 and 0.5 made with 0.1 N HCl and 0.2 N HCl, respectively.36

Colloidal iron also demonstrates acidic mucosubstances; at low pH it is adsorbed by negatively charged groups and is then visualized by conversion to ferric ferrocyanide. The main advantage of this technique over Alcian blue staining lies in its greater sensitivity and intensity of reaction, although it stains a broader spectrum of tissue components. Reaction with colloidal iron was carried out according to the method described by Rohlich.7 Periodic acid Schiff (PAS) was used as a counterstain for glycoproteins.39

General Protocol

Each group of control and experimental sections was stained in the same solution at the same time. After staining, sections were dehydrated in graded ethanol, cleared in xylene, and coverslip-mounted with Permount for observation. In all cases, the enzymatic and chemical digestion procedures were carried out at least three separate times. Since some variability inevitably occurs in IPM staining in rodents,15,20 we report our findings on maximal staining found in a given section.20

Results

Staining of either unincubated or buffer-incubated (control) retinal sections with colloidal iron or Alcian blue at pH 2.5 produced IPM staining that was predominantly at the apical surface of the RPE and at the basal photoreceptor inner and outer segment junction, with lesser staining of the interstices (Figs. 1a, 2a, 4a).
It should be noted that, in general, IPM staining was less with Alcian blue at pH 2.5 than it was with colloidal iron, particularly in the interstitial zone among the outer segments (20, 27, 28) (compare Figs. 1 and 2 with Figs. 3 and 4). With both stains, the interstitial zone was the most variable region of staining. For this reason, we have focused our attention primarily on the apical and basal zones. Alcian blue best demonstrates these different zones of the IPM, so it was used the most frequently in the digestion studies. Staining with Alcian blue at pH 0.5 produced an overall reduction of IPM stain, most noticeably in the apical and interstitial zones (Fig. 3a). At this low pH, the basal zone of the IPM appeared less diffuse and more distinctly localized as a band near the photoreceptor inner and outer segment junction (Fig. 3a) when compared to sections stained with Alcian blue at pH 2.5 (Fig. 4a). In sections stained with Alcian blue at pH 1.0 (not shown), we also found a reduction in IPM staining from that seen at pH 2.5; however, this was variable, and not as distinct as with Alcian blue at pH 0.5. It should also be noted that staining of Bruch’s membrane was observed at all pH values of Alcian blue (Figs. 3a, 4a).

**Single Digestions**

In the descriptions of single digestions and sequential digestions (next section), retinal sections were stained with either colloidal iron or Alcian blue at pH 2.5, unless otherwise stated. When sections were treated with Streptomyces hyaluronidase prior to staining, we found little or no reduction of stainable IPM in either the apical or basal zones (Fig. 1b) when compared to control sections incubated in buffer alone (Fig. 1a). Since Streptomyces hyaluronidase is specific for hyaluronic acid, this implies that little or no hyaluronic acid is present in these regions of the retina.

When testicular hyaluronidase was used, the enzyme removed little or no stainable material from the apical zone. However, it removed most of the interstitial material from among the outer segments and from the basal zone (Fig. 1c). We obtained the same results with chondroitinase AC (Fig. 2b), in which there was little or no reduction in apical IPM staining, but a great loss of stainable interstitial and basal IPM material when compared to control sections (Fig. 2a). Furthermore, when sections were treated with either enzyme and stained with Alcian blue at pH 0.5, basal IPM staining was totally removed (Fig. 3b) in contrast to control sections (Fig. 3a). Since digestion with either testicular hyaluronidase or chondroitinase AC produced the same results, we were confident that the same IPM components were being removed in both cases, and, therefore, used the two enzymes interchangeably in experimental procedures. These consistent observations...
suggest that most of the stainable IPM material in the basal zone is chondroitin SO₄ A and C, whereas little or no chondroitin SO₄ A and C is present in the apical zone. It should also be noted that neither enzyme significantly reduced the staining of Bruch’s membrane when sections were stained with colloidal iron or with Alcian blue at different pH values (i.e., Figs. 2b, 3b).

