PKC putative phosphorylation site Ser$^{235}$ is required for MIP/AQP0 translocation to the plasma membrane

Nady Golestaneh, Jianguo Fan, Peggy Zelenka, Ana B. Chepelinsky

Laboratory of Molecular and Developmental Biology, National Eye Institute, NIH, Bethesda, MD

Purpose: To investigate the functional significance of MIP/AQP0 phosphorylation at serine$^{235}$.

Methods: MIP/AQP0 expression and cellular localization was studied in rat lens epithelia explants induced to differentiate by FGF-2. MIP wild type (WT) and MIP (S235A) mutant expression plasmids were constructed and transiently expressed in RK13 cells. Subcellular localization of endogenous MIP in differentiating lens epithelia explants or of transfected MIP expression vectors in RK13 cells was analyzed by immunofluorescence confocal microscopy.

Results: MIP/AQP0 expressed in lens epithelia explants induced to differentiate by FGF-2 localizes to the plasma membrane of elongating cells. However, MIP/AQP0 translocation to the plasma membrane was prevented by inhibiting PKC activity with Go6976, resulting in retention in the cytoplasmic compartment. This effect was specific to MIP/AQP0; localization of AQP1 to the cell membrane was not affected by Go6976. When the consensus PKC phosphorylation site at MIP Ser$^{235}$ was mutated to alanine and transiently expressed in transfected RK13 cells, the mutant MIP was retained in the cytoplasmic compartment in contrast to WT MIP that localized to the plasma membrane of the transfected RK13 cells. Colocalization studies indicated that the mutant MIP was retained in the trans-Golgi network.

Conclusions: Our results indicate that serine$^{235}$ is required for proper intracellular transport of MIP/AQP0 from the trans-Golgi network to the plasma membrane. A PKC dependent phosphorylation event involving MIP at serine$^{235}$ is most likely involved in this process.

MIP/AQP0 is the major intrinsic protein of the ocular lens. It is specifically expressed in the lens fibers. Mutations in the MIP/AQP0 gene result in genetic cataracts in mice and humans [1-11]. In the last decade, great advances have been made in understanding the function of this protein, which is also known as “the founder of the MIP or aquaporin gene family.” Initially it was considered to be a gap junction protein. Even though it was later found not to be a member of the connexin gene family, it was demonstrated that MIP interacts transiently with some connexins and appears to be involved in gap junction formation [12-14]. It functions as a water channel when tested in various functional assays [2, 15-23]. However, MIP/AQP0 has also been demonstrated to have additional functions such as acting as an adhesion molecule [24,25], forming thin junctions [26-29], and playing a role in the correct formation of sutures in the ocular lens [30-33]. In this way, MIP/AQP0 contributes to the minimal intercellular space between the lens fibers and suture formation required for optimal focusing and transparency of the lens [7,8,22,30]. The MIP COOH-terminal domain interacts with other lens proteins such as gamma crystallins [34,35], filensin, CP49 [36], and connexins [13]. Posttranslational modifications of MIP such as proteolysis [26,37,38] and phosphorylation [15,36-41] may play a role in regulating the various functions that MIP is able to play in the lens for maintaining lens transparency.

However, regulation of the function of MIP/AQP0 by signaling pathways during lens differentiation is not understood as well. We have previously demonstrated that the ERK and JNK signaling pathways are involved in the regulation of expression of the MIP gene during the induction of lens epithelia differentiation by FGF-2 [42]. In this study, we demonstrate that although the PKC signaling pathway does not regulate MIP transcription, it does play an essential role in the trafficking of MIP/AQP0 from the Golgi apparatus to the plasma membrane to be able to perform its physiologic function in the ocular lens.

METHODS

Chemicals and reagents: Turbo Pfu DNA polymerase was obtained from Stratagene (La Jolla, CA). The Polymerase Chain Reaction (PCR) Purification Kit and The Plasmid Midi and Maxi Kits were purchased from Qiagen (Valencia, CA). PKC inhibitor, Go6976, was obtained from Calbiochem (San Diego, CA). Dimethylsulfoxide (DMSO) and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) were obtained from Sigma-Aldrich (St. Louis, MO). Alexa 555-streptavidin and Alexa 488-streptavidin were from Molecular...
Probes (Eugene, OR). All other chemicals were reagent grade and from standard commercial sources.

Antibodies: The MIP-specific rabbit polyclonal antiserum was obtained from Alpha Diagnostic (San Antonio, TX). Antibodies to Aquaporin 1 (mouse monoclonal), trans-Golgi network marker 38K (mouse monoclonal TGN38), goat anti-rabbit biotinylated, and anti-mouse biotinylated were purchased from Abcam (Cambridge, MA).

Plasmids: All plasmid DNAs used were propagated in E. coli strain, DH5α or DH10B, and were purified by ion exchange chromatography using Plasmid Midi Kits or Endonuclease-Free Maxi Kits from Qiagen (Valencia, CA).

