HHS Public Access

Author manuscript

Biochem Biophys Res Commun. Author manuscript; available in PMC 2019 November 17.

Published in final edited form as:

Biochem Biophys Res Commun. 2018 November 17; 506(1): 284–289. doi:10.1016/j.bbrc.2018.10.066.

Molecular mechanism of Aquaporin 0-induced fiber cell to fiber cell adhesion in the eye lens

Kulandaiappan Varadaraj[1,2,*] and **S. Sindhu Kumari**[1]

[1]Department of Physiology and Biophysics, Stony Brook University, Stony Brook, NY

[2]SUNY Eye Institute, New York, NY

Abstract

Cell-to-cell adhesion (CTCA), which is key for establishing lens transparency, is a critical function of Aquaporin 0 (AQP0). The aim of this investigation was to find out the possible mechanism by which AQP0 exerts CTCA between fiber cells, since there are two proposals currently, either a AQP0-AQP0 interaction or an AQP0-lipid interaction. We studied the mechanism of AQP0-induced CTCA in intact AQP0 and C-terminally cleaved AQP0 (CTC-AQP0) Assays showed CTCA between L-cells transfected with intact AQP0 or CTC-AQP0 and parental L-cells indicating AQP0-membrane interaction. Both forms of AQP0 significantly ($P<0.001$) promoted adhesion to negatively charged L-α-phosphatidylserine lipid vesicles signifying AQP0-lipid interaction. AQP0-expressing L-cells also promoted adhesion of WT and AQP0-KO mouse lens fiber cell membrane vesicles (FCMVs), significantly ($P <0.001$). However, when FCMVs of WT or AQP0-KO were plated over parental L-cells, only WT vesicles adhered significantly, corroborating AQP0-membrane interaction. After incubating with extracellular domain-specific AQP0 antibody, L-cells expressing intact AQP0 or CTC-AQP0 showed significant reduction ($P<0.001$) in the adhesion of AQP0-KO FCMVs indicating extracellular loop involvement in CTCA. WT FCMVs from outer cortex and inner cortex promoted adhesion to parental L-cells, without any statistically significant difference in adhesion efficiency ($P>0.05$). Ultrastructure studies of WT, AQP0-KO and transgenic lenses showed AQP0 is critical for fiber CTCA and compact packing. The data collected clearly demonstrate that the positively charged amino acids in the AQP0 extracellular loop domains interact with the negatively charged lipids in the plasma membrane to promote CTCA for compact packing of the fiber cells.

Keywords

AQP0; cell-to-cell adhesion; extracellular loop positive charges; plasma membrane lipids; electrostatic interaction; cataract

[*]Corresponding author Kulandaiappan Varadaraj, Physiology and Biophysics, School of Medicine, Stony Brook University, Stony Brook, NY 11794-8661; Fax: (631) 444-3442, Phone: (631) 444-7551, kulandaiappan.varadaraj@stonybrook.edu.

1. Introduction

Aquaporin (AQP) channel proteins play important roles in cellular homeostasis; alterations or lack of their expression in mammals lead to pathophysiological conditions. Three AQPs are expressed in the lens, AQP1 and AQP5 in the epithelial cells and AQP0 and AQP5 in the fiber cells. Fiber cell membranes show prolific expression of AQP0 which contributes ~45% [1] of the total membrane proteins in the lens. Mutation and knockout of one or both copies of AQP0 cause bilateral lens cataract and microphthalmia in mice [2,3], highlighting the importance of this protein for normal lens growth and transparency. Initially known only as a water channel protein, the single channel conductivity of AQP0 is 40% and 25% less, respectively, compared to that of the other two lens AQPs, AQP1 and AQP5. Further research on AQP0 recognizes it as multifunctional. Its roles include water permeability [3–5], cell-to-cell adhesion (CTCA; [6,7]), regulation of gap junctions [8–10], and establishment and maintenance of refractive index gradient (RING; [11]) and lens biomechanics [12]. AQP0 is also involved in fiber cell elongation, migration and maintenance of cell shape during differentiation [7,13,14].

AQP0, produced as monomers, assemble into homo-tetramers before trafficking to the plasma membrane. Each AQP0 monomer (Supplementary section, Appendix 1, Fig. S1a), which functions independently, consists of six alpha helical membrane-spanning regions (H1-H6) connected by three extracellular loops (LA, LC and LE) and two intracellular loops (LB and LD). Characteristic highly conserved NPA (Asn-Pro-Ala) motifs are present in LB and LE and they aid in forming the water pore. Carboxy (−COOH) terminus and amino (−NH2) ter minus are cytoplasmic.

To adjust the lens RING and biomechanics, strong CTCA and reduction in extracellular space are necessary. Lens switches AQP expression from the more efficient water channel AQP1 in the epithelial cells to 40 times less-efficient AQP0 in the differentiating fiber cells at the equator. This feature has been attributed as an adaptation to perform, perhaps, the most important function of CTCA, which AQP1 does not promote. AQP0 is synthesized as a 28 kDa protein and its N-terminus and C-terminus are progressively cleaved off in the maturing fiber cells. This is necessary to compactly pack the fiber cells and reduce extracellular space to minimize light scatter as well as to establish lens RING [11,15] and biomechanics [12] for proper image formation.

An ocular lens cross section reveals the unique architecture in fiber cell arrangement [13]. What is the molecular mechanism by which AQP0 exerts CTCA between two opposing fiber cell membranes? While there are a few studies pertaining to this question, the mechanism behind the CTCA is still debated. Lens electron microscopy [16], biochemical vesicular studies [17] and X-ray crystallographic studies [18] suggested that the positive charges of the extracellular loop interact with the negative charges in the plasma membrane to exert CTCA. Another school of thought reasoned it as the interactions between extracellular loop amino acids of the end-cleaved AQP0 present in the opposing fiber cells [19]. We explored the mechanism by which intact AQP0 and CTC-AQP0 (Supplementary section, Appendix 1, Fig. S1a, b) exert CTCA between fiber cells.

