The Retinal Pigment Epithelial-specific 11-cis Retinol Dehydrogenase Belongs to the Family of Short Chain Alcohol Dehydrogenases*

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We have isolated and partially characterized a 32-kDa membrane-associated protein (p32), which forms a complex with p63, an abundant membrane protein in bovine retinal pigment epithelium. The sequence of a cDNA clone for p32 revealed an open reading frame encoding 318 amino acid residues. Several hydrophobic regions could be identified, suggesting that p32 is an integral membrane protein. A search of data bases identified p32 as a member of the superfamily of short chain alcohol dehydrogenases. Transcripts for p32 were specifically expressed in retinal pigment epithelium. Overexpression of p32 in Cos cells produced a membrane-bound stereospecific 11-cis retinol dehydrogenase, active in the presence of NAD⁺ as cofactor but not in the presence of NADPH. We propose that p32 is the stereospecific 11-cis retinol dehydrogenase, which catalyzes the final step in the biosynthesis of 11-cis retinaldehyde, the universal chromophore of visual pigments.

Under normal physiological conditions, most cells obtain all-trans retinol as their major source of retinoid. The major physiologically active derivatives of retinol, retinoic acid in non-ocular tissues (1), and 11-cis retinaldehyde for ocular tissues (2) are then generated by specific cellular mechanisms. None of these mechanisms have been fully defined at the molecular level, and several of the enzymes involved have only been characterized as enzymatic activities (3–6).

Regarding retinoid metabolism, the polarized retinal pigment epithelial (RPE)² cells of the eye are unusual. These cells obtain all-trans retinol from two different pathways. Retinol, bound to plasma retinol-binding protein (7), is accumulated from the circulation through the basolateral plasma membrane, in order to support the general need of retinoids for synthesis of visual pigments. Furthermore, all-trans retinol, which has been generated in the photoreceptor cells after light exposure of the visual pigments, is taken up through the apical plasma membrane for regeneration of 11-cis retinaldehyde. At present, it is not known whether similar mechanisms are used with regard to cellular retinol uptake through the basolateral and the apical plasma membranes. However, available data show that membrane receptors for retinol-binding protein are expressed by RPE cells (8, 9), and retinol uptake by the apical plasma membrane is presumably mediated by the interstitial retinol-binding protein located in the space between the RPE and the photoreceptors (10–12).

Regardless of the origin of all-trans retinol, the synthesis and apical release of 11-cis retinaldehyde is the major pathway for retinol accumulated in the RPE. This pathway involves a specific isomerase, which converts all-trans retinylesters into 11-cis retinol (13). In the final step of this pathway, 11-cis retinol is oxidized to 11-cis retinaldehyde by a stereospecific 11-cis retinol dehydrogenase (3–6, 14).

In RPE cells, a major 63-kDa protein (p63) has been implicated as a component of the membrane receptor for retinol-binding protein (15). To get further information on the role of this protein in retinol uptake and metabolism, we have identified interacting proteins, including a 32-kDa protein (p32) that belongs to the family of short chain alcohol dehydrogenases. Enzymatic analyses showed that p32 is a stereospecific 11-cis retinol dehydrogenase.

EXPERIMENTAL PROCEDURES

Protein Labeling with Iodine-125, Immunoprecipitation, and Immunofluorescence Chromatography

Isolation of p32—RPE cells were isolated, and membrane fractions were prepared as described previously (8). RPE membranes were solubilized in PBS containing 1% CHAPS (1 mg of total membrane protein/ml of buffer), and insoluble material was removed by ultracentrifugation at 100,000 x g for 1 h. Detergent-solubilized membrane proteins were subjected to gel filtration using a Superose 6 column (1 x 30 cm) equilibrated in PBS containing 1% CHAPS at a flow rate of 0.2 ml/min. Proteins that eluted in fractions corresponding to globular proteins of M, 150,000–400,000 were radiolabeled with Na¹²⁵I (Amersham Corp.) using the Chloramin-T procedure (16). Nonincorporated iodine-125 was removed by gel filtration on Sephadex G-25 packed in a Pasteur pipette. Aliquots of the radiolabeled proteins were diluted in PBS containing 1% CHAPS and 1% bovine serum albumin and subjected to immunoprecipitation using the mAb A52 Ig against p63 (5 μg of Ig incubation) or two polyclonal rabbit antisera against p63 (3 μl of serum/ incubation) (15). Nonspecific immunoprecipitation was monitored in parallel incubations using an unrelated mAb Ig and preimmune rabbit serum. Fifty microtiter of a 50% alurity of protein A-Sepharose was added to the incubations for 30 min. The beads were subsequently carefully washed with PBS containing 1% CHAPS and then prepared for SDS-PAGE (17).