Plasmid constructions: The plasmid, pCMVScript, was purchased from Stratagene. The expression vector for wild type mouse MIP (pCMV-MIP) was constructed as indicated before [35]. The expression vector for Ala235 mutant MIP (pCMV-MIP Ala235) was constructed by site-directed mutagenesis of MIP in pCMV-MIP, which was accomplished by use of Quick Change II Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer’s instructions. The introduced mutation Ala235 was verified by DNA sequencing.

DNA sequencing: DNA sequencing of plasmid constructions was performed using a commercial system (CEQ DTCS-Quick Start Kit; Beckman-Coulter, Hialeah, FL) and an automated DNA analysis system (CEQ 2000XL; Beckman-Coulter) according to the manufacturer’s instructions.

Lens epithelia explant culture: Eyes were dissected from three-day-old Sprague Dawley rats (Charles River Laboratories, Wilmington, MA). The lens capsule with adhering epithelial cells was micro-dissected from the lens fibers and pinned down around its periphery with forceps onto the surface of either a 35 mm or 60 mm culture dish in Medium 199. The resulting lens epithelia explants were pre-incubated for 1 h in Medium 199 containing either 0.2% DMSO (control) or PKC inhibitor (4 μM Go6976%–0.2% DMSO) and from ion exchange chromatography using Plasmid Midi Kits or Endonuclease-Free Maxi Kits from Qiagen (Valencia, CA).

Confocal immunofluorescence: The lens explants and the transfected RK13 cell line were fixed in 4% formaldehyde in isotonic PBS (pH 7.4) buffer for 2 h at room temperature. Cellular localization of MIP, aquaporin 1, or TGN 38K either in the transfected cells or in the explants was examined by indirect immunofluorescence labeling. Brieﬂy, the explants or the cells were incubated in ICC buffer (PBS containing 0.20% Tween 20, 0.05% sodium azide, and 0.5% BSA, pH

Molecular Vision 2008; 14:1006-1014 <http://www.molvis.org/molvis/v14/a120> © 2008 Molecular Vision
7.3) for 20 min at room temperature. The rat lens explants were then incubated in ICC buffer with rabbit polyclonal anti-MIP (1:50) and mouse monoclonal anti-aquaporin 1 (1:50) for 1 h at 37 °C. The RK13 cells were incubated with rabbit polyclonal anti-MIP (1:50) and mouse monoclonal anti-TGN 38K (1:50) for 1 h at 37 °C. The explants or the cells were washed in ICC buffer followed by incubation for 1 h in ICC buffer containing fluorescent dye-conjugated secondary antibodies (goat anti-rabbit biotinylated and Alexa 555-streptavidin or goat-mouse biotinylated and Alexa 488-streptavidin) along with either DAPI or SYTOX green dye. Extensive washing was performed after each incubation step. After washing, the samples were mounted with TBS/glycerol (1:1) containing p-phenylenediamine (Sigma-Aldrich, St. Louis, MO) and covered by a coverslip. Samples were stored at 4 °C until they were imaged by confocal fluorescence microscopy.

The stained and mounted cells in 60 mm culture dishes were imaged using a Leica TCS SP2 confocal microscope (Leica Microsystems, Exton, PA) with a Leica 40X HCX Plan Apo CS (0.85 NA) and a Leica 40X HCX Plan Oil objective lens.

RESULTS

PKC inhibitor does not affect MIP transcription in rat lens explants: We have previously shown that induction of rat lens epithelia explant differentiation into lens fibers by FGF-2 involves the activation of transcription of the MIP/AQP0 gene through the activation of the FGF downstream signaling components, ERK and JNK [42].

To examine whether PKC signaling was also involved in activating MIP transcription during lens epithelia differentiation induced by FGF-2, we investigated whether the synthetic inhibitor to PKC, Go6976 [44,45], would affect MIP mRNA levels. We quantitated the MIP mRNA amounts in the untreated control and PKC inhibitor-treated explants using real-time PCR analyses. As shown in Figure 1, no significant change in MIP transcript levels was observed in the control and PKC inhibitor-treated explants. These results imply that unlike ERK and JNK, PKC signaling (at least through isoenzymes α, γ, and μ [44,45]) does not contribute to MIP transcription.

PKC inhibitor affects MIP plasma membrane localization in rat lens explants: Our results show that PKC is not involved in regulating MIP at the level of transcription. As PKC has been reported to have a possible role in MIP phosphorylation [46], we set up to investigate the role of PKC as a potential contributor to the subcellular localization of MIP. Thus, we studied the effect of the PKC inhibitor, Go6976, in the localization of expressed MIP in rat lens epithelia explant cultures induced to differentiate by FGF-2. As we previously demonstrated [42], MIP expressed in rat epithelia explants and induced to differentiate by FGF-2 localizes to the plasma membrane of elongating cells that characterize the first stages of lens epithelia differentiation as shown in Figure 2A,C and animation 1. However, when the PKC inhibitor, Go6976, is present during FGF-2-induced lens epithelia explant differentiation, MIP immunofluorescence shows that cells in explants treated with the PKC inhibitor retained MIP in the cytoplasmic compartment (Figure 2B,D and animation 2). These results implied the importance of PKC isoenzymes (α, γ, and/ or μ [44,45]) in MIP targeting to the plasma membrane in differentiating lens cells.