2. Materials and methods

2.1. Animals

WT, AQP0 heterozygous (AQP0-Htz), AQP0 knockout (AQPO-KO) as described in [10], and a transgenic model expressing AQP1 in the fiber cells of AQP0-KO mouse (TgAQP1/AQP0 [5,10]) were used (all in C57BL/6J strain). Animal procedures were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, National Institutes of Health's "Guide for the Care and Use of Laboratory Animals" and protocols approved by Stony Brook University Animal Care and Use Committee.

2.2. Generation of stable L-cell clones

Eukaryotic expression constructs of mouse intact AQP0, CTC-AQP0 (1–246 amino acids), AQP1, E-cadherin or empty vector were generated as described [11]. In brief, fibroblast L-cells of mouse (ATCC, Manassas, VA) were transfected with the expression constructs mentioned or empty vector. Stable clones were selected using G418 antibiotic and cloning cylinders.

2.3. Western blotting

Total Membrane proteins were extracted from L-cells expressing intact AQP0, CTC-AQP0 or AQP1, and from lenses of WT and AQP0-KO mice; Western blotting was done using AQP0 extracellular loop antibody, AQP0 C-terminal-specific antibody (Santa Cruz Biotechnology, Inc. Dallas, TX) or AQP1 antibody (Chemicon, Temecula, CA) as described [10–11].

2.4. Immunostaining

L-cells expressing intact AQP0 or CTC-AQP0 were immunostained using AQP0 extracellular loop antibody (Bioworld Technology, Inc., St. Louis Park, MN; details given in Supplementary section, Appendix 1). Lens sections were immunostained with AQP0 C-terminal antibody [10,11].

2.5. CTCA assay using an epifluorescent microscope

L-cells expressing empty vector, AQP1, E-cadherin, intact AQP0 or CTC-AQP0 were loaded with CellTracker green and overlaid on matching cells (without dye) or on parental L-cells, incubated for 1hr, washed, imaged and analyzed [6,11] (details given in Supplementary section, Appendix 1).

2.6. Lipid Vesicle adhesion assay

Unilamellar vesicles were prepared [4] using negatively charged L-α-phosphatidylserine (PS) and neutral phosphatidylcholine (PC) lipids purchased from Avanti Polar Lipids Inc., AL and subjected to adhesion assay (details given in Supplementary Section, Appendix 1).

2.7. Mouse lens fiber cell membrane vesicle adhesion (FCMV) assay

Lens FCMVs were prepared and assayed [3,4] (details given in Supplementary Section, Appendix 1).

2.8. Lens Ultrastructure

Transmission electron microscopy (TEM) of WT, $AQP0^{+/-}$ (AQP0 heterozygous) and $TgAQP1^{+/-}/AQP0^{+/-}$ (heterozygous for transgenic-AQP1 and AQP0) mouse lenses was done as described [7], at the Central Microscopy Imaging Center of Stony Brook University. For WT fiber cell junction characterization, membrane pellets from outer and inner nucleus were prepared and processed for TEM (details given in Supplementary section, Appendix 1).

2.9. Statistical Analysis

Sigma Plot 2000 software Version 6.10 was used to perform the Student's t-tests. P value ≤ 0.05 was considered significant.

3. Results and discussion

CTCA is critical, starting from primary fiber cell formation in the embryonic lens and secondary fiber cell differentiation in the outer cortex to compact packing of the mature fibers in the inner cortex and nucleus. Differentiating fiber cells from lens epithelial cells in the equatorial germinative zone, steadily and precisely migrate anteriorly and posteriorly to elongate and meet with opposing fiber cells at the respective poles to form sutures. Simultaneously, cell-to-cell and cell-to-matrix adherences are established and/or degraded, as appropriate; adhesion proteins and cytoskeletal proteins interact and regulate the adherence. Improper cell migration and elongation could cause anomalies in fiber cell shape and suture formation compromising lens transparency [20–25]. N-cadherin, an adhesion protein of the differentiating fiber cells in the outer cortex, is critical for lens differentiation and maintenance [21]. It is present at reduced levels in the inner cortex and is absent in the lens nucleus. However, inner cortex and nucleus contain connexin gap junctions (16–17 nm; [24,25]) and AQP0 thin junctions (11–13nm; [11,16,26]). During fiber cell differentiation, AQP0 could play a significant role in fiber cell elongation and CTCA in conjunction with other adhesion proteins in the cortex. In the nucleus, AQP0 and connexins could be responsible for fiber CTCA. We investigated the molecular mechanism of AQP0-induced CTCA using parental L-cells, L-cells transfected with and expressing intact AQP0 or CTC-AQP0, vesicles prepared using commercially available pure lipid, fiber cell membrane vesicles (FCMVs) prepared from WT and AQP0-KO mouse lenses, and ultrastructure studies of lenses from mouse models. AQP1, which lacks CTCA capability [6,11,27] and E-cadherin were used to compare and verify the results.

