

REVIEW ARTICLE

Retinoid-binding proteins: mediators of retinoid action

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Active vitamin A metabolites, known as retinoids, are essential for multiple physiological processes, ranging from vision to embryonic development. These small hydrophobic compounds associate *in vivo* with soluble proteins that are present in a variety of cells and in particular extracellular compartments, and which bind different types of retinoids with high selectivity and affinity. Traditionally, retinoid-binding proteins were viewed as transport proteins that act by solubilizing and protecting their labile ligands in aqueous spaces. It is becoming increasingly clear, however, that, in addition to this general role, retinoid-binding proteins have diverse and specific functions in regulating the disposition, metabolism and activities of retinoids. Some retinoid-binding proteins appear to act by sequestering their ligands, thereby generating concentration gradients that allow cells to take up retinoids from extracellular pools and metabolic steps to

proceed in energetically unfavourable directions. Other retinoid-binding proteins regulate the metabolic fates of their ligands by protecting them from some enzymes while allowing metabolism by others. In these cases, delivery of a bound retinoid from the binding protein to the 'correct' enzyme is likely to be mediated by direct and specific interactions between the two proteins. One retinoid-binding protein was reported to enhance the ability of its ligand to regulate gene transcription by directly delivering this retinoid to the transcription factor that is activated by it. 'Channelling' of retinoids between their corresponding binding protein and a particular protein target thus seems to be a general theme through which some retinoid-binding proteins exert their effects.

Key words: metabolism, retinoids, transport, vitamin A.

INTRODUCTION

Dietary vitamin A gives rise to a variety of active metabolites, collectively known as retinoids. Chemically, retinoids are composed of three distinct structural domains: a β -ionone ring, an isoprenoid tail, and a polar end-group with an oxidation state that may vary: a hydroxyl group in retinols, an aldehyde in retinals, and a carboxylic moiety in retinoic acids (Figure 1). These small lipophilic compounds exhibit multiple and diverse biological activities. One retinoid, 11-*cis*-retinal, is critical for the visual function [1]. Other vitamin A metabolites, i.e. all-*trans*- and 9-*cis*-retinoic acids, are potent regulators of gene transcription, and play important roles in regulating cell proliferation and differentiation [2]. Vitamin A-derived *retro*-retinoids were implicated in regulating immune function by controlling lymphocyte survival [3]. The multiple biological actions of retinoids are mediated by two classes of proteins: retinoid receptors and retinoid-binding proteins. Retinoid receptors are ligand-inducible transcription factors that belong to the superfamily of nuclear hormone receptors, and which are activated by stereoisomers of retinoic acid. Discussion of these proteins is beyond the scope of the present review. The term retinoid-binding proteins is used to describe soluble proteins that are found in a variety of tissues and that associate with specific retinoids. The aim of this review is to consider current knowledge on the spectrum of roles that retinoid-binding proteins play in mediating the biological activities of their ligands.

Retinoid-binding proteins

Multiple types of retinoid-binding proteins that associate with different chemical and isomeric forms of retinoids have been

identified both intracellularly and in particular extracellular compartments. Hence retinoids are found *in vivo* either associated with cellular membranes or bound to a specific retinoid-binding protein. The parent vitamin A molecule, all-*trans*-retinol, circulates in blood bound to serum retinol-binding protein (RBP). Inside cells, all-*trans*-retinol and its oxidation product, all-*trans*-retinal, are associated with one of the two isoforms of cellular retinol-binding proteins (CRBP-I and CRBP-II). all-*trans*-Retinoic acid is found intracellularly bound to one of the two cellular retinoic acid-binding protein isoforms (CRABP-I and CRABP-II). The visual pigment 11-*cis*-retinal and its precursor, 11-*cis*-retinol, associate in several cell types in the eye with cellular retinal-binding protein (CRALBP). Another ocular protein that can bind retinoids, the interphotoreceptor retinoid-binding protein (IRBP), is present in the extracellular space separating pigment epithelium and photoreceptor cells.

Retinoid-binding proteins are believed to share a common role, in that they act to solubilize and stabilize their hydrophobic and labile ligands in aqueous spaces. However, a growing body of information indicates that, in addition to this general role, specific retinoid-binding proteins have distinct functions in regulation of the transport, metabolism and action of the particular retinoids with which they associate. Of the numerous types of known retinoid-binding proteins, this review considers only those for which such specific functions have been documented (Table 1).

STRUCTURE

With the exception of CRALBP and IRBP, known retinoid-binding proteins belong to two protein families that were

Abbreviations used: ARAT, acyl-CoA:retinol acyltransferase; CRABP, cellular retinoic acid-binding protein; CRALBP, cellular retinal-binding protein; CRBP, cellular retinol-binding protein; DHA, docosahexaenoic acid; iLBP, intracellular lipid-binding protein; IPM, interphotoreceptor matrix; IRBP, interphotoreceptor retinoid-binding protein; LRAT, lecithin:retinol acyltransferase; RAR, retinoic acid receptor; RBP, serum retinol-binding protein. The terms retinol, retinal and retinoic acid designate the respective all-*trans*-isomers unless specified otherwise.

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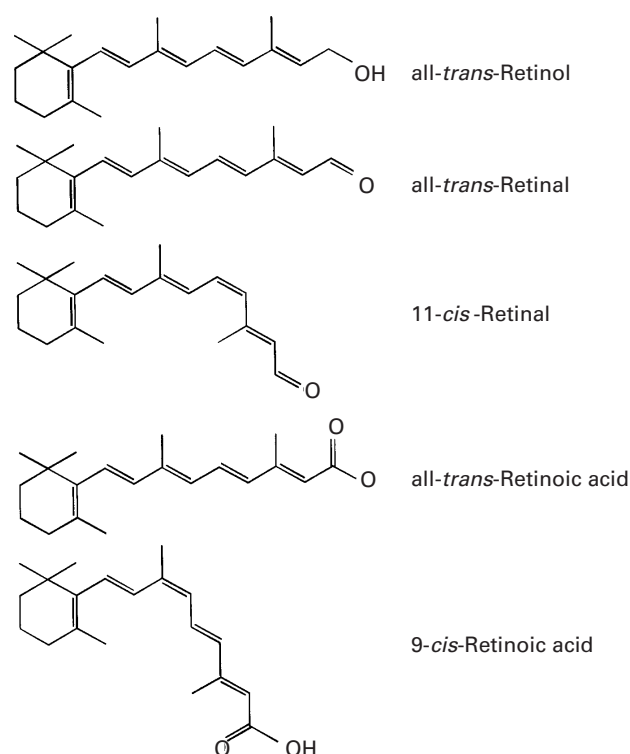


Figure 1 Chemical structures of some biologically active retinoids

Table 1 Characteristics of some retinoid-binding proteins

References: ^a [76] (rat RBP); ^b [57,60,149] (rat CRBP-I); ^c [150] (rat CRBP-II); ^d [101] (human CRABP-I, human CRABP-II); ^e N. Noy and J. C. Saari, unpublished work (bovine CRALBP); ^f [22]; N. Shaw and N. Noy, unpublished work (bovine IRBP).

Protein	Family	Molecular mass (kDa)	Major ligand(s)	K_d (nM)	No. of sites
RBP	Lipocalin	21.0	all- <i>trans</i> -Retinol	20 ^a	1
CRBP-I	iLBP	15.0	all- <i>trans</i> -Retinol	< 10 ^b	1
			all- <i>trans</i> -Retinal	50 ^b	
CRBP-II	iLBP	15.0	all- <i>trans</i> -Retinol	$\sim 100 \times K_d$ of CRBP-I ^c	1
			all- <i>trans</i> -Retinal	90 ^b	
CRABP-I	iLBP	14.0	all- <i>trans</i> -Retinoic acid	0.06 ^d	1
CRABP-II	iLBP	14.0	all- <i>trans</i> -Retinoic acid	0.13 ^d	1
CRALBP	—	36.0	11- <i>cis</i> -Retinol		1
			11- <i>cis</i> -Retinal	$\sim 15^e$	
IRBP	—	136.0	all- <i>trans</i> -Retinol	50–100 ^f	3 ^f
			11- <i>cis</i> -Retinal	50–100 ^f	

originally classified as transport proteins for hydrophobic ligands: the lipocalins and the intracellular lipid-binding proteins (iLBPs). Excellent reviews on the structural relationships between lipocalins and iLBPs have been published [4,5] and these issues are only discussed briefly here.

