Available online at www.sciencedirect.com
SCIENCE DIRECT®
Vision Research 43 (2003) 3021–3036
Vision Research
www.elsevier.com/locate/visres

Interphotoreceptor retinoid-binding protein—an old gene for new eyes

Federico Gonzalez-Fernandez *

Departments of Ophthalmology and Pathology, State University of New York at Buffalo and Medical Research Service, Veteran's Affairs Medical Center, 3495 Bailey Avenue, Buffalo, NY 14215, USA

Received 3 July 2003; received in revised form 11 August 2003

Abstract

Evolving 40 times independently, eyes are striking examples of convergent evolution in that 11-*cis* retinaldehyde is always used for photon capture, yet the mechanism for its regeneration may be dramatically different in between systems. In particular, insects, cephalopods and vertebrates show varying physical separation of the *cis* → *trans* photoisomerization and chromphore reisomerization. In the vertebrate retina, these two processes are actually distributed between *different* cells. This compartmentalization is made possible by the phylogenetic innovation of the two-layered optic cup of the vertebrate retina. This unprecedented design created the subretinal space as a novel anatomical compartment allowing photoreceptors access to the retinal pigment epithelium (RPE) and Müller cells, the two cell types which share the burden of 11-*cis* retinoid regeneration. To take advantage of this arrangement, early vertebrates appear to have recruited for retinoid binding, the ββα-spiral fold proven useful in enoyl-CoA isomerase/hydratases, and the carboxy-terminal proteases for stabilizing hydrophobic ligands. Quadruplication of this functional domain within a single polypeptide lead to the emergence of interphotoreceptor retinoid-binding protein (IRBP). IRBP is the main soluble component of the IPM, and is prevented from diffusing out of the subretinal space because its large size excludes it from the photoreceptor/Müller cell *zonulae adheretes*. Despite this physical entrapment, IRBP is rapidly turned over within the IPM through a process that coordinates secretion of the protein by the photoreceptors, and its removal from the matrix by RPE and photoreceptor endocytosis. The present review will summarize what is known about the structure and function of IRBP to anticipate future avenues of research.

Keywords: Visual cycle; Interphotoreceptor retinoid binding protein; Interphotoreceptor matrix; Retina; Molecular evolution

1. The invertebrate retinoid cycle is confined to the photoreceptor cell

Although the term "retinoid cycle" in this conference usually refers to the process of visual pigment regeneration in the vertebrate eye where it was first described, an analogous process is critical to the function of the invertebrate retina (Marmor & Martin, 1978). Insect eyes, are typically arranged in a faceted partial sphere consisting of as many as thousands of ommatidia, the basic unit of these compound eyes (Land, 1980) (Fig. 1A). Each ommatidia contains eight retinula cells with visual pigment contained in microvilli organized into rhabdomeres analogous to vertebrate outer segments.

In the rhabdomere of the insect retinula cells, as in the photoreceptors of all animals, vision begins with the photoisomerization of 11-*cis* retinaldehyde to all-*trans* retinaldehyde. However, in insects the all-*trans* isomer remains bound through its Schiff base to rhodopsin forming a thermally stable metarhodopsin. The G protein activation is simpler for invertebrate because unlike vertebrate rhodopsin, the active invertebrate photoproduct can be formed directly without Schiff base deprotonation (Hara, Hara, Tokunaga, & Yoshizawa, 1981; Nakagawa, Iwasa, Kikkawa, Tsuda, & Ebrey, 1999). From this metarhodopsin, the original rhodopsin is regenerated through the absorption of a second photon. Thus, insects have an elegant version of the visual cycle consisting of a rhodopsin/metarhodopsin photoequilibrium without the apparent need of accessory retinoid-binding proteins (Fig. 1A).

* Tel.: +1-716-862-6519; fax: +1-716-862-6526.
E-mail address: fg23@buffalo.edu (F. Gonzalez-Fernandez).

0042-6989/$ - see front matter © 2003 Published by Elsevier Ltd.
doi:10.1016/j.visres.2003.09.019

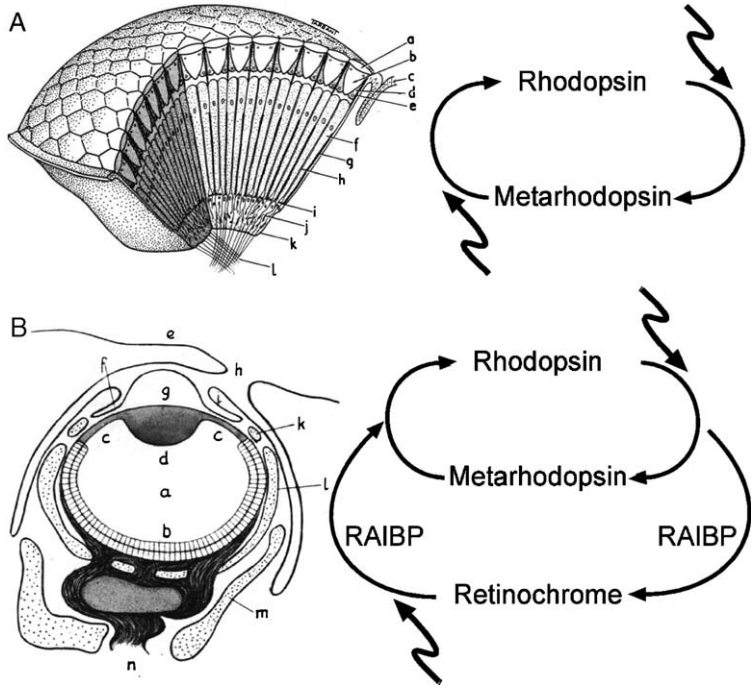

Fig. 1. The invertebrate visual cycle is an intracellular process. (A) Left, diagram of a compound eye of an insect with a sector exposed, within the photoreceptor cell (retinule; f in drawing), all-*trans* retinaldehyde photoisomerization from the 11-*cis* retinaldehyde remains bound to rhodopsin through its Schiff base forming a thermally stable metarhodopsin. The active photoproduct is formed directly without Schiff base deprotonation. Finally, the original rhodopsin is regenerated from metarhodopsin through the absorption of a second photon. Thus, the insect visual cycle consists of a rhodopsin/metarhodopsin photoequilibrium without the apparent need of accessory retinoid-binding proteins. (B) Left, diagram of the eye of a typical cephalopod. Although remeniscent of the vertebrate eye; the cephalopod retina (d) inverted with the photoreceptors position on the inner surface of the retina. Unlike the insect system, cephalopod metarhodopsin is ''Unstable'' resulting in hydrolysis of the Schiff base and release of all-*trans* retinaldehyde. The retinoid is picked up by retinaldehyde binding protein (RALBP) which translocates it to the photoreceptor inner segment myeloid bodies to complex with retinochrome. There photon capture returns the chromophore to the 11-*cis* configuration. The 11-*cis* retinaldehyde is returned to the rabdoms via RALBP regenerating the rhodopsin. Although the visual cycle retinoids are transferred via RALBP between rhodopsin and retinochrome, the process appears confined to the photoreceptor cell. The drawings are reprinted from Duke-Elder (1958) with permission from Elsevier.

Like insects, the dibranchiate cephalopods, which include squid, cuttlefish, and octopus, reform their 11-*cis* chromphore photochemically, but do so using a second visual pigment (Hara-Nishimura, Kondo, Nishimura, Hara, & Hara, 1993; Hara-Nishimura et al., 1990). These marine invertebrates, have a highly developed eye representing a classical example of convergent evolution. The cephalopod eye appears similar to vertebrate eyes in that it contains a single chamber with a single prominent lens (Fig. 1B). However, the cephalopod retina, which is composed mainly of only two types of cells, visual and supporting cells, has an inside-out arrangement compared to that of vertebrates (Messenger, 1981). In fact, their long photoreceptor outer segments (rhabdoms) are located in the inner retina close to the lens. In the rhabdoms, 11-*cis* retinaldehyde bound to rhodopsin is photoisomerized to the all-*trans* isomer forming metarhodopsin. However, unlike the insects system, cephalopod metarhodopsin is unstable resulting in hydrolysis of the Schiff base and release of all-*trans* retinaldehyde. The retinoid is picked up by retinaldehyde binding protein (RALBP) which translocates it to the photoreceptor inner segment myeloid bodies to complex with retinochrome (Molina et al., 1992; Ozaki, Terakita, Hara, & Hara, 1987; Terakita, Hara, & Hara, 1989). There photon capture returns the chromophore to the 11-*cis* configuration (Hara et al., 1981). The 11-*cis* retinaldehyde is returned to the rabdoms via RALBP regenerating the rhodopsin (Fig. 1B).

Thus, the insect and cephalopod systems both rely on photochemical mechanisms to reform 11-*cis* isomer. The difference is that cephalopods use a dual system of photosensitive chromoproteins with rhodopsin and retinochrome being stereospecific for 11-*cis* and all-*trans* retinaldehyde respectively (Hara-Nishimura et al., 1993). The recruitment of a retinoid-binding protein in the cephalopod visual cycle has been interpreted to provide a shuttle for visual cycle retinoids to traffick between the rhadoms and inner segment myoids (Hara & Hara, 1976; Terakita et al., 1989). Nevertheless, the cephalopod retinoid cycle is similar to that of insects as the entire cycle is contained within the same cell.

2. The vertebrate retinoid cycle introduces a new challenge—*retinoid trafficking between cells*

The embryology of the vertebrate eye is fundamentally different from that of invertebrates in that the eye arises from an out-pouching of the neuroectoderm, the optic vesicle. Involution of the vesicle results in a two-layered optic cup. The outer layer of the optic cups remains as a single layer of pigmented epithelium forming the outer epithelial layer of the iris and ciliary body, and the retinal pigment epithelium (RPE). The inner layer of the optic cup becomes the inner pigmented layer of the iris, the inner nonpigmented layer of the ciliary body, and the neural retina (Fig. 2A). At the level of the iris and ciliary body, the two epithelial layers are physically attached through junctional complexes. However the neural retina and RPE are separated only by the interphotoreceptor matrix (IPM), and therefore are susceptible to detachment, a not uncommon clinical problem.

The IPM provides a way that retinoids can be exchanged between the photoreceptors, RPE and Müller cells. Furthermore, unlike cephalopods where retinaldehyde is shuttled between rhodopsin and retinochrome, all-*trans* retinaldehyde released from vertebrate rhodopsin is first reduced to all-*trans* retinol by an outer segment retinol dehydrogenase. The all-*trans* retinol then leaves the outer segment, crosses the IPM, and then traverses the apical RPE cell membrane. Within the RPE, lecithin:retinol acyltransferase (LRAT) esterifies all-*trans* retinol to all-*trans* retinyl palmitate which becomes the substrate for a retinol isomerohydrolase (Rando, 1996). The product of this reaction is 11-*cis* retinol and palmitate. This generation of 11-*cis* chromophore appears to be supplemented by a light dependent pathway through RGR in the RPE (Chen, Hao, et al., 2001; Chen, Lee, & Fong, 2001; Maeda et al., 2003; Nakashima et al., 2003; Pepperberg & Crouch, 2001; Wolf, 2002). The 11-*cis* retinol is esterified for storage, or oxidized to 11-*cis* retinaldehyde by 11-*cis* retinol dehydrogenase. Finally, 11-*cis* retinaldehyde is released from the RPE, crosses the IPM, and enters the outer segments to associate with rhodopsin. The cycle is amazing given the lability and toxicity of the visual cycle retinoids, and the combination of extracellular, intracellular and transmembrane domains that must be traversed on each round-trip. Furthermore, the cycle accommodates large ranges in chromophore flux to operate in both dim and bright light conditions. Although the rate of movement of retinoids through aqueous compartments is rapid, the capacity for transfer is very low given the low solubilities of retinol and retinaldehyde.