Inhibition of PKC perturbs MIP/AQP0 translocation to the plasma membrane but does not affect AQP1 localization to the plasma membrane of rat lens explants: To distinguish whether the inhibition of PKC isoenzymes by Go6976 [44,45] specifically affects the localization of MIP to the plasma membrane or if it is the result of a general effect on membrane proteins due to other pathways being affected in the differentiating lens epithelia cells, we also looked at the possible effect of the PKC inhibitor Go6976 on the localization of AQP1. AQP1 is expressed in the anterior and equatorial lens epithelia. As the lens epithelial cells start differentiating into lens fibers, AQP1 expression is turned off and MIP/AQP0 expression is turned on [22]. The induction of lens epithelia differentiation by FGF-2 into lens fibers in the rat explant system allows us to study the expression of both proteins during this transition period. As shown in Figure 3A,B, both MIP/AQP0 and AQP1 are expressed and localized to the plasma membrane in the lens explant cells. When the explants are incubated in the presence of Go6976, MIP/AQP0 accumulates in the cytoplasmic compartment (Figure 3D).
whereas AQP1 localization into the cell plasma membrane is not affected (Figure 3E). These results clearly demonstrate that PKC isoenzymes inhibited by Go6976 (α, γ, and/or μ [44,45]) affect only MIP/AQP0.

Mutation of PKC putative phosphorylation site (Ser\textsuperscript{235}) prevents MIP targeting to the cell plasma membrane: It has been reported that PKC is involved in the phosphorylation of MIP [46]. We identified MIP Ser\textsuperscript{235} as a potential PKC phosphorylation site in the MIP protein by analyzing it with the Scansite program [47]. Serine\textsuperscript{235} coincides with the major phosphorylated site identified in MIP in vivo in the lens of several species [38,39,41]. Based on this identification, the putative PKC phosphorylation site, Ser\textsuperscript{235}, was mutated to Ala\textsuperscript{235} in the MIP cDNA. As we have previously demonstrated that MIP transiently expressed in RK13 cells localizes to the plasma membrane [35], we investigated whether the Ala\textsuperscript{235} mutation of MIP Ser\textsuperscript{235} would affect the localization to the plasma membrane when expressed in these cells. Therefore, RK13 cells were transfected with either the WT or mutant MIP cDNA expression vectors. Immunofluorescence of the transfected cells was performed to identify the localization pattern of the expressed MIP. In cells transfected with WT MIP cDNA, the translated MIP protein was localized to the cell plasma membrane (Figure 4A,E). However, in cells expressing the MIP-Ala\textsuperscript{235}, a cytoplasmic retention of the protein was evident; the bulk of the mutant protein shows a punctate expression in the intracellular compartment and no localization in the plasma membrane (see Figure 4J,N). These results show that mutation of a single PKC phosphorylation site in the MIP protein prevents its targeting to the plasma membrane.

Membrane proteins synthesized in the endoplasmic reticulum undergo proper folding and oligomerization before being routed through the Golgi apparatus and the Golgi vesicles to their final destination in the plasma membrane. Therefore, we investigated whether the WT and mutant MIP expressed in the transfected RK 13 cells were differentially processed in subcellular compartments such as trans-Golgi vesicles. We used an antibody to the trans-Golgi network (TGN) marker 38K along with the MIP antibody to determine...
whether they colocalized. The 38K TGN marker, an integral protein predominantly localized to the TGN, is also a component of the TGN-derived vesicles in route to the plasma membrane and can be observed at the plasma membrane. WT MIP colocalizes with the TGN vesicles in the plasma membrane as shown in Figure 4I (peaks indicated with blue lines) and Figure 4D (right peak indicated with blue line); it also shows colocalization with the trans-Golgi network in the cytoplasmic compartment in route to the plasma membrane (Figure 4; yellow pattern in Figure 4C and Figure 4G; left peak indicated with blue line in Figure 4D). In contrast, in cells expressing the MIP mutant Ala235, MIP is absent from the plasma membrane, showing a punctate distribution (Figure 4J,N). There is colocalization with the trans-Golgi network only in the cytoplasmic compartment (Figure 4; yellow pattern in Figure 4L and Figure 4P) besides localization in other cytoplasmic vesicles. There is no colocalization of MIP mutant Ala235 with the TGN vesicles at the cell plasma membrane (Figure 4M,Q). These results demonstrate that the PKC putative phosphorylation site Ser235 plays a prominent role in the process of MIP translocation from the TGN to the plasma membrane by the TGN vesicles.

