Colocalization of 11-cis Retinyl Esters and Retinyl Ester Hydrolase Activity in Retinal Pigment Epithelium Plasma Membrane

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PURPOSE. To identify the subcellular locale of 11-cis retinyl esters in bovine retinal pigment epithelium (RPE) and to characterize the enzymic mechanism responsible for liberation of 11-cis retinoids in this compartment.

METHODS. Endoplasmic reticulum (ER)-enriched and plasma membrane (PM)-enriched protein fractions were prepared from bovine RPE microsomes using sequential discontinuous sucrose and Percoll gradient fractionation. Enzyme markers for ER (such as carboxylesterase), and PM (such as 5′-nucleotidase [5′-ND]; alkaline phosphatase [AP]; and ouabain-sensitive Na⁺,K⁺-ATPase [ATPase]) were used to identify the subfractions. Membrane-associated retinoids were quantified by high-performance liquid chromatography (HPLC) and retinyl ester hydrolase (REH) activities were determined by radiometric and chromatographic (HPLC) means.

RESULTS. Chromatographic analyses of membrane-associated retinoids showed that 11-cis retinyl esters are localized mainly in PM-enriched fractions, whereas all-trans retinyl esters are associated predominantly with ER-enriched membranes; profiles of the distribution of 11-cis- and all-trans REH activities were consistent with the retinyl ester distribution. Further purification of the crude PM fraction yielded a fraction (P2) that was significantly enriched with 5′-ND (fivefold), ATPase (15-fold), AP (10-fold), and 11-cis retinyl ester hydrolase (11-cis REH; threefold) activities, but was relatively devoid of carboxylesterase and all-trans REH activities. Apparent kinetic constants (Kₘ and Vₐₘₚₚ) for 11-cis REH activity in P2 were 18 μM and 1800 picomoles/min per mg, respectively.

CONCLUSIONS. This is the first identification of an 11-cis-specific REH activity in RPE plasma membrane. Results from these studies demonstrate the capacity of RPE plasma membranes to accommodate and hydrolyze 11-cis retinyl esters. Plasma membrane storage and mobilization of 11-cis retinyl esters represents a novel compartmentalization of retinoid metabolism that is distinct from the sites where 11-cis retinoids are produced. The implication of these findings for present theories of visual chromophore biosynthesis are discussed. (Invest Ophthalmol Vis Sci. 1998;39:1312-1319)

Biosynthesis of 11-cis retinoids from dietary vitamin A (all-trans retinol) is a distinctive feature of the visual system, which relies on an isomerohydrolase enzyme in retinal pigment epithelium (RPE) membranes.1,2 The isomerohydrolase enzyme couples the free energy of all-trans retinyl ester hydrolysis to an isomerization reaction to generate 11-cis retinol.3 The liberation of 11-cis retinol represents a branchpoint in the visual cycle in which either 11-cis retinal1-6 or 11-cis retinyl esters may be produced.7 It is clear that 11-cis retinal biosynthesis in the RPE supports rhodopsin biosynthesis in the retina; however, the role of 11-cis retinyl esters stored in RPE membranes has not been examined.

There are various lines of evidence that suggest the presence of a regulated mechanism for 11-cis retinyl ester mobilization. For example, newly admitted all-trans retinol is steadily incorporated into the endogenous 11-cis retinyl palmitate pool during dark adaptation6; 11-cis retinyl ester concentration in the RPE also increases during this period.9,10 Moreover, it has been shown that 11-cis retinyl esters are selectively used during light adaptation.10-12 Thus, it is likely that stored 11-cis retinyl esters are used to produce visual chromophore through an accessory metabolic pathway.

