



Alternative splicing of the *APC* gene in the neural retina and retinal pigment epithelium

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Purpose: Hypertrophy and hyperplasia of the retinal pigment epithelium (RPE) is associated with an inherited predisposition to human familial adenomatous polyposis coli, suggesting that expression of the adenomatous polyposis coli (APC) tumor suppressor may regulate RPE proliferation/differentiation. Distinctive *APC* isoforms exist in different cell types due to alternative splicing of the *APC* transcripts. We hypothesize that differences in expression patterns of APC protein isoforms are critical to RPE proliferation/differentiation.

Methods: To investigate these relationships, *APC* gene expression was characterized in the retinas and RPE from fetal and adult human and mouse, and in the epiretinal membranes (ERM) from 5 patients with proliferative vitreoretinopathy (PVR). Expression patterns of alternative splice-forms of *APC* transcripts were evaluated by comparative quantitative RT-PCR. Exon 1 of *APC* encodes a heptad repeat that confers the ability of APC to homodimerize. APC protein isoforms containing or lacking this heptad were characterized by western blot analysis and immunohistochemistry.

Results: Comparative quantitative RT-PCR demonstrated a predominant exon 1 containing, conventional *APC* splice-form in the early developing fetal RPE and retina, and in all the tested ERM samples from patients with PVR. This method also demonstrated an increased level of exon 1 lacking *APC* splice-form in the mature RPE and retina. Western blot analysis and immunofluorescence microscopy demonstrated the conventional APC only in the RPE, and the APC isoform without the first heptad repeat in both the retina and RPE. Immunofluorescence microscopy also demonstrated only the conventional APC in the ERM samples tested.

Conclusions: These results suggest that alternative splicing of *APC* leads to differential APC expression with potentially unique functions. APC isoform without the first heptad repeat may play a role in cell cycle cessation in the adult retina and RPE, and the down regulation of this APC isoform may contribute to the potential of RPE to migrate and proliferate.

The morphologically and functionally polarized retinal pigment epithelium (RPE) has numerous functions including maintenance of adjacent retinal photoreceptor cells and formation of the outer blood-retinal barrier [1]. The stationary RPE cells in the normal adult eye, however, retain the ability to migrate and proliferate in response to a wide range of pathological insults [2]. In proliferative vitreoretinopathy (PVR), the most common cause of recurrent retinal detachment after successful surgical repair, epiretinal membranes (ERM) arise from dispersed vitreal RPE and may cause traction retinal detachment. Proliferative RPE may also be associated with exudative age-related macular degeneration (AMD), where choroidal neovascular membranes are often comprised of choroidal capillary endothelium and fibrovascular tissues surrounded by a rim of proliferating RPE [3]. In addition, the potential for successful RPE transplantation in various macular and retinal

degenerations may rely on an ability to control the proliferative nature of transplanted and/or native RPE. Thus, an understanding of potential molecular factors critical for the migration and proliferation of RPE is of great importance.

One of the factors that may affect the proliferation and migration of RPE is the adenomatous polyposis coli (APC) protein. APC (2843 amino acids, molecular weight about 310 kDa) is a ubiquitously expressed tumor suppressor protein that is particularly important in the colorectal epithelial cell proliferation and migration [4]. The importance of APC to colorectal epithelium and RPE cell physiology has been suggested by the association of APC gene mutations with a familial form of human colorectal carcinoma known as familial adenomatous polyposis coli (FAP) [5,6], also characterized by congenital hypertrophy/hyperplasia of the RPE, or CHRPE [7]. Similar phenotypes of intestinal tumors and proliferative RPE were recapitulated in a mouse model of FAP, and emphasize the important role of APC in RPE proliferation and development [8,9].

The conventional APC transcript includes 17 exons: 1, 2, 3, 4, 5, 6, 7, 8, 9, 9A, 10, 10A, 11, 12, 13, 14, and 15, with

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exons 1, 9, and 10A being alternatively expressed [10,11]. Exon 1 of APC encodes the first of a series of heptad repeats [12], which are required for homodimerization [5,13]. The role of the APC as a tumor suppressor protein may depend upon its ability to homodimerize [12,14]. Additional splice-forms of APC lacking exon 1 have been identified in neuronal cells and tissues rich in non-dividing cells such as cerebellum, cerebellum, skeletal muscle and heart [15]. These exon 1 lacking transcripts contain one or more additional exons located 5' of exon 1 (Figure 1). Two of these exons, brain-specific (BS) and 0.3, contain in-frame translation initiation codons [16]. Additionally, a stop codon located upstream of the original translation initiation codon in exon 1 prevents the in-frame translation of BS or 0.3 unless exon 1 is excluded by splicing. Therefore, amino acids encoded by exon BS or 0.3 are not included in the conventional APC. Exon 1 lacking 0.3-APC or BS-APC transcripts and BS-APC protein (encoded by exons BS and 2-15) have been identified in adult human brain, but not in tissues containing mitotic cells. These APC proteins without the first heptad repeat are most likely involved in cell cycle cessation or differentiation [15,17].

The splicing pattern of the APC gene in the neural retina and RPE has not been studied, despite the fact that APC may be important to the physiology of these cells. Although both neural retina and RPE share the same neural tube origin and are mitotically inactive, it is hypothesized that the APC splicing pattern may account for the ability of the RPE to become proliferative and migratory in pathologic conditions. In this report, we determined the alternative splice-forms of APC transcripts and isoforms of APC protein in developing mammalian retina, RPE and ERM from patients with PVR.

