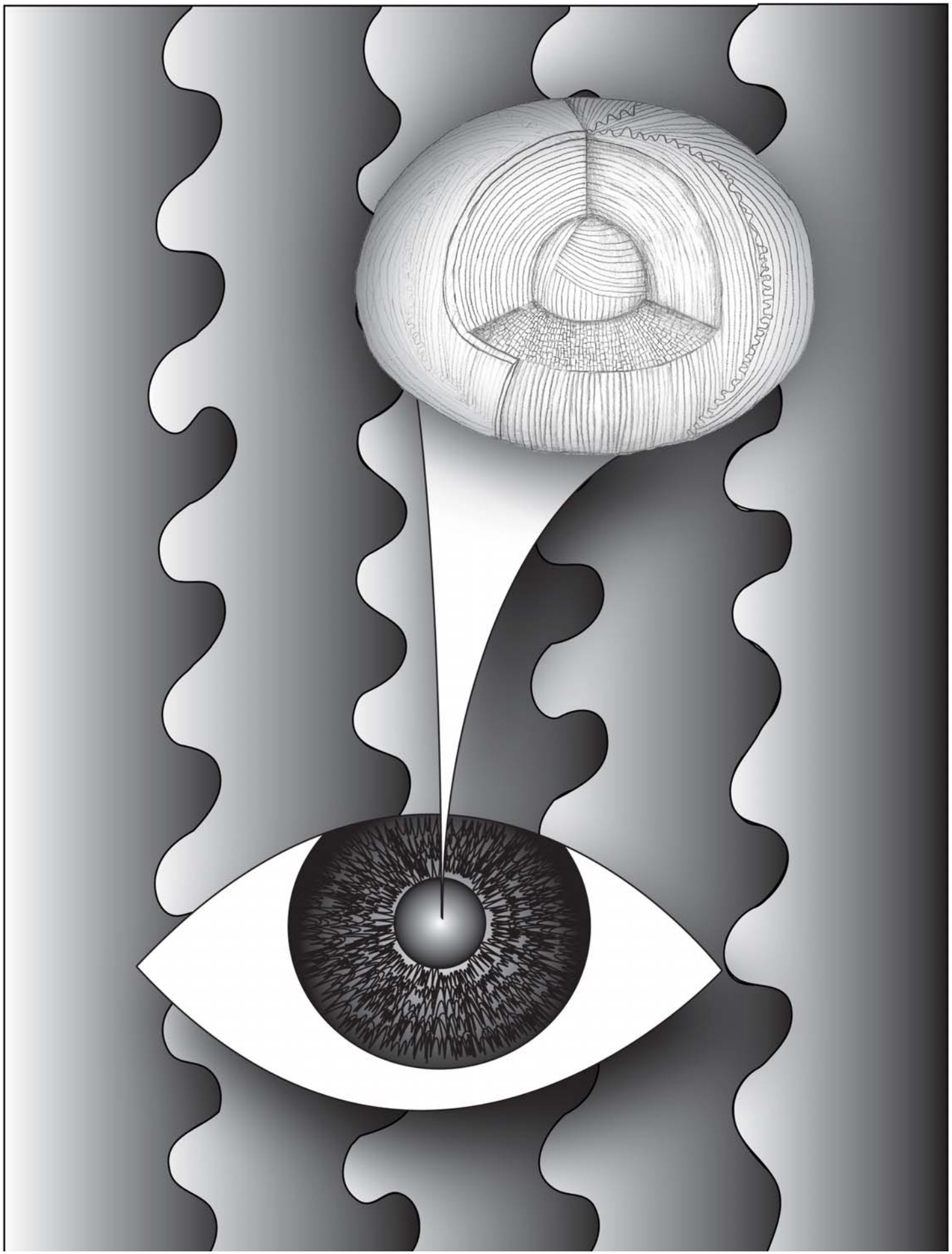


Chelsea Gordon, HSR

Obscured Crystal

Modeling the Microcirculation of the Mammalian Lens:

A Molecular Approach to Understanding Cataract Formation

By Ching Zhu

The lens of the eye is a natural marvel – an exemplar of the elegance achieved by millions of years of evolution. It is what keeps us connected to the visual world, its perfect transparency allowing for the transmission of light from the pupil to the retina. The slightest obscurity in the lens, such as that generated by a cataract, would significantly blur our visual perception. The distortion of vision caused by a cataract can be devastating, as in the case of the painter Claude Monet. Monet's poor eyesight, which was caused by cataracts in both eyes, meant that he could no longer see the true intensity of colors. This was a source of much frustration for the artist, who in 1922 wrote that he was no longer able to create anything of beauty (1).

