Aquaporin 0 Modulates Lens Gap Junctions in the Presence of Lens-Specific Beaded Filament Proteins

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iovs.arvojournals.org | ISSN: 1552-5783

The eye lens is a transparent, avascular organ that is responsible for approximately 40% of the focusing power for vision in humans. Structurally, a single layer of cuboidal epithelial cells covers the anterior hemisphere of the lens. Fiber cells constitute the bulk of the lens. They differentiate from the equatorial epithelial cells, elongate, and greatly change their cytoplasmic proteins. Approximately 15% of the distance into the lens, fiber cells undergo a second stage of maturation that eliminates cellular organelles and generates C-terminal cleavage of most membrane proteins, including Aquaporin 0 (AQP0)1,2 and gap junction (GJ) connexins (Cx).1-8 These modifications reduce light scattering and can allow central fibers to survive for the lifetime of the organism.9,10 In addition, these events help to adjust the refractive index gradient2,11,12 and biomechanics3,5 of the lens for proper focusing of light from nearby or far objects. The mammalian ocular lens contains approximately 66% water and approximately 33% proteins. To adjust the refractive index, lens fiber cell cytosolic protein concentration and free water content are maintained in a reciprocal gradient from the cortex to the nucleus.

Since the lens is avascular, to procure nourishment and dispose of metabolic waste, it creates a microcirculation involving water channels, ion transporters, and cotransporters.14-16 Intercellular GJ channels constituted by Cx membrane proteins form epithelial-to-epithelial, fiber-to-fiber, and epithelial-to-fiber cell connections at the plasma membrane.17-20 Small molecules, such as ions, metabolites, and second messengers, pass between two such connected cells1,22 and aid in intercellular communication. Three major GJ channel proteins are present in the lens. Cx43 (z1 gene) and Cx50 (z8 gene) are expressed in the epithelial cells; Cx46 (z3 gene) and Cx50 are expressed in the fiber cells.23,24 Along with GJ channels, lens water pores or aquaporin (AQP)s2,11-16,25-34 namely AQP0, AQP1, and AQP5 have significant roles in lens microcirculation and homeostasis. In a simplified version of the microcirculation model, sodium ions enter the extracellular spaces at the anterior and posterior poles of the lens.14-16,30 As the extracellular sodium flows toward the central part of the lens, the ions enter the fiber cells down their electrochemical potential across the fiber cell membranes. Once in the intracellular compartment, the flow reverses direction and moves from fiber cell to fiber cell through GJs back toward the lens equatorial surface. The Na-K-ATPase expressed in equatorial epithelial cells35 pumps the sodium out. Through the processes of osmosis and hydrostatic pressure (HP), water...
Aquaporin 0 Regulates Gap Junctions in the Lens

for the intracellular leg of the lens microcirculatory system,16 GJ channels appear to serve as the cell-to-cell outflow conduit accumulation of lactic acid as a result of anaerobic glycolysis. The presence of low pH in the center of the lens is due partly to the accumulation of lactic acid as a result of anaerobic glycolysis. GJ channels appear to serve as the cell-to-cell outflow conduit for the intracellular leg of the lens microcirculatory system,16 which carries lactic acid and possibly other waste products to surface cells where they are eliminated. AQP013–25,29,42 and GJ channels16 have significant roles in lens homeostasis. In vivo and in vitro studies were conducted by different groups to find out whether AQP0 has a role in GJ regulation producing mixed results. An in vivo study on an AQP0 knockout mouse model developed in the FVB strain that does not express beaded filaments (BFs) due to a mutation in the CP49 gene showed that 50% reduction in AQP0 does not alter lens GJ coupling. In vitro studies indicated that AQP0 facilitates GJ coupling; the cell-to-cell adhesion (CTCA) function of AQP0 might have promoted Cx50 GJ coupling.29,43 Moreover, several investigators have shown the possible interaction of AQP0 with lens Cx17,44,45 and cytoskeletal proteins (e.g., BF proteins CP49 and filensin46,47). Therefore, the role of AQP0 in lens GJ regulation is an open question. The difference in the results between in vivo and in vitro studies on the effect of AQP0 on GJ coupling could be due to several factors, such as the amount of AQP0 present, Cx expression levels or lack of other regulatory components in an in vitro environment.

AQP0 is the most abundantly expressed membrane protein in the plasma membranes of fiber cells, constituting approximately 44.8% of the total membrane proteins in the lens. Scientists were intrigued by the prolific expression of AQP0 and sought to determine the role(s) of this protein in the lens. Mutations in AQP0 result in autosomal dominant lens cataract in mice48–53 as well as humans.29,54,55 In vitro and in vivo studies demonstrated that AQP0 functions as a water channel25,27,56–58 and a CTCA molecule,26,28,43,59–61 both of which are important in maintaining lens transparency.26–28,62 refractive index gradient,2,11 biomechanics,13 and homeostasis,14–16,50 AQP0 interacts with other membrane proteins, such as Cx,44,65 and lens-specific cytoskeletal intermediate BF proteins CP49 (phakinin) and filensin (CP115).46 We suggested the involvement of BFs in the anchorage and distribution of AQP0 at the plasma membrane.2 Alteration in refractive index gradient, and increased spherical aberration and severity of cataract were observed in AQP0-heterozygous (Hz) FVB strain mice that lack BFs compared to similar lenses in the outer cortical region of the lens and glycolysis in the central nucleus. Experimental evidence shows that lens center has an acidic pH (6.81) compared to the outer cortex (7.2).39,40 The nucleus. Experimental evidence shows that lens center has an acidic pH (6.81) compared to the outer cortex (7.2).39,40 The inward extracellular fluid flow carries nutrients and antioxidants to central fiber cells (reviewed previously13) while the outward intracellular fluid flow carries waste products, such as lactic acid,39,40 from central fibers to surface cells that can eliminate them.36–38 The inward extracellular fluid flow carries nutrients and antioxidants to central fiber cells (reviewed previously13) while the outward intracellular fluid flow carries waste products, such as lactic acid,39,40 from central fibers to surface cells that can eliminate them.36–38 The inward extracellular fluid flow carries nutrients and antioxidants to central fiber cells (reviewed previously13) while the outward intracellular fluid flow carries waste products, such as lactic acid,39,40 from central fibers to surface cells that can eliminate them.36–38

Materials and Methods

Animals

The following mouse models were used: WT mouse strains FVB/N (FVB; Charles River Laboratories, Wilmington, MA, USA), C57BL/6j (C57; Jackson Laboratories, Bar Harbor, ME, USA), AQP0 heterozygous in both strains (AQP0-Hz/FVB, AQP0-Hz-C57), and AQP0-Hz mouse expressing AQP1 in the fiber cells in both strains (transgenic, TgAQP1/AQP0-Hz/FVB; TgAQP1/AQP0-Hz-C57). Animal procedures were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, the National Institutes of Health (NIH; Bethesda, MD, USA) “Guide for the Care and Use of Laboratory Animals” and protocols approved by Stony Brook University Animal Care and Use Committee.

