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Aquaporins and CFTR in Ocular Epithelial Fluid Transport

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Abstract. Aquaporins (AQPs) and the cystic fibrosis transmembrane conductance regulator (CFTR) provide the molecular routes for transport of water and chloride, respectively, through many epithelial tissues. In ocular epithelia, fluid transport generally involves secondary active chloride transport, which creates the osmotic gradient to drive transepithelial water transport. This review is focused on the role of AQPs and CFTR in water and ion transport across corneal/conjunctival epithelia, corneal endothelium, ciliary epithelium, and retinal pigment epithelium. The potential relevance of water and chloride transport to common disorders of ocular fluid balance is also considered. Recent data suggest AQPs and CFTR as attractive targets for drug development for therapy of keratoconjunctivitis sicca, recurrent corneal erosions, corneal edema, glaucoma, retinal detachment, and retinal ischemia.

Key words: Water channel — AQP — Chloride channel — CFTR — Eye — Epithelium

Introduction

The eye contains specialized fluid compartments and tissues that are avascular and low in protein so as to meet the needs of optical transparency, as in the cornea, and to maintain a suitable ionic environment for neural signal transduction, as in the retina. Regulated transfer of fluid and metabolites between extravascular spaces and adjacent tissues or the systemic circulation supports these highly specialized functions (reviewed by Hamann, 2002). It is now appreciated that Cl^- secretion provides a primary driving force for active, near-isosmolar water transport across the principal ocular epithelia: corneal/conjunctival epithelia, corneal 'endothelium', ciliary epithelium, and retinal pigment epithelium. HCO_3^- transport often plays a crucial, though indirect role in promoting Cl^- uptake across membranous barriers. AQPs and CFTR have been identified as the principal molecular pathways for water and chloride transport, respectively, across a variety of epithelial tissues. The roles of these channels in mammalian physiology have been elucidated from phenotype analysis of transgenic mice and/or humans with absent or mutated channels, and in the case of CFTR, from the use of small-molecule inhibitors.

AQP-type water channels make up a family of small (~30 kDa) proteins with six alpha-helical membrane-spanning domains that facilitate bidirectional osmotic water transport across cell plasma membranes (reviewed by Agre & Kozono, 2003; Verkman, 2005). A cell membrane osmotic water permeability coefficient of greater than ~0.01 cm/s generally indicates the presence of AQP water channels. Analysis of AQP knock-out mice has established the involvement of AQPs in extraocular epithelia in osmotically driven and near-isosmolar fluid transport, as in the urinary concentrating mechanism and in glandular fluid secretion (Verkman, 2005). Additionally, AQPs are involved in a growing list of less predictable epithelial cell functions, including cell migration and proliferation (Hara-Chikuma & Verkman, 2006; Levin & Verkman, 2005a). This review examines the roles of AQPs in ocular epithelial fluid absorption and secretion, with consideration of common human disorders of ocular fluid balance, such as dry eye syndrome, corneal swelling, and glaucoma.

CFTR, one of a growing list of Cl^- channels (reviewed by Jentsch et al., 2002), is expressed in many fluid-transporting epithelia in the airways, pancreas, and intestine (reviewed by Pilewsky & Frizzel, 1999; Sheppard & Welsh 1999). CFTR is a large (~180 kDa) cAMP-stimulated Cl^- channel

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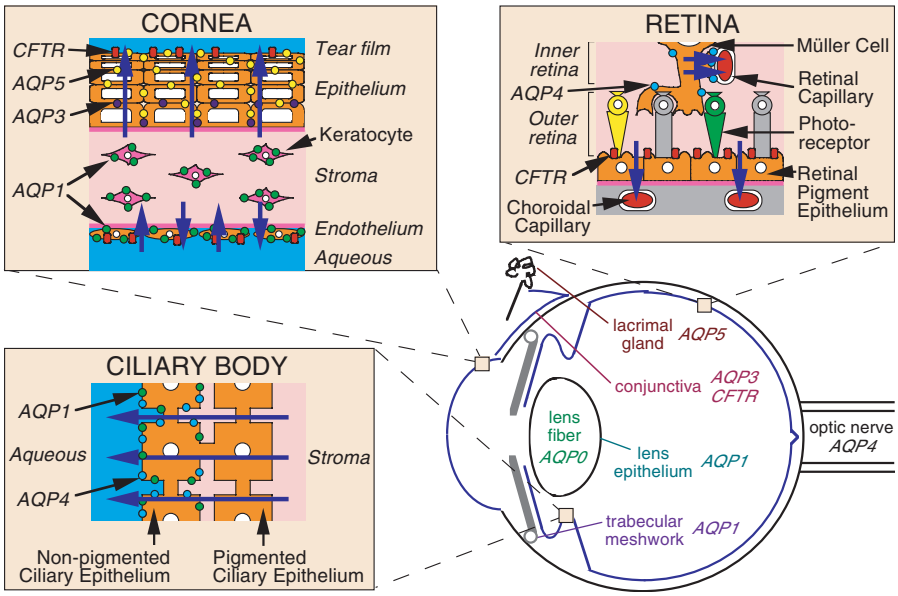

