Molecular Neurobiology
Copyright ©1993 Humana Press, Inc.
All rights of any nature whatsoever reserved.
ISSN0893-7648/93/7(1): 61–84/$3.80

Interphotoreceptor Retinoid-Binding Protein (IRBP)

Molecular Biology and Physiological Role in the Visual Cycle of Rhodopsin

David R. Pepperberg,*[1] Ting-Ing L. Okajima,[1] Barbara Wiggert,[2] Harris Ripps,[1,3] Rosalie K. Crouch,[4] and Gerald J. Chader[2]

[1]Lions of Illinois Eye Research Institute, Department of Ophthalmology and Visual Sciences; and [3]Department of Anatomy - Cell Biology, University of Illinois at Chicago College of Medicine, Chicago, IL 60612; [2]National Eye Institute, National Institutes of Health, Bethesda, MD 20892; and [4]Departments of Ophthalmology and Biochemistry, Medical University of South Carolina, Charleston, SC 29425

Contents

*Author to whom all correspondence and reprint requests should be addressed.

Abstract

The regeneration of visual pigment in rod photoreceptors of the vertebrate retina requires an exchange of retinoids between the neural retina and the retinal pigment epithelium (RPE). It has been hypothesized that interphotoreceptor retinoid-binding protein (IRBP) functions as a two-way carrier of retinoid through the aqueous compartment (interphotoreceptor matrix) that separates the RPE and the photoreceptors. The first part of this review summarizes the cellular and molecular biology of IRBP. Work on the IRBP gene indicates that the protein contains a four-fold repeat structure that may be involved in binding multiple retinoid and fatty acid ligands. These repeats and other aspects of the gene structure indicate that the gene has had an active and complex evolutionary history. IRBP mRNA is detected only in retinal photoreceptors and in the pineal gland; expression is thus restricted to the two photosensitive tissues of vertebrate organisms. In the second part of this review, we consider the results obtained in experiments that have examined the activity of IRBP in the process of visual pigment regeneration. We also consider the results obtained on the bleaching and regeneration of rhodopsin in the acutely detached retina, as well as in experiments testing the ability of IRBP to protect its retinoid ligand from isomerization and oxidation. Taken together, the findings provide evidence that, in vivo, IRBP facilitates both the delivery of all-*trans* retinol to the RPE and the transfer of 11-*cis* retinal from the RPE to bleached rod photoreceptors, and thereby directly supports the regeneration of rhodopsin in the visual cycle.

Index Entries: Interphotoreceptor retinoid-binding protein; IRBP; rhodopsin; retinoid; visual cycle; visual pigment; retinal pigment epithelium.

Introduction

It is well established that the bleaching and regeneration cycle of rhodopsin in the vertebrate eye depends critically on the movement of retinoid between the rod photoreceptors and the retinal pigment epithelium (RPE) (for recent reviews, *see* Saari, 1990; Bok, 1990; Rando et al., 1991). All-*trans* retinol, generated within the rods by the bleaching of rhodopsin (Wald, 1935; Futterman, 1963; Brin and Ripps, 1977; Sears and Kaplan, 1989; Ishiguro et al., 1991), is transferred from the rods to the RPE by passage through the interphotoreceptor matrix (IPM), the aqueous compartment that separates the RPE and the neural retina. Within the RPE, the retinol is enzymatically esterified (Krinsky, 1958; Hubbard and Dowling, 1962; Zimmerman, 1974; Bridges, 1975, 1976a; Berman et al., 1980; Saari and Bredberg, 1988a, 1989; Das and Gouras, 1988; Bongiorno et al., 1991) and isomerized from the all-*trans* to the 11-*cis* configuration (Bernstein et al., 1987; Deigner et al., 1989; Shi and Olson, 1990). 11-*cis* Retinoid formed within the RPE subsequently moves to the rods, where, as 11-*cis* retinal, it binds to opsin (Dowling, 1960; Perlman et al., 1982; Kawaguchi et al., 1986; Okajima et al., 1990). The resulting formation of rhodopsin directly regulates the recovery of visual sensitivity; ultimately, the full complement of photosensitive pigment is restored and sensitivity recovers to its fully dark-adapted, i.e., pre-bleach, level (Dowling, 1960; Rushton, 1961; Dowling and Ripps, 1970; Pepperberg et al., 1978; Jones et al., 1989).

The dependence of visual function on the regeneration of rhodopsin emphasizes the need to understand the mechanisms that support the transfer of retinoids between the rods and the RPE. Interest has focused on the possible activity in this process of interphotoreceptor retinoid-binding protein (IRBP), a glycoprotein ($M_r \approx 140$ kDa) that is uniquely localized in the IPM and is the major soluble protein of this intercellular compartment (Bunt-Milam and Saari, 1983; for a review, *see* Chader, 1989). The possibility that IRBP serves as a retinoid transport vehicle in the bleaching/regeneration cycle is clearly implied, for example, by the finding that light modulates the relative levels of all-*trans* and 11-*cis* retinoids associated with IRBP (Wiggert et al., 1979; Adler

and Martin, 1982; Liou et al., 1982; Bridges et al., 1984; Saari et al., 1985; Adler and Evans, 1985; Lin et al., 1989; Adler and Spencer, 1991a). Exposure to bright light favors the binding of all-*trans* retinol to IRBP, whereas in the course of dark adaptation, 11-*cis* retinal becomes the principal ligand.

Despite these observations, the need for an intercellular retinoid carrier within the IPM has been questioned. The relatively close apposition of the apical processes of the RPE with the plasma membranes of the rod outer segments, and the evidence that the intermembraneous transfer of retinol through aqueous medium is retarded by IRBP (Ho et al., 1989), have led to the suggestion that IRBP acts primarily to buffer the extracellular concentration of retinoid, thereby preventing both aqueous degradation of the retinoid and its potentially toxic effect on cell membranes.

It is important to note, however, that these diverse functions are not mutually exclusive, and as will become evident, it is likely that IRBP subserves multiple functions associated with the translocation of retinoids through the IPM. The present review summarizes a select body of work dealing with the molecular properties of IRBP, and its physiological role in the retinoid exchange process that underlies the rhodopsin cycle.

Molecular Biology and Expression of IRBP

cDNA Analysis

The analysis of cDNA has replaced the classical biochemical methods in determining the primary sequence of large proteins, such as IRBP; it also affords a quick insight into functional groupings of amino acids and their possible conformation. The first report of IRBP clones (Barrett et al., 1985) was followed by publication of the cDNA sequences of the bovine (Liou et al., 1986) and human (Fong and Bridges, 1988) proteins. The deduced protein sequence for bovine IRBP is consistent with a protein of about 140 kDa mol wt, and corresponds to a total of 1264 amino acids. The bovine and human proteins are 80–90% similar in sequence and in apparent structure. Although sequences of many other retinoid-binding proteins (opsin, serum retinol-binding protein, cellular retinol- and retinoic acid-binding proteins, cellular retinaldehyde-binding protein, and purpurin) have been reported, GenBank and PIR searches show IRBP to be unique, with no similarities to these proteins or to other members of the super families to which they belong.

One of the most striking features to emerge from the cDNA analysis is a four-fold repeat structure in the nucleic acid sequence that translates into a strong four-repeat structure in the protein moiety. Each repeat is approx 300 amino acids long with a short C-terminal extension of about 55 amino acids (Fong and Bridges, 1988). The sequence identity between any two of the repeats is 30–40%; many of the remaining positions exhibit conservative amino acid substitutions. Moreover, many of the identical amino acids are clustered together in the repeats, suggesting that these could be functionally important areas that are retained because of evolutionary constraints. These areas are therefore likely candidates for studies on retinoid and fatty acid binding. For example, Redmond et al. (1991) have recently used a prokaryotic expression system to synthesize 38, 64, and 82 kDa fragments of IRBP within repeats 1 + 2. Only the 82 kDa fragment exhibited binding activity for (^{3}H)retinol, suggesting that the full two repeats are necessary to constitute a binding domain. IRBP appears to have multiple binding sites for retinoids and for fatty acids, and this molecular biological approach should be profitable in mapping all of the binding sites.

A number of putative sites for covalent modification are apparent in the IRBP protein, including five sites that match the N-glycosylation site consensus sequence, NX (T/S). Three of these sites align closely within the repeat structure and may actually be glycosylated; the putatively glycosylated asparagine residues could not be detected by Edman degradation, suggesting

carbohydrate derivatization. There are 18 possible serine and threonine residues that can be phosphorylated in the bovine protein. Based on hydrophobicity analysis, many of these probably exist on the surface of the protein. Wiggert et al. (1988) demonstrated that IRBP can be phosphorylated by kinases endogenous to the IPM; the phosphorylated residues may be of importance in protein–protein or protein–membrane interactions in the IPM. Also of interest is the fact that the protein contains a stretch of five proline residues close to the middle of the peptide (residues 690–694); conceivably, these prolines may be involved in the flexible, bend area in the middle of the IRBP protein, as described by Adler et al. (1987).

mRNA Analysis

Using a bovine probe and Northern blot analyses, Inouye et al. (1989) examined retinal RNA from a number of species. They demonstrated that IRBP mRNA is relatively abundant in vertebrates except for birds, in which only relatively weak hybridization was detected. Although a single hybridizing band is observed in most species, rat retinal mRNA exhibits two discrete bands at 5.4 and 6.6 kb. A wide variation in band size is apparent; for example, mRNAs from human and monkey retinas exhibit bands at 4.6 kb, whereas the hybridizing band from hamster retinal mRNA is at 7.6 kb. This variation seems primarily to be due to differences in the 3'-untranslated region, since Si et al. (1989) demonstrated a 1.58 kb insertion/deletion in this region when dot matrix comparisons were made of the human and bovine cDNA sequences. Northern blot analysis also reveals that IRBP message is present in the pineal gland as well as in retina (Nickerson et al., 1991). It appears to be of the same size as the retinal mRNA but, as with the protein, is present at a much lower concentration than in the retina.

van Veen and coworkers (1986), using *in situ* hybridization techniques, found that the IRBP mRNA of bovine and monkey retinas is localized specifically in the photoreceptors (Fig. 1), but reaction product was found to be less intense over cones than over rods (Fig. 1D). These findings are consistent with recent results, obtained with light microscopic immunogold cytochemistry, showing that staining of the IPM around rod outer segments is denser than that around the outer segments of cones (Carter-Dawson and Burroughs, 1989a,b). The observation that IRBP mRNA appears early in the postnatal development of the rat retina suggests a possible role for the protein in retinal development as well as in the adult visual cycle (Gonzalez-Fernandez and Healy, 1990).

Genomic Analysis

A full genomic analysis was published by Borst and Nickerson (1988) and Borst et al. (1989) for bovine IRBP, and by Liou et al. (1989) and Fong et al. (1990) for the human protein. The human gene has been mapped to chromosome 10, and Liou et al. (1987) have assigned the gene to 10p11.2 to 10q11.2, with a secondary site at q24–25; Nickerson et al. (1991) make the assignment to 10q21.1. Restriction enzyme digests of bovine liver DNA reveal only 1–2 bands/lane, indicating the probability of only one IRBP gene per haploid genome. The nucleotide sequence indicates a surprisingly compact gene of only about 11.6 kb, containing only four exons and three introns (Fig. 2). Intron and exon lengths in the bovine and human proteins are roughly equivalent, except for variability in exon 4 as alluded to above, where, for example, the human sequence is about 1700 bp shorter than that of cow. The introns do not each code for one of the repeat units in the protein; rather, the first three repeats, as well as part of the fourth, lie within the first exon. This exon, at about 3.2 kb, is thus one of the longest yet reported.

