Differentiation-dependent changes in the membrane properties of fiber cells isolated from the rat lens

Kevin F. Webb and Paul J. Donaldson
Department of Physiology, School of Medical Sciences, University of Auckland, Auckland, New Zealand

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Webb KF, Donaldson PJ. Differentiation-dependent changes in the membrane properties of fiber cells isolated from the rat lens. Am J Physiol Cell Physiol 294: C1133–C1145, 2008; doi:10.1152/ajpcell.00315.2007.—Impedance measurements in whole lenses showed that lens fiber cells possess different permeability properties to the epithelial cells from which they differentiate. To confirm these observations at the cellular level, we analyzed the membrane properties of fiber cells isolated in the presence of the nonselective cation channel inhibitor Gd3+. Isolated fiber cells were viable in physiological [Ca2+] and exhibited a range of lengths that reflected their stage of differentiation. Analysis of a large population of fiber cells revealed a subgroup of cells whose conductivity matched values measured in the whole lens (1). In this group of cells, membrane resistance, conductivity, and reversal potential all varied with cell length, suggesting that the process of differentiation is associated with a change in the membrane properties of fiber cells. Using pharmacology and ion substitution experiments, we showed that newly differentiated fiber cells (<150 μm) contained variable combinations of Ba2+-and tetraethylammonium-sensitive K+ currents. Longer fiber cells (150–650 μm) were dominated by a low-level, nonselective conductance that was eliminated by the replacement of extracellular Na+ with N-methyl-D-glucamine, indicating that the lens contains both Gd3+-sensitive and -insensitive nonselective cation conductances. Fiber cell differentiation is therefore associated with a shift in membrane permeability from a dominant K+ conductance(s) toward larger contributions from anion and nonselective cation conductances as fiber cells elongate.

electrophysiology; potassium channel; anion channel; nonselective cation channel

THE OCULAR LENS grows throughout life via the addition of new cells at the surface that internalize the older cells, creating an inherent age gradient. Epithelial cells that cover the anterior surface of the lens differentiate into fiber cells that elongate, lose their cellular organelles, and express fiber-specific proteins (2). All cells in this differentiation continuum of epithelial cells, differentiating, and mature fiber cells are interconnected by a network of gap junctions (24) that ensures the lens functions as a nonuniform syncytium (39). This syncytial arrangement of the lens means that its membrane potential is set by the weighted average of the membrane permeability of all cells that comprise the tissue. Using ionic substitution experiments and whole lens impedance measurements, Mathias et al. have shown that the membrane properties at the lens surface are the net result of high Na+/K+-ATPase activity and a dominant K+ permeability, whereas the underlying fiber cells exhibit minimal Na+/K+-ATPase activity and are dominated by a membrane permeability to Na+ and Cl− (1, 38). This shift in membrane permeability results in spatially segregated ionic influx and efflux pathways (35). Since these pathways are connected by gap junctions, a circulating current is established that, in turn, drives an internal circulation system within the lens that is proposed to maintain lens homeostasis and transparency (37).

To date, efforts to confirm these spatial differences in membrane permeability have focused on lens epithelial cells since these surface cells are easy to obtain and maintain in culture and are therefore amenable to study using the patch-clamp technique. In isolated rodent lens epithelial cells, a Ba2+-sensitive inwardly rectifying K+ (KCa) channel has been shown to be active (43, 49), and the application of Ba2+ to these cells caused a depolarization of their membrane potential toward 0 mV (9). However, the application of Ba2+ to the intact rodent lens only causes a 10- to 20-mV depolarization (11, 48), indicating a nonexclusive role for KCa channels in setting overall lens potential. This suggests that either epithelial or fiber cells contain additional conductances that contribute to the resting potential of the intact lens (37). Consistent with this idea, a variety of other K+ conductances have been identified by patch-clamp studies conducted in the lens epithelium; however, not all of these channels are found in every species or even in any one cell (9). A delayed outwardly rectifying K+ conductance that is sensitive to tetraethylammonium (TEA) has been found in the lens epithelium of every species studied (for a review, see Ref. 37), but it is largely closed at potentials in the physiological voltage range, contributing only a fraction of its peak “active” conductance in cells at rest (43). TEA is only a weak depolarizer of whole lenses (34), a result consistent with the view that these channels play a minimal role in setting lens resting membrane potential. Ca2+-activated K+ (KCa) currents are ubiquitous in lens epithelia and are blocked by both Ba2+ and TEA (46, 50). KCa channels are normally closed at physiological intracellular Ca2+ concentrations but are open if levels rise into the micromolar range, with their open probability increasing further with membrane depolarization. Once again, this strong Ca2+ dependence indicates that KCa channels probably do not play a major role in the maintenance of the lens potential. Thus, despite considerable characterization of the permeability properties of the lens epithelium, no one conductance appears to set the lens membrane potential (Vm), which suggests that the fiber cells themselves may contribute significantly to the lens potential.

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Unfortunately, very little is known at the cellular level about the membrane properties of fiber cells. These elongated, highly differentiated cells are notoriously difficult to isolate and maintain in solutions that are amenable to electrophysiological analysis of their membrane properties, and past efforts to produce viable isolated fiber cell preparations have been only partially successful (13, 18, 19). Isolated lens cell preparations tended to contain only very short, newly differentiated fiber cells that exhibited an epithelial-like K\(^+\) conductance (13). Unfortunately, the membrane properties of isolated fiber cells degraded over time by activation of leak currents, often in midrecording, (13, 18, 41). In a single study, Jacob and co-workers (56) obtained short viable fiber cells from the bovine lens by enzymatic dispersion and plated them in physiological media embedded in the surface of low-temperature agar; however, difficulties in maintaining membrane integrity under whole cell patch clamp in the presence of Ca\(^{2+}\) makes such experimentation problematic. Bhatnagar and co-workers (6) obtained a preparation of highly elongated fiber cells from the rat lens by exposure to a nonionic trypsin solution that was heavily buffered to remove extracellular Ca\(^{2+}\). In this preparation, reintroduction of Ca\(^{2+}\) induced vesiculation, and this process could be significantly delayed by a variety of nonspecific cation and Ca\(^{2+}\) channel blockers, suggesting that Ca\(^{2+}\) influx is a key trigger of vesiculation. Based on these observations, Eckert et al. (18, 19) obtained a preparation of isolated fiber cells that were amenable for patch-clamp analysis by the use of physiological Ringer solutions that lacked Ca\(^{2+}\). Unfortunately, while removal of extracellular Ca\(^{2+}\) prevented vesiculation, subsequent electrophysiological analysis of these cells indicated that Ca\(^{2+}\) removal resulted in the activation of a large membrane leak that appeared to resemble currents mediated by Cx46 hemichannels expressed in oocytes (17, 19). A similar increase in membrane leak was observed in whole lenses upon the removal of extracellular Ca\(^{2+}\), which could be blocked by the addition of a variety of multivalent cations (48).