Fig. 1. AQP- and CFTR-dependent fluid transport across ocular epithelia. (*Top left*) Water balance in the corneal stroma, showing passive influx through the leaky endothelium and active efflux across endothelium and epithelium. (*Bottom left*) Aqueous fluid secretion across non-pigmented ciliary epithelium, involving transfer of water and solutes from pigmented ciliary epithelium via gap junctions. (*Top right*) Modulation of outer retinal fluid by retinal pigment epithelium and inner retinal fluid by astroglial Müller cells. (*Bottom right*) Locations of confirmed AQP- and CFTR-facilitated transport indicated. *See* text for explanations.

comprised of 12 transmembrane domains, and two cytoplasm-facing nucleotide-binding domains and a regulatory domain. CFTR is the only member of the ATP-binding-cassette (ABC) transporter superfamily known to conduct ions. Mutations in CFTR cause cystic fibrosis (CF), a relatively common life-shortening human genetic disease. Though CF is not commonly associated with overt eye diseases such as keratoconjunctivitis sicca (KCS, or dry eye), corneal edema, or retinal detachment, various subtle derangements have been reported in the eyes of CF subjects, including altered ocular surface hydration, corneal thickness, and visual transduction (Sheppard et al., 1989; Morkeberg et al., 1995; Ansari et al., 1999; Mrugacz & Minarowska, 2002). However, though reduced CFTR function produces only minor effects on ocular functions, increasing CFTR activity could be useful in the treatment of several important eye diseases, as discussed below.

AQP and CFTR Expression in Ocular Epithelia

Figure 1 depicts the sites of AQP and CFTR expression in the eye. The AQP/CFTR expression patterns in corneal/conjunctival epithelium, corneal endothelium, ciliary epithelium, and retinal pigment epithelium suggest their involvement in regulation of tear film volume, corneal hydration and transparency, aqueous fluid volume and intraocular pressure, and subretinal compartment size and ionic composition. Indeed, the functional significance of various AQPs in ocular tissues has been confirmed in AQP-deficient mice, including AQP1 in corneal endothelium and ciliary epithelium, AQP3 in conjunctival and corneal epithelium, AQP4 in ciliary epithelium and retinal Müller cells, and AQP5 in corneal epithelium (Zhang, Vetrivel & Verkman, 2002; Li, Patil & Verkman, 2002; Thiagarajah & Verkman, 2002; Levin & Verkman, 2004, 2005a; Da & Verkman, 2004). Also, recent studies have reported altered AQP expression in association with various human corneal disease states associated with fluid imbalance (Kenney et al., 2004; Macnamara et al., 2004; Rabinowitz et al., 2005). CFTR is functionally expressed in corneal and conjunctival epithelium, corneal endothelium, and retinal pigment epithelium (Turner, Bernstein & Candia, 2002; Shiue et al., 2002; Sun & Bonanno, 2002; Blaug et al., 2003; Levin & Verkman, 2005b; Reigada & Mitchell, 2005).

Ocular Surface Epithelia

The ocular surface is lined by stratified corneal and conjunctival epithelia, which lie in contact with the tear film. The water permeability of the ocular surface, together with the rates of evaporative water loss and tear fluid production and drainage, determine tear film volume and osmolality (Mathers et al., 1996), as well as corneal stromal water content (Mandell & Fatt, 1965). Active Cl^- secretion and

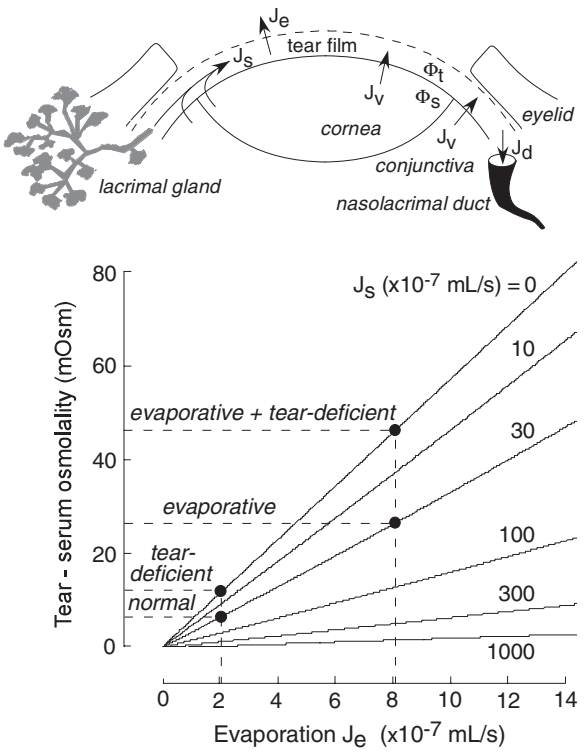

Fig. 2. Theoretical role of ocular surface water permeability in tear film dynamics. (*Top*) Schematic of ocular surface geometry and contributors of tear fluid balance. J_e, rate of evaporation at the exposed corneal surface; J_s, rate of fluid secretion by the lacrimal gland and ocular surface epithelia; J_v, osmotic volume flow across the corneal and conjunctival surfaces; J_d, tear fluid removal by nasolacrimal drainage; ϕ_t and ϕ_s, osmolarities of tear film and surface tissue, respectively. (*Bottom*) Theoretical dependence of tear film osmolarity on tear evaporation and secretion rates, computed from the model and plotted as the extent of hyperosmolarity ($\phi_t - \phi_s$). Adapted from Levin & Verkman (2004).