Treatment of retinal sections with neuraminidase before staining produced an image essentially opposite to that obtained with either testicular hyaluronidase or chondroitinase AC digestion. Neuraminidase removed most of the stainable material from the apical zone and from among the outer segments, but it removed little or no material from the basal zone (Fig. 4b). Deacetylation of retinal sections before neuraminidase digestion gave the same results as treatment with neuraminidase alone (Fig. 4c). Furthermore, when we carried out mild acid hydrolysis on retinal sections (Fig. 4d), the results were identical with those obtained with neuraminidase digestion alone or coupled with prior deacetylation. Thus, for all three experimental procedures, we found removal of most of the apical and interstitial IPM material, but little or no removal of basal zone material when compared to control sections (Fig. 4a). These consistent observations indicate that chemical treatment with mild acid hydrolysis and enzymatic digestion with neuraminidase removed the same IPM components, and we, therefore, used the two procedures interchangeably in sequential digestions. Our results suggest that sialic acid-containing glycoconjugates are predominantly localized in the apical zone of the IPM. It should also be noted that both procedures abolished the staining of Bruch’s membrane when sections were stained with Alcian blue at pH 2.5 (Fig. 4).

Sequential Digestions

As described above, testicular hyaluronidase (or chondroitinase AC) removed most of the basal and interstitial IPM material, but did not remove the apical band. It also failed to remove a small amount of residual IPM material in the basal zone near the junction of the photoreceptor inner and outer segments (Fig. 1c, 2b; arrows). Neuraminidase (or mild acid hydrolysis) removed the apical band. However, if neuraminidase also removed the residual basal material, the loss might not have been detected histochemically due to the abundance of heavily stained basal material that is still present after neuraminidase treatment of sections (Fig. 4b, 4c). In other words, if the residual material were a minor IPM component and neuraminidase-sensitive, its digestion could have been masked by the abundance of neuraminidase-insensitive IPM components staining in the same region. Therefore, to circumvent this problem, we carried out sequential digestions in which sections were first treated with testicular hyaluronidase (or chondroitinase AC) followed by mild acid hydrolysis (or neuraminidase). We found that this double treatment totally removed all stainable IPM material, including the residual material in the basal zone (Fig. 5a), when compared to single digestions.
Section 5. Sections of rat retinas at P25 that have been subjected to sequential digestion with testicular hyaluronidase (TH) followed by mild acid hydrolysis (MAH) (a), single digestion with either TH (b) or MAH (e), or reversed sequential digestion (MAH/TH) (d). Sequential digestion resulted in total removal of all stainable IPM material regardless of enzyme incubation order, including the residual basal material left after single digestion with TH (b, arrow). Alcian blue pH 2.5, posterior retina except for b, which is peripheral retina. (X1275)

(Figs. 5b, 5c). Furthermore, we obtained identical results when the steps of the sequential digestion were reversed, i.e., mild acid hydrolysis followed by testicular hyaluronidase (Fig. 5d). These results indicate that the residual basal material present after testicular hyaluronidase digestion is a neuraminidase-sensitive IPM component, and that its presence was masked by the abundance of neuraminidase-insensitive IPM material. Thus, sialoglycoconjugates are found not only in the apical zone in high concentration, but also in the basal zone in low concentration.

Discussion

Our histochemical findings suggest that the major constituents of the IPM in the normal rat retina are sialoglycoconjugates and chondroitin SO4 A and C, and that these constituents are differentially distributed in the IPM. The sialoglycoconjugates are found predominantly in the apical zone, whereas chondroitin SO4 A and C appear to be most concentrated in the basal zone (Fig. 6).

Histochemical Demonstration of IPM Composition

Our observations on the composition of the IPM appear to be in agreement with previous light microscopic studies that have used various histochemical stains to demonstrate the acid mucopolysaccharide nature of the matrix.15-17,20,25,40 Hall et al.41 and Occumpaugh and Young25 found that testicular hyaluronidase significantly reduced colloidal iron staining as well as 35SO4 autoradiographic label in the photoreceptor cell layer, thus suggesting that sulfated mucosubstances, presumably chondroitin SO4, are present in the IPM. Our finding of testicular hyaluronidase-labile material in the IPM of the rat retina is consistent with these observations. Earlier studies by Zimmerman et al.16,17,40 however, found the IPM to be insensitive to testicular hyaluronidase and, therefore, doubted the existence of sulfated components in the IPM. These discrepancies may be due to different enzyme preparations and digestion procedures. Moreover, they may reflect the fact that the quality of tissue fixation and histology has improved in recent years. This is particularly evident in our laboratory, since, with improved retinal histology, we now regularly find a more con-
concentrated region of basal IPM staining than we had previously seen. In addition, it should be noted that this new observation of basal staining is concomitant with our observation of testicular hyaluronidase sensitivity, which was undetected in our initial studies of the IPM.