**DISCUSSION**

The PKC pathway is one of the major signal transduction pathways that regulate a multitude of biologic functions. Most, if not all, membrane proteins continuously shuttle between several organelles along microtubules and actin cytoskeleton [48,49]. The dynamic equilibrium of this protein subcellular transport is regulated by several factors that include the activity of protein kinases and phosphatases; PKC is one of the protein kinases implicated in this trafficking [50-53]. Several members of the PKC family are expressed in the lens [54-56]. The role of the PKC γ isozyme on the lens gap junction function has been well documented [54-63], and PKC α isozyme may regulate the interaction of tropomodulin with cytoskeletal components in the lens [64].

PKC may be a possible contributor to MIP phosphorylation [46], and phosphorylated MIP is differentially distributed in the lens cortex and nucleus of the human lens [37]. In this study, we examined the role of active PKC on MIP expression and subcellular localization in differentiating lens cells. Our real-time PCR results which showed that the level of MIP transcripts in rat lens explants were unaffected by a PKC inhibitor to isozymes α, γ, and μ indicated that these PKC isozymes are not required for MIP gene expression. However, we found that active PKC isozymes (α, γ, and/or μ) play an important role in MIP translocation to the plasma membrane. PKC inhibition prevents MIP integration in the plasma membrane, resulting in retention in the cytoplasmic compartment of lens epithelia
Figure 4. Mutation of PKC putative phosphorylation site (Ser\(^{235}\)) prevents MIP translocation from the trans-Golgi network to the plasma membrane. Immunofluorescence of RK13 cells transfected with pCMV-MIP (WT; A-I) or pCMV-MIP Ala\(^{235}\) (S235A; J-Q) for 72 h is shown. A and E as well as J and N show the merged images of MIP red immunofluorescence with the corresponding images of their DAPI nuclear staining. B and F as well as K and O show the trans-Golgi network marker 38K (TGN) green immunofluorescence. C, G, L, and P show the merged images of MIP immunofluorescence and DAPI nuclear staining with their respective TGN green immunofluorescence (A and B; E and F, J and K; N and O, respectively). Spatial quantification was performed along a path across the plasma membrane, indicated by a white line with prominent end points in the merged images (C, G, L, and P). Red and green fluorescence was quantified separately and plotted as a function of distance along the path (D, I, M, and Q). Blue broken lines in the spatial quantification graphs indicate the approximate location of the plasma membrane (except the left line in D, which corresponds to the TGN region in C). Note that WT MIP and TGN vesicles colocalize at the plasma membrane (peaks are indicated with blue lines in I and right peak in D). MIP Ala\(^{235}\) mutant (S235A) does not colocalize with TGN vesicles at the plasma membrane (blue lines; M and Q). C and G show colocalization (yellow) of WT MIP (red immunofluorescence) and TGN 38K (green immunofluorescence) in the cytoplasmic compartment in addition to the localization of WT MIP in the plasma membrane. J and N show MIP Ala\(^{235}\) mutant punctate distribution in the cytosolic compartment (red immunofluorescence) and colocalization (yellow) with trans-Golgi network 38K (green) in L and P. A and E as well as J and N show cell images from either duplicate experiments or in different fields of the same cell culture of WT or MIP Ala\(^{235}\) mutant (S235A), respectively. Scale bars represent 10 μm. Note that three cells in A, B, and C (one cell at the right side and two cells in the upper part of the panels) that did not uptake the transfected MIP expression plasmid served as negative controls. They show no red immunofluorescence in contrast to two transfected cells showing the red immunofluorescence. All the cells in the panel (transfected and non-transfected) show green immunofluorescence for TGN38.
explants that were induced to differentiate by FGF-2. This effect was specific for MIP/AQP0; AQP1 membrane localization in the lens explants was not affected by the inhibitor to PKC isoenzymes α, γ, and μ.

We then focused on MIP serine\textsuperscript{235}, a putative PKC phosphorylation site that has also been identified as a major MIP phosphorylation site in rat, bovine, and human lens [38, 39,41]. Our results showed that mutation of MIP serine\textsuperscript{235} to alanine\textsuperscript{235} prevents translocation of MIP-Ala\textsuperscript{235} mutant from the trans-Golgi network to the plasma membrane of transfected RK13 cells. The additional punctate distribution of the mutant MIP in the cytoplasmic compartment probably represents its targeting from the trans-Golgi to the degradation pathways (i.e., lysosomal vesicles).

Natural mutations in MIP, either deletions or substitutions, have been linked to genetic cataracts with a dominant phenotype in mice and humans [1-11]. Some of those MIP mutants with mutations involving transmembrane domains are retained in the endoplasmic reticulum [5,6,8,9]. As MIP assembles as a tetramer [26,28], these MIP mutations prevent oligomerization and correct folding required for MIP transport from the endoplasmic reticulum to the Golgi apparatus. Our present results, which indicate that phosphorylation of MIP at serine\textsuperscript{235} is required for proper MIP translocation from the trans-Golgi network to the plasma membrane, reveal a novel control point in the MIP trafficking possibly serving as a sorting signal. PKC-dependent serine phosphorylation has also been identified as Golgi sorting domains that are involved in the trans-Golgi network to the plasma membrane of transfected RK13 cells. The additional punctate distribution of the mutant MIP in the cytoplasmic compartment probably represents its targeting from the trans-Golgi to the degradation pathways (i.e., lysosomal vesicles).