At the 3' end, the bovine gene contains a polyadenylation signal (AATAAA at position 11,613), and a sequence (TTGTTATCTTTT) 21 bases downstream that resembles a consensus sequence often found at the end of eukaryotic genes. At the 5' end, the cap site has been established by primer extension and S1 nuclease experiments (Borst et al., 1989). As IRBP is a secreted protein, it is not surprising that it contains a signal

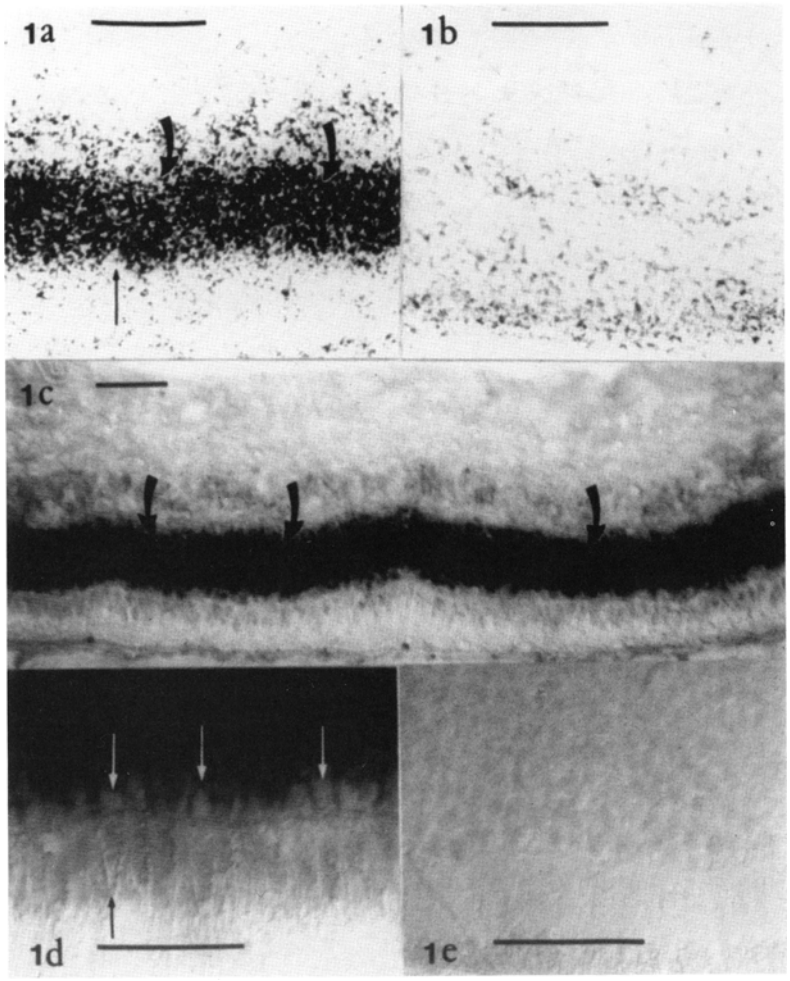


Fig. 1. *In situ* hybridization of bovine retinal tissue sections. **a:** ^{35}S-cRNA probe hybridization to photoreceptor layer (thick arrows) with less hybridization in cone perikaryon (thin arrow). **b:** Negative reaction using (+) sense cRNA strand. **c:** Hybridization of HRP-labeled probe in photoreceptor layer (arrows). **d:** Higher magnification of photoreceptor layer, showing cone perikarya (white arrows) or outer segments (black arrow) with little reaction product. **e:** Negative reaction with sense HRP-labeled probe. Reproduced from van Veen et al. (1986).

sequence coding for 17 amino acid residues and a pro-peptide of five amino acids positioned between the signal sequence and the authentic N-terminus of the protein.

Many genes contain sequences referred to as TATA boxes at about –25 to –30 bp and CAAT boxes at about –70 to –80 bp upstream from the start site. The bovine IRBP gene contains neither of these typical elements, although it does exhibit a TATA-like box (TTAAA) at about –50 bp and a CAAT-like box (CCACTT) at about –106 bp. Dot matrix analysis of the promoter regions within the vertebrate genes sequenced to date reveals two blocks of similarity: the first near the start of the gene at –1 to –350 bp, and the second well upstream at –1250 to –1500 bp. Thus, along with having an odd gene structure, the IRBP promoter signals are different from those present in most tissue-specific genes. The human S-antigen gene also lacks the typical transcrip-

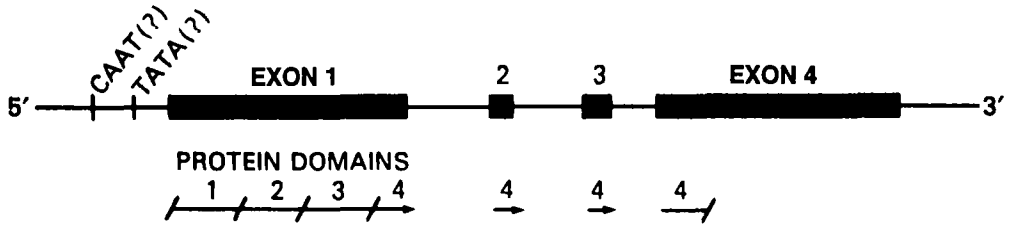


Fig. 2. General model of the IRBP gene.

tional regulatory elements (Yamaki et al., 1990), as does the gene for the transducin α-subunit in mouse rods (Raport et al., 1989). On the other hand, the opsin gene does contain conventional TATA and CAAT boxes (Nathans and Hogness, 1984). Future experiments with these genes should help to define the specificity of 5'-regulatory regions (or the lack thereof) in tissue-specific expression and in evolutionarily-related tissues such as retina and pineal.

Tissue-Specific Expression

The factors that govern the expression of IRBP have been examined in several recent investigations. In transgenic mice, Liou et al. (1990) have used the CAT reporter gene fused to segments of the human IRBP promoter region to begin to analyze the regulation of the IRBP gene. They determined that a 1.3 kb fragment that is 5'-upstream from the start site will allow for tissue-specific expression in retina and pineal. More recently, Liou et al. (1991) have used shorter chimeric constructs to establish that all elements necessary for tissue-specific expression are within the first 212 bps of the 5'-flanking region. It is well known that factors other than simple 5'-upstream DNA sequences are important in determining whether a gene is expressed. For example, the state of DNA methylation within a gene is thought to influence gene activation/inactivation in a reversible manner, allowing for tissue and developmental specificity of expression. Most often, methylation has been reported in CpG-rich promoter regions, with hypomethylation of specific CpG-rich islands associated with gene activation. Albini et al. (1990) have compared hypomethylation of the IRBP gene in cultured human retinoblastoma cells that express the IRBP protein, with that in cultured human lymphocytes in which no IRBP mRNA can be detected. Two sites of hypomethylation were observed in the retinoblastoma IRBP gene that were not present in its lymphocyte counterpart: a site within a CpG-rich region of the promoter (–1578 to –1108 bp) and sites within exon 1 (Fig. 3). Thus, hypomethylation may be a permissive factor that affords the binding of specific nuclear factors that control IRBP expression. Elucidation of the factors controlling the developmental timing of expression and tissue specificity in retina and pineal may allow for a better understanding of the functional roles of IRBP in the visual process.

Activity of IRBP in the Retinoid Visual Cycle

Abundance, Localization, and Ligand Binding in the IPM

The interphotoreceptor matrix (IPM) is bounded proximally by the external limiting membrane and distally by the apical surface of the RPE. Junctions between the cells forming these borders prevent diffusion of large molecules like IRBP beyond the confines of the IPM (Bunt-Milam et al., 1985). In the retina, IRBP is exclusively synthesized in the photoreceptor cells (Hollyfield et al., 1985a; van Veen et al., 1986:

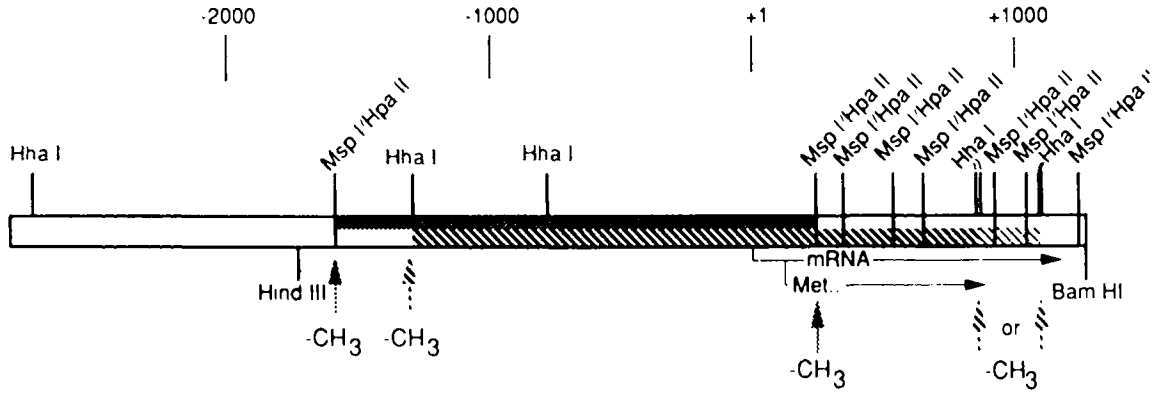


Fig. 3. Hypomethylation model of the IRBP promoter region. +1: mRNA cap site. Shaded region: 1.8 kb MspI/Hpa II fragment demethylated ($-CH_3$) in Y-79 cells as indicated by set of shaded arrows. Striped region: 2.1–2.3 Hha I fragment found in Y-79 cells due to demethylation at sites indicated by striped arrows. Reproduced from Albini et al. (1990) with permisssion from Oxford University Press.

Rodrigues et al., 1986), and there is indirect evidence indicating that it may be secreted into the IPM by a process of exocytosis (Bunt-Milam et al., 1985; Hollyfield et al., 1985b). IRBP has been isolated from the eyes of virtually every class of vertebrate, and its level within the IPM is to some extent species-dependent (Fong et al., 1984; Redmond et al., 1985; Wiggert et al., 1986; Bridges et al., 1986).

Interestingly, the quantity of IRBP recovered from light- and dark-adapted eyes appears to be independent of the state of adaptation (Lin et al., 1989; Adler and Spencer, 1991a). However, at least in the case of rat, photic exposure exerts a profound effect on the distribution of IRBP within the IPM. In dark-adapted rats, Uehara et al. (1990) observed that IRBP and other major constituents of the IPM (e.g., chondroitin sulfate) are distributed more or less uniformly throughout the interstitial space. In light-adapted animals, these substances were partitioned into bands: one near the distal ends of the photoreceptor outer segments, the other near the junction of the receptor inner- and outer-segments. In bovine eyes, on the other hand, IRBP exhibited a heterogeneous distribution within the IPM, but the localization of the protein was not affected by light (Adler and Spencer, 1991a). Although the basis for this discrepancy is not immediately apparent, it may reflect a species difference, or more likely, the very different experimental conditions in the two studies, i.e., the use of eyes from animals light- and dark-adapted in vivo (Uehara et al., 1990), as compared to the use of eyes enucleated prior to photic treatment (Adler and Spencer, 1991a). In the latter case, certain enzymatic or ATP-driven mechanisms required for protein translocation may have been inactivated.

When recovered from the IPM, IRBP is found to be associated with endogenous 11-*cis* and all-*trans* retinoids (*see* Introduction). The protein also binds a number of other hydrophobic ligands, such as α-tocopherol, cholesterol, and retinoic acid (Alvarez et al., 1987). Moreover, IRBP has been shown to bind fatty acids both covalently and noncovalently (Bazan et al., 1985); it has been hypothesized that the noncovalent binding reflects a carrier role for IRBP in the movement of fatty acids between the retina and RPE.

Electron microscopy of individual tungsten-coated IRBP molecules indicates the existence of both a bent (60–90° angle between the two arms of the molecule) and a straight configuration; the transition from the straight to the bent form may be induced by the binding of 11-*cis* retinoid (Adler et al., 1987). In vitro experiments analyzing the binding of retinoids by purified IRBP have yielded binding constants in the micromolar

range, i.e., affinities much weaker than those exhibited by other retinoid-binding proteins (Adler et al., 1985; Saari, 1990; Noy and Blaner, 1991; Adler and Spencer, 1991b). The previous studies have reported different values for the binding capacity of IRBP for all-*trans* retinol; the data of Adler et al. (1985) and Okajima et al. (1989) indicate a single (independent) binding site for this ligand, whereas results obtained by Fong et al. (1984), Saari et al. (1985), and Hazard et al. (1991) indicate the presence of at least two such sites (*see also* Adler and Spencer, 1991b). The basis of the disparity in the capacity for binding all-*trans* retinol remains unclear, but may reflect a dependence on experimental conditions, such as the composition of the physiological saline used in the incubations.

