



Localization of the ultraviolet-sensor *Opn5m* and its effect on myopia-related gene expression in the late-embryonic chick eye

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ABSTRACT

Recent studies show that exposure to ultraviolet (UV) light suppresses ocular elongation, which causes myopia development. However, the specific mechanisms of this process have not been elucidated. A UV-sensor, Opsin 5 (*Opn5*) mRNA was shown to be present in extraretinal tissues. To test the possibility that UV-signals mediated by *Opn5* would have a direct effect on the outer connective tissues of the eye, we first examined the expression patterns of a mammalian type *Opn5* (*Opn5m*) in the late-embryonic chicken eye. Quantitative PCR showed *Opn5m* mRNA expression in the cornea and sclera. The anti-*Opn5m* antibody stained a small subset of cells in the corneal stroma and fibrous sclera. We next assessed the effect of UV-A (375 nm) irradiation on the chicken fibroblast cell line DF-1 overexpressing chicken *Opn5m*. UV-A irradiation for 30 min significantly increased the expression of *Early growth response 1* (*Egr1*), known as an immediate early responsive gene, and of *Matrix metalloproteinase 2* (*Mmp2*) in the presence of retinal chromophore 11-*cis*-retinal. In contrast, expression of *Transforming growth factor beta 2* and *Tissue inhibitor of metalloproteinase 2* was not significantly altered. These results indicate that UV-A absorption by *Opn5m* can upregulate the expression levels of *Egr1* and *Mmp2* in non-neuronal, fibroblasts. Taken together with the presence of *Opn5m* in the cornea and sclera, it is suggested that UV-A signaling mediated by *Opn5* in the extraretinal ocular tissues could influence directly the outer connective tissues of the chicken late-embryonic eye.

1. Introduction

The rhodopsin family, consisting of an opsin protein and its chromophore retinal, are known as photon receptors functioning in the initial stages of vision [1]. There are 9 opsin family genes in the human genome: *Rod opsin* (*RHO*), three cone opsin genes (*OPN1SW*, *OPN1MW*, *OPN1LW*), *Encephalopsin/Panopsin* (*OPN3*), *Melanopsin* (*OPN4*), *Opsin 5* (*OPN5*), *Retinal G protein coupled receptor* (*RGR*), and *Retinal pigment epithelium derived rhodopsin homolog* (*RRH/peropsin*).

Opsin 5 (*Opn5*; formerly *neuropsin*), initially identified by genome mining in human and mouse DNA databases [2], has been found in a wide range of genomes from mammals to fish [3–7]. In addition to its

presence in the genome, *Opn5* was found to be an ultraviolet (UV) ($\lambda_{\max} = 380$ nm) sensor, which activates Gi-type G-protein and induces further signal transduction pathways via cAMP, Ca^{2+} , and phosphorylation of MAP kinases [5,8,9]. *Opn5* is present in the eyes and brains of birds, fish, rodents, and primates [2–8,10]. Its functions in the eye were partly elucidated recently: *Opn5* in the mouse retina and cornea has roles in the entrainment of their local photoperiodicity and *Opn5*-null mice exhibit impaired circadian photoentrainment [11,12]. Nevertheless, the functions of *Opn5* have not been studied from the viewpoint of ocular development.

The exponential increase in the number of people suffering from myopia globally, and especially in eastern Asia, is a matter of public

Abbreviations: cAMP, cyclic adenosine monophosphate; Egr1, Early growth response 1; Gapdh, Glyceraldehyde-3-phosphate dehydrogenase; MAP kinase, mitogen-activated protein kinase; Mmp2, Matrix metalloproteinase 2; Opn5, Opsin 5; Opn5m, mammalian type Opn5; qPCR, quantitative polymerase chain reaction; Tgfb2, Transforming growth factor beta 2; Timp2, Tissue inhibitor of metalloproteinase 2; UV, ultraviolet; UV-A, ultraviolet-A

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concern [13]. In myopia, objects viewed at a distance are blurred due to an abnormal elongation of the eyeball. Recent studies have shown that exposure to sunlight or more specifically violet light (360–400 nm), suppresses myopia progression [14,15]. However, the specific mechanisms of this process have not been elucidated.

