The ocular lens needs to be transparent for proper vision. Structurally, the lens has a monolayer of epithelial cells at the anterior surface, beneath which lie multiple layers of fiber cells. The epithelial cells at the equator transform and differentiate into the secondary fiber cells. It is a continuous process. Because there is no cellular turnover, all the fiber cells produced in a life span are contained within the lens. As new fiber cells are added over the older differentiating fiber cells in the outer cortex, inner cortical, and outer nuclear fiber cells undergo maturation and compaction for which loss of cellular nutrients and removing the metabolic wastes is essential. These processes help to organize the fiber cells in a precise manner to minimize light scattering. The oldest fiber cells occupy the center of the ellipsoidal crystalline lens, which grows peripherally. Constant addition of the fiber cells increases the bulk and could lead to spherical aberration (SA), which creates multiple focal points and blurred vision. To circumvent this, the lens adjusts the refractive index gradient (RING), which varies from 1.386 to 1.406 from the cortex to the nucleus in humans and 1.35 to 1.55 in mouse. RING adjustment is a cooperative process mediated by several proteins and post-translational modifications.

Aquaporin (AQP) water channels are integral membrane proteins belonging to the superfamily of Aquaporins. They are expressed in microbes, plants, and animals and are critical for cellular development, growth, and homeostasis. AQP5 allow transcellular passage of water (aquaporins) or water and small solutes like glycerol (aquaglyceroporins). Water channels, gap junction channels, ion transporters, and cotransporters are involved in creating a microcirculation within the lens to compensate for its avascular nature by providing the necessary nutrients and removing the metabolic wastes.

In mammals, 13 different AQP genes have been identified. The mammalian lens expresses AQP0, AQP1, and AQP5. Mammalian AQP0 is a 28-kDa protein that is profusely expressed in the lens contributing approximately 45% of the total membrane proteins of the fiber cells. It is a multifunctional protein. Water permeability and cell-to-cell adhesion (CTCA) functions of AQP0 are important for maintaining lens transparency, refractive index gradient, biomechanics, and homeostasis. AQP0 displays a characteristically low water permeability, which is several-fold less compared with its lens counterparts AQP1 and AQP5. Mutations and knockout of AQP0 alter ocular growth and result in dominant cataracts due to the loss of lens homeostasis,

Deletion of Seventeen Amino Acids at the C-Terminal End of Aquaporin 0 Causes Distortion Aberration and Cataract in the Lenses of AQP0AC/AC Mice

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Purpose. Investigate the effects of the absence of 17 amino acids at the C-terminal end of Aquaporin 0 (AQP0) on lens transparency, focusing property, and homeostasis.

Methods. A knockin (KI) mouse model (AQP0AC/AC) was developed to express AQP0 only as the end-cleaved form in the lens. For this, AQP0 was genetically engineered as C-terminally end-cleaved with amino acids 1 to 246, instead of the full length 1 to 265 of the wild type (WT). After verifying the KI integration into the genome and its expression, the mouse model was bred for several generations. AQP0 KI homozygous (AQP0AC/AC) and heterozygous (AQP0AC/+) lenses were imaged and analyzed at different developmental stages for transparency. Correspondingly, aberrations in the lens were characterized using the standard metal grid focusing method. Data were compared with age-matched WT, AQP0 knockout (AQP0−/−), and AQP0 heterozygous (AQP0AC/−) lenses.

Results. AQP0AC/AC lenses were transparent throughout the embryonic development and until postnatal day 15 (P15) in contrast to age-matched AQP0−/− lenses, which developed cataract at embryonic stage itself. However, there was distortion aberration in AQP0AC/AC lens at P5; after P15, cataract began to develop and progressed faster surpassing that of age-matched AQP0−/− lenses. AQP0AC/− lenses were transparent even at the age of 1 year in contrast to AQP0−/− lenses; however, there was distortion aberration starting at P15.

Conclusions. A specific distribution profile of intact and end-cleaved AQP0 from the outer cortex to the inner nucleus is required in the lens for establishing refractive index gradient to enable proper focusing without aberrations and for maintaining transparency.