The binding constants determined in vitro in principle afford determination of the percent loading of IRBP at a given concentration. However, the heterogeneous distribution of IRBP within the IPM, and the resulting uncertainty in the volume of this aqueous compartment, preclude an accurate determination of the (local) IRBP concentration, and thus, of percent loading, *in situ*. Average (i.e., bulk) concentrations of IRBP in human and bovine eyes are in the range of 30–100 μ*M* (Adler et al., 1985; Bridges et al., 1986). Using these numbers, the binding affinity is such that some free IRBP is expected. Adler and Spencer (1991a) estimate that there is about 3 nmole of IRBP in the bovine eye and that approximately 0.5 nmole of retinoid ligands are bound by IRBP in the dark-adapted eye. This implies that most of the IRBP is ligand-free under these conditions. By comparison, Lin et al. (1989) report that there is almost complete loading of IRBP (with 11-*cis* retinal and 11-*cis* retinol) in the frog eye after several hours of dark adaptation.

Little is known regarding the processes that underlie the degradation and removal of IRBP from the IPM. In vitro experiments have indicated that IRBP-containing particles can be taken up into the photoreceptors (inner segment region) (*see* Hollyfield et al., 1985b). Phagocytosis by the RPE is known to represent a major process of removal of disk membranes that have been shed from the outer segments (Young, 1976), but the role of phagocytosis in IRBP removal is unclear.

Delivery of Retinol to the RPE

The influence of light on the relative levels of endogenous 11-*cis* and all-*trans* retinoids bound to IRBP (*see* preceding section), the dependence of the conformation of IRBP on the form of retinoid bound (Adler et al., 1987), and the influence of illumination conditions on the localization of IRBP within the IPM (Uehara et al., 1990) clearly imply an involvement of the protein in the transfer of retinoid in the visual cycle. To test the hypothesis that IRBP functions as an intercellular shuttle of retinoid to and from the RPE, we have employed the "RPE-eyecup," i.e., the posterior half of the globe from which the vitreous and neural retina have been removed. The preparation serves as a vessel in which aqueous solutions (250 μL) containing IRBP or other test substances can be incubated in contact with the apical surface of the RPE. Procedures used to prepare the RPE-eyecup from the toad (*Bufo marinus*) eye, and to isolate and purify ligand-free IRBP from the bovine eye, as well as all other preparative and analytical procedures, have been described in previous reports (Okajima et al., 1989,1990; Pepperberg et al., 1991).

One example of the use of the RPE-eyecup is illustrated in Fig. 4, which shows the results of an experiment designed to test the possible activity of IRBP in the delivery of retinol to the RPE (Okajima et al., 1989). Because retinol is readily esterified in the RPE, the appearance of radiolabeled retinyl ester following incubation with (^{3}H)retinol in the RPE-eyecup provides a useful assay of the incorporation of retinol by the RPE. In the experiment illustrated in Fig. 4, RPE-eyecups were incubated with aqueous medium containing all-*trans* (^{3}H)retinol and IRBP. The medium delivered to each eyecup had been prepared in a test tube, by pre-incubating all-*trans* (^{3}H)retinol (55 pmol) with defined concentrations of IRBP in 250 μL of a physiological saline (Ringer's) solution. Following a 2-h incubation

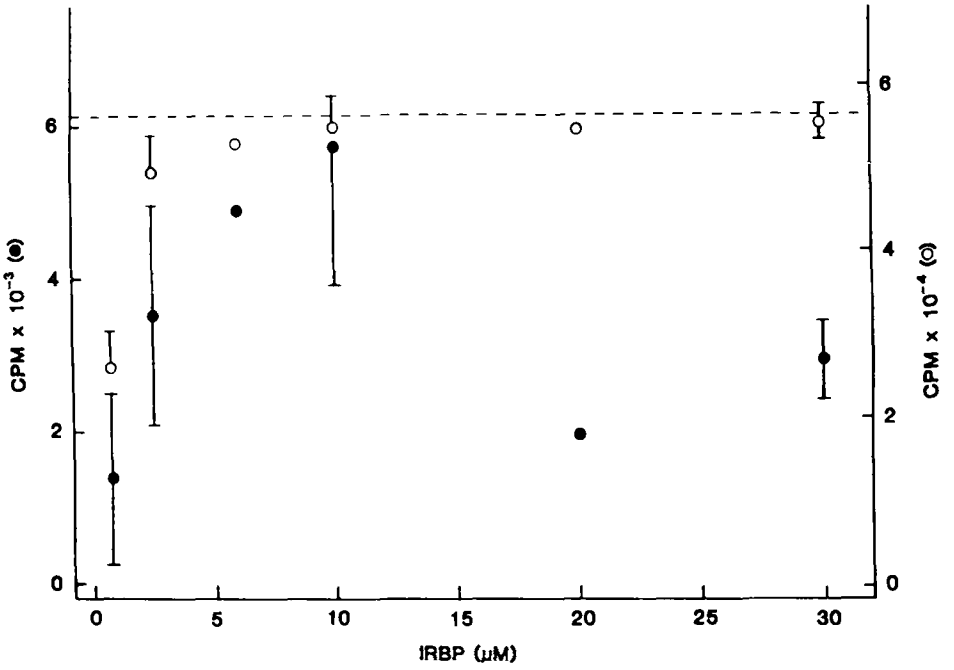


Fig. 4. Delivery of all-*trans* (^{3}H)retinol to the RPE; dependence on the concentration of IRBP. Aqueous medium (250 μL of a physiological saline solution) containing IRBP and all-*trans* (^{3}H)retinol was delivered to a group of RPE-eyecups. Following a 2-h incubation, (^{3}H)retinyl ester in the RPE was analyzed by isolation and extraction of the tissue, and by HPLC (normal phase separation in a Waters 5μ spherical silica column; elution with dioxane/hexane) and scintillation counting. Filled circles show mean levels ($n \geq 2$) of all-*trans* (^{3}H)retinyl ester in matched aliquots of the RPE extracts. Error bars indicate ± SD for ≥ 3 determinations; symbols with abscissa values of 0.8 μ*M* and 2.5 μ*M* combine results for, respectively, (1.0 μ*M* and 0.3 μ*M*) and (2 μ*M* and 3 μ*M*). Open circles show mean levels (and ± SD, where appropriate) of (^{3}H) contained in a 10 μL aliquot of the test mixture delivered to the RPE-eyecup; the dashed line indicates the predicted level of (^{3}H) in the aliquot, assuming complete solubilization of the (^{3}H)retinol originally added to the preparation tube. Reproduced from Okajima et al. (1989) with permission from Academic Press.

of the supplemented RPE-eyecups, each preparation was analyzed for the amount of (^{3}H)retinyl ester in the RPE. The observed levels of (^{3}H)retinyl ester are plotted in Fig. 4 (filled circles) as a function of the concentration of added IRBP; open circles in the Figure indicate the amount of (^{3}H)retinol contained in a 10 μL aliquot of the aqueous medium that had been delivered to the RPE-eyecup. In the range of about 1–10 μ*M* IRBP, the amount of radiolabeled ester formed in the RPE was found to increase with the concentration of IRBP; the amount of all-*trans* (^{3}H)retinol transferred from the pre-incubation tube to the RPE-eyecup also increased with the concentration of IRBP. The data obtained with IRBP concentrations of ≤10 μ*M* thus indicate a dependence of ester synthesis on the amount of all-*trans* retinol solubilized in the aqueous incubation medium, and a dependence of this aqueous level of retinol on the concentration of IRBP.

Interestingly, higher concentrations of IRBP (20–30 μ*M*) reduced both the amount of (^{3}H)retinyl ester formed (Fig. 4) and the overall molar quantity of retinyl ester in the RPE (data not illustrated; *see* Table I of Okajima et al., 1989). In light of the evidence that IRBP can retard the aqueous transfer of retinoid between membraneous particles (Ho et al., 1989; *see also* discussion below), it seems likely that the reduction observed with the high concentrations of IRBP may reflect a competition, for both tritiated (i.e., added) and native retinoid, between IRBP and the RPE.

Regeneration of Rhodopsin in the ROS/RPE-Eyecup

Another question that needs to be addressed concerns the role of IRBP in the transfer of 11-*cis* retinoid from the RPE to the rods, a *sine qua non* for rhodopsin regeneration. In this case, the evidence that IRBP facilitates the movement of 11-*cis* retinoid from the RPE to the rods comes from experiments involving the incubation of bleached rod outer segments (ROS) in the RPE-eyecup ("ROS/RPE-eyecup" preparation), both in the presence and absence of IRBP (Okajima et al., 1990).

Figures 5A–B illustrate the protocol for a representative experiment. A suspension of ROS was first subjected to irradiation that bleached most of the native rhodopsin ("preparative bleach"). Aliquots of the bleached ROS were supplemented with either Ringer's solution alone, or with Ringer's containing purified, initially ligand-free IRBP, and then incubated for 3 h in an RPE-eyecup. Subsequently, each ROS suspension was withdrawn from the RPE-eyecup, mixed with hydroxylamine, and analyzed by spectrophotometry before and after a 5-min irradiation to obtain the absorbance difference spectrum (hydroxylamine-difference spectrum) of the suspension.

Figure 5C shows the results obtained in the experiment just described. Consider first the data obtained from the ROS-RPE/eyecup that had received only Ringer's solution (filled squares). This spectrum was similar in magnitude to the baseline spectrum obtained from the ROS immediately after the preparative bleach (*see* horizontal arrow in the figure). Thus, there was little evidence of rhodopsin formation in the ROS; i.e., the final level of rhodopsin present in the Ringer's-supplemented RPE-eyecup was due almost entirely to the rhodopsin that remained after the preparative bleaching exposure. By contrast, the spectrum obtained from the IRBP-supplemented RPE-eyecup (filled circles) shows the formation within the ROS of a large quantity of light-sensitive pigment, spectrally indistinguishable from native rhodopsin (triangles).

The results shown in Fig. 5C demonstrate further the importance of the RPE to the process of rhodopsin regeneration. Shown by open squares and open circles in the figure are the data obtained when aliquots of the bleached ROS were incubated in test tubes, i.e., in the absence of the RPE, with Ringer's solution alone or with Ringer's containing IRBP. The hydroxylamine difference spectra of both of these RPE-deficient preparations were similar to the baseline difference spectrum, indicating the absence of substantial regeneration. It is apparent, therefore, that the RPE functions as the source of the 11-*cis* retinoid ultimately delivered to opsin.

The findings just summarized emphasize the need for both IRBP and the RPE in the delivery of 11-*cis* retinoid to opsin in ROS membranes. Interestingly, a similar result was seen when intact, previously isolated and bleached retina served as the target tissue in the RPE-eyecup. By comparison with bovine serum albumin (used as a control retinoid-binding protein), IRBP that had been introduced between the retina and RPE was substantially more effective in promoting the regeneration of rhodopsin in the retina (Okajima et al., 1990).