Lipocalins

Members of the lipocalin family, including the retinoid-binding proteins RBP, epididymal retinoic acid-binding protein and β -lactoglobulin, share a very low sequence identity but display a

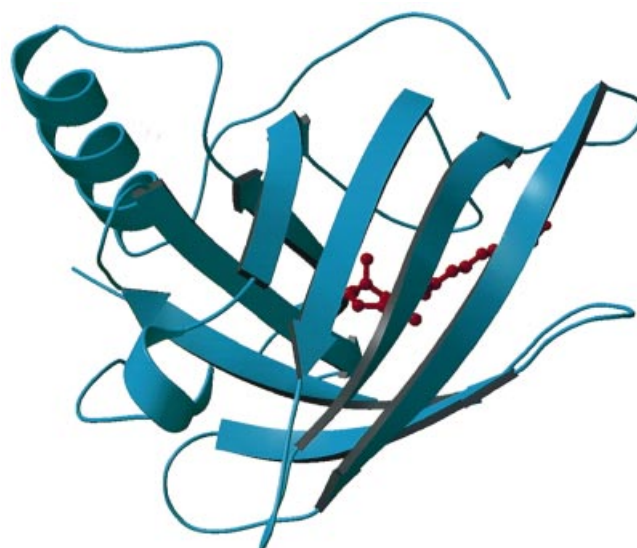


Figure 2 Ribbon drawing of the three-dimensional structure of bovine holo-RBP

Bound retinol is depicted in red. The figure was generated by using the co-ordinates of bovine holo-RBP reported in [148].

highly conserved overall fold. They are composed of an eight-stranded antiparallel β -sheet that is folded over itself to form a hydrogen-bonded β -barrel, which constitutes the ligand binding pocket (Figure 2). The *N*-termini of these proteins are folded around the back of the barrel, ‘capping’ that side of the pocket. In contrast, the front of the β -barrel is open to provide a portal for the ligand. The entrance is flanked by a single loop scaffold. The ligand-binding sites of different lipocalins differ in their structural details, conferring distinct ligand selectivity [4]. In RBP, the β -barrel completely encapsulates the bound retinol, with the β -ionone ring innermost and the hydroxy head-group reaching to the protein surface, where it is co-ordinated to a water molecule at the pocket entrance [6]. The interactions of retinol within RBP are stabilized mainly by hydrophobic interactions of the β -ionone ring and the isoprenoid chain with amino acid residues that line the interior of the pocket, whereas the hydroxyl group does not contribute significantly to ligand–protein interactions [6,7]. In addition to binding their hydrophobic ligands, many lipocalins interact with accessory proteins. RBP is bound in blood to transthyretin, a tetrameric protein with a molecular mass of ~ 55 kDa. This interaction is believed to protect the small RBP molecule from filtration by renal glomeruli, thereby retaining it in blood [8]. The interactions between RBP and transthyretin are mediated through the loop at the entrance of the ligand-binding pocket [9], and also involve amino acid residues at the RBP C-terminus [10].

iLBPs

By sequence identity, CRBPs and CRABPs belong to the family of iLBPs, which also includes the fatty acid-binding proteins. Like lipocalins, iLBPs bind their hydrophobic ligands within an antiparallel β -barrel structure. The β -barrel of iLBPs is constructed of two orthogonal, five-stranded, β -sheets that form a ligand binding pocket, with a helix–turn–helix motif at the entrance to the pocket [11–13] (see Figure 3). A striking difference between the mode by which lipocalins and iLBPs bind their

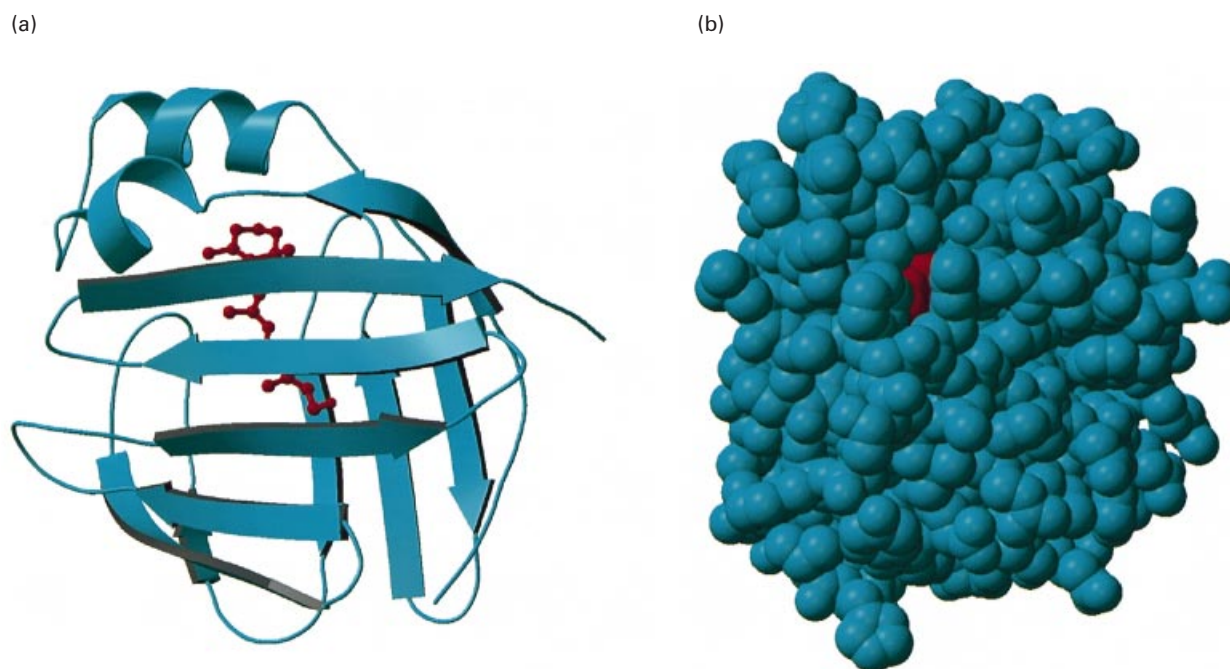


Figure 3 Three-dimensional structure of human holo-CRBP-I

(a) Ribbon drawing of the three-dimensional structure of CRBP-I with bound retinoid (red). (b) Space-filling model of holo-CRBP-I, emphasizing the limited accessibility of bound retinoid to the bulk solution. The figures were generated by using the co-ordinates of human holo-CRBP-I reported in [58].

ligands is the reversal of the binding orientation. Thus, in contrast with the orientation of retinol within the RBP binding site, iLBP ligands are oriented such that their polar groups are buried deep in the binding pockets and are co-ordinated to specific polar amino acid residues in this region. In both CRBPs and CRABPs, the β -ionone ring of retinol or retinoic acid is close to the surface of the respective protein, where it fits snugly within the entrance. Similarly to lipocalins, the N-terminus of the iLBP wraps around the back of the barrel, making that side inaccessible to the bulk solution. However, the front of the barrel in CRBPs and CRABPs is flanked by two α -helices that appear to limit access to the binding site [13] (Figure 3). An interesting question that thus arises is how the bound ligand exits the binding pocket to reach its sites of metabolism. Similarly, the closed structure of the binding pocket brings into question the mechanism by which the ligand can gain access to the site. The crystal structures of apo-CRABP-I [14] and -CRABP-II [11] indicate that these proteins crystallize as dimers, and that the pocket entrance is somewhat more open than that observed in the holoprotein. It was thus suggested that dimer formation constitutes a mechanism that allows better accessibility of the binding site. However, recent NMR studies have revealed that apo-CRABP-II is monomeric in solution, and that the entrance to the binding pocket of the apoprotein is greatly enlarged and readily accessible as compared with the holoprotein [12]. Similarly, NMR studies of the solution structure of apo-CRBP-II revealed the presence of an opening that can potentially accommodate initial entry of the polar end of the retinol molecule [15].

Ocular retinoid-binding proteins

The two retinoid-binding proteins that are found specifically in retina and neural tissue, CRALBP and IRBP (Table 1), do not

belong to either the lipocalin or the iLBP family, and their structural make-ups are poorly understood. CRALBP is notably similar to two other lipid-binding proteins: tocopherol transfer protein, a liver protein that interacts specifically with the α -conformer of tocopherol [16], and SEC14, which serves as a transfer protein for phosphatidylinositol in yeast [17]. Recent studies have indicated that the retinoid-binding pocket of CRALBP is located at the C-terminus, and have implicated residues Gln²¹⁰ and Lys²²¹ in serving to stabilize ligand binding by this protein [18].

IRBP is unique among retinoid-binding proteins in that it contains three binding sites for retinoids as well as one or two binding sites for long-chain fatty acids ([19–22]; N. Shaw and N. Noy, unpublished work). This protein is also unusual in that it can associate with multiple chemical and isomeric forms of retinoids although, in accordance to its proposed role, it displays the highest affinity towards the retinoids that are important in the visual cycle, i.e. 11-*cis*-retinal and all-*trans*-retinol [23]. Bovine IRBP can associate with 2 mol of all-*trans*-retinol or 2 mol of 11-*cis*-retinal per mol of protein. One of the protein's retinoid-binding sites displays a broad ligand selectivity and can accommodate either all-*trans*-retinol or 11-*cis*-retinal with similar affinities [23]. The two additional sites display narrower specificities for retinol and retinal, respectively ([22]; N. Shaw and N. Noy, unpublished work). The thermodynamic characteristics of the interactions of all-*trans*-retinol with IRBP indicate that association of retinol with both of its sites is stabilized mainly by hydrophobic interactions, that neither of the sites requires the hydroxy head-group for binding, and that, nevertheless, the two sites are structurally distinct [24].

The primary sequence of mammalian IRBP reveals four 300-residues-long repeats of homologous stretches. An additional 55-residue extension comprises the C-terminus of the protein [25,26].

