BEYOND CARRIER PROTEINS

Evolution of the visual cycle: the role of retinoid-binding proteins

F Gonzalez-Fernandez

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

(Requests for offprints should be addressed to F Gonzalez-Fernandez; Email: fg23@buffalo.edu)

Abstract

The trafficking of retinoids in the retina represents a model to study soluble hormone-binding proteins in a complex system subject to profound evolutionary adaptations. Although a remarkable illustration of convergent evolution, all visual systems detect light in the same way, that is through the photoisomerization of an 11-*cis* retinoid to a corresponding *trans* isomer. What is strikingly different between the systems, is the mechanism by which the 11-*cis* chromophore is reformed and visual pigment regenerated in a process known as the visual cycle. The variations of the cycle address a problem inherent to retinoids themselves. That is, the properties that make these molecules suited for light detection also account for their susceptibility to oxidative and isomeric degradation, and cellular toxicity. The cycle therefore provides an opportunity to examine the role of soluble hormone-

binding proteins within an integrative and evolutionary context. The present review focuses on interphotoreceptor retinoid-binding protein (IRBP), a controversial glycolipoprotein that recruits a protein fold common to C-terminal-processing proteases and the crotonase family. This unorthodox retinoid-binding protein is entrapped in the subretinal compartment of those eyes that translocate visual cycle retinoids between the photoreceptors and the retinal pigment epithelium. Recent studies suggest that we should look beyond a strictly carrier function if we are to appreciate the role of IRBP in the visual cycle. Here we draw lessons from other soluble hormone-binding proteins to anticipate avenues of future research likely to provide insight into the structure and function of IRBP in vision.

Journal of Endocrinology (2002) **175**, 75–88

The visual cycle is in the eye of the beholder

Evolving 40 separate times, the eye employs remarkable adaptations to environmental challenges. Although a striking example of convergent evolution, there are unique common genetic and biochemical themes played out in all known eyes (reviewed by Nilsson 1996, Fernald 2000). In particular, all visual systems rely on the photoisomerization of protein-bound retinoid for light detection (reviewed by Saari 1994). Furthermore, derivatives of 11-*cis* retinaldehyde serve as the chromophore of all known visual pigments and the initial event in vision is the photoisomerization of 11-*cis* retinaldehyde to all-*trans* retinaldehyde. What is remarkably different, is the way various eyes regenerate the 11-*cis* chromophore from its photoisomerization product. The process of photoisomerization followed by reisomerization and visual pigment regeneration is known as the visual cycle (Dowling 1987, Rando *et al.* 1991, Saari 2000, McBee *et al.* 2001a). Various types of eyes orchestrate the visual cycle in ingenious and fundamentally different ways made possible by soluble hormone-binding proteins.

Although uniquely suited for photon capture, the visual cycle is confronted by significant challenges. Not only are retinoids relatively insoluble, the very properties that make them highly suited for photon capture also make them vulnerable to degradation and potentially toxic to the retinal cells. How the eye addresses these challenges is different in various examples of ocular parallel evolution. The following paragraphs compare the visual cycle in the retinas of insects, cephalopods and vertebrates with special reference to the increasing separation of the photoisomerization reaction and the process of chromophore regeneration.

Same chromophore, different cycle

Although the term 'visual cycle' is often used to describe the process of visual pigment regeneration in the vertebrate eye where it was first described (Wald 1934, Dowling 1960, Marmor & Martin 1978), we here use the term more liberally, embracing the analogous processes in nonvertebrate systems. Through this comparative

approach we hope to obtain insight into how soluble hormone-binding proteins address the problems of retinoid solubility, stability and targeting.

Insect eyes, which often occupy a large portion of the head, are typically arranged in a faceted partial sphere facilitating a wide visual field (reviewed by Land 1981). The basic unit of these compound eyes is the ommatidium. As in dragonflies, there can be thousands of ommatidia in each compound eye. Each ommatidium contains eight photoreceptor or retinula cells with visual pigment contained in microvilli organized into rhabdomeres analogous to vertebrate outer segments. In the insect rhabdomere, 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 invertebrates, because unlike vertebrate rhodopsin the active invertebrate photoproduct can be formed directly without Schiff base deprotonation (Nakagawa *et al.* 1999). Simplicity is a virtue, and from this metarhodopsin, the original rhodopsin is regenerated through the absorption of a second photon. Thus, insects have an amazingly elegant version of the visual cycle consisting of a rhodopsin/metarhodopsin photoequilibrium without the apparent need of accessory retinoid-binding proteins (reviewed by Hardie 1986).

Like insects, the dibranchiate cephalopods reform their 11-*cis* chromophore photochemically, but do so using a second visual pigment (Hara-Nishimura *et al.* 1990, 1993b). These marine invertebrates, which include the familiar squid, cuttlefish and octopus, have a highly developed visual sense organ representing a classical example of convergent evolution. Unlike the compound eye of many other invertebrates, the cephalopod eye consists of a single chamber containing a single prominent lens. At first glance, their eyes appear morphologically very much like our own eye. However, closer inspection reveals interesting differences particularly with respect to the retina.

The cephalopod retina, which is composed mainly of only two types of cells, visual and supporting cells, has an inside-out arrangement compared with that of vertebrates (reviewed by Messenger 1981). That is, the long photoreceptor outer segments (rhabdoms) are located in the inner retina close to the lens. In contrast, vertebrate photoreceptor outer segments are located in the outer retina close the sclera. In the rhabdoms, 11-*cis* retinaldehyde bound to rhodopsin is photoisomerized to the all-*trans* isomer forming metarhodopsin. However, unlike the insect's 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 (Ozaki *et al.* 1987,

Terakita *et al.* 1989, Molina *et al.* 1992). There photon capture returns the chromophore to the 11-*cis* configuration (Hara & Hara 1973). The 11-*cis* retinaldehyde is returned to the rhabdoms via RALBP, regenerating the rhodopsin.

