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Experimental Eye Research 78 (2004) 689–698
EXPERIMENTAL EYE RESEARCH
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Review

# The lens: local transport and global transparency

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Received 13 June 2003; accepted in revised form 16 July 2003

**Abstract**

The perception of the lens changed remarkably during the career of David Maurice. The early view was that it was an inert sack of protein that assisted the cornea in focusing light on the retina. As investigators looked more carefully, more and more complexity was revealed and today we know the lens is a living, dynamic organ that carries out a host of biochemical and physiological processes necessary for homeostasis. We have worked on the lens over this period and have provided a small part of the data on lens physiology. This paper is an overview of our own contributions, in the context of the ever evolving view of the lens. Given this is a brief tribute to the career of David Maurice, there is not enough space nor is it appropriate to provide a complete review of all the work that has contributed to this evolving view.


*Keywords:* lens; ion channels; water channels; Na/K ATPase; gap junctions

## 1. Introduction

In the 1970s, there was some question whether the lens was actually a 'living' organ or more akin to a hair or nails. Intracellular microelectrode studies (Rae et al., 1970; Rae, 1973; Eisenberg and Rae, 1976) demonstrated a resting voltage and a low but measurable input resistance, suggesting a spark of life. However, the resting voltage was essentially the same wherever it was recorded, leading Duncan (1969) to speculate that the lens might be like a giant single cell, in which the inner cell membranes had degenerated. Many investigators implicitly adopted this view and experiments were interpreted without consideration of the lens cellular structure (Thoft and Kinoshita, 1965; Epstein and Kinoshita, 1970; Patmore and Duncan, 1980; Paterson, 1980; Delamere et al., 1980a,b; Hightower and Reddy, 1981). A slightly more complex variant of the single cell view was to treat the lens like a simple epithelium with anterior–posterior polarity. A number of studies (Kinsey and Reddy, 1965; Candia et al., 1970; and 1971; Delamere and Duncan, 1979; Platsch and Wiederholt, 1981) used an Ussing chamber to isolate the anterior and posterior surfaces, then measured the properties of the short circuit current induced by this protocol.

We know today that the uniform voltage occurs because all lens cells are interconnected through an extensive network of low resistance gap junction channels, which create a syncytium. This does not mean that all the cells are the same, but it does mean that any recorded response in the lens reflects the integrated properties of all the cells. Indeed, the lens is formed through a complex process of cell proliferation and differentiation (Menko, 2002), that results in at least three zones of cells (Fig. 1(A)): an outer layer of surface (red S) cells (epithelial and posterior foot like ends of new fibres), an intermediate layer of differentiating fibre cells (blue DF) that still have organelles and other distinct transport properties, and a core of mature fibre cells (green MF) that have lost their organelles and have post-translationally modified their transport proteins. Moreover, within the epithelium, as one looks from the anterior pole to equator there are several stages of cellular proliferation/differentiation and a large variation in membrane transport properties. Indeed, the transport properties of anterior epithelial cells are more similar to those of the posterior surface membranes than to those of the equatorial epithelial cells. Similarly, DF have a large variation in gap junction