Release of Retinoid by the RPE

A long-standing issue has been the identity of the 11-*cis* retinoid released by the RPE. Depending upon the state of visual adaptation *(see above)*, both 11-*cis* retinal and 11-*cis* retinol may be found in the IPM associated with IRBP (e.g., Lin et al., 1989; Adler and Spencer, 1991a), and indeed, in some species, both of these 11-*cis* retinoids support rhodopsin regeneration in the isolated retina preparation (Perlman et al., 1982). To identify the retinoid(s) released by the RPE of the toad, an RPE-eyecup was incubated for 3 h with 250 µL Ringer's solution that contained initially ligand-free IRBP. The aqueous solution was then extracted with organic solvent, and analyzed for its retinoid content by spectrophotometry and high performance liquid chromatography (HPLC) (Figs. 6A–B). The spectrophotometric data showed that the absorbance spectrum of the

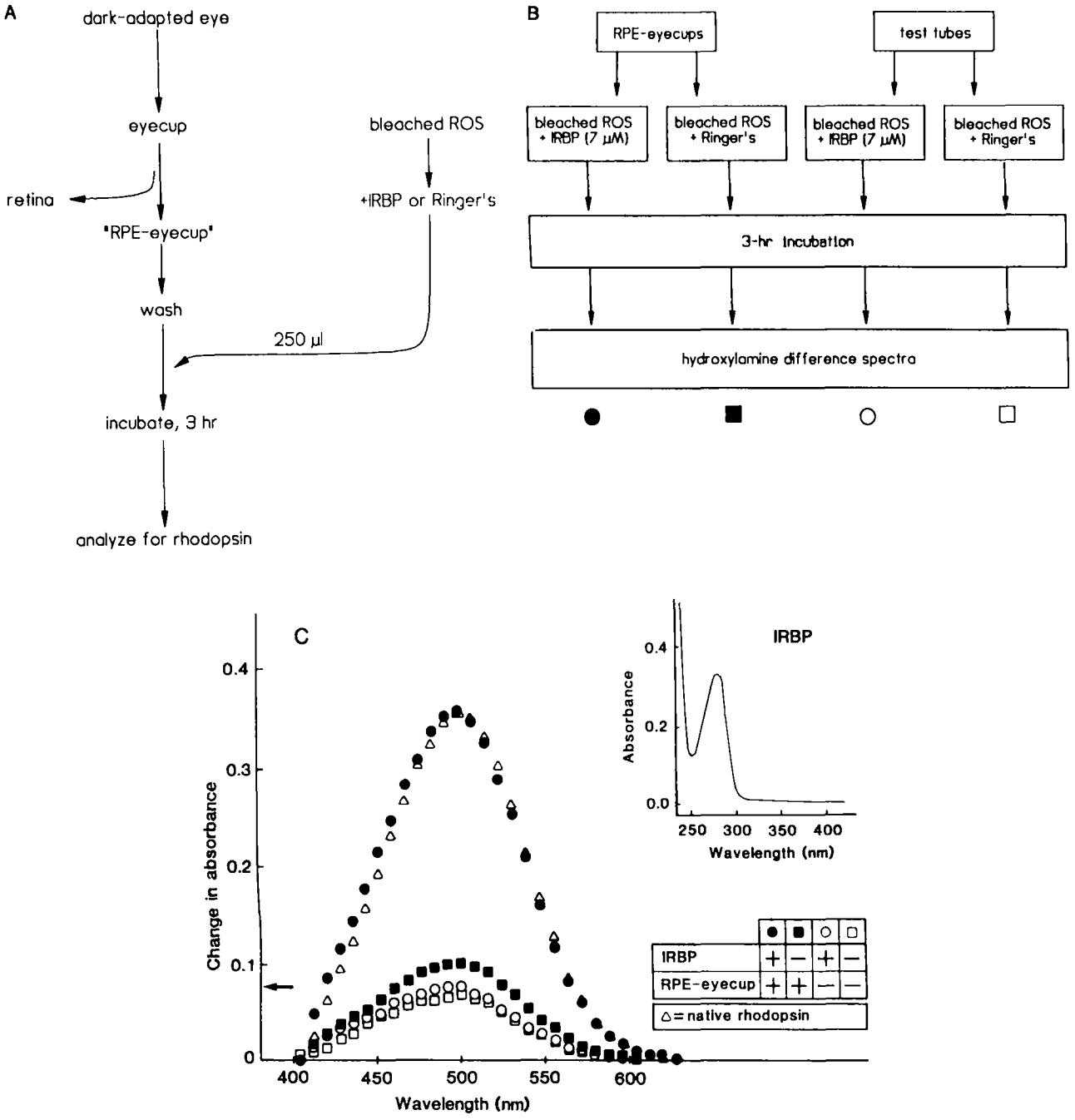
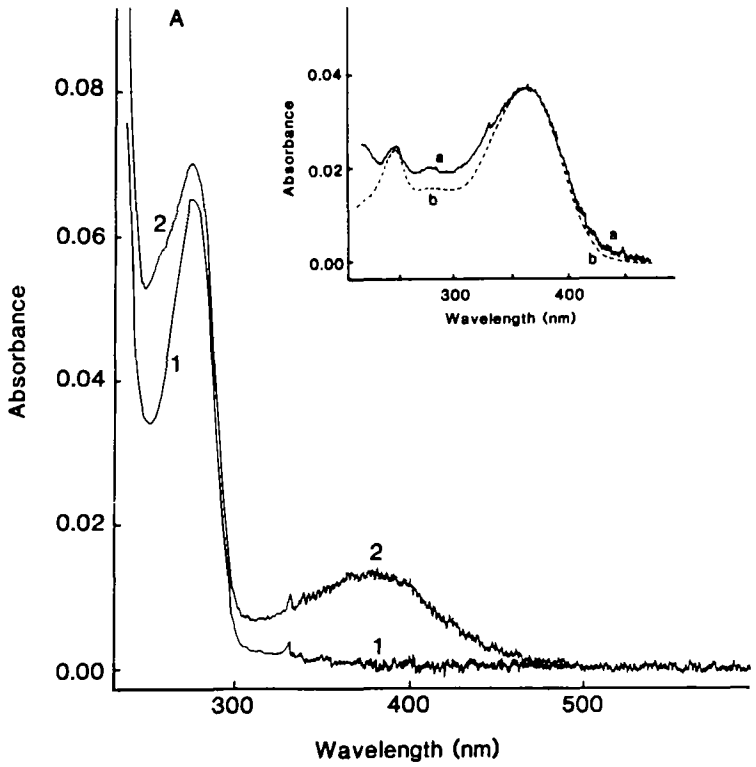
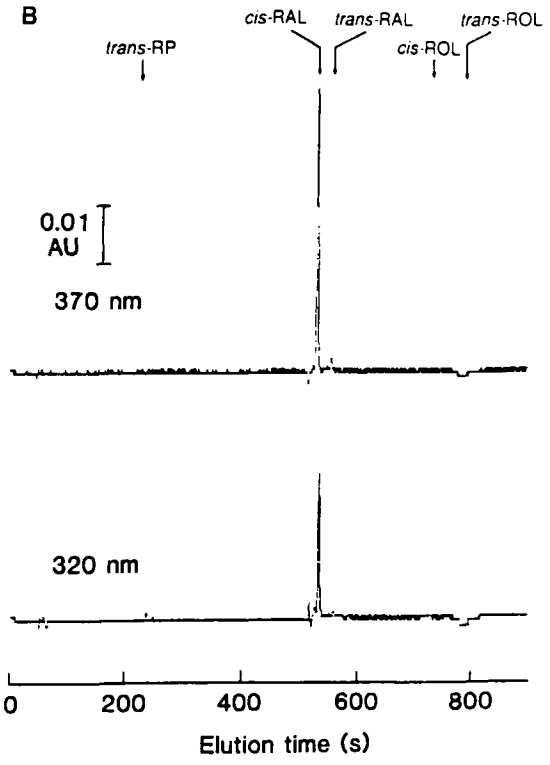


Fig. 5. Regeneration of rhodopsin in the IRBP-supplemented ROS/RPE-eyecup. **A** and **B:** Protocol for the experiment. Bleached ROS were supplemented with IRBP at a final concentration of 7.0 µ*M*, or with Ringer's solution only, and then incubated for 3 h in an RPE-eyecup or test tube. The RPE-eyecups were obtained from the same animal. **C:** Spectrophotometric results. Filled circles: IRBP, RPE-eyecup. Filled squares: Ringer's, RPE-eyecup. Open circles: IRBP, test tube. Open squares: Ringer's, test tube. Triangles: Relative difference spectrum of native rhodopsin in the parent ROS suspension; these data have been scaled downward by a factor of 1.6 for comparison with the other illustrated data. The horizontal arrow indicates the absorbance, at 500 nm, of the baseline difference spectrum obtained immediately after the preparative bleach. The inset shows the absorbance spectrum of IRBP in Ringer's solution. Panel **C** is reproduced from Okajima et al. (1990).


A
0.08
0.06
0.04
0.02
0.00
Absorbance
2
1
2
1
300
400
500
Wavelength (nm)
0.04
0.02
0.00
Absorbance
a
b
a
b
300
400
Wavelength (nm)


B
trans-RP
cis-RAL
trans-RAL
cis-ROL
trans-ROL
0.01
AU
370 nm
320 nm
0
200
400
600
800
Elution time (s)

extracted material was similar to that of authentic 11-*cis* retinal; the HPLC results indicated the presence in the extract of a single peak with retention time identical to that of 11-*cis* retinal. Thus, although the RPE contains several forms of both 11-*cis* and all-*trans* retinoids, only 11-*cis* retinal was detected in the incubation medium (Okajima et al., 1990). A selective release of 11-*cis* retinal is also exhibited by human RPE cells maintained in culture, following their incubation with all-*trans* retinol (Flannery et al., 1990).

Does the release of 11-*cis* retinal to the extracellular medium depend on the presence of IRBP? In other experiments (not illustrated), we examined this question by incubating RPE-eyecups with either IRBP-supplemented medium or Ringer's alone (3-h incubation), and then analyzing the aqueous media for 11-*cis* retinal. The results indicated a striking dependence of the release of 11-*cis* retinal on the presence of IRBP; little or no 11-*cis* retinal was released in preparations lacking IRBP (Table 1 of Okajima et al., 1990). The specific implications of this finding are discussed further below.

Protection of Retinoid in Aqueous Medium

Retinol, the form of retinoid that moves from bleached rods to the RPE, is sparingly soluble in aqueous medium (Hárosi and Szuts, 1991). Previous studies have shown that retinol initially contained in a lipid phase can move into the surrounding aqueous medium (Rando and Bangerter, 1982; Ho et al., 1989; *see also* Fex and Johannesson, 1987). Retinol is an allylic alcohol and as such, is expected to be unstable in an aqueous medium. Indeed, retinol in aqueous medium does undergo degradation (Futterman and Heller, 1972). One of the products of this degradation has been demonstrated to be retinal, most likely formed by auto-oxidation (Crouch et al., 1992). As shown in Fig. 7, rapid degradation can occur in phosphate buffer at 22°C. Bovine serum albumin (BSA), known to bind retinol (Futterman and Heller, 1972), protects the retinol to a small extent. On the other hand, IRBP provides good protection from degradation (Fig. 7). IRBP has also been shown to protect against the isomerization of retinol (Pepperberg et al., 1991; Crouch et al., 1992), although Timmers et al. (1991) report some (≤ 15%) isomerization of all-*trans* to 13-*cis* retinol in experiments involving incubation with IRBP. The results illustrated in Fig. 7 suggest that one role of IRBP in vivo may be to preserve the isomeric and oxidative integrity of retinoid in the aqueous environment (IPM) between the RPE and the photoreceptors. More generally, the findings indicate that IRBP is a useful agent for the protection of retinols in vitro, i.e., in experimental situations where there is concern regarding the formation of oxidation or degradation products of retinol.

Rhodopsin Regeneration in Detached Retina

The issue of whether the movement of retinoid across the IPM requires IRBP (or any transport protein) is not easily resolved. As mentioned ear-

Fig. 6. *(opposite page)* Release of retinoid from the RPE into IRBP-supplemented aqueous medium. **A:** Absorbance spectra of IRBP-supplemented medium following incubation in an RPE-eyecup for "zero time" (spectrum 1) and for 3 h (spectrum 2). Both the zero time control sample (delivered to the RPE-eyecup and then immediately removed), and the sample incubated for 3 h, consisted of 2.8 nmole of IRBP in 250 μL of Ringer's solution. The illustrated spectra were obtained following equal dilutions of the incubated solutions in Ringer's. *Inset:* Following incubation in the RPE-eyecup, an aliquot of aqueous sample 2 was supplemented with ethanol and hexane; the solid curve (spectrum a) shows the absorbance spectrum of material extracted into the hexane-dominated phase. The dashed curve (spectrum b) shows the absorbance spectrum of 11-*cis* retinal in hexane. **B:** Retinoid extracted from sample 2 (material contained in the hexane-dominated phase) was analyzed by HPLC. Arrows indicate the retention times of all-*trans* retinyl palmitate (*trans*-RP); of 11-*cis* and all-*trans* retinal (*cis*-RAL and *trans*-RAL, respectively); and of 11-*cis* and all-*trans* retinol (*cis*-ROL and *trans*-ROL, respectively). The vertical marker indicates the scale of absorbance (in absorbance units, AU) for both profiles. Reproduced from Okajima et al. (1990).