As a first step, we tested the different constructs for their expression in L-cells [11]. Protein expression at the membrane was verified by Western blotting using AQP1 antibody, or AQP0-extracellular loop antibody (Fig. 1A). Immunoreactive bands of ~28 kDa demonstrated the expression of AQP1 or AQP0 and a band of ~26.5 kDa substantiated the presence of CTC-AQP0. WT and AQP0-KO mouse fiber cell membrane proteins immunoblotted using C-terminal-specific AQP0 antibody recognized a band of ~28 kDa

only for the WT (Fig.1B). Immunostaining of L-cells expressing WT-AQP0 (intact AQP0) or CTC-AQP0 using AQP0-extracellular loop antibody confirmed the expression of the respective proteins (Fig.1C). Lens cross-sections of WT and AQP0-KO mice showed immunoreactive green fluorescence for WT only (Fig.1C)

CTCA assay was performed on L-cells stably expressing empty vector, AQP1, intact AQP0 or CTC-AQP0. Compared to the negative controls (empty vector or AQP1), intact AQP0 and CTC-AQP0 exhibited significantly higher ($P<0.001$) CTCA (Fig.2A, B; stars denote significance). Between intact AQP0 and CTC-AQP0, there was no statistically significant difference in CTCA ($P>0.05$). Using electron crystallography analysis, it has been suggested that intact AQP0 functions as an open water channel without facilitating CTCA; end-cleaved AQP0 is closed as a water pore but functions as a CTCA protein [19]. However, the present investigation and previous studies [5–9,11,12] show that both intact and cleaved forms of AQP0 facilitate CTCA.

Next, we examined whether the extracellular loops of one fiber cell could be interacting with the plasma membrane of the opposing cell (AQP0-lipid interaction) or with the extracellular loops of AQP0 present in the opposing fiber cell plasma membrane (AQP0-AQP0 interaction) to exert CTCA. L-cells expressing the same proteins as in Fig. 2B were dye-loaded and overlaid on parental L-cells to test CTCA (Fig. 2C). Quantification of the data showed that CTCA was comparable to that in Fig. 2B, showing AQP0-membrane interaction. When AQP0 is present both in the lawn of cells in the culture dish and in the overlaid cells, there was more efficient CTCA ($P<0.01$) than when AQP0 is present only in the overlaid cells. This indicates a bidirectional interaction i.e., AQP0 present in the culture cells interacting with the plasma membrane of the overlaid cells, and AQP0 from the overlaid cells interacting with the plasma membrane of the cells in the culture dish. This mechanism helps to bring the opposing membranes tightly closer to provide strong CTCA.

Is AQP0 interacting with negatively charged lipids in the plasma membrane or with other membrane protein/s in the parental L-cells? To find out, a lipid vesicle adhesion assay was performed. Quantification bar graph (Fig. 2D) shows the extent of adhesion of the negatively charged PS lipid vesicles. L-cells expressing intact AQP0 or CTC-AQP0 showed significantly higher ($P<0.001$) adhesion compared to L-cells expressing empty vector, AQP1 or E-cadherin, indicating AQP0-lipid interaction. The degree of adhesion exhibited by neutral PC lipid vesicles to L-cells expressing intact AQP0 or CTC-AQP0 was not significant ($P>0.05$) compared to L-cells expressing empty vector, AQP1 or E-cadherin (Fig. 2E). AQP1 and vector served as negative controls. E-cadherin exerts CTCA by the interaction of its extracellular loops present in two opposing plasma membranes [11]. Lack of adhesion by the lipid vesicles to E-cadherin corroborates the results obtained for intact AQP0 and CTC-AQP0. The lipid vesicle studies demonstrate that CTCA of L-cells expressing intact AQP0 or CTC-AQP0 to parental L-cells (Fig.2E) is due to extracellular loop positive charges of AQP0 from one fiber cell interacting with the negatively charged lipids in the adjacent fiber cell plasma membrane. Michea et al. [17] incorporated bovine AQP0 into negatively charged or neutral lipid vesicles, conducted resonance energy transfer and turbidity measurements, and reported that AQP0 could be promoting CTCA by electrostatic interaction of the positively charged amino acids in the extracellular loop, with

apposing cell's negatively charged lipid membrane. Among the thirteen aquaporins in mammals, only AQP0 is present at high levels in the plasma membrane and contains more positively charged amino acids (6) in the extracellular loops (Supplementary section, Appendix 1, Fig. S1), without any negative charge. AQP0 forms tetramer units in the fiber cells and thus each tetramer has 24-positive charges, 6/monomer. These positive charges electrostatically interact with the negative charges of the plasma membrane lipids to exert strong CTCA.

To explore the *in vivo* CTCA mechanism, fiber cell membrane vesicle (FCMV) adhesion assay was employed. CellTracker Green-loaded WT, AQP0-Htz or AQP0-KO [10] mouse lens FCMVs were plated over a monolayer of parental L-cells and incubated for 1hr. After washing, WT FCMVs exhibited significantly higher adhesion ($P<0.001$) to parental L-cells than AQP0-Htz or AQP0-KO FCMVs (Fig.3A). These data show that AQP0 in the WT FCMVs is responsible for the increased level of vesicle adhesion to the parental L-cells. FCMVs from AQP0-Htz, exhibited comparatively less adhesion ($P<0.05$) than those of WT, and significantly more adhesion ($P<0.05$) than those of AQP0-KO, indicating that AQP0-induced CTCA is due to the direct interaction of AQP0 with lipids in the plasma membrane. In another experiment, FCMVs of WT, AQP0-Htz or AQP0-KO were plated over a monolayer of L-cells expressing intact AQP0 (Fig.3B) or CTC-AQP0 (Fig.3C). The tested FCMVs adhered to the L-cells expressing intact AQP0 or CTC-AQP0, with WT adhering significantly higher than AQP0-Htz or AQP0-KO ($P<0.001$). Presence of AQP0 both in the FCMVs and in L-cells significantly increased the number of WT lens FCMV adhesion compared to when AQP0 is present only in the FCMVs, corroborating the conclusion that AQP0 of one fiber cell membrane interacts with the opposing fiber cell membrane.

For establishing lens RING [11] and biomechanics [12] to ensure proper focusing and accommodation, fiber cells should be packed compactly in a gradient manner in the inner cortex and nucleus for which N- and C-terminal end truncations are critical. There is a size-gradient of AQP0 from outer cortex to the center of the lens nucleus. To find out the mechanism of CTCA at the outer cortex that contains intact AQP0 and inner cortex that contains both intact and end-cleaved AQP0 [11,28], we prepared FCMVs of these regions from WT mouse, dye-loaded and overlaid separately on a monolayer of parental L-cells to test for adhesion. Both types of FCMVs exhibited adhesion without a statistically significant difference ($P>0.05$) between them (Fig.4A) showing that intact and cleaved forms of AQP0 promote CTCA by interacting with the plasma membrane.