METHODS

Human ocular tissues: Human ocular tissues were obtained from deceased donors and were distributed by the Cooperative Human Tissue Network (Philadelphia, PA). Donor ages ranged between 58 and 92 years. Donor eyes were kept at 4 °C and the average interval between death and retina dissection was 26 h. Human fetal eyes (gestational age 16-20 weeks) were supplied by Advanced Bioscience Resources, Inc

(Alameda, CA). Epiretinal membranes (PVR) were obtained from 5 consenting patients (P1 through P5). The research followed the tenets of the Declaration of Helsinki and was reviewed and approved by Human Assurance Committee of the Medical College of Georgia for using human post-mortem material.

Primary RPE cell culture from fetal human eyes: All reagents were purchased from Sigma unless otherwise indicated. Eyes were treated with 3 ml antibiotic-antimycotic solution 1 mg/ml gentamicin and 25 µg/ml Amphotericin B (Life Technologies, Rockville, MD) for 10 min [18]. After three washes with Hanks buffered salt solution, the anterior chamber and the vitreous were removed. The eyecup was incubated with 2 mg/ml Dispase (Life Technologies) at 37 °C for 30 min. After the retina was peeled off, sheets of RPE cells were peeled from the choroid and separated by repeated trituration in RPE culture medium, defined as follows: minimum essential medium Eagle (MEM), Alpha modification, supplemented with 5% heat-inactivated fetal bovine serum (FBS; Atlanta Biological, Atlanta, GA), 0.89 g/L L-alanine HCl, 1.5 g/L L-asparagine H₂O, 1.33 g/L aspartic acid, 1.47 g/L L-glutamic acid, 0.75 g/L glycine, 1.15 g/L L-proline, 1.05 g/L L-serine, 2 mM L-glutamine, 100,000 units/L penicillin, 20 mg/L streptomycin, 0.25 g/L taurine, 20 mg/L hydrocortisone, 13 ng/L triiodothyron, 5 mg/L insulin, 5 mg/L transferrin, 5 mg/L selenium, 16 mg/L putrescine, 7.3 mg/L progesterone. Cells were pelleted by centrifuging at 500 g for 5 min and washed with RPE culture medium. Finally, the cells were resuspended in 5 ml RPE seeding medium (same as RPE culture medium except with 15% FBS), seeded on a T25 Primaria tissue culture flask (Becton Dickinson and Co., Franklin Lakes, NJ) and cultured in a humidified incubator at 37 °C and 5% CO₂. Cells were switched to RPE culture medium (5% FBS) one day later and maintained in that medium. After 3-5 weeks, the confluent cells were trypsinized with 0.25% trypsin (Life Technologies).

Primary RPE cell culture from human donor eyes: The anterior portion of the human donor eye, as well as lens, vitreous and retina were removed to expose the RPE cell layer according to a previously published method [19]. The eyecup was rinsed with Hank's buffered salt solution, filled with a

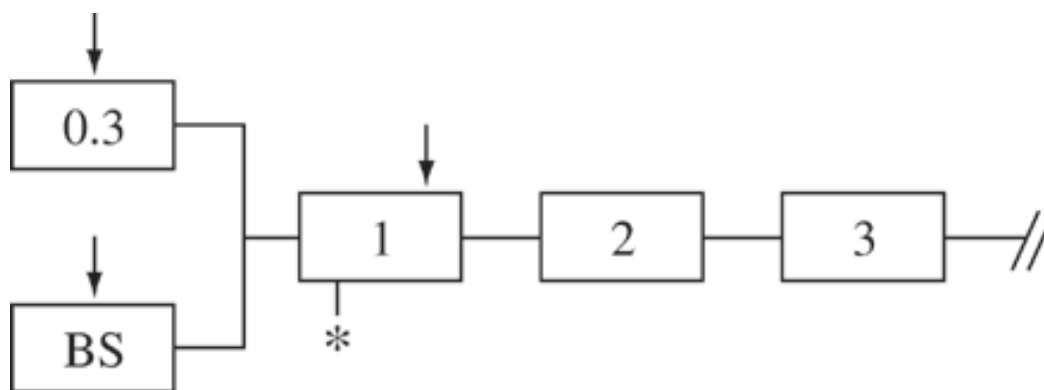


Figure 1. Schematic of APC alternative splice-forms. This figure is a partial schematic representation of the APC alternative splice-forms identified in the retina/RPE RNA. Exon 1 encodes a translational initiation site (arrow). The 5' exons 0.3 and BS also contain initiation codons that are in-frame with exon 2 (arrows). The upstream stop codon (*) in exon 1 will prevent the in-frame translations from the 5' exons 0.3 and BS unless exon 1 is excluded by splicing.

solution of 0.5 g trypsin/0.2 g EDTA/ml (Life Technologies) and incubated at 37 °C for 15 min. The dislodged cells were aspirated and the trypsin digestion procedure was repeated. Suspended cells were washed in Ham's F-10 medium containing 10% FBS, calcium chloride, ITS plus (Life Technologies) and antibiotics. The cells were resuspended in the same medium or D-MEM/F-12 with L-glutamine.