Keywords: AQP0, lens transparency, spherical aberration, cataractogenesis, C-terminal cleaved AQP0
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**Figure 1.** Strategy to generate a AQP0<sup>ΔCΔC</sup> by introducing a stop codon after amino acid 246. (A) WT: Schematic structure of WT mouse AQP0 gene showing exons 1–4 (as rectangular vertical or horizontal boxes) and the connecting introns. Vector: Exons 3 and 4 with introns (highlighted in blue and red) as well as a Neo selection gene were amplified by PCR and cloned into the vector (details in [B]). Black dotted lines on either side denote vector sequences. Asterisk indicates an in-frame translation stop codon predicted to truncate AQP0 after the amino acid Asparagine-246. KI-Neo: The recombinant vector (with vector sequences) and the Neo selection gene were transfected into mouse embryonic stem cells and positive clones were selected using the Neo selection marker. WT: Schematic structure of WT mouse in FVB strain was used as a positive control for AQP0<sup>ΔCΔC</sup> gene mutation. WT (AQP0<sup>ΔCΔC</sup> or AQP0<sup>ΔCΔC</sup>+) and heterozygous (AQP0<sup>ΔCΔC</sup>+) mouse model were used in this investigation are in C57BL/6J (The Jackson Laboratory, Bar Harbor, ME, USA) inbred strain which does not carry the CP49 gene mutation. WT (AQP0<sup>ΔCΔC</sup> or AQP0<sup>ΔCΔC</sup>+) and heterozygous (AQP0<sup>ΔCΔC</sup>+) mouse models were used in this investigation are in C57BL/6J (The Jackson Laboratory, Bar Harbor, ME, USA) inbred strain which does not carry the CP49 gene mutation. WT (AQP0<sup>ΔCΔC</sup> or AQP0<sup>ΔCΔC</sup>+) and heterozygous (AQP0<sup>ΔCΔC</sup>+) mouse models were used in this investigation are in C57BL/6J (The Jackson Laboratory, Bar Harbor, ME, USA) inbred strain which does not carry the CP49 gene mutation. WT (AQP0<sup>ΔCΔC</sup> or AQP0<sup>ΔCΔC</sup>+) and heterozygous (AQP0<sup>ΔCΔC</sup>+) mouse models were used in this investigation are in C57BL/6J (The Jackson Laboratory, Bar Harbor, ME, USA) inbred strain which does not carry the CP49 gene mutation.

**Materials and Methods**

**Animals**

The wild type (WT) and mouse models used in this investigation are in C57BL/6J (The Jackson Laboratory, Bar Harbor, ME, USA) inbred strain which does not carry the CP49 gene mutation. WT (AQP0<sup>ΔCΔC</sup> or AQP0<sup>ΔCΔC</sup>+) and heterozygous (AQP0<sup>ΔCΔC</sup>+) mouse models were used in this investigation are in C57BL/6J (The Jackson Laboratory, Bar Harbor, ME, USA) inbred strain which does not carry the CP49 gene mutation. WT (AQP0<sup>ΔCΔC</sup> or AQP0<sup>ΔCΔC</sup>+) and heterozygous (AQP0<sup>ΔCΔC</sup>+) mouse models were used in this investigation are in C57BL/6J (The Jackson Laboratory, Bar Harbor, ME, USA) inbred strain which does not carry the CP49 gene mutation.

**Generation of AQP0-1-246 Mutant Knockin (AQP0<sup>ΔCΔC</sup>) Mouse Model**

A truncated mutant AQP0 KI mouse model AQP0<sup>ΔCΔC</sup> was developed through inGenious Targeting Laboratory, Inc. (Ronkonkoma, NY, USA). This model expresses a major form of C-terminally end-cleaved AQP0 (that lacks amino acids 247–265), which is observed in human, bovine, and mouse lens nuclear regions. 22,43–45 The schematic diagram (Figs. 1A, 1B) depicts the strategy used for developing AQP0<sup>ΔCΔC</sup>- mutant knock-in mouse model (details on the KI mouse model development are given in the Supplementary Section).
proteins of WT, AQP0 validate CP49 protein expression, total lens membrane extracted from the lenses (at P5) of WT, AQP0þ prepared and immunoblotting was performed using CP49 marker.

To verify the expression of AQP0, membrane proteins were extracted from the lenses (at P5) of WT, AQP0+/−, or AQP0−/− mice and immunoblotting was performed using CP49 antibody.