It was proposed that conservative hydrophobic sequences located near the C-termini of each of the four repeats may be involved in binding of retinoids and/or fatty acids [26]. In contrast with the mammalian and amphibian proteins, IRBP in teleosts is composed of only two homologous repeats, corresponding to the N-terminal and C-terminal repeats of the mammalian protein [27]. Proteins that share sequence identity with IRBP have also been identified in bacteria and plants. One of these, Tsp, is a periplasmic protease of *Escherichia coli* that has been implicated in playing a role in fatty acid transport [28].

FUNCTIONS

It is becoming increasingly clear that the traditional view that retinoid-binding proteins act mainly as aqueous storage compartments for retinoids is far from being complete. Rather, accumulated evidence indicates that many of these proteins play specific roles in regulating the transport, metabolism and action of their ligands. Retinoid-binding proteins associate with their ligands with affinities in the nanomolar range (Table 1). An important general question that arises regarding their functions is: how do these proteins release the ligands at their sites of metabolism or action? Theoretically, a ligand can move from a donor (the respective binding protein) to an acceptor (the appropriate target) by one of two possible mechanisms. In the first model, the ligand dissociates from the donor, diffuses through the aqueous space, and binds to an acceptor, if such is

present (Figure 4a). In this model, the activity of the target will rely on the aqueous pool of the ligand, the transfer process will be driven by concentrations gradients, and its rate will be limited by the rate of dissociation of the donor–ligand complex. Alternatively, a donor may engage in direct interactions with an acceptor, transferring the ligand by ‘channelling’ between the two proteins (Figure 4b). Such a process will bypass the aqueous space and may result in facilitation of transfer. Available information indicates that, while some retinoid-binding proteins exert their effects simply by binding their ligands and releasing them to the bulk solution, thereby generating concentration gradients, some of these proteins can deliver their ligands to target proteins via direct protein–protein interactions. Direct targets that have been suggested include enzymes that utilize particular retinoids as their substrates, and, in one documented case, a transcription factor that is activated by a retinoid.

Functions of RBP

Retinol is secreted from its storage pools and circulates in blood bound to RBP. The main storage site for vitamin A and, correspondingly, the main site of synthesis of RBP, is the liver, although other tissues (including adipose tissue, kidney, lung, heart, skeletal muscle, spleen, eye and testis) express this protein. Secretion of RBP from the liver is tightly regulated by the availability of retinol. In vitamin A deficiency, RBP secretion is inhibited and the protein accumulates in the endoplasmic

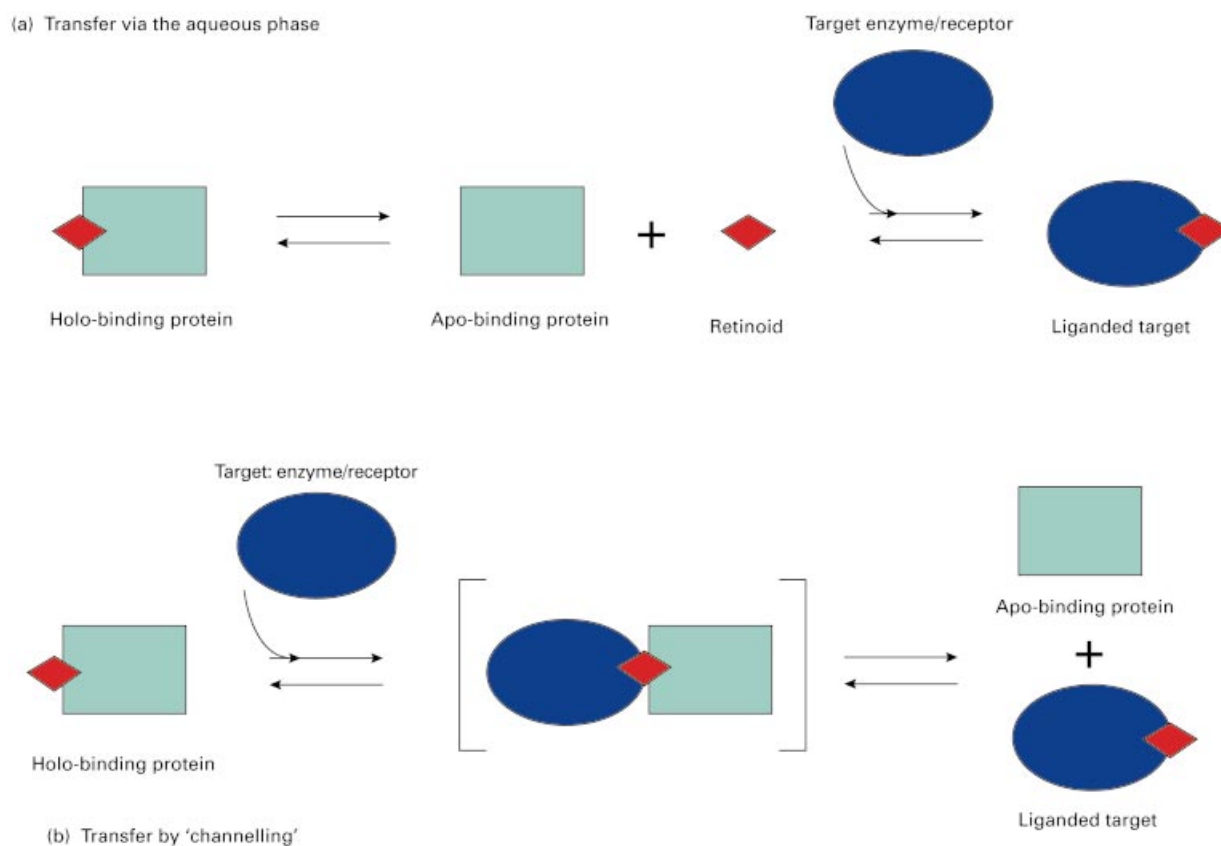


Figure 4 Pathways by which a ligand may transfer from a donor protein to an acceptor

(a) Transfer may require dissociation of the donor–ligand complex prior to diffusion of the ligand through the aqueous phase and its association with the acceptor. (b) Transfer may be mediated by direct interactions between the donor and the acceptor in a ‘channelling’ process. This process may result in facilitation of the formation of the acceptor–ligand complex.

reticulum of hepatic parenchymal cells. In the presence of retinol, RBP associates with retinol, moves to the Golgi apparatus and is secreted into blood in the form of the holoprotein [29]. The mechanism by which retinol initiates secretion of RBP from cells is unknown, but appears to be conserved in yeast ectopically expressing RBP [30]. In blood, RBP is bound to transthyretin, which, in addition to associating with RBP, functions as a carrier protein for thyroid hormones. Binding of RBP to transthyretin serves to prevent the loss of the smaller protein from the circulation by filtration in the renal glomeruli. The ternary complex transthyretin-RBP-retinol thus serves to transport retinol in the circulation and to deliver it to target tissues. It has been established that retinol enters most of its target cells (though perhaps not all, see [31,32]) in the form of free retinol, unaccompanied by the binding protein [8,29].

The exact role that RBP plays in regulating vitamin A uptake by extrahepatic cells is controversial, and two alternative views have been presented. On the one hand it has been proposed that a receptor embedded in the plasma membranes of target cells mediates transfer of retinol from RBP, across the membrane and into cytosol, or perhaps directly to the protein that binds retinol in cells, CRBP [33–39]. The function of the 'RBP receptor' has been assigned to a 65 kDa protein (Rpe65) which was originally identified in retinal pigment epithelium cells [36–40]. However, the ability of Rpe65 to act as a plasma membrane receptor for RBP has been questioned in view of its primary sequence, which lacks hydrophobic regions that would allow it to integrate into a membrane, and of immunolocalization studies which localized it intracellularly [41,42]. In addition, a recent study of Rpe65-deficient mice showed that the retinal pigment epithelium of these mice overaccumulates all-*trans*-retinyl esters but contains undetectable levels of 11-*cis*-retinyl esters [43]. These observations suggest that Rpe65 is not essential for uptake of retinol by the pigment epithelium, but rather that it is critical for conversion of all-*trans*-retinoids into the 11-*cis*-isomers in these cells.

An alternative view regarding the mechanism by which retinol leaves RBP in the plasma and enters target cells stemmed from the consideration that, because of the hydrophobic nature of retinol, cellular membranes do not constitute a barrier to it. Indeed, it was shown that retinol spontaneously crosses membranes at rates of the order of ms, that its dissociation from lipid bilayers is characterized by a $t_{1/2}$ of about 1 s, and that it rapidly partitions between proteins and membranes [7,44]. It was shown further that binding to RBP is not required for movement of retinol into or out of cells [45–47]. It was thus proposed that cellular uptake of retinol from RBP in blood occurs spontaneously and simply follows the concentration gradients of free retinol [45,46,48–50]. In support of this model, reported rates of uptake of retinol by several target cells are two to three orders of magnitude lower than the rate of the spontaneous dissociation of retinol from RBP [51]. Thus there does not seem to be a physiological need for facilitating the dissociation of the RBP-retinol complex by a distinct protein entity. These considerations suggest that the cellular uptake of retinol is not limited by events that occur in blood or at the level of the plasma membrane, but by downstream reactions such as the intracellular diffusion of retinol to sites of metabolism, and by subsequent metabolic steps. Accordingly, in most cells the pools of retinol in blood and in cytosol will be near equilibrium, and retinol will be selectively taken up only by cells in which a retinol concentration gradient, directed inwards, can be created. Such a gradient will be generated in cells that actively utilize retinol, or in cells in which CRBP is up-regulated, thereby decreasing the cytosolic concentration of free retinol (reviewed in [8,51]).