Animal care: All procedures concerning animals in this study adhered to the ARVO Statement for the Care and Use of Animals in Ophthalmic and Vision Research. One to two month old C57BL/6 or BALB/C mice were obtained from Jackson Laboratory (Bar Harbor, ME). Mice were permitted ad libitum rodent diet and water and maintained on a 12 h light/dark cycle. For studies using developing mice, inbred C57BL/6 are used. Mating cages were set up by placing two females with each stud male in the late afternoon. Pregnancy was determined by removing the females the following morning and checking for the presence of a copulation plug. When a copulation plug was found, that day was designated embryonic day 0.

RNA preparation and comparative quantitative RT-PCR: Human eyes were bisected at the ora serrata and the anterior chamber and vitreous gel were removed. The retinas and eye-

cups were rinsed with ice cold, diethylpyrocarbonate (DEPC) treated phosphate buffered saline (PBS) and the RPE cells were collected with a camel hair brush into 0.5 ml of DEPC treated PBS. Beginning at embryonic day 12, mouse eyes were dissected from each fetus. Adult mouse eyes were dissected to obtain the retinas and eyecups. RNA was prepared from cells in culture, or from human and mouse tissues using total RNA isolation reagent kit, RNawiz or ToTally RNA (Ambion, Austin, TX) according to the manufacturer's instructions. Both of these kits are based on the use of detergent, chaotropic salts and phenolic extraction to denature the RNase and to solubilize and extract the total RNA.

The identification and quantification of the alternative splice forms of *APC* transcripts, 0.3 *APC* or *BS-APC* containing or those forms lacking exon 1, was achieved by comparative quantitative RT-PCR (Ambion, Inc., Austin, TX) using mouse or human exons 0.3 and 3 or *BS* and 3 primer pairs [15]. The mouse exon 0.3 primer, GAG ACA GAA TGG AGG TGC TGC, and exon 3 antisense primer, TTT CAA GCT CTT CTA GAT ACC C, amplify a fragment of 471 and 318 bp in the presence and absence of exon 1, respectively. The human exon 0.3 primer (same as mouse) and exon 3 antisense primer, CTC TCT TTC TCA AGT TCT TCT A, amplify a fragment of