To verify the expression of AQP0, membrane proteins were extracted from the lenses (at P5) of WT, AQP0+/−, or AQP0−/− mouse pups. Mouse monoclonal AQP0 antibody (sc-376445; Santa Cruz Biotechnology, Inc.) was used (for details, please see the Supplementary Section).

**Immunohistochemistry**

Lens immunohistochemical studies were conducted as described.57,60 Lens sections were immunostained using mouse monoclonal AQP0 antibody (Santa Cruz Biotechnology, Inc.) (more information is given in the Supplementary Section).

**Evaluation of Lens Transparency and Aberration**

Lens transparency was assessed as described by Kumari et al.57 In brief, lenses of WT, AQP0+/−, AQP0−/−, AQP0+/−/−, or AQP0−/−/− mice were dissected out in prewarmed (37°C) mammalian physiological saline. Images of these lenses were captured under the same lighting and imaging conditions with the aid of a dark-field binocular microscope attached to a digital camera. Lens transparency was quantified from the dark-field lens images using ImageJ software (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). Qualitative evaluation of lens aberrations was performed using dark-field optical grid focusing. A copper electron microscope specimen grid was imaged through a whole lens placed on it. Quality of the grid lines focused was appraised for light scatter and aberrations due to refractive index gradient alteration.

**Cell Culture and Transfection of AQP0**

These procedures were performed basically as described by Varadaraj et al.27 (details are provided in the Supplementary Section).

**Statistical Analysis**

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

**RESULTS**

Introduction of a stop codon after amino acid 246 in mouse AQP0 was achieved by homologous recombination. Genomic sequencing showed that there was no disruption in the native AQP0 locus (data not shown). To verify the presence of the introduced mutation, genomic DNA from eight mouse chimeras with agouti coat color (#888, 889, 890, 891, 892, 893, 894, and 895) was PCR amplified using primers revneo3b located on the long homology arm. Among the eight chimeras tested, #889, 890, and 895 amplified a predicted 676-bp DNA segment (Fig. 2A); the amplicons were sequenced for verification of the introduced stop codon and deletion. To delete the Neo gene in the mutant chimeras, #889, 890, and 893 were crossed with C57 WT mice to eliminate FLP transgene. KI mice #889, 890, and 893 were FLP negative with no amplification product while FLP-positive mice were positive. The PCR product amplified by primers revneo3b and MOPE4 was sequenced to confirm the presence of the FLP transgene amplified a positive product of 233 bp for the WT allele and a 307-bp amplicon for the Neo-negative mice crossed with C57 WT to eliminate FLP transgene. KI mice #889, 890, and 893 were FLP positive with no amplification product while FLP-positive mice were positive. The PCR product amplified by primers revneo3b and MOPE4 was sequenced to confirm the presence of the introduced stop codon and deletion. To delete the Neo gene, which was identified originally in mouse 129 strains by Alizadeh et al.58 Genomic PCR was done using the primers and protocol as described previously.58,59

**Mass Spectrometric Analysis of Mouse Lens Membrane Protein**

Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) analysis was performed at our University Facility as described.22 The following lenses from WT, AQP0+/−, and AQP0−/− mice (P5) were analyzed (details of the protocol are provided in the Supplementary Section).

**Western Blotting**

Western blotting was done as described previously.60 To validate CP49 protein expression, total lens membrane proteins of WT, AQP0+/−, and AQP0−/− mice (P5) were prepared and immunoblotting was performed using CP49 antibody.

**Immunohistochemistry**

Lens immunohistochemical studies were conducted as described.57,60 Lens sections were immunostained using mouse monoclonal AQP0 antibody (Santa Cruz Biotechnology, Inc.) (more information is given in the Supplementary Section).
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amplicon indicating the absence of CP49 gene mutation. The FVB mouse genomic DNA amplified a 386-bp PCR product indicating the presence of the mutant CP49 alleles (Fig. 3A). Immunoblotting of total lens membrane proteins of WT, AQP0~+/~C, and AQP0~C/~C mice using CP49 antibody verified the expression of CP49 (Fig. 3B).

