Interphotoreceptor retinoid-binding protein (IRBP) was localized immunocytochemically in developing normal and RCS rat retinas. IRBP was present in normal and RCS neural retinas on the day after birth (postnatal day 2, P2) to P8 in the space between the neuroblastic layer and the retinal pigment epithelium (RPE). The presence of IRBP prior to the development of outer segments (OS) suggests that OS formation is not linked temporally with IRBP secretion. On P10, staining was confined to the interphotoreceptor space with an intense band of label adjacent to the RPE. This staining pattern persisted in normal rats throughout development and until P18 in RCS rats. On P18, anti-IRBP staining in the RCS was spread evenly throughout the OS layer with no intense band of label adjacent to the RPE and after P18, there was decreased staining with anti-IRBP. On P45 and later, no staining of the RCS retina was found with anti-IRBP. Immunoblots of normal and RCS retinas corroborated the results from immunocytochemical staining. These findings suggest that IRBP may be synthesized in the photoreceptors, but is not abnormal in amount or distribution prior to onset of retinal degeneration in the RCS rat. Invest Ophthalmol Vis Sci 26:775-778, 1985

The extracellular space between the retinal pigment epithelium (RPE) and neural retina contains the interphotoreceptor matrix. One of the glycoproteins present in this matrix is interphotoreceptor retinoid-binding protein (IRBP) that has the ability to bind a number of retinoids.1,2 It has been proposed that IRBP is involved in the transport of retinol between the RPE and neural retina.3,4 Biochemical evidence suggests that IRBP is secreted by the neural retina,1,5,6 perhaps by the photoreceptors.5,7 In this study, IRBP has been localized immunocytochemically in the developing rat retina. We also examined the appearance of IRBP in developing Royal College of Surgeons (RCS) rats since the interphotoreceptor matrix has been reported to be abnormal in rats with inherited retinal degeneration,8 and IRBP has been shown to decrease in amount after P23.5

Materials and Methods. Sprague-Dawley (SD) rats (Tyler Laboratories; Bellevue, WA) were used to study normal development. Nonpigmented RCS rats, from breeding pairs provided by Dr. Matthew LaVail (University of California, San Francisco), were used to study development in rats with inherited retinal degeneration. All procedures involving animals were performed in adherence to the ARVO Resolution on the Use of Animals in Research. The rats were kept in a 12-hr light/12-hr dark environment and ranged in age from P2 (postnatal day 2 or the day after birth) to adult. All rats were enucleated under ether anesthesia between 1:00 and 2:30 p.m. The cornea was slit, the lens and vitreous were removed, and the globe was immersed in 4% formalin in 0.13 M phosphate buffer (pH 7.4). After 6 hr in fixative at 4°C, the eyes were transferred to 30% sucrose in 0.13 M phosphate buffer and stored at 4°C overnight.

For immunofluorescence, 20 µm sections were cut with a cryostat at −20°C. The sections were mounted on chrome alum-gelatin coated slides and air-dried overnight at room temperature. Plastic rings (0.75 cm diameter) were mounted with fingernail polish around the sections to form incubation wells. Each well contained a section of RCS retina and an age-matched SD section. The sections were treated for 10 min at room temperature with 1% goat serum and 4% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) followed by 48 hr incubation at 4°C in anti-IRBP diluted 1:50 in PBS with 0.3% Triton X-100. Antibody production and further immunofluorescence techniques, including controls, were as previously described.3 For the peroxidase-antiperoxidase (PAP) technique, 40-µm free-floating sections and 20-µm sections mounted on slides as described above, were washed for 10 min at room temperature with 0.2 M Tris buffer (pH 7.4) followed by 48 hr incubation in anti-IRBP, diluted 1:50, at room temperature. The remainder of the protocol, including controls, has been described previously.3 The free-floating sections were postfixed with OsO4 and embedded in Epon for 5 µm sections and electron microscopy.

