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The molecular mechanisms underlying lens fiber elongation

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Abstract

Lens fiber cells are highly elongated cells with complex membrane morphologies that are critical for the transparency of the ocular lens. Investigations into the molecular mechanisms underlying lens fiber cell elongation were first reported in the 1960s, however, our understanding of the process is still poor nearly 50 years later. This review summarizes what is currently hypothesized about the regulation of lens fiber cell elongation along with the available experimental evidence, and how this information relates to what is known about the regulation of cell shape/elongation in other cell types, particularly neurons.

Keywords

cytoskeleton; tubulin; actin; cell shape; differentiation

Introduction

Lens Fiber Cell Morphology

The ocular lens is a remarkable structure. It is a transparent, cellular tissue which has numerous biochemical and structural specializations that form an exquisitely tuned refractive index gradient which efficiently refracts light (Figure 1A) (Bassnett et al., 2011). The earliest histological investigations of the lens noted that it is comprised of two morphologically distinct cell types surrounded by a thickened basement membrane, the lens capsule (Thin and Ewart, 1876). Lens epithelial cells are found on the anterior surface closest to the cornea, while the profoundly elongated lens fibers form the bulk of the lens (Figure 1B) (Zampighi et al., 2000). There is considerable species and developmental variation in the length of lens fibers. Chicken primary lens fibers are 200–400μm long measured from their apical tip to their basal attachment site on the lens capsule (Shestopalov and Bassnett, 2000), while adult bovine secondary lens fibers are up to 20mm in length (Kuszak et al., 2004a).

Lens fiber cells are organized into radial cell columns whose packing is optimized by their hexagonal cross sectional profile with two parallel sides ranging from 5 – 15μm wide and

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four shorter sides ranging from 1–5μm (Figure 2A) (Bassnett et al., 2011). The advent of electron microscopy revealed that lens fiber cell structure is even more complex, and can vary both between species and at different locations within the same lens (Kuszak et al., 2004a; Shestopalov and Bassnett, 2000). The straight sides of newly forming cortical fiber cells have “ball and socket” membrane specializations which are rich in gap junctions to allow for efficient cell communication in an avascular lens (Figure 3 A, B) (Bassnett et al., 2011; Kistler et al., 1986; Zhou and Lo, 2003). Each vertex at the intersection between these straight sides exhibits a very elaborate membrane structure, the membrane protrusion, which also greatly increases the surface area between cells, and may be critical for lens transparency (Figure 2B) (Kuszak et al., 1980, 2004a). In mice, fiber cells lose obvious ball and socket junctions and develop more elaborate membrane protrusions as they mature (Figure 3 C, D; Figure 4A). Overall, lens fibers at different depths within the lens have obvious differences in membrane architecture, and can develop even more complex membrane architectures, which can include the larger scale deformations of the lateral fiber cell membrane known as paddles and undulations (Figure 4) (Kuwabara, 1975). In contrast, lens fibers found in the center (the nucleus) of adult lenses tend to have only small membrane protrusions, but in many species, the straight sides develop a highly ordered membrane structure, the furrowed membrane (Figure 5), which allows lens fibers to tightly pack by reducing extracellular space, a process that may be critical to form the refractive index gradient (Al-Ghoul et al., 2001; Costello et al., 2008, 1989; Lo and Harding, 1984).

Lens differentiation

The lens derives exclusively from the head ectoderm which receives signals produced by the optic vesicle (an outpouching of the neural tube) (Chow and Lang, 2001; Donner et al., 2006; Grainger, 1992), to form the lens placode. This structure then invaginates to form the lens pit, and closes to form the lens vesicle (Cvekl and Piatigorsky, 1996). At this point, the lens vesicle is a hollow structure comprised of a basement membrane lined with polarized epithelial cells whose apical surfaces face the vesicle interior. The cells in the anterior portion of the lens vesicle (which faces the developing cornea) are fated to become the lens epithelium which will form the proliferative cell population solely responsible for any further lens cells that form throughout life (Bhat, 2001). In contrast, the lens cells in the posterior aspect of the lens vesicle (those closest to the developing retina), terminally leave the cell cycle (Griep, 2006), downregulate the expression of a subset of genes that are active in head ectoderm/lens epithelial cells (Manthey et al., 2014; Pontoriero et al., 2009; West-Mays et al., 1999), and turn on high level expression of numerous genes that will establish the lens fiber cell proteome that is necessary for lens transparency and its refractive index gradient (Duncan et al., 2004; Hawse et al., 2005; Hoang et al., 2014; Pierscionek and Regini, 2012). Simultaneously, these cells undergo a massive cell shape change where the cuboidal/flattened epithelial cell is remodeled into the elongated lens fiber cell (Bassnett and Winzenburger, 2003; Bassnett, 2005; Shestopalov and Bassnett, 2000). These “primary” lens fibers are the shortest mature lens fibers and are found in the center of adult lenses as there is little to no lens fiber cell turnover across the lifespan (Augusteyn, 2010; Bassnett and Winzenburger, 2003; Stewart et al., 2013). Further growth of the lens occurs as lens cells formed via lens epithelial cell proliferation are crowded out of the epithelium proper towards the lens equator (Shi et al., 2015). Upon reaching the lens equator, these nascent secondary