Two possible mechanisms for 11-cis retinyl ester utilization in the RPE are 11-cis retinyl ester hydrolysis, and lecithin: retinyl acyltransferase (LRAT) activity. The capacity of LRAT activity to catalyze the formation of retinyl esters has been well documented.13,14 In addition, LRAT activity also has been shown to catalyze an 11-cis retinyl ester-11-cis retinol exchange reaction in which the palmitate moiety of in situ synthesized 11-cis retinyl palmitate is transferred to 11-cis retinol.15 Thus, LRAT activity seems to have the capacity to synthesize 11-cis retinyl esters using an esterification reaction and also the capacity to liberate 11-cis retinol from the synthesized 11-cis retinyl ester pool by reversal of the esterification reaction. Alternatively, 11-cis retinol may be liberated using an
11-cis retinyl ester hydrolase enzyme. Biochemical properties of 11-cis retinyl ester hydrolysis have been investigated in human RPE and in bovine RPE. Studies designed to address the possibility that reversibility of the LRAT reaction (rLRAT) may have been responsible for 11-cis retinol liberation suggested no contribution from rLRAT to the observed 11-cis retinyl ester hydrolase (REH) activity. Further investigation of these two mechanisms will be required to reconcile the apparent discrepancies.

It is noteworthy that all the key enzymes involved in the visual cycle are intimately associated with membranes. Consequently, purification efforts have been largely unsuccessful, with the exception of a putative 11-cis-specific retinol dehydrogenase. This impasse has caused investigators to use microsomal membranes as an enzyme source for biochemical characterizations. In this report, we have addressed the subcellular localization of 11-cis retinyl esters and 11-cis REH activity. Findings from this investigation reveal that 11-cis REH activity and 11-cis retinyl esters are colocalized in plasma membranes of the RPE. The production of 11-cis retinoids from all-trans retinol occurs at a site that is spatially separated from the plasma membrane compartment. Thus, it is reasonable to suggest that 11-cis retinoids may be mobilized through two distinct subcellular pathways. Although the relationship between these two pathways is not presently clear, it is likely that these processes operate in a complementary fashion to provide visual chromophore for daily photopigment renewal and photopigment regeneration during periods of intense or prolonged light exposure.

MATERIALS AND METHODS

Materials

All-trans retinyl palmitate, bovine serum albumin, dithiothreitol, disodium EDTA, all-trans retinol, all-trans retinyl palmitate, 5'-nucleotidase (5'-ND), alkaline phosphatase (AP), and carboxylesterase assay reagents were purchased from Sigma Chemical (St. Louis, MO). Percoll was obtained from Pharmacia Biotech (Uppsala, Sweden). Ouabain was purchased from Research Biochemicals International (Natick, MA). [9,10-3H]Palmitic acid (specific activity 37 Ci/millimole) was purchased from DuPont-NEN (Rochester, NY). Purified, unlabeled 11-cis retinyl palmitate was a gift from Hoffman-La Roche (Nutley, NJ). All other reagents were purified by high-performance liquid chromatography (HPLC) and quantified by visible spectrophotometry before use in REH assays. Econo-L liquid scintillation cocktail and HPLC grade solvents were obtained from Fisher Scientific (Houston, TX). Quantitation of [3H] and [14C] was achieved with a liquid scintillation analyzer (model 2200CA; Packard Instrument, Downers Grove, IL).

Methods

Substrate Preparation. [3H]11-cis and [3H]all-trans retinyl palmitate substrates were prepared by reacting the respective vitamin A alcohols with [9,10-3H]palmitic acid anhydride as previously described. Specific activity of the retinyl ester substrates was adjusted to 65,000 to 75,000 dpm/nanomole by the addition of unlabeled 11-cis or all-trans retinyl palmitate. Substrate was routinely added to reaction mixtures in 10 μl ethanol.

Preparation of Subcellular Fractions. All procedures were performed on ice, or at 4°C. Microsomal protein was prepared from homogenates of fresh RPE according to methods previously described. Microsomal proteins were separated immediately on a discontinuous sucrose gradient into plasma membrane (PM)-enriched and endoplasmic reticulum (ER)-enriched fractions following methods described by Touster et al. Briefly, microsomal membranes, homogenized in 10 ml Tris-buffered sucrose (57%), were placed at the bottom of a clear polycarbonate centrifuge tube followed the addition of 18 ml 34% sucrose and 8 ml 8.5% (0.25 M) sucrose. The sucrose gradient was centrifuged for 22 hours at 75,500g. The resulting gradient fractions (S1-S5, from the top to the bottom of the centrifuge tube) were assayed for PM and ER enzyme markers (i.e., 5'-ND and carboxylesterase, respectively) and for 11-cis and all-trans REH activity. Protein fractions were stored at −85°C after dilution to less than 5% sucrose (vol/vol). All protein determinations were made by the dye-binding method using bovine serum albumin as a standard.