For biochemical analysis, retinas or whole-eye cups were homogenized in 50 mM Tris, pH 7.5, 200 mM NaCl containing 0.1 mM PMSF, and centrifuged at 110,000 × g. Portions of the supernatant were analyzed by SDS-PAGE and electrophoretic transfer to nitrocellulose followed by immunostaining with anti-IRBP and PAP. The details of the procedure have been published previously.3

Results. Immunocytochemistry: Anti-IRBP staining was similar in the RCS and SD retina from P2 to P18. On P2 through P8, there was a band of label between the neuroblastic layer and the RPE. On P5 through P8 the cytoplasm, but not nuclei, of some RPE cells also appeared to be labeled. In 20-µm sections, all RPE cells appeared to be stained with

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Immunocytochemical Localization of Interphotoreceptor Retinoid-Binding Protein in Developing Normal and RCS Rat Retinas

Amy J. Eisenfeld, Ann H. Bunt-Milam, and John C. Saari

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Fig. 1. Normal P6 retina reacted by the PAP technique for localization of IRBP. A, low magnification micrograph (×180) demonstrating anti-IRBP staining of the RPE and the space between the RPE and the retina (arrows); c: choroid; nb: neuroblasts. B, C, Higher magnification (×1,060, ×2,900, respectively) micrographs demonstrating labeling of some RPE cells (*). The space between the RPE and the retina (arrows) is labeled continuously throughout the retina. Erythrocytes (arrowheads) are stained dark brown due to endogenous peroxidase activity; c: choroid; nb: neuroblasts.

Anti-IRBP (Fig. 1A) but 5-μm sections revealed that not every RPE cell was positive for IRBP (Fig. 1B, C). On P10 the IRBP staining was confined to the interphotoreceptor space, with most intense staining in a band adjacent to the RPE (Fig. 2A). This pattern of staining persisted throughout development in the normal retina (Fig. 2B, C, E). Control sections showed no reactivity.

Beginning on P18 in the RCS retina, there was no band of intense staining adjacent to the RPE as seen in the normal retina (Fig. 2D). Instead, the staining was spread evenly throughout the outer segment layer. After P18, there was decreased staining with anti-IRBP. On P32 there was a fine line of staining along the apical border of the RPE, adjacent to the layer of outer segment debris (Fig. 2F). On P45 and later ages, no staining of the RCS retina was found with anti-IRBP. Control sections were negative as above.

Immunoblots: Immunoblots of SD retinas from P2, P4, P18, P25, and P32 revealed a small amount of IRBP on P2 and P4 (Fig. 3). Increased amounts were present on P18 through P32. In the RCS rat retina, the amount of IRBP closely resembled the SD on P12 through P25, but on P38 only a small amount of IRBP was present (Fig. 4).

Discussion. IRBP is present in the rat retina on P2. Only rudimentary inner segments are present at this age and there are no outer segments. This early appearance of IRBP is interesting in view of the evidence that IRBP is synthesized by the neural retina, and perhaps by the rod photoreceptors. The presence of IRBP prior to outer segment development suggests that outer segment formation is not linked temporally with IRBP secretion. Further study of IRBP in prenatal and early postnatal rats may provide information regarding the site of IRBP synthesis.

Anti-IRBP staining of the cytoplasm of some RPE cells in rats from P2 through P8 is not consistent with previous findings in adult animals that IRBP is localized only in the interphotoreceptor space and may be synthesized in the neural retina. These young retinas from P2 through P8 are difficult to fix; since RPE staining with anti-IRBP is not present at later ages when there is better fixation, it appears that the IRBP stained cells may have been damaged during the process of fixation, allowing leakage of IRBP into the cytoplasm. This interpretation is supported by the observation that not every RPE cell is labeled. Some immunocytochemical staining of cells that may have been damaged during preparation serves as a reminder that immunoreactivity within a cell may not necessarily prove that the antigen is present in that cell while still intact prior to fixation.