lens fiber cells come into contact with differentiation promoting FGF ligands, likely similar to those that drove primary fiber formation (Lovicu et al., 2011; de Iongh and Duncan, 2014). However, unlike primary fiber cell differentiation, secondary fiber differentiation does not appear to be as dependent on BMP (Faber et al. 2002) and Wnt/β-catenin signaling (Lovicu et al. 2011). While secondary lens fiber morphology appears more complex and tightly regulated than that of primary fibers (Bassnett et al., 2011), these cells do undergo many of the same molecular and morphological changes, leading to the formation of new fiber layers over the lens core which initially consists of only primary lens fibers (Bassnett, 2005). This process continues throughout the life span, with new lens fiber cells being added over those formerly produced to create a functional lens (Augusteyn, 2010).

Despite these significant changes in morphology during their differentiation, lens fibers initially maintain the classical apical/basal polarity characteristic of epithelial cells, maintaining a basal attachment to a basement membrane (the lens capsule) while making apical contacts with the apical side of lens epithelial cells (Zampighi et al., 2000). In primary lens fibers, this apical contact is lost as secondary lens fibers first establish apical contacts with the lens epithelium, then elongate while the apical fiber tip migrates along the apical side of the lens epithelium. When fiber cells from the same growth shell meet another fiber cell from the opposite side of the lens, they lose their apical-apical attachment with the lens epithelium and create an apical-apical interaction with another lens fiber, forming the anterior lens suture. Similarly, the basal surface of a newly formed lens fiber maintains its attachment to the lens capsule. As the fiber cell elongates, its basal tip simultaneously migrates towards the opposite side of the lens. This results in the first secondary lens fibers displacing the basal tips of primary lens fibers from the capsule. Eventually, the basal end of these secondary fibers will meet their partner from the opposite lens hemisphere, detach from the capsule, and generate the basal-basal cell contact of the posterior lens suture (Augusteyn, 2010; Joy et al., 2010; Kuszak et al., 2004a). It is notable that lens suture morphology varies between species, ranging from the umbilical sutures of birds and reptiles. the simple (line) sutures of frogs and rabbits, the Y-sutures of most mammals, and the star sutures of humans (Kuszak et al., 2004a; Kuszak et al., 2004b). The differences may arise from the constraints of lens growth (Kuszak et al., 2004a), the need to precisely position the refractive discontinuities generated by sutures in respect to the retina (Banh et al., 2006), the ability of the suture to facilitate the alterations in lens shape necessary for accommodation (Kuszak et al., 2006), and physiological considerations such as the need for fluid flow within the lens (Vaghefi et al., 2012).

Notably, while it is apparent that the hallmark of lens fiber cell differentiation is dramatic reorganization of cell shape from a cuboidal lens epithelial cell to the structurally complex fiber cell, our understanding of the molecular mechanisms underlying this lag far behind that for other critical aspects of fiber cell differentiation including the identity of the key transcription and growth factors critical for the process (Kawauchi et al., 1999; Lovicu et al., 2011; Nishiguchi et al., 1998; Wigle et al., 1999), the terminal exit of lens fibers from the cell cycle (Griep, 2006), the onset of crystallin expression (Cvekl et al., 2015), and the establishment of the lens circulation (Gao et al., 2011; Mathias et al., 2010). This review seeks to summarize our current understanding of the molecular mechanisms regulating lens fiber cell elongation.

Our current understanding of lens fiber elongation

Multiple hypotheses have been put forth to explain the profound changes in cell shape that occur during lens fiber differentiation. Here, each hypothesis and the associated evidence will be presented.

Do microtubules play a role in lens fiber cell elongation?

In 1964, Byers and Porter demonstrated that microtubules assemble at high densities during both lens placode thickening and primary lens fiber cell elongation. In primary lens fibers, these microtubules underlay the longitudinal membranes and are oriented along the axis of cell elongation. Polymerized microtubules are highly concentrated at the apical tip of the actively elongating fiber cell, while microtubules are less numerous at the posterior end of the cell and “seem to end freely”. These microtubules largely disappear as primary lens fiber cell elongation ends, but abundant microtubules are still seen in secondary lens fibers that are actively elongating. (Byers and Porter, 1964). These findings have been replicated in many other species, so the presence of organized microtubules underlying the membrane of elongating lens fiber cells is well accepted (Farnsworth et al., 1980; Lo et al., 2003; Piatigorsky, 1975). In 2003, Lo et al. reported that most lens microtubules are organized with their minus end towards the apical tip of lens fibers, while the plus ends face the basal end of the fibers. This finding is consistent with more recent studies that demonstrate the presence of microtubule organizing centers/centrosomes (MTOCs) at the apical tips of lens fibers, structures which are known to anchor the minus end of microtubules (Dahm et al., 2007; Dawes et al., 2013; Lodish, 2000; Manning et al., 2008; Sugiyama et al., 2016).