Taken together, the above studies suggest that while blockade of Ca\(^{2+}\) influx is key to preventing fiber cell vesiculation, this needs to occur in the presence of extracellular Ca\(^{2+}\) if the activation of leak conductances are to be avoided and physiologically relevant membrane properties recorded from isolated fiber cells. Recently, we have shown the inclusion of the multivalent nonsedative cation channel inhibitor Gd\(^{3+}\) in Ca\(^{2+}\)-containing Ringer solutions enables physiologically relevant membrane currents to be recorded from isolated elongated (>120 \(\mu\)m) lens fiber cells (54). In this study, we present our analysis of a larger data set of whole cell recordings from isolated fiber cells that cover a wider range of cell lengths and therefore different stages of fiber cell differentiation. Our analysis indicates that the membrane properties of fiber cells undergo differentiation-dependent changes as fiber cells elongate and become internalized. The differentiation of epithelial cells into elongated fiber cells is accompanied by a switch from dominant epithelial-like K\(^+\) conductances in short fiber cells to a more dominant contribution from Cl\(^-\) and Na\(^+\) conductances in longer, more differentiated fiber cells. These data represent the first confirmation at the cellular level of spatial variations in ion permeabilities within the lens cortex, a key postulate of the lens internal circulation model (35, 37).

**METHODS**

**Reagents.** Unless otherwise stated, all chemical were purchased from Sigma Chemical (St. Louis, MO). Collagenase dissociation buffer consisted of 170 mM Na-glucuronate, 4.7 mM KCl, 5 mM HEPES, 5 mM glucose, and 0.125% (wt/vol) Sigma type 1A collagenase, whereas artificial aqueous humor (AAH) consisted of 149 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl\(_2\), 5 mM glucose, and 5 mM HEPES (pH 7.4), with mannitol added to 300 mosmol/kg. Glutamate-, NO\(_3\) -, and I\(^-\)–based AAH solutions were prepared by equimolar substitution of NaCl with either Na-glucuronate, NaNO\(_3\), or NaI. Na\(^+\)-free AAH was similarly prepared by equimolar substitution of NaCl with N-methyl-D-glucamine (NMDG) and adjusted to pH 7.4 with 10 M HCl. All experiments were conducted at room temperature (~20°C).

**Isolation of lens fiber cells.** Three- to four-week-old Wistar rats were killed by CO\(_2\) asphyxiation and spinal dislocation in accordance with protocols approved by the University of Auckland Animal Ethics Committee. Enucleated eyes were placed into AAH on the stage of a dissecting microscope. Four radial cuts were made from the optic nerve head through the sclera as far as the border of the cornea. Two of the scleral flaps were grasped with forceps and vortexed to free the lens. The lens was then dissected from the ciliary body by cutting the suspensory ligaments. Sharpened forceps were then used to gently remove the capsule and epithelial and fiber cells attached to it. Capsules with adherent fiber cells were transferred immediately to an Eppendorf tube filled with 1 ml of collagenase dissociation buffer in a water bath at 35°C for 35–40 min. Fiber cells were lightly vortexed and centrifuged at 1,000 rpm for 2 min before gentle resuspension in a minimal volume of AAH (~200 \(\mu\)l) containing 2.5 mM Ca\(^{2+}\) with either 1 or 3 mM Gd\(^{3+}\). Isolated cells were plated into a recording chamber, the bottom of which was formed by a poly-l-lysine-coated glass coverslip. After cells were left to settle and adhere for ~5 min, the bath was overlaid with solution to give a bath volume of ~1 ml.

**Electrophysiological recording.** The chamber was mounted on the stage of an inverted microscope (Nikon Eclipse, Nikon, Melville, NY) fitted with differential interference contrast optics. The recording chamber was constantly perfused under gravity at a rate of ~1–2 ml/min. Test solutions were injected in 1.5-ml volumes via a custom-made low-deadspace manifold fabricated from 10-\(\mu\)l pipette tips using a TTL-driven peristaltic pump (Gilson Minipulse, Middleton, WI) and a network of multiway taps to control solution flow. Wash out of solutions was achieved via continuous aspiration of the bath surface from a connected bath compartment to give a constant bath volume. All single cell electrophysiological experiments were conducted at “room temperature” (~20°C) and used solutions that contained 1 mM GdCl\(_3\) to prevent fiber cell vesiculation. Patch clamping was performed using a Multiclamp 700A amplifier (Axon Instruments, Union City, CA) that was fitted with two matching high-impedance headstages. Patch electrodes were mounted directly on these headstages, which were positioned using a pair of piezo-electric manipulators (Burleigh PCS-5000, Exfo Life Sciences, Mississauga, Ontario, Canada). Because of their elongated morphology, there was an initial concern that voltage clamp in the distal parts of the fiber cell could be partially attenuated, leading to space clamp errors that may affect the accuracy of calculated membrane parameters (26, 28). In an effort to minimize such errors, seals were formed as close to the midpoint of a fiber cell as practicable, thereby halving the linear distance required to voltage clamp cell extremities, and series resistance (\(R_s\)) was kept to a minimum (17.21 \(\pm\) 0.76 \(\Omega\), n = 196). Data were digitized using a Digidata 1322A SCSI analog-to-digital converter (Axon Instruments) into a PC Pentium II 266-MHz computer running the pCLAMP version 8.1 software package (Axon Instruments). Images were acquired via a cooled charge-coupled device camera (Photometrics Imagepoint, Roper Scientific, Tuscon, AZ).
membrane potential. The bath was grounded by the connection of an AgCl-coated silver wire via a 3% agarose “salt bridge” made with pipette solution. Pipettes of optimal geometry had resistances of between 3 and 5 MΩ when filled with this solution. The bath was grounded by the connection of an AgCl-coated silver wire via a 3% agarose “salt bridge” made with pipette solution. 

Electrodes were pulled using a horizontal pipette puller (model P-77, Sutter Instruments), lightly fire polished, and backfilled with filtered pipette solution [containing (in mM) 10 NaCl, 130 K-gluconate, 1.3 CaCl2, 4.1 MgCl2, 5 HEPES (pH 7.4); 300 mosmol/kg]. Pipettes of optimal geometry had resistances of between 3 and 5 MΩ when filled with this solution. 

Current responses to voltage steps and ramps were usually filtered at 2 kHz using the inbuilt 4-pole Bessel filter and sampled at 10 kHz except in the case of capacitive transient analysis, where sampling was at 100 kHz with the anti-aliasing filter set at 10 kHz. Current records were analyzed offline using Clampfit version 9.0 (Axon Instruments). Curve fitting of capacitive transients were used to calculate membrane resistance (Rm), membrane capacitance (Cm), and Rm. Since membrane area is directly proportional to Cm and biological membranes have an approximate area capacitance of 1 μF/cm², cell membrane area (Am) was directly estimated from Cm (Am = Cm/k, where k = 1 μF/cm²). To enable comparison between fiber cells of vastly different membrane area, area-specific conductance (ρs) was derived by relating total membrane conductance (gm = 1/Rm) to Am, thus giving values (in units of pS/μm²) of the fiber cell membrane (ρs = gmAm, where Am is measured in units of μm²). 