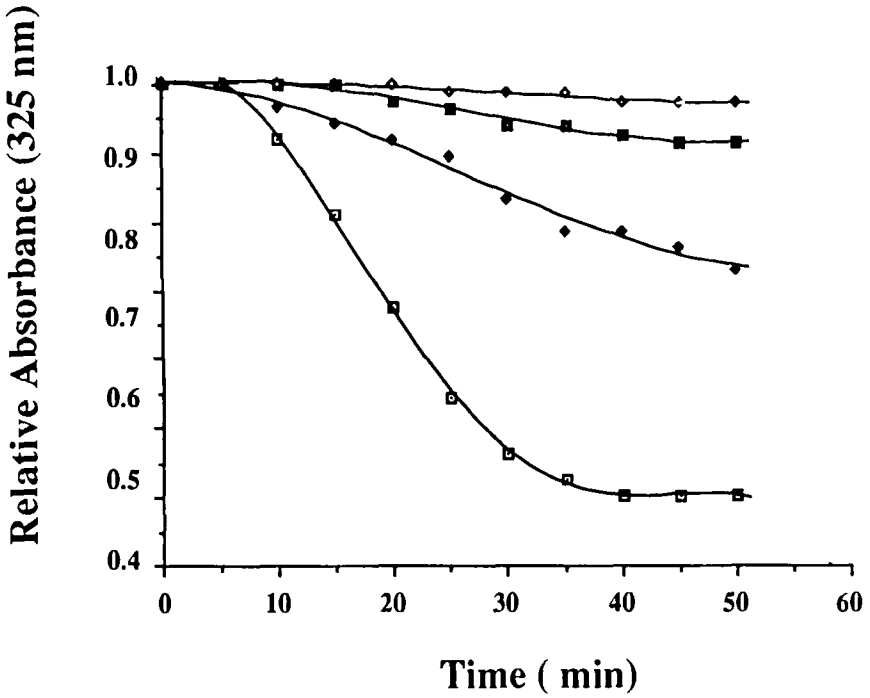


Fig. 7. Protection of retinol in aqueous medium by IRBP. At time zero, 10 µL of an ethanolic solution of 9-*cis* retinol was added to solutions (1 mL in volume) that consisted of 3 µ*M* IRBP, 3 µ*M* bovine serum albumin (BSA), and 90 µ*M* BSA (open diamonds, filled diamonds, and filled squares, respectively) in 100 m*M* potassium phosphate buffer (pH 7.4), or buffer alone (open squares). Each solution was gently vortexed for about 30 s, and then incubated in darkness (22°C). The concentration of 9-*cis* retinol in each sample at time zero was 3 µ*M*. The quantity of retinol remaining was analyzed spectrophotometrically at the times indicated. Reproduced with permission from Crouch et al. (1992).

lier, studies by Ho et al. (1989) have shown that a rapid, spontaneous transfer of (^{3}H)retinol from liposomes to ROS membranes occurs via the aqueous phase in the absence of IRBP, and that, in its presence, the transfer is about eight times slower. Indeed, the partial solubility of retinol, and its ability to cross an aqueous "barrier," have been well documented in other types of preparations (Rando and Bangerter, 1982; Fex and Johannesson, 1987; Creek et al., 1989), although it is important to note that corresponding information on isomers of retinal has yet to be obtained. The extent to which these observations relate to in vivo conditions is unclear, but as Ho et al. (1989) aptly note, the estimated distance (0.3 µm) separating the vesicular membranes in their experiments compares favorably with the distance (0.1 µm) separating the outer segment membranes from the ensheathing apical microvilli of the RPE (Hogan et al., 1971).

If close membrane apposition is a critical factor in allowing aqueous transfer of retinoids in the regenerative process, it is perhaps unlikely that such a transfer can be realized over macroscopic distances, e.g., as occurs often after retinal detachment (van Meel et al., 1984; Chuang et al., 1987). However, the question of whether rhodopsin regeneration takes place in the detached retina, and to what extent, can be readily tested experimentally. Studies were conducted on the skate eyecup using fundus reflectometry to measure rhodopsin kinetics in intact retinas and in retinas that had been detached and then replaced on the surface of the RPE (Sun and Ripps, 1992). Although the detachment procedure preserved (but probably diluted) the native IRBP, histologi-

cal examination of the tissue (Fig. 8A) showed that the normally intimate relation between the RPE and neural retina was severely disrupted; i.e., the apical projections that typically surround the rod outer segments were sheared to a fringe of short microvillar processes that no longer ensheathed the photoreceptors. Nevertheless, a comparison of the results obtained in normal and detached/replaced retina (Fig. 8B) showed that, after almost complete bleaching of the rhodopsin contained in a circumscribed test area, a substantial amount of photopigment was regenerated in the detached retina. Data from the intact preparation indicate that rhodopsin was fully regenerated after about 120 min in darkness, and that over most of its course, regeneration is well described by a linear function in which rhodopsin was reformed at a rate of about 0.875%/min (half-time of 55 min). In the detached/replaced retina, on the other hand, the bleached rhodopsin did not regenerate fully, and its resynthesis proceeded at a much slower rate (0.45%/min). However, a surprisingly large fraction (60%) of the bleached rhodopsin had regenerated after 140 min in darkness, and it appears unlikely that retinoid could have traversed the large subretinal space created by the detachment without the aid of IRBP.

Further evidence that rhodopsin regeneration occurs after detachment comes from electrophysiological data obtained in experiments that involved the acute, in vivo detachment of a localized portion of the rabbit retina (Mori et al., 1990). Electroretinographic recordings were obtained from the detached area ["local electroretinogram (LERG)"], as well as from the normal (attached) surrounding region ["vitreal electroretinogram (VERG)"], before and at various times after exposure to a bright adapting light that bleached > 60% of the native rhodopsin. The course of dark adaptation, i.e., the recovery of electroretinographic sensitivity, in the detached area was found to be almost identical to that exhibited by normal retina; thresholds for both the LERG and the VERG declined gradually to the fully dark-adapted level over a period of about 90 min. Previous studies have shown that, when fully isolated from the back of the eye, the retina remains permanently desensitized after a bleaching exposure, owing to interruption of the delivery of 11-*cis* retinoid to the photoreceptors (*see*, e.g., Pepperberg et al., 1978; Pepperberg and Masland, 1978). The spontaneous, complete recovery of LERG sensitivity observed in the locally detached retina is thus consistent with the notion that 11-*cis* retinal can move from the RPE to the photoreceptors through a greatly enlarged aqueous compartment and that it is assisted in this movement by IRBP.

Summary: Evolution and Physiological Function of IRBP

Evolutionary Considerations

Since most, if not all, vertebrate species studied to date have the IRBP gene, the protein in some form is at least 500 million years old. Lower on the evolutionary scale, relatively little is known about the presence of IRBP in invertebrates, although proteins with some characteristics of IRBP have been detected in cephalopods and in *Drosophila*. Fong et al. (1988) have reported that bovine and frog IRBP antibodies crossreact with a 132-kDa protein in six cephalopod species, although the protein does not bind added retinol. IRBP antibodies also recognize a 48-kDa squid (*T. pacificus*) retinal-binding protein (RALBP) that was originally described by Hara and coworkers (*see* Hara and Hara, 1991). RALBP specifically binds retinoid and has been postulated by Hara and Hara to be an intracellular shuttle of retinoid between visual pigment (rhodopsin) and a retinoid isomerizing system (retinochrome) in the squid photoreceptor cell. Based on antibody identification and close similarity in shuttle function, it is likely that the cephalopod and vertebrate proteins share a common ancestor that emerged before the divergence of vertebrates from invertebrates.

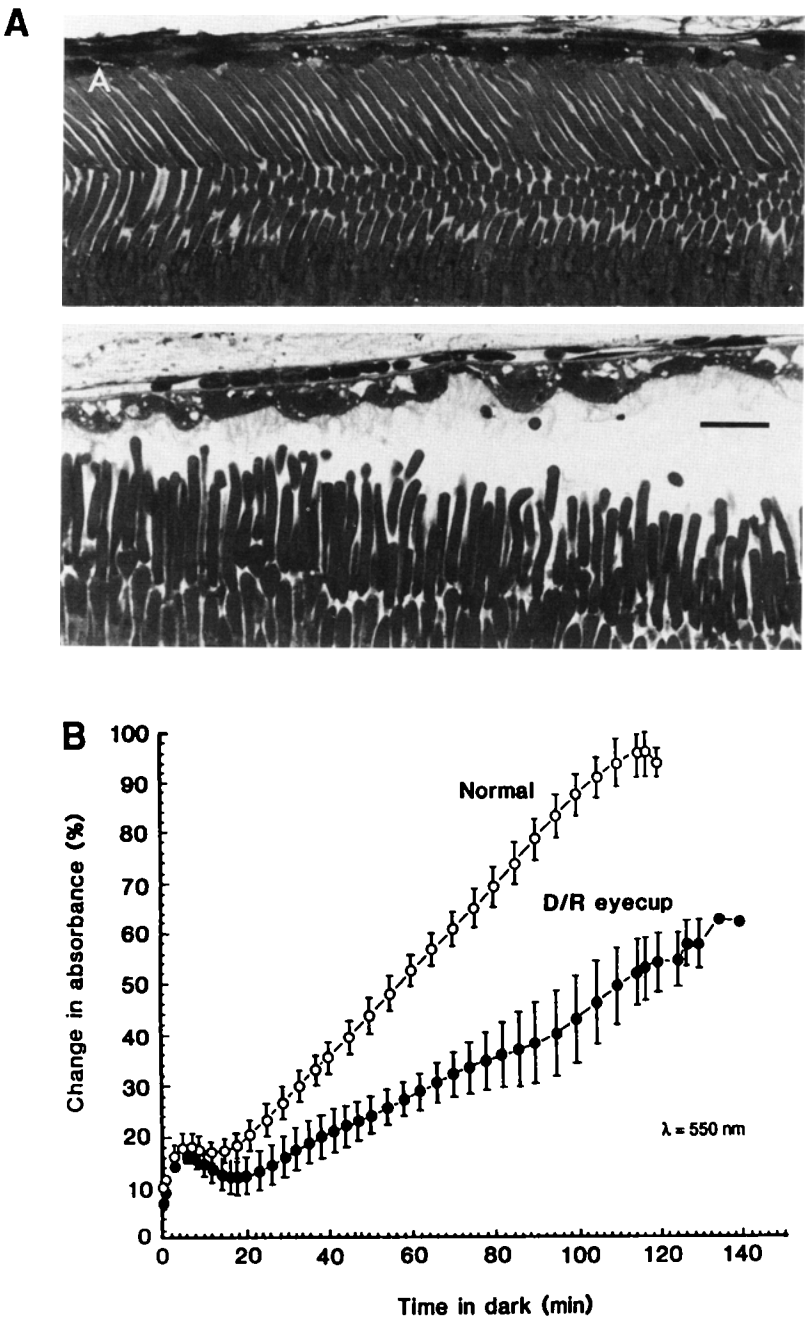


Fig. 8. Structural and functional changes associated with retinal detachment in the skate eye. **A:** Light micrographs of the RPE-neural retinal interface in normal (upper) and detached/replaced (lower) retinas. Note the intimate relation in the normal retina of the microvillar processes extending from the apical surface of the RPE with the membranes of the rod outer segments. After detachment, the apical projections appear shredded, and no longer ensheathe the photoreceptor outer segments. Bar = 20 μm. **B:** Rhodopsin kinetics in normal (open circles) and detached (filled circles) retinas; data are averages of five experimental runs with SEMs indicated by vertical bars. By comparison with normal, about 60% of the bleached rhodopsin regenerates in the detached retina, and the process occurs at about half the normal rate. Adapted from Sun and Ripps (1992) with permission of Academic Press.

In a similar vein, Wiggert and her collaborators (Kutty et al., 1991) have demonstrated the presence of an IRBP-like protein in *Drosophila* (*D. melanogaster*). This protein is a glycoprotein of about 140 kDa that is recognized by bovine IRBP antibody on Western blots, has the same retention time on HPLC as does bovine IRBP, and specifically binds (^{3}H)retinol. This retinol-binding activity is present in *Drosophila* heads, and absent both in fly bodies and in flies with the eyes absent (*eya*) mutation. The protein also readily binds (^{3}H)palmitic acid, as does vertebrate IRBP. The function of "fly IRBP" has yet to be determined; however, its presence in this animal, and the close analogy of cephalopod RALBP to vertebrate IRBP, suggest the evolutionary conservation of mechanisms for the transport of retinoids (and perhaps of fatty acids and other hydrophobic ligands as well), and the mediation of these processes by specific binding proteins.

Even though IRBP appears to be a relatively "new" protein, its repeat structure, exceptionally large first exon, and compact gene size indicate that the gene has gone through an active and relatively complex evolutionary process. Borst et al. (1989) have postulated the presence of an ancestral gene containing four exons that codes for a protein of about 300 amino acids. Insertion of a processed gene in front of the ancestral first exon, perhaps caused by viral reverse transcription, would generate a gene with two repeat units. Two unequal crossover events could then result in the gene structure as we know it. Genomic analyses of IRBP-like genes from *Drosophila*, cephalopods, and other invertebrates should give us more precise information as to the evolution of IRBP. Moreover, the analysis of IRBP mutants in *Drosophila* should lead to a much better understanding of the roles that IRBP plays in normal vision and in disease processes.