EM cytochemical studies have also established the mucoid nature of the IPM, as well as its extracellular localization. These reports, however, did not characterize specific IPM components, nor did they identify the heterogeneous IPM concentration and distribution to the extent that we observe in the rat retina. These differences may reflect the fact that, in thin sections (60–70 nm) of en bloc-stained tissue used for EM, the apparent concentration of visible IPM material is significantly reduced compared to the 10 μm thick wax sections we use for LM analysis. Indeed, the distribution differences of IPM that we observe with 10 μm sections are not as obvious even in 6 μm sections, presumably due to the relatively small amount of IPM present in the rat retina. In addition, the extent of penetration of stain is often a problem for those EM cytochemical methods that require en bloc staining of tissue before embedding and sectioning. Our histochemical methods, while not giving the resolution of EM cytochemistry, are not subject to the same sort of penetration problems, because the stains are applied after sectioning the tissue.

Chondroitin SO₄ in the Rat IPM

One major constituent of the rat IPM appears to be the glycosaminoglycan (GAG), chondroitin SO₄. Existing in situ as a component of proteoglycans, chondroitin SO₄ has been demonstrated in a variety of other tissues, including aorta, cornea, brain, and basement membrane. And, together with hyaluronic acid, constitutes part of the extracellular matrix (ECM), where it plays a morphogenetic role in numerous aspects of cell behavior. In many embryonic systems, hyaluronic acid is initially the major component, but chondroitin SO₄ increases in concentration coincident with cytodifferentiation and, eventually, overrides the concentration of hyaluronic acid. Morris et al have reported that chondroitin SO₄ A and C become the major GAGs in the mature chicken retina, and that hyaluronic acid synthesis remains low during differentiation. Autoradiographic studies have provided evidence that sulfated mucopolysaccharides become components of the IPM in the rat, and that their synthesis becomes significant after the photoreceptors are fully differentiated. Similarly, biochemical analysis of adult bovine retina by Bach and Berman has shown that a variety of chondroitin SO₄-containing glycoconjugates are present in the IPM, whereas hyaluronic acid is present in relatively small amounts. Our histochemical analysis of rat IPM is consistent with these reports, since we find apparent chondroitin SO₄ A and C in significant concentrations, but are unable to detect any hyaluronic acid within the limits of our methods. It should be noted that small amounts may be present in the IPM, but are masked by the staining of major constituents. Unfortunately, we have no comparable way of carrying out sequential digestions to detect hyaluronic acid, as we did to detect the residual basal material, since both testicular hyaluronidase and chondroitinase AC remove hyaluronic acid as well as chondroitin SO₄ A and C. Nonetheless, our findings are in agreement with the histochemical observations of Zimmerman et al, who also found no evidence for hyaluronic acid in human or mouse IPM.

Components of the IPM are thought to be produced by the photoreceptor inner segments, Müller cells, and the RPE, and their transfer into the extracellular space may involve the sloughing off of cell coats and/or secretion by these cells. With regard to chondroitin SO₄, autoradiographic studies on rat retina by Hall et al and Ocumpaugh and Young provided the first evidence that sulfated mucosubstances were synthesized by photoreceptor cells and distributed into the extracellular space. These reports are consistent with our results, since we find that chondroitin SO₄ A and C appear to be localized most prominently near the photoreceptor inner and outer segment junction. Thus the site of chondroitin SO₄ synthesis may also be the site of its highest concentration. To further address this issue, studies at the ultrastructural level will be required.

Sialoglycoconjugates in the Rat IPM

Our results suggest that the other major constituents of rat IPM are sialic acid-containing glycoconjugates. Sialic acids are ubiquitous among mammalian species, where they are predominantly found in epithelial mucins (i.e., glycocalyx) and as intrinsic membrane glycoconjugates. There are clear indications that sialic acids influence or even determine such cellular processes as recognition and adhesion, hormone action, hemostasis, antigenicity, transport, and secretion, and, in many cell types, they may constitute the main negative charge at the cell surface. In the retina, cytochemical binding studies have determined that the apical processes of the RPE bear a high negative charge that is due, in part, to the presence of sialic acid residues. Cohen and Nir found that digestion with neuraminidase abolishes colloidal iron staining of the RPE apical surface and of the rod outer segments. Furthermore, Essner et al observed
that testicular hyaluronidase and chondroitinase ABC had no effect on cationic ferritin binding to the RPE apical processes. Our digestion studies are consistent with these reports, since we found that the apical zone of the IPM was removed with neuraminidase, but not with testicular hyaluronidase or chondroitinase AC, suggesting that the stainable material in the apical zone is composed predominantly of sialoglycoconjugates. Our findings are also in agreement with the biochemical analyses of Bach and Berman, who described a soluble sialoglycan that is hyaluronidase-resistant and represents approximately one-third of the IPM in bovine retina. Essner et al., however, did not observe neuraminidase sensitivity and proposed that the sialic acid residues on the apical RPE surface were possibly of a neuraminidase-resistant class. This seems unlikely, since we obtained identical results with or without prior deacetylation to neuraminidase treatment, suggesting that little or no neuraminidase-resistant sialoglycoconjugates are present in the IPM. The inconsistency may be due to the fact that cationic ferritin binding at pH 1.8, as used by Essner et al., is not specific for sialic acid residues alone. Our results are also in disagreement with the recent cytochemical observations of McLaughlin and Boykins, who found little evidence for sialic acid on the RPE apical surface in the normal rat. These differences may reflect the choice of methodology as discussed above, where section thickness, embedding media, and staining specificity could influence the concentration and distribution of stainable IPM material.