Na^+ absorption determine net water secretion into tears across both corneal and conjunctival epithelia (reviewed by Dartt, 2002; Candia, 2004). The ocular surface, and the conjunctival epithelium in particular (covering 17 times more area than the cornea in humans; Watsky, Jablonski & Edelhauser, 1988), contributes to active tear fluid secretion under basal conditions, and even more so upon stimulation (Murakami et al., 2000; Yang et al., 2000). Keratoconjunctivitis sicca is a heterogeneous group of conditions with common features of reduced tear volume and tear hyperosmolarity, leading to inflammatory damage to the ocular surface (Lemp, 1995). A computational model of tear film balance demonstrated the sensitivity of tear film osmolarity to both excessive tear evaporation and inadequate tear secretion, the two general causes of dry eye syndrome (Fig. 2; Levin & Verkman; 2004). In this model, tear fluid generated by osmotic flux (J_v) and active isosmolar secretion (J_s) is removed by evaporation (J_e) and isotonic drainage (J_d), such that in the steady-state, $J_s + J_v = J_e + J_d$. Computed tear film osmolarity depended strongly on both passive water permeation and active fluid secretion, which are influenced by AQPs and CFTR, respectively.

AQPs and the Ocular Surface

The stratified corneal epithelium of mouse, rat, and human expresses a water-selective aquaporin, AQP5, and at relatively lower levels, a water- and glycerol-transporting aquaglyceroporin, AQP3. AQP3 is more highly expressed throughout the neighboring conjunctival epithelium (Patil et al., 1997; Hamann et al., 1998; Levin & Verkman, 2004, 2005a). Kang et al. (1999) demonstrated mercurial-sensitive water transport in cultured bovine corneal epithelial cells, which was attributed to AQP5 based on siRNA knockdown. Subsequent studies in AQP5-null mice revealed that AQP5-deficient corneas were substantially thicker than wild-type corneas by ~20 %, exhibiting both inter- and intraepithelial fluid accumulation at the ultrastructural level (Fig. 3*A*; Thiagarajah & Verkman, 2002; Levin & Verkman, 2005a). Functional measurements on living mice demonstrated AQP5 to be a significant epithelial pathway for stromal water uptake and extrusion. The rate of corneal swelling upon exposure of the epithelial surface to hypotonic saline was reduced 2-fold in AQP5-null compared to wild-type mice. Osmotically induced net fluid flux across the intact corneal barrier, measured by a steady-state 'dye-dilution' method, indicated a 5-fold slowing of transcorneal water movement in AQP5 deficiency.

Plasma membrane osmotic water permeability of corneal epithelial cells (P_f) was determined in mice utilizing an ocular surface perfusion method (Fig. 3*B*) involving microfluorimetric measurement of calcein quenching in surface cells. The high P_f (0.045 cm/s) measured in wild-type mice was in agreement with conclusions from the older literature that the cornea is highly water permeable (Mishima & Hedbys, 1967; Fischbarg & Motoreano, 1982). P_f was reduced 2-fold in AQP5 deficiency (Fig. 3*C*). The modest dependence of P_f on AQP5 was likely accounted for by the ~5-fold up-regulation of AQP3 in AQP5-null versus wild-type mouse corneas (Levin & Verkman, 2005a). Yet despite this compensation, AQP5-null corneas are grossly edematous, highlighting the exquisite sensitivity of corneal water balance to altered water permeation across multiple membrane barriers in series.

Recent studies have suggested a novel role for AQP3 in basal cells of the corneal epithelium that is unrelated to transepithelial corneal fluid transport (Levin & Verkman, 2006). AQP3-null mice had ~2-fold decreased P_f compared to that of wild-type mice, and (>10-fold) reduced glycerol transport. AQP3 deletion significantly delayed re-epithelialization following removal of the corneal epithelium by

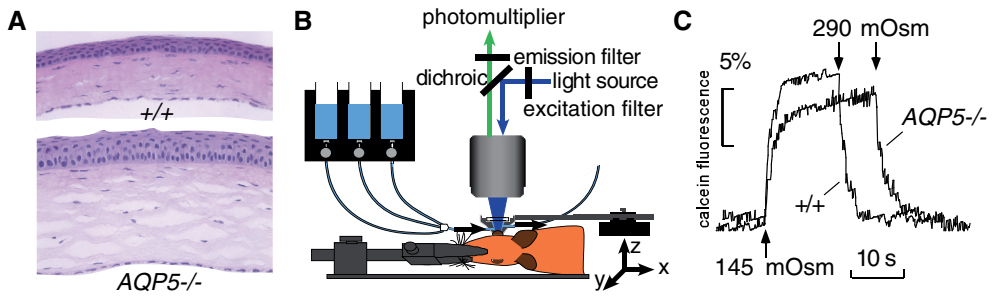

Fig. 3. AQP5 function at the ocular surface. (*A*) Increased stromal and epithelial thicknesses in AQP5-deficiency in paraffin-embedded central corneal sections. (*B*) Schematic of ocular surface perfusion for fluorescence measurements of cell volume changes. The instrumentation consisted of a microchamber positioned on the corneal surface, optical elements for calcein fluorescence measurement, rapid exchange perfusion system, and stereotaxic platform. (*C*) Representative time courses of corneal epithelial cell calcein fluorescence in response to hyposmolar osmotic gradient in wild-type and AQP5-deficient mice. Adapted from Levin & Verkman (2004).