MIP/AQP0 serine\textsuperscript{235} is located in the COOH-terminal domain, which is involved in interactions with other proteins in the lens such as gamma crystallins [34,35], filensin, CP49 [36], connexins [13], and calmodulin [71]. Phosphorylation of MIP COOH-terminal domain reduces its affinity for calmodulin [71], known to regulate water channel activity in functional assays [20,21,71]. Thus, phosphorylation of MIP/AQP0 at Ser\textsuperscript{235} may be involved in regulating its functions once integrated in the plasma membrane. We have now demonstrated in this study that MIP phosphorylation at Ser\textsuperscript{235} is required for correct trafficking to the plasma membrane; therefore, this phosphorylation event plays a primary role in MIP function by being required for proper targeting to the plasma membrane. Once MIP/AQP0 is correctly integrated in the plasma membrane, it becomes enabled to accomplish its functions in the lens, such as water channel, adhesion molecule and/or other possible functions required for the optical properties of the normal lens.

REFERENCES
1. Francis P, Berry V, Bhattacharya S, Moore A. Congenital progressive polymorphic cataract caused by a mutation in the major intrinsic protein of the lens, MIP (AQP0). Br J Ophthalmol 2000; 84:1376-9. [PMID: 11090476]
2. Francis P, Chung JJ, Yasui M, Berry V, Moore A, Wyatt MK, Wistow G, Bhattacharya SS, Agre P. Functional impairment of lens aquaporin in two families with dominantly inherited cataracts. Hum Mol Genet 2000; 9:2329-34. [PMID: 11001937]
3. Geyer DD, Spence MA, Johannes M, Flodman P, Clancy KP, Berry R, Sparkes RS, Jonsen MD, Isenberg SJ, Bateman JB. Novel single-base deletional mutation in major intrinsic protein (MIP) in autosomal dominant cataract. Am J Ophthalmol 2006; 141:761-3. [PMID: 16564824]
4. Lin H, Hejtmanek JF, Qi Y. A substitution of arginine to lysine at the COOH-terminus of MIP caused a different binocular phenotype in a congenital cataract family. Mol Vis 2007; 13:1822-7. [PMID: 17960133]
5. Okamura T, Miyoshi I, Takahashi K, Mototani Y, Ishigaki S, Kon Y, Kasai N. Bilateral congenital cataracts result from a gain-of-function mutation in the gene for aquaporin-0 in mice. Genomics 2003; 81:361-8. [PMID: 12676560]
6. Shiel A, Bassnett S. Mutations in the founder of the MIP gene family underlie cataract development in the mouse. Nat Genet 1996; 12:212-5. [PMID: 8563764]
7. Shiel A, Bassnett S, Varadaraj K, Mathias R, Al-Ghoul K, Kuszak J, Donoviel D, Lilleberg S, Friedrich G, Zambrowicz B. Optical dysfunction of the crystalline lens in aquaporin-0-deficient mice. Physiol Genomics 2001; 7:179-86. [PMID: 11773604]
8. Shiel A, Mackay D, Bassnett S, Al-Ghoul K, Kuszak J. Disruption of lens fiber cell architecture in mice expressing a chimeric AQP0-LTR protein. FASEB J 2000; 14:2207-12. [PMID: 11053241]
9. Sidjanin DJ, Parker-Wilson DM, Neuhauser-Klaus A, Pretsch W, Favor J, Deen PM. Ohtaka-MaruyamaC, Lu Y, Bragin A, Skach WR, Chepelinsky AB, Grimes PA, Stambolian DE. A 76-bp deletion in the Mip gene causes autosomal dominant cataract in Hfi mice. Genomics 2001; 74:313-9. [PMID: 11414759]
10. Gu F, Zhai H, Li D, Zhao L, Li C, Huang S, Ma X. A novel mutation in major intrinsic protein of the lens gene (MIP) underlies autosomal dominant cataract in a Chinese family. Mol Vis 2007; 13:1651-6. [PMID: 17893667]
11. Berry V, Francis P, Kaushal S, Moore A, Bhattacharya S. Missense mutations in MIP underlie autosomal dominant 'polymorphic' and lamellar cataracts linked to 12q. Nat Genet 2000; 25:15-7. [PMID: 10802646]
12. Yu XS, Jiang JX. Interaction of major intrinsic protein (aquaporin-0) with fiber connexins in lens development. J Cell Sci 2004; 117:871-80. [PMID: 14762116]
13. Yu XS, Yin X, Lafer EM, Jiang JX. Developmental regulation of the direct interaction between the intracellular loop of connexin 45.6 and the C-terminus of major intrinsic protein (aquaporin-0). J Biol Chem 2005; 280:22081-90. [PMID: 15802270]
14. Gruijters WT. A non-connexon protein (MIP) is involved in eye lens gap-junction formation. J Cell Sci 1989; 93:509-13. [PMID: 2691517]
15. Ball LE, Little M, Nowak MW, Garland DL, Crouch RK, Schey KL. Water permeability of C-terminally truncated aquaporin 0 (AQP0 1–243) observed in the aging human lens. Invest Ophthalmol Vis Sci 2003; 44:4820-8. [PMID: 14578404]
16. Chandy G, Zampighi GA, Kreman M, Hall JE. Comparison of the water transporting properties of MIP and AQP1. J Membr Biol 1997; 159:29-39. [PMID: 9309208]
17. Kalman K, Nemeth-Cahalan KL, Froger A, Hall JE. AQP0 -connexin 45.6 and the C-terminus of major intrinsic protein (aquaporin-0) with fiber connexins in lens development. J Cell Sci 1989; 93:509-13. [PMID: 1375651]
18. Nemeth-Cahalan KL, Hall JE. pH and calcium regulate the water permeability of aquaporin 0. J Biol Chem 2000; 275:6777-82. [PMID: 10702234]
19. Nemeth-Cahalan KL, Kalman K, Froger A, Hall JE. Zinc modulation of water permeability reveals that aquaporin 0 functions as a cooperative tetramer. J Gen Physiol 2007; 130:457-64. [PMID: 17938229]
20. Nemeth-Cahalan KL, Kalman K, Hall JE. Molecular basis of pH and Ca2+ regulation of aquaporin water permeability. J Gen Physiol 2004; 123:573-80. [PMID: 15078916]
21. Varadaraj K, Kumari S, Shiehs A, Mathias RT. Regulation of aquaporin water permeability in the lens. Invest Ophthalmol Vis Sci 2005; 46:1393-402. [PMID: 15790907]
22. Varadaraj K, Kumari SS, Mathias RT. Functional expression of aquaporins in embryonic, postnatal, and adult mouse lenses. Dev Dyn 2007; 236:1319-28. [PMID: 17577981]
23. Varadaraj K, Kushmerick C, Baldo GJ, Bassnasset S, Shiehs A, Mathias RT. The role of MIP in lens fiber cell membrane transport. J Membr Biol 1999; 170:191-203. [PMID: 10441663]
24. Michea LF, Andrinolo D, Ceppi H, Lagos N. Biochemical evidence for adhesion-promoting role of major intrinsic protein isolated from both normal and cataractous human lenses. Exp Eye Res 1995; 61:293-301. [PMID: 7556493]
25. Michea LF, de la Fuente M, Lagos N. Lens major intrinsic protein (MIP) promotes adhesion when reconstituted into large unilamellar liposomes. Biochemistry 1994; 33:7663-9. [PMID: 8011633]
26. Gonen T, Cheng Y, Kistler J, Walz T. Aquaporin-0 membrane junctions form upon proteolytic cleavage. J Mol Biol 2004; 342:1337-45. [PMID: 15351655]
27. Gonen T, Cheng Y, Sliz P, Hiroaki Y, Fujiyoshi Y, Harrison SC, Walz T. Lipid-protein interactions in double-layered two-dimensional AQP0 crystals. Nature 2005; 438:633-8. [PMID: 16319884]
28. Gonen T, Sliz P, Kistler J, Cheng Y, Walz T. Aquaporin-0 membrane junctions reveal the structure of a closed water pore. Nature 2004; 429:193-7. [PMID: 15141214]