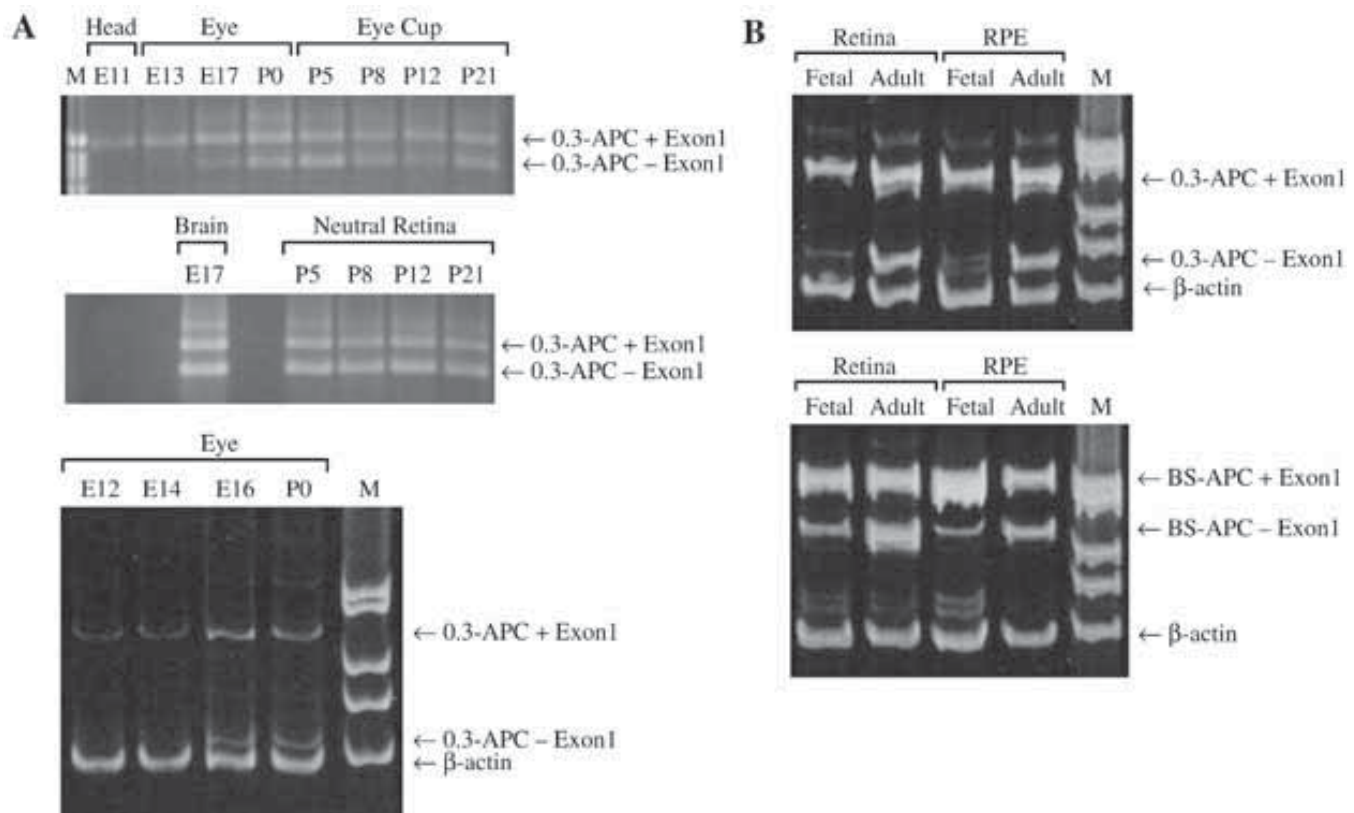


Figure 2. Alternative *APC* transcripts. RT-PCR in agarose gel (A, top) or comparative quantitative RT-PCR in polyacrylamide gel using mouse or human exon 0.3 and 3 primers, or human exons *BS* and 3 primers. The positions for exon 1 containing (0.3 *APC* + exon 1 or *BS-APC* + exon 1) or exon 1 lacking (0.3 *APC*-exon 1 or *BS-APC*-exon 1) *APC* transcripts, and internal standard β -actin are indicated. **A:** Alternative 0.3 *APC* transcripts in mouse ocular tissues during development. **B:** Alternative 0.3 *APC* and *BS-APC* transcripts in the developing (gestation 17 week fetal) and adult human retina and RPE.

477 and 324 bp in the presence and absence of exon 1, respectively. The human exon *BS* primer, GCT CTA CCC CAT TGA AAG GC, and exon 3 antisense primer amplify a fragment of 593 and 440 bp in the presence and absence of exon 1, respectively. Briefly, first-strand cDNA synthesis was performed on 2 µg DNase treated RNA. The individual cDNA species were amplified in a reaction containing a cDNA aliquot, PCR buffer, and MgCl₂. Reactions were initiated by incubating at 95 °C

for 15 min and PCR (94 °C, 40 s; 58 °C, 30 s; 72 °C, 30 s) performed for a suitable number of cycles to ensure that amplification was within exponential phase (0.3 *APC*, 25 or 26 cycles; *BS-APC*, 24 cycles), followed by a final extension at 72 °C for 7 min. The abundant internal standard, β-actin, was amplified to the levels comparable to 0.3 *APC* or *BS-APC* by using primer and competitor mixtures (Ambion) at various ratios. Interexperimental variations were avoided by performing all amplifications in a single run.

PCR products were separated in 1.6% agarose or 8% polyacrylamide gel electrophoresis. Gels were stained with ethidium bromide, photographed under ultraviolet illumination, and the appropriate product size determined by comparison with a 100 bp or 1 kb DNA ladder (Life Technologies).

Western blot analysis: Human donor eyes were bisected as above, and the retinas and eyecups were rinsed with ice cold phosphate buffered saline containing 0.5 mM phenylmethylsulfonylfluoride (PMSF). The RPE cells were collected with a camel hair brush into 0.5 ml of lysis buffer 50 mM tris-HCl, pH 7.5, 150 mM NaCl, 0.5% NP-40, 1 mM EDTA, 0.2 mM PMSF and 100 µl/ml of proteinase inhibitor cocktail containing 4-(2-aminoethyl) benzenesulfonylfluoride, pepstatin A, trans-epoxysuccinyl-L-leucylamido(4-guanidino) butane, bestatin, leupeptin and aprotinin (Sigma, St. Louis, MO). Retinal tissue and RPE cells were homogenized using a Dounce homogenizer with a tight fitting pestle and cell lysates were cleared by centrifugation at 14,000 rpm (16,000x g) for 20 min at 4 °C. Proteins of 100 µg, determined by Bio Rad Protein Assay (Detergent Compatible), were separated by SDS-PAGE on 4.5% to 9% gradient acrylamide mini-gels containing 25% glycerin and electrophoretically transferred

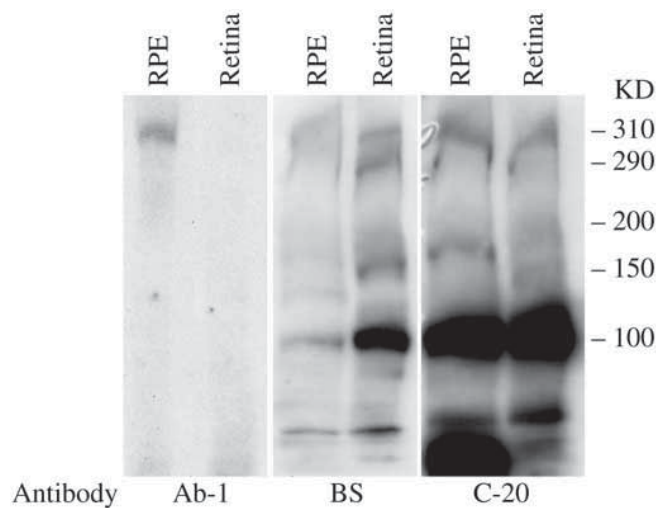


Figure 3. APC isoforms in the retina and RPE. Human retinal and RPE proteins (100 µg) were electrophoresed on a 4.5-9% gradient SDS-polyacrylamide gel, blotted to nitrocellulose membrane, incubated with antibody Ab-1, BS, or C-20, and developed with enhanced chemiluminescence. The positions of full-length and internal splice-variant isoforms of APC (kDa) are indicated.

