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Expression of a recombinant human RGR opsin in *Lentivirus*-transduced cultured cells

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Purpose: Our goals were to produce a functional recombinant RPE retinal G protein-coupled receptor (RGR) opsin for biochemical studies and to test the efficiency of a lentiviral vector for transgene expression of human RGR.
Methods: A human RGR cDNA was cloned into a replication-defective lentiviral vector, and recombinant hRGR-*Lentivirus* was prepared for transduction of the ARPE-19, a human retinal pigment epithelium (RPE) cell line, and COS-7 cells. Recombinant RGR expression was detected by Western blot analysis, and functionality of the protein was tested by a [^{3}H]all-*trans*-retinal binding assay.
Results: RGR protein was detected in each cell type after transduction with recombinant virus and was not observed in untreated cells. RGR expression in ARPE-19 cells increased steadily for up to 10 days after transduction and was stable for at least 6 months. The transduced ARPE-19 cells produced ~100-fold higher amounts of RGR protein than the transduced COS-7 cells. When cell membranes from the ARPE-19 cells were incubated with [^{3}H]all-*trans*-retinal, the chromophore bound specifically to the expressed protein. Uptake of [^{3}H]all-*trans*-retinol into the ARPE-19 cells was followed by specific binding of radiolabeled retinoid to RGR.
Conclusions: Using a *Lentivirus*-derived gene delivery system, we were able to express high amounts of human RGR protein in the ARPE-19 human RPE cell line. The transduced ARPE-19 cells remain able to process all-*trans*-retinol, and the expressed protein is capable of binding to the all-*trans*-retinal chromophore. The *Lentivirus*-based expression of functional RGR can be used to study RGR in cultured cells and to test in vivo transduction of quiescent RPE cells.

The retinal pigment epithelium (RPE) and Müller cells contain an abundant opsin that is homologous to the G-protein coupled receptors with seven transmembrane domains [1]. The RPE and Müller cell opsin, referred to as RPE retinal G protein-coupled receptor (RGR), represents a distant evolutionary branch of vertebrate opsins that are most closely related in amino acid sequence to invertebrate visual pigments and retinochrome, a photoisomerase that catalyzes the conversion of all-*trans*- to 11-*cis*-retinal in squid photoreceptors [2]. The RGR opsin is located in intracellular membranes of RPE and Müller cells and is capable of forming a stable photopigment with bound all-*trans*-retinal [3-5]. All-*trans*-retinal is the predominant endogenous chromophore bound to RGR protein that is purified in the dark, and illumination of RGR in vitro results in the stereospecific conversion of the bound all-*trans*-retinal to the 11-*cis* isomer [6]. These results provide evidence that RGR may function to generate 11-*cis*-retinal in vivo and participate in a light-dependent visual cycle.

The importance of RGR opsin to the health and viability of the neuroretina is shown by mutations in the human *RGR* gene that segregate with retinitis pigmentosa (RP) in patients with autosomal dominant or recessive RP [7]. The study of human RGR and mutants is hindered by the absence of an appropriate cell culture model. Unfortunately, cultured human RPE cells, as well as cultured RPE cells from other species, show a dramatic loss in their ability to produce RGR in vitro, and the RGR protein is available only in small quantities from any species. A spontaneously arisen human RPE cell line, named ARPE-19 cells [8], maintains many characteristics of normal RPE cells, but does not express the RGR opsin.

To express human RGR opsin in cultured cells, we used the replication-deficient *Lentivirus*-derived vector to deliver human RGR opsin cDNA into ARPE-19 cells. Here, we report the successful expression of a recombinant human RGR opsin that is capable of binding all-*trans*-retinal. The establishment of the stably-transformed ARPE-19 cells offers a potential model system for biochemical and functional studies of RGR and protein variants.