scraping. Tissue and cell culture studies suggested the involvement of AQP3 in corneal epithelial cell migration and proliferation, though the cellular mechanisms linking these functions to AQP3-dependent water and/or glycerol transport require further investigation.

CFTR and the Ocular Surface

Cl^--dependent fluid secretion has been found across ocular surface epithelia in several species. Electrophysiological measurements on rabbit cornea and conjunctiva, as well as in cultured cells, have demonstrated calcium- and cAMP-sensitive outward apical Cl^- currents, the latter likely due to activation of CFTR and other cAMP-sensitive channels, such as basolateral membrane K^+ channels that establish the electrochemical driving force for Cl^- efflux (Klyce, Neufeld & Zadunaisky, 1973; Klyce & Wong, 1977; Wolosin & Candia, 1987; Kompella, Kim & Lee, 1993; Turner, Alvarez & Candia, 2000; Al-Nakkash & Reinach, 2002; Shiue et al., 2002). CFTR is expressed in apical superficial cell membranes of corneal and conjunctival epithelia (Turner, Bernstein & Candia, 2002; Shiue et al., 2002), where it may participate in tear film homeostasis. Studies on CF subjects have shown mild ocular surface abnormalities suggestive of defective fluid secretion, including decreased tear production, increased corneal fluorescein staining, corneal and conjunctival metaplasia, and reduced tear film Na^+ content (Botelho, Goldstein & Rosenlund, 1973; Morkeberg et al., 1995; Ansari et al., 1999).

In recent experiments from our laboratory, CFTR function at the mouse ocular surface was demonstrated directly using an open-circuit potential difference (PD) technique, which involved perfusion of solutions over the ocular surface of anesthetized and immobilized mice (Fig. 4*A*; Levin & Verkman, 2005b). Electrogenic Cl^- secretion and Na^+ absorption across superficial cell apical membranes of corneal and conjunctival epithelia contribute to generate sizable steady-state transepithelial potentials (−23 mV on average, tear film with respect to the body), as measured with Ag/AgCl electrodes and a high-impedance voltmeter. These voltages were sensitive to imposed transepithelial Cl^- gradients and various Cl^- channel modulators, including CFTR activators and inhibitors. Comparative measurements were made on wild-type mice and transgenic mice lacking functional CFTR (representative PD tracings shown in Fig. 4*B*). CFTR Cl^- conductance was stimulated in wild-type mice by the cAMP agonist forskolin or by a selective activator (*not shown*), and this conductance was inhibited by $CFTR_{inh}$-172. The substantial and sustained activation of CFTR-mediated Cl^- secretion by CFTR-selective activators provides a rational basis for their evaluation as therapy for KCS. Indeed, the phosphodiesterase inhibitor isobutylmethylxanthine reduced tear osmolarity in a rabbit model of KCS and in humans with KCS (Gilbard et al., 1991; Gilbard, 1994). INS365, a long-acting UTP agonist that stimulates mucus and calcium-sensitive Cl^--driven fluid secretion (Li et al., 2001), is in phase III clinical trials for treatment of KCS. Direct measurement of CFTR-dependent ocular surface fluid secretion will be important in establishing the utility of CFTR activators as clinically useful secretagogues.

Corneal Endothelium

Maintenance of corneal stromal transparency requires precise regulation of extracellular water content (Maurice, 1957; Freegard, 1997). The aqueous-facing corneal endothelium, a misnomed leaky (low resistance) epithelium, permits passive hydrostatic flux of water and ions from the aqueous