Protein expression profile of WT, AQP0~+/~C, or AQP0~C/~C mouse lenses at P5 was characterized by MALDI-TOF MS and Western blotting analyses. Both modes of protein expression analyses showed that WT expressed the 28.2-kDa protein corresponding to intact AQP0, whereas AQP0~+/~C expressed 28.2 and 26.5 kDa polypeptides corresponding to intact and cleaved form; AQP0~C/~C lens expressed only the cleaved form of 26.5 kDa (Figs. 4A, 4B). Quantification of the immunoreactive bands representing the intact and cleaved forms in AQP0~+/~C mouse lens showed no significant statistical difference (P > 0.05) in antibody binding. Immunohistochemical analysis of lenses of P5 sagittal and cross sections of WT and AQP0~C/~C showed fiber cell membrane localization of C-terminal end-cleaved AQP0, very similar to the intact AQP0 expressed in the fiber cell plasma membrane of the WT lens (Fig. 4B); quantification bar graph showed comparable intensity in antibody binding between WT and KI lens cross sections.

After verifying normal trafficking and membrane localization of AQP0 KI protein, we imaged the lenses at different stages, such as embryonic day 18 (E18), P5, P10, P15, and P20 to confirm the expression of CP49 protein. Blot treated with CP49 antibody (arrow CP49, ~49 kDa).

FIGURE 3. (A) Genotyping of C57 WT and KI (AQP0~+/~C and AQP0~C/~C), and FVB WT mice to show the absence or presence of CP49 natural mutation. An amplicon of 320 bp indicates the presence of intact CP49 allele; 386-bp amplicon indicates the presence of mutant CP49 allele. (B) Western blotting of lens proteins of C57 WT and KI mice (AQP0~+/~C and AQP0~C/~C) to confirm the expression of CP49 protein. Blot treated with CP49 antibody (arrow CP49, ~49 kDa).

FIGURE 4. (A) Protein expression profile using MALDI/TOF/MS. Lenses of mouse pups at P5 from WT, AQP0~+/~C, and AQP0~C/~C were used. (B) (Top) Western blotting of P5 lens proteins of WT (lane 1), AQP0~+/~C (lane 2), and AQP0~C/~C (lane 3). The bar graph on the right shows quantification of the immunoreactive protein bands of AQP0~+/~C by densitometry. Values from five independent immunoblots of AQP0~+/~C lens membrane proteins were used. Levels of AQP0 C-terminal antibody binding were not significantly different (P > 0.05) between the immunoreactive bands of intact AQP0 and C-terminal end-cleaved AQP0. (Bottom) Immunostaining. P5 lens sagittal sections of WT and AQP0~C/~C at low magnification. Fluorescent signals in the whole-lens sections appear nonuniform at different areas under low magnification both in the WT and KI lenses due to the thickness of the cryosections, which does not permit uniformity in the imaging plane. White bar, 150 μm. Lens cross sections of WT and AQP0~C/~C are shown below at high magnification. Mutant AQP0 protein localized in the fiber cell membranes as in the case of WT lens. Yellow bar, 4 μm. The bar graph on the right shows quantification of the fluorescence intensity from four independent immunostainings of lens cross sections of WT and AQP0~C/~C mice. Level of fluorescence due to AQP0 C-terminal antibody binding was not significantly different (P > 0.05) between WT and AQP0~C/~C lens sections.
using pixel brightness intensity, which is inversely proportional to transparency (Fig. 5F). Development of lens opacity, which began after P5 in AQP0<sup>−/−</sup> and AQP0<sup>ΔC/ΔC</sup> mouse lenses at (A) E18 (white bar: 200 μm), (B) P5 (bar: 320 μm), (C) P10 (bar: 350 μm), (D) P15 (bar: 400 μm), and (E) P20 (bar: 425 μm). (F) Quantification of pixel brightness intensity to assess lens transparency in WT, AQP0<sup>−/−</sup>, and AQP0<sup>ΔC/ΔC</sup> mouse lenses at E18, P5, P10, P15, and P20. Higher the pixel brightness intensity, lower would be the lens transparency. Star indicates statistical significance, AQP0<sup>ΔC/ΔC</sup> compared with WT at P20 (P < 0.001); filled triangle indicates statistical significance, AQP0<sup>ΔC/ΔC</sup> compared with AQP0<sup>−/−</sup> (P < 0.05). Note that at P20 lens transparency of AQP0<sup>ΔC/ΔC</sup> lens is much less than that of AQP0<sup>−/−</sup> lens.