METHODS

Plasmid constructs: The pHR'-CMVLacZ plasmid, an HIV-based lentiviral vector with the human cytomegalovirus (CMV) immediate early promoter and *E. coli*β-galactosidase gene [9], was digested with *Bam*HI and *Xho*I to excise the *lacZ* reporter gene and generate pHR'-CMV. A full-length 1.4-kb human RGR cDNA fragment was inserted into pHR'-CMV by blunt-

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end ligation to create pHR'-CMV-hRGR (Figure 1). The lentiviral pHR'-CMV-hRGR vector was propagated in the Epicurian Coli SURE strain (Stratagene, La Jolla, CA) and purified using EndoFree Plasmid Maxi Kit (Qiagen Inc., Valencia, CA). Another human RGR expression vector, pcDNA3-hRGR, was constructed by insertion of the 1.4-kb human RGR cDNA into the *Eco*R1 cloning site of pcDNA3 (Invitrogen, Carlsbad, CA).

Cell culture: The ARPE-19 and COS-7 cell lines were maintained in DME/F12 (1:1) medium (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT) and 1% Glutamine-Penicillin-Streptomycin (Irvine Scientific, Santa Ana, CA) at 37 °C in a 5% CO_2 incubator. Human kidney 293T and mouse RPE cells were cultured with DME instead of DME/F12 medium.

Adult 129SV X C57BL/6 mice were euthanized, and RPE cell cultures were prepared as follows. Whole eyes were enucleated and bisected posterior to the limbus. The lens was removed, and the retina was peeled away from the eyecup. The eyecup was placed in 2% dispase (Life Technologies) solution for 50 min at 37 °C. The RPE cells were detached by brushing, and were sedimented by centrifugation. The pigmented RPE cells were plated on laminin-coated 6-well plates in DME 10% FBS culture medium.

All animals were treated, maintained, and euthanized in accordance with the ARVO resolutions on the use of animals in research and the guidelines of the U.S. Public Health Service, as delineated in its Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Preparation of Lentivirus*:* VSV-G-pseudotyped recombinant HIV-based virus was produced by three-plasmid co-transfection of 293T cells with 15 µg of pHR'-CMV-hRGR (Figure 1), the envelope protein-coding plasmid, pMD.G (3 µg), and the packaging construct, pCMVΔR8.91 (15 µg), using the calcium phosphate DNA precipitation method. The pMD.G construct provides the VSV G protein to pseudotype the vector, and the packaging construct, pCMVΔR8.91, provides all required vector proteins [10]. High titer stocks of recombinant HIV were prepared, as described previously [10]. Virus titers of 0.3 to 1 x 10^9transduction units (TU)/ml were usually obtained and were determined by transduction of confluent ARPE-19 cells, as described below. The absence of replication-competent virus was determined by the marker rescue assay and measurement of p24 Gag antigen levels by enzyme-linked immunosorbent assay, as described previously [9].

Figure 1. Diagrammatic structure of human RGR-encoding lentiviral transfer vector, pHR'-CMV-hRGR. The transducing plasmid, pHR'-CMV-hRGR, provides the vector genome packaged into the viral particle and includes a human RGR cDNA under control of the CMV promoter. Recombinant virus was generated by co-transfection of 293T human kidney cells with a three-plasmid expression system, including pHR'-CMV-hRGR, the packaging construct pCMVΔR8.91, and envelope protein-coding plasmid pMD.G. LTR, long terminal repeat; Ga, partial sequence of Gag gene; RRE, rev responsive element; SD, splicing donor; ψ, packaging signal [9,10].

Transduction of cells with Lentivirus*:* To determine the titer of a virus preparation, confluent ARPE-19 cells in chamber slides were cultured with serial dilutions of pHR'-CMV-hRGR recombinant virus overnight. After removal of the virus solution, the cells were maintained for 2 more days before fixation and immunohistochemical staining. Transduced ARPE-19 or COS-7 cells were detected by immunohistochemical staining with the DE7 antibody, which is directed against the carboxyl terminus of human RGR [11]. To establish cells that stably expressed human RGR, 1 x 10^6 ARPE-19 or COS-7 cells were incubated overnight with 1 x 10^7 TU pHR'-CMV-hRGR recombinant viral particles and then maintained in fresh culture medium.

Immunohistochemistry: Cultured cells were fixed in cold methanol for 5 min and incubated at room temperature for 30 min with blocking buffer containing 5% bovine serum albumin, 3% normal goat serum, and 0.1% Triton X-100 in phosphate-buffered saline (PBS). The cells were incubated with DE7 antibody, and immunohistochemical staining was performed with a peroxidase-based enzyme detection system, Vectastain ABC, using the Vector VIP substrate (Vector Laboratories, Burlingame, CA).