To address these challenges, the vertebrate visual cycle possesses a remarkable collection of enzymes and retinoid-binding proteins. The enzymology of the visual cycle is an exciting area of research and reader is referred to excellent reviews on the subject and other papers in this symposium (McBee, Palczewski, Baehr, & Pepperberg, 2001; Saari, 1994, 2000). A collection of mainly unrelated proteins are employed by retina to escort the retinoids through this sophisticated enzymatic machinery on their remarkable journey through the photoreceptor/RPE complex. Compartmentalization is a common theme in the distribution of these proteins. Retinol-binding protein (RBP), which is produced in the liver and responsible for mobilizing liver stores of

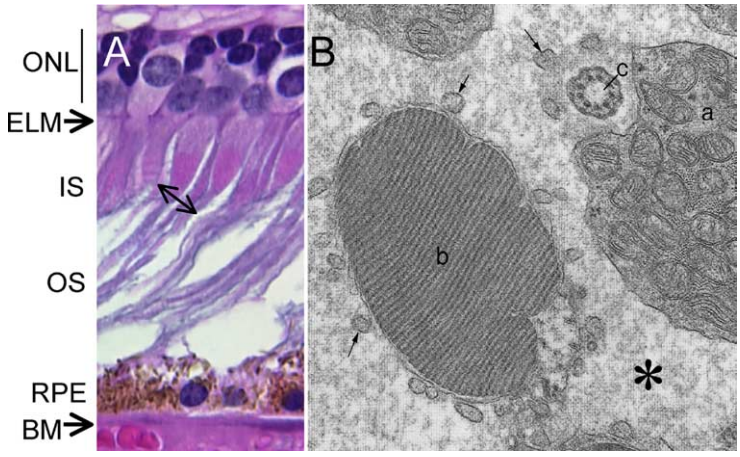

Fig. 2. The design of the vertebrate retina departs from that of nonvertebrates with the construction of a two layered system. This arrangement, derived embryologically from the involution of the optic vesicle, provides the rods and cones access to the RPE via the interphotoreceptor matrix (IPM). (A) In this haematoxylin and eosin stained paraffin section from the outer human retina, the IPM appears, due to a processing artifact, as empty spaces between the outer segments (OLN, outer nuclear layer; ELM, external limiting membrane; IS, inner segment; OS, outer segment; RPE, retinal pigment epithelium; BM, Bruch's membrane). This photomicrograph is an unpublished image from the author's laboratory. (B) Electron photomicrograph of two human photoreceptors in approximate cross-sectional orientation indicated by the slanted two sided arrow. The IPM (asterisk) appears as an amorphous extracellular material completely surrounding the outer (b) and inner (a) segments. This EM is from Hogan, Alvarado, and Weddell (1971) with permission from Elsevier.

vitamin A, is largely restricted to the serum and accesses the basal surface of the RPE to release all-*trans* retinol to the RPE. All-*trans* retinol also arrives at the apical surface of the RPE having been released from the outer segments and presented to the RPE by interphotoreceptor retinol-binding protein (IRBP). How retinol crosses either the basal or apical plasma membrane is not known, but once in the cytoplasm it is escorted to LRAT by cellular retinol-binding protein (CRBP). The 11-*cis* retinol released from the isomerohydrolase may either be oxidized to 11-*cis* retinaldehyde or esterified. How the reformed 11-*cis* retinaldehyde is released from the RPE is unknown.

3. The interphotoreceptor matrix—*a novel structure for the two layered vertebrate retina*

The double layered retina is an innovation bringing into physical proximity the photoreceptors, RPE and Müller cells (Bok, 1985, 1993; Mieziewska, 1996). Each of these cells borders the subretinal compartment which is filled with an interesting extracellular material termed the IPM (Hageman & Johnson, 1991). The IPM is a complex structure consisting of interphotoreceptor retinoid-binding protein (IRBP) (Fig. 3), growth factors (Hageman, Kirchoff-Rempe, Lewis, Fisher, & Anderson, 1991; Hewitt, Lindsey, Carbott, & Adler, 1990), metalloproteases (Plantner, Smine, & Quinn, 1998), hyaluronan and hyaluronan binding proteoglycans (Hollyfield, 1999), and sulfated glycoaminoglycans (Kuehn & Hageman, 1999). The IPM appears to mediate many of the critical interactions between the photoreceptors, RPE and Müller cells including retina/RPE adhesion, outer segment phagocytosis, outer segment structural stability, and nutrient exchange.

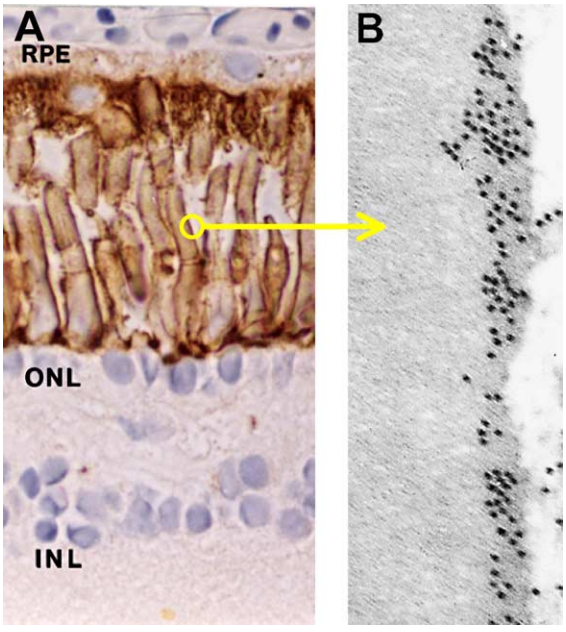

Fig. 3. Distribution of IRBP in the adult light adapted albino xenopus laevis retina. The primary antibody was a rabbit antixenopus IRBP serum. (A) Immunoperoxidase localization of IRBP to the light adapted adult albino xenopus retina. (B) Immuno-gold electron photomicrograph of the longitudinal edge of a rod outer segment. Labeling of IRBP is restricted to the extracellular material coating the surface of the outer segment. These images are adapted from Hessler, Baer, Bukelman, Kittredge, and Gonzalez-Fernandez (1996) and reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

It is important to appreciate that establishing that a protein is a component of the IPM may require a combination of biochemical and morphological approaches. For example, biochemical extracts of the IPM are usually contaminated by serum proteins, and proteins introduced by retinal cells damaged during the dissection. For example, the typical aqueous extract (retina soak) of the IPM shows a numerous proteins on Coomassie blue stained SDS-PAGE, with albumin and other proteins often dominating the profile. However, if the aqueous extract is obtained in a manner that does not disrupt the tissue significantly, a more accurate representation of the IPM protein profile may be obtained. Under such controlled conditions where the subretinal compartment is accessed by a microcannulation, IRBP is found to be the most prominent soluble component of the IPM as shown in Fig. 4. Therefore, if a protein is thought to be present in the IPM on the basis of typical biochemical extraction, immunohistochemical methods are often required to establish that the protein is truly localized to the IPM.

4. IRBP—an unusual retinoid-binding protein that is sequestered within the subretinal space

Compartmentalization is typically important for the function of retinoid-binding proteins. As discussed above, IRBP is apparently restricted to the subretinal space. How is this compartmentalization achieved? Although we cannot completely rule out that IRBP has an affinity for some component of the IPM, its sequestration within the IPM appears to be mainly related to its large overall molecular size and the particular anatomy of the outer retina. The subretinal space is bordered posteriorly by the RPE and anteriorly by the photoreceptors and Müller cells. Adjacent RPE cells are connected by *zonulae occludens* which prevent the passage of all proteins. However, the *zonulae adherentes* separating the photoreceptors and Müller cells allow the passage of proteins with a Stokes' radius under 30 Å (Bunt-Milam, Saari, Klock, & Garwin, 1985). Small proteins such as serum-retinol binding protein (RBP) would easily pass through the *zonulae* adherentes. In contrast IRBP (Stokes' radius = 55 Å) is larger than the *zonulae* adherentes exclusion limit.

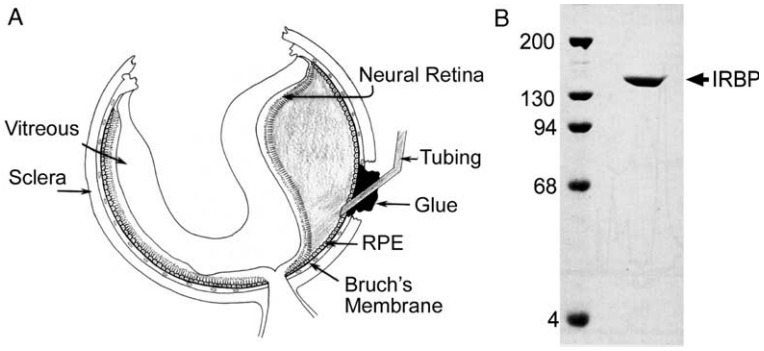

Fig. 4. Characterization of soluble interphotoreceptor matrix proteins by a gentle wash procedure. (A) A small canula is placed within the monkey subretinal space allowing the gradual introduction of saline into the space producing a retinal detachment. The fluid is then withdrawn and subjected to SDS-PAGE. The diagram is provided by Dr. Bruce Pefeffer and Dr. Barbara Wiggeret (the artist was Dr. John Roberts). (B) Coomassie blue stained gel showing molecular weight markers and the matrix extract. Note that IRBP is the major soluble component of the extract. Panel B is adapted from Pfeffer et al. (1983) and reprinted by permission of Wiley-Liss.

IRBP is unusually large for a retinoid-binding protein because it consists of four consecutive modules each ~300 amino acid residues in size. The gene appears to have arisen from the quadruplication of an ancient gene (Borst et al., 1989). Perhaps the innovation of the optic vesicle provided an evolutionary pressure toward selecting a protein that could be confined to the subretinal space. Indeed, the four-modular IRBP appears to have arisen early in the evolution of the vertebrate eye. Each module contains a structural domain recruited from enzyme systems having neither role in retinoid binding nor in vision (see below).

5. Function of IRBP

The function of IRBP is probably related to the structure and function of the IPM. As mentioned above, a number of functions have been attributed to this extracellular matrix including developmental interactions and nutrient exchange. In this context there is evidence that IRBP may have a role in both of these functions although most studies have focused on the latter in relation to the retinoid cycle.

Role in retinal development. The development of the vertebrate retina depends on retina-RPE interactions (Hollyfield & Witkovsky, 1974; Stiemke, Landers, al Ubaidi, Rayborn, & Hollyfield, 1994). IRBP accumulates in the subretinal space before the retinoid cycle is operational (Carter-Dawson et al., 1986; Carter-Dawson & Burroughs, 1989; Eisenfeld, Bunt-Milam, & Saari, 1985; Gonzalez-Fernandez & Healy, 1990; Hauswirth, Langerijt, Timmers, Adamus, & Ulshafer, 1992; Hessler et al., 1996). It is possible that IRBP may participate in retinal development by facilitating the transport of retinoids and/or other nutrients between the RPE and developing retina. The gene for IRBP is expressed early during rodent retinal development, and is upregulated before that of opsin (DesJardin, Timmers, & Hauswirth, 1993; Gonzalez-Fernandez & Healy, 1990; Gonzalez-Fernandez et al., 1993; Liou, Wang, & Matragoon, 1994). Targeted disruption of IRBP results in early photoreceptor degeneration in transgenic mice (Liou et al., 1998). In the *Xenopus* embryo, IRBP is first expressed by photoreceptors in the central retina, and a central-to-peripheral gradient of IRBP appears to be established by diffusion of IRBP through the subretinal space (Hessler et al., 1996). Such a gradient could allow IRBP to transport retinoid and fatty acids from the RPE to the developing peripheral retina.