Figure 5. Qualitative characterization of transparency (left column) and aberration (right column) of WT, AQP0<sup>−/−</sup>, and AQP0<sup>ΔC/ΔC</sup> mouse lenses at (A) E18 (white bar: 200 μm), (B) P5 (bar: 320 μm), (C) P10 (bar: 350 μm), (D) P15 (bar: 400 μm), and (E) P20 (bar: 425 μm). (F) Quantification of pixel brightness intensity to assess lens transparency in WT, AQP0<sup>−/−</sup>, and AQP0<sup>ΔC/ΔC</sup> mouse lenses at E18, P5, P10, P15, and P20. Higher the pixel brightness intensity, lower would be the lens transparency. Star indicates statistical significance, AQP0<sup>ΔC/ΔC</sup> compared with WT at P20 (P < 0.001); filled triangle indicates statistical significance, AQP0<sup>ΔC/ΔC</sup> compared with AQP0<sup>−/−</sup> (P < 0.05). Note that at P20 lens transparency of AQP0<sup>ΔC/ΔC</sup> lens is much less than that of AQP0<sup>−/−</sup> lens.

We tested the lenses for their ability to focus the lines of a copper grid. At E18, the transparent AQP0<sup>ΔC/ΔC</sup> lens focused
the gridlines well like the WT lens and in contrast to the AQP0+/− lenses showed SA due to the cataractous nature of the lens. The AQP0+/− lens, which appeared transparent produced distorted grid lines especially at the nuclear region. At P10 and P15, AQP0+/− displayed spreading of the central nuclear aberration to the periphery (Figs. 5C, 5D). AQP0+/− lenses showed increase in the severity of SA throughout the lens. By P20 (Fig. 5E), lens cataract was severe in AQP0+/− and AQP0+/−, notably more in the latter.

The changes we observed in the AQP0AC/AC lenses prompted us to examine the lenses of AQP0+/− mice. At early developmental stages, such as P5 and P10, AQP0+/− lenses showed one copy of intact AQP0 did not show lens opacity and aberration like AQP0+/− lenses (data not shown). At P15, dark-field images showed comparable lens transparency (Fig. 6A, left column). However, by P15, both AQP0+/− and AQP0+/− lenses showed aberration, which was apparent at a much higher degree in the former. AQP0AC/AC lenses showed a distinct pattern of central nuclear aberration as revealed by grid focusing (Fig. 6A, right column). Adult 2- (Fig. 6B) and 12-month-old (Fig. 6C) AQP0+/− lenses showed increased light scattering and central nuclear aberration (red circle), in contrast to global SA in AQP0+/− lens. Figure 6D shows that lens transparency of AQP0+/− continued to deteriorate with progression in age and the deterioration was statistically significant (P < 0.05) compared with the WT at each age shown; however, in AQP0+/− lens transparency was significantly higher than in AQP0+/−.

Optical quality of WT, AQP0+/−, AQP0+/−, AQP0AC/AC, and AQP0AC/AC lenses was analyzed from the images of the lenses focusing the electron microscopy grid. The grid line pattern magnified by WT lenses at the tested ages showed a positive barrel distortion aberration (Figs. 5, 6) in which the straight lines bend outward from the image center. The grid line pattern was significantly distorted throughout the lenses in AQP0+/− and AQP0+/−. In homozygous KI (AQP0AC/AC) lenses,
Membrane proteins showed multiple polypeptides binding to membrane proteins from mouse lenses at P10 showed two AQP0 indicating the absence of posttranslational truncation. As extracted from AQP0-transfected MDCK cells served as negative control for posttranslationally end-cleaved AQP0. As fiber cells of P10 pups and adult WT mice. Membrane proteins from P10 and 4-month-old adult C57 WT mice were tested. AQP0 C-terminal-specific antibody was used. The C-terminal antibody was raised using an epitope peptide having amino acids 220 to 263. Of note, here, the Ktruncated AQP0 has amino acids up to 246 at the C-terminus.

FIGURE 7. Distortion aberration zones in the lenses of AQP0/C0 mouse that expresses only C-terminal end-cleaved AQP0. (A) An early stage in lens development (P5) showing two distortion aberration zones. (B) A later stage (P15) showing three distortion aberration zones.