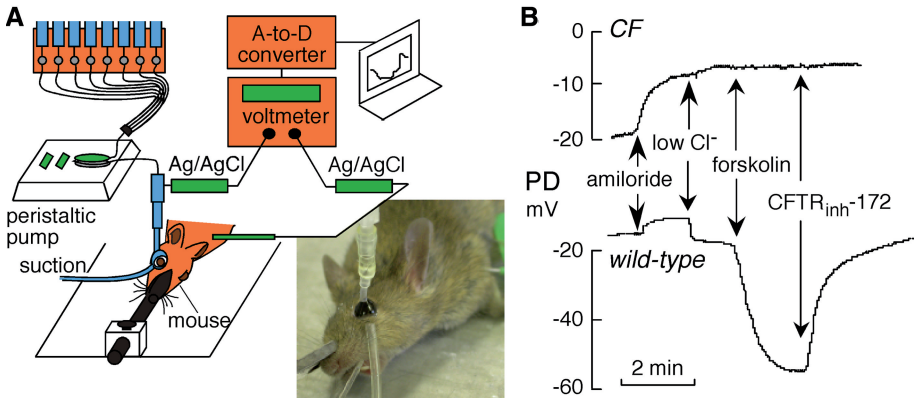

Fig. 4. CFTR function at the ocular surface. (*A*) Diagram of ocular surface perfusion and PD recording methods. Anesthetized mouse immobilized in a stereotaxic platform, with perfusion tubing attached to an electrical recording system and in contact with a droplet of constant size on the ocular surface. (*Inset*) Photograph of perfused ocular surface. (*B*) Ocular surface PDs in wild-type *vs.* CF mice in response sequentially to amiloride (100 μM), low Cl^-, forskolin (10 μM), and $CFTR_{inh}$-172 (10 μM). Adapted from Levin & Verkman (2005b).

compartment to the stroma. The endothelium also establishes opposing ion gradients by secondary active Cl^- and HCO_3^- transport, driving water movement to yield a relatively dehydrated stromal matrix of regularly spaced collagen fibrils. However, the corneal endothelial cell population is gradually depleted due to little or no cell proliferation in vivo (reviewed by Joyce, 2003). Corneal dystrophies and iatrogenic damage from ocular surgeries such as cataract extraction accelerate this age-related cell loss (Yi & Dana, 2002). Decreased total endothelial cell surface area results in reduced barrier and pump functions, potentially leading to corneal edema and opacity requiring corneal transplantation. Stimulation of stroma-to-aqueous fluid transport might reduce corneal swelling.

AQPs and Corneal Endothelium

AQP1 is expressed in mouse, rat, and human corneal endothelial cells (Patil et al., 1997; Hamann et al., 1998; Levin & Verkman, 2004). Corneal thickness is ~20% reduced in AQP1 deficiency (Fig. 5*A*) though baseline corneal transparency is normal (Thiagarajah & Verkman, 2002). AQP1 water transport function in corneal endothelium in vivo was demonstrated by slowed corneal swelling upon hypotonic challenge at the endothelial surface utilizing an anterior chamber microperfusion method (Fig. 5*B*). An important role for AQP1 in maintenance of corneal transparency was demonstrated in an experimental model of corneal edema produced by transient exposure of the corneal surface to hypotonic solution, in which AQP1 deficiency was associated with impaired recovery of corneal transparency and thickness (Fig. 5*C*). In primary corneal endothelial cell cultures, AQP1 deficiency reduced osmotically driven cell membrane osmotic water permeability, but did not impair active near-isosmolar transcellular fluid transport (Kuang et al., 2004). The commonly invoked mechanism of transcellular, AQP-facilitated fluid transport has been questioned in relation to the corneal endothelium, with Fischbarg and colleagues proposing a central role for electro-osmotic coupling of fluid transport to recirculating currents at the level of the intercellular junctions (Fischbarg, 2003; Fischbarg & Diecke, 2005). This model posits that AQP1 contributes primarily to cell volume regulation, a role that remains difficult to reconcile with the dramatic corneal swelling phenotype of AQP1-null mice and with the substantially slower rate of cell volume regulation vs. osmotic equilibration.

AQP1 is also highly expressed in stromal keratocytes (Hamann et al., 1998; Wen et al., 2001; Levin & Verkman, 2004), where its function is not known. Keratocytes, the primary resident stromal cell-type, perform many fibroblast-like functions, including extracellular matrix deposition during development and enhanced motility and signaling during inflammation and wound healing. AQP1 might be involved in keratocyte biosynthetic and/or cell migratory functions, and possibly in the regulation of stromal extracellular water content. The role for keratocyte/endothelial AQP1 in stromal dehydration may be revealed with an improved understanding of the evolution of the relatively thin AQP1-null corneas during development, similar to what was done for the lumican-deficient mouse (Song et al., 2003). Primary cultures of mouse keratocytes (Chakravarti et al., 2004) may also be informative in eludicating AQP1 function.

CFTR and Corneal Endothelium

Cl^- and HCO_3^- are important for stroma-to-aqueous fluid transport (reviewed by Bonanno, 2003). HCO_3^- is taken up at the stromal facing (basolateral) mem-