Further surprises were noted in the developing zebrafish retina (Stenkamp, Cunningham, Raymond, & Gonzalez-Fernandez, 1998). Remarkably, IRBP mRNA is expressed not only by the photoreceptors but also by the adult and embryonic RPE. In embryos, expression in both retina and RPE begins in a ventronasal patch and spreads to involve the entire eye (Fig. 5). In general, early IRBP mRNA expression was dominated by photoreceptors, but then RPE expression spread beyond the limit of photoreceptor expression. Double in situ hybridizations suggest that cones express IRBP mRNA before they express a specific opsin, while rods may express rod opsin prior to IRBP. These patterns of IRBP expression by the RPE and retina are consistent with a role in retinal development, and suggest coordination of RPE and photoreceptor differentiation. Further studies are need to determine what is the specific role of IRBP in retinal development.

Role in the retinoid-cycle. Most studies into the function of IRBP have focused on the visual cycle trafficking of 11-*cis* retinal and all-*trans* retinol between the photoreceptors and the RPE. Here, IRBP has been thought to promote the exchange of these retinoids by several mechanisms (Crouch, Chader, Wiggert, & Pepperberg, 1996; McBee et al., 2001; Pepperberg, Okajima, Ripps, Chader, & Wiggert, 1991; Pepperberg et al.,

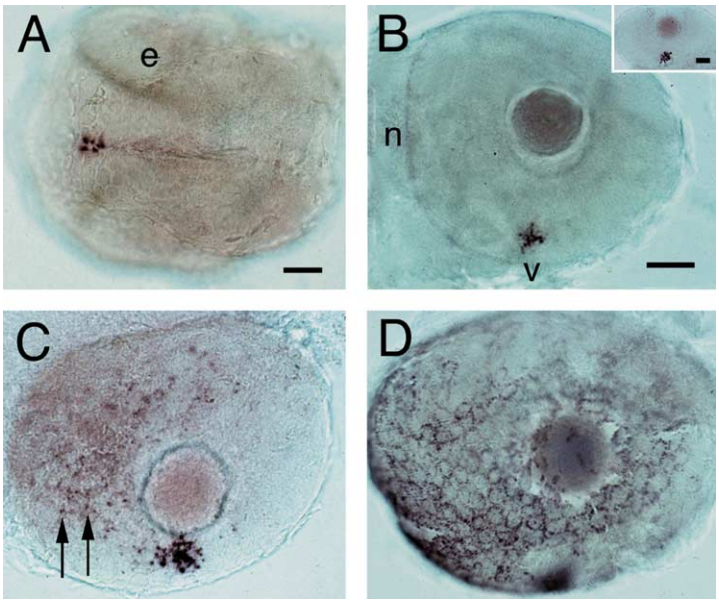

Fig. 5. IRBP mRNA expression in embryonic zebrafish whole mounts. (A) 31 hrs post-fertilization (hpf) zebrafish hybridized with IRBP cRNA. Five cells on dorsal midline surface of head are labeled; e, eye. (B) 53 hpf zebrafish eye, lateral view, hybridized with IRBP cRNA. Approximately 10 cells in ventral retina are labeled; v, ventral; n, nasal. Inset shows a 53 hpf zebrafish eye, same orientation, hybridized with rod opsin cRNA; approximately 25 cells are labeled. (C) 55 hpf zebrafish eye, same orientation as in B, hybridized with IRBP cRNA. Several additional photoreceptors outside of ventral patch (arrows) are labeled. (D) 60 hpf zebrafish eye, same orientation as B and C, hybridized with characteristic of RPE. Scale bars = 50 μm; bar in B applies to C and D; Reprinted from Stenkamp et al. (1998).

1993; Saari, 1994, 2000; Saari, Teller, Crabb, & Bredberg, 1985). First, since retinoids are insoluble compounds, IRBP is thought to solubilize retinoids within the IPM. Indeed, early studies emphasized the fact that IRBP contains endogenous visual cycle retinoids (Fig. 6). Second, IRBP is thought to target the delivery of all-*trans* retinol to the RPE (Okajima, Pepperberg, Ripps, Wiggert, & Chader, 1989), and promote the release of 11-*cis* retinal from the RPE (Carlson & Bok, 1992, 1999; Okajima, Pepperberg, Ripps, Wiggert, & Chader, 1990; Sun & Ripps, 1992). The molecular mechanism involved in these very interesting processes are unknown and likely to involve specific interactions with IRBP.

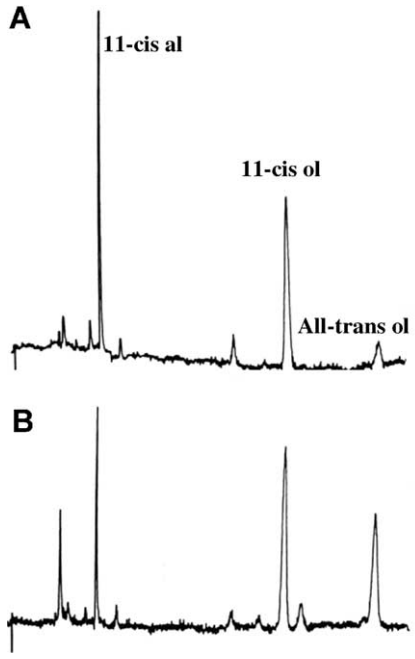

Fig. 6. High-performance liquid chromatography of retinoids extracted from IRBP. IRBP was purified from: (A) 24 h dark adapted, and (B) 0.5 h light-adapted Rana pipien frogs. Reprinted from Lin, Fong, and Bridges (1989) with permission from Elsevier.

6. New concepts regarding the potential role of IRBP in the visual cycle

In regards to the role of IRBP in the retinoid cycle, several new pieces to the puzzle have emerged in recent years. First, IRBP is not completely restricted to the IPM, but appears to be present within some of the cells that border the subretinal space particularly the IPM. Second, IRBP is rapidly turned over in the IPM. Third, regeneration of cone visual pigment depends on a retinoid cycle involving the Müller cells.

IRBP is not strictly an extracellular protein. Evidence is mounting that intracellular IRBP may have an important role in the function of IRBP. In *Xenopus*, the RPE contains 34 pmol, or ~20%, of the total IRBP in the eye (Cunningham & Gonzalez-Fernandez, 2003). This IRBP represents a fraction of IRBP that resists saline wash (Fig. 7). Within the RPE, immuno-electron microscopy localized IRBP to early RPE phagosomes

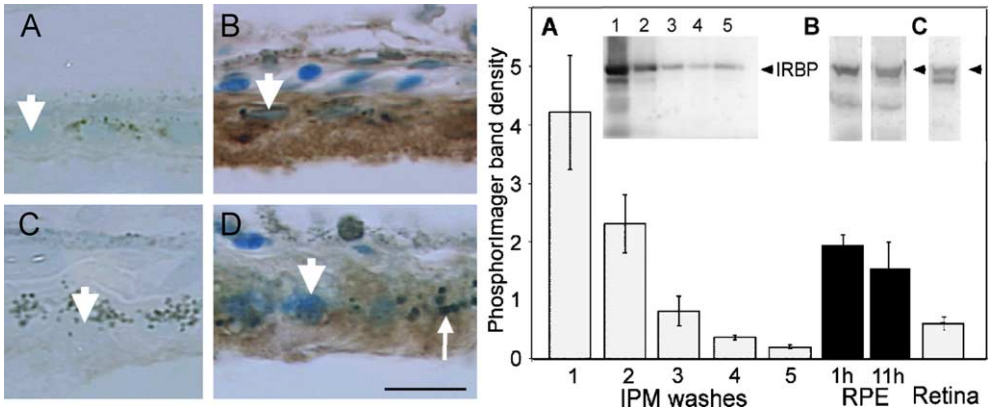

Fig. 7. Evidence for an intracellular pool of IRBP. (Left): Immunohistochemical localization of IRBP in the washed RPE eyecup (A–D). Transverse sections were incubated with a rabbit anti *Xenopus* IRBP serum diluted 1:1000 followed by a biotinylated goat anti-rabbit IgG before treatment with avidin:biotin peroxidase complex. The primary antibody was preadsorbed with recombinant *Xenopus* IRBP in the left panels (A, C). Panels A, B corresponds to mid-light; panels C, D correspond to mid-dark. All sections were counterstained with azure blue which metachromatically stains melanin granules blue-green (small arrows). Large arrows indicate RPE nuclei stained blue by azure blue. Calibration bar in H equals 25 μm in each panel. (Right): IRBP content of matrix extract, RPE and retina following multiple washes. Quantitative Western blot analysis was used to determine the relative amount of IRBP in each of 5 saline washes (A) and in the washed RPE eyecup (B) and retina (C). Washed RPE eyecups were collected just after photoreceptor outer segment disc shedding (1 h after light onset, 1 h) and just before light offset (11 h). The IPM, RPE and retina were found to contain ~75%, 18%, and 7% of the total IRBP in the eye, respectively. The bar graph shows the Phosphorimager band densities (mean ± SEM, $n = 5$ pairs of eyes) for each wash and the washed RPE eyecups and retinas. The data presented here is adapted from Cunningham and Gonzalez-Fernandez (2003) and reprinted by permission of Wiley-Liss.

and numerous endosomes. An example of the latter is shown in Fig. 8. These recent observations are not inconsistent with the older literature. An earlier immunoelectron microscopy study detected IRBP in RPE phagosomes and cytoplasmic vesicles and occasional myoid inner segment vacuoles in the mammalian and amphibian retinas (Schneider, Papermaster, Liou, Fong, & Bridges, 1986). An immunoperoxidase study of IRBP in the *Xenopus* retina noted RPE cytoplasmic staining, but could not determine whether the reaction product represents genuine cytosolic IRBP, nonspecific immunoreactivity, and/or melanin particles (Fig. 4 of Hessler et al., 1996; adapted here in Fig. 3A). Using adsorbed controls on intact retina and washed RPE eyecups counter stained with azure blue, which stains melanin granules metachromatically (Kamino & Tam, 1991), this cytoplasmic staining was found to be immunospecific (Fig. 7A) (Cunningham & Gonzalez-Fernandez, 2003). A previous electron microscopy study showed that the RPE and photoreceptors, but not Müller's cells, take up colloidal gold (CG) coated with IRBP (Hollyfield, Varner, Rayborn, Liou, & Bridges, 1985). Interestingly, the photoreceptors appeared to be more selective than the RPE in that they exclude ovalbumin-CG, while the

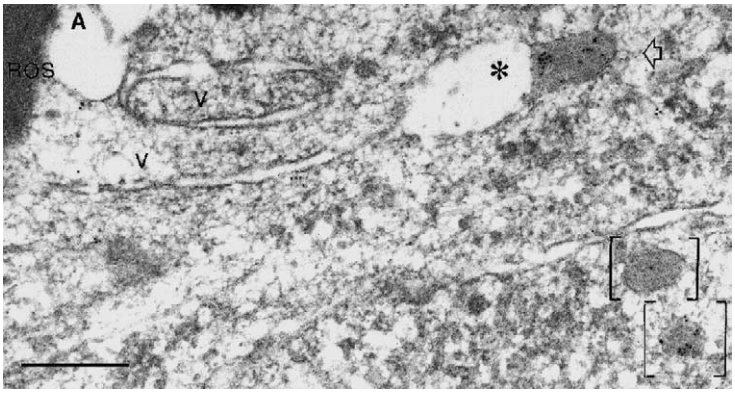

Fig. 8. Immuno-electron photomicrograph of an IRBP containing apical RPE endocytic pit and endosomes.Sections of undetached light-adapted albino *Xenopus* retinas were treated with anti-*Xenopus* IRBP serum followed by 10 nm gold protein A (panel A). The distal edge of a rod outer segment (ROS) abuts against two apical RPE villi each labeled "V". Between the two villi is a chalice shaped expansion (asterisk). The expansion ends in an endocytic pit containing immunogold labeled interphotoreceptor matrix (open arrow). Two immunogold label endosomes with no connection to the cell surface are marked by brackets. The calibration bar (lower left) equals 0.50 μm. The data presented here is adapted from Cunningham and Gonzalez-Fernandez (2003) with permission of Wiley-Liss.