This correlation between microtubule assembly and fiber cell elongation led to the hypothesis that microtubules play important roles in lens fiber cell elongation. This proposal was first supported experimentally when embryonic day 6 (E6) chicken lens epithelial explants induced to differentiate by treatment with vitreous humor failed to elongate when treated with the microtubule disassociating agents colchicine and vinblastine (Piatigorsky, 1975; Piatigorsky et al., 1973, 1972). However, David Beebe’s group suggested that microtubules were not required for the elongation of E6 chicken lens epithelium explants in response to vitreous humor as disruption of the microtubule network with a, then new, microtubule inhibitor, Nocodazole did not block the elongation of cultured chicken lens epithelial explants treated with vitreous humor (Beebe et al., 1979). Further, they showed that colchicine inhibited lens cell elongation in this model at doses too small to affect microtubule stability, suggesting that microtubules do not play a role in fiber cell elongation. Since then, little new experimental evidence has been published either supporting or refuting the idea that microtubules are critical for lens fiber cell elongation, however, this is an area needing new investigation.

When the current understanding of lens fiber elongation is considered, it is unclear whether the day six chick epithelial explant model, in which the initial investigations of microtubule function were performed, is the most appropriate one to study the mechanisms controlling lens fiber cell elongation. Under the best circumstances, cultured lens epithelial cells only double in length from 10 μm to 20 μm after 5 hours of vitreous humor treatment, while growth slows so that cells only reach 30 μm in length during the first day in culture (Beebe

and Feagans, 1981; Beebe et al., 1979). This is in stark contrast to chicken fibers *in vivo* whose secondary lens fibers elongate approximately 150 micrometers per day (Bassnett and Winzenburger, 2003). Further, the chick lens explant system never recapitulates the full elongation/structure of a mature secondary lens fiber cell, which has a complex lateral membrane structure and can reach 20 mm or more in length (Kuszak et al., 2004a).

Notably, lens fiber cells have been compared to neurons as they share some morphological features, including the need to greatly elongate during differentiation (Frederikse et al., 2012). This observation is supported by the discovery that lens fibers express several common neuronal markers (Bitel et al., 2010; Frederikse et al., 2015). Since the prior studies on microtubules in lens fiber elongation during the 1970s, it has been demonstrated that elongating bundled microtubules at the tip of an axonal growth cone can provide the motile force to move cell plasma membranes forward to drive neuronal elongation while also regulating axonal pathfinding during neural development (Etienne-Manneville, 2013; Laan et al., 2008; Suter and Miller, 2011), even in the absence of motile forces provided by actin and myosin (Bradke and Dotti, 1999; Das et al., 2015; Dehmelt et al., 2006; Etienne-Manneville, 2013; Laan et al., 2008; Tanaka et al., 1995). Future studies might evaluate whether the interplay of microtubules and actin at the apical tip of an elongating fiber cell is structurally analogous to the neuronal growth cone. While such an analog would likely be molecularly distinct from the neuronal growth cone since the orientation of the microtubules in the lens is opposite that of neurons, it could involve the interplay between the apically localized microtubule organizing centers of elongating lens fibers (Dahm et al., 2007) and the actin cytoskeleton via the recruitment of binding proteins that link actin and microtubule networks as is seen in other epithelia (Bazellières et al., 2012).

However, microtubules may have other important roles in lens fiber cell differentiation outside of just providing a motile force for membrane extension. Lens fiber cell elongation requires a large increase in membrane area (Bassnett, 2005; Borchman and Yappert, 2010; Piatigorsky, 1981), and the production/membrane insertion of fiber preferred membrane proteins such as aquaporin 0 (Bassnett et al., 2009; Chepelinsky, 2003; Sindhu Kumari et al., 2015). In neurons and other cell types, such membrane growth occurs via release of membranous vesicles from the endoplasmic reticulum/Golgi network, and trafficking of these vesicles to their site of membrane insertion. In neurons, it is established that such trafficking is, in part, driven by microtubules in partnership with motor proteins of the kinesin/dynein families which are able to move vesicular cargos to cellular locations distant from their synthesis (Hausott and Klimaschewski, 2015). While this mechanism has not been functionally investigated in the lens, the microtubules of elongating lens fiber cells are closely associated with the Golgi network in the central region of these cells, and contact numerous vesicles throughout the lens fiber, suggesting that microtubules can transport vesicular cargos necessary to increase fiber cell membrane area during cellular elongation (Lo et al., 2003). Notably, mitochondria, a microtubule associated cargo in other cell types (Suter and Miller, 2011), including elongating neurons, have been reported to move up to 18.5 μm/minute in lens fiber cells (Bantseev and Sivak, 2005). Alternatively, microtubules have been recently shown to provide key directional information needed for planar cell polarity (Matis et al., 2014), a process critical to regulate the migration of the apical lens

fiber cell tips necessary to form the anterior lens sutures during fiber cell elongation (Dawes et al., 2014; Sugiyama and McAvoy, 2012; Sugiyama et al., 2011).

Microtubules may also play other important roles in lens fiber cell function. Treatment of intact lenses with microtubule inhibitors results in lens opacities (Mikuni et al., 1981), while some human cataracts have been reported to lose microtubules (Kuwabara, 1968). Mutations in FYOC1, an autophagy associated protein which can bind to microtubule associated kinesin motors, results in human cataracts that may be caused by defects in vesicle transit and/or disposal of autophagosomes during lens fiber cell differentiation (Chen et al., 2011). Thus, microtubules may play important roles in lens fiber cells independent of fiber cell elongation per se. Overall, the complex functions that microtubules are likely to play in the lens are an important topic for future investigation.