We hypothesized that UV-A or violet light reception by *Opn5* might have a direct inhibitory effect on the elongation of the eyeball, whose wall consists of sclera and cornea, resulting in the suppression of myopia development. To test this hypothesis, we examined the expression levels and distribution of the mammalian type *Opn5* (*Opn5m*) in the sclera and cornea of chicken late-stage embryos. We also assessed the effect of UV-A (375 nm) irradiation on the chicken fibroblast cell line DF-1 overexpressing chicken *Opn5m*.

2. Materials and methods

2.1. Preparation of chicken late-embryonic eyes, RNA isolation, and synthesis of cDNA

Fertilized chicken eggs (*Gallus gallus*) were purchased from a commercial farm (Goto-furanjo, Japan; <http://www.gotonohiyoko.co.jp/>) and incubated at 37.5°C in a humidified incubator. According to the American Veterinary Medical Association Guidelines (<https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx>), we euthanized embryonic day 17 (E17) chicken embryos. Twenty eyes from 10 chicken embryos at E17 were removed and the cornea, fibrous sclera, cartilaginous sclera, chorior- etinal tissues, and a portion of liver were isolated in ice-cold phosphate-buffered saline (PBS). They were treated with ice-cold RNAlater stabilization solution (Thermo Fisher Scientific, Waltham, MA, USA), snap-frozen in liquid nitrogen, and stored at –80°C until further use. Total RNA was extracted from the tissues using an RNAqueous-Micro Total RNA Isolation Kit (Thermo). After treatment of DNase I, cDNA was synthesized using random hexamers and reverse transcriptase (SuperScript III First-Strand Synthesis System; Thermo) with completion by RNase H treatment. Polymerase chain reaction (PCR) was performed using Taq polymerase (Ex Taq; Takara, Kusatsu, Japan) and the primers shown in Table 1. The PCR products (459 bp for *Opn5m* and 551 bp for *Gapdh*) were sequenced and confirmed to be partial cDNAs for chicken *Opn5m* and *Gapdh*.

2.2. Quantitative PCR (qPCR) analysis

qPCR was performed using a LightCycler Nano (Roche Diagnostics, Penzberg, Germany). Primer sequences used are shown in Table 1. qPCR control genes for the quantification of relative gene expression were *Gapdh* for *Tgfb2* and cytoplasmic beta-actin (*Actb*) for *Opn5m*, *Egr1*,

Mmp2, and *Timp2*.

2.3. In situ hybridization (ISH)

Digoxigenin (DIG)-labeled RNA probes were synthesized using chicken *Opn5m* (*cOpn5m*) cDNAs of the coding region (1186 bp) and 3' untranslated region (UTR) (2679 bp) inserted into the pGEM-T Easy and pBluescript II KS (+) plasmids, respectively. E17 chicken embryonic eyes were dissected and fixed overnight in 4% paraformaldehyde (PFA)/PBS at 4°C. The eyes were then immersed overnight in 20% sucrose in PBS for cryo-protection at 4°C, embedded in optimal cutting temperature compound (Sakura Finetek, Tokyo, Japan), frozen, and stored at –80°C. Histology was confirmed by Hematoxylin and Eosin staining (Supplementary Fig. S1). Sixteen-micron thick frozen sections were cut and subjected to ISH as essentially described [7]. Briefly, rehydrated sections were treated for 15 min with a 2 µg mL⁻¹ proteinase K solution at room temperature, and hybridized with 0.34 µg mL⁻¹ of the DIG-labeled antisense or sense probe at 65°C for 2 days. Color development was performed with alkaline phosphatase reaction buffer (100 mM Tris-HCl, 50 mM MgCl₂, 100 mM NaCl, 0.1 % (v/v) Tween 20, 5% (w/v) polyvinyl alcohol (363081; Sigma-Aldrich, St Louis, MO, USA), pH 9.5) containing 50 µg mL⁻¹ Nitroblue Tetrazolium and 175 µg mL⁻¹ 5-Bromo-4-chloro-3-indolyl-phosphate for three days with buffer renewal after the first 2 days. Finally, the sections were counter-stained with nuclear fast red, dehydrated with methanol and tissue dehydration solution A (Wako Pure Chemical Industries, Ltd., Osaka, Japan), cleared with G-NOX (Genostaff, Tokyo, Japan), and mounted on coverslips using PARAmount-D mounting medium (Falma, Tokyo, Japan). Histological images were captured with a Nikon digital camera system (DS-Fi1, Nikon, Tokyo, Japan) mounted on a Leica light microscope (DM5000; Leica microsystems, Wetzlar, Germany).