RPE internalized both IRBP- and ovalbumin-CG. The investigators provided evidence that IRBP internalized by the photoreceptors is targeted for degradation (Hollyfield et al., 1985).

The endocytic uptake of IRBP may be related to its function(s). One possibility is that uptake is part of a mechanism to clear IRBP damaged during retinoid protection. IRBP is rapidly turned over in the *Xenopus* IPM (half-life, 10.7 h) (Cunningham, Yang, & Gonzalez-Fernandez, 1999). This is interesting in view of the fact that IRBP, as discussed above, cannot freely diffuse out of the IPM (Bunt-Milam et al., 1985). Although it is plausible that IRBP is enzymatically degraded within the IPM, proteolytic fragments are generally not observed on Western blots of matrix extracts. Furthermore, neither rat or human RPE-conditioned media has activity in degrading native bovine IRBP (Padgett, Lui, Werb, & LaVail, 1997). More likely, IRBP is removed from the IPM by a mechanism of uptake by the RPE and/or photoreceptors. Some IRBP may be removed during photoreceptor outer segment disc shedding (Cunningham & Gonzalez-Fernandez, 2003). Nevertheless, a recent study suggests that uptake during phagocytosis cannot account for the turnover of IRBP in the zebrafish retina (Cunningham & Gonzalez-Fernandez, 2000). Nonphagocytic RPE endocytosis could also contribute to the turnover of IRBP. IRBP was found in numerous endosomes near the apical RPE surface. Although RPE endosomes in *Xenopus* have not previously been described in detail, the structures described here are similar to those in the mammalian RPE (Akeo, Hiramitsu, Kanda, Yorifuji, & Okisaka, 1996; Heth & Bernstein, 1991; Hunt, Dewey, & Davis, 1989; Orzalesi, Fossarello, Carta, Del Fiacco, & Diaz, 1982; Perlman, Piltz, Korte, & Tsai, 1989). In zebrafish, where the expression of the IRBP mRNA is under circadian control (Fig. 9), the production and turnover of IRBP appears to be synchronized to achieve a constant amount of matrix IRBP throughout the light/dark cycle (Cunningham & Gonzalez-Fernandez, 2000).

Could the turnover of IRBP be linked to a function of the protein? We are currently considering two possibilities. The first, is that turnover may be part of a mechanism to clear damaged IRBP. As discussed below, IRBP may function to protect visual cycle retinoids from degradation. In fact, IRBP is particularly effective in protecting visual cycle retinoids from isomeric and oxidative degradation (Crouch et al., 1992). Interestingly, IRBP protects retinoids at the expense of its own integrity. Fedorovich, Semenova, Grant, Converse, and Ostrovsky (2000) showed that IRBP is damaged following irradiation by visible light in the presence of bound all-*trans* retinal. This damage involves photosensitization by the all-*trans* retinal, which is in turn protected from degradation. It is plausible that damaged IRBP would be continuously removed and replaced with new IRBP. It is therefore plausible that the rapid turnover of IRBP in the eye reflects an endocytic uptake mechanism to clear photodamaged IRBP from the IPM.

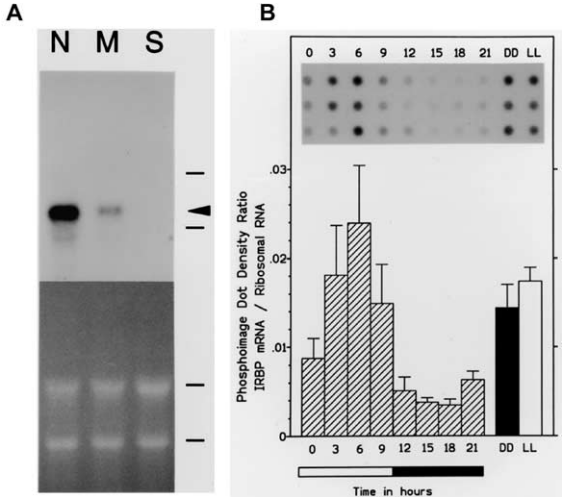

Fig. 9. Circadian expression of IRBP mRNA in the zebrafish retina. (A) Northern analysis of IRBP at noon (N) and midnight (M) under cyclic light. Top: autoradiogram of transfer, probed with ^{32}P-labeled zebrafish IRBP cDNA. On the basis of phosphoimage analysis, the radioactivity of the IRBP band (arrowhead) at noon is four times greater than at midnight. Bottom: ethidium bromide stained agarose gel showing the ribosomal subunits (dashes). 7 μg of total RNA was loaded in each lane. Lane S corresponds to skeletal muscle. (B) IRBP mRNA expression under cyclic and constant conditions. Zebrafish were entrained under a 12 h:12 h L:D cycle and were killed at 3 h intervals beginning at light onset. Other zebrafish, maintained for 8 days in constant dark (DD) or constant light (LL), were killed at subjective noon. For each time point and constant condition, six pairs of eyes were separately extracted and dotted. The ratio of [^{32}P]IRBP cDNA hybridization to that of each dot reprobed with a [^{32}P]18S ribosomal RNA cDNA is compared in each experimental condition. The inset is a representative autoradiogram of dots probed with the IRBP cDNA. Bar under graph: open, light; filled, dark. Error bars are standard errors of the mean, $N = 6$. Adapted from Rajendran et al. (1996).

A second possibility is that IRBP endocytosis is linked to retinoid delivery or release from the RPE. Many known extracellular nutrient transport proteins deliver their cargo to target cells via endocytic mechanisms that are often receptor-mediated. In any event, ongoing research should not only view IRBP as an extracellular protein, but should also consider its potential interaction and uptake by the cells that border the IPM.

IRBP a candidate for participation in the cone/Müller cell retinoid cycle. It has long been known that IRBP carries endogenous 11-*cis* retinol, however little attention has been given to that finding compared to the presence of bound 11-*cis* retinaldehyde and all-*trans* retinol. Nevertheless, the amount of bound 11-*cis* retinol is significant as shown in Fig. 6. The appreciation that vertebrate cones employ a private visual cycle separate from that used by rods may explain the presence of 11-*cis* retinol bound to IRBP.

An early suggestion that Müller cells have a role in the cone visual cycle was the finding that CRALBP (cellular retinaldehyde-binding protein) and CRBP are located in the Müller cell in addition to the RPE (Bok, 1993; Bunt-Milam & Saari, 1983; Saari, Bunt-Milam, Bredberg, & Garwin, 1984). Müller cell CRBP like that of the RPE carries endogenous all-*trans* retinol. Interestingly, Müller cell CRALBP carries both 11-*cis* retinaldehyde and 11-*cis* retinol at a 3:1 ratio (Saari, Bredberg, & Garwin, 1982). In contrast, RPE CRALBP carries only 11-*cis* retinaldehyde (Saari et al., 1982). Cones appear to utilize an isomerase that converts all-*trans* retinol directly to 11-*cis* retinol employing fatty acyl-coenzyme A as an energy source (Mata, Radu, Clemmons, & Travis, 2002). The location of the isomerase used in the cone cycle has not been established, however, isolated Müller cells can take up exogenous all-*trans* retinol and covert it to all-*trans* and 11-*cis* retinyl palmitate, and release 11-*cis* retinol into the culture medium (Das, Bhardwaj, Kjeldbye, & Gouras, 1992). The 11-*cis* retinyl esterase appears to be distinct from RPE LRAT (Mata et al., 2002).

Thirty-five years ago it had been observed that cones and not rods can regenerate visual pigment in detached retinas isolated from the RPE (Goldstein, 1967, 1970; Goldstein & Wolf, 1973). Nevertheless, cones are not self-sufficient in this process since they cannot regenerate when dissociated from other retinal cells (Liebman & Entine, 1964). A further difference between the receptor types, is the fact that bleached cones but not rods can regenerate visual pigment from exogenously applied 11-*cis* retinol (Jin, Jones, & Cornwall, 1994; Jones, Crouch, Wiggert, Cornwall, & Chader, 1989). This property was recently explained by the identification of a cone specific 11-*cis* retinol dehydrogenase and an all-*trans* retinol isomerase, and 11-*cis* retinyl-ester synthase thought to reside in the Müller cell (Mata et al., 2002). Further support for the existence of an alternative retinoid cycle in cones is the finding that RPE65, an enzyme essential for the formation of 11-*cis* retinol by the RPE (Redmond et al., 1998), is also found in cones but not in rods (Znoiko, Crouch, Moiseyev, & Ma, 2002). Given these observations, is plausible that IRBP plays a role in the cone retinoid cycle by transporting and protecting all-*trans* retinol and 11-*cis* retinol as they are exchanged between the cone and Müller cells.

7. Beyond carrier proteins

More than simple carriers serving to solublize retinoids, an emerging concept is that the central role of retinoid-binding proteins in the visual cycle is to achieve tight control of retinoid concentration, and to ensure their stability while protecting the retinal cells from the toxicity of free retinoids (Stenkamp & Adler, 1994).

Role of RALBP in the cephalopod retinoid cycle may be more than simply solubilizing retinoids. As discussed above, the cephalopod and vertebrate visual cycles have departed from that of insects in separating the processes of the *cis*/*trans* and *trans*/*cis* isomerizations. In both systems a soluble retinoid-binding protein is thought to be critical to transporting the visual cycle retinoids between the site of photoisomerization, and the site of chromophore regeneration. RALBP and IRBP have been thought to mediate these processes in the cephalopod and vertebrate retina respectively. In fact, early studies, which found an apparent cross reactivity of antibodies to IRBP with RALBP, suggested that the two proteins might be homologous (Fong et al., 1988). However, it is now clear that RALBP is a unique hydrophobic ligand-binding protein that is not similar to any known retinoid-binding protein including those involved in the vertebrate visual cycle (Ozaki et al., 1994).