In order to isolate p32 for structural studies, RPE membranes were solubilized in PBS containing 1% CHAPS as above and then incubated with mAb A52 Ig coupled to CNBr-activated Sepharose 4B beads (Pharmacia Biotechnology, Inc.) in a Bio-Rad poly prep column by end-over-end rotation at 4 °C. After a 2-h incubation, the beads were allowed to settle, and the column was quickly washed with 5 column volumes of PBS containing 1% CHAPS. Bound proteins were then eluted with 50 mM triethanolamine, pH 11.2, containing 1% CHAPS. The pH of the eluate was quickly adjusted to 8.0 by the addition of 1 M Tris-HCl buffer, pH 8.0, containing 1% CHAPS. The eluted fractions

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) X82062.

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‡ The abbreviations used are: RPE, retinal pigment epithelium; CHAPS, 3-(3-cholamidopropyl)dimethylammonio-1-propane sulfonate; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; SCAD, short chain alcohol dehydrogenase; PAGE, polyacrylamide gel electrophoresis; bp, base pair(s); kb, kilobase(s); HPLC, high performance liquid chromatography.
were subjected to SDS-PAGE, and the separated proteins were subse-
quently visualized by Coomassie Blue staining.

**Amino Acid Sequence Analysis of p32**—The Coomassie-stained bands containing the p32 protein (2–5 μg) were excised and treated according to Rosenfeld et al. (18), with minor modifications. The gel pieces were washed twice for 30 min at 30 °C with 100 μl of 0.2 mM ammonium bicarbonate and then equilibrated by incubating 50% ethanol and completely dried under a stream of nitrogen. The gel pieces were subsequent-

aly rehydrated with 0.2 M ammonium bicarbonate buffer containing 0.02% Tween 20 and 0.5 μg of modified trypsin (Promega Inc.). Rehydration was continued by the addition of 5-μl portions of the 0.2 M ammonium bicarbonate buffer until the gel pieces obtained their original sizes. The rehydrated gel pieces were then incubated overnight at 30 °C. The protease activity was inhibited by the addition of trisfluoro-

acetic acid to a final concentration of 1%. The supernatant was recov-
ered and combined with two extracts made with 0.1% trisfluoroacetic acid in 60% acetonitrile. The organic phase was reduced in a SpeedVac, and the digest was applied onto a mRCP C2/C18 SC 2.1×10 column operated in a SMART system (Pharmacia). The sample was eluted with a gradient of acetonitrile in 0.065% trisfluoroacetic acid, and fractions containing discrete peptides were collected, using the automatic peak fractionation option. Five peptides were selected for amino acid se-

quence analysis, and using an ABI 470A sequencer equipped with a model 120A PTI analyzer, the sequenced peptides were identified as follows:

**Isolation of a cDNA Clone Encoding p32**—Four degenerate oligonu-
cleotide mixtures derived from peptides p321 and p323 were synthe-
sized. The two sense mixtures (OM1 and OM3) were derived from the N-terminal amino acids 1–5 of p321 and 2–6 of p323. The anticodon mo-
etives for these mixtures were synthesized as described previously (10) and 10–15 of p323. All nucleotide mixtures were synthesized with a 4-5′ extension and an EcoRI site for subsequent cloning of the PCR products. The sequences of the oligonucleotide mixtures are given be-

low, and the EcoRI site is underlined. Positions containing all four bases are marked N. OM1: AGT GAA TTC TCT GTG GCC NTT (C/T/G/C); OM3: ACGT GAA TTC CCG GTG AAG (A/G/T/C); OM4: ACGT GAA TTC GC (T/C/G/A/N/A,A,G) NTT (C/T/C/T/C). To carry out the PCR amplifications, first-strand cDNA was synthe-
sized by standard procedures using avian myeloblastosis virus reverse transcriptase (Amersham). Twenty micrograms of total RNA from iso-
lated RPE cells were used, and the reaction was primed with a (dT)12 oligonucleotide. Aliquots corresponding to 2 μg of total RNA were used in each subsequent PCR reaction. The PCR amplifications were performed using Taq polymerase (Amersham) and a final concentration of 0.2 μM of the oligonucleotide mixtures in a 100-μl reaction. After 30 cycles (2 min at 95 °C, 1 min at 55 °C, and 2 min at 72 °C), aliquots of the reactions were analyzed on 4% NuSieve GTG agarose gel (FMC Bioproducts) containing 5 μg/ml of ethidium bromide. The amplified products using OM1–OM2 (61 bp) and OM3–OM4 (330 bp) were dig-

ested with EcoRI, gel purified, and cloned into EcoRI-cut pBS vectors (Stratagene). The respective 330-bp insert was used to create a RPE-specific a ZAP II cDNA library as described previously (19). Five positive λ clones were isolated, and the inserts were subcloned into plBluescript by in vivo excision as described (Stratagene). Clone p321 contained an insert of 1.1 kb, and both strands were fully se-

quenced using Sequenase (U.S. Biochemical Corp.) with T3/T7, or M13 universal primers or with internal primers. Standard molecular biology techniques were used (24). DNA probes were labeled, using random priming, to a specific activity of 5 × 106 to 1 × 107 cpm/μg DNA.

**Northern Blot Analyses**—Twenty micrograms of total RNA isolated from a number of tissues was electrophoresed on 1% agarose under denaturing conditions and transferred to a nylon-N filter (Amersham). The filter was hybridized with 32P-labeled full-length cDNA encoding p32 under stringent conditions. The isolation of total RNA, hybridization conditions, and washing procedure were identical to those previously described (19).

**Expression of p32 in COS Cells and Analysis of the Enzymatic Properties of the Recombinant Protein**—The EcoRI insert of p321 was cloned into the EcoRI-digested eucaryotic expression vector pSG5 (20). Cos cells were maintained in Dulbecco’s minimal essential medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and an-
tibiotics. Cell cultures were seeded into 10-cm Petri dishes at × 106 ce-

cells/dish and transfected with 5 μg of plasmid/dish using DEAE-
dextran. Control cells were transfected with equal amounts of the parental vector alone. After treatment with 10% methanol for 2 min, the cells were incubated for 72–96 h. To harvest the cells, the dishewere scraped with a rubber policeman and the cells collected by a low speed centrifugation. The cell pellets were resuspended in hypotonic buff-

(10 mM Tris-HCl, pH 7.5, containing 1 mM phenylmethylsulfonyl fluoride), put on ice for 20 min, and finally homogenized using a Dounce homogenizer. Unbroken cells and debris were removed by a 3,000 × g centrifugation for 15 min. The microsomes were subsequently collected by ultracentrifugation at 100,000 × g for 1 h. The membrane pellets were stored at −80 °C until further analysis.