Implications for the Function of IRBP In Vivo

The results obtained from the RPE-eyecup preparation, together with those obtained in studies of the endogenous ligands of IRBP (Adler and Evans, 1985; Saari et al., 1985; Lin et al., 1989; Adler and Spencer, 1991a), strongly suggest that IRBP functions as a carrier of retinoid between the rods and the RPE in vivo, and thereby plays a direct role in the regeneration of rhodopsin. The data specifically indicate that IRBP facilitates both the delivery of all-*trans* retinol from the rods to the RPE, and the delivery of 11-*cis* retinal from the RPE to the rods (Fig. 9). This conclusion is consistent with the resynthesis of 11-*cis* retinal in the RPE (Lion et al., 1975; Zimmerman, 1976; Bernstein et al., 1987; Saari and Bredberg, 1988b; Das et al., 1990), and with the long-recognized dependence of regeneration on the juxtaposition of the retina and the RPE (Kühne, 1877). Complete dark adaptation of the rod photoreceptors after bright illumination, a process known to depend on the regeneration of visual pigment (*see* Introduction), is thus linked intimately with the function of IRBP.

This conclusion might appear to conflict with the data cited above indicating that IRBP retards the aqueous transfer of retinoid, and the conclusion that IRBP serves primarily (or exclusively) as a protective agent and scavenger of excess retinoid (Ho et al., 1989). Moreover, there is general agreement that the delivery of retinoids to cells of both the RPE and the retina can be achieved using agents other than IRBP (Yoshikami and Nöll, 1978; Perlman et al., 1982; Jones et al., 1989; Okajima et al., 1989; Timmers et al., 1991).

The seeming paradox arises, we think, from the very different experimental conditions used to analyze the activity of IRBP. In the system employed by Ho et al. (1989), for example, the retention of retinoid by the target membranes is presumably facilitated only by a nonspecific, hydrophobic interaction of the retinoid with these membranes. By comparison, the IRBP-supplemented ROS/RPE-eyecup, in which we have examined the movement specifically of 11-*cis* retinal, incorporates a physiological "trap" for retinal, i.e., opsin, that binds 11-*cis* retinal with high affinity. In this respect, the ROS/RPE-eyecup preparation appears to mimic an essential

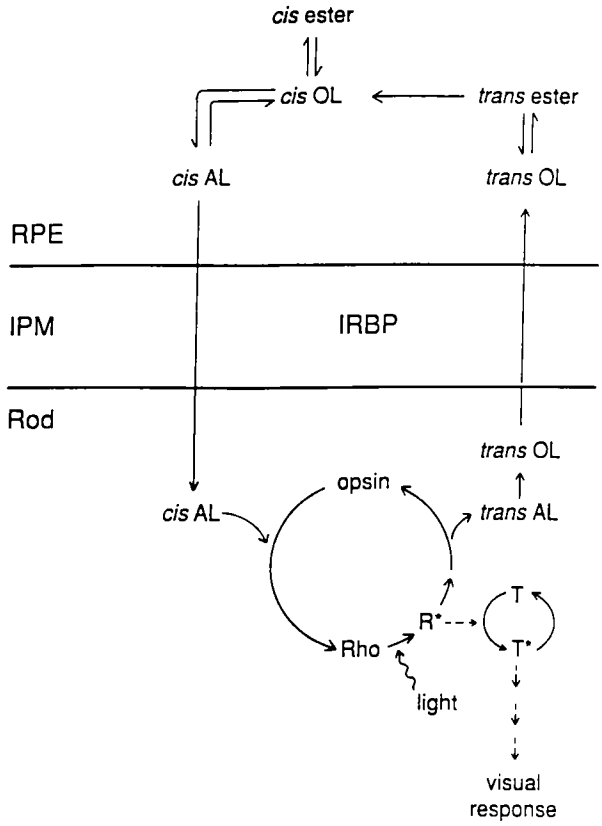


Fig. 9. Schematic figure summarizing the role of IRBP in the rhodopsin cycle of bleaching and regeneration. The absorption of light by rhodopsin (Rho) leads to the photoactivation (i.e., to the formation of R*) and ultimately, to the bleaching, of the visual pigment. The photoactivated form functions in the phototransduction process by catalyzing the activation of transducin (T → T* reaction) (for a review, *see* Chabre and Deterre, 1989). All-*trans* retinol (*trans* OL) is generated enzymatically within the rods from the all-*trans* retinal (*trans* AL) product of bleaching. Within the RPE, all-*trans* retinyl ester (*trans* ester), 11-*cis* retinol (*cis* OL), and 11-*cis* retinal (*cis* AL) are formed in sequential enzymatic reactions; 11-*cis* retinyl ester (*cis* ester) is thought to serve as a storage form of 11-*cis* retinoid. IRBP in the interphotoreceptor matrix (IPM) facilitates the transfer of all-*trans* retinol from the bleached rods to the RPE, and the transfer of 11-*cis* retinal from the RPE to the rods. The 11-*cis* retinal delivered to the rods combines with opsin to regenerate rhodopsin.

feature of the rhodopsin regeneration process in vivo, namely, a "directed flow" of 11-*cis* retinal (*see* Saari, 1990). That is, as a consequence of its very high affinity for 11-*cis* retinal, the free opsin produced in vivo by a bleaching irradiation favors a net flux of 11-*cis* retinal into the rods; in the ROS/RPE-eyecup, opsin in the bleached ROS similarly functions as a trapping agent for 11-*cis* retinal. In both the ROS/RPE-eyecup and the intact eye, the RPE (the source of 11-*cis* retinal), together with free opsin (a "sink" for 11-*cis* retinal), establish a driving force for the transfer of this retinoid. Similar considerations apply to the experiments involving the detached retinas, as well as those examining delivery of retinol to the RPE. In the latter case, ester synthesis from retinol within the RPE is expected to favor internalization of the added retinoid, although, as noted above (text accompanying Fig. 4), IRBP at relatively high concentration may compete with the target tissue for retinoid.

It thus appears likely that in these various systems IRBP facilitates movement through the aqueous medium by lowering a thermodynamic

barrier to a directed transfer, e.g., by enabling the passage of hydrophobic 11-*cis* retinal out of the RPE, and across an aqueous compartment to bleached photoreceptors (Okajima et al., 1990). On this view, only at high concentrations does the IRBP compete favorably for retinoid and thereby retard the transfer of retinoid through the aqueous phase. Although the existence of an active (i.e., energy-linked) transport of retinoid by IRBP is not ruled out, the available data can be fully explained by a passive, reversible binding of retinoid by the protein. The notion that IRBP in vivo supports the two-way movement of retinoid between the retina and RPE is in full accord with the abundance of IRBP in the IPM, and its affinity for hydrophobic ligands. That is, any explanation for the movement of these ligands through the IPM must take into account the binding activity of the IRBP present in high concentration in the extracellular space.

A number of additional questions relevant to the IRBP-dependent transfer of retinoids have yet to be resolved. For example, the data obtained from reconstituted preparations (e.g., the ROS/RPE-eyecup) do not establish how the rate of rhodopsin regeneration depends on the concentration of IRBP in vivo, where the outer segments of the rods are highly oriented and in close association with the RPE. Nor is it yet clear whether the uptake and release of retinoid by IRBP may involve specialized receptors in the plasma membrane of the RPE or the rods (*see* Politi et al., 1989). Of particular interest in this regard are the observations, in both the toad RPE-eyecup (Okajima et al., 1990) and in cultures of human RPE (Carlson and Bok, 1991), that the release of 11-*cis* retinal from the RPE to the extracellular medium depends critically on the presence of IRBP. By comparison with IRBP, bovine serum albumin exhibits notably little activity in this process. The available data furthermore indicate that the amount of 11-*cis* retinal released within a defined period of incubation increases with the level of IRBP in the apical medium (Okajima et al., 1990). However, it remains unclear whether an IRBP-mediated transfer of retinoid is rate-limiting for regeneration with the high concentrations of IRBP thought to exist in the living eye, and to what extent, in vivo, an IRBP-mediated release of 11-*cis* retinal from the RPE continues after the visual pigment in the rods is fully regenerated. Future studies of mutant animals that are deficient in IRBP may be helpful in addressing these questions (*see* van Veen et al., 1988).

A further unresolved issue concerns the nature of the visual cycle that supports the regeneration of visual pigment in *cone* photoreceptors. Cones, like rods, lack the capacity to form 11-*cis* retinal from all-*trans* bleaching product (Jones et al., 1989), and thus cannot "endogenously" regenerate a substantial quantity of visual pigment. However, previous data raise the possibility that the retinoid visual cycle of cones differs, in certain respects, from that of rods. For example, rhodopsin regeneration in bleached rods of the isolated retina ordinarily does not exceed the small extent attributable to free 11-*cis* retinoid initially present in the rods (Bridges, 1976b; Azuma et al., 1977; Cocozza and Ostroy, 1987). However, data obtained from the isolated retinas of frog (early receptor potential and aspartate-isolated photoreceptor response) and of turtle (responses of luminosity-type horizontal cells) indicate an ability of the retina to support a substantial degree of visual pigment regeneration in the cones (Goldstein, 1967, 1970; Hood and Hock, 1973; Normann and Perlman, 1990).

As yet, there is little information as to the immediate source or the mechanism of delivery of the 11-*cis* retinoid utilized by the cones for pigment regeneration. Indeed, the available data, including those just summarized, leave open the possibility that neither IRBP nor the RPE is immediately involved in this process. As previously noted, data reported by van Veen et al. (1986) and by Carter-Dawson and Burroughs (1989a,b) indicate that the extracellular milieu neighboring the cones contains less IRBP than does that surrounding the rods. However, the Müller cells of the retina are thought to contain 11-*cis* retinoid (Saari et al., 1982; Bunt-Milam and Saari, 1983; *see also* Bok et al., 1984; Bridges et al., 1984; Saari and

Bredberg, 1987), and it is known that cones can utilize 11-*cis* retinol for the regeneration of visual pigment (Jones et al., 1989). Furthermore, recent results obtained by Das et al. (1991) suggest that, for cone-dominated retinas, all-*trans* retinol is isomerized within the neural retina itself. Moreover, experiments on single, bleached photoreceptors indicate that in cones, but not in rods, 11-*cis* retinal introduced to the plasma membrane of the cell body can move to the outer segment and there induce the regeneration of visual pigment (Jin et al., 1991); in vivo, the cell bodies of the photoreceptors are situated close to the distal processes of the Müller cells. These findings are generally consistent with the possibility that visual pigment regeneration in the cones involves the direct, i.e., IRBP independent, transfer of all-*trans* retinol (from both rods and cones) to the Müller cells, and the direct, reciprocal movement, to the cone cell bodies, of 11-*cis* retinol or 11-*cis* retinal formed within the Müller cells. This exciting possibility is under active investigation.

Acknowledgments

Research in our laboratories is supported by grants EY-05494, EY-06516, EY-01792, and EY-04939 from the National Institutes of Health; by the Lions of Illinois Foundation, Maywood, Illinois; and by grants from Research to Prevent Blindness, Inc. H. Ripps is a Research to Prevent Blindness Senior Scientific Investigator.

References

Adler A. J. and Evans C. D. (1985) Proteins of the bovine interphotoreceptor matrix: retinoid binding and other functions, in *The Interphotoreceptor Matrix in Health and Disease* (Bridges C. D., and Adler A. J., eds.), Liss, New York, NY, pp. 65–88.

Adler A. J., Evans C. D., and Stafford III W. F. (1985) Molecular properties of bovine interphotoreceptor retinol-binding protein. *J. Biol. Chem.* **260,** 4850–4855.

Adler A. J. and Martin K. J. (1982) Retinol-binding proteins in bovine interphotoreceptor matrix. *Biochem. Biophys. Res. Commun.* **108,** 1601–1608.

Adler A. J. and Spencer S. A. (1991a) Effect of light on endogenous ligands carried by interphotoreceptor retinoid-binding protein. *Exper. Eye Res.* **53,** 337–346.