12. Yu XS, Jiang JX. Interaction of major intrinsic protein (aquaporin-0) with fiber connexins in lens development. J Cell Sci 2004; 117:871-80. [PMID: 14762116]
13. Yu XS, Yin X, Lafer EM, Jiang JX. Developmental regulation of the direct interaction between the intracellular loop of connexin 45.6 and the C-terms of major intrinsic protein (aquaporin-0). J Biol Chem 2005; 280:22081-90. [PMID: 15802270]
14. Gruijters WT. A non-connexon protein (MIP) is involved in eye lens gap-junction formation. J Cell Sci 1989; 93:509-13. [PMID: 2691517]
15. Ball LE, Little M, Nowak MW, Garland DL, Crouch RK, Schey KL. Water permeability of C-terminally truncated aquaporin 0 (AQP0 1–243) observed in the aging human lens. Invest Ophthalmol Vis Sci 2003; 44:4820-8. [PMID: 14578404]
16. Chandy G, Zampighi GA, Kreman M, Hall JE. Comparison of the water transporting properties of MIP and AQP1. J Membr Biol 1997; 159:29-39. [PMID: 9309208]
17. Kalman K, Nemeth-Cahalan KL, Froger A, Hall JE. AQP0 -connexin 45.6 and the C-terminus of major intrinsic protein (aquaporin-0) with fiber connexins in lens development. J Cell Sci 1989; 93:509-13. [PMID: 1375651]
18. Nemeth-Cahalan KL, Hall JE. pH and calcium regulate the water permeability of aquaporin 0. J Biol Chem 2000; 275:6777-82. [PMID: 10702234]
19. Nemeth-Cahalan KL, Kalman K, Froger A, Hall JE. Zinc modulation of water permeability reveals that aquaporin 0 functions as a cooperative tetramer. J Gen Physiol 2007; 130:457-64. [PMID: 17938229]
20. Nemeth-Cahalan KL, Kalman K, Hall JE. Molecular basis of pH and Ca2+ regulation of aquaporin water permeability. J Gen Physiol 2004; 123:573-80. [PMID: 15078916]
21. Varadaraj K, Kumari S, Shiehs A, Mathias RT. Regulation of aquaporin water permeability in the lens. Invest Ophthalmol Vis Sci 2005; 46:1393-402. [PMID: 15790907]
22. Varadaraj K, Kumari SS, Mathias RT. Functional expression of aquaporins in embryonic, postnatal, and adult mouse lenses. Dev Dyn 2007; 236:1319-28. [PMID: 17577981]
23. Varadaraj K, Kushmerick C, Baldo GJ, Bassnasset S, Shiehs A, Mathias RT. The role of MIP in lens fiber cell membrane transport. J Membr Biol 1999; 170:191-203. [PMID: 10441663]
24. Michea LF, Andrinolo D, Ceppi H, Lagos N. Biochemical evidence for adhesion-promoting role of major intrinsic protein isolated from both normal and cataractous human lenses. Exp Eye Res 1995; 61:293-301. [PMID: 7556493]
25. Michea LF, de la Fuente M, Lagos N. Lens major intrinsic protein (MIP) promotes adhesion when reconstituted into large unilamellar liposomes. Biochemistry 1994; 33:7663-9. [PMID: 8011633]
26. Gonen T, Cheng Y, Kistler J, Walz T. Aquaporin-0 membrane junctions form upon proteolytic cleavage. J Mol Biol 2004; 342:1337-45. [PMID: 15351655]
27. Gonen T, Cheng Y, Sliz P, Hiroaki Y, Fujiyoshi Y, Harrison SC, Walz T. Lipid-protein interactions in double-layered two-dimensional AQP0 crystals. Nature 2005; 438:633-8. [PMID: 16319884]
28. Gonen T, Sliz P, Kistler J, Cheng Y, Walz T. Aquaporin-0 membrane junctions reveal the structure of a closed water pore. Nature 2004; 429:193-7. [PMID: 15141214]
inhibition of protein kinase C isoforms by the indolocarbazole Go 6976. J Biol Chem 1993; 268:9194-7. [PMID: 8486620]