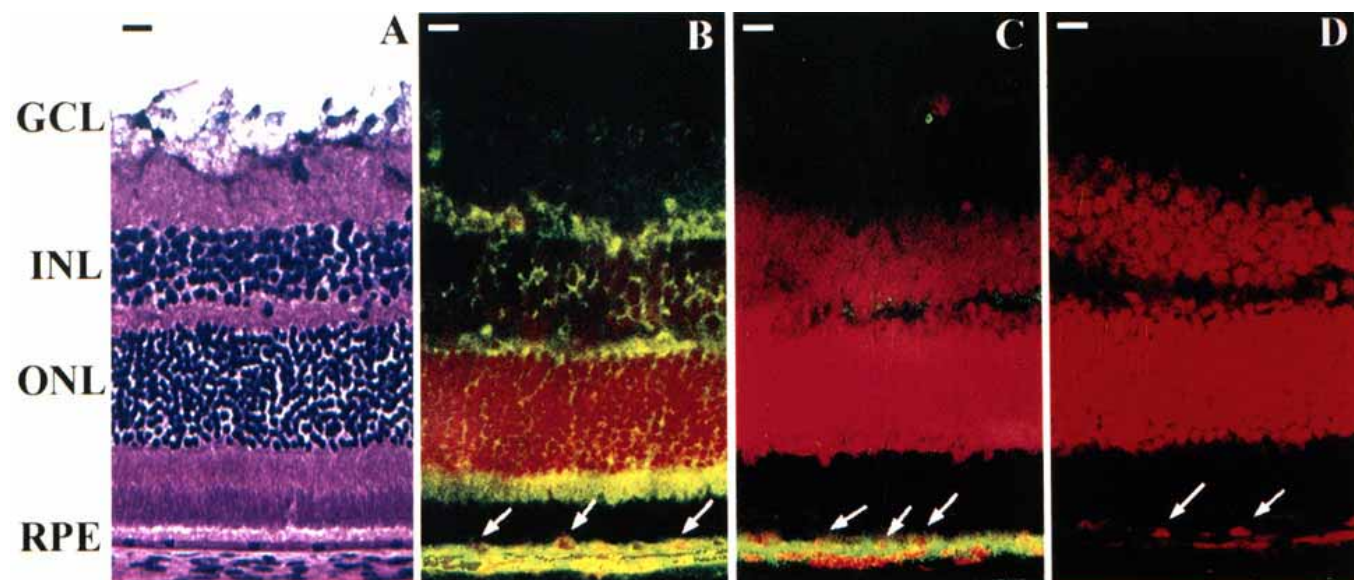


Figure 4. Retinal immunolocalization of conventional APC and BS-APC. Images are of laser scanning confocal microscopic immunolocalization of conventional APC and BS-APC in mouse retina. **A:** H & E stained cryosection depicting ganglion (GCL), inner and outer nuclear layer (INL and ONL), and RPE cells. **B-D:** Vertical sections (x and z) taken of cryosection of mouse eye incubated with different antibodies against APC and Oregon Green-conjugated secondary antibodies. **B:** Anti-BS. **C:** Anti-exon 1 (Ab-1). **D:** Normal serum. Cell nuclei were stained with propidium iodide. Arrows indicate RPE cell nuclei. Bar represents 10 µm.

to a nitrocellulose membrane (Schleicher and Shuell, Keene, NH). The antibodies used, mouse monoclonal antibodies Ab-1 (Oncogene Research Products, Cambridge, MA; Calbiochem, San Diego, CA) and C-20 (Santa Cruz Biotechnology Inc, Santa Cruz, CA), and rabbit polyclonal antibody anti-BS, are specific for the amino (N)-terminal 35 amino acids encoded by exon 1, C-terminus encoded by exon 15 and an undefined epitope encoded by exon BS [17], respectively. The antigen/antibody reaction was visualized by development with enhanced chemiluminescence (Amersham). For antibody stripping from the nitrocellulose membrane, the antibody stripping solution (Chemicon, Temecula, CA) was used.

Immunofluorescence of APC: Localization of APC in adult albino mouse (BALB/C) retina, human donor eye and ERM (PVR) was visualized by immunofluorescence with the Ab-1 and anti-BS antibodies described above. Mouse eyes and ERM were frozen in OCT. Human donor eye was fixed in 4% paraformaldehyde and paraffin-embedded. The 10 μ m thick sections on slides were fixed with ice-cold acetone and blocked with 4% normal goat serum. The slides were incubated overnight at 4 °C with a 1:50 dilution of Ab-1 or a 1:2000 dilution of BS. After rinsing, all samples were incubated overnight at 4 °C with a 1:1000 dilution of Oregon Green-labeled goat anti-mouse or goat anti-rabbit IgG secondary antibody (Molecular Probes, Eugene, OR). Controls were prepared in the same manner, except that the primary antibody was replaced with normal rabbit serum. Coverslips were mounted under

Permafluor mounting medium. Sections were optically sectioned (z series) using a Nikon Diaphot 200 Laser Scanning Confocal Imaging System (Molecular Dynamics, Sunnyvale, CA). Images were analyzed using the Image Space software package (Molecular Dynamics).