2.4. Immunostaining and confocal laser microscopy

Immunostaining was performed on the frozen sections mentioned above using anti-chicken *Opn5m* as reported [8]. Nuclei were stained with Hoechst 33342. Fluorescence images were captured by a Zeiss confocal laser microscope (Zeiss LSM 780 with LSM Software ZEN 2011; Carl Zeiss, Oberkochen, Germany). The color of the images was properly adjusted with Adobe Photoshop version 12.0 (Adobe systems, San Jose, CA, USA).

Table 1
Primer sequences for PCR used in this study.

Gene	Accession# (ref#)	Sequences (5'-3')	Amplicon size (bp)
<i>Opn5m</i>	AB368182	Forward:5'-ACTTAAAAATCTGTCACTTGGCTTATG-3' Reverse:5'-TGAATCTCTGTGTGAAGATAAGGTGA-3'	459
<i>Opn5m</i>	AB368182 ([31])	Forward:5'-GGGCTGGCTTCTTCTTGGCTGTGG-3' Reverse:5'-CAGGCAGATAAAGGCATGGTGT-3'	128
<i>Gapdh</i>	NM_204305.1	Forward: 5' -GAGAAATATGACAAGTCCCTGAAAAT-3' Reverse: 5' -CTGTATCCAACTCATTGTCATACCAG-3'	551
<i>Gapdh</i>	NM_204305.1 ([32])	Forward: 5' -AGATGCAGGTGCTGAGTATGTTG-3' Reverse: 5' -GATGAGCCCCAGCCTTCTC-3'	71
<i>Egr1</i>	AF026082 ([33])	Forward: 5'-ACTAACTCGTCACATTCGCA-3' Reverse: 5'-TGCTGAGACCGAAGTGCCT-3'	241/ for qPCR
<i>Tgfb2</i>	NM_001031045.2 ([25])	Forward: 5'-GCTGCGTGTCCAGGATTT-3' Reverse: 5'-TGGGTGTTTTGCCAATGATAGTAGA-3'	60 for qPCR
<i>Mmp2</i>	U07775 ([26])	Forward: 5'-TGGTGTGCTTCTACCAGCAG-3' Reverse: 5'-GAGTGCTCTAATCCCATCGC-3'	122 for qPCR
<i>Timp2</i>	AF004664 ([26])	Forward: 5'-GATGGAGAAGATCGTGGGCGG-3' Reverse: 5'-TGGGCTTTCCTACTGGCTACTG-3'	169 for qPCR
<i>Actb</i>	NM_205518.1 ([34])	Forward: 5'-GCGCTGGTGTGACAAT-3' Reverse: 5'-CATACCAACGTAGCTGTCTTT-3'	151 for qPCR