IRBP in the Rat IPM

Recently, a soluble, sialic acid-containing glycoprotein that binds retinol has been identified in a variety of mammalian eyes. Designated as interstitial retinol-binding protein, or IRBP, it is thought to be involved in the shuttling of retinol and its derivatives between the RPE and neural retina. Immunochemical studies have localized IRBP among the photoreceptor outer segments, and in greatest concentration along the apical surface of the RPE in the rat and bovine, monkey, and human retinas. This distribution of IRBP is virtually identical to our histochemical demonstration that sialic acid-containing glycoconjugates are found in highest concentration in the apical zone of the rat IPM. Thus, the IPM material that we have identified as sialoglycoconjugate may, in part, be the sialic acid-containing glycoprotein, IRBP. It should be noted, however, that our histochemical methods detect a broad spectrum of sialoglycoconjugates (i.e., sialylated membrane glycoproteins) of which IRBP may be only one (although IRBP constitutes about 70% of the readily soluble protein of the IPM). Biochemical studies have established that the neural retina synthesizes IRBP, whereas the RPE does not. More precisely, the photoreceptor inner segments appear to be primarily responsible for the synthesis and secretion of IRBP. They may also participate in the uptake and degradation of this glycoprotein. The results we have obtained with sequential digestion support these observations, since we find that the very small amount of residual IPM material located at the photoreceptor inner and outer segment junction (Figs. 1c, 2a, arrows) is also sialoglycoconjugate. IRBP that is synthesized basally by the photoreceptor inner segments must somehow be transferred to the region of the RPE apical surface. The ultimate distribution, seen histochemically and immunocytochemically, may reflect the functional properties of IRBP. For example, a gradient in IRBP concentration might be important for the mechanism by which retinoids are picked up at the apical surface of the RPE and transported to the photoreceptors. It is unclear at present, however, how such a gradient would participate in movement of retinoids in the opposite direction.

Spatial Heterogeneity of IPM Components

A differential compartmentalization of different components of the ECM, analogous to that which we observe in the IPM of the rat retina, has been observed in other systems as well. Johnson et al. have shown that, in pig, monkey, and human retinas, PNA-lectin binding is associated with discrete, cylindrical domains of IPM that ensheathe cone photoreceptors. Preliminary biochemical analysis of the sheaths suggests that glycoproteins are their major components, and that little or no heparin sulfate or chondroitin sulfate is present. The function of the cone matrix sheath is unknown; however, it might provide a unique microenvironment for cone photoreceptors or facilitate interactions with the RPE. Histochemical studies on peripheral nerve have found that the node of Ranvier also possesses a specific distribution of acidic mucosubstances, in which the macromolecules involved are suspected to be sialoglycoconjugates, hyaluronic acid, and chondroitin sulfate. These constituents are thought to provide a microenvironment for the axon membrane, where they may modify certain properties of the cell membrane, such as ion permeability. In the rat retina, and presumably in other species as well, the differential distribution of IPM components may provide a special microenvironment for the photoreceptors. For example, gradients of glycoconjugates throughout the IPM may facilitate the diffusion of metabolites from the RPE to the photoreceptors. Such gradients could also influence other metabolic and/or physical interactions between the RPE and the neural retina, such as retinal adhesion,
ion and water transport, or outer segment recognition for phagocytosis.

At present, we do not know if our observations of IPM heterogeneity are unique to the rat retina, because no comparable studies in other species have been published. We have observed that this heterogeneity also exists in the normal mouse retina (unpublished observations), but the mouse is a closely related species, and has an IPM staining pattern similar to the rat.

Although chondroitin SSO and sialoglycoconjugates are present in the IPM of many vertebrate retinas, their concentration and precise localization within the IPM remains to be determined.

**Key words:** retina, interphotoreceptor matrix, histochemistry, hyaluronic acid, chondroitin SSO, sialic acid, rat

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