Subfractionation of Plasma Membrane Proteins. Further purification of the PM-enriched fraction generated by discontinuous sucrose density gradient centrifugation (S2) was achieved by using self-forming gradients of Percoll as described by Ottonello and Mariani. Optimal resolution of the enzyme markers was achieved when the density of the isosmotic Percoll-protein solution was 1.045 and the centrifugation was performed at 12,000g for 20 minutes. The recovered fractions (P1-P18) were assayed for protein concentration, 5'-ND, carboxylesterase, ouabain-sensitive Na+,K+-ATPase (APase), AP, 11-cis, and all-trans REH activities.

Enzyme Marker Studies. Carboxylesterase activity was determined according to the methods of Mentel and Heymann. Briefly, production of o-nitrophenol was measured spectrophotometrically (420 nm) during the incubation of protein samples (10–250 μg) with o-nitrophenyl acetate (0.18 M stock in ice-cold methanol). Protein and assay buffer (2.7 ml 20 mM potassium phosphate, 1 mM EDTA, and 0.1% Triton X-100, pH 7.4) were preincubated in a quartz cuvette for 10 minutes at 22°C before the addition of o-nitrophenyl acetate (3 mM). Product abundance versus time measurements were then taken every 30 seconds for a period of 10 minutes. Rates were corrected for protein concentration and nonenzymatic activity. 5'-ND activity was determined as described in Sigma Procedure No. 265-UV or Procedure No. 675, which describe the quantitation of 5'-ND activity through the rate of nicotinamide adenine dinucleotide (NAD) formation and inorganic phosphorus production, respectively. Spectrophotometric analyses of rate versus time (340 nm for NAD production) and single-component analysis (660 nm for inorganic phosphorus production) were performed. 5'-ND activities were corrected for protein concentration and nonspecific phosphatase activity when quantitating inorganic phosphorus. Heat-denatured protein was used to correct for nonenzymatic carboxylesterase and 5'-ND activities. APase activity was determined in the methods of Bergmeyer.
volume = 200 μl). It should be noted that, although the concentration of ethanol in the REH reaction mixtures is relatively high (5%, vol/vol), the effect on the rate of hydrolysis is negligible when compared with REH reaction mixtures in which the ethanol concentration is 0.5% to 1.0%. Thus, the higher ethanol concentration was used because it greatly enhances the solubility of the retinyl ester substrates. After a timed incubation at 37°C, aqueous and lipid phases of the reaction mixtures were partitioned, as described by Belfrage and Vaughan. One milliliter of the aqueous ([3H]palmitic acid) phase was removed, placed in 10 ml Econo-1 scintillation cocktail, and was analyzed for [3H] using a liquid scintillation counter. REH activity is represented as the molar amount of tritiated free fatty acid liberated/minute per milligram of protein. Samples were analyzed in triplicate during each experiment, and nonenzymatic hydrolysis of the substrates was assessed in each analysis using heat-denatured protein.

**Extraction and High-Performance Liquid Chromatography Analysis of Membrane-Associated Retinyl Esters.** Protein samples (5–10 mg protein in a total volume of 1 ml) were mixed with 2 ml absolute ethanol and were left at room temperature for 15 minutes. Retinoids were partitioned into 15 ml of petroleum ether (three 5-ml extractions). The petroleum ether extract was evaporated to dryness under a stream of N₂ and then redissolved in 200 to 500 μl 0.2% dioxane/n-hexane. The samples were analyzed isocratically (flow rate = 2 ml/min) by normal-phase HPLC on a silica column (Microsorb; Rainin Instrument, Woburn, MA; 4.6 × 150 mm). Peak absorbance was monitored at 325 nm with a photodiode array detector (model 168; Beckman Instruments, Berkeley, CA); absorption spectra (450-210 nm) for all peaks were obtained simultaneously. Chromatographic data were integrated using PC software (System Gold; Beckman). The analysis of retinyl esters in sucrose gradient fractions was performed similarly after sedimentation of membrane proteins by dilution and ultracentrifugation (150,000g, 60 minutes), or by precipitation with 10% trichloroacetic acid and ultracentrifugation.