To study the function of p32, p32 was transfected into NIH 3T3 cells by using rabbits with p32 (amino acid residues 19–318) expressed as a fusion protein with glutathione S-transferase. The bacterial expression vector pGEX-2T was used, and induction and purification of the glutathione S-transferase fusion protein was as recommended by the supplier (Pharmacia). Each rabbit received a subcutaneous injection of 75 μg of fusion protein emulsified in Freund’s complete adjuvant. The rabbits were boosted with 50 μg of fusion protein emulsified in Freund’s incomplete adjuvant every 2nd week. Blood was collected every 2nd week. The immune rabbit sera were passed over a column containing the glutathione S-transferase fusion protein immobilized on CNBr-activated Sepharose beads. Bound Ig was eluted with 0.1 M sodium citrate buffer, pH 3.0, containing 0.5 M NaCl. To remove Ig to the glutathione S-transferase portion of the fusion protein, the eluted Ig was similarly incubated with glutathione S-transferase-coupled Sepharose beads, and the unbound Ig fraction was used. For immunoblot analysis of the overexpressed protein, the Ig was used at a concentration of 1 μg/ml. The details of the immunoblot-

procedure were as described previously (15).

11-cis retinol was synthesized from 11-cis retinaldehyde (a kind gift from Dr. R. Crouch, NED) using sodium borohydride (21) and stored under argon at −80 °C. HPLC analyses verified that 11-cis retinalde-

hyde was quantitatively reduced to 11-cis retinol. All manipulations with the retinol were done under subdued light. The enzymatic properties of p32, expressed in Cos cells, were assayed using conditions as de-

scribed previously (22) for the study of 11-cis retinol dehydrogenase activity in microsomal fractions of RPE cells. The final concentration of 11-cis retinol in the incubations was 100 μM. In some reactions, all-

trans retinol (SGT) was included to a final concentration of 100 μM. Twenty micrograms of total membrane protein from Cos cells expressing p32 or from control cells were used in each reaction. After a 30-min incubation at 37 °C, with or without the addition of NAD+ or NADP, the reaction mixtures were extracted once with n-hexane. The organic phases were carefully removed and dried under a stream of argon. The dried organic phases were then separately dissolved in ethanol, and aliquots were analyzed on a normal phase silica HPLC column (Chromasol KRS-Si-250 × 4.6 mm) developed with n-hexane containing 4% dioxane (23) at 1 ml/min. The effluent was monitored at 330 nm. Under the conditions specified here, 11-cis retinaldehyde and 11-cis retinol eluted at 8 and 23.0 min, respectively.

**Computer Analyses**—The amino acid sequence of p32 was analyzed with the GCG package (Genetics Computer Group).

**RESULTS**

**Identification, Isolation, and Partial Amino Acid Sequence of p32**—Aliquots of protein fractions from detergent-solubilized RPE membranes were radiolabeled with iodine-125 and sub-

dected to indirect immunoprecipitation and SDS-PAGE analy-

sis using the mAb A52 or rabbit antisera to highly purified p63. Autodigrams of the SDS-PAGE gels showed that both types of reagents reacted with p63, whereas an unrelated mAb or preimmune rabbit serum could not immunoprecipitate p63 (Fig. 1, A). In all lanes containing immunoprecipitated p63, there was an enrichment of a M, 32,000 protein. Because both mAb A52 and the rabbit antisera to p63 are highly specific for p63 (15), we conclude that the M, 32,000 protein coprecipitates in these analyses by binding to p63. Similarly, these analyses identified a double band of M, 50,000–52,000, which precipitated along with the 32-kDa protein and p63 (Fig. 1A, lanes d and e). The nature of the M, 50,000–52,000 proteins will not be further discussed in this article. Instead, we will focus on the 32-kDa protein, termed p32.

In order to isolate p32, we took advantage of the fact that p32 specifically interacts with p63. Thus, detergent-solubilized RPE membrane proteins were passed over an immunos affinity column containing A52 Ig. After a quick washing procedure, bound proteins were eluted at high pH in a CHAPS-containing buffer. SDS-PAGE analysis and Coomassie staining of the
eluted fractions revealed that p63 was specifically retained and eluted from the immunoaffinity column (Fig. 1B, lane b). In addition, a weakly stained band corresponding to p32 was observed in the eluate from the A52 column. A comparison of the total protein profile of solubilized RPE membranes and the eluted fraction from the A52 column indicate that p32 is not quantitatively retained on the A52 column (Fig. 1B). Nevertheless, the appearance of p32 in the eluted fraction from the A52 column, but not in the eluted fraction from the column containing an unrelated Ig, suggests a specific interaction with p63 (data not shown). This result is consistent with the previous immunoprecipitation data and shows that p32 is complexed to p63 and that it is retained on the immunoaffinity column due to this complex formation.