In cephalopods, RALBP is thought to transport 11-*cis* and all-*trans* retinaldehydes between rhodopsin in the rabdomeres and retinochrome in the receptor inner segments. This may be an oversimplification of a more complicated process involving interesting light dependent changes in the distribution of the key proteins. In a detailed scanning confocal microscopy study, Robles et al. (1995) showed that in addition to rhodopsin, retinochrome is present in the rhabdomal compartment. In the dark, opsin and retinochrome co-localize at the base of the rhabdomal microvilli. In the light, opsin redistributes itself along the length of the microvillar membranes, and retinochrome appears to become extracellular. The studies suggest that the metapigments can be close to one another, making possible the direct exchange of chromophore between the pigments. This raises the question as to what is the exact function of RALBP. Perhaps RALBP functions to transport additional chromophore from the inner segments, or to buffer retinoids unbound to either rhodopsin or retinochrome.

Transgenic and knock-out mouse models. Elegant biochemical and physiological studies have shown that the visual cycle is not interrupted in IRBP −/− mice (Palczewski et al., 1999). These interesting observations suggest that, if IRBP has a role in the retinoid cycle, we need to rethink what that role might be. Indeed, such negative results in other systems has helped to provide new important insights into physiology of the studied system. In fact, deletion of a single protein considered important to a physiological process, often reveals little phenotypic change (Garry, Meeson, Yan, & Williams, 2000; Gottesman, Quadro, & Blaner, 2001; Grange et al., 2001). This is illustrated by transgenic animals lacking a variety of proteins including transcription factors (Rudnicki, Braun, Hinuma, & Jaenisch, 1992; Weintraub, 1993) and ligand-binding proteins, for example myoglobin.

It is interesting that mice carrying a null mutation in cellular retinoic acid-binding protein appear indistinguishable from wild-type mice (Gorry et al., 1994; Lampron et al., 1995). Surprisingly, neither humans carrying mutations in RBP (Biesalski et al., 1999; Seeliger et al., 1999), the major carrier of all-*trans* retinol in the serum, nor transgenic mice lacking this protein altogether, have any disease, or severe visual deficits (Quadro et al., 1999). One explanation is that alternative pathway(s) are employed to carry the ligand. Although less efficient, the alternative mechanism may work under most conditions, particularly when the system is not stressed or particularly challenged.

Particularly instructive are the studies of Quadro et al. (1999) who noted that although RBP −/− animals are viable, fertile and have normal vision as adults, they cannot mobilize hepatic retinol stores (mobilization of retinol stored in the liver requires hepatocytic secretion of all-*trans* retinol bound to RBP). As a result, the vitamin A status is extremely tenuous and dependent on a regular vitamin A intake. Interestingly, although retinal function is impaired in young RBP −/− mice, the animals acquire normal vision by 5 months of age although blood retinol levels remain low. These observations may be explained by the existence of alternative pathway(s) which carry retinol to target tissues. Evidently, the alternative route(s) are less efficient as ocular retinol stores (retinyl ester) remain low in adult RPB −/− animals. These observations suggest that RBP is not simply a carrier to solubilize retinol in the blood, but is part of mechanism to ensure adequate availability of retinol in times of inadequate vitamin A intake (Quadro et al., 1999).

In the IRBP −/− mouse it is possible that another protein present in the IPM takes over the function of binding retinoids. Ripps (2001) suggested that albumin, could serve as such a surrogate. In fact, albumin is often detected by protein electrophoresis in the aqueous extracts of IPM of postmortem human retinas (Adler & Edwards, 2000). Furthermore, albumin mRNA can be detected in the mouse neural retina by reverse transcriptase PCR (Dodson et al., 2001). Recent studies from the author's laboratory have sought to define the location of albumin in the mouse eye. These studies, which employed immunofluorecence and immunohistochemical approaches, failed to identify albumin in the IPM in either wildtype or IRBP −/− mice (Liao & Gonzalez-Fernandez, in revision). Thus, albumin does not accumulate in the subretinal space as would be expected if it were to be IRBP's surrogate in IRBP −/− mice.

Finally, the appreciation that cones have a separate retinoid-cycle may have a bearing on the interpretation of the findings using the IRBP −/− mice. Mata et al. (2002) proposed a new function for IRBP, namely to mediate the exchange of retinoids between the cones and Müller cells. This would explain why IRBP typically contains significant quantities of bound endogenous 11-*cis* retinol (Fig. 6). In this role, IRBP would be expected to carry all-*trans* retinol and 11-*cis* retinol (Mata et al., 2002). Mata et al. (2002) pointed out that the physical distances between the cone outer segments and Müller cell villi are greater than that between the rod outer segment tips and the RPE. Biochemical studies are needed in the IRBP −/− mice that evaluate the cone retinoid-cycle.

8. Other functions for IRBP

IRBP may have a guardian role in the retinoid cycle rather than a strictly transport role. In fact, the transfer of all-trans-retinol between liposomes, and rod outer segment membranes can be accomplished rapidly via the aqueous phase. Furthermore, IRBP and not albumin retards rather than facilitates the transfer (Ho, Massey, Pownall, Anderson, & Hollyfield, 1989). Finally, not only is the visual cycle intact in IRBP −/− mice but dark adaptation recovery rates are even more rapid than those of IRBP +/+ mice (Ripps et al., 2000). This observation is consistent with the slowed *in vitro* intervesicle transfer of retinol (Ho et al., 1989). These findings suggest that IRBP has a buffering function.

In this connection, Crouch et al. (1992) made the interesting observation that IRBP has significant activity in preserving the isomeric and oxidation state of retinol. The mechanism of this activity is not known. However, it does appear that IRBP protects retinoids at the expense of its own integrity. Fedorovich et al. (2000) showed that IRBP is damaged following irradiation by visible light in the presence of bound all-*trans* retinal. This damage involves photosensitization by the all-*trans* retinal, which is in turn protected from degradation.

9. The structure of IRBP reveals the recruitment of an ancient hydrophobic ligand-binding domain for the vertebrate retina

Generating crystals of full length native bovine IRBP has been problematic. Post-translational modifications of the native protein may have interfered with crystal growth. IRBP is known to be glycosylated (Taniguchi, Adler, Mizuochi, Kochibe, & Kobata, 1986) and contains covalently and noncovalently bound fatty acids (Bazan, Reddy, Redmond, Wiggert, & Chader, 1985). Furthermore, the protein may exist in multiple conformational states as ligand is known to have a significant effect on its overall structure (Adler, Stafford, & Slayter, 1987). These considerations probably hindered early attempts to produce useable crystals of native IRBP.

To circumvent these problems, initial crystallographic work has focused on a single module of IRBP

(Loew, Baer, & Gonzalez-Fernandez, 2001). This was a reasonable place to start since, as discussed above, the modules of IRBP, which each consist of ~300 amino acid residues, appear to represent the basic fundamental unit of the protein. For example, each of the modules binds 1 to 2 mole equivalents of all-*trans* retinol or 11-*cis* retinaldehyde (Baer, Retief, Van Niel, Braiman, & Gonzalez-Fernandez, 1998; Nickerson et al., 1998; Gonzalez-Fernandez et al., 1998). These biochemical, and phylogenetic data suggest that the protein modules represent the functional unit of the protein, and that IRBP arose through the quadruplication of an ancestral monomeric binding protein (Borst et al., 1989; Liou et al., 1989; Rajendran et al., 1996).

The structure of an individual module of IRBP was recently solved (Loew & Gonzalez-Fernandez, 2002). The module, which is referred to as X2IRBP because it corresponds to the second repeat of *Xenopus IRBP*, is an elongated molecule consisting of two domains separated by a hydrophobic cleft (Fig. 10). Interestingly, this cleft and a second potential retinoid binding site are both restricted to the Exon 2–4 region shown previously to contain the module's ligand-binding activity (Baer et al., 1998).

The structural data revealed a homology between IRBP and two diverse protein families, namely the C-terminal transferases (CPTases) and the crotonases. Even before structural information was available, GenBank data-base searches had identified a similarity between IRBP and the CPTases (Baer et al., 1998). Although this similarity appeared limited to the C-terminal region of the CPTases, X-ray crystalographic analysis of IRBP revealed a more extensive similarity that included the N-terminus (Loew et al., 2001).

Photosystem II D1 C-terminal processing protease (D1P), a prototype CTPase whose structure has been solved (Liao, Qian, Chisholm, Jordan, & Diner, 2000), is involved in the process of renewal of the photosytem II D1 protein (Blankenship, 2002). The photosynthetic system must address the challenge of excess photons that can create damaging intermediates. Although the photosynthetic apparatus can often divert this energy to the antenna system, and has scavenging pathways to deal with oxygen free radicals, damage to the photosynthetic apparatus still takes place. The damage is largely confined to the D1 complex, the main protein component of the photosystem II reaction center. To address this problem, a repair system exists in which the photosynthesis complex is disassembled and the damaged D1 protein is replaced with new protein. A final step in this process is that D1P catalyzes the C-terminal cleavage of the D1 protein. This catalysis is an essential event for the assembly of a manganese cluster and consequent light-mediated water oxidation.

IRBP does not possess this enzymatic activity, because it lacks the D1P PDZ domain. Each module of IRBP is structurally similar to D1P domains A and C which correspond to N- and C-terminal X2IRBP domains respectively. The PDZ domain is inserted between D1P domains A and C. Because X2IRBP lacks the PDZ domain it exhibits no D1P like protease activity (Bruce Diner, personal communication). Why has the retina recruited the ligand binding fold but not the enzymatic activity? The answer to this question is not clear but may be related to the fact that the fold is suited for binding the hydrophobic C-terminus of D1 protein and therefore perhaps was adapted for binding hydophobic ligands in the retina.

A completely unexpected finding was that the C-terminal domain B of X2IRBP and domain C of D1P exhibit a significant structural homology with the enoyl Co-A hydratase/isomerase superfamily (Loew &

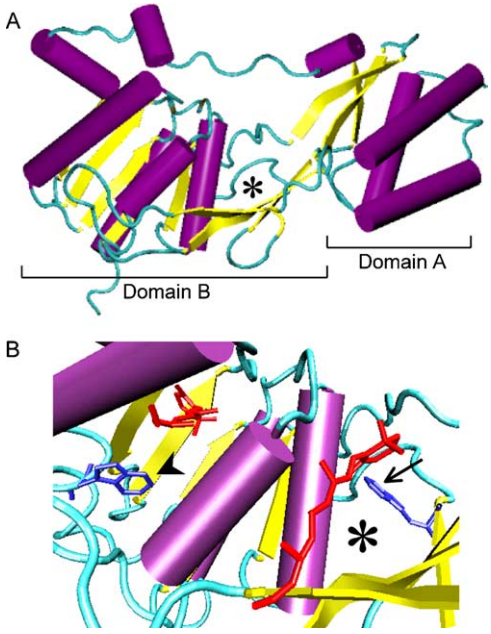

Fig. 10. X-ray crystal structure of the functional unit module of IRBP, and prediction of the retinoid-binding site(s). (A) Structure of the 2nd module of *Xenopus* IRBP (X2IRBP). Helices are in purple, and β strands are in yellow. The module can be divided into domains A and B according to truncation studies (Baer et al., 1998) (Appendix F). Those studies place the hydrophic ligand-binding site in Domain B. Indeed, domain B contains the $\beta\beta\alpha$-spiral fold (asterisk). This fold, which binds hydrophobic ligands in CptA and crotonases, is the candidate retinoid-binding site (diagram adapted from Loew & Gonzalez-Fernandez (2002) and reprinted with permission from Elsevier). (B) Expanded view of the X2IRBP structure showing the predicted hydrophobic ligand-binding domains for all-*trans* retinol (in red). Computer docking suggests two possible hydrophobic domains. We have shown that retinol binding quenches tryphtophan fluorescence in IRBP (Baer et al., 1998). The candidate tryptophan residues (arrows) are shown in blue. The molecular docking studies favor all-*trans* retinol to be located in the $\beta\beta\alpha$-spiral fold near tryptophan 272 (arrow), rather than in a small hydrophobic crater near tryptophan 135 (arrow head). This prediction is based on modeling using computer docking programs (diagram adapted from Gonzalez-Fernandez (2002) with permission from the Society For Endocrinology).