Important insights into the biological role of RBP have been

obtained recently by studies of mice and humans in which the RBP gene is disrupted. RBP-deficient mice display reduced blood retinol levels and impaired visual function during the first months of life. However, these mice are viable and fertile and, when maintained on a vitamin A-sufficient diet, they acquire normal vision by 5 months of age, even though their blood retinol level remains low [52]. A striking phenotype of the RBP-null mice is that they possess larger than normal hepatic vitamin A stores, but are nevertheless dependent on a continuous dietary intake of vitamin A [52]. A recent study of two human siblings that harbour point mutations in their RBP gene and exhibit undetectable plasma RBP levels revealed that these sisters suffer from night blindness and mild retinal dystrophy but do not exhibit other clinical symptoms of vitamin A deficiency [53,54]. Taken together, the observations obtained by examining RBP-deficient mice and humans support the conclusions that RBP is critical for the mobilization of retinol from hepatic storage pools in times of insufficient dietary intake, but that the protein is not essential for the delivery of retinol to tissues. Supply of vitamin A to target tissues in the absence of RBP is likely to be accomplished via newly absorbed retinyl esters, or perhaps β -carotene present in circulating chylomicrons.

Functions of CRBPs

CRBP-I and CRBP-II were the first intracellular retinoid-binding proteins to be discovered. The primary sequences of rat CRBP-I and CRBP-II show 56% identity [55], and each of these proteins is highly conserved among species, exhibiting 91–96% sequence identity among human, rat, mouse, pig and chick (reviewed in [5,56]). Both proteins interact with both all-*trans*-retinol and all-*trans*-retinal. It has been reported that the binding affinity of CRBP-I towards retinol is about 100-fold higher as compared with that of CRBP-II, that the two proteins display a similar binding affinity towards retinal, and that CRBP-II associates with retinol and retinal with similar affinities ([55,57]; see Table 1). The CRBPs belong to the family of iLBPs, and hence bind their ligands within a 10-stranded antiparallel β -barrel with the polar head-group directed innermost. The hydroxy group of retinol within the crystal structure of holo-CRBP is hydrogen-bonded to a Gln residue at the bottom of the barrel [58,59]. CRBP-bound retinol assumes a conformation in which its β -ionone ring is approximately planar in relation to the isoprene chain with the chain fully extended. The planarity of the molecule results in a 25 nm red-shift and in the appearance of fine structure in the absorption spectrum of retinol when bound to either CRBP-I or -II [60,61].

The two isoforms of CRBP display a strikingly different tissue distribution. CRBP-I is expressed in multiple tissues, both in the fetus and in the adult. In the adult, it is particularly abundant in the liver, kidney, lung and reproductive organs, and is also expressed in the choroid plexus in the brain and in pigment epithelium cells in the eye [62–66]. In contrast, in the adult, CRBP-II is restricted to the small intestine, where it is located in mature enterocytes on the villi of the mucosal epithelium [55,67,68]. CRBP-II in the fetus is mainly localized in the intestine, but is also transiently expressed in the liver where, in the rat, it appears several days before birth and disappears shortly after birth [55]. It is also expressed in the yolk sac between embryonic days 10.5 and 15.5 [69].

The high degree of sequence conservation of CRBPs indicate that they play important roles in retinoid biology. The amphipathic nature of retinol results in 'detergent-like' characteristics of the molecule, and its presence indeed affects various aspects of membrane structure and function [70–73]. CRBPs, and especially

CRBP-I with its high affinity for retinol, are believed to sequester this ligand, thereby protecting cells from its membranolytic effects. It has been shown in this regard that the predominant fraction of retinol in the liver is associated with CRBP, with very little of the compound partitioning into cellular membranes [74,75]. Up-regulation of the expression of CRBP is also expected to result in an equilibrium shift, i.e. in a decrease in the intracellular level of free retinol, thereby increasing the flux of retinol from the blood RBP-bound pool into cells [76].

In addition to these roles, the available information implicates CRBPs in regulation of the metabolism of retinol and retinal. A general consideration that should be kept in mind is that all known retinoid-metabolizing enzymes can utilize free retinoids as their substrates. Most of the evidence for the involvement of retinoid-binding proteins in retinoid metabolism thus stems from comparison of the kinetic parameters of key metabolic reactions in the presence and absence of a binding protein. Interpretation of such studies is hampered by the low aqueous solubility of retinoids and by their high affinity for cellular membranes. This caveat is of particular concern when the enzymes under study are integral membrane proteins. Retinoids in such assay mixtures partition between the binding protein, the aqueous space, the membranes and, when present at high concentrations, also between micelles and self-aggregates. Hence, neither the concentrations of retinoids in the different pools nor the potential rate-limiting steps for the observed activities can be readily assessed, complicating kinetic analyses. Nevertheless, accumulated evidence strongly supports the conclusion that some retinoid binding proteins act by allowing access of their ligands to particular enzymes, while restricting access to others.

Multiple enzymic activities that can catalyse metabolic conversions of free retinol and retinal have been reported [77,78]. However, only specific enzymes recognize CRBP-bound retinoids, suggesting that CRBPs limit access of retinoids to some enzymes, while allowing metabolism by others to proceed (reviewed in [5]). Such a role is deemed especially important in view of the need for tight regulation of cellular levels of the active vitamin A metabolite retinoic acid. The notion that a specific process, mediated by protein-protein interactions between CRBP and a target enzyme, may be necessary to allow an enzyme to gain access to bound ligand is also attractive in view of the three-dimensional structure of CRBPs, in which accessibility of bound-retinol to the bulk solution appears to be limited (Figure 3). Available information implicates both CRBP-I and -II in regulating several enzymic activities.

CRBP-I

(a) Regulation of vitamin A storage. The processes by which vitamin A is taken up into the liver, the subsequent metabolism and disposition of retinol in this tissue, and the enzymic steps that have been proposed to be under regulation of CRBP-I are depicted in Scheme 1. In vitamin A sufficiency, retinol accumulates in the liver, where it is stored in the form of retinyl esters. Two distinct enzymic activities that can catalyse the esterification of retinol with long-chain fatty acids to form retinyl esters have been identified: acyl-CoA:retinol acyltransferase (ARAT; EC 2.3.1.76), which is dependent upon the presence of CoA, and lecithin:retinol acyltransferase (LRAT; EC 2.3.1.135), for which the esterification reaction is coupled to hydrolysis of phospholipids [5,8]. It has been demonstrated that, while both enzymes can metabolize free retinol, esterification of CRBP-bound retinol by hepatic microsomal fractions is CoA-independent, implying that CRBP-bound retinol is metabolized by LRAT, but not by ARAT. Furthermore, the inclusion of CRBP-

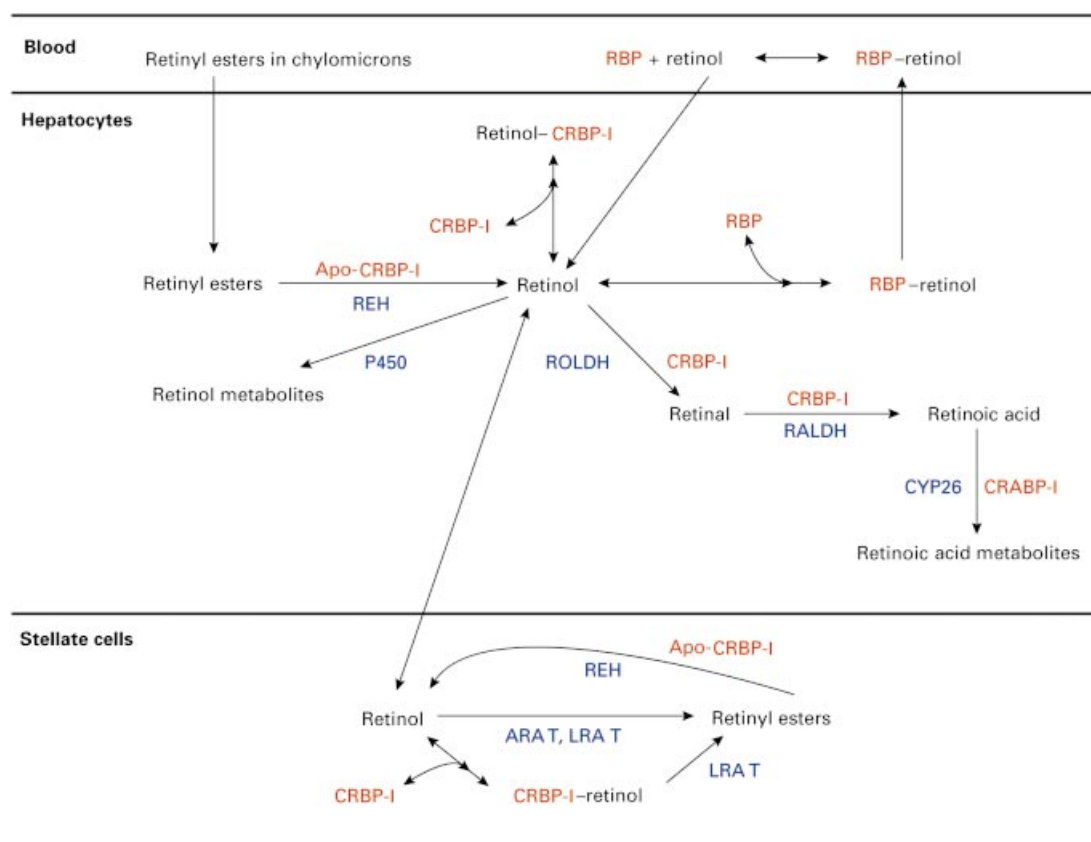
I in LRAT assay mixtures does not seem to significantly alter the observed K_m of the reaction [79]. As the concentration of free retinol is expected to be significantly reduced in the presence of the high-affinity binding protein, these observations were taken to indicate that LRAT can recognize retinol when bound to CRBP-I, suggesting that the substrate is 'channelled' between the binding protein and the enzyme via direct protein-protein interactions [5].