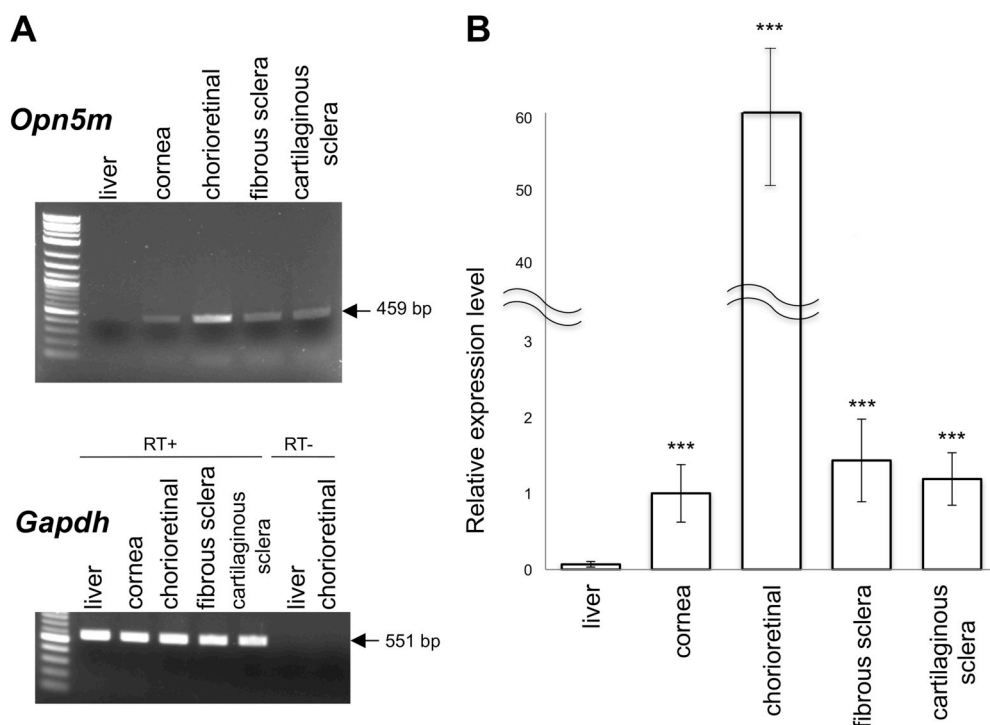


Fig. 1. Expression of *Opn5m* mRNA in E17 chicken ocular tissues.

A, Electrophoresis of PCR products for *Opn5m* and *Gapdh* amplified from cDNA of liver, cornea, chorioretinal tissues, fibrous sclera, and cartilaginous sclera. RT, reverse transcriptase. **B**, Quantitative PCR showing the relative expression of *Opn5m* mRNA in each tissue at E17. The level of *Opn5m* in the E17 cornea was standardized to $1(1.00 \pm 0.38)$. Relative expression levels are 1.43 ± 0.54 in fibrous sclera, 1.19 ± 0.34 in cartilaginous sclera, 60.12 ± 10.01 in chorioretinal tissues, and 0.068 ± 0.036 in liver. Error bars show standard error of the mean. Asterisks indicate a significant difference compared with the level in the liver ($p < 0.001$).

2.5. Retrovirus vector construction, cell culture, and UV irradiation experiments

The RCASBP(A) plasmid containing *cOpn5m* tagged with the epitope sequence (corresponding to amino acids, ETSQVAPA), recognized by an anti-bovine rhodopsin monoclonal antibody Rho1D4 at the C terminus, was constructed as follows. First, the tagged-*cOpn5m* cDNA was amplified from the insert in the mammalian expression vector pCAGGS [5] by PCR using the primers: 5'-CCGACCACCATGGCCATGAGTGGGATG GCATCGGA-3' (forward) and 5'-CTTATCGATAAGCTTTCAGGCAGGCG CCATCGGC-3' (reverse), containing recognition sites of the restriction enzymes *NcoI* and *HindIII*, respectively. After digestion with *NcoI* and *HindIII*, the DNA fragments were ligated to the *ClaI*2*Nco* adapter vector. After transformation of *E. coli* DH5 α competent cells, insertion of *cOpn5m* was confirmed by colony-direct PCR using the *cOpn5m* specific primer pair mentioned above. After digestion with *ClaI*, the tagged-*cOpn5m* was ligated to phosphatase-treated RCASBP(A). The insertion and orientation of *cOpn5m* in RCASBP(A) was confirmed by colony-direct PCR using the primers: 5'-ACGCTTTTGTCTGTGTGCTGC-3' (forward) and 5'-CGCTATGATGTCAGCTTCTC-3' (reverse), specific to the sequences in RCASBP(A) and *cOpn5m*, respectively. Finally, the plasmid RCASBP(A) containing the tagged-*cOpn5m* was confirmed by sequencing with the forward primer specific to RCASBP(A) shown above. A cell line derived from chicken embryonic fibroblasts, DF-1 [16] (CRL-12203; American Tissue Culture Collection) was cultured in Dulbecco's Modified Eagle Medium (049-32645; Wako) supplemented with 10% fetal bovine serum, 100 units of mL⁻¹ penicillin and 100 μ g mL⁻¹ streptomycin at 37°C under 5% CO₂ as described previously [17]. For transfection, DF-1 cells were seeded in a 3.5 cm dish, grown to 70% confluency, and transfected with 7.5 μ L per dish of Lipofectamine 3000 (Thermo) and 2.5 μ g per dish of RCASBP(A) containing the tagged-*cOpn5m*. After two passages, transfected-cells were seeded into 24-well black clear bottom plates (Perkin Elmer, Waltham, MA, USA) at the 80–90% confluency. Six days after transfection, 11-*cis*-retinal was supplied to a final concentration of 2 μ M under dim red light. The culture plate was installed on a light plate apparatus, which was made with a three-dimensional printer as described [18] and equipped with a UV-light emitting diode (peak wavelength:

375 nm) (S5UV30; Sousin Digital, Tokyo, Japan). The cells were irradiated with 700 μ W cm⁻² of UV light, as measured by a USB2000 + spectrometer (Ocean optics, Dunedin, Florida, USA), for half an hour (1.26 J cm⁻²), 1 h (2.52 J cm⁻²) or 4 h (10.1 J cm⁻²). UV irradiation was terminated 1 day after the addition of 11-*cis*-retinal, irrespective of the duration of exposure. After irradiation, the cells were harvested, lysed in Isogen II solution (Nippon Gene, Tokyo, Japan) and total RNA was extracted. cDNA was synthesized and analyzed by qPCR as mentioned. To estimate transfection efficiency, transfected DF-1 cells were fixed in 4% PFA in PBS and analyzed by standard immunofluorescence using anti-bovine rhodopsin monoclonal antibody Rho1D4 (P2194; Thermo). Through the propagation of the retroviral vector RCASBP, 100% of the transfected cells expressed the tagged-*cOpn5m* (See Supplementary Fig. S2).

2.6. Statistics

At least three independent replicates of each experiment were performed. The relative expression levels in qPCR were analyzed using Student's t-test comparison between two unpaired groups and differences with *P* values less than 0.05 were regarded as statistically significant (Fig. 4).

3. Results

3.1. PCR analysis of *Opn5m* expression in chicken embryonic ocular tissues

There are three *Opn5* subfamily members in *Gallus gallus* [3,8,19,20], and we focused on *Opn5m* as it was the most prominent *Opn5* receptor in the chicken embryonic retina and is also present in mammals. Reverse transcription PCR analysis showed that *Opn5m* was expressed in the cornea and sclera of E17 chicken embryos (Fig. 1A). There are two histologically distinct tissues in the chicken sclera; fibrous sclera and cartilaginous sclera and *Opn5m* was expressed in both. qPCR analysis showed that the expression levels of *Opn5m* in the cornea and sclera were approximately one sixtieth that of the chorioretinal tissue (Fig. 1B).

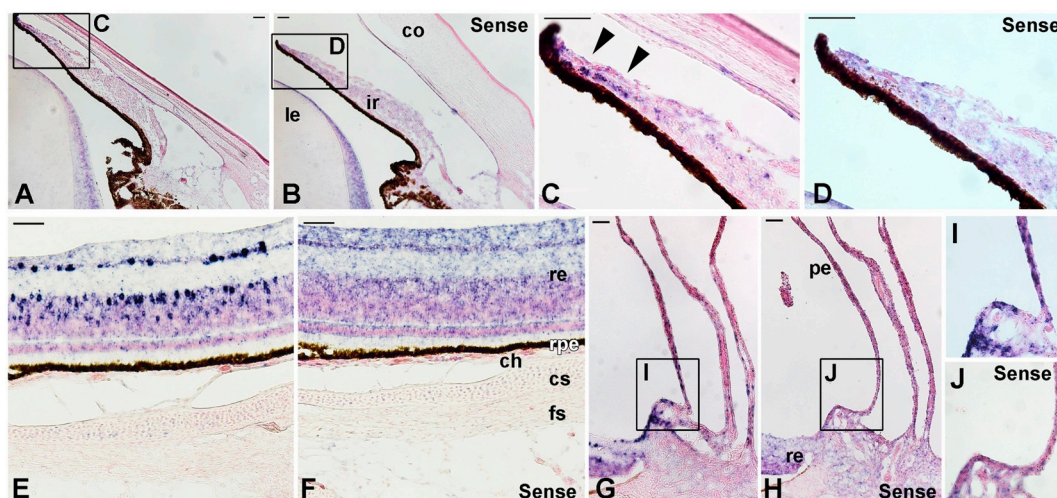


Fig. 2. In situ hybridization of *Opn5m* mRNA on E17 chicken ocular tissues.

Signals for *Opn5m* mRNA are dark blue and the sections are counter-stained with nuclear fast red. A sense probe was used as a negative control (B, D, F, H, J). A, anterior portion of the eye. Enlarged images of the iris (boxed area in A, B) are shown as C and D, respectively. C, *Opn5m* is expressed in the iris (arrows). E, posterior portion of the eye. There is no obvious staining for *Opn5m* mRNA in the sclera, while intense signals are observed in a subset of cells of the retina. F, non-specific staining is observed in the retina after a long period of color reaction. G, *Opn5m* is expressed in the pecten, where strong signals are observed at the base (boxed). Enlarged images of the pecten (boxed area in G, H) are shown as I and J. Abbreviations: ch, choroid; co, cornea (stroma); cs, cartilaginous sclera; fs, fibrous sclera; ir, iris; le, lens; pe, pecten; re, retina; rpe, retinal pigment epithelium. Scale bars, 50 μm . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Localization of *Opn5m* mRNA