**RESULTS**

**Subcellular Locale of 11-cis Retinyl Ester Hydrolase Activity**

Bovine RPE microsomes were subfractionated on a discontinuous sucrose gradient into five distinct membrane fractions (S1–S5). The membrane fractions were routinely analyzed for esterase (o-nitrophenyl-acetate esterase) and 5’-ND activity to confirm the enzymatic competence of these fractions and to assign a subcellular localization for 11-cis REH activity. The physical characteristics of the gradient, and the locale of PM and ER enzyme markers, were similar to those reported for subfractionated liver microsomes by Touster et al. Thus, the uppermost fraction (S1) contained very little protein and was relatively devoid of all measured enzyme activities (Fig. 1). Fraction S2 appeared as a thick band of white material, which migrated between the 34% (density = 1.15, 5°C) and the 0.25 M sucrose overlayer and contained the largest percentage of 5’-ND activity (48%; Fig. 1B). The intermediate fraction (S3) was relatively clear and contained moderate amounts of 5’-ND (20%) and carboxylesterase (30%) activities. The largest percentage of the protein recovered from the gradient was found in fraction S4 (44%; Fig. 1A); the specific activity and total recovery of carboxylesterase activity also was highest in this fraction (44%; Fig. 1C). The lowermost fraction (S5) was similar to S1 in that it was devoid of protein and enzyme activities.

**Analysis of REH activities revealed that 11-cis REH activity was primarily associated with the PM-enriched fraction (49%; Fig. 1D), whereas the greatest percentage of all-trans REH activity was recovered in the ER-enriched fraction (36%; Fig. 1E). It is noteworthy that the specific activity of 11-cis retinyl...**

**FIGURE 1. Distribution of 11-cis and all-trans retinyl ester hydrolase (REH) activities in sucrose gradient fractions. Freshly prepared bovine retinal pigment epithelium microsomal protein was separated on a discontinuous gradient of sucrose into five discrete protein fractions (S1-S5, from top to bottom of centrifuge tube). Gradient fractions were assayed for protein (A), 5'-nucleotidase (B), carboxylesterase (C), 11-cis REH activity (D), and all-trans REH activity (E). Conditions for the sucrose-density gradient and for the various enzyme assays are given in the text. In (A), the percentage of protein recovered in each fraction was determined by the amount of protein in that fraction divided by the total amount of protein recovered from all gradient fractions. Similarly, the percentages shown in (B, C, D, E) represent enzyme units in the indicated gradient fractions divided by the total enzyme units recovered in all gradient fractions. In (D) and (E), the total enzyme units recovered from all gradients were higher (up to 40%) than those loaded onto the sucrose gradient, probably as a result of the purification of enzymes from inhibitors. The data shown are mean values of six separate gradient preparations ± SD.**
ester hydrolysis was increased (twofold) in S2 relative to the microsomal value; all-trans REH-specific activity was comparable among fractions S2 to S4. These results, and the finding that approximately 25% of the all-trans REH activity is present also in fraction S2, prompted our focus on fractions S2 and S4.