To verify the involvement of extracellular loop in lens fiber CTCA, FCMVs prepared from AQP0-KO were plated over L-cells expressing intact AQP0 or CTC-AQP0 that were treated with extracellular loop-specific anti-AQP0 to block the interaction of extracellular loops with the plasma membrane. A similar set of L-cells expressing intact AQP0 or CTC-AQP0 without antibody treatment was tested as control. Cells treated with extracellular loop antibody showed significant reduction in FCMV adhesion compared to untreated L-cells (Fig.4B) corroborating the interaction of the extracellular loop domains of AQP0 with fiber cell plasma membrane.

Using transmission electron microscopy (TEM), we analyzed the ultrastructure [5] of mouse lens fiber cells and junctions in WT, $AQP0^{+/-}$ and in $TgAQP1^{+/-}/AQP0^{+/-}$ (Fig.4C, Top). Compared to WT, $AQP0^{+/-}$ with only 50% AQP0 showed disruption of cellular architecture, increase in extracellular space and loss of compact packing of fiber cells. Compensating the lost membrane P_f in the $AQP0^{+/-}$ by transgenic AQP1 in the lens fiber cells [5,7] did not restore the characteristic architecture and compact packing of fibers ($TgAQP1^{+/-}/AQP0^{+/-}$, Fig.4C). AQP0 forms thin junctions between plasma membranes. TEM images of WT lens outer nuclear fiber cell membrane junctions showed more thick junctions formed by other adhesion proteins (darker wavy lines, Fig. 4C, Middle Left) than thin junctions formed by AQP0 (light wavy lines); in contrast, inner nucleus showed more thin junctions than thick junctions (Fig. 4C, Middle Left), a specialization to exert strong CTCA for compact packing of the fiber cells by reducing extracellular space. The composite (Fig.4C, Middle Right) shows thick and thin junctions, from magnified TEM images of different areas of outer nucleus. Quantification bar graph showed the outer nuclear fiber cell membranes having significantly more thick junctions than thin junctions, and *vice versa* in the inner nuclear region of mouse lens (Fig.4C). Formation of thin junctions and square arrays by AQP0 was shown by ultrastructural studies and their involvement in CTCA was speculated [29]. Immunolabeling using AQP0 and connexin 50 antibodies showed AQP0 antibody binding to one side of the membrane and connexin antibody binding to adjacent membranes suggesting that positive charges in the extracellular surface of AQP0 interact with the negative charges of the membrane to form thin adhesion junctions whereas connexin 50 of one fiber cell membrane interacts with connexin 50 of the opposing membrane to form thick junctions [16]. The intercellular space is much narrower (0.5–0.7 nm) in the 11–13 nm thin junctions formed by AQP0 than that (3–5 nm) formed by 16–17nm thick gap junctions formed by connexins.

Gradient, compact and symmetrical packing of fiber cells from outer cortex to inner nucleus is critical for adjusting lens RING and biomechanics for proper accommodation. The current data demonstrate that the positive charges in the extracellular loops of AQP0 interact with the negative charges in the fiber cell plasma membrane lipids to establish CTCA. In the lens fiber cells, the copiously expressed AQP0 acts as a molecular velcro due to the electrostatic attraction between AQP0 and plasma membrane lipids. The abundant expression of AQP0 (~45% of the total membrane protein) is an adaptation to establish tight CTCA to cover ~90% of the protein-occupied membrane surfaces of adjacent mature fiber cells i.e., extracellular loops of AQP0 expressed in one fiber cell plasma membrane interact with the lipids in the adjacent plasma membrane and vice versa to provide strong CTCA. A human AQP0 mutation of a charged amino acid Arginine33 to Cysteine (R33C) in the first extracellular loop resulting in lens cataract [30] and the same mutant AQP0 showing reduced CTCA *in vitro* [9] further validate our conclusion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to Dr. Alan Shiels, Washington University, St. Louis, MO for providing mixed strain AQP0-KO mouse [3]. Funding for this investigation was through NIH-NEI R01 grants EY020506, EY026155 to K. Varadaraj.

Abbreviations:

AQP0	aquaporin 0
CTC-AQP0	C-terminally cleaved AQP0
CTCA	cell-to-cell adhesion
AQP0-Htz (AQP0$^{+/-}$)	AQP0 heterozygous
AQP0-KO	AQP0 knockout
TgAQP1$^{+/-}$/AQP0$^{+/-}$	heterozygous for transgenic-AQP1 and AQP0
P_f	water permeability
FCMV	fiber cell membrane vesicle

References

[1]. Bassnett S, Wilmarth PA, David LL, The membrane proteome of the mouse lens fiber cell, Mol. Vis 15 (2009) 2448–2463. [PubMed: 19956408]

[2]. Okamura T, Miyoshi I, Takahashi K, et al. Bilateral congenital cataracts result from a gain-of-function mutation in the gene for aquaporin-0 in mice, Genomics 81 (2003) 361–368. [PubMed: 12676560]

[3]. Shiels A, Bassnett S, Varadaraj K, et al. Optical dysfunction of the crystalline lens in aquaporin-0-deficient mice, Physiol. Genomics 7 (2001) 179–186. [PubMed: 11773604]

[4]. Varadaraj K, Kushmerick C, Baldo GJ, et al. The role of MIP in lens fiber cell membrane transport, J. Membr. Biol 170 (1999) 191–203. [PubMed: 10441663]

[5]. Varadaraj K, Kumari S, Mathias RT, Transgenic expression of AQP1 in the fiber cells of AQP0 knockout mouse: effects on lens transparency, Exp. Eye Res 91 (2010) 393–404. [PubMed: 20599966]

[6]. Kumari SS, Varadaraj K, Intact AQP0 performs cell-to-cell adhesion, Biochem. Biophys. Res. Commun 390 (2009) 1034–1039. [PubMed: 19857466]

[7]. Kumari SS, Eswaramoorthy S, Mathias RT, et al. Unique and analogous functions of aquaporin 0 for fiber cell architecture and ocular lens transparency, Biochim. Biophys. Acta 181(2011) 1089–1097.