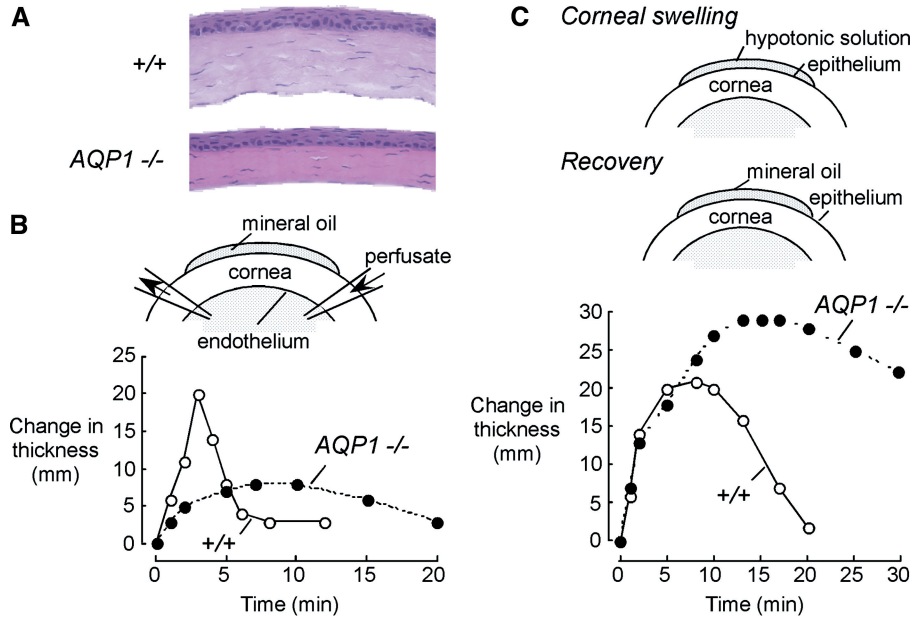

Fig. 5. AQP1 function in corneal endothelium. (*A*) Reduced corneal thickness in AQP1-deficient corneas in paraffin-embedded central corneal sections. (*B*) Osmotic water transport across the corneal endothelium. (*Top*) Schematic showing micropipette placement for anterior chamber perfusion. (*Bottom*) Time courses of corneal thickness following corneal endothelial exposure to hypotonic saline in wild-type (*empty circles*) and AQP1-null (*filled circles*) mice. (*C*) Restoration of corneal thickness after osmotic swelling. (*Top*) Procedure to induce corneal swelling and follow recovery of thickness. (*Bottom*) Representative time courses of corneal thickness after exposure of the corneal surface to hypotonic saline. Corneal thicknesses measured in vivo by z-scanning confocal microscopy. Adapted from Thiagarajah & Verkman (2002)

brane by an electrogenic Na^+/HCO_3^- co-transporter with 1:2 stoichiometry. Cl^- enters basolaterally via the NKCC co-transporter and across the aqueous-facing (apical) membrane via a Cl^-/HCO_3^- exchanger. CFTR was localized to the apical membrane in bovine corneal endothelial cell cultures, where efflux of Cl^- and HCO_3^- are cAMP-dependent (Sun & Bonnano, 2002). As found in other cell types (Paradiso, Coakley & Boucher, 2003; Welsh & Smith, 2001), CFTR is probably permeable to both Cl^- and HCO_3^- in corneal endothelium. Recent studies provide evidence for indirect HCO_3^- activation of CFTR-dependent Cl^- transport involving stimulation and up-regulation of soluble adenylyl cyclase (Sun et al., 2003; Sun, Cui & Bonnano, 2004). One study on CF subjects reported increased endothelial permeability and cell density, and slightly (4%) thicker corneas compared to control subjects (Lass et al., 1985). While these data support the possibility of CFTR activation to treat corneal edema, direct measurements of anion and fluid secretion in response to CFTR activators/inhibitors are needed to prove a role for CFTR in corneal endothelial fluid transport.

Ciliary Epithelium

The ciliary epithelium is a bilayered tissue consisting of pigmented ciliary epithelia (PCE) and nonpigmented ciliary epithelia (NCE), whose apical surfaces are juxtaposed and basolateral surfaces face the ciliary body and aqueous humor, respectively. Aqueous fluid production involves near-isosmolar water secretion across the ciliary epithelium into the posterior aqueous chamber. Aqueous secretion is crucial for providing nutrients to avascular ocular tissues of the anterior segment and for inflating the globe to maintain intraocular pressure (IOP). IOP is also influenced by aqueous drainage, which occurs by pressure-driven bulk fluid flow into the canal of Schlemm and across the sclera. Elevated IOP is associated with glaucoma, a disorder of progressive visual loss and a major cause of blindness worldwide (Weinreb & Khaw, 2004). Reduction of ciliary secretion is the primary pharmacologic strategy for treatment of elevated IOP in glaucoma, warranting examination of AQP- and CFTR-facilitated ciliary fluid transport as possible targets for inhibition.

AQPs and Ciliary Epithelium

NCE cells co-express AQP1 and AQP4 (Nielsen et al., 1993; Hasegawa et al., 1994; Frigeri et al., 1995; Hamann et al., 1998), suggesting their involvement in maintaining a high NCE cell water permeability for aqueous fluid production. An initial study on human NCE cultures reported AQP1 protein expression and a partial sensitivity of fluid

transport to Hg^{2+} and AQP1 siRNA, suggesting AQP1-dependent aqueous inflow (Patil et al., 2001). Measurements of IOP in mice using a fluid-filled microneedle inserted into the anterior chamber showed a modest reduction in IOP by 2–3 mm Hg in mice lacking AQP1 and/or AQP4 compared to wild-type mice (Zhang, Vetrivel & Verkman, 2002). AQP1 is also expressed in trabecular meshwork endothelium in the canal of Schlemm, where a role in cell volume regulation had been proposed (Stamer et al., 2001). However, direct measurement of aqueous fluid outflow in mice by a pulsed infusion method showed no effect of AQP1 deletion (Zhang, Vetrivel & Verkman, 2002). Together with measurements of aqueous fluid production by a fluorescein iontophoresis-confocal detection method, it was concluded that reduced IOP in AQP-deficient mice was due to reduced ciliary aqueous fluid production. It will be important to investigate the role of AQPs in IOP regulation in mouse models of glaucoma, such as an episcleral vein cauterization model developed recently by our lab (Ruiz-Ederra & Verkman, 2006), and when selective AQP inhibitors are available, in large animal models.