Thus, the insect and cephalopod visual systems are similar in that 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.* 1993a). RALBP is thought to facilitate the chromophore exchange since the two photopigments are largely compartmentalized in different regions of the photoreceptors (Hara & Hara 1976). Notwithstanding the dual photopigment mechanism, the cephalopod visual cycle is similar to that of insects as the entire cycle is contained within the same cell. The recruitment of a retinoid-binding protein in the cephalopod visual cycle has been interpreted to provide a shuttle for visual cycle retinoids to traffic between the rhabdoms and inner segment myoids (Terakita *et al.* 1989) (see also below).

The embryology of the vertebrate retina has a profound influence on its visual cycle. The vertebrate eye is radically different from that of both insects and cephalopods in that it usually develops 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. 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) (see below), and therefore are susceptible to separation (retinal detachment), a not uncommon clinical problem.

Both layers of the optic cup contribute to the innovative visual cycle of the vertebrate retina. Here, retinoids are exchanged between two different cell types, a clear departure from the invertebrate visual cycle, which is completely contained within a single cell. 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 does a remarkable thing. It 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 (Bernstein *et al.* 1987, Rando *et al.* 1991, Rando 1996). The product of

Figure 1 Immunohistochemical labeling of the IPM in the developing *Xenopus* retina. The IPM is the extracellular material that is contained within the subretinal space between the white arrows in panel B. The space is bordered by the outer nuclear layer and the RPE. Immunoperoxidase technique using IRBP as a marker for the matrix. L, lens. Adapted from Hessler *et al.* 1996.

this reaction is 11-*cis* retinol and palmitate. 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 where it associates 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 RBPs. The enzymology of the visual cycle is an exciting area of research and the reader is referred to excellent reviews on the subject (Saari 1994, 2000, McBee *et al.* 2001a). Germane to the present symposium is the collection of mainly unrelated proteins employed by the 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. RBP, which is produced in the liver and responsible for mobilizing liver stores of 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 RBP (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.

The interphotoreceptor (IPM) – more than meets the RPE

The double-layered retina is certainly an innovation allowing an intimate relationship between the photoreceptors and the RPE/choroidal complex (Bok 1993). To mediate the varied critical interactions between the RPE and neural retina, the two layers are separated by a fascinating extracellular matrix known as the IPM (reviewed by Bridges & Adler 1985, Hageman & Johnson 1991, Mieziowska 1996, Hollyfield 1999) (Fig. 1). This complex matrix is composed of IRBP, growth factors (Hewitt *et al.* 1990, Hageman *et al.* 1991), metalloproteinases (Plantner 1992), hyaluronan and hyaluronan-binding proteoglycans (Acharya *et al.* 1998, Hollyfield *et al.* 1998) and sulfated glycoaminoglycans (Kuehn & Hageman 1999). The emerging picture is that the IPM mediates many of the critical interactions between the photoreceptors and RPE/choroid complex including retina/RPE adhesion, outer segment phagocytosis, outer segment structural stability, and nutrient exchange (Hollyfield *et al.* 1989, Hageman *et al.* 1995).

An important interaction that occurs between the RPE and photoreceptors is the exchange of retinoids in the visual cycle. As discussed above, unlike the eyes of insects and coelenterates, in the two-layered vertebrate retina, 11-*cis* retinal photoisomerized to its all-*trans* isomer is not reisomerized in the photoreceptors, but is exported to the RPE for reisomerization and then returned to the photoreceptors. Notwithstanding the advantages of this arrangement, the retinoids themselves are now even more vulnerable to oxidative and isomeric degradation having to traffic between the two cell layers. Furthermore, the retina could be exposed to toxic membranolytic effects of free retinoids.

IRBP, a 145 kDa glycolipoprotein, has been thought to address the problems created by the necessity to transport visual cycle retinoids between the outer segments and the RPE (reviewed by Pepperberg *et al.* 1993, Saari 1994, McBee *et al.* 2001a). IRBP, which is the most abundant soluble protein component of the IPM, is an unlikely candidate for the job. Not only does it have no similarity with any known retinoid-binding protein, it is composed of four homologous segments each about 300 amino acid residues in length. Each module contains a structural domain recruited from enzyme systems having roles in neither retinoid binding nor vision (see below).

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. First, since retinoids are insoluble compounds, IRBP is thought to solubilize retinoids within the IPM (Pepperberg *et al.* 1993). Secondly, IRBP is thought to target the delivery of all-*trans* retinol to the RPE (Okajima *et al.* 1989), and promote the release of 11-*cis* retinal from the RPE (Okajima *et al.* 1990, Carlson & Bok 1992, 1999, Sun & Ripps 1992).

One of the remarkable features of IRBP, which undoubtedly is important to its function, is the fact that the protein is tightly restricted to the subretinal space yet rapidly turned over in the IPM (Bunt-Milam *et al.* 1985a, Cunningham *et al.* 1999). Could insight into the processes involved be drawn from what is known about serum retinol-binding protein (sRBP)? In the blood, sRBP is complexed to transthyretin as long as it is carrying a molecule of all-*trans* retinol. The size of the sRBP/transsthyretin complex is sufficient to exclude it from glomerular filtration. Release of all-*trans* retinol to a target tissue including the RPE results in the disassociation sRBP from transthyretin. As a result, due to its small size sRBP is now susceptible to glomerular filtration and is rapidly cleared from the serum.

Size may have also played an important role in sequestering IRBP in the subretinal space. The space is bordered posteriorly by the RPE and anteriorly by the photoreceptors and Müller cell glia. 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 *et al.* 1985b). Small proteins such as RBP would easily pass through the *zonulae adherentes*. In contrast IRBP (Stokes' radius=55 Å) is larger than the *zonulae adherentes* pore size. 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 (Nickerson *et al.* 1997). 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 (Fig. 2).