[8]. Liu J, Xu J, Gu S et al. Aquaporin 0 enhances gap junction coupling via its cell adhesion function and interaction with connexin 50, J. Cell Sci, 124 (2011) 198–206. [PubMed: 21172802]

[9]. Kumari SS, Gandhi J, Mustehsan MH et al. Functional characterization of an AQP0 missense mutation, R33C, that causes dominant congenital lens cataract, reveals impaired cell-to-cell adhesion, Exp Eye Res116 (2013) 371–385. [PubMed: 24120416]

[10]. Kumari S, Gao J, Mathias RT et al. Aquaporin 0 modulates lens gap junctions in the presence of lens-specific beaded filament proteins, Invest. Ophthalmol. Vis. Sci 58 (2017) 6006–6019. [PubMed: 29196765]

[11]. 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 1840 (2014a) 2862–2877. [PubMed: 24821012]

[12]. Kumari S, Gupta N, Shiels A et al. Role of Aquaporin 0 in lens biomechanics, Biochem. Biophys. Res. Commun 62 (2015) 339–345.

[13]. Al-Ghoul KJ, Kirk T, Kuszak AJ et al. Lens structure in MIP-deficient mice, Anat. Rec. A Discov. Mol. Cell Evol. Biol 273 (2003) 714–730. [PubMed: 12845708]

[14]. Lo WK, Biswas SK, Brako L et al. Aquaporin-0 targets interlocking domains to control the integrity and transparency of the eye lens, Invest. Ophthalmol. Vis. Sci 55 (2014) 1202–1212. [PubMed: 24458158]

[15]. Kumari SS, Varadaraj K, Aquaporin 0 plays a pivotal role in refractive index gradient development in mammalian eye lens to prevent spherical aberration, Biochem. Biophys. Res. Commun 45 (2014b) 986–991.

[16]. Zampighi GA, Hall HE, Ehring GR et al. The structural organization and protein composition of lens fiber junctions, J. Cell Biol 108 (1989) 2255–2275. [PubMed: 2738093]

[17]. Michea LF, de la Fuente M, Lagos N, Lens major intrinsic protein (MIP) promotes adhesion when reconstituted into large unilamellar liposomes, Biochemistry 33 (1994) 7663–7669. [PubMed: 8011633]

[18]. Harries WE, Akhavan D, Miercke LJ et al. The channel architecture of aquaporin 0 at a 2.2-A resolution, Proc. Natl. Acad. Sci. USA 101 (2004) 14045–14050. [PubMed: 15377788]

[19]. Gonen T, Sliz P, Kistler J et al. Aquaporin-0 membrane junctions reveal the structure of a closed water pore, Nature 429 (2004) 193–197. [PubMed: 15141214]

[20]. Lee A, Fischer RS, Fowler VM, Stabilization and remodeling of the membrane skeleton during lens fiber cell differentiation and maturation, Dev. Dyn 217(2000) 257–270. [PubMed: 10741420]

[21]. Logan CM, Rajakaruna S, Bowen C et al. N-cadherin regulates signaling mechanisms required for lens fiber cell elongation and lens morphogenesis, Dev. Biol 428(2017) 118–134. [PubMed: 28552735]

[22]. Logan CM, Bowen CJ, Menko AS, Functional role for stable microtubules in lens fiber cell elongation, Exp. Cell Res 362(2018) 477–488. [PubMed: 29253534]

[23]. Audette DS, Scheiblin DA, Duncan MK, The molecular mechanisms underlying lens fiber elongation, Exp. Eye Res 156 (2017) 41–49. [PubMed: 27015931]

[24]. White TW, Unique and redundant connexin contributions to lens development, Science 295 (2002) 319–320. [PubMed: 11786642]

[25]. Xia CH, Cheng C, Huang Q et al. Absence of alpha3 (Cx46) and alpha8 (Cx50) connexins leads to cataracts by affecting lens inner fiber cells, Exp. Eye Res 83 (2006) 688–696. [PubMed: 16696970]

[26]. Scheuring S, Buzhynskyy N, Jaroslawski S et al. Structural models of the supramolecular organization of AQP0 and connexons in junctional microdomains, J. Struct. Biol 160 (2007) 385–394. [PubMed: 17869130]

[27]. Hiroaki Y, Tani K, Kamegawa A et al. Implications of the aquaporin-4 structure on array formation and cell adhesion, J. Mol. Biol 355 (2006) 628–639. [PubMed: 16325200]

[28]. Ball LE, Garland DL, Crouch RK et al. Post-translational modifications of aquaporin 0 (AQP0) in the normal human lens: spatial and temporal occurrence, Biochemistry 43 (2004) 9856–9865. [PubMed: 15274640]

[29]. Zampighi GA, Eskandari S, Hall JE et al. Micro-domains of AQP0 in lens equatorial fibers, Exp. Eye Res 75 (2002)505–519. [PubMed: 12457863]

[30]. Gu F, Zhai H, Li D, Zhao L et al. A novel mutation in major intrinsic protein of the lens gene (MIP) underlies autosomal dominant cataract in a Chinese family. Mol. Vis 13, 1651 (2007) e1656.