Adler A. J. and Spencer S. A. (1991b) Interaction of IRBP with retinoids. *Invest. Ophthalmol. Vis. Sci. (ARVO Abst.)* **32,** 1250.

Adler A. J., Stafford III W. F., and Slayter H. S. (1987) Size and shape of bovine interphotoreceptor retinoid-binding protein by electron microscopy and hydrodynamic analysis. *J. Biol. Chem.* **262,** 13,198–13,203.

Albini A., Toffenetti J., Zhu Z., Chader G. J., and Noonan D. M. (1990) Hypomethylation of the interphotoreceptor retinoid-binding protein (IRBP) promoter and first exon is linked to expression of the gene. *Nuc. Acids Res.* **18,** 5181–5187.

Alvarez R. A., Liou G. I., Fong S.-L., and Bridges C. D. B. (1987) Levels of α- and γ-tocopherol in human eyes: evaluation of the possible role of IRBP in intraocular α-tocopherol transport. *Amer. J. Clin. Nutr.* **46,** 481–487.

Azuma K., Azuma M., and Sickel W. (1977) Regeneration of rhodopsin in frog rod outer segments. *J. Physiol.* **271,** 747–759.

Barrett D. J., Redmond T. M., Wiggert B., Oprian D. D., Chader G. J., and Nickerson J. M. (1985) cDNA clones encoding bovine interphotoreceptor retinoid binding protein. *Biochem. Biophys. Res. Commun.* **131,** 1086–1093.

Bazan N. G., Reddy T. S., Redmond T. M., Wiggert B, and Chader G. J. (1985) Endogenous fatty acids are covalently and noncovalently bound to interphotoreceptor retinoid-binding protein in the monkey retina. *J. Biol. Chem.* **260,** 13677–13680.

Berman E. R., Horowitz J., Segal N., Fisher S., and Feeney-Burns L. (1980) Enzymatic esterification of vitamin A in the pigment epithelium of bovine retina. *Biochim. Biophys. Acta* **630,** 36–46.

Bernstein P. S., Law W. C., and Rando R. R. (1987) Isomerization of all-*trans*-retinoids to 11-*cis*-retinoids *in vitro*. *Proc. Natl. Acad. Sci. USA* **84,** 1849–1853.

Bok D. (1990) Processing and transport of retinoids by the retinal pigment epithelium. *Eye* **4,** 326–332.

Bok D., Ong D. E., and Chytil F. (1984) Immunocytochemical localization of cellular retinol binding protein in the rat retina. *Invest. Ophthalmol. Vis. Sci.* **25,** 877–883.

Bongiorno A., Tesoriere L., Livrea M. A., and Pandolfo L. (1991) Distribution of vitamin A compounds in bovine eyes after bleaching adaptation. *Vision Res.* **31,** 1099–1106.

Borst D. E. and Nickerson J. M. (1988) The isolation of a gene encoding interphotoreceptor retinoid-binding protein. *Exper. Eye Res.* **47,** 825–838.

Borst D. E., Redmond T. M., Elser J. E., Gonda M. A., Wiggert B., Chader G. J., and Nickerson J. M. (1989) Interphotoreceptor retinoid-binding protein. Gene characterization, protein repeat structure and its evolution. *J. Biol. Chem.* **264,** 1115–1123.

Bridges C. D. B. (1975) Storage, distribution and utilization of vitamins A in the eyes of adult amphibians and their tadpoles. *Vision Res.* **15,** 1311–1323.

Bridges C. D. B. (1976a) Vitamin A and the role of the pigment epithelium during bleaching and regeneration of rhodopsin in the frog eye. *Exper. Eye Res.* **22,** 435–455.

Bridges C. D. B. (1976b) 11-*cis* vitamin A in dark-adapted rod outer segments is a probable source of prosthetic groups for rhodopsin biosynthesis. *Nature* **259,** 247–248.

Bridges C. D. B., Alvarez R. A., Fong S.-L., Gonzalez-Fernandez F., Lam D. M. K., and Liou G. I. (1984) Visual cycle in the mammalian eye. Retinoid-binding proteins and the distribution of 11-*cis* retinoids. *Vision Res.* **24,** 1581–1594.

Bridges C. D. B., Price J., Landers R. A., Fong S.-L., Liou G. I., Hong B.-S., and Tsin A. T. C. (1986) Interstitial retinol-binding protein (IRBP) in subretinal fluid. *Invest. Ophthalmol. Vis. Sci.* **27,** 1027–1030.

Brin K. P., and Ripps H. (1977) Rhodopsin photoproducts and rod sensitivity in the skate retina. *J. Gen. Physiol.* **69,** 97–120.

Bunt-Milam A. H. and Saari J. C. (1983) Immunocytochemical localization of two retinoid-binding proteins in vertebrate retina. *J. Cell Biol.* **97,** 703–712.

Bunt-Milam A. H., Saari J. C., and Bredberg D. L. (1985) Characterization of the interstitial space: immunocytochemical and biochemical studies, in *The Interphotoreceptor Matrix in Health and Disease* (Bridges C. D, and Adler A. J., eds.), Liss, New York, pp. 151–170.

Carlson A. and Bok D. (1991) Probing the mechanism for the polarized release of 11-*cis* retinal (RAL) using cultured retinal pigment epithelial (RPE) cells. *Invest. Ophthalmol. Vis. Sci. (ARVO Abst.).* **32,** 1250.

Carter-Dawson L. and Burroughs M. (1989a) Differential distribution of interphotoreceptor retinoid-binding protein (IRBP) around retinal rod and cone photoreceptors. *Curr. Eye Res.* **8,** 1331–1334.

Carter-Dawson L. and Burroughs M. (1989b) Interphotoreceptor retinoid-binding protein (IRBP) in the postnatal developing *rds* mutant mouse retina: EM immunocytochemical localization. *Exper. Eye Res.* **49,** 829–841.

Chabre M. and Deterre P. (1989) Molecular mechanism of visual transduction. *Eur. J. Biochem.* **179,** 255–266.

Chader G. J. (1989) Interphotoreceptor retinoid-binding protein (IRBP): a model protein for molecular biological and clinically relevant studies. *Invest. Ophthalmol. Vis. Sci.* **30,** 7–22.

Chuang E. L., Sharp D. M., Fitzke F. W., Kemp C. M., Holden A. L., and Bird A. C. (1987) Retinal dysfunction in central serous retinopathy. *Eye* **1,** 120–125.

Cocozza J. D. and Ostroy S. E. (1987) Factors affecting the regeneration of rhodopsin in the isolated amphibian retina. *Vision Res.* **27,** 1085–1091.

Creek K. E., Silverman-Jones C. S., and De Luca L. M. (1989) Comparison of the uptake and metabolism of retinol delivered to primary mouse keratinocytes either free or bound to rat serum retinol-binding protein. *J. Invest. Dermatol.* **92,** 283–289.

Crouch R. K., Hazard E. S., Lind T., Wiggert B., Chader G., and Corson D. W. (1992) Interphotoreceptor retinoid-binding protein and α-tocopherol preserve the isomeric and oxidation state of retinol. *Photochem. Photobiol.*, **56,** 251-255.

Das S. R., Bhardwaj N., and Gouras P. (1990) Synthesis of retinoids by human retinal epithelium and transfer to rod outer segments. *Biochem. J.* **268,** 201–206.

Das S. R., Bhardwaj N., Gouras P., and Kjeldbye H. (1991) Esterification and isomerization of all-*trans* retinol in cone and rod predominant retinas. *Invest. Ophthalmol Vis. Sci. (ARVO Abst.)* **32,** 1249.

Das S. R. and Gouras P. (1988) Retinoid metabolism in cultured human retinal pigment epithelium. *Biochem. J.* **250,** 459–465.

Deigner P. S., Law W. C., Cañada F. J., and Rando R. R. (1989) Membranes as the energy source in the endergonic transformation of vitamin A to 11-*cis*-retinol. *Science* **244,** 968–971.

Dowling J. E. (1960) Chemistry of visual adaptation in the rat. *Nature* **188,** 114–118.

Dowling J. E. and Ripps H. (1970) Visual adaptation in the retina of the skate. *J. Gen. Physiol.* **56,** 491–520.

Fex G. and Johannesson G. (1987) Studies of the spontaneous transfer of retinol from the retinol:retinol-

binding protein complex to unilamellar liposomes. *Biochim. Biophys. Acta* **901,** 255–264.

Flannery J. G., O'Day W., Pfeffer B. A., Horwitz J., and Bok D. (1990) Uptake, processing and release of retinoids by cultured human retinal pigment epithelium. *Exper. Eye Res.* **51,** 717–728.

Fong S.-L. and Bridges C. D. B. (1988) Internal quadruplication in the structure of human interstitial retinol-binding protein deduced from its cloned cDNA. *J. Biol. Chem.* **263,** 15330–15334.

Fong S.-L., Fong W.-B., Morris T. A., Kedzie K. M., and Bridges C. D. B. (1990) Characterization and comparative structural features of the gene for human interstitial retinol-binding protein. *J. Biol. Chem.* **265,** 3648–3653.

Fong S.-L., Lee P. G., Ozaki K., Hara R., Hara T., and Bridges C. D. B. (1988) IRBP-like proteins in the eyes of six cephalopod species—Immunochemical relationship to vertebrate interstitial retinol-binding protein (IRBP) and cephalopod retinal-binding protein. *Vision Res.* **28,** 563–573.

Fong S.-L., Liou G. I., Landers R. A., Alvarez R. A., and Bridges C. D. (1984) Purification and characterization of a retinol-binding glycoprotein synthesized and secreted by bovine neural retina. *J. Biol. Chem.* **259,** 6534–6542.

Futterman S. (1963) Metabolism of the retina. III. The role of reduced triphosphopyridine nucleotide in the visual cycle. *J. Biol. Chem.* **238,** 1145–1150.

Futterman S. and Heller J. (1972) The enhancement of fluorescence and the decreased susceptibility to enzymatic oxidation of retinol complexed with bovine serum albumin, β-lactoglobulin, and the retinol-binding protein of human plasma. *J. Biol. Chem.* **247,** 5168–5172.

Goldstein E. B. (1967) Early receptor potential of the isolated frog *(Rana pipiens)* retina. *Vision Res.* **7,** 837–845.

Goldstein E. B. (1970) Cone pigment regeneration in the isolated frog retina. *Vision Res.* **10,** 1065–1068.

Gonzalez-Fernandez F. and Healy J. I. (1990) Early expression of the gene for interphotoreceptor retinol-binding protein during photoreceptor differentiation suggests a critical role for the interphotoreceptor matrix in retinal development. *J. Cell Biol.* **111,** 2775–2784.

Hara T. and Hara R. (1991) Retinal-binding protein: function in a chromophore exchange system in the squid visual cell. *Progr. Ret. Res.* **10,** 179–206.

Hárosi F. I. and Szuts E. Z. (1991) Solubility of retinoids in water. *Arch. Biochem. Biophys.* **287,** 297–304.

Hazard E. S., Crouch R. K., Chader G., and Wiggert B. (1991) IRBP ligands: evidence for possible competition between all-*trans* retinol and palmitic acid. *Invest. Ophthalmol. Vis. Sci. (ARVO Abst.)* **32,** 1250.

Ho M.-T. P., Massey J. B., Pownall H. J., Anderson R. E., and Hollyfield J. G. (1989) Mechanism of vitamin A movement between rod outer segments, interphotoreceptor retinoid-binding protein, and liposomes. *J. Biol. Chem.* **264,** 928–935.

Hogan M. J., Alvarado J. A., and Weddell J. E. (1971) *Histology of the Human Eye,* Saunders, Philadelphia, 687 pp.

Hollyfield J. G., Fliesler S. J., Rayborn M. E., Fong S.-L., Landers R. A., and Bridges C. D. (1985a) Synthesis and secretion of interstitial retinol-binding protein by the human retina. *Invest. Ophthalmol. Vis. Sci.* **26,** 58–67.

Hollyfield J. G., Varner H. H., Rayborn M. E., and Bridges C. D. (1985b) Participation of photoreceptor cells in retrieval and degradation of components in the interphotoreceptor matrix, in *The Interphotoreceptor Matrix in Health and Disease* (Bridges C. D., and Adler A. J., eds.), Liss, New York, pp. 171–175.

Hood D. C. and P. A. Hock (1973) Recovery of cone receptor activity in the frog's isolated retina. *Vision Res.* **13,** 1943–1951.