Quantification of ρs also allowed estimation of cell cable properties in a manner similar to that applied to neurons with elongated processes (26, 27). This geometric approximation describes how voltage partitions along the intracellular conductive route [intracellular resistance (Ri; in Ωcm² or Ω/cm)] in the presence of parallel conductance pathways across the membrane (ρs) and depends directly on the cell (or process) diameter (d; in cm) and the ratio between membrane and intracellular resistivity per unit length. Thus,

![Figure 1](http://ajpcell.physiology.org/)

**Table 1. Improvement in fiber cell membrane properties in the presence of multivalent cations**

<table>
<thead>
<tr>
<th>Ca²⁺ Concentration</th>
<th>0 mM Ca²⁺</th>
<th>AAH</th>
<th>1 mM Ca²⁺</th>
<th>3 mM Ca²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>E_rev (mV)</td>
<td>-17.86±6.35 (10)</td>
<td>1.70±2.12 (10)</td>
<td>-32.55±5.09 (38)</td>
<td>-29.78±4.26 (53)</td>
</tr>
<tr>
<td>Rm (MΩ)</td>
<td>405.71±123.84 (14)</td>
<td>82.73±19.96 (11)</td>
<td>2366.46±371.36 (40)</td>
<td>2357.75±441.61 (72)</td>
</tr>
<tr>
<td>ρs (pS/μm²)</td>
<td>2.47±0.51 (17)</td>
<td>4.30±0.62 (40)</td>
<td>1.09±0.19 (36)</td>
<td>0.67±0.05 (103)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses are numbers of cells. E_rev, reversal potential; Rm, membrane resistance; ρs, area-specific conductance.

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where $\lambda$ is the membrane electrotonic length constant. $R_i$ has been estimated in rat lens fiber cells at $\sim 110 \, \text{M} \Omega \cdot \text{cm}$ by impedance methods (1).

The relative permeability of membrane conductance to different anions was assessed by measuring their effect on current reversal potential ($E_{\text{rev}}$), and the ratio of anion permeability to Cl$^-$ permeability ($P_X/P_{\text{Cl}}$) calculated as follows:

$$P_X = \exp \left( \frac{\Delta E_{\text{rev}}}{RT/2F} \right) \frac{[\text{Cl}^-]_o - [\text{Cl}^-]_i}{[\text{Anion}]_o} \tag{2}$$

where $R$ is the gas constant, $T$ is temperature, $z$ is electron valence, $F$ is Faraday’s constant, $[\text{Cl}^-]_o$ is the extracellular Cl$^-$ concentration, $[\text{Cl}^-]_i$ is $[\text{Cl}^-]_i$, in the new bathing solution, and $[\text{Anion}]_o$ is anion concentration. The “junction potential calculator” interface within Clampex version 8.1 (Axon Instruments) was used to correct $E_{\text{rev}}$ for junction potentials that arise during ion substitution experiments due to the symmetrical AgCl half-cell composition of the electrode connections.

Whole lens dissection and electrophysiological recording. Four posterior incisions were made in the extracted rat eyes to create four scleral flaps that were pinned out into a custom-made recording chamber that was transferred to the stage of a dissecting microscope (Stemi SV 11, Zeiss, Frankfurt, Germany). The pinned-out lens preparation was overlaid with warmed AAH (37°C) and continually perfused ($\sim 2 \, \text{ml/min}$) with AAH. The recording chamber (2 ml) was perfused using three solution reservoirs independently controlled by three-way taps via a manifold with $\sim 2 \, \text{ml}$ of downstream deadspace. Lenses were impaled with glass microelectrodes (150F-10, Clark Electromedical Instruments) manufactured using a pipette puller (P-77 Micropipette Puller, Sutter Instruments, Novato, CA) and had a resistance of between 3 and 5 M$\Omega$ when backfilled with 1 M KCl. Microelectrodes were attached to the headstage of a microelectrode amplifier (TEV-200, Dagan, Minneapolis, MN). The bath was grounded by connection to an AgCl-coated silver wire 1 M KCl half-cell via a 3% agarose-1 M KCl salt bridge. Lens potential recordings were sampled at 1 Hz using a DigiData 1200B analog-to-digital converter (Axon Instruments) and recorded using Axoscope version 8.1 (Axon instruments) running on a Celeron 500-MHz PC under Windows 2000. The headstage was mounted on a micromanipulator (World Precision Instruments, Sarasota, FL) that was attached to the stage of the dissecting microscope. This electrophysiological setup was sur-

![Fig. 2](http://ajpcell.physiology.org/) Identification of activated isolated fiber cells. A: plot of $\rho_s$ versus cell length for all isolated fiber cells in the data set ($n = 196$). The conductance ($g_s$; dashed line) calculated for internalized rat lens fiber membranes in whole lenses (1) was used to partition fiber cells into “activated” cells ($\bullet$; $\rho_s > g_s$) or cells of physiologically relevant conductance ($\bullet$; $\rho_s < g_s$). B: plot of $R_m$ versus length. Note the presence of $\rho_s < g_s$ cells ($\bullet$) among $\rho_s > g_s$ cells ($\circ$), indicating that $R_m$ alone is not a good indicator of fiber cell viability. C: plot of $\rho_s$ versus cell length replotted to show only the those cells where $\rho_s < g_s$ ($n = 126$). In this subset of the original data set, considerable variability in $\rho_s$ still existed among fiber cells of various lengths. D: plot of length-dependent changes in whole cell current $E_{\text{rev}}$ of fiber cells from the $\rho_s < g_s$ subset overlaid with calculated values for the equilibrium potentials for K$^+$ ($E_K$), Cl$^-$ ($E_{\text{Cl}}$), and nonselective cation ($E_{\text{NSC}}$) conductances. As cells elongated, there appeared to be a shift from $E_K$ in shorter cells to a more depolarized $E_{\text{rev}}$, dominated by $E_{\text{Cl}}/E_{\text{NSC}}$ in longer cells. Note, however, that a subset of cells of all lengths possessed this apparently depolarized characteristic despite displaying a physiological conductance level ($\rho_s < g_s$).
MEMBRANE PROPERTIES OF DIFFERENTIATING LENS FIBER CELLS

Table 2. Membrane properties of isolated fiber cells split by the $g_s$ criterion

<table>
<thead>
<tr>
<th></th>
<th>Nonactivated $p_s &lt; g_s$</th>
<th>Activated $p_s &gt; g_s$</th>
<th>$P$ Value (Activation Effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_m$, MΩ</td>
<td>3.597.70 ± 1.855.99 (126)</td>
<td>175.77 ± 32.07 (63)</td>
<td>0.628 (not significant)</td>
</tr>
<tr>
<td>$p_s$, pS/µm²</td>
<td>0.59 ± 0.04 (126)</td>
<td>7.05 ± 0.80 (61)</td>
<td>&lt;10⁻⁵*</td>
</tr>
<tr>
<td>$E_{rev}$, mV</td>
<td>-26.61 ± 2.53 (95)</td>
<td>-14.12 ± 2.52 (49)</td>
<td>0.060 (not significant)</td>
</tr>
<tr>
<td>Electrotonic decay lengths/cell, $\lambda$/length</td>
<td>0.07 ± 0.01 (126)</td>
<td>0.18 ± 0.01 (63)</td>
<td>&lt;10⁻⁵*</td>
</tr>
<tr>
<td>Applied $V_m$, %command</td>
<td>93.03 ± 0.52</td>
<td>84.17 ± 1.13</td>
<td></td>
</tr>
</tbody>
</table>

Values are means SE. Numbers in parentheses are numbers of cells. $g_s$, physiological value for conductance; $\lambda$, membrane electrotonic length constant; $V_m$, membrane potential. *Significant at the $P < 0.001$ level.

rounded by a Faraday cage positioned on a vibration isolation table (Newport, Irvine, CA). Mean lens potential was determined at baseline and both before and after exposure to solutions of a varying ionic composition. The relative permeability of the lens to different anions ($P_d/P_{cl}$) was assessed by measuring their effect on membrane potential and calculated by substitution of $\Delta V_m$ for $\Delta E_{rev}$ in Eq. 2. Summarized data were plotted in Microsoft Excel (Microsoft, Redmond, WA), and statistical analysis was performed using Origin version 7.5 (Originlab, Northampton, MA).