Highlights

- Intact and C-terminally cleaved AQP0 perform cell-to-cell adhesion
- Extracellular loop positively charged amino acids partake in cell-to-cell adhesion
- Negatively charged plasma membrane lipids partake in cell-to-cell adhesion

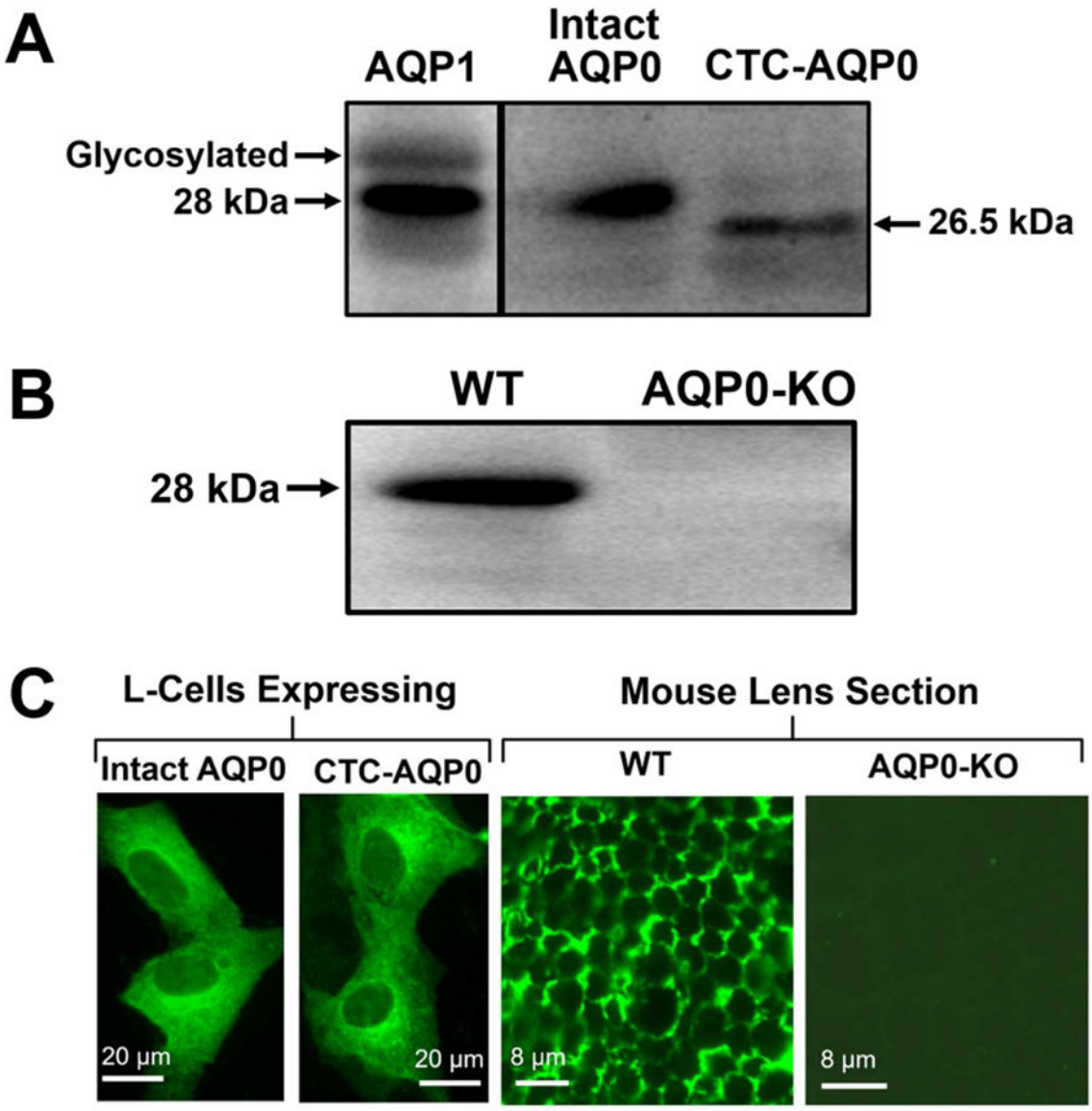

Fig. 1.

Western blotting and immunostaining. **A.** Aquaporin expression in L-cells transfected with AQP1, intact AQP0 or CTC-AQP0 as revealed by binding of AQP1 antibody or AQP0 extracellular loop antibody; **B.** Binding of C-terminal-specific AQP0 antibody by fiber cell membrane proteins in WT and lack of antibody binding in AQP0-KO mouse. **C.** AQP0 extracellular loop antibody binding to L-cells expressing intact AQP0 or CTC-AQP0; AQP0 C-terminal antibody binding (green) in WT lens section and lack of binding in AQP0-KO lens section.