RESULTS

Analysis of APC splice-forms in the retina and RPE: Alternative splice-forms of APC have been identified in different tissues using RT-PCR [15]. Transcripts encoding conventional APC have been identified in tissues with dividing cells, and those lacking exon 1 have been identified in tissues rich in non-dividing or terminally differentiated cells. To determine whether alternative splice-forms of APC exist in the retina and RPE that may regulate cell growth and development, we analyzed mouse and human 0.3 APC or BS-APC transcripts with or without exon 1 in these tissues during development and in the adult by RT-PCR. The composition of the splice-forms was confirmed by sequencing after cloning in pGEM-T Easy (Promega, Madison, WI; data not shown). As shown in Figure 2A, in the developing mouse eye prior to embryonic day 16-17 (E16-17), both non-quantitative (top panel) and comparative quantitative RT-PCR (lower panel) indicated that the exon 1 containing 0.3 APC transcript was the only splice-form. At E16 and later, the exon 1 containing and exon 1 lacking 0.3 APC transcripts were both evident in the brain, neural retina, eyecup and eye. To determine the APC transcripts specific to the RPE and neural retina, the splice-forms of 0.3 APC and BS-APC transcripts in the fetal (gestation time, 17 week) and adult human retina and RPE were analyzed by comparative quantitative RT-PCR. As shown in Figure 2B, the exon 1 lacking 0.3 or BS-APC transcript in the fetal human retina or RPE was much lower than that in the adult tissue. In the adult tissues, the exon 1 lacking BS-APC transcript in the RPE was lower than that in the retina. These results suggest that the exon 1 containing, conventional APC transcript plays a role in dividing cells during embryonic retinal/RPE development. On the other hand, higher levels of the exon 1 lacking APC transcripts in the adult retina and brain suggest a role for these isoforms in terminal differentiation or in the cessation of growth. The relatively lower level of the exon 1 lacking BS-APC transcript in mature RPE suggest that RPE cells may not be terminally differentiated and that APC may be critical to RPE migration and proliferation in pathologic conditions.

Identification of the APC protein isoforms in retina and RPE: Conventional APC protein has been identified in all epithelial tissues containing mitotic cells. BS-APC protein without the first heptad repeat has been identified in adult human brain, but not in tissues containing mitotic cells [17]. Conventional APC and BS-APC protein isoforms in adult human neural retina and RPE were determined by western blot analysis using antibodies specific for exon 1 (Ab-1), exon BS (BS) and exon 15 (C-20). Antibody C-20 is used to identify full-length APC as well as all the internal splice-variant isoforms. Antibodies BS and C-20 have identified full-length APC of about 310 kDa and internal splice-variant species of 290, 200, 150 and 100 kDa [17]. As shown in Figure 3, Ab-1

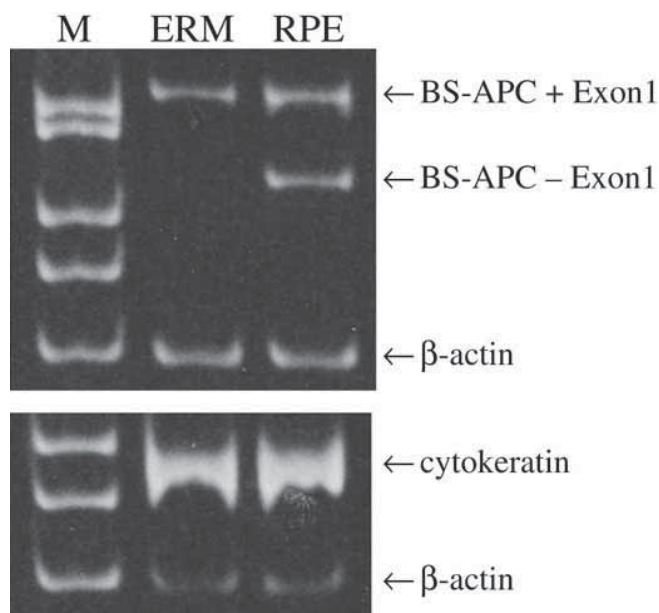


Figure 5. Alternative APC transcripts in the RPE and epiretinal membrane. Comparative quantitative RT-PCR in polyacrylamide gel using human exons BS and 3, and *cytokeratin-18* primers was used to identify APC isoforms in human RPE cells and from human ERM samples. The positions for exon 1 containing (BS-APC + exon 1) or exon 1 lacking (BS-APC-exon 1) APC transcripts, *cytokeratin-18*, and internal standard β -actin are indicated for RPE and ERM. Result of ERM represents one of three patients (P2).

detected a full-length, conventional APC band of about 310 kDa in the RPE, but not in the retina. Anti-BS antibody, on the other hand, detected multiple bands of 310, 290, 150 and 100 in the retina, but much lower levels of these bands in the RPE. Antibody C-20 detected almost comparable levels of these bands in the RPE and retina. These results suggest that while similar levels of total APC isoforms are expressed in the retina

and RPE, higher level of conventional APC protein is expressed in the RPE, and higher level of BS-APC protein is expressed in the retina.