Gonzalez-Fernandez, 2002) as represented by the known structures of dienoyl-CoA isomerase, (Modis et al., 1998) chlorobenzoyl-CoA dehydrogenase, and enoyl-CoA hydratase (Engel, Mathieu, Zeelen, Hiltunen, & Wierenga, 1996). X2IRBP shares with these enzymes a structural core composed of three helices and the large 5 stranded beta-sheet in domain B. These elements are arranged in a topological identical order in spite of peripheral insertions/deletions. However, none of the catalytic residues of the crotonase family appear to be conserved in IRBP. This unexpected topological similarity had not been predicted previously in published alignments of the primary structure.

Enoyl-CoA hydratase/isomerases, which are also known as the crotonase family, have a low sequence identity, but adopt the same overall fold (Engel et al., 1996). Both crotonases and CTPases utilize the same fold to stabilize unique ligands. The location of the hydrophobic ββα-spiral is indicated by the asterisk in Fig. 10. In the case of CTPases such as D1P the fold is used to stabilize the hydrophobic C-terminal end of a protein substrate. Substrate binding is mediated through the presence of an additional PDZ domain. This specific domain, which supports its proteolytic activity, is not present in either IRBP or the crotonases. In the case of dienoyl-CoA isomerase, the fold confers an ability to isomerize fatty acids to allow entry into the beta-oxidation pathway.

Although the active site of the crotonases is known, an IRBP structure with bound ligand is not yet available. Superposition of X2IRBP with one of the crotonase superfamily members suggests a similar ligand-binding domain. Interestingly, such comparisons place the ligand binding domain between the two domains of X2IRBP in the ββα-spiral. Furthermore, an equivalent superposition of 2-enoyl-CoA hydratase with D1P protease also places the ligand into the known active site of D1P. Although the catalytic residues and the function between the crotonase superfamily and CTPases are not conserved, the location of the substrate binding domain is. The structural homology between X2IRBP, crotonases and CTPases suggests that at least one binding site

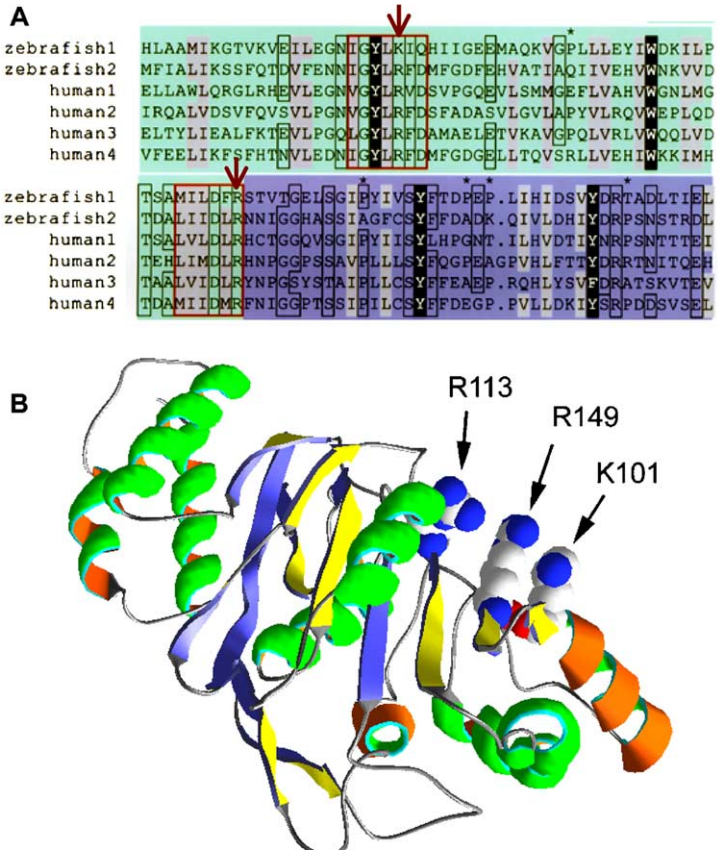

Fig. 11. Phyleogenetically conserved residues suggest a functional domain distinct from the retinoid-binding sites. (A) Sequence alignment of human and zebrafish IRBPs calls attention to conserved regions of the primary structure. Sequences corresponding to the second and third exons are highlighted in green and purple, respectively. Highly conserved regions are boxed in red. Chemically similar residues are indicated when five of the six are similar or identical (adapted from Rajendran et al. (1996)). (B) Ribbon structural diagram of the fourth module of *Xenopus* IRBP rotated ~90 degrees in the horizontal plane compared to Fig 10 to reveal that highly conserved arginine residues (red arrows in A) appear to participate in a surface positively charged surface domain. The relationship of these residues to the remainder of the protein, and possible role in intramolecular interactions is under study in our laboratory.

in X2IRBP is predicted to be located in the ββα-spiral fold. Computer docking studies are consistent with this prediction (Fig. 10B). In addition to the ββα-spiral fold, the docking studies suggest the existance of a second hydrophobic domain which could explain why the individual module binds >1 ligand equivalent in vitro. Site directed mutagenesis of nearby tryptophans (arrows in Fig. 10B) combined with crystal structures of the protein containing bound ligand will help to define the location of the retinoid-binding site, and which residues participate in stabilizing the bound ligand.

Previous sequence alignments have called attention to regions highly conserved between each of the modules of teleost, amphibian, and mammalian IRBPs (Baer et al., 1998; Rajendran et al., 1996) (Fig. 11A). Interestingly, most of these residues are located on the solvent exposed surface of the large β-sheet in domain B (Fig. 11B). The presence of both basic and acidic residues suggests that this cluster may represent a domain for intra- or intermolecular interactions. Since we do not yet know how each of the modules "fit" together, the above "surface" domain may represent sites through which the modules interact. Alternatively, the sites could be domains for docking with other proteins involved in the retinoid cycle, cell surface receptors, or components of the IPM.

To date, IRBP has not been identified in any invertebrate retina. Furthermore, we have not been able to identify the IRBP gene in the drosophila genomic database. The early suggestion that RALBP and IRBP are homologous (Fong et al., 1988) did not turn out to be correct (Ozaki et al., 1994). The lack of similarity with retinal proteins taken together with the homology to the CPTase and crontonase families suggests that IRBP represents the recruitment of a hydrophobic binding domain for a new function. The recruitment appears to be a solution to deal with the problem of intercellular retinoid trafficking required by the vertebrate retina. Hence, an "old gene for new eyes" (Nilsson, 1996).

10. Summary

Recent years have seen a shift away from viewing IRBP as simply a carrier that serves only to solubilize visual cycle retinoids in the IPM. The current need is to uncover detailed molecular mechanisms for how IRBP achieves specific interactions with the RPE, outer segments and possibly the IPM. Of particular importance is to evaluate the role of IRBP in the cone/Müller cell retinoid cycle. At the same time, other functions for IRBP outside of the retinoid cycle such as support of the photoreceptor maintenance and function should be considered. The structure of IRBP's ligand-binding domains, potential cooperative interactions between the modules, and identification of domains that mediate interactions with other players in the cycle, comprise the pieces of the puzzle that are yet missing. Uncovering the specific role of IRBP in the retinoid cycle will require open collaborations between disciplines of structural biology, retinal physiology, cell and molecular biology, and retinoid biochemistry.

References

Adler, A. J., & Edwards, R. B. (2000). Human interphotoreceptor matrix contains serum albumin and retinol-binding protein. *Experimental Eye Research, 70*, 227–234.

Adler, A. J., Stafford, W. F., III, & Slayter, H. S. (1987). Size and shape of bovine interphotoreceptor retinoid-binding protein by electron microscopy and hydrodynamic analysis. *Journal of Biological Chemistry, 262*, 13198–13203.

Akeo, K., Hiramitsu, T., Kanda, T., Yorifuji, H., & Okisaka, S. (1996). Comparative effects of linoleic acid and linoleic acid hydroperoxide on growth and morphology of bovine retinal pigment epithelial cells in vitro. *Current Eye Research, 15*, 467–476.

Baer, C. A., Retief, J. D., Van Niel, E., Braiman, M. S., & Gonzalez-Fernandez, F. (1998). Soluble expression in *E. coli* of a functional interphotoreceptor retinoid-binding protein module fused to thioredoxin: correlation of vitamin A binding regions with conserved domains of C-terminal processing proteases. *Experimental Eye Research, 66*, 249–262.

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

Biesalski, H. K., Frank, J., Beck, S. C., Heinrich, F., Illek, B., Reifen, R., Gollnick, H., Seeliger, M. W., Wissinger, B., & Zrenner, E. (1999). Biochemical but not clinical vitamin A deficiency results from mutations in the gene for retinol binding protein. *American Journal of Clinical Nutrition, 69*, 931–936.

Blankenship, R. E. (2002). *Molecular mechanisms of photosynthesis*. Williston: Blackwell Science.

Bok, D. (1985). Retinal hotoreceptor-pigment epithelium interactions. Friedenwald lecture. *Investigative Ophthalmology & Visual Science, 26*, 1659–1694.

Bok, D. (1993). The retinal pigment epithelium: a versatile partner in vision. *Journal of Cell Science Supplement, 17*, 189–195.

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

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

Bunt-Milam, A. H., Saari, J. C., Klock, I. B., & Garwin, G. G. (1985). Zonulae adherentes pore size in the external limiting membrane of the rabbit retina. *Investigative Ophthalmology & Visual Science, 26*, 1377–1380.

Carlson, A., & Bok, D. (1992). Promotion of the release of 11-*cis*-retinal from cultured retinal pigment epithelium by interphotoreceptor retinoid-binding protein. *Biochemistry, 31*, 9056–9062.

Carlson, A., & Bok, D. (1999). Polarity of 11-*cis* retinal release from cultured retinal pigment epithelium. *Investigative Ophthalmology & Visual Science, 40*, 533–537.

Carter-Dawson, L., Alvarez, R. A., Fong, S. L., Liou, G. I., Sperling, H. G., & Bridges, C. D. (1986). Rhodopsin, 11-*cis* vitamin A, and interstitial retinol-binding protein (IRBP) during retinal development in normal and rd mutant mice. *Developmental Biology, 116*, 431–438.

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

Chen, P., Hao, W., Rife, L., Wang, X. P., Shen, D., Chen, J., Ogden, T., Van Boemel, G. B., Wu, L., Yang, M., & Fong, H. K. (2001). A photic visual cycle of rhodopsin regeneration is dependent on Rgr. *Nature Genetics, 28*, 256–260.

Chen, P., Lee, T. D., & Fong, H. K. (2001). Interaction of 11-*cis*-retinol dehydrogenase with the chromophore of retinal g protein-coupled receptor opsin. *Journal of Biological Chemistry, 276*, 21098–21104.

Crouch, R. K., Chader, G. J., Wiggert, B., & Pepperberg, D. R. (1996). Retinoids and the visual process. *Photochemistry and Photobiology, 64*, 613–621.