Staining of RCS retinas with anti-IRBP is normal until P18, the age at which pyknotic photoreceptor
Fig. 2. Localization of IRBP in developing normal and RCS rats. A and B, Indirect immunofluorescence; C-F, PAP technique. A, Normal P10 retina. Note the line of staining in the interphotoreceptor space (arrowheads); PE: pigment epithelium; ON: outer nuclear layer. B, Normal P13 retina. Staining of the interphotoreceptor space (arrowheads) is similar to that seen in the P10 retina. C, Normal P18 retina. There is anti-IRBP staining in the interphotoreceptor space with an intense band of label along the apical border of the RPE (arrowheads); ON: outer nuclear layer; OS: outer segments. D, RCS P18 retina. The interphotoreceptor space (arrowheads) is stained with anti-IRBP. Note the absence of an apical band of staining. E, Normal P32 retina. The interphotoreceptor space is stained with anti-IRBP. There is an intense band of label along the apical border of the RPE (arrowheads). ON: outer nuclear layer; OS: outer segments. F, RCS P32 retina. There is a fine line of anti-IRBP staining (arrowheads) in the interphotoreceptor space along the apical border of the RPE. The outer nuclear layer (ON) is thinner than the normal retina; OS: outer segment layer; IN: inner nuclear layer (A and B, ×237; C-F, ×180).

nuclei are first present. Beginning on P18, instead of an intense band of staining along the apical border of the RPE, the IRBP is distributed evenly throughout the interphotoreceptor space. The outer segments in the RCS neural retina are disorganized at that time, due to the inability of the RPE to phagocytose shed outer segments. Because IRBP is localized in the space that surrounds the outer segments, their disorganization may underlie the altered staining pattern.

Fig. 3. Appearance of IRBP during postnatal development of the retina. Retinas from normal Sprague-Dawley rats were obtained at postnatal days 2, 4, 18, 25, and 32 (day 1 is the day of birth), homogenized, centrifuged, and supernatants analyzed by SDS-PAGE, electrophoretic transfer to nitrocellulose and immunostaining with anti-bovine IRBP. Equal amounts of protein were applied to each well. Bovine retinal IRBP (left lane, 5μg) was included as a standard. 400 μg of protein was applied to lanes marked 2 and 4; 50 μg to lanes 18, 25, and 32.

Fig. 4. Appearance of IRBP in normal (postnatal day 10-40) and RCS (postnatal day 12-38) rat retinas. The numbers indicate the age of the animal (day 1 is the day of birth). Twenty percent of the supernatant from one retina was applied to each of the numbered lanes and analyzed by SDS-PAGE, electrotransferred to nitrocellulose, and stained with anti-bovine IRBP. The lane marked IRBP contained 5 μg of purified retinal IRBP.
Unlike the results from mucopolysaccharide staining, no change is detected in staining for IRBP prior to photoreceptor degeneration. The intensity of anti-IRBP staining decreases in the RCS rat after P18, paralleling temporally the loss of photoreceptors. These findings suggest that IRBP may be synthesized in the photoreceptors but is not abnormal in amount or distribution prior to the disorganization of outer segments found in the RCS model of inherited retinal degeneration.

Key words: retinoid-binding protein, development, retina, RCS rat retina, intercellular retinoid-binding protein

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References

Structural Periodicities Observed in Mammalian Rod Outer Segments with Nomarski Optics

Lary D. Andrews

Structural periodicities have been observed in isolated rod outer segments of several mammalian species using differential interference contrast (Nomarski) optics. The spacing among the observed structural inhomogeneities is somewhat variable. If these measured spacings are corrected for shrinkage, estimated by comparing ROS widths in unfixed cells to those in light microscopic autoradiographs, the mean values correlate well with published rates of ROS renewal in rats and dogs. Invest Ophthalmol Vis Sci 26:778-782, 1985

The retinal rod outer segments (ROS) of a variety of vertebrate species have been studied intensively for many years. The relevance of this work to human ocular disorders has been diminished by the frequent use of ROS from nonmammalian species, which have the advantage of being several times larger in diameter than are mammalian ROS. Among the many consequences of the smaller diameter of mammalian outer segments is that they are very dim when viewed in the polarizing microscope. This makes it more difficult to detect any potentially interesting variations in their structure such as those reported in amphibians. 1,2 Kaplan 3 has reported observing a faint axial gradient of birefringence in monkey ROS, but no one has yet reported seeing the type of periodicities of birefringence in amphibia.

In this article, we report the existence of distinctive periodicities in mammalian ROS when viewed with Nomarski (differential interference contrast) optics. While these periodicities are considerably less regular than those seen in amphiban ROS, there is a correlation between their spacing and published data on the rate at which mammalian ROS are...