Localization of the APC protein isoforms in the retina and RPE: To localize the isoforms of APC protein in the neural retina and RPE in situ, confocal immunofluorescence microscopy of cryosectioned mouse eyes was performed. Sec-

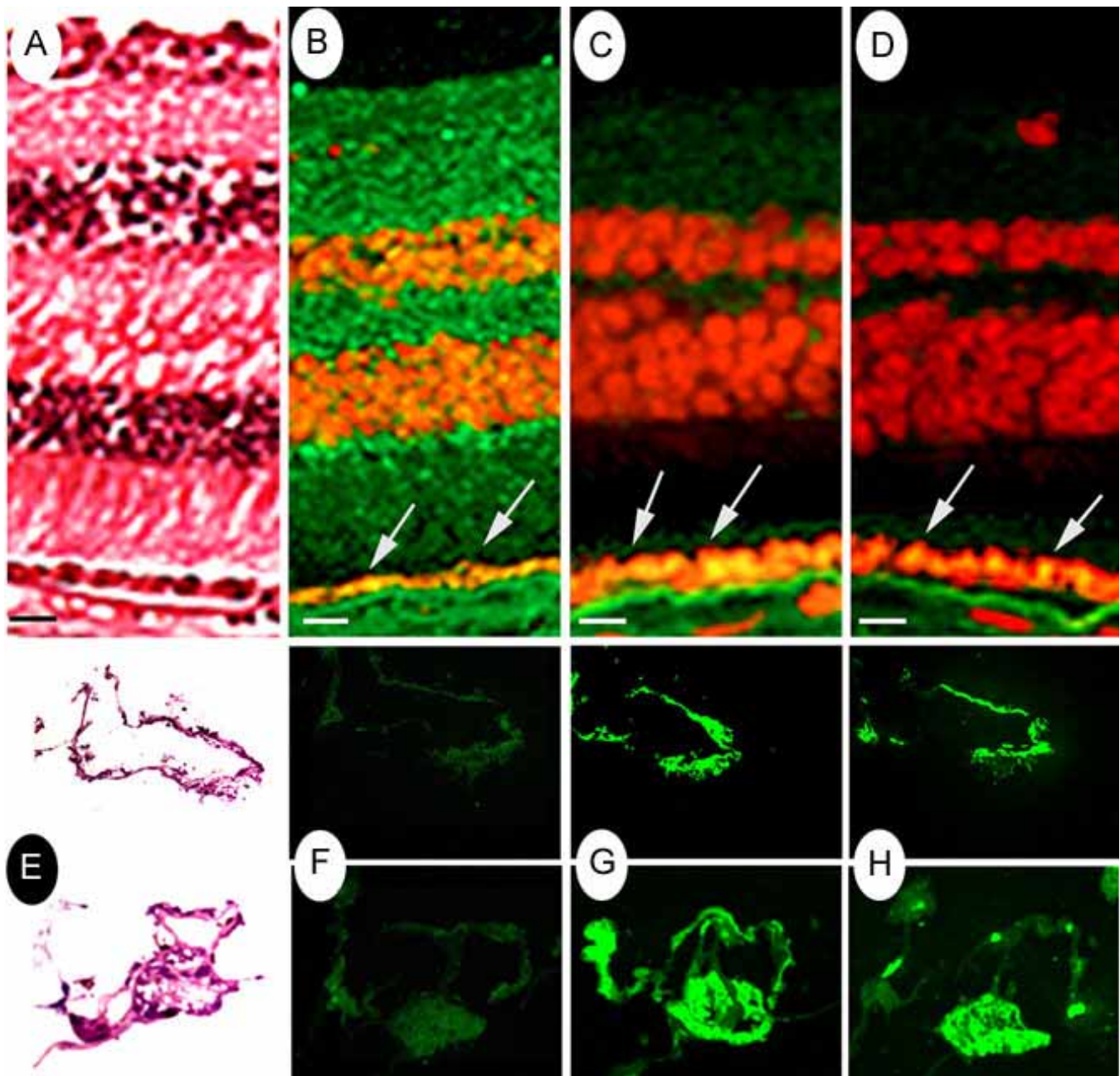


Figure 6. Immunolocalization of conventional APC and BS-APC in the epiretinal membrane. Laser-scanning confocal microscopic immunolocalization of conventional APC, BS-APC, and pan-cytokeratin is shown in human retina (A-D) and two different regions of an ERM from one patient (P5). E-H: Serial vertical sections (x and z) of formaldehyde fixed retina sections or cryosectioned ERM incubated with different antibodies and with Oregon Green-conjugated secondary antibodies. A,E: H & E stained. B,F: anti-BS-APC. C,G: anti-exon 1 (Ab-1). D,H: anti-pan-cytokeratin. Cell nuclei were stained with propidium iodide. Arrows indicate RPE monolayer, which is seen as an orange hue. Bar represents 10 μ m.

tions were compared to slides stained with hematoxylin and eosin (Figure 4A). Sections stained with BS antibody demonstrated immunofluorescence in both the neural retina and RPE (Figure 4B). In the neural retina, BS-APC is mainly localized in the inner and outer plexiform layers, and in the inner segment of the photoreceptors. In contrast, sections stained with Ab-1 antibody demonstrated an RPE-dominating immunofluorescence (Figure 4C). Sections stained with normal serum and with fluorescence-conjugated secondary antibody showed only minor immunofluorescence in the choroid/sclera (Figure 4D). These results demonstrated that while both BS-APC and conventional APC are present in the RPE, only BS-APC is expressed in the neural retina.

Identification of APC isoforms in the epiretinal membranes: The ERM of PVR contains migratory and proliferative cells of RPE origin [2]. To test the hypothesis that the proliferative nature of RPE in the ERM of PVR is associated with down-regulation of BS-APC, the expression of the alternative splice-forms of the *APC* gene and *cytokeratin-18* gene (epithelial cell marker) in adult human RPE and in the ERM of three patients with PVR (P1, P2, and P4) were analyzed by comparative quantitative RT-PCR. As shown in Figure 5, exon 1 containing and exon 1 lacking *BS-APC* transcripts are co-expressed in the RPE. However, exon 1 lacking *BS-APC* is not detected in the ERM from one of the patients (P2). Similar results are observed in the ERM of the other two patients (data not shown). Both the RPE and ERM express comparable levels of *cytokeratin-18* gene.