Originally, the AQP0-KO mouse model was established in a mixed background of 129S5 and C57BL/6j-albino.27 We transferred it to the FVB strain by backcrossing the AQP0-KO with WT FVB for more than 25 generations.26 Since FVB mice27 do not express CP49, a constituent of lens-specific BFs, due to a deletion mutation in the CP49 gene, the AQP0-KO mouse model developed in the FVB background26 was backcrossed with C57 for more than 25 generations to transfer the knockout genotype in this strain. Genotyping using PCR primers described by Alizadeh et al.1,71 (primer set I) and Simirskii et al.70 (primer set II) were used to distinguish the genotypes. These procedures were followed to ascertain that the data collected on lens fiber cell water permeability (Pf), CTCA, GJ coupling resistance and intracellular HP are strain-specific for data comparison.

Initially, we developed TgAQP1/AQP0-Hz in the FVB mouse strain.28 Restoring membrane Pf in TgAQP1/AQP0-Hz partially restored lens transparency in FVB.26,28 To see whether restoring Pf in the presence of BFs alter the outcome, we developed TgAQP1/AQP0-Hz in the C57 strain. For this, TgAQP1/AQP0-Hz in FVB was backcrossed with C57 WT for more than 25 generations and genotyped using PCR primers described by Simirskii et al.70 and Alizadeh et al.27 Strain transfer and genotyping were done to ensure that the data we collected using these mice were from the C57 genetic background.

Lens Transparency and Spherical Aberration

Lenses from 2-month-old mice (5 lenses/group) were dissected out in prewarmed (37°C) mammalian physiologic saline. Images were captured under the same lighting and imaging conditions using a dark field Zeiss binocular microscope attached with a Olympus digital camera.11 Lens transparency was quantified using the dark field lens images, which were converted to gray scale (Adobe Photoshop 9; Adobe Systems, San Jose, CA, USA) and processed using ImageJ software (NIH). An area from the lens image was drawn out as a square box to analyze and plot profile function and was selected to create values of pixel brightness intensity. Sigma Plot 10 software was used to plot histograms for pixel brightness intensity data.

Qualitative assessment of lens spherical aberration was performed using dark field optical grid focusing.11 A copper
Aquaporin 0 Regulates Gap Junctions in the Lens

Biotechnology, Dallas, TX, USA) were used. Antibody binding

Davis, Davis, CA, USA), Cx43, Cx46, and Cx50 (Santa Cruz

CP49, filensin (from Paul G. FitzGerald, University of California-

imaged by digital video microscopy and P

hypertonic saline (450 mOsm), and the rate of shrinkage was

vesicles were prepared. Each vesicle was incubated in

lenses from 2-month-old WT, AQP0-Htz, and TgAQP1/AQP0-

Lens GJ Coupling Measurement

Western Blotting

Fiber cell membrane proteins were extracted using 4M-urea-

buffer (4 mM Tris-HCl, pH 8.0; 5 mM EDTA, 4M Urea) from the

lenses of WT, AQP0-Htz, and TgAQP1/AQP0-Htz in C57 as well

as WT and AQP0-Htz in FVB strains. For Western blotting, lens

protein samples were resolved on 4% to 12% gradient NuPAGE

gels (Invitrogen, Carlsbad, CA, USA). Broad range Kaledoscope

markers (Bio-Rad Laboratories, Hercules, CA, USA) were loaded to

estimate the molecular sizes of the reactive bands. Proteins

were transferred to a nylon membrane. Since the colored

marker proteins do not bind to antibody and may fade during

processing, positions of the molecular size standards were

marked with a sharp pencil on the blot before removal of the

blot from the gel. This step helped us to find out the relative

molecular size of the protein band binding to the antibody.

Western blotting was done as described72,73 Antibodies to

proteins CP49 and Filensin (from Paul G. FitzGerald, University of California-

New York, New York, USA) were used. Antibody binding

detection was performed using an alkaline phosphatase kit (Vector Laborato-

ries, Burlingame, CA, USA).

Analysis of Localization of Lens AQP0 and BF

Proteins CP49 and Filensin

Lenses from 2-month-old WT and AQP0-Htz mice were dissected out, fixed for 12 hours in 2% and 24 hours in 4% paraformaldehyde, and rinsed in PBS. Lenses were cryosectioned (14 μm thickness) using a cryomicrotome (Leica, Wetzlar, Germany) and sections were stored at −20°C. Cryosections were permeabilized (0.2% vol/vol Triton X-100) for 30 minutes at room temperature, blocked with normal goat serum, and treated with rabbit polyclonal antibody raised against AQP0 (Santa Cruz Biotechnology), and mouse mono-

clonal antibody against filensin or CP49 in appropriate dilutions using 5% (wt/vol) BSA in PBS. After overnight incubation and three washes in PBS, the slides were incubated with FITC conjugated goat anti-rabbit IgG or Texas Red conjugated goat anti-mouse IgG in PBS containing 5% BSA. The slides with the treated tissue sections were washed again in PBS, mounted in anti-fade Vectamount (Vector Laboratories) and viewed under an epifluorescence confocal microscope. Optimized Z-sectional digital images were acquired with a Zeiss Axiovert 200 inverted microscope equipped with AxioCam and Zeiss AxioVision 5 software (Carl Zeiss Microimaging, Inc., Munich, Germany) and processed using Adobe Photoshop 9.0.

Lens Fiber Cell Membrane P2 Assay

Experiments for P2 measurement were performed using the rate of shrinking of fiber cell membrane vesicles, as described previously.25,27,28 In brief, lens capsules were removed, outer cortex fiber cell bundles were isolated and fiber cell membrane vesicles were prepared. Each vesicle was incubated in hypertonic saline (450 mOsm), and the rate of shrinkage was imaged by digital video microscopy and P2 was calculated as described by Varadaraj et al.25

Lens GJ Coupling Measurement

Lenses from 2-month-old WT, AQP0-Htz, and TgAQP1/AQP0-

Htz mice from C57 background were dissected out and

mounted as described previously.36 In brief, eyes were removed from euthanized mice and placed in a Sylgard-lined petri dish filled with normal Tyrode solution, pH 7.4 (in mM: 137.7 NaCl, 2.3 NaOH, 5.4 KCl, 2 CaCl2, 1 MgCl2, 5 HEPES, and 10 glucose). To mount the lens in the perfusion chamber for impedance studies, the anterior segment of the eye, cornea, and iris were dissected out. The optic nerve was removed from the posterior side of the eye and the sclera was cut into four flaps through the hole created while cutting the optic nerve. The lens was pinned to the Sylgard base present at the bottom of a perfusion chamber containing normal Tyrode solution. The perfusion chamber with lens was attached to a microscope stage. Normal Tyrode’s solution was used to perfuse the lens.