We next sought to identify the *Opn5m*-expressing cells using ISH on E17 chicken eye sections. In the anterior portion of the eye, however, we could not detect signals for *Opn5m* mRNA in the cornea (Fig. 2A; Fig. 2B as control), while it was detected in the distal marginal region of the iris (Fig. 2C; Fig. 2D as control). In the posterior portion of the eye, we could not detect obvious signals for *Opn5m* mRNA in the cartilaginous or fibrous scleral tissue by ISH, either (Fig. 2E; Fig. 2F as control). In addition to distinct signals in the retina, *Opn5m* mRNA was detected in the pecten, a vascular and pigmented structure unique to the avian species (Fig. 2G, I; Fig. 2H, J as control) [21].

3.3. Localization of *Opn5m* protein

We then determined the localization of Opn5m protein in the ocular tissues by immunostaining. In the anterior portion of the eye, immunoreactivity to chicken Opn5m (green fluorescence) was detected in a subset of the cells of the corneal stroma (Fig. 3A, B, a) as well as in the iris (Fig. 3A, B, b). In the posterior of the eye, low levels of green fluorescence were detected in the choroidal tissue and fibrous sclera (Fig. 3C, D, c, d). We also found signals in the pecten (Fig. 3E, F, e).

3.4. Expression analysis of myopia-related genes in chicken fibroblasts after UV irradiation

Myopia-related genes have been identified by experiments using animal myopia models and epidemiological data from human myopia patients. In this study, we focused on four genes, *Egr1* [22,23], *Tgfb2* [24,25], *Mmp2*, and *Timp2* [26]. To determine whether the expression of these four genes was altered after UV absorption by the Opn5m receptor, chicken *Opn5m* was overexpressed in the DF-1 fibroblasts. Initially, the *Opn5m*-expressing cells were UV-irradiated (375 nm) at an intensity of $700 \mu\text{W cm}^{-2}$ for half an hour, 1 h or 4 h. Since we found it difficult to obtain consistent results when the cells were UV-irradiated for longer durations, the irradiation time was fixed at 30 min. As it was reported that UV irradiation alone induces *Egr1* expression in mouse embryo fibroblasts [27], we saw a similar result in the DF-1 cells

(Fig. 4A). Of note, when the *Opn5m* gene was overexpressed in DF-1 cells and 11-*cis*-retinal, a chromophore of Opn5m, was added to the culture medium, the expression of *Egr1* was significantly increased after UV irradiation ($P = 0.01034$) (Fig. 4A). We also examined the relative expression levels of *Tgfb2*, *Mmp2*, and *Timp2* in each experimental condition. Expression of *Tgfb2* was significantly elevated after the forced expression of *Opn5m* without retinal, irrespective of UV irradiation ($P = 0.04060$ and $P = 0.03460$, respectively) (Fig. 4B). With the addition of retinal, *Tgfb2* expression tended to be elevated with irradiation, although the result was not statistically significant (Fig. 4B). *Mmp2* expression was significantly increased after UV irradiation in the presence of retinal ($P = 0.01765$) (Fig. 4C), and it was not elevated by UV irradiation alone. None of the conditions influenced the expression of *Timp2* (Fig. 4D).

4. Discussion

The Opn5m-immunoreactivity was detected in a subset of cells in the cornea and fibrous sclera, but ISH could not detect distinct mRNA signals in these tissues. We think that the levels of *Opn5m* mRNA in these tissues were too low to be detected by ISH but that the transmembrane receptor protein of Opn5m could be detected by immunostaining, as the structural membrane protein may not require high-abundance mRNA expression. Combined with the results from qPCR and immunostaining, we concluded that Opn5m is present in a subset of cells in the late-embryonic stage chicken corneal stroma and fibrous sclera. Since the expression profile of *Opn5m* might differ between late-embryonic and young chick, we also examined it in post-hatch day 1 chick (Supplementary Figs. S4–7). The relative expression level of *Opn5* was higher in the P1 cornea and sclera compared with E17 (Fig. S4). Immunostaining of the P1 eye tissues showed a similar localization pattern of Opn5m protein (Fig. S6) compared to that of E17. How the *Opn5m* expression around pre- and early post-hatch days might modulate ocular growth would be the next issue to be examined.