Preparation of cell membranes: Total cell membranes from ARPE-19, COS-7, and mouse RPE cells were prepared after homogenization of the cultured cells in a Dounce glass homogenizer. The homogenization buffer contained 67 mM phosphate, pH 6.6, 250 mM sucrose, and 100 µg/ml phenylmethylsulfonyl fluoride. The homogenates were centrifuged at 300 x g for 10 min at room temperature, and the supernatants were then centrifuged at 150,000 x g for 1.5 h at 4 °C. The membranes were collected and stored at -80 °C. Bovine RPE microsomal membranes were prepared from fresh eyes, as described previously [4]. Bovine eyes were obtained from a local abattoir. The isolation of RPE cells and preparation of RPE microsomes were performed under dim yellow light. The anterior segments, lens, vitreous and neural retina were removed, and the eyecups were rinsed with PBS. The RPE cells were removed by gently scraping the cell monolayer with a spatula. The cells were sedimented by low-speed centrifugation, resuspended and washed twice in 5-8 ml of ice-cold homogenization buffer containing 30 mM sodium phosphate, pH 6.5, and 250 mM sucrose. The cells were homogenized using a Dounce glass homogenizer, and the homogenate was centrifuged at 700 x g at 4 °C to remove nuclei and unbroken cells. The pellet was resuspended, and the homogenization and centrifugation steps were repeated four times. The supernatants from the homogenization steps were pooled and centrifuged in a Sorvall SS-34 rotor at 15,000 x g for 20 min at 4 °C. The 15,000 x g supernatant was removed and centrifuged in a Beckman 70 Ti rotor at 150,000 x g for 1 h at 4 °C. The membrane pellet was then stored at -80 °C.

Western blot assay: Cell membranes were resuspended in PBS, and protein concentration was measured by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). Samples were separated by 12% SDS-PAGE and then electro-transferred to Immobilon-P membrane (Millipore, Bedford, MA). The DE7 antibody was used to detect human RGR protein using ECL Western blot detection reagents (Amersham, Arlington Heights, IL). Prestained protein molecular weight standards, low range, were from Life Technologies.

*Preparation of [^{3}H]all-*trans*-retinal:* [^{3}H]all-*trans*-retinal (250 µCi) was produced in the dark by oxidation of [^{3}H]all-*trans*-retinol (NEN Life Science Products, Boston, MA) in the presence of 2.4 mg of MnO_2 in hexane solution saturated with retinoic acid, as previously described [4]. The product was purified by normal phase high performance liquid chromatography (HPLC) using a LiChrosorb RT Si60 silica column (4 x 250 mm, 5 µm; E. Merck, Darmstadt, Germany) and a Beckman Model 126 HPLC system (Beckman Instruments, Fullerton, CA). The running buffer was hexane with 2% dioxane. The [^{3}H]all-*trans*-retinal was dried and stored under nitrogen in -80 °C. An all-*trans*-retinal standard was purchased from Sigma (St. Louis, MO).

*Binding of [^{3}H]all-*trans*-retinal to RGR:* The [^{3}H]all-*trans*-retinal binding assay was performed, as described previously [4]. Briefly, cell membranes were resuspended in 67 mM phosphate, pH 6.6, 250 mM sucrose, and 100 µg/ml phenylmethylsulfonyl fluoride and incubated with [^{3}H]all-*trans*-retinal (2 x 10^5 cpm, 30-60 Ci/mmol) in the dark for 3 h at room temperature. After adjustment of the pH to 8.0 with 1 N NaOH, 38 mg/ml $NaBH_4$ was added to the suspension to reduce the Schiff base bond. The membranes were centrifuged at 150,000 x g for 1 h at 4 °C. The pellet was then dissolved in 0.1% SDS in PBS. After separation with 12% SDS-PAGE, the proteins were fixed, and the gel was soaked in ENLIGHTNING Rapid Autoradiography Enhancer (NEN Life Science Products). The gel was dried and exposed to Kodak X-omat AR 5 autoradiographic film (Eastman Kodak Co., Rochester, NY).