Measurement of lens GJ coupling resistance was performed as described previously.36 Briefly, a wide-band stochastic current was injected through a microelectrode that was inserted into a central fiber cell. A second microelectrode inserted into a peripheral fiber cell at a distance r (cm) from the center of a lens of radius a (cm) was used to record the induced voltage. Using a fast Fourier analyzer (Hewlett Packard, Palo Alto, CA, USA), the frequency domain impedance (induced voltage ÷ injected current) of the lens was recorded in real time. At high frequencies, the magnitude of the lens impedance asymptotes to the series resistance (R_S) which is due to all the GJs between the point of voltage recording and the lens surface. By measuring R_S*KΩ at multiple depths into lenses of each type, then curve fitting the data with Equation 1, the underlying effective intracellular resistivities (R_MF, R_MF*KΩ/cm) were determined. These effective resistivities were determined from the radial voltage drop, so they represent the radial component of GJ coupling resistance.

\[
R_S(r) = \begin{cases} 
\frac{R_{MF} \left( \frac{1}{a} - \frac{1}{r} \right)}{1 + \frac{R_{MF} \left( \frac{1}{a} - \frac{1}{r} \right)}{R_{SK} \left( \frac{1}{r} - \frac{1}{b} \right)}} & 0 \leq r \leq a \\
\frac{R_{MF} \left( \frac{1}{a} - \frac{1}{b} \right)}{1 + \frac{R_{MF} \left( \frac{1}{a} - \frac{1}{b} \right)}{R_{SK} \left( \frac{1}{r} - \frac{1}{b} \right)}} & b \leq r \leq b 
\end{cases}
\]

Fiber cells transition from peripheral differentiating fibers (DF - outer cortex containing intact proteins) to central mature fibers (MF) at a radial location b ≥ 0.85a, where r cm is the radial distance from the lens center and a cm is the lens radius. MF includes inner cortex, outer nucleus (both containing maturing fiber cells that undergo post-translational modifications (PTMs)) and inner nucleus (containing matured fiber cells). At inner cortex and outer nuclear regions of MF, the C-

terminal of Cx46 and Cx50 are cleaved gradually, resulting in a change in coupling resistance. The GJ coupling conductance per area of cell-to-cell radial contact can be estimated from the effective resistivities by:

\[
G_{DF,MF} = \frac{1}{w R_{DF,MF}} \frac{S}{cm^2}
\]

where w ≈ 3x10^-4 cm is the fiber cell width.

Lens Intracellular HP Measurements

Intracellular HP in intact lenses was measured as described by Gao et al.36 Briefly, microelectrodes were pulled with a resistance in the range of 1.5 to 2 MΩ when filled with 3 M KCl. A microelectrode was inserted into the intact lens. The positive intracellular pressure of the fiber cell forced cytoplasm into the microelectrode tip causing an increase in resistance. Pressure was applied to the interior of the microelectrode until its resistance just returned to the value measured outside of the lens. This pressure was measured using a manometer. Recording was performed for four to five depths in each lens. Data from at least eight lenses were pooled for each genotype.

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The average pressure gradient was estimated by curve-fitting Equation 3 to the pooled data.

\[
p_i(r) = \begin{cases} 
  p_i(b)(\frac{r-a}{r-b}) & b \leq r \leq a \\
  p_i(b) + \left[ p_i(0) - p_i(b) \right] \left( \frac{r-b}{r-a} \right) & 0 \leq r \leq b 
\end{cases}
\]

The quadratic r-dependence of pressure suggests the transmembrane entry of water into fiber cells essentially is uniform with depth into the lens.\(^{36}\) The change in slope of the r-dependence at \( r = b \) is thought to occur because the number of open GJ channels goes down in the MF relative to DF.

**Statistical Analysis**

SigmaPlot 10 software was used for Student’s t-tests. \( P \) values \( \leq 0.05 \) were considered significant.

**RESULTS**

**Genotyping, Protein Expression and Localization**

Pups were genotyped to authenticate the presence or absence of CP49 deletion mutation (Fig. 1A). Two sets of PCR reactions were assembled and performed; the primers used were as described by Alizadeh et al.\(^{71}\) (primer set I) and Simirskii et al.\(^{70}\) (competitive PCR; primer set II). Primer set I amplified a 320-base pair (bp) segment for WT, AQP0-Htz, and TgAQP1/AQP0-Htz in the C57 strain. A 386-bp product was amplified for FVB indicating the presence of mutant CP49 alleles. Primer set II\(^{70}\) amplified a 205-bp segment for all except FVB (Fig. 1A), which produced a 347-bp amplicon indicating the presence of mutant CP49 alleles; the 205 bp amplicon confirmed the absence of CP49 deletion mutation. These results ensured the selection of the desired genotypes for experiments and for appropriate analyses of the collected data.

Total membrane proteins of WT, AQP0-Htz, and TgAQP1/AQP0-Htz mice in C57 or FVB background were prepared and Western blotting was performed. Protein expression was confirmed using antibodies to BF proteins (CP49 or filensin). Western blot analyses showed binding of the antibodies to the respective polypeptide bands for filensin (approximately 95 kDa; arrow, Fig. 1B) and CP49 (approximately 49 kDa; arrow, Fig. 1B) in WT, AQP0-Htz, and TgAQP1/AQP0-Htz mouse lenses of C57 background. In FVB, there was no detectable immunoreaction with CP49 antibody. However, there was low level binding due to a low level of filensin; absence of CP49 causes instability of the filensin polypeptide\(^{71,75}\) despite FVB expressing filensin transcripts.\(^{38}\) These experiments showed the absence of BF s in FVB lenses in parallel with the presence of both BF proteins in C57 lenses.