While holo-CRBP-I presents its ligand directly to LRAT, apo-CRBP-I strongly inhibits this reaction. It was reported that a 2-fold excess of CRBP-I over retinol leads to a 80% decrease in LRAT activity. As the magnitude of the decrease could not be accounted for merely by the decrease in the concentration of the free ligand due to the excess binding protein, these data were taken to suggest that apo-CRBP-I is a direct inhibitor of the enzyme [79]. An additional enzymic activity that was reported to be affected by apo-CRBP-I is retinyl ester hydrolase. It was demonstrated that apo-CRBP-I stimulates the hydrolysis of endogenous retinyl esters in liver microsomal fractions in a saturable fashion. The molecular mechanisms underlying this effect have not been clarified [80].

The physiologically relevant scenario that emerges from these observations suggests that, in vitamin A sufficiency, CRBP-I will be largely saturated with retinol and will direct this ligand to storage by delivering it to LRAT. On the other hand, under conditions of vitamin A deficiency, the level of retinol and, consequently, the level of saturation of CRBP-I will decrease, resulting in an elevation in the level of the apoprotein. In turn, apo-CRBP-I will both inhibit retinol esterification and stimulate liberation of retinol from stored retinyl esters, thereby allowing retinol to be released into the circulation [5]. A recent study of mice in which the CRBP-I gene had been disrupted revealed that CRBP-I deficiency is accompanied by a 50% decrease in retinyl ester pools in hepatic stellate cells, although it does not affect the levels of enzymes that catalyse vitamin A metabolism. The decrease was found to stem from decreased retinyl ester synthesis and was accompanied by an accelerated rate of clearance of hepatic retinyl esters, strongly supporting the notion that CRBP-I is essential for maintenance of vitamin A storage [81].

(b) Synthesis of retinoic acid. Retinoic acid is produced from retinol by two enzymic steps. The first step, catalysed by retinol dehydrogenase, results in the formation of retinal, which is then converted, via the action of retinal dehydrogenase, to retinoic acid (Scheme 1). Free retinol can be oxidized by multiple forms of alcohol dehydrogenases with broad substrate specificities that are present in both cytosol and membrane fractions [77,78]. However, only distinct enzymes can gain access to CRBP-I-bound retinol, suggesting that the binding protein delivers its ligand to enzymes in a specific manner which is likely to involve direct protein-protein interactions. As CRBP-I binds not only retinol but also retinal, it was suggested that retinal that is produced following oxidation of retinol also associates with CRBP-I and that the protein-bound retinal serves as a substrate for the subsequent reaction by retinal dehydrogenase. It has been shown in regard to this that CRBP-I-bound retinal is not readily accessible to cytosolic retinal dehydrogenase activity, but is efficiently metabolized to retinoic acid by microsomal enzymes [78,82,83].

Overall, current information implicates CRBP-I in regulating vitamin A storage, as well as in determining the specificity of the two enzymic activities that produce retinoic acid. It is worth noting, however, that while the proposed function for CRBP-I in regulating vitamin A storage has been strongly supported by studies of CRBP-I-null mice, these animals do not display abnormalities related to retinoic acid deficiency, at least under



Scheme 1 Proposed involvement of retinoid-binding proteins in modulating the disposition and metabolism of retinoids in the liver

Following uptake of chylomicrons by the hepatocytes, retinyl esters incorporated in these particles are hydrolysed by retinyl ester hydrolase (REH) to yield free retinol. In vitamin A excess, retinol moves to stellate cells, where it is re-esterified by either ARAT or LRAT. In vitamin A deficiency, retinyl esters, stored in lipid droplets in the stellate cells, are hydrolysed by REH. Free retinol thus produced moves back to the hepatocytes, where it complexes with RBP, triggering secretion of holo-RBP into the blood. In the hepatocytes, retinol may be degraded by specific cytochrome P450 enzymes. Alternatively, retinol may be oxidized to retinal and, subsequently, to retinoic acid by processes sequentially catalysed by retinol dehydrogenase (ROLDH) and retinal dehydrogenase (RALDH). Degradation of retinoic acid is catalysed by specific cytochrome P450 enzymes (CYP26). CRBP-I draws retinol from blood into the cell by shifting the equilibrium between plasma and cellular retinol pools. This protein also controls the formation and hydrolysis of retinyl esters: CRBP-I-bound retinol is esterified exclusively by LRAT; apo-CRBP-I was reported to inhibit LRAT and to facilitate REH. It was also reported that CRBP-I directs retinol and retinal to specific ROLDH and RALDH isoforms, while limiting access to others. CRABP-I was shown to enhance retinoic acid degradation. Retinoid-binding proteins are shown in red; enzymic activities are shown in blue. See text for references.

conditions of maternal vitamin A sufficiency [81]. Hence, under these conditions, CRBP-I does not appear to be essential for retinoic acid synthesis during development.

CRBP-II

The exclusive expression of CRBP-II in the absorptive cells of the small intestine, and the high concentration of the protein in this location (comprising up to 1% of all soluble proteins [5]), suggests that CRBP-II has a role in the initial processing of retinol from food. Vitamin A is present in foods either as β -carotene, derived from plant sources, or as retinyl esters, originating from animal sources. The first step in the metabolism of β -carotene is an oxidative cleavage, generating retinal, which is subsequently reduced to retinol. Retinyl esters are hydrolysed to produce free retinol upon entry into the enterocytes. Retinol, derived either from β -carotene or from dietary retinyl esters, is then esterified and the resulting retinyl esters are packaged into chylomicrons and secreted into blood via the lymphatic system (reviewed in [8]). Similar to CRBP-I, CRBP-II seems to determine the specificity of enzymes that may gain access to retinol and retinal. For example, it has been shown that soluble enzymes

present in extracts of intestinal mucosa can reduce free retinal, but not retinal bound to CRBP-II. In contrast, retinal reductase in mucosal microsomal fractions readily produced retinol when presented with CRBP-II-bound retinal [84]. CRBP-II was also reported to block the re-oxidation of retinol to retinal, most likely because protein-bound retinol is not available to enterocyte retinol dehydrogenases [84]. CRBP-II also participates in regulation of the esterification of retinol in the enterocyte. Similar to hepatic ARAT, intestinal ARAT is incapable of processing CRBP-II-bound retinol. In contrast, LRAT catalyses retinol esterification more efficiently when the substrate is presented to the enzyme bound to CRBP as compared with free retinol [79]. Unlike apo-CRBP-I, apo-CRBP-II does not inhibit the activity of LRAT, and thus formation of retinyl esters in the intestine is expected to proceed in times of low intake of vitamin A [79].

Functions of CRABPs

Two binding proteins with a high affinity for all-*trans*-retinoic acid have been identified, CRABP-I and CRABP-II. The two isoforms are highly similar, displaying 74% sequence identity between human CRABP-I and -II, and are also extremely conserved between species. For example, rat, mouse and bovine

CRABP-I are all identical, and they differ from the human protein by a single amino acid residue [85–90]. In the three-dimensional structure of holo-CRABPs, retinoic acid is buried in the binding pocket such that its carboxy head group interacts with two arginine residues and one tyrosine residue at the bottom of the pocket. The β -ionone ring is twisted into a *cis*-like configuration relative to the isoprene tail, and fits at the entrance to the pocket such that only one edge is accessible to the solvent [13]. Hence, similarly to CRBPs (Figure 3), access to the entrance of the ligand-binding pocket of CRABP appears to be restricted, implying that significant conformational changes are required to allow the ligand to move out of the site.

