Molecular Solutions To Tissue Transparency

Joerg Kistler, Rachelle Merriman-Smith, Miriam Young, Tamir Gonen, Dougal Cowan, Kaa-Sandra Chee, Jun Sheng Lin[+], Colin Green[+], Lorenz Hasler[#], Andreas Engel[#], Paul Donaldson[*]
Cellular and Molecular Physiology Laboratory, School of Biological Sciences
[+]Department of Anatomy, [*]Department of Physiology, School of Medicine, University of Auckland, Auckland, New Zealand
[#]M.Mueller Institute, Biozentrum, Basel, Switzerland

Optimal tissue transparency of cornea and lens is essential for full visual acuity, and is often taken for granted. With an increasingly aging population the incidence of surgery to replace cataractous lenses has increased dramatically and imposes a significant burden on health systems world-wide. The spotlight therefore is on the molecular mechanisms involved in the maintenance of lens transparency. Such knowledge would give us the means to explore potential therapeutic interventions that could prevent or halt cataractogenesis.

At first glance the lens is a relatively simple tissue. The bulk of it consists of elongated fibre cells which have a hexagonal cross-profile, and have lost their organelles. These fibre cells differentiate from epithelial cells at the lens equator and are laid down with great precision to build the crystalline tissue architecture, which is the key to lens transparency (Figure 1A). The anterior lens surface is covered with an epithelial cell layer, which is enriched with membrane transporters and channels. It is generally agreed that precise homeostatic control is essential

fibre cells throughout the lens. Uncontrolled cell swelling even when spatially limited, increases intralenticular light scatter, and is often a precursor of lens cataract. Opinions diverge, however, on whether the epithelial cells alone are responsible for this homeostatic control, or whether the fibre cells also play a significant role.

The more widely established view is that the transport functions of the epithelium provide for the entire lens by postulating that nutrient and electrolyte exchange to deeper lens regions occurs via an extensive network of cell-cell (gap junction) channels. The problem with this 'freely communicating' lens model is that passive diffusion alone is unlikely to be sufficient to

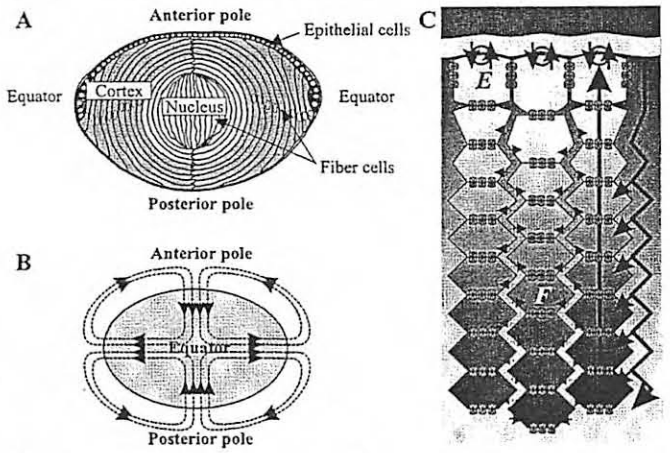

Figure 1. Structure and function of the mammalian lens. (A) Architecture of the lens showing the epithelial cell monolayer, the elongating nucleated fibre cells in the cortex, and the mature organelle-free fibre cells deeper in the lens. (B) Current flow through the lens which underpins the micro-circulation system. (C) Proposed pathway for the current and solute flow inwards via the extracellular space, crossing the fibre cell membranes, and outwards via an intracellular route mediated by gap junctions. The driving force for this flow is generated by the Na/K pump in the epithelial cell layer.

effectively reach the centre of the lens. This issue is addressed in an alternative model, which postulates that the lens operates an active micro-circulation system. Na/K pumps in the epithelium provide the driving force for a circulating current which consists of a potassium outflux in the epithelium, and a sodium and chloride influx deeper in the lens. Due to the asymmetric distribution of membrane conductances and gap junctions, a circular ion and water flow develops which is directed inwards at the poles and outwards at the equator (Figure 1B). The inward flow is via the extracellular space between the fibre cells. Solutes and water cross the fibre cell membranes and return to the lens surface via a cytoplasmic route mediated by gap junction channels (Figure 1C). Since the extracellular space between the fibre cells is narrow and tortuous, the solute flow also leads to convectional transport of nutrient molecules into the lens far beyond the distance that can realistically be expected for passive diffusion.

Common to both models is the fact that fibre cells are all intimately connected with each other via gap junction channels. Everyone agrees with this as we and others have demonstrated fibre cell coupling by patch clamp recording or dye transfer (Donaldson *et al.* 1995, Eckert *et al.* 1999). However, until recently there existed a puzzling enigma: the channels are pH sensitive and close around pH 6.5. This is the prevailing pH in the lens core which is due to an accumulation of lactate, the end product of anaerobic metabolism in the lens. How can fibre cells in the core region remain connected? We have recently solved this puzzle. The components that form the fibre cell gap junction channels, connexin46 and connexin50, are cleaved by calpain during fibre cell maturation (Lin *et al.* 1997). The lens core is composed of mature fibre cells with cleaved connexin. By determining the cleavage site in the carboxyl tail and expressing an appropriate truncation mutant in *Xenopus* oocytes, we have been able to demonstrate that cleavage abolishes the pH sensitivity (Lin *et al.* 1998). Hence, the lens has found an elegant solution to keep fibre cells coupled throughout the lens.

