mRNA From Frog Corneal Epithelium Increases Water Permeability in Xenopus Oocytes

Tamara Horwich,* Cristina Ibarra,† Paula Ford,‡ Aldo Zamudio,* Mario Parisi,† and Oscar A. Candia*

Purpose. To investigate the existence of a water channel in the frog corneal epithelium by studying the osmotic water permeability (Pw) of Xenopus oocytes expressing the mRNA message from frog corneal epithelium.

Methods. Total RNA was obtained from corneal epithelium by a single-step phase separation method, and poly A+ RNA was isolated using oligo-dT columns. This mRNA was injected into the oocytes. After a 48-hour incubation, oocyte volume changes elicited by a hypo-osmotic solution were measured with a computerized video system.

Results. Oocytes injected with 50 nl mRNA (1 µg/µl) showed a significant increase in Pw compared to water-injected controls (8.4 ± 1.5 to 17.5 ± 1.9 cm·sec⁻¹ × 10⁻⁴, P < 0.005). mRNA-injected oocytes exposed to a higher external [Cl⁻] showed a heightened permeability. Furthermore, Pw of oocytes exposed to a solution containing the recognized water-channel blocker HgCl₂ was significantly lower than the Pw of mRNA-injected oocytes not exposed to HgCl₂.

Conclusions. Evidence was found for a water channel in the frog corneal epithelium because oocytes injected with the epithelial mRNA manifested increased water permeability. The increase in water permeability was larger in the presence of external Cl⁻ and was inhibited by HgCl₂. This finding correlates with measurements of Pp in the intact epithelium in which apical Cl⁻ induced an increase in transepithelial water permeability prevented by HgCl₂. Invest Ophthalmol Vis Sci. 1995;36:2772-2774.

Evidence from previous physiological studies suggested the possibility of a Cl⁻-induced “water channel” at the apical membrane of the bullfrog corneal epithelium.² Experiments using ³H₂O to measure unidirectional diffusional water fluxes across isolated corneas showed a significant heightening (62%) of corneal water permeability when Cl⁻ was present on the cornea’s apical side. This diffusional flux was inhibited by HgCl₂, a mercurial agent well established as a water-channel blocker.³,⁴ Furthermore, in experiments designed to elicit net osmotic water flow, this flow was negligible unless Cl⁻ was present in the Ringer’s bathing the apical side, and this Cl⁻-activated net flow also was inhibited significantly by HgCl₂. Although previous reports suggested a relatively high water permeability for the rabbit corneal epithelium,⁵,⁶ these findings remained latent until the recent discoveries of water channels in many epithelia. Thus, the epithelium seems to possess a specific water transport pathway that may play an integral role in the maintenance of stromal dehydration and corneal transparency.

Interest in “water channels” has grown recently, beginning with the discovery of the CHIP28 protein in the red blood cell.⁷ Collectively termed aquaporins, these transmembrane channel-forming proteins are now recognized for their fundamental role in water transport in many biologic tissues, such as the renal collecting duct and the capillary endothelium.⁸ Of particular interest, aquaporins also have been located in several ocular tissues, such as the lens epithelium,⁹ iris epithelium,⁹ and corneal endothelium.⁵,⁶,¹⁰,¹¹ In addition, CHIP28 has been described in corneal endothelium, lens epithelium, ciliary processes, and trabecular meshwork of the human eye.¹² More recently, Raina et al.¹³ discovered a novel member of the aquaporin family (AQ5) in the salivary gland, and in situ hybridization using the AQ5 antisense riboprobe showed expression in the corneal epithelium.

To investigate further the possibility of a similar water channel in the frog corneal epithelium, we sought to examine a potential rise in water permeability of Xenopus oocytes that express mRNA from frog corneal epithelium.

METHODS. Epithelium Removal. Thirty bullfrogs (Rana catesbeiana) were double-pithed. Their eyes were enucleated and subsequently stored at −70°C. After thawing, corneal surfaces were rinsed with sterile, distilled water and dried with blowing air. The epithelium was removed by careful scraping using a dulled, sterilized blade, and the epithelial scrapings were immediately placed in the RNA isolation solution (Tri Reagent; MRC, Cincinnati, OH).