Partial amino acid sequence was generated from p32 that was isolated by SDS-PAGE of eluted fractions from the A52 immunoaffinity column. Five of the identified peptides were subjected to amino acid sequence analysis (Table I).

**Isolation and Analysis of a cDNA Clone Encoding p32**—Four degenerate oligonucleotide mixtures (OM1–OM4) were synthesized based on the amino acid sequences of two of the five sequenced peptides (p321 and p323). Reverse transcribed RPE mRNA and four combinations of the degenerate oligonucleotide mixtures were used in PCR. Amplifications using the oligonucleotide mixtures OM1 and OM2, both derived from peptide p321, resulted in an amplified 61-bp fragment (Fig. 2, lane a).

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<td>Amino acid sequence determinations of five peptides isolated from trypsin-digested p32</td>
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*The amino acid residues given within the parentheses are the residues deduced from the cDNA sequence in same positions.

This was the expected size of the fragment in this reaction. Amplifications using mixtures OM3–OM4 and OM1–OM4 did not yield any products, and amplification using OM3–OM2 resulted in an amplified 330-bp fragment (Fig. 2, lane b). Sequence analysis of the 61- and 330-bp fragments confirmed that we had amplified cDNA sequences corresponding to the peptide sequences generated in the previous amino acid sequence analysis. With the notable differences between the deduced amino acid sequences from the amplified PCR fragments and the generated amino acid sequence of peptide p321 (see below), we had generated specific probes suitable for the isolation of full-length cDNA clones encoding p32.

We screened a RPE-specific λ ZAP-II cDNA library with the 330-bp fragment as the probe. Five independent λ clones were isolated from approximately 200,000 clones and subcloned by in vitro excision. The cDNA clone p321 contained the longest insert (~1.1 kb), and this clone was selected for further studies. Both strands of p321 were fully sequenced, and the insert was 1122 bp long, excluding the linkers used to prepare the cDNA library. The insert contained one long open reading frame encoding 318 amino acid residues with a calculated mass of 35,041 Da (Fig. 3). The first methionine lies in a good context according to the rules for translational initiation and is likely to be the initiation codon (25). This suggestion is strengthened by the fact that in vitro translation of synthetic mRNA transcribed from p321 gives rise to a M, 32,000 protein in SDS-PAGE analysis (data not shown). However, there is no stop codon in-frame in the upstream 35-bp 5'-untranslated region of the cDNA. The 130-bp 3'-untranslated region ends with a poly(A) tract, and a putative polyadenylation signal could be identified in the upstream sequence (bp 1094–1099) (Fig. 3).

The deduced amino acid sequence of p321 and the amino
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Fig. 3. Nucleotide sequence of pa321 and the deduced amino acid sequence of p32. The nucleotides are numbered on the left and the amino acid residues on the right. Amino acid 1 is the initiation methionine. The partial amino acid sequences previously determined from peptides isolated from tryptic-digested p32 are underlined (see Table 1).

The deduced amino acid sequence, the first 18 residues are hydrophobic and have the characteristics of a classical signal sequence. However, a consensus site for signal peptidase cleavage cannot be clearly identified (26). The region between residues 132 and 152 are also hydrophobic, and there is a relatively long hydrophobic stretch near the C terminus of the protein (residues 289–310). Thus, p32 displays several hydrophobic regions, which are potential membrane-spanning segments. In light of the homology to the family of short chain alcohol dehydrogenases (see below), it is likely that the central hydrophobic region of p32 (residues 132–152) is not used as a membrane anchor. Instead, both the N- and C-terminal regions are potential membrane-anchoring domains.