*Incubation of ARPE-19 cells with [^{3}H]all-*trans*-retinol and analysis of radiolabeled proteins:* Transduced and normal ARPE-19 cells were preincubated overnight with serum-free RPMI1640 medium at 37 °C in 5% CO_2. The cells were washed with RPMI1640 medium and incubated in the dark with a mixture of [^{3}H]all-*trans*-retinol (10 µCi, 50 Ci/mmol), 500 µg/ml fatty acid-free bovine serum albumin, and 0.5% sucrose in RPMI1640 medium. After incubation for 3 h at 37 °C in 5% CO_2, the cells were washed with PBS, collected by scraping in 2 ml 67 mM sodium phosphate buffer, pH 6.7, and homogenized with a Dounce glass homogenizer. After adjustment of the pH to 8.0 with 1 N NaOH, 38 mg/ml $NaBH_4$ was added to the suspension to reduce Schiff base bonds. The membranes were centrifuged at 150,000 *g* for 1 h at 4 °C. [^{3}H]-Labeled proteins were analyzed by gel electrophoresis and fluorography, as described above.

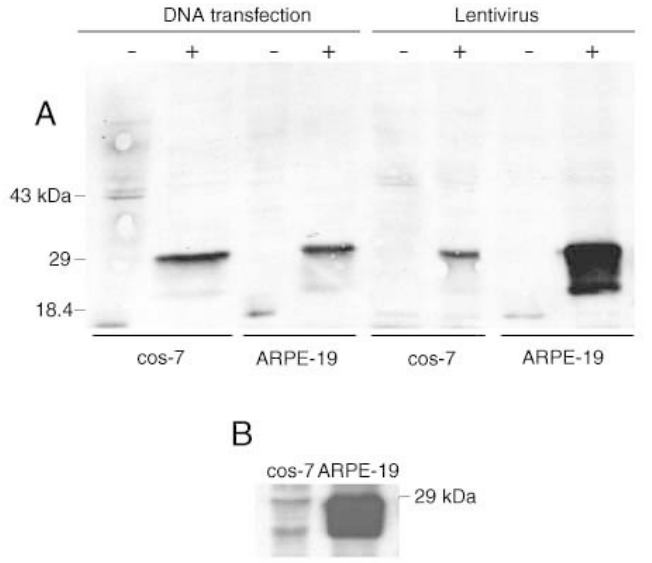

Figure 2. Expression of human RGR protein in COS-7 and ARPE-19 cells. (**A**) COS-7 and ARPE-19 cells were transfected with pcDNA3-hRGR (2 µg DNA/1 x 10^6 cells) using the Lipofectamine Plus agent (Life Technologies), according to the manufacturer's instructions. Alternatively, confluent cells were transduced with the pHR'-CMV-hRGR recombinant *Lentivirus* at equal transduction units (TU)/ml. Control cells were subjected to mock transfection or incubated without the *Lentivirus*. Each lane was loaded with 2 µg of total membrane protein. (**B**) In this experiment, the transduction frequency of the COS-7 and ARPE-19 cells was 10% and 30%, respectively. The transduction frequency was scored by immunohistochemical staining of transduced cells, and Western blot assays were performed with the DE7 antibody.

RESULTS

ARPE-19 and COS-7 cells were transfected with pcDNA3-hRGR, an expression vector that contained a human RGR cDNA under the control of the CMV promoter. Cell membranes were analyzed by Western blot assay two days after transfection, and the results showed that transfected ARPE-19 and COS-7 cells produced comparable amounts of human RGR protein (Figure 2A). A single ~30 kDa recombinant RGR protein was detected. The untreated control cells showed no expression of RGR.