To elucidate the pathophysiology of myopia, the chicken eye provides a good model system [28]. The chicken sclera consists of two layers, the fibrous and cartilaginous layers. Collagen degradation in the chicken sclera is confined to the fibrous layer during scleral remodeling

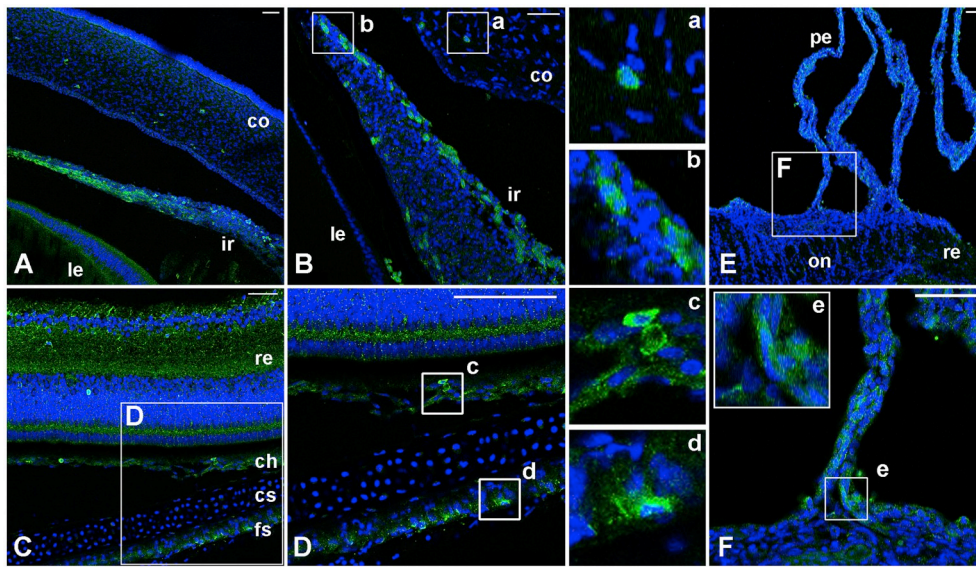


Fig. 3. Immunostaining for Opn5m protein on E17 chicken ocular tissues. Signals for *Opn5m* protein are green fluorescence and nuclei are shown in blue. The negative control, without primary antibody against chicken Opn5m, essentially shows no green fluorescence (Supplementary Fig. S3). The boxed area is enlarged and shown in a-e, D, F. A, anterior portion of the eye. Green fluorescence is observed in a subset of cells in the corneal stroma, and iris. Intense signals for Opn5m protein are observed in differentiating sphincter and dilator muscles of the iris. B, in this section, Opn5m protein in the cells just underneath the anterior lining cells and posterior pigmented epithelium. C, posterior portion of the eye. D, chorioretinal tissue and sclera. There is a low level of green fluorescence in the choroid and fibrous sclera. E, F, Opn5m protein is localized to the pecten. a, corneal stroma cells; b, iris stroma cells; c, choroidal stroma cells; d, fibrous scleral cells; e, basal part of pecten oculi. Abbreviations: ch, choroid; co, cornea (stroma); cs, cartilaginous sclera; fs, fibrous sclera; ir, iris; le, lens; pe, pecten; on, optic nerve disc; re, retina. Scale bars, 50 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

in myopia development [29]. Furthermore, during eyeball growth in chickens and humans, the diameter of collagen fibrils decreases and the structure of the fibrils is altered [24,30]. Thus, it is conceivable that alterations to the expression of genes involved in collagen remodeling such as *Mmp2* in the sclera have crucial roles in myopia progression.

We used a chicken fibroblast cell line to examine whether the expression levels of these four genes were altered after UV irradiation combined with the forced expression of *Opn5m*. We found that the expression of *Egr1* and *Mmp2* after UV-A exposure was significantly elevated in the *Opn5m*-overexpressing fibroblasts with 11-*cis*-retinal. This suggests that UV-A may alter the activity of fibroblasts as revealed by upregulation of *Egr1* and *Mmp2* via Opn5m. We attempted similar experiments in a primary culture of chicken scleral fibroblasts, but unfortunately the cells deteriorated after transfection with retroviral vectors.

