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A stochastic model of eye lens growth

Hrvoje Šikić [a,b], Yanrong Shi [b], Snježana Lubura [a], Steven Bassnett [b,*]

[a] University of Zagreb, Faculty of Science, Department of Mathematics, Croatia
[b] Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, USA

HIGHLIGHTS

- A stochastic model of lens growth is presented.
- Lens cell division is determined by proliferative fields associated with the lens surface.
- Numerical simulations are in accordance with empirical data sets.
- Surface areas of individual cells constrain the proliferative population and limit lens growth rate.
- Precise and reproducible radial growth can be generated using a stochastic growth engine.

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ABSTRACT

The size and shape of the ocular lens must be controlled with precision if light is to be focused sharply on the retina. The lifelong growth of the lens depends on the production of cells in the anterior epithelium. At the lens equator, epithelial cells differentiate into fiber cells, which are added to the surface of the existing fiber cell mass, increasing its volume and area.

We developed a stochastic model relating the rates of cell proliferation and death in various regions of the lens epithelium to deposition of fiber cells and radial lens growth. Epithelial population dynamics were modeled as a branching process with emigration and immigration between proliferative zones. Numerical simulations were in agreement with empirical measurements and demonstrated that, operating within the strict confines of lens geometry, a stochastic growth engine can produce the smooth and precise growth necessary for lens function.

1. Introduction

The mathematical relationships that determine the size and shape of tissues and organs are not well understood. The eye, and in particular the crystalline lens, appears to represent a promising model system with which to approach this difficult question. One can reason, *a priori*, that the dimensions of the image-forming tissues are likely to be regulated with particular precision. A lens that is too large or too small will not focus light sharply on the retina.

The vertebrate lens is a transparent, biconvex structure suspended in the eye behind the iris (Fig. 1A). In conjunction with the cornea, the lens serves to focus light onto the retina. The lens is composed of only two cell types: epithelial cells and fiber cells (Fig. 1B). The lens epithelium covers the anterior surface of the tissue and contains all of the mitotic cells (Hanna and O'Brien, 1961; Mikulicich and Young, 1963; Scullica et al., 1963). Epithelial cells differentiate into fiber cells at the edge of the epithelium, following exposure to growth factors in the vitreous humor (Lovicu et al., 2011). Newly differentiated fiber cells are added to the surface of the preexisting fiber cell mass. Fiber cell differentiation represents a profound biochemical and morphological transformation. For example, fiber cells withdraw from the cell cycle and undergo an enormous (10^2- to 10^3-fold) increase in length. They also express a distinct set of proteins, crystallins, which accumulate to extraordinary concentrations in the fiber cell cytoplasm and account for its high refractive index. The rate of cell death in the fiber population is undetectably low (Shi et al., 2015; Zandy et al., 2005). As a consequence, each lens retains a complete cellular history. Fiber cells that differentiated early in life are situated in the center of the lens, whereas newly-formed fibers are located near the surface. Thus, fiber cells at all stages of differentiation are perpetually present, arranged along the lens radius in the order formed.

Unlike most tissues in the body, the lens grows throughout life (albeit at a slower rate in later years; Augusteyn, 2010). Lens growth depends on the continuous production of new cells in the epithelial cell layer but the relationship between epithelial cell proliferation, fiber cell deposition and radial growth is unclear. In a companion study, we measured the proliferative index at various latitudinal

* Correspondence to: Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, 660 S. Euclid Ave., Box 8096 St., Louis, MO 63110, USA. Tel.: +1 314 362 1604.
E-mail address: Bassnett@vision.wustl.edu (S. Bassnett).

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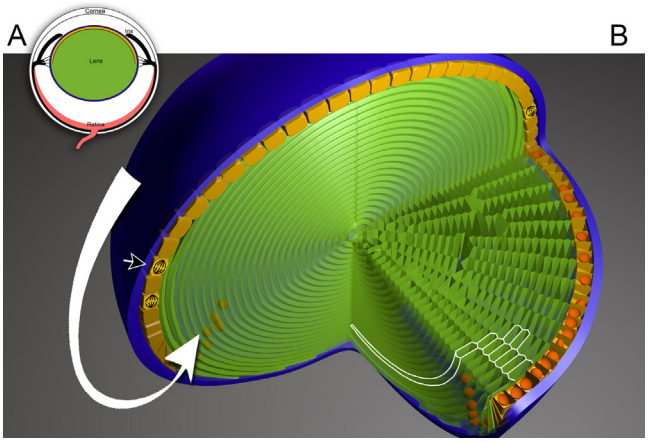

Fig. 1. Cellular arrangements of the vertebrate lens. (A) Schematic showing the location and orientation of the lens in the eye. (B) Three-dimensional cut-away model of the mouse lens. The anterior lens surface is shown uppermost. A thick basement membrane, the capsule (blue), envelops the lens. A mono-layered epithelium (yellow) runs beneath the anterior portion of the capsule. Proliferating epithelial cells (arrowhead) are numerous near the equatorial border of the epithelium but rare in the central epithelium. At the equator, epithelial cells differentiate into fiber cells (green) and are deposited on the surface of the existing fiber cell mass. Fiber cell differentiation involves a marked increase in cell length. The processes of proliferation and differentiation are continuous and together result in the collective flow of epithelial cells towards the lens equator (in the direction shown by the white arrow). There is no cell turnover in the fiber compartment. As a result, the addition of new fiber cells causes the lens to increase in volume and surface area. Fiber cells have an elongated, prismatic shape. Their intersection with the equatorial plane (highlighted in white) is hexagonal. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