**Subcellular Distribution of 11-cis Retinyl Esters**

Retinoids were extracted from RPE microsomal protein, fraction S2, and fraction S4 to determine whether a colocalization of REH activity and the respective retinyl ester substrate exists. Data obtained from HPLC analysis of extracts from microsomes, from S2, and from S4 are given in Figure 2 (left side). The identity of the retinyl esters found in these proteins was established by photo-diode array absorption spectra (shown on the right) and was confirmed by coelution with authentic retinyl ester standards. The quantitative data are provided in the figure legend. Microsomal protein contained 11-cis and all-trans retinyl esters (peak 1 and peak 2, respectively). Peak 2 also contains a retinoid species that demonstrates an absorption spectrum that is identical to that of all-trans retinyl palmitate (data not shown). A further investigation of peak 2 using different chromatographic conditions (i.e., 0.1% dioxane/n-hexane at a 1.5-ml/min flow rate) was effective to further separate the two coeluting retinoid species. The two peaks were collected from the HPLC eluate, mixed with authentic all-trans retinyl ester standards, and then were resolved on the 0.1% dioxane/n-hexane system. The chromatograms from these analyses indicated that the minor retinoid species was all-trans retinyl stearate and confirmed that the major component of peak 2 was in fact all-trans retinyl palmitate. The peak that precedes peak 2 in microsomes and S2 was determined to be 9-cis retinyl palmitate. The small peak that precedes peak 1 (retention time = 4.75 minutes) in these fractions was not identified. The localization of 11-cis and all-trans retinyl esters was found to be quite distinct. Using the microsomal 11-cis and all-trans retinyl ester concentrations as a reference (0.70 nanomoles/mg and 1.10 nanomoles/mg, respectively), 67% of the 11-cis retinyl esters were found in S2 and 70% of the all-trans retinyl esters were recovered in S4. Thus, 11-cis retinyl esters seem to be concentrated in a compartment where recovery of 11-cis REH activity is also greatest; a similar trend also was observed for all-trans retinyl esters and all-trans REH activity. The remaining gradient fractions demonstrated relatively insignificant recoveries (<5%) of retinyl ester. Additionally, neither 11-cis nor all-trans retinols were detected in any of the gradient fractions.

**Fractionation of Retinal Pigment Epithelium Plasma Membranes**

To achieve a further purification of plasma membranes, fraction S2 was subfractionated on self-forming gradients of Percoll. Eighteen fractions were collected from the gradient after centrifugation. Each of the eighteen fractions collected from the gradient (designated P1-P18) was analyzed for PM enzyme markers (5'-ND, ATPase, and AP) and for 11-cis REH activity. As shown in Figure 3, recovery of 11-cis REH activity was greatest in fraction P2 (density = 1.042, 5°C). Specific activities of the PM enzyme markers also were increased markedly in P2. Enrichment of the enzyme marker activities, relative to values obtained with S2 membranes, were as follows: 5'-ND (5-fold), ATPase (15-fold), and AP (10-fold).

Further characterization of P2 indicated relatively low carboxylesterase activity and no detectable all-trans REH activity (Fig. 3). This latter result is particularly significant because it represents the first physical separation of the two isomeric REH activities. The apparent kinetic constants ($K_m^{11-cis}$ and $V_m^{11-cis}$) for 11-cis REH activity in P2 were determined to be 18 μM and 1800 picomoles/min per mg, respectively (Fig. 4). Although these values closely approximate those reported in a previous characterization of 11-cis REH kinetics in microsomal membranes ($K_m^{11-cis}$ = 66 μM and $V_m^{11-cis}$ = 2100 picomoles/min per mg), the presence of a relatively nonspecific all-trans REH activity in the microsomal protein has since been determined to contribute to the 11-cis REH turnover number.29 Thus, the presently reported values represent the most accurate estimation of 11-cis REH kinetics to date.

**DISCUSSION**

We have characterized previously an 11-cis REH activity in bovine RPE microsomes.17 In this earlier report, studies were performed to determine whether the mechanism under study was indeed true ester hydrolysis and also to distinguish 11-cis REH activity from all-trans REH activity in RPE microsomes. Results from these studies led us to investigate the possibility that a distinct 11-cis REH may play a principal role in an accessory pathway of visual chromophore production; the presence of such a pathway would explain the physiologic relevance of 11-cis retinyl ester storage in RPE. In the present report, we sought to determine the subcellular locales of 11-cis REH activity and 11-cis retinyl esters and to characterize biochemical properties of 11-cis REH activity in these compartments. We have included analyses of all-trans REH activity in our studies in efforts to ascertain which experimental treatments are required to either chemically or physically separate the isomeric REH activities. These data will be useful during subsequent purification protocols for the 11-cis REH enzyme.