Isolation of mRNA (poly A+). Total RNA from the frog corneal epithelium was processed using a single-step, liquid-phase separation method (Tri Reagent; MRC). Phase separation occurred with the addition of chloroform, and the RNA-containing aqueous phase was removed by careful pipetting. The aqueous phase was run through oligo (dT) cellulose columns, and fractions containing only poly A+ RNA were obtained by elution.
Oocyte Injection and Incubation. Oocytes were extracted surgically from live *Xenopus* one day before oocyte injection with mRNA or water. Treatment of animals was in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. *Xenopus* were anesthetized with ice before surgery; a small incision on the lower abdomen was sewn after removal of the eggs. After a 2-hour treatment with collagenase solution (1 mg/ml Barth’s) to remove the follicular cell layer, oocytes were stored until injection at 4°C in sterile Barth’s buffer. Barth’s buffer, with a total osmolarity of 200 mOsm to match the oocytes’ internal osmolarity, has the following composition (in mM): 88 NaCl, 1 KCl, 0.8 MgSO₄· H₂O, 2 Ca (NO₃)· H₂O, 0.75 Tris, and 2.4 NaHCO₃.

Using an automatic oocyte injector system (Nanoject; Drummond Scientific, Broomal, PA) and micromanipulator, oocytes were inoculated by a sterile glass micropipette either with 50 nl of mRNA dissolved in water (1 /L/g//xl) or with 50 nl of water. Before measurement of the osmotically induced volume changes, injected oocytes were incubated at 18°C for 48 to 72 hours in sterile Barth’s buffer supplemented with antibiotics. Buffer solutions were changed after 48 hours.

**Measurement of Oocyte Volume and Calculation of Permeability.** Injected oocytes, both mRNA and controls, were submerged in 1 ml of a hypotonic solution to induce a condition in which volume changes could be monitored. A custom-built, laboratory-assembled computerized video microscope system was used for the calculation of oocyte water permeability ($P_w$).

Photographs, saved as images, were taken immediately after imposing a hypotonic osmotic gradient every 10 seconds for the first 3 minutes, and then they were taken at 7 and 10 minutes. After the experiment was complete, the photographs were analyzed in the following manner. The x- and y-axes of every photograph were measured, and, from this (considering the oocyte to be a quasisphere), the surface and volume of the oocyte at every point as a function of time were calculated. $P_w$ (cm/sec) was then calculated as the change in volume ($cm^3$) per unit area ($cm^2$) per time (sec) per difference in osmolarity (mol/cm³): $P_w = \frac{\Delta V}{(sec \cdot A \cdot \Delta C \cdot V_w)}$, where $A$ is the area of the oocyte surface ($cm^2$) and $V_w$ is the partial volume of water ($cm^3$/mol). This method was similar to that described by Echevarria et al.⁹

Oocyte volume changes were examined under three separate hypotonic conditions. For each experimental condition, oocytes were extracted from the same animal on the same day. Furthermore, only oocytes extracted together were used for statistical comparison. Both before and during measurement of volume changes, oocytes were maintained at 18°C by temperature-controlled water flow.

**RESULTS.** In the first set of experiments, a 180 mOsm gradient was created by placing the oocytes in a 20 mOsm bath (10% Barth’s) containing 8.8 mM [Cl⁻]. Mean permeability ($P_w$) calculated from oocytes’ swelling in mRNA-injected oocytes was more than two times greater than the $P_w$ of control oocytes injected only with water. The second set of experiments imposed a smaller gradient of 100 mOsm (50% Barth’s); however, the solution’s [Cl⁻] was higher, 44 mM. The heightened $P_w$ of oocytes expressing the epithelial mRNA was also true for the 100 mOsm gradient condition (Table 1).