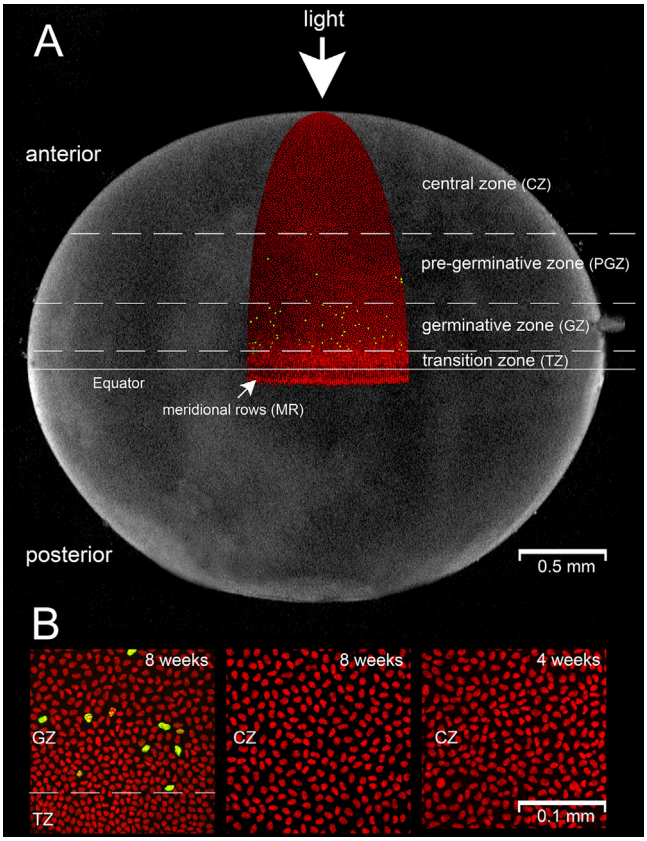

Fig. 2. Zonal organization of the lens epithelium. (A) The distribution of S-phase cells (green) is shown in an 8-week-old mouse lens (nuclei are counterstained red). Proliferating cells are most numerous in the germinative zone (GZ), although S-phase cells are also present, at lower frequency, in the pre-germinative zone (PGZ). S-phase cells are not detected in the central zone (CZ) or the transition zone (TZ). At the equator, fiber cell differentiation commences and nuclei become aligned in meridional rows (MR). Thus, the boundary between the TZ and MR marks the edge of the epithelium. (B) Cell size varies with age and latitudinal location. In 8-week-old mice, for example, cell area is smaller in the TZ and GZ compared to the CZ. The surface area of cells within the various zones increases with time (compare CZ cells at 4 weeks and 8 weeks, for example). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

positions in the mouse lens epithelium (Shi et al., 2015). On the basis of that analysis four distinct proliferative zones were identified. The highest concentration of S-phase cells was observed in a 300 μm-wide swath of cells encircling the lens above the equator (see Fig. 2A). In accordance with previous nomenclature (Harding et al., 1960) this region was called the germinative zone (GZ). At the equator, the reorganization of cell nuclei into meridional rows (MR) signified the onset of fiber cell differentiation, thus delineating the edge of the epithelium. Interposed between the GZ and the MR was a narrow strip of epithelium within which S-phase cells were not observed. This post-mitotic region was referred to as the transition zone (TZ). Our studies confirmed the existence of a pre-germinative zone (PGZ), a region extending approximately 400 μm from the anterior border of the GZ towards the apical pole. The proliferative index in the PGZ was several-fold lower than in the GZ. Proliferative cells were rare or absent in the central zone (CZ) of the epithelium. The size of individual cells was also found to vary significantly with latitude. Cells nearer to the equator (for example, those in the TZ) covered a much smaller area of the lens capsule than those in more anterior regions (Fig. 2B and Shi et al., 2015). The area of cells at all locations was also found to increase markedly with time (see Fig. 2B and Shi et al., 2015).

The migration of epithelial cells through the various zones can be visualized using a pulse-chase approach (Fig. 3 and Shi et al., 2015). If lenses are labeled with EdU (a thymidine analog incorporated into the DNA of S-phase cells) and examined immediately, proliferating cells are detected exclusively in the GZ and the PGZ (Figs. 2A and 3A). However, if lenses are examined 1 week after EdU incorporation, labeled cells are detected in the PGZ, GZ, TZ and MR (Fig. 3B). These observations support the notion that lens growth is characterized by cell division in the PGZ and GZ, migration of daughter cells through the TZ and MR and, ultimately, deposition of newly-differentiated fiber cells in the body of the lens.

The lifelong growth of the lens was first noted more than a century ago (Smith, 1883) but has yet to be modeled in detail. The growth process is of particular interest because unchecked lens growth has been implicated in the development of both presbyopia (Strenk et al., 2005) and cataract (Klein et al., 1998, 2000). A comprehensive model would necessarily incorporate the material properties of the tissue and cell biological parameters such as signaling networks or adhesive interactions. The goals of the current work were rather more modest. From a cursory inspection, it seems likely that the geometry of the lens (Fig. 1) will impose strong constraints on the growth of the system. Here we formulate and mathematically validate a simple and testable model of lens growth rooted in the unique geometry of the tissue. The model relates the production and migration of cells in the epithelial layer to the rate of fiber cell deposition and the resulting radial growth of the lens. The model derives from our own recently published measurements of lens growth kinetics (Shi et al., 2015). One might conclude from its evident precision that lens growth is a deterministic process. Here, however, growth is modeled as a stochastic process. Numerical simulations obtained from the model are in good agreement with published data sets and provide insights into the subtleties of the growth process. Perhaps surprisingly, we show that a stochastic growth engine can deliver the smooth and reproducible growth