The in situ localization of the isoforms of APC protein in adult human retina and RPE, and in the ERM of two patients with PVR (P3 and P5) was determined by immunohistochemical analysis. Confocal immunofluorescence microscopy demonstrated that cytokeratin, conventional APC and BS-APC are all expressed in the RPE, and only BS-APC is expressed in the neural retina (Figure 6, top panels). In the ERM of two patients, cytokeratin and conventional APC, but not BS-APC, are expressed in the RPE-like cells (Figure 6, lower panels, two sections from the ERM of patient P5; result for patient P3 not shown). These findings suggest down-regulation of BS-APC isoform may contribute to the potential of RPE to migrate and proliferate.

DISCUSSION

Mutation of the *APC* gene is responsible for familial adenomatous polyposis coli (FAP), an autosomal dominantly inherited disease of the colon and other polyposis syndromes [5,6]. Patients with FAP may present with ocular manifestations in the form of bilateral and multiple pigmented lesions of the RPE, which represent congenital hypertrophy/hyperplasia and hamartomatous proliferation of the RPE (CHRPE) [7]. The association of *APC* gene mutation and CHRPE implicates *APC* as a regulatory gene of RPE proliferation.

APC expression in the RPE or retina has not been extensively studied despite the fact that *APC* is more highly expressed in the central nervous neurons than in any other tissue as reported by Bhat et al. [20]. Analysis of *APC* expression in

brain with in situ hybridization by these authors showed that *APC* mRNA was expressed throughout the rat brain during development and remained abundant in the adult. In the embryonic rat brain, *APC* mRNA was particularly abundant in cortical, cerebellar, and retinal layers containing newly formed, post-mitotic neurons, suggesting that *APC* may contribute to suppressing neuronal proliferation. However, the study by these authors was carried out using probes to regions of *APC* downstream of exon 2, which did not allow determination of the temporal expression and distribution of transcripts likely to encode BS-APC or conventional APC proteins.

In this study, we have demonstrated that conventional *APC* (exon 1 containing *APC* transcript) is the predominant splice-form of *APC* transcript in the neural retinal and RPE cells during early embryonic eye development. As these tissues become mature, the exon 1 lacking splice-forms of *APC* transcript are also expressed. This is more evident for *BS-APC* in the neural retina. We have further demonstrated that conventional *APC* is the predominant protein isoform in the adult RPE, while *APC* protein lacking the first heptad repeat (*BS-APC*) is the predominant isoform in the adult neural retina. The presence of conventional *APC* transcripts in early developing neural retina and RPE is not unexpected, as conventional *APC* transcripts are present in dividing cells [15,17]. The expression of the conventional *APC* protein isoform in adult RPE cells is intriguing. RPE cells, like neural retinal cells, are derived from the same neuroectodermal precursors in the neural tube. Although RPE cells are stationary and remain largely non-proliferative, these cells in adult eyes are not terminally differentiated. The predominant BS-APC protein in the adult retina suggests a role in terminal differentiation. On the other hand, the co-expression of BS-APC and conventional *APC* isoforms in the adult RPE suggest that while BS-APC plays a role in maintaining cell differentiation, conventional *APC* may play a critical role in maintaining the potential for RPE proliferation and migration when BS-APC is down-regulated. We have demonstrated the association of BS-APC down-regulation with RPE proliferation in the proliferative RPE-like cells in the epiretinal membranes of PVR.

In this study, we have noticed that the presence of the transcripts for the *BS-APC* and conventional *APC* isoforms does not correlate with the corresponding proteins found. For example, in the adult neural retina or RPE, conventional *APC* and *BS-APC* transcripts are co-present, but only either the conventional *APC* protein or BS-APC protein is present in these tissues. A possible explanation is that postranscriptional/translational regulation may play a role in this discrepancy. In the mature neural retina, exon 1 lacking *BS-APC* transcript is preferentially translated whereas in the mature RPE, conventional *APC* transcript is preferentially translated. Further studies are required to resolve the mechanism of regulation.

APC functions through its interactions with, and down-regulation of, β -catenin [12,14]. The latter is a bi-functional molecule that mediates cellular adhesion when associated with E-cadherin at the plasma membrane, or, in the wnt/frizzled or hepatocyte growth factor/c-met signaling pathway, stimulates

the transcription of growth-promoting genes when associated with certain transcription factors [21]. APC may also be involved in cell migration when associated with microtubules in epithelial cell membranes [22]. Recently, the secreted frizzled-related protein-5 [23] and N- and E-cadherins [24] (unpublished observations) have been identified in the RPE. Moreover, hepatocyte growth factor/c-met [25-27] and β -catenin [27] may be involved in the regulation of RPE migration. Further study of the relationship between APC, cadherins and β -catenin may provide molecular insight into the regulatory role of APC in RPE proliferation.

In conclusion, our results in developing and adult retinal tissues suggest that splicing of APC may lead to differential APC expression with potentially unique functions. APC isoform without the first heptad repeat may play a role in cell cycle cessation in the adult retina and RPE, and the down-regulation of this APC isoform may contribute to the potential of RPE to migrate and proliferate.

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