In the RPE, endogenous levels of 11-cis and all-trans retinyl esters are affected largely by LRAT and isomerohydrolase activities. It has been reported that approximately 10% of newly internalized, apically derived, all-trans retinol is converted into 11-cis retinol, using all-trans retinyl esters as an intermediate.30 Liberated 11-cis retinol, which was observed to exhibit a steady-state level unaffected by further retinol supplementation, “triggered” synthesis of 11-cis retinyl esters and 11-cis retinal when supplementation was greater than 4 nanomoles/10^6 cells. These authors noted that the level of 11-cis retinyl esters subsequently increased to a level 20 times greater than that of 11-cis retinol. These findings demonstrate a functional relationship between LRAT and isomerohydrolase activities in which large quantities of 11-cis and all-trans retinyl esters are generated. Biochemical analyses of LRAT and isomerohydrolase activities have shown that these activities copurify by various chromatographic means and are similarly attenuated by diverse chemical reagents.32 Additionally, there are several lines of evidence that indicate that LRAT, isomerohydrolase, and dehydrogenase activities are localized primarily in the ER compartment.3,18,33 In fact, the measurement of LRAT activity in our gradient fractions has also revealed an ER locale for LRAT; PM-enriched fractions demonstrated no detectable LRAT activity (unpublished data). Thus, the ER possesses the complete functional complement of enzyme activities necessary to generate visual chromophore precursor from all-trans retinol. However, mobilization of the endogenous 11-cis retinyl ester pool does not seem to take place in the ER. We have found that 11-cis
The distribution of retinyl esters in plasma membrane (PM)-enriched and endoplasmic reticulum (ER)-enriched fractions. PM (S2)-enriched and ER (S4)-enriched membrane fractions were recovered from retinal pigment epithelium (RPE) microsomal membranes by discontinuous sucrose-density centrifugation. Retinoids were extracted from microsomal protein (M)-, PM (S2)-, and ER (S4)-enriched proteins using an organic-aqueous phase partitioning system. Neither retinols nor retinals were detected in the membrane extracts. The data shown are representative of a typical retinyl ester fraction, which was analyzed by high-performance liquid chromatography (HPLC; 0.2% dioxane/hexane mobile phase) with simultaneous photo-diode array detection. Representative HPLC chromatograms are shown on the left, and single-time point wavelength scans taken at each peak apex are shown on the right. The spectral and chromatographic data for a given sample share the same y-ordinate that is shown to the left of the chromatogram. Samples were quantified by reference to a standard curve relating retinoid concentration to peak area. The concentrations (nanomoles/mg) of 11-cis and all-trans retinyl esters in the examined fractions were as follows: M = 0.70 ± 0.01 and 1.10 ± 0.24, respectively; S2 = 0.47 ± 0.02 and 0, respectively; and S4 = 0 and 0.62 ± 0.05, respectively. These analyses reveal that 11-cis and all-trans retinyl esters are localized to distinct subcellular compartments within the RPE. 1, 11-cis retinyl ester; 2, all-trans retinyl ester.
The enrichment of enzyme markers and retinyl ester hydrolase (REH) activities in P2 membranes. Fraction S2 from the discontinuous sucrose-density gradient was subfractionated on a Percoll gradient into 18 fractions. One of these fractions was enriched significantly with plasma membrane enzyme markers (5'-nucleotidase, Na\(^+\)-K\(^+\)-ATPase, and alkaline phosphatase) and demonstrated virtually no carboxylesterase or all-trans REH activities. The figure shows the enrichment of these activities in P2 (mean ± SD, n = 4) relative to S2. The values were obtained by dividing specific activities determined with P2 by those of S2. The dashed line indicates a P2/S2 ratio of 1 (i.e., the point at which specific activities of P2 and S2 are equal).

Retinyl esters and an 11-cis-specific REH activity are localized in RPE PM. The enrichment factors of ATPase and AP activities and the fact that in RPE, ATPase activity is localized predominantly to the apical surface of the RPE\(^{34,35}\) indicate that 11-cis REH activity may be associated primarily with the apical PM region. This locale would be particularly advantageous for subsequent transport of visual chromophore to the retina. Our finding of membrane-associated retinyl esters is consistent with previous studies in which 11-cis and all-trans retinyl esters were found to be associated with membranous rather than cytosolic fractions of the RPE.\(^{36,37}\)