The much-distorted grid line pattern displayed two distinct zones at P5 (Fig. 5B). Zone I in the cortex showed a barrel distortion aberration (positive radial distortion) as in the WT lenses (Fig. 5B) and Zone II at the lens nucleus showed an abnormally increased barrel distortion aberration compared with WT-lens nucleus. These distortion aberration zones progressively increased in size with the age of the lenses (compare Figs. 5B and 5C). P10 (Fig. 5C) lens exhibited a tendency toward transitioning to a pincushion distortion aberration (negative radial distortion) in the nuclear region. At P15, AQP0/C0 showed three distinct aberration zones, I, II, and III (Fig. 5D). At this stage, the nuclear Zone II clearly exhibited a pincushion distortion aberration and a new Zone III was established between the Zones I and II (i.e., at the corticonuclear junction) with a greater barrel distortion aberration than that of cortical Zone I. In the heterozygous (AQP0/+/C0) lenses, only two distortion aberration zones start to appear at age P15 (Fig. 6A), as in the case of AQP0/C0/C0 lenses at P5 (Fig. 5B). These distortion aberration zones slowly progressed with the age of the lens (compare Figs. 6A–C). While P15 (Fig. 6A) lens showed more of a barrel distortion, the 2-month-old lens clearly showed a pincushion distortion (Fig. 6B). There was no distinct Zone III in AQP0+/C0 mouse lenses (Fig. 6C). The distortion aberration zones are shown as a schematic model (Fig. 7).

To find out whether the end-cleavage process is part of lens development or a consequence of senescence, we performed Western blotting of membrane proteins extracted from lens fiber cells of P10 pups and adult WT mice. Membrane proteins extracted from AQP0-transfected MDCK cells served as negative control for posttranslationally end-cleaved AQP0. As anticipated, protein samples from MDCK cells exhibited a specific band of 28.2 kDa for expression of the transfected AQP0 indicating the absence of posttranslationally truncation. Membrane proteins from mouse lenses at P10 showed two immunoreactive bands of 28.2 and 25.6 kDa. Adult lens membrane proteins showed multiple polypeptides binding to AQP0 antibody including the two peptide bands of 28.2 and 25.6 kDa, as observed for P10 lenses (Fig. 8). These results demonstrate that end-truncation begins at a very early age in life and is not a senescence-dependent phenomenon.

FIGURE 8. Western blot analysis. Membrane proteins extracted from MDCK cells transfected with mouse intact AQP0 or from the lenses of P10 and 4-month-old adult C57 WT mice were tested. AQP0 C-terminal-specific antibody was used. The C-terminal antibody was raised using an epitope peptide having amino acids 220 to 263. Of note, here, the Ktruncated AQP0 has amino acids up to 246 at the C-terminus.

DISCUSSION

Starting from the outer cortex and leading into the inner nucleus, lens has an array of gradients. Along this gradient, the intracellular pH reads 7.2 to 6.8,61,62 Ca2+ from 300 to 700 nM,63 protein gradient (less to more), water gradient (more to less) membrane proteins like AQP0, and cytosolic proteins like crystallins as intact soluble (more to less), N- and/or C-terminal end-cleaved insoluble forms (less to more), and refractive index gradient (RING: 1.386–1.406 in human).1,5 In accordance with this gradient trend, N- and/or C-terminal ends of AQP0, connexin, and crystallin proteins are progressively cleaved toward the center of the lens and remain there throughout life. The purpose behind these events remained puzzling. In a previous study using an AQP0−/− and a transgenic model that expresses AQP1 in the fiber cells of AQP0−/− mouse (TgAQPl+/−/AQP0−/−), we hypothesized that intact and end-cleaved AQP0 must be playing a crucial role in adjusting the lens RING.9,22 The current study corroborates that by demonstrating distortion aberration in the lenses of the AQP0/C0/C0 mouse model that expresses only cleaved AQP0. Arrangement of intact and cleaved forms in a gradient from the outer cortex to the inner nucleus must be the norm to ensure sharp focusing.

AQP0 is essential for embryonic and postnatal development. This is highlighted in AQP0−/− lens in which loss of lens transparency is fast and severe starting from the early developmental stages (Fig. 5). However, in AQP0/C0/C0 lens cataract development is either absent or slow in the initial developmental stages but is accelerated in the postnatal stages (Fig. 5E); absence of intact AQP0 during these stages, results in cataract that is more severe than in AQP0−/−, suggesting that presence of C-terminal end-cleaved AQP0 in the absence of the intact form could alter lens homeostasis significantly. It can be speculated that alterations in the expression level of intact AQP0 in the differentiating cortical fiber cells and abnormal accelerated N- and C-terminal end-cleavage during senescence could cause lens opacity and cataract due to the loss of homeostasis.