To maintain a steady state of transparency and simultaneously obtain nourishment, the lens, which lacks blood vessels, creates an internal microcirculation that transports water and nutrients throughout the entire tissue (2). Recent research has sought to create a clearer picture of this circulation and of the biological molecules that regulate it. A comprehensive model of lens microcirculation would serve not only to further our understanding of normal lens function and cataract formation, but also to generate ideas for non-surgical treatment strategies against cataracts.

Physiology of the Lens

The lens is the only transparent tissue in the human body, and is composed of about 1,000 layers of crystal clear cells (Figure 1). Such perfect clarity is achieved through a process of incomplete apoptosis, or programmed cell death. Making up the outermost tier of the lens is a layer of epithelial cells, from which new transparent lens cells continually arise throughout the lifetime of an individual. (Thus, the innermost cells of the lens are as old as the individual to which they belong.) Upon turning on their "death mechanism," dividing lens cells release enzymes within themselves that melt down most of their cellular organelles, leaving behind only a few membrane channels and a large quantity of soluble cytoplasmic proteins called crystallins (1).

It is the architecture of the crystallin lattice that is ultimately responsible for lens transparency and for the proper focusing of light. Crystallin concentration is highest in the center of the lens and decreases as a function of distance from the lens core. This creates a radial gradient in refractive index, which corrects inherent light scattering, a consequence of the curvature of the lens (2).

Figure 1. In early embryonic development, the lens is covered with a layer of stem cells which continuously differentiate, through incomplete apoptosis, into layer upon layer of transparent lens cells. This differentiation continues throughout an organism's lifetime, so that the cells in the very core of the lens are as old as the organism itself. While the epithelial lens cells have a nucleus and organelles such as mitochondria, mature core lens cells are barely alive, possessing only a few membrane proteins and a thick solution of crystallin proteins in the cytoplasm. This crystallin lattice creates a uniform index of refraction, which prevents light scattering (1).

Internal Lens Microcirculation

The transparent properties of the lens arise from its architecture at the molecular and cellular levels; however, one must bear in mind that this architecture must actively be maintained. Maintenance of transparency requires that lens cells be continuously supplied with water and nutrients (2). A wide range of recent research points to a model of a standing flow of ionic current which generates an internal microcirculation that is responsible for maintaining lens transparency (Figure 2) (2).

Recent data suggest that epithelial cells and newly forming lens cells near the surface of the lens have sodium-potassium pumps in their plasma membranes that generate a membrane potential which allows ions to pass across the membrane. In contrast, more mature cells located deeper inside the lens appear to lack these pumps (3). These inner cells are able to maintain their membrane potential by being connected with the surrounding surface cells via intercellular channels called gap junctions, which are made up of connexin protein subunits (4). This system of gap junctions and sodium-potassium pumps allows the standing ionic current to flow (Figure 3). Because water moves with the ions by osmosis, soluble nutrients can be circulated throughout the lens (2).

Glucose is the main metabolic fuel that allows the lens to maintain growth and homeostasis. Most glucose taken up by the lens is processed anaerobically by the core cells; only the newly forming lens cells at the surface can burn the glucose using oxygen because only they still possess mitochondria (2). Glucose uptake in the lens has been shown to be mediated by the GLUT family of glucose transport proteins. A recent study reveals that glucose channels are not limited to cells at the lens surface. Lens cells at the core appear to express GLUT proteins as well, though of a different form than is expressed in the outer lens layers (5). That both inner and outer lens cells possess glucose transporters is consistent with the total proportion of glucose taken up by the lens (2) and creates a clearer picture of the intricate system of membrane proteins in the lens (Figure 3).

Disruption of Microcirculation and Cataract Formation

The microcirculation created by the system of membrane pumps and channels maintains lens homeostasis and, consequently, transparency. The slightest disruption in internal circulation can lead to the formation of opaque cell masses, or cataracts. Because the epithelial cell layer of the lens never stops dividing during the lifetime of an individual, an interesting hypothesis exists regarding cataract development. It is believed that the maintenance of transparency cannot keep up with the ever-increasing lifespans of humans. Thus, the frequency of age-related cataract development is also increasing (1).