A consensus site for N-linked glycosylation (amino acid residues N-1-T) could be found in this deduced amino acid sequence at position 160–162 (Fig. 3).

p32 Shows Sequence Similarities to Short Chain Alcohol Dehydrogenases—A search through the Swissprot protein data base (release 28.0, 2/94), using the PASTA algorithm, demonstrated that p32 is structurally related to several previously sequenced proteins belonging to the short chain alcohol dehydrogenases superfamily (SCAD) (for a review, see Ref. 27). It is most closely related to a mitochondria matrix dehydrogenase, the p-β-hydroxybutyrate dehydrogenase (28) and shows less similarity to two other proteins, the 3-oxoacyl-[acyl carrier protein] reductase from Escherichia coli (FABG) (29) and the human estradiol 17 β-dehydrogenase (28) (30, 31). Aligned amino acid residues in all four sequences are boxed, and the invariant tyrosine residue is marked (open triangle).

Dehydrogenases—A search through the Swissprot protein data base (release 28.0, 2/94), using the PASTA algorithm, demonstrated that p32 is structurally related to several previously sequenced proteins belonging to the short chain alcohol dehydrogenases superfamily (SCAD) (for a review, see Ref. 27). It is most closely related to a mitochondria matrix dehydrogenase, the p-β-hydroxybutyrate dehydrogenase (28) and shows less similarity to two other proteins, the 3-oxoacyl-[acyl carrier protein] reductase from Escherichia coli (FABG) (29) and the human estradiol 17 β-dehydrogenase (28) (30, 31). Aligned amino acid residues in all four sequences are boxed, and the invariant tyrosine residue is marked (open triangle).
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**Fig. 5.** Expression of transcripts corresponding to p32. Total RNA isolated from RPE cells, liver, kidney, adrenal gland, lung, testis, brain, and muscle were fractionated on a 1% denaturing agarose gel using 20 µg of RNA/ lane. The RNA was transferred to a nitrocellulose membrane and probed with [32P]-labeled insert of p3321. A 1.4-kb mRNA is visualized in RPE. The migration of the 18 and 28 S ribosomal RNAs are indicated at the right.

Tissue-restricted Expression of p32—The tissue expression of p32 was analyzed by Northern blotting using total RNA isolated from bovine RPE, liver, kidney, adrenal, lung, testis, brain, and muscle (Fig. 5). Hybridization at high stringency, with the 1.1-kb insert of pA321 as the probe, revealed abundant expression of transcripts corresponding to p32 only in RPE but not at a detectable level in several other tissues. The size of the major transcript was 1.4 kb.

Expression of p32 in Cos Cells and Enzymatic Properties of the Recombinant Protein—The restricted expression of p32 in the RPE and biochemical properties similar to the previously identified 11-cis retinol dehydrogenase (14) encouraged us to investigate whether p32 was identical to this enzyme. To this end, p32 was overexpressed in Cos cells using a eucaryotic expression vector. To verify that the protein was expressed at high level, microsome fractions from transfected cells and from control cells were subjected to immunoblotting analysis. Expression of p32 was seen in cells transfected with the expression vector but not in control cells (Fig. 6A).

To investigate the enzymatic properties of p32, we incubated microsomal fractions from transfected cells and from control cells with different combinations of 11-cis retinol, all-trans retinol, and the cofactors (NAD⁺ or NADP⁺). Analyses by HPLC showed that microsomal fractions from transfected cells expressed an 11-cis retinol dehydrogenase that was active in the presence of NAD⁺, as indicated by the formation of 11-cis retinaldehyde (Fig. 6B). A second peak in the chromatogram coeluted with all-trans retinaldehyde. Control incubations with 11-cis retinaldehyde, in the absence of cellular membranes, showed that under the conditions used, a significant amount of the 11-cis retinaldehyde isomerized to all-trans retinaldehyde (data not shown). We conclude that all-trans retinaldehyde is generated during the incubation and extraction procedures and not during the enzymatic reaction. No enzymatic activity was detected with NADP⁺ as the cofactor (Fig. 6C). Control cells, not expressing p32, lack the ability to oxidize 11-cis retinol into 11-cis retinaldehyde under the same conditions (Fig. 6D). Incubations with all-trans retinol verified the stereospecificity of the enzyme, as no significant formation of all-trans retinaldehyde was detected (data not shown). These data show the stereospecificity of this enzyme and the requirement for NAD⁺ as the cofactor.