These data raise the question of how 11-cis retinyl esters become associated with the PM compartment. It has been suggested that the intracellular movements of long-chain fatty acids, acyl Co-A derivatives, and retinoids are affected by fatty acid-binding proteins (FABP).\(^{38,39}\) In fact, liver FABP has been shown to selectively remove fatty acids and retinyl esters from microsomal membranes.\(^{40}\) Binding analysis has revealed that FABP has a relatively high affinity (\(K_d = 1.4 \times 10^{-6} \text{M}\)) for retinyl palmitate. In contrast, the binding of retinyl palmitate to cellular retinol binding protein was barely detectable.\(^{41}\) Recently, we incubated \[^{3}H\]11-cis retinyl palmitate and \[^{3}H\]palmitic acid with bovine RPE cytosol and have identified a protein that binds reversibly with either labeled ligand.\(^{42}\) An earlier study by Wiggert et al.\(^{43}\) reported similar findings.

Although no data are available regarding FABP concentration in RPE, if one presumes its existence in the RPE at a concentration similar to that in the liver (i.e., 1000-fold molar excess relative to cellular retinol binding protein concentration\(^{44}\)), the limited solubility of 11-cis retinyl esters in an aqueous milieu (namely, in RPE cytosol) would be enhanced in vivo by FABP binding and would thereby facilitate intracellular movement of 11-cis retinyl esters from sites of synthesis to sites where 11-cis REH activity is localized (namely, plasma membrane). Interestingly, we have also recently identified an 11-cis
Figure 5. Compartmentalization of vitamin A metabolic pathways in the retinal pigment epithelium (RPE). The diagram shows two metabolic pathways by which visual chromophore may be synthesized. The incorporation of all-trans retinol bound to serum retinol-binding protein (SRBP) and subsequent intracellular binding to cellular retinol-binding protein (CRBP) has been well characterized (see Refs. 1 and 7 for review). In the endoplasmic reticulum (ER) pathway, the complete complement of enzymatic activities necessary to generate retinyl esters and 11-cis retinal are present (i.e., lecithin:retinol acyltransferase [LRAT]; isomerohydrolase [Iso-Hydro]; and 11-cis retinol oxidase [11-cis RO]). It is clear that all-trans retinyl esters serve as a precursor substrate for subsequent synthesis of 11-cis retinal, which presumably is shuttled to apical processes of the RPE conjugated to cellular retinaldehyde-binding protein (CRALBP). 11-cis retinyl esters, which are produced from 11-cis retinol using LRAT activity, are localized in the plasma membrane (PM) compartment. Therefore, a presumptive fatty acid-binding protein (FABP, indicated by an asterisk) is thought to facilitate intracellular transport of 11-cis retinyl esters from the site of synthesis (ER) to the PM, where 11-cis REH activity is also localized. Subsequent oxidation using 11-cis RO activity results in the liberation of 11-cis retinal directly at the apical RPE, thereby precluding the need for intracellular transport by CRALBP.

retinol oxidase activity in RPE PM (unpublished observations). Therefore, it is conceivable that visual chromophore may be generated within the PM compartment.

Data from the present report support the hypothesis that 11-cis retinoids may be mobilized from at least two separate subcellular compartments. Observations made by Okajima et al. during an investigation of the effects of interphotoreceptor retinoid-binding protein on the release of 11-cis retinal from amphibian RPE provide direct evidence that is supportive of this hypothesis. Results from the amphibian study clearly demonstrate that a single pool of retinoid precursor could not be responsible for the observed release of 11-cis retinal in the presence of interphotoreceptor retinoid-binding protein. Rather, the existence of discrete pools, or compartments, of all-trans and 11-cis retinyl esters, which are differentially mobilized in the presence of interphotoreceptor retinoid-binding protein, would render their findings interpretable. It is conceivable that an accessory metabolic pathway exists in the RPE to separate the mobilization of the readily available 11-cis retinoid pool (PM pathway) from the mobilization of 11-cis retinal through an all-trans retinyl ester intermediate (ER pathway). A diagram of the metabolic pathways that are theorized to contribute to visual chromophore biosynthesis in the RPE is shown in Figure 5. Proper integration of these pathways (presumably through specific binding proteins) may prove to be vital for photopigment renewal and regeneration. Future studies will be directed toward understanding the relationship of vitamin A metabolism in these subcellular compartments.

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References