This is where the consensus ends. Opinions diverge on whether gap junctions serve the 'freely communicating' lens model giving the epithelium full control over the whole lens, or whether they are a component of a micro-circulation system which relies on some active control by the fibre cells. Recent data from our laboratories strongly support the notion of an active micro-circulation system. Our approach has been to verify that the fibre cells are equipped with the types of channels and transporters required for the operation of such a system. For example, chloride ions are postulated to flow inwards extracellularly and enter the deeper lying fibre cells through selective channels. Water follows this ion flow, hence, if we stopped chloride from crossing the fibre cell membranes, water is expected to accumulate in extracellular pools. Confocal laser scanning microscopy of rat lenses treated with chloride channel blockers indeed reveals tissue lesions that are consistent with this scenario (Tunstall *et al.* 1999). Furthermore, through molecular screening of fibre cells we have detected transcript for the chloride channel ClC3 consistent with the type of blockers we employed for the physiological experiments.

Provided ions can cross the fibre cell membrane unhindered, as is the case in the normal lens, water follows by traversing the membrane through its own specific water channels. As it turns out, the most abundant protein in the fibre cell membrane, MIP, has recently been shown to act as a water channel. We have developed procedures for the purification of MIP and its reconstitution into 2D crystals, which are amenable to structure analysis by cryo-electron microscopy and image processing. Indeed, at 9Å resolution, the structure of MIP is almost indistinguishable from the archetype water channel AQP1 (Hasler *et al.* 1998).

Since a key role for the micro-circulation system is to import nutrient molecules deep into the lens we would expect that fibre cells possess their own nutrient transporters to mediate glucose uptake from the extracellular space. This is distinct from the 'freely communicating' lens model where such transporters would be found exclusively in the epithelium. To confirm this we have investigated the expression of facilitative glucose transporter isoforms (GLUT) in the lens, and the evidence is again in favour of the micro-circulation system. The high affinity isoform GLUT3 has been detected in significant amounts at transcript and protein levels in the fibre cells deep into the lens (Merriman-Smith *et al.* 1999). In contrast, epithelial cells express a different isoform GLUT1. This differential expression makes good sense: it indicates that both epithelium and fibre cells transport glucose, and that because fibre cells express the transporter isoform with the higher affinity they are better suited to extract glucose from the tortuous extracellular space.

Taken together, our data on gap junction channels, chloride channels, water channels, and glucose transporters strongly favour a model whereby the lens operates an active microcirculation system to maintain homeostasis deep into the lens rather than relying on passive diffusion from the epithelium through gap junction channels. By bringing together molecular and physiological data we have gained a firmer understanding of how tissue transparency is maintained in the lens. We are presently applying this knowledge to investigate what goes wrong in cataract, and to explore possibilities of therapeutic intervention to prevent or halt this increasingly frequent disease.

References

Donaldson, P; Dong, Y; Roos, M; Green, C; Goodenough, D A and Kistler, J. (1995). Changes in lens connexin expression lead to increased gap junctional voltage dependence and conductance. *Am. J. Physiol.* **269**: C590-C600.

Eckert, R; Adams, B; Kistler, J and Donaldson, P. (1999). Quantitative determination of gap junctional permeability in the lens cortex. *J. Membr. Biol.* **169**:91-102.

Hasler, L; Walz, T; Tittmann, P; Gross, H; Kistler, J and Engel, A. (1998). Purified lens major intrinsic protein (MIP) forms highly ordered tetragonal two-dimensional arrays by reconstitution. *J. Mol. Biol.* **279**: 855-864.

Lin, J S; Fitzgerald, S; Dong, Y; Knight, C; Donaldson, P and Kistler, J. (1997). Processing of the gap junction protein connexin50 in the ocular lens is accomplished by calpain. *Eur. J. Cell Biol.* **73**: 141-149.

Lin, J S; Eckert, R; Kistler, J and Donaldson, P. (1998). Spatial differences in gap junction gating in the lens are a consequence of connexin cleavage. *Eur. J. Cell Biol.* **76**: 246-250.

Merriman-Smith, R; Donaldson, P and Kistler, J. (1999). Differential expression of facilitative glucose transporters GLUT1 and GLUT3 in the lens. *Invest. Ophthalmol. Vis. Sci.* (submitted).

Tunstall, M J; Eckert, R; Donaldson, P and Kistler, J. (1999). Localised fibre cell swelling characteristic of diabetic cataract can be induced in normal rat lens using the chloride channel blocker NPPB. *Ophthalmic Res.* **31**: 317-320.