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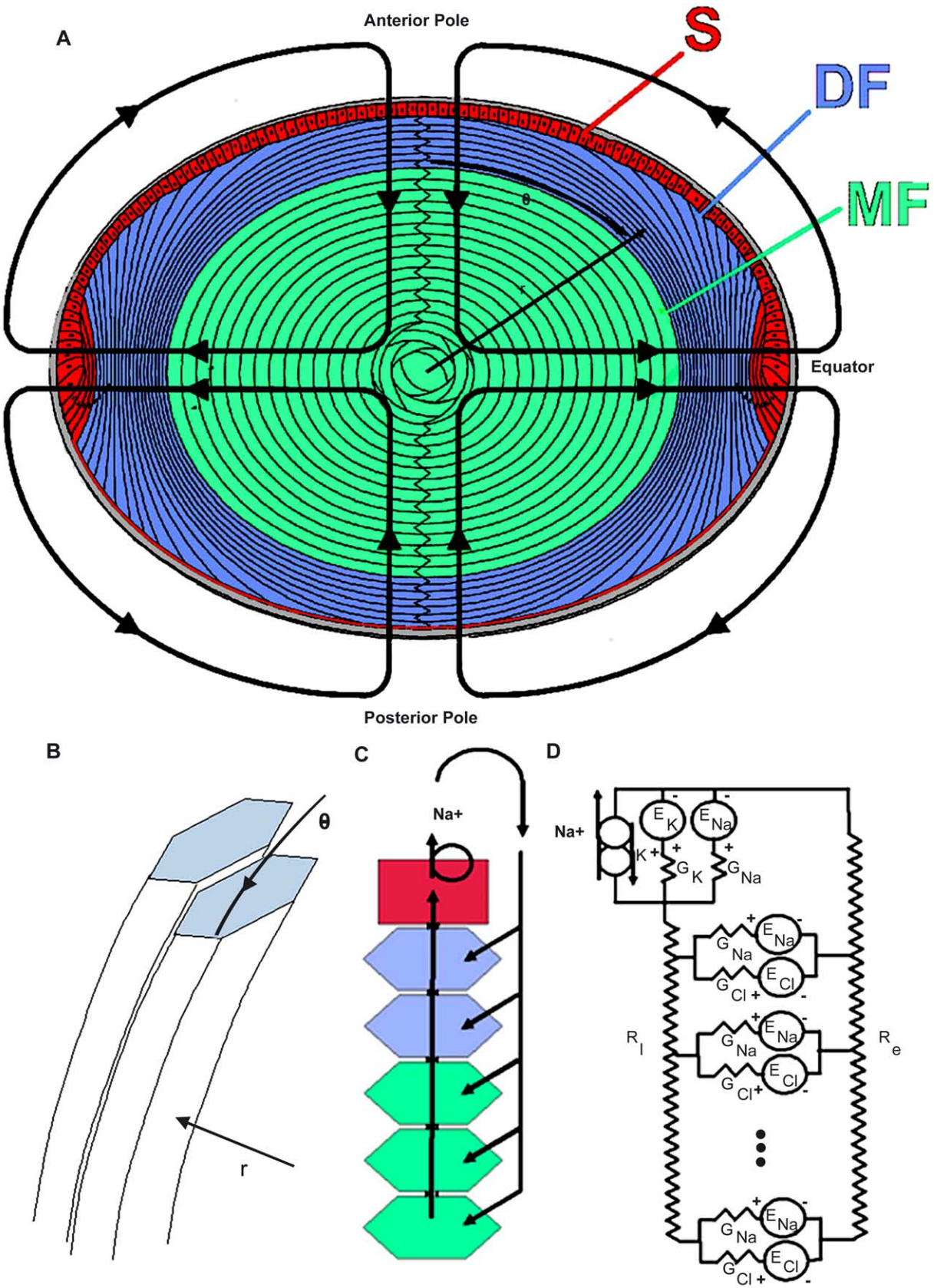
DOI:10.1016/j.exer.2003.07.001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coupling from the equator to either pole, with the conductance being concentrated at the equator. All of these spatially localized transport processes contribute to a standing, circulating current, which is illustrated by the lines of flow in Fig. 1(A). Mathias et al. (1997) review many of the transport properties that lead to this circulation, and suggest that it is coupled to fluid movement, resulting in an internal microcirculatory system for the avascular lens.

Our view of the lens physiology has therefore evolved from inert material to a complex and dynamic organ. This evolution has occurred in roughly three stages: Early Studies focused on whether the lens is truly 'living' tissue with a cellular structure; Transitional Studies provided an appreciation of the lens syncytial nature, and how spatial localization of membrane transport leads to the lens circulating current; Modern Studies have identified specific transport proteins, determined their localization to regions–cells of the lens, and have utilized molecular biological and genetic technology to evaluate protein-specific function. This paper is primarily intended to provide an overview of our contributions to this evolution in our perception the lens. Clearly, we have contributed only a small part of the picture, but given the nature of this series of papers, a complete review would not be appropriate.

## 2. Early studies

Morphological studies have played a major role in our understanding of lens physiology. Early electron microscopy studies (Wanko and Gavin, 1959; Cohen, 1965; Rafferty and Esson, 1974) suggested the lens was cellular in nature. Using scanning electron microscopy, Kuwabara (1975) and Kuszak et al. (1980) showed the cellular structure extended well into the lens, though they could not get views of the lens centre. Later, Rae et al. (1982, 1983) developed a new method of fixation that allowed one to section all the way to the centre of the lens. They showed that the lens cellular structure extended throughout. Thus, from a morphological point of view the lens appeared to be a multi-cellular organ, even though electrophysiological studies had failed to detect evidence of distinct cells.