**Fig. 6.** Expression of p32 in Cos cells and enzymatic properties of the recombinant protein. A, immunoblotting analyses of p32 expression in microsomal fractions from cells transfected with a p32 expression vector or from control cells. Ten micrograms of total microsomal protein was analyzed in each lane. B-D, HPLC analyses of products formed after incubations of microsomal fractions from p32 expressing Cos cells with 11-cis retinol (11-cis Rol) as the substrate. The incubations were carried out in the presence of NAD⁺ (B) or in the presence of NADP⁺ (C). As control, an incubation of a microsomal fraction from control cells was carried out in the presence of NAD⁺ (D). All-trans retinaldehyde (at RA1) formation in B was caused by isomerization of the 11-cis compound (11-cis RA1) during the incubation and the subsequent extraction procedures (see "Results" for details).

**DISCUSSION**

In this article, we have described a novel RPE-specific protein, p32, which forms a complex with p63 of the RPE. The primary structure of p32 has all the critical features of a functional SCAD, including a putative cofactor binding site and essential residues involved in the catalytic mechanism, namely the almost invariant tyrosine-containing sequence motif Y-X-X-X-K (27).

A major metabolic step in retinoid metabolism in RPE cells is the conversion of 11-cis retinol to 11-cis retinaldehyde, the universal chromophore of all visual pigments in higher animals. After its synthesis in the RPE, 11-cis retinaldehyde is transported to the photoreceptor cells and covalently attached to the opsins to form the visual pigments. The enzyme responsible for synthesis of 11-cis retinaldehyde is the stereospecific
11-cis retinol dehydrogenase (3, 5, 6, 14). We can demonstrate that p32 is such a stereospecific 11-cis retinol dehydrogenase. The identification and molecular cloning of this enzyme is important for further studies of the molecular and cellular mechanisms involved in synthesis and regeneration of visual pigments. Furthermore, the role of this enzyme in various pathological conditions of the retina can be investigated.

The identification of the 11-cis retinol dehydrogenase as a member of the SCAD superfamily is interesting from several points of view. SCADs are either cytosolic or membrane-bound enzymes that utilize a large number of substrates, including steroids and prostaglandins, but this is the first report that a membrane-bound retinol dehydrogenase belonging to the SCAD superfamily has been identified. Because the SCADs are oxidoreductases, i.e. depending on whether the cofactor is reduced or oxidized, these enzymes will oxidize or reduce their substrates, respectively. Thus, it would not be surprising to find that other retinol dehydrogenases belong to the SCAD superfamily. For example, the enzyme that reduces all-trans retinol to all-trans retinol in the photoreceptors after bleaching of the visual pigments may also be a SCAD (32). In several non-ocular tissues, all-trans retinol is oxidized to all-trans retinal for further synthesis to retinoic acid. It is generally accepted that the first step in this metabolic pathway is carried out by members of the medium chain alcohol dehydrogenases (33–35). An appealing possibility is that oxidation of all-trans retinol in such tissues could also be carried out by membrane-bound members of the SCAD family. The identification of novel retinol dehydrogenases in non-ocular tissues would be important for further understanding of the regulation of retinoic acid biosynthesis and action during embryonic development and during cellular growth and differentiation.

Like many other epithelial cells, RPE cells are polarized. Intracellular sorting of a membrane-bound 11-cis retinol dehydrogenase, to apically located cellular compartments, might occur in order to facilitate regeneration of the visual pigments. If so, then p63 with a putative function in cellular retinol uptake may be sorted along with p32 to apical compartments. The complex formation of p63 and 11-cis retinol dehydrogenase may be an indication that cellular uptake and metabolism of retinoids are coupled events in the RPE. Further experiments along these lines, including a detailed localization study of p63 and the 11-cis retinol dehydrogenase, might shed new light on the processes of general retinol uptake in RPE cells and chromophore regeneration during the visual cycle.

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