45. Gischwendt M, Dieterich S, Rennecke J, Kittstein W, Mueller HJ, Johannes FJ. Inhibition of protein kinase C mu by various inhibitors. Differentiation from protein kinase C isoenzymes. FEBS Lett 1996; 392:77-80. [PMID: 8772178]

46. Lampe PD, Johnson RG. Phosphorylation of MP26, a lens junction protein, is enhanced by activators of protein kinase C. J Membr Biol 1989; 107:145-55. [PMID: 2541249]

47. Obenauer JC, Cantley LC, Yaffe MB. Scansite 2.0: Proteome-wide prediction of cell signaling interactions using short sequence motifs. Nucleic Acids Res 2003; 31:3635-41. [PMID: 12824383]

48. Ghosh RN, Mallet WG, Soe TT, McGraw TE, Maxfield FR. An endocytosed TGN38 chimeric protein is delivered to the TGN after trafficking through the endocytic recycling compartment in CHO cells. J Cell Biol 1998; 142:923-36. [PMID: 9722606]

49. Rohn WM, Rouille Y, Waguri S, Hoflack B. Bi-directional trafficking between the trans-Golgi network and the endosomal/lysosomal system. J Cell Sci 2000; 113:2093-101. [PMID: 10825282]

50. Sabatini DD, Adesnik M, Ivanov IE, Simon JP. Mechanism of formation of post Golgi vesicles from TGN membranes: Arf-dependent coat assembly and PKC-regulated vesicle scission. Biocell 1996; 20:287-300. [PMID: 9031596]

51. Bao J, Alroy I, Waterman H, Schejter ED, Brodie C, Gruenberg J, Yarden Y. Threonine phosphorylation diverts internalized epidermal growth factor receptors from a degradative pathway to the recycling endosome. J Biol Chem 2000; 275:26178-86. [PMID: 10816576]

52. Molloy SS, Anderson ED, Jean F, Thomas G. Bi-cycling the furin pathway: from TGN localization to pathogen activation and embryogenesis. Trends Cell Biol 1999; 9:28-35. [PMID: 10087614]

53. Zehavi-Feferman R, Burgess JW, Stanley KK. Control of p62 binding to TGN38/41 by phosphorylation. FEBS Lett 1995; 368:122-4. [PMID: 7615064]