The morphological studies have also shown that less than 1% of the volume within a lens is extracellular and one assumes it would be difficult to get a microelectrode into such a small compartment. Furthermore, these studies had detected junctions between lens fibre cells, and if these were communicating (gap junctions), this would explain the uniform voltage. Given these possibilities, Rae (1974a,b) set out to make high resolution measurements of the lens resting voltage. An intracellular microelectrode was moved into the lens using finely controlled steps. He was able to show a blip in the voltage each time the microelectrode moved from one cell to the next. Moreover, a microelectrode would occasionally enter a 'low voltage' compartment, which had the expected properties of the extracellular spaces between fibre cells (Rae, 1974b). Dye injection, viewed through a light microscope, clearly showed that when the microelectrode was in the intracellular (−70 mV) compartment, the dye would diffuse along the axis of the fibre, whereas when in the extracellular (−30 mV) compartment, the pattern of dye diffusion was nearly spherical (Rae and Blankenship, 1973). In another series of dye injection studies (Rae, 1974c; Rae and Stacey, 1976), the tissue was fixed and sectioned for high resolution evaluation of the pattern of dye diffusion. Although dye preferentially diffused along the axis of the injected fibre, it also moved into the adjacent fibre cells, suggesting coupling via gap junctions. These studies, in parallel with those of

Fig. 1. The structure and electrical anatomy of the lens. (A) The cellular structure and functional zones of the lens. The anterior surface comprises a single layer of epithelial cells (S shown in red), which begin to elongate at the equator and then differentiate into the differentiating fibre cells (DF shown in blue). At this transition there is a dramatic alteration in the pattern of protein expression and an abrupt change in the membrane transport properties of the cells. There is also a gradient from equator to anterior pole in the transport properties of the epithelial cells. The foot like ends of the newest DF form the posterior surface membranes, which are more similar to the anterior epithelial cells than to either the equatorial epithelial or differentiating fibre cells. Thus, the membranes shown in red have distinct transport properties from the membranes of cells in the DF or in the mature fibre cell zone (MF shown in green), but the surface transport properties are not uniform and there is an approximate symmetry about the equator. Similarly, within the DF zone, there is a large gradient in gap junction coupling from the equator to either pole, again giving an approximate symmetry about the equator. About 15–20% of the distance into the lens, there is a transitional zone where there is significant cleavage of proteins, reorganization of gap junction plaques and probably many other post-translational modifications of proteins (DF to MF transition). In the MF, gap junction coupling becomes more spherically symmetric and the equator to poles symmetry appears to be lost. (B) The orientation of lens fibre cells. In panel A, the radial, $r$-direction, and the angular, $\theta$-direction, are defined in the intact lens. The distance from the lens centre is defined as $r$ and the angular distance from the anterior pole as $\theta$. In panel B, we illustrate the effect of the lens structure on transport of solutes in these two directions. The $\theta$ direction follows the axis of the fibre cells, hence solutes can move for long distances without crossing a gap junction. Consequently, the resistance to solute movement is much less in the $\theta$ vs $r$ direction, in which a solute must cross a gap junction every cell width (about every 3 μm). (C) The circulation of $Na^+$. In panel A, the overall pattern of circulating ionic current in a normal lens is illustrated by the lines of flow. In panel C, we illustrate our model of how this circulation is generated. Inward directed flow of $Na^+$ occurs along the extracellular clefts, outward directed intracellular flow through gap junctions, and transport out of the lens at the surface by the Na/K ATPase. This picture does not show the pole to equator pattern, which is thought to be due to the intracellular path being directed to the equator by a concentration of gap junction conductance in the equatorial region of the DF, and the transport of $Na^+$ out of the lens by a concentration of Na/K pump activity in surface equatorial cells. (D) The electrical equivalent circuit. This is the equivalent circuit analog of the $Na^+$-current flow shown in panel C. It is based on data suggesting the illustrated localization of conductances, but again, the angular component is omitted for clarity.

other investigators, supported the view that the lens is a living organ, made up of many intact cells, which are coupled by an extensive network of gap junctions.

## 3. Transitional studies

Given the view that the lens is a multi-cellular syncytial tissue, one is confronted with the problem of separating out the properties of individual cells. Eisenberg et al. (1979) provided the first paradigm when they published an electrical model of a spherically symmetric syncytium. This model explicitly depended on surface cell membrane conductance, inner fibre cell membrane conductance, fibre cell gap junction coupling conductance and the effective resistivity of the extracellular spaces between fibre cells. It predicted an equivalent circuit representation (reviewed in Mathias et al. (1985)), which has provided an intuitive understanding of many lens' complexities (Fig. 1D).