Changing views

Recent years have seen an increased complexity of the visual cycle. A complete summary of the cycle is beyond the scope of this review and the reader is referred to excellent recent reviews (see above). Of particular interest to the present discussion is the appreciation that cones metabolize retinoids in a cycle separate from the visual cycle used by rods. A second concept germane to this review is the emerging complexity and sophistication inherent in the family of retinoid-binding proteins that participate in the cycle. More than simple carriers serving to solubilize 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 ensure their stability while protecting the retinal cells from the toxicity of free retinoids.

Existence of a cone/Müller cell visual cycle

One of the most exciting emerging new concepts is that vertebrate cones employ a private visual cycle independent of that used by rods. Interestingly, the cone cycle appears to rely on a collaboration with the Müller cells instead of the RPE. The existence of this alternative cycle was first suggested over 35 years ago by the observation 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 (Jones *et al.* 1993, Jin *et al.* 1994). This property was recently explained by the identification of a cone-specific 11-*cis* retinol dehydrogenase (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

Figure 2 Evolution of the IRBP gene. The four-modular structure of the IRBP may have arisen through the quadruplication of an ancestral gene. A processed form of this ancestral gene is believed to have been inserted before the original gene (Borst *et al.* 1989). The four-repeat structure with the introns restricted to the fourth repeat may have arisen through two crossover events. The teleost IRBP gene consists of only two repeats presumably due to the loss of the middle two repeats during a crossover event during the evolution of the ray-finned fishes. From Rajendran *et al.* (1996).

the formation of 11-*cis* retinol by the RPE (Redmond *et al.* 1998), is also found in cones but not in rods (Znoiko *et al.* 2002).

An important early clue that Müller cells have a key role in the cone visual cycle was the finding that cellular RALBP (CRALBP) and CRBP are located in the Müller cell in addition to the RPE (Bunt-Milam & Saari 1983, Bok *et al.* 1984, Saari *et al.* 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 *et al.* 1982). In contrast, RPE CRALBP carries only 11-*cis* retinol (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 (Travis *et al.* 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 convert it to all-*trans* and 11-*cis* retinyl palmitate, and release 11-*cis* retinol into the culture medium (Das & Gouras 1988). The 11-*cis* retinyl esterase appears to be distinct from RPE LRAT (Travis *et al.* 2002).

CRALBP – more than just a carrier

CRALBP illustrates how a small hormone-binding protein can have a profound role in a physiological process beyond that which might be expected for a simple carrier protein. For example, an important role of CRALBP is to protect 11-*cis* retinol from UV light-induced reverse isomerization to all-*trans* and 13-*cis* retinol. Despite the presence of melanin pigment, the RPE cytosol is continuously exposed to incident light photons. Therefore, retinoids are susceptible to photodegradation at various points along the visual cycle. This is particularly true of the 11-*cis* retinol released from the isomerohydrotase. In a set of elegant experiments, McBee *et al.* (2001b) formally showed that this photoconversion is inhibited when 11-*cis* retinol is complexed with CRALBP.

Several lines of work suggest that in addition to its protective role, CRALBP has a critical function in regulating the metabolism of retinoids in the RPE and presumably in the Müller cell as well. The first suggestion of this was CRALBP's role in a key branch point of the visual cycle. 11-*cis* retinol produced by the isomerohydrotase can

either be enzymatically oxidized to 11-*cis* retinaldehyde and exported for visual pigment regeneration, or enzymatically esterified and stored. CRALBP appears to modify the substrate flow favoring the oxidation of 11-*cis* retinol to the aldehyde (Saari *et al.* 1994). The protein may also have a role in the isomerization reaction itself. Transgenic mice lacking CRALBP, although processing normal photosensitivity, have a >10-fold reduction in 11-*cis* retinal production and delayed dark adaptation. Interestingly, all-*trans* retinyl esters accumulate during the delay suggesting that the isomerization of all-*trans* to 11-*cis* retinol is markedly impaired (Saari *et al.* 2001). These and other observations suggest that CRALBP functions as an acceptor of 11-*cis* retinol in the isomerization reaction. In this role, CRALBP may drive the reaction by mass action, thus overcoming what is a thermodynamically unfavorable process (Winston & Rando 1998, Stecher *et al.* 1999).

Moving retinoids between cellular sites of cis→trans and trans→cis isomerization

As discussed above, the cephalopod and vertebrate visual cycles have departed from that of insects by 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 visual cycle (Ozaki *et al.* 1994).

In cephalopods, the classical view has been that RALBP functions to transport 11-*cis* and all-*trans* retinaldehydes between rhodopsin in the rhabdomeres 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 meta-pigments 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. In any event, further research into this fascinating system is needed.

Similarly, the specific role of IRBP in the visual cycle is not clear. In a manner analogous to RALBP, IRBP, since its first description in the early 1980s, has been thought to play some role in transporting visual cycle retinoids between their site of photoisomerization and chemical reisomerization in the vertebrate retina. The prevailing view has been that these retinoids need some carrier protein to effectively solubilize them in the aqueous milieu of the IPM through which they must cross during their continual trafficking between the outer segments and the RPE.

Transgenic and knock-out mouse models

It was therefore surprising when the visual cycle was reported not to be interrupted in IRBP^{−/−} mice (Palczewski *et al.* 1999 reviewed by Saari 2000, Ripps 2001). In interpreting the significance of these observations, it should be kept in mind that the deletion of a single protein considered vital to a critical physiological process often reveals little or even no phenotypic change. This principle is illustrated in the transgenic animals lacking a variety of proteins including transcription factors (Rudnicki *et al.* 1992, Weintraub 1993) as well as ligand-binding proteins, for example myoglobin (Garry *et al.* 2000, Grange *et al.* 2001).