After confirming the presence of BF proteins in C57 lens membranes by Western blotting, we tested whether AQP0 and BF proteins localize at the plasma membrane. Lens cryosections of WT, AQP0-Htz, and TgAQP1/AQP0-Htz mice in C57 and FVB genetic backgrounds were immunostained using AQP0, CP49, and filensin antibodies (Figs. 1C, 1D). In the C57 genetic background, fiber cell membranes of WT (Fig. 1C), AQP0-Htz (Fig. 1C), and TgAQP1/AQP0-Htz (Fig. 1C) displaying red indicate the binding of AQP0 antibody. Membrane images in green indicate binding of filensin or CP49 antibodies. In Figure 1C, the images at the bottom indicate AQP1 expression at the plasma membrane. In the FVB genetic background, fiber cell membranes of WT, AQP0-Htz, and TgAQP1/AQP0-Htz displaying red indicate the binding of AQP0 antibody (Fig. 1D).

Membrane images in Figure 1D did not show any specific binding of either filensin or CP49 antibodies for all genotypes tested. In Figure 1D, green indicates the AQP1-EGFP expression at the fiber cell membrane.

**Lens Transparency**

Lens transparency was assessed for WT, AQP0-Htz, and TgAQP1/AQP0-Htz in C57 and FVB genetic backgrounds using light microscopic images of 2-month-old lenses. Compared to AQP0-Htz and TgAQP1/AQP0-Htz lenses in the C57 strain, those of AQP0-Htz and TgAQP1/AQP0-Htz in the FVB strain showed relatively higher light scattering (Fig. 2A, top row). While AQP0-Htz and TgAQP1/AQP0-Htz lenses in both backgrounds focused the grid lines poorly as a result of light scattering and spherical aberration, the severity was more pronounced in the lenses in the FVB than those in the C57 strains (Fig. 2A, bottom row). Figures 2B and 2C show lenses of WT, AQP0-Htz, and TgAQP1/AQP0-Htz in C57 and FVB genetic backgrounds (five lenses each) used for quantifying lens transparency. Figure 2D shows quantification of lens transparency, which is calculated on the basis of it being inversely proportional to pixel brightness intensity. While WT lenses were clear with the lowest pixel brightness that translates to very low light scattering, AQP0-Htz and TgAQP1/AQP0-Htz lenses showed more pixel brightness and higher levels of light scattering (Figs. 2A–D). Lenses of AQP0-Htz and TgAQP1/AQP0-Htz mice in the FVB strain showed significant increase in light scattering (marked with an asterisk in Fig. 2D) and, hence, statistically significant reduction in lens transparency compared to their counterparts in the C57 strain.

**Lens Fiber Cell Membrane Water Permeability (\( P_f \))**

The \( P_f \) of WT, AQP0-Htz, TgAQP1/AQP0-Htz in C57 and FVB genetic backgrounds was studied using lens cortical fiber cell membrane vesicles. \( P_f \) was calculated by the rate of shrinking of each vesicle when subjected to a hypertonic bath solution. Fiber cell membrane vesicles of WT expressing AQP0 in C57 and FVB genetic backgrounds (Fig. 3; Table 1) had an average \( P_f \) of \( 40 \pm 10 \) and \( 38 \pm 8 \) \( \mu \)m/s, respectively, which was not affected by exposure to HgCl2 (C57, \( 43 \pm 11 \) \( \mu \)m/s; FVB, \( 42 \pm 10 \) \( \mu \)m/s; Table 1), an inhibitor of AQP1.\(^{25,76,77}\) Loss of 50% of AQP0 in AQP0-Htz mouse lens fiber cell membranes of C57 and FVB resulted in a \( P_f \) of approximately \( 20 \pm 11 \) and \( 21 \pm 10 \) \( \mu \)m/s, respectively, which also were unaffected by HgCl2 (C57, \( 22 \pm 10 \) \( \mu \)m/s; FVB, \( 22 \pm 8 \) \( \mu \)m/s; Table 1) as seen for the WT. The fiber cell membrane vesicles of transgenic AQP1 mice (TgAQP1/AQP0-Htz) showed 3-fold increase in \( P_f \) (C57, 63 \( \pm \) 14 \( \mu \)m/s; FVB, 62 \( \pm \) 13 \( \mu \)m/s), owing to the presence of the highly efficient AQP1, compared to those of AQP0-Htz lens and >1.5-fold increase compared to those of WT (\( P < 0.001 \); Fig. 3; Table 1). The increased \( P_f \) in TgAQP1/AQP0-Htz lens fiber cell was partially inhibited (C57: 36 \( \pm \) 8 \( \mu \)m/s; FVB: 35 \( \pm \) 11 \( \mu \)m/s) by HgCl2 (Fig. 3; Table 1). Previously, we reported that transgenic expression of AQP1 in the fiber cells of AQP0-Htz in the FVB strain restored \( P_f \) that was lost due to the KO of native AQP0, but lens transparency was not restored completely.\(^{26,28}\)

In our study in the C57 strain, even though lens transparency of the transgenic model showed significant improvement compared to that in the FVB strain, \( P_f \) did not differ significantly from that in the FVB background (Fig. 3; Table 1).

**Loss of One Copy of AQP0 in C57 Mouse Lenses Results in Decreased GJ Coupling Resistance and Intracellular HP**

Several investigations on mutant or KO genes in mouse lenses have revealed that an increase in GJ coupling resistance leads to an increase in intracellular HP.\(^{36,78-82}\) However, loss of 50%
**Figure 1.** (A) Genotyping to confirm the presence or absence of CP49 natural mutation. PCR using primers published by Alizadeh et al.\(^7\) (Primer set I); 320 bp, indicates the presence of intact CP49 allele; 386 bp, shows the presence of mutant CP49 allele. Competitive PCR as described by Simirskii et al.\(^7\) (Primer set II); 205-bp points to the presence of intact CP49 allele; 347-bp indicates the presence of mutant CP49 allele. M, Marker (50-bp Ladder). (B) Western blotting of filensin and CP49 in different genotypes as indicated. Arrows indicate immunoreactive bands. Blots treated with filensin antibody (arrow; filensin, approximately 95 kDa) or CP49 antibody (arrow; CP49, approximately 49 kDa). (C) Immunostaining of AQP0 and BF proteins in C57 genetic background lenses: WT, AQP0-Htz, and TgAQP1/AQP0-Htz. Cryosections of 2-month-old lenses were
of AQP0 in the FVB mice that lack BF, or expression of a mutated AQP0 protein (e.g., CatFR mouse) did not alter lens GJ coupling resistance or intracellular HP. These results motivated us to test whether there is any change in GJ coupling resistance and intracellular HP in AQP0-Htz and TgAQP1/AQP0-Htz in the C57 strain compared to the WT.