We also developed a lentiviral vector to express human RGR and tested the recombinant virus by transduction of ARPE-19 and COS-7 cells. The lentiviral vector contained

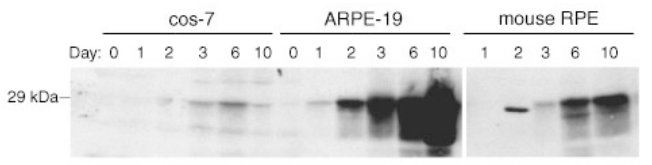

Figure 3. Time course of the production of human RGR protein in COS-7, ARPE-19, and primary mouse RPE cells in culture. Each cell type was inoculated with 10^6 TU recombinant *Lentivirus*. Human RGR protein was assayed at different time points after transduction. Aliquots of total cell protein from COS-7 (35 µg), ARPE-19 (14 µg) and primary mouse RPE cells (6 µg) were loaded in each group of lanes.

the human RGR cDNA again under the control of the CMV promoter. While both cell lines expressed human RGR by day 3 after transduction, the amount of RGR protein from transduced ARPE-19 cells was much higher than that from transduced COS-7 cells (Figure 2A,B). The *Lentivirus*-transduced COS-7 cells produced only the same amount of RGR as did ARPE-19 and COS-7 cells that were transfected with pcDNA3-hRGR DNA.

A time course for the production of human RGR protein in COS-7, ARPE-19, and cultured mouse RPE cells was determined over a 10-day period after transduction with recombinant virus (Figure 3). In each cell type, human RGR protein was detectable 1-2 days after transduction. In COS-7 cells, the expression of RGR reached a maximal level approximately 6 days after transduction. In ARPE-19 and mouse RPE cells, the amount of human RGR increased steadily up to 10 days after transduction. After 6 months of continuous passage, the transduced ARPE-19 cells continued to express the transgene and produced a high amount of human RGR protein, comparable to the amount produced 10 days after transduction.

The large difference in the amount of RGR produced by transduced ARPE-19 and COS-7 cells was investigated by comparison of transduction efficiencies for each cell type. ARPE-19 and COS-7 cells were transduced with the recombinant virus at three different concentrations, or transduction unit per cell (TU/cell), and transduced cells containing RGR were identified by immunohistochemical staining (Figure 4). As indicated in Table 1, the ARPE-19 cells were more efficiently transduced than COS-7 cells at each concentration of recombinant virus. In addition, other results suggested that the amount of human RGR protein produced per cell was higher in transduced ARPE-19 cells than in COS-7 cells. The level of RGR expression was compared in a population of ARPE-19 cells containing ~30% tranduced cells and a population of COS-7 cells containing ~10% transduced cells. Immunoblots showed that the amount of RGR protein from these cells was ~100x higher in ARPE-19 than in COS-7 cells (Figure 2B).

To determine whether recombinant human RGR expressed in ARPE-19 cells is capable of binding all-*trans*-retinal, cell membranes were prepared from transduced ARPE-19 cells and incubated with [^{3}H]all-*trans*-retinal. Results from fluorography demonstrated a single major radiolabeled band from the transduced ARPE-19 cells, but not from the nontransduced ARPE-19 cells (Figure 5). The position of this band in SDS-PAGE coincided with the position of radiolabeled bovine RGR from freshly isolated bovine RPE cells.

We have also demonstrated the uptake of exogenous all-*trans*-retinol into *Lentivirus*-transduced ARPE-19 cells and the incorporation of the retinoid into the chromophore of RGR. Transduced ARPE-19 cells with stable long term expression of human RGR and nontransduced ARPE-19 cells were incu-

Table 1. Transduction efficiency of human RGR lentiviral vector toward ARPE-19 and COS-7 cells

TU/cell	10.	1.	0.1
ARPE-19	98%	56%	10%
COS-7	29%	7%	2%

The cells were treated with equivalent amounts of recombinant Lentivirus at the indicated transduction units (TU)/cell. The transduction frequency, after 2 days of incubation with Lentivirus, was scored by immunocytochemical staining with the DE7 antibody. The results are presented as the mean percentage of total cells that were transduced with the lentiviral vector in three different fields. ARPE-19 cells were transduced more efficiently than COS-7 cells at each TU/cell.