Without proper hydration from the microcirculation of water, crystallin proteins likely become misfolded and precipitate, destroying the intricate architecture responsible for the uniform refractive index of the lens. In studies of genetically altered mice that lack connexin genes, loss of gap junction communication between lens cells resulted in the development of cataractous cells in the lens core (4). The outer epithelial cells and newly forming lens cells presumably remained transparent due to their close proximity to a hydrated environment. Thus, the absence of even one element of the microcirculation system is sufficient to sever the connections between cells that are so important for proper lens function.

Potential Treatments for Cataracts

Currently, the only treatment option for cataracts is surgery, which is often an uncomfortable experience for patients. If cataract formation is indeed explained by the loss of proper microcirculation, as is suggested by recent research, then it might be possible to develop less invasive treatment strategies that target the proteins that regulate this microcirculation. Recent work on sodium-potassium pumps suggests that pump activity may be regulated pharmacologically (6,7). In 2000, Mathias *et al.* reported that, in the guinea pig heart, sodium-potassium pumps are regulated by protein kinases, which attach phosphate groups onto their substrates (6). This discovery points to the possibility

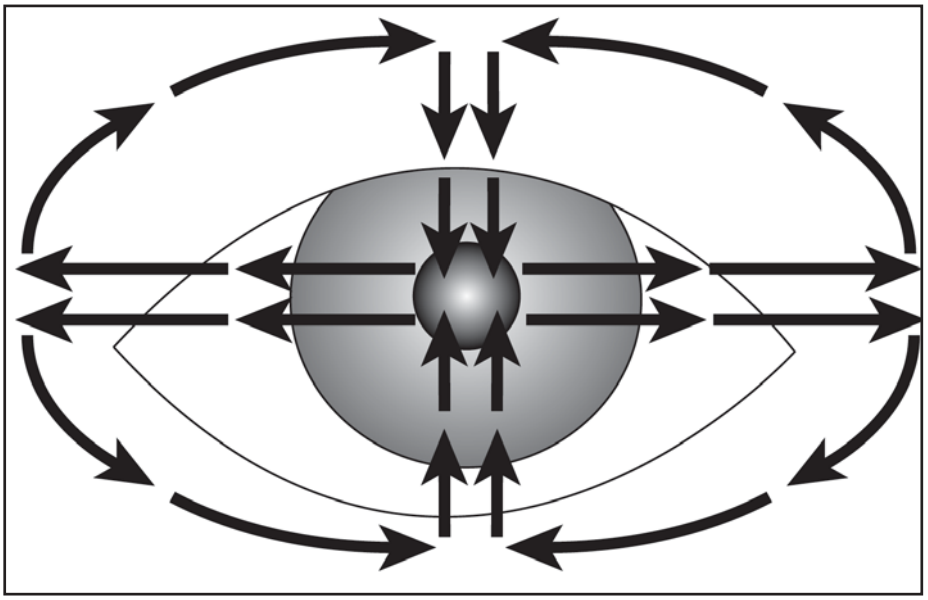
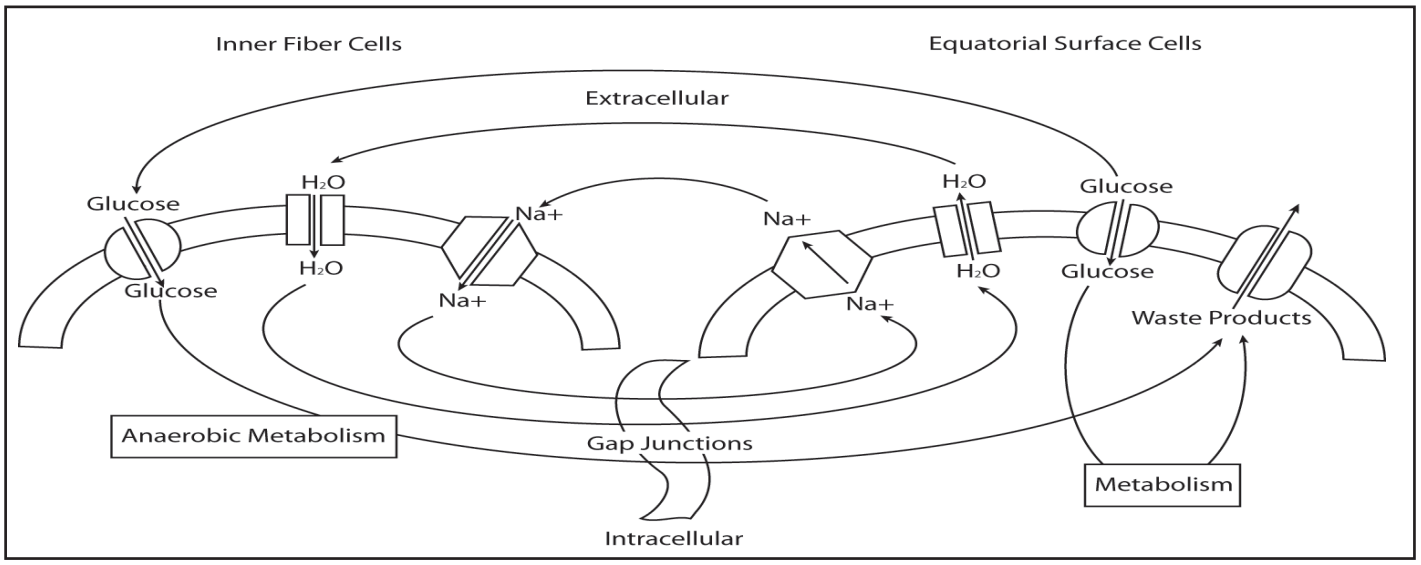