Fiber cell elongation driven by increases in cell volume?

After demonstrating that the ability of colchicine to block lens fiber cell elongation in the chicken epithelial cell explant model was unlikely to be due to its effect on microtubules (Beebe et al., 1982, 1979), David Beebe's group pursued the hypothesis that the initial elongation of lens fiber cells was driven by a net increase in cell volume in physically constrained cells (Beebe et al., 1979). They found that although sodium (Na+) and most metabolite concentrations did not differ significantly between lens epithelial and lens fiber cells, lens fibers had a significantly lower efflux of potassium (K+) which could increase fluid influx into these cells, resulting in cell swelling in cultured explants (Parmelee and Beebe, 1988). This was supported by their observation that culturing LECs in K+-free media blocked LEC elongation in response to treatment with vitreous humor, while culture of lens epithelial explants with an inhibitor of K+ efflux led to LEC elongation similar to the levels observed in LECs stimulated to differentiate in response to vitreous humor. This hypothesis was supported when they demonstrated that the three fold increase in chicken lens cell length observed in this culture model occurred in proportion to the increase in volume of these cells (Beebe et al., 1982). Notably, the Beebe group later demonstrated the feasibility of this mechanism as the elongation of head ectoderm cells into the thickened lens placode was largely driven by cell proliferation and the resulting crowding of the basal tips of these cells when they physically constrained to a restricted area of basement membrane (Huang et al., 2011). However, this model did not explain the additional factors or conditions that must be met to facilitate the 100 fold increase or more in cell length occurring during lens fiber cell differentiation (Bassnett and Winzenburger, 2003), nor does it explain the highly ordered membrane elaborations that lens fibers exhibit *in vivo*. Further, the initial stages of mouse secondary lens fiber differentiation *in vivo* occurred without an increase in lens cell volume (Bassnett, 2005), suggesting that either primary and secondary fiber cell elongation occur by different mechanisms or the chicken lens explant model does not recapitulate lens fiber cell elongation mechanisms *in vivo*. This could be either an intrinsic difference between *in vitro* and *in vivo* mechanisms or a difference between the initial stages of epithelial-fiber cell differentiation and those occurring later. It could also be a difference between mammals and birds, particularly since chicken lenses exhibit an exceptionally high fluid content in their developing lens as compared to other species, as well as a relatively depolarized membrane potential that could alter solute retention and osmosis in chicken lens

fibers (Bassnett et al., 1992). Overall, the role of volume regulation in driving lens fiber cell elongation is an open question, although it is unlikely to fully explain the differentiation of structurally elaborate lens fiber cells.

Does actin cytoskeletal dynamics drive fiber cell elongation?

In addition to microtubules, the lens fiber cell cytoskeleton consists of both F-actin containing microfilament networks and intermediate filaments (Rao and Maddala, 2006). Current data suggests that intermediate filaments do not play a role in lens fiber cell elongation as lens fiber cells elongate normally in animals lacking the lens preferred CP49/filensin beaded filament network (Alizadeh et al., 2002; Sandilands et al., 2004; Simirskii et al., 2006) despite the formation of cataracts in humans and animals with mutations in these genes (Song et al., 2009). Further, vimentin expression is largely restricted to the lens epithelium although lens fiber cell defects do arise in response to vimentin mutations (Matsuyama et al., 2013; Müller et al., 2009). Thus, only the potential roles for the actin cytoskeleton in lens fiber cell elongation will be discussed here.

The lens has a complex actin cytoskeletal network. In lens epithelial cells, F-actin is found in stress fibers along the basal cell membrane and in cortical rings along the lateral membranes (Weber and Menko, 2006). In some mouse strains, sequestered actin bundles are also seen at the apical tips of epithelial cells (Rafferty and Scholz, 1985). In lens fiber cells, cortical actin is found along the membranes while bundles of actin at the vertices are associated with fingerlike extensions (the “membrane protrusions”) (Kibbelaar et al., 1980; Lo et al., 1997; Lo, 1988). The basal tips of lens fibers exhibit actin networks that apparently are involved in stabilizing fiber cell/capsule interactions (Bassnett et al., 1999) which reorganize when the basal tips release from the lens capsule to form the posterior suture (Lu et al., 2008). Finally, the tips of lens fibers participating in lens sutures exhibit focal areas of actin, “the terminal web” which has been proposed to be important for stabilizing suture structure (Al-Ghoul et al., 2010). Thus, the actin cytoskeletal network of the lens reorganizes as lens fibers differentiate from lens epithelial cells, and again as fibers differentiate into their final form.