Data from our experiments in C57 background showed that there are significant differences in the values of series resistance ($R_s$) and coupling conductance (Figs. 4A, 4B; Table 2) in AQP0-Htz, and TgAQP1/AQP0-Htz mouse lenses compared to the WT lenses. In AQP0-Htz and TgAQP1/AQP0-Htz mice, coupling conductance per area of cell-to-cell contact of differentiating fibers was approximately 0.8 S/cm² for all types of lenses; however, the coupling conductance ($G_i$) of mature fibers increased dramatically in Htz lenses. It was 0.54 S/cm² in WT lenses but increased to 1.53 S/cm² in AQP0-Htz and 1.41 in TgAQP1/AQP0-Htz lenses (Table 2). These values were based on the best-fit curve (Equations 1 and 2) to $R_s$ data collected from eight lenses of the same genotype. Loss of 50% AQP0 in AQP0-Htz and TgAQP1/AQP0-Htz caused a significant decrease in resistance and approximately 3-fold increase in radial coupling conductance in the mature fibers (Fig. 4; Table 2). Increased $P_f$ in TgAQP1/AQP0-Htz did not alter GJ coupling resistance or HP compared to AQP0-Htz (Fig. 4; Tables 2, 3).
FIGURE 3. Lens fiber cell membrane \( P_f \) of 2-month-old WT, AQP0-Htz, and TgAQP1/AQP0-Htz mice in (a) C57 and (b) FVB genetic backgrounds. Exposure of fiber cell membrane vesicles to 1 mM \( \text{HgCl}_2 \) was to test for inhibition of \( P_f \). AQP1 is \( \text{Hg} \)-sensitive whereas AQP0 is not. Each bar represents mean ± SD. Eight membrane vesicles were used per experiment. *Significant reduction in \( P_f \) in AQP0-Htz compared to WT. **Significant increase in \( P_f \) in TgAQP1/AQP0-Htz compared to AQP0-Htz.

In previous studies, the HP at the center of the lens (\( p_i[0] \)) always varied with the GJ coupling resistance of the central mature fibers. If the resistance went up, pressure went up. In the Cx46 heterozygous KO lenses, the MF resistance doubled and \( p_i(0) \) essentially doubled, suggesting no significant change in water flow. Similarly, in Cx46 for Cx50 knockin lenses, the MF resistance was reduced to 50% and \( p_i(0) \) was reduced to approximately 50%, again suggesting no significant change in water flow. In the lenses studied here, the data on the radial distribution of intracellular pressure were fit with Equation 3 to determine the pressure at the lens surface \( p_i[a] \) at the transition from DF to MF (\( p_i[b] \)) and at the lens center (\( p_i[0] \); Figs. 4C, 4D, Table 3). \( p_i(0) \) decreased in the Htz lenses as expected given the reduced coupling resistance, but the change in \( p_i(0) \) was not nearly as great as the change in resistance. The value of \( R_{MF} \) decreased nearly 3-fold in the Htz lenses relative to WT, whereas \( p_i(0) \) was reduced by 34% to 46% compared to WT. Thus, there may be more water flow in the Htz lenses than WT. Increased \( P_f \) in AQP0-Htz (TgAQP1/AQP0-Htz) did not alter the HP compared to AQP0-Htz (Figs. 4C, 4D; Table 3).

Expression of Lens Cx

The increased coupling conductance seen in the C57 Htz lenses suggested there might be an increase in GJ protein expression. Therefore, we looked at expression levels of the major lens Cxs.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mouse Strain</th>
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<tbody>
<tr>
<td>C57</td>
<td>FVB</td>
</tr>
<tr>
<td>WT</td>
<td>39.75 ± 10.05</td>
</tr>
<tr>
<td>WT*</td>
<td>43.00 ± 11.24</td>
</tr>
<tr>
<td>AQP0-Htz</td>
<td>19.63 ± 7.60</td>
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<tr>
<td>AQP0-Htz*</td>
<td>22.38 ± 9.64</td>
</tr>
<tr>
<td>TgAQP1/AQP0-Htz</td>
<td>63.38 ± 14.13</td>
</tr>
<tr>
<td>TgAQP1/AQP0-Htz*</td>
<td>35.50 ± 8.38</td>
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</tbody>
</table>

* 1mM \( \text{HgCl}_2 \).

Immunoblotting of total lens membrane proteins extracted from WT, AQP0-Htz, and TgAQP1/AQP0-Htz was performed to ascertain whether there were changes in Cx43, Cx46, or Cx50 GJ protein expression levels (Fig. 5A). We tested AQP0-Htz to see the effect when only one copy of AQP0 is expressed. We also tested TgAQP1/AQP0-Htz, which has one copy of AQP0 and transgenically expresses the approximately 40-fold more efficient water channel AQP1\(^{83}\) in the fiber cells. Equal quantities of the membrane proteins extracted from the different genotypes (in the C57 strain) were tested using anti-Cx43, anti-Cx46, and anti-Cx50 antibodies. Western blotting (Fig. 5A) and protein quantification (Fig. 5B) studies on lens Cx showed no statistically significant difference (\( P > 0.05 \)) in the expression levels of Cx43, 46, or 50 among WT, AQP0-Htz, and TgAQP1/AQP0-Htz lenses. Thus, the increased GJ conductance in C57 AQP0-Htz probably is due to reduction in the abundance of AQP0 causing reorganization of GJs at the membrane in the presence of BF proteins, aided by the gradual post-translational covalent modifications that occur in the proteins involved.

DISCUSSION

A previous study on AQP0-Htz and TgAQP1/AQP0-Htz mouse models developed in FVB background showed no significant alteration in GJ coupling or HP.\(^{30}\) However, in our study using the same type of mouse models but in the C57 background, which expresses BFS, we found approximately 3-fold reduction in GJ coupling resistance and a 34% to 46% reduction in intracellular HP. Even though AQP1 in the fiber cells of the TgAQP1/AQP0-Htz restored \( P_f \) compared to the AQP0-Htz, it did not reduce the HP further, suggesting that HP might be determined by GJ coupling. We see the increase in GJ conductance was not due to an increase in GJ protein expression, though there clearly was an increase in the number of open GJ channels conducting radial ion and water flow. Our data suggested modulation of lens GJ coupling conductance by AQP0 in the presence of BFS.