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

Cunningham, L. L., & Gonzalez-Fernandez, F. (2000). Coordination between production and turnover of interphotoreceptor retinoid-binding protein in zebrafish. *Investigative Ophthalmology & Visual Science, 41*, 3590–3599.

Cunningham, L. L., & Gonzalez-Fernandez, F. (2003). Internalization of interphotoreceptor retinoid-binding protein (IRBP)—by the xenopus retinal pigment epithelium. *Journal of Comparative Neurology, 466*, 331–332.

Cunningham, L. L., Yang, L., & Gonzalez-Fernandez, F. (1999). Interphotoreceptor retinoid-binding protein (IRBP) is rapidly cleared from the *Xenopus* interphotoreceptor matrix. *Experimental Eye Research, 68*, 399–410.

Das, S. R., Bhardwaj, N., Kjeldbye, H., & Gouras, P. (1992). Müller cells of chicken retina synthesize 11-*cis*-retinol. *Biochemical Journal, 285*(Pt 3), 907–913.

DesJardin, L. E., Timmers, A. M., & Hauswirth, W. W. (1993). Transcription of photoreceptor genes during fetal retinal development. Evidence for positive and negative regulation. *Journal of Biological Chemistry, 268*, 6953–6960.

Dodson, C. S., Rengarajan, K., Gewant, H. D., Stodulkova, E., Nguyen, H. T., Boatright, J. H., & Nickerson, J. M. (2001). Extra-hepatic expression of serum albumin mRNA in mouse retina. *Current Eye Research, 22*, 182–189.

Duke-Elder, S. (1958). The Eye in Evolution. In S. Duke-Elder (Ed.), *System of Ophthalmology, Vol. I.* St Louis: C.V. Mosby Company.

Eisenfeld, A. J., Bunt-Milam, A. H., & Saari, J. C. (1985). Immunocytochemical localization of interphotoreceptor retinoid-binding protein in developing normal and RCS rat retinas. *Investigative Ophthalmology & Visual Science, 26*, 775–778.

Engel, C. K., Mathieu, M., Zeelen, J. P., Hiltunen, J. K., & Wierenga, R. K. (1996). Crystal structure of enoyl-coenzyme A (CoA) hydratase at 2.5 angstroms resolution: a spiral fold defines the CoA-binding pocket. *EMBO Journal, 15*, 5135–5145.

Fedorovich, I. B., Semenova, E. M., Grant, K., Converse, C. A., & Ostrovsky, M. A. (2000). Photosensitized light-induced damage of IRBP (interphotoreceptor retinoid-binding protein): effects on binding properties. *Current Eye Research, 21*, 975–980.

Fong, S. L., Lee, P. G., Ozaki, K., Hara, R., Hara, T., & Bridges, C. D. (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 Research, 28*, 563–573.

Garry, D. J., Meeson, A., Yan, Z., & Williams, R. S. (2000). Life without myoglobin. *Cellular and Molecular Life Sciences, 57*, 896–898.

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

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

Goldstein, E. B., & Wolf, B. M. (1973). Regeneration of the green-rod pigment in the isolated frog retina. *Vision Research, 13*, 527–534.

Gonzalez-Fernandez, F. (2002). Evolution of the visual cycle: the role of retinoid-binding proteins. *Journal of Endocrinology, 175*, 75–88.

Gonzalez-Fernandez, F., Baer, C. A., Baker, E., Okajima, T. I., Wiggert, B., Braiman, M. S., & Pepperberg, D. R. (1998). Fourth module of *Xenopus* interphotoreceptor retinoid-binding protein: activity in retinoid transfer between the retinal pigment epithelium and rod photoreceptors. *Current Eye Research, 17*, 1150–1157.

Gonzalez-Fernandez, F., & 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. *Journal of Cell Biology, 111*, 2775–2784.

Gonzalez-Fernandez, F., Van Niel, E., Edmonds, C., Beaver, H., Nickerson, J. M., Garcia-Fernandez, J. M., Campohiaro, P. A., & Foster, R. G. (1993). Differential expression of interphotoreceptor retinoid-binding protein, opsin, cellular retinaldehyde-binding protein, and basic fibroblastic growth factor. *Experimental Eye Research, 56*, 411–427.

Gorry, P., Lufkin, T., Dierich, A., Rochette-Egly, C., Decimo, D., Dolle, P., Mark, M., Durand, B., & Chambon, P. (1994). The cellular retinoic acid binding protein I is dispensable. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 9032–9036.

Gottesman, M. E., Quadro, L., & Blaner, W. S. (2001). Studies of vitamin A metabolism in mouse model systems. *Bioessays, 23*, 409–419.

Grange, R. W., Meeson, A., Chin, E., Lau, K. S., Stull, J. T., Shelton, J. M., Williams, R. S., & Garry, D. J. (2001). Functional and molecular adaptations in skeletal muscle of myoglobin-mutant mice. *American Journal of Physiology Cell Physiology, 281*, C1487–C1494.

Hageman, G. S., & Johnson, L. V. (1991). Structure, composition and function of the retinal interphotoreceptor matrix. *Progress in Retinal and Eye Research, 10*, 207–249.

Hageman, G. S., Kirchoff-Rempe, M. A., Lewis, G. P., Fisher, S. K., & Anderson, D. H. (1991). Sequestration of basic fibroblast growth factor in the primate retinal interphotoreceptor matrix. *Proceedings of the National Academy of Sciences of the United States of America, 88*, 6706–6710.

Hara, T., & Hara, R. (1976). Distribution of rhodopsin and retinochrome in the squid retina. *Journal of General Physiology, 67*, 791–805.

Hara, R., Hara, T., Tokunaga, F., & Yoshizawa, T. (1981). Photochemistry of retinochrome. *Photochemistry and Photobiology, 33*, 883–891.

Hara-Nishimura, I., Kondo, M., Nishimura, M., Hara, R., & Hara, T. (1993). Amino acid sequence surrounding the retinal-binding site in retinochrome of the squid, *Todarodes pacificus*. *FEBS Letters, 335*, 94–98.

Hara-Nishimura, I., Matsumoto, T., Mori, H., Nishimura, M., Hara, R., & Hara, T. (1990). Cloning and nucleotide sequence of cDNA for retinochrome, retinal photoisomerase from the squid retina. *FEBS Letters, 271*, 106–110.

Hauswirth, W. W., Langerijt, A. V., Timmers, A. M., Adamus, G., & Ulshafer, R. J. (1992). Early expression and localization of rhodopsin and interphotoreceptor retinoid-binding protein (IRBP) in the developing fetal bovine retina. *Experimental Eye Research, 54*, 661–670.

Hessler, R. B., Baer, C. A., Bukelman, A., Kittredge, K. L., & Gonzalez-Fernandez, F. (1996). Interphotoreceptor retinoid-binding protein IRBP: expression in the adult and developing *Xenopus* retina. *Journal of Comparative Neurology, 367*, 329–341.

Heth, C. A., & Bernstein, M. H. (1991). Mannose-sensitive HRP endocytosis by the retinal pigment epithelium. *Experimental Eye Research, 52*, 75–82.

Hewitt, A. T., Lindsey, J. D., Carbott, D., & Adler, R. (1990). Photoreceptor survival-promoting activity in interphotoreceptor matrix preparations: characterization and partial purification. *Experimental Eye Research, 50*, 79–88.

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

Hogan, M. J., Alvarado, R., & Weddell, J. E. (1971). *Histology of the Human Eye*. Philadelphia: W.B. Saunders.

Hollyfield, J. G. (1999). Hyaluronan and the functional organization of the interphotoreceptor matrix. *Investigative Ophthalmology & Visual Science, 40*, 2767–2769.

Hollyfield, J. G., Varner, H. H., Rayborn, M. E., Liou, G. I., & Bridges, C. D. (1985). Endocytosis and degradation of interstitial retinol-binding protein: differential capabilities of cells that border the interphotoreceptor matrix. *Journal of Cell Biology, 100*, 1676–1681.

Hollyfield, J. G., & Witkovsky, P. (1974). Pigmented retinal epithelium involvement in photoreceptor development and function. *Journal of Experimental Zoology, 189*, 357–378.

Hunt, R. C., Dewey, A., & Davis, A. A. (1989). Transferrin receptors on the surfaces of retinal pigment epithelial cells are associated with the cytoskeleton. *Journal of Cell Science, 92*(Pt 4), 655–666.

Jin, J., Jones, G. J., & Cornwall, M. C. (1994). Movement of retinal along cone and rod photoreceptors. *Visual Neuroscience, 11*, 389–399.

Jones, G. J., Crouch, R. K., Wiggert, B., Cornwall, M. C., & Chader, G. J. (1989). Retinoid requirements for recovery of sensitivity after visual-pigment bleaching in isolated photoreceptors. *Proceedings of the National Academy of Sciences of the United States of America, 86*, 9606–9610.

Kamino, H., & Tam, S. T. (1991). Immunoperoxidase technique modified by counterstain with azure B as a diagnostic aid in evaluating heavily pigmented melanocytic neoplasms. *Journal of Cutaneous Pathology, 18*, 436–439.

Kuehn, M. H., & Hageman, G. S. (1999). Expression and characterization of the IPM 150 gene (IMPG1) product, a novel human photoreceptor cell-associated chondroitin-sulfate proteoglycan. *Matrix Biology, 18*, 509–518.

Lampron, C., Rochette-Egly, C., Gorry, P., Dolle, P., Mark, M., Lufkin, T., LeMeur, M., & Chambon, P. (1995). Mice deficient in cellular retinoic acid binding protein II (CRABPII) or in both CRABPI and CRABPII are essentially normal. *Development, 121*, 539–548.

Land, M. F. (1980). Compound eyes: old and new optical mechanisms. *Nature, 287*, 681–686.

Liao, D. I., Qian, J., Chisholm, D. A., Jordan, D. B., & Diner, B. A. (2000). Crystal structures of the photosystem II D1 C-terminal processing protease. *Nature Structural Biology, 7*, 749–753.

Liebman, P. A., & Entine, G. (1964). Sensitive low-light-level microspectrophotometer: detection of photosensitive pigments of retinal cones. *Journal of the Optical Society of America, 54*, 1451–1459.

Lin, Z. S., Fong, S. L., & Bridges, C. D. (1989). Retinoids bound to interstitial retinol-binding protein during light and dark adaptation. *Vision Research, 43*, 864–869.

Liou, G. I., Fei, Y., Peachey, N. S., Matragoon, S., Wei, S., Blaner, W. S., Wang, Y., Liu, C., Gottesman, M. E., & Ripps, H. (1998). Early onset photoreceptor abnormalities induced by targeted disruption of the interphotoreceptor retinoid-binding protein gene. *Journal of Neuroscience, 18*, 4511–4520.

Liou, G. I., Ma, D. P., Yang, Y. W., Geng, L., Zhu, C., & Baehr, W. (1989). Human interstitial retinoid-binding protein. Gene structure and primary structure. *Journal of Biological Chemistry, 264*, 8200–8206.

Liou, G. I., Wang, M., & Matragoon, S. (1994). Timing of interphotoreceptor retinoid-binding protein (IRBP) gene expression and hypomethylation in developing mouse retina. *Developmental Biology, 161*, 345–356.

Loew, A., Baer, C., & Gonzalez-Fernandez, F. (2001). The functional unit of interphotoreceptor retinoid-binding protein (IRBP)—purification, characterization and preliminary crystallographic analysis. *Experimental Eye Research, 73*, 257–264.