The two isoforms of CRABP display different patterns of expression across cells and developmental stages. In the adult, CRABP-I is expressed almost ubiquitously [5], while CRABP-II is only expressed in the skin [5], uterus and ovary [66,91], and the choroid plexus [92]. Both CRABPs are widely expressed in the embryo, although they do not usually co-exist in the same cells [93]. The biological functions of CRABPs are not completely understood at present. It was reported that mice in which the genes for either CRABP-I or CRABP-II have been disrupted appear to be essentially normal, except for some defects in limb development [94,95]. It is interesting to note, in regard to these observations, that retinoic acid acts as a morphogen in vertebrate limb development. It was shown, for example, that exogenously generated concentration gradients of retinoic acid across the limb bud in the developing chick induced the formation of additional digits. Furthermore, pattern formation under these circumstances was found to be determined by the shape of the gradient rather than by the actual concentration at the source [96,97]. In view of the noted defects in limb development in CRABP-null mice, CRABPs may be involved in generation of appropriate retinoic acid concentration gradients in the limb bud. However, the molecular mechanisms underlying these effects remain to be clarified.

Similarly to other retinoid binding proteins, it has been proposed that CRABPs serve to solubilize and protect their ligand in the aqueous cytosol, and that they transport retinoic acid between different cellular compartments. It was thus suggested that CRABPs act to deliver their ligand to the nucleus, where it will bind and activate retinoid receptors [98]. In support of this notion, it was demonstrated that both CRABP isoforms are present not only in cytosol but also in the nuclei of cells [99]. The exceptionally high level of conservation of CRABPs across species, and the distinct expression pattern of the two isoforms, imply not only that they play critical roles in retinoic acid biology, but also that the two isoforms serve distinct functions. It was proposed that functional differences between the two isoforms may stem from differences in their binding affinity for retinoic acid [100]. However, the equilibrium dissociation constants of complexes of CRABP-I and -II with retinoic acid differ only by 2-fold ([101], see Table 1) and it is difficult to see how this small difference could account for distinct functions of the two proteins. The question thus remains: what are the different roles that CRABP-I and CRABP-II play in regulating retinoic acid action? Recent studies have begun to shed some light on this question.

CRABP-I

CRABP-I has been shown to modulate the activities of enzyme(s) that catalyse the metabolic transformation of retinoic acid. It was reported that, when bound to CRABP-I, retinoic acid serves as a substrate for enzymes in rat testes microsomes, giving rise to the polar metabolites 3,4-didehydro-, 4-hydroxy-, 4-oxo-,

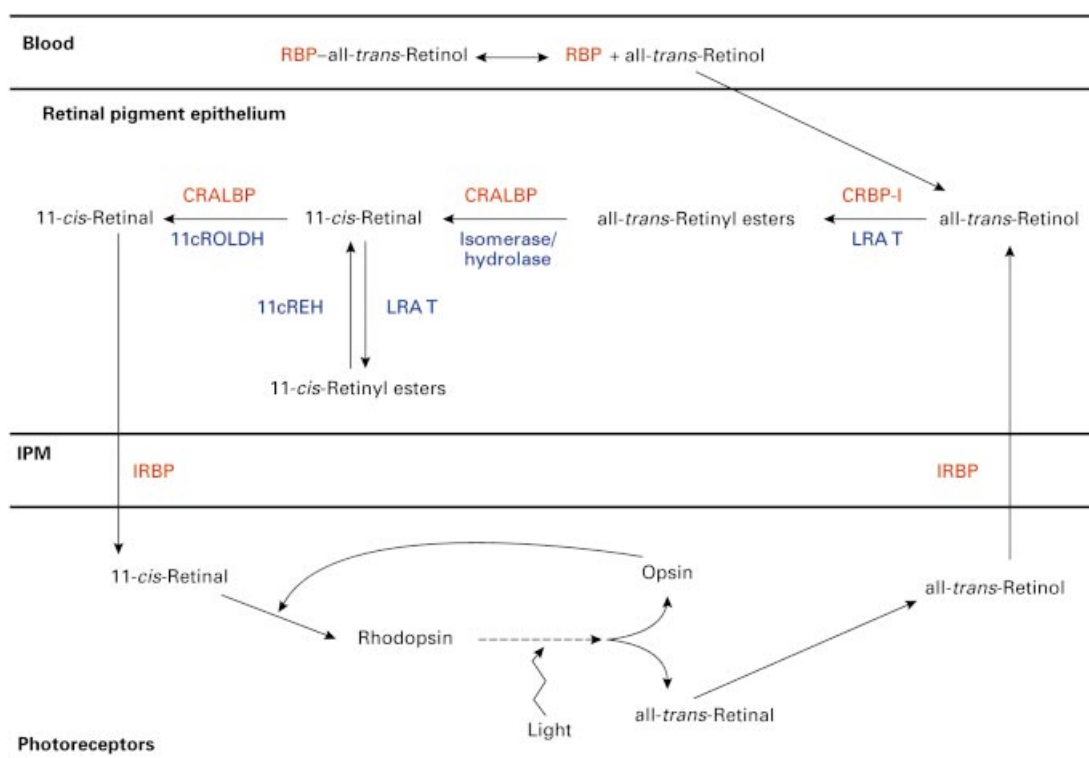
16-hydroxy-4-oxo- and 18-hydroxy-retinoic acids [102]. These metabolites are likely to be products of members of the CYP26 family, cytochromes P450 that specifically metabolize retinoic acid [103–106]. Additionally, it was shown that increased expression of CRABP-I in F9 teratocarcinoma cells enhanced the rate of formation of polar metabolites from retinoic acid [107], and that the sensitivity of F9 cells to retinoic acid-induced differentiation is inversely correlated with the cellular level of CRABP-I [108]. The details of the mechanisms by which CRABP-I regulates the activities of retinoic acid-metabolizing enzyme(s), and the identity of the specific enzyme(s) affected by this binding protein, remain to be clarified. Nevertheless, these observations suggest that CRABP-I acts to dampen cellular responses to retinoic acid, and that this effect reflects the ability of the binding protein to enhance the activity of an enzyme(s) that catalyses the degradation of the hormone.

CRABP-II

Both CRABP-I and -II are present in the nucleus, implying that both proteins may serve to deliver retinoic acid to the transcription factor that is activated by it, namely the retinoic acid receptor (RAR). However, it was recently shown that the mechanisms by which CRABP-I and -II deliver their ligand to RAR are fundamentally different [101]. As discussed above, transfer of retinoic acid from CRABP to RAR may occur either via the aqueous phase (Figure 4a) or by 'channelling' between the two proteins, i.e. through direct protein–protein interactions and in a way that bypasses the bulk solution (Figure 4b). Kinetic studies of the processes through which retinoic acid moves from CRABP-I or CRABP-II to RAR revealed that, while CRABP-I acts as a passive vehicle which binds and releases its ligand according to concentration gradients, CRABP-II delivers retinoic acid to the RAR in a direct collisional process. It was shown further that channelling of retinoic acid between CRABP-II and RAR results in a significant facilitation of the formation of holo-RAR [101]. These observations suggest that, in cells, expression of CRABP-II (but not of CRABP-I) would enhance the transcriptional activity of RAR. This conclusion has been supported by the observation that overexpression of CRABP-II (but not CRABP-I) results in a marked stimulation of transcription of a reporter gene driven by a RAR response element [101,109]. It was also reported that loss of expression of CRABP-II is correlated with resistance of mammary carcinoma cell lines to retinoic acid-induced growth arrest [110], and that expression of a CRABP-II antisense construct in SCC cells results in reduced sensitivity to retinoic acid-induced cell-cycle arrest [111]. Hence expression of CRABP-II appears to play a role in sensitizing cells to retinoic acid. Interestingly, the expression of CRABP-II is associated with cells that synthesize relatively large amounts of retinoic acid [92,112,113], suggesting that cells with an increased physiological requirement for retinoic acid require up-regulation of both the synthesis of the hormone and the level of expression of CRABP-II, allowing for efficient and rapid delivery of retinoic acid to the RAR.

Ocular retinoid-binding proteins

Quantitatively, the main user of vitamin A in the body is the eye, where the vitamin's metabolite 11-*cis*-retinal serves as the chromophore for the visual pigment rhodopsin. Scheme 2 depicts the major steps in the visual cycle through which 11-*cis*-retinal is produced in the eye, provided for regeneration of visual pigments, and replenished following bleaching of rhodopsin that occurs upon light absorption (for a detailed review of the visual cycle,



Scheme 2 Proposed involvement of retinoid-binding proteins in the visual cycle

all-trans-Retinol enters the eye from blood via retinal pigment epithelium cells, where it is esterified to *all-trans*-retinyl esters by LRAT. An isomerization activity, catalysed by isomerohydrolase [or, perhaps, by sequential catalysis by retinyl ester hydrolase REH and *all-trans*-retinol isomerase], leads to the formation of 11-*cis*-retinol. 11-*cis*-Retinol may either be esterified to form 11-*cis*-retinyl esters and stored, or oxidized by 11-*cis*-retinol dehydrogenase (11cROLDH) to the visual chromophore 11-*cis*-retinal. 11-*cis*-Retinal exits the pigment epithelium, and moves across the IPM to photoreceptor cells, where it binds to opsin to form the visual pigment rhodopsin. Light absorption by rhodopsin results in isomerization of rhodopsin-bound 11-*cis*-retinal, initiating visual signalling. *all-trans*-Retinol thus formed is enzymically reduced to *all-trans*-retinol. *all-trans*-Retinol is then transported back to retinal pigment epithelium for re-isomerization and re-oxidation. Similar to its role in the liver, CRBP-I was reported to deliver *all-trans*-retinol to LRAT of the retinal pigment epithelium. Due to its high affinity for 11-*cis*-retinol, CRALBP enables the *all-trans*- to 11-*cis*-isomerization reaction to occur. CRALBP also enhances the 11-*cis*-ROLDH reaction. This effect is likely to stem from direct interactions between CRALBP and 11-*cis*-ROLDH. Transport of 11-*cis*-retinal and *all-trans*-retinol across the IPM is mediated by IRBP. Retinoid-binding proteins are shown in red; enzymic activities are shown in blue. See text for references.