E18 lens showed severe cataract in AQP0−/− whereas the age-matched AQP0/C0/C0 lens was transparent without any aberrations (Fig. 5A). In the AQP0/C0/C0 mouse lens there was gradual development of distortion aberration until P15 followed by cataractogenesis (Figs. 5, 6). Absence of intact AQP0 does not appear to affect lens homeostasis significantly during early developmental stages in the AQP0/C0/C0 lens but is essential during the later stages of postnatal lens development and for transparency. A notable result is the presence of distortion aberration in early postnatal lenses, such as in P5,
even though there was no cataract. Because distortion aberration is a manifestation of alterations in the RING, it can be inferred that albeit the AQP0 WT lens manages to be transparent and prolong cataractogenesis, starting at an early age it is unable to establish and maintain the proper RING and homeostasis in the continually growing lens.

In AQP0WT/C mouse lens, presence of 50% of intact and cleaved forms caused loss of transparency, increased SA, and cataract. Presence of 100% of the cleaved forms (as in a WT lens) and 50% intact AQP0 in the AQP0WT/AC prolonged the onset of cataract. However, absence of intact AQP0 in the ratio as is present in the WT, did create distortion aberration at P15. AQP0WT/MC developed three distinct distortion (aberration) zones. This could be due to the loss of the arrangement of AQP0 and posttranslationally end-cleaved forms in a specific manner from the cortex (more intact AQP0 and less cleaved-AQP0) to the nucleus (less intact AQP0 and more cleaved-AQP0), which is required for lens RING development. Recent studies on a connexin 46 (Cx46) mutant mouse model (Cx46fs380) showed a barrel distortion and a pincushion distortion in the lenses. The phenotypic difference in distortion aberration between AQP0WT/C/AC and Cx46fs380 mouse models could be due to the functional difference of these proteins in the fiber cell membrane. Also, the mutant Cx46 did not traffic to the membrane whereas the C-terminal end-cleaved AQP0 trafficked to the plasma membrane (Fig. 4B). Our results show that intact and cleaved forms of AQP0 in the appropriate ratio in the lens is essential for the establishment and maintenance of RING, transparency, and homeostasis.

The lens grows continuously. Normal end-cleavage of AQP0 is not aging-related (senescence-related) but it is associated with fiber cell maturation process to adjust the lens RING. For example, in early-postnatal young lenses, such as in P10, differentiating fiber cells are more and contain only intact AQP0, the number of which is more than that in the mature fiber cells that carry both intact and cleaved AQP0. The opposite is true in the adult lenses. P10 lenses have less end-cleaved AQP0 due to the small volume of mature fiber cells than adult lenses that contain a large volume of mature fiber cells and more end-cleaved AQP0. The difference in the ratios of intact versus cleaved AQP0 between young and adult is not due to aging but due to the amount of differentiating and mature fiber cells in the lens. Mature adult contains more mature fiber cells in the inner cortex and nucleus and therefore the volume of cleaved forms is more. The 17 amino acid C-terminal end-cleaved AQP0 also showed a similar trend in mature fiber cells in the lens. Mature adult contains more mature fiber cells than adult lenses that contain a large volume of mature fiber cells and more end-cleaved AQP0. The difference in the ratios of intact versus cleaved AQP0 between young and adult is not due to aging but due to the amount of differentiating and mature fiber cells in the lens. Mature adult contains more mature fiber cells in the inner cortex and nucleus and therefore the volume of cleaved forms is more. The 17 amino acid C-terminal end-cleaved AQP0 also showed a similar trend in mature fiber cells in the lens. Mature adult contains more mature fiber cells than adult lenses that contain a large volume of mature fiber cells and more end-cleaved AQP0.

In conclusion, a conducive ratio of intact and C-terminal end-cleaved forms of AQP0 must be maintained in a spatial and temporal distribution manner to maintain lens transparency, RING and homeostasis in the continually growing lens for precise focusing. Until the questions of how the end-cleavages occur in the lens and what are the mechanisms involved are deciphered, developing a reciprocal mouse model with only intact AQP0 in the lens would remain a far-fetched goal.

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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. Biochim Biophys Acta. 2014;1840:2862–2877.