The disruption of actin interactions with N-cadherin, a transmembrane protein whose cytoplasmic tail can serve as a scaffold for actin assembly, disrupts the formation of F-actin and reduces, but does not abolish, fiber cell elongation in a chick lens explant model (Leonard et al., 2011). Removal of β1–integrin, another transmembrane protein able to serve as a scaffold for actin assembly, from either the entire lens or lens fibers exclusively, attenuates F-actin levels, and results in profound defects in lens fiber cell structure, although fiber cell elongation/differentiation per se proceeds normally (Scheiblin et al., 2014; Simirskii et al., 2007). Similarly, removal and/or inhibition of numerous regulators of actin cytoskeletal dynamics/organization such as Rho GTPases (Maddala et al., 2004), Rac1 GTPase (Maddala et al., 2011), tropomodulin (Nowak et al., 2009), and dystrophin (Fort et al., 2014), lead to disruptions in lens fiber cell structure without a profound effect on fiber cell elongation per se. Thus the role of actin in the regulation of lens fiber cell elongation is still an open question.

Treatment of cultured rat lens epithelial cells with the F-actin inhibitor, cytochalasin D blocked both their elongation and the onset of expression of the fiber cell differentiation

marker, γ-crystallin (Mousa and Trevithick, 1977). In cultured chick lenses explants, Beebe's group found that cytochalasin D treatment blocked fiber cell elongation. although they attributed the effect to a role in cytoskeleton in regulating potassium efflux and cell volume (see above) (Beebe and Cerrelli, 1989). More recently, Menko's laboratory found that transient disassembly of the actin stress fibers of the lens epithelium by cytochalasin D drove lens fiber cell differentiation as measured by the expression of the fiber cell preferred intermediate filament components, CP49 and filensin, although prolonged treatment resulted in apoptosis (Weber and Menko, 2006). All of these findings suggest an important role for actin cytoskeletal dynamics in lens fiber cell elongation/differentiation, although the direct relationship is not well understood.

If the actin cytoskeleton does play a role in lens fiber cell elongation, it could be via different, but not necessarily mutually exclusive, routes. In neurons, the tip of an elongating axon, the growth cone, develops structures that are analogous to filopodia and lammellipodia, and exhibit highly dynamic actin assembly which can drive the membrane forward, and disassembly which is critical to allow microtubules into the growth cone, suggesting that actin and tubulin networks could cooperate in fiber cell elongation (Ledesma and Dotti, 2003; Suter and Miller, 2011). Further, like microtubules, the actin cytoskeleton interacts with molecular motors, predominately of the myosin family, to move vesicular cargos within cells (Lu et al., 2014), Notably, actin-myosin motors often collaborate with tubulin-kinesin motors for final positioning of cargos such as organelles (Hammer and Sellers, 2012), again suggesting that microtubule and actin cytoskeletal networks may collaborate in lens fiber cell elongation.

Future Directions for Study

Our understanding of the molecular mechanisms driving lens fiber cell elongation lags behind that of many other aspects of lens biology, likely because we lack culture models that fully recapitulate the process of elongation that occurs *in vivo*. However, both chicken and rat lens explants do start the process when treated with factors known to drive lens fiber cell differentiation such as vitreous humor or bFGF (Beebe and Feagans, 1981; Iyengar et al., 2007; Menko and Boettiger, 1988; Musil, 2012; Piatigorsky et al., 1972; Zelenka et al., 2009), and could potentially be refined into quantitative models using modern imaging techniques. Alternatively, *in vivo* manipulation of early chick (Reza et al., 2007; Shestopalov and Bassnett, 2000) or frog embryos (Nakayama et al., 2015) could provide important insight into the mechanisms driving lens fiber cell elongation.

We have also underexploited mutant mice exhibiting defective lens fiber cell elongation as these animals have the potential to reveal fundamental regulators of this process. For instance, mutations in three different transcription factors, Prox1 (Wigle et al., 1999), cMaf (Kawauchi et al., 1999; Ring et al., 2000) and Sox1 (Nishiguchi et al., 1998), lead to a failure of lens fiber cell elongation, although why these transcription factors are important is unknown. Non-biased assessment of the transcriptome of these mutant lenses using microarrays or RNAseq could reveal novel regulators of lens fiber cell elongation. We recently used this technique to demonstrate that Prox1 regulates a large subset of the lens-preferred transcriptome, which includes many genes encoding functionally uncharacterized

cytoskeletal regulators (Audette et al., 2016). Similar techniques could be applied to study the abnormally shaped lenses of Sfrp2 transgenic mice (Chen et al., 2008) and the malformed sutures of Epha2 null mouse lenses (Shi et al., 2012), as these might elucidate the mechanisms involved in the organization and packing of lens fibers.

Similarly, removal of the fibroblast growth factor (FGF) receptors FGFR1, FGFR2 and FGFR3, from the mouse lens also leads to a failure of lens fiber cell elongation (Zhao et al., 2008). This could also result from changes in the expression of important cytoskeletal regulators as FGF signaling can alter transcription factor expression and function (Xie et al., 2016; Zhao et al., 2008, Audette et al., 2016). Alternatively, FGF signaling through MAPK, AKT and PI3-kinase pathways could change the function of actin regulators which can drive cell shape changes directly (Eswarakumar et al., 2005; Harding and Nechiporuk, 2012). Thus, investigation of such regulation in the lens could be a fruitful avenue of study.