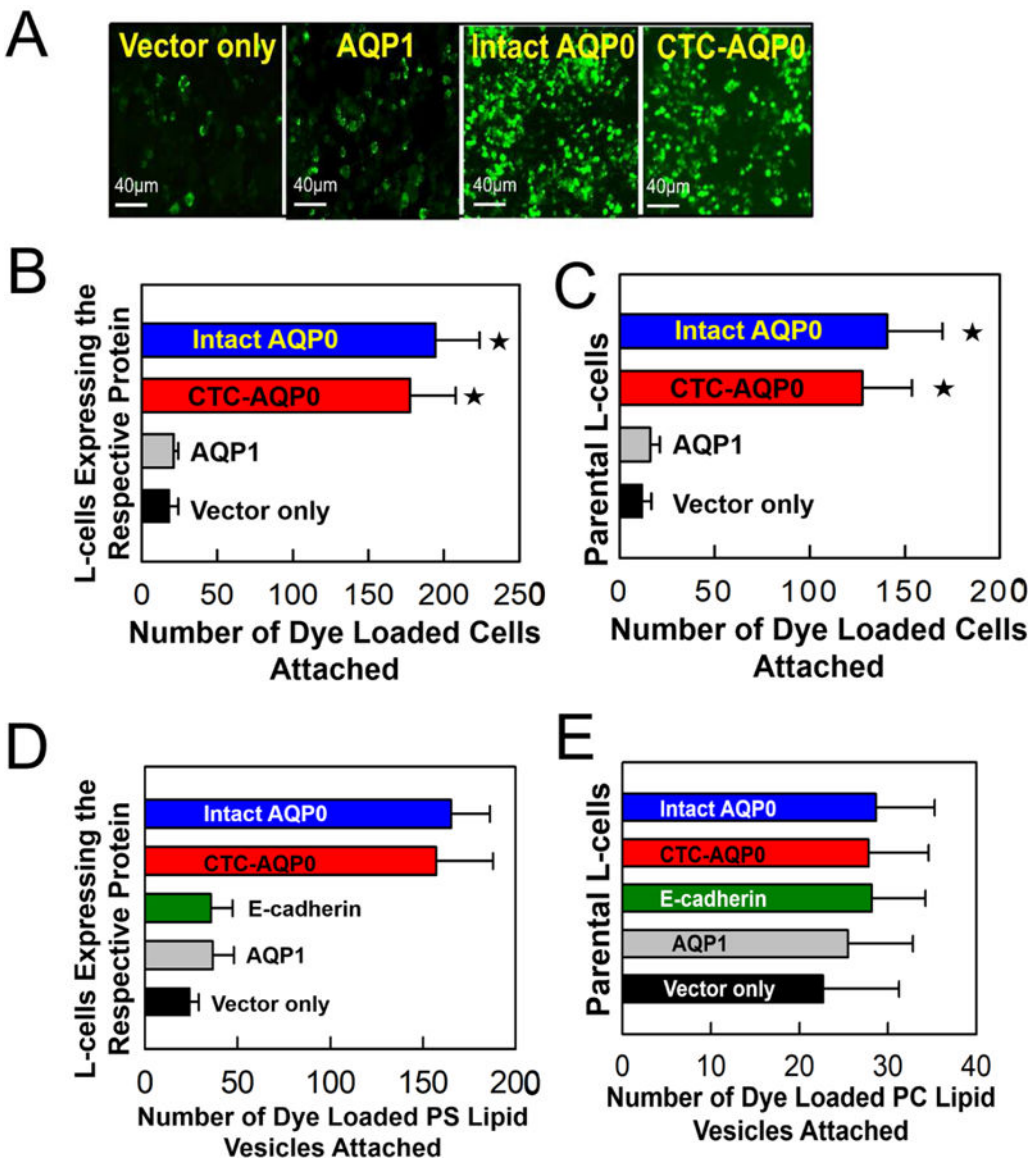

Fig. 2.

Adhesion assay using an epifluorescent microscope. A. L-cells expressing empty vector, AQP1, intact AQP0 or CTC-AQP0 were loaded with CellTracker green and overlaid on matching cells without dye and incubated for 1hr. Green fluorescence indicates cell-to-cell adhesion (CTCA); **B.** Adhesion assay quantification. Intact AQP0 and CTC-AQP0 exhibited significant adhesion compared to vector or AQP1; **C.** CTCA exhibited by CellTracker green loaded L-cells expressing the respective protein, when plated over parental L-cells; **D.** Bar graph showing positively charged PS lipid vesicles exhibiting significant adhesion to L-cells expressing intact AQP0 or CTC-AQP0 compared to L-cells expressing empty vector, AQP1 or E-cadherin; **E.** Bar graph showing no significant adhesion of neutral PC lipid vesicles to L-cells expressing intact AQP0 or CTC-AQP0 compared to empty vector, AQP1 or E-cadherin.

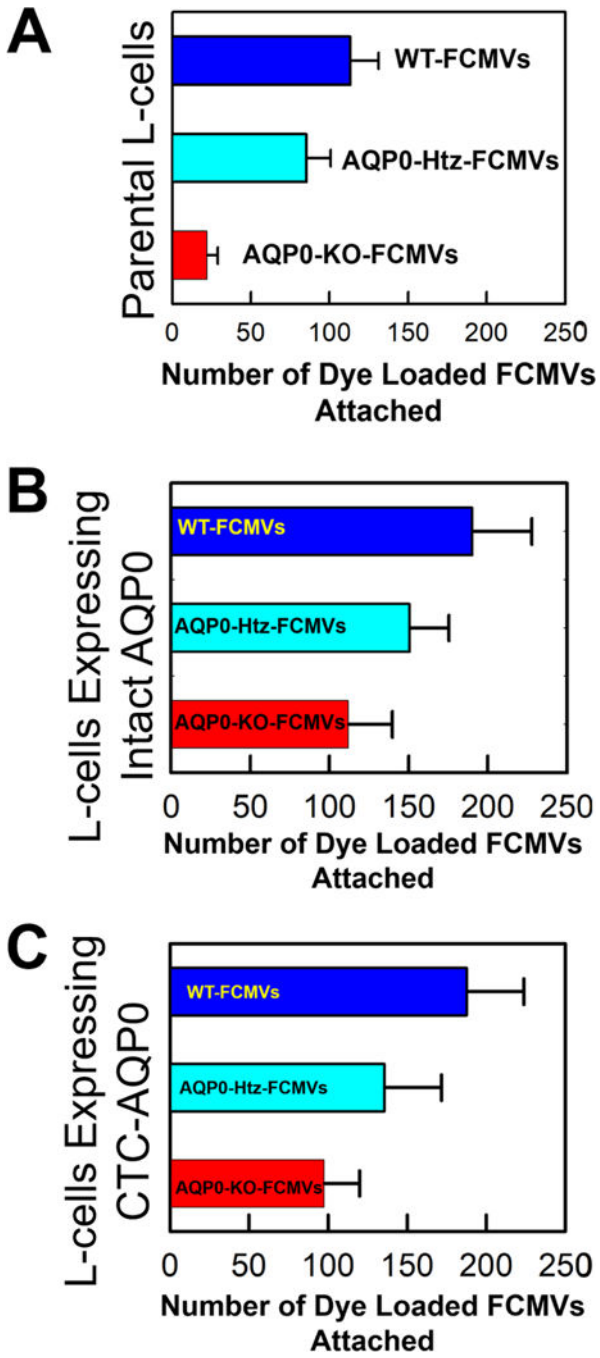

Fig. 3.