Transgenic mice lacking a retinoid-binding protein often lack the anticipated phenotype. For example, mice carrying a null mutation in one or more forms of CRBP appear indistinguishable from wild-type mice (Gorry *et al.* 1994, Lampron *et al.* 1995). Surprisingly, humans carrying mutations in sRBP (Biesalski *et al.* 1999, Seeliger *et al.* 1999), the major carrier of all-*trans* retinol in the serum, and transgenic mice lacking this protein altogether, have no disease or severe visual deficits (Quadro *et al.* 1999). One explanation for the lack of the expected phenotype 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. A second explanation for the lack of the expected effect is that our understanding of the system is incomplete.

Indeed, such knock-out studies are in many cases uncovering new insights into the physiological role of retinoid-binding proteins (reviewed by Gottesman *et al.* 2001). The sRBP^{−/−} mouse is an excellent illustration of this point. Quadro *et al.* (1999) noted that although sRBP^{−/−} 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 sRBP). 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 sRBP^{-/-} 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 sRBP^{-/-} animals. Taken together, these observations suggest that we should view sRBP not simply as an extracellular carrier to solubilize retinol in the blood, but as part of the mechanism to ensure adequate availability of retinol in times of inadequate vitamin A intake (Quadro *et al.* 1999).

Could similar insights for the function of IRBP be around the corner? That is, can the findings in the IRBP^{-/-} mouse be explained? One possibility is that another protein present in the IPM takes over the function of binding retinoids in the absence of IRBP. Ripps (2001) suggested that albumin, which binds a variety of hydrophobic molecules including retinoids, could serve as such a surrogate. This idea was advanced as it was thought that albumin might be present in significant quantities in the IPM. Indeed, albumin is often detected by protein electrophoresis in the aqueous extracts of IPM (Adler *et al.* 1988). However, the source of this albumin is unknown. That is, it may simply represent serum introduced as contamination during the dissection. Albumin has been detected in the IPM of postmortem human retinas (Adler & Edwards 2000). Furthermore, albumin mRNA can be detected in the mouse neural retina by RT-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 immunofluorescence and immunohistochemical approaches, failed to identify albumin in the IPM in either wild-type or IRBP^{-/-} mice (D-I Liao and F Gonzalez-Fernandez, unpublished observations). Thus, although albumin mRNA may be detected in the mouse neural retina, albumin does not accumulate in the subretinal space as would be expected if it were to be IRBP's surrogate in IRBP^{-/-} animals.

Although these studies appear to rule out albumin as an alternative route for retinoid trafficking in IRBP^{-/-} animals, it is possible that some other protein, even one not normally present in significant quantities in the subretinal space, is upregulated to take over retinoid transport in the absence of IRBP. Whether such changes in retinal gene expression occur in IRBP^{-/-} mice is not known.

Perhaps we should take a lesson from the sRBP knock-out mouse. That is, the function of IRBP may not be strictly related to transport *per se*, but rather to a mechanism that accommodates changes in retinoid availability. Perhaps IRBP's role is more closely linked to regulating the efficient bidirectional exchange of all-*trans* retinol and 11-*cis* retinaldehyde. Indeed, the system must

have sufficient capacity to accommodate large differences in retinoid flux caused by sudden changes in light intensity, while at the same time protecting the retinoids from degradation. In an analogous manner, serum transport of all-*trans* retinol can go on without sRBP. It is only when the system is challenged that the particular importance of sRBP becomes apparent.

Several observations do point to a guardian role for IRBP in the visual cycle rather than a strictly transport role. First, *in vitro* studies have shown that the transfer of all-*trans* retinol between liposomes and rod outer segment membranes can be accomplished rapidly via the aqueous phase. Surprisingly, IRBP and not albumin retards rather than facilitates this transfer process (Ho *et al.* 1989). This result would not be expected if IRBP's role is to facilitate retinoid diffusion by enhancing solubility of these molecules. The second observation comes from physiological studies of IRBP^{-/-} mice. 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). Taken together these observations suggest that IRBP has a buffering function which actually impedes intercellular retinoid transfer.

Why might it be necessary to buffer, or even retard the fluxes of the visual cycle retinoids across the IPM? 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. What happens to the photo-damaged IRBP? One possibility is that damaged IRBP is continuously removed from the IPM and replaced with newly synthesized IRBP. Whether a specific removal pathway for damaged IRBP exists is not known. However, it is known that IRBP is rapidly turned over within the IPM with a half-life of ~11 h in *Xenopus* (Cunningham *et al.* 1999). Furthermore, the turnover appears to be enhanced in light (Cunningham & Gonzalez-Fernandez 2000). Uncovering the mechanism and function of the turnover of IRBP could be a fruitful avenue for future research.

The structure of IRBP reveals an 'old gene for new eyes'

A recurrent theme in the evolution of the various visual systems is the recruitment of ancient genes. For insightful commentaries on this concept, the reader is referred to the reviews of Nilsson (1996) and Fernald (2000). The evolution of the gene for IRBP also appears to represent

Figure 3 Overall structure of the second module of *Xenopus* IRBP (X2IRBP). In this ribbon diagram, helices are shown in pink and beta-strands are in yellow. The disordered loop region, which is between residues 73 and 78, is marked by arrow heads. From Loew & Gonzalez-Fernandez (2002).

the recruitment of a successful hydrophobic ligand-binding domain with well-established functions unrelated to vision.

Of particular interest is the protein's fourfold repeat structure that is unprecedented among retinoid-binding proteins. What is the role of this apparent redundancy? Are the various modules selective for different forms of vitamin A, and/or do the modules interact modulating binding affinity analogous to the cooperative binding behavior of hemoglobin? Furthermore, do the modules contain functional domains that interact with other extracellular molecules or cell surface receptors?

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 large, hydrophobic cleft (Fig. 3). This cleft, which is presumably present in each of the four modules, appears to account for the retinoid-binding properties of the protein (Fig. 4).