This model was tested using linear frequency domain impedance techniques (Mathias et al., 1979) and by varying the resistivity of the extracellular solution between fibre cells (Rae et al., 1982). It provided accurate fits to the data, and sensible resistance and capacitance values for the various structural components represented in the model. We were mildly surprised by its utility, given we knew the lens was not spherically symmetric. In retrospect, the model worked well because the axis of each fibre provides a relatively low resistance path around the lens ($\theta$-direction in Fig. 1) and this reduces angular voltage gradients relative to radial ($r$-direction in Fig. 1) voltage gradients. As a consequence, at a given depth the induced voltage at any angular position is nearly the same and depends on the angular average of membrane transport properties at that depth.

Mathias et al. (1979) reported that, not only were fibre cells intact, they had very high resistance membranes, which were about three orders of magnitude more resistive than surface membranes. Also, fibre cells were electrically well coupled by gap junctions and the effective intracellular resistivity in the radial direction ($R_i$ in Fig. 1(D)) was about an order of magnitude lower than the effective extracellular resistivity in the same direction ($R_e$ in Fig. 1(D)).

To determine why the surface cell membrane conductance was so much higher than that of fibre cells, Mathias et al. (1985) used a series of ion substitutions in connection with impedance data. They found the fibre cell membranes (in frog lens) appeared to lack significant $K^+$-conductance, whereas surface cells were more typical with a relatively high $K^+$-conductance. A large number of studies by others (reviewed in Mathias and Rae (1985), or Mathias et al. (1997)) had shown that fibre cells lack Na/K ATPase activity, which is localized in the surface cells. The active uptake of $K^+$ into the lens and passive leak of $K^+$ out of the lens are therefore co-localized in surface cells. The handing of $Na^+$, however, appeared much more interesting. Mathias et al. (1985) reported that both fibre and surface cell membranes have a typically low $Na^+$-conductance. However, in a small frog lens, the area of surface cell membrane is about 1·0 $cm^2$ whereas the fibre cell membrane area is about 100 $cm^2$. Thus, even with a low $Na^+$-permeability per area of membrane, the total $Na^+$-current entering the fibre cells is significant. Moreover, the $Na^+$-entry occurs throughout the volume whereas active transport is localized to the surface, hence a circulation of $Na^+$ should exist.

Mathias (1985) used the equivalent circuit (Fig. 1(D)) for a spherically symmetric syncytium as the framework for modelling the predicted circulation of $Na^+$. However, one cannot model standing $Na^+$-current flow without including the possibility of $Na^+$ depletion in the small extracellular spaces within the lens. Similarly, one cannot model $Na^+$ depletion without including $Cl^-$ depletion to maintain electroneutrality, but NaCl depletion implies a transmembrane osmotic gradient that should induce water flow. Thus, while the equivalent circuit provided a relatively simple and intuitive picture of how the $Na^+$ would be expected to circulate, the actual model calculations had to include all of the ions as well as water flow. The prediction (Fig. 1(C)) was that $Na^+$ would move into the lens along the extracellular spaces, cross into a fibre cell down its electrochemical gradient, then move from cell to cell by gap junctions towards the surface, where the Na/K ATPase could transport it out of the lens to complete the current loop. The magnitude of the circulating $Na^+$-current was predicted to depend on the membrane water permeability: if water permeability was low, significant extracellular $Na^+$ depletion would occur and create a diffusion potential in opposition to the circulation, hence the current would be small; if water permeability was high, water would circulate with the $Na^+$ and wash out the $Na^+$-depletion, allowing a relatively large current under essentially isotonic conditions. Needless to say, this model was complex and based on rather indirect data, hence it was not very seriously considered except within a small circle of close colleagues.

At the same time we were developing these ideas, Robinson and Patterson (1982) used the vibrating probe to measure current around the lens. This technology uses a small voltage recording electrode, which is rapidly moved back and forth over a short distance. The voltage is recorded at the points of maximum excursion. When the direction of motion is perpendicular to a surface, the local voltage gradient is measured normal to that surface and, based on the resistivity of saline, one can estimate the normal component of current density. They used this technology to record the current density at the lens surface and show that a circulating current did indeed exist. The pattern of current circulation (shown in Fig. 1(A)), however, was completely counter intuitive to all previous views of the lens. The lens structure and Ussing chamber studies suggested an anterior to posterior polarity; our own view was that a more important polarity existed between surface

and inner cells; whereas the work from Patterson's lab demonstrated a large equator to poles polarity.