Figure 2. The arrows illustrate the path of ion flow through the lens. The ion flow brings water and metabolites to cells in the core, creating well-stirred cellular compartments and preventing dehydration of crystallin. The lens is elliptical in shape, with its anterior pole facing the pupil and posterior pole facing the back of the eye (2).

credit: Jimmy Yang, HSR



credit: Jimmy Yang, HSR

Figure 3. The various membrane transporters located in lens cells. While all lens cells possess sodium-potassium pumps, water channels, glucose transporters, and gap junctions, the isoforms of these proteins differ between surface lens cells and mature cells in the core, suggesting a high degree of organization in the maintenance of both cell types. Moreover, most of the glucose taken up by the lens is processed anaerobically by the core cells, which lack mitochondria. Only the surface cells, which still have mitochondria, utilize oxygen to metabolize glucose (2).

of manipulating these kinase pathways in order to restore normal levels of membrane pump activity and thereby reverse cataractogenesis.

Furthermore, studies on the connexin proteins making up gap junction channels are revealing the vast regulatory potential of the carboxy terminus, or end, of connexin proteins (8,9,10). For example, the PDZ protein zonula occludens-1 (ZO-1) has been observed to interact with the carboxy termini of lens connexins (8). Recent research on connexin-ZO-1 interactions in the heart reveals that ZO-1 plays a role in modifying cardiac gap junction signaling. Hunter *et al.* used peptides, or short protein constructs, to bind to the active sites of connexins and ZO-1 in cardiac muscle cells and thereby disrupt their interaction (9). This resulted in an increase in gap junction size, suggesting that ZO-1 binding to the carboxy termini of connexin proteins regulates gap junction size. It is likely that gap junction dynamics in the lens are also modulated by connexin-ZO-1 interactions and could thus be regulated with similar, peptide-based approaches.

In 2001, a study by Yin *et al.* also highlighted the importance of the connexin carboxy terminus (10). This study revealed that lens connexins are cleaved by caspases, which are enzymes involved in apoptosis that help generate lens transparency, at their carboxy termini, and that this cleavage is regulated by casein kinase II (CKII). Moreover, the truncated forms of lens connexins were localized exclusively to the lens core, implying that mature lens cells require a different type of gap junction than newly forming cells near the lens surface. While the physiological significance of connexin cleavage by caspases is not yet understood, the fact that CKII may regulate gap junction dynamics in the lens presents researchers with another possible target in the future development of non-surgical cataract treatments.

Recent research has revealed a great deal of information about the complex systems that work in concert to maintain lens transparency. These efforts at creating an accurate model of microcirculation have generated a host of possible targets for pharmacological cataract treatments. However, the current picture of internal circulation within the lens is not completely clear. The exact mechanisms of cataract development remain a mystery. The lens, while small and seemingly simple, is not at all easy to understand at the molecular level. Much work remains to be done in illuminating the dynamics of this important element of visual perception. H

—Ching Zhu '09 is a Biochemical Sciences concentrator in Matthews Hall.

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