An unexpected outcome of the structural data is the appreciation of a probable homology between IRBP and two diverse protein families previously not thought to be related, namely the C-terminal transferases (CPTases) and the crotonases (Fig. 5). Even before structural information

was available, GenBank database searches had identified a similarity between IRBP and the CPTases (reviewed in Baer *et al.* 1998). Although this similarity appeared limited to the C-terminal region of the CPTases, X-ray crystallographic analysis of IRBP revealed a more extensive similarity that included the N-terminus (Loew & Gonzalez-Fernandez 2002).

Photosystem II D1 C-terminal-processing protease (D1P), a prototype CTPase whose structure has been solved (Liao *et al.* 2000), is a fascinating enzyme involved in a process of continual renewal of the photosystem II D1 protein (reviewed by Blankenship 2002). Briefly, as with the retina, the photosynthetic system must address the challenge of excess photons whose energy must be dissipated to avoid toxic damaging intermediates. Although the photosynthetic apparatus can often divert this energy to the antenna system, and has scavenging pathways in place to deal with oxygen free radicals, the system is not perfect and damage to the photosynthetic apparatus still takes place. Repair is therefore required. The damage appears to be largely confined to the D1 complex, the main protein component of the photosystem II reaction center. If this damage is not repaired the system would rapidly lose all activity. To address this problem, a remarkable repair system exists in which the photosynthesis complex is disassembled and the damaged D1 protein is replaced with

Figure 4 Expanded view of the X2IRBP structure showing the predicted hydrophobic ligand-binding domains for all-*trans* retinol (red). This prediction is based on modeling using computer docking programs (unpublished data provided by Dr A Loew).

newly synthesized protein. Each of the modules of IRBP is structurally similar to D1P domains A and C which correspond to N- and C-terminal X2IRBP domains respectively. DIP has an additional domain known as PDZ, which is lacking in IRBP (Ponting 2001). The PDZ domain is inserted between D1P domains A and C. Because X2IRBP lacks the PDZ domain it exhibits no D1P-like protease activity (B 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 hydrophobic ligands in the retina.

A surprising structural homology was revealed when the structural coordinates of X2IRBP were subjected to three-dimensional database search programs. The C-terminal domain B of X2IRBP and domain C of D1P exhibit a significant structural homology with the enoyl-CoA hydratase/isomerase superfamily as represented by the known structures of dienoyl-CoA isomerase (Modis *et al.* 1998), chlorobenzoyl-CoA dehydrogenase (Benning *et al.* 1996) and enoyl-CoA hydratase (Engel *et al.* 1996). X2IRBP shares with these enzymes a structural core composed of three helices and the large five-stranded beta-sheet in domain B. These elements are arranged in a topologically identical order in spite of peripheral insertions/deletions. However, none of the catalytic

Figure 5 Comparison of X2IRBP and D1P. (a) D1P with helices in purple and strands in yellow. (b) Superposition of XIRBP (blue) with D1P (yellow). Note the $\sim 20^\circ$ offset of domain A in D1P compared with that in X2IRBP (curved arrow). (c) Structure-based alignment of the translated amino acid sequence of each of the four *Xenopus* IRBP modules with D1P. Secondary structure elements are shown in purple (helices), beige arrows (strands) and black (loops). Residues identical in all four modules of *Xenopus* IRBP (excluding D1P) are marked with asterisks. From Loew & Gonzalez-Fernandez (2002).

residues of the crotonase family appears to be conserved in IRBP. This unexpected topological similarity was not predicted by sequence alignments (Loew *et al.* 2001).

Enoyl-CoA hydratase/isomerases, which are also known as the crotonase family, have a low sequence identity, but adopt the same overall fold (Engel & Wierenga 1996). Both crotonases and CTPases utilize the same fold to stabilize unique ligands. 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 (Beebe *et al.* 2000). 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, the ligand-binding site of X2IRBP is yet uncharacterized. Superposition of X2IRBP with the crotonase superfamily members suggests a similar ligand-binding site. Interestingly, such comparisons place the ligand between the two domains of X2IRBP. 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 pocket is. It is therefore plausible from the structural homology between X2IRBP, crotonases and CTPases that at least one binding site in X2IRBP will be located in the large cleft between domains A and B. Further evidence for this is derived from a hydrophobicity surface map of X2IRBP, which reveals two potential retinoid-binding sites (Fig. 4).

Previous sequence alignments have called attention to regions highly conserved between each of the modules of teleost, amphibian and mammalian IRBPs (Rajendran *et al.* 1996, Baer *et al.* 1998). Interestingly, most of these residues are located on the solvent-exposed surface of the large beta-sheet in domain B. The presence of both basic and acidic residues suggests that this cluster may represent a potential interaction site with a thus far unidentified protein component 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, which is now virtually complete. The early suggestion that RALBP and IRBP are homologous (Fong *et al.* 1988) is not correct (Ozaki *et al.* 1994). The lack of similarity with retinal proteins taken together with the homology to the CPTase and crotonase families suggests that IRBP represents the recruitment of a hydrophobic binding domain for a new function. The recruitment appears to be an innovative solution to deal with the problem of intercellular retinoid trafficking required in the vertebrate retina. Hence, an 'old gene for new eyes' (Nilsson 1996).

Conclusions

Although evolving numerous times independently, all eyes utilize 11-*cis* retinaldehyde to capture incident light photons. However, the mechanism of visual pigment regeneration is fundamentally different in insects, cephalopods and vertebrates. The main differences are consequences of the compartmentalization of the cycle. Here, various hormone-carrier proteins have allowed the cephalopod and vertebrate retinas through convergent evolution to separate the initial photoisomerization reaction from the reisomerization machinery. Although compartmentalization does have advantages, it also brought problems of intra- and intercellular transport, significant issues given the limited solubility, and high lability and toxicity of retinoids. The retinoid-binding proteins described here address these challenges. To understand the role of IRBP in the retina, we should look to the various examples of hormone-binding proteins given in this symposium. This will provide insight to anticipate relationships between the structure and function of this interesting protein. So far,

we have seen that the common themes of compartmentalization, targeting, protection, and regulation of ligand concentration are forcing us to look beyond IRBP as carrier protein to future experiments that will uncover its specific role(s) in visual physiology.