Lens fiber cell membrane vesicle (FCMV) adhesion assay. Adhesion of WT, AQP0-heterozygous (AQP0-Htz) or AQP0-KO mouse lens FCMVs to a monolayer of parental L-cells (**A**), L-cells expressing intact AQP0 (**B**) or L-cells expressing CTC-AQP0 (**C**).

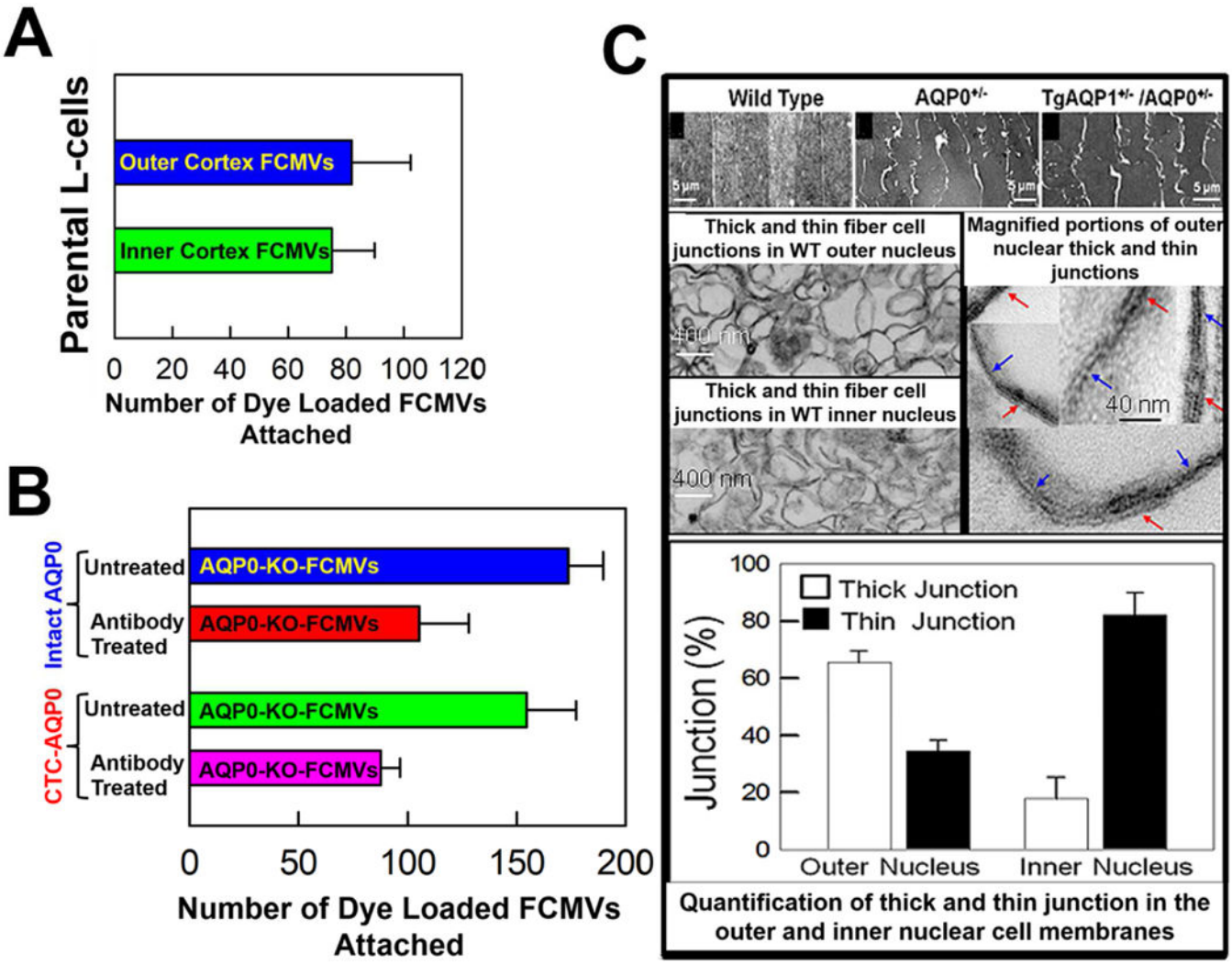

Fig. 4.

A. Adhesion of WT lens FCMVs from outer cortex or inner cortex to parental L-cells; **B.** Adhesion of lens AQP0-KO FCMVs to L-cells expressing intact AQP0 or CTC-AQP0 treated or not treated with AQP0 extracellular loop antibody; **C.** Transmission electron microscopy. Lenses of 2-month old mice were sectioned along polar axis. Top: WT, showing tightly packed fiber cells. $AQP0^{+/-}$ (AQP0 heterozygous), showing disruption of fiber cell arrangement TgAQP1+/−/AQP0+/− (heterozygous for transgenic-AQP1 and AQP0) lens section showing loss of fiber cell compactness even after restoring membrane Pf by transgene AQP1. Middle Left: Distribution of thick and thin fiber cell junctions in WT lens outer nucleus. Distribution of thick and thin fiber cell junctions in WT lens inner nucleus. Middle Right: Magnified portions of outer nuclear fiber thick junctions (darkly stained, red arrows) and thin junctions (lightly stained, blue arrows). Bottom: Quantification of thick and thin fiber cell junction distribution in the outer and inner nuclear fiber cell membranes.