Hubbard R. and Dowling J. E. (1962) Formation and utilization of 11-*cis* vitamin A by the eye tissues during light and dark adaptation. *Nature* **193,** 341–343.

Inouye L. N., Albini A., Chader G. J., Redmond T. M., and Nickerson J. M. (1989) mRNA for interphotoreceptor retinoid-binding protein (IRBP): distribution and size diversity in vertebrate species. *Exper. Eye Res.* **49,** 171–180.

Ishiguro S., Suzuki Y., Tamai M., and Mizuno K. (1991) Purification of retinol dehydrogenase from bovine retinal rod outer segments. *J. Biol. Chem.* **266,** 15520–15524.

Jin J., Jones G. J., and Cornwall M. C. (1991) The movement of retinal along cone and rod photoreceptors. *Invest. Ophthalmol. Vis. Sci. (ARVO Abst.)* **32,** 670.

Jones G. J., Crouch R. K., Wiggert B., Cornwall M. C., and Chader G. J. (1989) Retinoid requirements for recovery of sensitivity after visual-pigment bleaching in isolated photoreceptors. *Proc. Natl. Acad. Sci. USA* **86,** 9606–9610.

Kawaguchi T., Hamanaka T., and Kito Y. (1986) Kinetic study of transfer of 11-*cis* retinal between rod outer segment membranes using regeneration of rhodopsin. *Biophys. Chem.* **24,** 5–12.

Krinsky N. I. (1958) The enzymatic esterification of vitamin A. *J. Biol. Chem.* **232,** 881–894.

Kühne W. (1877) Zur Photochemie der Netzhaut. *Unters. Physiol. Inst. Univ. Heidelberg* **1,** 1–14.

Kutty G., Chader G. J., and Wiggert B. (1991) A retinoid-binding glycoprotein similar to IRBP is present in *Drosophila melanogaster. Invest. Ophthalmol. Vis. Sci. (ARVO Abst.)* **32,** 1250.

Lin Z.-S., Fong S.-L., and Bridges C. D. B. (1989) Retinoids bound to interstitial retinol-binding protein during light and dark-adaptation. *Vision Res.* **29,** 1699–1709.

Lion F., Rotmans J. P., Daemen F. J. M., and Bonting S. L. (1975) Biochemical aspects of the visual process. XXVII. Stereospecificity of ocular retinol dehydrogenases and the visual cycle. *Biochim. Biophys. Acta* **384,** 283–292.

Liou G. I., Bridges C. D. B., Fong S.-L., Alvarez R. A., and Gonzalez-Fernandez F. (1982) Vitamin A transport between retina and pigment epithelium—an interstitial protein carrying endogenous retinol (interstitial retinol-binding protein). *Vision Res.* **22,** 1457–1467.

Liou G. I., Fong S.-L., Beattie W. G., Cook R. G., Leone J., Landers R. A., Alvarez R. A., Wang C., Li Y., and Bridges C. D. B. (1986) Bovine interstitial retinol-binding protein (IRBP)—isolation and sequence analysis of cDNA clones, characterization and *in vitro* translation of mRNA. *Vision Res.* **26,** 1645–1653.

Liou G. I., Fong S.-L., Gosden J., van Tuinen P., Ledbetter D. H., Christie S., Rout D., Bhattacharya S., Cook R. G., Li Y., Wang C., and Bridges C. D. B. (1987) Human interstitial retinol-binding protein (IRBP): cloning, partial sequence, and chromosomal localization. *Somat. Cell Mol. Genet.* **13,** 315–323.

Liou G. I., Geng L., Al-Ubaidi M. R., Matragoon S., Hanten G., Baehr W., and Overbeek P. A. (1990) Tissue-specific expression in transgenic mice directed by the 5'-flanking sequences of the human gene encoding interphotoreceptor retinoid-binding protein. *J. Biol. Chem.* **265,** 8373–8376.

Liou G. I., Ma D.-P., Yang Y.-W., Geng L., Zhu C., and Baehr W. (1989) Human interstitial retinoid-binding protein. Gene structure and primary sequence. *J. Biol. Chem.* **264,** 8200–8206.

Liou G. I., Matragoon S., Yang J., Geng L., Overbeek P. A., and Ma D.-P. (1991) Retina-specific expression from the IRBP promoter in transgenic mice is conferred by 212 bp of the 5'-flanking region. *Biochem. Biophys. Res. Commun.* **181,** 159–165.

Mori T., Pepperberg D. R., and Marmor M. F. (1990) Dark adaptation in locally detached retina. *Invest. Ophthalmol. Vis. Sci.* **31,** 1259–1263.

Nathans J. and Hogness D. S. (1984) Isolation and nucleotide sequence of the gene encoding human rhodopsin. *Proc. Natl. Acad. Sci. USA* **81,** 4851–4855.

Nickerson J. M., Borst D. E., Redmond T. M., Si J.-S., Toffenetti J., and Chader G. J. (1991) The molecular biology of IRBP: applications to problems of uveitis, protein chemistry, and evolution, in *The Molecular Biology of the Retina: Basic and Clinically Relevant Studies* (Farber D. B., and Chader G. J., eds.), Wiley-Liss, New York, NY, pp. 139–161.

Normann R. A. and Perlman I. (1990) Background and bleaching adaptation in luminosity type horizontal cells in the isolated turtle retina. *J. Physiol.* **421,** 321–341.

Noy N. and Blaner W. S. (1991) Interactions of retinol with binding proteins: studies with rat cellular retinol-binding protein and with rat retinol-binding protein. *Biochemistry* **30,** 6380–6386.

Okajima T.-I. L., Pepperberg D. R., Ripps H., Wiggert B., and Chader G. J. (1989) Interphotoreceptor retinoid-binding protein: role in delivery of retinol to the pigment epithelium. *Exper. Eye Res.* **49,** 629–644.

Okajima T.-I. L., Pepperberg D. R., Ripps H., Wiggert B., and Chader G. J. (1990) Interphotoreceptor retinoid-binding protein promotes rhodopsin regeneration in toad photoreceptors. *Proc. Natl. Acad. Sci. USA* **87,** 6907–6911.

Pepperberg D. R., Brown P. K., Lurie M., and Dowling J. E. (1978) Visual pigment and photoreceptor sensitivity in the isolated skate retina. *J. Gen. Physiol.* **71,** 369–396.

Pepperberg D. R. and Masland R. H. (1978) Retinal-induced sensitization of light-adapted rabbit photoreceptors. *Brain Res.* **151,** 194–200.

Pepperberg D. R., Okajima T.-I. L., Ripps H., Chader G. J., andWiggert B. (1991). Functional properties of interphotoreceptor retinoid-binding protein. *Photochem. Photobiol.* **54,** 1057–1060.

Perlman J. I., Nodes B. R., and Pepperberg D. R. (1982) Utilization of retinoids in the bullfrog retina. *J. Gen. Physiol.* **80,** 885–913.

Politi L. E., Lee L., Wiggert B., Chader G., and Adler R. (1989) Synthesis and secretion of interphotoreceptor retinoid-binding protein (IRBP) by isolated normal and *rd* mouse retinal photoreceptor neurons in culture. *J. Cell. Physiol.* **141,** 682–690.

Rando R. R. and Bangerter F. W. (1982) The rapid intermembraneous transfer of retinoids. *Biochem. Biophys. Res. Commun.* **104,** 430–436.

Rando R. R., Bernstein P. S., and Barry R. J. (1991) New insights into the visual cycle. *Progr. Retinal Res.* **10,** 161–178.

Raport C. J., Dere B., and Hurley J. B. (1989) Characterization of the mouse rod transducin α subunit gene. *J. Biol. Chem.* **264,** 7122–7128.

Redmond T. M., Humayun M., Chader G. J., and Nickerson J. M. (1991) Retinoid binding to IRBP: is half a loaf better than none at all? *Invest. Ophthalmol. Vis. Sci. (ARVO Abst.)* **32,** 1250.

Redmond T. M., Wiggert B., Robey F. A., Nguyen N. Y., Lewis M. S., Lee L., and Chader G. J. (1985) Isolation and characterization of monkey interphotoreceptor retinoid-binding protein, a unique extracellular matrix component of the retina. *Biochemistry* **24,** 787–793.

Rodrigues M. M., Hackett J., Gaskins R., Wiggert B., Lee L., Redmond M., and Chader G. J. (1986) Interphotoreceptor retinoid-binding protein in retinal rod cells and pineal gland. *Invest. Ophthalmol. Vis. Sci.* **27,** 844–850.

Rushton W. A. H. (1961) Rhodopsin measurement and dark-adaptation in a subject deficient in cone vision. *J. Physiol.* **156,** 193–205.

Saari J. C. (1990) Enzymes and proteins of the mammalian visual cycle. *Progr. Ret. Res.* **9,** 363–381.

Saari J. C. and Bredberg D. L. (1987) Photochemistry and stereoselectivity of cellular retinaldehyde-binding protein from bovine retina. *J. Biol. Chem.* **262,** 7618–7622.

Saari J. C. and Bredberg D. L. (1988a) CoA- and non-CoA-dependent retinol esterification in retinal pigment epithelium. *J. Biol. Chem.* **263,** 8084–8090.

Saari J. C. and Bredberg D. L. (1988b) Purification of cellular retinaldehyde-binding protein from bovine retina and retinal pigment epithelium. *Exper. Eye Res.* **46,** 569–578.

Saari J. C. and Bredberg D. L. (1989) Lecithin:retinol acyltransferase in retinal pigment epithelial microsomes. *J. Biol. Chem.* **264,** 8636–8640.

Saari J. C., Bredberg L., and Garwin G. G. (1982) Identification of the endogenous retinoids associated with three cellular retinoid-binding proteins from bovine retina and retinal pigment epithelium. *J. Biol. Chem.* **257,** 13,329–13,333.

Saari J. C., Teller D. C., Crabb J. W., and Bredberg L. (1985) Properties of an interphotoreceptor retinoid-binding protein from bovine retina. *J. Biol. Chem.* **260,** 195–201.

Sears R. C. and Kaplan M. W. (1989) Axial diffusion of retinol in isolated frog rod outer segments following substantial bleaches of visual pigment. *Vision Res.* **29,** 1485–1492.

Shi H. and Olson J. A. (1990) Site of conversion of endogenous all-*trans*-retinoids to 11-*cis*-retinoids in the bovine eye. *Biochim. Biophys. Acta* **1035,** 1–5.

Si J.-S., Borst D. E., Redmond T. M., and Nickerson J. M. (1989) Cloning of cDNAs encoding human interphotoreceptor retinoid-binding protein (IRBP) and comparison with bovine IRBP sequences. *Gene* **80,** 99–108.

Sun Y. and Ripps H. (1992) Rhodopsin regeneration in the normal and in the detached/replaced retina of the skate. *Exper. Eye Res.* **55,** 679-689.

Timmers A. M., van Groningen-Luyben D. A. H. M., and DeGrip W. J. (1991) Uptake and isomerization of all-*trans* retinol by bovine retinal pigment epithelial cells: further clues to the visual cycle. *Exper. Eye Res.* **52,** 129–138.

Uehara F., Matthes M. T., Yasumura D., and LaVail M. M. (1990) Light-evoked changes in the interphotoreceptor matrix. *Science* **248,** 1633–1636.

van Meel G. J., Smith V. C., Pokorny J., and van Norren D. (1984) Foveal densitometry in central serous choroidopathy. *Am. J. Ophthalmol.* **98,** 359–368.

van Veen T., Ekstrom P., Wiggert B., Lee L., Hirose Y., Sanyal S., and Chader G. J. (1988) A developmental study of interphotoreceptor retinoid-binding protein (IRBP) in single and double homozygous *rd* and *rds* mutant mouse retinae. *Exper. Eye Res.* **47,** 291–305.

van Veen T., Katial A., Shinohara T., Barrett D. J., Wiggert B., Chader G. J., and Nickerson J. M. (1986) Retinal photoreceptor neurons and pinealocytes accumulate mRNA for interphotoreceptor retinoid-binding protein (IRBP). *FEBS Lett.* **208,** 133–137.

Wald G. (1935) Carotenoids and the visual cycle. *J. Gen. Physiol.* **19,** 351–371.

Wiggert B., Derr J. E., Fitzpatrick M., and Chader G. J. (1979) Vitamin A receptors of the retina. Differential binding in light and dark. *Biochim. Biophys. Acta* **582,** 115–121.