RESULTS

The enzymatic dissociation of the lens in Ca²⁺-containing AAH initially produces numerous isolated fiber cells of a range of lengths. However, over time, a destructive leak conductance becomes activated, which is permeable to Ca²⁺ and induces fiber cell vesiculation (6, 21). While this Ca²⁺-mediated vesiculation of fiber cells can be prevented by isolating cells in the absence of extracellular Ca²⁺ (6), the removal of extracellular Ca²⁺ activates a large nonphysiological leak conductance (18, 19) and compromises the adhesion of the isolated fiber cells to the recording chamber, making chamber perfusion difficult. Webb et al. (54) showed that the inclusion of Gd³⁺, a broad-spectrum inhibitor of nonselective cation channels (25) and gap junction hemichannels (20), to Ca²⁺-containing AAH prevented fiber cell vesiculation and promoted the adhesion of cells to the recording chamber. Whole cell patch analysis showed that cells incubated in Gd³⁺ exhibited an elevated $R_m$, lower $p_s$, and a more hyperpolarized $E_{rev}$ compared with cells incubated in either normal AAH or AAH in which Ca²⁺ had been removed (Table 1). Thus, the ability of Gd³⁺ to prevent vesiculation of isolated fiber cells is also associated with a generalized improvement in electrical properties of isolated fiber cells, suggesting that the inhibition of the nonselective leak conductance prevents the Ca²⁺ influx responsible for the activation of the cytoplasmic Ca²⁺-dependent proteases known to initiate vesiculation of isolated fiber cells (6, 52, 53).

Thus, in the presence of 1 mM Gd³⁺, the electrophysiological properties of a large number ($n = 196$) of isolated fiber cells of varying lengths (23–605 µm), and therefore different states of differentiation, could be studied in the presence of physiological extracellular Ca²⁺ (Fig. 1). Plotting $R_m$ (Fig. 1A), $p_s$ (Fig. 1B), and $E_{rev}$ (Fig. 1C) against fiber cell length indicated that these membrane parameters appeared to be related to fiber cell length. However, within this data set, there was a large variability in membrane parameters. This is highlighted by comparing the current profiles of two pairs of selected cells of similar length. The two short fiber cells presented display dramatically different membrane properties (Fig. 1, D–F) despite being of very similar length. One cell (Fig. 1D, 68 µm) exhibited a low $p_s$ (0.47 pS/µm²) and a hyperpolarized $E_{rev}$ (Fig. 1F, −88 mV). In contrast, another short fiber cell of similar length (Fig. 1E, 60 µm) exhibited a very high $p_s$ (3.21 pS/µm²), a linearization of the current-voltage ($I$-$V$) relationship, and a profound depolarization of $E_{rev}$ (Fig. 1F, −1 mV), which are parameters characteristic of the activation of a nonselective cation channel. A similar pattern was found when comparing longer fiber cells. While one cell selected from this length range (Fig. 1G, 357 µm) had a low $p_s$ (0.096 pS/µm²) and a hyperpolarized $E_{rev}$ (Fig. 1I, −53 mV), another cell of similar length (Fig. 1H, 383 µm) had a high $p_s$ (1.36 pS/µm²), a linearized $I$-$V$ relationship, and a

Fig. 3. Average $I$-$V$ relationships for isolated fiber cells. A: steady-state $I$-$V$ relationship for the $p_s < g_s$ subset of physiological relevant isolated fiber cells. ● Short fiber cells ($n = 32$); □ medium fiber cells ($n = 16$); ○, long fiber cells ($n = 10$). B: steady-state $I$-$V$ relationship of the $p_s < g_s$ subset of isolated fiber cells shown in A but normalized ($I_{scam}$) to membrane area (in pS/µm²).
depolarized $E_{rev}$ (Fig. 1I, +0.4 mV), again indicative of an increase in nonselective cation permeability.

Thus, it appears that a subset of fiber cells within our data set exhibit a nonphysiological leak conductance even in the presence of 1 mM Gd$^{3+}$, which is presumably activated by the dissociation process. The presence of these cells tends to exacerbate the variability observed in our data set, making it difficult to assess whether the fiber cells of different lengths, and therefore stages of differentiation, exhibit alterations in their membrane transport properties. To objectively exclude cells that exhibited this leak conductance, a “physiological” value for the conductance ($g_s$) of internalized rat lens fiber membranes of 2.02 pS/$\mu$m$^2$, originally calculated from whole lens impedance analysis (1), was adopted as a threshold for the acceptance of individual cells for further analysis (Fig. 2A). A comparison of fiber cell groups split according to the $g_s$ criterion highlights the drastic changes in membrane properties associated with fiber cell activation (Table 2). Activated ($\rho_s > g_s$) fiber cells exhibited markedly lower $R_m$ and a higher $\rho_s$ relative to $\rho_s < g_s$ fiber cells of the same length. These changes were associated with a depolarization of $E_{rev}$, indicating a loss of membrane selectivity concomitant with their activation. Because of their similar membrane properties recorded in vitro to those measured in lento, the $\rho_s < g_s$ group can be looked at as representing cells with properties as close as possible to those measured in healthy intact lenses. In contrast, the activated $\rho_s > g_s$ group displayed leaky membrane characteristics similar to those observed in vesiculating fiber cells. By plotting $R_m$ versus length, it can be seen that $\rho_s > g_s$ and $\rho_s < g_s$ cells overlap significantly on the $R_m$ axis (Fig. 2B), underscoring why membrane resistance alone is a poor marker of fiber cell membrane integrity. The use of $g_s$ as a selection criterion for activated cells cleanly split the fiber cell population independent of fiber cell length, allowing us to focus on cells with physiologically relevant membrane properties (Fig. 2, C and D). To gain an overview of how membrane permeability