Loss of BF proteins, CP49, and filensin, in the CP49 KO lenses did not cause a significant difference in the expression levels of Cx or GJ coupling conductance.\(^{44,82}\) Nevertheless, loss of BF proteins and Tropomodulin-1 (Tmod1, actin filament pointed end-capping protein) together resulted in increased GJ resistance and increased HP with no change in Cx expression.\(^{82}\) The investigators hypothesized that BF and Tmod1 acted like a corral to contain GJ channels on the broad faces of
FIGURE 4. Series resistance and HP in 2-month-old lenses of WT, AQP0-Htz, and TgAQP1/AQP0-Htz mice. (A, B) Series resistance ($R_s$) of lenses of: AQP0-Htz (A; $n = 8$) and TgAQP1/AQP0-Htz (B; $n = 8$) compared to corresponding data for WT (A, B; $n = 8$), as a function of distance from lens center ($r/a$), where ‘r’ (cm) is the actual distance and ‘a’ (cm) is the lens radius. Lenses of AQP0-Htz and TgAQP1/AQP0-Htz mice showed a significant decrease ($P < 0.001$) in resistance compared to WT. (C, D) Intracellular HP of lenses of AQP0-Htz (C; $n = 8$) and TgAQP1/AQP0-Htz (D; $n = 8$) as a function of normalized distance from lens center ($r/a$) showed a significant decrease ($P < 0.001$) compared to WT.

TABLE 2. Regional Values of Resistivity and Normalized Coupling Conductance of WT, AQP0-Htz, and TgAQP1/AQP0-Htz Lenses in C57 Compared to FVB Strain

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Zone</th>
<th>C57</th>
<th>FVB*</th>
<th>C57</th>
<th>FVB*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_i$, KΩ-cm</td>
<td>$R_o$, KΩ-cm</td>
<td>$G_i$, S/cm²</td>
<td>$G_o$, S/cm²</td>
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<tr>
<td>WT</td>
<td>DF</td>
<td>3.95</td>
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<tr>
<td>WT</td>
<td>MF</td>
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<td>5.16</td>
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<td>0.65</td>
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<tr>
<td>AQP0-Htz</td>
<td>DF</td>
<td>4.09</td>
<td>5.52</td>
<td>0.81</td>
<td>0.95</td>
</tr>
<tr>
<td>AQP0-Htz</td>
<td>MF</td>
<td>2.18</td>
<td>5.44</td>
<td>1.53</td>
<td>0.61</td>
</tr>
<tr>
<td>TgAQP1/AQP0-Htz</td>
<td>DF</td>
<td>4.09</td>
<td>4.36†</td>
<td>0.81</td>
<td>0.82†</td>
</tr>
<tr>
<td>TgAQP1/AQP0-Htz</td>
<td>MF</td>
<td>2.36</td>
<td>6.26**</td>
<td>1.41</td>
<td>0.53**</td>
</tr>
</tbody>
</table>

$R_i$, resistivity; $G_i$, conductance; DF, differentiating fibers; MF, mature fibers.

* Hall and Mathias.30
† This study was done at a different time than the comparison of WT with AQP0-Htz, hence they have a different set of control data with WT $R_i$(DF) = 4.09 and $R_o$(MF) = 6.03; WT $G_i$(DF) = 0.81 and $G_o$(MF) = 0.55.

TABLE 3. Regional Values of Intracellular HP in WT, AQP0-Htz and TgAQP1/AQP0-Htz Lenses in C57 Compared to FVB Strain

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Zone</th>
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<th>FVB*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$p_i$, mm Hg</td>
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<td>$p_i$(a)</td>
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<td>0</td>
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<tr>
<td>WT</td>
<td>$p_i$(b)</td>
<td>35</td>
<td>34</td>
</tr>
<tr>
<td>WT</td>
<td>$p_i$(0)</td>
<td>339</td>
<td>347</td>
</tr>
<tr>
<td>AQP0-Htz</td>
<td>$p_i$(a)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AQP0-Htz</td>
<td>$p_i$(b)</td>
<td>22</td>
<td>32</td>
</tr>
<tr>
<td>AQP0-Htz</td>
<td>$p_i$(0)</td>
<td>185</td>
<td>330</td>
</tr>
<tr>
<td>TgAQP1/AQP0-Htz</td>
<td>$p_i$(a)</td>
<td>0</td>
<td>0†</td>
</tr>
<tr>
<td>TgAQP1/AQP0-Htz</td>
<td>$p_i$(b)</td>
<td>23</td>
<td>32†</td>
</tr>
<tr>
<td>TgAQP1/AQP0-Htz</td>
<td>$p_i$(0)</td>
<td>225</td>
<td>332†</td>
</tr>
</tbody>
</table>

$p_i$(a), pressure at lens surface; $p_i$(b), pressure at the junction between differentiating fibers and mature fibers; $p_i$(0), pressure in the center of the lens.

* Hall and Mathias.30
† This study was done at a different time than the comparison of WT with AQP0-Htz, hence they have a different set of control data with WT $p_i$(a) = 0, $p_i$(b) = 35, $p_i$(0) = 339.
As indicated for the respective Cx. (mice lenses from different litters. of four or five independent Western blot analyses using 2-month-old quantification data shown for the different Cxs represent mean AQP0-Htz and TgAQP1/AQP0-Htz with those of WT. The protein by their respective antibodies. and Scheuring et al.91 are relevant to our notion. When another and atomic force microscopy studies by Buzhynskyy et al.88 In the maturing fiber cells, AQP0 forms thin junctional square arrays, except at some specific regions of the fiber cell membranes. In the differentiating fiber cells of the WT lens, AQP0 and GJs are distributed more or less uniformly (with respect to their expression levels in the lens) throughout the membrane aqueous lenses (AQP0-Htz, lack of 50% of AQP0 protein in the broader side of the fiber cells might be facilitating the small GJ plaques to form large ones in the presence of BF proteins to enable more efficient GJ coupling in the radial direction. Increased GJ coupling, consequently, could be creating a more efficient microcirculation to maintain lens homeostasis and reduce cataract severity. The AQP0-Htz lenses in FVB genetic background, that does not express BFs, develop severe cataract likely due to failure of small GJ junction plaques in the narrow sides of the fiber cells to migrate to the broader sides for increased GJ coupling and efficient microcirculation.

In the current study, increase in GJ coupling and reduction in HP are due to regulation of GJs by AQP0 in the presence of BF proteins, rather than indirect effects of the genetic manipulation causing membrane reorganization as a consequence of the loss of 50% of the profusely expressed AQP0. In the Cx46 AQP0-Htz, lacking 50% of AQP0 protein in the broader side of the fiber cells might be facilitating the small GJ plaques to form large ones in the presence of BF proteins to enable more efficient GJ coupling in the radial direction. Increased GJ coupling, consequently, could be creating a more efficient microcirculation to maintain lens homeostasis and reduce cataract severity. The AQP0-Htz lenses in FVB genetic background, that does not express BFs, develop severe cataract likely due to failure of small GJ junction plaques in the narrow sides of the fiber cells to migrate to the broader sides for increased GJ coupling and efficient microcirculation.