The presence of a large equator to poles circulation suggested that significant equator to poles gradients in some parameters must exist, hence Baldo and Mathias (1992) mapped the lens impedance as a function of angular position ($\theta$ in Fig. 1). The only observed parameter that varied significantly from equator to poles was the gap junction coupling conductance of the DF (Fig. 1(A)), which was maximum at the equator and fell to near zero at either pole. We had previously reported that the cell to cell coupling conductance in the MF was about half that of the average value in the DF (Mathias et al., 1981). Like other ion channels, gap junction channels can exist in either a closed or open state and transitions between these states is referred to as gating. Another difference between DF and MF is that DF gap junction channels gate closed in response to a drop in pH, whereas those in the MF were not able to gate (Mathias et al., 1991). Thus the DF gap junction conductance, which is concentrated at the equator, may be physiologically regulated, whereas the MF gap junction conductance is lower, it is more uniformly distributed around the lens, and it has lost the capacity for regulation. All of these factors may be important in the overall pattern of circulation, the effects of the circulation on the intracellular environment, and regulation of the circulation. Fibre cell gap junction coupling, like other aspects of lens physiology, appears to be far more complex than expected.

Rae and Kuszak (1983) demonstrated the presence of electrical coupling between the lens anterior epithelium and underlying fibre cells, but they had no way of quantifying the degree of coupling. Duncan et al. (1988) and Cooper et al. (1989) had shown a fairly high degree of electrical coupling between epithelial cells (this is represented by the $\theta$-dimension in Fig. 1). Rae et al. (1996) demonstrated extensive dye transfer from epithelial cell to epithelial cell but only about one in ten epithelial cells was dye coupled to its underlying fibre. This study was motivated by several experimental observations (reviewed in Mathias et al. (1997)) that had led to conflicting hypotheses on epithelial fibre cell coupling. In retrospect, the results not only resolved the controversy, but demonstrated that the preferential direction for current flow from an epithelial cell near the anterior pole is in the $\theta$-direction, toward the equator, rather than into a fibre cell and toward the lens centre (the $r$-direction). Again, significant intracellular current flow in the radial $r$-direction appeared to be relegated to the lens equator, where it is directed outward.

Mathias et al. (1997) review our expanding understanding of the complexities of lens physiology. This review also presents a model of the lens circulation. We believe this model is consistent with all available data and ties together a number of independent experimental results. In essence, it is a slightly more complex version of the (Mathias, 1985) first attempt to predict a circulation. Again, the idea is that $Na^+$ moves into the lens everywhere along the extracellular clefts, it moves down its electrochemical gradient into fibre cells (just as shown in Fig. 1(C)), but once in a fibre cell, the preferential path back to the surface is to the equator, since that is where gap junctions direct the current. In order for the model to generate the magnitude of the observed circulating current, Mathias et al. (1997) had to assume that the membrane water permeability is sufficiently large for water to isotonically follow the same flow pattern and maintain nearly constant osmolarity everywhere. Thus the prediction is that water moves into the lens along the extracellular clefts, convecting nutrients (like glucose) and anti oxidants (like ascorbate) to the inner fibre cells where they can be absorbed and used for homeostasis. The presence of this circulation allows the lens to maintain living cells deep inside of it, where there is no blood flow and distances are too great for diffusion.

## 4. Modern studies

The transitional studies suggested that localized expression of lens ion channels, Na/K pumps, gap junctions and water channels leads to an internal circulatory system that convects glucose, ascorbate and other molecules to the inner fibre cells, which rely on the circulation for homeostasis. This section will therefore focus on these four classes of transport proteins in the lens (reviewed in Donaldson et al. (2001)). In this regard, our role has been relatively minor, since we have simply followed the lead of a large number of researchers who, in other systems, developed and/or utilized the technology to identify, clone and characterize the many different ion channels (reviewed in Hille (2001, Chapter 5)), the various isoforms of the Na/K pump proteins (reviewed in Sweadner (1989)), the connexin family of gap junction proteins (reviewed in Harris (2001)) and the aquaporin family of water channels (reviewed in Agre et al. (2002)).