see [1]). *all-trans*-Retinol, the circulating form of vitamin A, enters the eye via the retinal pigment epithelium, where it is esterified to *all-trans*-retinyl esters. It is generally believed that these esters serve as a substrate for an isomerohydrolase that couples cleavage of the ester bond to isomerization across the retinoid 11–12 double bond, generating 11-*cis*-retinol [114]. Although the details of this model have been questioned by more recent observations [115], it is clear that isomerization occurs at the level of retinol, resulting in the generation of 11-*cis*-retinol. This compound may then be either esterified with long-chain fatty acids to form 11-*cis*-retinyl esters [116,117] or oxidized to the visual chromophore 11-*cis*-retinal [118]. As light sensing and subsequent visual transduction take place not in retinal pigment epithelium but in the outer segments of photoreceptor cells (where rhodopsin is embedded in intracellular membranous discs), regeneration of active visual pigments requires a continuous flux of 11-*cis*-retinal from pigment epithelium to photoreceptor cells. Within the photoreceptors, 11-*cis*-retinal binds to opsin to form active rhodopsin. Following absorption of light by rhodopsin, the visual chromophore is isomerized to *all-trans*-retinal, which is then enzymically reduced to *all-trans*-retinol. As photoreceptor cells do not contain the enzymic machinery necessary for regenerating 11-*cis*-retinal from the parent molecule, *all-trans*-retinol is transported back to the pigment epithelium for re-isomerization and oxidation. Thus the visual cycle

critically includes continuous shuttling of retinoids between photoreceptor and pigment epithelium cells via the aqueous space of the interphotoreceptor matrix which separates the two cell types.

RBP, CRBP and CRALBP are likely to play similar roles in the eye to those that they fulfil in other tissues. For example, retinol bound to CRBP-I was shown to be an efficient substrate for retinal pigment epithelium LRAT [119]. In addition to these proteins, the eye contains two unique retinoid-binding proteins. These proteins, CRALBP and IRBP, appear to have specific functions in the visual cycle.

Functions of CRALBP

A retinoid-binding protein that displays a high selectivity towards 11-*cis*-retinoids has been identified in retinal pigment epithelium and in the neural retina in the eye, and was termed CRALBP [120,121]. The unique role that 11-*cis*-retinoids play in vision, and the location of CRALBP in the retina, immediately suggested that this protein may be involved in the visual process. Indeed, mutations in the human CRALBP gene can result in recessive retinitis pigmentosa [122]. Interestingly, in addition to its presence in the retinal pigment epithelium and neural retina, CRALBP is also found in the pineal gland [123], ciliary body [124], cornea [125], optic nerve and brain [126]. It was reported that CRALBP

isolated from bovine brain, unlike CRALBP derived from the retina, is not associated with any endogenous retinoids [126]. Hence this protein may have other, as yet uncharacterized, functions that are unrelated to the visual process or to its retinoid-binding capabilities [1]. Available information implicates CRALBP in two separate functions in supporting the visual cycle.

Isomerization of all-*trans*-retinoids to 11-*cis*-retinoids

The process by which 11-*cis*-retinoids are generated in retinal pigment epithelium is not completely understood at present. Possible mechanisms include coupling the hydrolysis of all-*trans*-retinyl esters to isomerization, yielding 11-*cis*-retinol [114], or the presence of an enzyme that catalyses the direct isomerization of all-*trans*- to 11-*cis*-retinol [115]. It was recently shown that the formation of 11-*cis*-retinol by isolated retinal pigment epithelium microsomal membranes critically requires the presence of CRALBP [115,127]. It was proposed that CRALBP acts by sequestering 11-*cis*-retinol, thereby driving the energetically unfavourable isomerization reaction by mass action [115]. Alternatively, it was reported that the isomerase is subject to an end-product inhibition by 11-*cis*-retinol, suggesting that CRALBP facilitates the isomerization reaction by binding the product, thereby relieving the inhibition [127]. It was shown further that BSA, a 'non-specific' retinoid-binding protein, can also facilitate the isomerization reaction, albeit at a much higher concentration than the concentration at which CRALBP displays a similar effect [127]. Hence the ability of CRALBP to enable retinol isomerization in the retinal pigment epithelium does not appear to originate from specific interactions between the binding protein and the enzyme, but to stem from the strong binding affinity of the protein for 11-*cis*-retinol.

Regulation of the metabolic fate of 11-*cis*-retinol

11-*cis*-Retinol, generated in the retinal pigment epithelium, is at a metabolic branch point and can be acted upon either by LRAT, which catalyses its esterification to 11-*cis*-retinyl esters, or by 11-*cis*-retinol dehydrogenase, which converts it into 11-*cis*-retinal (Scheme 2). It has been demonstrated that CRALBP governs the partitioning of 11-*cis*-retinol between these alternative metabolic pathways. Hence it was reported that esterification of 11-*cis*-retinol is inhibited, while oxidation of this compound is stimulated, in the presence of CRALBP. The latter effect was shown to be specific to CRALBP and was not manifested when the substrate was presented bound to serum albumin [128]. The inhibition of the LRAT reaction by CRALBP can be readily explained by considering that introduction of a binding protein with a high affinity for 11-*cis*-retinol into reaction mixtures decreases the concentration of free substrate available to the enzyme. The molecular mechanism underlying the ability of CRALBP to stimulate the enzymic oxidation of 11-*cis*-retinol to 11-*cis*-retinal has not been clarified as yet, but is likely to involve protein-protein interactions between CRALBP and 11-*cis*-retinol dehydrogenase, resulting either in 'channelling' of the substrate to the enzyme or in allosteric activation of the enzyme by the binding protein [128].

Functions of IRBP

The regeneration of visual pigments in photoreceptor outer segments relies on the supply of 11-*cis*-retinal from the pigment epithelium; in turn, the maintenance of an adequate pool of 11-*cis*-retinal depends on rapid movement of all-*trans*-retinol, which

is generated in photoreceptors following bleaching of rhodopsin, back to the retinal pigment epithelium, where it can be re-isomerized and re-oxidized (Scheme 2). The question thus arises: how do retinoids, compounds that are poorly soluble in water, move across the interphotoreceptor matrix (IPM) at rates that are sufficient for sustaining visual function under light conditions?

The main soluble protein component of the IPM is IRBP, an ~ 140 kDa glycoprotein which is synthesized in photoreceptors and secreted into the IPM, where it binds two classes of ligands: retinoids and long-chain fatty acids. It has been repeatedly suggested that IRBP serves as a carrier for retinoids between photoreceptor and retinal pigment epithelium cells (reviewed in [129]). Such a function for IRBP is implied by the observations that the composition of retinoids that are endogenously associated with the protein is modulated by light [130], and that binding of retinoids to IRBP stabilizes them against degradation [131,132]. It was also reported that IRBP can take up 11-*cis*-retinal from the retinal pigment epithelium and efficiently deliver this chromophore to rod outer segments [133–135]. However, the exact role of IRBP in the transport process remains to be clarified. Theoretically, IRBP could serve simply as a storage compartment for retinoids in the IPM with the ability to bind and release these ligands according to their concentration gradients. Alternatively, the protein could function to specifically target particular retinoids to certain locations in the eye, i.e. to deliver 11-*cis*-retinal to photoreceptors and to transport all-*trans*-retinol to the retinal pigment epithelium. The latter possibility would require the existence of a mechanism that enables IRBP to 'sense' different locations within the IPM.

A potential mechanism that may allow IRBP to target 11-*cis*-retinal to photoreceptor cells has been suggested [22]. IRBP contains three retinoid-binding sites, as well as one or two sites for long-chain fatty acids. One of the retinoid sites can accommodate either all-*trans*-retinol or 11-*cis*-retinal [23]. The two additional sites appear to be selective towards all-*trans*-retinol and 11-*cis*-retinal respectively ([22]; and N. Shaw and N. Noy, unpublished work). It was reported that the long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA), but not more saturated fatty acids, inhibits the ability of IRBP to bind a second molecule of 11-*cis*-retinal. It was further shown that, while DHA facilitates the rate of dissociation of 11-*cis*-retinal from the regulatory site by about an order of magnitude, the fatty acid has little effect on binding of either all-*trans*-retinol or 11-*cis*-retinal in the remaining IRBP retinoid-binding sites [22]. DHA is highly enriched in photoreceptor cells, where it comprises approx. 50% of the acyl chains of phospholipids and, correspondingly, of non-esterified fatty acids [22,136]. Based on the specific effect of DHA on the interactions of IRBP with 11-*cis*-retinal, and considering the concentration gradient of this fatty acid across the IPM, it was suggested that DHA may serve as the 'switch' that allows IRBP to distinguish between different regions of the IPM, and which triggers the release of 11-*cis*-retinal at the correct location. As summarized in Figure 5, this model proposes that while IRBP is in the vicinity of the retinal pigment epithelium, where the concentration of DHA is low, it will possess a high affinity for 11-*cis*-retinal and associate with it. Movement of the protein to the vicinity of photoreceptor cells will expose it to high levels of DHA, resulting in rapid release of 11-*cis*-retinal from the regulated binding site at this location [22].