In the last condition, mRNA-injected oocytes were bathed in a 20-mOsm (10% Barth’s) bath containing 0.3 mM HgCl₂, and values were compared to a control

### TABLE 1. Water Permeability of *Xenopus* Oocytes Placed in Hypotonic Bath

<table>
<thead>
<tr>
<th>Gradient</th>
<th>180 mOsm</th>
<th>100 mOsm</th>
</tr>
</thead>
<tbody>
<tr>
<td>External [Cl⁻]</td>
<td>8.8 mM</td>
<td>44 mM</td>
</tr>
<tr>
<td>$P_w$ (cm·sec⁻¹ × 10⁻⁴)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mRNA injected</td>
<td>8.4 ± 1.5</td>
<td>11.2 ± 1.7</td>
</tr>
<tr>
<td>water-injected controls</td>
<td>(n = 6)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>mRNA injected</td>
<td>17.5 ± 1.9*</td>
<td>24.2 ± 3.1*</td>
</tr>
<tr>
<td>water-injected controls</td>
<td>(n = 10)</td>
<td>(n = 8)</td>
</tr>
</tbody>
</table>

Values represent mean ± SE.  
*Significantly higher than water-injected controls ($P < 0.005$).

### TABLE 2. Water Permeability of *Xenopus* Oocytes Placed in Hypotonic Bath: Effect of HgCl₂

<table>
<thead>
<tr>
<th>Gradient</th>
<th>180 mOsm</th>
</tr>
</thead>
<tbody>
<tr>
<td>External [Cl⁻]</td>
<td>8.8 mM</td>
</tr>
<tr>
<td>Control</td>
<td>20.0 ± 1.2</td>
</tr>
<tr>
<td>mRNA injected</td>
<td>5.4 ± 1.0*</td>
</tr>
</tbody>
</table>

Values represent mean ± SE.  
*Significantly lower than control without HgCl₂ in external solution ($P < 0.0001$).
HgCl₂-free condition. The presence of HgCl₂ in the bath significantly decreased the permeability of oocytes expressing mRNA. Average $P_f$ of HgCl₂-immersed oocytes was 27% of the $P_f$ of oocytes in an HgCl₂-free bath of the same osmolarity (Table 2).

**DISCUSSION.** Oocytes injected with frog corneal epithelial mRNA manifested a significantly increased water permeability when compared to the permeability of control oocytes injected only with water. Furthermore, HgCl₂ added to the bathing solution inhibited the increase in water permeability of mRNA-injected oocytes.

The presence of Cl⁻ appeared important for the activation of water channels, for the mean permeability at a low Cl⁻ concentration (17.5 ± 1.9 $\times$ 10⁻⁴ cm·sec⁻¹) was further increased when the oocytes were subjected to a larger external Cl⁻ concentration (24.2 ± 3.1 $\times$ 10⁻⁴ cm·sec⁻¹), although the significance of the difference between these two groups was borderline ($P < 0.0735$) (Table 1).

We found the $P_f$ of oocytes injected with total mRNA of frog corneal epithelium, on average, to be double that of controls. Studies of oocytes injected with a pure, isolated segment of mRNA, encoding an already "discovered" water channel, report a $P_f$ eight times greater than that of controls. When placed in an external hypotonic medium, these oocytes swelled and ruptured within 5 minutes. In the absence of an already-sequenced water channel, we inoculated oocytes with total mRNA and found that total mRNA expression significantly increased oocyte permeability.

The behavior of oocytes expressing frog corneal epithelial mRNA agree with physiological experiments studying both diffusional and net osmotic water flux across corneal epithelium, in which water permeability increased in proportion to apical [Cl⁻] and was blocked by HgCl₂. The need for water channels in cell membranes is obvious, and its importance in the epithelia of the eye has been reviewed recently. In epithelia, water not only has to be exchanged between the intracellular and extracellular compartments, it must traverse the basolateral and apical sides of the epithelium separating two different environments. To participate in stromal dehydration, the corneal epithelium must possess water channels in both cell surfaces. However, the control of the overall permeability must reside in only one of the two surfaces. Our findings suggest that mRNA-injected oocytes expressed functional water channels; thus, they provide further evidence for the existence of a Cl⁻-activated water channel possibly residing on the apical side of the frog corneal epithelium.

**Key Words**

aquaporins, membrane transport, mRNA, water permeability, Xenopus oocyte

**References**