Fig. 4. Ba$^{2+}$ blocks an inwardly activating current in short isolated fiber cells, whereas its effect on longer fiber cells is minimal. Whole cell currents were recorded from a short fiber cell in the presence of artificial aqueous humor (AAH) + 1 mM Gd$^{3+}$ (A), after the addition of 5 mM Ba$^{2+}$ (B), and after a wash with AAH + 1 mM Gd$^{3+}$ for 5 min (C). Note that the inward current (arrow) did not return in this cell despite protracted perfusion to remove the inhibitor. D: image of the fiber cell from which the currents were recorded (cell length = 37 $\mu$m). E: whole cell $I$-$V$ relationships extracted from the currents shown in A ($\bullet$), B ($\triangle$), and C ($\diamond$). In contrast, the effect of K$^+$ channel inhibitors on longer fiber cells was minimal. F: whole cell currents recorded from a longer cell (cell length = 165 $\mu$m) in AAH + 1 mM Gd$^{3+}$. G: currents from the same cell in 5 mM Ba$^{2+}$. Note the minor alteration in whole cell current. H: currents in the same cell in 5 mM tetraethylammonium (TEA). Note the persistence of the dominant outward current. I: presented isolated fiber cell under patch clamp (cell length = 165 $\mu$m). J: whole cell $I$-$V$ relationships extracted from the currents shown F ($\bullet$), G ($\triangle$), and H ($\diamond$).
changes as fiber cells elongate, we plotted \( E_{\text{rev}} \) recorded from \( \rho_s < g_s \), cells against cell length (Fig. 2D). The plot clearly illustrates that, despite the inherent variability in \( E_{\text{rev}} \), the observed values for \( E_{\text{rev}} \) tended to cluster near the expected equilibrium potential values for either \( K^+ (E_K) \), \( Cl^- (E_{Cl}) \), or nonselective conductances (\( E_{NSC} \)). From this analysis, it is apparent that while the majority of cells of all lengths clustered around or between \( E_{CI} \) and \( E_{NSC} \), only a subset of short fiber cells clustered near \( E_K \). Thus, cell elongation appears to be associated with a change in membrane properties, which can explain the observed variability in our data set.

However, it is also possible that the elongated morphology of the fiber cells could lead to space-clamp errors that caused the voltage clamping in the distal parts of the fiber cell to be attenuated, thereby affecting the accuracy of calculated membrane parameters (26, 27). While every effort was made to minimize such errors, by forming seals in the midpoint of a fiber cell to halve the linear distance required to voltage clamp the cell’s extremities and by minimizing \( R_s \), we thought it prudent to investigate the potential effects of space-clamp errors on recorded membrane parameters. To achieve this, the electrotonic length constant was calculated for each cell (Table 2), and the maximal voltage error experienced at the tips of the cell under voltage clamp was determined (26, 28). This analysis showed that even in the longest cells with the highest \( \rho_s \), the transmembrane voltage at membrane extremities of such cells exhibited only a 0.5 e-fold decrease in command potential, indicating that space-clamp errors were not the source of the observed length-related trends in membrane parameters. This analysis also showed that recordings from the activated fiber cells were more affected by space-clamp errors, since the average electrotonic decay length (\( \lambda \)) was half as long in this group of cells (Table 2), another objective reason for excluding this group of cells from further analysis.

Differentiation-dependent changes in membrane permeability of isolated fiber cells. At the equator lens, epithelial cells differentiate and elongate to form fiber cells. This process establishes a gradient of progressively longer and older cells that extends from the periphery to the center of the lens. Previously, we have shown that the length of isolated fiber cells is essentially equivalent to their length measured in the intact lens in vivo, and, therefore, the length of an isolated fiber is indicative of its differentiation state (54). To investigate whether the observed changes in the membrane properties of isolated fiber cells were differentiation dependent, cells were binned into three arbitrary length categories: short fiber cells (<150 \( \mu m \)), medium fiber cells (150 > medium fiber cell > 300 \( \mu m \)), and long fiber cells (>300 \( \mu m \)). Using these length groups, average \( I-V \) relationships for each of the three \( \rho_s < g_s \) groups were obtained and plotted as both absolute currents (Fig. 3A) and currents normalized to membrane capacitance (\( I/\text{Norm} \), Fig. 3B). The absolute current carried at all potentials by short fiber cells was found to be lower than for the two longer cell groups (medium and long fiber cells), which were indistinguishable in terms of both the shape and magnitude of their \( I-V \) responses (Fig. 3A). This similarity between the longer cell groups was preserved when currents were normalized to membrane capacitance (Fig. 3B), with both medium and long fiber cell groups overlying one another. However, normalization of currents to membrane area revealed that short fiber cells conducted approximately threefold more current per unit area in the outward direction than longer cells and also possessed an inwardly activating current component that altered the characteristic outwardly rectifying fiber cell \( I-V \) relationship at potentials below \(-80 \text{ mV} \) (Fig. 3B). The activation of an additional, inwardly activating conductance at potentials hyperpolarized to \(-80 \text{ mV} (=E_K) \) implies the presence of a conductance component in short fiber cells that is absent in longer fiber cells. The differences in \( I-V \) plots between shorter fiber cells and longer fiber cells (medium and long fiber cell groups), as shown in Fig. 3B, plus the wide variability in \( E_{\text{rev}} \) observed among cells of all lengths (Fig. 2D) implies that the underlying channel population giving rise to these currents may be more diverse than previously thought. Indeed, the gross morphology of whole cell currents was often observed to differ substantially even among cells of very similar lengths and baseline membrane properties. To further

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Fig. 5. TEA blocks an outwardly rectifying current in short isolated fiber cells. Whole cell currents were recorded from a short fiber cell in the presence of AAH + 1 mM Gd\(^{3+} \) (A), after the addition of 5 mM TEA (B), and then after the addition of 5 mM Ba\(^{2+} \) (C). D: image of the fiber cell from which the currents were recorded (cell length = 45 \( \mu m \)). E: whole cell \( I-V \) relationships extracted from the currents shown in A (●), B (○), and C (●).
investigate this diversity, pharmacological and ion substitution experiments were performed.

Characterization of K\(^+\) permeability in isolated fiber cells. Since Ba\(^{2+}\) and TEA are known to be effective inhibitors of K\(^+\)-selective channels in lens epithelial cells (9, 43, 44), these cationic compounds were used to probe for the presence of epithelial-like K\(^+\) channels in isolated fiber cells. The whole cell current morphologies of isolated fiber cells were highly variable and displayed varying sensitivity to the addition of Ba\(^{2+}\) (Fig. 4) and/or TEA (Fig. 5). Typical responses of a short fiber cell to exposure to 5 mM Ba\(^{2+}\) in the presence of 1 mM Gd\(^{3+}\) are shown in Fig. 4, A–E. This cell exhibited an inwardly activating conductance component (Fig. 4A, arrow). Ba\(^{2+}\) addition rapidly inhibited this inward conductance (Fig. 4B) and caused a depolarization of \(E_{\text{rev}}\) (Fig. 4E). While the effect of Ba\(^{2+}\) on an inward current component was a common response in short fiber cells, it was not always reversible (Fig. 4C). An example of a short fiber cell that lacked the Ba\(^{2+}\)-sensitive inwardly activating component but was sensitive to TEA is shown in Fig. 5. In this cell, exposure to 5 mM TEA reduced the outwardly rectifying conductance but did not alter cellular \(E_{\text{rev}}\) (Fig. 5, B and E). Washout of TEA did not always result in the return of the inhibited conductance, whereas the remaining whole cell current in this cell proved insensitive to Ba\(^{2+}\) (Fig. 5, C and E). In short fiber cells, Ba\(^{2+}\) addition inhibited an inwardly activating current component in 10 of 14 fiber cells tested (74.4%). In contrast, the addition of TEA to short fiber cells inhibited an outwardly rectifying conductance in 9 of 13 cells (69.2%). Of these TEA-sensitive cells, eight cells were also responsive to the addition of Ba\(^{2+}\), indicating that Ba\(^{2+}\)-sensitive and TEA-sensitive K\(^+\) conductances may or may not be coexpressed in individual cells. Thus, it appears that short fiber cells contain a similar heterogeneity of K\(^+\) conductances to that previously observed in the lens epithelium (47). In contrast to short fiber cells, longer fiber cells (from the medium and long fiber cell groups) were relatively insensitive to the addition of K\(^+\) channel inhibitors (Fig. 4, F–K), indicating that Ba\(^{2+}\)- and TEA-sensitive conductances in these longer cells were either absent or represent only a minor component that is overwhelmed by a dominant TEA-insensitive outwardly rectifying current.