Despite the body of evidence indicating that IRBP is likely to function in mediating the transport of retinoids across the IPM, it should be noted that it was recently reported that disruption of the IRBP gene in mice does not result in significant decreases in the rates of either the recovery of 11-*cis*-retinal or the regeneration

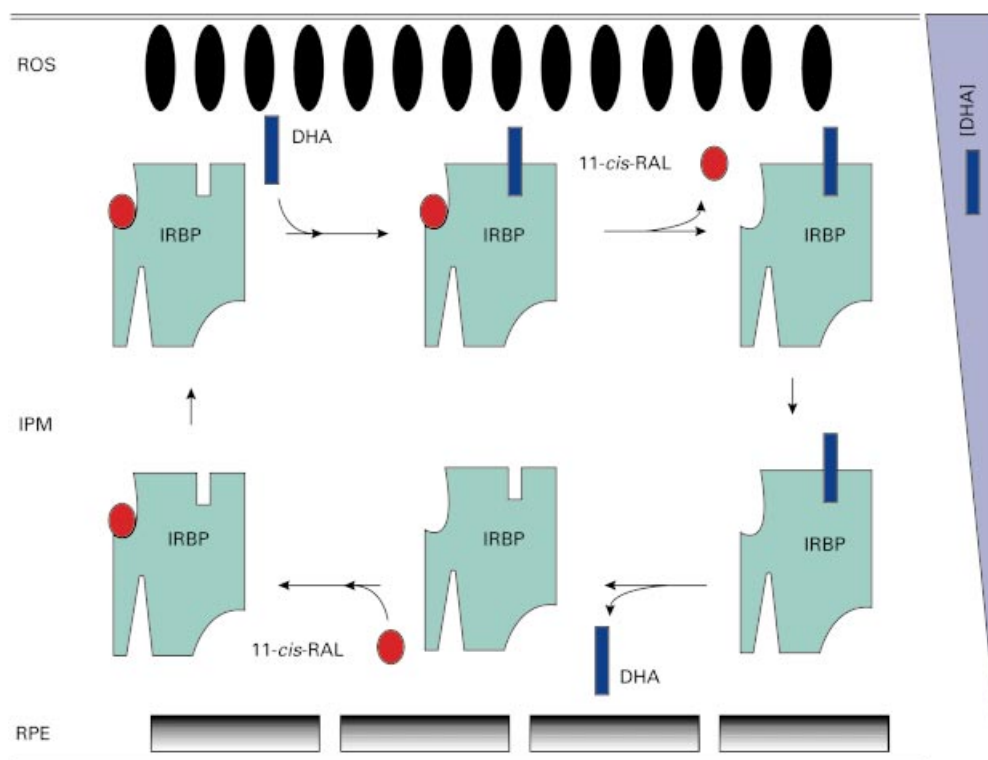


Figure 5 Model for targeting of retinoids by IRBP

The model is based on the findings that: (1) binding of the long chain fatty acid DHA to IRBP switches one of the protein's 11-*cis*-retinal (11-*cis*-RAL) binding sites from a state of a high affinity to a state of low affinity for this ligand, and (2) DHA is highly enriched in photoreceptor cells, resulting in a steep concentration gradient of this fatty acid across IPM. It has been proposed that, because the region of the IPM that is close to the retinal pigment epithelium (RPE) is relatively poor in DHA, IRBP possesses a high affinity for 11-*cis*-retinal at this location. Movement of IRBP, loaded with 11-*cis*-retinal, to the vicinity of photoreceptor cells exposes the protein to a high concentration of DHA. Binding of DHA to IRBP results in the release of 11-*cis*-retinal close to the photoreceptor surface. ROS, rod outer segment.

of rhodopsin following light exposure [137]. Nevertheless, the IRBP-deficient mice displayed progressive photoreceptor degeneration. These observations implicate IRBP in serving a role in photoreceptor survival, but question whether it is essential for a normal rate of visual pigment regeneration [137]. Hence the complete spectrum of the biological functions of IRBP remains ambiguous.

CONCLUSIONS AND PERSPECTIVES

Vitamin A metabolites are essential for numerous biological processes. They are critical for vision, play important roles in regulating proliferation and differentiation in both fetal and adult tissues, and have been implicated in modulating immune function. Retinoids are found *in vivo* primarily bound to various retinoid-binding proteins which utilize diverse mechanisms to modulate the biological activities of their cognate ligands (Table 2). Some retinoid-binding proteins govern the concentrations of retinoids in plasma and in cells, as well as the direction and magnitude of fluxes through which retinoids move between extra- and intra-cellular compartments. Other retinoid-binding proteins appear to act by specifically delivering their ligands to particular targets, such as enzymes or transcription factors. Kinetic evidence suggests that these activities are likely to be mediated by direct protein–protein interactions between the binding protein and the target, resulting in ‘channelling’ of

retinoids to the appropriate metabolic pathway or activity site. However, no molecular-level information on the interactions of retinoid-binding proteins with putative targets has been put forward to date. Progress on this important issue has long been hampered by the lack of availability of retinoid-metabolizing enzymes in pure forms, and hence most of the current information is based on work with crude cellular fractions. Recently the genes for LRAT [138] and for several isoforms of retinol and retinal dehydrogenases [139–147] have been cloned. The availability of these proteins should allow for better clarification of the mechanisms of action of specific retinoid-binding proteins.

Another issue on which virtually no information is currently available relates to the involvement of retinoid-binding proteins in modulating the transport, metabolism and action of bioactive isomers and metabolites of retinoids, such as 9-*cis*-, 13-*cis*- and 4-oxo-retinoids. Future goals also include delineating the structures and obtaining a better understanding of the functions of the less well characterized retinoid-binding proteins IRBP and CRALBP. Particularly intriguing in regard to the latter are the observations that CRALBP is present in areas in the brain that have no known function for the ligands of this protein, i.e. 11-*cis*-retinoids. Studies of retinoid-binding proteins in cultured cells and analyses of transgenic and null animal models for specific retinoid-binding proteins are expected to continue to allow for testing predictions derived from *in vitro* studies, and to provide additional insights into the roles that retinoid binding proteins play in different physiological states.

Table 2 Proposed functions of retinoid-binding proteins

See text for references. Abbreviations: ROLDH, retinol dehydrogenase; RALDH, retinal dehydrogenase; RPE, retinal pigment epithelium; 11cROLDH, 11-*cis*-retinol dehydrogenase.

Protein	Proposed function	Target (proposed mechanism)	Physiological role
RBP	Enable secretion of retinol from the liver Carry retinol in blood	Only holo-RBP is recognized by the secretion machinery in Golgi (?)	Maintenance of plasma retinol homeostasis
CRBP-I	Draw retinol from blood into cells Solubilize retinol and retinal, protect cells from membranolytic retinoid action Holo-CRBP-I: direct retinol to esterification by LRAT Apo-CRBP-I: inhibit retinol esterification Apo-CRBP-I: stimulate retinyl ester hydrolysis	(Shift of the intra/extracellular equilibrium of retinol) (Sequestration) LRAT (direct interactions) LRAT (direct interaction) Retinyl ester hydrolase (product sequestration? direct interactions?) ROLDH/RALDH (direct interactions)	Increase of intracellular retinol pools; regulation of vitamin A storage; perhaps involvement in regulating retinoic acid synthesis
CRBP-II	Direct retinol and retinal to specific dehydrogenases Direct retinal to reduction by microsomal enzymes	Microsomal retinal reductase (direct interactions)	Regulation of initial processing and production of retinyl esters from dietary vitamin A in the intestine
CRABP-I	Block re-oxidation of retinol to retinal Direct retinol to esterification by LRAT Enhance conversion of retinoic acid into polar metabolites	(Sequestration of retinol) LRAT (direct interactions) CYP26 family members (direct interactions)	Moderation of cellular response to retinoic acid
CRABP-II	'Channel' retinoic acid to RAR	RAR (direct interactions)	Enhancement of cellular response to retinoic acid
CRAALBP	Enable isomerization of all- <i>trans</i> -retinol to 11- <i>cis</i> -retinol Enhance oxidation of 11- <i>cis</i> -retinol to 11- <i>cis</i> -retinal	(Sequestration of 11- <i>cis</i> -retinol) 11cROLDH (direct interactions)	Control of generation of 11- <i>cis</i> -retinal in RPE
IRBP	Transport retinoids across the IPM Maintain photoreceptor integrity	?	Regeneration of rhodopsin

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