Egr1, also called ZENK or *zif268*, encodes a zinc finger transcription

factor, which regulates transcription of genes involved in tissue remodeling and fibrosis. Its target genes include those of Tgf betas, collagens, Timps, and other growth factors and matrix proteins. *Egr1* is one of the major mediators in fibroblasts or myofibroblasts not only in physiological tissue remodeling but also in pathological fibrosis. *Egr1* was the first gene to be identified whose synthesis is enhanced when ocular elongation is suppressed [22], and *Egr1*-knockout mice exhibit axial eye growth [23]. However, all these alterations of *Egr1* expression have been observed in the retina. Our study shows that UV-A reception through Opn5m in the presence of 11-*cis*-retinal significantly upregulates the expression of *Egr1* in fibroblasts, which supports the possibility of a direct effect on the sclera or cornea by UV reception via Opn5m.

Mmp2 encodes a protease that initiates degradation of collagens and the extracellular matrix (ECM) complex. In the remodeling of scleral tissue during ocular growth, continuous synthesis and degradation of ECM take place [26]. *Mmp2* is expressed in the scleral fibroblasts and

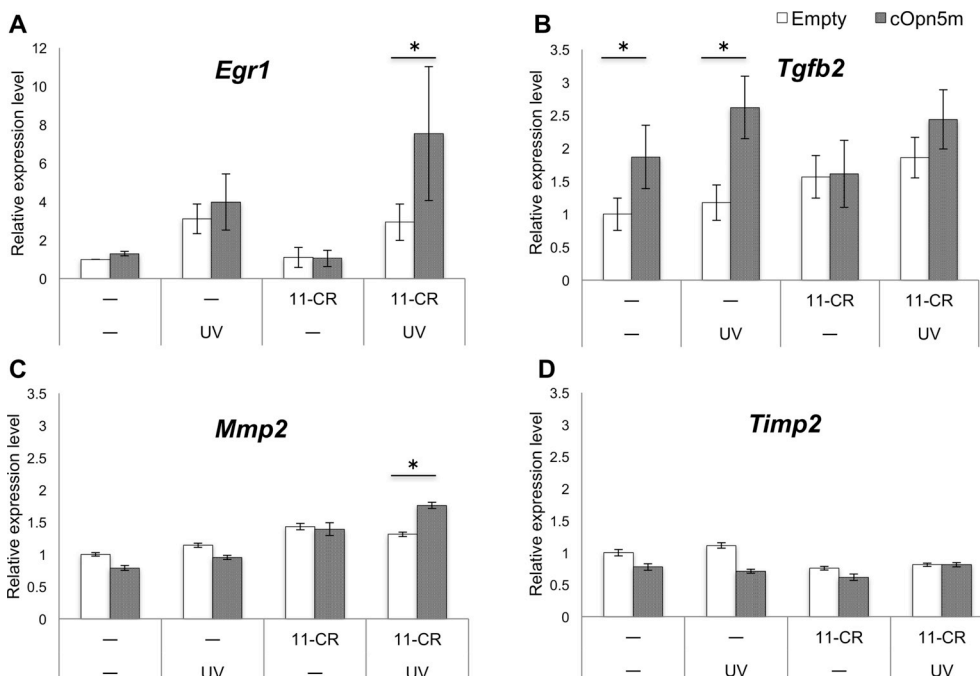


Fig. 4. Quantitative PCR analysis of myopia-related gene expression in DF-1 cells. Expression of *Egr1* (A), *Tgfb2* (B), *Mmp2* (C), and *Timp2* (D) was examined with or without forced-expression of chicken *Opn5m*, the addition of 11-*cis*-retinal (11-CR) to the culture medium, and UV irradiation. White columns show the negative control, in which the empty RCAS vector was transfected; gray columns show *cOpn5m* expression. Error bars show standard error of the mean. Asterisks indicate a significant difference between the two values ($p < 0.05$).

the expression of *Mmp2* is altered, increased or decreased after imposed myopic defocus or its recovery depending upon the experimental time course [26,31]. Our *in vitro* study shows that the expression of *Mmp2* in chicken fibroblasts is increased by short-time UV-A exposure in the presence of forced-expressed *Opn5m* and the retinal chromophore 11-*cis*-retinal. This suggests that UV-A absorption via *Opn5m* would have a direct effect on the fibroblasts of the sclera and cornea, the outer wall of the eye, for tissue remodeling involving *Mmp2* as well as *Egr1*. Whether biological responses induced by the UV-A-*Opn5m* phototransduction lead to changes in ECM of the sclera and cornea should be clarified in the next study.

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None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrep.2019.100665>.

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