### 4.1. Ion channels

Characterization of acutely isolated lens epithelial cells, using the whole cell and single channel patch clamp, revealed several types of $K^+$-channels in cells from each species and several species to species differences (Rae and Rae, 1992; Rae, 1994). The molecular identities of these $K^+$-channels were determined using RT-PCR (Shepard and Rae, 1998, 1999; Rae and Shepard, 1998a–c; Rae and Shepard, 2000a,b). Despite this variability, there was consistency in that every epithelium expressed a preponderance of $K^+$-channels over $Na^+$- or $Cl^-$ channels. The variability seemed to arise, at least in part, from size. Large lenses have a relatively large $Na^+$-leak, since the total $Na^+$-conductance depends on volume (radius$^3$) whereas total $K^+$-conductance depends on surface (radius$^2$). This means large lenses have a relatively depolarized resting voltage and $K^+$-channels must provide conductance in the appropriate voltage range to stabilize that voltage. Since

different $K^+$-channels have different voltage dependencies, there are lens size-dependent differences in expression. Other factors that lead to variety might be the environment in which the animal lives (salt water, fresh water or land), or hormonal and autonomic regulatory systems used by different species. We do not know the purpose of this variability, but the one consistency is that $K^+$-channels are localized to lens surface cells.

The molecular identities of the channels responsible for the $Na^+$-leak into fibre or epithelial cells are not yet known. Rae et al. (1992) showed that $Na^+$-permeability of fibre and epithelial cell membranes could be greatly increased by removal of external $Ca^{2+}$. Some cation channels seen with patch clamp increase their open probability when $Ca^{2+}$ is removed (Cooper et al., 1986), but so do hemi-channels made from lens Cx46 or Cx50 (Ebihara et al., 1995). Either hemi-channels or the cation channels have near zero open probability in normal physiological conditions, and this would be consistent with the low lens $Na^+$-permeability per unit area of membrane. There has not been a large scale effort to identify and classify the background $Na^+$-leak channels in other systems, so other candidate proteins, that have not yet been identified, probably exist in the lens. One should reserve judgment until this field has developed more fully. Conversely, there has been a recent surge of work on $Cl^-$-channels, leading to the classification of the ClC family of channels (reviewed in Hille (2001, Chapter 5)). Others (Reeves et al., 1998; Tunstall et al., 1999; Young et al., 2000; Cammarata et al., 2002) have shown these channels are found in lens fibre cells, but we have not been involved with these recent advances other than to submit the ClC lens nucleotide sequences to Genbank.

### 4.2. Na/K ATPase

The functional component of the Na/K pump is the alpha subunit, which binds $Na^+$ and $K^+$ and has the enzyme's ATPase activity. In the lens and most organs, three isoforms of the alpha subunit have been identified, with an alpha4 isoform being found in testis. Garner and Horowitz (1994) were the first to show that different isoforms were regionally expressed in bovine lens epithelium, with the alpha3 in the anterior cells and alpha1 in equatorial cells. Subsequent work from Delamere's lab (see the paper by Paterson and Delamere in this series) found that, as in the case of $K^+$-channels, Na/K pump isoform expression was species dependent, although in all species the ATPase activity is in the surface epithelium. Again, the purpose of this variability may relate to environment or regulation, we do not know.

Gao et al. (2000) separately isolated frog lens epithelial cells from the anterior and equatorial regions, and performed whole cell patch clamp studies of the Na/K pump current. In this species, they showed that anterior cells predominantly express the alpha2 isoform and the equatorial cells the alpha1. The pump current density was found to be twice as high in equatorial cells, which are also larger than anterior cells. Moreover, at the anterior pole the cells are flat and wide so they present a relatively small amount of total membrane area to a relative large surface area of the lens, whereas at the equator they are longer and narrower and present a larger amount of membrane area to a relatively smaller surface area of the lens (this is indicated in Fig. 1(A), although not to scale). As a consequence, the total Na/K pump current per area of lens surface was predicted to be about 20 times greater at the equator than the anterior pole (Gao et al., 2000). Candia and Zamudio (2002) used a special chamber and a series of o-rings to isolate various regions of the rabbit lens surface and measure the Na/K pump current. In these more direct experiments, the equatorial pump current density they measured was essentially the same as we had estimated, whereas at the anterior or posterior polar region they detected no Na/K pump current. We estimated the anterior current density per unit area of lens surface would be very low, so the observations appear to be consistent between rabbit and frog lenses. Tamiya et al. (2002) found the same pattern of Na/K pump current density, with essentially all of the current being at the equator of porcine lenses, which express only the alpha1 isoform. Thus, while isoform expression varies between species, the consistent result is that the Na/K pump current in the lens is highly concentrated in equatorial surface cells. This observation supports our hypothesis that the circulating current is carried by $Na^+$ and it explains how the $Na^+$-current, which is directed to the equator by gap junction conductance, is able to be transported out of equatorial surface cells. The Na/K pump stoichiometry is 3Na:2K, hence the outward $Na^+$-current density at the equator is about three times the Na/K pump current density, whereas the inward $K^+$-current density is two times the pump current. If one assumes that $K^+$-current generated by the Na/K pumps simply leaks out of the same cell (Fig. 1(D)) and does not contribute to the net current measured at the equatorial surface, then based on measured values of the net Na/K pump current, the outward $Na^+$-current density generated by the Na/K pumps at the equator is quantitatively consistent with the measured density of the circulating outward current at the equator. Thus, the hypothesis that $Na^+$ carries the circulating current is also quantitatively consistent with these two independent measurements of equatorial current densities.