References

- Acharya S, Rodriguez IR, Moreira EF, Midura RJ, Misono K, Todres E & Hollyfield JG 1998 SPACR, a novel interphotoreceptor matrix glycoprotein in human retina that interacts with hyaluronan. *Journal of Biological Chemistry* **273** 31599–31606.
- Adler AJ & Edwards RB 2000 Human interphotoreceptor matrix contains serum albumin and retinol-binding protein. *Experimental Eye Research* **70** 227–234.
- Adler AJ, Spencer SA, Heth CA & Schmidt SY 1988 Comparison of proteins in the interphotoreceptor matrix of vertebrates. *Ophthalmic Research* **20** 275–285.
- Baer CA, Retief JD, Van Niel E, Braiman MS & 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.
- Beebe KD, Shin J, Peng C, Chaudhury J, Khara J & Pei D 2000 Substrate recognition through a PDZ domain in tail-specific protease. *Biochemistry* **39** 3149–3155.
- Benning MM, Taylor KL, Liu R-Q, Yang G, Xiang H, Wesenberg G, Dunaway-Mariano D & Holden HM 1996 Structure of 4-chlorobenzoyl coenzyme A dehalogenase determined to 1.8 Å resolution: an enzyme catalyst generated via adaptive mutation. *Biochemistry* **35** 8103–8109.
- Bernstein PS, Law WC & Rando RR 1987 Isomerization of all-*trans* retinoids to 11-*cis* retinoids *in vitro*. *PNAS* **84** 1849–1853.
- Biesalski HK, Frank J, Beck SC, Heinrich F, Illek B, Reifen R, Gollnick H, Seeliger MW, 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 RE 2002 *Molecular Mechanisms of Photosynthesis*. Oxford: Blackwell Science.
- Bok D 1993 The retinal pigment epithelium: a versatile partner in vision. *Journal of Cell Science* **17** 189–195.
- Bok D, Ong DE & Chytil F 1984 Immunocytochemical localization of cellular retinol binding protein in the rat retina. *Investigative Ophthalmology and Visual Science* **25** 877–883.
- Borst DE, Redmond TM, Elser JE, Gonda MA, Wiggert B, Chader GJ & Nickerson JM 1989 Interphotoreceptor retinoid-binding protein. Gene characterization, protein repeat structure, and its evolution. *Journal of Biological Chemistry* **264** 1115–1123.
- Bridges CD & Adler AJ 1985 *The Interphotoreceptor Matrix in Health and Disease*. New York: Alan R. Liss, Inc.
- Bunt-Milam AH & Saari JC 1983 Immunocytochemical localization of two retinoid-binding proteins in vertebrate retina. *Journal of Cell Biology* **97** 703–712.
- Bunt-Milam AH, Saari JC & Bredberg DL 1985a Characterization of the interstitial space: immunocytochemical and biochemical studies. *Progress in Clinical and Biological Research* **190** 151–170.
- Bunt-Milam AH, Saari JC, Klock IB & Garwin GG 1985b Zonulae adherentes pore size in the external limiting membrane of the rabbit retina. *Investigative Ophthalmology and 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 and Visual Science* **40** 533–537.
- Crouch RK, Hazard ES, Lind T, Wiggert B, Chader G & Corson DW 1992 Interphotoreceptor retinoid-binding protein and alpha-tocopherol preserve the isomeric and oxidation state of retinol. *Photochemistry and Photobiology* **56** 251–255.
- Cunningham LL & Gonzalez-Fernandez F 2000 Coordination between production and turnover of interphotoreceptor retinoid-binding protein in zebrafish. *Investigative Ophthalmology and Visual Science* **41** 3590–3599.
- Cunningham LL, 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 SR & Gouras P 1988 Retinoid metabolism in cultured human retinal pigment epithelium. *Biochemical Journal* **250** 459–465.
- Dodson CS, Rengarajan K, Gewant HD, Stodulkova E, Nguyen HT, Boatright JH & Nickerson JM 2001 Extra-hepatic expression of serum albumin mRNA in mouse retina. *Current Eye Research* **22** 182–189.
- Dowling JE 1960 Chemistry of visual adaptation in the rat. *Nature* **168** 114–118.
- Dowling JE 1987 *The Retina – An Approachable Part of the Brain*. Cambridge, MA, USA: Harvard University Press.
- Engel CK & Wierenga RK 1996 The diverse world of CoA-binding proteins. *Current Opinion in Structural Biology* **6** 790–797.
- Engel CK, Mathieu M, Zeelen JP, Hiltunen JK & Wierenga RK 1996 Crystal structure of enoyl-coenzyme A (CoA) hydratase at 2.5 Å resolution: a spiral fold defines the CoA-binding pocket. *EMBO Journal* **15** 5135–5145.
- Fedorovich IB, Semenov EM, Grant K, Converse CA & Ostrovsky MA 2000 Photosensitized light-induced damage of IRBP (interphotoreceptor retinoid-binding protein): effects on binding properties. *Current Eye Research* **21** 975–980.
- Fernald RD 2000 Evolution of eyes. *Current Opinion in Neurobiology* **10** 444–450.