The Effect of GJ Coupling on the Lens Circulation and HP

Homeostasis in the avascular lens requires a microcirculation facilitated by AQPs, GJ channel, and ion channels. During the intracellular phase of the microcirculation, Na⁺ passes from the central fibers to the surface cells through GJ channels. As sodium enters fiber cells and flows to the surface, transmembrane osmotic gradients are generated and water enters the fiber cells through AQP0 and follows the flow of Na⁺ to the surface. To drive the cell-to-cell flow of sodium, there are intracellular diffusion and voltage gradients and to drive cell-to-cell water flow, there is an intracellular HP gradient. With a rise in GJ coupling, there is a decrease in the intracellular gradients for voltage, sodium diffusion, and HP.56,84 We documented a decrease in the HP gradient with the increase in open GJ channels; however, the pressure gradient did not go down as much as the coupling resistance. The HP gradient is dictated by water flow, which follows transmembrane sodium inflow. The reduction in GJ resistance will make the intracellular voltage more negative and reduce sodium radial-diffusion gradients. These changes could increase transmembrane sodium inflow and, thus, increase water flow. These simple indirect effects of reduced GJ resistance could lead to increased water flow, but the AQP0-Htz effect on water flow remains uncertain.

AQP0 Water Permeability Effect on Lens Water Flow and HP

Hall and Mathias50 provide an extensive discussion of the effect of membrane water permeability on water flow. The essence of their conclusions and data are that membrane Pₛ is much larger than salt permeability, so salt transport is rate-limiting for osmosis. Increasing fiber cell membrane Pₛ would not significantly increase water flow, which already is near maximal. Moreover, there would need to be a huge reduction in membrane Pₛ to significantly reduce water flow and, thus, reduce HP. They show that in FVB mice, expression of transgenic AQP1 (TgAQP1/AQP0-Htz) more than doubles fiber cell membrane Pₛ without appreciably affecting water flow or HP, and that in AQP0-Htz mice, fiber cell membrane Pₛ is reduced to approximately half that of WT mice without appreciably affecting water flow or HP. They also report no change in GJ coupling conductance in AQP0-Htz, FVB mice,
whereas here we reported a significant increase in GJ coupling conductance in AQP0-Htz, C57 mice. This increase in GJ coupling causes the decrease in HP in AQP0-Htz, C57 mice. GJs provide the path for intracellular outflow of water, so when the number of open GJ channels increases, the resistance for fluid outflow decreases and less pressure is required to drive the same water flow.

Possible Interactions Among AQP0, BFs, and GJs

In our study, loss of 50% of AQP0 caused a significant decrease in GJ coupling resistance and lens HP in the MF region, probably through effects on Cx46 rather than Cx50 GJs, since Cx46 is thought to be the only functional MF channel. Modulation of Cx46 by AQP0 could be direct. One such effect simply could be the abundance of AQP0 protein in the membrane; also, AQP0 forms large square arrays of thin junctions (11–13 nm) in the maturing fiber cells of MF region. As AQP0 thin junctions in this region appear to push the GJ plaques (16–17 nm) toward the periphery of the thin junctional square arrays. The observed increase in lens GJ coupling could be due to the increased availability of membrane space on the fiber cell surfaces, which mediate radial flows. If GJs were corralled by BFs in the broad faces, we would record a reduction in the radial GJ resistance as area normally filled with square arrays is filled with large GJ plaques. Thus, the increase in GJ coupling possibly could be caused by GJ channel plaque reorganization and distribution, which will be analyzed in the future using ultrastructural studies.

C-Terminal Truncation of AQP0 and Lens Cx

At the DF to MF transition, which occurs at approximately 15% of the distance into a mouse lens or 85% of the distance from the lens center, organelles are degraded and most membrane and cytosolic proteins begin to undergo PTMs (e.g., C-terminal truncation). C-terminal truncation of AQP0 does not appear to alter its water permeability, though it may induce formation of thin junctions, which are much more prevalent in the MF (inner cortex and outer nuclear regions) than DF. A number of studies (reviewed by Mathias et al.) suggest C-terminal truncation of Cx50 at the DF to MF transition causes the open probability of Cx50 channels to drop to near zero, so coupling conductance decreases, as seen for WT lenses in Table 2. C-terminal truncation of Cx46 does not affect the conductance of Cx46 channels, so coupling of MF region is thought almost exclusively to be due to Cx46 channels. Also, as seen in Table 2, expression of AQP0-Htz in lenses from C57 mice causes the Cx46 channels in the MF region to increase coupling conductance relative to those in DF region. There is no significant change in GJ coupling of the DF in AQP0-Htz lenses relative to WT. There also is no significant change in GJ coupling of either the DF or MF region in AQP0-Htz lenses from FVB mice, which lack BFs. Together, these studies suggest modulation of Cx46 GJ coupling conductance by AQP0 in the maturing fiber cells of the MF region, in the presence of BF proteins in the lens.

From our results, we hypothesized that the AQP0 tetramer scale square array density in the maturing fiber cells of inner cortex and outer nucleus could modulate lens GJ function for normal lens microcirculation to maintain lens transparency and homeostasis. Our hypothesis is depicted as models for WT and AQP0-Htz in Figures 6A and 6B. Due to the similarities in results for C57 TgAQP1/AQP0-Htz with AQP0-Htz, we did not attempt to provide a separate model for the former. The models are designed to represent the four putative regions of the lens, namely outer cortex, inner cortex, outer nucleus, and inner nucleus, because AQP0 and several other lens membrane and cytosolic proteins undergo gradual N- and C-terminal end cleavages, from the inner cortex to the inner nucleus.

In the WT (Fig. 6A, left column), intact AQP0 present at the outer cortex interacts with cytoskeletal proteins (e.g., BF proteins) and GJ proteins (Cxs), possibly aiding in the relatively uniform distribution of AQP0 in the fiber cell membranes. AQP0 constitutes approximately 45% of the total membrane proteins; it interacts with Cx, BF proteins, and crystallins through the C-terminus. During fiber cell maturation from inner cortex to outer nucleus, AQP0, Cx, and several other lens proteins undergo gradual deletion of N- and C-terminal ends, possibly resulting in the gradual loss of protein-protein interactions. This process could be triggering tetrameric AQP0 to group and form small square array thin junctions to provide strong adhesion, and in the process, be pushing the interspersed GJs to the periphery. The presence of GJ plaques at the periphery of AQP0 square array thin junctions has been reported by Buzhynskyy et al. As the distance to the lens interior advances, formation of more AQP0 large square array thin junctions occur in the nucleus, possibly to ensure tight holding of the fiber cells to reduce the extracellular space and aid in the compact packing of the maturing and matured fibers. The GJ plaques may reach a medium size into the lens but do not attain large sizes probably due to the abundant expression of AQP0 and presence of large AQP0 square array thin junctions.