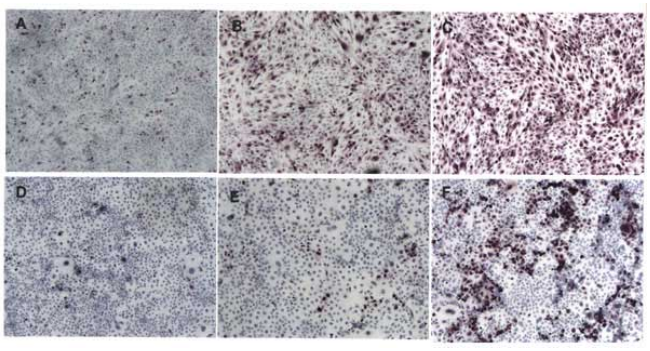

Figure 4. Immunohistochemical staining of *Lentivirus*-transduced ARPE-19 and COS-7 cells. ARPE-19 (**A-C**) and COS-7 cells (**D-F**) were transduced with the following different concentrations of recombinant *Lentivirus*: (**A** and **D**) 0.1 TU/cell; (**B** and **E**) 1 TU/cell; and (**C** and **F**) 10 TU/cell. After 2 days of incubation with *Lentivirus*, human RGR opsin was detected by immunohistochemical staining with the DE7 antibody [11]. Immunoreactivity was developed with the Vector VIP substrate, and the cells were counterstained with Mayer's hematoxylin.

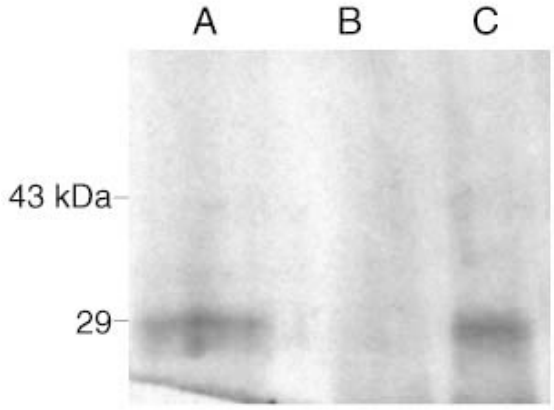

Figure 5. Binding of [^{3}H]all-*trans*-retinal to recombinant human RGR opsin. Bovine RPE microsomal (**A**) and total cell membranes from nontransduced (**B**) and transduced (**C**) ARPE-19 cells were incubated with [^{3}H]all-*trans*-retinal. The reaction products were separated by SDS-PAGE and analyzed by fluorography with 1-week exposure to autoradiographic film. Native bovine RGR and recombinant human RGR were capable of binding to [^{3}H]all-*trans*-retinal in a specific manner in vitro.

bated with [^{3}H]all-*trans*-retinol in serum-free RPMI1640 medium. Analysis of proteins by gel electrophoresis and fluorography demonstrated a single major radiolabeled band in the transduced ARPE-19 cells (Figure 6). The ~30-kDa protein was consistent in size to human RGR and was not detected in the nontransduced control cells.

DISCUSSION

RPE cells in culture can be used to study complex mechanisms of retinoid metabolism and the role of the RPE in the visual cycle [12-17]. Lipids and retinoid-binding proteins assist in the delivery of all-*trans*-retinol as a complex to the cultured RPE cells, and intracellular processing of all-*trans*-retinol has been analyzed using tracer [^{3}H]all-*trans*-retinol [12,14,18]. Often, RPE cells in culture undergo biochemical changes, such as loss of pigmentation, decreased ability to metabolize and store retinoids, and depletion of the cellular retinol-binding protein (CRBP), cellular retinaldehyde-binding protein (CRALBP), and RPE65 [19-22]. We have cultured human and bovine RPE cells in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and analyzed the expression of RGR mRNA in these cells by Northern blot hybridization. RGR mRNA was undetectable in these cultured human and bovine RPE cells, including ARPE-19 cells (results not shown). These findings indicate that an erstwhile active *RGR* gene promoter is dramatically repressed in cultured RPE cells.

To investigate RGR opsin in ARPE-19 cells, which retain unique characteristics of normal RPE cells, a *Lentivirus*-derived gene delivery system was used to produce high levels of recombinant human RGR opsin in transduced ARPE-19 cells. Lentivirus-derived vectors have been demonstrated to achieve an efficient and stable gene transfer in nondividing cells [9,10]. The efficient gene transfer is attributed to the VSV-G protein, which also enables broad cell type tropism of the recombinant virus. Integration of the transgene into the host cell genome leads to the stable transduction of cells. The long-term expression of human RGR in transduced ARPE-19 cells is consistent with previous results in which transgene expression was maintained for more than 6 months after transduction by the *Lentivirus*-derived vector [23,24]. Consequently, the lentiviral vector should also prove effective for in vivo transduction of quiescent RPE cells and potentially may be used to rescue the RGR-deficient mutant mouse [25].