- Fong SL, Lee PG, Ozaki K, Hara R, Hara T & Bridges CD 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 DJ, Meeson A, Yan Z & Williams RS 2000 Life without myoglobin. *Cellular and Molecular Life Sciences* **57** 896–898.
- Goldstein EB 1967 Early receptor potential of the isolated frog (*Rana pipiens*) retina. *Vision Research* **7** 837–845.
- Goldstein EB 1970 Cone pigment regeneration in the isolated frog retina. *Vision Research* **10** 1065–1068.
- Goldstein EB & Wolf BM 1973 Regeneration of the green-rod pigment in the isolated frog retina. *Vision Research* **13** 527–534.
- 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. *PNAS* **91** 9032–9036.
- Gottesman ME, Quadro L & Blaner WS 2001 Studies of vitamin A metabolism in mouse model systems. *Bioessays* **23** 409–419.
- Grange RW, Meeson A, Chin E, Lau KS, Stull JT, Shelton JM, Williams RS & Garry DJ 2001 Functional and molecular adaptations in skeletal muscle of myoglobin-mutant mice. *American Journal of Physiology. Cell Physiology* **281** C1487–C1494.
- Hageman GS, Kirchoff-Rempe MA, Lewis GP, Fisher SK & Anderson DH 1991 Sequestration of basic fibroblast growth factor in the primate retinal interphotoreceptor matrix. *PNAS* **88** 6706–6710.
- Hageman GS, Marmor MF, Yao XY & Johnson LV 1995 The interphotoreceptor matrix mediates primate retinal adhesion. *Archives of Ophthalmology* **113** 655–660.
- Hara T & Hara R 1973 Isomerization of retinal catalysed by retinochrome in the light. *Nature New Biology* **242** 39–43.
- Hara T & Hara R 1976 Distribution of rhodopsin and retinochrome in the squid retina. *Journal of General Physiology* **67** 791–805.
- 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.
- Hara-Nishimura I, Kondo M, Nishimura M, Hara R & Hara T 1993a Amino acid sequence surrounding the retinal-binding site in retinochrome of the squid, *Todarodes pacificus*. *FEBS Letters* **335** 94–98.
- Hara-Nishimura I, Kondo M, Nishimura M, Hara R & Hara T 1993b Cloning and nucleotide sequence of cDNA for rhodopsin of the squid *Todarodes pacificus*. *FEBS Letters* **317** 5–11.
- Hardie RC 1986 Photoreceptor array of the dipteran retina. *Trends in Neurosciences* **9** 419–423.
- Hessler RB, Baer CA, Bukelman A, Kittredge KL & Gonzalez-Fernandez F 1996 Interphotoreceptor retinoid-binding protein (IRBP): expression in the adult and developing *Xenopus* retina. *Journal of Comparative Neurology* **367** 329–341.
- Hewitt AT, Lindsey JD, Carbott D & Adler R 1990 Photoreceptor survival-promoting activity in interphotoreceptor matrix preparations: characterization and partial purification. *Experimental Eye Research* **50** 79–88.
- Ho MT, Massey JB, Pownall HJ, Anderson RE & Hollyfield JG 1989 Mechanism of vitamin A movement between rod outer segments, interphotoreceptor retinoid-binding protein, and liposomes. *Journal of Biological Chemistry* **264** 928–935.
- Hollyfield JG, Varner HH, Rayborn ME & Osterfeld AM 1989 Retinal attachment to the pigment epithelium. Linkage through an extracellular sheath surrounding cone photoreceptors. *Retina* **9** 59–68.
- Hollyfield JG, Rayborn ME, Tammi M & Tammi R 1998 Hyaluronan in the Interphotoreceptor matrix of the eye – species differences in content distribution, ligand binding and degradation. *Experimental Eye Research* **66** 241–248.
- Hollyfield JG 1999 Hyaluronan and the functional organization of the interphotoreceptor matrix. *Investigative Ophthalmology and Visual Science* **40** 2767–2769.
- Jin J, Jones GJ & Cornwall MC 1994 Movement of retinal along cone and rod photoreceptors. *Visual Neuroscience* **11** 389–399.
- Jones GJ, Fein A, MacNichol EF & Cornwall MC 1993 Visual pigment bleaching in isolated salamander retinal cones: microspectrophotometry and light adaptation. *Journal of General Physiology* **102** 483–502.
- Kuehn MH & Hageman GS 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 MF 1981 *Comparative Physiology and Evolution of Vision in Invertebrates*. Berlin: Springer-Verlag.
- Liao D-I, Qian J, Chisholm DA, Jordan DB & Diner BA 2000 Crystal structures of the photosystem II D1 C-terminal processing protease. *Nature Structural Biology* **7** 749–753.
- Liebman PA & 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.
- Loew A & Gonzalez-Fernandez F 2002 Crystal structure of the functional unit of interphotoreceptor retinoid binding protein. *Structure* **10** 43–49.
- Loew A, Baer CA & 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.