Overall, new studies on the mechanisms regulating lens fiber cell elongation are needed to answer this long standing question. The answers will reveal important basic knowledge of how a very complex, but tightly regulated, cell shape is regulated during development to yield a functional tissue. Further, this information may further our understanding of how lenses can regenerate after cataract surgery to yield transparent lenses, an approach that has been long proposed as a solution to the problem of posterior capsular opacification (Gwon and Gruber, 2010; Gwon, 2005). Notably, a recent report demonstrated that lens regeneration can be induced following surgery for congenital cataract in human infants, yielding lenses capable of supporting good visual acuity (Lin et al., 2016).

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Highlights

- We review and provide new data regarding lens fiber anatomy in the adult and developing lens
- Multiple hypothesis for the molecular mechanisms underlying lens fiber development are presented and contrasted
- We review the historical advances made in studying lens fiber elongation and suggest future strategies for study

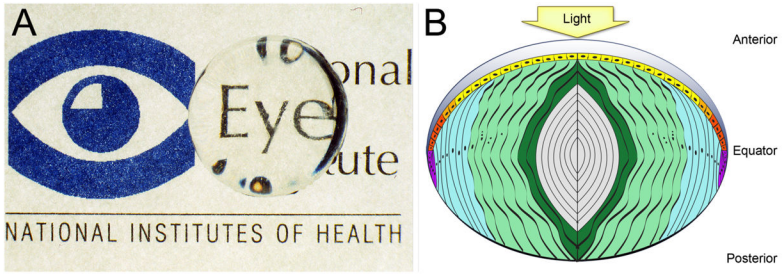

Figure 1.

A) A cow lens placed over the National Eye Institute logo showing both the clarity and refractive properties of the ocular lens. B) Parts of the lens. The lens is composed of two cell types: a monolayer of epithelial cells seen on the anterior surface, which proliferate and differentiate to fiber cells which make up the majority of the lens. Light yellow– light; dark grey– lens capsule; yellow– central epithelium; orange– germinative zone; red– transition zone; purple– meridional row region/bow region; light blue– outer cortical fiber cells; light green-inner cortical fiber cells; dark green– beginning nuclear fiber cells; light gray– nuclear fiber cells including the primary fiber cells which are found at the very center.

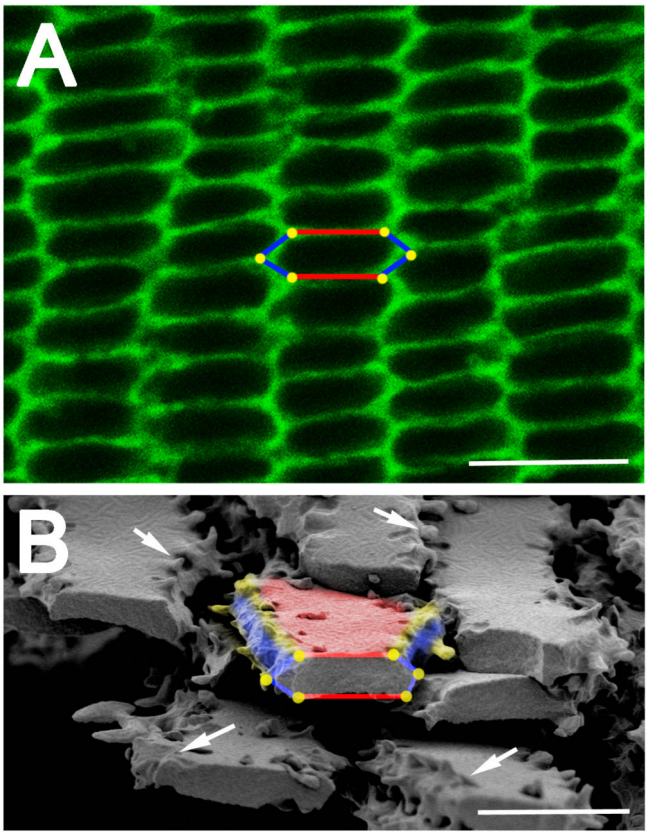

Figure 2.

Lens fiber cells have a hexagonal geometry when viewed in cross section. (A) An equatorial cross section of a mouse lens stained with fluorescent wheat germ and viewed with a confocal microscope. The hexagonal geometry of cells is highlighted in the center of the image showing the two broad sides of the cell labeled in red, the four short sides labeled in blue and the six vertices represented as yellow dots. Scale bar= 7.5μm. (B) A scanning electron micrograph showing lens fiber cells cut across their major axis allowing for viewing of their hexagonal geometry. The yellow vertices and white arrows show that there are membrane protrusions seen along these edges. Red– broad side; blue– short side; yellow– vertices/membrane protrusions; Scale bar= 5μm