Loew, A., & Gonzalez-Fernandez, F. (2002). Crystal structure of the functional unit of interphotoreceptor retinoid binding protein. *Structure (Cambridge), 10*, 43–49.

Maeda, T., Van Hooser, J. P., Driessen, C. A., Filipek, S., Janssen, J. J., & Palczewski, K. (2003). Evaluation of the role of the retinal G protein-coupled receptor (RGR) in the vertebrate retina in vivo. *Journal of Neurochemistry, 85*, 944–956.

Marmor, M. F., & Martin, L. J. (1978). 100 years of the visual cycle. *Survey of Ophthalmology, 22*, 279–285.

Mata, N., Radu, R., Clemmons, R., & Travis, G. (2002). Isomerization and oxidation of vitamin a in cone-dominant retinas. A novel pathway for visual-pigment regeneration in daylight. *Neuron, 36*, 69.

McBee, J. K., Palczewski, K., Baehr, W., & Pepperberg, D. R. (2001). Confronting complexity: the interlink of phototransduction and retinoid metabolism in the vertebrate retina. *Progress in Retinal and Eye Research, 20*, 469–529.

Messenger, J. B. (1981). *Comparative physiology and evolution of vision in invertebrates*. Berlin: Springer-Verlag.

Mieziewska, K. (1996). The interphotoreceptor matrix, a space in sight. *Microscopic Research and Technique, 35*, 463–471.

Modis, Y., Filppula, S. A., Novikov, D. K., Norledge, B., Hiltunen, J. K., & Wierenga, R. K. (1998). The crystal structure of dienoyl-CoA isomerase at 1.5 A resolution reveals the importance of aspartate and glutamate sidechains for catalysis. *Structure, 6*, 957–970.

Molina, T. M., Torres, S. C., Flores, A., Hara, T., Hara, R., & Robles, L. J. (1992). Immunocytochemical localization of retinal binding protein in the octopus retina: a shuttle protein for 11-*cis* retinal. *Experimental Eye Research, 54*, 83–90.

Nakagawa, M., Iwasa, T., Kikkawa, S., Tsuda, M., & Ebrey, T. G. (1999). How vertebrate and invertebrate visual pigments differ in their mechanism of photoactivation. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 6189–6192.

Nakashima, Y., Kusakabe, T., Kusakabe, R., Terakita, A., Shichida, Y., & Tsuda, M. (2003). Origin of the vertebrate visual cycle: genes encoding retinal photoisomerase and two putative visual cycle proteins are expressed in whole brain of a primitive chordate. *Journal of Comparative Neurology, 460*, 180–190.

Nickerson, J. M., Li, G. R., Lin, Z. Y., Takizawa, N., Si, J. S., & Gross, E. A. (1998). Structure-function relationships in the four repeats of human interphotoreceptor retinoid-binding protein (IRBP). *Molecular Vision, 4*, 33.

Nilsson, D. E. (1996). Eye ancestry: old genes for new eyes. *Current Biology, 6*, 39–42.

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

Okajima, T. I., Pepperberg, D. R., Ripps, H., Wiggert, B., & Chader, G. J. (1990). Interphotoreceptor retinoid-binding protein promotes rhodopsin regeneration in toad photoreceptors. *Proceedings of the National Academy of Sciences of the United States of America, 87*, 6907–6911.

Orzalesi, N., Fossarello, M., Carta, S., Del Fiacco, G., & Diaz, G. (1982). Identification and distribution of coated vesicles in the retinal pigment epithelium of man and rabbit. *Investigative Ophthalmology & Visual Science, 23*, 689–696.

Ozaki, K., Terakita, A., Hara, R., & Hara, T. (1987). Isolation and characterization of a retinal-binding protein from the squid retina. *Vision Research, 27*, 1057–1070.

Ozaki, K., Terakita, A., Ozaki, M., Hara, R., Hara, T., Hara-Nishimura, I., Mori, H., & Nishimura, M. (1994). Molecular characterization and functional expression of squid retinal-binding protein. A novel species of hydrophobic ligand-binding protein. *Journal of Biological Chemistry, 269*, 3838–3845.

Padgett, L. C., Lui, G. M., Werb, Z., & LaVail, M. M. (1997). Matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-1 in the retinal pigment epithelium and interphotoreceptor matrix: vectorial secretion and regulation. *Experimental Eye Research, 64*, 927–938.

Palczewski, K., Van Hooser, J. P., Garwin, G. G., Chen, J., Liou, G. I., & Saari, J. C. (1999). Kinetics of visual pigment regeneration in excised mouse eyes and in mice with a targeted disruption of the gene encoding interphotoreceptor retinoid-binding protein or arrestin. *Biochemistry, 38*, 12012–12019.

Pepperberg, D. R., & Crouch, R. K. (2001). An illuminating new step in visual-pigment regeneration. *Lancet, 358*, 2098–2099.

Pepperberg, D. R., Okajima, T. L., Ripps, H., Chader, G. J., & Wiggert, B. (1991). Functional properties of interphotoreceptor retinoid-binding protein. *Photochemistry and Photobiology, 54*, 1057–1060.

Pepperberg, D. R., Okajima, T. L., Wiggert, B., Ripps, H., Crouch, R. K., & Chader, G. J. (1993). Interphotoreceptor retinoid-binding protein (IRBP). Molecular biology and physiological role in the visual cycle of rhodopsin. *Molecular Neurobiology, 7*, 61–85.

Perlman, J. I., Piltz, J., Korte, G., & Tsai, C. (1989). Endocytosis in the rat retinal pigment epithelium. *Acta Anatomica (Basel), 135*, 354–360.

Pfeffer, B., Wiggert, B., Lee, L., Zonnenberg, B., Newsome, D., & Chader, G. (1983). The presence of a soluble interphotoreceptor retinol-binding protein (IRBP) in the retinal interphotoreceptor space. *Journal of Cellular Physiology, 117*, 333–341.

Plantner, J. J., Smine, A., & Quinn, T. A. (1998). Matrix metalloproteinases and metalloproteinase inhibitors in human interphotoreceptor matrix and vitreous. *Current Eye Research, 17*, 132–140.

Quadro, L., Blaner, W. S., Salchow, D. J., Vogel, S., Piantedosi, R., Gouras, P., Freeman, S., Cosma, M. P., Colantuoni, V., & Gottesman, M. E. (1999). Impaired retinal function and vitamin A availability in mice lacking retinol-binding protein. *EMBO Journal, 18*, 4633–4644.

Rajendran, R. R., Van Niel, E. E., Stenkamp, D. L., Cunningham, L. L., Raymond, P. A., & Gonzalez-Fernandez, F. (1996). Zebrafish interphotoreceptor retinoid-binding protein: differential circadian expression among cone subtypes. *Journal of Experimental Biology, 199*(12), 2775–2787.

Rando, R. R. (1996). Polyenes and vision. *Chemistry & Biology, 3*, 255–262.

Redmond, T. M., Yu, S., Lee, E., Bok, D., Hamasaki, D., Chen, N., Goletz, P., Ma, J. X., Crouch, R. K., & Pfeifer, K. (1998). Rpe65 is necessary for production of 11-*cis*-vitamin A in the retinal visual cycle. *Nature Genetics, 20*, 344–351.

Ripps, H. (2001). The rhodopsin cycle: a twist in the tale. *Progress in Brain Research, 131*, 335–350.

Ripps, H., Peachey, N. S., Xu, X., Nozell, S. E., Smith, S. B., & Liou, G. I. (2000). The rhodopsin cycle is preserved in IRBP "knockout" mice despite abnormalities in retinal structure and function. *Visual Neuroscience, 17*, 97–105.

Robles, L. J., Camacho, J. L., Torres, S. C., Flores, A., Fariss, R. N., & Matsumoto, B. (1995). Retinoid cycling proteins redistribute in light-/dark-adapted octopus retinas. *Journal of Comparative Neurology, 358*, 605–614.

Rudnicki, M. A., Braun, T., Hinuma, S., & Jaenisch, R. (1992). Inactivation of MyoD in mice leads to up-regulation of the myogenic HLH gene Myf-5 and results in apparently normal muscle development. *Cell, 71*, 383–390.

Saari, J. C. (1994). *Retinoids in photosensitive systems. The Retinoids*. New York: Raven Press.

Saari, J. C. (2000). Biochemistry of visual pigment regeneration: the Friedenwald lecture. *Investigative Ophthalmology & Visual Science, 41*, 337–348.

Saari, J. C., Bredberg, L., & Garwin, G. G. (1982). Identification of the endogenous retinoids associated with three cellular retinoid-binding proteins from bovine retina and retinal pigment epithelium. *Journal of Biological Chemistry, 257*, 13329–13333.

Saari, J. C., Bunt-Milam, A. H., Bredberg, D. L., & Garwin, G. G. (1984). Properties and immunocytochemical localization of three retinoid-binding proteins from bovine retina. *Vision Research, 24*, 1595–1603.

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

Schneider, B. G., Papermaster, D. S., Liou, G. I., Fong, S. L., & Bridges, C. D. (1986). Electron microscopic immunocytochemistry of interstitial retinol-binding protein in vertebrate retinas. *Investigative Ophthalmology & Visual Science, 27*, 679–688.

Seeliger, M. W., Biesalski, H. K., Wissinger, B., Gollnick, H., Gielen, S., Frank, J., Beck, S., & Zrenner, E. (1999). Phenotype in retinol deficiency due to a hereditary defect in retinol binding protein synthesis. *Investigative Ophthalmology & Visual Science, 40*, 3–11.

Stenkamp, D. L., & Adler, R. (1994). Cell-type- and developmental-stage-specific metabolism and storage of retinoids by embryonic chick retinal cells in culture. *Experimental Eye Research, 58*, 675–687.

Stenkamp, D. L., Cunningham, L. L., Raymond, P. A., & Gonzalez-Fernandez, F. (1998). Novel expression pattern of interphotoreceptor retinoid-binding protein (IRBP) in the adult and developing zebrafish retina and RPE. *Molecular Vision, 4*, 26.

Stiemke, M. M., Landers, R. A., al Ubaidi, M. R., Rayborn, M. E., & Hollyfield, J. G. (1994). Photoreceptor outer segment development in *Xenopus laevis*: influence of the pigment epithelium. *Developmental Biology, 162*, 169–180.

Sun, Y., & Ripps, H. (1992). Rhodopsin regeneration in the normal and in the detached/replaced retina of the skate. *Experimental Eye Research, 55*, 679–689.

Taniguchi, T., Adler, A. J., Mizuochi, T., Kochibe, N., & Kobata, A. (1986). The structures of the asparagine-linked sugar chains of bovine interphotoreceptor retinol-binding protein. Occurrence of fucosylated hybrid-type oligosaccharides. *Journal of Biological Chemistry, 261*, 1730–1736.

Terakita, A., Hara, R., & Hara, T. (1989). Retinal-binding protein as a shuttle for retinal in the rhodopsin–retinochrome system of the squid visual cells. *Vision Research, 29*, 639–652.

Weintraub, H. (1993). The MyoD family and myogenesis: redundancy, networks, and thresholds. *Cell, 75*, 1241–1244.

Wolf, G. (2002). A newly discovered visual cycle necessary for vision during continuous illumination. *Nutrition Reviews, 60*, 62–65.

Znoiko, S. L., Crouch, R. K., Moiseyev, G., & Ma, J. X. (2002). Identification of the RPE65 protein in mammalian cone photoreceptors. *Investigative Ophthalmology & Visual Science, 43*, 1604–1609.