- Marmor MF & Martin LJ 1978 100 years of the visual cycle. *Survey of Ophthalmology* **22** 279–285.
- Mata NL, Lee JS, Clemmons RS, Radu RA & Travis GH 2002 11-*cis* retinol dehydrogenase activity in the cone-dominant retina. *Investigative Ophthalmology and Visual Science* B593 (ARVO abstract).
- McBee JK, Palczewski K, Baehr W & Pepperberg DR 2001a Confronting complexity: the interlink of phototransduction and retinoid metabolism in the vertebrate retina. *Progress in Retinal and Eye Research* **20** 469–529.
- McBee JK, Van Hooser JP, Jang GF & Palczewski K 2001b Isomerization of 11-*cis*-retinoids to all-*trans*-retinoids *in vitro* and *in vivo*. *Journal of Biological Chemistry* **276** 48483–48493.
- Messenger JB 1981 *Comparative Physiology and Evolution of Vision in Invertebrates*. Berlin: Springer-Verlag.
- Miezewska K 1996 The interphotoreceptor matrix, a space in sight. *Microscopy Research and Technique* **35** 463–471.
- Modis Y, Filippula S, Novikov DK, Norledge B, Hiltunen JK & Wierenga RK 1998 The crystal structure of dienoyl-CoA isomerase at 1.5 Å resolution reveals the importance of aspartate and glutamate sidechains for catalysis. *Structure* **6** 957–970.
- Molina TM, Torres SC, Flores A, Hara T, Hara R & Robles LJ 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 TG 1999 How vertebrate and invertebrate visual pigments differ in their mechanism of photoactivation. *PNAS* **96** 6189–6192.
- Nickerson JM, Goodman M, Lin ZY, Czelusniak J & Mian IS 1997 Predicted structure, function and evolution of the repeated domain present in interphotoreceptor binding protein (IRBP) and its homologs. *Investigative Ophthalmology and Visual Science* **38** S3 (Abstract).
- Nilsson DE 1996 Eye ancestry: old genes for new eyes. *Current Biology* **6** 39–42.
- Okajima TI, Pepperberg DR, Ripps H, Wiggert B & Chader GJ 1989 Interphotoreceptor retinoid-binding protein: role in delivery of retinol to the pigment epithelium. *Experimental Eye Research* **49** 629–644.
- Okajima TI, Pepperberg DR, Ripps H, Wiggert B & Chader GJ 1990 Interphotoreceptor retinoid-binding protein promotes rhodopsin regeneration in toad photoreceptors. *PNAS* **87** 6907–6911.
- 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.
- Palczewski K, Van Hooser JP, Garwin GG, Chen J, Liou GI & Saari JC 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 DR, Okajima TL, Wiggert B, Ripps H, Crouch RK & Chader GJ 1993 Interphotoreceptor retinoid-binding protein (IRBP). Molecular biology and physiological role in the visual cycle of rhodopsin. *Molecular Neurobiology* **7** 61–85.
- Plantner JJ 1992 The presence of neutral metalloproteolytic activity and metalloproteinase inhibitors in the interphotoreceptor matrix. *Current Eye Research* **11** 91–101.
- Ponting CP 2001 Evidence for PDZ domains in bacteria, yeast, and plants. *Protein Science* **6** 464–468.
- Quadro L, Blaner WS, Salchow DJ, Vogel S, Piantadosi R, Gouras P, Freeman S, Cosma MP, Colantuoni V & Gottesman ME 1999 Impaired retinal function and vitamin A availability in mice lacking retinol-binding protein. *EMBO Journal* **18** 4633–4644.
- Rajendran RR, Van NE, Stenkamp DL, Cunningham LL, Raymond PA & Gonzalez-Fernandez F 1996 Zebrafish interphotoreceptor retinoid-binding protein: differential circadian expression among cone subtypes. *Journal of Experimental Biology* **199** 2775–2787.
- Rando RR 1996 Polyenes and vision. *Chemistry and Biology* **3** 255–262.
- Rando RR, Bernstein PS & Barry RJ 1991 New insights into the visual cycle. In *Progress in Retinal Research*, pp 161–178. Eds NN Osborne & GJ Chader. Oxford: Pergamon Press.
- Redmond TM, Yu S, Lee E, Bok D, Hamasaki D, Chen N, Goletz P, Ma JX, Crouch RK & 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 visual cycle – a twist in the tale. *Progress in Brain Research* **131** 335–350.
- Ripps H, Peachey NS, Xu X, Nozell SE, Smith SB & Liou GI 2000 The rhodopsin cycle is preserved in IRBP ‘knockout’ mice despite abnormalities in retinal structure and function. *Visual Neuroscience* **17** 97–105.
- Robles LJ, Camacho JL, Torres SC, Flores A, Fariss RN & Matsumoto B 1995 Retinoid cycling proteins redistribute in light-/dark-adapted octopus retinas. *Journal of Comparative Neurology* **358** 605–614.
- Rudnicki MA, 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 JC 1994 Retinoids in photosensitive systems. In *The Retinoids: Biology, Chemistry and Medicine*, pp 351–385. Eds MB Sporn, AB Roberts & DS Goodman. New York: Raven Press.
- Saari JC 2000 Biochemistry of visual pigment regeneration: the Friedenwald lecture. *Investigative Ophthalmology and Visual Science* **41** 337–348.
- Saari JC, Bredberg L & Garwin GG 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 JC, Bunt-Milam AH, Bredberg DL & Garwin GG 1984 Properties and immunocytochemical localization of three retinoid-binding proteins from bovine retina. *Vision Research* **24** 1595–1603.
- Saari JC, Bredberg DL & Noy N 1994 Control of substrate flow at a branch in the visual cycle. *Biochemistry* **33** 3106–3112.
- Saari JC, Nawrot M, Kennedy BN, Garwin GG, Hurley JB, Huang J, Possin DE & Crabb JW 2001 Visual cycle impairment in cellular retinaldehyde binding protein (CRALBP) knockout mice results in delayed dark adaptation. *Neuron* **29** 739–748.
- Seeliger MW, Biesalski HK, 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 and Visual Science* **40** 3–11.
- Stecher H, Gelb MH, Saari JC & Palczewski K 1999 Preferential release of 11-*cis*-retinol from retinal pigment epithelial cells in the presence of cellular retinaldehyde-binding protein. *Journal of Biological Chemistry* **274** 8577–8585.
- 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.
- 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.
- Travis GH, Radu RA, Lee J & Mata NL 2002 Identification of a new pathway for retinol isomerization in cone-dominant ground squirrel and chicken retinas. *Investigative Ophthalmology and Visual Science* B592 (ARVO abstract).
- Wald G 1934 Carotenoids and the vitamin A cycle in vision. *Nature* **134** 65.
- Weintraub H 1993 The MyoD family and myogenesis: redundancy, networks, and thresholds. *Cell* **75** 1241–1244.

- Winston A & Rando RR 1998 Regulation of isomerohydrolase activity in the visual cycle. *Biochemistry* **17** 2044–2055.
- Znoiko SL, Crouch RK, Moiseyev G & Ja J 2002 Identification of RPE65 protein in mammalian cone photoreceptors. *Investigative Ophthalmology and Visual Science* **43** 1604–1609.

Received 27 June 2002

Accepted 25 July 2002