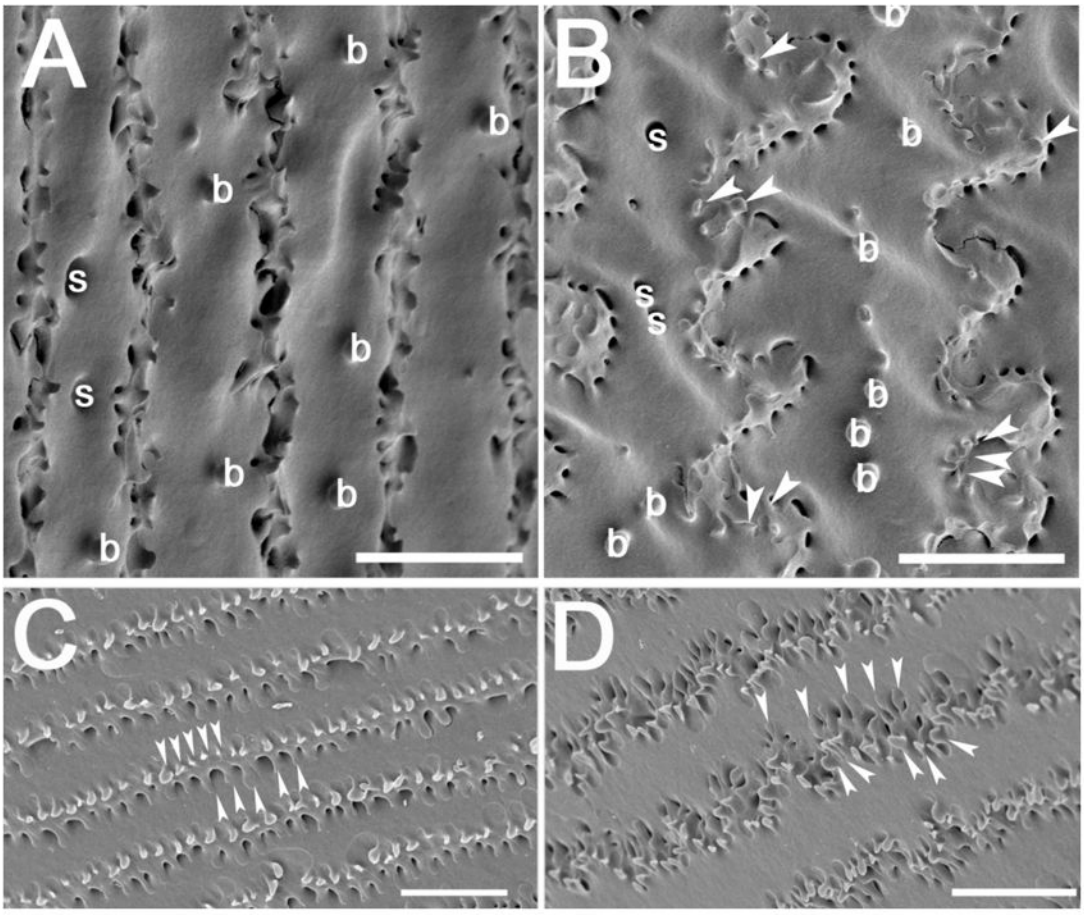

Figure 3.

Scanning electron micrographs showing ball and sockets on newly differentiated fiber cells and the formation of elaborate membrane protrusions in mouse lenses. (A) An electron micrograph showing straight fiber cells dotted with ball and sockets along the broad side of the cell in the youngest lens fiber cell layers. (B) As fiber cells mature, ball and sockets are seen along the broad sides of cells, and membrane protrusions (arrowheads) are first seen along the vertices of these cells. C) Deeper in the lens cortex, morphologically obvious ball and socket junctions are not seen, but the membrane protrusions become more obvious (arrowheads) D) In the deepest layers of the lens cortex, the membrane protrusions become even more elaborate (arrowheads). arrowheads- membrane protrusions; b– ball; s– socket; Scale bar for all panels= 5μm.

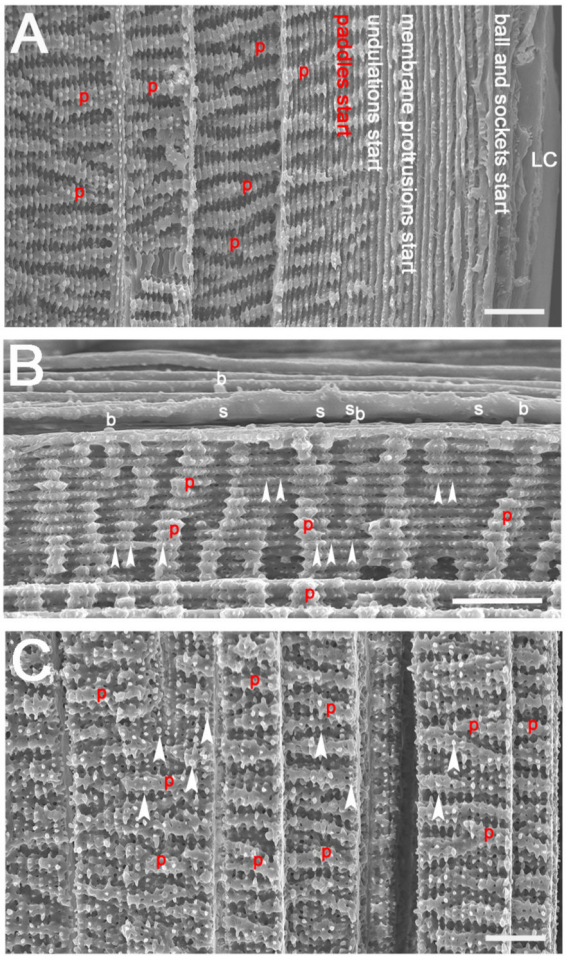

Figure 4.