### 4.3. Gap junctions

A large amount of work from a number of outstanding groups has shown the following: lens epithelial cells express Cx43 and Cx50; at the transition from equatorial surface epithelial to differentiating fibres (S to DF in Fig. 1(A)), Cx43 is degraded and Cx46 is expressed, hence DF express Cx46 and Cx50 but not Cx43; at the differentiating to mature fibre transition (DF to MF in Fig. 1(A)), both Cx50 and Cx46 are post-translationally modified but there is no further protein synthesis, hence MF express modified

versions of Cx46 and Cx50. Our earlier results on functional differences in gap junction coupling in the DF and MF were thought to relate to the modifications at the DF to MF transition. Development of the knockout mice lacking either Cx46 (Gong et al., 1997) or Cx50 (White et al., 1998) provided the opportunity to look in more detail at the molecular basis for these differences. Gong et al. (1998) and Baldo et al. (2001) studied gap junction coupling in lenses lacking either Cx46 or Cx50, respectively. Their results suggested two surprising hypotheses. (1) The modifications at the DF to MF transition appear to render channels made from Cx50 non-functional, hence coupling in the MF depends only on Cx46. (2) Lenses whose DF lacked Cx50 were unable to gate, suggesting that regulation of DF coupling conductance depends on Cx50. Most gap junction proteins turn-over with a half life of hours, but the MF gap junction channels remain functional for the lifetime of the animal without protein turn-over. Moreover, recall that these channels are insensitive to pH, which is rather low in the lens centre (Mathias et al., 1991). Thus the data suggest that the role of the lens specific connexin Cx46 is to provide unique channels, that are able to survive for years without protein turn-over, and these channels remain open in an acid environment. Lenses from the Cx50 knockout mice were smaller than normal as well as unable to regulate their coupling conductance. Thus the roles of the lens specific connexin Cx50 appear to relate to regulation, both by passing biochemical signals that regulate lens growth and by regulating the gating of coupling conductance in the DF.

### *4.4. Water channels*

Preston and Agre (1991) discovered the first transmembrane water channel, which is now called AQP1, and is now known to be a member of a large family of water channels called the aquaporins. Soon thereafter, it was realized that the major intrinsic protein of lens fibre cell membranes is a member of this family and it has been designated AQP0. Studies by others (reviewed in Mathias et al. (1997)), as well as our unpublished data, show that the lens expresses AQP1 in the surface epithelial cells, but at the surface epithelial cell to differentiating fibre cell transition (S to DF at the equator in Fig. 1(A)), AQP1 is degraded and AQP0 is expressed. Thus the DF express AQP0 and the MF express a modified version of AQP0.

Kushmerick et al. (1995), Mulders et al. (1995) and Chandy et al. (1997), all used exogenous expression of AQP0 in the oocyte system and showed that it is indeed a functional water channel, although its single molecule water permeability is lower than that of AQP1. Varadaraj et al. (1999) using large, right side out membrane vesicles that bud off of fibre cells, showed that fibre cell membranes have a significant water permeability that is reduced by about 80% in lenses from mice that express an AQP0 mutant, which does not traffic to the plasma membrane. A similar result was subsequently obtained from AQP0 knockout mice (Shiels et al., 2001). The lens epithelial cell membrane water permeability is also very significant; indeed it is 3- to 4-fold greater than that of fibre cell membranes, and it appears to be mediated by AQP1 (Varadaraj et al., 1999).