54. Wagner LM, Takemoto DJ. PKCalpha and PKCgamma overexpression causes lentoid body formation in the N/N 1003A rabbit lens epithelial cell line. Mol Vis 2001; 7:138-44. [PMID: 11436000]

55. Berthoud VM, Westphale EM, Grigoryeva A, Beyer EC. PKC isoenzymes in the chicken lens and TPA-induced effects on intercellular communication. Invest Ophthalmol Vis Sci 2000; 41:850-8. [PMID: 10711703]

56. Saleh SM, Takemoto LJ, Zoukhi D, Takemoto DJ. PKC-gamma phosphorylation of connexin 46 in the lens cortex. Mol Vis 2001; 7:240-6. [PMID: 11702063]

57. Lampe PD, TenBroek EM, Burt JM, Kurata WE, Johnson RG, Lau AF. Phosphorylation of connexin43 on serine368 by protein kinase C regulates gap junctional communication. J Cell Biol 2000; 149:1503-12. [PMID: 10871288]

58. Lin D, Boyle DL, Takemoto DJ. IGF-I-induced phosphorylation of connexin 43 by PKCgamma: regulation of gap junctions in rabbit lens epithelial cells. Invest Ophthalmol Vis Sci 2003; 44:1160-8. [PMID: 12601045]

59. Lin D, Lobell S, Jewell A, Takemoto DJ. Differential phosphorylation of connexin46 and connexin50 by H2O2 activation of protein kinase Cgamma. Mol Vis 2004; 10:688-95. [PMID: 15467523]

60. Lin D, Shanks D, Prakash O, Takemoto DJ. Protein kinase C gamma mutations in the C1B domain cause caspase-3-linked apoptosis in lens epithelial cells through gap junctions. Exp Eye Res 2007; 85:113-22. [PMID: 17493614]

61. Lin D, Zhou J, Zelenka PS, Takemoto DJ. Protein kinase Cgamma regulation of gap junction activity through caveolin-1-containing lipid rafts. Invest Ophthalmol Vis Sci 2003; 44:5259-68. [PMID: 14638725]

62. Lurtz MM, Louis CF. Calmodulin and protein kinase C regulate gap junctional coupling in lens epithelial cells. Am J Physiol Cell Physiol 2003; 285:C1475-82. [PMID: 12917107]

63. Zampighi GA, Planells AM, Lin D, Takemoto D. Regulation of lens cell-to-cell communication by activation of PKCgamma and disassembly of Cx50 channels. Invest Ophthalmol Vis Sci 2005; 46:3247-55. [PMID: 16123426]

64. Wagner LM, Fowler VM, Takemoto DJ. The interaction and phosphorylation of tropomodulin by protein kinase Calpha in N/N 1003A lens epithelial cells. Mol Vis 2002; 8:394-406. [PMID: 12419997]

65. Scott DB, Blanpied TA, Ehlers MD. Coordinated PKA and PKC phosphorylation suppresses RXR-mediated ER retention and regulates the surface delivery of NMDA receptors. Neuropharmacology 2003; 45:755-67. [PMID: 14529714]

66. Scott DB, Blanpied TA, Swanson GT, Zhang C, Ehlers MD. An NMDA receptor ER retention signal regulated by phosphorylation and alternative splicing. J Neurosci 2001; 21:3063-72. [PMID: 11312291]

67. van Tiel CM, Westerman J, Paasman MA, Hoebens MM, Wirtz KW, Snoek GT. The Golgi localization of phosphatidylinositol transfer protein beta requires the protein kinase C-dependent phosphorylation of serine 262 and is essential for maintaining plasma membrane sphingomyelin levels. J Biol Chem 2002; 277:22447-52. [PMID: 11953429]

68. Lan JY, Skeberdis VA, Jover T, Grooms SY, Lin Y, Araenda RC, Zheng X, Bennett MV, Zukan RS. Protein kinase C modulates NMDA receptor trafficking and gating. Nat Neurosci 2001; 4:382-90. [PMID: 11276228]

69. Haussler A, Storz P, Martens S, Link G, Toker A, Pfizenmaier K. Protein kinase D regulates vesicular transport by phosphorylating and activating phosphatidylinositol-4 kinase IIbeta at the Golgi complex. Nat Cell Biol 2005; 7:880-6. [PMID: 16100512]

70. Yeaman C, Ayala MI, Wright JR, Bard F, Bossard C, Ang A, Maeda Y, Seufferlein T, Mellman I, Nelson WJ, Malhotra V. Protein kinase D regulates basolateral membrane protein exit from trans-Golgi network. Nat Cell Biol 2004; 6:106-12. [PMID: 14743217]

71. Rose KM, Wang Z, Magrath GN, Hazard ES, Hildebrandt JD, Schey KL. Aquaporin 0-calmodulin interaction and the effect of aquaporin 0 phosphorylation. Biochemistry 2008; 47:339-47. [PMID: 18081321]