Fig. 6. Differential effects of K\(^+\) channel inhibitors on lens fiber cells of different lengths. Normalized average \(I-V\) relationships showing Ba\(^{2+}\)-sensitive (A) and TEA-sensitive (B) currents were extracted as difference currents (\(I_{\text{Diff, Norm}}\)) for each length group. • Short fiber cells (<150 \(\mu\)m); ○, medium fiber cells (150 \(\mu\)m > medium fiber cell > 300 \(\mu\)m); □, long fiber cell (>300 \(\mu\)m). Ba\(^{2+}\)- and TEA-sensitive currents were markedly different from those recorded from the medium and long fiber cell groups. In short fiber cells, the Ba\(^{2+}\)-sensitive current (A) exhibited a strong inwardly activating component and a negative \(E_{\text{rev}}\), whereas the TEA-sensitive current (B) was an outwardly rectifying current. In medium and long fiber cells, both difference currents were small, linear, and exhibited positive \(E_{\text{rev}}\).

Fig. 7. Variable expression of an outwardly rectifying Cl\(^-\) conductance in isolated fiber cells of different lengths. Whole cell currents were recorded in the presence of extracellular Cl\(^-\) and after its replacement with the impermeant anion gluconate in cells representative of the different length groups. A: short fiber cell (23 \(\mu\)m long); B: short fiber cell (25 \(\mu\)m long); C: medium fiber cell (165 \(\mu\)m long); D: long fiber cell (475 \(\mu\)m long).
To highlight the differential effects of K⁺ channel inhibitors on fiber cells of different lengths, inhibitor-sensitive difference currents were obtained by the subtraction of I-V relationships obtained in the presence and absence of two K⁺ channel inhibitors (Fig. 6). These difference currents were then normalized to cell capacitance, and the resultant I-V plots represent the average contribution of Ba²⁺-sensitive (Fig. 6A) and TEA-sensitive (Fig. 6B) currents to the whole cell current in the different length groups. The average normalized Ba²⁺-sensitive currents showed clear differences between the short fiber cell and medium/long fiber cell groups (Fig. 6A). In the short fiber cell group, the Ba²⁺-sensitive current was an inwardly activating conductance, which reverses at a potential indicative of a K⁺-selective channel (approximately −77 mV ≈ E_K). At potentials hyperpolarized to E_K (V_m < −80 mV), the conductance becomes strongly voltage activated. In the outward direction, the effect of Ba²⁺ is variable on this cell group, as evidenced by the larger error in this region. The E_rev and I-V relationship of this Ba²⁺-sensitive current in the short fiber cell group strongly indicates the inhibition of a Kᵢᵢ channel of a type similar to that reported in the lens epithelium in many species (47). The TEA-sensitive current present in short fiber cells was strongly outwardly rectifying, of relatively large magnitude, reversed near E_K, and activated at depolarized membrane potentials (Fig. 6B). The shape of the averaged difference current and the magnitude of the E_rev were compounded by the presence of non-TEA-sensitive cells within the data set, but the presence of an outwardly rectifying, epithelial-like K⁺ conductance was distinguishable on the basis of the I-V shape and E_rev (47).

In contrast to the epithelial-like K⁺ conductances observed in short fiber cells, Ba²⁺- and TEA-sensitive currents were relatively minor in longer fiber cells. Difference currents in longer fiber cells exposed to either inhibitor exhibited a relatively linear I-V relationship and positive E_rev (Fig. 6A,B), suggesting that the effects of Ba²⁺ and TEA on whole cell current are mediated via an interaction with some nonselective leak pathway, which is different from the currents inhibited in short fiber cells. This possibility is supported by previous experiments conducted in whole lenses (29, 48). In these experiments, a nonselective leak conductance induced in whole lenses by the removal of Ca²⁺ was partially attenuated by the addition of Ba²⁺. Although there is no documented interaction thus far of TEA with nonselective conductances in the lens, this linear, positive, low-magnitude difference current is clearly distinct from the outwardly rectifying K⁺ conductance inhibited by TEA in short fiber cells.

Thus, the pharmacological characterization of K⁺ channels in isolated fiber cells has revealed that newly differentiated short fiber cells contain K⁺-selective currents, which are reminiscent of those reported in the lens epithelium by numerous investigators (for a review, see Ref. 47). As seen in epithelial cells, outwardly rectifying K⁺ channels and Kᵢᵢ channels in short fiber cells appear to exist in parallel with each other, but neither are present to the same extent in all cells. In contrast, longer, more-differentiated fiber cells were dominated by other

Fig. 8. Anion selectivity of the outwardly rectifying Cl⁻ conductance in isolated fiber cells. Representative whole cell currents were recorded in AAH + 1 mM Gd⁺⁺ (A) and after equimolar replacement of extracellular Cl⁻ with NO₃⁻ (B), I⁻ (C), and gluconate (D). E: whole cell I-V relationships extracted for the membrane currents recorded in the presence of extracellular Cl⁻ (○), NO₃⁻ (●), I⁻ (△), and gluconate (○). Inset, image of the cell under whole cell patch clamp (cell length = 275 μm). F: average I-V relationships obtained using data pooled from all three length groups (means ± SE). G: average I-V relationships extracted for only the short fiber cell (≤150 μm) length group (shown without y-error bars for clarity).
conductances that were not inhibited by these classic K+ channel inhibitors, indicating that a shift away from a dominant K+ channel occurs as cells elongate.

Characterization of anion permeability in isolated lens fiber cells. Since the solubility of anion channel inhibitors was compromised by the presence of Gd³⁺ in the bathing medium (54), anion replacement experiments rather than pharmacological experiments were used to characterize the contribution of anion channels to the membrane properties of isolated fiber cells of different lengths. To initially determine the contribution anion channels make to overall membrane current, extracellular Cl⁻ was replaced with the impermeant anion gluconate. While gluconate replacement resulted in the reduction of an outwardly rectifying conductance recorded from the majority of fiber cells from all length groups, the magnitude of whole cell current alteration on gluconate replacement varied considerably between cells (Fig. 7). Fiber cells from the short fiber cell group appeared to display the most variability in their response to the removal of extracellular Cl⁻ (Fig. 7 A and B). Those SFCs that initially exhibited a large outwardly activating conductance displayed a large reduction in outward current upon the removal of extracellular Cl⁻ (Fig. 7A), whereas other cells of similar lengths but with a different current profile displayed a more modest reduction in current upon replacement of Cl⁻ with gluconate (Fig. 7B). In contrast, all longer fiber cells [from the medium (Fig. 7C) and long (Fig. 7D) fiber cell groups] consistently responded to the replacement of Cl⁻ with gluconate with a large inhibition of the outwardly rectifying current.