Scanning electron micrographs showing longitudinal views of C57Bl/6<har> mouse lens fiber cells which highlight the changes in fiber cell morphology that occur as fiber cells mature. (A) A longitudinal view of fiber cells from the lens capsule to a depth of about 150 μm within the lens showing where different structures arise. Note though, the locations of the structures is likely to differ between mouse genetic backgrounds. (B) The interface between cells developing from young cortical fiber cells with ball and sockets to more mature fiber cells which have prominent membrane protrusions. (C) A longitudinal view of the lens at a depth of about 200 to 350 μm showing that the paddles are now more prominent and distinct membrane protrusions can also be seen along these paddles. Red p= paddle; arrowheads= membrane protrusions; b= ball; s= socket; scale bar= 10 μm

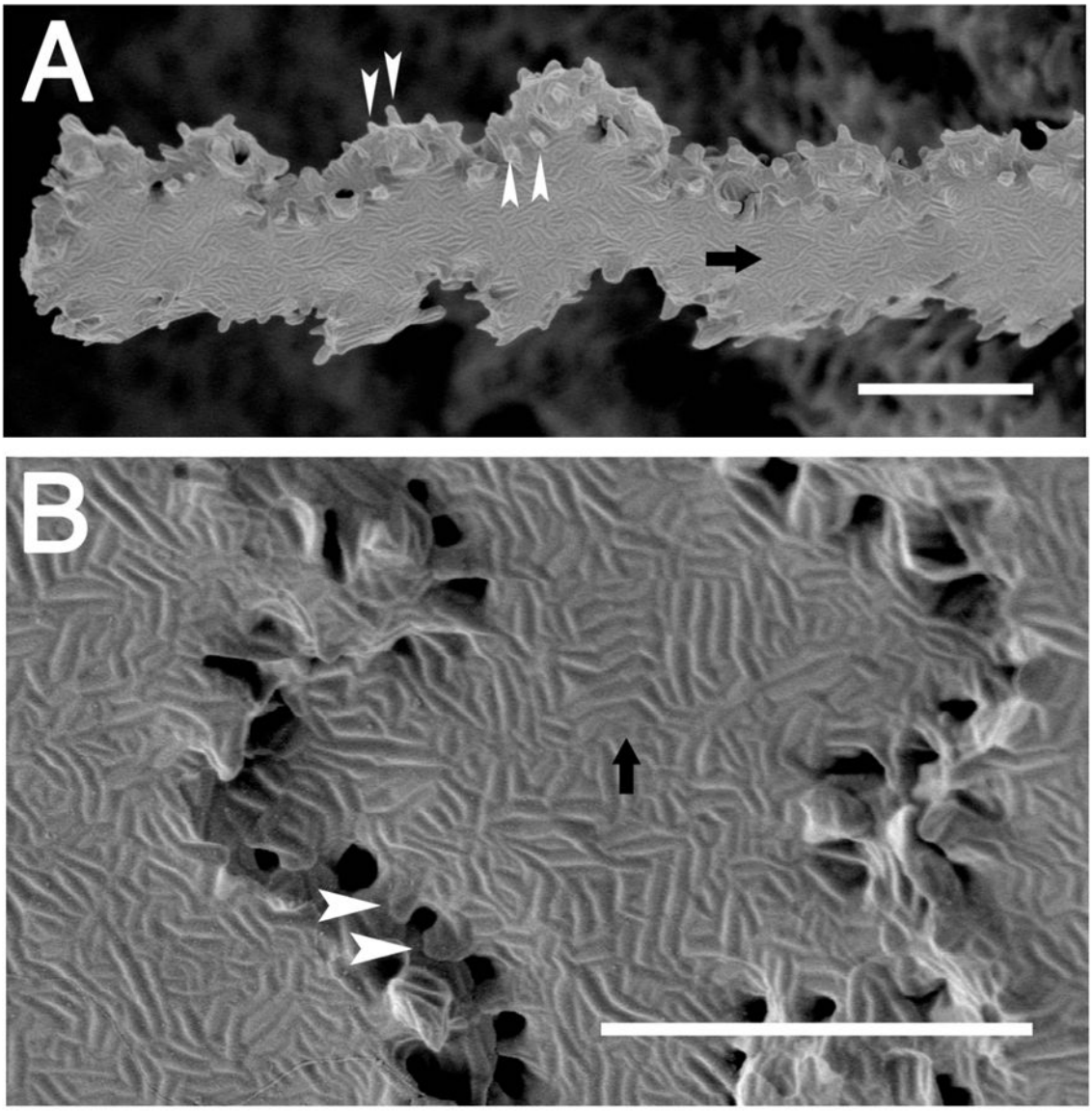

Figure 5.

Scanning electron micrographs showing that mouse lens nuclear fiber cells have elaborate "membrane furrows" on their broad sides. (A) A single nuclear fiber cell isolated from the beginning of the lens nucleus that has a slight undulation, along with membrane furrows on its broad sides and distinct membrane protrusions at the vertices. (B) A higher magnification image of the lens nucleus showing that membrane furrows even extend out to the membrane protrusions. arrow= membrane furrows; arrowheads= membrane protrusions; scale bar= 5μm