CFTR and Ciliary Epithelium

The clinical utility of acetazolamide in reducing aqueous fluid secretion had focused the bulk of attention regarding ciliary epithelial ion transport on HCO_3^-- rather than Cl^--driven fluid secretion. However, Cl^- but not HCO_3^- was found to concentrate within ciliary epithelial cells, leading to the realization that HCO_3^- plays a critical though secondary role in supporting Cl^- secretion (reviewed by Do & Civan, 2004). The NKCC co-transporter and Na^+/H^+ exchanger at the basolateral membrane of PCE cells have been shown to promote Cl^- uptake, with inhibition of cytoplasmic carbonic anhydrase by acetozolamide reducing aqueous fluid secretion by interfering with this secretion mechanism. Following Cl^- accumulation in PCE cells and subsequent spread through gap junctions to NCE cells, Cl^- release occurs through channel(s) of unknown molecular identity. There is no evidence for CFTR-dependent secretion across the ciliary epithelium. Aqueous fluid production in CF subjects was equivalent to that in non-CF controls (McCannel et al., 1992). While cAMP was shown to enhance NKCC symport, thereby increasing net Cl^- flux, forskolin-induced cAMP production was reported to, if anything, reduce aqueous fluid secretion (Do, Kong & To, 2004). More likely candidates for the basolateral NPE Cl^- channels have been proposed to be the swelling-activated ClCa-3 and pI_{Cln} channels, though their roles remain controversial.

Retinal Pigment Epithelium

The retinal pigment epithelium (RPE) is an epithelium separating the neural retina from the capillaries of the choroid. The RPE lines the outer blood-retinal barrier (BRB), preventing leak between compartments (the inner BRB consists of the tight-junction-laden endothelial cells of retinal blood vessels). Functionally, the RPE supplies the retina with nutrients critical for the visual cycle, phagocytoses photoreceptor outer segment discs, and absorbs fluid from the subretinal space in the outer retina. Fluid absorption from the subretinal space is important to maintain the volume and ionic composition of the extracellular space surrounding photoreceptor segments within a range that supports phototransduction. Passive forces, including IOP-driven bulk flow and choroidal osmotic pressure, prevent the build-up of subretinal fluid. However, in disease states when the BRB is disrupted and protein aberrantly enters the subretinal space, active RPE transport is crucial to oppose oncotic water accumulation (Marmor, 1999). Modulation of RPE absorptive function thus represents a logical strategy for treatment of retinal detachment (Marmor, 1990). A distinct retinal extracellular space, located in the inner retina, is maintained by the dehydrating actions of the glial-like Müller cells. Ischemic disorders, caused by retinal artery occlusion, diabetes, and hypertension, are associated with fluid accumulation in this second compartment (Bringmann et al., 2005).

AQPs and Retinal Pigment Epithelium

The presence and function of AQPs in the RPE remain in question. In one study, AQP1 was localized to human RPE in situ using an ultra-sensitive cell-surface biotinylation method and in cell culture by immunofluorescence using an affinity-purified AQP1 antibody (Stamer et al., 2003). AQP1 mRNA had been previously detected in cultured fetal RPE cells (Ruiz & Rok 1996). However, other studies on rat and human tissue have failed to identify AQP1 protein in RPE (Hamann et al. 1998, and our own unpublished data). AQP1 localization to photoreceptor, glycernic amacrine, and Müller cells has also been reported without demonstrated function (Kim et al., 1998, 2002; Iandiev et al., 2005).

AQP4 is strongly expressed in Müller cells, especially in perivascular and end-feet processes (facing the retinal capillaries and vitreous body), where it is thought to form multiprotein complexes involving the inwardly rectifying Kir4.1 K^+ channel (Nagelhus et al., 1998; Nagelhus et al., 1999; Connors & Kofuji, 2005). Analogous to its roles in brain astroglial cells and cochlear supportive cells (Manley et al., 2000; Li & Verkman, 2001), Müller cell AQP4

has been proposed to maintain extracellular space volume and K^+ concentration during bipolar cell neural activity. AQP4-null mice exhibited mildly defective retinal signal transduction as evidenced by reduced short full-field electroretinogram (ERG) b-wave amplitude and latency (Li, Patil & Verkman, 2002), suggesting functional coupling between water and K^+ clearance. AQP4 deletion in Müller cells also protected against edema and ganglion cell death following retinal ischemia (Da & Verkman, 2004). These results implicate AQP4 in both neuronal and glial cell swelling. AQP4 inhibitors might therefore limit inner retinal pathology following vascular occlusive and other ischemic diseases causing cytotoxic (cellular) edema (Bringmann et al., 2005).