Interestingly, the ARPE-19 cells were transduced more efficiently than COS-7 cells, and the amount of RGR per transduced cell was greater in ARPE-19 than in COS-7 cells. The results suggest that the ARPE-19 cells, like normal RPE cells in vivo, provide efficient synthesis, or stabilization of a functional human RGR protein. Since the CMV promoter in the lentiviral vector is not RPE-specific, the higher amount of human RGR protein found in ARPE-19 cells versus COS-7 cells cannot be explained by promoter activity. The ARPE-19 cells have been found to express endogenous RPE proteins [8], such as 11-*cis* retinyl ester hydrolase, CRALBP and RPE65. Since RGR is also found in microsomal membranes, the presence of other RPE proteins and a specialized smooth endoplasmic reticulum may enable interaction, stabilization and accumulation of the RGR protein. Cultured mouse RPE cells also produced a high amount of recombinant RGR.

The higher transduction efficiency of ARPE-19 epithelial cells in comparison to that of COS-7 fibroblast-like kidney cells may be due to the interaction of VSV-G protein with distinct cell types. The recombinant *Lentivirus* is pseudotyped with VSV-G protein, which may cause cell fusion and some interference with cell growth when attached to cell surfaces [26]. Polarized epithelial cells, and perhaps ARPE-19 cells, are relatively resistant to this fusogenic property of the VSV-G protein [26].

An important result of this study is that the expressed protein is capable of binding to all-*trans*-retinal, the endogenous chromophore of RGR purified from bovine RPE cells. This suggests that the recombinant RGR protein is able to fold into a proper conformation and may have functions of the native RGR protein. In contrast to RGR from ARPE-19 cells, recombinant bovine RGR that we have overexpressed in Sf9 cells of the baculovirus expression system failed consistently to bind all-*trans*-retinal (results not shown). By restoration of a functional RGR protein in ARPE-19 or RGR-/- mouse RPE cells, the biochemistry and role of RGR in retinoid metabolism and regulation can be studied in a cell culture system.

In the transduced ARPE-19 cells, the uptake and processing of [^{3}H]all-*trans*-retinol generated specific binding of the retinoid to RGR. Under the experimental conditions, no other membrane protein bound the radiolabeled retinoid. These results strongly suggest that the ARPE-19 cells are able to synthesize the chromophore of RGR from all-*trans*-retinol and that an all-*trans*-retinol dehydrogenase exists in the RPE. Such all-*trans*-retinol dehydrogenase activity in the RPE has not

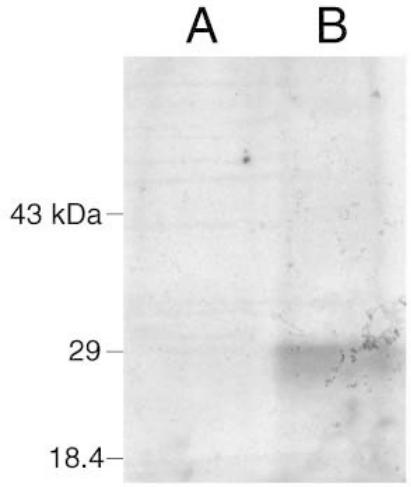

Figure 6. Uptake and incorporation of [^{3}H]all-*trans*-retinol into the chromophore of RGR. Normal (**A**) and *Lentivirus*-transduced (**B**) ARPE-19 cells were preincubated overnight in serum-free RPMI1640 medium and then incubated for 3 h with [^{3}H]all-*trans*-retinol (10 μCi, 50 Ci/mmol) in RPMI1640. Total membrane proteins (~15 μg per lane) were prepared and analyzed by gel electrophoresis and fluorography. The autoradiographic film was exposed for 10 days.

been characterized previously, but would be required to provide a ligand for RGR. The human RGR *Lentivirus*-transduced ARPE-19 cells provide a useful model for further study of the novel all-*trans*-retinol dehydrogenase and the retinoid metabolic pathways that lie upstream and downstream of the RGR opsin.

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