As previously mentioned, the model calculations presented in Mathias et al. (1997) assumed that the fibre and epithelial cell membrane water permeabilities are sufficiently high for an essentially isotonic solution to follow the circulating $Na^+$-flux. More specifically, this meant that the ratio of membrane salt to water permeability is small. The quantitative values of water permeabilities obtained in the above studies, in connection with electrophysiological characterization of the fibre and epithelial cell membrane salt permeabilities, are consistent with this assumption and predict that solutions within the intracellular and extracellular spaces of the lens will be within 0·1% of isotonic. Once again, the data are consistent with a significant velocity of fluid flow into the lens along the extracellular clefts, and because of the much larger volume of intracellular space, a much smaller intracellular flow velocity toward the surface of the lens. If the volume of water flow in each compartment is normalized to the surface area of the lens, the overall flow pattern is predicted to follow the lines of current density shown in Fig. 1(A).

## 5. Discussion

As one can see from the above overview, our imagination has been captured by the lens internal circulatory system, which we believe supports homeostasis of central fibre cells in a large (with respect to diffusion distances) avascular organ. The lens is the largest organ that lacks a blood supply. Our cardiovascular circulatory system evolved because of a simple physical law, which was first described by Einstein: the average time for diffusion to take place is proportional to the distance squared. This means diffusion is quite rapid over short distances such as cellular dimensions, but extremely slow over longer distances such as organ dimensions. For example, glucose will diffuse 10 μm in about 1 sec but it requires 11 days for glucose to diffuse 1 cm. In comparison, a 1 μm $sec^{-1}$ flow velocity, which is about the calculated rate of fluid entry into the lens (Mathias et al., 1997), will move glucose 1 cm in less than 3 hr. Obviously convection becomes essential when distances are significantly larger than cellular dimensions. The adult mammalian lens of all species is sufficiently large that diffusion of metabolites, such as glucose, to the central fibre cells would require at least many hours. Unlike other organs, the function of the lens precludes an external circulatory system, since blood vessels would diminish transparency. We believe that evolution has provided an elegant resolution to this problem by establishing the lens' internal circulatory system.

At this time, the circulation model is not universally accepted. However, one should make the distinction

between the circulating ionic current, which has been experimentally measured in rat lens (Robinson and Patterson, 1982), frog lens (Parmelee, 1986) and rabbit lens (Candia and Zamudio, 2002), and our model that the circulating current is carried primarily by $Na^+$ and generates a circulation of fluid (Mathias et al., 1997). The data on the existence of the circulating current are firm; the hypothesis that it is carried by $Na^+$, with the inflow occurring along the extracellular clefts and outflow occurring intracellularly is well supported by the data on localization of gap junction conductance to the equatorial region of the DF, localization of Na/K pump current to the equatorial surface, and localization of influx of $Na^+$ throughout the mass of fibre cells; whereas the model prediction that the circulation generates fluid movement needs further testing.

Fluid flow in the pattern shown in Fig. 1(A) is difficult to measure but some recent studies are consistent with its existence. Fischbarg et al. (1999) were able to detect translens (anterior to posterior) fluid movement when the lens was placed in an Ussing chamber that forced the pattern of current flow to be translens (anterior to posterior). The authors suggested that they might be measuring a new and different phenomenon, but their observation is consistent with our prediction, which is that fluid follows the path of $Na^+$-flux, so when the $Na^+$-flux is forced to be translens, the pattern of fluid flow should follow. A 2003 ARVO abstract from Candia's lab described data obtained using a series of o-rings to detect anterior to equator fluid movement in the bovine lens. The pattern of current circulation has not been measured in bovine lens but the data suggested that it might be more anterior to equator than symmetric about the equator as in other species. These are the first actual demonstrations that fluid movement occurs in the lens. The data were difficult to obtain and showed considerable variability, but they represent the beginning of a new era, in which researchers have to accept the complex and dynamic nature of the lens. Any studies of the biochemistry, physiology or cell biology of the lens need to consider regional differences in the properties of the cells and should interpret localized results in the context of how they fit with the properties of the entire lens.

## Acknowledgements

We would like to thank Dr Francisco Martinez-Wittinghan for his assistance in putting together Fig. 1. This work was supported by NIH grants EY06391, EY03282 and EY06005.

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