CFTR and Retinal Pigment Epithelium

Cl^- is transported into RPE cells across the retinal (apical) membrane against a concentration gradient through the NKCC co-transporter, and across the choroidal (basolateral) membrane via a Cl^-/HCO_3^- exchanger. Choroidal Cl^- exit takes place largely through a calcium-sensitive channel. Linkage of mutations in the Best1 gene to Best disease (autosomal dominant Best vitelliform macular dystrophy) led to the discovery of bestrophins (Marmorstein et al., 2000), which now appear to be the calcium-dependent Cl^- channels of the RPE and other epithelia. Interestingly, aspects of Best disease resemble the much more common disorder, age-related macular degeneration (reviewed by Hartzell et al., 2005). Basolateral Cl^- secretion is controlled by endogenously generated ATP, which initiates purinergic signaling at the $P2Y_2$ receptor on the apical membrane. In addition to expressing anion channels ClC-2, ClC-3, ClC-5, and the pCLCA1 Cl^- regulator (Loewen et al., 2003), the RPE also expresses CFTR. In human cell culture and bovine organ culture models, CFTR was proposed to enhance RPE fluid transport indirectly by ATP release and autocrine purinergic signaling (Reigada & Mitchell, 2005). In two studies, Vitamin A-corrected CF subjects exhibited decreased contrast sensitivity in visual testing, but this phenotype more likely reflects a defect in optic nerve rather than retinal neural processing (Morkeberg et al., 1995; Ansari et al., 1999). Pharmacologic studies on non-CF human fetal retinal explants provided evidence for cAMP regulation of the fast oscillatory component of the ERG (Blaug et al., 2003), which can be generated by Cl^- transport. However, in isolated frog retinal preparations, cAMP elevation dissipated solute gradients across the RPE, thereby inhibiting rather than stimulating fluid transport (Miller, Hughes & Machen, 1982). With only this conflicting data available, the precise role for CFTR in RPE transport is unclear. Nonetheless, modulation of net RPE Cl^- transport has considerable therapeutic promise, as demonstrated by reduced subretinal fluid bleb size in rat and rabbit following stimulation of purinergic receptors by INS37217 (Maminishkis et al., 2002; Meyer et al., 2002). CFTR activators may similarly enhance RPE fluid absorption.

Additional work is needed to establish roles for AQP1 and CFTR in RPE function. Recent advances in non-invasive measurement of slow potentials in mice allow for study of electrical activity derived from non-neuronal components of the visual cycle (Wu, Peachey & Marmorstein, 2004). Differences in Müller cell-generated slow potentials have been identified in Kir4.1 heterozygous mice (Wu et al., 2004). The slow light peak in the electrooculagram also reflects RPE basolateral membrane Cl^- conductance (Gallemore, Hughes & Miller, 1997), and is decreased as a diagnostic feature of Best disease. Advances in mouse ERG measurements may thus be valuable in evaluating the electrophysiological consequences of AQP and CFTR channel gene disruption.

Summary and Perspective

Ocular epithelia have the capacity for high rates of fluid transport, which often depends on active Cl^- secretion or absorption, producing near-isosmolar fluid transport. From a variety of approaches, including analysis of AQP- and CFTR-deficient mice, there is now compelling evidence for involvement of AQPs and CFTR in ocular epithelial fluid transport. Small-molecule modulators of ocular AQP and CFTR function or expression might thus be exploited clinically, as summarized in Table 1. At the ocular surface, AQP3 or AQP5 up-regulation could accelerate wound healing and reduce corneal edema, while CFTR activators are predicted to stimulate tear secretion. Corneal endothelial AQP1 and CFTR activators/inducers might also reduce corneal edema and associated opacity. AQP1/AQP4 inhibition represents a promising strategy in reducing IOP associated with glaucoma. In the retina, AQP4 inhibitors might be neuroprotective following retinal ischemia, and activation of RPE CFTR might be useful in treating retinal detachment. Potent small-molecule CFTR activators and inhibitors have already been identified, and the therapeutic potential of CFTR inhibitors has been demonstrated in rodent models of CFTR-dependent secretory diarrhea (Ma et al., 2002a,b; Muanprasat et al., 2004). When available, non-toxic AQP inhibitors will prove useful in confirming analyses of knockout mice, and may provide new approaches for treatment of eye diseases associated with abnormalities in fluid balance.

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Table 1. Proposed AQP- and CFTR-based therapies for common ocular disorders

Clinical Disorder	Treatment Strategy	Intervention
Keratoconjunctivitis Sicca (Tear film deficiency)	↑ Corneal and conjunctival fluid secretion	↑ CFTR
Corneal Edema/Opacity (Dystrophy, trauma)	↑ Endothelial and epithelial fluid transport	↑ CFTR, AQP1, AQP5
Recurrent Corneal Erosions (Persistent abrasions)	↑ Epithelial migration and proliferation	↑ AQP3
Glaucoma (Elevated IOP)	↓ Ciliary epithelial aqueous humor secretion	↓ AQP1, AQP4
Retinal Detachment (BRB break-down & outer retinal edema)	↑ Retinal pigment epithelial fluid absorption	↑ CFTR
Retinal Ischemia (Vascular occlusion & inner retinal edema)	↓ Neuronal and Müller swelling	↓AQP4

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