To further classify the type of anion channel expressed in isolated fiber cells, the relative permeability of the conductance to the permeant anions Cl⁻, NO₃⁻, and I⁻ was assessed (30, 31, 42). While there was variability in the magnitude of current alteration caused by ion replacement among isolated fiber cells, the majority of cells examined displayed current alterations similar to those shown in Fig. 8. In this representative cell from the middle of the length range (275 μm), sequential exposure to extracellular Cl⁻, NO₃⁻, I⁻, and gluconate altered whole cell current and induced shifts in Erev. Exposure to NO₃⁻ and I⁻ triggered a graded increase in outward current relative to Cl⁻ (Fig. 8, B and C), and a hyperpolarization of Erev. In contrast, gluconate replacement in this cell greatly reduced outward current (Fig. 8D) and caused significant depolarization of Erev (Fig. 8E).

The pooled results from all anion substitution experiments over a range of fiber cell lengths (n = 33, lengths of 23–475 μm) are shown in Fig. 8F and were used to calculate the relative permeability of the outwardly rectifying conductance to anions (Pₓ/PCl⁻; Table 3). From the analysis of this pooled data set, it is evident that the fiber cell anion conductance has a permeability sequence of NO₃⁻ > I⁻ > Cl⁻ ≫ gluconate. Interestingly, we observed some variability in the anion replacement responses between fiber cells of different lengths. While replacement of extracellular Cl⁻ with either I⁻ or NO₃⁻ always hyperpolarized Erev in fiber cells from all length groups, gluconate exposure caused a depolarization in Erev in medium and long fiber cells. In short fiber cells, gluconate replacement caused a hyperpolarization of Erev toward EK (Fig. 8G) and a reduction in the whole cell current magnitude. Thus, the variability in the magnitude of whole cell current alteration on replacement of extracellular Cl⁻ observed in short fiber cells (Fig. 7, A–D) likely reflects the observed variability in the degree of the activation of the anion conductance in this group of cells. The earlier demonstration of dominant K+ conductances in a subgroup of short fiber cells (Fig. 2D) suggests that the reduction in anion conductance caused by gluconate replacement leads to the reemergence of K+ conductances as the main contributor to Erev in these shorter, younger cells. In longer cells (medium and long fiber cells), removal of extracellular Cl⁻ leads to almost complete depolarization of Erev, suggesting that the membrane properties of longer fiber cells are dominated by an anion conductance. Thus, the slightly negative Erev of the characteristic outwardly rectifying fiber cell whole cell current is set by the activity of an anion conductance whose influence on fiber cell membrane properties appears to increase as cells differentiate.

To directly test whether the observed anion conductance in isolated fiber cells is a physiological feature of the lens at rest or an artifact of the dissociation process, whole lenses were impaled with a microelectrode and the response of Vm to changes in the anion composition of the bath solution was monitored. Vm was found to be modulated by extracellular anion replacement in a substantially similar fashion to that observed for Erev in isolated fiber cells (Table 3). Replacement of extracellular Cl⁻ with either NO₃⁻ or I⁻ caused a hyperpolarization of Vm, whereas replacement with gluconate caused a depolarization. Thus, it appears that fiber cells within the intact lens possess a constitutively active anion conductance that displays a similar anion selectivity to that observed in isolated fiber cells (PX/PCl⁻; Table 3). This conductance appears to make only a minor contribution to the membrane properties of shorter, more epithelial-like fiber cells, which are dominated by K+ conductance(s) at rest.

### Table 3. Ionic selectivity of anion conductances in isolated fiber cells and intact lenses probed by extracellular anion replacement

<table>
<thead>
<tr>
<th></th>
<th>Cl⁻</th>
<th>I⁻</th>
<th>NO₃⁻</th>
<th>Gluconate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated fiber cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erev, mV</td>
<td>-20.28±3.30 (33)</td>
<td>-34.76±4.94 (25)</td>
<td>-40.00±6.50 (13)</td>
<td>-9.54±4.70 (9)</td>
</tr>
<tr>
<td>ΔErev, mV</td>
<td>-16.58±4.39</td>
<td>-23.07±4.09</td>
<td>2.59</td>
<td>11.89±9.26</td>
</tr>
<tr>
<td>Pₓ/PCl⁻</td>
<td>1.00</td>
<td>1.99</td>
<td>2.96 (7)</td>
<td></td>
</tr>
<tr>
<td>Intact lenses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vm, mV</td>
<td>-60.36±3.26 (11)</td>
<td>-67.13±2.96 (7)</td>
<td>-77.04±3.89 (5)</td>
<td>-57.12±4.42 (10)</td>
</tr>
<tr>
<td>ΔVm, mV</td>
<td>-7.21±7.31</td>
<td>-8.24±0.86</td>
<td>7.04±9.54</td>
<td></td>
</tr>
<tr>
<td>Pₓ/PCl⁻</td>
<td>1.00</td>
<td>1.35</td>
<td>1.44</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses are numbers of cells. PX, anion permeability; PCl⁻, Cl⁻ permeability.
Characterization of nonselective permeability in isolated lens fiber cells. Although the inhibition of a nonselective cation conductance with Gd$^{3+}$ was a prerequisite for obtaining viable isolated fiber cells, it is evident that a residual leak conductance remains in the presence of Gd$^{3+}$, since in many isolated fiber cells $E_{rev}$ was depolarized from $E_K$ and $E_{Cl}$ and clustered near $E_{NOSC}$ (Fig. 2D). This contribution of nonselective leak conductance to overall membrane current is shown in Fig. 9. Membrane currents recorded from a long fiber cell were dominated by an outwardly rectifying anion conductance (Fig. 9B); however, $E_{rev}$ of this cell was substantially depolarized from $E_{Cl}$ (Fig. 9D). The subsequent replacement of extracellular Na$^+$ with the impermeant cation NMDG (Fig. 9C) resulted in a hyperpolarization of $E_{rev}$ toward $E_{Cl}$ (Fig. 9D), suggesting that both Cl$^-$ and nonselective cation conductance contribute to the membrane properties of this cell. A different cell, which was dominated by an outwardly rectifying anion conductance (Fig. 9E), also exhibited a minor inward current component that was abolished by Ba$^{2+}$ (Fig. 9F). This indicates that a residual K$^+$ conductance still persists in this cell in parallel with the fiber cell anion conductance. Interestingly, the replacement of extracellular Na$^+$ with NMDG (Fig. 9G) reduced overall current and hyperpolarized $E_{rev}$ toward $E_K$ (Fig. 9H). Thus, it appears that longer fiber cells can also possess a nonselective cation conductance that is insensitive to Gd$^{3+}$ and may contribute to the membrane properties of isolated fiber cells.