In the AQP0-Htz (Fig. 6A, right column) outer cortex, intact AQP0 may follow the same distribution in principle as in the WT, but with only 50% AQP0 present, there is more space in the membrane. During fiber cell maturation in the inner cortex, outer nucleus, and inner nucleus, availability of more membrane space possibly allows the GJs to move and form larger plaques and more open channels; this could be the reason for the increased GJ conductance in AQP0-Htz. Higher GJ coupling conductance has been reported for larger compared to smaller plaques. The increase in GJ conductance might have facilitated a decrease in HP. However, even the increase in GJ conductance and decrease in HP were not enough to restore complete lens transparency and homeoeostasis, possibly due to the reduction in cell-to-cell adhesion, which could be a consequence of the lack of 50% AQP0.

A portion of a fiber cell membrane surface representing only AQP0 and GJ proteins is modeled in Figure 6B for WT (top row) and AQP0-Htz (bottom row) lenses in C57 background. At the outer cortex differentiating fibers of the WT, AQP0 tetramers and GJs are distributed throughout the membrane. In the maturing fiber cell nuclei, Cx46 containing GJs and several other lens proteins undergo gradual deletion of N- and C-terminal ends, possibly due to the gradual loss of N- and C-terminal ends of lens proteins causing rearrangement of proteins at the membrane surface. This process results in the formation of more and more AQP0 square array thin junctions and GJ plaques from the outer to the inner nucleus. In AQP0-Htz (Fig. 6B, bottom row), reduction in 50% of AQP0 provides more free space, and post-translational N- and C-terminal end cleavages of proteins allow movement of AQP0 to form collective square array thin junctions, and the GJs to form much larger plaques with more conductance. Formation of large GJ plaques could increase radial GJ coupling and reduce HP.

FVB WT lenses that lack BFs are as transparent as those of C57 WT mice. However, the severe lens cataract phenotype in FVB AQP0-Htz compared to C57 AQP0-Htz could be due to the absence of BFs causing loss of interactions with AQP0 and/or other proteins. The less severe lens cataract phenotype in the
Aquaporin 0 (AQP0) Regulates Gap Junctions in the Lens

Figure 6. (A) Schematic model describing the possible regulation of lens GJ channel distribution and function by AQP0, in WT and AQP0-Htz C57 mouse lens fiber cells. Cross-sectional views of fiber cells at outer cortex, inner cortex, outer nucleus, and inner nucleus are presented. WT (left column) lens fiber cells show BFs, AQP0, and GJ plaques and other cytoskeletal proteins in the outer cortex. AQP0 is distributed at the membrane interspersed with GJs. As fiber cells mature (inner cortex and outer nucleus), the cell nucleus is lost, and BFs, AQP0, GJ, and other proteins begin to lose their N- and C-terminal ends; reorganization of the cell membrane proteins occur. AQP0 group to form larger square array thin junctions and the small GJ plaques are pushed to the periphery of the square arrays. GJs probably are prevented from forming very large plaques due to the prolific
expression of AQP0 and thin junctions. At the inner nucleus in the terminally differentiated fiber cells large patches of square array thin junctions and small to medium GJ plaques are seen. AQP0-Htz (right column) mouse lens fiber cells express BFs, 50% AQP0, and 100% Cx and other proteins. The outer cortex may follow the same type of distribution of the proteins but with only 50% of AQP0, which clears more membrane space. Cx GJ channels form larger aggregates of junctional plaques due to the availability of more surface area as a result of the reduction in AQP0 and consequent possible reduction in large patches of AQP0 square array thin junctions. The differentiated fiber cells at the inner nucleus have AQP0 large square array thin junctions and large Cx GJ plaques. Compared to small GJ plaques, large GJ plaques have higher conductance.\textsuperscript{82} (B) Schematic representations of only AQP0 and GJs at fiber cell membrane surfaces of WT and AQP0-Htz C57 mice lenses. WT (top row) outer cortex shows AQP0 tetramers, and Cx distributed optimally. Formation of more GJ plaques, grouping of AQP0, and thin junction formation occur at the inner cortex and outer nucleus probably due to the gradual loss of N- and C-terminal ends of the proteins. These events happen to a greater extent at the inner nucleus and increase in GJ plaque size and AQP0 square array thin junctions occur. AQP0-Htz (bottom row) has fewer tetramers compared to the WT with more free space due to the loss of 50% AQP0. Availability of more surface area probably stimulates the smaller GJ plaques to move and aggregate to form larger plaques in the inner cortex, outer nucleus, and inner nucleus. The large GJ plaques with increased GJ coupling might be responsible for the decrease in HP in the AQP0-Htz condition.

AQP0-Htz in C57 mouse strain compared to the cataract phenotype in FVB could be due to the increased efficiency in the lens microcirculation.

**Conclusions**

In the presence of BF proteins, AQP0 plays a significant role in modulating lens GJ coupling and HP to regulate the microcirculation. Our data are consistent with the hypothesis that the improved transparency in C57 AQP0-Htz lenses is due to modulation of GJ coupling by AQP0 in the presence of BF proteins to increase the circulation of fluid in the lens and support homeostasis. To our knowledge, this is the first report on an aquaporin water channel regulating gap junctions in the lens.

**Acknowledgments**

The authors thank Alan Shiels, PhD, for providing the original AQP0 knockout mouse model in a mixed background and Paul G. FitzGerald, PhD, for providing antibodies for BF proteins CP49 and CP115.

Supported by funding from National Institute of Health-NEI, Grants R01: EOY20506, EOY26155 (KV), and R01: EOY6391 (RTM).

Disclosure: S. Kumari, None; J. Gao, None; R.T. Mathias, None; X. Sun, None; A. Eswaramoorthy, None; N. Browne, None; N. Zhang, None; K. Varadaraj, None

**References**


2. Kumari SS, Varadaraj K. Intact and N- or C-terminal end truncated AQP0 function as open water channels and cell-to-cell adhesion proteins: end truncation could be a prelude for adjusting the refractive index of the lens to prevent spherical aberration. *Bioclin Biophys Acta*. 2014;1840:2862–2877.


Aquaporin 0 Regulates Gap Junctions in the Lens