**DISCUSSION**

Here, we have performed the first in-depth characterization of the membrane properties of isolated lens fiber cells maintained in the presence of physiological Ca$^{2+}$. The success of this study was critically dependent on the ability of extracellular Gd$^{3+}$ to prevent the vesiculation of fiber cells isolated from the whole lens. By inhibiting a Ca$^{2+}$-permeable nonselective leak conductance, Gd$^{3+}$ prevented fiber cell depolarization and the influx of Ca$^{2+}$ that has previously been shown to initiate fiber cell vesiculation (6, 52). In the presence of Gd$^{3+}$, a viable population of isolated fiber cells was obtained that was amenable to whole cell patch-clamp recordings for several hours in the presence of physiological concentrations of extracellular Ca$^{2+}$. Fiber cells isolated in the presence of Gd$^{3+}$ exhibited a range of lengths that represented different degrees of differentiation from their progenitor cells in the anterior epithelium (54). Our analysis shows that the shortest fiber cells, and therefore the least differentiated cells, exhibit epithelial-like permeability properties that are dominated by a varying combination of inwardly and/or outwardly rectifying K$^+$ conductances (Figs. 4–6). In contrast, longer, more-differentiated fiber cells were dominated by an outwardly rectifying Cl$^-$ conductance, which exhibited a lyotropic anionic selectivity (Fig. 8). Although these experiments were performed in the presence of the nonselective cation channel inhibitor Gd$^{3+}$, a variable contribution from an additional nonselective cation conductance was evident either as a depolarization of $E_{rev}$ at baseline (Fig. 2D) or as a hyperpolarization of $E_{rev}$ after the replacement of extracellular Na$^+$ with NMDG (Fig. 9). Taken together, our findings indicate that the processes of fiber cell differentiation and elongation are associated with a switch in the dominant membrane permeability from a K$^+$ conductance(s) in short fiber cells to a mixture of Cl$^-$ and nonselective cation conductances in long fiber cells, a result that is consistent with the original macroscopic impedance measurements performed in whole lenses (1).

The dominance of K$^+$ conductances we observed in short fiber cells is not only consistent with their differentiation from...
equatorial epithelial cells known to express a variety of K+ channels (9, 10, 45, 49) but also with the finding that these differentiating cells contain active Na+/K+-ATPase (23). At the equator, epithelial cells and differentiating fiber cells exhibit a pump current density per area that is ~20 times larger than that measured at the anterior pole (23). This equatorial concentration of Na+ pump activity is responsible for active Na+ efflux and sets the lens resting potential (37), whereas the elongation of fiber cells increases the surface area available for the extrusion of Na+ (22). Since the Na+ pump couples Na+ efflux to K+ uptake, it makes sense that these newly differentiating fiber cells initially retain a K+ conductance to facilitate the K+ recycling necessary to maintain pump activity. However, as fiber cell differentiation proceeds, it is evident that the contribution of K+ channels to overall fiber cell conductance (Fig. 6) and Na+/K+-ATPase activity (12) both diminish as cells elongate. Whether the decrease in channel and pump activity occurs in parallel remains to be determined, but a dissociation is possible since we have recently shown that the rat lens expresses an alternative K+ efflux pathway that is mediated by an electroneutral KCl cotransporter (8). Furthermore, a reduction in K+ permeability has been postulated to help drive the increase in cellular volume that occurs during the rapid phase of fiber elongation seen in explant models of fiber cell differentiation (4, 5).

While the differentiation of epithelial cells into fiber cells has long been thought to be associated with the downregulation of K+ conductance (for a review, see Ref. 37), the mechanism by which this is achieved is less clear. Are the K+ channels inactivated/degraded in longer fiber cells or are they still present but become diluted/overwhelmed by other conductances that are activated during the course of fiber cell differentiation? Our results tend to suggest the latter, since some fiber cells from medium/long fiber cell length groups were shown to contain a K+ conductance that appears to be buried beneath an outwardly rectifying lyotropic anion conductance and a nonselective cation conductance (Fig. 9). This sporadic detection of apparent Ba2+- and TEA-sensitive current components suggests that K+ channels can persist in longer cells, but these are often difficult to detect against a background of more dominant conductances that emerge during fiber cell differentiation.

Our finding that an anion conductance dominates the membrane properties of longer isolated fiber cells is consistent with earlier experiments that reported modulation of resting lens voltage by extracellular Cl− removal (1, 3, 15), a series of morphological studies on whole lens organs cultured under isosmotic conditions that were exposed to a variety of anion channel inhibitors (8, 40, 51, 55), and patch-clamp studies that previously detected anion channel activity in isolated fiber cells and excised membrane patches (54, 56). In this study, we demonstrate, for the first time, that isolated fiber cells and whole lenses share a common lyotropic anion selectivity sequence (Table 3). Our results therefore indicate that the Cl− conductance we identified in isolated fiber cells is not an artifact of the dissociation process but that it also contributes to setting of the resting voltage in the intact lens. In other cell types, this lyotropic selectivity sequence (NO3− > I− > Cl− >> gluconate) has been associated with both volume-sensitive anion conductances and members of the Ca2+-activated Cl− channel family (30). While in this study we have made no attempt to distinguish between these two families, a proportion of the variability in the observed basal activity and kinetics of this Cl− conductance observed in differentiating fiber cells (Fig. 7) could be potentially attributable to cell-to-cell variations in cell volume and/or intracellular Ca2+ levels. The ongoing identification of signaling pathways in the lens that mobilize intracellular Ca2+ (14) suggests that these Cl− channels may potentially be modulated to control the overall contribution of Cl− conductance to the electrical properties of the lens, again indicating that lens fiber cells contribute more to the overall properties of the lens than originally anticipated.

A subpopulation of isolated fiber cells displayed Erev values more depolarized than Ec1 in the presence of Gd3+, suggesting that a Gd3+-insensitive Na+-permeable leak pathway remains active at rest even in cells that display a physiologically relevant conductance (ρc < ρg). Thus, individual isolated fiber cells appear capable of existing in a stable state in vitro with a variable level of basal Na+ leak. From the results shown in Table 2, it is apparent that this baseline level of cation channel activity is grossly elevated in isolated cells that undergo vesiculation. Lens depolarization and elevation of intracellular Na+, both signs of the activation of a cation conductance, have been observed in animal models of diabetic cataract (32, 33) and in the aging human lens (16). In the diabetic lens, the activation of these nonselective cation channels is associated with dramatic changes in membrane potential (33) and ionic homeostasis (32) that manifest in tissue damage in the lens cortex (7). In contrast, the changes observed in human lenses occur more gradually in an age-dependent manner over many decades of life but ultimately result in age-related nuclear cataract (16). Obviously, further study into the regulation of cation channels is required to shed further light on the changes in membrane permeability known to accompany these different types of lens cataracts.

In summary, the trends in ionic selectivity with length directly measured here in viable isolated fiber cells confirm the original predictions of Mathias et al. (36) in whole lenses. While epithelial cells and short fiber cells are dominated by K+ conductance, longer fiber cells become increasingly dominated by an anion conductance with a low level of concomitant cationic leak. As such, this study provides the first direct confirmation at the single cell level of the spatial differences in ionic permeability believed to drive the lens internal circulation system. Further characterization of the membrane conductances described in this study should shed new light on how membrane permeability is regulated in the normal lens and altered during cataractogenesis.

GRANTS

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